MYCOFLORA AND AFLATOXINS STATUS OF SOME SPICES AND HERBS COMMONLY CONSUMED IN TAIZ GOVERNORATE, REPUBLIC OF YEMEN

Mohamed A. Abdel-Sater⁽¹⁾; Abdul-Rahman H. Al-Zubeiry⁽²⁾; Fahd A. Al-Sharjabi⁽²⁾ and Mufedah A. Gazem⁽²⁾

- (1) Botany Dept., Faculty of Science, Assiut Univ., Assiut, Egypt.
- (2) Applied Microbiology Dept., Faculty of Applied Sci., Taiz Univ., Taiz, Yemen.

Abstract: The present work was carried out on 105 samples representing 21 kinds of spices (5 samples each) commonly consumed in Taiz city. Ajawan, black cumin. Yemen. coriander, cumin, fenugreek, ginger, laurel and thyme were highly polluted. Whereas black mustard, caraway, fennel, cardamom. garden cress, nutrneg and red pepper moderately contaminated by mould and yeasts. On the other hand, anise, black pepper, cinnamon, linseed and safflower were less contaminated by moulds and yeasts. Clove samples were free from mould and yeasts. The mycological survey of the different spices revealed that they were heavily contaminated

with mould and yeasts, where about 91.42 % of the samples examined were contaminated with fungi. Ninety-one species and two varieties appertaining to 25 genera were isolated. Aspergillus, Rhizopus and Penicillium species contributed the broadest spectra of species. Aspergillus was the most prevalent genus, it occurred in 90.5 % of the samples constituting 65.1 % of total fungal count. Among fungi recovered in these work, 14 species were new records in Yemen. The results proved the presence of aflatoxin B₁ in seven samples while the rest 14 tested samples were free from any detectable amount of aflatoxins.

Key words: Fungi, spices, aflatoxins.

Introduction

Spices are the various strongly flavored or aromatic substances of vegetable origin; they are commonly used as condiments or employed for other purposes on account of their fragrance and preservation qualities (Oxford English Dictionary, 1989). These

are parts of plants such as dried leaves, seeds, fruits, bark, roots, stems, buds and flowers (Sherman and Billing, 1998). Spices have two main components: volatile oils (essential oils), which are responsible for the characteristic aroma of spices and oleoresins (non volatile extracts), which are

Received on: 21/2/2009 Accepted for publication on: 12/3/2009

Referees:: Prof.Dr. Naaem E. Al-Keltawi Prof.Dr. Abd-Al-Naser Al-zohari

responsible for the typical taste and flavor, these substances also have preserving action, that are toxic to microorganisms (Pruthi 1980). In addition, many of these spices have medicinal properties that alleviate symptoms or prevent diseases because they possess antimicrobial properties. Other spices contain potent phytochemical that provides significant protection against cancer (Aruoma 1998, and Sherman and Flaxman 2001). In fact, the adverse effects of long-term herbal and spices use, adulteration with toxic compounds and contamination by pathogenic microorganisms natural toxins like mycotoxins have been reported for spices, herbal products and medicinal plants (Aziz et al. 1998, Abou-Arab et al. 1999, Efuntoye 1999, Freire et al. 2000, Tassaneevakul et al. 2004 and Mandeel 2005). Most spices are susceptible to invasion by fungi during some stage of production, processing, transport, harvesting or storage (Salunkhe et al. 1980, Thompson et al. 1989, Giese 1994, Kneifel and Berger 1994, McKee Elshafie 1995. et alAbdulkadar et al. 2004 and Bircan 2005). Additionally, spices may be attacked by fungi in the field or while in storage. (Julseth and Deibel 1974, Flannigan and Hui 1976, Ayres et al. 1980 and Eman 1990). Many fungi are capable of producing toxic substances. If mold growth occurs on the spices, there is possibility of mycotoxin production in the field before harvest and or during transportation, storage and

remain in the commodities for several years (Salunkhe et al. 1980, Thompson et al. 1989, Giese 1994, Kneifel and Berger 1994 and Youssef 1995). Contamination of spices with aflatoxins may takes place in the field, and or during drying, storage and processing stages (Elshafie et al. 2002). Several investigations have been carried out on the mycoflora (distribution, composition, density and frequency of occurrence) and aflatoxins production on the herbal and several kinds of spices in many countries (Abdel-Hafez and El-Maghraby 1992, El-Kady et al. 1992 & 1997, Aziz et al. 1998, Freire et al. 2000, El-Bazza et al. 2001, Tassaneevakul et al. 2004, Mandeel 2005, Martin et al. 2005, Bugno et al. 2006 and Romagnoli et al. 2007). They recorded that many spices are heavily contaminated with fungal populations. In addition, there are numerous studies in different countries and regions have established that spices will support fungal growth and subsequent aflatoxins production (Aziz and Youssef 1991, Takahashi 1993, Ragab 1994, Youssef 1995, Akiyama et al. 2001, El-Bazza et al. 2001, Martins et al. 2001, Abdulkadar et al. 2004. Bircan 2005, Zinedine et al. 2006 and Romagnoli et al. 2007).

This investigation was carried out in an attempt for isolation, identification, frequency, distribution, occurrence and composition of the mycoflora of 21 kinds of spices. Screening for the natural occurrence of aflatoxins in the different investigated spices was also evaluated.

Materials and Methods

Collection of Spices and Herbs Sample:

One hundred and five samples related to twenty one kinds of spices and herbs, mentioned in Table (1), were gathered from different retail supermarkets at Taiz Governorate (five samples for each kind). Each sample (500 g) was put in a sterile polyethylene bag then transferred immediately to the Laboratory for fungal determination and aflatoxins analysis.

