

## Isolation and Identification of Fungi from Spices and Medicinal Plants

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**Abstract:** This investigation was designed to throw light on the microbial status of some crude herbal materials. A total of 16 samples, representing different types of spices and medicinal plants were collected from common market in the Erbil city. Ten different fungal genera and 16 species were isolated and identified as *Alternaria alternata*, *Aspergillus* spp., *Gliocladium* sp., *Hyalodendron diddeus*, *Memmoniella* sp., *Penicillium* spp., *Rhizopus* spp., *Syncephalastrum* sp., *Cladosporium lignicolum* and *Ulocladium botrytis*. The total number of isolated fungi from the all sixteen selected samples was serially diluted and plated on Potato Dextrose Agar (PDA) medium was  $(203 \times 10^3)$  cfu/g. samples. *Aspergillus* spp. and *Penicillium* spp. were more frequently detected, while *Stachybotrys* sp., *Syncephalastrum racemocum*, *Uocladium botrytis*, *Alternaria alternata*, *Cladosporium lignicolum* and *Gliocladium catenulatum* were less frequently detected. Detection of mycotoxin on Dichloran Rose Bengal Chloramphenicol agar (DRBC) for fungi isolated from spices and medicinal plant samples, *A. flavus*, *A. Niger* and *A. ochraceous* show positive results on the culture for mycotoxin production. Estimation of natural occurrence of Aflatoxin (AT) and Ochratoxin (OT) in some selected dried samples by using ELISA method, the high result of aflatoxin and ochratoxin show in Red tea (150.5, 387.3) ppb while the low result of aflatoxin and ochratoxin show in Garlic (1.4, 0) ppb respectively.

**Keywords:** Aflatoxin, ELISA, fungi, medicinal plants, ochratoxin, *Penicillium* spp., spices

### INTRODUCTION

Spices and herbs are valued for their distinctive flavors, colors and aromas and are among the most versatile and widely used ingredient in food preparation and processing throughout the world (Ayres *et al.*, 1980). They are widely used as raw materials for pharmaceutical preparations (Galenic products) and as a supplement for dietetic products, especially for "self medications" in public (Weiser *et al.*, 1971). As with many other agricultural products, spices and herbs may be exposed to a wide range of microbial contamination during pre- and post-harvest. 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 (McKee, 1995; Koci-Tanackov *et al.*, 2007). The potential for spoilage and mycotoxin production depends upon the types of fungi present, the composition of the food and the conditions of handling and storage. For example, dried foods are susceptible to spoilage and toxin production if storage temperature is suitable for fungal growth (Misra, 1981). Moreover, spices are collected in tropical areas by simple methods

and are commonly exposed to many contaminants before, being dry enough to prevent microbial growth. They are also stored in conditions favoring contamination by insects, rodents and other vermin (Sharma *et al.*, 1984). The mycological quality of some spices on the market, especially of pepper, is quite poor, bearing many genera and species of fungi. Most fungi are present on pepper of the post-harvest and storage type, which develop after harvest if relative humidity is not controlled during storage (Aziz *et al.*, 1998). Fungi are the predominant contaminants of spices, but most such microbial populations are probably regarded as commensal residents on the plant that survived drying and storage. Soil and air is the main inoculum source for causing contamination in crude spices in field (Kneifel and Berger, 1994). The microbial flora on many spices and related materials is generally dominated by aerobic spore-forming microorganisms. It was found that celery seed, paprika, black and white pepper and Ginger usually show total plate counts in millions per gram (Krishnaswamy *et al.*, 1971). Also, total plate counts above few hundred thousand per gram have been noted in Cassia, Mace and Nutmeg (Guarino, 1974). Spices are commonly heavily contaminated with xerophilic storage moulds and bacteria (Dimic *et al.*, 2000; Romagnoli *et al.*, 2007). There are more and more indications that primary liver carcinoma and other serious diseases may be induced by consuming food or using raw materials for food processing contaminated

Table 1: Common, scientific and part used of spices and medicinal plant

Sample no	Common name	Plant part used	Scientific name
1	Red tea	Flower	<i>Hibiscus sabdariffa</i>
2	Saffron	Flower	<i>Crocus sativus</i>
3	Peppermint	Leaves	<i>Mentha piperita</i>
4	Nutmeg	Peeled seeds	<i>Myristica fragrans</i>
5	Black cumin	Seeds	<i>Nigella sativa</i>
6	Garlic	Clove	<i>Allium sativum</i>
7	Clove	Flower buds	<i>Syzygium aromaticum</i>
8	Black pepper	Dried fruits	<i>Piper nigrum</i>
9	Cumin	Seeds	<i>Cuminum cyminum</i>
10	Ginger	Dry rhizomes	<i>Zingiber officinale</i>
11	Cardamom	Seeds	<i>Elleteria cardamomum</i>
12	Cinnamon	Bark	<i>Cinnamomum zeylanicum</i>
13	Dry lemon	Fruit	<i>Citrus spp</i>
14	Sumac	Dried fruit	<i>Rhus coriaria</i>
15	Bay leaf	Leaves	<i>Laurus nobilis</i>
16	Thyme	Leaves plus stems	<i>Thymus vulgaris</i>

with fungi or mycotoxins. Aflatoxins, ochratoxin and sterigmatocystin proved resistant to heat and have an ability to accumulate in the organism. In the laboratory, both *A. flavus* and *A. ochraceus* are reported to produce mycotoxins. Other species of molds are frequently isolated from spices, including those of *Penicillium*, *Scopulariopsis* and *Sporendonema* (Galvano *et al.*, 2005; Zinedine *et al.*, 2006).

