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Research Article

Biocontrol of tomato gray mold disease by *Trichoderma harzianum* and *Bacillus subtilis*

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Abstract: This study aimed to evaluate the antagonistic activity of some fungal and bacterial isolates against *Botrytis cinerea*, the causal agent of tomato gray mold disease. For this purpose, out of six fungal isolates obtained from the gray mold symptoms on tomato and melon, isolates B1 and B2 were selected based on the pathogenicity test result for the *in vitro* and *in vivo* experiments. These isolates were identified as *Botrytis cinerea* based on morphological and molecular information (ITS sequence). In dual culture test of two bacterial and six antagonistic fungal isolates, *Trichoderma harzianum* T1 and *Bacillus subtilis* B43 with up to 60% and 71.54% of inhibition levels, respectively, were the most efficient treatments to limit fungal growth. In volatile compounds tests, isolates T1 and B43 inhibited pathogen mycelia growth up to 95.98 and 100%, respectively. The results of the secondary metabolites test showed that *B. subtilis* B43 inhibited pathogen mycelium growth by 98%. *In vivo* experiments showed that the isolates T1 and B43 controlled gray mold of tomato effectively, and the average inhibition rates were more than 60%. None of the antagonistic isolates significantly affected the height, fresh and dry weight of whole parts of the plants compared to healthy control.

Keywords: Antagonist, biological control, *Botrytis cinerea*, tomato

Introduction

Tomato *Solanum lycopersicum* is one the most widely grown and highly consumed fresh vegetable crops globally (Al-Saleh, 2011). Fungal diseases are a major limiting factor for tomato production. *Botrytis cinerea*, the causal agent of gray mold disease, is a necrotrophic fungus infecting more than 200 host plants (Chen *et al*., 2019). Gray mold is one of the most critical and widespread pre-and post-harvest diseases attacking flowers, fruits, leaves, and stems of tomato plants grown in greenhouses, and it causes massive crop losses (Shtienberg *et al*., 1998).

Control of *B. cinerea* is difficult due to its several attack modes, diverse hosts, and inoculum sources, and because it can survive as mycelia, conidia, and sclerotia in crop debris (Herrera-Téllez *et al*., 0219). Chemical fungicides are mainly used for preventing and treating tomato gray mold disease (Correa and Soria, 2010). Nevertheless, there is a global trend to find alternative methods to chemical fungicides to manage plant diseases, reduce the disease's damage, and reduce the effects of chemical compounds on human health and the environment (Chen *et al*., 2019). In this regard, biological agents constitute an excellent alternative to replace chemicals for disease control (Herrera-Téllez *et al*., 2019). The genera *Trichoderma* and *Bacillus* include a promising pool of organisms with potential for *B. cinerea* control (Gao *et al*.,

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2017; Vos *et al*., 2015). They are widely used in the biocontrol of different diseases.

