

Microbiological Quality and Aflatoxinogenesis of Egyptian 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 303 samples, representing different types of spices and medicinal plants were collected from random sources in Egypt. Microbiological analysis was carried out for the detection and enumeration of microorganisms using standard media. Also, samples were investigated for the mycological point of view and aflatoxins analysis was performed. Representative figures for the microbial status of dried herbal materials including an aerobic bacterial count, spore-forming, coliform, *E. coli*, *Staphylococcus aureus*, Salmonella, yeast and mold were (10^3 to 10^3), (10 to 10^4), (10 to 10^3), (3 to 10^2), (10 in positive samples), (not detectable), (10 to 10^4) and (10 to 10^4 CFU/g), respectively. Moreover, fungi were found in all of the collected samples. *Aspergillus*, *Fusarium* and *Penicillium* genera were more frequently detected than other genera (*Alternaria*, *Absidia spp.*, *Mucor spp.*, *Rhizoctonia* and *Cladosporium spp.*). The collected samples were found to be free of aflatoxins (B1, B2, G1 and G2). It was concluded that spices and medicinal plants may be high risk products and therefore, more studies are necessary to find methods of decontamination.

Key words: Spices • medicinal plants • bacteria • fungi • aflatoxins

INTRODUCTION

Spices and medicinal plants are widely used as raw materials for pharmaceutical preparations (Galenic products) and as a supplement for dietetic products, especially for “self medications” in public. These plants are normally carrying a great number of bacteria and molds, often of soil origin. Current practices of harvesting, handling and production often cause additional contamination and microbial growth [1, 2]. The microbial flora on many spices and related materials is generally dominated by aerobic spore-forming bacteria [3]. It was found that Celery seed, paprika, black and white pepper and Ginger usually show total plate counts in millions per gram [1, 4, 5]. Also, total plate counts above few hundred thousands per gram have been noted in Cassia, Mace and Nutmeg [5]. Spices having essential oils which exhibit antimicrobial effects, generally show the lowest microbial populations [1].

During the cleaning and processing of spices there is a progressive reduction in the number and types of microorganisms. Those organisms remaining after physical cleaning operations are generally mixtures of aerobic sporforming bacteria and common molds [5, 6].

Coliform bacteria, among other species in the Enterobacteriaceae family, occur sporadically and usually in small populations [5,7] and are associated with fecal contaminations. Bacterial spores of the *Bacillaceae* family are resistant to thermal treatment usually applied in infusion preparation and this thermal shock may stimulate spore germination. Some of these bacteria like *Bacillus cereus* and *Clostridium perfringens* are recognized as having potential pathogenicity and have been incriminated in food poisoning [8, 9] and are occasionally present but at very low levels [6]. Pathogens such as salmonella, shigella and coagulase-positive staphylococci are rarely found in spices [5, 10]. Yeasts and mold densities vary considerably with the individual spices, but are usually quite low. They range from less than 10 per gram in the case of such spices as Mace, Mustard seed and Cloves [11] to greater than 10,000 per gram for a variety of other spices, mainly Basil, Black pepper, Capsicum, Celery seed and Cinnamon [5, 6].

Keeping quality of spices is directly related to the condition of the product at harvest. Post harvest processing and properly storage of most harvested seeds will require no further drying, whereas roots, barks and certain berries may require various drying time. When

properly dried and stored, spices are generally resistant to microbial spoilage. However, spices are raw agricultural materials and if the moisture content is too high, toxigenic molds, like *Aspergillus spp.*, *Penicillium spp.* and *Fusarium spp.* [12,13], may grow offering the opportunity for aflatoxins production [14-16].

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. Also, investigation of the microbiological quality, bacteria and fungi and the toxicity of isolated fungi is another target of this study. Also, analysis of aflatoxins will be considered.

MATERIALS AND METHODS

Samples collection: A total of 303 samples, representing different types of spices and medicinal plants, are collected from random sources in Egypt. Many of these herbs grow in different growing seasons and each with its own agricultural practices. Eleven types were belonging to leafy group, six to fruity group and three to the flowery group. The samples were analyzed directly after collection. Table 1 presents the different collected samples.

Standard media: For the detection and enumeration of microorganisms, standard media are prepared. For aerobic and sporeformer bacterial count, the medium was standard plate count agar. For coliform bacteria count, the medium was lauryl sulfate broth and brilliant green bile broth. For *E. coli*, the medium was EC and EMB agar. For *Staphylococcus aureus*, the medium was Baird parker, brain heart infusion and trypticase soy broth with 10% NaCl. For Salmonella, the medium was selenite cystine broth and selective agars (bismuth sulfite, brilliant green, triple sugar iron agar and lysine iron agar). For yeast and mold, the medium was potato dextrose agar. Gram stain and microscopic examination was used for confirmation.

