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RESEARCH

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Molecular characterization and biocontrol potential of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. dianthi in carnation

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Abstract

Background Carnation, a major cut flower product cultivated economically in Iran, faces economic challenges due to the devastating *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *dianthi* (*Fod*). To address this issue, twenty-five *Trichoderma harzianum* and *T. viride* isolates were collected from the rhizosphere soil of three Iranian provinces: Tehran, Markazi, and Fars. RAPD-PCR was applied to analyze the genetic relatedness of the isolates.

Results The RAPD profiles showed genetic diversity among the isolates, with two major clusters. The antagonistic potential of the twenty isolates was evaluated against the carnation wilt caused by *Fod*. The results showed that Th1, Th7, and Th2 isolates of *T. harzianum* significantly inhibited *Fod* mycelial growth (58, 56.5, and 48.6%, respectively). Among *T. viride* isolates, Tv5, Tv4, and Tv7 exhibited the highest antifungal ability to inhibit mycelial growth. All investigated isolates of *Trichoderma* secreted volatile compounds that hindered *Fod* mycelial growth, with isolates of *T. harzianum* ranging from 9.3 to 67.5% inhibition and those of *T. viride* from 25.2 to 50.2%. Additionally, the experiment on competitive saprophytic ability indicated that maximum colonization occurred with Th1, Th7, and Th2 isolates at 78.2, 70.8, and 69.8%, respectively. Lastly, the greenhouse experiment showed a complete pathogen eradication or significant inhibition in the infected carnation after *T. harzianum* and *T. viride*. Conversely, control treatment with the *Fod* pathogen died after 90 days.

Conclusions The investigation suggested that *Trichoderma* spp. could be a potential biocontrol agent to mitigate *Fusarium* wilt in carnation and improve production quality, replacing chemical pesticides.

Keywords *Trichoderma*, Soil-borne pathogens, *Fusarium oxysporum*, Biocontrol, Carnation wilt, Antifungal

Background

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *dianthi* (*Fod*) is a soil-borne fungal disease that affects a wide range of economically important plants worldwide, from crops to ornamental flowers (Cer et al. 2022). The first report of *Fod* races in Iran was in 2011 (Zahiri et al. 2013). Carnation is among the most popular commercially cut flower, after roses and chrysanthemums, due to its varied colors and exquisite shape (Migheli et al. 1998). The most devastating pathogenic fungi affecting carnation (*Dianthus caryophyllus* L.) and a major limiting factor for cultivation and export in Iran and worldwide is *Fusarium* wilt, caused by *Fod* (Zandyavari et al. 2013). There are various methods for controlling this disease, but using *Trichoderma* strains for biocontrol is considered safer and more preferable (Coşkuntuna and Özer 2008).

The nonpathogenic and saprophytic filamentous fungus *Trichoderma* is a joint antagonist agent capable of

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producing a variety of hydrolytic enzymes and antifungal metabolites that can colonize and suppress many phytopathogens, such as those from *Rhizoctonia, Pythium, Phytophthora*, *Sclerotium*, and *Fusarium* genera (Filizola et al. 2019). *Trichoderma* spp. have been characterized as promising BCAs against plant nematodes and soil-borne pathogens, such as *F. oxysporum, Rhizoctonia solani*, and *Botrytis cinerea* (Mukhopadhyay and Kumar 2020), and they are considered the potential biocontrol alternatives for plant disease management due to the diverse range of a plethora of metabolites they possess (Phoka et al. 2020). They contribute to inhibiting up to 80% of economically important plant pathogens (Sánchez-Montesinos et al. 2020). Several investigations have found that *Trichoderma* spp. exhibit a variety of biocontrol mechanisms. These mechanisms include mycoparasitism, antibiosis, competition for nutrients and space, stress tolerance via increased root and plant development, inorganic nutrient solubilization and sequestration, systemic acquired resistance, and enzyme inactivation (Guzmán-Guzmán et al. 2023).

Biocontrol is an environmentally friendly approach to managing plant diseases without leaving chemical residues on living organisms (Alvarado-Marchena and Rivera-Méndez 2016). With the global challenge of food scarcity, efficient BCAs are in demand for improving crop production and disease management. *Trichoderma* is a multipurpose BCA with multiple antagonistic properties that can mitigate many plant diseases and promote plant growth (Woo et al. 2023). This study was carried out to evaluate the antagonistic potential of *Trichoderma harzianum* and *T. viride* against *Fusarium* wilt disease in carnation flowers.

