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An Attempt at Biological Control of Blossom Blight of Rose Caused by *Botrytis cinerea* Using some Local *Trichoderma* spp. Strains

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The northern of Khuzestan province in Iran is mainly considered as one of the major areas of miniature rose production. Blossom blight caused by *Botrytis cinerea* has recently become a serious limiting factor in rose production in pre and post-harvest. In current study, an attempt was made to evaluate the inhibitory potential of some local *Trichoderma* spp. strains against *B. cinerea* under *in vitro* and *in vivo* conditions. The *in vitro* results showed that all *Trichoderma* spp. strains were significantly able to reduce the mycelial growth of the pathogen in dual culture, volatile and non-volatile compounds tests compared with control, with superiority of *T. atroviride* Tsafi than others. Under *in vivo* condition, the selected strain of *T. atroviride* Tsafi had much better performance than *T. harzianum* IRAN 523C in reduction of disease severity compared with the untreated control. Overall, the findings of this study showed that the application of *Trichoderma*-based biocontrol agents such as *T. atroviride* Tsafi can be effective to protect cut rose flowers against blossom blight.

Keywords: Biocontrol, botrytis blight, *Rosa* × *hybrida* L. cv. Ilona, *Trichoderma* spp.

Rose (*Rosa* spp.), an ornamental plant belongs to the family Rosaceae is widely grown for multiple uses including production of petals, extraction of perfumes, extraction of vitamin C from hips, medicinal uses and for sale as cut flowers (Tabassum et al., 2002). The development of rose production is commonly limited because of perishable nature of flowers and susceptibility to different abiotic and biotic factors including mechanical damages, physiological disorders, insects and diseases. Botrytis blossom blight of rose caused by *Botrytis cinerea* Pers.: Fr., a serious disease of greenhouse-grown roses, affects the production, marketing and postharvest quality of cut roses worldwide (Redmond et al., 1987). The pathogen mainly attacks the flowers appearing as small flecks or blisters on petals and also induces stem cankers and lesions on the leaves and vegetative buds (Horst, 1983; Elad, 1988). Progress of these symptoms under favorable conditions can cause a significant economic damage on cut flowers particularly during shipment and storage (Volpin and Elad, 1991). The pathogen can express multiple modes of invasion, colonize diverse hosts as inoculum sources and survive as mycelia and/or conidia for long periods as sclerotia in crop debris leading to failure in control of the disease (Williamson et al., 2007). Various control strategies including sanitation, cultural practices, fungicide sprays and heating are

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commonly used for disease management (Morandi et al., 2000). Resistance of *B. cinerea* to different fungicides including carbendazim and azoxystrobin has been reported, necessitating adoption of alternative management practice such as biological control using microbial biopesticides (Nakkeeran et al., 2019). The biocontrol potential of various fungal antagonists including *Trichoderma harzianum*, *Clonostachys rosea*, *Ulocladium atrum* and different yeasts has previously been shown against *B. cinerea* on rose (Yohalem, 2004). However, the efficacy of different *Trichoderma* spp. has not so far been assessed against *B. cinerea* on rose. Considering serious concerns associated with rose production caused by *B. cinerea* in Iran, our aim in this study was to assess the inhibitory potential of some local *Trichoderma* spp. against *B. cinerea* under *in vitro* and *in vivo* conditions.

Material and Methods

Microorganisms

Botrytis cinerea strain N7, as a virulent pathogen, isolated from rose flowers in Khuzestan province in Iran (Zadehdabagh et al., 2010) was used in this study. A total of nine *Trichoderma* spp. strains listed in Table 1. were assessed for their inhibitory potential against the pathogen. All stock cultures of fungal strains were grown on potato dextrose agar at 25 °C in the dark (PDA, Merck Company, Hamburg).

Dual culture assay

Mycelial plugs (5 mm diameter) of the *Trichoderma* spp. strains were taken from four-day-old cultures and placed on one side of the plates (9 cm) on PDA, 0.5 mm from the border. Similar plugs were taken from the four-day-old culture of *B. cinerea* strain N7 and placed on the opposite side to *Trichoderma* spp. strains at the same distance from the border. Four replicates were assessed for the performance of each fungal antagonist. Plates were incubated at 25 °C for 10 days. Plates inoculated only with the pathogen were used as control. The inhibition percentage was calculated using the following formula: $(C\text{-}T_n)/C \times 100$, where C is the colony radius in the control and T_n is the colony radius of the pathogen in each treatment (Karimi et al., 2017).

