

Research Paper

Ochratoxigenic fungi associated with green coffee beans (*Coffea arabica* L.) in conventional and organic cultivation in Brazil

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Abstract

The genera *Aspergillus* comprises species that produce mycotoxins such as aflatoxins, ochratoxins and patulin. These are cosmopolitan species, natural contaminants of agricultural products. In coffee grains, the most important *Aspergillus* species in terms of the risk of presenting mycotoxins belong to the genera *Aspergillus* Section *Circumdati* and Section *Nigri*. The purpose of this study was to assess the occurrence of isolated ochratoxigenic fungi of coffee grains from organic and conventional cultivation from the South of Minas Gerais, Brazil, as well as to evaluate which farming system presents higher contamination risk by ochratoxin A (OTA) produced by fungi. Thirty samples of coffee grains (*Coffea arabica* L.) were analysed, being 20 of them of conventional coffee grains and 10 of them organic. The microbiological analysis was done with the Direct Plating Technique in a Dichloran Rose Bengal Chloramphenicol Agar (DRBC) media. The identification was done based on the macro and micro morphological characteristics and on the toxigenic potential with the Plug Agar technique. From the 30 samples analysed, 480 filamentous fungi of the genera *Aspergillus* of the *Circumdati* and *Nigri* Sections were isolated. The ochratoxigenic species identified were: *Aspergillus auricomus*, *A. ochraceus*, *A. ostianus*, *A. niger* and *A. niger* Aggregate. The most frequent species which produces ochratoxin A among the isolated ones was *A. ochraceus*, corresponding to 89.55%. There was no significant difference regarding the presence of ochratoxigenic *A. ochraceus* between the conventional and organic cultivation systems, which suggests that the contamination risk is similar for both cultivation systems.

Key words: *Aspergillus ochraceus*, ochratoxin A, arabica coffee.

Introduction

Like other agricultural products, coffee fruits and beans are subject to contamination by microorganisms during different development stages, from the crop field to storage (Batista *et al.*, 2009). The presence of fungi in the coffee beans does not only affect quality in terms of flavor and aroma of the beverage, but also presents a safety risk

for the final product, due to the production of toxic secondary metabolites, the mycotoxins, which can be harmful to consumers at certain concentrations (Bernnett and Klich, 2003, Vilela *et al.*, 2010).

The contamination by ochratoxigenic fungi and production of ochratoxin A in the beans only occur in the presence of specific conditions like weather, plant susceptibility, environmental factors (such as temperature, damage

caused by insects, pest attacks, contamination by other genera of fungi), chemical composition of beans, product cultivation and later handling, substrate nutrients, genetics of the microorganisms, and deficient storage (Paterson and Lima, 2010).

The difference between the conventional and organic cultivation for coffee production is mainly based on the products used during coffee growth. The organic coffee is produced without the utilization of high solubility pesticides and chemical fertilizers, which are replaced by sub-products originated from recycling plant and animal organic matter, animal dejects, bio-fertilizers, coffee pulp and husks, composting, worm humus, and so on (Theodoro and Guimarães, 2003).

Jestoi *et al.* (2004), analysing the levels of mycotoxins in cereal grains, have not found a significant difference for the concentration of the toxin between the conventional and organic cultivation systems, although the total mean concentration was slightly higher in the organic products. Since the organic products do not receive chemical supplies, the fruits and grains are exposed to fungi contamination, including potentially toxigenic fungi (Jestoi *et al.*, 2004; Juan *et al.*, 2008). The concentrations of ochratoxin A and ochratoxigenic *Aspergillus* species in grapes cultivated in the conventional and organic system were analysed by Ponsone *et al.* (2007). These authors observed that the presence of these fungi is not influenced by the cultivation system, but by the maturation stage of the fruits. In fact, most studies have concluded that more investigations are needed so that the safety of agriculture products can be assessed (Jestoi *et al.*, 2004).

