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The Effect of in Vitro and in Vivo Trichoderma Sp (TR2) on the Reduction of Infection of the Tomato Variety (Elgon) Contaminated with Fusarium Oxysporum F. Sp .Lycopersici

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ABSTRACT

In vitro antagonistic activity was studied according to the method of confrontation by direct contact on culture medium, between Fusarium oxysporum f. sp. lycopersici and Trichoderma sp that have been considered as biological control agents against fungal diseases of plants. It could inhibit 95.09% from the mycelial growth of Fusarium oxysporum f. sp. lycopersici in comparison with test reference during seven days of the incubation at 25 ° C. Interesting results were also obtained in vivo: spraying tomato plants with a spore suspension of Fusarium oxysporum f.p. lycopersici and Trichoderma sp reduced the incidence of root and neck fusariosis compared to the untreated plants and inoculated ones by the pathogen. In addition, plants treated with Trichoderma sp had a greater vegetative development.

Keywords: *Fusarium oxysporum f. sp. lycopersici, Trichoderma sp, biological control, antagonist, Lycopersicum esculentum MILL.*

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1. INTRODUCTION

Biological control involves the use of beneficial micro-organisms in order to attack and control plant pathogens and the diseases they cause. It is an acceptable environmental approach to disease management. Among the fungal biological control agents, *Trichoderma spp* has been the most important one (Papavizas 1985, Sreenivasaprasad & Manibhushanrao, 1990).

Trichoderma spp are fungi that exist all over the world. Recent studies have shown that they are not only parasites of fungal plant pathogens, but they can also produce antibiotics. In addition, some strains can induce systemic and localized resistance to several plant pathogens, and enhance plant growth and development (Ha, 2010). Fusarium wilt is a vascular disease caused by *Fusarium oxysporum f.sp.lycopersicis*.

The latter is a telluric fungus with a strict specificity of hosts. It is able to invade the entire vascular system of the plant causing its obstruction, and eventually the weakening of the plant that eventually dies.

The objective of this study was testing the ability of *Trichoderma sp* in suppressing *Fusarium oxysporum f. sp. lycopersici* in the tomato plant under in vitro and in vivo conditions.

2. MATERIALS AND METHODS

In this experiment, the soil collected from the technical institute of vegetable and industrial crops of Oum El-Bouaghi. (Algeria) in which the geographic coordinates have been 35 ° 83'39.43 '' north latitude and 7 ° 26'62.61 '' east longitude, was used. The soil was undergone drying and sieving (5mm diameter) to obtain a particle size neither too fine nor too big. The physico-chemical characteristics of the soil showed that it had an argillaceous texture with pH 8.08.

2.1. The cultivar of tomato

The study was carried out on tomato variety (Elgon) as a host plant and *Fusarium oxysporum f. sp. lycopersici*. The choice of this variety was based on its sensitivity to this pathogen.

2.2. Fungal isolates

The isolates of *Fusarium oxysporum f. sp.lycopersici* were obtained from tomato plants showing typical symptoms of vascular wilting.

2.3. Antagonist isolates

The antagonist agent used to control *Fusarium oxysporum f. sp. lycopersici* was *Trichoderma sp* (TR2) which was obtained from the laboratory of the NIAR (National Institute of Agronomic Research Constantine, Algeria). The in vitro effect of *Trichoderma sp* on *Fusarium oxysporum f. sp. Lycopersici was examined.*

2.4. Direct confrontation

The antagonist activity of *Trichoderma sp* was tested in vitro on a culture medium, according to the method of (Hiba et al. 2005, Perveen & Boukhari 2012), by the direct confrontation

with the phytopathogenic agent. This technique involved placing the material in a Petri dish of 9 cm containing the PDA medium of two mycelial disks of 5 mm in diameter; one contained *Trichoderma sp* and the other contained the phytopathogenic agent. The two fragments were placed equidistantly from the center of the dish along a diametrical axis of 30 mm. For the control dishes, a mycelial disk of the 5 mm diameter pathogen was deposited in the center of a dish containing the PDA. The incubation was carried out at 25 ° C during ten days. The notations concerning the inhibition of diametric growth colonies of *Fusarium oxysporum f. sp. lycopersici* and their invasion by the mycelium of *Trichoderma sp* were realized every day.

