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An Exposure and Intake Assessment of Ochratoxin a from Imported Coffee Beans in Egypt

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Abstract: Ochratoxin A (OTA) is a hepatonephrotoxic and carcinogenic mycotoxin drives. Its name from the producing fungi; *Aspergillus ochraceus*.OTA has been known as intermittent contaminant of coffee beans and other stored cereals. Analysis of OTA in 30 locally roasted ground (R) and 45 green (G) coffee bean samples imported from seven coffee producing countries were carried out in this work. Of the green coffee samples 60% were contaminated with OTA at levels reached 5.66 μg kg^{-1} with an average of 1.55 μg kg^{-1} while 66.67% of the roasted ground samples showed levels ranging from <5 to 8.35 and average 6.1 μg kg^{-1}. Five media were used for the determination of the total fungal counts and /or the percentages of fungal infection on green and ground roasted coffee beans. Eight fungi genera were found, *Aspergillus* predominated followed by *Penicillium.*, *Fusarium* and *Alternaria* but in far more lower percentages in both (G) and (R) coffee beans. Five species of *Aspergillus* were detected. *A. niger* predominated followed by *A. flavus*, *A. ochraceus* in both green and roasted coffee beans. The 75 isolates of *A. ochraceus* from both G and R coffee beans 47 isolates were potential producers of OTA on both YES and SM media. The average yield of OTA elaborated in the latter medium was significantly higher than that in the former one.

Key words: Coffee · processing · decaffeination · ochratoxin A · intake assessment

INTRODUCTION

Mycotoxin contamination of agricultural commodities has became a natural phenomenon in many parts of the world. This may be due to the favorable environmental conditions prevalent in those regions coupled with the traditional methods of crop cultivation, harvesting, handling and storage, all of which ultimately lead to severe mold growth and mycotoxin production in these agricultural commodities Soares and Rodriguez-Amaya [1]. The most frequently contaminated foods with mycotoxins producing molds include coffee, beer, wine, grains and spices [2]. Mycotoxigenic fungi are widespread in nature and have been implicated in many crop-related diseases. The major mycotoxin producing fungi are members of the genera *Aspergillus*, *Fusarium* and *Penicillum* Betina [3]. *Aspergillus ochraceaus* is a common mold of stored grains. It has been isolated from a variety of agricultural commodities. Many *A. ochraceus* strains regularly form Sclerotia which may be a major cause of foodstuff contamination [4]. Many strains belonging to the *A. ochraceus* group are capable of producing ochratoxin A (OTA) the most common of ochratoxins [5] which drives its name from *A. ochraceus*, the first and /or the best known species of *Aspergillus* from which it was isolated. [6,7]. OTA has been know as intermittent contaminant of stored cereals, cereal products and coffee beans for 30 years [2,8]. Coffee seeds are liable to mould attack specially when they are not dried to safe moisture level of 11% [2].

OTA has shown several biological effects such as immunosppression, tetratagenicity,genotoxicity and carcinogenicity [9-12]. It has also been implicated in a disease in humans called endamic nephropathy [13].

Green coffee beans are imported into Egypt from Seven coffee producing countries; i.e. Uganda, Ethiopia, Indonesia, Portugal, Brazil, Yemen and India. The imported green beans are later roasted and grounded by individual coffee retailers. Toxigenic fungi, which may be present on green beans, may be destroyed by roasting, however, any toxins produced by these fungi could remain. However further contamination of roasted coffee beans with the toxigenic fungi ought to be considered. It is worthy to report that as coffee is one of the most popular and favorable beverages in Egypt, questions arise concerning the detection and /or

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the prevalence of ochratoxin A and/or toxigenic fungi in green and roasted ground coffee beans. This study attempted to asses the market situation with respect to OTA through detecting the residues of this toxin as well as the incidence of toxigenic and other fungi in both green and roasted ground coffee beans. However attention was focused on *A. ochraceus* being the most common fungi responsible of OTA production. In addition the toxigenicity of isolated *A. ochraceus* was studied.

MATERIALS AND METHODS

Materials

Coffee samples: Forty five green and thirty roasted ground coffee bean samples were collected from local markets at different regions in Cairo Governorate. The samples were stored at -20°C to arrest any toxins formation up to the time of analysis.

Ochratoxin A standard: The standard of ochratoxin A was purchased from Sigma, Chemical Co. P.O. Box 4508 (St. Louis, MO, USA).

