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QUALIFICATION AND QUANTIFICATION OF THE AFLATOXINS IN FOOD PRODUCTS CONSUMED IN KHARTOUM STATE, COMPARING WITH AFLATOXIN ALLOWANCE IN FOOD, SUDAN

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ABSTRACT

The present study is aim to detect and quantify an aflatoxin namely B1, B2 (Aflatoxin produce blue colour), G1, G2 (Aflatoxin produce green colour) and AF M (Aflatoxin in milk) in milk, egg, banana and onion in locations (Bahri. Omdurman and Khartoum) in Khartoum State Sudan. The results reflect that AFB1 in onion in three locations in Khartoum State is high. AFB2 for egg and banana in three locations are detected, but in small amount. Egg, milk, onion and banana are free from AFG1 and AFG2. AFM in milk was detected in three locations in Khartoum State, in high amount.

KEYWORDS

AFB1, AFB2, AFGI, AFG2 and AFM, Egg, Onion, Banana and Milk.

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INTRODUCTON

Aflatoxins are a group of structurally a toxic compounds produced by certain strains of the fungi (*Asperillus flavus*), this fungi is produce only B Aflatoxins and A parasitic us; in addition it is produces both B and G Aflatoxins. The major Aflatoxins are designated B1, B2, G1 and G2 Aflatoxin B is the aflatoxin which produces a blue color under ultraviolet while Aflatoxin G produces the green color. AFM1 produces a blue-violet fluorescence while AFM2 produces a violet fluorescence (PPSHS, 2016)[1]. Aflatoxins are

genotoxic, carcinogenic and immunosuppressive substances, and cause both acute and chronic toxicity (Battilani *et al,* 2016)[2].

Crop growth and its interaction with beneficiary and pathogenic and/or toxigenic micro-organisms vary from year to year, mainly depending on local weather, making the agricultural sector particularly exposed to climate change (Moore and Lobell, 2015)[3]. These fungal metabolites are important causes of chronic toxicity from exposure via food (De Boevre *et al*, 2015)[4].

These toxins are usually found together in various foods and feeds in various proportion. Aflatoxins M1 and M2 are oxidative metabolic product of Aflatoxins B1, and B2 produce by animals and is usually excreted in the milk, urine and faces of dairy cattle and other mammalian species that have consumed Aflatoxins contaminated food or feed. Aflatoxicol is reductive metabolite of Aflatoxins B1 (Bakirici, 2001)[5]. National dietary surveys are used for monitoring the food consumption and nutrient intakes of population groups and for assessing potential foodborne risks, which arise from the intake of harmful substances such as toxins or microbes, or the excessive intake of natural food components. Food risk assessment is used to characterize the potential adverse effects on health resulting from exposure to food-borne risks over a specified time period (FAO/WHO, 2006)[6]. In recent years, the idea of risk based food safety management has increased the need for food risk assessment. Risk assessment based food safety measures are designed to reduce risks to a target level (FAO/WHO, 2006)[6]. These measures are planned in order to achieve an established level of human health protection. In order to qualify food-borne risks, an exposure assessment, as a part risk assessment, is essential. For exposure assessment, demographically and geographically representative food consumption data is needed. In most countries, Risk analysis is a powerful tool for carrying out science-based analysis and for reaching sound, consistent solutions to food safety problem. The term of risk analysis can promote ongoing improvement in public health and produce a basis for expanding international trade in food. Mycotoxins are toxic secondary metabolites produced by filamentous fungi, most commonly of the genera Aspergillus, Fusarium and Penicillium, other important producers being Claviceps and Alternaria (Bhat *et al,* 2010)[7].