The present study aimed to throw light on the safety of spices and medicinal plants for direct human use as well as for pharmaceutical purposes. The present investigation reports the association of mycoflora with medicinal plant samples and spices, their screening for mycotoxin producing ability and mycotoxin occurrence in samples under Erbil environmental conditions.

## MATERIALS AND METHODS

**Sample collection:** A total of sixteen dried samples, representing different types of spices and medicinal plants, were collected randomly from different places of famous (Shekhalla) market in the Erbil city during the months of March and July 2011. These products of spices and medicinal plant were chosen on the basis of their availability in the market and popularly of usage. Spice samples were usually found outside, kept in metal or plastic containers, wooden boxes or gunny bags or on the bare ground. Care was taken to avoid old stocks and visibly contaminated samples. For each spice and medicinal sample, 3 replicates were taken and mixed to prepare one composite sample. A total of 3 composite samples were prepared for each sample. In the laboratory, samples were individually finely ground in a common household blender. The blender's cup was rinsed in 85% alcohol between samples. The powder kept tightly packed in a new paper bag and stored at 4°C for further analysis. The common names, scientific name and used parts of each sample are presented in Table 1.

**Mycological analysis (isolation techniques):** In collection of samples, the method described by Neergaard (1973) has been adopted. Accordingly,

samples mycoflora was isolated by using Agar Plate Method (APM) and Standard Moist Blotter method (SMB) as recommended by ISTA (1966) and Neergaard (1973).

### Isolation of fungi by direct plating method:

**Isolation of sample-borne mycoflora:** The method used for isolation of fungi was previously described by Abdullah *et al.* (2002). Ten gm of each sample were surface sterilized by 6% sodium hypochlorite solution (NaOCl) in a sterile conical flask for 1-2 min, and then washed by distill water 3 times for 1.5-2 min to removing the toxic activity of the chemical agent on the samples. The disinfected samples transferred with sterile forceps into Petri dish contain sterilized Czapek Dox Agar (CDA), at the rate of (5-10) pieces per plate, depending on the size of the particles, larger samples cut into small pieces. CDA supplemented with 0.5 mg chloramphenicol/mL to restrict bacterial growth. Three replicates were made and the plates were incubated at 25°C for 5-7 days. Fungi colonies were identified according to morphological and microscopic characteristics.

**Isolation of sample surface mycoflora:** The samples transferred with sterile forceps into Petri dish contain sterilized CDA. Three replicates were made and the plates were incubated at 25°C for 5-7 days. Fungi colonies were identified according to morphological and microscopic characteristics (Pitt *et al.*, 1992).

**Isolation on moist blotting paper (sterile filter plate method):** Plates are sterilized by oven on 80°C over night with 2-3 layers of filter paper of 90 mm size (Whatman No.1), the filter papers saturated with 10-15 mL sterile distilled water.

**Isolation of sample-borne mycoflora:** The samples submerge in the sodium hypochlorite 6% concentration for 1-2 min and then washed by distill water 3 times for 1.5- 2 min, then transfer by using sterilized forceps into three layers of moistened 9 cm diameter filter paper in sterilized Petri dishes, sterilized samples were evenly placed at the rate of 10 pieces per Petri plate at equal distance in each Petri plate, samples of each variety were tested by employing standard blotter method in 3 replications. The plates were incubated for 5-7 days at 25°C; fungi developing on samples were examined and transferred to PDA for identification and pathogenicity studies.

**Isolation of sample surface mycoflora:** Non-sterilized samples were evenly placed at the rate of 10 pieces/Petri plate at equal distance in each Petri plate on three layers of moistened 9 cm diameter filter paper in sterilized Petri dishes. The plates were incubated for 5-7 days at 25°C; after incubation the samples were

examined under microscope for the associated fungi and they were identified based on “habit characters” (Anonymous, 1996).

