Article

Microbial antagonists for the biocontrol of wilting and its promoter effect on the performance of serrano chili

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Abstract

Fusarium oxysporum and *Rhizoctonia solani* are causal agents of chili wilt and cause losses up to 80% in the crop in Mexico. The inadequate use of chemical fungicides has generated problems of resistance, this was not observed with microbial agents, so they were evaluated in the biocontrol of wilt of chili in experimental plots where *F. oxysporum* and *R. solani* were identified as causal agents of the illness. *Trichoderma* spp., *Bacillus* spp., mixture of microbial propagative ferment, thiabendazole and control, were applied on a plot of 800 m^2 with six varieties of chili. The treatments *Trichoderma* and propative ferment showed low percentages of incidence and severity, high yield with *Trichoderma* in HS-52 and Coloso (15.67 and 13.89 t ha^{-1}). *Trichoderma* treatments and propagative fermentation promote the biocontrol of chili wilt caused by *F. oxysporum* and *R. solani* and increase yield.

Keywords: *Trichoderma*, *Bacillus*, chili wilt, propagative ferment.

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Introduction

Chili wilt is one of the main biological limitations in the production of this crop and is caused by *Phytophthora capsici*, *Rhizoctonia solani* and *Fusarium oxysporum* (Albañil *et al*., 2015). There are reports of chill wilt where it is frequently associated with *F. oxysporum*, which takes advantage of the mechanical damage and natural openings of the plant to infect, however, *P. capsici* is considered the most important pathogen due to its capacity to penetrate the epidermis of the roots and invade the vascular bundles (Zapata *et al*., 2012).

This disease is reported throughout Mexico, estimating losses up to 80% root rot to invade the vascular system of plants (González *et al*., 2009). Chemical control is the most used method to control the disease, it is common to reduce the inoculum by disinfecting the soil with Metam Sodium, fungicide applications are also made with the active ingredients 2Tiocianomethyl (TCMTB), Metalaxyl, Azoxystrobin and Propanocar to control *P. capsici* (Pérez *et al*., 2003), *R. solani* and *Fusarium* spp., are controlled with Tebuconazole, Carbendazim, Thiabendazole and Methyl Thiophanate (Yossen and Conles, 2014).

The high genetic variability that exists in the fungal complex associated with the disease and the inadequate use of fungicides has led to resistance problems; in this context, biological control through microbial agents does not report resistance of phytopathogens, which makes them a feasible alternative for the management of the disease (Bardin *et al*., 2015). Different species of *Trichoderma* and *Bacillus* are reported for the management of the genera *Phytophthora*, *Sclerotium*, *Fusarium*, *Rhizoctonia*, *Alternaria*, among others, these antagonists promote the production of biomass and the yield of crops (Astorga *et al*., 2014; Mamani *et al*., 2016; Arenas *et al*., 2017); as well as, they also activate the defense response of the plants, where they involve ethylene, jasmonate and salicylic acid (Manganiello *et al*., 2018), colonize the rhizosphere and produce secondary metabolites that generate antagonism (Fan *et al*., 2017; Saravanakumar *et al*., 2017) and parasitism when colonizing and penetrating the phytopathogen (Druzhinina *et al*., 2018).

The efficiency of microorganisms for the management of diseases has been shown to obtain a greater effect with formulations of *B. subtilis* and *T. asperellum* compared with chemical products of active ingredients such as Propanocarb, Aluminum Fosetil and Etridiazol (Villanueva, 2018). It was proposed to evaluate the effectiveness of microbial agents as biocontrol of the wilt of the chili crop and effect on the yield of different varieties of serrano chili.

Materials and methods

Location of the experiment

The research work was established in the year 2017 in the El Bajío Experimental Field, where there is a history of the presence of wilt of chili, also worked in the phytopathology laboratory of the Agrarian Autonomous University Antonio Narro (UAAAN) of Saltillo, Coahuila, Mexico.

Experimental establishment

The materials of serrano chili HS-52, Coloso, HS-44, Centauro, Paraiso and Tampiqueño 74 were evaluated, provided by the Experimental Field 'Las Huastecas' INIFAP of Villa Cuauhtémoc, Tamaulipas, Mexico. The plant was developed in polystyrene trays of 200 cavities, which were disinfected with 3% sodium hypochlorite, packed with a mixture of peatmoss-perlite in a ratio of 2-1 and maintained in greenhouse conditions for 40 days. Planting in the field was done with seedlings of 10 cm in height, transplanted in beds at 1.5 m to double row.

