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Enhancing *Freesia* (*Freesia armstrongii*) Growth and Yield through *Trichoderma*-Mediated Biocontrol of *Fusarium* Wilt

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ABSTRACT

The current research explored the biocontrol ability of two strains of *Trichoderma* fungi on infected *Freesia* corm by *Fusarium* disease. Enhancements in plant growth and performance were evaluated in response to the *Trichoderma* fungi in laboratory and greenhouse conditions. Laboratory experiments included five *Trichoderma strains*, i.e., T1, T20, and Th4, related to *T. harzianum* species, T2 related to *T. atroviride* species, and Tvi related to *T. virens* species. A dual culture test and investigation of the inhibition and colonization rate were carried out with 11 treatments in three replications. Results revealed that strains T1 and T2 completely covered the pathogen in 96 h of culture by 70.02% and 68.98% and successfully inhibited the colonization of the disease agent by 84.53% and 82.72%, respectively, compared to the other strains. Then, a greenhouse experiment was performed with eight treatments, using suspensions of *Trichoderma* strains separately or combined with the presence of *Fusarium* fungus isolate on *Freesia* cultivation. The results showed that the strains at different levels were able to increase the growth and yield of *Freesia* plants and control *Fusarium* disease. In general, the T2 strain was more effective in increasing plant growth and flower stem height in Freesia. The T1 strain specifically improved the corm diameter. Both T1 and T2 strains demonstrated effectiveness in controlling and reducing the severity of *Fusarium* disease and enhancing the growth quality of *Freesia*.

Introduction

Freesia armstrongii var. Red Bi-Color is considered one of the most important plants within the Iridaceae family (Manning et al., 2010). The *Freesia* genus has about 20 species, mainly grown as cut branches and suitable for cultivation

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in borders and pots. *Freesia* is propagated by corms. The leaves of this plant are sword-shaped and light green, and its stems grow 20 to 30 cm. Also, the arrangement of this flower is unique. Trumpet-shaped cluster flowers occur vertically on the stems. *Freesia* flowers have stunning colors of pink, yellow, red, blue, violet, orange, gold, purple or white (Wang, 2007). Inflorescences and flowers usually bloom horizontally at a 90-degree angle on the rest of the stem. Secondary stems may grow from the leaf axis of the main stem, each of which has flowers. This flower is mostly cultivated as a cut flower due to its pleasant fragrance (Azimi, 2023).

Freesia usually produces 4-8 fragrant funnel-shaped flowers, making it one of the most popular flowers. Compared to other cut flowers, the *Freesia* plant requires a shorter growing period in the greenhouse and lower temperatures during the winter months (Wülfinghoff, 2016; Wang, 2007). These two advantages, along with the special attention of consumers, have caused a significant increase in the production of *Freesia* flowers in recent years in Iran. In commercial production, breeders need assurance of flowering time, tall flower stems, high performance, and quality flowers. However, compared to other cut flowers, *Freesia* has a shorter flowering stem, less stem strength, and smaller flower size (Khan et al., 2012).

The cultivation and commercial production of this flower date back to 1873 AD, but these flowers were known in Europe in 1945. Currently, in Europe, large areas of about 600 hectares have become famous for freesia cultivation (Wülfinghoff, 2016). In Iran, it is estimated that the planted area under *Freesia* cultivation could be increased from 20 to 50 hectares. *Fusarium* wilt disease in *Freesia*, caused by *Fusarium* oxysporum, is considered one of the most destructive diseases of *Freesia* in most of the production areas of this flower worldwide (Ben and Shtienberg, 1997).

Fusarium species are active in all fields worldwide, where moisture and organic matter exist. These microorganisms are related to the ecosystem in nature. As such, they can live in all areas of the world, in oceans, and on all land, even in desert areas. Wherever there is soil and a plant, a species of *Fusarium* fungus will usually live as a pathogen or saprophyte around the atmosphere of the plant root. Basically, like other fungi, *Fusarium* is heterotrophs and without chlorophyll. Their vascular system develops weakly. Instead of primary carbon hydrate and starch, they have glycogen. Their vegetative part is through hyphae, and they assimilate nutrition by absorption. The filaments contain a transverse wall or septum that can form one or more single cells with a thick wall called chlamydospore (Sarmi, 2005).

Planting material, water, soil particles, tools, footwear, and machinery can efficiently disseminate the pathogen. The fungus and chlamydospore can survive in the soil for 20 years or more, exhibiting a long latent period, detectable only long after introduction. There are no symptomatic differences among races. Early detection of symptoms in the field and fast laboratory diagnostics are essential steps to eradicate or contain an eventual outbreak (Pérez-Vicente et al., 2014).

In Iran, many plants are susceptible to *Fusarium* wilt, making it one of the most problematic diseases. This fungus is highly resistant to environmental disinfection, and a specific or general chemical that can completely control it from the environment has not yet been introduced (Sarmi, 2005). So far, no accurate statistics on the percentage of damage caused by vascular wilt disease to *Freesia* plants have been reported in Iran. Therefore, testing and finding relevant solutions can be essential due to the ineffectiveness of the usual methods to control and prevent this disease. Until now, for the control of *Fusarium oxysporum*, the cause of *Fusarium* wilt of *Freesia*, agricultural management has been absent in controlling the disease via biological control and resistant cultivars.

