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Mycoparasitism of *Trichoderma* spp. Against *Phytophthora capsici* and *Rhizoctonia solani*

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ABSTRACT

In this study three Trichoderma species; T. afro-harzianum (T8A4), T. reesei (T9i12) and T. guizouhense (T4) were investigated for their mycoparasitic potential in vitro against Phytophthora capsici and Rhizoctonia solani in dual culture test. Antagonistic activity was assessed by calculating the percentage of inhibition of radial growth of pathogens' mycelium (PIRG %). T. afro-harzianum showed outstanding inhibition of pathogens' mycelium in dual plates. The highest PIRG % recorded was 84.7 % when T. afro-harzianum (T8A4) confronted Phytophthora capsici whereas the lowest PIRG % recorded was 57.0 % when T. afro-harzianum antagonized Rhizoctonia solani. Signs of mycoparasitism like coiling and degradation of pathogens hyphae were observed.

Key words: *Trichoderma, Antagonistic activity, Mycoparasitic potential, Soil-borne phytopathogens*

INTRODUCTION

Common root diseases like blight pepper caused by *Phytophthora* and *Rhizoctonia* disease may lead to severe damages in different crops and vegetables, and causing important losses of different cash crops. Phytosanitary management programs are used to control soil-borne pathogens infection and/or infestation in different crops. Biological control agents are more and more applied in integrated common root diseases caused by soil-borne pathogens[1].

Not surprisingly, *Trichoderma* is recognized by its diverse antagonistic lifestyle which makes it highly efficient at suppressing different soil-borne plant pathogens. Mycoparasitic and rhizosphere colonizing lifestyle define the wide range of antagonism performances of *Trichoderma* spp. against diverse phytopathogenes. Mycoparasitism is the ancestral lifestyle of *Trichoderma* genus with a major function; cell wall lysis of the prey[4].

Previous works on antagonistic efficacy and mycoparasitic potential of *Trichoderma* against diverse phytopthogens including *Rhizoctonia solani* and *Phytophthora capsici*.

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Different direct antagonistic tests were revealed significant decrease in mycelia growth of the pathogen *in vitro* and *in vivo*[4]. The present work goal is to evaluate antagonistic activity of *Trichoderma* against two plant pathogens. Therefore, we investigated dual culture test of *Trichoderma* against *Phytophthora capsici* and *Rhizoctonia solani*. This paper research aims to discover mechanisms of action possibly implicated in the antagonistic interaction of *Trichoderma* against the pathogens.

MATERIAL AND METHODS

Obtaining fungi for test pathogens

Phytophthora capsici and *Rhizoctonia solani* were provided from mycotheque of mycology laboratory at IAV CHA, Ait-melloul in Morocco.

Rhizoctonia and *Phytophthora* cultures were grown and maintained on potato dextrose agar medium (PDA, Difco).

Obtaining *Trichoderma* isolates for antagonistic evaluation

Three *Trichoderma* isolates T8A4, T4 and T9i12 previously identified at the species level as *T. afro-harzianum* T8A4, *T. guizouhense* T4 and *reseei* T9i12 respectively[6] were used in this study to evaluate their antagonistic activity in vitro against *Phytophthora capsici* and *Rhizoctonia solani*.

Dual culture plates

Trichoderma isolates were tested *in vitro* for their antagonistic activity against *P. capsici* and *R. solani* using dual plate method as ascribed by Rahman *et al*.[9], and Matarese *et al*.[8], Confrontation assay allowed assessing capacity of *Trichoderma* isolates to inhibit mycelia growth of phytopathogenic fungi mentioned above[9,8].