The evaluation of the inhibition rate exerted by *Trichoderma sp* was estimated by calculating the percentage of inhibition of mycelial growth according to the following formula (Hamouni et al. 1996):

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

Or:

I (%): the percentage inhibition of mycelial growth.

Cn: the average diameter of the colonies in the presence of the antagonist.

Co: the average diameter of control colonies.

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

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

Or:

VCM: mycelial growth rate in mm / hour.

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

Te: the incubation time.

The in vivo effect of *Trichoderma sp* on the vegetative development parameters of tomato variety (Elgon) contaminated with *Fusarium oxysporum f. sp. lycopersici.*

2.5. Preparation of inoculated seedlings

The disinfection of seeds was carried out according to the technique of Messiaen et al. (1991), by soaking them in alcohol (70%) for 1 min, and then rinsing them firstly and abundantly in a sterile distilled water to eliminate the rests of the pesticides used in the treatment of seeds (Benhamou et al.1997), secondly in sodium hypochlorite solution (2%) for 10 min, and finely four times with sterile distilled water. After that they were dried by a filter paper.

The experiment was realized at the T I IVC (Technical Institute of Industrial Vegetable Crops) under a greenhouse.

The semi was carried out in cell plates containing peat which was previously disinfected.

The plants were grown in a greenhouse at 23 °C.

The transplantation of seedlings of each tomato cultivar was carried out when they reached the well-spread two-leaf stage (Woo et al.1996).

The seedling transplantation was carried out in nursery plastic bags of 20 cm in high and 13 cm in diameter and with a capacity of 1 kg. The bags were filled by the substrate (1/3 soil +1/3 peat + 1/3 of sand), and in each bag, a seedling was put. A daily watering during the period of the experimentation was carried out.

2.6. Preparation of inoculum

Monosporous culture of *Fusarium oxysporum f. sp. lycopersici* was seeded on PDA medium and incubated at

25 ° C for 7 days. The contents of each dish were milled for 30 seconds in 50 ml of sterile distilled water, and then they were filtered through a layer of the filter paper (Zaim et al.2013). The concentration of the suspension in *Fusarium oxysporum f. sp. lycopersici* conidia (macro-and microconidia), was adjusted by the dilution with sterile distilled water to give a concentration of 106 conidia / ml, that was found to be sufficient to cause the symptoms of the disease (Westerlund et al. 1974).

By the same technique, the concentration of the conidial suspension of *Trichoderma sp* (TR2) isolates was adjusted to 108 conidia / ml that was found to be sufficient for the protection (Bailey et al. 2008).

2.7. The measured parameters

After 26 days of treatment, the vegetative growth parameters were measured:

a) **The height of the stem (HS, in cm):** It was measured from the neck to the upper end of the plants;

b) **The length of the root system (LR in cm):** The root system of the tomato plants was thoroughly rinsed with clear water and dried with filter paper. LR was measured from the neck to the lower end of the main root;

c) **The fresh weight of the aerial part and the roots (FWA and FWR, in g):** The aboveground and underground biomass of the plants were weighed fresh.

d) **The dry weight of the aerial part and the roots (DWA and DWR, in g):** The aboveground biomass and the root mass of the plants were estimated after they were carefully rinsed with water, dried with filter paper then placed at oven at 75 ° until the weight was stabilized.

e) **The root fineness (RF, in cm / g):** The ratio of the length of the root system by the weight of the dry matter of plant roots was estimated (Windhem et al. 1986 cited by Ben Mebarek 2011).

2.8. Statistical processing of data

The statistical significance of the results obtained during this experiment was tested by variance analysis which was carried out using IBM SPSS statistics 24. The least significant difference was employed to test the significant difference between treatments at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Direct confrontation on culture medium between Fusarium oxysporum f. sp. lycopersici and Trichoderma sp.

