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In Vitro Antagonist Action of *Trichoderma* Strains Against *Sclerotinia sclerotiorum* and *Sclerotium cepivorum*

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Abstract: Problem statement: *Sclerotinia sclerotiorum* and *Sclerotium cepivorum* are soil pathogens which have generated resistance to synthetic fungicides. One biological alternative to this problem is to select novel strains of *Trichoderma* with different mechanisms of action against these plant pathogens to ensure efficient control. **Approach:** In the present research was determined the antagonism effect of Mexicans *Trichoderma* strains on *S. sclerotiorum* and *S. cepivorum in vitro*. It was used dual culture technique by determining the percentage of mycelia growth inhibition, days to contact and antagonism levels proposed by Bell. It was also determined the effect of volatile compounds and metabolites compound from *Trichoderma* produced in solutions. **Results:** The mycelia growth inhibition of *S. sclerotiorum* and *S. cepivorum* were 45-63.8 and 50.9-81.5% respectively by the effect of *Trichoderma* strains. The *T. ghanense* and *T. longibrachiatum* (T15 and T10) inhibited in higher proportion to *S. sclerotiorum*. On the other hand *T. inhamatum* and *T. asperellum* (T32 and T11) inhibited in higher proportion to *S. cepivorum*. The days to contact between the *Trichoderma* and phytopathogen species were between two to three days. The levels of antagonism according to the Bell's scale were different between the two species of phytopathogen and for *Trichoderma* strains were grouped into I and II class. The maximum inhibition effect by volatiles compound was occasioned by *T. longibrachiatum* with 31.5 (T3) and 59.2% (T10) on *S. sclerotiorum* and *S. cepivorum* respectively. Respect to metabolites, these shown high effects on phytopathogen growth, where *T. asperellum* is the more outstanding specie which produce totals mycelia growth inhibition of two phytopathogen species. **Conclusion:** *T. longibrachiatum* (T3 and T10) and *T. asperellum* (T1 and T11) were the most efficient species with the highest antagonist effects against *S. sclerotiorum* and *S. cepivorum*.

Keywords: Maximum inhibition, dual culture technique, phytopathogen species, *Sclerotinia sclerotiorum*, *Sclerotium cepivorum*, higher proportion, *Trichoderma* strains, different mechanisms

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary, is a phytopathogen with worldwide distribution, with wide host range and has been located in different soil types and environmental conditions. *Sclerotium cepivorum* Berk is a specific fungus pathogen associated to *Allium* generous, it is present in all onion and garlic production areas, symptoms in affected plants are yellowed basal leaves, foliage necrosis, damping off, rot of lower stem and roots and wilting. This pathogen may cause losses that may reach up to 100% (Adams and Ayers, 1979; Baniasadi *et al*., 2009; Beheshti *et al*., 2011). The fungus survives as sclerotia in the soil for decades and germinates in response to exudates from plant roots and if the pathogen is not properly controlled, it is incremented up large populations of sclerotia, with increased levels of the disease (Johnson and Atallah,

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2006). The control of plant pathogens in soil with some synthetic fungicides has been producing problems of fungi resistance, ecosystem imbalance by toxic effects of residues and human and animal health hazards (Johnson and Atallah, 2006). The biological control of diseases caused by soil fungi has been how considered a management alternative for their ability in reducing root rot of various crops (Baniasadi *et al*., 2009; Bell *et al*., 1982; Benhamou and Chet, 1993; Cherif and Benhamou, 1990; Cooney and Lauren, 1998; Dennis and Webster, 1971; Dubey and Suresh, 2006; Etebarian, 2006; Johnson and Atallah, 2006; Metcalf *et al*., 2004). More than 30 species of fungi and bacteria are antagonistic to *Sclerotinia* and *Sclerotium*, among these antagonist fungi are include species of the genus *Trichoderma*. This fungus is considered as one of the most attractive micro-organisms for biological control because it has different mechanisms of action against plant pathogens, these mechanisms include competition for nutrients, mycoparasitism and antibiosis by hydrolytic enzymes and metabolites also produces substances that promote plant growth (Mendez-Vilas 2010; Valencia *et al*., 2011). In several studies have determined the presence of hyphae, chlamydospores and conidia of *Trichoderma* on species of sclerotium forming pathogens including *Sclerotinia* and *Sclerotium*, there has been found that *Trichoderma* species cause destruction and lysis of sclerotia of these infectious agents, considering the *Trichoderma* efficiency on control of soil plant pathogens, the goal of this study was to select *in vitro* novel strains of *Trichoderma* by their antagonism effects against *S. sclerotiorum* and *S. cepivorum*.

