Incidence of mycobiota and aflatoxins during storage of paddy and milled rice grown in Uganda

H. K. Taligoola[1], M. A. Ismail*[1,2] and S. K. Chebon[1,3]

[1]Department of Botany, Faculty of Science, Makerere University, P.O. Box 7062, Uganda, e-mail: botany@africaonline.co.ug

[2]Department of Botany, Faculty of Science, Assiut University, P.O. Box 71516, Assiut, Egypt;

[3]Moi High School-Kabarak, P.O. Box, 7049, Nakuru, Kenya.

*[2]Corresponding author: e-mail: madyismail@yahoo.com
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Abstract: Paddy and milled rice grown in Uganda were investigated during storage for natural contamination by various types of fungi and aflatoxins. Direct plating method using five isolation media including dichloran rose-bengal chloramphenicol agar (DRBC), dichloran 18 % glycerol agar (DG18), *Aspergillus flavus/parasiticus* agar (AFPA), pentachloronitrobenzene rose-bengal yeast extract sucrose agar (PRYES), and pentachloronitrobenzene potato sucrose agar media (PCNB-PSA) were used to determine the fungal contamination. Fungi were isolated and identified to species or genus level and percentage contamination level was calculated. During storage over a 270-day period between Nov.1998 and Aug. 1999, paddy rice recorded a total of 58 species belonging to 33 genera while the milled rice recorded 50 species belonging to 30 genera. The broadest spectrum of species on both types of rice were from the genera *Fusarium, Aspergillus and Penicillium* on all isolation media whereby, 11, 9, and 4 species respectively were recorded on the paddy, while 4, 10 and 4 species respectively, occurred on the milled rice. *Eurotium* was represented by 3 species; *Cladosporium, Cochliobolus* and *Humicola* were each represented by 2 species, while the remaining genera each had one species on the paddy rice. *Eurotium* was similarly represented by 3 species on the milled rice, *Chaetomium, Cladosporium, Humicola, Paecilomyces* and *Scopulariopsis* each had 2 species while the remaining genera each had only one species. Field fungi including *Cochliobolus miyabeanus, Fusarium lateritium, F. stilboides, Khuskia oryzae, Pestalotiopsis guepinii,* and *Scytalidium lignicola* were all predominantly isolated on the paddy rice grains but only during the first half of the storage period, declining thereafter except for *S. lignicola*. Storage fungi including *Aspergillus candidus, A. flavus, Eurotium* spp., *Paecilomyces variotii, Penicillium* spp. and *Talaromyces* spp. occurred sporadically. The majority of the paddy samples (60 %) screened for aflatoxins recorded contamination but none above 20 ppb, the maximum level allowed in foodstuffs internationally. Increase in moisture content of the paddy was recorded during storage, attaining a maximum of 12.9 %. The milled rice was however, comparatively less contaminated by fungi, and with both the field fungi and storage fungi recording relatively equal distribution. Milling (dehusking) removed most of the fungal propagules from these grains. Aflatoxin contamination was similarly scarcely recorded with 60 % of the milled samples not having indicated any contamination. Increase in moisture content occurred during storage, attaining a maximum of 13.98 %.

Key words: Paddy rice, milled rice, field fungi, storage fungi, aflatoxins

Introduction

Consumption of rice (*Oryza sativa* L.) has been increasing in Uganda for the last 20 years. The high nutritional value of rice together with its ability to mix well with most local stable diets such as matooke (banana) account for this diet shift (Juliano 1993, World Bank 1993). However, the grains' nutritive value and its high hygroscopicity subject it to natural contamination by ecologically diverse fungal groups particularly during such postharvest operations as drying and storage (Bencini and Walston 1991). Fungal invasion may also occur prior to harvest such at harvest, when the grains' moisture content is 20-24 %. Field fungi including *Acremonium, Alternaria, Aureobasidium, Cladosporium, Curvularia, Drechslera, Epicoccum, Fusarium, Humicola, Phoma, Nigrospora (*Teleomorph: *Khuskia), Trichorderma,* and yeasts already exist as predominant contaminants in freshly harvested paddy (Kurata *et al.* 1968, Christensen and Kaufmann 1969, Majumdar 1974, Kuthubutheen 1979, Abdel-Hafez *et al.*1987, Pitt *et al.* 1994, Lapmak *et al.* 2009, Reddy *et al.* 2009).

Field fungi usually disappear from the grain upon drying, however, inadequate drying or leaving the harvests unthreashed for several weeks out in the field worsen their deteriorative effects. The shelf-life of improperly dried or unhygienically dried and stored grain is thus greatly reduced. Drying is recommended to a moisture content of 13.0 % prior to storage of paddy rice while for milled rice, the ideal moisture content for safe storage is 14 % (Christensen and Kaufmann 1969, Bencini and Walston 1991). However, dehusking the grains prior to their storage enhances exit of moisture from the grain, thus reducing the viability of fungal spores trapped within the husk layers (Flannigan 1970, Gariboldi 1973). Improper drying enhances fungal damage of the grains by such storage and xerophilic fungi including *Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A. versicolor, A. wentii, Eurotium amstelodami, E. chevalieri, E. repens, E. rubrum* and *Penicillium* spp., all of which have frequently been isolated from stored rice (Abdel-Azim and Khalil 1979, Mheen *et al.* 1982, Magan *et al.* 1984, Pitt *et al*, 1994, Reddy et *al*. 2009). These storage fungi are therefore of greater significance due to their toxicity on the rice since the water stress created by the low moisture content of the dried grain is ideal for the formation of toxic fungal metabolites (Bullerman 1979, FAO 1990).

Fungi render contaminated grains unpalatable, less nutritive, less viable and discoloured, but also reduce their dry weight and make them unsafe for human and animal consumption by producing mycotoxins. Mycotoxins evoke acute and chronic pathological changes upon ingestion. Currently, over 200 mycotoxins are known but only those that occur naturally in foods are significant in human food poisoning or mycotoxicoses. Species of *Aspergillus*, *Penicillium* and *Fusarium* are the main toxigenic fungi associated with cereals. They produce such mycotoxins as aflatoxins, ochratoxins, trichothecenes and zearalenone. Mycotoxicoses in humans include growth retardation, impaired immunity, tumour growth, and death in acute cases (Cole and Cox 1981, Council for Agricultural Science and Technology 1989, Lindsay 1997, Paterson and Lima 2010).

The most serious effects of consumption of mycotoxin-contaminated food and feeds are thought to occur in sub-Saharan Africa. Studies in Uganda and Kenya have linked the highest incidence of liver cancer in the world to the presence of high levels of aflatoxins in foods and beverages (Alpert *et al*. 1971, Peers and Linsell 1973, Ocama *et al.* 2009).

