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# *Fusarium verticillioides* and its fumonisin production potential in maize meal

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## ABSTRACT

This study aimed to identify the presence of fungi of the genus *Fusarium* and to evaluate the presence of contamination of fumonisin in maize meal destined to human consumption in the city of Teresina, Piauí, Brazil. It was used 30 samples of maize meal of six different brands sold in supermarkets. Mycological evaluation was carried out immediately. Then aliquots were stored at - 4 °C for later analysis of fumonisins. It was obtained 34 isolates from *Fusarium verticilloides*. These isolates had the ability to produce fumonisins B1, B2 and B3, with values ranging from 48.2 to 1190.1 µg $g^{-1}$ for B1; from 6.7 to 311.5 µg $g^{-1}$ for B2; and from 23 to 667 µg $g^{-1}$ for B3. The fumonisin concentrations for isolates ranged from 84.3 to 2168.6 µg $g^{-1}$. All the samples presented fumonisins with values ranging from 0.10 to 2.13 µg $g^{-1}$. Isolates from strains of *F. verticillioides* were obtained in the maize meal samples, and the lots examined had different levels of fumonisins, which may represent risks to consumers.

**Key words:** elisa, fungi, mycotoxins

# *Fusarium verticillioides e seu potencial de produção de fumonisinas em farinha de milho*

## RESUMO

Objetivou-se identificar a presença de fungos do gênero *Fusarium* e avaliar a presença quanto à contaminação de fumonisina em farinha de milho, destinados ao consumo humano na cidade de Teresina, Piauí, Brasil. Foram utilizadas 30 amostras de farinha de milho a partir de seis diferentes marcas comercializadas em supermercados. A avaliação micológica foi realizada imediatamente, e em seguida foram armazenadas a - 4 ºC, alíquotas, para posteriormente ser feita a análise de fumonisinas. Foram obtidos 34 isolados de *Fusarium verticilloides*. Esses isolados apresentaram capacidade de produzirem fumonisinas B1, B2 e B3, com valores que variaram de 48,2 para 1190,1 µg $g^{-1}$ de B1; 6,7 para 311,5 µg $g^{-1}$ de B2; e 23 para 667 µg $g^{-1}$ de B3. As concentrações de fumonisinas por isolados variaram de 84,3 a 2168,6 µg $g^{-1}$. Todas as amostras analisadas apresentaram fumonisinas, com valores que variaram de 0,10 a 2,13 µg $g^{-1}$. Nas amostras de farinha de milho, foram obtidos isolados de *F. verticillioides* e os lotes analisados apresentaram diferentes níveis de fumonisinas e podendo representar riscos ao consumidor.

**Palavras-chave:** elisa, fungos, micotoxinas

## Introduction

Among the leading crops in Brazil, corn (*Zea mays*) is the second most produced grain, behind only to soybeans, according to research done by Conab (2010). Corn is highlighted for being used as food for humans and animals; its derivatives have effective participation in the diet of the poorest sections of the population (Melo-Filho & Richetti, 1997). Tropical and subtropical climates Brazil promote fungal growth, which under suitable conditions can produce mycotoxins. These toxins have been frequently reported by several researchers (Bittencourt et al., 2005; Caldas & Silva, 2007).

Among the fungi, the major pathogen of plants belongs to genus Fusarium. These fungi can produce mycotoxins before or immediately after harvest (Sartori et al., 2004). Associated with corn, there are Fusarium species causing rot of stem and ear, besides the presence of the endophytic colonization *F. verticillioides*, *F. proliferatum* and *F. subglutinans,* in which *F. verticillioides* is the predominant species, particularly in the tropics (Leslie & Summerell, 2006).

