International Journal of Microbiological Research 2 (2): 124-128, 2011
ISSN 2079-2093

In vitro Biocontrol Using the Antagonist *Trichoderma harzianum* Against the Algerian Isolates of *Ascochyta rabiei* (Pass.) Labr., the Agent of *Ascochyta* Blight in Chickpea (*Cicer arietinum* L.)

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Abstract: This study has the objective to evaluate the effect of the antagonist *T. harzianum* on the mycelial growth of the fungus *Ascochyta rabiei*, the agent of *ascochyta* blight on chickpea (*Cicer arietinum* L.). Sixteen isolates of *A. rabiei* were collected from various areas in the north west of Algeria, then preserved in a medium gélosé containing chickpea (CDA) at 4°C to use them elsewhere. An inhibiting action was observed on the mycelial growth of the isolates by the effect *T. harzianum* with a creation of a zone of inhibition which stopped the mycelial growth of the isolates. Even for the values of the rates of inhibition, they also show the effect of this antagonist compared to the witnesses.

Key words: *Ascochyta rabiei* · *Trichoderma harzianum* · Antagonism · Growth inhibition · Biocontrol

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the world's most important grain legumes and it's the major source of protein for humans [1, 2]. World chickpea production has increased stedly in the past two decades and in 2009 production reached 9 MT ranking third behind dry bean (*Phaseolus vulgaris* L.) at 19 and field pea (*Pisum sativum* L.) at 10,3 MT [3]. The average seed yield of chickpea varies from 390 to 3600 kg/ha, depending on environmental conditions and crop management for biotic and abiotic constraints [3].

Ascochyta blight, a disease caused by *Ascochyta rabiei* (Pass.) Labr., is the major constraint limiting chickpea productivity worldwide [5- 9].

All the farming, chemical and genetic means of fight knew limits to decrease the damage caused by this pathogen [1, 2,10]. Several research was made on the program of screening of the chick-pea lines in the whole world for the goal to find lines resistant, did not give any stable levels of resistance to *A. rabiei* at these line [1, 11- 13].

However, the biological fight by the use of antagonistic mushrooms can be understood like another means to fight the disease with its integration in the program of fight integrated.

The aim of this study is to evaluate *in vitro* effect of the antagonist *Trichoderma harzianum* on the mycelial growth of *Ascochyta rabiei* isolates.

MATERIALS AND METHODS

Fungal Material: The isolates of *Ascochyta rabiei* used in this study, were obtained by isolation from samples of stems, sheets an chickpea pods presenting of the symptoms of *ascochyta* blight (table 1). The antagoinste was isolated from soil sample in the rhizosphere and its identification was done by microscope.

Purification and Cultural Conservation: The isolates were conserved in petri dishes contained CDA medium (-Chickpea Seed Meal Dextrose Agar) [14]. The isolates were maintened on CDA medium at 20±2°C [14,15].

Confrontation Test: The method of Howell (2003) was carried out [16] to evaluate the inhibiting action of *T. harzianum* on the mycelial growth of *A. rabiei*. In Petri dishes containing 15 ml of CDA medium, two explants of 5mm in diameter colonies of *A. rabiei* and *T. harzianum* were lied out, each one in with dimensions of limps. Each treatment is repeated 4 times. the witnesses are colonies of the isolates of *A. rabiei* in limp of $petri alone without

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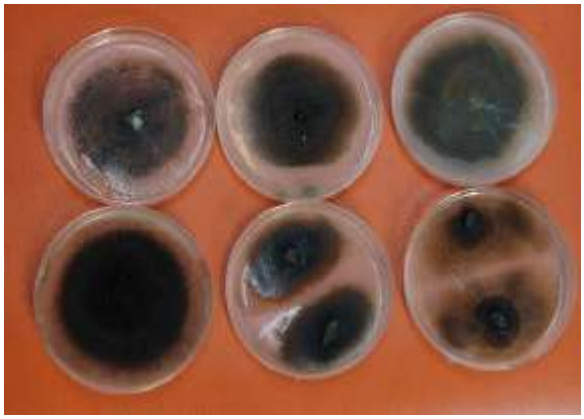

Fig. 1: Cultural aspects of *A. rabiei* isolates in CDA medium.

Fig. 2: Colony of *Trichoderma harzianum* in CDA medium.

