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RESEARCH ARTICLE

EFFECT OF TWOTRICHODERMA HARZIANUM SPECIES IN FUSARIUMSOLANI AND CYLINDROSPORIUMSP, PATHOGENS OF LENS CULINARIS

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ABSTRACT

The aim of this study was to evaluate in vitro inhibitory activity of Trichoderma harzianum 127a and 127b Trichoderma harzianum. For this,two kinds of samples were made: of soil samples at different depth (5, 10 and 15 cm), and samples of the different plant organs (roots, stems and leaves). Direct and indirect confrontation techniques have served tested for different metabolites. While the technique has served, inserts evaluated the effect of the opposing competitor strains. The results of the various confrontations showed that the inhibition rate was around 90% for the two antagonistic strains T-127a and 127b-T. Furthermore, comparison tests showed a significant reduction in germination. The two strains of Trichoderma harzianum tested in this study showed a high efficiency vis-à-vis Cylindrosporium sp and Fusarium solani pathogenic lenses in the Constantine region.

INTRODUCTION

The presence of fungal diseases on Lens culinaris plant and its economical consequences require the use of many pesticides. Chemical pesticides that are widely used to control plant pathogens do not degrade completely and leave toxic residues in food chain (Chet, 1987; Lynch, 1990). However, biological control agents to fight plant diseases become more and more widespread. Several Trichodermaspecies are recognized from 1930 as biocontrol (Biljana Gveroska and Jugoslav Ziberoski, 2012) and today there are modern technologies for including them in the biological control of various diseases.

Trichodermaspwas described in 1794, including anamorphic fungi isolated primarily from soil and decomposing organic matter (Persoon, 1794), and approximately 130 species have been classified (Degenkolb, 2008). Trichodermaspare free-living fungi that are highly interactive in root, soil and foliar environments. The most commonly used strains of Trichodermabio control agents are Trichodermavirens, Trichodermaviride and Trichodermaharzianum (Grondona I. and *et al*., 1997).

The strains of Trichoderma used as biocontrol agents show different mechanisms of action in their antagonistic interactions with fungal pathogens. These include antibiosis, through the production of a variety of compounds with antibiotic activity (Ghisalberti and Sivasithamparam,1991), mycoparasitism or hyperparasitism (Ayers and Adams, 1981), competition for nutrients (Chet, 1987), cell wall-lytic enzyme activity (Lorito *et al*., 1996; Lorito 1998) and induction of systemic resistance to pathogens in planta (Harman and *et al*., 2004).

Results from different studies showed that several strains of Trichodermaexerted significant reducing effects on plant diseases caused by pathogens such as Rizoctonia, Pythium, Fusarium, Gaeumannomyce under greenhouse and field conditions (Sivan and Chet 1993; Chet and Baker 1981). According to Rosado *et al*. (2007), the main factor for ecological success of this genus is a combination of very active mycoparasitism mechanisms and an effective efensive strategy, induced in the plants. Our work is oriented to study the effect of two Trichoderma species in Fusariumsolani and Cylindrosporiumsp pathogens of Lens culinaris.

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MATERIAL AND METHODS

Screening of Trichodermaharzianum from agriculture soil

Soil samples taken at 5, 10 and 15 cm in depth were collected from four locations in the vicinity of the two areas (AIN SEMARA and BARAOUIA), in sterile paper bags. The soils collected from each area were mixed thoroughly and Trichodermaharzianum spices were isolated using dilution plate and soilplate techniques on PDA and malt extract agar (MEA) with antibiotics.

