Aflatoxigenic contamination of freshly harvested white maize (*Zea mays* L.*)* from some selected Ugandan districts

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Abstract

The moisture content and total aflatoxin (AF) content of 27 samples of freshly harvested white maize (*Zea mays* L.) from Mubende (n = 3), Ibanda (n = 3), Jinja (n = 3), Mayuge (n = 3) , Buikwe (n = 3), Hoima (n = 3), Mpigi (n = 3), Masindi (n = 3) and Bugiri (n = 3) districts of Uganda representing the agroecological zones: Lake Victoria crescent, Western Highlands, South East and Lake Albert Crescent were determined in the second season harvest of January 2019 to March 2019. Moisture content ranged from 12.9 to 18.8% (mean moisture content varied from 13.9±0.35-17.2±1.55%) with the highest moisture recorded in maize from Ibanda. The highest mean AF contamination of 11.0±3.01 µg/kg was recorded in maize from Hoima while the lowest AF content of 3.8±1.30 µg/kg was recorded in maize from Mpigi. Despite the fact that all the samples had detectable aflatoxins, none of the maize samples had aflatoxin greater than WHO regulatory limit of 20 µg/kg. White maize in Uganda are precontaminated by aflatoxins prior to harvest. Whereas the spectre of aflatoxigenic contamination of foods remains a ticklish challenge to address, strategic adaptation and deployment of appropriate interventions can help secure a safe harvest. Farmers should plant maize varieties with established maturity periods to ensure timely harvesting. Further research should assess the presence of other mycotoxins as zearalenone, sterigmatocystin, ochratoxin A, citrinin, vomitoxin and diacetoxyscirpenol that may co-occur with aflatoxins in freshly harvested maize.

Introduction

Maize (*Zea mays* L.) is a non-aboriginal crop that was introduced in Uganda in 1861 [1]. It is the most important cereal in Uganda [2][3], followed by millet and sorghum [4]. It flourishes in nearly all districts of Uganda [5][6]. In 2017, maize production in Uganda was estimated at 3.02

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million metric tons, an increase from 341, 884 metric tons in 1968 that represents an average annual rate of 6.07% increase in production [7].

Maize is consumed as green maize (young and soft corn prepared by either roasting or boiling immediately after harvest), dry maize roasted as popcorns (*eberenge*) and as maize flour prepared for a variety of meals, making porridge and maize flour-based cakes [8][9].

Maize yields improved in Uganda following political stability after 1986 and sustained yearly economic growth estimated at 7%, the adoption of improved seeds like hybrid and open pollinated varieties, timely planting, proper spacing and timely weeding and harvesting. This has been unfortunately followed by fluctuations in the prices of the cereal. This excess production has been associated with high incidences of aflatoxins (AFs) as some farmers keep their grains until when prices rise up [10]. Due to poor storage practices and management of maize in storage, the maize gets pest damaged, further exacerbating the chances of AF contamination.

Aflatoxins are a family of toxins produced predominantly by filamentous fungi-*Aspergillus flavus* and *A. parasiticus.* AFs are sought to be an evolution as anti-insect or anti-rodent products for protection by the producing fungal species. Unfortunately, the toxins have made most food crops unacceptable in the regional and international markets given the health risks they pose to humans and animals after ingesting them. In Uganda, several studies have reported that AFs are high in both maize and maize products. The occurrence of AFs and *Aspergillus* species in staple Ugandan foods and poultry feeds were evaluated by Sebunya and Yourtee [11]. Of the 54 samples of maize, peanuts, soybean and poultry feed taken and precultured on *A. flavus/parasiticus* selective agar (AFPA) and analyzed for their fungal content on a coconut agar medium under UV light with a subsequent confirmatory scrutinization for AF production in a pure culture, 25 were analyzed for AFB_1, AFG_1, zearalenone, sterigmatocystin, ochratoxin A, citrinin, vomitoxin and diacetoxyscirpenol. *A. flavus/parasiticus* were reported in 77% of maize, peanuts (36% human food; 83.3% animal feed) and 66.6% in poultry feed. No fungus was detected in soybeans whereas 8% (two) samples of the 25 mycotoxins scrutinized samples had 20.0μg/kg of AFB_1 (4 times the statutory limit of 5.0 μg/kg for AFB_1 in Ugandan foods).

