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Occurrence of Aflatoxin B1 and Zearalenone in Corn based Food Products

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ABSTRACT: Mycotoxins contaminated food products are emerging as a major food safety concern throughout the world. The purpose of this study was to identify the occurrence of zearalenone and Aflatoxin B1 mycotoxins in corn based food products which are commercially available across Chennai, Tamilnadu, India. As maize is a good source of dietary fibre, which serves as a quality energy product for human consumption. Abundant availability of maize to the humans, makes a major food consumption through various food products. Considering the above impact, this study was conducted for 30 samples for aflatoxin B1 detection and 40 samples for Zearalenone, comprising 10 samples of corn chips, 10 Samples of corn flakes and 10 samples of processed and dried corn for Aflatoxin B1 and 20 samples for Zearalenone which are sold commercially as corn based food products in Chennai, India. The study using Romer's all-purpose method was carried out for extraction of Aflatoxin B1 and similarly multi toxin method was used for extraction of Zearalenone from various corn products and were detected by HPTLC (High performance thin layer chromatography) technique. Outcome of this study reveals, the presence of aflatoxin B1 in food samples was nearly 30%, ranging from 5 µg/kg to 48.54 µg/kg and zearalenone were found in about 35% of the total samples, ranging from a minimum of 33 µg/kg to maximum limit of 218 µg/kg. About 10% of the sample were detected Aflatoxin B1, exceeding the EU limits, similarly 15% of the sample detected Zearalenone exceeding the EU legal limits. Thus, Mycotoxins contaminated food products remains as a food safety concern which is emerging throughout the world, thus this study focus on the levels of toxins present in corn based food products present in the market .

Keywords: Aflatoxin B1, HPTLC (High-Performance Thin Layer Chromatography), Oestrogenic toxin, Zearalenone.

I. INTRODUCTION

Mycotoxins are basically a secondary fungal metabolites which occurs universally in various food and feed [7-8]. The term mycotoxin, derives from the Greek word for fungus as 'Mykes' and Latin word 'Toxicum' meaning poison. Food and feeds contaminated by mycotoxins may cause at present, there more than hundred different mycotoxins that have been discovered and these exhibit different structural diversity with various chemical and physiochemical properties. Several studies conducted to study in detail about the substance interaction and occurrence, but mycotoxins remain challenging to categorize, owing to their diverse chemical structures, biosynthetic origins and their production by a wide number of fungal species. Few mycotoxins do not immediately degrade at higher temperature and therefore are resistant to food processing and may present in the human and animal food supplies [1].

Poisoning caused by mycotoxins is called mycotoxicosis [24]. The most important mycotoxin associated with human body and veterinary diseases, includes aflatoxins and ochratoxin (*Aspergillus* sp.), fumonisins, trichothecenes, and zearalenone. The Maximum limits of these mycotoxins have been defined in several countries and regions [20]. In China, the maximum levels of residue are fixed at 60µg/kg for Zearalenone (ZEN) in cereal and their products (National Health and Family Planning Committee of China, 2017). Also for Aflatoxin (AFB1), the maximum residue limit in wheat & Barley was 5 µg/kg. While for corn, cornmeal and corn products it was 20 µg/kg. Similarly, EU has regulated the maximum residual limit for AFB1 as 2 µg/kg and ZEN at 75 µg/kg in all cereal and products produced from cereals [21]. In India according to FSSAI standards has regulated the maximum permissible limits for aflatoxin b1as 15 µg/kg for cereal based products.

Maize is one of the primary and most versatile emerging crops having wider adaptability under varied agro-climatic conditions. Globally, maize is highest genetic yield potential among the other cereals (APEDA). In India, Maize is the third highest grown cereal crop. In terms of market value, it is one of the most important cereal crops of the world. Maize is utilised as food, feed, and fodder and is a source of products including specialized maize like quality protein maize (QPM), baby corn, cornflakes, corn chips etc. There are five major mycotoxins associated with ear rot diseases of corn [23]. Aflatoxins are mostly found in corn with *Aspergillus* ear rot. Similarly, zearalenone are traced in corn with *Gibberella* ear rot while Ochratoxin is produced in corn ears infected by *Penicillium verrucosum*, and by few *Aspergillus* species.

