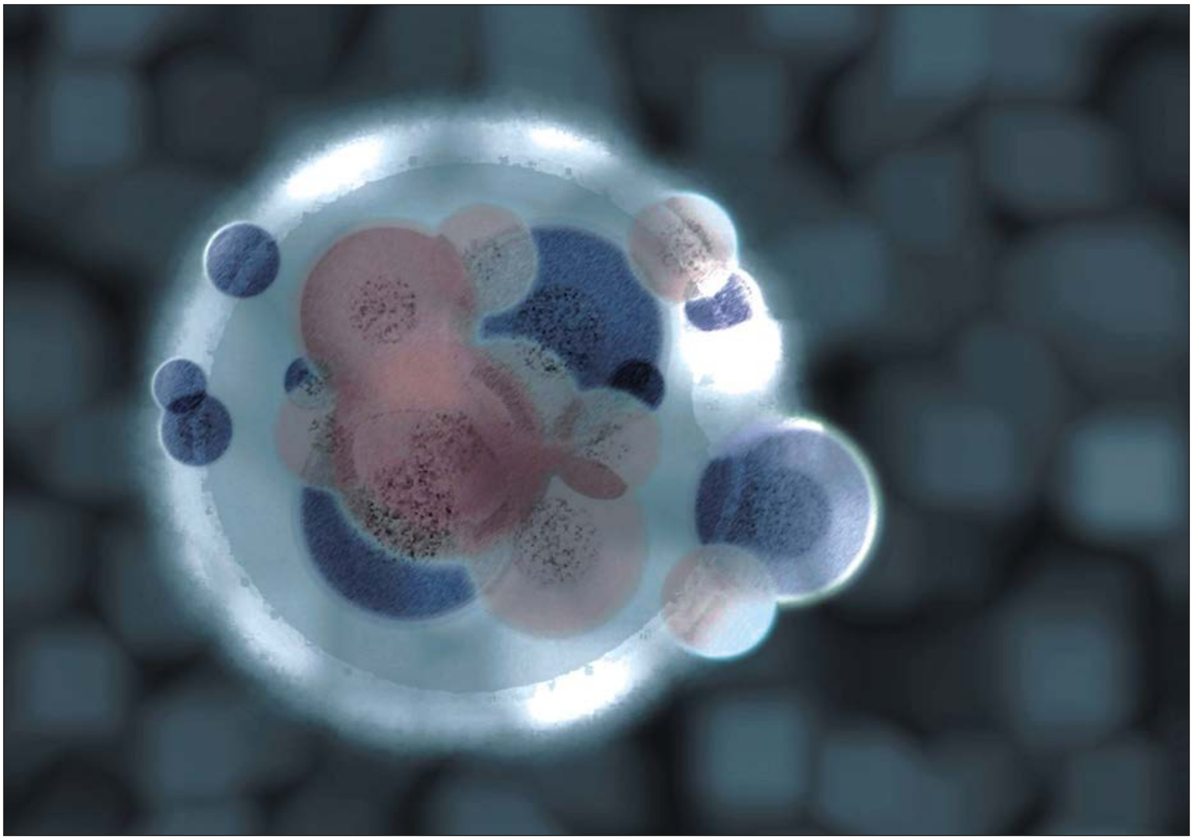

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Mycobiota Associated with Rice Grains Marketed in Uganda

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Abstract: A comparative investigation was made for the natural fungal contamination of imported and locally grown milled rice grains. Twenty four samples of each type of rice were obtained from various market centers within and around Kampala capital city. Contaminating fungi were enumerated by direct plating method on two isolation media including dichloran rose-bengal chloramphenicol agar (DRBC) and dichloran 18% glycerol agar (DG18) media. The locally grown rice recorded a total of 62 species belonging to 34 genera while the imported rice had 61 species belonging to 31 genera, on both isolation media. The broadest species spectrum on both types of rice grains were from the genera *Aspergillus, Penicillium, Eurotium* followed by *Fusarium, Cladosporium* and *Cochliobolus*. Both types of rice grains were predominantly contaminated by *Aspergillus candidus, A. flavus, A. niger, Eurotium amstelodami, E. rubrum, Penicillium citrinum, P.oxalicum* and *Talaromyces* spp. on the two isolation media used. The incidence levels of xerophilic fungi especially *A.candidus* and *Eurotium* spp. were however, comparatively higher on the imported rice than the local rice. Field fungi including *Acremonium strictum, Fusarium moniliforme, F. solani, Scytalidium lignicola* and *Trichorderma harzianum* occurred only on the local rice while xerophilic fungi including *Aspergillus penicillioides, A. wentii, Eurotium repens* and *Paecilomyces lilacinus* occurred only on the imported rice, on both isolation media. The imported rice grain samples recorded comparatively higher moisture contents than the local rice whereby 15.50% was the highest level for the import while for the local it was 14.60%. 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.

