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Aflatoxin Contaminants Determination in Cold Storage Red Chilli and Ground Nut Using UHPLC for Safety Assessment

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A B S T R A C T

Export of groundnut and chilli products from India is ever-growing and necessitates continuous safety assessment. This study aimed to assay aflatoxin (AFs) contamination from the export quality groundnut and chilli produce using a UHPLC method. Categorized as branded, non-branded and cold storage samples were collected from retail shops and open markets of the Raichur region of South India. As a separate aim, we validated the method for AFB1 in both the groundnut and chilli matrices for linearity (0.993, 0.999), LOD (0.5 & 0.5µgkg^{-1}), LOQ (5.0 & 2.0µgkg^{-1}), recovery (90.66 per cent & 113.33 per cent), robustness (93.33 per cent & 112.66 per cent) and per cent residual (8.48 & 112.66) respectively. Standard linearity generated was in the range of 5- 50.0 ηgg^{-1} in groundnut and 0.5-10ηgg^{-1} in chilli matrix. Sixty-five per cent of the total chilli samples collected were infected by *Aspergillus spp.* and one sample showed AFB1 above the regulatory limit. The levels of AFB1 and total AFs in the contaminated dried chillies from branded, unbranded, cold storage and Radiofrequency treated were in the range of 1.895-29.653µgkg^{-1}, 4.747-6.972µgkg^{-1}, and 7.23-13.17µgkg^{-1}, respectively. In contrast to chilli samples, seventeen per cent of the branded groundnut samples showed an AFB1 concentration of13.59µgkg^{-1}. This study showed that the cold storage chilli and groundnut were aflatoxin-free and thereby safe compared to the branded and non branded samples.

Introduction

The permitted level of aflatoxin in groundnut and red chilli for human consumption, according to European Union and US standards is 4 ppb and 20 ppb respectively (European Commission 2010). India, being largest producer of groundnut and chilli, face difficulties in accessing these markets as the level of aflatoxin is high and is considered unfit for human consumption. In accordance with Food Safety and Standards Authority of India (FSSAI) standards, the permitted level of aflatoxin in India is 30 ppb. The most important aflatoxinare AFG2, AFG1, AFB2 and AFB1probably the immensely studied mycotoxins in the world. Their hepato-toxicity and carcinogenicity is well documented

(IARC 1993). Apart from humans, livestock also suffer from aflatoxicosis (Frisvad *et al.,* 2006).

Since the last decade, numerous qualitative and quantitative techniques have been developed for the quantification of aflatoxin residues in a variety of agricultural products, animal feeds, meat products, milk and milk products. HPLC-FLD using immuno-affinity column clean-up and ELISA methods are the well-known (Aydin *et al.,* 2007; Golge *et al.,* 2013). The aflatoxin isomers *viz.,* AFG2, AFG1, AFB2 and AFB1 were quantified by HPLC to a linearity concentration of 0.2-0.4 μgkg^{-1} with a LOQ of 0.115,0.140,0.111 and 0.110$mgkg^{-1}$ respectively (Bircan, 2005; Golge *et al.,* 2013). A thin layer chromatography method having LOQ of 1.0μgkg^{-1} (Khan *et al.,* 2014) whereas HPLC method with a range of LOQ from 0.05 to 2.5ηg (Rosas-Contreras *et al.,* 2016) and 0.2μgkg^{-1}in cumin samples (Bircan, 2005) is another method available so far.

The fungal growth on stored chilli leads to aflatoxin production and therefore requires monitoring using sensitive and reproducible methods. A study assessed the AFB1 level by ELISA in over 100 samples of powdered red pepper market samples and proved the contamination level in 18 per cent of the samples (Aydin *et al.,* 2007). Similarly, the indirect competitive ELISA used to analyse AFB1 content in different grades of dry red chillies found AFB1 to be approximately 9 per cent containing above the permissible limit (Reddy *et al.,* 2001). Aflatoxin contamination in red chilli collected from different parts of Pakistan by thin-layer chromatography revealed that 46 per cent of powdered and 52 per cent of crushed, showed above MRL of 20μgkg^{-1} (Khan *et al.,* 2014).

