J. Crop Prot. 2020, 9 (4): 577-589

Research Article

The reaction of some apple rootstocks to biocontrol of white root rot *Rosellinia necatrix* by *Trichoderma harzianum* in greenhouse

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Abstract: Three *Trichoderma harzianum* isolates, were evaluated for their antagonistic effect on *Rosellinia necatrix* causal agent of white root rot (WRT). According to *in vitro* evaluations, *T. harzianum* T20A isolate showed the most pathogen growth inhibition. The inoculum of T20A isolate was applied to control WRT disease on four commercial apple rootstocks: Malling (M7, M25) and Malling Merton (MM111, MM106) in greenhouse experiments. Root rot and leaf fall indices were measured 70 days after pathogen inoculation. The biocontrol agent had a significant effect ($p < 0.01$) on the reduction of pathogen indices but the effect of rootstocks was not significant. Root rot reduction on MM111, MM106, M25 and M7 rootstocks were 63.84%, 61.13%, 28.63% and 17.47%, respectively. The antagonist also caused reduction of leaf drop symptom on MM106, M7, MM111 and M25 infected apple rootstocks by 57.4%, 56.06%, 44.09% and 40.24%, respectively. Disease indices were also measured for fungicide treatment and the results were compared with disease indices in antagonist treatments. The most biological control was observed on MM111 (63.84%) and MM106 (57.4%) according to the reduction in root rot and leaf drop, respectively. The reactions of apple rootstocks to WRT were also evaluated. The results showed that all the rootstocks were susceptible to WRT in the greenhouse condition. The MM106 rootstock which showed 100% root rot and 78% leaf drop was the most susceptible and M7with 43.5% root rot and 84.56 leaf drop was the least susceptible in our experiment. This was the first study of reaction and biocontrol of white root rot disease on apple commercial rootstocks in Iran and the results suggest a better insight to disease management either by integrating resistance and biocontrol measures or replacing chemical control by antagonist application to soil.

Keywords: *Malus domestica*, *Trichoderma harzianum*, apple rootstock, *Rosellinia necatrix*, white root rot, biological control

Introduction

Rosellinia necatrix Prill. (anamorph *Dematophora necatrix* R. Hartig), the causal agent of white root rot is a soil-borne and threatening fungal pathogen that can attack more than 170 plant species mainly woody plants in temperate, tropical and subtropical areas of the world (Schena *et al*., 2008; Pliego *et al*., 2012). The pathogen has been recognized to cause losses in many economically important fruit trees such as apple in Italy (Pasini *et al*., 2016), Japan (Ten Hoopen and Krauss, 2006) and India (Agarwala and Sharma, 1966), avocado in South

Handling Editor: Vahe Minassian

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Received: 20 January 2020, Accepted: 05 February 2020
Published online: 05 August 2020

Africa, Italy and Spain (Pliego *et al*., 2012), grapevines in Japan (Ten Hoopen and Krauss, 2006), mango in Mediterranean countries (Pérez-Jiménez, 2006). *R. necatrix* is also one of the most important causes of root rot in poplars in Italy (Anselmi and Giorcelli, 1990) and wild cherry, jasmine, narcissus and peony in France (Guillaumin *et al*., 1982). Infected roots in advanced stages are characterized by white fluffy mycelium or black and white mycelium on root surface and under root bark in fan-shaped patterns or mycelial strands or cords without any special pattern (Pliego *et al*., 2012). After colonizing the root surface, the invading hyphae penetrate roots mostly through lenticels or wounds, although direct penetration by infectious sclerotium was also reported (Pasini *et al*., 2016). Invasion is followed by the growth of hyphal strands in one or more directions, colonizing both epidermal and cortical cells and finally, degrading the vascular system of the plant (Pliego *et al*., 2009). Infested soil, plant debris and irrigation water contribute to the spread of disease (Anselmi and Giorcelli, 1990). The cytochalasin E found in this pathogen has a highly pathogenicity direct effect on photosynthesis (Kshirsagar *et al*., 2001). Favorable characteristics such as resistance to drought stress, tolerance to different soil acidities (pH:4-9), broad host range, strong saprophytic survival capability, ability to move deeply in the soil and resistance to common fungicides, render white root rot difficult to control (Pérez-Jiménez, 2006; Lo´pez-Herrera and Zea-Bonilla, 2007; Schena *et al*., 2008; Pliego *et al*., 2012; Pasini *et al*., 2016). Thus white root rot control is almost completely summarized in disease prevention strategy to restrict pathogen dissemination and early disease control (Pasini *et al*., 2016). In Iran, *R. necatrix* has been previously reported on 36 different plant species from different provinces including, Tehran, Fars, Hamedan and Tabriz (Behdad, 1979). Following the climate changes in recent years, now the disease is scattered in some of the previously reported provinces like Isfahan and South Khorasan mostly in old orchards of apple, pear and grapevine (personal observation). Current apple rootstocks are mostly susceptible to white root rot (Modgil *et al*., 2012). Considering disease control difficulties and serious concerns over chemical pesticides, an integrated approach, including all environmentally friendly strategies must be adopted to decrease white root rot incidence in orchards and nurseries. Biological control is one of the promising and alternative strategies for chemical application in disease management. As a successful biocontrol agent, *T. harzianum* Rifai (Ascomycota, Hypocreales, Hypocreaceae) has a long history to control a variety of plant-pathogenic fungi (Sztejnberg *et al*., 1987). It is cosmopolitan soil fungus that has been used in the biological control of soil-borne diseases caused by *Fusarium oxysporum f. sp. melonis*, *Pythium* spp., *Rhizoctonia solani* and *Sclerotium rolfsii* (Ortiz and Orduz, 2001; Krupke *et al*., 2003). *T. harzianum* propagules are capable to produce cheap formulations of bio-pesticides, bio-protectants, bio-stimulants and bio-fertilizers in large quantities and high concentration, whether in liquid (water or oil) or dry (granules, pellets and wettable powder) state, and can also be stored for long periods (Harman and Kubicek, 1998; Woo *et al*., 2006).