Non-volatile toxic compounds

All *Trichoderma* spp. strains were grown in 250 ml Erlenmeyer flasks containing 100 ml home-made sterilized potato dextrose broth (PDB) for 10 days with periodical shaking. Cultures were filtered through Whatmann filter no. 42 filter paper into sterilized flasks. The filtrated cultures were centrifuged at 6000 rpm for 10 min, sterilized by passing it through Cellulose Millipore membrane filter paper (0.4 μm pore size). Culture supernatants were added to melt PDA at a ratio of 15% and poured into plates evenly followed by inoculation with mycelial plug (5 mm diameter) of four-day-old culture of *B. cinerea* strain N7. Non-amended medium was served as control. (Dennis and Webster, 1971b). Plates were incubated at 25°C for 10 days. Four replicates were assessed for each treatment and inhibition percentage was calculated using the formula presented before.

Table 1

Trichoderma spp. strains used in the study to assess their inhibitory potential against *Botrytis cinerea* causing the blossom blight of rose

Fungal strains	Strain	country	source
Trichoderma harzianum	A	Iran	SCUA
T. harzianum	IRAN 523C	Iran	AREEO
T. virens	IRAN 199C	Iran	AREEO
T. atroviride	Tsafi	Iran	SARENRC
T. virens	Tsafi	Iran	SARENRC
T. atroviride	H	Iran	BASU
T.koningiopsis	H	Iran	BASU
T. asperellum	H	Iran	BASU
T. brevicompactum	H	Iran	BASU

SCUA: Shahid Chamran University of Ahvaz; **AREEO**: Agricultural Research, Education and Extension Organization; **SARENRC**: Safiabad Agricultural Research and Education and Natural Resources Center; **BASU**H: Bu-Ali Sina University of Hamdan.

Volatile toxic compounds

In this test, plates containing PDA were inoculated with a mycelial plug (5 mm diameter) of each *Trichoderma* spp. strain and incubated at 25 °C three days earlier. Afterwards, each of the inoculated plates was placed face-to-face with plates inoculated with a mycelial plug (5 mm diameter) of four-day-old culture of *B. cinerea* strain N7 and free-pathogen PDA plugs were used as a control. Both half plates were firmly sealed with parafilm to prevent any loss of volatile compounds. Plates were maintained at 25 °C for 10 days. Four replicates were assessed for the performance of each fungal antagonist. Inhibition percentage was calculated using the formula shown for the dual culture assay (Dennis and Webster, 1971a).

In vivo *assay*

Based on the combined analysis of *in vitro* tests, inhibitory potential of *T. harzianum* IRAN 523C and *T. atroviride* Tsafi were assessed against *B. cinerea* as selected strains under *in vivo* condition. Two independent representative experiments were performed. Instead of the inoculation of rose plants using pathogen inoculums, naturally infected rose plants (*Rosa* × *hybrida* cv.Ilona) grown in a private greenhouse were collected. The selected plants were treated with *Trichoderma* spp. strains. In brief, 50 ml conidial suspensions of *T. atroviride* Tsafi and *T. harzianum* IRAN 523C containing tween 80 (0.1% v/v) were prepared at a concentration of 1×10^6 conidia/ml and sprayed onto the two blocks of rose plants at early flowering stage in greenhouse. The remaining third block was sprayed with sterilized distilled water containing tween 80 (0.1% v/v) as untreated control. After three days, a total of 20 cuttings were harvested for each treatment and transferred into glass bottles half filled with sterile tap water. Glass bottles were kept in another section of the greenhouse at 20°C with the relative humidity of > 90% and 16 h of light-day. Disease severity was measured after five, seven and nine days of treatment using a scale of 0 to 9 as 0 = healthy, 1 = 0-2%; 2 = 2-5%; 3 = 5-10%; 4 = 10-15%; 5 = 15-25%; 6 = 25-50%; 7 = 50-75%; 8 = 75-100% and 9 = 100% (Capdeville et al., 2005).

Statistical analyses

All data were analysed using one-way ANOVA with SAS® software ver. 9.1. The means were compared with the Least Significant Difference (LSD) test.