The chilli samples in Turkey analysed by HPLC-FLD followed by Immuno-affinity column (IAC) clean-up showed 5kg or above the MRL (Golge *et al.,* 2013). Silica bound monolithic column in HPLC was used to find the contamination of aflatoxin in Malaysian chilli and nine samples showed one or other aflatoxin and the total ranging above the MRL (Khayoon *et al.,* 2012). The market cayenne pepper samples of Portugal were contaminated with AFB1 levels ranging from 2-32μgkg^{-1} (Martins *et al.,* 2001). Moroccan red chilli samples showed a national occurrence of AFB1 detected by HPLC (Zinedine *et al.,* 2006). Survey reports on imported chilli and paprika samples in Australia, showed that 91 per cent of the samples did not pass their requirements which were less than 5μgkg^{-1} (Klieber, 2001). Samyal and Sumbali, (2013) reported 19.0 and 29.0 per cent of the twenty-one red chilli samples contaminated with AFB1 and AFB2, respectively (Samyal & Sumbali, 2013). Some of the reviews on the analysis of contaminated red chilli in Turkey showed the presence of aflatoxin in 61per cent of samples (Bircan, 2005). Four out of seven samples were tested positive for AFG1, AFG2 and AFB2 in the survey conducted at Guntur region of India (Sailaja, 2018).

Even though the aflatoxin analysis on different types of equipments has certain limitations, particularly the use of different non-polar organic solvents in TLC, additional column clean up as well as post-column derivatization in HPLC and usage of high cost mass spectrometers. The present method of UHPLC has the highest sensitivity in terms of quantification limits falling below the tolerance limit of 30μgkg^{-1} besides having control charts, uncertainty measurements and per cent residuals calculations as additional data to claim. These parameters are presently required by the regulatory laboratories to comply while conducting experiments particularly on aflatoxin contaminant analysis. Per cent residuals play a prominent role in the selection of linearity range and followed by optimization of the quantification limits. Though the scenario concerning AFs contamination in India is well documented, however there is need for analysing AFs concentration in different regions of India. With these experimental data, the present paper discusses a simple Ultra HPLC method showing a clear separation of aflatoxin isomers and its reproducibility in terms of robustness in red chilli and groundnut matrices. This method was employed to screen the samples collected from market places of the Raichur region of South India and found

suitable for routine monitoring of the samples for aflatoxin isomers in red chilli, groundnut and its products.

Materials and Methods

Chemicals and reagents

Aflatoxin standard mixture solution (AFG2, AFG1, AFB2, AFB1; Product code: 33415) of 99.0 per cent purity was procured from Sigma Aldrich. AflaTest immune-affinity columns photochemical reactor and four stand position pump solid substrate to hold the column were purchased from Waters, USA. Whatman No.1 filter papers (Cat. No. 1001-110), Nexflo nylon syringe filters (0.22µm) were purchased from Sigma-Aldrich, Bangalore, India. AR grade acids and solvents were procured from J T Baker, NJ, USA. HPLC grade water (18MΩ) was obtained from the Merck Millipore water purification system.

Collection of chilli and groundnut samples from cold storage

Ten commercially cultivable genotypes of Chilli samples were collected from Cold Storage Private Limited Unit, Askihala, Raichur. The samples were kept in cold storages (Conditions: 10-15^{o}C and RH of 45-55%). Samples were drawn at three month intervals as per the standard sampling methods followed by International Seed Testing Association (ISTA).

Collection of chilli and groundnut samples from open Market

Commercial branded and non-branded samples were purchased directly from the retail shops and open markets. Four branded red chilli powders treated with radiofrequency waves were collected from Food Engineering department, UAS, Raichur. *A. flavus* cultured groundnut pastes were collected from the Plant Pathology Department UAS Raichur. Samples (1kg) were used in the study except *A. flavus* cultured groundnut paste. Powdered samples were sieved through 20µm mesh and was transferred to an airtight container, labelled and stored at 4^{o}C until further use.

