

## Aflatoxin Contamination of Spices Sold Collected from Local Market in Tripoli

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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. Conducted a survey to assess the level of contamination of spices items poisons aflatoxins B1, B2, G1 and G2, and in the local market in Tripoli in Libya during (2014), a (Caraway, Mixed spices, Cinnamon, Black pepper, Red pepper and Cumin). Aflatoxins were determined by High Performance Liquid Chromatography (HPLC). The results were obtained as follows: the average concentrations of aflatoxins B1, B2, G1 and G2 in Caraway ( $7.1 \pm 0.1$ ,  $4.9 \pm 0.08$ ,  $2.4 \pm 0.9$ ,  $3.1 \pm 0.07$   $\mu\text{g}/\text{kg}$ ) respectively, in Mixed spices the average concentrations ( $3.4 \pm 0.2$ ,  $1.9 \pm 0.04$ ,  $1.2 \pm 0.03$ ,  $0.9 \pm 0.09$   $\mu\text{g}/\text{kg}$ ) respectively, in the cinnamon, the average concentrations ( $6.4 \pm 0.06$ ,  $2.5 \pm 0.03$ ,  $3.1 \pm 0.8$ ,  $0.7 \pm 0.01$   $\mu\text{g}/\text{kg}$ ) respectively, in Black pepper, the average concentrations ( $12.1 \pm 0.7$ ,  $7.8 \pm 0.09$ ,  $3.4 \pm 0.08$ ,  $5.1 \pm 0.04$   $\mu\text{g}/\text{kg}$ ) respectively, in Red pepper, the average concentrations ( $5.2 \pm 0.2$ ,  $4.4 \pm 0.08$ ,  $2.2 \pm 0.06$ ,  $3.1 \pm 0.1$   $\mu\text{g}/\text{kg}$ ) respectively, and in Cumin concentrations were an average of ( $10.2 \pm 0.2$ ,  $9.8 \pm 0.1$ ,  $12.12 \pm 0.09$ ,  $7.6 \pm 0.1$   $\mu\text{g}/\text{kg}$ ), respectively. These results indicate the contamination of spices to aflatoxins B1, B2, G1 and G2 were in various concentrations, this difference depends on the type of the fungus and environmental conditions, as well as the availability of nutrients to the fungus.

#### Keywords

Spice,  
Tripoli,  
Aflatoxins,  
HPLC.

#### Article Info

Accepted:  
22 February 2017  
Available Online:  
10 March 2017

### Introduction

Foods are exposed to certain species of fungi that secrete organic compounds as their metabolites which mostly are poisonous for humans, animals and other microorganisms. The most important recorded fungi as toxin producers are *Aspergillus*, *Fusarium* and *Penicillium* species that produce various kinds of toxins the most important of are aflatoxins, ochratoxins, zearalinon and others. Those

toxins are highly toxic compounds that cause many kinds of diseases including cancers.

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). Fungi grow on crops in the field and after crop harvest and storage, storage fungi grows on it. Fungi also

grow within wide range of temperatures (15-35 °C) and causes the damage of 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) changes in addition to the aflatoxins that they secrete. That did not necessarily mean that every fungus infected commodity is containing aflatoxins because fungal 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 for fungus to grow and produce toxins. However, not every fungus has the genetic capacity to produce aflatoxins even he belongs to one species known with aflatoxins production due to the differences between the isolated strains from the same species which are accompanied with differences in the capability of toxin (s) production according to the genetic capacity. The same aflatoxins also may be produced by more than one of toxic fungal species (Anonymous, 2002). The popularity of hot peppers (*Capsicum annum* L.), also known as chili peppers, as spices or vegetables and for other uses increases every year, powdered red pepper is one of the favorite spices in South Asia and is commonly used for flavoring, seasoning, and impart in aroma or coloring to foods. Hot peppers are the principal component of curry and chili powder and can be used to make pepper sauce, red pepper, and paprika (Iqbal *et al.*, 2011).

