# Natural Occurrence of Fumonisin $B_1$ and Its Co-occurrence with Aflatoxin $B_1$ in Indian Sorghum, Maize, and Poultry Feeds

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A total of 138 samples consisting of 19 rain-affected sorghum, 25 rain-affected maize, 35 normal maize, 44 normal sorghum, and 14 poultry feed samples collected from six different places were analyzed for fumonisin $B_1$ and aflatoxin $B_1$. All the rain-affected sorghum and maize samples were contaminated with fumonisin $B_1$ in the range 0.07−8 and 0.04−65 mg/kg, respectively. On the other hand 26 normal maize samples and 2 normal sorghum samples were contaminated with fumonisin $B_1$ at levels ranging from 0.01 to 5 and 0.15 to 0.51 mg/kg. Fumonisin $B_1$ contamination in the poultry feed samples ranged from 0.02 to 0.26 mg/kg. All of the rain-affected samples and poultry feed samples were contaminated with aflatoxin $B_1$. 20% of normal sorghum and 89% of normal maize samples also contained aflatoxin $B_1$ in the range of 5−125 and 0.38−109 $\mu$g/kg, respectively. This study reports for the first time the natural co-occurrence of fumonisin $B_1$ and aflatoxin $B_1$ in Indian maize and poultry feeds.

**Keywords:** *Fumonisin $B_1$; aflatoxin $B_1$; Fusarium moniliforme; co-occurrence; sorghum; maize; poultry feeds*

## INTRODUCTION

*Fusarium moniliforme* is a ubiquitous fungus occurring in cereals, millets, and other hosts worldwide (Marasas *et al.*, 1984) that produces a group of closely related mycotoxins called fumonisins, which were isolated and purified recently (Gelderblom *et al.*, 1988). Within a decade of its discovery, it has been shown that fumonisins are of importance second only to aflatoxins. Although several disease outbreaks in animals have been associated with consumption of *F. moniliforme* contaminated maize, recent studies have shown that leukoencephalomalacia in horses (Marasas *et al.*, 1988) and pulmonary edema and hydrothorax in pigs are attributed to fumonisin contamination in feeds (Ross *et al.*, 1992). In addition, the high incidence of human oesophageal cancer in certain parts of the world such as Transkei of South Africa (Rheeder *et al.*, 1992) and Linxian China (Luo *et al.*, 1990) has been epidemiologically correlated with fumonisins. The target organ of fumonisin toxicity is different in different animals. It causes brain lesions in horses, pulmonary toxicity in swine, liver toxicity and liver cancer in rats, atherosclerosis in monkeys, rickets and immune suppression in poultry (Norred, 1993), and nephrosis in sheep (Edrington *et al.*, 1995). Fumonisin ingestion elevates sphingosine to sphinganine ratios, and these changes occurred more quickly than did the elevation of serum enzymes (Wang *et al.*, 1992).

The natural occurrence of fumonisin in maize and maize-based products has been reported from different parts of the world such as South Africa (Sydenham *et al.*, 1990, 1991), North America (Murphy *et al.*, 1993), South America (Julian *et al.*, 1995; Chulze *et al.*, 1996; Sydenham *et al.*, 1992, 1993), Europe (Pittet *et al.*, 1992; Sanchis *et al.*, 1994), and Asia (Yamashita *et al.*, 1995; Ueno *et al.*, 1993). High levels of fumonisin $B_1$ were reported in maize ears visibly infected with *F. moniliforme* collected from a local field in India (Chatterjee and Mukherjee, 1994). In another study (Chourasia and Shelby, 1996), maize ears collected randomly from 13 different fields showed fumonisin $B_1$ and $B_2$ levels ranging from 1200 to 2500 and 120 to 950 $\mu$g/kg, respectively. High naturally occurring levels of fumonisin were reported from the high human oesophageal cancer incidence areas of South Africa (Sydenham *et al.*, 1990) and China (Yoshizawa *et al.*, 1994). Fumonisins were found to co-occur with aflatoxin $B_1$ (Ueno *et al.*, 1993; Chamberlain *et al.*, 1993) and other *Fusarium* mycotoxins (Chu and Li, 1994; Yamashita *et al.*, 1995) or with both (Wang *et al.*, 1994). Different levels of fumonisins were found in maize-based feeds of horses and pigs associated with disease outbreaks (Thiel *et al.*, 1991b; Ross *et al.*, 1991, 1992). A chicken feed sample associated with diarrhoea outbreak had 5000 $\mu$g/kg of fumonisin $B_1$ (Sydenham *et al.*, 1992). In another study of poultry feeds from Swiss market, 6/22 feed samples had fumonisin $B_1$ at up to 480 $\mu$g/kg (Pittet *et al.*, 1992).