More recently, partnership for aflatoxin control in Africa (PACA) [12] reported that maize from some agroecological zones had AF levels of 65*u*g/kg while Tororo situated in Kioga plains (one of the agroecological zones of Uganda) had 20*u*g/kg AF content.

From the aforeacknowledged reports, it is imperative to note that AFs are distributed in all the maize growing agroecological zones of Uganda [18]. The present study augmented the foregoing reports, reporting the moisture content and total AF content of maize in some districts in Uganda with no reports of AFs in maize.

Materials and Methods

Brief description of the districts studied

Samples were collected from Mubende, Ibanda, Jinja, Mayuge, Buikwe, Hoima, Mpigi, Masindi and Bugiri districts of Uganda representing the agroecological zones: Lake Victoria crescent, Western Highlands, South East and Lake Albert Crescent.

These were selected because they are among the most prominent maize producing districts in the country. More so, some previous studies recorded alarming AF levels in maize from some of the districts [13].

Mayuge

Mayuge is bordered by Iganga to the north, Bugiri to the northeast, Namayingo to the east, Tanzania to the south and Jinja to the west (**Figure 1**). The district lies at 00º20N', 33º 30' E. It lies at approximate altitude of 1070m and 1167m above sea level, with rainfall total of 2200mm per year. Temperatures are high at over 21°C [14]. Owing to the fertile soils and favorable climate, the district has great agricultural potential. Unfortunately, open water of Lake Victoria and natural forest reserves occupy an estimated 87% of the surface area of the district, exerting a significant pressure on the natural environment due to the increasing population.

Ibanda

Created on 1st July 2005, Ibanda was a county of Mbarara district. It is bordered by Kamwenge, Kiruhura, Mbarara and Buhweju, and Rubirizi districts in the west and north, east, south, and southwest respectively. It is located at 00°07′S 30°30′E. Agriculture is the backbone of the economy of the district, with the largest farm (Kiburara Prison Farm) for Kiburara Prison. Maize, passion fruits, jack fruits, mangoes, bananas, matooke, groundnuts, water melons, coffee and tea are the major crops grown [15].

Jinja

Jinja has a tropical climate marked by significant rainfall with an average annual temperature of 22°C. The warmest month of the year is March, with an average temperature of 22.9°C while the lowest average temperature of 20.8°C is experienced in July. The difference in precipitation between the driest month and the wettest month is 136 mm with an average rainfall of 1,317 mm [16].

Bugiri

Originally a county, Bugiri was created in 1997 from Iganga district. It is bordered by Iganga in the North and West, Mayuge in the South West, Busia and Lake Victoria in the East, Tororo and Kenya in the North (**Figure 1**). The main economic activities include agriculture with emphasis on food crops: maize, rice, finger millet, sorghum, bananas, sweet potatoes, beans, peas and cassava [17]. The climate in Bugiri is warm, humid and overcast. Over the course of the year, the temperature typically varies from 16.7°C to 30.6°C. Bugiri experiences seasonal variation in the perceived humidity [18].

Mubende

Named after the ancient Mubende hill (the seat of the first capital of the famous Chwezi empire of Bunyoro Kitara), Mubende is located in central Uganda at 00°36′N 31°24′E (**Figure 1**). It is at 106m to 154m above sea level with lowland surfaces covering a great proportion while the upland surfaces are evident to the Northern border East of Butoloogo and Bukuya. Tors and