The ochratest immunoaffinity column: The ochratest immunoaffinity columns were purchased from Vicam (Somerville, MA, USA)

Cultivation and isolation of fungi:
The following six media were used for cultivation and isolation of different fungi: (Table 1)

***Cultivation*:**

- Malt Extract Agar (MEA) [14]
- Synthetic Medium Agar (SM A) [4]
- Czapek Yeast Extract Agar with 20% sucrose CY20S [15]
- Czapek Yeast Extract Agar (CYA) [16]
- Czapek Dox Agar (CDA)[17]

***Isolation*:**

- Potato Dextrose Agar (PDA) [18]

OTA production: The following media were used for the production of OTA (Table 1).

Table 1: Composition of the cultivate and isolate media

Medium	Composition (per litre)	Reference
1- Malt Extract Agar (MEA)	Powder malt extract 20g ; Peptone 1.0g ; lucose 20g ; Agar 20g	*Harrigan and Margare, [14])*
2- Czapek Yeast Extract Agar with 20% sucrose (CY20S)	K_2HPO_4 1.0g ; *Czapek concentrate 10ml ; powder yeast extract 5.0g; sucrose 200g ; agar 20g	*Pitt and Hocking [15]*
3- Czapek Yeast Extract Agar (CYA)	K_2HPO_4 1.0g ; *Czapek concentrate 10ml ; powder yeast extract 5.0g; sucrose 30g ; agar 20g	*Pitt [16]*
4- Czapek Dox Agar (CDA)	Sucrose 30g ; $MgSO_4$. 7 H_2O 0.5g ; KCL 0.5g ; $NaNO_3$ 2.0g; K_2HPO_4 1.0g ; $FeSO_4$ 0.01g ; agar 20g	*Misraand Misra[17])*
5- Synthetic medium Agar (SMA)	Xylose 30g; KCL 0.2g; K_2HPO_4 0.9g ;NH_4NO_3 1.0g ; $ZnSO_4$ 0.002g; $MgSO_4$. 7 H_2O 0.2g ; $FeSO_4$ 0.002g ; $MnCL_2$ 0.002g ; Thiamine chloride 0.001g; Bacto- Agar (Difco) 20g	*Paster and Chet [4]*
6- Potato dextrose Agar (PDA)	Potato 200g ; glucose 15g ; Agar 20g	*ATCC(19)*
7-Yeast Extract Sucrose Broth medium (YES)	Sucrose 150g ; Powder yeast extract 20g	*Davis et al., [20]*
8- Synthetic medium Broth (SMB)	NH_4NO_3 3.0g ; Phosphate buffer 0.15M ; KCL 1.0g ; glucose 50g ; $MgSO_4$. 7 H_2O 1.0g ; $Na_2B_4O_7.10H_2O$ 0.7mg; $Fe_2(SO_4)_3.6H_2O$10.0mg ; $CuSO_4$. 5H_2O 0.3mg; $MnSO_4$. 6H_2O 0.11mg ; ZnSO4.7H2O17.6mg $(NH_4)_{56}Mo7O_{24}.4H_2O$ 0.5mg	*Lai et al., [21])*

* Czapek concentrate (with trace metals):

$NaNO_3$ 30.0 g ; KCl 5.0 g ; $MgSO_4$. 7 H_2O 5.0 g; $FeSO_4.7H_2O$ 0.1 g ; $ZnSO_4$. 7H_2O 0.1 g ; $CuSO_4$. 5H_2O 0.05 g ; Distilled water 100 ml

Yeast Extract sucrose broth (YES) [20]
Synthetic medium broth (S M B)[21]

Methods

Isolation and Identification of fungi associated with green and/or roasted ground coffee beans samples:

Isolation of fungi: Fungi associated with green and roasted ground coffee beans were isolated according to the International Groundnut *Aspergillus flavus* Nursery IGAFN [22].

Isolation of fungi from green coffee beans: Each green coffee bean sample (25 beans) was immersed in 70% ethanol for 2 minutes. Ethanol was drained off and 2.5% Sodium hypochlorite solution was added. After 3 min the Sodium hypochlorite solution was drained off and the sterilized beans washed twice with sterilized distilled water. Distilled water was drained off, then the beans were dried between two layer of sterilized filter paper. Disinfected beans of each sample were plated Petri dishes containing each kind of the five media used (five beans/ dish).

