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Biological Control of Tomato Damping-off and Potato Black Scurf by Seed Treatment with *Trichoderma harzianum*

Naffaa Walid[1,*], Lubana Al-Jaramany[2], Ahed Elbenay[2] , Reema Al-Mhethawi[2]

[1]*Damascus University, Faculty of Agriculture, Department of Plant Protection, 30621 Damascus – Syria;*[2] *Damascus University, Second Faculty of Agriculture, Sweida – Syria*

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Abstract

During the present study, the antagonistic potential of *Trichoderma harzianum* Rifai against *Phytophthora infestans* (Mont.) de Bary and *Rhizoctonia solani* Ktihn. was evaluated by dual culture technique. Its efficacy to inhibit pathogenic effects caused by these two pathogens was also evaluated on tomato and potato, under artificial inoculation conditions. On PDA medium, *T. harzianum* showed high level of antagonistic activity against all tested isolates of two pathogens. Appressorium – like structures were observed on *R. solani* hyphae. Treatment of tomato seeds with spore suspension of *T. harzianum* showed considerable decrease in pre-emergence damping-off disease incidence caused by *P. infestans* and *R. solani*, with biological control efficacy of 49.3% and 64.33% respectively. Seed treatment with *T. harzianum* also showed biological control efficacy of 66.03% for stem canker disease of tomato seedlings caused by *R. solani*. *Trichoderma* treatment significantly (≤ 0.05) reduced black scurf disease incidence caused by *R. solani* on potato during pot test experiments by 77.95% over untreated control. Although 0.2% carbendazim treatment highly protected tomato and potato plants, but it had a phytotoxic effect, however *Trichoderma* treatments had a positive effect on growth parameters studied as compared to the untreated control.

Keywords: biological control, black scurf disease, *Trichoderma*, damping-off

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important and widespread vegetable crops in the world (FAOSTAT, 2019). Tomato production is very important in Syria as a cash crop due to its relatively low expenditure and high productivity. Moderate temperatures in Syria allow farmers to produce tomato in open fields. Also, due to the mild climate in the coastal area, greenhouses can be used with minimal heating in winter (Annual Agricultural Statistical group, 2016). Potato (*Tuberosum solanum* L.) is one of the most important food and industrial crops in the world. Potato production has increased enormously in developing countries in the past few decades, and has now overtaken that in the developed countries, underlining the growing importance of potato as a staple food crop to meet the demands of increasing human populations (Birch *et al*., 2012). In Syria, potato production was estimated to be 507,384 tons with a cultivated area of 22,369 hectares in 2016 (Annual Agricultural Statistical Group, 2016).

Damping off, a destructive disease of plant seedlings, is caused by a number of seed- and soil-borne fungi and fungus-like Oomycetes, including *Pythium* spp., *Phytophthora* sp. (Joo, 2005), *Rhizoctonia solani* (Asaka and Shoda, 1996), *Sclerotium rolfsii* (Errakhi *et al*., 2007), and *Fusarium oxysporum* (Getha and Vikineswary, 2002). *Phytopthora infestans* (Mont.) *de Bary* causes pre- and post-emergence damping-off and late blight of potato and tomato (Ston, 2009). The disease incidence varied and ranged from 0 to 85.71% in all survived areas throughout the interior of Syria (Naffaa *et al*., 2017). *Rhizoctonia solani* Kühn. [telemorph *Thanatephorus cucumeris* (A.B. Frank) Donk] infects many crops, including tomatoes, causing seed decay as well as pre- and post-emergence damping-off of tomato seedlings (Rehman *et al*., 2012). Stem canker and black scurf also caused by *R. solani* is a serious disease of potato grown in cooler regions of the world (Yanar *et al*., 2005). Yield losses of up to 50% caused by black scurf, in severely affected potato areas have been reported (Keiser, 2008). Studies have confirmed the spread of the disease in most potato cultivation areas in Syria, where the overall average incidence of the disease in autumn season reached 60.46%, while in the spring season, infection rate was higher than in autumn with an average incidence of 64.19% (Abdo *et al*., 2012).

The use of fungicides, besides being expensive and representing a potential risk for the environment and for human health, is not totally effective and may lead to the

* Corresponding author. e-mail: walid1851966@yahoo.com or ray-dya@scs-net.org.

** **Abbreviations used:** (RAM) rye agar medium, (PDA) potato dextrose agar, (AGs) anastomosis groups, (BCE) biological control efficacy, (CE) control efficacy, (T) treatment.

appearance of new resistant strains of pathogens (Alwathnani and Perveen 2012; Tomlin, 2006). It is therefore, necessary to explore alternative control strategies (Boogert Van der and Luttikholt, 2004; Rauf *et al*., 2007). One such alternative is biological control, in which living organisms are selected for their ability to prevent or reduce damage caused by pathogens. Species of the genus *Trichoderma* are considered as potential biological control agents (BCAs) (Vinale *et al*., 2014). *Trichoderma* is a fast growing, secondary opportunistic invader, with efficient sporulation rate, antibiotic and cell wall degrading enzyme producing ability (Francesco *et al*., 2008). The success of strains as bio-control agents may be attributed to their high reproductive ability, survival under adverse conditions, efficient utilization of nutrients, rhizosphere modifying capacity, aggressiveness against plant pathogenic fungi and plant growth promotion. Therefore, *Trichoderma* spp. are the most investigated fungal biocontrol agents, which are available commercially as bio-pesticides (Harman, 2000; Sawant, 2014).

The aim of this study was to determine the antagonistic potential of *T. harzianum* against *P. infestans* and *R. solani* by dual culture technique, and to evaluate the efficacy of seed treatment with *T. harzianum* in controlling diseases caused by two pathogens on tomato and potato in pot experiment under artificial inoculation conditions.

