

**DETECTION OF FUNGI AND TOTAL AFLATOXINS IN FOOD ADDITIVES  
AND SOME MEAT PRODUCTS BY SEROLOGICAL AND MOLECULAR  
BIOLOGICAL METHODS**

By

**Gehad N.M.Abdo<sup>1,3</sup>, Nagwa I. M. Khafaga<sup>2</sup>, El- Hariri M.<sup>3</sup>  
and Mohamed K. Refai<sup>3</sup>**

<sup>1</sup>Animal Health Research Institute, Agriculture Research Center, Giza branch, Egypt.

<sup>2</sup>Animal Health Research Institute, Agriculture Research Center, Dokki, Egypt.

<sup>3</sup>Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt.

**ABSTRACT**

Spices and natural flavors are basic elements that go into food, especially meat and poultry products. In countries with hot and humid climate, contamination of spices and other food additives with moulds especially aflatoxigenic species is commonly encountered. There are great potential risks on human health via consumption of such spices or foods which contain these spices. Hence, creating awareness among consumers regarding aflatoxins is of great importance for food safety. From this point of view, this investigation was designed to evaluate the quality of 14 kinds of spices and 6 meat products. A total of 200 samples of food additives (Black pepper, Cumin, Coriander, Turmeric, Chili powder, Ginger, Garlic powder, Paprika, Curry, Cinnamon, Thyme, Clove, Cardamom and Onion powder) and meat products (Basterma, Beef burger, Frankfurter, Hot dog, Kofta and Luncheon) representing 10 each were collected from local markets at Cairo and Giza Governorates. All samples were analyzed for moulds count, predominant mould genera especially aflatoxins producing species and total aflatoxins level. All examined spices and meat products showed positive result for moulds with highest contamination percentage (100%) in black pepper, cumin, coriander and turmeric, while in meat products kofta and Hot dog showed contamination percentages of 80 & 70%, respectively. The lowest percentage of contamination (40%) was in clove and Luncheon. Moulds load in spices ranged from  $2.47 \pm 1.09$  in clove to  $3.61 \pm 1.67$  in chili powder  $\log_{10}$  CFU/g, whereas in meat products it was  $3.53 \pm 2.12$  in frankfurter and  $3.95 \pm 2.63$  in beef burger. *Aspergillus* and *Penicillium* species were generally the most predominant species recovered from the examined samples, followed by *Mucor Cladosporium* and *Altrnaria*

species. Further characterization of *Aspergillus* species proved that *A. niger* was the most prevalent followed by *A. flavus*, *A. ochraceus*, *A. candidus* and *A. glaucus*. Almost fifteen percent (14.9%) of the isolated *A. flavus* isolates were aflatoxogenic. The total aflatoxin residues were maximum in chili powder ( $40.94 \pm 9.28$  ug/kg) for spices and in hot dog ( $8.23 \pm 1.52$  ug/kg) for meat products. In consistence with ELISA findings, the molecular study on genomic DNA using PCR technique confirmed presence of *Aspergillus* species in isolated samples.

**Key words:**

Spices - aflatoxins - ELISA - Food additives - Meat products- PCR.

Corresponding Author: Nagwa Khafaga, Animal Health Research Institute, Agriculture.

Research Center, Dokki-Giza, Egypt. E-mail: Nagwawarda86@hotmail.com.

**INTRODUCTION**

Food and microorganisms have long and interesting associations which developed long before the beginning of recorded history. Foods are not only nutritious to consumers, but are also excellent source of nutrients for microbial growth. On the other hand, foods also can act as a reservoir for disease transmission, and thus detection and control of pathogens and spoilage organisms are important areas of food microbiology. During the entire sequence of food handling from the producer to the final consumer, microorganisms can affect food quality and human health. Mould contamination in food is a useful indicator to evaluate the food quality, the degree of deterioration is an essential component for microbiological assurance programs (Taniwaki *et al.*, 2001). Developed countries considered the mould counts as a standard test for hygienic condition due to its economic and public health effects. Fungi are ubiquitous plant pathogens and so are major spoilage agents of foods and feedstuffs. Mould not only cause deterioration of food and feed but also adversely affect the health of humans and animals since they are capable of producing toxic metabolites known as mycotoxins causing food poisoning and liver cancer in human (Brera *et al.*, 1998). The ingestion of such mycotoxin contaminated grains by animals and human beings has enormous public health significance because these toxins are nephrotoxic, immunotoxic, teratogenic and mutagenic which are capable of causing acute and chronic effects in man and animals ranging from disorder of central nervous, cardiovascular and pulmonary systems and intestinal tract to death (Paterson and Lima, 2010; Refai and Hassan, 2013). Of greatest concern is the relevance of these toxins in human hepatoma and oesophageal cancer, increased susceptibility

to diseases especially in children and childhood pre-five mortality and reduced life expectancy (**Marasas, 2001**). The significant economic and health hazards caused by fungi and mycotoxin especially in developing countries that have poor food storages are of great concern. So, to ensure a healthy food supply thereby minimizing consequences to food security, international trade and animal and human health, there is a need to monitor fungal and mycotoxin contamination periodically so as to meet international and national mycotoxin regulatory standards. Spices have been defined as a natural compound, or a mixture of natural compounds that is extracted from the seeds, fruits, flowers, or trunks (skins, roots, leaves) of several plants that indigenous or exotic origin, aromatic or with strong taste, used in minute quantities, and added to food preparation and processing throughout the world in order to provide colour, taste, smell, or flavor (**Škrinjar et al., 2012**). They are essentially flavoring agents reported to have both beneficial effect and antimicrobial properties, if properly stored (**Atanda et al., 2006**). As with many other agricultural products, spices may be exposed to a wide range of microbial contamination during pre- and post-harvest (**Hashem and Alamri, 2010**). Although spices are present in foods in small amounts, they are recognized as important carriers of microbial contamination mainly because of the conditions in which they were grown, harvested and processed. In addition, because of possible neglects during sanitation or processing, foods containing spices are more likely to deteriorate and also could exert harmful effects, having in mind health risks associated with mycotoxins produced by some fungal genera (**Koci -Tanackov et al., 2007**). Fungi are the predominant contaminants of spices, but most such microbial populations are probably regarded as commensally residents on the plant that survived drying and storage. Soil and air are the main inoculum source for causing contamination in crude spices in field (**Kneifel and Berger, 1994**). Such contamination may occur during processing storage, distribution, sale and/or use (**McKee, 1995**). Meat is considered the main source of animal protein and a high source of iron, zinc and several vitamins, and so meat products are considered a favorable food as it is easy to buy, fast to cook, delicious to eat. Rich nutrient matrix meat and meat products are the first-choice source of animal protein for many people all over the world (**Heinz and Hautzinger, 2007**). Meat products may be contaminated with one of the most dangerous microbial hazard represented in moulds. Moulds contamination of these meat products may occur at different stages at which the products are prepared. It may occur during animal slaughter under bad hygienic condition by using of contaminated water, equipments and

