Journal of Fundamental and Applied Sciences

Research Article

ISSN 1112-9867

Available online at http://www.jfas.info

BIOLOGICAL CONTROL OF *TRICHOTHECIUM ROSEUM* BY USING INDIGENOUS STRAINS OF *TRICHODERMA HARZIANUM* ISOLATED IN ALGERIA

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Received: 31 December 2019 / Accepted: 25 August 2020 / Published online: 01 September 2020

ABSTRACT

The present study focuses on the *in vitro* antagonistic effect of *Trichoderma harzianum* against tomato cryptogrammic disease in the region of Wadi Righ; in order to introduce them into a biological control program. The fungus *Trichotecium roseum* was isolated from tomato plants that have fungal diseases symptoms; they were confronted directly with antagonistic strains of *T. harzianum* at a temperature of 26° C. The confrontation revealed that at the end of six days maximum, strains inhibited the mycelia growth of phytopathogenic species.

Beyond the 6th day, *T. harzianum* invades the colonies of *T. roseum* with 57.9, 48.29 and 32.8% obtained with the strains (ST1, ST2, ST3) respectively, revealing its highly inhibitory activity. *T. harzianum* strains have a real antifungal potential against *T. roseum* and can constitute an alternative to chemicals and used as a biological control because they have succeeded in inhibiting this phytopathogenic agent growth.

Keywords: *In vitro*, antagonist, indigenous strains, *T. harzianum*, *T. roseum*.

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doi: http://dx.doi.org/10.4314/jfas.v12i3.21

1. INTRODUCTION

Organic farming is a mode of production limited by a regulation that prohibits the use of synthetic chemical products (fertilizers, pesticides, etc.) and encourages the use of physical and biological means [1,2]. In addition, the intensive use of chemicals in agriculture is leading to biological soil depletion, groundwater pollution and the development of resistance in plant pathogens and pests. In Wadi Righ region, the tomato is exposed to attack by several phytopathogenic fungi, which can reduce the quality and the yield of this crop, while *Trichotecium roseum* is frequently found on this solanaceae in our locality.

To move towards a more sustainable mode of production, two categories of micro-organisms are of interest, present in the soil and can be applied at the level of the root system to contribute to the growth of the surrounding plants. The latter are mainly bacteria and antagonistic fungi [3]. Since a long time, *Trichoderma* species have been known by its potency in controlling fungi and can improve both plant growth and resistance to diseases, and colonizes the roots, which improves plant height and leaf area [4].

Trichothecium roseum has been reported on tomato fruits produced under shelter [5] and on tomato leaves [6], which caused circular lesions on fruits that can be haloed from a brown area and they are covered with a powdery pale pink mold. This disease is one of the most important post-crop pathogens in arid and semi-arid areas [7]. This fungus produces a wide variety of metabolites, including mycotoxins, such as roseotoxins B, and trichothecenes [8, 9]. Trichothecenes are responsible for the bitter taste of infected fruit and used as basis in establishment of an entire class of mycotoxins: Epoxy-trichothecins [10-12], Mycosporinglutaminol [13], andprotease enzyme [14].

The present study aims to test the ability of three indigenous strains of *Trichoderma harzianum* to colonize the environment and valorize their antagonist potency to eliminate a phytopathogenic agent of tomato in Wadi Righ region. The interaction between *T. harzianum* and *T. roseum* able to grow on open fields culture was studied *in vitro.*

2. RESULTS

This study allowed us to distinguish colonies of fungus (Figure 1). Their characterization was

done by examining the color and texture of the mycelium formed on the surface of the dishes.

2.1. Appearance of isolated strains of *Trichothecium roseum*

After four days, the colony of *T. roseum* was initially white and became pale pink. Then, it develops rapidly a pinkish powdery color with velvety appearance (Figure 1.B). Conidiophores are upright and conidia are long, thin, smooth, bicellular and hyaline (Figure 1.A), bearing, bicellular conidiophores sand hyaline that arise alone or in loose groups [15], [16].