Estimation of Spices and Herbs Borne Fungi:

Direct plate technique, adopted and recommended by Pitt et al. 1992, was employed for the enumeration and isolation of fungi associated with spices and herbs sample. Ten segments (0.5 cm²) or seeds sample 10 per immersed on the surface of agar plates (3 plates each sample). The plates were incubated at 28° C for 7-10 days and the developing moulds were examined, counted, isolated and identified. The numbers of colonies were calculated per 30 segments or seeds for each sample.

Medium Used for Isolation of Spices or Herbs Borne Fungi:

Modified Czapek's Dox agar medium was used in which the 3% sucrose was substituted with 2% glucose. The composition of the medium (g/L) was (glucose, 20; NaNO₃, 3; KH₂PO₄.7H₂O, 0.5; MgSO₄.7H₂O, 0.5; KCl, 0.5; FeSO₄.7H₂O, 0.01; Agar Agar, 15). Ros-bengal (1/15000) combined with Chloramphenicol (0.5 mg/ml) were used as bacteriostatic agents according to Smith and Dawson (1944) and Al-Doory (1980).

Identification of Fungal Genera and Species:

Identification of fungi genera (based on macro and microscopic characteristics) was performed as outlined in following keys: Raper and Thom (1949), Raper and Fennell (1965), Simmons (1967), Rifai (1969), Booth (1971 & 1977), Ellis (1971 & 1976), Christensen and Raper (1978), Pitt (1979 & 1985), Domsch *et al.* (1980), Ramirez (1982), Sivanesan (1984) and Moubasher (1993).

Estimation of Aflatoxins in Spices:

Aflatoxins of species samples were extracted (as crude extracts) according to the method described by Pons et al. (1972). The crude extracts were purified by column chromatography according method modified by Zohri (1990). The thin layer chromatographic technique described by El-Kady and Moubasher (1982) was carried out to qualitative determination of aflatoxins, and the procedure of Ibrahim (1983) was employed for chemical confirmatory tests for aflatoxins.

Table(1): Code No. of samples collected from different retail supermarkets.

Code No.	Latin name	Plant part	English name	Arabic name
1-5	Trachyspermum ammi	Seeds	Ajwan	النخوة (الكمون الملوكي)
6-10	Pimpinella anisum	Seeds	Anise	ينسون
11-15	Nigella sativa	Seeds	Black cumin	الحبة السوداء (حبة البركة)
16-20	Brassica nigra	Seeds	Black mustard	خردل اسود
21-25	Piper nigrum	Fruits	Black pepper	ظفل أمنو د
26-30	Carum carvi	Fruits	Caraway	كراوية
31-35	Elettaria cardamomum	Fruits	Cardamom	هيل (حبهان)
36-40	Coriandrum sativum	Flower buds	Coriander	كزيرة
41-45	Syzygium aromaticum	Flower buds	Clove	فرنقل .
46-50	Cinnamomum verum	Bark	Cinnamon	قر فة
51-55	Cuminum cyminum	Flower buds	Cumin	كمون
56-60	Foeniculum vulgare	Flower buds	Fennel	شمر
61-65	Trigonella foenum-graecum	Flower buds	Fenugreek	حلبة
66-70	Lepidium sativum	Seeds	Garden cress	الحلف (حب الرشاد)
71-75	Zingiber officinale	Rhizomes	Ginger	زنجييل
76-80	Laurus nobilis	Leaves	Laurel	غار
81-85	Linum usitatissimum	Seeds	Linseed	كتان
86-90	Myristica fragrans	Fruits	Nutmeg	جوز الطيب
91-95	Capsicum frutescens	Fruits	Red pepper	الظفل الأحمر
96-100	Carthamus tinctorius	Flowers	Safflower	القرطع
101-105	Thymus vulgaris	Leaves	Thyme	الزعز

Results and Discussions

The mycological analysis of the 21 kinds of spices revealed that most of them were heavily contaminated with mould and yeasts. From the 105 samples tested, 96 samples (91.4 %) were polluted with fungi. Ajawan, black cumin, coriander, cumin, fenugreek, ginger, laurel and thyme were highly polluted, containing

150 - 197 colonies/150 seeds or segments. Whereas, black mustard, caraway, cardamom, fennel, garden cress, nutmeg and red pepper were moderate contaminated having 126-148 colonies/150 seeds or segments. On the other hands, anise, black pepper, cinnamon, linseed and safflower were less contaminated having 8-120 colonies/150 seeds or segments.

Only clove samples were free from mould and yeasts and that was agreement with the results of Garrido et al. (1988 & 1992), El-Kady et al. (1992), Youssef (1995), EL-Bazza et al. (2001), Elshafie et al. (2002), El-Shanawany et al. (2002), Hemida (2004) and Mandeel (2005).

Tabulated data presented in Table (2) revealed that 2720 colonies representing ninety-one fungal species and two varieties appertaining to 25 genera were identified from the tested samples at 28° C. on Czapek's agar Aspergillus, Rhizopus and Penicillium were the most common fungi, isolated in high or moderate frequency of occurrence. Our results were greatly harmony with those obtained by numerous researchers all over the world. They reported that, these genera were quite common on crude and herbal drugs, spices and umbelliferous seeds (Shrivastava and Jain 1992, Banerjee et al. 1993, Halt 1998, Freire et al. 2000, Omafuvbe and Kolawole 2004 and Rizzo et al. 2004).

Aspergillus was the most prevalent genus. It was occurred in 90.5 % of the samples constituting 65.1 % of total count. The mean total counts of Aspergillus widely varied from 21 - 162 colonies/150 seeds or segments of spice, showing the maximum in samples of laurel leaves and the minimum in linseed seeds. Most of spices sample were highly contaminated

with Aspergillus but clove and cinnamon bark were free from Aspergillus. In addition, Aspergillus was the first predominant genus among fungi isolated from herbs, spices or medicinal plants. In agreement, Aspergillus agreed the main component of the mycoflora of spices (Martinez-Magana et al. 1989, Ismail 2000, El-Shanawany et al. 2002, Mandeel 2005 and Bugno et al. 2006).

