Agricultural Journal 2 (2): 222-225, 2007

Geographic Distribution and Frecuency of *Fusarium* and Fumonisin B_1 in Mexican Corn

[1]P. Cudberto Contreras, [1]M. Rene Rosiles, [1]R. Claudia Ríos,
[2]R. Carlos Muñoz and [3]O. Victor Fuentes
[1]Instituto Nacional de Diagnóstico y Referencia Epidemiológicos, S.S.A, Mexico
[1]Laboratorio de Toxicología, Facultad de Medicina Veterinaria, U.N.A.M, Mexico
[2]Centro Interdisciplinario de Investigación para el desarrollo Integral
Regional. I.P.N. Michoacán, Mexico
[3]Centro Universitario de los Altos, Universidad de Guadalajara,
Km 7.5 Carretera a Yahualica. Tepatitlan, Jalisco, Mexico 47600

Abstract: The geographic distribution and frequency of *Fusarium* (*Liseola* sp.) and fumonisin B_1 presence in corn in Mexico was studied. For this purpose corn samples were obtained from 26 states of Mexico. *F. verticillioides* was detected in 13.22% of corn samples, there was a correlation between fungal species and the presence of fumonisin B_1 (FB_1) production (44.00%); low levels of FB were detected after 20 days of incubation (less than 7 PPM).

Key words: Fumonisins, *Fusarium*, corn, geographic, NTD, Mexico

INTRODUCTION

Maize (*Zea mays* L.) is one of the main components of the diet in Mexican people. Corn is harvested and kept until used for food. During this period of storage the fungus *Fusarium* finds and excellent media for growing in the maize plant and consequently reported as the main fungus associated with maize (Proctor *et al*., 2006).

Corn is also fed to domestic animals such as the horse, in this species Equine leukoencephalomalacia (ELEM) has being associated with the feeding of moldy corn infested with *Fusarium verticillioides* (Carson and Poppenga, 2002; Rosiles *et al*., 1996). This fungus has also been isolated from corn in other countries, such as the Linxian County of The People's Republic of China and also in the Transkei regions of South Africa (Marasas *et al*., 1979). It is interesting to ad that in these regions a high incidence of human esophageal cancer also has being reported. The incidence of Oesophageal Cancer (OC) is very high in the Transkei where home-grown maize has been shown to contain high levels of FB_1. A similar situation exists in Linxian in China, high incidence rates of Oesophageal Cancer and Neural Tube Deffect (NTD) together with high levels of FB_1 contamination of home-grown maize have being reported (Rheeder, *et al*., 1992; Sydenham *et al*., 1990; Thiel *et al*., 1992; Yoshizawa *et al*., 1994).

Fusarium vertcillioides and *Fusarium proliferatum* (Matsushima) Niremberg are two species of *Liseola* that a lso occur worldwide associated with corn derived diseases. Both species are known to produce mycotoxines, among the most outstanding are the fumonisins (Zenteno and Ulloa, 1977; Pittet *et al*., 1992). Fumonisins B_1, B_2 and, B_3(FB_1, FB_2 and FB_3), defined as fungal metabolites. They are propane-1,2,3-tricarboxilic acid diesters of an amino-dimethyl-tetra or amino-dimethyl-pentapentahydroxyeicosane (Gelderbrom *et al*., 1988; Nelson *et al*., 1993).

FB_1 is the major micotoxin produced by *F. verticillioides* and all related fungi, they are most frequently found in corn. It has been reported that they are potent cancer promoters and also responsible of outbreaks of ELEM (Carson and Poppenga, 2002; Rosiles *et al*., 1996).

A preliminary report of the occurrence of fumonisin in corn grown in Mexico was carried out by Desjardins and coworkers (1994) while studying maize collected only from the state of Nuevo León in northeast of México. Although some FB_1 is removed during the alkali treatment of maize to prepare tortillas (nixtamalisation), high levels of FB_1 have been found in some tortillas, particularly home-made tortillas in isolated rural areas. Rural areas in Africa, America and Asia are characterised by high maize consumption and poor economic status. The maize-based diet and a FB_1 intake that is several fold higher than the Provisional Maximum Tolerable Daily Intake (PMTDI) of 2 g kg^{-1} body weight/day set by the JECFA of the WHO/FAO, may play a role in NTD and/or OC In view of this report we considered of importance to study the presence of this fungal toxins in samples of corn of different states of the Mexican Republic.

