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FUNGAL PROFILE OF SOME SELECTED EGYPTIAN MEDICINAL PLANTS AND SPICES AND ESTIMATION OF MYCOTOXIN PRODUCTION

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ABSTRACT

Hundred and fifty samples of five medicinal plants and spices (Basil, Chamomile,Dry mint,Marjoram and Fennel) were screened for the isolation of mold and yeast flora. The prevalence of molds ranged from 10.04% in Fennel to 33.95% in Dry mint. *Aspergillus niger* was the most prevalent mold. It was represented by 428 isolates (32.08%) in Basil, 190 isolates (28.83%) in Chamomil, 729 isolates (34.45%) in Dry mint, 546 isolates (36.45%)in Marjoram and 45 isolates (7.19%) in Fennel. The prevalence of yeasts ranged from 0.0% in Basil to 38.9% in Chamomile. *Saccharomyces cerevisiae* was the most prevalent yeast. The anti fungal activity of ethanolic extracts of Cinnamon, Curcuma and Ginger were estimated against the derived molds and yeasts isolated from the selected spices and medicinal plants(60 isolates of molds and 18 isolates of yeast). All mold isolates were resistant to the Curcuma and Ginger extracts. In case of Cinnamon extract, only nine mold isolates were intermediate, while eighteen mold isolates were sensitive. All yeast isolates were resistant to the ethanolic extract of each of Curcuma and Cinnamon, while the majority of yeast isolats were sensitive to Ginger extract. Seven mold isolates out of sixty produced mycotoxins(11.7%). *Aspergillus flavus* from Marjoram produced aflatoxin B1(160µg/ml) and aflatoxin B2(50µg/ml), while *A.flavus* from Basil produced aflatoxin G1(25µg/ml). Each of *A.glaucus*, *A.versicolor* from Dry mint and *A.nidulans* from Basil produced zeralenone.

Key words*: antifungal activity, fungal flora, medicinal plants, mycotoxins.*

1. INTRODUCTION

Medicinal plants and herbs were known to modern and ancient civilization, particularly ancient Egyptians, for their healing properties(El-Sawi, 2000).Medicinal plants are of increasing importance in Egypt, they were used as human remedies and raw materials for pharmaceutical and cosmetic industries. The source of contamination of medicinal plants referred to the exposure of public as consumers during cultivation and the unhygienic ways of storage. Bacterial or fungal flora play an important role in the contamination of medicinal plants. These microbial flora were responsible for lowering yield of medicinal plants(El Tahan & Fahmy, 2005). Fungi are the predominant contaminants of spices (Kneifel & Berger, 1994), but most of such microbial populations were probably regarded as commensal residents on the plants that survived drying and storage. This referring to the uncontrolling of humidity during storage (Aziz *et al.,* 1998). Strains and species of *Aspergillus flavus* group were widely distributed in many different therapeutic herbal medicines. *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp. and *Mucor* sp. are regular elements of spice fungal flora, while yeast isolates were absent (Wojcik and Jakubowska, 2005). Walker, (2002) stated that prevention of contamination at source is considered to be the most effective public health measure. Secondary metabolites of certain ubiquitous moulds growing on dried herbs may lead to toxic effects(El Tahan and Taha,1996). The chlorinated isocoumarin compound, ochratoxin A (OTA), together with some related derivatives (ochratoxins B, C, alpha, beta) were produced by *Penicillium verrucosum* and by several species of *Aspergillus*, most notably *Aspergillus ochraceus*. *Penicillium verrucosum* was the principal source of OTA contamination of

stored foods in temperate climates, while *Aspergillus* sp. predominated in warmer countries(Walker, 2002). The contamination of raw materials of spices and medicinal plants by microorganisms is one of the major reasons for decline any country's share in the global spices and medicinal plants market. Also,the risk assessment of the microbial load of medicinal plants has become an important subject in the establishment of modern Hazard Analysis and Critical Control Point (HACCP) schemes(Kneifel *et al.,* 2002). Accordingly, the aim of this study was to determine the fungal profile(mold,yeast) in 150 samples of the selected five spices and medicinal plants(Basil, Marjoram, Drymint, Fennel, Chamomile) and to evaluate the capability of sixty molds to produce mycotoxins. Also, this study was concerned with the estimation of the antifungal activity of ethanolic extract of some spices and medicinal plants(curcuma, cinnamon and ginger)against fungal flora (mold,yeast) isolated from the selected medicinal plants.

2. MATERIALS AND METHODS

2.1. Spices and medicinal plant samples

Thirty samples of each of the selected five spices and medicinal plants (Basil, Chamomile, Dry mint, Marjoram and Fennel) were brought to the Central Laboratory for food and feed [Agriculture Research Center] for making chemical and microbial analysis before they were exported to European countries. These samples (150 samples) were screened for the isolation of fungal flora(molds & yeasts).