In addition, five species and one variety could produce sexual spores (Teleomorphs) which related to two genera namely: Emericella (tree species + one variety) and Eurotium (two species). From this genus, 38 species and one variety were identified of which A. flavus and A. niger were the most common species. They were isolated in high frequency of occurrence emerging in 56.2 % and 63.8 % of the samples comprising 17.0 % and 33.5 % of total Aspergillus and 11.1 % and 21.8 % of total fungi, respectively. It was noticed that samples of cumin and laurel were highly polluted with the above two species where as, linseed samples was less contaminated with them. Several studies reported that A. niger or A. flavus was the most frequently encountered and widely distributed fungus in herbal drugs and spices (Vardavakis 1988. Abdel-Gawad and Saber 1989, Moharram et al. 1989, Shrivastava and Jain 1992, Zohri et al. 1992, Youssef 1995, Freire et al. 2000 and Omafuvbe and kolawole 2004). Eight species and one variety from

Aspergillus, were isolated and identified in moderate or low frequency of occurrence and these were: A. foetidus, A. oryzae, A. flavo furcatis, A. flavus var. columnaris. **A**. ochraceus. A. parasiticus, A. pulverulentus, A. tamarii and A. unguis. These Aspergillus species were previously isolated in moderate, low or rare frequency from different kinds of spices and medicinal plants (Abdel-Gawad and Saber 1989, Moharram et al. 1989, El-Kady et al. 1992, Shrivastava and Jain 1992, Youssef 1995, Aziz et al. 1998, Freire et al. 2000, El-Shanawany et al. 2002, Mandeel 2005 and Bugno et al. 2006). The remaining Aspergillus species were isolated in rare frequency of occurrence matching collectively about 12.1 % of total Aspergillus and 7.9 % of total fungi. These species were previously isolated in different densities from several kinds of medicinal spices and plant (Moharram et al. 1989, Eman 1990, Youssef 1995, Abdel-Hafez and El-Maghraby 1992, El-Kady et al. 1997, Ismail 2000, El-Shanawany et al. 2002 and Hemida 2004).

Rhizopus (R. stolonifer) was the second common genus came behind Aspergillus in the number of cases of isolation and total counts. It was recovered from 61.9 % of the samples comprising 13.8 % of total counts. The mean total counts of Rhizopus were varied from 2 - 44 colonies/150 seeds or segments. Samples of coriander, red pepper, cumin, anise and ajowan were the

most contaminated, whereas thyme, ginger, black cumin and laurel were less contaminated with *Rhizopus stolonifer*. On the other side, the clove and cinnamon samples were free from *Rhizopus stolonifer*. In general these results were in agreement with those of El-Kady et al. (1992), Shrivastava and Jain (1992), Youssef (1995), Ismail (2000), El-Bazza et al. (2001), Elshafie et al. (2002), Martin et al. (2005) and Bugno et al. (2006).

According to the number of isolates. Penicillium occupied the third order. It was recovered from 30.5 % of the samples contributing 5.0 % of total fungi. The mean total counts of this genus varied from 1 -23 colonies/150 seeds or segments. Spices sample of thyme, fenugreek, garden cress, linseed were heavily polluted. while red pepper, caraway, ajowan, cumin, black pepper, black cumin, ginger, laurel and cardamom were less polluted species. with Penicillium contrast, samples of clove, fennel, coriander and cinnamon were free from Penicillium species. Fifteen species of Penicillium were identified belonging three sections and three subsections described by Raper and Thom (1949) as follows: Monoverticillata species) P (two namely. decumbens Thom. and P. waksmanii Zaleski. Biverticillata Symmetrica (three species) namely, P. funiculosum Thom P. islandicum Sopp Р. variabile Sopp. Asymmetrica Velutina subsection (seven species) namely.

brevicompactum Dierckx Р. chrysogenum Thom P. citrinum Thom P. corylophilum Dierckx P. oxalicum Currie and Thom P. roquefortii Thom P. steckii Zaleski. Asymmetrica Lanata subsection (one species) P. lanosum Westling. Finally, Asymmetrica Fasciculata subsection (two species) namely, P. ex Grav P. expansum Link griseofulvum Dierckx. From those isolate P. brevicompactum, P. chrysogenum and P. citrinum were the most prevalent. These three species were isolated from 10.5 %, 7.6 % and 6.7 % of the samples constituting 13.3 %, 11.8 % and 12.4 % of total Penicillium and 0.6 %, 0.8 % and 0.7 % of total fungi. respectively. The remaining Penicillium species were isolated in rare frequency of occurrence (from 1 - 4 samples) representing collectively about 55.5 % of total Penicillium and 2.8 % of total fungi. Penicillia were recorded as the main components of mold counts in spices sample medicinal plants (Hashmi and Ghaffar 1991. Shrivastava and Jain 1992, Baneriee et al. 1993, Abdel-Hafez and El-Said 1997, Ismail 2000 and Martin et al. 2005).

Ulocladium was isolated from spices sample in low frequency of occurrence. It was recovered from 22.9 % of the samples contributing 1.2 % of total fungi. From the genus, 3 species were identified of which U. alternaria was the commonest species. U. atrum and U. chlamydosporum were recovered in rare frequency of

occurrence. This genus was also isolated from different substrates including spices and herbs in low and rare frequencies of occurrence. In this respect, El-Kady et al. (1992) isolated U botrvtis in occurrence from 120 samples belonging to 24 kinds of spices. Youssef (1995) isolated two species from the genus namely U. atrum and *U. botrvtis* from 165 samples of medicinal plants. In addition, El-Kady et al. (1997) could isolate U. atrum and U. microsporium from, phyllosphere of some herbal plants. However, El-Shanawany et al. (2002) could isolate U. atrum in low frequency of occurrence from 48 samples of 12 types of spices.