Fig.1. Micro (A) and macroscopic (B) aspect of *Trichotecium roseum*

2.2. Mycelial growth rate of *T. roseum* in the presence of *T. harzianum*

The results of this test indicate that the development of phytopathogenic fungus was inhibited by the three strains of *T. Harzianum* (ST1, ST2, ST3). The daily mycelial growth values, of this species, are less important in the presence of antagonist. Indeed, *T. roseum*, recorded values of 0.5, 0.5 and 0.4 mm /h respectively. On the other hand, the control showed a greater growth rate with 0.99, 0.7 and 0.6 mm / h respectively.

2.3. *In vitro* antagonism test between *T. harzianum* strains and *T. roseum*

The antifungal activity of the three strains of *T. harzianum* was revealed by the inhibition rate of *T. harzianum* against tomato phytopathogenic agent.

After six days of incubation, the control of *T. roseum* is totally invaded by the mycelium of *T. harzianum*. The rate of inhibition on the last day of the test gives an idea about the growth kinetics of each fungus, antagonist or phytopathogen. The results indicate that ST1, ST2 and ST3 inhibited mycelial growth of *T. roseum* with 57.9, 48.3, and 32.8% respectively.

Statistical analysis confirms the sensitivity of *T. roseum* to *T. harzianum* strains. Analysis of the variance showed a very highly significant effect of *T. harzianum* (ST1, ST2, ST3) on the fungus with ($Pr < 0.0001$), obtaining observed F of the order of 32.805, 12.790, 37.379 for the three strains respectively.

On the other hand, the variance analysis between the three strains of *T. harzianum* showed a very highly significant difference ($Pr < 0.0001$) with an observed F of 10.211.

The Fisher test revealed two homogeneous groups, recording the two most effective strains of *T. harzianum* (ST2) followed by the first strain (ST1), and then the 3rd strain (ST3).

3. DISCUSSION

The present study showed that *T. harzianum* has a real antifungal potential against *T. roseum*. Indeed, the rate of mycelial growth of the tested fungus was slowed down by the three strains of the antagonist. It was observed in our study that at the end of 6 days in maximum, the colonies of *T. harzianum* overlap those of the fungus, thus revealing their inhibitory power.

This situation has also been advanced by many researchers who have followed the same technique of direct confrontation and used the same culture medium (PDA). However, there is no *in vitro* previous study of the antagonistic effect of *T. harzianum* on *T. roseum*.

In fact, the phenomenon of antagonism is brought into play by various mechanisms such as mycoparasitism [17,18], which is widely exploited in biological control [4].

This phenomenon occurs when the hyphae of *T. harzianum* are wrapped around the phytopathogenic fungus [19], presenting an initial phase of interaction with mycelia, an intermediate phase to overcome the inhibitory effect of the fungus and a final phase of parasitism [4].

Another mechanism is provided by the secretion of volatile and diffusible metabolites [20], which cause lyses of spores and mycelium [21,22].

The competition for nutrition and space may also be involved when the fungus fragments are placed in contact with *Trichoderma* [23], this latter has the advantage of space and causes abnormalities in the morphology of phytopathogenic agents [24].

4. MATERIAL AND METHODS

4.1. Study area

This study was conducted in the region of Sidi Mehdi, which is an area located in the Southeastern Algeria. It is a Saharan region with one dry period throughout the year. This very low region is located at an altitude of 69 m at 06°4’ E and 33°7’N. This area is approximately 7 km of Touggourt on the road leading to the airport [25].

4.2. Isolation of antagonistic fungus

Trichoderma harzianum is the antagonistic agent used in this bioassay with three strains. They were isolated from different crops (potato leaves (ST1), durum wheat seeds (ST2) and date palm leaf (ST3). The macroscopic appearance of *T. harzianum* is manifested by a mycelium at first white and after sporulation been green (Figure 2) with regular concentric circles (ST1, ST3) or relatively scattered circles (ST2).

