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## Fungal Microflora Causing Maize Ear Rots in Uganda and Associated Aflatoxins

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**Abstract:** Freshly harvested dry maize ears were sampled from farmers in 2002A and 2002B seasons in ten major maize growing districts of Uganda and kernels plated onto malt extract or malt salt agar for mould growth and were also analysed for moisture and aflatoxin content. *Fusarium*, *Aspergillus*, *Penicillium*, *Phomopsis*, *Acremonium*, *Stenocarpella* and *Rhizopus* were the genera identified. *Fusarium verticillioides* and *Aspergillus flavus* were the most common mould species identified and were isolated from both symptomatic and asymptomatic maize samples in both seasons. Overall, mean aflatoxin levels of both asymptomatic and symptomatic samples were lower than the FDA/WHO 20 ppb regulatory limit. Samples from Masindi district had the highest mean aflatoxin levels (13 ppb) followed by those from Mayuge (11.8 ppb) while those from Kapchworwa had the least mean levels (0.7 ppb). Samples collected during 2002A season had generally higher moisture content, mould incidence and aflatoxin levels than the 2002B samples. Although the findings from the study were only able to show aflatoxins, there is a strong likelihood of other mycotoxins occurring in maize grain in higher proportions. There is therefore the need to quantify these toxins to form a basis for the next course of action.

**Key words:** Ear rots, microflora, asymptomatic, symptomatic, mycotoxins, safety

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### INTRODUCTION

Maize (*Zea mays* L.) in Uganda doubles both as a food and cash crop. Its production however, is curtailed by a number of factors both biotic and abiotic. Many of these constraints have been given a lot of attention as attested by considerable research carried out for their containment. However, the ear rots for a long time have been given low priority and following increased occurrences (Kikafunda-Twine *et al.*, 2001), their attention can no longer be overlooked. Among the common fungal species commonly reported to cause maize ear rots are *Fusarium* spp., *Stenocarpella* spp., *Nigrospora* spp. and *Aspergillus* spp. (Kapindu *et al.*, 1999) and their importance is well documented in some parts of the world including America, Asia and some countries of Africa (Latterell and Rossi, 1983; Flett and van Rensburg, 1992; McDonald and Chapman, 1997) but hardly any from Uganda.

The other quality aspect that has been associated with maize ear rots is their causal agents' ability to produce mycotoxins. The most commonly associated

mycotoxin is aflatoxin caused mainly by *Aspergillus flavus* Link which has been reported to be carcinogenic and immuno-suppressor in both human and animals (Sétamou *et al.*, 1998; Williams *et al.*, 2004). In Uganda, aflatoxins have been reported to occur in both pre and post harvested maize grain (Sebunya and Yourtee, 1990; Kaaya *et al.*, 2005). Consequently, the quality of maize produced in the country is often queried on regional markets.

The objectives of the study were two fold (i) to identify the fungal microflora responsible for the observed maize ear rots in Uganda; (ii) to determine the aflatoxin levels in the maize kernels. This information is very important in initiating maize ear rot management strategies in Uganda as well as reducing mycotoxin contamination in the food and feeds.

### MATERIALS AND METHODS

**Sampling:** Maize ears used in this study were sampled from ten districts of Uganda namely; Kamuli, Iganga, Mayuge, Tororo, Masaka, Masindi and Mubende for mid-

altitude areas (900-1500 m above sea level) and Mbale, Sironko and Kapchorwa, for high altitude areas (above 1500 m above sea level). These districts were purposely chosen because they are the major maize producers in the country (Rwabwoogo, 2002). The samples were collected over two seasons; first season 2002 (July - October) and second season (December 2002-January 2003). These seasons are subsequently referred to as 2002A and 2002B, respectively. However, no sampling was done in Kapchworwa during 2002B season because maize in this district is harvested once a year, during the month of October. Forty samples were collected from farmers in five different villages in each district, spaced approximately 8-10 km apart. During sampling, 4 healthy (asymptomatic) and 4 infected (symptomatic) ears (all of almost same size), were picked from each farmer's field when the maize was dry and ready for harvesting. The ears were sampled at this stage because field drying of maize is a practice carried out by all farmers in Uganda. Thus, twenty healthy and twenty symptomatic maize ears were sampled in each district. Each cob was put in a polyethylene bag, labeled and transported same day to the Department of Food Science and Technology, Makerere University for laboratory analyses. The samples were stored at -20°C to prevent postharvest accumulation of moulds and aflatoxins until analysis (Anderson *et al.*, 1995).

