Advanced Journal of Microbiology Research Volume 2013
Available online at http://advancedresearchjournals.org/AJMR

Full Length Research Paper

Incidence of *Toxigenic fusaria* in feeds of Godavari belt area of Andhra Pradesh, India

Narasimha Rao Kollu*, Sivadevuni Girisham and Solipuram Madhusudhan Reddy

Department of Microbiology, Kakatiya University, Warangal – 506 009, Andhra Pradesh, India.

Accepted 20 February, 2012

Mycoflora and mycotoxin contamination of different feeds (cattle and poultry) from different regions of Godavari belt was analysed. In all, five species of *Fusarium* were detected with varying percentages of incidence. A high percentage of isolates of *Fusarium* were mycotoxigenic and produced one or more mycotoxins. Zearalenone, T_2 toxin, nivalenol (NIV) and deoxyscripenol (DAS) were some of the mycotoxins detected.

Key words: Poultry feed, cattle feed, moulds, mycotoxins, *Fusarium* spp.

INTRODUCTION

Poultry feed used in most parts of India comprise of approximately 50% of maize and 25% of groundnut cake, which are very good substrates for mould growth. Moulds, besides depleting the nutrients, may also produce toxic substances that are potential health hazards to animals and, in turn, to humans (Fazekas et al., 1996, Trucksess, 2001) . The oestrogenic syndrome in swine, poultry and dairy cattle, due to ingestion of mould-infested feed, is well documented (Gimeno and Quintanialla, 1981).

There are several studies dealing with the mycoflora of feeds and mycotoxicoses of livestock from different countries (Burdit et al., 1983; Westalake and Dutton, 1985; Morenoroma and Fernandez, 1986). Such reports are also available from India (Neelakantan et al., 1978; Sudarshan Singh, 1971; Krishna Reddy et al., 1987). However, little or no studies on the incidence of fusaria in poultry feeds and fusarial mycotoxin contamination is available from the Godavari belt area of Andhra Pradesh, India. Hence, different feeds employed in this region were analyzed for the presence of *Fusarium* species.

MATERIALS AND METHODS

Isolation of fungi

Mycoflora of different feeds (cattle and poultry) was analysed by the dilution plate technique (Waksman, 1922). Ten grams of each sample was placed in 250 ml conical flasks, containing 100 ml of sterilized water, and subjected to horizontal shaking for 30 min. From this solution, dilutions were prepared and 0.5 ml of the dilutions was poured aseptically into sterilized and cooled Asthana and Hawker's medium A (glucose 5 g; KNO_3 3.5 g; KH_2PO_4 1.75 g; $MgSO_4.7H_2O$ 0.75 g; Agar-agar 16 g; distilled water 1000 ml). Gentle rotational movements of Petri dishes were made so as to ensure uniform spreading of the samples. The Petri dishes were incubated in an inverted position at 27 - 29°C for 7 days. To suppress bacterial growth and restrict fungal colonies, streptomycin and rose Bengal, respectively, were added. The fungal colonies were isolated from the third day until the seventh day, and identified by standard monographs (Ellis, 1971; Samson et al., 1984). Special attention was paid to the isolation and identification of different species of *Fusarium* (Nelson et al., 1983). The percentage incidence of individual fungi was calculated using the following formula.

$$\text{Percentage of incidence} = \frac{\text{No. of colonies of species in all the plates}}{\text{Total No. of colonies of all the species in all the plates}} \times 100$$

Identification of mycotoxins

Fusarial mycotoxins were analysed using thin layer chromatography (TLC) . For this purpose, fusarial culture filtrates were extracted twice with 100 ml of chloroform. The combined extracts were passed through an anhydrous Na_2SO_4 bed to remove moisture and then evaporated to dryness before dissolving in 1 ml of chloroform and spotting onto the TLC plates. The toxins were identified by spraying the plates with different spray reagents, (Table 1) as suggested by Kamimura et al. (1981) and Rao et al. (1985), and the compounds thus separated were identified based on the colour of the fluorescence of the spot and by the R_f values, as compared with standards. The R_f was calculated by using formula.

*Corresponding author. E-mail: kollu2006@yahoo.com.

Table 1. Detection of trichothecenes and other mycotoxins produced by *Fusarium*.

