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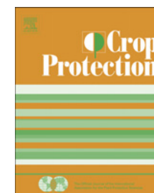

# Mycobiota and aflatoxins in a peanut variety grown in different regions in the state of São Paulo, Brazil

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ABSTRACT

The aim of this study was to characterize the mycobiota and to determine the occurrence of aflatoxins in peanut samples (husks and kernels) originating from four production regions in the State of São Paulo, Brazil. Analysis of soil samples showed that *Penicillium* spp. was the most frequent fungal species (52.1%) in the four regions studied. *Aspergillus flavus* was the most frequent species of the genus *Aspergillus* (13.4%). In husks and kernels, *Fusarium* spp. presented the highest frequency of isolation (70.2%) and *A. flavus* was the most frequently isolated species (9.8%) among the four *Aspergillus* species detected. High-performance liquid chromatography revealed the presence of aflatoxins in 5% of kernel samples, with concentrations ranging from 1.0 to 12.7 μg/kg, and in 13.8% of husk samples, with concentrations ranging from 1.0 to 117.8 μg/kg. Samples originating from the Tupã region presented higher contamination of husks and kernels with *A. flavus* before and after plant uprooting than samples obtained from the other regions. Although *A. flavus* and *Aspergillus parasiticus* were isolated from peanuts, few samples were contaminated with aflatoxins, demonstrating that the presence of these fungi does not necessarily indicate the presence of aflatoxins in the substrate. Isolation of these species from soil confirmed that this is the main route of peanut contamination.

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## 1. Introduction

Peanut is a dicotyledonous plant and the only species cultivated is *Arachis hypogaea* L. Peanuts are annual, herbaceous, pubescent, erect or low-growing plants. Their peculiarities are the aerial flowers and subterraneous fruits. After fertilization, the flowers form gynophores that grow downwards into the soil. Fruit development starts under the soil and lasts about 40–50 days until harvest (Câmara, 1998). In commercial plantations, once the plants are uprooted, the pods are placed to dry in the sun in a windrow (Godoy and Minotti, 2005). This is one of the most important stages of production since poor drying can provoke a significant increase in fungal contamination (Fonseca, 2010). The increase in the level of fungal contamination does not only occur in the field, but also during the process of kernel formation, harvesting, drying, transport, and storage (Rossetto et al., 2005), as well as during handling (Santos et al., 2001). The economic impact of fungal invasion includes reduced seed germination rates and, more importantly, compromised product quality such as mold growth, discoloration, unpleasant odor, loss of dry fabric heating, cooking, chemical and nutritional alterations, and mycotoxin production, all of which may make peanut products unsuitable for consumption (Christensen, 1982; Paster and Bullerman, 1988).

Soil is the primary reservoir for many fungi, including those of the genus *Aspergillus* (Smith and Ross, 1991). Fungi of the genera *Aspergillus*, *Penicillium*, *Rhizopus*, and *Fusarium* are frequently detected in peanut kernels. In addition, *Alternaria*, *Nigrospora*, *Trichoderma*, *Dothiorella*, and *Pestalotia* have also been identified (Rossetto et al., 2005).

Water activity ($a_w$) of the substrate also plays an important role in fungal growth and is defined as the ratio between the vapor pressure of a material ($p$) and the vapor pressure of pure water (po) under the same conditions ($a_w = p/\text{po}$) (Pitt and Hocking, 1997). The minimum $a_w$ required for growth of most fungi, especially toxigenic ones, ranges from 0.78 to 0.80. No fungal growth is generally observed below $a_w$ of 0.60. The water activity necessary for fungal growth is below that required for the production of mycotoxins (Lacey, 1989).

The contamination of peanuts with aflatoxins, secondary metabolites mainly produced by *Aspergillus flavus* and *Aspergillus*

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*parasiticus*, is one of the main factors that compromise product quality (Horn et al., 2000). Chemically, aflatoxins are characterized by the presence of a polycyclic structure derived from a coumarin nucleus linked to a bifuran system on one side and to a pentone (B series aflatoxins) or six-membered lactone (G series aflatoxins) on the other. At present, 18 different compounds are known, with aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ being the ones most commonly found in nature (Oga, 1996). Aflatoxins are identified according to the fluorescence they emit when exposed to UV light (B for blue, G for green). They possess a low molecular weight, are very soluble in moderately polar solvents such as chloroform, methanol and dimethylsulfoxide, but are poorly soluble in water. Aflatoxins are stable at high temperatures (the fusion point of $AFB_1$ is 269 °C), but can be destroyed by autoclaving in the presence of ammonia and treatment with hypochlorite (WHO, 1979). Aflatoxin $B_1$ is the most toxic to mammals and exhibits hepatotoxic, teratogenic, and mutagenic properties (Santos et al., 2001), causing toxic hepatitis, hemorrhage, edema, immunosuppression, and hepatic carcinoma (Smith and Ross, 1991). This toxin has been classified as a class one human carcinogen by the International Agency for Research on Cancer (IARC, 1993). The Brazilian Ministry of Health (Resolution RDC 274, ANVISA, 15/10/02) and the Ministry of Agriculture (Decree MAARA No. 183, 21/03/96) have established a limit of 20 μg/kg for the sum of aflatoxins $B_1$, $B_2$, $G_1$, and $G_2$ (Brazil, 1996, 2002).