Preparation of aflatoxin standard solution

A primary intermediate standard stock solution of 1µgmL^{-1} was prepared to a final volume of 10mL with acetonitrile. A secondary intermediate stock of concentration 0.1µgmL^{-1} was prepared and the working standards ranging from 5-50 and 0.5-10ηgmL^{-1} were prepared freshly for conducting solvent linearity, matrix specific linearity and recovery studies. All the prepared solutions were protected from light by wrapping the volumetric flasks with aluminium foil. Safety measures were taken to combat the toxic nature of the aflatoxin by soaking the glassware in 5per cent Sodium hypochlorite solution.

Instrumentation

UHPLC (NexeraX2 Shimadzu, Tokyo, Japan) assembled with SIL 30AC auto-sampler, DGU-20A 3R and LC 30AD binary pump was used for the separation of aflatoxin. Purospher STAR RP 18 e column at 40^{o}C with a mixture of acidic and organic solvents was a used. The resolved peaks were detected by a fluorescence detector coupled with a photochemical reactor (PhCR). The automatic and base to base integration of symmetric peaks in post-run were made possible through the inbuilt Lab Solutions software version 5.60 SP2. All the quantifiable samples and standard analysis was done in an isocratic mode at injection volume of 10µL.The fluorescence detector was supported with a photochemical reactor (PhCR) for the detection of Aflatoxin G1 and B1 in the solvent and matrix samples. The fluorescence quenching activity of organic solvents on the aflatoxin molecules was avoided for better resolution and identification by UV irradiation of analyte solution at 254nm. The photochemical derivatization process resulted in the hydroxylation of aflatoxin B and G enabling for detection at minimum concentrations (Joshua, 1993).

Preparation of control matrix, groundnut and red chilli samples

Five grams of the finely ground matrix was transferred into a centrifuge tube. The aflatoxin was extracted and protein precipitated by adding 25 ml of methanol-water (7:3, V/V) and 1 g of NaCl, respectively. Upon vortexing the matrix for 3 min, the mixture was aggregated by gravity filtration on Whatman No.1 filter paper and the aqueous extractant was collected in a clean polypropylene tube. Due to the complexity of the matrix components and the presence of other interfering macromolecules further, one portion of the sample was diluted with two portions of MilliQ water. Before the clean up by immuno-affinity column, the sample solution was ensured free of un dissolved particles by filtering through a 0.22 µm syringe filter in order to avoid damage to agarose bed and to facilitate for continuous flow of sample solution without destruction of the column outlet. Solid phase extraction clean-up was optimized by positioning the four stand position pump with clean glass syringe barrels of 10 mL volume and the immuno-affinity column was attached down to the barrel.

Five millilitre of the 1:2 diluted matrix sample solution was loaded on to the barrel by filtering with a 0.25µm syringe filter and covered the top of the column with pump tubing. Matrix solution was allowed to pass through the column drop by drop to allow the selective binding of aflatoxin to the anti-aflatoxin antibody.

The column was washed twice with 10 ml of water to remove the unbound sample components and dried the column completely at high pressure for a minute after the washing step. The bound aflatoxin to the antibody on agarose bed was eluted using 1ml of methanol, filtered and collected for analysis. A standard addition method for matrix linearity and trueness was performed by adding an appropriate volume of aqueous standard solution to the control matrix, in the linear range 5-50 and 0.5-10ηgg^{-1} in individual matrix.

Method validation

Control matrix specificity was evaluated by screening the matrix (n=6) for the presence of aflatoxin contamination in groundnut and red chilli. The matrix were found free from contamination even at the low level chosen and assessed for selectivity of the matrix towards aflatoxin by spiking an appropriate volume of aflatoxin mix solution.

Further, the extraction procedure was optimized using the screened matrix for linearity and recovery studies. The linearity was drawn from 5-50ηgg^{-1} (5, 10, 15, 25, and 50) in groundnut and 0.5-10ηgg^{-1} (0.5, 1, 2, 5, and 10) in chilli matrix.

As well as solvent linearity of respective concentrations in acetonitrile was performed and coefficient of determination (r^2) was derived. Further, the regression equation was applied in calculating the per cent residuals.

The LOD and LOQ for aflatoxin mixture in the control matrix was calculated based on the S/N ratio for each isomer peak, wherein the lowest concentration of aflatoxin mixture in the matrix was detected and quantified with an S/N ratio of 3.3 considered as LOD and S/N ratio of 10.0 considered as LOQ.

