https://doi.org/10.29321/MAJ.10.A02055

SURVEILLANCE OF TOXIGENIC FUNGI AND AFLATOXINS IN RICE AND PADDY GRAINS

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ABSTRACT

A total of 337 samples of paddy grain and rice were surveyed for the incidence of toxigenic fungi and aflatoxins in a few districts of Tamil Nadu. The fungal contamination on paddy was greater than on rice and were mostly field fungi. The major toxigenic fungi included species of **Aspergillus** and **Penicillium**. The aflatoxin contamination was at a low percentage in the samples surveyed. The aflatoxin B1 level ranged from trace to 40 µg/kg rice and up to 20 µg/kg paddy. However rice and paddy grain supplied through the public distribution system did not show aflatoxin B1 above tolerance level.

KEY WORDS: Toxigenic fungi, Aflatoxin, Rice.

Rice is the staple food grain for millions of people especially in Asia. The per capita rice consumption ranges from 170 to 440 g daily supplying up to 70 per cent of the calorie intake and over 50 per cent of the dietary protein. The contamination of rice with different fungi occurs at any stage from the standing crop through harvest and post-harvest handling processes until it reaches the consumer. The health hazards due to mycotoxicoses, especially aflatoxicoses, have been reported from various countries. According to Saito et al. (1971) yellow rice disease is caused by more than 15 kinds of fungi, and important among these are **Aspergilli** and **Penicillia**. They further reported that consumption of heavily contaminated rice has also resulted in paralysis, respiratory and circulatory disturbances, renal damage, liver cirrhosis and even death.

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The extent of fungal and aflatoxin contamination depends on the per cent moisture of the grain, temperature and relative humidity during drying and storage. In Tamil Nadu, rice is extensively grown in Thanjavur and Tiruchirapalli districts. The temperature and relative humidity are usually high and the harvest usually coincides with the monsoon rains in these regions. The moist grain provides a favourable medium for the fungi to grow until it is dried to safe moisture level (12%). Non-availability of godowns, blending of high and low moisture grains, parboiling, insect attack etc., influence the development of storage fungi and consequently toxin elaboration. The information on the toxigenic fungi and mycotoxins on rice is very limited. A study has been made to investigate the extent of fungal contamination, the presence of toxigenic fungi and aflatoxin content in rice and paddy grains in Tamil Nadu.

MATERIALS AND METHODS

Collection of samples

Samples of rice and paddy grains were collected from the godowns of public distribution system such as Food Corporation of India, Central Warehousing Corporation and Tamil Nadu Civil Supplies Corporation in Coimbatore, Erode, Madurai and Tirupur cities. The samples were withdrawn using a probe from alternate peripheral bags. A minimum of 500 g material was drawn from at least 25 bags. The physical characteristics such as colour, off-flavour, caking, insect and visible fungal attack were recorded. The moisture content of the samples was also determined. Then the samples were stored in polythene bags at room temperature until analysis. The study was conducted for a period of over three years: April 82 through June 84 in other cities; July 8 through December 86 in Madurai.

Isolation of fungal flora

A sample of 10 g was washed with sterile water and plated on Czapek-Dox agar at room temperature (30°C). A minimum of 100 grains from each sample was used. After 5-7 days of incubation, the fungi grown were identified and the per cent contamination worked out. The identification of individual fungus was made after bringing it into pure culture following the procedure of Raper and Fennel (1973). The toxin producing ability of the isolates was

Table 1. Incidence of aflatoxin producing fungi on rice and paddy grain from Coimbatore and Madurai regions.

Places	Samples collected	Contaminated %	toxigenic %	Aflatoxin contamination		
				Contaminated with aflatoxin B1	% of contamination	Aflatoxin B1 range µg/kg
I. Coimbatore						
Raw rice	20	20.00	20.00	2	10.00	0-40
Parboiled	80	41.25	36.25	5	6.25	30-35
Paddy	25	84.00	12.00	2	8.00	0-20
Total	125	46.40	28.80	9	7.20	0-40
II. Madurai						
Raw rice	29	6.80	6.80	1	3.45	Trace
Parboiled	161	14.20	3.12	1	0.62	35
Paddy	22	100.00	13.60	1	4.55	Trace
Total	212	22.16	9.72	3	7.42	Tr-35
Grand Total	337	31.45	13.39	12	3.56	0-40

Table 2. Fungal incidence and aflatoxin contamination in rice and paddy

Commodity	Number of samples analysed	Total contamination %	With toxigenic fungi %	Aflatoxin contamination %	Extent of toxigenic fungi %	
Raw rice	49	12.24	10.20	6.30	Aspergillus flavus	8.0
					A. niger	2.2
Parboiled rice	241	23.65	14.93	2.49	A. flavus	6.0
					A. niger	5.4
					A. parasiticus	1.0
					A. versicolor	1.0
					Penicillium urticas	1.5
Paddy	47	91.48	12.76	6.38	A. flavus	8.0
					A. niger	1.8
					A. versicolor	0.5
					A. parasiticus	1.5
					Penicillium expansum	1.0
Total	337	31.45	13.94	3.56		

assessed on autoclaved rice medium according to Shotwell et al. (1966).

Aflatoxin analysis

Fifty gram portions of powdered sample were extracted with aqueous chloroform in duplicate as described by Jones (1972). The chloroform extracts were concentrated by distillation in vacuo and stored under refrigeration in amber coloured vials until analysis. The seperation of aflatoxins was carried out on silica gel G by thin layer chromotography method. Initially, a qualitative screening of each extract was carried out to detect the presence of UV florescent substance including aflatoxins on chromotoplates. Estimation of aflatoxin was carried out with internal and external standards and by diluting each extract to extinction.

