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Toxigenic fungi and aflatoxins associated with marketed rice grains in Uganda

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Abstract: The natural contamination of rice grains from Ugandan locally grown crop or imported from Pakistan by toxigenic fungi and aflatoxins was investigated and assessed. Twenty four samples of each type of rice were obtained from various markets within 3 different districts. Contaminating fungi were enumerated by direct plating method on three isolation media including pentachloronitrobenzene rose-bengal yeast extract sucrose agar (PRYES), pentachloronitrobenzene potato sucrose agar (PCNB-PSA) and *Aspergillus flavus/ parasiticus* agar (AFPA). Fungi including toxigenic *Penicillium, Fusarium* and aflatoxigenic *Aspergillus* spp. were isolated and identified to species level and the percentage contamination levels were calculated. Locally grown rice had a comparatively narrower species spectrum of *Penicillium, Fusarium* and *Aspergillus* genera, whereby it recorded 5, 5 and 7 species respectively while the imported rice recorded 8, 6 and 11 species respectively. Among the *Penicillium, P. chrysogenum, P. novae-zeelandiae, P. oxalicum, P. pinophilum* and *P. purpurogenum* occurred on both types of rice grains while among the *Fusarium, F. graminearum, F. verticillioides* and *F. oxysporum* also occurred on the two grains. Similarly occurring on the two types of rice were *Aspergillus flavus, A. candidus, A. fumigatus, A. niger, A. oryzae* and *A. terreus. Aspergillus parasiticus* was recorded on only the local rice but *A. candidus* was the most frequent *Aspergillus* on both types of rice grains. *Penicillium oxalicum* was among the most frequent *Penicillium* species on the local rice while *P. chrysogenum* and *P. citrinum* were prevalent on the imported rice. *F. solani* was the most frequent *Fusarium* species on the local rice whereas the imported rice recorded scarce contamination by *Fusarium* spp. The locally grown rice recorded a comparatively lower incidence of aflatoxins whereby, 44.4 % of its samples were contaminated while 77.8 % of the imported rice samples were contaminated. Similarly, the local rice had comparatively lower moisture contents, in which, 14.60 % was its highest level while 15.5 % was the highest level for the imported rice. A half of the imported rice samples had moisture content above the recommended level for safe storage of milled rice while for the local rice only 12.5 % of the samples moisture content were above this level. A significantly positive correlation coefficient existed between incidence of aflatoxigenic *Aspergillus* spp. and moisture content of the rice grain samples screened for aflatoxin contamination.

Key words: Fungi, natural contamination, milled rice, toxigenic, aflatoxigenic, aflatoxins, Pakistan, Uganda

Introduction

Food contamination by fungi and their toxic metabolites (mycotoxins) still remains a serious global problem. It is estimated that as much as 25 % of the worlds' cereals are contaminated with fungi and known mycotoxins, while a higher percentage could be contaminated with toxins are yet unidentified (Mannon & Johnson 1985). Cereals including rice are considered among the most consumed foods in Uganda as well as the rest of the world (ICMSF 1980). Cereals are known to be good substrates for fungal and mycotoxin development. Cereals are foods of intermediate moisture content, thus contamination may occur at the farm or at the site of storage, affects the yield, quality and nutritive value of the products. Moreover, some grains may contain mycotoxins as a result of fungal growth (Council for Agricultural Science and Technology 1989, FAO 1990).

The potential impact on human health has, particularly caused health agencies to act. In warmer climates including sub-Saharan Africa where Uganda falls, aflatoxins is a major problem (Bullerman 1979, FAO 1990).

However, studies on aflatoxins in food crops in Uganda in the past 10 years have not been documented, nor have extensive comparative studies ever been conducted on the incidence of various toxigenic fungi on locally grown and imported cereals including rice grains. In a previous paper (Taligoola *et al.* 2004), the contamination of locally grown and imported rice by various types of fungi, particularly xerophilic fungi is documented. In this research, fungal contamination of locally grown and imported rice grains by toxigenic *Penicillium, Fusarium* and *Aspergillus* have been determined with the aim of establishing those which are potential mycotoxin producers. Similarly, incidence of aflatoxin on rice grains at consumption levels in Ugandan markets were also determined. The identification of the contaminating fungi will also help to estimate the probable types of mycotoxins which might be produced.

Materials and Methods

Rice samples

A total of 48 milled rice grain samples each weighing 2 kg were bought periodically from different retailers in shops and open-air markets within and around Kampala, Mukono and Mpigi districts of Uganda between Nov. 1998 and Aug. 1999. Random method of sampling (Mojica & Gomez, 1994) was used for the collection of the rice samples such that an equal number of samples, 24, each of a locally grown rice variety called Super and an imported rice grain from Pakistan were collected from each of the three districts, 8 from each district. These samples were analysed for fungal and aflatoxin, as well as for their moisture content.

