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FUMONISINS IN CORN CULTIVARS IN THE STATE OF SÃO PAULO

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ABSTRACT

Twenty three samples, belonging to 19 corn cultivars with distinct types of germoplasms, endosperm and length of vegetative cycle, were analyzed for fumonisins B_1 and B_2. The cultivars were grown in experimental fields in three locations (Votuporanga, Ribeirão Preto and Capão Bonito) within the State of São Paulo, Brazil, during the 97/98 crop. All samples were contaminated with fumonisins with concentrations ranging from 1.63 µg/g to 25.69 µg/g with an average of 5.61 µg/g for FB_1 and from 0.38 µg/g to 8.60 µg/g with an average of 1.86 µg/g for FB_2. In terms of fumonisins, these high levels put the corn cultivated in São Paulo among the most contaminated in the world reported to date.

Key words: mycotoxins, fumonisins, *Fusarium*, corn.

INTRODUCTION

Corn is the most produced grain in Brazil and the one with the largest cultivation area. In São Paulo, the Instituto Agronômico de Campinas (IAC) and the Coordenadoria de Assistência Técnica Integral (CATI), both under the Secretaria de Agricultura e Abastecimento, Brazil, and the commercial seed producing companies have developed a partnership to test commercial maize cultivars in terms of productivity, resistance to diseases and adaptability to the maize growing areas of the state.

Corn is constantly exposed to toxigenic fungi (26). Several fungi species have been associated to corn, mainly the ones from the genera *Fusarium*, *Aspergillus* e *Penicillium*. This is a cause of concern because these same genera have species capable of producing a wide array of compounds shown to be toxic to man and animals (8).

Fumonsins are a group of mycotoxins produced mainly by *Fusarium moniliforme* and *F. proliferatum* and to a lesser extent by other species of the Liseola section. *F. moniliforme* is ubiquitous and can produce asymptomatic infections in corn (6). Among the fumonsins characterized so far, FA_1, FA_2, FA_3, FB_1, FB_2, FB_3, FB_4, FC_1, FC_3, FC_4, FAK_1, FP_1, FP_2, FP_3, FPH_{1a} and FH_{1b} (10,16,19), FB_1 is the most toxic and the most abundant comprising from 60 to 90% of the fumonsins found in lab cultures and corn samples (10,16,23). It has been shown that FB_1 causes leukoencephalomalacia in horses (ELEM) (15), pulmonary edema in swine (SPE) (11), brain hemorrhage in rabbits (2) and liver cancer in mice (5). Esophageal cancer in humans has been related to the presence of fumonsins in the diet of populations in China (25) and in South Africa (20).

In Brazil, ELEM has been reported in the states of Rio Grande do Sul, São Paulo, Santa Catarina, Minas Gerais (9), and Paraná (19, 24). Corn stored in silos in the states of Paraná, Mato Grosso do Sul and Goiás has shown to be contaminated with FB_1 and FB_2 (7). More information is needed about the possible presence of fumonisins in corn being cultivated in the country in order to allow the necessary measures to be taken by the government agencies involved with the production, regulation and control of foods and feeds.

The present work aimed to check for FB_1 and FB_2 in freshly harvested commercial cultivars corn planted in different locations of the state of São Paulo. The locations chosen

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belong to areas where corn constitutes an important commercial crop.

MATERIALS AND METHODS

Samples: Twenty three samples of corn corresponding to 19 commercial cultivars (Table 1) were harvested at the Experimental Stations of Capão Bonito, Ribeirão Preto and Votuporanga, belonging to the Agronomic Institute of Campinas. All ears were harvested in the two central rows of each cultivar plot. The kernels were shelled and combined. The samples were homogenized and reduced by either quartering or riffling to 500g. The samples were kept in plastic bags at –18°C until analysis. The grain was ground to 20 mesh prior to analysis.