RESULTS AND DISCUSSION

The rice and paddy samples surveyed at Coimbatore and Madurai for toxigenic fungi and aflatoxins are given in Tables 1 and 2 respectively. These included 290 rice and 47 paddy samples. Most of these stocks were procured and transferred from Thanjavur district to these places for storage and distribution. Among the Coimbatore samples tested, seven were collected from imported rice stocks and four from stocks declared unfit for human consumption and these were stored away from the main stocks. The predominant fungi isolated from rice and paddy samples are given in Tables 3. The per cent contamination on paddy (91%) was greater than that on rice (24%). However, the fungi encountered on paddy were mostly field fungi **viz., Curvularia lunata, Drechslera oryzae, Pyricularia oryzae** and **Cephalosporium roseum** (Table 1).

The incidence of toxigenic fungi on rice ranged from 2.5-6.3 per cent. It is seen that species of **Aspergilli** and **Penicillia** and **Rhizopus oryzae** were the major storage fungi recorded both on rice and paddy. The samples of imported rice showed little contamination with any toxigenic fungi. However, samples discarded as unfit for human consumption were found highly contaminated with **A. flavus** and **Penicillium uriticae.**

Though 13.94 per cent of samples were contaminated with toxigenic fungi it was found that only 3.56 per cent were

contaminated with aflatoxins (Table 2). It is found that 3.10 per cent of rice and 6.38 per cent of paddy samples showed the presence of aflatoxin B1. In rice, 6.3 per cent raw rice and 2.49 per cent parboiled rice contained aflatoxin B1. The aflatoxin B1 level ranged from trace to 40 µg/kg of the material (Table 1). The samples did not show any other toxins. However four paddy samples exhibited the presence of unidentified spots in addition to aflatoxin B1. The aflatoxin content of cyclone-affected paddy in Andhra Pradesh ranged from trace to 1130 µg/kg (Bhatt and Tulpule, 1978). It is interesting to note that though 13.94 per cent of rice and paddy samples (Table 2) were contaminated with toxin producing fungi, none of them contained aflatoxins above tolerance limit. On the other hand, samples from stocks declared unfit for human consumption contained 30 to 40 µg/kg of aflatoxin B1.

A comparison of fungal flora and aflatoxin levels in various samples showed that a large number of paddy samples (91.48%) were contaminated mostly by field as well as non-toxigenic storage fungi. Further it may be seen that although 12.76 per cent samples were contaminated with different toxigenic fungi, only 6.38 per cent samples were contaminated with aflatoxin B1 (Table 2), the toxin content ranging from trace to 20 µg/kg of the material (Table 1). In both rice and paddy, the moisture content greatly influenced the occurrence of toxigenic fungi and aflatoxin contamination.

The incidence of fungi on both paddy and rice grains collected from Coimbatore was 46 per cent while it was 22 per cent in samples from Madurai (Table 1). This is possibly due to weather conditions during harvest and post-harvest handling, drying and storage. The fungal contamination in raw rice was lower than in parboiled rice, while aflatoxin contamination in raw rice was greater than in parboiled rice (Table 2).

The health hazards caused by ingestion of mycotoxin contaminated foodstuffs is a matter of great concern. Since rice is the major cereal in South Indian diets, the information on the extent to which it is contaminated with toxigenic fungi and mycotoxin is essential. The information on the extent of contamination of rice with different toxigenic

Table 3. Incidence of fungi in rice grain and paddy (290 samples).

Fungal flora	Mean per cent incidence in rice		
	Raw	Parboiled	Paddy grain
Aspergillus flavus *	2.0	23.0	4.0
A. niger *	1.0	19.0	1.5
A. parasiticus *	0.5	2.0	1.0
A. versicolor	0.5	0.5	0.5
Penicillium urticae	0	8.0	-
P. digitatum	1.0	0.0	-
P. expansum	0.0	0.5	1.0
Rhizopus oryzae	2.0	5.0	3.0
Mucor	0	2.0	1.5
Cladosporium fulvum	1.0	-	2.0
Cepholosporium roseu	-	-	2.0
Dreschslera oryzae	-	-	17.0
Pyricularia oryzae	-	-	2.0
Tricothecium sp.	-	-	0.5
Fusarium moniliforme	-	-	2.0
Alternaria longissim	-	-	3.0
Curvularia lunata	-	-	22.0
Diplodia oryzae	-	-	1.5
Cladosporium fulvum			2.0
Choetomium roseum	-	-	0.5
Sclerotium rolfsii	-	-	2.5
Colletotichum sp.	-	-	0.5

* aflatoxin producing fungi

fungi and mycotoxins in this part of the country is limited and inadequate (Sreenivasamurthy, 1977.

The occurrence of various fungi on rice grains both domestic and imported was studied by a number of Japanese workers (Saito et al., 1971). Tsunoda (1970) reported that **Aspergilli** and **Penicillia** are the major aflatoxin producing fungi in rice which caused intoxication in man and livestock. The rice gets contaminated with fungi at various stages-in the fields, at harvest and post-harvest processes. The moisture content of paddy grain at harvest ranged from 18-25 per cent and sometimes even more. The paddy grain harvest usually coincides with the on-set of monsoon rains especially in coastal regions of Tamil Nadu which delays drying of grains. Non-availability of good threshing floors and drying yards makes it still difficult to dry the wet produce to safe moisture level and as a result the fungal contamination is greater. Further parboiled paddy grains are more vulnerable for fungal invasion unless rapidly dried to safe moisture level. The susceptibility to insect infestation in turn increases the susceptibility to fungal contamination (Majumdar, 1974). Thus all the harvest and post-harvest handling processes make the commodity vulnerable for fungal invasion and toxin elaboration.

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