

OCHRATOXIN A AND AFLATOXINS QUANTIFICATION IN GREEN COFFEE BEANS: MYCOLOGICAL AND ANALYTICAL STUDY

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ABSTRACT

This study investigated the contamination of 22 selected green coffee bean samples grown in South and Central America by microscopic filamentous fungi and their potential for mycotoxin (ochratoxin A, aflatoxins) production. A total of 2860 isolates belonging to 11 genera, including *Aspergillus*, *Penicillium*, *Fusarium*, *Syncephalastrum*, and *Rhizopus*, were identified. The genus *Aspergillus* was present in all samples (frequency of occurrence – Fr 100.00%, relative density – RD 88.49%) and exhibited the highest frequency, with sections *Nigri* (Fr 95.45%, RD 49.86%), *Aspergillus* (Fr 90.90%, RD 8.36%), and *Flavi* (Fr 77.27%, RD 27.94%). Toxicogenic screening by thin layer chromatography (TLC) revealed that 27.8% of *Aspergillus* and 71.4% of *Penicillium* tested isolates were capable of producing mycotoxins, including ochratoxin A and aflatoxins. While aflatoxins were not detected in the green coffee beans by HPLC-FLD, ochratoxin A was found in 68.2% of them at low concentrations (from 0.375 to 1.487 ng/kg). These findings emphasize the importance of monitoring fungal contamination and mycotoxin levels in green coffee beans to ensure food safety and quality in the coffee industry.

Keywords: *Aspergillus*, *Penicillium*, green coffee, micromycetes, mycotoxins, toxinogenicity

INTRODUCTION

Coffee is the most widely used beverage in the world, and numerous scientific studies have shown that it has health benefits (Chu, 2012). However, different toxigenic fungi and bacteria can contaminate coffee beans during the various stages of production, including harvesting, processing (washing, fermentation, and drying), transportation, and storage (Batista et al., 2009; Munyendo et al., 2017). Green coffee bean mycocenosis involves a complex community of fungi that colonise coffee during its growth, processing and storage, particularly in South and Central America - regions known for coffee production. This microbial ecosystem significantly affects the overall quality of coffee and poses health risk due to the possible presence of mycotoxins, toxic compounds that can lead to serious health problems in humans. Studies on the microbiology of coffee cherries and beans have shown that the genera *Aspergillus* and *Penicillium* are natural coffee contaminants (Viegas et al., 2017). These micromycetes are important producers of mycotoxins in green coffee beans. Ochratoxin A (OTA) is one of the most widespread mycotoxins that can be found in coffee beans and its beverage (Velmourougane et al., 2010). OTA is a mycotoxin considered to be nephrotoxic, carcinogenic and, as it can cross the placenta, also embryotoxic and teratogenic (WHO, 2003). This toxin is a secondary metabolite produced mainly by species of *Aspergillus* genera (e.g. *A. ochraceus*, *A. carbonarius*, *A. sulphureus*, *A. niger*, *A. sclerotiorum*, *A. westerdijkiae*) (Frisvad et al., 2004; Samson et al., 2004) and species of *Penicillium* genera (*P. verrucosum* and *P. nordicum*) (Larsen et al., 2001). OTA is the only mycotoxin for which regulatory limits have been established in coffee (5 µg/kg in roasted coffee beans and ground roasted coffee, 10 µg/kg in soluble coffee products in the EU) (EC, 2006). In addition to OTA, aflatoxins and fumonisin B₂ have also been identified in coffee beans (Nielsen et al., 2015; Soliman, 2002). Bessaire et al. (2019) reported OTA, fumonisin B₂, sterigmatocystin, beauvericin, and enniatin A as the most frequently detected mycotoxins with the highest levels in green coffee samples, with maximum levels of 12.2, 76.3, 19.8, 4.4, and 1.7 µg/kg, respectively. Contamination with aflatoxin B₁ in the study by Bessaire et al. (2019) was sporadic with a maximum level of 1.2 µg/kg.

The aim of our study was to analyse the contamination of green coffee beans by microscopic filamentous fungi, identify microscopic fungi genera, sections of

Aspergillus genera and species of *Penicillium* genera, and assess the ability of selected isolates to produce specific mycotoxins. Additionally, the study focused on the quantification of aflatoxins and ochratoxin A by HPLC-FLD method to evaluate potential health risks associated with their presence in green coffee beans.

