

## Occurrence and toxigenicity of *Fusarium moniliforme* from freshly harvested maize ears with special references to fumonisin production in Egypt

Ezzat M. Fadl Allah

Botany Department, Faculty of Science, Minia University, Minia, Egypt

Received 31 July 1997, accepted in final form 1 January 1998

### Abstract

Using the seed-plate technique, 18 different isolates of *Fusarium moniliforme* were isolated on pentachloronitrobenzene (PCNB) agar medium from 18 samples of a local variety of corn collected from locations in Minia Governorate.

The isolates of *F. moniliforme* were screened for their ability to produce fumonisins on polished rice grains using the solid state fermentation technique. Based on thin layer chromatographic (TLC) analyses using silica gel plates, 14 of the 18 isolates tested produced FB<sub>1</sub> and FB<sub>2</sub> with  $R_f$  (0.17) and (0.24), respectively.

Concentration of FB<sub>1</sub> was estimated using high performance liquid chromatography (HPLC). Production of FB<sub>1</sub> by the 14 isolates of *F. moniliforme* tested ranged from 69 to 4495 ppm indicating that mouldy corn may represent a health hazard to consumers.

**Key words:** Maize, *F. moniliforme*, fumonisins, Egypt.

### Introduction

Maize (*Zea mays*) is considered one of the most important cereal crops in Egypt [1]. It is mostly utilized as food (cooking and bread-making in rural areas) and livestock and to a lesser extent as a raw material in industrial products such as starch, flour, cooking oils and others.

Maize ears are naturally contaminated with different fungi including *Fusarium* spp., e.g. *Fusarium moniliforme* Sheldon, *F. moniliforme* var. *subglutinans* (Woln. & Reink) and *F. graminearum* Schwabe; *F. oxysporum* Schlecht. emend. Snyd. & Hans) and *F. solani* Mart. (Appl. & Wollenw.) [2–4]. These fungi are generally classified as field pathogens.

*Fusarium* spp. infesting maize ears have a less marked effect on weight loss, but may cause discoloration and necrosis of grains. In addition to loss of production, these pathogens can produce a range of toxins which have been linked with a number of mycotoxicoses and carcinomas of humans and domestic livestock including oesophageal cytological abnor-

malities in humans [5–7], pulmonary edema (PPB), hyperoestrogenism and hydrothorax in swine, leukoencephalomalacia (ELEM) in horses and intoxication and paralysis in cattle [7–9].

Toxin production by *Fusarium* is species specific and strain dependent. *F. moniliforme* isolated from corn kernels were found to produce fumonisin B<sub>1</sub> (FB<sub>1</sub>), fumonisin B<sub>2</sub> (FB<sub>2</sub>) and fumonisin B<sub>3</sub> (FB<sub>3</sub>) [10]. In Korea, Soo et al. [11] isolated 6 strains of *F. moniliforme* from corn samples all of which were able to produce FB<sub>1</sub> and FB<sub>2</sub> with maximum levels of 80.7 to 180.9 µg/g. In USA, Ross et al. [12] isolated 12 strains of *F. moniliforme* from corn samples and reported that 9 isolates of these strains produced FB<sub>1</sub> and FB<sub>2</sub> with levels ranging from 960–2350 µg/g for FB<sub>1</sub> and from 130 to 350 µg/g for FB<sub>2</sub>. In Taiwan, 11 isolates out of 25 strains were reported to produce FB<sub>1</sub> and FB<sub>2</sub> [13]. Corn and corn-based products collected from stores in Egypt were found to be contaminated with a high level of fumonisins [9]. Because of the toxicological importance of the fumonisins, the potentiality of several strains of *F. moniliforme*, isolated

Table 1. Concentrations of fumonisin B<sub>1</sub> produced by different isolates of *Fusarium moniliforme* isolated from maize kernels at Minia Governorate, Egypt.

Local strain no.	Fumonisin conc.		Locality
	FB <sub>1</sub> (μg/g)	FB <sub>2</sub> <sup>a</sup>	
<i>F. moniliforme</i>			
FM1	114	+	Minia city
FM2	146	+	Derma was city
FM3	173	+	Minia city
FM4	215	+	Abuqurquas city
FMS	127	+	Abuqurquas city
FM6	530	+	Abuqurquas city
FM7	155	+	Minia city
FM8	69	+	Minia city
FM9	-ve	-ve	Minia city
FM10	-ve	-ve	Minia city
FM11	165	+	Minia city
FM12	-ve	-ve	Minia city
FM13	538	+	Minia city
FM14	107	+	Matai city
FM15	-ve	+	Mallawi city
FM16	388	+	Benimazar city
FM17	126	+	Maghagha city
FM18	4495	+	Matai city

<sup>a</sup> Based on TLC.

