FUNGI ASSOCIATION AND TOXIN PRODUCTION IN STORED MILLED RICE

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ABSTRACT

T. H. Ansari, M. S. Hossain, M. A. Ali, S. Banik, M. A. Siddique S. Hosen and M. Ahmed. Fungi association and toxin production in stored milled rice. Bangladesh J. Plant Pathol. 32 (1&2): 67-74

Association of fungi in stored milled rice grain and the production of aflatoxin cause food intoxication. Therefore, infection level of storage fungi and aflatoxin production in stored milled rice procured locally or imported outside the country was investigated. Samples were collected from the government storages in different districts. *Aspergillus, Penicilium, Fusarium, Rhizopus, Curvularia, Bipolaris, Tricochonis* and *Alternaria* were detected as infecting fungi. Irrespective of storage type, the mean percentage of fungal association was low that ranged from 0.57-2.43% in the Central Storage Depoe and 0.66-2.53% in the Local Storage Depoe. Regardless of origin, infection level ranged from 0.38-2.90%. Probably proper drying before storing, good storage conditions and quarantine regulations were the key factors for low infection. Local milled rice was comparatively more infected with storage fungi *Penicilium, Fusarium, Rhizopus,* than imported milled rice. *Aspergillus* incidence was 2.25% in Indian, 2.12-3.66% in local samples which was comparatively more than Thailand (0.35-0.69%) and Pakistan (0.19%) samples. Among the tested 41 samples randomly selected from the collected samples, five samples contaminated with aflatoxin B_1 (5.39-8.08 µg kg^{-1}) and/or aflatoxin B_2 (0.06-0.13 µg kg^{-1}) at below permissible limit of WHO/UNICEF/FAO. The study provides information on the infection of storage fungi mentioned above and aflatoxin contamination level of stored milled rice in the government storage.

Kew words: Fungi, Toxin, Production, Stored milled rice.

INTRODUCTION

Bangladesh produces a large amount of rice and the government (Govt.) procure rice/milled rice during both dry and wet seasons from the local market for food crisis management. In order to maintain sustainable food securities, sometimes the country also imports milled rice from India, Myanmer, Pakinstan and Thailand (ricepedia.org/Bangladesh, USDA 2012).

Rice is generally harvested at high moisture contents. A full range of microbes, including bacteria, fungi, and actinomycetes, are found on freshly harvested rice (BRRI 1996, BRRI 1997). Delays in drying occur frequently that promotes the growth of field microorganisms at the elevated moisture contents (Teunisson 1954a, b). Some rice lots are dried within a few hours of harvest. Sometimes it may be harvested in the late afternoon, held overnight at the field or yard, and dry in the next morning. Consequently longer period of conserving moisture enhances both field and storage fungi. In India, the bulk of rice is grown in Kharif or wet season. Frequent heavy rainfall and floods particularly near harvest in coastal areas in eastern, southern and western regions of India wet the crop and make panicles more prone to invasion by *Aspergillus* spp. (Reddy *et al.* 2004).

The fungi especially *Aspergillus, Fusarium* and *Penicillium* are common in stored products or developing grains (Ansari *et al.* 1996, BRRI 1997, Magro *et al.* 2006). They are responsible for quantitative and qualitative losses. Under certain conditions these species can cause weight loss, seed discoloration, heating and mustiness as well as develop toxic metabolites called mycotoxins. The mycotoxins are hazardous to animal and human health and constitute a factor for economic losses in food products worldwide (Christensen and Saucer 1992).

