British Biotechnology Journal
4(4): 387-399, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antibiosis *In vitro* of *Trichoderma* Strains Metabolic Extract on Mycelial Growth and Reproductive Capacity of *Fusarium oxysporum* Isolated from Pepper Plants (*Capsicum annuum L.*)

Hernández Castillo Francisco Daniel[1*], Flores Flores Wilfredo[1], Castillo Reyes Francisco[2], Gallegos Morales Gabriel[1] and Castro Del Ángel Epifanio[1]

[1]Department of Agricultural Parasitology Antonio Narro Agrarian Autonomous University Buenavista, Saltillo, México.
[2]Experimental Station-INIFAP Saltillo, Zacatecas-Saltillo Road, Colonia Hacienda de Buenavista, Saltillo, Coahuila, México.

Authors' contributions

This work was carried out in collaboration between all authors. Author HCFD designed the study, wrote the protocol and wrote the first draft of the manuscript. Author FFW performed the statistical analysis. Author CRF managed the analyses of the study. Authors GMG and CDAE managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

Received 9th October 2013
Accepted 4th December 2013
Published 26th March 2014

ABSTRACT

Aims: To inhibit the mycelia growth inhibition and reproductive capacity of important phyto-pathogen fungus: *Fusarium oxysporum* by cell-extracts from submerged cultures of *Trichoderma.*
Study Design: A complete randomized experimental design with factorial fix was used.
Place and Duration of Study: Laboratory of plant-pathology, Department of Agricultural Parasitology, Universidad Autónoma Agraria Antonio Narro (UAAAN), Mexico, between August 2012 and March 2012.
Methodology: Metabolic extract of *Trichoderma asperellum* produced in liquid medium and *Trichoderma* dual cultures on *F. oxysporum* isolated infected plant pepper was

**Corresponding author: Email: fdanielhc@hotmail.com;*

evaluated; both strains were subsequently activated in PDA and tested in dual culture and poisoned culture with *Trichoderma* strains and metabolic extracts against *F. oxysporum* to determine their growth inhibition potential.
Results: Strains *Trichoderma* were able to inhibit the growth of the plant pathogen, demonstrating to be an attractive alternative for biological control assays. Similar results were obtained with metabolic extracts, where the inhibition was affected up to 29%, the conidiogenesis by 30% and spore viability by 60% at the highest concentration tested.
Conclusions: Metabolites produced have the power to reduce the reproductive capacity of *F. oxysporum,* decreasing sporulation and inhibit the germination of conidia, and this is extremely important, as reducing the quantity and viability of conidia, it is reducing the secondary inoculum of the pathogen.

Keywords: Metabolic extract; Trichoderma; mycelial inhibition; conidiogenesis inhibition.

1. INTRODUCTION

Root rots are the main problem phyto-pathologic in the pepper (*Capsicum annuum* L.) crop in North-Central region of Mexico [1,2,3], where are reported between 40 to 70% mortality in the initial population of plants [4]. In field studies conducted during 2000 in the states of Aguascalientes and Zacatecas, was detected the fungi presence *Rhizoctonia* spp., *Fusarium spp*., *Phytophthora* spp., *Verticillium* spp. and *Pythium* spp. on roots of infected plants with a frequency of isolation of 34.1, 31.6, 14.1, 2.5 and 1.0% respectively [1]. The symptoms associated in chilli root rot are defoliation, discoloration and curling foliage, damage to reproductive structures, advanced and irregular ripening, root rot, and other symptoms such as depressed knots [1]. The mortality in experimental plots has been estimated between 26 and 40% for mirasolchilli varieties [5]. In favorable conditions the pathogens involved in wilt, cause devastating economic losses by affecting 60 to 100% of the cultivated area. In Mexico there are regions that have lost up to 80% and states such as Aguascalientes and San Luis Potosi the chilli sowing area has fallen by 60% because of wilting [3,6]. The disease results in a reduction of 50% in performance without application of fungicides [7]. The control method used for management of this disease is the chemical, and but its use is questioned by many because the constant use of some products, can cause fungus resistance, and they can also cause damage to human health and environment. Currently the system production is focused on sustainable agriculture where natural resources are optimized, uses least amount of chemicals and increase using microbial products, this falls in the context of organic agriculture, where the antagonistic microorganisms play an important role in the production scheme. There is an important group of fungi and bacteria that have antagonistic effects on other microorganisms; this action can be harnessed as a form of biological control of plant pathogens [8]. Within the group of antagonistic fungi is the genus *Trichoderma*, the species of this fungus are the most studied and used throughout the world as agents of biological control of plant pathogens. The successful antagonistic capacity of *Trichoderma* spp. on fungal plant pathogens is given by their different action modes, such as mycoparasitism, excretion of lytic enzymes [9], competition by space and nutrients [10], promotes growth and induces systemic resistance [11,12], inhibits enzymes produced by the pathogen necessary to penetrate the plant [11] and antibiosis by the producing volatile and nonvolatile secondary metabolites that impeding growth of the pathogen [13]. *Trichoderma* spp. as a natural antagonist of biological control of plant pathogens, shows a wide range of plant pathogenic species affected and within these are the economically important plant pathogenic fungi, such as *Fusarium oxysporum* f. sp*. cubense, Fusarium roseum, Fusarium subglutinans, Fusarium oxysporum* f. sp*. passiflorae, Fusarium oxysporum* f. sp*. dianthi,*

