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BIOLOGICAL CONTROL OF *FUSARIUM* CROWN AND ROOT ROT DISEASE OF TOMATO BY *TRICHODERMA HARZIANUM* IN THE WEST OF ALGERIA

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ABSTRACT
Fusarium oxysporum is a mushroom of telluric origin very ubiquiste, which presents a very big genetic and ecological diversity and which has the capacity to cause diseases on numerous botanical species cultivated by economic interest. Crown and root rot disease of tomato caused by *Fusarium oxysporum* (Schlechtendahl: Fries) f. sp. *radicis-lycopersici* (Jarvis and Shoemaker) is the most frequent disease observed in Algeria west tomato production areas and the pathogen seriously threatens greenhouse tomato production. FORL is able to invade the entire vascular system of the plant causing its obstruction and subsequently the weakening of the plant eventually dies. *Trichoderma harzianum* was tested to determine its effect on the mycelial growth of *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) in dual culture and its control of root rot disease on tomato seedlings grown in pots.

KEY WORDS: *Fusarium oxysporum*, *Trichoderma harzianum*, tomato, *Fusarium oxysporum f. sp. radicis-lycopersici*.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the world's most cultivated vegetable crops, and China is by far the first world producer with a little more than a quarter of the total (33.6 million tons); production was essentially intended (approximately 85%) for the internal market for the consumption cool (expenses). China is followed by five countries producing more than five million tons: the United States, Turkey, India, Egypt, Italy and Iran. Tomato plants are affected by several diseases including *Fusarium oxysporum* Schltdl. which is a soil borne fungus that includes both non-pathogenic and pathogenic strains. *Fusarium* wilt, caused by *F. oxysporum f. sp. lycopersici* (Sacc.) W.C. Snyder and H.N. Hansen, which can cause serious economic losses and *Fusarium* crown and root rot (FCRR) caused by *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) (Jarvis and Shoemaker) is one of the most damaging soil-borne diseases of tomato and becoming more common in greenhouse tomato production. Early symptoms of the disease in seedlings include stunting, yellowing and premature loss of cotyledons and lower true leaves. Methods used to control vascular wilt are either not very efficient and difficult to apply. The best way to control the disease is by selecting resistant varieties of tomatoes or by employing biological control with several antagonists already known to fight FCRR. However, biocontrol agents such us *Trichoderma harzianum and Trichoderma viride* (Perveen & Boukhari, 2012), non-pathogenic *Fusarium* (Gbongué *et al*., 2012; Diabate *et al*., 2013), *Bacillus subtilis* (Chen *et al*., 2008; Baysal *et al*., 2009) and *Pseudomonas fluorescens* (Ramamoorthy *et al*., 2002; Kamilova *et al*., 2006) have been reported to reduce the incidence of FCRR.

MATERIALS &METHODS

Fungal isolates

Eighty stumps of *F. oxysporum* and four strains of *F. oxysporum* f. sp. *radicis lycopersici* were recovered from tomato plants showing typical symptoms of *F. wilt and F.* crown and root rot in various western regions of Algeria during 2007 to 2009 (Table 1). All strains were isolated on potato dextrose agar (PDA) and incubated at 28°C. Plates were regularly checked for 1 month to confirm isolate identification and culture purity.

TABLE 1. Pathogen and antagonist isolates used in this study

Species	Isolate	Origin	Isolation year
	F1	Mascara	2007
F. oxysporum f. sp. *radicis-lycopersici* FORL	F4	SBA	2007
	F7	Oran	2007
	F8	Mostaganem	2007
Trichoderma harzianum	Trh1	Soil	2008
Trichoderma harzianum	Trh2	Soil	2008

Antagonist isolates

Trichoderma spp. was isolated from Algeria soil samples by using PDA medium. Samples were inoculated over plates by multiple tube dilution technique (MTDT) and the plates were incubated at 26°C for 4 days. The fungal colonies were picked up and purified by streaking and incubated at 26°C for 7 to 8 days.