MATERIALS AND METHODS

***Trichoderma* isolates:** Forty one *Trichoderma* strains isolated from soils which come from different Mexican agricultural regions. These isolates were purified by the monospore cultures technique using serial dilutions and Potato Dextrose Agar (PDA)culture medium, after that the *Trichoderma* strains were identified by 18S rDNA sequencing using the primer pair PN3 (5'-3-CGTTG GTGAACCAACCAACCAGCGGAGGATC-3') and PN10 (5'-TCCGCTTATTGATATGCTTAAG-3'). These species were identifying how: *Trichoderma harzianum* eight strains (T4, T9, T22, T23, T34, T35, T36, T37), twelve strains as *Trichoderma asperellum* (T1, T11, T16, T20, T21, T24, T25, T26, T28, T29, T30, T38), Two as *Trichoderma koningiopsis* (T31, T33), seven as *T. longibrachiatum* (T2, T3, T8, T10, T18, T40, T41), one as *T. citrinoviride* (T27), one as *T. yunnanense* (T6), two as *T. inhamatum* (T19, T32), one as *T. ghanense* (T15), three as *T. atroviride* (T14, T17, T39) and four as *Trichoderma* spp. (T5, T7, T12, T13).

Antagonistic activity: The antagonistic activity of nine species of *Trichoderma* was studied on *S. sclerotiorum* and *S. cepivorum* by dual culture technique (Cherif and Benhamou, 1990). On Petri dishes with PDA and placing equidistantly a disk (5 mm in diameter) with mycelium of the plant pathogen and on the other side of the Petri dish, a disk of mycelium of the same diameter of *Trichoderma* strains under study. The plates inoculated were incubated at 27 ± 1°C until the growth of control treatment (with only plant pathogen disk), covered the Petri dish.

The effect of *Trichoderma* strains on plant pathogens was determined by the percentage of mycelia growth inhibition in cm calculated with the follow formula: inhibition (%) = [(D1-D2) / D1] ×100, where D1 = growth of the phytopathogen in the absence of antagonist and D2 = growth of the phytopathogen in the presence of antagonist. The days of contact between plant pathogen-antagonistic and antagonistic ability of *Trichoderma* isolates according to the methodology proposed by Bell *et al*. (1982) were also determined. Bell *et al*. (1982) classified the antagonism produced by *Trichoderma* as follows: Class I *Trichoderma* overgrows completely to pathogen and covers the whole surface of the medium, Class II *Trichoderma* overgrows two-thirds of the surface of the medium and Class III *Trichoderma* and pathogen colonized each half of the surface and nobody seems to dominate the other, Class IV the pathogen colonizes the 2/3 parts of the media surface and resists invasion by *Trichoderma* and Class V the plant pathogen overgrows completely to *Trichoderma* covers an area total culture media.

Volatile compounds: The effect of volatile compounds produced by *Trichoderma* on plant pathogens mycelia growth inhibition was determined as follow: In the center of a Petri dish having only PDA medium a disk of 5 mm in diameter with active mycelia of each plant pathogens (five days old) was placed and the top of the dish was replaced with another Petri dish in which disks with mycelia of each of the 41 strains of *Trichoderma*, in this case the lid was pierced with a punch (10 mm in diameter), the Petri dishes was joined and were sealed with parafilm paper and incubated at 26 ± 1°C until each pathogen covered the Petri dish. The effect of volatile compounds was measured considering the diameter of pathogen colonies and was expressed as percentage inhibition mycelia growth (Dennis and Webster, 1971; Hernandez *et al*., 2010).