Biocontrol activities of *Trichoderma* spp. can be exerted through different mechanisms, such as mycoparasitism, the production of extracellular hydrolytic enzymes, competition for nutrients, and secondary metabolites with antifungal activity (Benítez *et al*., 2004). Moreover, *Trichoderma* also exerts an indirect control against pathogens through the induced systemic response (ISR) in plant cells, resulting in an enhanced defense (Bigirimana *et al*., 1997). Freeman *et al.* (2004) used three species of *Trichoderma*, including *T. harzianum*, *T. atroviride*, and *T. longibrachiatum*, against gray mold disease on strawberry plants under greenhouse conditions and their results showed significant control of the disease. In Zavvari (2010) studies, 13 isolates of *Trichoderma* belonging to three species of this genus were screened for β- 1,3 glucanase activity and biological potential to control cucumber root rot *Phytophthora drechsleri in vitro* and *in vivo*. All antagonist isolates inhibited mycelia growth of the pathogen in dual culture and through the production of volatiles. According to the results, *T. harzianum* T1 showed a maximum rate of enzyme activity. The effects of *Trichoderma* isolates on the biocontrol of cucumber root rot were investigated in greenhouse conditions, and *T. harzianum* T1 was the best isolate. Antagonistic activity of *T. asperellum* and *T. harzianum* against genetically diverse *B. cinerea* isolates was evaluated by Kuzmanovska *et al.* (2018) *in vitro*. Both antagonists significantly inhibited the mycelia growth and conidia germination of pathogen isolates (Kuzmanovska *et al.*, 2018). Also, several modes of action have been described to explain *Bacillus* antagonism toward fungal pathogens. They include colonizing the surface or wounds of plants and producing broad-spectrum antibiotics that suppress various plant pathogens (Stein, 2005). Khosro-Anjam (2015) evaluated the biocontrol ability of some antagonist strains of *Trichoderma* sp., *Pseudomonas* spp., and *Bacillus* spp. against root rot disease of bean caused by *Fusarium solani* f. sp. *phaseoli* in both laboratory and greenhouse conditions. *Bacillus subtilis* B43 showed more than 60% inhibitory rate on mycelia growth of the pathogen *in vitro*. In Wang *et al.* (2018) studies, *Bacillus subtilis* reduced the gray mold disease on tomato plants by more than 80% and significantly promoted plant growth.

In this study, the antagonistic activities of some fungal and bacterial agents have been evaluated against *B. cinerea* mycelial growth *in vitro*. Out of them, isolates *T. harzianum* T1 and *B. subtilis* B43 were selected to be assessed against tomato gray mold disease in greenhouse conditions.

Materials and Methods

Sampling and pathogen isolates

Tomato and melon plants with gray mold symptoms were collected from some greenhouses in Pakdasht, Tehran province, during December 2019 and January 2020. Six isolates of the fungal pathogen were isolated and purified from infected tomato and melon plants and stored on a PDA slant at 4 °C.

Fungal isolates were identified based on morphological features on PDA at 25 °C under continuous light. Fungal attributes such as conidiophores and conidia shape and size were taken from microscopic slides using an Olympus BH2 light microscope (Olympus, Japan). Sclerotia were produced on PDA at 20 °C under continuous dark after seven days.

Pathogenicity tests

Tomato seeds (cultivar Valero, Rijk Zwaan Company, Netherland) were surface-sterilized in 0.5% sodium hypochlorite for five min and rinsed three times with distilled water. Seeds were sown on a seedling tray filled with sterile Perlite and Coco Peat. After three weeks, individual seedlings were transplanted into plastic pots filled with sterile field soil, coco peat, and sand (1: 1: 1 ratio). Plants were maintained at 18-20 °C in the greenhouse for two weeks. Conidial suspensions (1×10^5 conidia/ml) of each *B. cinerea* isolate (B1, B2, B3, B4, B5, and B6) were sprayed on tomato plants with three replications (each pot considered as one replication). Water was used as the negative control. The pots were individually covered with plastic bags and incubated in a greenhouse for 48 h to maintain a relative humidity of 100%. Then the

pots were maintained at approximately > 80% humidity at 18-20 ℃. Evaluations were performed daily to verify the appearance of symptoms.

For the molecular identification of *B. cinerea* isolates, the whole genomic DNA was extracted from seven-day-old mycelia using the Cenis (1992) method. ITS regions were amplified using ITS1 and ITS4 primers (White *et al.*, 1990). Ebrahimi and Fotouhifar (2016a) protocol for PCR amplification and sequencing was followed. Molecular analysis was performed according to Ebrahimi and Fotouhifar (2016a).

Antagonistic isolates

Bacillus subtilis B43 and B44, and fungal isolates including *T. harzianum* T1 and T14, *Clonostachys rosea* IR4, *Sarocladium strictum* IR6, and *Sarocladium kiliense* IR5 were obtained from the fungal collection of Department of Entomology and Plant Pathology, College of Aburaihan, University of Tehran. The antagonistic activity of *B. subtilis* and *T. harzianum* isolates has been proved against different pathogens such as *Fusarium solani* f. sp. *phaseoli* (Khosro-Anjam, 2015) *Phytophthora drechsleri* (Zavvari, 2010) in the Department of Entomology and Plant Pathology, College of Aburaihan. The isolates IR4, IR5, and IR6 have been isolated from apple and pear leaves (Ebrahimi and Fotouhifar, 2016b), and their antagonistic activity against *B. cinerea* was investigated.

