

BIOLOGICAL CONTROL OF TOMATO WILT FUSARIUM UNDER PLASTIC-HOUSE CONDITIONS

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ABSTRACT

Tomato (*Lycopersicon esculentum* Mill.) is considered as one of the most important vegetable crops in the world and Jordan. Fusarium wilt of tomato, caused by *Fusarium oxysporum* f. sp. *lycopersici* is one of the most prevalent serious diseases of tomato. The experiment was set up in a completely randomized design (CRD), in which six different treatments were used. Fresh and dry weights of shoot and root, plant heights, root colonization, disease severity, disease incidence and disease control percentage were recorded. Results indicated that there were no symptoms observed in the control plants treated with *Trichoderma harzianum* alone or in plants grown in un-inoculated soil (tap water treatment). *F. oxysporum* f. sp. *lycopersici* caused a significant decrease in plant growth of both shoot and root fresh weights. The decrease in fresh shoot weight caused by the pathogen was 44.0% and 45.8%, respectively, as compared with using *T. harzianum* as spore suspension or mycelium. Inoculation with *T. harzianum* increased shoot and root fresh weights as compared with un-inoculated plants or plants treated with *F. oxysporum* f. sp. *lycopersici* treatment. *T. harzianum* also achieved a significant increase in plant height as compared with the control. Plants height significantly decreased with *F. oxysporum* f. sp. *lycopersici* treatment. The results also showed a reduction in both disease severity and incidence in the bio-control treatment as compared with the plants treated with *T. harzianum* mycelium and *T. harzianum* spore suspension. *T. harzianum* mycelium treatment has significantly increased colonization percentage than *T. harzianum* spore suspension treatment. Healthy plants increased with application of *T. harzianum*, and this percentage increased and reached 55% and 64% with spore and mycelium treatments, respectively.

Key words: *Trichoderma harzianum*, *Fusarium oxysporum* f. sp. *Lycopersici*, biological control, tomato wilt, tomato, Jordan.

1. INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is considered as one of the most important vegetables in the world (Rick, 1979). This economic crop is considered the second commonly grown vegetable in the world and the first one in Jordan for local consumption or exportation purposes. Jordan produced 419.230 thousand tons of tomato from a planted area of about 820,890 dunums (Agricultural Statistics, 2013). However, tomato is affected by several diseases, reflecting negative effect on plant growth and yield. *Fusarium* spp. are among the most important plant pathogens in the world. Out of these, pathogenic fungi especially the wilt caused by *Fusarium* remains to be a challenging task in terms of management (Agrios, 2000 and Srinon *et al.*, 2006).

Fusarium oxysporum is responsible for wilt and cortical rot diseases of more than 100 economically important plants (Swift *et al.*, 2002). Fusarium wilt of tomato caused by *F. oxysporum* [(Schlecht.) f. sp. *lycopersici* (Sacc.)] Snyder et Hansen is one of the most prevalent serious diseases of tomato (Reis *et al.*, 2005 and Sudhamoy *et al.*, 2009).

Currently, controlling this pathogen is mainly done by chemical control (fungicides), which creates serious health hazard to applicators as well as to consumers of the treated plants. In addition to target organism, pesticides also kill various beneficial organisms. Their toxic forms persist in the soil and contaminate the environment (Hayes and Laws, 1991). Thus, alternative control measures are needed. Biological control involves the use of beneficial microorganisms, such as specialized fungi and

bacteria. Two of the major bio-control agents, which reduce soil borne diseases of various crops, include isolates of the bacterium fluorescent, *Pseudomonas* spp. and the fungus, *Trichoderma* spp. (Howell and Stipanovic, 1995).

Therefore, the objectives of the present work were set as an attempt to evaluate the relative bio-control efficiency of *T. harzianum* in controlling tomato wilt caused by *F. oxysporum* f. sp. *lycopersici*, and to investigate the effect of *T. harzianum* in plants grown in pots, and test the use of *T. harzianum* treatment to induce any like-disease symptoms or abnormalities in the plants under plastic house conditions.

2. MATERIALS AND METHODS

2.1. Fungal isolation and purification

The fungus, *F. oxysporum* f. sp. *lycopersici* was isolated from infected tomato plants obtained from the Agricultural Research Station fields, Faculty of Agriculture, Mu'tah University. The fungus was identified on the basis of their culture, morphological and microscopic characteristics. To obtain the inoculums, stem sections of wilted plants were placed onto Potato Dextrose Agar (PDA) at $25\pm 2^{\circ}\text{C}$ in the incubator. The cfu of *F. oxysporum* f. sp. *lycopersici* was obtained from the wash off the agar surface with sterile distilled water; and filtered through Whatman No.1 filter paper (Maidstone, England) and repeated three times, and washed to be free of nutrients before adding to the soil at 5×10^6 cfu/ml concentration.

