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Full Length Research Paper

Natural occurrence of fungi and aflatoxins of cinnamon in the Saudi Arabia

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This investigation was designed to throw light on the microbial status of cinnamon (*Cinamomum zeylanicum* blume). A total of 1126 fungal isolated, representing 8 species of 2 genera, *Aspergiluus* and *Penicillium*, were the most common genera. They were recovered and identified from the fifty samples that were dried and ground. Fungi were found in all of the collected samples, *Aspergillus terreus, Aspergillus glaucus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus clavatus, Aspergillus niger, Penicillium restrectum* and *Penicillium* sp. Direct determination of mycotoxins in samples revealed aflatoxin B_1, B_2 and G_1,. They were detected in low concentration which did not exceed 4.67 µg/ kg from 62% of samples. Nevertheless, 17 samples were found to be free of aflatoxins (B_1, B_2 and G_1). Atmospheric relative humidity and storage methods were increased in the infected samples.

Key words: Aflatoxins, cinnamon, *Aspergilus niger*, fungi.

INTRODUCTION

Cinnamon has a long history both as spice and as a medicine. It is the brown bark of the cinnamon tree, which is available in its dried tubular form known as a quill or as ground powder. The two varieties of cinnamon, Chinese and Ceylon, have similar flavor, however the cinnamon from ceylon is slightly sweeter and more refined. Cinnamon has versatile and widely used ingredient in food preparation especially in cakes and sweets (Ensminger and Esminger, 1986).

As with many other agricultural products, cinnamon may be exposed to a wide range of microbial contamination during pre- and post- harvest. And agricultural commodities is a major problem in the tropics and subtropics, where climatic conditions and agricultural and storage practices are conductive to fungal growth and toxin production. Mycotoxins are secondary metabolites of mould fungi identified in toxigenic molds (Van Egmond, 1981; Aziz, 1987; Clevsrton and Ljunggren, 1985; Hashem and Alamri, 2009) *Aspergillus parasiticus, Aspergillus flavus, Aspergillus ochraceus, Aspergillus versicolor, Aspergillus niger* were reported as the most common fungi isolated from herbal and cinnamon (Morozumi, 1978; Aziz et al., 1998; Elshafie et al., 2002). It is commonly and heavily contaminated with xerophilic storage molds and bacteria (Dimic et al., 2000; Roamgnoli et al., 2007).

Food containing spices are more likely to deteriorate and also could exert harmful effects, having in mind health risks associated with mycotoxins produced by some fungal genera (Wary, 1981; Refai, 1988; Aziz et al., 1998; Koci-Tanackov et al., 2007; Hashem and Alamri, 2009). This paper aims at assessing the intensity and frequency of mould's contamination of cinnamon in public markets and the potential producer of mycotoxins to highlight its risk assessment.

MATERIALS AND METHODS

Sampling

Fifty samples of cinnamon bark (*Cinamomum zeylanicum* blume) were collected from various retailers from Eastern Region (Dammam, Al-Jubail - and Al-Khobar) of Saudi Arabia. Samples (100 g /samples) were collected in sterilized polyethylene bags and stored at 4°C to arrest any mycotoxin formation before analysis.

Moisture content

In preparation for the assay, each sample was mixed while in the bag and the required amount weighed for moisture content determination. Moisture content was measured prior to rinsing in distilled water. For moisture content, prepared samples were dried at 60°C for 24 h or until their weight remain constant and the

difference in weight was calculated. Each sample was analyzed in triplicate (Aziz, 1987).

Mycological studies

Ten grams of sample were added to 90 ml portion of sterile saline solution (0.35%) in 500 ml Erlenmeyer flask and was homogenized thoroughly on an electric shaker at constant speed for 15 min. Ten fold serial dilutions were then prepared (Aziz and Youssef, 1991). One milliliter portion of suitable dilutions were used to inoculate Petri dishes containing 15 ml dextrose agar fortified by 0.5 mg chloromphnicol/ml medium plates were incubated at 28°C for 7 to 15 days and the growth of molds was examined. Fungi were isolated and identified according to Raper and Fennel (1977) and Domsch et al. (1981).

Aflatoxins analysis

The method of the associated of official analytical chemists (AOAC) was used for column preparation, clean up and analysis of aflatoxins (Helrich, 1988). Aflatoxins were determined by high pressure liquid chromatography (HPLC) having a water autoinjectore 717, water 626 quadratic pump, millennium 32 software integrator, photo diode detector PDA 996, C18 reverse column white water: methanol: acetonitrile (60:25:15) as a mobile phase at a rate of 0.1 ml/min. Standard aflatoxins (Sigma) were used (Ellis, 1976). Data were collected and represented in tables.