Standard toxins: Standard aflatoxins were obtained from Sigma Co.

Microbiological analysis: Microbiological analysis for spice and medicinal plant samples was carried out according to Official Microbiological Methods of World Health Organization [17]. The samples were prepared as many decimal dilutions as necessary depending on the expected bacterial load of the material being examined. After dilutions prepared, appropriate media were inoculated and incubated at specific temperature.

Table 1: Spices and medicinal plant samples collected from several selected shipments in Egypt

Plant type	No. of collected samples	Scientific name
Leaves		
Geranium	20	<i>Pelargonium graveolens</i> L.
Basil	20	<i>Ocimum basilicum</i> L.
Marjoram	20	<i>Marjorana hortensis</i> L.
Peppermint	20	<i>Mentha piperita</i> L.
Spearmint	20	<i>Mentha viridis</i>
Jews mallow	10	<i>Corchorus oritorius</i>
Dill	10	<i>Anethum graveolens</i> L.
Celery	10	<i>Apium graveolens</i> Mill.
Parsley	10	<i>Petroselinum sativum</i> Hoffm.
Cumin	10	<i>Cuminum cyminum</i> L.
Tea	6	<i>Thea sinensis</i> L.
Fruits		
Caraway	15	<i>Carum carvi</i> L.
Anise	15	<i>Pimpinella anisum</i> L.
Fennel	15	<i>Foeniculum vulgare</i> L.
Coriander	15	<i>Coriander sativum</i> L.
Dill	5	<i>Anethum graveolens</i> L.
Black pepper	5	<i>Piper nigrum</i>
Flowers		
Chamomile	66	<i>Matricaria chamomila</i> L.
Karkade	6	<i>Rosella jamica</i>
Saffron	5	<i>Crocus sativus</i> Linn

Mycological studies: Ten grams of each sample were added to 90 ml portion of sterile saline solution (0.85%) in 500 ml Erlenmeyer flask and homogenized thoroughly on an electric shaker at constant speed for 15 minutes. Ten fold serial dilutions were then prepared [18]. One ml portion of suitable dilutions were used to inoculate petri dishes containing 15 ml dextrose agar fortified by 0.5 mg chloromphenicol/ml medium. Plates were incubated at 28 °C for 7-15 days and examined for the growth of molds. Fungi were isolated and identified according to [19-21].

Aflatoxins analysis: Twenty five grams from samples of ground spices and medicinal plants were extracted according to the method of [22]. Thin layer chromatography (TLC) of aflatoxins (B1, B2, G1 and G2) was performed according to [23].

Data were collected and represented in tables.

RESULTS

Bacterial contamination: The microbiological quality of spice and medicinal plant samples collected from Egyptian shipments is shown in Table 2. Among different bacteria in the present study, total viable counts were noticed in different plants at different levels. The highest mean

Table 2: Mean counts (CFU/g) of microorganisms detected in Egyptian spices and medicinal plants

