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A Review on Contamination, Effects and Analysis of Aflatoxins in Different Food Products

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Abstract

Humans are continuously exposed to varying amounts of chemicals that have been shown to have carcinogenic or mutagenic properties in environmental systems. Food stuffs have been found contaminated with substances having carcinogenic, mutagenic, teratogenic and allergenic properties. Among them the present paper reviews the Aflatoxins contamination, effects and analysis in different food products. The present paper deals with Aflatoxin types, contamination different in food items. The previous studies reported the presence and effects of Aflatoxins in India also presented. The qualitative and quantitative analysis of different Aflatoxins in food samples by High performance liquid chromatography by various authors also discussed. In conclusion this paper provides adequate information about Aflatoxin contamination instrumental analysis which helps to apply to study the Aflatoxin contamination.

Keywords

Food products, Aflaoxins, *Aspergillus flavus and Aspergillus parasiticus.*

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INTRODUCTION:

Humans are continuously exposed to varying amounts of chemicals that have been shown to have carcinogenic or mutagenic properties in environmental systems. Exposure can occur exogenously when these agents are present in food, air or water, and also endogenously when they are products of metabolism or pathophysiologic states such as inflammation. For the past two decade's great attention is focused on environmental health as a consequence of the increasing awareness over the quality of life due to major environment pollutants that affect it. Food stuffs have been found contaminated with substances having carcinogenic, mutagenic, teratogenic and allergenic properties. As these substances can be supplied with food throughout the entire life-time of a person. The International Agency for Research on Cancer (IARC)

has concluded that naturally occurring aflatoxins are carcinogenic to humans (group 1), with a role in etiology of liver cancer, notably among subjects who are carriers of hepatitis B virus surface antigens. Among the 18 different types of aflatoxins identified, the major members are aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), M1 (AFM1) and M2 (AFM2). AFB1 is normally predominant in amount in cultures as well as in food products.

High amount of aflatoxins uptake in a very short time can cause liver damage, Liver cancer, mental impairment, Abdominal Pain, Vomiting, Convulsions, Edema, Pulmonary Edema, Haemorrhaging, Disruption of food digestion, absorption or metabolism, Coma, Death. If aflatoxins take at a time, but over a long period leads to Liver cancer due to DNA mutation caused by aflatoxins. Acute high-level exposure can progress to potentially lethal hepatitis with vomiting, abdominal pain, jaundice, fulminant hepatic failure and death (1). The aflatoxin LD50 rate (the dosage level that causes 50% of a group to die) for animals is between 0.5 and 10 mg/kg of the animal's weight. Aflatoxins are quite stable compounds and survive relatively high temperatures with little degradation. Various reports have been presented that presence of Aflatoxins in various food products that can affect human, birds and animal health.

Aflotoxin contamination studies in India:

Various studies have been reported the food contamination by Aflatoxin in India. **Najeeb S. Al-Zoreky et al (2017),** found **c**oncentrations of 0.123 mg/kg of aflatoxin B1 and 2.58 mg/kg of total AFS in imported rice and ground rice subsamples. Analysis was conducted with RP-HPLC-FLD using post column electrochemical derivatization with a Kobra Cell. *Aspergillus parasiticus* is observed at 25^{0}C and high concentrations of aflatoxin B1 observed at fungal growth at 37^{0}C in rice stored for three weeks [2]. **Maryam Jalili (2016),** determined the aflatoxin B1, B2, G1 and G2 content in 80 different spices samples (red pepper, black pepper, turmeric and cinnamon). Analysis was conducted with HPLC with fluorescence detector with a mixture of acetonitrile–methanol–water (17:29:54; v/v). Recovery of aflatoxin is ranged from 76.4±5.6 to 98.3±3.2 for AFG1 in cinnamon (spiked at 1ng/g) and AFB2 in turmeric (spiked at 10ng/g) respectively. Thirty-two out of 80 (40%) samples were contaminated with aflatoxins ranged from 0.85±0.10 to 24.60±0.12 [3]. **Vasanthi Siruguri et al (2012),** collected the stored rice variety PAU 201 in Punjab that was not permitted for milling and public distribution due to the presence of damaged grains at levels exceeding the regulatory limits of 4.75 per cent and analyzed for presence of aflatoxin contamination by Scanning electron microscopy (SEM), energy dispersive X-ray (EDX) analysis and Prussian blue staining. Results of the study indicates that none exceeded the Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011 tolerance limit of 30 µg/kg and majority of the samples had levels <15µg/kg [4]. **Harish Chandr et al (2013),** reported the occurrence of total aflatoxin contamination in Indian maize samples collected from local market of Lucknow city by using investigated by competitive ELISA technique. The result showed total aflatoxin content ranges from 9.0 to 250 ppb [5]. **Amir Sasan Mozaffari Nejad et al (2014),** analyzed 36 spices samples of Iran and India that include chilli powder (*n* = 12), black pepper powder (*n* = 12) and whole black pepper (*n* = 12) for determination of aflatoxin content. Aflatoxin content in Iranian samples was found ranged from 63.16 to 626.81ng/kg and in Indian samples was ranged from 31.15 to 245.94ng/kg when analyzed with enzyme-linked immunosorbent assay [6].

