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# INCIDENCE OF MYCOTOXIGENIC FUNGI IN LIVESTOCK ANIMAL FEED CAKES OF GODAVARI BELT REGIONS OF A.P (INDIA)

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**ABSTRACT** Incidence of mycotoxigenic fungi and elaboration of different mycotoxins in feed cakes in the Godavari belt region of A.P was assessed. In all 23 fungal species representing 12 genera could be detected. Many of the feed cakes ware highly infested by species of Aspergillus, Penicillium and Fusarium, besides other fungi. Groundnut cake supported maximum number of fungi with 23 fungal species representing 12 genera, while coconut cake and cotton seed cake with supported comparatively less number of moulds. No positive correlation could be observed among percentage of incidence, frequency and abundance of different fungi. Mycotoxins like aflatoxins, sterigmatocystin, ochratoxin A, patulin, citrinin, cyclopiazonic acid, zearalenone, satratoxin, trichothecene mycotoxins were potential contaminates of these cakes.

**KEYWORDS :** Feed cakes, *Aspergillus, Penicillium, Fusarium,* mycotoxins and mycotoxigenic fungi.

## INTRODUCTION

Exposure of livestock to variety of mycotoxins has been common due to nutritionally richness of formulated feeds. Moulds proliferate in these feeds and elaborate variety of mycotoxins which are responsible for variety of health hazards in animal including man. Contamination of feeds and feed ingredients with mycotoxins has been reported from different parts of the world (Reddy *et al.*, 2000; Kranjay *et al*., 2008; Sultana and Hanif, 2009; Saleemi *et al*., 2010 and Koteshwar Rao et al., 2011). Yiannikouris and Jouany (2002) and Lanier *et al*., (2009) have discussed various aspects of the incidence of mould infestation and mycotoxin contamination of oil seed cakes and consequential health effects on farm animals. Presence of such mycotoxins in feeds may also decrease feed intake and affect animal performance. In addition to possible presence of these toxic residues in edible products (milk, meat etc) may have detrimental effects on human health. Therefore, preventing fungal infestation of feed ingredients and elimination of mycotoxins from contaminated feed, use of adsorbents to reduce bioavailability of toxins in the digestive track of animals is of vital importance. In the present investigations detection of fungi in different feed cakes which are commonly used in feed formation in the Godavari belt region which is warm and humid conditions prevail during most part of the year.

## MATERIALS AND METHODS

Regular survey of feed cakes (groundnut cake, ginger cake, coconut cake and cotton seed cake) which generally used as feed ingredients of livestock in the Godavari belt regions of A.P. (Fig 1) was conducted. The details of the condition of samples, age of samples and place of collection was recorded carefully. The sample was subjected to analysis of mycoflora by employing dilution plated method (ISTA, 1993) and dilution plate method (Waksman, 1922). The fungi appeared were isolated, purified and identified with the help of standard manuals (Buck and Cote, 1991; Singh et al., 1999; Mathur and Kongsdal, 2003 and Lislie and Summerel, 2006).

The fungal isolated were grown in Czapek's dox medium (Sucrose-30g; NaNo3-3.0g; KH2Po4-1g; KCl-0.5g; FeSo4 7 H2O-0.01g; MgSo4 7H2O-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 (table 1).

**Fig 1: Site of survey presence of mould in different feed cakes**

**Table 1: Detection of different mycotoxins 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 | - | - |
| Sterigmatocystin | C:M:A (1:1:1) | 20% $AlCl_3$ | y | - |
| Zearalenone | C:M (97:3) | $Ce(SO_4)_2$ 1% in 6N $H_2SO_4$ | -,-,-,br,ch,bl | br,do,lp,-,-,- |
| | | 2,4-DNP, $FeCl_3$ 3% in ethanol | | |
| | | 50% $H_2SO_4$ in methanol, $H_2SO_4$, 20% $AlCl_3$ | | |
| Citrinin | T:Ea:F (6:3:1) | $Ce(SO_4)_2$ 1% in 6N $H_2SO_4$, 2,4-DNP | y | y,by,lo |
| | | $FeCl_3$ 3% in ethanol | | |
| Cyclopiazonic acid | T:Ea:F (6:3:1) | $Ce(SO_4)_2$ 1% in 6N $H_2SO_4$, 2,4-DNP | y | bl,rb,br |

| | | | | |
|---|---|---|---|---|
| | | $FeCl_3$ 3% in ethanol | | |
| Satratoxin | C:M (97:3) | Phloroglucinal | - | pi |
| Trichothecenes | 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

