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Aflatoxin-producing fungi associated with pre-harvest maize contamination in Uganda

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Abstract

Maize is an important staple crop for the majority of the population in Uganda. However, in tropical and subtropical climates, maize is frequently contaminated with aflatoxins, a group of cancer-causing and immuno-suppressive mycotoxins produced by *Aspergillus* section Flavi fungi. In Uganda, there is limited knowledge about the causal agents of aflatoxin contamination. The current study determined both the aflatoxin levels in pre-harvest maize across Uganda and the structures of communities of aflatoxin-producing fungi associated with the maize. A total of 256 pre-harvest maize samples were collected from 23 major maize-growing districts in eight agro-ecological zones (AEZ). Maize aflatoxin content ranged from 0 to 3,760 ng/g although only around 5% of the samples contained aflatoxin concentrations above tolerance thresholds. A total of 3,105 *Aspergillus* section Flavi isolates were recovered and these were dominated by the *A. flavus* L morphotype (89.4%). Densities of aflatoxin-producing fungi were negatively correlated with elevation. Farming systems and climatic conditions of the AEZ are thought to have influenced communities' structure composition. Fungi from different AEZ varied significantly in aflatoxin-producing abilities and several atoxigenic genotypes were identified. The extremely high aflatoxin concentrations detected in some of the studied regions indicate that management strategies should be urgently designed for use at the pre-harvest stage. Atoxigenic genotypes detected across Uganda could serve as aflatoxin bio-control agents to reduce crop contamination from fields conditions and throughout the maize value chain.

Keywords: *Aspergillus* section Flavi, morphotype, aflatoxins, agro-ecology, maize, Uganda

1. Introduction

In Uganda, maize (*Zea mays* L.) is one of the most important crops in terms of production, consumption, and income generation. Because of its relevance, maize was one of the food security crops–and the only cereal–selected by the Ministry of Animal Industry and Fisheries of Uganda to implement a 5-year, multi-million USD agricultural intervention as part of the Development Strategy and Investment Plan (DSIP) during 2010–2015 (MAAIF 2010). The aim of the intervention was to significantly reduce the number of people suffering from extreme hunger. Production of maize in Uganda has also increased due to development and dissemination of improved varieties. The National Agricultural Research Organisation (NARO) of Uganda has developed, released, and promoted over 20 improved maize varieties. However, maize produced in Uganda is prone to aflatoxin contamination, and sometimes it harbors dangerous aflatoxin concentrations (Kaaya et al., 2005; Kaaya and Kyamuhangire, 2006; Simyung et al., 2013). In 2016, Uganda exported 215,000 tons of maize valued at US$ 53.9 million (FAOSTAT 2016). Increased production has led farmers and maize traders in Uganda to seek external markets in the Democratic Republic of Congo, Kenya, South Sudan, and Tanzania. Those countries, except Kenya occasionally, typically do not impose strict safety standards at the border due to lack of infrastructure, qualified personnel, and sampling procedures. Therefore, populations in Uganda and countries importing maize grown in Uganda could be exposed to high aflatoxin content (Kaaya and Kyamuhangire, 2006; Kaaya et al., 2005; Simyung et al., 2013). Apart from maize, other highly susceptible crops include groundnut, cottonseed, tree nuts, and chili peppers.

Aflatoxins are toxic and carcinogenic mycotoxins produced by fungi belonging to *Aspergillus* section Flavi, primarily *A. flavus* and *A. parasiticus* (Amaike and Keller, 2011; Baranyi et al., 2013; Cotty et al., 1994; Mahuku et al., 2019). Within section *Flavi*, there are 18

species that produce aflatoxins but most of them have little economic or agricultural importance (Frisvad et al., 2019). Aflatoxin pose serious health threats to both human and livestock (Baranyi et al., 2013; Bennett and Klich, 2003). Because of diverse factors, human populations in Asia and sub-Saharan Africa (SSA) have a higher risk of developing hepatocellular carcinoma (Groopman et al., 2005). Many people have lost their lives due to acute aflatoxicosis in two countries (Kenya and Tanzania) bordering Uganda (Kamala et al., 2018; Nyikal et al., 2004; Probst et al., 2007; Probst et al., 2011).

Aspergillus fungi typically infect crops in the field and may produce aflatoxins at pre- and/or post-harvest stages (Cotty, 2006). Due to their highly toxigenic nature, over 100 countries impose aflatoxin tolerance levels in foods and feeds (van Egmond et al., 2007). When exceeding tolerance thresholds, the crops cannot enter premium local and/or international markets and this results in serious negative economic impacts.

In Uganda, several environmental conditions and practices that exacerbate crop aflatoxin contamination occur frequently in the field and in storage (Kaaya et al., 2005; Kaaya and Kyamuhangire, 2006). Erratic rainfall, high temperatures and high humidity favour aflatoxin contamination in maize (Cotty and Jaime-Garcia, 2007; Mutegi et al., 2009) and these occur across Uganda. Wet, humid areas are linked to higher incidence of aflatoxin-producing fungi in various African nations (Atehnkeng et al., 2008; Kaaya and Kyamuhangire, 2006). Smallholder farmers produce most of the maize in Uganda and most of them use poor harvest techniques, and inadequately dry and store their crops. Also, cultivation of local maize varieties, which are susceptible to both insect damage and diseases, and are less drought-tolerant, predispose the maize to infection by aflatoxin-producing fungi during crop development and maturation.

Despite availability of improved varieties, local varieties are still planted by a significant portion of maize farmers.

Even though there is knowledge of the occurrence of aflatoxin accumulation in maize collected in markets and farmer stores across Uganda (Simyung et al., 2013; Sserumaga et al., 2015) little is known of the aflatoxin levels when the maize is still in the field (pre-harvest maize) and the composition of community structures of *Aspergillus* section Flavi associated with the maize in Uganda. Obtaining knowledge of structures of aflatoxin-producing fungi associated with maize in Uganda would aid to design appropriate aflatoxin management strategies for that nation.

