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Biological control of Fusarium wilt in *Phalaenopsis* orchids by *Trichoderma harzianum* isolates

Phalaenopsis orkidelerinde Fusarium solgunluğunun *Trichoderma harzianum* izolatları ile biyolojik kontrolü

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ABSTRACT

Moth orchids (*Phalaenopsis* spp.) are widely cultivated ornamental plants due to their high flower production and long flowering period. This study aimed to identify fungal agents associated with Phalaenopsis orchids grown in Türkiye and to evaluate the biocontrol potential of *Trichoderma harzianum* (TH) isolates against pathogenicity-confirmed *Fusarium oxysporum* isolates. *Fusarium* spp. were the dominant fungal agents, and *F. oxysporum* was one of the most common species. In addition, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Macrophomina phaseolina* were identified as important phytopathogens. Samples were collected from Antalya, Mersin, Yalova, and Istanbul. A total of 15 *T. harzianum* isolates and three pathogenicity-confirmed *F. oxysporum* isolates were used in *in vitro* assays. Based on these results, selected isolates and a commercial *T. harzianum* product were further tested in *in vivo* experiments under controlled conditions. *In vitro* assays showed that volatile metabolites of TH isolates reduced the colony diameter of *F. oxysporum* by 54.1%, while hyperparasitic effects resulted in up to 76.36% inhibition. *In vivo* applications reduced disease severity by 77.3% for orchid-derived isolates and 81.8% for the commercial product. Both treatments also increased root length, root fresh weight, and shoot biomass. These results demonstrate that *Trichoderma harzianum* isolates are effective in the biological control of *Fusarium oxysporum*-related diseases in orchid cultivation.

INTRODUCTION

The Orchidaceae family is one of the largest plant families, comprising about 10% of the world's flowering plants, and orchids are among its most valuable ornamental species(Roberts and Dixon 2008, Srivastava et al. 2018). Among them, Phalaenopsis (moth) orchids are highly popular in the global floriculture market due to their long-lasting flowers and extended flowering period (Hsu et al. 2018). The global orchid market was valued at US$5.152.1 million in 2020 and is projected to reach US$7.051.3 million by 2027, with a growth rate of 4.6% between 2021 and 2027 (Anonymous 2021). This growth is also reflected at the national level. In Türkiye, orchids are commercially grown

under greenhouse conditions due to climatic requirements. Orchid production has increased markedly over the years, showing a nearly ninefold rise between 2011 and 2025 (TÜIK 2025). Many fungal species, especially soilborne ones, are pathogenic in ornamental plants and can cause significant yield and quality losses. Among Fusarium species in orchids, *Fusarium oxysporum* is the most common and destructive (Swett 2007). *Fusarium solani*, isolated from *Phalaenopsis* sp., caused severe leaf yellowing and was subsequently identified as *F. solani* (Mart.) Sacc. f. sp. *phalaenopsis* (Chung et al. 2011, Kim et al. 2002, Laurence et al. 2016). *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr., as the pathogen causing collar rot in orchids, was reported in Korea and Taiwan (Chiu et al. 2021, Han et al. 2011).

Trichoderma species are antagonistic and hyperparasitic fungi against other fungi (Harman et al. 2004, Marra et al. 2006). The use of *Trichoderma* species as biological control agents (BCA) is associated with several different characteristics, such as their high reproductive capacity, ability to survive in adverse conditions, dominance in competition for nutrients and colonization sites, capacity to modify the rhizosphere, dominance over phytopathogenic agents, and effectiveness in promoting plant growth and defense mechanisms (Benítez et al. 2004, Chet et al. 1997). Several studies have demonstrated the antagonistic potential of different *Trichoderma* species against important fungal pathogens of ornamental plants. Arslan (2019) reported that *T. harzianum* reduced Fusarium wilt caused by *F. oxysporum* f.sp. *dianthi* in carnations by 86.2% under in vivo conditions, mainly through mycoparasitism and the production of secondary metabolites. *T. viride* has also been reported to suppress *Rhizoctonia solani* in orchids. In a dual culture test, *T. viride* reduced the mycelial growth of the pathogen by 79.08% (Pallavi and Purshotam 2012). The application of *T. pseudokoningii* strain SMF2 to Phalaenopsis has been reported to increase both shoot and root biomass and to induce systemic resistance against gray mold caused by *Botrytis cinerea*. When applied to roots or leaves, the strain enhanced the activity of defense-related enzymes, including peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase, as well as antioxidant enzymes such as superoxide dismutase and catalase. At the same time, malondialdehyde levels decreased. These results suggest that induced systemic resistance plays a key role in disease suppression (Zhao et al. 2018). *Trichoderma* species have been reported to be biostimulants in the growing medium of ornamental plants (Prisa 2012). These results further support the potential of *Trichoderma* species, including *T. harzianum*, as effective biological control agents in orchid production.