In dual plates, malt extract agar (MEA) discs of 6 mm diameters, cut from the edge of an actively growing colony of every *Trichoderma* and pathogen, were placed at opposite sides (4.5 cm from each other) on fresh MEA medium plates (Fig. 1b). In control plates, only pathogens' monocultures were deposited (figure 1a). To evaluate antagonistic activity of *Trichoderma* we started measuring radii of developing pathogen's mycelium three times a day in the direction of antagonist's colony in dual plate (R2) until contact. Simultaneously, pathogens' radial growth is measured in mm (R1) in the absence of *Trichoderma* in control plates. Each antagonist/pathogen combination was set up in 5 replicates and inoculated plates were incubated at 24±2 °C.

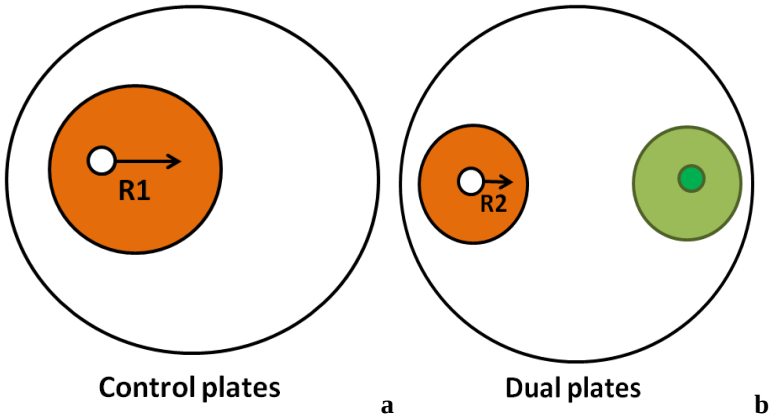

Figure 1: Dual plate culture[8]

a: control plates where pathogen is growing without *Trichoderma*

b: dual plates where *Trichoderma* isolate confront pathogen

Orange disc is positioning of pathogen plug and mycelium and green disc is positioning of *Trichoderma* plug and mycelium

Assessment of antagonistic activity

Antagonistic activity of *Trichoderma* species was evaluated by measuring percentage of inhibition of radial growth (PIRG %) of pathogens mycelium and assessing mycoparasitic signs.

Percentage of Inhibition of Radial Growth (PIRG %)

Inhibition of mycelia growth was estimated by measuring Percentage of Radial Inhibition Growth (PIRG %) and calculated using equation (1) approved by Skidmore and Dickinson[10].

$$PIRG\% = \frac{R_1 - R_2}{R_1} * 100 \qquad (1)$$

Where R_1: Radius (mm) of growing mycelia of pathogenic fungus in control plates (without *Trichoderma*) and R_2: Radius (mm) of growing mycelia of pathogenic fungus in the presence of *Trichoderma*.

Overgrowth and proliferation

After seven days of incubation, overgrowth and sporulation of *Trichoderma* spp. were assessed over pathogens' in dual plates. These mycoparasitic signs were examined to conduct antagonistic activity mechanisms established against pathogens by *Trichoderma* spp. Other signs were determined like *Trichoderma* spore proliferation over pathogens and colony and spore colors changes of both *Trichoderma* and pathogen in a dual plate.

Slide culture assay

To visualize mycoparasitic interaction of *Trichoderma* spp. against pathogens, slide culture assay was performed.

Thin layer of fresh PDA was deposited on glass slide and small plugs of *Trichoderma* and pathogen were placed on the opposite sides. Slide culture represents a miniaturized confrontation assay.

Microscopic observation

Microscopic visualization of co-cultures in slide assay was performed to look for mycoparasitic ability of *Trichoderma* spp to degrade, coil and/or develop short loops around pathogens' hyphae. All microscopic observations were realized at 40x, 100x and 400x magnification.

RESULTS

Percentage of Inhibition of Radial Growth (PIRG %)

We found that all of three *Trichoderma* species were able to inhibit significantly pathogens mycelium growth (P = 0.000). The highest PIRG% means = 84.7 % when *T. afro-harzianum* T8A4 confronted *Phytophthora capsici* and the lowest PIRG% means = 52.4% when *T.guizouhense* T4 confronted *Rhizoctonia solani* as indicated in figure 2.