Numerous study has significantly showed the presence of Mycotoxins in corn based products [22] detected aflatoxin and zearalenone. A study conducted on crude and powdered herbal drugs using TLC method showed Aflatoxin B1 presence in 22.2% and 25% of fresh Amla and powdered baheda [6]. Test was conducted for around 208 maize samples using GC-MS and of that 71 samples were showed AFB1 and 20 samples were detected for Zearalenone. This study revealed the occurrence of two carcinogenic Mycotoxins fumonisins and aflatoxins together with *Fusarium mycotoxins* in corn based products in Asian tropics [22]. A study conducted on detection of AFB1 in rice, showed the contamination of AFB1 for 2% samples out of 1200

samples above the permissible limits (>30 µg/kg 0 [13]. Similarly, test was conducted on 25 rice sample under storage condition using TLC and 72% of the sample showed presence of Aflatoxin B1 and B2 [7]. Another study In china, study was conducted for 103 maize ear or kernel samples and ZEN was detected in 11 out of 16 contaminated samples using UHPLC – MS. Therefore remains a food safety and quality issue on top priority. A concern for food safety in food products contaminated with mycotoxins is emerging throughout the world, thus this study focus on the levels of toxins present in corn based food products present in the market in India. This study was conducted to detect the levels of aflatoxin B1 and zearalenone in various corn products in Indian market as no there is no comprehensive study been conducted. Experiments were conducted to study the qualitative parameters and microbial activity in corn based food products. Screening and detection of aflatoxin B1 and zearalenone in corn-based products done by using High Performance Thin Layer Chromatography.

II. MATERIALS AND METHODS

A total of 40 samples comprising of 10 commercially available corn flakes, 10 corn chips and 10 processed and dried corn were randomly collected from supermarkets, groceries and vendors during the period of October to December 2019. About 250 g of samples was collected and were stored under room temperature in a sterilized bags until analysis. A minimum of 75 g collected products were blended individually using an electric mixer to a powdered form and were stored at room temperature for further analysis. Each grounded sample was put in a sterilized bag and was divided into two parts in the laboratory. One part was used to study qualitative parameters such as moisture content and water activity along with microbial contamination and the second part was kept for mycotoxin analysis.

Aliquots of 25 gms of sample weighed before mycotoxins extraction and about 5g each to determine qualitative parameters and microbial contamination. All reagents were of analytical grade (Emerck). The mycotoxin standards aflatoxin B1 and zearalenone were procured from Sigma Aldrich, USA. These standards were dissolved in specific solvents to get a desired stock concentration: 10ng/µl (10ppm) Aflatoxin B1 in benzene: acetonitrile (98:2) and 1mg/5ml zearalenone in chloroform (200 ppm).Working standards used in the present study were prepared from the stock solution by diluting in their specific solvents: Aflatoxin B1 -1µg/kg and 20 µg/kg for Zearalenone. All the standards were stored in amber color vials at 4℃ and away from bright light source.

All the samples were analysed in triplicates and the results were expressed as an average of three repetitions for qualitative parameters and microbial contamination which were determined using AOAC method [2]. The water-activity was measured using Resistive Electrolytic Hygrometer. The samples were simultaneously analyzed for aflatoxin B1 by Romers all-purpose method [16], while zearalenone were analyzed using multi mycotoxin screening method [18]. The samples after extraction, cleanup, concentrated dried were subjected to High performance thin layer chromatography. After extraction, the samples were placed on a preheated silica gel plate (E.Merck) and to develop using solvents such as toluene, ethyl acetate and 90% formic acid (6:3:1,V/V/V") for zearalenone and acetone: chloroform (1:9,V/V) for Aflatoxin B1. Apparatus concentration of aliquots of mixed working standards were prepared and applied on the TLC plates in the range 1µl- 25µl to give a series of hands covering the range from 1ng to 500ng/ band with the help of Camag Linomat-5 HPTLC applicator. The plates were developed under the specified conditions, dried, scanned densitometrically with the help of Camag scanner-3. The peak area obtained for each concentration level was recorded. Calibration curves were prepared by plotting integrated areas on Y-axis versus concentration on X-axis.

