CZECH MYCOL. 63(1): 93–107, 2011

Mycobiota and aflatoxins associated with imported rice grains stored in Uganda

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Taligoola H. K., Ismail M. A., Chebon S. K. (2011): Mycobiota and aflatoxins associated with imported rice grains stored in Uganda. – Czech Mycol. 63(1): 93–107.

Milled rice grains imported into Uganda from Pakistan were investigated for natural contamination by fungi and aflatoxins. The direct plating method using five isolation media was used to enumerate and isolate the fungi during a 270-day storage period. Fungi were isolated and identified to species level and the percentage contamination levels were calculated. A total of 35 species belonging to 16 genera were recorded. The broadest species spectrum were found in the genera *Aspergillus*, *Penicillium*, *Eurotium* and *Fusarium*, which were represented by 11, 7, 4, and 3 species, respectively. Throughout the storage period, xerophilic fungi including *Aspergillus candidus*, *Eurotium amstelodami* and *E. chevalieri* were predominantly isolated. Species of the genus *Penicillium* (particularly *P. pinophilum*) and its teleomorph *Talaromyces* ranked second in predominance, while *Aspergillus flavus*, *Fusarium* spp. and other field fungi occurred only sporadically. Aflatoxins were recorded in rice samples during most storage periods with one sample recording 20–50 ppb. The moisture content increased in rice grains attaining values of over 14 % from the 180th day of storage onwards. A positive correlation was observed between moisture content and incidence of xerophiles, including *A. candidus* and *E. amstelodami*.

Key words: rice grain, xerophilic fungi, nephrotoxigenic penicillia, *Fusarium*, aflatoxins.

Taligoola H. K., Ismail M. A., Chebon S. K. (2011): Mykobiota a aflatoxiny v rýži importované do Ugandy. – Czech Mycol. 63(1): 93–107.

Mletá rýže importovaná z Pakistánu do Ugandy byla studována s ohledem na výskyt hub a aflatoxinů. Bylo zaznamenáno 35 druhů z 16 rodů. Druhově nejpočetnější byly rody *Aspergillus*, *Penicillium*, *Eurotium* a *Fusarium* (11, 7, 4, 3 druhy). Aflatoxiny byly zaznamenány ve vlhkých obdobích. Byla pozorována pozitivní korelace mezi vlhkostí a výskytem xerofilních druhů, včetně *A. candidus* a *E. amstelodami*.

INTRODUCTION

Developing countries including those in sub-Saharan Africa, which includes Uganda, have considerable import and export trade in agricultural commodities. The imports from the Asiatic countries, including Pakistan, are cereal grains, par-

ticularly rice (*Oryza sativa*). These agricultural products are transported from farm to collection centre, from inland to port, from port of the exporting country to that of the importing one, and also from port to areas of consumption in the country of import. In each of these stages, the commodities have to be stored, the period of storage depending on such factors as distance involved, mode and speed of transport, customs clearance, weather conditions like monsoons or the El-Niño, and conditions of storage at the various transit points as well as during transportation (Bhat 1988, Milton & Pawsey 1988).

The time lag involved during transportation particularly includes long journeys overseas lasting several weeks to months during which time the moisture content of the otherwise sufficiently dried grain increases considerably to levels ideal for growth of xerophilic fungi. Further, the metabolic moisture and heat resulting from respiration of both the fungi and rice grain may become ideal for the growth of less xerotolerant fungi. This triggers a chain of reaction that ultimately results in massive colonisation of the grain bulk by various types of fungi (Magan et al. 1984, Bhat 1988, Makun et al. 2007, Reddy et al. 2009, Taligoola et al. 2010).

The study of fungi in cereal grains which have undergone transport is significant due to the presence of mycotoxins and the relationship of these toxins to mycotoxicoses of domestic animals as well as humans, when they consume contaminated grains as food. Several studies on fungal contamination of cereals in storage and those having undergone prolonged transportation have been made worldwide (Wallace & Sinha 1975, Hesseltine 1982, Sidik & Pedersen 1986, Bhat 1988, Milton & Pawsey 1988, Mossel 1988, Wareing 1997, Makun et al. 2007, Reddy et al. 2009). In Uganda, several studies on fungi and mycotoxins, particularly aflatoxin contamination, have been focused on local staple foods including rice, corn, soybean and peanuts (Lopez & Crawford 1967; Apert et al. 1971; Sebunya & Yourtee 1990; Ismail et al. 2003; Taligoola et al. 2004, 2010). However, studies on fungal and aflatoxin contamination of imported foods which are a significant part of diets in Uganda, particularly cereals including rice grain, are yet undocumented.