In view of the health and economic risks associated with fungal and mycotoxin contamination of foods, it is important that realistic goals for microbial food quality are established. Incidence and toxicological data, from which some baseline of food hazard analysis can be derived is therefore essential yet it is scarce in Uganda. This research intended to contribute to that goal.

Materials and methods

Sampling of rice

A set of 4 bags of freshly harvested rice, each weighing 60 kg, were collected from the rice growing regions of Eastern Uganda, around Kibimba rice irrigation scheme. A rice variety locally called super, was involved in the study such that two bags each of paddy (rice with husks) and milled (dehusked) rice were acquired. Random method of sampling (Mojica and Gomez 1994) was used for the collection of the rice samples. All the bags were stored in one room at temperature of 25 ± 2 ºC for a period of 270 days, during which time the rice samples were periodically analysed for fungal and aflatoxin contamination, as well as determination of their moisture content. Sub-samples, each weighing 2 kg, to be used for analysis were withdrawn from the top, middle and bottom of each of the bags using a nobble trier into a clean conical flask and carefully mixed.

Isolation of fungi

The seed-plate (direct plating) method was used to determine the rice grain-borne fungi whereby, a general purpose enumeration medium and 4 selective agar media were used to detect and isolate the following groups of fungi: (i) Fungi in general using dichloran rose-bengal chloramphenicol agar, DRBC (King *et al* 1979, Pitt and Hocking 2009), (ii) Xerophilic fungi using dichloran 18 % glycerol agar, DG18: (Hocking and Pitt 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 and Synder 1962, Booth 1971). Prior to plating, the rice of each sub-sample were first surface sterilized using 70 % ethanol pre-rinse prior to a 0.8 % chlorine treatment for 2 minutes (Andrews 1996). The rice was then plated at a plating rate of 10 rice grains per each agar plate onto the isolation media used. Five agar plates (including 50 rice grains) per sub-sample were plated for DRBC and PCNB-PSA, while 10 agar plates (including 100 rice grains) for the other remaining selective media were plated. Thus, a total of 100 grains were plated from the two bags for DRBC and PCNB-PSA, and a total of 200 grains for DG18, AFPA and PRYES agar media. The plates were then incubated under natural conditions of light and darkness for 7-8 days except for those containing AFPA which were incubated at 30 ºC for 42-48 hrs, DG18 which were incubated for 14-20 days and PCNB-PSA which were incubated under continuous light from a fluorescent tube. The incidence of fungi from each of the two bags of rice grain was used to find the mean incidence of fungi 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 and Fennell (1965), Booth (1971), Ellis (1971), Pitt (1979), Moubasher (1993), Domsch *et al.* (2007), Pitt and Hocking (2009). Five identification media were used including Czapek yeast extract agar supplemented with 20 % sucrose: CY20S (Thom and 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. and Potato sucrose agar: PSA (Booth 1971, Leslie and Summerell 2006) for identification of *Fusarium* spp., and CYA and MEA for other groups of fungi.

Extraction and estimation of aflatoxins

Aflatoxin determination was done between the 135th and 270th day of storage such that 10 samples

of each type of rice were screened. Semi-quantitative test for the determination of total aflatoxins in the rice grains was done whereby a commercial immunological test kits, 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 rice was determined according to the procedure outlined in the aflascan. Samples to be analyzed were at least 1 kg of rice, aseptically and thoroughly ground to fine powder, from which a 50g sub-sample was withdrawn for the aflatoxin assay. A filtered 50-100 ml extract consisting of a blend of 4g of sodium chloride and 250 ml of 60 % high performance 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 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 below the column, into which 1.0 ml each, 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 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 ultraviolet 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 rice sample was determined by finding the loss in weight of the rice upon heating for a 24-hour period in an oven at 110 ^{o}C and expressing it as a percentage of the fresh weight (Gariboldi 1973). Triplicate sub-samples of 50g each per each sample of rice were used. The average of the triplicates became the moisture content. Moisture contents of the rice were periodically determined during the 270-day storage period, whereby, the average value of the two bags of a given type rice represented the moisture content at a certain period of storage.

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 was used to determine the relationship between the various variables.

Results and discussion

Incidence of fungi on rice during storage (recovered on DRBC)

A total of 36 species belonging to 23 genera were isolated from paddy on dichloran rose-bengal chloramphenicol agar medium (DRBC), while for milled rice 31 species belonging to 24 genera were isolated. The contaminated rice during the whole storage period was 89.4 % of the total number of paddy, but it was only 14.5 % for the milled rice. Similarly, the highest incidence level at any one occasion of plating was 68 % for paddy but for milled rice, it was generally not more than 5 %, except yeasts which had 15% incidence level on one occasion, day 180, of the storage period (Table 1).

Cochliobolus miyabeanus, *Khuskia oryzae* (anamorph: *Nigrospora oryzae*), *Pestalotiopsis guepinii* and *Scytalidium lignicola*, all of which are field fungi (Christensen and Kaufmann 1974) occurred predominantly on the paddy but not on milled rice (Table 1). The first 3 species were also the most frequent on paddy rice, particularly during the first half period of storage. *Fusarium* including: *F. lateritium, F. verticillioides* and *F. stilboides* were also frequent, but their incidence levels were comparatively lower with 8 % being the highest level recorded. Other species which also occurred at moderate frequencies were *Geotrichum candidum*, *Wallrothiella subiculosa* anamorph and *Phialophora verrucosa*, but their incidence levels were also very low, each had less than 10 % (Table 1).

The remaining species, whose incidence levels were also low, each occurred in less than 5 % of the kernels in the samples in which they occurred. These included *Beauveria* sp., *Byssochlamys fulva*, *Cochliobolus lunatus, Chrysosporium* spp., *Doratomyces* sp., *Monascus ruber, Paecilomyces variotii, Penicillium* spp., *Phoma* spp., *Scopulariopsis brevicaulis, Trichoderma harzianum, Thermoascus aurantiacus, Rhodotorula mucilaginosa* and other unidentified yeasts.

The incidence levels of *Cochliobolus miyabeanus*, *Khuskia oryzae* and *Pestalotiopsis guepinii* on paddy (super rice) were high only during the first half period of storage. A decline occurred thereafter resulting in lower incidence levels or disappearance (Table 1). This trend of incidence was tested and found statistically significant whereby at $p<0.05$; F_{test} =2.84 while F_{cnt} =2.66. Milled super rice was

similarly found to exhibit a change in species diversity in the fungi that inhabited them whereby *Khuskia oryzae* and *Pestalotiopsis guepinii* were isolated within the first half period of storage, but disappeared thereafter. This observation is consistent with the decrease in the incidence of field fungi including *Alternaria* and *Curvularia* in fresh harvests of sorghum from Nigeria during a 12-month storage (Elegbede *et al.* 1982). These species, being field fungi, usually invade cereal grains before harvest at moisture content of 22-23 %. Other field fungi that have been established to be predominantly harboured by paddy include *Alternaria*, *Curvularia*, *Drechslera*, *Fusarium* and *Cladosporium* spp. (Fanse and Christensen 1969, National Institute of Nutrition 1977, Abdel-Hafez *et al*. 1987, Tonon *et al.* 1997, Makun *et al.* 2007, Lapmak *et al.* 2009, Reddy *et al.* 2009). The husks of other cereals including barley, wheat, and oats have similarly, been found to be heavily contaminated by these field fungi (Flannigan 1970, Mulinge and Chesters 1970).