**Standard dilution plate:** For fungal analysis, dilution method was used to determine total fungal counts in spice and medicinal plant samples. Ten grams of each composite sample (fine powder) were transferred into 250 mL screw-capped medicinal bottle containing 90 mL of sterile distilled water and were mechanically homogenized at constant speed for 15 min. The sample-water suspension was allowed to stand for 10 min with intermittent shaking before being plated. Appropriate tenfold serial dilutions (1:10) were prepared and 1 mL portions of suitable dilutions of the resulting samples suspension were used to inoculate Petri dishes each containing 15 mL Potato Dextrose Agar (PDA). Plates were then incubated for 7 days at 28°C. Three replicates plates per medium were used for each sample and the developing fungi were counted and the number per mg dry sample was determined and identified according to several key processes. Data expressed are average of all these media. After incubation, the results were expressed in Colony-Forming Units (CFU) /g of samples; all plates were examined visually, directly and with a microscope (Aziz *et al.*, 1998).

#### **Diagnosis:**

**Identification of the fungal genera:** The fungal isolates were transferred to sterilized plates for purification and identification. The grown fungi were mounted on a slide, Stained with lactophenol-cotton blue to detect fungal structures (Basu, 1980), covered with a cover slip, examined under microscope and identified on the basis of their colony morphology and spore characteristics (Ronhede *et al.*, 2005; Rajankar *et al.*, 2007).

The texts (books) used for identification of fungi, depending on their taxonomic keys are as follows; Moubasher (1993), Larone (1995), Pitt and Hocking (1997), Guarro *et al.* (1999), Howard (2002), Watanabe (2002), Ulhan *et al.* (2006) and Pornsuriya *et al.* (2008).

#### **Determination of toxigenic potential of fungi in culture media:**

**Dichloran rose bengal chloramphenicol agar test:** DRBC is a selective medium that supports good growth of fungi. It is formulated as described by King *et al.* (1979) is a modification of DRBC (Jarvis, 1973). The substances in the DRBC are dichloran (is added to the medium to reduce colony diameters of spreading fungi), rose Bengal (suppresses the growth of bacteria and restricts the size height of colonies of the more rapidly growing moulds) and chloramphenicol (is included in this medium to inhibit the growth of bacteria present in environmental and food samples). The reduced pH of

the medium from (7.2 to 5.6) helps inhibition of the spreading fungi (Jarvis, 1973). The isolated fungi were inoculated in the solidified DRBC medium after incubation for 7 days at 25°C search for pigmentation and color change observed in compare to CDA medium control.

#### **Sample preparation for natural aflatoxin and ochratoxin determination by ELISA method:**

Samples were homogenized and kept in glass bottle and stored at 2-8°C until further analysis. For the quantitative analysis of mycotoxins (aflatoxin and ochratoxin). Enzyme Linked Immunosorbent Assay technique (ELISA) was used. Samples (10 g) were taken in 50 mL of 70% methanol for aflatoxins and ochratoxin analysis. Sample powder was blended individually with high speed blender for three minutes. After blending the material was filtered with Whatman filter paper number 1, the filtrate was used for further analysis. Commercially available immunoassay kit Veratox for quantitative analysis of aflatoxin and ochratoxin test-NEOGEN Crop, Lansing, MI was used. The assay kit was based on Competitive Direct Enzyme Linked Immunosorbent Assay (CD-ELISA) (Stoloff *et al.*, 1991). The antibodies captured the analyze and conjugated to the enzyme (horse reddish peroxidase). Tetra methylbenzidine/hydrogen peroxide was used as a substrate for color development. Finally stopping solution was added to stop the reaction. The color intensity was inversely proportional to the mycotoxin concentration and measured with the ELISA reader. All necessary reagents were present in the kit. Concentration of mycotoxins was calculated by Log/logit Software Awareness Technology Inc. (Anonymous, 2000; Stoloff *et al.*, 1991).

## **RESULTS AND DISCUSSION**

Common and scientific names, plant part used of the spices and herbal drugs were tabulated in Table 1. Totally 16 samples were analyzed for enumeration of fungal isolates.

Fungal genera were isolated by different method on the different media. The results presented in Table 2 show fungi were isolated by using Standard Moist Blotter method (SMB) and Agar Plate Method (APM). Blotter and Agar plate methods were employed for this study and two sets of samples were analyzed i.e., unsterilized and surface sterilized spices and medicinal plant. A total of 10 different fungal genera and 16 species were isolated. All fungi were identified on the basis of their cultural and morphological characteristics. These were identified as *Alternaria alternata*, *Aspergillus aculeatus*, *A. candidus*, *A. flavus*, *A. niger*, *A. ochraceous*, *A. tamari*, *A. terreus*, *Gliocladium* sp.,

Table 2: The isolated fungi from herbs and spices samples by blotting paper (filter paper) and czapex dox agar