Considering the importance of *Freesia* as a cut flower and the necessity of finding a more suitable, environmentally friendly, and effective disease control method, this research investigates the potential of biological control for vascular wilt disease in *Freesia*. The *Trichoderma* genus, with its various species, has demonstrated considerable success in establishing both parasitic and symbiotic relationships in different substrates and living organisms, including plants and microorganisms, especially soil fungi. These species exhibit successful mechanisms and specifications for biocontrol, coupled with the ability to reproduce quickly in soil and produce strong antibiotics. Additionally, they are among the most resistant microorganisms to synthetic or natural chemicals and toxins (Chaverri et al., 2015). Therefore, in this study, different species of this genus were used as biological control agents for the vascular wilt fungus affecting *Freesia*.

Materials and Methods

Isolation of pathogenic fungi

Corms were collected from a greenhouse in the

Department of Horticultural Sciences and Landscape Engineering, Faculty of Agriculture, University of Tehran, 2018. The wilt-affected corm samples showing signs of *Fusarium* infection with dry rot were carefully placed in disposable envelopes and transferred to the laboratory.

Then, the isolation of fungi was done by direct culture method. *Freesia* with dry rot signs were selected, and to remove soil particles and suspended materials, they were immersed in sterile water for 5 min. The lower half of the plants were then sectioned into 0.5 to 1 cm pieces, followed by a 30 s soak in 75% ethanol for disinfection, and then plants were washed in sterile distilled water for 30 s. After air-drying, the corm samples were placed on sterile filter paper and transferred to the specialized Peptone PCNB Agar (PPA) Basic Culture Medium, with four pieces per petri dish. The Petri dishes were then incubated for 3 to 5 days in an incubator with a temperature of 25 °C and a dark environment, maintaining conditions until the appearance of the disease agent. *Fusarium* fungi grown on the culture medium were purified by a single-fungal hyphae method (Nelson et al., 1983).

Identification of fungal isolate

To identify the isolates, PDA and CLA commercial culture media were used. In the PDA culture medium, we determined colony characteristics (macroscopic features). In the CLA culture medium, we determined microscopic characteristics. The species identification of *Fusarium* isolates was conducted based on reliable sources, utilizing *Fusarium* identification keys (Nelson et al., 1983; Leslie et al., 2006). After the initial diagnosis, species identification was further validated by the Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran. To prove their pathogenicity, all *Fusarium* isolates underwent testing on susceptible *Freesia* cultivars using the pathogen suspension immersion method in the greenhouse campus of the Department of Horticultural Sciences and Landscape Engineering, Faculty of Agriculture, University of Tehran.

Proving the pathogenicity agent on Freesia

In this experiment, a plate (disc) with a diameter of 5 mm was obtained from a four-day culture of Fusarium head isolates grown in 250 mL Erlenmeyer flasks containing 150 mL of sterile LB (Luria-Bertani Broth) culture medium. The corm surface was disinfected with 1% sodium hypochlorite for 15 min. After three washes with sterile distilled water and drying with sterile filter paper, these corm pieces were placed inside a basket containing twice-sterile perlite. The basket was autoclaved at a temperature of 121 °C and a pressure of 1.5 bar for two hours. Subsequently, it was kept in a refrigerator at a temperature of 5 °C until the plant roots began to grow (Rajik, et al., 2012). The experiment was carried out in three repetitions. The Freesia corm was cultivated in a pot containing a well-mixed combination of garden soil (sandy loam texture), cocopeat, and perlite in an equal volume ratio (1:1:1). The lower third of each pot was filled with peat moss. Subsequently, after being moistened, the medium was placed in double-walled plastic bags and underwent complete sterilization in an autoclave at 121 °C and a pressure of 1.5 bar for two hours.

Corms were treated in two ways. In the first method, immersion was done for one minute in a suspension of the pathogenic fungi *Fusarium oxysporum*. For the second method, a suspension with 90 mL k^{-1} of culture media was added to the pot surface. Then, the pot containing the sterile culture media together with the *Freesia* plants at the two-leaf stage were placed in a greenhouse for 30 days. The plants were irrigated as needed. After data collection, calculations followed the equation below (Etebarian et al., 2006).

$$Percentage\ of\ infected\ Plants = \frac{NP1 - NP2}{\mathrm{NP1}} \times 100$$

Where NP1 was number of plants in control and NP2 was the number of plants in the treatment.

To confirm the specific contamination, the fungus responsible for the disease was isolated from the plants exhibiting relevant signs. After isolating the causative fungus, the characteristics were compared with a previously isolated sample, and their taxonomic similarity was investigated. After carrying out the pathogenicity test, an isolate of *Fusarium oxysporum* was selected as the superior isolate based on the intensity of pathogenicity. This selected isolate was then used in tests related to the antagonistic effect of *Trichoderma*, as well as in a greenhouse experiment.

Pathogenic symptoms

As a result of the activity of this fungus, the leaves of the *Freesia* plant were affected by rot near the soil surface, and rot signs also spread to the roots. Infected leaves are light green, sometimes appearing as necrotic spots with red lines. Yellowing, shriveling, or dryness progresses from the tip to the bottom of the leaf, eventually leading

to leaf death.

Preparation of fungus antagonist

In this research, five strains of *Trichoderma* fungi originated from the Department of Plant Protection, University of Tehran. The strains included three *T. harzianum* (T1, T20, and Th4), *Trichoderma atroviride* (T2), and *T. virens* (Tvi).