**Mould isolation and identification:** The direct plating technique (Pitt and Hocking, 1997) was used to isolate the moulds. Grain in each sample was shelled, divided into 3 sub-sample lots and 10 kernels were taken at random from each sample lot, surface disinfected for 2 min with 10% sodium hypochlorite (commercial bleach-Jik), washed three times with sterile distilled water and placed directly on malt extract agar (Becton Dickinson Microbiological Systems, Becton Dickinson and Company, Sparks, MD 21152, USA) prepared by mixing 33.6 g in 1 L of distilled water as recommended by manufacturers. In addition, malt salt agar media prepared by mixing 68 g of sodium chloride, 10 g of malt extract, 20 g of agar and 1 L of distilled water was used for growing mould species requiring a high osmotic concentration (Hanlin and Uiloo, 1979). The plates were incubated upright at 30°C for 42-72 h. After sufficient growth, some of the cultures that could not be identified were transferred onto Potato Dextrose Agar several times for purification and mould species were identified using the recommended keys (Hanlin and Uiloo, 1979; Tuite, 1992). The mould incidence, defined as the proportion of plated grains showing mould growth, was calculated (Kapindu *et al.*, 1999).

**Determination of moisture content:** The moisture content of each of the maize samples was determined by the standard air oven method (AOAC, 1999). The samples were dried at 100°C to a constant weight and moisture content calculated as a percentage of the fresh weight.

**Aflatoxin analysis:** Each of the samples was divided into two replicate lots and aflatoxins were extracted using methanol-water solution (80:20 vol) and quantified in parts per billion (ppb) using Aflatest® Fluorometer following the manufacturer's instructions (VICAM L.P., 313 Pleasant Street, Watertown, MA 02472, USA). The lowest detection limit was set at 0 parts per billion (ppb) and highest at 50 ppb. The range and mean aflatoxin content (ppb) of the samples were computed.

**Data analysis:** Data for moisture content, aflatoxin levels and mould incidence were subjected to analysis of variance (ANOVA) using Genstat Statistical Programme (Genstat, 1995). The means were separated using LSD ( $p = 0.05$ ).

## RESULTS

**Moisture content:** During the 2002A and 2002B seasons, moisture content varied significantly among the maize grain from the different districts of Uganda (Table 1). Dry freshly harvested maize samples had moisture levels between 17.5 and 23.4%. In 2002A season, grain from both healthy and symptomatic samples from Masindi district had the highest moisture content levels (23.4%; 22.9%) while in 2002B grain from Mubende district had the highest moisture content levels (20.9%; 20.1%). However, in both seasons moisture content of the healthy and

Table 1: Moisture content of maize kernels sampled from farmers in different districts of Uganda during 2002A and 2002B seasons

District	Mean moisture content (%) <sup>a,b</sup>			
	2002A kernels		2002B kernels	
	Infected <sup>c</sup>	Healthy <sup>d</sup>	Infected <sup>e</sup>	Healthy <sup>d</sup>
Iganga	19.6	19.2	18.4	18.3
Kamuli	18.7	18.9	17.8	17.5
Mayuge	22.4	21.8	20.6	19.9
Tororo	19.5	19.2	18.9	18.1
Mbale	18.7	17.9	17.5	18.0
Sironko	20.5	20.0	18.8	18.1
Masindi	23.4	22.9	19.8	19.3
Mubende	21.5	21.1	20.9	20.1
Masaka	22.6	21.7	19.4	19.6
Kapchworwa	18.5	18.4	-	-
LSD ( $p \leq 0.05$ )	1.15	1.11	1.09	1.09
CV (%)	15.4	18.1	10.4	12.5

<sup>a</sup>Means are for twenty samples in each of the infected and asymptomatic maize categories in each season. <sup>b</sup>In each season, mean moisture content levels were not significant among the infected and asymptomatic kernels <sup>c</sup>Infected kernels refer to symptomatic. <sup>d</sup>Healthy kernels refer to asymptomatic <sup>e</sup>No maize samples were collected during this season

Table 2: Major mould species and percentage of mould-infected maize kernels sampled at harvest from different districts of Uganda during 2002A season