Several researches have been carried out to analyze the presence of ochratoxigenic fungi in coffee (Noonim *et al.*, 2008; Silva *et al.*, 2008; Batista *et al.*, 2009, Vilela *et al.*, 2010). The main ochratoxin A-producing species for beans coffee belong to the genera *Aspergillus* Section *Circumdati* and Section *Nigri* (Batista *et al.*, 2003; Batista *et al.*, 2009, Gil-Serna *et al.*, 2011). The ochratoxin A-producing species of the genera *Aspergillus* are *A. ochraceus* (Taniwaki *et al.*, 2003; Frisvad *et al.*, 2004; Perrone *et al.*, 2007; Batista *et al.*, 2009; Gil-Serna *et al.*, 2011;), *A. niger* (Taniwaki *et al.*, 2003; Samson *et al.*, 2004; Perrone *et al.*, 2007), *A. carbonarius* (Taniwaki *et al.*, 2003; Samson *et*

al., 2004; Perrone *et al.*, 2007), *A. sulphureus* (Batista *et al.*, 2009), *A. sclerotiorum* (Batista *et al.*, 2009), *A. westerdijkiae* (Frisvad *et al.*, 2004, Gil-Serna *et al.*, 2011). *A. ochraceus* is commonly found in coffee and is an important ochratoxin A producer (Suarez-Quiroz *et al.*, 2004b; Batista *et al.*, 2009; Vilela *et al.*, 2011). *A. carbonarius* is common in grape and in robusta coffee. Its occurrence in coffee beans is not frequent in Brazil, unlike in Thailand, where this species is commonly isolated (Taniwaki *et al.*, 2003; Noonim *et al.*, 2008). Ochratoxin A is possibly carcinogenic to humans, as well as nephrotoxic, immunotoxic and teratogenic (IARC,1993).

The purpose of this study was to identify toxigenic fungi in organically and conventionally cultivated coffee beans in the South of Minas Gerais, Brazil, as well as to evaluate which farming system presents higher contamination risk by ochratoxin A produced by fungi.

Materials and Methods

Sampling

A total of 30 green coffee beans samples (*Coffea arabica* L.), harvest of the year 2009/2010, divided into 10-bean organic coffee sample and a 20-bean conventional coffee samples (Table 1). The samples were collected from the southern city of Minas Gerais - Brazil, (Poço Fundo: latitude -21°46'51"; longitude -45°57'54"; altitude 836 m); (Santo Antônio do Amparo: latitude -20°56'47"; longitude -44°55'08"; altitude 1013 m); (Lavras: latitude -21°14'43"; longitude -44°59'59"; altitude 919 m). These samples were analysed in the Laboratory of Food Microbiology - Mycology and Mycotoxins - Department of Food Science, Federal University of Lavras (Lavras, MG, Brazil).

Mycological analysis

For isolation of fungi associated with green coffee beans, the direct plating technique was applied in DRBC medium - Dicloran Rose de Bengal Chloramphenicol (glucose 10.0 g; peptone 5.0 g; KH₂PO₄ 1.0 g; MgSO₄.7H₂O 0.5 g; Agar 15.0 g; bengal rose 25.0 mg; dicloran 2.0 mg; chloramphenicol 100.0 mg; distilled water 1.0 L). A total of 100 coffee beans were plating directly without surface disinfection and 100 beans were plated with surface disinfection

Table 1 - Studied coffee samples.

| Samples | Cultivation system | Harvest | Locations |
|---------|--------------------|--------------------|-------------------------|
| 5 | Organic | Harvest onto cloth | Poço Fundo |
| 3 | Organic | Swept from ground | Poço Fundo |
| 1 | Organic | Harvest onto cloth | Santo Antônio do Amparo |
| 1 | Organic | Swept from ground | Santo Antônio do Amparo |
| 4 | Conventional | Swept from ground | Poço Fundo |
| 4 | Conventional | Harvest onto cloth | Poço Fundo |
| 12 | Conventional | Harvest onto cloth | Lavras |

tion with 70% alcohol and 1% sodium hypochlorite according to Samson *et al.* (2000). The plates were incubated for 5-7 days at 25 °C. The overall percent contamination was expressed as the percentage of particles yielding visible growth of fungi.

Isolation and identification of fungi

The isolated fungi were purified and identified according to Klich (2002), Frisvad *et al.* (2004) and Samson *et al.* (2004).

The isolates were incubated in CYA medium - Czapek yeast Agar (K₂HPO₄ 1.0 g; concentrate Czapek NaNO₃ 30.0 g; KCl 5.0 g; MgSO₄.7H₂O 5.0 g; FeSO₄.7H₂O 0.1 g; ZnSO₄.7H₂O 0.1 g CuSO₄.5H₂O 0.05 g; distilled water 100mL) in MEA - Malt Extract Agar (malt extract 20.0 g; peptone 1.0 g; glucose 30.0 g; Agar 20 g; distilled water 1 L) at 25 °C and CYA at 25 °C and 37 °C. After incubation for 7 days, the microscopic and macroscopic characteristics described by Klich (2002b) were observed.

