

Full Length Research Paper

Incidence and severity of potentially toxigenic *Aspergillus flavus* in maize (*Zea mays* L.) from different major maize growing regions of Uganda

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Maize is grown in most regions of Uganda. Aflatoxin contamination of maize occurs in Uganda but there is lack of information on the distribution of *Aspergillus flavus* in major maize production regions of the country. The objective of this study was to determine the incidence and severity of *Aspergillus flavus* in major maize growing regions of Uganda. Hierarchical sampling procedure was used to randomly collect maize samples from all different fields in 16 districts in two cropping seasons of 2013. Samples were assayed for *A. flavus* incidence and severity. Results revealed significant ($P < 0.001$) variation among regions and districts within regions for *A. flavus* incidence and severity. The highest *A. flavus* incidence and severity were recorded for Pallisa (74.2% and 4.8, respectively), one of the leading maize producing districts in Uganda. Among regions, the highest *A. flavus* incidence and severity were registered in eastern region at 62.4% and 4.6 respectively. These results reveal the presence of *A. flavus* and provide information on its distribution in the key maize producing regions in Uganda. There is need for regular monitoring of aflatoxin levels in maize grain from the major maize production districts in Uganda in order to establish proper aflatoxin management guidelines.

Key words: *Aspergillus flavus*, incidence, severity, Uganda, maize.

INTRODUCTION

Aspergillus flavus is a saprophytic fungus that belongs to *Aspergillus* section *Flavi*. *A. flavus* produces a diverse

array of secondary metabolites, among which the most important are mycotoxins. A type of mycotoxin produced

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by *A. flavus* that occurs frequently and is of agricultural and health significance is aflatoxin. Maize infection by *A. flavus* and subsequent aflatoxin accumulation, poses a serious threat to both human and animals because of aspergillosis and aflatoxicosis effects (Krishnan et al., 2009). *Aspergillus flavus* can invade several types of crops which include cereals, legumes, cotton, and nut trees as well as other crops either before or after harvest hence results into food and feed contamination due to aflatoxin accumulation (Cotty et al., 1994). Losses due to aflatoxin contamination are attributed directly to crop and livestock and from the cost of regulatory programs which are designed to moderate risks to human and animal health. Estimates by Food and Agricultural Organisation (FAO) indicate that up to 25% of the world's crops are affected by mycotoxins, with aflatoxins being the most notorious (Moreno and Kang, 1999, FAO, 2002). Aflatoxin contamination in feed for livestock and poultry may result into death, suppression of immune system, growth rates reduction, and losses in feed efficiency (Sisson, 1987, Kaaya and Warren, 2005). In crops, aflatoxin contamination results in lower yields for food and fiber crops (Simyung et al., 2013).

Maize is a major crop grown in most geographic regions of Uganda. In 2011, maize occupied 19% of the total land area under food crops in Uganda (Simyung et al., 2013). In 2011/12 the total maize production was 3,150,000 MT with total export of 787,000 MT totaling USD 46.9 million (MFPED, 2014). Marketing of maize in Uganda is hampered by contamination with different mycotoxins that reduce quality (Rodrigues and Naehrer, 2002). Uganda is characterized by diverse climate in the different agro ecologies, and this may result into variability among mycotoxin causing pathogens, including *A. flavus*.

In Uganda, aflatoxin contamination of maize has been reported (Kaaya and Warren, 2005, Simyung et al., 2013) but there is lack of information on the distribution of either *A. flavus* or other fungi of *Aspergillus* section *Flavi* across the major maize growing regions in Uganda. Comparisons of aflatoxin-producing potential among *Aspergillus* section *Flavi* communities from different maize growing regions is important for understanding population dynamics and suitable control measures for field reduction of pre-harvest aflatoxin contamination (Cotty, 1997; Horn and Dorner, 1999; Calvo et al., 1999). This study was conducted to establish the incidence and severity of *A. flavus* in all the major maize growing regions of Uganda.

MATERIALS AND METHODS

Survey and sampling strategy

Field survey were conducted to collect samples and determine the occurrences of the potential toxigenic strain of aspergillus flavus form maize in different maize growing districts in different regions of Uganda (Figure 1). The surveys were carried out during two

cropping seasons: 2013A (March to July) and 2013B (August to December), which are characterized by longer and shorter rainy periods, respectively. Five districts in the western region (Hoima, Masindi, Kiryandongo, Kyenjojo, and Kabarole), four districts in the central region (Wakiso, Luwero, Mityana, and Mubende), two districts in the northern region (Lira and Oyam) and five districts in the eastern region (Iganga, Bugiri, Kumi, Pallisa, and Soroti) were surveyed. Districts were chosen to capture the different maize growing areas in Uganda with diverse cropping patterns and environmental conditions which have an influence on the occurrence of the fungus.

