Effect of Volatile and Non-Volatile Metabolites from *Trichoderma spp*. against Important Phytopathogens

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The aim of this study was to assess the potential of volatile and non-volatile metabolites from antagonistic Trichoderma spp. against phytopathogenic fungi. The biocontrol activity was tested versus the pathogens responsible for important losses in agriculture, such as: Fusarium graminearum, Rhizoctonia solani and Pythium ultimum. In vitro studies have demonstrated that the volatile and non-volatile metabolites produced by the strains Trichoderma T36 and T50 displayed inhibitory effects on pathogens growth. The volatile metabolites assay revealed that Trichoderma T36 produced a higher inhibitory effect on pathogens growth as comparative with T50. Maximum inhibition (100%) by the volatile compounds occurred in R. solani - Trichoderma T36 interaction. Mycelial growth of target fungi was retarded by non-volatile metabolites from antagonistic strains. The pathogens growth was significantly restricted at higher concentrations of culture filtrate and a total inhibition of R. solani and P. ultimum was obtained with 50% (v/v) concentration of filtrate from Trichoderma T36. The strain of Trichoderma T36 used in current study was capable to inhibit the growth of fungal pathogens and may be used as an efficient agent to control a broad spectrum of plant pathogens.

Keywords : biocontrol, non-volatile metabolites, pathogen, Trichoderma, volatile metabolites

Importance of *Trichoderma* in biological control of pathogens has been discussed in several works [1-4]. *Trichoderma* spp. has been successfully used for the management of plant diseases caused by *R. solani* [5-7], *Pythium* [8], *Sclerotium* [9-10], *Fusarium* [11-12] and *Alternaria* [13]. The antagonistic activity of *Trichoderma* is based on direct and indirect mechanisms, and their role in biocontrol process depends on the *Trichoderma* strain, pathogen, plant characteristics and environmental conditions. Production of metabolites such as volatile and non-volatile compounds leads to an improvement of biological control of plant pathogens.

The present investigation was carried out to examine the effect of volatile and non volatile metabolites produced by two *Trichoderma* strains against fungal plant pathogens with economic importance, such as *F. graminearum, R. solani* and *P. ultimum,* under laboratory conditions. For exemple, *F. graminearum* is producing the major economically losses in many vital crops, *R. solani* is responsible for the diseases on important crop plants of the world (potato, tomato, onion, lettuce etc) as well as ornamental plants and forest trees, *Pythium ultimum* is producing a devastating disease that affects a wide range of hosts and impacts on harvest yields.

Experimental part

Materials and methods

Antagonistic strains

The tested *Trichoderma* T36 and T50 isolates from different soil types belong to Microbial Collection of INCDCP-ICECHIM. The strains were maintained on potato dextrose agar (PDA) slants at 4°C. *Trichoderma atroviride* used as standard strain to evaluate the antagonistic activity against pathogens was purchased from BCCM/MUCL (Agro) Industrial Fungi & Yeasts Collection (Belgium).

Microorganisms (pathogens)

Plant phytopathogens *Fusarium graminearum*, *Rhizoctonia solani* and *Pythium ultimum* were purchased from Microbial Collection of DSMZ (Germany).

Assay for volatile compounds from *Trichoderma strain*

The effect of volatile metabolites against fungal pathogens was assessed according to the method of Dennis and Webster [14]. Petri plates containing PDA medium were centrally inoculated with a 5 mm diameter disc of antagonistic strains and pathogens and covered with parafilm (Breathe-Easy™ sealing membrane, Sigma) to avoid the volatilization of compounds. Composition of PDA medium was (g/L): 250, potato; 20, glucose; 18, agar. The plates were incubated for 3 days at 28° C. After incubation time, the lids were removed aseptically and a plate containing pathogen was placed over a plate containing *Trichoderma* strain. The plates were enclosed by three layers of parafilm to prevent the loss of volatile substances and inocubated for 5 days at 28° C. The average diameter of the treatments was measured as comparative with standard strain of *Trichoderma atroviride.* The Petri plate containing PDA without antagonist serves as control. Each assay was performed in triplicate. The percent inhibition was obtained using the following formula [14]:

$$\text{Inhibition (\%)} = (\mathbf{D1 - D2})/\mathbf{D1 \times 100};$$

where D1 represents the diameter of radial growth of pathogen in control and D2 represents the diameter of radial growth of pathogen in *Trichoderma* tests.

