Plant Pathol. J. 24(1) : 74-79 (2008)

The Plant Pathology Journal

Temporal Changes of Fungal and Bacterial Populations in Rice under Indoor Storage Conditions

Ji Yeon Oh[1], Mee Kyung Sang[1], Mun Il Ryoo[2] and Ki Deok Kim[1]*

[1]Division of Biotechnology and [2]Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-713, Korea

(Received on December 17, 2007; Accepted on February 20, 2008)

This research was conducted to evaluate fungal and bacterial populations in unhulled and brown rice under indoor storage conditions, and to examine the relationship between microbial populations and environmental conditions such as temperature and relative humidity. The temperature and relative humidity of the storage room ranged from 22.6°C to 27.0°C and 23.3% to 44.2%, respectively. Total fungal and bacterial populations remained relatively stable over the storage period. Predominant fungi included *Aspergillus candidus*, *A. flavus*, *A. fumigatus*, and *Penicillium* spp.; the predominant bacteria were *Bacillus*, *Microbacterium*, *Sphingomonas*, and *Methylobacterium* spp. Total fungi and bacteria were not significantly correlated with either unhulled (r=0.448, P=0.372) or brown (r=0.466, P=0.351) rice. In unhulled rice, total fungi showed positive correlations with total *Aspergillus* (r=0.994, P<0.001) and total *Penicillium* (r=0.906, P<0.05); *A. flavus* was positively correlated with total *Aspergillus* (r=0.913, P<0.05) and total fungi (r=0.868, P<0.05). In brown rice, *Bacillus* spp. was also positively correlated with total bacteria (r=0.998, P<0.001). Mean temperature was negatively correlated with *A. candidus* (r=−0.852, P< 0.05) and total fungi (r=−0.961, P<0.01), and mean relative humidity was positively correlated with total *Penicillium* spp. (r=0.884, P<0.05) in brown rice. Hence these results could provide basic information on the fungal and bacterial populations in unhulled and brown rice stored under room conditions, and on the effect of environmental conditions on the populations of fungi and bacteria, especially *Aspergillus* and *Penicillium* spp.

Keywords : *Aspergillus*, *Penicillium*, relative humidity, rice, storage bacteria, storage fungi, temperature

Rice is a widely consumed food crop that contains high quality nutrients and is also a good substrate for fungal growth before and after harvest (Wicklow et al., 1992). Some fungal infections occur in the field; however, after harvest, populations of field fungi decrease while storage fungi increase due to the loss of moisture content (Williams et al., 1983). These storage fungi cause numerous adverse effects in that their mycelia and spores weaken the rice by invading the germ or embryo (Williams et al., 1983) and reduce the germination rate of rice (Misra et al., 1995). The invaded rice also loses its high nutritional value; the total water soluble sugar and total free amino acids are lower in infected rice than in healthy rice (Misra et al., 1995; Prange et al., 2005). Water content and temperature in invaded bulk rice are also increased, which lead to infestation by other pests, such as insects and mites. Furthermore, storage fungi produce undesirable odors and discolor the rice, which reduce the quality of rice and consequently lead to economic losses (Bhattacharya et al., 2002). Some storage fungi such as *Aspergillus* and *Penicillium* spp. produce mycotoxins that are toxic to humans and animals (Sweeney et al., 1998; Miller et al., 1995). These mycotoxins are secondary metabolites that have many types of toxicities such as carcinogenic, nephrotoxic, teratogenic, hepatotoxic and hepatocarcinogenic toxicities (Sweeney et al., 1998).