Baranitharan Krishnan et al (2015), studied on 40 samples of local and branded corn flour that are marketed in Chennai, Tamil Nadu, India for determination of contamination levels of aflatoxins. Among all samples 23 samples (57.5%) have shown positive for aflatoxin (35% samples have shown positive for G1, 5% samples have shown positive for G2, 50% samples have shown positive for B1, 32.5% samples have shown positives for B2). B1 type aflatoxin was found high in all samples [7]. **Jayaramachandran Ramesh et al (2013),** studies on food grain samples (Bengal gram (2), bajra / cumbu (6), maize (10) and jowar / sorghum (1) and grain flour (3) collected from local market for determination aflatoxin B1. Thin layer chromatography and high performance thin layer chromatography applied for analysis and results found that contamination of aflatoxin B1 was found to be 68.18% in food grains whereas 100% in grain flour, which might be due to improper post-harvest technology and storage condition [8]. **Nadeem A. Siddiquea et al (2013),** studied on presence of aflatoxins in medicinal plants, namely *Mucuna pruriens*, *Delphinium denudatum* and *Portulaca oleraceae*. The aflatoxins were extracted, purified by immunoaffinity column chromatography and analysed by HPLC–MS/MS with Agilent XDB C18-column. Results found that AFB1 and AFB2 were in trace amounts below the detection limit in *M. pruriens* whilst they were not detected in *D. denudatum*. *P. oleraceae* was found to be contaminated with AFB1 and AFB2 [9]. **Punam Jeswal**

et al (2015), has analyzed for fungal contamination as well as aflatoxin (AFs), ochratoxin A (OTA), citrinin (CTN) contamination in nine different Indian spices (red chilli, black pepper, turmeric, coriander, cumin, fennel, caraway, fenugreek, and dry ginger) in India. ELISA and LC-MS/MS techniques were used for analysis. All spices samples found contamination with Aspergillus flavus and Aspergillus niger. Aflatoxin content was found in Red chilli samples (85.4%), dry ginger (77.7%). 56% Aspergillus flavus from red chilli and 45% Aspergillus ochraceus from black pepper were toxigenic and produced aflatoxins and ochratoxin A, respectively. Penicillium citrinum produced citrinin in red chilli, black pepper, coriander, cumin, fenugreek, and dry ginger samples. The highest amount of AFs was found in red chilli (219.6 ng/g), OTA was in black pepper (154.1 ng/g), and CTN was in dry ginger samples (85.1 ng/g) [10].

Aflotoxin contamination studies other countries:

Waenlor W et al (2003), presented the aflatoxin contamination in various foods and products in Thailand between 1967-2001. Thirteen available international and local reports (n=3,206 samples) focused on type of food, season and geographic areas, and have been collected for statistical analysis. The accumulated data showed 1,248 (38.9%) of 3,206 samples were highly contaminated with aflatoxin. Over half (728) of the contaminated samples (1,248) were peanuts, milk, and poultry [11].

FF Ilesanmi et al (2011), reported the descriptive cross-sectional study on awareness and knowledge of aflatoxin contamination in groundnut and the risk of its ingestion among 417 health workers in Ibadan. The study reports that 95% of the respondents had previous awareness of aflatoxin and class room lectures was the most common source of information (56%). Knowledge regarding aflatoxin contamination in groundnut and the risk of its ingestion was obtained showing knowledge score range of 0 to 14. In all, 80.6% had good scores of 11 to 14. None of the respondents had ever told their patients about the risk of aflatoxin ingestion [12].