Corresponding Author: P. Cudberto Contreras, Instituto Nacional de Diagnóstico y Referencia Epidemiológicos, S.S.A., Mexico

MATERIALS AND METHODS

Sampling: Samples from the studied states contained 20 corn kernels of the 1996-1997 and 50 corn kernels from the 2002-2003 crop from 26 states of Mexico. They were either hybrid or native varieties of maize destined for human consumption. Corn samples with 10-12% moisture harvested in 1997-98 and 1998 samples were collected from different states of México, they were collected by the epidemiological net of the National Institute for Diagnosis and Epidemiological Reference (INDRE). Through INDRE it was possible that in some states samples from different locations in different times were collected and studied.

Fungal isolation and identification: Samples were further divided to sub samples of 8 kernels in order to determine fungal contamination. Each subsample was surface disinfected with 0.5% NaClO for 3 min, rinsed in distilled water and blotted dry with paper towels. These sub samples were cultured on SNA (Niremberg, 1981) medium (4 kernels per Petri dish). These SNA plates were incubated at 26°C for 6 days. *Fusarium* sp. that developed from the kernels were then grown on Potato Dextrose Agar (PDA) medium. From this medium, single spore isolation was carried out on water agar (1.5%), mass transferred to Carnation Leaf Agar (CLA) and PDA and identified.

Production of fumonisin: Flasks containing 25 g of corn grains with 12% of humidity and 9 mL of distilled water were autoclaved twice for 30 min at 121°C. After cooling, corn was inoculated with an aqueous suspension of conidia (1 mL of 10^7 from the CLA culture) and incubated in dark at 24°C for 20 days. To avoid clump formation, the cultures were hand-shaken every week. Fumonisin was extracted from corn grains after washed, with 50 mL of methanol-water (3: 1) and finely ground with a laboratory mill by 15 seconds, filtered through a Watman no. 4 filter paper. An aliquot (4 mL) of the filtered extract was applied to a Bond-Elut Strong Anion-Exchange (SAX) cartridge (Varian Harbor City, CA. USA), previously conditioned by the passage of 0.5 mL methanol/water (3: 1) and then was centrifugated at 250 (CPM) for 1 min.

The cartridge was washed twice by centrifugation 250 CPM with 3 mL methanol/water and 3 times with methanol. And 3 more times with acetic acid/methanol 5% to elute the mycotoxine. The eluate was evaporated to dryness at 40°C under a mild stream of nitrogen.

The residue after cleanup was redissolved in 100 μL of methanol. An aliquot (20 μL) of this solution was placed on the thin layer chromatoplate reverse phase, previously activated at 120°C by 10 min. At the same time spots of the FB_1 standard solution (10 μL) at 100 PPM were also included.

The run was carried out in a mobile phase methanol/demineralized water (70: 30, v:v) allowing to reach 90% of the plate.

The plate was dried by air flow and atomized with vainillina 0.5% in sulfuric acid 97%/ ethanol 4: 1. The plates were dried again by air and 3 min at 80°C to detect FB_1 that when present forms violet bands.

This methodology has been compared against high performance liquid chromatography showing a 20% variation coefficient.

Fumonisin analysis: The content of fumonisin in the corn samples that were *Fusarium* positive, was compared with the first samples and with known *Fusarium* species incubated on kernels, using a modified version of Pittet's thin layer chromatography (TLC) (Pittet *et al*., 1992).

RESULTS AND DISCUSSION

In Table 1 listed are the samples obtained from the different states of Mexico during the crops of 1996-1997 and 2002-2003 and the results of the mycological study obtained through the isolation of 36 *Fusaria* strains, showing that in a great percentage of the samples studied the isolated fungus belonged to *F. vertilloides* and to *F. subglutinas*.

The high frequency of recovery of *F. verticilioides* is consistent with previous observations of the presence of this fungus in maize kernels and stalks in all of the Americas after harvest (Chulze *et al*., 1996; Desjardins *et al*., 1994; Leslie *et al*., 1990; Neish *et al*., 1993).

Of the 36 strains isolated 16 (44%) produced fumonisin B_1, after incubation in the laboratory. This finding represents a low incidence, but it was similar to the levels of fumonisin B_1 detected in corn samples obtained from areas of high risk of human esophageal cancer as reported in China (Yang, 1980) and Argentine (Chulze *et al*., 1996). It was impossible to detected Fumonisins B_1 in non incubated samples.