Evaluation of the antagonistic property of Trichoderma strains on Fusarium wilt fungus Freesia in dual culture test

To measure the inhibition of growth of the superior isolate of the disease-causing fungus by the two superior agents of the antagonistic fungus, the simultaneous dual culture test was used. In this method 11 treatments containing five strains of *Trichoderma* including *T. harzianum* (T1), *T. harzianum* (T20), *T. atroviride* (T2), *T. virens* (Tvi), *T. harzianum* (T4), and one isolate of *Fusarium oxysporum* (Fo) as control treatment plus these treatments dually with *Fusarium oxysporum* (Fo). Each isolate was positioned and cultured on both sides of a separate petri dish consisting of three replicates. For this, oa 5 mm diameter disc was taken from the four-day culture of *Trichoderma strains*, and placed in the center of a Petri dish containing a PDA culture medium. At the same time, the best isolate of the *Fusarium* fungus was extracted from the four-day culture on the PDA culture medium and placed in the same plate, one centimeter away from the edge and on the opposite side of the *Trichoderma* fungi. In the control treatments, a 5 mm disc diameter from a four-day *Fusarium* culture was placed in the middle of the PDA culture medium after only four days of cultivation. Petri dishes were kept at a temperature of 25 °C, and the linear growth of *Fusarium oxysporum* colonies and *Trichoderma* strains (5 treatments of Trichoderma strains and one control) were measured at 24, 48, 72, and 96 h intervals. The growth inhibition percentage was calculated using the formula provided below (Jeyaseelan et al., 2012).

$$Percentage\ of\ inhibition = \frac{R1-R2}{\mathrm{R1}} \times 100$$

Where R1 was radial growth of colony in control and R2 was the radial growth of colony in the treatment group.

Evaluating the ability of hyper-parasitism (colonization) of Trichoderma strains

To evaluate the colonization ability and hyper-parasitism of *Trichoderma* strains, the growth diameter of *Fusarium oxysporum* was measured 24, 48, 72, and 96 h after counter-cultivation. The percentage of *Fusarium* colonization by *Trichoderma* and an evaluation of the hyper-parasitic ability of *Trichoderma* strains were calculated using the following formula (Ibarra-Medina et al., 2010).

$$IRM = \frac{\mathrm{GAnS}}{\mathrm{D}} \times 100$$

Where IRM was the percentage of colonization, GAnS was the rate of growth of *Trichoderma* strains in the given treatment (mm) and D was the diameter of the Petri dish (mm).

Greenhouse experiment

Preparation of plant material, treatments, and experiment conditions

Corms were planted twice in early September 2019 and then in May 2020. Uniform corms of *Freesia* (*Freesia armstrongii*) with a diameter of 25-30 mm were planted in two-liter pots containing the same proportion of perlite, cocopeat, peat moss, and soil, with the corms planted at a depth of 5 cm. The experiment was conducted in a randomized complete block design with treatments including the control (without receiving any *Trichoderma* or *Fusarium*, C), *F. oxysporum* (Fo), *T. harzianum* (T1), *T. atroviride* (T2), a mixture of T. harzianum and *T. atroviride* (T1T2), a mixture of *F. oxysporum* and *T. harzianum* T1 (T1Fo), a mixture of *F. oxysporum* and *T. atroviride* T2 (T2Fo), and a mixture of *T. harzianum* T1, *T. atroviride* T2, and *F. oxysporum* (T1T2Fo). To apply treatments with *Trichoderma* or *Fusarium*, 90 mL of the respective suspensions were added to the irrigated pots at the two-leaf growth stage of *Freesia*. The greenhouse temperature during cultivation was maintained around 18-22 ± 3 °C.

Morphological evaluation

Vegetative traits, including plant height, number of leaves, leaf width, leaf surface, plant fresh weight, and plant dry weight, were measured via routine procedures. The diameter of corm, diameter of cormels, number of cormels, weight of corm, weight of cormels, dry weight of corm, and dry weight of cormels were measured to check corm growth. The flower stem height, number of lateral branches, number of flowers per stem, flower diameter, flower fresh weight, and flower dry weight were investigated as reproductive traits. Also, the time between the

germination stage and the flower bud growth stage was measured as the flower bud emergence time. The number of days from the germination stage to the flowering stage was considered the flowering time. The end of *Freesia* flower life was recorded as flower durability based on the time of observing the last flower in each cluster.

Biochemical investigation

Chlorophyll and carotenoids were measured by the method of Wright and Wickard (1998). Fifty mg of fresh leaves were ground in 80% acetone and then filtered and brought to a volume of 5 mL. The absorption value was read using a spectrophotometer at A645, A663, and A470 nm, to measure chlorophyll (a, b, c, and total) and carotenoids.
To measure carbohydrates, leaves (50 mg) were treated with 1 N HCl for 24 h. The filtered solution was mixed with 5% phenol and sulfuric acid, and heated at 30 °C for 30 min. Spectrophotometry at 488 nm was used for calculating glucose concentration in micrograms per gram of fresh weight (Kerepesi & Galiba, 2000).
Proline content was measured in fresh *Freesia* tissue using sulfosalicylic acid 3%, ninhydrin, and glacial acetic acid according to Bates et al. (1973) method with some modifications. The absorbance was read by a spectrophotometer at a wavelength of 520 nm, and proline values were calculated.
The level of lipid peroxidation in *Freesia* was quantified as malondialdehyde (MDA) concentration according to Li (2000), using the TBARS (thiobarbituric acid reactive substances) technique. A spectrophotometer was applied to measure the quantity of MDA at absorbance wavelengths of 600 (non-specific) and 532 nm (specific).