Angele N. Tchana et al (2010), determined the presence of aflatoxins in eggs, milk, urine, and blood samples collected from various sources and periods in Cameroon. Aflatoxin was found in eggs (45.2%), cow raw milk (15.9%), breast milk (4.8%), urine from kwashiorkor and marasmic kwashiorkor children (45.5%), and sera from primary liver cancer patients (63.9%) [13].

Carlos A. F. Oliveiraet al (2009), detected aflatoxin levels in peanut (240 samples) products traded in the Northeast region of Sao Paulo, Brazil by using HPLC. Results showed 44.2% samples positive for AF at levels of 0.5 to 103.8 µg·kg-1. Nine of the positive samples (3.7% of the analysed samples) had total aflatoxin concentrations higher than the limit established by Brazilian regulations (20 µg·kg-1) [14].

H. A. Makun et al (2010), analyzed 343 samples of five different food commodities from three States in Nigeria. Maize samples from Niger (43) and Kogi (50) States and dried yam chips (50) from Niger State and also imported powdered milk (100) from in Lagos metropolis were also analyzed using column chromatography for clean-up and thin layer chromatography coupled with a densitometer equipped with win CATs software for quantification. Toxicity screening results showed that 68.57% of the fungi tested were toxigenic and were mostly isolates of Aspergillus, Fusarium, Penicillium, Rhizopus and Mucor. AFM1 was detected in 19 samples out of the 100 milk samples analyzed at levels ranging from 0.02 - 0.41 µg/kg. AFB1 was found in 29 out of the 50 beans (63.5 - 106.2 µg/kg) samples analyzed while 54% of the 50 marketed wheat samples (102.9 - 198.4 µg/kg) were also contaminated with the toxin [15].

Nisa, A et al (2016), analyzed 50 samples of local and import quality brown rice in Lahore, Pakistan for determination of quality difference with respect to the limits set by European Commission i.e.10 ppb. Thin Layer Chromatography was applied for analysis and among all samples 92% local brown rice samples were contaminated, 56% were contaminated above permissible limits; whereas, 36% were below permissible limits. In import quality only 48% of samples were contaminated where 44% were below and only 4% were above permissible limits [16].

Fadia. F. Hassan et al (2014), investigated AFB1 in the incidence of mycoflora and presence of aflatoxin in 24 samples of local stored maize collected from Iraqi governorates using TLC and ELISA techniques. Aflatoxin B1 was present in 12 samples of stored maize and the concentration of toxin ranged between 2.30 to 30 ppb using TLC technique and 270 to 500 ppb using ELISA technique [17].

Younis M H Younis et al (2003), analyzed 400 samples of peanuts and peanut products collected from local market over two seasons from different sites in the Central Region (Sudan). HPLC and TLC techniques are used for study the percentage aflatoxin contamination in all samples and results found 2%, 64%, 14% and 11% aflatoxin in peanut kernels, peanut butter, peanut cake and roasted peanuts respectively. The highest aflatoxin levels were detected in peanut butter (32-54 g/kg) and the lowest in peanut kernels (3-8 g/kg) [18].

Matome Gabriel Thathana et al screened the 540 isolates obtained from maize and soil in Kenya for the

production of Aflatoxins. Among the isolates the incidence of Aspergillus was found to be 63% and aflatoxin production was concluded as 28% [19]. **Edmond Panariti et al (2001),** reported the levels of aflatoxin M1 in the farm-gate milk in Albania. Total 120 evenly distributed samples collected in winter and summer from various farms all over the country and quantitative analysis carried out by thin layer chromatography. 13% of the winter samples resulted above the 0.5 µg/kg level, as compared to 3% of the summer samples exceeding that level [20]. **Salah Eldeen Abass Ali Ahmed et al (2016),** analyzed 30 samples of groundnut and 15 groundnut products from three states (Khartoum, Kordofan and Gadarif) of Sudan for AFB1, AFB2, AFG1, and AFG2 using HPLC method with fluorescence detection. The frequency of contaminated groundnut samples with AFB1 from Khartoum, Gadarif and Kordofan state was 58.3%, 57.1%, and 66.7%, respectively. No sample of groundnut or groundnut product was contaminated with AFG1 or AFG2 [21]. **Shami Elhaj Alssafi Bakhiet et al (2011**), 60 samples of stored peanut kernels were collected from four different locations in Sudan namely Mayo city, Umbaddah city in Khartoum state, Al-Helalia city, and Al-Managel city in Al-Jazeera state to study the contamination with aflatoxin. By TLC technique. Thirty-five samples (58.33%) gave positive and concentration of aflatoxin B1 in these samples was ranged from low to very high, in range of (17.57-404.00 µg/Kg kernel) [22]. **Zahra Nazari Khorasgani et al (2013),** analyzed peanut samples of supermarkets in Ahvaz, Iran for the determination of aflatoxins concentration by TLC scanner. In total, 59.26% of samples were contaminated with aflatoxins, 14.8% of samples were found to contain above of 20 ppb, above the maximum level of total aflatoxins permitted in Iran [23].