Trichoderma employed various mechanisms in biological control, including antibiosis, competition, mycoparasitism, plant growth promotion and induction of plant resistance against pathogens (Freeman *et al*., 1986; Benitez *et al*., 2004; Ruano-Rosa and Lopez-Herrera, 2009; Ruano-Rosa *et al*., 2014). This study aims to evaluate the potential of *T. harzianum* antagonistic properties against *R. necatrix* in petri dish tests and also to survey the biocontrol potential of selected isolates on different apple rootstocks: Malling Merton (MM111, MM106) and East Malling (M7, M25) which were inoculated with *R. necatrix* virulent isolate.

Materials and Methods

Survey for white root rot symptoms and root sample collection

Between, 2014 and 2015, a survey to identify apple, pear and grapevine orchards, potentially infected with *R. necatrix* was

conducted in different provinces including South Khorasan, Isfahan, Shahr-e-Kord, Alborz, Markazi and Guilan, first by interviewing plant protection advisors and local growers. Then potentially infected orchards were inspected for symptoms of white root rot. Underground parts of trees with aerial symptoms including stunted growth, pale or chlorotic and wilted leaves, death of shoots and branches were inspected and rotten roots with or without signs of white cottony mycelium or mycelial strands, were sampled. Samples were contained in paper bags individually and transported in an ice box to the laboratory for the isolations of putative pathogens. The site information for each sample was recorded. In sum fifty six samples were collected.

Pathogen isolation

R. necatrix was isolated from root samples based on Ruano-Rosa (2010) method. Firstly, the soil was removed gently and root segments were carefully washed with tap water then surface sterilization was conducted using sodium hypochlorite (0.5% solution) for 3 min followed by rinsing three times with sterile distilled water and drying on sterile paper towels. Segments of bark and outer wood (0.5 × 0.5 cm) were placed on Petri dishes containing malt extract agar (MEA) medium amended with streptomycin sulfate at 0.05 g/liter. Cultures were incubated at 24C° in the dark condition. Emerging colonies were purified using the hyphal tip culture (Castro *et al*., 2013). *R. necatrix* colonies were identified initially under the light microscope by presence of the typical hyphal pear-shaped swellings adjacent to the cell walls in four- weeks cultures (Francis, 1985). Isolates of *R. necatrix* were identified based on the sequence of ITS-rDNA in advance. The isolates had 98% or more similarity with *R. necatrix* sequences in NCBI (White *et al*., 1990). We used specific PCR by ITS-based primers, R2/R8 (R2: CAAAACCC ATGTGAACATACCA; R8: CCGAGGTCAA CCTTTGGTATAG) for pathogen surveys in the next steps (Schena *et al*., 2002, 2003).

The antagonist

Three isolates of *T. harzianum* were used as antagonists in this study. T20 and T20A *T. harzianum* isolates, recently proved by their antagonistic properties against a wide range of soil-borne pathogens, were supplied by the fungal collection in the department of Plant Pathology in Tarbiat Modares University and T. H isolate, was isolated from roots of Williams pear rootstock. Isolates of *T. harzianum* were identified based on sequence of the amplified fragment using ITS1/ITS4 primers (White *et al*., 1990). Isolates had more than 98% similarity with *T. harzianum* sequences in NCBI.

In vitro antagonist evaluation

Antagonistic properties of *T. harzianum* isolates against *R. necatrix* were evaluated through different in vitro experiments. All experiments were conducted in a completely randomized design with three replications and repeated twice. Treatments were compared by Duncan's test ($p < 0.01$) and results were plotted in Excel software.

