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CONTAMINATION OF SPICES BY FUNGI AND THEIR MYCOTOXINS

BY

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ABSTRACT

A total of 205 spices samples (including the following types : Black pepper corns, cumin, anise, caraway and coriander "25 samples of each").

Ground black pepper, ground cumun, fennel and spearmint (20 samples of each) were collected from various retailers of Alexandria city and their fungal flora was studied and the ability of the isolated fungi to produce aflatoxins was investigated.

Seventeen genera of contaminating fungi, were detected A. flavus and A. parasiticus represented the highest percentage (46.66%, 40% respectively).

Direct determination of aflatoxins in spices samples revealed aflatoxin G_1 contamination in 2 samples of black peppers and aflatoxin B_1 contamination in 2 cumin samples.

INTRODUCTION

Recently, the field of food mycology has gained tremendous attention throughout the world due to the occasional contamination of foodstuffs by mycotoxins, arising from the secondary metabolic processes of contaminant moulds, (Joffe, 1978).

The potential significance of such food-borne mycotoxins is concerned in their connection with human and animal health, for instance outbreaks of fatal toxicosis of man and animal which have been correlated with the consumption of mouldy foods (Angusbhakoru et al., 1981 and Colvin et al., 1984).

In addition, some mycotoxins have been found to be carcinogenic, teratogenic, tremorgenic, haemorrhagic and dermatitic (wray, 1981 and Garner, 1984). While others are now known to be capable of causing mutations in susceptible organisms (Bullerman, 1979 and Dietert et al., 1983).

The present investigation aimed at studying the fungal flors in different spices and investigating the sbility of the isolated fungi to produce aflatoxins, in addition to the detection of the possibility of aflatoxing occurrence in spices samples.

MATERIAL AND METHODS

A total of 205 spices samples (including the following types: Black pepper corns, cumin, anise, caraway and coriander "25 samples of each").

Ground black pepper, ground cumin, fennel and spearmint (20 samples of each) were collected from various retailers of Alexandria city in clean labelled packets.

Each sample was subjected to the following procedures:

1. Mycological examination:-
   a. Enumeration of moulds in the samples : (Refai, 1979).
   b. Isolation and identification of fungal isolates : using micro-technique, according to the methods described in Ajello et al., (1963); Raper and Fennll (1965), and Al-Doory (1980).
      The isolated yeast genera were characterised using Api 200 kits.

2. Screening of the isolated fungi for aflatoxin production using thin layer chromatography (T.L.C.) (Mashaly and El-Deeb, 1983).

3. Determination of aflatoxins from spices samples. In parallel with mycological examination of spices samples, direct extraction and estimation of aflatoxins were done on representative samples of Black pepper corn and cumin (10 each) according to the methods of Awe % Schranz (1981).

RESULTS

The investigation of 205 samples of spices revealed 17 genera of contaminating fungi, of which the most commonly isolated genera in order of frequency were *Aspergillus*, *Penicillium* and *Rhizopus* species (96.59, 69.76 and 64.39% respectively). While the percentages of the other genera were 25.85% of *Absidis*, 10.73% of *Mucor*, 9.29% of *Alternaria*, 6.34% of *Paciliomyces*, 5.85% of *Cladosporium*, 4.88% of *Cephalosporium*, 4.39% of *Scopulariopsis*, 3.90% of *Candida*, 2.44% of *Fusarium* and *Stemphylium* 1.46% of *Epicoccum* and *Geotricum* and 0.98% of *Saccharomyces*. (Table 1).

Ten species of *Aspergillus* were identified, (Table 2). Four species of *Candida* (*C. tropicalis*, *C. Krusei*, *C. guilliermondii*, *C. parapsiliosis*) were isolated from 8 samples of spieces (Black pepper corn, cumin, anise, caraway, coriander and ground cumin).

Table (1) The different of fungi isolated from 205 samples of spices

Genera of fungi	Number of samples yielded fungal isolates									Total	
	Black pepper corn	Cumin	Anise	Caraway	Cori-sander	Ground black pepper	Ground cumin	Fennel	Spear-mint	No.	%
Aspergillus	24	24	23	24	24	20	20	19	20	198	96.59
Penicillium	15	21	20	23	22	7	12	15	8	143	69.76
Phizopus	12	18	20	15	22	5	15	18	7	132	64.39
Absida	13	3	6	7	5	6	1	4	8	53	25.85
Mucor	5	3	2	3	2	-	-	2	5	22	10.73
Trichoderma	-	2	4	3	5	-	3	2	-	19	9.29
Alternaria	-	3	3	3	3	1	2	2	-	17	8.29
Paciliomyces	1	2	1	3	1	-	1	4	-	13	6.34
Cladosporium	-	2	3	-	1	-	1	-	5	12	5.85
Cephalosporium	-	-	4	1	3	-	-	2	-	10	4.88
Scopulariopsis	-	1	2	4	-	-	-	2	-	9	4.39
Candida species	1	1	2	1	1	-	2	-	-	8	3.90
Funnrium	1	-	-	-	-	-	-	-	4	5	2.44
Stemphyling	-	-	1	1	-	-	-	1	2	5	2.44
Epicoccum	-	-	1	-	1	-	-	-	1	3	1.46
Ceotrium	-	-	-	-	1	-	2	-	-	3	1.46
Saccharomyces	-	1	1	-	-	-	-	-	-	2	0.98