utensils or during processing through adding of contaminated meat additives with mould spores or during packing, handling, transportation and storage. Contamination of meat products with different mould species is considered a real hazard as it affects the quality of these meat products by increasing the opportunity for their spoilage and deterioration (Comi *et al.*, 2004). The most important aspect about mould spoilage of food is, however, the formation of mycotoxins, more than 400 mycotoxins are known today. The more common and the most dangerous types of mycotoxins are aflatoxins. Aflatoxins are the main toxic secondary metabolites of some *Aspergillus* species such as *A. flavus*, *A. parasiticus* (Alcaide-Molina *et al.*, 2009). More than 18 different types of aflatoxins are identified; AFB1 is the most potent toxic metabolite and is classified as human carcinogen (Talebi *et al.*, 2011). The potential for spoilage and mycotoxin production primarily depends on genetic factors; however, environmental conditions at the site of mould growth (temperature, water activity, matrix composition, moisture content, pH of the medium, contamination and physical destruction of the substrate, antifungal properties and other factors) are considered highly significant (Škrinjar *et al.*, 2012). Therefore, the present study aimed at assessing the intensity and frequency of moulds contamination in common spices and some meat products in public markets and the potential producers of mycotoxins to highlight their risk assessment, screening the isolated fungi for aflatoxin production as well as determination of aflatoxin residues.

## MATERIAL AND METHODS

### Sampling:

Two hundred random samples of food additives (Black pepper, Cumin, Coriander, Turmeric, Chili powder, Ginger, Garlic powder, Paprika, Curry, Cinnamon, Thyme, Clove, Cardamom and Onion powder) and meat products (Basterma, Beef burger, Frankfurter, Hot dog, Kofta and Luncheon) representing 10 each were collected from local markets at Cairo and Giza Governorates. Samples were aseptically transferred into sterile polyethylene bags without undue delay, and transported to the laboratory for mycological examination and aflatoxins detection (Charoenpornsook. and Kavisarasai.P. 2006).

### Enumeration of total mould count:

#### **Preparation of samples (ICMSF, 1978).**

Ten grams of each sample were transferred aseptically into sterile blender jar, to which 90 ml of 1% peptone water were added and homogenized in a sterile warring blender for 2 minutes,

and tenfold serial dilutions of the homogenate were prepared. One milliliter quantities of the previously prepared serial dilutions were inoculated separately into Petri dish plates and mixed with rose Bengal agar medium and incubated at 25°C for 3-5 days. The counts of mould colonies were recorded. Estimation of the Total Mould Count was carried out according to (FDA, 2001) by using dichloran Rose-Bengal chloramphenicol (DRBC) agar medium.

**Identification of isolates:**

The identification of mould species was carried out by careful observation and measurements macroscopically and microscopically according to (Pitt and Hocking, 2009).

**Aflatoxicity testing of isolates:**

Seventy-four isolates of *Aspergillus* section *Flavi* obtained from the examined spices and meat products were tested for their capacity to produce aflatoxins on neutral red desiccated coconut agar (NRDCA). The agar medium was prepared as described by Atanda *et al.* (2011). Each of the isolates were inoculated at the center of triplicate NRDCA Petri dishes and incubated at 31°C for 5 days in the dark. On the third day of incubation the plates were checked under UV at 365nm for blue fluorescence characterizing aflatoxin production.

**ELISA test procedure (Sahar *et al.*, 2013):**

The quantitative analysis of total aflatoxins was determined through a competitive direct enzyme linked Immunosorbent Assay (ELISA) method. The method is based on the accurate monitoring of mycotoxins and is suitable for screening large number of samples. The veratox test kits (Neogen Corp., Lansing, MI. USA approved by the AOAC research institute (certificate No 950702) and the USDA-GIPSA (2008 - 011) were used for the analysis. The analysis was done according to the manufacturer's instructions. Concentration of aflatoxins was calculated by Log/log it Software Awareness Technology Inc. (Anonymous, 2000; Stoloff *et al.*, 1991).

**Calculation:**

Qualitative result could be derived by visual comparison of the sample color to the standard wells. Samples containing few colors than the standard well had greater concentration of aflatoxin than the standard well. In contrast, the sample containing more color had lower aflatoxin concentration. For quantitative result, plotting the standard curve on the semi logarithmic graph paper, placing the value of standards on x-axis and the corresponding

absorbance value on Y-axis. Read AFB1 concentration in the sample directly from the standard curve in ppb Fig. (1).

**Genomic DNA analysis:**

Fungal genomic DNA was extracted from cultured *aspergillus flavus* using Quigen genomic DNA extraction kit (QIAamp® DNA Mini Kit).

The purity and concentration of isolated DNA samples were measured using Nanodrop 1000 (Thermoscientific).

**Primers design:**

The forward and reverse primers used to probe the presence of *Aspergillus flavus* species were designed from the previously deposited gene sequence of *Aspergillus flavus* (*Aspergillus flavus* NRRL3357scf\_1106286417600, whole genome shotgun sequence with accession number NW\_002477238; version NW\_002477238.1. Primer3 software was used to design the primers and the primers were designed via by HVD LIFE SCIENCES (Eurofins, Germany) and their specificity was previously tested (Oligo Analyzer program ver. 1.0.3).

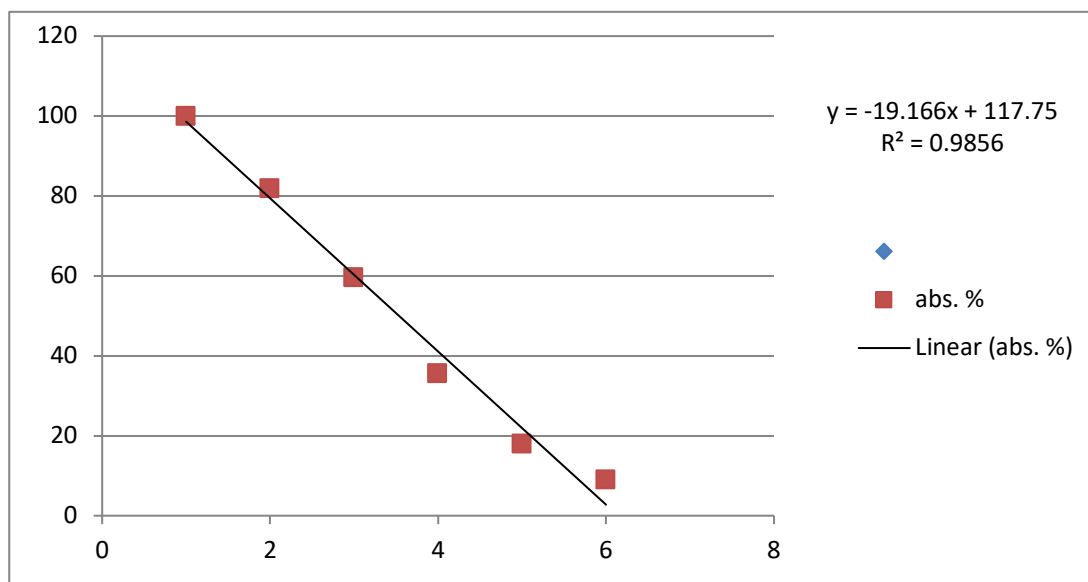
Primer pair

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self comp.	Self 3' comp.
Forward primer	ATGATTGGCCGTAAGTGC GA	Plus	20	2484	2503	60.11	50.00	4.00	2.00
Reverse primer	GTAGGTTTCGATGCCGAGGAG	Minus	20	3129	3110	59.97	60.00	4.00	0.00
Product length	646								
Range: 2400 - 3200									