Key words: Local rice, imported rice, milled rice grains, storage fungi, xerophilic fungi, Uganda

INTRODUCTION

Consumption of rice (*Oryza sativa* L.) has been increasing in Uganda for the last 15 years. The domestic output is however, inadequate for the countries demand. Thus, importation has been a supplementary measure. Its high nutritional value together with its ability to mix well with most local staple diets including matooke(banana) account for this diet shift[1-3]. However, the grains nutritive value and its high hygroscopicity subjects it to natural contamination by fungi, particularly during such postharvest operations as drying, transportation and storage. Fungal invasion may also occur before harvest[4-6]. These fungi have their spores suspended in the air, thus prevention of grain contamination cannot be totally eliminated[7-10] . However, by drying the grains to moisture content of 14.0%, the recommended level for storage of milled rice, the time taken for storage fungi to develop on the grains will delay[4,11].

Fungi cause organoleptic changes that results in the grains discoloration, mould odour, mustiness and reduced germinability by destroying the embryo. Similarly, respiration of the stored nutrients by the fungal enzymes reduces not only the grains weight but also its nutritional and market value. Further, it will be unsafe for human consumption owing to its ability to cause food poisoning or mycotoxicoses[12,13]. In the developing world where food availability still remains a serious problem, fungal contamination is of greater significance since it not only reduces the food availability, food quality is also reduced[5,14]. The formation of some toxic fungal metabolites (mycotoxins), is the most deleterious effect of fungal invasion of cereals by fungi. The low moisture content of the dried grain is ideal for mycotoxin development by storage fungi including species of *Aspergillus* and *Eurotium* and rarely, *Penicillium* and *Emericella*, all of which are significant storage fungi in the tropics. Indeed, these fungi including

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Aspergillus candidus, A.restrictus, A. wentii and *Eurotium* spp. are xerophiles capable of initiating spoilage of otherwise sufficiently dried grain[4,15].

Studies on fungal and mycotoxin contamination of foods in Uganda have not been documented in the past 10 years despite prevalence of environmental conditions optimum for fungal and aflatoxin development characteristic of the tropics[14]. Similarly, comparative studies on the incidence of various fungi on local and imported foods including rice is yet undocumented. However, Sebunya and Yourtee[16], established Ugandan staple foods (peanuts and corn) and poultry feed to have contamination of *Aspergillus flavus/ parasiticus* and aflatoxin B_1 level of 20 ppb. Similarly, studies in Uganda and Kenya established a link between the highest incidence of liver cancer in the world in this sub-Saharan region to the presence of high levels aflatoxins in foods and beverages[17,18]. This implies that the conditions associated with the possibility of consuming mycotoxin contaminated food exist in this region. This include lack of food variety and food supply, agricultural practices and environmental conditions which favour fungal development in crops and foods and absence of reliable mycological and mycotoxin quality programmes[15,19]. In the present study, locally grown milled rice grain and rice imported into Uganda from Pakistan were analyzed for fungal contamination with the aim of establishing those which are potential producers of mycotoxins.

MATERIALS AND METHODS

Sampling of rice grains: A total of 48 milled rice grain samples each weighing 2 kg were periodically bought from different retailers in shops and open-air markets within and around Kampala, Mukono and Mpigi districts of Uganda between Nov. 1988 and Aug. 1999. Random method of sampling[20] was used for the collection of the rice samples such that an equal number of samples, 8 each, of a locally grown rice variety called super and an imported rice grain from Pakistan were collected from each of the three districts. Thus, 24 samples each of the two types of rice were involved in the study. These samples were analysed for fungal contamination and their moisture content.

Isolation of fungi: The seed-plate (direct plating) method was used to determine the seed-borne fungi on the rice grains. Prior to plating, a 500 g sub-sample was first surface sterilized using 70% ethanol pre-rinse prior to a 0.8% chlorine treatment for 2 min[21]. Excess disinfectant was drained off from the grains after which sterilized distilled 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. A general purpose enumeration agar medium, dichloran rose-bengal chloramphenicol agar: DRBC[22]; modified by Pitt and Hocking[23] and a selective isolation medium, dichloran 18% glycerol agar: DG18[24] were used to detect and isolate fungi, in general and xerophilic fungi, respectively. Fifty grains per sub-sample were plated for DRBC, while 100 grains were plated on DG18. All plates were incubated at under natural conditions of light and darkness for 7-8 days, but plates containing DG18 were incubated for 14-20 days.