Trueness or recovery of the method was proved to comply with the regulatory guidelines at three fortification levels viz., 5, 15 and 50ηg g^{-1} in groundnut and 2, 5 and 10 ηgg^{-1} in red chilli. The optimum recoveries were attained by extracting the analyte into the methanol-water system and clean up through affinity column chromatography.

The repeatability and robust reproducibility of the method was explored by carrying out the analysis with a different analyst and with a different analytical column (Phenomenex Luna C18, 250x4.6 mm and 5µ) respectively. The determined per cent RSD and recovery in case of robustness between two analytical columns were comparable and the data was within the compliance.

Results and Discussion

Validation of optimized method in groundnut and chilli matrix

Various lots of groundnut and red chilli samples (n=6) collected from different sources were screened using the modified AOAC official method 991.31 for the analysis of aflatoxin using the Immuno-affinity column (Trucksess *et al.*, 1991). Method validation was carried out for the following parameters in compliance with the European Commission document 2019, SANTE/12682/2019 (European Commission, 2020). The matrix with no interference of aflatoxin residues was used as a control for the validation procedure. The separated aflatoxin AFG2, AFG1, AFB2 and AFB1 in both the matrices were qualitatively confirmed based on the retention times at 9.489 ± 0.019, 10.699 ± 0.026, 11.685 ± 0.031 and 13.411 ± 0.042 min respectively (Figure 1A & 1B).

Linearity was drawn for the concentrations of each aflatoxin AFB1, AFB2, AFG1 and AFG2 ranging from 5-50ηgg^{-1}in groundnut matrixwith a coefficient of regression of 0.995, 0.996, 0.995 and 0.993 respectively. Similarly linear concentrations ranging from 0.5-10ηgg^{-1} in red chillimatrix for AFG2, AFG1, AFB2 and AFB1 with a coefficient of regression of 0.999, 0.999, 0.999 and 0.999 respectively. The R^2, LOD, LOQ and per cent residuals for all molecules are reported (Table 1). The quantification limit optimized at 5ηgg^{-1}is based on the signal-to-noise ratio and reproducible per cent RSD of 130 and 2.30 for AFG2, 64 and 16.49 for AFG1, 306 and 16.89 for AFB2 and 97 and 19.61 for AFB1 respectively in groundnut matrix while, 2ηg g^{-1}in red chilli with signal-to-noise ratio and reproducible per cent RSD of 128 and 1.40 for AFG2, 62 and 0.93 for AFG1, 283 and 0.47 for AFB2 and 89 and 0.75 for AFB1 respectively. Accuracy of aflatoxin in groundnut and red chilli matrix was reported at LOQ level was 90.66-116.83 per cent and 97.50-113.33 per cent respectively (Table 1). The repeatability (RSDr) of the method was verified using statistically derived per cent RSD for both the retention time and peak area responses at recovery levels. The average per cent RSD for RT and the peak area in groundnut matrix is reported as 0.09 and 1.85 for AFG2, 0.08 and 1.72 for AFG1, 0.08 and 1.30 for AFB2 and 0.08 and 2.28 for AFB1respectively at 5ηgg^{-1} in groundnut. Whereas 0.19 and 3.63 (AFG2), 0.23 and 1.64 (AFG1), 0.26 and 2.16 (AFB2) and 0.31 and 1.98 (AFB1) at 2ηg g^{-1} respectively in red chilli. The reproducibility (RSDwr) in terms of matrix robustness of the method was evaluated at LOQ levels on Phenomenex Luna C_{18} column (250x 4.6 mm, 5µm) and verified the per cent recovery. At 5ηgg^{-1}of Aflatoxin (AFG2, AFG1, AFB2 and AFB1) spiking to thegroundnut matrix the per cent recovery was 116.66, 97.33, 95.16, 93.33and at 2ηgg^{-1}spiking to red chillithe per cent recovery was 95.16, 103.16, 110.83, and 112.66 respectively.

