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

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A B S T R A C T

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 25.16-104.51ppm respectively. It indicated widespread prevalence of fumonisin B1 in maize, poultry and livestock feeds in different parts of Tamil Nadu, India.

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 Fumonisin 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 Chromato-graphy (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–ELSD. All samples were analyzed in triplicate.

Preparation of standards

Fumonisin standards: Fumonisin standards were prepared in acetonitrile: water (1:1) and stored at 4^{o}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 FB_1 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 FB_1 at level ranging from 27.24 to 113.9 ppm. Most of the post-harvest maize kernels samples were contaminated with FB_1 at level ranging from 38.16 to 118.21 ppm. five samples contained FB_1 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	Sathyamangalam	Erode	Live stock	0
S20	Attur	Salem	Poultry	72.07
S21	Kandampalayam	Namakkal	Post-H	0
S22	Rasipuram	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	Viruthunagar	Poultry	93.75
S38	Rajapalayam	Viruthunagar	Live stock	104.51
S39	Mamsapuiram	Viruthunagar	Poultry feed	70.48
S40	Devathanam	Viruthunagar	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, FB_1 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 FB_1 at level ranging from 25.16 to 104.51 ppm. Among all the samples, the highest level 118.21 ppm of FB_1 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 FB_1 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 FB_1 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 FB1at 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) analyzed a total of 104 corn kernel samples obtained from local households, granaries, wholesale markets (central markets), and retail markets (stores and supermarkets) and reported that the Fumonisin B1 (FB1) concentration was found with a positive rate of 61.5%, 50%, 33.3%, and 17%, respectively. No fumonisin was detected in samples from the supermarket. The highest FB1 levels (0.30–3.20 ppm; mean, 1.42 ppm) were found 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). The author concluded that the variation of FB1 content among different surveys in corn from households in Linxian County may be related to rainy during and after harvest as well as the improper storage and humidity. It was also reported that the toxins could be formed during post-harvest storage, especially when corn was inadequately stored at high temperature and high relative moisture (Jackson and Jalonski, 2004). It is well known that production of Fumonisin in maize is dependent upon a number of factors such as temperature, humidity, insect injury, handling during harvesting and storage (Wang *et al.*, 2008). The level of FB_1 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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