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Incidence and Mycotoxigenic Fungi Associated with Cattle Feeds in North Telangana Region, India

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Mycoflora and mycotoxins contamination of different cattle feed samples of north Telangana region was analysed both quantitatively and qualitatively. The feed samples were highly infested by *A. flavus*, *A. terreus* and *Penicillium* species. The incidence of the rest of the fungi varied with the type of sample and the place of collection. The toxigenic potential of different mycotoxigenic fungi varied with substratum. The strains of *A. flavus*, *A. terreus* and *Penicillium citrinum* elaborated aflatoxin, patulin, terreic acid and citrinin respectively. The significance of occurrence of mycotoxigenic fungi in different fungi in different cattle feed is discussed.

Introduction

Microscopic fungi and their metabolites of mycotoxins are often found as contaminants in agricultural products before or after harvest as well as during transportation or storage. Animal feeds are routinely subject to contamination from diverse sources, including environmental pollution and activities of insects and microbes. Feed toxins include compounds of both plant and microbial origin, among microbes, fungal contamination is a concerning issue in animal nutrition products for human. There are consistent reports of worldwide contamination of feeds with fungi and their spores. *Aspergillus* is the predominant genus in dairy and other feeds (Mohammad *et al.*, 2015) other species include *Penicillium*, *Fusarium* and *Alternaria*, which are also important contaminants of cattle feeds and cereal grains (Alonso *et al*, 2013 and Surekha *et al.*, 2017) when cereal grains and animal feed are colonised by moulds there is a significant risk of contamination with the secondary metabolites of these fungi (Goncalves *et al.*, 2015). These fungi contaminate wide range of agricultural products mainly cereal gains, during pre and

post-harvest stage (Khoury *et al.,* 2011). Rafai *et al.,* (2000), Eriksen and Pettersson (2004), Wu *et al.,* (2007), Adamse *et al.,* (2012) and Elisabeth *et al.,* (2012) have discussed various aspects of the incidence of mould infestation and mycotoxin contamination of cattle feed and significant health effects on farm animals. Presence of mycotoxins in feeds may also decrease feed intake and affect animal performance. In addition to possible presence of toxic residues in edible products (milk, meat etc) may have harmful effects on human health. Contamination of cattle feeds and feed ingredients with mycotoxins has been reported from different parts of the world (Reddy *et al.,* 2000; Sultana and Hanif, 2009 and Surekha *et al.,* 2017). In the present investigations detection of fungi in different cattle feeds which are commonly used in feed formulation in the Telangana region which is warm and humid conditions prevail during most part of the year.

Materials and Methods

Habitual survey of cattle feeds (Green gram, Legume mixture, Mixed Feed and Commercial feed) which generally used as feed ingredients of livestock in the Telangana regions was conducted. The details of the condition of samples, age of samples and place of collection were recorded carefully. The sample was subjected to analysis of mycoflora by dilution plate method (Waksman, 1922). The fungi appeared were isolated, purified and identified with the help of standard manuals (Singh *et al.,* 1999, Mathur and Kongsdal, 2003 and Lislie and Summerel, 2006). The fungal isolated were grown in Czapek's dox medium (Sucrose30g; NaNo3-3.0g; KH_2PO_4-1g; KCl-0.5g; $FeSO_4$ 7 H_2O-0.01g; $MgSO_4$ $7H_2O$-0.5g; distilled water-1000ml) and assessed for their mycotoxin producing potential. Old culture was harvested and culture filtrate was employed for detection of different mycotoxin Liquid - liquid extraction was employed using appropriate solvent. The extractant was concentrated and subjected to TLC separations. The TLC plates thus developed were observed under long wave UV light (360 nm) and they were further confirmed with help of colour tests and spray reagents.

Results and Discussion

Sixteen fungal species representing 10 genera were recorded in green gram feed samples (Table 1). *Aspergillus flavus, A. niger A. nidulans, A. terreus, Fusarium* and *Penicillium* were associated with all the samples collected from different North Telangana Region. While *Paecilomyces variotii, Rhizoctonia solani* and *Syncephalastrum racemosum* were associated only with samples collected from Karimnagar, Peddapalli and Asifabad respectively. *Drechslera nodulasa, D. spicifera, Memnoniella echinata* and *Rhizopus stolonifer* were recorded in samples collected from only a few places. The percentage of incidence of *A. flavus* was highest in most of the samples.

