BIODIVERSITY OF FILAMENTOUS FUNGI IN COFFEE BEANS GROWN IN AN ORGANIC AND CONVENTIONAL SYSTEM

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ABSTRACT: The fruits of organically and conventionally produced coffee are subject to contamination from several species of fungi, and that may be related to poor beverage quality and mycotoxin production. The aim of this study was to identify the biodiversity of isolated filamentous fungi in the coffee beans produced on organic and conventional farms within the same area. Two hundred and twelve isolates belonging to eleven different genders were identified from the fifteen samples analyzed. The main gender found was Aspergillus, with isolation of fungi from the Sections Circumdati, Nigri, Flavi and Versicolores. The samples that obtained the greatest index of contamination were those that didn't pass through the disinfection process with 1% sodium hypochlorite. The samples of coffee beans from organic cultivation exhibited the greatest degree of richness and diversity within a single location with very similar climatic conditions. Thus, organic coffee production requires greater care due to the increased incidence of filamentous fungi.

Index terms: Aspergillus, farming system, disinfection.

BIODIVERSIDADE DE FUNGOS FILAMENTOSOS EM GRÃOS DE CAFÉ CULTIVADOS EM SISTEMA ORGÂNICO E CONVENCIONAL

RESUMO: *Os frutos de café produzidos de forma orgânica ou convencional estão sujeitos à contaminação de diversas espécies de fungos que podem estar relacionados à má qualidade da bebida e à produção de micotoxinas. Realizou-se este estudo para identificar a biodiversidade de fungos filamentosos isolados nos grãos de café produzidos em fazendas orgânicas e convencionais de uma mesma localidade. Das 15 amostras analisadas, foram identificados 212 isolados, pertencentes a 11 diferentes gêneros. O principal gênero encontrado foi o Aspergillus, sendo isolados fungos das Seções Circumdati, Nigri, Flavi e Versicolores. As amostras que obtiveram o maior índice de contaminação foram as que não passaram pelo processo de desinfecção com hipoclorito de sódio a 1%. As amostras de grãos de café de cultivo orgânico apresentaram o maior índice de riqueza e diversidade dentro de uma mesma localidade, com condições climáticas muito próximas. Sendo assim, a produção de café orgânico necessita de maiores cuidados devido ao aumento na incidência de fungos filamentosos.*

Termos para indexação: *Aspergillus, sistema de cultivo, desinfecção.*

1 INTRODUCTION

Brazil is the world's biggest coffee producer and exporter (Coffea arabica L.). Coffee production in 2009 reached 28,9 million sacs and the 2010 production was estimated 47,04 million processed coffee sacs. Such result represents an increase of19,2% which is justified by the year of positive biannuality, along with favorable weather conditions (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2009).

Coffee fruits and beans are subject to filamentous fungi contamination, which can be related to beverage quality and the risk of mycotoxin incidence. These fungi contaminate coffee fruits and beans during all development and processing stages (BATISTA et al., 2003, 2009). Fungi diversity in coffee beans depends on many factors such as coffee variety, geographic region, weather and processing method (PERRONE et al., 2007).

The diversity of filamentous fungi found in coffee beans produced in conventional crop system has been reported in diverse studies (BATISTA et al., 2003, 2009; SILVA; BATISTA; SCWUAN, 2008; SILVA et al., 2000).

Due to the search for healty and chemical contaminant free foods, organic agriculture became an expanding market. This crop system maintains biodiversity, promoting biological benefits that compensate conventional practices (LETOURNEAU; BOTHWELL, 2008).

Recently there has been suggested that organic food would be more willing to mycotoxin contamination than conventional food, since

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they are not treated the same way by antifungal agents (KHOUBA, 2003; LAIRON, 2010). However, there are no evidence that organic food is more willing to mycotoxin contamination than conventional (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAO, 2000).

Organic coffee production in Brazil, generally, is performed by small farm owners, who gather in cooperatives, to commercialize the products. Even though there is a growing demand for the organic cultivation system, still there are not enough research information about filamentous fungi in organic coffee beans (BETTIOL et al., 2002; MIRANDA et al., 2010). For such reason, the present study was performed to analise the biodiversity of filamentous fungi in processed coffee beans, produced in traditional and organic systems in the municipality of Poço Funredo, MG.

2 MATERIALS AND METHODS

2.1 Material

Fifteen samples of processed coffee beansQuinze amostras de grãos de café beneficiados (aproximately 500g) were analised in this experiment, being 8 of beans grown in organic system and 7 of beans grown in conventional system. The examined samples were obtained from Poço Fundo, located 21° 46' South latitude and 45° 57' West longitude. The area is 475 Km, altitude tropical weather, average yearly temperature of 20ºC, average yearly rainfall of 1592,7 mm an maximum altitude of 1435m (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA - IBGE, 2011).