Microbial control agents were provided by the Department of Parasitology of the UAAAN, as a research product, *Trichoderma asperellum*, *T. harzianum* and *T. yunnanense* (Osorio *et al*., 2011; Jimenez *et al*., 2018), *Bacillus amyloliquefaciens*, *B. liqueniformis* and *B. subtilis* (Hernandez *et al*., 2014) and a mixture of microbial propagative ferment (MFPM) based on *Trichoderma* spp. - *Bacillus* spp. The mixture of three species of *Trichoderma* ($1x10^8$) (treatment 1), MFPM (treatment 2), mixture of three species of *Bacillus* ($1x10^8$) (treatment 3), Thiabendazole (60%) (treatment 4) and an absolute control (treatment 5), at a dose of 1 L ha^{-1} for treatments 1, 2 and 3, while for thiabendazole they were 0.5 kg ha^{-1}.

The application was made to the drench with a manual sprayer at 7, 28 and 49 days after the transplant (DDT). Subsequently to 85, 105, 125 and 145 ddt the yield per block (4.5 m^2) was determined and transformed to t ha^{-1}, from samples of 10 fruits the fruit weight (g) and size (mm) were determined. In the first and last cut the incidence transformed to percentage was evaluated, the severity was evaluated through the visual scale shown in Figure 1, where 0= no visible symptoms; 1= initial. Light chlorosis, presence of flowers and fruits; 2= intermediate. Partial withering, severe chlorosis, premature ripening of fruits and 3= advanced. Total withering without recovery, the leaves and fruits stay stuck to the stem.

0. No symptoms 1. Initial 2. Intermediate 3. Advanced

Figure 1. Photographs used to determine the severity of chili wilt.

The data of the scales obtained in the assessments of the degree of severity of the disease were transformed by using the following formula cited by Carrión (2016):

$$s=\left(\frac{\sum(a*b)}{(n*k)}\right)*100$$

Where: s= severity; $\Sigma(a*b)$= summation of the degree of affectation (0, 1, 2, 3); n= number of plants evaluated; k= greater degree of the scale (3).

Isolation of phytopathogenic fungi

Plants with apparent symptoms of the disease were collected and transferred to the laboratory, where their roots were washed with running water and cross sections were made at the base of the stem. Subsequently, they were superficially disinfected with a 1% sodium hypochlorite solution, where the stem sections were immersed for three minutes and rinsed in three sterile water passes. The tissues were dried on sterile paper and deposited in Petri dishes with the culture medium potato dextrose agar (PDA), incubated seven days for the development of fungi and colonies with different characteristics were isolated.

The purification was performed at 24 h by the technique of tip of hypha, were incubated for 15 days to make observations of the growth in PDA and under the microscope the characteristics of the mycelium, structures of reproduction and resistance, were used taxonomic keys of Sneh *et al*. (1991); Barnett and Hunter (1998); Leslie and Summerell (2006).

Pathogenicity test

It was carried out using the technique proposed by Sánchez *et al*. (1975), with slight modifications, using seeds of serrano chili of the variety Tampiqueño 74. The seeds were disinfected for three minutes in a solution of sodium hypochlorite at 1%, rinsed in three sterile water passes, and then dried in sterile paper and transfer them to Petri dishes containing water agar (AA) at 2% and incubated at 28 °C with 12-hour light cycles. They were checked every 24 h for three days to select seeds free of the growth of phytopathogens, to then transfer them in groups of three germinated seeds to Petri dishes with the same medium where they were inoculated near the root system with a fragment of the fungi of 0.5 cm of diameter approximately. They were incubated at 28 °C, for 7 days and the mortality of the seedlings was evaluated.

Statistical analysis

It was carried out by means of a completely randomized design with three repetitions, through an analysis of variance (Anva) and a comparison test of means according to Fisher's supported by LSD test ($p< 0.05$), using the statistical program R (R Development Core Team, 2007).