Isolation of toxigenic fungi

The seed-plate (direct plating) method was used to determine the seed-borne fungi on the rice grains. Prior to plating, a 500g sub-sample was first surface sterilized using 70 % ethanol prior to a 0.8 % chlorine treatment for 2 minutes (Andrews 1996). Excess disinfectant was drained off from the grains after which sterilized distilled tap water was used to rinse the grains three times. Excess water on the grains was mopped using sterile filter paper. The grains were then plated on a suitable isolation media at a plating rate of 10 rice grains per plate. Three selective isolation agar media were used to detect and isolate the following groups of toxigenic species: (i) nephrotoxigenic *Penicillium* spp. using pentachloronitrobenzene rose-bengal yeast extract sucrose agar; PRYES (Frisvad, 1983), (ii) *Fusarium* spp. using pentachloronitrobenzene-potato sucrose agar; PCNB-PSA (Nash & Synder 1962, Booth 1971) and (iii) aflatoxigenic *Aspergillus* spp. using *Aspergillus flavus*/ *parasiticus* agar; AFPA (Pitt *et al.* 1983). Fifty grains per sub-sample were plated for PCNB-PSA while for the other two selective media, 100 grains were plated. Plates of PRYES were incubated under natural conditions of light and darkness for 7-8 days while those containing AFPA were incubated at 30 ºC for 42-48 hrs in dark and PCNB-PSA plates were incubated under continous light from a flourescent tube, for 7-8 days.

Identification of fungi

Fungi were identified on the basis of their macroscopic and microscopic features using the keys of Raper and Fennell (1965), Booth (1971), Pitt (1979), Pitt and Hocking (1997).

Four identification media were used including Czapek yeast extract agar; CYA (Pitt 1973), Malt extract agar; MEA (Blakeslee 1915) and 25 % glycerol nitrate agar; G25N (Pitt 1973) for identification of *Penicillium* spp. and potato sucrose agar; PSA (Booth 1971) for identification of *Fusarium* spp.

Extraction and estimation of aflatoxins

Incidence of aflatoxins was done using 18 rice grain samples (9 from each type of rice). Semi-quantitative tests for the determination of total aflatoxins in the rice grains were done whereby a commercial immunological test kit, aflascan (from Rhône Diagnostics and Technologies Ltd., Glasgow, U.K.) were used. A comparator card, a component of the aflascan, was used in the determination of the levels of aflatoxins in μg/Kg (ppb). The total aflatoxin level (aflatoxin B_1, B_2, G_1 and G_2) in the grain was determined according to the procedure outlined in the aflascan. Samples to be analyzed were at least 1 kg of rice grain, aseptically and thoroughly ground to fine powder, from which a 50 g sub-sample was withdrawn for the aflatoxin assay. The sub-sample was blended with 4 g of sodium chloride (NaCl) and 250 ml of 60 % high performance liquid chromatography (HPLC) analytical methanol. The extract was diluted with 250 ml of distilled water and then filtered using Whatman filter paper No. 4. Twenty five to fifty ml of the filtrate was collected from which 10 ml was pumped through an antibody-containing immunoaffinity column at 2-3 ml / minute, a component of the aflascan, using the glass syringe, of the aflascan. Residues in the column were washed by pumping 10 ml of distilled water three times at 5 ml / minute. Any aflatoxins bound on to the antibodies in the immunoaffinity column were extracted during elution, a process that involved pumping HPLC analytical grade methanol (eluant) through the column at maximum flow rate of 1 drop per second.

The eluant, containing aflatoxins, was collected in a glass tube put below the column, into which 1.0 ml each, of distilled water and chloroform were later added. Upon shaking the liquid mixture, two separate layers resulted, chloroform being at the bottom. A florisil tip, a component of the aflascan, was attached to the bottom of a glass syringe and a carefully pipetted chloroform layer was pumped slowly through it.

To estimate the aflatoxin level, the florisil tip was placed under an ultra-violet light box at 360 nm. Comparison of the intensity of any blue and/or green fluorescence on the florisil tip with the fluorescent comparator card provided a semi-quantitative estimation of the total aflatoxin in ppb of the original sample. For 10 ml filtrate, the comparator card was viewed on a scale of 0 ppb, 10 ppb, 20 ppb, 50 ppb and 100 ppb.

Determination of moisture content

The moisture content of each rice grain sample was determined by finding the loss in weight of the rice grain upon heating for a 24-hour period in an oven at 110 °c and expressing it as a percentage of the fresh weight (Gariboldi 1973). Triplicate sub-samples of 50g each per each rice sample were used.