Further the competence of the method performance was evaluated by participating in the proficiency test program for aflatoxin in groundnut matrix conducted by National Research Laboratory for Grapes, Pune, India. The performance score (Z score) was below ±2.0 which is acceptable as the aflatoxin molecules B1 and G1 with total aflatoxin showed observed concentrations of 5.44, and 7.487 respectively (Table 2). Thus the method demonstrated its competence. The validation results of the present study is superior with the findings of Rosas-Contreras *et al.*, 2016, in Mexican chilli where the per cent recovery was 81.0 (AFG2), 96.0 (AFG1), 75.0 (AFB2) and 83.0 (AFB1). The recoveries ranged from 75.0-96 per cent while our method showed per cent recovery of 90.66 & 113.33 for groundnut and chilli matrices. Özkan *et al.*, (2015) reported LOD and LOQs as 0.1µgkg^{-1} and 0.35 µgkg^{-1} respectively. The calibration curves of AFB1, AFB2, AFG1 and AFG2 were linear between 1 and 100µgkg^{-1}.

Estimation of aflatoxin content inmarket samples

In the present study, 10 of each the branded, non-branded and cold storage groundnut and chilli samples were collected from retail shops and open

markets of the Raichur region of South India. The investigation found that most of the commercial branded chilli powders were contaminated with AFB1 than the cold storage ones. The percentage of AFB1 contamination in chilli is 65.7 and the levels of AFB1 and total AFs in the contaminated dried chillies from branded, unbranded, cold storage and radiofrequency treated were in the range of 1.895-29.653µg/kg, 4.747-6.972µg/kg, and 7.23-13.17µg/kg, respectively. In contrast to chilli samples, 3.3per cent of the branded groundnut samples showed an AFB1 contamination of 13.59µg/kg (Figure.2). The total aflatoxin concentration (B1+B2) in one of the branded red chilli powder is 31.086µg/kg and non-compliant to the FSSAI maximum residue limit of 15 µg/kg (Ashwani, 2020) (Table 3).

Apart from the market samples, the method was utilized to detect the amount of toxins in a groundnut paste sample inoculated with *Aspergillus flavus*. This study was conducted to know the highest production of aflatoxin at different moisture content recorded in the rainy season. The higher moisture content of 40-50 per cent and temperature above 25^{o}C present during rainy season (July to August) in Raichur region favoured the highest production of aflatoxin in groundnut. TheAFB1 and AFB2 concentrations detected on the seventh day of growth period were 2524.40 and 297.40µg/kg respectively to a dilution factor of 200 fold. The quantity of AFG2 and AFG1 was negligible at that dilution.

The outcome of the market sample analysis aligned with the findings of Bircan 2005, where out of seventy-five spices collected for evaluation of aflatoxin contamination, all the fifteen chilli powder samples were shown positive for the AFB1contamination in the range of 1.6-80.4µgkg^{-1} and four chilli powder samples were found above the regulatory limits. Rosas-Contreras *et al.,* 2016, estimated the AFB1 contamination in 64 powdered red chilli samples and revealed that 40-60 per cent samples were detected with AFB1. Similarly Samyal and Sumbali, (2013) studied the aflatoxin contamination in twenty-one Kashmiri loose red chilli powders procured from the market and found that 19 per cent of collected samples were detected with AFB1and and 29 per cent with AFB2, respectively.

The study conducted by Golge *et al.,* 2013, determined the aflatoxin in 180 red chilli samples of Turkey also revealed that 82 per cent of red chilli samples were contaminated with AFB1, 46.66 per cent samples with AFB2, 17.77 per cent samples with AFG1 and none of the samples showed the presence of AFG2. Among the 331 red chilli samples of Pakistan, 62.4 per cent of whole, 26.1 per cent of powder and 19.4per cent of crushed chilli showed AFB1 in the range of 10-20µgkg^{-}1 (Khan *et al.,* 2014). Sixty seven per cent of Sri Lankan red chillies showed AFB1 in the range of 5µgkg^{-1} (Yogendraraja *et al.,* 2014). Among the 7 samples of Guntur, India, showed positive for AFB2 (28.57 per cent) in the range of 6.68-34 µgkg^{-1}, AFG1 (14.28 per cent) in the range 21.30µgkg^{-1}and AFG2 (57.14 per cent) in the range 8.71-19.39µgkg^{-1} (Sailaja, 2018). The chilli samples from south eastern India reported occurrence of AFG2 in all the samples and AFB2 was found very rarely in one sample with the contamination level of 34.02µgkg^{-1} (Sailaja, 2018). The highest mean intake per day observed for consumption of red chilli powder is 3.19 g with a range of 0.35–5.23 g among an urban Indian family and 2.41 g with range 0.25-3.75 g for a rural family. This data is noteworthy when our survey shows that 62per cent of the branded chillies samples sold and used across India are contaminated with AFB1 (Table 4). This study showed that the cold storage chilli and groundnut were aflatoxin-free and thereby safe compared to the branded and non-branded samples and was in contrast to the contamination found in red chilli stored at 4^{o}C (Guru Prasad *et al.*, 2018).