Several hundred different mycotoxins have been discovered so far, exhibiting different structural diversity, with various chemical and physicochemical properties, but only a few present significant food safety challenges (Cole and Cox, 1981)[8]. Aflatoxins and ochratoxins are produced by Aspergillus sp., fumonisins, trichothecenes and zearalenone are produced by Fusarium sp., patulin is produced by Penicillium sp., and ergot alkaloids are produced in the sclerotia of Claviceps sp., They are the most frequent occurring mycotoxins with the most severe effects in humans and animals (Richard et *al,* 1993)[9].

Mycotoxins remain challenging to classify due to their diverse chemical structures, biosynthetic origins and their production by a wide number of fungal species. A first approach can be to classify them according to their differences in their fungal origin, chemical structure and biological activity. Also the classification can be done according to how frequently they occur and in what amounts. This is a more complicated task because mycotoxin contamination of food and feed depends on environmental and climatic conditions, harvesting techniques, storage conditions and some others factors. Typically, the classification schemes reflect the scientific background of the person doing the categorizing. For clinicians the classification is done depending on the organ they affect: hepatotoxins, nephrotoxins, neurotoxins, immunotoxins. For cell biologists the classification is done according to generic groups such as teratogens, mutagens, carcinogens and allergens. Organic chemists tend to classify mycotoxins according to their chemical structures e.g. Lactones, coumarines, biochemists according to their biosynthetic origins such as polyketides, amino acid-derived, physicians by the illnesses, they cause such as St. Anthony's fire, stachybotrytoxicosis, and mycologists by the fungi that produce them e.g. *Aspergillus toxins*, Penicillium toxins (Scott-Craig *et al*, 1992)[10]. The major mycotoxins are Aflatoxins (AFs) (Vargas *et al*, 2007[11] and Klich, 2002)[12]. Zearalenone (ZEN) (Hussein and Brasel, 2001)[13] and Ochratoxins (OT) (Zinedine, 2010)[14].

After extensive investigation into the deaths, a link was observed that the feed had come from the same shipment of peanut meal from Brazil (Daly, 2000)[15] which had become moldy during transport. Further investigations showed that the peanut meal was heavily contaminated with the organism Aspergillus flavus, hence the name Aflatoxin (Smith, 19997)[16] and that the poultry died from liver cancer since the aflatoxins were highly carcinogenic.

In particular, Aflatoxins, which have the highest acute and chronic toxicity of all mycotoxins (Flores-Flores *et al*, 2015)[17].

The topic is of great economic and societal interest both for the quantitative and qualitative effects on crop yield and the impact on the occurrence of mycotoxins (Magan *et al,* 2011)[18]. Very low Aflatoxin contamination was reported in the few studies published on wheat (Alkadri, 2014)[19].

Indeed, new strategies supported by predictions should be adopted (Atehnkeng *et al,* 2014)[20], such as biological control using atoxigenic *A. flavus* strains, able to displace the toxigenic populations of the fungus, as largely applied in risk areas in the USA and Africa (Hayat and Idris, 2000)[21].

Objectives of this study are

1. To monitoring the analysis the contents of undesirable Aflatoxin (contaminant) in specific foods such as Milk, Egg, Onion and Banana.
2. To study the particular implications (risk assessment) of targeted food consumed by individual or family per day using a cross-sectional population survey.
3. To Assessment the contaminate substance such as Aflatoxins in foods and analysis it by using HPLC method comparing with control.

MATERIAL AND METHODS

Materials

Seventy two samples (4 Foods X 3 locations X 6 samples) namely, Milk; Egg, Onion and Banana were collected from different locations (Khartoum, Omdurman and Bahri) in Khartoum State.

Preparation of sample

All samples were kept in polyethylene bags and the milk put in glass bottles, then they were taken to the laboratory for analysis. The dry samples were crushed in mortar to a fine powder and put in sterile containers until all chemicals analysis will be started. A serial dilution technique was employed where 1g of the sample was diluted in 9ml peptone water and vortexed sample of 1ml of this suspension was transferred to sterile petrin dishes mixed with potato dextrose Agar (PDA) at 28^{o}C for 3-5 days. After the incubation period the growing fungal culture were examined micro scenically using lacto phenol cotton blue (LPCB) stain and classified by reporting the culture characteristics at the face and reverse side of the inoculated petri dishes (Hayat. and Idris, 2000)[21]. Standard solution was prepared by dissolving 0.4ml of stock standard solution in methanol to produce a concentration of 50mg/ml in a 10ml volumetric flask, other working standard solutions were prepared by diluting this standard solution with methanol to achieve different concentrations of aflatoxins mixtures.

