

## Fungal Infection and Mycotoxins Contamination in Organic and Conventional Maize

Julio Cesar Peres<sup>1</sup>; Christiane Ceriani Aparecido<sup>2</sup>, Daiane Hansen<sup>3</sup> Joana D'arc Felicio<sup>4</sup>;  
Roberto Carlos Felicio<sup>5</sup>; & Edlayne Gonçalez<sup>6</sup>

### 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: *Aspergillus* 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<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, 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).

<sup>1</sup>Centro de Pesquisa e Desenvolvimento em Sanidade Animal, Instituto Biológico, São Paulo, Brazil.

E-mail: [goncalvez@biologico.sp.gov.br](mailto:goncalvez@biologico.sp.gov.br)

<sup>2</sup>Centro de Pesquisa e Desenvolvimento em Sanidade Vegetal, Instituto Biológico, São Paulo, Brazil.

<sup>3</sup>Centro de Pesquisa e Desenvolvimento em Sanidade Animal, Instituto Biológico, São Paulo, Brazil.

E-mail: [goncalvez@biologico.sp.gov.br](mailto:goncalvez@biologico.sp.gov.br)

<sup>4</sup>Centro de Pesquisa e Desenvolvimento em Sanidade Animal, Instituto Biológico, São Paulo, Brazil.

E-mail: [goncalvez@biologico.sp.gov.br](mailto:goncalvez@biologico.sp.gov.br)

<sup>5</sup> Departamento de Ciências Exatas e Tecnológicas, Universidade Estadual de Santa Cruz, Ilhéus, Brazil

<sup>6</sup>Centro de Pesquisa e Desenvolvimento em Sanidade Animal, Instituto Biológico, São Paulo, Brazil.

E-mail: [goncalvez@biologico.sp.gov.br](mailto:goncalvez@biologico.sp.gov.br)

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 *Penicillium* 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<sub>1</sub> (AFB<sub>1</sub>), B<sub>2</sub> (AFB<sub>2</sub>), G<sub>1</sub> (AFG<sub>1</sub>) and G<sub>2</sub> (AFG<sub>2</sub>), 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 potato 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<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, 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<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, 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 Fumonisin 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  $\mu\text{g/g}$  to 2.0  $\mu\text{g/g}$ .

### 2.5.2 HPLC conditions

Two hundred microliters of the final extract were derivatized with 50  $\mu\text{L}$  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  $\mu\text{L}$  of mercaptoethanol). The product of this reaction was analyzed by a reverse phase isocratic HPLC system using a 150 $\times$ 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: fumonisin B1: 0.25  $\mu\text{g/mL}$ ; 0.5  $\mu\text{g/mL}$ ; 1.0  $\mu\text{g/mL}$  and 2.0  $\mu\text{g/mL}$  ( $r_2 = 0.996$ ); fumonisin B2: 0.125  $\mu\text{g/mL}$ ; 0.25  $\mu\text{g/mL}$ ; 0.5  $\mu\text{g/mL}$  and 1.0  $\mu\text{g/mL}$  ( $r_2 = 0.997$ ). The HPLC quantification and detection limits were: 0.25  $\mu\text{g/mL}$  and 0.025  $\mu\text{g/mL}$  for fumonisin B1 and 0.125  $\mu\text{g/mL}$  and 0.0125  $\mu\text{g/mL}$  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 ( $A_w$ ) 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).

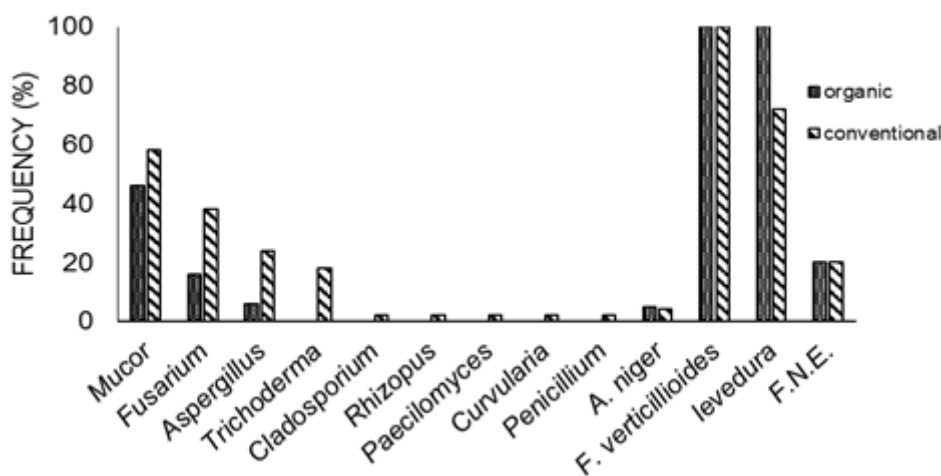


Figure 1. Fungal frequency in organic and conventional corn samples collected in São Paulo city, Brazil.