MATERIAL AND METHODS

Samples information

In the study, 22 samples of a selected green Arabica coffee (*Coffea arabica*) from South and Central America were analysed. Information on the samples is given in Table 1. The water activity (a_w) of the samples was measured using the LabMaster-aw Novasina water activity meter (Novasina, Switzerland).

Determination of green coffee endogenous mycocenosis

For determination of endogenous mycocenosis of green coffee beans the method of direct placing of surface-sterilized grains on cultivation media was used. Green coffee beans were surface sterilized with 0.4% Chloramine-T (Gavax, Slovakia) solution for 2 minutes. Subsequently, the beans were rinsed 3 times with sterile distilled water and dried on sterile filter paper. Surface sterilized green coffee beans were directly plated on DRBC (Dichloran Rose Bengal Chloramphenicol agar, HiMedia M1000, India) and DG18 (Dichloran Glycerol agar, Biolife 4013942, Italy) media plates. Plastic petri dishes with a diameter 150 mm were used for the analysis. Ten beans were placed on each medium plate. A total of 100 beans from each sample were analysed, i.e. 50 beans on DRBC (Figure 1) and 50 beans on DG18. Cultivation was carried out for 5-7 days at 25 ± 1 °C in a dark, thermostat-controlled environment.

Mycological analysis was evaluated on the 7th day of cultivation. The micromycetes were subsequently classified based on the assessment of macroscopic and microscopic features. Lactic acid with cottonseed blue was used for preparation of slides according to Tančinová et al. (2012). The preparations were observed with Olympus CX21FS1 and Olympus BX51TF microscopes with Nomarski contrast.

Table 1 Analysed samples information

Continent	Country	Sample	Processing method	a _w	Continent	Country	Sample	Processing method	a _w
South America	Columbia	1	Fully washed	0.458	Central America	Honduras	12	Washed	0.516
	Columbia	2	Washed	0.453		Mexico	13	Fully washed	0.464
	Peru	3	Fully washed	0.488		Mexico	14	Washed	0.454
	Peru	4	Natural	0.484		Nicaragua	15	Fully washed	0.501
	Brazil	5	Pulped natural	0.417		Nicaragua	16	Washed	0.438
	Ecuador	6	Washed	0.529		Panama	17	Washed	0.425
Central America	Costa Rica	7	Yellow honey	0.463		Panama	18	Washed	0.450
	Costa Rica	8	Fully washed	0.450		Dominican r.	19	Washed	0.489
	Guatemala	9	Fully washed	0.462		Cuba	20	Washed	0.467
	Guatemala	10	Washed	0.452		El Salvador	21	Washed	0.446
	Honduras	11	Fully washed	0.471		El Salvador	22	Washed	0.441

Legend: a_w – water activity

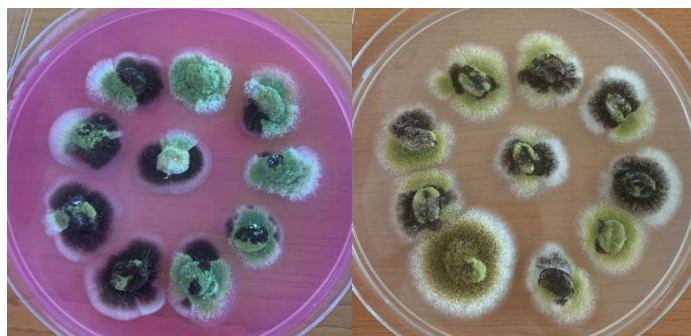


Figure 1 Microscopic filamentous fungi on green coffee beans (left DRBC, right DG18)

Aspergillus and Penicillium species identification

Isolates of the genus *Aspergillus* were identified at the section level and isolates of the genus *Penicillium* were identified at the species level. The identification was based on the observation of macromorphological, micromorphological, chemotaxonomic and physiological characters of the microscopic filamentous fungi on identification media according to Samson et al. (2002, 2010, 2014, 2019), Samson and Frisvad (2004), Varga et al. (2011), Visagie et al. (2014a, b), Yilmaz et al. (2014).

