Mycoflora of Herbal Drug Plants from Bahrain

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ABSTRACT

Mycoflora profile were evaluated in crude unstored samples of twenty herbal drug plants, in which half were collected from cultivated habitats and the rest from desert locations. Microfungal colonies were singled out from a total of 130 samples using standard dilution plate method on several media. The highest fungal spectra and diversity was encountered in materials of cultivated habitats with a total of 2707 isolates, representing 39 species compared to only 965 isolates and 25 species from desert locations. The most heavily contaminated herbal drug samples examined from cultivated and desert habitats were observed in parts of Atriplex leucoclada and Erodium laciniatum var pulverulentum, with an average of 636 and 259 cfu/g, respectively. The maximum relative contamination frequency from five random samples infected with ≥ 5 fungi was also found associated with A. leucoclada (93.2%) followed by Trigonella anguina (86.7%) in cultivated and desert habitats, correspondingly. Moreover, among both habitats, the medicinal herb A. leucoclada from the cultivated habitat exhibited the highest species diversity based on the SIMPSON (0.930) and SHANNON-WIENER (2.856) indices. The most predominant mould genera detected from both habitats, based on relative density values (≥ 5%), were Chaetomium, Aspergillus, Rhizopus and Cladosporium. Moreover, these taxa revealed an overall frequency of occurrence rates ≥ 70% and hence contributed substantially to the total population density load of the recovered fungi in this study. The incidence of toxigenic moulds represented mainly by A. flavus in ≥ 5 random samples fluctuated between 31.3 – 1.9% in cultivated habitat plants and 11.7 – 3.2% in desert habitat plants. Similarly, data are consistent with previously reported findings that mould spoilage incidence of herbal drug plants investigated in this study constitute a health hazard, especially if destined for human consumption. Thus, there is an urgent need for quality control and hygienic measures before the finished products are channeled to consumers or end up in drug preparations.

KEYWORDS: Herbal drug plants, mould contamination, mycotoxins, mycoflora, spoilage fungi.

INTRODUCTION

Bahrain is a small island nation in the eastern part of Arabian Gulf with rich and versatile vegetation resources. El-Oqlah and Abbas (1994) have identified more than 350 plant species in which about 80 species are considered as frequently used herbal drugs. Their role as antimicrobial therapeutic agents has received considerable attention in the recent years (Mandeel and Taha, 2005). Abbas and Al-Saleh (2002) were first to establish their significance as folk remedies used by native people in the treatment of various alignments. The plant parts commonly used consist of roots, stems and leaves with few seeds or fruits either alone (decoctions) or in combination with other parts (concoctions). These plant parts are used to treat most of the prevalent diseases in the island like pneumonia, fever, renal problems, diarrhea, internal helminthes, bone fractures, eczema, rheumatic arthritis, bronchitis and skin as well as many stomach disorders (Abbas and Al-Saleh, 2002; Mandeel and Taha, 2005). Locally, herbal drug remedies are commonly consumed daily as herbal tea or added in drinks from concentrated distillates. The kingdom of Bahrain is reputable, among

neighboring Arabian Gulf countries, as one of the main attraction centers for herbal medicine therapy.

Herbalists in Bahrain usually collect the various plant parts from cultivated and/or desert locations throughout the year, but mostly after the rainy season in spring, and preserve them by air-drying for few days in open space prior sale in herbal "Hawaj or Attar" market. In fields, environmental dust settled on different plant parts in addition with other contamination sources, often from soil, can load significant amount of mould spores or structures. Such unclean plant raw parts are always stored in aseptic conditions in small conventional metal, cloth, wooden or plastic containers and packaged without an effective hygienic and sanitary control procedure. Moreover, lengthened storage in low ventilated, humid (75-85%), and high temperature (30-40 C) regimes characteristic of arid conditions can generate favorable microenvironments thus, enhancing microbial activities, especially fungal growth (Misra, 1981; Mandeel, 2005). The importance and hazard of the latter may be overlooked by the consumer assumption that medicinal plants are naturally safe and healthy.

Fungal attacks, ordinarily, constitute a major contamination source and spoilage of food commodities, primarily, in unprocessed foodstuff during harvest, transit and storage (Efuntoye, 1996; Pitt and Hocking, 1997; Mandeel, 2005; Garcia *et al.*, 2001). According to Pitt *et al.* (1993), Aziz *et al.* (1998), El-Shafie *et al.* (1999), Abou-Arab *et al.* (1999) and Martins *et al.* (2001) the most commonly associated moulds with raw herbal drug plants are naturally occurring saprophytic or pathogenic species of *Aspergillus, Penicillium, Rhizopus, Chaetomium, Cladosporium* and *Fusarium.* An important mould species represented by *A. flavus* and *F. moniliforme* are well-recognized producers of many mycotoxins like aflatoxin and fumonisins, respectively. The incidence of these mycotoxins by associated moulds in medicinal plants has already been well established (Halt, 1998; Abeywickrama and Bean, 1991; Chourasia, 1995). Mycotoxins, specifically like aflatoxin, were reported to be highly carcinogenic, teratogenic, tremorogenic and hemorrhagic metabolites to many prokaryotic and eukaryotic organisms (Aziz *et al.*, 1988; Chourasia, 1995; Lwellyn *et al.*, 1990). Their presence in herbal crude drug samples remains a major risk to consumers, if taken even in small amounts (Halt, 1998; Abou-Arab *et al.*, 1999). Therefore, quality control of herbal drugs is essential.

Information on mycobiota associated with crude herbal drug plants is limited (Aziz *et al.*, 1998; El-Shafie *et al.*, 1999; Abou-Arab *et al.*, 1999) or lacking from this part of the world (Mandeel, 2005). The present paper reports on the incidence of natural epiphytic mycoflora contaminating crude herbal drug materials from Bahrain. The study may serve as a reliable bioindicator for the production of mycotoxins by associated potential toxigenic mould fungi to assure public health safety and quality control to the consumers of herbal preparations.

MATERIALS AND METHODS
Plant material collection and preparation
Twenty medicinal plant species were collected during the months of March to April 2002, in their natural habitats from various regions of Bahrain. Based on their frequency of occurrence, ten herbal plant species were collected from cultivated habitats and another ten from desert or uncultivated sites. Each plant material (about 20-25 cm) was gently handpicked using autoclaved paper towel tissue and immediately placed in a sterile polyethylene bag. Sample sizes ranged from 200-500 g, based on their occurrence. These plant species were chosen on the basis of their availability in fields and popularly of usage. Care was taken to avoid badly deteriorated and visibly diseased materials. In the laboratory, plant parts, mostly leaves and stems, were aseptically shade-dried at room temperature (25-30

°C) for five days until complete dryness. The plant parts were individually finely ground in a common household blender. The blender's cup was rinsed in 85% alcohol between samples. The powder was sieved through No. 50 mesh sieve, kept tightly packed in a new paper bags and stored at 5 °C for further analysis, usually within a week. Dr. Dawood Al-Esawi (Department of Biological Sciences, Faculty of Science, University of Jordan, Amman, Jordan) verified and identified the plant materials according to the checklist of El-Oqlah and Abbas (1994). Voucher specimens of dry plant samples were retained at the Herbarium of the Department of Biology, University of Bahrain. Acquisition code numbers, plant parts used and number of samples per plant medicinal plant examined along with other botanical data are listed in Table 1.