2.2 Isolation of filamentous fungi

The isolation of filamentous fungi associated to processed coffee beans was performed according to the direct plating technique in culture mean Dicloram Rosa de Bengala Chloramphenicol (DRBC- 10g glucose, 5g bacteriological peptone; 1g KH2PO4; 0,5g MgSO4 7H2O ; 15g agar ; 1 L distilled water; 25mg rosa de bengala, 2mg dicloran; 100g chloramphenicol), according to Sansom et al. (2004). From each sample were collected 200 random beans, being 100 beans plated with superficial disinfection and 100 beans without superficial disinfection. In the disinfection process, the samples were submersed in a 70% alcohol solution, followed by a 1% Sodium hypochlorite solution, during 30 seconds and then washed with distilled water.

The isolated fungi were purified in Malt Extract at 2% (MA- 20g Malt Extract, 20g Agar and 1L distilled water) and kept at 25°C during seven days. Later on, purification and identification of filamentous fungi were performed in the Food Microbiology Laboratory of the Phytopathology Department of the Lavras Federal University (Laboratório de Microbiologia de Alimentos do Departamento de Ciência dos Alimentos e no Laboratório de Micologia do Departamento de Fitopatologia da Universidade Federal de Lavras). The beans contamination was expressed in percentage of contaminated beans.

2.3 Identification of isolated fungi

From pure cultures, the Aspergillus and Penicillium species were incubated in CYA (Czapek Yeast Agar – 1g K2HPO4 ; 10 mL Czapek concentrate; 5g yeast extract; 15g agar; 1L distilled water) and MEA (20g malt extract; 1g peptone; 30g glucose; 20g agar; 1L distilled water) at 25°C and at 37°C and, after 7 days of incubation, the microscopic and macroscopic characteristics were observed. the Aspergillus species were identified according to Klich (2002) and the Penicillium according to Pitt (2000). The species of the genus Fusarium were identified according to Nelson, Toussoun and Marasas (1983). The fungi were inoculated in plated containing SNA (1g KH2PO4; 1g KNO3; 1g MgSO4 7H2O; 0,5g KCl; 0,2g glucose; 0,2g saccharose; 20g agar and 1L distilled water) and MA, to analyse the microscopic characteristics and BDA to observe the colonies color. The plates were kept in BOD with photoperiod, at 21°C, for 10 days. The isolated from the genera Mucor, Rhizopus, Cladosporium and Trichoderma were grown in MEA (malt extract) at 25°C, for 7 days and the identification followed the protocol of Samson et al. (2004). The fungi from the genera Colleototrichum, Glomerella, Bipolaris and Epicoccum were grown in MA (Malt extract Agar) at 25°C, for 7 days and the identification of these fungi was performed according to Ellis (1971). The species belonging to the genera Phoma and Alternaria were inoculated in AO (Agar Oat) - (30g oat, 1L distilled water, 15g agar) for 7 days, at temperature of 25°C and identified according to Ellis (1971).

2.4 Statistic analyses

The statistic methodology used in this work consisted in the construction of a relying interval of 95% for the standard error, aiming to inferr the dispersion of filamentous fungi occurrence in organic and conventional coffee samples.

2.5 Calculation of biodiversity indexes

To assess the diversity of fungal colonies in organic and conventional coffee beans, the indexes described by Magurran (1988) were used: the Margalef (Rm) richness index, being the formula Rm = (S - 1) / (ln (N)), where S= number of species and N = number of identified individuals and the Shannon-Weiner (H') diversity index, where H' = - Σ (pi. ln pi), in which pi is the proportion of individuals from each species i in relation to the total number of individuals (ODUM, 1983).

3 RESULTS AND DISCUSSION

3.1 Isolation of fungal microbiota

From the 15 samples of coffee beans analysed, 212 fungal isolates were obtained, being 23 species belonging to 11 genera. The main species found in coffee samples from organic and conventional crops can be observed in Table 1.

The genera Aspergillus, Penicillium and Fusarium stood out. Such results are close to the ones obtained in filamentous fungi biodiversity studies in coffee beans (BATISTA; CHALFOUN, 2007; BATISTA et al., 2003; SILVA; BATISTA; SCWUAN, 2008; SILVA et al., 2000; VISOTTO et al., 2001).

Besides those genera, Cladosporium, Mucor, Rhizopus, Trichoderma, Colleototrichum, Gliocladium, Epicoccum and Alternaria were also found in coffee samples. All of the genera in this study have been identified in conventional coffee samples in Brazil (BATISTA et al., 2003; PEREIRA, 2002; TANIWAKI et al., 2003). However, there are no records about the incidence of filamentous fungi in organic coffee beans. Therefore, the fungi Aspergillus flavus, A. foetidus, A. ochraceus, A. oryzae, A. sulphureus, A. versicolor, Cladosporium cladosporioides, Colleototrichum gloesporioides, Fusarium oxysporum, F. semitectum, F. solani, Gliocladium sp., Mucor hiemalis, Penicillium brevicompactum, P. hirsutum, P. solitum and Trichoderma harzianum are quoted for the first time in organic coffee in the country.

