

## Biological Control of *Phytophthora capsici* by Native *Trichoderma* of the Rhizosphere of Serrano Pepper, *In vitro*

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The oomycete "*Phytophthora capsici*" is the main cause of the blight of pepper (*Capsicum annuum* L.), wilt a disease that can cause yield losses of up to 100%. For its control, it mainly resorts to the use of fungicides that harm the environment, however, the use of biological control is a tool that can be useful for combating this disease. Fungi of *Trichoderma* spp have the potential to antagonize to *Phytophthora capsici*, however, studies should be performed on native strains that may be established in different soils from which they were isolated. In this work it was evaluated the antagonistic activity of three native strains of rhizosphere from chili peppers from Puebla Tetela de Ocampo Mexico and isolated strains from other environments against a native strain of *P. capsici* extracted from pepper plants that presented the disease. The development rate (DR) and concentration of conidia (con/mL) of the strains of *Trichoderma* were evaluated in culture medium malt extract agar (EMA), likewise, it was evaluated the presented inhibition percentage. The highest rate of development (DR) and concentration of conidia was presented in the strain "S3A4", with 33.7 mm and  $1.73 \times 10^6$  / mL respectively. The *Trichoderma* strains showed different qualities of parasitism in the scale of Bell, however there was some homogeneity in the percent inhibition of the strains from pepper soil.

**Key words:** Pepper wilt, biological control, inhibitory activity.

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The most important disease of chili cultivation worldwide is wilt, caused by *Phytophthora capsici*, a microorganism capable of infecting any underground or aerial parts of the plants<sup>1,2</sup>.

In Mexico a variety of types of chilies is grown, including the Serrano pepper (*Capsicum annuum* L.), which is one of the most economically important for its large consumption, high profitability and high demand for labor<sup>3</sup>. It is also the eighth most important crop in national agriculture with averaging 2.2 million tons, of which about 900 thousand tons are exported in fresh, dried chilies and preparations. Most pepper

production (72.5%) is concentrated in Chihuahua, Sinaloa, Zacatecas, San Luis Potosi and Michoacan<sup>4</sup>. Today the production of chili is focusing towards agroecological management, which aims to recover the ecological balance, and the preservation of natural diversity. In this regard, crop rotation techniques, application of organic fertilizers, use of mulches, greenhouse production, application of antagonists and application of microorganisms that promote growth and root developments are used<sup>5</sup>. Biological control antagonistic organisms is a valuable tool "non-chemical" in the crop protection against pathogens<sup>6,7</sup>. Species of the *Trichoderma* genus are the most commonly used to control plant diseases, due to their qualities as an antagonist<sup>8,9</sup>; however, its effectiveness may vary when species are introduced and natural soil homeostasis limits its establishment and antagonistic growth<sup>10,11</sup>.

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In this paper we report the in vitro inhibitory activity of native strains of *Trichoderma* spp isolated or not isolated from the rhizosphere of Serrano peppers in the municipality of Tetela de Ocampo, Puebla Mexico, where *Phytophthora capsici* has caused high wilt incidence.

## MATERIALS AND METHODS

### Isolation of strains

It was isolated a strain of *Phytophthora capsici* (PC-A) from the root of Serrano pepper criollo (*Capsicum annuum* L.) from Tetela de Ocampo that showed signs of wilting and three strains of *Trichoderma* spp from the rhizosphere of the crop itself.

These strains were compared with six strains of *Trichoderma* spp., including three native of eroded soils of the reference area "TP1S1, T4-(3) and T5-(2)" and three strains from tomato roots (TJIM-I, TJIM-II and TMIX) the state of Morelos, Mexico.

