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# High Incidence of *Fusarium verticillioides* in Animal and Poultry Feed Mixtures Produced in Karnataka, India

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**Abstract:** The aim of present study was to determine the species incidence of genus *Fusarium* in animal and poultry feed mixtures and to know the extent of potential risk of feed contamination by *Fusarium* mycotoxins. One hundred and seven different animal feed samples and (71) poultry-feed mixtures were collected from Karnataka (India) during April 2004 to April 2005. All samples were analyzed for the incidence of *Fusarium* species on PDA, DCPA and MGA 2.5 media. A total frequency of the *Fusarium* species isolated was determined to be 50% and their counts ranged from $9.5\times10^{1}$ to $4.4\times10^{5}$ CFU $g^{-1}$ of poultry feed and $5.728\times10^{1}$ to $2.088\times10^{5}$ CFU $g^{-1}$ of animal feed sample. Of the total number of *Fusarium* isolates (330) from animal and poultry feedstuffs, *F. verticillioides* recorded 89.09%, followed by *F. pallidoroseum* (6.66%), *F. oxysporum* (3.63%) and *F. solani* (0.6%). The results of this study showed a high incidence of *F. verticillioides* in poultry feed mixtures while animal feeds especially cotton seeds, fine wheat bran and maize pellets showed high incidence of *F. verticillioides*. Bengal gram husk, coarse horse gram powder, groundnut seed cake, sunflower seed cake and wheat flakes showed very low incidence of *F. verticillioides*. The study not only reveals a high incidence of the potentially toxigenic *F. verticillioides*, in the local feeds of Karnataka region but also represents the possibility of occurrence of fusarial mycotoxins, especially fumonisins.

**Key words:** *Fusarium* species, *F. verticillioides*, mycotoxins, animal feeds, poultry feed mixtures

## INTRODUCTION

There are thousands of poultry farms and local animal feed manufacturing units in India, producing mixed feeds for poultry and animal nutrition. In general, the mixed feeds constitute maize and sorghum as major ingredients, which represent an excellent substrate for growth and reproduction of numerous fungi, under favourable conditions such as high moisture and increased temperature (Labuda *et al*., 2003). A number of morphologically related *Fusarium* species namely: *Fusarium verticillioides* (Syn. *F. moniliforme*), *F. proliferatum*, *F. napiforme*, *F. anthophilum*, *F. dlamini*, *F. thapsinum* and *F. globosum* occur world-wide and are capable of producing a group of structurally related mycotoxins such as fumonisins (Gelderblom *et al*., 1988). Fumonisins are a group of secondary metabolites produced by *F. verticillioides* (Nelson *et al*., 1991). Fumonisin $B_1$ is the most common, most toxic compound occurring on wheat seedlings (Chelkowski *et al*., 1995) and mainly on maize and maize products (Gutema *et al*., 2000). Fumonisin containing culture material of *F. verticillioides* was found to be hepatotoxic and carcinogenic (Kellerman *et al*., 1990) and nephrotoxic in rats (Suzuki *et al*., 1995). Fumonisin $B_1$ causes leucoencephalomalacia in horses (Kellerman *et al*., 1990), pulmonary oedema and hydrothorax in swine (Harrison *et al*., 1990). Acute hepatic and renal toxicity with significant mortality was found in lambs dosed with fumonisin $B_1$ (Gelderblom *et al*., 1991). Fumonisins are toxic to fish where enteritis was observed in catfish (*Ictalurus punctatus*) fed with fumonisin $B_1$ (Lumlertdacha *et al*., 1995). Feeding *F. verticillioides* culture material to broilers led to reduced performance, increased organ weights, hepatic necrosis, black sticky diarrhoea, rickets and altered serum constituents and enzyme activities (Weibking *et al*., 1993). Acute mycotoxicosis caused by the fumonisins or their toxic effects in poultry were studied for instance by other scientific reports (Prathapkumar *et al*., 1997;

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Weibking *et al*., 1993). In spite of the worldwide natural occurrence of fumonisins in maize (*Zea mays*), which is a major dietary ingredient in poultry feeds, no natural disease outbreak in poultry attributable to fumonisin was reported in India till 1994. However, during October 1995, a poultry disease outbreak was reported from two layer farms more than 100 miles apart (Prathapkumar *et al*., 1997). Not much work has been done on high-energetic cereals such as maize, sorghum, wheat, which form the bulk ingredients in the production of animal and poultry feed mixtures in Karnataka. Hence the aim of this present study was therefore to determine the species incidence of the genus *Fusarium* in animal and poultry feed mixtures produced in the region of Karnataka, India and to know the extent of potential risk of feed contamination by *Fusarium* mycotoxins, especially fumonisins.

