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TOXIGENIC FUNGI AND MYCOTOXINS IN HARVESTED RICE FODDER SAMPLES OF NORTH TELANGANA REGION

A. Bhagya, K. Satheesh Kumar, S. Kiran and M. Surekha*
Department of Botany, Kakatiya University, Warangal, Telangana

***Corresponding Author Email:** magantirekha@gmail.com

ABSTRACT

Rice (Oryza sativa L.) fodder samples (260) collected from different regions of Telangana were analysed both qualitatively and quantitatively, variety of moulds were associated with rice fodder, which varied with the place and time of collection. In all 65-fungal species representing 25 genera were isolated Aspergillus flavus, A. niger, R. stolonifer and Penicillium spp were associated with all the samples collected. The incidence of fungi varied with place of collection and many of the isolates of A. flavus, A. terreus, Stachybotrys atra, Penicillium citrinum and Fusarium spp were toxigenic and elaborated aflatoxin, terreic acid, satratoxin, citrinin and zearalenone respectively. The significance of occurrence of these toxins is discussed.

KEY WORDS

Rice fodder, aflatoxins, terreic acid and satratoxin

INTRODUCTION

Dairy farming once a means of bare subsistence, now has become an important agribusiness. Millions of farmers use crop residues and natural herbage as feed to the livestock. The availability and type of fodder depends on the available areas, climatic and edaphic factors as well as the socio-economic conditions of the area.

Paddy, maize and sorghum straws left after harvesting of the grains, form the main bulk of livestock feed throughout the country. Though these straws are poor in nutritive value, containing about 3% protein and 40% total digestible nutrients (TDN), these straws along with small quantities of protein supplements are used as a sole feed. It is a usual practice in the country side to store the straw under open conditions. Tropical conditions, harvesting practices, unseasonal rains and high moisture content of straw during baling and open storage promote fungal proliferation in the fodders.

Fungi from mouldy fodders may cause infections, provoke allergic responses and poison with toxic metabolites (Hussein and Brasel, 2001, Williams *et al.,* 2004 and Shukla, 2013). Dairy cattle exposed to many spores from fodders may often develop subclinical diseases (Lanyasunya *et al.,* 2005; Boudra *et al.,* 2007 and Masoero *et al.,* 2007). Ali and Khan (2006) have isolated fungi from out breaks of mycotic abortion in cattle, including species not commonly pathogenic, but most common in mouldy fodders.

Many fungi found in fodders produce toxic metabolites, but a few of their toxins have been identified in fodders on which they grew (Sharma *et.al*, 2010, Dolan *et al.,* 2010) Fungi may also cause animal diseases indirectly in ways that mimic mycotoxicoses. Fungal growth may also deplete the nutritive value and can alter the availability of micronutrients (Kabak *et al.,* 2006). There are several reports of death and mycotoxicoses of livestock (Abidin *et al.,* 2012, De Oliveira *et al*., 2014 and Dell'Orto *et al*., 2015). Hence, an attempt has been made to study the mycoflora associated with rice fodder commonly used in this region.

MATERIALS AND METHODS

Two sixty rice fodder samples were collected from different regions of North Telangana Region. These samples were examined for mycological profile before storage at 20^{0}C. Isolation, enumeration and identification of fungi were made following the standard methodology and manuals (Lislie and Summerell 2006, Mathur and Kongsdal 2003). The fungi associated with samples were isolated using different media (malt extract agar, potato dextrose agar (PDA) and Asthana & Hawkers media). Twenty fungal strains were grown on malt extract agar and PDA, for 7 days at 20^{0}C and examined for production of mycotoxins using TLC technique as suggested by (Hans and Walter 1986) and assessed for their mycotoxin producing potential. Culture filtrate was employed for detection of different mycotoxins Liquid-liquid extraction was employed using appropriate solvent. The extract was concentrated and subjected to TLC separations. The TLC Plates thus developed were observed under long wave UV light (360 mm) and they were further confirmed with help of colour tests and spray reagents (Table.1).