Samples no	Samples name	Blotting paper		Czapex dox agar	
		Sterilized surface	Unsterilized surface	Sterilized surface	Unsterilized surface
1	Red tea	Negative	<i>Aspergillus niger</i> <i>Penicillium</i> spp	Negative	<i>A. niger</i> <i>Penicillium</i> spp
2	Saffron	Negative	<i>A. niger</i>	Negative	<i>A. niger</i> <i>Penicillium</i> spp
3	Peppermint	<i>A. niger</i>	<i>A. niger</i>	<i>A. niger</i>	<i>A. niger</i> <i>Gliocladium</i> sp. <i>Rhizopus oryzae</i> <i>Ulocladium botrytis</i>
4	Nutmeg	Negative	<i>A. niger</i>	Negative	<i>A. niger</i> <i>Sterile mycellium</i>
5	Black cumin	Negative	<i>Penicillium</i> spp	<i>A. aculeatus</i>	<i>Alternaria alternata</i> <i>A. aculeatus</i> <i>Penicillium</i> spp
6	Garlic	Negative	<i>Penicillium</i> spp	Negative	<i>A. flavus</i> <i>Penicillium</i> spp
7	Clove	Negative	Negative	Negative	<i>A. flavus</i> <i>A. niger</i>
8	Black pepper	Negative	<i>Asp. flavus</i>	<i>A. flavus</i>	<i>Alternaria alternata</i> <i>A. candidus</i> <i>A. flavus</i> <i>A. tamarii</i> <i>A. flavus</i> <i>A. Niger</i> <i>Penicillium</i> spp
9	Cumin	Negative	<i>Penicillium</i> spp	Negative	<i>A. flavus</i> <i>A. Niger</i> <i>Penicillium</i> spp
10	Ginger	Negative	<i>A. flavus</i> <i>A. niger</i> <i>A. terreus</i>	<i>A. flavus</i>	<i>A. flavus</i> <i>A. niger</i> <i>Penicillium</i> spp
11	Cardamom	Negative	<i>Rhizopus arrhizus</i>	Negative	<i>A. aculeatus</i> <i>A. ochraceous</i> <i>Rhizopus arrhizus</i>
12	Cinnamon	Negative	Negative	Negative	<i>Syncephalastrum</i> sp.
13	Dry lemon	<i>A. niger</i>	<i>A. niger</i>  <i>Penicillium</i> spp	Negative	<i>A. niger</i> <i>Memmoniella echinata</i> <i>Penicillium</i> spp <i>Rhizopus stolonifer</i>
14	Sumac	<i>A. niger</i>	<i>A. niger</i>	<i>A. niger</i>	<i>A. niger</i>
15	Bay leaf	Negative	<i>A. niger</i>	Negative	<i>A. aculeatus</i> <i>A. flavus</i> <i>A. niger</i>
16	Thyme	Negative	Negative	Negative	<i>Hyalodendron diddeus</i> <i>Penicillium</i> spp

*Hyalodendron diddeus*, *Memmoniella* sp., *Penicillium* spp., *Rhizopus arrhizus*, *R. oryzae*, *R. stolonifer*, sterile mycelium, *Syncephalastrum* sp. and *Ulocladium botrytis*. It was observed that treated samples (with sodium hypochlorite) yielded less population of samples-borne fungi than the untreated samples, indicating partial elimination of some contaminating fungi. In this table, it was found that both the agar and blotter paper methods of fungal isolation are effective, routinely and consistently applicable and provide reliable results. A total of 10 fungal genera were isolated by agar plate method and 3 fungi by blotting paper method under unsterilized conditions. Out of 3 samples, *Aspergillus* spp. was isolated from all sample, showed samples infection in both methods and as such it appeared as the most predominant fungus of spices and medicinal plants. The results agree at large with many of the investigators working on seed pathology. Sumanth *et al.* (2010), who isolated fungal genera from

tested spices, found that the most common fungi isolated were *Aspergillus* spp. followed by *Alternaria alternata*, *Cladosporium*, *Curvularia*, *Fusarium* spp., *Helminthosporium* and *Trichoderma* show maximum incidence on Agar plate method. Many developing countries have been trying to increase the quality of their seed production. 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 Dimic *et al.* (2008) twenty three different fungi were isolated from the test spices. It indicates the ability of fungi in developing association with broad spectrum of seeds, irrespective of their types. The similar reports regarding the incidence of fungi have been given by Sharma and Sharma (1984) and Regina and Raman (1992) in Ammi and cumin, *A. niger*, *A. flavus* and *Cladosporium* sp., have been recorded as most dominant. Agar plate method is proved to be superior for the highest

Table 3: The isolated fungi from herbs and spices samples by standard dilution plate method