District	Infected kernels <sup>a</sup>		Healthy kernels <sup>b</sup>	
	Mould species	Mouldy kernels <sup>c</sup> (%)	Mould species	Mouldy kernels <sup>c</sup> (%)
Iganga	<i>Fusarium verticillioides</i>	35.0	<i>Fusarium verticillioides</i>	20
	<i>F. solani</i>	10.0	<i>Aspergillus flavus</i>	5.0
	<i>Aspergillus flavus</i>	15.0	<i>Acremonium strictum</i>	16.6
	<i>A. wentii</i>	20.0	<i>Stenocarpella maydis</i>	20.5
	<i>A. penicillioides</i>	6.7		
	<i>A. fumigatus</i>	10.0		
	<i>Acremonium strictum</i>	25.5		
	<i>Stenocarpella maydis</i>	40.0		
	<i>Rhizopus</i> spp.	10.5		
	<i>Fusarium graminearum</i>	10.0	<i>Fusarium verticillioides</i>	8.5
Kamuli	<i>F. verticillioides</i>	26.7	<i>Phomopsis</i> spp.	30.0
	<i>F. solani</i>	36.0	<i>Aspergillus flavus</i>	6.7
	<i>Aspergillus flavus</i>	9.7	<i>A. niger</i>	12.5
	<i>Penicillium atramentosum</i>	3.3		
	<i>P. citrinum</i>	6.0		
	<i>Fusarium verticillioides</i>	25.0	<i>Fusarium verticillioides</i>	16.7
	<i>F. graminearum</i>	6.0	<i>Aspergillus flavus</i>	4.5
	<i>F. solani</i>	45.0	<i>A. niger</i>	5.4
	<i>Aspergillus flavus</i>	12.0		
	<i>A. niger</i>	30.0		
Mayuge	<i>A. tamaraii</i>	3.3		
	<i>Acremonium strictum</i>	15.0		
	<i>Rhizopus</i> spp.	12.5		
	<i>Fusarium verticillioides</i>	50.0	<i>Fusarium verticillioides</i>	30.0
	<i>Aspergillus flavus</i>	12.0	<i>F. solani</i>	10.0
	<i>A. aculeatus</i>	6.7	<i>P. atramentosum</i>	3.3
	<i>Phomopsis</i> spp.	10.0	<i>Aspergillus flavus</i>	9.6
	<i>Fusarium verticillioides</i>	100	<i>Fusarium verticillioides</i>	40.0
	<i>Penicillium atramentosum</i>	3.3	<i>Aspergillus flavus</i>	4.5
	<i>Phomopsis</i> spp.	12.0	<i>Stenocarpella maydis</i>	10.0
Sironko	<i>Stenocarpella maydis</i>	15.0	<i>Phomopsis</i> spp.	9.6
	<i>Aspergillus flavus</i>	5.4		
	<i>Fusarium verticillioides</i>	45.0	<i>Fusarium verticillioides</i>	10.0
	<i>F. solani</i>	36.0	<i>A. flavus</i>	5.4
	<i>Aspergillus flavus</i>	7.5	<i>A. niger</i>	10.0
	<i>A. penicillioides</i>	9.0	<i>Stenocarpella maydis</i>	15.0
	<i>Penicillium atramentosum</i>	16.6		
	<i>Phomopsis</i> spp.	21.3		
	<i>Stenocarpella maydis</i>	18.0		
	<i>Penicillium atramentosum</i>	6.7	<i>P. atramentosum</i>	10.0
Masindi	<i>Aspergillus flavus</i>	15.3	<i>Aspergillus flavus</i>	
	<i>A. wentii</i>	12.5	<i>A. niger</i>	
	<i>A. fumigatus</i>	20.0	<i>Acremonium strictum</i>	7.5
	<i>Stenocarpella maydis</i>	15.0	<i>Fusarium verticillioides</i>	10.0
	<i>Acremonium strictum</i>	18.7		
	<i>Fusarium verticillioides</i>	30.0		
	<i>F. solani</i>	20.0		
	<i>Aspergillus flavus</i>	33.3	<i>Aspergillus flavus</i>	10.0
	<i>A. tamaraii</i>	24.6	<i>A. niger</i>	3.3
	<i>A. niger</i>	20.0	<i>Fusarium verticillioides</i>	6.0
Mubende	<i>Fusarium verticillioides</i>	9.6	<i>Stenocarpella maydis</i>	15.0
	<i>F. solani</i>	33.3		
	<i>F. graminearum</i>	12.0		
	<i>Stenocarpella maydis</i>	20.0		
	<i>Penicillium atramentosum</i>	6.7		
	<i>Aspergillus flavus</i>	10.0	<i>Aspergillus flavus</i>	10.0
	<i>A. niger</i>	15.0	<i>A. penicillioides</i>	5.5
	<i>A. penicillioides</i>	6.7	<i>Fusarium verticillioides</i>	14.0
	<i>Fusarium verticillioides</i>	10.0	<i>F. solani</i>	7.5
	<i>F. solani</i>	20.0		
Masaka	<i>Penicillium atramentosum</i>	5.4		
	<i>Rhizopus</i> spp.	18.7		
	<i>Phomopsis</i> spp.	30.0	<i>Fusarium verticillioides</i>	15.0
	<i>Fusarium verticillioides</i>	60.5	<i>F. solani</i>	10.0
	<i>F. solani</i>	8.6	<i>Stenocarpella maydis</i>	10.0
	<i>Aspergillus flavus</i>	12.6	<i>Aspergillus flavus</i>	7.5
	<i>Stenocarpella maydis</i>	9.0		
	CV (%)	30.8		25.4