Antioxidant enzymes activity (APX, PAL, and SOD)

Peroxidase enzyme activity was measured according to Polle et al. (1997). Guaiacol absorption at 470 nm was monitored in a reaction mixture with enzyme extract, potassium phosphate buffer, EDTA, guaiacol, and hydrogen peroxide.
Phenylalanine ammonia lyase (PAL) was extracted from leaf tissue (Beaudoin-Eagan and Thorpe, 1985) using Tris-HCI buffer and 2-mercaptoethanol. PAL was purified through Biogel P2 column, NH_4KSO_4 fractionation, and chromatography on Biogel CM-100 and Sephadex G-25/G-200. Protein content was determined by the Coomassie blue method. PAL enzymatic assay measured by trans-cinnamic acid and p-coumarate formation at 290 nm.
Superoxide dismutase (SOD) enzyme activity was measured based on the inhibition effect of this enzyme by the photoreduction of nitroblue tetrazolium NBT, followed by spectrophotometric readings at 560 nm, according to the method (Giannopolitis and Ries, 1977).

Statistical analysis

Data were statistically analyzed using SAS (version 9.4) software using a randomized complete block design (RCBD), and mean values were compared using Duncan's multiple range test.

Results

Identifying the Fusarium species and determining the percentage of its isolates

Sampling was on *Freesia* corms exhibiting signs of rot or dryness from the Tehran region, and 15 pure isolates of *Fusarium* were obtained using the single hyphal tip method (Nelson et al., 1983). Following the initial identification of disease-causing isolates based on macroscopic and microscopic characteristics, about 47% of the isolates were *Fusarium oxysporum*, 27% were *Fusarium proliferatum*, and 26% were *Fusarium fujikuroi*, all demonstrating pathogenetic properties on *Freesia* corm.

Evaluation of pathogenicity of Fusarium isolates

To assess the pathogenicity of the isolates, a scale ranging from 0 to 5 was used, as described by Naseri and Marefat (2011). Zero indicated no wilting, while the severity of wilting ranged from weak (1-10%) to moderate (11-25%), moderate to severe (26-50%), severe (51-75%), and very severe to complete plant death (76-100%).

Pathogenicity experiment and selection of superior Fusarium isolates for antagonistic experiment

Based on the preliminary test, isolates F1-1, F1-5, F1-3, and F1-4 of *Fusarium oxysporum* species have shown strong pathogenicity on *Freesia* plants. However, the isolates F3-1, F3-2, and F3-3 of *F. oxysporum* had severe pathogenicity on *Freesia* plants. After that, isolates F5-1, F5-2, and F5-3 of *Fusarium proliferatum* had moderate to severe pathogenicity. Moderate pathogenicity appeared on isolates F2-1 and F5-4 of *Fusarium fujikuroi* and *F. proliferatum*, respectively. F2-2, F4-1, and F4-2 isolates of *F. fujikuroi* species showed the lowest signs of pathogenicity on *Freesia* plants. For this reason, to conduct a

pathogenicity test on the *Freesia* plant at the greenhouse, the F1-3 isolate was selected as the severe pathogenic isolate. The results (Table 1) showed the average percentage of *Freesia* plants infected with different isolates of *Fusarium spp.* in the pathogenicity test.

Table 1. Mean percentages of infected plants of *Freesia armstrongii* by different isolates of *Fusarium* spp. in pathogenicity tests.

Isolate*	Species	Infected plant (%)
F1-1	*F. oxysporum*	**70.00**
F3-1	*F. oxysporum*	**60.33**
F3-2	*F. oxysporum*	**66.67**
F3-3	*F. oxysporum*	**66.67**
F1-5	*F. oxysporum*	**73.33**
F1-3	*F. oxysporum*	**100.00**
F1-4	*F. oxysporum*	**86.67**
F2-1	*F. fujikuroi*	**31.33**
F2-2	*F. fujikuroi*	**26.67**
F4-1	*F. fujikuroi*	**26.67**
F4-2	*F. fujikuroi*	**26.67**
F5-1	*F. proliferatum*	**46.67**
F5-2	*F. proliferatum*	**43.33**
F5-3	*F. proliferatum*	**40.00**
F5-4	*F. proliferatum*	**33.33**

*Codes for different isolates of *Fusarium* spp. indicated in the second column of the table.

Simultaneous dual culture test

Comparison of mean values regarding average growth of the pathogenic isolate and inhibition percentage of the *Trichoderma* growth after 96 h of simultaneous culture showed that the lowest average growth of the *Fusarium* colony was against *Trichoderma atroviride* (T2) and *T. harzianum* (T1), so that these two strains were significantly the most successful isolates in competition with *F. oxysporum*, which had the highest percentage of inhibition. The lowest inhibition percentage was related to the *T. virens* strain (Table 2).

Table 2. Colony growth inhibition percentage of *F. oxysporum* by different *Trichoderma* strains after 24, 48, 72, and 96 h of simultaneous dual culture.