The growth and survival of storage fungi and bacteria are affected by number of factors, such as moisture content of the grain, degree of grain damage, temperature, oxygen, aeration competition, storage period, foreign material, types and cultivars of grain, harvest conditions, insects, mites, and interference competition (Miller et al., 1995). Among these, temperature and relative humidity have been reported as primary factors to affect the growth of storage fungi (Filipello Marchisio et al., 2001; Magan et al., 2003). In our previous study (Oh et al., 2007), we evaluated the populations of fungi and bacteria in unhulled and white rice sampled from rice processing complexes (RPC) of the National Agricultural Cooperative Federation in 11 regions in Korea during 2005 and 2006. Although this survey showed the population tendency of fungi and bacteria in unhulled and white rice, it could not provide enough information regarding to their population dynamics during storage. Therefore, we have evaluated the fungal and bacterial populations in unhulled and brown rice under indoor

*Corresponding author.
Phone) +82-2-3290-3065, FAX) +82-2-925-1970
E-mail) kidkim@korea.ac.kr

storage conditions, and examined the correlations among fungal and bacterial populations and environmental factors including temperature and relative humidity.

Unhulled rice and brown rice each in a ton bag were stored under indoor storage conditions on July 11, 2005 in a room at the College of Life Sciences and Biotechnology, Korea University, Seoul, Korea. A total of 100 adult Indian meal moths (*Plodia interpunctella*) and 100 adult maize weevils (*Sitophilus zeamais*) were added to the bags of the unhulled and brown rice to simulate natural environments. During rice storage, temperature and relative humidity of the room were monitored from December 12, 2005 (155 days after storage) using the HOBO H8 Temp/RH Data Logger (Onset Computer Corporation, Bourne, USA) and the software BoxCar for Windows version 3.7.3. (Onset Computer Corporation, Bourne, USA).

To sample rice from the stored bags, 2 g each of unhulled and brown rice were taken by stabbing into each ton bag 18 times with a sampler (National Agricultural Products Quality Management Service, Anyang, Korea). The 18 stabbings consisted of nine stabs (three per sub-sample) from the middle and the bottom of the ton bag, respectively. These sub-samples were separately placed into plastic bags [six bags (replications) per sampling]. Four grams from each sub-sample were finely ground with the analytical mill (IKA A11 basic, IKA® Works, Inc. Wilmington, USA); one gram was used for dry weight calculation and three grams for microbial population tests. The samples were suspended in sterile distilled water and incubated for 30 min at 28°C with shaking at 120 rpm. These samples were then placed on dichloran 18% glycerol agar (DG18) (31.6 g dichloran glycerol, 220 g anhydrous glycerol, 10 mg $ZnSO_4 \cdot 7H_2O$, 5 mg $CuSO_4 \cdot 5H_2O$, 50 mg chloramphenicol in 1L H_2O) amended with 50 mg chlortetracycline for fungi and on nutrient agar (NA, Difco, Detroit, USA) amended with 50 mg NaCl and 50 mg cycloheximide for bacteria. The treated media were incubated in the dark at 28°C for 2 and 5 days for bacteria and fungi, respectively. The populations of fungi and bacteria in the ton bags were evaluated for temporal microbial changes from December 27, 2005 to May 26, 2006, which corresponded to 170 to 320 days after storage in the room, respectively. Distinct fungal colonies that appeared on the media were isolated onto malt extract agar (MEA) (20 g malt extract, 1 g peptone, 20 g glucose, 20 g agar in 1L H_2O). *Aspergillus* and *Penicillium* spp. were identified by the methods of Pitt (1985) and Klich (2002). The results were confirmed by comparing the isolates with reference isolates, *A. candidus* KACC 41846, *A. flavus* KACC 40244, and *A. fumigatus* KACC 41390, obtained from Korean Agricultural Culture Collection (KACC), Suwon, Korea. Representative bacterial strains having typical morphology were isolated and incubated on NA at 28°C for 48 hr. All bacterial strains were stored in NB with 20% glycerol at −70°C. Predominant bacterial strains were identified by Biolog and FAME analysis to the genus level as described by Oh et al. (2007). Correlation analyses were conducted to investigate the relationships between total fungi, total bacteria, each fungal and bacterial species, and environmental conditions, such as temperature and relative humidity. The mean temperature and mean relative humidity were determined from the data between one sampling date and the previous sampling date for the analysis. Data were analyzed by SAS version 9.1.3. (SAS Systems, Cary, USA).

