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Article

Husk tomato cultivars susceptible to wilt in Sinaloa

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Abstract

The wilt or damping-off of husk tomato is a disease caused by fungi originating in the soil, among which *Fusarium oxysporum*, *Macrophomina phaseolina,* and *Rhizoctonia solani* stand out, causing losses due to the lack of resistant varieties. This study aimed to determine the response of husk tomato (*Physalis ixocarpa* Brot.), Dalí, Siqueiros, and Tamayo hybrids, as well as cv. Gabriela, Puebla, San Miguel, and Tecozautla, to pathogenic isolates of *F. oxysporum*, *M. phaseolina,* and *R. solani*. The study was conducted under greenhouse conditions; sterilized substrate of river sand + peat (1:3 v/v) was placed in pots and then inoculated with three isolates of *F. oxysporum* $1.6x10^5$ CFU, two of *M. phaseolina*, and three of *R. solani* (8 g of infested sorghum seed/pot, of each fungus). Five seeds of the corresponding husk tomato cultivar were deposited in each pot, then covered with the same substrate and incubated for 30 days. A completely randomized design was used, with three repetitions (pots). In the control treatment, the seeds were sown on soil without fungus. The emergence of seedlings was estimated nine days after sowing (das), and the severity of infection for each of the pathogens (scale of 0-5) at 30 das. Data were analyzed using nonparametric statistics. The seven cultivars tested were susceptible to the species *F. oxysporum*, *M. phaseolina,* and *R. solani*; some of the isolates tested exerted a significant differential effect of virulence on some of the varieties studied.

Keywords:

Fusarium oxysporum, *Macrophomina phaseolina*, *Rhizoctonia solani*, husk tomato cultivars, pathogenicity.

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Introduction

Husk tomato (*Physalis ixocarpa* Brot.) is a nightshade that, in 2020, was cultivated in an area of 39 865 ha nationwide. Sinaloa is the primary producer of this crop, with 140 752 t of harvested fruits in a cultivated area of 6 114 ha with an average yield of 23 t ha^{-1} (SIAP, 2020). The main husk tomato-growing municipalities in Sinaloa are Escuinapa with 1 610 ha sown, Guasave 1 175 ha, Ahome 884 ha, Angostura 720 ha, and Mazatlán 438 ha (SIAP, 2020).

In Mexico, husk tomato has a productive potential of 80 t ha^{-1} (Castro-Brindis, 2000). However, the production of the crop is limited by the presence of fungal diseases, among which the following stand out: white smut (*Entyloma australe*) (Moncayo-Pérez *et al*., 2020), leaf spot, *Cercospora physalidis*, powdery mildew *Podosphaera xanthii* (Félix-Gastélum *et al*., 2007) and yellowing-wilting caused by *Fusarium* sp. (Soto-Zarazúa *et al*., 1998).

In Sinaloa, wilt is, after the virus disease, the most important disease, with losses that can reach 50% of the yield (Ayala-Armenta *et al*., 2020). The disease called husk tomato wilt presents severity that can be estimated in total or in proportion and manifests itself as chlorosis and flaccidity of the foliage, the fruits often detach prematurely, the roots show a light brown to dark brown rot and sometimes pink, reddish or violet tones are also observed, the rot can extend to the neck and base of the stem (Apodaca-Sánchez, 2005; Apodaca-Sánchez *et al*., 2008; Vásquez-López *et al*., 2009; Ayala-Armenta *et al*., 2020).

Wilt is of complex etiology since, in Sinaloa, a group of fungi has been reported as associated with the disease, among which the most frequent are *Fusarium oxysporum*, *Fusarium solani,* and *Rhizoctonia solani* (Apodaca-Sánchez, 2005; Apodaca-Sánchez *et al*., 2008), whose pathogenicity has been proven (Flores-Sánchez *et al*., 2009), but the identity of the fungi has been determined only based on their morphology. To date, there is little information related to the response of husk tomato varieties sown in Mexico to fungi originating in the soil associated with wilt.

