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Research Article

Fungal infestation and mycotoxins contamination of paddy of flooded areas of Godavari belt region of Telangana State, India

S. Kiran*, M. Surekha and S.M. Reddy

Department of Botany, Toxicology Laboratory, Kakatiya University
Warangal 506 009, Telangana State, India
*sainik244@gmail.com

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Abstract

Fungal infestation and mycotoxin contamination of paddy of flood affected region during (2012-2013) was analysed. In all 34 fungal species representing 16 genera could be isolated. However, the incidence of different fungi varied with the flood affected area and condition of sample. Significant percentage of moulds was mycotoxigenic and elaborated variety and varying amount of mycotoxins. Aflatoxins, patulin, CPA, citrinin, zearalenone, deoxynivalenol and ochratoxin A were detected in one or other paddy sample.

INTRODUCTION

The bulk of paddy (*Oryza sativa* L.) is grown in both extensively and intensively in *kharif* season. During this time frequent and heavy rainfall and flash floods is a common feature and make it ideal substratum for the growth of moulds. Annual loss due to spoilage of high moisture paddy is estimated to be 10-15% of the total production of paddy (Yasan, 1980). Variety of fungi are reported to colonize which include both plant pathogens and mycotoxigenic. The heavy rains during the its harvesting season promote variety of mycotoxigenic moulds (Blandino *et al.*, 2004; Magan and Aldred, 2007). Species of *Aspergillus* are the most predominant fungi on grain (Begum and Samajpati, 2000; Reddy *et al.*, 2009 and Kandhare, 2014; Mogle and Maske, 2012). Waghray *et al.*, (1988) reported number of species of *Aspergillus* on the grains of flood affected paddy collected from standing crop, threshing floors, and storage sites in the Nellore district, Andhra Pradesh. Generally tropical conditions such as high temperature and moisture, un-seasonal rains and flash floods during harvest create ideal condition for mould infestations of food grains (Bhat and Vasanti, 2005). Tulpule *et al.* (1982) reported that heavy rains during the harvesting season favours aflatoxin contamination of paddy. Reddy *et al.* (2004) feel that the paddy crop exposed to heavy rainfall and floods, is vulnerable to infestation by *Aspergillus* sp. (Chary and Reddy, 1987). In the present investigations fungal infestation and mycotoxin contamination of paddy affected by floods was analyzed.

MATERIALS AND METHODS

An extensive and intensive survey of paddy from flood affected Godavari belt region (Fig.1) Telangana State, for the presence of fungi was analysed by blotter technique (ISTA, 1985) and dilution plate method (Waksman, 1922). The fungi growing on seed surface were isolated and identified with the help of standard manuals (Singh *et al.*, 1999; Mathur and Kongsdal, 2003; Leslie and Summerell, 2006). The percentage of incidence, frequency and abundance of individual fungus was calculated.

Species of *Aspergillus, Penicillium, Fusarium, Myrothecium* and *Stachybotrys* which are known to be mycotoxin producers were screened for production of different mycotoxins as described in AOAC (1984). The mycotoxigenic fungi were grown in 25 ml of rice flour medium contained in 250 ml erlenmeyer conical flask at 29±2°C for 15 days. At the end of incubation period, the culture filtrate was employed for the extraction and detection of different mycotoxins. Liquid-liquid extraction was employed by using suitable solvent. They were separated with the help of TLC and identified, on the basis of fluorescence under long wave (360 nm) UV light. They were further confirmed with help of colour tests (Surekha *et al.*, 2011).

Fig. 1: Sampling site of flood affected regions of Godavari belt

RESULTS AND DISCUSSION

From table 1 and Fig. 2 it is clear the paddy that the collected from flooded area of Godavari belt region harboured variety of moulds which, differed in their percentage of incidence with the place and time of collection. In all 34 fungal species representing 16 genera could be spotted on the surface of paddy collected from different flooded regions when dilution plate technique was employed. Species of *Fusarium, A. flavus* and *P. citrinum* were highest in their percentage of incidence. *A. fumigatus, A. nidulans, A. terreus, A. niger, P. islandicum, P. oxalicum and M. roridum* were next highest in their incidence. On the other hand, *C. globosum, A. alternata, N. oryzae, Aureobasidium pullulans* and *D. spicifer* were with low percentage of incidence. The degree of infestation by *Fusarium verticillioides* (20.8%), *A. flavus* (15.3%), *F. equiseti* (13.6%) *F. oxysporum* (8.33%) and *F. heterosporum* (4.47%) and *F. semitectum* (3.84%) was in decreasing order in the flood affected regions.