Determination of OTA-producing fungi by the plug agar method

The isolates tested were inoculated in YES medium - Yeast Extract Sucrose Agar (yeast extract 20.0 g; sucrose 150.0 g; Agar 20.0 g; MgSO₄.7H₂O 0.5 g; distilled water 1 L) with metallic solution 1 mL (ZnSO₄.7H₂O 1%; CuSO₄.5H₂O 0.5%) for 7 days at 25 °C, according to Filtenborg and Frisvad (1980). The following was used: ochratoxin A standard (Sigma-Aldrich), thin layer chromatography plates (Merk-Silica Gel 60, 20x20) as mobile phase; toluene, ethyl acetate and formic acid 90% (60:30:10 v/v/v). After elution, the plates were air dried. Mycotoxin production was confirmed by green fluorescence in ultraviolet light with $\lambda = 366$ nm in chromatovisor CAMAG (UF-BETRACHTER). The isolates considered as OTA producers presented an RF (refection factor) and a fluorescence spot similar to that of OTA standard.

Statistical analysis

To correlate the levels of OTA contamination with the different coffee samples, simple correspondence analysis was used, as described by Greenacre (1993). This technique consists of applying the main components such as the contingency table, in this case, a table displaying the ochratoxigenic fungi frequency in one line and the coffee sample in columns. The proportion of the coffee sample totals corresponds to the profile of the variables displayed in the column. Analogously, the profile for the variables lines is produced. For the comparison between the species *Aspergillus* Section *Circumdati* and Section *Nigri* in the two different cultivation systems, a simple descriptive analysis of media was carried out.

Results and Discussion

From the samples analysed, 100% were contaminated by filamentous fungi and approximately 50% contamination with yeasts. The main genera found in this study (Table 2) have also been reported in coffee beans in other researches carried out in Brazil and in other countries (Joosten *et al.*, 2001; Pardo *et al.*, 2004; Bokhari, 2007; Leong *et al.*, 2007; Silva *et al.*, 2008; Batista *et al.*, 2009; Vilela *et al.*, 2010).

Analysing the samples plated without disinfection, the genera *Aspergillus* was found in about 62.3% of the samples. The *Aspergillus* genera was found in an incidence of 93% of coffee beans (Pardo *et al.*, 2004), and was predominant in coffee beans in the Saudi Arabia (Bokhari, 2007).

Aspergillus Section *Circumdati* and *Nigri* corresponded to 56.54% of the total of contaminant fungi of coffee beans. This result was similar to the of Batista *et al.* (2003), who observed 54.78% for the same sections of the genera in Brazilian coffee beans. These two sections was responsible for 75% of the Vietnamese green coffee beans contamination (Leong *et al.*, 2007). In this work, fungi of the genera *Aspergillus* Section *Circumdati* corresponded to 27.29% and Section *Nigri* to 29.25% of the contaminant fungi of the beans. In a study carried out by Batista *et al.*

Table 2 - Percentage of fungi found in conventional and organic cultivated system of coffee beans after plating with or without surface disinfection.

| Fungi | Organic system with disinfection | Organic system without disinfection | Conventional system with disinfection | Conventional system without disinfection |
|------------------------|----------------------------------|-------------------------------------|---------------------------------------|--|
| <i>Aspergillus sp</i> | 3.6 | 49.8 | 7.6 | 62.3 |
| <i>Cladosporium sp</i> | 0.9 | 0.08 | 6.9 | 5.0 |
| <i>Eurotium sp</i> | 0.24 | 0 | 0.4 | 1.8 |
| <i>Fusarium sp</i> | 53.1 | 33.3 | 28.5 | 24.1 |
| <i>Mucor sp</i> | 0.5 | 0 | 0 | 0.9 |
| <i>Penicillium sp</i> | 5.3 | 11.8 | 3.9 | 4.9 |
| <i>Rhizopus sp</i> | 0 | 0.3 | 0.7 | 0.2 |
| <i>Trichoderma sp</i> | 0 | 0 | 0 | 0.16 |