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Pathogen	Antagonist *Trichoderma* strain	Growth inhibition (%)*
F. graminearum	*Trichoderma* T36	40.0
	Trichoderma T50	39.5
	*Trichoderma atroviride***	45.0
R. solani	*Trichoderma* T36	40.0
	Trichoderma T50	32,5
	*Trichoderma atroviride***	40.0
P. ultimum	*Trichoderma* T36	32.5
	Trichoderma T50	28.5
	*Trichoderma atroviride***	37.5

Table 1
INHIBITION OF PATHOGEN GROWTH BY VOLATILE METABOLITES FROM *TRICHODERMA* STRAINS

**Each value is an average of three replicates; **standard strain to evaluate the pathogen inhibition*

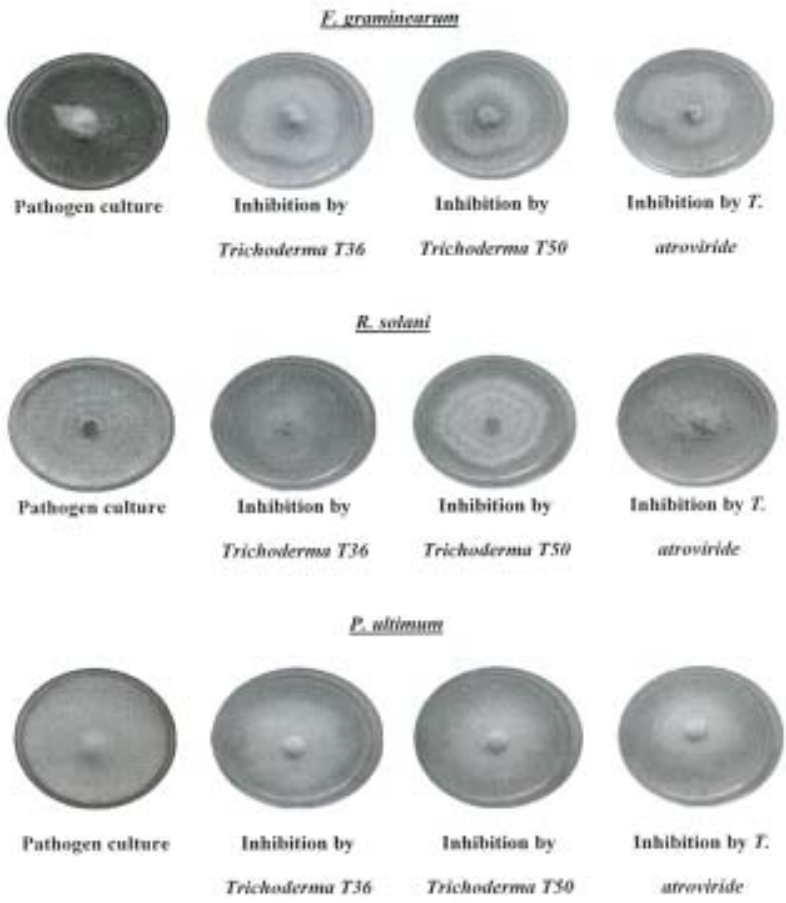

Fig. 1. Plate assay for the influence of volatile metabolites from *Trichoderma* strains on the mycelial growth of fungal pathogens

Assay for non-volatile compounds from Trichoderma strains

The testes were performed according to method of Dennis and Webster [15]. *Trichoderma* strains were inoculated in Erlenmayer flasks with 100 mL PDB medium and incubated for 15 days, at 25°C, under agitation at 100 rpm on an orbital incubator Heidolph Unimax 1010. Composition of PDB medium was (g/L): 250, potato; 20, glucose. The culture broth was filtered through sterile filter paper for removing the mycelial mats and then was sterilized by passing through a 0.22 μm pore biological membrane filter (Rotilabo spritzenfilter, steril). The *Trichoderma* filtrate was mixed with molten PDA medium (40 ± 3°C) to obtain 10, 25 and 50 % (v/v) concentration in Petri plates. A mycelial disc of 5 mm diameter of each pathogen was put in the center of the Petri plates containing the blend. The cultures were incubated at 25°C for 4-5 days. The plates without filtrate served as control. The colony diameter was measured and the percentage inhibition of the radial growth was calculated. Each assay was performed in triplicate.