Trichoderma* strain	**Inhibition percentage on simultaneous dual cultures**			
	24	**48**	**72**	**96**
T1	44.21[h]	66.22[b]	69.68[a]	70.02[a]
T20	28.42[j]	48.78[g]	58.27[de]	62.31[c]
T2	53.19[f]	62.82[c]	65.33[b]	68.98[a]
Tvi	40.57[i]	57.15[e]	57.21[e]	60.70[cd]
Th4	51.84[f]	61.76[c]	62.50[c]	64.91[b]

*T1, T20, and Th4 strains of *Trichoderma harzianum*, T2 strain of *Trichoderma artoviride*, Tvi strain of *Trichoderma virens*.

Colonization ability of strains of Trichoderma spp. against Fusarium oxysporum

The colonization ability of *Trichoderma* strains was assessed by measuring the diameter of *Trichoderma* growth after 24, 48, 72, and 96 h of dual culture. On the fourth day after dual culture, T. atroviride (T2) and *T. harzianum* (T1) strains had the highest colonization of *F. oxysporum*, so that they covered the petri dish in a shorter time after dual culture, indicating a higher colonization ability compared to the other strains. *T. virens* (Tvi) and *T. harzianum* (T20) strains on the fourth day had a lower colonization ratio compared to the previous strains, which had completely colonized the *Fusarium* species on the fourth day (Table 3).

Table 3. Average percentage of the colonization ability of *Trichoderma* spp. against *Fusarium oxysporum* after 3, 4, 5, and 6 days of culture.

Trichoderma strain*	Inhibition growth on simultaneous dual cultures			
	3	4	5	6
T1	66.91^{g}	78.53^{c}	81.67^{a}	84.53^{a}
T20	45.52^{k}	60.98^{h}	72.85^{ef}	77.89^{c}
T2	55.27^{i}	77.77^{b}	80.10^{b}	82.72^{a}
Tvi	50.72^{j}	71.44^{f}	74.51^{de}	75.88^{cd}
Th4	64.81^{g}	73.20^{c}	78.13^{c}	80.14^{b}

*T1, T20, and Th4 strains of *Trichoderma harzianum*, T2 strain of *Trichoderma atroviride*, and Tvi strain of *Trichoderma virens*.

Effect of Trichoderma on vegetative growth indicators of Freesia plant

In the presence of two strains of *Trichoderma*, separately T1 and T2, and simultaneously T1T2, a significant increase was observed in *Freesia* growth indicators such as plant height, leaf number, leaf width, leaf surface, and fresh and dry weight compared to the control (Table 4). In addition, in the treatments where the wilt-causing fungus was utilized along with *Trichoderma* (T1Fo and T2Fo), *Freesia* plants exhibited higher values for plant height, number of leaves, leaf width, leaf area, fresh weight, and dry weight compared to *Fusarium* wilt fungus (Fo) and the control treatments.

Table 4. Effects of different *Trichoderma* strains on vegetative growth indicators of *Freesia*.

Treatment*	plant height (cm)	Number of leaves	Leaf width (mm)	Total leaf area (cm^2)	Leaf fresh weight (g)	Leaf dry weight (g)
C	31.83 ± 0.64^{d}	7.42 ± 0.36^{b-d}	13.38 ± 0.27^{d}	97.99 ± 7.73^{e}	57.98 ± 0.06^{g}	7.70 ± 0.28^{e}
Fo	27.00 ± 0.14^{e}	5.47 ± 0.14^{e}	11.04 ± 0.75^{f}	49.75 ± 3.74^{f}	20.15 ± 0.44^{h}	3.29 ± 0.26^{g}
T1	73.27 ± 0.62^{b}	9.17 ± 0.62^{b}	16.07 ± 0.17^{b}	143.34 ± 4.11^{c}	113.70 ± 1.29^{b}	13.62 ± 0.59^{c}
T2	86.67 ± 1.30^{a}	10.67 ± 0.94^{a}	16.71 ± 0.14^{a}	198.30 ± 5.82^{a}	122.45 ± 2.61^{a}	21.13 ± 1.33^{a}
T1T2	68.37 ± 0.31^{c}	9.00 ± 1.01^{b}	15.10 ± 0.48^{c}	158.84 ± 4.94^{b}	104.32 ± 0.78^{c}	15.33 ± 0.45^{b}
T1Fo	64.33 ± 0.41^{c}	8.00 ± 0.41^{b}	12.07 ± 0.15^{e}	103.80 ± 4.36^{e}	83.08 ± 1.13^{e}	10.20 ± 0.18^{d}
T2Fo	73.93 ± 0.92^{b}	8.33 ± 0.62^{b}	14.56 ± 0.47^{c}	130.33 ± 5.28^{d}	94.34 ± 2.56^{d}	12.47± 0.34^{c}
T1T2Fo	67.97 ± 0.25^{c}	7.75 ± 0.21^{bc}	13.40 ± 0.77^{d}	102.79 ± 3.36^{e}	78.02 ± 1.04^{f}	6.15 ± 0.03^{f}

**Trichoderma* or *Fusarium*, (C as control), *F. oxysporum* (FO), *T. harzianum* (T1), *T. atroviride* (T2), mixture of *T. harzianum* and *T. atroviride* (T1T2), mixture of *F. oxysporum* and *T. harzianum* T1 (T1FO), mixture of *F. oxysporum* and *T. atroviride* T2 (T2FO), and mixture of *T. harzianum* T1, *T. atroviride* T2, and *F. oxysporum* (T1T2FO).