***Trichoderma* crude extracts effect on plant pathogens mycelia growth inhibition:** Plant pathogens mycelia growth inhibition by substances secreted by *Trichoderma* in liquid medium was determined as follow: liquid medium composed of an infusion of 200 g L^{-1} of potato and 20g L^{-1} of dextrose was placed in Erlenmeyer flasks of 250 mL which were inoculated with two disks of active mycelium of the 41 *Trichoderma* strains and shaken at 100 rpm for 10 days at 27°C. The supernatant was filtered with Whatman No. 1 and sterilized by Millipore membrane filtration of 0.25 μm. To determine the effect of the filtrate, the surface of the Petri dishes with PDA culture medium was inoculated with 500μL of the filtered supernatant of each *Trichoderma* strain and then in the center of each dish was placed a disk (5 mm) with active mycelium of *S. sclerotiorum* and *S. cepivorum*, then plates were incubated at 27 ± 1°C. When the control Petri dish with only fungal pathogens mycelia was covered, mycelia growth was measured in all treatments and the results were expressed as the percentage of mycelia growth inhibition.

Statistical analysis: Test for antagonism effects, production of volatile compounds and inhibition by *Trichoderma* filtrates were established under a completely randomized design with a control and four replications for each pathogen tested. Data were analyzed on SAS System version 9.0. Mean separation was tested using the Tukey Multiple Range Test. The percentage data of mycelia growth inhibition were transformed by arcsine ($\sqrt{X}$ +0.5).

RESULTS

Antagonistic activity: Significant differences ($p<0.01$) among the different *Trichoderma* strains were found for percentage of plant pathogens mycelia growth inhibition when both microorganisms were under dual cultures. The plant pathogens growth inhibition ranged from 45-63.8 and 50.9-81.5% for *S. sclerotiorum* and S. *cepivorum*, respectively (Table 1and 2), the results indicated that *T. ghanense* (T15) and *T. longibrachiatum* (T10) were statistically similar to induce the maximum inhibition of *S. sclerotiorum* (63.8%). The results obtained against *S. cepivorum* indicated that *T. inhamatum* (T32) and *T. asperellum* (T11) were statistically similar and showed the highest inhibition rates (81.5 and 81.2% respectively). The treatment with the lowest inhibition was T19 (*T. inhamatum*) for the two plant pathogens (Table 1and 2).

The days of contact between *Trichoderma* species and *S. sclerotiorum* were statistically similar ($p<0.01$). The contact between the antagonist and the plant pathogens occurs in two days (Table 2) except for the treatment T19 (*T. inhamatum*) that made contact on the third day. For *S. cepivorum* the days of contact were two to three days, in general (Table 1),

According to the Bell *et al*. (1982) classification, *Trichoderma* species were placed in Class I, II and III, with significant differences ($p<0.01$) between treatments when were confronted with the two pathogen species. *T. yunnanense* (T6) had the highest antagonistic activity against *S. sclerotiorum* to fully colonize the plant pathogen in five days, reaching the Class I, 64% of the isolates were classified as Class II presenting good antagonist potential for use as control agents. The remaining isolates were placed in Class III. After confronting *Trichoderma* species with *S. cepivorum*, it was observed that 31.7% of the *Trichoderma* strains showed good antagonist activity to be placed in Class I, the remaining 58.3% fell into class II.

Volatile compounds: The plant pathogens mycelia growth inhibition by volatile compounds of *Trichoderma* species showed significant differences ($p<0.01$). The maximum inhibition of *S. sclerotiorum* and *S. cepivorum* was obtained with volatile compounds of *T. longibrachiatum* with 28.1 (T3) and 73.8% (T10) respectively, followed by *T. harzianum* (T4 and T9) with 12.5 and 62.5% respectively. The 41 isolates showed variability in the production of volatile compounds. *S. sclerotiorum* maximum inhibition by volatile compounds was 28.1%, observed that 85.3% of isolates do not induce mycelia growth inhibition of this phytopathogen, however, the mycelia growth was poor and less dense than the control (Table 1).