***In vitro* assays**

Dual culture

Dual culture tests were performed based on Dennis and Webster (1971). Plugs (5 mm in diameter) of each antagonistic fungus and pathogenic isolates were seeded opposite on PDA. For dual culture test of bacteria based on Thomashow and Weller (1996), two loops of the 24 hour-old bacteria were streaked on half plates and sterile deionized water as a control. Then a plug cut from the leading edge of a one-week-old culture of *B. cinerea* on PDA was placed on the other half of the plate. Plates with three replicates for each treatment were incubated at 25 ℃ for five days. The growth inhibition percentage was calculated using the formula $n = \frac{a-b}{a} \times 100$, where n is the growth inhibition percent; a is the colony diameter of uninhibited *B. cinerea*, and b is the colony diameter of fungus/bacterium-treated *B. cinerea* (Etebarian *et al*., 2005).

Volatile compounds

Antifungal activity of volatile compounds of fungi and bacteria was assessed by inoculating a plate containing PDA medium with a plug of the pathogen. The second plate with an antagonistic fungus and/or plate containing nutrient agar (NA) was cultured by four loops of 24-hour-old bacterium and sterile deionized water as control. Two plates were wrapped together with parafilm and incubated at 25 ℃ for five days (Lillbro, 2005). Three replications were considered for each treatment. The growth inhibition percentage was determined by the formula described above.

Secondary metabolites

In antibiosis tests, four loops of bacterial strains were cultured on PDA and incubated at 25 ℃ for 72 h. After three days, the bacterial strains were washed from plates and treated with chloroform for 30 min. A plug of a seven-day-old pathogen culture was placed at the center of the plate (Kraus and Loper, 1992). The test was performed with three replications for each treatment. Plates were incubated at 25 ℃ for five days, and the growth inhibition was calculated based on the mentioned formula above.

Biocontrol assessments under greenhouse conditions

Tomato seedlings after one month of seeding were inoculated with *B. subtilis* B43 and *T. harzianum* T1 with a final concentration of 10^8 cfu/ml. *Botrytis cinerea* isolates suspension (10^5 conidia/ml) were sprayed on the whole aerial parts of plants 24 h after antagonist inoculation. The plants were covered with plastic bags to maintain a relative humidity of 100% and incubated in a greenhouse at 18-20 ℃. Mancozeb (2 kg/1000 L) was used as a positive control, and water was used as the negative control and applied simultaneously with the antagonists. Four pots (each containing

two seedlings) were considered for each treatment. Disease severity was evaluated 14 days after inoculation, and its assessment was conducted based on visual evaluation of lesions caused by *B. cinerea.* Also, the effects of different treatments on plant growth parameters, including fresh and dry weights (g) (whole parts of the plant) and aerial parts height (mm) of the plants, were evaluated after two weeks of pathogen inoculation. Fresh and dry weights of plants were measured with a precision analytical balance. All measurements were recorded for eight plants of each treatment with two independent repetitions.

Statistical analysis

Experiments were conducted in a completely randomized design and randomized complete block design for *in vitro* and *in vivo* tests, respectively. The analysis of variance was performed using SAS ver. 9.1 software. Mean comparisons were performed using Fisher's Least Significance Difference (LSD) Test.

Results

Pathogen isolates

Five tomato plants and one melon stem with gray mold symptoms were collected, and six fungal isolates were purified and identified as *B. cinerea* based on morphological features.

Botrytis cinerea Pers. Ann. Bot. (Usteri) 1: 32 (1794)

Specimen examined. IRAN, Tehran Province, Pakdasht, on tomato stem, 31 Dec 2019, *H. Jalali* & *L. Ebrahimi* B1 (accession number: B37); IRAN, Tehran Province, Pakdasht, on the melon stem, 11 Dec 2019, *H. Jalali* & *L. Ebrahimi* B2 (accession number: B38).

