






***Fusarium* species in maize grains and stems (*Zea mays* L.) from subsistence and commercial systems Especies de *Fusarium* en granos y tallos de maíz (*Zea mays* L.) de sistemas comerciales y de subsistencia**

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Abstract

The objective of this research was the morphomolecular identification of species of the genus *Fusarium* associated with native and commercial maize genotypes in municipalities of Puebla and Morelos, Mexico, as well as to describe the socioeconomic and cultural importance of maize cultivation. *Fusarium* species were isolated from grains and stem cuttings with purple, brown, and dark brown pigmentations. They were identified morphomolecularly by using taxonomic keys and the Dinocapture 2.0 software, and by DNA extraction by the CTAB method and amplification of the ITS1 and ITS4 region by PCR. Four species were identified: *Fusarium equiseti*, *Fusarium verticillioides*, *Fusarium incarnatum* and *Fusarium napiforme* with an identity greater than 98 %. The genotypes with the highest incidence of *Fusarium* spp. were: Pioneer 30F35 in San Miguel, Teotlalco, Puebla by *F. verticillioides* (A2). The genotypes with the lowest incidence were: Shark from Tetelilla, Morelos by *F. incarnatum* (A1), *F. verticillioides* (A6) and *F. napiforme* (A7). Maize is the main food in many locations in Mexico, however, due to the high consumption of this grain and its derivatives, as well as the high consumption of meat presumably fed with this grain, it could represent a route of ingestion of mycotoxins.

Keywords: *Fusarium equiseti*; *Fusarium incarnatum*; *Fusarium napiforme*; *Fusarium verticillioides*; incidence; stem rot; ear rot; severity; genotypes; cultivation; pigmentations

Resumen

El objetivo de esta investigación fue la identificación morfomolecular de especies del género *Fusarium* asociadas a genotipos de maíces nativo y comercial en municipios de Puebla y Morelos, México, así como describir la importancia socioeconómica y cultural del cultivo del maíz. Se aislaron especies de *Fusarium* a partir de granos y esquejes de tallo con pigmentaciones moradas, pardas y pardas oscuras. Se identificaron morfomolecularmente mediante el uso de claves taxonómicas y el software Dinocapture 2.0, y mediante extracción de ADN por el método CTAB y amplificación de la región ITS1 e ITS4 por PCR. Se identificaron cuatro especies: *Fusarium equiseti*, *Fusarium verticillioides*, *Fusarium incarnatum* y *Fusarium napiforme* con una identidad superior al 98 %. Los genotipos con mayor incidencia de *Fusarium* spp. fueron: Pioneer 30F35 en San Miguel, Teotlalco, Puebla por *F. verticillioides* (A2). Los genotipos con menor incidencia fueron: Tiburón de Tetelilla, Morelos por *F. incarnatum* (A1), *F. verticillioides* (A6) y *F. napiforme* (A7). El maíz es el principal alimento en muchas localidades de México, sin embargo, debido al alto consumo de este grano y sus derivados, así como al alto consumo de carne presumiblemente alimentado con este grano, podría representar una vía de ingestión de micotoxinas.

Palabras clave: *Fusarium equiseti*; *Fusarium incarnatum*; *Fusarium napiforme*; *Fusarium verticillioides*; incidencia; pudrición del tallo; pudrición de la mazorca; severidad; genotipos; cultivo; pigmentaciones

1. Introduction

Among diseases that frequently occur in corn crops, which affect root, stem, and ear, are caused by fungi (White, 1999); mainly, stem and ear rot caused by *Gibberella/Fusarium* Link (Nectriaceae) (Levin et al., 2003). The lesions on corn stalk caused by *Fusarium* appear oval or in narrow, vertical areas with a cloudy appearance that start reddish-brown and then turn black, and in advanced stages of rot, the stem breaks at the node, characterized by white mycelium in the bark (Pioneer, 2020a). In maize program of the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) México (2004) described that *Fusarium* wilted plants remain erect when dried and small dark brown lesions are observed on lower internodes. Carmona & Scandiani (2010) add that first symptoms appear on stems that are still green, but as a yellow spot and later turn brown in the lower internodes.

The *Fusarium* genus includes species that infect cereals and are of high concern for agricultural production and food/feed safety worldwide (Desjardins, 2006). The species of this genus are characterized by being the causal agent of diseases in a wide range of host plants, with significant economic impacts (Wang et al., 2011). Among the species that cause ear rot and corn kernels, *Fusarium verticillioides* (Sacc.) Nirenberg, *Fusarium graminearum* Schwabe, *Fusarium oxysporum* Schltdl., *Fusarium equiseti* (Corda) Sacc., *Fusarium subglutinans* (Wollenw & Reinking) P.E. Nelson, Toussoun & Marasas (Nectriaceae) (Görtz et al., 2008; Qin et al., 2014; Ammar et al., 2013; Rahjoo et al., 2008; Macdonald et al., 1997) stand out. *Fusarium verticillioides* is the most common isolated species of corn in the world (Munkvold & Desjardins, 1997), causing seedling blight, stem, root, and ear rot (Shurtleff, 1980). The contamination level and population structure of *Fusarium* spp. in grains could predict the presence of certain mycotoxins (Miller, 1995; Bottalico, 1998) which are toxic to humans or animals that consume them (Brown et al., 2004; Desjardins, 2006; Alexander et al., 2009; Sánchez-Rangel & Plasencia, 2010; Yin et al., 2016), which is why they are strictly regulated throughout the world (Van Egmond & Jonker, 2005). Climatic conditions such as temperature, rainfall, and relative humidity during flowering are critical for primary infection, as well as for toxin accumulation during flowering and prior to harvest (De La Campa et al., 2005; Maiorano et al., 2009; Cao et al., 2014). The accurate knowledge of water amount in grains and seeds is important for commercialization, mainly due to the moisture content, since it influences the fungi growth in storage stage (Christensen & Kaufmann, 1969), while, in the post-harvest stage, the fungi development and mycotoxins synthesis can be well controlled, respecting the maximum limits of relative humidity (Bottalico & Perrone, 2002; Logrieco 2003).

Phytopathogens can cause serious losses in economic terms and production, therefore, the crucial step in disease management under natural field conditions is to properly detect the pathogen (Tewari, 2019). In addition, mycotoxins are a latent threat to human health, as they can act as a silent killer since their consumption in very small doses does not induce obvious clinical symptoms, but over time it can have serious consequences on life quality (Borja & Calvo, 2021). Therefore, the aim of this research was to identify morpho-molecular *Fusarium* species associated with nine genotypes of native and commercial maize in municipalities of Puebla and Morelos states, México, as well as the description of socioeconomic importance and cultural of this crop.

