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DETECTION OF FUNGI ASSOCIATED WITH SOME SPICES IN ORIGINAL FORM

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ABSTRACT

This study was conducted to detection fungi associated with some spice were selling in local market such as Cumin, Anise, Cinnamon, Sesame, Fennel, Black pill, Nutmeg, Coriander, Black pepper, Turmeric, Ginger, Galangal, Caraway, Cardamom. Results were recorded twenty four fungus belonging to fifteen genus. The most predominant fungal genera encountered were *Aspergillus spp, Penicillium spp, Alternaria spp,* and *Fusarium spp*. Yeasts were also frequently recovered, but not identified. All fungi were isolated on Agar plate method than Blotter paper. The highly number of isolated fungi was recorded in Coriander but the lowest observed in Cumin.

Keywords: *Spice, fungi, Plate Agar, Blotter paper, Libya.*

INTRODUCTION

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 (Ayres, 1980, Anonim, 2000, Bulduk, 2004). Spices occupy a prominent place in the traditional culinary practices and are indispensable part of daily diets of millions of people all over the world. Spices such as pepper, paprika, cumin, ginger, saffron and clove are extensively used in Libya to flavoring foods as well as for medication and are highly valuable due to their preservative and antioxidant properties. They are essentially flavoring agents used in small amounts and are reported to have both beneficial effect and antimicrobial properties, if properly stored (Atanda, 2006). However very little information is available on the mycoflora of spices worldwide (.Elshafie, 2002). Soil and air is the main inoculums source for causing contamination in crude spices in field (Kneifel and Berger, 1994). As with many other agricultural products, spices 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, 2007). Spices are very heavily contaminated at import (Yasair and Williams, 1942; Bokhari, 2002; Julseth and Deibel, 1974; Qaher, 2005) but few published data are available on the microbiology of spices at retail (El-Kady, 1992; Martins, 2001; Abdulkadir, 2003; Fazekas, 2005). The present study aimed to throw light on the investigation a detailed survey of mycoflora of fourteen selected spices from local market under environmental conditions of El-Beida region in Libya.

MATERIAL AND METHODS

Sample collection:

A total of fourteen dried samples, representing different types of spices were purchased from different places of local markets in the El-Beida city. These products of spices 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. For each spice 3 replicates were taken and mixed to prepare one composite sample. A total of 3 composite samples were prepared for each sample. Each sample (100 g) was put in a new paper bag and transferred immediately to the laboratory and stored in cool place at 4°C for fungal determination. The common names, scientific name and used parts of each sample are presented in Table (1).

Mycological studies:

In collection of samples, the Mycoflora of selected spices was isolated by two different methods Viz. Standard Blotter method and 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 ISTA (1966) and Neergaard (1973).

Isolation on Moist Blotting Paper:

Non-sterilized samples were evenly placed at the rate of (5-25) seeds or 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. Five replicates were made and the plates were incubated for 5-7 days at 25°C; after incubation the samples were examined under microscope and the fungi developing on samples were transferred to PDA for purification and identification.

Isolation on Agar Plate Method:

Each sample were surface sterilized by 1% sodium hypochlorite solution (NaOCl) 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 Potato Dextrose Agar (PDA), at the rate of (5-10) seeds/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. Fungi colonies were identified according to morphological and microscopic characteristics.

Identification of the fungal genera:

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). Also research articles and other related literature fungal Identification and illustrations were made up to the Genera and Species level.

Recorded of results:

After incubation and identification percentages of isolated fungi, seed/part infection (contamination) in each sample were calculated according to the formula

$$\text{percentage of isolated fungus: } \frac{\text{Count of detected fungal colonies appeared}}{\text{Count of colonies}} \times 100$$

$$\text{Percentage of seed/part infection: } \frac{\text{Number of infected seed/part}}{\text{Number of seed/part per samples}} \times 100$$

RESULTS AND DISCUSSION

RESULTS

Common and scientific names, plant part used of the spices were tabulated in Table 1. Totally 14 samples were analyzed for enumeration of fungal isolates and percentage of infection.

Table 1. Botanical name of spices and used plant parts

S. No	Name of spices	Botanical Name	Plant Part Used	% of fungal infection	Bacteria	Yeast
1	Galangal	Alpinia officinarum	Rhizomes	81.2		
2	Turmeric	Curcuma longa	Rhizomes	*40*		+
3	Coriander	Coriandrum sativum	Seed	*100*		
4	Fennel	Foeniculum vulgare	Seed	*80*		+
5	Anise	Pimpinella anisum	Seed	*2*		+
6	Cinnamon	Cinnamomum zeylanicum	Bark	*70*	+	
7	Ginger	Zingiben officinale	Rhizomes	*75*		
8	Black pill	Nigeria sativa	Seed	*50*		+
9	Nutmeg	Myristica fragrans	Fruit	*25*		
10	Caraway	Carum carui	Seed	*13.3*		+
11	Sesame	Sesamum indicum	Seed	*15*	+	
12	Cardamom	Elettaria cardamemum	Fruit	*65*		
13	Black pepper	Piper nigrum	Seed	*45*		+
14	Cumin	Cuminum cyminum	Seed	*100*		

From the data tabulated in Table (1) noticed that all samples of spices were infected with fungi. The highly heavily contaminated spice samples examined were observed in Coriander and Cumin in order of magnitude of 100% for both types, followed by Galangal 81.2% then Fennel 80% and Ginger 75%. While the lowest contaminated in Anise 2%. Bacteria and Yeasts were also frequently recovered, but not identified.