This study aimed to identify fungal agents associated with Phalaenopsis orchids cultivated in Türkiye and to evaluate the biocontrol efficacy and plant growth-promoting effect of *T. harzianum* against *F. oxysporum* in moth orchids.

MATERIALS AND METHODS

Survey and isolation

This study was carried out between 2020 and 2022. Plants showing symptoms such as leaf spots, yellowing, wilting, root rot, and collar rot were collected from commercial moth orchid (*Phalaenopsis*) greenhouses in Türkiye. Sampling was carried out in Antalya, Mersin, Yalova, and İstanbul, which are important regions for indoor ornamental plant production (TAGEM, 2020). In total, 87 symptomatic plants were collected from Antalya (34 samples), Mersin (18), Yalova (22), and İstanbul (13). These regions represent a substantial proportion of commercial orchid production in Türkiye. Yalova accounts for approximately 64% of the country's orchid production (Yalova Provincial Directorate of Agriculture and Forestry, 2022). Isolations were performed following standard mycological methods. Roots and leaves from symptomatic plants were brought to the laboratory for fungal isolation. Plant samples were washed under running tap water and dried. Small pieces were cut from infected tissues, including both diseased and nearby healthy parts. The pieces were surface sterilized in 2% sodium hypochlorite for 2 minutes, rinsed twice with sterile distilled water, and left to dry. The dried tissues were placed on Potato Dextrose Agar (PDA, Merck, 39 g/l). The medium was sterilized by autoclaving at 121 °C for 20 min before use and incubated at 24 °C for 5 days. Pure cultures were obtained by transferring hyphal tips from each colony onto fresh PDA plates. Developing fungal colonies were examined under a microscope. *Trichoderma* isolates were obtained from root tissues using the same procedure and purified by hyphal tip transfer to obtain pure cultures (Uysal and Erkılıç 2022). Fungal species developed in pure culture were identified according to genus-specific morphological characteristics using mycological identification keys. *Fusarium* species were identified based on hyphal branching patterns, phialide formation, and the morphology of micro- and macroconidia, as well as the presence of chlamydospores and sporodochia (Samson et al. 1995, Leslie and Summerell 2006). Conidial measurements were performed under a 40× objective lens. *Trichoderma* species were identified based on conidiophore branching patterns, phialide arrangement, and conidial morphology (Harman and Kubicek 1998, Rahman et al. 2011, Kumar et al. 2019). *Rhizoctonia* species were diagnosed based on colony characteristics on PDA, vegetative hyphal morphology, and the number of nuclei per

cell, with Safranin O used for nuclear staining (Ogoshi 1975, Bandoni 1979, Sneh et al. 1991). *Macrophomina* spp. were identified based on colony morphology on PDA and the characteristics of microsclerotia (Ashby 1927, Goidanich 1947). *Sclerotinia sclerotiorum* was identified based on the formation of black sclerotia in culture and characteristic hyphal features (Wong and Willetts 1975, Ordóñez-Valencia et al. 2015).Fungi from various genera were isolated from commercial moth orchid production greenhouses. The fungi were identified to the genus and species level based on certain morphological and microscopic characteristics. The incidence of the fungi was determined at the genus level with the formula Incidence (%) = (Number of Diseased Parts / Total Number of Parts) × 100 (Bora and Karaca 1970).

Pathogenicity test

Pathogenicity tests were conducted in a climate room (25±2 °C, 70% relative humidity, and a 16:8h light/dark photoperiod) with five replicates (one plant per pot). *Fusarium oxysporum* isolates were grown on sterilized wheat grains, and 3 g of colonized wheat inoculum was placed into the root zone of each healthy plant. Control plants received sterile wheat grains without fungal growth (Atakan 2014). Three *F. oxysporum* isolates were selected to represent different sampling locations.The isolates that were found to be pathogenic in the tests were reisolated from the roots of the test plants. Plants were maintained under greenhouse conditions and observed for at least 4 weeks for symptom development. Wilt, yellowing, and root rot symptoms were recorded. The pathogen was re-isolated from symptomatic plants, and identification was confirmed by comparing colony morphology and microscopic characteristics with the original isolates.