Dual culture method

Three isolates of *T. harzianum* were screened for their mycoparasitic ability. Petri dishes (9 cm in diameter) containing PDA medium were seeded with fungal active colony discs (7 × 7 mm) from pathogen isolate (ESKA1) and antagonists isolates opposite to each other and were incubated at 24 °C for 4 days (Dennis and Webster, 1971a). Inhibition of radial growth (%) of the pathogen was determined according to the following formula in which dc is colony diameter of ESKA1 isolate in single culture as control and dt is colony diameter of ESKA1 isolate in dual culture (Morton and Stroube, 1955).

$GI\% = dc\text{-}dt/dc \times 100$

Volatile compound evaluation

A disc (7 × 7 mm) cut from active edge of *T. harzianum* culture (4 days old) was placed in the center of Petri dishes containing PDA medium and the lid of Petri dish containing *T. harzianum* was replaced by an inverted new culture plate of ESKA1 isolate, then the two plates were held together and sealed with

parafilm. The inhibition of radial growth (%) of ESKA1 isolate was determined after 7 days (Dennis and Webster, 1971b).

Extracellular compound evaluation

The extracellular fluid of *T. harzianum* isolates against the pathogen was evaluated by using Dennis and Webster (1971a) method. Firstly, potato dextrose broth in Erlenmeyer flask (100 ml PDB without antibiotics) was inoculated by a disc (7 × 7 mm) cut from the active edge of 4 days old *T. harzianum* culture. Then the flasks were incubated for 12 days at 25 °C in a shaker incubator (70 rpm). The liquid culture of *T. harzianum* was filtered through filter paper and then through a 0.22µm filter and the culture filtrate added to melted PDA (42 °C) to a final concentration of 10% (V/V) and dispensed in new Petri dishes, a disc (7 × 7 mm) cut from active edge of ESKA1 culture (10 days old) was placed in the center of Petri dishes containing PDA + *T. harzianum* fluid. Inhibition of radial growth (%) of ESKA1 isolate was determined after 7 days of incubation at 24 °C (Dennis and Webster, 1971b).

Biocontrol evaluation in greenhouse

Preparation of pathogen Inoculum

To prepare pathogen inoculum, several autoclaved apple wooden chips (10 × 5 mm) were directly placed on the fully grown colony of ESKA1 isolate and incubated at 25 °C. After 2 weeks, the ESKA1 mycelium grew over the wooden chips completely. Infected wooden chips, the inocula, were placed in soil in contact with the roots of apple seedlings, ten pieces of chips per pot (Negishi *et al*, 2011).

Biocontrol inoculum preparation

Wheat seeds in containers were soaked overnight and autoclaved twice in two days, then sterile wheat seeds were inoculated by *T. harzianum*. Eight discs (5 × 5mm) cut from an active edge of 4 days old culture per 200 grams of wheat seeds were used. Inoculated wheat seeds containers were kept at 24 °C in the dark condition for 15 days (Sztejenberg and Madar, 1980).

Plant materials

Pathogen free apple rootstocks: Malling (M7, M25) and Malling Merton (MM106, MM111) which had been propagated by tissue culture method were provided by Itasadra company. Two-year-old rootstocks were planted individually in pots (two kg.) containing peat moss, perlite and autoclaved field soil (3:3:3 V). A factorial experiment was conducted in randomized complete block design with four replications.

Greenhouse experiments

T. harzianum isolate T20A, selected based on in vitro experiments results, was used as a biocontrol agent in greenhouse pot experiments. *T. harzianum* inoculum on wheat seeds was added (10 g per kg) near the roots on the first day and thirtieth day after transplanting to the soil. Pathogen inoculum was added a week later to soil near the roots. Leaf fall percentage was recorded weekly for 70 days after pathogen inoculation (Knudsen *et al*., 1997; Sallam *et al*, 2009). Four pots of each rootstock, inoculated by pathogen and uninoculated by T20A isolate, were used as the positive control. Four healthy rootstocks (without pathogen or antagonist) were used as negative control for each rootstock. At the end of the experiment, all the plants were uprooted and the roots of inoculated and non-inoculated plants were carefully washed with tap water and visual necrosis symptoms on roots were scored according to the Rupple scaling method (Rupple *et al.*, 1979). Thiophanate Methyl (2 g/l) was used as fungicide stock and 200 µl of stock was added to irrigation water in each fungicide application. Fungicide was applied on the first and 30th day after pathogen inoculation.

Results

In Vitro experiments

All the *T. harzianum* isolates showed antagonistic effects on the pathogen isolate in In Vitro experiments: dual culture, volatile and extracellular compound evaluations. Analysis of variance of pathogen growth

inhibition showed that biocontrol isolates were significantly different ($p < 0.01$). In all the *in vitro* experiments, the growth rate of *T. harzianum* isolates was more than the pathogen growth rate.

Dual culture

Radial growth inhibition of pathogen was significantly different ($p < 0.01$), in the presence of different *T. harzianum* isolates in dual culture. The T.H isolate caused the highest inhibition of pathogen radial growth by 73.51%. Other *T. harzianum* isolates T20A, T20 caused 59.27%, 58.75% growth inhibition respectively which were not significantly different (Fig. 1, Table 1).