Results

Dual culture

In dual culture test all *Trichoderma* strains were able to inhibit the mycelial growth of the pathogen significantly compared with control (Fig. 1a). However, there was no significant difference between the tested *Trichoderma* strains except between *T. atroviride* Tsafi and *T. koningiopsis* H (Fig. 1a).

Non-volatile toxic compounds

In this test all *Trichoderma* strains significantly reduced the mycelial growth of the pathogen compared with control (Fig. 1b). Two strains of *T. atroviride* Tsafi and *T. harzianum* A showed the highest inhibition percent against the pathogen. No significant difference was detected between these latter two (Fig. 1b).

Volatile toxic compounds

All *Trichoderma* strains were able to reduce the mycelial growth of the pathogen significantly compared with control except *T. virens* (Fig. 1c). *Trichoderma harzianum* IRAN 523C showed the highest inhibition percent compared with other *Trichoderma* strains besides *T. atroviride* Tsafi and *T. virens* Tsafi (Fig. 1c).

In vivo *assay*

In the first experiment, cut roses treated with *T. atroviride* Tsafi and *T. harzianum* IRAN 523C showed lower means of disease severity compared with untreated control five, seven and nine days after the treatment (Fig. 2a). A significant reduction of disease severity was detected only in cut roses treated with *T. atroviride* Tsafi three and nine days after the treatment compared with untreated control (Fig. 2a). In the second experiment, *T. atroviride* Tsafi could keep the mean of disease severity low compared with the untreated control in all given times, with a significant effect on the ninth day of the experiment, while cut roses treated with *T. harzianum* IRAN 523C showed no signs of inhibition (Fig. 2b). Altogether, a combined analysis of both experiments revealed that the means of disease severity in cut roses treated with selected *Trichoderma* strains were lower compared with untreated control (Fig. 2c, Fig. 3). In combined analysis, a statistically significant reduction of disease severity was detected between *T. atroviride* Tsafi and untreated control on the ninth day after application of the biocontrol agent (Fig. 2c, Fig. 3).

Discussion

In this study the inhibitory effect of some local *Trichoderma* spp. strains was evaluated against *B. cinerea* causing blossom blight of rose plant under *in vitro* and *in vivo* conditions. *Trichoderma* spp. are commonly appreciated because of their potential in suppression of detrimental plant pathogens using multiple mechanisms including antibi-

