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EFFECT OF NON VOLATILE COMPOUNDS OF *TRICHODERMA SPP.* AGAINST *FUSARIUM GRAMINEARUM, RHIZOCTONIA SOLANI* AND *PYTHIUM ULTIMUM*

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Abstract

Biological control represents an important approach of agricultural biotechnology for controlling many fungal plant pathogens. Trichoderma spp. are the most promising and effective bioagents against many plant pathogenic fungi. In present paper, two strains of Trichoderma aspellerum isolated from soil were screened for their efficacy against some common soil borne plant pathogens by dual culture technique. Trichoderma strains were grown in potato dextrose broth and collected metabolites were amended to PDA medium to obtain 5, 10, 25 and 50% concentration in Petri plates. The solidified agar plates were inoculated with pathogen and incubated at 25 ± 2ºC for 7 days. The colony diameter was measured and percentage inhibition of radial growth was calculated. Both antagonists' strains produced non-volatile metabolites and inhibit the mycelial growth of Fusarium graminearum, Rhizoctonia solani and Pythium umtimum.

Key words : *biocontrol, non volatile, phytopathogens, Trichoderma*

INTRODUCTION

Biological control represents a viable alternative to the use of chemical fungicides and it is considered to be a safe, effective and eco friendly method for plant disease management [1].

Trichoderma is known to be one of the best candidates of biocontrol agents. Modes of action of this fungus include mycoparasitism, antibiosis, competition for nutrients and space, tolerance to stress through enhanced root and plant development [1; 2]. Many *Trichoderma* strains, mainly *T. harzianum*, *T. asperellum, T. viride* and *T. virens* have been identified as having potential applications in biological control. The list of genera of plant pathogenic fungi affected by *Trichoderma* includes: *Armillaria, Botrytis, Colletotrichum, Dematophora, Endothia, Fulvia, Fusarium, Fusicladium*, *Monilia, Nectria,Phytophthora, Plasmopara, Pythium, Rhizoctonia, Rhizopus, Sclerotinia, Venturia Sclerotium, Verticillium,* and wood rot fungi [3-4].

In present study, two strains as *Trichoderma aspellerum* T75 and *T. aspellerum* T83 were evaluated against pathogens in dual culture techniques and through production of non-volatile compounds. The targeted pathogens were some common soil borne plant pathogens, such as *Rhizoctonia solani, Fusarium graminearum* and *Pythium ultimum*

MATERIALS AND METHODS

Microorganisms

Potential biocontrol agents *Trichoderma asperellum* T57 and *T. aspellerum* T83 were provided from Microbial Collection of ICECHIM. The strains isolated from forest soil

were maintained on potato dextrose agar (PDA) slants at 4°C. Plant pathogens were obtained from Microbial Collection of DSMZ (Germany).

Evaluation of antagonistic activity through production of antifungal non-volatile metabolites

The effect of non-volatile substances on pathogen was studied following the method elaborated by Dennis and Webster [5], developed by Kamala and Indira [6], and Mishra [7]. *Trichoderma* isolates were inoculated in 100 ml sterilized potato dextrose broth (PDB) in 250 ml conical flasks and incubated at 25 ± 1° C on a rotatory shaker Heidolph Unimax 1010 at 150 rpm for 14 days. The mycelium was collected after 14 days and filtered through Whatman filter paper. The culture filtrate was sterilized by passing through a microbiological membrane filter. Different volumes of filtrates were added to the molten PDA medium (at 40 ± 3 °C) to obtain final concentrations of 10, 25 and 10% (v/v). The medium placed into Petri plate was inoculated with mycelial plug of pathogen at the centre. The plates were incubated at 25±1°C for 7 days. Control plates were maintained without culture filtrate. All experiments were done in triplicate. The colony diameter was measured and percentage inhibition of radial growth was calculated by following formula:

Inhibition (%) = D1 – D2/D1 x 100,

where: D1 is the diameter of radial growth of plant pathogens in control; D2 is the diameter of radial growth of plant pathogens in treatment

RESULTS AND DISCUSSIONS

The visual observations of antagonist microorganisms and pathogen cultures carried out on solid medium in Petri plates are presented.