In the present study an attempt is made to assess the natural occurrence of fumonisin $B_1$ and its co-occurrence with aflatoxin $B_1$ in sorghum, maize, and feed samples collected from different places in India.

## MATERIALS AND METHODS

**Samples.** Sorghum and maize samples were collected from households and retail shops from six different places from South and North India. Samples also included rain-affected sorghum and maize from Andhra Pradesh. Feeds were collected from local poultry farms. Details of samples collected are given in Table 1.

**Mycology.** Grains were surface sterilized with 0.4% sodium hypochlorite solution and plated on selective medium for *Fusarium* and dematiaceous hypomycetes in cereals (Andrews and Pitt, 1986). *Fusarium* colonies were picked up and transferred to PDA slants. Isolates belonging to section Lisiola were identified with the differential medium of Clear and Patrick (1992).

**Analytical Standards.** Fumonisin $B_1$ and aflatoxin $B_1$ standards were purchased from Sigma (St. Louis, MO).

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**Table 1. Place, Type, and Numbers of Samples Collected**

| place of collection | commodity | number of samples |
|---|---|---|
| North India | | |
| Delhi town | maize | 5 |
| Hapud city | maize | 8 |
| South India | | |
| Hyderabad town | maize | 8 |
| | sorghum | 44 |
| | poultry feeds | 14 |
| Laxmapur village | maize | 14 |
| Domakonda village | rain-affected maize | 19 |
| Noorlapoor village | rain-affected sorghum | 25 |

**Determination of Fumonisin $B_1$.** Maize and poultry feed samples were analysed for fumonisin $B_1$ by the method of Stack and Eppley (1992). In brief, finely ground samples were extracted with methanol−water (3:1), cleaned up on Supelclean strong anion exchange catridge (LC-SAX Supleco) followed by precolumn derivatization with *o*-phthalaldehyde and high-performance liquid chromatography (HPLC) on a reverse phase column (Bondclone RP-18, 150 × 3.9 mm, Phenomenex). The mobile phase consisted of acetonitrile−water−acetic acid (50:50:1). The same method was also used for the sorghum samples as the recoveries were comparable.

**Determination of Aflatoxin $B_1$.** The standard AOAC procedure (Scott, 1990) was used for aflatoxin extraction and cleanup which involves extraction with acetone−water (85:15), cleanup on a silica gel column by eluting with chloroform−acetone (9:1), and further derivatization with trifluoroacetic acid. The derivative was detected by HPLC analysis according to the method of Park *et al.* (1990).

**Statistical Analysis.** Regression analysis was carried out to assess the correlation between the occurrence of fumonisin $B_1$ and aflatoxin $B_1$. Mean values presented are geometric means.

## RESULTS AND DISCUSSION

Incidence and levels of fumonisin $B_1$ and aflatoxin $B_1$ in sorghum, maize, and poultry feed samples are shown in Table 2. The highest contamination of fumonisin $B_1$ is seen in the rain-affected maize and lowest is observed in the feeds. Rain-affected samples showed higher contamination with fumonisin $B_1$ than the normal samples. 74% of normal maize and 5% of normal sorghum samples were contaminated with fumonisin $B_1$ in levels ranging from 0.01 to 4 mg/g, but all of the rain-affected samples were contaminated with fumonisin $B_1$. The levels were in the range 0.07−8 and 0.04−65 mg/kg for sorghum and maize, respectively. 36% of feed samples were contaminated with fumonisin $B_1$ in the range 0.02−0.26. This appears to be the first report of natural occurrence of fumonisin $B_1$ in sorghum, although many strains isolated from sorghum have been shown to prouce fumonisin $B_1$ in the laboratory (Bacon and Nelson 1994). The method reported for maize and maize products was successfully applied to the sorghum samples in the present study which gave similar (70% ± 4%) recoveries although the detection limit was higher (25 ng instead of 10 ng).