Botrytis cinerea, Rhizoctonia solani, Sclerotium rolfsii, Sclerotinia spp*., Pythium* spp*., Phytophthora* spp., *Alternaria* spp*.,* among others [14,15,16,17]. The aim of this study was to determine the effect *In vitro* from antagonistic capacity and activity from metabolic extract of *Trichoderma asperellum* on inhibition of mycelia growth and reproductive capacity of *Fusarium oxysporum.*

2. MATERIALS AND METHODS

2.1 Isolation and Identification of *Fusarium oxysporum*

The pathogen *F. oxysporum* was obtained from plants of pepper (*Capsicum annum* L) growth an experimental plot associated with wilt symptoms as mentioned by Velasquez [1], and located in an Area from Universidad Autónoma Agraria Antonio Narro (UAAAN). The isolation was carried out in the laboratory of plant pathology, from the Department of Parasitology of the Universidad Autónoma Agraria Antonio Narro, according to the methodology mentioned by Agrios [18]. The fungus isolated was purified according to monosporic culture technique and characterized phenotypically according to the morphological characteristics described by Leslie et al, [19]

2.2 Antagonistic Fungal Strains

Two *Trichoderma* strains coded how T2-11 and T2-31 from the species of *Trichoderma* sp. and *T. asperellum* were taken from fungal collection in Parasitology Department UAAAN. These strains were selected due their study because they showed great antagonistic capacity against the soil fungus *Phytophthora capsici* in dual cultures, filtered toxic and volatile compounds. Such strains were characterized and evaluated previously by Osorio et al., [20].These antagonist strains were identified based on 18S ribosomal gene sequencing. Therefore *Trichoderma* as *F. oxysporum* strains were increased in Petri dishes with culture medium Potato-Dextrose-Agar (PDA), placed in the center an explant 5 mm in diameter. The boxes were incubated in the dark at a temperature of 28±2ºC.

2.3 Antagonism of *Trichoderma* in Dual culture on *Fusarium oxysporum*

The antagonistic capacity was evaluated using the technique proposal by Cherif and Benhamou [21], studying quantitatively the area of intersection or overlap between the antagonist and the pathogen fungus. Petri dishes with PDA medium was placed an disc of 5 mm diameter with *Fusarium oxysporum* mycelia of ten days of age, the fungus was grown without the antagonist by three days in this because preliminary tests showed slow growth. After three days, was placed at the opposite end a disc of 5 mm in diameter (PDA+ mycelia) of *Trichoderma* with seven days old, immediately incubated at 28±2ºC. Using a vernier was measured the radius of the intersection zone and / or overlapping each of the treatments. The strains were compared with respect to antagonistic ability, according to the scale proposed by Bell et al. [22], the number of days to contact between the antagonist and the plant pathogen, and according to the percentage inhibition of radial growth (PICR) of the strains *F. oxysporum*, which is determined with the formula proposal by Ezziyyani et al. [23]. PICR=R1-R2 / R1x100. Where R1 is the growth of the pathogen without antagonist (Control) and R2 is the growth of the pathogen in confrontation with the antagonist. We used a completely randomized design with four replications. Data were analyzed with the statistical program SAS System Version 9.1 and media differences was obtained applied the Tukey test.

2.4 Effect of Metabolic Extract of *Trichoderma asperellum* T2-31 on the Mycelial Growth Inhibition of *Fusarium oxysporum*

The metabolic extract was obtained in submerged culture; we prepared liquid media used 200gr.L^{-1} freshpotato infusions shelled, 20 gr.L^{-1} sucrose and 10 gr.$L^{-1}$of malt. From this broth 100 ml was placed in Erlenmeyer flask 250 ml and sterilized at 120ºC for 15 minutes in an autoclave, then in each flask were placed three *T. asperellum* T2-31 explants of 5 mm diameter and incubated in constant shaking at 150 rpm at 25±2ºC for 6 days. After time, the contents of the flasks were filtered using Watman No. 1 paper and the filtrate was placed in centrifuge tubes of 50 ml and centrifuged at 4000 rpm, for a time of 10 minutes, the supernatant was immediately sterilized filter twice using Millipore of 0.20 microns. The extract remained sterile was refrigerated at 4ºC until use, which was prepared in five concentrations equivalent to 100, 75, 50, 25 and 0% in sterile distilled water. Each of these concentrations was placed 1000 ul before addicting the PDA culture medium, after extract and PDA mix in the petri dish were gently shaken 10 times in a circular motion and left observation for 24 hours to verify that there was no growth of the antagonist or contaminant. After standing time, was placing a disc (5 mm diameter) PDA with mycelia of *F. oxysporum* of 10 days old was placed in center Petri Dish and were incubated at 28±2ºC for 15 days. Daily was measuring mold growth with vernier, the values were transforms to mycelia growth inhibition percentage using the formula above. For each concentration were established four replicates and a control. The inhibition data were analyzed under a completely randomized design using the statistical package SAS System Version 9.1, also performed Tukey means comparison.