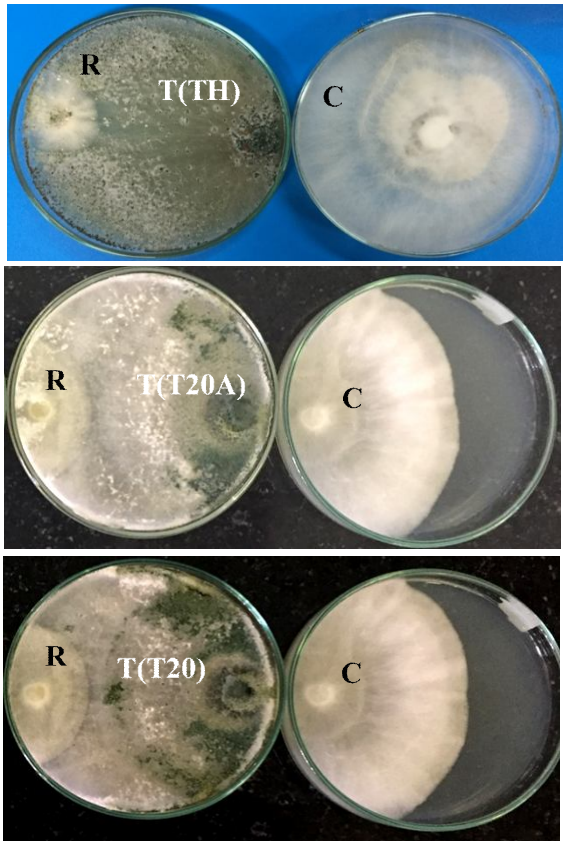

Figure 1 Growth inhibition of *Rosellinia necatrix* ESKA1 isolate in the presence of different *Trichoderma harzianum* isolates in dual culture. T: *T. harzianum* isolates, R: *R. necatrix* ESKA1 isolate, C: *R. necatrix* ESKA1 isolate on PDA with no antagonistic agent.

Table 1 Radial growth inhibition of the ESKA1 colony by *Trichoderma harzianum* isolates.

Isolates	Radial growth inhibition (%)		
	Dual culture	Volatile compound	Extracellular compound
T. H	73.51 a	51.06 c	25.24 b
T20A	59.27 b	87.38 a	100 a
T20	58.75 b	70.71 b	100 a

Volatile compounds

T. harzianum isolates were significantly different in volatile compound evaluation ($p < 0.01$). The T20A isolate caused the most inhibition of pathogen radial growth by 87.38%. Other isolates T20 and T. H, prevented the ESKA1 isolate radial growth by 70.71%, 51.06% respectively (Fig. 2, Table 1)

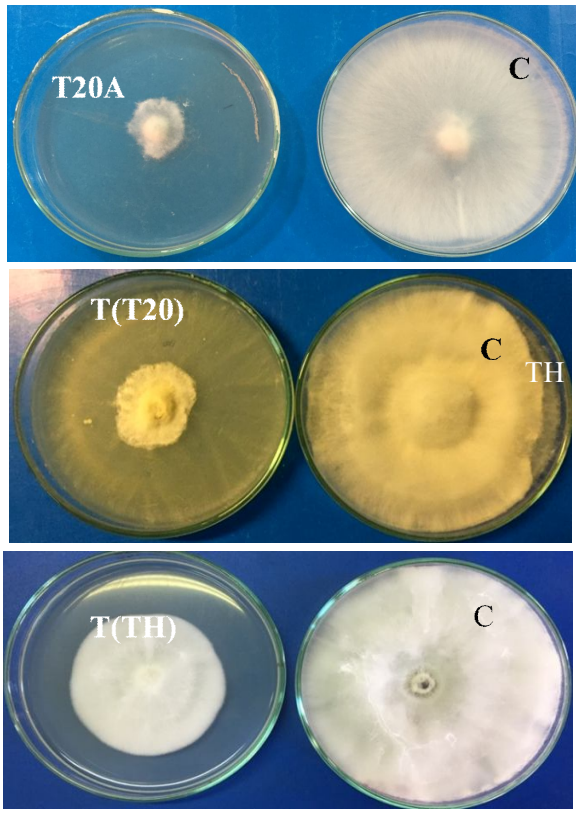

Figure 2 Growth inhibition of *Rosellinia necatrix* ESKA1 isolate in response to the volatile compounds of different *Trichoderma harzianum* isolates. T: *T. harzianum* isolates, C: *R. necatrix* ESKA1 isolate on PDA.

Liquid extracellular compounds

Liquid extracellular compounds of both isolates T20 and T20A could completely prevent growth of the pathogen isolate (Fig. 3). The pathogen isolate, ESKA1 showed only 25.24% decrease in colony radial growth in response to extracellular compounds filtrated from T.H isolate liquid culture (Table 1). Based on In Vitro results, T20A isolate had the most antagonistic effect in sum and was selected as a biocontrol agent for the greenhouse experiments.