Currently, in Uganda there are no efficient, practical, cost-effective intervention measures to prevent aflatoxin contamination right at the source of contamination, the field. A better understanding of geographical divergence, adaptation, and aflatoxin-producing abilities of *Aspergillus* section Flavi fungi in the major maize-producing AEZ of Uganda may be useful in identifying fungi that can be used to alter compositions of *A. flavus* communities and reduce aflatoxin contamination (Cotty, 2006). In addition, it is necessary to investigate aflatoxin at the field level in major maize-producing areas of the country in order to identify areas in more need of intervention. Therefore, the current study was undertaken to i) establish baseline aflatoxin levels in physiologically mature maize across major maize-growing areas in Uganda; ii) identify compositions of communities of *Aspergillus* section Flavi associated with the maize; and iii) assess aflatoxin-producing potentials of the recovered fungi to identify atoxigenic (non-toxin producing) genotypes for further evaluation as aflatoxin biocontrol agents. Results of the current study will serve as the basis to design and implement tailored aflatoxin management strategies for use in Uganda. Also, this information will be essential for identifying whether maize

contamination in the field is a critical point along the maize value chain in Uganda to prevent future outbreaks of aflatoxin poisoning (Mahuku et al., 2019).

2. Material and methods

2.1. Field survey and description of sampled areas

Maize samples were collected in the major maize-producing areas of Uganda. Samples included at least one main maize-producing district from each AEZ (Table 1). The AEZ are: 1) Eastern Savannah, 2) Busoga Farming System, 3) Eastern Highlands, 4) Lake Albert Crescent, 5) Lake Victoria Crescent, 6) Northern Farming System, 7) South Western Highlands, and 8) Western Range Lands. Ten pre-harvest maize cobs were randomly selected from each field of the 23 examined districts. Global Positioning System (GPS) readings were taken at each field using a GPS receiver model 315 (Magellan Navigation, Inc., Tulsa, OK, US). A total of 256 pre-harvest maize samples were collected and transferred to the Regional Laboratory for Mycotoxin Research and Capacity Development (RLMRCD), in Katumani, Kenya, for further processing.

2.2. Sample preparation and aflatoxin quantification of pre-harvest maize

Maize ears were hand-shelled, bulked, and 500 g were sampled and dried in an oven (130°C, 38 h). Final maize moisture content was around 8%. Samples were then ground in a coffee mill grinder (Bunn-O-Matic Corporation, Springfield, IL, US) and passed through a #12 sieve. Total aflatoxins were quantified with a GIPSA-approved lateral flow immunochromatographic assay (Reveal Q+ for Aflatoxin with AccuScan testing system, Neogen Corporation, Lansing, MI, US) following modifications to the manufacturer's instructions recommended by GIPSA. Briefly, 10 g of maize were combined with 500 ml 70% ethanol and shaken for 3 min using an orbital shaker

(HS501, IKA-Werke Company, Staufen, Germany). The mixture was filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England) and aflatoxin content determined with AccuScan testing system. Samples exceeding the upper quantification limit (150 ng/g), were diluted and quantified again. The limit of quantification of Reveal Q+ for Aflatoxin is 2 ng/g.

2.3. Fungal isolation and identification

Fungi were recovered from ground maize using dilution plate technique on modified rose Bengal agar (MRBA) (Cotty, 1994a). Ground maize (0.1 g to 10 g) was shaken in 50 ml sterile distilled water for 20 min (100 rpm) on an orbital shaker. Aliquots (100 µl per plate) of the resulting suspension were spread on three MRBA Petri plates. After incubation (3 d, 31°C, dark), up to eight colonies of suspicious *Aspergillus* section Flavi fungi were transferred to 5-2 agar (5% V8 juice (Campbell Soup Company, Camden, NJ, US); 2% Bacto-agar (Difco Laboratories Inc., Detroit, MI, US); pH 6.0) and incubated (7 d, 31°C). Isolations were performed at least twice for each sample. A total of 10 isolates were randomly selected per maize sample. Species and morphotypes were delineated using morphological (colony characteristics and spore ornamentation) and physiological (aflatoxin-producing profile) criteria (Klich and Pitt, 1988), into *A. flavus* L morphotype (avg. sclerotia diameter > 400 µm), fungi with S morphotype (avg. sclerotia diameter < 400 µm), *A. parasiticus*, and *A. tamarii* (Cotty, 1989; Klich, 2002; Klich and Pitt, 1988). There are several species with phenotype similar as to that of the *A. flavus* S morphotype (Frisvad et al., 2019); since molecular characterization was not conducted, we refer to all those morphologically similar isolates as fungi with S morphotype.

2.4. Evaluation of aflatoxin-producing ability of the recovered fungi

Fungal isolates were evaluated for aflatoxin-producing potential on maize fermentations. The fermentations were conducted at RLMRCD. All 2,192 isolates of *A. flavus* L morphotype and a randomly selected set of fungi consisting of 15 *A. parasiticus* isolates, 73 isolates of fungi with S morphotype, and 184 *A. tamarii* isolates were inoculated onto undamaged maize kernels (10 g in sterile 250 ml Erlenmeyer flasks). Maize was previously soaked in sterile water, washed, and autoclaved for 60 min. Then, maize was cooled at room temperature in a biosafety cabinet and moisture content was adjusted to 30%. Sterilized, moistened kernels were independently inoculated with 500 µl of a suspension containing approximately 10^6 spores of each of the evaluated isolates. Inoculated maize was incubated at 100% RH (7 d, 31°C, dark). Maize inoculated with 500 µl sterile distilled water served as negative control. After incubation, samples were combined with 50 ml 70% methanol and ground in a blender (Waring commercial, Springfield, MO, US) at high speed for 20 sec. The mixture was filtered using Whatman No. 4 filter paper into a 250 ml separatory funnel and 25 ml distilled water was added to ease separation. Methylene chloride, 6.5 ml, was added to the extract which was then passed through a bed of anhydrous sodium sulphate (25 g) into a Tri-Pour® beaker and later to Eppendorf® tubes. Extracts were evaporated to dryness in a fume hood chamber (dark, room temperature) and transferred to IITA-Ibadan, Nigeria for aflatoxin quantification.