Evaluation of the occurrence of micromycetes

To assess the abundance of micromycetes in the samples, the frequency of occurrence and relative density at the genera, species, or section level was used. Frequency of occurrence (Fr) was defined as the percentage of samples in which a given genus/species/section occurred at least once. The formula for calculation follows González et al. (1999):

$$Fr (\%) = (ns/N) \times 100$$

where ns - number of samples in which the genus/species/section was detected; N - total number of samples.

Relative density (RD) is the percentage of isolates of a particular genus/species/section occurring in the analysed samples. The formula for calculation is:

$$RD (\%) = (ni/Ni) \times 100$$

where ni - number of isolates of genus/species/section; Ni - total number of detected isolates.

During the evaluation, the percentage of grains contaminated with micromycetes was calculated, i.e. how many grains out of the total number of analysed grains were infested with micromycetes.

Determination of potential toxigenicity of micromycete isolates

Representative isolates of potentially toxigenic species of the genera *Aspergillus* and *Penicillium* were tested *in vitro* for their ability to produce selected isolates by thin-layer chromatography (TLC) according to the methodology of Samson et al. (2002), which was adapted by Labuda and Tancinova (2006). Cultures for the screening of cyclopiazonic acid, penitrem A, and roquefortin C were carried out on Czapek Yeast Autolysate agar (CYA) and for the screening of aflatoxins, ochratoxin A, and griseofulvin on Yeast Extract Sucrose medium (YES) for 14

days at 25 ± 1 °C. Chloroform:methanol solution (2:1, v/v) was used as extraction reagent and toluene:ethyl acetate:formic acid solution (5:4:1, v/v/v) was used as developing system. Visualization of mycotoxins was performed according to Samson et al. (2010) and Barboráková et al. (2010, 2023).

Samples preparation for HPLC-FLD analysis

The preparation of green coffee samples was carried out according to the modified methodology of Arduini et al. (2016). Green coffee bean samples (50 g) were ground on a Sencor SCG 1050 BK type coffee grinder and stored at -18 °C until HPLC analysis. To 5 g of the ground green coffee bean sample, 0.5 g of NaCl and 10 mL of methanol:water extraction reagent (80:20, v/v) were added. The centrifuge tube containing the sample, NaCl and extraction reagent was shaken on a shaker (QS-10, Biosan) for 30 min at 220 rpm. The sample was filtered through filter paper (Whatman no. 1, 90 mm diameter). From the filtrate obtained, 1 mL was taken to which 4 mL of ultrapure water was added and prepared using a Millipore system (Millipore, Bedford, USA). The extract (5 mL) was quantitatively filtered through a Nylon syringe filter (0.2 µm, Agilent Captiva, USA) into an immunoaffinity column (AflaOchra, LC VICAM, Waters, USA) at a rate of 1 - 2 drops/second. The immunoaffinity column was subsequently washed with 10 mL of ultrapure water. To obtain a sample for HPLC analysis, toxins from the immunoaffinity column were washed with 1 mL of ethanol into HPLC vials (2 mL, Agilent, USA).

HPLC-FLD analysis of aflatoxins

HPLC analysis was performed on an Agilent 1260 Infinity system (Agilent, USA). An Agilent Poroshell 120 EC-C18, 4.6 x 100 mm, 2.7 µm column with an Agilent Zorbax Eclipse XDB-C18, 4.6 x 15 mm, 3.5 µm pre-column (Jakabová et al., 2022) was used for the determination of aflatoxins (B₁, B₂, G₁, G₂) in green coffee bean samples. A mixture of 60% H₂O (A) and 40% methanol (B) was used as the mobile phase in isocratic mode with a flow rate of 1 mL/min. The injection volume was 10 µL. Samples were analysed in triplicate. The detection of aflatoxins was carried out at an excitation wavelength of 362 nm and an emission wavelength of 455 nm using a fluorescence detector (FLD). Reference standards AFB₁ (Sigma-Aldrich Laborchemikalien GmbH, Germany), AFB₂ (Sigma-Aldrich Laborchemikalien GmbH, Germany), AFG₁ (Cyaman Chemical Company, USA), and AFG₂ (Cyaman Chemical Company, USA) were used in the analysis. The limits of detection (LOD) for aflatoxins were: AFB₁ - 0.1234 µg/mL, AFB₂ - 0.00058 µg/mL, AFG₁ - 0.3464 µg/mL, and AFG₂ - 0.0010 µg/mL. The limits of quantification (LOQ) for aflatoxins were: AFB₁ - 0.3728 µg/mL, AFB₂ - 0.00175 µg/mL, AFG₁ - 1.0389 µg/mL, and AFG₂ - 0.0030 µg/mL.