The No. of examined samples : 25 samples of each spices 20 samples of each spices

Table (2): Nembers of Aspergillus groups isolated from 205 samples of spices

Aspergillus group	Number of samples yielded different groups of Aspergillus										
	Black* pepper	Cumin*	Anise*	Caraway*	Cori-* ander	Ground. black pepper	Ground** curin	Fennel**	Spear-** mint	No.	%
A.flavus-oryzae group	20	18	16	16	20	20	18	18	11	157	76.58
A.flavus	9	9	8	7	9	11	10	8	6	77	37.56
A.parasiticus	5	4	5	3	6	5	4	5	3	40	19.51
A.oryzae	4	5	3	5	5	3	4	5	2	36	17.56
A.tararrii Kita	2	-	-	1	-	1	-	-	-	4	1.95
A.niger	23	22	20	21	23	20	20	19	18	186	90.73
A.ochrecous	3	4	2	1	4	12	2	3	1	32	15.61
A.fumigatus	2	1	3	9	-	1	-	6	1	23	11.22
A.Terreus	2	3	1	2	1	-	-	-	1	10	4.88
A.candidus	1	-	2	-	-	-	-	-	1	4	1.95
A.nidulanus	-	1	-	-	-	-	-	-	-	1	0.49
Other spp.	-	4	5	9	13	-	5	2	1	39	19.04

The no. of examined samples
* 25 samples of each spices.
** 20 samples of each spices.

The fungal spore counts per gram of spices samples were variable from genus to the other and even within the same species (Table 3).

Results of aflatoxins production by isolated fungi from spices:

A. flavus represented the highest percentage (46.66%), A. parasiticus was the second one (40%), followed by A. oryzae (16.66%), and A. higher and Penicillium (10%). But none of the tested strains of A. tamarrii kita produced aflatoxins (Table 4).

Aflatoxin B_1 was the most frequent type produced by the tested organisms, next was aflatoxin B_2.

Results of direct determination of aflatoxins in spices samples :

Out of 20 spices samples 2 samples of Black peppers were contaminated with aflatoxin G_1 with a level of 1.72 and 3.18 ppb., and 2 samples of cumin were contaminated with aflatoxin B_1 in level of 0.29 and 0.96 ppb. At the same time the 4 positive samples of aflatoxin contamination, were also contaminated with toxigenic strains of A. flavus and A. parasiticus.

DISCUSSION

The domination of Aspergillus and Penicillium spp. in all examined spice samples was in accord with the results of Flannigan and Hui (1976) who stated that Aspergillus and Penicillium spp. were the main components of ground spices (black, white and red pepper, caraway, cumin, fennel, coriander, aniseed and fenugreek) in United kingdom.

A. higher and A. flavus-oryzae group especially A. flavus were the most frequent Aspergillus species yielded from our spices samples. This was in agreement with many authors who isolated A. flavus as a prominent component of black pepper mycoflora (Christensen et al., 1967 and Moreno-Martinez and Christensen, 1973) and Indian spices (coriander, cumin and fennel) (Misra, 1981). Considering that the examined spices samples were stored with different long averages at retailers the domination of

Table (3) : Colony count of contaminating fungi in spices samples

Spices	Colonies / gram of spices				
	Total	All Aspergillus spp.	<u>A.flavus-oryzae</u> group	b- <u>A. niger</u>	Penicillium spp.
Black pepper corn	5.7×10^3	3.3×10^3	1.3×10^3	1.4×10^3	2.3×10^3
Cumin	1.1×10^4	4.9×10^3	1.7×10^3	2.6×10^3	5.3×10^3
Anise	6.8×10^3	4×10^3	ε.6 x 10^2	2.4×10^3	2.5×10^3
Caraway	1.5×10^4	7.6×10^3	1.3×10^3	1×10^3	7.3×10^3
Coriander	1.2×10^4	8.9×10^3	1.9×10^3	3.4×10^3	2.9×10^3
Ground black pepper	1.6×10^4	1.5×10^4	7×10^3	7.6×10^3	5.7×10^2
Ground cumin	6.4×10^3	6.4×10^3	4.7×10^3	1.7×10^3	1.5×10^3
Fennel	6.6×10^3	2.6×10^3	7.4×10^2	1.4×10^3	3.4×10^3
Spearmint	2.6×10^3	1.4×10^3	2.4×10^2	1×10^3	6×10^2