The results of the direct confrontation between *Trichoderma sp* and the pathogen, showed that the mycelial growth of the control strains was more important compared to those obtained by the different confrontations (Pathogen - Antagonist).

After 10 days of incubation, *Trichoderma sp* showed a good inhibitory activity towards the test pathogenic strains, by the appearance of an area of inhibition followed by the arrested growth of all the pathogenic strains (Fig. 1).

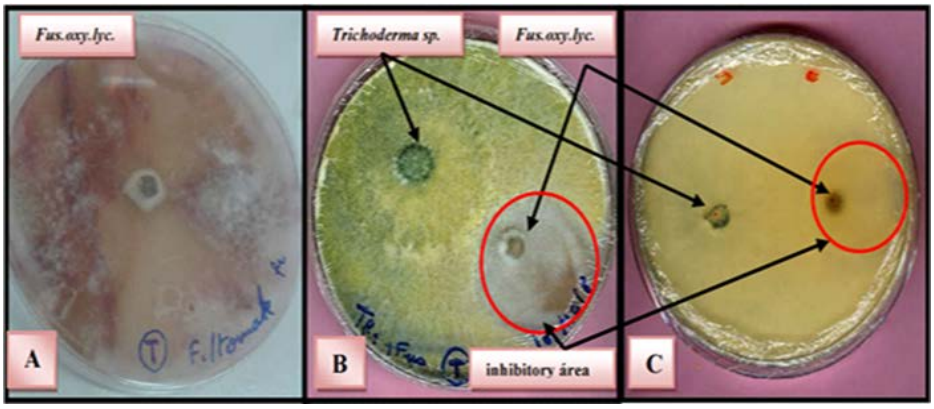

Figure 1. Remote inhibitory effect of Trichoderma sp on mycelial growth of Fusarium. oxysporum f. sp. lycopersici for an incubation period of seven days at 25 ° C. (A) control Fusarium oxysporum f sp. lycopersici (B) front and (C) back of the dish.

The mycelial growth of the pathogen was daily evaluated by measuring the diameter of the petri dish in which the radius of the pathogen was found next to the antagonist. This evaluation was realized every 24 hours for 10 days.

The simultaneous transplantation of *Trichoderma sp* isolates and others of *Fusarium oxysporum f sp. lycopersici* showed a faster growth of *Trichoderma sp* compared with the phytopathogen. The confrontation was done taking into account the rate's growth of tested pathogenic fungus. The *Fusarium* isolate occupied a diametric growth ranging only from 0.4 to 0.8 mm after the days of incubation; which corresponded to an inhibition of mycelial growth ranging from 91.11% to 97.61%, depending on the tested isolates **(Fig 2).**

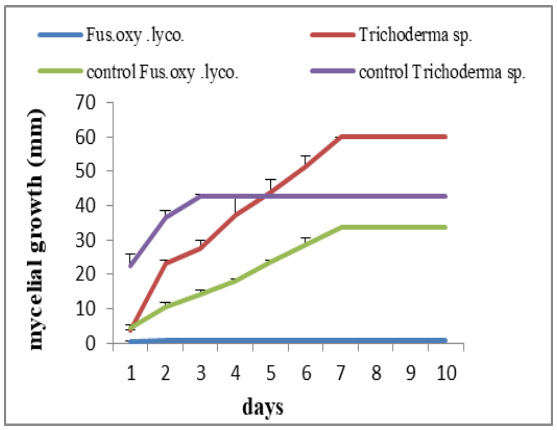

Figure 2. Influence of Trichoderma sp. On mycelial growth of Fusarium

In this test, after a maximum of 7 days, it was observed that the colonies of *Trichoderma sp* recovered those of the fungi thus revealing their inhibitory power. This situation has also been advanced by many researchers who followed the same direct confrontation technique and used the same culture medium (PDA).