2.2. Preparation of medicinal plant samples

Ten grams of each of the spices and medicinal plant samples were mixed with 90 ml diluent (maximal recovery diluent) in the mixer (Stomacher 400). The mixing process was extended for 10 min . Ten fold serial dilutions were prepared. Six levels spacing one logarithmic unit were investigated by pipetting one ml from each level into duplicate sterile petri dishes containing the specific medium.

2.3. Isolation of molds and yeasts (ISO 21527 2001)

Each sample of the medicinal plants (1ml) was mixed carefully with 15 ml of Dichlorane 18% Glycerol agar medium (DG18 ISO /CD 21527(2001) and allowed to solidify. The plates were incubated at 30 $\pm$ 2 ^{O}C for 48 hr for the isolation of yeast and for 3- 4 days for the isolation of molds. After three days of incubation, the colonies in each plate were counted. The plates containing fewer than 150 colonies were discarded. A control plate with 15 ml medium was prepared to check the sterility. The identification of the fungal isolates was carried out on the basis of growth and microscopic morphology using the following universal manuals (Samson, 1974; John, 1979 and Kendrick, 2000), while the yeast isolates were identified using the routine laboratory methods recommended by (Wickerham, 1951; Lodder, 1971; Ahearn, 1978 and Barnett *et al.,* 2000).

2.4. Statistical analyses

All data were subjected to statistical analysis of variance (F-test) "one way ANOV". It was noted that if the means of the subgroups are greatly different, the variance of the combined groups is much larger than the variance of the separate groups. The analysis of variance format for the differences in means is based on this fact.Duncan's multiple range test is one of the multiple- comparison procedures. It uses the "t" distribution corresponding to the number of degrees of freedom for error mean square.The significance of the measured data was considered as follows :

not significant (N.S) when $P > 0.05$
significant (S) when $P < 0.05$
highly significant(H.S) when $P < 0.01$

where P is the probability (reflect of null hypothesis). Details of the formulae used are given by (Armitage, 1971).

2.5. Ethanolic extraction of the medicinal plants (cinnamon, curcuma and ginger) and estimation of its antifungal activity(Velickvic *et al*., 2002)

Fifty grams of the medicinal plants were added to 500 ml of ethanol (70%) and left for 2 days. The extract was filtered using Buchner filter. The filtrate was added into Rota vapor to evaporate alcohol and then left in the incubator at 44.5 ºC to evaporate the remaining water. The antifungal activity was estimated using the hole plate diffusion technique (Clark *et al*., 1981). Muller Hinton agar medium (g/L);Beef infusion(300), Casein hydrolysate(17.5), starch(1.5), Agar(17.0), pH/7.3± 0.2 was prepared and allowed to solidify in plates. From the fungal flora of the selected five spices and medicinal plants, the suspensions of eighteen Yeast isolates and sixty mold isolates were prepared and matched with a McFarland 0.5. The suspensions were swabbed on the surface of Hinton agar medium using sterile cotton swab. Each of the ethanolic extract of the medicinal plants (Cinnamon,Curcuma and Ginger) was added to each hole plate and allowed to diffuse at room

temperature for 20 minutes. The plates were incubated aerobically overnight at 30˚C. The antifungal activity was recorded as the diameter of the resulting inhibition zones measured in millimeters. According to Bauer *et al.,* (1966), the microbicidal activity was classified into resistant if the diameter of inhibition zone is less than 8mm. Intermediate (9-11mm) and sensitive (≥12 mm).

2.6. Mycotoxins production

Sixty mould isolates from the selected five medicinal plants (Majoram, Dry mint, Chamomile, Basil and Fennel) were evaluated for the production of Aflatoxines, Zerianone and Ochratoxines.The inoculum was prepared by inoculating potato dextrose agar medium (PDA) with the selected mould isolates and incubated for 7 to 21 days at 28ºC. Flasks of liquid yeast media(g/l); yeast extract(20),sucrose(200), magnesium sulphate(0.2),calcium carbonate (0.2),pH 7. were inoculated with the selected mould isolates and incubated at 15ºC in the first day, at 21ºC in the 2nd day and at 28ºC in the 3rd day till the end of incubation period (from 15 days to 3weeks). The flasks were steamed by autoclaving at 121˚ C for 15 min to destroy the fungus and then filtered to separate the spores from the liquid media (El Tahan and Taha, 1996).

2.7. Qualitative assay of mycotoxins

Ten ml of the filterate were shaked in separating funnel with 250 ml chloroform for 30 min. The chloroform layer was filtered using filter paper (Whatman no. 1), anhydrous sodium sulfate (25g) was added to remove water. The filterate was clarified using buchner funnel and concentrated under vacuum using rotary evaporator. The residue was made up to 10ml with chloroform for chromatography on thin-layer plates. 1,2,3,4,5 µl of the standers of aflatoxin (B1,B2, G1, G2 and zeralenone) were spotted on thin layer plates and developed on ether-methanol-water(96-3-1). The spots were examined under U.V. lamb at wave length of 365 nm(El Tahan and Taha,1996). The quantitative performance of aflatoxin B1, B2 and G1 was carried out in the central laboratory for food and feed [Agriculture Research Center] using TLC Denistometer schmatzo 9000.