2.2. Source of bio-agent and preparation

Wild type of Jordanian isolate *T. harzianum* was isolated from rhizosphere soil of infected tomato roots. The isolate was identified according to Rifai (1969), and it was mutagenized by Benomyl tolerant as described by Ahmad and Baker (1987) containing $10\ \mu\text{g}$ a.i. benomyl/ ml. Spores' suspension was obtained from culture of the fungus grown on PDA, and the plates were harvested by flooding with a sterile distilled water. The resulting suspension was then strained through cheesecloth to remove mycelia fragments, spores collected and filtered through Whatman No.1 filter paper (Maidstone, England) repeated three times, and washed to be free of nutrients before adding to the soil at 5×10^8 spores/ml (Al-Ameiri, 2007).

T. harzianum mycelium obtained from a culture of the fungus were grown on PDB. The

medium was autoclaved, then cooled, seeded with 0.5 cm of *T. harzianum* disc, and incubated at shaking incubator for seven days. The culture was then strained through cheesecloth to remove mycelia fragments, the obtained mycelium was filtered through filter paper, repeated three times, and washed to be free of nutrients before adding to the soil (Al-Ameiri, 2007).

2.3. Plastic house experiment

2.3.1. The effect of *Trichoderma harzianum* and *F. oxysporum* f. sp. *lycopersici* on tomato growth

Two week old tomato seedlings cv. GS12 averaging 5 cm in height were grown in a plastic house at the Agricultural Research Station, Faculty of Agriculture, Mu'tah University, and used in the experiment.

Plastic pots (30x25 cm) were separately filled with 5 kg of sterilized soil. GS12 (cv) susceptible to Fusarium wilt was used in the experiment; in which five tomato seedlings were planted in each pot. The pots were placed on a plastic house bench, where the temperature was $27\pm 5^{\circ}\text{C}$. Two weeks post planting, each pot was inoculated with 60 ml/pot of *F. oxysporum* f. sp. *lycopersici* (5×10^6 cfu/ml) into 3 cm deep furrows, uniformly round sides of the seedlings. The pathogen inoculums were applied after this period to avoid developing damping-off of the seedling. At the time of pathogen inoculation either *T. harzianum* (50 ml of spore suspension) with a final concentration of 5×10^8 spores/ml or mycelium (5 gm/kg of soil) were equally distributed around the seedlings in the respective treatments.

The experiment was set up in a completely randomized design (CRD), and conducted at the Agricultural Research Station, Faculty of Agriculture, Mu'tah University, in which each treatment consisted of three replicates (pots), with five plants/pot and the experiment was repeated twice. The treatments employed in the experiment were: (1) un-inoculated seedlings (drenched with tap water); (2) *F. oxysporum* f. sp. *lycopersici* only; (3) *T. harzianum* (spore suspension); (4) *T. harzianum* (mycelium) (5g /kg of soil); (5) *F. oxysporum* f. sp. *lycopersici* + *T. harzianum* (spore suspension); and (6) *F. oxysporum* f. sp. *lycopersici* + *T. harzianum* (mycelium).

The plants were carefully watered by hand daily, and fertilized with a soluble feed plant-NPK (12-10-12) at 10-day-interval after the antagonist-pathogen inoculation. After four weeks, the plants were exposed to water stress to

enhance the development of *F. oxysporum* in the vascular system and produce the symptoms of wilt.

2.3.2. The effect of *Trichoderma harzianum* on disease incidence and severity caused by *F. oxysporum* f. sp. *lycopersici*

The bio-control activity was measured by the incidence of wilt produced by the pathogen on the treated plants upon appearance of > 20 % disease symptoms on the pathogen treatment. Disease was monitored for 7 to 8 weeks and assayed as the total percentage of seedlings (disease incidence) showing any wilt symptoms resulted from the pathogen (i.e. yellowing and dropping of leaves, vascular discoloration wilting). Stem sections of wilted seedlings were surface-disinfested in 0.5% sodium hypochlorite then washed with a sterile distilled water and plated on PDA to confirm the presence of the *F. oxysporum* f. sp. *lycopersici*.

