Article

Biocontrol of Damping off and promotion of vegetative growth in plants of *Capsicum chinense* (Jacq) with *Trichoderma* spp.

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Abstract

The objective of the study was to determine the effectiveness of *Trichoderma* spp. in the reduction of the incidence of Damping off and promotion of vegetative growth of *Capsicum chinense* (Jacq.) var. 'Chichen Itza'. The foliar application was evaluated, through a complete randomized design, of two native strains of *Trichoderma* sp. (SP6 and Clombta), the co-application of both, a commercial product (Tri-HB®: *Trichoderma harzianum* and *Bacillus subtilis*) and a chemical fungicide (Captan®). During the study period, plants treated with *Trichoderma* sp. Clombta and with the co-inoculation of *Trichoderma* sp. Clombta + *Trichoderma* sp. SP6 showed no symptoms of Damping off. In contrast, plants treated with Captan® and Tri-HB® showed the highest cumulative incidence percentages with 5 and 4.5%, respectively. Also, at 28 days after germination, plants treated with *Trichoderma* sp. Clombta had a higher height (11 cm), stem diameter (2.6 mm), aerial biomass (fresh= 0.8 g plant^{-1} and dry= 0.13 g plant^{-1}) and root volume (fresh= 0.13 g plant^{-1} and dry= 0.04 g plant^{-1}), in comparison to the rest of the treatments evaluated. For the formation of leaves (9.1 leaves plant^{-1}), leaf area (10.2 cm^2) and chlorophyll index (Clombta= 209.9) the application of *Trichoderma* sp. Clombta stood out again with the highest values ($p< 0.05$), with respect to the applications of Captan® and Tri-HB®. According to the results obtained, it was determined that the strain of *Trichoderma* sp. Clombta at a concentration of 1x10^{13} conidia mL^{-1} was effective for the management of Damping off and promotion of vegetative growth of *C. chinense* var. 'Chichen Itza'.

Keywords: biological fungicide, chlorophyll index, habanero pepper, incidence.

Reception date: January 2019
Acceptance date: March 2019

Introduction

Mexico stands out in the production of pepper (*Capsicum* spp.) in the world and is the fifth supplier of consumption worldwide, which reaches a production of 2.3 million tons. In the country, pepper is the third most important horticultural crop considering the area sown. In 2018 about 26 300 ha of pepper were established, of the harvested production, Sinaloa occupied the first place (15 625 ha), followed by Chiapas (2 451 ha), Veracruz (1 952 ha), Sonora (1 814 ha) and Oaxaca (1 364 ha) (SAGARPA, 2018a). While Colima (269.5 ha) occupied the twentieth place with 9 172 t of production and a yield of 34.03 t ha^{-1} (SIAP, 2018).

Regarding the production of habanero pepper (*Capsicum chinense* Jacq.) it is estimated that Mexico produces 9 072 t per year, the states with the highest production are Tabasco, Yucatan and Campeche with 4 546, 2 615 and 578 t, respectively (Ocampo-Thomason, 2014). The state of Colima produces between 9 and 28 t per year, this low production could be due to various reasons; for example, there are no technological packages adapted to the area, the response of commercial varieties to the climate of the state is not known, not yet promote the benefits of consumption of habanero pepper in the consumer society and its potential use in the industry (Ocampo-Thomason, 2014; SAGARPA, 2018b).

The commercial production of habanero pepper plants in the greenhouse or during their transplant can be severely affected by phytopathogenic fungi and oomycetes from soil, water and substrates. Among them are the genera *Fusarium*, *Rhizoctonia*, *Pythium* and *Phytophthora* (Mojica *et al*., 2009), which are the main causal agents of Damping off. This pathology is characterized by a rot at the base of the stem of the plants at ground level, which causes wilting and death of the same, causing significant losses (Cárdenas *et al*., 2005).

The handling of the Damping off is carried out; through, of the control of diverse abiotic factors like the relative humidity and temperature, which favor the development of the causal agents. In addition, producers have to resort to the use of chemical fungicides, which has caused resistance in the phytopathogens, environmental contamination in soil, water, fruits and toxicity in plants. These reasons are the reason for the search for other more effective methods that are not harmful to the environment and human health (Mojica *et al*., 2009).