HPLC-FLD analysis of ochratoxin A

HPLC analysis was performed on an Agilent 1260 Infinity system (Agilent, USA). An Agilent Poroshell 120 EC-C18, 4.6 x 100 mm, 2.7 µm column was used for the determination of OTA in green coffee bean samples. The column thermostat was heated to 25 °C, 55% H₂O (A) and 45% acetonitrile (B) were used as mobile phase in isocratic mode. The flow rate was 1 mL/min. The injection volume was 40 µL. They were analyzed in triplicate. The excitation wavelength of 333 nm and emission wavelength of 450 nm were used to detect OTA using a FLD detector. An OTA reference standard (Supelco, Bellefonte, USA) was used for the analysis. The LOD and LOQ were 0.0807 µg/L and 0.2421 µg/L, respectively.

RESULTS AND DISCUSSION

Percentage of green coffee beans contaminated with microscopic fungi

The percentage of micromycete-contaminated coffee beans ranged from 10% (sample 9, Guatemala) to 100%. In samples 1, 3, 4, 11, 13, 14, 18, 19, 20, all green

coffee beans analysed (100%) were contaminated with micromycetes on both media used (Figure 2). **Viegas et al. (2017)** reported that in their study 64% of green coffee beans (Arabica coffee) were contaminated with micromycetes.

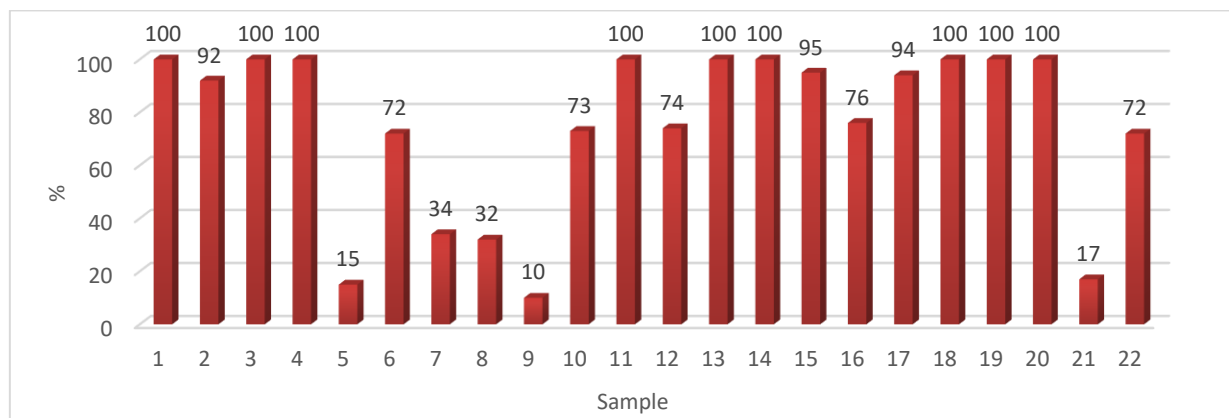


Figure 2 Percentage of micromycete-contaminated green coffee beans on DRBC and DG18

Filamentous fungi genera identification

A total of 2860 isolates of microscopic filamentous fungi belonging to 11 genera and *Mycelium sterillum* (fungi that did not form fructifying structures) were isolated on DRBC and DG18 medium (Table 2). The following genera of

filamentous fungi were isolated: *Aspergillus*, including xerophilic species formerly belonging to the genus *Eurotium*, *Absidia*, *Alternaria*, *Cladosporium*, *Chaetomium*, *Fusarium*, *Paecilomyces*, *Mucor*, *Penicillium*, *Rhizopus*, and *Syncephalastrum*.