III. RESULTS AND DISCUSSION

A. Moisture content

The moisture content on the wet basis of the grounded corn based food samples were tabulated below. All the samples were analysed in triplicates and the results were expressed as an average of three repetitions. The standard value ranges: Sample A<16%, Sample B<7.5%, Sample C<16%. The mean values are listed in the Table 1.

B. Water-activity

The water-activity for corn based samples were determined and tabulated below. All the samples were analysed in triplicates and the results were expressed as an average of three repetitions. The standard value ranges: Sample A- 0.6-0.65, Sample B- 0.3-0.5, Sample C- 0.6-0.65. The mean values are listed in the Table 2.

C. Total plate count

The microbial contamination of the sample A, B, C after 24 hours are listed in the Table 3.

D. Yeast and mould count

The yeast and mould contamination of the sample A, B, C after 72 hours are listed in the Table 4.

E. Quantification of mycotoxins

The detection of mycotoxin showed that Aflatoxin B1 and Zearalenone has equally contaminated the food stuffs. 30 samples were analysed for Aflatoxin B1 by HPTLC in which about 9 food samples were contaminated similarly 40 samples were analysed for Zearalenone by HPTLC in which 15 of the samples were found to be contaminated.

Table 1: Moisture content.

Sample	Mean value of MC %
A	8.54±1.64
B	3.5±1.33
C	11.2±0.585

Table 2: Water-Activity.

Sample	Water activity	Temperature ℃
A	0.601±0.028	29.7
B	0.362±0.09	29.3
C	0.625±0.012	29.0

Table 3: TPC (the dilution factor - 10^{-1}, TNTC- Too Numerous To Count).

Sample A	24 hours	Sample B	24 hours	Sample C	24 Hours
A1	6	B1	—	C1	10
A2	TNTC	B2	—	C2	27
A3	TNTC	B3	—	C3	TNTC
A4	TNTC	B4	—	C4	8
A5	14	B5	—	C5	TNTC
A6	TNTC	B6	—	C6	TNTC
A7	TNTC	B7	—	C7	TNTC
A8	TNTC	B8	—	C8	-
A9	TNTC	B9	—	C9	TNTC
A10	TNTC	B10	—	C10	-

(The above data's are mean values of triplicate determinations)

Table 4: Yeast and mould count (the dilution factor - 10^{-1},TNTC- too Numerous to Count).

Sample A	72 hours	Sample B	72 hours	Sample c	72 hours
A1	-	B1	—	C1	TNTC
A2	TNTC	B2	—	C2	1
A3	TNTC	B3	—	C3	2
A4	2	B4	—	C4	—
A5	2	B5	—	C5	2
A6	TNTC	B6	—	C6	TNTC
A7	3	B7	—	C7	TNTC
A8	-	B8	—	C8	—
A9	TNTC	B9	—	C9	—
A10	TNTC	B10	—	C10	TNTC

(The above data's are mean values of triplicate determinations)

Table 5: Data summary on mycotoxin analysis by HPTLC.

Food	Mycotoxins	No. of samples tested	No. of samples contaminated	% contaminated	Range ($\mu g\ Kg^{-1}$)		Mean±S.E ($\mu g\ Kg^{-1}$)
					Low	High	
A	AFLATOXIN- B1	10	7	70	5	22	13.28± 6.82
	ZEARALENONE	10	2	20	30	42	36± 8.48
B	AFLATOXIN- B1	10	ND	ND	ND	ND	ND
	ZEARALENONE	10	ND	ND	ND	ND	ND
C	AFLATOXIN- B1	10	2	20	37.5	48.54	43.02± 7.81
	ZEARALENONE	20	12	60	33	218	99.39± 76.13

(The above data's are mean values of triplicate determinations)

The mean range of contamination analysed by HPTLC (High Performance Thin Layer Chromatography) for the above mentioned samples were listed in the Table 5. Aflatoxin B1 were found to be present in about 30% of the sample in which about 10% of the sample were found to be contaminated above the European Union permissible limit (20 µg/kg) alongside zearalenone were found to contaminate about 35% of the food sample from which 15% of the sample exceeded the European Union legal limit (50-100µg/kg).