In this regard, biological control has taken great relevance in recent years. Within this method of control, one of the alternatives consists of the application of antagonistic microorganisms of soil pathogens, such as the use of fungal species of the genus *Trichoderma* (Hernández-Mendoza *et al*., 2011). Some isolates and species of this fungus have been shown to be antagonists of *Pythium*, *Rhizoctonia*, *Sclerotium*, *Fusarium* and *Phytophthora*, which is why several commercial products contain it (Naseby *et al*., 2000; Ezziyyani *et al*., 2004; Hoyos-Carbajal *et al*., 2008; Michel-Aceves *et al*., 2009).

Trichoderma carries out its antagonism against plant pathogens by degradation and subsequent assimilation of its cellular content. This antifungal activity involves the production of antibiotics, including compounds that affect the integrity of the fungal membranes, competition for key nutrients and the production of enzymes that degrade the cell wall of fungi (López and González,

2004). In addition to displacing and controlling phytopathogenic root fungi and oomycetes; through mycoparasitism and antibiosis, different species of *Trichoderma* increase the radical growth and development of plants through a series of mechanisms such as the solubilization of inorganic nutrients ($Ca_3(PO_4)_2$ and $FePO_4$), production of organic acids, siderophores and phytohormones (Mukherjee *et al*., 2012; Chirino-Valle *et al*., 2016).

In previous studies, Candelero *et al*. (2015) reported an increase in the height of *C. chinense* plants due to the inoculation of *Trichoderma* sp. Th05-02 (55.57%) and *Trichoderma virens* (47.62%). While *Trichoderma harzianum* increased the length (41.57%) and root volume (550%) with respect to the control (without inoculation). Likewise, the authors reported the ability of some strains to control juveniles (J2) of the *Meloidogyne incognita* nematode, the strains with the highest control (immobility) were *T. vires* Th43-13 and *Trichoderma* sp. Th43-14, both with 100% immobility. The bioprospecting and evaluation of microorganisms that promote plant growth and biofungicides is of great importance in organic agriculture (Chirino-Valle *et al*., 2016).

Therefore, the objective of the present work was to determine the effectiveness of *Trichoderma* spp. in the reduction of the incidence of Damping off and promotion of the vegetative growth of *C. chinense* var. 'Chichen Itza'.

Materials and methods

The research was carried out in the Biological Control Laboratory of the Faculty of Biological and Agricultural Sciences of the University of Colima (FCBA-UCOL). While the experimentation with habanero pepper plants was carried out in a greenhouse located in the postgraduate area of the same Faculty, located at km 40 of the Colima-Manzanillo highway in the municipality of Tecoman, Colima. The predominant climate of the region is warm subhumid (AW_1), with rains in summer, average annual temperature of 26.3 °C and its location is between the coordinates 18º 57' 13.4'' North latitude, 103º 53' 42.6'' West longitude a height of 56 masl (Cigales and Pérez, 2011).

Strains of *Trichoderma*

Two strains of *Trichoderma* spp. previously isolated by Sánchez-Rangel *et al*. (2016): *Trichoderma* sp. SP6 native to the rhizosphere of papaya (*Carica papaya* L.), isolated from a crop in the ranch 'Las Mercedes' (Tecoman-El Real highway, km 9, Tecoman, Colima 18° 50' 26.05'' North latitude and 103° 55' 13.79'' West longitude) and *Trichoderma* sp. Clombta native to the rhizosphere of the melon crop (*Cucumis melo* L.) isolated in the municipality of Armeria, Colima (19° 06' 04.91'' North latitude and 104° 00' 43.74'' West longitude).

Mass production of spores of *Trichoderma* spp.

The production was carried out in whole rice grain (*Oryza sativa* L.) in polyurethane plastic bags. The rice was washed with potable water and soaked in 500 ppm of chloramphenicol (Lab. Sophia SA de CV), for 30 min. After that time, 250 g of rice were placed in plastic bags and sterilized in an autoclave at 120 °C for 45 min at 18 pounds of pressure. The bags were inoculated with 10 mL

of a conidia suspension at a concentration of $1x10^6$ conidia mL^{-1}. The inoculated rice bags were incubated at 25 °C with 12 h light: dark for 21 days. Subsequently, the conidia were harvested through the procedure described by Lezama-Gutiérrez *et al*. (2006).