3. RESULTS AND DISCUSSION

3.1. Prevalence and frequency of the isolated molds and yeasts from themedicinal plants

The results in Table (1) show the prevalence of molds in different herbs, which ranged from 10.04% in Fennel to 33.95% in Dry mint , while the highest prevalence of yeasts (38.9%) was represented in Chamomile (Table, 3). These results coincide with Martins *et al.,* (2001)who revealed that many yeasts were found in Chamomile and Dry mint. From the total samples (150), twelve fungal isolates were detected; seven species of *Aspergillus* [*A. glaucus* (13.48%), *A.versicolor* (3.05%), *A.nidulaus* (7.78%),*A.niger* (31.09%), *A.flavus* (3.29%), *A.terreus* (1.97%) and *A.flavipes* (2.04%)], *Alternaria alternata (7.24%), Acremonium strictum*(23.34%), *Rhizopus orrhizus* (2.15%), *Fusarium solni*(4.09%) and *Absidia corymbifeza* (0.48%) (Table,1). *Aspergillus niger* was the most prevalent mold. It was represented by four hundred and twenty eight isolates (32.08%) in Basil, 190 isolates (28.83%) in Chamomile, 729 isolates (34.45%) in Dry mint, 546 isolates (36.45%) in Marjoram and 45 isolates (7.19%) in Fennel. Mandeel (2005) found that *Penicillium* sp was the highly prevalent mold in Chamomile, Dry mint samples. Hashem and Alamri, (2010) found that the ginger samples were the most heavily contaminated spice samples and *Aspergillus*, *Penicillium*, and *Rhizopus* were the most predominant fungal isolates. Eighteen yeast isolates were isolated from the collected 30 samples of each of the selected five spices and medicinal plants (Marjoram, Dry mint, Chamomile, Basil and Fennel). Seven yeast species were detected ; *Saccharomyces rosinii* (1.3%), *Candida rugosa* (2.0%), *Debaromyces hansenii* (2.0%), *Lipomyces libofore* (1.3%), *Trichosporon jirovecii* (0.7%), *Saccharomyces cerevisiae* (2.7%) *and Candida glabrata* (2.0%) (Table 3) . *Saccharomyces cerevisiae* was the most prevalent yeast. It was represented by one isolate (3.3%) in Chamomile, three isolates in Fennel (10.0%). In the Basil samples, 1000, 300 and 34 mold isolates were isolated.Ten mold species were detected; *Aspergillus glaucus* (22.86%), *A.versicolor* (4.27%), *A.nidulaus* (15.67%), *A. niger* (32.08%), *A. flavus* (4.05%), *A. terreus* (16.34%), *Alternaria alternata* (0.60%), *Acremonium strictum* (1.65%), *Rhizopus orrhizus* (1.35%) and *Fusarium solani* (1.12%) (Table, 1). No yeast isolates were detected in Basil samples (Table, 3). The highest number of mould isolates was represented in Dry mint. Eleven mold species were identified as; *Aspergillus glaucus(*7.75%), *Aspergillus versicolor (*4.82%), *Aspergillus nidulaus (*1.89%) , *Aspergillus niger(*34.45%), *Aspergillus flavus(*3.64%), *Aspergillus terreus (*4.82%) *Aspergillus flavipes(*5.43%), *Alternaria alternate(*7.94%), *Acremonium strictum(*20.42%), *Rhizopus orrhizus(*1.04%) and *Fusarium solani*