Table:1. Detection of different mycotoxins from fodders

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% phenyllhydrazine hydrochloride	-	y
Terreic acid	T: Ea:F (6:3:1)	Quantitative estimation	-	-
Sterigmatocystin	C:M: A (1:1:1)	20% $AlCl_2$	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, 20% $AlCl_3$	br,ch,bl	br,do,lp,
MPA	T: Ea:F (6:3:1)	*p*-anisaldehyde, 1% $Fecl_3$ in ethanol	Pb, pb	g, lo
Nivalenol	C:M (97:3)	*p*-anisaldehyde, H_2SO_4 20% $Alcl_3$	ch, b	y
Fusarenone-X	C:M (97:3)	20% $Alcl_3$	bl	-
DON	C:M (97:3)	*p*-anisaldehyde, H_2SO_4, 20% $ClCl_3$	ch,bl	y
HT – toxin	C:M (97:3)	20% H_2So_4	bg	-
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
Satratoxin	C:M (97:3)	Phloroglucinal	-	pi

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

RESULTS AND DISCUSSION

Table 2 reveals that varieties of moulds were associated with the rice fodder which varied with the place of collection and time of collection. In all 65-fungal species representing 25 genera were isolated from different samples. The incidence of different fungi varied with the place of collection.

Aspergillus flavus, A. niger, Rhizopus stolonifer and Penicillium spp, were associated with all the samples collected, while *Rhizoctonia solani*, *Melanospora damnsoa,* and *Alternaria tenuissima, Aureobasidium pullulans* were associated with the samples collected from Bhupalpally and Peddapally respectively. *Allescheriella* sp. was isolated from samples collected from Mancherial and Peddapally. Similarly, *Sclerotium rolfsii* was isolated only from samples collected from Warangal and Asifabad *Nigrospora oryzae* was traced in samples collected from Peddapally and Bhadradri Kothagudem, while *Chaetomium brasiliense* was recorded only in samples collected from Warangal, Nirmal and Peddapally. While *Curvularia ovoidea* was recorded in samples of Khammam and Peddapally *Curvularia tuberculata* was recorded in samples of Nirmal, and Jagtial, *Neurospora crassa* was recorded in

samples of Nirmal, Mancherial and Jagtial. Species of *Fusarium* could not be recorded in samples collected from Asifabad and Rajanna Sircilla, while *Alternaria alternate* was absent in samples collected from Karimnagar, Adilabad, Peddapally and Bhadradri Kothagudem. *Chaetomium globosum* could not be detected in samples collected from Adilabad and Mancherial. *Cladosporium cladosporioides* could not be traced in samples of Warangal, Karimnagar, Mancherial and Jagtial, while species of *Drechslera* (*D. spicifer* and *D. rostrata*) were absent in samples of Asifabad. *Myrothecium roridum* was absent in samples collected from Adilabad, Nirmal, Asifabad and Jagtial. *Memnoniella echinata* could not be traced in samples collected from Karimnagar, Nirmal, Asifabad, Mancherial, Peddapally and Bhadradri Kothagudem. While *S. atra* could not be spotted in samples of Khammam, Adilabad, Asifabad and Peddapally. Similarly, *Trichothecium roseum* was not found in samples collected from Karimnagar, Adilabad, Nirmal and Asifabad.

The percentage of incidence of *A. flavus* was highest in almost all the samples except in samples collected from Rajanna sircilla, Bhadradri Kothagudem and Peddapally in which the incidence of *A. niger* was highest. Species of *Penicillium* were next highest in their incidence. The population of *R. stolonifer* was also quite high in all the samples collected. Surekha *et al.* (2011) and Rekha *et al.* (2015) have also recorded variety of fungi from different fodder samples analysed by them.

The mycoflora associated with the rice fodder differed significantly with the isolation method. Interestingly, when mycoflora of rice fodder was analysed by dilution plate method. *Allescheriella sp. Aureobasidium pullulans, Melanospora damnosa, Cladosporium sphaerospermum, Rhizoctonia solani, Nigrospora oryzae* and *Sclerotium rolfsii* could not be detected. On the other hand, fungi like *Chaetomium brasiliense, Curvularia ovoids, Myrothecium verrucaria, Neurospora crassa*, *Syncephalastrum racemosum* and *Phoma sorghina* which could be detected in dilution plate method were not traced in agar plate method. *A. alternate, A. flavus, A. niger, Chaetomium globosum, C. cladosporioides, C. pallescens, D. spicifer, R, stolonifer*, species of *Fusarium* and *Penicillium* were detected by both the methods employed with significant percentage of incidence and frequency. The percentage of incidence and abundance were different in different methods employed. In general, the fungi detected were more both qualitatively and quantitatively when dilution plate method was employed. The detection of few fungi by agar plate method may be attributed to the lack of nutrients in the medium or some of the fungi specific to straw might have dominated by other sensitive fungi. Thus, it is clear that more than one technique should be employed in order, to get a complete picture of the microflora of fodders.

