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IDENTIFICATION, PATHOGENICITY AND IN VITRO ANTAGONISM TESTS OF THE CAUSAL AGENT OF VASCULAR WILT OF TOMATO (SOLANUM LYCOPERSICUM L.)

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Abstract: A problem that limits the production of tomato crops worldwide and nationally is vascular wilting. The objectives of the present investigation were: to identify the causal agent of vascular wilt of tomato and find the race of Fusarium oxysporum to which it belongs, evaluate its pathogenicity and determine its biological control in vitro.

Tomato stems were collected from the municipality of Parácuaro, Michoacán that showed symptoms of wilting, isolations were carried out in PDA culture medium to identify the pathogen. The determination of the physiological race of Fusarium oxysporum f. sp. lycopersici was carried out using varieties such as: El Rey, Patria, 4853, 387, 386 and Primus FL. For antagonism tests, five biological products were used, at doses of the product formulated from: Bacillus subtilis 0.625 mL, B. pumilus 1.5 mL, combination of: Bacillus subtilis + B. polymyxa + B. megaterium + Pseudomonas fluorescen + Trichoderma harzianum+ T. reesei+ T viride+ Gliocladium virens.0.25 g and two products based on Trichoderma harzianum at 0.12 g and 1g. A completely randomized experimental design was used with five treatments, five repetitions and an absolute control without fungicide. Fusarium oxysporum f. sp. lycopersici as the causal agent of vascular wilting in Tomato, the race found was race 3 (R3). The products formulated based on T. harzianum present different degrees of antagonism against F. oxysporum f. sp lycopersici R3. T. harzianum is the major in vitro antagonist of F. oxysporum f. sp lycopersici R3.

Keywords:*Fusarium oxysporum*F. sp. lycopersici, physiological race, inhibition.

INTRODUCTION

One of the most consumed vegetables in the world is the tomato (Solanum lycopersicum L.), it is grown in more than 100 countries for fresh and industrial consumption; 76% of production is concentrated in China, India, the United States, Turkey, Egypt, Iran, Italy, Spain, Brazil and Mexico (FAOSTAT, 2015), however a limitation for production and profitability are pests and diseases; 48 important diseases are reported, such as powdery mildew caused by Oidiopsis taurica, late blight caused by Phytophthora infestans, and Rhizoctonia solani Kühn, which, although it does not cause plant death, reduces growth (50%) and produces generalized yellowing (Fernández et al. 2013) and vascular wilt and stem disease, caused by Fusarium oxysporum f. sp. lycopersici (Sacc.) WC Snyder & HN Hansen, one of more than 100 known special forms of Fusarium oxysporum (Gordon, 2017).

According to Arie (2010), F. oxysporum f. sp. lycopersici (Fol) has been grouped into three physiological races (Race 1, 2 and 3), Fol enters through the root system and moves through the vascular tissue to inhabit and obstruct the xylem vessels, which leads to severe water stress. in the plant, whose symptoms are manifested in the yellowing of the leaves, starting from the lower third of one side in the leaflets, accompanied by vascular discoloration, wilting and finally the death of the plant (Srinivas et al., 2019).

The three physiological races of Fusarium oxysporum f. sp. lycopersici can be detected by PCR-based methods and/or according to their ability to infect a set of differential tomato cultivars and the susceptibility and resistance response to the isolation of Fol (Kashiwa et al., 2016).

In Mexico, in the states of Sinaloa, San Luis Potosí, Nayarit and Baja California Sur, the three races 1, 2 and 3 have been reported, while in Morelos only races 2 and 3 have been

recorded (Carrillo et al., 2003, Holguín, 2005). On the other hand, F. oxysporum radicis-lycopersici has only been found causing damage in the state of Sinaloa (Apodaca et al., 2004). One of the possible causes is the use of hybrid materials susceptible to the breeds already reported in Mexico and the lack of knowledge of the type of breed present in the valley. The magnitude of the damage caused by this disease is very varied. In some fields it is barely perceptible, while in others it is very severe causing losses between 21 and 47% in open field and covered crops (Ramyabharathi et al. 2012; Enespa & Dwivedi, 2014).

Given the importance of F. oxysporum radicis-lycopersici and the damage it causes not only in the field but also in the greenhouse, the objectives of this work were to identify the causal agent of vascular wilt of tomato crops in the municipality of Parácuaro Michoacán and find Through the use of plants to differentiate the race of F. oxysporum, evaluate its pathogenicity and determine in vitro the effect of different biological agents to control this pathogen.

MATERIALS AND METHODS

ISOLATION AND IDENTIFICATION OF THE PATHOGEN

To obtain the plant material, four stem samples were collected from tomato plants of the varieties V 38-6 and V 38-7, both resistant to race 1 of F. oxysporum f. sp. lycopersici in Parácuaro, Michoacán. To do this, targeted sampling was carried out on plants that showed symptoms of wilting. After collection, the stems of the tomato plants were placed in brown paper bags measuring 26 x 12.5 x 6.5 cm to prevent dehydration and were transferred to the Phytopathology Laboratory of the "Presidente Juárez" Faculty of Agrobiology, UMSNH.