<sup>a</sup>Infected kernels refer to symptomatic, <sup>b</sup>Healthy kernels refer to asymptomatic, <sup>c</sup>Means are for thirty kernels per sample

Table 3: Major mould species and percentage of mould-infected maize kernels sampled at harvest from different districts of Uganda during 2002B season

District	Infected kernels <sup>a</sup>		Healthy kernels <sup>b</sup>	
	Mould species	Mouldy kernels <sup>c</sup> (%)	Mould species	Mouldy kernels <sup>c</sup> (%)
Iganga	<i>Fusarium verticillioides</i>	25.0	<i>Fusarium verticillioides</i>	15.6
	<i>F. solani</i>	10.0	<i>Aspergillus flavus</i>	9.5
	<i>Aspergillus flavus</i>	15.0	<i>Rhizopus</i> spp.	12.5
	<i>Acremonium strictum</i>	5.5		
	<i>Stenocarpella maydis</i>	20.0		
Kamuli	<i>Fusarium verticillioides</i>	30.7	<i>Fusarium verticillioides</i>	20.5
	<i>Sternocarpella maydis</i>	36.0	<i>Aspergillus flavus</i>	10.0
	<i>Aspergillus flavus</i>	9.7	<i>A. niger</i>	18.0
	<i>A. niger</i>	35.7	<i>Penicillium expansum</i>	3.3
	<i>Rhizopus</i> spp.	6.0		
	<i>Penicillium expansum</i>	5.5		
Mayuge	<i>Fusarium verticillioides</i>	55.0	<i>Fusarium verticillioides</i>	30.5
	<i>F. graminearum</i>	16.5	<i>Aspergillus flavus</i>	10.6
	<i>Aspergillus flavus</i>	11.4	<i>A. niger</i>	9.5
	<i>A. niger</i>	30.0		
	<i>A. penicillioides</i>	9.7		
Tororo	<i>Fusarium verticillioides</i>	40.5	<i>Fusarium verticillioides</i>	35.0
	<i>F. graminearum</i>	30.0	<i>Phomopsis</i> spp.	25.5
	<i>Aspergillus flavus</i>	15.0	<i>Aspergillus flavus</i>	12.5
	<i>Stenocarpella maydis</i>	20.6	<i>Stenocarpella maydis</i>	6.0
	<i>Phomopsis</i> spp.	9.7		
Sironko	<i>Fusarium verticillioides</i>	90.0	<i>Fusarium verticillioides</i>	45.5
	<i>Phomopsis</i> spp.	15.6	<i>Aspergillus flavus</i>	4.5
	<i>Stenocarpella maydis</i>	20.0	<i>A. niger</i>	15.0
	<i>Aspergillus flavus</i>	25.0	<i>Phomopsis</i> spp.	9.5
	<i>A. ochraceous</i>	15.4		
Mbale	<i>Fusarium verticillioides</i>	36.0	<i>Fusarium verticillioides</i>	20.0
	<i>F. graminearum</i>	25.5	<i>A. flavus</i>	15.6
	<i>Aspergillus flavus</i>	10.5	<i>A. niger</i>	20.5
	<i>A. wentii</i>	20.0		
	<i>Penicillium atramentosum</i>	15.0		
Masindi	<i>Stenocarpella maydis</i>	12.0		
	<i>Fusarium verticillioides</i>	90.6	<i>Fusarium verticillioides</i>	40.0
	<i>Aspergillus flavus</i>	30.0	<i>Aspergillus flavus</i>	15.5
	<i>A. niger</i>	25.5	<i>A. niger</i>	20.0
	<i>Penicillium expansum</i>	9.0	<i>P. expansum</i>	6.6
Mubende	<i>P. citrinum</i>	5.6		
	<i>Aspergillus flavus</i>	30.5	<i>Fusarium verticillioides</i>	20.0
	<i>A. niger</i>	15.6	<i>Aspergillus flavus</i>	18.6
	<i>Fusarium verticillioides</i>	40.0	<i>A. niger</i>	10.0
	<i>F. graminearum</i>	18.0	<i>Rhizopus</i> spp.	12.8
Masaka	<i>Rhizopus</i> spp.	10.5		
	<i>Fusarium verticillioides</i>	40.0	<i>Fusarium verticillioides</i>	35.6
	<i>F. solani</i>	6.0	<i>Aspergillus flavus</i>	15.5
	<i>Aspergillus flavus</i>	15.0	<i>A. niger</i>	20.5
	<i>A. wentii</i>	10.0		
CV (%)		25.7		
		30.4		35.0

