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Discovery of Fungi Supplementary with Some Spices Collected from Iraqi Markets

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Abstract
The mycoflora association of 10 spices (Coriander, Black cumin, Fennel, Bay leaf, Sumac, Cardamom, Clove, Sesame, Cinnamon and Caraway) were purchased randomly from different places of many local markets in Iraq. the Mycoflora of selected spices was isolated by Agar Plate Method used Potato Dextrose Agar (PDA). The maximum percentage of infected samples was found in Coriander and Fennel While the lowest percentage was found in Cardamom..The species of fungi that were isolated belonged to fourteen (10) genera (*Aspergillus niger , A.flavus , A. aculeatus, Alternaria alternate, A. solani , Rhizopus oryzae , R. stolonifer, R. nodosus, R. arrhizus , Fusarium culumorum, F. solani,Penicillium spp , Phoma sp, Gliocladium sp,Mucor hiemalis, Colletotrichum dematium, Ulocladium botrytis*). *A. niger* and *A. flavus* were more frequently detected than other genera of fungi. *A. niger* was found in all examined spices and medicinal plant samples except Sumac while, *A. flavus* was found in all examined spices except Black cumin and Cinnamon.Other species of fungi were isolated from different spices samples, were also frequently improved.The equal of mycoflora connected in spices propose a essential for regulator in all features of the production , dispensation and practice of these spices from decay and food diseases outstanding to contamination of spices.
Keywords: Spice, Fungi, Potato Dextrose Agar, Contamination, Iraqi

1- INTRODUCTION

In recent years increasing shopper consciousness has stressed the need for microbiologically safe food. Since the human food source comprises basically of plants and animals or products resultant from them, it is reasonable that our food supply can encompass micro-organisms in interaction with the foods. When the micro-organisms involved are pathogenic, their connotation with our food is serious from community health point of view Serious health dangers due to the presence of pathogenic microbes in food can principal to food exterminating eruptions (Atanda *et al*.,2007).Spices are imperative agricultural possessions, because of their provisions, it occupy a protruding place in the outdated and food practices all over the world (Oluwafemi,2000). Spices have been defined as a natural composite, or a combination of natural compounds that is removed from the seeds, fruits, flowers, or trunks (skins, roots, leaves) of numerous plants that original or exotic origin, aromatic or with robust taste, used in miniature numbers (Bulduk, 2004). By way with many other agricultural harvests, spices and herbs may be unprotected to a wide range of microbial contamination during pre- and post-harvest, while spices are present in foods in small quantities, they are documented as important transporters of microbial contamination mainly because of the conditions in which they were grown, harvested and treated. In adding, because of possible abandonments during cleanliness or processing, foods containing spices are more likely to deteriorate and also could exert destructive effects, having in mind health risks connected with mycotoxins produced by some fungal genera (McKee, 1995; Koci-Tanackov *et al*., 2007). When appropriately dried and stored, spices are normally resistant to microbial spoilage. However, spices are fresh agricultural materials and if the moisture content is moreover high, toxigenic fungi like *Aspergillus* spp, *Penicillium* spp. and *Fusurium* spp.,may grow contribution the opportunity for aflatoxin production (Halt, 1998; Aziz *et al*.,1998; Reddy *et al*.,2001) . The greatest recurrent fungal pathogen of spices is from genera *Aspergillus* and *Penicillium* (Koci *et al*., 2007). Fungi are normal component of food mycoflora and cause spoilage and mycotoxin production. It is well known fact that numerous fungi causes significant damage to spices under storage condition and also reduction in germination ability staining of parts, beating in weight and production of toxin and it be contingent upon the type of fungi current, the arrangement of food, storage and management (Mandeel, 2004). The contemporary study designed to throw light on the investigation a comprehensive examination of mycoflora of selected spices samples from local market under environmental conditions of many region in Iraq.