A three-level hierarchical sampling method was used to collect infected maize cob samples. This sampling method was used to facilitate capture of diversity of the fungus in different maize cobs by stratifying and focusing on collecting enough samples in fields within a districts and also representative districts with different regions. The samples were collected from different maize fields in each district within different regions in the country. Within each district 30 fields were selected. Within each fields a quadrant of 20*20 m was drawn and then on average 5 maize cobs were randomly sampled within the quadrant. All diseased maize cob samples were taken from all varieties in each farmer's fields in cases where farmers planted a mixture because of farmer's lack of knowledge of the varieties they cultivate. Climate data during the sampling period was accessed from the website (<http://me.awhere.com/>) (Table 1).

Fungal infection assessment

Ears in a sample collected from each field were put in the paper bags to reduce the evaporation and also absorb all the moisture from the samples, then they were sun dried with in their respective paper bags (Figure 2) for 7 days and then shelled as a bulked. One hundred sun dried kernels from each sample from each location were assayed for fungal mold using direct plating technique for internal infestation (Zhang et al., 1997; Moreno and Kang, 1999). All the Kernels were surface sterilized for 1 min in 2.5% NaOCl, washed three times in sterile distilled water, and fifty kernels were plate Standard 90mm Petri Dish in two replicates on the surface of 1/4 strength Potato Dextrose Agar (PDA) containing 9.75 g/l Potato Dextrose Broth (Difco) and 20 g/l agar, amended with 2 ml/l lactic acid to suppress bacterial contamination. Plated kernels were incubated at 31°C for 3 days. All cultures that developed from the kernels were identified on the plates based macromorphological features which included; conidial and mycelial colour, colony diameter, reverse colony colour, presence of sclerotia (Klich, 2002). *Aspergillus* ear rot disease incidence and severity were assessed using percentage kernel infection method (Zhang et al., 1997). The number of infected kernels was counted and the percent kernel infection calculated Incidence of *A. flavus* in each sample was determined as:

$$\text{Incidence} = \frac{(\text{Number of kernels infected with } A. \text{flavus}) \times 100}{\text{Total number of kernels incubated}}$$

Severity of *A. flavus* infection in each sample was visually rated on a scale of 1-5 where 1 = 0-1%; 2 = 2-5%; 3 = 6-10%, 4 = 11-49%, and 5 = 50-100% (Corkidi et al., 2006).

Statistical analysis

Incidence and severity data were subjected to analysis of variance using MINITAB version 14 (www.minitab.com). The general linear model (GLM) option was used to ascertain the influence of region, district and field on incidence and severity of *A. flavus*.

