Food Control 113 (2020) 107204

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Fungi associated to beans infested with coffee berry borer and the risk of ochratoxin A

Sabrina Alves da Silva[a,*], Rosemary Gualberto Fonseca Alvarenga Pereira[a], Nathasha de Azevedo Lira[b], Eduardo Micotti da Glória[c], Sara Maria Chalfoun[d], Luís Roberto Batista[a]

[a] Department of Food Science, Federal University of Lavras (Departamento de Ciência de Alimentos, Universidade Federal de Lavras), Post Office Box (Caixa Postal) 3037, Zip Code (CEP), 37200-000, Lavras, Minas Gerais, Brazil
[b] Department of Biology, Federal University of Lavras (Departamento de Biologia, Universidade Federal de Lavras), Post Office Box (Caixa Postal) 3037, Zip Code (CEP), 37200-000, Lavras, Minas Gerais, Brazil
[c] Department of Biological Science, Luiz de Queiroz College of Agriculture, University of São Paulo (Departamento de Biologia, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo), Av. Pádua Dias 11, Post Office Box (Caixa Postal) 9, Zip Code (CEP), 13418-900, Piracicaba, São Paulo, Brazil
[d] EPAMIG – Agricultural and Livestock Minas Gerais State Institution (Empresa Mineira de Pesquisa Agropecuária), Rodovia Lavras/ Ijaci Km 02, Post Office Box (Caixa Postal) 176, Zip Code (CEP), 37200-000, Lavras, Minas Gerais, Brazil

ARTICLE INFO

Keywords:
Agricultural pest
Coffee
Fungal contamination
OTA
Toxigenic potential
Demaged beans

ABSTRACT

Coffee berry borer (*Hypothenemus hampei*) is the main pest of coffee crop. Its damage starts when insect pierces coffee berries. Perforations may compromise microbiological quality and consumption safety of beans. This study aimed to identify toxigenic fungi associated to damaged coffee beans and quantify ochratoxin A content. Coffee beans from two Brazilian producing areas were collected and the damaged beans in the sample were classified by the level of infestation. There is a relationship between coffee berry borer infestation and the increase in fungal contamination percentage. Twenty fungal species were identified, where those from the *Fusarium* and *Aspergillus* genera were more incident. Among the twenty isolated species four were potential OTA producers. OTA presence was detected only in samples from the "Cerrado" producing region, where high mycotoxin concentrations were found in "Dirty I and II" levels.

1. Introduction

Coffee berry borer (*Hypothenemus hampei*) is the most important pest in coffee crop, causing losses in every producing country worldwide. Annually, about US$ 500 million are accounted in losses for the coffee production sector caused solely by the coffee berry borer. (Vega et al., 2002, 2015). The damage starts when the female penetrates the berries while still in the plant. Female lays eggs inside the berries and when larvae hatch they start to feed from the endosperm, carving galleries in the seed (Rodriguez et al., 2013, 2017; Vega, Infante, Castilho, & Jaramillo, 2009).

Beyond the direct economic damage due to the loss of bean weight, perforations may also favor fungal contamination. When insects perforate coffee berries in field, the lesions may favor the infection of external microorganisms. Additionally, the insect itself may serve as transport for contaminants such as toxigenic fungi (Amézqueta, González-Peñas, Murillo-Arbizu, & Cerain, 2009; Paterson & Lima, 2010).

Inside the berries, microorganisms find favorable conditions to develop and induce several changes, such as change of bean color, especially internally and around the holes, and compromise sensorial quality of the beverage (Rezende et al., 2013; Vilela, Pereira, Silva, Batista, & Schwam, 2010). Moreover, some fungal species have the ability to produce toxic extracellular metabolites, such as mycotoxins (Ramirez, Cendoya, Nichea, Zachetti, & Chulze, 2018).

Among frequent mycotoxins that may occur in coffee bean, the ochratoxin A (OTA) is the most studied and most found one. This toxin is produced by some fungal species of *Aspergillus* and *Penicilium* genera (Batista et al., 2009; Rezende et al., 2013; Taniwaki, Pitt, Teixeira, & Iamanaka, 2003) and it can cause nephrotoxic, genotoxic, hepatotoxic, immunosuppressive and carcinogenic effects (Ha, 2015; Sorrenti et al., 2013). The International Agency for Research on Cancer – IARC (1993) classified such mycotoxin as a group 2B compound: possibly carcinogenic substance (Ostry, Malir, Toman, & Grosse, 2017). Due to its high

* Corresponding author.
E-mail address: ssalvessilva@gmail.com (S. Alves da Silva).

https://doi.org/10.1016/j.foodcont.2020.107204
Received 30 December 2019; Received in revised form 22 February 2020; Accepted 24 February 2020
Available online 25 February 2020
0956-7135/

risk to human health, regulatory entities have stablished maximum level of such substance in food prone to contamination, e.g. 5 μg/kg in processed coffee in the European Union and 10 μg/kg in Brazil (Commission Regulation (EU), 2010; Geremew, Abete, Landsschoot, Haesaert, & Audenaert, 2016; BRASIL, 2011).