Quantification and Detection of Aflatoxin

Then Extraction, clean up and determination of aflatoxins were done according to method described by (AOAC, 1995)[22].

Fifty grams of a representative powdered of different type of samples were transferred into a blender Jar, containing 200 ml methanol and 50ml 0.1N hydrochloric acid and blundered for 3 min at high speed. The solution was filtered through 24cm Whatman No.1. Filter paper, then 50ml of the filtrate was transferred into a 250ml separation funnel, 50ml of 10% sodium chloride solution was added and the solution was swirled. Fifty milliliters of hexane was added and the solution was shaken gently for 20 seconds. The two phases were separated and the lower layer was drained into a 250ml separation funnel and extracted three times with 25ml of dichloromethane. The dichloromethane extracts were combined and concentrated to approximately 2ml. The concentrated extract was carefully transferred into a silica gel chromatography column and washed with 30ml of ether: hexane solution (3:1) (v/v) 2.0ml of the dichloromethane extract was poured into the column and the beaker was washed with 0.5ml of dichloromethane. The column was cleaned with 25ml of benzene: acetic acid (9:1) (v/v), then 30ml of ether. Aflatoxins (AFs) were eluted from the column with 100ml of dichloromethane: acetones

(90:10) (v/v), the solvents were evaporated. To derivative the AFs, 200µl hexane and 50µl triflouroacetic acid (TFA) were added. The mixture was shaken vigorously using a Vortex for 30 seconds and left to stand for 5min. 1.95ml of acetonitrile; water (1:9) (v/v) was added; the mixture was shaken for 30 seconds and left for 10min to separate. The lower aqueous layer was collected by automatic pipette and used for HPLC analysis; for the blank test, similarly, a working standard mixture was derivatived. The HPLC conditions used were Supercoil LC 18 column, 150 x 4.6mm internal diameter (I.D), 5 micron particle size; oven temperature 40ºC, fluorescence detector at excitation 360nm and emission 476nm, mobile phase consisted of water: acetonitrile: methanol (700:170:170) was used. The flow rate of the mobile phase was maintained at 1.0ml min-1 and then volume of sample solution was injected about 20µl.

Statistical analysis

Statistical analysis was performed using SPSS package for windows version 21.0 Data are expressed as Mean±SD, One way ANOVA and T - test were used to analyze differences among groups.

RESULTS AND DISCUSSION

Table No.1 indicated that calculate values ofAFB1 (Aflatoxin bluish) in Bahri, Khartoum and Omdurman in onion were 9.58±1.12, 3.80±0.76 and 6.75±1.48 ppb, respectively. These findings are observed the AFB1 are found in three locations only for anion crop, but it is still observed that calculated value of FB1 are not detect in Egg, Banana and Milk in three locations in Khartoum State. But calculated value of AFB1 in Bahri is highly significant ($P<0.01$) compared with maximum residue limit value (MRL) reported by (ACS, 2014)[23], whereas calculated value of AFB1 in Khartoum location is significantly low ($P < 0.05$) compared with maximum residue limit value (MRL) reported by (ACS, 2014)[23]. While in Omdurman location is high than those findings by (ACS, 2014)[23]. In three locations (Bahri, Khartoum and Omdurman) is still lower than thus value reported by (EFS, 2018)[24]. The calculated value of AFB2 (Aflatoxin bluish) in egg and Banana for Bahri location was 1.78±0.12 and 2.70±0.32 ppb, respectively. While calculated value of AFB2 in Khartoum for Banana was 2.18±0.67ppb, but it is not detected in egg, but it is observed that the AFB2 in three locations were not detected in onion and milk. These findings are lower than those values reported by (ACS, 2014[23] and EFS, 2018)[24]. Therefore, the egg, banana, onion and milk are less contaminated with AFB2 in three locations. Contamination of milk, egg, onion and banana with AFB1 and AFB2 can cause potential carcinogenic effects if ingested even in small amounts (EFS, 2017)[25].