For isolation of pathogens in the laboratory, tomato stems were washed with tap water to remove soil particles. Subsequently, 20 2 mm cuts were made, which were placed, for 15 s, in a porcelain capsule containing a 3% sodium hypochlorite solution. After this time, the sections were rinsed three times with sterile distilled water, and placed on sterile absorbent paper to remove excess moisture. Finally, five cuts of the stems were planted at equidistant points in Petri dishes (9 cm in diameter) containing PDA (potato dextrose agar) Bioxon® nutrient medium by Becton Dickinson. Once the growth of the pathogen was observed, it was purified to the hyphal tip. Pathogens that did not belong to the genus Fusarium were discarded. Two Fusarium strains FV7 and FV8 were obtained.

The identification of the pathogen was carried out based on cultures of the fungus in PDA. To do this, the mycelium of the seven-day-old fungus was placed on a slide with a drop of lactophenol and covered with a coverslip. The identification of the fungus was carried out with the help of a Leica DM500M microscope (complete equipment data), and with the keys of Barnett and Hunter (1998) and the keys of Toussoun and Nelson (1976).

PATHOGENICITY TESTING AND PATHOGEN RACE DETERMINATION

The pathogenicity tests were carried out in a non-technical greenhouse of the "Presidente Juárez" Faculty of Agrobiology. The work area was disinfected with a 3% sodium hypochlorite solution to prevent the development of pathogens. Six differential varieties of this vegetable were used: El Rey, Patria, 4853, 387, 386 and Primus FL, which were inoculated 15 days after transplanting, the substrate used was sterilized oak soil. For the inoculation of the differential plants, two of the strains obtained from F. oxysporum f. sp. lycopersici, making a suspension of

conidia at a concentration of 1x106/mL, using the Neubauer chamber the conidia count was determined. Inoculation was carried out directly in a drench type (in the soil near the stem), placing 16 mL of the concentrated suspension of conidia to the substrate in which each differential plant developed. The experimental unit was 10 plants of each variety and three as controls, inoculating five with the FV7 isolates and five with the FV8 isolates. After inoculation, the plants were moved to the laboratory for two weeks. The plants were then placed under greenhouse conditions and watered every other day for two weeks, then as needed. The inoculated plants as well as the controls were observed for three months every third day to determine the presence or absence of the characteristic symptoms of the disease in each plant of each differential variety. From the inoculated plants with symptoms of the disease, re-isolations were carried out to complete Kotch's postulates.

IN VITRO BIOASSAYS

Sensitivity tests of the fungus F. oxysporum f were carried out in BD Bioxon® PDA medium. sp. lycopersici to five biological products with doses of the formulated product: Baktillis® (B. subtilis) by Biokrone 0.625 mL, Sonata® by Bayer (B. pumilus strain) 1.5 mL, Bactiva® by TNI (Bacillus subtilis + B. polymyxa + B megaterium + Pseudomonas fluorescen + Trichoderma harzianum + T. reesei + T viride + Gliocladium virens) 0.25 g, Labrador® by Grupo Versa (T. harzianum) 0.12 g and NatuControl® by Biokrone (T. harzianum) 1 g. The products were diluted in 500 mL of water, 1 cm diameter filter paper discs were placed there for 3 minutes so that they were impregnated with the product, then equidistantly these were placed in each Petri dish with PDA culture medium and in A disk with fungal mycelium approximately 1 cm in diameter was placed in the center.

The boxes were sealed with kleen pack paper, placed in a bioclimatic chamber at a temperature of 28 ºC and a humidity of 100%. Every 24 h, the growth of the fungus was measured with a vernier until the control covered the entire area of the Petri dish.

The percentage of inhibition of mycelium (PIM) was obtained with the following formula: (Pandey et al., 1982). $PIM=dc–dt/dc\times100$. Where: PIM = Percentage of inhibition of the mycelium, dc = diameter of the control colony, dt = diameter of the treatments. PIM data were subjected to analysis of variance (ANOVA) and Tukey comparison of means ($P < 0.05$). The SAS program was used.

RESULTS

ISOLATION AND IDENTIFICATION OF THE PATHOGEN

The isolated fungus was identified as Fusarium oxysporum Schl. F. sp lycopersici. The colonies of this fungus were pink and white with a purple hue, plush and cottony, they had abundant microconidia of 6.72-11.46 x 2.64 – 3.48 µm of 0-1 septum, macroconidia of 25.2- 29.28 × 3.58- 4.44 µm, with 3-5 slightly curved septa with the presence of a foot cell; yy Chlamydospores are abundant, terminal or intercalated and solitary. This coincides with what was reported by Toussoun and Nelson (1976) and Hafizi and Salleh (2013).