Methods

Pathogen isolation

Soil samples were collected from the Tehran, Markazi, and Fars provinces of Iran for isolation. Ninety-six isolates, used in this study, were maintained in the culture collections of the Department of Plant Pathology, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University (Table 1). These isolates were identified as *Fod* based on a pathogenicity test (Zandyavari et al. 2013). The *Fod* isolates were cultured on PDA media for 5 days and then purified using the single-spore method.

Pathogen identification

Total DNA from pathogen isolates was extracted using the cetyltrimethylammonium bromide (CTAB) method (Turaki et al. 2017). For the PCR techniques, the components: 17.5-µl ddH2O, 2.5-µl PCR buffer (10X), 0.75-µl MgCl2 (50 mM), 0.5-µl dNTPs mix (10 mM), 0.3-µl Taq DNA polymerase (5 units/µl), 2-µl DNA, 0.5 µl of ITS1 primer (5′-TCCGTAGGTGAACCTGCGG-3′), and 0.5 µl of ITS4 primer (5′-TCCTCCGCTTATTGATATGC-3′) were utilized (Baayen et al. 1997). Bio-Rad thermal cycler was used for the PCRs, using the following PCR program: initial denaturation at 94 °C for 3 min, 40 cycles at 94 °C for 1 min, 55 °C for 45 s, and 72 °C for 1 min, with a final extension at 72 °C for 2 min. PCR products were purified using polyethylene glycol and analyzed on a 1.2% agarose gel, demonstrating that the isolates with a vicinity of 600 bp were *Fusarium* spp. (Baayen et al. 1997).

Isolation of antagonists

Twenty-five rhizospheres soil samples containing antagonistic fungi, including *Trichoderma*, were collected from carnation fields in greenhouses. The soil samples weighing 1 g were obtained using the serial dilution technique (Marraschi et al. 2019). *Trichoderma* selective medium (TSM) was used to isolate the *Trichoderma* from the samples (Marraschi et al. 2019). The colonies emerged in the Petri plates and were subsequently purified using the hyphae tip isolation technique. For this, 1 ml of the 10–3 dilution was plated on the aforementioned selective medium.

Molecular identification of *Trichoderma* isolates

The *Trichoderma* spp. isolates were grown on a PDA medium for 10 days at 25 °C, and DNA was extracted using the CTAB method (Turaki et al. 2017). To amplify the translation elongation factor 1-alpha gene, polymerase chain reaction (PCR) was performed with a pair of primers, tef 71F (5′-CAAAATGGGTAAGGAGGASAAGAC-3′) and tef 997R (5′CAGTACCGGCRGCRATRATSAG-3′), producing products of approximately 930 bp in size (Marraschi et al. 2019). The PCR was carried out

Table 1 *Trichoderma* spp. and *Fod* isolates from three Iranian provinces

Provinces	No. of isolates	No. of soil samples	Trichoderma isolates
Tehran	34	9	Th1, Th2, Th7, Th8, Tv2, Tv4, Tv7, and Tv10
Markazi	31	8	Th3, Th5, Th6, Th9, Th10, Tv6, and Tv9
Fars	31	8	Th4, Tv1, Tv3, Tv5, and Tv8

in the same thermal cycler, with an initial denaturation at 94 °C for 2 min, 40 cycles at 94 °C for 30 s, 54 °C for 30 s, 72 °C for 60 s, and a final extension at 72 °C for 4 min.

The PCR products were purified using polyethylene glycol precipitation, following the protocol described by Marraschi et al. (2019). *Trichoderma* isolates were stored on PDA media. RAPD-PCR was applied to identify the antagonist isolates based on the method by Navyashree et al. (2018). Seven primers—OPC05, A11, OPA04, AA04, OPA01, OPD06, and OPA16—along with 20-µl ddH2O, 3-µl PCR buffer 10X, 0.75-µl MgCl2 (50 mM), 0.5-µl dNTPs mix (10 mM), 0.5-µl Taq DNA polymerase (5 units/µl), and 2-µl DNA were used (Table 2). The PCRs were conducted with the following program without changing the thermal cycler settings: The first denaturation was at 94 °C for 4 min, followed by 35 cycles of 94 °C for 60 s, 36 °C for 60 s, and 70 °C for 90 s, followed by a final extension at 72 °C for 7 min. The PCR products were purified by polyethylene glycol precipitation and analyzed on a 2% agarose gel (Navyashree et al. 2018).

