Article

Antagonism of *Trichoderma* spp. *vs* fungi associated with wilting of chilli

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Abstract

In Mexico, more than 100 varieties of chili (*Capsicum annuum* L.) are planted, it is a highly profitable crop and represents an economic activity of national importance. Currently, it faces serious phytosanitary problems due to the presence of diseases such as the wilting of chili, caused by a complex of soil pathogens. To control these diseases, toxic and residual fungicides are used, which pollute the environment and induce genetic resistance in phytopathogens. Therefore, it is necessary to look for control alternatives to solve this problem, therefore, the objective of this work was to evaluate the antagonistic effect of *Trichoderma* spp., against the causal agents of wilting in *in vitro* confrontations by means of dual cultures. The study was carried out in 2016, at the Intercultural University of the State of Puebla, Huehuetla, Puebla, Mexico. Three *Trichoderma* isolates from avocado tree rhizospheres, *T. viride*, *T. harzianum* and *T. asperellum* and three isolates of the phytopathogens *P. capsici*, *F. oxysporum* and *R. solani* obtained from the roots of plant plants were evaluated chili with wilting symptoms. The isolates were morphologically characterized for identification. The three antagonistic fungi presented a high percentage of inhibition, *T. asperellum* showed 88.25%, *T. viride* 87.22% and *T. harzianum* with 87.8%. Under the conditions of the study, *T. asperellum*, *T. viride* and *T. harzianum*, were efficient antagonistic microorganisms against the studied phytopathogens. It is suggested to carry out biocontrol evaluations with these isolates in the greenhouse and in the open field.

Keywords: *Capsicun annum*, *Phytophthora capsici* and *Trichoderma* spp.

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Introduction

The cultivation of chili (*Capsicum annuum* L.) in Mexico, faces serious phytosanitary problems that limit its production due to diseases such as wilting of chili, induced by a complex of soil phytopathogens, including *Phytophthora capsici* Leo (Hernández-Castillo *et al*., 2014), *Rhizoctonia solani* Kühn and *Fusarium oxysporum* (Vásquez *et al*., 2009). The main symptoms caused by this disease are the premature death of the plants, causing the obstruction and imbalance of the functioning of the vascular bundles, defoliation, color changes, foliage curl, damage to reproductive structures, advanced and irregular maturation, rot of the root, stem necrosis and occurrence of regrowths (Granke *et al*., 2012; Rivera *et al*., 2018).

To control the incidence and severity of these pathogens, highly toxic and residual fungicides are used that cause a negative environmental impact, contaminating the soil, air and aquifers, also poison the human being, create genetic resistance in the phytopathogens to the ingredients assets (Matar *et al*., 2009; Osorio *et al*., 2016; Hernández-Hernández *et al*., 2018) and increase production costs. Another consequence is the reduction of the planting area due to the infestation of the soil is the increase in the severity and incidence of the disease and reduction of the yields obtained by planted area (García, 2010).

The relevance of the genus *Trichoderma* lies in the efficiency it must fight diseases, the impact on root rotting has been reported in multiple studies, a problem that is found among diseases that cause significant economic losses in different agricultural crops. Although chemicals are still the main control tool for these diseases, biological agents are an effective way to provide faster and safer control (Verma *et al*., 2007).

The biological control mechanisms of *Trichoderma* spp. species have been investigated for more than 70 years (Martínez *et al*., 2013), its antagonistic capacity is widely documented as biocontrol agents of various pathogens that affect crops of agricultural and economic importance (Infante *et al*., 2009; Mayo *et al*., 2015). *Trichoderma* strains produce extracellular enzymes (Osorio-Hernández *et al*., 2016), compete with pathogenic fungi for space and nutrients by completely reducing or stopping the development of phytopathogens (Sánchez-García *et al*., 2017), promoting growth of plants and induce systemic resistance, mycoparasitism and antibiosis through direct restrictive action to phytopathogenic fungi.

These mechanisms are favored by the ability of *Trichoderma* isolates to colonize the rhizosphere of plants. (Leandro *et al*., 2007; Woo and Lorito, 2007; Guedez *et al*., 2012). The different species of *Trichoderma* are an alternative for biocontrol against various soil phytopathogens (Singh *et al*., 2011) including *P. capsici*, *F. oxysporum* and *R. solani*, in addition to stimulating the increase in root biomass and area foliar (Ezziyyani *et al*., 2005; Ezziyyani *et al*., 2007). Therefore, the objective of this research was to determine the antagonistic effect of *Trichoderma* spp., as a biocontrol method for the complex of phytopathogens that cause wilting of chili in *in vitro* evaluations using dual cultures.

Materials and methods

Study location

This work was carried out in the Biology Laboratory of the Intercultural University of the State of Puebla, Huehuetla, Puebla, Mexico. Its geographical coordinates are the parallels 20º 01' 48" and 20º 09' 12" of north latitude and the meridians 97º 35' 00" and 97º 40' 24" of west longitude.

Fungal material

Three species of *Trichoderma* were isolated from soil in potato dextrose-agar medium (PDA). 1 g of wet soil was dispersed in boxes with PDA medium and incubated at 25 ±2 °C in the dark for two days, then removed from the incubator and kept under a white light lamp for two days to induce sporderma sporulation sp., from the development of *Trichoderma*, a portion was taken to transfer to PDA culture medium and incubate at 25 ±2 °C, this was done until obtaining axenic and pure growths, free of other fungi or bacteria. After the last sporulation in this medium, they were preserved in sterile water and mineral oil.

The pathogens *Rhizoctonia* sp., *Fusarium* sp. and *Phytophthora capsici* were isolated from chili roots, the roots were washed with running water; subsequently, the tissue portion was deflated by immersion for 1 min in 1.5% solution of sodium hypochlorite and rinsed with sterile distilled water, the roots were drained on sterile sanitas, finally 1 cm fragments were seeded in PDA medium for isolation and purification of *Rhizoctonia* sp. and *Fusarium* sp.