Isolation of fungi from roasted ground coffee: Ten grams of roasted ground coffee were transferred to a wide mouth reagent bottle containing 90 ml sterile distilled water. The bottles were shacked for 15 min. This gave an approximate dilution of 1/10. Sterile dilutions from $1/10^2$ to $1/10^3$, were made using test tubes containing 9 ml sterilized distilled water. Then one ml of each of selected dilution was put in a sterile petri dish and about 10 ml of the used medium were added and the plate was moved gently for homogenous distribution. The plates were incubated at 28°C for 7 to 14 days. Five replicates from each sample were used. The bacterial growth was inhibited by using the antibacterial streptomycin in the concentration of 300 ppm. To each medium used sodium bicarbonate ($NaHCO_3$ 0.11M) was added to exhibit substantial inhibition toward the growth of *A. niger* according to David *et al.* [23].

The colonies of fungi were observed and calculated for total fungi count (TFC) then isolated and finally purified on Potato dextrose agar (PDA) slant. Number of colonies / g was determined and the percentages of different fungi were calculated according to the following equation:

Fungal species % = (No of each fungal species/Total fungi count)×100

Identification of the isolated fungi: The purified *A. ochraceus* strains maintained on PDA slants were identified according to Nelson *et al.* [24].

Production of OTA by isolated A. ochraceus strain: The ability of OTA production by *A. ochraceus* strains using various liquid media (YES and SMB) was investigated according to Tsubouchi *et al.* [25].

Analysis of OTA

Extraction of OTA from YES and SMB broth media: OTA was extracted from YES and SMB broth media according to Tsubouchi *et al.* [25]and determined by HPLC according to the AOAC [26].

Extraction chromatographic separation and determination of OTA from green and roasted ground coffee beans: The OTA was extracted from green and roasted coffee beans using the modified extraction technique followed by the immunoaffinity clean up procedure of Pittet *et al.* [27].Whereas OTA separation and determination was carried out by HPLC equipment with solvent delivery systems (Shimadzu) and a reverse phase analytical column packed with C18 material (spherisorb 5 μm ODS2, 15 cm×4.6 nm). The mobile phase consisted of acetonitrile: water: acetic acid (99:99:2). The separation was performed at ambient temperature at a flow rate of 1.0 ml/min, the injection volume was 20 μl for both standard solutions and sample extracts. The fluorescence detector was operated at an excitation wave length of 330 nm and an emission wave length of 460 nm A.O.A.C. [26].

Statistical analysis: All data were subjected to Statistical Analysis using the General Linear Mediel Procedure of the Statistical Analysis System SAS [27]. The significant of the differences among treatment groups was determined by Waller- Duncan K-ratio Waller and Duncan [28]. All statements of significantly were based on probability of ($P > 0.05$).

RESULTS AND DISCUSSION

The total fungal counts and percentages of fungal infection in green and roasted coffee beans: The total fungal counts (TFC) and percentages of fungal infection on green and roasted coffee beans on the five media used (MEA, SMA, CY20S, CYA & CDA supplemented by (0.11 M) Sodium bicarbonate are given in Table 2. The

Table 2: The total fungal count (TFC) and percentage of infection in green (G) and roasted (R) ground coffee beans cultivated on different media

Medium used		MEA	SMA	CY20S	CYA	CDA
	(G)	710	809	642	505	430
TFC	R)	1663	1231	1973	1624	1504
% of infection with						
A. niger	(G)	65.40	55.10	61.10	42.40	61.20
	(R)	29.60	28.43	18.85	27.00	24.20
A. flavus	(G)	8.60	11.80	14.50	30.00	16.00
	(R)	29.20	24.78	15.50	21.18	23.40
A. parasiticus	(G)	3.70	3.30	2.96	5.70	3.20
	(R)	1.44	2.60	2.48	1.97	1.73
A. ochraceus	(G)	6.05	19.50	8.09	14.80	6.04
	(R)	12.50	23.07	12.80	15.70	9.71
A. versicolor	(G)	0.42	0.37	2.20	0.20	0.00
	(R)	2.10	2.60	2.38	3.14	2.40
Alternaria spp	(G)	0.28	0.00	0.30	0.40	1.86
	(R)	3.73	0.49	1.57	2.40	2.19
Fusarium spp	(G)	8.02	1.73	2.50	1.58	2.32
	(R)	6.43	6.17	8.31	4.47	14.96
Penicillum spp	(G)	7.60	11.70	8.40	5.15	9.50
	(R)	14.97	11.86	38.06	23.80	21.41