In vitro biocontrol assays

In vitro studies were conducted using dual culture (Mokhtari et al. 2017) and sealed plate (Mahalakshmi and Raja 2013) methods to evaluate the antagonistic activity of 15 *T. harzianum* isolates against 3 *F. oxysporum* isolates (H8, G3 and YL3-3B). For each *F. oxysporum* isolate, five different *T. harzianum* isolates were screened to identify the most effective antagonist, considering possible isolate-specific variability in antagonistic interactions.

In both *in vitro* test methods, control plates consisted of *Fusarium oxysporum* cultures grown alone without *Trichoderma* treatment. Separate control plates were established for each *F. oxysporum* isolate to ensure accurate comparison of treatment effects.

The dual culture assay was used to assess direct mycoparasitic interaction by observing the confrontation of hyphae and the inhibition of pathogen growth on the same plate. The sealed plate method was performed to evaluate the effect of volatile compounds produced by *Trichoderma* on pathogen growth without direct physical contact. In the sealed plate method, *T. harzianum* was inoculated on the bottom plate, while *F. oxysporum* was inoculated on the upper plate.

The experiment was conducted on 90 mm Petri dishes containing PDA and arranged in a completely randomized design with five replicates, each replicate consisting of one Petri dish. The Petri dishes were incubated at 24 °C for 4 days, after which colony diameters were measured and the percentage of growth inhibition was calculated. In the dual culture test, fungal growth was evaluated on the 4th day of incubation, and the efficacy rate (%) was calculated using the Abbott formula. In the sealed plate method, colony diameters and growth inhibition rate were measured on the 4th day of incubation.

Inhibition percentage (%) = $((A_1 - A_2) / A_1) \times 100$ (A1: Pathogen colony diameter in control (mm) A2: Pathogen colony diameter in test culture (mm) (Sreedevi et al. 2011)

In vivo biocontrol assay

An *in vivo* trial was conducted in a climate room maintained at 25±2 °C, 70% relative humidity, and a 16:8 h light/dark photoperiod. For the experiment, ten-month-old potted *Phalaenopsis* plants with 2–3 leaves were obtained from a commercial ornamental plant company engaged in both potted and cut orchid production. This study evaluated the biological control potential of the most promising *Trichoderma harzianum* isolate obtained from moth orchids, as determined by in vitro assays, against *Fusarium oxysporum* isolate H8, in comparison with a commercial *T. harzianum* (Planter Box) product. Isolates showing the highest efficacy in the *in vitro* assays were subsequently selected for the *in vivo* trials.The experiment followed a completely randomized design with six treatments, each replicated four times with four plants per replicate. The spore suspension of the *T. harzianum* isolate was prepared by cultivating the fungus on PDA to obtain a pure culture. The culture surface was scraped with sterile distilled water, and the resulting suspension was filtered through a double layer of cheesecloth. The spore concentration was adjusted to 10^6 spores/ml using a Thoma slide. 50 ml of the suspension was applied to the roots of each plant (Islam et al. 2022). The Planter Box was prepared at a concentration of 2 g/l, as recommended by the manufacturer, and applied at a volume of 50 ml per plant. The pathogen *F. oxysporum* was applied 48 hours after *Trichoderma* application. For pathogen inoculation, *F. oxysporum* was cultured on sterilized wheat grains as described above. After full colonization, 3 g of

wheat-based inoculum was placed near the root collar of each plant. The inoculum amount was adapted from previous studies (Atakan 2014) and adjusted according to plant size. The experimental treatments were: 1. Negative control with no application, 2. Positive control with *Fusarium oxysporum* inoculation, 3. *Trichoderma harzianum* isolated from moth orchid, 4. Isolated *T. harzianum* isolated from moth orchid + *F. oxysporum* inoculation, 5. Commercial *T. harzianum* application, 6. Commercial *T. harzianum*+ *F. oxysporum* application.