Pathogenicity test

The determination of the special shape of the pathogen was realized by the pathogenic test using a series of different cultivars of tomato: Saint Pierre (dory), Heintz, Rio grande and Dijon. Cultures from 5-day-old on a solid medium of potato dextrose broth were scraped and rinsed with deionised water. Conidia suspension was adjusted to about 1×10^6 spores/ml for FORL. Tomato seedlings were grown in vermiculite for 2 weeks when they reached the stage to spread out on sheet. The root dip method of inoculation was employed as described by Hibar *et al*. (2005). The disease severity was recorded on 0 to 3 visual scales, according to Vakalounakis and Fragkiadakis (1999): 0, No symptoms; 1, Light yellowing of leaves; light or moderate rot on taproot and secondary roots and crown rot; 2, moderate or severe yellowing of leaves with or without wilting, stunting, severe rot on taproot and secondary roots, crown rot with or without hypocotyls rot, and vascular discoloration in the stem; 3) Dead seedlings. Disease incidence was determined using the following formula (Song et al., 2004):

$$\text{Disease incidence (\%)} = \frac{\sum \text{scale} \times \text{number of plants infected}}{\text{Highest scale} \times \text{total number of plants}} \times 100$$

The antagonistic effect of *T. harzianum in vitro*

Direct confrontation

This technique consists of placing two active mycelia disc (6 mms in diameter) in the same plate containing PDA medium, one carrying *T. harzianum* or *T. viride* and the other the *F. oxysporum* f. sp. *radicis- lycopersici*. The two pastilles are placed along a diametrical axis to 3 cm in the center of the plate (Figure 1); the sowing of stumps was made at the same time (Benhamou and Chet, 1996; Hibar *et al*., 2005; Perveen and Boukhari, 2012). Incubation was carried out at 25°C during six days. Notations concerning the inhibition of the diametrical growth of the colonies of *F. oxysporum* f. sp. *radicis-lycopersici* and their invasion by the mycelium of *T. harzianum* were carried out every two days. Moreover, microscopic observations relating to the direct effect of the antagonistic agent on the state of the mycelium of *F. oxysporum* f. sp. *radicis-lycopersici* was made. The control consists of a transplanting the pathogenic isolates in the center of the box.

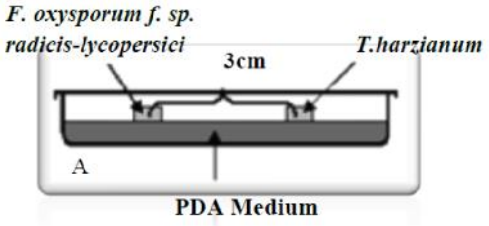

FIGURE 1. Equidistant confrontation of *F. oxysporum f. sp. radicis-lycopersici* and *T. harzianum* (Hibar *et al*., 2005).

Indirect confrontation

This method consists in mending the antagonist and the pathogenic one in two separate boxes; thereafter, an assembly is carried out by superposition of the two boxes; *Trichoderma* on the bottom and *Fusarium* on top (Figure 2). The junction between the two boxes was ensured by layers of Parafilm in order to avoid any loss of the volatile substances (Daami-Remadi and El Mahjoub, 2001; Hibar *et al*., 2005).

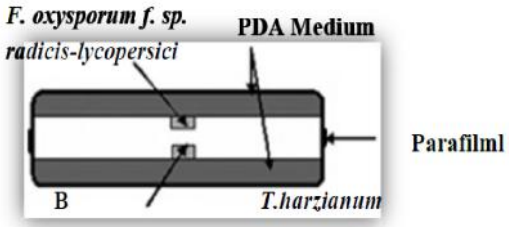

Figure 2. Remote confrontationbetween *F. oxysporum f. sp. radicis-lycopersici* and *T. harzianum* (Hibar *et al*., 2005).

The control is formed by the superposition of two boxes: the upper part containing a pellet of *F. oxysporum* f. sp. radicis-lycopersici, whereas the lower part contains only the PDA medium. The evaluation of the inhibition exerted per *T. harzianum* was estimated by the calculation of the percentage of inhibition of the mycelia growth according to the formula (Singh *et al*., 2002)

$$[I\ (\%) = (C - T/C) \times 100]$$

Where, I = Inhibition (%), C = Colony diameter in control plate and T= Colony diameter in treated plate.

The antagonism effect of *T. harzianum in vivo*

In this experiment, for evaluating of the effect of Trichoderma isolates on tomato seedling vigor and growth improvement, four steps are very necessary to keep track of.