Identification of isolated fungi: Fungi were identified on the basis of their macroscopic and microscopic features using the keys of Raper and Fennell[25]; Booth[26]; Ellis[27]; Pitt[28]; Pitt and Hocking[29]. Five identification media were used including Czapek yeast extract agar supplemented with 20% sucrose: CY20S[30], for the identification of *Eurotium* spp., Czapek yeast extract agar: CYA[31], Malt extract agar: MEA[32] and 25% glycerol nitrate agar: G25N[31] for identification of *Penicillium* spp. and Potato sucrose agar; PSA[26] for identification of *Fusarium* spp.

Determination of moisture content: The moisture content of each rice grain sample was determined by finding the loss in weight of the rice grains upon heating for a 24 h period in an oven at 110°C and expressing it as a percentage of the fresh weight[33]. Triplicate sub-samples of 50 g per each rice sample were used. The average of the triplicates became the moisture content of a rice sample.

Statistical analyses: Data were subjected to analysis of variance (ANOVA), t-test and F-test. Statements of significance are based on P 0.05[34]. Correlation was used to determine the relationship between the various variables.

RESULTS AND DISCUSSION

Relationship between moisture content and incidence of fungi: The locally grown super rice had the lowest moisture content of 11.60% while 14.60% was its highest level. Only 3 out of 24 (12.5%) of its samples had moisture contents above 14.0%, the recommended level for safe storage of milled rice grains[11,35]. In contrast, the imported Pakistan rice had the lowest moisture content of 11.60%, while 15.50% was its highest, in which case, 12 out of 24 (50%) of its samples had moisture contents above the recommended level for safe storage of milled rice grains (Table 1). This difference in moisture contents of the two

Table 1: Moisture content (MC) and percentage incidence (%IG) of fungi on 24 samples, each of local milled super rice and imported milled Pakistan rice grains, on dichloran rose-bengal chloramphenicol agar (DRBC) and dichloran 18% glycerol agar (DG18) media

Milled super rice			Milled Pakistan rice		
	%IG			%IG	
MC±SD	DRBC	DG18	MC±SD	DRBC	DG18
11.60±0.26	26	27	11.60	40	44
11.60±0.15	14	5	11.65±0.05	10	19
11.65±0.15	12	3	12.45±0.05	30	22
11.93±0.06	32	31	12.47±0.15	18	35
12.23±0.06	22	57	13.40±0.03	26	12
12.36±0.06	6	9	13.53±0.06	6	17
12.45±0.25	12	6	13.47±0.12	60	81
12.50±0.26	22	11	13.63±0.06	10	10
12.53±0.15	12	64	13.65±0.05	46	82
12.70±0.10	22	5	13.70±0.20	34	97
12.70±0.10	24	14	13.87±0.12	12	36
12.75±0.05	62	7	13.90	10	24
12.80±0.20	42	46	14.03±0.06	58	42
12.95±0.05	10	7	14.27±0.31	12	22
12.96±0.05	16	32	14.33±0.06	36	59
12.53±0.15	8	68	14.33±0.15	48	90
13.20	8	7	14.55±0.05	56	75
13.15±0.15	54	44	14.53±0.06	76	99
13.23±0.06	32	92	14.55±0.05	88	100
13.46±0.05	8	33	14.67±0.06	18	52
13.97±1.10	10	14	14.97±0.25	28	95
14.15±0.15	18	13	15.20±0.20	82	97
14.20±0.17	52	45	15.47±0.06	4	16
14.60±0.17	18	39	15.50	30	10

types of rice is consistent with the difference in the incidence of fungi on the two rice, on both DRBC and DG18 agar media. The local rice had 13 out of 24 (54.20%) of its samples with incidence level of below 20%, with only 3 out of 24 samples (12.50%) having had incidence level of above 50%, on DRBC agar (Table 1). In contrast, the imported rice had 9 out of 24 (37.50%) of its samples with incidence level of below 20%, with 6 out of 24 samples (25%) having had incidence level of above 50%, on DRBC agar. Similarly, the local rice had 11 out of 24 (45.80%) of its samples with incidence level of below 20%, with only 4 out of 24 samples (16.70%) having had incidence level of above 50%, on DG18 agar. In contrast, the imported rice had 6 out of 24 (25%) of its samples with incidence level of below 20%, with 11 out of 24 samples (45.80%) having had incidence level of above 50%, on DRBC agar. Thus, the local rice had majority of its samples recording incidence levels of less than 20% on both isolation media, while the percentage of samples which had incidence levels of more than 50% were relatively higher on the imported rice than the local rice, on both isolation media.