2. Materials and Methods

2.1. Isolation and identification of plant pathogenic fungi

Samples of tomato leaves showing late blight symptoms, and potato tubers showing typical symptoms of black scurf were collected from five different tomato and potato fields in Sweida governorate (southern of Syria). Infected leaves were superficially sterilized for 2 minutes in 1% sodium hypochlorite, washed 3 times with sterile water, cut into small pieces, dried between two sterilized filter papers, placed under potato slices in Petri dishes and incubated at 20 ± 2°C for 7 days until abundant sporulation appeared on the upper side of the slice. The clean pure mycelium was placed on Rye Agar Medium (RAM) (60 g of rye grain, 15 g of agar and 20 g of dextrose in 1L of distilled water) amended with 12 mg/L of rifampicin in Petri dishes. Incubation was carried out for at least two weeks at 20°C (Jmour and Hamada, 2006). *P. infestans* identification was based on colony, sporangiophores and sporangia morphological characteristics.

Infected potato tubers were carefully washed under running tap water to remove the associated soil particles, surface sterilized with 1% sodium hypochlorite for 2 min, rinsed three times with sterile water and then dried on sterilized filter papers. Infected parts were cut using sterilized scalpel into small pieces (3-5 mm), transferred to plates of PDA supplemented with streptomycin sulfate (120 mg l^{-1}) to suppress bacterial growth, then incubated at 25±1ºC for 7 days. Plates were observed daily for mycelial growth. Hyphal tips of mycelium emerging from the infected pieces were transferred to fresh plates of PDA. Pure cultures of *R. solani* isolates were identified microscopically as described by Ogoshi (1987).

2.2. Preparation of antagonistic fungus

Spore suspension of *Trichoderma harzianum* was prepared from a commercial product (1.15% BIO-TH WP, 1 x 10^7 cfu/g) obtained from the Trichoderma laboratory in Hama (Syria). 0.5 ml of the spore suspensions were added to each Petri dish containing PDA medium and incubated at 25 ± 1ºC for 7 days.

2.3. Antagonistic test by dual culture technique

The antifungal activity of *T. harzianum* against 5 isolates of each plant pathogenic fungi was evaluated on PDA medium using a dual culture technique. An agar disc (0.5 cm) of 7 days-old colony of *T. harzianum* was placed on one end of the Petri dish (9 cm) and an agar disc with one of plant pathogenic isolates was placed on the opposite end. The control test was set up without placing the disc of the antagonist, only pathogen was kept for comparison. Experiment was carried out in three replicates. Paired cultures were incubated at 25 ± 1 ºC till the fungal growth in the control test reached the edge of the plate. Scoring for the degree of antagonism was carried out on a scale of 1 – 5 classes: class 1= *Trichoderma* completely overgrew the pathogen and covered the entire medium surface, class 2 = *Trichoderma* overgrew at least 2/3 of the medium surface, class 3 = *Trichoderma* and the pathogen each colonized approximately 1/2 of the medium surface (more than 1/3 and less than 2/3) and neither organism appeared to dominant the other, class 4 = the pathogen colonized at least 2/3 of the medium surface and appeared to withstand encroachment by *Trichoderma*, class 5 = the pathogen completely overgrew the *Trichoderma* and occupied the entire medium surface. *Trichoderma* was considered to be antagonistic to the pathogen if the mean score for a given comparison (when rounded to the nearest whole class number) was ≤ 2, but considered less antagonistic if the mean score was ≥ 3 (Bell *et al*., 1982).

2.4. Preparation of the plant pathogenic fungi inoculum

Culturing of pathogens was carried out according to Jayaraj *et al*. (2006) on broken maize-sand medium (broken maize 37.5 g; black sand 3550 g; tap water 360.0 ml), then sterilized at 121ºC / 1.5 bar for 20 min and inoculated with mycelial discs (5 mm diameter) taken from 7 days-old PDA culture of the fungal pathogens. The inoculated medium was transferred to 500 ml flasks (300 g in each). The flasks were sealed with the help of cotton and incubated at 25±1°C for 15 days with periodical mixing to avoid formation of clump. When the medium were fully covered with test fungus, it was used in the biological control test.

2.5. Preparation of T. harzianum spore suspension and tomato seed treatment

10 ml of distilled water was added to each plate containing 15 days-old colony of *T. harzianum.* Plates were carefully sealed and incubated at room temperature about 2 h. The spores were harvested as previously described (Perelló *et al*., 2009). The spore concentration was measured with a hemocytometer and a suspension with a concentration of 5 × 10^8 spores per ml was selected and used immediately (Biam and Majumder, 2019). Tomato seeds (Marmande cv.) were surface disinfested in 1% sodium hypochlorite for 3 min, washed three times in sterilized distilled water, dried between filter papers and dipped into the spore suspension for 5 min. The treated

seeds were then spread on a cleaned blotting paper and allowed to air dry.

2.6. Efficacy of bio-control against R. solani and P. infestans on tomato in pot experiment

A biological control test against *P. infestans* isolated from tomato, and *R. solani* isolated from potato was performed for both the pathogens on tomato. Soil (clay, sand and peat 1: 1: 2 v) was sterilized twice in autoclave at 121ºC for 30 min. Soil mix was inoculated with *P. infestans* and *R. solani* inoculum individually at the rate of 5 g/kg soil. Inoculated soil was shifted into 15 cm pots (1 kg/pot) and incubated at 28°C, subjected to darkness and watered for 4 days before sowing. Tomato seeds treated with *T. harzianum* were sown in pots (5 seeds / pot). Treatment details are furnished as follows: T1 = treated seeds in inoculated soil, T2 = untreated seeds in inoculated soil (untreated control), T3 = untreated seeds in un-inoculated soil (healthy control), T4 = seeds dipped into 0.2% carbendazim (Bavistin) for 1 min and sown in inoculated soil. Total eight replicates were set up for all treatments. Plants were watered as and when required. The disease incidence of pre-and post-emergence of tomato plants were recorded after 15 and 45 days respectively using the standard procedure (Omokhua, 2011). Percentage disease incidence was calculated using following formula:

$$\text{Percentage of disease incidence (pre-emergence)} = \frac{\text{Number of seeds not germinated}}{\text{Total number of seeds sown}} \times 100$$

$$\text{Percentage of disease incidence (post-emergence)} = \frac{\text{Number of infected seedlings}}{\text{Total number of seeds germinated}} \times 100$$

Control efficacy was calculated by using the following formula (Zhang *et al*., 2012)

$$\text{Control efficacy} = \frac{\text{Disease incidence of control - disease incidence of treatment}}{\text{Disease incidence of control}} \times 100$$

The average plant lengths were calculated.

