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Incidence of *Fusarium verticillioides* and Levels of Fumonisins in Corn from Main Production Areas in Iran

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A total of 52 corn samples collected in 2000 from four main corn production provinces of Iran (Fars, Kermanshah, Khuzestan, and Mazandaran) were analyzed for contamination with *Fusarium verticillioides* and fumonisins (FB_1, FB_2, FB_3, and 3-*epi*-FB_3). The mean incidence of *F. verticillioides* (percent of kernels infected) for these four areas was 26.7, 21.4, 24.9, and 59.0%, respectively. The incidence in Mazandaran was significantly ($p < 0.05$) above that of the other areas. All samples from Mazandaran were contaminated with fumonisins with a mean level of total fumonisins of 10674 μg/kg. In contrast, the incidence of fumonisin contamination above 10 μg/kg was 53 (8/15), 42 (5/12), and 57% (8/14) in the samples from Fars, Kermanshah, and Khuzestan, respectively, and the corresponding mean total fumonisin levels were 215, 71, and 174 μg/kg, respectively. No statistical differences ($p > 0.05$) were observed in the fumonisin levels of the corn samples from these three provinces, which were significantly ($p < 0.05$) lower than the fumonisin contamination in samples from Mazandaran.

KEYWORDS: Corn (*Zea mays*); maize; fumonisin; *Fusarium verticillioides*; Iran

INTRODUCTION

The fumonisins are a group of allied mycotoxins produced mainly by *Fusarium verticillioides* (Sacc.) Nirenberg (= *Fusarium moniliforme* Sheldon) and *Fusarium proliferatum* (Matsushima) Nirenberg, which infect corn (*Zea mays* L.) crops worldwide (*1*). Fumonisins can be found almost wherever corn is grown, probably with the exception of cold climatic areas such as the northeastern parts of Europe and Canada (*2*), although some samples from these areas have been shown to contain fumonisins (*3*). FB_1, FB_2, and FB_3 have been reported to occur naturally at high levels in corn-based animal feeds associated with field outbreaks of leukoencephalomalacia (LEM) in horses and pulmonary edema syndrome (PES) in pigs (*4*). On the basis of available toxicological evidence, the International Agency for Research on Cancer in 2002 classified FB_1 as possibly carcinogenic to humans (group 2B carcinogen) (*5*). The fumonisins have been statistically associated with a high incidence of esophageal cancer (EC) in certain areas of the former Transkei, South Africa (*6*), and have been shown to occur at high levels in corn in EC endemic areas in China (*7*), Zimbabwe (*8*), the United States (*9*), Italy (*3*), Iran (*10*, *11*), Kenya (*12*), and Brazil (*13*). It has been suggested that they may also play a role in the promotion of primary liver cancer in certain endemic areas of China (*14*) and have been implicated in the etiology of neural tube defects in a high corn-consuming population in the southeastern United States (*15*).

In Iran, corn is one of the most important agricultural commodities produced and imported annually, because it is the major component of animal feed. However, only limited data are available on the fungal and mycotoxin contamination of Iranian corn and on the ability of local fungal strains to produce mycotoxins. The fumonisin contamination levels in the corn of

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Mazandaran, a high EC endemic area (*16*), were higher than those in corn sampled in Isfahan (*10*, *11*). Recent mycotoxin studies on corn samples from Iran have shown contamination with different mycotoxins including fumonisins, aflatoxins, and ochratoxin A (*10*, *11*, *17*). Previous analyses of farm rice, wheat, and corn kernels showed the presence of *F. verticillioides* and *F. proliferatum* as the most common seed-borne fungi in the Mazandaran province in northern Iran (*18*, *19*). A recent study on the fungal mycoflora associated with corn kernels in the main corn-producing areas of Iran revealed that the incidence of *F. verticillioides* in Mazandaran was significantly higher than two other provinces, Khuzestan and Kermanshah (*20*). Furthermore, production of fumonisins and aflatoxins by Iranian fungal isolates in culture has been demonstrated (*19*, *21*).

Unlike northern Iran, little is known about fungal flora and mycotoxin contamination of corn and other cereals in other parts of Iran. In this study, corn intended for human and animal consumption in Iran in 2000 was collected from four main corn production areas (Fars, Khuzestan, Kermanshah, and Mazandaran Provinces) in order to investigate the natural occurrence of *F. verticillioides* and fumonisins.

MATERIALS AND METHODS

Corn Samples. A total of 52 samples (1.5−5 kg) of corn kernels harvested in 2000 were collected from the main corn-producing areas of Iran at 18 locations in four provinces, i.e., Fars (15 samples), Khuzestan (14 samples), Kermanshah (12 samples), and Mazandaran (11 samples). Most of the samples were intended for animal feed and some for human consumption. The corn cultivar in all provinces was the same, i.e., Yellow Single Cross 704.