The average of the triplicates became the moisture content.

Statistical analyses
Data were subjected to analysis of variance (ANOVA), t-test, and F-test. Statements of significance are based on $P \leq 0.05$ (Erricker 1979). Correlation and X^2 were used to determine the relationship between the various variables.

Results and discussion

Incidence of *Penicillium* species
The incidence of *Penicillium* spp. on both the local and imported rice grains were generally low as determined on pentachloronitrobenzene rose-bengal yeast extract sucrose agar medium (PRYES), in which case, less than 1 % of the grains were contaminated by each of the isolated species (Table 1). The species diversity was, however, broader on the imported rice, which recorded 8 species while the local rice had only 5 species isolated from its samples. The species that were recorded on both types of rice grain included *P. chrysogenum*, *P. novae-zeelandiae, P. oxalicum, P. pinophilum* and *P.purpogenum. Penicillium oxalicum* ranked the most frequent species on the local rice, occurring on 16.7 % of the samples while *P.chrysogenum* and *P. novae-zeelandiae* both ranked second, each occurred on 8.3 % of the samples. *P. chrysogenum* and *P.citrinum* ranked the most frequent species on the imported rice, each having been recorded on 12.5 % of the samples while *P.islandicum* ranked second occurring on 8.3 % of the samples. Similarly, *P.chrysogenum*, which was the most frequent species on both types of rice, was comparatively more predominant on the imported rice than the local rice. The remaining species on both types of rice each occurred on only 4.2 % of the samples (Table 1). In addition, *Penicillium* teleomorphs including *Talaromyces flavus* and other unidentified *Talaromyces* spp. were recorded on 8.3 % of the samples but at incidence levels of only 0.1 % and 0.2 % of total grains tested respectively (Table 1). *Talaromyces flavus*, the most common *Talaromyces* in nature has occasionally been reported in cereals including wheat (Pelhate 1968). The well-known nephrotoxigenic *Penicillium* spp. including *P. viridicatum* and *P. verrucosum* were, however, not recorded.

The observed prevalence of *P. chrysogenum, P. citrinum, P. islandicum* and *P. oxalicum* on both types of rice grains has also been reported on other cereals.
P. chrysogenum and *P. citrinum* were frequently encountered on corn and sorghum from Burundi (Munimbazi & Bullerman 1996), barley, wheat, maize and sorghum from Egypt (El-Kady *et al*. 1982), corn from Spain (Jimenez *et al.* 1985), wheat and rice from Turkey (Aran & Eke 1987). *P. citrinum* and *P. islandicum* were also frequently encountered in milled rice from Argentina (Tonon *et al*. 1997) while milled rice from Iran was predominantly contaminated by *P. islandicum* (Lacey 1988). The comparatively higher prevalence of *Penicillium* spp. on the imported rice than the local rice suggests that mycotoxin contamination could similarly be higher on the foreign rice. Studies on the relationship of the incidence of *Penicillium* and human mycotoxicoses have been reported including *P. chrysogenum* which has repeatedly been isolated from urinary tract infection and is reported to cause endophthalimitis (Eschete *et al.* 1981), and fatal cases of oesophagitis in a patient with acquired immunodeficiency syndrome (Hoffman *et al.* 1992).

Incidence of *Fusarium* species
The incidence of *Fusarium* spp. on both the local and imported rice grains were generally low as determined on Potato sucrose-pentachloronitrobenzene (PSA-PCNB) agar medium, with less than 1 % of the grains having been contaminated by each of the isolated species (Table 1). Similarly, the highest frequency recorded was only 4 out of 24 samples (16.7 %). The species that occurred on both types of rice grains included *F. graminearum*, *F. verticillioides* and *F. oxysporum. F. solani* was the most predominant and frequent species on the local rice having been recorded on 16.7 % of the samples, while *F. graminearum* ranked second having been recorded on 8.3 % of the samples.
Fusarium dimerum ranked the most predominant and frequent species on the imported rice having occurred on 8.3 % of the samples. The remaining species each occurred on 4.2 % of the samples on both types of rice. The species diversity was closely similar on both types of rice with the local rice having recorded 5 species, while the imported rice had 6 species (Table 1). The 3 species commonly occurring on both the local super and imported Pakistani rice including: *F. graminearum*, *F. moniliforme* and *F. oxysporum* are reported to contain toxigenic strains (Bullerman 1979, Davis & Diener 1987).