Such results demonstrate that, in coffee beans grown under organic and conventional systems, the mais identified genera were Aspergillus, Fusarium and Penicillium. Besides the filamentous fungi, conventional coffee samples showed a high level of yeast infection. Similarly, Urbano et al. (2001) found 100% yeast contamination and fungi in coffee samples, analysed in different maturation stages and processing.

The coffee samples that were disinfected with Sodium hypochlorite at 1% had a 22% reduction of contamination by filamentous fungi. This occurred because the disinfection of the samples eliminated most of the fungi present outside the beans. Noonim et al. (2008) demonstrated that, after disinfection method, the coffee beans contamination reduced from 98% to 60%.

The coffee beans that did not undertake disinfection process showed higher contamination with filamentous fungi and yeast. The genus Aspergillus was responsible for the contamination of most of the coffee beans samples grown under organic and conventional systems. Therefore, Aspergillus was the dominant genus, with approximately 34,43% of the beans contaminated by species of the section Circumdati, Nigri, Flavi and Versicolor (Table 1).

The isolates from the genus Aspergillus contaminated 44,32% of the organic coffee bean samples that did not undertake the disinfection process with Sodium Hypochlorite at 1%. This indicates that the species belonging to these genera contaminate mostly the exterior of the coffee beans. The presence of fungi from the genus Aspergillus in coffee is concerning, since these organisms have the capacity to produce many toxic compounds, named mycotoxins. The species from the genus Aspergillus belonging to the section Circumdati and Nigri are the main producers of ochratoxin A in coffee samples (BATISTA et al., 2003; CLOUVEL et al., 2008; DUARTE et al., 2010; FRISVAD et al., 2004; FRISVAD; SAMSON, 2000; PERRONE et al., 2007; URBANO et al., 2001). In this study, the Aspergillus species belonging to the sections Nigri (A. foetidus, A. niger, A. tubingensis) and Circumdati (A. ochraceus, A. sulphureus) were found abundantly.

The presence of filamentous fungi in organic and conventional coffee beans is harmful, since the genera Aspergillus, Penicillium and Fusarium are the ones that are more frequently associated with the production of mycotoxins in agricultural products. However, the presence of these fungi does not indicate necessarily the presence of mycotoxins (SIDHU, 2002; YIANNIKOURIS; JOUANY, 2002).

Many factors involved in organic and conventional coffee culture may favor or not the development of toxigenic species. The microbial biodiversity is one of them, either being able to prevent the production of mycotoxins, degrading the toxin in a natural way, or even favoring the formation of a competitive environment.

As for the genus Fusarium, it was observed that these isolates contaminated mostly the samples that were not disinfected with Sodium hypochlorite at 1%. However, they were also found abundantly in coffee beans were superficial disinfection was performed. Analyzing the fungi associated to different cultivars of Coffea arabica L. Pasin et al. (2009) the high incidence of fungi from the genus Fusarium in different coffee cultivars was noticed.

The samples contamination with the Penicillium isolates was also significantly larger in organic coffee beans that did not undertake the superficial disinfection process, being responsible for 12,2% contamination. In conventional coffee, the genus Penicillium was rtesponsible for only 1,32% contamination, mostly of the disinfected analyses. According to Chalfoun et al. (2003), the species P. variabile Sopp, P. citrinum Thom and P. minioluteum Dierckx were the species of Penicillium that were mostly found in organic coffee beans. The presence of this genus in coffee can be considered positive, since they are not mycotoxin producers and can also be used as phosphate solubilizers.

3.2 Biodiversity of fungi in conventional and organic coffee

In Table 1 there are the main species of isolated fungi in organic and conventional coffee.

According to Table 1, the larger number of species found in coffee beans belonged to the genus Aspergillus. The genus Aspergillus from section Circumdati was the dominant group, with 41,09% contamination (A. ochraceus, A. sulphureus), followed bu section Nigri with 27,39% (A. niger, A. foetidus, A. tubingensis), 27,39% from the section Flavi (A. flavus and A. oryzae) and 4,1% from the section Versicolor (A. versicolor). Ferreira et al. (2011) analized fungi associated to coffee beans in Southwestern Bahia and also reported that species from the genus Aspergillus were the dominant in coffee. According to Klich (2002), species from the genus Aspergillus showed wide distribution, being frequently found in hot regions and its distribution is related to the weather, vegetation and soil. Such species has also been repeatedly associated to coffee beans (CHALFOUN; BATISTA, 2003; PIMENTA; VILELA, 2003).