In the case of the *Phytophthora capsici* oomycete, 1.5 cm long chili roots were seeded in agar-agar® medium with V8® juice (8 vegetable juice) and antibiotics were added (Pimaricin 10 µg L^{-1}, Ampicillin 292 µg L^{-1}, Rifampicin 10 µg L^{-1}, Pentachloronitrobenzene 0.1 g L^{-1} and Himexazol 0.25 µg L^{-1}), subsequently incubated at 28 ±2°C for three days (Andrade *et al*., 2012), previously purified pathogens from the chili root was kept under conservation in sterile water for the purpose of preservation (Molina-Gayosso *et al*., 2016). Prior to dual confrontation, microorganisms were activated in PDA culture medium plus lactic acid (Figure 1).

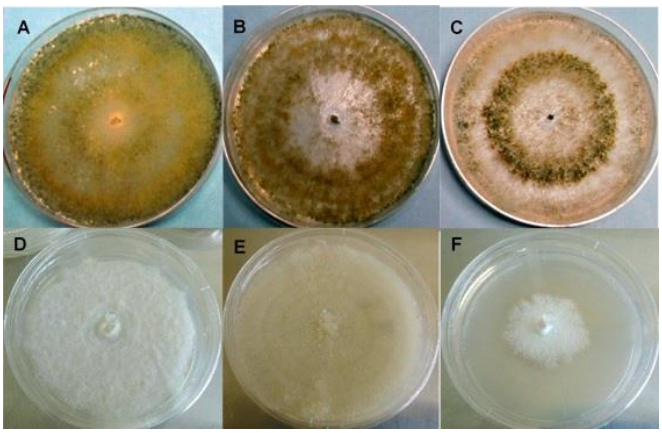

Figure 1. Growth in PDA culture medium of antagonists and pathogens of the wilting of chili. A= *T. viride*; B= *T. harzianum*; C= *T. asperellum* (C2); D= *Rhizoctonia* sp.; E= *P. capcisi*; and F= *Fusarium* sp.

Morphological characterization

To study the morphological characteristics, both pathogen and *Trichoderma* isolates were grown in PDA medium and incubated at 24 ±2 °C for 12 h under white light (Rivera-Jiménez *et al*., 2018). In the identification of the genus *Fusarium* sp. the description of Burgess *et al*. (1994) and the identification of the species was made using the keys of Seifert (1996). *Rhizoctonia* species was identified based on Sneh *et al*. (1991) and that of the oomycete *Phytophthora* sp. It was characterized by the keys of Erwin and Ribeiro (1996); Gallegly and Hong (2008).

Finally, in the morphological characterization of the isolates of *Trichoderma* spp. the taxonomic keys of Barnett and Hunter (1972) were used. For the *Trichoderma* isolates, monoconidial cultures were used in combination with the wet chamber technique (Harris, 1986). A 5 mm diameter PDA disc was inoculated with the *Trichoderma* spore and placed between a slide and coverslip and this preparation on a sterile rod triangle inside a petri dish and incubated at 25 ±2 °C for 24 h.

The macro and microscopic characteristics to be considered were colony texture, presence of mycelium, concentric rings, colony staining on the reverse, shape and size of conidia and phyloid (Chaverri *et al*., 2015; Jang *et al*., 2018; Nawaz *et al*., 2018; Du Plessis *et al*., 2018), the structures of the *Trichoderma* spp. at 40X in an optical microscope (Zeiss Axiosiop plus).

Growth rate

In determining the growth rate of mycelium in antagonists and pathogens, grown in PDA, measurements were made every eight hours.

Trichoderma antagonistic activity on pathogens

The dual culture technique was used to determine the antagonistic activity of the different *Trichoderma* isolates on *Rhizoctonia* sp., *Fusarium* sp. and *P. capsici* (Sonnenbichler *et al*., 1983). A completely randomized design was used (with two factors: *Trichoderma* and pathogens), with eight repetitions.

The tests were carried out evaluating dual cultures of *Trichoderma* spp., Contrasted with three phytopathogens, three isolates of *Trichoderma* against *Rhizoctonia* sp., *Fusarium* sp. and *P. capsici*. In the test of dual cultures, petri dishes with antibiotic-free PDA were used, a 5 mm diameter disc with active mycelium of the phytopathogen (previously grown in PDA for five days) was placed on one end and an equidistant end was placed Mycelium disc (previously grown in PDA for five days) of 5 mm in diameter with *Trichoderma* spp., the boxes were incubated at 28 ± 2 °C and observed every 8 h to record the number of days at the first contact between the antagonist and the phytopathogen, the growth of both colonies (cm) was measured and the percentage of inhibition of radial mycelial growth was evaluated, based on the formula proposed by Ezziyyani *et al*. (2007), calculating as follows:

$$PICR\% = \frac{R1-R2}{R1} * 100$$

Where: PICR= percentage of radial growth inhibition; R1= radial growth (mm) of the pathogen without *Trichoderma* spp., R2= radial growth (mm) of the pathogen with *Trichoderma* spp.

Trichoderma mycoparasitism on pathogens

To know the presence of parasitism of the *Trichoderma* strains towards *Rizoctonia* sp., *Fusarium* sp. and *P. capsici*, the Riddel microculture technique described by Paul (1999) was used. This technique consists of placing a V-shaped glass rod inside a petri dish and on top of it, a sterile slide, on which a 10 mm diameter PDA disc was placed. The arrangement of each fungus was made in two cardinal points, after placing the mycelium of both fungi on the PDA, with a difference of 24 h, the pathogens were first placed with the help of a sterile needle, which have a slow growth compared with *Trichoderma* and a sterile coverslip was placed, it was incubated at 25 ±2 °C.