Results and discussions

Trichoderma spp. produces over 100 different secondary metabolites including polyketides, pyrones, terpenes, metabolites with antibiotic properties, fungicides,

Pathogen	Antagonist *Trichoderma* strain	Metabolite concentration % (v/v)	Growth inhibition* (%)
F. graminearum	*Trichoderma* T36	10	58.8
		25	63.9
		50	63.9
	Trichoderma T50	10	63.9
		25	63.9
		50	69.4
R. solani	*Trichoderma* T36	10	65.0
		25	72.5
		50	100.0
	Trichoderma T50	10	60.0
		25	70.5
		50	98.5
P. ultimum	*Trichoderma* T36	10	18.2
		25	37.5
		50	100.0
	Trichoderma T50	10	0
		25	0
		50	0

**Each value is an average of three replicates.*

Table 2
INHIBITION OF PATHOGEN GROWTH BY NON-VOLATILE METABOLITES FROM *TRICHODERMA* STRAINS

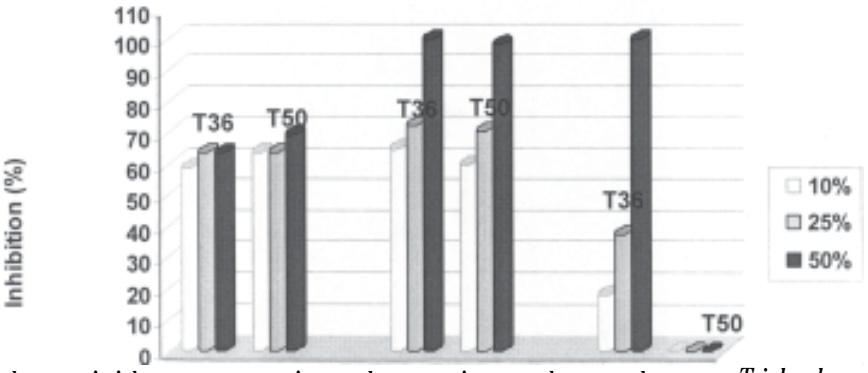

Fig. 2. Effect of non-volatile metabolites of *Trichoderma* spp on radial growth of pathogens

bactericides, mycotoxins, phytotoxins and growth regulators. Some of these compounds may contribute to the ability of *Trichoderma* spp to act as biocontrol agent against plant pathogens.

In vitro antifungal activity of *Trichoderma* strains against several selected pathogens was evaluated as production of the volatile and non volatile metabolites. The tested pathogens *F. graminearum, R. solani* and *P. ultimum* are known to be among the most destructive fungal species.

The results for volatile metabolites activity against pathogens are presented in table 1 and figure 1.

The volatile metabolites from T36 inhibited the growth of pathogens as follows: *R. solani* (100%) > *F. graminearum* (88%) > *P. ultiumum* (86.6%), by reference to standard strain of *T. atroviride.* All tested pathogens were less susceptible to inhibitory effect of volatile metabolites from *Trichoderma T50*, that produced 87, 81 and 76% inhibition in the growth of *F. graminearum, R. solani* and *P. ultiumum*, respectively, by reference to *T. atroviride*.

The pathogens grew covering whole plates in control cultures, while in contact with volatile compounds from antagonistic strains the microbial growth of pathogen was significantly restricted (fig. 1.). The maximum inhibition was produced by standard strain of *T. atroviride.*

Several studies have shown the effect of volatile compounds of antagonistic *Trichoderma* spp. against soil pathogens affecting their growth and development. It is considered that the production of volatile metabolites by *Trichoderma* spp. depends on strain characteristic in connection with growth conditions and stage of development. Also there are reports indicating that each plant pathogen responds differently to volatile compounds [16-18].

Trichoderma strains secreted non-volatile metabolites into the liquid medium and its filtrates inhibited the growth of pathogens. Evaluation of produced non volatile components also showed an acceptable performance on inhibiting mycelial growth of pathogens. The tested *Trichoderma* strains showed differences in inhibition level, the strain of *Trichoderma* T36 being more active against all pathogens (table 2 and fig. 2).

The pathogens growth was significant inhibited in the presence of higher concentrations of the culture filtrates. The maximum inhibition (100%) of the growth of fungal pathogen *R. solani* and *P. ultimum* was obtained at 50% (v/v) concentration of culture filtrate from *Trichoderma* T36. The weakest effect of *Trichoderma* T36 was recorded against *F. graminearum* with 63.9% inhibition at 50% metabolite concentration. Despite the fact that the activity of *Trichoderma* T50 against *R. solani* was comparable with T36 at the similar metabolite concentration, the strain T50 failed in the inhibition of *P. ultimum* and no effect was recorded. As a general behaviour, *R. solani* showed the strongest sensitivity to both antagonistic strains.

