International Journal of Applied Agricultural Research
ISSN 0973-2683 Volume 7, Number 2 (2012) pp. 83-98

http://www.ripublication.com/ijaar.htm

Incidence and Distribution of Filamentous Fungi during Storage of Coffee Beans in Eastern Region, Kingdom of Saudi Arabia

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Abstract

This Study Aimed to determine and identify fungi associated with four varieties of coffee beans (*Coffea arabica* L.) were collected from different grocery stores and retail markets in an eastern region of the Kingdom of Saudi Arabia: harary, lukkimy, habbashy and barry. However the highest percentage of fungal infection was associated with harary (36.26%of infection) followed by lukkimy (29.13%), habbashy (20.50%) and barry (14.12%). Several fungi associated with four varieties were isolated at the following frequencies: *Aspergillus niger* (74.71%), *Aspergillus alliaceus* (7.33%), *Aspergillus melleus* (4.32%), *Aspergillus tubingensis* (4.27%), *Fusarium solani* (3.56%), *Aspergillus flavus* (2.01%), *Penicillium oxalicum* (1.61%), *Alternaria alternata* (1.2%), *Emericella nidulans* (0.85%) and *Paecilomyces variotii* (0.15%). The potency of the first five most frequent fungi to infect all studied seeds was ranked, in decreasing order, as: *A. niger, A. alliaceus* and *A.melleus* (100%) however *Fusarium solani* (94%) and *A. flavus* (71%). Processing methods such as roasting reduced to a large extent the degree of fungal contamination in coffee beans, where it decreased the total fungal load in coffee beans after roasting in general for all varieties studied, where almost all samples of coffee were empty of fungi, with different degrees of roasting. The exception was one sample only that is habbashy ($1x10^1$cfu/g). The total fungal load of coffee beans of rubesta (*Coffea canephora* L.) collected from different retail markets, the results was determined by plate count technique. A representative sample from the grounded coffee beans was drawn using sterile metal scoop and a decimal serial dilution was made under sterile conditions, where fungal colonies were isolated in pure culture, that Mocha coffee have

the highest average viable mould count (20 x 10^1 cfu/g), followed by Cappuccino (3 x 10^1 cfu/g), Turkish Coffee (2 x 10^1 cfu/g), Nescafe without caffeine and French Hazelnut Coffee (1 x 10^1 cfu/g), while Nescafe caffeine, French Coffee with milk or vanilla or with coconut and espresso were free of fungi.

Keywords: *Coffee* beans, *Aspergillus, Penicillium*, Filamentous Fungi

Introduction

Coffee (*Coffea arabica* L.), a native of Africa, is grown in more than 50 countries throughout the tropics by more than 20 million coffee farming families [1]. Green coffee is prepared from berries of coffee trees by a relatively complex series of process steps carried out entirely within the producing countries. The fundamental purpose of green coffee processing is the recovery of the beans, by removing the various covering layers and drying to produce green beans with a moisture content below 12%. Drying process and wetting process, are similar processes including grading, cleaning and polishing are performed [2, 3].

Arabic coffee is considered one of the major beverage popular in many parts of the world, like Saudi Arabia, which is one of the main countries that import coffee from countries such as Brazil, Ethiopia, and Yemen in recent times. According to the statistics of 2007, Saudi Arabia imports annually 45477.84 tons of coffee [4].

Coffee beans, like other crops, can be contaminated by microorganisms during different stages of growing, harvesting, processing, transport and storage. Many studies revealed that the important toxigenic fungal genera (Aspergillus and Penicillium) are natural coffee contaminants, and are present from the field to storage [5, 6, 7]. Ochratoxin A (OTA) is a mycotoxin naturally found in various food products including green coffee bean, roasted coffee and instant coffee. Ochratoxin A OTA has carcinogenic, teratogenic, genotoxic, immuno suppressive and potentially cause harm to animals including humans [8, 9]. Ochratoxin A (OTA) in coffee are probably produced by species of Aspergillus section Circumdati and Nigri [10, 11, 12].

No coffee producing country is free from fungal contamination [13]. Extensive studies have been carried out on the mycobiota of coffee in African, Latin American, Middle East, and Asian countries [12, 13, 14, 15, 16, 17, 18, 19, 20]. It is not currently known however, at which point during coffee growth, harvest and processing most fungal contamination occurred and more likely that levels increase when drying and storage are inadequate [13, 18, 21, 22, 23]. Fungal contamination in coffee and an associated ochratoxin A (OTA) problem was due to faults in harvesting and storage practices [24]. Ochratoxin A is an important hepatotoxic, nephrotoxic, teratogenic and carcinogenic toxin [25]. Ochratoxin A (OTA) production was earlier believed to be restricted to *Penicillium verrucosum* [25, 26, 27], and *Aspergillus ochraceus* [28, 29], with *P. verrucosum* predominating in temperate regions and *A. ochraceus* producing OTA in warmer areas [30]. It is likely that environmental conditions in the eastern region are frequently conducive to fungal development in

coffee beans. Taking all this information into account, this study enumerated the mycoflora in coffee beans from areas of major retailers and at the retail market outlets.