Similarly, *Trichoderma* isolate extracted from commercial product (TC) inhibited mycelium growth of three pathogens at the same PIRG% range of that of our three *Trichoderma* isolates.

Mycelium of *Rhizoctonia* was significantly inhibited at 57.04% when confronting T9i12 and 52.43% by T4 while commercial *Trichoderma* significantly reduced mycelium growth of *Rhizoctonia* at 76.13%.

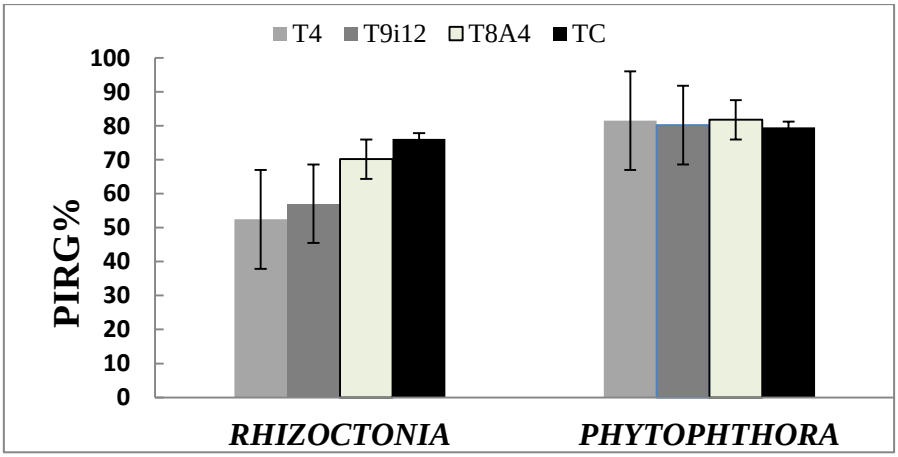

Figure 2: PIRG % of *Rhizoctoni* and *Phytophthora* mycelium against *Trichoderma* isolates: T4, T8A4 & T9i12. TC is the commercial *Trichoderma*

Tow-way ANOVA has demonstrated interaction effect of antagonistic *Trichoderma* and pathogens. Data of two-way ANOVA suggest that PIRG % is highly dependent on *Trichoderma* species locally isolated and commercial *Trichoderma*.

Mycoparasitic signs detection

Coiling around pathogens hyphae

Microscopic observation was investigated to detect mycoparasitic signs of *Trichoderma* species such as mycoparasitic coils around pathogens hyphae and degradation of pathogens' mycelium. *Trichoderma afro-harzianum* produces coils around hyphae of both *Rhizoctonia solani* in figures 2 (a) and (b) and *Phytophthora capsici* observed in figures 2 (c) and (d). T8A4; *T. afro-harzianum* created tight windings along *Phytophthora* and *Rhizoctonia* mycelia as demonstrated in figures 2 (b), (c) and (d) and produce high number of coils around *Rhizoctonia solani* (see figure 2. b).

