

Toxigenic fungi collected in maize fields from four states of Mexico

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Abstract

Maize is the most important crop for the Mexican society. Phytopathogenic fungi pose a major challenge to this cereal. These fungi are harmful to people and animals, mainly as a result of their mycotoxins. The objective of this research was to identify the fungi found in 18 maize genotypes collected in fields from the states of Morelos, Coahuila, Chiapas, and Puebla, Mexico. Pathogens were isolated in potato dextrose agar (PDA) culture medium and identified using morphological criteria. The pathogens were purified using monoonidial cultures and sheltered at 4 °C ± 2 °C. An analysis of variance was carried out using the SAS[®] 9.1 statistical software. Fungi from the following genus and species were identified: *Aspergillus*, *Penicillium*, *Alternaria*, *Bipolaris*, *Trichothecium roseum*, and *Fusarium verticillioides*. It is worth mentioning that *F. verticillioides* and *Penicillium* sp. are the most common pathogens in maize genotypes, with an incidence of 63.68 and 6.57%, respectively. Moreover, along with genus *Aspergillus*, they are considered to be the most important toxicological fungi, given the harm that they cause to humans and animals. Therefore, management strategies are needed to guarantee that grains can be safely consumed by society.

Keywords: Seeds, harvest, fungi, storage, genotype.

Abbreviations: PDA_potato dextrose agar; SAF_seed-associated fungi; DON_deoxynivalenol; NIV_nivalenol; DAS_diacetoxyscirpenol

Introduction

Seed-associated fungi (SAF) cause quality loss, because they affect the viability and diminish germination (Ghangaokar and Kshirsagar, 2013), a core feature of the life cycle of plants (Fenner, 1985; Thompson, 1987; Luzuriaga *et al.*, 2005). The quality of the seeds sown must meet physical, physiological, sanitary, and genetic standards (McDonald, 1975). Seed-borne pathogens are contaminants attached to the seed cover or carried within the seed (Neergaard, 1977). Every year, fungi cause losses in maize fields: they cause approximately 125 diseases. In order to recognize and manage them, they are classified according to the part of the plant they infect: foliage, spike, stem, or cob (Rodríguez and De Leon, 2008). Pathogens associated with rot in maize crops are a complex formed by necrotrophic fungi and occasionally bacteria (Carmona *et al.*, 2006; Marinelli *et al.*, 2010; Diaz, 2011; Couretot *et al.*, 2016). The most frequently detected fungi are: *Fusarium graminearum* Schwabe,

Fusarium verticillioides (Saccardo) Nirenberg, *Stenocarpella macrospora* (Earle) Sutton, *Stenocarpella maydis* (Berkeley) Sutton, *Colletotrichum graminicola* (Cesati) GW Wilson, and *Macrophomina phaseolina* (Tassi) Goidanich. The fungi associated with seeds are commonly those that cause the spike to rot. The most frequently detected fungi of this kind are: *F. graminearum*, *F. verticillioides*, *S. maydis*, *Aspergillus* spp., and *Penicillium* spp. (De Rossi *et al.*, 2017). When animals or humans ingest food contaminated by the different types of toxigenic fungi that grow on hay, silage, grains, by-products, and other stored foods they can suffer mycotoxicosis as result of the mycotoxins that the fungi contain (Perusia and Rodríguez, 2001). The objective of this research was to identify the fungi found in 18 maize genotypes collected in field from the states of Morelos, Coahuila, Chiapas, and Puebla, Mexico.

Results and discussion

Fungi from the following genera and species were identified: *Aspergillus*, *Penicillium*, *Alternaria*, *Bipolaris*, *Fusarium*, *Trichothecium roseum* (Persoon) Gray, and *Fusarium verticillioides* (Table 1, Figure 1).

F. verticillioides and *Alternaria* sp. belong to the so-called field fungi, while *Penicillium* sp. is part of the most common storage fungi category, together with *Aspergillus* sp. However, this was not the case of all genotypes, perhaps because the seeds were not sheltered for long periods or because the environmental conditions were unsuitable for the said pathogens, high temperatures, high moisture content in grains, and genetic variability. The appearances of diseases in maize are subject to environmental conditions that favor the infection by the pathogen and its multiplication; as well as the source of the inoculum and the susceptibility of genotypes (Varón and Sarria-Villa, 2007).