*TFC (Total fungal count) = No of colonies /gm

Table 3: Mean ±SE for the total fungal counts (TFC) and the different Spp. of fungi isolated from green and roasted ground coffee beans

Coffee beans	TFC	*Aspergillus*	*Pencillium*	*Alternaria*	*Fusarium*
Green	619.2±68.42[a]	86.56±2.15[c]	8.47±1.08[e]	0.57±0.33[g]	3.23±1.21[i]
Roasted	1599±130.58[b]	67.75±5.14[d]	22.02±4.55[f]	2.076±0.53[h]	8.122±1.80[j]

Means with the same letter are not significantly different Within each, column ($p>0.05$)

CY20S medium showed higher TFC on the roasted coffee beans compared with the other media tested, where the SMA one showed the highest TFC on green coffee beans. This might be probably due to the different species of fungi prevailing and / or to the composition of the medium as well. Moreover, the average count of the five different media used was 1599 ±130.58 for the roasted coffee beans where it was 619.2 ± 68.42 for the green coffee ones (Table 3). This can be probably attributed to the bad storage conditions as storage of the ground roasted coffee is critical as the chance of rewetting exists and bad circumstances of storage caused such comparatively higher average count [29].

It is quite evident that all green and roasted ground coffee beans samples are invaded with different fungi. Eight species belonging to four genera were isolated and identified (Table 2). *Aspergillus* was the most frequently encountered and was detected in significantly higher counts ($p>0.05$) than the other genera particularly on the green ones. The genus *Penicillium* followed as it was also encountered although the percentage of its occurrence was substantially lower than that of *Aspergillus*, but significantly higher ($p>0.05$) than the *Alternaria* and *Fusarium* for both green and roasted coffee beans. However, the genera *Fusarium* and *Alternaria* were also detected but in far more lower percentages than those of *Aspergillus* and *Penicillium* (Table 3). The rare detection of *Alternaria* and *Fusarium* indicated that these two genera do not readily invade green and / or roasted coffee beans. The trend of fungal detection was similar for both the green and roasted ground coffee beans indicating the presence of both storage and field fungi. The same sequence of the four genera detected in the current study was also shown by Levi *et al.* [30], Mislevic *et al.* [31] and Vasanthi and Baht [29] on green coffee beans.

It is obvious from the current results that the flora was dominated by five species of *Aspergillus*. *A. niger* was the predominant followed by *A. flavus* and *A. ochraceus* in green and ground roasted coffee beans. *A. parasiticus* and *A. versicalor* were also present but in comparatively far lower percentages on both green and ground roasted coffee beans (Table 2).

It should be mentioned here, that there was an over growth of *A. niger* on green and roasted coffee beans which led to obscure the growth of other fungi.

Table 4: Production of OTA by *A. ochraceus* isolated from green (G) and roasted ground (R) coffee beans

	OTA Concentration μL^{-1}			
	G		R	
	SMB	YES	SMB	YES
Maximum	14.47	9.40	16.00	13.50
Minimum	6.32	2.07	5.46	2.82
Average	10.42	6.77	11.92	8.49
No of isolates	45.00	45.00	30.00	30.00
% of positive isolates	57.70	57.70	70.00	70.00

Supplementation with sodium bicarbonate 0.11 M (SB) in the fore- mentioned media was effective against *A. niger* as it reduced its viability and exhibited substantial inhibition towards its growth thus giving the way to other mycotoxigenic fungi such as *A. ochraceus* present to grow and enumerate. In this regard David *et al.* [23] reported that inhibition of SB may be caused by bicarbonate anions and the presence of SB induced alterations in fungal morphology and cause pigment accumulation in the other mycotoxigenic fungi. Moreover the changes in pigmentation which accompanied growth in the presence of bicarbonate - survivors are significant because many intermediates in mycotoxin biosynthetic pathways are pigmented.

Our findings on the predominance of the particular *Aspergillus* species encountered on green coffee beans imported from seven coffee - producing countries (Uganda, Ethiopia, Indonesia, Portugal, Brazil, Yemen and India) are in general agreement with those of the mold flora of green coffee given by Corte Dos Santos *et al.* [32] on coffee beans from Angola, Levi *et al.* [30] on 5 improperly stored samples of undetermined origin, with those of Mislevic *et al.* [31] on 944 samples representing 31 coffee - producing countries and those of Vasanthi and Bhat [28] on 27 Indian (Kamataka) green coffee beans. However no reports on the total mold flora of roasted ground coffee beans are existing in the literature.