2.5 Effect of Metabolic Extract of *Trichoderma asperellum* T2-31 on *Fusarium oxysporum* Conidiagenesis Inhibition

Effect of the metabolic extract on conidia-genesis *F. oxysporum* was determining count the spore production by the fungus under growth conditions with extract at different concentration after 15 days of growth. Spores obtaining were made by the harvest of these by placing 10 ml of distilled water in each petri dish and scraping the mycelium gently with a glass rod, the conidial suspension was placed in a threaded test tube and stirred a vortex for 10 seconds. With a micropipette, we took a small amount of the mixture, placed in a Neubauer chamber and spores were counted. Results are expressed in number of conidia per milliliter. The spore production data were analyzed under a completely randomized design using the statistical package SAS System Version 9.1, also performed Tukey means comparison.

2.6 Effect of Metabolic Extract of *Trichoderma asperellum* T2-31 on Conidial Viability of *Fusarium oxysporum*

The effect of the extract on the germination of conidia of *F. oxysporum*, was determined using the culture medium poisoned technique based on the use water-agar (AA) at 2% and extract concentration at 100, 75, 50, 25 and 0%. After the Petri dishes were left to stand for 24 hrs, and time elapsed, 1 ml of a conidial suspension of $1x10^6$ conidia/ml of a culture vigorous *F. oxysporum* of ten days age was added, the suspension was dispersed homogeneously in the petri dish with a glass rod, then, the dishes were incubated at 28±2ºC in the dark. The germination percentage was determined at 24 hours using a compound microscope at 40X magnification. It was counted ten conidia at randomly for each repetitionand data was recorded into two categories: Normal Germination (germ tube length

similar in traits using metabolic extract and not metabolic extract) and Not germinated. The Data were analyzed with the statistical program SAS System Version 9.1., under completely randomized design with four replications, also performed Tukey means comparison.

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of *Fusarium oxysporum*

The morphological characteristics on PDA medium observed were cottony mycelium and variable color according to age, from a pinkish to a pale violet at 15 days. The phenotypic characteristic on inverted microscope at 40X, and it was observed that conidiophore is individual with irregular growth and without forming sporodochia and lacked macroconidia. The microconidia without septa, oval, elliptical to reniform, formed in abundance in monophyalides in bottle shaped short called "false head", this is the feature with which *F. oxysporum* differs from *F. solani* who form the microconidia in false heads on long monophyalides. The chlamydospores of intercalary or terminal position, singly or in pairs, with a circular double wall smooth appearance Fig. 1. On carnation-agar media (CLA) showed hyaline mycelium, growing very close to the surface of the medium, at 12 days it was observed on carnation leaf sporodochia. In a microscopic preparation were observed fusoid and allantois shaped macroconidia, with three to five septa, basal cell was just apical curve and the hook-shaped. It was also observed the presence of microconidia and chlamydospores, in this latter the macroconidia are formed in some cases within. This characteristic corresponds at described by Leslie et al. [19].

3.2 Antagonism of *Trichoderma* in Dual Culture on *Fusarium oxysporum*

The results showed that the two strains possess the ability to inhibit mycelia growth of *F. oxysporum*, managing to stop the growth on contact. At 14 days in confrontation, T2-31 strain radial growth inhibited in 86.5%, whereas T2-11 strain only inhibited in 69.8%. The variance analysis detected highly significant difference between the two treatments (P <.0001); the media test (Tukey 0.05) indicates that the strain of *Trichoderma* that best inhibits the mycelia growth of *F. oxysporum* is *T. asperellum*T2-31 strain Table1. These results contrast with those obtained by Ramos [19], who found differences in the percentages of overgrowth of *Trichoderma* spp. on strains of *Fusarium oxysporum* in dual cultures from 2.75% and 56.25 after 12 days of incubation, the results obtained in this work are better, since the percentage of overgrowth of the strains tested ranged between 69.8% and 86.5% at 14 days of incubation. Likewise contrast results from Guigon et al. [24] Who report that the strains of *T. asperellum* (TC74, T341 and T359) have a greater ability to inhibit the growth of *R. solani*and *B. cinerea*than *T. longibrachiatum* with values of 44 to 64% but little effect on reducing the growth of *Fusarium* spp. these results indicate that only certain strains of *Trichoderma* species are able to inhibit growth of certain pathogenic fungi. Respect at contact day between the two species of *Trichoderma* and *F. oxysporum* was filled within three days, according to the classification of Bell et al. [22], we locate the two strains on scale 2, where T2-31 and T2-11 covered two thirds of the surface of the medium Table 1 and Fig. 2.

