v. 2, n. 6, 2022

International Journal of Biological and Natural Sciences

IDENTIFICATION AND in vitro CONTROL OF THE CAUSAL AGENT OF VASCULAR WILT IN TOMATO (Solanum lycopersicum L.) ISOLATED FROM PARACUARO, MICHOACÁN

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Abstract: One of the phytosanitary problems limiting the production of the tomato crop (Solanum lycopersicum) at a global and national level is vascular wilting, which causes economic losses to the producer of up to 60%. Given the importance of this disease, the objectives of this study were to identify the causal agent of tomato vascular wilt and to find the effective chemical and biorational product for control under in vitro conditions. Samples of tomato plant stems were collected in the valley ofParacuaro, the causal agent was isolated and identified macroscopically and microscopically. For the in vitro tests, the treatments wereAzoxystrobin, Folpet, Thiabendazole, Benomyl, Mancozeb, Copper Octanoate, Larrea tridentata and a control, three doses of each treatment were evaluated: 100, 500 and 1000 ppm, in a completely randomized design with four repetitions. The causal agent was identified as Fusarium oxysporum f. sp. lycopersici (Fol). The determination of the special form and race of F. oxysporum was through the use of differential tomato plants (S. lycopersicum), which was identified as race 3 of Fol. In the bioassays, the best treatments were: Folpet, Tiabendazol and Benomyl in the three doses used (100, 500 and 1000 ppm) and Mancozeb and Folpet only in the medium and high doses (500 and 1000 ppm), statistically significant.
Keywords: *Fusarium oxysporum*F. sp. lycopersici (Fol) race 3, differential plants, effectiveness.

INTRODUCTION

The tomato is one of the most consumed vegetables worldwide, in addition to being one of those with the highest economic value, its demand is continuously increasing and with it its cultivation, production and trade. The tomato is considered one of the most important vegetables in many countries around the world, due to the large number of products obtained (Hernández et al., 2007). Due to its level of production, it ranks second among vegetables, surpassed by potato cultivation (Ascencio et al., 2008).

According to FIRA (2014), Mexico is in tenth place in tomato production with a 2.1% share. Michoacán contributing 6.4% of which Parácuaro contributes 13 thousand tons (SAGARPA 2017).

This vegetable is strongly affected by diseases that considerably reduce the production and quality of the product, this frequently causes the total loss of the crop if the necessary control measures are not taken, which significantly raise production costs (López, 2012). Among the most important diseases that affect this crop is vascular wilt, responsible for losses of up to 60% in yield, which also affects the quality of the product. This disease affects tomato crops in at least 32 countries and occurs in a wide variety of conditions. Up to now, three races are known, which are distinguished by their virulence in materials that contain resistance genes (González et al., 2012).

The symptoms initially appear with foliar chlorosis in a sector of the plant and as the disease progresses, yellowing is gradually observed in most of the foliage, causing wilting and later the death of the plant, without producing fruit. or sometimes it is scarce. The vascular tissue at the base of the stem is dark brown in color, extending to the apical end of the stem. The brown color in the inner part of the stem is characteristic of the disease, so it is used for identification, while the pith remains free of infection. The infection of the fruit appears occasionally through dark brown coloration in the vascular tissue (Báez et al., 2010).

In addition to the symptoms described, others may appear such as: growth retardation, epinasty of the leaves (curvature and flaccidity of the leaves without losing

their green color), formation of buttons and adventitious roots in the stem (López, 2012).

This pathogen is more severe in acidic soil conditions. The severity of the infection increases when ammonium-based nitrogen fertilizers are applied, so irrigation with saline water and fertilization with ammonium sulfate predispose the plant to attack by the fungus (FAO, 2003).

The objectives of the following work were; isolate and identify the causal agent of vascular wilt of tomato plants, in the municipality of Parácuaro, Michoacán and determine the physiological race. In addition to evaluating in vitro the efficacy and/or sensitivity of fungicides used in conventional and biorational agriculture for their control.