Spices are often contaminated with mycotoxins. The climatic conditions prevailing in the tropics are especially favorable for mold contamination and mycotoxin production. Of the different mycotoxins, aflatoxin is the commonest contaminant in spices (Fazekas *et al.*, 2005). Peppers are very susceptible to aflatoxin

contamination, which is affected by atmospheric temperature, humidity, insects, and drying and processing conditions. Mold contamination can occur in the field during crop production and during storage when conditions are favorable. Sun drying is a common post harvest practice in some countries, which involves spreading peppers on soil in a single layer. Because of the drying processes are on the soil, some peppers are contaminated with fungus (Iqbal *et al.*, 2011 and Erdogan, 2004).

### **Materials and Methods**

In this research, total of 54 samples of crushed spices (caraway, mixed spices, cinnamon, black pepper, red pepper and cumin) were collected from private markets at Tripoli city (Table 1). Samples were collected randomly and put into LDPE bags. Sample weight at collection was 1 kg and information about sample name, weight and date of collection were recorded on each bag. Samples were transferred under suitable conditions for storage and stored at 4°C until tests were carried out to assess the kinds of aflatoxins.

The assessment steps begin with subsampling, preparation and good 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 immunoaffinity 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 autoinjector 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: acetonitril (65:25:10) with a flow rate of 1 ml/min.

### Results and Discussion

Spices are considered from the agricultural crops that expose to the fungal infection especially with aflatoxins producing fungi. In this research, aflatoxins were assessed and the results in table 2 and figure 1 show that the maximum concentration of Afla B1 was in Black pepper samples ( $12.1 \pm 0.7 \mu\text{g/kg}$ ) and the minimum concentration was in Mixed spice samples ( $3.4 \pm 0.2 \mu\text{g/kg}$ ). The maximum concentration of Afla B2 was in Cumin samples ( $10.2 \pm 0.1 \mu\text{g/kg}$ ) as well as the maximum concentrations of Afla G1 and G2 that was found in Cumin samples also ( $12.12 \pm 0.09$  and  $7.6 \pm 0.1 \mu\text{g/kg}$ , respectively).

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. From table 3 and figure 2, it indicated that extracting

aflatoxins by immunoaffinity columns is a sensitive and highly accurate method with a recovery rate up to 86%. The climatic conditions prevailing in the tropics are especially favorable for mold contamination and mycotoxin production. Of the different mycotoxins, aflatoxin is the commonest contaminant in spices (Fazekas *et al.*, 2005).

Peppers are very susceptible to aflatoxin contamination, which is affected by atmospheric temperature, humidity, insects, and drying and processing conditions. Mold contamination can occur in the field during crop production and during storage when conditions are favorable. Sun drying is a common postharvest practice in some countries, which involves spreading peppers on soil in a single layer. Because of the drying processes are on the soil, some peppers are contaminated with fungus (Iqbal *et al.*, 2011 and Erdogan, 2004).

Substantial aflatoxin contamination of ground red pepper has been reported from Ethiopia, where eight of 60 samples collected from markets, shops and storage facilities were contaminated with AFB1 in concentrations of 250-525  $\mu\text{g kg}$  (Fufa and Uрга, 1996).

**Table.1** The common and scientific names of spices that sampled from private markets

Common name	Scientific name
Caraway	<i>Carum carvi</i> L.
Mixed spices	<i>Eucalyptos globulus</i> L.
Cinnamon	<i>Cinnamonum verum</i> Presel
Black pepper	<i>Piper nigrum</i> L
Red pepper	<i>Capsicum frutescens</i> L.
Cumin	<i>Cuminum cuminum</i>