The low levels of Fumonisin B1 here observed are consistent with the reported low frequency of esophageal cancer in Mexican people. But it is interesting to point out that, in The annals of The National Institute of Oncology of this country; this cancer form, is not considered among the 20 more important forms of cancer. According to this study oesophageal cancer it is considered as an infrequent finding in Mexico. One explanation would be that during the process of nixtamalization in Mexico, corn when boiling during cooking, calcium hydroxide is added and thereafter washed with cold water until the corn skin is washed off. It is a national costume to prefer white masa and white tortillas. This latter assumption is based in

Table 1: Number of strains isolated from *Fusaria* (*Liseola* section) and fumonisin concentration μg g^{-1} of culture material during 1996-1997 and 2002-2003 crops of maize in different states of the Mexican republic

State	Strains	Species	---------Fumonicin B_1 (PPM)---------	
M1Colima	2	*F. moniliforme*	2.35 (96-97)	(2002-03)
M2 Campeche	1	*F. moniliforme*		2
M3 Nuevo León	1	*F. moniliforme*		
M4 Edo. De México	1	*F. subglutinans*		1.9
M5 Colima	2	*F. moniliforme*		
		F. subglutinans		
M6 Baja California Sur	0			
M7 Guanajuato	1	*F. subglutinans*		2.1
M8 Tlaxcala	1	*F. moniliforme*		
M9 Aguascalientes	2	*F. moniliforme*		
		F. subglutinans		1
M10 Morelos	2	*F. moniliforme*	2.70	
			6.57	4.2
M11 Chihuahua	1	*F. moniliforme*	2.97	2
M12 Chihuahua	2	*F. moniliforme*	2.23	2.5
M13 Colima	2	*F. moniliforme*	3.22	2.9
M14 Colima	2	*F. moniliforme*		3
M15 Jalisco	1	*F. moniliforme*	3.12	3
M16 Jalisco	2	*F. moniliforme*		2.5
M17 Durango	2	*F. moniliforme*	2.87	
M18 Durango	1	*F. moniliforme*		2
M19 Durango	1	*F. moniliforme*		
M20 Hidalgo	1	*F. subglutinans*	2.87	2.5
M21 Tamaulipas	2	*F. moniliforme*		
M22 Oaxaca	2	*F. moniliforme*		
		F. subglutinans	4.85	5
M23 Chiapas	1	*F. moniliforme*		3.9
M24 Quintana Roo	2	*F. moniliforme*		
M25 Tabasco	2	*F. moniliforme*		3
M26 Nayarit	1	*F. moniliforme*	2.35	2
M27 Nayarit	1	*F. moniliforme*	2.19	2
M28 Sonora	1	*F. moniliforme*		
M29 Guerrero	1	*F. moniliforme*		1.5
M30 San Luis Potosi	1	*F. moniliforme*	2.06	2
M31 Zacatecas	1	*F. moniliforme*	2.08	2
M32 Queretaro	1	*F. moniliforme*	2.45	3
M33 Michoacan	1	*F. moniliforme*		
M34 Sinaloa	1	*F. monilifomre*	2.01	2

previous reports in which masa and tortillas, made with maize in Mexico, presence of mycotoxins was low or non detectable (Dombrink-Kurtzman and Dvorak, 1999; Sydenham *et al*., 1995; Bennett and Richard, 1996; Stack, 1998).

In this research it can be observed that samples from Oaxaca and Chiapas showed higher concentrations of fumonisin B1 and this might be due to the preponderant humid clima of this Mexican states.

When comparing with the costumary consumption of maize in the African transkei regions of South Africa and in the Lixiang provice of China. In this latter countries Maize porridge is the staple diet (up to 100% of calories). And in the transkei adults also consume beer deliberately made from mouldy maize selected by the housewife from the harvest. These forms of maize consumption has been found to contain up to 118 mg kg^{-1} fumonisins.

It is interesting to mention that in México ELEM has been reported in horses and donkeys due to the consumption of moldy corn (Rosiles *et al*., 1996). Therefore for practical reasons (trade and storage) corn should be dried until a minimum of 12% of moisture is reached in order to restrict fungal growth, furthermore we have observed that this management with time, decreases the viability and growth of *Fusaria* in corn kernels.

It was concluded that the incidence of fumonisin B_1 should be continually monitored in order to establish a future control in the comsumption of fungus infested corn in the daily diet of the Mexican population.

REFERENCES

Bennett, G.A. and J.L. Richard, 1996. Influence of processing on *Fusarium* mycotoxins in contaminated grains. Food Tech., pp: 235-238.

Carson, T.L. and R.H. Poppenga, 2002. Equine Leucoencephalomalacia. The 5-Min Vet. Consult (Brown CM and Bertone J. Eds.), pp: 624-625.