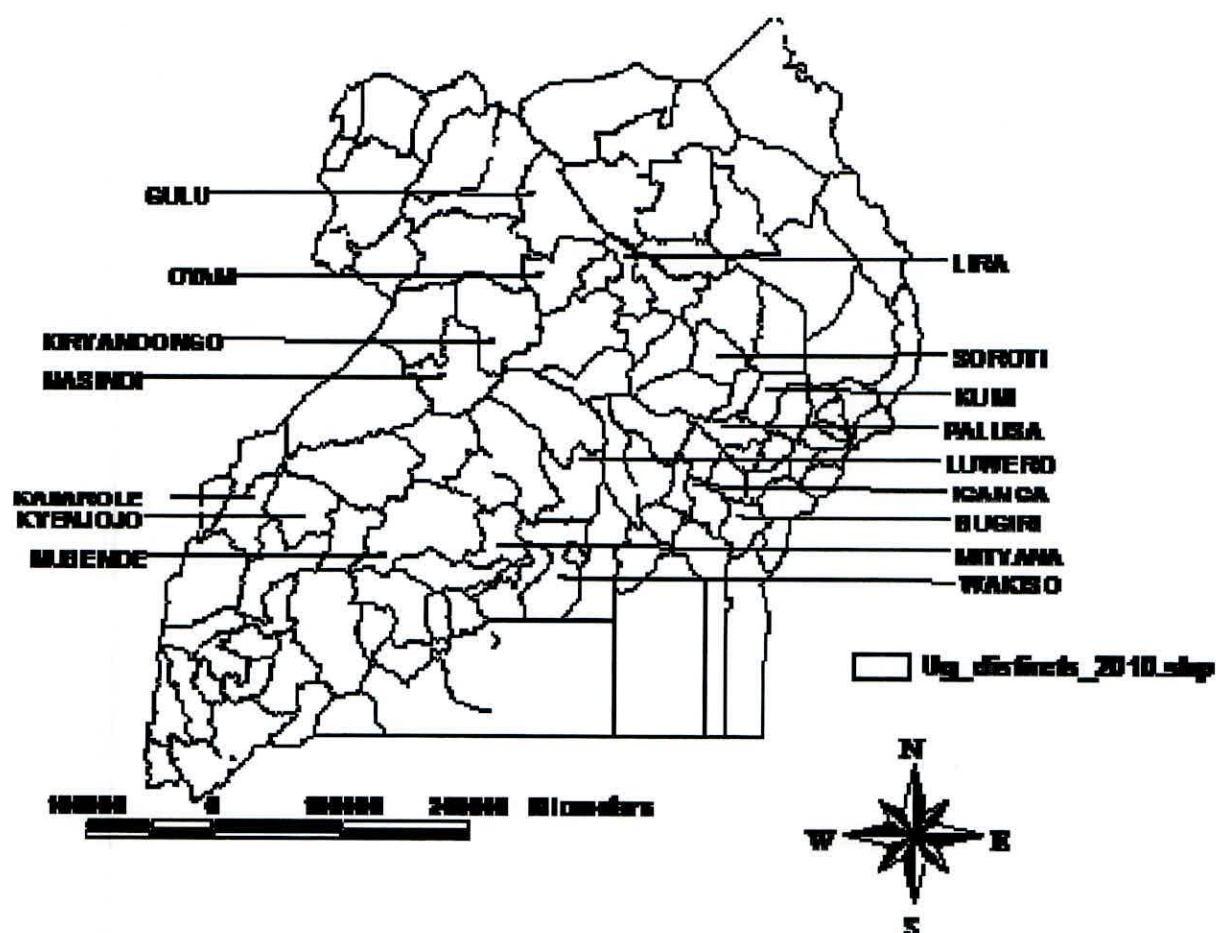


Figure 1. Map of Uganda showing sampled districts.

Table 1. Climatic conditions of districts in 2013 and number of samples collected per district.

Districts	Annual precipitation (mm)	Temperature (°C)		Relative humidity (%)	Number of samples collected
		Min	Max		
Hoima	608.0	19.6	27.7	71.4	15.0
Masindi	560.3	19.2	27.4	70.7	29.0
Kabarole	598.4	18.0	26.2	72.0	13.0
Kyenjojo	610.0	18.5	26.7	72.2	18.0
Kiryandongo	615.8	20.1	28.3	70.4	19.0
Wakiso	356.3	18.8	27.4	73.3	5.0
Luwero	484.8	19.4	27.9	73.1	17.0
Mityana	399.1	18.9	27.4	73.2	15.0
Mubende	804.6	18.2	26.6	72.5	18.0
Lira	687.6	20.0	28.6	68.6	23.0
Oyam	1120.6	20.3	28.7	68.6	14.0
Iganga	709.6	19.0	28.1	73.0	21.0
Bugiri	776.9	18.9	28.1	72.9	18.0
Kumi	527.5	19.2	28.4	70.2	13.0
Pallisa	535.7	19.1	28.3	71.2	12.0
Soroti	792.5	19.5	28.5	69.7	7.0

Source for weather data: <http://me.awhere.com>.