Biocontrol tests

Evaluation of Trichoderma spp. antifungal effect against Fod in vitro

This experiment was conducted on PDA medium using the dual culture method as described by Unartngam et al. 2020. Ten isolates of *T. harzianum* and *T. viride* were cultivated with the pathogen isolates on PDA for a week. Disks measuring 5 mm in diameter from the growing borders of the colonies were selected and transferred to other PDA dishes. Two disks of *Trichoderma* spp. and the plant pathogen mycelium were placed opposite each other near the periphery of the Petri plate and incubated at room temperature for 7 days. In control plate, only the pathogen was placed as no antifungal isolate was applied. The experiment was replicated three times, and the proportion of mycelium inhibition was measured using the method described by Dugassa et al. 2021.

$$[I = (R_1 - R_2)/R_1 \times 100]$$

where I = percentage inhibition; R_1 = radial growth of pathogen in control plate (cm); and R_2 = radial growth of pathogen in dual culture plate (cm).

Table 2 RAPD primers and their sequences

Primer	Sequence (5′–3′)	Primer	Sequence (5′–3′)
OPC05	GATGACCGCC	A11	AGGGGTCTTG
OPA04	AATCGGGCTG	AA04	CAGGCCCTTC
OPA01	CAGGCCCTTC	OPD06	GGGGTCTTGA
OPA16	AGCCAGCGAA		

Evaluation of the volatile compounds

Properties of volatile substances secreted by *Trichoderma* spp. on the mycelial growth of *Fod* using the technique of paired Petri dishes described by Mahalakshmi and Raja 2013 were evaluated. Disks measuring 9 mm in diameter, obtained from 4 days cultured antagonistic isolates, were placed on PDA plates. The bottom of each PDA dish, inoculated with the mycelia disk of *Fod*, was then placed on top of Petri dishes flipped over *Trichoderma* spp. dishes, and the setup was incubated at 25 °C for 4 days. The PDA plates were also inoculated with *Fod* alone and paired without *Trichoderma* spp. as a control. Three replications were conducted for each isolate. After the incubation period, the mycelial growth of the pathogen was measured and expressed as a percentage of inhibition over the control. The ability of volatile compounds to control pathogen growth was evaluated using the mentioned formula (Dugassa et al. 2021):

$$[I = (R1 - R2)/R1 \times 100]$$

Competitive saprophytic ability (CSA) assay

To conduct the colonization success assay (CSA) experiment with *Trichoderma* spp. isolates, the procedure described by Mahalakshmi and Raja 2013 was applied. All isolates of *Trichoderma* spp. were cultured on molasses yeast medium, and the conidial concentration was adjusted to 106 per gram, which was then mixed with unsterilized soil and placed in plastic pots. Each pot was prepared with 21-cm segments of clean paddy straw buried in the soil. The plastic pots were covered and incubated in a dark environment for a week to maintain moisture.

After the incubation period, twenty pieces of each isolate were retrieved from the plastic pots, washed, and disinfected using $NaCl_2$ and ethanol. The desired pieces were then plated on the TSM and incubated at 25 °C for 5 days. The experiment was replicated three times for each isolate, as mentioned in Marraschi et al. 2019. The results of the CSA were calculated using the same formula: $[I = (R1 - R2)/R1 \times 100]$ (Dugassa et al. 2021).

Biocontrol evaluation under greenhouse

The effectiveness of *T. harzianum* and *T. viride* isolates in controlling *Fusarium* wilt in carnation cultivated in pots was evaluated under greenhouse conditions, following the methods outlined in Locke et al. 1985 with some modifications. For this experiment, standard carnation rendezvous cuttings were selected due to their high susceptibility to *Fusarium* wilt. The cuttings were

individually planted in pots containing a sterilized soil mixture of sand, peat, and silt (2:1:2 by volume) and kept in a greenhouse at 25 °C with natural light and 55–60% R.H. The treatments were 106 CFU/ml spore suspension of each antagonist isolate to each pot, along with 106 CFU/ml spore suspension of *Fod* isolates. A total of 288 pots were used to evaluate the antagonist isolates, and twelve pots were used as control (without antagonist isolates against *Fod*). After an incubation period of 90 days, the occurrence of *Fusarium* wilt in the carnation was assessed and recorded.

Statistical analysis

The data were statistically analyzed using analysis of variance (ANOVA), followed by post hoc analysis. Duncan's multiple range test was used to determine the significance of the treatments compared to the control, and a significance level of $p < 0.05$ was applied. The greenhouse treatment was analyzed using the randomized complete block design (RCBD) test. The data were subjected to normality and heterogeneity tests before analysis of variance. All statistical analyses were done using SPSS software (version 26) (Levesque 2007).