Effect of crude extracts of *Trichoderma*: The effect of *Trichoderma* metabolites produced in liquid medium was statistically significant ($p<0.01$). The isolates of *T. asperellum* (T1, T16, T20, T21 and T25) caused 100% of *S. sclerotiorum* mycelia growth inhibition followed by isolates T2 and T8 of *T. longibrachiatum* with an inhibition of 86.6 and 89.4% respectively (Table 1). For *S. cepivorum* the highest mycelia growth inhibition (100%) was obtained with *T. asperellum* (T1, T11), *T. longibrachiatum* (T41), *T. atroviride* (T39) and *Trichoderma* sp. (T7) (Table 2).

Table 1: Effect of isolates of *Trichoderma* spp. in inhibiting the mycelia growth of *Sclerotinia sclerotiorum* in dual culture, production of volatile compounds and metabolites produced in liquid media.

			Inhibition (%) *Sclerotinia sclerotiorum*			
Trichoderma species	Strains	Dual cultures*	Days contact*	Scale Bell*	Volatile compounds*	Crude extracts *
T. *harzianum*	T4	60.3 abcd	2 a	2 b	12.5 b	86.9 b
	T9	54.7 cdefgh	2 a	2 b	0.0 d	16.3 fghi
	T22	55.6 cdefgh	2 a	2 b	0.0 d	0.0 j
	T23	58.2 abcdef	2 a	2 b	0.0 d	0.0 j
	T34	54.4 defgh	2 a	3 c	0.0 d	0.0 j
	T35	55.3 cdefgh	2 a	3 c	0.0 d	15.6 ghi
	T36	54.7 cdefgh	2 a	3 c	0.0 d	0.0 j
	T37	56.8 bcdefg	2 a	2 b	0.0 d	29.4 f
T. *asperellum*	T1	57.6 bcdefg	2 a	3 c	0.0 d	100 a
	T11	56.8 bcdefg	2 a	2 b	0.0 d	84.7 bc
	T16	57.9 abcdef	2 a	2 b	0.0 d	100 a
	T20	57.6 bcdefg	2 a	2 b	0.0 d	100 a
	T21	56.5 bcdefgh	2 a	2 b	0.0 d	100 a
	T24	55.3 cdefgh	2 a	2 b	0.0 d	0.0 j
	T25	52.9 fgh	2 a	3 c	0.0 d	100 a
	T26	56.2 bcdefgh	2 a	2 b	0.0 d	12.5 i
	T28	55.9 bcdefgh	2 a	2 b	0.0 d	86.3 b
	T29	55.3 cdefgh	2 a	2b	0.0 d	82.8 b
	T30	57.6 bcdefg	2 a	2 b	0.0 d	81.9 b
	T38	58.5 abcdef	2 a	2 b	0.0 d	46.3 de
T *longibrachiatum*	T31	59.4 abcde	2 a	2 b	0.0 d	0.0 j
	T33	60.6 abc	2 a	2 b	0.0 d	0.0 j
	T2	60.6 abc	2 a	3 c	0.0 d	86.6 b
	T3	61.8 ab	2 a	3 c	28.1 a	15.6 hi
	T8	57.6 bcdefg	2 a	3 c	0.0 d	89.4 b
	T10	63.8 a	2 a	2 b	0.0 d	40.0 e
	T18	54.1 efgh	2 a	3 c	6.3 c	79.1 bc
	T40	57.9 abcde	2 a	3 c	0.0 d	82.5 b
	T41	61.8 ab	2 a	3 c	0.0 d	0.0 j
T. *atroviride*	T14	58.2 abcdef	2 a	2 a	0.0 d	0.0 j
	T17	54.4 defgh	2 a	2a	0.0 d	33.4 ef
	T39	56.5 bcdefgh	2 a	2 a	0.0 d	0.0 j
T. *citrinoviride*	T27	50.6 h I	2 a	3 c	0.0 d	33.1 efg
	T6	58.8 abcdef	2 a	1 a	0.0 d	62.5 cd
	T19	45.0 I	3 b	3 b	0.0 d	33.8 ef
	T32	58.5 abcdef	2 a	2 b	0.0 d	0.0 j
T. *ghanense*	T15	63.8a	2 a	2 b	0.0 d	0.0 j
Trichoderma Spp.	T5	55.3 cdefgh	2 a	2 b	6.3 c	0.0 j
	T7	60.6 abc	2 a	2 b	0.0 d	0.0 j
	T12	51.8 gh	2 a	2 b	10.3 b	14.7 hi
	T13	60.3 abcd	2 a	2 b	0.0 d	17.8 fghi