Muna T. AL-Mossawei et al (2016), analyzed 130 samples of liquid and powder milk, white and soft cheese, yoghurt was randomly collected from Baghdad markets from September 2014 to June 2015 and distributes into imported and local samples. The aflatoxin M1 (AFM1) was analyzed by using TLC, HPLC and ELISA. The AFM1 contamination showed as 50 (38.5%), 65 (50 %) and 70 (53.8%) respectively, furthermore, yogurt and cheese showed more contamination with AFM1 than other products and the highest concentration of AFM1 in the local cheese reached 300.7ng/L and 939.67ng/L [24]. **Fardos M Bokhari (2002),** analyzed 51 foodstuff samples contaminated with Aspergillus flavus and screened for aflatoxin production by TLC and HPLC. Results found that 26.1% of the total isolates were toxic strains and the main toxin was found to be aflatoxin B1 of 46 foodstuffs commodities. Most contaminated samples were poultry feed, cereal grains and oil seeds. The presence of four aflatoxins B1, B2, G1 nad G2 was detected only in two of the tested samples [25].

Aflatoxin analysis by different methods:

There are different qualitative and quantitative methods are available for determination and separation and quantification Aflatoxins. Thin layer chromatography (TLC), high-performance thin layer chromatography (HPTLC), enzyme-linked immunosorbent assay (ELISA) and High performance liquid chromatography (HPLC) techniques are mostly used for Aflatoxisn analysis in food samples. Recently High performance liquid chromatography coupled with mass spectrometry (LC-MS) is also applying for using for analysis of Aflatoxins. Various studies reported by using these techniques for determination Aflatoxin contamination in various food samples are described below.

Korrapati Kotinagu et al (2015), reported the aflatoxin B1 contamination in Livestock Compound Feed and feed ingredients from different livestock farms and farmers by HPTLC. Out of 48 livestock compound feed samples, aflatoxin B1 could be detected in 16 samples representing 33%, whereas in livestock feed ingredients out of 49 samples, 13 found positive for aflatoxin B1 representing 24.5% [26]. **J. Ramesh et al (2014),** analyzed feed ingredients and feed for aflatoxin B1 carried by HPTLC method and compared with TLC method. Out of 38 samples of nine types of feed ingredients analyzed. Aflatoxin B1 was found to be ranging from 1.61 ppb to 630.73 ppb of 77.42% positive samples, whereas by TLC method it was from 05 ppb to 140 ppb in 70.97% positive samples. While 4 samples of wheat bran analysed and 50% (2 samples) found to be positive with HPTLC method with concentration ranging from 2.73 to 17.88. Similarly, out of 59 feed samples analyzed, 47 and 46 samples were positive for Aflatoxin B1 representing 79.66% and 77.97% of the samples, with concentration ranging from 0.54 ppb to 204.72 ppb and from 05 ppb to 710 ppb by HPTLC and TLC respectively [27]. **H. Marina Martins et al (2007),** analyzed 128 samples of several types of hard cheese from five different European countries were acquired from supermarkets in Lisbon, Portugal. Eight samples (6.25%) contained levels of AFM1 at the maximum permissible level (0.05µg/kg) [28]. **Seikichi Tsuboi et al (1984),** was reported AFB1 presence in serum samples of healthy Japanese males by radioimmunoassay and HPLC. AFB1 was detected in 5 of 20 fasting blood samples [20 to 56 pg/ml of serum; 33.6 Â± 14.6 (S.D.)] and in 29 of 80

serum samples taken after lunch (20 to 1169 pg/ml of serum; 218.1 Â± 268.3) [29].

