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Fungal Infection and Mycotoxins Contamination in Organic and Conventional Maize

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Abstract

The conventional agriculture is the model user the world, but consumer concern over the quality and safety of conventional food has intensified in recent years, and primarily the increasing demand for organically grown food, which is perceived as healthier and safer. This study aimed to evaluate the presence of fungi and mycotoxins (aflatoxins, ochratoxin, zearalenone and fumonisins) in samples of organic and conventional corn collected from markets in São Paulo city, Brazil. The fungi isolated from organic corn samples were: *Mucor* spp., *Fusarium* spp., *Aspergillus niger, Aspergillus* spp and yeast. In the conventional corn were isolated: *Aspergullus* spp., *Fusarium* spp., *Trichoderma* spp., *Cladosporium* spp., *Rhizopus* spp., *Paecilomyces* spp., *Curvularia* spp., *Penicillium* spp. and yeast. *F. moniliforme* was isolated in all samples of organic and conventional corn. Aflatoxins B_1, B_2, G_1 and G_2, ochratoxin A and zearalenone were not detected in the samples studied, but the fumonisins were detected in 96% of organic corn in 100% of conventional corn. The *F. moniliforme* presence in all samples and the detection of fumonisins indicate the importance of quality control the organic and conventional corns before their utilization.

Keywords: organic agriculture; aflatoxins, fumonisins, ochratoxin, zearalenone, corn

1. Introduction

The benefits of agriculture have been immense, and the conventional food production is the model used in the world. By the 1940s one of the key innovations in the arable sector was the potential use of chemicals in combating both weeds and pests (Morgan and Murdoch, 2000). Organic farming has been suggested as an alternative to conventional farming systems in order to enhance environmental quality (Tilman et al., 2001). When comparing both production systems with regards to food safety, it appears that, for the well-known toxicants (pesticides, nitrates), organic products present some clear advantages, but it is also recognized that natural toxicants need to be better identified within this mode of production. Environmental and food processing contaminants are present in both organic and conventional products (Pussemier et al., 2006). Considering the difficulty in preventing contamination by toxigenic fungi or in maintaining low contamination levels in conventional crops, it is reasonable to expect that the problem occurs even more clearly in organic crops where antifungal and chemical agents may not be used (Armorini et al., 2016).

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However, the study of the specialized literature by the FAO (2000) has led to the conclusion that there is no evidence to indicate that organic food is more prone to mycotoxin contamination than conventional food (Kouba, 2003). The presence of molds and mycotoxins in food commodities is a potential health threat to humans and animals. Many agricultural products are invaded by fungi before, during and after harvest, in drying, transport and / or storage. In general, peanuts, corn, cotton seeds are among the grains with the highest risk of contamination (Ismail, 2000).

Fusarium, *Aspergillus* and *Penicililum* are often isolated toxigenic genera in maize samples. Aflatoxin and fumonisin were the primary mycotoxin contaminants in maize (Torres et al., 2015).

The aim of this study was to obtain data on the occurrence of aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2), zearalenone (ZEA), ochratoxin A (OTA) and fumonisin, and identify the mycobiota from organic and conventional maize grain purchased on markets of São Paulo city, Brazil.

2. Materials and Methods

2.1 Samples

A total of 100 samples of corn cobs (800g each), 50 from organic and 50 from conventional agriculture, were collected from markets in São Paulo city, Brazil, in 2015.

2.2 Water activity determination

The water activity (a_w) of goat feed and forage samples was determined by automatic analysis using Aqualab 4TE (Decagon Devices Inc., Pullman, WA).

2.3 Recovery, identification and enumeration of the mycobiota from corns samples

2.3.1 Disinfection of corns

Approximately 30 g subsamples of each sample were disinfected by immersion in 2.0 % sodium hypochloride solution for 3 min, followed by three rinses with sterile distilled water.

2.3.2 Isolation of mycobiota from corns

Some 33 disinfected grains (11 kernels per dish) were sampled for mycobiota isolation. The same procedure was done for seed, flowers and peg. Based on Pitt et al. (1993), the first isolation was done on potate dextrose agar (PDA) with chloramphenicol 500 mg/L. The fungal colonies recovered were identified according to recommended methods for each genus (Raper and Fennel, 1965; Barnett and Hunter, 1998).