**2.5.2. Polymerase Chain Reaction and Sequencing:**

The reaction mixture for PCR was prepared by adding 25.5 µl of nuclease free water, 5 µl of DreamTaq™ DNA polymerase buffer, 5µl (100 µM) dNTPs, 5 µl of each primers (20 µM), 5 µl of DreamTaq™ DNA polymerase (Fermentas) and genomic DNA (50 ng) in each tube. The run was for 30 cycles in a Thermo-cycler (Biometra), with initial denaturation at 95°C for 5 min followed by 1min denaturation at 95°C; 2 min annealing at 66°; 2 min extension at 72°C. The run was then terminated by a final extension at 73°C for 9 min. The amplification products were separated by electrophoresis in 1.5% agarose gel at 100 volts for 1 hr. and stained with ethidium bromide and photographed using Gel documentation system (Bhatnagar et al. 2006).

## RESULTS



**Fig. (1):** Total aflatoxin standard curve.

**Table (1):** Mean total mould count ( $\log_{10}$  CFU/g), and total aflatoxins level ( $\mu\text{g}/\text{kg}$ ) of examined food additives.

Food additives	Positive		Total Mould Count	Total Aflatoxin (average)
	No.	(%)	Mean $\pm$ SE*	
Black pepper	10/10	(100)	3.23 $\pm$ 1.21	21.83 $\pm$ 3.39
Cumin	10/10	(100)	3.12 $\pm$ 1.22	30.33 $\pm$ 3.88
Coriander	10/10	(100)	3.25 $\pm$ 1.24	12.34 $\pm$ 1.09
Turmeric	10/10	(100)	3.21 $\pm$ 1.28	27.72 $\pm$ 6.62
Chili powder	9/10	(90)	3.61 $\pm$ 1.67	40.94 $\pm$ 9.28
Ginger	7/10	(70)	3.38 $\pm$ 1.31	8.85 $\pm$ 1.26
Garlic powder	7/10	(70)	3.20 $\pm$ 1.62	7.13 $\pm$ 1.71
Paprika	6/10	(60)	3.04 $\pm$ 1.16	28.68 $\pm$ 3.99
Curry	8/10	(80)	3.44 $\pm$ 1.70	29.25 $\pm$ 3.92
Cinnamon	5/10	(50)	3.36 $\pm$ 1.66	29.22 $\pm$ 4.46
Thyme	9/10	(90)	3.47 $\pm$ 1.39	19.71 $\pm$ 3.44
Clove	4/10	(40)	2.47 $\pm$ 1.09	7.99 $\pm$ 1.20
Cardamom	7/10	(70)	3.54 $\pm$ 1.54	3.41 $\pm$ 0.73
Onion powder	7/10	(70)	3.45 $\pm$ 1.44	17.65 $\pm$ 1.17

\* SE: Standard Error

**Table (2):** Incidence of isolated mould genera and species from examined food additive samples (n=10 each).

	Black pepper	Cumin	Coriander	Turmeric	Chili powder	Ginger	Garlic powder	Paprika	Curry	Cinnamon	Thyme	Clove	Cardamom	Onion powder	Total Percent
<i>Aspergillus</i>	60	60	65.2	56.5	62.5	78.5	50	61.5	25	64.3	54.5	80	53.8	42.8	59.4
<i>A. flavus</i>	38.8	44.4	33.3	46.2	30	45.4	25	37.5	50.0	33.3	41.6	37.5	42.8	33.3	38.7
<i>A. niger</i>	38.8	38.8	33.3	38.5	60	18.1	75	62.5	50	55.5	50	50	57.1	33.3	45.1
<i>A. candidus</i>	5.5	16.7	13.3	15.4	10	9.1	-	-	-	-	-	-	-	-	7.0
<i>A. glaucus</i>	5.5	-	-	-	-	-	--	-	-	-	-	-	-	-	0.7
<i>A. ochraceus</i>	11.1	-	3	-	-	27.3	-	-	-	11.1	8.3	-	-	33.3	8.5
<i>Penicillium</i>	30	26.6	13	30.4	12.5	21.4	18.8	30.7	25	35.7	9.1	12.5	15.4	28.7	21.8
<i>Cladosporium</i>	-	-	-	-	-	-	-	-	-	-	9.1	-	7.7	-	1.3
<i>Alternaria</i>	3.3	6.6	8.7	-	-	-	-	-	-	-	4.5	-	-	-	2.5
<i>Mucor</i>	6.6	6.7	13	13	25	-	31.3	7.6	50	-	22.7	20.	23.1	28.5	15.1

**Table (3):** Mean total mould count (log<sub>10</sub> CFU/g), and total aflatoxins level (µg/kg) of examined meat products containing food additives.

Meat Products Varity	Positive		Total Molds Count	Total Aflatoxins
	No.	(%)	Mean ± SE*	average ±SE*±±
Basterma	6/10	(60)	3.62 ± 1.83	4.58 ± 0.55
Beef burger	8/10	(80)	3.95 ± 2.63	4.17 ± 0.66
Frankfurter	5/10	(50)	3.53 ± 2.12	3.79 ± 0.41
Hot dog	7/10	(70)	3.77 ± 1.58	8.23 ± 1.52
Kofta	8/10	(80)	3.67 ± 1.52	6.35 ± 0.87
Luncheon	4/10	(40)	3.67 ± 1.19	5.44 ± 0.39