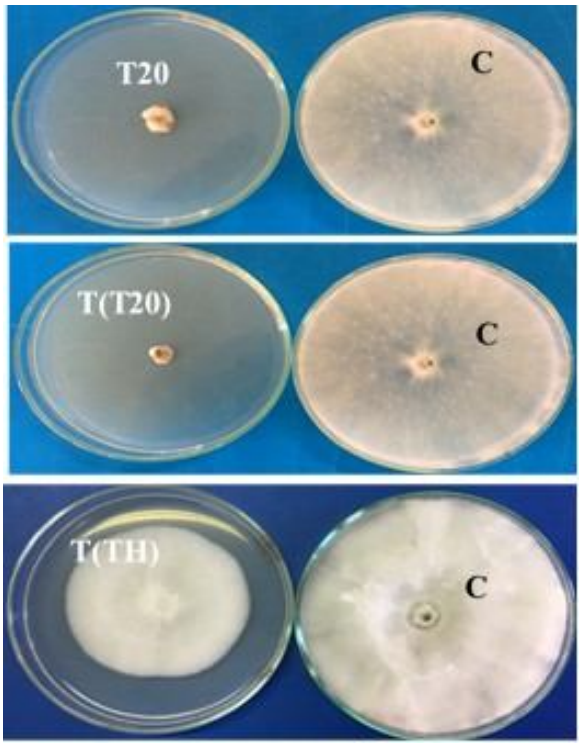

Figure 3 Growth inhibition of *Rosellinia necatrix* ESKA1 isolate in response to the liquid culture filtrate of different *Trichoderma harzianum* isolates. T: *T. harzianum* isolates, C: *R. necatrix* ESKA1 isolate on PDA.

Greenhouse experiments

Biological control of white root rot

Based on scoring root rot, the T20A isolate had the most biocontrol effect on MM111 apple root rot by a 63.84% reduction in disease. The biocontrol effect on MM106, M25 and M7 infected rootstocks were 61.13%, 28.63% and 17.47% reduction of root rot respectively (Fig. 4, Fig. 5).

The chemical control of white root rot

Thiophanate Methyl caused a reduction of white root rot on different rootstocks. Root rot reductions after applying fungicide on MM106, MM111 and M25 rootstocks were 94.11%, 92.32% and 70.89% respectively. Fungicide had the least reduction of root rot index on M7 rootstock (38.45%) (Fig. 5, Table 2).

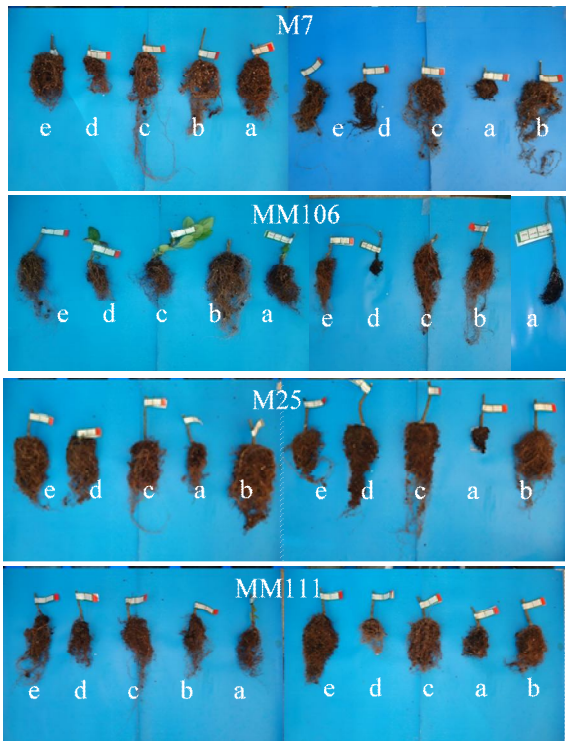

Figure 4 Root rot on two-year-old stocks of M7, M25, MM111, MM106, a: positive control, b: negative control, c: antagonist, d: antagonist + pathogen, e: fungicide + pathogen.

The biocontrol effect on leaf fall index

The antagonist caused a reduction of leaf fall symptom on apple rootstocks differentially. *T. harzianum* T20A isolate caused the most decrease (57.4 %) in leaf fall, on MM106 rootstock and on other rootstocks: M7, M25 and MM111, the reductions of leaf fall were 56%, 40.23%, and 26.42% respectively (Fig. 6).