Name of the toxin	Solvent system	Spray reagent	Detection		Reference
			UV	Visible	
Deoxynivalenol (DON)	C : M (97 : 3)	4, 7, 8	-, ch, bl	y, -, -	A
Diacetoxy scirpenol (DAS)	C : M (97 : 3)	6, 9	bg, -	-, br	B,A
HT-2 toxin	C : M (97 : 3)	6	bg	-	B
Nivalenol (NIV)	C : M (97 : 3)	4, 7, 8	-, chl, bl	y, -, -	B
T-2 Toxin	C : M (97 : 3)	6, 9	bg, -	-, p	B
Zearalenone	C : M (97 : 3)	1, 2, 3, 5, 7, 8	-, -, -, br, ch, bl	br, do, ip, -, -, -	C,D,E

Solvent systems: C = Chloroform; M = Methanol;
Spray reagents: 1 = $Ce(SO_4)_2$ 1% in 6H.H_2SO_4; 2 = 2,4-DNP; 3 = $FeCl_3$ 3% in ethanol;
4 = p-anisaldehyde; 5 = 50% H_2SO_4 in methanol; 6 = 20% H_2SO_4; 7 = H_2SO_4;
8 = 20% $AlCl_3$; 9 = Chromatropic acid; 10 = 0.1% methanolic ninhydrdin

Detection colours: bl = Blue; ch = Charring, y = Yellow; bg = Blue green, br = Brown; p = Purple; do = Dark orange; lp = light purple; pi = Pink; bb = Bright blue.

References: A = Ramakrishna and Bhatt (1987); B = Kamimura et al. (1981);
C = Gorst – Allman and Steyn (1979); D = Mirocha et al. (1974); E = Pathre et al. (1979).

Table 2. Incidence of mycotoxigenic fungi in feeds (Poultry and cattle) of Godavari belt.

Name of the fungus	Warangal		Khammam		Karimnagar		Adilabad		Nizamabad		East Godavari		West Godavari	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
F. moniliforme	2.3	2.8	1.8	2.2	4.3	5.6	1.6	1.9	--	--	4.2	3.8	4.9	5.2
F. equiseti	--	--	1.6	1.9	1.4	1.7	0.7	1.2	--	--	2.1	2.7	3.2	4.1
F. oxysporum	0.5	1.2	1.2	0.8	1.8	2.4	0.3	0.4	1.1	1.4	3.6	2.9	4.4	3.9
F. solani	1.2	1.6	0.3	0.6	0.6	0.9	--	--	--	--	--	--	1.6	2.1
F. semitectum	--	--	--	--	--	--	0.1	1.9	--	--	--	--	1.2	1.8
A. flavus	28.2	22.3	25.6	19.2	29.6	24.8	17.6	19.8	24.2	25.2	19.8	172	21.4	18.4
A. niger	18.6	14.7	11.4	10.8	21.7	18.4	21.2	27.2	19.8	14.6	21.2	14.6	22.5	19.7
A. Japanicus	1.8	0.6	3.7	4.2	14.6	4.8	6.4	6.7	--	--	--	--	3.2	1.8
P. citrinum	4.2	6.4	7.6	10.2	4.9	3.6	11.7	10.8	1.2	2.4	--	--	4.2	3.6
P. chrysogenum	1.7	0.8	1.4	2.8	0.7	0.1	4.5	1.4	--	--	1.8	3.2	--	--
Myrothecium roridum	2.4	2.8	1.5	2.2	0.5	1.3	--	2.1	1.5	2.1	--	--	1.4	0.7
Trichoderma viridae	0.6	0.8	1.2	1.8	0.3	0.6	--	1.6	0.4	1.2	1.8	1.2	--	--
Stachybotrys atra	--	--	--	--	--	2.3	2.8	1.2	2.1	0.5	--	--	--	--
Other fungi	10.6	11.2	18.8	14.8	4.4	2.8	13.3	9.6	11.4	9.8	14.2	9.2	12.4	9.7

A - Poultry feed; B - Cattle feed.

$$R_f = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent}}$$

RESULTS AND DISCUSSION

In addition to species of *Aspergillus*, *Penicillium* and other fungi, five species of *Fusarium* was recorded with varying percentages of incidence (Table 2). Poultry feeds collected from Warangal were comparatively more infested by fusaria, while feeds from Nizamabad contained low infestation. Samples from Karimnagar and East Godavari come next with regard to their degree of fusarial infestation. Of all the *Fusarium* species, *F. moniliforme* was dominant and could be isolated from all the samples collected, except in samples from Nizamabad. *F. oxysporum* was also isolated from all the samples with a high percentage of incidence. *F. equiseti* could not be isolated from feed samples of Warangal and Nizamabad. Similarly, *F. solani* could not be isolated from samples of Adilabad, Nizamabad and East Godavari, and its incidence in samples of other places was low.