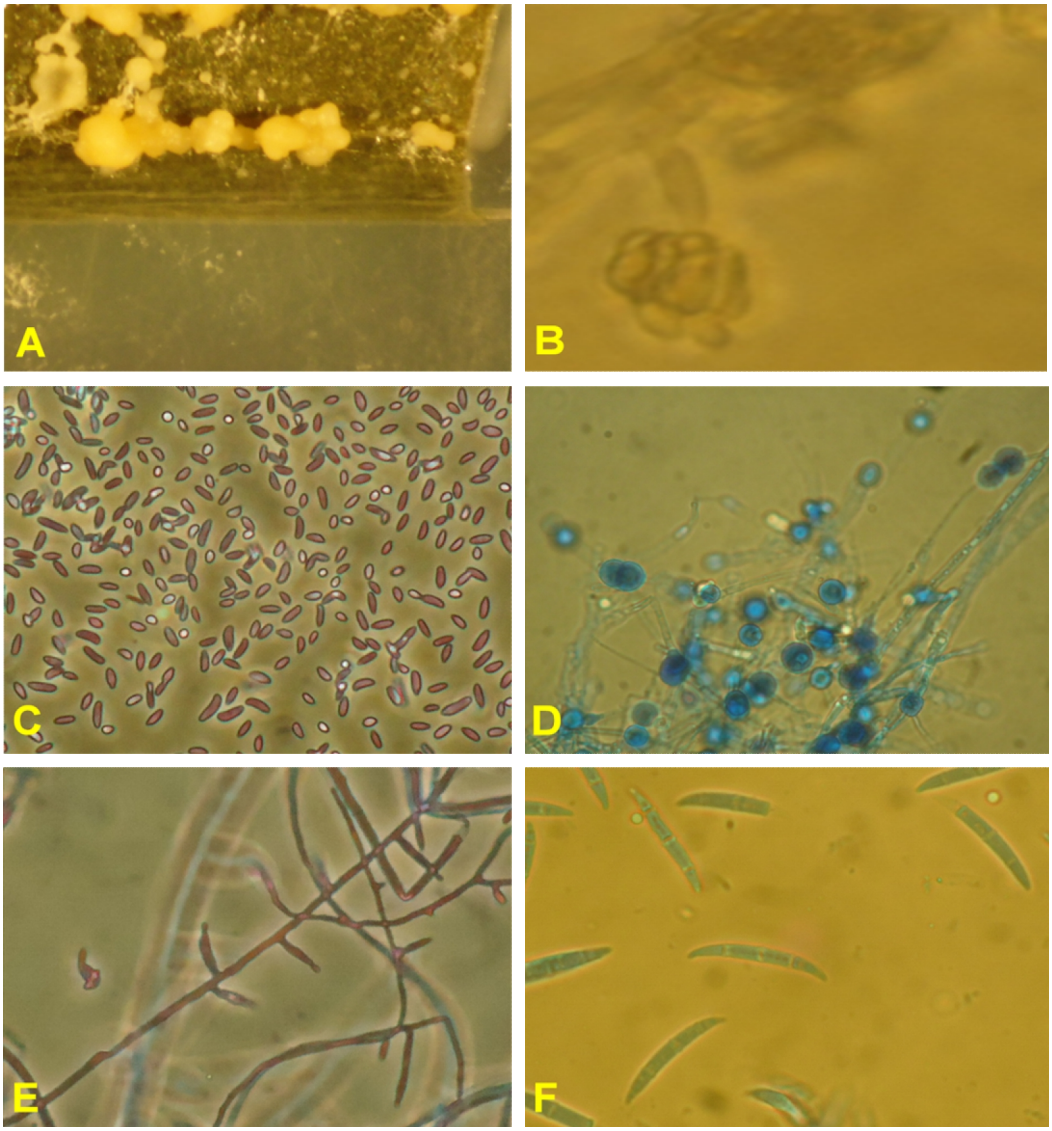

Fig. 1. Phenotypic characteristics of *Fusarium oxysporum*. A) Sporodochia in CLA, B) Microconidia in monophyalides short (false head), C) Microconidia, D) Chlamydospores, E) Monophyalids, F) Macroconidia

Table 1. Overlap (cm) of mycelia growth of strains T2-31 and T2-11 on the mycelia growth of *Fusarium oxysporum*, days to first contact between microorganisms and class antagonism

Strains	Overlap (cm)	Day to Contact	Antagonism type**
T2-11	3.4 A*	3	2
T2-31	4.1 B	3	2

**Values with the same letter are not statistically different according to Tukey mean test (0.05)*
*** Class antagonism according to Bell et al. [22]*