2.7. Efficacy of bio-control against black scurf disease on potato in pot experiment

Potato tubers (cv. "Spunta"), almost identical in size with 4-5 buds, were surface sterilized by soaking in 1% sodium hypochlorite for 3 min, washed three times with sterilized water, air dried and dipped into the *Trichoderma* spore suspension for 5 min. The treated tubers were then spread on a cleaned blotting paper and allowed to air dry. Soil (clay, sand and peat 1: 1: 2 v) was sterilized twice in autoclave at 121ºC for 30 min, and inoculated with *R. solani* as described earlier. Three tubers were planted at a depth of approximately 5 cm in a 50-cm plastic pot containing 6 kg of soil mix. The treatments given in the pots were as follows: T1 = treated tubers in inoculated soil, T2 = untreated tubers in inoculated soil (untreated control), T3 = untreated tubers in un-inoculated soil (healthy control), T4 = tubers were dipped into 0.2% carbendazim (Bavistin) for 1 min and sown in inoculated soil. Eight pots were used as replicates for each treatment. The plants were grown in spring season at ambient temperature. The plants were fertilized with a balanced NPK (2-3g/ liter of water), and watered as and when needed. Average plant height at the beginning of flowering stage, stem canker incidence (%), number and weight of tubers and the ratio of infected progeny tubers were calculated 120 days after planting (Woodhall *et al*., 2008).

2.8. Statistical analysis

Analyses of variance were carried out using SPSS15 statistical program. The least significant difference (*LSD*) was employed to test significant differences between treatments at $P \leq 0.05$ (Gomez & Gomez, 1984).

3. Results and Discussion

Five isolates of both pathogens *P. infestans* and *R. solani* were obtained and identified according to their morphological characteristics.

3.1. Antagonistic activity of T. harzianum

The dual culture method used to investigate the antagonistic potential of *T. harzianum* against *P. infestans* and *R. solani* indicated that *T. harzianum* had a high level of antagonism (rating ≤ 2) against all tested isolates of both pathogenic fungi. Different susceptibility patterns against the antagonism were observed between isolates of both pathogens with significant differences (≤ 0.05) (Table 1). Previous study showed that significant differences in antagonism susceptibility of *R. solani* to *Trichoderma* have been noticed between isolates from different anastomosis groups (AGs) and between isolates within an AG (Bell *et al*., 1982). In another study, *T. harzianum* was consistently found to be the effective inhibitor of radial growth of all isolates of *P. infestans* with the highest inhibition rate (85 %) for isolate P10 and less inhibition rate (57%) for isolate P19 (Kerroum *et al*., 2015). Elad *et al*. (1983) suggest that the lectin of the host plays a major role in the recognition of host hyphae by *Trichoderma* spp., and it may also be involved in the direct attachment of *Trichoderma* spp. to its host, so the differences in the antagonistic susceptibility between pathogens and isolates may be due to the lectin level in the host. The most antagonistic susceptible isolates *R. solani* (Rh 3) and *P. infestans* (P 5) were chosen for biological control tests.

Table 1. Antagonistic potential of *T. harzianum* against plant pathogenic fungi

Pathogen	Percentage (%) of the medium surface occupied by *T. harzianum*					
	Isolate1 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Mean
P. infestans	^{ab}96.67	^{c}90	^{d}83.33	^{bc} 91.67	^{a} 100	92.33
R. solani	^{b}90	^{d}70	^{a}100	^{c}83.33	^{c}80	82.67

*Values followed by the same letter in the same row do not differ significantly according to (LSD) least significant difference (P ≤ 0.05).

Microscopic examination showed that in dual culture plates, the hyphae of the two cultures grew towards each other, came into contact, and the *Trichoderma* hyphae overgrew the hyphae of the plant pathogens. The pathogen colony became entirely covered by the antagonistic fungi and was hardly visible (Figure 1A). The diameter of hyphae from *Trichoderma* was about 1.3 µm, where it ranged from 5.3 to 7.9 µm for *R. solani*, and from 4.8 to 7.1 µm for *P. infestans*. The differences in diameter made them easily distinguishable from each other. Furthermore, the hyphae of *T. harzianum* produced appressorium-like structures on *R. solani* hyphae (Figure 1B).

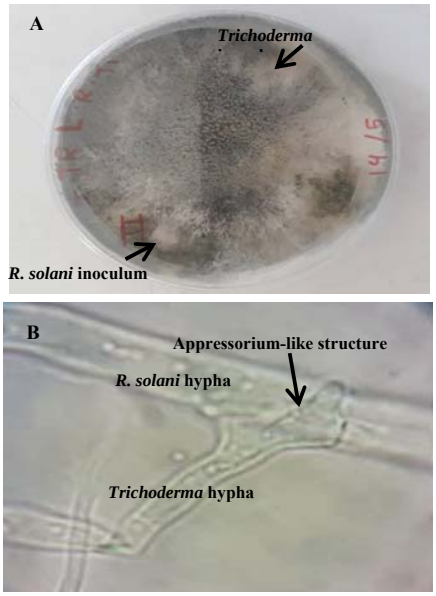

Figure 1. Antagonistic potential of *T. harizianum*: (A) antagonistic fungal hyphae overgrew hyphae of *R. solani*; (B) Appressorium-like structures formed by *T. harzianum* on *R. solani* hyphae.