Given the high infestation of coffee berry borer in the last years and the capacity of the insect of being a facilitator of bean contamination by toxin-producer fungi, this study aimed to identify toxigenic fungi associated to damaged coffee bean and quantify ochratoxin A content in these beans.

2. Material and methods

2.1. Sample collection and preparation

Coffee bean samples used in this study were collected in two different producing regions in the state of Minas Gerais, Brazil, which present high levels of coffee berry borer incidence: Cerrado and Sul de Minas (Fundação Procafé, 2016). A total of 60 kg of processed coffee was collected from each region, which were manually classified for selection and categorization of level of infestation. Classification was performed based on the number of perforations in the bean and internal presence/absence of color change inside the galleries, totaling four categories for each region (Fig. 1):

Control: perfect beans, absence of any kind of defect;
Damaged clean: beans with up to two perforations free from any internal color change;
Damaged dirty I: beans with up to two perforations and internal color change;
Damaged dirty II: beans with three to six perforations and internal color change.

2.2. Percentage of fungal contamination

The assessment of the fungal contamination was performed using direct plating technique (Samsom, Hoekstra, Frisvad, & Filtenborg, 2000). For this, 200 coffee beans were randomly sampled from each level of infestation of both regions. From this total, 100 coffee beans were equidistantly plated in Dicloran Rose Bengal Chloramphenicol culture medium (DRBC) (10.0 g of glucose; 5.0 g of bacteriological peptone; 1.0 g of KH_2PO_4; 0.5 g of $MgSO_4{\cdot}7H_2O$; 0.5 mL of 5% solution of Bengal rose; 1.0 mL of Dichloran; 1.000 mL of distilled water; 15.0 g of agar; 1.0 mg of chloramphenicol) for assessment of fungal contamination in nondisinfected beans. The other 100 coffee beans were superficially disinfected in ethanol 70% (1 min) and sodium hypochlorite 1% (30 s), washed three times in sterile water, and plated in DRBC to assess contamination by internal fungi. Plates were stored at 25 °C for 7 days and results were expressed in percentage of contaminated beans, according to Pitt and Hocknig (1997).

Fig. 1. Bean damage classification according to number of perforations and internal color change: control (A); damaged clean (B); damaged dirty I (C); damaged dirty II (D), in the sequence.

Fig. 1. (*continued*)

Fig. 1. (*continued*)

Fig. 1. (*continued*)

2.3. Isolation and identification of fungi

The isolation and purification of fungi were performed by transferring colonies from DRBC to malt extract agar medium (MA) and incubation at 25 °C for seven days. The isolates were then grown in specific media according to each genus and incubated at 25 and 37 °C for seven days. Macro and microscopic characteristics of each colony were assessed according to Klich (2002), Pitt (2000), Pitt and Hocknig (1997), Samsom et al. (2000), and others.

2.4. Fungi toxigenic potential

In order to determine the toxigenic potential of each identified species (*Aspergillus* section *Nigri* and *Circumdati*), a thin-layer chromatography (TLC) was run following Filtenborg and Frisvad (1980) methodology. To detect OTA production, the CzapeckYeast Agar medium (CYA) was used for species from the *Aspergillus* section *Nigri*,

Table 1
Infection variance (IV) (%) of fungi in nondisinfected (ND) and disinfected (DB) bean samples.

IV	Clean/Sul de Minas		Dirty I/Sul de Minas		Dirty II/Sul de Minas		Control/Sul de Minas		Clean/Cerrado		Dirty I/Cerrado		Dirty II/Cerrado		Control/Cerrado	
	ND	DB	ND	DB	ND	DB	ND	DB	ND	DB	ND	DB	ND	DB	ND	DB
	54–70	6–10	98–100	3–11	83–92	13–19	67–100	0–4	99–100	19–28	95–100	56–62	95–100	41–77	99–100	7–18

and Yeast Extract Sucrose Agar medium (YES) for the *Aspergillus* section *Cirbumdati* species, both incubated for 10 days at 25 °C. Mobile phase used was composed of toluene, ethyl acetate and formic acid 90% (60:30:10), OTA standard solution (Sigma-Aldrich), and thin-layer chromatography plates (CCD) (Merk-Silica Gel 60, 20 × 20). The confirmation of OTA production was performed in a CAMAG chromatovisor (UF-BETRACHTER) under 366 nm-ultraviolet light. Microorganisms considered to be OTA-producers exhibited retention factor (RF) and purple fluorescent spots similar to the toxin standards.

2.5. Quantification of ochratoxin A in coffee beans

To assess OTA in coffee beans, 5 g of grounded coffee (fine particles, 40 mesh) was placed in a 250 mL-Erlenmeyer with 100 mL of sodium bicarbonate 1%. The suspension was mechanically stirred for 1 h and centrifugated at 3600 g for 10 min at 25 °C. From the supernatant, 15 mL was mixed with 65 mL of phosphate-buffered saline pH 7.4. The resulting solution was filtered through a microfiber filter, and 50 mL of the filtered solution was passed through the immunoaffinity column Ochratest (Vican, USA) to purify the extract and concentration of mycotoxin. After the passage of the filtrate through the column, cleaning was proceeded with 15 mL of distilled water and elution of mycotoxin linked to the antibodies with 3 mL of methanol. From the solution, 2.7 mL was collected for drying and later diluted in 500 μL of a water-acetonitrile-acetic acid solution (49.5:49.5:1). Fifty microliters of the solution were injected in the chromatography system. The ochratoxin was identified and quantified using a Shimadzu HPLC system (Shimadzu, Kyoto, Japan). The system consisted of an LC-20 AT pump fitted with an FCV-10AL quaternary valve, a SIL-20A autosampler, a DGU-20A5 degasser, a CTO-20A column oven maintained at 40 °C, a fluorescence detector (FLD) RF-10AXL and the software (LC Solution v. 1.21).