-ve: not detected.

from freshly harvested samples of maize ears collected from Minia Governorate in Egypt, to produce FB<sub>1</sub> and FB<sub>2</sub> was determined.

## Materials and methods

**Maize samples.** Freshly harvested hand-shelled maize ears were collected during the 1996 season. Eighteen samples of moldy ears were collected from the major producing areas (4 ears per sample) of corn at Minia Governorate (Table 1). Corn samples were visibly infected by molds. All samples of corn seeds selected were of the white hybrids (local variety) except one sample which was of the yellow sweet corn. Samples were brought to the laboratory in clean plastic bags and kept at 4 °C.

**Isolation and identification of *F. moniliforme*.** Visibly infected kernels from each sample were surface sterilized using 1.5% sodium hypochlorite solution for 3 min, then washed thoroughly with sterile distilled water and completely dried using sterile filter paper. Five surface sterilized grains were plated on agar

plates containing a modified pentachloronitrobenzene medium selective for isolation of *Fusarium* [14] and incubated at 25 °C in the dark for 7 days. *Fusarium* colonies were transferred to freshly prepared plates containing potato dextrose agar (PDA) medium. The colonies were incubated at 25 °C in the dark for 8 days. The isolated *F. moniliforme* were identified according to the taxonomic system of Nelson et al. [15]. For culture preservation, mycelia and conidia from wild strains grown on PDA were transferred aseptically in PDA slants, incubated at 25 °C in the dark and kept in the refrigerator for fermentation studies.

**Fermentation conditions.** The isolated strains of *F. moniliforme* were screened for their ability to produce fumonisins using solid state fermentation on rice medium according to Fadl-Allah et al. [16]. Fifty grams of polished rice were moistened overnight with 50 ml distilled water. Excess water was decanted and flasks containing rice were autoclaved at 121 °C for 15 min. for 2 consecutive days. Autoclaved rice was inoculated with 2 ml of prepared spore suspensions of each of the tested isolates in sterile distilled water containing approximately 10<sup>7</sup> conidia/ml. Inoculated flasks were incubated at 25 °C for 3 weeks in the dark. Flasks were shaken once every day to prevent rice adhering and to distribute the inoculum. At the end of the incubation period, cultures were transferred from flasks and dried on the bench at room temperature, then finely ground using an analytical electric mill (Tekinar A-10) and the fine powder was kept at 4 °C for fumonisins analyses. Uninoculated autoclaved rice treated in the same trend served as control.

**Extraction and clean up.** Five grams of ground rice of each sample and 0.5 g sodium chloride were placed in blender jar and 100 ml methanol – water (8 : 2 v/v) were added and the mixture was blended for 2 min at high speed and filtered through prefolded filter paper (Whatman No. 1).

The filtrate was cleaned following the method of Rottinghaus et al. [17]. A C<sub>18</sub> clean up column (Sep-Pak Cartridge) was preconditioned with 5 ml methanol followed by 5 ml 1% aqueous potassium chloride (KCl). Two ml of the filtrate was combined with 5 ml 1% aqueous KCl and applied to the column. The column was washed with 5 ml 1% aqueous KCl followed by 2 ml acetonitrile:1% aqueous KCl (1 : 9 v/v) and the eluants were discarded. The fumonisins were eluted with 4 ml acetonitrile : water (7 : 3 v/v), and the column eluant was evaporated to dry-

ness under a stream of nitrogen at 60 °C. The residue was redissolved in 0.1 M sodium borate (200  $\mu$ l) and aliquots (50  $\mu$ l) of this solution were used for derivatization. The clean extract was kept at 4 °C for fumonisins examination by thin layer chromatography (TLC) according to Cawood et al. [18] and analyses by high performance liquid chromatography (HPLC) following the method of Shephard et al. [19].