Fumonisins are important due to the occurrence of mycotoxicosis in domestic animals and humans. Some important events occurred because of the consumption of foods containing fumonisin. In South Africa, specifically in the Transkei area, it was found the presence of high levels of moldy beans. Consequently, the local people who ate this product had esophageal cancer (Sydenham et al., 1990). In this same country it was confirmed the presence of equines with leukoencephalomalacia. The analysis of moldy corn samples eaten by these equines found the presence of fumonisin (Wilson et al., 1990). Fumonisins (FBS) are a group of mycotoxins produced mainly by *Fusarium verticillioides*, most prevalent, and *F. proliferatum*, which are the species most frequently associated with contamination in maize meal (Leslie & Summerell, 2006). This contamination by both fungi and mycotoxins, have been previously reported in maize grain around the world (Rheeder et al., 2002).

Fumonisin B1 is classified as a possible carcinogen for human (IARC, 2002), the consumption of food contaminated with this toxin has been related to the incidence of liver cancer and esophageal (Quieroga & Pernambuco, 2006; Sun et al., 2007), and also neural tube defects (Torres-Sanchez & Lopez-Carrillo, 2010). Moreover, the FBS can cause a variety of diseases in animals, which may reduce the productivity of them. The fumonisin levels in products from corn are dependent on the degree of contamination and transformation processes used in the production of the final product (Saunders et al., 2001). The fumonisins are heat stable and survive under cooking and frying conditions (Humpf & Voss, 2004).

Earlier studies conducted in Brazil evaluated the exposure of the Brazilian population to fumonisins by means of maize flour consumption (Bittencourt et al., 2005; Caldas & Silva, 2007). It was recently demonstrated that *F. verticillioides* is the prevalent species in some of producing regions of corn in Brazil; isolated from this study showed different levels of fumonisin production (Lanza et al., 2014). There are several work related to the *F. verticillioides* and maize; however, there are few studies related to *F. verticillioides* and maize by-products. Furthermore, it is necessary to evaluate the level of fumonisins present in foods marketed nationwide. These studies are important to define secure levels of contamination by mycotoxins in the food chain and to define priorities for monitoring programs (Join FAO & WHO, 2002).

This study aimed to identify isolates of Fusarium, using the concept of morphological species and evaluate the fumonisins levels in maize by-products purchased in markets of Teresina, PI.

## Material and Methods

It was used 30 samples (500 g) maize meal from six different brands sold in supermarkets in the city of Teresina, Piauí, Brazil. After collection, the samples were homogenized, mixed and fractionated to obtain samples of 100g that were forwarded to the laboratory of the Center for Studies, Research and Food Processing of the Federal University of Piauí, Teresina, Piauí, for isolation of the genre. The identification of the isolates of *Fusarium* and production, detection and quantification of fumonisins by its isolates was held at the Mycology Laboratory of the National University of Río Cuarto, Córdoba, Argentina. The quantification of fumonisins in maize meal was made at the Center for mycological and mycotoxicologic research of the Rural University of Rio de Janeiro.

It was added 25 grams of each sample to 225.0 mL of 0.1% peptone water, which formed the dilution of $10^{-1}$ this mixture was homogenized and diluted to final concentrations of $10^{-2}$ and $10^{-3}$. It was spread 0.1 ml of each dilution (in duplicate) in the solid medium surface, Dicloran Rose Bengal Chloramphenicol agar (DRBC). The plates were incubated for seven days at 25 ° C. On the last day of incubation, the genders of the colonies were identified according to criteria proposed by Pitt & Hocking (2009). All Fusarium strains were transferred to Spezieller Nalvistof Agar (SNA), and incubated at 25 ° C for seven days for species identification. After growing in SNA, the colonies of *Fusarium spp.* were plated on monosporic cultivation and later cultivated in the media Carnation Sheets Agar (CLA) and Potato Dextrose Agar (PDA). The colonies were then incubated for 14 days at 24 ° C in cyclo of 12 hours of white light and 12 hour of dark light. After this period, the species identification was made by both macroscopic and microscopic features according to Leslie & Summerell (2006).