2. Methods, techniques, and instruments

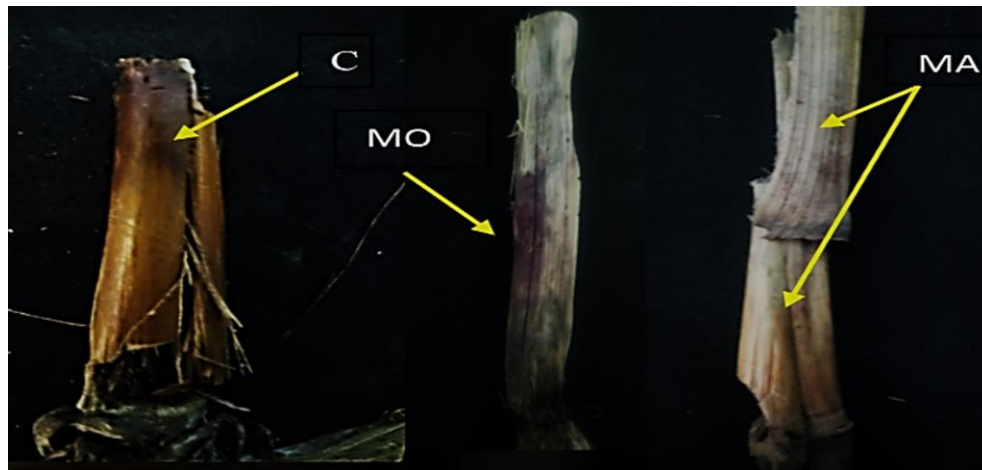
Genetic material

The materials under study were obtained at the end of the production cycle (November 2019) of nine corn plots destined for grain (human and animal consumption) in the municipalities of Contla, San Miguel, Teotlalco Puebla and Tepalcingo and Tetelilla in Morelos state (table 1).

From each genotype, 15 ears (with and without *Fusarium* symptoms) of 10 central rows and 15 stem cuts with pigmentation on the outside were randomly taken (figure 1) between the fourth and fifth internodes, the samples were placed in paper bags, protected in expanded polystyrene coolers, identified, and transferred to Parasitology Department of Universidad Autónoma Agraria Antonio Narro in Saltillo, Coahuila, México.

Table 1. Native and hybrid corn genotypes used in this research.**Tabla 1.** Genotipos de maíz nativo e híbrido utilizados en esta investigación.

Genetic material origin	Genotypes	Coordinates	Height above sea level (m)	Company
Tetelilla, Morelos	Pioneer P4028W	18.611667, -98.770000	1164	Pioneer
Tepalcingo, Morelos	Pioneer P4028W	18.620266, -98.813735	1160	Pioneer
Tetelilla, Morelos	Tiburón	18.611667, -98.770000	1164	Syngenta
San Miguel, Teotlalco, Puebla	Black Native	18.512159, -98.820937	1044	Native
San Miguel, Teotlalco, Puebla	Pioneer 30F35	18.512159, -98.820937	1044	Pioneer
San Miguel, Teotlalco, Puebla	Zapata 2	18.512159, -98.820937	1044	Agrícola el caudillo
Contla, Teotlalco, Puebla	White Native	18.517819, -98.811872	1055	Native
Contla, Teotlalco, Puebla	Black Native	18.517819, -98.811872	1055	Native
Contla, Teotlalco, Puebla	Pioneer 30F35	18.517819, -98.811872	1055	Pioneer

**Figure 1.** Exterior pigmentation on corn stalks.

Note: C = Brown color. Mo = Purple color. MA = Dark brown color.

Figura 1. Pigmentación exterior en tallos de maíz.

Nota: C = Color marrón. Mo = Color morado. MA = Color marrón oscuro.

***Fusarium spp.* isolation from stalk and ear of corn**

The corn stalk and grain samples were collected from plants with a complete phenological cycle. The stem samples were sectioned into 1cm² sections of healthy and infected plants, under a laminar flow hood, which was superficially disinfected with 3 % sodium hypochlorite for 2 min (three times) and in distilled water for 1 min (three times), then they were left to dry for 10 min, and then three sections were placed in a Petri dish with culture medium Spezieller Nährstoffarmer Agar (SNA) (1 g KH₂PO₄, 1 g KNO₃, 0.5 g MgSO₄·7H₂O, 0.5 g KCL, 0.2 g glucose, 0.2 g sucrose and 20 g agar in 1 L of distilled water) + antibiotics (Gentamicin, 1 mL L⁻¹). The Petri

dishes were sealed and kept in incubation at 26 °C for 168 h, 15 replicates were made per genotype, and where one repetition consisted of a Petri dish with three sections.

Fungal isolation from grains was made according to the National Seed Health System (SNHS 2020). For this purpose, grains were disinfected with 3 % sodium hypochlorite (two times) and subsequently they were rinsed with distilled water for 1 min (two times). The sowing was carried out in polyethylene trays on two layers of sterile blotting paper (previously moistened), 50 grains (with and without *Fusarium* symptoms) were randomly selected and uniformly distributed in the tray, giving a total of 20 replicates per genotype. Finally, the trays were labeled and kept at 25 ± 2 °C for 48 h in a bioclimatic chamber, later, they were frozen at -20 °C for 24 h, and finally they were kept at room temperature (25 °C ± 2) per 264 h, alternating 12:12 h white light and darkness (figure 2).



Figure 2. Sample of tagged stem and ear corn used for *Fusarium* spp. isolation.

Figura 2. Muestra de maíz de tallo y mazorca marcado utilizado para el aislamiento de *Fusarium* spp.

Pathogens purification by monoconidial cultures

Three 6 mm diameter explants were extracted from each isolated fungus colony and were placed in test tubes with 9 mL of sterile distilled water and 1 mL was extracted and then deposited in a Petri dish with SNA medium, and with a dispersion rod it was spread uniformly over the entire box, 24 h later only one germinated conidium was taken and placed in a new Petri dishes with SNA and kept at 25 ± 2 °C for 120 h.

Morphological identification

The macroscopic identification of colonies was carried out according to the shape, color and texture, and their microscopic identification was based on the structure and composition of 100 conidia, phialides and chlamydospores using a digital microscope with an integrated camera and with the support of measurement DinoCapture software 2.0 (Dinolite, 2020), and for morphological identification the taxonomic keys of Leslie & Summerell (2006) and the interactive key for *Fusarium* of Seifert (1996) were used.