RESULTS AND DISCUSSION

Fungal contamination

The microbiological quality of cinnamon samples collected from eastern province of Saudi Arabia had been shown in Table 1.

One thousand one hundred and twenty six isolates represent 8 species of 2 genera were isolated from samples. *Aspergiluus* and *Penicillium* were the most common genera, where they were represented by 2 and 6 species, respectively. Among these species, *A. flavus* and *A. clavatus* had the highest occurrence remarks and emerged between 32.7 and 19.9 of samples, respectively. The most common *Aspergilli* were *A. fumigatus*, *A. niger*, *A. glaucus* and *A. terreus*. They were detected in 16.9, 13.2, 3.3 and 1.2%, respectively. *Penicillium restrectum* of samples was detected in 9.6% of samples. The other *Penicillium* sp. was observed rarely in less than 3.2% of samples. The emergence of *Aspergilli* and *Penicillia* on the media greatly indicates the presence of these fungi as the dominant mycoflora of different spices. This observation was greatly in agreement with other investigator who deals with mycoflora of species (El-Kady et al., 1995; Bugno et al., 2006; Dimic et al., 2008; Hashem and Alamri, 2009).

The dominant of *Aspergillus* and *Penincillium* spp. in all examined samples 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 cinnamon and other spices which are common in the food industry. Mirsa (1981) and Roy et al. (1988) isolated *A. flavus, A. niger, A. fumigatus, A. ochraceus, A. candicus, A. sydowi, Chaetomium dolicholrichumy, Fusarium moniliforme, Penicillium oxalicum, Alternaria curvularia,* and *Rhizopus* from the seeds of *Amomum subulatum, Coriandrum sativum, Cuminum cyminum, Foeniculum vulagare, Piper nigrum, Cinnamomum zeylanicum* and from the bark of *Acacia catechu* strains of *A. niger* and *A. flavus* or the Oryzae group especially *A. flavus* were the most frequent *Aspergillus* species yielded in all examined samples in this investigation. This was in according with the results of Roy and Chourasia (1990) who stated that *A. flavus* was the main contaminant of different herbal drug samples.

Moisture content

Moisture content is shown in Table 2. The moisture levels in the examined samples ranging from 1 to 9% of the samples were usually found exposed to the outside environment. Atmospheric relative humidity is generally high in the eastern region of Saudi Arabia (average 60 to 80%) (Mandeel, 2005). The contamination with fungal species resulted from neutral extraneous contamination by dust following storage in humid conditions (Domsch et al., 1981; Mandeel, 2005). Fungi fall into two ecological categories, for example, field and storage fungi. Field fungi were observed to invade developing or mature seeds while it is on the plant, the major field fungi genera are, *Alternaria*, *Fusarium* and *Cladosporium*. On 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* (Abou, 2008). The species could be subject for contamination with fungi mainly spice processing, storage and transport (Dimic et al., 2008).

Aflatoxins productions

Aflatoxin production in cinnamon samples is shown in Table 3. Only 31 samples were found to be contaminated with aflatoxin B_1, one sample contained afalatoxin B_2 (0.33 µg/kg) and five samples contained aflatoxin G_1. The samples that had a content of aflatoxin B_1 did not exceed 4.67 µg/kg. Similar results were obtained by Aziz et al. (1998) and Hashem and Alamri (2009). Different samples of spices contaminated by *Aspergiluus* species were analyzed for aflatoxins and it was found free of aflatoxins. This means that the presence of toxigenic moulds in the food does not directly imply the presence of mycotoxins and vise versa (Abou, 2008). This could be explained in light of those spices that are not ideal substrate for aflatoxins formation. Due to the presence of essential oils with antimycotic effects, they may inhibit the production of aflatoxins or reduce fungal infestation and/or subsequent

Table 1. Distribution of mycoflora among cinnamon samples.