F. verticillioides and *Penicillium* sp. are pathogens found in maize genotypes, with an incidence of 63.68 and 6.57%, respectively (Table 2, Figure 2). *F. verticillioides* is one of the most important species and it is widely distributed in all tropical and subtropical areas where it causes the cob and stem of maize to rot (McKeen, 1953; McGee, 1988; Romero, 1993; Visconti and Doko, 1994; De la Torre-Hernández *et al.*, 2014; Arispe-Vazquez *et al.*, 2023). The various mycotoxins are mainly produced by the following four genera of fungi: *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* (Desjardins and Hohn, 1997; Frisvad *et al.*, 2006; Richard, 2007; Brase *et al.*, 2009; Abrunhosa *et al.*, 2014; Adam *et al.*, 2015). The major toxicological fungi genera are: *Fusarium*, *Aspergillus*, and *Penicillium*. They can develop on a wide range of stored grains and maize seeds (Christensen and Kaufmann, 1976; Richarson, 1979; Sauer, 1984; FAO, 2022), making them a serious danger to Mexican society, of which maize is the staple food.

Fusarium spp. and their mycotoxins are related to diseases in the humans and the animals that consume them (Tuite *et al.*, 1974; Shurtleff, 1980; Sutton, 1982; Charmley *et al.*, 1994; Jelinek *et al.*, 1997; Macías and Peraza, 2008). The main *Fusarium* mycotoxins that can develop in cereals and cereal-based products are: deoxynivalenol (in wheat, corn, barley, oats, and rye), T-2 and HT-2 toxins (in oats, wheat, and barley), zearalenone (in corn and wheat), and fumonisins (in maize), as well as trichothecenes (*e.g.*, deoxynivalenol (DON), nivalenol (NIV), and diacetoxyscirpenol (DAS) (Pleadin *et al.*, 2013). The presence of this genera of fungi may be related to other pathogens. Arispe-Vazquez *et al.* (2021) reported that the incidence of *Fusarium* spp. is related to the fall armyworm (FAW) (*Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)) which increases cob rot in maize crops. El-Behadli *et al.* (1998) analyzed ten isolates of *T. roseum* (a saprophytic pathogen) to determine the production of the T-2 toxin and obtained positive results for all cases. Furthermore, this pathogen has been reported as an agent that causes cob rot in northern China (Xue *et al.*, 2016). In this research, it was recorded in maize seeds from the Trinitaria region in Chiapas, which had been stored for at least 6 months and were mainly used for human consumption.

Materials and methods

Genetic material

Samples of maize (native and hybrids) were obtained at the end of the maize production cycle in plots from Tepalcingo (Morelos), Trinitaria (Chiapas), Saltillo (Coahuila), and the state of Puebla. A total of 18 genotypes were collected (Table 3, Figure 3).

Blotting paper and freezing test

The test was conducted in accordance with the National Seed Health System (NSHS, 2020). The seeds were disinfected for 3 min in a 2% sodium hypochlorite (NaClO) solution and then rinsed with distilled water for 1 min. Subsequently, 40 to 50 seeds were distributed evenly in plastic trays, which contained a double layer of previously moistened stencil paper, obtaining a total of 4 to 8 repetitions with a total of 200 to 400 seeds per genotype (Table 4). The trays were kept at 25 °C for 2 days in the bioclimatic chamber of a phytopathology laboratory. Subsequently, they were kept in an ultrafreezer at -20°C for 24 h. Finally, they were removed from the ultrafreezer and kept at 25°C ± 2°C during 11 days, alternating 12 h of white light with 12 h of darkness.

Pathogen isolation

After observing the full development of the fungal colonies in the trays, we isolated the fungi in Petri dishes with PDA which were kept at 25°C ± 2°C.

Purification of phytopathogens

For each of the pathogen colonies, 5-mm wide discs were taken and placed in test tubes with 9 ml of sterile distilled water, which were stirred constantly, while 50 µL were poured in petri dishes with a PDA culture medium. After 20 h, a germinated conidia was taken and placed in a petri dish with PDA, where it was kept at 25°C ± 2°C for 120 h.