Morphological features of the isolates (Fig. 1) were according to the description provided by Ellis (1971) and Jarvis (1977). Molecular data of ITS regions of B1 and B2 isolates (Genebank accession numbers: MW653742 and MW653743, respectively) confirmed the morphological identification as *B. cinerea*. The NCBI blast of ITS sequences of our isolates showed 100% identity to *B. cinerea* isolates in GenBank.

Pathogenicity

In the greenhouse, gray mold symptoms were observed on leaves, and stem for all pathogen isolates one week after inoculation. There was no symptom in control after the same time. Isolates B1 and B2 infected aerial parts of the plants (Fig. 2) and were selected for the *in vitro* and *in vivo* experiments. The fungal agents were isolated from the rotten tissues of the plants again and identified based on morphological characteristics but not from control treatments, confirming Koch's postulates.

***In vitro* assays**

The dual culture and volatile compounds tests showed that *T. harzianum* T1 and *B. subtilis* B43 had the most mycelia growth inhibitory rate against pathogen isolates (Figs. 3, 4). In secondary metabolites tests, *B. subtilis* B43 and B44 were inhibited by 98% *B. cinerea* mycelia growth (Table 1). Based on these results, isolates *T. harzianum* T1 and *B. subtilis* B43 were selected to be used in greenhouse assessments.

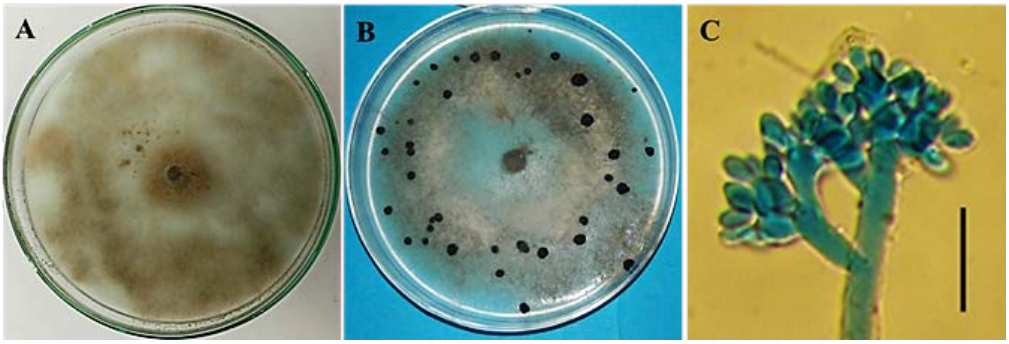

Figure 1 *Botrytis cinerea*, isolate B1. **A** colony on PDA after seven days at 25 °C in the continuous light condition, and **B** sclerotia on PDA after seven days at 20 °C in the continuous dark condition. **C** conidiophore and conidia. Bar = 10 µm.

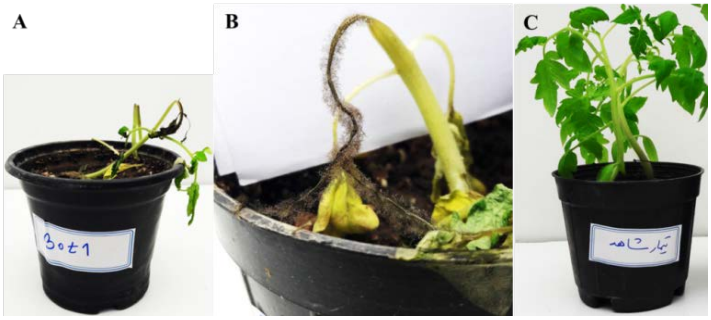

Figure 2 Symptoms of gray mold disease on tomato seedlings inoculated with *Botrytis cinerea* isolates A B1 and B B2 compared to **C** control after seven days incubation at 20 ºC under greenhouse conditions.