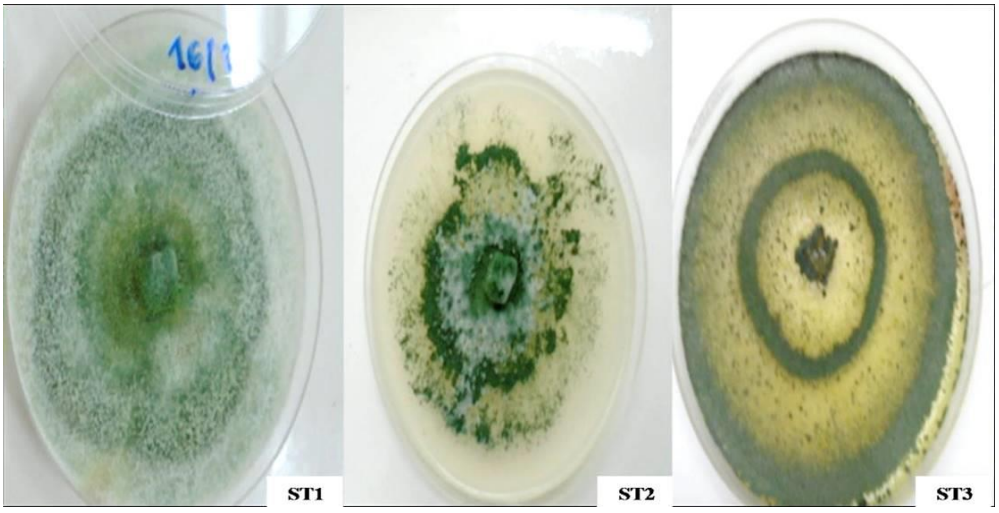

Fig.2. Morphological aspect of three strains of *T.harzianum* grown on PDA medium

4.3. Isolation and identification of phytopathogenic agent

Samples from roots, stems and leaves of cherry tomato, carrying disease symptoms (morphological or chlorotic deformations, spots, burns, rotting ...), were brought to laboratory for examination. The attacked subjects were rinsed with tap water, cut to small pieces and disinfected with Hypochlorite Sodium (2%) for three minutes (to eliminate saprophytic flora). Then, they were rinsed twice with distilled water and dried on a sterile filter paper near the

Bunsen spout. These fragments were seeded aseptically in PDA agar medium placed in a Petri dish, then incubated at a temperature of 25° C under a hemeroperiodic regime (12 h dark / 12 h light), in order to promote sporulation.

The identification of fungi essentially requires the morphological characteristics, the pigmentation and the shape of the colonies (powdery, fluffy, cottony...). In addition, we based on the microscopic aspect of these microorganisms, taking in consideration the shape of the mycelium, the presence or absence of partitions and the mode of branching, size, morphology, coloring and segmentation of spores.

4.4. *In vitro* bioassay of antagonism

The antagonistic activity of *T. harzianum* was tested *in vitro* according to the method of Benhamou & Chet (1996) [17], on a culture medium, by direct confrontation with phytopathogenic agent. This technique consists in placing two agar mycelial discs of 5 mm in diameter for each of them, one carrying *T. harzianum* and the other carrying the phytopathogenic agent, in 9 cm Petri dish containing 18 ml of PDA medium. The two fragments are placed along a diametral axis of 3 cm and in equidistant from the center of the dish (Figure 3). For the control dishes, a mycelial disk of the pathogen of 5 mm diameter was deposited in the center of a dish containing the PDA. Five replicates were selected by phytopathogenic agent and as much for the controls. The incubation was conducted in an oven at 25° C.