Molecular identification

The cetyl-trimethyl ammonium bromide (CTAB) method was used to extract DNA fungal (Almeyda et al., 2001), to do this, starting from axenic strains, mycelium of each *Fusarium* spp. the strain was macerated with pistil in a porcelain mortar previously sterilized, to which 500 µL of extraction were added with: NaCl 1.4 mM, EDTA 20 mM, PVP 1 % Tris Base 100 mM (pH 8) and 200 µL B- mercaptoethanol. The maceration product was placed into sterile 1.5 mL microtubes, incubated at 65 °C for 45 min at 15 rpm and vortexed for 30 s. Next, 500 µL of SEVAG (chloroform-isoamyl alcohol, 24: 1) were added to the sample and subsequently it was centrifuged at 1200 g for 15 min. The supernatant was recovered and transferred to another microtube, and an equal volume of cold isopropanol was added and kept at -20 °C for 24 h. After that time, it was centrifuged at 1200 g for 10 min, the microtubes were decanted and finally the DNA pellet obtained was resuspended into 60 µL nuclease-

free water and stored at 4 °C until use. The ITS1 and ITS4 regions were amplified. Mixing was performed for amplification in a final volume of 15 µL, composed of 5.8 µL MQ water, 2.5 µL buffer (10X), 0.5 µL dNTP's (10mM), 2.5µL of each primer ITS1 (5'TCC GTA GGT GAA CCT GCG G3') and ITS4 (5'TCC TCC GCT TAT TGA TAT GC3'), 0.2 µL Taq-polymerase, 0.2µL DNA Taq-polymerase and 1µL of DNA. The amplification reactions were carried out using a thermal cycler under the following conditions: 1 cycle 95°C 3 min, followed by 35 cycles to 95°C by 10 s, 57 °C by 30 s and 72 °C by 45 s, ending with a polymerization cycle of 72 °C for 5 min. The PCR products were verified on 1 % agarose gels and visualized under UV light. Finally, the PCR products were purified using the isolate II PCR product and gel kit. The samples were sequenced at Potosino Institute for Scientific and Technological Research (IPYCIT) with the labeled dideoxynucleotide method on the 3130 Genetic Analyzer sequencer, and the sequence obtained was assembled and compared with those available in the database of the National Center for Biotechnology Information (NCBI), using the BLAST tool for highly similar sequences.

Socioeconomic and cultural study of the corn crop

To obtain information on consumers of corn and its derivatives, as well as the methods of its storage in Puebla and Morelos states, a semistructured survey was designed with quantitative and qualitative questions to 200 families per state (table 2).

Table 2. Survey performed with families in towns of Puebla and Morelos states Mexico.

Tabla 2. Encuesta realizada a familias en municipios de los estados de Puebla y Morelos, México.

On the consumption of corn and its derivatives
How old are you?
How many members there are in the family?
Do you consume native or hybrid corn?
What color is the corn you eat?
Do you consume products derived from corn?
How many times a week do you consume corn products?
In what quantity do you consume products derived from corn?
Do you consume meat and/or livestock derivatives?
Do they consume meat and/or derivatives of cattle that incorporate corn in their diet?
How many times a week do you eat meat?
How much do you eat meat?
About storing corn
Do you produce corn?
Do you store corn for consumption or sell it?
What do you store corn in?
Do you apply any product when the corn is stored?
What kind of product do you use in the storage of corn?
How long do you store corn?

Data analysis

The data analysis process of surveys obtained by the families was carried out using Excel® software version 16.0 and R software (R Core Team, 2013).

3. Results and discussion

3.1. Morpho-molecular identification

In general, the fungal strains (all strains were identified in corn stem and grains) isolated in grains and corn stalks from nine localities, flattened colonies of slow and rapid growth were observed, of white, orange, purple colors, with hyaline microconidia in the shape of a club and slightly flattened at each end from 6.597 to 45.788

µm long and 2.537 to 4.760 µm wide. Macroconidia curved to nearly straight; 3-9 septa with foot-shaped basal cell 34.36 to 79.555 µm long and 3.383 to 6.800 µm wide. Chlamydospores (absent in some species), determinants for species of *Fusarium*.

The PCR amplification ranged from 690 to 800 bp (figure 3) identifying four species: *Fusarium equiseti*, *Fusarium verticillioides*, *Fusarium incarnatum* (Roberge) Sacc. (*Fusarium semitectum*) and *Fusarium napiforme* Marasas, Nelson and Rabie (without sexual reproduction) with macro and microscopic characteristics of each species (figures 4 and 5), with a percentage of molecular identity of 98 to 100 % (table 3).

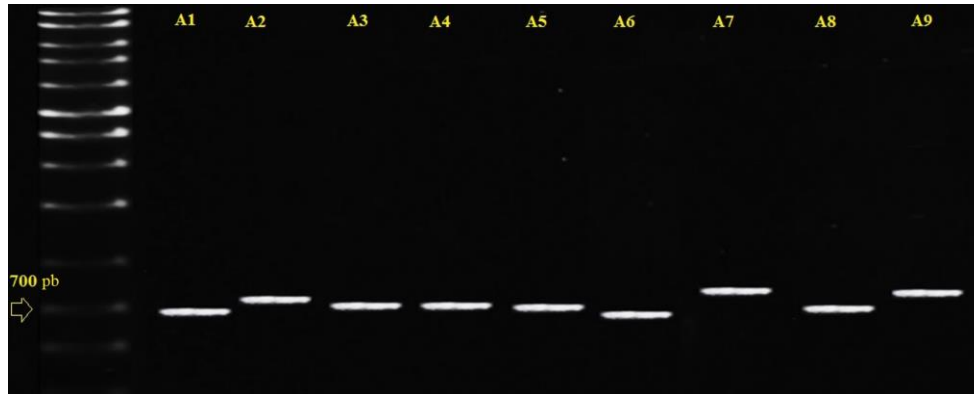


Figure 3. Molecular markers of identified *Fusarium* species.

Note: A1 = *F. incarnatum*. A2 = *F. verticillioides*. A3 = *F. verticillioides*. A4 = *F. verticillioides*. A5 = *F. incarnatum*. A6 = *F. verticillioides*. A7 = *F. napiforme*. A8 = *F. equiseti*. A9 = *F. verticillioides*.

Figura 3. Marcadores moleculares de especies de *Fusarium* identificadas.

Nota: A1 = *F. incarnatum*. A2 = *F. verticillioides*. A3 = *F. verticillioides*. A4 = *F. verticillioides*. A5 = *F. incarnatum*. A6 = *F. verticillioides*. A7 = *F. napiforme*. A8 = *F. equiseti*. A9 = *F. verticillioides*.



Figure 4. Top view of *Fusarium* species colonies isolated from corn.

Note: A1 = *F. incarnatum*. A2 = *F. verticillioides*, A3 = *F. verticillioides*. A4 = *F. verticillioides*. A5 = *F. equiseti*. A6 = *F. verticillioides*. A7 = *F. napiforme*. A8 = *F. equiseti*. A9 = *F. verticillioides*.

Figura 4. Vista superior de colonias de especies de *Fusarium* aisladas de maíz.

Nota: A1= *F. incarnatum*. A2= *F. verticillioides*. A3 = *F. verticillioides*. A4 = *F. verticillioides*. A5 = *F. equiseti*. A6 = *F. verticillioides*. A7 = *F. napiforme*. A8 = *F. equiseti*. A9 = *F. verticillioides*.