Storage fungi of the genera *Aspergillus* and *Penicillium* may also appear with high moisture. These fungi produce aflatoxins which destroy the liver and induce carcino-, muta- and teratogenesis [31]. Identification of fungi associated with such seeds gives an idea of some of the problems faced by seeds in the cultivation of coffee.

The Aim of this study was to isolate fungi associated with the seeds of four varieties of coffee beans in markets in the eastern region of Saudi Arabia, namely: harary, lukkimy, habbashy and barry. The percentage infection and potency of the more frequent fungi were studied.

Materials and Methods

Coffee Samples

Twenty samples of coffee beans (*Coffea arabica* L) were collected from different places of eastern region of Saudi Arabia, to determine and identify fungal population. Samples of four types of coffee beans were collected from different grocery stores for the following studies.

Estimation of Relative Humidity

The "two-stage air oven method" [32] was used. A known seed weight was placed in an oven at 70°C. After 24 h its weight was recorded. Samples were redried and every hour the sample weight was recorded until a constant weight was obtained.
The moisture content was calculated as

$$\text{moisture content} = \frac{\text{moist weight - dry weight}}{\text{moist weight}} \times 100$$

Percentage External Infection

One hundred seeds, chosen at random from each seed batch was used to calculate the percentage infection according to the [33] method using the equation:

$$\%\ \text{External infection} = \frac{\text{number of external infected seeds}}{\text{total number of seeds}} \times 100$$

External infection appeared as coloured, irregular or atrophic seeds.

Isolation and Identification of Fungi

Coffee Beans

The coloured or irregular and atrophied seeds were chosen, disinfected by immersing in 10% household chlorine bleach ($NaClO_2$) for 3 min, rinsed in distilled water for 5 min and dried for almost 1 min [34]. Seeds were plated on malt extract agar (MEA) [35]. Five seeds were placed in each Petri dish with ten replicates and incubated at 28 ± 2°C for five days.

Ground Coffee

Each sample (half kg) was toasted with different degrees (light, medium, dark) and ground using a mill and thoroughly mixed for one hour by dough mixer. Fungal load in coffee beans was determined by plate count technique, serial dilution was made under sterile conditions. From the serially diluted solution, 1 ml each was pour plated in malt extract agar (MEA) with rose bengal and chloramphenicol agar. Fungal population was accounted after 5−7 days incubation at 25 °C. All samples were analyzed twice with five replicated plates.

The kind and number of fungi were recorded using the hyphal tip technique suggested by [36]. The isolated fungi were identified according to their morphological characters. Identification of genus and species was confirmed with help of the Department of Mycology at the Agricultural Research Institute, A.R.E.

Frequency of Fungal Infection

The frequency of infection was calculated to determine the most susceptible seeds to fungal infection.

$$\% \text{ Frequency } = \frac{\text{Total No. of fungal isolate \ crop}}{\text{total number of fungal isolates}} \times 100$$

Pathogenicity Tests

Preparation of Fungus-Free Coffee Beans

Complete ripe spikes, that free from diseases and insect infestation were handly picked, threshed and cleaned. The seeds were surface disinfected as mentioned before, washed thoroughly several times in sterilized distilled water dried in an air oven at 42^{o}C for two days, and then kept under aseptic conditions till use. These samples were tested relatively fungus-free as no more than 3% of its seeds yielded fungi when plated on either PDA or malt extract agar.

Pathogenicity Tests of Isolated Mold Fungi

Five fungal genera, representing the most frequently isolated seed pathogens, named *Aspergillus niger* (frequency 74.43%), *Aspergillus alliaceus* (7.28%), *Aspergillus melleus* (4.29%), *Aspergillus tubingensis* (4.24%) and *Fusarium solani* (3.54%) were grown on Potato Dextrse Agar (PDA) media. incubating at 25 ± 2°C for 15 days. Spores from pure culture suspended in sterilized distilled water containing 0.1% agar were used as an inoculums. The spore density was determined by counting 10 samples of each using hemacytometer. Spore suspension sufficient to give final concentration of approximately 2000-2500 spores/g were added to the calculated amount of water needed to raise the seeds moisture content to 20%. After 45days of storage at 25+2^{o}C both seed invasion and external infection were determined.

Statistical Analysis

Data obtained were statistically analyzed using SPSS Version 6. Treatment averages were compared at the 0.05 level of probability using LSD. [37].

Results

Twenty samples of coffee beans were collected from different places of eastern region, Saudi Arabia to determine and identify fungal population. Ten species belonging to 5 genera were isolated by using malt extract agar (MEA) media at 27±2°C. The most prevalent genera were *Aspergillus.*

The mycoflora of coffee beans in an eastern region were determined in green beans and after roasted coffee from retail markets. Tent species from 5 genera were recovered. *Aspergillus* was prevalent with five species such as *Aspergillus niger*, *A. alliaceus*, *A. melleus* , *A. tubingensis* and *A. flavus* dominated coffee at : *Aspergillus niger* (74.71%), *Aspergillus alliaceus* (7.33%), *Aspergillus melleus* (4.32%), *Aspergillus tubingensis* (4.27%), *Aspergillus flavus* (2.01%) and *Emericella nidulans* (0.40%). The species of *Penicillium* were *P. oxalicum* (1.60%) and *Paecilomyces variotii* have less than 1% incidence. Other filamentous fungi were *Fusarium solani* (3.54%) and *Alternaria alternata* (1.30%) as it is written in the table 6.