Pathogen identification

Identification was based on the structures of each pathogen and was carried out with a microscope using the taxonomic keys of Barnett and Hunter (2006) and Warham *et al.* (2003).

Statistical analysis

The incidence was reported in terms of percentage and the resulting data was subjected to the analysis of variance, using the SAS version 9.1[®] statistical software (SAS 2002; version 9.1, SAS Institute, Cary, North Carolina, USA).

Conclusion

The identified fungi belonged to the following genus and species: *Aspergillus*, *Penicillium*, *Alternaria*, *Bipolaris*, *T. roseum*, *F. verticillioides*, and *Penicillium* sp. The two latter are the most common pathogens in maize genotypes, with an incidence of 63.68 and 6.57%, respectively. They are also considered to be the most important toxicological fungi that harm people and animals, along with genus *Aspergillus*. Therefore, management strategies are needed to guarantee that grains are safe for human consumption.

Table 1. Incidence (%) of fungi present in the grains of the maize genotypes.

Genotype	<i>F. verticillioides</i>	<i>Penicillium</i> sp.	<i>Alternaria</i> sp.	<i>Bipolaris</i> sp.	<i>Aspergillus</i> sp.	<i>T. roseum</i>
Elotero 7573	79.5	20.5	0	0	0	0
Native Pozolero	90	10	0	0	0	0
Pioneer P4082W	87	13	0	0	0	0
Black Native	84	16	0	0	0	0
Zapata 6	97.5	0.5	1	0	0	0
Accesion 2 Red Native	79.5	0	11.5	0	0	0
Hibryd H-515	98	0.5	1.5	0	0	0
Híbryd H-516	83.5	4	11.5	0	0	0
Zapata 2	77	0.3	3.2	3	0	0
Accesion 1 Red Native	53.7	0.7	4	5.3	0	0
Yellow Native	77.8	0.2	4	2	0	0
Purple Native	31.25	6.5	0	0	5	0
Jaguan	33.5	3	0	0	2	0.75
Accesion 1 HC8	43	3	0	0	2	0
Accesion 1 Faisán	16	9.5	0	0	2.5	0
Accesion 2 HC8	40	4.5	0	0	1.5	0
Accesion 2 Faisán	38.25	13.5	0	0	2.75	0
Antonio Narro 4-47	36.75	12.475	0	0	3.5	0