*: Means with the same letter, in the same column, are not significantly different according Tukey range Test ($p \leq 0.05$)

Table 2: Effect of isolates of *Trichoderma* spp. in inhibiting the mycelia growth of *Sclerotium cepivorum* in dual culture, production of volatile compounds and metabolites produced in liquid media

			Sclerotinia sclerotiorum Inhibition (%)			
Trichoderma species	Strains	Dual cultures*	Days contact*	Scale Bell*	Volatile compounds*	Crude extracts *
T. *harzianum*	T4	64.1 defgh	2 a	1 a	0.0 i	83.1 cdef
	T9	76.8 abcd	3 b	1 a	62.5 ab	44.7 hij
	T22	55.6 cdefgh	2 a	2 b	27.5 bcdefgh	6.3 m
	T23	64.7 cdefg	2 a	1 a	29.7 bcdefhg	0.0 m
	T34	59.4 fgh	3 b	2 b	35.9 abcdef	45.6 hij
	T35	65.3 bcdefg	3 b	2 b	62.5 ab	20.3 kl
	T36	66.5 bcdefg	2 a	2 b	31.3 bcdefgh	0.0 m
	T37	60.3 fgh	2 a	2 b	23.8 bcdefghi	0.0 m
T. *asperellum*	T1	61.5 fgh	3 b	2 b	60.9 ab	100 a

Table 2: Continue

	T11	56.8 bcdefg	3 b	1 a	42.5 abcdef	100 a
	T16	57.9 abcdef	2 a	2 b	55.3 abc	0.0 m
	T20	57.6 bcdefg	3 b	2 b	5.3 hi	88.1 cd
	T21	56.5 bcdefgh	3b	2b	19.4 defghi	
	T24	55.3 cdefgh	3 b	2 b	37.8 abcdefg	0.0 m
	T25	52.9 fgh	3 b	3 c	35.6 abcdefg	84.7 cde
	T26	56.2 bcdefgh	3 b	2 b	36.9 abcdefg	0.0 m
	T28	55.9 bcdefgh	3 b	2 b	29.7 bcdefgh	0.0 m
	T29	55.3 cdefgh	3 b	2b	25.0 bcdefgh	64.1 fgh
	T30	57.6 bcdefg	3 b	2 b	26.6 cdefghi	52.21 hi
	T38	58.5 abcdef	3 b	1 a	28.8 bcdefgh	8.1 lm
T. koningiopsis	T31	76.5 abcde	2 a	1 a	0.0 i	7.5 lm
	T33	60.6 fgh	2 a	2 b	17.2 fghi	25.9 k
T. *longibrachiatum*	T2	61.5 fgh	2 a	2 b	28.1 bcdefgi	65.6 efgh
	T3	70.0 abcdefg	2 a	2 b	27.2 bcdefgi	3.1 m
	T8	78.5 ab	2 a	1 a	49.1 abcd	75.6 defg
	T10	77.9 abc	2 a	1 a	73.8 a	86.3 cd
	T18	59.7 fgh	2 a	2 b	7.8 ghi	55.9 gh
	T40	59.7 fgh	2 a	2 b	28.1 bcdefgh	36.8 ijk
	T41	69.7 abcdefg	2 a	2 b	0.0 i	100 a
T. *atroviride*	T14	71.5 abcdef	2 a	2 b	28.1 bcdefgh	20.3 kl
	T17	70.3 abcdefg	3 b	2 b	10.9 fghi	80.6 cdef
	T39	65.0 bcdefg	3 b	1 a	12.5 fghi	100 a
T. *citrinoviride*	T27	57.6 gh	2 a	2 b	36.9 abcdefg	0.0 m
T. *yunnanense*	T6	69.4 abcdefg	2 a	1 a	50.0 abcd	92.2 bc
T. *inhamatum*	T19	50.9 h	3 b	3 c	34.4 bcdefgh	0.0 m
	T32	81.5 a	2 a	1 a	26.9 bcdefgh	0.0 m
T. *ghanense*	T15	63.8a	2 a	2 b	59.1 ab	0.0 m
Trichoderma	T5	63.8 defgh	2 a	2 b	23.4 cdefghi	74.4 defg
Spp.	T7	68.8 abcdefg	2 a	1 a	59.1 ab	100 a
	T12	62.9 efgh	2 a	1 a	55.3 abc	6.3 m
	T13	60.3 abcd	2 a	2 b	31.6 bcdefgh	96.6 ab