When the agar disk was covered with the growth of both fungi, the slide was removed from the petri dish to prepare the smears. The coverslip was placed on another clean slide and provided with a drop of dye known as cotton blue. On the other hand, the agar disc was removed from the original slide, a drop of the dye and a coverslip were placed. Once the excess dye was removed, the preparations were sealed and evaluated microscopically, that is, 10 samples were observed to determine whether or not there was parasitism by the *Trichoderma* sp. strains, 40X was observed in an optical microscope (Zeiss Axiosiop plus).

Statistic analysis

The PICR data, expressed as a percentage, were transformed with the angular arcsine $\sqrt{x+1}$ (Steel *et al*., 1986) and in the mycelial growth in the biocontrol test, a completely randomized design was used (three strains of *Trichoderma* sp and three phytopathogens) with eight repetitions. The data obtained for each of the trials were subjected to an analysis of variance and a comparison test of Tukey means ($p \leq 0.05$). These data were analyzed with the statistical package SAS version 9.0 for Windows (SAS, 2002).

Results and discussion

Morphological characterization

The pathogens causing the wilting of chili corresponded with what was reported by Rivera-Jimenez *et al*. (2018). Similarity was found with *F. oxysporum*, the growing colonies in PDA had abundant aerial mycelium and pale pink to deep purple to magenta with a cottony texture. The macro-conidia presented one to five septa, the conidia measured 23-54 × 3-4.5 µm, respectively. In the identification of *Rhizoctonia solani*, it corresponded with that reported by Sneh *et al*. (1991) and Lozano *et al*. (2015), *R. solani* was characterized by forming aerial mycelium, brown, in PDA culture medium formed mycelium hyaline.

The hyphae generally showed a right angle and after this a septum formed, the diameters of the hyphae presented 4.1 to 8.9 µm, which corresponds to Barnett and Hunter (1998). The isolation of *Phytophthora capsici* was characterized by forming ovoid, elongated, ellipsoid sporangia with one or two papillae, the size of the sporangia was 35.8 to 64.7 x 19.6 to 25.2 µm., finally the apical thickness was 1.85 to 4.3 µm (Erwin and Ribeiro, 1996; Gallegly and Hong, 2008).

According to the taxonomic identification criteria of Barnett and Hunter (1972), *Trichoderma* species were compared with taxonomic keys and identified by their morphological characteristics. The species of *T. harzianum* presented light-to-dark green colonies with a dusty-cottony texture, with mycelium, conidophore and green-looking conidia (Figure 2, Table 1) show various branches in pyramidal form, in some cases the formation of two to three lateral branches and presents aerial mycelium. In *T. asperellum* and *T. viride*, an absent coloration can be seen on the back of the petri dish.

Table 1. Morphological characterization of *Trichoderma* spp.

Trichoderma spp.	Colony color	Colony texture	Aerial mycelium	Rings	Color on the back	Shape of conidia	Size conid (µm)	Phyloid form	Size phialides (µm)
T. harzianum	Dark green to grayish color	Cottony	Abundant	No presence	Colorless	Subglobose	3.8-4x 3.1-3.7	Globose	6.3-15.6x 2.7-3.4
T. asperrellum	Light green to dark green	Dusty	No presence	3 to 4 rings	Colorless	Ovoid to elisoipse	4-4.4x 3-3.5	Slightly globose	2.3-2.8x 10.2-11.8
T. viride	Dark green	Cottony	Abundant	No presence	Colorless	Ellipsoidal globose	4-4.8x 3.5-4	Lageniformes	8-14 x2.4-3

The spores and phyloids, characteristic structures correspond to *Trichoderma* species, presented forms and sizes typical of the species of *T. harzianum*, *T. asperellum* and *T. viride* corresponding to what was reported by different authors (Barnett *et al*., 1972; de Hoog, 2000; Romero-Arenas *et al*., 2009; Chaverri *et al*., 2015; Jaklitsch *et al*., 2015).

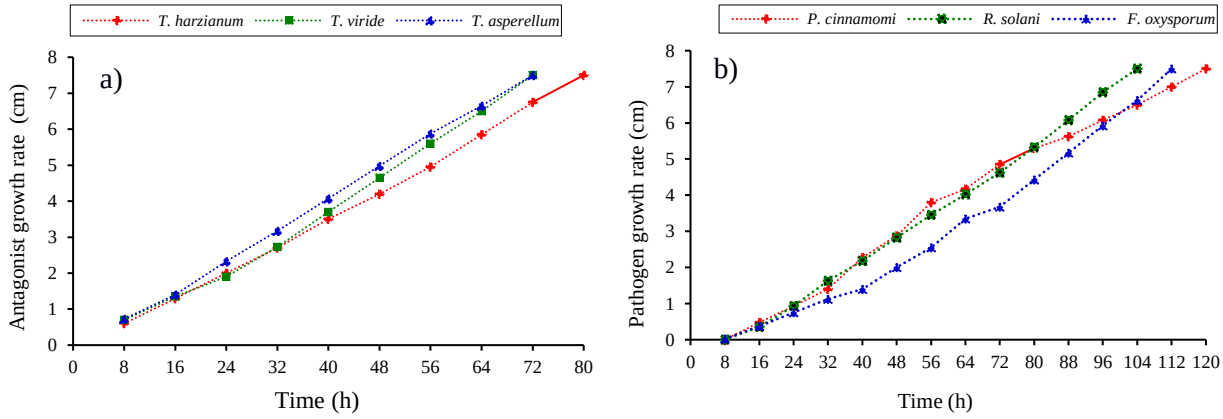

Figure 2. Growth rate, A) *T. harzianum, T. viride* and *T. asperellum*; and B) *P. capsici, R. solani* and *F. oxysporum*.