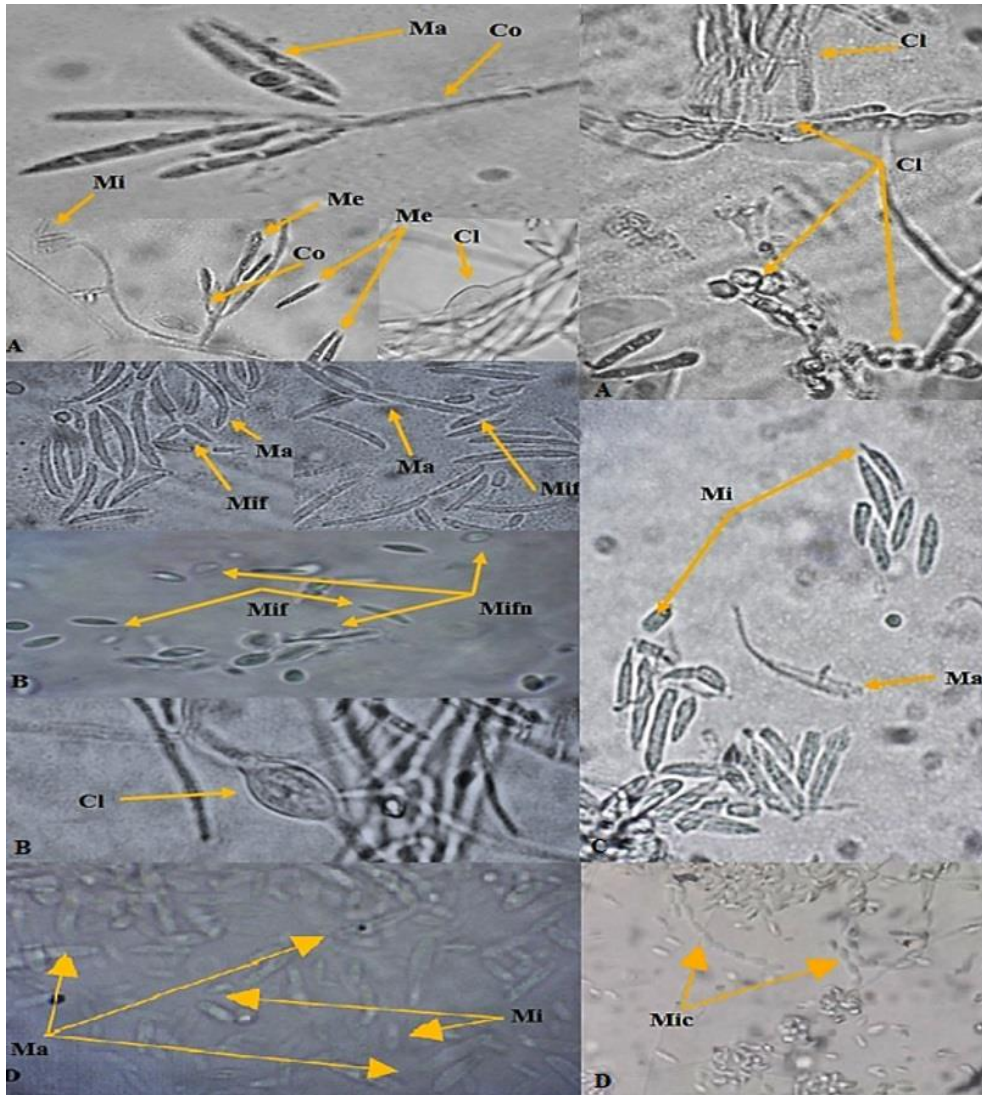


Figure 5. Typical microscopic structures of *Fusarium* species.

Note: A = *F. incarnatum*. B = *F. napiforme*. C = *F. equiseti*. D = *F. verticillioides*. Ma = Macroconidia. Mi = Microconidia. Me = Mesoconidia. Co = Conidiophore. Cl = Chlamydospore. Mif = Spindle-shaped microconidia. Mifn = Spindle and napiform microconidia. Mic = Chain microconidia.

Figura 5. Estructuras microscópicas típicas de especies de *Fusarium*.

Nota: A = *F. incarnatum*. B = *F. napiforme*. C = *F. equiseti*, D = *F. verticillioides*. Ma = Macroconidios. Mi = Microconidios. Me = Mesoconidios. Co = Conidióforo. Cl = Clamidospora. Mif = Microconidios fusiformes. Mifn = Microconidios fusiformes y napiformes. Mic = Microconidios en cadena.

Table 3. Morpho-molecular character values for *Fusarium* species.

Table 3. Valores de caracteres morfomoleculares para las especies de *Fusarium*.

Code	Pathogen	Macroconidia (µm)		Microconidia (µm)		PCL	BP	Max score	Total score	% identity	Key*
		Long	Wide	Long	Wide						
A1	<i>F. incarnatum</i>	39.03	6.33	12.0	5.05	Yes	710	915	1095	98.60	MW53 4564.1
A2	<i>F. verticillioides</i>	40.04	3.98	7.8	2.98	No	700	926	926	100.00	MK79 0050.1
A3	<i>F. verticillioides</i>	34.04	3.38	6.6	2.54	No	750	689	689	98.48	MN87 1798.1
A4	<i>F. verticillioides</i>	40.08	3.98	7.8	3.01	No	750	915	915	100.00	MN12 1060.1
A5	<i>F. equiseti</i>	72.45	6.19	41.7	4.34	No	690	865	865	98.00	MT51 5832.2
A6	<i>F. verticillioides</i>	44.05	4.38	8.5	3.28	No	680	909	909	99.40	MN04 9928.1
A7	<i>F. napiforme</i>	53.38	5.88	8.9	3.11	Yes	800	915	915	99.80	MH86 2670.1
A8	<i>F. equiseti</i>	79.56	6.80	45.8	4.76	No	690	920	1100	100.00	KR819 405.1
A9	<i>F. verticillioides</i>	39.82	3.96	7.7	2.97	No	750	926	926	99.80	MT50 5436.1

Note: PCL = Chlamydospores presence. BP = Base pairs. *NCBI-BLAST comparative basis.

Nota: PCL = Presencia de clamidosporas. BP = Pares de bases. *Base comparativa NCBI-BLAST.