2.4 Aflatoxins, ochratoxin and zearalenone analysis

The sample of corn (50 g each) was used for extraction of aflatoxins B_1, B_2, G_1 and G_2, ochratoxin A and zearalenone through the method of Soares and Rodriguez-Amaya (1989). The mycotoxins were extracted with chloroform and the solvent evaporated until 1.0 mL in a volumetric flask. An aliquot (40 µL) of each sample was spotted on silica gel-G thin layer plate (Merck, Germany) and then developed with chloroform:acetone 9:1 (v:v) as a solvent system. The concentration of aflatoxin B_1, B_2, G_1 and G_2, ochratoxin A and zearalenone were determined by photodensitometry (Shimadzu, CS9000) comparing the area of the spot samples with mycotoxin standards (SigmaAldrich, USA). The quantification and detection limits for aflatoxins were 2,0 and 4,0 µg/Kg; 5,0 µg/Kg and 10 µg/Kg for ochratoxin A and 55 µg/Kg and 165 µg/Kg for zearalenone, respectively.

2.5 Fumonisins analysis

2.5.1 Fumonisin extraction

The samples of corn were analyzed for fumonisins B1 and B2 according to the method of Visconti et al. (2001). Briefly, 50 mL of acetonitrile/methanol/water (25:25:50 v/v/v) solution was added to each 20 g samples of corn and the mixture was shaken for 20 min in an orbital shaker. The mixture was then centrifuged for 10 min at 2500 ×g, and the supernatant filtered through filter paper (Whatman No. 4). The process was repeated again with the remaining solid material. The two filtrates were combined and 10 mL aliquots were mixed with 40 mL of phosphate buffered saline (PBS). This diluted extract was microfiber filtered (Whatman No. 4) and 10 mL aliquots were submitted to cleanup by immunoaffinity column (Fumonitest, Vicam, Somerville, MA, USA) at a flow rate of 1–2 drops/s.The fumonisins were eluted with 1.5 mL of methanol HPLC grade, at a flow rate of 1 drop/s.

The quantification limit was agreed Visconti et al., (2001), at total levels from 0.5 μg/g to 2.0 μg/g.

2.5.2 HPLC conditions

Two hundred microliters of the final extract were derivatized with 50 μL of *o*-phthaldialdehyde (OPA) solution (40 mg of OPA dissolved in 1.0 mL of methanol and diluted in 5.0 mL of 0.1 M sodium tetraborate containing 50 μL of mercaptoethanol). The product of this reaction was analyzed by a reverse phase isocratic HPLC system using a 150×4.6 mm C18 column (5ODS-20, Phenomenex) at a wavelength of 355 nm excitation and 440 nm emission. The mobile phase consisted of acetonitrile/water/acetic acid (520:480:5 v/v/v) solution at the 1.2 mL/min flow rate. The calibration curve was done by external standard method using four concentrations for each fumonisin: fuminisin B1: 0.25 μg/mL; 0.5 μg/mL; 1.0 μg/mL and 2.0 μg/mL (r2 =0.996); fumonisin B2: 0.125 μg/mL; 0.25 μg/mL; 0.5 μg/mL and 1.0 μg/mL (r2 =0.997). The HPLC quantification and detection limits were: 0.25 μg/mL and 0.025 μg/mL for fumonisin B1 and 0.125 μg/mL and 0.0125 μg/mL for fumonisin B2.

2.6 Statistical Analysis

The experiments were analyzed by ANOVA and Tukey's multiple range tests with a significance level $P < 0.05$.

3. Results

3.1 Water activity

Samples of conventional corn showed water activity (Aw) between 0.99 and 1.0, while organic corn samples showed values ranging from 0.94 to 1.0.

