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Contamination of some common medicinal plant samples and spices by fungi and their mycotoxins

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Abstract. A total of 84 medicinal plant samples and spices were examined for the contamination of molds and mycotoxins. Ten fungal genera of different taxonomic groups were detected. *Aspergillus flavus*, *A. parasiticus*, *A. niger*, *Fusarium oxysporum*, and *Penicillium viridicatum* occurred most often on the medicinal plant samples. Direct determination of mycotoxins in medicinal plant samples revealed aflatoxin B_1 in 17 samples at an average of from 10 to 160 μgkg^{-1}, ochratoxin-A in 3 samples at an average of from 20 to 80 μgkg^{-1}, and no detection of penicillic acid, zearalenone, or T-toxin. *Aspergillus flavus*, *A. parasiticus*, and *A. oryzae* were aflatoxin-producers, whereas, *A. ochraceus*, *P. viridicatum*, and *P. variable* were ochratoxin-A producers. In addition *P. viridicatum*, *P. chrysogenum*, and *P. commune* were penicillic acid-producers. The molds produced high concentrations of mycotoxins in synthetic medium and low to zero concentrations in the medicinal plants.

Keywords: Medicinal plants; Mycoflora; Mycotoxins; Spices; Toxigenic fungi.

Introduction

Mycotoxin contamination of various foodstuffs and agricultural commodities is a major problem in the tropics and subtropics, where climatic conditions and agricultural and storage practices are conducive to fungal growth and toxin production. Mycotoxins are secondary metabolites of mold fungi identified in many agricultural products screened for toxigenic molds (Aziz, 1987; Clevsrton and Ljunggren, 1985; Van Egmond, 1981). *Aspergillus flavus*, *A. candidus*, *A. niger*, *A. luchuensis*, *A. ochraceus*, *A. nidulans*, *F. moniliforme*, *F. oxysporum*, *Alternaria alternata*, *Curvularia* spp., *Chaetomium* sp., *Penicillium citrinum*, and *Rhizopus stolonifer* were reported as the most common fungi isolated from drug plants (Ayres et al., 1980; Aziz and Youssef, 1991; Misra, 1981; Roy et al., 1988; Takatori et al., 1977). The occurrence of aflatoxin in medicinal plants has already been established (Rani and Singh, 1990; Roy and Chourasia, 1990). Mycotoxins have been reported to be carcinogenic, teratogenic, tremorogenic, haemorrhagic, and dermatitic to a wide range of organisms and to cause hepatic carcinoma in man (Refai, 1988; Wary, 1981).

In Egypt, different medicinal plant samples and spices have been imported from India during the last five years. Due to the rise of an Egyptian market for these plants, the present studies have been carried out in the interests of public health and safety.

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The present investigation reports the association of mycoflora with medicinal plant samples and spices, their screening for mycotoxin producing ability, and mycotoxin occurrence in plant samples under Egyptian environmental conditions.

Materials and Methods

Samples

A total of 84 samples of fourteen different medicinal plants and spices imported from India were collected from various retailers in the city of Cairo in clean labelled packets, each containing 50 g. The samples were sent to the laboratory as soon as they were collected, finely ground in a Buhler mill, and either tested on arrival or stored at 4°C to arrest any mycotoxin formation before analysis. Table 1 shows the botanical names, the part of plant used, and the number of samples examined for each medicinal plant.

Moisture Content

Medicinal plant and spice samples were dried at 60°C under vacuum for 8 to 12 h until their weight remained constant. The weight difference after drying was considered the moisture content (Aziz, 1987).