Incidence of fungi on dichloran rose-bengal chloramphenicol agar (DRBC): Locally grown rice grain marketed in Uganda were found contaminated by 45 species of fungi that represented 29 genera while rice imported from Pakistan had 51 species belonging to 26 genera, as determined on DRBC agar medium. The broadest species spectrum on both types of rice grain was from the genus *Aspergillus* that had 7 species on the local rice and 9 species in the imported rice. *Eurotium, Cladosporium, Fusarium* and *Penicillium* genera were each represented by 3 species on the local rice, while on the imported rice, *Eurotium, Cochliobolus* and *Fusarium* each had 4 species. *Cochliobolus* and *Rhizopus* each had 2 species on the local rice while *Cladosporium* and *Penicillium* each had 3 species on the imported rice. The remaining genera were each represented by only one species (Table 2).

The two types of rice grains were predominantly contaminated by *Aspergillus candidus, Eurotium chevalieri, E. rubrum, Mucor circinelloides* and yeasts, which occurred on 1.1, 2.7, 1.4, 0.3 and 4.2%, respectively on the local rice, while on the imported rice they occurred on 5.3, 6.8, 2.7, 2.5 and 4.3% of the rice grains, respectively (Table 2). These fungi except, *M. circinelloides* were similarly the most frequently recorded contaminants, occurring on 20.8, 25, 20.8, 4.2 and 54.1%, respectively, of the local rice samples while on the imported rice they occurred on 29.2, 41.7, 33.3, 8.3 and 58.3% of the samples, respectively (Table 2; Fig. 1). Thus, the imported rice had comparatively higher species diversity and percentage frequency of the predominant fungi, most of which were xerophiles. *Eurotium amstelodami,* also a xerophile, occurred more predominantly and frequently on the imported rice whereby, it was occurred on 3.7% of the grains and 37.5% of the samples while on the local rice it occurred only on 0.2% of the grains and 8.3% of the samples. Together with these xerophilic fungi, other storage fungi including *E. repens, Penicillium citrinum* and *Talaromyces* spp. occurred more frequently on the imported rice than on the local rice. In contrast, field fungi including *Acremonium strictum, Aspergillus niger, Aureobasidium pullulans, Chaetomium* spp.*, Fusarium moniliforme, F. solani, Geotrichum candidum, Scytalidium lignicola and Cladosporium sphaerospermum,* a common field fungus[36], all occurred at a relatively higher frequency on the local rice than on the imported rice (Table 2). *Chaetomium* spp. and *Geotrichum candidum,* both of which occurred more frequently on the local rice require a moisture content of 20-25% for their growth[37]. The local rice, having been freshly harvested were thus ideal substrates for their growth.

The prevalence of *Aspergillus* on both types of rice grains, whereby it ranked the first genus with the widest species spectrum is consistent with other findings, in

Table 2: Percentage incidence (% IG) ,frequency (F) of isolation (from 24 samples) and percentage frequency (% F) of various types of fungi on local milled super rice and imported milled Pakistan rice grains marketed in Uganda, on dichloran rose-bengal chloramphenicol agar medium (DRBC)