Detection of Fungal External Symptom

lukkimy coffee beans had the highest percentage of the external infection (40.99%) followed by habbashy (40.48%), harary (35.02%) and finally barry (30.78%) (Table 1). There was great variation between external symptoms of infection and moisture content of seeds (Table 1).

Table 1: Average Percentage of External symptoms and percentage moisture content of some varieties of coffee beans in the different governorates at the Eastern Province of Saudi Arabia Kingdom. (A: % External symptoms, B:% Moisture content).

Governorate	Coffee Beans Cultivars								Mean
	harary		lukkimy		habbashy		barry		
	A	B	A	B	A	B	A	B	
Al-Dammam	30.09	6.01	45.83	6.09	46.5	8.52	31	6.38	38.36
Al-Hassa	21.2	6.27	29.98	5.92	44.5	4.83	34.33	4.73	32.45
Ras Tanura	37.53	8.31	48.53	7.84	45.63	8.46	-	-	43.9
Qatif	42.32	6.18	33.3	6.21	25.3	7.77	27	6.80	31.98
Al-Khobar	43.66	5.05	47.33	5.82	-	-	27	5.84	39.33
Mean	35.02	6.36	40.99	6.38	40.48	7.4	30.78	5.16	

Fungal Isolation

Harary variety were contaminated with *Aspergillus niger* greatly (74.28%) (Table 2), followed by *A. alliaceus* (11.28%), *Fusarium solani* (4.40%), *A.melleus* (4.00%), *A. flavua* and *Emericella nidulans* (1.38), *Paecilomyces variotii* and *A. tubingensis* and finally *Penicillium oxalicum* (083%).

Table 2: Frequency of Isolated Fungi Obtained From Harary Variety of Coffee Beans from Eastern Province of Saudi Arabia.

No.	Isolated fungi	Total No. of fungal isolate/ governorate					Total No. of fungal isolates	% Frequency
		Al-Dammam	Al-Hassa	Ras Tanura	Qatif	Al-Khobar		
1	Aspergillus niger	109	146	150	90	45	540	74.28
2	Aspergillus alliaceus	31	40	-	11	-	82	11.28
3	Fusarium solani	25	1	-	1	5	32	4.4
4	Aspergillus melleus	14	9	-	3	3	29	4
5	Emericella nidulans	-	-	-	10	-	10	1.38
6	Aspergillus flavus	8	2	-	-	-	10	1.38
7	Aspergillus tubingensis	9	-	-	-	-	9	1.24
8	Paecilomyces variotii	9	-	-	-	-	9	1.24
9	Penicillium oxalicum	6	-	-	-	-	6	0.83
10	Alternaria alternata	-	-	-	-	-	-	-
Total No. of fungal isolates at each governorate		211	198	150	115	53	727	
% of fungal isolates at each governorate		29.02	27.24	20.63	15.82	7.29		

Results in Table 3 proved also that the fungus *Aspergillus niger* greatly infected of lukkimy variety (78.42%), followed by *A. tubingensis* (7.02%), *A. alliaceus* (5.83%), *A.melleus* (3.25%), *Penicillium oxalicum* (2.74%), *Fusarium solani* (1.54%), *A. flavus* (0.69%), *Paecilomyces variotii* (0,34%) and *Emericella nidulans* (0,17%). While habbashy variety (Table 4) were contaminated with *Aspergillus niger* greatly (73.97%), followed by *A. alliaceus* and *Alternaria alternata* (6.33%), *A.melleus* (5.11%), *A. flavus* (4.38%), *Fusarium solani* (3.41%), *Penicillium oxalicum* and *A. tubingensis* (0.24%).

Table 3: Frequency of Isolated Fungi Obtained From Lukkimy Variety of Coffee Beans From Eastern Province of Saudi Arabia.

No.	Isolated fungi	Total No. of fungal isolate/ governorate					Total No. of fungal isolates	% Frequency
		Al-Dammam	Al-Hassa	Ras Tanura	Qatif	Al-Khobar		
1	Aspergillus niger	81	87	150	90	50	458	78.42
2	Aspergillus tubingensis	2	-	-	39	-	41	7.02
3	Aspergillus alliaceus	11	11	7	5	-	34	5.83
4	Aspergillus melleus	7	-	6	6	-	19	3.25
5	Penicillium oxalicum	-	1	-	5	10	16	2.74
6	Fusarium solani	1	6	-	2	-	9	1.54
7	Aspergillus flavus	-	4	-	-	-	4	0.69
8	Paecilomyces variotii	-	-	2	-	-	2	0.34
9	Emericella nidulans	-	1	-	-	-	1	0.17
10	Alternaria alternata	-	-	-	-	-	-	-
Total No. of fungal isolates at each governorate		102	110	165	147	60	584	
% of fungal isolates at each governorate		17.5	18.84	28.30	25.17	10.27		

Table 4: Frequency of Isolated Fungi Obtained From Habbashy Variety of Coffee Beans From Eastern Province of Saudi Arabia.