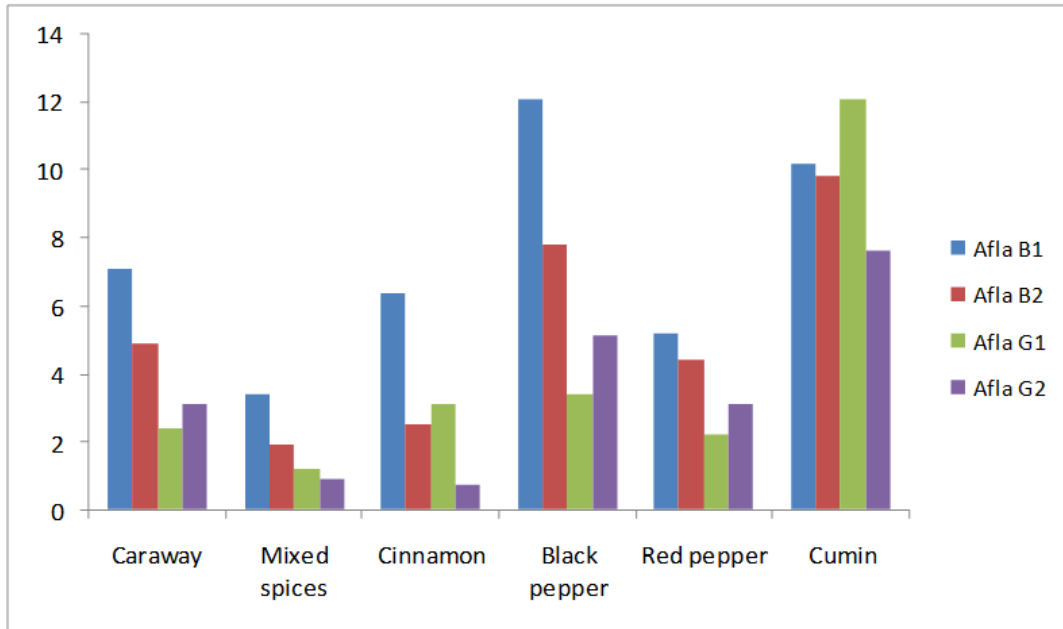
**Table.2** Average concentrations of positive samples within the different kinds of spices

Sample	Positive samples	Average concentration of aflatoxins µg/kg			
		Afla B1	Afla B2	Afla G1	Afla G2
Caraway	5	7.1 ± 0.1	4.9 ± 0.08	2.4 ± 0.9	3.1 ± 0.07
Mixed spices	4	3.4 ± 0.2	1.9 ± 0.04	1.2 ± 0.03	0.9 ± 0.09
Cinnamon	6	6.4 ± 0.06	2.5 ± 0.03	3.1 ± 0.8	0.7 ± 0.01
Black pepper	4	12.1 ± 0.7	7.8 ± 0.09	3.4 ± 0.08	5.1 ± 0.04
Red pepper	6	5.2 ± 0.2	4.4 ± 0.08	2.2 ± 0.06	3.1 ± 0.1
Cumin	4	10.2 ± 0.2	9.8 ± 0.1	12.12 ± 0.09	7.6 ± 0.1

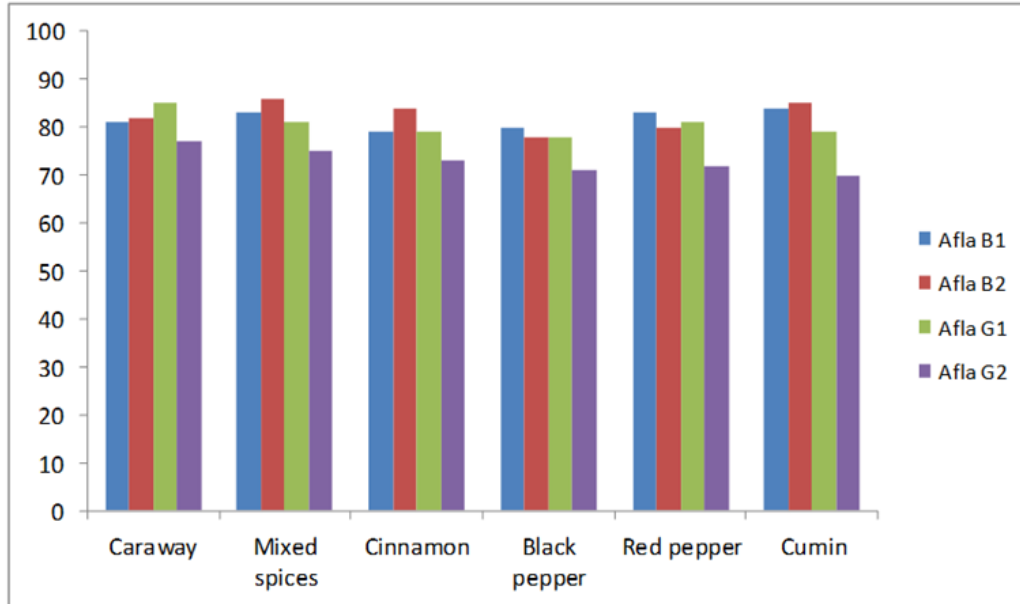
**Table.3** Average recovery rate for each aflatoxin (B1, B2, G1 and G2) that was added to red pepper sample at a concentration of 5 µ/kg