To proceed the determination and quantitation of fumonisins production by isolates of *F. verticillioides*, it was used Erlenmeyer flasks with 100 g of maize and 40 ml of distilled water, which was autoclaved twice for 30 minutes at 121 ° C. After cooling down, it was inoculated in maize a conidial aqueous suspension of spores (1.0 mL) obtained in the culture of carnation leaf agar (CLA) incubated in the dark at 25 ° C for 28 days. To avoid the formation of lumps, the cultures were shaken during the first days of incubation, and thereafter only when it was necessary. Maize crops were dried at 50 ° C and finely ground in a laboratory mill and stored at 4 ° C for later analysis of the presence of fumonisin. For each 15 g of the maize culture sample, it was added 50 mL of acetonitrile: water (1:1). Subsequently they were agitated in a shaker for 30

minutes and filtered through Whatman # 4 filter paper. It was taken aliquots of the extracts (1000µL) for high performance liquid chromatography (HPLC).

An aliquot of 50 µL of this extract was derivatized with 200 µL of an S-phthaldialdehyde solution (OPA). The derivatives of OPA fumonisin (solution of 20 µL) were analyzed by reverse phase HPLC / fluorescence detection system. The HPLC system consisted of a pump 1050 Hewlett Packard (Palo Alto, CA, USA) connected to a fluorescence detector 1046A programmable of Hewlett Packard and a Hewlett Packard Kayak XA data module (HP ChemStation Rev. A.06.01). Chromatographic separations were performed on C18 reversed phase column (150 x 4,6 mm id, particle size of 5 µm; Luna-Phenomenex, Torrance, CA, EUA), connected to the cartridge safety guard (4 id × 3 mm, particle size of 5 µm; Phenomenex, Torrance, CA, EUA). The mobile phase used was methanol: 0.1 M sodium dihydrogen phosphate (75:25 v $v^{-1}$) adjusted to pH 3.35 with phosphoric acid, at a flow rate of 1.5 ml $min^{-1}$. The derivative fluorescence of OPA Fumonisin was recorded at excitation and emission in wavelengths of 335 and 440 nm, respectively. The fumonisins were evaluated by the peak heights and compared with standard solutions of fumonisin references B1, B2 and B3 (Sigma Chemical Co., St. Louis, MO, USA). The detection limit of the analytical method was 0.01 µg $g^{-1}$.

For detection and quantification of total fumonisins in maize meal samples, it was used AgraQuant® commercial kits produced by Romer Labs Singapore Pte Ltd. The kit is based on the direct competitive ELISA in plaques. The mycotoxin sample is extracted by agitation it with 70% methanol. The extract is filtered and then tested by immunoassay, following the manufacturer guidelines. The determination of the toxin was performed by comparison with standards of different concentrations provided by the kit. The quantitative analysis was performed on a specific computer program provided by the manufacturer.

It was performed the analysis of variance followed by the test to compare means, SNK, with significance ($p < 0.05$) using the Sigma Stat Statistical Package (1994) for the results of the amount of fumonisins in maize meal.

## Results and Discussion

Thirty-four isolates showed microconidia produced in long chains and in monofialides. The produced macroconidia had 3-5 septa, with little apparent foot and apical cell, and it was not observed the formation of chlamydospores in the tested isolates. In BDA the color of the cultures ranged from orange to purple, and the color tone were evident over the last days of evaluation. According to the characteristics observed the isolates were identified as *F. verticillioides*. The culture of color pattern is not a good marker for characterization of *Fusarium* and for complex species as *Fusarium fujikuroi* (Seifert, 1996). *Fusarium thapsinum* shares the same morphological markers with *F. Verticillioides*; however, this species is predominantly associated with sorghum and millet (Leslie & Summerell, 2006). In Brazil and other countries, it is observed that the species predominantly associated with the cultivation of maize and maize byproducts is *F. verticillioides*, though other species of the *Fusarium fujikuroi* complex are also present as *F. proliferatum* e *F. subglutinans* (Lanza et al., 2014, Leyva-Madrigal et al., 2015).