Siahi Shadbad Mohammad Reza et al (2012), determined the contamination levels of aflatoxins in 142 samples including 35 almonds, 26 walnuts, 4 seeds of apricot, 6 sunflower seeds kernel, 6 sesame seed, 6 peanuts, 32 pistachios, 13 hazelnuts and 14 cashews and conclude that the aflatoxin contamination rate higher than 15 ppb were observed in 28.1% of pistachios, 5.1% of walnuts and 7.1% of cashews [30]. **S. T. Ubwaet al (2012),** was carried screening of aflatoxin B1 levels in maize sold in some markets of Benue State, Nigeria using thin layer chromatography and confirmed that 7.64 to 13.40% of samples contains aflatoxin B1 in less than the permission limits of National Agency for Food and Drug Administration and Control [31]. **Mohamad Sadeghet al (2012),** studied the aflatoxin B1 levels in feed-stuff samples using enzyme linked immunosorbent assay (ELISA) and high performance liquid chromatography. The aflatoxin B1 level was found to be 2.21±2.25 and 10.76±0.86 µg/kg for HPLC and ELISA techniques, respectively [32]. **Shotwell OLet al (2014),** analyzed the aflatoxin levels in corn samples using modified AOAC method and confirms that the modified method can detect the 0.5-1.0 ng aflatoxin B1 on TLC plate making the limit of detection about 9ng/g for 0.1g samples [33].

Marcela Martínez-Mirandaa et al (2015), analyzed the aflatoxin content in 144 corn arepas samples using HPLC method and confirms that 9.72% samples were found to be contaminated with aflatoxin in the concentration range of 0.95 and 11.56 µg kg−1 [34]. **Wejdan Shakir Khayoon et al (2010),** estimated the aflatoxins B1, B2, G1 and G2 content in 42 animal feed samples using HPLC fluorescence detection method. The results confirm that eight samples (19%) were contaminated with aflatoxins, ranging from 6.5 to 101.9 ng g-1. Total aflatoxin levels in three samples exceed the legal limits of many countries of 20 ng g-1 [35]. **Kai Peng et al (2015),** determined the aflatoxin B1 in tea by using HPLC-FLD with photochemical derivatization method [36]. **Joao Augusto et al (1996),** developed an easy and inexpensive method to screen for aflatoxins in peanut and corn by enhancing the fluorescence of aflatoxin B1 with cyclodextrins for detection using a fluorometer and aflatoxin B1 content was estimated [37]. **Ghalia et al (2009),** has been used a reversed-phase high-perfsormance liquid chromatographic method for the determination of aflatoxins B1, B2, G1 and G2 in Tunisian sorghum and pistachios. Aflatoxin recoveries in sorghum and pistachios samples spiked at 0.5 and 2ng/g varied from 68.3 to 87.7%. The incidences were 52.5 and 62%, respectively, for pistachios and sorghum samples, with respective average contamination levels of 21.8 ± 38.0 and 9.9 ± 11.5 ng/g [38].

Sai Gopalakrishna Yerneni et al (2012), analyzed for aflatoxin contamination in cloves, pepper, cardamom and red chillies samples by using TLC and HPLC analysis respectively. Among all the samples, red chillies were found to be positive for AFG and AFB aflatoxins by TLC analysis. HPLC analysis of the red chillies sample detected the presence of AFG2 and AFB1 types of aflatoxin within the 6 min of the baseline separation [39]. **Baranitharan Krishnan et al (2015),** reported Aflatoxins contamination in 40 samples of local and branded corn flour that are marketed in Chennai, Tamil Nadu, India by Romar's all-purpose extraction method followed by HPLC analysis. Among 40 samples 23 samples (57.5%) have shown positive for aflatoxin (35% samples have shown positive for G1, 5% samples have shown positive for G2, 50% samples have shown positive for B1, 32.5% samples have shown positives for B2) [40]. FDA establishing regulations to protect their citizens and livestock from potential harm caused by mycotoxins. The limits of Aflatoxins B1, B2, < G2, and M1 in foods and feed stuffs varies from (0-40) ppb for foods & 0-1000ppb for food) [41]. However, not only the FDA in USA, but also some European countries have been establishing special committees and commissions to create and recommend guidelines, test standardized assay protocols, and maintain up-to-date information on regulatory statutes of aflatoxins and other mycotoxins. Estimates of "safe doses" are usually stated as a "tolerable daily intake". For example, in the United States, the Food and Drug Administration guideline is 20 ppb total aflatoxin in food destined for human consumption and 100 ppb is the limit for breeding cattle and mature poultry (42]. Hence the systematic determination of the foreign substances in nutritional products and feedstock plays an important role. The choice of the method of analysis depends on the sample, the analyte to be assayed, accuracy, limit of detection, cost and time to complete the analysis [43].