However, the activity of these field fungi is arrested upon drying and subsequent storage of the grain or seed (Christensen and Kaufmann 1974, Bullerman 1979). Despite the low moisture contents of the paddy grains, propagules of these fungi may have persisted on the husks shortly after harvest and could be isolated at high incidence levels particularly during the first half of the 270-day storage period (Table 1). In contrast, xerophilic fungi including *Aspergillus candidus*, *Eurotium amstelodami*, and *E. rubrum* occurred frequently on the milled rice only during the second half period of storage but just, sporadically as compared to the field fungi (Table 2). These xerophiles are among the pioneer agents of seed deterioration during storage whereby they may produce mycotoxins including citrinin and sterigmatocystin apart from reducing seeds viability (Sanchis *et al*. 1982). Paddy grains from Egypt were also found to show increasing incidence levels of various fungi including: *A. candidus*, *A. flavus*, *A. sydowii*, *A. tamarii*, *P. chrysogenum*, *F. oxysporum* and *C. cladosporioides* during a 4-month storage (Mazen *et al*. 1993).

Milled rice, unlike the paddy, was comparatively less contaminated by fungi during storage as determined on DRBC medium (Table 1a). Milling may thus have caused the observed low incidence of fungi on the milled. Fanse and Christensen (1969) found that 21.3% of the paddy grains samples yielded storage fungi from more than 50% of the grains, but germination of these samples exceeded 90%, indicating that the invasion by storage fungi probably was superficial. *A. flavus* was similarly isolated on only the husks of the kernels from which they grew. Flannigan, (1970) found that fungi deposited as spores on wheat grains late in the season penetrate the husk, but not the pericarp or even remain as superficial contaminants of the husks. The 5% of the hull (husk) and pericarp removed during dehulling of barley for human consumption were similarly found to contain the most viable inoculum of fungi (Flannigan and Dickie 1972). Field fungi were similarly established more frequently on hulls than in caryopses of moist stored barley grains (Mulinge and Chesters 1970).

However, the distribution of the field and storage fungi on the milled rice, unlike, on the paddy was relatively similar. Evidently, storage fungi including *Aspergillus*, *Eurotium* and *Chaetomium* were more frequent on the milled than on the paddy rice. Dehusking and drying probably reduced the propagules of the field fungi deposited on the husks prior to harvesting, thus creating conditions ideal for the invasion of the milled rice grain by storage fungi. The scarcity of storage fungi on samples of freshly harvested cereals has also been established in barley grains by Abdel-Kader *et al.* (1979) whereby, low frequency of only 20 % and 2.5 % were recorded for *E. amstelodami and E.chevalieri*, respectively. Aran and Eke (1987) also recorded *Eurotium* spp. to contaminate only 4.1% of the samples of cereal grains including rice, barley, corn and wheat. *Chaetomium*, a fungus reported to cause advanced decay of cereal grains (Bullerman 1979) also occurred only rarely on the paddy grains during storage (Table 1).

The fungi that were commonly isolated from the milled rice may thus, have penetrated into the kernel before dehusking. *C. sphaerospermum*, a common field fungus, and yeasts were the most frequently isolated fungi, in which case *Rhodotorula mucilaginosa* was the commonest yeast (Table 1). These findings are consistent with observations by Clarke and Hill (1981) whereby field fungi alongside yeasts, particularly *R. mucilaginosa* were the predominant fungi isolated from moist barley during storage. *Rhodotorula* spp. were similarly found to be the commonest yeasts in freshly harvested barley and wheat grains (Flannigan 1974), and field, marketed and stored rice in Nigeria (Makun *et al*. 2007). Other fungi that occurred on milled rice grains on DRBC medium but at moderate frequencies included *Aspergillus niger*, *Chaetomium brasiliense*, *Eurotium chevalieri* and *Penicillium* spp. (Table 1). *Aspergillus niger* has been reported as a common fungus in both field conditions and stored foods, particularly in warmer climates (Pitt and Hocking 2009). *E. chevalieri*, a xerophile involved in food spoilage at the initial phase of grain storage, together with *Penicillium* spp. are both storage fungi while, *Chaetomium* spp. are advanced decay fungi which grow on stored foods after considerable deterioration by other microbes (Bullerman 1979).

The diversity of species for various genera varied whereby *Fusarium* was the commonest genus on the paddy rice grains. It was represented by 7 species and some others unidentified, whereby *F. lateritium*, *F. verticillioides* and *F. stilboides* were the most frequent species (Table 1). Closely similar findings

were reported by Abdel-Hafez *et al.* (1987), whereby *Fusarium* ranked the third most frequent genus on paddy grains from Egypt, with *F. oxysporum* being the commonest species. El-Kady *et al.* (1982) also found *Fusarium* the second most common species on barley and wheat from Egypt, whereby *F. oxysporum* and *F. solani* were the commonest species. *Fusarium* was also established to be the most common genus in fresh harvests of wheat, maize and sorghum in Egypt (Moubasher *et al.* 1972, Abdel-Kader *et al.* 1979), maize in Spain (Jimenez *et al.* 1985) and rice in Thailand (Lapmak *et al.* 2009). Samples of maize grains from Central America, Asia and some sub-Saharan countries including Kenya were found to have predominant contamination by *Fusarium* spp. (Macdonald and Chapman 1997).

The prevalence of *Fusarium*, which is a field fungus, on paddy rice confirms that the rice grains were indeed fresh harvests yet to be massively invaded by storage fungi. This observation is consistent with other findings whereby, field fungi including *Alternaria, Curvularia, Drechslera, Fusarium* and *Cladosporium* spp. have similarly been established to predominate on fresh harvests of sorghum and corn (Fanse and Christensen 1969, National Institute of Nutrition 1977, Diener *et al.* 1981, Abdel-Hafez *et al.*1987, Lacey 1988), paddy rice (Tonon *et al.* 1997, Lapmak *et al.* 2009) or polished rice (Tonon *et al.* 1997, Taligoola *et al.* 2004). The husks of other cereals including barley, oats and wheat have similarly been found to have heavy contamination by these fungi (Flannigan 1970, Mulinge and Chesters 1970). Flannigan (1974) also established closely similar findings, in which, field fungi and yeasts including *R. mucilaginosa* were the most frequent fungi on freshly harvested barley and wheat grains.