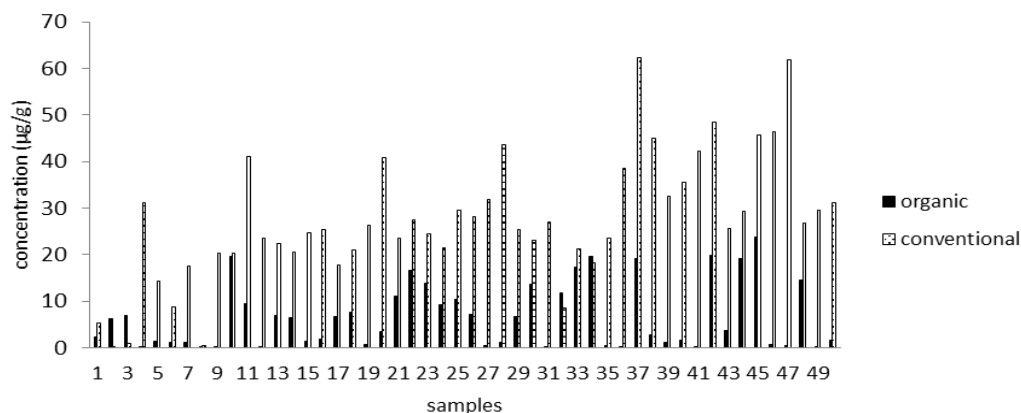


Figure 2. Concentration ( $\mu\text{g/g}$ ) of fumonisin B1 in organic and conventional corn samples collected in São Paulo city, Brazil.

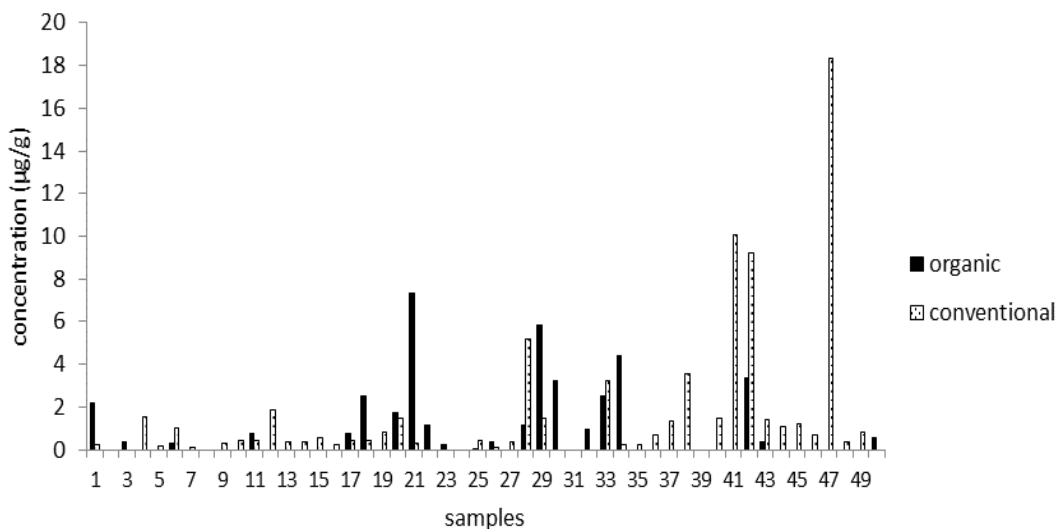


Figure 3. Concentration ( $\mu\text{g/g}$ ) of fumonisin B2 in organic and conventional corn samples collected in São Paulo city, Brazil.