Fusarium verticillioides, an endemic fungus on maize worldwide, produces fumonisins, mycotoxins associated with oesophageal cancer (Gelderblom *et al.* 1988, Sydenham *et al.* 1990, Munimbazi & Bullerman1996)). Milled rice from Burundi markets were found contaminated with fumonisins (Munimbazi & Bullerman 1996). *Fusarium solani*, the comparatively predominant species on the local Super rice grains, was the commonest *Fusarium* species on sorghum from Nigeria, Lesotho and Zimbabwe (Onyike & Nelson 1992). *Fusarium oxysporum* was the commonest *Fusarium* on cereals in storage from Egypt including wheat, barley, maize and sorghum (El-Kady *et al*. 1982). *Fusarium oxysporum* and *F. solani* have also been recorded from mouldy foods, and both of them produce trichothecenes (Bullerman 1979).

Table (1): Percentage infested grains (% IG), Frequency (F) and percentage frequency (% F) of toxigenic *Penicillium*, *Fusarium* and aflatoxigenic *Aspergillus* species on local Super and imported Pakistani rice grains marketed in Uganda, on pentachloronitrobenzene rose-bengal yeast extract sucrose agar (PRYES), pentachloronitrobenzene potato sucrose agar (PCNB-PSA) and *Aspergillus flavus*/*parasiticus* agar (AFPA) media.

Toxigenic fungi	Super rice			Pakistani rice		
	%IG	F	%F	%IG	F	%F
***Penicillium* species (on PRYES medium)**						
P. chrysogenum Thom	0.1	2	8.3	0.2	3	12.5
P. citrinum Thom	0	0	0	0.2	3	12.5
P. fellutanum Biourge	0	0	0	0.04	1	4.2
P. islandicum Sopp	0	0	0	0.1	2	8.3
P. novae-zeelandiae	0.1	2	8.3	0.04	1	4.2
P. oxalicum Currie & Thom	0 .04	4	16.7	0.04	1	4.2
P. pinophilum Hedgcock	0.04	1	4.2	0.04	1	4.2
P. purpurogenum Stoll	0.04	1	4.2	0.04	1	4.2
Penicillium spp.	0.5	7	29.2	0.2	2	8.3
Talaromyces flavus (Klöcker) Stolk & Samson	0.1	2	8.3	0	0	0
Talaromyces spp.	0.2	2	8.3	0.3	4	16.7
***Fusarium* species (on PCNB-PSA medium)**						
F. culmorum (Smith) Saccardo	0	0	0	0.2	1	4.2
F. dimerum Penzig	0	0	0	0.3	2	8.3
F. equiseti (Corda) Saccardo	0.1	1	4.2	0	0	0
F. graminearum Schwabe	0.3	2	8.3	0.1	1	4.2
F. verticillioides (Saccardo) Nirenberg	0.1	1	4.2	0.1	1	4.2
F. oxysporum Schlechtendal	0.2	1	4.2	0.1	1	4.2
F. solani (Martius) Saccardo	0.4	4	16.7	0	0	0
F. tricintum (Corda) Saccardo	0	0	0	0.2	1	4.2
Fusarium spp.	0.3	2	8.3	0.8	2	8.3
Aflatoxigenic *Aspergillus* spp. (on AFPA medium)						
A. flavus Link	0.7	8	33.3	3.2	7	29.2
A. parasiticus Speare	0.3	3	12.5	0	0	0
Other aspergilli						
A. candidus Link	3.0	12	50	11.3	16	66.7
A. clavatus Desmazieres	0	0	0	0.04	1	4.2
A. fumigatus Fresenius	0.1	1	4.2	0.1	1	4.2
A. niger van Tieghem	0.3	4	16.7	0.8	10	41.7
A. ochraceus Wilhelm	0	0	0	0.04	1	4.2
A .oryzae (Ahlburg) Cohn	0.04	1	4.2	0.1	2	8.3
A. penicilloides Spegazzini	0	0	0	0.04	1	4.2
A. tamarii Kita	0	0	0	0.1	1	4.2
A. terreus Thom	0.3	2	8.3	0.1	1	4.2
A. wentii Wehmer	0	0	0	0.2	1	4.2

Incidence of aflatoxigenic *Aspergillus* species

Aflatoxigenic *Aspergillus* spp. were found to occur sparsely on both the local and imported rice grains as determined on *Aspergillus flavus/parasiticus* agar medium (AFPA). The local rice had only 33.3 % and 12.5 % of its samples contaminated by *A. flavus* and *A.parasiticus*, respectively. Similarly, the incidence level of both species on the local rice were equally low with *A. flavus* having contaminated 0.7 % of the rice (Table 1). The incidence of aflatoxigenic *Aspergillus* spp., particularly *A. flavus* which is both a field and storage fungus in foods, is of health concern due to its ability to produce aflatoxins the most potent mycotoxins involved in food spoilage (Bullerman 1979, Sanchis *et al.* 1982, Pitt & Hocking 1997).