**Analytical method.** The clean extract (10, 20, 30  $\mu$ l) was spotted on TLC plate (Aluminium sheet, silica gel 60, F254, Merck Art. 5554, Germany) along with 5  $\mu$ g each of FB<sub>1</sub> and FB<sub>2</sub> standards (Dr. R. Eppley, FDA, Washington DC, USA). Fumonisin standards were dissolved in acetonitrile : water (1 : 1 v/v). The spots on the plate were dried with a heat gun to remove solvent from the spotting zone and then developed in ethyl acetate : acetic acid : water (60 : 30 : 10 v/v/v) to within 1–2 cm of the plate top. The plate was air-dried and sprayed with *p*-anisaldehyde (0.5% in methanol : sulfuric acid : acetic acid (90 : 5 : 5 v/v/v) and incubated at 100 °C for 5 min.

O. phthalaldehyde (OPA) reagent was prepared by dissolving OPA (40 mg) in methanol (1 ml) and adding 5 ml 0.1 M sodium borate and 50  $\mu$ l 2-mercaptoethanol.

HPLC injections (10  $\mu$ l) were made between 1 and 2 min after derivatization. The derivatized samples were analyzed by a reverse phase, isocratic HPLC system (Water model 6000 A solvent delivery system) equipped with a fluorescence detector (excitation 338 nm, emission 420 nm), a 6 k injector and analytical column (Spherisorb 5 ODS, Dimi 250  $\times$  4.60 nm). The mobile phase was methanol: 0.1 M KH<sub>2</sub>PO<sub>4</sub> (76 : 24 v/v) adjusted to pH 3.35 with *o*.phosphoric acid. The flow rate was 0.6 ml/min.

Since FB<sub>1</sub> was indicated to be the major toxin responsible for the hepatotoxicological effects caused by *F. moniliforme* in rats [20], quantification of only FB<sub>1</sub> in the extracts of the tested isolates of *F. moniliforme* was achieved from the peak area measurement using a Waters 720 data module. FB<sub>2</sub> was estimated only visually on TLC plates.

## Results and discussion

### *Occurrence of F. moniliforme in infected maize kernels*

The seed-plate method was used for isolation of *F. moniliforme* from infected corn kernels on PCNB-

agar medium selective for *Fusarium*. Eighteen isolates of only *F. moniliforme* (Sheldon) were isolated from the 18 corn samples collected from 7 locations at Minia Governorate. *F. moniliforme* was found to be the dominant species isolated from all the tested samples.

The presence of one or more *Fusarium* species in corn seeds of infected ear kernels was reported earlier from different countries. In Egypt, El-Maghraby et al. [21] isolated four species of *Fusarium* from white hybrids of corn grains. They observed that *F. moniliforme* was the dominant species. In Italy, Logrieco et al. [3] reported the occurrence of *F. moniliforme* in preharvest maize ears (yellow hybrids) and it was the predominant species in infected ear kernels. *F. moniliforme* is considered to be the most common *Fusarium* species occurring in tropical and subtropical climates [22], and the most prevalent fungus associated with corn in USA [23].

### *Toxigenicity*

Fumonisin B<sub>1</sub> and B<sub>2</sub> were initially detected on TLC plates by the presence of reddish spots after spraying with *p*-anisaldehyde at the locations compatible to those of the standards of FB<sub>1</sub> ( $R_f$  0.17) and FB<sub>2</sub> ( $R_f$  0.24) at the condition of our laboratory. The ratio of the  $R_f$  value of FB<sub>1</sub>/FB<sub>2</sub> was found to be 0.71. This ratio is in agreement with the results previously reported by Tseng et al [13] as equal to 0.72.

The concentrations of FB<sub>1</sub> produced by the tested isolates of *F. moniliforme* grown on autoclaved rice as quantified by comparing the peak heights of FB<sub>1</sub> in HPLC chromatograms with those of standard FB<sub>1</sub> solution are shown in Table 1. The results indicate that 14 of the 18 isolates of *F. moniliforme* examined produced FB<sub>1</sub> whereas four isolates were not able to produce fumonisin. Actually the data presented in Table 1 revealed that the *F. moniliforme* isolates tested produced FB<sub>1</sub> at concentrations ranging from 69–4495  $\mu$ g/g in addition to FB<sub>2</sub>.