3.2. *Fusarium* spp. incidence in grains and corn stalks

In all locations studied there was an incidence of *Fusarium* species, although not all of them were equally present in grains and stems. The genotypes that showed the highest *Fusarium* spp. incidence was Pioneer 30F35 in San Miguel, Teotlalco, Puebla and the Native white in Contla, Puebla, with 35.2 and 39.6 % in grains and 44.4 and 26.7 % in stems by *F. verticillioides* (A2). The genotypes that showed lowest incidence were Tiburón in Tetelilla, Morelos, with three associated *Fusarium* species and only with an incidence in grains with 1.5, 3.7 and 29.2 % by *F. incarnatum* (A1), *F. verticillioides* (A6) and *F. napiforme* (A7), respectively. On the other hand, Pioneer P4028W genotype in Tepalcingo, Morelos, showed low incidence in grains with 2.2 % by *F. verticillioides* (A3), and 13.7 and 28.9 % in grains and stems respectively by *F. equiseti* (A5) (figure 6).

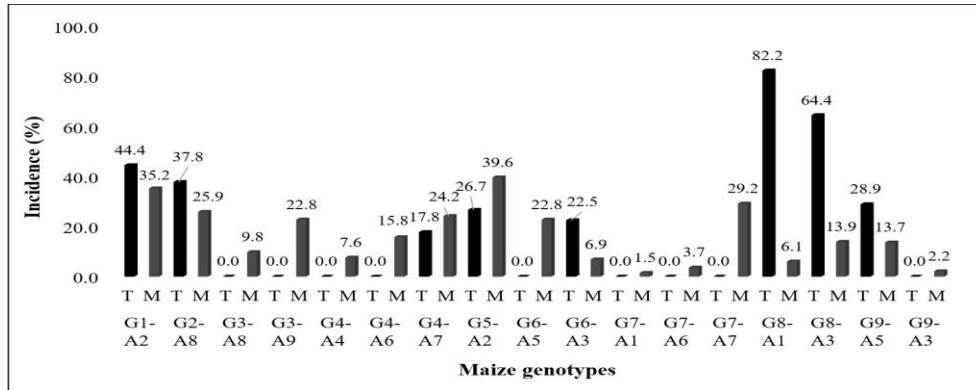


Figure 6. *Fusarium spp.* incidence in corn (maize) stem and grain.

T = Stem incidence. M = Grains incidence. G1 = Pioneer 30F35 from San Miguel, Teotlalco, Puebla. G2 = Native black from San Miguel, Teotlalco, Puebla. G3 = Zapata 2 from San Miguel, Teotlalco, Puebla. G4 = Native black from Contla. G5 = Native white from Contla. G6 = Pioneer 30F35 from Contla, Puebla. G7 = Tiburón from Tetelilla, Morelos. G8 = Pioneer P4028W from Tetelilla, Morelos. G9= Pioneer P4028W from Tepalcingo, Morelos. A1 = *F. incarnatum*. A2 = *F. verticillioides*. A3 = *F. verticillioides*. A4 = *F. verticillioides*. A5 = *F. equiseti*. A6 = *F. verticillioides*. A7 = *F. napiforme*. A8 = *F. equiseti*. A9 = *F. verticillioides*.

Figura 6. Incidencia de *Fusarium spp.* en tallo y granos de maíz.

T = Incidencia en tallo. M = Incidencia en granos. G1 = Pioneer 30F35 de San Miguel, Teotlalco, Puebla. G2 = Negro nativo de San Miguel, Teotlalco, Puebla. G3 = Zapata 2 de San Miguel, Teotlalco, Puebla. G4 = Negro nativo de Contla. G5 = Blanco nativo de Contla. G6 = Pioneer 30F35 de Contla, Puebla. G7 = Tiburón de Tetelilla, Morelos. G8= Pioneer P4028W de Tetelilla, Morelos. G9= Pioneer P4028W de Tepalcingo, Morelos. A1 = *F. incarnatum*. A2 = *F. verticillioides*. A3 = *F. verticillioides*. A4 = *F. verticillioides*. A5 = *F. equiseti*. A6 = *F. verticillioides*. A7 = *F. napiforme*. A8 = *F. equiseti*. A9 = *F. verticillioides*.

According to pigmentation, it should be noted that genotype with the highest incidence due to brown color was Zapata 2 from San Miguel, Teotlalco, Puebla, for dark brown it was a Black native from Contla, Puebla, and for purple pigmentation the White native from Contla, Puebla (figure 7). The genotypes that showed the lowest incidence due to brown pigmentation were the Black native from San Miguel, Teotlalco, and Puebla, for the dark brown color they were Zapata 2 from San Miguel, Teotlalco, Puebla and White native from Contla, Puebla, and for purple color the Black native from San Miguel, Teotlalco, Puebla (figure 7).

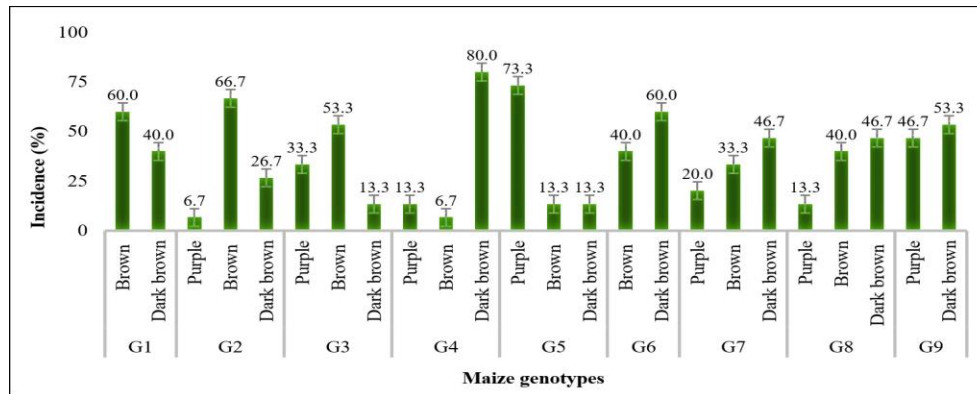


Figure 7. Pigmentation color on the outside of corn stalks.

Note: T = G1 = Pioneer 30F35 from San Miguel, Teotlalco, Puebla. G2 = Black native from San Miguel, Teotlalco, Puebla. G3 = Zapata 2 from San Miguel, Teotlalco, Puebla. G4 = Black native from Contla, Puebla. G5 = White native from Contla, Puebla. G6 = Pioneer 30F35 from Contla, Puebla. G7 = Tiburón from Tetelilla, Morelos. G8 = Pioneer P4028W from Tetelilla, Morelos. G9 = Pioneer P4028W from Tepalcingo, Morelos.

Figura 7. Color de pigmentación en el exterior de tallos de maíz.

Nota: T = G1 = Pioneer 30F35 de San Miguel, Teotlalco, Puebla. G2 = Negro nativo de San Miguel, Teotlalco, Puebla. G3 = Zapata 2 de San Miguel, Teotlalco, Puebla. G4 = Negro nativo de Contla, Puebla. G5 = Blanco nativo de Contla, Puebla. G6 = Pioneer 30F35 de Contla, Puebla. G7 = Tiburón de Tetelilla, Morelos. G8 = Pioneer P4028W de Tetelilla, Morelos. G9 = Pioneer P4028W de Tepalcingo, Morelos.