In addition, the data presented in Table (2) showed that the nonfilamentous fungi (yeasts) were isolated in moderate frequency of occurrence. It emerged in 24.8 % of the samples contributing 4.9 % of total fungi. Their total counts varied from 2 - 30 colonies/150 seeds or The segments. samples cardamom, red pepper, caraway, garden cress, linseed and ajowan were highly polluted with yeasts but, ginger, thyme, anise and black mustard were less contaminated with non filamentous pseudohypha fungi. On contrast, fenugreek, nutmeg, cinnamon, cumin, black pepper, clove, fennel, coriander and laurel were free from yeasts. Several reports revealed that yeast were present in crude herbal and spices sample (Beuchat et al 1991, Omafuvbe and kolawole

2004, Martin *et al.* 2005 and Mandeel 2005).

Non-sporing filamentous fungi (sterile mycelia) were isolated in frequency of occurrence represented in 14.3 % of the samples constituting 2.4 % of total fungi. The mean total counts of these filamentous fungi widely varied from 1 - 24 colonies /150 seeds or segments. Sterile mycelia were isolated but with different incidence from numerous substances including spices and herbs reported as by some researchers (Eman 1990, El-Kady et al. 1992 & 1997 and Youssef 1995)

Thirty-four and one variety belonging to twenty-one genera were isolated in rare frequency of occurrence. They were occurred in 0.9 - 9.5 % of the samples matching collectively about 7.6 % of total fungi. Most of these fungi were previously isolated from herbal drugs and spices in variable numbers and frequencies (Moharram et al. 1989, Abdel-Hafez and El-Maghraby 1992, El-Kady et al. 1992, Youssef 1995, Ismail 2000, Freire et al. 2000, Hemida 2004, Mandeel 2005, Bugno et al. 2006 and several others).

Fourteen species were new records in Yemen and these are Alternaria dianthi, Aspergillus ambiguus, A. caespitosus, A.

ellipticus. A. multicolor. A. ostianus, A. pulverulentus, A. pulvinus. puniceus. \boldsymbol{A} . A. recurvatus. speluneus, A. A. tubingensis, A. viridi-nutans, and Paecilomyces farinosus. Numerous of these fungal genera and species isolated previously, but in different counts and frequencies from some spices sample in many parts of the world (Garrido et al. 1988. Moharram et al. 1989, Abdel-Hafez and El-Maghraby 1992, El-Kady et al. 1992. Chourasia 1995. Youssef 1995, Aziz et al. 1998 and others).

The analysis of extracts of samples of the twenty-one kind of spices proved the presence of aflatoxin B1 in five spices named anise (Pimpinella anisum), caraway (Carum carvi), fennel (Foeniculum vulgare), laurel (Laurus nobilis) safflower and (Carthamys tinctorius). In addition, traces of aflatoxin B1 were detected in another two kinds named ajawan (Trachyspermum ammi) and cumin (Cuminum cyminum). In contrast, the tested samples of other 14 spices were free from aflatoxins. Findings similar to the results obtained in the present study, have been reported by several workers (Takahashi, 1993; Ragab, 1994; Youssef, 1995; Aziz et al., 1998; Akiyama et al., 2001; El-Bazza et al., 2001; Martins et al., 2001; Abdulkadar et al., 2004; Bircan, 2005; Zinedine et al., 2006 and Romagnoli et al., 2007).

Table (2): Total counts (TC, calculated /3150 seeds or segments in all samples), Number of cases of isolation (NCI, out of 105 samples) and occurrence remarks (OR) of fungal genera and species recovered from 105 spice samples on Czapek's agar at 28°C.

Genera and species	TC	NCI and OR	Keys of samples contained that isolates
Alternaria	26	10 R	6, 13, 14,15, 16, 17, 20, 21
A. alternata	5	3 R	17, 20, 21
A. chlamydospora	3	I R	21
A. dianthi	7	3 R	13.21
A. tenuissima	11	7 R	6, 13, 14,15, 16, 17, 21
Aspergillus	1771	90 H	All samples except (9, 10)
A. aculeatus	7	4 R	4. 7. 14. 17
A. alliaceus	.8	3 R	15, 16
A. ambignus	2	1 R	13
A. aureolatus	4.	1 R	4
A. awamori	41	8 R	2, 4, 6, 15, 20, 21
A. caespitosus	2	2 R	17, 20
A. candidus	j	İR	14
A. ellipticus	8	IR	2
A. ficuum	21	6 R	5, 6, 7, 19
A. Sla vipes	3	2 R	11, 14
A. flavo-furcatis	42	15 L	1, 3, 4, 5, 7, 8, 14 15, 16, 18, 19, 20
A. flavus	301	59 H	All samples except (9, 10, 17)
A. flavus var. columnaris	23	12 L	1,2, 3, 4, 6, 13, 14, 17, 18, 19
A. foetidus	134	26 M	1, 2, 4, 8, 13, 14, 15, 16, 18, 19, 21
A. fumigatus	5	4 R	2,4,6
A. jamıs	2	2 R	17, 19
A. japonicus	21	6 R	1, 7, 19
A. multicolor	1	1 R	20
A. niger	594	67 H	All samples except (9, 10)
A. ochraceus	51	12 L	8, 11, 12,13, 14, 15, 17, 18, 20
A. oryzae	103	28 M	1. 2, 4, 6, 11. 13, 14. 16, 17. 18, 20, 21