(2009), 41% of the isolated identified belonged to Section *Circumdati* and 25% to Section *Nigri*. Pardo *et al.* (2004) found 67.40% of beans, from different origins, infected with *Aspergillus* Section *Nigri*. In the present study, we observed a uniform distribution of filamentous fungi of the genera *Aspergillus* Section *Circumdati* and Section *Nigri* among organic and conventional coffee samples (Figures 1 and 2; Tables 3 and 4). The uniform distribution of the filamentous fungi in coffee samples from different regions have also reported for Batista *et al.* (2003). The presence of these fungi in coffee beans in Brazil is due to the geographical distribution of this genus, including latitudes 26° - 35° Klich (2002a), as well as environmental conditions and type of coffee processing.

Figures 1 and 2 and Tables 3 and 4 shows the results of contaminations by genera *Aspergillus* Section *Circumdati* and Section *Nigri* in coffee bean samples regarding the cultivation system (conventional and organic). It was possible to observe that there were no significant differences. Regarding the harvesting type, the harvest on

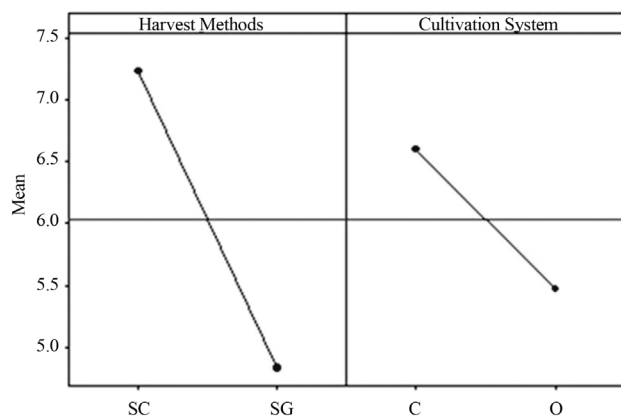


Figure 1 - Graphic of the main effects of the harvest method and type of coffee factors averages for the fungi of genus *Aspergillus* Section *Nigri* (data transformed by the square root). SC - harvest onto cloth SG- swept from ground. C - conventional. O - organic.

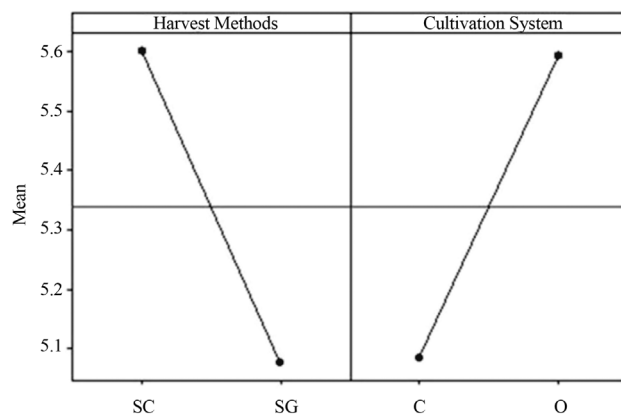


Figure 2 - Graphic of the main effects of type of coffee and harvest method factors averages for fungi of the genus *Aspergillus* Section *Circumdati* (data transformed by the square root). SC - Harvest onto cloth SG- swept from ground. C - conventional. O - organic.

cloth presented a significant difference for fungi of the genera *Aspergillus* Section *Nigri*. The harvesting system directly affects the incidence of fungi in fruit and coffee beans (Batista *et al.*, 2009). The coffee berries harvest onto a cloth are fruits that were in the tree, which can be ripe, unripe or dried on the tree (Batista *et al.*, 2009); these fruits are exposed to more insulation. *Aspergillus* Section *Nigri* are more resistant to UV light due to the spore colors, which confers a larger capacity of competition for the substrate, and can justify the presence of fungi of the genera *Aspergillus* Section *Nigri* onto the cloth (Abarca *et al.*, 2003; Romero *et al.*, 2005; Duarte *et al.*, 2010). The cloth used for harvest could also be contaminated with fungal spores of the genus *Aspergillus* Section *Nigri*, and favors the contamination of coffee fruits and beans.