Table 1: *Ascochyta rabiei* isolates with their origin and date of isolation

Isolates	Origins	Dates of isolation
At0108	Ain Temouchent	March 2008
Sba0108	Didi Bel Abbes	March 2008
Sba0208	Didi Bel Abbes	March 2008
Msc0108	Mascara	April 2008
Ad0108	Ain Defla	April 2008
Mos0108	Mostaganem	June 2008
Mos0208	Mostaganem	June 2008
Msc0208	Mascara	November 2008
Msc0308	Mascara	November 2008
Msc0408	Mascara	November 2008
At0208	Ain Temouchent	November 2008
At0308	Ain Temouchent	November 2008
Rel0109	Relizane	September 2009
Rel0209	Relizane	September 2009
Rel0309	Relizane	September 2009
Chl0110	Chlef	July 2010

antagonist. The mycelial growth of isolates *A. rabiei* is evaluated by measuring the ray of the colony each day until the seventh day.

Evaluation of Mycelial Growth: To estimate the mycelial growth, the technique used is that indicated by Rapilly [17]. This method initially consists in measuring the mycelial growth linear day laborer of the colonies until the seventh day, according to the formula:

$$L = (D - d) / 2 ;$$

L : Mycelial growth (mm),
D : Colony diameter (mm),
d : Explant diameter (5mm).

The averages of mycelial growth are calculated by theformula:

$$V(mm/day) = \Sigma (L_n - L_{n-1}) / n;$$

V : Mean of mycelial growth (mm/day),
L_n, L_{n-1} … are the mycelial growths on the day n;
n : Days number.

While, the rate of inhibition (%), is calculated as follows:

$$RGI (\%) = (Lw - L) \times 100 / Lw;$$

RGI : Rate of growth inhibition (%),
Lw : Mycelial growth of witness isolates (without *T. harzianum*),
L : Mycelial growth of isolates (without *T. harzianum*).

Statistical Analysis: The variances (σ^2), averages and standard deviation (SD) of various repetitions were calculated and analyzed by the software of statistics (STAT BOX 6.0.4. GRIMMERSOFT) and the device used, are the unifactorielle total Randomization (a studied factor) by the test of Newman and Keuls ($P_{0,05}$ and $P_{0,01}$).

RESULTS AND DISCUSSION

Highly significant effect of antagonism of *T. harzianum* on mycelial growth of *A. rabiei* isolates is observed (Table 2).

Table. 2: Mean values of mycelial growth of *A. rabiei* isolates by *T. harzianum* effect

Isolates	Mycelial growth (Mean ± SD) Without *T. harzianum* (mm/day)	With *T. harzianum* (mm/day)	Test F	C.V.
At0108	3,43^{a}±0,4	2,2^{b}±0,26	19,55**	5,36%
Sba0108	3,1^{a}±0,1	1,96^{b}±0,15	115,5**	5,1%
Sba0208	3,36^{a}±0,15	2,46^{b}±0,05	91,12**	3,96%
Msc0108	3,86^{a}±0,32	1,23^{b}±0,25	124,82**	11,32%
Ad0108	5,06^{a}±0,11	3,83^{b}±0,76	7.64*	12,27%
Mos0108	4,06^{a}±0,05	2,56^{b}±0,4	40,5**	8,7%
Mos0208	3,53^{a}±0,25	2,63^{b}±0,15	28,03**	6,75%
Msc0208	3,46^{a}±0,25	1,46^{b}±0,2	112,5**	9,36%
Msc0308	2,93^{a}±0,11	1,3^{b}±0,17	184,69**	6,95%
Msc0408	7,5^{a}±0,5	4,16^{b}±0,76	40**	11,07%
At0208	3,06^{a}±0,11	2,06^{b}±0,11	112,5**	4,5%
At0308	3,43^{a}±0, 2	2,26^{b}±0,05	87,5**	5,3%
Rel0109	5,06^{a}±0,11	3^{b}±0,5	48,65**	9%
Rel0209	4,9^{a}±0,36	3,1^{b}±0,28	42,25**	8,1
Rel0309	4,06^{a}±0,11	3,13^{b}±0,11	98**	3,21
Chl0110	6,16^{a}±0,28	3,4^{b}±0,17	202,61**	4,98

C.V.: Coefficient of variation ; SD: standard deviation ; * Significant effect (at P≤0,05) ; ** Highly significant effect (at P=0,01).

The averages of the mycelial growth of the isolates of *A. rabiei* under the effect *T. harzianum* were always lower than those without *T. harzianum* (Figure 3). With regard to the rate of inhibition (RGI), it is very important for all the isolates (Figure 4). What explains and confirms the existence of antagonist an action against the isolates of *A. rabiei* on *T. harzianum*.