Fourteen imported raw spice samples obtained from retail outlets were examined for spoilage mould profile. A total of 24 species, belonging to 15 genera were recovered and identified from dried spice samples using PDA medium and Blotter paper methods. All fungi were identified on the basis of their cultural and morphological characteristics. These were identified as Acremonium sp, Alternaria alternata, A. radicina, A. solani, A. longissima, Aspergillus aculeatus, A. flavus, A. niger, A. fumigatus, A. terreus, Cheatomium sp, Cheatomium spinosum, Choanephora sp, Cephalosporium sp, Drechslera sp, Colletotrichum dematium, Curvularia sp, Fusarium culumorum, Fusarium solani, Penicillium spp, Phoma sp, Stemyphylium sp and Stachybotrys sp. all isolated fungi were grouped in different classes depending to taxonomy (Table 2).

Table 2. Different classes of fungi isolated from selected Spices

Fungal genera and species	Class	Fungal genera and species	Class
Acremonium sp	Deuteromycetes	Cephalosporium sp	Deuteromycetes
A. alternata	Deuteromycetes	Cladosporium sp	Deuteromycetes
A. radicina	Deuteromycetes	Drechslera sp	Deuteromycetes
A. solani	Deuteromycetes	Colletotrichum dematium	Deuteromycetes
A. longissima	Deuteromycetes	Curvularia sp	Deuteromycetes
A. flavus	Ascomycetes	Fusarium culumorum	Deuteromycetes
A. fumigates	Ascomycetes	Fusarium solani	Deuteromycetes
A. niger	Ascomycetes	Penicillium sp	Ascomycetes
A. terrus	Ascomycetes	Penicillium sp	Ascomycetes
Cheatomium sp	Ascomycetes	Phoma sp	Ascomycetes
Cheatomium spinosum	Ascomycetes	Stemyphylium sp	Deuteromycetes
Choanephora sp	Zygomycetes	Stachybotrys sp	Deuteromycetes

Isolation of Fungi with respect to the different isolation methods shows highest number of fungal species isolates belongs to different fungal classes viz. Deuteromycetes (14), Ascomycets (9), Zygomycetes fungi (1) species from both methods. A total of 7 genera and 7 species belonging to Class Deuteromycetes, 4 genera and 5 species belonging to Class Ascomycetes and one genus alone belonging to Class Zygomycetes.

The results in Table (3) showed that, in general the agar plate method generally is more efficient to isolate the different fungi associated with spice samples than blotter test. A total of 20 different fungal species were isolated on agar plate compared with 10 species were isolated on blotter paper. Some of isolated fungi were appeared only on blotter paper such as Acremonium sp, A. flavus, A. niger and Cheatomium spinosum, and some appeared on agar plate only such as Alternaria spp, Aspergillus spp, Phoma sp , Stachbotrys, Stemyphylium sp, Fusarium solani, etc., while others fungi were appeared on both methods. The total percentage of fungi isolated by agar plat technique was higher in number than blotter technique.

Table 3. Percentage of isolated fungi from spices samples by Blotter method and Agar plate method

Fungal genera and species	% isolated fungi	
	Blotter paper	Agar plate
Acremonium sp	*2.20*	*0*
A. alternata	*22.2*	*11.1*
A. radicina	*0*	*5.55*
A. solani	*0*	*5.55*
A. longissima	*0*	*1.8*
A. flavus	*1.50*	*0*
A. fumigates	*0*	*5.55*
A. niger	*77.7*	*0*
A. terrus	*0*	*11.1*
Cheatomium sp	*16.6*	*5.55*
Cheatomium spinosum	*11.1*	*0*
Choanephora sp	*0*	*0*
Cephalosporium sp	*16.6*	*5.55*
Cladosporium sp	*5.55*	*5.55*
Drechslera sp	*0*	*11.1*
Colletotrichum dematium	*0*	*11.1*
Curvularia sp	*0*	*1.8*
Fusarium culumorum	*5.55*	*5.55*
Fusarium solani	*0*	*11.1*
Penicillium sp	*11.1*	*38.8*
Penicillium sp	*33.3*	*61.1*
Phoma sp	*0*	*5.55*
Stemyphylium sp	*0*	*5.55*
Stachybotrys sp	*0*	*11.1*

Table (4) showed that the frequency of fungi in samples of spices. The most predominant fungal genera encountered were Aspergillus and Pencillium in all samples. Acremonium sp, A. radicina, Drechslera sp and Stachybotrys sp were isolated from Fennel samples alone, A. longissima from Black pepper alone, Cladosporium sp from Coriander and Stemyphylium sp from Anise alone. The maximum number of fungal diversity was detected in Coriander (7 genera) followed by Fennel (6 genera). Whereas the minimum number of fungal diversity was found in Sesame (Penicillium sp. alone).