(7.80%)(Table, 1). One yeast isolate was identified as *Candida rugosa* (3.3%) (Table, 3). Martins *et al.,* (2001) and Abou-Donia, (2008) reported that, *Fusarium* sp., *Penicillium* sp., *Aspergillus flavus* and *A. niger* were the predominant fungal isolates in almost all medicinal plant samples and many yeasts were found in Chamomile and Dry mint. In Marjoram samples, 1000, 498 mold isolates were isolated ; Twelve mold species were detected; *Aspergillus glaucus*(19.69%), *A. versicolor*(1.27%), *A. nidulaus(*7.74%), *A. niger*(36.45%), *A.flavus(*2.40%), *A.terreus*(0.20%) *A. flavipes* (0.53%), *Alternaria alternate*(3.14%), *Acremonium strictum* (22.83%), *Rhizopus orrhizus*(0.87%), *Fusarium solani* (2.94%) and *Absidia corymbifeza* (1.94%) (Table, 1). Three yeast isolates were identified as *Saccharomyces rosinii* (3.3%) , *Lipomyces libofore* (3.3%) , and *Candida glabrata* (10.0%) (Table 3). In Chamomile,659 mold isolates were isolated. Twelve mold species were identified as; *Aspergillus glaucus*(7.28%), *A.versicolor*(1.21%), *A.nidulaus*(15.78%), *A.niger*(28.83%), *A.flavus*(1.67%), *A.terreus*(1.37%) and *A.flavipes(*0.30%), *Alternaria alternate* (13.05%), *Acremonium strictum* (23.98%), *Rhizopus orrhizus*(1.67%), *Fusarium solni*(4.70%) and *Absidia corymbifeza* (0.15%)(Table, 1). The highest yeast count was detected in Chamomile; five yeast species were identified (23.3%); *Candida rugosa(*6.6%), *Debaromyces hansenii(*6.6%), *Lipomyces libofore(*3.3%), *Trichosporon jirovecii(*3.3%) *and Saccharomyces cerevisiae(*3.3%) (Table 3). In Fennel **,** the lowest total fungal count was detected, ten mold species were identified; *A.glaucus* (*4.47%) , A.versicolor* (*0.65%), A.nidulaus* (*2.56%) A.niger* (*7.19%), *A.flavus* (*4.31%), A.terreus* (*0.16%), *A.flavipes* (*0.32%), Alternaria alternate* (*20.45%), *Acremonium strictum* (*48.72%) and Rhizopus orrhizus* (*11.18%*) (Table 1). Three yeast species were identified as *Saccharomyces rosinii(*3.3%), *Debaromyces hansenii(*3.3%)and *Saccharomyces cerevisiae(*10.0%) (Table 3). The high levels of microbial contamination in spices and herbs were reported by many studies and suggested a need for better control in all aspects of the production, processing and usage of these products to prevent potential food spoilage and food-borne illnesses(McKee, 1995). Quality control of the exported and imported food products is a vital process. Herb-exporting countries will receive more revenues following the widening market for herbal products in developed countries(Dubey *et al*., 2008). The high prevalence of fungal contamination in herbs and spices was documented and referred to the unscientific methods of collection, storage, transportations and congenial climatic condition(Dubey *et al*., 2008).

The results in Table (2) show the significant difference which was represented by the difference in letters (Duncan grouping). A highly significant p values were obtained with *Aspergillus glaucus*, *A.versicolor,A.flavus* (.001,.003 and .001, respectively). According to Duncan test ,in each row any two means taking the same letter there is no significant difference between them).

3.2.Estimation of antifungal activity of the ethanolic extracts of three medicinal plants (curcuma, cinnamon and ginger)

In this study,the ethanolic extracts of three medicinal plants (Curcuma, Cinnamon and Ginger) were used to inhibit the growth of the fungal contaminants in the selected five spices and medicinal plants. All mould isolates were resistant to the Curcuma and Ginger extracts(the inhibition zones ranged from 1-3mm)(Table 4). In the case of Cinnamon extract, nine mold isolates were intermediate (the inhibition zones ranged from 9-11mm), eighteen mold isolates were sensitive (the inhibition ranged from12-22 mm) and other mold isolates were resistant to Cinnamon extract (Table, 3). Simić *et al*. (2004) reported that the *trans*-cinnamaldehyde is the essential oil of *Cinnamomum zeylanicum* ,which showed the strongest antifungal activity. While the GC and GC-MS chemical analysis of volatile oils of *Curcuma longa*, *C. zedoaria*, *C. aromatica* and *C. amada*, revealed the presence of ar-turmerone, ar-turmerol, myrcene, β - pinene, , cineol, cymene, α-phellandrene. These compounds are used as antifungal materials (Singh *et al.,* 2002). All yeast isolates were resistant to the ethanolic extract of each of Curcuma and Cinnamon (the inhibition zone ranged from one to six mm). In the case of ethanolic extract of Ginger, only two yeast isolates were intermediate (*Lipomyces lipofore* and *Debaromyces hansenii*) with inhibition zone of 10mm. Other yeast isolates were sensitive with inhibition zones ranged from 12mm to 30mm (Table, 4). These results matched with Lopez *et al.* (2005)who tested the antimicrobial activity of essential oils of cinnamon and ginger against *Candida albicans* and two moulds(*Penicillium islandicum* and *Aspergillus flavus*)and found that Cinnamon gave the strongest inhibition in the case of molds, while ginger gave the strongest inhibition in the case of yeasts. It is clear that

Table (1): Frequency and percentage of the isolated molds from the collected thirty samples of each of the selected five spices and medicinal plants (basil, chamomile, dry mint, marjoram and fennel).