### 3.4 Mycotoxins analysis

Fumonisin B1 and B2 were detected in all samples of conventional corn at concentrations ranging from 0.54 to 62.20  $\mu\text{g/g}$  and from 0.2 to 18.31  $\mu\text{g/g}$ , respectively (figure 1 and 2). In organic corn, fumonisins were detected in 48 samples at concentrations ranging from 0.16 to 23.73  $\mu\text{g/g}$  for fumonisin B1 and from 0.12 to 7.30  $\mu\text{g/g}$  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<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, 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. Fumonisin 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  $\mu\text{g}/\text{kg}$  (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  $\text{ng}/\text{g}$  respectively. While 10% of the organic corn samples contained fumonisins in concentrations lower than 35  $\text{ng}/\text{g}$  (FB1) and 19  $\text{ng}/\text{g}$  (FB2). Cirillo et al. (2003), carried out a study of the Italian market and obtained higher mean concentrations of fumonisin B1 (345  $\text{ng}/\text{g}$ ) in maize from conventional planting system while fumonisin B2 (210  $\text{ng}/\text{g}$ ) 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<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> contamination in 3 samples, 2 samples with AFB<sub>2</sub>, 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<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, 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.

## Acknowledgements

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

## References

- Alborch, L., Bragulat, M. R., Castellá, G., Abarca, M. L., & Cabañes, F. J. (2012). Mycobiota and mycotoxin contamination of maize flours and popcorn kernels for human consumption commercialized in Spain. *Food Microbiology*, 32, 97-103.
- Almeida, A. P., Fonseca, H., Fancelli, A. L., Direito, G. M., Ortega, E. M., & Corrêa, B. (2002). Mycoflora and fumonisin contamination in brazilian corn from sowing to harvest. *Journal Agricultural and Food Chemistry*, 50, 3877-3882.
- Ariño, A., Estopañan, G., Juan, T., & Herrera, A. (2007). Estimation of dietary intakes of fumonisins B1 and B2 from conventional and organic corn. *Food Control*, 18, 1058-1062.
- Armorini, S., Altafini, A., Zaghini, A., & Roncada, P. (2016). Occurrence of aflatoxin M1 in conventional and organic milk offered for sale in Italy. *Mycotoxin Research*, 32, 237-246.
- Asevedo, I. G., Gambale, W., Corrêa, B., Paula, C. R., Almeida, R. M. A., Barnett, H. L., & HunteR, B.B. (1998). *Illustrated Genera of Imperfect Fungi*. Minneapolis: Burgess Publishing Company.
- Bhat, R., & Reddy, K. R. N. (2017). Challenger and issues concerning mycotoxins contamination in oil seeds and their edible oils: Updates from last decade. *Food Chemistry*, 215, 425-437.
- Brazil (2011). Agência Nacional de Vigilância Sanitária, resolução RDC nº 7, 18 de fevereiro de 2011, DOU Nº 46.
- Cirillo, T., Ritieni, A., Visone, M., & Cocchieri, R. A. (2003). Evaluation of conventional and organic Italian foodstuffs for deoxinivalenol and fumonisins B1 and B2. *Journal of Agricultural and Food Chemistry*, 51, 8128-8131.
- Conceição, R. R. P., Queiroz, V. A. V., Saraiva, J. S. C., Alves, G. L. O., Ferreira, P., Mendes, S. M., & Costa, R. V. (2010). Teores de ocratoxinas em milho armazenado com palha na região Central de Minas Gerais. XXVIII Congresso Nacional de Milho e Sorgo 2010, Goiânia: Associação Brasileira de Milho e Sorgo.
- Covarelli, L., Beccari, G., Salvi, S. (2011). Infection by mycotoxigenic fungal species and mycotoxin contamination of maize grain in Umbria, central Italy. *Food and Chemical Toxicology*, 49, 2365-2369.
- Cruz, J. C., Pereira-Filho, I. A., Matrangolo, W. J. R., Marriel, I. E., & Moreira, J. A. A. (2011). Milho orgânico. Ageitec Agência Embrapa de Informação Tecnológica. [Online] Available: <http://www.agencia.cnpia.embrapa.br/gestor/milho/arvore/CONT000fz5e6zsp02wx5ok0cpoo6agwc2gy1.html> (September 11, 2013)
- FAO (2004). Worldwide regulations for mycotoxins in food and feed in 2003. Food and agriculture organization of the united nations, FAO food and nutrition paper 81, fao, Rome,
- Galarreta, J. I. R., Butron, A., Ortiz-Barredo, A., Malvar, R. A., Ordas, A., Landa, A., & Revilla, P. (2015). Mycotoxins in maize grains in organic and conventional agriculture. *Food Control*, 52, 98-102,