Results and discussion

Two phytopathogenic fungi associated with the wilting of the chili culture of the experimental plot established in Saltillo, Coahuila, *Fusarium oxysporum* and *Rhizoctonia solani* were recovered. The growth of *F. oxysporum* in PDA showed white color and microscopically unicellular and bicellular microconidy were observed, from ovoid to ellipsoid on short fialides grouped in falas heads and microconidy of three septa with slightly curved apical cells. *R. solani* in PDA presented growth of light brown mycelium, under the microscope the hyphae tended to branch at right angles with a slight constriction near the point of formation of the produced angle.

The recovered fungi were confirmed as causative agents by means of the pathogenicity tests, where a smaller amount of absorbent hairs and necrosis were observed in the root. *R. solani* was more aggressive observing death of the seedlings at 8 days and *F. oxysporum* until 12 days. In the symptomatology of the disease, yellowness, flower abortion, wilting, necrosis in the root system and premature maturity of fruits were observed, which were adhered to the plant (Figure 2).

Figure 2. Symptomatology of wilting in field conditions. a) Healthy plant; b) Yellowing in the foliar stratum of the plant; c) Yellowing, flower abortion and premature ripening of fruits; and d) Fruits ripe and attached to plants killed by drought.

As in the present investigation, Albañil *et al*., (2015) reported *F. oxysporum* and *R. solani* as causal agents of chili wilt in the Bajio and southwest of Guanajuato. The symptomatology in the severity scale agrees with the report in the states of Aguascalientes and Zacatecas, where these phytopathogens were found in the greater percentage of the isolations causing wilting, defoliation, changes in foliage color, root rot, among other symptoms (Velásquez *et al*., 2001).

In the Table 1 shows the incidence of the disease, where statistical differences were observed ($p \leq 0.05$) in the materials HS-52 and Coloso. The *Trichoderma* treatment with the variety HS-52 has an incidence of the disease of 10.67% that represented a decrease with respect to the control of 21.16%.

The Coloso variety showed low percentages of incidence with thiabendazole where 6.83% existed, this shows excellent response of the variety to the application of the chemical. The varieties HS-44, Centauro, Tampiqueño 74 and Paraíso did not present significant statistical differences, placing them in the same statistical group.

Table 1. Incidence of the disease (%) in serrano chili with respect to each treatment.

	Varieties of serrano chili					
Treatments	HS- 52	Coloso	HS- 44	Centauro	Tampiqueño 74	Paraiso
Trichoderma	10.67 ^{a}	18.17^{ab}	16.84^{a}	19.17^{a}	12.5^{a}	10^{a}
MFPM	26.67^{ab}	15.5^{ab}	10.5^{a}	15.33^{a}	19.83^{a}	10.67^{a}
Bacillus	29.17^{ab}	29.67^{b}	20.07^{a}	20.5^{a}	21.83^{a}	19.5^{a}
Tiabendazol	21^{ab}	6.83^{a}	19.51^{a}	24.17^{a}	10.33^{a}	16.17^{a}
Control	31.83^{b}	21.33^{ab}	21.51^{a}	23.33^{a}	24.67^{a}	22.33^{a}

Average values in the same column with different lowercase letters significant statistical difference ($p< 0.05$) according to Fisher's supported by the LSD test; NS= not significant. Mean values in the same column with equal letters.

In the Figure 3 showed significant statistical differences ($p\leq 0.05$) in percentages of incidence of the disease between treatments in the six evaluated materials, where *Trichoderma* and MFPM had low percentages of incidence (14.39 and 16.39%), being control and *Bacillus* the who presented high levels of the presence of symptoms (24.08 and 23.36%).