The attachment of *Trichoderma* hyphae to the host hyphae occurred by numerous means. The formation of hyphal coiling by *Trichoderma* is common (Dennis and Webster, 1971). *T. harzianum* formed hooks, but their presence was infrequent (Murmanis *et al*., 1988). The hyphae of *T. polysporum* formed appressorium-like structures, which pressed against the host hyphae. At the end of the parasitic activity, the cytoplasmic contents of host fungi appeared totally evacuated, and the hyphae looked almost "translucent" as if only the hyphal "exoskeleton" had remained (Murmanis *et al*., 1988). Ibrahim (2017) showed that five isolates of *T. harzianum* were found antagonistic to the growth of *R. solani* in dual culture on PDA. The method of myco-parasitism was sparse to intense coiling followed by disintegration, disorganization and death of *R. solani* mycelium.

Antagonistic activity of *Trichoderma* spp. could also be due to the secretion of antifungal substances, some enzymes that degrade the fungal cell walls including protease, β-1,3- glucanase and chitinase, various toxic and antibiotic metabolites (Limon *et al*., 2004; Sood *et al*., 2020) which are involved in the inhibition and lysis of pathogenic fungi. Singh and Islam (2010) affirmed that the *in vitro* culture of *Phytophthora nicotianae* and *T. harzianum* together led to a variety of interactions. *P. nicotianae* growth was inhibited and the hyphae severely parasitized by *T. harzianum* were lysed.

Isolates of *Trichoderma* spp. have been reported to produce fungicidal antibiotics, regarding their production there is a considerable variation among the isolates (Lee and Wu, 1984; Dennis and Webster, 1971). In this study, however we did not observe inhibition zones in any of the dual culture interactions, corroborating the absence of any antifungal substance in the medium, and hyphal interactions had taken place between the two fungi (Dennis and Webster, 1971; Tronsmo and Dennis, 1978; Murmanis *et al.,* 1988).

3.2. Biological control of pre-emergence damping-off of tomato seeds and seedlings

The effect of tomato seed treatment with *T. harzianum* on pre-emergence damping off and its ability to reduce the disease incidence caused by *P. infestans* and *R. solani* under artificially inoculated conditions was studied. The results showed that *Trichoderma* treatment (T1) significantly (≤ 0.05) reduced the disease incidence caused by *P. infestans* by 24.3%, where the pathogen caused seed decay before germination and pre-emergence seedling damping-off. Among the treatments, the lowest disease incidence was recorded in T4 (carbendazim seed treatment) that resulted in high percent of seed germination (92.3%) (Figure2). The biological control efficacy (BCE) was 49.3% compared with control efficacy (CE) of 84.38% in T4 (Table 2).

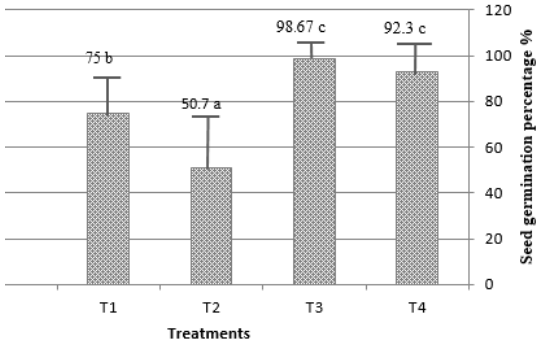

Figure 2. Efficacy of *T. harzianum* in controlling seed rot and damping-off diseases of tomato caused by *P. infestans* under artificial inoculation conditions. T1; *Trichoderma* treatment, T2; untreated control, T3; healthy control, T4; fungicide treatment. Values followed by the same letter do not differ significantly according to (LSD) least significant difference (P ≤ 0.05).

Likewise tomato seed treatment with spore suspension of *T. harzianum* (T1) significantly increased the seed germination rate from 41.67% (T2) to 79.33% under artificial inoculation by *R. solani*, with no significant

differences as compared to the fungicide treatment (T4) (87.6%%). The highest pre-emergence damping off incidence was recorded in the untreated control T2 (58.23%) (Figure 3). The biological control efficacy (BCE) was 64.56% compared to CE of 78.74% in T4 (Table 2).

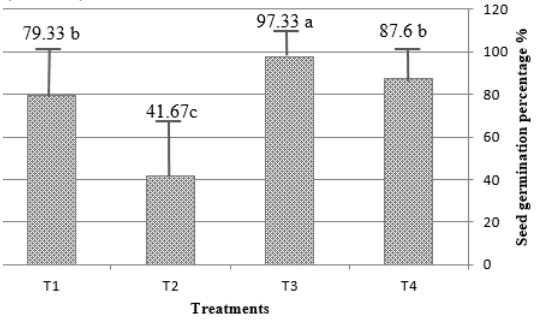

Figure 3. Efficacy of *T. harzianum* in controlling seed rot and damping-off diseases of tomato caused by *R. solani* under artificial inoculation conditions. T1; *Trichoderma* treatment, T2; untreated control, T3; healthy control, T4; fungicide treatment. Values followed by the same letter do not differ significantly according to (LSD) least significant difference ($P \leq 0.05$).

Table 2. Effect of tomato seed treatment with *T. harzianum* on pre-emergence damping-off disease incidence and biological control efficacy against *P. infestans* and *R. solani* under artificial inoculation conditions.

Pathogen	Treatment	Percentage of pre-emergence disease incidence (%)	Control efficacy (CE) %
P. infestans	*T. harzianum*	25	49.3
	Carbenazim	7.7	84.38
R. solani	*T. harzianum*	20.67	64.56
	Carbendazim	12.4	78.74

This study showed that tomato seed treatment with *T. harzianum* decreased significantly the incidence of pre-emergence damping off disease caused by *P. infestans* and *R. solani* under artificial inoculation conditions. Our results are in accordance with those of Biam and Majumder (2019), where tomato seed treatment with four *Trichoderma* isolates showed considerable increase in germination percentage, and reduction in pre-emergence damping off incidence, with biological control efficacy ranged from 20.66% to 39.23% against *R. solani*, and from 32.39% to 64.46% against *Pythium* sp. depending on the *Trichoderma* isolate. Hassan *et al*. (2015) revealed a noticeable reduction in tomato damping off caused by *R. solani* in different substrates amended with *Trichoderma* spp.