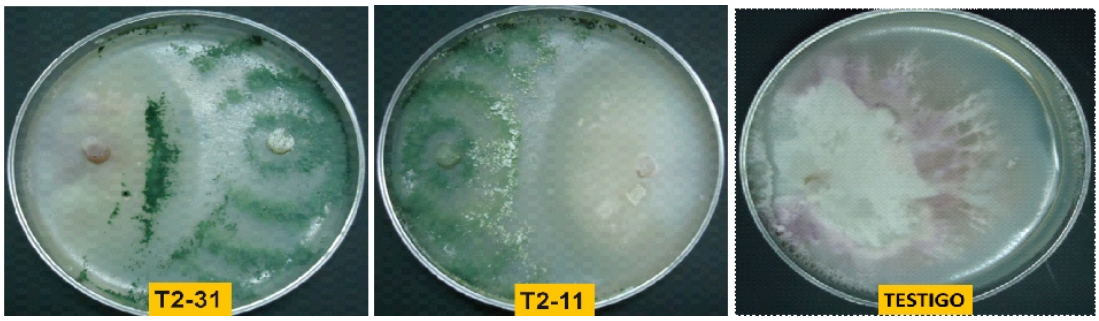

Fig. 2. Comparing the growth of *Fusarium oxysporum* in the presence of antagonists *Trichoderma asperellum* T2-31 and *Trichoderma* sp. T2-11 and *Fusarium oxysporun* without antagonist

Osorio et al. [20] reported days to contact between T2-31 strains of *T. asperellum* on *Phytophthora capsici* in dual cultures of two days and classify in Class 1 of the scale of Bell, similarly found that T2-11 strain of *Trichoderma* sp. require two days for contact with the pathogen and was classified in class 2 of Bell. The data obtained in this investigation are not consistent with those obtained by Osorio et al. [20], this may be because phytopathogen in test are different specie or the production of any compounds by the phytopathogen, Sharma et al. [25] reported that *Fusarium* spp. produce a phytotoxin call fusaric acid (FA) and*Tichodermaharzianum*S17TH is able to detoxify this toxin in an concentration of 400 ppm in a period of 7 days, but the production of chitinase is significantly reduced, this may be an explanation why *T. asperellum* T2-31 takes longer time in make contact with *F. oxysporum* that against *Phytophthora capsici.*

3.3 Effect of Metabolic Extract of *Trichoderma asperellum* T2-31 on The Mycelial Growth of *Fusarium oxysporum*

The results shown that all extract concentrations affect the mycelial growth of *F. oxysporum* (Fig. 3). The variance analysis for inhibition percentage of mycelial growth (PICM) showed a highly significant difference between different concentrations of extract tested ($P<0.0001$).

The Table 2 shows that the PICM between concentration ranged from 17.39% to 29.76%, we can see that there is a correlation between the concentration of the extract and the mycelial growth inhibition, to high extract concentration, larger is the PICM. The extract concentrations were shown to be best according to the Tukey means comparison (0.05) were 75 and 100%, however, no statistical significant difference between treatments Table 2.The concentration 75% was achieved inhibitmycelial growth in 26.08 % while 100% was 29.76%.

These results agree with those obtained by Ramos [26] who found similar percentages of inhibition, reported that extracts of *Trichoderma* spp. inhibit mycelial growth of strains of *F. oxysporum*, in a range of 0 to 43.07%, and those of Sharfuddin and Mohanka [27] who tested different concentrations (10, 25 and 50%) of extracts from *Trichoderma* spp. Against *F. oxysporum* and found that at 50% concentration, shows a strong inhibition values in the inhibition percentage between 66.7% and 83.3%, this may mean that the percentage of inhibition obtained in this study are within the range of obtained by these authors. Moreover Castro [28] in a study of antibiosis, using filtered *T. asperellum* against *F. verticillioides*,

reports that doses tested showed no difference, only able to observe that inhibit 28.86% at 500µL, while to 1000µL inhibited 28.72%, these results are very close to those obtained on work as a 1000µL (100%) was achieved inhibit growth an 29.76%. Studying the same species Ayodeji et al. [29] found that filtrate of *T. asperellum* NG-T161 reduced the mycelial growth of *F. oxysporum* at 49.7% and *T. asperellum* NG-T158 reduced it by 8.6%. Perveen and Bokhari [30] reported that filtrates of *Trichoderma* strains incubated at 25°C showed a higher growth inhibition of *F. oxysporum*. Moreover, *T. viride* (TvDPs) inhibited 25.57%, followed by TDP strain (*T. harzianum*) with 20.59% and T1 (*T. harzianum*) with 17.43%. These three studies show that the extracts or filtrates of *Trichoderma* spp. inhibit the greatly growth of *Fusarium* spp., coinciding with the results obtained in this investigation. The growth dynamics of *F. oxysporum* is affected at different levels Table 3.being the average daily slower growth at concentrations of 75 and 100% (3.68 mm / day and 3.5 mm/day respectively) that compared with the control (4.98 mm/day).