The chromatography separation was performed with a C18 chromatographic column of 150 mm in length and 4.6 mm of internal diameter (Synergi Fusion RP, Phenomenex, USA) at 40 °C. The mobile phase was maintained in an isocratic system of water-acetonitrile-acetic acid (49.5:49.5:1) and pumped at a rate of 1.0 mL/min. For OTA detection we used a fluorescent detector set with wavelengths of 333 nm (excitation) and 443 nm (emission). The quantification was performed with an external calibration curve built with six concentration levels of the OTA standard (Sigma-Aldrich, USA).

Our method was checked to assess the OTA recovery rate and repeatability of the results. To evaluate the recovery rate, samples of OTA-free immature coffee beans were added with OTA standard rates to get concentration levels of 2.85 and 9.15 μg kg^{-1} (three samples of each were prepared). Repeatability was calculated using the concentration values observed during the recovery rate assessment and expressed in terms of the coefficient of variation.

2.6. Scanning electron microscopy

We randomly selected 10 coffee beans from each level of infestation of each region and horizontally sliced each of them in two 2-cm-wide pieces. Pieces were immersed in fixing solution (modified Karnovsky's) pH 7.2 for 24 h, washed in 0.05 M sodium cacodylate buffer, and dehydrated in acetone (gradient of 25, 50, 75, 90, and 100%, three times each). Samples were then critical point-dried in a CPD 050 (Bal-Tec), mounted on stubs and sputter coated with gold (Bozzola & Russell, 1998). Scanning electronic microscopy was performed in a LEO EVO 40 XVP microscope (Carl Zeiss).

2.7. Statistical analysis

The experiment was conducted under a completely randomized experimental design, with four treatments (infestation levels: control, clean, dirty I and dirty II), and three replications for each region (Cerrado and Sul de Minas). Data were analyzed by descriptive statistics and analysis of variance followed by the Student Newman Keuls test (SNK), at a level of 5% of probability, was performed for mean values of OTA, using SPEED stat 1.0 statistical software.

3. Results and discussion

3.1. Fungal contamination percentage

For nondisinfected coffee beans (ND), the Cerrado region presented a high fungal contamination percentage regardless of the level of infestation, from 95 to 100% of the infested beans. For Sul de Minas, there was a greater variance, where damaged clean beans showed lower contamination (54–70%), while the other levels varied from 67 to 100% of infestation (Table 1). For disinfested coffee beans (DB), the more severe the level of insect infestation in Cerrado the greater was the percentage contamination. The same behavior was seen for coffee beans from Sul de Minas although on a lower scale.

The microbiota present in coffee bean depends on many factors, such as climate, coffee susceptibility, postharvest handling, pest infestation, drying, storage, etc. The greater percentage of contamination in coffee beans from Cerrado may be associated with the climate, since these two regions present a distinct annual average temperature, rainfall, and humidity. However, cultural practices such as disease and pest control, and good agricultural practices in pre- and post-harvest also plays as a determining factor on bean contamination (Paterson, Lima, & Taniwaki, 2014).

3.2. Identified fungi

A total of 374 fungi were isolated from coffee beans (Table 2), 239 from nondisinfected grains, which came from internal or external contamination, and 135 from superficially disinfected beans (internal contamination). The isolated fungi were all deposited in the microbial culture collection of the Universidade Federal de Lavras (LATAX – DCA/UFLA). Most of the isolated fungi were from the *Aspergillus* genus, well distributed through all infestation levels, as showed in Table 2. This genus was also isolated from coffee bean (roasted or not) in many other studies, and it is considered as the most frequent fungi in coffee beans (Chalfoun & Batista, 2003; Geremew et al., 2016; Iamanaka et al., 2014; Taniwaki, Teixeira, Teixeira, Copetti, & Iamanaka, 2014; Vilela et al., 2010). Another microorganism very incident and well distributed in all infestation levels was *Fusarium stilboides*. Such genus is commonly associated with coffee beans and some species may cause a disease in coffee plants known as fusariosis. Pérez et al. (2003), when isolating fungi associated with the galleries formed by the coffee berry borer,

Table 2
Occurrence frequency[a] (%) and toxigenic potential** (%) of fungi identified with isolation of nondisinfected (ND) and disinfected (DB) beans.