Mycological Studies

Ten grams of each sample were added to a 90 ml portion sterile 0.85% saline solution in 500 ml Erlenmeyer flasks and homogenized thoroughly on an electric shaker

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

English name	Scientific name	Part of plant used	No. of samples
Black cumin	*Nigella sativa* L.	Seeds	7
Fennel	*Foeniculum vulgare* Miller	Dry fruit	7
Lime tree	*Tilia parvifalie* Ehr.	Dry inflorescence	5
Absinthium	*Artemisia absinthium* L.	Whole herb & dry leaves	5
Ginger	*Zingiber officinale* Rosoe	Rhizome	5
Cinnamon	*Cinnamomum cassia* Blume	Bark or cinnamomum bark	7
Pepper mint	*Mentha spicata* L.	Leaves	7
Carob tree	*Ceratonia siliqua* L.	Dry fruits	7
Chamomile	*Cymbopogom schoerenthus* (L.) sperg	Leaves and stems	5
Saffron	*Crocus sativus* L.	Dry parts of stylles and stigma	5
Curcuma	*Curcuma longa* L.	Rhizomes	7
Worm wood	*Artmisia heba*	Succlent branches	5
Rose	*Rosa canina* L. H	Dry buds	7
Lesser galangel	*Alpinia officinarum* Hance	Rhizome	5

at constant speed for 15 min. Tenfold serial dilutions were then prepared (Aziz and Youssef, 1991). One ml portions of three suitable dilutions of the resulting medicinal plant suspension were used to inoculate Petri dishes each containing 15 ml Sabouroud's dextrose agar containing 0.5 mg chloramphenicol/ ml medium to suppress bacterial growth. Plates were then incubated for 7–15 days at 28°C and examined visually and microscopically for the growth of molds. The isolated molds were subcultured on the above medium for identification of *Aspergilli* (Raper and Fennel, 1977), *Penicillia* (Pitt, 1979; 1985), and other molds (Domsch et al., 1981).

Screening of Aspergillus flavus and Aspergillus parasiticus Strains

Identification of presumptive *A. flavus* and *A. parasiticus* strains depends on the reserve colony colour and gross morphology of conidial heads. Isolates were tested on a new medium, *Aspergillus flavus* and *parasiticus* agar (AFPA) of Pitt et al. (1983). The production of an intense orange yellow pigment after inoculation at 30°C for 2 days, is said to be indicative of *A. flavus* and *A. parasiticus*. The presumptive production of aflatoxin by individual isolates was demonstrated using the aflatoxin production agar (APA) of Hara et al. (1974). After incubation at 27°C for 10 days, the plate cultures were examined for blue fluorescence under long wave ultraviolet light (365 nm) (UV lamp Emita-Poland).

Analysis of Mycotoxins

Extraction of mycotoxins from medicinal plant samples and spices. Samples (25 g) of ground medicinal plant samples and spices were extracted according to the method of Grabarkiewicz-Szczesna et al. (1985). In cases with large amounts of impurities and pigments, preliminary silica gel chromatography column in chloroform or in n-hexane was performed to clean up the samples.

Cultivation of molds in medicinal plant and liquid medium. Erlenmeyer flasks (250 ml) containing 50 g of sterile mycotoxin-free medicinal plants and spices or 50 ml of yeast extract sucrose medium (2% yeast extract and 20% sucrose) were inoculated with about 10^6 spores per ml and incubated with shaking at 28°C for 7 days.

After incubation, the contents of each flask were mixed with 120 ml of chloroform: water (100:10, v/v) and were shaken vigorously by rotary shaker (200 rpm) overnight. The extracts were sequentially filtered through anhydrous sodium sulfate. The chloroform extracts were collected for dryness.

Thin layer chromatography (TLC) of mycotoxins. TLC was performed in all instances with pre-coated glass plates (20 × 20 cm of silica gel D.G.60 Merck, Darmstadt) using chloroform : acetone (9:1, v/v) and toluene : ethyl acetate : 90% formic acid (6:3:1, v/v/v) as development solvents (Golinski and Grabarkiewicz-Szczesna, 1984). The intensity of the mycotoxins was measured with a flurodensitometer CD-60 at an excitation wavelength of 365 nm and emission wavelength of 443 nm. The amount of mycotoxin extraction given is the mean of three replicates on one TLC plate, and each spot was scanned twice.