The possible reasons for the occurrence of aflatoxin contamination in chilli samples collected from the retail shops are due to the storage conditions such as high temperature and relatively high humidity.

Table.1 Validation parameters : Co-efficient of regression, LOD ,LOQ, % Recovery, Repeatability (%RSD), Robustness and per cent residuals of the aflatoxin AFB1, AFB2, AFG1 and AFG2 spiked to the groundnut and red chilli matrix determined using the developed method (n=3)

Groundnut Matrix	r^2	LOD (µg/kg)	LOQ (µg/kg)	Accuracy (per cent Recovery)	Repeatability (per cent RSD)	Robustness (per cent Recovery)	per cent Residual
AFB1	0.993	0.5	5.0	90.66	2.28	93.33	8.48
AFB2	0.995	0.5	5.0	94.16	1.30	95.16	5.68
AFG1	0.996	0.5	5.0	102.00	1.72	97.33	7.70
AFG2	0.995	0.5	5.0	116.83	1.85	116.66	-0.49
Red chilli Matrix							
AFB1	0.999	0.5	2.0	113.33	1.98	112.66	-3.29
AFB2	0.999	0.5	2.0	112.33	2.16	110.83	-1.70
AFG1	0.999	0.5	2.0	106.10	1.64	103.16	0.30
AFG2	0.999	0.5	2.0	97.50	3.63	95.16	1.84

Table.2 Proficiency test results of aflatoxin determination in groundnut matrix conducted by Referral lab, ICAR NRC-Grapes (2020), Pune, India.

Analyte	Determined Conc.(µg/kg)	Actual Conc. (µg/kg)	Z score	Remark
AFB1	5.440	7.98	-1.33	Acceptable
AFG1	7.487	11.12	-1.14	Acceptable
AFB1+AFG1	12.927	19.06	-1.26	Acceptable

Table.3 Natural aflatoxin prevalence and ranges in the branded, non-branded, cold storage, and radio frequency (RF) treated red chilli samples and branded, non-branded, cold storage and infected groundnut samples.

	No. of samples Tested	No. of samples contaminated	No. of contaminated samples above the regulatory limit	Range of AFB1 (µg/kg)	Range of total aflatoxin (µg/kg)
Red chilli					
Branded	10	8	01*	1.895-29.653	1.895-31.086
Non branded	10	9	Nil	4.747-6.972	4.747-6.972
Cold storage	10	0	Nil	Nil	Nil
RF treated	8	8	Nil	7.23-13.17	7.76-13.95
Total	38	25 (65.78per cent)			
Groundnut					
Branded	10	1	Nil	13.59	15.66
Non branded	10	0	Nil	Nil	Nil
Cold storage	10	0	Nil	Nil	Nil
Total	30	1(3.33per cent)			
A. flavus infected Groundnut	5	5	01*	250-2524.40	290-2821.80

*Above the regulatory limit (15µg/kg)

Fig.1 UHPLC chromatograms of aflatoxin mixture in spiked groundnut (A) and red chilli matrix(B).