The minimum and maximum mean temperatures of the storage room over the storage period were 22.6 and 27.0°C, while the minimum and maximum relative humidities were 23.3 and 44.2%, respectively (Fig. 1). Since we sought to simulate the uncontrolled storage of an individual house or retail market, the temperature was designed to be affected by ventilation in the room. Thus, even though the samplings had begun early in January, the temperatures were relatively high due to the heating for the winter. In contrast, the temperatures were relatively low at the time of the last sampling at the end of June due to the air-conditioning for the summer. However, relative humidity was not affected by the ventilation system, but rather by outdoor relative humidity being low in winter and high in summer.

The morphologies and populations of different fungi and bacteria from unhulled and brown rice during storage are presented in Figures 2 and 3, respectively. The total fungal population was remained relatively stable over time; however, the population of individual species varied (Fig. 2). The population and diversity of fungi were significantly (P=0.05) different between unhulled and brown rice with approximately 10^3~10^4 CFU/g and 10^1~10^2 CFU/g,

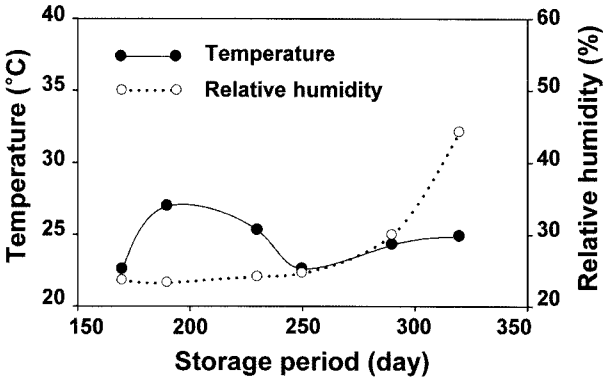

Fig. 1. Mean temperatures and mean relative humidity at the sampling date between sampling dates 170~320 days after indoor storage. The means of temperature and relative humidity are determined from the data between one sampling date and the previous sampling date.