Mycological Analyses. *F. verticillioides* counts were determined by dilution plating. Finely ground corn (1 g) was mixed thoroughly with sterile distilled water (9 mL), followed by 10-fold serial dilutions up to 10^{-8}. An aliquot (1 mL) of each dilution was added to a Petri dish containing melted potato dextrose agar (15 mL). The dishes were agitated, allowed to set, and incubated at 25 °C for 7 days. The appropriate dilution factor was selected by choosing the Petri dishes containing 10−30 colonies. The results were expressed as colony-forming units per gram (cfu/g) of sample.

F. verticillioides incidence was evaluated by direct plating. Briefly, kernels from each sample were surface disinfected for 1 min with a 1% sodium hypochlorite solution, rinsed twice in sterile distilled water, and dried in a safety cabinet. One hundred kernels per sample were plated onto Nash−Snyder agar containing 500 mg/L of chloramphenicol (*22*). The plates were incubated at 28 °C for 5−7 days. Final identification of *F. verticillioides* colonies recovered in both methods was made following Nelson et al. (*22*).

Analyses of Fumonisins in Corn Samples. Fumonisins were extracted from corn by the method of Sydenham et al. (*23*). Briefly, samples were extracted by homogenization with methanol/water (3:1). After centrifugation at 1500*g* for 10 min at 4 °C, the mixture was filtered and the pH was adjusted to 6.0 with 1 M sodium hydroxide. An aliquot was applied to a strong anion exchange solid-phase extraction cartridge (Bond-Elut, Varian, Harbor City, CA) containing 500 mg of sorbent, which had been conditioned with methanol and methanol/water (3:1). After they were washed with methanol/water (3:1) and methanol, the fumonisins were eluted from the cartridge with 1% acetic acid in methanol. The eluates were evaporated to dryness at <60 °C under a stream of nitrogen gas and redissolved in 200 μL of methanol immediately prior to derivatization and high-performance liquid chromatography (HPLC) analysis.

Fumonisin standards were isolated and purified according to the method of Cawood et al. (*24*). Standard solutions were prepared containing FB_1 (245 μg/mL), FB_2 (200 μg/mL), and FB_3 (285 μg/mL) in acetonitrile/ water (1:1). Fumonisins (FB_1, FB_2, FB_3, and 3-*epi*-FB_3) were determined by the HPLC method with fluorescence detection described by Sydenham et al. (*23*). An aliquot of the purified extract was derivatized with *o*-phthaldialdehyde solution and injected into a

Table 1. Incidence of *F. verticillioides* Contamination in Corn from Four Main Production Areas in Iran in 2000

		F. verticillioides (cfu/g[a] × 10^2)		kernels infected (%)	
province	*n*	mean	range	mean	range
Fars	15	17.9 ± 43.2[b] a[c]	ND[d]–153	26.7 ± 43.2[b] a	2–88
Khuzestan	14	96 ± 304 a	ND–1150	24.9 ± 32.8 a	2–100
Kermanshah	12	5.1 ± 7.9 a	ND–25	21.4 ± 17.4 a	4–48
Mazandaran	11	425 ± 457 b	1–1200	59 ± 37.2 b	10–100

[a] Colony forming units/g milled corn. [b] Standard deviation. [c] Means followed by the same letter are not statistically different ($p > 0.05$), and means followed by a different letter are statistically different ($p < 0.05$). [d] Not visibly detected at lowest dilution.

HPLC system consisting of a Waters model 590 pump (Milford, MA) connected to a Waters 470 scanning fluorescence detector. The separations were performed on a reversed-phase Ultracarb 5 ODS column (150 mm × 4.60 mm id, Phenomenex, Torrance, CA). The mobile phase was methanol/0.1 M sodium dihydrogen phosphate (75:25) adjusted to pH 3.35 with *o*-phosphoric acid and pumped at a flow rate of 1 mL/min. Fumonisins were quantified by peak area measurement in comparison with fumonisin standards. Recoveries were determined in corn samples spiked at levels of 1037, 1058, and 1015 μg/kg. Mean recoveries were 78.3 ± 3.05 (SD), 81.2 ± 2.7, and 77.6 ± 3.4 for FB_1, FB_2, and FB_3, respectively. Analytical values were not corrected for recovery.

Statistical Analysis. Statistical analyses were performed using SPSS version 9.0 (SPSS Inc. Chicago, IL). The results were statistically analyzed using one-way analyses of variance.

RESULTS

Table 1 shows the incidence (% kernels infected) and cfu/g of *F. verticillioides.* Contamination with *F. verticillioides* was significantly higher ($p < 0.05$) in Mazandaran with a mean incidence of 59%, while the incidences in the samples from the other three provinces ranged from 21.4 to 26.7%. Similarly, the mean number of cfu/g for *F. verticillioides* in corn from Mazandaran province was significantly higher ($p < 0.05$) than the values in the samples from the other provinces.