2- Material and Methods

2-1-Sample collection

A total of 50 dried samples, representing 10 types of spices Table (1), were purchased randomly from different places of many local markets in Iraq . For each spice fife replicates were taken and mixed to prepare one composite sample. Half the amount of Each sample (100 g) In the laboratory were individually finely ground in a common household blender. The blender's cup was rinsed in 70% alcohol between samples. The samples were put in a new paper bag and stored in cool place at 4°C for fungal determination .

2-2-Isolation of Fungi

In collection of samples, the Mycoflora of selected spices was isolated by Agar Plate Method used Potato Dextrose Agar (PDA) supplemented with 0.5 mg Chloramphenicol/ml antibiotic is used to inhibit the bacterial growth as recommended by Neergaard (1973). Each sample were surface sterilized by 1% sodium hypochlorite solution (NaOCl) for 1-2 min, and then washed by distill water 3 times for 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 Potato Dextrose Agar (PDA), at the rate of (5-10) pieces per plate, depending on the size of the particles, lager samples chopped into small pieces (1 mm). Five replicates were made and the plates were incubated at 25°C for 5-7 days.

2-3- Standard dilution plate

Dilution method was used to determine total fungal counts ,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 fife serial dilutions (1:5) 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 5- 7 days at 25°C. fife 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.

2-4-Identification of the fungal

The fungal isolates were transferred to sterilized plates for purification and identification. Identification of different fungi was done with help of slides prepared by direct mount from the culture. The examined under microscope and identified on the basis of their colony morphology and spore characteristics (CMI, 1966; Nelson, *et al*. 1983; Samson and Hoektra, 1988; Singh *et al*. 1991; Malone and Muskett, 1997; Mathur and Kongsdal, 2003).

Table 1: Public name , scientific name and part name of spices that used .

Number of sample	Public name	Scientific name	part name of spices
1	Coriander	Coriandrum sativum	Seeds
2	Black cumin	Nigella sativa	Seeds
3	Fennel	Foeniculum vulgare	Seeds
4	Bay leaf	Laurus nobilis	Leaves
5	Sumac	Rhus coriaria	Dried fruit
6	Cardamom	Elleteria cardamomum	Fruit
7	Clove	Syzygium aromaticum	Flower buds
8	Sesame	Sesamum indicum	Seeds
9	Cinnamon	Cinnamomum zeylanicum	Bark
10	Caraway	Carum carui	Seed

3-RESULTS AND DISCUSSION

Coriander, Black cumin, Fennel, Bay leaf, Sumac, Cardamom, Clove, Sesame, Cinnamon and Caraway are purchased randomly from different places of many local markets in Iraq . The current study discloses the isolation of 17 fungal species belongs to 10 genera, out of which 5 species going to 2 genera of Zygomycota ,7 species going to 5 genera of Ascomycota and 5 species going to 3 genera of Deuteromycota , (Table 2).

Table 2: groups of fungi isolated from studying Spices samples

Phylum	Genes	Species
Ascomycota	5	7
Deuteromycota	3	5
Zycomycota	2	5

Our results were in well contract with those found by Bokhari (2007), Hashem and Alamri (2010). Bugno, (2006) expression that the major mycoflora obtained was distributed in 10 genera . Toma and Nareen (2013) found total of 10 fungal genera were isolated by agar plate method.Mohamed(2010) studied fifteen spices for their fungal contamination and isolated a total of 57 species. E- Kady *et al*(1992) isolated 81 species goes to 38 genera of fungi from 24 different of spices composed from Egypt .

From the data formulated in Table (3) detected that all samples of spices were infected with fungi .