Initial disease symptoms were observed within 3–4 weeks after inoculation. Disease severity was evaluated three months after inoculation using a 0–4 rating scale, when symptoms were clearly established in inoculated plants (Prodos-Ligero et al. 2007). According to the scale: 0: Healthy plant, 1: Chlorosis in the lower parts of the plant, 2: Chlorosis or wilt in the lower part of the plant and 1/3 of the plant, 3: Wilt in a few leaves in the upper part of the plant, 4: Dead plant.

At the end of the trial, root length, wet weight, and stem weight were measured to evaluate plant growth parameters. Disease severity (%) was calculated based on the scale values using the Townsend–Heuberger (1943) formula, according to the following equation:

$$\text{Disease severity (\%)} = [\Sigma(n \times v) / (N \times V)] \times 100$$

The data obtained were subjected to analysis of variance, and the differences between treatments were evaluated according to the Tukey test ($P<0.05$).

RESULTS

Fungal isolates were identified to genus and species level based on morphological and microscopic characteristics using standard identification keys. *Fusarium* was the predominant genus across all surveyed provinces, with isolation frequencies of 14.00% in Mersin, 56.00% in Yalova, 64.60% in Antalya, and 28.80% in Istanbul. *Macrophomina* sp. (0.80%) was detected only in Yalova, whereas *Rhizoctonia* sp. (0.40%) and *Pythium* sp. (0.80%) were found exclusively in Mersin. *Sclerotinia* sp. (2.00%) was isolated only from Antalya. Other genera identified in the survey included *Aspergillus*, *Bipolaris*, *Chaetomium*, and *Penicillium* (Table 1).

Table 1. Species and isolation frequencies (%) collected from moth orchid greenhouses

Species	isolation frequencies (%)				
	Mersin	Yalova	Antalya	İstanbul	number of isolates
Fusarium acutatum	3.2	-	1.6	-	3
Fusarium chlamydosporum	-	-	11.4	-	7
Fusarium oxysporum	8.1	9.8	16.3	6.5	25
Fusarium proliferatum	-	4.9	3.2	1.6	6
Fusarium poae	-	8.1	1.6	-	6
Fusarium redolens	-	-	1.6	-	1
Fusarium solani	6.5	4.9	1.6	1.6	8
Fusarium verticilloides	-	6.5	-	-	4
Macrophomina phaseolina	-	0.80	-	-	1
Rhizoctonia solani	0.80			-	1
Sclerotinia sclerotiorum	-	-	2.00	-	1
Trichoderma atroviride	-	2.2	-	-	1
Trichoderma harzianum	16.6	6.6	40	36.6	30
Trichoderma koningii	-	40	-	60	5
Trichoderma virens	62.5	25	-	12.5	8
Other species*	9.60	14.57	3.61	2.20	58

**Bipolaris* sp., *Aspergillus* sp., *Penicillium* sp., *Chaetomium* sp.

Fusarium oxysporum is the most common species, with an incidence rate of 6.5–16.3%, and was isolated from all provinces in the study area. *Fusarium solani* was also isolated in all provinces, and its incidence rate varied between 1.6% and 6.5%. *Fusarium redolens* Wollenw. and *F. chlamydosporum* Wollenw. & Reinking had an incidence of 1.6% and 11.4%, respectively, and were isolated only in Antalya province. *Fusarium verticilloides* (Sacc.) Nirenberg, with an incidence of 6.5%, was isolated only in Yalova. The incidence of species classified as "other species" ranged from 2.2% to 14.5%.

A total of 44 Trichoderma isolates were obtained. Species identification was performed based on morphological criteria using standard identification keys (Harman and Kubicek 1998, Rahman et al. 2011, Kumar et al. 2019). Accordingly, 30 isolates were identified as *Trichoderma harzianum*, eight as *T. virens* (J.H. Miller, Giddens & A.A. Foster) Arx, five as *T. koningii* Oudem., and one as *T. atroviride* P. Karst. The most frequently isolated species was *T. harzianum* (68.1%), whereas *T. atroviride* was the least common (2.2%).

Pathogenicity tests

Pathogenicity tests were conducted for three selected *Fusarium oxysporum* isolates (H8, G3, and YL3-3B), which were chosen from a total of 25 isolates based on their representative characteristics. All tested isolates induced disease symptoms on orchid plants, confirming their pathogenicity. Although minor differences in symptom severity were observed under the experimental conditions, a detailed quantitative comparison of virulence among isolates was beyond the scope of this study.