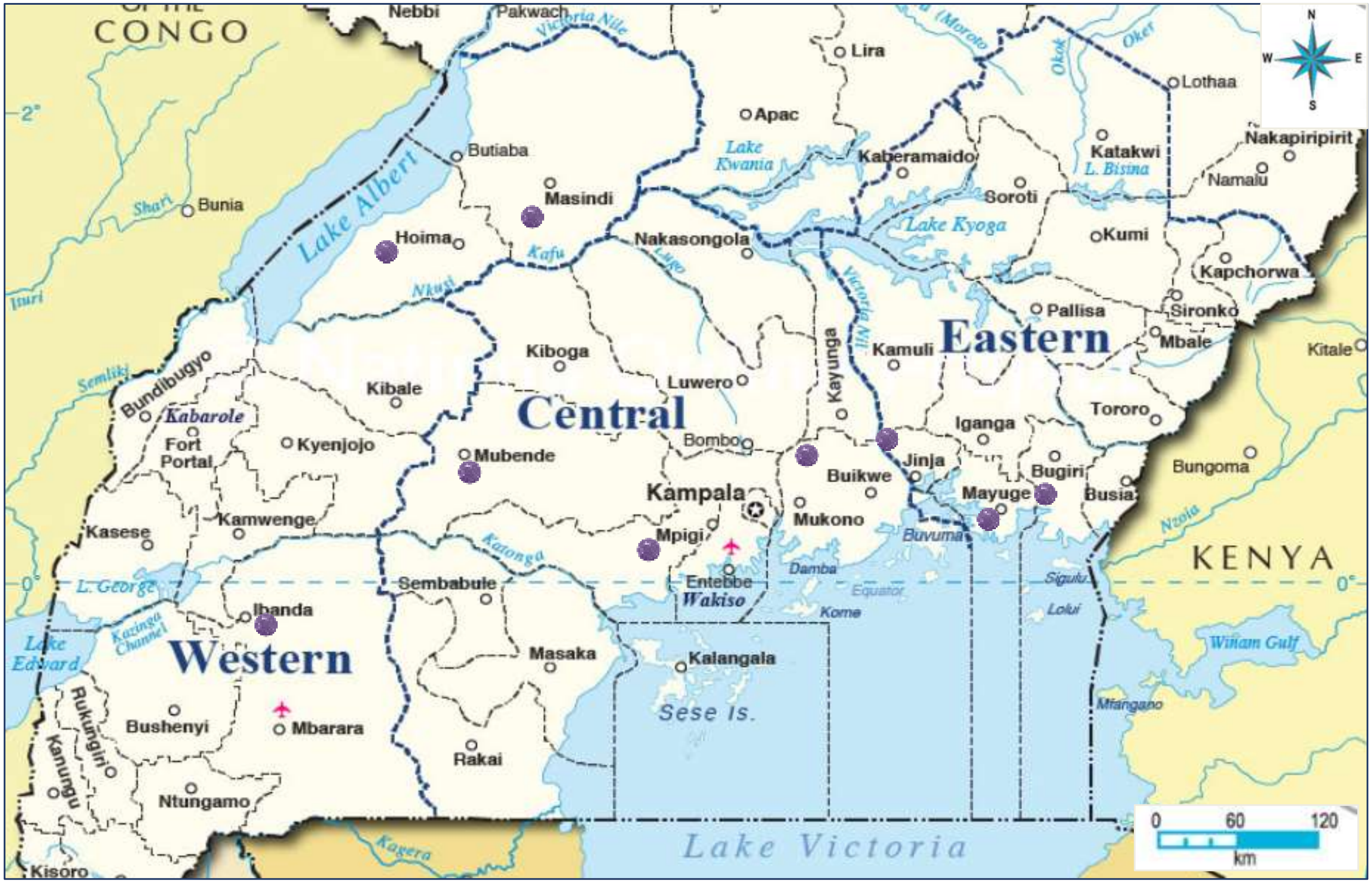

Figure 1. Map of Uganda showing the location of the studied districts where maize samples were obtained

inselbergs are common thus it experiences bimodal rainfall from March to May and September to November (up to 1,272mm). The high altitude ensures favorable climate with average annual of 21.5°C and average rainfall of 1,090mm. 70% of population depends on cultivation of bananas, sweet potatoes, cassava, Irish potatoes, beans, maize and groundnuts [19]. The driest month is January, with 42 mm of rain. The greatest amount of precipitation occurs in October, with an average of 148 mm. February is the warmest month of the year. The temperature in February averages 22.6 °C. The lowest average temperatures in the year occur in July, when it is around 20.8 °C. There is a difference of 106 mm of precipitation between the driest and wettest months. The variation in temperatures throughout the year is 1.8°C.