In Ibadan, extracts were dissolved in 1 ml methylene chloride and subjected to scanning densitometry. Extracts were directly spotted (4 µl) alongside aflatoxin standards (4 µl, Supelco, Bellefonte, PA, US) on thin layer chromatography (TLC) Aluminum (20 cm × 10 cm) Silica gel 60 F254 plates (Merck, Darmstadt, Germany). Plates were developed with diethyl ether-

methanol-water (96:3:1) (Cotty, 1997) and visualized under ultraviolet light (365 nm) for presence or absence of aflatoxins. Aflatoxins were quantified directly on TLC plates with a scanning densitometer (CAMAG TLC Scanner 3) and quantification software (winCATS 1.4.2, Camag, AG, Muttenz, Switzerland) (Probst et al., 2011). Extracts from which aflatoxins were not detected were evaporated to dryness and residues were solubilized in an appropriate volume of methylene chloride (200 µl) for accurate densitometry and quantified as above. The limit of quantification for all experiments was 20 ng/g. Over 85% the aflatoxins present in the samples are recovered when using the described method (Atehnkeng et al., 2008).

2.5. Data analysis

Maize samples were grouped into five categories based on total aflatoxin limits imposed by the European Union (EU; 4 ng/g), the Uganda National Bureau of Standards (UNBOS; 10 ng/g), and the United States Food and Drug Administration (FDA; 20 ng/g). The categories are as follows: samples with i) no detectable aflatoxins, ii) <4 ng/g, iii) <10 ng/g, iv) <15 ng/g, and v) >15 ng/g. Densities of section Flavi fungi from each sample were calculated as Colony-Forming Units per g of sample (CFU/g). Frequencies of section Flavi fungi were calculated as proportions of the recovered section Flavi fungi. Values for aflatoxin, fungal type incidence, aflatoxin-producing potentials, and frequencies of atoxigenic fungi were subjected to analysis of variance (ANOVA) with the general linear model (GLM) suitable for unbalanced data. All statistical tests were performed with Genstat 15th Edition (Payne et al., 2012). Means were compared using paired *t*-tests and multiple comparisons were done using Tukey's honestly significant difference test to fit data as implemented in Genstat ($\alpha = 0.05$). Data were tested for normality using Kolmogorov-Smirnov test and, if required, transformed to normalize

distributions before analysis. However, actual means are presented for clarity. Aflatoxin and CFU/g values were first fractional ranked and later transformed using inverse distribution function normal, while fungal frequencies were arcsine square root transformed. Values for both fungal densities and aflatoxin-producing potentials were log transformed prior to analysis to normalize the variance. Where transformation did not achieve normality and equal variances, the non-parametric methods, Wilcoxon's Rank Sum and Signed-Rank tests were used. Districts and AEZs were treated as independent variables. Non-parametric Spearman's correlation coefficients with 2-tailed level of significance were generated to assess relationships between ecological and biological variables.

3. Results

3.1. Prevalence of aflatoxin contamination in pre-harvest maize in Uganda

Most pre-harvest maize samples evaluated in the current study were not contaminated with aflatoxins beyond thresholds set by UNBOS (Table 2). However, 16.4%, 4.5%, and 0.8% of maize samples exceeded total aflatoxin regulatory limits set by EU, UNBOS, and FDA, respectively. The averages of total aflatoxin concentrations in maize from Lake Albert Crescent and South Western Highlands were above tolerance thresholds (Table 3). Mean total aflatoxin concentration in Eastern Highlands, Lake Victoria Crescent, and Western Rangelands was <4 ng/g. In Busoga Farming System, Eastern Savannah, and Northern Farming System, mean total aflatoxin concentration was <10 ng/g. In Lake Albert Crescent and South Western Highlands mean total aflatoxin content was >15 ng/g. The highest average total aflatoxin concentration (3,760 ng/g) was detected in Hoima district while Kapchorwa and Bundibugyo had the lowest (Table 4). On average, there were no significant differences ($P < 0.05$) in total aflatoxin content

among AEZ and districts (Tables 3 and 4). Total aflatoxin levels had a relatively weak but significant positive correlation with CFU/g (r = 0.19, *P* = 0.003) (Supplementary Table 1). Total aflatoxin content was not correlated (r = 0.09, *P* = 0.21) with altitude (Supplementary Table 1).

3.2. Distribution of **Aspergillus** *section Flavi across Uganda*

From all the 256 pre-harvest maize samples, a total of 3,105 *Aspergillus* section Flavi isolates were recovered. These included *A. flavus* L morphotype, fungi with S morphotype, *A. parasiticus*, and *A. tamarii*. Across AEZ, the *A. flavus* L morphotype was the most prevalent (89.4%) followed by *A. tamarii* (6.6%; Table 5). Incidences of *A. tamarii*, fungi with S morphotype, and *A. parasiticus* were low and inconsistent in all AEZ. At district level, Iganga, Kasese, Hoima, and Kumi had the highest association with the L morphotype, fungi with S morphotype, *A. parasiticus*, and *A. tamarii*, respectively (Table 6). On average, there were significant differences ($P < 0.05$) in fungal type incidences among AEZ and districts (Tables 5 and 6).

Fungal densities were highly variable among AEZ and districts ranging from 0 CFU/g to 95,000 CFU/g (Tables 5 and 6). Only the *A. flavus* L morphotype had a significant positive correlation with CFU/g (r = 0.21, *P* = 0.001). None of the biological variables were significantly correlated with altitude. Densities of *A. flavus* L morphotype, fungi with S morphotype, and *A. parasiticus* had a negative correlation with elevation (Table 7).