MATERIALS AND METHODS

SAMPLE COLLECTION

The samples were taken in the field within the San Silviano property of Mr. Mardoqueo Bautista, located in the Tenencia "Los Bancos" municipality of Parácuaro, Michoacán. Stems of tomato plants were collected, with symptoms of wilting, generalized yellowing and internal necrosis in the vascular bundles. The selected tomato cultivar was of the determinate growth saladette type of two differential varieties, the variety 386 F1 and 387 F1, both of which were in the reproductive stage. Two samples of each variety were collected and processed in the laboratory.

ISOLATION AND IDENTIFICATION

The culture medium used was synthetic PDA. The collected plant material (both varieties 386 F1 and 387 F1) was washed with drinking water and cut into sections of approximately 0.5 cm in diameter from the inner part of the stem crown. They were disinfected by shaking with a 3% sodium hypochlorite solution for 30 seconds and triple washed with sterile distilled water. They were then placed equidistantly in Petri dishes.they were sealed with Clean Pack and incubated at 25 °C. Once the growth of the colonies in the different isolates was present, the morphological identification was carried out, for which the specialized keys according to Toussoun and Nelson were used. Once the colonies were identified, pure cultures were obtained at the tip of the hypha, obtaining four strains of each of the varieties.

DETERMINATION OF THE PATHOGENIC RACE OF f. Oxysporum

For the determination of f. sp. and physiological race of F. oxysporum, the methodology reported by Carrillo et al., (2003) was used, in which differential tomato plants (S. lycopersicum) were used, where the following differential tomato varieties were used: El rey F1, 4853 F1, 387 F1, 386 F1 and Rio Grande (Table 1).

The differential varieties were germinated in polyethylene trayswith Sphagnum substrate (Peat Moss). The seedling was disinfected with a 3% sodium hypochlorite solution, while the 2 kg substrate was sterilized in an autoclave. The seedling was placed under greenhouse conditions and maintained at 80% humidity, irrigated with sterile distilled water until seedling growth.When the differential plants presented the first two true leaves, they were transplanted into pots previously disinfected with 3% sodium hypochlorite. The substrate used was oak soil which was sterilized in the autoclave. Once the transplant is done and 7 days after the day after the transplant(DDT), each seedling was irrigated with 250 mL of nutrient solution until obtaining a substrate in its field capacity, in order to avoid nutritional deficiencies that could be confused with the characteristic symptoms of the disease. The

differential variety	Endurance			Comercial House
	race 1	race 2	race 3	
The F1 King	high resistance	high resistance	high resistance	Harris Moran
4853 F1	Resistant	Resistant	Resistant	Harris Moran
387F1	Resistant	Resistant	not resistant	Vilmorin
386F1	Resistant	Resistant	not resistant	Vilmorin
Rio Grande	not resistant	not resistant	not resistant	heat

Table 1. Description of the differential cultivars of tomato *(S. lycopersicum)* used for the identification of the physiological race of Fol.

scale level	incidence and severity
1	no symptoms
2	Slight chlorosis, slight wilting and slight stunting
3	Moderate chlorosis, moderate wilting and moderate stunting
4	Severe chlorosis, severe wilting and severe stunting.
5	dead plant

Table 2. Scale used to determine the degree of the disease in differential tomato plants *(S. lycopersicum)* inoculated with strains of Fol race 3.

Tradename	Active Ingredient (AI)	Concentration
bankit	Azoxystrobin	Equivalent to 250 g ai/kg.
folpan	Folpet	Equivalent to 800 g ai/kg.
roof 60	Thiabendazole	Equivalent to 600 g ai/kg.
Promyl	Benomyl	Equivalent to 500 g ai/kg.
Manzate	Mancozeb	Equivalent to 800 g ai/kg.
Cave	copper octanoate	Equivalent to 104 g ai/L.
governor extract	*L. tridentata*	Equivalent to 900 g ai/L.
Witness		

Table 3. Treatments.

inoculation was carried out at 15 DAT. 250 mL of a conidia suspension was prepared at a concentration of 1x106 spores/mL for each differential plant. The inoculation was indirect through the saturation of the concentrated suspension of conidia to the substrate. The inoculated and control plants were placed under greenhouse conditions and watered every third day with sterile distilled water. The symptoms present were compared with the characteristic damage described by Marlatt et al., (1996),