In Mexico, chemical and cultural management measures are usually ineffective against wilt-associated fungi. The comprehensive management of the disease should be based on sowing resistant or tolerant genotypes, but the behavior of commercial varieties in relation to possible resistance or tolerance is unknown. The present work aimed to evaluate, in the greenhouse, the response of seven commercial genotypes of husk tomato to different isolates of the fungi *F. oxysporum* (Family: Nectriaceae), *M. phaseolina* (Family: Botryosphaeriaceae) and *R. solani* (Family: Ceratobasidiaceae), which cause the wilt of husk tomato, in Sinaloa.

Materials and methods

Site description

The work was carried out in the greenhouses of the Faculty of Agriculture of Valle del Fuerte (25° 44' north latitude, 108° 48' west longitude, and 14 masl) in 2019. The following were tested: six isolates of *F. oxysporum* with access code of the gene bank F-131 (MN270353), 135 (MN270354), 140 (MN270356), 143 (MN270360), 145 (MN270358) and F-146 (MN270362), two isolates of *M. phaseolina* M-147 (MN270374) and M-150 (MN270377) and three of *R. solani* from the anastomosis group AG-4 HGI R-115 (MN264605), 116 (MN264606) and R-118 (MN264607) (Ayala-Armenta *et al*., 2020). Seven commercial husk tomato cultivars with wide distribution in Mexico were sown: Dalí (Harris Moran Mexico, HM Clause®), Gabriela (United Genetics Mexico), Puebla (Semillas Caloro Mexico), San Miguel (LatamSeeds Mexico), Siqueiros (Harris Moran Mexico, HM Clause®), Tamayo (Harris Moran Mexico, HM Clause®) and Tecozautla (Suprema Seeds Mexico).

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Inoculation and development of the trial

Expanded polystyrene (EPS) pots, with a capacity of 2 L, were filled with a sterile substrate of sand + peat moss 1:3 v/v previously infested with the corresponding isolate of the three species under study. The inoculum of *F. oxysporum* was reproduced in corn kernel (Gilchrist-Saavedra *et al*., 2005; López-Valenzuela *et al*., 2014). In contrast, *R. solani* and *M. phaseolina* were multiplied in grains of sorghum (*Sorghum bicolor*), cited by Fernández-Herrera *et al*. (2013). In both cases the grain was washed with distilled water and soaked for 24 h, then the excess water was absorbed with absorbent paper. The grain was sterilized in 1 L bottles for two one-hour periods at 121 °C at 15 pounds of pressure. The isolates were grown in potato dextrose agar (PDA) at room temperature (24 ±3 °C) until the mycelium covered the box. The mycelial growth in PDA was transferred in strips to the bottles with grains and stirred until homogenized. The inoculum was incubated for 30 days in darkness at room temperature (24 ±3 °C) with daily manual stirring, prior to inoculation in the substrate, cited by Fernández-Herrera *et al*. (2013), with slight modifications.

The fungal isolates were inoculated prior to sowing the husk tomato; for *F. oxysporum*, 8 g of infested corn was applied in each pot, whose density was $1.6x10^5$ CFU, similar to that reported by Chehri *et al*. (2011), while for *R. solani* and *M. phaseolina*, 8 g of infected sorghum grain was used, cited by Fernández-Herrera *et al*. (2013) with minimal modifications. For *M. phaseolina*, the density was 115 CFU/g soil (Torrealba *et al*., 2015), and for *R. solani*, 100 CFU/g soil (Hernández-Castillo *et al*., 2014). Five seeds were sown in each pot, covered by a 2 cm layer of the same substrate, then the pot was covered with a plastic lid, and a hole was left for ventilation.

Description of treatments and experimental design

Each treatment was made up of a combination of a fungal isolate and a variety. The trial was adjusted to a completely randomized design with three replications. Seeds were sown on the substrate without inoculum as a control. The pots were incubated for five days at laboratory temperature (20-24 °C) and then in the greenhouse for 30 days at 15-30 °C. The water and the nutrient solution were applied timely according to the requirements to avoid stress of the plants; from each inoculated plant, a sample of roots in PDA was analyzed in order to corroborate Koch's postulates (Agrios, 2005). The strains obtained were analyzed under the microscope to confirm that their identity corresponded to the isolate inoculated at first.