Item	Aerobic bacteria	Sporformer bacteria	Coliform bacteria	<i>E. coli</i>	<i>Staphylococcus aureus</i>	Salmonella	Yeast	Mold
Leaves								
1.Geranium	3.1 x 10 ⁶	2.0 x 10 ³	2.4 x 10 ²	2.1 x 10 ²	-	-	3.0 x 10 ³	3.5 x 10 ³
2.Basil	1.0 x 10 ⁷	2.1 x 10 ³	2.4 x 10 ²	1.5 x 10 ²	6.0 x 10	-	8.0 x 10 ²	3.0 x 10 ³
3.Marjoram	5.6 x 10 ⁶	3.4 x 10 ²	4.6 x 10 ²	2.1 x 10 ²	-	-	3.1 x 10 ²	1.5 x 10 ⁴
4. Peppermint	2.6 x 10 ⁸	1.4 x 10 ⁴	1.1 x 10 ³	4.6 x 10 ²	3.0 x 10	-	1.1 x 10 ⁴	2.0 x 10 ⁴
5.Spearmint	3.0 x 10 ⁷	2.3 x 10 ³	1.1 x 10 ³	2.1 x 10 ²	2.0 x 10	-	3.0 x 10 ³	2.0 x 10 ³
6.Mulokhia	2.0 x 10 ⁷	4.0 x 10 ³	1.1 x 10 ³	2.1 x 10 ²	-	-	4.1 x 10 ³	3.0 x 10 ²
7.Dill	3.1 x 10 ⁶	4.1 x 10 ²	1.1 x 10 ³	1.5 x 10	-	-	1.2 x 10 ³	1.3 x 10 ³
8.Celery	4.8 x 10 ⁶	2.0 x 10 ³	1.1 x 10 ³	1.5 x 10	-	-	2.1 x 10 ²	4.4 x 10 ²
9.Parsley	2.5 x 10 ⁷	3.6 x 10 ²	1.1 x 10 ³	4.6 x 10 ²	-	-	1.1 x 10 ³	3.5 x 10 ³
10.Cumin	2.8 x 10 ⁶	8.4 x 10 ²	1.1 x 10 ³	1.5 x 10	-	-	3.1 x 10 ³	2.9 x 10 ²
11.Tea	6.0 x 10 ⁵	2.4 x 10 ²	1.5 x 10	nd	-	-	2.2 x 10 ²	1.2 x 10 ²
Fruits								
1.Caraway	1.8 x 10 ⁶	1.8 x 10 ²	2.1 x 10	1.5 x 10	-	-	3.0 x 10 ²	1.0 x 10 ³
2.Anise	7.0 x 10 ⁶	6.1 x 10 ²	1.1 x 10 ³	1.5 x 10	-	-	1.2 x 10 ³	1.6 x 10 ³
3.Fennel	8.1 x 10 ⁶	4.1 x 10 ²	2.4 x 10 ²	9.3 x 10	-	-	4.1 x 10 ³	2.4 x 10 ³
4.Coriander	4.0 x 10 ⁵	1.6 x 10 ²	2.3 x 10	3	-	-	1.2 x 10 ³	2.2 x 10 ³
5.Dill	6.0 x 10 ⁵	2.6 x 10 ²	1.5 x 10	3	-	-	2.0 x 10 ³	3.0 x 10 ³
6.Black pepper	4.0 x 10 ³	1.1 x 10	-	-	-	-	3.4 x 10 ²	2.1 x 10 ²
Flowers								
1.Chamomile	3.6 x 10 ⁷	1.4 x 10 ³	1.1 x 10 ³	4.6 x 10 ²	-	-	1.1 x 10 ⁴	7.4 x 10 ³
2.Karkade	8.1 x 10 ⁵	6.1 x 10 ²	1.5 x 10	-	-	-	6.0 x 10	8.1 x 10 ²
3.Saffron	3.1 x 10 ⁴	2.3 x 10	-	-	-	-	4.0 x 10 ²	3.0 x 10 ²

nd = not detected

Table 3: Percentage of fungi in positive infected samples of leafy medicinal plants

Fungal species	Geranium n=20	Basil n=20	Marjoram n=20	Peppermint n=20	Spearmint n=20	Jews n=10	Dill n=10	Celery n=10	Parsely n=10	Cumin n=10	Tea n=6
<i>Asp. fumigatus</i>	2 (10%)	2 (10%)	3 (15%)	1 (5%)	2 (10%)	4(40%)	2 (20%)	- (-)	1 (10%)	1 (10%)	- (-)
<i>Asp. niger</i>	3 (15%)	4 (20%)	4 (20%)	5 (25%)	4 (20%)	2 (20%)	2(20%)	2(20%)	1 (10%)	1 (10%)	1(16.7)
<i>Asp. candidus</i>	2 (10%)	2 (10%)	1 (5%)	5 (25%)	2 (10%)	2 (20%)	2(20%)	2(20%)	2 (20%)	1 (10%)	1(16.7)
<i>Asp. ochraceus</i>	4 (20%)	4 (20%)	2 (10%)	2 (10%)	2 (10%)	2 (20%)	2(20%)	1(10%)	1 (10%)	1 (10%)	1(16.7)
<i>Asp. flavus</i>	4 (20%)	5 (25%)	3 (15%)	5 (25%)	4 (20%)	2 (20%)	1(10%)	1(10%)	1 (10%)	- (-)	1(16.7)
<i>Asp. terreus</i>	1 (5%)	2 (10%)	2 (10%)	5 (25%)	6 (30%)	1 (10%)	1(10%)	1(10%)	1 (10%)	- (-)	- (-)
<i>Asp. crothecium</i>	1 (5%)	2 (10%)	2 (10%)	- (-)	- (-)	1 (10%)	1(10%)	1(10%)	1 (10%)	1 (10%)	- (-)
<i>Fusarium spp.</i>	2 (10%)	2 (10%)	2 (10%)	5 (25%)	3 (15%)	4 (40%)	2(20%)	2 (20%)	2 (20%)	1 (10%)	1(16.7)
<i>Penicillium spp.</i>	2 (10%)	3 (15%)	2 (10%)	5 (25%)	1 (5%)	3 (30%)	1(10%)	1 (10%)	1 (10%)	- (-)	1(16.7)
<i>Alternaria</i>	1 (5%)	- (-)	- (-)	1 (5%)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
<i>Trichoderma</i>	1 (5%)	- (-)	- (-)	1 (5%)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
<i>Absidia spp.</i>	1 (5%)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
<i>Mucor spp.</i>	2 (10%)	1 (5%)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
<i>Rhizoctonia</i>	1 (5%)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
<i>Cladosporium spp.</i>	1 (5%)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)