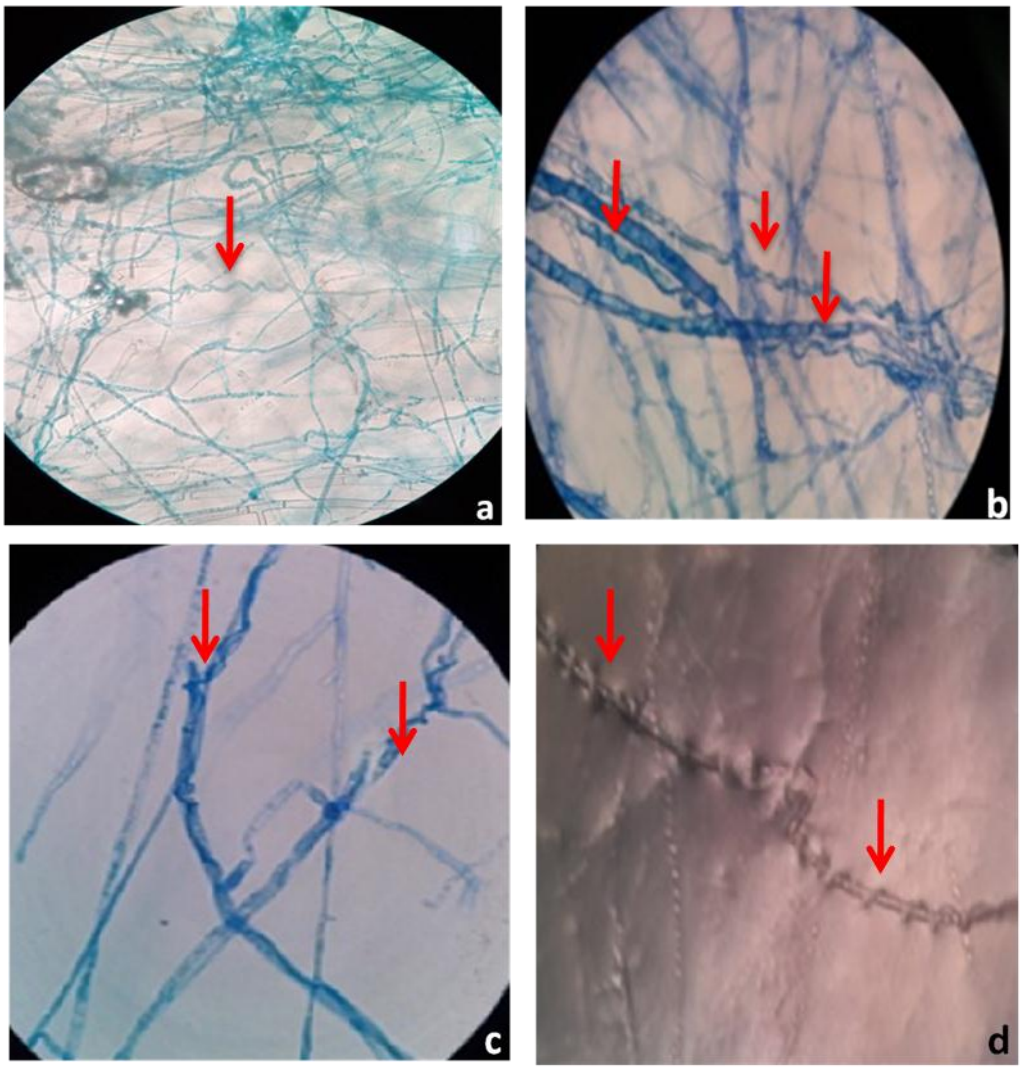

Figure 2: Coiling of mycelium of pathogens by *Trichoderma* hyphae T8A4.
a and b: Coiling of mycelium of *Rhizoctonia* solani by *Trichoderma* hyphae T8A4
c and d: Coiling of mycelium of *Phytophthora capsici* by *Trichoderma* hyphae T8A4

Slide culture confrontation of *Trichoderma* and pathogens resulted in distinguished high density of coils of *T. harzianum* T8A4 hyphae around *Rhizoctonia* mycelium. In addition, as can be seen in figure 26 (d), there was a tight coil around *Phytophthora* mycelium.

Presence of coils, their high density and consistent attachment around pathogens' hyphae may suggest *Trichoderma* hyphae penetration inside pathogenic mycelium.

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Mycelium degradation of *Rhizoctonia solani*

It appears that *Rhizoctonia* mycelium is degraded only in the presence of T8A4 mycelium as observed in figures 3a and 3b.