In vitro biocontrol assay

In the dual culture and sealed plate assays, three *Fusarium oxysporum* isolates (H8, G3, and YL3-3B) were used as pathogens. In the dual culture test, inhibition rates ranged from 37.8% to 76.36%, with the highest inhibition observed for isolate B2.2. In addition, 9 out of 15 *Trichoderma* isolates formed inhibition zones, and the largest inhibition zone was recorded for isolate FKBK1 (9 mm) (Figure 1).

In contrast, the sealed plate method, which reflects the effect of volatile compounds, showed a different pattern. Although isolate B1.1 exhibited limited inhibition in dual culture assays, it showed a relatively higher inhibitory effect (54.1%) in the sealed plate method. However, all tested isolates exhibited similar levels of inhibition in this method, ranging between 47.3% and 54.1%. These results indicate that, under sealed plate conditions, the inhibitory effects of *Trichoderma* isolates were relatively comparable. Therefore, the observed inhibition may not be attributed solely to the activity of individual isolates, but could also be influenced by differences in the sensitivity of *F. oxysporum* isolates to volatile compounds (Table 2).

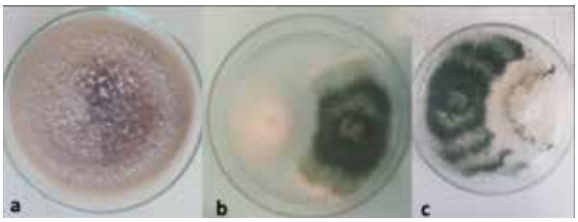


Figure 1. Control growth of *Fusarium oxysporum* G3 isolate (a), Dual culture test of *Fusarium oxysporum* G3 isolate with *Trichoderma harzianum* B2.2 isolate (b), Dual culture test of *Fusarium oxysporum* G3 isolate with *Trichoderma harzianum* A5.2 isolate (c)

Sealed plate data evaluated collectively revealed significant differences among *Trichoderma* isolates ($P<0.05$). The inhibitory effects varied widely, with mean colony diameters ranging from 3.54 cm to 6.68 cm. Some isolates (e.g., B1,1, A5,2 , and B2,2) exhibited significantly stronger antifungal activity, whereas others (e.g., YL1,A and FKBK8) showed limited inhibitory effects (Table 3).

Table 3. Overall comparison of *Trichoderma* isolates in the sealed plate assay

Trichoderma isolate	**% Mycelial growth - Sealed plate method (Overall comparison)**
YL1, A	60.68±0.12 a
FKBK8	60.50±0.16 a
FKBK1	60.24± 0.23 a
KBK8	60.05±0.22 a
FKBK5	50.76±0.28 ab
3.5	50.10±0.25 bc
5.3	40.89±0.19 bc
4.4	40.71±0.24 cd
8.3	40.71±0.19 cd
1.3	40.28±0.18 cde
F1,1	40.25±0.11 cde
D1,1	30.92±0.07 de
B2,2	30.72±0.13 e
A5,2	30.70±0.07 e
B1,1	30.54±0.20 e

*Averages following by different letters in the same column are significantly different ($P<0.05$) according to Tukey test

In vivo biocontrol assay

The isolate used in the *in vivo* experiments was selected based on its *in vitro* performance. Among the tested isolates,

Table 2. Dual culture and sealed plate method results of *Trichoderma* isolates

Trichoderma isolate	% Mycelial growth		% Effect		Inhibition zone (mm)
	Dual culture	Sealed plate method	Dual culture	Sealed plate method	Dual culture
			***Fusarium oxysporum* isolate H8**		
Control	40.80±0.20 a	40.87±0.16 a	-	-	-
4.4	30.32± 0.23 b	40.71±0.24 a	55.5	45.1	-
8.3	20.99±0.10 b	40.71±0.19 a	54.9	43.5	-
1.3	20.80 ±0.16 b	40.28±0.18 a	68.5	45.6	-
5.3	20.76±0.08 b	40.89±0.19 a	37.8	41.1	-
3.5	20.02± 0.13 c	50.10±0.25 a	68.6	41.4	-
			Fusarium oxysporum isolate G3		
Control	50.30±0.13 a	50.30±0.13 a	-	-	-
B2,2	30.70 ±0.28 b	30.70±0.13 c	76.36	52.1	5
A5,2	30.43±0.23 b	30.70±0.07 c	51.85	52.3	5
D1,1	30.73±0.18 b	30.92±0.07 bc	45.45	50.0	5
F1,1	30.71±0.18 b	40.25±0.11 b	46.15	47.3	3
B1,1	30.02±0.10 b	30.50±0.20 c	50.94	54.1	-
			Fusarium oxysporum isolate YL3,3B		
Control	40.04±0.26 a	50.26±0.05 c	-	-	-
YL1, A	20.89±0.29 b	60.60±0.24 a	48.28	20.2	5
FKBK5	20.82±0.24 b	50.70±0.28 bc	57.14	29.7	6
FKBK8	20.66±0.15 b	60.50±0.16 ab	45.95	24.0	5
FKBK1	30.45±0.23 ab	60.20±0.23 ab	45.83	25.2	9
KBK8	30.22±0.19 ab	60.05±0.22 abc	60.38	28.6	7