The disease severity of the pathogen infection on the plants was individually assessed at the end of the experimental time. A rating scale of 0 to 5 was used after modifying the scale of Grattidge and O'Brien (1982); where: 0: healthy plants; 1: <24% of the leaves yellowed and wilted; 2: 25–49% of the leaves yellowed and wilted; 3: 50–74% of the leaves yellowed and wilted; 4: 75–99% of leaves yellowed and wilted; and 5: 100% the dead plants. The percentage of disease severity was calculated using the following scheme:

$$\text{Disease severity}\% = \frac{\text{Sum of the rating value}}{\text{Total no. of plant/ replicate} \times 5 (\text{highest rating value})} \times 100$$

At harvesting time, the plants were collected and fresh and dry weights of shoots and roots were recorded. In addition, the colonization of *F. oxysporum* and *T. harzianum* fungus in the vascular system was determined by plating 25 segments of plant roots (5/plate), surface sterilized by immersing the root pieces in 1% sodium hypochlorite solution for 5 min, and then washed several times in a sterilized distilled water to remove any residues of sodium hypochlorite. The segments of plant roots were plated on PDA plates using the method described by El-Hassan and Gowen (2006). After 7 days, the number of segments that produced *F. oxysporum* and *T. harzianum* colonies of each plant/plate was counted using a microscope. The competitive colonization percentage (%) was calculated as follows:

CI = [number of root segments colonized by bio-control agent or *F. oxysporum*/total number of root segments] x 100. Where: CI = Colonization index.

The efficiency of endophytic *T. harzianum* (disease percentage control) was presented as a reduction percentage in colonized vascular tissues by *F. oxysporum*. The disease percentage control was calculated according to Abbot (1925) formula:

$$\% \text{Disease control} = \frac{\text{Disease index of control (pathogen)} - \text{Disease index of treatment}}{\text{Disease index of control (pathogen)}} \times 100$$

2.4. Statistical Analysis

Data obtained were statistically analyzed using MSTAT-C statistical package, disease severity, *F. oxysporum* and *T. harzianum* colonization percentage were transformed to $\sqrt{x + 1}$ to reduce heterogeneity of variances and to overcome zero readings (Steel and Torrie, 1960). Analysis of variance was determined using Least Significant Differences (LSD) test for means separation. The level of significance was calculated at a probability level of 0.05 (Clewer and Scarisbrick, 2001).

3. RESULTS

3.1. The effect of *Trichoderma harzianum* and *F. oxysporum* f. sp. *lycopersici* on tomato growth

There were no symptoms observed in the control plants treated with *T. harzianum* alone or in the plants grown in un-inoculated soil (tap water treatment). The pathogen was not isolated from tomato vascular tissues of those treatments. The perusal of data presented in Fig. (1: A and B) clearly shows that *F. oxysporum* f. sp. *lycopersici* causes a significant decrease in plant growth of both shoot and root fresh weights. The decrease in fresh shoot weight caused by the pathogen was 44.0% and 45.8%, respectively as compared with used *T. harzianum* as spore suspension and mycelium, respectively. A significant decrease (33%) in shoot fresh weight was found between pathogen and uninoculated treatments.

Single inoculations of *T. harzianum* increased shoot and root fresh weights as compared to the un-inoculated treatment and *F. oxysporum* f. sp. *lycopersici* treatment. Shoot dry weight was significantly affected by all treatments rather than *F. oxysporum* f. sp. *lycopersici* treatment (Fig. 1). There were no significant differences