Altogether 23 fungal species representing 12 genera were recorded in groundnut cake samples (Table 2). *Aspergillus flavus, A. niger,* species of *Fusarium* and *Penicillium* were associated with all the samples collected from different Godavari belt regions of A.P. while *Stachybotrys atra* could be recorded only in samples collected from Medak. Similarly *D. rostrata, T. roseum, P. sorghina, M. roridum, A. ochraceus, A. nidulans and A. alternata* were recorded in samples collected from only a few places. The percentage of incidence of *A. flavus* was highest in most of the samples. In samples of Warangal it was more than 50 percent. Verma, and Agarwal (2000) have also noted the dominance of species of *Aspergillus, Fusarium* and *Penicillium* in the groundnut cake.

*Aspergillum flavus, A. niger* species of *Fusarium* and *Penicillium* were with highest percentage of frequency, while *S. atra* was with lowest percentage of frequency. *R. stolonifer* was next highest in its frequency. Percentage of abundance of *A. flavus* was highest followed by *A. niger* and species of *Penicillium*. *A. alternate* and *D. rostrata* were lowest in their abundance. Interestingly, though the incidence and frequency of *S. atra* was low, its abundance was more. In general incidence of *A. flavus* and *A. niger* was more. Similarly species of Fusarium and Penicillium were also considerably high in their percentage of incidence and can be much significance in the livestock health.

Fourteen fungal species representing 9 genera were recorded in coconut cake collected from different Godavari belt regions of Andhra Pradesh. *A. flavus, A. niger, C. pallescens* and species of *Fusarium* and *Penicillium* were associated with all the samples, while *M. roridum* was isolated only from samples of Nizamabad. *P. varioti, R. stolonifer, T. roseum, D. spicifer,* and *A. terreus* were recorded in samples of only some places. The incidence of A. flavus was highest followed by *A. niger, species* of *Penicillium* and *Fusarium* in a descending order.

*A. flavus, A. niger, C. pallescens, species* of Fusarium and Penicillium were with highest percentage of frequency, while it was least with *M. roridum, P. varioti, R. stolonifer* and *T. roseum. A. flavus* followed by species of *Penicillium, A. niger* and A. terreus were with highest percentage of abundance. On the other hand, *M. roridum* and P. *varioti occurred* with lowest percentage of abundance.

Gingelly cake collected from different regions of Godavari belt of Andhra Pradesh supported 12 fungal species representing 8 genera (Table 2) *A. flavus, A. niger,* species of Fusarium and Penicillium were recorded in all the samples, while *M. roridum* and *D. spicifer* were associated only with samples collected from Nalagonda and Warangal respectively. *R. stolonifer, S. racemosum, C. lunata* and *A. terreus* were recorded in samples of only some places. The incidence of *A. flavus* followed by species of Penicillium was highest in all the samples collected. *A. niger* was next dominant fungus. The highest incidence of *A. flavus* may pose health hazard to livestock and intrun to man. *A. flavus* was highest in percentage of frequency and abundance followed by *A. niger, A. terreus* and species of Penicillium in a descending order, while it was least with *M. roridum.*

Cotton seed cake was reported to be an ideal substratum for the toxigenic moulds (Reddy *et al.*, 1986). The cotton seed cake harboured 14 fungal species representing 9 genera (Table 1). *A. flavus, A. niger* and species of Penicillium were associated with almost all the samples collected from different places of *A.P. M. roridum* and *P. varioti* were associated only with the samples collected from Khammam, while R. stolonifer and *S. racemosum* was recorded in Warangal samples. Species of Fusarium could not be detected in samples from *Nalagonda* and *Nizamabad*, while *C. pallescens* was traced in samples collected from *Khammam, Nizamabad* and *Ranga Reddy. A. terreus* could be detected only in samples of *Khammam, Karimnagar, Medak* and Hyderabad. *D. spicifer* was traced in samples of Warangal, Khammam, Nizamabad and *Ranga Reddy.* The percentage of frequency and abundance of *A. flavus*, followed by species of Penicillium and *A. niger* was high, while *M. roridum* and *P. varioti* were least.