The conidia were recovered in 250 mL of water with 0.1% Tween 80® (Sigma-Aldrich, Toluca, Mexico). To separate the grain of rice from the conidia, they were passed through a sieve of 200 meshes and a sieve. The suspension of conidia obtained was centrifuged at 3 600 rpm, for 15 min, in order to concentrate the conidia and separate them from the liquid. Once the conidia were obtained, they were allowed to dry for two days in a laminar flow chamber at 25 °C and stored at 5 °C until their use in the experiment. To determine and adjust the concentration of the conidia mL^{-1} to be used in the bioassays, 0.1 g of the dry powder (conidia) was taken and suspended in 100 mL of sterile distilled water. Through a Neubauer® chamber (Marienfeld, Germany) the concentrations of the conidia for the bioassays were recorded and adjusted (Lezama-Gutiérrez *et al*., 2006).

Plant production

Ten unicel trays were used, which were separated in pairs to establish five different treatments; each tray consisted of 200 plants. BM2 pedestrians (Martin's®, Shippensburg, PA, USA) were used as substrate, at a rate of 4 kg $tray^{-1}$ with 60% humidity. A seed of *C. chinense* var. 'Chichen Itza' (Seminis®, Mexico City) by cavity to a depth of 1 cm. The trays were wrapped in black polyethylene plastic separated by treatments and left in the dark until germination for five days.

Application of treatments

Five treatments were used, each one contemplated two trays of 200 cavities and 30 plants were measured per tray. A plant was considered as an experimental unit, resulting in an n of 60 plants per treatment. The treatments evaluated are described in Table 1.

Table 1. Treatments and concentrations applied in *Capsicum chinense* var. 'Chichen Itza'.

Number	Treatment	Dose	Concentration
1	*Trichoderma* sp. (SP6)	250 g 200 L^{-1}	$1x10^{13}$ conidia mL^{-1}
2	*Trichoderma* sp. (Clombta)	250 g 200 L^{-1}	$1x10^{13}$ conidia mL^{-1}
3	*T.* SP6 + *T.* Clombta (co-inoculation)	250 g 200 L^{-1}	$1x10^{13}$ conidia mL^{-1}
4	Tri-HB® (*Trichoderma harzianum* + *Bacillus subtilis*, Abiosa®, Mexico)	500 g 200 L^{-1}	$1x10^{13}$ UFC mL^{-1}
5	Captan (Captan® 50 PH, Adama®, Mexico)	400 mL 200 L^{-1}	500 ppm

The application of the treatments was done in a foliar way with the help of a 10 L capacity garden sprinkler, then a sprinkler irrigation was carried out with potable water to lower the product to the root zone. Applications were made in the cool hours of the morning (7:00-8:00 am) at 7, 14, 21 and 28 days after the emergence of the plants.

Response variables

The incidence of the disease was evaluated as an epidemiological parameter, and the following agronomic variables were determined: height, diameter of plants, number of leaves, chlorophyll index, aerial biomass (fresh and dry), root biomass (fresh and dry) and area foliar. The variables were measured as described below. The incidence was calculated with the formula: inc.= (number of diseased plants/numbers of total plants) ∗100. Plant height was measured with a ruler (Truper®, Mexico) graduated in millimeters, every seven days after germination. Stem diameter was determined with a vernier (Truper®, Mexico) graduated in millimeters, every seven days after germination.

The number of leaves was counted manually, taking into account only the true leaves and at the end of the experiment (Aguirre-Medina and Espinosa-Moreno, 2016). The leaf area determined with the maximum length from the base of the petiole to the end of the central leaflet and the maximum width of the leaves perpendicular to the maximum length at the end of the experiment (Cabezas-Gutiérrez *et al*., 2009). The chlorophyll index was quantified with a spectrophotometer (FieldScout 1000, Spectrum Technologies, Inc., USA) with a light reflectance measurement system at 700 and 840 nm, the units of measure was the index of the relative chlorophyll content, with values ranging from 0 to 999 (Mahdavi *et al*., 2017).