Results

Molecular identification and phylogenetic analysis

The genomic DNA of the selected isolates was amplified using ITS1 and ITS4 primers targeting the rDNA region. The ITS PCR was utilized to identify polymorphisms within the ITS region of rDNA among the *Fusarium* isolates. The results demonstrated that 96 Iranian *Fusarium* isolates showed a band of approximately 600 bp in size (with a fragment size ranging from 550 to 578 bp) on a 1.2% agarose gel, and they were characterized as *Fod* (Fig. 1). The twenty *Trichoderma* spp. isolates displayed amplified products at approximately 900 bp (Fig. 2).

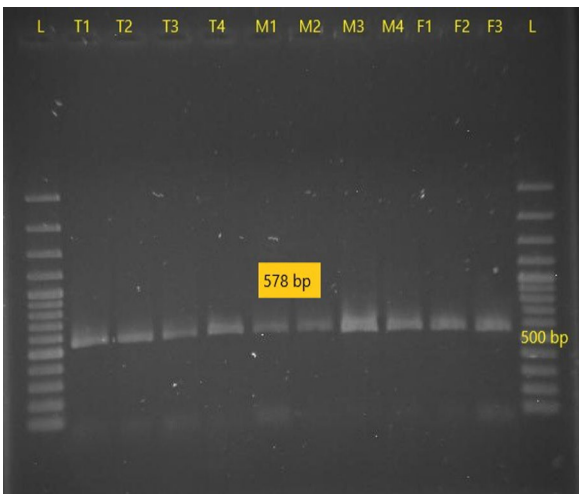

Fig. 1 *Fusarium oxysporum* isolates on 1.2% agarose gel; *L*: 100-bp Fermentas

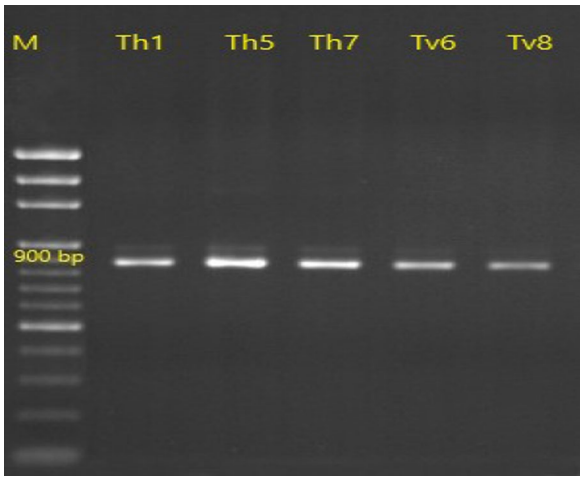

Fig. 2 *Trichoderma* spp. isolates on 2% agarose gel; *M*: 100-bp Fermentas

All amplified products generated with the RAPD primers showed polymorphic and distinguishable bands, indicating the genetic diversity of *Trichoderma* isolates. Among the twenty *Trichoderma* isolates, seven primers produced 104 reproducible polymorphic bands, ranging in size from approximately 100 to 3200 bp (Table 3). The RAPD profiles showed that primers A11 and AA04 generated the highest number of bands, ranging from almost 410 to 2000 bp and 400 to 3000 bp, respectively. Moreover, the molecular characterization of *T. viride* and *T. harzianum* isolated from Iranian soils revealed polymorphic bands ranging from 296 to 3300 bp, with primer A05 producing the highest number of bands among the RAPD primers.

Cluster analysis of the data was performed using the similarity matrix to identify relationships among the isolates (Additional file 1: Table S4). The similarity

Table 3 Analysis of the polymorphism obtained with seven RAPD markers in 20 *Trichoderma* isolates

Primer	Total bands	Amplification product range (bp)	Polymorphic bands	Polymorphism (%)
OPC05	14	300–3000	11	79
A11	21	400–2300	21	100
OPA04	12	300–1500	11	91
AA04	21	400–3100	21	100
OPA16	13	290–3200	11	85
OPA01	9	100–1000	9	100
OPD06	14	100–1000	14	100

coefficients ranged from 0.15 to 1.00 among the isolates. The similarity matrix revealed that isolates Th1 and Tv6 were genetically distinct, showing only 15% similarity. The dendrogram was generated using the UPGMA and NTSYSpc software based on the similarity matrix (Fig. 3). According to the results obtained, all twenty *Trichoderma* spp. isolates could be grouped into two major clusters: One cluster comprised of *T. viride* and the other of *T. harzianum*. Different numbers of isolates were present in each sub-cluster.