Detection of *A. ochraceus* and other molds on green and roasted ground coffee beans coincide with those reported by many investigators (Levi *et al.* [33, 30], Mislevic *et al.* [31], Stack *et al.* [34], Tsubouchi *et al.* [2, 35], Vasanthi and Bhat [28] for green coffee beans and Tsubouchi *et al.* [36] for roasted coffee beans. It is obvious from the obtained results (Table 2) that the percentage of *A. ochraceus* recorded for green and roasted coffee were higher on the SMA medium and which was found to be the best medium for enumeration of *A. ochrauceus* than the other media tested. This might be probably due to the composition and / or the nutritional constituents of the medium which effects the growth of *A. ochroaceus*. In this concern Paster and Chet [4] reported that nutritional factors affect the morphogenetic process of *A. ochraceus* group and that good growth and sclerotium formation occurred on such medium containing sulpher compounds. It can thus be concluded that the Paster and Chet [4] medium is an efficient one for isolation and enumeration of *A. ochraceus* from green and roasted coffee beans.

Production of OTA by isolated strains of *A. ochraceus* from green and roasted ground coffee beans: In connection with investigations of an occurrence of potential OTA producing fungi in the green and ground roasted coffee beans, we have followed the occurrence of *A. ochraceus* strains and the ability of the isolated strains to produce OTA in vitro (Table 4). Two different liquid nutritional media namely yeast extract sucrose agar (YES) Davis *et al.* [20] and thesynthetic medium (SMB) Lai *et al.* [21] were used to select the one that leads to the highest production of OTA. A total of 75 strains of *A. ochraceus* were isolated from green and roasted ground coffee beans. Their toxigenic potential was assessed. After the desired period of incubation (14 days of 25- 28°C) culture filtrates were extracted and analyzed by HPLC. Our data indicated that 26 (57%) out of 45 isolates from green coffee beans were capable to produce OTA in both YES and SMB media at levels 2.07 to 9.40 $\mu g\ L^{-1}$ with an average of 6.77 and from 6.32 to 14.47 averaging 10.42 $\mu g\ L^{-1}$ in YES and SMB media respectively. Among the 30 strains isolated from the roasted ground coffee beans 21 (70 %) were potentially OTA producers in both the two liquid media used SMB and YES at levels ranging from 5.46 to 16 $\mu g\ L^{-1}$ with an average of 11.92 $\mu g\ L^{-1}$ and from 2.82 to 13.5 $\mu g\ L^{-1}$ averaging 8.94 $\mu g\ L^{-1}$ in this order. The present data showed that the percentage of OTA producers isolated from the roasted ground coffee

Table 5: Distribution of Ochratoxin A in green and roasted ground coffee beans imported into Egypt

		Distribution of Ochratoxin A						
	Total No. of	Detected		1-5 μg kg^{-1}		>5 μg kg^{-1}		Highest level of OTA found
Sample type	samples	No.	%	No.	%	No.	%	(μg kg^{-1})
Green	45	27	60.0	24	88.9	3	11.1	5.66
Roasted	30	20	66.6	10	50.0	10	50.0	8.35

beans was relatively higher (70 %) than those of the green ones (57 %). This might be probably attributed to the comparatively higher incidence of OTA producer in the roasted ground coffee beans due to the storage and environmental conditions that favors the growth of OTA producers than that of the green ones [28]. It is also evident that although the total OTA - producing strains (47) produced the toxin in both culture media used yet the average yields of OTA detected in the SMB was significantly ($P>0.05$) higher than that in YES medium and the maximum yield being 16 μg L^{-1} was obtained in the SMB media as well. This might be probably attributed to the variation in the composition of the two culture media used. The synthetic medium (SMB) of Lai *et al.* [21] might probably contain the sufficient concentrations of the different ingredients and/or trace elements required by *A. ochraceus* for maximal growth and/or maximum toxin yield production. This is supported by the earlier findings of Lai *et al.* [21] who found that with different salt concentrations; maximum OTA production was obtained in the presence of K^{+2}, P^{+5}, they explained that the amounts of $MgSO_4$ in the medium were sufficient as the sole source of Mg^{+2} and S^{-6} required for maximum toxin yield. Moreover, they added that trace elements, Fe^{+3}, Zn^{+2}, Cu^{+2}, B^{-1} and Mo^{+6} and Mn^{+2} in such media lead to maximal growth and toxin production and were needed as well for the synthesis of ochratoxin A. This was also proved by Delgadello [37] for Zn^{+2}, Cu^{+2}. Furthermore, Lai *et al.* [21] indicated that small and large amounts of the toxin elaborated depended on the carbon and sulfur sources and on the near - optimal quantities of sulfur, magnesium, potassium and phosphorus contain in the growth media.