Chemical confirmation of mycotoxins. Chemical confirmation of mycotoxins was performed directly on the developed TLC plate. The plates were then sprayed with either 20% $AlCl_3$ solution or 20% sulfuric acid and heated 10 min at 110°C and observed under 365 nm UV light. These spraying reagents were used for visualization and to increase the fluorescence intensity of the mycotoxins. The spray reagents recommended for developing or changing the colour of mycotoxin fluorescence are presented in Table 2 (Grabarkiewicz-Saczesna et al., 1985).

Results and Discussion

Fungal Contamination

The differences in fungal populations isolated from the medicinal plant samples and spices are shown in Table 3. In all cases, a total of 20 species of fungi belonging to 9

Table 2. Colour of fluorescence of mycotoxins before and after treatment with different reagents.

Toxin	Colour in UV-360 nm without Spraying	Colour of fluorescence in UV-360 nm	
		$AlCl_3$[a]	H_2SO_4[b]
Aflatoxin B_1	Dark-blue	Dark-blue	Yellow-beige
Aflatoxin B_2	Dark-blue	Dark-blue	Yellow-beige
Aflatoxin G_1	Greenish-blue	Greenish-blue	Beige
Aflatoxin G_2	Greenish-blue	Greenish-blue	Beige
Ochratoxin A	Greenish-blue	Dark-blue	Greenish-blue
Penicillic acid	Dark-blue	Dark-blue	Pink-violet
Zearalenone	Gray-blue	Dark-blue	Gray-blue
T-2 toxin	Gray-blue	Dark-blue	Light-greenish-blue

[a]$AlCl_3$ 20% solution (W/V) in ethanol.
[b]H_2SO_4 20% solution (W/V) in ethanol, sprayed plates were heated for 15 min at 110°C.

Table 3. Percentage of samples contaminated by different fungal species isolated from different medicinial plants.

Fungal species	% of samples yielding different species of fungi													
	Black cumin	Fennel	Lime tree	Absin-thium	Ginger	Cinn-amon	Pepper mint	Carob tree	Cham-omile	Safforn	Curc-uma	Worm wood	Rose	Lesser galangel
Absidia corymbifera	71	100	20	40	20	85	57	–	–	–	14	–	28	20
Aspergillus flavus	100	72	43	100	80	71	100	86	60	80	86	80	71	80
Aspergillus parasiticus	86	57	71	80	100	43	71	71	80	80	71	80	100	60
Aspergillus niger	57	86	100	60	80	71	86	57	60	60	71	60	86	60
Aspergillus oryzae	43	29	–	20	–	29	29	–	40	20	–	–	43	80
Aspergillus ochraceus	71	57	40	–	60	57	–	43	60	–	57	40	–	–
Aspergillus terreus	29	–	60	20	–	14	–	14	–	40	14	–	29	40
Aspergillus tamarii	14	29	–	–	60	–	43	29	–	20	–	40	14	60
Cladosporium herbarum	–	42	40	20	60	–	42	–	–	–	–	–	14	20
Penicillium viridicatum	43	71	20	–	80	–	14	–	60	–	57	60	–	–
Penicillium chrysogenum	14	–	60	40	40	29	–	43	40	–	43	–	57	40
Penicillium nigricans	–	14	–	20	–	14	29	57	–	–	43	20	14	20
Penicillium variable	86	–	–	60	60	43	–	–	20	40	–	–	29	–
Penicillium commume	–	29	20	–	–	14	29	–	–	–	14	–	–	20
Paecilomyces variotii	–	57	20	20	20	–	14	–	–	–	43	20	57	60
Fusarium oxysporum	29	43	60	40	–	–	14	–	40	20	–	40	43	–
Fusarium solani	14	–	–	20	40	14	–	25	–	20	14	–	–	40
Rhizopus stolonifer	85	100	20	20	20	57	71	–	–	–	24	–	29	20
Mucor pusillus	85	85	20	20	40	28	85	–	–	–	28	–	–	20
Scopulariopsis brevicaulais	42	29	40	20	20	14	29	–	–	–	14	–	14	20