Aspergillus ranked second most frequent genus on paddy rice, it had 4 species whereby *A. niger* and *A. flavus* were comparatively predominant (Table 1). These observations are consistent with other findings whereby storage fungi including *A. niger* and *A. flavus* predominated on wheat and maize grains under storage (Wallace and Sinha 1975). *Aspergillus* was also the consistently frequent genus in barley, wheat, maize and sorghum grains from Egypt, in which *A. niger* occurred in all the four types of cereal grains, followed by *A. flavus* (El-Kady *et al.* 1982). *Aspergillus* was similarly reported to predominantly contaminate fresh harvests of corn (Jimenez *et al.*1985), stored maize in the sub-Saharan Africa, in which *A. flavus* occurred (Wareing 1997), in milled or paddy rice (Tonon *et al.* 1997, Taligoola *et al.* 2004, 2010). Freshly harvested barley from Egypt (Abdel-Kader *et al.* 1979), and sorghum from Alabama (Dierner *et al.* 1981), paddy and milled rice in Argentina, Paraguay and Nigeria (Tonon *et al.* 1997, Makun *et al.* 2007) were also found to have frequent contamination by *Aspergillus*, whereby, *A. niger* was among the most common species

Aspergillus and *Fusarium* were also the most frequent genera on milled rice during storage, but *Fusarium* was represented by only *F. culmorum*. The genera which were represented by only 2 species on paddy rice included *Cladosporium* which had *C. cladosporioides* and *C. sphaerospermum; Cochliobolus* had *C. lunatus* and *C. miyabeanus*, while *Scopulariopsis* had *S. brevicaulis* and *S. brumptii.* The genera which were also represented by 2 species on milled super rice were *Chaetomium* and *Cladosporium.* The remaining genera on the two types of rice were each represented by only 1 species (Table 1).

Incidence of xerophilic and/or xerotolerant fungi on rice grains during storage

Paddy in storage had 6 species belonging to 3 genera of xerophilic fungi as determined on dichloran 18 % glycerol agar (DG18), while milled rice had 8 species that belong to 5 genera. However, several xerotolerant fungi were also isolated from the types of rice on this medium (Table 2). The two types of rice were sparsely contaminated by xerophilic fungi, whereby 2.5% and 4.5% were the highest incidence levels recorded on paddy and milled rice, respectively throughout the 270-day storage period (Table 2). These low incidence levels of xerophiles as opposed to the predominance of xerotolerants particularly, on the paddy confirms that these grains were indeed fresh harvests, yet to be invaded by storage fungi. The paddy rice grains were thus generally free of xerophilic fungi especially, during the first 135 days of storage. In contrast, field fungi including: *Cladosporium sphaerospermum*, *Cochliobolus miyabeanus*, *Khuskia oryzae*, *Scytalidium lignicola*, *Wallrothiella subiculosa* and *Phialophora verrucosa* tolerated this medium (Table 1b). Storage fungi have similarly been found to be virtually absent from fresh harvests of other cereals including barley, wheat (Flannigan 1970, 1974, Warnock 1972, Clarke and Hill 1981, Lacey 1988) and rice grains (Makun *et al.* 2007)

Among the xerophilic fungi, *A. candidus* and *E. rubrum* were comparatively frequent in the second half of the storage period on the paddy rice, while *E. chevalieri, E. amstelodami* and *E. rubrum* were prevalent on the milled rice. These observations are consistent with other findings whereby *Eurotium* spp. have been found to precede *A. candidus* in the ecological succession of fungi on stored fresh harvests of cereal grains including paddy rice, milled rice and corn (Fanse and Christensen 1969, Lopez and Christensen 1966). The incidence levels of *A. candidus* and *A. fumigatus* together with other storage fungi including *A. sydowii*, *A. tamarii*, *A. versicolor* and *P. chrysogenum* were also found to

increase with storage time on paddy rice from Egypt (Abdel- Hafez *et al*. 1992, Mazen *et al*. 1993).

The species diversity (as revealed on DG18) was such that *Aspergillus* recorded the highest number of species on both types of rice; 9 species were isolated from paddy, while 6 species occurred on milled rice. The species that occurred on both types of rice included *A. candidus, A. penicillioides, A. flavus, A. fumigatus, A. melleus, A.niger, A. tamarii, A. terreus, A.ustus, A. versicolor* and *A. wentii* (Table 2). Similarly, *Fusarium* was also prevalent on paddy rice whereby 9 species were isolated but only 2 *Fusarium* species occurred on milled rice. *Eurotium* was represented by 3 species: *E. amstelodami, E. chevalieri* and *E. rubrum*, on both types of rice grains. *Penicillium* was represented by *P. islandicum* and *P. pinophilum* on paddy rice but milled rice had only *P. pinophilum*. Milled rice also had 2 species of *Scopulariopsis; S. brumptii* and *S. candida.* The remaining genera were each represented by only 1 species (Table 2). It is worthed to mention that, in addition to the six *Aspergillus* species occurred on milled rice and isolated on DG18, 4 more species of *Aspergillus* (*A. candidus, A. flavus, A. niger* and *A. oryzae)* were recorded on DRBC and AFPA media (Tables 1 & 3).

Incidence of aflatoxigenic *Aspergillus* species on rice grains during storage

The two types of rice in storage: paddy and milled were sparsely contaminated with aflatoxigenic *Aspergillus* spp. as determined on AFPA medium (Table 3). The incidence of *A. flavus*, the only aflatoxigenic species isolated, was sporadic. Paddy grains, however, had an incidence of 45 % only on day 135 of storage. This relatively high incidence compared to other low incidence levels obtained (2.5%) is consistent with other findings whereby fungal spoilage has been demonstrated to begin in small pockets within large consignments or bulks of cereal grains (Christensen and Kaufmann 1975). These localized spots thus, must have had a moisture content of 18.0%, which is the lower limit for *A. flavus* to invade the cereal grains (Christensen 1987, Sauer 1988). The highest moisture content recorded for the various samples of the two rice types during the entire period of storage was 12.9 % for paddy and 13.98 % for the milled rice (Table 3).