3.3. Socioeconomic and cultural study of the corn crop

The Black and White native and the Pioneer 30F35 in Contla, Puebla (natives and hybrids) represent a serious problem, due to these genotypes are used mainly as food for people and livestock, with a high consumption of corn per day, as well as its derivatives.

According to the interviews carried out with farmers in the study locations, they show that few people know that they consume hybrid and native maize, from white, black, and red colors, and to a lesser extent they do not know what type of maize they are consuming. The mean was obtained by adding all the data on the consumption (corn and derivatives) of each person (mean per family) and the result was divided by the total number of people. The consumption of corn and its derivatives for these study locations is 1.15 and 1.13 kg per day, in an average of 6 days (days in which they consume corn ()), that is, if we do the interpolation at 7 days a week (complete week), it would be an average per capita consumption of 0.9554 and 0.9416 kg per day at Puebla and Morelos, respectively.

3.4. Identification and incidence of *Fusarium* spp. in grains and corn stalks

The *Fusarium* incidence in grains and corn stalks may be related to the fact that fungicide applications are not carried out in study locations for the prevention (on grains) or control of phytopathogenic fungi in plants. This is mainly due to the lack of information on part of the farmer, as mentioned in the interviews, "we do not apply any fungicide because we do not know it and we do not see the pest", referring to fungi, they said. The latter are microorganisms that constitute the group to which the largest number of agents that cause devastating diseases in animals and plants belong (Dean et al., 2012), being the most numerous groups of pathogens that affect crops (UNL, 2017) and one of the greatest challenges in modern agriculture to achieve its effective and lasting control (Collinge et al., 2010). In addition, they have a great impact, because they cause pre- and post-harvest diseases (Agrios, 2005) and specifically cause losses mainly in five important foods worldwide, rice, wheat, corn, potatoes, and soybeans (Carreras et al., 2021). Regarding losses, these can be quantitative and/or qualitative (taste, texture, color, and shape) (Ashworth et al., 1981; Agrios 1988). Strange & Scott (2005) commented that despite the continuous release of cultivars with some degree of resistance and chemical fungicides, it is

estimated that 10 % of crop yields are lost due to fungal diseases, one of several reasons why farmers in the study localities barely have a production approximately 3-6 tons per ha⁻¹ in corn.

Fusarium can infect any part of the plant, from the beginning to the end of the growing season. Stem rot is a common and severe symptom in corn causing reduced growth, rotten leaf sheaths and inner stem tissue with brown stripes on the lower internodes, and in mature plants, it causes a salmon-pink discoloration of tissues of the medulla inner of stem (Shaner & Scott, 2022). Stem rots cause the vascular bundles to discolor to a yellow-dark brown hue and then the appearance of reddish-brown stripes on lower internodes, with symptoms progressing to the fifth internode or more (Sabet et al., 1966), such as happened in the present investigation, whose pigmentations on the outside of the stems with the highest incidence were dark brown.

Stem rot caused by the *Fusarium* genus is commonly associated with two species, *F. verticillioides* and *F. graminearum* (De León, 1984). The *Fusarium* genus is the most common cob pathogen worldwide (García-Lara et al., 2007) and *F. verticillioides* is the main species, considered as the causal agent of ear rot (Logrieco et al., 2002; Munkvold, 2003; Summerell et al., 2003; Adejumo et al., 2007; Folcher et al., 2009; Nayaka et al., 2009; Woloshuk & Wise, 2014; Dekalb, Asgrow & Deltapine, 2017; Kuki et al., 2020; Pioneer, 2020b; Syngenta 2022; Ramos, 2022) and stem (White, 1999; Liu et al., 2012; Gai et al., 2018), furthermore, this pathogen is found everywhere where corn is grown (Pioneer, 2021). In the present research work, the identified species were *F. equiseti*, *F. verticillioides*, *F. incarnatum* and *F. napiforme*, and it should be noted that the outcomes are largely similar in morphometric characteristics to those reported by other authors (table 4).

Table 4. References in similar morphometric characters in species found in the present research work.

Tabla 4. Referencias en caracteres morfológicos similares en especies encontradas en el presente trabajo de investigación.

Code	<i>Fusarium</i> species	RMOR	RMIC
A1	<i>F. incarnatum</i>	Khoa et al. (2004) Wang et al. (2020) Song et al. (2014)	Lezcano et al. (2012) Mao et al. (2020) Shen et al. (2018) Wonglom & Sunpapao (2020) García-Estrada et al. (2020)
A2, A3, A4, A6, A9	<i>F. verticillioides</i>	Rahjoo et al. (2008) Figueroa-Rivera et al. (2010) Rosas-Guevara et al. (2014) De la Torre-Hernández et al. (2014) Walker et al. (2016) Leyva-Mir et al. (2017) Kiranjot et al. (2020) Giraldo-Arias et al. (2018) Aparecido & Rosa (2019) Solano-Báez et al. (2011)	
A7	<i>F. napiforme</i>	Marasas et al. (1988) Morales-Rodríguez et al. (2007)	
A5, A8	<i>F. equiseti</i>	Goswami et al. (2008) Rodríguez & Menezes (2005) Kosiak et al. (2005) Hami et al. (2021)	

Note: RMOR = Similar reference in morphology. RMIC= Similar reference in micrometry.

Nota: RMOR = Referencias similares en morfología. RMIC= Referencias similares en micrometría.

In the present research, *F. verticillioides* was the species with the highest incidence in stems and grains in all locations studied. There are biotic and abiotic factors that contribute to increasing the incidence and severity of *F. verticillioides* infections in the field and under storage conditions that alter the physical integrity of the grain, such as insect and bird pests (Díaz, 2012). The use of glyphosate -based herbicide to weed control and a nitrogen source as fertilizer are factors that can also promote or inhibit the development of *Fusarium* communities (Leplat et al., 2013). Also, the pH and nutritional composition, as well as the water activity, oxygen presence, time, and temperature of storage (Auerbach, 2003). However, the *Fusarium* spores can be spread to new plants by wind and water; this pathogen spreads over short distances, through water, agricultural equipment and infested tools, and over long distances, through diseased plants or soil attached to them, in addition, it is important to emphasize that once the soil is infested, it remains that way indefinitely (Dixon & Tilston, 2010). In the study locations, some producers do not carry out adequate pest control, except for glyphosate use for weeding control due to its low cost. For example, in Mexico one liter of glyphosate (Faena®) it has a cost that ranges from MX \$ 115.00 in Morelos state to MX \$ 1,250.00 in Tamaulipas state (SE, 2021), in addition to this, producers use nitrogen as the main source of fertilizer in crops. The importance of controlling fungal diseases not only lies in reducing losses in yield, but also consists in avoiding the production of which has implications for grains quality, in public and animal health (Mazzani et al., 1999; García et al., 2006). On the other hand, fumonisins have toxic effects when consumed by humans and animals (Levin et al., 2003; Bush et al., 2004). There are multiple alternatives to prevent colonization of molds in grains and seeds, and thus avoid mycotoxins presence, however, genotype selection and the improvement for resistance to fungal incidence turns out to be the most favorable, due to the great variety of factors that influence the grain deterioration (Chavarri, 2014; taken from Chavarri et al., 2017).