From the present investigations it is clear that feed cakes are ideal substrates for mould infestation. Groundnut cake being highly preferred, while coconut cake is least preferred substratum for *A. flavus* infestation. Cotton and coconut cakes are preferred substrates for the growth of Penicillium species. Groundnut cake was the best substrate for the growth of Fusarium species. The incidence of other fungi in different cake samples was not only inconsistent but also sporadic.

Considerable number of fungi associated with livestock feed cake samples, were mycotoxigenic and elaborated variety of mycotoxins (Table 3). However, the toxigenic potential of different fungi varied with the feed cake. Out of 168 strains of *A. flavus* of groundnut cake 154 were positive for aflatoxins production, 74 isolates out of 83 of coconut cake were positive for aflatoxins. When 40 and 30 strains of *A. flavus* isolated from gingelly and cotton cakes respectively were screened 25 each were positive for aflatoxins production. Nusrath and Nahdi (1983) and Charoenpornsook and Kavisarasai (2006) have also reported the aflatoxins contamination of feed cakes examined by them. Out of 23 strains of *A. nidulans* isolated from groundnut cake samples, only 3 produced sterigmatocystin. None of the isolates of *A. nidulans* isolated from cotton seeds cake elaborated sterigmatocystin. On the other hand, Neelakantan *et al,* (1979) recorded sterigmatocystin in cotton seed cake. None of the isolates of *A. terreus* isolated from different cakes could elaborate patulin. However, 43 out of 82 strains of A. terreus isolated from different cakes could elaborate terreic acid. Only 2 out of 12 isolates of A. ochraceus isolated from groundnut cake elaborated ochratoxin *A. Toxigenic potential* of *P. citrinum* isolated from different cakes varied from 30-60%. Groundnut cake samples recorded the highest incidence of citrinin producing strains, where as coconut cake samples recorded lowest incidence of citrinin producing strains. None of the isolates of *P. griseofulvum* of coconut cake samples elaborated CPA, while the toxigenic potential of P. griseofulvum isolated from other cakes ranged from 15-25%. Both the species of Fusarium (*F. oxysporum and F. moniliforme*) isolated from groundnut cake and coconut cake samples elaborated only zearalenone. Similarly *F. oxysporum and F. moniliforme* isolated from gingelly cake and cotton seed cake respectively elaborated zearalenone. Saleemi *et al.* (2010) have also reported aflatoxins and zearalenone in groundnut meal. Of 3 isolates of S. atra and T. roseum isolated from groundnut cake, only one isolate each elaborated satratoxin and trichothecene. Thus cattle feed cakes can be potentially health hazardous to livestock if proper attention is not paid during storage or utilization and inturn to man.

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**Table 2: Mycoflora of animal feed cakes**