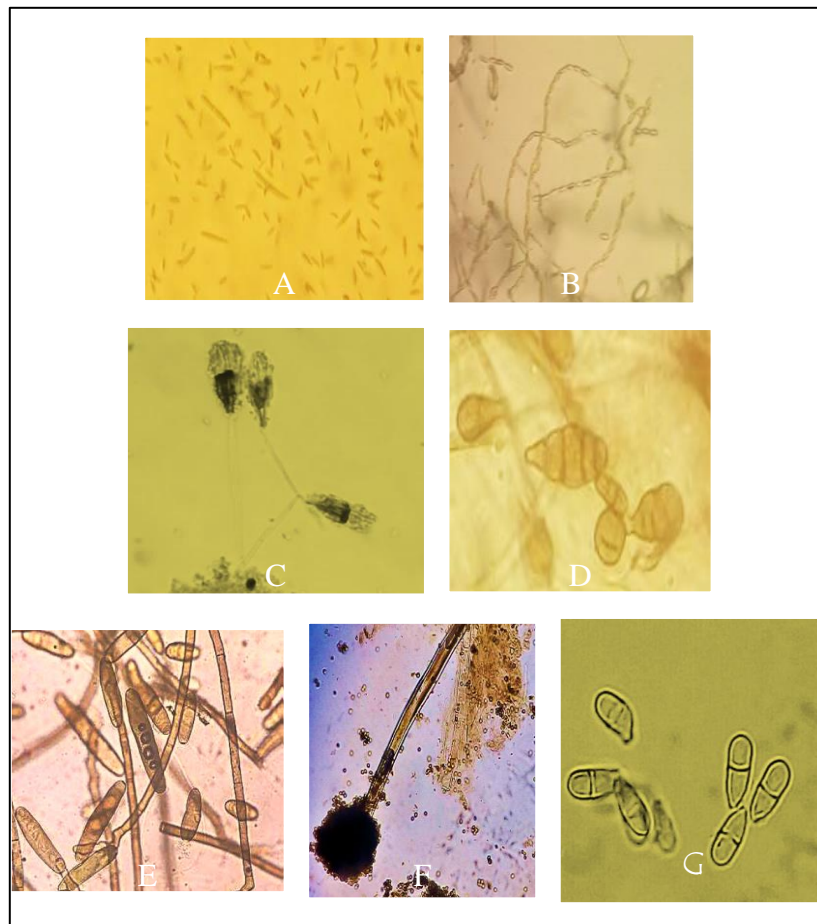


Figure 1. Identified fungi. A and B= Macro and microconidia of *F. verticillioides*, C= *Penicillium* sp., D= *Alternaria* sp., E= *Bipolaris* sp., F= *Aspergillus* sp., G= *T. roseum*.

Table 2. General incidence (%) for each pathogen.

P1	P2	P3	P4	P5	P6
64.48	6.57	2.04	0.57	1.07	0.04

P1= *F. verticillioides*, P2= *Penicillium* sp., P3= *Alternaria* sp., P4= *Bipolaris* sp., P5= *Aspergillus* sp., P6= *T. roseum*.

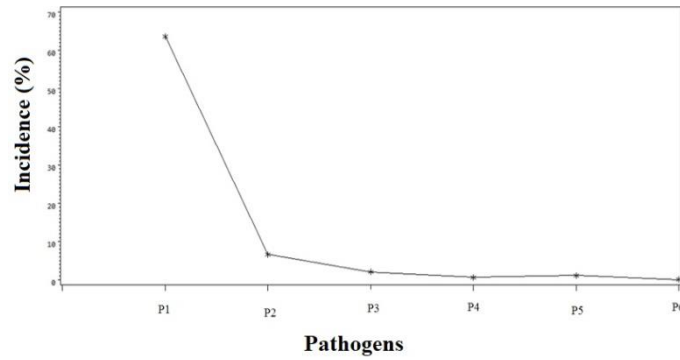


Figure 2. General incidence (%) of the pathogens present in the seeds of the different maize genotypes. P1= *F. verticillioides*, P2= *Penicillium* sp., P3= *Alternaria* sp., P4= *Bipolaris* sp., P5= *Aspergillus* sp., P6= *T. roseum*.

Table 3. Location of the genotypes under study in localities of different states.

Location	Genotype	Cultivar	Color
Tepalcingo, Morelos	Elotero 7573	Hybrid	White
	Native Pozolero	Native	White
	Pioneer P4082W	Hybrid	White
	Black Native	Native	Black
	Zapata 6	Hybrid	Yellow
	Accesion 2 Red Native	Native	Red
	Hibryd H-515	Hybrid	White
	Hibryd H-516	Hybrid	White
	Zapata 2	Hybrid	Yellow
	Accesion 1 Red Native	Native	Red
Trinitaria, Chiapas	Yellow Native	Native	Yellow
	Purple Native	Native	Purple
Saltillo, Coahuila	Jaguan	Maize variety	White
Puebla	Accesion 1 HC8	Hybrid	White
	Accesion 1 Faisán	Hybrid	White
	Accesion 2 HC8	Hybrid	White
	Accesion 2 Faisán	Hybrid	White
	Antonio Narro 4-47	Hybrid	White



Figure 3. Corn genotypes used in this research.

Table 4. Genotypes and replicates using in research.

N° genotype	Genotype	Seeds used	Seeds per tray	Replicate
1	Elotero 7573	200	50	4
2	Native Pozolero	200	50	4
3	Pioneer 4082	200	50	4
4	Black Native	400	50	8
5	Zapata 6	400	50	8
6	Accesion 2 Red Native	400	50	8
7	Hibryd H-515	200	40	5
8	Hibryd H-516	200	40	5
9	Zapata 2	200	40	5
10	Accesion 1 Red Native	200	40	5
11	Yellow Native	400	50	8
12	Purple Native	400	50	8
13	Jaguan	200	50	4
14	Accesion 1 HC8	200	50	4
15	Accesion 1 Faisán	200	50	4
16	Accesion 2 HC8	400	50	8
17	Accesion 2 Faisán	400	50	8
18	Antonio Narro 4-47	200	50	4

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