The producers of food should use the pre harvest and post-harvest control measure to avoid Aflatoxin contamination. Pre-harvest control of aflatoxins is best achieved through general Good Agricultural Practice (GAP) to include Land preparation, crop waste removal, fertilizer application and crop rotation, Use of fungus and pest-resistant crop varieties, Control of insect pests, Control of fungal infection, Prevention of drought stress by irrigation,

Harvesting at the correct moisture level and stage of maturity. Post-harvest handling and storage is the control of moisture content and hence, the water activity of the crop is the most important and effective control measure. It is also important to ensure that the moisture content does not vary too much in a bulk-stored crop [44].

The control measures for food processors include physical separation of contaminated material. Density segregation, mechanical separation and the removal of fines and screenings from grain and nut shipments can also be effective measures. Chemical decontamination methods have been investigated, especially for material used in animal feed, but most of the methods investigated are impractical, or produce toxic by-products. Biological decontamination has also been considered, and a single bacterial species, *Flavobacterium aurantiacum*, has been shown to remove aflatoxin B1 from peanuts and corn. A number of analytical methods have been developed based on TLC, HPLC and ELISA and there are also rapid screening kits available. Applying of control measures and analysis of suspected food samples for determination of Aflatoxins to take necessary action can control the Afltatoxins effects.

REFERENCES:

1. Walderhaug M. (1992). Ciguatera. Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. Food and Drug Administration, U.S.
2. Najeeb S. Al-Zoreky, Farag A. Saleh, Limited survey on aflatoxin contamination in rice, Saudi Journal of Biological Sciences, 2017, 26(2), 225-231.
3. Maryam Jalili, Natural Occurrence of Aflatoxins Contamination in Commercial Spices in Iran, Iranian Journal of Health, Safety & Environment, 2016, 3(2), 513-517.
4. Vasanthi Siruguri, P Uday Kumar, P. Raghu, M Vishnu Vardhana Rao, B. Sesikeran, G. S. Toteja, Priyanka Gupta, Spriha Rao, K. Satyanarayana, V.M. Katoch, T.S. Bharaj, G.S. Mangat, Neerja Sharma, J.S. Sandhu, V.K. Bhargav, and Shobha Rani, Aflatoxin contamination in stored rice variety PAU 201 collected from Punjab, India, Indian J Med Res. 2012, 136(1), 89–97.
5. Harish Chandra, Jyotsana Bahuguna and Ajay Singh, Detection of Aflatoxin in Zea mays L. from Indian Markets by Competitive ELISA, Octa Journal of Biosciences, 2013, 1(1), 62-68.
6. Amir Sasan Mozaffari Nejad, Masoud Sabouri Ghannad & Abolfazl Kamkar, Determination of aflatoxin B levels in Iranian and Indian spices by ELISA method, Journal Toxin Reviews. 2014, 33(4), 151-154.
7. Baranitharan krishnan, Sarathchandra Ghadevaru, Manimehalai, N. Athmaselvi, K.A. & Padmavati, R, Determination of Aflatoxin B1 in Corn Flour Using Highperformance Liquid Chromatography, I.J.A.B.R, 2015, 5(2): 172-176.
8. Jayaramachandran Ramesh, Ghadevaru Sarathchandra and Veerapandian Sureshkumar, Survey of market samples of food grains and grain flour for Aflatoxin B1 contamination, Int.J.Curr.Microbiol.App.Sci. 2013, 2(5), 184-188.\
9. Nadeem A. Siddiquea, Mohd Mujeeba, Sayeed Ahmada, Bibhu P. Pandab, Mohd Makhmoor, Determination of Aflatoxins in Medicinal Plants by High-Performance Liquid Chromatography–Tandem Mass Spectrometry, J Pharm Pharm Sci. 2013, 16(2) 321 – 330.
10. Punam Jeswal and Dhiraj Kumar, Mycobiota and Natural Incidence of A□atoxins, Ochratoxin A, and Citrinin in Indian Spices Confirmed by LC-MS/MS, International Journal of Microbiology, 2015, Volume 2015, 8 p.
11. Waenlor W, Wiwanitkit V, Aflatoxin contamination of food and food products in Thailand: An Overview, Southeast Asian J Trop Med Public Health, 2003, 34(Suppl 2) 184-190.
12. FF Ilesanmi and OS Ilesanmi, Knowledge of aflatoxin contamination in groundnut and the risk of its ingestion among health workers in Ibadan, Nigeria, Asian Pac J Trop Biomed, 2011, 1(6), 493–495.
13. Angele N. Tchana, Paul F. Moundipa and Felicity M. Tchouanguep, Aflatoxin Contamination in Food and Body Fluids in Relation to Malnutrition and Cancer Status in Cameroon, international journal of environmental research and public health, 2010, 7(1), 178-188.
14. Carlos A. F. Oliveira, Natália B. Gonçalves, Roice E. Rosim and Andrezza M. Fernandes, Determination of Aflatoxins in Peanut Products in the Northeast Region of São Paulo, Brazil, International Journal of Molecular Sciences, 2009, 10(1), 174-183.
15. H. A. Makun, S. T. Anjorin, B. Moronfoye, F. O. Adejo, O. A. Afolabi, G. Fagbayibo, B. O. Balogun and A. A. Surajudeen, Fungal and aflatoxin contamination of some human food commodities in Nigeria, African Journal of Food Science, 2010, 4(4), 127-135.
16. Nisa, A, Zahra N. and Yasha, N. B. Comparative study of aflatoxins in brown rice samples of local and import quality, International Food Research Journal, 2016, 23(1), 243-247.
17. Fadia. F. Hassan, Muna. H. Al- Jibouri Abdul. Kareem J. Hashim, Isolation and Identification of Fungal Propagation in Stored Maize and detection of aflatoxin B1 Using TLC and ELISA Technique, Iraqi journal of science, 2014, 55, 634-642.
18. Younis M H Younis and Kamal Malik, TLC and HPLC assays of aflatoxin contamination in Sudanese peanuts and peanut products, Kuwait journal of science & engineering, 2003, 30(1), 79-94.
19. Matome Gabriel Thathana, HunjaMurage, Akebe Luther King Abia ID and Michael Pillay, Morphological Characterization and Determination of Aflatoxin-Production Potentials of Aspergillusflavus Isolated