Effect of Trichoderma strains on reproductive indicators of Freesia

The results showed that in plants treated with *Trichoderma* strains (T1 and T2) separately and in combination with each other (T1T2), flower bud formation and flowering occurred significantly earlier compared to the control plants (Table 5). This acceleration in flower bud formation and flowering in plants treated with *Trichoderma* strains (T1 and T2) separately and in combination with each other (T1T2) occurred in comparison with treatment groups exposed to *Fusarium* wilt (T1Fo and T2Fo). This process happened faster in T1T2Fo than in T1Fo and T2Fo (Table 5). When applied separately, *Trichoderma* (T2) caused a significant increase in indicators such as the number of flowers on the main cluster, the diameter of the first flower, and fresh weight and dry weight of *Freesia* flower stems compared to the control (Table 5). In addition, in treatments suspended with *Fusarium* fungus in the presence of *Trichoderma* control agent (T2Fo), the number of flowers, diameter of the first flower, fresh weight, and dry weight of *Freesia* plants were higher than in the treatment infected with *Fusarium* fungus alone (Table 5, Fig. 1A and B). However, the effect of *Trichoderma* T1 treatment on the number of lateral branches, the diameter of the first flower, and the dry weight of the *Freesia* flower stem resulted in a significant increase compared to the control and other treatments. In addition, in treatments where *Fusarium* disease fungus was suspended in the presence of *Trichoderma* control agent (T2Fo), higher values were observed in the number of flowers on the main cluster, the diameter of the first flower, and fresh and dry weights of *Freesia* flower stems than in the infection treatment group with only *Fusarium* disease fungus and the control (Table 5, Fig. 1A-G).

Trichoderma alone (T2) resulted in a significant increase in the *Freesia* flower branch height compared to the control. In addition, in the treatment where *Fusarium* fungus was suspended in the presence of the *Trichoderma* control agent (T2Fo), the height of the *Freesia* flower branch was higher than in the control treatment with *Fusarium* fungus alone (Fig. 2).

The effect of *Trichoderma* (T2) treatment on the longevity of *Freesia* flower was significantly increased compared to the control and other treatments. In addition, the *Trichoderma* (T1) treatment and combined (T1T2) treatment ranked second in the flower durability compared to other treatments. In addition, in treatments where *Fusarium* disease fungus was suspended in the presence of the *Trichoderma* fungus control agent (T2Fo), the longevity of *Freesia* flower was higher than in the treatment with only *Fusarium* fungus (Fo) and the control treatment (Fig. 3).

Table 5. Effect of *Trichoderma* strains on reproductive indicators of *Freesia*.

Treatment	Time of flower budding (days)	Flowering (day)	Number of lateral stems	Number of florets on main spike	Diameter of first flower (mm)	Fresh weight of flower stem (g)	Dry weight of flower stem (g)
C	172.33 ± 2.06^{c}	184.33 ± 1.63^{d}	1.00 ± ^{b}	9.00 ± 0.82^{ab}	35.63 ± 1.24^{e}	22.98 ± 0.25^{f}	2.35 ± 0.18^{d}
Fo	183.33 ± 1.25^{a}	204.33 ± 1.25^{a}	0.33 ± 0.47^{b}	3.67 ± 0.47^{c}	30.98 ± 0.47^{f}	16.19 ± 0.41^{g}	1.30 ± 0.02^{e}
T1	136.33 ± 2.49^{f}	163.33 ± 0.47^{f}	3.83 ± 0.47^{a}	10.00 ± 1.00^{a}	46.22 ± 0.36^{a}	36.22 ± 0.56^{b}	3.59 ± 0.12^{a}
T2	133.67 ± 1.25^{f}	164.00 ± 0.82^{f}	2.33 ± 0.47^{ab}	11.00 ± 0.47^{a}	47.29 ± 0.59^{a}	38.33 ± 1.00^{a}	3.72 ± 0.02^{a}
T1T2	141.33 ± 1.00^{e}	175.00 ± 0.82^{e}	3.33 ± 0.24^{a}	11.00 ± 0.82^{a}	43.65 ± 1.50^{b}	34.76 ± 0.73^{b}	3.30 ± 0.14^{b}
T1Fo	166.33 ± 1.25^{d}	191.00 ± 0.99^{c}	1.67 ± 0.47^{b}	8.33 ± 1.25^{ab}	39.96 ± 1.88^{d}	27.67 ± 0.58^{d}	3.06 ± 0.03^{b}
T2Fo	163.00 ± 2.16^{d}	186.67 ± 0.94^{d}	1.00 ± ^{b}	11.67 ± 0.82^{a}	41.23 ± 0.73^{c}	31.77 ± 1.88^{c}	3.14 ± 0.01^{b}
T1T2Fo	176.33 ± 2.07^{b}	201.67 ± 2.06^{b}	2.00 ± 0.82^{ab}	8.00 ± 0.82^{b}	38.93 ± 0.59^{d}	24.91 ± 0.06^{e}	2.69 ± 0.17^{c}