All the rain-affected maize and poultry feed samples were contaminated with aflatoxin $B_1$ in the range 5.0−125 and 0.38−109 $\mu$g/kg, respectively. All of the rain-affected sorghum samples except one were contaminated with aflatoxin $B_1$ (range 2.0−830 $\mu$g/kg). 20% of normal sorghum samples and 89% of normal maize samples were contaminated with aflatoxin $B_1$. As high as 4030 $\mu$g/kg of aflatoxin $B_1$ was found in one sample of normal maize. Even though high levels of aflatoxin $B_1$ were found in normal maize and rain-affected sorghum, the mean level of fumonisin $B_1$ was highest in the poultry feeds.

Fumonisin $B_1$ and aflatoxin $B_1$ were found to co-occur in all of the rain-affected maize samples and in all but one sample of rain-affected sorghum samples. 63% of normal maize and 36% of poultry feed samples contained both fumonisin $B_1$ and aflatoxin $B_1$. The two normal sorghum samples which had fumonisin $B_1$ were also contaminated with aflatoxin $B_1$. The rain-damaged maize sample containing the highest fumonisin $B_1$ level had a very low level (20 $\mu$g/kg) of aflatoxin $B_1$. On the other hand, the normal maize sample with highest aflatoxin $B_1$ level had a high level of fumonisin $B_1$ (2 mg/kg).

The frequency distribution of fumonisin $B_1$ and aflatoxin $B_1$ in the contaminated samples is given in Table 3. Most of the normal maize and sorhum samples contained low levels of both fumonisin $B_1$ (less than 1.0 mg/kg) and aflatoxin $B_1$ (<40 $\mu$g/kg). More than 50% of the rain-affected maize samples contained more than 1 mg/kg of fumonisin $B_1$ and more than 40 $\mu$g/kg of aflatoxin $B_1$. All of the poultry feed samples contained fumonisin $B_1$ levels lower than 0.5 mg/kg, and more than 50% of the samples contained aflatoxin $B_1$ levels higher than 40 $\mu$g/kg.

A total of 26 strains of *Fusarium* sp. were isolated, of which 16 strains are from maize, 7 strains are from sorghum, and 3 strains are from poultry feeds. Nineteen of the isolates belonged to *F. moniliforme* of which 11 were from maize, 6 were from sorghum, and 2 were from poultry feeds. From maize samples, four isolates and from sorghum samples and one isolate of *F. proliferatum* were obtained. One isolate each was obtained from maize and feeds.

Although the occurrence of fumonisin $B_1$ of *F. subglutinans* in standing maize plants has been reported from India, this is the first report of occurrence of fumonisin in maize grains in stored conditions and the co-occurrence of fumonisin $B_1$ and aflatoxin $B_1$ in maize. Although outbreak of aflatoxic hepatitis due to consumption of maize contaminated with aflatoxin was reported earlier by our Institute (Krishnamachari *et al.*, 1975), information on the occurrence of fumonisin $B_1$ in those maize samples is not available.

*F. moniliforme* is basically a preharvest fungus. Therefore, occurrence of fumonisin is more of a preharvest problem. Toxin production can occur during or immediately after harvest in case of unseasonal rains.

**Table 2. Incidence and Levels of Fumonisin $B_1$ and Aflatoxin $B_1$ in Different Commodities**

| | fumonisin $B_1$ | | | aflatoxin $B_1$ | | |
|---|---|---|---|---|---|---|
| commodity | incidence (positives/total) | range (positives) (mg/kg) | mean (mg/kg) | incidence (positives/total) | range (positives) ($\mu$g/kg) | mean ($\mu$g/kg) |
| normal sorghum | 2/43 | 0.15−0.51 | 0.27 | 9/44 | 0.18−30.34 | 1.97 |
| rain-affected sorghum | 25/25 | 0.07−7.8 | 0.48 | 24/25 | 2.0−830.0 | 24.01 |
| normal maize | 26/35 | 0.01−4.74 | 0.62 | 31/35 | 0.11−4030.47 | 2.6 |
| rain-affected maize | 19/19 | 0.04−64.7 | 1.17 | 19/19 | 5.0−126.0 | 14.38 |
| poultry feeds | 5/14 | 0.02−0.26 | 0.10 | 14/14 | 0.38−108.61 | 37.88 |