The growth inhibition of *F. graminearum* in presence of different concentrations of *T. asperellum* culture extracts as non-volatile compounds is presented in Fig. 1 and Fig. 2.

These results showed that culture filtrates from *Trichoderma* T83 was more active against pathogens than the other strain. The lowest inhibition was produced by *T. asperellum* T57 at a concentration of 10% (69.44%). The highest inhibition of 88.88% was produced by *T. asperellum* T83 at 50% filtrate concentration.

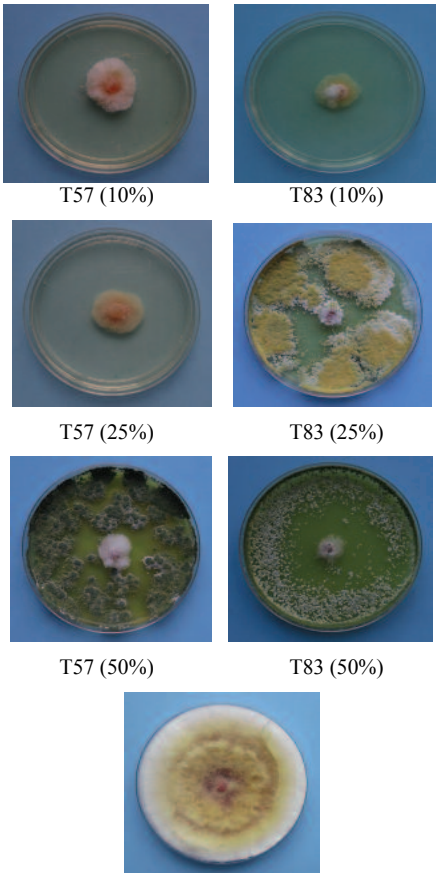


Figure 1 Results of *in vitro* antagonism testing for *T. aspellerum* T57 and T83 against *F. graminearum*

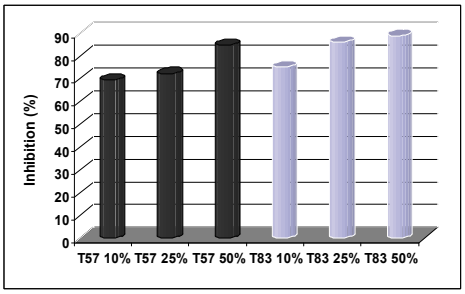
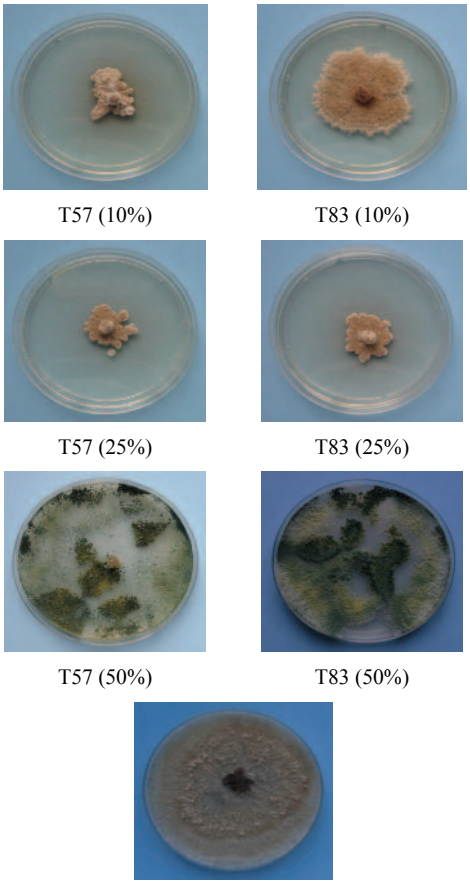


Figure 2. *In vitro* inhibition of *F. graminearum* by non-

volatile compounds from *T. asperellum* T57 and T83

The performance of antagonistic strains against *R. solani* is presented in Fig. 3. In the case of this pathogen, the inhibition induced by non-volatile metabolites varied from 48.71% for 10% filtrate concentration at *T. aspellerum* T83, to total inhibition at 50% filtrate concentration for both *Trichoderma* strains (Fig. 4).