Of all the samples analysed, 480 isolates of the genus *Aspergillus* were obtained and identified, based on the morphological characteristics. Of these isolates, 277 were of the genera *Aspergillus* Section *Nigri* and 203 Section *Circumdati*. Of the Section *Circumdati*, the most common species, both for conventional and organic cultivation coffee was *A. ochraceus* (n = 169) representing 35.20% of the total of isolates, a similar proportion as the ones reported in other studies with coffee fruits and beans (Batista *et al.*, 2003; Suarez-Quiroz *et al.*, 2004b; Silva *et al.*, 2008). Due to the special relevance of this species for coffee quality and safety, in function of its large distribution and toxigenic

Table 3 - Variance analysis for the calculation of filamentous fungi of the Genus *Aspergillus* Section *Nigri*.

| VS ^a | FD ^b | SS ^c | MS ^d | F ^e | p-value ^f |
|-----------------|-----------------|-----------------|-----------------|----------------|----------------------|
| Harvest | 1 | 22.342 | 22.342 | 4.69 | 0.049 |
| Coffee | 1 | 4.935 | 4.935 | 1.04 | 0.329 |
| Coffee harvest* | 1 | 0.009 | 0.009 | 0.00 | 0.967 |
| Error | 12 | 57.136 | 4.761 | | |
| Total | 15 | 82.191 | | | |

OBS: Data transformed by the square root. ^aVS - Variation source. ^bFD - Freedom degree. ^cSS - Sum of squares. ^dMS - Mean square. ^eF - Test of F. ^fp-value - level of significance.

Table 4 - Variance analysis for the calculation of filamentous fungi of the genus *Aspergillus* Section *Circumdati*.

| VS ^a | FD ^b | SS ^c | MS ^d | F ^e | p-value ^f |
|-----------------|-----------------|-----------------|-----------------|----------------|----------------------|
| Harvest | 1 | 1.071 | 1.071 | 0.19 | 0.672 |
| Coffee | 1 | 1.016 | 1.016 | 0.18 | 0.680 |
| Coffee harvest* | 1 | 0.007 | 0.007 | 0.00 | 0.973 |
| Error | 12 | 68.377 | 68.377 | | |
| Total | 15 | 70.811 | 70.811 | | |

OBS: Data transformed by the square root. ^aVS - Variation source. ^bFD - Freedom degree. ^cSS - Sum of the squares. ^dMS - Mean square. ^eF - Test of F. ^fp-value - Significance level.

potential, *A. ochraceus* has been the target of many researches with coffee beans and fruits (Silva *et al.*, 2000; Batista *et al.*, 2003; Taniwaki *et al.*, 2003; Suarez-Quiroz *et al.*, 2004a; Vilela *et al.*, 2010).

Considering all the samples analysed, the most common species of the genus *Aspergillus* Section *Nigri* in the conventional cultivation system was *A. tubingensis* with 42.70% of the isolates. In the organic cultivation system, the main species was *A. foetidus* with 35.80%. Noonim *et al.* (2008) isolated, identified and evaluated the ochratoxigenic potential of *Aspergillus* species in coffee beans in two regions of Thailand, and they also found the non-ochratoxigenic species *A. foetidus* and *A. tubingensis* in the beans analysed. However, similar works with green coffee beans, only in the conventional cultivation system, were carried out in Brazil (Taniwaki *et al.*, 2003) and in other countries, like Saudi Arabia (Bokhari, 2007), and Vietnam (Ilic *et al.*, 2007; Leong *et al.*, 2007). In all these studies *A. niger* predominated. *A. niger* is largely distributed in the environment (Urbano *et al.*, 2001) and is isolated not only from coffee beans, but also from other foods like grape and derivatives (Magnoli *et al.*, 2003; Perrone *et al.*, 2007). The concentration of ochratoxin A and ochratoxigenic *Aspergillus* species in grapes cultivated in conventional and organic system were analysed by Ponsone *et al.* (2007). These authors observed that the presence of these fungi is not influenced by the cultivation system, but by the maturation stage of the fruits.

The other species found in this work have also been identified in other coffee researches, such as *A. auricomus* (19 isolates) and *A. sulphureus* (8 isolated), which have also been reported by Batista *et al.* (2009), *A. niger* and *A. niger* Aggregate reported by Silva *et al.* (2008) and Batista *et al.* (2009).

All the 480 isolated were tested for the capacity of producing ochratoxin A with the Plug Agar technique. Considering the 30 coffee bean samples, the species *A. ochraceus* was the main producer of this mycotoxin. Similar results were found by Batista *et al.* (2003).