Asp. = Aspergillus

Table 4: Percentage of fungi in positive infected samples of fruity medicinal plants and spices.

Fungal species	Caraway n=15	Anise n=15	Fennel n=15	Coriander n=15	Dill n=5	Black pepper n=5
<i>Asp. fumigatus</i>	1 (6.7%)	1 (6.7%)	1 (6.7%)	1 (6.7%)	1 (20%)	----
<i>Asp. niger</i>	3(20.0%)	10 (66.7%)	10 (66.7%)	2 (13.3%)	1 (20%)	----
<i>Asp. candidus</i>	1(6.7%)	1 (6.7%)	2 (13.3%)	1 (6.7%)	1 (20%)	1 (20%)
<i>Asp. ochraceus</i>	4(26.7)	1 (6.7%)	5 (33.3%)	1 (6.7%)	2 (40%)	1 (20%)
<i>Asp. flavus</i>	4(26.7)	6 (40.2%)	4 (26.7%)	1 (6.7%)	2 (40%)	1 (20%)
<i>Asp. terreus</i>	3(20.0%)	1 (6.7%)	1 (6.7%)	1 (6.7%)	1 (20%)	1 (20%)
<i>Asp. crothecium</i>	3(20.0%)	1 (6.7%)	1 (6.7%)	1 (6.7%)	1 (20%)	----
<i>Fusarium spp.</i>	3(20.0%)	6 (40.2%)	4 (26.7%)	2 (13.3%)	1 (20%)	1 (20%)
<i>Penicillium spp.</i>	4(26.7)	4 (26.7%)	5 (33.3%)	1 (6.7%)	1 (20%)	1 (20%)
<i>Alternaria</i>	----	3 (20.1%)	----	----	----	----
<i>Trichoderma</i>	----	----	----	----	----	----
<i>Absidia spp.</i>	----	----	4 (26.7%)	----	----	----
<i>Mucor spp.</i>	----	----	2 (13.3%)	1 (6.7%)	1 (20%)	----
<i>Rhizoctonia</i>	1(6.7%)	----	2 (13.3%)	----	----	----
<i>Cladosporium spp.</i>	----	----	----	----	----	----

Asp = Aspergillus

Table 5: Incidence of fungal species (%) in flowery medicinal plants

Fungal species	Chamomile n=66	Karkade n=6	Saffron n=5
<i>Asp. fumigatus</i>	20 (30.3%)	1 (16.7%)	1 (20.0%)
<i>Asp. niger</i>	15 (22.7%)	1 (16.7%)	2 (40.0%)
<i>Asp. candidus</i>	20 (30.3%)	1 (16.7%)	1 (20.0%)
<i>Asp. ochraceus</i>	22 (33.3%)	1 (16.7%)	1 (20.0%)
<i>Asp. flavus</i>	25 (37.9%)	2 (33.3%)	3 (60.0%)
<i>Asp. terreus</i>	12 (18.2%)	1 (16.7%)	2 (40.0%)
<i>Asp. crothecium</i>	2 (3.0%)	1 (16.7%)	1 (20.0%)
<i>Fusarium spp.</i>	12 (18.2%)	2 (33.3%)	1 (20.0%)
<i>Penicillium spp.</i>	6 (9.1%)	2 (33.3%)	2 (40.0%)
<i>Alternaria</i>	----	----	----
<i>Trichoderma</i>	----	----	----
<i>Absidia spp.</i>	----	----	----
<i>Mucor spp.</i>	4 (6.1%)	1 (16.7%)	1 (20.0%)
<i>Rhizoctonia</i>	----	----	----
<i>Cladosporium spp.</i>	2 (3.0%)	----	----

Asp= *Aspergillus*

Table 6: Requirements for the microbial purity of herbal drugs established in the 1989 supplement to DAB g, the German Pharmacopeia [30]