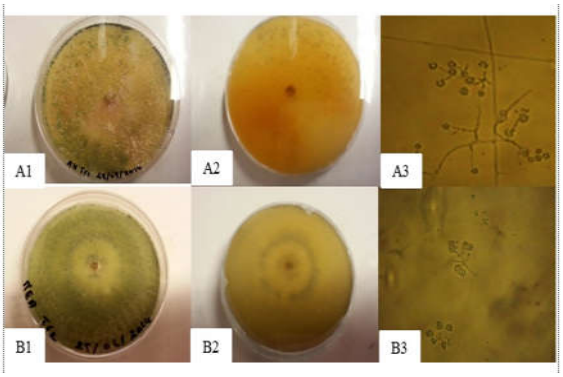

Fig 1 The two strains of Trichoderma: (A): Trichoderma harzianum 127a, (B): Trichoderma harzianum 127b- Les deuxsouchesanatgonistes de Trichoderma : (A): Trichoderma harzianum 127a, (B): Trichoderma harzianum 127b.

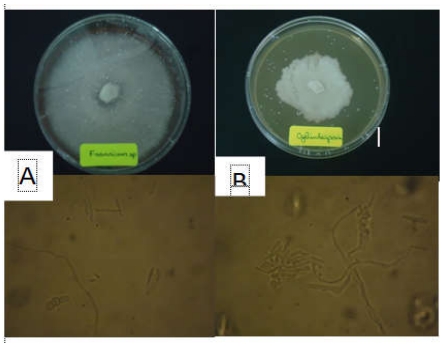

Fig 2 Macroscopic and microscopic characteristics of Fusarium solani (A) and Cylindrosporium sp (B)-Caractèresmacroscopiqueset microscopiquesde Fusarium solani (A) *ET* Cylindrosporium sp (B)

Isolation of pathogens spices from plants

Fusariumsolani and Cylindrosporiumsp pathogens of Lens culinaris were isolated from different hosts showing typical disease symptoms. Isolation of pathogens was done by cutting 0.5 cm pieces of diseased samples and surface sterilizing them in with Sodium Hypochlorite (2%) for 3 min, then rinsed several times with sterile distilled water and ethanol (70%) and dried between sterile filter paper. The pieces treatment was transferred to sterilized PDA in petriplates. The Petriplates were incubated at 27 ° C for 4 days (Belabid and *et al.,* 2000). Finally, myceliums obtained are purified in sterilized malt extract agar (MEA).

Identification of spices

Microscopy

Strains isolated and purified in malt extract agar were examined under optic Zeiss Axioskop 2 MOT microscope where morphology was noted besides hyphal structure, spore size, shapes andspores bearing structures. They were compared with the standard works of Samson *et al* Haesks (1988); Hawkswarth *et al*. (1994); HoogandGurro (1995) and Gams *et al*. (1998).

Identification by sequencing segment of the Tef 1-α gene

The two strains of Trichoderma isolated were identified in ULC (Catholic University of Louvain-La-Neuve- Belgium). The DNA of the segment of the Tef 1-α gene was sequenced and compared with reference sequences deposited in public database, cross checked with the morphology.

Antagonist tests

Diffusible metabolitestests (DM Tests)

The effect of non-volatile metabolites from Trichoderma harzianum species against Fusarium solani and Cylindrosporiumsp was tested by the method described by Dennis and Webster (1971), and Lundberg and Unestan (1980). This technique consists of placing, in the same Petri dish containing PDA medium, two agar pellets (6 mm diameter), one bearing the T. harzianum and the other gold C. sp or F. solani. The two wafers are positioned along a diametral axis to 3cm and equidistant from the center of the box; subcultures are performed simultaneously. Incubation was carried out at 30°C for six days. Colony size in each treatment was recorded and percent inhibition was calculated by using the formula as proposed by Vincent (1947).

- I = (C–T /C) x 100.

Where:

- I = Inhibition of mycelial growth (%).
- C = Growth of pathogen in control (mm).
- T = Growth of pathogen in treatment (mm).

Volatile metabolites tests (VM Tests)

In this technique, the two pellets (6mm diameter) (as I, Diffusible metabolites tests), are placing in two different Petri dishes containing PDA medium. The two Petri dishes are sealed with parafilm in a spetic condition and incubate for six days at 30°C. The percent of inhibition was calculated as in the last test.