## MATERIALS AND METHODS

**Sample collection:** A field trip was undertaken, in order to collect 71 samples of local poultry feed mixtures from various poultry farms in and around Mysore district and also from different districts of Karnataka State during the period of April 2004 to April 2005. One hundred and seven animal feed samples were also collected from local merchants and brought to the laboratory in sterile polythene bags during the same period. Representative samples were obtained by hand sampling for further analysis.

**Mycological analysis of poultry and animal feeds:** All the samples were subjected to serial dilution and plating. Each sample (1 g) was added to 9 mL of sterile saline and vortexed for 10 min. One mL suspension was spread onto an agar plate in dilution of $10^{-2}$ to $10^{-5}$ in duplicates. For isolation and enumeration of *Fusarium* species, Dichloran Chloramphenicol Peptone Agar (DCPA) was used (Leslie and Summerell, 2006). Plates were incubated at 25±2°C, for a period of 6 days with alternating periods of 12 h light and 12 h darkness. The colonies of *Fusarium* were counted and expressed as Colony Forming Units (CFU) $g^{-1}$ of the sample. Malachite Green Agar 2.5 (MGA 2.5) medium was also used for selective isolation of *Fusarium* species (Bragulat *et al*., 2004). Cottonseeds from 100 g samples were surface sterilized with 1% NaOCl for 30 sec and then plated on Malachite Green agar (MGA 2.5) media. Percent incidence of *Fusarium* species was calculated using the formula.

$$\text{Percent incidence (\%)} = \frac{\text{No. of samples positive for } \textit{Fusarium} \text{ species}}{\text{Total No. of samples screened}} \times 100$$

*Fusarium* isolates were sub-cultured and incubated at 25°C, for 10 days in alternating periods of 12 h darkness and light using a carbohydrate-rich medium like Potato Dextrose Agar (PDA) that contains 20 g of dextrose, 20 g of agar and the broth from 250 g white potatoes made up to 1 L with water, supplemented with antibiotics to inhibit bacterial growth. The potatoes were peeled, washed, diced and boiled until soft. The boiled potatoes were filtered through a single layer of cheesecloth and the broth was used. Individual species were identified on the basis of their macro morphological and micro morphological characters in accordance with previously described *Fusarium* keys (Booth *et al*., 1977; Leslie and Summerell, 2006).

## RESULTS AND DISCUSSION

Out of 107 animal feed samples that were screened, 35 samples were found to be positive (Table 1) and 54 poultry feed mixture samples out of 71 samples tested positive for *Fusarium* species contamination (Table 2). Their counts ranged from $7.33\times10^{2}$ to $4.58\times10^{5}$ CFU $g^{-1}$ of animal feed sample. Out of the 175 isolates of the genus *Fusarium*, four *Fusarium* species were recorded. *F. verticillioides* was the most frequently isolated species among the 34 positive samples (Table 3).

On the other hand the total counts of *Fusarium* species from poultry feed mixture samples ranged from $4.04\times10^{2}$ to $1.95\times10^{5}$ CFU $g^{-1}$. Of the 155 isolates of the genus *Fusarium*, 4 species of *Fusarium* were isolated (Table 3). The most frequently isolated species in all the 54 positive samples was found to be *F. verticillioides*. The other Fusaria isolated included *F. pallidoroseum*, *F. oxysporum* and *F. solani*.

All the *Fusarium* species found were determined to belong to some section from the infrageneric point of view. *F. verticillioides* belonged to section Liseola (Saccardo) Nirenberg, *F. oxysporum* belonged to section Elegans (Schlecht: Fr.), *F. pallidoroseum* belonged to section Arthrosporiella (Berkeley and Ravenel) and *F. solani* belonged to section Martiella (Martius) Appel and Wollenweber emend. Snyder and Hansen.

*Fusarium verticillioides* was most frequently isolated from all positive samples, i.e., in 88 samples. This represented a part of 89.09% (in total 330 isolates). The colonies on PDA were creamish to peach to vinaceous on the obverse and pale cream to salmon to violet or blue on the reverse (Fig. 1A). This taxon was characterized by mostly zero-septate, clavate microconidia with a flat base produced on monophialides in chains (Fig. 1B) in the aerial mycelium. The macroconidia were observed rarely which were mostly 3-septate in some isolates.