Mycotoxin contamination in rice is generally lower compared to other cereals (Tanaka *et al.* 2007). But it is prone to contaminate with mycotoxins like aflatoxins B_1, B_2, G_1, G_2; citrinin, ochratoxin A, fumonisins B_1, B_2, B_3 etc. (Bulleman and Bianchini 2007, Tanaka *et al*. 2007). Aflatoxins are potentially toxic secondary metabolites produced mainly by *Aspergillus* spp (Reddy *et al.* 2005). Under favourable conditions, these fungi can grow and produce aflatoxins during harvest, handling, shipment and storage (Bushby Wagon 1979, Peraica *et al.* 1999, Giray *et al.* 2007 and Reddy *et al.* 2009). Among the aflatoxins, AFB_1 is the most toxic form for mammals (Peraica *et al.* 1999; Kotsonis *et al.* 2001; Speijers and Speijers 2004) and considered as class-I human carcinogen by the International Agency for Research on Cancer (IARC 1993). Several diseases outbreaks of aflatoxicosis in humans and animals have been reported due to consumption of aflatoxin contaminated food and feed (Reddy and Raghavender 2007). Several countries have been carried out surveys and monitoring programs to obtain a general pattern of aflatoxin contamination in rice (Reddy *et al.* 2008). Regulations for major mycotoxins in commodities and food exist in at least 100 countries, most of which are for aflatoxins, maximum tolerant levels differ greatly among countries (FAO 2004; van Egmond and Jonker 2004; van Egmond *et al.* 2007). In this background, the study was undertaken to determine the storage fungi association and toxin production level in stored milled rice procured from both local market and foreign countries.

MATERIALS AND METHODS

Site selection and sample collection. The government storages for food grain were considered for this study. Therefore, food storages like Silo, Central Storage Depoe (CSD) and Local Storage Depoe (LSD) of Khulna and Satkhira, districts in the south-western part of the country were selected for sampling. Specifically Khulna and Moheshwarpasha CSDs in Khulna CSDs and Satkhira sadar upazilla, Parulia of Debhata , Patkel ghata of Tala, Kaligonj and Nawabeki of Shyamnagar upazillas in Satkhira district were selected for sample collection. One hundred and sixty four milled rice samples were collected from different CSD and LSD food storages. Five hundred gram milled rice were collected for each sample from different lots in each store from the above mentioned storages. During sample collection, information was collected on origin of rice procured eg., procured locally or imported from foreign countries, parboiled or unparboiled, storage period, storage management and quality management of food grain.

Detection of fungal association. The collected samples were examined following the standard seed testing protocol of ISTA (1966). The collected stored milled rice samples were examined for the identification of fungal pathogens especially the stored fungi by blotter method following the International rules for Seed Testing (ISTA 1966). Four hundred grains of each sample were invetigated to determine the fungal association with milled rice. Twenty five grains were placed on water soaked three layered blotter paper (Whatman No. 1) in a glass petridishes sterilized previously. The petridishes were incubated at 25±1°C under 12/12 hrs light and darkness cycle for 7 days. Then, each seed was observed under strereomicroscope in order to record the presence of fungal colony 7 days after incubation based on growth habit. The results were recorded as number of infected grain for individual pathogen. Per cent grain infection was calculated.

Estimation of aflatoxin. Milled rice samples collected from different CSD and LSD storages of Khulna and Satkhira districts were also investigated for toxin estimation. Forty one milled rice samples were selected and tested for the presence of aflatoxin. The moisture content of the selected samples was estimated by using a Kett PBTK moisture meter. The samples sealed in polythene bags were stored at -58 ºC for aflatoxin estimations. Milled rice samples were ground in Udy cyclone mill. Measurement of aflatoxin was done following methods of Jones (1972). Aflatoxins were extracted by the modified 70% aqueous acetone procedure. HPLC grade standard aflatoxin reagent was purchased from Sigma (Aldrich, Madrid, Spain). Estimation of aflatoxin was done using HPLC (Experimental apparatus: Column ZORBAX Eclipse XDB- C18, 4.6 mm×150mm, 3.5 µm). Instrumental condition was HPLC system Agilent-1100 series with Flourosence detector, HPLC column was C18. Mobile phase was 630 mL water, 220 mL methanol, 150 mL acetonitrile in isocratic mode with 1 mL flow rate. Total run time was 15 minutes and injection volume 20 µL. Column oven temperature was 30 ^{0}C.

Quality control of analysis. Recovery was calculated for aflatoxins (B_1, B_2, G_1 and G_2) fortified at 2 µg kg^{-1}. 10 µg kg^{-1}, 20 µg kg^{-1}, 100 µg kg^{-1} and 200 µg kg^{-1} levels using peak area of chromatograms was found 90-92%. Suitable seven point calibration curve was done, preferably on matrix at 0.5 ng mL^{-1}, 2 ng mL^{-1}, 10 ng mL^{-1}, 25 ng mL^{-1}, 50 ng mL^{-1}, 100 ng mL^{-1} and 250 ng mL^{-1} i.e µg kg^{-1} level. Linear regression was 0.99. The detection limit was 0.5 µg kg^{-1}, decision limit (CC) was 4.34 µg kg^{-1} and detection capacity (CC) was 4.64 µg kg^{-1}.