Preparation of seedlings to be inoculated

The seeds of every cultivar (Table 02) are superficially disinfected by soaking in some ethanol absolved within 5 min, then rinsed abundantly in the sterile distilled water to eliminate the rests of pesticides used in treatment of seeds.

TABLE 2. Tomato Cultivars.

Cultivars	Sensible/Resistance
Saint pière	S
Heintz	S
Riogrand	S
Dijon	R

After drying, seeds are aseptically put in sterile Petri dishes containing filter papers soaked with sterile distilled water, at the rate of 30 seeds distributed uniformly on all the surface of the box; the seeding of seeds is assured further to the incubation of these boxes in a steam room settled in 20°C during four in five days. Once these pre-germinated, the transplanting of seeds is realized in jars containing some beforehand disinfected peat.

Preparation of conidia suspension

For the preparation of the spore suspension, 5 mm diameter mycelia disc of *Trichoderma* or FORL was centrally placed on potato dextrose agar medium PDA in the Petri dish then incubated at 25 ± 1°C for 7 days. After the incubation period, 30 ml of double distilled water was added to each isolates for the purpose to scrape and filter the mycelium which contains spores. The concentration of antagonist and pathogen spores was counted using haemocytometer and was adjusted to 10^6-10^7 (sp/ml) spores per ml.

Treatment of seedlings

The transplanting of the seedlings of every cultivar of tomato is realized when the latter affect the stage two well spread leaves.

Tomato plant treatment

Antagonists were added to the media (a mixture of peat and perlite) one week before the pathogen spores were added (Hibar *et al*., 2005). Tomato plants treated with FORL were used as controls. All treated tomato plants were put on growth in a cell of greenhouse glazed under a temperature of approximately 23°C and a photoperiod of 12 h. Three rehearsals were used in this study.

The reduction of the incidence of the disease (%) was calculated according to the following formula (Song *et al*., 2004):

$$\frac{\text{Incidence of the disease of witness inoculated - incidence of the disease of plants treated}}{\text{Incidence of the disease of witness inoculated}} \times 100]$$

RESULTS

All plants inoculated with FORL (F1, F4, F7 and F8) showed characteristic brownish lesions on the root system, moderate or severe yellowing of leaves and stunting, severe rot on taproot and secondary roots, crown rot and vascular discoloration in the stem when we spent a sensitive cultivars of tomato (Heintz, saint pièrre and Riogrande) but no symptoms appeared when resistance cultivar was used (Dijon) in the inoculation of plants.

Direct interaction between *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *T. harzianum*

The direct interaction of *T. harzianum* and *F. oxysporum* f. sp. *radicis-lycopersici* showed a faster growth of *T. harzianum* than isolates of *F. oxysporum* f. sp. *radicis-lycopersici*. At the end of three days of incubation, the box was entirely invaded by the antagonist, while the isolates of *F. oxysporum* f. sp. *radicis-lycopersici* occupied only a surface 1. 7 cm in diameter; what corresponds to an inhibition of the mycelial growth superior to 78%. The witness (only FORL) occupied a surface about 7.9cm in diameter after 7days of incubation. In the other direct confrontation between *Trichoderma harzianum* and FORL in the same box shows the appearance a zone of inhibition which indicates the effect antagonism of *Trichoderma* by the secretion of antifungal substances which inhibit in their turn the growth of the pathogenic agent, thus we speak about antibiosis.

Indirect confrontation between *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *T. harzianum*

This technique enabled us to even remotely highlight the inhibiting effect of *T. harzianum* exerted on the isolates of *F. oxysporum* f. sp. *radicis-lycopersici*; this effect is evaluated by measurement of the diameters of the colonies of pathogen in presence or absence of the antagonist (Figure 3).