In the present study, milled rice grains imported into Uganda from Pakistan were analysed for fungal contamination under storage conditions. Aflatoxin levels in the rice grain were also monitored.

MATERIALS AND METHODS

Rice samples. A set of two nylon bags of milled rice grains (each weighing 50 kg) imported from Pakistan into Uganda were bought from markets in the capital city Kampala. The date of packaging as indicated on the bags was 22nd April 1998. The rice was stored in a room at a temperature of 25 ± 2 °C for a period of

270 days between Nov. 1998 and Aug. 1999. During this time the rice grains were periodically sampled 8 times: on the 1st day upon arrival from the market, and then on the 45th, 90th, 135th, 180th, 210th, 240th and 270th day, to analyse fungal and aflatoxin contamination, as well as the moisture content of the rice grains.

Sampling and surface sterilisation. After every storage period, a representative sub-sample of 2 kg, was withdrawn for analysis from the top, middle and bottom of each of the two bags using a nobble trier into a clean conical flask and carefully mixed. Two further working sub-samples of 500 g each from these sub-samples, to be used for determination of fungal contamination, were obtained and first surface-sterilised using 70 % ethanol pre-rinse prior to a 0.8 % chlorine treatment for 2 minutes (Andrews 1996). Excess disinfectant was drained off from the grains, followed by rinsing the grains three times with sterilised tap water. Excess water on the grains was mopped using sterile filter paper. The grains were then plated on suitable isolation media at a plating rate of 10 rice grains per plate in all the media used.

Isolation of fungi. The direct plating method was used to determine seed-borne fungi. A general purpose enumeration medium and four selective agar media were used to detect and isolate the following groups of fungal species: (i) fungi in general, using dichloran rose-bengal chloramphenicol agar, DRBC (King et al. 1979), modified by Pitt & Hocking (1985); (ii) xerophilic fungi, using dichloran 18 % glycerol agar, DG18 (Pitt & Hocking 1980); (iii) aflatoxigenic *Aspergillus* spp., using *Aspergillus flavus/parasiticus* agar, AFPA (Pitt et al. 1983); (iv) nephrotoxigenic *Penicillium* spp., using pentachloronitrobenzene rose-bengal yeast extract sucrose agar, PRYES (Frisvad 1983); and (v) *Fusarium* spp., using pentachloronitrobenzene-potato sucrose agar, PCNB-PSA (Nash & Synder 1962, Booth 1971). Fifty grains per sub-sample were plated on DRBC and PCNB-PSA, while 100 grains were plated for the other remaining selective media. All plates were incubated under natural conditions of light and darkness at 25 ± 2 °C for 7–8 days except for those containing AFPA, which were incubated at 30 °C for 42–48 hrs, DG18, which was incubated for 14–20 days, and PCNB-PSA, which was incubated under continuous light from an ordinary 40-watt, fluorescent tube for 7–8 days. The occurrence of fungi for each sub-sample of the two bags was calculated per total number of rice grains plated on each medium and the resulted incidences were used to calculate the mean occurrence of fungi (as a percentage) on grains from the two bags, since the second bag was a duplicate.

Identification of fungi. Fungi were identified on the basis of their macroscopic and microscopic features using the keys of Raper & Fennell (1965), Booth (1971), Ellis (1971), Pitt (1979), Moubasher (1993), Pitt & Hocking (1997), Leslie & Summerell (2006). Six identification media were used including Czapek yeast extract agar supplemented with 20 % sucrose (CY20S – Thom & Raper 1941) for the identification of *Eurotium* spp., 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., potato dextrose agar (PDA – Booth 1971, Leslie & Summerell 2006) for identification of *Fusarium* spp., and malt extract agar (MEA – Pitt & Hocking 1997) for identification of yeasts.