Results of the research done by Barari (2016) also indicated that *Trichoderma harzianum*, isolate N-8, effectively inhibited the radial mycelial growth of *Fusarium oxysporum f. sp. Lycopersici* L-6 (68.22%). In a work on the effect of *T. harzianum* on *B. cinerea,* Bendahmane et al. (2011) obtained a recorded result of 47%, and Hamitou & Dehimat (2012) noted a value close to 56.52%. Also, Ibarra-medina et al. (2010) tested the antagonistic effect of this species on *Sclerotinia sclerotiorum*, and they marked more interesting results (between 91 and 100%) close to the results obtained in this study. In fact, *Trichoderma harzianum* has a very remarkable invasiveness because it is able to grow and sporulate even on the *sclerotia* of the phytopathogenic fungus. Hamitou & Dehimat (2012) tested this species against *Alternaria sp.,* they obtained a higher inhibition value (57%) than the value obtained in the present study (43%). This strain was also tested against *Fusarium oxysporum f. sp. radicis lycopersici* by Hibar et al. (2005), the inhibition rate reported was 65% while that obtained in this trial was only 95%.

The application of *Trichoderma* species can control a large number of foliar and telluric fungi *Fusarium spp.* Several authors have attributed the inhibition or the destruction of pathogenic mycelia to one or more fungus by *Trichoderma spp*. Daami-Remadi (2001) showed the strongly opposite effect of *T. harzianum* on *Fusarium* responsible for dry rot on potato tubers. This inhibition was more marked (about 93%) if the antagonist was brought in the form of a suspension of conidia in the culture medium.

These results were in perfect conformity with those obtained by Alabouvette et al. (1983), Dubot (1985) , Davet (1996) who showed the growth of *Trichoderma sp.* Singh & Islam (2010) found an inhibition of mycelial growth in *Phytophthora*

nicotianae caused by the antagonist strain of *Trichoderma spp*., with 61% of radial reduction of the growth of the pathogen on the control which indicated that among these isolates, there were physiological differences, and these variations could be due to the mechanism involved in the antagonist activity by the differential secretion of antifungal substances.

The current study was based on the selection of *Trichoderma* which is able to protect tomato plants vis-à-vis *Fusarium. oxysporum f. sp. lycopersici* .The use of the native fungi in biological control can not only help preserve the balance of the agrosystem, but also the character of yield. Several studies indicated that *Trichoderma* species can effectively limit the pathogenicity of *Fusarium* (Gajera et al., 2013), and the other phytopathogenic agents (Benitez et al., 2004, Carvalho, et al. 2014, Naher et al. 2014).

Sivan et al. (1987) who showed that coating tomato seeds with this antagonist reduced the attack of *Fusarium* root and crown wilt by 80% have also reported the beneficial effects of *Trichoderma harzianum*.

3.2. Rate of mycelia growth of Fusarium oxysporum f. sp. Lycopersici in the presence of Trichoderma sp

This experiment revealed that this strain was also exerted an antagonistic effect on the phytopathogenic fungi of tomato. Indeed, *Fusarium oxysporum f. sp. lycopersici* recorded the value of 0.583 mm / h **(Fig 3).** However, *Trichoderma sp* showed a higher rate of growth ranked at 20.22 mm / h.

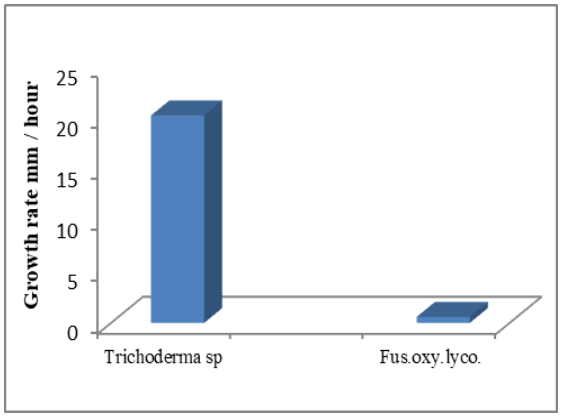

Figure 3. Rate of mycelial growth Fusarium oxysporum f. sp. lycopersici in the presence of Trichoderma sp.