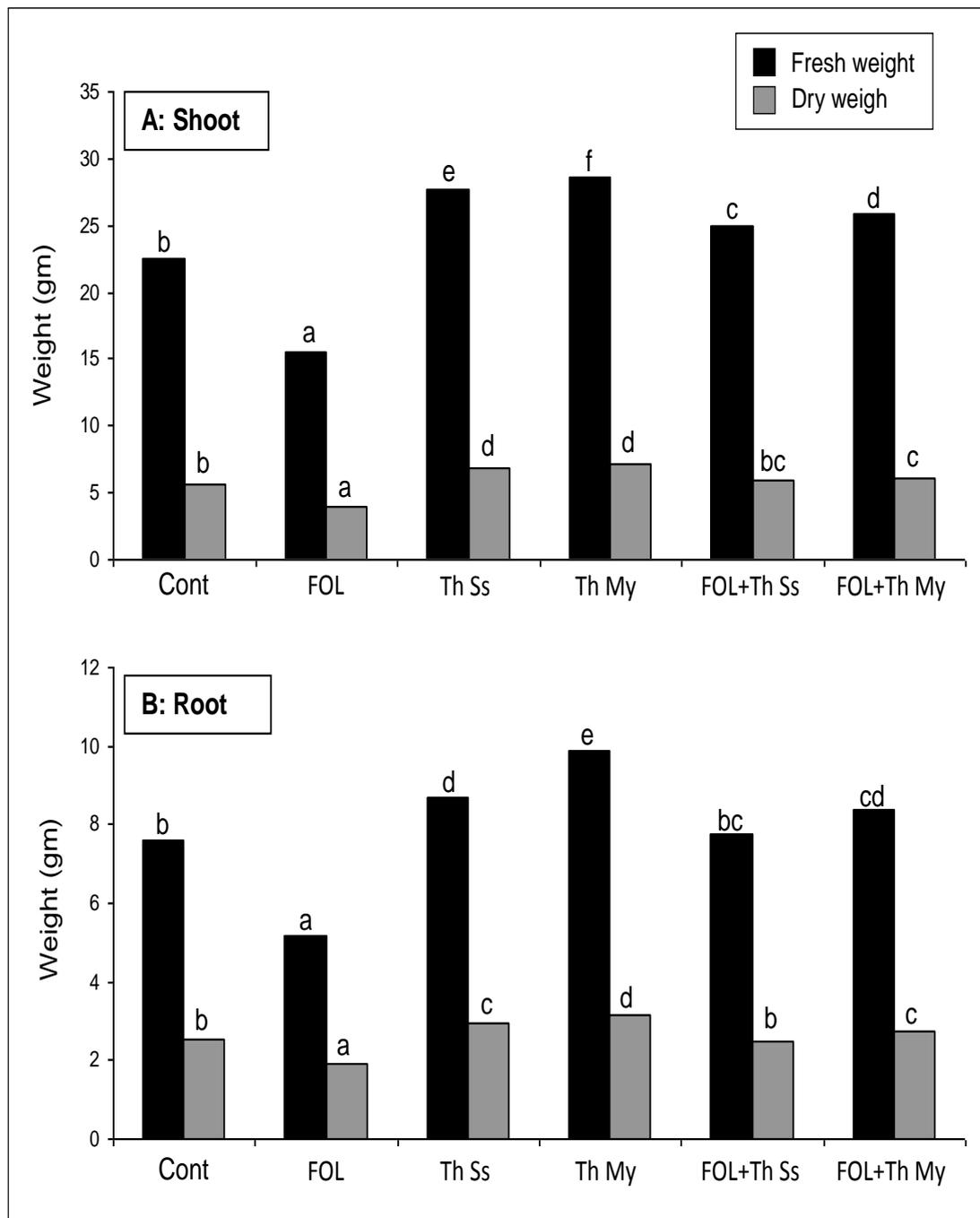


Fig. (1): Effects of *Fusarium oxysporum* f.sp. *lycopersici* and *Trichoderma harzianum* (spore suspension and mycelium) on shoot fresh and dry weights (A) root fresh and dry weights (B) of tomato. *Where: 1- (Cont) = control, 2- (FOL) = *F. oxysporum* f.sp. *lycopersici*, 3- (Th Ss) = *T. harzianum* Conidia suspension, 4 - (Th My) = *T. harzianum* Mycelium, 5- (FOL+Th Ss) = *F. oxysporum* f. sp. *lycopersici*, + *T. harzianum* Conidia suspension, 6 – (FOL+Th My)= *F. oxysporum* f.sp. *lycopersici*, + *T. harzianum* Mycelium. **Different small letters above bars indicated significant differences among the five different treatments at $p < 0.05$ (one-factor analysis of variance).

between application methods of *T. harzianum* when applied with or without *F. oxysporum* f. sp. *lycopersici*. Root dry weight showed significant differences between *F. oxysporum* f.

sp. *lycopersici* and all treatments, either a significant differences between the two applications of *T. harzianum* with or without *F. oxysporum* f. sp. *lycopersici*.

In the potted culture under plastic-house conditions, *T. harzianum* only applied into pot soil caused a high significant increase in plant height as compared to the control treatment (Fig. 2). The method of applying antagonist had a significant effect between the two. *T. harzianum* mycelium treatment, and it has increased plant height than *T. harzianum* spore suspension treatment with or without *F. oxysporum* f. sp. *lycopersic*. Plant height significantly decreased in *F. oxysporum* f. sp. *lycopersic* treatment compared with all the treatments used.

between the *T. harzianum* treatments, where *T. harzianum* mycelium treatment significantly reduced disease severity than *T. harzianum* spore suspension treatment.