No.	Isolated fungi	Total No. of fungal isolate/ governorate					Total No. of fungal isolates	% Frequency
		Al-Dammam	Al-Hassa	Ras Tanura	Qatif	Al-Khobar		
1	Aspergillus niger	49	55	150	50	-	304	73.97
2	Aspergillus alliaceus	26	-	-	-	-	26	6.33
3	Alternaria alternata	26	-	-	-	-	26	6.33
4	Aspergillus melleus	2	14	4	1	-	21	5.11
5	Aspergillus flavus	11	-	1	6	-	18	4.38

6	Fusarium solani	4	10	-	-	-	14	3.41
7	Penicillium oxalicum	-	-	1	-	-	1	0.24
8	Aspergillus tubingensis	-	-	-	1	-	1	0.24
9	Emericella nidulans	-	-	-	-	-	-	-
10	Paecilomyces variotii	-	-	-	-	-	-	-
Total No. of fungal isolates at each governorate		118	79	156	58	-	411	
% of fungal isolates at each governorate		28.71	19.22	37.96	14.11	-		

Barry variety were contaminated with *Aspergillus niger* (66.1%) (Table 5), followed by *A. tubingensis* (12.02%), *A.melleus* (6.01%), *Fusarium solani* (5.65%), *A. alliaceus* (5%), *Penicillium oxalicum* (3.20%), *A. flavus* (2.83%), *Emericella nidulans* (2.47%), and finally *Paecilomyces variotii* (0.35%).

Table 5: Frequency of Isolated Fungi Obtained From Barry Variety of Coffee Beans From Eastern Province of Saudi Arabia.

No.	Isolated fungi	Total No. of fungal isolate/ governorate					Total No. of fungal isolates	% Frequency
		Al-Dammam	Al-Hassa	Ras Tanura	Qatif	Al-Khobar		
1	Aspergillus niger	59	44	-	47	37	187	66.1
2	Aspergillus tubingensis	-	34	-	-	-	34	12.02
3	Aspergillus melleus	4	10	-	3	-	17	6.01
4	Fusarium solani	7	3	-	3	3	16	5.65
5	Penicillium oxalicum	-	-	-	4	5	9	3.20
6	Aspergillus flavus	1	7	-	-	-	8	2.47
7	Emericella nidulans	1	-	-	5	1	7	2.47
8	Aspergillus alliaceus	-	-	-	4	-	4	5
9	Paecilomyces variotii	1	-	-	-	-	1	0.35
10	Alternaria alternata	-	-	-	-	-	-	-

Total No. of fungal isolates at each governorate	73	98	-	66	46	283	
% of fungal isolates at each governorate	25.8	34.63	-	23.32	6.25		

Pathogenicity of Isolated Fungi

The obtained data are presented in Table 7 The pathogenicity of the five frequent fungi in isolation, regarding *Aspergillus niger* was isolated from harary which has 150 from 540 isolates followed by *Aspergillus alliaceus*, from harary (40 from total 82 isolates), *SclerotiA. melleus* from harary (14 from 29 isolates), and *Fusarium solani* from harary (25 from 32 isolates) and *A.flavus* from habbashy (11 from 18 isolates). The pathogenicity of the five fungi was measured against all tested varieties. Percentage of infection was estimated by measuring the number of infection severity after storing for 45 days at 30^{o}C, the results were *A. niger*, *A. alliaceus* and *A.melleus* (100%), *Fusarium solani* (94%) and *A. flavus* (71%). However harary was the highest susceptible seeds with infection percentage (83.67%) followed by lukkimy (80.33%), habbashy (80%) and barry (71.67%).

Table 6: Number of Isolated Fungi Obtained From Four Varieties of Coffee Beans From Eastern Province of Saudi Arabia.

No.	Isolated fungi	The varieties				Total No. of fungal isolates	% Frequency
		harary	lukkimy	habbashy	barry		
1	Aspergillus niger	540	458	304	187	1489	74.43
2	Aspergillus alliaceus	82	34	26	4	146	7.28
3	Aspergillus melleus	29	19	21	17	86	4.29
4	Aspergillus tubingensis	9	41	1	34	85	4.24
5	Fusarium solani	32	9	14	16	71	3.54
6	Aspergillus flavus	10	4	18	8	40	2.00
7	Penicillium oxalicum	6	16	1	9	32	1.60
8	Alternaria alternata	-	-	26	-	26	1.30
9	Emericella nidulans	10	1	-	7	18	0.40
10	Paecilomyces variotii	9	2	-	1	12	0.60
Total No. of fungal isolates at each governorate		727	584	411	283	2005	
% of fungal isolates at each governorate		36.26	29.13	20.50	14.12		

Table 7:- Effect of the Artificial Inoculation With Most Frequent Isolated Fungi From Coffee Beans Tested on the Infection Severity of Four Cultivars After 45 Days of Storage At 30°c. (Pathogenicity Test).