(–) No detection fungal species.

genera were isolated and identified. Sixteen species were isolated from black cumin, fennel, absinthium, and lesser galangel samples with a moisture content of 6%; 15 species from lime tree cinnamon, peppermint, ginger, curcuma, and rose samples had a moisture level 4%; and 9 species were isolated from carob tree, chamomile, worm wood and saffron with a moisture content of 2.5%. In this study, the isolated species of fungi belong to the genera *Absidia*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Paecilomyces*, *Fusarium*, *Rhizopus*, *Mucor*, and *Scopulariopsis*. The greater number of species was held to the genus *Aspergillus*. Seven species were recovered namely: *A. flavus*, *A. parasiticus*, *A. niger*, *A. oryzae*, *A. ochraceus*, *A. terreus*, and *A. tamarii*. Five species of *Penicillium* were isolated, namely *P. viridicatum*, *P. chrysogenum*, *P. nigricans*, *P. variable*, and *P. commune*. Two species were isolated from the genus *Fusarium*, and only one species was found in the different plant samples and spices from the other genera of fungi. The most prevalent fungi isolated from the medicinal plant samples and spices were *A. flavus*, *A. parasiticus*, and *A. niger*. Domsch et al. (1981) postulated that the contamination of feedstuffs with fungal species was as a result of natural extraneous contamination by dust following storage in humid conditions. Fungi fall into two ecological categories: field and storage fungi. Field fungi were observed to invade developing or mature seed while it is on the plant, the major field fungi genera are: *Alternaria*, *Helminthosporium*, *Fusarium*, and *Cladosporium*. On the other hand, storage molds are those encountered on plants at moisture conditions routinely found in stored products, these fungi are principally species of *Aspergillus* and *Penicillium*.

The dominant of *Aspergillus* and *Penicillium* spp. in all examined medicinal plant samples and spices was in accord with the results of Takatori et al. (1977) and Ayres et al. (1980), who stated that *Aspergillus* and *Penicillium* spp. were the main components of cardamon, cinnamon, fennel, coriander, cumin, black cumin, and white pepper, all of which are common in the food industry. They found

a high degree of contamination in all samples. Misra (1981) and Roy et al. (1988) isolated *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *A. orchaceus*, *A. candidus*, *A. sydowi*, *Chaetomium dolicholrichum*, *F. moniliforme*, *Penicillium oxalicum*, *Alternaria*, *Curvularia*, and *Rhizopus* from the seeds of *Amomum subulatum*, *Coriandrum sativum*, *Cuminum cyminum*, *Foeniculum vulgare*, *Piper nigrum*, *Cinnamomum zeylanicum*, and from the bark of *Acacia catechu*, all of which are commonly used drug plants.

Aspergillus niger and *A. flavus* or the *oryzae* group—especially *Aspergillus flavus*— were the most frequent *Aspergillus* species yielded in all examined medicinal plant samples in this investigation. This was in accordance with the results of Roy and Chourasia (1990), who stated that *A. flavus* was the main contaminant of different herbal drug samples.

