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Spices in Local Market and Their Contamination by Aflatoxins, Tripoli - Libya

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Abstract: Aflatoxins are considered the most carcinogenic, mutagenic and teratogenic substances found naturally in foods and feeds. These metabolites cause liver damage to humans and to most experimental animal species tested. This study was conducted to assess the level of contamination of spices such as caraway, mixed spices, cinnamon, black pepper, red pepper and cumin sold in Tripoli market with poisons aflatoxins. The results of this study showed that the highest contamination of Afla B1 was in black pepper samples (12.1 ± 0.7 µg/kg) and the lowest contamination was in mixed spice samples (3.4 ± 0.2 µg/kg). The highest contamination of Afla B2 was in cumin samples as 10.2 ± 0.1 µg/kg, whereas the highest contamination of Afla G1 and G2 were also found in cumin samples 12.12 ± 0.09 and 7.6 ± 0.1 µg/kg, respectively. The contaminations of spices with aflatoxins were in various concentrations.

Keywords: Aflatoxins; Spices; Tripoli; Libya.

INTRODUCTION

Foods are exposed to certain species of fungi that secrete organic compounds as their metabolites that are mostly poisonous for humans, animals and other microorganisms (Abdulkadar, Al-Ali, Al-Kildi, & Al-Jedah, 2004). The most important recorded fungi as toxin producers are *Aspergillus*, *Fusarium* and *Penicillium* species that produce various kinds of toxins (Agaoglu, 1999). The most important myotoxins are aflatoxins, ochratoxins, zearalinon and others (Akiyama, Goda, Tanaka, & Toyoda, 2001). These toxins are highly toxic compounds that cause many kinds of diseases including cancers (Bircan, 2005). Aflatoxins are natural and very toxic to the human being (Songsermsakul & Razzazi-Fazeli, 2008). Aflatoxins are occurring carcinogenic substances and recognized as hepatotoxic and carcinogenic agents to humans and capable of inducing liver cancer and cirrhosis (Trombete, Santos, Direito, Fraga, & Saldanha, 2014).Fungi have the capability to grow on all foods without an exception, whether their moisture content was high or low (although fungi growth requires moisture) (El-Kady, El-Maraghy, & Mostafa, 1995). Fungi grow on crops in the field and after crop harvest and during storage (Erdogan, 2004). Fungi also grow within wide range of temperatures (15-35 ºC) and causes the damage to these commodities due to what they exposed to from physical (in shape, texture, color, aroma, and taste) and chemical (due to the fungal consumption of nutrients so lowering the food content of organic matter) (Fazekas, Tar, & Kovacs, 2005).

That did not necessarily mean that every fungus infected commodity is producing aflatoxins because fugal growth needs conditions different from that needed to produce toxins such as the moisture of the infected commodity, medium temperature, medium content of oxygen, and other required conditions (Garrido, Jodral, & Pozo, 1992; Romagnoli, Menna, Gruppioni, & Bergamini, 2007). However, not every fungus has the genetic capacity to produce aflatoxins even if belongs to one species known with aflatoxins production due to the differences between the isolated strains from the same spe-

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cies which are accompanied with differences in the capability of toxin (s) production according to the genetic capacity (Hazir & Coksoyler, 1998). The same aflatoxins also may be produced by more than one of toxic fungal species (Macdonald & Castle, 1996), which indicates the similarity of genetic capacity between fungal species.Many studies have been conducted concerning about public health for safety and to determine maximum aflatoxin levels for food and feed. The maximum levels for aflatoxins in various nuts, grains, dried figs, and milk are in the range of 0.5 to 15 µg/kg (a µgram is one millionth [1×10^{-6}] of a gram) (WHO, 2018).

The main objectives of this study were: (i) to survey the levels of Aflatoxins in Libyan spices by applying a HPLC method; (ii) to evaluate the differences regarding co-occurrence and the level of concentrations. To the best of our knowledge, this is the first survey concerning the presence of aflatoxins in Libyan Spices, collected from different local markets in Tripoli, Libya.

MATERIALS AND METHODS

In this research, a total of 154 samples of crushed spices (caraway, mixed spices, cinnamon, black pepper, red pepper, and cumin) were collected from private markets at Tripoli city. Samples were collected randomly and put into LDPE bags. Sample weight at collection was 100-200 g and information about sample name, weight, and date of collection were recorded on each bag. Samples were transferred under suitable conditions and stored at 4-20 ºC until tests were carried out to assess the kinds of aflatoxins (Martins, Martins, & Bernardo, 2001).The assessment steps began with subsampling, preparation and blending of 25 g subsample, extraction with methanol: water (70:30) mixture and filtration through Watman no 4-filter paper. The resulted filtrate was passed through immune affinity column and eluted with methanol 100%. Quantitative assessment was carried out on HPLC chromatograph with the following technical specifications: The HPLC equipment was a Shiseido (SI-2) system with 3023 pump, 3023 auto - injector and fluorescence detector set at 360 nm for excitation and 460 nm for emission. A Capcellpack C18 column (4.6- 250 mm, 5µm particle size, Shiseido, Japan) was used. The mobile phase was distilled water: methanol: acetonitrile (65:25:10) with a flow rate of 1 ml/min. The recovery experiments were carried out in samples which, the four aflatoxins were not found, with the concentration of 7 µg/ kg for each aflatoxin (B1, B2, G1 and G2) and stored for 12 hours at room temperature, prior to the extraction procedure.