3.3. The in vivo effect of some isolates of Trichoderma sp on the vegetative development parameters of tomatoes

The treatment of plants by *Trichoderma sp* has had a beneficial effect on its growth by promoting the development of it in the presence of pathogen and delaying the onset of symptoms. The observation of the state of the plants was inoculated by the pathogen and the antagonist, compared to that of the inoculated control, and showed that the plants treated with *Trichoderma sp* exhibited a greater vegetative development **(Fig 4).**

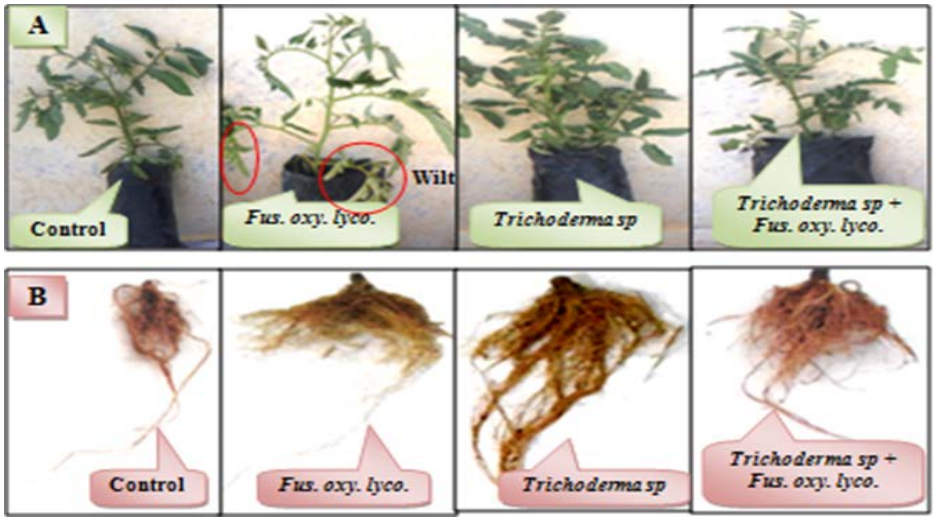

Figure 4. Comparison between plants inoculated by Trichoderma sp. and Fusarium. oxysporum f. sp. lycopersici with control . (A) growth and development of tomato plants (B) development of the root system.

According to **Figure 5,** the treatment with *Trichoderma sp* resulted an increase in the height of the plant, root length, fresh weight of the aerial part, the fresh weight of the roots, the dry weight of the aerial part, the dry weight root and fineness of the root system.

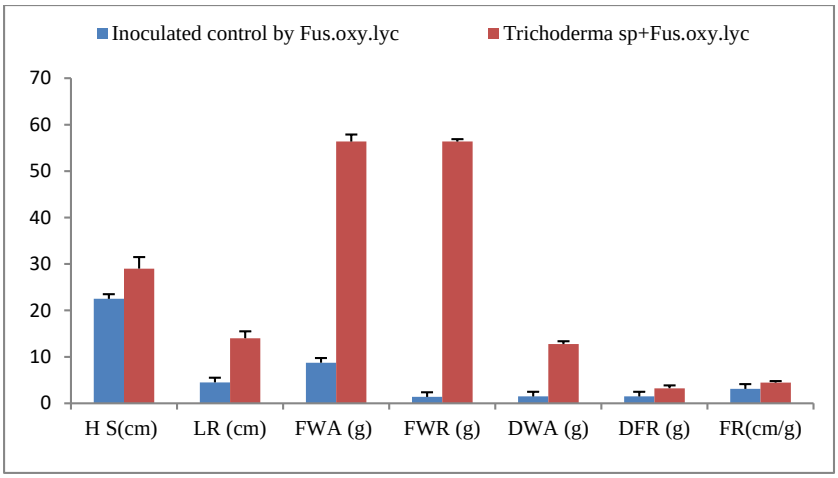

Figure 5. Comparison between plants inoculated by Trichoderma sp.and Fusarium. oxysporum f. sp. lycopersiciwith control .(A) growth and development of tomato plants (B) development of the root system.

