Contamination by fusariotoxins in Zea Mays L. (maize) for human consumption

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Abstract. In Mexico, mycotoxin contamination had been studied in maize grain and its products destined for human consumption, particularly aflatoxins and fumonisins [1]. The national regulation establishes a level maximum of 20 µg kg for unprocessed maize [2]. We have investigated in maize the physical and chemical composition, resistance to *Fusarium sp* and mycotoxins contamination. The results showed that *Fusarium sp* is a common fungus in agricultural soil in the central states of the country, whose species are *Fusarium proliferatum*, *F. roseum, F. oxysporum* and *F. poae*. Fumonisins, Fusarenone X, Enniatin A and mycophenolic acid that are the most prevalent fusariotoxins. The detected levels of fumonisins and mycophenolic acid are low but in a high frequency. It is concluded that the maize genotypes evaluated are susceptible to fungus *Fusarium sp* and fusariotoxins, under the environmental conditions of central regions in Mexico. The aflatoxin contamination was presented in both genotypes, yellow and white. It is important to consider fusariotoxins in a commercial maize regulation for human consumption; due to the long periods and high consumption of maize it is a risk to hepatotoxicity and cancer development.

Keywords: fusariotoxins; maize

1. Introduction

Mexico has been considered the origin of maize, crops are grown in 7 million of ha, principally in tropical and subtropical zones, being the principal varieties white and yellow, with a national production about 12 million tons per year [1, 2]. The corn is the base of 300 hundred by-products as fructosed honey, nixtamalized fluors, tortillas and tortilla chips for human consumption. It is estimated that the annual consumption of corn is 300 kg per person, in which more than 500g per day as tortillas; however, an excessive consumption may contribute to obesity in children [3]. In the field, maize crop can be infected by fungus pathogens, as *Fusarium sp*, which synthetizes fusariotoxins, due to water stress conditions, nitrogen excess, acidity in soil, lack of oxygen. Grain storage for long periods coupled with poor hygiene and high temperatures contributes to *Aspergillus sp*, infestation, causing a fungal mycoflore magnification and mycotoxin contamination [4, 5].

In Mexico aflatoxins research in maize began with an alert in 1989 in the north of the country, where aflatoxins were detected at levels not suitable for human consumption. Ten years later, in 1999, 95% of white corn still had aflatoxins; In 2013, it was mentioned a frequency of 56%, in allowed average levels of 1 to 18 µg kg, so it has been regulated the presence of total aflatoxins in a maximum permitted level of 20 µg kg [6].

Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius, are microscopic saprophytic fungi that develops at a relative humidity between 70 and 90% and over a wide range of temperatures, ranging from 0 ˚C to 45 ˚C, so they are able to infect the maize plant from the field and continue during harvesting and storage. These pathogenic fungi have been detected in the agricultural soils of central Mexico (Guanajuato, Colima, Nayarit), which can synthesize aflatoxins [7]; they are chemical substances, product of the secondary fungus metabolism, which are produced from simple intermediates of primary metabolism such as acetate, malonate and certain amino acids, which possess great thermal stability, that confers dangerous physical and chemical properties to human health [8]. The International Agency for Research on Cancer considered aflatoxins as substances with a high carcinogenic, mutagenic and teratogenic capacity [9]. In Asian and African populations, aflatoxin B1 has been epidemiologically related to liver cancer. Aflatoxin M1, is genotoxic, mutagenic, teratogenic and carcinogenic; it is found in the milk of cattle that have been fed with aflatoxin contaminated feed [10].