Growth rate

The evaluation of the initial growth rate of the antagonists and pathogens turned out to be completely related at the beginning of mycelial growth at 8 h; however, the three species of *Trichoderma* have the ability to antagonize the completion of the filling of the petri dish in 80 h. These results indicate the importance of knowing the growth rate of pathogens, as well as the antagonists of *T. harzianum*, *T. viride* and *T. asperellum*.

The isolates of *Trichoderma* spp. showed a faster growth than the pathogens, this behavior is promising in the control of pathogens of the root, also allowed this trial allowed to consider it for the PICR tests and give *Fusarium oxysporum*, *Rhizoctonia solani* and *Phytophthora capsici* two days advantage.

Antagonistic activity of *Trichoderma* on pathogens

The antagonistic activity of *T. viride* against *P. capsici* showed a significant difference ($p \leq 0.05$), because *T. viride* presented 0.516 mm in comparison to *P. capsici*, which obtained 0.241 mm of growth in a petri dish with culture medium, is say *T. viride* is suitable for use as a biological control agent, as it is effective against *P. capsici*, since hyperparasitism was also observed by the pathogen (Figure 3). The foregoing is consistent with that reported by Bouziane *et al*. (2016), when evaluating *T. viride* against *P. infestans* in *in vitro* tests, they found that the beneficial fungus showed inhibitory capacity between 58 and 68%, similar results were presented when evaluating in plants.

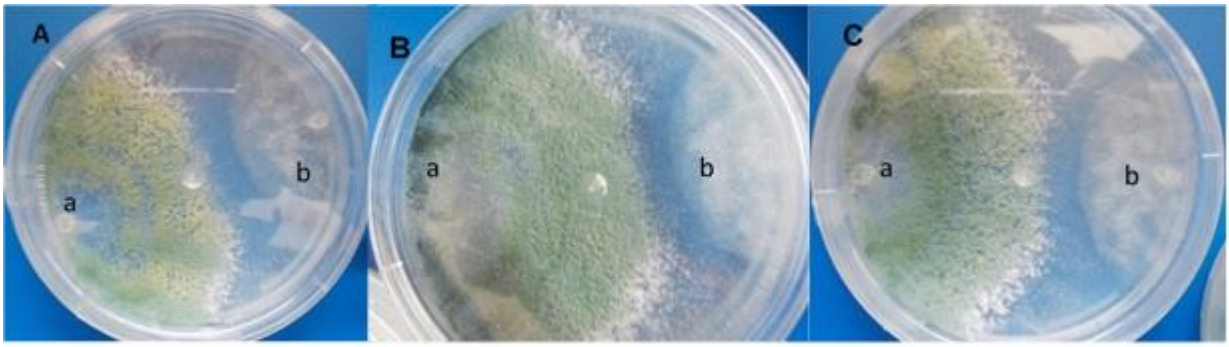

Figure 3. Dual growth, *in vitro* confrontations with *Trichoderma* against pathogens, A) *Trichoderma viride* vs. *P. capsici*; B) *Trichoderma asperellum* vs *Fusarium oxysporum*; and C) *Trichoderma harzianum* vs *Rhizoctonia solani*.

Similarly, Zegeye *et al*. (2011), found that *T. viride* showed a complete inhibition in the radial growth of *P. infestans* in *in vitro* tests. In addition, they mentioned that the foliar application of *T. viride* has a good potential to control *P. infestans* in greenhouse conditions. On the other hand, the evaluation of dual antagonism of *T. viride* against *F. oxysporum* showed growth of 0.656 mm of the antagonist and 0.221 mm by the pathogen, this evidences how *T. viride* is suitable for use in the control of *F. oxysporum*.

In the dual confrontation *T. viride* against *R. solani* obtained a growth of the 0.746 mm antagonist and the 0.112 mm pathogen, the results of growth by the antagonist were higher

compared to the pathogen (Figure 3). In both cases similar results have been found where the two fungi are mycoparasited or destroyed by *T. viride* (Figure 3) since it has an anti-fungal effect with this pathogen (Jhon *et al*., 2010; Perveen, 2012; Sánchez-García *et al*., 2017).

It is important to mention that in the first 8 h the growth of the antagonists showed an accelerated growth that facilitates the encounter with the pathogen in less time, indicative that the antagonist grew or covered more surface in culture *in vitro*.

Similar results mentioned several authors where they demonstrated that *Trichoderma* spp. and *T. viride* are used for the biological control of *R. solani* (Sánchez-García *et al*., 2017). An antifungal effect was observed, in which it inhibits growth and causes lysis of this pathogen. In the confrontations of *T. viride* against the causative agents of the wilting of chili. The evaluation of the antagonism of *T. harzianum* against *P. capsici*, *F. oxysporum* and *R. solani* (Figure 4) showed greater growth by the antagonist compared to the pathogen, denoting hyperparasitism by completely invading the pathogen, a similar result was obtained by Osorio-Hernández *et al*. (2011) in which he observed that *Tricoderma* spp., is an effective antagonist for the control of *P. capsici*, inhibiting this pathogen up to 48%.