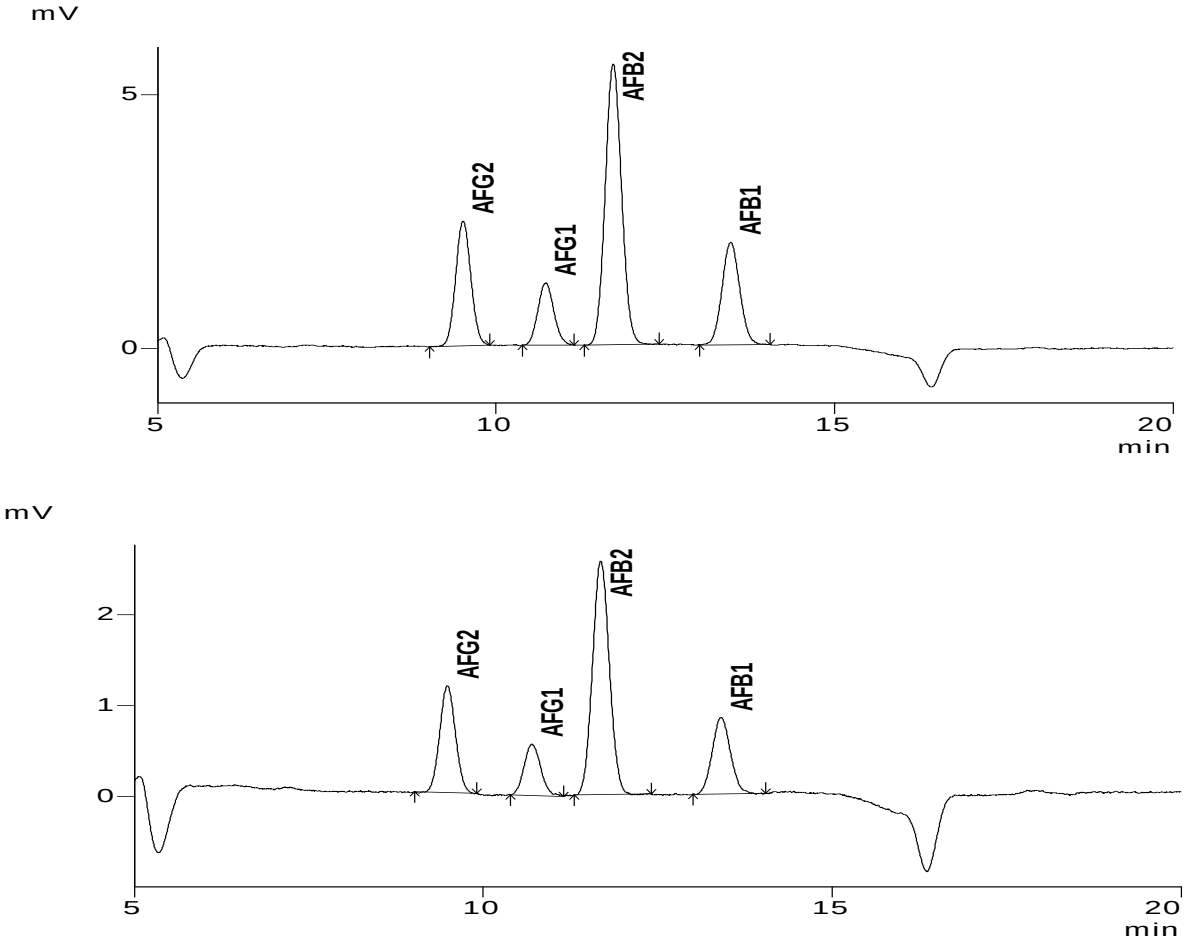
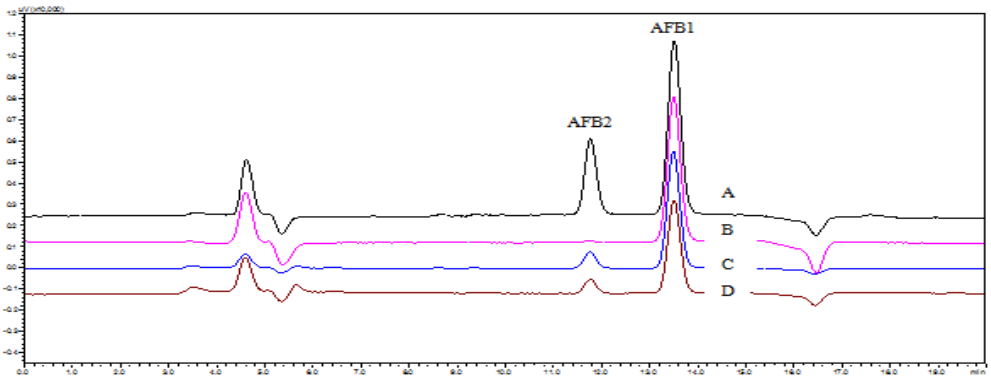


