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SEASONAL VARIATION IN MYCOFLORA OF UNMILLED RICE IN RELATION TO MYCOTOXINS CONTAMINATION

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ABSTRACT

About 240 samples from different regions of Warangal district of Andhra Pradesh was analysed by employing dilution plate method and seed plating. Most of the samples of unmilled rice were heavily infested. However, fungi associated varied with the condition of the sample and place of collection. In all 30 fungal species be longing 19 genera could be reported in unmilled rice. Species of *Aspergillus*, *Penicillium* and *Fusarium* were dominant. *Nigrospora oryza*, *Phoma sorghina* and *Stachybotrys atra* could be recorded in Kharif season samples only, while *Myrothecium roridum* could be recorded in Rabi season samples. In general samples of Kharif season were more mould infested than Rabi season samples. Considerable percentage strains of mycotoxigenic fungi were toxigenic and elaborated aflatoxins, patulin, terreic acid, ochratoxin A, citrinin, zearalenone, DON, roridin, fumonisins and trichothecenes.

Key words: Unmilled rice, Kharif crop, Rabi crop, mycoflora, *Aspergillus, Penicillium, Fusarium*, mycotoxins.

INTRODUCTION

Unmilled rice (*Oryza sativa* L.) is the most important staple food crop in India and the bulk of rice is grown in Kharif or the wet season in Andhra Pradesh. Unmilled rice is the major crop cultivated along the Godavari belt of Andhra Pradesh, India. More relative humidity (%) and warm conditions during rainy season (June to October) favour the mould infestation. Further, seed harvested with high moisture and not dried before storage deteriorates fast due to increased mould activity. The harmful effects of fungal invasion of grain are seed discoloration (Reddy *et al.,* 2005), loss of viability (Reddy *et al.,* 2004; Enikuomehin, 2005) and quality and mycotoxins contamination such as aflatoxins (Liu *et al.,* 2006; Mangala *et al.,* 2006), fumonisins (Silva *et al.,* 2000), trichothecenes (Buck and Cote, 1991), zearalenone and DON (Megalla *et al.,* 2007), citrinin (Nguyen *et al.,* 2007), CPA (Trung *et al*., 2001), patulin (Rao *et al*., 2005), ochratoxin A (Makun *et al*., 2007; Reddy *et al*., 2007) and Sterigmatocystin (Engelhart *et al*., 2002). Therefore in the present investigations the seed mycoflora of unmilled rice in relation to season and mycotoxins contamination was studied and discussed in this communication.

MATERIALS AND METHODS

An extensive and intensive survey of Warangal district (Fig 1) for fungi associated with unmilled rice during two crop seasons (Kharif and Rabi) in Warangal, Andhra Pradesh (India) was analysed by employing dilution plate method and agar plate method (ISTA, 1993). The fungi thus isolated were subcultured and identified with the help of standard manuals (Samson *et al.,* 1984; Keith, 1996; Singh *et al.,* 1999; Mathur and Kongsdal, 2003; Leslie and Summerell, 2006). The percentage of incidence, frequency and abundance of individual fungus was calculated using the formulae (Ghiasian *et al.,* 2004). The results are obtained are statistically analysed using SPSS software (Version 17.0).

$$\text{Incidence (\%)} = \frac{\text{No. of colonies of a species in all the plates}}{\text{Total no. of colonies of all the species in all the plates}} \times 100$$

$$\text{Frequency (\%)} = \frac{\text{No. of observations in which a species appeared}}{\text{Total no. of observation}} \times 100$$

$$\text{Abundance (\%)} = \frac{\text{Total no. of colonies of a species in all observations}}{\text{Total no. of colonies in all observations}} \times 100$$

Species of *Aspergillus, Penicillium, Fusarium, Myrothecium* and *Trichothecium* which are known to be mycotoxin producers were screened for production of different mycotoxins by as suggested AOAC (1984).

The mycotoxigenic fungi were grown in 25 ml of rice flour medium at 27±2°C for 15 days. At the end of the incubation period, the culture filtrate was employed for the detection and characterization of different mycotoxins. Liquid-liquid extraction was employed for separation of mycotoxins and different mycotoxins were detected with the help of Thin Layer Chromatography (TLC). On the basis of fluorescence under long wave UV light (360 nm) different mycotoxins were identified. They were further confirmed with help of colour tests and spray reagents as detailed in table 1 (Surekha *et al.,* 2011).