| Name of the fungus | Percentage of Incidence | | | | | | | | | Percentage of frequency | Percentage of abundance |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | | |
| **Groundnut cake** | | | | | | | | | | | |
| *Alternaria alternata* | 5.10 | - | - | 4.40 | - | 2.40 | 0.80 | - | - | 44.4 | 0.93 |
| *Aspergillus flavipes* | - | - | 3.70 | - | - | 6.40 | - | 3.00 | 6.50 | 44.4 | 1.22 |
| *A.flavus* | 62.2 | 35.0 | 27.8 | 37.8 | 40.3 | 15.3 | 11.8 | 9.80 | 6.30 | 100.0 | 23.8 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. nidulans* | - | 3.50 | - | - | 3.80 | - | - | 18.0 | - | 33.3 | 6.35 |
| *A. niger* | 10.2 | 8.20 | 6.30 | 11.80 | 6.50 | 17.3 | 16.8 | 3.20 | 5.60 | 100.0 | 20.1 |
| *A.ochraceus* | - | 5.00 | - | - | 7.00 | - | - | - | 0.80 | 33.3 | 8.32 |
| *A. terreus* | 3.20 | - | 3.70 | - | - | 12.8 | - | - | 13.5 | 44.4 | 10.2 |
| *Curvularia lunata* | - | 5.80 | 6.50 | - | 4.50 | 2.60 | 8.80 | 1.20 | 0.50 | 74.7 | 1.02 |
| *C. pallescens* | - | 1.20 | - | 9.40 | - | - | 1.20 | - | - | 33.3 | 0.85 |
| *Drechslera spicifer* | - | 8.00 | 4.00 | 5.50 | - | 1.20 | 3.60 | - | - | 55.6 | 1.32 |
| *D.rostrata* | - | - | - | 0.30 | - | - | - | 10.20 | - | 22.2 | 0.85 |
| *Fusarium spp. ( F. moniliforme, F.oxysporum,F.solani)* | 12.0 | 10.0 | 15.5 | 10.2 | 12.3 | 10.5 | 18.8 | 18.3 | 28.6 | 100.0 | 18.4 |
| *Myrothecium roridum* | 0.50 | -- | - | - | - | 1.00 | - | 3.50 | 0.80 | 44.4 | 3.65 |
| *Penicillium spp. (P.citrinum, P.oxalicum,P.griseofulvum)* | 6.80 | 15.0 | 18.5 | 13.8 | 15.6 | 10.50 | 18.2 | 15.5 | 13.2 | 100.0 | 18.3 |
| *Phoma sorghina* | - | - | - | - | 3.50 | - | 1.80 | - | 3.20 | 33.3 | 2.34 |
| *Rhizopus stolonifer* | - | 3.30 | 10.5 | 6.80 | 5.50 | 9.50 | 1.50 | 16.0 | 10.0 | 88.9 | 10.2 |
| *Stachybotrys atra* | - | - | - | - | - | 3.00 | - | - | - | 11.1 | 1.02 |
| *Trichothecium roseum* | - | - | - | - | 1.00 | - | 3.50 | - | 4.20 | 33.3 | 3.12 |
| *Sterile mycelium* | - | 5.00 | 3.50 | - | - | 7.50 | 3.40 | 1.30 | 5.80 | 66.7 | 2.38 |
| *Coconut cake* | | | | | | | | | | | |
| *Aspergillus flavus* | 27.8 | 28.6 | 46.2 | 25.3 | 28.8 | N | N | N | N | 100.0 | 28.3 |
| *A. niger* | 22.9 | 25.9 | 18.2 | 10.2 | 10.2 | N | N | N | N | 100.0 | 18.2 |
| *A. terreus* | - | 5.50 | 3.40 | 3.80 | 3.80 | N | N | N | N | 80.0 | 15.6 |
| *Curvularia pallescens* | 1.20 | 1.30 | 2.20 | 3.20 | 3.20 | N | N | N | N | 100.0 | 3.50 |
| *Drechslera spicifer* | - | - | 1.80 | 3.80 | 3.80 | N | N | N | N | 60.0 | 1.20 |
| *Fusarium spp. ( F. moniliforme, F.oxysporum)* | 12.80 | 10.30 | 6.80 | 15.60 | 4.20 | N | N | N | N | 100.0 | 10.5 |
| *Myrothecium roridum* | - | - | - | - | 0.20 | N | N | N | N | 20.0 | 0.40 |
| *Penicillium spp. (P.citrinum, P.oxalicum,P.griseofulvum)* | 18.6 | 12.7 | 18.5 | 18.8 | 18.8 | N | N | N | N | 100.0 | 21.3 |
| *Paecilimyces varioti* | 3.10 | - | - | - | - | N | N | N | N | 20.0 | 0.80 |
| *Rhizopus stolonifer* | 10.10 | - | - | - | - | N | N | N | N | 20.0 | 3.20 |
| *Sterilemycelium* | 3.60 | - | - | - | - | N | N | N | N | 20.0 | 1.20 |
| *Gingelly cake* | | | | | | | | | | | |
| *Aspergillus flavus* | 41.4 | 48.8 | 53.5 | 36.4 | 44.2 | 30.8 | N | N | N | 100.0 | 20.3 |
| *A. niger* | 10.6 | 18.2 | 12.5 | 18.2 | 12.8 | 18.2 | N | N | N | 100.0 | 18.2 |
| *A. terreus* | - | 15.2 | 9.4 | 15.6 | 12.3 | 10.5 | N | N | N | 83.3 | 15.6 |
| *Curvularia lunata* | 2.60 | - | 0.30 | 7.30 | 6.40 | - | N | N | N | 66.6 | 3.40 |
| *Drechslera spicifer* | 4.40 | - | - | - | - | - | N | N | N | 16.6 | 1.20 |
| *Fusarium spp. ( F. moniliforme, F.oxysporum)* | 9.40 | 6.30 | 4.40 | 8.60 | 6.80 | - | N | N | N | 83.3 | 10.8 |
| *Myrothecium roridum* | - | - | - | 0.20 | - | - | N | N | N | 16.6 | 0.20 |
| *Penicillium spp. (P.citrinum, P.oxalicum,)* | 23.6 | 28.2 | 12.8 | 22.8 | 25.6 | 15.0 | N | N | N | 100.0 | 13.6 |
| *Rhizopus stolonifer* | 5.00 | - | - | - | - | 10.5 | N | N | N | 33.3 | 7.30 |
| *Syncephalastrum racemosum* | 3.00 | - | - | - | - | 5.50 | N | N | N | 33.3 | 2.50 |
| **Cotton seed cake** | | | | | | | | | | | |
| *Aspergillus flavus* | 60.2 | 53.8 | 40.2 | 55.0 | 72.3 | 38.2 | 40.0 | 36.3 | N | 100.0 | 26.2 |
| *A. niger* | 8.80 | 6.20 | 10.8 | 10.0 | 10.0 | 11.8 | 10.0 | 13.7 | N | 100.0 | 15.2 |
| *A. terreus* | - | 15.50 | 10.30 | - | - | 15.0 | - | 10.0 | N | 50.0 | 12.4 |
| *Curvularia pallescens* | - | 6.40 | - | - | - | 9.30 | 9.30 | - | N | 50.0 | 8.20 |
| *Drechslera spicifer* | 1.00 | 3.60 | - | - | - | - | 1.70 | - | N | 50.0 | 6.50 |
| *Fusarium spp. ( F. moniliforme, F.oxysporum)* | 5.50 | 3.50 | 8.30 | - | - | 12.0 | 8.00 | 11.5 | N | 75 | 15.3 |
| *Myrothecium roridum* | - | 1.50 | - | - | - | - | - | - | N | 12.5 | 0.92 |
| *Penicillium spp. (P.citrinum, P.oxalicum,P.griseofulvum)* | 15.0 | 5.00 | 20.0 | 36.8 | 10.0 | 18.3 | 15.5 | 16.4 | N | 100.0 | 20.2 |
| *Paecilimyces varioti* | - | 4.50 | - | - | - | - | - | - | N | 12.5 | 1.60 |
| *Rhizopus stolonifer* | 5.50 | - | - | - | - | - | - | - | N | 12.5 | 4.30 |
| *Syncephalastrum racemosum* | 6.40 | - | - | - | - | - | - | - | N | 12.5 | 3.80 |
| A= Warangal, B= Khammam, C= Karimnagar, D=Nalagonda, E=Nizamabad, F= Medak, G=Ranga reedy, H=Hyderabad. | | | | | | | | | | | |
| I= Vijayanagaram,N= not Collected. | | | | | | | | | | | |