F. verticillioides, a saprophytic fungus in agricultural soils, with high global prevalence, and great virulence, causes a systemic infection known as fusariosis, which is a devastating disease for crops It is capable to synthesize fusariotoxins, among which, fumonisins that are chemically polyketides, are toxic substances capable of disturbing phospholipids metabolism, which are found in many tissues mainly in nerve tissue and fat tissue, whose function is cell protection and cell apoptosis signaling. Fumonisins prevent the formation of the enzyme sphinganine N-acyl transferase and de novo synthesis of sphingolipids, triggering a buildup of sphinganine. In animals the lack of phospholipids causes a demyelination of neurons, occurring necrosis of nerve cells and polioencephalomalacia [11]. In humans the consumption of fumonisin contaminated maize has been epidemiologically related to neural tube defects and esophageal cancer [12], due to the lipid peroxidation induced on the cell membrane causing cell death and inhibition of macromolecule synthesis, so the International Cancer Research Center classified them as possible human carcinogens for unprocessed maize it is recommended a maximum content of 1.0 mg kg, and in corn flours for consumption of 750

mg kg. It has been documented the contamination by fumonisins in corn flour and in tortillas in amounts of 1.80 mg/kg, even fumonisin metabolites (FB1H, FB2H, FB3H), of greater toxicity have also been identified. The Food Codex Commission established the daily intake of 2 ng g for fumonisins [13, 14].

The analytical method used to isolate and identify mycotoxins is High Performance Liquid Chromatography (HPLC), due to its high sensitivity of 5 µg/g [15], the lateral flow immunoassay (IFL) is a green technology and environmentally friendly. Liquid chromatography tandem mass HPLC/MS/MS is now used [16]. The Laboratory of Toxicology of UAM-X University, has works with diferents genotypes of maize from Mexico City, Morelos and Hidalgo, zone with sutropical and tropical climate. The mycotoxins identification was made with a cromatographic identification (HPTLC, HPLC-FD) and immunochromatographic test [17].

2. Sampling, extraction and analysis

During 2013-2014, twenty nine samples were evaluated; twenty five samples of maize (sixteen white maize and nine yellow maize) were obtained from Zacatepec, in the state of Morelos, located between the coordinates of 18 ° 37 'and 18 41' altitude N and 99 ° 10 'and 99 ° 14' longitude O; at a height between 900 and 1200 m above sea level, with a minimum temperature of 24°C and maximum of 40°C, with an average annual precipitation of 82 mm.

A random AFB_1 monitoring was performed on eight maize samples selected for their greater commercialization in the state of Morelos. Eight samples were processed using the High Resolution Liquid Chromatography (HPLC) technique, with fluorescence detector (FD). An isocratic pump brand Varian, model Polaris was used. Column of C18, size of 150 mm x 4.6 mm and of 5 microns of diameter. 50 g of pulverized flour was weighed and mixed with 100 mL of 80% methanol, leaving on mechanical agitation for 30 min. The extract was filtered through Waltham paper (No. 41); 10 mL was extracted and passed through a C18 silica column, from which 25 µL were taken and injected in triplicate to the chromatograph. The mobile phase was composed of water, methanol and acetonitrile (60:20:20 v/v/v). The fluorescence detector at a excitation length (λ 360 nm) and emission (λ 440 nm), with a mobile phase flow of 1 mL per minute. The calibration curve ($y = 3128699.71x + 218056.30$) showed a significant linearity ($p < 0.05$) in a range of 0.10; 0.25; 0.5; and 1 µg / mL with a regression coefficient of 0.99. The limits of detection and quantification were 0.241 and 0.43 µg/kg, respectively. The recovery (accuracy) was 87%. In addition, two samples were collected from Hidalgo State, and the rest directly from maize producers in Mexico City were analyzed by UHPLC/MS/MS [16].

Twenty five samples were analyzed for fumonisins in a lateral flow immunoassay technique, starting from 5 g of the sample and an extraction with 50 mL of the buffer solution (PBS), the suspension was mechanically mixed for two minutes and the supernatant was placed in the immunological strip where the Ag-Ac reaction concludes, by identifying two bands as positive test. The quantification was done by inserting the strip into reader [17].