Many studies revealed the efficacy of biological control against tomato pre-emergence damping off disease. When tomato and chilli seedlings that colonized with antagonistic *Streptomyces rubrolavendulae* S4 were grown in *P. infestans* artificially inoculated peat moss, the percentage of survival of tomato and chilli seedlings significantly increased from 51.42% to 88.57% and from 34.10% to 76.71% respectively (Loliam *et al*., 2012). Other studies showed that *T. harzianum*, *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Streptomyces* species were reported as commercial biocontrol agents for controlling *Phytophthora* species (Xiao *et al*., 2002; Lozoya-Saldana *et al*., 2006; Fialho de Oliveira *et al*., 2010). In one of the recent studies, *Bacillus subtilis* subsp. *subtilis*, and *T. harzianum* significantly enhanced tomato plant growth and immunity when exposed to *P. infestans* (Bahramisharif and Rose, 2019), and *T. harzianum* proved effective in controlling damping off disease of chilli (*Capsicum annuum* L.) caused by *Pythium aphanidermatum* (Tekale *et al*., 2019).

It was observed that, in T2 (untreated seeds in inoculated soil), the post-emergence damping-off disease incidence was approximately 37.3% compared with 12.67% and 0% in the *Trichoderma* treatment (T1) and fungicide treatment (T4) respectively. The affected seedlings emerging from the soil have developed dark brown lesions (0.5 – 1.3 cm) at the collar region leading of seedlings death. The biological control efficacy of post-emergence damping off disease was 66.03%.

The effect of tomato seed treatment with *T. harzianum* on the plant growth was studied in pots under artificial inoculation with *P. infestans* and *R. solani*, and the results are presented in Figure 4. It was observed that both pathogens reduced the plant height significantly (≤ 0.05) by 15.9% and 30.1% respectively than in the healthy control. In contrast, *Trichoderma* treatment (T1) had a positive effect on tomato plant growth, where it increased the plant height significantly by 12.49% and 64.33% under artificial inoculation with *P. infestans* and *R. solani* respectively. T1 (*Trichoderma* / *R. solani*) had the highest plant lengths, with significant increase of 14.8% than the healthy plants. It was also observed that seed treatment with carbendazim reduced the plant growth significantly.

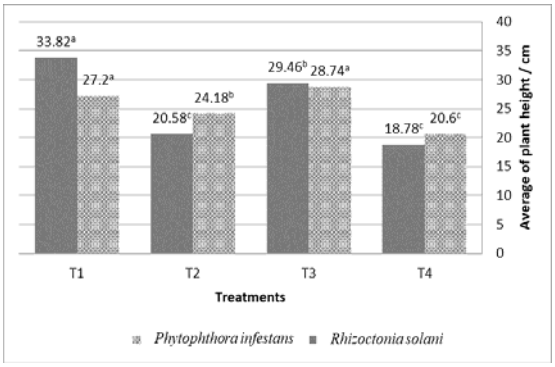

Figure 4. Effect of different treatments on tomato plant height under artificial infection by *R. solani* and *P. infestans*. Values followed by the same letter for each pathogen do not differ significantly according to (LSD) least significant difference ($P \leq 0.05$).

Trichoderma spp. are known as a biocontrol agent against many plant pathogens, and improve the plant growth and yield by enhancing the growth hormones and increment of plant beneficial microbiome (Dubey *et al*., 2007; Khatabi *et al*., 2012; Hussein, 2019). Biam and Majumder (2019) showed that plant height, number of leaves and flowers per plant, dry and fresh weight, root length and yield of tomato was significantly superior in *Trichoderma* treated plants and low in control. *T. harzianum* improved the overall tomato plant growth in the presence of *Pythium ultimum* and *Phytophthora capsici*, while both pathogens reduced the plant height by 28.6% and 42% respectively than in the healthy control (Uddin *et*

al., 2018). Improvement in agronomic traits of host plant in the presence of pathogen was attributed to *T. harzianum* that increased water uptake and translocation of nutrients (Hoyos-Carvajal *et al*., 2009).

On the contrary, beside tomato seed treatment with carbendazim almost completely protected the plants against the two pathogens, but it significantly decreased the plant height. It seems that carbendazim concentration (0.2%) used in this study was inappropriate for tomato seed treatment. Dhanamanjuri *et al*. (2013) showed that the plant growth was slightly affected according to the difference in crops and fungicide concentration used for seed treatment. The fungicide Bavistin (Carbendazim) at 10 ppm concentration was the best among the treatments of *Cicer arietinum*, while in case of *Zea mays*, 1ppm concentration of carbendazim has shown better stimulating effect on the plant growth as compared to control (Dhanamanjuri *et al*., 2013). Many studies showed that carbendazim could have some phytotoxic effects especially when used at dosage higher than recommended. It caused a decrease in dry weight and in all of the foliar pigments of tobacco plants *Nicotiana tabacum* L. cv. *tennessee* 86 (Garcia *et al*., 2002).

3.3. Efficacy of T. harzianum in controlling potato black scurf disease caused by R. solani

Black scurf incidence was significantly reduced by *T. harzianum* under artificial inoculation conditions. Biocontrol treatment had significantly low black scurf incidence (12.7%), and low percent of stem canker (7.33%) compared with 57.6% and 47.3% in untreated control (T2). No sclerotia were observed on new tubers in the carbendazim treated pots, while 1.52% of plants showed stem canker symptoms.