Table 2 Occurrence of fungal genera in green coffee beans

Genus	Sample										
	1	2	3	4	5	6	7	8	9	10	11
<i>Absidia</i>	11	1	-	-	3	-	-	-	-	-	-
<i>Aspergillus</i>	162	122	141	116	3	55	9	23	4	61	171
<i>Aspergillus/Eurotium</i>	6	1	39	41	5	21	27	5	1	2	24
<i>Cladosporium</i>	1	-	-	-	1	-	-	2	-	-	-
<i>Fusarium</i>	-	-	-	-	-	1	-	-	-	-	1
<i>Mucor</i>	-	-	-	-	-	3	-	-	-	-	-
<i>Mycelium sterillum</i>	-	-	-	-	-	-	2	-	-	-	1
<i>Penicillium</i>	-	-	-	-	1	1	-	2	-	2	-
<i>Rhizopus</i>	1	7	-	36	3	-	-	-	-	10	1
<i>Syncephalastrum</i>	-	-	-	3	-	3	-	2	-	-	-

Genus	Sample										
	12	13	14	15	16	17	18	19	20	21	22
<i>Absidia</i>	-	-	-	-	-	6	-	-	-	-	14
<i>Alternaria</i>	-	-	-	-	-	1	-	-	-	-	-
<i>Aspergillus</i>	58	155	248	92	92	94	186	227	170	10	93
<i>Aspergillus/Eurotium</i>	36	1	-	4	1	-	1	3	7	5	9
<i>Cladosporium</i>	-	-	-	-	-	-	-	-	-	2	-
<i>Chaetomium</i>	-	-	-	-	-	-	-	-	-	-	1
<i>Mycelium sterillum</i>	1	-	-	-	2	-	-	-	-	-	-
<i>Paecilomyces</i>	-	-	-	-	-	-	-	-	-	1	-
<i>Penicillium</i>	-	-	51	7	2	7	-	-	-	-	-
<i>Rhizopus</i>	-	16	-	7	49	1	-	1	-	-	-
<i>Syncephalastrum</i>	-	-	16	7	-	35	2	-	-	-	-

Lemessa et al. (2015) reported that the green coffee beans in their study were contaminated mainly by the genera *Aspergillus*, *Penicillium*, *Fusarium*, and *Mucor*. **Vieira et al. (2015)** mention the genera *Aspergillus*, *Fusarium*, and *Penicillium* as being among the natural contaminants of green coffee beans during processing. **Viegas et al. (2017)** isolated 15 different genera, with *Aspergillus* and *Penicillium* having the highest occurrence. Of the 18 samples of green coffee beans analysed in the study by the authors, 16 samples were contaminated with isolates of the genus *Aspergillus*. These authors suggest that the higher prevalence of *Aspergillus* may be due to better penetration into the bean structures as well as better growth at high temperatures. In a study by **Martins et al. (2003)**, the genus *Aspergillus* was also the dominant genus, followed by the genera *Cladosporium* and *Penicillium*, which were less abundant. **El Aaraj et al. (2015)** isolated micromycetes of the genera *Aspergillus*, *Penicillium*, *Rhizopus*, and yeasts.

***Aspergillus* sections and *Penicillium* species**

The genus *Aspergillus* had the highest frequency of occurrence (100.00%, Table 3), this genus was present in all samples. The RD of this genus was 88.49%. A total of 2531 isolates of this genus were isolated (Table 4), with the highest number of isolates found in sample no. 14 (248, Mexico). Representatives of 6 *Aspergillus* sections were isolated, with the highest Fr and RD belonging to *Nigri* (Fr 95.45%, RD 49.86%), *Aspergillus* (Fr 90.90%, RD 8.36%), and *Flavi* (Fr 77.27%, RD 27.94%) section. **Bessaire et al. (2019)** reported that green coffee beans worldwide are infected by the genus *Aspergillus*, with *Aspergillus* from the *Circumdati* and

Nigri sections being the most common aspergilli, which are the main OTA producers in coffee. **Viegas et al. (2017)** report that in their study the predominant *Aspergillus* section detected in green coffee beans was *Nigri* (39%), followed by *Aspergillus* section *Circumdati* (29%). In our study, aspergilli section *Circumdati* had Fr 18.18% and RD 0.94%. Filamentous fungi from the Mucoromycota had higher Fr, especially the *Rhizopus* (Fr 50.00%, RD 4.62%) and *Syncephalastrum* (Fr 31.82%, RD 2.38%) genera.