The presence of Penicillium species in processed coffee beans was also reported by Batista and Chalfoun (2007), who identified P. brevicompactum Dierckx, P. citrinum Thom, P. commune Thom, P. minioluteum Dierckx, P. variabile Sopp, P. expansum Link and P. corylophilum Dierckx, associated to coffee beans collected in 11 municipalities from the Southern region of Minas Gerais.

According to Chalfoun and Batista (2003), the Aspergillus and Penicillium species are of cosmopolitan occurrence and are among the most abundant microorganisms, being usually associated to stored or damaged beans.

It was noticed, by Margalef index calculation and Shannon index, that the organic coffee samples have larger species and biodiversity richness. The values referring to the indexes can be observed at Table 2.

Therefore, organic coffee samples have larger species and biodiversity richness when compared to conventional coffee. So far, there are no studies about filamentous fungi diversity in coffee beans on both growing systems. However, these results concur with the studies performed by Oehl et al. (2004) who observed larger diversity of mycorrhyzal fungi in conventional system.

A larger fungal diversity in organic system is fundamental for the agroecosystem, since it maintains the biologic balance and enables less disease and pest problems in plantations (HYDE, 2001). The adopted crop system will influence the coffee quality. In the last years, several works were developed under conventional and organic systems and the results have shown increase in organic matter, activity and microbial biomass in organically managed soils (EDMEADES, 2003; GLOVER; REGANOLD; ANDREWS, 2000; MELERO et al., 2005; TU; RISTAINO; HU, 2006). Thus, the organic growing system stimulates biodiversity and biologic activity on the ground, keeping the ecosystem balanced to give to the plant conditions for its development.

The reduction of soil biodiversity is considered negative, since the alterations in biologic diversity can reduce the sources of food, fuels, medicinal or genetic resources (BRUSSAARD et al., 2010).

TABLE 1- Number of fungal isolates present in coffee beans organic and conventional in the fractions: 1- cloth and 2- sweeping (+ D: With disinfection, - D: Without disinfection).

SPECIES	CONVENTIONAL				ORGANIC			
	[1] + D	[1]- D	[2]+ D	[2]- D	[1] + D	[1]- D	[2]+ D	[2]- D
Alternaria								
A. alternata Keissl.	6	-	-	-	-	-	-	-
Aspergillus								
A. flavus Link	-	-	-	-	7	6	4	2
A. foetidus Thom e Raper	-	-	-	-	-	9	-	3
A. niger Tiegh.	3	-	-	-	-	-	-	-
A. ochraceus K. Wilh.	-	-	-	-	5	14	-	4
A. oryzae Cohn	-	-	-	-	-	-	-	2
A. sulphureus Thom e Church	-	-	-	-	2	-	-	-
A. tubingensis Mosseray	-	5	-	-	-	-	-	-
A. versicolor Tiraboschi	-	-	-	-	-	3	-	-
Cladosporium								
C. cladosporioides G.A deVries	4	-	-	3	5	5	-	-
Colleototrichum								
C. gloesporioides (Penz.) Sacc.	-	-	-	-	3	4	-	-
Epicoccum								
E. purpurascens Link	6	-	-	-	-	-	-	-
Fusarium								
F. oxysporum Schltdl.	1	-	-	6	-	-	-	2
F. semitectum Berk e Ravenel	-	5	-	8	-	-	-	8
F. solani (Mart) Sacc.		6						9
***Gliocladium* sp.**	-	-	-	-	-	-	1	-
Mucor								
M. hiemalis Wehmer	-	-	-	-	4	-	-	7
Penicillium								
P. brevicompactum Dierckx	8	11	-	-	1	11	-	-
P. citrinum Thom	2	-	-	-	-	-	-	-
P. hirsutum Dierckx	-	-	-	-	-	-	3	-
P. solitum Westtling	-	-	-	-	-	-	2	-
Rhizopus								
R. stolonifer Lindner	8	1	-	-	-	-	-	-
Trichoderma								
T. harzianum Rifai	-	-	-	-	-	-	-	4

TABLE 2 - Results of Indices of Richness and Diversity of filamentous fungi in beans from organic and conventional cultivation.

Planting system	Indices of richness –Da (Margalef)	Indices of diversity – H' (Shannon- Winer)
Coffee Organic	3,29	4,42
Coffee Conventional	2,26	2,91

4 CONCLUSIONS

The results obtained in this study demonstrate that the main genus of fungi found in organic and conventional coffee beaqns were Aspergillus, Penicillium and Fusarium. The richness and biodiversity of filamentous fungi were larger in organic coffee samples than in conventional coffee samples.

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