Extraction and estimation of aflatoxins. Aflatoxin was determined between the 135th and 270th day of storage such that 10 samples of rice were screened. A semi-quantitative test for the determination of total aflatoxins in the rice grains was carried out whereby a commercial immunological test kit, aflascan (from Rhône Diagnostics and Technologies Ltd., Glasgow, U.K.) was 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 analysed 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. A filtered 50–100 ml extract consisting of a blend of 4 g of sodium chloride and 250 ml of 60 % high perfomance liquid chromatography (HPLC) analytical methanol 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 a glass syringe, also a component of the aflascan. Residues in the column were washed off by pumping 10 ml of distilled water three times at 5 ml/minute. Any aflatoxins bound onto the antibodies in the immunoaffinity column were extracted during elution, a process that involved pumping HPLC analytical grade methanol (eluant) through the column at a 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 of distilled water and chloroform was 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 the 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 periodically detemined during the 270-day storage period 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 50 g each per each rice sample from each bag were used. The mean moisture content value of rice from the two bags at a certain period of storage was then calculated.

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

RESULTS

Occurrence of various types of fungi on DRBC

A total of 28 species belonging to 15 genera were isolated from Pakistani rice grains in storage as determined on DRBC medium. These fungi contaminated 72.5 % of the grains. The dominant fungi causing contamination were species of *Eurotium*, *Aspergillus* and *Penicillium* (Tab. 1). *Eurotium* species including *E. amstelodami* and *E. chevalieri* were consistently isolated at relatively higher incidence levels than *E. repens* and *E. rubrum*. *E. amstelodami* and *E. chevalieri* occurred on the rice grains throughout the storage period, but *E. repens* and *E. rubrum* occurred sporadically.

Among aspergilli, *A. candidus* was consistently isolated during the storage period except at day 135 of storage. *A. fumigatus* recorded the highest incidence level (18 %) among aspergilli at the end of storage period (Tab. 1). Among penicillia, *P. pinophilum* had the highest number of isolates while *P. islandicum*, *P. variabile* and *P. oxalicum* contaminated only very few rice grains. *Talaromyces* occurred sporadically, occurring in 36 % of the grains on day 180. Other species coming after the former ones in incidence were *A. niger*, *Chaetomium globosum* and *Rhodotorula mucilaginosa*. The remaining species had low incidence levels. These included *Aspergillus tamarii*, *A. terreus*, *Paecilomyces variotii*, *Phoma* spp., *Pseudogliomastix protea* (teleomorph: *Wallrothiella subiculosa*), *Scopulariopsis brumptii*, *S. candida*, and *Penicillium* spp., apart from those mentioned in Tab. 1.

Occurrence of various xerophilic fungi on DG18

Milled imported rice grains in storage contained 10 species from 6 genera of xerophilic fungi as determined on dichloran 18 % glycerol agar. Several xerotolerant fungi were also isolated. Among the xerophiles, *Eurotium amstelodami*, *E. chevalieri*, *E. rubrum* and *Aspergillus candidus* were isolated in high incidences (Tab. 2). Other species such as *A. wentii*, *Chrysosporium farinicola* and *Polypaecilum pisce* occurred occasionally and at comparatively lower incidence levels than the *Eurotium* spp. *Eurotium amstelodami* and *E. chevalieri* were encountered throughout the storage period, occurring at incidence levels ranging from 30.0–52.5 % and 8.0–27.5 %, respectively.

Tab. 1. Percentage occurrence of fungi (mean number of colonies calculated per 100 grains tested from the two bags) on milled Pakistani rice during a 270-day storage on dichloran rose bengal chloramphenicol agar medium (DRBC).

Fungi*	Storage period (days)							
	0	45	90	135	180	210	240	270
Acremonium strictum	–	–	–	–	–	–	3	–
Aspergillus candidus	14	8	5	–	3	1	3	11
A. flavus	1	–	–	–	–	–	–	–
A. fumigatus	–	1	–	3	–	–	–	18
A. niger	–	6	–	–	1	1	2	–
A. oryzae	1	3	–	2	–	–	–	–
A. sydowii	–	3	–	–	3	–	–	–
Chaetomium globosum	–	1	2	1	2	2	3	–
Cladosporium sphaerospermum	–	–	2	–	–	1	–	1
Cochliobolus bicolor	1	–	–	–	–	–	–	1
Eurotium amstelodami	40	26	22	32	44	38	33	35
E. chevalieri	24	10	14	15	4	14	17	12
E. repens	–	–	11	–	–	–	3	1
E. rubrum	11	7	–	–	–	4	7	3
Fusarium graminearum	–	–	–	2	–	–	4	–
Monascus ruber	–	–	–	–	–	7	2	–
Penicillium islandicum	–	4	–	–	–	–	2	1
P. oxalicum	–	–	–	–	–	–	4	–
P. pinophilum	–	2	4	19	–	3	1	–
P. variabile	–	2	–	–	–	–	1	–
Polypaecilum pisce	–	–	–	–	5	–	–	–
Talaromyces spp.	–	–	19	–	36	2	3	–
Sterile mycelia (white)	2	–	–	–	–	–	–	1
Rhodotorula mucilaginosa	1	–	3	8	3	4	–	–
Other yeasts	–	–	–	1	–	2	5	–

*Fungi which occurred at very low incidence levels are not included in the table.