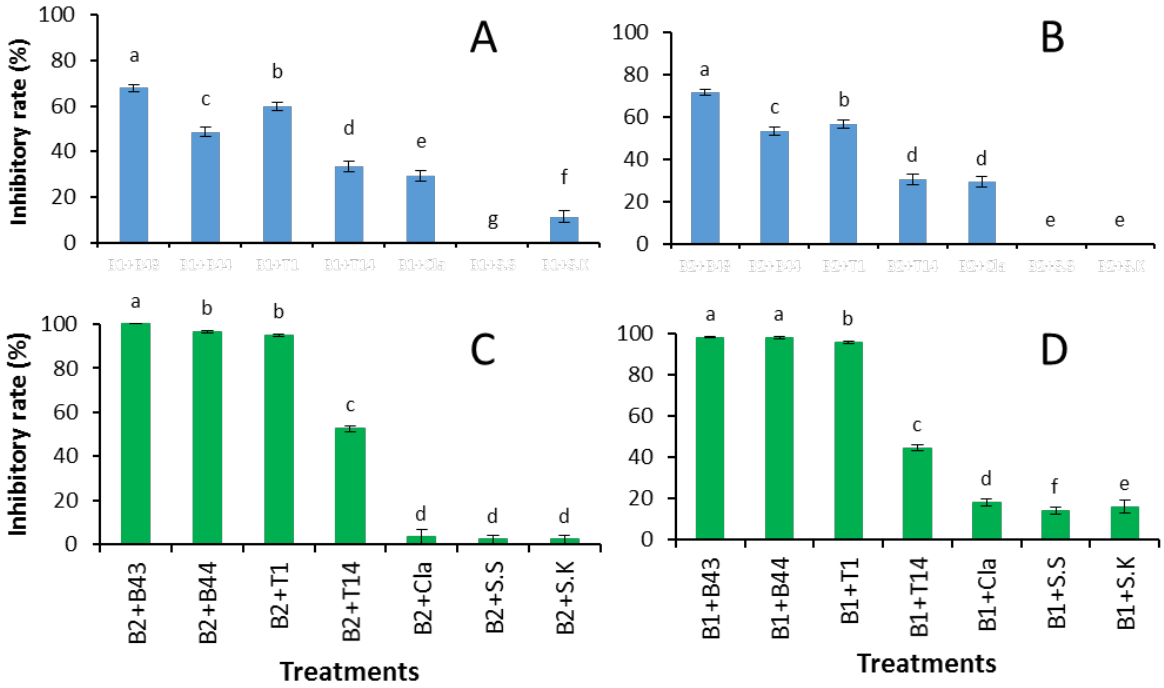

Figure 3 Effects of antagonistic agents on mycelium growth of *Botrytis cinerea* isolates B1 (**A** and **C**) and B2 (**B** and **D)** in dual culture (**A** and **B**) and volatile compounds (**C** and **D**) tests after five days. Data are the means ± SE of three replications. Columns followed with the same letter are not significant at 1% probability level (LSD test). B1, B2: *Botrytis cinerea*; B43, B44: *Bacillus subtilis* isolates; T1, T14: *Trichoderma harzianum*; Cla: *Clonostachys rosea* IR4; S.S: *Sarocladium strictum* IR6; S.K: *Sarocladium kiliense* IR5.

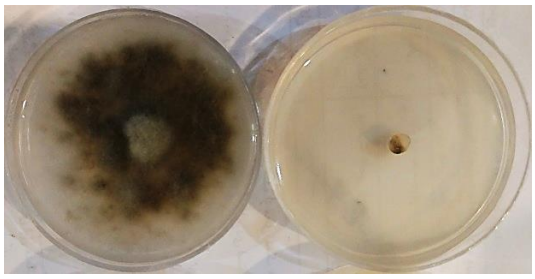

Figure 4 Effect of *Bacillus subtilis* B43 on mycelia growth of *Botrytis cinerea* B2 in volatile compounds tests (left: control treatment; right: pathogenic isolate treated by volatile compounds of antagonist).