Fig 1: Unmilled rice collected from different pleases of Warangal district

RESULTS

Variety of moulds were associated with the stored paddy during different times of the year which, however, varied with the age and place of collection of sample (Table 2). During Kharif season unmilled rice seeds supported 30 fungal species representing 19 genera, while during Rabi season it supported 22 fungal species representing 14 genera.

The incidence of species of *Aspergillus, Penicillium* and *Fusarium* occurred through out the year (Fig 2). On the other hand, *A. clavatus, A. flavipes, A. ochraceus, Aureobasidium pullulans, Drechslera spicifer, Paecilomyces lilacinus, Nigrospora oryzae* and *Phoma sorghina* could not be detected during Rabi season. *Aspergillus nidulans* and *Myrothecium roridum* were absent in the spermosphere of unmilled rice during Kharif season. The incidence of *A. flavus, A. ustus, A. terreus* and *C. herbarum* were recorded during both the crop seasons. On the other hand, *A. niger, C. lunata* and *A. parasiticus* could be recorded with comparatively more percentage of incidence during Rabi season, while species of *Fusarium* were

recorded with more percentage of incidence during Kharif season.

A. flavus was highest in its percentage of frequency, while *Thielavia terricola* and *Drechslera rostrata* were with least percentage of frequency. *A. terreus, A. niger* and species of *Penicillium* were next highest in their percentage of frequency. Rest of the fungi occurred with intermediate percentage of frequency. *A. flavus* was with highest percentage of abundance, while *C. herbarum, D. rostrata, N. oryzae* and *T. terricola* were with least percentage of abundance. *A. niger, A. terreus,* species of *Fusarium* and *Penicillium* were next highest in their percentage of abundance.

Table 1: Detection of different mycotoxins in unmilled rice on TLC

Name of the toxin	**Solvent system**	**Spray reagent**	**Detection**	
			UV	**Visible**
Aflatoxins	C:A (95:5)	-	bl & g	-
Ochratoxin A	T:Ea:F (6:3:1)	20% $AlCl_3$	bb	-
Patulin	T:Ea:F (6:3:1)	2% phenylhydrazine hydrochloride	-	y
Terreic acid	T:Ea:F (6:3:1)	Quantitative estimation	-	-
Deoxynivalenol	C:M (97:3)	P-anisaldehyde, H_2SO_4, 20% $AlCl_3$	-, ch, bl	y,-,-
Zearalenone	C:M (97:3)	$Ce(SO_4)_2$ 1% in 6N H_2SO_4 2,4-DNP, $FeCl_3$ 3% in ethanol 50% H_2SO_4 in methanol, H_2SO_4, 20% $AlCl_3$	-,-,-,br,ch,bl	br,do,lp,-,-,-
Roridin E	C:M (97:3)	Phloroglucinal	-	pi
Citrinin	T:Ea:F (6:3:1)	$Ce(SO_4)_2$ 1% in 6N H_2SO_4, 2,4-DNP $FeCl_3$ 3% in ethanol	y	y,by,lo
Cyclopiazonic acid	T:Ea:F (6:3:1)	$Ce(SO_4)_2$ 1% in 6N H_2SO_4, 2,4-DNP $FeCl_3$ 3% in ethanol	y	bl,rb,br
Ochratoxin A	T:Ea:F (6:3:1)	2,4-DNP, $FeCl_3$ 3% in ethanol, Ammonia fumes	-,-,bb	y,pb,-
Sterigmatocystin	C:M:A (1:1:1)	20% $AlCl_3$	y	-
Satratoxin H	C:M (97:3)	Phloroglucinal	-	pi
Trichothecenes	C:M (97:3)	Phloroglucinal	-	pi

Solvent system: C=chloroform, A=acetone, M=methanol, T=toluene, Ea=ethyl acetate, F=formic acid.
Detection colours: g=green, bl=blue, y=yellow, bb=bright blue, ch=charring, pb=purple brown, by=brown yellow, lo=light orange, br=brown, do=dark orange, lp=light purple, pi=pink

Statistical analysis: F-Test (ANOVA)

	Kharif season	**Rabi season**
Mean	3.41	3.24
SE	0.942	0.946
Mean ±SE	3.41±0.942	3.24±0.946
p-Value	0.43	
Result	Not Significant	