Table (2): continue

Conom and energies	тс	NCI and OR	Keys of samples contained that isolates
Genera and species	+	 	
A. ostianus	2	2 R	4,7
A. parasiticus	73	24 L	1, 3, 4, 5, 6, 7, 11, 12, 13, 18, 19
A. petrakii	19	8 R	3, 4, 7, 11, 13, 15
A. pulverulentus	153	18 L	1, 3, 6, 7, 12, 13, 15, 16, 18, 19
A. pulvinuis	6	I R	19
A. recurvatus	2	2 R	13
A. speluneus	2	1 R	14
A. subolivaceus	2	1 R	11
A. subsessilis	10	6 R	4, 12, 13, 20. 21
A. sydowi	14	4 R	4, 11, 17
A. tamarii	41	13 L	1, 4, 5, 8, 15, 18, 20, 21
A. terreus	6	5 R	4. 5. 11. 16
A. tubingensis	13	4 R	4, 14, 15, 21
A. unguis	28	16 L	1, 4, 5, 11, 12, 13, 14, 16, 18, 21
A. ustus	2	2 R	21
A. versicolor	8	2 R	8, 12, 13, 15, 21
A. viridi-nutans	3	2 R	12. 14
A. wentti	13	6 R	3. 5, 8. 12
Botryotrichum piluliferum	2	2 R	2.6
Cladosporium cladosporioides	28	4 R	13, 17, 20
Cochliobolus	17	8 R	2, 4, 21
C. lunatus	2	2 R	21
C. ovoidea	5	3 R	21
C. spicifer	10	3 R	2.4
Curvularia tuberculata	ı	1 R	4
Emericella	9	3 R	4, 8
E. nidulans	5	2R	4, 8
E. nidulans var. dentata	1	1 R	4
E. quadrilineata	2	I R	4

Table (2): continue

Genera and species	TC	NCI and OR	Keys of samples contained that isolates
E. rugulosa	1	1 R	4
Eurotium	7	6 R	4, 17 18
E. amstelodami	5	5 R	4, 17
E. chevalieri	2	I R	18
Fusarium	9	4 R	7, 17, 21
F. dimerum	1	I R	17,21
F. oxysporum	8	3 R	7, 17, 21
Gibberella pulicaris	10	3 R	21
Humicola	3	3 R	3, 4
H. fuscoatra	2	2 R	3
H. grisea	1	1 R	4
Memnoniella echinata	1	I R	21
Monodictys paradoxa	1	1 R	21
Mucor	13	4 R	6, 10, 14
M. circinelloides	4	1 R	10
M. hiemalis	9	3 R	6. 14
Nectria haematococca	9	2 R	21
Paecilomyces farinosus	6	2 R	2, 20
Penicillium	135	32 M	All samples except (8, 9, 10, 12)
P. brevicompactum	18	11 R	1, 2, 6, 7, 13. 16, 17, 18, 21
P. chrysogenum	23	8 R	2, 14, 16, 17, 19, 20
P. citriman	19	7 R	1, 2, 4, 13, 14, 17
P. corylophilum	1	1 R	17
P. decumbens	2	1 R	3
P. expansum	ı	1 R	13
P. funiculosum	10	4 R	4. 15, 17
P. griseofulvum	1	I R	. 2
P. islandicum	19	3 R	13.21
P. lanosum	2	2 R	17, 18
P. oxalicum	5	2 R	5, 14

Table (2): continue

Genera and species	TC	NCI and OR	Keys of samples contained that isolates
P. roquefortii	8	4 R	2, 13, 17, 18
P. steckii	7	4 R	3, 11, 13, 21
P. variabile	7	3 R	5, 18, 21
P. waksmani	12	2 R	13, 20
Rhizopus stolonifer	376	.65 H	All samples except (9, 10)
Scopulariopsis brevicaulis	15	I R	8
Stemphylium vesicarium	4	1 R	2
Syncephalastrum racemosum	23	6 R	6, 10, 14
Teteracoccosporium paxianum	1	1 R	11
Trichoderma	12	4 R	2,21
T. hamatum	3	3 R	2,21
T. koningii	9	3 R	2.21
Trimmutostroma	10	6 R	2, 6, 7, 20, 21
T. betulinum	8	5 R	2, 7. 20, 21
T. salicis	2	1 R	6
Ulocladium	33	24 L	1, 2, 5, 6, 7, 8, 11, 12, 16, 21
U. alternaria	17	13 L	1, 5, 6, 7, 8, 11, 12, 16, 21
U. atrum	6	6 R	2, 6, 7, 8, 11, 21
U. chlamydosporum	10	8 R	1, 2, 6, 8, 11, 12, 16, 21
Sterile mycelia	64	15 L	3, 4, 5, 7, 8, 12, 14,15, 17,20
Yeast	134	26 M	. 1, 2, 4, 6, 7, 14, 15, 17, 19, 20, 21
Total count	2720		
Number of genera	25		
Number of species	91 + 2 var		

Occurrence remarks (OR) H = High occurrence > 51 samples; M = Moderate occurrence, 25 - 50 samples; L = Low occurrence, 12 - 24 samples; R = rare occurrence < 11 samples.