Table 1. Description of medicinal plant samples used and number of examined samples.

Serial code	Accession Number[a]	Botanical name	Family	Plant part used	Life form	Collection site	Habitat type[b]	Number of samples
1	DEQ11	*Alhagi maurorum* Medik.	Leguminosae	Leaves &stems	Shrub	Saqyia	C	8
2	DEQ12	*Amaranthus viridis* L.	Amaranthaceae	Leaves	Herb	Ain Adhari	C	6
3	DEQ13	*Andrachne telephioides* L.	Euphorbiaceae	Leaves &stems	Grass	Hamad Town	D	5
4	DEQ14	*Atriplex leucoclada* Boiss.	Chenopodiaceae	Leaves	Herb	Mugshaa	C	9
5	DEQ15	*Chenopodium murale* L.	Chenopodiaceae	Leaves	Herb	Jid Hafs	C	6
6	DEQ16	*Heliotropium kotschyi* (Ledeb.) Gurke	Boraginaceae	Leaves &stems	Herb	Mugshaa	C	8
7	DEQ17	*Prosopis farcta* Macbride	Leguminosae	Leaves	Shrub	Jid Hafs	C	7
8	DEQ18	*Salsola villosa* Det. ex Roem	Chenopodiaceae	Leaves	Herb	Mugshaa	C	5
9	DEQ19	*Sesuvium verrucosum* Raf.	Aizoaceae	Leaves &stems	Shrub	Saqyia	C	8
10	DEQ20	*Suaeda aegyptica* (Hasselq.) Zoh.	Chenopodiaceae	Leaves	Shrub	Zinj	D	6
11	DEQ21	*Suaeda vermiculata* Forssk.	Chenopodiaceae	Leaves	Shrub	Zinj	D	6
12	DEQ22	*Aizoon hispanicum* L.	Aizoaceae	Leaves &stems	Herb	Saar	D	5
13	DEQ23	*Erodium laciniatum* (Cav.) Willd. var. *pulverulentum* (Cav.) Boiss.	Geraniaceae	Leaves	Herb	Sakheer	D	7
14	DEQ24	*Plantago notata* Lag.	Plantaginaceae	Leaves	Herb	Saar	D	6
15	DEQ25	*Fagomia indica* Burm.f.	Zygophyllaceae	Leaves &stems	Herb	Janabya	D	5
16	DEQ26	*Paronychia arabica* (L.) DC.	Caryophyllaceae	Leaves &stems	Herb	Saar	C	8
17	DEQ27	*Neurada procumbens* L.	Rosaceae	Leaves &stems	Herb	Sakheer	D	5
18	DEQ28	*Pulicaria crispa* (Forssk.) Benth. & Hook.f.	Compositae	Leaves &stems	Shrub	Sakheer	D	7
19	DEQ29	*Trigonella anguina* Del.	Leguminosae	Leaves &stems	Herb	Allie	D	5
20	DEQ30	*Malva parviflora* L.	Malvaceae	Leaves	Herb	Karaneh	C	7

[a]Accession numbers are preceded by DEQ for the name of collectors, Drs. Dawwod, Al-Esawi and Qaher.

MYCOLOGICAL ANALYSIS

The total number of fungal colonies was determined using the method of Aziz *et al.* (1998). The method is also in accordance with the recommended methods for mycological examination of foods (Pitt *et al.,* 1992). Ten grams of each plant sample were aseptically transferred into 250 ml screw-capped medicinal bottle containing 90 ml of sterile 0.85% saline solution and were mechanically homogenized at constant speed for 15 minutes. The plant-water suspension was allowed to stand for 10 minutes with intermittent shaking before being aseptically plated. Appropriate ten folds serial dilutions (1:10) were prepared according to the anticipated contamination load, usually till 10^{-4}. One ml aliquots of each dilution were aseptically surface plated onto sterile plastic Petri dishes (9 cm) and distributed uniformly on isolation media with a sterilized glass rod. For isolation, general purpose enumeration media used were freshly prepared Potato Dextrose Agar (PDA), dehydrated Malt Extract Agar (MEA), Dichloran Rose Bengal Chlorotetracycline agar (DRBC) and Czapek Dox Agar (CDA) medium all from Oxoid Ltd., England. To each medium, 0.5 mg chloramphenicol/ml was added to suppress bacterial growth. The control treatment consisted of plating a sterilized plant-water suspension or sterilized water only onto the above media. Five replicates plates per medium were used for each medicinal plant sample. Data expressed are average of mean number of fungal colonies per five plates per medium. Total colony-forming-units per gram dried (cfu/g) samples were also determined. Selection of five random samples tested found infected with ≥5 fungi or *A. flavus* were used to measure total or toxigenic contamination frequency (%), respectively. Plates were incubated in the upright position at 28±1°C for 7- 15 days and examined daily; but counts were recorded only after 4-5 days. After incubation, all plates were examined visually, directly and with a stereo- microscope. Mould colonies representative of all morphologically different types present were individually sub-cultured by hyphal tip method onto PDA, corn yeast agar, CDA and water agar for identification.

IDENTIFICATION

Identification was carried out by cultural and morphological characteristics and followed many taxonomic schemes. For the genus *Aspergillus,* Raper and Fennel (1977) were used, for the genus *Penicillium,* Pitt (1985), and for the genus *Fusarium,* Leslie and Summerell (2005) were used. For most other genera, Domsch *et al.* (1980) and Moubasher (1993) were the primary source texts. Because of their importance as mycotoxigenic moulds, isolates belonging to *F. moniliforme* and *A. flavus* were identified with great care, primarily based on their morphology.

DATA ANALYSIS

To quantify fungal abundance and diversity in the various herbal drugs, data were analyzed as outlined by Mandeel (2005). The abundance of species is expressed as the number of individual occurrences of a species per plant at a given habitat. Relative density (%) of a species is defined as the number of isolates of a given species divided by the total number of fungal isolates. To measure the recovery of species from plants, percentage recovery rates were determined. Percentage recovery is defined as the number of occurrences of a species for each plant divided by the total number of occurrences recovered from all plants at each habitat.

Analysis of biodiversity was based on dominance indices of the Simpson index (*D*) and Shannon- Wiener index (*H*). The Simpson index, $D = 1 - \Sigma (p_i)^2$ and the Shannon-WEAVER index, $H = \Sigma s \ (p_i \ \log_2 p_i)$, where "$p_i$" is the proportion of individuals that species "*i*" in a sampling plots contributes to total. Evenness (*J*), which was defined as, $J = H/H_{max}$, where H_{max} is the maximum value of "*H*" for the number "*S*" of species present. To evaluate

similarity among fungal communities Jaccard similarity index was used. The Jaccard coefficient, $a/(a + b + c)$, where "a" is number of species common to both plants, "b" is number of species found only in soil of the first plant, and "c" is the number of species found only in soil of the second plant.