Chulze, N.S., M.L. Ramírez, M.C. Farnochi, M. Pascale, A. Visconti and G. March, 1996. *Fusarium* and fumonisin occurrence in argentinian corn at different ear maturity stages. J. Agric. Chem., 44: 2797-2801.

Desjardins, A.E., R.D. Plattner and P.E. Nelson, 1994. Fumonisin production and other traits of *Fusarium moniliforme* in northeast Mexico. Applied Environ. Microbiol., 60: 1695-1697.

Dombrink-Kurtzman, M.A. and T.J. Dvorak, 1999. Fumonisin content in masa and tortillas from Mexico. J. Agric. Food Chem., 47: 622-627.

Gelderblom, W.C.A., K. Jaskiewicz, W.F.O. Marasas, P.G. Thiel, R.M. Horak, R. Vleggaar and N.P.J. Kriek, 1988. Fumonisins-novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Applied Environ. Microbiol., 54:1806-1811.

Leslie, J.F., C.A.S. Pearson, P.E. Nelson and T.A. Toussoun, 1990. *Fusarium* sp. From corn, sorghum and soybean fields in the central and eastern United States. Phytopathol., 80: 343-350.

Marasas, W.F.O.,N.P.J. Kriek, V.M. Wiggins, P.S. Steyn, D.K. Towers and T.J. Hastie, 1979. Incidence, geographic distribution and toxigenicity of *Fusarium* sp. In South African corn Phytopathol., 69: 1181-1185.

Neish, G.A., E.R. Farnworth, R. Grenhalgh and J.C. Young, 1983. Observations on the occurrence of *Fusarium* sp. and their toxins in corn in Eastern Ontario. Can. J. Plant Pathol., 5: 11-16.

Nelson, P.E., Desjardins, A.E. and R.D. Plattner, 1993. Fumonisins mycotoxins produced by *Fusarium* sp. Biology, chemistry and significance. Annu. Rev. Phytopathol., 31: 233-252.

Niremberg, H.I, 1981.A simplified method for identifying *Fusarium* sp. ocurring on wheat. Can. J. Bot., 59: 1599-1609.

Pittet, A., V. Parisod and M. Schellenberg, 1992. Occurrence of fumonisins B1 and B2 in corn based products from the Swiss market. J. Agric. Food Chem., 40: 1352-1354.

Proctor, R.H., R.D. Plattner, A.E.Desjardins, M. Busman, R.A.E. Butchko, 2006. Fumonisin production in the corn pathogen *Fusarium* verticillioides: The genetic basis of naturally occurring chemical variation. J. Agric. Food Chem., 54: 2424-30.

Rheeder, J.P., W.F.O. Marasas, P.G. Thiel, E.W. Sydenham, G.s. Shephard and D.J. van Schalkwyk, 1992 *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. Phytopathol., 82: 353-357.

Rosiles, M.R., M.G. Torres and F.P. Ross, 1996. Confirmación fisicoquímica de la fumonicina B_1 en maíz y alimento para équidos que murieron por leucoencefalomalacia. Vet. Méx. 27: 111-113 .

Stack, M.E., 1998. Analysis of fumonisin B_1 and its hydrolysis product in tortillas. J. AOAC Int., 81: 737-740.

Sydenham, E.W., P.G. Thiel, W.F.O. Marasas, G.S. Shephard, D.J. Van Schalkwyk and K.R. Koch, 1990. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence in areas of the Transkei, Southern Africa. J. Agric. Food Chem., 38: 1900-1903.

Sydenham, E.W., S. Stockenström, P.G. Thiel, G.S. Shephard, K. Koch and W.F.O. Marasas, 1995. Potential of alkaline hydrolysis for the removal of fumonisins from contaminated corn. J. Agric. Food Chem., 43: 1198-1201.

Thiel, P.G., W.F.O. Marasas, E.W. Sydenham, G.S. Shephard and W.C.A. Gelderblom, 1992. The implication of naturally occurring levels of fumonisins in corn for human and animals health.Mycopathol., 119: 3-9.

Yang, C.S., 1980. Research on esophageal cancer in China: A review. Cancer Res., 40: 2633-2644.

Yoshizawa, T., Yamashita and Y. Luo, 1994. Fumonisin Occurrence in corn from high- and low-risk areas for human cancer in China. Applied Environ. Micobiol., 60: 1626-1629.

Zenteno-Zevada, M. and M. Ulloa, 1977. Microflora en mazorcas de maíz (*Zea mays* L). Rev. Latinoam. Microbiol., 19: 27-31.