Masindi

Located in mid-western part of Uganda, Masindi is bordered by Nwoya in the north, Kiryandongo in the east, Nakasongola and Nakaseke districts in the southeast, Kyankwanzi to the south, Hoima to the southwest, Kiboga in the south and Bulisa to the northwest (**Figure 1**). The coordinates of the district are: 01°41’N, 31°44’E. Upto 73.1% of the population in the district are agriculturalists. The district is the leading producer of maize in the western region [20]. The district is at an average altitude of 1295 m above sea level, situated between 1^{0} 22’ and 2^{0} 20’ North of the equator, longitude 31^{0} 22’ and 32^{0} 23’ East of Greenwich [21].

Buikwe

Buikwe (coordinates: 00^{0} 21’N, 33^{0} 02’E) is a district in central Uganda bordered by Kayunga, Jinja, Buvuma and Mukono districts in the north, east, southeast and west respectively (**Figure 1**). To the south is Tanzania. Previously part of Mukono, Buikwe became a district by the Act of Parliament and it commenced operations in July 2009. In Buikwe, the average annual temperature is 21.4 °C and the average annual rainfall is 1371 mm. The driest month is January, with 69 mm of rain. The greatest amount of precipitation occurs in April, with an average of 193 mm. Most of Buikwe district lies on a high plateau (1000-1300) above sea level with some areas along Sezibwa River below 760m above sea level, Southern Buikwe is a raised plateau (1220-2440m) drained by rivers of Sezibwa and Musamya. The mean annual rainfall is 11,000mm distributed over 106 rain days, with peaks in March to May and September to November. Temperatures range between 16^{o}C and 28^{o}c throughout the year. Both relief and the climate provide a good potential for investment in cash and food crop, horticulture and floriculture on a commercial basis.

Mpigi

Mpigi district (coordinates: 00^{o}14’N, 32 ^{o}20’E) boarders the districts of Mubende in the North, Wakiso in the East, Kalangala and Masaka in the South and Sembabule in the West. The major economic activity is agriculture with food crops like sweet potatoes, beans, cassava, maize, bananas, ground nuts etc. Cash crops include coffee and cotton. More so fruits and vegetables like tomatoes, onions and cabbages are grown in the Mpigi [22].

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Hoima

Hoima district is located in Western Region of Uganda between 1°00'-2°00'N and 30°30'-31°45' E. It is bordered by Lake Albert in the west, Bundibugyo and Kibaale in the south, Masindi in the northeast and Kiboga in the east (**Figure 1**). The district lies within an altitude range of 621m and 1,158m above sea level, making it one of the lowest and hottest areas in the country. Annual rainfall ranges between 700 and 1,000 mm with a bi-modal distribution, March-May and August-November. The district is the largest producer of sweet potatoes and pigeon peas and the second largest producer of simsim in the region. Other food crops grown include bananas, finger millet, sorghum, maize, cassava, beans, soya beans, cowpeas, ground nuts, yams and Irish potatoes. Despite the ample surface water resources, agriculture is limited by low rainfall amounts.

Sample collection and preparation

Three 1kg composite freshly harvested maize samples were obtained from three local maize farmers from Mubende, Ibanda, Jinja, Mayuge, Buikwe, Hoima, Mpigi, Masindi and Bugiri districts between January 2019 to March 2019 using GIPSA sampling protocol [23].

The grains, initially on the cobs, were shelled manually. The grains were thoroughly mixed and divided into four equal working samples. An analytical sample was obtained from one of the four separated samples.

Determination of moisture content

Moisture content of the samples were determined on-site in triplicate using Superpoint handheld moisture analyzer (Supertech Agroline, Hestchaven 5, DK-5400 Bogense, Denmark; ±0.5% accuracy) following the manufacturer's instructions [24].

Quantification of total aflatoxins

Analytical samples (100±0.05g) were ground in a 500g Mulry Function Disintegrator WF-10 (Professional Chef Plus®, China) at 25, 000 rpm so that > 95% of the ground samples passed through a 20-mesh sieve.

