Detection of Aflatoxins Present in Some Types of Nuts are Available In Local Markets

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ABSTRACT
Aflatoxins are a group of mycotoxins and secondary metabolites of various species of *Aspergillus.* These are various forms of aflatoxins. Aflatoxins cause important health problems and have a high potential effect on live cancer. Many studies have been conducted during last three decades about contamination of Nuts by aflatoxins. The aim of this work was to investigate the contamination levels of nuts used by confectionaries in Baghdad. A total of fifteen samples including (3Almonds, 3Pistachio, 3Hazelnut , 3Peanuts and 3Walnut) were collected from local market in Baghdad city. ELISA (enzyme linked immunosorbent assays) technique was employed for screening of total aflatoxins in nuts. The contamination rate was higher than 10 ppb was observed in (one sample of Almonds, two samples of hazelnut, and in two samples of Walnuts). The result of tested samples showed that the rate of contamination of hazelnut is higher than other tested samples.

الكشف عن السموم الفطريه المتواجدة في بعض انواع المكسرات المتوافرة في الاسواق المحليه

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الكلمات المفتاحية: السموم الفطريه, المكسرات, فحص ELISA

الخلاصة
الأفلاتوكسين مجموعة من السموم الفطرية والمركبات الثانوية لمختلف أنواع الفطريات مثل Aspergillus flavus . وتسبب السموم الفطريه مشاكل صحية خطيرة مثل سرطان الكبد. وقد أجريت العديد من الدراسات خلال العقود الثلاثة الماضية. والهدف من هذا البحث هو التحري عن مستويات التلوث بالسموم الفطريه في مختلف انواع المكسرات التي يتناولها المستهلك العراقي في بغداد. تم جمع ما مجموعه خمسة عشر عينة منها (3 اللوز، 3الفستق، 3 البندق،3فستق العبيد و 3 الجوز) من الاسواق المحليه في مدينة بغداد .أستخدمت طريقه ELISA (المقايسة المناعية المرتبط بالإنزيم) لفحص إجمالي الأفلاتوكسين في المكسرات. وكان معدل التلوث أعلى مما هو لوحظ (10) اجزاء في البليون في (عينة واحدة من اللوز، وعينتان من البندق، وعينتان من الجوز).وأظهرت نتيجة العينات المفحوصه أن معدل تلوث في البندق أعلى من العينات المفحوصه الأخرى.

1. INTRODUCTION

Aflatoxins (AFs) are a group of mycotoxins and secondary metabolites of strains of the fungi Aspergillus spp**.[1].** There are various forms of aflatoxins including B1, B2, G1, G2, M1 and M2 types **[2].**Fungi produce several metabolites and develop rapidly on different kinds of foods **[3**]. These microorganisms can easily spread by wind, insects and raining, thus, survive in the environment **[4]**. The most toxic form of mycotoxins is called aflatoxin (AF), Aflatoxins are toxic secondary metabolites produced mostly by *Aspergillus flavus* and *Aspergillus parasiticus*,. *Aspergillus flavus* and *Aspergillus parasiticus* colonize a wide variety of food commodities including maize, oilseeds, spices, groundnuts, tree nuts, milk, and dried fruit **[5]**.

Aflatoxin B1 exhibits the highest toxicity and carcinogenicity and it can be found in many markets in (Almond, pistachio, hazelnut, pistachio field, coconut) **[6]**. The International Agency for Research on Cancer (**IARC**) classified aflatoxin B1 as carcinogenic to humans **[7].** The fungi produce aflatoxin depend on drought stress and rainfall, suitability of crop genotype for its climate, insect damage, and agricultural practices**[6]**.

These fungi can also produce aflatoxin depending on conditions including storage, transportation, and food processing. Aflatoxin contamination is a particular problem in maize, oilseeds, spices, peanuts, tree nuts (almonds, pistachios, hazelnuts, Peanuts, and walnuts)**[8]**. Almonds, pistachios, hazelnuts, walnuts are the main sources of human exposure to aflatoxin because they are so highly consumed worldwide and unfortunately are also the most susceptible crops to aflatoxin contamination and the pathway by which aflatoxin accumulates in food and contributes to various adverse human health effects **[9]**. Molds naturally produce a wide range of metabolites, called mycotoxins. Mycotoxins can have toxic effects on human health and human tissues and organs **[10]**. All studies have shown that aflatoxin contaminates some kinds of foods such as almond, pistachio, hazelnut, pistachio field, coconut, dried fruits, cereals and tea, since these groups are often exposed to the fungal infestations [**11,12,13**]. According to the food and agriculture organization (**FAO**) on all the worlds more than 50% of the foodstuff are contaminated with Aflatoxin [**14**,**15**].