3.3. Aflatoxin-producing potentials

Aflatoxin production assessment revealed that 1,771 of the 2,438 (72.6%) isolates did not produce aflatoxins. Toxigenic isolates (667) included 11 of *A. parasiticus*, 41 of fungi with S

morphotype, and 615 of *A. flavus* L morphotype. Since atoxigenicity in *A. parasiticus* and fungi with S morphotype fungi is rare, the four isolates of *A. parasiticus* and 32 of fungi with S morphotype that did not produce aflatoxins were evaluated twice and their inability to produce aflatoxins was confirmed.

Toxigenic isolates varied in their aflatoxin-producing potential (Supplementary Table 2). Toxigenic *A. flavus* L morphotype produced only B aflatoxins, as expected. When comparing total aflatoxin-producing abilities among types of fungi, significant ($P < 0.05$) differences were detected in each AEZ (Supplementary Table 2). Fungi with S morphotype (41 of 73 evaluated isolates) and *A. parasiticus* (11 of 15 evaluated isolates) with aflatoxin-producing abilities produced both B and G aflatoxins. None of the *A. tamarii* isolates produced aflatoxins, as expected (Supplementary Table 2). Within AEZ, aflatoxin-producing fungi varied largely in their ability to produce B aflatoxins. Isolates of *A. parasiticus* produced significantly ($P < 0.05$) higher amounts of aflatoxin B_1 than fungi with S morphotype and L morphotype. In general, isolates from Oyam districts produced higher aflatoxin concentrations than isolates recovered from other districts.

3.4. Distribution of toxigenic and atoxigenic isolates of *Aspergillus flavus* L morphotype

Distribution of toxigenic and atoxigenic L morphotype isolates varied across AEZ. Atoxigenic isolates were significantly ($P < 0.01$) more prevalent than toxigenic isolates (Fig. 1). In all, 66.6% of tested L morphotype isolates did not produce detectable aflatoxin concentrations and were therefore classified as atoxigenic. Incidence of atoxigenic and aflatoxin producers varied significantly ($P < 0.01$) across AEZ (Fig. 1a) and districts (Fig. 1b).

4. Discussion

The current study documents total aflatoxin levels in pre-harvest maize across major AEZ of Uganda. In addition, structures of communities of aflatoxin-producing fungi and their toxigenic potentials are reported. A relatively small fraction of the maize contained unsafe aflatoxin levels; however, in some cases the aflatoxin levels were extremely dangerous. Furthermore, we detected that most of the maize was associated with large numbers of atoxigenic fungi. The atoxigenic fungi may be useful as bio-control agents to mitigate aflatoxin contamination, from field to plot. Deaths as a result of consumption of highly contaminated food have occurred in the East Africa region, particularly in Kenya and Tanzania. There is increased recognition of the need to understand the etiologic agents of aflatoxin contamination in areas at high risk of aflatoxin contamination, including most nations in SSA (Probst et al., 2007; Shirima et al., 2013). Knowledge obtained from the current study will aid in the development of aflatoxin management strategies for use in Uganda, especially for those areas in which aflatoxin contamination was high.

4.1. Prevalence of aflatoxin contamination in pre-harvest maize in Uganda

The aflatoxin concentrations found in most of the examined samples were relatively low. In all, 25.8% of pre-harvest maize samples were contaminated with aflatoxins, but concentrations varied among and within AEZ. Results from our investigations suggest that most maize produced in Uganda is at a high risk of aflatoxin contamination once harvested and stored in an environment conducive for fungal growth, as suggested previously by Simyung et al. (2013).

In the current study, the overall total aflatoxin mean level was twice that of UNBOS threshold (Table 2). However, total aflatoxin levels in some samples were higher than those

previously reported in Uganda (Simyung et al., 2013). Similar high aflatoxin levels in maize have been reported in neighboring Kenya, where acute aflatoxicosis outbreaks and death have been linked to high aflatoxin exposure (Lewis et al., 2005). A report examining aflatoxin contamination in pre-harvest maize in Kenya reported lower levels than those found in the current study (Mahuku et al., 2019). Therefore, in Uganda, in some regions aflatoxin contamination at extremely high levels occurs during field conditions while in others the contamination results after sub-optimal storage practices in farmers stores or in market stores. We argue that we detected a wide range of aflatoxin levels, sometimes extremely high, because a well-designed strategic sampling strategy was used. Protocols used in previous studies (Sserumaga et al., 2015) were improved and allowed conducting a more robust sampling. Our sampling allowed detecting several maize samples with unsafe aflatoxin concentrations, even in areas that we found to be naturally dominated by atoxigenic fungi. Some of the maize contained high aflatoxin levels and the maize was associated with over 30% of aflatoxin-producing strains. Thus, the maize may continue accumulating aflatoxins in storage as earlier reported (Kaaya et al., 2005), if storage conditions are sub-optimal. The lower the frequencies of aflatoxin producers associated with a susceptible crop, the lower the risk of aflatoxin contamination throughout the value chain. In several nations across SSA, crops treated with atoxigenic strains become associated with a high proportion of the applied fungi (>80%) and the aflatoxin levels are drastically reduced compared to nontreated crops, which are associated with higher proportions of toxigenic fungi (Senghor et al., 2019; Ezekiel et al., 2019; Agbetiameh et al., 2019). Biocontrol products under the trade name Aflasafe containing native atoxigenic strains as active ingredient fungi have been registered in Nigeria, Kenya, Senegal, The Gambia, Ghana, Burkina Faso, Tanzania, Zambia, and Mozambique (Bandyopadhyay et al., 2016; Schreurs et al., 2019).

A strategy employing atoxigenic strains native to Uganda as biocontrol agents to limit maize aflatoxin content from field to plate, could result in reduced incidences of aflatoxin contamination events in Uganda.