Under artificial inoculation conditions, *R. solani* adversely affected the growth of host plant resulting in a significant decrease in number and weight of new tubers by 49.93% and 54.21% compared to 6.28% and 14.03% respectively in T1 (*Trichoderma* treatment). Although carbendazim showed high efficiency to prevent the potato black scurf disease, but it caused a significant decrease (17.19%) in tuber number compared to *Trichoderma* treatment (6.28%), and it also caused an obvious reduction in tuber weigh by 20.46% with no significant differences compared to 14.03% in T1 (biocontrol treatment). On the other hand, potato plant height was not significantly affected by the pathogen compared to the healthy plants, while *T. harzianum* (T1) increased significantly the plant height compared to T2 (pathogen alone), but this increase in plant height was not significant as compared to T3 (healthy control). T4 (carbendazim) significantly reduced plant height by 24.03% (Table 3).

Table 3. Efficacy of *T. harzianum* in controlling potato black scurf and stem canker diseases caused by *R. solani* under artificial inoculation conditions

Treatment	Percentage of progeny tubers infection (%)	Percentage of stem canker %	Number of tubers	Relative reduction in tubers Number %	Mean weight of tubers (g)	Relative reduction in tubers weight %	Mean length of plants (cm)	Relative reduction in plant length %
T1	b12.7	7.33 a	6.87	a6.28	337.55	14.03 b	41.29	+a 3.23
T2	57.6 c	47.3 b	3.67	c49.93	179.8	c54.21	39.33	b 1.68
T3	- a	- a	7.33	- a	392.65	- a	40.0	- ab
T4	a-	1.52 a	6.07	b17.19	312.33	b20.46	30.39	c 24.03
LSD at P=5%	9.63	12.8	-	8.95	-	6.54	-	4.8

T1; *Trichoderma* treatment, T2; untreated control (*R. solani*), T3; healthy control, T4; fungicide (carbendazim). Values followed by the same letter in the same column do not differ significantly according to (LSD) least significant difference (P = 0.05).

It appears that there are many factors affecting the effectiveness of biological control, among them the isolates of the fungus used in the biological control. It was determined that both *in vitro* and *in vivo* the isolates of *T. harzianum* and *T. virens* have shown different efficiency against *R. solani*. Some isolates of these two antagonistic fungi have significantly reduced the severity of the potato black scurf and stem canker diseases, and they raised the development of the plant (Durak, 2016).

Increased growth response of several plants, following the treatment of pathogen – free soil by *Trichoderma* has been documented (Baker, 1989; Chang *et al*., 1986; Kleifeld and Chet, 1992). This was explained by the ability of *Trichoderma* to inhibit pathogens in the rhizosphere which might cause seed rots and pre-emergence damping off (Kleifeld and Chet, 1992). Some studies reported that the increased growth response caused by *Trichoderma* isolates resulted in large increase in the root area and root lengths and may be related to the effect on root system. Yedidia *et al*. (1999) suggested a direct role for *T. harzianum* in mineral uptake by the plant at a very early stage of fungal-plant association. In addition, Harman (2000) demonstrated that *Trichoderma* spp. are opportunistic plant colonizers that affect plant growth by promoting healthy and abundant plant roots, probably via the production or control of plant hormones.

4. Conclusion

The present study clearly indicated that *T. harzianum* had strong antagonistic activity against *P. infestans* and *R. solani* on PDA medium. Tomato seed treatment with *T. harzianum* spore suspension showed higher seed germination, lower pre and post emergence damping off disease incidence caused by *P. infestans* and *R. solani,* and higher biological control efficacy than the untreated control. Seed treatment with *T. harzianum* also showed effective biological control for stem canker disease of

tomato seedlings caused by *R. solani*. *Trichoderma* treatment significantly reduced black scurf and stem canker disease incidence caused by *R. solani* on potato as compared to untreated control. From the present findings, recommendation could be made for further evaluation of local *Trichoderma* isolates under different climatic condition of Syria for development of effective Trichoderma formulation as a component of integrated disease management practice to manage damping-off of tomato and potato, and black scurf disease of potato.

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References

Annual Agricultural Statistical Abstract. 2016. Agricultural Statistics Directorate, Ministry of Agriculture and Agrarian Reform, Damascus, Syria.

Abdo RH, Bayaa B and Abbas A. 2012. Determination of anastomosis groups within population of *Rhizoctonia solani* Kuhn in potato in Syria. *Arab J. Plant Prot.*, **30**: 1-10.

Alwathnani HA and Perveen K. 2012. Biological control of Fusarium wilt of tomato by antagonist fungi and cyanobacteria. *Afr. J. Biotechnol.*, **11(5)**:1100-1105.

Asaka O and Shoda M. 1996. Biocontrol of *Rhizoctonia solani* damping-off of tomato with *Bacillus subtilis* RB14. *Appl. Environ. Microbiol.*, **62(11)**: 4081– 4085.

Bahramisharif A and Rose LE. 2019. Efficacy of biological agents and compost on growth and resistance of tomatoes to late blight. *Planta*, **249**:799–813. Doi.org/10.1007/ s00425-018-3035-2

Baker R. 1989. Improved *Trichoderma* spp. for promoting crop productivity. *Trends Biotechnol.*, **7**: 34-38.

Bell DK, Wells HD and Markham CR. 1982. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. *J. Phytopathol.*, **72:** 379-382.

Biam M and Majumder D. 2019. Biocontrol efficacy of *Trichoderma* isolates against tomato damping off caused by *Pythium* spp. and *Rhizoctonia solani* (Kuhn.). *Int. J. Chem. Stud.*, **7(3)**: 81-89.

Birch PRJ, Bryan G, Fenton B, Gilroy EM, Hein I, Jones JT, Prashar A, Taylor MA, Torrance L and Toth IK. 2012. Crops that feed the world 8: Potato: are trends of increased globa production sustainable? *Food Secur.*, **4:** 477-508.

Boogert van den H and Luttikholt AJG. 2004. Compatible biological and chemical control systems for *Rhizoctonia solani* in Potato. *Eur. J. Plant Pathol.*, **2:** 110. doi:10.1023/B:EJPP.0000015325. 33299.e0.

Chang YC, Chang YC, Baker R, Kleifeld O and Chet I. 1986. Increased growth of plants in presence of biological control agent *Trichoderma harzianum. Plant Dis.*, **70**: 145-148.