Table 1: Per cent incidence of *F. verticillioides* in animal feeds collected from different districts of Karnataka

| Animal feed type | Place of sample collection | No. of samples screened | No. of positive *Fusarium* sp. samples | No. of samples positive for *F. verticillioides* | Percentage incidence of *Fusarium* species | CFU ($g^{-1}$) |
|---|---|---|---|---|---|---|
| Bengalgram husk | Tamilnadu | 13 | 0 | 0 | 00.00 | - |
| Coarse horsegram powder | Mysore | 10 | 1 | 1 | 10.00 | $1\times10^{1}$ |
| Cottonseeds | Davangere | 5 | 3 | 3 | 60.00 | - |
| Cottonseeds | Dharwad | 1 | 1 | 1 | 100.00 | - |
| Fine wheat bran | Mysore | 22 | 11 | 11 | 50.00 | $10.25\times10^{1}$ |
| Groundnut seed cake | Mandya | 12 | 1 | 0 | - | $0.5\times10^{4}$ |
| Maize meal | Mysore | 1 | 0 | 0 | 00.00 | - |
| Maize meal | Mandya | 2 | 1 | 1 | 50.00 | $2.5\times10^{1}$ |
| Maize pellets | Mysore | 7 | 3 | 3 | 42.85 | $67.5\times10^{1}$ |
| Maize pellets | Mandya | 7 | 7 | 7 | 100.00 | $23.7\times10^{1}$ |
| Maize powder | Mysore | 10 | 6 | 6 | 60.00 | $1.45\times10^{1}$ |
| Sunflower seed cake | Mysore | 1 | 1 | 1 | 100.00 | $8.5\times10^{2}$ |
| Sunflower seed cake | Mandya | 2 | 0 | 0 | 00.00 | - |
| Wheat flakes | Mysore | 13 | 0 | 0 | 00.00 | - |
| Wheat flakes | Mangalore | 1 | 0 | 0 | 00.00 | - |
| Total | | 107 | 35 | 34 | | |

Table 2: Per cent incidence of *Fusarium* species in poultry feeds collected from different districts of Karnataka

| Place | No. of samples screened | No. of positive samples | No. of positive samples for *F. verticillioides* | Percentage incidence | CFU ($g^{-1}$) |
|---|---|---|---|---|---|
| Mysore | 55 | 44 | 44 | 80 | $14.75\times10^{1}$ |
| Mandya | 1 | - | - | - | - |
| Bangalore | 2 | 2 | 2 | 100 | $0.5\times10^{1}$ |
| Chitradurga | 4 | 1 | 1 | 25 | $1.5\times10^{2}$ |
| Hubli | 3 | 3 | 3 | 100 | $1.5\times10^{1}$ |
| Bellary | 2 | 1 | 1 | 50 | $0.5\times10^{5}$ |
| Bhagalakote | 1 | 1 | 1 | 100 | $1.5\times10^{2}$ |
| Gadag | 1 | 1 | 1 | 100 | $1.3\times10^{1}$ |
| Hospet | 1 | 1 | 1 | 100 | $0.5\times10^{2}$ |
| Tumkur | 1 | - | - | - | - |
| Total | 71 | 54 | 54 | | |

Table 3: Frequency of *fusarium* species in poultry feed mixtures

| Species | Total isolates | *Fusarium* species | Relative frequency(%) |
|---|---|---|---|
| **Poultry feed mixtures** | | | |
| *Fusarium verticillioides* | 155 | 135 | 87.00 |
| *Fusarium pallidoroseum* | 155 | 17 | 10.96 |
| *Fusarium oxysporum* | 155 | 2 | 01.29 |
| *Fusarium solani* | 155 | 1 | 00.60 |
| **Animal feeds** | | | |
| *Fusarium verticillioides* | 175 | 159 | 90.85 |
| *Fusarium oxysporum* | 175 | 10 | 05.71 |
| *Fusarium pallidoroseum* | 175 | 5 | 02.85 |
| *Fusarium solani* | 175 | 1 | 00.57 |

*Fusarium* species have been one of the most economically important groups of fungi. Reducing the realization value of cereals as food, feed and grain cereal, toxins produced by the fungi cause chronic and acute poisoning and allergic signs both to animals and humans. Therefore, throughout the world great attention is paid to investigating *Fusarium* species and elaborating means for controlling them. Contamination of cereals with *Fusarium* toxins is a global problem, occurring in Europe, the Americas, Asia and Australia (Placinta *et al*., 1999).