Table 2 shows the incidence of fumonisin contamination and the levels of FB_1, FB_2, FB_3, and 3-*epi*-FB_3 (*25*) as well as total fumonisins determined in corn samples in this study. FB_1, FB_2, FB_3, and 3-*epi*-FB_3 were detected in 61.5 (32/52), 34.6 (18/52), 30.8 (16/52), and 19.2% (10/52) of all 52 corn samples, respectively (detection limit = 10 μg/kg). FB_1, FB_2, and FB_3 were present in all of the corn samples obtained from Mazandaran with levels ranging from 1687 to 11015, 383 to 3364, and 52 to 900 μg/kg, respectively. For samples obtained from the other three provinces, fumonisin levels ranged from not detected to 949 μg/kg for FB_1, not detected to 329 μg/kg for FB_2, and not detected to 64 μg/kg of FB_3. The incidence of contaminated samples was 21/41 (51%), 7/41 (17%), and 5/41 (12%) for FB_1, FB_2, and FB_3, respectively. Overall, 10 samples (seven from Mazandaran) were found to contain the minor fumonisin analogue 3-*epi*-FB_3, although the maximum level found was 191 μg/kg determined in one sample from Mazandaran. It accounted on average for only 1.8% (maximum 2.8%) of the total fumonisin in these samples. Mean fumonisin (FB_1, FB_2, FB_3, and 3-*epi*-FB_3) levels were significantly higher ($p < 0.0001$) in corn from Mazandaran than those in corn from the other three provinces. The highest level of total fumonisins (15447 μg/kg) was determined in a corn sample collected from Mazandaran. There were no statistical differences in the fumonisin levels between the corn samples of Fars, Khuzestan, and Kermanshah ($p > 0.05$). Whereas only 7.3% (3/41) of corn

Table 2. Incidence and Levels of Fumonisins in Corn from Four Main Production Areas in Iran in 2000

	incidence (positive/total)				μg/kg									
					FB_1		FB_2		FB_3		3-*epi*-FB_3		total FB	
province	FB_1	FB_2	FB_3	3-*epi*-FB_3	mean[a]	range	mean	range	mean	range	mean	range	mean	range
Fars	8/15	2/15	2/15	1/15	173 ± 303[b]	ND[c]–949	33 ± 91	ND–306	7 ± 20	ND–64	1 ± 4	ND–15	215 ± 404	ND–1228
Khuzestan	8/14	3/14	2/14	1/14	139 ± 197	ND–732	28 ± 88	ND–329	5 ± 13	ND–48	2 ± 7	ND– 29	174 ± 297	ND–1138
Kermanshah	5/12	2/12	1/12	1/12	57 ± 104	ND–342	11 ± 30	ND–106	3 ± 10	ND–33	1 ± 4	ND–13	71 ± 143	ND– 494
Mazandaran	11/11	11/11	11/11	7/11	7811 ± 3070	1687–11015	2158 ± 948	383–3364	596 ± 283	52–900	110 ± 87	ND–191	10674 ± 4283	2123–15447

[a] Mean of all samples. [b] Standard deviation. [c] ND, not detected (<10 μg/kg).

samples obtained from these areas had total fumonisin levels higher than 1000 μg/kg, 91% (10/11) of corn samples obtained from Mazandaran had total fumonisin levels higher than 5000 μg/kg and 64% (7/11) had total fumonisin levels higher than 10000 μg/kg.

DISCUSSION

The potential presence of fumonisins in cereals and processed foods is a serious threat to public health. Surveys of the natural occurrence of fumonisins in corn are necessary to accurately assess human and animal exposure to these toxins. The significance of *F. verticillioides* lies not only in its symptomless infection of corn kernels but also in its toxigenicity (*5*). The high incidence of *F. verticillioides* in Mazandaran supports a previous report on the high incidence of this fungus (*26*), as well as high levels of fumonisins in corn from this area (*10*, *11*). The high incidence of *F. verticillioides* as well as the high fumonisin levels in Mazandaran are in agreement with its climatic conditions, which from 1981 to 2000 relatively among the areas in this study, had the highest mean rainfall (672.6 mm; $p < 0.001$), highest mean relative humidity (68.8%; $p < 0.001$), and the lowest minimum altitude (*20*). Such findings match the theory proposed by Shelby et al. (*27*) whereby the levels of fumonisins in raw corn are influenced by environmental factors such as temperature, humidity, and rainfall during preharvest and harvest periods. The dominance of *F. verticillioides* in Iranian corn is in accordance with other reports that *F. verticillioides* is regarded as the most common seed-borne fungus of corn worldwide (*1*). The Mazandaran province on the Caspian littoral of Iran is an area of high EC incidence (*16*). It is interesting to note that similar to other areas of high EC incidence, such as the former Transkei region of South Africa (*6*), corn grown in Mazandaran is significantly more contaminated with *F. verticillioides* than the corn in Fars, Khuzestan, and Kermanshah, areas of low EC incidence.