Sample	Fungal species							
	P. restrectum	*Penicillium* sp.	*A. terreus*	*A. glaucus*	*A. flavus*	*A. fumigatus*	*A. clavatus*	*A. niger*
1	-	-	-	-	-	-	-	-
2		-	-	-	-	-	20	4
3	-	-	-	-	-	4	20	-
4	-	-	-	6	4	6	2	-
5	-	-	-	6	4	-	18	-
6	-	-	-	-	8	10	4	18
7	-	-	-	21	6	4	-	-
8	20	-	-	-	-	8	-	-
9	-	-	-	-	-	-	-	-
10	-	-	-	-	--	10	-	-
11	-	-	-	4	14	-	-	-
12	-	-	-	-	-	-	-	--
13	-	-	-	-	-	-	6	10
14	4	-	-	-	14	-	-	-
15	-	-	-	-	-	-	-	-
16	--	--	-	-	-	-	--	-
17	-	-	-	-	-	-	-	-
18	2	14	-	-	18	-	-	22
19	-	-	-	-	4	-	-	-
20	-	--	-	-	-	12	-	12
21	-	8	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-
23	8	4	-	-	18	-	-	-
24	-	-	-	-	20	-	20	-
25	-	-	-	-	24	-	-	-
26	-	-	-	-	12	-	-	-
27	-	-	-	-	20	-	-	-
28	-	-	-	-	14	-	-	-
29	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-
31	18		-	-	18	-	-	-
32	10	10	10	-	24	-	-	-
33	14		-	-	-	-	-	-
34	-		-	-	-	-	-	-
35	-		-	-	14	-	-	-
36	14		-	-	-	-	-	22
37	-		-	-	20	14	81	-
38	18		-	-	-	-	-	-
39	-	-	-	--	12	80	-	20
40	-	-	-	-	20	10	4	-
41	-	-	-	-	-	-	-	-
42	14	-	-	-	10	14	-	-
43	-	-	-	-	8	8	-	20
44	-	-	-	-	20	10	-	-
45	-	-	4	-	14	--	-	-
46	-	-	-	-	4	-	-	-
47	-	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	-
49	-	-	-	-	10	-	80	20
50	-	-	-	-	-	-	-	-
Total	108	36	14	37	368	190	225	148
%	9.6	3.2	1.2	3.3	32.7	16.9	19.9	13.2

Table 2. Moisture contents of cinnamon samples.

Sample No	Moisture (%)	Sample No	Moisture (%)
1	5.7	26	6
2	6	27	6
3	7.5	28	7
4	8	29	6
5	7	30	2
6	4	31	8
7	8	32	7
8	7	33	6
9	4.4	34	4
10	6	35	4
11	8	36	9
12	3	37	5
13	8	38	5
14	6	39	8
15	7	40	5
16	8.4	41	2
17	1.4	42	3
18	7	43	3
19	6	44	5
20	7	45	7
21	5.6	46	5
22	4.7	47	7
23	5.1	48	1
24	3	49	7
25	9	50	6

Table 3. Aflatoxin production (µg/kg) in cinnamon samples.

Sample No	Type of AFS	Quantity detected (µg/kg)	Sample No	Type of AFS	Quantity detected (µg/kg)
1	B1	0.4	26	B1	3.33
				G1	2
2	=	0.33	27	B2	0.33
3	=	3	28	B1	0.2
4	=	3.33	29	=	0.13
5	=	0.27	30	=	0.33
6	=	0.33	31	=	Trace
7	=	0.33	32		-
8	=	0.33	33		-
9		-	34	B1	0.13
10	B1	0.27	35	=	0.13
11	=	0.13	36	=	0.27
12	=	-	37	=	0.33
13	B1	1.67	38	=	0.53
14	=	0.53	39	=	0.47
15	=	1	40	B1	3.33
				G1	2.67
15	=	-	41		-
17	B1	0.53	42	B1	0.33
18	=	-	43		-

Table 3. Contd.

19	=	-	44		-
20	B1	1	45	B1	1.33
				G1	3.33
21	=	-	46		-
22	=	-	47		-
23	B1	4.67	48		-
24	=	-	49	G1	1.33
25	B1	2.67	50		-
	G1	2.33			

aflatoxins production.

The important compounds and essential oils in cinnamon are cinnamldehyde, eugenol, cinnamylacetate methoxxcinnamaldehyde, cinnamylakoh, cinnamic acid, cinnzelanol, cinnzeylanin, oligomeric and proanthocyanidins. This agrees with the studies of Lwellyn et al. (1992), Bartine and Elaraki (1997), Chang et al. (2001), Juglal et al. (2002), Wang et al. (2005), Giordani et al. (2006), Ooi et al. (2006) and Lopez et al. (2007), which were indicative of causal contamination after harvesting and drying. The antifungal effects are dependent on the concentration of essential oils and this concentration is further dependent on some factors, such as the part of plant, plant species and environment growth conditions (FAO, 1999); Gowda et al., 2004). Actually, it is important to note that fungal growth is weaker in spices and herbs than other commodities (Roamgnoli, 2007). Most of the identified moulds have been reported to have the ability to produce mycotoxins (Bugno et al., 2006). These results showed that a potential risk for mycotoxins contamination may be caused especially during prolonged storage in poor conditions without temperature and moisture control that usually render plants more susceptible to mould growth and mycotoxins production. More studies are needed to find different methods of using cinnamon as a preservation matrix in some food, due to the role of its essential oils that are highly effective against moulds.

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