During storage, a change in species diversity among the xerophilic fungi was recorded. Six species were encountered during the initial 135 days of storage, but all the 10 species occurred between 210 and 270 days of storage on the grains (Tab. 2). Similarly, marginal xerophiles including *Aspergillus fumigatus* and *A. penicillioides* also occurred during the second half of the storage period. The incidence of xerophiles, particularly *A. candidus* and *E. amstelodami*, increased during storage so that the highest incidence levels were recorded during the second half of the storage period (Tab. 2).

Tab. 2. Percentage occurrence of fungi (mean number of colonies; calculated per 200 grains tested from the two bags) on milled Pakistani rice during a 270-day storage on dichloran 18 % glycerol agar medium (DG 18).

	Storage period (days)							
Fungi*	0	45	90	135	180	210	240	270
Xerophilic fungi								
Aspergillus candidus	–	4.5	–	2	3	3.5	2.5	22.5
A. wentii	1	–	–	–	–	–	2	–
Chrysosporium farinicola	–	–	–	–	–	0.5	3.5	–
Eurotium amstelodami	35	30	50.5	42	45	47.5	41	52.5
E. chevalieri	25	16.5	15	15	14.5	27.5	16	8
E. repens	–	6.5	–	–	–	–	2	0.5
E. rubrum	4.5	5	4.5	3	3	–	4.5	3
Monascus ruber	–	–	–	–	–	3	0.5	–
Polypaecilum pisce	–	–	–	–	1.5	2.5	–	–
Wallemia sebi	–	–	–	–	–	–	1	–
Xerotolerant fungi								
Aspergillus flavus	–	–	–	–	–	–	1.5	–
A. fumigatus	–	–	1	–	–	–	–	36.5
A. niger	0.5	–	–	–	0.5	0.5	2.5	–
A. penicillioides	–	–	–	–	–	–	1.5	–
Cladosporium sphaerospermum	1	–	1	3	–	–	2	0.5
Penicillium pinophilum	0.5	1	8	11.5	6	2.5	0.5	0.5
Penicillium spp.	0.5	4	–	–	–	–	–	–
Rhodotorula mucilaginosa	–	–	–	7.5	–	–	–	–
Other yeasts	0.5	–	–	0.5	–	0.5	–	–

*Fungi which occurred at very low incidence levels are not included in the table.

The increasing incidence levels of xerophiles including *A. candidus* and *E. amstelodami* on the imported Pakistani rice were positively correlated with increasing moisture content during storage (Tabs. 1, 2).

Occurrence of aflatoxigenic *Aspergillus* species on AFPA

Rice grains in storage were scarcely contaminated by aflatoxigenic *Aspergillus* spp. Only *A. flavus* was recorded on the rice grain and it occurred sporadically, only twice out of the 8 occasions of plating. Among the other seven *Aspergillus* species found to contaminate rice, *A. candidus* and *A. niger* were the most frequent, occurring on 7 and 4 out of 8 occasions, respectively. The remaining species each occurred on only 2 or 1 occasion out of 8. However, all species of *Aspergillus* except *A. candidus* had incidence levels of less than 10 %. The highest incidence of *A. candidus* was 32 % (Tab. 3).

Tab. 3. Percentage occurrence of fungi on milled Pakistani rice during a 270-day storage period (calculated as mean number of colonies per total number of grains tested from two bags) on *Aspergillus flavus/parasiticus* agar (AFPA, 200 grains), pentachloronitrobenzene yeast extract sucrose agar (PRYES, 200 grains) and pentachloronitrobenzene potato sucrose agar (PCNB– PSA, 100 grains).