The measurements were made every seven days after germination. The aerial biomass was determined by the fresh weight and dry weight of the vegetative part starting from the base of the stem using an analytical balance (OHAUS®, Mexico). For the root biomass, the fresh weight and dry weight of the root part from the base of the stem were taken into account taking into account the whole root (Tavera-Zavala *et al*., 2017). These variables were evaluated at the end of the experiment.

Experimental design and data analysis

The experiment was established under a completely randomized design, with five treatments and two replications each. Each replica consisted of a 200 cavity tray. 30 plants were measured per tray as an experimental unit (n= 60). Only for the variable of incidence of the Damping off, 100 plants were evaluated per replication (n= 200). The variables evaluated were analyzed; through an analysis of variance (Andeva), when finding a significant difference, a multiple range comparison was made using the statistic of the minimum significant difference (DMS) with an α= 0.05. Given that all the data presented normality according to the Levene test (p> 0.05), there was no need to use transformations. All analyzes were performed with the StatGraphics Plus® and Prism® software.

Results

Incidence of Damping off

Seven days after germination (application of treatments) there was no significant difference (p> 0.05) regarding the incidence of Damping off among the treatments evaluated, only the application of Captan® presented a diseased plant (Figure 1).

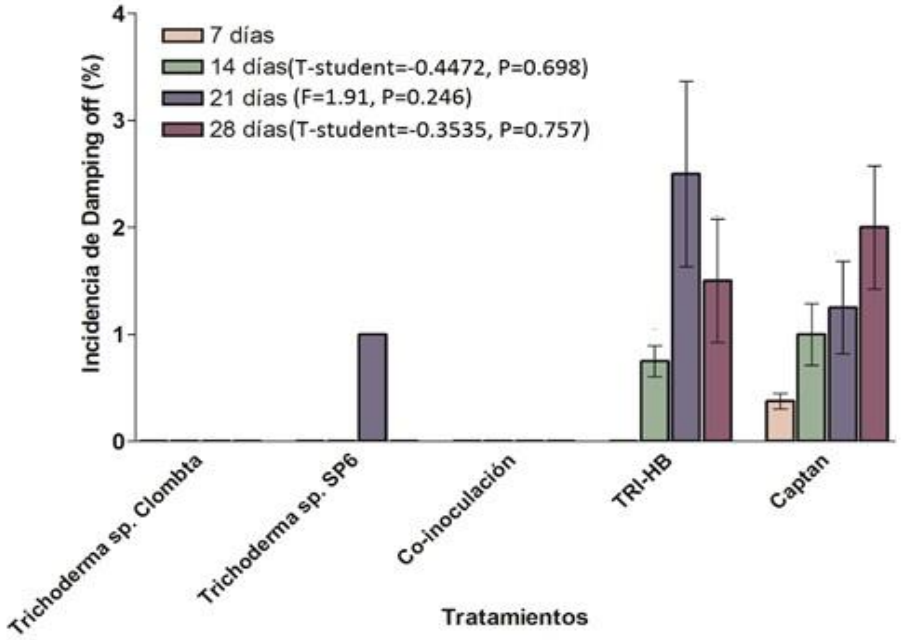

Figure 1. Incidence of Damping off in *Capsicum chinense* var. 'Chichen Itza' inoculated with different strains of *Trichoderma* sp. and with application of Captan® (n= 200).

In the second measurement (14 days), Andeva revealed that the Tri-HB® and Captan® treatments showed symptoms of Damping off with two (1%) and three diseased plants (1.5%), respectively. At 21 days, the incidence of said disease showed no significant difference (p> 0.05), since *Trichoderma* sp. SP6, Captan® and Tri-HB® presented two (1%), three (1.5%) and four (2%) diseased plants, respectively.

Finally, in the last measurement (28 days) there was no significant difference (p> 0.05), since only the treatments of Captan® and Tri-HB® presented four (2%) and three (1.5%) diseased plants each.

During the entire study period, in *Trichoderma* sp. Clombta and the co-inoculation of *Trichoderma* sp. Clombta and *Trichoderma* sp. SP6, did not register plants with symptoms of Damping off (Figure 1).

Plant height

The Andeva indicated that seven days after germination (application of the treatments) there was a significant difference (F= 8.65, p= 0.00001) in plant height due to the application of the treatments. The co-inoculation allowed greater height of plant (5.8 cm) in comparison with the rest of the treatments, where the values ranged between 5.2 to 5.5 cm (Table 2).