Natural occurrence of OTA in green and roasted ground coffee beans: OTA was detected in 27/45 (60%) and 20/30 (66.75%) for green and roasted ground coffee beans in this respective order. OTA levels ranged from 0 - 5.66, average 1.55 and from 0 - 8.35 average. 6.1 μg kg^{-1} for green and roasted coffee respectively (Table 5). Eighty eight and fifty percent of the positive green and / or roasted samples contained less than 5 μg kg^{-1} which is currently the limit being proposed by the European Union (EU) for OTA levels in coffee..

Low levels of OTA detected in the present investigation coincides with those given by Trucksess *et al.* [38] and Vasanthi and Ramesh [29] who reported levels ranging from 0.1 to 4.6 and less than 5 μg kg^{-1}, respectively and at an incidence of 52.5% (Table 6). This can be probably attributed to the appropriate conditions prevailing during production and / or good storage, transport and handling conditions of the green coffee beans, which prevents the production of OTA [39,9]. Moreover, Tsubouchi *et al.* [25] reported that caffeine had an inhibitory effect on the growth and OTA production by *A. ochraceus.* They added that caffeine is a major factor in detecting low levels of OTA in green coffee beans.

It is worthy to report that the detected maximum levels and the given ranges as well of OTA in the green coffee beans of the current study are far more lower than those observed by other authors from various countries. In this concern; Norton *et al.* [40], Tsubouchi *et al.* [35,2], Studer -Rohr *et al.* [8] Nakajima *et al.* [41], Blanc *et al.* [42] and Romani *et al.* [43] revealed ranges between 0-200 μg kg^{-1} at a total incidence of (61.4 %) (Table 6). These authors attributed their detected high levels in green coffee beans to variety of environmental factors. In this regard Vasanthi and Bhat [29] found that the methodology of harvesting, processing, storage and transport can have considerable impact on mould contamination and OTA production as well. They reported that coffee seeds are liable to mould attack, especially when they are not dried to a safe moisture level (11%). The same authors added that, it is well recognized that mould damage occurs during post-harvest stages due to improper drying at the drying yard or during storage of highly moist seeds at the curing works and which encourage and leads to OTA production.

Moreover, the presence of OTA in commercial roasted coffee beans has been observed by other authors

Table 6: Reviewed estimates for Ochratoxin A levels in the coffee (1982-2000)

Author & Country	Year	Incidence	Product	Concentrations ofOTA
High level				
Norton et al. U.K [40]	1982	9/ 9	Green coffee beans	< 10 up to 200 μg kg^{-1}
Tsubouchi et al. Japan [35]	1984	4/22	Green coffee beans	9.9 - 46.0 μg kg^{-1}
Tsubouchi et al. Japan [2]	1992	11/22	Green coffee beans	0.08 - 72.7 PPb
StuderRohr et al. Switzerland [8]	1995	13/25	Green coffee beans	1.2 - 56.0 μg kg^{-1}
Nakajima et al. Japan [41]	1997	14/47	Green coffee beans	0.1 - 17.4 μg kg^{-1}
Blanc et al. Switzerland [42]	1998	50/50	Green coffee beans	4.0 - 22.1 μg kg^{-1} average 7.3 μg kg^{-1}
Romani et al. Italy [44]	2000	106/162	Green coffee beans	0 - 48 μg kg^{-1}
Overall		175/337 (61.4%)		
Low level				
Truckcess et al. USA [38]	1999	9/19	Green coffee beans	0.1 - 4.6 μg kg^{-1}
Vasanthi and Baht. India [29]	2000	84/158	Green coffee beans	> 5 μg kg^{-1}
Overall		93/177 (52.5%)		
Tsubouchi et al. Japan [36]	1988	5/68	Roasted coffee	3.2 - 17 μg kg^{-1}
MAFF UK [45]	1995	17/20	Roasted coffee	0.2 - 2.1 μg kg^{-1}
Koch et al. Germany [46]	1996	20/30	Roasted coffee	0.3 - 7.5 μg kg^{-1}
Burdaspal and Legarda Spain [47]	1998	28/26	Roasted coffee	0.22 - 5.64 μg kg^{-1}
Jorgensen Denmark [48]	1998	11/11	Roasted coffee	0.51 - 3.2 μg kg^{-1}
Truckcess et al. USA [38]	1999	9/13	Roasted coffee	0.1 - 1.2 μg kg^{-1}
Leoni et al. Brazil [49]	2000	23/34	Roasted coffee	0.3 - 6.5 μg kg^{-1}
Overall		113/202(55.9 %)		