Type of commodity / Mold isolates	Basil (30 samples)		Chamomile (30 samples)		Dry mint (30 samples)		Marjoram (30 samples)		Fennel (30 samples)		Total (150 samples)	
	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%
Aspergillus glaucus	305	22.86	48	7.28	164	7.75	295	19.69	28	4.47	840	13.48
Aspergillus versicolor	57	4.27	8	1.21	102	4.82	19	1.27	4	0.64	190	3.05
Aspergillus nidulaus	209	15.67	104	15.78	40	1.89	116	7.74	16	2.56	485	7.78
Aspergillus niger	428	32.08	190	28.83	729	34.45	546	36.45	45	7.19	1938	31.09
Aspergillus flavus	54	4.05	11	1.67	77	3.64	36	2.40	27	4.31	205	3.29
Aspergillus terreus	8	0.60	9	1.37	102	4.82	3	0.20	1	0.16	123	1.97
Aspergillus flavipes	0	0. 00	2	0.30	115	5.43	8	0.53	2	0.32	127	2.04
Alternaria alternate	22	1.65	86	13.05	168	7.94	47	3.14	128	20.45	451	7.24
Acremonium strictum	218	16.34	158	23.98	432	20.42	342	22.83	305	48.72	1455	23.34
Rhizopus orrhizus	18	1.35	11	1.67	22	1.04	13	0.87	70	11.18	134	2.15
Fusarium solani	15	1.12	31	4.70	165	7.80	44	2.94	0	0.00	255	4.09
Absidia corymbifeza	0	0.00	1	0.15	0	0.00	29	1.94	0	0.00	30	0.48
Total	1334	21.40	659	10.57	2116	33.95	1498	24.03	626	10.04	6233	

Table (2): Mean value of isolated molds from the collected thirty samples of each of the selected five spices and medicinal plants (basil, chamomile, dry mint, marjoram and fennel).

Type of commodity / Mold isolates	Basil	Chamomile	Dry mint	Marjoram	Fennel	F-test	
						F-value	P-value
1-Aspergillus glaucus	**101.66 a**	**16.00 c**	**59.33 b**	**98.33 a b**	**9.33 c**	**12.071**	**0.001**
2-Aspergillus versicolor	**19.00 b**	**2.66 c**	**35.33 a**	**6.33 bc**	**1.33 c**	**8.850**	**0.003**
3-Aspergillus nidulaus	**69.66 a**	**34.66b**	**13.33 c**	**38.66 b**	**5.33 c**	**35.304**	**0.000**
4-Aspergillus niger	**142.66 ab**	**63.33c**	**243.00 a**	**182.00 bc**	**15.00 bc**	**21.322**	**0.000**
5-Aspergillus flavus	**18.00 ab**	**3.66c**	**25.66 a**	**12.00 bc**	**9.00 bc**	**6.544**	**0.001**
6-Aspergillus terreus	**2.66b**	**3.00b**	**34.00 a**	**1.00 b**	**0.330 b**	**11.026**	**0.000**
7-Aspergillus flavipes	**0.00 b**	**0.067b**	**38.33a**	**2.66 b**	**0.660 b**	**17.316**	**0.000**
8-Alternaria alternate	**7.33 c**	**28.66 b**	**56.00 a**	**15.66 bc**	**42.66 a**	**21.684**	**0.000**
9-Acremonium strictum	**72.66c**	**52.66c**	**144.00a**	**114.00 b**	**101.66 b**	**22.533**	**0.000**
10-Rhizopus orrhizus	**6.00b**	**3.66b**	**7.33 b**	**4.33 b**	**23.33 a**	**24.262**	**0.000**
11-Fusarium solani	**4.00bc**	**10.33bc**	**55.00 a**	**14.66 b**	**0.000 c**	**30.411**	**0.000**
12-Absidia corymbifeza	**0.000 b**	**0.30 b**	**0.000b**	**9.66 a**	**0.000 b**	**59.107**	**0.000**

Where P-value is the probability (reflect of null hypothesis) P-value < 0.01 highly significant between plants

Table (3): Frequency and percentage of the isolated yeasts from the collected thirty samples of each of the selected five spices and medicinal plants.

Type of commodity / Yeast isolate	Basil (30 samples)		Chamomile (30 samples)		Dry mint (30 samples)		Marjoram (30 samples)		Fennel (30 samples)		Total (150 samples)	
	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%
Saccharomyces rosinii	0	0.0	0	0.0	0	0.0	1	3.3	1	3.3	2	1.3
Candida rugosa	0	0.0	2	6.6	1	3.3	0	0.0	0	0.0	3	2.0
Debaromyces hansenii	0	0.0	2	6.6	0	0.0	0	0.0	1	3.3	3	2.0
Lipomyces libofore	0	0.0	1	3.3	0	0.0	1	3.3	0	0.0	2	1.3
Trichosporon jirovecii	0	0.0	1	3.3	0	0.0	0	0.0	0	0.0	1	0.7
Saccharomyces cerevisiae	0	0.0	1	3.3	0	0.0	0	0.0	3	10.0	4	2.7
Candida glabrata	0	0.0	0	0.0	0	0.0	3	10.0	0	0.0	3	2.0
Total	0	0.0 0.0	7	23.3 38.9	1	3.3 5.6	5	16.7 27.8	5	16.7 27.8	18	12

Table (4): Estimation of antifungal activity of ethanolic extracts of three medicinal plants (curcuma, cinnamon and ginger)against the isolated molds from the selected five spices and medicinal plants (basil, chamomile, dry mint, marjoram and fennel).