### Growth rate (GR) and concentration of conidia of *Trichoderma* spp

The native strains of *Trichoderma* spp were cultivated separately on malt extract agar (EMA-Difco®). Four replications were prepared for each strain in 140 mm Petri dishes and were incubated at 26 °C with a photoperiod of 12 hours. The development rate of the mycelium was calculated was calculated, based on the formula of Romero <sup>12</sup>: DR = growth final - growth initial /

number of days. Additionally it was estimated the conidia concentration reached by each of the *Trichoderma* spp strains. For this purpose, they were added 20 mL of sterile distilled water in each box of 4 days of incubation, with a sterile glass spatula conidia spores were recollected and deposited in a glass beaker (KIMAX®), to this suspension was added a drop of liquid soap as surfactant and it was taken two samples of 10 iL to be evaluated in a hemacytometer (MARIENFELD™) and a compound microscope (Leica Inc., USA). The samples were observed at 10x and 40x, the counting process of conidia was done by triplicate, with the following formula:

concentration Total = No. of spores / 8 x 10<sup>4</sup>.

### Antagonism of *Trichoderma* spp against *Phytophthora capsici*

The procedure of Cherift and Benamou 13 was followed to evaluate antagonism. The nine *Trichoderma* strains were confronted against *P. capsici* (PC-A). In each experimental unit a disk of 5 mm diameter was used with *P. capsici* with mycelium of 8 days old and each experimental unit was placed at one end of the Petri dish with EMA. The oomycete was allowed to proceed for 3 days due to slow growth. At the end of this period it was placed at the opposite end of each box another disc of 5 mm with mycelium of *Trichoderma* spp. Thereafter the Petri dishes were incubated in a controlled environment chamber at 25 °C and 40% relative humidity with a 12 hours photoperiod. On the tenth day, it was evaluated the kind of

**Table 1.** Rate of development (DR) and concentration of conidia of *Trichoderma* spp in culture EMA

Culture Medium	key	Rate of development (mm/day)		Concentration of spores and conidia x 10 <sup>6</sup> con/mL	
EMA	S3A4	33.7	a	1.73	a
EMA	S3A3	31.3	a	1.33	c
EMA	A2S2P3	33.7	a	0.08	d
EMA	TP1S1	26.3	bc	1.53	b
EMA	T5-(2)	25.7	c	0.33	e
EMA	T4-(3)	24.4	c	0.46	e
EMA	TJIM-I	14.1	d	0.26	e
EMA	TJIM-II	27.5	c	0.70	d
EMA	TMIX-I	26.4	c	0.41	e

\* Means with different letters in the column indicate significant differences with the Tukey test ( $\alpha = 0.05$ ).

antagonism according to the scale proposed by Bell<sup>14</sup> and the percent inhibition of mycelial growth, based on the formula of Vincent<sup>15</sup>:

$$I = (CT / C) * 100$$

Where;

I = Percentage inhibition.

C = Growth (mm) of *P. capsici* without *Trichoderma* spp.

T = Growth (mm) of *P. capsici* with *Trichoderma* spp.

## RESULTS AND DISCUSSION

In the bioassay, significant differences ( $p < 0.05$ ) in the development rate (DR) of *Trichoderma* spp strains were observed. The strain "S3A4" showed the highest DR (33.7 mm), in 4 days of growth, compared with the strain "TJIM" it had the lowest DR (table 1). It notes that the three strains isolated from the rhizosphere of Serrano pepper floors of Tetela de Ocampo, accumulated the highest values and no significant differences between them. The development rate (DR) is an indirect measure of saprophytism and a useful tool in the characterization of the ability of biocontrol strains of *Trichoderma* spp<sup>16</sup>, which allows to determine the optimal time for performing experiments in planting crops and evaluate the potential of secondary metabolites of the antagonist's fungi<sup>13, 17</sup>.

