



Screening of Fungi and Mycotoxins Associated with Stored Rice Grains in Himachal Pradesh

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ABSTRACT: The present paper explores fungi and mycotoxins associated with rice grains during storage. Total 25 rice samples collected from different locations of district Mandi, Himachal Pradesh were analysed. All the samples were found contaminated with one or more fungal genera like *Aspergillus*, *Rhizopus*, *Penicillium*, *Fusarium*, *Curvularia*, *Cladosporium* and *Alternaria*. *Aspergillus flavus*, *A. niger* and *Rhizopus stolonifer* were isolated with highest frequency and density. After mycotoxins analysis only Aflatoxins B1 and B2 were detected in about 72% samples. Presence of Aflatoxins B1 was reported in 28% samples while B2 in 48%. The present study suggests that detection of fungi and aflatoxins poses a risk for consumer's health and it is necessary to check the rice grains before allowing distribution for public use.

Keywords: Fungi, mycotoxins, rice, contamination.

I. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most cultivated food crops globally. About 593 million tones (Mt) rice is produced annually (FAO, 2002). It is one of the main food crops after wheat and corns in India. It is used in variety food products. Cooked rice, breakfast cereals, desserts, and rice flour are some important food products of rice. It is also used to prepare local beer, while rice hull is used as fuel, fertilizer, packing and insulation. Rice bran is also used as a suitable substrate in mushroom cultivation. Straw from the leaves and stems is used as bedding for animals. Rice flour is used to prepare many traditional foods on some special occasions (Giddel and Jivani, 2007).

The major practices used for rice cultivation in India are sowing, harvesting and storage. The diverse climatic conditions in India with respect to altitude, moisture, temperature and relative humidity provide variation in rice cultivation practices. Apart from these, frequent and heavy rainfall and floods, particularly near harvest, in coastal areas in eastern, southern, and western regions of the country wet the crop and make panicles more prone to invasion by filamentous fungi and bacteria (Makun *et al.*, 2007; Reddy *et al.*, 2005, 2009). This contamination leads to the secretion of many secondary metabolites known as mycotoxins.

Contamination of food grains as, rice is important issue for grain quality and from consumer's health

point of view. Therefore, present study was carried out for the isolation and identification mycotoxins and their producing fungi associated with rice grains during storage.

II. MATERIAL AND METHODS

Sample collection

A total of 25 rice samples (about 250gm) were collected from different locations of district Mandi, Himachal Pradesh. Collected samples were transported immediately to laboratory for labelling, like date and place of collection and kept in cool place for further mycological analysis.

Isolation and identification of fungi

Fungal isolation from collected rice samples was carried by using direct plating method. About 6-7 grains were inoculated randomly in each of petriplates containing PDA medium. An antibacterial agent Chloramphenicol (50ppm) was used to inhibit the growth of bacteria. Petri plates were incubated at $27 \pm 2^\circ\text{C}$ for 6-7 days and examined daily for fungal growth. Fungal colonies grow on inoculated samples were counted and subculture on PDA for identification. Morphological and cultural characteristics of the growing cultures were evaluated for preliminary identification. Then fungal colonies were subjected to microscopic identification (Gilman, 2001).

Analysis of mycotoxins and mycotoxigenic fungi

The collected samples were evaluated for the presence of aflatoxins. Extraction of mycotoxins (Singh, 1988) and the qualitative detection of mycotoxins were done on the basis of their fluorescence and Rf values (Scott *et al.*, 1970). About 50gm powder of each collected rice samples were extracted with chloroform. About 50 ul of chloroform extract was applied on silica gel plates together with specific standards (Sigma, chemical, St Louis, USA) developed with mobile phase benzene: methanol: acetic acid (24:2:1) and observed under long wavelength UV light at 365nm.

III. RESULTS AND DISCUSSION

Mandi is one of the twelve districts of Himachal Pradesh located in north-west Himalayas. The average altitude and rainfall of the area is 1,044 metres and 758.3 mm respectively. Average temperature typically ranges between 6.7- 39.6 °C over the course of a year. The average temperature during summer is between 18.9 - 39.6 °C and between 6.7 - 26.2 °C in winter. The variable climatic conditions of the area may favour the chances of fungal and mycotoxin contamination of storage food grains (Essono *et al.*, 2007; Murthy *et al.*, 2009).

Total 25 samples were analysed in the present study. Colour variation was observed during morphological analysis. White to light yellow coloured rice samples were observed depending upon the storage conditions. After mycological analysis, all the rice

samples were found infested to various degrees with storage fungi while aflatoxins were detected in 72% of samples (Fig.1). A total of seven fungal genera belonging to twelve species namely *Aspergillus flavus*, *A. niger*, *A. nidulans*, *A. terreus*, *A. parasiticus*, *Rhizopus*, *Penicillium chrysogenum*, *P. citrinum*, *Fusarium* sp., *Curvularia lunata*, *Cladosporium* sp. and *Alternaria alternata* were identified from rice samples. Some unidentified fungi were also recorded during the study.