4.2. Distribution of **Aspergillus** *section Flavi across Uganda*

Communities of *Aspergillus* section Flavi consist of a complex assemblage of individuals that vary widely in their phenotypic and genotypic characteristics (Agbetiameh et al., 2018; Cotty et al., 1994; Mehl and Cotty, 2010). Communities' compositions largely influence incidences and severities of contamination (Probst et al., 2010). In the current study, across AEZ, four types of fungi belonging to *Aspergillus* section Flavi were identified as in studies conducted in other SSA countries (Agbetiameh et al., 2018; Atehnkeng et al., 2008; Donner et al., 2009; Kachapulula et al., 2017). Fungal densities (CFU/g) in pre-harvest maize varied across AEZ. In the evaluated AEZ, the mean monthly temperatures and relative humidity usually exceed 25°C and 70%, respectively (Sserumaga et al., 2015). Such conditions are conducive for infection and growth of aflatoxin-producing fungi (Diener et al., 1987) and thus the population of *Aspergillus* section Flavi was expected to be high in the maize. The observed variation in populations of *Aspergillus* section Flavi across AEZ and districts could be attributed to the type of farming systems, as has been noticed earlier (Jaime-Garcia and Cotty, 2010). Many crops grown in Eastern Region Farming System are also susceptible to infection by aflatoxin-producing fungi. On the other hand, farmers in the Southern Region alternate maize with other crops like common beans and potatoes that support little to no growth of aflatoxin-producing fungi. More research is necessary to determine which specific cropping systems favor reduced incidences of aflatoxin-producing fungi.

4.3. Distribution of the toxigenic and atoxigenic isolates of **Aspergillus** *section Flavi and their aflatoxin-producing potential*

In all AEZ, the incidence of atoxigenic strains was higher than that of toxigenic strains. This may explain the low proportion of maize containing high aflatoxin levels in most of the evaluated samples (Hamidou et al., 2014). Similar findings have been reported in Nigeria and Kenya (Atehnkeng et al., 2008; Okun et al., 2015). Differences in farming systems, temperature, and humidity, among other factors are thought to have played a role in the observed aflatoxin concentrations. The *A. flavus* L morphotype was the species most frequently associated with the maize. This is similar to other studies examining maize aflatoxin-producing communities in Kenya, Nigeria, and Ghana (Agbetiameh et al., 2018; Atehnkeng et al., 2008; Okun et al., 2015). For maize produced in Uganda, aflatoxin management strategies should be directed to this species. Average aflatoxin-producing potential of *Aspergillus* section Flavi communities varied greatly across regions, as in other studies. In both Argentina (Vaamonde et al., 2003) and Iran (Razzaghi-Abyaneh et al., 2006), less than 30% of *A. flavus* isolates were found to produce aflatoxins, while in the southern US, Kenya, Zambia, Ghana, Nigeria, and Mexico the majority of *A. flavus* isolates are aflatoxin producers (Agbetiameh et al., 2018; Atehnkeng et al., 2008; Horn and Dorner, 1999; Kachapulula et al., 2017; Okun et al., 2015; Ortega-Beltran et al., 2015; Probst et al., 2011). The average aflatoxin-producing potential of the examined communities appeared to be influenced by latitude, as reported in other studies. Cotty (1997) reported a negative correlation between latitude and *A. flavus* toxigenicity, and Horn and Dorner (1999) observed greater proportions of L morphotype isolates producing aflatoxins in southern than in northern peanut-growing regions. In the current study, most *A. flavus* L morphotype isolates

were unable to produce aflatoxins. However, the toxigenic L morphotype isolates produced relatively high aflatoxin concentrations (> 1,000 ng/g aflatoxin B_1). Surprisingly, we detected that a high proportion of fungi with S morphotype (43.8%) and *A. parasiticus* (26.6%) did not produce detectable amounts of aflatoxins; isolates of both fungal types are typically extremely high aflatoxin producers. Atoxigenicity in those two types of fungi has been reported sporadically (Agbetiameh et al., 2018). The importance of atoxigenicity in those fungal types is unknown and demands further investigation.

The current study is the first that determined that pre-harvest maize is a potential source of aflatoxin exposure in certain regions of Uganda. We compared aflatoxin levels in pre-harvest maize in different AEZ following the recommendation of a previous study (Sserumaga et al., 2015). Our results indicate that high densities of aflatoxin-producing fungi are associated with pre-harvest maize although in some areas atoxigenic fungi dominated the communities. Hotspot regions for aflatoxin contamination of these crops were identified based on both environmental conditions favorable for aflatoxin contamination and high frequencies of highly toxigenic fungi, suggesting areas for preferential aflatoxin management efforts. Those areas are Lake Albert Crescent and South Western Highlands. However, studies in additional years should be conducted to determine if crops grown in these regions are perennially at risk of aflatoxin contamination.

A fairly large collection of atoxigenic *A. flavus* L morphotype isolates was identified. These atoxigenic isolates are currently being characterized to develop aflatoxin biocontrol management programs for Uganda. We have presented snapshot data of both aflatoxin contamination and fungal community structures. Since *Aspergillus* section Flavi communities are highly dynamic across areas and years (Ortega-Beltran et al., 2015; Ortega-Beltran and Cotty, 2018) more

research should be conducted to determine the stability of the communities detected in the current study.

The current study contributed towards identifying native, widely distributed, and competitive atoxigenic genotypes of *A. flavus* associated with crops and/or soils of target AEZ. The atoxigenic strains native to Uganda could be used to develop a biocontrol product for use in Uganda. If atoxigenic genotypes native to Uganda are also common in other neighboring nations then a regional biocontrol product could be designed for use in multiple target nations (Bandyopadhyay et al., 2016). Atoxigenic biocontrol of aflatoxins offers an economical, environmentally sound, cost-effective method of aflatoxin mitigation throughout the value chain (Atehnkeng et al., 2014; Bandyopadhyay et al., 2016; Cotty, 2006; Dorner, 2004; Mehl et al., 2012; Wu et al., 2008). Implementing aflatoxin biocontrol management strategies to reduce aflatoxin contamination in the field and throughout storage would result in improved health, enhanced trade, increased income, and welfare of farmers and consumers in Uganda.

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Conflict of interest

The authors have no conflict of interest to declare.