RESULTS
Mycoflora incidence of cultivated habitat herbal drug plants
A total of 2707 colonies (average 271) from 73 crude plant samples were singled out and identified during the present study (Table 2). The counts fluctuated between as low as 174 in *Prosopis farcta* to as high as 511 in *Atriplex leucoclada*, with 8 and 9 samples of each plant, correspondingly. Plant samples like *Salsola villosa* (356 isolates), *Amaranthus viridis* (332 isolates) and *Chenopodium murale* (301 isolates) also yielded a substantial fungal count, whereas *Paronychia arabica* (180 isolates), *Malva parviflora* (184 isolates) and *Alhagi maurorum* (192 isolates) showed merely comparatively low occurrence level of mycological contamination.

The recovered fungi were classified into 39 species belonging to 17 genera and one non-sporulating fungus; 33 (84.6%) were Hyphomycetes, 3 (7.7%) Zygomycetes and 3 (7.7%) Ascomycetes. The identified species per medicinal plant varied between 15 in *P. arabica* to 24 in *A. leucoclada* with an average of 19.4 species per plant. *A. viridis, C. murale* and *S. verrucosum* all retained 22 species. Toxigenic fungi represented mainly by *Aspergillus flavus* comprised 2.5% of all fungi recorded and in relation to other species of *Aspergillus* genera, by 12.5%.

On the basis of colony appearance, species like *Aspergillus, Penicillium, Chaetomium, Cladosporium* and *Rhizopus* were found to be commonly present in most samples examined. The highest species occurrence was manifested by the genus *Aspergillus* with eight species, followed by Penicillium (4 species), *Cladosporium, Alternaria, Ulocladium, Chaetomium* and *Phoma* (3 species), *Fusarium* (2 species) while, all remaining mycobiota with one species. Other species having frequent association but lower incidence were *Mucor, Trichoderma and Stachybotritis*. The widest spectrum of fungal species was detected in crude plant parts of *A. leucoclada* (24 species), followed by *A. viridis, C. murale* and *Sesuvium verrucosum* (22 species). *P. farcta* and *P. arabica* showed the narrowest species diversity among all herbal drug samples with 16 and 15 species, respectively.

In Table 2, the relative density values (RD) were estimated to determine the abundance classes of isolates of a given species among all herbal drug materials. Accordingly, *C. thermophilum* was the most predominant species with $\geq$ 5% RD (16.43), followed in descending rank by *C. elatum* (9.71), *A. flavus* (7.94) *R. stolonifer* (7.46), *A. niger* (6.87) and *C. sphaerospermum* (5.54). Moderate class fungi with $\geq$ 1-4.99 % RD are revealed, for instance, by species like *M. hiemalis* (3.11), *U. chlamydosporum* (2.77) and *P. herbarum* (1.95). Low occurrence class moulds with $\geq$ 0.1-0.99 RD varied from *S. verrucosum* and *T. viride* to *Helminthosporium nodulosum* with 0.99 and 0.18 RD, respectively. Among all the recovered fungi, only *C. thermophilum* showed 100% occurrence frequency in all the tested plant samples, followed by *A. flavus, R. stolonifer, A. niger* and *C. sphaerospermum* (90%).

A summary account of fungal counts and contamination frequency are presented in Table 3. The highest average total fungal population density was observed in raw samples of *A. leucoclada* (636 cfu/g), followed by *S. villosa* (421 cfu/g). The lowest colony counts were encountered in *P. arabica* (212 cfu/g) and *M. parviflora* (218 cfu/g). The maximum relative contamination frequency from five random plant samples infected with $\geq$ 5 fungi was found

associated with *A. leucoclada* (93.2%), followed in decreasing order by *S. villosa* (89.2%) and *A. viridis* (87.6%), while *P. arabica* showed the minimum mould frequency (46.5%). Also, an appreciable fungal count was recovered from *C. murale* (83.8%) and *S. verrucosum* (72.4%). The incidence of toxigenic moulds *A. flavus* in ≥ 5 random samples fluctuated between as low as 1.9% in crude parts of *S. verrucosum* to as high as 31.3% in *H. kotschyi*.

Table 3. Fungal counts (fu/g) and contamination frequency (%) of cultivated habitat herbal drug plants.

Cultivated Habitat Plants	Fungal colony counts (cfu/g)			Total Species	Total Contamination[a] Frequency (%)	Toxigenic Contamination[b] Frequency (%)
	Min.	Max.	Average			
A. maurorum	136	460	226	19	68.3	NR[c]
A. viridis	283	491	304	22	87.6	11.4
A. leucoclada	447	837	636	23	93.2	7.7
C. murale	219	415	371	22	83.8	17.2
H. kotschyi	198	326	298	18	63.4	31.3
P. farcta	97	310	248	16	52.5	5.8
S. villosa	283	521	421	19	89.2	24.6
S. verrucosum	135	385	236	22	72.4	1.9
P. arabica	88	356	212	15	46.5	6.3
M. parviflora	112	279	218	17	57.2	8.2

[a]Percentage of five random samples tested found contaminated with five or more fungi.
[b]Percentage of five random samples tested found contaminated with *A. flavus*.
[c]NR, Not recovered.

Mycoflora incidence of desert habitat herbal drug plants
A total of 965 colonies from 57 samples were recovered and identified from desert habitat herbal drug plants (Table 4). The lowest fungal load was detained in *Suaeda vermiculata* (42 isolates) while the highest in *Erodium laciniatum* var *pulverulentum* (173 isolates), with 6 and 7 samples of each plant, in that order. The tested plant materials like *Trigonella anguina* (159 isolates), *Andrachne telephioides* (148 isolates) and *Neurada procumbens* (110 isolates) yielded substantial fungal counts, whereas *S. aegyptica* (58 isolates), *Plantago notata* (68 isolates) and *Aizoon hispanicum* (70 isolates) showed merely somewhat low occurrence level.

The singled out colonies were classified into 25 species belonging to 12 genera; 20 (80%) were Hyphomycetes, 2 (8%) Zygomycetes and 3 (12%) Ascomycetes. The identified species per medicinal plant varied between 6 in *S. aegyptica* and *S. vermiculata* to 13 in *E. laciniatum var pulverulentum*. *P. notata, N. procumbens* and *Fagonia indica* all retained 22 species. Mycotoxigenic fungi represented by *A. flavus* comprised 4% of all fungi recorded and in relation to other species of *Aspergillus* genera, by 20%.

Morphologically, mould genera like *Aspergillus, Penicillium, Chaetomium, Cladosporium* and *Rhizopus* were frequently detected in most samples examined. The highest species occurrence arranged in the order of magnitude was apparent by the genus *Aspergillus* with 5

species, followed by *Cladosporium* and *Chaetomium* (3 species), *Penicillium, Fusarium, Alternaria, Ulocladium* and *Phoma* (2 species). Other species having low incidence rates were *H. nodulosum* and *C. herbarum*. The widest spectrum of fungal species was detected in plant parts of *E. laciniatum* var *pulverulentum* (13 species), followed by *A. telephioides* and *A. hispanicum* (12 species). *S. aegyptica* and *S. vermiculata* showed the narrowest species diversity among all herbal drug samples with 6 species only.