The colonization percentage of root segments was not significantly different between the method of applied antagonist when the pathogen was absent. It was found that only *T. harzianum* spore suspension treatment with *F. oxysporum* f. sp. *lycopersici* present decreased significantly colonization percentage compared with the other treatments. *F. oxysporum* f. sp. *lycopersici* added

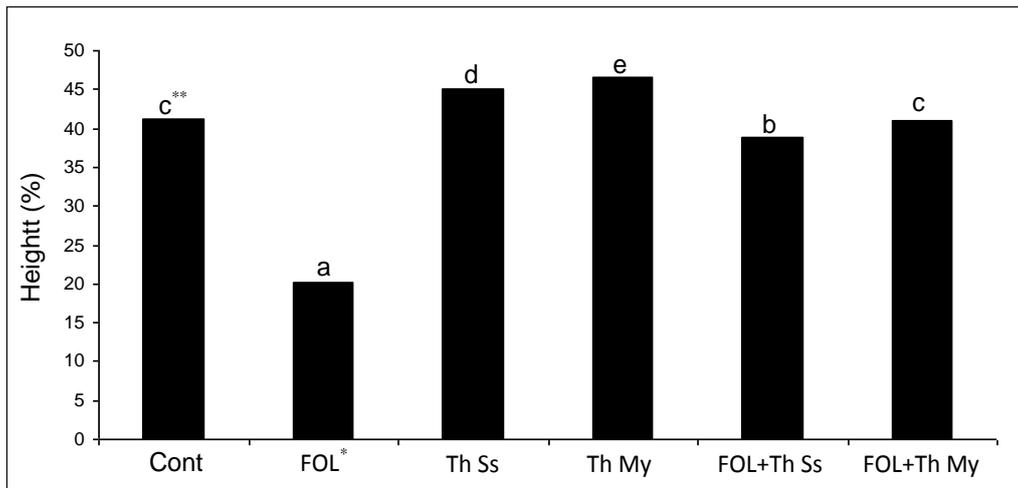


Fig. (2): Effects of *F. oxysporum* f.sp. *lycopersici* and *Trichoderma harzianum* (spore suspension and mycelium) on tomato heights. *Where: 1- (Cont)= control, 2- (FOL) = *F. oxysporum* f. sp. *lycopersici*, 3- (Th Ss) = *T. harzianum* Conidia suspension, 4 - (Th My) = *T. harzianum* Mycelium, -, 5- (FOL+Th Ss) = *F. oxysporum* f. sp. *lycopersici*, + *T. harzianum* Conidia suspension, 6 – (FOL+Th My)= *F. oxysporum* f. sp. *lycopersici* + *T. harzianum* Mycelium. **Different small letters above bars indicated significant differences among the five different treatments at $p < 0.05$ (one-factor analysis of variance).

3.2. The effect of *Trichoderma harzianum* on disease incidence and severity caused by *F. oxysporum* f. sp. *lycopersici*

The antagonist colonization percentage was developed as a general assessment of the ability of *T. harzianum* to establish an endophytic relationship with tomato plants in an attempt to protect the plants directly from the initial *F. oxysporum* f. sp. *lycopersic* infection. Isolating *T. harzianum* from vascular tissues indicated that the isolate was living inside the plant tissues and it is, therefore, an endophyte of tomato root (Fig. 3). The results clearly show a reduction in disease severity in the bio-control treatment as compared with the pathogen treatment (Fig. 4). Disease severity was significantly differed

to the soil had significantly higher colonization of root segments than the treatments that have both of pathogen and bio-control. *T. harzianum* mycelium treatment significantly increased colonization percentage than *T. harzianum* spore suspension treatment.

Bio-control agent tested significantly reduced Fusarium wilt of tomato plants (Table 1). Disease control percentage increased with application of two types of *T. harzianum*. This percentage was highly increased and reached 55% and 64% with *T. harzianum* spore suspension and mycelium treatments, respectively.