Micro-organism	Limits per drug category	
	Category 4a ^a	Category 4b ^b
Aerobic bacteria	10 ⁷ /g	10 ⁵ /g
Yeasts and molds	10 ⁴ /g	10 ³ /g
<i>Escherichia coli</i>	10 ² /g	10 ¹ /g
Other enterobacteria	10 ⁴ /g	10 ³ /g
Salmonella	None	None

^aDrug category 4a: Dried herbs and dried herbal mixtures for the preparation of medicinal teas, which undergo a germ reduction before use (e.g., by pouring boiling water on the material), as well as preparations for topical use that contain dried herbs

^bDrug category 4b: Other preparation for internal use that contain dried herbs

count was detected in Peppermint followed by Chamomile flowers, Spearmint and dried Parsley. However, the lowest mean counts were detected in Black Pepper followed by Saffron.

Sporforming bacteria were detected in all the analyzed samples. The highest mean count was detected in Peppermint, followed by Mulokhia and spearmint. On the other hand, coliform bacteria were detected in all samples except black Pepper and Saffron. *E. coli*, also detected in all analyzed samples except Tea, Black Pepper, Karkade and Saffron. The highest mean count was detected in Peppermint, Parsley and Chamomile. However, the lowest mean counts were found in Coriander and Dill. Salmonella was not detectable in all the analyzed samples. However, *Staphylococcus aureus* was detected only in Basil, Peppermint and Spearmint.

Regarding to yeast, Peppermint and Chamomile contained the highest mean counts. However the lowest mean counts were detected in karkade. On the other hand, the other types contained moderate counts (Table 2).

Mold was detected in all different plants. The highest mean counts were detected in Peppermint and Marjoram. The lowest mean counts were detected in Tea.

Fungal contamination and aflatoxins production:

Fungal populations isolated from spices and medicinal plant samples are shown in Tables 3-5. Fungi were found in all of the collected samples. The lowest percent of mycological contamination were detected in Coriander and Cumin. In contrast, Saffron and Fennel, presented the highest infections of fungi. *Aspergillus*, *Fusarium* and *Penicillium* genera were more frequently detected than other genera of fungi. *Aspergillus* spp. was found in all examined medicinal plant samples under investigation while, *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus crothecium* were not detected in Black pepper samples (Table 4). Strains of *Aspergillus flavus* and *Aspergillus niger* were the most dominant in the collected samples.

Fusarium and *Penicillium spp.* were isolated from all the collected samples. Other species of moulds were isolated from different medicinal plant samples, such as, *Alternaria*, *Trichoderma*, *Absidia spp.*, *Mucor spp.*, *Rhizoctonia* and *Cladosporium spp.* (Table 4-6). Different samples of spices and medicinal plants that contaminated by *Aspergillus* species were free of aflatoxins.

DISCUSSION

The microbiological quality of spice and medicinal plant samples collected from Egyptian shipments had been demonstrated in Table 2. However, it was reported that microbial counts vary according to the region, the year of production and the harvest and storage conditions prior to drying. So, the observed counts are thus a reflection of the original bioload, of growth, as well as of die-off which are probably enhanced by oxidation and the presence of active compounds in herbs and spices [24]. Similar results were obtained by [25–28]. [25] reported that 10% of analyzed samples from 95 different crude herbal drugs contained <10² CFU/g molds, 38% contained 10²-10³ CFU/g, 28% 10³-10⁴ and 24% >10⁴ CUF/g. [26] analyzed 548 samples of seeds and 221 samples of 38 other crude herbal materials (barks, flowers, leaves, fruits, herbs, roots and rhizomas). He found that

total count of aerobic bacteria in seed samples up to $>10^7$ CFU/g (mean values in the range of 10^6 CFU/g). The other samples contained $<10^2$ To $>10^7$ CFU/g ($>80\%$ of the mean values in the range of 10^4 - 10^6 CFU). He detected also *E. coli* in 37 samples (6.8%) of seeds and 17 samples (7.7%) of other types. On the other hand, [27] analyzed 184 samples of 56 different herbs. He found that 85% ($>10^4$ CFU) and 52% ($>10^6$ CFU) total bacterial count. He reported also that yeasts and molds found in 36% $>10^4$ and 3% $>10^6$ and coliform bacteria detected in 58% $>10^4$ and 19% $>10^6$. However *Staph. aureus* and Salmonella were not detected. [28] analyzed 578 samples of crude plants. He reported that total viable count $<10^3$ to $>10^8$ CFU/g, yeasts and molds up to 10^7 CFU/g, coliform bacteria up to 10^8 , *Staph. aureus* detected in $<1.3\%$ of the samples and Salmonella were detected in 1.3% of the samples. [29] reported that 96.8% of the collected medicinal plants were contaminated with *Bacillus cereus*, 19.2% of them contained levels higher than 10^3 spores/g.