Colony abundance evaluated by RD values (Table 4) conveniently facilitates the grouping of the recognized fungi into three classes. Consequently, a high abundance class with ≥ 5% RD encompass the most predominant species *C. thermophilum* (20.41), followed in descending rank by *C. elatum* (11.81), *R. stolonifer* (7.15) and *A. niger* (6.52). Moderate class fungi with ≥ 1-4.99 % RD are manifested, for instance, by species like *P. herbarum* (4.35), *S. verrucosum* (2.27) and *A. chlamydospora* (1.86). Low prevalence class moulds with ≥ 0.1-0.99 % RD varied from *U. chlamydosporum* to *H. nodulosum* with 0.93 and 0.41 RD, respectively. No species were common to all tested plant samples. However, the highest frequently recovered fungus was revealed by *R. stolonifer* (70%), followed by, *C. elatum* and *C. sphaerospermum* (60%), *C. thermophilum A. niger* and *A. flavus* (50%).

The highest average total fungal population density was observed in raw samples of *E. laciniatum* var *pulverulentum* (259 cfu/g; range 210-314 cfu/g) that caused a notable elevation in total fungal number, followed by *N. procumbens* (244 cfu/g) and *A. telephioides* (172 cfu/g) (Table 5). The lowest colony counts were encountered in S. *vermiculata* (39 cfu/g) and *S. aegyptica* (42 cfu/g). The maximum relative contamination frequency was found associated with *T. anguina* (86.7%), followed in decreasing order by *E. laciniatum* var *pulverulentum* (81.6%) and *A. telephioides* (73.8%), while *S. vermiculata* also showed the minimum mould frequency (38.7%). The incidence of toxigenic mould *A. flavus* varied between the least (3.2%) in crude parts of *S. vermiculata* to highest (11.7%) in *E. laciniatum* var *pulverulentum*.

Table 5. Fungal counts (cfu/g) and contamination frequency (%) of desert habitat herbal drug plants

Desert Habitat Plants	Fungal colony counts (cfu/g)			Total Species	Total Contamination[a] Frequency (%)	Toxigenic Contamination[b] Frequency (%)
	Min.	Max.	Average			
A. telephioides	103	297	172	13	73.8	4.1
A. hispanicum	46	123	136	12	70.1	NR
E. laciniatum var. *pulverulentum*	210	314	259	12	81.6	11.7
P. notata	33	161	87	10	63.3	NR
F. indica	52	147	128	10	57.3	NR
N. procumbens	49	332	244	10	60.9	8.8
P. crispa	28	95	76	10	52.0	NR
T. anguina	96	223	150	14	86.7	NR
S. aegyptica	38	111	42	6	44.8	4.9
S. vermiculata	15	74	39	6	38.7	3.2

Similarity and diversity characteristics of both habitat plants
Pairwise comparisons to determine similarity among species composition between the various herbal drugs plants were based on Jaccard similarity coefficients. For medicinal plants from cultivated habitats (Table 6), the highest similarity coefficient combinations were

mainly noted between *A. viridis* and the following plants, in the order of, *S. villosa* (0.27), *H. kotschyi* (0.26) and *C. murale* (0.25). Maximum similarity coefficients in species composition between plants from desert locations (Table 7) were observed in the *P. crispa* and *F. indica* combinations (0.23).

Table 6. Jaccard's Similarity Coefficients for the mycobiota of medicinal plants from cultivated habitats

	S. villosa	A. viridis	C. murale	H. kotschyi	S. verrucosum	A. maurorum	M. parviflora	P. arabica	P. farcta
A. leucoclada	0.22	0.20	0.21	0.21	0.24	0.22	0.25	0.17	0.24
S. villosa		0.27	0.24	0.23	0.20	0.22	0.20	0.24	0.15
A. viridis			0.25	0.26	0.23	0.21	0.22	0.22	0.17
C. murale				0.15	0.24	0.21	0.18	0.22	0.19
H. kotschyi					0.22	0.18	0.20	0.23	0.17
S. verrucosum						0.23	0.20	0.16	0.22
A. maurorum							0.18	0.22	0.17
M. parviflora								0.20	0.18
P. arabica									0.18

Table 7. Jaccard's Similarity Coefficients for the mycobiota of medicinal plants from desert habitats

	T. anguina	E. laciniatum var. pulverulentum	N. procumbens	F. indica	A. hispanicum	P. notata	S. aegyptica	P. crispa	S. vermiculata
A. telephioides	0.20	0.21	0.20	0.20	0.16	0.20	0.17	0.17	0.13
T. anguina		0.21	0.22	0.14	0.16	0.20	0.09	0.11	0.13
E. laciniatum var. pulverulentum			0.15	0.18	0.11	0.18	0.21	0.15	0.21
N. procumbens				0.00	0.15	0.2	0.16	0.04	0.15
F. indica					0.21	0.09	0.11	0.23	0.11
A. hispanicum						0.15	0.05	0.21	0.10
P. notata							0.11	0.16	0.11
S. aegyptica								0.11	0.14
A. telephioides									0.13

Two separate indices, the Simpson index (*D*) and the Shannon-Wiener (*H)* diversity index, were used to determine the fungal species diversity in the various studied plants (Tables 8 and 9). Mycobiota species of the of the herbal drug plant *A. leucoclada* from the cultivated habitat (Table 8) exhibited the highest SIMPSON (0.930) and Shannon-Wiener index (2.856), followed by *M. parviflora* (0.913, SIMPSON) and *S. verrucosum* (2.648, SHANNON-Wiener). Likewise, the recorded Shannon-Wiener index was also high in *C. murale* (2.602) with evenness (*J*) measure of (0.841). Medicinal plants from desert areas exhibited somewhat lower diversity indices compare to plants from cultivated habitats (Table 9). The herbs *A. hispanicum* followed by *A. telephioides* encountered the highest Simpson index value (0.892 and 0.872), Shannon-Wiener (2.328 and 2.262) and evenness (0.937 and 0.881).

Table 8. Biodiversity characteristics of mycobiota of medicinal plants from cultivated habitats

	Species Richness	Isolate Range	Simpson's Diversity (*D*)	Shannon-Wiener Index (*H*)	Evenness (*J*)
A. leucoclada	23	1-69	0.930	2.856	0.910
S. villosa	19	1-82	0.884	2.500	0.849
A. viridis	22	1-89	0.881	2.574	0.832
C. murale	22	2-72	0.894	2.602	0.841
H. kotschyi	18	1-62	0.875	2.376	0.822
S. verrucosum	22	1-34	0.912	2.648	0.856
A. maurorum	19	1-44	0.892	2.512	0.853
M. parviflora	17	1-26	0.913	2.569	0.906
P. arabica	15	2-48	0.866	2.305	0.851
P. farcta	16	1-33	0.884	2.386	0.860

Table 9. Biodiversity characteristics of mycobiota of medicinal plants from desert habitats

	Species Richness	Isolate Range	Simpson's Diversity (*D*)	Shannon-Wiener Index (*H*)	Evenness (*J*)
A. telephioides	13	1-43	0.872	2.262	0.881
T. anguina	14	1-47	0.836	2.108	0.799
B. laciniatum var. pulverulentum	12	1-68	0.740	1.793	0.721
N. procumbens	10	2-23	0.848	2.021	0.877
F. indica	10	1-23	0.845	2.004	0.870
A. hispanicum	12	1-12	0.892	2.328	0.937
P. notata	10	1-15	0.867	2.123	0.922
S. aegyptica	6	1-22	0.725	1.423	0.794
P. crispa	10	1-9	0.867	2.141	0.930
S. vermiculata	6	2-12	0.801	1.687	0.941