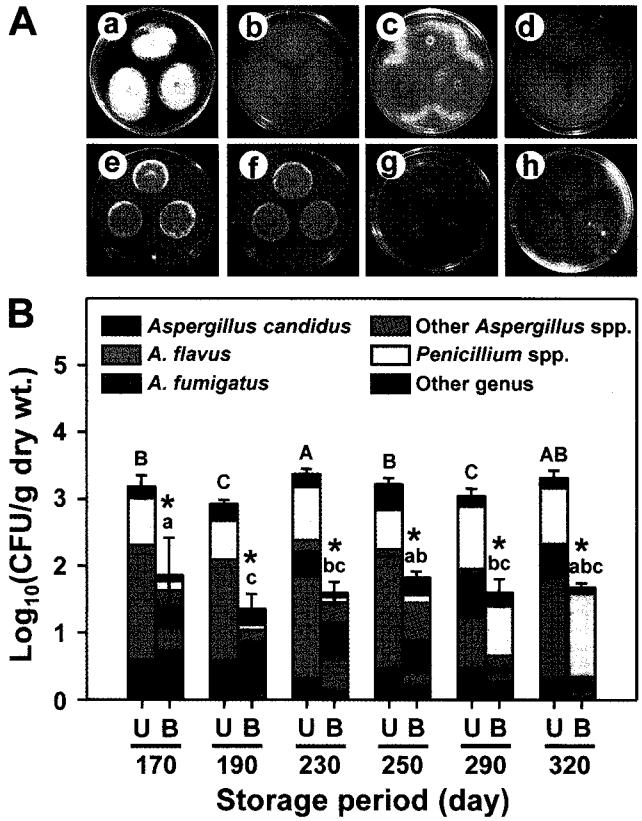

Fig. 2. A, Various morphologies of fungal species isolated from unhulled and brown rice. (a) *Aspergillus candidus*, (b) *A. flavus*, (c) *A. fumigatus*, (d) *A. terreus* belonged to "other *Aspergillus* spp.", (e, f) *Penicillium* spp., and (g, h) other genus. Fungal isolates were grown on malt extract agar at 25°C for 7 days. B, Population dynamics of different fungal species in (U) unhulled and (B) brown rice from 170~320 days after indoor storage. Each bar represents a standard deviation of the mean of six replicates. Asterisks on the bars of brown rice indicate significant (P=0.05) differences from unhulled rice on a given sampling date. The different capital and small letters on the bars indicate that fungal population among unhulled and brown rice samples, respectively, at each sampling date are significantly (P=0.05) different.

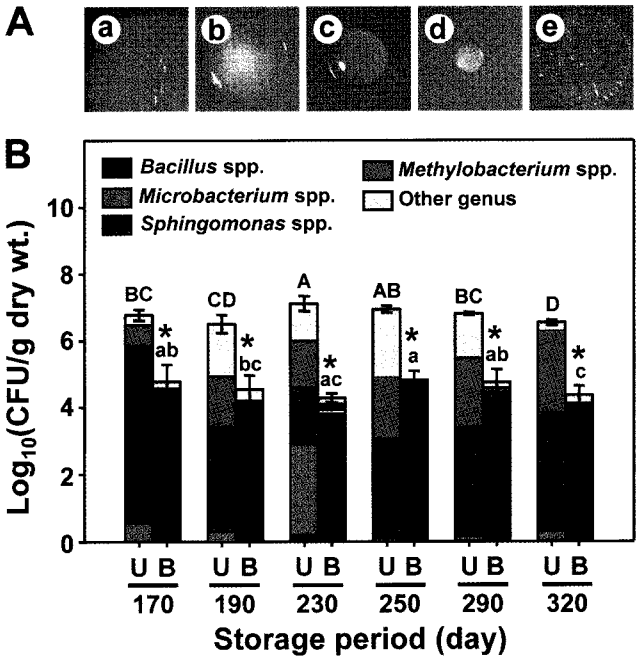

Fig. 3. A, Various morphologies of different bacterial strains isolated from unhulled and brown rice. (a) *Bacillus* spp., (b) *Microbacterium* spp., (c) *Sphingomonas* spp., (d) *Methylobacterium* spp. and (e) other genus. Bacterial isolates were grown on nutrient agar at 25°C for 7 days. B, Population dynamics of different bacterial strains in (U) unhulled and (B) brown rice from 170~320 days after indoor storage. Each bar represents a standard deviation of the mean of six replicates. Asterisks on the bars of brown rice indicate significant (P=0.05) differences from unhulled rice on a given sampling date. The different capital and small letters on the bars indicate that bacterial populations among unhulled and brown rice samples, respectively, at each sampling date are significantly (P=0.05) different.

respectively (Fig. 2). The predominant fungi included *A. candidus, A. flavus, A. fumigatus*, and *Penicillium* spp. *A. flavus* was relatively predominant in unhulled rice, while *A. candidus* was predominant in brown rice. These results were similar to our previous results obtained from rice samples from RPC in 11 regions in Korea (Oh et al., 2007). *Aspergillus* and *Penicillium* spp. were the predominant genera in most studies, particularly *A. candidus* and *A. flavus* (Park et al., 2005; Taligoola et al., 2004). At the same time, bacterial population was significantly (P=0.05) different in unhulled and brown rice with approximately 10^6~10^7 CFU/g and 10^5 CFU/g, respectively (Fig. 3). The predominant bacteria included *Bacillus cereus* (similarity index=0.304), *Microbacterium chocolatum* (similarity index =0.735), *Sphingomonas paucimobilis* (similarity index= 0.822), and *Methylobacterium rhodesianum* (similarity index=0.910) according to Biolog and FAME analyses. *Sphingomonas* spp. were relatively predominant in unhulled rice, and *Bacillus* spp. in brown rice. This result was similar to that of Cottyn et al. (2001), who described *Bacillus, Microbacterium, Sphingomonas,* and *Methylobacterium* spp. in harvested rice in the Philippine.

