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EFFECT OF SECONDARY METABOLITES PRODUCED BY DIFFERENT *TRICHODERMA* SPP. ISOLATES AGAINST *FUSARIUM OXYSPORUM* F.SP. *RADICIS-LYCOPERSICI* AND *FUSARIUM SOLANI*

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Abstract

Secondary metabolites produced by filamentous fungi have different structure and function, and they are a source of novel compounds with pharmaceutical, agricultural and medicinal importance. Trichoderma spp. are considered to be an abundant source of secondary metabolites, some of them with applications in biological control, plant growth promotion, like aroma constituents or in plant immunity. The aim of this study was to assess the potential of volatile and non-volatile metabolites released from some antagonistic Trichoderma spp. isolates against pathogens Fusarium oxysporum f.sp. radicis-lycopersici and Fusarium solani which causes wilting for more cultures. In vitro studies have demonstrate that volatile compounds produced by T49 (67.69%), Tk14 (64.61%), T50 (61.53 %), T85 (60%) showed strong inhibitory effect on FORL growth compared with M14 isolate. The non-volatile tests revealed that three isolates of Trichoderma (T85, T50, Tal12) are the best which inhibited the growth of F.solani in vitro.

Key words: *volatile compounds, non-volatile compounds, biocontrol, antifungal effects.*

INTRODUCTION

Fusarium sp. is a soil borne fungal pathogen that attacks plants through roots at all stages of plant growth, is considered as one of the main soil-borne systemic diseases and the major limiting factor in the production of tomato both in greenhouse and field-grown (Srivastava et al., 2010; Borrero et al., 2004).

Various species of fungi described as antagonists of phytopathogenic fungi produce secondary metabolites with strong antifungal activity. *Trichoderma* species have been used widely as biocontrol agents because produce many antifungal metabolites, volatile and non-volatile that adversely affect growth of different fungi phytopathogens (Li et al., 2016; Barakat et al., 2014; Nagendra et al., 2011; Ajith and Lakshmidevi, 2010; Srivastava et al., 2010; Faheem et al., 2007; Vinale et al., 2006; Dennis and Webster 1971a; Dennis and Webster 1971b). Also, the potential of *Trichoderma* spp. to produce many volatile (e.g. pyrones, sesquiterpenes) and non-volatile secondary metabolites (e.g.) has been reviewed by Reino et al., 2008. *Trichoderma* spp. differ in their abilities to produce volatile and non-volatile secondary metabolites and their production varies greatly between species and between isolates of the same species, depends on environmental conditions (Vinale et al., 2009).

Many previous studies revealed that antimicrobial metabolites produced by *Trichoderma* spp. are effective against a wide range of phytopathogenic fungi, *Botrytis fabae*, *Fusarium* spp, *Rhizoctonia solani*, *Macrophomina phaseolina* (Chen et al., 2015; Barakat et al., 2014; Sreedevi et al., 2011).

Dubey et al., 2011 reported that secondary metabolites from culture filtrates with higher concentration of *Trichoderma viride, Trichoderma virens and Trichoderma harzianum* inhibited mycelial growth of *Fusarium oxysporum* f.sp. *ciceris*. Vinale et al., 2006 reported that volatile secondary metabolites play a key role not only in mycoparasitism by *Trichoderma harzianum* and *Trichoderma atroviride*, but also in their interactions with tomato and canola seedlings. Ajith and Lakshmidevi, 2010 reported the effect of volatile and non-volatile compounds produced by *Trichoderma* spp. against *Colletotrichum capsici*, a fungal pathogen responsible for anthracnose disease in bell peppers. The results of this authors showed that the volatile compounds produced by *Trichoderma* spp.

showed 30 to 67% inhibition of *Colletotrichum capsici,* whereas non-volatile compounds have the ability to control growth of *Colletotrichum capsici* by 21 to 68% at a concentration of 50% culture filtrate. In Romania there is little information available on the implication of secondary metabolites produced by *Trichoderma* with inhibitory effect on different pathogens (Raut et al., 2014a; Raut et al., 2014b).