Biocontrol activity of *Trichoderma* spp. in vitro

This study examined twenty isolates of *T. harzianum* and *T. viride* (ten isolates of each species) for their antifungal activity against the Iranian soil-borne pathogen, *Fod*. These antagonists demonstrated varying degrees of antagonistic activity isolates against *Fusarium* wilt in dual culture plates, volatile compounds, and CSA (competitive saprophytic ability) tests.

Among the tested isolates in this study, *T. harzianum* (Th1) revealed the highest mycelia growth inhibition proportion of 58%, followed by Th7 with 56.5% and Th2 with 48.6% inhibition of mycelia growth. The lowest percentage of mycelia growth inhibition was observed in *T. viride* (Tv3) and Th5, with only 15% (Table 4) showing a significant variation ($P \leq 0.05$). Figure 4 demonstrates the control of *Fusarium* mycelium growth by *Trichoderma* spp.

The volatile compounds of *T. harzianum* isolates Th1, Th7, and Th2 showed the highest growth inhibition

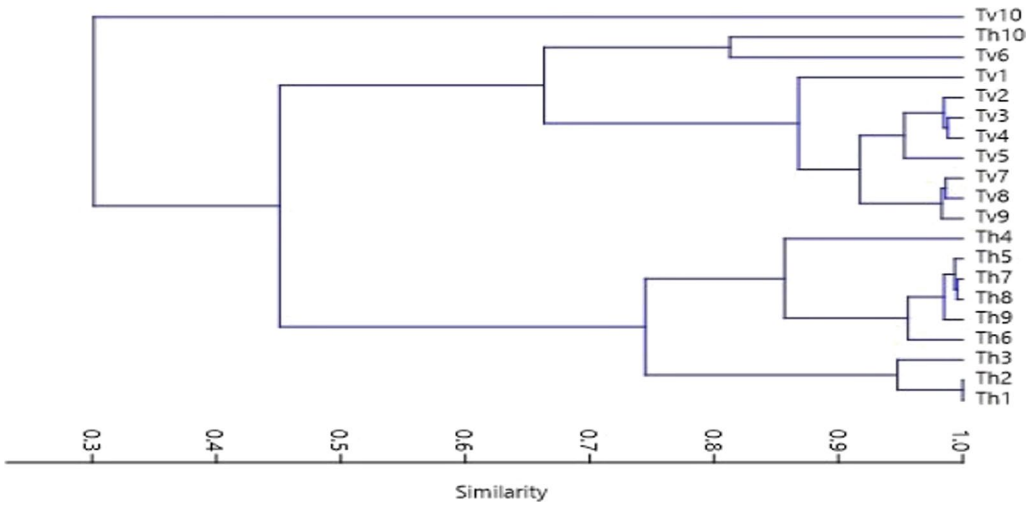

Fig. 3 The genetic relationship between the twenty *Trichoderma* isolates based on RAPD analysis

Table 4 Antifungal activity of *Trichoderma* spp. against the mycelia growth of the pathogen

Trichoderma harzianum	Mycelia growth (cm)*	Reduction over control (%)	*Trichoderma viride*	Mycelia growth (cm)*	Reduction over control (%)
TH 1	4.20	58.0	TV 1	6.80	32.0
TH 2	5.14	48.6	TV 2	6.92	30.8
TH 3	5.22	47.8	TV 3	8.22	17.8
TH 4	5.20	48.0	TV 4	6.20	38.0
TH 5	8.53	15.0	TV 5	6.17	38.3
TH 6	7.20	28.0	TV 6	7.10	31.0
TH 7	4.35	56.5	TV 7	6.30	33.2
TH 8	7.22	27.8	TV 8	8.20	18.0
TH 9	8.42	15.8	TV 9	7.18	28.2
TH 10	8.31	16.9	TV 10	7.35	26.5
Control	10.0	–	Control	10.0	–