The relatively high incidence levels of *A. flavus* on only the paddy grains (45 %) as compared to the milled super rice which had 2% as the highest incidence, implies that contamination was probably only superficially on the husks. Milling of the rice may thus, have resulted in the loss of most of the *A. flavus* propagules. Confinement of *A. flavus* on only the husks of grains has similarly been established by Fanse and Christensen (1969), who found that, though 20% of rough rice (paddy) kernels had *A. flavus*, their germination was 80 %. Thus, *A. flavus* was present only on the hulls. Milled rice from Argentina and Paraguay has similarly been reported to have low levels of *A. flavus*, than the paddy grains (Tonon *et al*. 1997). Predominance of *A. flavus* as the main aflatoxigenic *Aspergillus* spp. has similarly been found in various cereal grains including rice from Thailand (Lapmak *et al.* 2009), India (Reddy *et al.* 2009) and Uganda (Taligoola *et al.* 2010), corn from Burundi (Munimbazi and Bullerman 1996), sorghum and barley from Ethiopia (Abate and Gashe 1985), and maize that has undergone transport in tropical countries including sub-Saharan African (Milton and Pawsey 1988, Wareing 1997, Ismail *et al.* 2003).

Incidence of *Penicillium* species on rice grains during storage

The two types of rice in storage were sparsely contaminated with species of *Penicillium* as determined on pentachloronitrobenzene rose-bengal yeast extract sucrose agar (PRYES), whereby 1% was the highest incidence level recorded (Table 4). Similarly, on this medium only 3 species of *Penicillium* were recorded on the paddy rice (and one more species: *P. pinophilum* and some others unidentified on DRBC), while milled super rice had 4 species. Of these, only *P. oxalicum* occurred on both rice. However, such nephrotoxigenic *Penicillium* species as *P. aurantiogriseum* and *P. viridicatum* were not among the isolated species. An increase in the incidence of contamination by *Penicillium* was not recorded at all during storage. *Talaromyces intermedius* and other unidentified species of *Talaromyces* were found contaminating the paddy rice (Table 4).

The comparatively low incidence levels of *Penicillium* species on the locally grown rice confirms that they were freshly harvested whereby field fungi, and not storage fungi including *Penicillium*, predominate (Christensen and Kaufmann 1974, Bullerman 1979). *Penicillium* being a storage fungus, normally occur only sporadically on fresh harvest of cereal grains. However, they replace field fungi upon storage of the fresh harvests, or succeed xerophilic fungi such as *Eurotium* spp. upon prolonged storage of the cereal grains including: paddy (Abdel-Hafez *et al.* 1992, Mazen *et al*. 1993), milled rice (Sidik and Pedersen 1986, Taligoola *et al.* 2004), sorghum (Elegbede *et al.* 1982), barley and wheat (Flannigan 1970, Clarke and Hill 1981, Lacey 1988) and maize (Sauer 1988).

The general scarcity of *Penicillium* species on all the rice grains may also be attributed to their comparatively low moisture contents during storage whereby the highest moisture content recorded was 13.98 ± 0.12 % (Table 1). Most *Penicillium* species, however, require 16.5-19.0% as the lower limit of

moisture for optimal growth in cereal grains (Christensen, 1987). Thus, the moisture contents of the rice grains were not favourable for growth of *Penicillium* spp.

Among the *Penicillium* species that occurred on the two types of rice in storage, *P. oxalicum* was common. However, *P. pinophilum* was the most frequent species on milled rice. *Penicillium chrysogenum*, *P. citrinum*, *P. islandicum* and *P. restrictum* were comparatively less common (Table 4). In this respect, Tonon *et al.* (1997) reported *P. citrinum, P. islandicum* and *P. aurantiogriseum* as dominant on paddy rice, while milled rice showed the major species as *P. citrinum* and *P. islandicum*. The presence of these *Penicillium* species on rice grains suggests a potential for mycotoxin contamination, particularly by *P. citrinum* which produces citrinin, a nephrotoxin known to cause kidney malfunctioning upon prolonged ingestion by humans (Bullerman 1979, Frisvad 1983). *Penicillium islandicum* also produces nephrotoxins, hepatoxins and carcinogens known to cause kidney and liver malfunctioning (Pitt and Hocking 2009).

Incidence of *Fusarium* species on rice during storage

The incidence of *Fusarium* species on the locally grown rice during storage was such that paddy rice was found contaminated with 8 species of *Fusarium* and others unidentified as determined on pentachloronitrobenzene potato sucrose agar (PCNB-PSA). *F. stilboides*, *F. lateritium* and *F. verticillioides* were the most frequent species, occurring on 7, 5 and 5 out of 8 occasions of plating respectively (Table 5). However, milled rice was comparatively almost free from contamination by *Fusarium* species. Only 3 species were isolated. Similarly, species of *Fusarium* were recorded at a higher incidence level on the paddy than on milled rice whereby their highest incidence levels were 11 % and 2 % respectively (Table 5). During storage, the incidence levels of some *Fusarium* species on paddy rice showed a decline, but only marginally. *F. lateritium* and *F. stilboides* had lower incidence levels during the second half of the storage period. The incidence levels of the various species of *Fusarium* were however, generally less than 7 % except *F. stilboides*, which contaminated 11% of the grains at day 135 (Table 5).

The paddy rice, having been freshly harvested, recorded a wider species spectrum of *Fusarium* among them *F. verticillioides*, which has recently been established to produce fumonisins alongside, moniliformin, and fusaric acid. Fumonisins have now been linked with the incidence of oesophageal cancer in humans in southern Africa (Bacon and Nelson 1994, Dmello and Macdonald 1997) and in sub-Saharan Africa (Williams *et al.* 2010).

Field fungi including *Fusarium* species have been found to grow at high grain moisture content of 20-25% (Bullerman 1979). However, since 13.98 % was the highest moisture content recorded in the two types of rice in storage (Table 1), growth of *Fusarium* species was thus not enhanced particularly on the paddy rice, which was freshly harvested. The increase of moisture content with the lengthening of storage period was found to cause increase in incidence of *Fusarium* spp. on paddy grains from Egypt whereby moisture contents of 11.5%, 17%, 22.5% and 28% were recorded (Abdel-Hafez *et al.* 1992, Mazen *et al.* 1993) and sorghum from Nigeria (Elegbede *et al.* 1982).

F. oxysporum was the only species which occurred in both types of rice under storage. Trichothecenes, moniliformin and zearalenone have been found to be produced by *F. oxysporum* (Leslie and Summerell 2006). *F. lateritium* which was among the predominant species on the paddy (Table 5), is also known to produce trichothecenes whose presence in foods is of human health concern (Bullerman 1979, Abbas *et al.* 1989). It is worthed to mention that in addition to the 8 species of *Fusarium* identified on PCNB-PSA from paddy, other 3 different species were recorded on DRBC (*F. graminearum, F. proliferatum),* and on DG18 (*F. decemcellulare).* On the other hand, only one more (*F. culmorum)* was recorded on DRBC from milled rice in addition to the 3 species on PCNB-PSA.