Fungi	Clean/Sul de Minas		Dirty I/Sul de Minas		Dirty II/Sul de Minas		Control/Sul de Minas		Clean/Cerrado		Dirty I/Cerrado		Dirty II/Cerrado		Control/Cerrado		Toxigenic potential
	ND	DB	ND	DB	ND	DB	ND	DB	ND	DB	ND	DB	ND	DB	ND	DB	
A. brasiliensis	2.8	–	–	–	–	–	–	–	3.5	–	–	–	–	–	–	–	NT
A. flavus	–	–	–	–	4.1	–	–	–	–	–	–	–	–	–	–	–	NT
A. lanosus	–	–	–	–	–	–	5.8	–	–	–	–	–	–	–	–	–	NT
A. niger	28.5	22.2	37	–	20.8	23	28.4	–	14.3	–	34	11.1	22.2	15.3	20.6	–	11,3
A. ochraceus	34.7	33.5	29.6	–	37.7	7.7	16.4	–	20.9	55	38	29.6	55.5	23	44.7	68.1	40
A. oryzae	–	–	–	–	–	–	–	–	3.4	–	–	–	–	–	–	–	NT
A. ostianus	–	–	–	–	–	–	6	–	–	–	–	–	–	11.5	–	–	100
A. sulphureus	–	–	–	–	–	–	2.2	–	–	–	–	–	–	–	–	–	NT
A. tamarii	–	–	–	–	4.1	–	–	–	–	–	–	–	3.7	–	3.4	–	NT
A. tubingensis	2.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NT
Aspergillus sp seção *Circumdati*	–	11.1	11.1	–	–	23.1	–	–	34.3	15	20	25.9	7.5	19.2	10.3	–	38,5
A. niger agregado	11.4	–	14.9	–	16.6	–	–	–	16.6	–	4	–	–	–	7.1	9	–
Chaetomium globosum	–	–	–	25	–	–	–	–	–	10	–	7.4	–	–	3.4	9	NT
Cladosporium cladosporioides complexo	–	–	–	–	–	–	11.8	60	–	–	–	–	–	3.8	–	–	NT
Eurotium amstelodami	–	–	–	–	–	–	–	–	–	4.9	–	3.8	–	–	–	4.6	NT
F. stilboides	–	27.7	–	75	4.3	23.2	–	40	3.4	10	–	7.4	3.7	15.6	3.7	4.8	NT
P. brevicompactum	14.2	–	–	–	12.4	15.4	23.5	–	3.6	5.1	–	11.1	–	4	6.8	–	NT
P. citrinum	–	–	–	–	–	–	–	–	–	–	–	–	7.4	–	–	4.5	NT
P. commune	2.8	–	7.4	–	–	–	–	–	–	–	4	3.7	–	7.6	–	–	NT
P. italicum	2.8	5.5	–	–	–	7.6	5.9	–	–	–	–	–	–	–	–	–	NT

[a] Occurrence frequency: number of species isolates/total of species isolated in the sample; **Toxigenic potential: number of isolates toxin-producers/total of species isolates; NT: not tested.

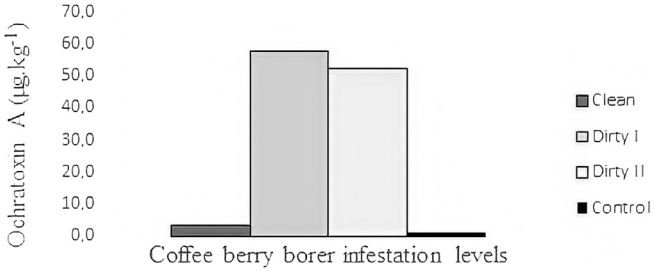

Fig. 2. Average Ochratoxin A content (µg.kg $^{-1}$) in coffee beans in different levels of coffee berry borer infestation, from Cerrado region.

reported two *Fusarium* species, which corresponded to 64% of their isolates.

The relation between the level of infestation of coffee berry borer and the increase in fungal contamination percentage proves that the insect may induce a greater level of bean contamination. Pérez et al. (2003) studying the microbiota associated with this pest in producing areas of Mexico, isolated 187 fungi from the cuticle, intestine, and feces of the insect, where *Fusarium*, *Penicillium*, and *Aspergillus* were the most abundant and the most present in the insect cuticle. They also isolated potentially toxigenic fungi, such as *Aspergillus niger* and *A. ochraceus* from the cuticle of insects of two Mexico regions, showing that insects may carry fungi that may be a threat to human health.

3.3. Toxigenic potential of identified fungi

Among the isolated fungi, four species presented toxigenic potential for OTA production: *A. niger*, where 11.3% were OTA producers; *A. ochraceus* (40%); *Aspergillus* section *Circumdati* (38.5%); and *A. ostianus* (100%) (Table 2).

Velmourougane, Bhat, and Gopinandhan (2010), studying microbiota associated with healthy- and coffee berry borer-damaged beans, observed that the greatest fungal contamination was recorded in damaged beans, with a significant presence of potentially toxigenic fungi (*A. niger* and *A. ochraceus*). It was also noticed that among damaged berries, those collected in the ground and from the plant after harvest presented higher OTA content than the properly harvested-damaged berries.

3.4. Ochratoxin A quantification

The analytic method for ochratoxin A determination had a detection limit of 0.6 µg kg^{-1} and a quantification limit of 1.0 µg kg $^{-1}$. The average recovery rate ranged from 86.9 to 90.4%, with a coefficient of variation between 2.6 and 16.3%.