Variables assessed

Seedling emergence was assessed at nine days after inoculation (dai). The severity of the symptoms was estimated with the modified scale of Correll *et al*. (1986), where the following categories were considered: 0= healthy plant; 1= slight discoloration of the root; 2= extensive discoloration of root vascular tissue; 3= slight root rot; 4= extensive root rot; and 5= ungerminated seed or dead plant. In the case of the average severity values for each treatment, when relevant, the data were converted to percentage by rule of three, taking the maximum value of the scale as 100% (average severity values ≥40% are considered susceptible).

Statistical analysis

Data analysis was performed using the Shapiro-Wilk test (Shapiro and Wilk, 1965) in order to verify normality and the F test for homogeneity of variances. However, the assumptions were not fulfilled, even after the transformation, so the analysis of variance was done with the Kruskal-Wallis test (Kruskal and Wallis, 1952) and in the variables where there were significant differences ($p \leq 0.05$), the comparison of Conover's mean ranks (Conover *et al*., 1981) was carried out.

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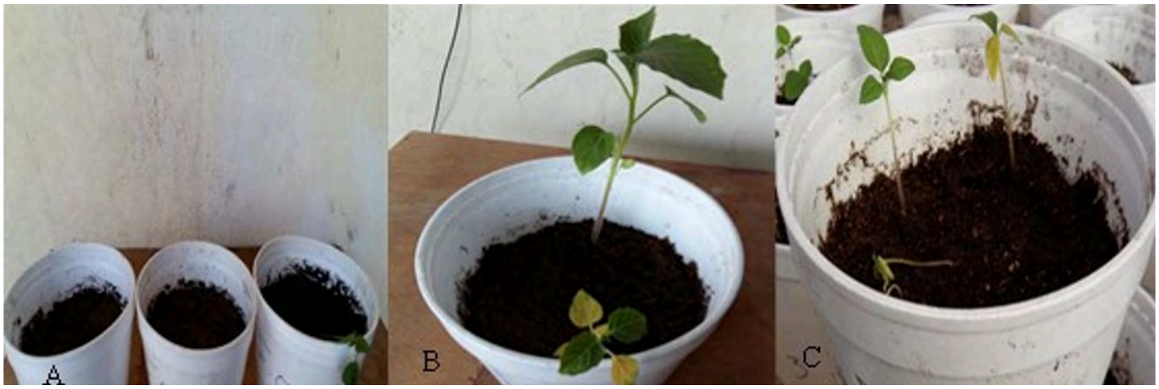
Results and discussion

General symptoms

F. oxysporum

Seeds sown in soil infested with *F. oxysporum* showed rot, which prevented the germination of many seeds; likewise, there was post-emergent death of seedlings due to root rot, which turned brown to pinkish (Figure 1A). The surviving seedlings presented yellowing and reduced radical development (Figure 1B), as well as wilt and gradual death (Figure 1C) similar to that reported in husk tomato (*Physalis ixocarpa* Brot.) (Soto-Zarazúa *et al*., 1998; Gómez-Camacho *et al*., 2006).

Figure 1. Husk tomato seedlings with symptoms of *F. oxysporum* wilt at 30 dai. A) Gabriela isolate F-131; B) Tamayo isolate F-140; and C) Gabriela isolate F-145.



Regarding the emergence of seedlings inoculated with *F. oxysporum*, the Dalí hybrid and the Tecozautla variety did not present significant differences (p> 0.05) between the isolates and the control. The Gabriela variety and the Siqueiros hybrid reduced this variable when inoculated with isolates F-140, F-131, F-135 and F-145, which were different (p# 0.05) to the control, while F-146 and F-143 were the same. For the Puebla variety and the San Miguel material, this variable also decreased with isolates F-140 and 131, which were different from the control (p# 0.05). Likewise, the seedlings of the Tamayo hybrid showed statistical differences with the isolates F-140, 146 and F-131, compared to the control (Table 1).

Table 1. Ranks of seedling emergence in husk tomato cultivars caused by *F. oxysporum* in greenhouse.