The aim of the present study was to determine, *in vitro*, effect of volatile and nonvolatile compounds produced by some *Trichoderma* isolates against fungal plant pathogens such as *Fusarium oxysporum* f.sp. *radicis-lycopersici* and *Fusarium solani.*

MATERIALS AND METHODS

The tested *Trichoderma* isolates as well as phytopahogens *Fusarium oxysporum* f.sp. *radicis-lycopersici (FORL)* and *Fusarium solani* (*F.solani*) used in this experiment belong to Microbial Collection of RDIPP.

Effect of volatile compounds produced by Trichoderma isolates on the mycelial growth of Fusarium

The effect of volatile metabolites produced by *Trichoderma* against *Fusarium* was tested using the inverted plate technique described by Dennis and Webster, 1971a. The mycelial disk (5 mm) of *Trichoderma* excised from the edge of 5 days old cultures was inoculated into the center of a Petri dish which containing PDA medium. The lid of each plate was replaced by the bottom of a plate containing PDA medium inoculated with a 5-mm-diameter mycelial disk of *FORL and F solani* so as test pathogens were directly exposed to antagonistic environment created by *Trichoderma*. Then, the two plates were sealed together with parafilm and incubated at 28°C for 6 days in the dark. The control sets did not contain the antagonist. Radial growth of pathogens was recorded after 6 days of incubation and percentage inhibition was calculated in relation to control.

Effect of non-volatile compounds produced by Trichoderma isolates on the mycelial growth of Fusarium

The production of non-volatile compounds by *Trichoderma* isolates against *Fusarium* was studied using the method described by Dennis and Webster (1971b). Initially, mycelia agar plugs (5mm diameter) removed from the edge of a 5 days old *Trichoderma isolates* mycelium were inoculated in 100 ml sterilized Potato Dextrose Broth in 250 ml conical flasks, and incubated at 28±2°C on a rotatory shaker set at 100 rpm for 10 days. The culture filtrate was filtered through Whatman paper for removing mycelial mats and then centrifugated at 3000 rpm 10 minutes.

The filtrate was added to molten PDA medium (at 40±3°C) to obtain a final concentration of 10% (v/v), 25% (v/v), 50% (v/v). Then PDA containing Petri dishes were inoculated with mycelial plugs (5 mm diameter) of *FORL* and *F. solani* at the centres. The dishes were incubated at 26±2°C until the colony reached the plate edge. Plates without filtrate served as control. There were three replicates for each treatment and the experiment was repeated two times. The percentage inhibition was calculated in relation to the control by the formula:

$$L=C-T/Cx100$$

where: L – inhibition of radial mycelial growth; C – the radial growth measurement of the pathogen in the control; T– the radial growth measurement of the pathogen in the presence of antagonists.

RESULTS AND DISCUSSIONS

A large variety of volatile secondary metabolites could be produced by *Trichoderma* spp. such as ethylene, hydrogen cyanide, aldehydes and ketones, which play an important role in controlling various plant pathogens (Faheem et al., 2010; Siddique et al., 2012; Chen et al., 2015). The results for volatile metabolites activity against pathogens are presented in table 1 and figure1.

From our results, it is evident that volatile compounds produced by *Trichoderma* isolates studied decreased the mycelial growth of *FORL* and *F.solani* (table 1). The effects of volatiles produced by the *Trichoderma* isolates studied over the 6-day incubation period were different for *FORL* and *F. solani*. *In vitro* studies showed that the volatile compounds produced by T49, T50, Tk14, T85 and Tal12 significantly reduced mycelial growth and inhibited spore germination of *FORL* with inhibition percent between 58.46% and 67.69%.