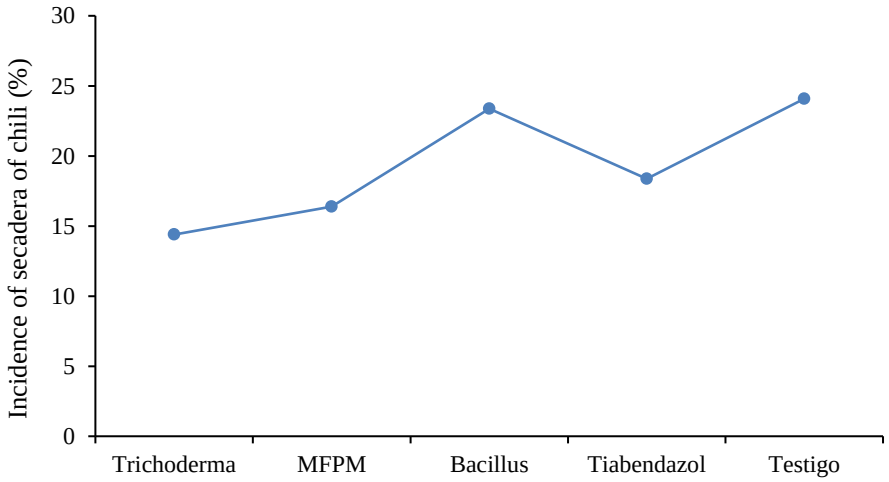

Figure 3. Incidence of the disease in the treatments with respect to all varieties of serrano chili.

The severity of the disease transformed to percentage (Table 2) showed statistical differences ($p\leq 0.05$), where the MFPM treatment with the material HS-52 expressed the best results with 8.33%, in this same variety the *Trichoderma* treatment presented low percentage of incidence and severity of 11.30%. In the variety Tampiqueño 74 the application of thiabendazole had 6.54% severity, followed by *Trichoderma* with 7.83% and control with 17.96%, observing response of the material to the application of the treatments.

On the other hand, Paraíso with application of the mixture of MFPM and *Trichoderma* showed low levels of severity (6.46 and 6.93%), representative and inferior to the control, which allowed a better harvest. When testing with different *Bacillus* species in the chili culture Guillén *et al*. (2006) reported that the application of these microorganisms increased plant biomass and yield, this is possibly related to the type of clay-loam soil where good results were obtained, such effect was not found in this investigation where the control of the disease and yields were low.

Table 2. Severity of the disease (%) in serrano chili with respect to treatments.

Treatments	Varieties of serrano chili					
	HS- 52	Coloso	HS- 44	Centauro	Tampiqueño 74	Paraiso
Trichoderma	11.3^{ab}	18.43^{a}	10.8 ^{a}	6.83^{a}	7.83^{ab}	6.93^{a}
MFPM	8.33^{a}	16.28^{a}	24.45^{a}	12.76^{a}	14.35^{ab}	6.46^{a}
Bacillus	14.4^{ab}	19.16^{a}	15.09^{a}	11.54^{a}	17.6^{b}	16.22^{ab}
Tiabendazol	20.04^{b}	14.07^{a}	17.97^{a}	15.74^{a}	6.56^{a}	18.24^{b}
Control	19.45^{ab}	24.35^{a}	24.07^{a}	13.65^{a}	17.96^{b}	18.43^{b}

Average values in the same column with different lowercase letters significant statistical difference ($p< 0.05$) according to Fisher's supported by the LSD test; NS= not significant. Mean values in the same column with equal letters.

The biofungicidal efficiency of *Trichoderma* spp. it is attributed to antagonism (Reyes *et al*., 2012) and mycoparasitism to phytopathogenic fungi, which is appreciable in this investigation where the incidence and severity of wilt of the chili culture was reduced (Atanasova *et al*., 2013).

In Table 3 it was observed that the fruit size was statistically higher ($p\leq 0.05$) in the Centauro variety with the MFPM (73.44 mm), *Bacillus* (67.94 mm) and *Trichoderma* (66.44 mm) treatments, with an increase of 28, 23 and 21%, with respect to the witness. In the variety Tampiqueño 74, *Bacillus*, MFPM and *Trichoderma* treatments had sizes of 78.74, 74.73 and 73.63 mm, respectively. For the fruit weight variable, the material HS-44 was found to be statistically different, with a better response in *Bacillus* (10.83 g), MFPM (10.5 g) and *Trichoderma* (10.00 g) with respect to the control.

In the crop yield (Table 3) the statistical difference ($p\leq 0.05$) was studied in varieties HS-52, Paraiso and Centauro, in this context it was observed that the *Trichoderma* treatment presented a better behavior with 15.67 and 13.22 t ha^{-1} in the variety HS-52 and Centauro. The application of Trichoderma increases the production of 62% and 61% in the varieties mentioned above, with respect to the control. The application of the MFPM reports high yields in the variety Centauro (11.52 t ha^{-1}), Paraíso (10.59 t ha^{-1}) and HS-52 (10.37 t ha^{-1}), the control was the lowest yield.