Tested fungi	The varieties				Mean
	harary	lukkimy	habbashy	barry	
Aspergillus niger	100	100	100	100	100
Aspergillus alliaceus	100	100	100	100	100
Aspergillus melleus	100	100	100	100	100
Fusarium solani	100	98	100	78	94
Aspergillus flavus	100	82	60	42	71
Control	2	2	20	10	8.5
Mean	83.67	80.33	80	71.67	
L.S.D. at 0.05%	7.6	7.5	8.67	6.89	

Results in Table 8 proved the virulence of the five fungi to infect all tested seeds external infection appeared as coloured and speckled with different degrees were lukkimy (85.88%), barry (84.33%), harary (83.83%) and habbashy (70.39%).

Table 8: Effect of the Artificial Inoculation With Most Frequent Isolated Fungi from Coffee Beans Tested on the External Infection of four Cultivars After 45 Days of Storage At 30°c. (Pathogenicity Test).

Tested fungi	The varieties				Mean
	harary	lukkimy	habbashy	barry	
Aspergillus niger	98	100	100	100	99.5
Aspergillus alliaceus	100	100	100	100	100
Aspergillus melleus	100	100	100	100	100
Fusarium solani	100	100	35.33	100	83.83
Aspergillus flavus	100	100	78	100	94.5
Control	5	15.3	9	6	8.83
Mean	83.83	85.88	70.39	84.33	
L.S.D. at 0.05%	7.88	9.02	6.54	7.98	

Table 9 gives quantification of the total fungal load of coffee beans collected from Dammam and Qatif province. Empty coffee almost all samples of fungi, and different degrees of roasting, with the exception of only one sample is habbashy from Dammam have average viable mould count (1×10^1 cfu/g). The viable mould count decreased or disappeared in all samples after roasting.

Table 9: Quantification of total Fungal Load (Cfu/G) Of Coffee Beans From Various Varieties and Different Degrees Of Roasting.

Sample origin	The varieties	Degree of roasting			% Average water content
		light	medium	dark	
Al-Dammam	harary	0.00	0.00	0.00	5.47
	lukkimy	0.00	0.00	0.00	5.67
	habbashy	$1x10^1$	0.00	0.00	8.1
	barry	0.00	0.00	0.00	6.1
Al-Qatif	harary	0.00	0.00	0.00	2.83
	lukkimy	0.00	0.00	0.00	4.27
	habbashy	0.00	0.00	0.00	6.32
	barry	0.00	0.00	0.00	3.15
	Harary with ginger	0.00	0.00	0.00	5.3

Table 10 gives quantification of the total fungal load of coffee beans of rubesta collected from different retail markets, the results where that Mocha coffee have the highest average viable mould count (20 x 10^1 cfu/g), followed by Cappuccino (3 x 10^1 cfu/g), Turkish Coffee (2 x 10^1 cfu/g), Nescafe without caffeine and French Hazelnut Coffee (1 x 10^1 cfu/g), while Nescafe caffeine, French Coffee with milk or vanilla or with coconut and espresso were free of fungi, microbial action detrimental to the quality and safety of the final product will depend on environmental conditions as well as crop and product management.

Table 10: Quantification of Total Fungal Load (Cfu/G) Of Coffee Beans from Various Varieties and Different Flavored Coffee.

Type of coffee	Total Fungal Load (cfu/g)	% Average water content
Nescafe caffeine	0.00	5.47
Nescafe without caffeine	$1x10^{1(A.niger)}$	6.1
Cappuccino	$3x10^{1(A.niger,\ A.nudulans)}$	5.67
Mocha coffee	$20x10^{1(A.niger,A.\ melleus)}$	8.1
French Hazelnut Coffee	$1x10^{1(A.\ niger)}$	2.83
French Coffee with milk	0.00	4.27
French Vanilla Coffee	0.00	6.32
French Coconut Coffee	0.00	3.15
Turkish Coffee	$2x10^{1(Alternaria\ alternata)}$	4.74
Espresso	0.00	1.89

Discussion

The coloration of seeds may be a reaction between nitrogenous compounds and reduced sugars, and the loss of seed viability may be due to microbial infection, especially by fungi, also internal changes due to bad storage conditions such as oxidation of some compound fats [38, 39, 40].

From results it appeared that the most frequently isolated fungal species are not the most pathogenic. Some fungi can infect seeds before harvest but not at the storage stage while others have reverse effect. Similar results were observed by several authors [41, 42].

Differences in susceptibility to infection could also be attributed to the difference in the genetic structure of each seed, as also reported by several groups [43, 44, 45].

Differences in isolation frequency may be due to environmental factors and variation in each cultivated land, especially relative humidity. The type of soil and fertilization system plays a role in fungal infectivity [46, 47].

These results are disagreement with those obtained by [48]Alvindia and Acda (2010) who reported that the processing methods such as drying and roasting substantially affected the degree of fungal contamination in coffee beans, and the total fungal load in coffee beans increased after drying but was reduced significantly by 93 to 97% after roasting.