2. MATERIALS AND METHODS

Collection of sampling: A total of fifteen samples including (**Almond, Pistachio, hazelnut**, **Peanuts** ,**Walnut**) were collected randomly (250g of each sample) from different region in Baghdad local markets, from February to October 2014. All samples delivered in sterile packets, moved to a laboratory and kept in a cool place (3 - 5°C) maximum for three days and analysis at Market Research and Consumer Production Center in Baghdad University. Nuts were surface-sterilized in 4% sodium hypochlorite for two minutes, diluted with distilled water three times to a concentration of 2%, rinsed in 100 mL distilled water and then let to dry (Table1) .

Table :(1) The collected samples from local markets

No	Sample	origin	Date of production	Date of expired	Weight (gram)
1	Almond	Mansour	2013/11/1	2014/10/1	250
		Karada	2014/1/12	2015/1/11	
		Shorja	2013/10/2	2014/9/1	
		Mansour	2014/2/1	2015/1/1	

2	Pistachio	Karada	2014/2/2	2015/2/1	250
		Shorja	2014/3/22	2015/2/21	
3	hazelnut	Mansour	2014/3/1	2015/2/1	250
		Karada	2013/12/1	2014/11/1	
		Shorja	2014/1/29	2015/1/1	
4	Peanuts	Mansour	2014/11/1	2015/10/2	250
		Karada	2014/4/1	2015/3/1	
		Shorja	2014/2/1	2015/1/6	
5	Walnut	Mansour	2013/12/29	2014/11/1	250
		Karada	2014/2/25	2015/1/24	
		Shorja	2014/10/1	2015/9/2	

Samples extraction:

Nuts samples to be tested were collected according to accepted sampling techniques. The samples were ground and thoroughly mixed prior to proceeding with the extraction. Samples were stored at 2-8° C (35-46°F) until analyzed. First samples extractions were carried out according to (AOAC Methods) .

1. 70% methanol solution was prepared by mixing 7 parts ACS Grade methanol with 3 parts distilled water for each sample to be tested.

2. A representative sample was obtained. Grinded the entire sample, so that at least 75% of the ground material passes through a 20 mesh sieve.

For this purpose, 50 g of grinded samples was taken and 250 ml of 70% methanol solution (methanol: distilled water, 30:60) added, shaken for three minutes and then let settle for 15 minutes at room temperature. Then, the extract was filtered through a Whatman (No 1 filter paper), the clear supernatant was diluted 1:2 with 70% methanol solution were transferred to microtubes and stored at (2 -8° C) until analysis be started. Samples were tested through ELISA Neogen's Extraction Kits [**15**].

Enzyme – linked immunosorbent assay (ELISA) analysis:

Analysis of samples done by ELISA, (Neogen's Extraction Kits) and all reagents warmed to room temperature (18- 30°C) before use. First removed 1 red marked mixed well for each

sample to be tested plus 4 red-marked wells for controls, and placed in the well holder, than removed an equal number of antibody –coated wells. Returned antibody wells which will not be used immediately to the foil pack with desiccant. Resealed the foil pack to protected the antibody. Mark one end of strip with a "1", and placed strip in the well holder with the marked end on the left, than mixed each reagent by swirled the reagent bottle prior to use, than placed 100 µL of conjugated from the blue –labeled bottle in each red-marked mixed well and used anew pipette tip for each, transferred 100 µL of controls and samples to the red-marked mixed wells as described below.