DISCUSSION

Data interpretation of most studies concerning mould spoilage incidences of food commodities are usually based on the origin of fungal contamination source (Aziz *et al.*, 1998; Abou-Arab *et al.*, 1999). The source can either be open (field fungi) or closed (transit and storage fungi and packaging method) system. Field fungi infect and colonize developing or mature plant parts like seeds while in the field. Mould genera like *Alternaria, Helminthosporium, Fusarium* and *Cladosporium* represent this category (Tables 3 and 5). Storage moulds, nevertheless, are those that can be encountered on plant parts at relatively high moisture conditions found in store products. Those fungi are principally species of *Aspergillus, Rhizopus, Mucor* and *Penicillium*. Evidently, the singled out mycobiota in this study are principally field fungi, since the crude plant samples were not allowed for storage. However, few of the storage fungi were commonly recovered among most of the crude samples. The source(s) of some of these fungi, in particular, *A. flavus* and *R. stolonifer* with high occurrence frequency (Tables 2 and 4), is not precisely known. It is probable that due to some factors like cross contamination or an insect damage and lack of surface disinfection of the material during processing may have accounted for such species of storage category. Pitt *et al.* (1993) extensively investigated mycoflora of food commodities from Thailand and compared the fungal profile of peanuts kernels before and at retail. They attributed the sharp decline in flora assemblage (e.g. *Chaetomium, Fusarium, Penicillium* and *Aspergillus*) prior

to retail to the infection and penetration of kernels before or soon after harvest, but not during storage. These findings are in harmony with those reported by Chourasia (1995) who indicated that contamination of crude herbal drugs samples with *A. flavus* occurred as a result of insect infestation in the field and during transportation. Insects in such cases operate as biological vectors to transmit and spread fungal propagates. Nonetheless, Efuntoye (1996) found that fungi associated with fresh drug plants were less and disappeared gradually, whereas high and different mycobiota incidence appeared during storage that was considered as post harvest contaminants rather than being normal flora of the plant parts in the field. Moreover, method and type of packing had profound effect on mould concentration and contamination level. In this regard, Abou-Arab *et al.* (1999) while contrasting the effect of packing on mould contamination found that mould genera like *Penicillium* spp., especially, were recovered more frequently and were also predominant in packed samples. The higher contamination levels in these packed samples were attributed to rise of humidity inside the pack and also unsuitable hygienic methods of handling and storing of the pack.

Data in this report clearly revealed that mould profile and intensity of crude herbal drug plants from arid cultivated habitats (Tables 2) are much higher (2707 isolates) and diverse (39 species) than those of desert habitat samples (965 isolates and 25 species) (Tables 4). Moreover, all species found associated with desert habitat plants were essentially correspondingly encountered in cultivated habitat plants, except *F. chlamydosporum* in desert location was substituted by *F. oxysporum* in cultivated habitat plants. All the three *Chaetomium* spp., *C. globosum*, *C. thermophilum* and *C. elatum* were of common occurrence to both habitats. Given the nature of limited geographical area of the island of Bahrain, the dominated vegetation cover, and the prevailing arid environmental conditions may, in part, explain the co occurrence of similar phylloplane microbial spectrum in both habitats. Contamination of standing plant parts in the field is mainly influenced by soil and air. Domsch *et al.* (1980) concluded "contamination of foodstuffs with spoilage fungi was a result of natural extraneous pollution with dust particles containing spores following storage at normal conditions". Also, other practices like harvesting, handling and transit instigate additional spore load (Pitt and Hocking, 1997). These findings are in agreement with those reported by Abou-Arab *et al.* (1999) who indicated that pollution in irrigation water, atmosphere, soil, sterilization methods and storage conditions, all play an important role in contamination of medicinal plants by mould flora.

From the samples examined in this study, the highest average population density of the recovered moulds from cultivated habitat plants was noted in *A. leucoclada* and the lowest in *P. arabica* (Table 3), while from desert habitat plants varied from 259–39 for *E. laciniatum* var *pulverulentum* and *S. vermiculata,* respectively. These total fungal counts of crude plant parts appear to be analogous or lower than those reported for other similar herbal drug materials in other countries (King *et al.*, 1981; Garcia *et al.* 2001; Halt, 1998; Pitt *et al.,* 1993; El-Shafie *et al.,* 1999). However, variations in the encountered species assemblages observed by other workers and this study can be explained due to the differences in the plant origin examined (Pitt *et al.,* 1993), harvesting and storage conditions (Misra, 1981; Efuntoye, 1996), mycological assay used (Pitt *et al.,* 1992; Kneifel and Berger, 1994) and differences in the chemical composition of the herbal plants (Abbas and Al-Saleh, 2002; Mandeel and Taha, 2005).

Patterns of species prevalence in the current study as measured by relative density (%) values facilitated categorizing of the singled out mycobiota into three main classes. Consequently, *C. thermophilum* was the most predominantly recovered field fungus, followed by *C. elatum*

representing the foremost class from crude plant parts of both habitats (Tables 2 and 3). *C. thermophilum* manifested an absolute occurrence frequency (100%) in cultivated habitat plant parts (Table 6) and half of that in desert habitat plants. Both fungi substantially increased the overall load of fungal counts. These species were also reported from various food commodities (King *et al.*, 1981; Pitt *et al.*, 1993), spices (Chourasia, 1995; Mandeel, 2005) and herbal medicinal plants (El-Shafie *et al.*, 1999). They are very vigorous ascomycetous saprophytic cellulose-decomposers soil fungi, coprophilous on the dung of herbivorous animals and phylloplane inhabitants especially at crown zone area. *C. thermophilum,* especially, is a thermo-xerophilic fungus that has a maximum limit of up to 55 C (Moubasher, 1993). In addition to the above findings, species of *A. flavus*, *R. stolonifer A. niger* as well as *C. sphaerospermum* were also dominant and frequently recovered moulds among most of the evaluated samples from both habitats (Table 6). Comparable findings were noted elsewhere (Abeywickrama and Bean, 1991; Aziz *et al.*, 1998; Halt, 1998; Efuntoye, 1996; Garcia *et al.*, 2001; Martins *et al.*, 2001). Mycobiota with moderate occurrence class are restricted to 14 and 15 species in desert and cultivated habitat crude plant parts, respectively. Some of the most important commonly shared notorious genera are dominated by, *A. clavatus, A. alternata, F. moniliforme, P. chrysogenum, A. fumigatus* and *A. terrus,* all being air-borne cosmopolitan mycoflora characterized by massive sporulation capacity. Fast growing sugar fungi like *Mucor hiemalis* was present in low numbers except in *A. leucoclada* (23 isolates) and *S. verrucosum* (22 isolates). Among the most notable moulds within the lowermost occurrence class from both desert and cultivated habitat plants are species of *Cladosporium* and *Ulocladium*. Reports showed that these are emblematic phylloplane microbes on various plant families and are worldwide in distribution (King *et al.*, 1981; Martins *et al.*, 2001; Mandeel, 2005). They are primary saprophytic decomposers of organic matter in soil with broad nutritional range of substrates. In arid zone these species are featured by slow vegetative growth but spore germination at low water potential and high temperature and salinity ranges, thus regarded as thermo-osmotolerant (Domsch *et al.*, 1980; Moubasher, 1993).