Table (4) : Types of aflatoxins produced by fungi isolated from spice samples

Types of screened fungi	Total No. of fungi	Positive strains for aflatoxi production	Types of aflatoxins produced					
			B_1	B_2	B_1 & B_2	G_1	G_2	B_1 & G_1
A. flavus	30	14 (46.66%)	7	-	4	1	-	2
A. parasiticus	20	8 (40 %)	6	-	-	2	-	-
A. Oryzae	18	3 (16.66%)	2	-	1	-	-	-
A. tamarrii kita	4	0 (0%)	-	-	-	-	-	-
A. niger	20	2 (10 %)	1	1	-	-	-	-
Penicillium spp.	20	2 (10 %)	2	-	-	-	-	-
Total	112	29 (25.89%)	18	1	5	3	-	2

Aspergillus and Penicillium species may be attributed to the suitability of the substrate for their growth. The mean total fungal spore of spices ranged from 2.6 X 10^3 colonies/ /gram in spearmint to 1.6 X 10^4 colonies/gram in ground black pepper. There is a great controversy concerning this point, and most authorities reported variable results; for instance Flannigan and Hui (1976), in Scotland, recorded that the mean mould counts of ground spices (cumin, caraway, fennel, Aniseed, coriander and black pepper) has ranged from 1.5 X 10^3 colonies/gram in cumin to 6.4 X 10^5 colonies/gram in black pepper.

Insects can cause damage in the spices, which assist in the fungal inoculation as they carry fungal spores and act as vectors for fungi (Fennell et al., 1975).

The isolation of Candida and Saccharomyces species from our spice samples (cumin, ground cumin, anise and coriander) was in accord with the results of Flannigan and Hui (1976), who isolated yeast from anised and cumin in a count of 3.0 X 10^2 and 8.0 X 10^2 colonies/gram respectively.

Thin layer chromatographic analysis of chloroform extracts of the present isolated fungal cultures showed that only, 89% of the tested fungi yielded from spices, produced aflatoxins and the main type detected was aflatoxin B_1.

This was in accord with El-Khadem et al., (1983), who recorded that A. flavus, yielded from spices (red and white pepper, ginger and jamaica pepper), produced aflatoxin B_1, in addition to small percentages of B_2 and G_1.

Several studies reported the production of aflatoxins by standard strains of A. flavus and A. parasiticus using different fermentation media as chemically defined medium, synthetic medium, liquid medium and buffered yeast extract medium (Mashaly and El-Deeb, 1983).

In our study the casein-hydrolyzate medium (which was not previously used as a fermentation medium) was used successfuly for production of aflatoxins. The presence of casein-hydrolyzate as a component of the medium besides other essential elements and trace metals may be a definitive factor in the production of aflatoxins especially by the poor aflatoxin - producer strains.

In favour of such assumption, Ismail et al., (1983) stated that mycelium growth of standard strains of A. flavus and A. parasiticus, and their production of aflatoxins B_1, B_2, G_1, M_1 and M_2, were greatly influenced by the compositional and structural properties of the protein as well as accompanied constituents with protein and their pH. The yielding of aflatoxin G_1 in a small amount from black pepper samples, was in agreement with Seenappa and Kempton (1980).

In contrast, Scott and Kennedy (1973), failed to detect aflatoxins in a number of black pepper samples.

Hitokoto et al., (1978) and Mabrouk and El-Shayeb (1980) recorded the inhibitory effect of black pepper and cumin on aflatoxin formation rather than on mycelial growth. But they did not investigate the natural contamination of these spices by moulds and their toxins. They added their powders to medium contaminated with aflatoxigenic strains. Thus they neglected the natural parameters of spices as natural barriers of substrate like seed coat, pH, water content, temperature and length of time for the mould growth.

So, some spices contain inhibitory substances to aflatoxin production, but this may be in small quantities that decrease the quantity of the toxin produced, but can not stop its production entirely.

It is necessary to protect our foods from mould growth and mycotoxin elaboration, to avoid the public health hazards and to limit economic losses. This can be accomplished by proper storage (under dry conditions and low temperature).

Egyptian standards limits of aflatoxins concentration in the local and imported spices as well as food crops should be formulated and imposed.

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