CONTROL *IN VITRO*

There was a completely randomized experimental design where seven fungicides, five chemical fungicides and two fungicides of natural origin were evaluated, before an absolute control, four repetitions were used for each treatment. Three different doses were evaluated for each treatment: 100 ppm, 500 ppm and 1000 ppm.(table 3). To give a total of twenty-two treatments, which included a witness. This in order to compare the inhibition of treatments against the fungus. Once 24 hours had elapsed after the application of the treatments, data collection began, for which the growth of the mycelium was measured before the different treatments. An analysis of variance and comparison of means were performed on the data obtained using the Tukey test.

For the application of the treatments, the poisoned culture medium technique was used. In which the different fungicides were added with their respective standardized doses in the culture medium. It was allowed to solidify and was placed in refrigeration at a temperature of 4 °C for 24 h; With the help of a punch, a 9 mm diameter disc of the Fol strains was placed in the center of each of the different treatments.

RESULTS AND DISCUSSION

IDENTIFICATION OF THE CAUSAL AGENT AT THE GENUS AND SPECIES LEVEL

The strains obtained were identified within the genus Fusarium while the species corresponded to F. oxysporum, being able to observe the presence of microconidia, macroconidia, chlamydospores and phialides. The microconidia were unicellular with very varied shapes, both cylindrical, oval and slightly curved, these are very abundant and in turn produced in small unbranched monophyalides. The macroconidia presented a maximum of five septa, these are less abundant with the appearance of thin walls and slightly curved.The chlamydospores presented a round appearance with a smooth surface, with different locations, both terminal, intermediate and intercalary (Figure 1). Arbeláez, (2000) reports the same characteristics of the structures of F. oxysporum.

*F. oxysporum*It is often confused with the species Fusarium solani, however, Gene and Guarro (1992), mention that a way to clearly differentiate between the species of F. oxysporum and F. solani is observed in the growth size of their phialides, the phialides of F.oxysporum are short8-14 µm compared to the solani species, since those of this species are longer growing (45-80 µm) and slightly narrower. The phialides of both strains presented a short growth of 9 µm in length (Figure 2) with a thickened appearance, which generally contained a microconidium at its apex.

The macroscopic characteristics that both strains presented at the beginning of their development were pink and white, with a matted and cottony texture of regular growth with a stringy appearance on the back of the Petri dish. The most developed strains presented dark purple coloration with white sporulations (Figure 3).