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	TCFU/gm.s.
<i>Alternaria alternata</i>	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	2
<i>Aspergillus aculeatus</i>	-	-	-	-	4	-	-	-	-	2	-	1	1	1	-	-	9
<i>A. candidus</i>	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	2
<i>A. flavus</i>	6	2	4	1	-	2	8	3	1	4	-	8	15	12	2	-	68
<i>A. niger</i>	1	1	3	1	-	1	1	-	1	2	-	-	1	1	1	-	14
<i>A. ochraceous</i>	2	1	-	2	-	-	-	3	-	-	6	15	16	2	2	-	49
<i>A. tamarii</i>	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
<i>A. terreus</i>	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
<i>A. versicolor</i>	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	6
<i>Cladosporium lignicolum</i>	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	2
<i>Emericella rugolosa</i>	-	-	2	-	-	-	-	-	-	-	-	4	-	-	-	-	6
<i>E. wentii</i>	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	3
<i>Gliocladium catenulatum</i>	-	-	1	-	-	-	-	-	-	-	-	2	-	-	-	-	3
<i>Penicillium</i> spp	1	1	-	-	1	8	-	-	1	6	-	-	1	-	-	1	20
<i>Rhizopus arrhizus</i>	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1
<i>R. oryzae</i>	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
<i>R. stolonifer</i>	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
<i>Stachybotrys</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
<i>Syncephalastrum racemocum</i>	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	3
<i>Uocladium botrytis</i>	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Yeast	-	-	5	-	-	-	-	-	4	-	-	-	-	-	-	-	9
Total	10	5	17	12	9	11	9	10	3	17	9	32	36	16	6	1	203×10 <sup>3</sup>

incidence of fungi and the results are similar to the reports of Bilgrami and Ghaffar (1993) and Motta *et al.* (1996). The highest incidence of fungi was observed in ammi and coriander followed by cardamom, caraway and cumin respectively according to Seema and Monica (2003). It is interesting to note that the species of *A. alternata*, *A. flavus*, *A. Niger* and *C. cladosporidies* have dominant association, a similar results also reported by Ayres *et al.* (1980). The overall result reveals that the agar method is a more supporting medium than blotter method used for the isolation of fungi.

The results presented in Table 3 show the identity and the total Colony Forming Units (CFU) of fungi were found in all of the collected samples, they were serially diluted and plated on PDA medium. The total number of isolated fungi from the all sixteen selected samples was (203×10<sup>3</sup>) cfu/g. samples. The minimum number of fungi was detected in Thyme (1×10<sup>3</sup>) followed by Saffron (5×10<sup>3</sup>) and Bay leaf (6×10<sup>3</sup>). In contrast, Dry lemon (36×10<sup>3</sup>) and Cinnamon (32×10<sup>3</sup>) presented the highest infections of fungi. *Aspergillus* spp. and *Penicillium* genera were more frequently detected than other genera of fungi. *Aspergillus* spp. was found in all examined spices and medicinal plant samples except Thyme while, *Rhizopus stolonifer* and *Stachybotrys* sp., found only in Dry lemon, *Syncephalastrum racemocum* in Cinnamon and *Uocladium botrytis* in Peppermint. Other species of moulds were isolated from different spices and medicinal plant samples, such as, *Alternaria alternata*, *Cladosporium lignicolum*, *Gliocladium catenulatum* and Yeasts were also frequently recovered, but not identified.

Our results were in well agreement with those found by Bokhari (2007) were isolated fungi from spices samples during the investigation. *Aspergillus*

was the most common genus in the different spices tested. *Aspergillus flavus* and *A. Niger* were the most prevalent. The result less agreement to this finding by Srivastava and Chandra (1985) recorded that *Aspergillus* followed by *Fusarium* were the most frequent members of the mycobiota of coriander, cumin, fennel and fenugreek. Our results were in well agreement with those found by Hassan (1984) reported that members of *Fusarium* was completely absent in 12 kind of spices tested. Hashem and Alamri (2010) the most predominant fungal genera encountered were *Aspergillus*, *Penicillium* and *Rhizopus*. Samples obtained from sumac encountered very rare colony counts indicating its antifungal prosperities. *Alternaria* was represented by *Alternaria alternate*. Ath-Har *et al.* (1988) reported that *A. flavus*, *A. Niger*, *Aspergillus nidulans*, *A. sydowii*, *A. ochraceous*, *Penicillium* and *Rhizopus* spp. were most frequently isolated from spices and drug plants. Bugno *et al.* (2006) show that the predominant mycoflora obtained was distributed in 10 genera. The genus *Aspergillus* was the most dominant genus recovered (179 isolates) followed by *Penicillium* (44 isolates). The presence of a wide range of storage fungi indicates that considerable improvements could be made during post-harvest storage. The dominant of *Aspergillus* and *Penicillium* spp. in all examined medicinal plant samples and spices was in accord with the results of Takatori *et al.* (1977) and Ayres *et al.* (1980), who stated that *Aspergillus* and *Penicillium* spp. were the main components of cardamom, cinnamon, fennel, coriander, cumin, black cumin and white pepper, all of which are common in the food industry. They found a high degree of contamination in all samples.