Table1 Effect of volatile compounds produced by different *Trichoderma* strains on *Fusarium* mycelial growth

Pathogen	Antagonist *Trichoderma* strain	Growth inhibition (%)
Fusarium oxysporum f.sp. *radicis-lycopersici*	T50	61.53
	T49	67.69
	T85	60
	Tal12	58.46
	Tk14	64.61
	TK20	56.15
	M14	31.53
Fusarium solani	T50	55.38
	T49	32.30
	T85	33.84
	Tal12	66.15
	Tk14	48.76
	TK20	38.46
	M14	29.23

Volatile metabolites produced by Td49 were more efficient in reducing the mycelial growth of *FORL* by 67.69%, after 6 days of incubation, respectively than M14 being 31.53%. The data obtained show that the Tal12 (66.15%), T50 (55.38%) and Tk14 (48.76%) strains producing volatile compounds with significant effect in reducing growth of phytopatogenic fungi *F.solani.* Other strains studied (T49, T85, Tk20, M14) inhibit very weak the growth of *F.solani,* inhibition percentages ranging from 29.23% (M14) to 38.46% (Tk20).The high degree of growth inhibition of *FORL* by all of the strains suggests that the inhibitory effect observed in dual culture could mostly be attributed to volatile metabolites.

Many strains of *Trichoderma* have been reported to produce volatile compounds that inhibit the growth of pathogen fungi significantly. Studies of Zhang et al. (2014) showed that the volatile compounds produced by *Trichoderma harzianum* T-E5 have an significant inhibition of *FOC* mycelial growth but not by killing *FOC*. This investigation suggests that metabolites released by these *Trichoderma* species are toxic and fungistatic to *Fusarium.*

Some *Trichoderma* species (*T. viride* and *T.asperellum*) tested by Qualhato et al., (2013) produced volatile metabolites having significant effects on the mycelial growth and development of the *S. sclerotiorum*, *F.solani.*

Tapwal et al. (2011) reported that volatile compounds of *Trichoderma* spp. isolates significantly inhibited the mycelial growth and spore germination of *F.oxysporum.*

Research of Calistru et al. (1997) revealed that volatile metabolites produced by *Trichoderma harzianum* species can significantly suppress the growth of *Aspergillus flavus and Fusarium moniliforme* rather than mycoparasitism. Also, studies of Raut et al. (2014a) demonstrated that volatile metabolites produced by two *Trichoderma* strains displayed inhibitory effects on *R. solani* and *P.ultimum* pathogens growth.

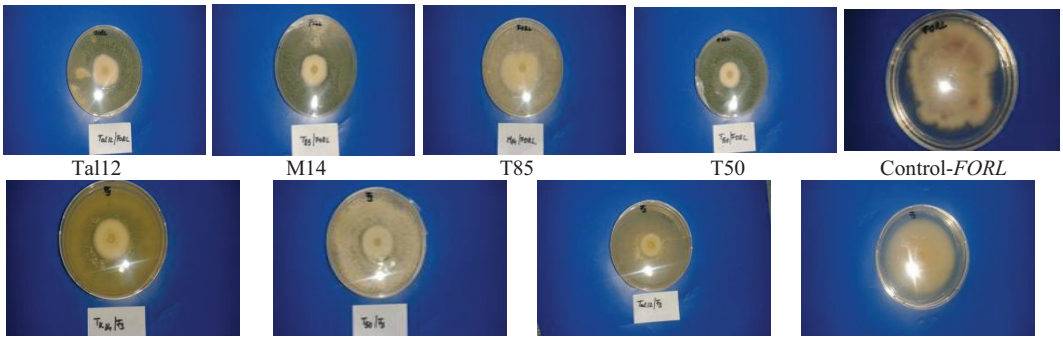

Figure 1. Plate assay for the influence of volatile metabolites from *Trichoderma* isolates on the mycelial growth of fungal pathogens

The effect of 10%, 25%, 50% filtrate concentration of *Trichoderma* isolates on *F. solani* radial growth has been show in figure 2. All concentrations of the metabolites reduced radial growth of *F.solani* in different percent.T50 and T85 and Tal12 isolates showed the highest inhibition of *F.solani* radial growth, however Tk20 and M14 had the lowest effect on growth of this pathogen. The efficiency of the non-volatile metabolites on the mycelial growth of the pathogenic strain of *F.solani* varied from 30% and 70%. This results are in accordance with of Raut et al., 2014b that supported that 25% and 50% filtrate

concentration of *Trichoderma* isolate produced between 70-80% inhibition of *F.solani.*

The non-volatile secondary metabolites produced by *Trichoderma* isolates used in this study were found to be more effective in suppressing the mycelia growth of *Fusarium solani* when compared to FORL.