Many strains of *F. verticillioides*, isolated not only from corn and feeds but also from other substrates such as sorghum and millet, have been shown to produce fumonisins (Nelson *et al*., 1991; Norred *et al*., 1993). Since the number of *F. verticillioides* isolated from poultry feed mixtures and animal feedstuffs were very high, it poses a

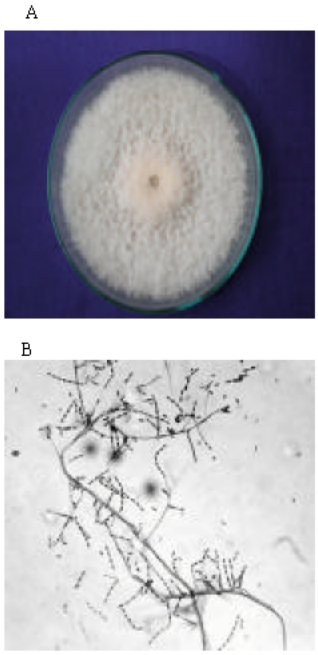


Fig. 1: *F. verticillioides*: (A) Colony on PDA after 7 days of incubation and (B) Microconidia in chains on monophialides under 450X

potential toxigenicity in these samples from the region of Karnataka, India. Studies conducted in the region of Karnataka have reported the occurrence of potential fumonisin-producing *Fusarium* species (Sreenivasa *et al*., 2006) from freshly harvested maize samples. In India, Janardhana *et al*. (1999) has reported mycotoxin contamination in maize grains. Nevertheless, it is corn that is used as one of the major ingredients in the production of poultry feed mixtures as well as a variety of animal feedstuffs like maize meal, maize pellets, maize powder, etc. This is evident from our investigation where, maize pellets showed a high incidence of *Fusarium* species whose major component is corn. *F. verticillioides*, the major species isolated in our investigation is also the most prolific fumonisin-producing fungus.

Sixteen out of the thirty-three samples of poultry mixed feeds collected from the region of Nitra, Slovakia, assayed for the incidence of *Fusarium* species revealed that *F. proliferatum* was the most frequently isolated species (Labuda *et al*., 2003).

However, samples assayed by us recorded *F. verticillioides* as the most frequently isolated species. Pieckova and Jesenska (2001) has reported the production fumonisin $B_1$ by strains of *F. verticillioides* from maize products in Slovakia. One of the major concerns about fumonisins in animals is that it has been involved in Equine Leukoencephalomalacia (ELEM) (Thiel *et al*., 1991), pulmonary oedema in swine and toxic effects in poultry. Toxicoses have been observed in at least two species (Equidae and swine) and are suspected of being caused by consumption of moldy feeds. Studies to determine whether *F. verticillioides* contamination and fumonisins might cause disease in other farm animals, especially poultry, are being conducted by several investigators. Mycotoxicoses arising from the consumption of food containing ingredients contaminated with mycotoxins has been a major problem for poultry producers in many countries (Bhat *et al*., 1997; Prathapkumar *et al*., 1997). In India, aflatoxicosis has been well recognized as a major problem in poultry mainly because of the popularity of maize (Gutema *et al*., 2000) and sorghum as a dietary ingredient. Poultry are apparently more resistant to fumonisins than are swine and equines. In India corn and sorghum are used as bulk ingredients in the production of animal as well as poultry feeds. Also sunflower seed cake and groundnut seed cake are used in the production of poultry feed mixtures. Hence, the chances of *Fusarium* contamination in such feeds can be expected beyond doubt. The occurrence of *F. verticillioides* species in animal and poultry feed mixtures in India, can pose a threat to animal and poultry health (Prathapkumar *et al*., 1997). The fusaria could thus be a potential source of *Fusarium* mycotoxins and in turn the animal and poultry feed mixtures harboring such fusaria. This study warrants the need for analyzing the samples for *Fusarium* mycotoxins, especially fumonisins and also to design effective management strategies to prevent the occurrence *Fusarium* and fumonisin contamination in cereals widely used in production of animal and poultry feeds.

## CONCLUSIONS

The study highlights a potential risk of animal and poultry feedstuffs getting contaminated with hazardous toxic compounds, thus making it necessary for further analysis and continual monitoring and evaluation of such feeds before they reach the consumer for animal and poultry nutrition, respectively.

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## REFERENCES

Bhat, R.V., H.S. Prathapkumar, P.A. Rao and R.V. Sudershan, 1997. A food borne disease outbreak due to consumption of moldy sorghum and maize containing fumonisin mycotoxins. Clin. Toxicol., 35: 249-255.

Booth, C., 1977. Laboratory Guide to the Identification of Major Species. Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey.

Bragulat, M.R., E. Martinez, G. Castella and F.J. Cabanes, 2004. Selective efficacy of culture media recommended for isolation and enumeration of *Fusarium* species. J. Food. Prot., 67: 207-211.

Chelkowski, J., A. Visconti, B. Dokko and H. Wisniewska, 1995. *F. verticillioides* Sheldon-Pathogenicity to Wheat seedlings and ability to produce fumonisins. J. Phytopathol., 143: 491-493.