Aspergillus flavus was recovered with highest percentage frequency (37.77%) followed by *A. niger* (16.88%) and *Rhizopus Stolonifer* (22.22%). Other fungal isolates were recovered in the range of 3.55%-1.7%. Similarly, relative density was found at higher level in *Aspergillus flavus* (22.66) followed by *A. niger* (10.21) and *Rhizopus Stolonifer* (15.59). The density of other fungal species was in the range of 2.95%-1.07%. Reddy *et al.*, (2005) characterized AFB₁ produced by *Aspergillus flavus* isolated from discoloured rice grains. Sales and Yoshizawa (2005a&b) studied occurrence of *Aspergillus* section *Flavi* in rice from Philippines. Makun *et al.*, (2007) also described the similar fungi as mycoflora associated with Nigerian rice. Similar findings of mycoflora associated with rice grains were also reported by Trung *et al* (2001) from South Vietnam. Taligoola *et al* (2010; 2011) also isolated toxigenic fungi from stored rice grains in Uganda.

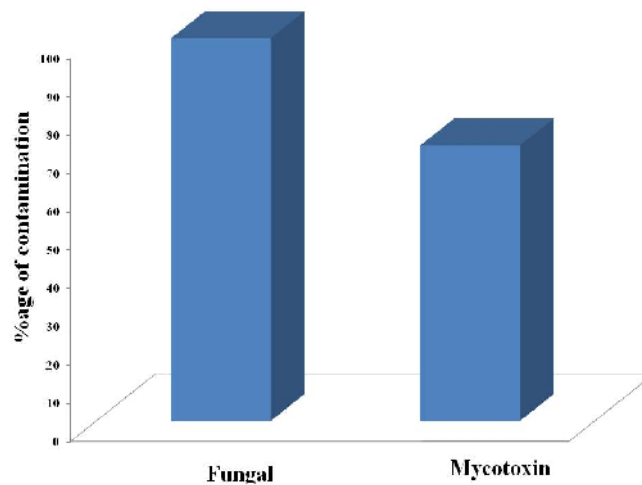
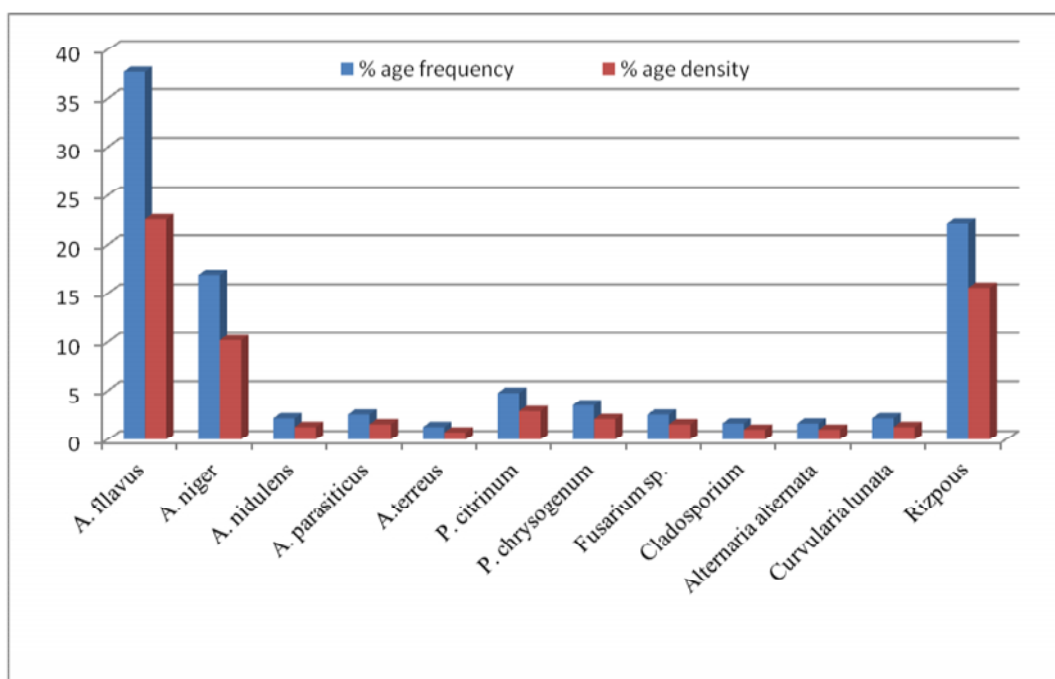


Fig. 1: Fungal and mycotoxin contamination of rice.