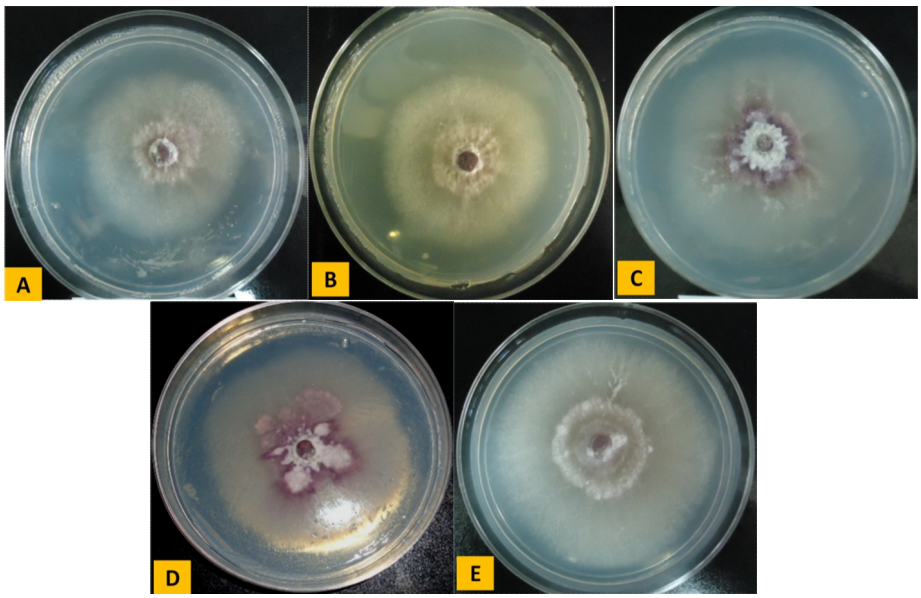

Fig. 3. Inhibition of mycelial growth of *Fusarium oxysporum* by metabolic extract from *Trichoderma asperellum* T2-31 at concentrations 100% (A) , 75% (B) , 50% (C) , 25% (D) and control (E)

Table 2. Mycelial growth Inhibition of *Fusarium oxysporum* with metabolic extracts of *Trichoderma asperellum* (T2-31) at different concentrations, in a time of 15 days incubation

Concentration	ICM (%) **
25%	17.39 B*
50%	19.06 B
75%	26.08 A
100%	29.76 A
Control	0 C

***Inhibition of mycelial growth*

** Values with the same letter are not statistically different according to Tukey mean test (0.05)*

Table 3. Effect of the concentrations of the extract on average daily growth *Fusarium oxysporum*

Concentration	Radial Growth (mm/day)
25%	4.12
50%	4.03
75%	3.68
100%	3.50
Control	4.98

3.4 Effect of Metabolic Extract of *Trichoderma asperellum* T2-31 on *Fusarium oxysporum* Conidiogenesis

The Tukey means test (0.05) showed that treatments are not significantly different Table 4.but to nevertheless it can be noted that at 100% concentration reduce the formation of conidia of $3x10^7$ to $1.2x10^7$ which represents the 59.82% in conidiogenesis reduction, while at 25% concentration was reduced only 27.87%, which is the lowest value Table 4. The extracts used in this research, are mixed hypothetically enzymes and antibiotics (uncharacterized) produced by *T. asperellum*T2-31 in liquid medium, such extracts had a negative effect on conidiogenesis in *F. oxysporum*, this results coincides with reported by Michel et al. [32], who mention that chitinase produced by *Trichoderma* spp. fail to reduce conidiation of *F. oxysporum*at 94.5%. The antibiotic 6-pentyl-a-pyrone produced by *Trichoderma* spp. Reduce conidiation of *F. oxysporum* up to 44.4% Michel et al. [15]. Subsequent research work, are focused to study the compounds that affect the development of phytopathogen *F. oxysporum*.

Table 4. Effect of extract concentration on conidiogenesis in *Fusarium oxysporum*

Concentration	Conidia/mL	Conidia-genesis Inhibition (%)
25%	$2.2 x10^7$	27.87 A*
50%	$1.5 x10^7$	51.12 A
75%	$1.5 x10^7$	49.43 A
100%	$1.2 x10^7$	59.82 A
Control	$3.0 x10^7$	0.00 B

** Values with the same letter are not statistically different according to Tukey mean test (0.05)*

3.5 Effect of Metabolic Extract of *Trichoderma asperellum* T2-31 on the Conidia Viability of *Fusarium oxysporum*

The results showed that the conidia germination is reduced gradually by the concentrations of the extract evaluated, and the percentage of germination of conidia ranged from 55 to 82.5%, with significant difference between treatments ($P<0.0004$) Table 5. The lowest percentage of germination was presented to the 100% concentration of the extract, this coincides with that reported by Ubalua and Oti [31] who found that at concentration of 100% of the filtrate of *Trichoderma viride*, there is a lower percentage of conidia germination of *Fusarium solani* (0% germination).