For the Sul de Minas region, no ochratoxin A was detected in the coffee beans, regardless of the level of infestation. But in beans from Cerrado, OTA content was detected in all levels of infestation, and dirty I and dirty II treatments presented the greatest content, with averages of 57.8 and 52.3 µg kg^{-1}, respectively (Fig. 2).

With the higher incidence of toxigenic fungi in beans from Cerrado (Table 2), the OTA content results corroborate with fungal incidence, since coffee beans from Sul de Minas presented low fungal incidence and therefore low toxin content. According to Pitt and Hocknig (1997) and Serra, Lourenço, Alípio, and Venâncio (2006) species of *Penicillium* are commonly found in regions of temperate/cold climate, while *Aspegillus* species tend to be more common in tropical/hot conditions. The two studied regions have different climate conditions, while Cerrado is hot and dry, Sul de Minas is a region with lower temperatures and higher rainfall rate. However, the OTA production by a fungus does not depend only on its ability. External and environmental factors, such as temperature, humidity, nutrient availability, and interspecific competition, may influence on the expression of the gene responsible to produce the toxin (Dijksterhuisd & Samson, 2007).

The Brazilian legislation (BRASIL, 2011) does not define a maximum level of OTA in immature or raw coffee beans, but there is a limit of 10 µg kg^{-1} for processed beans. Taking into consideration that OTA is a chemical compound with relative thermal stability, later processing of the bean, such as soft or clear roasting, will not reduce the toxin content, posing threat to consumer health (Castellanos-Onorio et al., 2011; Oliveira, Silva, Pereira, Paiva, & Batista, 2013; Paterson et al.,

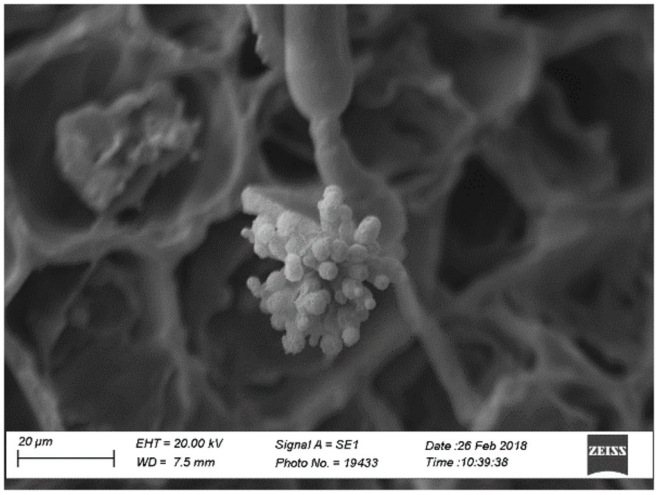

Fig. 3. Electronic scanning micrographs. A – reproductive structure of a fungus; B – Coffee berry borer inside a gallery of a Dirty I treatment coffee bean from Cerrado; C – Coffee bean cells of Cerrado control treatment. D – Coffee bean cells of Dirty II treatment from Cerrado; E – Coffee bean cells of control treatment from Sul de Minas; F – Coffee bean cells of Clean treatment from Sul de Minas, in the sequence.

2014).

3.5. Scanning electron microscopy

Images from scanning electron microscopy were performed in order to analyze the internal cellular structure of coffee beans, especially near the galleries formed by the coffee berry borer during its infestation. With the micrographs is possible to compare cellular structures of coffee beans with different levels of infestation.

In Fig. 3A we can see the reproductive structure of a fungus. Fig. 3C and E shows coffee bean cells from the control treatment (Cerrado and Sul de Minas, respectively). Fig. 3D and F shows cells of the Dirty II treatment from Cerrado and the Clean treatment from Sul de Minas, respectively. Comparing micrographs from control (Fig. 3C and E) and other treatments (Fig. 3D and F) we observe significant cell disorganization in infested beans, as well as the presence of hyphae and other microbial structures, indicating that beans attacked by the insect are contaminated.

Another important factor observed during the image analysis was the presence of a coffee berry borer inside one perforation in a bean from the dirty I treatment of Cerrado (Fig. 3B). The insect, besides of being a contaminant vehicle, may die inside the galleries after agricultural procedures for pest control and remains inside the coffee bean throughout the whole process. There are no studies yet proving the toxic potential of such insects to human health. However, the insect acting as a contaminant vehicle in beans may bring fungi that produce

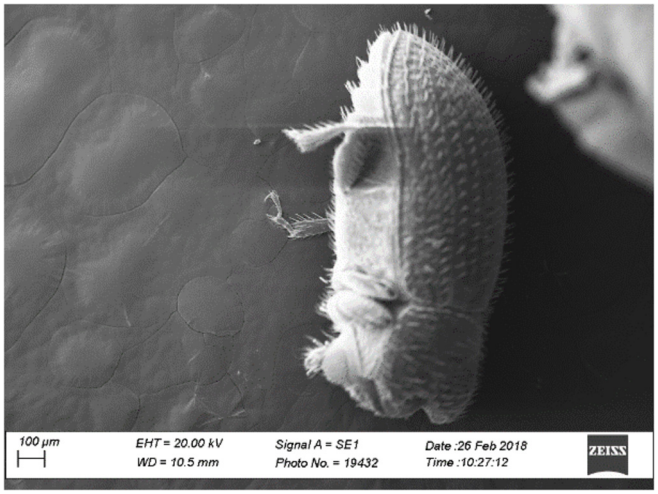

Fig. 3. (*continued*)