\* SE: Standard Error



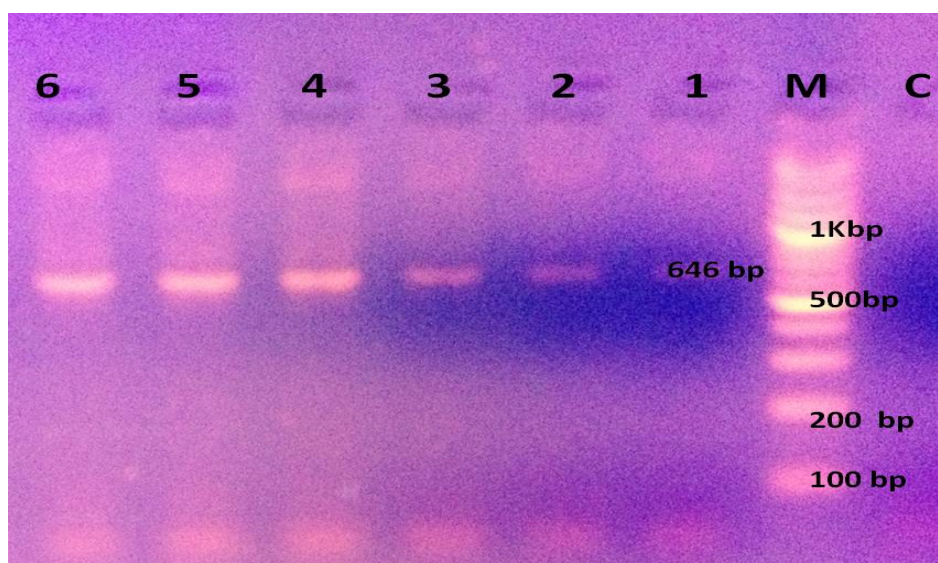
## DETECTION OF FUNGI AND TOTAL AFLATOXINS IN FOOD

**Table (4):** Incidence of isolated mould genera in examined meat products containing food additives (n=10 each)

	Basterma	Beef burger	Frankfurter	Hot dog	kofta	Luncheon	Total Percent
<i>Aspergillus</i>	53.3	44	46.2	61.5	33.3	46.1	48.4
<i>A. flavus</i>	25.0	45.5	50.0	50.0	60.0	33.3	43.2
<i>A. niger</i>	50.0	54.5	33.3	50.0	40.0	50.0	47.7
<i>A. candidus</i>	-	-	-	-	-	-	-
<i>A. glaucus</i>	12.5	-	-	-	-	-	2.3
<i>A. ochraceus</i>	12.5	-	16.7	-	-	16.7	6.8
<i>Penicillium</i>	26.6	12	38.5	15.4	53.3	21.4	24.2
<i>Cladosporium</i>	-	4	-			15.3	3.3
<i>Alternaria</i>	-	-	7.7				1.1
<i>Mucor</i>	20	32	-	23	13.3	38.5	23.1

**Table (5):** Screening of *Aspergillus flavus* isolates for aflatoxin production.

Total no. of examined samples	No. of tested isolates	Toxigenic <i>A. flavus</i> isolates	
		No	%
200	74	11	14.9 %



**Fig. (2):** The expected DNA fragment length with 646 bp were obtained from 6 food samples.

Agarose gel electrophoresis of the resulting amplification of PCR products of *Aspergillus flavus* specific genome sequence: C is a negative control, M is the DNA ladder sequence, and lanes 1 to 6 are the expected specific products length of 646 bp from the genome of toxigenic isolated *Aspergillus flavus* samples.

**Table (6):** FDA’s Action Levels for aflatoxins present in human food and animal feed.

<b>Chart 1: FDA Action Levels for Aflatoxin in Human Food, Animal Feed and Animal Feed Ingredients</b>		
<b>Intended Use</b>	<b>Grain, Grain By-Product, Feed or other Products</b>	<b>Aflatoxin Level [parts per billion (p.p.b.)]</b>
<b>Human consumption</b>	<b>Milk</b>	<b>0.5 p.p.b. (aflatoxin M1)</b>
<b>Human consumption</b>	<b>Foods, peanuts and peanut products, brazil and pistachio nuts</b>	<b>20 p.p.b.</b>
<b>Immature animals</b>	<b>Corn, peanut products, and other animal feeds and ingredients, excluding cottonseed meal</b>	<b>20 p.p.b.</b>
<b>Dairy animals, animals not listed above, or unknown use</b>	<b>Corn, peanut products, cottonseed, and other animal feeds and ingredients</b>	<b>20 p.p.b.</b>

### DISCUSSION

Since food additives are possible source of contamination of meat products and potential producers of mycotoxins, therefore it is necessary to conduct an adequate and continuous control of food additives on presence of the moulds. Food additives have been reported to be heavily contaminated by a wide array of storage fungi including *Aspergillus*, *Penicillium*, *Rhizopus*, *Eurotium*, *Cladosporium*, *Trichoderma*, *Mucor* and *Stachybotrys* (**Elshafie et al., 2002; Bokhari, 2007; Hashem and Alamri, 2010**). In this study, the mycological investigation of the examined food additives showed high contamination in black pepper, cumin, coriander, and turmeric in order of magnitude of 100%. This followed by chili and thyme 90% for each, curry 80%, ginger; garlic powder, cardamom and onion powder 70% for each, paprika 60%, and cinnamon 50% while the lowest contamination was in clove samples 40%, with an average mould contamination level of  $2.47 \pm 1.09 - 3.61 \pm 1.67$  (table 1). It was detected that

clove was the least mould- contaminated spices, though clove is well known for its antimicrobial properties with essential oils highly effective against moulds. (**Guyenot et al., 2003**). Results given in (Table 1) revealed that total aflatoxin residues as deduced from Fig. (1) could be detected from the examined Black pepper, Cumin, coriander, Turmeric, Chili powder, Ginger, Garlic powder, Paprika, Curry, Cinnamon, Thyme, Clove, Cardamom and Onion powder with average value of  $21.83 \pm 3.39$ ,  $30.33 \pm 3.88$ ,  $12.34 \pm 1.09$ ,  $27.72 \pm 6.62$ ,  $40.94 \pm 9.28$ ,  $8.85 \pm 1.26$ ,  $7.13 \pm 1.71$ ,  $28.68 \pm 3.99$ ,  $29.25 \pm 3.92$ ,  $29.22 \pm 4.46$ ,  $19.71 \pm 3.44$ ,  $7.99 \pm 1.20$ ,  $3.41 \pm 0.73$  and  $17.65 \pm 1.17$   $\mu\text{g}/\text{kg}$  respectively. These results were found to be higher than those reported by **Cho et al., (2008)** who investigated 88 spice samples and found aflatoxin contamination in only 13.6% at a concentration below 5  $\mu\text{g}/\text{kg}$  and **Santos et al. (2010)** found aflatoxins in 40% of 35 chili samples, all at concentrations below the maximum allowable limits. The present results are nearly similar to that of **Mac Donald and Castle, (1996)**, who detect aflatoxins (48 $\mu\text{g}/\text{kg}$ ) in chili powder samples, another analysis of spice samples for aflatoxins showed that highest contamination up to 120  $\mu\text{g}/\text{kg}$  was found in red chilies while in coriander samples that contamination ranged between 2-75  $\mu\text{g}/\text{kg}$ . (**Llewellyn et al., 1992**), **Fufa and Urga (1996)** stated that out of 60 samples of ground red pepper, 8 (13.3%) were positive for aflatoxins, the contamination levels ranged from 250-515 ppb. In this study most food additives samples were above the detectable aflatoxin limit (20  $\mu\text{g}/\text{kg}$ ), this is in contrary with **Farid and Nareen (2013)**, who stated that the quantity of mycotoxins in most of the samples was not detected within the detectable limits. However, in few samples, values of mycotoxins were above the permissible safe limits for human consumption. Mostly, spices are grown in tropical and subtropical regions and harvested in poor sanitary conditions. These improper conditions are convenient for the biosynthesis of aflatoxins. Therefore, growing conditions, harvesting, processing methods, storage conditions and postharvest treatments should be carefully controlled in order to prevent aflatoxins risks due to contaminated spices. In addition, training programs should be presented for producers. The isolated fungi belonged to five genera: *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria* and *Mucor*. *Aspergillus* was the most predominant (59.4%) genera in the examined food additive samples followed by *Penicillium*, *Mucor*, *Alternaria* and *Cladosporium*, (Table 2). The trend of fungal predominance which we observed agreed with the reports of **Bokhari (2007)**, **Hashem and Alamri (2010)**, **Sumanth et al. (2010)**, and were less in agreement to this finding by **Srivastava and Chandra (1985)** who recorded that *Aspergillus* followed by *Fusarium* were the most frequent