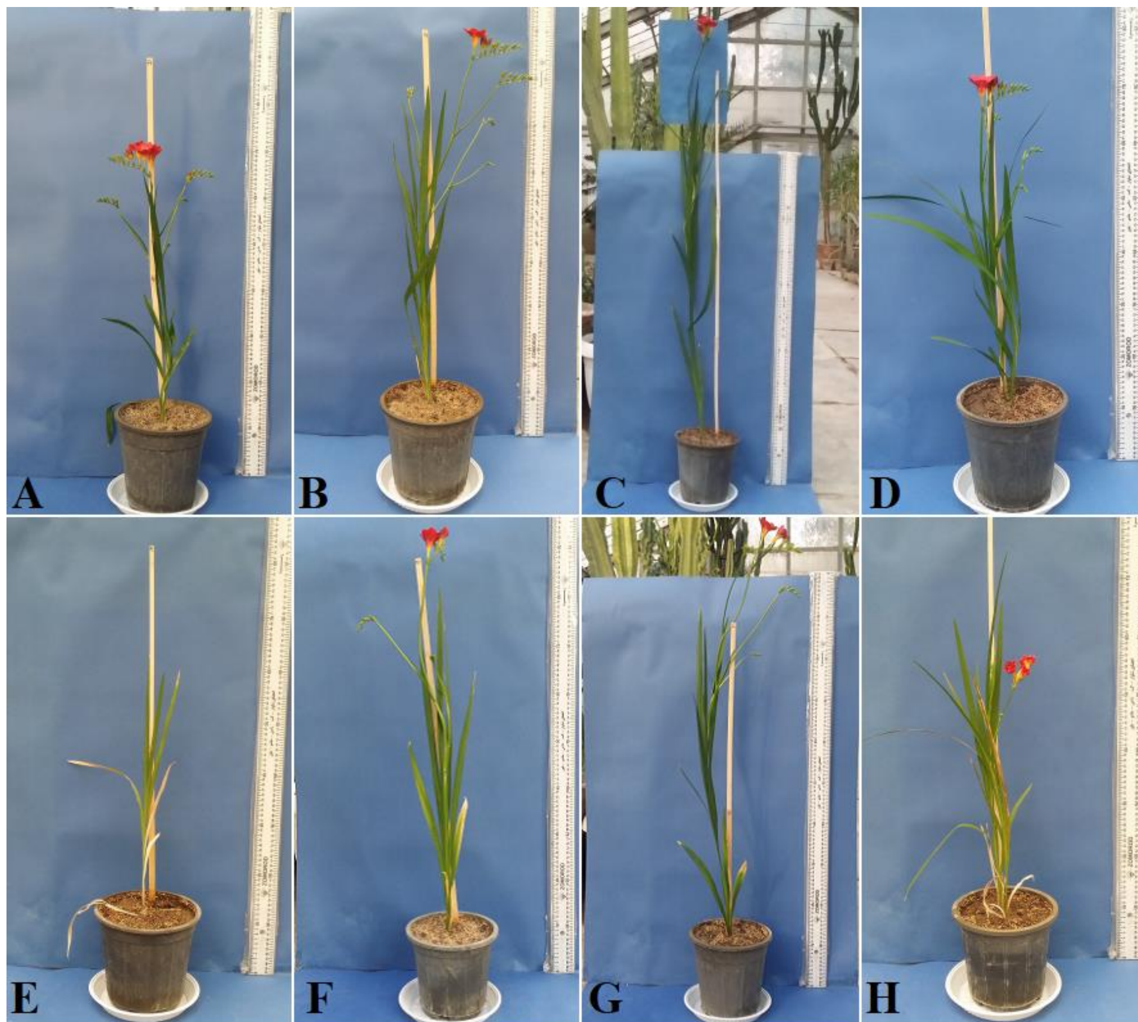

Fig. 1. *Freesia* plants at the flowering stage. (A) Control plant, (B) Plant treated with *T. harzianum* (T1), (C) Plant treated with *T. atroviride* (T2), (D) Plant treated with *T. harzianum* and *T. atroviride* (T1T2), (E) Plant treated with *F. oxysporum* (Fo), (F) Plant treated with *T. harzianum* and *F. oxysporum* (T1Fo), (G) Plant treated with *T. atroviride* and *F. oxysporum*, (H) Plant treated with *T. harzianum, T. atroviride*, and *F. oxysporum* (T1T2Fo).

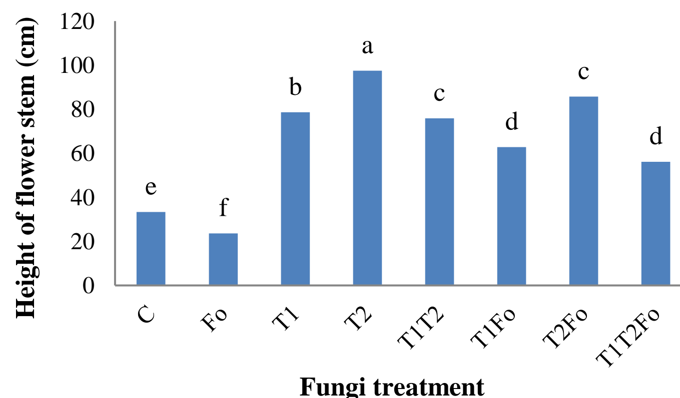

Fig. 2. Performance of *T. harzianum* (T1), *T. atroviride* (T2) separately and the combination with each other in the presence of the disease agent *F. oxysporum* (Fo) on the *Freesia* height flower stem. More clear explanation: *Trichoderma* or *Fusarium*, (C as control), *F. oxysporum* (FO), *T. harzianum* (T1), *T. atroviride* (T2), mixture of *T. harzianum* and *T. atroviride* (T1T2), mixture of *F. oxysporum* and *T. harzianum* T1 (T1FO), mixture of *F. oxysporum* and *T. atroviride* T2 (T2FO), and mixture of *T. harzianum* T1, *T. atroviride* T2, and *F. oxysporum* (T1T2FO).