Table 3. Toxigenic potential of different speices of *Fusarium* associated with different feeds.

Name of the fungi	Warangal		Khammam		Karimnagar		Adilabad		Nizamabad		E. Godavari		W. Godavari		Name of the toxin
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	
Poultry Feed															
F. moniliforme	12	7	9	2	11	5	12	3	6	2	11	5	9	3	ZEA(11), T2(5), DON(2), DAS (4) HT2(2) NIV(1)
F. oxysporum	8	3	6	2	4	--	7	2	4	1	8	3	6	2	ZEA(6), T2(2), DON(2), DAS(1), NIV(2)
F. solani	4	--	6	1	--	--	9	3	5	--	6	2	8	3	ZEA(4), T2(3), NIV(2)
F. equiseti	7	2	--	--	--	--	11	4	9	4	5	--	4	1	T2(3), HT2(1), DON(2), ZEA(1), DAS(2), NIV(1)
Cattle Feed															
F. moniliforme	16	8	13	5	14	7	14	8	9	2	10	4	6	2	ZEA(11), T2(8), HT2(1), DAS(6), NIV(1)
F. oxysporum	11	6	8	3	4	--	9	3	7	3	8	2	6	1	ZEA(12), T2(3), DAS(2), NIV(3)
F. solani	5	--	6	2	8	2	11	4	4	--	6	2	4	--	ZEA(5), T2(2), NIV(2), DAS(1)
F. equiseti	4	1	7	4	4	--	3	--	3	--	5	1	4	1	ZEA(3), T2(1), DAS(2), NIV(1)

F. semitectum could be isolated only from feed samples of Adilabad and West Godavari. The very low incidence of fusarial species in feeds collected from Nizamabad may be attributed to the prevailing dry climate. In comparison, the observed higher incidence of different *Fusarium* species in feeds of West Godavari may be due to the warm humid climate prevailing in that region. The dominance of *A. flavus* and *A . niger* may be another reason for low incidence of *Fusarium*. However, more samples, during different times of the year and from different regions, should be collected and analysed before a definitive conclusion can be reached.

In all, five *Fusarium* species could be recorded in cattle feed samples collected from the Godavari belt. Besides species of *Penicillium*, *Aspergillus* and other fungi, the feeds collected from West Godavari were rich, both qualitatively and quantitatively, for *Fusarium* species. *F. semitectum* could be recorded in feed samples of Adilabad and West Godavari with a low percentage of incidence. *F. equiseti* and *F. moniliforme* could not be recorded in the samples of both Warangal and Nizamabad. *F. oxysporum* could be recorded in all the samples collected from different places. However, its incidence was high only in samples of West Godavari, followed by East Godavari. *F. equiseti* could be recorded with a high percentage in samples of East Godavari and West Godavari. The incidence of *F. moniliforme* was high in samples collected from Karimnagar and East Godavari. The incidence of species of *Fusarium* was comparatively higher in cattle feeds than in poultry feeds.

Table 3 reveals that of the 397 *Fusarium* iso-lates, 131 were mycotoxigenic. Many of the strains could produce more than one mycotoxin. Screening of 70 strains of *F. moniliforme* indicated that 27 were mycotoxigenic, ofwhich 11, 5 and 4 strains produced Zearalenone, T_2 toxin and DAS, respectively. Only one strain could produce NIV, while HT_2 toxin was produced by two strains. DON could be produced by two strains of *F. monili-forme*. Screening of 43 strains of *F. oxysporum* indicated that 13 were mycotoxigenic and pro-duced one or more mycotoxins. Of these strains, 6, 2, 2 and 1 strain, respectively, produced Zea-ralenone, T_2 toxin, DON and DAS. NIV could be produced by two strains of *F. oxysporum*. Of the 38 strains of *F. solani* screened, 9 were toxigenic and 4 produced Zea, 3 produced T_2 toxin, and 2 produced NIV. Screening of 36 strains of *F. equiseti*, indicated that 11 were mycotoxigenic and produced T_2 toxin, HT_2 toxin, DON, DAS and NIV.

