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# SURVEY OF MONILIFORMIN IN CORN CULTIVATED IN THE STATE OF SÃO PAULO AND IN CORN PRODUCTS COMMERCIALIZED IN THE CITY OF CAMPINAS, S.P.

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**SHORT COMMUNICATION**

## ABSTRACT

Moniliformin, a toxin produced by *Fusarium*, was investigated in 22 samples of corn, 17 coming from districts of the state of São Paulo and 4 from experimental plots of the Campinas Institute of Agronomy, Brazil. The toxin was also investigated in 68 samples of corn products commercialized in Campinas, SP. Moniliformin was not detected in any sample.

**Key words:** mycotoxins, moniliformin, corn, *Fusarium* toxins

Moniliformin is a toxin produced mostly by *Fusarium subglutinans* (Wollenw. & Reink.) Nelson, Toussoun & Marassas (22,26). It was first isolated and characterized by Cole *et al.* (7), who reported a $LD_{50}$ of 4.0 mg/kg b.w. (p.o.) for 1 day old cockerels and described plant-growth regulating and phytotoxic effects of moniliformin on plants. A larger $DL_{50}$ (5.4 mg/kg) was found for day old cockerels by another group of workers (5). Kriek *et al*. (14) described the toxic symptoms of moniliformin in ducklings and rats as progressive muscular weakness, respiratory distress, cyanosis, coma and death. The oral $LD_{50}$, reported by this group of researchers, was 3.68 mg/kg in 7 day old ducklings and 50.0 and 41.57 mg/kg in male and female rats, respectively.

Very low levels of moniliformin were observed to cause chromosomal aberration in liver cells in vitro (12). However, moniliformin showed negative results for the Ames test for mutagenicity (12,33).

The presence of 50 mg moniliformin/kg in the feed of broiler chickens caused increased heart weight (19). Moniliformin and fumonisin $B_1$ in broiler chick feed has shown an additive effect in causing mortality (15), but a somewhat less than additive effect happens between deoxynivalenol and moniliformin inducing smaller feed intake and weight gain in broiler chicks (10).

Some experiments indicate moderate heat stability for this toxin. Up to 68 to 77% of the toxin was observed to remain when the potassium salt of moniliformin present in corn and wheat kernels was kept at room temperature during 6 days and when the grain was heated at 50ºC during 2 hours. At higher temperatures, such as 100 to 150ºC, only 38% of the salt was present after 2 hours (27,28).

Moniliformin producing *Fusarium* spp. have been reported in several parts of the world, such as, Argentina, Austria, Canada, Germany, Italy, Poland, New Zealand, Peru, South Africa, U.S. and parts of Asia and Africa (2,3,4,8,9,17,21, 24,26,29,30). Besides *F. subglutinans*, also strains of *F. acuminatum*, *F. anthophilum F. avenaceum*, *F. concolor*, *F. denticulatum, F. equiseti*, *F. fujikuroi, F. fusarioides*, *F. moniliforme (*now called *F. verticilioides)*, *F. oxysporum*, *F. proliferatum, F. ramigenum, F.sambucinum, F. semitectum, F. succisae, F. tricinctum,* and *F. thapsinum*, were detected. The mentioned reports plus others found in the literature indicate that strains of *F. moniliforme* (*F. verticilioides*) rarely produce the toxin (1,11,16,22,25,28). Some authors even question its toxigenicity (23,29,32).

Moniliformin have been found in corn in Canada, Germany, and New Zealand (18,28,31), and in wheat in Austria and Poland

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(2,20). Low levels of the toxin (< 0.25 mg/kg) were determined in samples of corn products commercialized in the UK. The levels of toxin in corn samples from Africa, Asia and the European Union, varied from not detectable to 3.16 mg/kg. Samples of *Fusarium* damaged oat, wheat and triticale were also found contaminated at levels from 0.05 to 399.3 mg/kg (30).

There have been no reports of surveys for the presence of moniliformin in Brazilian commodities. The present work aimed to investigate the presence of moniliformin in commercial corn planted in the state of São Paulo and corn products commercialized in the city of Campinas, SP.

Eighteen samples of freshly harvested corn were collected by personnel from the "Coordenadoria de Assitência Técnica de Pessoal de Nível Superior" (CATI) during transfer of corn to commercial silos located at the corn producing districts of Valentin Gentil, Bariri, Reginópolis, São Manuel, Votuporanga, Cafelândia, Olímpia, Cuna, Analândia, Casa Branca, Lutécia, Salto de Pirapora, São Sebastião da Grama, Santópolis do Aguapeí, Jales, Lorena (2 samplings), and Taubaté, all within the state of São Paulo, Brazil. The sampling was conducted by taking about 200 g at time intervals suitable to the amount of corn being transferred. The bulk sample was mixed and reduced by quartering and a 500 g sample was destined to moniliformin determination. All samples were stored in plastic bags at –18ºC, and prior to analysis the grain was ground to 20 mesh.