Among the sample chosen for studies, sample B (corn flakes) detected no contamination whereas sample A (corn chips) and sample C (processed and dried corn) where found to be contaminated at the higher rates. The maximum contamination of Aflatoxin B1 was found at concentration rate of 48.54 µg/kg and zearalenone was found at the maximum concentration rate of 218 µg/kg. On comparison within the samples, showed that sample C were contaminated at higher rate.

Since 1994 many countries have developed regulations for Aflatoxins, ochratoxin and zearalenone in animal feed and human food but the regulations may vary from country to country [17]. The Food Safety and Standards Authority of India (FSSAI) has set a maximum level of Aflatoxin B1 in cereals and cereal based food products as 15 µg/kg whereas in US, the FDA has regulated 20 µg/kg in food and feed [3]. Similarly EU has set a maximum level of 2-12 µg/kg for Aflatoxin B1. There are no limits set by fssai or FDA for zearalenone but EU has different levels for various food stuffs ranging from 50-1000 µg/kg and for Maize intended for direct human consumption (sample C), maize-based snacks(sample A)and maize based cereals(sample B) the maximum level of zearalenone has been set as 100 µg/kg [5].

During 2002-2003, a study was conducted on occurrence of Aflatoxin B1, Zearalenone and Ochratoxin A in maize food samples collected from the commerce of *Maringá* City, Paraná State, Brazil. Out of 121 maize based food products, 3 samples were contaminated with Aflatoxin B1 ranging from the minimum of 8 µg/kg to maximum of 59 µg/kg and one sample detected the presence of Zearalenone with an higher level of 448 µg/kg. The author iterated that lower frequency of maize products were traded in the region but Probable Average Daily Intake (PDI) of aflatoxin B1 was higher [19].

The result obtained from the present study indicates the contamination of mycotoxins in few samples at an higher rate which indicates the potential chronic risk for human health as humans are directly exposed to corn based products in the form of breakfast cereals and snacks.

A study from Bulgaria, during 2007 conducted a study on incidence of Zearalenone and Fuminosins in a Bulgarian cereal production unit. 91 small grains included wheat, barley and maize were detected for

Zearalenone and Fuminosins by Liquid Chromatography. The study revealed that Zearalenone in cereals were low and only single evidence were found with concentration upto 148 µg/kg for maize and Fuminosins were contaminated at higher rates (94% samples were positive) [12].

Our findings were similar to the previous study from Spain, the author has chosen the samples from Spanish commercially available trademarks were 25 samples of corn based foods was chosen according to their higher consumable rate. Zearalenone was detected by HPLC under fluorescence detection with the level ranging from 34-216 µg/kg. The author also cited risk for consumers of corn products and an need for monitoring of products before consumption [4].

Numerous studies has revealed the occurrence of mycotoxins in cereals and cereal based food products. Our findings along with previous data clearly shows that mycotoxins are ubiquitously present in cereals and cereal based food products throughout the world [11].

Purified mycotoxin analysis involved majority of studies, while multitoxin occurrence may require important explanation [17]. However agricultural crops and food stuffs are often contaminated with more than one mycotoxin from where human beings are exposed to multiple mycotoxins at the same time thus humans are exposed to greater toxicity on comparison with single mycotoxin. Aflatoxin B1 and *Fusarium* generally found to cooccur naturally in cereal grains as they share a common target organ [11]. Our findings also showed the cooccurrence of Aflatoxin B1and Zearalenone in 3 out of 30 samples were Aflatoxin B1 were found in higher concentration whereas Zearalenone were found to be under the limit. Thus agricultural practices need to be given importance in mycotoxin detection, as mycotoxin can produce at any stages of practicing. Several control points, such as Good Manufacturing Practices (GMP), selection and development of resistant cultivars with conventional breeding or biotechnology, Good Agricultural Practices (GAP), chemical and biological control during the cultivation, and proper management during the storage, HACCP, may reduce the fungal infestation and growth as well as mycotoxin production in cereal grains[11].