Table 1 Inhibitory effect of *Bacillus subtilis* B43 and B44 isolates on mycelia growth of *Botrytis cinerea* isolates B1 and B2 after five days in secondary metabolites tests.

Treatments	Inhibition (%)	
	Botrytis cinerea B1	*Botrytis cinerea* B2
Bacillus subtilis B43	98.89 ± 0.40 a	98.69 ± 0.56 a
Bacillus subtilis B44	98.42 ± 0.30 a	98.30 ± 0.50 a

Data are the means of three replications ± standard error. Means in a column followed with the same letter are not significant at 5% level of probability (LSD test).

Biocontrol of tomato gray mold disease by *Trichoderma harzianum* and *Bacillus subtilis* under greenhouse conditions

In the greenhouse, the gray mold symptoms 14 days after inoculation were reduced in T1- and B43- inoculated plants compared to the untreated plants (Figs. 5, 6). These results were concordant with the reduction in fresh and dry weight loss triggered by pathogen infection in antagonist-treated plants about the untreated plants (Table 2). Mancozeb completely inhibited the disease symptoms (Fig. 5). None of the antagonistic isolates and mancozeb significantly affected the height, fresh and dry weight of the plants compared to control plants (Table 2).

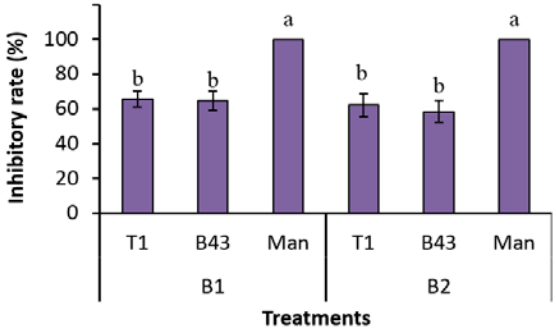

Figure 5 The effect of antagonists and mancozeb fungicide against gray mold disease on tomato plants after 14 days of pathogen inoculation under greenhouse conditions. Data are the means ± SE of eight replications (four pots containing eight plants). Columns with the same letter are not significant at 1% probability level (LSD test). B1, B2: *Botrytis cinerea* isolates; T1: *Trichoderma harzianum*; B43: *Bacillus subtilis*; Man: mancozeb.

Figure 6 Symptoms of gray mold disease on tomato plants treated by; **A** *Botrytis cinerea* B2 and **B** *B. cinerea* B2 + *Trichoderma harzianum* T1 at 20 °C after 14 days of pathogen inoculation in the greenhouse.

Table 2 Height (mm) of aerial parts**,** fresh and dry weight (g) of whole parts of tomato plants in different treatments of *Botrytis cinerea*, *Trichoderma harzianum*, *Bacillus subtilis* and mancozeb, 14 days after pathogen inoculation under greenhouse conditions.

Treatments	Shoot height (mm)		Fresh weight (g)		Dry weight (g)	
	B1	B2	B1	B2	B1	B2
Pathogen + T1	174 ± 5.8 b	162 ± 9.2 b	45.27 ± 1.5 b	44.77 ± 4.3 b	3.82 ± 0.05 bc	3.27 ± 0.07 c
Pathogen + B43	166 ± 13.7 b	153 ± 10.4 bc	43.25 ± 4.0 b	42.25 ± 1.9 b	3.20 ± 0.19 c	2.90 ± 0.17 cd
Pathogen + Man	203 ± 3.8 a	204 ± 4.9 a	56.67 ± 4.3 a	59.90 ± 3.5 a	4.74 ± 0.99 ab	4.37 ± 0.86 b
T1	202 ± 9.2 a	202 ± 9.2 a	58.27 ± 2.9 a	58.27 ± 2.9 a	4.68 ± 0.96 ab	4.68 ± 0.96 ab
B43	201 ± 4.3 a	201 ± 4.3 a	57.50 ± 1.5 a	57.50 ± 1.5 a	4.62 ± 0.80 ab	4.62 ± 0.80 ab
Man	201 ± 11.7 a	201 ± 11.7 a	57.12 ± 5.1 a	57.12 ± 5.1 a	4.88 ± 0.89 a	4.88 ± 0.89 ab
Pathogen	98 ± 8.5 c	141 ± 19.3 c	17.00 ± 0.8 c	25.32 ± 2.5 c	1.52 ± 0.03 d	2.00 ± 0.06 d
Control	204 ± 4.9 a	204 ± 4.9 a	59.60 ± 6.9 a	59.60 ± 6.9 a	5.51 ± 1.05 a	5.51 ± 0.05 a

