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Mycobiota and mycotoxins in Brazilian peanut kernels from sowing to harvest

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Abstract

The total mycobiota and the mycotoxin contamination of peanuts were analyzed in plants collected at different stages of the pod maturity sampled in Junqueirópolis, at São Paulo State (Brazil). The prevalent peanut mycobiota were *Fusarium* spp. and *Aspergillus flavus*, present in 26% and 17% respectively of the samples analyzed. In soil, the genus *Penicillium* and *Fusarium* were most frequently detected, and *A. flavus* was detected in 8% of the samples. The screening of mycotoxins indicated that aflatoxins and cyclopiazonic acid were present the highest incidence, being detected in 32% of the samples, in concentrations, respectively, from 4.20 μg/kg to 198.84 μg/kg and from 260 μg/kg to 600 μg/kg. Fumonisins were not detected by HPLC. All data were correlated with the occurrence of wind-dispersed fungi and the environmental and soil conditions. Results indicate that good management of the agricultural environment may offer a way to reduce mycotoxins and the toxigenic fungal contamination in peanuts preharvest because the pods are exposed to different environmental conditions during their formation until harvest, and the optimal conditions for mycotoxin production and fungal growth are frequently found in the crop fields.

Keywords: Aflatoxins; Cyclopiazonic acid; Fumonisin; Fungi; *Aspergillus*

1. Introduction

Mycotoxins are secondary metabolites of certain filamentous fungi that can be produced in foods as a result of fungal growth (Sweeney and Dobson, 1998). Since the recognition of hazards of mycotoxin contamination in food and feed commodities as a result of the discovery of aflatoxin in the early 1960s, some countries have conducted surveys on the incidence of mycotoxins in their agricultural products (El-Maghraby and El-Maraghy, 1987). The mycotoxigenic fungi involved in the human food chain belong mainly to the *Fusarium*, *Aspergillus*, *Penicillium* and *Alternaria* genera. The simultaneous presence of different mycotoxins in the same commodity produced by fungi belonging to different genera is not uncommon (Aresta et al., 2003).

Examples of mycotoxins of great public health and agroeconomic significance include aflatoxin, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins and ergot alkaloids (Hussein and Brasel, 2001).

In Brazil, the presence of aflatoxins in peanuts and peanut products has been an alarming problem, although the extent and quantities found have been mostly low (Rodriguez-Amaya and Sabino, 2002). Peanuts are often invaded before harvest by *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare, fungi which produce the carcinogenic aflatoxins (Horn et al., 2000). Cyclopiazonic acid may also be produced by some *Aspergilli*, including *A. flavus*, and *Penicillium* species. This toxin occurs naturally in agricultural commodities such as peanuts and corn, mostly as a co-contaminant with aflatoxin (Lisker et al., 1993).

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Environmental conditions and management practices during production, harvest, handling, and storage may affect the nature and degree of mycobiota contamination (Hill et al., 1983). Soil serves as a major source of inoculum of the *A. flavus* and *A. parasiticus* in crops (Horn et al., 1994).

Mycotoxins may produce adverse effects on humans and animals, and since peanuts are an important food commodity, the objectives of this study were: 1) to determine the mycloflora of soil, air, peanuts flowers, peg, seed and peanut kernels collected in the field at different maturity stages; 2) to determine the occurrence of aflatoxins B_1, B_2, G_1 and G_2, cyclopiazonic acid and fumonisin B_1 and B_2 in the seeds, at different stages of maturity of peanut kernels and the kernels after drying.

2. Materials and methods

2.1. Peanuts development stage

The six samplings were based on peanuts phenological events. 1) During sow planting, before germination (DS): for seed, air and soil; 2) Beginning Bloom (BB): for flowers, air and soil; 3) Peg (PG),at the Beginning of Pod formation (BP) and Beginning of Pod Filling (BPF): for pegs, pods, air and soil; 4) Pod Filling (PF): for pods, air and soil; 5) Full Pod Maturity (FPM): for pods, air and soil; 6) Dried Pod (DP): for pods after drying.

2.2. Peanut samples

The peanut seeds (Runner IAC 886) were cultivated in a farm located at Junqueiropolis, São Paulo State, Brazil. The peanuts were sampled in four different maturity stages and after drying. The area was divided into 10 uniform parcels of 80 m^2, and five parcels were chosen for each sampling. Five nearly 1.0 kg samples were collected at the same time. In the all material collected, the shells of the pods were removed and the peanut kernel samples were assayed for mycobiota, aflatoxin, cyclopiazonic acid, fumonisin and water activity.

2.3. Soil samples

Twenty-five samples were collected from the surface (0–5 cm depth), containing approximately 1.0 kg at each maturity stage. The bulk five soil samples were homogenized and assayed for mycobiota and water activity.

2.4. Water activity determinations

The water activity (a_w) of soil, seeds, flowers, peg, and kernels of peanut samples was determined by automatic analysis, using Aqualab CX-2 (Decagon Devices Inc., Pullman, WA).

2.5. Climatic conditions of the peanuts field

The determination of the prevailing climatic factors was done with specific equipment such as thermometers and rain gauges. Soil temperatures were measured by thermometer, and the moisture (%) and pH were measured at 5 cm deep by a soil tester (Demetra E.M. System Soil Tester, Tokyo, Japan).

2.6. Recovery, identification and enumeration of the mycobiota from soil, seed, flowers, peg and peanuts samples

2.6.1. Disinfection of seed, flowers, peg and pod of peanuts

From each sample, approximately 30 g subsamples of each vegetal material was disinfected by immersion in 2% sodium hypochloride solution for 3 min, followed by three rinses with sterile distilled water.

2.6.2. Isolation of mycobiota from seed, flowers, peg and kernels of peanuts

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 Dichloran Rose Bengal Chloramphenicol agar media (DRBC). Further selection of fungi producing aflatoxins was done on *A. flavus* and *A. parasiticus* Agar (AFPA). The fungal colonies recovered were identified according to recommended methods for each genus (Raper and Fennel, 1965; Barnett and Hunter, 1972; Nelson et al., 1983).

2.6.3. Isolation of mycobiota from soil samples

Soil samples were analyzed according to the method described by Swanson et al. (1992) using the Martin medium for mycobiota isolation. The plates were incubated at 25 °C for ten days, but the observations were made daily. The colonies were recovered and identified according to the recommended methods for each genus (Raper and Fennel, 1965; Barnett and Hunter, 1972; Nelson et al., 1983).

2.7. Determination of wind-dispersed fungi

The samplings of wind-dispersed fungi were done before silk emergence. For each period, 10 Petri dishes containing sterile Sabouraud agar had the lid removed, were put at 1.50 cm above the ground lids were removed and the dishes kept open for 15 min to allow deposition of wind-dispersed fungal spores (Gambale et al., 1983). The plates were then incubated at 25 °C for 7 days, and the fungal colonies identified according to the recommended methods for each genus (Raper and Fennel, 1965; Barnett and Hunter, 1972; Nelson et al., 1983). The analysis of wind-dispersed fungi was carried out throughout the period of soil and peanut samplings.

2.8. Aflatoxin analysis

The aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2) in peanut kernel samples were extracted according to the method of AOAC 49.12.18 (AOAC, 2000). Briefly, 25 g of peanut kernels were added to 5 g NaCl and 125 mL of methanol/water solution (7:3 v/v) and stirred for 30 min. The extract was filtered through filter paper (Whatman 2V) and 30 mL of water