References

- Abdel-Gawad K. M. and Sabah M. Saber. 1989: Mycoflora and mycotoxins producing fungi of Karkade calyx in Egypt. Bulletin Faculty of Science, Assiut University 18 (2-D): 111 125.
- Abdel-Hafez A. I. I. and O. M. O. EL-Maghraby. 1992. Fungal flora and aflatoxin associated with cocoa, roasted coffee and tea powders in Egypt. Cryptogamie Mycologie 13 (1): 31 45.
- Abdel-Hafez S. I. I. and A. H. M. El-Said 1997. Effect of garlic, onion and sodium benzoate on the mycoflora of pepper, cinnamon and rosemary in Egypt. International Biodeterioration and Biodegradation 39 (1): 67 77.
- Abdulkadar W. H. A., A. A.Al-Ali, M. A. Al-Kildi and H. J. Al-Jedah, 2004. Mycotoxin in food products available in Qatar. Food Control 15: 543 548.
- Abou-Arab A. Α. K.. M. S.Kawther, M. E.El-Tantawy, R. I. Badeaa and N. Khavria. 1999. Quantity estimation of contaminants some in used medicinal commonly plants in the Egyptian market. Food Chemistry 67: 357 - 363.
- Akiyama H., Y. Goda, T. Tanaka and M. Toyoda.2001. Determination of aflatoxins B₁, B₂, G₁ and G₂ in spices using a

- multifunctional column cleanup. Journal of Chromatography A 932: 153 – 157.
- Al-Doory Y. 1980. Laboratory Medical Mycology. Lea and Febiger, Philadelphia Kimpton publishers, London 410.
- Aruoma O. I. 1998. Free radicals, oxidative stress and antioxidants in human health and disease. Journal of the American Chemists Society 75 (2): 199 212.
- Ayres G. I., I. Mudto and E. W. Sondine.1980. Microbiology of food, spices and condiments. (pp. 249) A series of Books in Food and Nutrition (ED.) Scheigret.
- Aziz N. H. and Y. A. Youssef.1991. Occurrence of aflatoxins and aflatoxinproducing molds in fresh and processed meat in Egypt. Food Additives and Contaminants 3: 321 331.
- Aziz N. H., Y. 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. Botanical Bulletin Academia Sinica 39(4): 279 285.
- Banerjee A., R. P.Mathews, H. S. Prakash and H. S. Shetty. 1993. Mycobiota and toxigenic Aspergillus flavus associated with developing cardamom and

- pepper. Mycological Research 97 (11): 1403 1406.
- Beuchat L. R., B. V. Nail, R. E. Brackett and T. L. Fox. 1991. Comparison of the petrifilm TM yeast and mold culture film method to conventional methods for enumerating yeasts and molds in foods. Journal of Food Protection 54: 443 447.
- Bircan C. 2005. The determination of aflatoxins in spices by immunoaffinity column extraction using HPLC. International Journal of Food Science and Technology 40: 929 934.
- Booth C. 1971. The genus Fusarium (PP. 237). Commonwealth Mycological institute, Kew, Surrey, England.
- Booth C. 1977. Fusarium laboratory guide to the major species (PP.58). Commonwealth Mycological institute, Kew, Surrey, England.
- Bugno A., A. A. B.Almodovar, T. C.Pereira, T. J. O. A. Pinto and M. Sabino. 2006. Occurrence of toxigenic fungi in herbal drugs. Brazilian Journal of Microbiology 37: 47 51.
- Chourasia H. K.1995. Mycobiota and mycotoxins in herbal drugs of Indian pharmaceutical industries. Mycological Research 99: 697 703.
- Christensen C. M. and Raper K. P. 1978. Synoptic key to Aspergillus nidulans group

- species and related *Emericella* species. Transaction of the British Mycological Society 71: 177 191.
- Domsch K. H., W. Gams and T. Anderson. 1980. Compendium of soil fungi (PP. 859). Academic Press, London.
- Efuntoye M. O. 1999 Mycotoxins of fungal strains from stored herbal plants and mycotoxin contents of Nigerian crude herbal drugs. Mycopathologia 147: 43 48.
- El-Bazza Z. E., A. F. Hala, M. Z. El-Fouly and S. M. El-Tablay. 2001 Inhibitory effect of gamma radiation and Nigella sativa seeds oil on growth, spore germination and toxin production of fungi. Radiation Physics and Chemistry 60: 181-182.
- El-Kady I. A. and M. H. Moubasher. 1982. Toxigenicity and toxin of *Stachybotrys chartarum* isolated from wheat straw samples in Egypt. Experimental Mycology 6 (1): 25 31.
- El-Kady I. A., El-Maraghy S. M. and Eman Mostafa M. 1992. Contribution of mesophilic fungi of different spices in Egypt. Mycopathologia 120: 93 101.
- El-Kady I. A., A. A.Zohri, M.Eman Mostafa and R. S. M. Kotby. 1997. Phyllosphere and phylloplane fumgi of some

- herbal plants belonging to Labiates, Solanaceae and Umblliferae in Egypt. Qatar University Science Tournal 17 (2): 247 264.
- Ellis M. B. 1971. Dematiaceus Hyphomycetes, (PP.608). Commonwealth Mycological institute, Kew, Surrey, England.
- Ellis M. B. 1976. More Dematiaceus Hyphomycetes, (PP. 507). Commonwealth Mycological institute, Kew, Surrey, England.
- ElShafie E. A., A. T.Al-Rashd, N. S. Al-Bahry and S. C. Bakheit. 2002. Fungi and aflatoxins associated with spices in Sultanate of Oman. Mycopathologia 155: 155 160.
- El-Shanawany A. A., L. A.Naser and A.Barakat. 2002. Mycobiota and antifungal effect of some spices in Riyadh, Saudi Arabia. Bulletin of Pharmceulical Science, Assiut University 25(1): 95 104.
- Eman Mostafa M. 1990. Mycoflora and mycotoxins of some spices.
 M. Sc. Thesis. Botany department, Faculty of Science, Assiut University, Assiut, Egypt.
- Flannigan B. and S.C. Hui.1976.

 The occurrence of aflatoxin producing strains of Aspergillus flavus in the mould flora of ground spices. Journal of Applied Bacteriology 41: 411 418.