The aspects of inhibition of radial growth of pathogens by non volatile metabolites are presented in figure 3.

In plates with total inhibition of pathogen growth, antagonistic *Trichoderma* spp. has developed the characteristic mycelium of greenish color covering the entire solid medium surface. On the other hand, when antagonistic *Trichoderma* T50 was unable to inhibit *P. ultimum* growth, the pathogen has grown undestroyed on solid medium.

In similar studies, species of *Trichoderma* have been demonstrated to act against different plant fungal diseases by producing diffusible volatile metabolites which

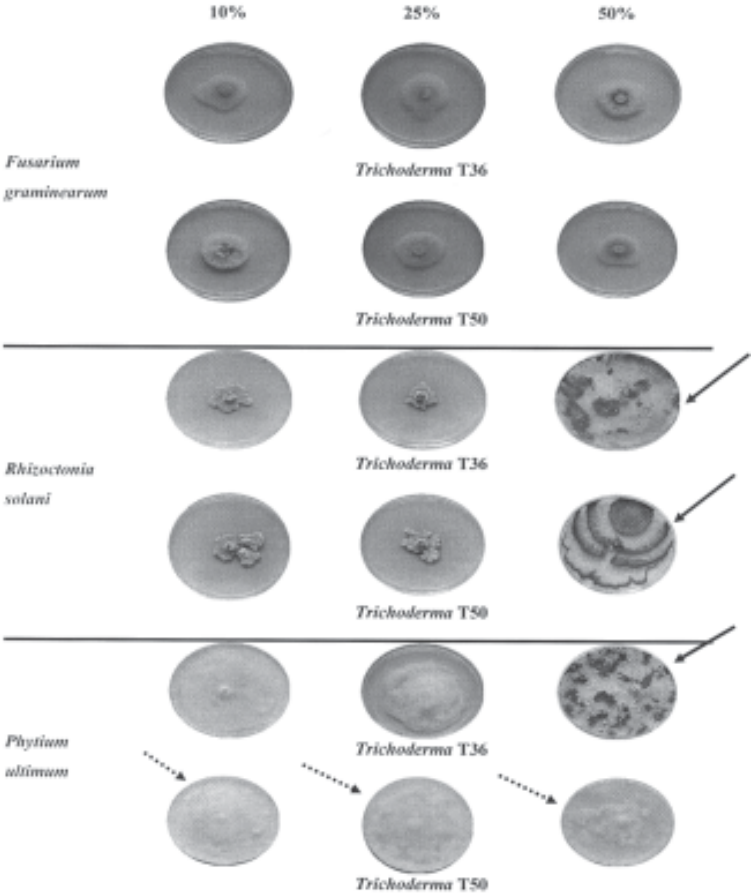

Fig. 3. Plate assay for the influence of non-volatile substances secreted by *Trichoderma* strains on the mycelial growth of fungal pathogens (black arrow marks total inhibition of pathogen growth with *Trichoderma* covering the plate; dotted arrow marks the absence of inhibition and pathogen is covering the plate)

suppressed the hyphal growth of fungal pathogens [19-20].

Conclusions

Our studies demonstrated that the volatile and non-volatile metabolites produced by the strains *Trichoderma* T36 and T50 displayed inhibitory effects on pathogens growth. The degree of effectiveness of these metabolites varies according to the nature, quality and quantity of inhibitory substances secreted by the antagonistic strains.

The volatile metabolites assay revealed that *Trichoderma T36* produced a higher inhibitory effect on pathogens growth as comparative with T50. Maximum inhibition by volatile compounds occurred in *R. solani - Trichoderma T36* interaction with 100% inhibition. Mycelial growth of target fungi was retarded also by non-volatile metabolites from antagonistic strains. The pathogens growth was significantly inhibited at higher concentrations of culture filtrates and the maximum inhibition of 100% was obtained against *R. solani* and *P. ultimum* at 50% (v/v) concentration of filtrate from *Trichoderma* T36. The strain of *Trichoderma T50* was inefficient against *P. ultimum*. Further studies will be focused on the identification of the compounds secreted in culture broth by selected antagonistic strains.

The results reported here suggest that the strain of *Trichoderma* T36 was capable to inhibit the growth of fungal pathogens and may be used as an efficient agent to control a broad spectrum of plant pathogens.

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