In the second measurement (14 days), the analysis indicated that plants treated with *Trichoderma* sp. Clombta, *Trichoderma* sp. SP6 and co-inoculation had higher height (F= 37.1, p= 0.00001) with 6.3, 6.3 and 6.4 cm, respectively; while treatments with lower height were the Tri-HB® (5.4 cm) and Captan® (5.3 cm, Table 2). In the penultimate (21 days) and last measurement (28 days),

the application of *Trichoderma* sp. Clombta increased significantly (21 days: F= 90.23, *p*= 0.00001, 28 days: F= 165.91, *p*= 0.00001) the height of *C. chinense* plants compared to the rest of the treatments, with average values of 10 and 11 cm at 21 and 28 days, respectively.

Table 2. Height of plants (cm) of *Capsicum chinense* var. 'Chichen Itza' inoculated with different strains of *Trichoderma* sp. and with the application of Captan®.

Treatment	Days after germination			
	7	14	21	28
Trichoderma sp. (Clombta)	5.2 ±0.05 c	6.3 ±0.09 a	10 ±0.25 a	11 ±0.21 a
Trichoderma sp. (SP6)	5.5 ±0.08 b	6.3 ±0.07 a	8.3 ±0.06 b	9.8 ±0.09 b
T. SP6 + *T.* Clombta	5.8 ±0.07 a	6.4 ±0.06 a	7.3 ±0.07 c	7.9 ±0.07 c
Tri-HB®	5.2 ±0.07 c	5.4 ±0.07 b	6.9 ±0.08 d	7.4 ±0.06 d
Captan® (control)	5.2 ±0.14 c	5.3 ±0.12 b	6.7 ±0.15 d	7.6 ±0.1 cd
CV (%)	14.9	12.7	13.7	10.6
F	8.65	37.1	90.23	165.91
p	0.00001	0.00001	0.00001	0.00001

Means (± standard error) with different literal in one column are statistically different from each other (DMS, $p \leq 0.05$, n= 60); CV= coefficient of variation.

In contrast, the Tri-HB® and Captan® treatments showed the lowest height values of *C. chinense* plants in the last two measurements, with 6.7 to 6.9 and 7.4 to 7.6 cm at 21 and 28 days, respectively (Table 2).

Stem diameter

Seven days after germination (application of the treatments) there was a significant difference (F= 107.28, *p*= 0.00001) in the stem diameter of *C. chinense* plants. The co-inoculation allowed a greater diameter of stem with 1.4 mm in comparison with the plants inoculated with the other treatments, which ranged between 1.2 and 1.3 mm (Table 3). in the last three measurements, oscillating between 1.4 mm and 1.9 mm stem diameter (Table 3).

Table 3. Stem diameter (mm) of *Capsicum chinense* var. 'Chichen Itza' inoculated with different strains of *Trichoderma* sp. and Captan® application.

Treatment	Days after germination			
	7	14	21	28
Trichoderma sp. (Clombta)	1.3 ±0.03 b	1.8 ±0.03 a	2.4 ±0.05 a	2.6 ±0.04 a
Trichoderma sp. (SP6)	1.2 ±0.03 c	1.5 ±0.03 b	1.8 ±0.04 b	2.3 ±0.06 b
T. SP6 + *T.* Clombta	1.4 ±0.02 a	1.6 ±0.03 b	1.8 ±0.03 b	2 ±0.04 c
Tri-HB®	1.3 ±0.03 b	1.4 ±0.04 c	1.6 ±0.02 c	1.9 ±0.04 cd
Captan® (control)	1.2 ±0.02 c	1.3 ±0.03 c	1.4 ±0.02 d	1.8 ±0.04 d
CV (%)	9.2	10.27	11.25	12.33
F	107.28	25.17	107.07	41.04
p	0.00001	0.00001	0.00001	0.00001

Means (± standard error) with different literal in one column are statistically different from each other (DMS, $p \leq 0.05$, n= 60); CV= coefficient of variation.