Studies on the microbiology of coffee cherries and beans have shown that the main toxigenic fungal genera (*Aspergillus*, *Penicillium* and *Fusarium*) are natural coffee contaminants and are present from the field to the warehouse [6, 7, 49,50]. The most important pathogenic genera of fungi which Infect green coffee beans and dominant were *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria* and *Paecilomyces* species. However differences in isolation frequency may be due to variation in the moisture content of seeds, varietal differences and seed susceptibility to infection besides specific environmental conditions in each seed store. Due to the nature of coffee processing, which is done in the open in the same areas where the coffee berry is harvested, it is not surprising that a high number of fungi have been isolated from green coffee seeds from various countries: Brazil [7, 50, 51, 52, 53, 54, 55], Cameroon [56], Indonesia and Mexico [51], and India [57]. Fungi have also been isolated from coffee berries from Brazil [7, 18] and Mexico [58].

Many studies revealed that *Aspergillus* and *Penicillium* are natural coffee contaminants, and are present from the field to storage (6, 7]. *Aspergillus niger* and *A. ochraceus* are the two species reported to be capable of producing OTA [10, 12, 13, 18, 59, 60, 61, 62]. likewise, investigation of other fungi which produce enzymes that can reduce coffee quality is also encouraged.

Conclusion

All varieties of Arabic coffee that tested were contaminated with many fungal species with varying rates. The roasting process is a key factor in the disappearance of the fungi in the tested samples of coffee

References

[1] Cardenas J The world coffee crisis. World Coffee Conference, London, UK, May 2001. Available at http://www.ico.org/event_pdfs/cardenas.pdf

[2] Clarke RJ Green coffee processing, pp. 230-250. In M.N. Clifford and K.C. Wilson, eds. Coffee Botany Biochemistry and Production of Beans and Beverage. *West Port, CN. AVI Publishing Co., Inc*., 1985.

[3] Varnam HA and PJ Sutherland Beverage technology chemistry and microbiology. *New York: Chapman & Hall,* 1994.

[4] International Coffee Organization-ICO Total production of exporting members. *http://www.ico.org/proddoc.htm*/, 2007.

[5] Food and Agriculture Organization [FAO] OTA in foodstuffs and associated regulations. http://www.coffee-ota.org/ota_foodstuffs.asp (Downloaded June 30, 2010.

[6] Nakajima M, Tsubouchi H, Miyabe M and Y Ueno Survey of aflatoxin B1 and ochratoxin A in commercial green coffee beans by high- performance liquid chromatography linked with immunoaffinity chromatography. *Food and Agricultural Immunology*. 1997; 9: 77-83.

[7] Silva CF, Schwan RF, Sousa-Diaz E and AE Wheals Microbial diversity during maturation and natural processing of coffee cherries of Coffea arabica in Brazil. *Intl. J. Food Microbiol*.2000; 60: 251-260.

[8] Holzhauser D, Delatour T, Marin-Kuan M, Junod S, Guignard G, Piguet D, Richoz J, Bezencon C, Schilter B and C Cavin Ochratoxin A: toxicity and carcinogenicity. *Toxicology Letters*. 2003; 144: s65.

[9] Schlatter C, Studer RJ and T Rasonyi Carcinogenicity and kinetic aspects of ochratoxin A. *Food Additives and Contaminants.* 1996;13 :43–44.

[10] Abarca ML, Accensi F, Bragulat MR and FJ Cabañes Current importance of ochratoxin A-producing *Aspergillus* spp. *Journal of Food Protection*. 2001; 64: 903–906.

[11] JECFA. "Fifty-sixth meeting of the Joint FAO/WHO Expert Commiatee on Food Additives Ochratoxin A" in: Safety evaluation of certain mycotoxins in food. *WHO Additives Series 47 and FAO Food and Nutrition Paper.* 2001; 74: 281–416.

[12] Joosten HMLJ, Goetz J, Pittet A, Schellenberg M and P Bucheli Production of ochratoxin A by *Aspergillus carbonarius* on coffee cherries. *Inter. J. Food Microbiol*.2001; 65: 39-44.

[13] Taniwaki An update on ochratoxigenic fungi and ochratoxin A in coffee, pp 189-202. In Hocking, A.D., J.I. Pitt, R.A. Samson, and U. Thrane (eds.). Advances in Food Mycology. Springer, New York, 2006.

[14] Abdel-Hafez AII and OMO El-Maghraby Fungal flora and aflatoxin associated with cocoa, roasted coffee and tea powders in Egypt. *Crypt. Mycol*.1992; 13:31-45.

[15] Bokhari FM Mycotoxins and toxigenic fungi in Arabic coffee beans in Saudi Arabia. *Adv. Biol. Res*.2007;1:56-66.

[16] Ilic Z, Bui T, Tran-Dinh N, Dang MHV, Kennedy I and D Carter Survey of Vietnamese coffee beans for the presence of ochratoxigenic Aspergilli. *Mycopathol*.2007;163:177-182.