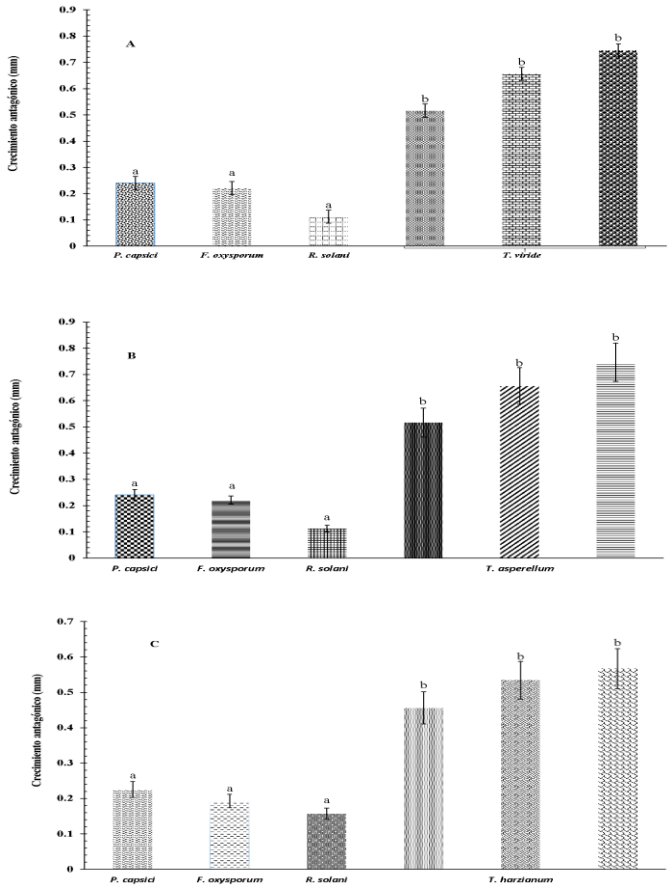

Figure 4. Mycelial growth (mm) in the biocontrol test of A) *T. viride vs P. capsici*, *F. oxysporum* and *R. solani*; B) *T. asperellum*. *vs P. capsici*, *F. oxysporum* and *R. solani*; and C) *T. harzianum* *vs P. capsici*, *F. oxysporum* and *R. solani*.

Also, in the confrontation of *T. viride* against *F. oxysporum* and *R. solani*, the same tendency was found. A significant value ($p \leq 0.05$) was found in the susceptibility assessment based on the PICR of the three pathogens responsible for the wilting of chili; however, the highest susceptibility was presented by *Fusarium oxysporum* with 92.68%, followed by *Rizoctonia solani* and *P. capsici* (Table 2), results that prove the effectiveness of the antagonists (Osorio *et al*., 2016) against pathogens that affect the root of the chilli from seedlings and plants in production (Romero *et al*., 2017).

Table 2. Evaluation of the susceptibility of pathogens to *Trichoderma* and percent inhibition of *Trichoderma* sp. against the pathogens causing the wilting of chili.

Pathogens	Susceptibility of pathogens to *Trichoderma* PICR (%)	*Trichoderma*	Antagonistic activity *Trichoderma* PICR (%)
Fusarium oxysporum	92.68 a	*T. asperellum*	88.25 a
Rhizoctonia solani	87.86 b	*T. viride*	87.22 a
Phytophthora capsici	83.33 c	*T. harzianum*	87.8 a

a, b, c= denotes the average treatments that are significantly the same or different according to the Tukey test in $p < 0.05$. PICR= percentage of radial growth inhibition.

In the evaluation of the percentage of radial growth inhibition (PICR) of the pathogens causing the wilting of the chili, there were no significant differences between the antagonists *T. asperellum*, *T. viride* and *T. harzianum*; therefore, it is suggested to use any of the three *Trichoderma* as a biological control agent; that is, they are effective in inhibiting more than 85% of the pathogens causing the wilting of chili (Table 2), the response of inhibition of the growth of phytopathogens is due to the synthesis of secondary metabolites and to the different mechanisms of action of mycoparasitism (Harman, 2006; Infante, 2009).

The similar inhibition behavior of the three treatments are favorable results found by those who report that above 80% inhibition is acceptable in the biocontrol of pathogens that are transmitted by the soil and by the efficacy of fighting root diseases (Hermosa *et al*., 2012; Sabbagh *et al*., 2017).

The results of mycoparasitism of the antagonists frequently had a more favorable growth, it should be mentioned that the first 8 h were significant ($p \leq 0.05$) showing that the antagonist had an accelerated growth that favors that the encounter can be had in a short time. Likewise, it was observed that not only did it present the inhibition of the growth of these pathogens, but it also presented a total invasion (hyperparasitism) of the antagonist towards the pathogen even sporulating on the pathogen. In a study with selections of 25 *Trichoderma* strains including *T. harzianum*, of which they presented inhibition in *F. oxysporum* on mycelium growth in 33 and 35%.

Similarly, Sinuco *et al*. (2017) indicate that volatile organic compounds of *T. viride* affected the growth halos of *Fusarium* spp. The growth of *T. asperellum* showed significant differences ($p \leq 0.05$) when confronted with the different pathogens causing the wilting of chili caused by the pathogens of *P. capsici*, *F. oxysporum* and *R. solani* ($p \leq 0.001$) since at to analyze the data, it was observed that the growth rate of the pathogen was reduced by almost half when confronted with *T. asperellum*.

In addition, this antagonist overgrown up to 100% on the pathogens, covering the box completely and thus causing hyperparasitism, thus showing a very efficient antagonistic ability to each of the pathogens.

These results coincide with other authors who mentioned that *Trichoderma* ssp. overgrowth *Phytophthora* causing hyperparasitism (Bae *et al*., 2016). Similarly, *Trichoderma* spp. it develops against *R. solani*, causing the inhibition of *in vitro* growth of *R. solani* from 58 to 86%. *Trichoderma* spp., has the ability to induce the expression of genes related to the defense of plants and produce a higher level of ergosterol, indicating its ability to grow at a higher rate in the soil, this explains its positive effects on the growth and defense of the plant in the presence of the pathogen (Mayo *et al*., 2015).