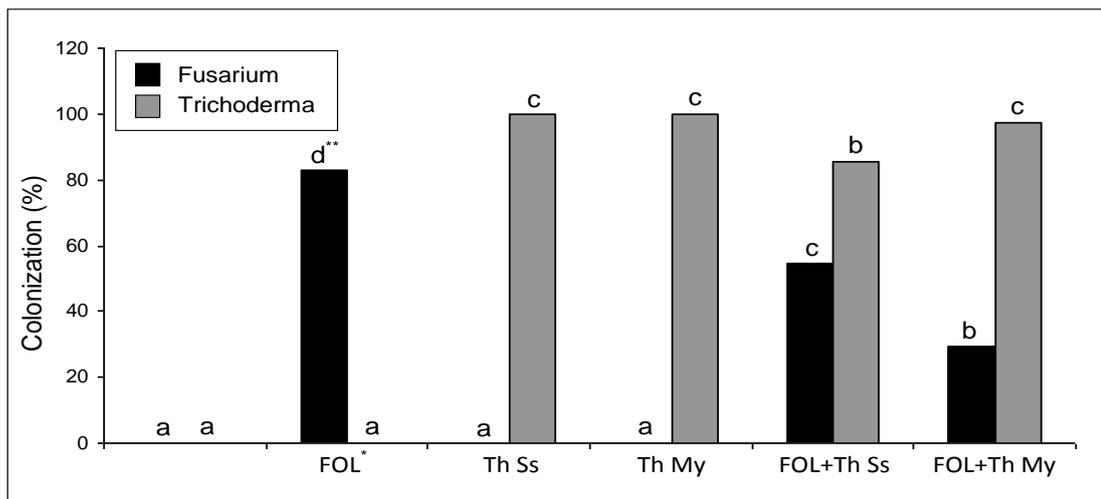


Fig. (3): Percent colonization of roots tomato plant by *F. oxysporum f.sp. lycopersici* and *Trichoderma harzianum* (spore suspension and mycelium) * Where: 1- (Cont)= control, 2- (FOL) = *F. oxysporum f. sp. Lycopersici*, 3- (Th Ss) = *T. harzianum* Conidia suspension, 4 - (Th My) = *T. harzianum* Mycelium, -, 5- (FOL+Th Ss) = *F. oxysporum f. sp. lycopersici* + *T. harzianum* Conidia suspension, 6 – (FOL+Th My)= *F. oxysporum f. sp. Lycopersici* + *T. harzianum* Mycelium **Different small letters above bars indicated significant differences among the five different treatments at $p<0.05$. (one-factor analysis of variance).