Incidence of aflatoxins in rice during storage

The incidence of aflatoxins on the rice was such that 6 out of 10 samples of the paddy rice and 4 out 10 samples of milled rice screened for presence of aflatoxins were found contaminated. However, the levels of aflatoxins in the two types of rice did not show any consistent increase during storage (Table 6).Three levels of contamination were recorded: 0 ppb, 0-10 ppb, and 10-20 ppb. All the positive samples for aflatoxins had levels that were below or at the maximum level allowed in foods of 20 ppb by the Food and Drug Administration (FDA) of the United States (Davis and Diener 1987). Uganda abides by this limit (Uganda National Bureau of Standards1999).

The data on the relationship between the incidence of aflatoxins and aflatoxigenic *Aspergillus* spp. (*A. flavus*) on the various samples of rice grains and their respective moisture contents show that while an increase in moisture content was recorded, a concomitant increase in the incidence of *A. flavus* and aflatoxins was not recorded (Table 6).

Table 1: Percentage incidence of fungi (out of 100 rice grains, 50 grains per subsample) on paddy and milled rice grains during a 270-day storage period on dichloran rose Bengal chloramphenicol agar (DRBC)*.

Fungal taxa	Paddy								Milled rice							
	Storage period (days)								Storage period (days)							
	0	45	90	135	180	210	240	270	0	45	90	135	180	210	240	270
Acremonium muromum (Corda) W. Gams	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Aspergillus candidus Link	-	-	-	-	-	1	-	-	-	-	5	-	-	-	2	-
A. flavus Link	-	-	-	3	-	-	-	-	-	-	-	1	-	1	-	-
A. melleus Yukawa	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-
A. niger van Tieghem	8	-	2	-	-	-	-	-	2	2	-	3	-	-	1	-
A. oryzae (Ahlburg) Cohn	-	-	-	-	-	-	-	-	4	-	-	-	1	-	-	-
Aureobasidium pullulans (de Bary) Arnaud	9	8	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Beauveria sp.	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Byssochlamys fulva Ollivr & Smith	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Chaetomium brasiliense Batista & Pontual	-	-	-	-	-	-	-	-	-	1	-	1	-	1	-	3
C. globosum Kunze	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1
Chrysosporium spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-
Cladosporium cladosporioides (Fresenius) de Vries	-	-	1	-	-	-	-	-	-	3	-	-	-	-	-	-
C. sphaerospermum Penzig	-	-	-	2	1	-	-	-	1	5	1	1	2	2	1	3
Cochliobolus lunatus Nelson & Haasis	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. miyabeanus (Ito & Kuribayashi) Drechsler ex Dastur	43	48	43	52	36	18	6	-	1	-	-	3	-	1	-	2
Doratomyces sp.	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Epicoccum nigrum Link	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Eurotium chevalieri Mangin	-	-	-	-	1	-	-	-	5	-	-	1	1	3	2	-
Fusarium culmorum (Smith) Saccardo	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
F. dimerum Penzig	2	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
F. equiseti (Corda) Saccardo	-	-	-	3	-	2	5	-	-	-	-	-	-	-	-	-
F. graminearum Schwabe	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
F. lateritium Nees	3	1	-	-	2	-	1	4	-	-	-	-	-	-	-	-
F. proliferatum (Matsush.) Nirenberg	-	-	-	2	-	-	-	4	-	-	-	-	-	-	-	-
F. stilboides Wollenweber	4	-	3	3	1	4	2	8	-	-	-	-	-	-	-	-
F. verticillioides (Sacc.) Nirenberg	-	3	1	2	2	-	1	-	-	-	-	-	-	-	-	-
Unidentified *Fusarium* spp.	-	2	-	1	2	-	-	-	-	-	-	-	-	-	-	-
Geotrichum candidum Link	-	1	-	7	2	-	-	-	-	-	-	1	-	-	-	-
Humicola grisea Traaen	2	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-

Khuskia oryzae Hudson	54	16	33	9	22	2	4	-	1	1	-	-	-	-	-	-
Microascus spp.	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Microdochium nivale (Fries) Samuels & Hallett	-	-	-	2	-	-	-	-	-	-	-	1	-	-	-	-
Mucor spp.	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Paecilomyces variotii (Thom) Samson	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Paecilomyces spp.	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Penicillium pinophilum Hedgcock	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unidentified *Penicillium* spp.	-	-	-	-	-	-	-	1	2	2	-	1	-	-	-	1
Pestalotiopsis guepinii (Desm.) Stey.	37	12	13	-	1	-	-	1	1	-	-	-	1	-	-	-
Phialophora verrucosa Medlar	-	-	7	3	-	-	3	3	-	-	-	-	-	-	-	-
Phoma spp.	-	-	-	-	1	-	2	-	-	-	-	-	-	-	-	-
Pichia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Rhizopus oryzae Went & Prinsen-Geerligs	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Scolecobasidium terreum E.V. Abbott	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Scopulariopsis brevicaulis (Saccardo) Bainier	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
S. brumptii Salvanet- Duval	-	-	-	-	-	2	-	-	-	1	-	-	-	-	-	1
Scytalidium lignicola Pesante	8	11	48	58	68	46	63	50	-	-	-	-	-	-	-	-
Talaromyces spp.	-	-	1	-	1	1	-	-	-	-	-	-	-	-	1	-
Thermoascus aurantiacus Miehe	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Wallemia sebi (Fries) von Arx	-	-	-	-	-	-	-	-=	-	-	-	-	1	-	-	1
Wallrothiella subiculosa Höhnel	1	-	-	6	-	1	-	3	-	-	-	-	-	-	-	-
Rhodotorula mucilaginosa (A. Jorgensen) F. C. Harrison	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	2
Other yeasts	-	-	-	-	-	-	-	-	-	4	2	-	15	2	5	3
Rice moisture content	12.5 ± 0.1	12.7 ± 0.2	12.6 ± 0.05	12.7 ± 0.1	12.63 ± 0.25	12.58 ± 0.06	12.67 ± 0.06	12.9 ± 0.17	12.38 ± 0.03	13.4	13.02 ± 0.1	13.71 ± 0.1	13.65	13.98 ± 0.13	13.98 ± 0.13	13.78 ± 0.03

* Numbers in the columns are the mean percentage of grains infected by the respective fungal species at a time, calculated from the two samples tested of each kind of rice grains.

Table 2: Percentage incidence of xerophilic and/or xerotolerant fungi (out of 200 grains, 100 grains per sub-sample) on paddy and milled rice during a 270-day storage period on dichloran 18% glycerol agar (DG18)*.