**Table 3. Frequency Distribution of Fumonisin $B_1$ and Aflatoxin $B_1$ in Contaminated Sorghum, Maize, and Poultry Feeds**

| | fumonisin $B_1$ (mg/kg) | | | | | aflatoxin $B_1$ ($\mu$g/kg) | | | |
|---|---|---|---|---|---|---|---|---|---|
| commodity | <0.5 | 0.5−1.0 | 1.0−1.5 | 1.5−2.0 | >2.0 | <20 | 20−40 | 40−60 | >60 |
| normal sorghum | 1 | 1 | 0 | 0 | 0 | 8 | 1 | 0 | 0 |
| rain-affected sorghum | 13 | 14 | 3 | 1 | 4 | 11 | 5 | 2 | 6 |
| normal maize | 12 | 5 | 1 | 1 | 7 | 23 | 2 | 2 | 3 |
| rain-affected maize | 8 | 0 | 0 | 1 | 10 | 6 | 2 | 2 | 9 |
| poultry feeds | 5 | 0 | 0 | 0 | 0 | 2 | 4 | 2 | 6 |

Production of fumonisin is very sensitive to water activity. When the water activity increases from 0.9 to 1, there can be an increase in fumonisin production as high as 300-fold (Cahagnler *et al.*, 1995). During harvesting the ears are heaped in the field, and if there is rainfall during this period, favorable conditions will be created for toxin production. Significant amounts of toxin may be formed either in the preharvest stage or during harvest, and these might just get carried on to the storage stage. Chulze *et al.* (1996) showed that in the field most of the toxin production occurs toward the later stage of maturity.

No correlation was found between fumonisin $B_1$ and aflatoxin $B_1$ contamination in maize and sorghum samples ($r = 0.0178$ and $r = 0.16897$, $P < 0.05$). This result is in agreement with the earlier report (Chamberlain *et al.*, 1993) and differs from a recent report (Yoshizawa *et al.*, 1996) in which a negative correlation was found. *Aspergillus flavus* and *F. moniliforme* infect maize ears by different routes. *A. flavus* is nonpathogenic fungus colonizing through the silk ears and cracks in the pericarp of maize grain and need not be seedborne. *F. moniliforme* is endophytic to maize, entering the kernel through the pedicle to occupy the internal space distal to tip cap and is primarily seedborne (Chamberlain *et al.*, 1993). *F. moniliforme* was shown to inhibit the kernel infection with *A. flavus* and aflatoxin production (Zummo and Scott, 1992) in laboratory conditions. In the present study in maize and sorghum naturally contaminated with both the fumonisin- and aflatoxin-producing fungi, the concentrations of aflatoxin $B_1$ and fumonisin $B_1$ in the kernels were independent. In the natural conditions, colonization and distribution of the two fungi in the grain might be different and the condition may not be truly simulated in the artificially inoculated grain.

When compared with the earlier report (Pittet *et al.*, 1992), both incidence (35%) and level of contamination in the poultry feeds (36 $\mu$g/kg mean) were much lower in the present study (27% and 235 $\mu$g/kg, respectively). This is the first report of co-occurrence of fumonisin $B_1$ and aflatoxin $B_1$ in poultry feeds. Co-contamination of fumonisin and aflatoxins at varying levels in feeds is of concern to the poultry industry. It should also be noted that presently poultry farmers are aware of only aflatoxins, and only aflatoxins are checked routinely in feeds. In view of the known effects of fumonisin on poultry (Brown *et al.*, 1992; Ledoux *et al.*, 1992) and possible synergestic action of fumonisin $B_1$ with other mycotoxins like aflatoxins, due importance should be given to fumonisin contamination in feeds and proper safe limits need to be established.

In conclusion, this paper reports for the first time the occurrence of fumonisin $B_1$ in sorghum and its co-occurrence with aflatoxin $B_1$ and the occurrence and co-occurrence of fumonisin $B_1$ and aflatoxin $B_1$ in Indian maize and poultry feeds.

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