*F. moniliforme* has been reported to produce various mycotoxins such as fusarin C [24], FB<sub>1</sub> and FB<sub>2</sub> [25]. In this study attention was focused on the fumonisin-producing ability of *F. moniliforme* infesting corn grains in Egypt. Results obtained were consistent with those reported earlier by other investigators who demonstrated that many strains of *F. moniliforme*, isolated from corn in different countries, produce fumonisins [25, 11]. Nelson et al. [26] reported that FB<sub>1</sub> was produced by *F. moniliforme* isolates at concentrations ranging from less than 1 ppm

to greater than 6000 ppm and Theil et al. [27] reported that maximum production of FB<sub>1</sub> produced by isolate of *F. moniliforme* was 7100 ppm.

It must be noted that although *F. moniliforme* has been reported as a fumonisin producer, some isolates were found to be non-toxicogenic. This difference indicate variation among *F. moniliforme* isolates with respect to fumonisins production. This suggestion is confirmed by the work of Tseng et al. [13] who reported that not all the strains of *F. moniliforme* isolated from corn kernels in Taiwan produce fumonisins. In conclusion, *F. moniliforme* is a predominant fungus contaminating our local variety of corn grown in Minia Governorate in Egypt. Production of fumonisin by *F. moniliforme* isolates from infested corn kernels is reported here which, as far as we know, is the first record in Egypt.

Since the toxigenic isolates of *F. moniliforme* which were isolated in this study from corn kernels produced fumonisin on polished rice, it is not known whether the toxin production by these isolates is universal on starchy substrates and whether resistance to toxin production occurs among different varieties of these starchy grains. These points needs further investigation.

### Acknowledgments

The author is grateful to Prof. Dr. M. A. El-Naghy, Minia University (Egypt) for reading and revising the manuscript and to Prof. Dr. G. A. Bean, University of Maryland (USA) and Dr. R. Epply, FDA (USA) for providing the mycotoxin fumonisin standard which made this research possible. Also, I thank the staff members at Central Laboratory of Mycotoxin in National Research Center, Dokki, Cairo for their technical assistance of using HPLC.