Until recently, there were no special pharmacopoeial or other official regulations providing concrete upper limits for the microbial contamination of phytotherapeutical materials. For lack of something better, most investigators compared their results to the so-called FIP requirements for the microbial purity of non-sterile medicines which were proposed in the 1970s by the committee of Official Laboratories and Control Services of the Federation International Pharmaceutique (Table 6). The FIP proposals have been considered to be unsuitable for medicinal plants [28]. Even when bacteria have been killed by sterilisation processes it is still possible that endotoxins (liposaccharides from bacterial cell walls) are present in dried medicinal plants Endotoxins may produce pyrogenic effects such as elevated temperature, headache and joint pain in humans. Substantial amounts of endotoxin have been found in commercial samples of injection fluids obtained from plant extracts [31].

Representative figures for the microbial status of dried herbal materials is in the same range as that of vegetal foods. It is not so much caused by secondary contamination during processing, but is primarily due to the fact that plants have their own microbial flora. This was elegantly demonstrated by [31], who compared samples of the same three herbs in their fresh state and after work-up to dried material. Due to this natural contamination, a majority of dried medicinal herb samples fails to meet the FIP requirements for microbial purity. This is particularly true for so-called mass drugs, such as Peppermint, Chamomile, Melissa and Valerian [27, 28, 32]. On the other hands, monographs of the US pharmacopeia

[33] for products that contain raw materials of natural origin establish a maximum fungal contamination limit of 2×10^3 CFU/g of the products.

Fungi were found in all of the collected samples. Strains of *Aspergillus flavus* and *Aspergillus niger* were the most dominant in the collected samples. These results are in agreement with that reported by [15, 34-36]. *Fusarium* and *Penicillium spp.* were isolated from all the collected samples. In other studies, *Penicillium spp.* was also found to be highly prevalent in Camomile, Pennyroyal mint and Garden sage samples [2, 37]. The contamination with fungal species resulted from neutral extraneous contamination by dust following storage in humid conditions [21]. Fungi fall into two ecological categories, e.g., field and storage fungi. Field fungi were observed to invade developing or mature seeds while it is on the plant, the major field fungi genera are, *Alternaria*, *Fusarium* and *Cladosporium*. On the other hand, storage moulds are those encountered on plants at moisture conditions routinely found in stored products, these fungi are principally species of *Aspergillus* and *Penicillium*.

Different samples of spices and medicinal plants that contaminated by *Aspergillus* species were analyzed for aflatoxins and it was found free of aflatoxins. This means that the presence of toxigenic moulds in the food does not mean directly the presence of mycotoxins and vice versa. This could be explained in light of that spices and medicinal plants are not ideal substrate for aflatoxins formation, due to the presence of essential oils with antimycotic effects, may inhibit the production of aflatoxins or reduce fungal infestation and/or subsequent aflatoxins production. This agrees with [38-40] and is indicative of causal contamination after harvesting and drying. The antifungal effects are dependent on the concentration of essential oils and this concentration is further dependent on some factors, such as the part of the plant, plant species and environment growth conditions [41-43]. Actually, it is important to note that fungal growth is weaker in spices and herbs than other commodities [44]. For example, although the fungus *Aspergillus flavus* grows well on the spices, the production of aflatoxins is lower than in cereals [45]. So, it seems that not all *Aspergillus* strains are aflatoxigenic on this type of matrix [46].

Most of the identified moulds have been reported to have the ability to produce mycotoxins [47]. According to [48], 70-80% of the *Penicillia* are potential mycotoxins producer (citrinin, patulin, cyclopiasonic acid and penitrem). These results showed that a potential risk for mycotoxins contamination may be caused, especially

during prolonged storage in poorly conditions without temperature and moisture control that usually render medicinal plants more susceptible to mould growth and mycotoxins production [47].

It was concluded that spices and medicinal plants may be high risk products as it contained many pathogenic bacteria and fungi and therefore, more studies are necessary to find methods of decontamination. Also, processing methods such as harvest, drying, transportation and storage must be improved.