[17] Pardo, E, Marin S, Ramos AJ and V Sanchis Occurrence of ochratoxigenic fungi and ochratoxin A in green coffee from different origins. *Food Sci. Technol. Inter*.2004; 10:45-49.

[18] Taniwaki MH, Pitt JI, Teixeira AA and BT Iamanaka The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. *Int. J. Food Microbiol*.2003; 82:173-179.

[19] Téren J, Palágyi A and J Varga Isolation of ochratoxin producing aspergilli from green coffee beans of different origin. *Cereal Res*.1997; 25:303-304.

[20] Visotto LE, Costa MD, Cavalcanti Coelho JL, Chaves-Alves VM, Oliveira MGA and FQ Mendes Isolamento de fungos toxigênicos em grãos de café (*Coffea arabica* L.) E avaliação da produção in vitro de ocratoxina A. *R. Brasil Armaz Especial Café – Viçosa MG*, 2008; 10:49-57.(abstract).

[21] Bucheli P and MH Taniwaki Research on the origin and the impact of postharvest handling and manufacturing on the presence of ochratoxin A in coffee – Review. *Food Addit. Contam*.2002; 19:655-665.

[22] Bucheli P, Kanchanomai C, Meyer I and A Pittet Development of ochratoxin A during robusta (*Coffea canephora*) coffee cherry drying. *J. Agric. Food Chem*.2000; 48:1358-1362.

[23] Bucheli P, Kanchanomai C, Pittet A, Goetz J and H Joosten Development of ochratoxin A (OTA) during Robusta (*Coffea canephora*) coffee cherry drying and isolation of *Aspergillus carbonarius* strains that produce OTA in vitro on coffee cherries. 19th ASIC Coffee Conference, Trieste, Italy, 2001.

[24] Urbano GR, Taniwaki MH, Leitao MF and MC Vicentini Occurrence of ochratoxin Aproducing fungi in raw Brazilian coffee. *J. Food Prot*.2001; 64:1226-1230.

[25] Pitt JI *Penicillium viridicatum*, *Penicillium verrucosum*, and production of ochratoxin A. *Appl. Environ. Microbiol*. 1987; 53:266-269.

[26] Pitt JI and AD Hocking Significance of fungi in stored products, pp 16-21. In ACIAR Proc. 36. Champ, B.R., Highley, E., A.D. Hocking, and J.I. Pitt (eds.). Fungi and Mycotoxins in Stored Products.1991.

[27] Pitt JI and AD Hocking Fungi and Food Spoilage, 2nd edition, Blackie, Academic and Professional, London, 1997.

[28] Ciegler A Bioproduction of ochratoxin A and penicillic acid by members of the *Aspergillus ochraceus* group. *Can. J. Microbiol*.1972; 18:631-636.

[29] Hesseltine CW, Vandergraft EE, Fennel DI, Smith ML and OL Shotwell Aspergilli as ochratoxin producers. *Mycologia*. 1972; 64:539-550.

[30] Moss MO Mode of formation of ochratoxin A. *Food Addit. Contam.*1996; 13:5-9.

[31] Pereyra GML, Pereyra CM, Ramírez ML, Rosa CAR, Dalcero AM and LR Cavaglieri Determination of mycobiota and mycotoxins in pig feed in central Argentina. *Letters in Applied Microbiology.* 2008; 46: 555-561.

[32] Anonymous Association of Official Analytical Chemists, Official Methods of Analysis (A.O.A.C.) (10th Edn), The Association, *Washington D.C.*1975; pp 462-482.

[33] Saber MM The changes that occur in wheat grains infected with some fungal during storage. MSc thesis, Faculty of Agriculture, Cairo University. 1984; pp 22-24

[34] Elmer WH, Yang HA and MW Sweetingham Characterization of *Colletotrichum gloeosporioides* isolates from ornamental lupines in Connecticut. *Plant Disease.* 2001; 85, 216-219

[35] Samson RA, Houbraken JAMP, Kuijpers AFA, Frank M and C Frisvad New ochratoxin A or sclerotium producing species in *Aspergillus* section *Nigri*. *Stud. Mycol*.2004; 50:45-61.

[36] Dhingra OD and JB Sinclair Basic Plant Pathology Methods, *CRC Press, Inc*., *Boca Raton, Florida, USA*, 1985.

[37] Norusis MJ SPSS/PC + Statistics 6.0 for the IBM PC/XT/AT and PS/2. *Library of Congress, USA*., 1999.

[38] Bothast RJ Fungal deterioration and released phenomenal in cereals, legumes and oil seeds. *Phytopathology.* 1978; 73, 312-315

[39] Christensen CM Loss of viability in storage micro flora. *Seed Science and Technology.* 1973; 1, 547-562

[40] Osman HYA Studies on fungi associated with sorghum grains during storage. PhD thesis, Faculty of Agriculture, Cairo University. 1982; 80 pp

[41] Khan MSA, Ramsey MD and ES Scott Host range studies with an Australian isolate of *Ascochyta rabiei*. *Australasian Plant Pathology.* 1999; 28: 170-173.