### References

1. FAO. FAO RAAPA Publications 1993; 1992/60.
2. Julian AM, Wareing PW, Phillips SI, Medlock VFP. Fungal contamination and selected mycotoxins in pre and post harvest maize in Honduras. *Mycopathologia* 1995; 129: 5–16.
3. Logrieco A, Moretti A, Ritieni A, Bottalico A, Corda P. Occurrence and toxigenicity of *Fusarium proliferatum* from preharvest maize ear rot and associated mycotoxins in Italy. *Plant Dis* 1995; 79 :727–731.
4. Abbas HK, Mirocha CJ, Meronuk RA, Pokorny JD, Gould SL, Kommedahl T. Mycotoxins and *Fusarium* spp. associated with infected ears of corn in Minnesota. *Appl Environ Microbiol* 1988; 54: 1930–1933.
5. Marasas WFO, Wehner FC, Van Rensburg SJ, Van Schalkwyk DJ. Mycoflora of corn produced in human esophageal cancer areas in Transkei, South Africa. *Phytopathology* 1981; 71: 792–797.
6. Marasas WFO, Jaskiewicz K, Venter FS, Van Schalkwyk DJ. *Fusarium moniliforme* contamination of maize in human esophageal cancer areas in Transkei. *S Afr Med J* 1988; 24: 110–114.
7. Sydenham EW, Thiel PG, Marasas WFO, Shephard GS, Van Schalkwyk DJ, Koch, KR. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence areas of the Transkei, Southern Africa. *J Agric Food Chem* 1990; 38: 1900–1903.
8. Ross PF, Rice LG, Plattner RD, Osweiler GD, Wilson TM, Nelson HA, Richard JL. Concentrations of fumonisin B<sub>1</sub> in feeds associated with animal health problems. *Mycopathologia* 1993; 114: 129–135.
9. Thiel PG, Marasas WFO, Sydenham EW, Shephard GS, Gelderblom WCA. The implications of naturally occurring levels of fumonisins in corn for human and animal health. *Mycopathologia* 1992; 117: 3–9.
10. Alberts JF, Gelderblom WCA, Marasas WFO. Evaluation of the extraction and purification procedures of the maleyl derivatization HPLC technique for the quantification of the fumonisin B mycotoxins in corn cultures. *Mycotoxin Research* 1992; 8: 2–12.
11. Soo LU, Yur LM, Sop SK, Sik MY, Min CC, Ueno Y. Production of fumonisin by B<sub>1</sub> and B<sub>2</sub> by *Fusarium moniliforme* isolated from Korean corn kernels for feed. *Mycotoxin Research* 1994; 10 : 67–72.
12. Ross, PF, Nelson, PE, Richard JL, Osewiler GD, Rice LG, Plattner RD, Wilson TM. Production of fumonisin by *Fusarium moniliforme* and *Fusarium proliferatum* isolates associated with equine leukoencephalomalacia and a pulmonary edema syndrome in Swine. *Appl Environ Microbiol* 1990; 56(10): 3225–3226.
13. Tseng TC, Lee KL, Deng TS, Liu CY, Huang JW. Production of fumonisins by *Fusarium* species of Taiwan. *Mycopathologia* 1995; 130: 117–121.
14. Nash SM, Synder WC. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. *Phytopathology* 1962; 52: 567–572.
15. Nelson PE, Toussoun TA, Marasas WFO. *Fusarium* Species: An Illustrated Manual for Identification. Pennsylvania State University Press, University Park, USA, 1983.
16. Fadl-Allah EM, Stack M, Goth R, Bean GA. Production of fumonisin B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub> by *Fusarium proliferatum* isolated from rye grains. *Mycotoxin Research* 1997; 13 : 43–48.
17. Rottinghaus GE, Coatney CE, Minor CH. A rapid, sensitive thin layer chromatography procedure for the detection of fumonisin B<sub>1</sub> and B<sub>2</sub>. *J Vet Diagn Invest* 1992; 4: 326–329.
18. Cawood ME, Gelderblom WCA, Vleggaar R, Behrend Y, Thiel PG, Marasas FO. Isolation of the fumonisin mycotoxins: A quantitative approach. *J Agri Food Chem* 1991; 39: 1958–1962.
19. Shephard GS, Sydenham, EW, Thiel PG, Gelderblom WCA. Quantitative determination of fumonisin B<sub>1</sub> and B<sub>2</sub> by high performance liquid chromatography with fluorescence detection. *J L Chromatography* 1990; 13: 2077–2087.
20. Gelderblom WCA, Kriek NPJ, Marasas WFO, Thiel PG. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite fumonisin B<sub>1</sub>, in rats. *Carcinogenesis*, 1991 (in press); cited in *Mycopathologia* (1992).

21. El-Maghraby OMO, El-Kady IA, Soliman S. Mycoflora and *Fusarium* toxins of three types of corn grains in Egypt with special reference to production of trichothecene toxins. *Microbiol Res.* 1995; 150: 225–232.
22. Smith JE, Moss MO. *Mycotoxin Formation Analyses and Significance*. John and Sons. Chichester, New York, 1985.
23. Kommedahl T, Windels CE. Root, stalk and ear infecting *Fusarium* species on corn in the USA. In: Nelson PE, Toussoun TA, Cook RJ (eds.), *Fusarium: Disease, Biology and Taxonomy*. Pennsylvania State Univ Press, Univ. Park and London 1987, 94–103.
24. Marasas WFO, Nelson PE, Toussoun TA. *Toxigenic Fusarium Species*. Pennsylvania State University Press, University Park. USA, 1984.
25. Ross PF, Rice LG, Osweiler GD, Nelson PE, Richard JL, Wilson TM. A review and update of animal toxicoses associated with fumonisin-contaminated feeds and production of fumonisins by *Fusarium* isolates. *Mycopathologia* 1992; 117: 109–111.
26. Nelson PE, Plattner RD, Shackelford DD, Desjardins AE. Production of fumonisins by *Fusarium moniliforme* strains from various substrates and geographic areas. *Appl Environ Microbiol* 1991; 57: 2410–2412.
27. Thiel PG, Marasas WFO, Sydenham EW, Shephard GS, Gelderblom WCA, Nieuwenhuis JJ. Survey of fumonisin production by *Fusarium* species. *Appl Environ Microbiol* 1991; 57(4): 1089–1093.

*Address for correspondence:* Dr. Ezzat M. Fadl-Allah, Department of Botany, Faculty of Science, Minia University, Minia, Egypt.  
Phone: 02-86-345228