Sample	Average recovery rate (%)			
	Afla B1	Afla B2	Afla G1	Afla G2
Caraway	81±0.31	82± 0.85	85± 0.92	77± 0.30
Mixed spices	83±0.53	86± 0.30	81±0.26	75±0.55
Cinnamon	79± 0.47	84±0.22	79±0.45	73±0.61
Black pepper	80±0.35	78±0.48	78±0.65	71±0.67
Red pepper	83± 0.46	80±0.57	81±0.23	72± 0.34
Cumin	84±0.52	85±0.54	79±0.83	70±0.75

**Figure.1** Average concentration of aflatoxins ( $\mu\text{g}/\text{kg}$ ) in the different kinds of spices



**Figure.2** Average recovery rate (%) for aflatoxins



In Turkey, 24% and 13% of samples of different pepper types contained 7.5-200 (*Demircioglu and Filazi, 2010*) and 1.1-97.5  $\mu\text{g}/\text{kg}$  (*Erdogan, 2004*) total aflatoxins, respectively. *Elshafie et al., (2002)* were

surveyed one hundred and five samples of seven spices (cumin, cinnamon, clove, black pepper, cardamom, ginger, and coriander) for the mycoflora and AFs in the Sultanate of Oman. Twenty fungal species were isolated in

which *A. flavus*, *A. niger*, *Penicillium*, *Rhizopus*, and *Syncephalastrum racemosum* were the most dominant. Of the seven spices studied, clove was found to be the least contaminated, while cumin was the most contaminated. None of the 15 selected samples of the spices contaminated by *A. flavus* were found to contain AFs (Elshafie *et al.*, 2002). Cooking revealed that the aflatoxin content of spices did not decrease (MacDonald and Castle, 1996).

Although the mold *A. flavus* grows well on the spices, the production of AFs is lower than in cereals. This indicates that spices are not an ideal substrate for AF formation. It has been shown that essential oils extracted from spices, e.g. cloves, can inhibit mold growth and AF production completely. According to some reports, fungal growth was weak on curcumin, black pepper and white pepper and no AF was detected in black or white pepper after 10 days at 25 °C. According Martins *et al.*, (2001), the results of the survey indicate that there is little evidence for significant AF contamination in spices. The majority of samples contained AFs at low levels and others were negative (cardamom, cloves, ginger and mustard). In the meat industry (sausage, dry cured ham, luncheon meat) and in confection of ethnic dishes, AF contamination of spices is probably not relevant as a direct health hazard (Martins *et al.*, 2001).

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### How to cite this article:

Essawet, N., H. Abushahma, S. Inbaia, O.H. Elkwil and Amra, H.A. 2017. Aflatoxin Contamination of Spices Sold Collected from Local Market in Tripoli. *Int.J.Curr.Microbiol.App.Sci*. 6(3): 1468-1473. doi: <https://doi.org/10.20546/ijcmas.2017.603.168>