RESULTS AND DISCUSSION

Fungal association. Fungi associated with stored milled rice were identified as *Aspergillus, Penicillium, Fusarium, Rhizopus, Curvularia, Tricochonis and Alternaria*. Among those the first four fungi is known as stored grain fungi that cause food contamination and produce toxins (Reddy *et al.* 2009a). Generally fungal infection was found low. Average incidence of *Aspergillus* was 1.87% followed by *Penicillium*

(1.60%), *Rhizopus* (1.26%) and *Fusarium* (0.79%) irrespective of the storage.

Infection level at CSD and LSDs. Fungal infection ranged from 0.57-2.43% in the CSDs and 0.66-2.53% in the LSDs (Fig. 1). Incidence of *Aspergillus* was the highest both in the Khulna CSD and Local LSDs. The highest incidence of *Aspergillus* in the local storages was due to its higher incidence in the locally collected Boro rice. In Moheshwarpasa CSD, *Penicillium* (2.43%) infected rice grains was more followed by *Aspergillus* (1.40%). This was also due to highest infection of those fungi in the Boro rice locally collected from northern part of the country. More infection in the locally collected boro rice might be due to poor drying during the procurement. Early monsoon rain might be one of the factors during drying that cause the poor drying or excess moisture in the rice. High moisture might increase the higher incidence of both the fungus. Phillips (1984) reported that the high moisture content of paddy and inability to dry it rapidly resulted in high incidence of yellowing of rice in storage because of fungal infection. The difference of incidence among different fungi in each kind of storage and among the storages was less besides the incidence of *Penicillium* in Maheshwarpasha and *Aspergillus* in local storages. Therefore, the results suggested that the fungal incidence in CSDs and LSDs did not differ much.

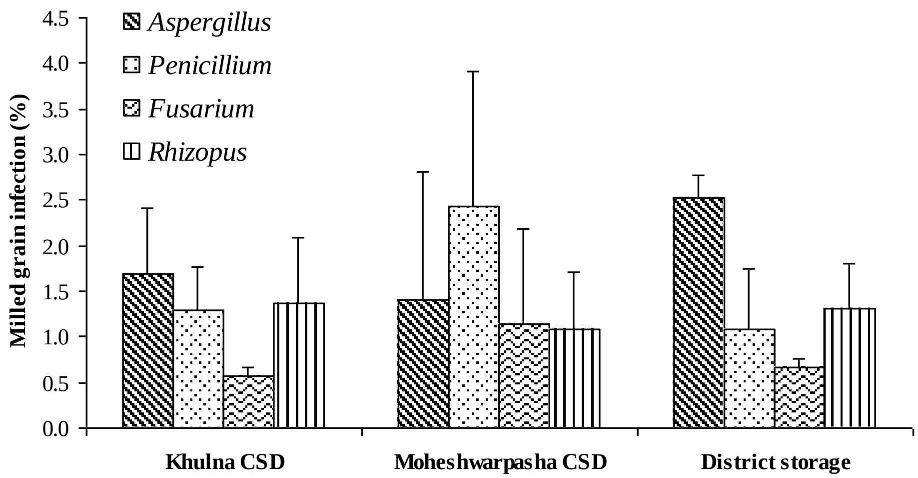

Fig. 1. Milled rice grain infection (%) at different storages

Incidence of fungi in LSDs at upazilla. Stored rice samples were collected from different local storages at four upazillas in Satkhira district. The results indicated that maxium 6.50 % and 2.90% incidence of *Aspergillus* and *Rhyzopus* were found in Satkhira sadar upazilla store (Fig. 2). These were the highest incidence compare to other local storages even from CSD storages (Fig. 1 & 2). Imported rice from India was infected more (18.25% for *Aspergillus* and 6.00% for *Rhyzopus*) and hence the average incidence was more. Incidence of other fungi ranged from 0.20-2.44%.