0 5 15 50 S1 S2 S3 S4 S5 S6 S7 S8 Strip1

S9 S10 S11 S12 S13 S14 S15 S16 S17 S18 S19 S20 Strip2

Than used 12 channel pipettor, mixed the liquid in the wells by pipetted it up and down 3 times. Transfered 100 µL to the antibody-coated wells, Discarded the red- marked mixed wells. And set timer for 2 minutes, mixed the wells for the first 10-20 seconds of the room temperature incubated by slide the microwell holder back and forth on a flat surface without splashing reagents from wells. Then shacked out the contents of the antibody wells, filled the wells with distal water and dumped them out. Repeated this step 5time, then turned the wells upside-down and tapped out on a paper towel until the remaining water removed. With new tips on the 12 channel pipettor, primed and pipetted 100 µl substrate into the wells then set timer for 3 minutes, mixed the wells for the first 10-20 seconds by slide back and forth on a flat surface. Discarded the remaining substrate and rinsed the reagent boat with water. Then poured red stop solution from the red-labeled bottle into the red-labeled reagent boat. Ejected the excess substrate from the 12 channel pipttor, primed the tips, and pipetted 100 µl of red stop to each well. Mixed by slide back and forth on a flat surface. Discarded the tips. Wiped the bottom of the microwells with a dry cloth and read in a mirowell reader using a (650nm) filter. Air bubbles was eliminated because they could be effected on analytical results. Results was viewed within 20 minutes after the addition of red stop, then read and calculated result using Neogen's Stat fax microwell reader [**16**].

3. Statistical Analysis: Statistical significance was assessed by using least significant differences – LSD (T-test), P – value < 0.05 was considered significance.

4. RESULTS

In this study shows the contamination in different kinds of nuts by aflatoxin B1. Tables (**2,3,4,5,6**) and figures (**1,2,3,4,5**) presents the concentrations of total aflatoxin B1 in analyzed samples.

Table (2) Determination of aflatoxin B1 in almonds by ELISA method.

NO	Sample	Origin	Aflatoxin B1 (µg/kg)	Normal range (µg/kg)
1	Almonds	Mansour	14	10
		Karada	8	
		Shorja	7.5	

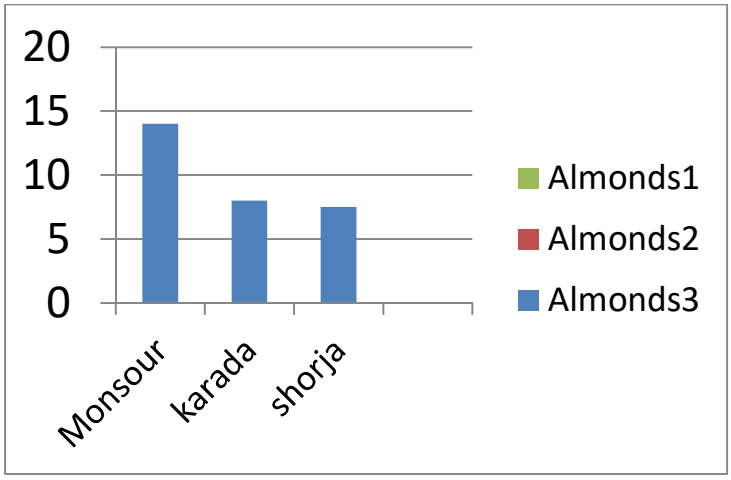

Figure (1) Determination of aflatoxin B1 in almonds samples.

Table (3) Determination of aflatoxin B1 in pistachio by ELISA method.

NO	Sample	Origin	Aflatoxin B1 (µg/kg)	Normal range (µg/kg)
2	Pistachio	Mansour	3.6	10
		Karada	5.0	
		Shorja	9.0	

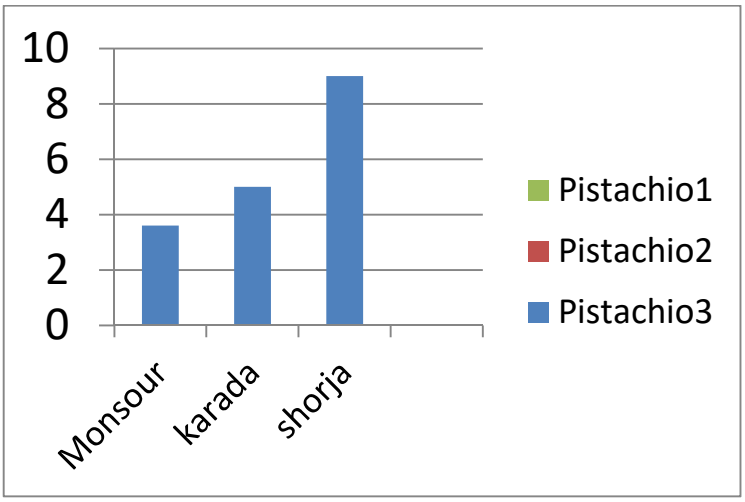

Figure (2) Determination of aflatoxin B1 in pistachio samples.

Table (4) Determination of aflatoxin B1 in hazelnut by ELISA method.

