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Original article

Antagonic effectiveness *in vitro* of *Trichoderma* sp. in front of *Stemphylium lycopersici*

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ABSTRACT

Stemphylium lycopersici is one of the causal agents of the gray spot on the tomato leaves, a recurrent and widespread pathology in tomato crops in Holguín. The *in vitro* antagonism of *Trichoderma harzianum* strains A-34, A-53 and *Trichoderma viride* TS-3 was evaluated against *Stemphylium lycopersici*. The confrontation was carried out by means of an

antagonism assay in Papa Dextrose Agar PDA culture medium. The indicator evaluated was the mycelial inhibition percentage IM (%).The three strains of *Trichoderma* sp. showed high antagonistic effectiveness *in vitro* against *Stemphylium lycopersici*.

Key words: tomato; biocontrol, mycelium, inhibition, pathogen

INTRODUCTION

Trichoderma sp. is as a biopesticide used, because of its properties to inhibit fungal phytopathogens. This fungus can be found distributed in different areas and habitats, especially in sites that contain abundant organic matter [(1)]. Its main antifungal activity is the result of antagonistic competition, a mechanism of vital importance and defined as the differentiated behavior of two or more organisms against the same requirement, so that one of the organisms reduces the space or the available nutrients of the other [(2-3)]. Certain characteristics such as the specific speed of growth or development and external factors: temperature, humidity or pH, influence antagonistic competition [(4)].

The global production of tomato (*Lycopersicum sculentum*) for consumption is estimated at 108 000 000 t, with an average yield of 36 t ha^{-1}. China is the largest producer in the world, followed by the US, Turkey, India, Egypt and Italy [(5)]. Annual tomato production in Cuba represents more than 50 % of the total area cultivated by vegetables, with more than 200,000 t commercialized [(6)]. For 2017, FAO estimated that 48713 hectares of tomato were grown in Cuba with a production of 58 4072 t and a yield exceeding 11 t ha^{-1} [(7)].

• A high incidence of *Stemphylium lycopersici* has been in tomato crops observed in many regions of the world. Recently an increase in this disease has been reported in South America [(8)]. This pathogen is widely distributed in Cuba, mainly affecting the West of the country [(9)]. It causes wilting of leaves and stems in plants and consequently shortens the normal biological cycle and reduces yields [(10)]. In the Provincial Laboratory of Plant Health of Holguín there has been, in the last 10 years, a recurrent and massive affectation of this pathogen, which causes great losses of tomato crops. So far, the causes that could influence the increase of this disease are unknown.

• Therefore, the objective of this work was to evaluate the antagonistic effectiveness *in vitro* of *T. harzianum* strains A-34, A-53 and *T. viride* TS-3, against *S. lycopersici*.

MATERIALS AND METHODS

Three strains were used: *Trichoderma harzianum* Rifai strain A-34, *Trichoderma harzianum* Rifai strain A-53 and *Trichoderma viride* Persoon strain TS-3, from the Center for Reproduction for Biological Control (CREE), of Ciudad Jardín, Agricultural Company of Holguin.

From leaves of *Lycopersicon sculentum*, Mill variety HC-2580, with symptoms of *Stemphylium lycopersici*, leaves were collected that had dark spots or small spots of about 2 to 4 mm, brown or black and circular with irregular contours and surrounded by a yellowish halo. In some cases, the collected leaves presented gray spots with a hole in the center of the sheet [10]. The leaves were from tomato crops obtained from the municipalities of Gibara, Holguín and Banes in Holguín province.

The antagonism tests were carried out in the Provincial Laboratory of Plant Health of Holguín, in the mycology department. *Stemphylium lycopersici* was isolated from infected leaves of *L. sculentum*. The pathogen was reproduced by inoculation of mycelial suspension in Petri dish, with Papa Dextrose Agar (APD) culture medium at 28 °C, for 72 hours in an incubator [11].

The confrontation of *S. lycopersici* and *Trichoderma sp* was performed by the antagonism test [12]. Petri dishes of 90 mm diameter were used, with APD culture medium. The mycelium of *S. lycopersici* and the strains of *Trichoderma s*p were obtained from the center of colonies grown on Petri dishes on the sixth day. A 7 mm diameter disc of the mycelium of the pathogen was placed at one end of the plate and at the opposite end another 5 mm disc with mycelium of each strain of *Trichoderma* sp, according to the different treatments. Pathogen samples not inoculated with *Trichoderma* sp. They were used as a control. Six Petri dishes were used for each treatment for each of the *T. harzianum* A-34, A-53 and *T. viride* TS-3 strains. From the seventh day of starting the confrontation, the percentage of mycelial inhibition MI (%) was measured [13,14]. To determine the area of mycelial growth of *Trichoderma* sp. NHI Image J software was used [15].

Statistical analysis

To compare the MI (%) between the strains, a PERMANOVA was performed, with 10,000 permutations, using the Euclidean distance, with a significance level of 5 %, the value of p without correction. The PAST 3.18 statistical software [16] was used.