*The mean of three replications

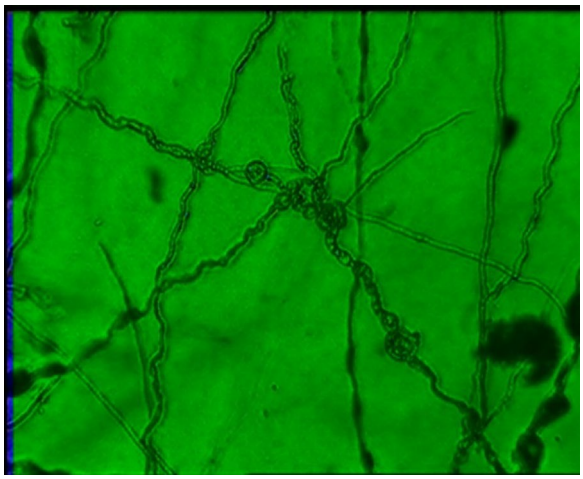

Fig. 4 The *Fusarium* mycelium growth inhabitancy by *Trichoderma* spp.

against *Fod*, with percentages of 67.5, 65, and 63.8%, respectively (with $p \leq 0.05$ variation). On the other hand, Th5 isolate exhibited the smallest growth inhibition with 9.3% (Table 5 and Fig. 5). *Trichoderma* isolates' inhibition of *Fusarium* strains ranged from 20.8 to 78.2% (Table 6). The results revealed a direct correlation between the colonization proportion of *Trichoderma* spp. and their CSA ability. Isolates Th1 and Th7 demonstrated the highest level of CSA.

The fungi recovered from plant material buried in soil fields showed the ability to colonize these substrates as competitive saprophytes. Similarly, the greenhouse evaluation study revealed significant differences between *T. harzianum, T. viride*, and control treatments. These differences were observed in disease symptoms indications, with either slight disease symptoms or complete eradication of the pathogen in the carnation stems or vascular system. After 90 days of inoculation, the *Fod* control treatment resulted in complete mortality (Fig. 6).

Discussion

Fusarium wilt of carnation caused by *Fod* is one of the challenging soil-borne pathogens that devastate carnation cultivation around the world (Wolcan et al. 2016). Mycoparasitism, nutrient competition, induced plant

Table 5 Inhibitory effect of the volatile component of *Trichoderma* spp. against *Fod* growth in vitro

Isolates	4 days after incubation		8 days after incubation		12 days after incubation	
	Mycelial growth(cm)*	Percent reduction over control (%)	Mycelial growth (cm)*	Percent reduction over control (%)	Mycelial growth (cm)*	Percent reduction over control (%)
Trichoderma harzianum						
TH I	2.12	78.8	2.28	77.2	3.25	67.5
TH 2	3.07	69.3	3.31	66.9	3.62	63.8
TH 3	4.14	58.6	4.72	52.8	4.92	50.8
TH 4	3.45	65.5	3.62	63.8	3.81	61.9
TH 5	8.40	16.0	8.96	10.4	9.07	9.3
TH 6	5.52	44.8	5.90	41.0	6.12	38.8
TH 7	2.82	71.8	2.96	70.4	3.42	65.8
TH 8	5.68	43.2	6.07	39.3	6.32	36.8
TH 9	8.22	17.8	8.49	15.1	8.88	11.2
TH 10	7.38	26.2	7.62	23.8	8.00	20.0
Trichoderma viride						
TV 1	5.02	49.8	5.28	47.2	5.41	45.9
TV 2	5.25	47.5	5.63	43.7	5.82	41.8
TV 3	7.18	28.2	7.32	26.8	7.48	25.2
TV 4	4.45	55.5	4.73	52.7	5.00	50.0
TV 5	4.32	56.8	4.62	53.8	4.98	50.2
TV 6	5.18	48.2	5.42	45.8	5.60	44.0
TV 7	4.68	53.2	4.86	51.4	5.08	49.2
TV 8	6.42	35.8	6.71	32.9	6.97	30.3
TV 9	5.34	46.6	5.75	42.5	5.92	40.8
TV 10	6.31	36.9	6.42	35.8	6.53	34.7
Control	10.0	–	10.0	–	10.0	–