<sup>a</sup>Infected kernels refer to symptomatic, <sup>b</sup>Healthy kernels refer to asymptomatic, <sup>c</sup>Means are for thirty kernels per sample

symptomatic maize sampled from the same district did not differ significantly. On the other hand, the majority of grain sampled during the 2002B season had slightly lower moisture content levels than grain sampled during 2002A season (Table 1).

**Mould incidence in maize grain samples:** During 2002A season, a total of 16 different mould species were identified from maize samples at harvest (Table 2) namely; *Fusarium verticillioides*, *F. graminearum*, *F. solani*, *Aspergillus flavus*, *A. niger*, *A. aculeatus*, *A. fumigatus*, *A. penicillioides*, *A. wentii*, *A. tamari*, *Acremonium strictum*, *Penicillium atramentosum*, *P. citrinum*, *Stenocarpella maydis*, *Phomopsis* spp. and *Rhizopus* spp. Species of four genera, *Fusarium*, *Aspergillus*,

*Stenocarpella* and *Acremonium* were the most common moulds isolated while *Phomopsis* and *Rhizopus* species occurred less frequently in both asymptomatic and symptomatic samples. *F. verticillioides* and *A. flavus* occurred in all symptomatic and asymptomatic samples. For example, the highest incidence among the symptomatic samples was 100% in Sironko district due to *F. verticillioides* infection (Table 2). Overall, in each district, fewer mould species were isolated in samples from asymptomatic maize kernels compared to symptomatic maize kernels and the incidence of the former was low (Table 2).

During the 2002B season, no samples were collected from farmers in Kapchworwa district. Results indicate that a total of 15 mould species were found infecting maize

Table 4: Aflatoxin levels in maize grain sampled from farmers in different districts of Uganda

Aflatoxin levels (ppb)												
District	2002A kernels						2002B kernels					
	Infected <sup>a</sup>			Healthy <sup>b</sup>			Infected <sup>a</sup>			Healthy <sup>b</sup>		
	Range	Mean	Positive (%)	Range	Mean	Positive (%)	Range	Mean	Positive (%)	Range	Mean	Positive (%)
Iganga	0-32	10.8	90	0-27	5.8	40.0	0-15	8.9	77.5	0-15	5.3	47.5
Kamuli	0-27	10.2	85	0-5	2.6	45.0	0-10	7.2	75.0	0-8	2.8	37.5
Mayuge	5-25	11.8	100	0-10	2.8	60.0	0-22	10.4	82.5	0-8	2.9	45.0
Tororo	0-15	7.5	75	0-4.5	3.5	50.0	0-10	5.6	40.0	0-5.5	3.3	37.5
Mbale	0-14	4.8	50	0-5	3.3	20.0	0-10	4.2	37.5	0-4	2.0	30.0
Sironko	0-13	8.5	75	0-7	1.8	17.5	0-12	5.8	45.0	0-6	3.2	40.0
Masindi	11-15	13.0	100	0-9	7.2	60.0	0-15	10.2	87.5	0-10	4.9	52.5
Mubende	0-14	8.4	80	5-9	3.0	100	0-9	6.0	75.0	0-10	4.1	47.5
Masaka	0-21	10.1	80	0-6	1.3	40.0	0-16	10.2	75.0	0-8	1.4	30.0
Kapchworwa	0-22	5.4	40	0-4	0.7	7.5	-	-	-	-	-	-
LSD (p≤0.05)		2.64			1.02			1.14			1.08	
CV (%)		12.2			12.8			14.1			12.5	

<sup>a</sup>Infected kernels refer to symptomatic, <sup>b</sup>Healthy kernels refer to asymptomatic

grain and these were; *Fusarium verticillioides*, *F. graminearum*, *F. solani*, *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *A. penicillioides*, *A. wentii*, *Acrimonium strictum*, *Penicillium atramentosum*, *P. citrinum*, *P. expansum*, *Stenocarpella maydis*, *Phomopsis* spp. and *Rhizopus* spp. (Table 3). Species of *Fusarium* and *Aspergillus* were the most common moulds isolated with *F. verticillioides* and *A. flavus* occurring in all infected and asymptomatic samples. The highest incidence (90.6%) was in kernels from symptomatic samples obtained from Masindi district due to *F. verticillioides* infection while the lowest incidence (3.3%) was due to *P. expansum* in kernels from asymptomatic samples obtained from Kamuli district. However, in each district, fewer mould species were isolated in samples from asymptomatic maize kernels compared to symptomatic maize kernels and their incidence was quite low (Table 3).