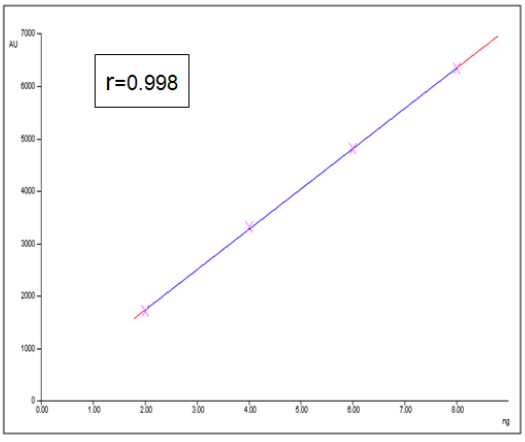

Fig. 1. HPTLC linearity for Aflatoxin B1.

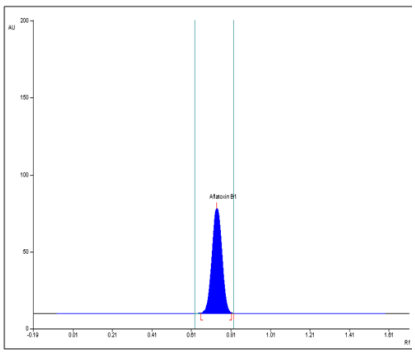

Fig. 2. HPTLC Std. peak for Aflatoxin B1.

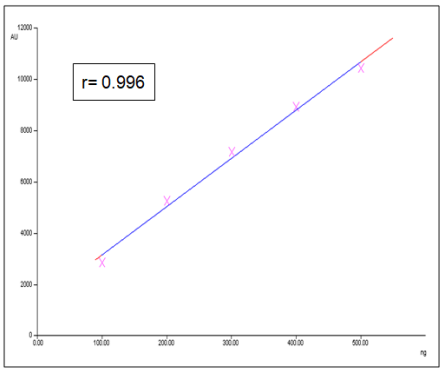

Fig. 3. HPTLC linearity for Zearalenone.

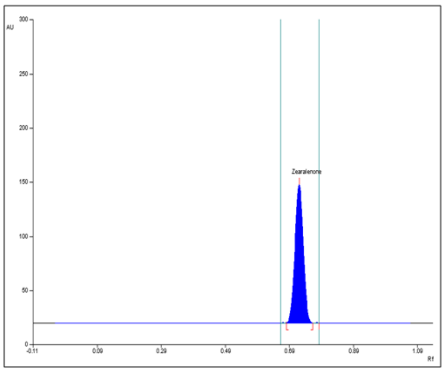

Fig. 4. HPTLC Std. peak for Zearalenone.

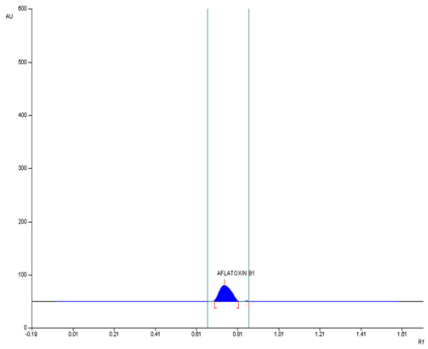

Fig. 5. Aflatoxin B1sample- C peak.

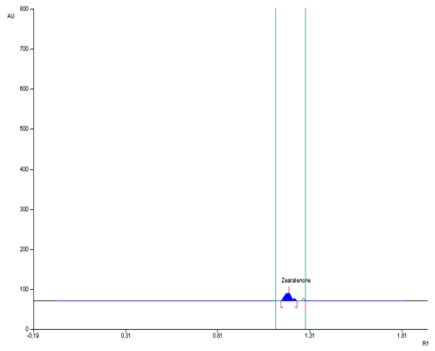

Fig. 6. Zearalenone sample C peak.

IV. CONCLUSION

HPTLC method detection was simple and accurate and also the observations made in our recent study showed the importance for systematically monitoring the levels of Aflatoxin B1 and Zearalenone in food stuffs. This warrants the need for an expanding monitoring programme to generate database on the suspected mycotoxins and establish regulatory permissible levels for setting up of mycotoxin safety standards [17]. Thus, Mycotoxins can be managed to safe levels in corn and corn products through an integrated approach that involves pre-harvest and post-harvest interventions; however, this can be challenging, especially in developing countries where corn is a staple food, and conditions are very favourable for mycotoxin contamination [13].

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Conflict of Interest. The authors declare that there are no conflicts of interest.

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