Fig.2 UHPLC chromatograms ofnaturally contaminated groundnut samples, (A) Branded samples (AFB_2-2.07 µg/kg and AFB_1-13.59 µg/kg), (B) *A. flavus* infected sample (AFB_2- 297.40µg/kg and AFB_1-2524.40µg/kg) Redchilli samples (C) branded (AFB_2-1.43 µg/kg and AFB_1-29.65 µg/kg) and (D) non-branded (AFB_2-0.574µg/kg and AFB_1-10.460 µg/kg).



During harvest season, improper practices such as drying on the bare ground, lack of proper storage conditions, unsafe packing, insufficient care taken during the transport, limited conditions for marketing in the retail shops are the probable reasons. Additionally, lack of knowledge on good agricultural practices regarding the removal of moulded chilli fruits from the lots and preventing the contact of the red chillies with soil particles (Khan *et al.*, 2014).

Over the last few years, groundnut and red chilli powder safety concern has resulted in reduction of exports to many developed and developing countries. In the present investigation, we report a detailed method validation procedure, for aflatoxin analysis in both the groundnut and red chilli powder using UHPLC-FLD coupled with the PhCR detection. The method is sensitive and reproducible. The detection of aflatoxin contamination in 65 per cent of red chilli collected as branded and non-branded samples enables to check of the authenticity of red chilli powder released in the markets. It also necessitates continuous monitoring to safeguard the consumer's health and export as per the quality standards, not permitting morethan30.0 µg kg^{-1} as prescribed under FSSAI.

The key question from consumer point of view is that if the high level of aflatoxin in groundnut/ chillimakes it unfit for human consumption in many countries, then it is unfit for Indian consumers too. According to Indian Council of Medical Research (ICMR),21 per cent of groundnut in India is unfit for human consumption due to aflatoxin and our study reveals only 3.3 per cent as we have taken cold storage and freshly harvested unbranded samples.

Aflatoxin accumulating fungi can infect groundnut during the production as well as post-harvest season. Pre-harvest infection by *Aspergillus flavus* and consequent aflatoxin contamination happens more frequently in the semi-arid tropics, because of the likelihood of drought before the harvest season. Drought-stressed plants lose moisture from seeds and physiological activity is significantly reduced. Mutually these factors augment susceptibility to fungal attack. The solution would be taking up resistant varieties and good agricultural practices (GAPs) to reduce the aflatoxin level to 20 ppb. Breeding efforts to reduce groundnut maturity periods to escape the season end drought and the identification of short-duration lines with resistance to *Aspergillus sps*. would be preferred. Applications of lime solution on the crop alone can reduce aflatoxin contamination by 72 per cent, while application of farm yard manure reduces aflatoxin by 42 per cent under field conditions. When combined, aflatoxin contamination can be reduced by up to 84 per cent. Harvesting at appropriate period, embracing of proper drying method, reducing crop moisture level to 8 per cent put a stop to the accumulation of aflatoxin significantly. Some of the measures such as crop rotation with onion or garlic, selection of short and medium-term varieties, advanced sowing, supplemental irrigation during end of the season, etc. are the measures to reduce aflatoxin. The response of farmers towards adoption of these practices, however, has been meagre and is even unaware of the health issues related to aflatoxin consumption. The unavailability of premium price for aflatoxin-free chilli or groundnut is probable reason. There is need for implementation of mass level sensitisation programme for farmers and consumers to deal with the issue right from farm to processing and to consumption. Awareness about aflatoxin can lead to demand for aflatoxin-free groundnut/chilli and thus farmers may also fetch better price for taking the pain of improving their field and storage level practices.

Conflict of Interest Statements

Authors declare no conflicts of interest in publishing the paper.

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