PREVALENCE OF AFLATOXIN B_1 CONTAMINATION IN PRE- AND POST-HARVEST MAIZE KERNELS, FOOD PRODUCTS, POULTRY AND LIVESTOCK FEEDS IN TAMIL NADU, INDIA

*Ayyathurai Vijayasamundeeswari, Munusamy Mohankumar, Muthusamy Karthikeyan, Selvaraj Vijayanandraj, Vaikuntavasen Paranidharan, Rethinasamy Velazhahan**

Department of Plant Pathology, Centre for Plant Protection Studies,
Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India

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Abstract: Aflatoxins, a group of mycotoxins mainly produced by *Aspergillus flavus* and *A. parasiticus*, have adverse health effects on humans and livestock that ingest aflatoxin- contaminated food products and feeds. To secure the safety of food and feed, regular monitoring of aflatoxin levels is necessary. In order to understand the magnitude of aflatoxin contamination, a survey was conducted in different agro-ecological zones of Tamil Nadu, India and 242 samples consisting of pre- and post-harvest maize kernels, food products, poultry and livestock feeds were collected from farmers' fields, poultry farms, retail shops and supermarkets and analyzed for aflatoxin B_1 (AFB_1) contamination by enzyme- linked immunosorbent assay (ELISA) using antiserum raised against aflatoxin B_1-Bovine serum albumin (AFB_1-BSA). The results indicated that 61.3% of the maize kernel samples were contaminated with AFB_1 and the levels of AFB_1 in 26% of the pre- and post-harvest maize kernels exceeded 20 μg/kg. The highest level of AFB_1 (245 μg/kg) was recorded in post-harvest maize kernel samples. In food products AFB_1 was detected only in two samples out of 30 samples tested. Furthermore, the levels ranged from 0.6 to 3.7 μg/kg. In poultry feeds, AFB_1 was detected in 30 out of 53 samples and the levels ranged from 0.7 to 31.6 μg/kg. Among the 40 livestock feed samples evaluated 29 samples were contaminated with AFB_1 at level ranging from 1.8 to 244.9 μg/kg.

Key words: Aflatoxin B_1, enzyme-linked immunosorbent assay, *Zea mays*, feed

INTRODUCTION

Aflatoxins are a group of mycotoxins that are produced by strains of *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare while growing on groundnuts, maize, cotton, chilli and many other agricultural commodities. Aflatoxins are acutely toxic, carcinogenic, mutagenic, teratogenic, and immunosuppressive to most mammalian species (Eaton and Gallagher 1994). Currently, 18 different types of aflatoxins have been identified, with aflatoxin B_1, B_2, G_1, G_2, M_1, and M_2 being the most common. Aflatoxins B_1 and B_2 are produced by *A. flavus*, whereas B_1, B_2, G_1 and G_2 are produced by *A. parasiticus* (Ong 1975). Aflatoxin M_1, a major metabolite of aflatoxin B_1, may occur in milk and its products if obtained from livestock that have consumed aflatoxin B_1 contaminated feed (Polan *et al*. 1974). Aflatoxin contaminated diet has been linked with the high incidence of liver cancer (Bababunmi *et al.* 1978). Li *et al.* (2001) reported that the levels of aflatoxins B_1, B_2 and G_1 were significantly higher in corn from high incidence areas of human hepatocellular carcinoma. Hence the U.S Food and Drug Administration has set an aflatoxin limit of 20 parts per billion (ppb) for foods and for most feeds and feed ingredients. The European Union has enacted very severe aflatoxin tolerance level of 2 μg/kg aflatoxin B_1 and 4 μg/kg total aflatoxins in nuts and cereals for human consumption (Bankole and Adebanjo 2003). The regulations on the import and sale of aflatoxin contaminated food products results in huge losses each year to the agriculture and feed industries.

Maize and peanut are excellent substrates for the growth of *A. flavus* and aflatoxin production. Furthermore, maize and peanut are the major ingredients in poultry and livestock feeds. Bhat *et al.* (1997) reported that 26% of maize kernels collected from different parts of India were contaminated with AFB_1 beyond the level of Indian standard for consumption (30 μg/kg). Setamou *et al.* (1997) reported that up to 42.5% of maize samples in Benin were contaminated with aflatoxin. Kpodo (1996) reported that the maize samples collected from Ghana contained aflatoxins at levels ranging from 20 to 355 μg/kg. Waliyar *et al.* (2003) reported that 43% of maize samples collected from retail shops or supermarkets in and around Hyderabad, Andhra Pradesh, India were contaminated with toxin with the highest AFB_1 level of 806 μg/kg. Therefore, much emphasis has been focused on the control or elimination of these fungi and/or their

*Corresponding address:
velazhahan@hotmail.com, Ph. 091-422-6611226, Fax: 091-422-6611437

toxic metabolites in food grains and livestock feeds. To secure the safety of food and feed, regular monitoring of aflatoxin levels is necessary. The present study was conducted to understand the level of aflatoxin contamination in maize kernels, food products and feeds in different agro-ecological zones of Tamil Nadu, India.