Corn samples were also collected at four experimental plots of the Campinas Institute of Agronomy (IAC). They belonged to the cultivars "Cateto" (Jundiaí and Campinas), "Moroti" (Monte Alegre do Sul), "Taioba" (Campinas). A random block experimental design with three repetitions was used for each cultivar. The plots had 5 plants per $m^2$ aligned in four 5-meter rows with 0.9 m distance between rows. All ears were harvested in the two central rows of each plot. The kernels were shelled and combined. Samples were homogenized and reduced by quartering to 500g, stored in plastic bags at –18ºC, and ground to 20 mesh prior to analysis.

Sixty-eight samples of corn products (25 corn flour, 24 popcorn, 19 corn for "canjica") were acquired at retail points in the city of Campinas, state of São Paulo, in amounts of at least 500g. The samples were stored in plastic bags at -18ºC. Prior to analysis, the samples of "canjica" and popcorn were ground to 20 mesh.

Moniliformin, sodium salt, was obtained from the Council for Scientific and Industrial Research, South Africa. Concentrated solutions were prepared by weighing the salt to the nearest 0.001 mg and dissolving it in a volumetric flask using methanol (Merck, Lichrosolv grade). Working standards were then prepared by dilution according to the need. Standard solutions were sonicated before use.

Five grams of sample were weighed and extracted with 50 mL methanol/water (95+5) in a blender at medium speed during 5 minutes. The mixture was filtered through fluted filter paper. An aliquot of 35 mL of the filtrate was collected and transferred to a separatory funnel. The sample extract was deffated twice with portions of 100 mL and 50 mL hexane. An aliquot of 35 mL was taken from the methanolic fraction and dried in a rotary evaporator at 40ºC. The dried residue was dissolved in 3 mL water and sonicated prior to the liquid chromatography step.

The extract was injected into a liquid chromatograph (model 9010, with integrator 4400, Varian) with the following conditions: injector loop, 20 µL; analytical column, Spherisorb ODS-1, 5 µm, 4.6 mm x 250 mm (Altech); mobile phase, 42% of 350 mg citrimide and 500 mg zinc sulfate hepta hydrate/L plus 48% methanol, at a flow rate of 0.8 mL/min; ultraviolet diode array detector set at wavelength, 263 nm (Polichrom 9065, Varian).

The detection limit of the analytical method was 0.3 mg/kg, determined by visually establishing the smallest peak that the analyst could comfortably see in the chromatogram. The calibration curve was linear up to 14 mg/mL. The average recovery of the method was 84%.

The samples of corn collected at silos right after harvest numbered 18. The corn storing silos were located at 17 districts of the state of São Paulo. The silos stored the commercial production of the area and were sampled during the process of filling the silos with the freshly harvested corn. The samples were all negative for moniliformin. The degree of infection of the samples by *Fusarium* spp was high, and varied between 16% and 80%, as previously reported (6).

The four samples of corn harvested at experimental plots of the Campinas Institute of Agronomy (IAC) were also negative for moniliformin.

The samples of corn products commercialized in the city of Campinas, SP were also negative for moniliformin.

Results indicate that moniliformin is not a common contaminant in corn planted within the state. The samples of commercial corn products followed the same pattern. The origin of the corn used in these products is not known and it may have included imported corn. However, it should be noted that mycotoxin contamination can vary according to many factors. The genus *Fusarium*, although capable of growing on cereals during storage, it is mostly a field fungi. So, factors like weather conditions, *Fusarium* species, strains predominating during kernel growth and maturing stages, and types of corn hybrids being planted, may change the picture reported by the present work.

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## RESUMO

### Investigação de moniliformina em milho cultivado no estado de São Paulo e em produtos de milho comercializados na cidade de Campinas, SP

Moniliformina, uma toxina produzida por *Fusarium*, foi pesquisada em 22 amostras de milho, sendo 18 provenientes de 17 municípios do Estado de São Paulo, e 4 de estações experimentais do Instituto Agronômico de Campinas. A toxina foi investigada também em 68 amostras de produtos de milho comercializados em Campinas, SP. Moniliformina não foi detectada em nenhuma das amostras estudadas.


**Palavras-chave:** micotoxinas, moniliformina, milho, toxinas de *Fusarium*.

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