**Aflatoxin contamination:** Aflatoxin levels significantly varied in both the asymptomatic and symptomatic dry freshly harvested maize samples from the different districts of Uganda during 2002A and 2002B seasons (Table 4). During 2002A season, results of the kernels from symptomatic samples indicate that Masindi had the highest mean aflatoxin levels (13.0 ppb) followed by Mayuge (11.8 ppb) while Mbale and Kapchworwa districts had the least aflatoxin levels of 4.8 and 5.4 ppb, respectively. In Masindi and Mayuge districts all samples (100%) from symptomatic grain tested positive for aflatoxin contamination (Table 4). In addition, during this first season, kernels from the asymptomatic maize samples were less contaminated with aflatoxins compared to kernels from infected samples. It was found that the highest mean aflatoxin levels in asymptomatic grain were 7.2 ppb obtained from Masindi where 60% tested positive while the lowest were 0.7 ppb in grain from Kapchworwa where 40% of the samples tested positive. Although all

(100%) asymptomatic samples from Mubende district tested positive for aflatoxins, their mean levels (3.0 ppb) were significantly lower than those of samples from Masindi and both had an upper range limit of 9 ppb (Table 4).

During 2002B season, no kernels from both the asymptomatic and symptomatic maize samples were found to be 100% contaminated with aflatoxins (Table 4). Among the symptomatic samples, the highest mean aflatoxin levels (10.4 ppb) were recorded in samples from Mayuge district where 82.5% tested positive while the lowest levels (4.2 ppb) were from Mbale district with 37.5% positive samples. The aflatoxin levels in samples from asymptomatic dry freshly harvested maize were lower than those of the symptomatic lot and, the highest levels were 5.3 ppb in samples from Iganga where 47.5% tested positive, while the lowest levels (1.4 ppb) were in samples from Masaka with only 30% positive samples.

## DISCUSSION

Field drying of maize in Uganda normally lasts 4-7 weeks beyond physiological maturity (Odogola and Henriksson, 1991). During this period, the moisture of the grain is reduced from the 40-35% level at physiological maturity to 20-16% agreeing with the findings of present study. The majority of maize sampled during the 2002B season had lower moisture content than those harvested during the 2002A season. Similar results were reported by Kaaya *et al.* (2005) and this was attributed to the continued rainfall during the harvest period of the first season.

Several fungi were found to cause maize ear rots in Uganda, the predominant ones were noted to belong to genera *Fusarium*, *Aspergillus*, *Stenocarpella* and *Acrimonium* with *Fusarium* spp. having the highest incidence. These results are comparable to what has been reported elsewhere in Africa (Flett and Wehner, 1991;

Kapindu *et al.*, 1999). Physical appearance of a maize cob or kernels can be misleading as fungi could be isolated from both symptomatic and asymptomatic kernels. This perhaps explains why maize samples for trade should be subjected to thorough inspection and analysis. In this study, it was common to find fungi/moulds in freshly harvested samples implying that infection starts from the field as earlier reported (Kaaya *et al.*, 2000; Kaaya *et al.*, 2005). Fungal incidences of as high as 46% were recorded in asymptomatic kernels which were on average 30% less than what was got from infected samples. In 1992, Bacon *et al.* (1992) also reported presence of *Fusarium verticillioides* within asymptomatic corn kernels. High incidences of moulds especially *Fusarium verticillioides* and *Acremonium strictum* have also been reported on both infected and asymptomatic maize grain from small holder farms in Central Malawi, with the former mould species having higher incidence (Kapindu *et al.*, 1999).