[42] Pande S, Siddique KHM, Kishore GK, Bayaa B, Gaur PM, Gowda CLL, Bretag TW, and JH Crouch (2005). Ascochyta blight of chickpea (*Cicer arietinum* L.): a review of biology, pathogenicity, and disease management. *Australian Journal of Agricultural Research.* 2005; 56: 317-332.

[43] Al-Shebel S and S El-Hussieni S Susceptibility of some legume crops to *Rhizoctonia solani* AG-4 recovered from bean plants. The international conference on the Arabian oryx in the Arabian Peninsula, the 23rd Meeting of the Saudi Biological Society. 2007; 21-23.

[44] Kohpina S, Knight R and FL Stoddard Evaluating faba beans for resistance to ascochyta blight using detached organs. *Australian Journal of Experimental Agriculture.* 2000; 40:707-713.

[45] Nguyen TT, Taylor PWJ, Redden RJ and R Ford Resistance to *Ascochyta rabiei* (Pass.) Lab. in a wild *Cicer* germplasm collection. *Australian Journal of Experimental Agriculture.* 2005; 45, 1291-1296.

[46] Baker KF and CA Martinson Seed transmission of *Rhizoctonia solani* in relation to control of seedling damping-off. *Phytopathology.* 1970; 37: 912-924.

[47] Kimati H and HAA Mascarenhas Incidence of diseases in trials with bean varieties in wet cultivation in Sao Paulo state. *Bragantia.* 1967; 26: 17-25 (c.f. *Review of Applied Microbiology* 48, 2072).

[48] Alvindia DG and MA Acda Mycoflora of coffee beans in the Philippines *J. ISSAAS*. 2010; 16:116 -125

[49] Bokhari FM and N Gum Gum Jee Studies of Some Factors Affecting Production of Aflatoxin B by *Aspergillus parasiticus*. *Saudi J. Biol. Sci*.2002; 9: 40-56.

[50] Mislivec PB, Bruce VR and R Gibson Incidence of toxigenic and other molds in green coffee beans. *J. Food Protect*.1983; 46: 969-973.

[51] Tsubochi H, Yamamoto K, Hisada K and Y Sakabe A survey of occurrence of mycotoxins and toxigenic fungi in imported green coffee beans. *Proceedings of the Japanese Association of Mycotoxicology.* 1984; 19: 16-21.

[52] (de) Carvalho VD, Chalfoun SM and SJ Chagas Rezende Relação entre classificação do café pela bebida e composição físico química, química e microflora do grão beneficiado. In: 15 Congreso Brasileiro de Pesquisas Caffeiras, 15, Maringa, Parana.1989; 25-26.(abstract).

[53] Batista LR, Chalfoun SM, Prado G, Schwan RF and AE Wheals Toxigenic fungi associated with processed (green) coffee beans (*Coffea arabica* L.). *Inter. J. Food Microbiol*.2003; 85:293-300.

[54] Martins ML, Martins HM and A Gimeno Incidence of microflora and of ochratoxin A in green coffee beans (*Coffea arabica*). *Food Addit. Contam*. . 2003;20:1127-1131.

[55] Reynaud DT, Pimentel IC, Homechim M, Kania CE and C Dykstra Fungos isolados dos grãos do café (*Coffea arabica* L. – Rubiaceae) variedade Mundo Novo. *Estudios de Biologia Curitiba*. 2003; 25: 49-54.(abstract).

[56] Tagne A and SB Mathur Fungi associated with seeds of *Coffea arabica*. *Plant Genetic Resources Newsletter.*2003; 135: 44-46.

[57] Tharappan B and R Ahmad Fungal colonization and biochemical changes in coffee beans undergoing monsooning. *Food Chemistry.* 2006; 94: 247-252.

[58] Roussos S, Aquia´huatl MA, Trejo-Hernandez MR, Gaime Perraud I, Favela E, Ramakrishna M and Raimbault Biotechnological management of coffee pulp-isolation, screening, characterization, selection of caffeine-degrading fungi and natural microflora, present in coffee pulp and husk. *Appl. Microbiol. Biotechnol.*1995; 42:756-762.

[59] Bayman P, Baker JL, Doster MA, Michailisdes TJ and NE Mahoney Ochratoxin production by the *Aspergillus ochraceus* group and *Aspergillus alliaceus. Appl. Environ. Microbiol*.2002; 68:2326-2329.

[60] Mantle PG and AM Chow Ochratoxin formation in *Aspergillus ochraceus* with particular reference to spoilage of coffee. *Intl. J. Food Microbiol*.2000; 56: 105-109.

[61] Perrone G, Susca A, Cozzi G, Ehrlich K, Varga J, Frisvad JC, Meijer M, Noonim P, Mahakarnchanakul W and SA Samson Biodiversity of *Aspergillus* species in some important agricultural products. *Stud. Mycol*.2007; 9:53-66.

[62] Noonim P, Mahakarnchanakul W, Nielsen JKF, Frisvad C and RA Samson (2008). Isolation, identification and toxigenic potential of ochratoxin A-producing *Aspergillus* species from coffee beans grown in two regions of Thailand. *Inter. J. Food Microbiol*.2008; 128:197-202.