In the following three measurements (14, 21 and 28 days), the analysis showed that the application of *Trichoderma* sp. Clombta increased significantly (14 days: F= 25.17, *p*= 0.00001, 21 days: F= 107.07, *p*= 0.00001 and 28 days: F= 41.04, *p*= 0.00001) the stem diameter of *C. chinense* plants compared to the rest of the treatments, with average values of 1.8, 2.4 and 2.6 mm at 14, 21 and 28 days, respectively. In contrast, plants treated with Tri-HB® and Captan® showed the lowest values.

Number of leaves and foliar area

The results indicated that there was a significant difference (F= 4.6, *p*= 0.0023) in the number of leaves in *C. chinense* plants due to the application of the treatments. *Trichoderma* sp. Clombta significantly increased the number of true leaves of *C. chinense* plants, showing a value of 9.1 leaves per plant. Plants treated with co-inoculation, Tri-HB® and Captan® showed the lowest number of leaves with 8.3, 8.3 and 8.5, respectively (Table 4). In the same way for the foliar area, *Trichoderma* sp. Clombta favored a higher value compared to plants inoculated with the other treatments, with an average per leaf of 10.2 cm² (F= 60.17, *p*= 0.00001), while treatments that allowed lower leaf area were Captan® and Tri-HB® with 5.8 and 6.4 cm², respectively (Table 4).

Table 4. Number of leaves and foliar area (cm²) of *Capsicum chinense* var. 'Chichen Itza' inoculated with different strains of *Trichoderma* sp. and with the application of Captan®.

Treatment	Num. of leaves	Leaf area
Trichoderma sp. (Clombta)	9.1 ±0.2 a	10.2 ±0.2 a
Trichoderma sp. (SP6)	8.9 ±0.2 bc	7.3 ±0.2 c
T. SP6 + *T.* Clombta	8.3 ±0.1 d	8.4 ±0.2 b
Tri-HB®	8.3 ±0.2 d	6.4 ±0.2 d
Captan® (control)	8.5 ±0.2 cd	5.8 ±0.2 d
CV (%)	1.42	2.22
F	4.6	60.17
p	0.0023	0.00001

Means (± standard error) with different literal in one column are statistically different from each other (DMS, $p \leq 0.05$, n= 60); CV= coefficient of variation.

Relative chlorophyll index

In the first evaluation (seven days after germination) a significant difference was found (F= 9.71, *p*= 0.00001) in the chlorophyll index. Plants inoculated with *Trichoderma* sp. Clombta showed higher chlorophyll index with an average value of 162.5 (on a scale of 0-999), this value was higher in comparison with the rest of the plants inoculated with the other treatments, which ranged from 100 to 123.7 (Table 5). In the second measurement (14 days) no significant differences were found (*F*= 1.93, *p*= 0.1215), the values oscillated between 139.6 (Captan®) and 155.7 (*Trichoderma* sp. Clombta) (Table 5).

At 21 days, the Andeva indicated that the application of *Trichoderma* sp. Clombta again increased the chlorophyll index (F= 90.23, *p*= 0.00001) in *C. chinense* plants. While in the last measurement (28 days), the application of *Trichoderma* sp. Clombta (209.9) and *Trichoderma* sp. SP6 (204.0) significantly increased (F= 165.91, *p*= 0.00001) the chlorophyll index of the plants compared to the rest of the treatments, where the average values ranged between 149.9 and 153.9. In contrast, plants treated with Captan® and Tri-HB® showed the lowest chlorophyll indexes in the last two measurements, with values between 120 to 149.9 and 151.3 to 153.9 at 21 and 28 days, respectively (Table 5).

Table 5. Relative chlorophyll index of *Capsicum chinense* var. 'Chichen Itza' (on a scale of 0 to 999) inoculated with different strains of *Trichoderma* sp. and with the application of Captan®.