T57 (10%) T83 (10%)

T57 (25%) T83 (25%)

T57 (50%) T83 (50%)

Control – *R. solani* culture

Figure 3. Results of *in vitro* antagonism testing for *T. aspellerum* T57 and T83 against *R. solani*

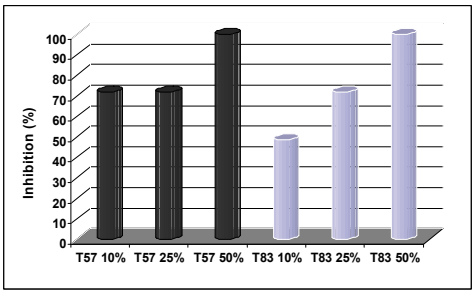
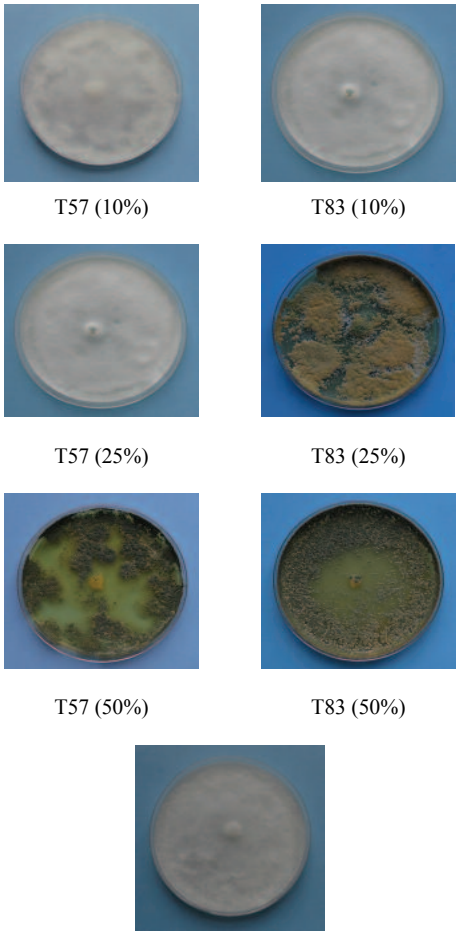


Figure 4. *In vitro* growth inhibition of *R. solani* by non-volatile compounds from *T. asperellum* T57 and T83



T57 (10%) T83 (10%)

T57 (25%) T83 (25%)

T57 (50%) T83 (50%)

Control – *P. ultimum* culture

Figure 5. Results of *in vitro* antagonism testing for *T. aspellerum* T57 and T83 against *P. ultimum*

The non-volatile metabolite activity against *P. ultimum* is presented in Fig. 5 and Fig. 6.
In test with *T. aspellerum* T57 strain, the growth inhibition of pathogen was zero for 10% and 25% filtrate concentrations, while the increase of filtrate concentration to 50% produced 65% inhibition.
T. aspellerum T83 was more active against *P. ultimum* producing 75% growth inhibition even at 25% filtrate concentration. For 50% filtrate concentration, the pathogen inhibition was 75%.

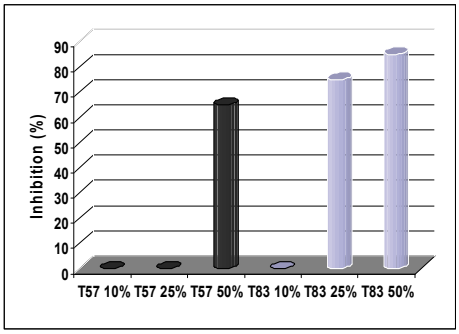


Figure 6. Effect of non volatile compounds produced by *Trichoderma* strains on the growth of *P. ultimum*

CONCLUSIONS

This study demonstrated the efficacy of isolates of *Trichoderma asperellum* T57 and T83 in controlling *Fusarium graminearum*, *Rhizoctonia solani* and *Pythium ultimum.* The antagonist strains produced non-volatile metabolites that inhibit the mycelial growth of plant phytopathogen. The antifungal activity of the culture filtrate was dose-dependent. At higher concentrations, the culture filtrate of the antagonist restricted the growth of all pathogens with better efficacy. The tests showed that *T. aspellerum* T83 was more active in controlling all pathogens tested. Out of tested pathogens, *R. solani* was more sensitive to the inhibitory effect of tested biological agents.

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