Data are means of eight replications (four pots containing eight plants) ± standard error. Means in a column followed with the same letters are not significant at 1% probability level (LSD test). B1, B2: *Botrytis cinerea* isolates; T1: *Trichoderma harzianum*; B43: *Bacillus subtilis*; Man: Mancozeb.

Discussion

Biocontrol of plant pathogens instead of synthetic fungicides provides adequate protection for plants, animals, humans, and the natural environment. There are many reports of antagonistic microorganisms such as *Bacillus* (Gao *et al*., 2017), *Clonostachys rosea* (Gong *et al*., 2017), *T. harzianum* (Shtienberg *et al*., 1998), and yeast strains (Parafati *et al*., 2015) suppressing the development of *B. cinerea*. In the present study, we evaluated the biocontrol potential of some fungal and bacterial isolates, including *T. harzianum* T1 and T14, *C. rosea* IR4, *S. strictum* IR6, and *S. kiliense* IR5, *B. subtilis* B43 and B44 against *B. cinerea* B1 and B2 isolates *in vitro*. Based on the results of dual culture, volatile and non-volatile compounds tests, the isolates *T. harzianum* T1 and *B. subtilis* B43 with a high biocontrol potential were selected for further assays. Under greenhouse conditions, both antagonistic isolates were efficient against gray mold disease.

In recent years, the application of *Bacillus* species to control plant diseases has been reported worldwide (Kilani-Feki *et al*., 2016; Zhang *et al*., 2017; Wang *et al*., 2018; Chen *et al.*, 2019). The *Bacillus* main biocontrol modes of action include biofilm formation, competition for iron, and production of volatile organic compounds (VOCs), cell-wall-degrading enzymes, lipopeptide antibiotics, secondary metabolites, which are greatly affected by the environment (Zhang *et al*., 2017). *Bacillus subtilis* V26 displayed high antifungal activity against several tomato post-harvest pathogens, including *B. cinerea*, with a potent chitosanase activity (Kilani-Feki *et al*., 2016). In Wang *et al*. (2018) studies, *B. subtilis* showed 95.28% inhibitory rate on *B. cinerea* mycelial growth *in vitro*. The pot experiments showed that *B. subtilis* effectively controlled gray tomato mold with an efficiency of 74.7%. Furthermore, in their study, *B. subtilis* stimulated the seed germination and seedling growth of tomatoes (Wang *et al*., 2018). But in the present study, *B. subtilis* B43 did not display a promotion effect on plant' height, fresh and dry weight. Chen *et al.* (2019) evaluated the inhibitory ability and impact of *B. subtilis* VOCs and secondary metabolites against the gray mold on the strawberry, grape, and tomato fruit. Their results showed acceptable biocontrol efficiency for all bacterial strains. All the strains displayed potent cellulase and protease activities but no chitinase activity. All the strains showed biofilm formation, fruit

colonization, and lipopeptide production, which may be the main modes of action of the antagonists against *B. cinerea* on the fruit (Chen *et al.*, 2019).