*: Means with the same letter, in the same column, are not significantly different according Tukey range Test ($p \leq 0.05$)

DISCUSSION

Antagonistic activity: The ability of *Trichoderma* species to inhibit the growth of *S. sclerotiorum* and *S. cepivorum* in dual culture varies between *Trichoderma* species and even among strains of the same species. These results indicate that the observed antagonistic capacity is attributed to the antagonist potential of each strain rather than species differences. Although, Shaigan *et al*. (2008) found that among five species of this generous, *T. viride* showed greater inhibition of *Sclerotium rolfsii* Sacc. than *T. harzianum*, *T. hamatum*, *T. longibrachiatum* and *T. paraseramosum.* While, respect El-Hasan *et al.* (2007) selected too strains of *Trichoderma* with highest mean inhibition values for their control on *F. moniliforme*, the specie was identify as: *T. harzianum.*

In addition Dubey and Suresh (2006) tested 10 *Trichoderma* isolates of *T. viride*, *T. harzianum* and *T. virens* against *Fusarium oxysporum* f. sp. *ciceris* being *T. viride* and *T. harzianum* which showed the highest ability to inhibit the fungus in percentages of 61.1 and 60% respectively. In the same way, he results of this study and other research indicates that the level of antagonism by *Trichoderma* varies when this is confronted with different pathogens making necessary a specific selection of *Trichoderma* isolates for each plant pathogen.

The days to contact and antagonism levels according to Bell's scale among species of *Trichoderma* these shown rapid growth, this indicate a good level of competition, in general, the results obtained place the 41 isolates of *Trichoderma* as organisms with high antagonistic ability against *S. Sclerotiorum* and *S. cepivorum* while more short are the days to contact higher is antagonist competition for space and nutrients (Shaigan *et al*., 2008). Studies conducted by Benhamou and Chet (Benhamou and Chet, 1993) indicate that the days to contact between *T. harzianum* and *Rhizoctonia solani* occurs in two days, while Michel *et al*. (2005) reported a range of 3-6 days for to contact between native strains of *Trichoderma* spp. and *F. oxysporum* and 3-10 days for contact with *F. subglutinans*.

Volatile compounds: The production of volatile compounds was different between the nine species of *Trichoderma*. *T. longibrachiatum* was the specie that inhibited in mayor proportion the growth of *S.*

sclerotiorum and *S. cepivorum*, in this sense, Shaigan *et al*. (2008) reported the effect of volatile metabolites produced by *T. viride, T. harziamun* and T. *longibrachiatum* on *S. rolfsii* with mycelia growth inhibition of 60.8, 58.8 and 58.4% respectively. Dubey and Suresh (2006) reported the effect of volatile compounds produced by *T. hamatum, T. viride* and *T. virens* on soil pathogens affecting growth and development of the pathogens, just as happened in this bioassay.

In general the phytopathogen mycelia growth inhibition by *Trichoderma* volatile compounds was very heterogeneous, even in strains of the same species, this trend was reported by Dennis and Webster (Dennis and Webster, 1971) whom indicated that the production of metabolites by *Trichoderma* strains is variable and that a particular strain produces different metabolites in different stages of development depending on growing conditions. By example, Cooney and Lauren (Cooney and Lauren, 1998) mentioned that *T. harzianum* produces higher levels of 6-pentyl-α-pyrone (6PAP) in response to specific pathogens, which could be due to direct interaction of recognition and response of *T. harzianum* to presence pathogen, considering the possibility that some pathogens can produce extracellular enzymes, proteins or metabolites that lead *Trichoderma* to produce high levels of volatile metabolites.

