Occurrence of Fumonisin B1 in Maize Kernels, Poultry and Livestock Feeds in Tamil Nadu, India

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ABSTRACT

The level of mycotoxin fumonisin B1 in maize kernels, poultry and livestock feeds collected from various parts of Tamil Nadu, India has been analyzed using High performance Liquid Chromatographic coupled with evaporative light scattering detector (HPLC-ELSD). A total of 41 Maize samples (21 pre and Post-harvest maize kernels, 12 poultry feeds and 8 livestock feeds) obtained from field, Poultry farm and retail shops. The results indicated that 71% maize kernels, 75% poultry feeds and 62% livestock feed samples contains FB1 with the levels ranged from 27.24 – 118.21 ppm 31.56 – 93.75 ppm and 25.16 – 104.51 ppm respectively. It indicated widespread prevalence of fumonisin B1 in maize, poultry and livestock feeds in different parts of Tamil Nadu, India.

Keywords

Mycotoxin, Fumonisin, HPLC-ELSD

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Introduction

Fumonisins are Mycotoxins produced mainly by the fungus Fusarium verticillioides a primary fungal contaminant of Maize and maize based products throughout the world (Shephard et al., 1996). It was first discovered in South Africa in 1988 (Gelderblom et al., 1988; Marasas et al., 2001) FB1 is the most commonly found, not only in maize and maize-based foods, but also in beer, rice, sorghum, triticale, cowpea seeds, beans, soybeans and asparagus. FB1 can cause two diseases in farm animals. i.e leucoencephalomalacia and porcine pulmonary oedema in horses. It is also carcinogenic, hepatotoxic, nephrotoxic and embryotoxic in laboratory animals. In humans, fumonisins are associated with oesophageal cancer and neural tube defects based on studies conducted in Transkei and Texas (Marasas et al., 2001). The International Agency for Research on Cancer (IARC) designated FB1 in Group 2B as ‘possibly carcinogenic to humans’ (IARC 1993). Till now, twenty-eight types of fumonisins have been isolated and they can be classified into A, B, C and P series. FB1, FB2 and FB3 are the principal fumonisins analyzed as natural contaminants of cereals (Soriano et al., 2005; Wang et al., 2008). F. verticillioides produces several mycotoxins,
the most prominent of which is called fumonisin B1 (FB1). The U.S. Food and Drug Administration guidelines for fumonisin in human foods and animal feeds is 4 µg/g and <50 µg/g respectively.

Mycotoxin contamination in agricultural commodities has considerable economic implications. Losses from rejected shipments and lower prices for inferior quality can devastate the export markets of developing country. In India, more than one-quarter of tested maize samples exceeded the Indian tolerance limit of 30 ppb, and that if Codex standards were applied, nearly one-half (47 per cent) of the samples would have to be rejected from export (Van Egmond, 2002; Bhat et al., 2000) indicating high levels of contamination.

Several surveys have been conducted concerning the natural occurrence of fumonisin in corn samples collected from households in Linxian County. Chu and Li (1994) detected high level (18–155 ppm; mean, 74 ppm) and lower level (20–60 ppm; mean, 35.3 ppm) of fumonisin in household moldy samples. However, lower incidence and level of FB1 were also reported in other surveys conducted in Linxian County by (Yoshizawa et al., 1994) and (Wang and Zhu, 2002). The 91 percent maize samples contains the fumonisin level 0.1 ppm to 87.0 ppm and 84 percent of poultry feeds contain 0.1 ppm to 87.0 ppm level of fumonisin could be observed in Hariyana, India by N. Jindal et al., 1999). The high level FB1 (0.30–3.20 µg/g; mean, 1.42 µg/g) in samples from the granary, followed by household (0.25–1.80 ppm; mean, 0.73 ppm), central market (0.25–1.10 ppm; mean, 0.51 ppm), and store (0.22–0.34 ppm; mean, 0.28 ppm) in china could be detected by (Wang. J. et al., 2008)

The aim of this study was to investigate the level of Fumonisins FB1 contamination in Pre and post-harvest maize kernels, Poultry feeds and livestock feed samples collected from different parts of Tamil Nadu, India using High performance Liquid Chromatographic coupled with evaporative light scattering detector (HPLC-ELSD)

**Materials and Methods**

**Survey and collection of samples**

A Surveys was conducted in different agro-ecological zones of Tamil Nadu, India and a total of 41 Maize samples (21 pre and Post harvest maize kernels, 12 Poultry feeds and 8 live stock feeds) obtained from field, Poultry farm and retail shops in order to understand the magnitude of fumonisin contamination in maize kernels, Poultry and livestock feeds.