	Super rice			Pakistan rice		
Taxa	%IG	F	%F	%IG	F	%F
Acremonium strictum	0.8	3	12.5	0.2	1	4.2
Actinomucor elegans	0.3	1	4.2	0.0	0	0.0
Alternaria chlamydospora	0.0	0	0.0	0.2	2	8.3
Aspergillus candidus	1.1	5	20.8	5.3	7	29.2
A. clavatus	0.0	0	0.0	0.3	2	8.3
A. flavus	0.3	3	12.5	2.1	2	8.3
A. niger	0.4	5	20.8	0.6	4	16.7
A. ochraceus	0.4	3	12.5	0.1	1	4.2
A. oryzae	0.0	0	0.0	0.2	2	8.3
A. parasiticus	0.9	1	4.2	0.0	0	0.0
A. penicillioides	0.0	0	0.0	0.1	1	4.2
A. sydowii	0.0	0	0.0	0.2	1	4.2
A. tamarii	0.1	1	4.2	0.0	0	0.0
A. ustus	1.2	2	8.3	0.0	0	0.0
A. wentii	0.0	0	0.0	0.1	1	4.2
Aureobasidium pullulans	0.2	2	8.3	0.3	1	4.2
Byssochlamys nivea	1.1	2	8.3	0.0	0	0.0
Chaetomium globosum	0.0	0	0.0	0.2	2	8.3
Chaetomium spp.	0.8	5	20.8	0.0	0	0.0
Chrysosporium farinicola	0.1	1	4.2	0.0	0	0.0
Cladosporium cladosporioides	0.3	3	12.5	0.3	2	8.3
C. herbarum	0.3	1	4.2	0.3	2	8.3
C. sphaerospermum	0.8	8	33.3	1.5	5	20.8
Cochliobolus bicolor	0.0	0	0.0	0.3	2	8.3
C. lunatus	0.2	1	4.2	0.6	3	12.5
C. miyabeanus	0.2	2	8.3	0.4	1	4.2
C. pallesceus	0.0	0	0.0	0.3	1	4.2
Epicoccum nigrum	0.1	1	4.2	0.0	0	0.0
Eurotium amstelodami	0.2	2	8.3	3.7	9	37.5
E. chevalieri	2.7	6	25.0	6.8	10	41.7
E. repeus	0.0	0	0.0	1.0	4	16.7
E. rubrum	1.4	5	20.8	2.7	8	33.3
Fennellia flavipes	0.2	1	4.2	0.0	0	0.0
Fusarium decemcellulare	0.0	0	0.0	0.1	1	4.2
F. dimerum	0.0	0	0.0	0.1	1	4.2
F. graminearum	0.1	2	8.3	0.0	0	0.0
F. moniliforme	0.5	3	12.5	0.0	0	0.0
F. oxysporum	0.0	0	0.0	0.1	1	4.2
F. solani	0.3	2	8.3	0.1	1	4.2
Geotrichum candidum	0.4	4	16.7	0.2	2	8.3
Gliomastix state of *Wallrothiella subiculosa*	0.1	1	4.2	0.0	0	0.0
Monographella nivalis	0.3	2	8.3	0.0	0	0.0
Mucor circinelloides	0.3	1	4.2	2.5	2	8.3
Mucor spp.	0.2	1	4.2	0.1	1	4.2
Neosartorya fischeri	0.3	1	4.2	0.0	0	0.0
Paecilomyces lilacinus	0.0	0	0.0	0.1	1	4.2
Penicillium chrysogennm	0.2	1	4.2	0.0	0	0.0
P. citrinum	0.7	1	4.2	0.5	4	16.7
P. islandicum	0.0	0	0.0	0.3	1	4.2
P. oxalicum	0.5	3	12.5	0.0	0	0.0
P.pinophilum	0.1	1	4.2	0.6	4	16.7
P. purpurogenum	0.0	0	0.0	0.2	1	4.2
Penicillium spp.	0.7	3	12.5	0.7	1	4.2
Pestalotiopsis guepinii	0.1	1	4.2	0.3	2	8.3
Phoma spp.	0.1	1	4.2	0.0	0	0.0
Rhizopus oryzae	0.2	1	4.2	0.0	0	0.0
Scopulariopsis candida	0.0	0	0.0	0.3	2	8.3
Scytalidium lignicola	0.3	2	8.3	0.1	1	4.2
Talaromyces spp.	0.4	3	12.5	0.6	5	20.8
Trichoderma harzianum	0.0	0	0.0	0.1	1	4.2
Trichothecium roseum	0.0	0	0.0	0.2	2	8.3
Pichia sp.	0.0	0	0.0	0.1	1	4.2
Wallemia sebi	0.1	1	4.2	0.0	0	0.0
Rhodotorula mucilaginosa	0.9	5	20.8	1.0	5	20.8
Other yeasts	3.3	8	33.3	3.2	8	33.3

which, stored cereal grains have been established to have frequent contamination by *Aspergillus*[35,38]. Cereal grain in storage among them corn from Spain[39]; barley, wheat, maize and sorghum from Egypt[40] and stored maize in sub-Saharan Africa[6] were found to show predominant contamination by *Aspergillus.*

The comparatively higher incidence levels of storage fungi and species diversity on the imported rice than on the local rice may be attributed to the repeated handling of the imported grain from its harvesting in 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 the storage environment and period that the grain is subjected to[41]. 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[13, 42]. Subsequently, the grains moisture content may have increased to levels suitable for growth of xerophilic 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[8,43].

The difference in moisture contents between the imported and the local rice (Table 1) is consistent with the higher incidence levels and frequencies of the predominant fungi, together with the broader species diversity on the imported rice as compared to the locally grown super rice (Table 2). The comparatively low incidence levels of storage fungi on the local rice but prevalence of *Cladosporium sphaerospermum,* a field fungus, is similar to findings from Burundi whereby less than 5% of rice grains sampled from various markets were found contaminated with field fungi including: *Cladosporium* spp., *Alternaria* spp. and *Pyricularia oryzae*[44]. Rice grains from Turkey and Iran were also found to be comparatively free of fungal contamination as compared to other cereals including barley, wheat and corn[45,46].