**Table 3: Toxigenic potential of fungi of livestock cakes:**

| Name of the fungus | Groundnut cake | Coconut cake | Gingelly cake | Cotton cake | Name of the toxin | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | A | B | A | B | A | B | A | B | |
| *Aspergillus flavus* | 168 | 79.4 | 83 | 72.7 | 40 | 62.5 | 30 | 83.3 | Aflatoxins |
| *A. nidulans* | 23 | 13.0 | - | - | - | - | 4 | - | Sterigmatocystin |
| *A. ochraceus* | 12 | 16.7 | - | - | - | - | - | - | Ochratoxin A |
| *A. terreus* | 28 | - | 15 | - | 19 | - | 20 | - | Patulin |
| *A. terreus* | 28 | 42.9 | 15 | 53.3 | 19 | 63.2 | 20 | 55.0 | Terreic acid |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Penicillium citrinum* | 15 | 53.3 | 28 | 28.6 | 15 | 33.3 | 16 | 37.5 | Citrinin |
| *P. griseofulvum* | 25 | 20.0 | 20 | - | 18 | 16.1 | 22 | 27.7 | CPA |
| *Fusarium oxysporum* | 28 | 50.0 | 25 | 60.0 | 12 | 33.3 | 14 | - | Zearalenone |
| *F. moniliforme* | 12 | 33.3 | 10 | 20.0 | 8 | - | 12 | 50.0 | Zearalenone |
| *Stachybotrys atra* | 3 | 33.3 | - | - | - | - | - | - | Satratoxin |
| *Trichothecium roseum* | 3 | 33.3 | - | - | - | - | - | - | Trichothecene |
| **A=Number of strains screened, B=Percentage of toxigenic strains** | | | | | | | | | |

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