Table 3 Frequency of occurrence and relative density of fungal genera isolated from green coffee beans

Genus	Fr [%]	RD [%]
<i>Absidia</i>	22.72	1.22
<i>Alternaria</i>	4.55	0.03
<i>Aspergillus</i> (+ <i>Eurotium</i>)	100.00	88.49
<i>Cladosporium</i>	18.18	0.21
<i>Fusarium</i>	9.09	0.07
<i>Chaetomium</i>	4.55	0.03
<i>Mucor</i>	4.55	0.11
<i>Mycelium sterillum</i>	18.18	0.21
<i>Paecilomyces</i>	4.55	0.03
<i>Penicillium</i>	36.36	2.55
<i>Rhizopus</i>	50.00	4.62
<i>Syncephalastrum</i>	31.82	2.38

Legend: Fr – frequency of occurrence, RD – relative density

We isolated 73 isolates of 9 *Penicillium* species from green coffee beans (Table 5). Fr of the genus *Penicillium* was 36.36% and RD 2.55%. The highest RD had species *P. chrysogenum* (1.78%). The highest abundance of *Penicillium* species

was again in sample 14 (51 isolates, Mexico). Hlebová et al. (2022) reported a higher Fr 90% and RD 24.6% of *Penicillium* genus.

Table 4 Overview of *Aspergillus* sections isolated from green coffee beans

Section	Sample										
	1	2	3	4	5	6	7	8	9	10	11
<i>Aspergillus</i>	6	1	39	41	5	21	27	5	1	2	24
<i>Flavi</i>	60	38	41	16	-	-	1	23	-	-	76
<i>Fumigati</i>	-	-	-	-	-	-	4	-	-	1	-
<i>Nigri</i>	102	84	100	100	2	54	4	-	4	60	95
<i>Terrei</i>	-	-	-	-	1	-	-	-	-	-	-
<i>Aspergillus</i> sp.	-	-	-	-	-	1	-	-	-	-	-

Section	Sample										
	12	13	14	15	16	17	18	19	20	21	22
<i>Aspergillus</i>	36	1	-	4	1	-	1	3	7	5	9
<i>Circumdati</i>	-	-	17	5	-	2	-	-	-	-	3
<i>Flavi</i>	7	25	92	39	26	13	99	129	71	-	43
<i>Fumigati</i>	2	30	-	-	1	-	-	-	-	-	-
<i>Nigri</i>	49	100	139	48	65	79	87	98	99	10	47

Martins et al. (2003) reported that the genus *Penicillium* was represented at a lower frequency, which agrees with our results. Nasser (2008) isolated 5 species of the genus *Penicillium* from green coffee beans namely *P. funiculosum* (2 isolates), *P. glabrum* (1 isolate), *P. islandicum* (1 isolate), *P. oxalicum* (1 isolate), and *P. chrysogenum* (3 isolates). An important OTA-producing species that can occur in green coffee beans is *P. verrucosum* (Jović et al., 2009). However, this species was not isolated in the green coffee samples analysed by us.

In our green coffee samples 1, 2, 7, 8, 9, 10, and 17 the presence of OTA (<LOD) was not detected. In 68.2% of the samples analysed, the presence of OTA (Table 6) was detected in very low concentrations (from 0.375 to 1.487 ng/kg). The largest amount of OTA was detected in sample 19 (1.487 ng/kg) from the Dominican Republic. According to Bessaire et al. (2018), the highest value of OTA that was measured in their study, was 12.2 µg/kg. Vaclavik et al. (2013) indicate the presence of OTA in green coffee beans in a concentration from 1.0 to 136.9 µg/kg.

Table 5 Overview of *Penicillium* species isolated from green coffee beans

Species	Sample							
	5	6	8	10	14	15	16	17
<i>P. aethiopicum</i>	-	-	-	-	-	-	1	-
<i>P. brevicompactum</i>	-	-	-	-	-	7	-	-
<i>P. crustosum</i>	-	-	-	-	-	-	1	-
<i>P. freii</i>	-	-	1	-	-	-	-	-
<i>P. glabrum</i> clade	-	-	1	-	-	-	-	6
<i>P. hirsutum</i>	1	-	-	-	-	-	-	-
<i>P. chrysogenum</i>	-	-	-	-	51	-	-	-
<i>P. oxalicum</i>	-	1	-	-	-	-	-	-
<i>P. palitans</i>	-	-	-	2	-	-	-	-
<i>Penicillium</i> sp.	-	-	-	-	-	-	-	1