Authors Contribution

Ranajit Bandyopadhyay, Charity Mutegi and Julius P. Sserumaga conceived and designed the study; John M. Wagacha and Julius P. Sserumaga conducted sampling activities, fungal characterization, and toxin analyses. Alejandro Ortega-Beltran conducted aflatoxin quantification analyses. Julius P. Sserumaga and Alejandro Ortega-Beltran analyzed and interpreted the data. Julius P. Sserumaga, Alejandro Ortega-Beltran, and John M. Wagacha drafted and edited the manuscript and all authors helped to review and approved the final manuscript.

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Figure 1a: Distribution and incidence of toxigenic and atoxigenic strains of *Aspergillus* section *Flavi* associated with pre-harvest maize collected in diverse agroecological zones of Uganda. The error bars accompanying each bar graph represent the standard error of the mean.

Figure 1b: Distribution and incidence of toxigenic and atoxigenic strains of *Aspergillus* section *Flavi* associated with pre-harvest maize collected in diverse districts of Uganda. The error bars accompanying each bar graph represent the standard error of the mean.

Table 1: Characteristics of the eight agro-ecological zones (AEZ) under study

No.	AEZ (and districts)	Description of AEZ	Agricultural practices
1	Eastern Savannah (Soroti, Pallisa, and Kumi)	Rainfall: 800-1500 mm; 1,200-1,340 masl. Generally flat with undulating hills, moderate to good soils.	Rainfed agriculture, consisting of cereals, oil crops and pulses with moderate livestock rearing. Paddy rice grown in drained swamps.
2	Busoga Farming System (Iganga and Bugiri)	East of R. Nile and north of L. Victoria. Rainfall: 1,000-1,350 mm; 1,215-1,320 masl. Flat and swampy in places, soils poor to moderate.	Mostly rainfed crop cultivation of cereals, oilseeds, and pulses. Paddy rice grown in drained swamps.
3	Eastern Highlands (Mbale, Bulambuli, Sironko, and Kapchorwa)	Eastern, covering the ranges of Mt Elgon. Rainfall: over 1,400 mm; 1,300-3,600 masl. Mostly rich volcanic soils.	Rainfed mixed farming involving mostly stall-fed cattle, small ruminants, vegetable production, cereals such as barley and wheat in Kapchorwa, and Arabica coffee.
4	Lake Albert Crescent (Bundibugyo, Kyenjojo, Masindi Kiryandongo, and Hoima)	Rainfall of 800-1,400 mm, 620-1,585 masl, generally flat with undulating hills. Soils are good to moderate.	Rainfed mixed farming of maize, pulses, root crops, coffee and livestock rearing.
5	Lake Victoria Crescent (Wakiso, Luweero, Mityana, and Masaka)	Rainfall of 1,200-1,450 mm, 1,000-1,800 masl, hilly and flat areas, some with wetlands and forest. Soils good to moderate.	Mixed cropping of bananas, coffee, vegetables, maize and moderate dairy farming. Mostly rainfed.
6	Northern Farming System (Lira, Oyam, and Gulu)	Average rainfall 1200 mm, 975-1,520 masl, generally flat with isolated hills, fairly heavy fertile soils.	Rainfed crop cultivation, consisting of sorghum, pearl millet, cassava, sesame, and pulses. Some rearing of cattle and small ruminants.
7	South Western Highlands (Kabale)	Rainfall >1400 mm, altitude 1,300-3,960 m mountainous areas of Mt. Muhavura with mostly volcanic rich soils.	Rainfed mixed farming involving mostly stall-fed cattle, small ruminants, and vegetables, tuber crops such as potato.
8	Western Range Lands (Kasese)	Midwestern Uganda, average rainfall 915-1020 mm, 600-1,524 masl, rolling hills with some flat areas, soils are moderate to poor.	Cattle rearing is predominant mixed in places with banana production

Source: http://www.fao.org/agriculture/seed/cropcalendar/searchbycountry.do

Table 2. Percent and numbers of pre-harvest maize grain samples from Uganda with different levels of aflatoxin concentration

Category based on biological and/or economic relevance (µg/kg)	Percent (number) of crop samples
ND (<2)	74.2 (190)
<4	16.4 (42)
<10	4.5 (11)
<15	0.8 (2)
>15	4.3 (11)

Table 3. Levels of aflatoxin contamination in pre-harvest maize grain samples collected from eight agroecologies in Uganda.

AEZ	N	Range (ng/g)	Arithmetic Mean (ng/g)	Geometric Mean (ng/g)	Percent exceeding 10 ng/g
Busoga Farming System	22	<2 – 41.9	4.4±9.7[a]	1.6	13.6
Eastern Highlands	35	<2 – 20.7	1.8±3.5[a]	1.0	8.7
Eastern Savannah	30	<2 – 174.4	9.7±33.0[a]	1.7	4.4
Lake Albert Crescent	58	<2 – 3,760.0	66.5±493.5[a]	1.5	1.7
Lake Victoria Crescent	50	<2 – 7.7	1.7±1.4[a]	1.3	0.0
Northern Farming System	39	<2 – 1,81.3	7.4±29.5[a]	0.0	5.1
South Western Highlands	9	<2 – 1,287.7	145.5±428.3[a]	2.9	22.2
Western Range Lands	13	<2 – 2.6	1.7±0.8[a]	1.5	0.0
Total	**256**		**23.5**		

Table 4. Levels of aflatoxin contamination in pre-harvest maize grain samples collected from 23 districts in Uganda.