Elgon variety of 29 cm, 14 cm, 56.37g 12.75g, 3.23 g et 4.46 cm / g sequentially compared to the inoculated control of 22.5 cm, 4.5 cm, 8.74 g, 1.38 g, 1.49g, 1.48 g and 3.11cm / g ; respectively.

It should be noted that the vegetative development parameters were measured at the end of the pot trial. The effect of *Trichoderma* on the vegetative growth parameters of tomato plant has been shown in **(Tab1).**

The variance analysis showed a quite significant effect on stem height, root length, fresh weight of aerial part, fresh weight of roots, dry weight of aerial part, dry weight of roots and root fineness.

Table 1. Variance analysis of vegetative development parameters of tomato plants treated with Trichoderma sp in the presence of Fusarium. oxysporum f. sp. lycopersici.

	Settings						
Treatments	**Height of the stem (HT cm)**	**Length of the root system (LR cm)**	**Fresh biomass of the aerial part (BFA g)**	**Fresh biomass of the root part (BFA g)**	**Dry biomass of the aerial part (BSA g)**	**Dry biomass of the root part (BSR g)**	**Root fineness (FR cm/g)**
Trichodermasp	29,00± 0,87	14,00±1,00	56,37±1,62	56,37±1,62	12,75±1,04	3,24±0,48	29,00±0,87
Inoculated control	23,67±3,28	8,00±3,10	16,04±5,77	2,71±1,26	2,290±,69	1,20±0,56	14,08±6,84
P	0,022*	0,009**	0,000***	0,000***	0,000***	0,000***	0,004**

The results of the current study showed that *Trichoderma* isolates were able to stimulate tomato plant growth parameters to varying degrees. This stimulation has mainly resulted in better axial growth and a larger biomass, which corroborated with the results of several authors (Gravel et al. 2007, Contreras-Cornejo et al. 2009, Sofo et al. 2011, Salas-Marina et al. 2011). The stimulation of biomass has been observed not only in the aerial parts, but also in the root parts. Indeed Gupta et al. (2006) showed that *Trichoderma viride* treatment of chickpea seeds improved biomass, dry weight and yield compared to the untreated control. The improvement of plant vegetative growth by *Trichoderma spp.* was observed by Windham et al. (1986) who showed that the application of two *Trichoderma* species to the sterilized culture substrate can improve the dry weight of roots and aerial parts of tomatoes and tobacco. Root fineness plays a key role in drought resistance. The flexibility of the root system is translated by the accumulation of little dry matter at root level under favorable conditions and the improvement of the growth of its root system under water deficit condition by the allocation of more dry matter (Daaloul et al. 2007 cited by Ben Mebarek 2011). This increase was noted more for fresh weight than the dry one, indicating that this was a general increase in metabolism and not just excessive water uptake. Better still; Hibar et al. (2005) compared the features of tomato plants inoculated by a pathogenic strain of *Fusarium oxysporum* and others treated by *Trichoderma harzianum* with uninoculated and untreated healthy control plants. They found that the plants inoculated with the pathogen and treated with the antagonist had greater vegetative and root development than the control plants.

4. CONCLUSION

The increase in the production of biomaterials might be due to the production of plant growth factors or indirect stimulation of nutrient uptake and siderophore or antibiotic production to protect plants from the harmful rhizosphere organisms. Therefore, the *Trichoderma* antagonist has been chosen to be the most promising bio-control agent for *Fusarium oxysporum f. sp. lycopersici*. On the basis of this study, biological control agents against plant diseases could be exploited for sustainable disease management programs to reduce the environmental

risks. *Trichoderma* antagonists were shown to have a strong ability to inhibit pathogen growth during in vitro challenge tests, and to prevent the effect of *Fusarium oxysporum f. sp. lycopersici* by a strong reduction of the disease in vivo.
This study showed that the action of *Trichoderma* tested has been not only the protection of the plant but also the stimulation of plant growth.

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