- Ismail, M. A. (2000). Deterioration and spoilage of peanuts and desiccated coconuts from two sub-saharan tropical East African countries due to the associated mycobiota and their degradative enzymes. *Mycopathologia*, 150, 67-80.
- Karami-Osboo, R., Mirabolfathy, M., Kamran, R., Shetab-Boushehri, M., & Sarkari, S. (2012). Aflatoxin B1 in maize harvested over 3 years in Iran. *Food Control*, 23, 271-274.
- Krout-Greenberg, N. D., Puschner, B., Davidson, M. G., & Depeters, E. J. (2013). Preliminary study to assess mycotoxin concentrations in whole corn in the California feed supply. *Journal of Dairy Science*, 96, 705–2712.
- Lazzaro, I., Moretti, A., Georni, P., Brera, C., & Battiloni, P. (2015). Organic vs conventional farming: differences in infection by mycotoxin-production fungi on maize and wheat in Northern a Central Italy. *Crop Protection*, 72, 22-30.
- Kouba, M. (2003) Quality of organic animal products. *Livestock Production Science*, 80 33–40.
- Masoud, W., & Kalsoft, H. C. (2006). The effects of yeasts involved in the fermentation of *Coffea arabica* in East Africa on growth and ochratoxin A (OTA) production by *Aspergillus ochraceus*. *International Journal of Food Microbiology*, 106, 229-234.
- Morgan, K., & Murdoch, J. (2000). Organic vs. conventional agriculture: knowledge, power and innovation in the food chain. *Geoforum*, 31, 159-173.
- Torres, O., Matute, J., Gelineau-Van Waes, J., Maddox, J. R., Gregory, S. G., Ashley-Koch, A. E., Showker, J. L., Voss, K. A., & Riley, R.T. (2015). Human health implications from co-exposure to aflatoxins and fumonisins in maize based foods in Latin America: Guatemala as a case study. *World Mycotoxin Journal*, 8, 143-159.
- Orris, G. D. (1997). *Animal Diseases of Public Health Importance*. Food and Agriculture Organization of the United Nation (FAO), Rome, Italy. [Online] Available: <http://wwwnc.cdc.gov/eid/content/3/4/contents.htm> (July 29, 2015)
- Petersson, S., & Schnürer, J. (1998). *Pichia anomala* as a biocontrol agent of *Penicillium roqueforti* in high moisture wheat, rye, barley, and oats stored under airtight conditions. *Canadian Journal of Microbiology*, 44, 471-476,
- Pitt, J. I., Hocking, A. D. (1997). *Aspergillus* and related teleomorphs. In: Pitt, J. and Hocking, A. D. (Eds.). *Fungi and food spoilage* (pp. 339-416). London: Chapman & Hall.
- Pussemier, L., Larondelle, Y., Van Peteghem, C., & Huyghebaert, A. (2006). Chemical safety of conventionally and organically produced foodstuffs: a tentative comparison under Belgian conditions. *Food Control*, 17, 14–21.
- Ramos, D. M. B., Silva, C. F., Batista, L. R., & Schwan, R. F. (2010). Inibição *in vitro* de fungos toxigênicos por *Pichia* sp. e *Debaryomyces* sp. isoladas de frutos de café (*Coffea arabica*). *Acta Scientiarum Agronomy*, 32, 397-402.
- Raper, K. B., FENNEL, D. I. (1965). *The genus Aspergillus*. Baltimore, Willians Wilkins.
- Souza, V. M. (1994). Mycoflora and aflatoxigenic species of *Aspergillus* ssp isolated from stored maize. *Revista de Microbiologia*, 25, 46-50.
- Roigé, M. B., Aranguren, S. M., Riccio, M. B., Pereyra, S., Soraci, A. L., & Tapia, M. O. (2009). Mycobiota and mycotoxins in fermented feed, wheat grains and corn grains in Southeastern Buenos Aires Province, Argentina. *Revista Iberoamericana de Micologia*, 26, 233–237.
- Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., & Polasky, S. (2001). Agricultural sustainability and intensive production practices. *Nature*, 418, 671-677.
- Soares, L. M. V, & Rodriguez-Amaya, D. B. (1989). Survey of aflatoxins, ochratoxin A, zearalenone, and sterigmatocystin in some Brazilian foods by using multi-toxin thin-layer chromatographic method. *Journal of the Association of Analytical Chemists*, 7, 22-26.
- Visconti, A., Solfrizzo, M., & Girolamo, A. (2001). Determination of fumonisins B<sub>1</sub> and B<sub>2</sub> in corn and corn flakes by liquid chromatography with immunoaffinity column cleanup: collaborative study. *Food Chemical Contaminants*, 84, 1828-1837.