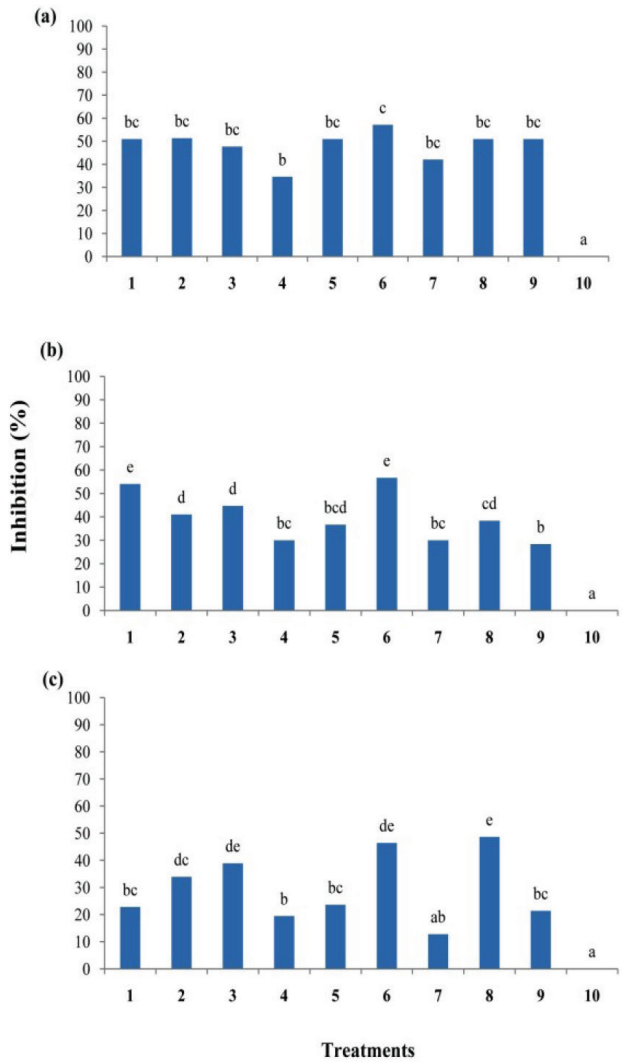

Fig. 1. Biocontrol potential of *Trichoderma* spp. in inhibition of the mycelial growth of *Botrytis cinerea* causing the blossom blight of rose in dual culture (a), non-volatile toxic compounds (b) and volatile toxic compounds (c) tests. 1: *Trichoderma harzianum* A, 2: *T. atroviride* H, 3: *T. virens* Tsafi, 4: *T. koningiopsis* H, 5: *T. asperellum* H, 6: *T. atroviride* Tsafi, 7: *T. virens* IRAN 199C, 8: *T. harzianum* IRAN 523C, 9: *T. brevicompactum* H, 10: Control. Treatments with the same letter are not significantly different

osis, mycoparasitism or, competition (Harman et al., 2004). Supply of various *Trichoderma*-based biological products in recent years such as AkTRIvator®, Trichosan®, Vitalin® and Promot® WP (Chaverri et al., 2015) highlights the importance of *Trichoderma* spp. as biocontrol agents. In present study, *Trichoderma* spp. strains significantly inhibited the mycelial growth of *B. cinerea* compared with the control under *in vitro* condition

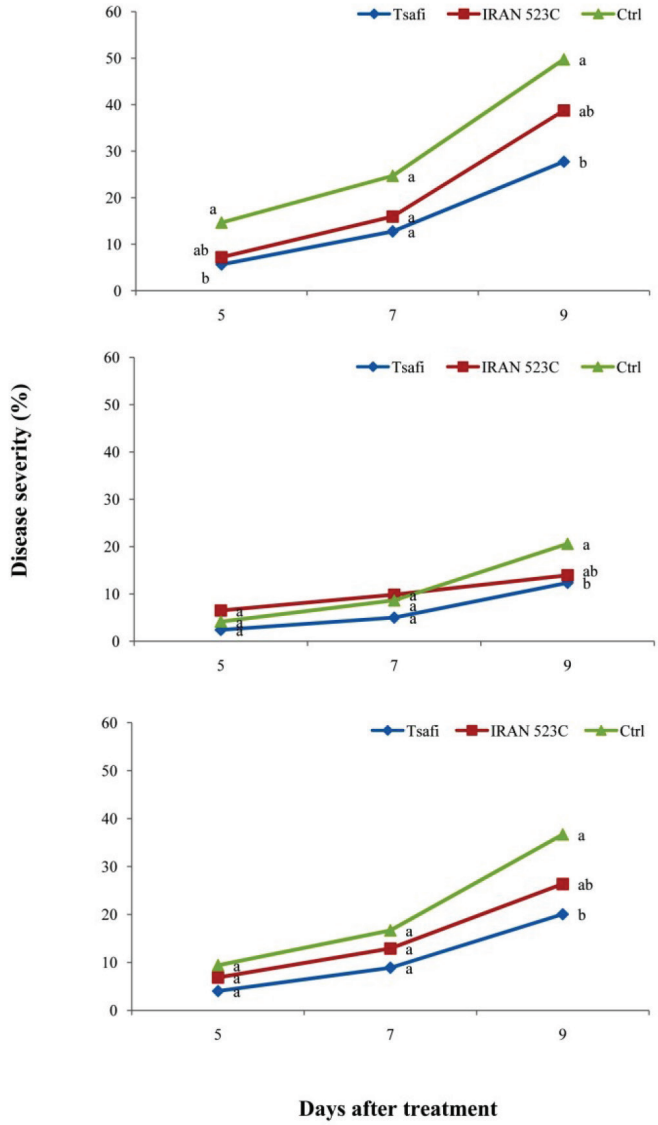

Fig. 2. Effect of selected strains of *Trichoderma harzianum* IRAN 523C and *T. atroviride* Tsafi in reduction of the disease severity caused by *Botrytis cinerea* on cut roses (*Rosa-hybrida* cv. Ilona) five, seven and nine days after treatment. The graphs show the results of first experiment (a), second experiment (b) and combined experiments (c). The treatments with the same letter are not significantly different