Trichoderma isolates confronted with *P. capsici* exerted a highly significant antagonistic effect ($p \leq 0.001$), causing inhibition in oomycete growth under dual conditions. *T. harzianum*, *T. viride* and *T. asperellum*, also had a similar behavior against *F. Oxysporum* and *R. solani* (Figure 4). The antagonists continued to grow up to six days later, showing hyperparasitism by invading or growing completely on the pathogen. In this regard, Segarra *et al*. (2013), mentioned that *T. asperellum* strain T34, is able to reduce *P. capsici* up to 71%, when applied to pepper plants at different stages of growth.

Mycoparasitism

In vitro tests the inhibition of the three species of *Trichoderma* was evaluated, the results showed the efficacy in competition for space and nutrients, in addition to appreciating the parasitic ability against *Rhizoctonia solani*, *Fusarium oxysporum* and *P. capsici*. In addition, was observed by light microscopy and corroborate in microcultures and double-confrontation cultures at the crossing point between the native isolates of *Trichoderma* spp. and pathogens causing the wilting of chili the different types of mycoparasitism (Figure 5).

Figure 5. Mycoparasitism of *Trichoderma* sp. A) *T. harzianum* surrounding the mycelium of *Rhizoctonia solani*; B) *T. viride* and *T. asperellum* surrounding the mycelium of *P. capsici*; and C) massive winding of *T. asperellum* covering *Fusarium oxysporum*.

In some early investigations, myco-parasitism, antibiosis has been observed, in addition to Trichoderma having a growth behavior parallel to the pathogen until it is wound avoiding the different survival mechanisms of the pathogen (Chet *et al*., 1981; Harman, 2006; Infante, 2009).

Conclusions

T. asperellum, *T. viride* and *T. harzianum* are efficient antagonists of the phytopathogens that cause the wilting of chili. The tests with these antagonistic microorganisms have demonstrated the existence of biological alternatives for the control of phytopathogens of chili root in laboratory conditions, therefore, it is suggested to carry out biocontrol evaluations with these isolates and cultivation of chili in the greenhouse and in the open field. This will help to know the behavior in competitive conditions with a greater diversity of microorganisms and environmental factors different from those studied.

Cited literature

Andrade, H. P.; De León, C.; Espíndola, B. M. C., Alvarado, R.D.; López, J. A. y García, E. R. 2012. Selección de porta-injertos de aguacate para tolerancia-resistencia a *Phytophthora cinamomo* Rands. Usando temperaturas controladas. Spanish J. Rural Development. 3(4):23-30.

Bae, S. J.; Mohanta, T. K.; Chung, J. Y.; Ryu, M.; Park, G.; Shim, S.; Hong, S. B.; Seo, H.; Bae, D. W.; Bae, I., Kim, J. J. and Bae, H. 2016. *Trichoderma* metabolites as biological control agents against *Phytophthora* pathogens. Biological Control. 92:128-138. Doi:10.1016/ j.biocontrol.2015.10.005.

Barnett, H. and Hunter, B. 1972. Illustrated genera of imperfect fungi. EE. UU. Burgess Publ. Co., 241p. Doi:10.2307/3757954.

Barnett, H. L. and Hunter, B. B. 1998. Illustrated genera of imperfect fungi. 4ª. Ed. American Phytophatology Society, MN. 217 p.

Bouziane, Z.; Dehimet, L. and Kacem, C. N. 2016. Inhibitory activity of *Trichoderma viride* against *Phytophthora infestans* that affects the Spunta potato (*Solanum tuberosum* L.) variety. Afr. J. Microbiol. Res. 29(10):1121-1127.

Burgess, L. W.; Summerell, B. A.; Bullock, S.; Gott, K. P. and Backhouse, D. 1994. Laboratory manual for *Fusarium* research. 3rd (Ed.). University of Sydney, Sydney, Australia. 132 p.

Chaverri, P.; Branco, R. F.; Jaklitsch, W.; Gazis, R.; Degenkolb, T. and Samuels, G. J. 2015. Systematics of the *Trichoderma harzianum* species complex and the re-identification of commercial biocontrol strains. Mycologia. 107(3):558-590.

Chet, I.; Harman, G. E. and Bake, R. 1981. *Trichoderma hamatum*: Its Hyphal Interactions with *Rhizoctonia solani* and *Pythium* spp. Microbial Ecol. 7(1):29-38.

de Hoog, G. S.; Guarro, J.; Gene, J. and Figueras, M. J. 2000. Atlas of clinical fungi, 2nd edition. entraalbureau voor Schimmelcultures, Utrecht. Mycological research. 106(4):507-511. Doi:10.1017/S0953756202226167.

Du Plessis, I. L.; Druzhinina, I. S.; Atanasova, L.; Yarden, O. and Jacobs, K. 2018. The diversity of *Trichoderma* species from soil in South Africa, with five new additions. Mycologia. 110(3):559-583.

Erwin, D. C. and Ribeiro, O. K. 1996. Phytophthora diseases worldwide. Department of Plant Pathology, University of California, Riverside, USA. American Phytopathol. Society. 562 p.

Ezziyyani, M.; Requena M. E.; Egea-Gilabert C. and Candela M. E. 2007. Biological Control of Phytophthora Root Rot of Pepper Using Trichoderma harzianum and Streptomyces rochei in Combination. Journal of Phytopathology. 155(6):342-349. Doi:10.1111/j.1439-0434.2007.01237.x.

Ezziyyani, M.; Requena, M. A. y Candela, M. A. 2005. Producción de proteínas-PR en la inducción de resistencia a *P. capsici* en plantas de pimiento. Anales de Biología. 27:143-154.

Gallegly, M. E. and Hong, M. C. 2008. Phytophthora: identifying species by morphology and DNA fingerprints. American Phytopathol. Society. St. Paul, M. N. 158 p.

García, E. R. 2010. Agroecología y enfermedades de la raíz en cultivos agrícolas. Colegio de Postgraduados, estado de México, México. 130 p.