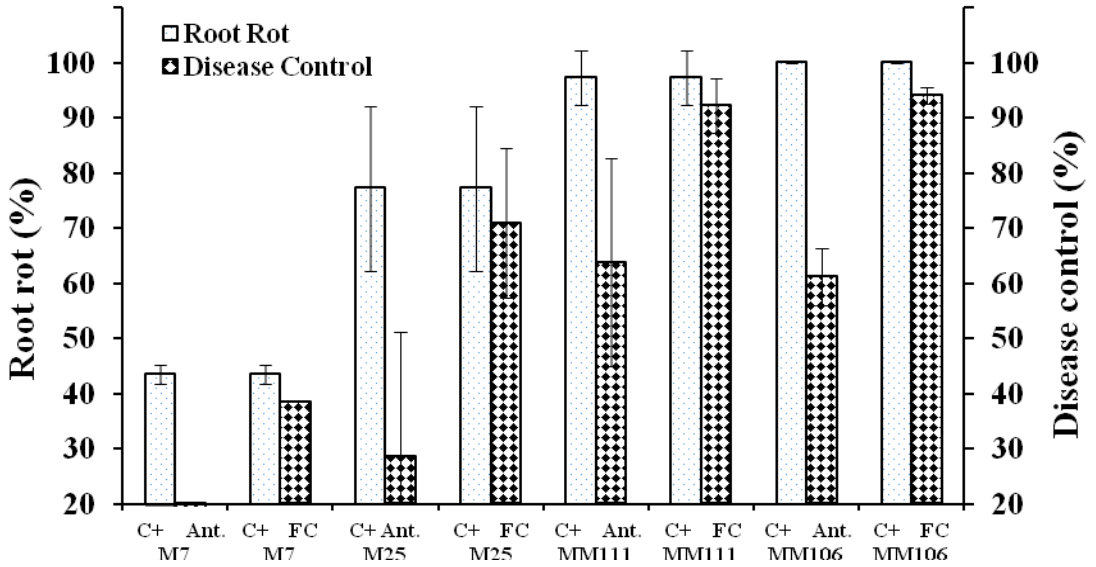

Figure 5 White root rot percentage in control treatments of antagonist (T20A) and fungicide in four apple rootstocks M7, M25, MM111, MM106.
C+: Positive control, Ant.: Antagonist, FC: Fungicide.

Table 2 The effect of antagonist, fungicide and rootstocks on root rot index compared with non-infected treatment.

Treatments	Root rot (%)	Treatments	Root rot (%)
P + A + M7	26.03 bc	A + M7	0 d
P + A + M25	48.60 b	A + M25	0 d
P + A + MM111	33.53 b	A + MM111	0 d
P + A + MM106	38.92 b	A + MM106	0 d
M7 C^+	43.50 b	M7 C^-	0 d
M25 C^+	77.23 a	M25 C^-	0 d
MM111 C^+	97.37 a	MM111 C^-	0 d
MM106 C^+	100.06 a	MM106 C^-	0 d
P + F + M7	5.05 cd	F + M7	0 d
P + F + M25	6.25 cd	F + M25	0 d
P + F + MM111	5.05 cd	F + MM111	0 d
P + F + MM106	5.95cd	F + MM106	0 d

P: Pathogen, A: Antagonist, F: Fungicide, C^+: Pathogen positive control, C^-: Pathogen negative control.

The fungicide effect on leaf fall index

Fungicide caused reduction of leaf fall symptom on different rootstocks. Thiophanate Methyl caused the most decrease (94 %) of leaf fall, on M25 rootstock and on other rootstocks MM111, M7 and MM106, the reductions of leaf fall were 81.76%, 81.27%, and 72.55% respectively (Fig. 6, Table 3).

The reaction of apple rootstocks to the ESKA1 isolate

Based on the root rot index, MM106 with 100%, MM111 with 97.37% and M25 with 77.23% root rot index showed susceptibility to the pathogen and M7 with 43.5% root rot index had the least susceptibility to the pathogen (Table 2). All rootstocks were also susceptible based on leaf fall index, M25, M7 and MM106 showed 100%, 84.56% and 78% leaf fall respectively. MM111 with 58.48% leaf fall, had the least susceptibility to leaf fall following infection (Table 3).