**High performance Liquid Chromatography (HPLC) analysis of Fumonisin (FB1)**

The HPLC system is an “Agilent 1200 series”, consists of an isocratic unit with a quaternary pump capable of a flow rate of 0.2 - 10ml/min and a suitable manual injector with Rheodyne 7725i 7-port sample injection valve capable of 10µl injections where the sample is loaded into the external 20 µl sample loop through the injection port. A 15 cm long reversed-phase column containing C18 or C8 modified silica packing material of 3 to 5 µm particle size is present inside the system for the separation of the compounds. The HPLC system is equipped with an evaporative light scattering detector.

The HPLC–ELSD method conditions were performed according to procedures with some modifications (Bojja et al., 2004). The mobile phases were (A) water– trifluoroacetic acid (TFA) (100:0.025, v/v) and (B) acetonitrile–TFA (100:0.025, v/v), with a gradient of 0–20% B in A in the first 5 min, 20–40% B
from 5 to 10 min, 40–80% B from 10 to 15 min, 80% B from 15 to 20 min, and 80–0% B from 20 to 25 min. The flow rate was 1.0 ml/min.

The conditions set for ELSD (Agilent 1260 infinity ELSD) were 45 °C of drift tube temperature, 2.0 l/min nitrogen gas flow and gain value of 1 in the impactor-on mode.

**Extraction and clean-up**

10g of fined maize samples were placed in a flask containing 25 ml Acetonitrile/water (1:1, v/v), then was placed in an orbital shaker overnight, and filtered with Whatman No. 1 paper under vacuum.

Ten milliliters filtrate was transferred into 50-ml centrifugal tube and placed on the ice for 15 min. and centrifuged at 7000 rpm for 10 min at 4°C, then transferred to a new 50-ml centrifugal tube containing 300 mg Amberlite XAD-4 (37380-42-0, Sigma–Aldrich Co., USA) which had been activated with 2 ml methanol and washed with deionized water, and the tube was stirred for 5 h or overnight in an orbital shaker after adding 40 ml deionized water. The XAD-4 beads were then washed with 200 ml deionized water, then transferred the XAD-4 beads to a Bond Elute column without stuffing by deionized water and the toxins were eluted with 3 ml 100% methanol. The eluent was dried under vacuum with freezing at 65°C and dissolved in 200 µl deionized water. The solution was filtered through a 0.2 µm syringe-filter and 20 µl was injected directly into the HPLC system. Calibration plot was prepared and checked for linearity.

**Preparation of standards**

Fumonisin standards: Fumonisin standards were prepared in acetonitrile: water (1:1) and stored at 4°C. Standards stored for long periods in methanol undergo slow degradation. Stock solution of individual fumonisin standards of concentration 250 µg/ml were used, from which a working standard was prepared with a concentration of 10 µg /µl and diluted into four different concentration 0.15, 0.3, 0.6, 1.2 µg /µl. The calibrant stock solutions of individual FB1 acetonitrile-water (50+50, v/v) was prepared. Fumonisin calibrant solutions are stable for 6 months when stored at 4 °C. A calibration curve was prepared from the four different dilutions of 0.15, 0.3, 0.6, 1.2 µg /µl concentration of 10 µg /µl. 20 µl of dilution was directly injected to the HPLC system. Calibration plot was prepared and checked for linearity.

**Determination**

The established condition standard FB1 gave a peak at a retention time of 11.33 min. The peak areas for fumonisin in the sample chromatogram are determined and the amount of each fumonisin analogue injected is determined from the calibration plot. From the calibration curve the amount of Fumonisin level in nanogram in the aliquot of solution injected into the HPLC was read from the calibration curve.

**Results and Discussion**

In the present study 41 samples consisting of pre and post-harvest maize kernels, poultry and livestock feeds collected from farmer’s fields, poultry farms and retail shops were analyzed for FB1 contamination. As shown in Table 1, Fumonisin contamination in maize kernels and feeds was observed in more than 70 per cent of the samples tested. The pre harvest maize samples were contaminated with FB1 at level ranging from 27.24 to 113.9 ppm. Most of the post-harvest maize kernels samples were contaminated with FB1 at level ranging from 38.16 to 118.21 ppm. five samples contained FB1 above 100 ppm.
Table 1: Fumonisin (FB1) contamination in maize kernels, poultry and livestock feed samples in Tamil Nadu, India