No.	Sample code	Fungal isolate	Type of commodity	Inhibition zone (mm)		
				Curcuma	Cinnamon	Ginger
1	1.1A	*Aspergillus glaucus*	Basil	10	6	2
2	4.20E	*Aspergillus flavus*	Marjoram	0	1	0
3	1.18A	*Aspergillus nidulaus*	Basil	0	12	0
4	1.3A	*Aspergillus nidulaus*	Basil	0	6	1
5	1.7E	*Aspergillus flavus*	Basil	0	3	1
6	2.5A	*Aspergillus glaucus*	Dry mint	0	9	1
7	1.5A	*Aspergillus versicolor*	Basil	0	12	0
8	2.23A	*Alternaria alternata*	Dry mint	3	8	1
9	2.50A	*Alternaria alternata*	Dry mint	2	17	1
10	1.24E	*Aspergillus flavus*	Basil	0	4	1
11	4.8D	*Aspergillus niger*	Marjoram	2	0	1
12	1.10A	*Aspergillus nidulaus*	Basil	2	5	1
13	1.3A	*Aspergillus versicolor*	Basil	0	6	0
14	4.10A	*Aspergillus versicolor*	Marjoram	2	17	1
15	2.33A	*Aspergillus glaucus*	Dry mint	0	22	0
16	2.37A	*Alternaria alternata*	Dry mint	0	10	1
17	2.36A	*Aspergillus versicolor*	Dry mint	2	15	1
18	2.38A	*Alternaria alternata*	Dry mint	0	19	0
19	1.16A	*Alternaria alternata*	Basil	0	10	3
20	2.14B	*Acremonium strictum*	Dry mint	5	4	0
21	6.2B	*Acremoniumstrictum*	Fennel	0	2	0
22	2.18G	*Fusarium solani*	Dry mint	0	3	0
23	2.8B	*Acremoniumstrictum*	Dry mint	2	8	0
24	5.8G	*Fusarium solani*	Chamomile	0	10	1
25	2.16G	*Fusarium solani*	Dry mint	0	9	1
26	4.4F	*Rhizopus orrhizus*	Marjoram	4	2	3
27	1.11B	*Acremonium strictum*	Basil	0	6	2
28	5.9F	*Rhizopus orrhizus*	Chamomile	2	0	0
29	2.2G	*Fusarium solani*	Dry mint	0	6	0
30	2.5G	*Fusarium solani*	Dry mint	0	12	0
31	2.42A	*Alternaria alternata*	Dry mint	0	14	2
32	5.29A	*Alternaria alternata*	Chamomile	0	2	2
33	2.15F	*Rhizopus orrhizus*	Dry mint	2	0	2
34	1.4B	*Acremonium strictum*	Basil	0	5	0
35	2.46A	*Alternaria alternata*	Dry mint	2	9	2
36	2.36A	*Alternaria alternata*	Dry mint	3	11	1

Cont. Table (4):

No.	Sample code	Fungal isolate	Type of commodity	Inhibition zone (mm)		
				Curcuma	Cinnamon	Ginger
37	5.16A	*Alternaria alternata*	Chamomile	0	15	2
38	2.9B	*Acremonium strictum*	Dry mint	0	6	3
39	2.44	*Absidia corymbifeza*	Dry mint	2	16	1
40	5.27E	*Aspergillus flavus*	Chamomile	0	13	1
41	6.26B	*Acremonium strictum*	Fennel	0	5	4
42	1.2G	*Fusarium solani*	Basil	0	3	0
43	1.2B	*Acremonium strictum*	Basil	0	16	4
44	2.17D	*Aspergillus niger*	Dry mint	0	0	1
45	2.50D	*Aspergillus niger*	Dry mint	0	0	0
46	1.2F	*Rhizopus orrhizus*	Basil	0	1	2
47	6.17A	*Alternaria alternata*	Fennel	0	13	3
48	6.18B	*Acremonium strictum*	Fennel	0	12	0
49	2.25I	*Aspergillus flavipes*	Dry mint	0	2	0
50	4.25B	*Acremonium strictum*	Marjoram	0	11	0
51	2.33E	*Aspergillus terreus*	Dry mint	0	9	0
52	4.19A	*Aspergillus glaucus*	Marjoram	2	0	2
53	4.27	*Absidia corymbifeza*	Marjoram	0	7	6
54	2.4F	*Rhizopus orrhizus*	Dry mint	5	2	2
55	1.14F	*Rhizopus orrhizus*	Basil	0	0	2
56	5.27B	*Acremonium strictum*	Chamomile	0	13	1
57	2.26B	*Acremonium strictum*	Dry mint	0	15	0
58	2.42F	*Rhizopus orrhizus*	Dry mint	0	2	1
59	2.7D	*Aspergillus niger*	Dry mint	0	0	0
60	2.47I	*Aspergillus flavipes*	Dry mint	0	22	3