Fungal taxa	Paddy								Milled rice							
	Storage period (days)								Storage period (days)							
	0	45	90	135	180	210	240	270	0	45	90	135	180	210	240	270
Xerophilic fungi																
Aspergillus candidus	-	-	-	-	1	0.5	-	1	-	-	-	-	-	-	-	-
A. penicillioides	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-
A. wentii	2.5	-	-	-	-	-	-	-	4.5	-	-	0.5	-	-	-	-
Chrysosporium farinicola	-	-	-	-	-	-	-	-	0.5	-	-	-	-	3	-	-
Chrysosporium sp.	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
Eurotium amstelodami	-	-	-	-	-	-	-	1	-	-	-	-	1.5	2	1	4.5
E. chevalieri	1	-	-	-	0.5	-	-	-	-	1	1	-	-	1	0.5	1
E. rubrum	-	-	-	-	0.5	0.5	0.5	-	-	-	-	1	1.5	1	1	-
Monascus ruber	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.5	2
Wallemia sebi	-	-	-	-	-	-	-	-	-	0.5	-	-	-	0.5	-	-
Xerotolerant fungi																
Aspergillus flavus	1.5	-	-	12	-	-	0.5	-	-	-	-	-	-	-	-	-
A. fumigatus	-	-	-	-	-	-	-	-	0.5	1	-	-	-	-	-	-
A. melleus	-	-	-	-	-	0.5	-	0.5	-	1	-	0.5	-	-	-	-
A. niger	7	0.5	1	-	-	-	-	-	-	-	-	-	-	-	-	-
A. tamarii	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-
A. terreus	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
A. ustus	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	0.5
A. versicolor	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-
Aureobasidium pullulans	-	-	1	-	-	1	-	0.5	-	1.5	-	-	-	-	-	-
Chaetomium brasiliense	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-	1
C.globosum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Chaetomium spp.	-	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cladosporium sphaerospermum	0.5	2.5	0.5	-	-	-	1.5	0.5	2.5	3	2	2.5	0.5	1	0.5	0.5
Cochliobolus miyabeanus	48	35	37.5	39	38.5	2.5	-	-	-	0.5	1	0.5	4	0.5	-	-
Curvularia ovoidea	-	-	-	-	-	-	-	-	1.5	-	-	-	-	-	-	-
Doratomyces sp.	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-
Epicoccum nigrum	-	-	-	-	-	-	-	-	0.5	-	1.5	2	2.5	0.5	-	-
Fusarium dimerum	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
F. decemcellulare	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F. equiseti	-	2	-	3.5	-	-	1	-	-	-	-	-	-	-	-	-

F. graminearum	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
F. lateritium	2.5	-	-	0.5	1.5	-	1.5	0.5	-	-	-	-	-	-	-	-
F. oxysporum	-	-	-	3.5	-	0.5	-	-	-	-	-	-	-	-	-	-
F. proliferatum	-	-	-	-	-	-	0.5	0.5	-	-	-	-	-	-	-	-
F. solani	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
F. stilboides	-	4	5.5	4	5.5	-	0.5	1.5	-	-	-	-	-	-	-	-
F. verticillioidese	-	1	1.5	-	-	1.5	1	-	-	-	-	1	-	-	-	-
Geotrichum candidum	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-
Humicola grisea	-	-	0.5	2	-	1	1.5	0.5	-	-	-	-	-	-	1	-
Humicola spp.	3.5	2	-	-	2.5	-	-	-	-	-	-	-	-	-	-	-
Khuskia oryzae	65	26	22.5	3	3.5	1	1	-	-	-	-	1.5	-	-	-	-
Lasiodiplodia theobromae	-	-	0.5	-	-	-	-	-	-	-	-	0.5	-	-	-	-
Microascus spp.	0.5	-	-	-	-	-	-	3.5	-	-	-	-	-	-	-	-
Microdochium nivale	-	-	0.5	1	-	0.5	-	3.5	-	-	-	2	-	-	-	-
Mucor sp.	-	-	-	-	-	-	-	-	-	0.5	-	-	-	-	-	-
Paecilomyces lilacinus	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-	-	-
P. variotii	-	-	-	-	-	2	1.5	-	-	-	-	-	-	-	-	-
Paecilomyces sp.	-	-	-	-	-	-	-	-	-	0.5	-	-	-	-	-	-
Penicillium islandicum	-	-	-	-	-	-	-	-	-	0.5	-	-	-	-	-	-
P. pinophilum	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-
Penicillium spp.	1	-	2	-	-	0.5	-	0.5	-	1	-	0.5	-	-	-	1
Pestalotiopsis guepinii	23	3	4	-	-	-	-	1	4.5	-	2	1	-	-	-	-
Phialophora verrucosa	-	3	0.5	2.5	4.5	-	2	47.5	-	-	-	-	-	-	-	-
Phoma spp.	5	-	10	-	1	-	-	-	-	-	-	-	-	-	-	-
Rhodotorula mucilaginosa	-	1	-	0.5	-	-	0.5	0.5	1.5	2	1.5	-	-	-	-	-
Scolecobasidium terreum	-	-	-	1.5	-	-	-	-	-	2	-	-	-	-	-	-
Scopulariopsis brumptii	-	-	-	-	-	-	-	-	-	0.5	-	-	-	-	-	-
S. candida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Scytalidium lignicola	4	7	16	44.5	42	58	64	-	0.5	1	0.5	7	1	-	-	-
Syncephalastrum racemosum	-	-	-	-	-	-	-	-	1	-	-	-	-	-	5	-
Talaromyces spp.	-	0.5	1	-	1.5	1.5	2	3	-	0.5	-	1.5	-	1.5	1	-
Thermoascus aurantiacus	-	-	-	0.5	-	0.5	-	-	-	-	-	-	-	-	-	-
Trichordema harzianum	-	0.5	0.5	-	-	-	-	-	-	1.5	-	-	-	0.5	-	-
Wallrothiella subiculosa	2	2	0.5	3	2	1	-	1.5	-	-	-	-	-	-	-	-

*footnotes as below Table (1).

Table 3: Percentage incidence of aflatoxigenic and other *Aspergillus* species (out of 200 grains, 100 grains per sub-sample) on paddy and milled rice during a 270-day storage period on *Aspergillus flavus/parasiticus* agar (AFPA)*.

Fungal taxa	Paddy rice								Milled rice							
	Storage period (days)								Storage period (days)							
	0	45	90	135	180	210	240	270	0	45	90	135	180	210	240	270
Aflatoxigenic *Aspergillus* spp.																
Aspergillus flavus	2.5	-	-	45	2	-	-	-	-	1	-	2	-	-	-	-
Other *Aspergillus* spp.																
A. candidus	-	-	-	-	2	-	0.5	-	0.5	0.5	1.5	1.5	-	1	-	-
A. niger	8	-	0.5	-	0.5	1	-	-	1.5	2	-	0.5	-	1	-	-
A. oryzae	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
A. quercinus	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-

* Footnotes as below Table (1).