Aspergillus flavus, A.terreus and *Penicillium* were with highest percentage of frequency, while *Paecilomyces variotii, Rhizoctonia solani and Syncephalastrum racemosum* was with lowest percentage of frequency. Percentage of abundance of *A. flavus* was highest followed by *A. terreus* and species of *Penicillium, Syncephalastrum racemosum* and *Memnoniella echinata* were lowest in their abundance. (Interestingly, thought the incidence and frequency of *Syncephalastrum racemosum* was low, its abundance was more). In general incidence of *A. flavus* and *A. terreus* was more. Similarly specie of *Penicillium* was also considerably high in their percentage of incidence and can be much significance in the livestock health.

Eighteen Fungal species respecting six genera were recorded in legume mixture feed samples collected from different north Telangana region. *A. flavus, A. niger, Cladosporium herbarum* and *Penicillium* were associated with all the samples, while *A. ochraceus, Cladosporium herbarum* and *Paecilomyces variotii* was isolated only from samples of Warangal and Peddapalli. *A. flavipes, A. nidulans, A. terreus* and *Syncephalastrum racemosum* were recorded in samples of only some places. The incidence of *A. flavus* was highest followed by *A. niger* species of *C. lunata and Penicillium* in a descending order. *A. flavus, A. niger, C. lunata* and species of *Penicillium* were with highest percentage of frequency, while it was least with *A. ochraceus, Cladosporium herbarum* and *Paecilomyces variotii*. *A. flavus* followed by species of *Fusarium, A. niger, Penicillium* and *A. terreus* were with highest percentage of abundance on the other hand, *Paecilomyces variotii* and *Cladosporium herbarum* occurred with lowest percentage of abundance.

Mixed feed samples collected from different region of Telangana supported fifteen fungal species representing six genera. *A. flavus, A. terreus, Fusarium* and *Penicillium* were recorded in all the samples, while *A. nidulans* and *Drechslera nodulasa* were associated only with samples collected Adilabad, Asifabad, Peddapalli and Jagityal respectively. *Alternaria alternate, A. flavipes, A. ochraceus* and *Rhizopus stolonifer* were recorded in samples of only some places. The incidence of *A. flavus* followed by species of *Fusarium* and *Penicillium* was highest in all the samples collected. *A terreus* was next dominant fungus. The highest incidence of *A. flavus* may pose health hazard to livestock and in turn to man. *A flavus* was highest in percentage of frequency and abundance followed by *A. terreus* and species of *Fusarium* and *Penicillium* in a descending order, while it was least with *Alternaria alternate*.

Fifteen fungal species representing 5 genera were recorded in commercial feed collected from different north Telangana region. *A. flavus A. niger* and species of *Fusarium* and *Penicillium* were associated with almost all the samples collected from different places of Telangana. *A. ochraceus* was isolated only from samples of Bhupalpally respectively. *A. flavipes, A. nidulans, A. terreus* species of *Cladosporium* and *Trichoderma* were recorded in samples of only some places. The percentage of frequency and abundance of *A. flavus* followed by species of *Fusarium, Penicillium* and *A. niger* was high, while *A. ochraceus* and *A. flavipes* were least.

From the present investigations it is clear that cattle feeds are ideals substrates for mould infestation commercial feed being highly preferred, while green gram feed is least preferred, substratum for *A. flavus* infestation legume mixture and mixed feeds are preferred substrates for the growth of *Penicillium* species, commercial and mixed feed was the best substrate for the growth of *Fusarium* species. The incidence of other fungi in different cattle feed samples was not only inconsistent but also sporadic.

The toxigenic potential of different fungi varied with the substratum (Table 2). Among the fungi associated with green gram feed only *A. flavus A. terreus* and *Penicillium citrinum* were able to elaborate aflatoxins, patulin, terreic acid and citrinin, respectively.

About 68% of *A. flavus* isolated were positive for aflatoxins production, while 66% of isolates of *P. citrinum* were positive for citrinin production. None of the isolates of *P. griseofulvum* and *A. nidulans* were capable of producing cyclopiazonic acid (CPA) and sterigmatocystin, respectively.