PATHOGENICITY TESTING AND PATHOGEN RACE DETERMINATION

The first symptoms that were observed were 45 days after inoculations, some plants were in flowering and others were in fruit formation, mainly yellowing was observed in the leaves, which withered and remained attached to the plant and later fell. Which agrees with Srinivas et al. (2019) who mention that the first sign appears at the beginning of flowering

or fruit formation, are symptoms of yellowing on the lower leaves. From the inoculation of F. oxysporum in differential tomato plants (S. lycopersicum L.), all the plants of the Primus LF, Patria, 386 and 387 varieties presented characteristic symptoms of the disease. This indicates that the strain obtained from the field corresponds to race 3 (R3), since the four aforementioned varieties have resistance to R1 and R2 of Fol, with susceptibility to R3 of Fol. On the other hand, varieties 4853 and El Rey did not present characteristic symptoms of the disease, being these with resistance to R1, R2, R3.

BIOASSAYS

The highest percentage of mycelium inhibition against F. oxysporum f. sp. lycopersici was obtained with the product NatuControl® (T. harzianum), followed by BactivaMR® (Bacillus subtilis + B. polymyxa + B. megaterium + Pseudomonas fluorescen + Trichoderma harzianum + T. reesei + T viride + Gliocladium virens). While the biofungicides Baktillis® (Bacillus subtilis), Sonata® (Bacillus pumilus strain) and Labrador® (Trichoderma harzianum) there were no significant differences compared to the control treatment (Figure 1).

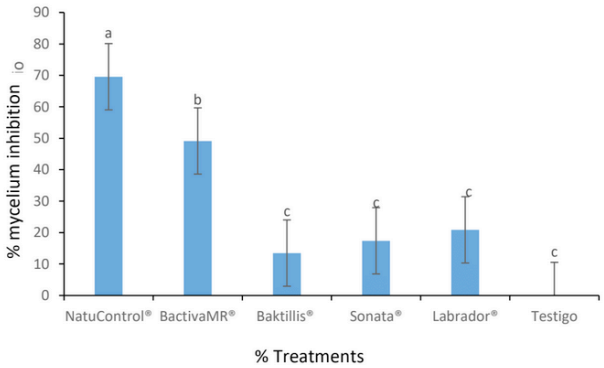

Figure 1. Percentage of mycelium inhibition when confronting five biological fungicides with Fusarium oxysporum Schl. F. sp lycopersici from Parácuaro Mich. Different letters indicate significant difference.

The inhibitory intensity of Trichoderma sp against Fusarium sp. It has been observed in various works such as: Gonzales et. to the. (2005) who tested different concentrations of Trichoderma spp. against Fusarium oxysporum in papaya seedlings and the concentration of 1x106 was effective. Suarez et. to the. (2008) evaluated in vitro a native strain of Trichoderma and a commercial strain where both strains showed strong antagonism against Fusarium solani. Manayay et. to the. (2016) verified in vitro the effect of Trichoderma spp. against Fusarium sp. isolated from tomato, with an inhibition percentage between 85-100%. On the other hand, Michel-Aceves et al., (2020) showed that the percentage of inhibition of dual cultures of T. inhamatum against Phytophthora parasitica. F. oxysporum, was 95 and 85% respectively.

These authors report that the intense inhibition of Trichoderma spp. is due to the higher growth rate of Trichoderma spp. on Fusarium spp., competing for substrate and nutrients, in addition to Trichoderma spp. They produce antibiotics and enzymes: (p-1,3-glucanase, chitinase, protease and cellulase) that degrade the cell wall and play an important role in Mycoparasitism.

The production of antifungal compounds produced by Trichoderma sp. They affect the cell wall of the pathogen that causes mycoparasitism, stop its growth and increase its inhibition (González et al., 2011).

In the present work it agrees with the aforementioned authors, clearly observing the greater inhibition against Fusarium oxysporum by Trichoderma harzianum. However, a difference can also be observed in the percentage of inhibition of the products that contained Trichoderma. Such is the case of the great difference between the product NatuControl® and Labrador®, both with active ingredient Trichoderma harzianum.

The difference between products that

contain Trichoderma as an active ingredient could be due to the additives, inert ingredients or adjuvants that each manufacturer uses that can modify the active ingredient (Ramírez and Lacasaña, 2001).

It is recommended to perform molecular tests to corroborate the morphological identity of F. oxysporum Schl. F. sp lycopersici, R3 since this race emerged with a point mutation that occurred in the avr2 gene, the different races of the fungus carry in various combinations three virulence genes avr1, avr2 and avr3 that are of great importance to distinguish through molecular tests.

In addition, it is also of utmost importance to evaluate the products in the field to observe their effectiveness and thus propose integrated management of the crop.

CONCLUSIONS

The causal agent of wilting in the plants evaluated was F. oxysporum Schl. F. sp lycopersici. In the pathogenicity tests, it was proven that this fungus causes the symptoms when the plant is flowering or forming the first fruits. The Primus LF, Patria, 386 and 387 varieties evaluated were resistant to races R1 and R2 and susceptible to R3. This indicates that the strain obtained in the field belongs to race R3 of F. oxysporum f. sp lycopersici.

Products formulated based on Trichoderma harzianum present different degrees of antagonism against F. oxysporum f. sp lycopersici R3. Trichoderma harzianum is the major in vitro antagonist of F. oxysporum f. sp lycopersici R3.

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