Regard to the production of fumonisin by *F. verticilloides* isolated from maize meal, the data showed that all 34 isolates tested were positive for FB1 (Fumonisin B1), FB2 (Fumonisin B2) and FB3 (Fumonisin B3), the concentration produced ranged from 48.2 to 1190.1 µg $g^{-1}$ for FB1; from 6.7 to 311.5 µg $g^{-1}$ for FB2; and from 23 to 667 µg $g^{-1}$ for FB3. Given the three types of fumonisins, the concentrations for each strain ranged from 84.3 to 2168.6 µg $g^{-1}$. The industry processes maize grains eventually without symptoms of rot; however, *F. verticillioides* is a species with endophytic character. This means that the beans even not presenting symptoms, may be lodged with the fungus inside of it. From a total of 49 isolates of *F. verticillioides* from the Philippines, 70% of its isolates were potentially producers of fumonisins (Magculia et al., 2011). Fumonisin production has generally varying according to the origin of the isolate. Isolates from northern Mexico produced high levels of toxins, while isolated from the central region of Brazil showed low production of fumonisin (Stumpf et al., 2013; Lanza et al., 2014).

Brazilian law had no limits for fumonisin in food by 2010, however, in February 2011 came into effect a new resolution (N° 07, of February 18, 2011 developed by the Agency ANVISA). This Resolution has limits for various classes of food and several mycotoxins previously covered by other reference legislations, such as the US and several European countries, the maximum allowable limits for fumonisin B1 and B2 is 2.5 µg $g^{-1}$ to maize meal (Brasil, 2011). All samples studied in this work were within of the standard recommended by the Brazilian legislation.

It was detected fumonisin in all samples of maize meal, but there was no significant difference ($P > 0.05$) for the concentration of fumonisin among the surveyed brands (Table 1). The highest concentration of fumonisin was detected in a sample of the brand E, with 2.13 µg $g^{-1}$. The highest average fumonisin was also found in samples of the brand E with 1.12 µg $g^{-1}$. The average of all samples analyzed in this work was 0.83 µg $g^{-1}$.

Caldas & Silva (2007) detected fumonisins in maize meal samples in Brasilia-FD, and some of its samples had values of 6.17 µg $g^{-1}$, and the average of the samples was 1.68 µg $g^{-1}$, this results were higher than those found in our study. This may be a result of the region to which this maize was originated or the time when it was harvested (Stumpf et al., 2013; Lanza et al., 2014). The incidence of fumonisin contamination in maize grown in the South and Southeast of Brazil can reach 100% (Van Der Westhuizen et al., 2003). Fumonisin has been

**Table 1.** Mean levels of fumonisins in maize meal of different brands studied

| Brands | Mean (µg $g^{-1}$) | Standard deviation (µg $g^{-1}$) |
|---|---|---|
| A | 0.89 | 0.51 |
| B | 0.51 | 0.41 |
| C | 0.82 | 0.31 |
| D | 0.90 | 0.41 |
| E | 1.12 | 0.61 |
| F | 0.75 | 0.45 |

P = 0,295. µg $g^{-1}$= microgram per gram

frequently detected in maize meal and its by-products in the United States, China, European countries, South American countries and Africa (Rhedder et al., 2002).

In Campinas, SP, Machinski & Soares (2000) evaluated various derivatives of maize and demonstrated that the majority of the samples were contaminated with fumonisin. From these derivatives, only cornmeal was identified with fumonisins in all investigated samples with an average of 2290 μg $kg^{-1}$ (2.29 μg $g^{-1}$) of fumonisin B1. In research with maize meal in the city of Recife, PE, the fumonisin levels found ranged from undetectable to 150 μg/kg (0.15 μg $g^{-1}$) (Kawashima & Soares, 2006). These results were inferior to those reported in this work. In another study in the city of São Paulo, it was found fumonisin B1 and B2 in 60 samples of maize meal and cornmeal (Bittencourt et al., 2005). Other authors also found contamination by fumonisin B1, B2 and B3 in maize samples used for human consumption in the state of Santa Catarina (Van Der Westhuizen et al., 2003).

## Conclusion

In this study was observed that the maize meal samples tested were contaminated with fumonisin, and it is likely that the source of this contamination is the presence of *F. verticillioides.*

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