3.5. Socioeconomic and cultural study of the corn crop

Corn consumption in Puebla and Morelos is 0.9554 and 0.9416 kg per day, equivalent to an annual consumption of 348.72 and 343.68 kg (by for example, only the tortilla (made hand) weighs approximately between 30 to 40 g (mean 35 g), people said that they had lunch with a minimum of 5 to 6 tortillas (approximately 210 g) to eat 6 to 7 tortillas (approximately 245 g) and for 3 to 4 tortillas (approximately 140 g), that is, an mean of 595 g in tortillas alone, plus the consumption of corn derivatives) (figure 8), while in México the national level there is an annual *per capita* consumption of 196.4 kg of white corn (PAN, 2016). The consumption of foods prepared with cereals contaminated with fumonisin B, produced mainly by *F. verticillioides* (the pathogen with the highest incidence in this research) has been associated with acute poisonings, in which gastrointestinal manifestations such as vomiting, diarrhea, abdominal pain occur and dehydration (Peraica et al., 1999).

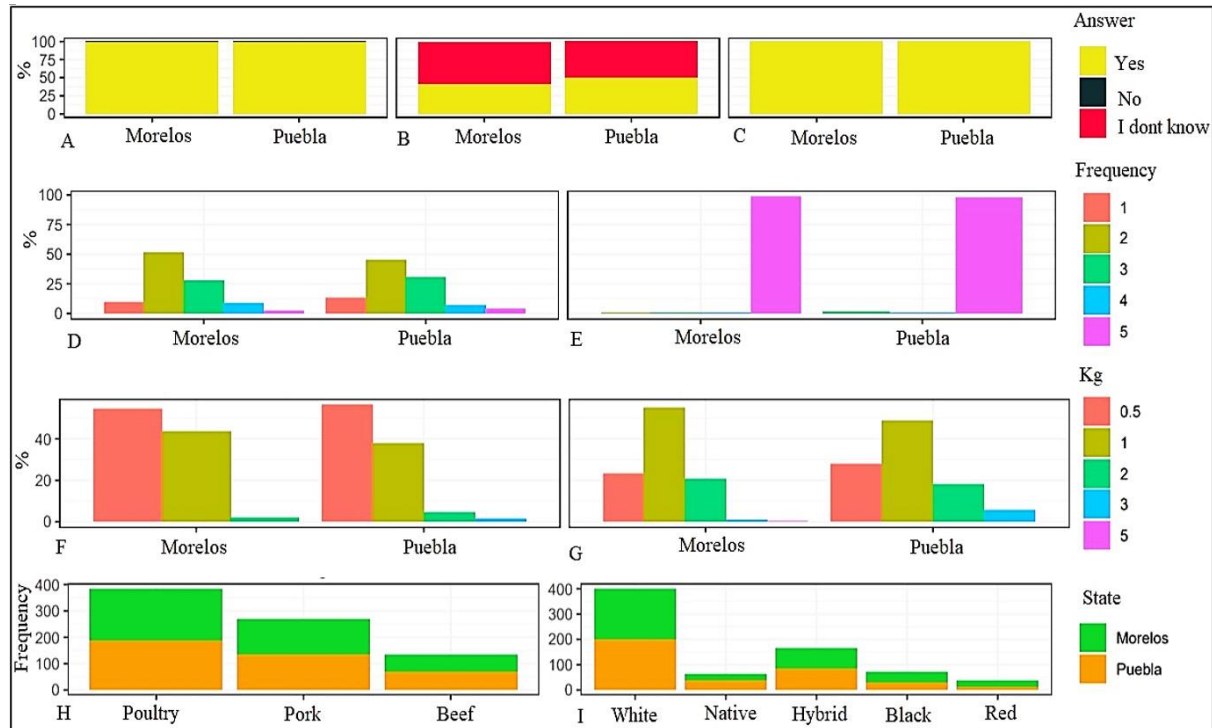


Figure 8. Means of corn consumption in Puebla and Morelos states y consumption of products derived from corn, as well as meat and livestock derivatives in Puebla and Morelos states.

Note: A = Do you consume meat and/or livestock derivatives? B = You consume native or hybrid corn? C = You consume products derived from corn? (Answers). D = Do you consume meat and/or livestock derivatives? E = You consume products derived from corn? (Frequency's). F = Do you consume meat and/or livestock derivatives? G = You consume products derived from corn? (kg). H = Do you consume meat and/or livestock derivatives? (Types). I = You consume native or hybrid corn? (Color and type of corn).

Figura 8. Formas de consumo de maíz en los estados de Puebla y Morelos y consumo de productos derivados del maíz, así como carnes y derivados pecuarios en los estados de Puebla y Morelos.

Nota: A = ¿Consumes carne y/o derivados pecuarios? B = ¿Consumes maíz nativo o híbrido? C = ¿Consumes productos derivados del maíz? (Respuestas). D = ¿Consumes carne y/o derivados pecuarios? E = ¿Consumes productos derivados del maíz? (Frecuencias). F = ¿Consumes carne y/o derivados pecuarios? G = ¿Consumes productos derivados del maíz? (kg). H = ¿Consumes carne y/o derivados pecuarios? (Tipos). I = ¿Consumes maíz nativo o híbrido? (Color y tipo de maíz).

Especially in corn dough, global fumonisin concentrations range from <10 ppm in the United States of America to > 100 ppm in some locations in China and Africa (Marasas, 2001), however, subsequent results indicate that fumonisins role depends on the environment and genetic context in plant-pathogen interaction (Desjardins et al., 2007). For example, in Africa considering that per capita consumption of corn is among the highest in the world (400 g/d), Shephard et al. (2007) estimated that the average daily intake of fumonisin B1 of an average adult (60 kg) is between 3.43 and 8.67 mg/kg of body weight per day, greatly exceeding the provisional maximum tolerable intake of 2 mg/kg of body weight per day established by the World Health Organization (WHO, 2002). The high corn consumption and its derivatives in México for the localities of this study, would be a strong problem when consuming more than doubled *per capita* consumption than in Africa, in addition to consuming beef, pork and poultry on a daily basis, highlighting that the consumption of mycotoxins in people is facilitated by the consumption of products of plant and animal origin, which are contaminated when animals consume significant amounts of mycotoxins and are transported to final products (meat, milk or eggs) (Duarte

& Villamil, 2006; Battacone et al., 2010; Bandera et al., 2011; Baliukoniene et al., 2012), however, these studies are necessary.