To weighed 10.0±0.1g of the sieved samples in 250ml Erlenmeyer flasks were added 100ml of 65% Ethanol (Neogen item 8073). The solutions were vigorously agitated on a vortex agitator for 3 minutes. The pH of the resultant solutions were adjusted to between 6.0 to 8.0 and the samples were recovered by filtration through Whatmann No. 1 filter paper.

Exactly 500µL of the sample diluent was pipetted into a red dilution cup followed by 100µL of the sample. The sample and the diluent were mixed thoroughly by pipetting up and down ten times.

100µL of the resultant solution was transferred into a new clear sample cup and a new Reveal Q+ test strip (lot no. 25252) with the sample end down was placed into the sample cup and allowed to develop for 6 minutes.

Total AF in the samples were quantified by a single step lateral flow immunoassay utilizing the developed Reveal Q+ test strip for aflatoxin (Neogen Item 8085) [25] read on a calibrated

AccuSan Gold reader (Neogen Corporation, 620 Lesher Place, Lansing, MI 48912 USA-Neogen item 9595) at 18-22^{o}C [26].

Statistical analysis of results

Analytical data was subjected to statistical evaluation using GraphPad Prism (v7.05.237, GraphPad software, USA). Results were thus presented as mean ± standard deviations of triplicate analyses. One-way analysis of variance followed by Turkeys` pairwise post hoc test was used for comparison of the differences between the moisture and aflatoxin contents of the samples from the different districts. Analyses were performed at a 95% confidence interval.

Results

The moisture and total aflatoxin contents of the maize samples with their descriptive characteristics are given in **Table 1**.

Table 1. Moisture and total aflatoxin content of the maize samples

District	Moisture content (%)					Total aflatoxin (µg/kg)				
	Mean±S.D	**Range**	**S.E**	**Variance**	**EAS 2** [25]	**Mean±S.D**	**Range**	**S.E**	**Variance**	**EAS 2** [25]
Mubende^{LVC}	16.3±1.27	14.8-17.1	0.74	1.62		9.6±4.20	5.5-13.9	2.43	17.67	
Ibanda^{WH}	17.2±1.55	15.7-18.8	0.89	2.41		**10.1±3.10**	6.9-13.1	1.79	9.63	
Jinja^{SE}	14.9±0.80	14.2-15.8	0.46	0.64		9.1±4.35	4.4-13.0	2.51	18.97	
Hoima^{LAC}	14.5±0.35	14.1-14.8	0.20	0.12		**11.0±3.01**	7.9-13.9	1.73	9.03	
Mayuge^{SE}	14.7±0.30	14.4-15.0	0.17	0.09	**13.5**	**10.6±1.63**	8.8-10.9	0.94	2.64	**10**
Buikwe^{SE}	15.5±0.61	15.0-16.2	0.35	0.37		6.5±0.60	5.9-7.1	0.35	0.36	
Mpigi^{LVC}	13.9±0.35	13.5-14.2	0.20	0.12		3.8±1.30	2.6-5.2	0.75	1.70	
Masindi^{LAC}	15.3±0.96	14.3-16.2	0.55	0.92		7.2±1.99	5.5-9.4	1.15	3.94	
Bugiri^{SE}	14.3±1.35	12.9-15.6	0.78	1.82		8.5±2.56	6.7-8.9	0.67	6.74	

Agroecological zones: LVC-Lake Victoria crescent, WH- Western Highlands, SE-South East, LAC = Lake Albert Crescent [26], n = 3 for every district, S.D-Standard deviation, S.E.-Standard error.

Discussion

The moisture content of the maize samples ranged from to 12.9% to 18.8%. The mean moisture contents of the maize samples ranged from 13.9±0.35 to 17.2±1.55 (**Table 1**). Only two samples (7.4%; one from Migi and one from Bugiri) of the freshly harvested maize had of the average moisture contents within the East African standard of 13.5% safe for direct storage without drying.