Analysing the eight samples of coffee from the conventional cultivation and the eight samples of coffee from the organic cultivation, provenient from the Poço Fundo, it was possible to observe that of the 107 isolates of the genus *Aspergillus* Section *Circumdati*, 62.62% (n = 67) produced ochratoxin A (Table 5).

The values obtained in this study were close to the ones obtained by Batista *et al.* (2003), who identified 74.6% of the Section *Circumdati* species as ochratoxin A-producing, in conventional coffee beans. Of the ochratoxin A-producing fungi, the major producer species was *A. ochraceus* 89.55% (Table 5). In the species of *A. auricomus* and *A. ostianus* we observed isolates capable of producing mycotoxin (Table 5) these species as potentially ochratoxigenic also were reported by Batista *et al.* (2003, 2009).

Table 5 - Identification of potentially toxigenic species and isolates. Samples from the Poço Fundo.

| Genus <i>Aspergillus</i> (number of same) | N. of isolates identified | N. of potentially toxigenic ^a isolates |
|--|------------------------------|--|
| Organic coffee (8) | | |
| Section <i>Circumdati</i> | | |
| <i>A. auricomus</i> | 7 | 4 |
| <i>A. ochraceus</i> | 49 | 32 |
| <i>A. ostianus</i> | 1 | 1 |
| <i>A. sulphureus</i> | 2 | ND |
| Section <i>Nigri</i> | | |
| <i>A. foetidus</i> | 22 | ND |
| <i>A. niger</i> | 7 | ND |
| <i>A. niger</i> Aggregate | 10 | 2 |
| <i>A. tubingensis</i> | 12 | ND |
| Conventional coffee (8) | | |
| Section <i>Circumdati</i> | | |
| <i>A. auricomus</i> | ND | ND |
| <i>A. ochraceus</i> | 45 | 28 |
| <i>A. ostianus</i> | 2 | 2 |
| <i>A. sulphureus</i> | 1 | ND |
| Section <i>Nigri</i> | | |
| <i>A. foetidus</i> | 20 | ND |
| <i>A. niger</i> | 33 | 4 |
| <i>A. niger</i> Aggregate | 15 | ND |
| <i>A. tubingensis</i> | 13 | ND |

^aND - not detected by the Plug Agar method.

Although the correspondence analysis does not establish the statistical significance of the associations and does not evaluate the independent effect of each characteristic, this analysis combines the advantages of non-linear and multi-dimensional methods, which allows the description of the characteristics that occur simultaneously in the coffee culture, and the identification of the different profiles of the ochratoxigenic species found. With the map (Figure 3) generated from the correspondence analysis, it was possible to observe in the coffee samples from the Poço Fundo, that *A. ochraceus* was the toxin-producing species found more often in both cultivation systems. The similarity of frequency of this species was notorious in both cultivation systems evaluated, as well as the harvest methods (harvest onto cloth and swept from the ground). Regarding the other species (*A. niger* and *A. niger* Aggregate), we verified that no cultivation system or type of harvesting was characterized according to the presence of the isolate.

Regarding the ochratoxin A-producing species reported by other authors, Batista *et al.* (2003) found *A. ochraceus* and *A. sulphureus* as the main species, while Pardo *et al.* (2004) found 23.1% of ochratoxin A-producing *Aspergillus ochraceus*. Urbano *et al.* (2001) obtained the

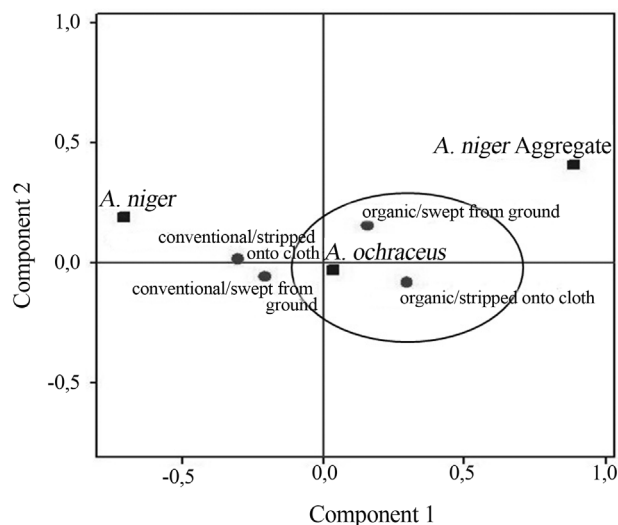


Figure 3 - Perception map of the associations between filamentous fungi and the harvest method of the sample coffee from Poço Fundo.