MATERIALS AND METHODS

Survey and collection of samples

A survey was conducted in different agro-ecological zones (Coimbatore, Dindigul, Madurai, Salem, Theni, Trichy, Namakkal, Perambalur, Viruthunagar and Thoothukudi districts) of Tamil Nadu, India over three years (2005–2007) in order to understand the magnitude of aflatoxin contamination of maize kernels, food products and feeds. Pre- and post-harvest maize kernel samples were collected from farmers' fields. Poultry feed samples were collected from poultry farms from different parts of Tamil Nadu. Food products (Hollow corn, groundnut candies, raw groundnuts) and livestock feeds were obtained from retail shops or supermarkets in different locations of Tamil Nadu.

Antibody production

Polyclonal antibodies against aflatoxin B_1-Bovine serum albumin (AFB_1-BSA) were raised in rabbit as described by Reddy *et al.* (2001). Eighty μg of AFB_1-BSA (Sigma, USA) in 0.25 ml of 0.1 M phosphate buffer (pH 7.0) was emulsified with an equal volume of Freund's complete adjuvant and injected intramuscularly at multiple sites into the hind leg of a New Zealand White inbred rabbit. For the subsequent four injections at weekly intervals, AFB_1-BSA was emulsified with Freund's incomplete adjuvant. The rabbit was bled and the titer of the antiserum was determined by indirect competitive ELISA.

Detection of AFB_1 by indirect competitive ELISA

Samples (2 g) were powdered in a coffee grinder and then mixed with 10 ml of solvent containing 70 ml methanol + 30 ml water + 0.5 g KCl. The mixture was incubated on a rotary shaker for 30 min at 250 rpm at room temperature (28±2°C). The extract was filtered through Whatman No. 41 filter paper and the filtrate was used for determination of AFB_1 content by indirect competitive ELISA following the method of Reddy *et al*. (2001). Briefly, the wells of microtiter plates were coated with 150 μl per well of AFB_1-BSA at a concentration of 100 μg/ml in carbonate coating buffer. The plates were washed with phosphate buffered saline (PBS) containing Tween-20 (PBST) and treated with PBST-BSA. One hundred μl of sample extract or AFB_1 standard was mixed with 50 μl of AFB_1-BSA antiserum (1:6000) in 0.2% PBST-BSA and added into the wells. This step was followed by the addition of alkaline phosphatase labelled goat antirabbit IgG conjugate diluted to 1:4000 in PBST-BSA. *P*-nitrophenyl phosphate prepared in 10% diethanolamine was used as substrate. The plates were incubated at room temperature and then read in an ELISA reader at 405 nm. The concentration of AFB_1 in the samples was calculated based on the absorbance of the AFB_1 standard.

RESULTS AND DISCUSSION

In the present study 242 samples consisting of pre- and post-harvest maize kernels, food products, poultry and livestock feeds collected from farmers' fields, poultry farms, retail shops and supermarkets from different agro-ecological zones of Tamil Nadu, India were analyzed for AFB_1 contamination. As shown in table 1, aflatoxin contamination in maize kernels was observed in more than 61.3% of the samples tested and the levels of AFB_1 in 26% of the pre- and post-harvest maize kernels exceeded 20 μg/kg. The highest level of AFB_1 (245 μg/kg) was recorded in post-harvest maize kernel samples. In food products, AFB_1 was detected only in two samples out of 30 samples tested. Furthermore the levels of AFB_1 ranged from 0.6 to 3.7 μg/kg. In poultry feeds, AFB_1 was detected in 30 out of 53 samples and its levels ranged from 0.7 to 31.6 μg/kg. Among the 40 livestock feed samples evaluated, 29 samples were contaminated with AFB_1 at the level ranging from 1.8 to 244.9 μg/kg (data not shown). The occurrence of high levels of AFB_1 in food and feed stuffs has been reported by several workers (Reddy *et al.* 1984; Singh *et al.* 1984; Balasubramanian 1985; Selvasubramanian *et al.* 1987; Dhavan and Chaudary 1995; Dutta and Das 2001). Dutta and Das (2001) reported that AFB_1 content in feed samples collected from different parts of Northern India was very high with an average of 0.412–0.514 ppm. It is well known that growth of *Aspergillus* spp. and subse-