Treatment	Days after germination			
	7	14	21	28
Trichoderma (Clombta)	162.5 ±6.8 a	155.7 ±4.5 a	206.5 ±5.1 a	209.9 ±4.3 a
Trichoderma (SP6)	123.7 ±6 b	151.6 ±5.8 b	174.9 ±9 b	204 ±5.9 a
T. SP6 + *T.* Clombta	120.8 ±7.5 bc	152.2 ±8 b	154.1 ±5.2 c	153.4 ±9.9 b
Tri-HB®	100 ±10.6 c	134.1 ±4.2 c	151.3 ±4.3 c	153.9 ±6.5 b
Captan® (control)	113 ±5.7 bc	139.6 ±9.2 c	120 ±4.4 d	149.9 ±8.9 b
CV (%)	4.15	2.87	2.48	2.03
F	9.71	1.93	29.09	14.21
p	0.00001	0.1215	0.00001	0.00001

Means (± standard error) with different literals in a column are statistically different from each other (DMS, $p \leq 0.05$, n= 10); CV= coefficient of variation.

Aerial and radical biomass (fresh and dry)

At the end of the experiment (28 days) a significant difference was found in fresh aerial biomass (F= 25.87, *p*= 0.00001) and dry biomass (F= 40.14, *p*= 0.00001). The fresh aerial biomass in plants inoculated with *Trichoderma* sp. Clombta showed greater weight in comparison with the plants inoculated with the other treatments, with 0.8 g plant^{-1}, while the treatments with the least weight were Co-inoculation and Tri-HB® with 0.5 g plant^{-1} in both cases (Table 6). For the dry aerial biomass, in the same way, plants inoculated with *Trichoderma* sp. Clombta showed greater weight (0.13 g plant^{-1}) compared to the other treatments, the lowest dry aerial biomass was found in the co-inoculation and Tri-HB® with 0.07 and 0.05 g plant^{-1}, respectively (Table 6).

For fresh root biomass, Andeva indicated a significant difference (*F*= 3.26, *p*= 0.0164) between treatments. Plants inoculated with *Trichoderma* sp. Clombta (0.12 g plant^{-1}) and *Trichoderma* sp. SP6 (0.11 g plant^{-1}) obtained greater weight in comparison with plants inoculated with Tri-HB® (0.05 g plant^{-1}); however, both treatments were statistically equal to the non-inoculated plants treated with Captan® (0.09 g plant^{-1}). Finally, for the dry root biomass, a significant difference was found (F= 25.47, *p*= 0.00001) between the treatments, plants inoculated with *Trichoderma* sp.

Clombta (0.04 g plant^{-1}) showed a higher dry root weight compared to the rest of the treatments; on the contrary, the treatments with the lowest weight were Captan® and Tri-HB® with 0.01 and 0.02 g plant^{-1}, respectively (Table 6).

Table 6. Aerial and radical biomass (fresh and dry in g) of *Capsicum chinense* var. 'Chichen Itza' inoculated with two strains of *Trichoderma* sp. and with the application of Captan®.

Treatment	Fresh biomass		Dry biomass	
	Aerial	Radicular	Aerial	Radicular
Trichoderma (Clombta)	0.8 ±0.02 a	0.12 ±0.01 a	0.13 ±0.003 a	0.04 ±0.001 a
Trichoderma (SP6)	0.6 ±0.01 b	0.11 ±0.03 a	0.09 ±0.003 b	0.02 ±0.001 bc
T. SP6 + *T.* Clombta	0.5 ±0.02 c	0.08 ±0.01 ab	0.07 ±0.003 cd	0.03 ±0.001 b
Tri-HB®	0.5 ±0.03 c	0.05 ±0.01 b	0.05 ±0.003 d	0.01 ±0.0006 c
Captan® (control)	0.7 ±0.04 b	0.09 ±0.02 a	0.08 ±0.006 c	0.02 ±0.002 c
CV (%)	2.43	8.56	3.08	3.22
F	25.87	3.26	40.14	25.47
p	0.00001	0.0164	0.00001	0.00001

Means (± standard error) with different literals in a column are statistically different from each other (DMS, $p \leq 0.05$, n= 10); CV= coefficient of variation.

Discussion

In the present study it was found that the evaluated treatments showed different abilities to avoid the incidence of Damping off. The application of the *Trichoderma* Clombta strain and its co-inoculation with *Trichoderma* sp. SP6 did not allow the appearance of symptoms and death of *C. chinense* plants by Damping off. However, the incidence of the disease was low in the chemical control (5% in Captan®), despite this, the strain of *Trichoderma* sp. they provided protection to the plants.