The fumonisin levels determined in corn samples from these four provinces follow the same trend as the *F. verticillioides* contamination, namely, relatively low levels in Fars, Kermanshah, and Khuzestan and significantly higher levels in Mazandaran. The mean levels in the former three provinces reflect the low levels previously found in Isfahan (*10*, *11*; **Table 3**) and are comparable with good corn produced in other corn-growing regions (*28*). In contrast, the fumonisin levels in the harvest from Mazandaran were significantly higher than in the other three areas. The mean FB_1 level (7811 μg/kg**)** in corn samples from Mazandaran in the current study also exceeded by 3.4 and 2.5 times the mean values of 2270 and 3180 μg/kg observed by Shephard et al. in samples from Mazandaran in 1998 and 1999, respectively. Overall, the mean levels of FB_1, FB_2, and FB_3 from Mazandaran in the current study were higher than those found in previous studies in Iran (*10*, *11*; **Table 3**) and are comparable with the high levels previously reported in areas of high EC incidence. Maximum total fumonisin levels in corn associated with high EC areas of the former Transkei in South Africa were 10150 (mean, 2100 μg/kg) and 6700 μg/kg (mean, 1960 μg/kg) in healthy corn for the 1985 and 1989 crop years, respectively (*6*). The maximum total fumonisin level (15447 μg/kg) in corn sampled in the current study from Mazandaran, which had no visible signs of mold contamination, was much higher than the healthy corn from Transkei region (*6*). Chu and Li found FB_1 in 15 healthy corn samples collected from Cixian and Linxian Counties, the regions with high incidences of EC in China, at maximum levels of 60000 μg/kg (mean, 35300 μg/kg), which was much higher than FB_1 levels in Iran and South Africa (*7*). Ueno et al. detected FB_1, FB_2, and FB_3 at levels of 160−26000, 160−6770, and 110−4130 μg/kg, respectively, in 40 corn samples collected in Haimen, a high risk area for primary liver cancer in China (*14*).

Table 3. Fumonisin Levels in Corn from Mazandaran and Isfahan Provinces

			mean fumonisin levels (μg/kg)			
province	year	incidence (positive/total)	FB_1	FB_2	FB_3	ref
Mazandaran	1998	11/11	2270	510	360	*10*
	1999	20/20	3180	NM[a]	NM	*11*
Isfahan	1998	8/8	170	60	60	*10*
	1999	6/10	220	NM	NM	*11*

[a] NM, not measured.

The U.S. Food and Drug Administration has set guidance levels for fumonisins ($FB_1 + FB_2 + FB_3$) in which the recommendations for corn products intended for human foods, such as flaking grits, corn grits, corn meal, and cleaned corn for popcorn or masa production, range between 2000 and 4000 μg/kg (*29*). Guidance levels in animal feed that have been recommended in the United States range from 5000 μg/kg total fumonisins (no more than 20% of diet) in equids and rabbits to 100000 μg/kg (no more than 50% of diet) in poultry being raised for slaughter. Iran is one of a few countries that have legislated a maximum tolerated level (MTL) for fumonisins in corn, which in the case of Iran is 1000 μg/kg for $FB_1 + FB_2$ (*30*). Clearly, corn harvested in Fars, Khuzestan, and Kermanshah in 2000 mostly meets this MTL, whereas corn from Mazandaran was contaminated with levels an order of magnitude greater than the Iranian MTL. According to Ross et al., a concentration of FB_1 greater than 10 mg/kg in horse feed could be related to LEM (*4*), whereas Thiel et al. (*31*) suggested that lower FB_1 concentrations (7.7 mg/kg) may be associated with LEM. Sixty-four percent (7/11) of the Mazandaran corn samples in this study were above this latter level for FB_1. In comparison with the naturally occurring fumonisin levels reported in the literature, it is highly probable that corn containing FB_1 at the levels found in the 2000 Mazandaran corn samples would cause LEM.

This is the first report on the natural occurrence of fumonisins (including 3-*epi*-FB_3) in corn from such a large corn production region including different meteorological areas of Iran. The high levels of *F. verticillioides* and fumonisin contamination found in corn from Mazandaran are a cause for concern, especially if it is consumed by local populations and livestock on a regular basis. The final end use of this corn should be carefully considered. The results emphasize the importance of continued research on mycotoxins in Iranian cereals.

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