Table 6 Ochratoxin A occurrence in green coffee beans

Sample	OTA [ng/kg]*	Sample	OTA [ng/kg]*
1	<LOD	12	1.095
2	<LOD	13	1.004
3	1.094	14	1.068
4	1.296	15	0.995
5	0.999	16	0.375
6	1.063	17	<LOD
7	<LOD	18	1.140
8	<LOD	19	1.487
9	<LOD	20	1.120
10	<LOD	21	1.055
11	0.731	22	0.690

Legend: * - average value from three measurements, LOD - limit of detection

Toxicogenicity of selected isolates of the *Aspergillus* and *Penicillium* genera

We tested 133 isolates of the genus *Aspergillus* and 7 isolates of the genus *Penicillium* for their ability to produce selected mycotoxins by TLC method. The ability to produce at least one mycotoxin was detected in 27.8% of *Aspergillus* and 71.4% of *Penicillium* isolates. We tested the ability to produce aflatoxins and cyclopiiazonic acid (CPA) in 65 isolates from section *Flavi*, AFB₁ produced 11 isolates (16.9%), AFB₂ 3 isolates (4.6%), AFG₁ 5 isolates (7.7%), AFG₂ 2 isolates (3.1%) and CPA produced 20 isolates (30.8%). We tested the potential of *Aspergillus* from the *Nigri* section to produce OTA and CPA. Totally, we tested 59 isolates of this section, 6 (10.2%) isolates produced CPA, and 8 isolates produced OTA (13.6%). All tested (9) *Aspergillus* isolates from *Circumdati* section had the ability to produce OTA. Velmourougane et al. (2010) reported that *A. ochraceus* (section *Circumdati*), can rapidly contaminate coffee beans and is among the main producers of OTA in green coffee. In the study by the authors mentioned, 408 samples of green coffee were tested, of which 872 isolates of this species were isolated and of which 269 isolates produced OTA. Reddy et al. (2010) reported that the main source of OTA in green coffee is *A. ochraceus*, *A. carbonarius* (section *Nigri*), and *A. niger* (section *Nigri*). This study demonstrated a high percentage of the ability of *A. ochraceus* to produce OTA (75-90%).

Using the TLC method, we tested the toxinogenicity of 7 isolates of the genus *Penicillium*, while 5 isolates (71.4%) produced at least one mycotoxin. Roquefortin C was produced by all tested isolates of *P. palitans* (3), *P. hirsutum* (1), and *P. crustosum* (1). The *P. crustosum* isolate produced penitrem A, too. The assessed *P. aethiopicum* isolate did not produce griseofulvin.

Aflatoxins and ochratoxin A quantification by HPLC-FLD

The presence of aflatoxins (AFB₁, AFB₂, AFG₁, AFG₂) was not detected by the selected method in any of the analysed green coffee samples. Nasser (2008) reports the presence of aflatoxins in green coffee beans at the level of 3-12 µg/kg (AFB₁). Coffee samples from Yemen had measured AFB₁ values from 110-600 µg/kg and 360-600 µg/kg. Vieira et al. (2015) report that aflatoxins are rare in green coffee beans. Bessaire et al. (2019) conducted a study on the occurrence of mycotoxins in green coffee beans and found that these mycotoxins were rarely detected in coffee, too. AFB₁ contamination was at the level of 1.2 µg/kg.

CONCLUSION

In this study, we confirmed the widespread presence of micromycetes in green coffee beans, with *Aspergillus* being the most frequently occurring genus. The ability of certain isolates of *Aspergillus* and *Penicillium* genera to produce mycotoxins highlights potential health risks and underscores the need for stringent quality control measures in coffee production and storage. Although aflatoxins were not detected in green coffee bean samples, the low concentrations of OTA in the majority of samples warrant ongoing monitoring. Regular intake of low concentrations of OTA leads to its accumulation in the body, especially in the kidneys. Many studies suggest that chronic exposure may be associated with nephropathies, DNA damage, and increased risk of kidney cancer. These findings contribute valuable insights into fungal contamination patterns and mycotoxin risks associated with green coffee beans, supporting future research aimed at enhancing food safety and reducing exposure to harmful contaminants.

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