Gelderblom, W.C.A., K. Jaskiewiez, W.F.O. Marasas, P.G. Thiel, R.M. Horak, R. Vleggaar and N.P.J. Kriek, 1988. Fumonisins-Novel mycotoxins with cancer-promoting activity produce by *F. verticillioides*. Applied Environ. Microbiol., 54: 1806-1811.

Gelderblom, W.C.A., N.P.J. Kriek, W.F.O. Marasas and P.G. Thiel, 1991. Toxicity and carcinogenicity of *F. verticillioides* metabolite, fumonisin $B_1$ in rats. Carcinogenesis, 12: 1247-1251.

Gutema, T., C. Munimbazi and L.B. Bullerman, 2000. Occurrence of fumonisins and moniliformin in corn and corn based food products of US origin. J. Food Prot., 63: 1732-1737.

Harrison, L.R., B.M. Colvin, J.T. Greene, L.E. Newman and J.R. Cole, 1990. Pulmonary oedema and hydrothorax in swine produced by fumonisin $B_1$, a toxic metabolite of *F. verticillioides*. J. Vet. Diagn. Invest., 2: 217-221.

Janardhana, G.R., K.A. Raveesha and H.S. Shetty, 1999. Mycotoxin contamination of maize grains grown in India (Karnataka). Food Chem. Toxicol., 37: 863-868.

Kellerman, T.S., W.F.O. Marasas, P.G. Thiel, W.C.A. Gelderblom, M. Cawood and J.A.W. Coetzer, 1990. Leucoencephalomalacia in two horses induced by oral dosing of fumonisin $B_1$. Onder. J. Vet. Res., 57: 269-275.

Labuda, R., D. Tancinova and K. Hudec, 2003. Identification and enumeration of *Fusarium* species in poultry feed mixtures from Slovakia. Ann. Agric. Environ. Med., 10: 61-66.

Leslie, J.F. and B.A. Summerell, 2006. The *Fusarium* Laboratory Manual. 1st Edn., Blackwell Pub. USA.

Lumlertdacha, R.T., R.A. Lovell, S. Shelby, D. Lenz and B.W. Kempainen, 1995. Growth, hematology and histopathology of channel catfish, *Ictalurus punctatus* fed toxins from *F. verticillioides*. Aquaculture, 130: 201-218.

Nelson, P.E., R.D. Plattner, D.D. Shackelford and A.E. Desjardins, 1991. Production of fumonisins by *F. verticillioides* strains from various substrates and geographic areas. Applied Environ. Microbiol., 57: 2410-2412.

Norred, W.B., 1993. Fumonisins: Mycotoxins produced by *F. verticillioides*. J. Toxicol. Environ. Health., 38: 309-328.

Pieckova, E. and Z. Jesenska, 2001. *F. moniliforme*, *Fusarium subghutinans* and *Aspergillus flavus* in maize products in Slovakia. Czech. Mycol., 53: 229-235.

Placinta, C.F., J.P. D'Mello and A.M. MacDonald, 1999. A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. Anim. Feed Sci. Technol., 78: 21-37.

Prathapkumar, S.H., V.S. Rao, R.J. Paramkishan and R.V. Bhat, 1997. Disease outbreak in laying hens arising from the consumption of fumonisin contaminated food. Br. Poult. Sci., 38: 475-479.

Sreenivasa, M.Y., R.S. Dass, A.P. Charith Raj and G.R. Janardhana, 2006. Molecular detection of fumonisin producing *Fusarium* species of freshly harvested maize kernels using Polymerase Chain Reaction (PCR), Taiwania, 51: 251-257.

Suzuki, C.A.M., L. Hierlihy, M. Barker, I. Curran, R. Mueller and G.S. Bondy, 1995. The effects of fumonisin $B_1$ on several markers of nephrotoxicity in rats. Toxicol. Applied Pharmacol., 133: 207-214.

Thiel, P.G., G.S. Shephard, E.W. Sydenham, W.F.O. Marasas, P.E. Nelson and T.M. Wilson, 1991. Levels of fumonisin $B_1$ and $B_2$ in feeds associated with confirmed cases of equine leucoencephalomalacia. J. Agric. Food. Chem., 39: 109-111.

Weibking, T.S., D.R. Ledoux, A.J. Bermudez, J.R. Turk, G.E. Rottinghaus, E. Wang and A.H. Merrill, 1993. Effects of feeding *F. verticillioides* culture material, containing known levels of fumonisin $B_1$ on the young broiler chick. Poult. Sci., 72: 456-466.

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