*The mean of three replications

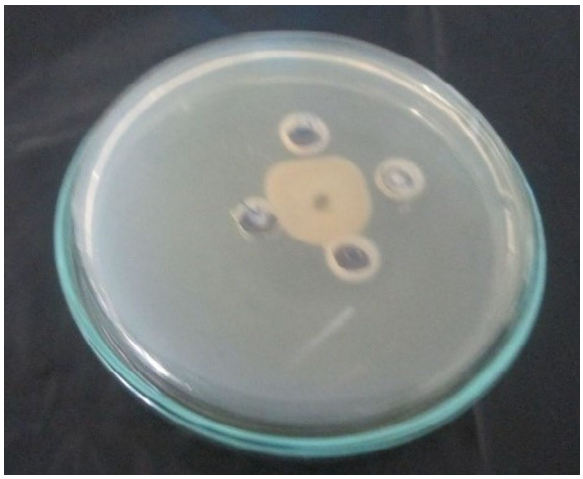

Fig. 5 The inhibitory effect of volatile compounds on the pathogen growth

resistance, and the synthesis of antibiotic and hydrolytic enzymes biocontrol mechanisms of *Trichoderma* spp. make them an excellent candidate for biocontrol application against many plant pathogens (Manzar et al. 2022). Our investigation aimed to explore the potential of *Trichoderma* isolates from Iranian soils for combating the *Fusarium* wilt of carnation. The results confirm the characterization of these isolates as *Fod*. These results align with Baayen et al. (1997), who studied and identified the genetic diversity in *F. oxysporum* f. sp. *dianthi* and *Fusarium redolens* f. sp. *dianthi* isolates. Distinguishing features in the morphology of *Trichoderma* strains, such as copious conidial pigments in white or bright green, along with conidiophores that are repetitively branched and lack clear definition, enable their recognition at the genus level (Rifai 1969).

The BCAs *Trichoderma* spp. have been widely considered excellent alternative control agents against plant pathogens such as *Fusarium* strains (Filizola et al. 2019), brown spot of rice (Charoenrak and Chamswarng 2015), *Phytophthora infestans*, the agent of potato late blight (García-Núñez et al. 2017), and other plant pathogens like *Fusarium* wilt on carnation (Marraschi et al. 2019). *Trichoderma* spp. can adapt to various living environmental factors and conditions (García-Núñez et al. 2017). The present study revealed that *Fusarium* mycelium was effectively controlled by *T. harzianum* (Th1) exhibited the highest proportion of mycelial growth inhibition at 58%, followed by Th7 with 56.5%, and Th2 with 48.6% inhibition. Pratibha (2000) reported that *T. harzianum* had inhibited the mycelia growth of *Fod*. Similarly, it has been reported in India that *T. harzianum, T. viride*,

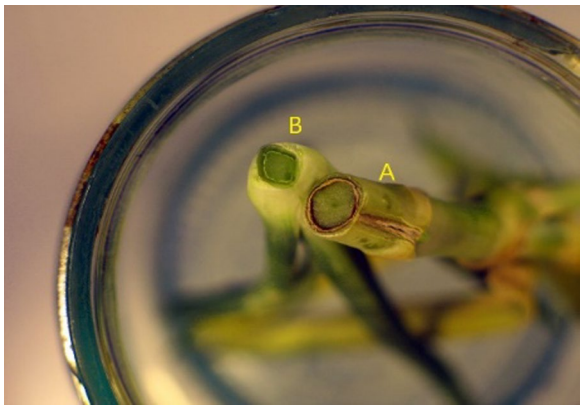

Fig. 6 Comparison of vascular structures in an unprotected plant treated with an antagonist (**A**) and a protected plant (**B**)

Table 6 The competitive saprophytic ability (CSA) of *T. harzianum* and *T. viride* isolates

Treatments	Mycelium growth (cm)*	Percentage of colonization (%)	Treatments	Mycelium growth (cm)*	Percentage of colonization (%)
TH 1	2.18	78.2	TV 1	5.02	49.8
TH 2	3.02	69.8	TV 2	5.82	41.8
TH 3	3.62	63.8	TV 3	7.02	29.8
TH 4	3.35	66.5	TV 4	4.35	56.5
TH 5	8.19	18.1	TV 5	4.20	58.0
TH 6	6.18	38.2	TV 6	5.32	46.8
TH 7	2.92	70.8	TV 7	4.63	53.7
TH 8	6.32	36.8	TV 8	6.85	31.5
TH 9	7.92	20.8	TV 9	6.00	40.0
TH 10	7.28	27.2	TV 10	6.42	35.8
Control	10	–	Control	10	–

*The mean of three replications

and *T. ressei* showed significant efficiency against *Fod* (Mahalakshmi and Raja 2013). The antagonistic impact of *Trichoderma* spp. against other plant pathogens has also been reported (Gupta and Misra 2009).