Mycotoxin Production by Isolated Fungi from Medicinal Plant Samples and Spices

Table 4 shows the type of aflatoxins produced by *Aspergillus* spp. isolated from medicinal plant samples and spices. Aflatoxins were produced by 38 out of l09 isolates of *Aspergillus* in synthetic medium. *Aspergillus flavus*, *A. parasiticus*, and *A. oryzae* were observed to be the most common aspergilli producing aflatoxins. Table 5 makes clear that, out of 49 strains of *A. flavus*, 22 produced aflatoxins in synthetic medium and only 8 produced aflatoxins in medicinal plants. Roy et al. (1988) demonstrated that *A. flavus* strains obtained from drug plants produced aflatoxin B_1 from 0.86 to 5.24 µg/ml of culture filtrates. Recently Aziz and Youssef (1991) isolated *A. flavus* and *A. parasiticus* with a high tendency for aflatoxin production from some common herbal drugs and spices. The production of ochratoxin A and penicillic acid by *Aspergillus* and *Penicillium* sp. isolated from medicinal plants was recorded in Table 6. *Aspergillus ochraceus* and *P. variable* were ochratoxin A producers, whereas *P. viridicatum*, *P. commune*, and *P. chrysogenum* were penicillic acid producers. It was noticed that ochratoxin A and penicillic acid producing isolates were widespread on medicinal plant samples (Wyllie and Morehouse, 1977). *Aspergillus ochraceus* was reported to be one of the major organisms producing ochratoxin A (Aziz, 1987). Leistner and Pitt (1977) found that, out of 442 *Penicillium* isolates, 44 synthesized penicillic acid, 17 ochratoxin A, 11 penitrem, 10 citrinin, 6 patulin, and 3 produced both patulin and citrinin.

On the basis the present investigation, it may be concluded that the contamination of herbal drugs and spices with mycotoxins is alarming, and such products need thor-

Table 4. Distribution of aflatoxin–producing fungi among medicinal plant samples and spices.

Type of fungi screened	Total no. of fungi examined	Positive strains for aflatoxin production	Type of aflatoxin produced				
			B_1	B_2	B_1 & B_2	B_1 & G_1	B_1 & G_2
Aspergillus flavus	49	22 (44.90%)[a]	10	3	4	2	3
Aspergillus parasiticus	37	14 (37.85%)[a]	50	1	6	1	1
Aspergillus oryzae	15	2 (13.33%)[b]	2	–	–	–	–
Aspergillus tamarii	8	0 (0 %)	0	–	–	–	–
Total	109	38 (34.86%)	17 (15.6%)	4 (3.67%)	10 (9.17%)	3 (2.75%)	4 (3.67%)

[a]Aflatoxins ranged from 320–750 µgl^{-1}.
[b]Aflatoxin ranged from 80–220 µgl^{-1}.

Table 5. Types of aflatoxin produced by *A. flavus* artificially inoculated to synthetic medium and medicinal plant samples and spices.

Medicinal plant samples	Number of fungal isolates	No. of +ve isolates (average µgL^{-1}) in synthetic medium					No. of +ve isolates (average µgkg^{-1}) in medicinal plant				
		No. of +ve strains	B_1	B_1 & B_2	B_1 & G_1	B_1 & G_2	No. of +ve strains	B_1	B_1 & B_2	B_1 & G_1	B_1 & G_2
Black cumin	4	2	1 (4500)	–	–	1 (1600)	1	1 (20)	–	–	–
Fennel	6	4	2 (1500)	2 (14400)	–	–	1	–	–	–	1 (250)
Lime tree	6	2	2 (8200)	–	–	–	1	–	1 (150)	–	–
Absinthium	3	2	1 (600)	–	1 (14500)	–	1	1 (200)	–	–	–
Ginger	3	1	–	1 (11200)	–	–	0	–	–	–	–
Cinnamon	3	1	–	–	–	1 (1150)	0	–	–	–	–
Pepermint	6	3	2 (15500)	1 (4400)	–	–	1	1 (80)	–	–	–
Carob tree	3	2	1 (14800)	1 (60)	–	–	0	–	–	–	–
Chamomile	3	1	1 (4950)	–	–	–	1	1 (45)	–	–	–
Safforn	2	0	–	–	–	–	0	–	–	–	–
Curcuma	2	1	–	–	1 (1200)	–	0	–	–	–	–
Worm wood	0	0	–	–	–	–	0	–	–	–	–
Rosse	4	2	1 (440)	–	–	1 (3500)	1	1 (175)	–	–	–
Lesser glange	4	1	1 (600)	–	–	–	1	1 (100)	–	–	–
Total	49	22	12	5	2	3	8	6	1		1

Table 6. Ochratoxin–A and penicillic acid produced by *Aspergillus* and *Penicillium* species isolated from medicinal plant samples and spices on synthetic medium.