Ear rot fungi produce mycotoxins, which are poisonous to animals and humans (Pittet, 1998). Identification of a fungus from seed, particularly if frequent in high levels probably indicates that the seed sample is potentially toxic (Tuite *et al.*, 1985). On the other hand, absence of mould symptoms on a seed does not imply absence of toxins. From this study, both asymptomatic and symptomatic maize kernels were found to be contaminated by aflatoxins. Although the mean aflatoxin levels in both asymptomatic and symptomatic kernels in both seasons were lower than the 20 ppb FDA/WHO regulatory limit (Mphande *et al.*, 2004), considering the aflatoxin range, some symptomatic samples from Iganga, Kamuli, Mayuge, Masaka and Kapchorwa in 2003A season had 32, 27, 25, 21 and 32 ppb respectively, while in 2002B samples from Mayuge had up to 22 ppb implying all these samples were not suitable for consumption. In addition, although the levels of aflatoxin contamination of asymptomatic kernels were lower than symptomatic kernels, some of the asymptomatic samples were found to be 100% contaminated with aflatoxins. This may imply that grain physically judged to be safe for consumption and trade could be contaminated. It is for this reason that grain traders in Uganda have occasionally been disappointed to have their produce rejected within the region because of having aflatoxin levels beyond acceptable limits. The Uganda Grain Traders Ltd., a company which sells grain outside Uganda has of recent set a limit for aflatoxin to be less than 25 ppb and does the testing before the grain is destined for sale (Rates, 2003). Farmers whose produce has been rejected have been advised how best to reduce or avoid mould and aflatoxin contamination.

According to the findings of this study it is anticipated that the mould incidence and aflatoxin levels in maize samples could have been influenced by season

and altitude. Samples collected during 2002A season had generally higher moisture content, mould incidence and aflatoxin levels than the 2002B samples. On the other hand, samples obtained from mid-altitude districts had generally higher aflatoxin levels than samples from high altitude districts. Temperatures and amount of rainfall in Uganda tend to vary from season to season and also with location. The occurrence of mycotoxins is governed by existence of conditions that favour the fungi concerned and, temperature and moisture content are predominant factors (Magan and Lacey, 1988). In Uganda, the first season tends to have more rainfall than the second season in most districts thus influencing moisture content of the grain and subsequent ear rot infection. Since higher mould contamination of grain has been associated with high levels of aflatoxins (Miller, 1995), this could also explain why maize samples with high moisture content had high aflatoxin levels. High temperatures (26-38°C) during grain filling are associated with ear rot infection and are particularly important for pre-harvest aflatoxin contamination (Jones *et al.*, 1980; Widstrom *et al.*, 1990; Vincelli, 2003). This may explain why maize samples from the mid-altitude districts of Uganda whose maximum temperatures vary between 25 and 32°C during the growing season had higher aflatoxin levels.

Owing to inadequate storage conditions of maize by farmers and traders in Uganda, aflatoxin levels can build up to unacceptable limits in a short spell of time. However, under proper storage, especially under low moisture level, consumption of maize may probably pose little ill-health effects to the consumers. By and large it is important to investigate the presence and levels of other mycotoxins and to conduct sensitization programs to the public about mycotoxins and how to avoid them through good pre and postharvest practices. Establishing if there are variety differences in mould infection on varieties and period of storage would also help in designing management strategies.

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#### REFERENCES

- Anderson, W.F., C.C. Holbrook, D.M. Wilson and M.E. Matheron, 1995. Evaluation of peanut aflatoxin contamination in several potentially resistant peanut genotypes. *Peanut Sci.*, 22: 29-32.
- AOAC, 1999. Official methods of analysis of AOAC international. AOAC Int. Publ., Suite 500, 481 North, Fredric Av. Gaithersburg, Maryland 20877. USA.