In the correlation analysis, the populations of total fungi and total bacteria were not significantly correlated in either unhulled (r=0.448, P=0.372) or brown (r=0.466, P= 0.351) rice. Since total *Aspergillus* (r=0.994, P<0.001) and total *Penicillium* (r=0.906, P<0.05) were the predominant genera in unhulled rice, both of them showed positive correlations with total fungi (Table 1). *A. flavus* as the most predominant species was also positively correlated with total *Aspergillus* (r=0.913, P<0.05) and total fungi (r=0.868, P<0.05) in unhulled rice. Total *Penicillium* also exhibited positive correlations with total *Aspergillus* (r=0.865, P<0.05). In brown rice, total *Aspergillus* and other *Aspergillus* spp. showed positive correlation (r=

Table 1. Pearson correlation coefficients between populations of various fungal species from unhulled and brown rice, temperature, and relative humidity under indoor storage conditions

Sample	Factor[a]	*Aspergillus*					Total *Penicillium*	Total Fungi	Mean Temp.[b]	Mean R.H.[c]
		Total	*A. candidus*	*A. flavus*	*A. fumigatus*	Others				
Unhulled rice	Total *Aspergillus*	1.000								
	A. candidus	0.619	1.000							
	A. flavus	0.913*[d]	0.677	1.000						
	A. fumigatus	0.075	−0.567	−0.200	1.000					
	Other species	0.491	0.688	0.594	−0.227	1.000				
	Total *Penicillium*	0.865*	0.378	0.589	0.718	0.189	1.000			
	Total fungi	0.994***	0.581	0.868*	0.116	0.441	0.906*	1.000		
	Mean Temp.	−0.327	−0.750	−0.287	0.945	−0.188	−0.267	−0.343	1.000	
	Mean R.H.	0.288	−0.282	0.128	0.456	−0.674	0.490	0.324	0.085	1.000
Brown rice	Total *Aspergillus*	1.000								
	A. candidus	0.797	1.000							
	A. flavus	0.760	0.752	1.000						
	A. fumigatus	0.746	0.224	0.342	1.000					
	Other species	0.898*	0.686	0.693	0.630	1.000				
	Total *Penicillium*	−0.567	−0.060	−0.084	−0.857*	−0.408	1.000			
	Total fungi	0.626	0.798	0.784	0.164	0.656	0.259	1.000		
	Mean Temp.	−0.659	−0.852*	−0.720	−0.132	−0.745	−0.220	−0.961**	1.000	
	Mean R.H.	−0.740	−0.439	−0.260	−0.709	−0.581	0.884*	0.032	0.085	1.000

[a]Population of fungal species from unhulled and brown rice samples from a ton bag were determined for 170~320 days after indoor storage.
[b]Temp. indicates mean temperature between sampling dates in the storage room.
[c]R.H. indicates mean relative humidity between sampling dates in the storage room.
[d]Asterisks (*, **, ***) indicate significant differences at $P<0.05$, 0.01, and 0.001, respectively.

Table 2. Pearson correlation coefficients between populations of various bacterial genera from unhulled and brown rice, temperature, and relative humidity under indoor storage conditions