These facts, associated with the growing demand for peanuts for human and animal consumption, and the importance of the worldwide control and prevention of aflatoxins have encouraged the present study. The objectives of the study were 1) to characterize the mycobiota and to determine the occurrence of aflatoxins in peanut samples (husks and kernels) originating from four production regions in the State of São Paulo, Brazil, 2) to characterize the mycobiota in soil samples collected in the regions studied, and 3) to correlate the mycobiota data with the $a_w$ levels detected in the samples analyzed.

## 2. Materials and methods

### 2.1. Samples

Commercial peanuts of the Runner IAC 886 variety, the most widely grown variety in these regions, planted in four peanut producing municipalities in the State of São Paulo, Brazil (Jaboticabal, Rosália, Tupã, and Cafelândia) were used. The plants were cultivated between the first half of December 2007 and the second half of January 2008 according to common practices currently used for peanut production (Godoy and Minotti, 2005).

At the time of seeding, 10 completely randomized sections (repetitions) were defined, which consisted of 10 rows of plants (0.9 m between rows) measuring 20 m in length and comprising an area of 180 $m^2$ per section.

Two samples of peanut pods were collected per section, for a total of 5 kg per sample. The samples were collected using sterile gloves and were stored in sterile packages. The first sampling was performed 2 weeks prior to plant uprooting at 110 days after seeding and the second sampling immediately after uprooting, while the plants were windrowing in the field during the natural drying process. For each sample, the pods were removed from the plants at different sites within each section. The pod samples were stored in ventilated packages and sent to the Postharvest Unit of the Campinas Agronomic Institute (Instituto Agronômico de Campinas, IAC), São Paulo, Brazil. The samples were submitted to a process of complementary natural drying *in loco* and monitored until they reached a uniform humidity of approximately 11%. Next, the samples were temporarily stored at 15 °C before being sent to the laboratory. Natural, controlled drying and storage at 15 °C permit the preservation of microorganisms without their proliferation. The pods were dehusked manually and divided into 80 husk samples and 80 kernel samples. These samples were subsequently analyzed regarding $a_w$ and presence of fungi and aflatoxins. Manual dehusking was performed with sterile gloves to prevent cross-contamination.

Two soil samples were collected in the cultivation areas. The first sample was obtained immediately after plant emergence (10–15 days after planting) and the second sample was collected 2 weeks prior to uprooting (harvest), i.e., 110 days after planting. Each sample consisted of 500 g soil taken from the area close to the plant stem at a depth of up to 15 cm below the soil surface, where pods are formed. Ten subsamples were collected throughout each section, from five of the previously defined sections, which were homogenized and pooled.

### 2.2. Determination of water activity

Water activity ($a_w$) in the peanut samples (determined separately for husks and kernels) and soil was measured with an AQUALAB CX-2 equipment (Decagon Devices Inc., Pullman, WA, USA).

### 2.3. Isolation of mycobiota from soil samples

Ten gram of each soil sample was diluted in 90 mL sterile 0.085 g/L sodium chloride solution to obtain a dilution of $10^{-1}$. Serial decimal dilutions were then prepared from this dilution up to $10^{-6}$. An aliquot (0.1 mL) of each dilution was placed on the surface of Petri dishes containing 10–15 mL Martin agar (Martin, 1950) and the plates were incubated at 25 °C for 5 days. All samples were processed in duplicate (Pitt and Hocking, 1997). After incubation, all colonies were counted and multiplied by the dilution factor in order to obtain the number of colony-forming units per gram substrate (CFU/g).

All colonies were subcultured on potato dextrose agar (Oxoid, Basingstoke, UK) at 25 °C for 7 days. The colonies were identified to genus level and those belonging to the genus *Aspergillus* were identified to species level according to Barron (1972) and Pitt and Hocking (1997).

### 2.4. Isolation of mycobiota from seeds and peanut samples (Berjak, 1984)

After collection, approximately 30 g of peanuts (and seeds) were separated from each previously homogenized sample and disinfected by immersion in 20 mL/L sodium hypochlorite solution for 3 min, followed by vigorous washing with sterile distilled water to eliminate external contaminants. Next, the husks and kernels were separated and randomly seeded onto Petri dishes containing *Aspergillus flavus–A. parasiticus* agar, which is recommended for the isolation of these species (Pitt et al., 1983). Four repetitions consisting of 4 open husks per plate were prepared. For kernels, mycobiota analysis was performed in triplicate, with each Petri dish containing 11 kernels. The plates were incubated at 25 °C for 5 days and the results are expressed as the percentage of the total number of seeded husks or kernels. The seeds were plated and the results were analyzed as described for kernels. Next, colonies presenting different morphologies were subcultured on potato dextrose agar (Oxoid) at 25 °C for 7 days. All colonies were identified to genus level and those belonging to the genus *Aspergillus* were identified to species level according to Barron (1972) and Pitt and Hocking (1997).

### 2.5. Analysis of aflatoxins in peanut samples

For extraction of aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ from the peanut husks and kernels of the 160 samples collected [80 during seed

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