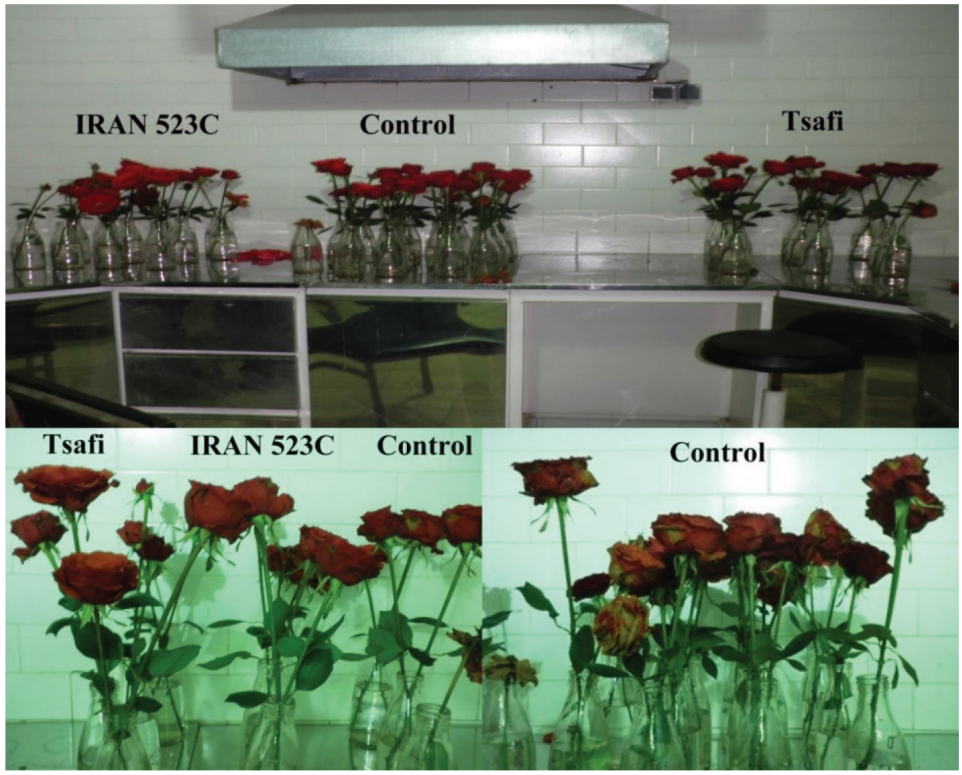

Fig. 3. Disease severity caused by *Botrytis cinerea* in cut roses (*Rosa×hybrida* cv. Ilona) treated with the selected strains of *Trichoderma atroviride* Tsafi and *T. harzianum* IRAN 523C compared with untreated control nine days after treatment

(Fig. 1a, b, c). Under *in vivo* condition, two *Trichoderma* strains of *T. atroviride* Tsafi and *T. harzianum* IRAN 523C could reduce the mean of disease severity after five, seven and nine days of treatment compared with the untreated control. Although a significant effect was observed only in cut roses treated with *T. atroviride* Tsafi nine days after application of the biocontrol agent (Fig. 2a, b, c). The results showed that the rate of disease severity was gradually decreasing over given times after treatment with *T. atroviride* Tsafi and *T. harzianum* IRAN 523C enhancing the longevity of the rose cuttings compared with the untreated control (Fig. 2, 3). This observation could have been due to the gradual colonization of petals by *Trichoderma* spp. strains and consequently the reduction of sporulation of the pathogen relative to untreated controls, similar to the results published by Yohalem (2004). Since *B. cinerea* conidia need to be germinated in the presence of nutrients, therefore competition for nutrients might be considered as a limiting factor where biocontrol activity is involved (Elad, 1996). However, the occurrence of other mechanisms including mycoparasitism can also be involved in suppression of disease development. Generally, various types of *Trichoderma* spp. show different capabilities in the biocontrol process and their activity and survival are dependent on biotic and abiotic environmental factors (Mukherjee et al., 2008).

Overall, the results of this study showed that *Trichoderma*-based biocontrol of blossom blight of rose seems to be a promising approach for disease management. Incorporation of *Trichoderma*-based biocontrol into the management strategy can be increasingly effective for control of Botrytis blight in cut rose flowers.

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