Penicillium sp had a highly frequency (100%) in Sesame and the lowest frequency (10%) was recorded in Fennel by Acremonium sp, A. radicina, Drechslera sp and Stachybotrys sp.

Table 4. Frequency of isolated fungi from different spices

Fungal genera and species	Spices													
	Ga	Tu	Co	Fe	An	Ci	Gi	Bpi	Nu	Ca	Se	Car	Bp	Cu
Acremonium sp				10										
A. alternata			12.5											25
A. radicina				10										
A. solani			12.5					16.6						25
A. longissima							16.6						50	
A. flavus						16.6			28.5	20				
A. fumigates										20				
A. niger	55.5	50.0	12.5	30	40	16.6	50		28.5			21		
A. terrus	22.2	33.3												
Cheatomium sp	11.1					16.6								
Cheatomium spinosum			25.0							20				
Choanephora sp							33.3							
Cephalosporium sp						16.6								
Cladosporium sp			12.5											
Drechslera sp				10										
Colletotrichum dematium	+		12.5											
Curvularia sp			12.5											
Fusarium culumorum										40				
Fusarium solani						16.6								25
Penicillium sp	11.1			30	40	16.6		33.0	28.5			42.2	50	
Penicillium sp		16.6						33.0	14.2		100	36.8		25
Phoma sp								16.6						
Stemyphylium sp					20									
Stachybotrys sp				10										

Ga: Galangal, Tu: Turmeric, Co: Coriander, Fe: Fennel, An: Anise, Ci: Cinnamon, Gi: Ginger, Bpi: Black pill, Nu: Nutmeg, Ca: Caraway, Se: Sesame, Car: Cardamom, Bp: Black pepper, Cu: Cumin.

DISCUSSION

Plant spices have been traditionally used since ancient time for the preservation of food product as they have been reported to have antiseptic and disinfectant properties. Plant diseases are serious problem which decrease production and result in great losses specially those caused by seed borne fungi and transmission the pathogen into human food which effect of health. Several studies in other countries reported that the spices are very heavily contaminated at import (Yasair and Williams, 1942; Bokhari, 2002; Julseth and Deibel, 1974; Qaher, 2005; Ramesh and Jayagoudar, 2013; Ramesh, 2013).

The results obtained on prevalence, isolations and identification of seed mycoflora of different spices conducted on storage fungi parasitizing Cumin, Anise, Cinnamon, Sesame, Fennel, Black pill, Nutmeg, Coriander, Black pepper, Turmeric, Ginger, Galangal, Caraway, Cardamom are presented for El-Beida region. These seeds were brought into the laboratory and analyzed. The highest percentage of infected samples was found in Coriander and Cumin While the lowest percentage was found in Anise. These results may be due to different components in different spices and storage environment. Most fungi are present on spice of the post-harvest and storage type, which develop after harvest if relative humidity is not controlled during storage (Aziz, 1998). 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.

As well as fungi the results indicated to occurrence bacteria and yeast in some of spice samples. Spices are commonly heavily contaminated with xerophilic storage moulds, bacteria (Dimic, 2000; Romagnoli, 2007) and yeast (Qaher, 2005).

Regarding seed health testing, the agar plate test was more efficient to isolate the different fungi associated with spice samples than the blotter paper test. the agar method is more supporting for isolation fungi due to the food abundance. This finding confirm those found by ….. who reported that all the methods yielded more or less the same set of fungi but the greatest percentage of mycoflora was detected with agar plate method.

The results presented in Table 3 show the frequency of fungi were found in all of the collected samples, they were blotter paper and plated on PDA medium. Aspergillus spp. and Penicillium genera were more frequently detected than other genera of fungi. Aspergillus niger. was found in all examined spices samples except Black pill, Caraway, sesame, Black pepper and Cumin while, Penicillium spp. were dominant in all samples except Coriander, Ginger, and Caraway. Other species of moulds were isolated from different spices, such as, Alternaria alternata, Cladosporium sp, Fusarium spp, Stemyphylium sp, and others fungi. Yeasts were also frequently recovered, but not identified. Our results were in well agreement with those found by Onesirosan, (1982), Srivastava and Chandra (1985), Jeyanandarajah, (1991), Bokhari (2007), Hashem and Alamri (2010). Bugno, (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. Takatori, (1977) and Ayres, (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 industry. They found a high degree of contamination in all samples. In general, the most of isolated fungi are able to produce toxins which reported hazard on human health.

In conclusion, the results indicated that many samples of spices, although pure in the sense that they are not grossly adulterated with foreign matter, are far from pure microbiology. Occurrence of mycotoxins fungi such as Aspergillus spp, Fusarium spp and Penicillium spp led to production of toxins in foods, feeds and other materials. Although toxins present as a natural contaminant of spices is of minor concentrations, the health risk is increased because sume of spices such as Anise and Cumin are used as carminative, as expectorant, treatment colic and flatulence for children..

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