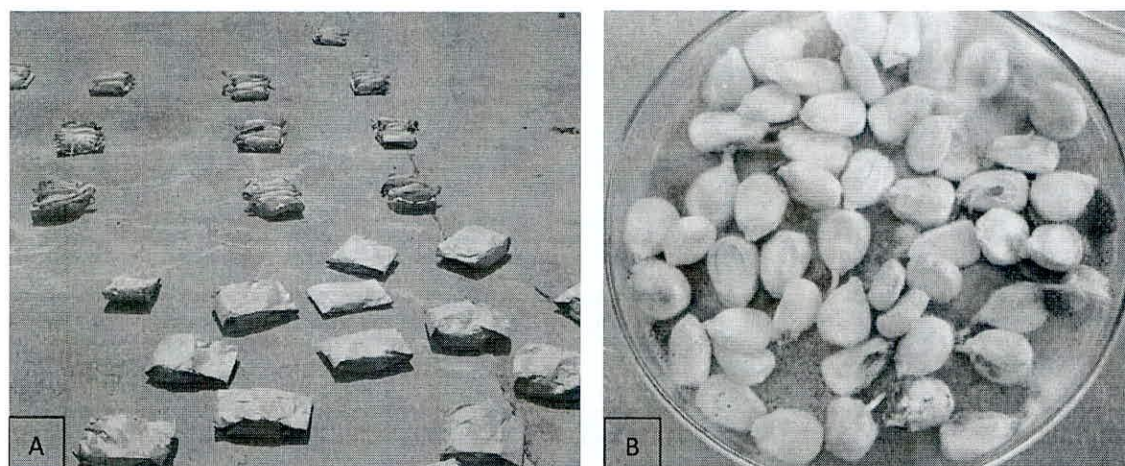


Figure 2. Sun drying of the field samples (A) and cultured maize grain on Potato Dextrose Agar (B).

Table 2. Effect of region, district, and field on *Aspergillus flavus* incidence and severity in maize samples from four regions in Uganda.

Source	df	Incidence		Severity	
		Mean square	F value	Mean square	F value
Region	3	7812.67	5.72***	20.09	18.83***
District (Region)	12	4168.58	3.05*	7.23	6.78***
Field (Region District)	226	1107.79	0.81 ^{ns}	1.92	1.80 ^{ns}
Error	15	1364.87		1.07	

*Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$.

RESULTS

There was highly significant ($P < 0.001$) variation among regions for incidence and severity of *Aspergillus flavus* (Table 2). Incidence and severity of *Aspergillus flavus* differed significantly ($P < 0.05$ or $P < 0.001$) among districts within regions but not among fields with regions and districts. The mean incidence and severity of *A. flavus* in the districts were 48.3% and 3.9, respectively (Table 3). Among the districts, Pallisa had the highest incidence (74.2%) followed by Bugiri (73.9%) among the districts surveyed. These two districts also recorded some of the highest severity values of *A. flavus*. Kumi and Soroti districts had significantly lower incidence than both Pallisa and Bugiri that are in the same region (Table 3). Both Masindi and Hoima districts had significantly lower incidence than other districts in the same region. Wakiso district had the lowest incidence (7%) and least severity (2.0) of *A. flavus* among the districts in central region and overall. At the regional level, the highest *A. flavus* incidence (62.4%) was registered in the eastern region (Table 4). The eastern region had the two districts with the highest incidence. The northern region registered the lowest incidence (28.9%) but this did not differ

significantly from the incidence recorded for the western region. Severity of *A. flavus* infection was highest in the eastern region (4.6) and lowest in the northern region (3.0).

DISCUSSION

This study was conducted in the major maize growing regions and samples analyzed revealed occurrence of *A. flavus*. *A. flavus* was identified in all the samples collected in different districts of Uganda. These results indicate that *A. flavus* is widely distributed in the country and that most of the commonly grown maize varieties are susceptible to fungal spoilage and aflatoxin contamination. These results corroborate earlier reports that aflatoxin accumulation in different food and feed commodities are a major threat to not only maize production in the country but also to both human and animal health (Kaaya and Warren, 2005). In a study by Simyung et al. (2013), about 11% of samples collected from different districts of Uganda were contaminated with aflatoxin that ranged from 12.7 to 123.5 $\mu\text{g/Kg}$. In addition, 9% of samples exceeded the range of 4 $\mu\text{g/kg}$ to

Table 3. *Aspergillus flavus* incidence and severity in maize samples from 16 districts of Uganda.

Districts	Incidence (%)	Severity (1-5)
Hoima	31.3	3.2
Masindi	16.7	2.4
Kabarole	60.0	4.2
Kyenjojo	58.1	4.4
Kiryandongo	63.9	4.5
Wakiso	7.0	2.0
Luwero	62.9	4.2
Mityana	55.7	3.6
Mubende	59.7	4.1
Lira	28.3	2.9
Oyam	30.0	3.3
Iganga	61.4	4.6
Bugiri	73.9	4.6
Kumi	48.5	4.7
Pallisa	74.2	4.8
Soroti	41.4	4.8
Mean	48.3	3.9
Standard error of difference (SED)	20.2	0.9

10 or 15 µg/kg as per the new European Union aflatoxin maximum level guidelines (EU, 2010). Because we could not obtain the names of the varieties planted by the farmers, it was not possible to ascertain whether high disease incidence was related to adoption of elite maize varieties or the use of older maize varieties in the study areas. The variability of both incidence and severity of *A. flavus* observed among district and regions may be attributed to the different weather patterns prevailing during the sampling periods.