There was a varied efficiency of volatile compounds in inhabiting the growth of *Fod*, particularly the volatile compounds from *T. harzianum* isolates Th1, Th7, and Th2 demonstrated the most significant growth inhibition against *Fod.* The previous studies have shown that *Trichoderma* spp. produce both volatile and non-volatile compounds that can affect the growth of different plant pathogenic fungi (Mahalakshmi and Raja 2013). In line with that, Mahalakshmi and Raja (2013) reported that *T. harzianum* and *T. viride* isolates exhibited biological efficacy against the vascular wilt pathogen in carnation. Khaledi and Taheri (2016) found that volatile compounds production is one of the major antagonistic mechanisms against *Macrophomina phaseolina* pathogenic fungi. Similarly, volatile and non-volatile compounds from *T. harzianum* and *T. viride* have been reported to be associated in controlling *Fusarium* spp. and *Alternaria* spp. (Meena et al. 2017).

A clear correlation between the colonization proportion of *Trichoderma* spp. and their ability was to compete for space and nutrients. This correlation between colonization and CSA was confirmed and reported by García-Núñez et al. (2017). It was clear that these fungi have the capacity to thrive and compete in the soil environment from the recovered plant material from soil. Locke et al. (1985) reported that the increased antagonistic potential of *T. viride* isolates was attributed to enhance CSA. Furthermore, Thiruvudainambi et al. (2010) demonstrated that the percentage of mycelia growth among *Sclerotium rolfsii* isolates declined due to the robust CSA exhibited by *T. viride* isolates. Moreover, several investigations have reported a correlation between the proportion of colonization among *Trichoderma* spp. isolates against *Fod* and other pathogens with their CSA activity (Dugassa et al. 2021). Hermosa et al. (2000) reported that conducting experiments in natural environments is essential to validate further the antagonistic effect of a biocontrol strain selected through a laboratory protocol for potential biocontrol against plant pathogens.

A significant impact of *Trichoderma* treatments on disease symptoms and pathogen eradication was observed in the greenhouse experiment; these findings imply that *Trichoderma* spp. isolates in this study profoundly eliminate the *Fusarium* wilt in carnation under laboratory conditions and greenhouse setting. Comparable results were obtained in the greenhouse, where *Trichoderma* spp. effectively controlled the *Fusarium* wilt of chrysanthemums (Locke et al. 1985). According to a report by Fattahi (2014), it was confirmed that the cultivation of greenhouse-grown carnation in Iran's Markazi province was significantly impacted by *Fusarium* wilt. The versatile qualities of *Trichoderma* spp., from effective defense mechanisms and the highest canalizations and competition in their habitats through potent degradation enzymes, make them promising biocontrol fungicides and potential candidates for industrial production (Schuster and Schmoll. 2010). The study by Martinez (2023) highlighted the use of carrier substances, drying techniques, and microencapsulation to create stable, high-performing products from *Trichoderma* spp. for developing effective and life shelf fungicides. *Trichoderma* spp. are complex plant hosts that communicate with their hosts and provide multifaceted benefits. They function as a plant growth promoter, inducer of local and systemic defense responses against biotic and abiotic stresses and activator of transcriptional memory affecting future stress responses (Woo et al. 2023).

Conclusion

The potential of *T. harzianum* and *T. viride* isolates as BCAs against *Fusarium* wilt caused by *Fod* in carnation was evaluated. In vitro assays showed isolates Th1, Th7, and Th2 of *T. harzianum* and Tv5, Tv4, and Tv7 of *T. viride* exhibited the highest antifungal activity against the pathogen. Treatments with these efficient isolates in the greenhouse led to significant *Fusarium* wilt reduction or eradication in carnation. The study found that *Trichoderma* isolates are a highly effective biological control agent for managing this disease. Further field studies to establish their efficacy before implementing their use in agriculture are recommended.

Abbreviations

Fod	*Fusarium oxysporum* f. sp. *dianthi*
BCA	Biocontrol agent
CSA	Competitive saprophytic ability
PDA	Potato dextrose agar
RAPD	Random amplification of polymorphic DNA
CTAB	Cetyltrimethylammonium bromide
TSM	Trichoderma selective medium
ITS	The nuclear ribosomal internal transcribed spacer

Supplementary Information

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Additional file 1. **Table S4**: Average percentage RAPD-based genetic similarity within groups

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Author contributions
NZ conceptualized the research idea, conducted the experiments, performed statistical analysis, and wrote the first draft of the manuscript. MAS critically reviewed and edited the manuscript and gave his valuable suggestions. NH assisted in experiment design, data analysis, and interpretation. All authors read and approved the final version of the manuscript.

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Availability of data and materials
The data supporting this study are not publicly available. Readers are welcome to contact the corresponding author for further information regarding the data used in this research.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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