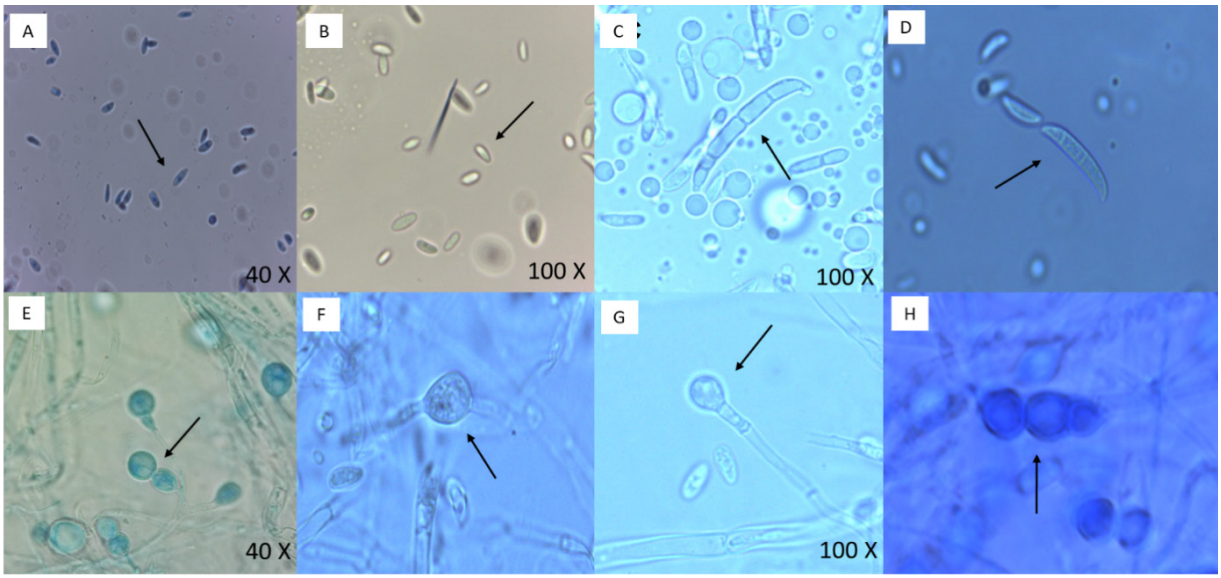

Figure 1. A) Microconidia with varied appearance, cylindrical, oval to slightly curved, B) Appearance of microconidia C) Macroconidia with five slightly curved septa, D) Macroconidia with the presence of three septa, E) Terminal chlamydospore with double cell, F) Intermediate Chlamydospore, G) Terminal Chlamydospore, and H) Intercalary Chlamydospore with three cells.

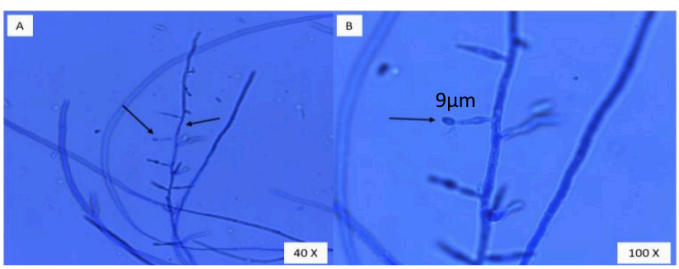

Figure 2. A) Phialides at 40x magnification, and B) Phialides at 100x magnification.

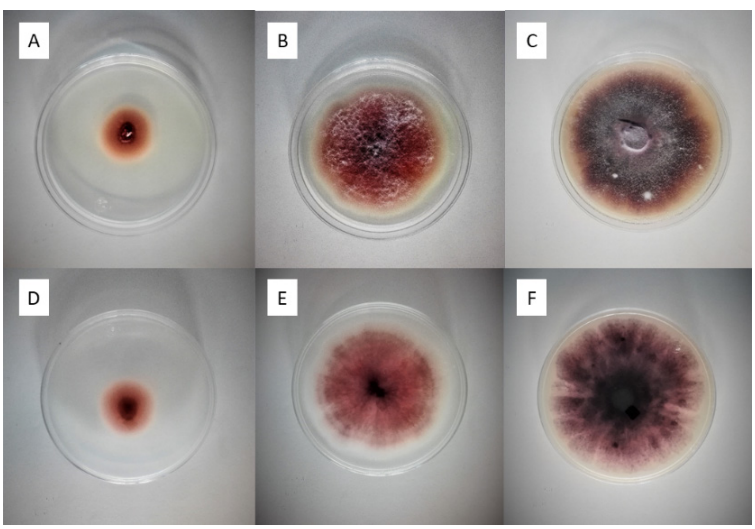

Figure 3. A) Appearance after 7 days of culture, B) 15 days of culture, C) 22 days of culture. Cross-sectional view: D, E and F) At 7, 15 and 22 days of culture.

DETERMINATION OF THE PATHOGENIC RACE

Of the strains of F. oxysporum inoculated in the different differential tomato plants, characteristic symptoms of the disease only appeared in the Rio Grande, 386 F1 and 387 F1 varieties. This indicates that when presenting the characteristic symptoms of the disease in the Rio Grande variety, the damage by said pathogen is confirmed and indicates that the F. oxysporum strain corresponds to the special form (f. sp.) lycopersici. These results are supported by the publishedbyArbeláez (2000), who mentions that of each special form that exists within the fungus F. oxysporum, this is determined based on the susceptibility it has to a certain plant to parasitize it (Figure 4).