District	N^{λ}	Range (ng/g)	Arithmetic Mean (ng/g)	Geometric Mean (ng/g)	Percent exceeding 10 ng/g
Bugiri	13	<2 – 41.9	5.8±12.3[a]	1.7	20.0
Bulambuli	10	<2 – 20.7	2.9±6.3[a]	1.2	10.0
Bundibugyo	13	NA	0.7±0.3[a]	0.6	0.0
Gulu	13	<2 – 3.3	1.0±0.9[a]	0.0	0.0
Hoima	13	<2 – 3,760.0	290.7±1042.4[a]	2.3	10.0
Iganga	9	<2 – 13.1	2.4±4.0[a]	1.4	10.0
Kabale	9	<2 – 1,287.7	145.5±428.3[ab]	2.9	20.0
Kapchorwa	10	NA	1.0±0.7[a]	0.8	0.0
Kasese	13	<2 – 2.6	1.7±0.5[a]	1.5	0.0
Kiryadongo	10	<2 – 60.2	1.7±0.5[a]	1.6	0.0
Kumi	10	<2 – 60.2	7.9±18.4[a]	2.7	10.0
Kyenjojo	10	<2 – 4.1	1.7±0.9[a]	1.4	0.0
Lira	13	<2 – 181.3	20.1±49.9[a]	3.4	30.0
Luwero	13	<2 – 2.3	1.3±0.6[a]	1.2	0.0
Masaka	13	<2 – 2.3	1.6±0.9[a]	1.3	0.0
Masindi	13	<2 – 37.9	2.9±2.7[a]	2.0	0.0
Mbale	6	<2 – 6.5	2.2±2.2[a]	1.5	0.0
Mityana	10	<2 – 5.0	2.3±1.6[a]	1.8	0.0
Oyam	13	<2 – 2.6	1.1±0.6[a]	0.9	0.0
Pallisa	10	<2 – 2.2	1.0±0.8[a]	0.8	0.0
Sironko	9	<2 – 4.0	1.1±1.2[a]	0.7	0.0
Soroti	10	<2 – 174.4	20.3±54.4[a]	2.4	10.0
Wakiso	13	<2 – 7.7	1.9±2.1[a]	1.2	0.0
Total	**256**		**23.5**		

N/A: not applicable were all the samples aflatoxin content was below detection level

N^{λ} =Number of maize samples collected and tested for aflatoxin content

Table 5 Proportion of *Aspergillus* section *Flavi* comprising of major taxa, and colony forming units in pre-harvested maize samples from 8 agroecologies in Uganda

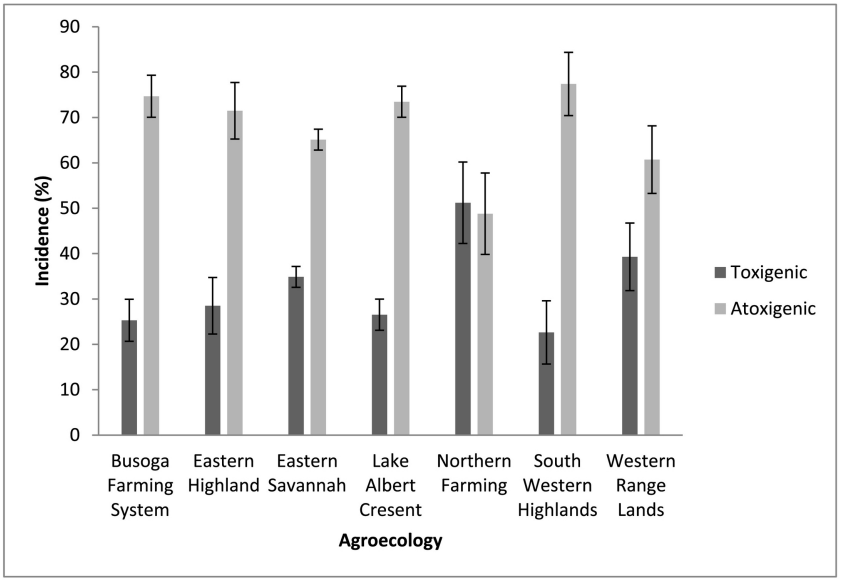
AEZ	*A. flavus* L morphotype (%)	Fungi with S morphotype (%)	*A. parasiticus* (%)	*A. tamarii* (%)	CFU/g	
					Range	Mean
Busoga Farming System	98.5±0.7[a]	0.3±0.3[a]	0.6±0.4[a]	0.6±0.4[a]	62 – 48,000	9,233[bc]
Eastern Highlands	88.5±3.8[a]	0.9±0.5[a]	0.7±0.4[a]	9.9±3.7[a]	8 – 32,500	2,216[ab]
Eastern Savannah	85.7±5.5[a]	0.8±0.6[a]	0.3±0.3[a]	13.2±5.5[a]	0 – 36,000	3,768[abc]
Lake Albert Crescent	88.7±3.3[a]	3.3±1.3[a]	1.6±1.3[a]	6.5±2.5[a]	8 – 24,000	628[a]
Lake Victoria Crescent	90.0±3.3[a]	1.0±0.5[a]	0.0±0.0[a]	9.0±3.2[a]	10 – 20,000	1,145[a]
Northern Farming System	92.2±3.0[a]	5.4±2.8[a]	0.4±0.3[a]	1.92±1.0[a]	21 – 25,000	3,712[abc]
South Western Highlands	95.4±3.7[a]	0.0±0.0[a]	0.0±0.0[a]	4.6±3.7[a]	47 – 95,000	13,435[c]
Western Range Lands	82.1±7.2[a]	16.0±6.3[b]	1.9±1.9[a]	0.0±0.0[a]	11 – 27,000	2,452[abc]
Grand Mean	**89.8±1.4**	**2.8±0.7**	**0.7±0.3**	**6.6±1.2**	**0 – 95,000**	**3,066**

Table 6 Proportion of *Aspergillus* section *Flavi* comprising of major taxa, and colony forming units in pre-harvest maize samples from 23 districts of Uganda