	Storage period (days)							
Fungi*	0	45	90	135	180	210	240	270
On AFPA medium								
A. flavus	2.5	–	–	–	–	–	2	–
Other *Aspergillus* spp.								
A. candidus	0.5	12.5	32	11	5	–	2.5	8
A. fumigatus	–	5	–	–	–	–	–	9
A. niger	2	–	–	–	0.5	–	1	1
A. ochraceus	–	0.5	–	–	–	–	–	–
A. oryzae	0.5	1.5	–	–	–	–	–	–
A. penicillioides	0.5	–	–	–	–	–	–	–
A. sydowii	–	–	–	–	1.5	–	6.5	–
On PRYES medium								
Penicillium chrysogenum	0.5	0.5	–	–	–	–	–	–
P. fellutanum	–	–	–	–	–	0.5	–	–
P. jensenii	–	–	–	–	–	0.5	–	–
P. oxalicum	–	–	–	–	–	–	0.5	0.5
P. pinophilum	–	1.5	0.5	1	4	2	1.5	2.5
Penicillium sp.	–	0.5	–	–	–	–	–	–
Talaromyces spp.	–	4.5	4.5	1	–	2.5	0.5	–
On PCNB–PSA medium								
Fusarium graminearum	–	–	–	–	2	–	–	–
F. oxysporum	–	–	4	–	–	–	–	–
F. solani	–	–	8	–	–	–	–	–
Moisture content (%)	13.2 ± 0.1	13.6 ± 0.09	13.53 ± 0.03	13.97 ± 0.12	14.03 ± 0.13	14.23 ± 0.12	14.27 ± 0.06	14.48 ± 0.12

*Fungi which occurred at very low incidence levels are not included in the table.

Occurrence of *Penicillium* species on PRYES

Rice grains were found to be contaminated by 5 *Penicillium* species and one unidentified fungus on the nephrotoxigenic *Penicillium* agar medium, pentachloronitrobenzene rose-bengal yeast extract sucrose (PRYES). Several *Talaromyces* species were also isolated. Well-known nephrotoxigenic *Penicillium* spp. including *P. aurantiogriseum* and *P. viridicatum* were not among the isolated species. However, the incidence levels of *Penicillium* spp. on the imported rice were low with only 4.0 % being the highest level recorded in one sample (Tab. 3). Among the *Penicillium* species occurring on Pakistani rice grain in storage, *P. pinophilum* was predominant. *Penicillium chrysogenum* and *P. oxalicum* were less frequent,

while *P. fellutanum* and *P. jensenii* were rare (Tab. 3). *Talaromyces* spp. (teleomorphs of penicillia) scored 4.5 % as the highest incidence level.

Occurrence of *Fusarium* species on PCNB-PSA

Milled rice grains in storage were found contaminated with only 3 species of *Fusarium* on pentachloronitrobenzene potato sucrose agar (PCNB-PSA). These *Fusarium* spp. were each found only in 1 out of 8 occasions (Tab. 3). The percentage of rice grains contaminated by these *Fusarium* species was equally low; *F. solani* had the highest occurrence level of only 8 %, while *F. oxysporum* and *F. graminearum* contaminated 4 % and 2 % of the grains, respectively. However, despite the increase in moisture content of the rice grains from 13.2 to 14.48 %, the incidence levels of fusaria did not increase during storage because this range of moisture contents is not ideal for the growth of *Fusarium* spp. on rice.

Occurrence of aflatoxins and their relationship between incidence of aflatoxigenic aspergilli, and the moisture content of rice grains.

Eight samples out of the 10 milled Pakistani rice samples screened for aflatoxins were found to be contaminated. Four levels of contamination were recorded: 0 ppb, 0–10 ppb, 10–20 ppb and 20–50 ppb (Tab. 4). The aflatoxin level of 20–50 ppb recorded is above the maximum level internationally allowed in foods (20 ppb). It was noted that all the positive samples for aflatoxins had moisture contents above the recommended level of 14.0 % for safe storage of milled rice grains. Of the important toxigenic fungi only *A. flavus* was found to contaminate the rice grains. It occurred sporadically, only at the start of the study and on the 240th day on each of the two samples of rice studied (Tab. 4).

Tab. 4. Occurrence of aflatoxins, aflatoxigenic aspergilli on *Aspergillus flavus/parasiticus* agar (AFPA) and moisture content of milled Pakistani rice during a storage period of 270 days.