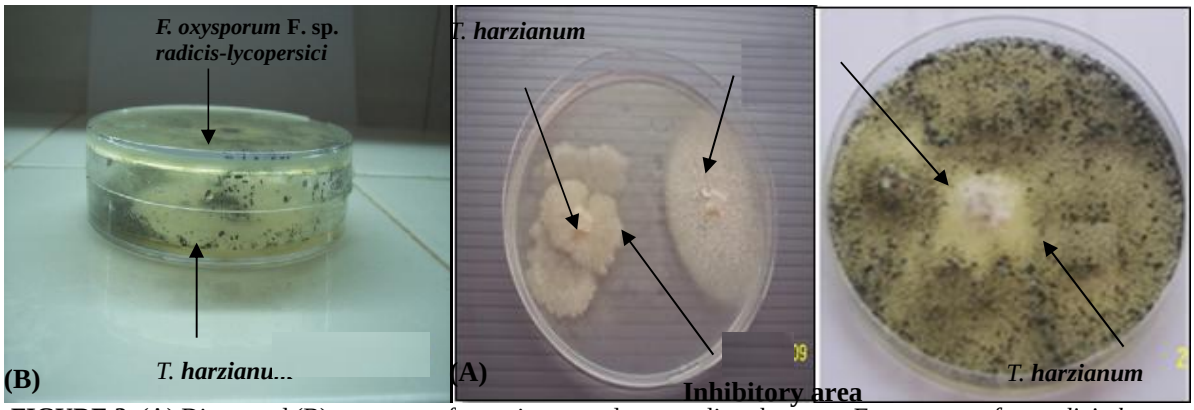

FIGURE 3: (A) Direct and (B) remote confrontation on culture medium between *F. oxysporum f.sp.radicis-lycopersici* and *T. harzianum*

Effect of *T. harzianum* on the incidence of the disease

The observation of the state of the seedlings mended in the mixture of peat and pearlite inoculated by the antagonist and the pathogenic, compared to those of the healthy witness (not inoculated and untreated), showed that the seedlings treated per *T. harzianum* present a more

significant vegetative development (Figure 4) with 77% reduction of the incidence of the disease. However, for the seedlings only mended in the mixture inoculated by the pathogenic one, the seedlings show a higher aggressiveness degree of the sensitive cultivars.

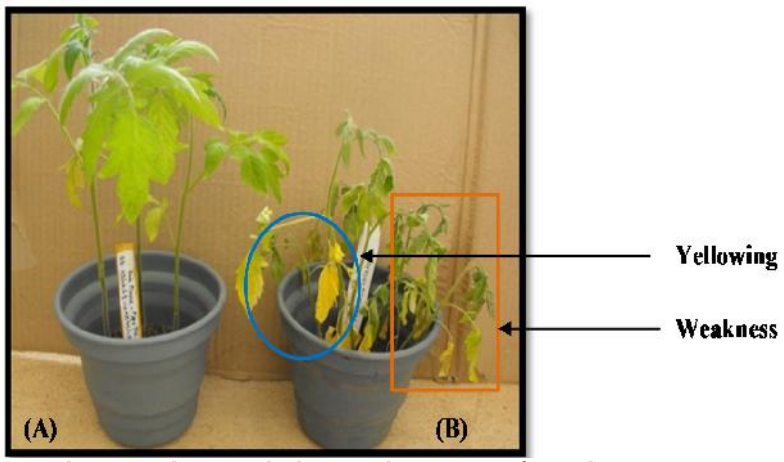

FIGURE 4. Comparison between plants pricked out in the mixture of inoculum. (A), *Fusarium Oxysporum* f. sp. *radicis-lycopersici* (B), *T. harzianum* and the pathogenic FORL.

DISCUSSION

The application of *Trichoderma* species can control a large number of foliar and soil borne fungi that is *Fusarium spp., R. solani, Pythium spp., S. sclerotium, and S. rolfsii*, in vegetables, field, fruit and industrial crops (Ngo *et al*., 2006; Trun, N. Ha 2010). Several authors have attributed the inhibition or destruction of pathogen mycelia to one or several by *Trichoderma* spp. Daami-Remadi (2001) showed the strongly opposing effect of the *T. harzianum* towards *Fusarium* responsible for the dry rot on tubers of potatoes. This inhibition was more marked (about 93 %) if the antagonist was brought in the form of a conidia suspension in the culture medium. Benhamou and Chet (1996) showed a deterioration of the mycelium of *Sclerotinium rolfsii* caused per *T. harzianum*, resulting in an aggregation, a retraction and a vacuolization of the cytoplasm which illustrates the highly mycoparasitic capacity well that *T. harzianum* has. Similar results were obtained by *Hibar et al.* (2005) by comparing plants of tomato inoculated by *F. oxysporum* f. sp. *radicis-lycopersici* and by *T. harzianum* with others inoculated by pathogenic alone.