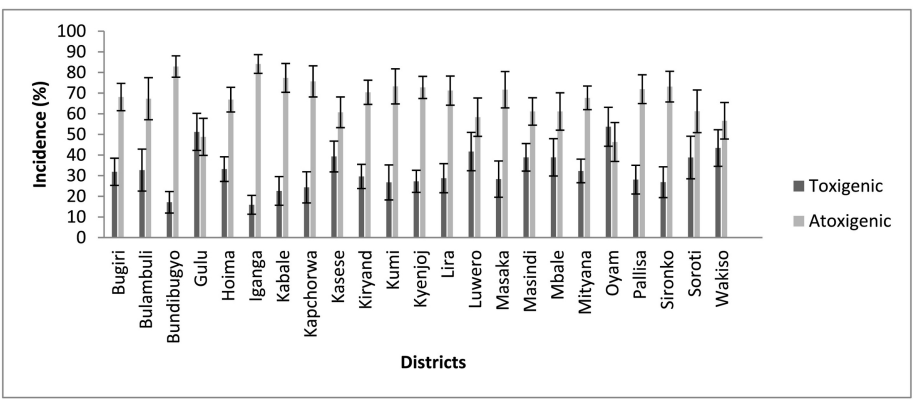
Districts	*A. flavus* L morphotype (%)	Fungi with S morphotype (%)	*A. parasiticus* (%)	*A. tamarii* (%)	CFU/g	
					Range	Mean
Bugiri	97.5±1.1[a]	0.6±0.6[abc]	1.0±0.7[a]	0.9±0.6[a]	63 – 16,000	2,703[a]
Bulambuli	92.3±5.0[a]	3.0±1.8[abcd]	0.8±0.8[a]	3.9±2.6[a]	38 – 32,500	4,718[ab]
Bundibugyo	79.5±9.1[a]	0.6±0.6[abc]	0.6±0.6[a]	19.2±9.3[a]	21 – 750	213[a]
Gulu	86.3±8.1[a]	13.7±8.1[abcd]	0.0±0.0[a]	0.0±0.0[a]	47 – 16,000	1,732[a]
Hoima	88.5±7.6[a]	5.8±2.4[abcd]	5.8±5.8[a]	0.0±0.0[a]	33 – 24,000	2,043[a]
Iganga	100.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	3,000 – 48,000	18,667[b]
Kabale	95.4±3.7[a]	0.0±0.0[a]	0.0±0.0[a]	4.6±3.7[a]	47 – 95,000	13,436[ab]
Kapchorwa	80.8±11.0[a]	0.0±0.0[a]	0.0±0.0[a]	19.2±11.0[a]	8 – 2,750	538[a]
Kasese	82.1±7.2[a]	16.0±6.3[bd]	1.9±1.9[a]	0.0±0.0[a]	11 – 27,000	2,453[a]
Kiryadongo	88.3±9.2[a]	0.0±0.0[a]	0.0±0.0[a]	11.7±9.2[a]	8 – 688	203[a]
Kumi	76.7±13.0[a]	1.7±1.7[abcd]	0.8±0.8[a]	20.8±13.2[a]	21 – 36,000	5,696[ab]
Kyenjojo	94.1±2.5[a]	4.2±2.6[abcd]	0.8±0.8[a]	0.8±0.8[a]	13 – 1,000	185[a]
Lira	96.2±2.2[a]	0.0±0.0[a]	0.0±0.0[a]	3.9±2.2[a]	29 – 22,000	6,644[ab]
Luwero	86.5±27.9[a]	1.9±1.0[abcd]	0.0±0.0[a]	11.5±7.8[a]	31 – 1,750	698[a]
Masaka	92.3±7.7[a]	0.0±0.0[a]	0.0±0.0[a]	7.7±7.7[a]	25 – 3,667	685[a]
Masindi	94.9±5.1[a]	5.1±5.1[abcd]	0.0±0.0[a]	0.0±0.0[a]	9 – 1,000	252[a]
Mbale	98.6±1.4[a]	0.0±0.0[ab]	1.4±1.4[a]	0.0±0.0[a]	42 – 1,000	434[a]
Mityana	90.8±4.9[a]	2.5±1.8[abcd]	0.0±0.0[a]	6.7±4.9[a]	21 – 13,000	1,437[a]
Oyam	94.2±2.9[a]	2.6±1.7[abcd]	1.3±0.9[a]	1.9±1.9[a]	21 – 25,000	2,760[a]
Pallisa	83.9±9.6[a]	0.0±0.0[abc]	0.0±0.0[a]	16.1±9.6[a]	8 – 14,000	1,467[a]
Sironko	86.1±6.1[a]	0.0±0.0[abc]	0.9±0.9[a]	13.0±5.8[a]	8 – 20,000	2,489[a]
Soroti	96.7±2.6[a]	0.8±0.8[abcd]	0.0±0.0[a]	2.5±2.5[a]	0 – 30,000	4,143[a]
Wakiso	89.6±5.1[a]	0.0±0.0[abc]	0.0±0.0[a]	10.4±5.4[a]	10 – 20,000	1,912[a]
Grand mean	**89.8±1.4**	**2.8±0.7**	**0.7±0.3**	**6.6±1.2**	**0 – 95,000**	**3,067**

Highlights

1. *Aspergillus flavus* L morphotype was the predominant fungus associated with maize in Uganda.
2. Densities of aflatoxin-producing fungi were negatively correlated with elevation.
3. Farming systems and climatic conditions of the AEZ are thought to have influenced communities' structure composition.
4. Aflatoxin concentrations in certain areas reached extremely unsafe levels, under field conditions.
5. Atoxigenic genotypes detected across Uganda could serve as aflatoxin biocontrol agents.


Incidence (%)
90
80
70
60
50
40
30
20
10
0
Toxigenic
Atoxigenic
Busoga Farming System
Eastern Highland
Eastern Savannah
Lake Albert Cresent
Northern Farming
South Western Highlands
Western Range Lands
Agroecology

a


Incidence (%)
100
90
80
70
60
50
40
30
20
10
0
Toxigenic
Atoxigenic
Bugiri
Bulambuli
Bundibugyo
Gulu
Hoima
Iganga
Kabale
Kapchorwa
Kasese
Kiryand
Kumi
Kyenjoj
Lira
Luwero
Masaka
Masindi
Mbale
Mityana
Oyam
Pallisa
Sironko
Soroti
Wakiso
Districts

b

Figure 1