Table 5. Effect of extract concentration on the conidia germination of *Fusarium oxysporum*, at 24 hours

Metabolic Extract Concentration (%)	Conidia		Total Conidia evaluated	Conidia germination Inhibition (%)
	Germinated	Not Germinated		
0	38	2	40	95 A*
25	33	7	40	82.5 AB
75	31	9	40	77.5 AB
50	28	12	40	70 BC
100	22	18	40	55 C

** Values with the same letter are not statistically different according to Tukey mean test (0.05)*

At the highest concentration (100%) of the extract there are reduction in the viability of conidia by 45%, which was similar to those results reported by Michel et al, [15], likewise these authors report compound 6 pentyl--pyrone(6PAP) produced by *Trichoderma* spp. on the viability of conidia of *F. oxysporum*, this compound could reduce the viability of conidia in 45.9%. Furthermore it has been reported that chitinases and glucanases produced by *Trichoderma* spp., conidial viability inhibit of*F. Oxysporum* [32]. Aneja et al. [33] reports effects on various genera of fungi with use the species *Trichoderma harzianum*, which inhibits spore germination of *Moniliophthora roreri* in 70% and *Crinipellis pernicious* in 75%. Moreover Reshu and Mohd [34] indicate that the filtrates of *Trichoderma viride*, inhibit the conidia germination of *Alternaria brassicae* and *Alternaria brassicicola* in 71 and 69%, respectively. Thus it is demonstrated that metabolic extracts *Trichoderma* spp. inhibit the germination of spores of several fungal genera.

4. CONCLUSION

The two *Trichoderma* strains tested in possess dual cultures shown antagonistic activity against *Fusarium oxysporum*, but only *T. asperellum*T2-31 showed an increased in the antagonic effect, indicating that the strain is more prominent for inhibiting the growth of the pathogen. The T2-31 strains produced secondary metabolites in liquid fermentation, these metabolites have the power to reduce the reproductive capacity of *F. oxysporum*, reducing their sporulation and conidia germination, this is very important, as reducing the quantity and viability of conidia, it is reducing the secondary inoculum of the pathogen.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Velasquez VR, Medina MMM, Luna RJJ. Symptoms and genera of pathogens associated with root rot of chile (*Capsicum annuum* L.). In the North-Central Mexico. Rev Mex Fitopatol Spanish. 2001;19:175-181.
2. Velasquez VR, Medina MMM. The root rot of chile (*Capsicum annum* L). In north central Mexico. IEstudios basic producers Brochure No 14. Campo Experimental Calera-INIFAP-SAGARPA-CIRNE Spanish; 2003.

3. Silva RHV, Fernández PSP, Góngora CC, Macías LBC, Ávila QGD. Temporal Distribution Space Wilt Chile (*Capsicum annuum* L.) Chihuahua and Identification of the Causal Agent Phytophthora capsici Leo Rev Mex Fitopatol Spanish. 2009;27(2):134-147.
4. Velasquez VR, Medina MMM, Mena CJ. Guide to identify and manage the major parasitic diseases chili Aguascalientes and Zacatecas. Pamphlet No 20 producers. Campo Experimental Calera-INIFAP-SAGARPA-CIRNE Spanish; 2002.
5. Velasquez VR, Rincon VF, Lopez FLC. Guide to control root rot of chile in Zacatecas and Aguascalientes. Pamphlet No 25 producers. Campo Experimental Calera-INIFAP-SAGARPA-CIRNE Spanish; 2000.
6. González PE, Yañez MM,De J, Santiago SV, Montero PA. Fungal biodiversity in chile wilt and some factors involved in Tlacotepec Joseph Manzo, green, Puebla. Agrociencia Spanish. 2004;38:653-661.
7. Guillen CR, Hernández CFD, Gallegos MG, Rodriguez HR, Aguilar GCN, Padrón CE. *Bacillus spp.* as biocontrol in infested soils with *Fusarium* spp*., RhizoctoniasolaniKühn and Phytophthora capsici* Leonina and its effect on development and yield of pepper *(Capsicum annuum L.).* Rev Mex Fitopatol Spanish. 2006;23:105-113.
8. Orietta F, Larrea V. Antagonistic microorganisms for phytosanitary control. Pest Manejo Integrado Spanish. 2001;62:96-100.
9. Schirmbock M, Parrot M, Wang YL, Hayes CK, Arisan-Atac I, Scala FG, Harman E, Kubicek CP. Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular mechanisms flag involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. Journal of Applied and Environmental Microbiology. 1994;60:4364-4370.
10. Howell CR. Mechanisms employed by *Trichoderma* species in the biological monitoring of plant diseases the history and evolution of current concepts. Journal of plant disease. 2003;87:1-10.
11. Harman GE. Overview of mechanisms and uses of *Trichoderma* spp. Symposium, the nature and application of biocontrol microbes II *Trichoderma* spp. Journal of Phytopathology. 2006;96:190-194.
12. Abeysinghe S. Systemic resistance induced by *Tichoderma harzianum* RU01 against *Uromyces appendiculatus* on *Phaseolus vulgaris*. J Natn Sci Sri Lanka Foundation. 2009;3:203-207.
13. Stefanova M, Lewis A, Larrinaga L, Coronado F. Metabolic activity of strains of Trichoderma spp. for the control of phytopathogenic fungi in the soil. Light Revista Facultad Agronomía Spanish. 1999;16:509-516.
14. Infante D, Martinez B, Gonzalez N, Reyes Y. Mechanism of action of Trichoderma fungi fitopatógenos front. Rev. Protection Veg Spanish. 2009;1:14-21.
15. Michel AAC, Reyes CA, Otero SMA, Rebolledo DO, Lezama GR. Production and antibiotic activity from 6 pentyl- a -pyrone from *Trichoderma* spp. on *Fusarium* species. Journal of Phytopathology. 2004;22(1):14-21.
16. Fernandez B, Smith M. Antagonismo *In vitro* of *Trichoderma harzianum* Rifaisobre *Fusarium oxysporum* Schlecht f. sp. *Passifloraee*n maracuyá (*Passiflora edulis* Sims var. Flavicarpa) Colombian banana-growing area of the municipality. Magazine SPANISH National College of Agriculture. 2009;62:4743-4748.