The low incidence of *Aspergillus flavus* on both the local Super rice and the imported Pakistani rice is similar to other findings including milled rice from Burundi markets which were free of contamination by both *A. flavus* and *A. parasiticus* (Munimbazi & Bullerman 1996). Incidence levels of *A. flavus* in milled rice from Thailand were also very low, from Indonesia, 3% of the grains were contaminated while from Philippines, 9% of the grains were contaminated (Pitt *et al.* 1994). These south-east Asian countries whose stable food is rice, have been reported to contain the most severe contamination of its stable foods by aflatoxins (Bullerman 1979). In studies on contamination of Uganda's stable foods by *A. flavus/parasiticus*, corn had 77% of its samples contaminated while peanuts had 36% of its samples contaminated. Also, soyabean seeds from Uganda were free of any contamination (Sebunya & Yourtee 1990).

Incidence of aflatoxins

The samples of rice grains had a total of 11 samples out of 18 (61.1 %) contaminated with aflatoxins while 7 samples of the 18 (38.9 %) were uncontaminated. Four levels of contamination were recorded; 0ppb, 0-10ppb, 10-20ppb and 20-50ppb with each having had a total of 7, 7, 3 and 1 samples out of 18 respectively. Therefore, few samples (4 out of 18) were recorded in the aflatoxin levels above 10_{ppb}. However, only 1 sample, from milled Super rice grains, had aflatoxin level above the maximum level of 20ppb allowed in foodstuffs (Table 2).

Milled Super rice grains had 5 out of its 9 samples analyzed (55.6%) free from aflatoxin contamination while milled Pakistan rice grains had only 2 out of 9 samples (22.2%) uncontaminated. Therefore most of the Pakistani rice grain samples, 7 out of 9 (77.8%) were contaminated with aflatoxins. However, when tested statistically using the x^2-test, these results reveal that the incidence of aflatoxins is independent of the type of rice grain whereby at P=0.05, x_{cnt} =7.815 while x^2_{test} = 3.9.

Relationship between the incidence of aflatoxins and aflatoxigenic *Aspergillus* species

The relationship between the incidence of aflatoxins and aflatoxigenic *Aspergillus* spp. reveals that the variables are interdependent, with a weak positive correlation coefficient of r=0.0147 (Table 2; Fig.1). The various samples screened for contamination indicated presence of aflatoxins even when the aflatoxigenic *Aspergillus* spp. lacked. In contrast, aflatoxins were not detected or occurred in relatively low levels in some samples despite the high incidence of aflatoxigenic *Aspergillus* spp. In samples of rice where both *A. flavus* and *A. parasiticus* existed, aflatoxins were also present. This is clearly revealed in sample 9 of milled Super rice, which had the highest level of aflatoxins. However, though the imported rice recorded comparatively higher incidence of aflatoxins than the local rice, the incidence of aflatoxigenic *Aspergillus* on both types was almost equal with 55.5 % of the imported and 44.4 % of the local rice samples having been contaminated respectively.

Relationship between incidence of aflatoxigenic *Aspergillus* species and moisture content

The relationship between incidence of aflatoxigenic *Aspergillus* spp. and moisture content of rice grain samples screened for aflatoxins reveals that the two variables are strongly positively correlated with a correlation coefficient of r = 0.5101 (Table 2, Fig. 2). This implies that high moisture contents are associated with high incidences of aflatoxigenic *Aspergillus* species. The highest moisture content among all the 18 samples screened for aflatoxin presence was 15.2 %; it was recorded on sample 4 of Pakistani rice. This sample also had the highest level of aflatoxigenic *Aspergillus* spp. on Pakistani rice (Table 2).The comparatively higher incidence of aflatoxins, and

Table (2): Summarised relationship between incidence of aflatoxins, aflatoxigenic *Aspergillus* spp. (on AFPA agar) and moisture contents of various market samples of milled Super and Pakistani rice (9 samples each).

Sample No.	Aflatoxin level (ppb)		Aflatoxigenic *Aspergillus*		Moisture content	
	Super rice	Pakistani rice	Super rice	Pakistani rice	Super rice	Pakistani rice
1	0	0	0	0	13.15 ± 0.15	11.80 ± 0.2
2	0	0	0	3	12.95 ± 0.05	13.70 ± 0.20
3	0	0-10	0	0	12.75 ± 0.05	11.60
4	0	0-10	3	62	14.15 ± 0.15	15.2 ± 0.20
5	0	0-10	2	4	12.80 ± 0.20	13.65 ± 0.05
6	0-10	0-10	2	2	11.65 ± 0.15	11.65 ± 0.05
7	0-10	0-10	0	0	12.70 ± 0.10	14.55 ± 0.05
8	10-20	10-20	0	0	12.70 ± 0.10	12.45 ± 0.05
9	20-50	10-20	5	1	12.45 ± 0.25	14.55 ± 0.05