Fumonisins B_1 and B_2 standard solutions: Individual stock solutions of FB_1 and FB_2 (Sigma Chemical Company, St. Louis, MO) were prepared dissolving 1 mg in acetonitrile/water (1+1) according to Visconti *et al.* (23). Working solutions were then prepared with concentrations of 50 µg/ml of FB_1 and FB_2, respectively. All standard solutions were kept in amber flasks at -18°C when not in use.

Fumonisins determination: The determinations of fumonisins were conducted by HPLC with fluorescence detection according to Shephard *et al.* (17) with modifications as described by Camargos *et al.* (3).

Analytical quality control: Samples were analyzed in batches of 10 and each batch included a recovery test. Duplicates were analyzed in different days and the results were corrected for the recovery of the batch. Relative standard

Table 1. Agronomic characteristics of some corn commercial cultivars planted during the growing season of 97/98 in the Experimental Stations of Capão Bonito, Ribeirão Preto and Votuporanga, from the Agronomic Institute of Campinas, São Paulo, Brazil.

Experimental Station	Cultivars	Type of cross	Endosperm type	Cycle lenght
Capão Bonito	1	double	yellow semi dent	normal
	2	double	yellow dent	normal
	3	triple	yellow semi dent	precocious
	4	double	yellow semi flint	normal
	5	triple	orange semi flint	precocious
	6	triple	orange flint	precocious
	7	single	orange flint	precocious
Ribeirão Preto	8	double	yellow dent	normal
	2	double	yellow dent	normal
	5	triple	orange semi flint	precocious
	9	variety	yellow semi dent	precocious
	10	triple	orange semi flint	normal
	11	double	yellow semi dent	precocious
	12	single	orange semi dent	precocious
Votuporanga	13	double	yellow semi dent	normal
	14	double	orange semi flint	precocious
	15	double	yellow semi dent	normal
	1	double	yellow flint	super precocious
	16	double	yellow semi dent	precocious
	17	double	orange semi dent	precocious
	18	single	orange semi flint	precocious
	19	triple	yellow semi dent	precocious
	11	double	yellow semi dent	precocious

Normal cycle - masculine flowering around 70 days after planting;
precocious cycle - around 65 days; super precocious - under 60 days.

Table 2. Levels of fumonisins in corn from the cultivars grown in the Experimental Stations of Capão Bonito, Ribeirão Preto and Votuporanga, São Paulo, Brazil, during the 97/98 crop.

Experimental Station	Cultivars	Fumonisins (µg/g)	
		B_1	B_2
Capão Bonito	1	9.49	2.76
	2	6.82	2.50
	3	3.37	0.65
	4	1.78	0.38
	5	1.63	0.45
	6	4.48	1.17
	7	8.00	2.73
Ribeirão Preto	8	12.25	3.68
	2	7.18	2.09
	5	2.57	1.01
	9	25.69	8.60
	10	8.33	2.88
	11	5.28	2.00
	12	2.76	1.02
Votuporanga	13	2.40	0.89
	14	4.13	1.62
	15	4.82	1.90
	1	4.58	1.37
	16	3.83	1.89
	17	2.31	0.56
	18	1.86	0.82
	19	2.85	0.69
	11	2.60	1.08
	Average	5.61	1.86

The results represent the average of two determinations

deviations between duplicates were considered acceptable up to 30%.

RESULTS AND DISCUSSION

Analytical quality control: the standard curves for FB_1 and FB_2 were linear in the concentrations from 0.02 to 10 µg/g and from 0.04 to 10 µg/g, respectively. The limits of detection were 0.02 µg/g for FB_1 and 0.04 µg/g for FB_2. The average recovery and the relative standard deviation were 92% and 0.6% for FB_1 and 72% and 2.2% for FB_2, respectively.

Incidence of fumonisins in freshly harvested corn: All samples analyzed contained FB_1 and FB_2 (Table 2). The ratio of FB_2/FB_1 ranged from 0.21 to 0.44 showing FB_1 by far prevailing over FB_2. FB_1 predominating over FB_2 have been more frequently reported in the literature and are considered more typical for these toxins (10,16). The highest levels of contamination were found in Ribeirão Preto, both for FB_1 and FB_2.