From table 3 it is evident that a considerable number of fungi associated with rice fodder elaborated various mycotoxins. However, the incidence and toxigenic potential of different fungi varied. Out of 238 isolates of *A. flavus* screened, 160 strains elaborated one or other aflatoxins. None of the isolates elaborated all the four aflatoxins. Fifteen isolates of *A. nidulans* elaborated sterigmatocystin, when 53 isolates were screened. Out of 78 isolates of *A. terreus*, only 18 were positive for patulin, while all of them elaborated terreic acid. Ochratoxin A was elaborated by 2 isolates out of 36 isolates. Out of 32 isolates of *P. citrinum* screened, only 18 were positive for citrinin. Fifteen isolates of *P. griseofulvum* elaborated CPA out of 48 strains screened. Only four isolates of *P. oxalicum* were positive for production of MPA. None of the isolates of *P. islandicum* and *P.funiculosum* were able to elaborate any toxin. Species of *Fusarium* elaborated most of the fusarial toxins. However, the type of toxin and the toxigenic potential of different species varied. Out of 38 isolates of *F. oxysporum* screened, 20 and 8 isolates elaborated zearalenone and nivalenol respectively. When 58 isolates of *F. moniliforme* were screened 32, 8 and 5 were positive for zearalenone, fusarenon-x and deoxynivalenol respectively. Similarly, 8, 2 and 2 isolates of *F. semitectum* elaborated zearalenone, HT-toxin and nivalenol respectively when 16 isolates were screened. None of the isolates of *F. solani* were positive for *zearalenone*. Out of 58 strains of *S. atra* screened, 48 elaborated *satratoxin*.

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Table 2. Mycoflora of rice fodder

Name of the fungi	A	B	C	D	E	F	G	H	I	J	K	L	% of frequency	% of abundance
Allescheriella	-	-	-	-	-	-	0.23	-	-	0.8	-	-	16.66	0.21
Alternaria alternate	1.29	0.82	-	-	2.89	1.82	1.2	0.27	-	1.25	-	0.6	75	2.34
A. tenuissima	-	-	-	-	-	-	-	-	0.83	-	-	-	8.33	0.12
Asprgillus flavipes	0.91	-	-	-	-	1.02	2.08	1.32	-	-	-	-	83.33	1.86
A. flavus	20.21	20.32	16.65	22.3	28.0	17.35	28.32	18.3	6.03	10.21	3.25	1.8	100	22.5
A. nidulans	4.26	3.01	2.02	-	-	-	10.24	1.75	2.78	-	4.23	-	58.33	12.30
A. niger	10.49	12.86	6.6	13.05	6.08	11.28	7.34	8.44	7.32	4.32	6.83	11.02	100	18.64
A. ochraceus	3.06	-	2.02	2.02	3.24	3.24	3.38	3.38	-	-	-	-	58.33	5.31
A. sydowii	0.86	-	-	-	1.02	-	0.34	-	-	0.21	-	-	33.33	3.61
A. terreus	3.32	4.54	2.63	6.82	0.85	3.62	16.24	9.26	-	-	1.38	-	75	14.36
A. ustus	-	0.82	-	-	-	-	0.21	-	-	-	-	-	16.66	1.35
A. versicolor	-	-	3.88	-	-	-	0.21	-	0.31	-	1.65	-	33.33	2.1
Aureobasidium pullulans	-	-	-	-	-	-	-	-	0.1	-	-	-	8.33	0.13
Basidiomycetes	-	-	-	0.8	-	--	-	-	-	-	-	-	8.33	0.13
Chaetomium globosum	6.02	7.82	5.97	-	9.34	4.62	-	1.52	2.38	1.38	2.02	0.38	83.33	11.4
C. brasiliense	-	1.08	-	-	-	-	-	-	-	0.89	-	-	16.66	3.12
Cladosporium cladosporioides	-	5.34	-	3.68	6.08	5	-	2.78	1.62	-	6.02	3.82	66.66	5.62
C. sphaerospermum	-	-	0.38	-	-	-	-	0.48	-	1.02	-	062	33.33	1.85
Curvularia clavata	1.13	-	-	3.28	-	3.24	-	1.19	-	-	-	0.05	41.66	2.1
C. ovoidea	-	1.38	-	-	-	-	-	-	0.38	-	-	-	50	3.14
C. lunata	1.28	3.08	-	-	4.08	-	1.38	2.78	0.38	0.25	-	-	50	1.28
C. pallescens	2.24	-	4.64	7.32	-	-	4.32	-	1.2	0.92	6.63	0.92	66.66	1.63
C. tuberculata	-	-	-	-	3.8	-	-	-	-	0.92	-	-	16.66	1.24
Drechslera halodes	-	-	-	-	-	-	-	0.62	-	-	0.81	-	16.66	1.24