Trichoderma species reduce the negative effects of plant pathogens by different mechanisms (Herrera-Téllez *et al*., 2019). Their biocontrol mechanisms involve various modes of action, including mycoparasitism, antibiosis, competition, and induction of plant resistance (Reino *et al*., 2008; Vos *et al*., 2015). These mechanisms may act coordinately, and their importance in the biocontrol process depends on the *Trichoderma* strain, the pathogenic fungus, the host plant, and the environmental conditions (Benítez *et al*., 2004; Golafrouz *et al*., 2020). Also, many reports have shown that *Trichoderma* spp. improve many plant species growth parameters, including plant absolute growth rate and fresh and dry weight with a significant difference relative to mock treatment (Bhattacharyya and Basu, 1982). However, in our study, *T. harzianum* T1 had no promotion effect on tomato plant growth parameters, including height, fresh and dry weight of plants. Kuzmanovska *et al*. (2018) evaluated antagonistic activity of *T. asperellum*, and *T. harzianum* against 18 genetically diverse *B. cinerea* isolates *in vitro*. The results showed considerable antagonistic abilities of both *Trichoderma* species against all tested *B. cinerea* isolates (Kuzmanovska *et al*., 2018). Freeman *et al*. (2004) used several *Trichoderma* species, including *T. harzianum* against *B. cinerea*. Their results showed that the isolates were effective in controlling gray mold on strawberries under controlled and greenhouse conditions.

In conclusion, our results indicated that *T. harzianum* T1 and *B. subtilis* B43 were beneficial for controlling tomato gray mold disease caused by *B. cinerea* under greenhouse conditions. Further assessment of these isolates alone or combined with some other controlling procedures for biocontrol of different *B. cinerea* isolates are needed along with application to other tomato cultivars.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

Authors' Contributions

Hossein Jalali performed the project and analyzed the data.

Leila Ebrahimi designed the experiments, analyzed the data and wrote the paper.

Hassan Reza Etebarian revised the paper.

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مهار زیستی بیماری کپک خاکستری گوجه‌فرنگی با استفاده از *Trichoderma harzianum* و *Bacillus subtilis*

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چکیده: در این پژوهش، هدف ارزیابی خاصیت آنتاگونیستی چند جدایه قارچی و باکتریایی علیه قارچ عامل بیماری کپک خاکستری گوجه‌فرنگی می‌باشد. بدین‌منظور، شش جدایه قارچ عامل بیماری از محل علائم بیماری کپک خاکستری روی گیاهان گوجه‌فرنگی و طالبی جداسازی شد. با انجام آزمون بیماری‌زایی در شرایط گلخانه روی گیاهچه‌های یک ماهه گوجه‌فرنگی دو جدایه B1 و B2 برای آزمون‌های مهار زیستی آزمایشگاهی و گلخانه‌ای انتخاب شدند. در آزمون کشت متقابل از بین شش جدایه قارچی و دو جدایه باکتریایی آنتاگونیست، جدایه‌های *Trichoderma harzianum* T1 و *Bacillus subtilis* B43 با بازدارندگی تا حدود ۶۰٪ و ۷۱/۵۴٪ از رشد جدایه‌های قارچ بیمارگر، مؤثرترین تیمارها در مهار بیمارگر بودند. در آزمون ترکیبات فرّار نیز جدایه‌های T1 و B43 به‌ترتیب تا ۹۵/۹۸ و ۱۰۰درصد از رشد میسلیومی قارچ عامل بیماری جلوگیری کردند. در آزمون تولید متابولیت‌های ثانویه، جدایه باکتریایی B43 تا ۹۸درصد مانع رشد میسلیومی هر دو جدایه قارچ بیمارگر شد. آزمون‌های گلخانه‌ای نشان دادند که جدایه‌های T1 و B43 در مهار قارچ عامل بیماری کپک خاکستری مؤثر بودند و متوسط بازدارندگی از بیماری بیش از ۶۰ درصد برآورد شد. هیچ‌یک از جدایه‌های آنتاگونیست تأثیر معنی‌داری روی طول قسمت‌های هوایی گیاهچه، وزن تر و وزن خشک گیاهچه‌ها در مقایسه با شاهد سالم نداشتند.

واژگان کلیدی: آنتاگونیست، گوجه‌فرنگی، مهار زیستی، *Botrytis cinerea*