5- Study of competition activity of Trichoderma In vitro

Competition for nutrition was studied in vitro by using a non-destructive method developed by Janisiewicz and *et al*. (2000). The device consists of cell culture 24-well plates (TC-tests plats) and of cylindrical inserts (BD Falcon Cell Culture Inserts, 0.4µm). In this method, the antagonist effect of Trichodermaharzianum on Cylindrosporiumsp and Fusarium solani was tested at different glucose concentraction of MEA medium (0, 5, 10, 15, 20 g / L) and M2 medium. The effect of strains of T. harzianum on C. spand F. solani in the different environments was evaluated by comparing the percentage of germination of conidia in each case with the corresponding control.

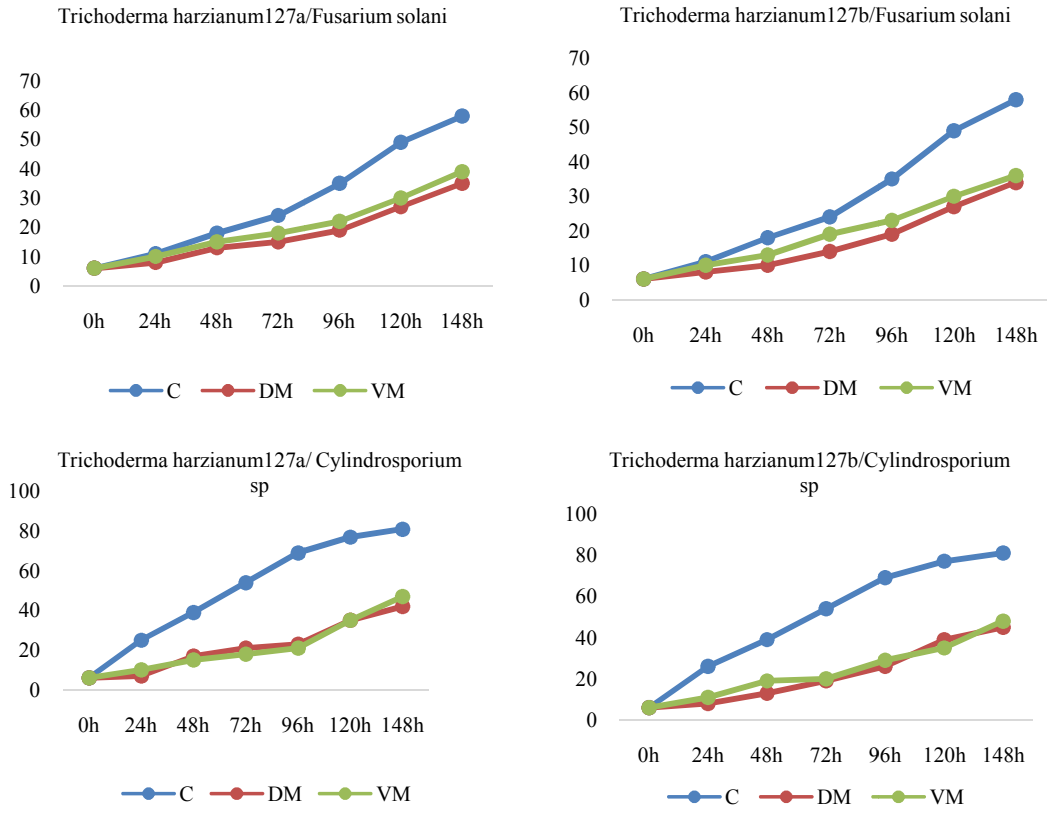

Fig 3 Inhibition of mycelia growth of Fusarium solani and Cylindrosporium sp by diffusible metabolites (DM) and volatile metabolites (VM) produced by T-127a and T-127b- Inhibition de la croissance de Fusarium solani et de Cylindrosporium sp par les métabolites diffusibles (DM) et les métabolites volatiles (VM) produits par T-127a et T-127b.