Ginger contains a vast and complex array of chemicals that, in combination, provides a powerful aid to healing. The enzyme zingibain was believed to improve digestion as well as kills parasites and their eggs. Furthermore, zingibain enhanced antibacterial and antifungal actions (Tajkarimi *et al*., 2010).

There was a significant difference in using the ethanolic extract of each of Curcuma, Cinnamon and Ginger against *Aspergillus versicolor*, *A.nidulans*, *Acremonium strictum* and *Fusarium solani* (the mean values of inhibition zones take different letters) (Table,5).

3.3. Estimation of mycotoxins production from fungal contaminants of the selected five spices and medicinal plants

Mycotoxin contamination is a worldwide food safety problem. It can cause significant economic

Table (5): Estimation of antifungal activity of ethanolic extracts of three medicinal plants (curcuma, cinnamon and ginger)against the isolated yeasts from the selected five spices and medicinal plants (basil, chamomile, dry mint, marjoram and fennel).

No.	Code number	Yeast isolate	Type of commodity	Inhibition zone (mm)using ethanolic extract of		
				Curcuma	Cinnamon	Ginger
1	4.6	*Lipomyces lipofore*	Marjoram	0	2	10
2	2.11	*Candida rugosa*	Dry mint	0	1	12
3	5.23	*Candida rugosa*	Chamomile	0	6	17
4	5.9	*Lipomyces lipofore*	Chamomile	0	2	15
5	5.10	*Saccharomyces cerevisiae*	Chamomile	0	0	19
6	5.3	*Candida rugosa*	Chamomile	0	0	23
7	5.9	*Trichosporon Jirovecii*	Chamomile	0	3	20
8	5.30	*Debaromyces hansenii*	Chamomile	0	1	30
9	5.8	*Debaromyces hansenii*	Chamomile	0	1	10
10	6.28	*Saccharomyces cerevisiae*	Fennel	2	3	15
11	6.15	*Debaromyces hansenii*	Fennel	3	3	16
12	6.26	*Saccharomyces cerevisiae*	Fennel	4	3	15
13	6.16	*Saccharomyces cerevisiae*	Fennel	0	5	26
14	6.17	*Saccharomyces rosinii*	Fennel	0	3	20
15	4.10	*Candida glabrata*	Marjoram	0	0	15
16	4.6	*Candida glabrata*	Marjoram	0	1	15
17	4.1	*Candida glabrata*	Marjoram	2	0	15
18	4.5	*Saccharomyces rosinii*	Marjoram	0	0	25

Table (6): Mean value of antifungal activity(inhibition zone) of three medicinal plants ethanolic extracts (curcuma, cinnamon and ginger)against the isolated molds from the selected five spices and medicinal plants (basil, chamomile, dry mint, marjoram and fennel).

Fungal isolate	Inhibition zone (mm)			F-test	
	Curcuma	Cinnamon	Ginger	F-value	P-value
1-Aspergillus glaucus	000.3 a	9. 250 a	1.250 a	1.932	0.200
2-Aspergillus versicolor	1.000 b	12.500 a	0.500 b	22.419	0.000
3-Aspergillus nidulaus	1.000 b	5.500 a	1.000 b	16.200	0.020
4-Aspergillus niger	0.500a	0.000 a	0.250 a	0.600	0.560
5-Aspergillus flavus	0.000a	5.250 a	0.750 a	3.395	0.080
7-Aspergillus flavipes	0.000a	12.000 a	1.500 a	1.254	0.400
8-Alternaria alternate	0.9090 a	11.6364 a	1.6364 b	48.204	0.000
9-Acremonium strictum	0.5830 b	8.5833 a	1.667 b	27.022	0.000
10-Rhizopus orrhizus	1.8571 a	1.0000 a	1.7143 a	0.7320	0.490
11-Fusarium solani	0.000 b	7.167a	0.330 b	20.404	0.000
12-Absidia corymbifeza	1.000 a	11.5000 a	3.500 a	3.282	0.170