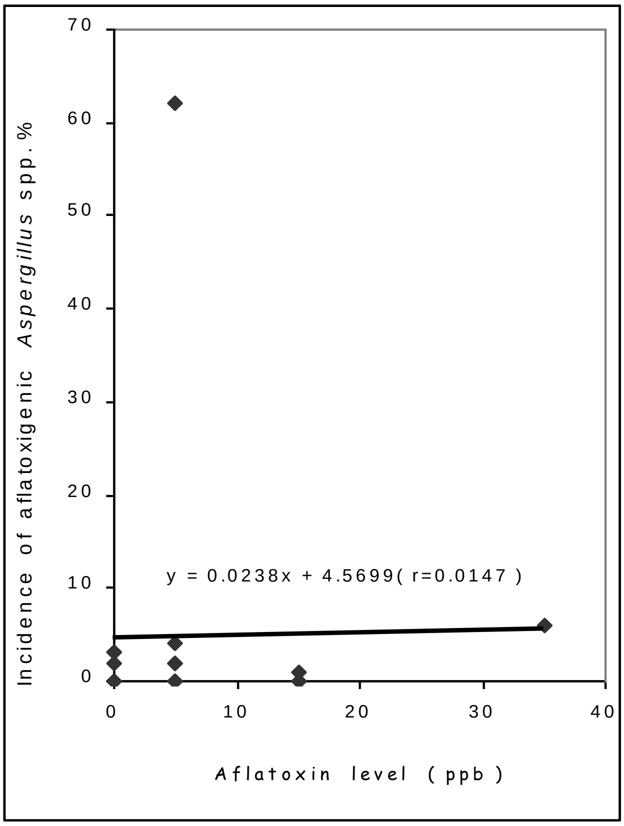

Fig.1: Relationship between incidence of aflatoxigenic *Aspergillus* spp. and incidence of aflatoxins on rice grains marketed in Uganda.

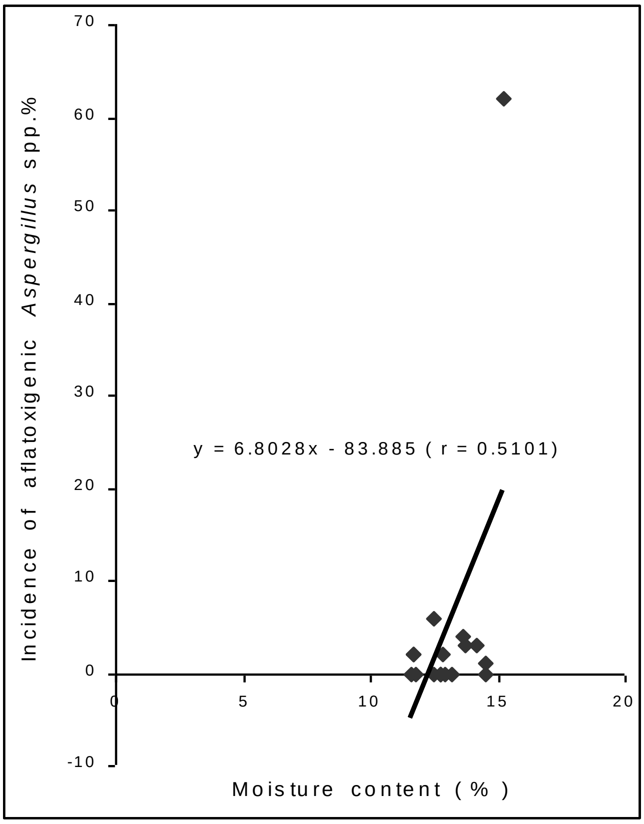

Fig.2: Relationship between moisture content and incidence of aflatoxigenic *Aspergillus* spp. on rice grains marketed in Uganda.

aflatoxigenic *Aspergillus* spp. together with moisture contents on the imported Pakistani rice than the locally grown Super rice may be attributed to the repeated handling and prolonged period of time involved in transporting the grain from Pakistan until its arrival in Ugandan markets. Fungal population and diversity have been found to reflect the kind and efficiency of post harvest handling, conditioning and storage environment, and period that the grain is subjected to (Justice & Bass 1978). The difference between the locally grown rice and the imported rice is that the imported rice has been subjected to prolonged transport over the seas during which time the grains took up moisture from the atmosphere and from metabolic water of the respiring grain (Harris & Lindblad 1978, Pillaiyar 1988). Subsequently, the grains moisture content may have increased to levels suitable for growth of storage fungi. Moisture contents ranging from 12.5% to 16.0% have been found to allow occurrence of an ecological succession of fungi on stored cereal grains from extreme xerophiles to xerotolerants, which include aflatoxigenic *Aspergillus* spp. (Sidik and Pedersen 1986, Milton & Pawsey 1988).