No variation is found in between two crop seasons. Since there is no significance between the mean incidences of two crops and p value is not <0.05

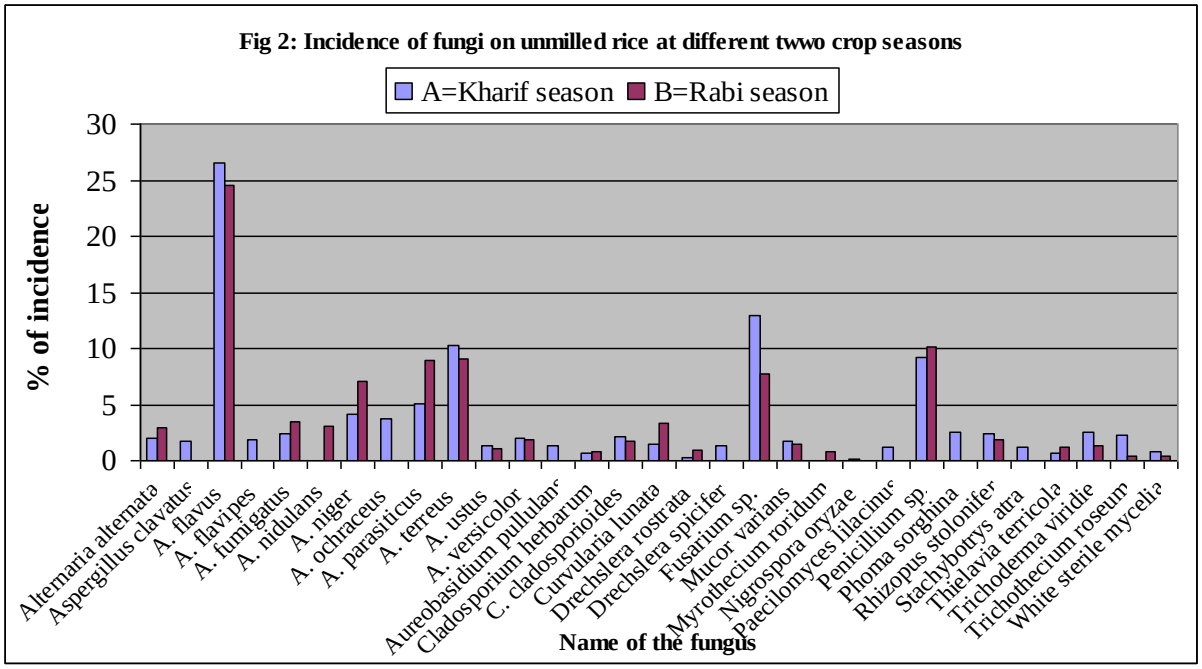
Table 2: Incidence of different fungi on unmilled rice during two crop seasons (Kharif and Rabi)