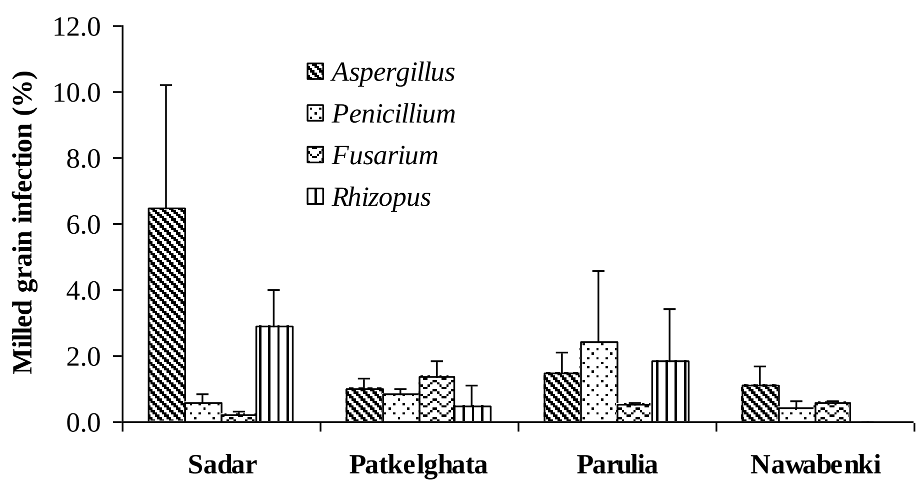

Fig. 2. Incidence of fungi associated with milled rice at different upazilla storages of Satkhira.

Incidence of fungi in imported rice. Bangladesh import rice from different foreign countries eg., Thailand, India, Pakistan, Vietnam and Myanmar. Rice samples of different foreign origin in CSD storages were investigated which results that rice of Bangladesh origin was more infected with fungi than Thailand, India and Pakistan (Fig. 3 & 4). All four stored grain fungi *Aspergillus*, *Penicillium*, *Fusarium* and *Rhyzopus* were more prevalent in Bangladesh rice than Thailand, India and Pakistan rice in Maheshwarpasha CSD. Similar results were also found in Khulna CSD both for *Penicillium* and *Rhyzopus*. But *Aspergillus and Fusarium* were found more in Indian rice. However, rice imported from any of the country like Thailand, India and Pakistan was less infected and may not above the acceptable limit.

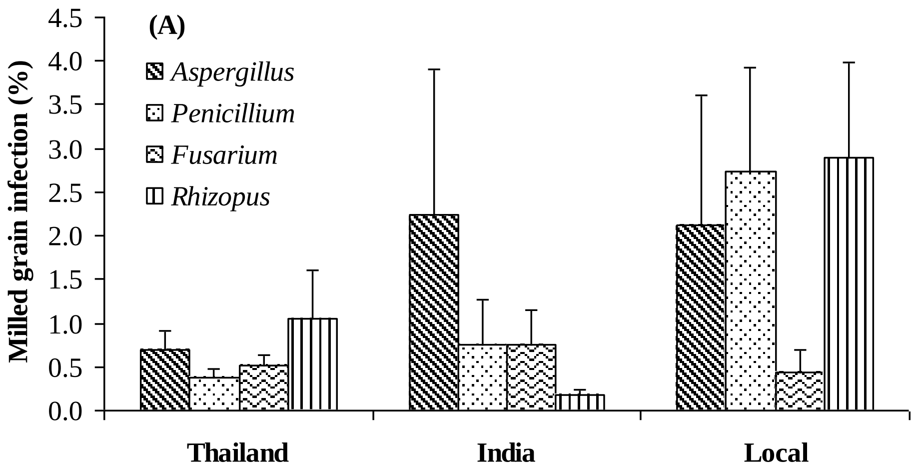

Fig. 3. Infection (%) of milled rice grain collected from different origin in Khulna CSD