| Sample ID | Place     | District | Sample          | FB1 (ppm) |
|-----------|-----------|----------|-----------------|-----------|
| S1        | TNAU      | Coimbatore | Poultry feed   | 37.09     |
| S2        | Kuppanur  | Coimbatore | Post-H         | 0         |
| S3        | Devarayapuram | Coimbatore | Pre-H         | 113.9     |
| S4        | Irugur    | Coimbatore | Post-H         | 41.42     |
| S5        | Karamadai | Coimbatore | Poultry feed   | 0         |
| S6        | Annur     | Coimbatore | Live stock     | 69.42     |
| S7        | Ukkadam   | Coimbatore | Post-H         | 0         |
| S8        | Thethipalayam | Coimbatore | Post-H       | 38.16     |
| S9        | Sulur     | Coimbatore | Pre-H          | 27.24     |
| S10       | Udumalpet | Coimbatore | Poultry feed   | 44.72     |
| S11       | Uthukuli  | Coimbatore | Pre-H          | 34.26     |
| S12       | Pollachi  | Coimbatore | Poultry       | 31.56     |
| S13       | Palladam  | Thirupur  | Live stock     | 0         |
| S14       | Senjerimalai | Thirupur  | Live stock    | 25.16     |
| S15       | Gopichettipalayam | Erode | Post-H | 0         |
| S16       | Moolanur  | Erode    | Poultry feed   | 0         |
| S17       | Bhavanisagar | Erode    | Post-H        | 42.06     |
| S18       | Ammapetai | Erode    | Post-H        | 58.19     |
| S19       | Sathymangalam | Erode    | Live stock    | 0         |
| S20       | Attur     | Salem    | Poultry       | 72.07     |
| S21       | Kandampalayam | Namakkal | Post-H | 0         |
| S22       | Rasiapuram | Namakkal  | Pre-H         | 76.24     |
| S23       | Thiruchenkode | Namakkal  | Poultry feed  | 73.3      |
| S24       | Paramathi | Namakkal  | Live stock    | 41.91     |
| S25       | Vagarai   | Ariyalur  | Post-H        | 67.42     |
| S26       | Thuraiyur | Ariyalur  | Pre-H         | 0         |
| S27       | Jeyankondam | Ariyalur  | Poultry feed  | 89.36     |
| S28       | Padalur   | Perambalur| Post-H       | 82.97     |
| S29       | Thuraimangalam | Perambalur| Live stock | 78.54     |
| S30       | Karai     | Perambalur| Pre-H         | 0         |
| S31       | Ammapalayam | Perambalur| Post-H  | 114.4     |
| S32       | Alanthur  | Perambalur| Poultry feed  | 75.81     |
| S33       | Siruganur | Trichy    | Live stock    | 0         |
| S34       | Srirangam | Trichy    | Pre-H         | 71.08     |
| S35       | Andipatti | Madurai   | Poultry feed  | 0         |
| S36       | Madurai   | Madurai   | Post-H        | 118.21    |
| S37       | Srivilliputhur | Virutchanaged | Poultry | 93.75     |
| S38       | Rajapalayam | Virutchanaged | Live stock | 104.51    |
| S39       | Mamsapuiram | Virutchanaged | Poultry feed | 70.48    |
| S40       | Devathanam | Virutchanaged | Pre-H    | 107.6     |
| S41       | Ottachanthiram | Dindugal  | Post-H     | 72.58     |

Pre-H: Pre harvest maize kernels. Post-H: Post harvest maize kernels
In poultry feeds, FB1 was detected in 9 out of 12 samples and the levels ranged from 31.56 to 93.75 ppm. Among the 8 livestock feed samples evaluated, 5 samples were contaminated with FB1 at level ranging from 25.16 to 104.51 ppm. Among all the samples, the highest level 118.21 ppm of FB1 was observed in the post-harvest maize kernel obtained from the Madurai district. The high level of Fumonisin in maize kernels and feeds present a risk for human and animal consumption. The occurrence of high levels of FB1 in food and feed stuffs has been reported by several workers (Chu and Li, 1994; Yoshzawa et al., 1994; Jindal et al., 1999, Wang and Zhu, 2002). Chu and Li 1994 reported that FB1 content was high with an average level of 19-107.5 (mean 54.65) ppm in maize samples collected from households of Linxian country. However the lower incidence and level of 0.872 ppm were found in the house hold maize samples (Yoshzawa et al., 1994). The work done by Wang and Zhu (2002) reported that FB1 at the concentrations ranged from 1.07 to 2.56 ppm was detected in 50% moldy corns obtained from maize field (Jindal et al., 1999) analyzed a total of 100 maize and 50 poultry feed samples collected from nine and eight districts of Haryana and reported that 91% of maize samples, 84% of poultry feed samples contain a fumonisin levels of 0.1–87.0 ppm and 0.02–28.0 ppm respectively (Wang et al., 2008). The level of FB1 in more than 70 % of the contaminated pre- and post-harvest maize kernel samples exceeded the tolerance level fixed by the World Health Organization. Hence, regular monitoring of the level of fumonisin in maize and its feeds is very important to ensure the safety and quality of maize and its poultry, animal feeds. In the present study the fumonisin concentration in general are exceeded the admissible limits. Inappropriate storage conditions and field contamination of grains could be implicated the fumonisin production.

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