The presence of symptoms in varieties 386 F1 and 387 F1 indicates that the strain belongs to race 3 since both varieties have resistance to R1 and R2 of Fol, but are susceptible to R3 of Fol. These results are verified with the resistance expressed to the disease in the El Rey F1 and 4853 F1 varieties, since both have resistance to R1, R2 and R3 of Fol. Kings. These data are supported by what was published by Gutiérrez, (2004), who mentions that the best control method against wilting has been the development of cultivars resistant to new strains of the Fusarium genus.

CONTROL *IN VITRO*

It was possible to determine that Fol race 3 is susceptible to the treatments used, since they presented significant differences between the treatments ($p<0.0001$) for the variable percentage of growth of the fungus mycelium (Table 4).

The best treatments werefolpet, Thiabendazole and Benomyl in their three doses used (100, 500 and 1000 ppm), and Mancozeb in their medium and high doses (500 and 1000 ppm) together with Copper Octanoate in their medium and high doses (500 and 1000 ppm). Statistically these treatments were equal to each other according to Tukey's grouping. But if highly superior to the treatment used as a control, which presented a growth of 100% (Figure 5).

López (2012), López et al., (2005), obtained good results when testing Benomyl through in vivo bioassays in tomato plants inoculated with strains of F. oxysporum f. sp. radicis-lycopersici with 7.75% damage on a 100% scale. Therefore, these results support the use of Benomyl as an alternative in the control of Fol race 3. While Yossen (2014) when comparing the efficiency of Thiabendazole and Mancozeb at three different doses in the in vitro control of F. oxysporum isolated from the oregano cultivation, a greater control was found with Thiabendazole than with Benomyl. With Mancozeb at its highest dose, 93.07% growth inhibition was obtained, while Thiabendazole at its highest dose inhibited 99.80%. Regarding the use of L. tridentata, ópez et al. (2005) showed contradictory results to this research, obtaining a percentage of inhibition in mycelial growth of 87.5% and 96.2%. Similarly, Peñuelas et al. (2017) evaluated the in vitro inhibitory capacity of the L. tridentata extract at different concentrations (0-2000 ppm) and solvents (dichloromethane, methanol, ethanol and water) for the control of F. oxysporum radicis-lycopersici; where the best treatment was at 500 ppm with the ethanol solvent with a 97% inhibition of mycelial growth. This can be justified by what was published by Lira et al. (2002), which reports that the percentage of inhibition of the extract of L. tridentata differs from the geographical location where the plant material of the governor plant is collected.

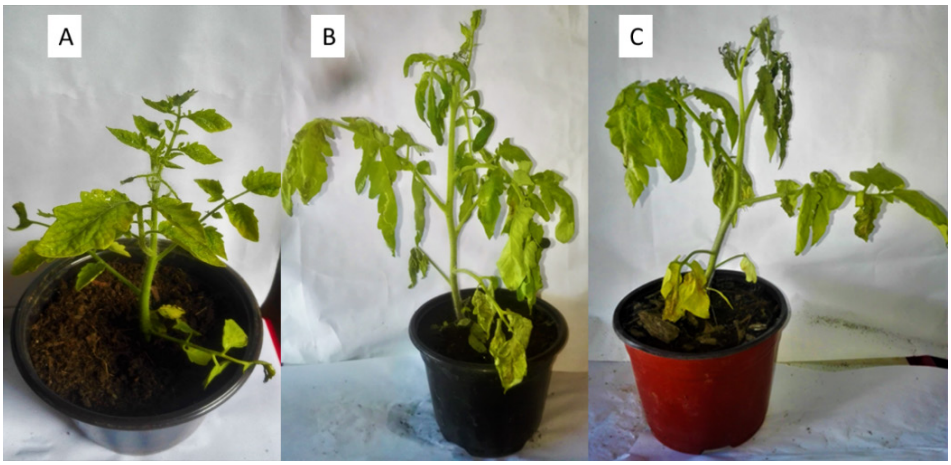

Figure 4. Symptomatology in susceptible varieties. A) Rio Grande variety, B) 386 F1 variety, and C) 387 F1 variety.