Table 3. Fungi isolated from selected Spices samples

Number of sample	Samples name	Isolated fungi
1	Coriander	*Penicillium spp. , Alternaria alternate, Aspergillus niger , Aspergillus flavus , Rhizopus oryzae, Aspergillus aculeatus, Gliocladium sp.*
2	Black cumin	*Phoma sp., Fusarium culumorum, Alternaria solani , A. niger, A. aculeatus*
3	Fennel	*R. stolonifer, Fusarium solani, A. niger, Colletotrichum dematium, Ulocladium botrytis, A. flavus*
4	Bay leaf	*R. oryzae, Penicillium spp, A. niger, F. culumorum, A. flavus*
5	Sumac	*F.solani, R. oryzae, A. flavus, Gliocladium sp.*
6	Cardamom	*A. flavus, A. niger, A. alternate, R. oryzae*
7	Clove	*Penicillium spp., Phoma sp., R. nodosus, U. botrytis A. niger, , A. flavus*
8	Sesame	*A. niger , A. flavus, R. arrhizus , F. culumorum, R. nodosus*
9	Cinnamon	*C. dematium, Mucor hiemalis, Penicillium spp., A. niger*
10	Caraway	*A. niger , A. flavus, Penicillium spp., R. arrhizus , R. nodosus, Gliocladium sp.*

The results obtained on commonness, isolations and identification mycoflora of different spices showed fungi isolated 17 fungal species out of which species like; in this study we are (*Aspergillus niger , A.flavus , A. aculeatus, Alternaria alternate, A. solani , Rhizopus oryzae , R. stolonifer, R. nodosus, R. arrhizus , Fusarium culumorum, F. solani,Penicillium spp , Phoma sp, Gliocladium sp,Mucor hiemalis, Colletotrichum dematium, Ulocladium botrytis*).

Many developing countries have been annoying to intensification the quality of their seed creation. Inappropriately due to the lack of correct post-harvest protection techniques, large percentage of annual yield gets damaged by fungal action according to Dimic *et al.* (2008) twenty three dissimilar fungi were isolated from the test spices. It designates the ability of fungi in emerging association with extensive spectrum of seeds .The other workers partitioned mycoflora of different spices and informed the major fungal genera , like Elshafie *et al*(2002) isolated twenty fungal species out of which species like *Aspergillus flavus A. niger, Penicillium* Sp., and *Rhizopus* spp.were predominately isolated ; Abou Donia (2008)screened 303 samples representing different types of spices and conveyed genera like *Aspergillus*, *Fusarium*, *Cladosporium*, *Penicillium*, *Mucor* ,*Absidia* and *Rhizoctonia*. Sumanth *et al*. (2010), who isolated fungal genera from verified spices, originate that the most communal fungi isolated were *Aspergillus* spp. ,*Alternaria* sp., *Cladosporium*, *Curvularia*, *Fusarium* spp., *Helminthosporium* and *Trichoderma* .

The results in Table (4) presented The total incidence percentage of fungi isolated from spices samples .

Table 4: incidence percentage of isolated fungi from spices samples

Isolated fungi	incidence percentage									
	Number of spices samples									
	1	2	3	4	5	6	7	8	9	10
A. niger	51.2	13.4	25	15	-	5.3	11.5	16.1	14.6	44.7
A. flavus	31.2	-	12.3	6.4	15.3	17.7	2.5	9.4	-	4.2
Ulocladium botrytis	-	-	14.9	-	-	-	20.3	-	-	-
Rhizopus oryzae	13.1	-	-	10.4	11.2	12	-	-	-	-
Penicillium spp	21.3	-	-	7.4	-	-	11.3	-	4.2	15
Gliocladium sp	14.3	-	-	-	2.7	-	-	-	-	28.9
Alternaria alternata	19.6	-	-	-	-	9.5	-	-	-	-
Phoma sp	-	3.2	-	-	-	-	9.3	-	-	-
Fusarium solani	-	-	29 .5	-	8.2	-	-	-	-	-
Mucor hiemalis	-	-	-	-	-	-	-	-	3.8	-
A. aculeatus	22.3	9.1	-	-	-	-	-	-	-	-
Colletotrichum dematium	-	-	3.5	-	-	-	-	-	11.6	-
Rhizopus arrhizus	-	-	-	-	-	-	-	23.6	-	5.6
Fusarium culumorum	-	20.6	-	10.6	-	-	-	11.7	-	-
Rhizopus stolonifer	-	-	33.9	-	-	-	-	-	-	-
Rhizopus nodosus	-	-	-	-	-	-	11.7	25.6	-	14