Maize has been known to be highly susceptible to AF contamination in Uganda and this was also observed in this study. The maize samples analyzed had AF levels ranging from 2.6 µg/kg to 13.9 µg/kg. The mean AF contents of the maize samples were from 3.8±1.30 µg/kg to 11.0±3.01 µg/kg. The total AF content of maize grains in this study is lower than reported by other studies in Uganda probably because the maize was still fresh. It is worth noting that most investigations in Uganda assessed maize stored after harvest [3], maize sold in markets [27, 28] or maize products meant for consumption [29]. Bad agronomic practices such as drying maize on bare

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ground, mats or on polyethylene sheets have been linked to higher incidences of *Aspergillus flavus* and AFs in Uganda [3] but these have been excluded in this study.

In a preceding study by Osuret *et al.* [29], about 35% of maize samples were reported positive with AFs with nearly 60% of the positive samples exceeding the statutory maximum compliance of 10 μg/kg [25]. The total aflatoxin results reported in this study is lower than that reported by Lee *et al.* [27] in which 11% of fifty five (55) maize samples collected in a survey were contaminated with AF in the range of 12.7-123.5 mg/kg, 9 % of which exceeded the maximum regulatory limit. There were significant differences ($p = 0.05$) in the moisture content and total aflatoxin content of maize samples. This may be attributed to variations in the time of planting and climatic conditions of the different agroecological zones [3][30][31]. More humid zones encourage the growth and proliferation of aflatoxigenic fungi and subsequent production of AFs than the drier zones. In a similar study on fuminonsins (a member of the mycotoxins family to which aflatoxins belongs) in Uganda, Atukwase *et al.* [32] reported that maize samples from high altitude zone registered higher mean total fumonisins content of 4.93 $mgkg^{-1}$ vis-a-vis those from the mid-altitude-moist and mid-altitude-dry zones which registered fumonisins content of 4.53 $mgkg^{-1}$ and 4.50 $mgkg^{-1}$ respectively. More so, the time of this study was not a rainy season. A comparative study in Nigeria by Aghimien and Ikenebomeh [31] recorded the highest AF levels at 64 μg/kg in maize grains from Edo State (South-South agroecological zone). Unprecedentedly high AF levels of 2,072 μg/kg have also been reported in maize from Croatian farms and feed factories and this was attributed to a high weather temperatures and drought during the period of cultivation of the maize [33].

The AF content of maize from Mubende and Masindi reported in this are lower than 75.2 μg/kg and 45.99 μg/kg reported by PACA [12]. This could be due to the fact that the maize samples used in this study were freshly harvested. In a similar study elsewhere, Karthikeyan *et al.* [34] observed a range of AF level of 0.0 to 149.32 μg/kg in pre- and post-harvest maize sampled in Tamil Nadu, India. In other African countries, AF content of maize grains have been recorded in excess of 100 μg/kg [35][36].

Despite the fact that all the maize samples had detectable AF levels, none of the AF contents exceeded the maximum AF content of 20 μg/kg recommended by WHO/FDA for maize meant to be further processed prior to consumption. It is essential therefore, that maize used for consumption should be first dehulled, though studies reveal that this generally reduces the nutritional value of the maize product [37].

This study agrees well with that of Wu and Khlangwiset [38] which hinted that most aflatoxin problems begin and develop in the field. Strategies are needed to prevent infection of growing plants by toxigenic moulds. Pre- and peri harvest management interventions should seek to reduce or eliminate fungal infection in the field. Damage by insects, birds and stress such as drought predispose crops to aflatoxigenic *A. flavus* infection and these should be minimized.

Mycotoxins such as zearalenone, sterigmatocystin, ochratoxin A, citrinin, vomitoxin and diacetoxyscirpenol have been reported to occur simultaneously in matrices [39]. Therefore, there is need to screen for the possibility of co-occurrence of aflatoxins with other mycotoxins in maize and other foods on Ugandan markets and farms.

Conclusion

White maize in this study were precontaminated by AFs prior to harvest. Therefore, AF contamination of maize in Uganda starts from the farms. Whereas the spectre of aflatoxigenic contamination of foods remains a ticklish challenge to address, strategic adaptation and deployment of appropriate interventions can help secure a safe harvest. Farmers should plant maize varieties with known maturity periods, time planting and perform timely harvesting.

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