Statistical Analysis: The results from aflatoxins analyses were subjected to statistical analysis using GenStat (Release 8.1, Rothamsted Experimental Station, and UK). Analyses of variance were run to evaluate the average of readings, and standard deviation was also calculated for each individual toxin.

RESULTS

Spices are considered as agricultural crops that might expose to the fungal infection especially with aflatoxins producing fungi. In this research, aflatoxins were assessed and the results in table (2) showed that the highest contamination of Afla B1 was in black pepper samples (12.1 ± 0.7 µg/kg) and the lowest contamination was in mixed spice samples (3.4 ± 0.2 µg/kg).

Table:(1). Average recovery rate (± sd) for total aflatoxins (B1, B2, G1, and G2) that were added to spice samples at a concentration of 7 µ/kg.

Sample	Average recovery rate (%)			
	Afla B1	Afla B2	Afla G1	Afla G2
Caraway	81±3	82±5	85±2	77±3
Mixed spices	83±5	86±3	81±6	75±5
Cinnamon	79±4	84±2	79±4	73±6
Black pepper	80±3	78±4	78±5	71±6
Red pepper	83±6	80±3	81±3	72±4
Cumin	84±2	85±4	79±3	70±5

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The highest contamination of Afla B2 was in cumin samples as 10.2 ± 0.1 µg/kg, whereas the highest contamination of Afla G1 and G2 were also found in cumin samples 12.12 ± 0.09 and 7.6 ± 0.1 µg/kg, respectively. The contamination concentrations were different from spice to another.

Table:(2). Average concentrations (± sd) and positive samples within the different kinds of spices.

Sample	Positive samples	Average concentration of aflatoxins (µg/kg)		
		Afla B1	Afla B2	Afla G1
Caraway	24 (5)	7.1 ± 0.1	4.9±0.08	2.4 ± 0.9
Mixed spices	29 (4)	3.4 ± 0.2	1.9±0.04	1.2±0.03
Cinnamon	22 (6)	6.4±0.06	2.5±0.03	3.1 ± 0.8
Black pepper	31 (4)	12.1±0.7	7.8±0.09	3.4±0.08
Red pepper	27 (4)	5.2 ± 0.2	4.4±0.08	2.2±0.06
Cumin	21 (6)	10.2±0.2	9.8 ± 0.1	2.1±0.09

DISCUSSION

Aflatoxins occur in nature, but four aflatoxins B1, B2, G1 and G2 are dangerous to humans and animals as they have been found in all major food crops (WHO, 2018). Aflatoxins are considered the most carcinogenic, mutagenic and teratogenic substances found naturally in foods and feeds. Fungal toxins are highly toxic compounds cause many kinds of diseases including cancers and mutagens (Bircan, 2005). This paper studied the levels of aflatoxins in Libyan spices applying a HPLC method to evaluate the differences regarding co-occurrence and the level of contamination.

The results demonstrated that aflatoxins were present in various concentrations in the different kinds of spice samples as a result of the growth of the productive fungi and the availability of suitable moisture and temperature for fungal growth, which agrees with (A Zinedine et al., 2006; Abdellah Zinedine et al., 2007). The results in this survey showed that the concentration of aflatoxins is in the range of the standard for aflatoxins as set by WHO. The concern about aflatoxins due to their important role as a source of disease outbreaks associated with food consumption is increasing and more studies should be carried out in Libya.

CONCLUSION

The contaminations of spices sold in Tripoli local markets with aflatoxins B1, B2, G1, and G2 were found in various concentrations. Therefore, regular monitoring of spices is strongly recommended. This study contributes to increasing the knowledge of Libyan public to ensure safety and quality of food.

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التوابل في السوق المحلي وتلوثها بسموم آلفا، طرابلس، ليبيا

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المستخلص : تعتبر الأفلاتوكسين من أكثر المواد المسببة للسرطان والطفرات الموجودة طبيعيا في الأطعمة والأعلاف. هذه الأيضات تسبب تلف الكبد للبشر ومعظم أنواع حيوانات تجارب المختبرات، أجرت هذه الدراسة لتقييم مستوى تلوث عناصر التوابل بسموم الأفلاتوكسينات B1 و B2 و G1 و G2 ،والتي تباع في السوق المحلي بطرابلس في ليبيا ، (كروية و التوابل المختلطة والقرفة والفلفل الأسود والفلفل الأحمر و الكمون) . نتائج الدراسة أظهرت أن أعلى تركيز للأفلاتوكسين B1 كان في عينات الفلفل الأسود بنسبة 12.4 ± 0.7 ميكروجرام / كجم وأن أقل مستوى للتلوث كان في عينات التوابل المختلطة بتركيز 3.4 ± 0.2 ميكروجرام / كجم. أعلى تركيز للتلوث بالأفلاتوكسين B1 كان في عينات الكمون بنسبة 0.1±10.2ميكروجرام / كجم. بينما أعلى تركيز للتلوث بالأفلاتوكسين G1 and G2 أيضا كان في عينات الكمون بنسبة 0.09±12.12 و0.17.6±ميكروجرام / كجم على التوالي. نستنتج من الدراسة أن تلوث التوابل بالأفلاتوكسين B1 و B2 و G1 و G2 كان بتركيزات مختلفة.

الكلمات المفتاحية: سموم آلفا، التوابل، طرابلس، ليبيا.

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