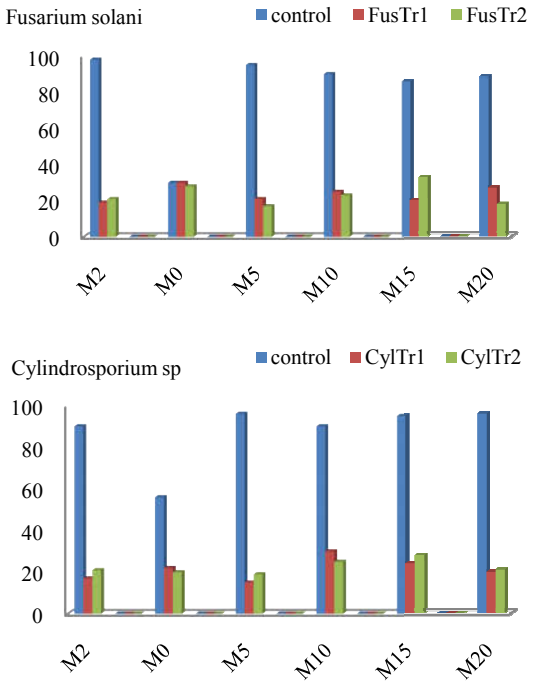

Fig 4 Percentage of conidial germination of Fusarium solani and Cylindrosporium sp in the absent and present of antagonists T-127a and T-127b- Pourcentage de la germination des conidies de Fusarium solani et de Cylindrosporium sp en présence et en absence de T-127a *et* T-127b.

DISCUSSION

The idea of a sustainable agricultural practice and environmental protection enhances the importance of biocontrol. One of the key elements of such sustainable agriculture is the application of biocontrol agents (Cook and *et al*., 1989; Deacon and *et al*., 1992). The genus Trichoderma is most important in achieving that and, at the same time, sustaining a favourable environment, instead of using chemicals.

The results obtained during the various confrontations (antagonism tests: VM, DM) conducted in vitro, showed a highly significant inhibition (60 to 92%) of pathogens growth during the study. Indeed, species of Trichoderma develop, exactly on other fungi's hyphae, coils around them and degrade the cell's walls. This action of parasitism restricts the development and activity of pathogenic fungi. Additionally, or together with mycoparasitism, some Trichoderma species release antibiotics (Harman, 1996). When the Trichodermaharzianum 127a and T-127b, are placed in Petri dishes, they have a space advantage to develop rapidely and to stop pathogen development, and to develop its mechanisms of antagonistic action. In a short time, it significantly reduces growth of Fusariumsolani and Cylindrosporium sp. The pathogen develops until the moment of contact with the antagonist; simultaneously it sporulates and changes the colony's color, acting as a super–parasite (Mirkova, 1983). Küçük and Kivanç (2003) showed that in general, the diffusible

metabolites have a bigger reducing effect than the volatile ones.

In vitro-made competition tests showed that in the absence of antagonists strains (control), the germination of conidia of Fusariumsolani and Cylindrosporiumsp was better in the presence or absence of glucose in the medium. This confirms that F.solani and C. sp are dependent nutrient environment and need enough nutrients to germinate and become infectious. Indeed, Trichodermaharzianum127a and T.harzianum 127b has grown faster than Fusariumsolani and Cylindrosporiumsp, in MEA culture. This rapid growth gives Trichoderma species an important advantage in the competition for space and nutrients with plant pathogenic fungi (Barbosa, Rehm *et al.,* 2001).

The efficacity of the genus Trichoderma strains, and especially those belonging to the genus Trichodermaharzianum, was also proved by the different results of studies of Basin H. *et al.* (1999), Faheem Amin *et al*. (2010), Inbar *et al*. (1994), Sivan and Chet (1993) and Ubalua *et al*. (2007), which reported that Trichoderma has significant inhibitory effects on pathogenic strains which Fusariumsolani and Cylindrosporium sp.

CONCLUSION

This study confirms In vitro biological activity of Trichodermaharzianum 127a and T- harzianum 127b towards Fusarium solani and Cylindrosporium sp. It has also shown an important reduction effect on the development of these pathogens of Lens culinaris. The diffusible metabolites have shown more reducing effects than the volatile ones.

Knowledge

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