- Bacon, C.W., R.M. Bennett, D.M. Hinton and K.A. Voss, 1992. Scanning electron microscopy of *Fusarium moniliforme* within asymptomatic corn kernels and kernels associated with equine leukocephalomalacia. *Plant Dis.*, 76: 144-148.
- Flett, B.C and F.C. Wehner, 1991. Incidence of *Stenocarpella* and *Fusarium* cob rots in monoculture maize under different tillage systems. *J. Phytopathol.*, 133: 327-333.
- Flett, B.C. and J.B.J. van Rensburg, 1992. Effect of *Busseola fusca* on the incidence of maize ear rot caused by *Fusarium moniliforme* and *Stenocarpella maydis*. *S. Afric. J. Plant Soil*, 9: 177-179.
- Genstat, 1995. Genstat 5, Release 3.2, PC/Windows NT, Copyright 1995, Lawes Agricultural Trust, Rothamsted Experimental Station, USA.
- Hanlin, R.T. and M. Uilola, 1979. Atlas of introductory mycology. Hunter Publishing Company, Winston-Salem, North Carolina, USA., pp: 3-8.
- Jones, R.K., H.E. Duncan, G.A. Payne and K.J. Leonard, 1980. Factors influencing infection by *Aspergillus flavus* in silk-inoculated corn. *Plant Dis.*, 64: 859-863.
- Kaaya, A.N., H.L. Warren and E. Adipala, 2000. Molds and aflatoxin contamination of maize and groundnuts in Mayuge and Kumi districts of Uganda. *MUARIK Bull.*, 3: 33-41.
- Kaaya, A.N., H.L. Warren, S. Kyamanywa and W. Kyamuhangire, 2005. The effect of delayed harvest on moisture content, insect damage, moulds and aflatoxin contamination of maize in Mayuge district of Uganda. *J. Sci. Food. Agric.*, 85: 2595-2599.
- Kapindu, S.J., V.W. Saka, A.M. Julian, R. Hillocks and W.A.B. Msuku, 1999. The significance and management of maize cob rots in smallholder farms in central Malawi. *Afric. Crop Sci. J.*, 7: 531-537.
- Kikafunda-Twine, J., D.T. Kyetere, G. Bigirwa, T. Kalule and M. Wamaniala, 2001. Maize. In: *Agriculture in Uganda. Volume II: Crops.*, Mukiibi, J.K. (Ed.). Fountain Publishers/CTA/NARO, pp: 55-69.
- Latterell, F.M and A.E. Rossi, 1983. *Stenocarpella macrospore* (= *Diplodia macrospore*) and *S. Maydis* (= *D. maydis*) compared as pathogens of corn. *Plant Dis.*, 67: 725-729.
- MacDonald, M.V. and R. Chapman, 1997. The incidence of *Fusarium moniliforme* on maize from Central America, Africa and Asia during 1992-1995. *Plant Pathol.*, 46: 112-125.
- Magan, N. and J. Lacey, 1988. Ecological determinants of mould growth in stored grain. *Intl. J. Food Microbiol.*, 7: 245-256.
- Miller, J.D., 1995. Fungi and Mycotoxins in grain: Implications for stored product research. *J. Stored Prod. Res.*, 31: 1-6.
- Mphande, F.A., B.A. Siame and J.E. Taylor, 2004. Fungi, aflatoxins and cyclopiazonic acid associated with peanut retailing in Botswana. *J. Food Prot.*, 67: 96-102.
- Odogola, W.R. and R. Henriksson, 1991. Postharvest management and storage of maize. Technical systems for agriculture. UNDP/OPS regional programme on agricultural operations technology for small holders in East and Southern Africa, pp: 162.
- Pitt, J.I. and A.D. Hocking, 1997. *Fungi and food spoilage*. Blackie Academic and Professional. London, Great Britain. 2nd Edn., pp: 21-32.
- Pittet, A., 1998. Natural occurrence of mycotoxins in foods and feeds. An updated review. *Rev. Med. Vet.*, 149: 479-492.
- Rates, 2003. Regional Agricultural Trade Expansion Support Program. Maize market assessment and baseline study for Uganda. Regional Trade Center, Rates Program, USAID/REDSO Project Report, pp: 1-36.
- Rwabwoogo, M.O., 2002. Uganda districts information handbook. Fountain Publishers Ltd. P.O. Box 488, Kampala, Uganda. 5th Edn., pp: 184.
- Sebunya, T.K. and D.M. Yourtee, 1990. Aflatoxigenic *Aspergilli* in foods and feeds in Uganda. *J. Food. Qual.*, 13: 97-107.
- Sétamou, M., K.F. Cardwell, F. Schulthess and K. Hell, 1998. Effect of insect damage to maize ears, with special reference to *Mussidia nigrivenella* (Lepidoptera: Pyralidae), on *Aspergillus flavus* (Deuteromycetes: Moniliales) infection and aflatoxin production in maize before harvest in the Republic of Benin. *J. Econ. Ent.*, 91: 433-438.
- Tuite, J., 1982. Examining and Identifying Fungal Cultures Growing out from Corn Kernels. A Laboratory Manual. Perdue University, USA.
- Tuite, J., C. Koh-Knox, R. Strohshine, F.A. Cantone and L.F. Bauman, 1985. Effect of physical damage to corn kernels on the development of *Penicillium* species and *Aspergillus glaucus* in storage. *Phytopathology*, 75: 1137-1140.
- Vincelli, P., 2003. Ear rot of corn caused by *Stenocarpella maydis* (= *Diplodia maydis*). Online Publications. <http://search.epnet.com>.
- Widstrom, N.W., W.W. McMillian, R.W. Beaver and D.M. Wilson, 1990. Weather-associated changes in aflatoxin contamination of preharvest maize. *J. Prod. Agric.*, 3: 196-199.
- Williams, J.H., T.D. Phillips, P. Jolly, J.K. Styles, C.M. Jolly and D. Aggarwal, 2004. Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences and interventions. *Am. J. Clin. Nutr.*, 80: 1106-1122.