Isolate	Dalí	Gabriela	Puebla	San Miguel	Siqueiros	Tamayo	Tecozautla
				Ranks			
F-131	9.2	7.5 ab	9 bc	4.5 ab	8.3 ab	7.3 ab	10
F-135	11.3	9.5 ab	3.2 a	12.7 bc	5.7 a	9.7 ac	11.8
F140	9	3.5 a	3.8 ab	2.5 a	4.3 a	4.2 a	2.7
F-143	13.5	15.8 bc	10 c	14.5 c	16.2 bc	15.7 bc	11.5
F-145	3.5	9.3 ab	53	11.8 ac	8.5 ab	12 ac	8.7
F-146	10.5	11 ac	9 bc	12.5 bc	13.5 ac	8.7 ab	13.3
Control	20	19.5 c	3.2 a	18.5 c	19.5 c	19.5 c	19
CV	55.5	55.6	3.8	55.1	55.6	55.6	55.3

Average ranks that do not share the same letter in each column show significant differences. Kruskal-Wallis test and comparison of Conover's mean ranks ($p\leq$ 0.05). CV= coefficient of variation.

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For the estimation of the severity in seedlings inoculated with *F. oxysporum*, it was found that, in the Dalí hybrid, the isolates were virulent, although F-135 and 143 did not differ from the control. For the Gabriela variety, the isolate F-143 did not show enough severity to differentiate itself from the control. In the Puebla variety, the isolates with the greatest virulence and different from the control (*p*# 0.05) were F-140, 131, 145 and 146. In the San Miguel material, the most virulent isolates were F-145, 140, 146 and 131, with significant differences (*p*# 0.05) with respect to the control. In the Siqueiros hybrid, the most virulent isolates were F-140, 135, 131, 145 and F-146, which were different (*p*# 0.05) from the control. The Tamayo hybrid and the Tecozautla material showed that the most virulent isolates were F-145, 140, 146, and F131 and they were also different from the control (Table 2).

Table 2. Severity of *Fusarium oxysporum* isolates on husk tomato (*Physalis ixocarpa* Brot.) cultivars 30 das.

Isolate	Dalí	Gabriela	Puebla	San Miguel	Siqueiros	Tamayo	Tecozautla
				Ranks			
F-131	21 b	30.5 b	22.3 bc	22.8 bc	19.3 b	22 b	16.3 bc
F-135	13.7 ab	22.8 b	11.5 ab	10.7 ab	14 b	13.8 ab	6.8 ab
F-140	23.5 b	30.5 b	25.8 c	25.3 c	20 b	24.7 b	19.7 c
F-143	13.3 ab	20.3 ab	5 ab	14.3 ac	8.3 ab	12.2 ab	6.8 ab
F-145	23.3 b	28.7 b	20.4 bc	20.3 bc	15.8 b	21.3 b	13 bc
F-146	16.7 b	27.7 b	16.8 bc	16 bc	14 b	17 b	12.3 bc
Control	5 a	9.5 a	6 a	5.5 a	3.5 a	5 a	2 a
CV	55.4	53.2	54.6	55	55.8	55.5	56.1

Average ranks that do not share the same letter in each column show significant differences. Kruskal-Wallis test and comparison of Conover's mean ranks ($p \leq 0.05$). CV= coefficient of variation.

In a work carried out by Soto-Zarazúa *et al*. (1998), there were differences in the susceptibility of 95 husk tomato genotypes in Mexico to natural infection by *Fusarium* sp. under field conditions. In a study conducted with 24 husk tomato materials to determine resistance or susceptibility to white smut (*Entyloma australe* Speg.), *Fusarium* spp. was found with symptoms of stunting and plant death (Moncayo-Pérez *et al*., 2020). In another nightshade such as tomato (*Solanum lycopersicum*), 144 materials (Pérez-Almeida *et al*., 2016) and nine commercial genotypes were susceptible to *F. oxysporum* (Montiel-Peralta *et al*., 2020). To date we have no previous work in which specific isolates of *F. oxysporum* have been tested and correctly identified, in different husk tomato genotypes.

In the plants that emerged from seeds that were sown in the substrate infested with *M. phaseolina*, there was pre-emergent damping-off and the emergence of seedlings decreased (Figure 2A), the root turned black, the foliage showed a yellow coloration, while the stems were thin and brown (Figure 2B), the leaves detached and the seedlings died prematurely (Figure 2C). The control plants remained healthy until the end of the trial. The reisolated fungi were of morphology similar to the respective inoculated pathogens, which fulfilled Koch's postulates.

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Figure 2. Husk tomato seedlings with symptoms of *Macrophomina phaseolina* wilt at 30 dai. A) Tamayo isolate M-147; B) San Miguel isolate M-147; and C) Tecozautla isolate M-147.