The antagonism which exists between the microoganisms is possible to use it to fight against the parasites. Navas-Cortés [18] reported that when he buries a fungus *A. rabiei* in a sterile soil there is an enormous production of pycnids and pseudothecia that in an original soil. The study concluded that the fungus is affected by other microorganisms.

Wang *et al*. [19] reported that the antagonistic fungus, verdant *Trichoderma* influences the development and the survival of *A. rabiei*. The native *Rhizobium* bacterium produces a fungic anti acid, this acid limits the development of *A. rabiei* in ground [20]. Dugan *et al*. [21] found that the two forms, *Ascochyta rabiei* and *Didymella rabiei* are inhibited by *Aureobasidium pullulans* and *Clonostachys rosea*.

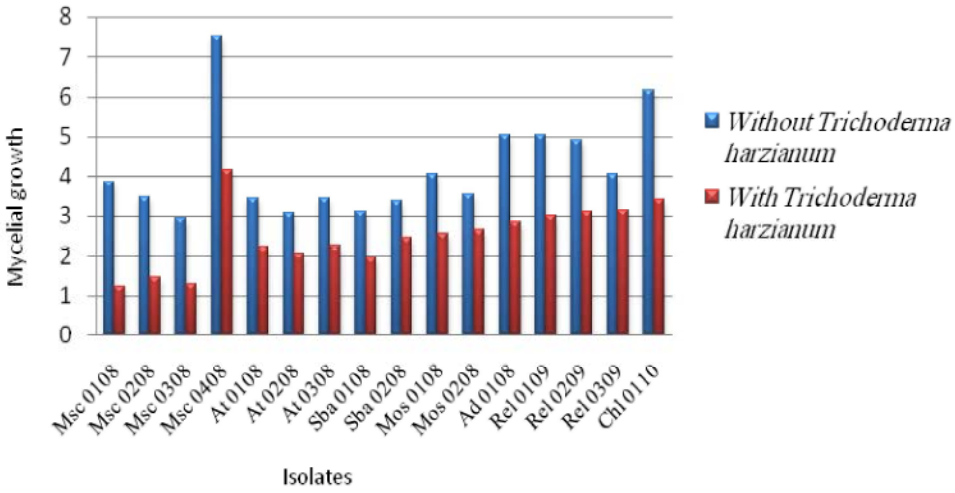

Fig. 3: Effect of *T. harzianum* on mycelial growth of *A. rabiei* isolates.

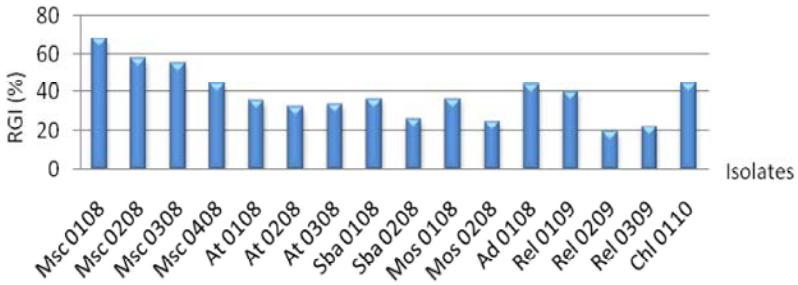

Fig. 4: Rates of growth inhibition (RGI) of *A. rabiei* colonies by *T. harzianum* effect.

The potential of *Trichoderma* species as biocontrol agent of plant diseases was first recognized in the earlier 1930s [22]. In subsequent years, the antagonisme of *T. harzianum* is possible in many diseases has been added to the list [23- 29].

Howell [16] announced that *T. harzianum* attacks the fungi phytopathogens by mycoparasitism and production of antibiotics. Elad et al. [30] showed that *T. harzianum* produces enzymes like β1,3 glucanase and chitinase which hydrolize the cellular walls of parasitic fungi.

In conclusion, the present study studied the antagonism effect of *Trichoderma harzianum* on the mycelial growth of the isolates of *A. rabiei*, agent responsible for the anthracnose of chick-pea. An inhibiting action on the mycelial growth of the isolates of *Ascochyta rabiei* was observed, followed by a complete stop of growth after the 7th day. These results indicates the existance of an antagonistic effect carried out per *T. harzianum* comparedwith its absence. Therefore, it is possible in future to integrate the biological control by biopesticides containing *Trichoderma harzianum* in the program of management against the *ascochyta* blight in chickpea.

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