Table 1. Aflatoxin content in maize kernels and feeds as assessed by indirect competitive ELISA

Sample	No. of samples analyzed	No. of samples with aflatoxin B_1 in the range [μg/kg]				
		0	1–20	20–50	50–100	> 00
Pre-harvest maize kernels	59	12	28	13	3	3
Post-harvest maize kernels	60	34	13	2	3	8
Food samples	30	28	2	0	0	0
Poultry feeds	53	23	29	1	0	0
Livestock feeds	40	11	22	2	2	3

quent production of aflatoxins in maize is dependent on a number of factors such as temperature, humidity, insect injury, handling during harvest and storage (Hell *et al.* 2003). A significant positive correlation between moisture content of maize seeds and *A. flavus* population and aflatoxin production has been reported (Oyebanji and Efiuvwevwere 1999). Karthikeyan *et al.* (2009) reported wide variability in aflatoxin production potential of different isolates of *A. flavus* from maize. Hence the variations in aflatoxin content among different samples may be mainly the result of unsatisfactory storage conditions, high moisture content and the occurrence of aflatoxigenic fungi.

Aflatoxin affects the quality of the commodities thereby hitting the export trade in the international market particularly in the post World Trade Organization (WTO) period. The importing countries impose Sanitary and Phytosanitary specifications (SPS) and Hazard Analysis Critical Control Point (HACCP) before importing agricultural commodities to their countries according to the WTO agreement. In the present study, the level of AFB_1 in more than 26% of the contaminated pre- and post-harvest maize kernel samples exceeded 20 μg/kg, the tolerance level fixed by the World Health Organization. The presence of aflatoxin in maize kernels and feeds presents a risk for human and animal health. Therefore, proper post-harvest handling of maize and proper storage of feeds can greatly help in reducing infection by *Aspergillus* spp. and subsequent contamination with aflatoxins.

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POLISH SUMMARY

WYSTĘPOWANIE PRZED- I POZBIOROWEGO SKAŻENIA ZIARNIAKÓW KUKURYDZY, PRODUKTÓW SPOŻYWCZYCH, DROBIU I PASZY ZWIERZĘCEJ AFLATOKSYNĄ B_1 W TAMIL NADU W INDIACH

Aflatoksyny, grupa mykotoksyn wytwarzanych głównie przez *Aspergillus flavus*, ma szkodliwy wpływ na zdrowie ludzi i zwierząt domowych spożywających produkty spożywcze i paszę skażone aflatoksyną. Aby zapewnić bezpieczeństwo żywności i paszy, potrzebny jest regularny monitoring zawartości w nich aflatoksyny. W celu rozpoznania zakresu skażenia aflatoksyną przeprowadzono badania w różnych agroekologicznych strefach Tamil Nadu w Indiach. Pobrano 242 próby ziarniaków kukurydzy przed i po zbiorze, produkty spożywcze, drób i paszę zwierzęcą uzyskano z pól farmerskich, farm drobiu, sklepów detalicznych i supermarketów, a następ-

nie analizowano je na obecność zakażenia aflatoksyną B_1 (AFB_1) przy wykorzystaniu serum albuminy (AFB_1-BSA). Wyniki badań wskazywały, że 61,3% prób ziarniaków kukurydzy było skażone AFB_1, a poziom AFB_1 w 26% ziarniakach pobranych przed i po zbiorze kukurydzy przekraczał 20 μm/kg. Najwyższy poziom AFB_1 (945 μm/kg) wykryto w próbach ziarniaków pobranych po zbiorze. W produktach spożywczych AFB_1 wykryto tylko w dwóch próbach z 30 prób badanych. Ponad to, poziom ten zamykał się w granicach od 0,6 do 3,7 μm/kg. W paszy dla drobiu, AFB_1 wykryto w 30 próbach z 53 badanych, a poziom AFB_1 wynosił od 0,7 do 31,6 μm/kg. Spośród 40 prób badanej paszy zwierzęcej 29 prób było skażonych AFB_1 na poziomie od 1,8 do 244,9 μm/kg.