Granke, L. L.; Quezada, O. L.; Lamour, K. and Hausbeck, M. K. 2012. Advances in research on *Phytophthora capsici* on vegetable crops in the United States. Plant disease. 11(96):1588-1600.

Guédez, C.; Cañizalez, L. M.; Castillo, C. y Olivar, R. 2012. Evaluación *in vitro* de aislamientos de *Trichoderma harzianum* para el control de *Rhizoctonia solani, Sclerotium rolfsii* y *Fusarium oxysporum* en plantas de tomate. Rev. de la Sociedad Venezolana de Microbiología. 3(32):44-49.

Harman, G. E. 2006. Overview of Mechanisms and uses of Trichoderma spp. Phytopathology. 96(2):190-194. Doi: 10.1094/PHYTO-96-0190.

Harris, I. J. 1986. Modified method for fungal slide culture. J. Clinical Microbiol. 24(3):460-461.

Hermosa, R.; Viterbo, A.; Chet, I. and Monte, E. 2012. Plant-beneficial effects of Trichoderma and of its genes. Microbiology. 158(1):17-25. Doi: 10.1099/mic.0.052274-0.

Hernández, C. F. D.; Lira, S. R. H.; Gallegos, M. G.; Hernández, S. M. and Solis, G. S. 2014. Biocontrol of pepper wilt with three *Bacillus* species and its effect on growth and yield. Phyton. 83(1):49-55.

Hernández, H. E. J.; Hernández, R. I.; Almaraz, S. J. J.; López, L. A.; Torres, A. M. y Morales, F. F. J. 2018. Caracterización *in vitro* de rizobacterias y su antagonismo con hongos causantes del damping off en chile. Rev. Mex. Cienc. Agríc. 9(3):525-537.

Infante, D.; Martínez, B.; González, N. y Reyes, Y. 2009. Mecanismos de acción de *Trichoderma* frente a hongos fitopatógenos. Revista de Protección Vegetal. 1(24):14-21.

Jaklitsch, W.M. and Voglmayr, H. 2015. Biodiversity of Trichoderma (Hypocreaceae) in Southern Europe and Macaronesia. Studies in Mycology. 80:1-87. Doi.org/10.1016/j.simyco. 2014.11.001.

Jang, S.; Kwon, S. L.; Lee, H.; Jang, Y.; Park, M. S.; Lim, Y. W.; Kim C. and Kim, J. J. 2018. New report of three unrecorded species in *Trichoderma harzianum* species complex in Korea. Micology. 46(3):177-184.

Jhon, R. P.; Tyagi, R. D.; Prévost, D.; Brar, S. K.; Pouleur, S. and Surampalli, R. Y. 2010. Mycoparasitic *Trichoderma viride* as a biocontrol agent against *Fusarium oxysporum* f. sp. adzuki and *Pythium arrhenomanes* and as a growth promoter of soybean. Crop Protection. 12(29):1452-1459.

Leandro, L. F. S.; Guzman, T.; Ferguson, L. M.; Fernandez G. E. and Louws F. J. 2007. Population dynamics of *Trichoderma* in fumigated and compostamended soil and on strawberry roots. Appl. Soil Ecol. 1(35):237-246.

Lozano, A. N.; Guzmán, P. R.; Zavaleta, M. E.; Aguilar, R. H.; Ayala, V. V. 2015. Etiology and evaluation of control alternatives for wilt in chile de árbol (*Capsicum annuum* L.) in La Vega, Metzitlán, Hidalgo, México. 33(1):31-53.

Martínez, B.; Infante, D. y Reyes, R. Y. 2013. *Trichoderma* spp. y su función en el control de plagas en los cultivos. Revista de Protección Vegetal. 1(28):1-11.

Matar, S. M.; El-Kazzaz, S. A.; Wagih, E. E.; El-Diwan, A. I.; Moustafa, H. E.; Abo-Zaid, G. A.; Abd-Elsalam, H. E. and Hafez, E. E. 2009. Antagonistic and inhibitory effect of *Bacillus subtilis* against certain plant pathogenic fungi, I. Biotechnology. 8(1):53-61.

Mayo, S.; Gutiérrez S.; Malmierca M. G.; Lorenzana A.; Campelo M. P.; Hermosa R. and Casquero P. A. 2015. Influence of Rhizoctonia solani and Trichoderma spp. In growth of bean (Phaseolus vulgaris L.) and in the induction of plant defense-related genes. Frontiers Plant Science. 6(685):1-11. Doi: 10.3389/fpls.2015.00685.

Molina, G. E.; Andrade, H. P.; García, E. R. y Sosa, H. C. M. 2016. Capacidad de sobrevivencia de tres especies de *Phytophthora* y dos de *Pythium* preservados en dos sustratos a corto y largo plazo. Rev. Mex. Cienc. Agríc. 7(7):1759-1764.

Nawaz, K.; Shahid, A. A.; Bengyell, L.; Subhani, M. N.; Alia, M.; Anwar, W.; Iftikhar, S. and Ali, S.W. 2018. Diversity of Trichoderma species in chili rhizosphere that promote vigor and antagonism against virulent *Phytophthora capsici*. Scientia Horticulturae. 239:242-252. Doi.org/10.1016/j.scienta.2018.05.048.

Osorio, H. E.; Hernández, C. F. D.; Gallegos, M. G.; Rodríguez, H. R. and Castillo, R. F. 2011. *In-vitro* behavior of *Trichoderma* spp. Against *Phytophthora capsici* Leonian. Afr. J. Agric. Res. 19(6):4594-4600.

Osorio, H. E.; Hernández, F. D. C.; Rodríguez, R. H.; Varela, S. D. F.; Estrada, B. D. y López, J. A. S. 2016. Actividad antagónica de *Trichoderma* spp. sobre *Rhizoctonia solani in vitro*. Investigación y Ciencia. 67(24):5-11.