losses associated with the adverse effects of mycotoxins on human and animal health, food security and international trade. Mycotoxins are toxic substances produced mostly as secondary metabolites by fungi that grow on seeds and feed in the field, or in storage (Trucksess and Diaz-Amigo, 2011). The major mycotoxin-producing fungi are species of *Aspergillus*, *Fusarium* and *Penicillium* (Kumar *et al.*, 2008). In the present study, seven mold isolates out of sixty produced mycotoxins (11.7%). *Aspergillus flavus* from Marjoram produced aflatoxin B_1 (160µg/ml) and aflatoxin B_2 (50µg/ml), while,each of *A.glaucus*, *A.flavus* from Basil and *A.niger* from Marjoram produced aflatoxin G_1 as 3.3µg/ml,25 µg/ml and 7.5µg/ml ,respectively(Fig,1). Each of *A.glaucus*, *A.versicolor* from Dry mint and *A.nidulaus* from basil produced Zeralenone. A significant part of aflatoxin B1 in the Egyptian food could come from nine essential food spices and dried herbs, most of them are probably infested with *Aspergillus flavus* during drying and storage (Selim *et al.*,1996 & Kumar *et al.*, 2009). There had been a growing body of circumstantial evidence that aflatoxin B_1 is carcinogenic, as well as acutely toxic to humans, but in 1987 the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) accepted that aflatoxin should be classified as a Group 1 carcinogen. Regulatory levels set by the governments of many countries were based on the premise that aflatoxin is indeed carcinogenic and the European Community agreed on 16 July 1998 a limit of 2 µg kg^{-1} for aflatoxin B_1 in a range of foods for human consumption. Bungo *et al*. (2006)and Ajose (2007) reported that, a potential risk for mycotoxins contamination may occur especially during prolonged storage in poor condition without tempreture and moisture control that usually render medicinal plants more susceptible to mould growth and mycotoxins production.

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المحتوى الفطرى لبعض النباتات الطبية والبهارات المصرية وتقييم قدراتها لانتاج السموم الفطرية

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**المعمل المركزى لتحليل متبقيات المبيدات والعناصر الثقيلة فى الاغذية – مركز البحوث الزراعية– الجيزة – مصر

ملخص

تم عمل مسح على مائة و خمسون عينة من الخمس بهارات و النباتات الطبية (ثلاثون عينة من كل من الريحان والشيح والنعناع المجفف والبردقوش و الشمر) وذلك لعزل الفطريات والخميرة منها. تم عد العزلات وتعريفها. كان تواجد وانتشار الفطريات يتراوح بين 10.04% فى الشمر الى 33.95% فى النعناع المجفف. اثبتت النتائج أن الاسبرجلس نيجر هو الأكثر سيادة بين الفطريات، و هو ممثل باربعمائة و ثمانى وعشرون عزلة (32.08%) فى عينات الريحان ومائة وتسعون عزلة (2.83%) فى عينات الشيح و سبعمائة و تسع وعشرون عزلة (34.45%) فى عينات النعناع المجفف و خمسمائة و ست و أربعون عزلة (36.45%) فى عينات البردقوش وخمس واربعون عزلة (7.19%) فى عينات الشمر . تم التعرف على أجناس و أنواع مختلفة من الخمائر ويتراوح تواجد الخمائر ضمن النباتات الطبية المختلفة بين 5.5% فى عينات الريحان الى 38.9% فى عينات الشيح . تم الكشف عن النشاط المضاد للفطريات للمستخلص الايثانولى لكل من القرفةو الكركم و الزنجبيل باستخدام اختبار الحساسية ضد عزلات الفطريات و الخميرة المعزولة من البهارات و النباتات الطبية (60 عزلة من الفطريات و 18 عزلة من الخميرة). كانت كل عزلات الفطريات مقاومة للمستخلص الايثانولى لكل من الكركم والجنزبيل. وجد بالنسبة لمستخلص القرفة أن تسع عزلات من الفطريات كانت وسطية، بينما كانت ثمانية عشر عزلة من الفطريات حساسة لمستخلص القرفة. كانت كل عزلات الخميرة مقاومة للمستخلص الايثانولى لكل من الكركم و القرفة، بينما كانت باقى عزلات الخميرة حساسة لمستخلص الزنجبيل. بدراسة نتيجة السموم الفطرية للفطريات المعزولة اتضح أن سبع عزلات من الفطريات من أصل ستون عزلة (11.7%) انتجوا أنواع مختلفة من الميكوتوكسن. اسبرجلس فلافس من البردقوش انتج افلاتوكسن B1 (160ميكروجرام/مل) و افلاتوكسن B2(50ميكروجرام/مل) اما الاسبرجلس جلواكس و الاسبرجلس فلافس من الريحان و الاسبرجلس نايجر من البردقوش انتجوا افلاتوكسن G1 كالاتى: 3.3 ميكروجرام/مل، 25 ميكروجرام/مل و 7.5 ميكروجرام/مل ،على التوالى. انتج اسبرجلس نديولانس من الريحان واسبرجلس جلواكس من النعناع المجفف واسبرجلس فرسيكلر من النعناع المجفف الزيريانون.

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