It is widely documented that *Trichoderma* can inhibit the growth of different phytopathogenic microorganisms. The inhibitory effect of *Trichoderma* strains in phytopathogenic fungi can be associated with the production of enzymes that act against their cell wall (Guédez *et al*., 2012). In the literature, benefits of biofertilization have been reported in *C. chinense* Jacq. For example, Candelero *et al*. (2015) reported increases of 55.57 and 47.62% in the height of *C. chinense* plants inoculated with *Trichoderma* sp. Th05-02 and *T. virens*. In the same way, *T. harzianum* was able to increase the length and volume of *C. chinense* root, 41.57 and 55%, respectively.

In the cultivation of *Capsicum annuum*, Guigón-López and González-González (2004) reported the ability of six strains of *Trichoderma* to control the causal agent of Damping off (*Phytophthora capsici*) *in vitro*. Strains TS01, TC74 and TvB from *Trichoderma* showed higher mycoparasitic activity *in vitro*. While in greenhouses, strains TC74 and TS01 at concentrations of 1.3×10^7 conidia mL^{-1}, reduced the growth rate and the severity of wilt of *C. annuum* plants caused by *P. capsici*. As in this study, the strains evaluated by Guigón-López and González-González (2004) increased the height (30%), number of leaves (20%), leaf area (30%) and aerial biomass (60%) and radical (38) of plants of *C. annuuum*.

In another study, Mehetre and Kale (2011) reported the ability of *Trichoderma harzianum* to parasitize *Pythium aphanidermatum* under *in vitro* conditions in dual cultures, in addition to experiments in pots, *T. harzianum* inhibited 83.16% the progress of damping off caused by *P. aphanidermatum* in plants of *C. annuum*. Likewise, Cárdenas *et al*. (2005) compared the efficiency of the fungus *Trichoderma* spp. against *Fusarium oxysporum*, the causative agent of Damping off in papaya (*C. papaya* L.).

The bioassays revealed that the application of *Trichoderma* sp. at a concentration of 1×10^6 conidia mL^{-1} it controlled the disease. Reyes *et al*. (2012) suggest that *Trichoderma* sp. it acts as an agent of biological control and that its action mechanisms are based on the activation of multiple metabolic pathways that promote competition for nutrients and space, the modification of environmental conditions, the stimulation of growth and the activation of defensive mechanisms of plants for antibiosis and mycoparasitism.

In addition to the displacement and control of deleterious microflora of the root, different species of *Trichoderma* increase the root growth and development of the plants. This affirmation was confirmed in the plants of *C. chinense*, since the application of *Trichoderma* sp. Clombta increased the height, diameter and radical biomass of the same. Each strain and species of *Trichoderma* has different ability to promote plant growth, therefore it was observed that *Trichoderma* sp. Clombta was superior to *Trichoderma* SP6 in the three plant growth variables. Possibly, both strains are different species and therefore have different biochemical abilities (eg production of auxins and organic acids and solubilization of inorganic phosphates) that allow one strain to be a better promoter of growth compared to another (Ortuño *et al*., 2013).

Likewise, inter-specific and intra-specific fungal interactions play an important role when developing biological products or inoculations with more than one strain or species (Ortuño *et al*., 2013; Moo-Koh *et al*., 2018). The present work is the preamble for future research in vegetables with the strain of *Trichoderma* sp. Clombta, for example, its evaluation in the field or greenhouse to know its effectiveness in the inhibition of phytopathogenic fungi in the stage of transplantation and production of *C. chinense*. It is necessary to identify at the species level this strain and to know what the particular mechanisms are involved in the processes of promotion of plant growth of this strain.

Conclusions

The weekly application of *Trichoderma* sp. Clombat at a concentration of 1×10^{13} conidia mL^{-1} gave indications to reduce the incidence of the causative agents of 'Damping off' in *C. chinense* Var. 'Chichen Itza' and was able to promote vegetative growth by increasing the height, stem diameter, aerial and root biomass, number of leaves and chlorophyll index of the inoculated plants. *Trichoderma* sp. Clombta is a good candidate to be studied as biofungicide and biofertilizer in plantations and nurseries of habanero pepper under the climatic conditions of Tecoman, Colima, Mexico.

Acknowledgments

The authors are grateful for the funding granted by the SEP-PRODEP program for the development of this study.

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