3.2 Fungal Identification

The study of the fungal microbiota of the 50 samples of conventional corn grains presented the following results in descending order of percentage of contamination: yeast (68%), *Mucor* spp. (58%), *Fusarium* spp. (38%), *Aspergillus* spp. (24%), F.N.E. (Non-spore fungus) (20%), *Trichoderma* spp. (18%), *Aspergillus niger* (4%), *Cladosporium* spp. (2%), *Rhizopus* spp. (2%), *Paecilomyces* spp. (2%), *Curvularia* spp. (2%) and *Penicillium* spp. (2%) (figure 1). In the study of the fungal microbiota of the 50 samples of organic corn grains, the following results were presented in order of decreasing percentage of contamination: yeast (100%), *Mucor* spp. (46%), F.N.E. (20%), *Fusarium* spp. (16%), *A. niger* (10%) *Aspergillus* spp. (4%) and it was not possible identified 16% of the fungi (figure 1).
F. moniliforme was isolated in all samples of organic and conventional corn grains (figure 1).

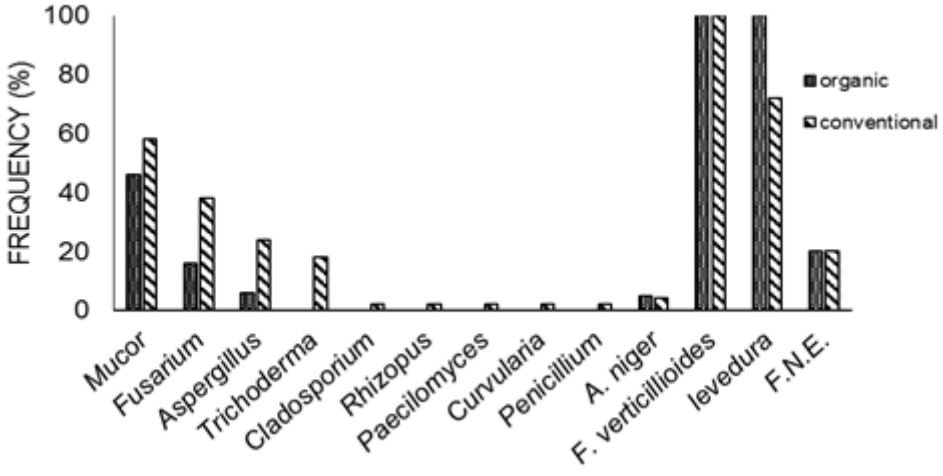

Figure 1. Fungal frequency in organic and conventional corn samples collected in São Paulo city, Brazil.

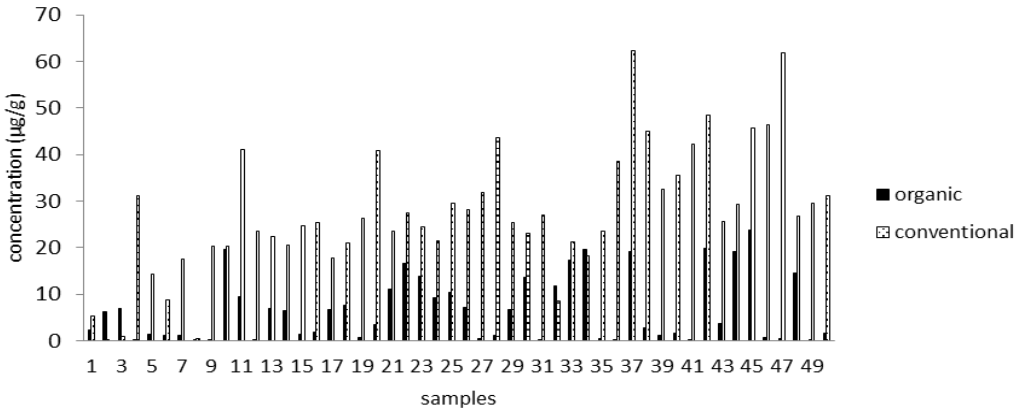

Figure 2. Concentration (µg/g) of fumonisin B1 in organic and conventional corn samples collected in São Paulo city, Brazil.