*Averages following by different letters in the same column are significantly different (P <0.05) according to Tukey test

isolate B1,1 exhibited the highest antifungal activity in the sealed plate method, reducing mycelial growth by 54.1%. Since this method reflects the effect of volatile organic compounds, isolate B1,1 was considered the most promising candidate and was therefore chosen for the *in vivo* assays.

The effects of *T. harzianum* on Fusarium wilt in orchids are shown in Table 4. *In vivo* experiments were conducted using isolate B1,1, which was the most effective isolate *in vitro*. *T. harzianum* isolated from moth orchid decreased the disease severity by 77.3%, while the commercial *Trichoderma* preparation (Planter Box) reduced disease severity by 81.78%.

Table 4. Effects of *Trichoderma harzianum* on Fusarium wilt

Application	Disease index	Disease incidence (%)	Effect (%)
FO	2.8 a	88	-
TH+FO	0.6 b	20	77.3
TTH+FO	0.5 b	16	81.8

Averages following by different letters in the same column are significantly different (P < 0.05) according to Tukey test(Fo: *Fusarium oxysporum* positive control, TH+Fo: *Trichoderma harzianum* + *Fusarium oxysporum*, TTH+Fo: Commercial *Trichoderma harzianum*+ *Fusarium oxysporum*).

The effect of *T.harzianum* on plant growth parameters is presented in Table 5. Infection with *F. oxysporum* (FO) significantly reduced plant growth, with root length decreasing from 21.4 cm in the control to 14.3 cm and shoot fresh weight from 12.7 g to 5.9 g. In contrast, *Trichoderma* treatments improved all growth parameters. In the absence of the pathogen, both TH and TTH increased plant growth compared with the control, with the highest values observed in TTH treatment (30.2 cm root length and 29.9 g shoot fresh weight). Under pathogen pressure (TH+FO and TTH+FO), both treatments alleviated the negative effects of FO. Root length increased to 21.6 cm (TH+FO) and 29.1 cm (TTH+FO), while shoot fresh weight reached 25.2 g and 24.1 g, respectively.

Overall, both *Trichoderma harzianum* applications promoted plant growth and reduced the negative effects of *F. oxysporum*, with the commercial formulation generally showing higher performance.

Table 5. Effects of *Trichoderma harzianum* on root length, root and green parts fresh weight

Application	Root length (cm)*	Root wet weight (g)	Shoot wet weights (g)
Negative control	21.4 b	40.4 b	12.7 d
FO	14.3 c	28.6 c	5.9 e
TH	24.9 b	52.9 a	19.4 c
TH+FO	21.6 b	48.6 a	25.2 b
TTH	30.2 a	52.2 a	29.9 a
TTH+FO	29.1 a	50.2 a	24.1 b

Averages following by different letters in the same column are significantly different ($P < 0.05$) according to Tukey test(Fo: *Fusarium oxysporum* positive control, TH+Fo: *Trichoderma harzianum* + *Fusarium oxysporum*, TTH+Fo: Commercial *Trichoderma harzianum*+ *Fusarium oxysporum*).

DISCUSSION

Pathogenic, non-pathogenic, mutualistic, and plant growth-promoting fungal species are known to be associated with orchids. Among the Fusarium species reported as orchid pathogens, *F. oxysporum* is considered the most widespread and destructive (Swett 2007). In the present study, *F. oxysporum* was again the most frequently isolated species and was confirmed as a pathogen through pathogenicity tests. Similarly, *Fusarium solani* was isolated from diseased moth orchid plants. While molecular identification was not conducted in the present study, the observed disease symptoms are comparable to those reported for *F. solani* infections in orchids by Chung et al. (2011).