NO	Sample	Origin	Aflatoxin B1 (µg/kg)	Normal range (µg/kg)
3	Hazelnut	Mansour	24	10
		Karada	15	
		Shorja	8.2	

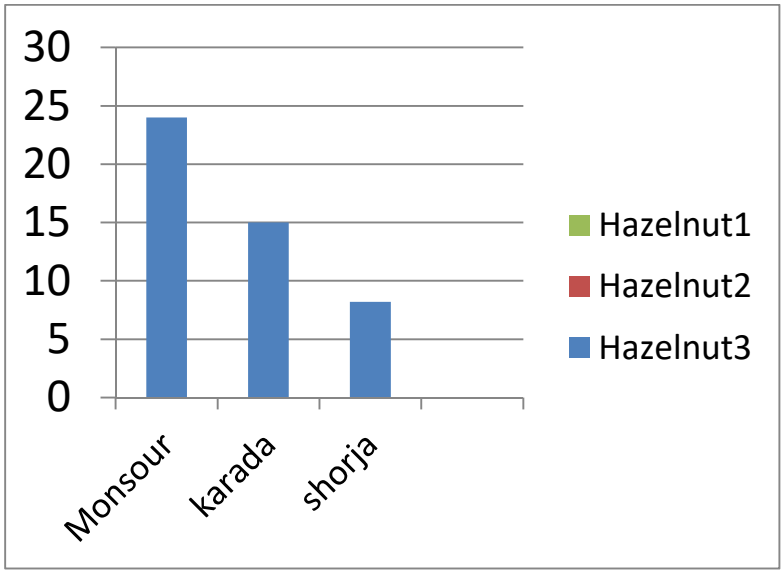

Figure (3) Determination of aflatoxin B1 in hazelnut samples

Table (5) Determination of aflatoxin B1 in peanuts by ELISA method.

NO	Sample	Origin	Aflatoxin B1 (µg/kg)	Normal range (µg/kg)
4	Peanuts	Mansour	6.1	10
		Karada	2.1	
		Shorja	10	

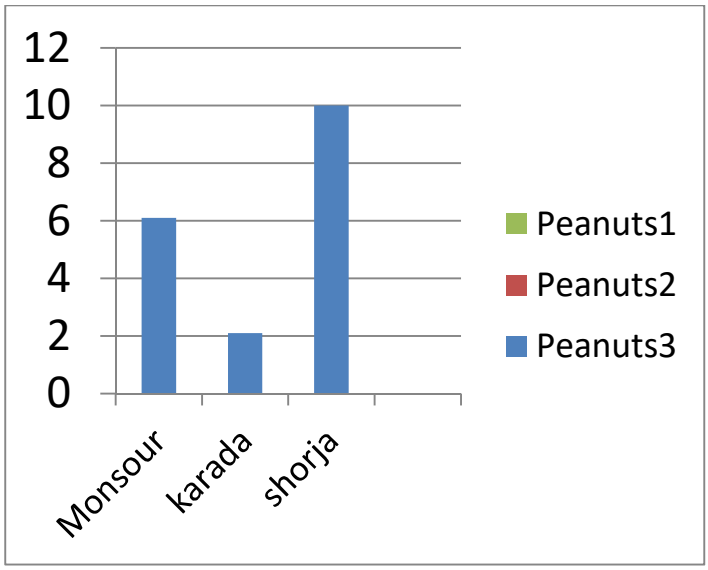

Figure (4) Determination of aflatoxin B1 in peanuts samples.

Table(6) Determination of aflatoxin B1 in walnuts by ELISA method.

NO	Sample	Origin	Aflatoxin B1 (µg/kg)	Normal range (µg/kg)
5	Nut	Mansour	8.4	10
		Karada	22	
		Shorja	11.5	