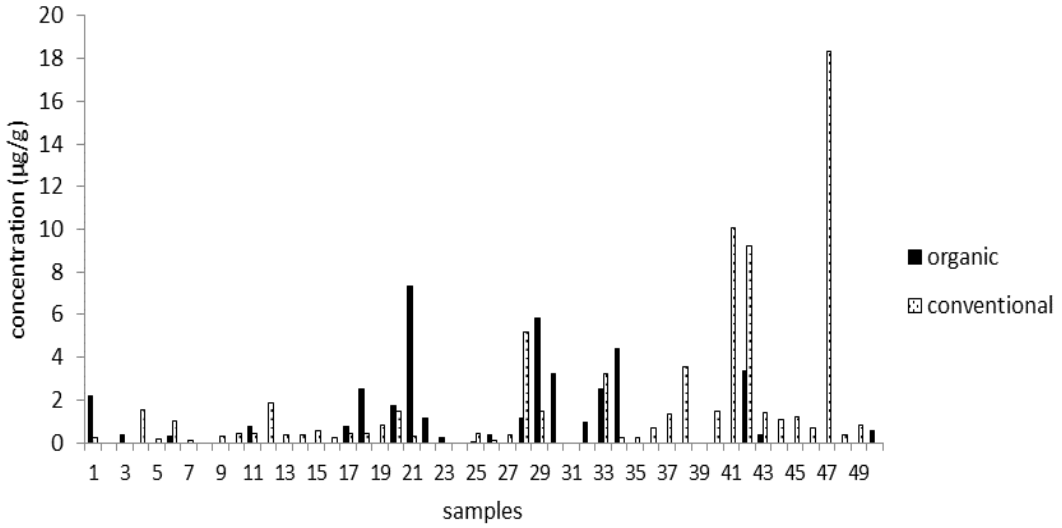

Figure 3. Concentration (µg/g) of fumonisin B2 in organic and conventional corn samples collected in São Paulo city, Brazil.

3.4 Mycotoxins analysis

Fumonisins B1 and B2 were detected in all samples of conventional corn at concentrations ranging from 0.54 to 62.20 µg/g and from 0.2 to 18.31 µg/g, respectively (figure 1 and 2). In organic corn, fumonisins were detected in 48 samples at concentrations ranging from 0.16 to 23.73 µg/g for fumonisin B1 and from 0.12 to 7.30 µg/g for fumonisin B2 (figure 1 and 2).

When compared to fumonisin concentrations between organic and conventional maize, there was a statistically significant difference ($P<0.05$) between them. Aflatoxins B_1, B_2, G_1 and G_2, ochratoxin A and zearalenone were not detected in the samples of conventional and organic corn.

4. Discussion

Maize/corn is an extensively studied crop for the presence of toxigenic fungi and mycotoxins. Infection of corn kernels by toxigenic fungi like *Aspergillus flavus*, *A. parasiticus*, *Fusarium verticillioides* and *F. proliferatum* is well established (Bhat and Reddy, 2017).

In the samples of corn, both conventional and organic, we verified the genera of toxigenic fungi with higher incidence were *Fusarium* spp. and *Aspergillus* spp. (figure 1). The conventional corn showed higher incidence of *Fusarium* spp. and *Aspergillus* spp. than organic corn (figure 1).

Ariño et al. (2007) also found lower Fusarium contamination on organic maize than conventional. On the other hand, Lazzaro et al. (2015) found more contaminated by *Fusarium* spp. on organic maize than conventional.

F. moniliforme was found in all samples of organic and conventional corn (figure 1). In Brazil was reported a higher incidence of *F. verticillioides*, *Penicillium* spp. and *A. flavus* in maize samples at different stages of maturity (Almeida et al., 2002). These results were also reported by Covarelli et al. (2011), in corn grains from different locations in Umbria (central Italy).

Asevedo et al. (1994), when evaluating the incidence of fungi in stored corn, found more frequently the genus *Aspergillus* followed by *Penicillium* and *Fusarium*.

In this study, a greater diversity of filamentous fungi species was verified in the form of conventional culture when compared to organic (Figure 1). According to Cruz et al. (2011) organic agriculture relies on the biodiversity of key components, such as pollinators, natural enemies, earthworms, microorganisms, among others. Through their ecological roles, these groups mediated important processes, such as natural control of insect populations, nutrient cycling, biological nitrogen fixation, synchronization between nutrient release and plant demand, carbon sequestration, the integration between plant and animal production, etc. The use of non-selective chemicals that cause the death of many natural enemies, causing the imbalance in the environment, causing the growth of different microorganisms.
The yeasts were present in 100% of the samples of organic maize and 68% of the samples of conventional maize (figure 1), and in 4 samples of conventional maize and in 15 of organic samples only showed growth of these organisms. In addition, the action of yeasts on the growth of filamentous fungi could explain the lower diversity of fungal genera found in organic maize. The influence of the yeast species on the development of filamentous fungi has been explored by some authors, especially for use in stored grains (Masoud and Kaltof, 2006; Petersson and Schnurer, 1998). Ramos et al. (2010) observed that the yeasts UFLACF 710 and UFLACF 951 belonging to the species *Pichia anomala* and the isolates UFLACF 889 and UFLACF 847 of the species *Debaryomyces hansenii* inhibit the sporulation of *A. ochraceus*, *A. parasiticus* and *P. roqueforti*, but do not interfere in the mycelial growth.