members of the mycobiota of Coriander, Cumin, Fennel and Fenugreek. Unfortunately, due to the lack of proper post-harvest preservation techniques, large portion of annual yield gets damaged by fungal action according to **Abou Donia (2008)** and the variation in frequency of mycopopulation of spices cultivated is most probably related to the strain type within one species. Environmental factors also have significant effect and can induce the growth of mycopopulation at lower  $a_w$  values (optimal temperature and type of nutritive components in the medium). The incidence of identified *Aspergillus* species declared that *A. niger* had the highest incidence rate 45.1, followed by *A. flavus* 38.7 *A. ochraceus* 8.5 *A. candidus* 7.0 and *A. glaucus* 0.7. These findings are nearly similar to those obtained by **Hussien (2008) and El-diahy et al. (2013)**. Moulds fall into two ecological categories, e.g., field and storage moulds. Field moulds were observed to invade developing or mature seeds while they are on the plant, the major field moulds genera being *Alternaria*, *Fusarium* and *Cladosporium*. On the other hand, storage moulds are those encountered on plants in conditions of moisture commonly found in stored products. These moulds principally belong to species *Aspergillus* and *Penicillium* (**Abou Donia, 2008**). *Aspergillus flavus* and *A. niger* were the most frequently encountered and widely distributed in spices and herbal drugs (**Abdulkadir et al., 2003**). From *Aspergillus* 7 species and one variety (*A. ochraceus*, *A. fumigatus*, *A. flavus* var. *columnaris*, *A. nidulans*, *A. terreus*, *A. versicolor*, *A. sydowii* and *A. tamarii*) were isolated in moderate or low frequency of occurrence. These species were previously isolated from different kind of spices by several researchers (**Abdulkadir et al., 2003**). On the overall, moulds could be detected in Basterma, Beef Burger, Frankfurter, Hot Dog, Kofta and Luncheon with 60%, 80%, 50%, 70%, 80% and 40% with mean values of  $3.62 \pm 1.83$ ,  $3.95 \pm 2.63$ ,  $3.53 \pm 2.12$ ,  $3.77 \pm 1.58$ ,  $3.67 \pm 1.52$  and  $3.67 \pm 1.19$  respectively (Table 3). Our surveyed investigation showed lesser degree of contamination than those previously reported (**Freire and Offord, 2002, Bokhari 2007 and Salari, R. 2012**). Nearly such finding substantiates what has been reported by **Lamada and Nassif (2008), Javadi et al., (2011), Eman and Sherifa (2012), El-diahy et al. (2013) and Alaa-Eldin (2015)**. Such variations may be attributed to unsanitary measures and hygienic differences in which the meat products were prepared and/or using of unsterilized spices (untreated food additives) which usually carry mould spores used in manufacture of these meat products especially Beef Burger as these fresh products are usually manufactured under absence of hygienic conditions in addition to using of inferior quality raw materials. The heat treatment used in luncheon processing which affects the

fungal spore, results in decrease of the mould contamination in this product, while the lower incidence of moulds in Basterma and Frankfurter samples may be attributed to the lower water activity ( $a_w$ ) in these products and presence of garlic, garlic essential oils showed strong inhibitory effects against *A. niger* (Benkeblia, 2004). The results presented in (Table 3) revealed that, the total aflatoxin residues could be detected from the examined Basterma, Beef Burger, Frankfurter, Hot Dog, Kofta and Luncheon samples with mean values of  $4.58 \pm 0.55$ ,  $4.17 \pm 0.66$ ,  $3.79 \pm 0.41$ ,  $8.23 \pm 1.52$ ,  $6.35 \pm 0.87$  and  $5.44 \pm 0.39$  ppb, respectively. These different mean values of aflatoxins residues may be related to the amount of additives used in the processing, level of additives contamination with aflatoxin and the amount of aflatoxin residues which may be present in animal muscles. Aflatoxins are the most documented mycotoxins. Several studies indicated that dry cured meats can be contaminated with toxigenic *A. flavus* strains, especially when products are processed in countries with hot climate (FAO 2004). Moreover, it has been demonstrated that the processing conditions during ageing of meat products may allow aflatoxin synthesis. Therefore; it is of public health importance to evaluate the possible production of aflatoxin during meat processing and ageing. Higher values were found by Refai *et al.*, (2003) and El-diahy (2013). Our results are higher than those reported by Alaa-Eldin (2015), however the level of aflatoxin residues detected in the examined samples was lower than the permissible limit (20ppb) recommended by FAO (2004) but this low amount of toxins may result in the carcinogenic, mutagenic and immuno - suppressive effect on human health in the long term exposure. Few studies were carried out but they all demonstrated that, the frequency of contamination of processed meat with aflatoxin is low and that, the level of toxin within meat is usually below 10 ng /kg. However, it is not clear whether aflatoxin was produced during meat processing or was present before at the residual level in muscles. Indeed, it seems that there is no relationship between the presence of toxigenic strains of *A. flavus* and aflatoxin contamination of meat samples. The frequent contamination of spices and additives used in such meat processing may also represent a source of mycotoxins. Moreover, it has been demonstrated that the use of food additives contaminated with toxigenic mould strains as ingredient in meat products making may lead to a secondary contamination of the final product with aflatoxins (Francis *et al.*, 2009). The results of mould identification (Table 4) declared that the most predominant mould genera in meat products samples were; *Aspergillus* and *Penicillium* species 48.4% and 24.2% respectively, followed by *Mucor* 23.1%, *Cladosporium* 3.3% and *Alternaria* 1.1%.