Table 4: Percentage incidence of *Penicillium* species (out of 200 grains, 100 grains per sub-sample) on paddy and milled rice during a 270-day storage period on pentachloronitrobenzene yeast extract sucrose agar (PRYES)*.

Fungal taxa	Paddy rice								Milled rice							
	Storage period (days)								Storage period (days)							
	0	45	90	135	180	210	240	270	0	45	90	135	180	210	240	270
Penicillium chrysogenum	-	-	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-
P. citrinum	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-
P.islandicum	-	-	-	-	-	-	-	-	1.5	0.5	-	-	-	-	-	-
P. oxalicum	-	-	-	-	0.5	1	0.5	-	1	-	-	-	0.5	-	-	-
P. pinophilum	-	-	-	-	-	-	-	-	1	0.5	-	-	-	0.5	-	-
P. restrictum	-	-	-	-	-	-	-	-	1	-	-	0.5	-	-	-	-
Penicillium spp.	-	-	-	-	-	-	-	-	0.5	-	-	-	-	-	-	-
Talaromyces intermedius	-	-	-	-	-	1	1	0.5	-	-	-	-	-	-	-	-
Talaromyces spp.	-	-	4.5	0.5	3.5	45	1	-	-	-	-	1.5	-	0.5	2	-

*Footnotes as below Table (1).

Table 5: Percentage incidence of *Fusarium* species (out of 100 rice grains, 50 grains per subsample) on paddy and milled rice grains during a 270-day storage period on pentachloronitrobenzene potato sucrose agar (PCNB-PSA)*.

Fungal taxa	Paddy rice								Milled rice							
	Storage period (days)								Storage period (days)							
	0	45	90	135	180	210	240	270	0	45	90	135	180	210	240	270
Fusarium acuminatum	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
F dimerum	-	-	5	-	-	1	-	-	-	-	-	-	-	-	-	-
F. equiseti	1	-	-	2	-	3	-	-	-	-	-	-	-	-	-	-
F. lateritium	5	6	-	2	1	-	3	-	-	-	-	-	-	-	-	-
F. oxysporum	-	3	-	-	-	3	-	2	-	-	-	-	-	-	2	-
F. poae	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-
F. solani	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-
F. stilboides	4	-	6	11	4	1	2	3.5	-	-	-	-	-	-	-	-
F. verticillioides	2	-	1	-	3	-	4	1	-	2	-	-	-	-	-	-
Fusarium spp.	-	3	-	-	2	-	-	-	-	-	-	-	-	-	-	-

* Footnotes as below Table (1a).

Table 6: Percentages of infected grains with aflatoxigenic *Aspergillus* species (on AFPA), aflatoxins and moisture content of paddy and milled rice during a 270-day storage period

Type of rice	Analysis	Storage period (days)				
		135	180	210	240	270
Paddy	Aflatoxigenic *Aspergillus* spp.	20	2	-	-	-
	Aflatoxin level (ppb)	10-20 ppb	0-10 ppb	0 ppb	0-10 ppb	0-10 ppb
Sample 1	Moisture content (%)	12.8 ± 0.2	12.7 ± 0.1	12.7 ± 0.1	12.7 ± 0.1	13.2 ± 0.2
	Aflatoxigenic *Aspergillus* spp.	70	2	-	-	-
	Aflatoxin level (ppb)	0- 10 ppb	0 ppb	0 ppb	0 ppb	0- 10 ppb
Sample 2	Moisture content (%)	12.6	12.6 ± 0.4	12.5	12.7 ± 0.1	12.8 ± 0.4
Milled rice	Aflatoxigenic *Aspergillus* spp.	-	-	-	-	-
	Aflatoxin level (ppb)	0 ppb	10-20 ppb	0 ppb	0 ppb	10 – 20 ppb
Sample 1	Moisture content (%)	13.7 ± 0.1	13.95 ±0.05	14.2 ± 0.2	13.95 ± 0.05	13.8
	Aflatoxigenic *Aspergillus* spp.	4	-	-	-	-
	Aflatoxin level (ppb)	0 ppb	10- 20 ppb	0 ppb	0 ppb	10 -20 ppb
Sample 2	Moisture content (%)	13.7 ± 0.1	13.5	13.95 ± 0.05	14.05 ± 0.15	13.8

ppb= Parts per billion (micrograms per kilogram)

The highest moisture content recorded for the various samples of the two types of rice during the entire period of storage was 13.2 % for paddy rice and 14.2 % for the milled super rice. Thus, these rice samples had moisture contents which are ideal for their safe storage whereby for paddy rice it is 13.0 %, while for milled rice it is 14.0 % (Christensen and Kaufmann1969, Bencini and Walston1991). Milled rice had only 2 out of 10 (20 %) of its samples with moisture content above 14.0 %. Similarly, paddy rice had most of its samples: 9 out of 10 with moisture content below 13 %, which is the recommended level for its safe storage (Table 6). However, the moisture contents of all the samples were considerably below 18 %, which is the lower limit for *A. flavus*, the main aflatoxigenic species, to grow in cereal grains (Christensen 1987, Sauer 1988, Reddy *et al*. 2009).

Conclusion

The current study revealed that the rice (paddy and milled rice) were contaminated by various types of fungi and by aflatoxins during the 270-day storage period. The two types of rice were predominantly contaminated by field fungi including *Cochliobolus miyabeanus*, *Cladosporium sphaerospermum*, *Fusarium* spp., *Pestalotiopsis guepinii*, *Khuskia oryzae and Scytalidium lignicola*. However, the milled rice had comparatively lower levels of contamination than the paddy. Milling (de-husking) may have drastically reduced fungal propagules in the milled rice. During the 270-day storage period, field fungi gradually disappeared on both types of rice such that xerophilic fungi began emerging especially towards the second half period of storage, though only sporadically. It is thus recommended that both types of rice be stored for not more than 1 year after harvest. Thereafter, xerophilic fungi including *Eurotium* spp. and *Aspergillus candidus* both of which produce mycotoxins including sterigmatocystin and citrinin, respectively, are thus of human concern. Paddy had majority of its samples (60 %) contaminated with aflatoxins, while the milled rice had only 40 % of its samples contaminated, with 10-20 ppb being the highest incidence of aflatoxins on both types of rice. Thus, the contamination with aflatoxins, which did not record any significant increase during storage, was not above 20 ppb, the maximum contamination level recommended in foods internationally. The moisture contents of both types of rice, however, recorded significant increase with paddy attaining a maximum of 13.2 %, which is slightly above 13.0 %, the recommended level for safe storage of paddy rice. Milled rice attained a maximum of 14.2 %, which is also slightly above 14 %, the recommended level for safe storage of milled rice.

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