The percentage of frequency of *F. equiseti* (76.8%), followed by *F. verticillioides* (69.8%), *F. oxysporum* (59.6%) and *F. solani* (44.5%). *A. flavus* (42.2%), *F. heterosporum* (42.1%) and *P. citrinum* (38.6%) were high in their percentage of frequency. On the other hand, *M. variotii* and *T. viride* (3.25%), *C. globosum, D. spicifer, P. variotii* (1.75%), *N. oryza* and *R. stolonifer* (1.25%) were low in their percentage of frequency. Rest of the fungi occurred with intermediate percentage of frequency.

F. verticillioides was with highest percentage of abundance followed by *A. flavus, F. equiseti, F. oxysporum, P. citrinum* and *A. terreus*. On the other hand, *A. niger, A. fumigatus, A. nidulans, F. heterosporum, F. semitectum, A. parasiticus, F. solani, P. islandicum, Myrothecium roridum, P. griseofulvum, P. oxalicum, A. ochraceus, Mucor varians, Drechslera spicifer, Paecilomyces variotii, Rhizopus stolonifer, Nigrospora oryzae* and *A. alternata* were with least percentage of abundance. Rest of the fungi were associated with intermediate percentage of abundance.

Table 1 : Incidence, frequency and abundance of fungi on paddy collected from flood affected areas of Godavari belt

Name of the fungi	Percentage of incidence	Percentage of frequency	Percentage of abundance
Alternaria alternata	0.23	5.42	0.27
A. tenuissima	0.34	5.13	0.31
A. flavipes	0.86	9.25	1.65
A. flavus	15.3	42.2	7.82
A. fumigatus	3.62	32.5	4.21
A. japonicus	1.11	8.25	1.19
A. nidulans	2.42	23.2	3.81
A. niger	1.50	21.7	4.94
A. ochraceus	0.33	10.5	2.17
A. parasiticus	0.88	17.2	3.34
A. terreus	1.24	30.5	5.26
Aureobasidium pullulans	0.21	4.50	0.92
Chaetomium globosum	0.25	1.75	0.73
Cladosporium cladosporioides	0.82	16.5	1.75
Curvularia lunata	0.86	11.7	1.25
Drechslera spicifera	0.02	1.75	0.73
Fusarium equiseti	13.6	76.8	7.04
F. verticillioides	20.8	69.8	9.76
F. oxysporum	8.33	59.6	5.82
F. heterosporum	4.47	42.1	3.94
F. semitectum	3.84	36.5	3.84
F. solani	2.69	44.5	3.16
F. paladoroseum	1.24	33.6	1.61
Mucor varians	0.91	3.25	0.82
Myrothecium roridum	1.27	14.2	2.35
Nigrospora oryzae	0.22	1.25	0.41
Paecilomyces variotii	0.51	1.75	0.54
Penicillium citrinum	5.41	38.6	5.75
P. griseofulvum	1.15	11.2	2.52
P. islandicum	1.48	11.5	3.07
P. oxalicum	1.33	9.75	2.24
Rhizoctonia solani	0.66	5.50	1.46
Rhizopus stolonifer	0.55	1.25	0.54
Trichothecium viride	0.76	3.25	1.09
Sterile mycelium	0.44	7.25	2.47

Toxigenic potential of different fungi isolated from flood affected paddy was determined and the results are presented in Table 2. Many fungi colonizing paddy seeds affected by floods were toxigenic. However, the degree of mycotoxicity varied with the fungus. Out of 53 and 21 isolates of *A. flavus* and *A. parasiticus* screened, 15 and 12 respectively were positive for aflatoxins production. Only 2 isolates of *A. nidulans* were positive for sterigmatocystin when 26 isolates were screened. Out of 32 isolates of *A. terreus* screened, 5, 6 and 3 isolates elaborated terreic acid, patulin and territrem B respectively. Six isolates of *A. ochraceus* elaborated ochratoxin-A when 28 isolates were screened. Reddy *et al.* (2007) have reported *A. ochraceus* infestation in forty six paddy samples collected from flooded affected areas. *A. ochraceus* could be spotted in the seed samples exposed to rain was 35.5% at ArundathinagerTripura) and 28.5% at Aduthurai (Tamil Nadu).