Our results is consistent with the results of Kavitha and Nelson, 2013 which supported that non-volatile compound of *Trichoderma* inhibited growth of *Fusarium javanicum* and *Fusarium oxysporum.* This results is supported by the previous reports of Hasan et al., 2012 which found that *Trichoderma harzianum* inhibited the radial growth of *Fusarium graminearum* by 43.33%.

The concentrated solutions (50%) of the *Trichoderma* culture filtrates suppressed the growth of *FORL* but weaker compared to *F.solani*. Neverthelss culture filtrates of *Trichoderma* diluted (10%, 25%) showed very low inhibition level (20%) of this pathogen.

Some investigations suggest that metabolites released by *Trichoderma* species are the most effective on *Fusarium culmorum* and can be used successfully to control *Fusarium* foot rot in wheat seedlings (El-Hasan et al., 2008). Also, Barakat et al. (2014) founded that the non-volatile secondary metabolites of *Trichoderma* species were more effective in suppressing the mycelial growth of *Botrytis fabae* when compared to volatile compound.

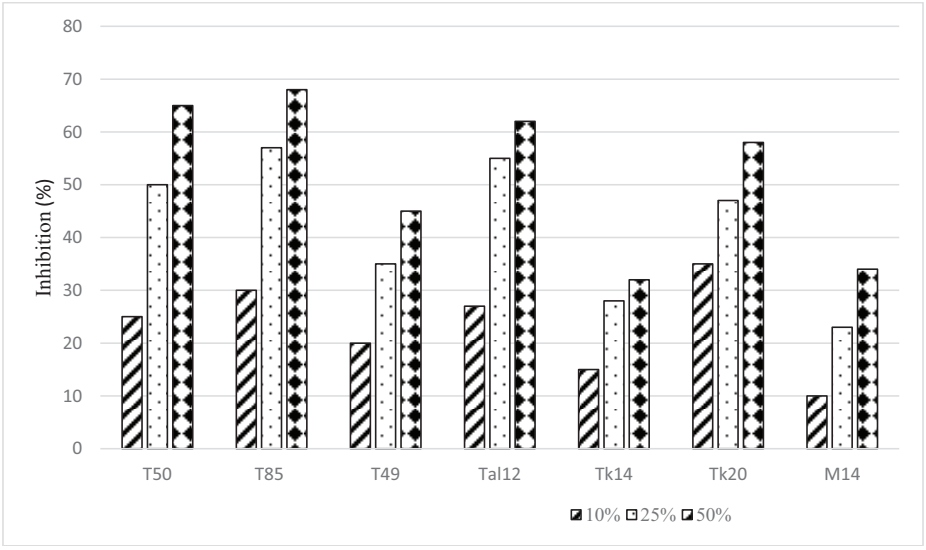

Figure 2. Effect of different concentrations of non-volatile metabolites produced by *Trichoderma* isolates on radial growth of *Fusarium solani* after 6 days of inoculation

CONCLUSIONS

Our results demonstrated the involvement of volatile and non-volatile compounds in the inhibition of *FORL* and *F.solani*

All *Trichoderma* isolate produced volatile compounds having significant effect in reducing the growth of *Fusarium.*

The volatile compounds produced by T49, T50, T85 and Tk14 isolates significantly reduced the radial growth of *FORL*.

The non-volatile compounds produced by filtrate concentrations (25% and 50%) of T85, T50 and Tal12 inhibited between 50-70% radial growth of *F.solani.*

The present results showed that the ability of secondary metabolites production is different among isolates studied .

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