Data represented in Table 4 show the result of mycotoxin detection on DRBC media for fungi isolated from spices and medicinal plant samples, *A. flavus*, *A. niger* and *A. ochraceous*, show Positive results on

Table 4: Determination of aflatoxin and ochratoxin content in fungal culture by dichloran rosebengal chloramphenicol agar media

No	Fungi	Dichloran rosebengal chloramphenicol agar media
1	<i>Aspergillus flavus</i>	Positive
2	<i>Aspergillus niger</i>	Positive
3	<i>Aspergillus ochraceus</i>	Positive
4	<i>Penicillium</i> sp.	Negative
5	<i>Rhizopus</i> sp.	Negative

Table 5: Total aflatoxins and ochratoxin content in herbs and spices samples by ELISA method

Sample	Natural aflatoxin content (ppb)		Natural ochratoxin content (ppb)	
	OD	Results	OD	Results
Sumac	0.210	58.1	0.120	49.3
Ginger	1.039	5.2	0.669	3.1
Garlic	1.406	1.4	1.133	0.0
Black pepper	1.055	5.0	0.551	5.8
Thyme	0.913	7.2	0.470	9.0
Dry lemon	0.221	54.9	0.105	162.5
Cinnamon	0.489	20.8	0.086	224.6
Red tea	0.085	150.5	0.061	387.3
Bay leaf	0.677	12.7	0.363	16.6
Peppermint	0.598	12.7	0.689	4.8
Nutmeg	0.887	4.5	0.157	82.8
Cardamon	1.085	1.2	0.627	6.2

the culture for mycotoxin production and show change in Colony Diameter and color change in compare with control (CDA media), while *Penicillium* sp. and *Rhizopus* sp., show no any change in Colony Diameter and color in compare with control (CDA media). Our results were in Contrary to this finding with Rajab (2011) show that the result of aflatoxin detection on DRBC media for fungi isolated from dried fruit samples, all the fungi culture were negative for mycotoxin production and show no any change in Colony Diameter or color change in compare with control (CDA media).

Dichloran Rose Bengal Chloramphenicol (DRBC) is an antibacterial media containing compounds to inhibit or reduce spreading growth of moulds such as *Mucor* sp. and *Rhizopus* sp., (Samson *et al.*, 1992; Hocking *et al.*, 2006). Dichloran and rose Bengal effectively slow down the growth of fast-growing fungi, thus readily allowing detection of other yeast and mold propagules, which have lower growth rates. Media containing rose Bengal are light-sensitive; relatively short exposure to light will result in the formation of inhibitory compounds; therefore it should kept in a dark, cool place until used (Tournas *et al.*, 2001).

Table 5 shows results of Estimation of natural occurrence of Aflatoxin (AT) and Ochratoxin (OT) in dried sample by ELISA method some dried samples selected by using ELISA method, the samples include (Sumac, Ginger, Garlic, Black pepper, Thyme, Dry lemon, Cinnamon, Red tea, Bay leaf, Peppermint, Nutmeg and Cardamon) the concentrations of aflatoxin and ochratoxin were (58.1, 5.2, 1.4, 5, 7.2, 54.9, 20.8, 150.5, 12.7, 12.7, 4.5 and 1.2), (49.3, 3.1, 0, 5.8, 9, 162.5, 224.6, 387.3, 16.6, 4.8, 82.8 and 6.2) ppb

respectively. The results show high AF and OT content in most sample exceeds the normal rang for aflatoxin (20 ppb) for human consumption.

For the detection of different types of mycotoxins, 12 samples were analyzed quantitatively by CD-ELISA technique (Competitive Direct Enzyme Linked Immunosorbent Assay). 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 and health. Mycotoxins can cause severe damage to liver, kidney and nervous system of man even in low dosages (Rodricks, 1976). *Fusarium* and *Aspergillus* species are common fungal contaminants of maize and also produce mycotoxins (Bakan *et al.*, 2002; Verga and Teren, 2005).

## REFERENCES

- Abdullah, S.K., I. Al-Saad and R.A. Essa, 2002. Mycobiota and natural occurrence of sterigmatocysin in herbal drugs in Iraq. *Basrah J. Sci. B.*, 20: 1-8.
- Abou Donia, M.A., 2008. Microbiological quality and aflatoxinogenesis of Egyptian spices and medicinal plants. *Global Vet.*, 2(4): 175-181.
- Anonymous, 1996. International rules for seed testing. *Seed Sci. Tech.*, 24: 1-335.
- Anonymous, 2000. Veratox Software for Windows. Version 2, 0, II. Technology Inc., Palm City, FL.
- Ath-Har, M.A., H.S. Prakash and H.S. Shetty, 1988. Mycoflora of Indian spices with special reference to aflatoxin producing isolates of *Aspergillus flavus*. *Indian J. Microbiol.*, 28: 125-127.
- Ayres, G.I., T.I. Mund and E.W. Sondin, 1980. Microbiology of Food Spices and Condiments. A Series of Books in Food and Nutrition, Schmeigert, pp: 249.
- Aziz, N.H., Y.A. Youssef, M.Z. El-Fouly and L.A. Moussa, 1998. Contamination of some common medicinal plant samples and spices by fungi and their mycotoxins. *Bot. Bull. Acad. Sinica*, 39(4): 279-285.
- Bakan, B., D. Richard, D. Molard and B. Cahagnier, 2002. Fungal growth and *Fusarium* mycotoxin content in isogenic traditional maize and genetically modified maize grown in France and Spain. *J. Agric. Food Chem.*, 50(4): 278-731.
- Basu, P.K., 1980. Production of chlamydospores of phytophthora megasperma and their possible role in primary infection and survival in soil. *Can. J. Plant Pathol.*, 2: 70-75.
- Bilgrami, A. and A. Ghaffar, 1993. Detection of seed borne mycoflora in Pinus Gera diama. *Pak. J. Bot.*, 25(2): 225-231.
- Bokhari, F.M., 2007. Spices mycobiota and mycotoxins available in Saudi Arabia and their abilities to inhibit growth of some toxigenic fungi. *Korean Soc. Mycol.*, 35(2): 47-53.