Osorio, H. E.; Hernández, M. J.; Conde, M. V.; Michel, A. A. C.; López, S. J. A. and Torres, C. J. A. 2016. *In vitro* activities of *Trichoderma* species against *Phytophthora parasitica* and *Fusarium oxysporum*. Afr. J. Microbiol. Res. 15(10):521-527.

Paul, B. 1999. Pythium periplocum, an aggressive mycoparasite of Botrytis cinerea causing the gray mould disease of grape-vine. FEMS Microbiology Lett. 181(2):277-80. Doi:10.1111/j.1574-6968.1999.tb08855.x.

Perveen, K. and Bokhari, N. A. 2012. Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* isolated from soil of date palm field against *Fusarium oxysporum*. African J. Microbiol. Res. 13(6):3348-3353.

Rivera-Jiménez, M. N.; Zavaleta-Mancera, H. A.; Rebollar-Alviter, A.; Aguilar-Rincón, V. H.; García de los Santos, G.; Vaquera-Huerta, H.; Silva-Rojas, H. V. 2018. Phylogenetics and histology provide insight into damping-off infections of 'Poblano' pepper seedlings caused by Fusarium wilt in greenhouses. Mycological Progress. 17(11):1237-1249. Doi.org/10.1007/s11557-018-1441-2.

Romero-Arenas, O.; Amaro, J. L.; Damián, M. A.; Valencia de Ita, M. A.; Rivera, A. y Huerta, M. 2017. Biopreparados de *Trichoderma* spp. para el control biológico de *Phytophthora capsici* en el cultivo de tomate de Puebla, México. Información Técnica Económica Agraria.113(4):313-324. Doi.org/10.12706/itea.2017.019.

Romero, A. O.; Huerta, L. M.; Damián, H. M. A.; Domínguez, H. F. y Arellano, V. D. A. 2009. Características de *Trichoderma harzianum*, como agente limitante en el cultivo de hongos comestibles. Rev. Colomb. Biotecnol. 11(2):143-151.

Sabbagh, S. K.; Roudini, M. and Panjehkeh, N. 2017. Systemic resistance induced by *Trichoderma harzianum* and *Glomus mossea* on cucumber damping-off disease caused by *Phytophthora melonis*. Arch Phytopathol. Plant Protec. 50(5-8):375-3788.

Sánchez, G. B. M.; Espinosa, H. E.; Villordo, P. E.; Rodríguez, G. R. y Mora, A. M. A. 2017. Identificación molecular y evaluación antagónica *in vitro* de cepas nativas de *Trichoderma* spp. sobre hongos fitopatógenos de raíz en frijol (*Phaseolus vulgaris* l.) cv. Montcalm. Agrociencia. 1(51):63-79.

SAS Institute Inc. 2002. SAS/STAT User guide. Release 9.0. North Carolina, USA. 1289.

Segarra, G.; Avilés, M.; Casanova, E.; Borrero, C. and Trillas, I. 2013. Effectiveness of biological control of *Phytophthora capsici* in pepper by *Trichoderma asperellum* strain T-34. Phytopathol. Mediterránea. 1(52):77-83.

Seifert, K. 1996. *Fusarium* interactive key. Agric. Agri-Food. Canada. 65 p.

Singh, B. N.; Singh, A.; Singh, S. P. and Singh, H. B. 2011. *Trichoderma harzianum*-mediated reprogramming of oxidative stress response in root apoplast of sunflower enhances defence against *Rhizoctonia solani*. European Journal Plant Pathology. 1(131):121-134.

Sinuco, D. C.; Pérez, A. C.; Moreno, N. 2017. Evaluación de la actividad fungicida e identificación de compuestos orgánicos volátiles liberados por *Trichoderma viride*. Rev. Colomb. Biotecnol. 1(19):63-70.

Sneh B.; Burpee L. and Ogoshi A. 1991. Identification of *Rhizoctonia* species. American Phytopathology Society, MN. 133 p.

Sonnenbichler, J.; Lamm, V.; Gieren, A.; Holdenrieder, O, and Lotter, H. 1983. A Cyclopentabenzopyranone produced by the fungus Heterobasidion annosum in dual cultures. Phyrochemistry. 22(6):489-1491. Doi.org/10.1016/S0031-9422(00)84043-1.

Steel, R. G. D. y Torrie, J. H. 1986. Bioestadística: principios y procedimientos. 2ª (Ed.). México: McGraw-Hill. 228 p.

Vásquez, L. A.; Tlapal, B. B.; Yáñez, M. M.; Pérez, R. P. y Quintos, M. E. 2009. Etiología de la marchitez del 'chile de agua' (*Capsicum annuum* L.) en Oaxaca, México. Rev. Fitotec. Mex. 2(32):127-134.

Verma, M.; Brar, S. K.; Tyagi, R. D.; Sahai, V.; Prévost, D.; Valéro, J. R. and Surapalli, R. Y. 2007. Bench-scale fermentation of *Trichoderma viride* on wastewater sludge: rheology, lityc enzymes and biocontrol activity. Enzyme and Microbial Technology. 41(6):764-771.

Woo, S. L. and Lorito, M. 2007. Exploiting the interactions between fungal antagonists, pathogens and the plant for biocontrol. *In*: ovel biotechnologies for biocontrol agent enhancement and management. Vurro, M; Gressel, J. N. (Eds.). Springer, Dordrecht. 107-130 pp.

Zegeye, D. E.; Santhanam A.; Gorfu, D.; Tessera, M. and Kassa, B. 2011. Biocontrol activity of *Trichoderma viride* and *Pseudomonas fluorescens* against *Phytophthora infestans* under greenhouse conditions. Inter. J. Agric. Technol. 6(7):1589-1602.