Among the detected mycotoxigenic moulds, the predominance of *A. flavus* in most of the examined herbal drug samples coincide with those reported by Halt (1998), Aziz *et al.* (1998), Abou-Arab (1999) and El-Shafie *et al.* (2002). Overall, higher contamination incidence of *A. flavus* was observed in cultivated habitat plants than in desert habitat plants (Tables 3 and 5). Also, *A. flavus* alone accounted for 2.5% of the total fungi recovered from all samples of cultivated habitat materials. The presence of other equally important toxigenic moulds e.g. *A. niger, T. viride* and *F. moniliforme,* reported in this study, is alarming. Toxigenic strains of these fungi have been known to produce several toxic and carcinogenic metabolites like aflatoxins, protocatechuic acid, oxalic acid and fumonisins (Lwellyn *et al.*, 1990; Abeywickrama and Bean, 1991; Aziz *et al.*, 1998; Halt, 1998). This study did not attempt to examine medicinal plants and their co-occurring fungi for the production of mycotoxins. However, a potential health hazard exists for spoilage and mycotoxigenesis if an inappropriate handling occurs during harvest, transport and storage (8, 9). Roy and Chourasia (1990), Chourasia (1995), Aziz *et al.* (1998) and Halt (1998) and have recently documented the occurrence of aflatoxins B1, B2, G1, G2, zearalenone and other toxins in herbal drugs contaminated samples by similar fungi. On the contrary, several reports revealed the absence of aflatoxins in crude plant samples, even though these samples were heavily contaminated with *Aspergillus spp.* Similarly, the presence of toxigenic moulds in food commodities does not necessarily validate the presence of mycotoxins. For example, Halt (1998), Abou-Arab *et al.* (1999) and El-Shafie *et al.* (2002) while screening spices and medicinal plants for the occurrence of toxins have not detected aflatoxins on the examined samples but isolated

aflatoxigenic strains of *A. flavus*. They concluded that samples are not ideal substrates for aflatoxin formation, due to their essential oils, which may prevent the toxin production. These results are substantiated by the findings of Lwellyn *et al.* (1990) who concluded that toxin accumulation in plants is indicative of contamination following harvesting and drying.

In conclusion, this survey has shown that mycoflora density and diversity profile of the examined herbal drug plants is analogous to or lower than those reported elsewhere in the world. The contamination incidence was mainly dominated by field fungi. Also, the presence of potentially mycotoxigenic fungi, especially like *A. flavus* and *F. moniliforme* in these crude samples constitute a health hazard. Care in processing, packaging, storage and handling of plant materials, destined for human use, is vital to reduce possible toxigenic moulds and the production of hazardous and alarming mycotoxins. Consequently, such products should pass through strict quality control inspection and decontamination methods to reduce the likelihood that such plant materials remain free of mycotoxins. Although several potentially mycotoxigenic fungi were isolated during the present study, neither the foodstuff nor the fungi were assayed for the presence of these toxins. Chourasia (1995) concluded that it is rather unexpected to eliminate all traces of mycotoxins from crude and finished herbal drugs, but definite hygienic measures coupled with modern storage facilities will without doubt confine it to the minimal level.

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REFERENCES

1. Abbas, J. A. and Al-Saleh, F. S. (2002) "Medicinal plants of Bahrain". Bahrain, University of Bahrain Press, Bahrain, pp. 290.
2. Abeywickrama, K. and Bean, G. A. (1991) "Toxigenic Aspergillus Flavus and Aflatoxins in Sri Lankan Medicinal Plant Material". Mycopathologia 113: 187-190.
3. Abou-Arab, A. A. K., Kawther, M. S., El Tantawy, M. E., Badeaa, R. I. and Khayria, N. (1999) "Quantity Estimation of Some Contaminants in Commonly Used Medicinal Plants in the Egyptian Market". Food Chemistry 67: 357-363.
4. Aziz, N. H., Youssef, Y. A., El-Fouly, M. Z. and Moussa, L. A. (1998) "Contamination of Some Common Medicinal Plant Samples and Spices by Fungi and their Mycotoxins". Botanical Bulletin of Academica Sinica 39: 279-285.
5. Chourasia, H. K. (1995) "Mycobiota and Mycotoxins in Herbal Drugs of Indian Pharmaceutical Industries". Mycological Research 99: 697-703.
6. Domsch, K. H., Gams, W. and Anderson, T. H. (1980) "Compendium of Soil Fungi". (Vol. 1), pp. 857 & (Vol. II), pp. 405, Academic Press, London.
7. Efuntoye, M.O. (1996) "Fungi Associated with Herbal Drug Plants During Storage". Mycopathologia 136: 115-118.
8. El-Oqlah, A. A. and Abbas, J. A. (1994) "A Checklist of Vascular Plants of Bahrain". Dirasat 6: 95-118.
9. El-Shafie, E. A., Al-Lawatia, T. and Al-Bahry, S. (1999) "Fungi Associated with Black Tea in the Sultanate of Oman". Mycopathologia 145: 89-93.
10. Elshafie, E. A., Al-Rashdi, T. A., Al-Bahry, S. N. and Bakheit, C. S. (2002) "Fungi and Aflatoxins Associated with Spices in the Sultanate of Oman". Mycopathologia 155: 155-160.

11. Garcia, S., Iracheta, F. and Heredia, N. (2001) "Microbiological Survey of Retail Herbs and Spices from Mexican Markets". Journal of Food Protection 54: 99-103.

12. Halt, M. (1998) "Moulds and Mycotoxins in Herb Tea and Medicinal Plants". European Journal of Epidemiology 4: 269-274.

13. King, A. D., Hocking, A. D. and Pitt, J. I. (1981) "The Mycoflora of Some Australian Food". Food Technology 33: 55-60.

14. Kneifel, W. and Berger, E. (1994) "Microbial Criteria of Random Samples of Spices and Herbs Retailed on the Australian Market". Journal of Food Protection 57: 893-901.

15. LESLIE, J. F. and Summerell, B. (2005) "The Fusarium Laboratory Manual". Blackwell Publication, Iowa, USA, pp 300.

16. Lwellyn, G., Mooney, C., Cheatle, R. L. and Flanningan, B. (1990) "Mycotoxin Contamination of Spices" – An update. International Biodeterioration and Biodegradation 29: 111-121.

17. Mandeel, Q. A. and Taha, A. (2005) "Assessment of in Vitro Antifungal Activities of Various Extracts of Indigenous Bahraini Medicinal Plants". Pharmaceutical Biology 43: 164-172.

18. Mandeel, Q. A. (2005) "Fungal Contamination of Some Imported Spice". Mycopathologia 159: 291-298.

19. Martins, H. M., Martins, M. L., Dias, M. I. and Bernardo, F. (2001) "Evaluation of Microbiological Quality of Medicinal Plants Used in Natural Infusions". International Journal of Food Microbiology 68: 149-153.

20. Misra, N., (1981) "Influence of Temperature and Relative Humidity on Fungal Flora of Some Spices in Storage". Z. Lebensm Unters Forsch 172(1): 30-31.