Incidence of fungi on dichloran 18% glycerol agar (DG18): Locally grown rice marketed in Uganda was found contaminated by 11 species belonging to 6 genera of xerophilic fungi among other 52 species of xerotolerant fungi as determined on DG18 agar medium. The imported rice had a comparatively narrower species spectrum of 9 species belonging to 3 genera of xerophilic fungi among other 40 species of xerotolerant fungi. Among the xerophilic fungi, *Aspergillus candidus, E. chevalieri* and *E. rubrum* were the most frequently isolated species,

Table 3: Percentage incidence (%IG), frequency (F) of isolation (from 24 samples) and percentage frequency (%F) of xerophilic fungi on milled super and milled Pakistan rice grains bought from various markets, on dichloran 18% glycerol agar medium (DG18)

	Super rice			Pakistan rice		
Taxa	%IG	F	%F	%IG	F	%F
Xerophilic fungi						
Aspergillus candidus	2.50	13	54.2	3.9	14	58.3
A. penicillioides	0.30	2	8.3	0.2	4	16.7
A. restrictus	0.70	2	8.3	1.0	5	20.8
A. wentii	0.80	7	29.2	0.6	6	25
Chrysosporium farinicola	0.10	2	8.3	0.5	4	16.7
Chrysosporium spp.	0.00	0	0.0	0.5	4	16.7
Eurotium amstelodami	0.80	6	25.0	7.4	14	58.3
E. chevalieri	2.70	12	50.0	15.0	20	83.3
E. cristatum	0.20	1	4.2	1.7	4	16.7
E. repeus	0.00	0	0.0	1.5	6	25
E. rubrum	3.60	11	45.8	5.5	16	66.7
Monascus ruber	0.10	1	4.2	0	0	0
Polypaecilum iusolitum	0.04	1	4.2	0	0	0
Wallemia sebi	0.30	3	12.5	0.2	3	12.5
Xerotolerant fungi:						
Actinomucor elegans	0.04	1	4.2	0.04	1	4.2
Aspergillus clavatus	0.00	0	0.0	0.04	1	4.2
A. flavus	2.30	7	29.2	2.90	4	16.7
A. fumigatus	1.30	5	20.8	4.90	6	25.0
A. niger	3.90	11	45.8	1.00	6	25.0
A. ochraceus	0.30	5	20.8	0.00	0	0.0
A. tamarii	0.40	1	4.2	0.00	0	0.0
A. terreus	0.04	3	12.5	0.10	2	8.3
A. ustus	0.10	1	4.2	0.30	1	4.2
A. versicolor	0.30	4	16.7	0.04	1	4.2
Aureobasidium pullulans	0.10	2	8.3	0.10	1	4.2
Chaetomium globosum	0.00	0	0.0	0.10	2	8.3
Chaetomium spp.	0.10	3	12.5	0.00	0	0.0
Cladosporium cladosporioides	0.30	5	20.8	1.20	12	50.0
C. sphaerospermum	0.70	10	41.7	1.60	11	45.8
C. herbarum	0.10	3	12.5	0.10	2	8.3
Cochliobolus miyabeanus	0.00	0	0.0	0.10	2	8.3
C. pallesceus	0.00	0	0.0	0.20	2	8.3
Drechslera perpendorfii	0.10	1	4.2	0.00	0	0.0
Emericella nidulans	0.00	0	0.0	0.04	1	4.2
Epicoccum nigrum	0.00	0	0.0	0.10	1	4.2
Fusarium culmorum	0.04	1	4.2	0.00	0	0.0
F. moniliforme	0.20	2	8.3	0.00	0	0.0
F. solani	0.04	1	4.2	0.00	0	0.0
F. tricinctum	0.10	1	4.2	0.00	0	0.0
Fusarium spp.	0.10	1	4.2	0.00	0	0.0
Geotrichum candidum	0.10	2	8.3	0.30	4	16.7
Khuskia oryzae	0.00	0	0.0	0.30	1	4.2
Lasiodiplodia theobromae	0.00	0	0.0	0.30	1	4.2
Monographella nivalis	0.00	0	0.0	0.10	1	4.2
Mucor circinelloides	0.30	2	8.3	3.20	2	8.3
M racemosus	0.00	0	0.0	0.10	1	4.2
Paecilomyces lilacinus	0.00	0	0.0	0.04	1	4.2
P. variotii	0.10	1	4.2	0.00	0	0.0
Penicillium chrysogenum	0.20	2	8.3	0.20	3	12.5
P. citrinum	0.30	2	8.3	0.50	3	12.5
P. dnclauxii	0.04	1	4.2	0.00	0	0.0
P. islandicum	0.04	1	4.2	0.10	2	8.3
P. miczyuskii	0.10	1	4.2	0.00	0	0.0
P. novae-zeelandiae	0.20	2	8.3	0.00	0	0.0
P. oxalicum	0.60	5	20.8	0.50	3	12.5
P. paxilli	0.04	1	4.2	0.00	0	0.0
P. purpurogenum	0.00	0	0.0	0.04	1	4.2
P. variabile	0.00	0	0.0	0.04	1	4.2
Penicillium spp.	2.30	10	41.7	1.20	8	33.3
Scopulariopsis candida	0.20	1	4.2	0.00	0	0.0
S. brumptii	0.00	0	0.0	0.20	2	8.3
Scytalidium lignicola	0.10	2	8.3	0.10	1	4.2
Talaromyces intermedius	0.10	1	4.2	0.00	0	0.0
Talaromyces spp.	0.20	1	4.2	0.40	2	8.3
Thermoascus aurantiacus	0.00	0	0.0	0.04	1	4.2
Trichoderma harzianum	0.04	1	4.2	0.04	1	4.2
Rhodotorula mncilaginosa	0.70	5	20.8	0.50	5	20.8
Other yeasts	0.10	1	4.2	0.04	1	4.2