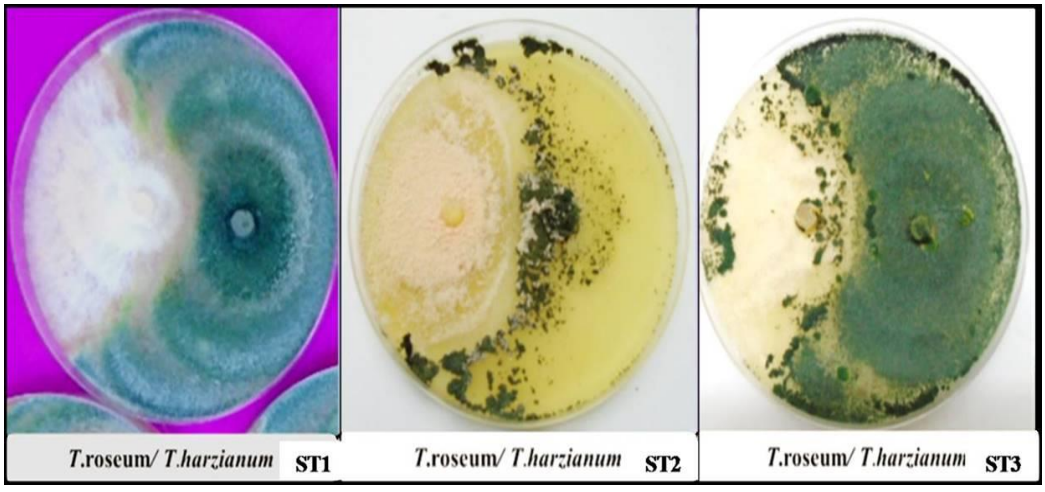

Fig.3. Inhibitory effect of (ST1, ST2, ST3) on *T. roseum* after 6 days of incubation

Daily radial measurements are estimated in millimeters of fungus mycelia and antagonist agent. The test was completed when the mycelia covered all the control dishes.

The evaluation of inhibition rate exerted by *T. harzianum* was estimated by the formula of Yong & Wolf (1995) [26]:

$$I\ (\%) = (1- Cn/Co) \times 100$$

I (%): is the percent inhibition of mycelial growth.

Cn:is the mean diameter of the colonies in the presence of the antagonist.

Co:is the mean diameter of the control colonies.

4.5. Estimation of mycelial growth rate

The mycelial growth rate of each fungus was determined by the formula of Cahagnier & Molard (1998) [27].

$$VC = [D1/Te1] + [(D2-D1)/Te2] + [(D3-D2)/Te3] +\ldots + [(Dn-Dn-1)/Ten]$$

D: is the diameter of the daily growth zone in mm.

Te: is the incubation time per hour.

4.6. Statistical analysis

The statistical analysis used is ANOVA (analysis of variance), made by the EXCEL STAT for all tests. The comparison of the averages is done on the basis of FISHER test.

5. CONCLUSION

The three indigenous antagonistic fungal strains of *T. harzianum* tested in this study present an inhibition potential against *T. roseum* and constitute an important mean of control which can replace the traditional method of controlling the phytopathogenic fungi of tomato by using chemicals. Encouraging results deserve to be complemented by the study of the antagonistic effect of *T. harzianum* strains by *in vivo* tests on whole tomato plants which can be used in the formulation of bio-fungicides capable of being applied to treat tomato diseases. Also, it would be exciting to test these strains against other fungal species of tomato, as well as other crops, in order to maximize this biological control tool.

6. ACKNOWLEDGEMENT

This work was supported by SINAL's Company of Oran (Algeria) and the General Direction of Scientific Research and Technological Development (DGRSDT) who funded our study as part of a mixed project of research, conducted with the National Institute of Agronomic Research of Algeria (INRAA).

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How to cite this article:
Lakhdari W, Dehliz A, Benlamoudi W, Mlik R, Mekhadmi NH, Hammi H, Ouargli D. Biological control of *trichothecium roseum* by using indigenous strains of *trichoderma harzianum* isolated in Algeria. J. Fundam. Appl. Sci., 2020, *12(3), 1327-1336.*