All samples had FB_1 above 1.0 µg/g and 35% had FB_1 above 5.0 µg/g. According to the Mycotoxin Committee of American Association of Veterinary Diagnosticians this is a level of concern for animal feed. Levels below 5.0 µg/g are recommended for horses, below 10.0 µg/g for swine and 50.0 µg/g for cattle and chickens (13). Levels above 10.0 µg/g may cause ELEM in horses (14), but levels below 7.7 µg/g FB_1 have been recorded as causing ELEM (21). Ross *et al.* (15) reported SPE cases when the feed used contained 10 µg/g de FB_1. Sydenham *et al.*(19) described cases of swine, fowl and rabbits suspected of mycotoxicoses after ingestion of feed contaminated with 7 µg/g de FB_1. Bane *et al.* (1), however, found levels of FB_1+ FB_2 higher than 31 µg/g in cases of swine mycotoxicoses. At least seven samples analyzed in the present work had levels of FB_1 and FB_2 within ranges not recommended for animal feed (Table 2).

The levels of contamination found in the maize examined in the present work (Table 2) compare with those reported in Transkei, South Africa, in areas where the high incidence of esophageal cancer have been correlated with the presence of the toxin in corn used as food (0.45-18.90 µg/g for FB_1 e de 0.15-6.75 µg/g for FB_2) (20). Linxian, province of China, is considered an area of high risk for esophageal cancer. The levels of FB_1 and FB_2 in the corn consumed by the population ranges from 0.19 to 2.96 µg/g and from 0.30 to 0.55 µg/g, respectively (25). Shephard *et al.* (18) reviewed the data on the contamination of corn by fumonsins and the upper value and the mean value of the present survey is higher than the results of the surveys reported from Benin, Canada, China, Croatia, Gambia, Italy, Nepal, Portugal, Romania, South Africa and USA. The data reported in the present work clearly indicates a threat to human health for frequent consumers of corn products and strongly suggests the subject should be further studied to assess the risk the consumption of corn products may represent for the public health.

In this study, high levels of contamination have been found in all types of cultivars (variety, single, double, and triple cross hybrids), as well as all types of vegetative cycle (normal precocious and super precocious) and types of endosperm (flint, dent, semi flint and semi dent) (Table 1 and 2) although no statistic calculation was attempted due to the low number of samples examined.

The possible geographical nature of the contamination of corn by fumonsins has been pointed out. Visconti (22) examined samples of corn genotypes grown in several parts of the world. He found that four main groups could be recognized in terms of fumonisins contamination level: Eastern Europe (Poland, Romania and Croatia) would be the group with lowest contamination, Western Europe (Italy and Portugal) with slightly higher contamination, Africa (Benin and Zambia) with still higher contamination and finally Argentina with the highest levels of contamination. The levels of fumonisins found in the present work are comparable with the levels reported for Argentinian freshly harvested corn (4,12) and indicate that high levels of contamination by fumonisins in corn might be a South American problem or at least, a problem for the south tip of the continent.

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RESUMO

Fumonisinas em cultivares de milho no Estado de São Paulo

Vinte e três amostras, representando 19 cultivares de milho com diferentes tipos de germoplasma, de endosperma e ciclo vegetativo, foram avaliadas quanto ao teor de fumonisinas em três Estações Experimentais do Instituto Agronômico (Capão Bonito, Ribeirão Preto e Votuporanga) em São Paulo, Brasil, durante a safra de 97/98. Todos os cultivares analisados estavam contaminados com fumonisinas em níveis que variaram de 1.63 µg/g a 25.69 µg/g e uma média de 5.61 µg/g FB_1 e de 0.38 µg/g a 8.60 µg/g e uma média de 1.86 µg/g FB_2. Estes níveis tão elevados colocam o milho cultivado no Estado de São Paulo entre os mais contaminados do mundo em termos de fumonisinas.

Palavras-chave: Micotoxinas, fumonisinas, *Fusarium*, milho.

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