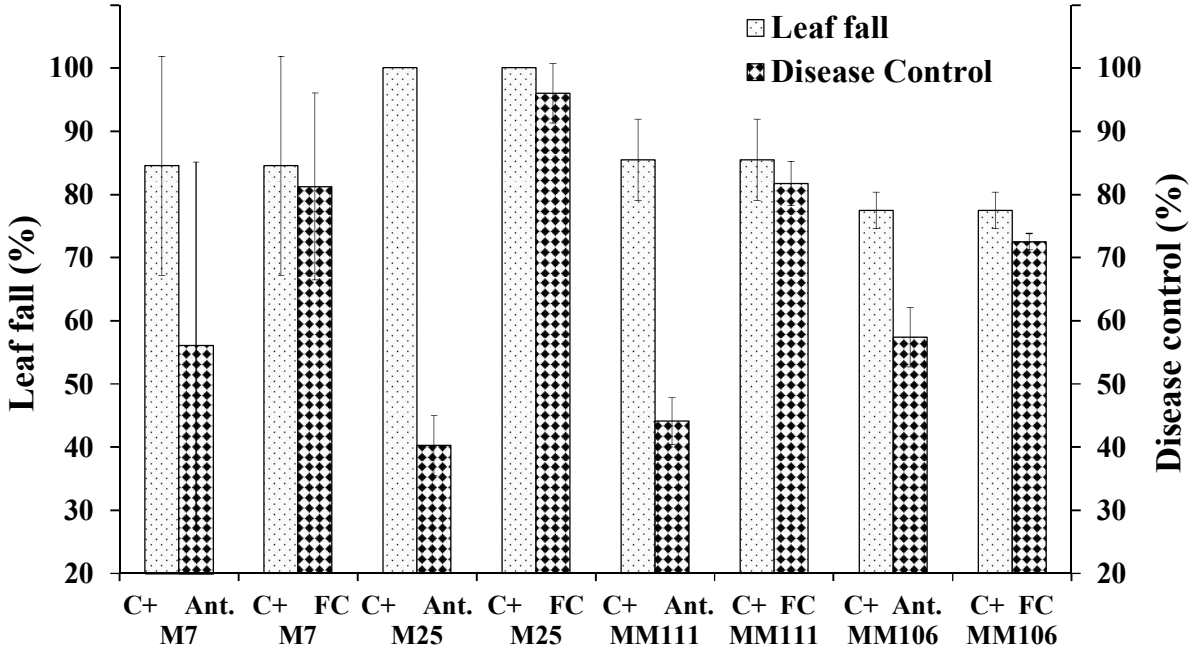

Figure 6 Leaf fall percentage caused by white root rot disease in control and treatments of antagonist and fungicide on four apple rootstocks M7, M25, MM111, MM106.
C+: Positive control, Ant.: Antagonist, FC: Fungicide

Table 3 The effect of antagonist, fungicide and rootstocks on leaf fall index compared with non-infected treatment.

Treatments	Leaf fall (%)	Treatments	Leaf fall (%)
P + A + M7	28.49 cde	A + M7	0 e
P + A + M25	59.83 abc	A + M25	0 e
P + A + MM111	41.39 bcd	A + MM111	0 e
P + A + MM106	20.05 de	A + MM106	0 e
M7 C+	84.56 a	M7 C-	0 e
M25 C+	100.0625a	M25 C-	0 e
MM111 C+	85.4896 a	MM111 C-	0 e
MM106 C+	77.4641ab	MM106 C-	0 e
P + F + M7	3.28 e	F + M7	0 e
P + F + M25	4.02 e	F + M25	0 e
P + F + MM111	3.72 e	F + MM111	0 e
P + F + MM106	4.90 e	F + MM106	0 e

P: Pathogen, A: Antagonist, F: Fungicide, C^+: Pathogen positive control, C^-: Pathogen negative control.

Discussion

There is a huge amount of research aimed at trying to exploit antagonisms against plant pathogens. The genus *Trichoderma* comprises a great number of fungal strains that act as biological control agents and amongst them, *T. harzianum* is well-known and promising which has been used to control soil-borne plant pathogens in an eco-friendly manner for a long

time (Asad *et al*., 2014; Singh *et al.,* 2018; Benítez *et al*., 2004). In a research, 56 *T. harzianum* isolates, collected from different regions of Spain were evaluated for biocontrol potential on avocado white root rot as an immediate result, they had different potential to inhibit the growth of the pathogen. They suggested different *Trichoderma* isolates use different mechanism against fungal pathogens based on their genetic diversities and environmental conditions (Ruano-Rosa *et al*., 2010). The growth rate of the biocontrol agent is critical in antagonistic properties definition. The higher growth rate the more biocontrol potential (Rey *et al*., 2001). The antagonistic properties of *Trichoderma* are based on the activation of multiple mechanisms which are either indirectly by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth or plant defensive mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism. These indirect and direct mechanisms may act coordinately and their importance in the biocontrol process depends on the *Trichoderma* strain, the antagonized fungus, the crop plant, and the environmental conditions, including nutrient availability, pH, temperature, and iron concentration. Activation of each mechanism implies the production of specific compounds and metabolites, such as plant growth factors, hydrolytic enzymes, siderophores, antibiotics, and carbon and nitrogen permeases (Benítez *et al*., 2004). Considering the complexity of soil environment, limited accessibility to selective and efficient control for soil-borne root pathogens, concerns and difficulties involved in soil fumigation, and last but not least high cost of fumigant and fungicides (Grube *et al*., 2011), there is an urgent and growing demand for biologically based soil-borne pathogen management practices and approaches. There are a lot of reports on biological control of a wide range of soil-borne pathogens by *T. harzianum* in Iran (Mohammadi *et al*., 2009; Sadravi and Haji, 2019) but there is no report on white root rot biological control in the country. The greenhouse results showed that the biocontrol efficiency of *T. harzianum* strain decreased in the greenhouse in comparison with lab evaluation results. The most efficient biocontrol effect was observed in MM111 rootstock by 63.84% reduction in white root rot index compared to infected control without antagonist treatment and for leaf fall index reduction, the most efficiency of the antagonist was observed on MM106 rootstock by 57.4%, reduction in leaf fall. MM111 a Malling Merton rootstock has been bred and developed to provide resistanceto drought and tolerance to apple fire blight. Our result showed that MM111 is not resistant to white root rot (94% root rot index) but its susceptibility to white root rot may be of different degrees depending on soil conditions, however this issue was not addressed in this research. MM106, a semi-dwarf and early bearing rootstock is very susceptible to fire blight and *Phytophthora* root rot. It also proved susceptible to *R. necatrix* attack according to our results (100% root rot index). On the contrary, M7 rootstock had the least root rot index (43.5 %) and was less susceptible to the pathogen. White root rot is one of the most destructive root system diseases in fruit trees that is spreading in the last few decades in the world. In Iran, although the disease occurrence seems to be decreased in recent years following the precipitation reduction, it's a potential and important threat to orchards and nurseries especially in heavy soils with poor drainage conditions. Few studies are made on *R. necatrix* and only a report was published on the rapid detection of the pathogen from the stone fruit trees in recent years (Mohammadi Meymand *et al*., 2010) but recently there was no report on white root rot in pome fruit trees. This is the first study of apple rootstocks reaction and biological control of white root rot disease in the country and hope to provide a more efficient, eco-friendly and well-balanced plant health management for apple orchards and nurseries either by integrating resistance and biocontrol measures or replacing chemical control by antagonist application to soil.