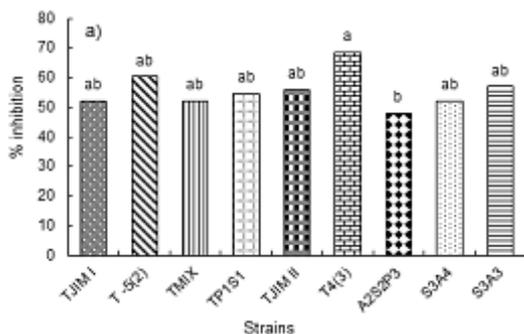
Moreover, the concentration of conidia can serve as an estimate of the degree of biocontrol as part of the survival qualities, colonization and dispersion of the antagonist. According to Papavizas<sup>18</sup>, the genus *Trichoderma* only produces three types of propagules: hyphae

chlamydospores and spores; however, of these, the conidia or spores are the most viable propagules in biocontrol programs<sup>19, 20</sup>. In this research, the highest values of sporulation were obtained with the strain "S3A4" ( $1.73 \times 10^6$  with / mL) and the lowest concentration was  $0.26 \times 10^6$  with con / mL with the "TJIM-I" strain, see table 1. **Antagonism of *Trichoderma* spp. against *Phytophthora capsici***

The type of antagonism caused by the *Trichoderma* strains against *P. capsici* varied and depended on their antagonistic capacity. The "A2S2P3" and "S23A3" strains presented a type antagonism 1. The "T4-(3)", "S3A3", "S3A4", "A2S2P3" and "T5-(2)" strains showed better biocontrol qualities, since all the cases had a type 2 antagonism.

The "TJIM-I" strain was the only one that showed a type 3 antagonism, against *P. capsici*, which was related to its slow development. According to Dennis and Webster<sup>21</sup>, the overgrowth is an advantageous character in the dispute to colonize the area, completely for space and nutrients, which in turn is part of the biocontrol strategy, as it can reduce or completely stop mycelium development. According to Michel<sup>22</sup>, isolates with antagonistic class 1 and 2 are considered very efficient antagonists and have potential enough for being further evaluated in a greenhouse. Percent inhibition (PI) exerted by the *Trichoderma* strains presented statistically significant differences and ranged from 47.8 to 68.7% for PC-A (Fig. 1); where, the strain "T4-(3)" had the highest PI with 68.7%.

The percentage of inhibition is a parameter that has been evaluated in several studies as a variable to characterize the biological fitness of antagonists against the pathogen<sup>13,23,24</sup>. The implications of this interaction are important characteristics where the action mechanisms of biological control agents and the qualities of resistance and survival of the pathogen involved. The nine strains of *Trichoderma* spp employed in this study showed certain homogeneity in inhibiting *P. capsici*. It should be noted, the nine strains of *Trichoderma* showed antagonistic effect, which contrasts with the obtained by Osorio<sup>25</sup>, who evaluated 30 isolates of *Trichoderma* spp against *P. capsici* and reported results inhibitions lower that 50% on the eighth day; where the strain T25



**Fig. 1.** Percentage of inhibition exercised by strains of *Trichoderma* spp on *Phytophthora capsici* in culture medium EMA to 10 days after planting the pathogen.

(*T. hamatum*) stand out obtaining 49% inhibition.

Furthermore, some authors<sup>26,27 and 28</sup>, have reported that the selection of the antagonists by their good qualities displayed by dual culture, does not guarantee a similar behavior in greenhouses. For example Ezzyyani<sup>29</sup>, determined that 100% inhibition of *P. capsici* with *Trichoderma harzianum* in cultures in vitro and decreased by 56% in greenhouse.

### CONCLUSIONS

The highest rate of development (DR) and concentration of conidia was presented in the native strain "S3A4" with 33.7 mm and  $1.73 \times 10^6$  / mL, from the rhizosphere from chili peppers in the region of Tetela de Ocampo Puebla-Mexico. Eight native strains of *Trichoderma* evaluated in this study showed antagonistic effect of the 50% against *P. capsici* and are in a range of 1 and 2 in Bell scale, with the exception of the strain "A2S2P3" that obtained the lower percent of inhibition. These results demonstrate the feasibility of using native strains of *Trichoderma* for biological control in the pepper-producing region of Tetela de Ocampo, Puebla-Mexico.

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