Sample	Factor[a]	Total bacteria	*Bacillus*	*Micro-bacterium*	*Sphingo-monas*	*Methylo-bacterium*	Mean Temp.[b]	Mean R.H.[c]
Unhulled rice	Total bacteria	1.000						
	Bacillus	0.625	1.000					
	Microbacterium	0.686	0.396	1.000				
	Sphingomonas	0.564	0.445	−0.207	1.000			
	Methylobacterium	0.939*[d]	0.664	0.260	0.881*	1.000		
	Mean Temp.	−0.405	0.708	−0.445	−0.041	−0.031	1.000	
	Mean R.H.	−0.608	−0.231	−0.136	−0.578	−0.796	0.085	1.000
Brown Rice	Total bacteria	1.000						
	Bacillus	0.998***	1.000					
	Microbacterium	−0.731	−0.725	1.000				
	Sphingomonas	0.646	0.622	0.366	1.000			
	Methylobacterium	0.936	0.925	–	0.991	1.000		
	Mean Temp.	−0.655	−0.648	−0.090	−0.905*	−0.800	1.000	
	Mean R.H.	−0.389	−0.374	0.356	−0.198	−0.429	0.085	1.000

[a]Population of bacterial species from unhulled and brown rice samples from a ton bag were determined for 170~320 days after indoor storage.
[b]Temp. indicates mean temperature between sampling dates in the storage room.
[c]R.H. indicates mean relative humidity between sampling dates in the storage room.
[d]Asterisks (*, **, ***) indicate significant differences at $P<0.05$, 0.01, and 0.001, respectively.

0.898, $P<0.05$), while total *Penicillium* and *A. fumigatus* exhibited negative correlation ($r=-0.857$, $P<0.05$). Total fungi was also negatively correlated with mean temperature ($r=-0.961$, $P<0.01$) in brown rice (Table 1). These results

were similar to those of Filipello Marchisio et al. (2001), who found a negative correlation of *Aspergillus* and *Penicillium* spp. with temperature in a study on the temporal trends of airborne fungi and the relationships with environment in a suburban site in Italy. Among *Aspergillus* spp., *A. candidus* was negatively correlated (r=–0.852, P<0.05) with mean temperature in brown rice. This might explain why the total population of *Aspergillus* spp. and the temperature showed negative correlation. Moreover, this is also supported by the observation of DiĞrak et al. (2001), who reported that the biomass of *A. candidus* was greater at 15°C than at 25°C in a study of the relationship between fungal biomass and temperature, time, pH and sugars. On the other hand, total *Penicillium* showed positive correlation (r=0.884, P<0.05) with mean relative humidity in brown rice (Table 1). Mislivec et al. (1970) investigated the growth of 14 species of *Penicillium* at different relative humidities and reported that *Penicillium* spp. germinated and sporulated faster at higher relative humidity. Misra et al. (1981) also evaluated the effect of relative humidity on the incidence of storage fungi by storing spice seeds at five different relative humidities. At higher relative humidity 20 species of storage fungi appeared; however, at lower relative humidity, eight of these species, including *Penicillium* spp., did not appear. This significant relationship among fungal population and environmental condition existed in brown rice, but not in unhulled rice. This may be due to the rice bran which protects the inside of the rice from environmental stresses; therefore, storage fungi existed in the inside of the rice may be less affected by temperature and relative humidity.

In correlation analysis of population of bacterial genus and environmental conditions, *Methylobacterium* spp., one of the most predominant species, showed positive correlation with total bacteria (r=0.939, P<0.05) and *Sphingomonas* spp. (r=0.881, P<0.05) in unhulled rice (Table 2). In brown rice, *Sphingomonas* spp. also showed negative correlation (r=–0.905, P<0.05) with mean temperature. On the other hand, *Bacillus* spp., as the most predominant species composing approximately 90% of total bacterial population (Fig. 3), were positively correlated with total bacteria (r=0.998, P<0.001) in brown rice (Table 2). The population of all other bacterial genera or even of total bacteria did not correlate with temperature or relative humidity.

Based on this study, it is not possible to give a perfect explanation regarding the changes of populations of fungi and bacteria simply based on temperature and relative humidity, as the populations may have been affected by numerous factors (Filipello Marchisio et al., 2001). Although this indoor storage condition cannot fully represent the real situation, the results might help to predict the occurrence of fungi and bacteria in unhulled and brown rice, and even in white rice, stored in uncontrolled room conditions in retail markets and individual homes. Moreover, this study shows the effect of environmental conditions on populations of fungi and bacteria and provides primary information on which environmental factor should be controlled to inhibit the deterioration of rice caused by storage fungi, *Aspergillus* and *Penicillium* spp.