Table.1 Mycoflora of cattle feeds

Name of the fungus	Percentage of Incidence										
	A	B	C	D	E	F	G	H	I	Percentage of Frequency	Percentage of abundance
Green gram feed											
Alternaria alternate	0.80	0.60	-	8.00	2.60	1.80	N	N	N	83.30	10.50
A. flavus	38.20	30.30	38.60	20.30	35.20	21.30	N	N	N	100.00	28.40
A. nidulans	3.80	4.20	6.30	-	-	8.70	N	N	N	66.60	6.30
A. niger	12.40	2.10	10.40	28.60	11.30	-	N	N	N	83.30	9.60
A. terreus	20.30	28.20	25.80	36.20	38.30	32.20	N	N	N	100.00	25.80
Curvularia lunata	2.30	2.80	-	-	3.60	4.40	N	N	N	66.60	8.80
Drechslera nodulasa	-	-	2.40	-	4.80	-	N	N	N	33.30	6.50
D. spicifera	-	-	-	2.00	-	2.80	N	N	N	33.30	1.80
Memnoniella echinata	-	2.20	-	0.80	-	-	N	N	N	33.30	0.60
Paecilomyces variotii	1.80	-	-	-	-	-	N	N	N	16.60	3.60
Penicillium spp (P. citrinum, P. viridicatum, P. islandicum)	16.30	12.20	11.80	3.20	2.50	15.80	N	N	N	100.00	18.30
Rhizopus stolonifer	5.10	17.20	-	-	-	18.00	N	N	N	66.60	14.30
Rhizoctonia solani	-	-	-	1.70	-	-	N	N	N	16.60	0.80
Syncephalastrum racemosum	-	-	-	-	1.70	-	N	N	N	16.60	0.40
Legume mixture feed											
Aspergillus flavipes	-	3.60	-	6.30	N	4.10	N	N	N	60.00	8.30
A. flavus	36.50	20.30	28.20	30.60	N	38.60	N	N	N	100.00	25.60
A. nidulans	-	3.80	2.50	1.60	N	-	N	N	N	60.00	11.30
A. niger	10.30	15.30	12.50	10.30	N	10.20	N	N	N	100.00	20.30
A. ochraceus	-	1.20	-	-	N	-	N	N	N	20.00	6.50
A. terreus	6.20	-	8.30	12.20	N	4.10	N	N	N	80.00	15.30
Cladosporium herbarum	-	0.20	-	-	N	-	N	N	N	20.00	0.80
C. lunata	6.30	1.10	4.50	4.00	N	6.80	N	N	N	100.00	2.90
Fusarium spp. (F. moniliforme, F.oxysporum, F solani, F. equiseti)	21.20	18.50	19.50	-	N	18.20	N	N	N	80.00	23.60
Paecilomyces variotii	-	-	-	1.30	N	-	N	N	N	20.00	0.30
Penicillium spp. (P. citrinum, P. viridicatum, P. funiculosum, P. griseofulvum,)	18.50	26.80	24.20	28.30	N	11.20	N	N	N	100.00	18.60
Syncephalastrum racemosum	1.20	3.50	-	-	N	1.80	N	N	N	60.00	1.40
Mixed Feed											
Alternaria alternate	-	-	1.20	-	4.00	0.80	-	-	N	37.50	0.80
Aspergillus flavipes	-	8.80	-	1.60	-	-	3.40	-	N	37.50	1.80
A. flavus	28.30	26.30	28.80	35.60	25.00	24.00	25.80	22.50	N	100.00	33.30
A. nidulans	-	-	13.20	-	7.80	-	-	-	N	25.00	1.80
A. ochraceus	10.00	-	-	1.20	-	-	8.50	13.60	N	50.00	3.60
A. terreus	13.50	22.80	12.20	15.60	15.80	16.00	14.20	17.50	N	100.00	11.30
Drechslera nodulasa	-	-	-	5.60	-	6.20	-	-	N	25.00	1.20