Type of fungi screened	Total number of moulds	Positive strains	Ochratoxin-A	Penicillic acid
Aspergillus ochraceus	15	3	3[a]	0
Aspergillus terreus	5	0	0	0
Penicillium viridicatum	23	5	2[a]	3[b]
Penicillium chrysogenum	18	2	0	2[c]
Penicillium commune	3	2	0	2[c]
Penicillium variable	13	3	3[a]	0
Total	77	15	8	7

[a]Average concentration (80–225 μgL^{-1}).
[b]Average concentration (120–185 μgL^{-1}).
[c]Average concentration (70–95 μgL^{-1}).

Table 7. Average value (μgkg^{-1}) of mycotoxins present in medicinal plant samples and spices.

Medicinal plants	No. of samples examined	No. of samples contaminated	Mycotoxins concentration (μgkg^{-1})				
			Aflatoxin B_1	Ochratoxin–A	Penicillic acid	Zearalenone	T-2
Black cumin	5	3	30	35	–	–	–
			20				
Fennel	6	3	160	80	–	–	–
			80				
Lime tree	7	1	75	–	–	–	–
Absinthium	7	2	25	20	–	–	–
Ginger	5	2	10	–	–	–	–
			10				
Cinnamon	5	0	–	–	–	–	–
Pepermint	7	3	25				
			35	–	–	–	–
			45				
Carob tree	4	1	10	–	–	–	–
Chamomile	6	3	145	–	–	–	–
			80	–	–	–	–
			75				
Safforn	5	0	–	–	–	–	–
Curcuma	3	0	–	–	–	–	–
Worm wood	4	2	90	–	–	–	–
			50				
Rosse	7	0	–	–	–	–	–
Lesser glangel	5	0	–	–	–	–	–
Total	76	20 (26.3%)	17 (22.4%)	3	–	–	–

(–) No detection of mycotoxins under the experimental conditions.

ough inspection before being channeled to the drug and food industries.

Mycotoxin Production in Medicinal Plant Samples and Spices

As shown in Table 7, only 17 samples were found contaminated with aflatoxin B_1 and three samples with ochratoxin A. Three samples of peppermint and chamomile, two samples of black cumin, fennel, ginger, and worm wood and one sample of lime tree, absinthium, and carob tree contained aflatoxin B_1. The highest level was 160 μgkg^{-1} found in the fennel. Ochratoxin A was detected in one sample of black cumin, fennel, and absinthium with an average level of 35, 80, and 20 μgkg^{-1}, respectively. No mycotoxins were detected in any sample of cinnamon, saffron, curcuma, rose, or galangel. All 84 tested medicinal plants and spices were free from penicillic acid, zearalenone, and T-2 toxin. Detection limits of mycotoxins ranged from 20 to 100 μgkg^{-1}. *Aspergillus*, *Penicillium*, and *Fusarium* are toxigenic to human beings and animals (Wyllie and Morehouse, 1977). As stated earlier, mycotoxins are produced by fungi on plants in the field before harvest or later after harvest during long storage under favourable conditions (Gedek, 1985). As these plant materials are used for preparation of traditional medicines, the possibility of aflatoxin contamination in these is implied. This is certainly a matter of great concern because humans use these medicines to treat diseases. In previous study, Rani

and Singh (1990) found that 89% of samples of fennel, coriander, cumin, and ammi were contaminated with aflatoxin B_1 at the levels 3000 ppb, 1640 ppb, 1580 ppb, and 2550 ppb, respectively. In addition, Roy et al. (1988) and Roy and Chourasia (1990) determined that the seeds of *Piper nigrum* and *Mucuna prurians*, and the barks of *Acacia catechu*, *Coriandrum sativum*, and *Elettaria cardamomun* were contaminated with aflatoxin B_1 at levels below 20 μgkg^{-1}.

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