In the dual culture test, the zone of inhibition around the treatment areas was measured. The zone of inhibition, the area in the Petri dish where mycelial growth is inhibited formed by antagonist fungi in dual culture is actually due to their antibiotic production (Bhargav et al. 2016, Tunabaş and Erkılıç 2022). This method has been used in various studies to evaluate the antimicrobial effects of *Trichoderma* spp. (Anwar and Iqbal 2017, Sharma 2011). Vizcaíno et al. (2005) examined the effects of *T. harzianum*, and *T. atroviride* isolates on different fungal and bacterial agents such as *F. oxysporum*, and *Botrytis cinerea* Pers. The observation of zones of inhibition was associated with positive antimicrobial activity. The zones around the application points were classified as either greater than or less than 15 mm. In that study, *F. oxysporum* was the most resistant fungus, as it was inhibited by only three strains each of *T. asperellum* and *T. harzianum* out of a total of 24 isolates. Low antimicrobial activity does not necessarily imply a weak biocontrol effect, as a particular strain may be more mycoparasitic than antibiotic producing. Similarly, in our study, the KBK8 isolate showed low activity through volatile metabolites in sealed plate method; however, the formation of a 9 mm inhibition zone and a 60.38% inhibition rate in dual culture indicated a strong mycoparasitic effect.

Girma (2022) reported that *T. viride* strain showed 93.57% activity against *F. oxysporum* f.sp. *capsici* radial growth in a dual culture test, followed by *T. harzianum* with 90.12% and *T. asperellum* with 89.45%. Although a relatively lower effect was observed in our study, the isolate still acted as a hyperparasite, showing an inhibitory effect on fungal growth, with a maximum inhibition rate of 76.36%.

Meena et al. (2017) reported that, using the sealed plate method to examining the effect of volatile compounds released by *Trichoderma* spp., *T. harzianum* inhibited the growth of *F. oxysporum* by 17.14% on the seventh day, while it inhibited *A. alternata* by 40.00%. In contrast, the sealed plate method, which reflects the effect of volatile

compounds, showed a different pattern. Although some isolates exhibited limited inhibition in dual culture assays, a relatively higher inhibitory effect (up to 54.1%) was observed in the sealed plate method. However, all tested isolates exhibited comparable inhibition levels ranging between 47.3% and 54.1%, indicating a generally uniform response under volatile exposure conditions.

Sealed plate data evaluated collectively revealed significant differences among *Trichoderma* isolates ($P<0.05$). The inhibitory effects varied, with mean colony diameters ranging from 3.54 cm to 6.68 cm. Some isolates (e.g., B1.1, A5.2, and B2.2) exhibited significantly stronger antifungal activity, whereas others (e.g., YL1A and FKBK8) showed comparatively limited inhibition.

These observations suggest that the inhibitory response in the sealed plate assay is not solely dependent on the intrinsic activity of individual *Trichoderma* isolates. Instead, it may also be influenced by variation in the sensitivity of *F.oxysporum* isolates to volatile organic compounds. Such isolate-dependent sensitivity can lead to a partial "response attenuation" or "effect breakage" phenomenon, where differences among *Trichoderma* isolates appear reduced under uniform exposure conditions. This pattern is consistent with reports indicating that *F. oxysporum* isolates may differ in their susceptibility to antifungal volatiles, thereby affecting the overall magnitude of growth inhibition under identical experimental conditions (Ghosal et al. 2020).

Trichoderma spp., with their high reproductive capacity, ability to survive under adverse conditions, efficient nutrient utilization, rhizosphere modification capacity, suppression of phytopathogenic fungi, and effectiveness in promoting plant growth and defense mechanisms, are preferred biological control agents (Misra and Prasad 2003, Pandya et al. 2011). For this reason, *T. harzianum* isolates were selected for biological control. To evaluate their efficacy, the test isolate was compared with the commercial preparation T-22 Planter Box. *In vitro* assays showed that volatile metabolites of TH isolates reduced the colony diameter of *F. oxysporum* by 54.1%, while hyperparasitic effects resulted in up to 76.36% inhibition. *In vivo* applications reduced disease severity by 77.3% for orchid-derived isolates and 81.8% for the commercial product. Both treatments also increased root length, root fresh weight, and shoot biomass. These results demonstrate that *T. harzianum* isolates are effective in the biological control of *F. oxysporum*-related diseases in orchid cultivation.