Fusarium spp. (F. moniliforme, F. Oxysporum, F solani, F. equiseti)	10.30	12.50	15.60	22.40	26.30	24.40	18.60	12.30	N	100.00	26.50
Penicillium spp. (P. citrinum, P. viridicatum, P. griseofulvum,)	3.30	29.60	28.00	13.60	21.10	24.80	20.80	25.10	N	100.00	20.30
Rhizopus stolonifer	7.70	-	-	4.40	-	3.80	10.50	9.00	N	62.50	6.50
Commercial feed											
Aspergillus flavipes	2.60	-	-	8.30	-	-	2.80	-	-	33.30	0.80
A. flavus	20.30	25.80	26.80	10.80	35.60	25.80	32.50	20.20	14.80	100.00	38.60
A. nidulans	-	3.20	1.80	-	1.60	2.50	-	3.80	18.20	66.66	11.30
A. niger	11.30	12.80	13.30	10.80	6.30	11.60	3.80	18.60	12.80	100.00	10.30
A. ochraceus	-	-	-	-	-	-	-	1.20	-	11.10	0.20
A. terreus	-	-	1.20	-	2.50	-	8.80	-	-	33.30	1.50
Cladosporium herbarum	1.60	-	2.30	-	-	10.90	-	-	4.60	44.40	2.70
Fusarium spp. (F. moniliforme, F. oxysporum, F solani,)	26.40	28.20	15.80	25.60	23.00	25.00	24.50	22.90	26.60	100.00	30.30
Penicillium spp. (P. citrinum, P. oxalicum, P. griseofulvum,)	28.20	16.40	32.50	32.60	20.60	15.00	21.30	25.00	28.00	100.00	18.30
Trichoderma viride	1.80	-	-	3.40	-	-	3.50	-	3.60	44.40	8.20

A= Karimnagar, B= Warangal, C= Adilabad, D= Peddapalli, E= Asifabad, F= Jagityal, G= Nirmal, H= Bhupalpally, I= Mancherial

Table.2 Toxigenic potential of fungi of cattle feeds

Name of the fungus	**Green gram feed**		**Legume mixture feed**		**Mixed feed**		**Commercial feed**		**Name of the Toxin**
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**	
Aspergillus flavus	38	68	43	74	40	70	33	84	Aflatoxins
A. nidulans	20	-	18	44	12	25	15	53	Sterigmatocystin
A. ochraceus	-	-	-	-	-	-	8	25	Ochratoxin A
A. terreus	34	35	21	-	24	8	12	-	Patulin
A. terreus	34	58	21	28	24	75	12	83	Terreic acid
Penicillium citrinum	12	66	8	50	25	40	18	44	Citrinin
p. griseofulvum	4	-	-	-	-	-	12	33	CPA
p. islandicum	-	-	6	16	8	12	-	-	Islandicin
Fusarium oxysporum	-	-	4	75	3	-	13	30	Zearalenone

A = Number of strains screened, B = percentage of Incidence

The fungi associated with legume mixture were potential of elaborating mycotoxins. However, the percentage of toxigenic isolates varied. None the isolates of *A. terreus* could elaborate patulin out of 4 isolates of *F. oxysporum*, 3 were positive for zearalenone production one isolate out of 6 strains of *P. islandicum*, screened elaborated islandicin. Interestingly mixed feeds and commercial feeds proved to be more susceptible to mould infestation and supported elaboration of various mycotoxins that about 70%, 25% and

40% of isolates of *A. flavus*, *A. nidulans* and *P. citrinum* respectively of mixed feeds were toxigenic. Two out 3 isolates of *F. oxysporum* screened elaborated zearalenone. Isolates of *P. islandicum* (12%) elaborated islandicin respectively. Similarly commercial feeds were also ideal substrates for toxigenic moulds. About 84% of *A. flavus* isolates were positive for aflatoxins production, while 53% and 25% of isolates of *A. nidulans* and *A. ochraceus* respectively were able to elaborate *sterigmatocystin* and ochratoxin *A* respectively. None of the isolates of *A. terreus* could elaborate patulin, while 83% of *A. terreus* strains elaborated *terreic acid*, when *P. citrinum* and *P. griseofulvum* strains were screened. 44% and 33% of strains were positive for citrinin and CPA respectively.

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