- Freire F. C. O., Z. Kazakiewicz and R. R. M. Paterson. 2000. Mycoflora and mycotoxins in Brazilian black pepper, white pepper and Brazil nuts. Mycopathologia 149: 13 19.
- Garrido M. D., R. Jordano and R. Pozo. 1992. Mold flora and aflatoxin- producing strains of Aspergillus flavus in spices and herbs. Journal of food Protection. 55: 451 452.
- Garrido M. D., J. R. ordano, P.Martinez, M. Jodral and R. Pozo. 1988. Fungal contamination of commercial spices. Alimentaria 8 I: 83 - 84.
- Giese J. 1994. Spices and seasoning blends: A taste for all seasons. Food Technology 48 (4): 87 -98.
- Halt M. 1998. Moulds and mycotoxins in herb tea and medicinal plants. Europian Journal of Epidemiology 14: 269 274.
- Hashmi M. H. and A. Ghaffar 1991. Seed-borne mycoflora of *Coriandrum sativnm* L. Pakistan Journal of Botany 23: 165 - 172.
- Hemida S. K. 2004. Influence of some commercial oils on growth of fungi and their degrading enzymes. Journal of Biomedical Science 16: 365 379.
- Ibrahim M. N. M. 1983. Studies on some mycotoxins in beans. M. Sc. Thesis, Food Technology,

- Faculty of Agriculture; Cairo University, Cairo, Egypt.
- Ismail M. A. 2000. Mycological criteria and antifungal properties of cardamom and black mustard from two east African tropical countries. Bulletin of the Faculty of science. Assiut University 29 (2-d): 233 254.
- Julseth R. M. and R. H. Deibel 1974. Microbial profile of selected spices and herbs at import. Journal of Milk Food Technology 37 (8): 414 - 419.
- Kneifel W. and E. Berger 1994.
 Microbial criteria of random samples of spices and herbs retailed on the Austrian market.
 Journal of Food Protection 57: 893 901.
- Mandeel Q. A. 2005. Fungal contamination of some imported spices. Mycopathologia 159: 291 298.
- Martin A., Aranda E., M. J. Benito, F.Pěrez-Nevado and M. G. Cŏrdoba.2005. Identification of fungal contamination and determination of mycotoxigenic molds by micellar electrokinetic capillary chromatography in smoked paprika. Journal of Food Protection 68 (4): 815 822.
- Martinez-Magana P., M. Jodral-Villarejo and R. Pozo-Lora. 1989. Mycoflora and Aspergillus flavus in pepper on sale in Spain. Microbiologie-Aliments- Nutrition 7: 311-314.

- Martins L. M., Martins M. H. and Bernardo F. (2001): Aflatoxins in spices marketed in Portugal. Food Additives and Contaminations 18: 315 - 319.
- McKee L. H.1995.Microbial contamination of spices and herbs: A Review. Lebensmiltte-Wissenschaft und Technologie 28: 1 11.
- Moharram A. M. M., A. Y. Abel-Mallek and A. I. I. Abdel-Hafez. 1989. Mycoflora of anise and fennel seeds in Egypt. Journal of Basic Microbiology 29 (7): 427 435.
- Moubasher M. H.1993. Soil Fungi in Qatar and Others Arab countries. Pulblished by Center for Scientific and Applied Research, University of Qatar, Doha, Qatar (pp. 566).
- Omafuvbe B. O. and D. O. Kolawole 2004. Quality assurance of stored pepper (*Piper guineense*) using controlled processing methods. Pakistan Journal of Nutrition 3 (4): 244 249.
- Oxford English Dictionary. 1989. Oxford English Dictionary 2nd Ed, Oxford University Press, Oxford, England.
- Pitt J. I. 1979. The genus Penicillium and its teleomorphic Eupenicillium states and **Talaromycces** (PP. 634). Commonwealth Scientific and Industrial Research Organization. Division ofFood

- Research, North Ryde, N. S. W. Australia.
- Pitt J. I. 1985. A laboratory guide to common *Penicillium* species. Commonwealth Scientific and Industrial Research Organization. Division of Food Research, North Ryde, N. S. W. Australia. (PP. 184).
- Pitt J. I., A. D. Hocking, R. A. Samson and A. D. King. 1992. Recommended methods for mycological examination of foods, In: Modern Methods in Food Mycology, eds. Samson R. A., Hocking A. D., Pitt J. I. and King A. D. Amsterdam: Elsevier. (pp. 365 368).
- Pons W. A., A. F. Cucullu and A. O Franz. 1972. Rapid quantitative TLC method for determining aflatoxins B1. 1- Development of the method. J. Association Official Analytical Chemists 55: 768 - 774.
- Pruthi J. S. 1980. Spices and condiment: chemistry, microbiology and technology. Advance in Food Research Supply 4. Academic Press, New York.
- Ragab W. S. M. 1994. Studies on some mycotoxins in certain foodstuffs. Ph. D. Thesis. Food Science and Technology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.
- Ramirez C. 1982. Manual and Atlas of Penicillia. Elsevier

- Biomedical Press, Amsterdam, The Netherlands (PP. 874).
- Raper K. B. and D. J. Fennell 1965.
 The genus *Aspergillus*.
 Williams and Wilkins, Baltimore USA (pp. 686).
- Raper K. B. and C. Thom 1949. A manual of *Penicillium*. Williams and Wilkins, Baltimore USA. (PP. 75).
- Rifai M. A. 1969. A revision of the genus Trichoderma. Mycological Papers 166: (pp.56).
- Rizzo I., G. Vedoya, S. Maurutto, M. Haidukowski and E. Varsavsky 2004. Assesment of toxigenic fungi on Argentinean medicinal herbs. Microbiology Research 159: 291 - 298.
- Romagnoli B., V. Menna, N. Gruppioni and C. Bergamini. 2007. Aflatoxins in spices aromatic herbs, teas and medicinal plants marketed in Italy. Food Control 18: 697 701.
- Salunkhe D. K. M. T. Wu, J. Y. Do and M. R. Mass. 1980. Mycotoxins in foods and feeds. In Safety of Foods, 2nd edition, Ed by H. D. Graham. AVI Publishing Company Inc. Westport, Ct.: 198 268.
- Sherman W. P. and J. Billing. 1998. Antimicrobial function of spices. Quarterly Review of Biology 73 (3): 1-47.
- Sherman W. P. and S. M.Flaxman 2001. Protecting our selves from