Nearly similar results were obtained by **Mizakova et al., (2002)**. The presence of such moulds may cause spoilage of meat products by breaking down their components and liberating different acids and gas with subsequent change of their odor and flavor. Moreover, mould growth on meat products causes economic losses from discoloration, poor appearance and off flavors. In addition, some moulds are capable of producing toxic metabolites known as mycotoxins such as aflatoxins which are known carcinogens (**Pitt and Hocking, 2009**). From the public health point of view, *Aspergillus* species were incriminated in pulmonary aspergillosis, pulmonary allergy, skin infections, sinusitis and otitis for meat handlers. It has been stated that some species of *Penicillium* were found to be associated with pulmonary and urinary tract infections as well as yellow "rice disease" causing several deaths in man. *Mucor* and *Rhizopus* species may cause lesions in lungs, gastrointestinal infection, skin infections, intraocular infections, external otomycosis, and cellulitis and deep wound infections (**Banwart, (1980)**). *Cladosporium* species may induce chromatomycosis and brain abscesses (**Edris, 1986**). The isolated fungi are extremely considered as a major factor in the spoilage of food, leading to great economic losses and constitute a public health hazards by production of wide variety of mycotoxins causing, food poisoning and have carcinogenic effects in human (**Foster et al., 1983**). These findings indicate that processing may increase the contamination rate of meat especially when additives of low quality as flavorings, especially, spices were used or manufactured under lack of proper handling and sanitary practices during processing and storage (**Refai et al., 1990 and Yassein et al., 1991**). Food and Drug Administration (**FDA, 2000**) established regulatory working guidelines on the acceptable levels of aflatoxins in human foods set at 20 ppb for total aflatoxins. Large number of mould species including mycotoxigenic fungi contaminated several sources of foods rendering them unpalatable and unsafe for human consumption, (**Munimbazi and Bullerman 1996**), the incidence of identified *Aspergillus* species declared that *A. niger* had the highest incidence rate 21 (47.7%) followed by *A. flavus* with incidence rate 19 (43.2), *A. ochraceus* 3 (6.8), *A. glaucus* 1 (2.3) and *A. candidus* 0 (0). These findings are nearly similar to those obtained by **Hussien (2008) and El-diasty et al. (2013)**. When the capacity of each toxigenic strain was tested for aflatoxin production on neutral red desiccated coconut agar NRDC, we found that only 11 (14.9%) out of 74 *Aspergillus* section Flavi isolates had the ability to produce aflatoxins (Table 5). This was in lower extent compared with some authors as **Elshafie et al., (2002)** who found 45% of their 20 *A. flavus* isolates to be aflatoxigenic, **Ostry, (2001)**, who reported

that 50 out of 78 species of moulds reported to be toxicogenic have already been isolated from meat and various meat products, and in contrary with **Rajab (2011)**, who showed that all the fungi cultures were negative for mycotoxin production on DRBC media. Black pepper alongside other spices has been reported to inhibit aflatoxin biosynthesis but not the growth of the toxigenic fungi (**Madhyastha and Bhat, 1984; Bokhari, 2007**). It is also important to note that spices may support fungal growth (e.g. *Aspergillus flavus*) but inhibit the production of aflatoxins than in cereals (**MacDonald and Castle, 1996**). Therefore, not all *A. flavus* strains are aflatoxigenic (**Elshafie et al., 2002**). Although toxins present as a natural contaminant of spices are of minor concentrations, the health risk is increased because some of spices such as Anise and Cumin are used as carminative, as expectorant, treatment colic and flatulence for children. Moulds are capable of producing mycotoxins under suitable conditions. According to (**Ostry, 2001**), the production of mycotoxins in meat and meat products can be fostered by the presence of oxygen, temperatures between 4 and 40°C, pH values between 2.5 and 8.0, a minimum  $A_w$  of 0.80, and a maximum salt concentration of 14%. The molecular characterization of *Aspergillus Flavus* strains Fig. (2) was carried out by isolation of genomic DNA from previously inoculated *Aspergillus* mould that was isolated from contaminated samples. The PCR was performed on these genomic DNA using specific primers and the results agreed with the obtained data by ELISA detection. As aflatoxins pose more serious risks for public health, certain limits of total aflatoxins in food and feed were determined according to **FDA (2000)** as shown in (Table 6).

### CONCLUSION

In conclusion, this study warrants the global public health hazards of consumption of mould-contaminated meat products. This requires a strict control against possible meat products contaminations with mould and total aflatoxins. It is also extremely important to maintain a safety low mode level of aflatoxin B<sub>1</sub> in the feeds of meat producing and dairy animals. The internationally-compatible continuous training and surveillance programs uprising among correspondent personnel are at most important to cope against possible contamination. More importantly, storage conditions of feeds must be widely available and be subjected to regular and serious investigation.

It is necessary, however, to apply an ideal recommended limit of possible aflatoxins contamination in meat products. Application of Good Agricultural Practices and Good Veterinary Practices by agriculture and also the Hazard Analysis and Critical Control Points

(HACCP) system as a draft code of practice for pre-harvest and postharvest control of dairy cow's feed and in spices and meat products processing is effective.

## REFERENCES

- Abdulkadir, E.; Tahiya, A.; Saif, A. and Charles, B. (2003):** Fungi and aflatoxins associated with spices in the sultanate of Oman. *Mycopathologia* 155: 155-160.
- Abou Donia, M.A. (2008):** Microbiological quality and aflatoxicogenesis of Egyptian spices and medicinal plants *Global Veterinaria*, 2 (4), 175-181
- Alaa-Eldin M.A. Morshdy, Mohamed A. M. Hussien, Mohamed T. El-Abbasy and Radwa R.M. Elzwahery (2015):** Aflatoxins Residues in Some Meat Products, 2nd Conference of Food Safety, Suez Canal University, Faculty of Vet. Med. Vol. I, 90-95.
- Alcaide-Molina, M.; Ruiz-Jiménez, J.; Mata-Granados, J.; and Luque de Castro, M. (2009):** High through-put aflatoxin determination in plant material by automated solidphase extraction on-line coupled to laser-induced fluorescence screening and determination by liquid chromatography-triple quadrupole mass spectrometry. *Journal of Chromatography A*, 1216 (7):1115-1125.
- Anonymous (2000):** Veratox Software for Windows. Version 2, 0, II. Technology Inc., Palm City, FL.
- AOAC "Association of Official Analytical Chemists" (2000):** Official methods of analysis of Association of Official Analytical Chemists (AOAC) International. Gaithersburg, MD, USA.
- Atanda, O.O.; Akpan, I. and Oluwafemi, F. (2006):** The potential of some spice essential oils in the control of *A. parasiticus* CFR 223 and aflatoxin production. Elsevier 1-7.
- Atanda, O.O.; Ogunrinu, M.C. and Olorunfemi, F.M. (2011):** A neutral red desiccated coconut agar for rapid detection of aflatoxigenic fungi and visual determination of aflatoxins. *World Mycotoxin Journal* 4 (2): 147-155.
- Aziz, N.H.; Youssef, Y.A.; El-Fouly, M.Z. and Moussa, L.A. (1998):** Contamination of some common medicinal plant samples and spices by fungi and their mycotoxins. *Bot. Bull. Acad. Sin.* 39, 279- 285.
- Banwart, G.J. (1980):** Basic food microbiology. A text book. Ed. Avi. Publishing CO. INK. Westport, Coeecticut, U.S.A.
- Bhatnagar D, Cary JW, Ehrlich K, Yu J and Cleveland TE (2006):** Understanding the genetics of regulation of aflatoxin production and *Aspergillus flavus* development. *Mycopathologia* 262:255-266.
- Benkeblia, N. (2004):** Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *Lebensm. - Wiss.u-Technol.* 37: 263-268.
- Bokhari, F.M. (2007):** Mycotoxins and toxigenic fungi in Arabic coffee beans in Saudi Arabia. *Adv Biol Res* 1:56-66.