3. Results and Discussion

The presence of pathogenic fungal flora in white maize adapted to areas with a tropical and subtropical climate indicates the susceptibility of genotype maize to *Fusarium sp*, as *Fusarium proliferatum*, *F. roseum, F. oxysporum* and *F. poae* and other fungus, in low incidence, as *Alternaria sp* or *Penicillium sp*, in samples from Hidalgo. The results of fumonisins contamination in twenty nine maize samples are presented in Table 1, where it is observed that all the samples presented fumonisins contamination. The white maize with levels of 0.37 mg kg, with a minimum value of 0.23 mg kg and a maximum of 1.20 mg kg, in yellow maize with an average level of 0.51 mg kg, with a minimum of 0.23 mg kg and a maximum of 1.7 mg kg; being the P 2844 and P4032 genotypes of the greater contamination. Fumonisin contamination has been linked to esophageal cancer and neural tube defects [18].

The aflatoxins contamination was found in both genotypes, yellow with a mean of 8.16 µg kg and 6.7 µg kg for white maize. The NB genotype was the most contaminated from the state of Morelos; all within the national regulation for aflatoxins, however outside the European regulation, which is 5 µg kg. On the other hand, observing the co-contamination of both toxins, it suggests a synergistic effect between the hepatotoxic activity of aflatoxin and the cancer promoting effect of fumonisins, coupled with the daily exposure due to tortilla consumption, probably the maximum intake (IDA), would be achieved and may be a factor to be considered for the development of liver cancer in the Mexican population [19].

In the two samples analyzed by UHPC/MS/MS, 22 mycotoxins with an average content of 956.9 µg kg were detected, of which 55.38% corresponded to mycophenolic acid (530.7 µg kg), 20.58% to trichothecenes A and B (Nivalenol, DON, NEO, Fusarenone X, DAS, HT-2, T2, Zearalenone, Zearalenol), 13.77% to fumonisins and the rest to Ochratoxin A with 8.90 µg kg, total aflatoxins 17.0 µg kg, being AFG2 the highest with 8.5 µg kg, Sterigmatocystine with 6.50 µg kg, Roquefortine C with 3 µg kg, Enanthyne 8.65 µg kg, alternarol 17.4 µg kg, methyl alternariol 16.7 µg kg. The micophenolic acid is synthesized by *Penicillum sp*., which can be considered as an emergent mycotoxin, that has recognized immunosuppressive activity [20].

In table 3 it is observed that the mycophenolic acid was the mycotoxin with the highest concentration in both evaluated samples, also the aflatoxins levels between both samples was very similar and those levels are within the national regulation. It is observed that fumonisins levels are within the permitted levels by the European Legislation.

The level of fumonisins and aflatoxins detected in genotype white and yellow maize was variable, in no case outside the national maximum allowable limit for aflatoxins, but it is important to be controlled both mycotoxins due their capacity to affect the biochemical process, in breast cells, even in small amounts and the co-exposure to develop hepatotoxicity; also the unbalance of ceramide has been related by the presence of fumonisins with a greater resistance to insulin, so it can be considered a risk in the development of type 2 Diabetes [21].

4. Conclusions

The presence of *Fusarium verticillioides, Aspergillus flavus, Penicillium sp, Alternaria sp* in maize from Hidalgo, a central state of Mexico, was demonstrated in two agricultural cycles.

The co-contamination of aflatoxins and fumonisins total, had been demonstrated in maize from central states. In addition, the mycophenolic acid as the principal fusariotoxin, present in maize was detected for the first time in the region.

It is necessary to continue the study of the fusariotoxins in maize for human consumption and its interaction with the metabolic syndrome in the Mexican population, since it is suspected that the contamination by fumonisins favors insulin resistance and the development of type 2 Diabetes.

Table 1 Total fumonisin content in 29 samples of white and yellow corn.