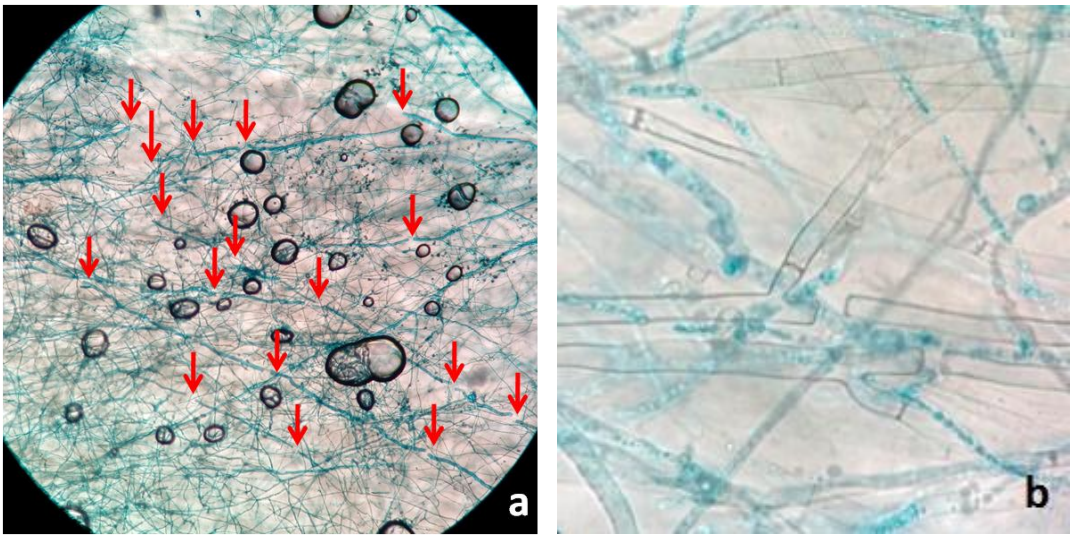

Figure 3: Mycelium degradation of *Rhizoctonia solani*.

a: Degradation of mycelium of pathogen in the presence of T8A4, magnification 40X and ref arrows pointed degradation ends.

b: Degradation of mycelium of pathogen in the presence of T8A4, magnification 400X

DISCUSSION AND CONCLUSION

It appears that *Trichoderma* spp. showed antagonistic performance against both pathogens. *T. afro-harzianum* showed high inhibition of *Phytophthora* in dual plates with the highest PIRG % = 84.7 %. Mycoparasitic-coils were observed around both pathogens hyphae; *Phytophthora capsici* and *Rhizoctonia solani*. These results were in line with previous research work on evaluating antagonistic activity of *Trichoderma* species against *Rhizoctonia solani*[11]. Hyperparasitic activity in dual culture test was established when testing *Trichoderma* species like *T. harzianum* and *T gamsii* isolated from Tenerife islands. Coiling was the most revealed signs of mycoparasitism of the antagonistic *Trichoderma gamsii* evaluated[2].

Mycoparasitism is a mechanism that implicates mycoparasitic signals such as coiling and degradation. Perceiving of signals, compounds and pathways inducing mycoparasitic potential in *Trichoderma* spp is crucial to understand how *Trichoderma* antagonists recognize, redirect and attach and consume the pathogen. *T. afro-harzianum* coils and mycelium degradation observed in *Rhizoctonia* mycelium provides compelling evidence of mycoparasitic interaction of *Trichoderma afro-harzianum* against the pathogen. Moreover, the inhibition of both pathogens mycelium growth in the co-culture plates was tightly correlated to the presence of *Trichoderma* spp. based on two-way ANOVA analysis. That is, both antagonists and pathogens are implicated in the effective antagonistic interaction of *Trichoderma* species. Researchers have reported the implication of recognition compounds on the surface of pathogens cell wall such as "Lectins" on the induction of *Trichoderma* coiling around the pathogens' hyphae[3,5]. In fact, *Trichoderma*-pathogens interaction established in parasitic zone in dual plates may give more insights on the hyperparasitic potential of *Trichoderma* in specific and its

biocontrol potential in general. Therefore, *Trichoderma* parasitic interaction towards its host is a key point to focus on during screening process of an effective antagonist potential among *Trichoderma* species[3,5,12].

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