Name of the fungus	Kharif Season			Rabi Season		
	Incidence (%)	Frequency (%)	Abundance (%)	Incidence (%)	Frequency (%)	Abundance (%)
Alternaria alternata (Fr.) Keissl	1.97	18.3	2.10	2.99	21.1	3.39
Aspergillus clavatus Desm	1.76	14.2	1.47	-	-	-
A. flavus J.H. Friedich Link.	26.6	84.7	20.6	24.5	86.5	26.1
A. flavipes Bain & Sart	1.89	14.2	2.10	-	-	-
A. fumigatus Fresenius	2.36	18.3	3.78	3.41	17.3	2.54
A. nidulans Winter	-	-	-	3.03	19.2	3.39
A. niger Van Tieghem	4.14	40.8	7.78	7.10	30.7	6.36
A. ochraceus Wilhelm	3.77	20.4	3.15	-	-	-
A. parasiticus Spears	5.09	22.4	5.68	8.92	44.2	9.55
A. terreus Thom	10.3	66.5	7.42	9.01	63.5	7.00
A. ustus (Bainier) Thom & Church	1.31	21.7	0.89	1.12	18.0	0.14
A. versicolor (Vuill.)Tiraboschi	1.97	15.8	1.01	1.88	12.6	0.26
Aureobasidium pullulans (De Bary) Arn and herbarum	1.38	14.2	1.10	-	-	-
Cladosporium. herbarum (Pres.) Link.	0.71	1.55	0.11	0.86	1.70	0.19
C. cladosporioides (Fresen.) G.A. de Vries	2.10	16.3	2.31	1.71	7.69	1.69
Curvularia lunata Boedijn	1.43	12.2	1.68	3.36	8.80	4.88
Drechslera rostrata Drechsler	0.23	8.76	0.11	0.98	6.56	0.12
Drechslera spicifer Nelson	1.27	14.2	2.10	-	-	-
Fusarium spp. (F. moniliforme (J. Sheld), *F. oxysporum* (Schlecht), *F. equiseti* (Wollenw)*)*	13.0	55.1	10.1	7.71	34.6	8.49
Mucor varians Povah	1.69	14.2	1.47	1.47	11.5	1.27
Myrothecium roridum Tode	-	-	-	0.76	7.69	0.84
Nigrospora oryzae (Berk.& Br.) Petch	0.13	7.12	0.45	-	-	-
Paecilomyces lilacinus Thom	1.24	10.2	1.05	-	-	-
Penicillium spp. (P. citrinum(Thom), *P. expansum* Link), *P. griseofulvum* (Dierckx))	9.20	46.9	11.3	10.1	42.3	10.8
Phoma sorghina Breda de Haan	2.52	12.2	1.47	-	-	-
Rhizopus stolonifer (Ehrenb.Fr) Vuill	2.38	22.4	2.94	1.82	30.7	3.82
Stachybotrys atra Corda	1.18	9.61	1.27	-	-	-
Thielavia terricola J.C. Gilman & Abbott	0.64	6.12	0.63	1.24	9.61	1.27
Trichoderma viride Pers	2.48	16.3	2.73	1.36	11.5	1.48
Trichothecium roseum (Pers.) Link.	2.28	16.3	2.73	0.43	3.84	0.42
White sterile mycelia	0.76	6.12	0.89	0.45	6.05	0.69

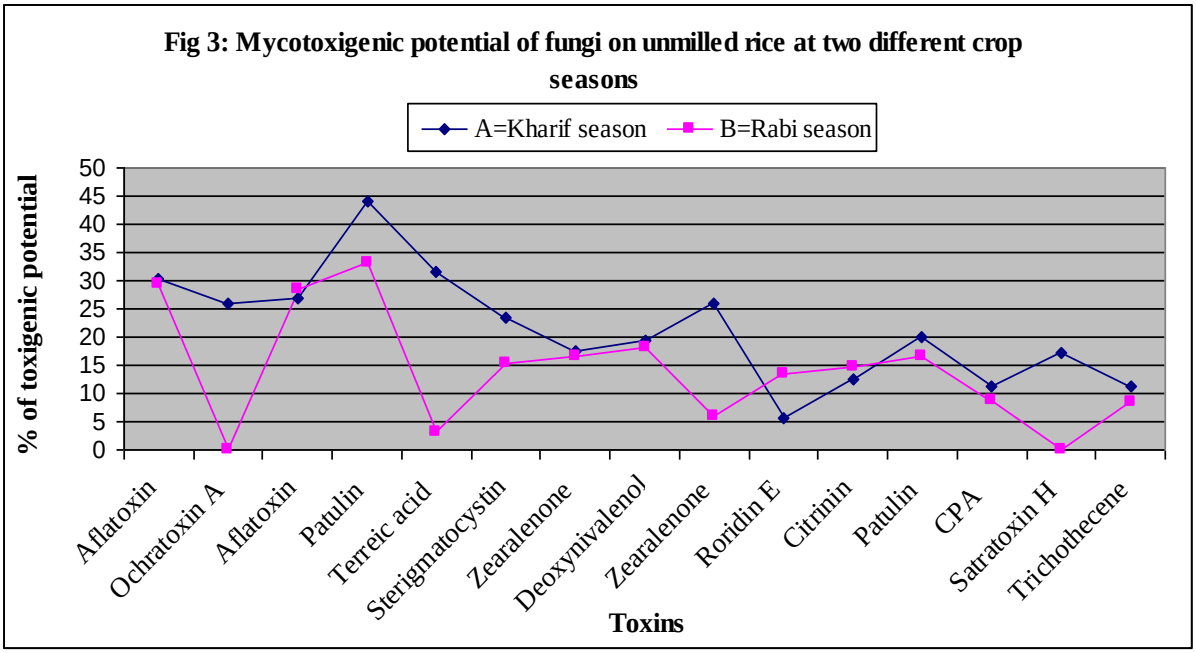
Table 3: Toxigenic potential of unmilled rice in two crop seasons