- food. American Scientist 89 (2): 142.
- Shrivastava A. and P. C.Jain. 1992. Seed mycoflora of some spices. Journal of Food Science and Technology 29: 228 - 230.
- Simmons E. G. 1967. Typification of *Alternaria*, *Stemphylium* and *Ulocladium*. Mycologia 59: 67 92.
- Sivanesan A. 1984. The bitunicate Ascomycetes and their anamorphs. Strauss and Cramer Gmbh, Hirschberg ISBN, Germany (PP. 701).
- Smith N. R. and V. T. Dawson.1944. The bacteriostatic action of Rose-bangal media used for the plate counts of soil fungi. Soil Science 58: 467 - 471.
- Takahashi T. (1993): Aflatoxin contamination in nutmeg: analysis of interfering TLC spots. Journal of Food Science 58: 197 198.
- Tassaneeyakul W., E.Razzazi-Fazeli, S. Porosnphtana and J. Bohm 2004. Contamination of aflatoxin in herbal medicinal products in Thailand. Mycopathologia 158: 239 - 244.
- Thompson S. S., J. P Beasley. and J. A. Baldwin 1989. Preventing of aflatoxin in farmer stock peanuts, Leaflet. Cooperative

- Extension Service, University of Georgian Athens, Ga.
- Vardavakis E. 1988. Seasonal fluctuation of non-parasitic mycoflora associated with living leaves of *Cistus incanus*. *Arbutus unedo* and *Quercus coccifera*. Mycologia 80 (2): 200 210.
- Youssef M. S. 1995. Mycoflora and mycotoxins of some medicinal plants and their antimicrobial activities in Egypt. Ph. D. Thesis, Botany Department, Faculty of Science, Sohage, South Valley University, Egypt.
- Zinedine A., C. Brera, S. Elakhdari, C. Catano, F. Debegnach, S. Angelini, B. De Santis, M. Faid, M. Benlemlih, V. Minardi and M. Miraglia. 2006. Natural Occurrence of mycotoxins in cereals and spices commercialized in Morocco. Food Control 17: 868 874.
- Zohri A. A. 1990. Mycoflora and mycotoxins of some meat products. Ph. D. Thesis, Botany Department, Faculty of Science, Assiut University, Assiut, Egypt.
- Zohri A. A., M. Sabah Saber and K. M. Abdel-Gawad 1992. Fungal flora and mycotoxins associated with onion (*Allium cepa* L.) in Egypt. Korean Mycology 20: 302 308.

الحالة الفطرية وسموم الافلاتوكسينات لبعض البهارات والأعشاب شائعة الاستخدام في محافظة تعز، الجمهورية اليمنية

محمد أحمد عبد الساتر $^{(1)}$, عبد الرحمن حمود الزبيري $^{(2)}$, فهد عبد الحميد الشرجبي $^{(2)}$ و مغيدة أحمد هزاع جازم $^{(2)}$

(1) قسم النبات, كلية العلوم, جامعة أسيوط, أسيوط, جمهورية مصر العربية

(2) قسم الميكروبيولوجي التطبيقي, كلية العلوم التطبيقية, جامعة تعز, تعز, الجمهورية اليمنية

أجريت الدراسة على ١٠٥ عينة تنتمي إلى ٢١ نوع من البهارات والأعــشاب شــائعة الاستخدام في محافظة تعز، الجمهورية اليمنية. كانت أكثر البهارات تلوثا هي النخوة (الكمون الملوكي), الحبة السوداء (حبة البركة), الكزبرة, الكمون, الحلبة, الزنجبيل, الغار والزعتر. في حين كانت بهارات الخردل الأسود, الكراوية, الهيل, الشمر, الحلف (حسب الرشاد), جوز الطيب والفلفل الأحمر متوسطة التلوث بالأعفان والخمائر. على الجانسب الأخر كان كل من الينسون, الفلفل الأسود, القرفة, الكتان والقرطم أقل تلوثـــا بالأعفـــان والخمائر. بينما كانت عينات القرنفل خالية تماماً من الأعفان والخمائر. دراسمة الفلورا الفطرية المتواجدة في البهارات المختلفة أظهرت أن معظمها عالية التلوث باللاعفان والخمائر, حيث وجد أن ما نسبته 91,42 % من العينات المختبرة ملوثة بالفطريات. تـم عزل إحدى وتسعون نوعا بالإضافة إلى صنفين ينتمون إلى 25 جنسا من الفطريسات. كانت أجناس الأسبر جلس, الريزوبس والبنيسليوم هي الأكثر شيوعا حيث مثلــت العــدد الأكبر من الفطريات المعزولة. كان جنس الأسبرجلس هو الأكثر ســيادة وكانــت نــسبة تواجده في العينات 90,5 % بنسبة 65,1 % من العدد الكلى للفطريات المعزولة. من بين الفطريات المعزولة في هذه الدراسة كان هنالك 14 نوعاً تم عزلها وتعريفها لأول مرة في اليمن. وقد أظهرت النتائج وجود سم الافلاتوكيسن B_i في سبعة أنسواع مــن البهـــارات المدروسة في حين خلت البقية من أي كميات محسوسة من سموم الافلاتوكسينات.