in similar concentration ranges to that detected in the current study. In this regard Maff [45] in UK analyzed 20 samples of which 17 were positive and contained levels between 0.2 and 2.1 μg kg^{-1}, Koch *et al.* [46] found 20 out of 30 samples containing 0.3 -7.5 μg kg^{-1}, Burdaspal and legarda [47] in Spain detected levels ranging from 0.22 to 5.64 μg kg^{-1}, Jorgensen [48] in Denmark found 11 samples contained OTA mean content of 0.51 and highest value of 3.2 μg kg^{-1} and Leoni *et al.* [49] in Brazil found that 23 samples out of 34 were contaminated with OTA with levels ranging between 0.3 and 6.5 μg kg^{-1} and the total incidence being 55.9%.

It is obvious from the current data that the analyzed ground roasted coffee samples showed relatively higher concentrations of OTA than the green ones. This might be probably due to the relatively higher incidence (70%) of toxigenic strains of *A. ochraceus* isolated from roasted ground than those of green coffee beans (57%) which leads to higher OTA production as given before (Table 4). Moverover, other environmental factors particularly storage of highly moist roasted ground beans [29] which encourage the growth of toxigenic strains and leads to comparatively higher OTA production as well. The differences in storage temperature and time and their interaction between both green and roasted beans ought to be considered Ramos *et al.* [44]. However, it is worthy to report that if green and /or roasted ground coffee beans are appropriable dried to a safe moisture level (11%), it can be prevented and /or protected from mould growth and OTA production [29].

Assessment of OTA intake: OTA was detected in local commercial roasted ground coffee bean samples at a high incidence of 50% recording levels ranging from <5 to 8.35 μg kg^{-1} and averaging 6.1μg kg^{-1}. These contaminated levels are higher than the accepted permissible levels proposed by the European Union for OTA in coffee. From the reached data and as coffee is one of the two extensively consumed beverages along with tea, regular coffee consumption may probably contribute to exposure of human to OTA [6]. To clarify this important point, in view of our results an attempt was made to estimate and / or calculate the intake of OTA from drinking coffee. Assessing coffee intake will be based on the data concerning the quantity of Turkish coffee ingested in terms of numbers of cups consumed per week, the strength of roasted coffee /cup and the average level of OTA detected in roasted ground coffee. In this concern the regular Turkish coffee consumption in Egypt ranges between four to twelve cups/day /person,

with an average typical strength of six gm toasted coffee /cup. Accordingly, the quantity of roasted ground coffee consumed is 168 and /or 504 g /person /week. It is worthy to report that based on toxicological properties, the Joint FAO/ WHO Expert Committee on Food Additives [50] has established a Provisional Tolerable Weekly Intake (PTWI) of 100 ng /kg body weight per person / week for OTA. In view of the present data (mean level of OTA, being 6.1 $\mu g\ kg^{-1}$), applying the PTWI set up by JECFA, the OTA intake through coffee consumption was observed to be 17.18 and /or 51.24 ng OTA /kg body weight per person (60 kg) from consuming 168 and /or 504 gm of roasted ground coffee/week, respectively. Taking in our consideration that in the Turkish coffee method frequently used in Egypt the OTA is essentially fully extracted [38]. Consequently consumption of 28 and / or 84 cups of coffee per week (168 and / or 504 gm roasted ground coffee) contribute up to 17.18 and 51.24 %, respectively to the PTWI limits. Thus the average level observed in the present study is well below the limits established by JECFA. However, for the benefit of human health, surveys of OTA in green and/ or roasted ground coffee beans should be continued.

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