Milled Pakistani rice	Analysis	Storage period (days)				
		135	180	210	240	270
Sample 1	Aflatoxin level (ppb)*	0	0	0–10	0–10	0–10
	Aflatoxigenic aspergilla**	2	–	–	2	–
	Moisture content (%)	13.9±0.1	13.9±0.1	14.2±0.2	14±0.5	14.3±0.0
Sample 2	Aflatoxin level (ppb)*	10–20	20–50	0–10	0–10	10–20
	Aflatoxigenic aspergilli**	3	–	–	2	–
	Moisture content (%)	14.1±0.1	14.15±0.15	14.3±0.1	14.35±0.14	14.6±0.2

*ppb: Parts per billion (micrograms per kilogram)

**calculated as percentage occurrence of isolates (out of 100 grains tested at the end of each storage period).

DISCUSSION

Pakistani rice, being an imported commodity from Pakistan, is subjected to diverse environmental conditions during its transport oversea whereby the grains absorb moisture during its long distance transportation to Uganda. Even before its transportation, the rice may have been subjected to repeated handling between its harvesting and its packaging for export. The date of packaging as indicated on the bags was 22nd April 1998; thus its harvesting and transportation to Uganda was probably around the 1997/98 El-Niño wet weather phenomena. Rice grains imported into the country from Pakistan, Vietnam and India around this period were reported to have been unfit for human consumption (Mukanga 1999).

Milled Pakistani rice was predominantly contaminated by storage fungi including species of *Eurotium*, *Aspergillus* and *Penicillium* as determined on DRBC. Cereal grains which have undergone transport have been shown to be commonly contaminated with these fungi (Wallace & Sinha 1975, Milton & Pawsey 1988, Taligoola et al. 2010).

The heavy contamination of the imported Pakistani rice with xerophiles right from the start of the study in November 1998 suggests that the rice was already contaminated upon its arrival in Uganda. The period of only 7 months which elapsed between its packaging in Pakistan (April 1998 as was indicated on the bags) and the start of the study in November 1998 strongly supports this suggestion. It has been demonstrated that it takes 12 months before extreme xerophilic fungi including *Eurotium* spp. appear in cereal grains including rice and corn with an original moisture contents of 13.5 % (Sauer & Christensen 1968, Sidik & Pedersen 1986). Respiration by the extreme xerophiles on the grains and by the grain itself produces metabolic water which increases the grains' moisture. This subsequently enhances growth of various fungi on the grains (Harris & Lindblad 1978, Bhat 1988, Reddy et al. 2009).

Under storage, in absence of ventilation of the vessels (containers), cereal grains which have undergone shipment have been found to create conditions ideal for growth of extreme xerophiles including *A. restrictus* and *Eurotium* spp. (Bhat 1988). An ecological succession occurs to result in subsequent contamination of the grains by less xerophilic and then xerotolerant fungi including yeasts, *A. candidus*, other *Aspergillus* spp. and *Penicillium* spp. Most of these fungi are involved in grain heating during storage (Christensen 1987, Sauer 1988). Shipment of food-aid grain and from large bag stacks of maize stored in sub-Saharan Africa have been found to be predominantly contaminated by fungi including *Aspergillus candidus*, *A. fumigatus*, *A. flavus*, and *Thermoascus aurantiacus* (Wareing 1997).

On AFPA, the aflatoxigenic species *A. flavus* was recorded at incidence levels of only 2.5 % at the start of the storage period, and 2 % on the 240th day. Therefore,

Pakistani rice grains had few grains and samples contaminated by *A. flavus*. This sparse contamination may be attributed to the moisture contents of the rice grains, which were found unsuitable for the growth of *A. flavus*. The highest moisture content of the various samples of Pakistani rice grains during the entire period of storage was 14.48 ± 0.12 %. However, *A. flavus* has been found to require a lower limit of moisture content of 18.0 % for its growth (Christensen 1987; Sauer 1988). *Aspergillus flavus* as the main aflatoxigenic *Aspergillus* species has similarly been found in various cereal grains including rice from Uganda (Taligoola et al. 2004, 2010), Nigeria (Makun et al. 2007), Thailand (Lapmak et al. 2009), and India (Reddy et al. 2009), corn from Uganda (Ismail et al. 2003) and Burundi (Munimbazi & Bullerman 1996), sorghum and barley from Ethiopia (Abate & Gashe 1985), and in cereal grains, particularly maize, which have undergone transport in tropical countries including sub-Saharan Africa (Milton and Pawsey 1988, Wareing 1997).