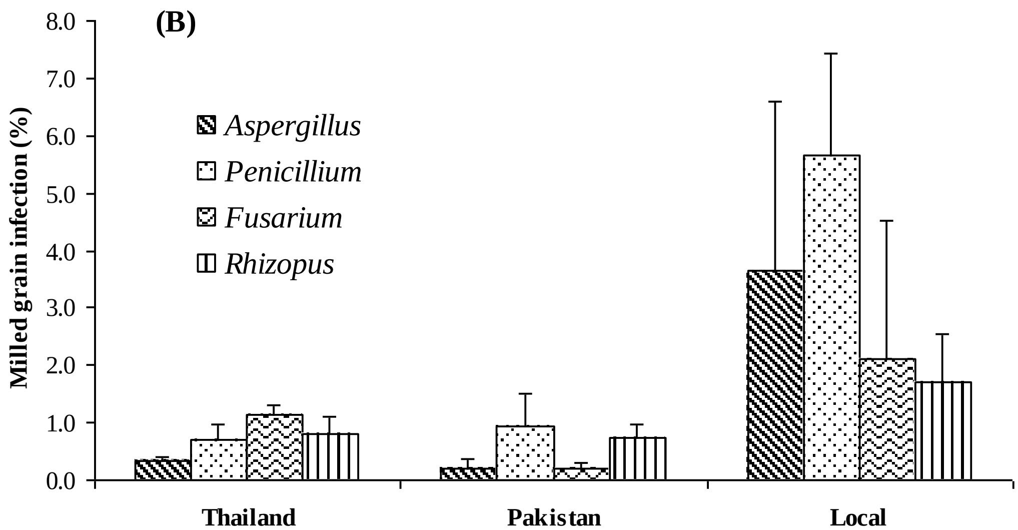

Fig. 4. Infection (%) of milled rice grain collected from different origin in Moheshwarpasha CSD (B).

Grain infection at different storage period. Grain infection for any fungus at different period of storage showed no consistent result irrespective of country of origin in both Khulna and Moheshwarpasha CSDs (Table 1 & 2). Similar results were also observed in the local storage samples with different period of storage. The infection level did not increase with storage period irrespective of storages and country of origin.

Table 1. Grain infection at different storage periods in Khulna CSD with their source.

Source/ Origin	Storage period (month)	% Grain infection			
		Aspergillus	*Penicillium*	*Fusarium*	*Rhizopus*
Thailand	7	0.38	0.25	0.25	0.00
	5	0.73	0.60	0.40	2.42
	4	0.00	0.13	0.75	0.00
	3	0.96	0.36	0.60	0.86
India	<1	2.25	0.75	0.75	0.19
Local	5	2.00	0.00	0.33	0.67
	3	0.42	4.46	0.22	2.36
	2	13.00	0.00	0.00	2.75
	1	3.00	0.19	0.44	1.88
	<1	0.00	4.00	1.25	6.63

CSD: Central Storage Depoe

Table 2. Grain infection at different storage periods in Moheshwarpasha CSD with their source.

Source/ Origin	Storage period (month)	% Grain infection			
		Aspergillus	*Penicillium*	*Fusarium*	*Rhizopus*
Bangladesh	<1	5.85	5.05	0.50	0.80
Bangladesh	3	0.00	6.67	4.75	3.25
Pakistan	10	0.19	0.94	0.19	0.75
Thailand	10	0.35	0.70	1.14	0.80

CSD: Central Storage Depoe

Infection level of any fungus was not increased with the increase of storage period which indicates the good storage management for food preservation in all kind and level of store (Fig. 5).

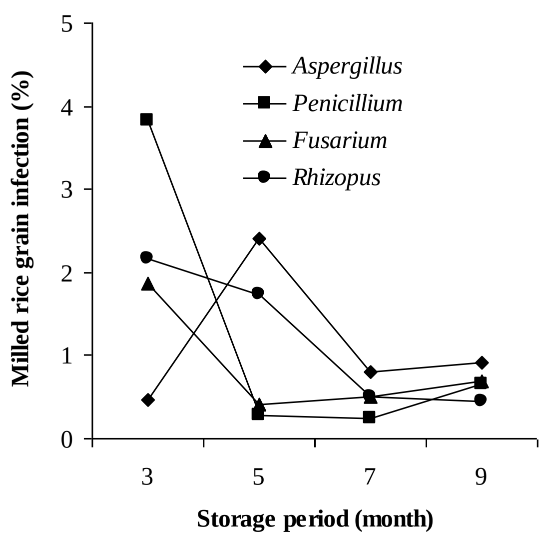

Fig. 5. Grain infection at different duration irrespective of origin and storage

Estimation of aflatoxin

None of the samples contained aflatoxin B_1 above the maximum permissible level 30 µg kg^{-1} (ppb) in foods for human consumption (UNICF/WHO/FAO). Aflatoxin B_1 was not detected in 36 tested samples. Only five samples contained aflatoxins B_1 ranged from 5.39-8.51 µg kg^{-1} (Table 3). Only three samples contained 0.06-0.13 µg kg^{-1} aflatoxin B_2. These samples was more likely to be colonized by the fungi might be due to high moisture levels caused by improper drying.

