

In Vitro Investigation of Trichoderma Strain Potential Against Fusarium Wilt of Tomato

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Abstract. Three modes of action of Trichoderma strain are evaluated, with in vitro tests, in order to verify the potential of this antagonistic against *Fusarium oxysporum* f. sp. *lycopersici* isolated from tomato plant in Oued Righ region, Algerian southeast. Inhibition rates of Fusarium wilt of the order of 56, 65 and 70% are respectively obtained with antibiosis, competition and mycoparasitism mechanisms. Results, analyzed by ANOVA, are confirmed that the biological agent showed significant fungistatic effect towards Fusarium wilt of tomato most importantly by mycoparasitism that constitutes the most effective mechanism among all the tests applied.

Keyword. Trichoderma, *Fusarium oxysporum* f. sp. *lycopersici*, inhibition mechanisms, in vitro .

I. INTRODUCTION

In Oued Righ region (southeastern of Algeria) controlling of *Fusarium* diseases by using chemicals is the first and the only solution the farmer looks for, due to the aggressiveness of this fungus and the lack of biological alternatives. However, under certain conditions *Fusarium* diseases can be controlled by using natural performant antagonistic. *Trichoderma* spp. are worldwide known for their antifungal inhibitor power against a large range of soil born pathogenic fungi [1-4]. This potency is expressed by several mechanisms involved in antagonistic interactions like competition, antibiosis and mycoparasitism.

Screening of biocontrol agents needs to monitor secondary metabolites and enzymes [5], because they play the same function with chemical pesticides. Indeed, over 100 peptaibols synthesized by *Trichoderma* spp. can undeniably suppress the resistance development of pathogens [6-8]. Among them, trichokonin VI can induce extensive apoptosis of mycelia of *Fusarium oxysporum* [7]. Moreover, chitinase, oxidase, protease and glucanase are enzymes associated with fungal cell wall lysis that are produced by *Trichoderma* species in order to increase plant defence against the pathogen infection [9,10].

On the other hand, competition on nutrients and space could be used by a potent antagonistic strain. As a result, this may later lead to reduce the virulence of the pathogen [11].

Evaluation of those modes of action serves to predict the reliability of *Trichoderma* spp., as well as estimate their potential in the biocontrol of many diseases of different crops [12].

The aim of this study is to investigate the ability of a *Trichoderma* isolate to produce volatile and non-volatile compounds as well as study the competitive power applies against *F. oxysporum* f. sp. *lycopersici*. That is why, in vitro approaches by direct and indirect confrontations of two fungus, biological and pathogen, are used to investigate the potency of *Trichoderma* isolate in competition, antibiosis and mycoparasitism mechanisms.

II. MATERIAL AND METHODS

A. Pathogenic fungal material

In earlier study, *F. oxysporum* f. sp. *lycopersici* pathotype is isolated from leaves of tomato (Chifa hybrid) that presented typical symptoms of disease [13]. High pathogenicity of this fungal agent is recorded on infected tissues after an intense downpour.

B. Antagonistic fungal material

Trichoderma strain is isolated from leaves of tomato (Berberana hybrid). This later is conducted under a greenhouse installed in National Institute of Agronomic Research, station of Sidi Mehdi, Touggourt, Algeria.

C. Antagonism evaluation

1. Competition (direct confrontation)

This mechanism is tested according to the method described in [14]. In 90 mm Petri dish containing PDA-agar, two mycelial explants of 5 mm of diameter, one is carrying *Trichoderma* and the other is carrying the pathogenic fungus, are placed along a diametrical axis of 30 mm. After mycelia growth of fungus, microscopic observations on confrontation area are carried out in order to investigate spore morphology and hypha interaction between fungi.

2. Mycoparasitism (diffusion of non-volatile compounds)

According to the method detailed in [15], a sterile cellophane sheet are laid on Petri dish previously containing PDA-agar. *Trichoderma* explant of 5 mm of diameter are deposited in the center of plate then stand for three days in order to excrete and diffuse the non-volatile compounds, through the cellophane sheet, on the plate culture before any installation of the pathogenic agent. At the end of time, the cellophane membrane carrying the *Trichoderma* explant is removed and the disk of *F. oxysporum* f. sp. *lycopersici* of 5 mm has been immediately deposited in the center of the Petri dish to control its mycelia growth.

3. Antibiosis (emission of volatile compounds)

This test is conducted according to the method described in [16]. A mycelia disc of 5 mm of diameter, taken from a pure culture of *Trichoderma*, is deposited in the center of a Petri dish containing the PDA. The same is realized for the pathogenic agent. Then the two bottoms of plates are attached to each other using a parafilm.

One single control for all the three tests is conducted. A pathogen disc of 5 mm is placed in the center of Petri dish containing previously PDA medium. Tests are replicated five times, incubated at 26 °C and achieved when all control batches are filled.

D. Inhibition rate of pathogen fungus

At the end, relative inhibitory activity is calculated by the following formula [14]:

$$R = (A - B) / B \times 100$$

Where:

R: inhibition rate (%); A: mean diameter of colonies in presence of *Trichoderma* (mm); B: mean diameter of control colonies (mm).