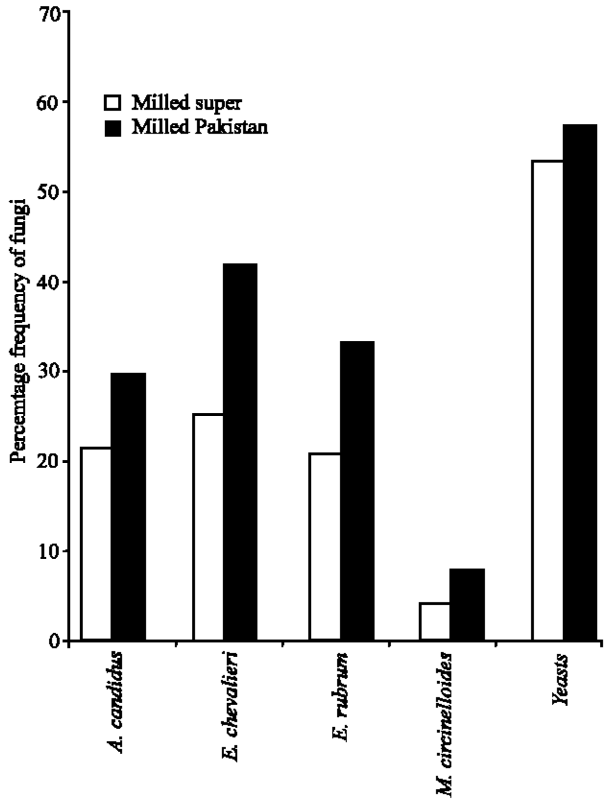

Fig. 1: Percentage frequency of the predominant fungi on local super and imported milled Pakistan rice grains marketed in Uganda, on dichloran rose-bengal chloramphenicol agar medium (DRBC)

occurring on 54.2, 50 and 45.8% of the local rice samples and 58.3, 83.3 and 66.7% of the imported rice grain samples, respectively. *Aspergillus wentii* and *E. amstelodami* were also frequently isolated on both types of rice occurring on 29.2 and 25% of the local rice samples, while on the imported rice they occurred on 25 and 58.3% of the samples, respectively (Table 3, Fig. 2).

These xerophiles except *A. wentii,* were similarly the most predominant species on both types of rice, whereby *A.candidus, A.wentii, E. amstelodami, E. chevalieri* and *E. rubrum* occurred on 2.5, 0.8, 0.8, 2.7 and 3.6% of the local rice grains, respectively while on the imported rice they occurred on 3.9, 0.6, 7.4, 15.0 and 5.5% of the grains, respectively (Table 3). The remaining species, except *E. repens* which occurred only on the imported rice, contaminating 1.5% of its grains and 25% of its samples, occurred only sparsely. However, other species of xerophilic fungi which were recorded on both types of rice include *A. restrictus, E. cristatum* and *Wallemia sebi* (Table 3).