The water activity was high for all samples, above 0.94. According to Orris (1997) data related to environmental factors, especially temperature and relative humidity, storage time and mainly food water activity, are important factors that influence the fungal growth and mycotoxin production in the substrate, making important for the establishment of a program for the prevention and control of this agent.

Advocates of the conventional farming system point out those products from organic farming may present a much higher risk of the presence of mycotoxins. Fumonisins are often found in maize and maize-based foods. All samples of conventionally grown maize were contaminated with fumonisins and only two samples of organic maize did not present this toxin (Figures 2 and 3). The concentrations found in conventional corn were higher than those of organic maize (Figures 2 and 3), and this difference was statistically significant ($P<0.05$). Despite the difference in concentration between the two forms of cultivation, the levels found are in accordance with the Brazilian legislation that stipulates as maximum limit for FB1 + FB2 in maize grain of 5000 μg/kg (Brazil, 2011). Ariño et al. (2007), which studied the presence of mycotoxins in 60 maize samples from conventional and organic systems, and evaluated that 13.3% of the corn samples from the conventional system presented fumonisins B1 and B2 at a concentration of 43 and 22 ng/g respectively. While 10% of the organic corn samples contained fumonisins in concentrations lower than 35 ng/g (FB1) and 19 ng/g (FB2). Cirillo et al. (2003), carried out a study of the Italian market and obtained higher mean concentrations of fumonisin B1 (345 ng/g) in maize from conventional planting system while fumonisin B2 (210 ng/g) appeared in maize obtained from organic production system. Other study reported difference was not significantly for fumonisin contamination in maize under organic or conventional conditions (Galarreta et al, 2015).

No aflatoxins B_1, B_2, G_1 and G_2, ochratoxin A and zearalenone were detected in the samples of conventional and organic maize grains analyzed. Almeida et al. (2002) also did not detect the presence of aflatoxins in maize samples grown in different regions of the State of São Paulo. Already, Roigé et al. (2009) analyzed maize grains and detected zearalenone in 36% of samples and aflatoxin B_1 in each 4% of maize samples. Alborch et al. (2012) evaluated 30 samples of corn meal and 30 samples of corn popcorn, and identified 14 samples with AFs and 4 samples with OTA. In the samples of popcorn 2 samples were contaminated by AFs and 10 with OTA, and ZEA was not detected in any of the samples. Karami-Osboo et al. (2012) reported the presence of AFB_1 in 43.6% of 373 maize samples analyzed. Ochratoxin was detected in only four of the 40 samples of corn stored collected in the Central region of Minas Gerais (Conceição et al., 2010).

Samples of maize-based foods (121), which were collected in the city of Maringá, PR, Brazil, showed AFB_1 contamination in 3 samples, 2 samples with AFB_2, 1 sample with OTA and 1 sample with ZEA. Krout-Greenberg et al. (2013) evaluated 50 random samples of whole maize and aflatoxins were detected in 14 samples, where only one sample contained a high concentration.

5. Conclusion

The absence of aflatoxins B_1, B_2, G_1 and G_2, ochratoxin A and zearalenone and fumonisin concentrations found according to Brazilian legislation in the samples of green maize grains analyzed demonstrate the good quality of organic and conventional corn produced in relation of these toxins.

However, fungal grow in organic and conventional food and can produce mycotoxins in both of them. There are fundamental differences in organic and conventional production practices, but it is necessary more studies about mycotoxin contamination in organic food to conclude if this practice is safer than conventional form.

Conflict of Interest

The authors have no conflict of interest to declare.

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