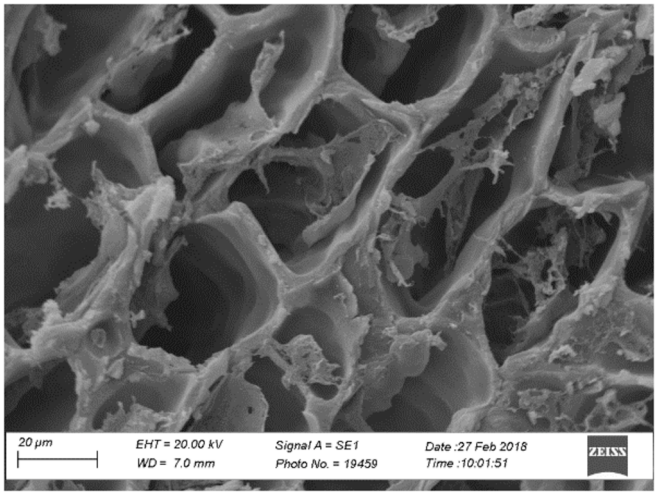

Fig. 3. (*continued*)

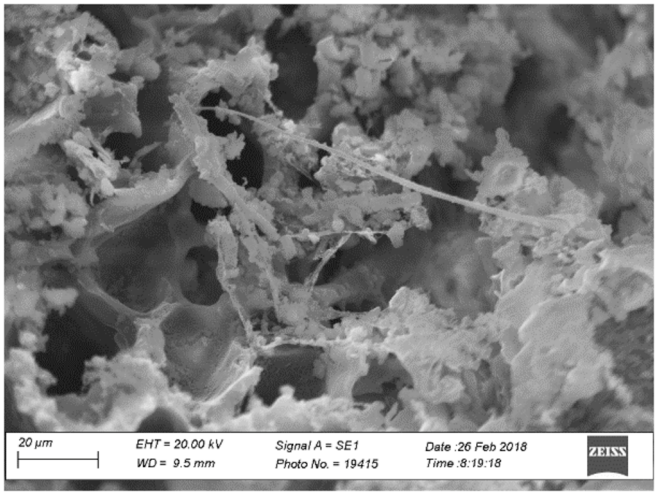

Fig. 3. (*continued*)

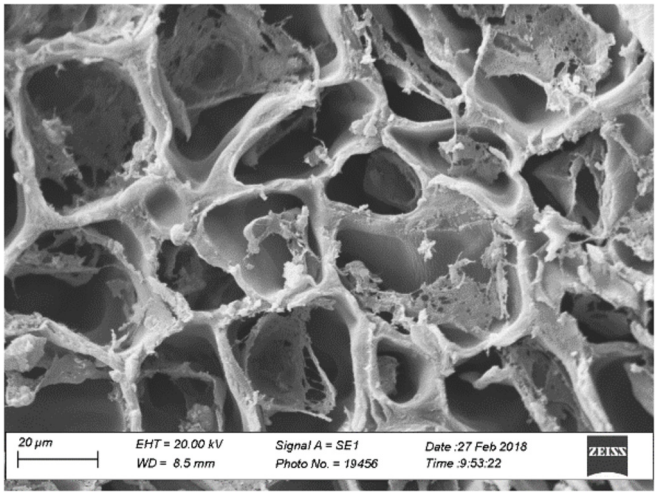

Fig. 3. (*continued*)

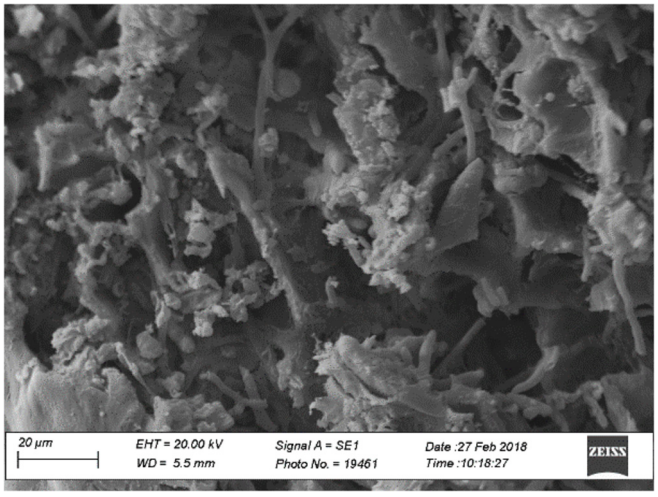

Fig. 3. (*continued*)

human health-threatening toxins attached to its cuticle (Pérez et al., 2003).

According to the Brazillian legislation (BRASIL, 2014), up to 60 fragments of an insect are allowed in 25 g of roasted/toasted coffee, which may be a result of failures in good agricultural and industrial practices. In the case of coffee berry borer, high tolerance may lead to the acceptance of highly contaminated beans, which may contain significant OTA concentrations. It is noteworthy that the visual aspect of a coffee bean is not an indication of mycotoxins presence since in this study the highest OTA concentration was found in not-too-damaged coffee beans, as illustrated in Fig. 1C.