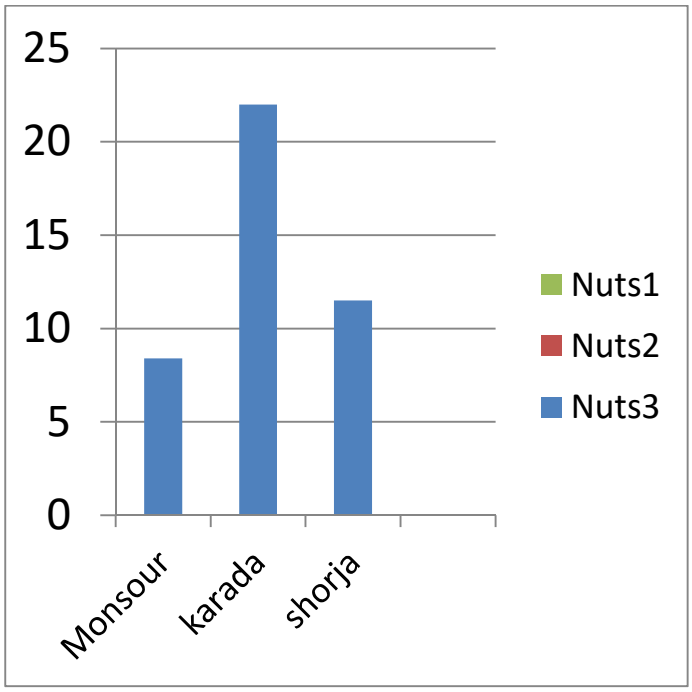

Figure (5) Determination of aflatoxin B1 in walnuts samples.

5. DISCUSSION

The contamination food with aflatoxins is a very important case of focus of health scientists. Walnuts, almonds, pistachio, hazelnut and peanuts are food samples which are susceptible for this contamination because of their composition and storage condition. The contamination level of aflatoxins in different types of nuts and nutty products has been studied by several scientists from different countries [**17**].

In this study ELISA method was used for detecting contaminated samples ELISA methods is suitable for determination of contamination in large number of samples in a shorter time. Besides, ELISA is rapid, cost- effective, accurate, sensitive, easy to use and convenient [**18**].

Results obtained in this study showed that more than five samples were contamination above the maximum tolerated levels (10µg/kg). The most contaminated sample was hazelnut (24.0µg/kg), followed by walnut (22.0 µg/kg),almonds (14µg/kg),and the least amount of contamination found in pistachio (9.0 µg/kg). This result is similar to the findings [**19,20**], while the results incompatible with **[21]** that indicate to concentration of aflatoxin in peanuts was (7.6 µg/kg) but in our results was (10µg/kg). In another study in Saudi Arabia, the concentration of AF in peanuts was 28 µg/kg [**15**,**22**].

Tables (**2,3,4,5,6**) shows the number of samples containing Aflatoxin more than 10 µg/kg (Parts per billion). According to these tables, hazelnut containing aflatoxin of more than 10 µg/kg were considered contamination with aflatoxin. High levels of aflatoxin in hazelnut and walnuts are probably due to the old poor methods manufacturing storage, transition and marketing [**23, 24**].

It seems that long- term consumption of the aflatoxins contaminated nuts has carcinogenic and toxigenic on the human Health.[**25, 26]**

Among the nut products, pistachios are extremely contaminated with Aflatoxin , In an Iranian study, the mean level of Aflatoxin in pistachios was recorded as 7.3 ± 53.2 ng/g which was lower than the Aflatoxin maximum tolerated level (MTL) of Iran [**27**].

Currently the worldwide MTL for Aflatoxin B1 and total Aflatoxins are 1-20 ng/g and 0-35 ng/g respectively[**28**]. European Commission regulating set limits for Aflatoxin B1 and total Aflatoxins 2 and 4 ng/g respectively in nuts, groundnuts, dried fruit and cereals since 1998 [**29, 30**]. Research on Food products has demonstrated that AFs are still being found frequently in food product at level that are of significant concern for consumers [**16**, **31**] . Due to the significant health risks associated with the presence of aflatoxins in foods, it is important to establish a data collection on the occurrence of these toxins in nuts as valuable foods. The aim of this study was to screen the content of Aflatoxins in nut used in the confectionary products in Baghdad markets.

The contamination of aflatoxin of the samples in our study were higher compared to intact one, probably that is due to storage condition in stores which make samples more susceptible for the fungal growth and contamination [**19**].

In Iraq, necessary steps should be taken by health organization and other related agencies to minimize the aflatoxin contamination and also educate people about the danger of aflatoxins in nuts, which are favorable products and used on nourishing and safe snacks in the Iraqi society. The standard requires in Iraqi products including (almonds, walnuts, hazelnuts, pistachios field) should be free from microbes and their toxins,aflatoxin B1 is the most carcinogenic, the toxic effects include acute hepatitis ,immunosuppression and hepatocellular carcinoma for human and animals [**32,33**].

6. CONCLUSIONS:

It was concluded that aflatoxin content of nuts should be monitored regularly to minimize the risk of aflatoxin hazard and ensure the food safety and quality.

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