The effect of *Trichoderma* volatile compounds is more noticeable against *S. cepivorum* than on *S. sclerotiorum,* in this latter phytopathogen only *T. longibrachiatum* (T3) and T. *harzianum* (T4) presented an effect, this indicates that each plant pathogen responds differently to volatile compounds, which is based on the results of Cooney and Lauren (1998) and Pezet *et al*. (1999) whom indicated that the levels of 6PAP produced by *T. harzianum* was increased from 300 to 700% in the presence of *B. cinerea* and to a lesser extent with *F. culmorum* and *R. solani* to unlike *T. koningii* with which it is not detected 6PAP production but produces small amounts of volatile metabolites identified as koninginins when is confronted with the same pathogens.

The 6PAP is the best known *Trichoderma* volatile component in addition is the one with the most antagonistic activity, as 6PAP concentration increase so does the inhibition exerted on phytopathogens, in evaluations conducted by El-Hasan *et al*. (2007) the species *T. harzianum* produce high levels of 6PAP and have a greater *in vitro* effect on inhibiting mycelia growth of *F. moniliforme*, similar to this study, where mycelia growth inhibition by effect of *Trichoderma* volatile compounds on *S. cepivorum* and *S. sclerotiorum* was probably caused by 6PAP produced by *T. longibrachiatum* and *T. harzianum*.

Effect of crude extracts of *Trichoderma*: The mycelia growth inhibition of *S. sclerotiorum* and *S. cepivorum* by extracts produced in liquid medium varied from 0.0-100% among *Trichoderma* strains and species, this is accord to Sivasithamparam and Ghisalberti (2002), which indicated that different species of the same family and different strains of the same species, often can produce significantly different compounds which suggests that secondary metabolites express the individuality of species in chemical terms.

The effect of secondary metabolites produced by *Trichoderma* on the development of plant pathogens has been extensively studied, The presence of non-volatile metabolites with antifungal activity in four isolates of *Trichoderma* on the development of *Phytophthora nicotianae* and *R. solani* which suggest that the metabolites of *Trichoderma* cause vacuolation, granulation, coagulation, disintegration and cell lysis, meanwhile Etebarian (2006) suggests fungicidal effect of metabolites produced by strains of *T. harzianum* and *T. virens* that caused inhibition of mycelia growth of 100% on *Macrophomina phaseolina*. Antibiotic production by fungi and bacteria was mentioned by Dianez *et al*. (2007) and cell free extracts exhibited a limited antagonist capacity in comparison of those extracts with cells, which showed an excellent capacity to inhibit the growth of *C. michiganensis, X. axonopodis* and *E. carotovora*, demonstrating the intracellular nature of the bioactive metabolites associated to phytopathogens growth inhibition (Cruz-Quiroz *et al*., 2011).

Currently there are reported more than 120 secondary metabolites produced by *Trichoderma* spp. including polyketides, pyrones, terpenes, metabolites derived from amino acids and polypeptides which are characterized by having antibiotic properties, fungicides, bactericides, mycotoxins, phytotoxins and growth regulators (Sivasithamparam and Ghisalberti, 2002), also compounds related to control of plant pathogens soil, such as antagonistic activity of *T.* (= *Gilocladium*) *virens* by the production of glyotoxin and viridine that is coagulating the protoplasm of *P. ultimum* and in the presence of this compound does not grow well, also pacibasine, trichodermine and other antibiotics and enzymes that have been shown to be involved in reducing the inoculum of fungal pathogens, from *T. harzianu* were obtained furanone, trichorziamines that are secondary metabolites with antibiotic effect and may be producing change in the

morphogenetic pattern of the mycelium of *S. rolfsii* (Reino *et al*., 2008).

CONCLUSION

The results obtained indicate that the 41 *Trichoderma* isolates showed excellent levels of antagonism toward *S. sclerotiorum* and *S. cepivorum* either by competition for nutrients, antibiosis by volatile compounds or effect by filtering toxic. Stick out the species *T. longibrachiatum* (T3 and T10) and *T. asperellum* (T1 and T11) that's were the treatments most efficient in inhibiting the development of *S. sclerotiorum* and *S. cepivorum*, by direct competition and other mechanism of action against these pathogens.

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