REFERENCES

1. Weiser, H.H., G.J. Mountney and W.A. Gould, 1971. Practical Food Microbiology and Technology. 2nd Edn. AVI Publishing Co., Westport, Conn. (BOOK)
2. Hitokoto, H., S. Morozumi, T. Wauke, S. Sakai and H. Kurata, 1978. Fungal contamination and mycotoxins detection of powdered herbal drugs. Applied. Environmental Microbiology, 36: 252-256 (Journal).
3. Goto, A., K. Yamazaki and M. Oka, 1971. Bacteriology of radiation sterilization of spices. Food Irrad. Shokuhin-Shosha, 6: 35-42.
4. Krishnaswamy, M.A., J.D. Patel and N. Parthasarathy, 1971. Enumeration of microorganisms in spices and spice mixtures. J. Food Sci., Technol., 8: 191-194.
5. 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.
6. Powers, E.M., R. Lawyer and Y. Masuoka, 1975. Microbiology of processed spices. ASTA, Englewood Cliffs, N.J. 07632, 1976.
7. Mundt, J.O., 1976. Streptococci in dried and frozen foods. J. Milk Food Technol., 39: 413-416.
8. Kunene, N.F., J.W. Hastings and A. Von Holy, 1999. Bacterial populations associated with a sorghum-based fermented weaning cereal. Int. J. Food Microbiol., 49: 75-83.
9. Miwa, N.T., K. Masuda, T. Katsuda, K. Kawamura Otani and H. Hiyamoto, 1999. Bacteriological investigation of an outbreak of *Closteridium perfringens* food poisoning caused by Japanese food without animal protein. Int. J. Food Microbiol., 49: 103-106.
10. Foster, E.M., 1971. The control of salmonella in processed foods: A classification system and sampling plan. J. AOAC, 54: 259-266.
11. Julseth, R.M. and R.H. Deibel, 1974. Microbial profile of selected spices and herbs import. J. Milk Food Technol., 37: 414-419.
12. Halt, M., 1998. Moulds and mycotoxins in herb tea and medicinal plants. European J. of Epidemiology, 14: 269-274.
13. Martins, H.M., I.F. Dias, M.L. Martins and F.M. Bernardo, 1999. Fumonisin B1 e B2 em plantas medicinais para infuses naturais. Actas de 4 Encontro de Quimica de Alimentos Coimbra, Portugal, pp: 237-239.
14. Roy, A.K., K.K. Sinha and II.K. Chourasia, 1988. Aflatoxin contamination of some common drug plants. Applied and Environmental Microbiology, 54: 842-843.
15. Aziz, N.H., T.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. Sin., 39: 279-285.
16. Reddy, S.V., M.D. Kiram, R.M. Uma, K. Thirumala-Devi and D.V.R. Reddy, 2001. Aflatoxins B1 in different grades of chillies (*Capsicum comum*. I.) in India as determined by indirect competitive-ELISA. Food Additives and Contaminants, 18: 553-558.
17. WHO, 2005. Quality control methods for medicinal plant materials. World Health Organization. Working document CeAS/ 05.131/ Rev.1.
18. Aziz, N.H. and Y.A. Youssef, 1991. Occurrence of aflatoxins and aflatoxins-producing moulds in fresh and processed meal in Egypt. Food Addit. Contam., 3: 321-331.
19. Raper, K.P. and D.I. Fennel, 1977. "The Genus *Aspergillus*" R.E. Krieger Publishing Company, II Untington, New York.
20. Pitt, J.I., 1985. A laboratory guide to common Penicillium species. Commonwealth mycological institute, kew, Surrey, England, pp: 184.
21. Domsch, K.H., W. Gams and T.H. Anderson, 1981. Compendium of soil fungi, Vol. 1 and 2, Academic Press, London.
22. Grabarkiewicz-Szezesna, J.P., J. Golinski, Chelkowski and K. Szebiotko, 1985. Mycotoxins in cereal grain, Part XI, Simple multidetection procedure for determination of 11 mycotoxins in cereals. Nahrung Food, 3: 229-240.
23. Golinski, P. and J. Grabarkiewicz-Szezesna, 1984. Chemical confirmatory tests for ochratoxin A, citrinin, penicillic acid, sterigmatocystin and zearalenone performed directly on thin-layer chromatographic plates. J. Assoc. Off. Anal. Chem., 67: 1108-1110.