- Brera C, Miraglia M, Colatosti M. (1998):** Evaluation of the impact of mycotoxins on human health: sources of errors. *Microchemical J.* 5:45 - 49.
- Charoenpornsook, K. and Kavisarasai, P. (2006):** Mycotoxins in animal feedstuffs of Thailand. *KMITL Science and Technology Journal*, 6: 25-28.
- Cho, S.; Lee, C.; Jang, M.; Son, Y.; Lee, S.; Choi, I.; Kim, S. and Kim, D. (2008):** Aflatoxins contamination in spices and processed spice products commercialized in Korea. *Food Chemistry*, Vol.107, No.7, pp. 1283 - 1288, ISSN 0308-8146
- Comi, G., Orlic, S. and Redzepovic, S., (2004):** Bacterial starter cultures for meat fermentation. In: Kniewald, Z. (Ed.): *Current Studies of Biotechnology. Food Vol. III.* Croatian Society of Biotechnology and Medicinska naklada, pp. 87-92. ISBN: 953-176-209-0.
- Edris, A.M. (1986):** Estimation of mould and yeasts in imported frozen meat and chicken. Ph.D. Fac. Vet. Med. Alex., Edfina University.
- El-diahy, E.M.; Ismail, A. S. and Shehata, A. A. (2013):** Microbiological Quality of Some Meat Products in Local Markets with Special Reference to Mycotoxins. *Global Veterinaria* 10 (5): 577-584.
- Elshafie, E.A.; Al-Rashdi, T.A.; Al-Bahry, S.N. and Bakheit, C.S. (2002):** Fungi and aflatoxins associated with spices in the Sultanate of Oman. *Mycopathologia* 155 (3), 155 -160.
- Eman M. Sharaf and Sherifa M. Sabra (2012):** Microbiological Loads for Some Types of Cooked Chicken Meat Products at Al-Taif Governorate, KSA, and *World Applied Sciences Journal* 17 (5): 593-597, 2012
- FAO, (2004):** Worldwide regulations for mycotoxins in food and feed in 2003. *FAO food and nutrition papers*, Rome, Italy, 81.
- Farid M.; Toma and Nareen, Q. Faqi Abdulla (2013):** Isolation and identification of fungi from Spices and medicinal plants *Research Journal of Environmental and Earth Sciences*, 5 (3), 131-138.
- Food and Drug Administration (FDA) (2000):** Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed, Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5001 Campus Drive, College Park, MD 20740.
- Food and Drug Administration (FDA) (2001):** *Bacteriological Analytical Manual*, 8th Ed., Rev. A, AOAC INTERNATIONAL, Gaithersburg, MD
- Foster, G.M.; Nelson, F.K.; Speck, M.L.; Doestscli, R.N. and Olson, J. C. (1983):** *Dairy Microbiology*, Ridgeview Publication Co. California.

- Francis, F.B., R.C. Bruce, W.D. Catherine, H. Paul, D.H. Ailsa, J.M. Thomas and R.B. Tompkin, (2009):** Safety of Meat and Processed Meat. Fidel Toldra', CSIC, Instituto de Agroquímica y Tecnología de Alimentos(IATA) 46100 Burjassot Valencia Spain. Springer Science + Business Media, LLC
- Freire, C. O. and Offord, L. (2002):** Bacterial and Yeast Counts in Brazilian Commodities and Spices. Braz. J. Microbiol., 33: 145-148.
- Fufa, M. and Urga, K. (1996):** Screening of aflatoxins in shiro and ground red pepper in Addis Ababa. Ethiopian Med. Jr. 34 (4) 243-249.
- Guyenot, M. E., Ramos, A. J., Seto, L., Purroy, P., Sanchis, V. and Marin, S. (2003):** Antifungal activity of volatile compounds generated by essential oils against fungi commonly causing deterioration of bakery products. J. of Appl. Microbiol. 94, 893-899.
- Hashem, M. and Alamri, S. (2010):** Contamination of common spices in Saudi Arabia markets with potential mycotoxin-producing fungi, Saudi Journal of Biological Sciences 17, 167-175
- Heinz, G., and Hautzinger, P. (2007):** Meat Processing Technology. For Small-To Medium scale Producers. Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific. Retrived on 1st June 2010, from <ftp://ftp.fao.org/docrep/fao/010/ai407e/ai407e00.pdf>.
- Hussein, A. M. (2008):** mycological aspects of fresh and processed meat with a special respect to proteolytic and lipolytic mould. Ph .D. Thesis, Fac. Vet.Med.Zagazig Univ., Egypt.
- ICMSF (International Commition on Microbiological Specifications for foods) (1978):** Microbiology in foods, their significance and methods of enumeration, 2nd Ed. University of Toronto press, Canada.
- Javadi, A., R. Zarrin and S. Safarmashaei, (2011):** Microbiological Study of Cocktail Sausage during Shelf Life. Middle-East Journal of Scientific Research, 7 (6): 1056-1056.
- Koci-Tanackov, S.D., Dimi, G.R. and Karali, D. (2007):** Contamination of spices with moulds potential producers of sterigmatocystine. APTEFF, 38: 29-35.
- Kneifel W. and Berger, E. (1994):** Microbiological criteria of random samples of spices and herbs retailed on the Austrian Market. J. Food Protec..., 57: 839-901.
- Lamada, H.M. and Nassif, M.Z. (2008):** Mycological profile of meat product, with special reference to aflatoxins. 9th Vet. Med. Zag. Conference (20 -22 Auguset 2008) Port-Said.
- Llewellyn, G. C.; Mooney,R. L.; Cheatle, T. F.and Flannigan, B.(1992):** Mycotoxin contamination of spices - an update. Inter. Biodeterioration and Biodegradation 29: 1 1 1-1 21.
- Madhyastha, M.S. and Bhat, R.V. (1984):** *Aspergillus parasiticus* growth and aflatoxin production on black and white pepper and the inhibitory action of their chemical constituents. Appl Environ Microbiol. ; 48:376 -379.