Of the 110 isolates of cattle feed samples screened, 71 were toxigenic (Table 3). *F. moniliforme* could be recorded in all of the samples with a high percentage of mycotoxigenicity. Of the 83 strains of *F. moniliforme* isolated from cattle feeds, 11, 8 and 6 strains, respectively, produced Zearalenone, T_2 toxin and DAS. Only one strain each produced HT_2 and NIV. Similarly 12, 3 and 2 of the *F. oxysporum* strains produced Zearalenone, T_2 toxin, NIV and DAS, respectively. Of the 44 strains of *F. solani* screened, 10 were toxigenic. Of these, 5, 2, 2 and 1 strain produced Zearalenone, T_2 toxin, NIV and DAS, respectively. Screening of 30 strains of *F. equiseti* indicated that 7 were toxigenic of which 3 strains produced Zearalenone, 1 strain produced T_2 toxin, 2 strains produced DAS and 1 strain produced NIV. None of the strains of *F . oxysporum* isolated from Karimnagar, *F. solani* from Warangal and Nizama-

bad were toxigenic. Similarly, *F. equiseti* isolated from samples of Karimnagar failed to produce any mycotoxin. In general, about 25 - 45% of the strains of different species of *Fusarium* isolated from samples of Godavari belt region were mycotoxigenic and produced toxic substances that are health hazards to animals and, in turn, to humans.

ACKNOWLEDGEMENT

Our thanks are due to Head, Department of Microbiology for providing necessary facilities.

REFERENCES

Burdit SJ, Hagler WM, Hamilton PB (1983). Survey of moulds and mycotoxin for their ability to cause feed refusal in chickens. J. Poult. Sci. 63(11): 2187-2191.

Ellis MB (1971). Dematiaceous Hypomycetes, Kew, Surrey, Common wealth Mycological Institute, London.

Fazekas B, Kis M, Haidu ET (1996). Data on the contamination of maize with fumonisins B_1 and other fusarial toxins in Hungary. Acta Vet. Hung. 44: 25-37.

Gimeno A, Quintanialla JA (1981). International Symposium and Workshop on Mycotoxins, Cairo Dokki, Egypt (Abstracts) 50.

Gorst-Allman ChP, Steyn PS (1979). Screening methods for the detection of common mycotoxins. J. Chromatogr. 175: 325-331.

Kamimura H, Nishijima M, Yasuda K, Saito K, Ibe A, Nagayama T, Yoshiyama H, Naoi Y (1981). Simulataneous detection of fusarial toxins. J. Assoc. Off. Anal. Chem. 64**:** 1067-1073.

Krishna RV, Girisham S, Reddy SM (1987). Incidence of trichothecenes producing fusarium on standing crop of maize. Perspectives in Mycology Research, Prof. G.P. Agarwal Fest Schrift, Today and Tomorrow Printers and Publishers, New Delhi. pp. 177-185

Mirocha CJ, Schauerames B, Pathre CV (1974). Isolation, detection and quantification of zearalenone in maize and barley. J. Assoc. Off. Anal. Chem. 57: 1104-1110.

Moreno RMA, Fernandez GS (1986). Mycoflora of commercial poultry mixed feeds. Poult. Sci. 65**:** 284-287.

Neelakantam SR, Swaminathan R, Balasubramaniyan T, Indira Jasmin (1978). Indian Poult. Gaz. 62: 40-44.

Nelson PE, Toussoun TA, Marasas WFO (1983). *Fusarium* Species. An illustrated manual for identification, Pennsylvania state university, University park.

Pathre SV, Mirocha CJ (1979). Trichothecens natural joccurrence and potential hazard. J. Amer. Oil Chem. Soc. 56: 820-823.

Ramakrishna Y, Bhat RV (1987). Comparison of different spray reagents for identification of trichothecenes. Curr. Sci. *56***:** 524-526.

Rao GV, Rao PS, Girisham S, Reddy SM (1985). A Novel spray reagent for chromatographic detection of trichothecene toxins. Curr. Sci. 54: 507-509.

Samson RA, Moekstra E, Van CN (1984). Introduction to food borne fungi. Institute of Royal Netherlands Academy of arts and science.

Sudarshan S (1971). Incidence of aflatoxin in poultry feeds. Poult. Advisor. 20: 25.

Trucksess W (2001). Joint mycotoxin technical committee reports. J. AOAC. 83**:** 2.

Waksman SA (1922). A method of counting the number of fungi in the soil. J. Bact. 7: 339-341.

Westalake K, Dutton MF (1985). The incidence of mycotoxins in litter feed and livers of chickens in Natal South Africa. S. Aft. Tydskr. Veekd. 15**:** 175-177.