Name of the fungus	Kharif Season		Rabi Season		Name of the Toxin
	S.S.	T.S. (%)	S.S.	T.S. (%)	
Aspergillus flavus	79	30.3	68	29.4	Aflatoxin
A. ochraceus	23	26.0	-	-	Ochratoxin A
A. parasiticus	48	27.0	49	28.5	Aflatoxin
A. terreus	38	44.0	33	33.0	Patulin
A. terreus	38	31.5	33	3.03	Terreic acid
A. versicolor	17	23.5	13	15.3	Sterigmatocystin
Fusarium moniliforme	29	17.4	24	16.6	Zearalenone
F. oxysporum	31	19.3	22	18.1	Deoxynivalenol
F. equiseti	27	25.9	17	5.88	Zearalenone
Myrothecium roridum	18	5.55	15	13.3	Roridin E
Penicillium citrinum	24	12.5	27	14.8	Citrinin
P. expansum	25	20.0	24	16.6	Patulin
P. griseofulvum	27	11.1	23	8.69	CPA
Stachybotrys atra	29	17.2	-	-	Satratoxin H
Trichothecium roseum	18	11.1	12	8.33	Trichothecene

S.S. = Strains Screened, T.S. = Toxigenic Strains



Rest of the fungi occurred with intermediate percentage of abundance. Almost same trend in the percentage of frequency and abundance was observed during Rabi season. Most of the fungi which are known to be mycotoxigenic elaborated respective mycotoxins (Table 3 and Fig 3). However, percentage of toxigencity varied with the species. About of 30% and 29% of strains of *A. flavus* and *A. parasiticus* respectively elaborated aflatoxins during two crop seasons. *A. ochraceus* which was spotted only during Kharif season, and above 26% strains elaborated ochratoxin A. Similarly 36% of strains *A. terreus* were toxigenic and produced patulin and terreic acid, while 14% of *A. versicolor* strains were positive for production sterigmatocystin. Strains of different species of *Fusarium* elaborated zearalenone and DON. Similarly about 10% and 17% strains of *M. roridum* and *S. atra* were positive for roridin E and Satratoxin H production respectively. *T. roseum* exhibited its potential to elaborate trichothecene.



About 13%, 18% and 9% strains of *P. citrinum*, *P. expansum* and *P. griseofulvum* strains were toxigenic and elaborated citrinin, patulin and CPA respectively. From the present investigations it is clear that most of the spermosphere fungi of unmilled rice stored under faulty conditions were toxigenic and could elaborate different mycotoxins. Kharif season was more favorable for mould infestation and mycotoxin contaminations.

DISCUSSION

The present investigation reveals the wide variations in mycoflora of unmilled rice with place of collection, age of sample and condition of the sample which can be attributed to environmental conditions such as humidity and temperature. Such variation in seed mycoflora has also been reported for different crop seeds studied by them (Fakhrunnisa and Ghaffar, 2006). More mould infestation of unmilled rice collected from flooded area is in agreement with Chary and Reddy (1987) who also recorded many species of *Aspergillus* with comparatively high incidence. Sinha (1987) has also recorded number
aflatoxigenic strains on maize in flooded areas of Bihar. The comparatively more percentage of incidence of moulds as Kharif crop than Rabi crop again may be attributed to warm and humid conditions during rainy season. Sinha (1983) have also recorded more number of fungi on maize crop during Kharif season in Bihar. Comparatively more incidence of fungi during Kharif season may be due humid atmosphere and frequent rain fall. The more incidence of *A. niger*, *R. stolonifer* during Rabi season may be attributed to dry condition prevail during the crop season. No variation is found in between two crop seasons, since there is no significance between the mean incidences of two crops and p value is not <0.05.

CONCLUSION: From the present investigations it can be concluded that unmilled rice is prone to moulds infestation which varied with the environmental conditions and condition of stored seeds. The moulds associated with unmilled rice contributed to seed deterioration and constitutional changes. Some of the fungi associated with unmilled rice were mycotoxingenic and potential of elaborating aflatoxins, citrinin, fumonisins, trichothecenes, ochratoxin A, patulin etc.

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