Dennis C and Webster J. 1971. Antagonistic properties of species groups of *Trichoderma*. *Trans. Br. Mycol. Soc.*, **57**: 25-39.

Dhanamanjuri W, Thoudam R and Dutta BK. 2013. Effect of some pesticides (Fungicides) on the germination and growth of seeds/seedlings of some crop plants, (i.e. *Cicer arietinum* and *Zea mays*). *Middle East J. Sci. Res.*, **17:** 627-632. Doi: 10.5829/idosi.mejsr.2013.17.05.12221.

Dubey SC, Suresh M and Birendra SS. 2007. Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilts. *Biol. Control*, **40**:118–127.

Durak E. 2016. Biological control of *Rhizoctonia solani* on potato by using indigenous *Trichoderma* spp. AIP Conference Proceedings 1726, 020020. Doi.org/10.1063/ 1.4945846

Elad Y and Kapat A. 1999. The role of *Trichoderma harzianum* protease in the bio-control of *Botrytis cinerea*. *Eur. J. Plant. Pathol*., **105**:177-189.

Errakhi R, Bouteau F, Lebrihi A and Barakate M. 2007. Evidences of biological control capacities of *Streptomyces* spp. against *Sclerotium rolfsii* responsible for damping-off disease in sugar beet (*Beta vulgaris* L.). *World J. Microbiol. Biotechnol.*, **23(11)**: 1503–1509.

FAOSTAT, 2019. Food and Agriculture Organization of the United Nations (Syria).

Fialho de Oliveira M, Germano da Silva M and Van Der Sand ST. 2010. Anti-phytopathogen potential of endophytic actinobacteria isolated from tomato plants (*Lycopersicon esculentum*) in southern Brazil, and characterization of *Streptomyces* sp. R18(6), a potential biocontrol agent. *Res. Microbiol.*, **161(7)**: 565–572.

Francesco VK, Sivasithamparam EL, Ghisalberti RM, Sheridan L and Woo ML. 2008. *Trichoderma* plant pathogen interactions. *Soil Biol. Biochemist*, **40**: 1-10.

García P, Ruiz J, Rivero R, López-Lefebre L, Sánchez E and Romero L. 2002. Is the Application of Carbendazim Harmful to Healthy Plants? Evidence of Weak Phytotoxicity in Tobacco. *J. Agric. Food Chem.*, **50:** 279-83. Doi:10.1021/jf010748g.

Getha K and Vikineswary S. 2002. Antagonistic effects of *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum* f. sp. *cubense* race 4: indirect evidence for the role of antibiosis in the antagonistic process. *J. Ind. Microbiol. Biotechnol.*, **28(6):** 303–310.

Gomez KA and Gomez A. 1984. Statistical Procedure for Agricultural Research—Hand Book. John Wiley & Sons, New York.

Harman GE. 2000. Myths and dogmas of bio-control: changes in perceptions derived from research on *Trichoderma harzianum* T22. *Plant Dis.*, **84:** 377-393.

Hassan WA, Taher IE, Saido KA and Ali AS. 2015. Antagonistic succession of *Trichoderma* against Rhizoctonial damping- off on tomato in composted media. Bull. *Iraq nat. Hist. Mus.*, **13 (4)**: 41-49

Hussein SN. 2019. Biological control of root rot disease of cowpea *Vigna unguiculata* caused by the fungus *Rhizoctonia solani* using some bacterial and fungal species. *Arab J. Plant Prot.*, **37(1)**:31-39. Doi.org/10.22268/AJPP-037.1. 031039.

Hoyos-Carvajal L, Ordua S and Bissett J. 2009. Growth stimulation in bean (*Phaseolus vulgaris* L.) by *Trichoderma*. *Biol. Control*, **51:**409–416

Ibrahim ME. 2017. *In vitro* Antagonistic activity of *Trichoderma harzianum* against *Rhizoctonia solani* the causative agent of potato black scurf and stem canker. The 7th Inter. Conf."Plant & Microbial Biotech. & their Role in the Development of the Society"pp.173 -185. Doi:10.21608/ejbo.2017. 903.1067.

Jayaraj J, Radhakrishnan NV and Velazhahan R. 2006. Development of formulations of *Trichoderma harzianum* strain M1 for control of damping-off of tomato caused by *Pythium aphanidermatum*. *Arch. Phytopathol. Pflanzenschutz*, **39(1)**:1-8.

Jmour W and Hamada W. 2006. First report of A2 mating type of *Phytophthora infestans* in Tunisia using molecular markers and some observations on its metalaxyl resistance. *Tunis. J. Plant Protect.*, **1:** 85-91.

Joo GJ. 2005. Production of an anti-fungal substance for biological control of *Phytophthora capsici* causing phytophthora blight in red-peppers by *Streptomyces halstedii*. *Biotechnol. Lett.*, **27(3):** 201–205.

Keiser A. 2008. *Rhizoctonia solani* a fungal disease with multiple symptoms: means of preventive and curative control. *In*: Potato Research for a Production of Quality. Information Day, February 2008, Changins

Kerroum F, Noureddine K, Henni J and Mebrouk K. 2015. Antagonistic effect of *Trichoderma harzianum* against *Phytophthora infestans* in the North-west of Algeria. *Int. J. Agric. Res.*, **6:** 44-53.

Khatabi B, Molitor A, Lindermayr C, Pfiffi S, Durner J, Wettstein D, Kogel KH and Schäfer P. 2012. Ethylene supports colonization of plant roots by the mutualistic fungus *Piriformospora indica*. PLoS One 7:e35502

Kleifeld O and Chet I. 1992. *Trichoderma harzianum* – interaction with plants and effect on growth response. *Plant Soil*, **144:** 267-272.

Lee ET, Lim SK, Nam DH, Khang YH and Kim SD. 2003. Pyoverdin 2112 of *Pseudomonas fluorescens* 2112 inhibits *Phytophthora capsici*, a red-pepper blight-causing fungus. *J. Microbiol. Biotechnol.*, **13(3):** 415–421.