D. spicifer	0.21	0.05	8.62	0.63	6.83	-	1.1	1	2.02	0.63	1.82	2.1	58.33	6.31
D. rostrata	0.36	2.05	0.03	1.82	0.12	-	0.78	2	1.08	0.92	1.8	2.03	50	3.21
Fusarium spp. (F. oxysporum, F. moniliforme, F. Semitectum, F. solani, F. equiseti, F. pallidoroseum)	12.35	7.03	11.08	3.62	4.04	-	8.93	0.47	5.32	9.98	9.98	-	83.33	15.61
Melanospora damnosa	-	-	-	-	-	-	-	0.25	-	-	-	-	6.35	1.08
Memnoniella echinata	1.35	2.8	-	1.62	-	-	-	1.01	-	4.13	-	1.83	58.33	2.25
Myrothecium roridum	3.58	0.6	0.68	-	-	-	0.25	1.81	0.02	-	0.82	1.01	58.33	1.38
M. verrucaria	-	0.21	-	-	-	-	-	-	-	0.02	-	-	16.66	0.18
Neurospora crassa	-	-	-	-	0.82	-	0.1	-	-	0.03	-	-	25	0.23
Nigrospora oryzae	-	-	-	-	-	-	-	-	0.02	-	1.02	-	16.66	0.15
Paecilomyces varioti	1.48	-	-	-	1.02	-	-	-	1.81	-	-	-	25	1.63
Penicillium spp (P. funiculo Penicillium spp. (P. funiculosum, P. oxalicum, P. citrinum, P. islandicum. P. griseofulvum, P. strialifon, P. aurantiogriseum)	13.32	14.86	16.03	18.62	11.21	13.32	6.04	13.01	5.69	4.13	2.38	6.05	100	15.65
Phoma sorghina	0.25	-	0.38	1.03	-	1.82	-	-	0.13	-	-	-	41.66	3.24
Rhizoctonia solani	-	-	-	-	-	-	-	0.05	-	-	-	-	8.33	0.02
Rhizopus stolonifer	5.28	6.68	10.82	9.38	5.05	6.82	4.13	5.69	2.78	3.32	6.03	2.08	100	8.54
Sclerotium rolfsii	0.75	-	-	-	-	1.02	-	-	-	-	-	-	16.66	1.28
Syncephalastrum racemosum	-	0.8	-	0.62	-	-	0.03	0.12	-	-	-	-	33.33	2.35

Stachybotrys atra	3.24	-	4.28	-	2.62	-	1.22	0.53	-	1.35	0.82	1.03	83.33	1.64
Trichoderma viridie	1	-	-	-	-	-	1.9	0.18	-	1.8	-	-	33.33	1.03
Trichothecium roseum	2.02	1.08	-	-	-	-	1.46	1.79	2.02	0.81	0.82	12	83.33	2.06

A=Warangal, B=Khammam, C=Karimnagar, D=Adilabad, E=Nirmal, F=Asifabad, G=Mancherial H=Bhupalpally, I=Peddapally, J=Jagtial, K=Bhadradri Kothagudem and L=Rajanna sircilla

Table.3. Toxigenic potential of fungi associated with rice fodder samples

Name of the fungus	Number of strains screened	Number of toxin producing strains	% of incidence	Name of the toxin
Aspergillus flavus	238	160	80.00	Aflatoxins
A. nidulans	53	15	28.30	Sterigmatocystin
A. terreus	78	78	100.00	Terreic acid
		18	23.20	Patulin
A. ochraceus	36	2	12.50	Ochratoxin
Penicillium citrinum	32	18	56.25	Citrinin
P. griseofuluvm	48	15	31.25	CPA
P. oxalicum	53	4	7.69	MPA
Fusarium oxysporum	38	8	21.05	Nivalenol
	38	20	55.17	Zearalenone
F. moniliforme	58	8	13.79	Fusarenone – X
		5	8.62	Deoxynivalenol
		32	55.17	Zearalenone
F. solani	22	--	--	Zearalenone
F. semitectum	16	2	12.50	HT – Toxin
		2	12.50	Nivalenol
		8	50.00	Zearalenone
Stachybotrys atra	58	48	82.75	Satratoxin

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***Corresponding Author:**
M. Surekha*
Email: magantirekha@gmail.com