It is also observed that AFG1 and AFG2 (Aflatoxin greenish) in Egg, Banana, Onion and milk are not detected in three locations. These results are reflecting that Egg, Banana, Onion and milk were free from AFG1 and AFG2; this might be due to following the proper hygienic operations during handling, transport and storage of those egg, milk, onion and banana. Therefore, those foods in three Locations in Khartoum state was less contaminated with AFG1 and AFG2.

It is still observed that the calculated value of AFM (Aflatoxin in milk) for the milk in three locations (Bahri, Khartoum and Omdurman) was 2.27±0.69, 4.35±0.69 and 3.48±0.62 ppb, respectively. These results are indicated that calculated value of AFM for milk in three locations was highly significant than those values reported by (JECFA, 2016)[26].

It is also clearly observed that AFM are not detected in egg, banana and onion in three locations. This might be climate Change, highlighting how changes in temperature, humidity, rainfall and carbon dioxide production impact on fungal behaviour and consequently on mycotoxin production (EFS, 2017)[25].

The panel estimates that raising the maximum level could increase the risk of aflatoxin-induced cancers for consumers of peanuts and peanut-based processed products (JECFA, 2016)[26].

Table No.1: Aflatoxin concentration (ppb) of products collected from different sites in Khartoum State and correlated with the maximum residue limit (MRL)

S.No	Aflatoxin types	AFB1	AFB2			AFG1	AFG2	AFM		
1		CV		MRL	MD				MRL	MD
2	Bahri									
3	Egg	ND	1.78±0.12	5.0	-3.22*	ND	ND	ND		
4	Banana	ND	2.70±0.32	5.0	-2.30*	ND	ND	ND		
5	Onion	9.58±1.12	ND	5.0	4.58**	ND	ND	ND		
6	Milk	ND	ND	5.0	------	ND	ND	2.27±0.69	0.50	1.77**
7	Khartoum									
8	Egg	ND	ND	5.0	------	ND	ND	ND		
9	Banana	ND	2.18±0.67	5.0	- 2.82*	ND	ND	ND		
10	Onion	3.80±0.76	ND	5.0	-1.2*	ND	ND	ND		
11	Milk	ND	ND	5.0	-------	ND	ND	4.35±0.69	0.50	3.85**
12	Omdurman									
13	Egg	ND	1.38±0.57	5.0	- 2.82*	ND	ND	ND		
14	Banana	ND	4.32±0.48	5.0	-2.68*	ND	ND	ND		
15	Onion	6.75±1.48	ND	5.0	1.75*	ND	ND	ND		
16	Milk	ND	ND	5.0	------	ND	ND	3.48±0.62	0.50	2.98**

CV (Calculated value), MD (Mean difference), and ND (Not detected). Values are means of 6 samples ±SD. ** P<0.01, * P<0.05. Mean difference= calculated value- maximum residue limit (MRL). (ACS, 2014)[23].

CONCLUSION

The results are reflected that AFB1 in onion was observed in three locations in Khartoum State, in addition the value obtained is high. AFB2 for egg and banana in three locations are detected, except AFB2 for egg in Khartoum is not detected. AFG1 and AFG2 for egg, milk, onion and banana in three location were not detected, therefore, egg, milk, onion and banana are free from AFG1 and AFG2. In addition AFM in milk was detected in three locations in Khartoum State, in addition the values obtained for AFM is high.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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