During the study period, annual rainfall ranged from 356.3 to 1120 mm (Table 1) which was low compared to the normal rainfall amounts characteristic of Uganda which range from 1000 to 1400 mm, but the mean temperature was as high as 28°C with average relative humidity of up to 73.2%. Environment conditions characterized with high temperatures and drought stress have an impact on the physiology of both the host and fungus, with high temperature which favour fungal growth may affect the infected plant. In addition, there are compounds present within the kernel that are induced when infected by *Aspergillus* spp. which affect its development hence the low severity (Chanda et al., 2009, Atehnkeng et al., 2008). Studies have demonstrated that kernel pericarp wax has been associated with resistance to *Aspergillus* infection and inhibited growth (Gembeh et al., 2001; Brown et al., 2013). In addition Gas chromatography/mass spectroscopy (GC/MS) analysis of the whole was component showed a higher percentage of phenol-like compounds in the resistant genotypes than in the susceptible lines (Gembeh et al., 2001). Another study which examined kernel proteins revealed

differences between genotypes resistant and susceptible to aflatoxins contaminations (Huang et al., 1997)

Earlier studies have indicated that *A. flavus* in maize may differed between the different agro-ecological zones due to the prevailing climatic conditions (Cotty and Jaime-Garcia, 2007), the cultivars grown in each zone, the cultural practices and / or the storage methods (Sétamou et al., 1997). Land management strategies and, particularly, crop rotation systems and factors such as genotype may influence crop infestation by *Aspergillus* section Flavi and the aflatoxin content of maize.

Although rainfall received was low but temperature were high during the seasons when this study was carried out (Table 1), it created an environment characteristic of warm and humid. Such conditions are conducive for *Aspergillus* growth. Similar results were reported for maize in the USA (Anderson et al., 1975) but are contrary to results by Sisson (1987) which revealed that minimum temperature above 21°C was negatively correlated with aflatoxin incidence in maize in the USA. *Aspergillus* strains have been reported to survive in a wider range of temperature from 19 to 35°C (Northolt and Van Egmond, 1981), although 28°C is the most conducive for aflatoxin production (Sanchis and Magan, 2004; Simyung et al., 2013). The mean temperature in the districts covered by the study was 28°C for most of the districts, and this probably enhanced the development of *A. flavus*. This is exemplified by the high incidence and severity in Pallisa district which experienced moderate rainfall, high temperatures (28°C) and high relative humidity (71%) (Table 1).

In addition to temperature, availability of moisture has a

significant impact on *A. flavus* growth and aflatoxin production. This combination of susceptible genotypes and good weather (warm-humid) supports high pathogen severity and incidence. Besides the climatic factors, the high incidence of *A. flavus* in samples from Pallisa, Bugiri, Kiryandongo, and Iganga, could be explained by the common farming practice of leaving maize in the field for more than three weeks after physiological maturity, which leads to increased incidence of *A. flavus* (Kaaya et al., 2005).

These results provide for the first time, key evidence about the presence and distribution of *A. flavus* in the key maize producing regions in Uganda. The implication of these results is that maize grain from these districts may place consumers at risk of exposure to aflatoxin. It is necessary to establish the levels of aflatoxin contamination in maize grain from farmers' fields and maize grain dealers in these districts and correlate these with the incidence and severity of *A. flavus*. This information will be useful for the formulation of guidelines on aflatoxin management in Uganda. From the earlier studies (Gardner et al., 1987; Campbell and White, 1995), combined use of management control strategies should be considered to reduce *Aspergillus* infection and hence aflatoxin control.

Incidences of over 50% in samples from central and eastern regions suggest that *A. flavus* occurrence is widespread in some of the important maize production regions in Uganda. Our study demonstrates presence of *A. flavus*, and these are useful resources for identifying and developing biological control technology to manage aflatoxins (Atehnkeng et al., 2008). However, more research is required to evaluate these atoxigenic strains for identifying a few effective and adapted atoxigenic vegetative compatibility groups that can be pursued in developing biological control technology in Uganda. Also further work is needed to understand the relationship between pathogen occurrence and aflatoxin levels along the maize supply chain.

Conflict of Interest

The authors have not declared any conflict of interest.

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