- Macdonald, S. and Castle, L. (1996):** AUK retail survey of aflatoxins in herbs and spices and their fate during cooking. *Jr. of Food Addit. and contam.* 13 (1) 121-128.
- Marasas, Walter F.O. (2001):** Discovery and occurrence of the fumonisins: a historical perspective. *Environmental Health Perspectives.* Volume 109.
- McKee, L.H.(1995):** Microbial contamination of spices and herbs: A review. *Lebensm. Wiss. Technol.*, 28: 1- 11.
- Mizakova, A.; Pipova, M. and Turek, P. (2002):** The occurrence of moulds in fermented raw meat products. *Czech Food Sci.* 20, (3):89 -94.
- Munimbazi, C. and Bullerman, L.B. (1996):** Moulds and mycotoxins in foods from Burundi. *J. Food Protect.* 59: 864.
- Ostry, V. (2001):** Vyskyt plisni v mase a masnych vyrobcich (I). *Vliv Na zdravi clove' ka.* Maso 12, 20-24.
- Paterson R.R.M. and Lima N. (2010):** Molecular, clinical and environmental toxicology. *Clinical Toxicology.* Vol. 2. Basel (Switzerland): Springer. Toxicology of mycotoxins. p. 31-63.
- Pitt, J.I. and Hocking, A.D. (2009):** Fungi and Food spoilage. 3rd Ed. Published by Springer Dordrecht Heidelberg London New York.
- Rajab, N.N. (2011):** Isolation of fungi from dried and concentrated fruits and study the effect of some plant extracts on the growth of aflatoxigenic fungi. M.Sc. Thesis, College of Science, Salah-ud-Din University, Erbil.
- Refai, M.K.; Mansour, N; Elnagar. A. and Abdel Aziz, A. (1990):** Fungal flora in modern Egyptian abattoirs. *Vet. Med.* J.38, 588-597.
- Refai, M.K.; Niazi, Z.M.; Aziz, N.H. and Khafaga, N.E.M. (2003):** Incidence of aflatoxin B1 in the Egyptian cured meat basterma and control by  $\gamma$ -irradiation. *Nahrung/Food* 47, 377-382.
- Refai, M.K. and Hassan, A. (2013):** Monograph on Mycotoxigenic Fungi and Mycotoxins in Foods and Feeds with Synopsis of studies done by authors on mycotoxigenic fungi and mycotoxins in foods and feeds. [http:// Cairo, academic.edu/Mohamed Refai](http://Cairo.academic.edu/MohamedRefai).
- Sahar, N.; Arif, S.; Afzel, Q.; Ahmed, M.; Ara, J. and Chaudhry, Q. (2013):** Impact of discoloration and picking practices of red chilies on aflatoxin levels, *Pak. J. Bot.* 45 (5):1169-1672.
- Salari R, Habibi MB Najafi, Boroushaki MT, Mortazavi SA and Fathi MN (2012):** Assessment of the microbiological quality and mycotoxin contamination of Iranian red pepper spice. *J. Agric. Sci. Technol.* 14:1511-1521.
- Santos, L.; Marín, S.; Sanchis V. and Ramos, A.J. (2010):** Co-occurrence of aflatoxins, ochratoxin A and zearalenone in Capsicum powder samples available on the Spanish market. *Food Chem.*, 122: 826-830

- Škrinjar, M.; Janković, V.; Vesković Moračanin, S. and Vukojević, J. (2012): Xerophilic moulds isolated from spices used in meat industry as potential producers of mycotoxins. *Zbornik Matice srpske za prirodne nauke*, 123, 7-16.
- Srivastava, R. K. and Chandra, S. (1985): Studies on seed mycoflora of some spices in India: Qualitative and quantitative estimations. *International Biodeterioration* 21: 19 -26.
- Stoloff, L.; Egmond, H.P. and Park, D.L. (1991): Rationales for the establishment of Limits and regulations for mycotoxins. *Food Addit. Contam*, 8: 213-216.
- Sumanth, G.T.; Bhagawan, M.W. and Surendra, R.S. (2010): Incidence of mycoflora from seeds of Indian main spices *African Journal of Agriculture Research*, 5 (22), 3122-3125.
- Talebi, E.; Khademi, M. and Rastad, A. (2011): An Over Review on Effect of Aflatoxin in Animal Husbandry. *Asian J. Exp. Biol. Sci.* Vol. 2 (3): 754 - 757.
- Taniwaki, M.R; Silva, N.D.; Banhe, A. A. and lamariaika, B.I. (2001): Comparison of culture media, simplate and petrifilm for enumeration of yeasts and moulds in foods. *J. Food Protect* .64 (10): 1592-1596.
- Yassein, N; Darwish, A; Elsherif, A. and Eldaly, E. (1991): Food borne fungi in sparrows. *Vet. Med. J.*, 39, 201.

الكشف عن الفطريات و سموم الافلاتوكسين الكلية فى اضافات الاغذية و بعض منتجات اللحوم

بالطرق السيرولوجية و البيولوجية الجزيئية

\* جهاد نبيل محمد \*\* نجوى ابراهيم محمد خفاجة \*\*\* محمود الحريرى \*\*\* محمد كمال رفاعى

\* معهد بحوث صحة الحيوان- مركز البحوث الزراعية- فرع الجيزة

\*\* معهد بحوث صحة الحيوان- مركز البحوث الزراعية- الدقى

\*\*\* قسم البكتيرولوجيا - كلية الطب البيطرى- جامعة القاهرة

### الملخص العربى

تعتبر التوابل و المنكهات الطبيعية مكون اساسى فى الغذاء كما تعتبر المناطق الحارة وسط مناسب للنمو الفطرى و على صدهه التلوث بسموم الافلاتوكسين الكلية مما يؤثر على الصحة العامة للانسان باستهلاك مثل هذه الاغذية و لذا وجب التنبيه للصحة العامة و اخذ باسباب تقليل مثل هذا التلوث وقد اجريت هذه الدراسة على 14 نوع من اضافات اللحوم (التوابل) ممثلة فى الفلفل الاسود و الكمون و الكزبرة و الكركم و الفلفل الحار و الزنجبيل و بودرة الثوم و البابريكا و الكارى و القرقة و الزعتر و القرنفل و الهيل و بودرة البصل و كذا 6 انواع من منتجات اللحوم المختلفة ممثلة فى البسطرمة و البيف برجر و الفرانكفورتر و الهوت دوج و اللانشون التى تحتوى على مثل هذه الاضافات من الاسواق المختلفة فى مدينتى القاهرة و الجيزة و قد تعرضت جميع العينات لعزل و تصنيف الفطريات و تقدير كمية سموم الافلاتوكسين الكلية باستخدام جهاز الاليزا التنافسى المباشر حسب تعليمات الشركة المجهزه لمواد الاختبار لكونها مخاطر جسيمة على الصحة العامة و قد تم اختبار سمية عترات الاسبيرجلس بالطرق الكيمائية و البيولوجية الجزيئية و قد اسفرت نتائج الفحص ان جميع العينات كانت ملوثة بالفطريات و ان اعلى تركيز ( 100%) كان فى عينات الفلفل الاسود و الكمون و الكزبرة و الزعتر اما بالنسبة لعينات الكفتة و الهوت دوج كانت نسبة التلوث 80% , 70% على التوالى بينما كانت اقل نسبة تلوث ( 40 %) فى كل من القرنفل و اللانشون. و كان متوسط العد الفطرى من 2.47 ± 1.09 الى 3.61 ± 1.67 فى اضافات اللحوم بينما كان من 2.12 ± 3.53 الى 2.63 ± 3.95 فى منتجات اللحوم . و دلت النتائج ان اكثر العترات تواجدا هى الاسبيرجلس و البنيسيليوم المعزولة من العينات محل الدراسة كما اثبتت النتائج ان حوالى 15% من عترات الاسبيرجلس كانت مفرزة للسموم و قد اتفقت مع دراستها بالطرق البيولوجية الجزيئية. و قد اتضح ان اعلى تقدير لكمية الافلاتوكسين الكلية 9.28 ± 40.94 ميكروجرام لكل كيلو جرام كانت ممثلة فى عينات الفلفل الحار و كانت 1.52 ± 8.23 ميكروجرام لكل كيلو جرام فى عينات الهوت دوج. و قد تم مناقشه اهمية تواجد الفطريات و كذلك سموم الافلاتوكسين من الناحية الصحية للمستهلك و كذلك التوجيه باتخاذ الممارسات الصحية السليمة للحفاظ على صحة المستهلك بتقديم منتجات لحوم مأمونه خاليه من مسببات الامراض .