The maximum percentage of infected samples was found in Coriander and Fennel While the lowest percentage was found in Cardamom. These results may be outstanding to different mechanisms in different spices and storage environment. Most fungi are current on spice of the post-harvest and storage nature, which advance after harvest if relative humidity is not measured during storage (Aziz, 1998). Fungi are the principal contaminants of

spices, but most such infectious populations are maybe regarded as commensally residents on the plant that endured drying and storage (Romagnoli, 2007).

The results presented in Table 5 show the individuality and the total Colony Forming Units (CFU) of fungi were found in all of the collected samples, The total number of isolated fungi from the all 50 selected samples was (190×103) cfu/g. samples. The minimum number of fungi was detected in Black cumin (8×103) followed by Cinnamon (11×103). In contrast, Fennel (34×103) and Coriander (22×103) presented the highest infections of fungi. A. niger and A. flavus were more frequently detected than other genera of fungi. A. niger was found in all examined spices and medicinal plant samples except Sumac while, A. flavus was found in all examined spices except Black cumin and Cinnamon. Phoma sp, found only in Black cumin and Clove, Ulocladium botrytis in Fennel and Clove, Mucor hiemalis racemocum in Cinnamon. Other species of fungi were isolated from different spices samples, were also frequently improved.

Our results were in well covenant with persons found by Bokhari (2007) were isolated fungi from spices samples during the study. *Aspergillus* was the most common genus in the different spices tested. *Aspergillus flavus* and *A. niger* were the most prevalent. Hashem and Alamri (2010) the most major fungal genera encountered were *Aspergillus, Penicillium* and *Rhizopus*. Samples obtained from sumac encountered very rare colony counts indicating its antifungal prosperities. Bugno *et al*. (2006) show that the genus *Aspergillus* was the most dominant genus recovered followed by *Penicillium*. The occurrence of a wide range of loading fungi indicates that considerable improvements could be made during post-harvest storage. The leading 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 cardamon, cinnamon, fennel, coriander, cumin, black cumin and white pepper, all of which are common in the food manufacturing. They found a high gradation of contamination in all samples.

Table 5. Frequency of isolated fungi from spices samples

Isolated fungi	Frequency of isolated fungi										CFU/gm.s.
	Number of spices samples										
	1	2	3	4	5	6	7	8	9	10	
A. niger	1	1	3	5	-	9	4	6	1	1	31
A. flavus	4	-	6	5	2	3	2	3	-	1	26
Ulocladium botrytis	-	-	7	-	-	-	2	-	-	-	9
Rhizopus oryzae	4	-	-	5	3	1	-	-	-	-	13
Penicillium spp	5	-	-	3	-	-	6	-	3	3	20
Gliocladium sp	1	-	-	-	4	-	-	-	-	6	11
Alternaria alternata	5	-	-	-	-	3	-	-	-	-	8
Phoma sp	-	1	-	-	-	-	1	-	-	-	2
Fusarium solani	-	-	4	-	9	-	-	-	-	-	13
Mucor hiemalis	-	-	-	-	-	-	-	-	5	-	5
A. aculeatus	2	3	-	-	-	-	-	-	-	-	5
Colletotrichum dematium	-	-	1	-	-	-	-	-	2	-	3
Rhizopus arrhizus	-	-	-	-	-	-	-	2	-	3	5
Fusarium culumorum	-	3	-	4	-	-	-	1	-	-	8
Rhizopus stolonifer	-	-	13	-	-	-	-	-	-	-	13
Rhizopus nodosus	-	-	-	-	-	-	3	5	-	10	18
total	22	8	34	22	18	16	18	17	11	19	103 ×190

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