Maize genotype	Adaptation zone	Total fumonisins (mg kg)	Variety	Aflatoxins (µg kg)
NB-1	Tropical/subtropical	0.54	white	12
NB-11	Tropical/subtropical	0.54	white	17.7
Eros	Tropical/subtropical	0.23	white	2.1
Py063	Tropical/subtropical	0.23	white	4.0
Zapata	Tropical	0.23	white	3.0
Costeño	Tropical/subtropical	0.23	white	Nd
P4052	Tropical	0.23	white	8.0
Orion	Tropical	0.23	yellow	Negative
NA-35	Tropical	0.23	yellow	Nd
AmCC	Tropical/subtropical	0.40	yellow	Nd
305-49	Tropical/subtropical	0.23	yellow	8.5
H-443	Tropical/subtropical	0.23	yellow	2.1
P-2844	Tropical/subtropical	1.70	yellow	15.2
Tundra	Tropical/subtropical	0.35	yellow	Nd
H382	Tropical	0.23	yellow	15.0
P4032	Tropical	1.0	yellow	Nd
H-515	Tropical	0.23	white	4.5
H-516	Tropical	0.79	white	1.8
30A60	Tropical	0.23	white	14.5
P3055	Tropical/subtropical	0.23	white	5.0
V5335	Tropical/subtropical	1.20	white	6.33
H-377	Tropical/subtropical	0.23	white	2.5
H-374-c	Tropical/subtropical	0.23	white	3.5
ARES	Tropical/subtropical	0.23	white	Negative
San Andrés	Tropical/subtropical	0.43	white	Negative
P-4082	Tropical/subtropical	0.23	white	5.0
30V46	Tropical/subtropical	0.37	white	2.5
Oso	Tropical/subtropical	Nd	white	13
Leopardo	Tropical/subtropical	Nd	white	13

5. Statistic analysis

Table 2 Total fumonisin content in white and yellow native maize.

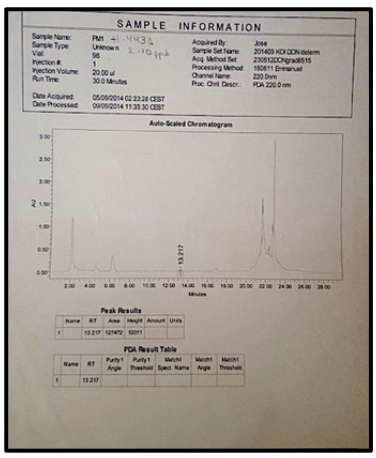
Maize variety	Total Aflatoxins		Total fumonisins Ppm	
	$\bar{x}$	d.e	$\bar{x}$	d.e
Yellow (n=9)	8.16	7.06	0.51	0.51
White (n=20)	6.23	5.26	0.37	0.26
P			<0.01	

$\bar{x}$ = Mean. s.d.= Mean Standard deviation, P=probabilty, 0.05. ppm=parts per million

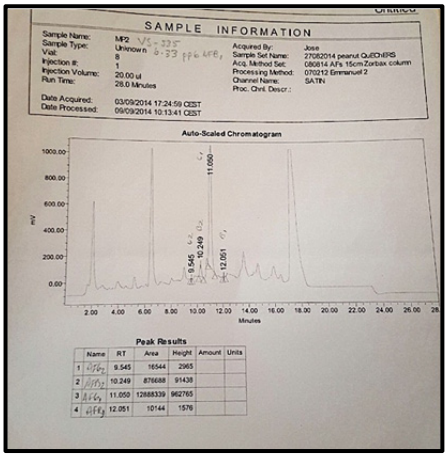
Table 3 Multimycotoxins analysis.

Sample	Fumonisin (µg/kg)	Ochratoxin A (µg/kg)	Aflatoxin (µg/kg)	Trichothecenes (µg/kg)	Mycophenolic acid (µg/kg)
1	139.30	8.90	16.4	242.90	581.8
2	132.97	8.90	17.7	271	479.7

Fig. 1 Immunochromatographic strip.



a)



b)

Fig. 2 Auto-Scaled HPLC Chromatograms.

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