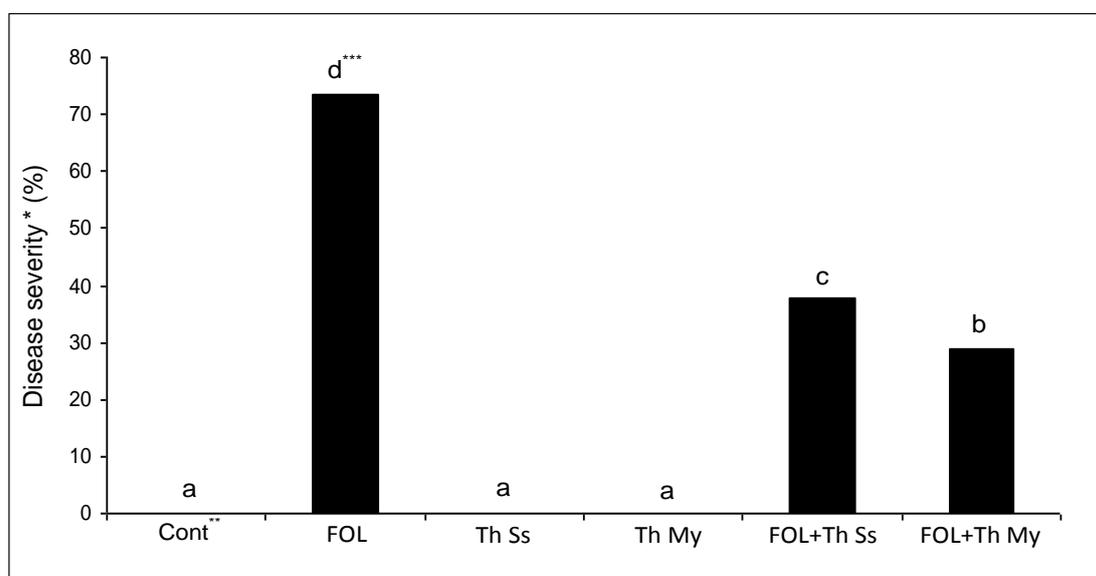


Fig. (4): Effect of *Trichoderma harzianum* (spore suspension and mycelium) on disease severity% caused by *F. oxysporum f. sp. lycopersici* on tomato. * Disease severity was based on a scale (0-5) where: 0, (healthy plant; 1, (>24%) of leaves yellowed and wilted; 2, (25–49%) of leaves yellowed and wilted; 3, (50–74%) of leaves yellowed and wilted; 4, (75–99%) of leaves yellowed and wilted; 5, (100%) dead plan ** Where: 1- (Cont) = control, 2- (FOL) = *F. oxysporum f. sp. Lycopersici* 3- (Th Ss) = *T. harzianum* Conidia suspension, 4 - (Th My) = *T. harzianum* Mycelium, - 5- (FOL+Th Ss) = *F. oxysporum f. sp. lycopersici* + *T. harzianum* Conidia suspension, 6 – (FOL+Th My) = *F. oxysporum f. sp. lycopersici* + *T. harzianum* Mycelium. ***Different small letters above bars indicated significant differences among the five different treatments at $p<0.05$ (one-factor analysis of variance).

Table (1): Disease incidence of tomato wilt *Fusarium* as affected by *Trichoderma harzianum* treatments.

Treatments	Wilt (%)*	Reduction (%)
Un-inoculated seedlings(control)	0.0 d	100
<i>F. oxysporum</i> f. sp. <i>lycopersici</i> only	80 a	0.0
<i>T. harzianum</i> spore suspension	0.0 d	100
<i>T. harzianum</i> mycelium	0.0 d	100
<i>F. oxysporum</i> f. sp. <i>lycopersici</i> + <i>T. harzianum</i> (spore suspension)	36 b	55
<i>F. oxysporum</i> f. sp. <i>lycopersici</i> + <i>T. harzianum</i> (mycelium)	29 c	64

*Values followed by different letters within a column differ significantly < 0.05.

4. DISCUSSION

The results of the current study indicated that under plastichouse conditions all methods of *T. harzianum* application whether single or in combination with *F. oxysporum* f. sp. *lycopersic* caused a significant increase in shoot and root fresh and dry weights as compared with the un-inoculated control and *F. oxysporum* f. sp. *Lycopersici* (Fig. 1, A and B). In addition, there was a significant difference in plant height in all *T. harzianum* treatments (Fig. 2). The general mechanism of biological control can be divided into direct and indirect effects of the bio-control agent (BCA) on the plant pathogen. Direct effects include competition for nutrients or space, production of antibiotic and lytic enzymes, inactivation of the pathogen’s enzymes and parasitism. Indirect effects include all those aspects that produce morphological and biochemical changes in the host plant, such as tolerance to stress through enhanced root and plant development, solubilization or sequestration of inorganic nutrients, and induced resistance (Viterbo *et al.*, 2002). The rhizodeposition of the plants influences the surrounding soil and its microflora (Garbeva *et al.*, 2011). The use of the bio-agent also results in an increase in shoot dry weight in steamed; this means that the bio-agent is able to increase plant height and weight through protection from infection with the pathogens (Mohamed and Abo-Raya, 1993; Adm, 2000; Al-Ameiri, 2007). These results are in agreement with those of Morsy *et al.* (2009), who found that the dual inoculation by *T. viride* and *F. solani* gave the highest records of growth parameters.

Various species of *Trichoderma* have received the most attention. *Trichoderma harzianum* is a fungal bio-agent that attacks a range of phytopathogenic fungi. *T. harzianum* alone or in combination with other *Trichoderma* species can be used in biological control of several plant diseases (Papavizas, 1985; Amini and Siddovich, 2010 and Al-Amreiri, 2014). It has been also reported to be effective in controlling *Fusarium* crown and root rot under

greenhouse and field conditions (Meraj-UI and Nandkar, 2012).

When the two fungi were co-inoculated, both were observed on the root. The presence of one of them on the root did not prevent its colonization by the other fungus. The amount of root colonization by *T. harzianum* was reduced with the two methods of application when the soil was infested with *F. oxysporum* f. sp. *lycopersic*. This reduction in colonization is consistent with a reciprocal competitive interaction for nutrients. Similar results were also reported by Olivain *et al.* (2006), who found that when strains of *Fusarium oxysporum* pathogenic and non pathogenic were introduced together, they both colonized the root surface and were observed at the same locations and this occurred for nutrients rather than for infection sites.