from Maize and Soil in Kenya, Agriculture, 2017, 7(10), 1-14.
20. Edmond Panariti, seasonal variations of aflatoxin M1 in the farm milk in Albania, Arh Hig Rada Toksikol, 2001, 52(1), 37–41.
21. Salah Eldeen Abass Ali Ahmed, Abdalla Ahmed Elbashir, Determination of Aflatoxins in Groundnut and Groundnut products in Sudan using AflaTest and HPLC, Mem. Inst. Investig. Cienc. Salud. 2016, 14(2), 35-39.
22. Shami Elhaj Alssafi Bakhiet and Ahmed Altayeb Ahmed Musa, Survey and Determination of Aflatoxin Levels in Stored Peanut in Sudan, Jordan Journal of Biological Sciences, 2011, 4(1), 13-20.
23. Zahra Nazari Khorasgani, Azadeh Nakisa & Nadereh Rahbar Farshpira, The Occurrence of Aflatoxins in Peanuts in Supermarkets in Ahvaz, Iran, Journal of Food Research, 2013, 2(1), 94-100.
24. Muna T. AL-Mossawei, Labeeb A. AL-Zubaidi, Isam S. Hamza, Saja Y. Abduljaleel, Detection of AFM1 in Milk and Some Dairy Products in Iraq using different techniques, Advances in Life Science and Technology, 2016, 41, 74-81.
25. Fardos M Bokhari, Aflatoxins Production by Aspergillus flavus, isolated from Different Foodstuffs Commonly Used in Jeddah Region, Saudi Arabia, Pakistan Journal of Biological Sciences, 2002, 5(1), 69-74.
26. Korrapati Kotinagu, T. Mohanamba and L. Rathna Kumari, Assessment of aflatoxin B1 in livestock feed and feed ingredients by high-performance thin layer chromatography, Veterinary World, EISSN, 2015, 8(5), 2231-0916.
27. J. Ramesh, Ghadevaru Sarath Chandra and V. Suresh Kumar, Validated HPTLC Method FOR Aflatoxin B1 Detection in Feed Ingredient and Feed Samples, Pharmacovigilance Laboratory for Animal Feed and Food Safety, 2014, 43 (2), 159 – 165.
28. H. Marina Martins, Salomé A. Magalhães1, Inês Almeida2, Marta Marques1, M. Manuela Guerra, F. Bernardo4 Aflatoxin M1 determination in cheese by immunoaffinity column clean-up coupled to high-performance liquid chromatography, Determinação de Aflatoxina M1 em queijos por HPLC com purificação por colunas de imunoafinidade, 2007, 102 (563-564), 321-325.
29. Seikichi Tsuboi,1 Toshitaro Nakagawa, Masahiro Tornita, Toshihiko Seo, Hidenori Ono, Koji Kawamura, and Noboru Iwamura, Detection of Aflatoxin B1 in Serum Samples of Male Japanese Subjects by Radioimmunoassay and High-Performance Liquid Chromatography, Cancer Research, 1984, 44, 1231-1234.
30. Siahi Shadbad Mohammad Reza, Ansarin Masoud, Tahavori Ali, Ghaderi Faranak, Nemati Mahboob, Determination of aflatoxins in nuts of Tabriz confectionaries by ELISA and HPLC methods, Advanced Pharmaceutical Bulletin, 2012, 2(1), 123-126
31. S. T. Ubwa, K. Asemave and G. O. Igbum, Preliminary screening of aflatoxin level in maize (Zea mays L.) in some selected markets in Benue State, Nigeria, African Journal of Pure and Applied Chemistry, 2012, 6(14), 159-163