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بررسی واکنش برخی از پایه‌های سیب به کنترل بیولوژیک پوسیدگی سفید ریشه *Rosellinia necatrix* توسط *Trichoderma harzianum* در شرایط گلخانه

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چکیده: در این پژوهش نخست اثر سه جدایه از *Trichoderma harzianum* در کنترل بیولوژیکی بیمارگر *Rosellinia necatrix* عامل بیماری پوسیدگی سفید ریشه در آزمایشگاه بررسی شد. جدایه T20A در آزمون‌های کشت متقابل، تولید ترکیبات فرّار ضدقارچی و مواد مایع خارج سلولی، به‌ترتیب با ۵۹/۲۷، ۸۷/۳۸ و ۱۰۰درصد بازدارندگی از رشد پرگنه قارچ بیمارگر، مؤثرترین جدایه از *T. harzianum* در این پژوهش شناسایی و اثر آن در کنترل بیماری پوسیدگی سفید ریشه روی چهار پایه از پایه‌های تجاری سیب در گلخانه مورد آزمون قرار گرفت. شاخص‌های درصد پوسیدگی ریشه و ریزش برگ پس از گذشت ۷۰ روز از مایه‌زنی بیمارگر تعیین شد. اثر جدایه T20A بر کاهش پوسیدگی ریشه نسبت به شاهد آلوده در پایه‌های MM106, MM111، M25 وM7 به‌ترتیب، ۶۳/۸۴، ۶۱/۱۳، ۲۸/۶۳ و ۱۷/۴۷ برآورد گردید. اثر عامل بیوکنترل بر کاهش ریزش برگ در پایه‌های MM106، M7، MM111 و M25 به‌ترتیب ۵۷/۴، ۵۶/۰۶، ۴۴/۰۹ و ۴۰/۲۴ درصد برآورد گردید. تأثیر تیمار قارچ‌کش نیز در کاهش پوسیدگی ریشه و کاهش ریزش برگ بر این پایه‌ها در گلخانه مورد ارزیابی و مقایسه قرار گرفت. بنا براین نتایج بیش‌ترین اثر آنتاگونیست به‌ترتیب مربوط است به کنترل بیماری در پایه MM111، با۸۴/۶۳ درصد کاهش در پوسیدگی ریشه و پس از آن در پایه MM106 که با ۵۷/۴ درصد کاهش در ریزش برگ، در کنترل بیماری نقش داشت. در بین پایه‌های سیب مورد ارزیابی، MM106 به‌طور میانگین با پوسیدگی ریشه ۱۰۰ درصد و ۷۸ درصد ریزش برگ بیش‌ترین حساسیت را به بیماری و پایه M7 با ۴۳/۵ درصد پوسیدگی ریشه و ۸۴/۵۶ درصد ریزش برگ از حساسیت کم‌تری نسبت به پوسیدگی سفید ریشه برخوردار بودند. پایه MM111 با ۵۸/۴۸ درصد ریزش برگ، کم‌ترین درصد ریزش برگ را در اثر آلودگی به بیماری نشان داد. این پژوهش روی پایه‌های تجاری سیب برای اولین بار در کشور انجام می‌شود. نتایج این پژوهش پیشنهاد می‌کند که برای مدیریت بیماری می‌توان از مدیریت تلفیقی مقاومت و بیوکنترل بهره گرفت و یا استفاده از آنتاگونیست را جایگزین سموم شیمیایی در خاک نمود.

واژگان کلیدی: *Trichoderma harzianum*، *Rosellinia necatrix*، پایه‌های سیب، پوسیدگی سفید ریشه سیب