The general scarcity of *Penicillum* spp. isolated on PRYES from Pakistani rice grains may also be attributed to their comparatively low moisture contents during storage, whereby the highest moisture contents recorded was 14.48 ± 0.12 %. Most *Penicillium* spp., however, require a minimum moisture of 16.5–19.0 % for optimal growth in cereal grains (Christensen 1987).

Among *Penicillium* species isolated, *P. pinophilum* and *P. chrysogenum* have commonly been reported to occur in food grains which have undergone transport including milled rice (Tonon et al. 1997, Taligoola et al. 2010) and soybean (Bothast et al. 1979). *Penicillium chrysogenum* has also been found to occur in barley (Frisvad 1983). *Penicillium pinophilum* has been reported as an active agent of biodeterioration (Pitt 1979) and has been isolated from maize and peanuts (Pitt et al. 1994, Pitt & Hocking 1997).

Fusarium species, being field fungi, have been found to grow at high grain moisture contents of 20–25 % (Bullerman 1979). However, since 14.48 ± 0.12 % was the highest moisture content recorded in Pakistani rice grains under storage, growth of *Fusarium* spp. was thus inhibited and only three species were recorded on PCNB-PSA agar. However, increase in moisture content and storage period were found to cause an increase in incidence of *Fusarium* spp. on paddy grains from Egypt, whereby moisture contents of 11.5 %, 17 %, 22.5 % and 28 % were found in two studies (Abdel-Hafez et al. 1992, Mazen et al. 1993), and on sorghum from Nigeria (Elegbede et al. 1982).

The presence of *Fusarium* spp. on rice grains suggests a potential for mycotoxin contamination. Trichothecenes, moniliformin and zearalenone have been found to be produced by *F. oxysporum*. *F. graminearum* is also known to produce trichothecenes, whose presence in foods is of human health concern (Bullerman 1979, Abbas et al. 1989).

Data on the relationship between incidence of aflatoxigenic *Aspergillus* spp. (*A. flavus*), aflatoxins on the various samples of Pakistani rice grains and their respective moisture contents show that while an increase in moisture content was recorded, a consistent increase in the incidence of *A. flavus* and aflatoxins was not observed. The highest moisture content recorded for the two samples of rice grains during the entire period of storage was 14.6 %. However, this was considerably below 18.0 %, which is the minimum moisture content for *A. flavus*, the main aflatoxigenic species, to grow in cereal grains (Christensen 1987, Sauer 1988). However, it was noted that all positive samples for aflatoxins had moisture contents above the recommended level for safe storage (14 %) (Christensen & Kaufmann 1965, Bencini & Walston 1991, Taligoola et al. 2010).

CONCLUSION

The current study revealed that milled rice grain imported from Pakistan into Uganda recorded fungal contamination predominantly by xerophilic fungi including *Eurotium* spp. and *Aspergillus candidus* throughout a 270-day storage period, while field fungi including *Fusarium* spp. occurred scarcely. Other storage fungi including aflatoxigenic *Aspergillus flavus*, *Penicillium* spp. and *Talaromyces* spp. were isolated only sporadically. The presence of these fungi, some of which are toxigenic, might enabe mycotoxin contamination of the rice. Aflatoxin incidence on the rice grains did not record any consistent increase, but the majority of the samples (80 %) were found contaminated with one sample recording 20–50 ppb, which is above the maximum limit of 20 ppb internationally allowed in foods. Uganda abides by this limit. From the current data, it is apparent that the presence of fungi and aflatoxins are not due to storage and were most likely present before the start of the study, i.e. at the source. Moisture contents of the rice grains increased significantly during storage attaining values of over 14 %, which is the recommended safe storage level for milled rice, as from the 180th day of storage onwards. A positive correlation was established between increase of moisture content of the rice grains and their contamination level by xerophilic fungi, particularly *A. candidus* and *E. amstelodami*, during storage.

ACKNOWLEDGEMENTS

The authors are deeply indebted to Prof. Bukenya–Ziraba, Head of the Botany Department, Makerere University, Kampala for the facilities he provided during this research. Our gratitude also goes to Managing Director, Uganda Bureau of Standards for the aflatoxin screening facilities he provided. Grateful acknowledg-

ment is due to the Egyptian Fund for Technical Cooperation with Africa for sponsoring Prof. Ismail at Makerere University, giving him the opportunity to act as a supervisor of Mr. S. K. Chebon.

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