The symptoms mentioned coincide with those reported in Gran Esmeralda husk tomato (Ayala-Armenta *et al*., 2020). General symptoms, in its various hosts, include chlorosis, severe root and neck rot, early wilt or death of adult plants (Kaur *et al*., 2012). The results of *M. Phaseolina* found on husk tomato cited above also coincide with the symptoms reported for sunflower (*Helianthus annuus*) (Aboshosha *et al*., 2007); (Khan, 2007); (Monaliza and Soares, 2014), corn (*Zea mays* L.) (Ortiz-Bustos *et al*., 2015) and soybeans (*Glycine max*) (Ahmad *et al*., 1997; Gupta *et al*., 2012; Hemmati *et al*., 2018).

The effect of the inoculation of *M. phaseolina* in the Dalí, San Miguel, Siqueiros, Tamayo and Tecozautla hybrids showed no significant differences between the isolates, but the isolate M-147 was different (*p*# 0.05) from the control. In the Gabriela and Puebla varieties, no statistically significant differences ($p > 0.05$) were found in seedling emergence (Table 3).

Table 3. Ranks of emergence of husk tomato cultivar seedling caused by *M. phaseolina* in greenhouse.

Isolate	Dalí	Gabriela	Puebla	San Miguel	Siqueiros	Tamayo	Tecozautla
	Ranks						
M-147	2.3 a	3.7	2.5	2 a	3 a	2.2 a	2 a
M-150	4.7 ab	4.3	5.5	5.5 ab	4 ab	5.3 ab	5.5 ab
Control	8 b	7	7	7.5 b	8 b	7.5 b	7.5 b
CV	52.7	49	5.5	52	52.7	52	52.2

Average ranks that do not share the same letter in each column show significant differences. Kruskal-Wallis's test and comparison of Conover's mean ranks ($p \leq 0.05$).

Coefficient of variation (CV)

In all commercial materials, the severity reflected the virulence of the isolates, which were different ($p \leq 0.05$) from the control (Table 4).

Table 4. Severity of *Macrophomina phaseolina* isolates on commercial materials of husk tomato (*Physalis ixocarpa* Brot.) 30 das.

Isolate	Dalí	Gabriela	Puebla	San Miguel	Siqueiros	Tamayo	Tecozautla
	Ranks						
M-147	14 b	6.5 b	10.5 b	14 b	10 b	13.8 b	14 b
M-150	11 b	6.5 b	8.5 b	11 b	9 b	11.2 b	11 b

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Isolate	Dalí	Gabriela	Puebla	San Miguel	Siqueiros	Tamayo	Tecozautla
				Ranks			
Control	5 a	2 a	3.5 a	5 a	3.5 a	5 a	5 a
CV	49.5	51.2	50.8	49.4	51.6	49.4	49.4

Average ranks that do not share the same letter in each column show significant differences. Kruskal-Wallis test and comparison of Conover's mean ranks ($p \leq 0.05$).

Coefficient of variation (CV)

The symptoms in the plants exposed to *R. solani* AG-4 HGI showed seed rot and brown roots (Figure 3A), as well as damping-off at the base of the stem, wilt and chlorosis in leaves and branches (Figure 3B); finally, at 30 das, premature death of the seedlings occurred (Figure 3C). The symptoms observed in plants emerged from seeds agree with those reported in different crops, such as: root rot, damping-off at the base of the stem and general wilt. In early stages, it causes damping-off, death of seedlings, and in adult stage, death of plants (Agrios, 2005).

Figure 3. Husk tomato seedlings with symptoms of wilt by *Rhizoctonia solani* AG-4 HGI, at 30 dai. A) Dalí isolate R-116; B) San Miguel isolate R-118; and C) Puebla isolate R-118.

The inoculation of *R. solani* AG-4 HGI in the hybrids Dalí and Gabriela showed effect on the emergence of seedlings, with significant differences only between the control and the isolates R-116 and 118 ($p \leq 0.05$). The Puebla and San Miguel varieties showed reduction in the emergence of seedlings, the isolate R-116 presented significant differences with respect to the control ($p \leq 0.05$). In the pots with inoculated substrate, the Siqueiros variety showed no differences between the isolates; however, the isolate R-116 was different ($p \leq 0.05$) from the control regarding seedling emergence. In the Tamayo + *R. solani* treatments, the isolate R-116 induced the least emergence of seedlings and was different ($p \leq 0.05$) with respect to the control. In the Tecozautla material, no significant differences were found between the isolates, but the isolates R-118 and R-116 were different from the control ($p \leq 0.05$) (Table 5).