Table 1. Relative frequency, density and incidence of fungi isolated form stored rice grains.

Fungi isolated	% age frequency	% age density	Relative incidence (%)
<i>Aspergillus flavus</i>	37.77	22.66	41.6
<i>A. niger</i>	16.88	10.21	
<i>A. nidulans</i>	2.22	1.34	
<i>A. terreus</i>	1.33	0.80	
<i>A. parasiticus</i>	2.6	1.61	
<i>P. chrysogenum</i>	3.55	2.15	16.6
<i>P. citrinum</i>	4.8	2.95	
<i>Fusarium</i>	2.6	1.61	8.3
<i>Curvularia lunata</i>	2.22	1.34	8.3
<i>Cladosporium</i>	1.7	1.07	8.3
<i>Alternaria alternata</i>	1.7	1.07	8.3
<i>Rizopus stolonifer</i>	22.22	15.59	8.3

**Fig. 2.** Percentage frequency and density of fungi isolated form stored rice samples.

Natural occurrence of mycotoxins

After analysis collected rice samples for natural occurrence of mycotoxins, about 72% of the samples were found contaminated with mycotoxins. Only two mycotoxins viz. AFB₁ and AFB₂ was detected on the basis of their fluorescence and retention factor (Rf) values. Presence of each mycotoxin was confirmed long wave UV light at 365 nm, spots giving bluish

fluorescence indicated the presence of aflatoxins B as described by Scott *et al.*, (1970) and Ono *et al.*, (2010).

Out of 25, 28% (07) were found associated with AFB₁ whereas, 48% (12) of samples were with AFB₂ (Table 2). The retention factors of all the mycotoxins produced were determined. This was done by thin layer chromatography.

Table 2. Mycotoxin contamination in rice sample.

Rice Sample	No. of Sample Analysed	No. of Samples Contaminated (%)	Mycotoxin	Positive Samples (% age fq)
	25	18 (72%)	AfB ₂	12 (48)
			AfB ₁	7 (28)

Detection of aflatoxin as a primary mycotoxin in stored rice was also reported by demonstrated time to time by Reddy and his associates (Reddy *et al.*, 2004; 2005; 2009) when they analyse the rice grains from all over India. The detection of mycotoxins particularly aflatoxins from rice was also reported worldwide. Carlos *et al.*, (2000) isolated aflatoxigenic species from derivatives of milled rice while Fouzia and Samajpati (2000) from rice, pulses and oilseeds. Similarly, Desjardins *et al.*, (2000) detected different *Fusarium* sp. from Napalese rice and observed the production of mycotoxins and gibberellic acid by selected species. Abd-Allah and Ezzat (2005) reported natural occurrence of citrinin in rice grains and studied its biocontrol by *Trichoderma hamatum*. Concurrently, Liu *et al.*, (2006) and Konishi *et al.*, (2006) studied aflatoxins and other mycotoxins in rice from China and Japan respectively whereas, Mangala *et al.*, (2006) in India. Subsequently, Tanaka *et al.*, (2007); Taligoola *et al.*, (2010; 11) and Surekha *et al.*, (2011) also reported Toxigenic fungi and mycotoxins in rice.

Since, *A. flavus* is one of the major producers of aflatoxin; therefore, detection of aflatoxins in 72% of samples of rice justifies the isolation of *A. flavus* with high frequency and density. Presence of *A. flavus* is of main concern because fungus is a potent aflatoxin producer. The aflatoxins are reported to be carcinogenic, hepatogenic, nephrogenic and cause various nervous disorders (Shephard, 2008). Similarly, presence of *Aspergillus niger* is also alarming, due to allergic efficiency of its spores (Schuster *et al.*, 2002; Noonimabc *et al.*, 2009; Edwin *et al.*, 2010; Gautam and Bhadauria, 2009; Gautam *et al.*, 2011). *Penicillium citrinum* is a main citrinin producer. This mycotoxin has ability to cause nervous and carcinogenic effects (Benneth, 2003; Yu

et al., 2006; Singh *et al.*, 2007). Fusarial toxins are produced by various *Fusarium* sp. which can cause various health effects. Therefore, detection of *Fusarium* sp. is also the matter of concern. Likewise, detection of other fungi cannot be ignored due to their various health disorders. Fungal and mycotoxin contamination of rice samples during storage poses a danger to the consumers because majority of the population of the area consuming rice as major food grain. Therefore, it is necessary to analyse the rice grains after storage for fungal and mycotoxin contamination and then allowed to distribute for public use if found free from contaminations.

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