21. Moubasher, A. H. (1993) "Soil Fungi in Qatar and Other Arab Countries". University of Qatar, Qatar, PP. 566.

22. Pitt, J. I., Hocking, A. D., Bhudhasamai, K., Misccamble, B. F., Wheeler, K. A. and Tanboon-Ek, P. (1993) "The Normal Mycoflora of Commodities from Thailand". 1. Nuts and oilseeds. International Journal of Food Microbiology 20: 211-226.

23. Pitt, J. I., Hocking, A. D., Samson, R. A. and King, A. D. (1992) "Recommended Methods for Mycological Examination of Foods". In: Modern Methods in Food Mycology (R. A. Samson, A. D. Hocking, J. I. Pitt and A. D. King, Editors), pp. 365-368, Elsevier, Amsterdam.

24. Pitt, J. I. and Hocking, A. D. (1997) "Fungi and Food Spoilage". Aspen Publishers, Gaithersburg, USA, pp. 593.

25. Pitt, J. I. (1985) "A Laboratory Guide to Common Penicillium Species". North Ryde, Australia, pp. 184 pp.

26. Raper, K. B. and Fennel, D. P. (1977) "The Genus Aspergillus". Krieger Publishing Company, Huntington, New York.

27. Roy, A. K. and Chourasia, H. K. (1990) "Mycoflora, Mycotoxin Producibility and Mycotoxins in Traditional Herbal Drugs from India". Journal of Genetics and Applied Microbiology 36: 295-302.

Table 2. Distribution of mycobiota in samples of crude herbal plant parts collected from cultivated habitats.

Fungal Species[a]	Cultivated Habitat Plants										Total	Relative Density (%)
	A. leucoclada	*S. villosa*	*A. viridis*	*C. murale*	*H. kotschyi*	*S. verrucosum*	*A. maurorum*	*M. parviflora*	*P. arabica*	*P. farcta*	Abundance	Density (%)
Chaetomium thermophilum	69 (8/9)[b]	82 (4/5)	89 (6/6)	72 (6/6)	8 (4/8)	22 (6/8)	13 (6/8)	10 (5/7)	48 (7/8)	32 (7/8)	445	16.43
Chaetomium elatum	46 (7/9)	63 (2/5)	51 (4/6)	25 (4/6)		34 (6/8)	44 (7/8)	17 (4/7)	10 (5/8)	8 (6/8)	263	9.71
Aspergillus flavus	16 (5/9)	42 (3/5)	26 (4/6)	32 (4/6)	62 (4/8)	2 (3/8)	26 (4/8)		23 (6/8)	24 (6/8)	215	7.94
Rhizopus stolonifer	28 (5/9)	9 (3/5)	15 (5/6)	31 (4/6)	27 (6/8)	19 (6/8)	26 (4/8)	3 (3/7)	23 (6/8)	24 (6/8)	202	7.46
Aspergillus niger	17 (6/9)	12 (2/5)	20 (5/6)	13 (4/6)	26 (5/8)	8 (4/8)	11 (5/8)		14 (4/8)	33 (5/8)	186	6.87
Cladosporium sphaerospermum	37 (4/9)	14 (4/5)	12 (4/6)	22 (4/6)	43 (6/8)	5 (4/8)	10 (5/8)	20 (6/7)	7 (3/8)		150	5.54
Aspergillus clavatus	23 (5/9)	14 (2/5)	12 (4/6)	9 (3/6)	14 (5/8)			26 (3/7)	21 (5/8)	33 (5/8)	133	4.91
Chaetomium globosum	22 (4/9)	17 (3/5)	11 (3/6)	19 (3/6)		14 (4/8)				8 (5/8)	92	3.39
Penicillium chrysogenum	23 (4/9)		15 (3/6)		17 (3/8)	4 (2/8)	8 (3/8)	19 (4/7)			85	3.14
Mucor hiemalis	23 (4/9)		6 (2/6)		4 (5/8)	22 (5/8)	13 (4/7)	13 (4/7)		16 (3/8)	84	3.11
Ulocladium chlamydosporum	20 (3/9)	13 (2/5)	7 (2/6)	2 (2/6)			15 (4/8)	18 (5/7)			75	2.77
Stachybotrys chartarum	20 (4/9)			9 (2/6)		26 (4/8)	13 (1/8)			6 (3/8)	74	2.73
Alternaria alternata	40 (5/9)	8 (3/5)	6 (4/6)		6 (3/8)				3 (3/8)	4 (5/8)	67	2.47
Aspergillus fumigatus	26 (5/9)	9 (3/5)				3 (4/8)	14 (5/8)	12 (5/7)			64	2.36
Fusarium moniliforme		21 (3/5)		10 (2/6)			14 (4/8)	15 (3/7)	4 (5/8)	5 (3/8)	58	2.14
Phoma herbarum	19 (5/9)		8 (3/6)		3 (3/8)	8 (4/8)			23 (5/8)		53	1.95
Alternaria tenuissima			2 (3/6)	16 (3/6)	6 (2/8)	19 (6/8)					43	1.58
Fusarium oxysporum	19 (5/9)	17 (3/5)	11 (3/6)		23 (3/8)	1 (3/8)					43	1.58
Stemphylium botryosum			12 (3/6)		10 (3/8)					10 (4/8)	38	1.42
Aspergillus terreus	8 (3/9)	12 (3/5)		8 (3/6)		5 (3/8)				10 (4/8)	35	1.29
Humicola grisea	9 (3/9)							12 (4/7)		13 (4/8)	33	1.21
Penicillium verrucosum	6 (3/9)			3 (2/6)		2 (4/8)	7 (4/8)			6 (3/8)	27	0.99
Trichoderma viride	6 (3/9)	10 (1/5)			3 (3/8)		1 (2/8)		7 (2/8)		27	0.99
Cladosporium herbarum	8 (4/9)					4 (4/8)	2 (2/8)	4 (3/7)		6 (4/8)	24	0.88
Alternaria chlamydospora	14 (4/9)							7 (3/7)		1 (3/8)	22	0.81
Ulocladium atrum		6 (1/5)	10 (2/6)		3 (3/8)		4 (3/8)				19	0.71
Penicillium citrinum		7 (2/5)	3 (1/6)	4 (3/6)			4 (3/8)		6 (4/8)		18	0.66
Phoma leveillei		2 (1/5)	7 (1/6)		1 (2/8)		1 (2/8)		6 (4/8)		17	0.62

Species											Total number of isolates	%
Ulocladium chartarum				4 (3/6)		13 (4/8)					17	0.62
Phoma sorghina		9 (2/5)		5 (2/6)							14	0.51
Aspergillus egyptiacus			5 (1/6)	2 (3/6)			1 (2/8)		5 (2/8)		13	0.48
Aspergillus versicolor	1 (2/9)	1 (2/5)		2 (1/6)			3 (2/8)	1 (/7)	5 (3/8)		13	0.48
Cladosporium cladosporioides			3 (2/6)	2 (3/6)		1 (2/8)	3 (4/8)	1 (3/7)	2 (3/8)		12	0.44
Fennellia flavipes				8 (3/6)		3 (2/8)					11	0.46
Aspergillus sulphureus	4 (3/9)				2 (4/8)			2 (4/7)			8	0.29
Paecilomyces variotii	4 (2/9)			3 (2/6)						1 (4/8)	8	0.29
Penicillium expansum			1 (1/6)		2 (3/8)	1 (3/8)		4 (3/7)			8	0.29
Sterile mycelia	5 (1/9)					1 (2/8)					6	0.22
Helminthosporium nodulosum							2 (3/8)		2 (3/8)	1 (2/8)	5	0.18
Total number of isolates	511	356	332	301	260	217	192	184	180	174	2707	100%

[a] Species are arranged in decreasing order of the total abundance obtained.
[b] The numbers in parentheses are the number of samples positive to fungi/total samples examined.