The *Trichoderma* isolate obtained from orchids exhibited an efficacy value comparable to that of Planter Box, suggesting its potential use as a biological control agent against the disease.

On the other hand, both *Trichoderma* treatments increased root length, root fresh weight, and shoot weight to varying degrees among the plant growth parameters. Planter Box was found to be more effective than the Trichoderma isolate obtained from orchids.Similar to the positive effect of *T. harzianum* on plant growth, a two-year study conducted by Özçelik et al. (2008) reported that *T. harzianum* significantly increased the flower stem length and diameter of moth orchids in the second year, while no positive effects were observed during the first year. The commercial product performed better in both cases. This may be attributed to its formulation advantages and the fact that it was selected as the most effective strain based on field analyses.

This study demonstrates that *T. harzianum* isolates exhibit effective biocontrol activity against *F. oxysporum* associated with orchid diseases, with varying levels of inhibition depending on the isolate and assay conditions. Both dual culture and sealed plate methods confirmed antifungal activity, while in planta results further supported disease suppression and plant growth promotion. The observed differences among treatments suggest that both antagonist efficacy and pathogen isolate-specific sensitivity contribute to the overall interaction outcome. Overall, the findings indicate that orchid-derived *Trichoderma* isolates have promising potential as sustainable biological control agents in orchid cultivation systems.

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Author's Contributions

Authors declare that each author's contribution is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Güve orkideleri olarak bilinen *Phalaenopsis* türleri, çok sayıda çiçek oluşturması ve uzun çiçeklenme süresi nedeniyle yaygın olarak yetiştirilen süs bitkilerindendir. Bu çalışmanın amacı, ülkemizde yetiştirilen *Phalaenopsis* orkideleri ile ilişkili fungal etmenlerin belirlenmesi ve güve orkidesinden elde edilen *Trichoderma harzianum* (TH) izolatlarının, patojenitesi doğrulanan *Fusarium oxysporum* izolatlarına karşı biyokontrol etkinliğinin ortaya

konulmasıdır. Orkidelerden izole edilen fungal etmenler arasında başta *Fusarium* spp. olmak üzere, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* ve *Macrophomina phaseolina* gibi önemli fitopatojenlerin yer aldığı ve özellikle *Fusarium oxysporum*'un en yaygın türlerden biri olarak öne çıktığı belirlenmiştir. Çalışmada Antalya, Mersin, Yalova ve İstanbul illerinden alınan örnekler incelenmiştir. Orkidelerden izole edilen 15 adet *T. harzianum* izolatı ve patojenitesi doğrulanan 3 adet *F. oxysporum* izolatı ile *in vitro* testler gerçekleştirilmiştir. Elde edilen sonuçlara göre seçilen bir izolat ve bir ticari *T. harzianum* preparatı *in vivo* denemelerde kullanılmıştır. Denemeler kontrollü koşullarda iklim odasında, tesadüf parselleri deneme desenine göre 4 tekerrürlü olarak yürütülmüştür. *In vitro* testlerde, TH izolatlarının sekonder uçucu metabolitlerinin *F. oxysporum* koloni çapını kontrole kıyasla %54.1 oranında azalttığı, hiperparazitik etki ile bu oranın %76.36'ya kadar ulaştığı belirlenmiştir. *In vivo* denemelerde ise orkide kökenli TH izolatlarının uygulamaları, yalnızca *F. oxysporum* inokule edilmiş pozitif kontrole kıyasla hastalık şiddetini %77.3 oranında azaltırken, ticari TH preparatında bu oran %81.8 olarak saptanmıştır. Ayrıca, hem orkide kökenli TH izolatları hem de ticari preparat uygulamaları bitkilerde kök uzunluğu, kök yaş ağırlığı ve yeşil aksam ağırlığını artırmıştır. Elde edilen bulgular, *T. harzianum* izolatlarının orkide yetiştiriciliğinde *F. oxysporum* kaynaklı hastalıkların biyolojik mücadelesinde etkili bir şekilde kullanılabileceğini açıkça ortaya koymaktadır.

Anahtar kelimeler: süs bitkileri, fungal hastalıklar, *Trichoderma harzianum*, hastalık şiddeti

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