- Bugno, A., A.B. Almodovar, T.C. Pereira, T.J. Andreoli Pinto and S. Myrna, 2006. Occurrence of toxigenic fungi in herbal drugs. *Braz. J. Microbiol.*, 37: 47-51.
- Dimic, G., S. Krinjar and M.V. Dosen-Bogic Evic, 2000. Dance, a potential producer and sterigmatocystin in spices (Croatian). *Tehnol. Mes.*, 41(4-6): 131-137.
- Dimic, G.R., D. Suncica, T. Kocic, N.T. Alcksandra, L.V. Biserka and M.S. Zdravko, 2008. Mycopopulation of spices. *Acta Period. Technol.*, 39: 1-9.
- Galvano, F., A. Ritieni, G. Piva and A. Pietri, 2005. Mycotoxins in the Human Food Chain. In: Duarate Diaz (Ed.), *The Mycotoxin Blue Book*. Nottingham University Press, England, pp: 187-225.
- Guarino, P.A., 1974. Microbiology of spices, herbs and related materials. *Proceedings of 7th Annual Symposium, Fungi and Foods. Special Report No. 13*, New York State Agric. Exper. Station, Geneva, NY, pp: 16-18.
- Guarro, J., J. Gene and A.M. Stchigel, 1999. Developments in fungal taxonomy. *Clin. Microb. Rev.*, 12(3): 454-500.
- Hashem, M. and S. Alamri, 2010. Contamination of common spices in Saudi Arabia markets with potential mycotoxin-producing fungi. *Saudi J. Biol. Sci.*, 17: 167-175.
- Hassan, H., 1984. Effect of gamma radiation on the microflora of some spices. M. Sc. Thesis, Faculty of Science, Ain Shams University, Cairo, Egypt.
- Hocking, A.D., J.I. Pitt, R.A. Samson and U. Thrane, 2006. *Advances in Food Mycology*. Springer, New York.
- Howard, D.H., 2002. *Pathogenic Fungi in Humans and Animals*. 2nd Edn., Marcel Dekker Inc., New York. USA, 16: 790.
- ISTA, 1966. International rules for seed testing. *Prof. Inst. Seed test. Assoc.*, 31: 1-152.
- Jarvis, B., 1973. Comparison of an improved rose-Bengal chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in food. *J. Appl. Bacteriol.*, 36: 723-727.
- King, D.A., A.D. Hocking and J. Pitt, 1979. Dichloran-rose Bengal medium for enumeration and isolation of moulds from foods. *J. Appl. Environ. Microb.*, 37: 959-964.
- Kneifel, W. and E. Berger, 1994. Microbial criteria of random samples of spices and herbs retailed on the Austrian market. *J. Food Prot.*, 57: 893-901.
- Koci-Tanackov, S.D., G.R. Dimi and D. Karali, 2007. Contamination of spices with moulds potential producers of sterigmatocystine. *APTEFF*, 38: 29-35.
- Krishnaswamy, M.A., J.D. Patel and N. Parthasarathy, 1971. Enumeration of microorganisms in spices and spice mixtures. *J. Food Sci.*, 8: 191-194.
- Larone, D.H., 1995. *Medically Important Fungi: A Guide to Identification*. ASM Press, Washinton, DC, pp: 274.
- McKee, L.H., 1995. Microbial contamination of spices and herbs: A review. *Lebensm. Wiss. Technol.*, 28: 1-11.
- Misra, N., 1981. Influence of temperature and relative humidity on fungal flora of some spices in storage. *Z. Lebensm. Unter. Forsch.*, 172(1): 30-31.
- Motta, E., T. Annesi and R.K. Balonas, 1996. Seed borne fungi in Norway spruce: Testing methods and pathogen control by seed dressing. *Eur. J. Trust Path.*, 26(6): 307-314.
- Moubasher, A.H., 1993. *Soil Fungi in Qatar and other Arab Countries*. Center for Scientific and Applied Research, University of Qatar, Doha, Qatar, pp: 566.
- Neergaard, P., 1973. *Seed Pathology*. Vol. 1, John Willey and Sons, NY.
- Pitt, J.I. and A.D. Hocking, 1997. *Fungi and Food Spoilage*. 2nd Edn., Gaithersburge, Chapman and Hall, Maryland, pp: 593.
- Pitt, J.I., A.D. Hocking, R.A. Samson and A.D. King, 1992. *Recommended Methods for the Mycological Examination of Foods*. Modern Methods in Food Mycology. Elsevier, Amsterdam, pp: 365-368.
- Pornsuriya, C., F.C. Lin, S. Kanokmedhakul and K. Soyong, 2008. New record of *Chaetomium* sp., isolated from soil under pineapple plantation in Thailand. *J. Agric. Technol.*, 4(2): 91-103.
- 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.
- Rajankar, P.N., D.H. Tambekar and S.R. Wate, 2007. Study of phosphate solubilization efficiencies of fungi and bacteria isolated from saline belt of purna river basin. *Res. J. Agric. Biol. Sci.*, 3(6): 701-703.
- Regina, M. and T. Raman, 1992. Species of *Chaetomium* isolated from spices. *Ind. Phytopath.*, 41(4): 628-629.
- Rodricks, J.V., 1976. *Mycotoxins and other Fungus related Food Problems*. Advance in Chemistry Series 149. American Chemicals Society, Washington, DC, pp: 239.
- Romagnoli, B., V. Menna, N. Gruppioni and C. Bergamini, 2007. Aflatoxins in spices, aromatic herbs, herbs-Teas and medicinal plants marketed in Italy. *Food Control*, 18: 697-701.
- Ronhede, S., B. Jenesen, S. Rosendahl, B.B. Kragelund, R.K. Juhler and J. Amand, 2005. Hydroxylation of the herbicide isoproturon by fungi isolated from agricultural soil. *Appl. Environ. Microb.*, 71(12): 7927-7932.