Table 5. Ranks of emergence of husk tomato cultivar seedlings caused by *R. solani* AG-4 HGI in greenhouse.

Isolate	Dalí	Gabriela	Puebla	San Miguel	Siqueiros	Tamayo	Tecozautla
				Ranks			
R-115	8 ab	9 bc	9 b	7.3 ab	7.8 ab	8 bc	8.2 ab
R-116	2.8 a	3.2 a	2.5 a	2 a	2.8 a	2 a	3 a
R-118	4.7 a	3.8 ab	4.5 ab	5.7 ab	4.8 ab	5 ab	4.3 a

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Isolate	Dalí	Gabriela	Puebla	San Miguel	Siqueiros	Tamayo	Tecozautla
				Ranks			
Control	10.5 b	10 c	10 b	11 b	10.5 b	11 c	10.5 b
CV	54.2	53	53.3	54.2	53.9	54.8	54.1

Average ranks that do not share the same letter in each column show significant differences. Kruskal-Wallis test and comparison of Conover's mean ranks ($p \leq 0.05$).

In the specific case of *R. solani* subgroup HGI, it has been reported in nightshades such as potato (*Solanum tuberosum* L.) (Kanetis *et al*., 2016) and tomato (*Solanum lycopersicum* L.) (Kuramae *et al*., 2003; Taheri, 2011), in sesame (*Sesamum indicum*) (Cochran *et al*., 2018), beans (*Phaseolus vulgaris* L.) (Çebi-Kiliço#lu and Özkoç, 2010), melon (*Cucumis melo* L.) (Kuramae *et al*., 2003). In a work, it was determined that some isolates of *R. solani* AG-4 subgroup HGI severely affected the germination and emergence of husk tomato cv. Great Emerald (Ayala-Armenta *et al*., 2020).

Coefficient of variation (CV)

For severity, it was found that, in all commercial materials, the isolates were virulent and different from the control (*p*# 0.05) (Table 6).

Table 6. Severity of isolates of *Rhizoctonia solani* AG-4 HGI on commercial materials of husk tomato (*Physalis ixocarpa* Brot.) 30 das.

Isolate	Dalí	Gabriela	Puebla	San Miguel	Siqueiros	Tamayo	Tecozautla
				Ranks			
R-115	14.8 b	17 b	17 b	17.2 b	12 b	17 b	17 b
R-116	19.2 b	22.2 b	21.7 b	21 b	15.8 b	23 b	21.8 b
R-118	17 b	20.8 b	21.3 b	21.8 b	14.2 b	20 b	21.2 b
Control	6.5 a	8 a	8 a	8 a	5 a	8 a	8 a
CV	50.8	49.2	49.2	49.1	52.5	49.2	49.1

Average ranks that do not share the same letter in each column show significant differences. Kruskal-Wallis test and comparison of Conover's mean ranks ($p \leq 0.05$).

Coefficient of variation (CV)

Given the lack of cultivars resistant to soil fungi that cause wilt, coordinated work in plant genetics with phytopathologists is required to obtain materials resistant to soil fungi, such as those studied here. In other nightshades such as tomatoes, the basis for the management of *F. oxysporum* wilt is through the use of resistant varieties (González *et al*., 2012; Leyva-Mir *et al*., 2013; Morales-Palacios, 2014). Currently, the commercial materials under study are commonly used by farmers in central and northern Mexico and at least three of them (Dalí, Siqueiros and Tamayo) are genotypes that are considered new and important in Sinaloa for being of high yield and quality.

Conclusions

Commercial husk tomato materials Dalí, Gabriela, Puebla, San Miguel, Siqueiros, Tamayo and Tecozautla were susceptible (severity # 40%) to *F. oxysporum* and *R. solani*, while Dalí, Siqueiros, Tamayo and Tecozautla were also susceptible to *M. phaseolina*, 30 days after sowing under greenhouse conditions.

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