17. Aquino M, Vazquez G, Reyes R. Biocontrol *In vitro* e *In vivo* of *Fusarium oxysporum* Schlecht. f. sp. *dianthi* (Prill. y Delacr.) Snyder and Hans. native antagonistic fungi the flower Near Villa Guerrero, State of Mexico. Rev Mex Fitopatol Spanish. 2007;26:127-137.
18. Agrios GN. Plant pathology. Elsiver Editorial. 2005;523:19.
19. Leslie JF, Summerell BA, Bullock S. The *Fusarium* laboratory manual. Ed Blackwell Publishing. 2006;212.
20. Osorio HE, Hernandez CFD, Gallegos MG, Rodriguez HR, Castillo RF. *In vitro* behavior of *Trichoderma* spp. against *Phythoptora capsici* Leonian. African Journal of Agricultural Research. 2011;6(19):4594-4600.
21. Cherif M, Benhamou N. Cytochemical aspects of chitin breakdown during the parasitic action of a *Trichoderma* sp. on *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Phytopatology. 1990;80:14061414.
22. Bell DK, Well HD, Markham CR. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology. 1982;73:379-382.
23. Ezziyyani M, Perez SC, Sid AA, Emilia RA, Maria E. *Trichoderma harzianum* as fungicide for biocontrol of Phytophthora capsici in pepper plants (*Capsicum annuum* L.). Annals of Biology Spanish. 2004;26:35-45.
24. Guigon LC, Guerrero PV, Vargas AF, Carvajal ME, Avila QGD, Bravo LL, Ruocco ML, Woo S, Parrot M. Molecular identification of native strains *Trichoderma* spp. *In vitro* rate of growth and antagonism against phytopathogenic fungi. Rev. Mex. Fitopatol Spanish. 2010;28:87-96.
25. Sharma V, Bhandari P, Singh B, Bhatacharya A, Shanmugan V. Fusaric acid detoxification and chitinase expression in an antagonistic *Trichoderma harzianum* S17TH, P, 18 in books of abstracts, *Trichoderma* molecular mechanisms flag and applications of biocontrol in agriculture. Technion, Haifa, Israel; 2010.
26. Ramos HMM. Antagonistic effect *In vitro* isolates *Trichoderma* spp. sobre *Fusarium oxysporum*. Tesis Licenciatura, Coahuila, Mexico Spanish. 2008;28.
27. Sharfuddin C, Mohanka R. *In vitro* antagonism of indigenous *Trichoderma* isolates against phytopathogen causing wilt of Lentil. Int J Lifesci Pharma Res. 2012;2:195-202.
28. Castro AE. Interaction of *Fusarium verticillioides* (Sacc.) Niremberg (Sin. *F. moniliforme* Sheld.) corn different materials and evaluation *In vitro* with *Trichoderma* spp. como biocontrol. Tesis Licenciatura, Coahuila, México Spanish. 2011;31.
29. Ayodeji AA, Adegboyega OC, Maria AA. Control of postharvest rots of banana fruits by conidia and culture filtrates of *Trichoderma asperellum*. Journal of Plant Protection Research. 2009;49:302-308.
30. Perveen K, Bokhari N. Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* isolated from soil of date palm field against *Fusarium oxysporum*. African Journal of Microbiology Research. 2012;6(13):3348-3353.
31. Ubalua AO, Oti E. Antagonistic properties of *Trichoderma viride* on postharvest cassava root rot pathogens. African Journal of Biotechnology. 2007;6(21):2447-2450.
32. Michel A, Otero S, Rebolledo D, Lezama G, Ochoa MME. Production and antagonistic effect of chitinases and glucanases by *Trichoderma* spp., in inhibiting of *Fusarium subglutinans* y *F. oxysporum In vitro*. Revista Chapingo Spanish. 2005;11:273-278.
33. Aneja M, Gianfagna TJ, Hebbar PK. *Trichoderma harzianum* produces nonanoic acid, an inhibitor of spore germination and mycelial growth of two cacao pathogens. Journal of Physiological and Molecular Plant Pathology. 2005;67:304-307.

34. Reshu, Mohd MK. Role of different antifungal microbial-origin bioactive compounds against *Alternaria* spp. causing leaf blight of mustard. Plant Pathology Journal. 2012;11(1):1-9.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=444&id=11&aid=4126