The common malpractice amongst traders of withholding the imported grain as they speculate upon higher demand and prices may also greatly contribute to the incidence of aflatoxigenic *Aspergillus* spp. and subsequent development of aflatoxins in the grain. This malpractice is particularly common when special festivities including the Christian Christmas, and the Muslem fasting period, and Eid approach. Further, the unhygienic practice among retail traders of exposing the rice grain to the atmosphere in a bid to attract customers predisposes the rice grain in the markets to increased moisture content through the grains' hygroscopicity (Harris & Lindblad 1978). Subsequently, aflatoxigenic *Aspergillus* spp. and aflatoxins may develop on this rice. These unhygienic storage and marketing practices among traders have been reported to greatly enhance food spoilage by fungi and mycotoxins (Bullerman 1979).

Conclusion

The current study revealed that both the locally grown and the imported rice grains were contaminated by toxigenic *Penicillium*, *Fusarium*, and *Aspergillus*, including *P. chrysogenum* which is reported to cause endophthalimitis and oesophageal cancer, *F. verticillioides* which is known to produce fumonisins, mycotoxins also associated with oesophageal cancer

(Gelderblom *et al.* 1988, Sydenham *et al.* 1990, Munimbazi & Bullerman 1996). *Fusarium graminearum* which was also isolated on both types of rice is known to produce numerous mycotoxins including trichothecenes (Abbass e*t al.* 1989), while *A. flavus* and *A. candidus,* also occurring on the two rice, produce aflatoxins and citrinin respectively. *Aspergillus parasiticus* which also produces aflatoxins was encountered only on the local rice. However, *P. citrinum,* which is known to produce the nephrotoxigenic citrinin (Frisvad 1983) was recorded only on the imported rice. The local rice recorded comparatively lower moisture contents and incidence of aflatoxins whereby, only 12.5 % of its samples had moisture content above 14.0 %, the recommended storage level of milled rice. In contrast, the imported rice had a half of its samples with moisture content above the recommended storage level. Similarly, the local rice had only 44.4 % of its samples contaminated with aflatoxins with one sample recording 20-50 ppb, a level already above the maximum internationally recommended limit in foods of 20 ppb. In contrast, the imported rice had 77.8 % of its samples contaminated with aflatoxins.

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References

Abbass HK, Mirocha CJ, Kommedal T, Vesonder RF and Golinski P (1989): Production of trichothecene and non-trichothecene mycotoxins by *Fusarium* species isolated from maize in Minnesota. Mycopathol 108: 55-58.

Andrews S (1996): Evaluating of surface disinfection procedures for enumerating fungi in foods: a collaborative study. Int J Food Microbiol 29 (2-3): 177-184.

Aran N and Eke D (1987): Mould mycoflora of some Turkish cereals and cereal products. Mircen J 3: 281-287.

Blakeslee AF (1915): Linders roll tube method of separation cultures. Phytopathol 5: 68-69.

Booth C (1971): The genus *Fusarium.* Commonwealth Mycological Institute, Kew, Surrey, England.

Bullerman LB (1979): Significance of mycotoxins to food safety and human health. J Food Prot 42: 65-86.

Council for Agricultural Science and Technology (1989): Mycotoxins: Economic and Health risks. Task Force Report (U.S.A.) 116: 28-52.

Davis ND and Diener UL (1987): Mycotoxins. In; Food and Beverage Mycology 2nd ed, pp 517 - 570. Beuchat, L.R. (ed). New York: Van Nostrand Reinhold.

El-Kady IA, Abdel-Hafez SII and El-Maghraby OM (1982): Contribution to the fungal flora of cereal grains in Egypt. Mycopathol 77:103-109.

Erricker BC (1979): Advanced general statistics. Williams, Clowes and Sons. Ltd. London.

Eschete ML, King J, West BC and Orbele A (1981): *Penicillium chrysogenum* endophthalmitis. First report case. Mycopathol 4: 125-127.

FAO (1990): Training in mycotoxin analysis. Manuals of food quality control; FAO, Rome No. 14 (10).

Frisvad JC (1983): A selective and indicative medium for groups of *Penicillium viridicatum* producing different mycotoxins in cereals. J Appl Bacteriol 54: 409-416.

Gariboldi F (1973): Rice testing method and equipment. pp. 20-24. FAO, Rome.