The ability of *Trichoderma* to protect plants against root pathogens has long been attributed to an antagonistic effect against the invasive pathogen (Chet *et al.*, 1997). However, these root-fungus' associations also stimulate plant defensive mechanisms. Data presented in Figure (4) reveal that soil infested with *F. oxysporum* f. sp. *lycopersici* significantly increased disease severity of tomato plants (75%) more than those treated with *F. oxysporum* and *T. harzianum* spore suspension and mycelium treatments with only 40% and 35%, respectively. However, the lowest percentage of disease severity of tomato plants was attained in response to treatment with *T. harzianum*. Similar results were reported by Getha *et al.* (2005), who observed that *T. harzianum* and *B. subtilis* were effective antagonists against *F. oxysporum*. Al-Ameiri (2014) found that *T. harzianum* decreased disease severity to 16% in mycelial preparation treatment as compared to *Pythium aphanidermatum* treatment, where it reached up to 73%. *Trichoderma* strains are always associated with plant root and root ecosystems. Some authors have defined *Trichoderma* strains as plant symbiotic opportunistic virulent organisms, which are able to colonize plant

roots by mechanisms similar to those of mycorrhizal fungi and to produce compounds that stimulate growth and plant defense mechanisms (Franken *et al.*, 2002).

In conclusion, the current study demonstrated that the bio-agent, *T. harzianum* has the ability to suppress tomato wilt caused by *F. oxysporum* f. sp. *lycopersici* as indicated by reducing severity, incidence of disease and pathogen root colonization under plastic-house conditions, and increasing plant growth and no symptoms were observed in the control plants (*T. harzianum* alone).

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المكافحة الحيوية لمرض الذبول الفيوزاري الذي يصيب الطماطم تحت ظروف البيوت البلاستيكية

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ملخص

يعتبر نبات الطماطم من أهم نباتات الخضر إقتصاديا في العالم، وفي الاردن يحتل المرتبة الاولى من ناحية المساحة المنزرعه والانتاج والاستهلاك. وتصاب بالعديد من الامراض ويعتبر مرض الذبول الفيوزاري والمتسبب عن الفطر *Fusarium oxysporum* f. sp. *lycopersici* من الامراض الهامة. أجريت تجربة بالتصميم العشوائي الكامل تحت ظروف الصوب البلاستيكية في محطة الربة الزراعية لكلية الزراعة/جامعة مؤتة. تم استخدام الفطر المضاد *Trichoderma harzainum* لمكافحة مرض الذبول الفيوزاري الذي يصيب نبات الطماطم على هيئة غزل فطري أو معلق جراثيم، واستخدمت ست معاملات لتنفيذ ذلك. حيث كان الهدف من التجربة دراسة تأثير الفطر المضاد على كل من النمو الخضري والجاف وارتفاع النبات وكذلك قدرته على استعمار جذور النباتات بوجود الكائن الممرض وايضا عدم وجوده وكذلك حساب كل من نسبة الاصابة وشدة الاصابة ونسبة مكافحة للمرض. أظهرت نتائج الدراسة انه لا يوجد اي تأثير سلبي للفطر المضاد على احداث الذبول او انخفاض في نمو النبات عند المعاملة به، فقد أظهرت النتائج تفوق معنوي في كل من النمو الطازج والجاف لكل من المجموع الخضري والجذري وكذلك ارتفاع النباتات مقارنة مع جميع المعاملات. كما أن وجود الفطر الممرض بشكل منفرد قد خفض معنويا النمو الطازج والجاف وارتفاع النبات. كما أظهرت النتائج ان استخدام الفطر المضاد بشكل غزل فطري تفوق معنويا في زيادة النمو الطازج والجاف للمجموع الخضري والجذري عن معاملة معلق الجراثيم في وجود الفطر الممرض او عدم وجوده. وكان هناك تفوق معنوي في معاملات الفطر المضاد في استعمار الجذور على هيئة غزل فطري او معلق جراثيم على الفطر الممرض بشكل منفرد ومعهما. وأظهرت النتائج أن معاملة الفطر المضاد على هيئة غزل فطري تفوقت معنويا على معاملة معلق الجراثيم في استعمار الجذور في وجود الفطر الممرض. وأظهرت النتائج ان المعاملات بالفطر المضاد لها قدرة معنوية في خفض شدة الاصابة حيث كانت مع الغزل الفطري 35% ومع معلق الجراثيم 40% بينما كانت مع الفطر الممرض 75%. وأظهرت النتائج خفض نسبة الاصابة الى 29% مع الغزل الفطري و 36% لمعلق الجراثيم في وجود الفطر الممرض، حيث كانت مع الفطر الممرض بمفرده 80%.

وأدت المعاملات الى مكافحة المرض حيث وصلت نسبة مكافحة المرض الى 64% و 54% مع الغزل الفطري ومعلق الجراثيم على التوالي.