E. Statistical analysis

Means of latest diameter measurements of pathogenic fungus colonies, in optimal inhibition, are described statistically and treated according to Shapiro-Wilk normality and Levene's homoscedasticity test. The effect of *Trichoderma* on the mycelia development of pathogenic fungus is verified on five repetitions in each treatment: T₁ (competition), T₂ (mycoparasitism), T₃ (antibiosis) and T_m (control). All data are treated with ANOVA at a significance level of (P < 0.05) by using SPSS (version 20).

III. RESULTS AND DISCUSSIONS

Trichoderma is an efficient fungistatic agent control against *F. oxysporum* f. sp. *lycopersici*. It is able to inhibit mycelia growth of vascular fungus in all of three studied approaches. Interesting values of inhibition of the order of 56, 65 and 70% are respectively obtained with T₃, T₁ and T₂ mechanisms. Statistical analysis proved this significant inhibition in all of tests with $F(3,16) = 38.13$, $P = 0.001$. Results revealed a credible null hypothesis that the development of colonies has a significant normal distribution under all the applied treatments. Over 4 days, descriptive statistics results showed that T₂ comes in first position with daily mean of 2.48 ± 0.46 (min = 2; max = 3). Values obtained of 2.92 ± 1.42 (min = 0.60; max = 4) and 3.60 ± 1.35 (min = 1.80; max = 5) respectively with T₁ and T₃ while to control measure was of the order of 8.5 ± 0 (min = 8.5; max = 8.5). A Shapiro-Wilk's test manifested significant values: $P = 0.01$, 0.25 and 0.40 recorded respectively with the applied treatments: T₁, T₂ and T₃. Likewise, significant Levene's test based on means assumed the null hypothesis that there is homogeneity of variances between tests with a significance of the order of $P = 0.06$.

Moreover, there were particular obvious mechanisms to eliminate this pathogen because the latter is revealed more sensitive to T₁ and T₂ than T₃. Among the three modes of action of applied *Trichoderma*, mycoparasitism is the most effective mechanism against the *Fusarium* wilt of tomato.

It revealed that the development of *F. oxysporum* f. sp. *lycopersici* colony didn't really started until the sixth day of its submission to the excrements of *Trichoderma* diffused into the medium. Beyond this period, color and aspect of all

the colonies of pathogen are altered to the wet and blue mycelium “Fig. 1. B.” compared with the control white mycelia “Fig. 1. A.”. This note is confirmed in others studies [17,18] where authors reported alterations in cultural characteristics of pathogens like color, moist aspect and scanty sporulation. In addition, microscopic observations revealed morphological alteration of macroconidia that are turned to inflated sticks with visible slight swelling in the middle.

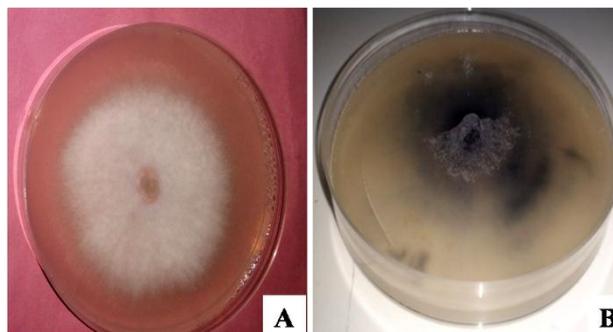


FIGURE 1. Morphological alteration of color and aspect of *Fusarium oxysporum* f. sp. *lycopersici* colony in mycoparasitism test A. Control pathogen B. pathogen exposed to *Trichoderma* excrements.

This observation is also noted with pathotype colonies in competition test where *Trichoderma* sporulated on the pathogen in later stage of interaction. Confrontation area resulted in this test presented in-tense yellowing due possibly to enzymes diffused by the antagonistic against the pathogen. Microscopic observations over this zone revealed an invasion of *Trichoderma* hyphae as ropes that coiling the pathogen mycelia. In fact, *Trichoderma* seems to sense the presence of target hyphae fungi, coils around them to degrade their cells walls [16,19] and produces some peroxidase, polyphenol oxidase and phe-nylalanine ammonia lyase [20,21]. In addition, modest inhibition rate of *F. oxysporum* f. sp. *lycopersici* colony is recorded by antibiosis mechanism test. Result explained that pathogen exerted probably a tiny resistance against *Trichoderma* species. But this hypothesis is denied because at the end of test, *Trichoderma* is able to invade the bottom bearing the pathogen and turn the antibiosis mechanism to a competition level. However, using in vitro assays to check the potential of an antagonistic strain is questionable. Authors in their work [11], mentioned that secondary metabolites, produced by *Trichoderma* in laboratory conditions, probably may be influenced by in vivo culture.

IV. CONCLUSION

The study of the *in vitro* inhibitory effect of *Trichoderma* against *Fusarium* wilt of tomato is an essential approach to valorize this strong antagonistic power that will subsequently be able to effectively eliminate a fungal disease in greenhouses conditions. This potency is referred to non-volatile antimicrobial peptides (peptaibols) and volatile metabolites belonging to various structural classes namely: mono and sesquiterpenes, pyrones, alcohols, ketones, lactones, esters or organic C8 compounds. Results must be reinforced by *in vivo* tests where mechanisms are dependent to severity of pathogen used, target organism, and environmental conditions.

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