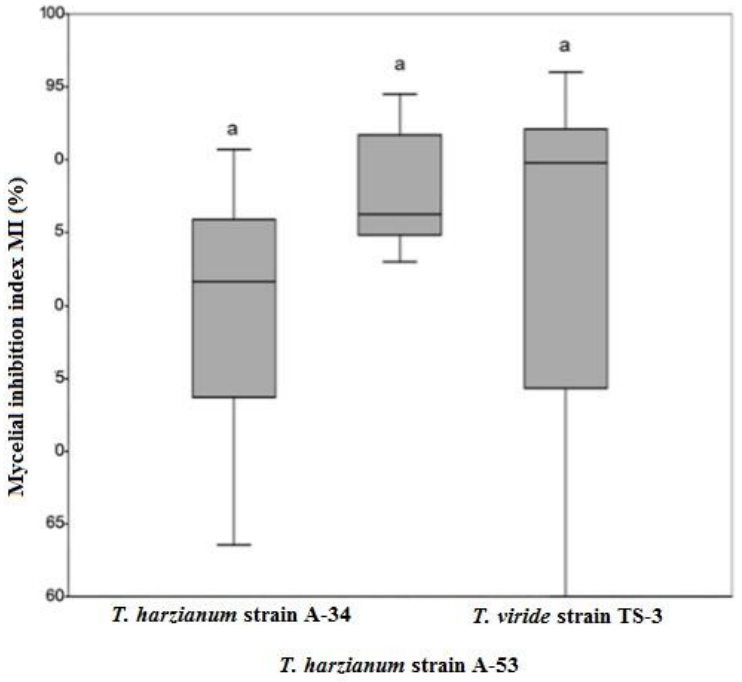
RESULTS AND DISCUSSION

In vitro antagonism of the three strains of *Trichoderma* sp. used against *S. lycopersici*. No statistical differences were obtained in the percentage of mycelial inhibition of *S. lycopersici* by the different *Trichoderma* strains used (Figure 1). The values obtained were in *T. harzianum* strain A-53 MI (%) = 87.2 ± 4.53, followed by *T. viride* strain TS-3 MI (%) = 81.13 ± 13.80. The lowest value was obtained by *T. harzianum* strain A-34 MI (%) = 80.28 ± 9.78.

The antagonistic activity shown by the *Trichoderma* sp. It could be due to a higher growth rate, competition for space and for nutrients or other factors such as the production of enzymes, highlighting chitinases, glucanases and proteases [17,18].

It has been shown that *Trichoderma* sp. It presents extracellular enzymes with lytic activity on the hyphae of the pathogen. Some studies have shown evidence of chitinase production, which contribute to the degradation of the cell walls of pathogenic microorganisms [19].

The antagonistic intensity of *Trichoderma* sp is considered high because it inhibited more than 80 % the growth of *S. lycopersici*. One of the most common forms of antagonism reported by *Trichoderma* sp. It is due to hyperparasitism, where the hyphae of *Trichoderma* cover those of the pathogen, intertwining or intersecting with it occupying much of the living space [20].



Equal letters represent that there is no difference between the means, p≤0.05

Figure 1. Percentage of myelial inhibition of *Stemphylium lycopersici* against the strains of *Trichoderma* sp. A-34, A-53 and TS-3 seven days after the PERMANOVA test (p = 0.29 and F = 1.31)

Other works show that *Trichoderma* sp. It presents an effective mycelial inhibition of *Fusarium* sp., pathogen in onion seeds. This result was maintained in different reproductive cycles of *Trichoderma* sp., mainly during sporulation [21]. Strains of *T. harzianum* and *Trichoderma yunnanense* also showed a growth inhibition of *Phytophthora capsici* of 10.3 and 22.1 % on the sixth day of starting the confrontation [22].

On the other hand, *Trichoderma longibranchiatum* showed inhibition percentages of 21.9 and 22.5 % against *Colletotrichum gloeosporioides* and 23.86 and 43.14 % against *Fusarium verticillioides* [23,24]. These values are lower than those obtained in the present study are.

Similar tests of antagonism performed *in vitro* with *T. harzianum* strain CCIBP-T4 demonstrated a high activity of hyperparasitism at six days of inoculation against *Mycosphaerella fijiensis*, foliar pathogen in banana crops in Cuba. *Trichoderma* caused the breakdown in the cell wall of the hyphae of the pathogen and the outflow of extracellular content [25].

Other authors evaluated the *in vitro* antagonistic effect of 31 strains of *Trichoderma* against *Phytophthora capsici*, with the strains showing 97 % mycelial inhibition [26].

Cladosporium fulvum leaf mold is a disease that affects tomato crops in Peru. To control this pathogen, confrontations were carried out *in vitro* with *Hansfordia pulvinata*, *T. harzianum*, *T. viride* and *Trichoderma virens*. It was found that at 24 °C and at 72 h, *T. harzianum* presented a percentage of mycelial inhibition statistically higher than the remaining three species, and at 28 °C it was similar to *T. viride* [27].

Mycoparasitism is established as the most common mechanism of action of *Trichoderma* sp. during mycelial inhibition in different pathogens and it is adduced as an optimal temperature of inhibition in the range of 20 to 30 °C [27]. In this sense, it is possible that temperature and pH could be the two factors that most influenced not finding differences between strains A-34, A-53 and TS-3, in the mycelial inhibition of *S. lycopersici* in this investigation. Both chemical-physical parameters remained constant during the clashes and several authors report a strong relationship between them and the antagonistic effectiveness of *Trichoderma* sp. [21-27].

The results demonstrate, for the first time, the *in vitro* antagonistic activity of *Trichoderma harzianum* (strains A-53 and A-34) and *Trichoderma viride* (strain TS-3) against *Stemphylium lycopersici*, foliar pathogen in tomato crops in the province from Holguin.

CONCLUSION

Strains A-53, A-34 and TS-3 of *Trichoderma* sp. they have *in vitro* antagonism against the *Stemphylium lycopersici* pathogen.

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