Limón MC, Chacón MR, Mejías R, Delgado-Jarana J, Rincón AM, Codón AC and Benítez T. 2004. Increased antifungal and chitinase specific activities of *Trichoderma harzianum* CECT 2413 by addition of a cellulose binding domain. *Appl Microbiol Biotechnol*, **64(5)**:675-85. Doi: 10.1007/s00253-003-1538-6.

Loliam B, Morinaga T and Chaiyanan S. 2012. Biocontrol of *Phytophthora infestans*, fungal pathogen of seedling damping off disease in economic plant nursery. *Hindawi Publishing Corporation Psyche*. Doi:10.1155/2012/ 324317

Lozoya-Saldana H, Coyote-Palma MH, Ferrera-Cerrato R and Lara-Hernandez ME. 2006. Microbial antagonism against *Phytophthora infestans* (Mont) de Bary. *Agrociencia*, **40(4)**: 491–499.

Murmanis L, Highley TL and Ricard J. 1988. Hyphal interaction of *Trichoderma harzianum* and *Trichodema polysporum* with wood decay fungi. *Material Organismen*, **23**:271 – 279.

Naffaa W, Ibrahim S, Abou-Alfadil T and Dawood A. 2017. Incidence of the potato late blight pathogen, *Phytophthora infestans*, in Syria and its mating type. *J. Plant Dis. Prot.*, **124 (6)**: 533-537. Doi.org/10.1007/s41348-017-0130-8

Ogoshi A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. *Ann. Rev. Phytopathol*., **25:** 125–143.

Omokhua GE. 2011. Pre and post-emergence damping-off of *Chrysophyllum albidum* in Port Harcourt. *Int. Multidiscip. J.*, **5(6)**:411-421.

Perelló AE, Moreno MV, Mónaco C, Simón MR and Cordo C. 2009. Biological control of *Septoria tritici* blotch on wheat by *Trichoderma* spp. under field conditions in Argentina. *Biocontrol*, **54:**113–122. Doi.org/10.1007/s10526-008-9159-8

Rauf C, Ashraf M and Ahmad I. 2007. Management of black scurf disease of potato. *Pak. J. Bot.*, **39(4):** 1353.

Rehman SU, Lawrence R, Kumar EJ and Badri ZA. 2012. Comparative efficacy of *Trichoderma viride, T. harzianum* and carbendazim against damping-off disease of cauliflower caused by *Rhizoctonia solani* Kuhn. *J. Biopestic.*, **5(1):**23-27.

Sawant I. 2014. Trichoderma- Foliar Pathogen Interactions. *Open Mycol. J.*, **8:** 58-70. Doi: 10.2174/1874437001408010058.

Singh A and Islam MN. 2010. *In vitro* evaluation of *Trichoderma* spp. against *Phytophthora nicotianae*. *Int. J. Expt. Agric*, **1(1):** 20-25.

Sood M, Kapoor D, Kumar V, Sheteiwy MS, Ramakrishnan M, Landi M, Araniti F and Sharma A. 2020. *Trichoderma*: The "Secrets" of a Multitalented Biocontrol Agent. *Plants*, **9:** 762.

Ston A. 2009. Organic Management of Late Blight of Potato and Tomato (*Phytophthora infestans*). Sustainable Agriculture Research and Education. Oregon State University. Available on: http://www.extension.org/pages/ 18361/Accessed on 22/03/2014.

Tekale AG, Guldekar DD, Kendre VP, Deshmukh AP and Potdukhe SR. 2019. Efficacy of fungicides and bioagents against damping off in Chilli caused by *Pythium aphanidermatum*. *Int. J. Curr. Microbiol. App. Sci.*, **8(6):** 637-648. Doi. org/10. 20546

Tomlin CDS. 2006. The Pesticide Manual: A World Compendium, 14th ed.; British Crop Protection Council: Surrey, UK.

Tronsmo A and Dennis C. 1978. Effect of temperature on antagonistic properties of *Trichoderma* species. *Trans Brit. Mycol. Soc*., **71(3):**469-474.

Vinale F, Sivasithamparam K, Ghisalberti E, Woo S, Nigro M, Marra R, Lombardi N, Pascale A, Ruocco M, Lanzuise S, Manganiello G and Lorito M. 2014. *Trichoderma* secondary metabolites active on plants and fungal pathogens. *Open Mycol. J.,* **8**. 10.2174/1874437001408010127.

Uddin MN, Rahman UU, khan W. *et al.* 2018. Effect of *Trichoderma harzianum* on tomato plant growth and its antagonistic activity against *Phythium ultimum* and *Phytopthora capsici*. *Egypt. J. Biol. Pest. Control*, **28**, 32. Doi.org/10.1186/s41938-018-0032-5

Woodhall JW, Lees AK, Edwards SG and Jenkinson P. 2008. Infection of potato by *Rhizoctonia solani*: effect of anastomosis group. *Plant Pathol.*, **57:**897-905.

Xiao K, Kinkel LL and Samac DA. 2002. Biological control of *Phytophthora* root rots on alfalfa and soybean with *Streptomyces*. *Biol. Control*, **23(3):** 285–295.

Yanar Y, Yilmaz G, Cesmeli I and Coskun S. 2005. Characterization of *Rhizoctonia solani* isolates from potatoes in Turkey and screening potato cultivars for resistance to AG-3 isolates. *Phytoparasitica*, **33:** 370-376.

Yedidia I, Benhamou N and Chet I. 1999. Induction of defense responses in cucumber plants (*Cucummis sativus* L.) by the Biocontrol agent *Trichoderma harzianum*. *Appl. Environ. Microbiol.*, **65:** 1061- 1070.

Zhang RS, Liu YF, Luo CP, Wang XY, Liu YZ, Qiao JQ *et al*., 2012. *Bacillus amyloliquefaciens* Lx-11, A potential biocontrol agent against rice bacterial leaf streak. *J. Plant Pathol*., **94(3)**:609-619.