4. Conclusions

According to results from the present study, the increase in fungal contamination is directly linked to the level of infestation by coffee berry borer.

Among the isolated and identified fungi, those from the *Fusarium* and *Aspergillus* genera were present in greater proportions and in all levels of insect infestation. Four of the 20 isolated species were potential producers of ochratoxin A and they were present in all levels of infestation, except for *Aspergillus ostianus*. Ochratoxin A was detected only in samples from the Cerrado region, where Dirty I and Dirty II treatments presented the highest concentration levels of the compound.

CRediT authorship contribution statement

Sabrina Alves da Silva: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing. **Rosemary Gualberto Fonseca Alvarenga Pereira:** Conceptualization, Supervision, Funding acquisition. **Nathasha de Azevedo Lira:** Investigation, Writing - original draft. **Eduardo Micotti da Glória:** Investigation, Validation. **Sara Maria Chalfoun:** Resources, Supervision. **Luís Roberto Batista:** Resources, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknoledgments

The authors are thankful for the COOXUPÉ for providing coffee bean samples and to the financing agencies CAPES, FAPEMIG, CNPq, and FINEP for scholarships and financial support.

References

Amézqueta, S., González-Peñas, E., Murillo-Arbizu, M., & Cerain, A. L. (2009). Ochratoxin A descontamination: A review. *Food Control, 20*, 326–333.

Batista, L. R., Chalfoun, S. M., Silva, C. F., Cirillo, M., Vargas, E. A., & Schwan, R. F. (2009). Ochratoxin A in coffee beans (*Coffea arabica* L.) processed by dry and wet methods. *Food Control, 20*, 784–790.

Bozzola, J. J., & Russell, L. D. (1998). *Microscopia eletrônica: Princípios e técnicas para biólogos* (2th ed.). Sudbury: Jones & Barlett.

BRASIL. Resolução RDC nº 7, de 18 de fevereiro de 2011. Aprova do regulamento técnico sobre limites máximos tolerados (LMT) para micotoxinas em alimentos. Órgão emissor: ANVISA - agência nacional de Vigilância sanitária. (2011). http://portal.anvisa.gov.br/documents/10181/2968262/RDC_07_2011_COMP.pdf/afe3f054-bc99-4e27-85c4-780b92e2b966 /Acessed 12 March 2018.

BRASIL. Resolução RDC nº 14, de 28 de março de 2014. Aprova do regulamento técnico que dispõe sobre matérias estranhas macroscópicas e microscópicas em alimentos e bebidas, seus limites de tolerâncias e dá outras providências. Órgão emissor: ANVISA - agência nacional de Vigilância sanitária. (2014). http://portal.anvisa.gov.br/documents/10181/2966692/RDC_14_2014_.pdf/2ae304af-8f2b-446b-a964-2d13ef295569 /Acessed 20 March 2018.

Castellanos-Onorio, O., Gonzalez-Rios, O., Guyot, B., Fontana, T. A., Guiraud, J. P., Schorr-Galindo, S., et al. (2011). Effect of two different roasting techniques on the ochratoxin A (OTA) reduction in coffee beans (*Coffea arabica*). *Food Control, 22*, 1184–1188.

Chalfoun, S. M., & Batista, L. R. (2003). *Fungos associados a frutos e grãos do café: Aspergillus e Penicillium.* Brasília: Embrapa.

Commission Regulation (EU) (2010). Nº 105/2010 of 5 February 2010 amending Regulation (EC) Nº 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards ochratoxin A. *Official Journal of the European Union, L, 35*, 7–8.

Dijksterhuisd, J., & Samson, R. A. (2007). *Food mycology: A multifaceted approach to fungi and food.* New York: CRC Press.

Filtenborg, O., & Frisvad, J. C. (1980). A simple screening method for toxigenic moulds in pure cultures. *Lebensmittel-Wissenschaft und - Technologie, 13*, 128–130.

Fundação Procafé. Boletim de Avisos fitossanitários. (2016). http://fundacaoprocafe.com.br Acessed 13 July 2016.

Geremew, T., Abete, D., Landsschoot, S., Haesaert, G., & Audenaert, K. (2016). Occurrence of toxigenic fungi and ocratoxin A in Ethiopian coffee for local consumption. *Food Control, 69*, 65–73.

Ha, T. H. (2015). Recent advances for the detection of ochratoxin A. *Toxins, 7*, 5276–5300.

Iamanaka, B. T., Teixeira, A. A., Teixeira, A. R. R., Copetti, M. V., Bragagnolo, N., & Taniwaki, M. H. (2014). The mycobiota of the coffee beans and its influence on the coffee beverage. *Food Research International, 62*, 353–358.

IARC – International Agency for Research on Cancer. Monographs on the evaluation of carcinogenic risks to humans. Some naturally occurring substances: Food items and constituints, heterocyclic aromatic amines and mycotixins. (1993). https://monographs.iarc.fr/wp-content/uploads/2018/06/mono56.pdf Accessed 2 August 2018.