It was analyzed that the application of the MFPM increased 76% the yield with respect to the control, with statistically significant difference ($p\leq 0.05$) regarding the treatments Tiabendazol and control (Table 3). It was observed that *Trichoderma* increased the yield, this has been shown by different Trichoderma species in habanero chili plants (*Capsicum chinense*) (Candelero *et al*., 2015), lettuce (*Lactuca sativa*), radish (*Raphanus sativus*) (Ortuño *et al*., 2013) and pea (*Pisum sativum*) (Camargo and Avila 2014). In the same context, Cubillos *et al*. (2009) tested with *Trichoderma harzianum* in the cultivation of passion fruit (*Passiflora edulis*) where they could determine that it is an antagonist to *F. oxysporum* and *F. solani*, besides stimulating germination, increase of biomass and root length.

Table 3. Length, fruit weight and yield with the different evaluated treatments of the serrano chili culture.

Treatments	Size (mm)	Weight (g)	Yield (t h^{-1})	Size (mm)	Weight (g)	Yield (t h^{-1})
	HS-52			Coloso		
Trichoderma	72.37^{a}	12.83^{a}	15.67^{a}	74.3^{a}	14.33 ^{a}	13.89^{a}
MFPM	78.78^{a}	14.67^{a}	10.37^{ab}	74.49^{a}	14.17 ^{a}	10.04^{a}
Bacillus	76.18^{a}	14.17^{a}	7.26^{b}	75.95^{a}	14.17 ^{a}	8.75^{a}
Tiabendazol	73.89^{a}	12.33^{a}	10.02^{ab}	74.13^{a}	13.17^{a}	9.02^{a}
Control	78.8^{a}	13.67^{a}	5.98^{b}	72.56^{a}	12.67 ^{a}	8.06^{a}
	Centauro			HS-44		
Trichoderma	66.44^{abc}	10.67^{a}	13.22^{a}	64.73^{a}	10^{ab}	7.55^{a}
MFPM	73.44^{a}	10.67^{a}	11.52^{ab}	67.44^{a}	10.5^{ab}	13.04^{a}
Bacillus	67.94^{ab}	9.83^{a}	8.18^{ab}	64.29^{a}	10.83^{a}	10.3^{a}
Tiabendazol	59.25^{bc}	7.50^{a}	8.69^{ab}	61.36^{a}	9^{ab}	10.62^{a}
Control	52.58^{c}	8.33^{a}	5.15^{b}	64.76^{a}	8.5^{b}	6.94^{a}
	Paraiso			Tampiqueño 74		
Trichoderma	66.13^{a}	10.17^{a}	8.48^{b}	73.63^{abc}	11^{a}	12.26^{a}
MFPM	63.94^{a}	10.5^{a}	10.59^{a}	74.73^{ab}	11.67^{a}	13.3^{a}
Bacillus	66.18^{a}	11^{a}	5.41^{b}	78.74^{a}	10.5^{a}	8.74^{a}
Tiabendazol	61.18^{a}	8.17^{a}	7.44^{b}	68.1^{c}	10.33^{a}	9.77^{a}
Control	61.02^{a}	8.5^{a}	2.59^{b}	71.19^{bc}	11^{a}	6.26^{a}

Average values in the same column with different lowercase letters significant statistical difference ($p< 0.05$) according to Fisher's supported by the LSD test; NS= not significant. Mean values in the same column with equal letters.

The positive interaction between *Trichoderma* and the host plant is attributed to a complex chemical activity of volatile and diffusible secondary metabolites, release of phytohormones and antibiotics in the rhizosphere, which promote the development of the root and a greater absorption of nutrients, which help control phytopathogens and increase yield (López *et al*., 2015), which explains the effect produced in this investigation. Microbial extracts as biofertilizers have the capacity to generate hormones that stimulate the development and increase the yield (Martínez *et al*., 2017), which could be verified with the application of the MFPM of the growth of *Trichoderma* spp., and *Bacillus* spp., which showed an effect in the control of the disease and in the development of the crop in equal or better percentage than when using the microorganisms.

Conclusions

The application of *Trichoderma* and the MFPM are excellent alternatives for the control of the wilt of the chili caused by *Fusarium oxysporum* and *Rhizoctonia solani*, besides increasing the yield of the different materials of serrano chili under the conditions of the Experimental Field El Bajío of the UAAAN in Saltillo Coahuila.

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