The contamination of food with mycotoxins is considered a process that begins in the field and continues during the harvest, the grains drying and the storage of field products (CAST, 2003), mainly in cereals, which are the basis of food in México and developing countries (Santillán-Mendoza et al., 2017). Among mycotoxins, fumonisins are produced mainly by *F. verticillioides* and produce fumonisins B1, B2, B3 and B4 (Proctor et al., 2006) with an important role as a potential Group 2B cancer promoting agent (Ueno, 1993; IARC, 2002), in equine leukoencephalomalacia, porcine pulmonary edema, and rat hepatocarcinoma (Marasas, 1995; Marasas et al., 1988; Gelderblom et al., 1998; Covarelli et al., 2012), furthermore, it is one of the species frequently implicated in human infections together with *F. solani* and *F. oxysporum* (Dignani & Anaissie, 2004).

Fusarium napiforme is one of the species that together with *F. subglutinans* are a problem by causing mycosis in humans (De Souza et al., 2014), it also causes hypersensitivity pneumonitis (Lee et al., 2000) and hyalohyphomycosis (Melcher et al., 1993). On the other hand, *F. incarnatum* is a field fungus that affects crops such as sorghum, rice, and corn, and mainly occurs in temperate and subtropical regions, in addition, it produces toxins such as deoxynivalenol and fumonisin (Riddell et al., 2010; Gupta, 2017), while *F. equiseti* is a weak pathogen in cereals and is occasionally associated with grains infected by other *Fusarium* species (Goswami & Kistler, 2004).

Currently, the toxicological risk associated with mycotoxins has become a central aspect of the problem of fungal invasion of crops or stored grains (Devries et al., 2002), since, during corn storage, fungi mostly cause problems due to heating, compaction, and deterioration of grains; and the use of fungal inhibitors delays heating, reduces the growth rate of fungi and keeps the grain dry (Bolivar, 2007). The relative humidity and temperature of the grain must be controlled to avoid the development of fungal pathogens that cause grains deterioration (Antonio-Bautista et al., 2021). Several studies have shown that species composition of *Fusarium* is influenced by climatic conditions, as well as cultural practices (Arino & Bullermann, 1994; Flett et al., 1998; Mansfield et al., 2005; Scala et al., 2016; Fernández et al., 2008; Edwards & Jennings, 2018).

In the study locations, there is a minimum of people who apply a product when the corn is in storage; in the people interviewed by state, in Puebla only 16 % store corn and of these, 6 % apply aluminum phosphide and the largest number of people store it for less than 6 months, while in Morelos state only 10 % of the people interviewed store corn, of which 1 % apply the previous product, and store it for a period of between 6 to 12 months in inappropriate conditions, such as; black polyethylene bags, water tanks, unventilated rooms, polyethylene barrels and gallons, sacks and even wooden sheds (figure 9), what causes an increase in damage, for example, direct damage; when insects consume the grain, causing weight loss, reduced germination, and fewer nutrients and indirect damage; heating and humidity and the transmission of parasites to people and animals (Agroware, 2016), as fungi development and mycotoxins (Fusé et al., 2013).

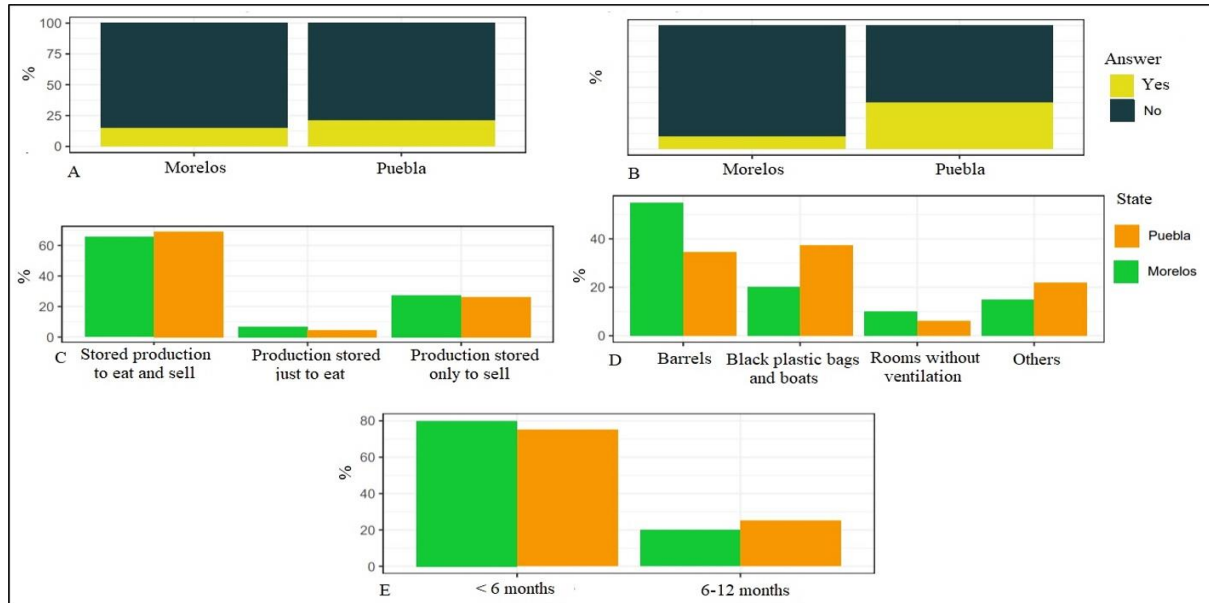


Figure 9. Corn production in Puebla and Morelos states.

Note: A = Do you produce corn? B = Do you apply any product when the corn is stored? C = Do you store corn for consumption or sell it? D = What do you store corn in? E = How long do you store corn?

Figura 9. Producción de maíz en los estados de Puebla y Morelos.

Nota: A = ¿Usted produce maíz? B = ¿Aplica algún producto cuando se almacena el maíz? C = ¿Almacena maíz para consumo o lo vende? D = ¿En qué almacena el maíz? E = ¿Cuánto tiempo almacena el maíz?

4. Conclusions

Four species of *Fusarium* fungus were found associated with stem and ear rot that were identified as *F. equiseti*, *F. verticillioides*, *F. incarnatum* and *F. napiforme* in hybrid and native maize with an identity of 98 %. Corn is the main food for the study locations, however, due to the high corn consumption and its derivatives, there is a need to implement quality measures that guarantee safe grains production for animal and human consumption and avoid possible diseases due to direct and indirect mycotoxin contamination.

5. Supplementary information

No.

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Interest conflict

The authors declare that there is no conflict of interest.

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