24. Farkas, J., 2000. Spices and herbs, in the microbiological safety and quality of foods. Lund, B.M., T.C. Baird-Parker and G.W. Gould (Eds.). Aspen Publication, Vol: 1.
25. Lutomski, J. and B. Kedzia, 1980. Mycoflora of crude drugs. Estimation of mould contaminations and their toxicity. *Planta Med.*, 40: 212-217.
26. Schilcher, H., 1982. Ruckstande und verunreinigungen bei und Drogenzubereitungen. 19. Mitteilung: zur Wertbestimmung und Qualitätsprüfung von Drogen. *Planta Med.*, 44: 65-77.
27. Leimbeck, R., 1987. Teedrogen-Wie steht es mit der mikrobiologischen Qualität? *Dtsch Apoth Ztg.*, 127: 1221-1226.
28. Frank, B., 1989. Mikroorganismen in Drogen. Der mikrobiologische Status von Drogen und Drogenzubereitungen und seine Beurteilung. *Dtsch Apoth Ztg.* 129: 617-623.
29. Martins, H.M., M.L. Martins, M.I. Dias and F. Bernardo, 2001. Evaluation of microbiological quality of medicinal plants used in natural infusions. *International J. of Food Microbiology*, 68: 149-153.
30. Kruger, D., 1989. Was ist neu aus mikrobiologischer Sicht? *Dtsch Apoth Ztg.*, 129 (Suppl 16): 21-24.
31. Schneider, E., 1987. Keimbesiedlung von frischen Arzneipflanzern und von Drogen. Veränderungen der Keimzahlen von Drogen Während der Gewinnung und Verarbeitung. *Dtsch Apoth Ztg.*, 127: 1683-1686.
32. Hartling, C., 1987. Beitrag zur Frage des mikrobiellen Zustandes pflanzlicher Drogen-Fakten und Folgerungen. *Pharm. ztg.*, 132: 643-644.
33. The United States Pharmacopeia, 2005. 28 ed. Rockville: United States Pharmacopeial Convention.
34. Abou-Arab, A.A.K., K.M. Soliman, M.E. El-Tantawy, B.R. Ismail and K. Naguib, 1999. Quantity estimation of some contaminants in commonly used medicinal plants in Egyptian market. *Food Chemistry*, 67: 357-363.
35. Elshafie, A.E., T. Al-Lawatia and S. Al-Bahry, 1999. Fungi associated with black tea and tea quality in the Sultanate of Oman. *Mycopathologia*, 145: 89-93.
36. Mandeel, Q.A., 2005. Fungal contamination of some imported spices. *Mycopathologia*, 159: 291-298.
37. 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 Edn. Schmeigert, pp: 249.
38. Lwellyn, G.C., R.L. Mooney, T.F. Cheatle and B. Flannigan, 1992. Mycotoxin contamination of spices-an update. *International Biodeterioration and biodegradation*, 29: 111-121.
39. Martini, I.I., M. Weidenborner, S. Adams and B. Kunz, 1996. Eugenol and carvenol: The main fungicidal compound in clove and savory. *Italian J. of Food Science*, 1: 63-67.
40. Bartine, H. and T.A. Elaraki, 1997. Growth and toxigenesis of *Aspergillus flavus* isolated on selected spices. *Journal of Environmental Pathology, Toxicology and Oncology*, 16: 61-65.
41. FAO, 1999. Use of spices and medicinals as bioactive protectants for grains. By Golob, P., Moss, C., Dales, M., Fidgen, A., Evans, J. and Gudraps, I., FAO, Agriculture Services Bulletin no. 137, FAO Publications, Rome, Italy.
42. Soliman, K.M. and R.I. Badea, 2002. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food and Chemical Toxicology*, 40: 1669-1675.
43. Gowda, N.K.S., V. Malathi and R.U. Suganthi, 2004. Effect of some chemical and herbal compounds on growth of *Aspergillus parasiticus* and aflatoxins production. *Animal Feed Science and Technology*, 116: 281-291.
44. Romagnoli, B., V. Menna, N. Gruppioni and C. Bergamini, 2007. Aflatoxins in spices, aromatic herbs, herb-teas and medicinal plants marketed in Italy. *Food Control*, 18: 697-701.
45. Mac Donalds, S. and I. Castle, 1996. A UK retail survey of aflatoxins in herbs and spices and their fate during cooking. *Food Additives and Contaminants*, 13: 121-128.
46. Elshafie, A.F., T.A. Al-Rashdi, S.N. Al-Bahry and C.S. Bakheil, 2002. Fungi and aflatoxins associated with spices in the Sultanate of Oman. *Mycopathologia*, 155: 155-160.
47. Bugno, A., A.A.B. Almodovar, T.C. Pereira, T.A. Pinto and M. Sabino, 2006. Occurrence of toxigenic fungi in herbal drugs. *Brazilian J. of Microbiology*, 37 (1): 1-7.
48. Pohland, A.E. and G.E. Wood, 1987. Occurrence of mycotoxins in food. I. (Ed.), *Mycotoxins in food*, pp: 35-64.

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