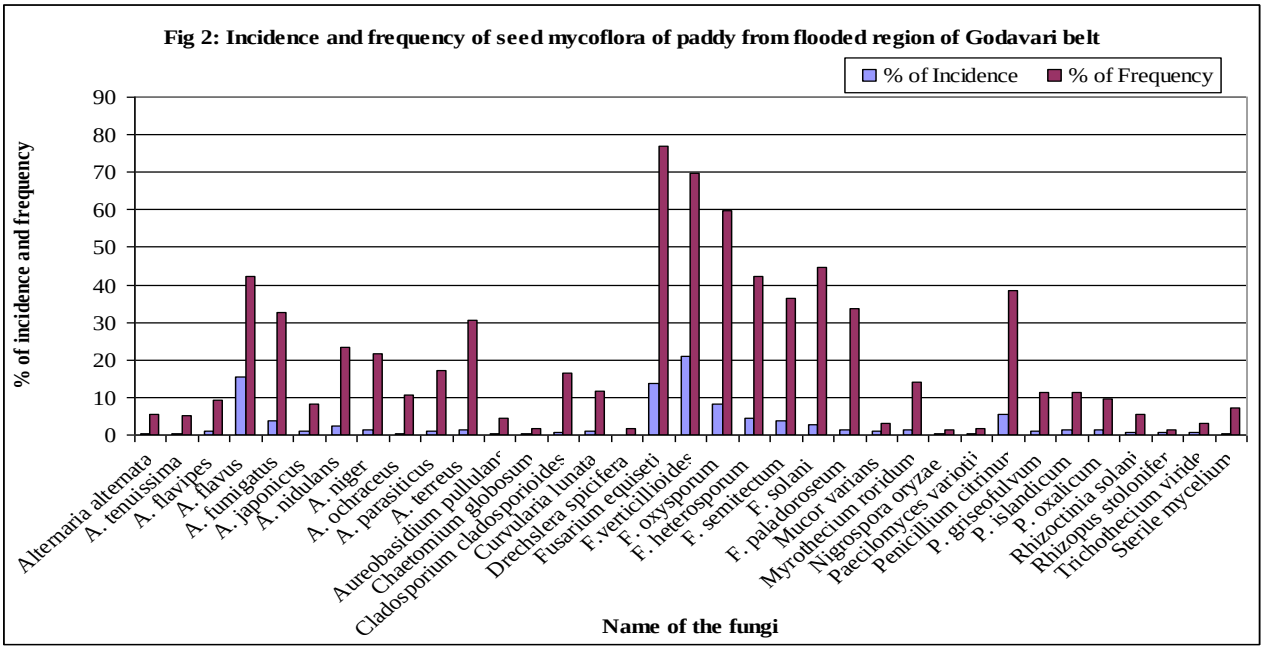

Table 2: Mycotoxigenic potential of fungi isolated from paddy of flood affected areas of Godavari belt

Name of the fungi	No. of strains screened	No. of positive strains	% of incidence	Name of the toxin
Aspergillus flavus	53	15	28.3	Aflatoxin
A. nidulans	26	2	7.69	Sterigmatocystin
A. parasiticus	21	12	19.0	Aflatoxin
A. terreus	32	5	15.6	Terreic acid
		6	18.7	Patulin
		3	9.37	Territrem B
A. ochraceus	28	6	21.4	Ochratoxin
Fusarium oxysporum	48	26	54.1	Nivalenol
		11	22.9	Zearalenone
		10	20.8	Fusarenone-X
F. verticillioides	65	41	63.0	Deoxynivalenol
		22	33.8	Zearalenone
F. equiseti	61	37	60.6	Zearalenone
F. solani	28	2	7.14	Zearalenone
F. semitectum	30	5	16.6	HT-Toxin
		2	6.66	Zearalenone
Penicillium citrinum	38	12	31.5	Citrinin
P. griseofulvum	27	6	22.2	CPA
P. oxalicum	21	3	14.2	MPA
P. islandicum	26	2	7.69	Islandicin

(Out of 48 isolates of *F. oxysporum*, 26, 11 and 10 respectively were positive for nivalenol, zearalenone and fusarenone-X production. On the other hand, out of 65 strains of *F. verticillioides* 41 and 22 were positive for production of deoxynivalenol and zearalenone respectively. Zearalenone was elaborated by 37 isolates of *F. equiseti* when 61 isolates were screened.

On the other hand, only two isolates of *F. solani* could elaborate zearalenone when 28 strains were screened. Out of 30 isolates of *F. semitectum*, 5 and 2 were positive for zearalenone and HT-toxin production respectively. Similarly, Megalla *et al*. (2007) have reported contamination of paddy with fusarial toxins. Twelve isolates of *P. citrinum* elaborated citrinin when 38 isolates were screened. Out of 27 isolates of *P. griseofulvum* screened, 6 isolates elaborated cyclopiazonic acid. Mycophenolic acid MPA was elaborated by 3 strains when 21 strains of *P. oxalicum* were screened. Only 2 isolates of *P. islandicum* could elaborate islandicin. Sakai *et al.* (2005), Kiran Saini *et al. (*2012) and Maria Pinciroli *et al*. (2013) have reported that domestic rice samples analysed were contaminated with *P. islandicum.*

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