In the same way, Btissam *et al*. (2013) tested the effect of *T. harzianum* on the abolition of the verticilliose of the tomato and they showed that the isolates of *T. harzianum* which were used in their study, although effective, led to a reduction of the disease of more than 69%; remain the least favorable treatments. Other studies (Osorio-Hernández *et al*., 2011) mentioned that the inhibition of *P. capsici* mycelia growth induced by the volatile compounds produced by *Trichoderma* strains ranged between 4.3 to 48.8%. In addition, Michel *et al.* (2004) stated that *Trichoderma* spp. produce volatile compounds like 6-pentyl-α-pyrone (6PP). These authors mentioned that as 6pentyl-α-pyrone (6PP) concentration increases so does the *Fusarium spp*. mycelia inhibition (2 to 42% of inhibition). Also, LeLay *et al*. (2007) tested six *Trichoderma* strains that inhibited *Rosellinia necatrix* mycelia growth in a range of 14 to 27%; this inhibition was attributed to concentration of several metabolites.

Singh and Islam (2010) found in *Phytophthora nicotianae* mycelial growth inhibition caused by the antagonistic strains of *Trichoderma* spp. with 61% reduction in radial growth of pathogen over control which indicates that among these isolates there are physiological differences, and these variations could be due to the mechanism involved in the antagonistic activity by differential secretion of antifungal substances. Srivastava and Tiwari (2003) reported that seed treatment with *T. viride*, followed by its soil application, reduced damping-off disease in onion seedlings. Alike experiments tested in *T. harzianum* inhibited the growth of *Fusarium* basal rot infection of onion isolate at the rate of 73. 3% in dual culture under pot and field conditions (Coşkuntuna and Özer, 2008). Similar results with other fungi had been reported by Moayedi et al. (2009). When inoculated with *phytophthora root rot* of Sugar Beet isolates caused by *P. drechsleri* and *P. cryptogeaalone*, roots became extensively discolored, both externally and internally 2-4 weeks after inoculation. Roots inoculated with both *phytophthora root rot* and *Trichoderma (T. asperellum, T. atroviride, T. brevicompactum, T. harzianum, T. longibrachiatum, T. spirale, T. tomentosum and T. virens)* isolates showed less discoloration than the control. Our results agree with those published by Ezziani *et al.* (2009) who showed that *T. harzianum* represented a clearly antagonistic effect against *P. capsici*, especially on PDA medium enriched with laminarine-glucose (3:1,v/v), which is reported to increase the antifungal activity through secretion of the hydrolytic enzyme, β-1,3 glucanase. The intensity of *P. capsici* inhibition by *T. harzianum in vitro* varied with the culture medium, temperature and pH. Among *Trichoderma* species, therefore, *T. harzianum*-8, *T. atroviride* PTCC5220 and *T. longibrachiatum* PTCC5140 showed the highest biocontrol activity against the two strains of phytopathogenic *Sclerotinia sclerotiorum* (S1 and S2) tested by Matroudi *et al.* (2009).

CONCLUSION

This study shows the definitely antagonistic effect of *T. harzianum* against *F. oxysporum* f. sp. *radicis-lycopersic*i, responsible for the crown and root rot of tomato. Indeed, tests of confrontations between *F. oxysporum* f. sp. *radicis-lycopersici* and *T. harzianum* that it is in a direct way on culture medium or remotely, revealed an inhibition of the mycelia growth of pathogenic tested. That proves that in addition to, its capacity myco-parasitic, *T. harzianum* can act by the secretion of volatile or enzymatic substances which are able to stop the development of the disease-causing agent remotely. In the case of seedlings treated by *T. harzianum*; the addition of this agent to the substrate of culture stimulated the vegetative growth of the tomato seedlings. While being based on these results, it is of paramount interest to use *Trichoderma* spp. as a biological agent to fight against the *Fusarium* crown and root rot of tomato caused by *F. oxysporum* f. sp. *radicis-lycopersici.*

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