Acknowledgment

This study was carried out with the support of "Specific Joint Agricultural Research-Promoting Projects (Project No. 20070101-033-025-001-03-00)", RDA, Republic of Korea.

References

Bhattacharya, K. and Raha, S. 2002. Deteriorative changes of maize, groundnut and soybean seeds by fungi in storage. *Mycopathologia* 155:135-141.

Cottyn, B., Regalado, E., Lanoot, B., De Cleene, M., Mew, T. W. and Swings, J. 2001. Bacterial populations associated with rice seed in the tropical environment. *Phytopathology* 91:282-292.

DiĞrak, M. and Özçelik, S. 2001. Determination of some fungal metabolites as influenced by temperature, time, pH and sugars by biomass method. *Turk. J. Biol.* 25:197-203.

Filipello Marchisio, V. and Airaudi, D. 2001. Temporal trends of the airborne fungi and their functional relations with the environment in a suburban site. *Mycologia* 93:831-840.

Klich, M. A. 2002. *Identification of Common* Aspergillus *Species. Centraalbureau voor Schimmelcultures*, Utrecht, The Netherlands , 116pp.

Magan, N., Hope, R., Cairns, V. and Aldred, D. 2003. Post-harvest fungal ecology: Impact of fungal growth and mycotoxin accumulation in stored grain. *Eur. J. Plant Pathol.* 109:723-730.

Miller, J. D. 1995. Fungi and mycotoxins in grain: Implications for stored product research. *J. Stored Prod. Res.* 31: 1-16.

Mislivec, P. B., Dieter, C. T. and Bruce, V. R. 1975. Effect of temperature and relative humidity on spore germination of mycotoxic species of *Aspergillus* and *Penicillium*. *Mycologia* 67:1187-1189.

Misra, J. K., Gergon, E. B. and Mew, T. W. 1995. Storage fungi and seed health of rice: A study in the Philippines. *Mycopathologia* 131:13-24.

Misra, N. 1981. Influence of temperature and relative humidity on fungal flora of some spices in storage. *Z. Lebensm. Unters. Forsch.* 172:30-31.

Oh, J. Y., Jee, S. N., Nam, Y., Lee, H., Ryoo, M. I. and Kim, K. D. 2007. Populations of fungi and bacteria associated with samples of stored rice in Korea. *Mycobiology* 35:36-38.

Park, J. W., Choi, S. Y., Hwang, H. J. and Kim, Y. B. 2005. Fungal mycoflora and mycotoxins in Korean polished rice destined

for humans. *Int. J. Food Microbiol.* 103:305-314.

Pitt, J. I. 1985. *A Laboratory Guide to Common* Penicillium *Species.* Food Science Australia, Australia, 197pp.

Prange, A., Modrow, H., Hormes, J., Krämer, J. and Kähler, P. 2005. Influence of mycotoxin producing fungi (*Fusarium, Aspergillus, Penicillium*) on gluten proteins during suboptimal storage of wheat after harvest and competitive interactions between field and storage fungi. *J. Agric. Food Chem.* 53:6930-6938.

Sweeney, M. J. and Dobson, D. W. 1998. Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* species. *Int. J. Food Microbiol.* 43:141-158.

Taligoola, H. K., Ismail, M. A. and Chebon, S. K. 2004. Mycobiota associated with rice grains marketed in Uganda. *J. Biol. Sci.* 4:271-278.

Wicklow, D. T. 1992. The Mycology of stored grain: An ecological perspective. In: *Stored-grain Ecosystems.* Marcel Dekker, Inc. pp757.

Williams, R. J. and McDonald, D. 1983. Grain molds in the tropics: Problems and importance. *Ann. Rev. Phytopathol.* 21:153-178.