32. Mohamad Sadegh, Parya Rahnama, Ali Mohamadi Sani Comparison of ELISA and HPLC techniques in determination of AFB1 in feedstuff, An Indian Journal, 2014, 9(10), 429-434.
33. Shotwell OL, Burg WR, Diller T, Thin layer chromatographic determination of aflatoxin in corn 64(5): dust. J Assoc off Anal Chem, 1981, 64(5), 1060-3.
34. Marcela Martínez-Mirandaa, Gonzalo Taborda-Ocampob and Milton Rosero-Moreanob, Validation of a High Performance Liquid Chromatography Method for Aflatoxins Determination in Corn Arepas, Journal of the Brazilian Chemical Society, 2015, 26(4), 797-803.
35. Wejdan Shakir Khayoon, Bahruddin Saad, Yan Chew Bee, Nor Hasani Hashim, Abdussalam Salhin, M. A., Muhammad Idiris Salleh and Baharuddin Salleh, Analytical Methods Determination of aflatoxins in animal feeds by HPLC with multifunctional column clean-up, Food Chemistry, 2010, 118(3), 882-886.
36. Kai Peng, Meisheng Jiang, Xiong Kang, Kai Yang, Shunxi Wang and Cailing Liu, A Cooperative Method for Detemination of Aflatoxin B1 in Tea by Immunoaffinity Column Purification and HPLC-FLD with Post-Column Photochemical Derivatization, American Society of Agricultural and Biological Engineers, 2015.
37. Joao Augusto, Under Direction of David M. Wilson, Development of a screening method for determination of Aflatoxins, B.S., Universidade Eduardo Mondlane, Mozambique, 1996.
38. R. Ghalia and Belouaera, S.Hdiria, H.Ghorbela, K.Maaroufib, A.Hedillia, Simultaneous HPLC determination of aflatoxins B1, B2, G1 and G2 in Tunisian sorghum and pistachios, Journal of Food Composition and Analysis, 2009, 22(7/8), 751-755.
39. Sai Gopalakrishna Yerneni, Seenivasan Sree Hari, Ragupathy Jaganathan, Monoharan Senthamarai, Narasinganallur Sankaranarayanan Vasanthi and Kilavan Packiam Kannan, Determination of the level of aflatoxin present in the marketed spices, IWSoSrNld: J2o2u3r1n a–l 2o5f 8S7cience and Technology, 2012, 2(12), 31-34.
40. Baranitharan krishnan, Sarathchandra Ghadevaru, Manimehalai, N. Athmaselvi, K.A. & Padmavati, R, determination of aflatoxin b1 in corn flour using high performance liquid chromatography, IJABR, 2015, 5(2), 172-176.
41. Papiya Mitra Mazumder and D. Sasmal, Mycotoxins – Limits and Regulations, Anc Sci Life. 2001, 20 (3): 1–19.
42. FDA. (1988) Food and Drug Administration, USA. (1988) Action levels for added poisonous or deleterious substances in food. Notice Fed. Register 53, 5043–5044.

43. Bennett JW, Kale S & Yu J. (2007) Aflatoxins: Background, Toxicology, and Molecular Biology. In: Simjee S. Infectious Disease: Foodborne Diseases. Humana Press Inc, Totowa NJ, 355-373.

44. Strosnider H, Azziz-Baumgartner E, Banziger M, Bhat RV, Breiman R, et al. (2006). Public Health Strategies for Reducing Aflatoxin Exposure in Developing Countries: A Workgroup Report. Environmental Health Perspectives, 12:1898-1903.