Table 3. Aflatoxins content in stored milled rice grain collected from CSD/LSD.

Code no.	Aflatoxin B_1 (μg kg^{-1})	Aflatoxin B_2 (μg kg^{-1})	Aflatoxin G_1 (μg kg^{-1})	Aflatoxin G_2 (μg kg^{-1})	Total (μg kg^{-1})
1	5.71	0.13	-----	-----	5.95
2	5.39	0.07	-----	-----	5.46
3	8.51	0.06	-----	-----	8.57
18	7.52	-----	-----	-----	0.11
20	8.08	-----	-----	-----	-----

Max. permissible limit 30 μg/kg (ppb) in foods for human consumption (UNICEF/WHO/FAO).
CSD: Central Storage Depoe, LSD: Local Storage Depoe

The incidence of seed borne storage fungi was detected on milled rice irrespective of storage type or origin. *Aspergillus*, *Penicilium*, *Fusarium*, *Rhizopus*, *Curvularia*, *Bipolaris*, *Tricochonis* and *Alternaria* were detected as infecting fungi. However, the infection level was very low. Previous studies indicated that storage fungi infect rice seed not only in storage condition but also at field condition (Ora *et al*. 2011, Islam *et al*. 2012, BRRI 1997). The fungi sometimes invade and infect the endosperm resulting the discoloration and deterioration of milled rice quality. Therefore, seed infection at field or its origin is very important to consider during the procurement of milled rice for ensuring the quality of food grain. Rice grain infection could be more at high grain moisture and storage humidity which are the most important factors in storage conditions (Monajjem *et al*. 2014). The investigated milled rice was not infected much probably due to proper drying during processing (12% moisture) and maintaining the proper humidity in storage. On the other hand, quarantine regulations for importing milled rice might have effective for procuring good quality milled rice from other countries. None of the fungicides used in the storage to control fungal infection but sometimes drying different rice lots to prevent absorbed moisture which might be effective for less infection. Chemical fungicide spray may not control the storage fungi effectively (Butt *et al.* 2011) rather it could be disastrous to human health. The fungi *Aspergillus* produced aflatoxins in stored milled rice. The infection level was not high and as such toxin production was insignificant as well as confined to a limited number of samples. However, it is very important to be cautious during importing, local procurement, processing and storing of rice to avoid toxin producing fungi. These could be done in different ways like- i) Frequent analytical surveillance program by food control agencies is highly recommended to control the incidence of storage fungi and aflatoxins contamination in food grains to ensure food safety and to protect consumer's health, ii) Farmers should improve knowledge and practice of drying rice grains to the required moisture content immediately after harvest to avoid aflatoxin problem in food grain, iii) Proper drying as well as spraying fungicides or some other chemicals is needed to control *Aspergillius* and other storage fungi like *Fusarium*, *Penicillium* and *Rhizopus* infection and subsequent toxin accumulation on food grains under storage conditions especially in the government CSD and LSD storages and iv) Up gradation of quarantine regulation for prevention of major mycotoxins especially aflatoxins in milled rice is necessary.

CONCLUSION

Aspergillus, Penicillium, Fusarium and *Rhyzopus* were detected in both domestic and imported milled rice in all categories of rice storage. Some of them are known to be mycotoxin producers. Aaflatoxin produced by *Aspergillus* spp. was also detected in the stored rice. Fungi infection was less and none of the samples was toxic over the recommended level of FAO/WHO/UNICEF. The information about storage fungi is essential to describe the status of food quality in the government storage and to take control measures for prevention of mycotoxin formation.

ACKNOWLEDGEMENT

The project activities were done with the financial help of Bangladesh Agricultural Research council (BARC) through SPGR-NATP project. We also acknowledge the Department of Food, Ministry of Food for their cooperation in getting rice samples from different government storages.

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