Gelderblom WLA, Jaskiewicz K, Marasas WFO, Thiel PG, Horak RM, Vleggar R and Kriek NPJ (1988): Fumonisins-novel mycotoxins with cancer promoting activity produced by *Fusarium moniliforme*. Appl Environ Microbiol 54:1806-1811.

Harris KL and Lindblad CJ (1978): Postharvest grain loss assessment methods: A manual of methods for the evaluation of postharvest losses. The Amer Ass Cer Chem, pp. 95-99.

Hoffman M, Bash E, Berger SA, Burke M and Yust I (1992): Fatal necrotizing oesophagitis due to *Penicillium chrysogenum* in a patient with acquired immunodeficiency symptom. Eur J Clin Microbiol Infect 11: 1158-1160.

ICMSF (1980): Cereals and Cereal products. In Microbiol Ecology and Foods, Vol. II: Food Commodities, pp. 669-730. New York, Academic Press.

Jimenez M, Sanchis V, Santamarina P and Hernandez E (1985): *Penicillium* in preharvest corn from Valencia (Spain) I: Influence of different factors on the contamination. Mycopathol 92: 53-57.

Justice LO and Bass NL (1978): Principles and practices of seed storage. Science and Education Administration Federal Research Staff. Washington. pp. 81- 87.

Lacey J (1988): Microbiology of cereal grains from areas of Iran with a high incidence of oesophageal cancer: J Stored Prod Res 24 (1): 39-50.

Mannon J and Johnson E (1985): Fungi down the farm. New Scientists 105 (1446): 12-16.

Mojica J and Gomez KA (1994): Samples and sampling. In: A manual of rice Seed Health Testing. International Rice Research Institute (I.R.R.I.).

Milton RF and Pawsey RK (1988): Spoilage relating to the storage and transport of cereals and oil seeds. Int J Food Microbiol **7**: 211-217.

Munimbazi, C and Bullerman LB (1996): Molds and mycotoxins in foods from Burundi. J Food Prot 59 (8): 869-875.

Nash SM and Snyder WC (1962): Quantitative estimation by plate counts of the bean root rot *Fusarium* in field soils. Phytopathol 52: 567-572.

Onyike NBN and Nelson PE (1992): *Fusarium* species associated with sorghum grain from Nigeria, Lesotho and Zimbabwe. Mycopathol 84: 452-458.

Pelhate J (1968): Inventaire de la mycoflore des blés de conservation. Bull Trimest Soc Mycol Fr 84: 127-143.

Pillaiyar P (1988): Rice: Rice Production Manual. Wiley Eastern Ltd, New Delhi. pp. 9-41; pp. 230-292.

Pitt JI (1973): An appraisal of identification methods for *Penicillium* species: novel taxonomic criteria based on temperature and water relations. Mycologia 65: 1135-1157.

Pitt JI (1979): The Genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press, London.

Pitt JI, Hocking AD and Glenn DR (1983): An improved medium for the detection of *Aspergillus flavus and A. parasiticus*. J Appl Bacteriol 54: 109-114.

Pitt JI, Hocking AD, Budhasamai K, Miscamble BF, Wheeler KA and Tanboon EKP (1994): The normal mycoflora of commodities from Thailand 2: Beans, rice, small grains and other commodities. Int J Food Microbiol 23: 35-53.

Pitt JI and Hocking AD (1997): Fungi and food spoilage. Blackie Academic and Professional. Australia.

Raper KB and Fennell DJ (1965): The genus *Aspergillus*. Williams and Wilkins, Baltimore, USA (pp. 686).

Sanchis V, Vinas I, Jimenez M, Calvo MA and Hernandez E (1982): Mycotoxin - producing fungi isolated from bin-stored corn. Mycopathol 80: 89-92.

Sebunya TK and Yourtee OM (1990): Aflatoxigenic *Aspergillus* in foods and feeds in Uganda. J Food Quality 13: 97-107.

Sidik M and Pedersen JR (1986): The extent of damage to stored milled rice due to insect infestation. ASEAN Food Handling Bureau, Kuala Lumpur. Malaysia.

Sydenham EW, Thiel PG, Marasas WFO , Shepard G S, Van Schalkwyk DJ and Koch KR (1990): Natural occurrence of some *Fusarium* mycotoxins in corn from low and high oesophageal cancer prevalent areas of Trankei, Southern Africa. J Agric Food Chem 38: 1900-1903.

Taligoola HK, Ismail MA and Chebon SK (2004): Mycobiota associated with rice grains marketed in Uganda. J Biol Sci 4 (1): 271-278.

Tonon SA, Marucci RS, Jerke G and Garcia A (1997): Mycoflora of paddy and milled rice produced in the region of N.E. Argentina and Southern Paraguay. Int J Food Microbiol 37 (2-3): 231-235.