- Samson, R.A., A.D. Hocking, J.I. Pitt and A.D. King, 1992. *Modern Methods in Food Mycology*. Elsevier, Amsterdam.
- Seema, K. and B. Monica, 2003. Evaluation of aflatoxin contamination in different spices. *Ind. Phytopath.*, 56(4): 457-459.
- Sharma, A.K. and K.D. Sharma, 1984. Studies on seed mycoflora of some umbeliferous spices. *Agri. Sci. Digest.*, 4(2): 71-73.
- Sharma, A.K., A.S. Ghanekar, S.R. Podwal-Desai and G.P. Nadkarni, 1984. Microbiological status and antifungal properties of irradiated spices. *J. Agric. Food Chem.*, 32: 1061-1063.
- Srivastava, R.K. and S. Chandra, 1985. Studies on seed mycoflora of some spices in India: Qualitative and quantitative estimations. *Int. Biodeterior.*, 21: 19-26.
- Stoloff, L., H.P. Egmond and D.L. Park, 1991. Rationales for the establishment of Limits and regulations for mycotoxins. *Food Addit. Contam.*, 8: 213-216.
- Sumanth, G.T., B.M. Wagmare and S.R. Shinde, 2010. Incidence of mycoflora from the seeds of Indian main spices. *Afr. J. Agric. Res.*, 5(22): 3122-3125.
- Takatori, K., K. Watanabe, S. Udagawa and H. Kurata, 1977. Mycoflora of imported spices and inhibitory effects of the spices on the growth of some fungi. *Proc. Jpn. Assoc. Mycotoxicol.*, 9: 36-38.
- Tournas, V., M.E. Stack, B.P. Mislivec, H.A. Koch and R. Bandler, 2001. Yeasts, Molds and Mycotoxins. In: *Bacteriological Analytical Manual*. 8th Edn., Center for Food Safety and Applied Nutrition. Revision A. Chapter 18, US Food and Drug Administration. Retrieved from: <http://www.cfsan.fda.gov/~ebam/bam-18.html> (Accessed on: March 15, 2011).
- Ulhan, S., R. Demurel, A. Asan, C. Baycu and E. Kinaci, 2006. Colonial and morphological characteristics of some microfungi species isolated from agricultural soils in Eskişehir Province (Turkey). *Turk. J. Bot.*, 30: 95-104.
- Verga, B.T. and J. Teren, 2005. Mycotoxin producing fungi and mycotoxins in foods in Hungary. *J. Act. Alim. Akad.*, 34(3): 267-275.
- Watanabe, T., 2002. *Morphologies of Cultured Fungi and Key to Species*. 2nd Edn., CRC Press, Hoboken, pp: 484.
- Weiser, H.H., G.J. Mountney and W.A. Gould, 1971. *Practical Food Microbiology and Technology*. 2nd Edn., 54: AVI Publishing Co., Westport, Conn.
- Zinedine, A., C. Brera, S. Elakhdari, C. Catano, F. Debegnach, S. Angelini, B. DeSantis, M. Faid, M. Benlemlih, V. Minardi and M. Miraglia, 2006. Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. *Food Control*, 17: 868-874.