Table 4. Distribution of mycobiota in samples of crude herbal plant parts collected from desert habitats.

Fungal Species[a]	Desert Habitat Plants										Total Abundance	Relative Density (%)
	A. telephioides	T. anguina	E. laciniatum var. pulverulentum	N. procumbens	F. indica	A. hispanicum	P. notata	S. aegyptica	P. crispa	S. vermiculata		
Chaetomium thermophilum	43 (6/7)[b]	47 (4/5)	68 (5/5)		17 (4/5)			22 (4/6)			197	20.41
Chaetomium elatum	23 (5/7)	29 (4/5)	22 (4/5)	18 (4/5)				16 (3/6)		6 (3/6)	114	11.81
Rhizopus stolonifer	4 (3/7)	22 (4/5)	2 (2/5)	8 (3/5)		12 (4/5)	15(4/6)			6 (4/6)	69	7.15
Aspergillus niger.	15 (3/7)			21 (2/5)		9 (3/5)	6 (4/6)	12 (4/6)			63	6.52
Aspergillus flavus	21 (5/7)		6 (2/5)	18 (3/5)				6 (3/6)		9 (2/6)	60	6.21
Cladosporium sphaerospermum	13 (3/7)	17 (3/5)		10 (3/5)		8 (2/5)	7 (2/6)		5(4/7)		60	6.21
Chaetomium globosum	16 (5/7)	13 (3/5)	19 (4/5)		10 (5/5)						58	6.01
Phoma herbarum			7 (3/5)		23 (3/5)		8 (2/6)	1 (2/6)	3(3/7)		42	4.35
Aspergillus clavatus		5 (3/5)		23 (3/5)			8 (2/6)				36	3.73
Phoma sorghina	2 (2/7)		4 (1/5)		14 (3/5)	1 (1/5)	1 (1/6)		7(3/7)		29	3.00
Aspergillus fumigatus			9 (3/5)				2 (2/6)		3(3/7)	12 (2/6)	26	2.69
Stemphylium botryosum	7 (1/7)	6 (3/5)	3 (1/5)				9 (2/6)				25	2.59
Penicillium verrucosum	6 (2/7)				4 (2/5)	3 (1/5)			9(2/7)		22	2.27
Fusarium moniliforme		6 (3/5)			1 (1/5)	8 (2/5)			7(3/7)		22	2.27
Mucor hiemalis	11 (5/7)				3 (2/5)	7 (3/5)			1(2/7)		22	2.27
Ulocladium atrum		4 (2/5)		6 (2/5)			9 (3/6)				19	1.96
Alternaria alternata.					12 (4/5)	3 (1/5)			3(2/7)		18	1.86
Alternaria chlamydospora					9 (3/5)	7 (2/5)				2 (1/6)	18	1.86
Cladosporium cladosporioides	11 (4/7)	1 (2/5)					3 (1/6)		2(2/7)		17	1.76
Aspergillus terreus		2 (1/5)	4 (1/5)		1 (1/5)					7 (2/6)	14	1.45
Penicillium chrysogenum		3 (2/5)	1 (2/5)	2 (1/5)		4 (2/5)					10	1.03
Ulocladium chlamydosporum	1 (2/7)			2 (1/5)		6 (4/5)					9	0.93
Fusarium chlamydosporum			3 (2/5)					1 (2/6)	3(4/7)		7	0.72
Cladosporium herbarum		2 (2/5)				2 (1/5)					4	0.41
Helminthosporium nodulosum		2 (2/5)		2 (1/5)							4	0.41
Total number of isolates	173	159	148	110	94	70	68	58	43	42	965	100%

[a]Species are arranged in decreasing order of the total abundance obtained.

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الفلورا الفطرية للنباتات الطبية في البحرين

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ملخص

يعنى هذا البحث بدراسة الفلورا الفطرية لعشرين نوعاً من النباتات العشبية الطبية غير المصنعة جمع نصفها من المناطق الزراعية ونصفها الأخرى من بيئات صحراوية. وقد تم عزل المستعمرات الفطرية من مائة وثلاثين عينة لنباتات طبية باستخدام طريقة التخفيف العشري زرعت على أوساط استنبات مختلفة. وتوصلت الدراسة إلى أن أكثر كثافة عددية ونوعية للفطريات وجدت في العينات المأخوذة من مناطق زراعية بمجموع 2707 عزلة/جرام توزعت على 39 جنسا فطريا مقابل 965 عزلة/جرام توزعت على 25 جنسا فطريا لعينات البيئات الصحراوية. ولوحظ أن أكثر النباتات الطبية تلوثاً بالفطريات في المناطق الزراعية هو الرغل بمعدل 636 مستعمرة/جرام، ومن النباتات الصحراوية الكرش بمعدل 259 مستعمرة/جرام. كما لوحظ أن أعلى نسبة تقريبية للتلوث لخمس عينات نباتية عشوائية مصابة بخمسة أو أكثر من الفطريات وجدت في نفس النبات السابق من المناطق الزراعية بنسبة 93,2% ونبات الحلبة البرية بنسبة 86,7% من البيئات الصحراوية. ووجد أن الفطريات من النوع الحقلي سائدة على بقية الأنواع. إضافة لذلك كان نفس النبات السابق الذكر الأكثر تنوعاً حيوياً استنادا لمؤشر سمبسون (0,93) وشانون – واينر (2,865). ووجد أيضاً أن أكثر الأجناس الفطرية شيوعاً في المناطق الزراعية والصحراوية هو فطر الشيتاميوم يليه الأسبرجلس ثم الرايزوبس ثم الكلادوسبوريوم. وحيث إن هذه الأجناس وجدت بنسبة 70%، لذا فإن تواجدها أدى للزيادة الإجمالية للفطريات المعزولة في هذه الدراسة. أن نسبة الإصابة بالفطريات السامة متمثلة في فطر الأسبرجلس فلافس في خمس أو أكثر عينات عشوائية تتفاوت بين 1,9% إلى 31,3% لنباتات المناطق الزراعية و 2,3% إلى 11,7% لنباتات المناطق الصحراوية. وتخلص النتائج إلى أن نوعية الفطريات المعزولة في هذه الدراسة وكثافتها تتشابه مع الفلورا الفطرية لعينات من نباتات طبية مماثلة في دراسات أخرى. كما أن الفطريات الملوثة للنباتات الطبية تشكل خطراً صحياً محتملا لاسيما عندما تستخدم للاستهلاك البشري. وبالتالي فإن هناك حاجة لوجود رقابة نوعية ميكروبيولوجية على النباتات الطبية المصنعة قبل وصولها للمستهلك أو استعمالها في الصناعات الدوائية.