Klich, M. A. (2002). *Identification of commom Aspergillus species.* Utrecht: Centraalbureau Voor Schimmelcultures.

Oliveira, G., Silva, D. M., Pereira, R. G. F. A., Paiva, L. C. P. A., & Batista, L. R. (2013). Effect of different roasting levels and particle sizes on ochratoxin A concentration in coffee beans. *Food Control, 34*, 651–656.

Ostry, V., Malir, F., Toman, J., & Grosse, Y. (2017). Mycotoxins as human carcinogens – the IARC *Monographs* classification. *Micotoxin Research, 33*, 65–73.

Paterson, R. R. M., & Lima, N. (2010). How will climate change affect mycotoxins in food? *Food Research International, 43*, 1902–1914.

Paterson, R. R. M., Lima, N., & Taniwaki, M. H. (2014). Coffee, mycotoxins and climate change. *Food Research International, 61*, 1–15.

Pérez, J., Infante, F., Vega, F. E., Holguín, F., Macías, J., Valle, J., et al. (2003). Mycobiota associated with the coffee berry borer (*Hypothenemus hampei*) in Mexico. *Mycological Research, 107*, 879–887.

Pitt, J. I. (2000). *A laboratory guide to commom Penicillium species.* Melbourne: Food Science Australia.

Pitt, J. I., & Hocknig, A. D. (1997). *Fungi and food spoilage.* London: Blackie Academic and Professional.

Ramirez, L. M., Cendoya, E., Nichea, M. J., Zachetti, V. G. L., & Chulze, S. N. (2018). Impact of toxigenic fungi and mycotoxins in chickpea: A review. *Current Opinion in Food Science, 23*, 32–37.

Rezende, E. F., Borges, J. G., Cirillo, M. A., Prado, G., Paiva, L. P., & Batista, L. R. (2013). Ochratoxigenic fungi associated with green coffee beans (*Coffea arabica* L.) in conventional and organic cultivation in Brazil. *Brazilian Journal of Microbiology, 44*, 377–384.

Rodriguez, D., Cure, J. R., Gutierrez, A. P., & Cotes, J. M. (2017). A coffee agroecosystem model: III. Parasitoides of the coffee berry borer (*Hypothenemus hampei*). *Ecological Modelling, 363*, 96–110.

Rodriguez, D., Cure, J. R., Gutierrez, A. P., Cotes, J. M., & Cantor, F. (2013). A coffee agroecosystem model II: Dynamics of a coffee berry borer. *Ecological Modelling, 248*, 203–214.

Samsom, R. A., Hoekstra, E. S., Frisvad, J. C., & Filtenborg, O. (2000). *Indroduction to food and air borne fungi* (4th ed.). Wageningen: Central Bureau Voor Schimmel Cultures.

Serra, R., Lourenço, A., Alípio, P., & Venâncio, A. (2006). Influence of the region of origin on the mycobiota of grepes with emphasis of *Aspergillus* and *Penicilium* species. *Mycological Research, 110*, 971–978.

Sorrenti, V., Di Giacomo, C., Acquaviva, R., Barbagallo, I., Bognanno, M., & Galvano, F. (2013). Toxicity of ochratoxin A and its modulation by antioxidants: A review. *Toxins, 5*, 1742–1766.

Taniwaki, M. H., Pitt, J. I., Teixeira, A. A., & Iamanaka, B. T. (2003). The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. *International Journal of Food Microbiology, 82*, 173–179.

Taniwaki, M. H., Teixeira, A. A., Teixeira, A. R. R., Copetti, M. V., & Iamanaka, B. T. (2014). Ochratoxigenic fungi and ochratoxin A in defective coffee beans. *Food Research International, 61*, 161–166.

Vega, F. E., Franqui, R. A., & Benavides, P. B. (2002). The presence of the coffee berry borer, *Hipothenemus hampei*, in Puerto Rico: Fact or fiction? *Journal of Insect Science, 2*, 1–3.

Vega, F. E., Infante, E., Castilho, A., & Jaramillo, J. (2009). The coffee berry borer, *Hypothenemus hampei* (ferrari) (Coleoptera: Curculionidae): A short review, with recent findings and future research directions. *Terristrial Arthropod Reviews, 2*, 129–147.

Vega, F. E., Infante, F., & Johnson, A. J. (2015). The genus *Hypothenemus*, with emphasis on *H. hampei*, the coffee barry borer. In F. E. Vega, & R. W. Hoffstetter (Eds.). *Bark beetles: Biology and ecology of native and invasive species* (pp. 427–494). San Diego: Academic Press.

Velmourougane, K., Bhat, R., & Gopinandhan, T. N. (2010). Coffee berry borer (*Hypothenemus hampei*) - a vector for toxigenic molds and ochratoxin A contamination in coffee beans. *Foodborne Pathogens and Disease, 7*, 1279–1284.

Vilela, D. M., Pereira, G. V. M., Silva, C. F., Batista, L. R., & Schwam, R. F. (2010). Molecular ecology and polyphasic characterization of the microbiota associated with semi-dry processed coffee (*Coffea arabica* L.). *Food Microbiology, 27*, 1128–1135.