The predominant xerophilic fungi occurring on both the local milled super and imported Pakistan rice were

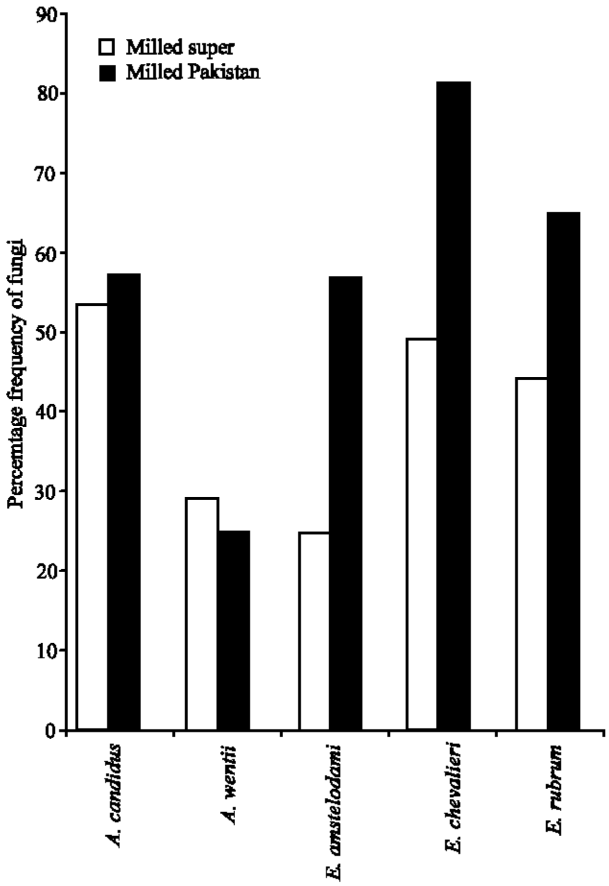

Fig. 2: Percentage frequency of the predominantxerophilic fungi on local super and imported milled Pakistan rice grains marketed in Uganda, on dichloran 18% glycerol agar medium (DG18)

thus: *Eurotium* spp. and *Aspergillus* spp. Among the *Eurotium* spp., *Eurotium amstelodami, E. chevalieri* and *E. rubrum*, followed by *E. repens* were predominant, while among the *Aspergillus* spp., *A. candidus* was the most predominant species followed by *A. wentii* and *A. restrictus* (Table 3, Fig. 2). These observations are consistent with other findings whereby *Eurotium chevalieri* has been reported as the commonest spoilage fungi in stored cereal grains while *E. amstelodami* and *E. rubrum* have also been reported to occur frequently, but the latter is more common in warmer regions[29].

Foodstuffs in retail outlets and markets have been reported to be actually under storage and depending on the conditions and time that the commodities are stored awaiting customers, fungal contamination may occur[37]. As a biological product, aging of the rice grain with subsequent increase in its hygroscopicity is highly significant in the storability of the rice. Absorption of atmospheric moisture by the rice grains increases its moisture content. Subsequently, this enhances fungal growth on the rice[42].

The comparatively higher moisture contents and prevalence of xerophiles on the imported rice grain than the local rice grain (Tables 1 and 3; Fig. 2) may be attributed to the prolonged period of time involved in transporting the imported rice grain from Pakistan to Uganda. Further, more time is involved in distributing it within the various local wholesale and retail markets. Similarly, the common malpractice among traders of withholding the rice as they speculate for increased demand and higher prices may also provide more time for the grains' moisture content to increase resulting in further fungal contamination of the imported Pakistan rice. In contrast, the locally grown super rice does not undergo prolonged transportation and thus comparatively little time is available for the grains' moisture content to increase.

The current study revealed that both the locally grown and imported rice grains were predominantly contaminated by xerophilic fungi including *Eurotium amstelodami, E. chevalieri, E. rubrum* and *Aspergillus candidus*. The presence of these toxigenic fungi on the rice grains suggest contamination by mycotoxins including sterigmatocystin by *E.amstelodami* and citrinin by *A.candidus*. The incidence of these xerophilic fungi was however, higher on the imported rice. Further, the imported rice had a relatively wider species spectrum of xerophilic fungi including *A. penicilloides, A. wentii, E. repens* and *Paecilomyces lilacinus,* all of which were not encountered on the local rice. Instead, field fungi including *Acremonium strictum, Fusarium moniliforme, F. solani, Scytalidium lignicola* and *Trichoderma harzianum* occurred only on the local rice. The presence of these fungi including *F. moniliforme* and *F. solani* on the rice grains also suggest presence of mycotoxins including fumonisins, which have been associated with esophageal cancer[47,48]. Similarly, the imported rice, recorded a comparatively higher moisture content, whereby, 15.5% was its highest level while for the local rice 14.6% was the highest level. Further, a half of the imported rice samples had moisture content above 14.0%, the ideal level for storage of milled rice, while the local rice had only 12.5% of its samples with moisture above 14.0%.

The comparatively higher moisture contents of the imported rice correspond to its higher contamination levels by fungi, particularly storage fungi, most of which are associated with the production of mycotoxins. Thus, there is a high possibility that the imported rice could have more mycotoxin contamination than the local rice.

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