same results as the present work, with 88.1% of *A. ochraceus* producers of the toxin. Batista *et al.* (2009) analysed ochratoxigenic fungi in coffee beans conventionally processed by dry and wet methods; of fungi of the genera *Aspergillus* Section *Circumdati*, 92.69% were identified as *A. ochraceus* and of these, 95% produced ochratoxin A. Taniwaki *et al.* (2003) found 75% of these species with capacity of producing this toxin.

Besides these species reported as ochratoxin A-producing, recent studies also show that other species may be potentially producers of mycotoxin in coffee, like *A. elegans*, *A. steynii* and *A. westerdijkiae* (Batista *et al.*, 2009; Gil-Serna *et al.*, 2011; Prado *et al.*, 2006). Gil-Serna *et al.* (2011) stated that the species of *A. steynii* and *A. westerdijkiae* can represent a higher risk of producing ochratoxin A, due to their capacity of producing a great amount of it and the diversity of foods that these species may contaminate. However, these authors do not ignore the possibility that *A. elegans* and *A. ochraceus* produce the same toxin.

Comparing the results of the coffee beans samples from the Poço Fundo, of the 132 isolates of the genera *Aspergillus* Section *Nigri*, 4.54% were capable of producing ochratoxin A (Table 5). Pardo *et al.* (2004) found 7.3% of isolates of the Section *Nigri* as ochratoxin A producers, while Urbano *et al.* (2001) found 11.5% in a similar study. In contrast with these results, Batista *et al.* (2003; 2009) have not found any ochratoxigenic isolates of the Section *Nigri*.

Of the ochratoxin A-producing isolates belonging to Section *Nigri* analysed in this work (Table 5), 10% were *A. niger* (n = 4) and 8% *A. niger* Aggregate (n = 2). This result agrees with the one reported by Noonim *et al.* (2008), who found only 13% of the isolates of *A. niger* as ochratoxin A producers.

This research did not find *A. carbonarius*, which is described by other researchers as an important ochratoxin A-producing species. *A. carbonarius* is the species of the genus *Aspergillus* Section *Nigri* with the highest ochratoxin A production potential (Prado *et al.*, 2004). However, it is not a common species of arabica coffee beans as *A. niger* (Noonim *et al.*, 2008; Palacios-Cabeira *et al.*, 2005). This statement was reinforced by the studies of Taniwaki *et al.* (2003), who found 62.95% of *A. niger* and 6.19% of *A. carbonarius* in coffee beans and identified as ochratoxigenic only 3% of the isolates of *A. niger* while in the isolates of *A. carbonarius* this proportion was of 77%. That the contamination with *A. carbonarius* is slightly higher in robusta coffee than in arabica coffee (Pardo *et al.*, 2004; Noonim *et al.*, 2008).

The eventual divergence about ochratoxin A production by the genus *Aspergillus* suggests that the synthesis of ochratoxin A is dependent on the interaction of several environmental factors (Mühlencoert *et al.*, 2004), more than the simple fact of growth, that is, the inability to produce ochratoxin A in determined conditions does not justify any conclusion about the general ability to produce the mycotoxin (Bakutis *et al.*, 2006). The production of secondary metabolites such as mycotoxins are not essential for the microorganism. The environmental factors will regulate the genes and enzymes involved in the production of ochratoxin A (Mühlencoert *et al.*, 2004).

Based on the results it was possible to observe that the organic and conventional coffee cultivation systems did not differ in terms of the contamination of beans by species of the genera *Aspergillus* Section *Nigri* and Section *Circumdati*. Likewise, there was no difference in terms of the occurrence of *A. ochraceus*, which was the main ochratoxigenic species found, which allows us to affirm that there is no influence of the cultivation system on the contamination by *Aspergillus* species, neither on the occurrence of ochratoxigenic species that belong to this genus. Both cultivation systems present the same risks of contamination. Not all the isolates of the *A. ochraceus* species were ochratoxin A producers; however, just the presence of this ochratoxigenic species does not imply that the production of the mycotoxin will necessarily occur.

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