Variation Source	Degrees of freedom	Sum of squares	middle square	Calculated F	Pr › F
TREATMENTS	twenty-one	140.7636	6.7030	82.23**	<0.0001
MISTAKE	66	5.3800	0.0815		
TOTAL	87	146.1436			

CV = 26.28%

Table 4. Analysis of variance on the in vitro control of Fol race 3

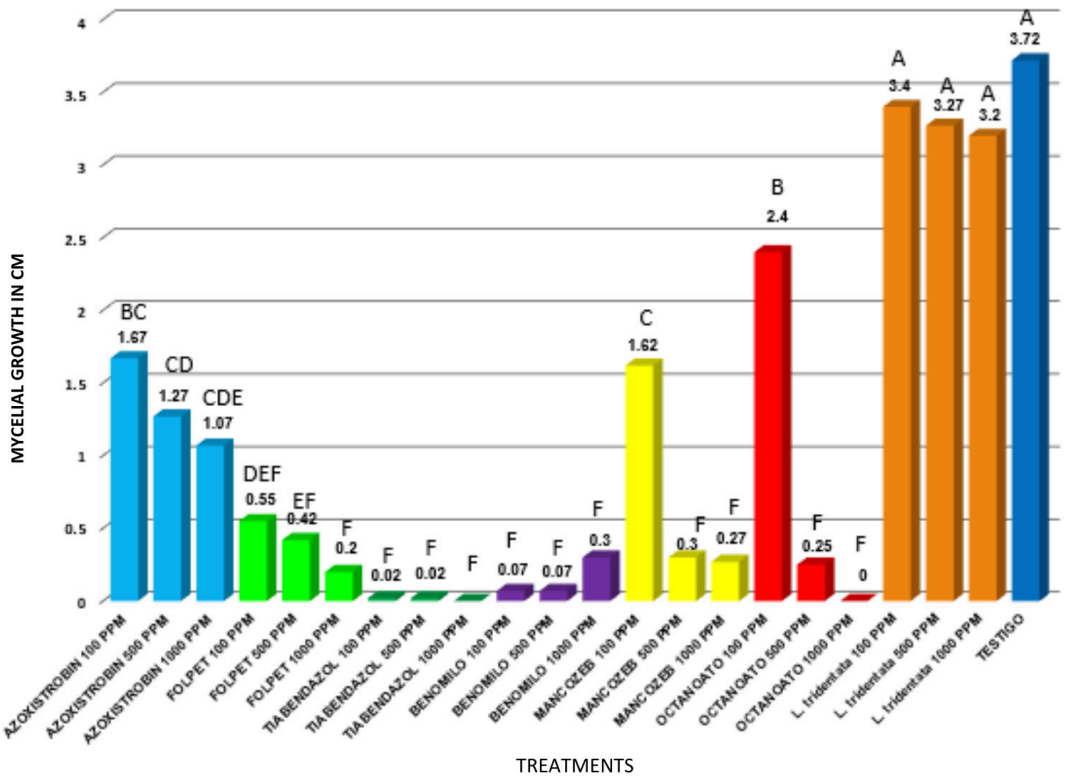

Figure 5. Effect of controlin vitro of the fungicides on Fol race 3 and comparison of means between treatments according to Tukey's grouping (0.05) (AF).

CONCLUSIONS

1. The causal agent of the disease known as vascular wilt of tomato (S. lycopersicum) in the municipality of Parácuaro, Michoacán is the fungus F. oxysporum

2. The physiological race of the F. oxysporum strain isolated from the municipality of Parácuaro, Michoacán, belongs to race 3 of F. oxysporum in its special form lycopersici.

3. The use of varieties resistant to certain taxonomic groups of fungi is an alternative to control highly devastating diseases in agriculture.

4. The fungus F. oxysporum f. sp. lycopersici race 3, is highly susceptible to Thiabendazole, Benomyl and Folpet as it manages to control the growth of the fungus in its three different doses, so it is recommended to use the lowest dose in both fungicides. The fungicide copper octanoate and Mancozeb that presented a control with the high and medium dose, it is recommended to use the medium dose in both fungicides.

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