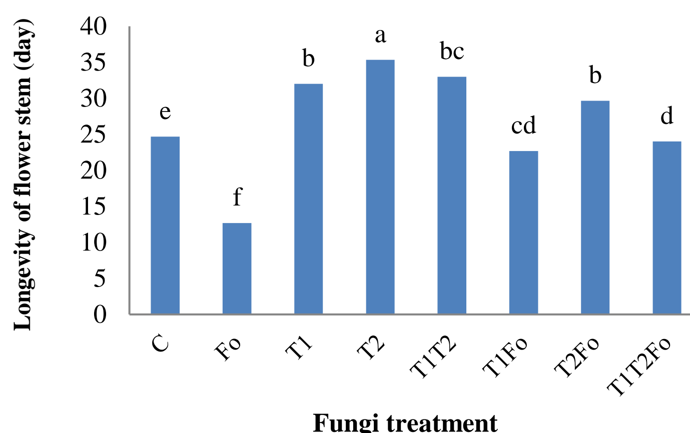

Fig. 3. Performance of *T. harzianum* (T1), *T. atroviride* (T2) separately and the combination of T1T2 in the presence of the disease agent (Fo) *F. oxysporum* on the longevity of *Freesia* flowers. More clear explanation: *Trichoderma* or *Fusarium*, (C as control), *F. oxysporum* (FO), *T. harzianum* (T1), *T. atroviride* (T2), mixture of *T. harzianum* and *T. atroviride* (T1T2), mixture of *F. oxysporum* and *T. harzianum* T1 (T1FO), mixture of *F. oxysporum* and *T. atroviride* T2 (T2FO), and mixture of *T. harzianum* T1, *T. atroviride* T2, and *F. oxysporum* (T1T2FO).

The effect of Trichoderma strains on bulb formation of Freesia

Table 6 showed that *Trichoderma* T1 treatment led to a significant increase in corm diameter, cormel diameter, fresh weight of corm, fresh weight of corm, dry weight of corm, and dry weight of cormels compared to the control treatment and other treatments. However, in treatments with *Fusarium* isolates in the presence of *Trichoderma* antagonist (T2Fo), there was a significant increase in corm diameter, cormels diameter, number of cormels, fresh weight of corm, and dry weight of corm than the treatment using separate infections by *Fusarium* isolates and control treatment. Additionally, the treatment of *Freesia* plants with *Trichoderma* T2 resulted in a significant increase in the number of cormels compared to the control treatment.

Table 6. Mean growth characteristics of *Freesia* corm.

Treatment	Diameter of corm (mm)	Diameter of cormels (mm)	Number of cormels	Fresh weight of corm (g)	Fresh weight of cormels (g)	Dry weight of corm (g)	Dry weight of cormels (g)
C	18.01 ± 0.02^{g}	10.64 ± 1.05^{cd}	2.33 ± 0.21^{a}	6.68 ± 047^{e}	1.12 ± 0.11^{bc}	0.66 ± 0.09^{e}	0.11 ± 0.01^{bc}
Fo	8.69 ± 1.95^{h}	3.23 ± 0.97^{e}	0.33 ± 0.47^{c}	3.93 ± 1.30^{f}	0.34 ± 0.02^{cd}	0.34 ± 0.05^{f}	0.03 ± ^{cd}
T1	54.15 ± 0.44^{a}	24.98 ± 0.46^{a}	2.67 ± ^{a}	18.05 ± 0.21^{a}	2.38 ± 0.13^{a}	1.80 ± 0.04^{a}	0.23 ± 0.01^{a}
T2	49.19 ± 0.67^{b}	17.07 ± 0.81^{b}	3.000 ± 0.47^{a}	16.40 ± 0.43^{b}	1.76 ± 0.26^{b}	1.62 ± 0.09^{b}	0.18 ± 0.02^{b}
T1T2	35.05 ± 1.31^{d}	16.18 ± 0.82^{b}	3.00 ± ^{a}	12.3 ± 0.91^{c}	1.70 ± 0.08^{b}	1.21 ± 0.04^{c}	0.17 ± 0.01^{b}
T1Fo	27.56 ± 0.19^{e}	14.13 ± 0.21^{b}	1.33 ± 0.47^{b}	9.19 ± 0.65^{d}	1.49 ± 0.06^{b}	· 0.94 ± 0.07^{d}	0.16 ± 0.01^{b}
T2Fo	38.08 ± 1.37^{c}	13.68 ± 0.82^{bc}	2.67 ± 0.47^{a}	11.68 ± 0.59^{c}	1.44 ± 0.84^{b}	1.17 ± 0.15^{c}	0.14 ± 0.01^{b}
T1T2Fo	25.51 ± 1.13^{f}	7.48 ± 1.04^{d}	1.33 ± 0.47^{b}	8.50 ± 1.05^{d}	0.79 ± 0.11^{c}	0.90 ± 0.11^{d}	0.86 ± 0.01^{c}

At the same time, the treatment of *Freesia* plants with *Fusarium* wilt fungus isolates in the presence of *Trichoderma* antagonist treatment (T2Fo) significantly increased the diameter of corm, number of cormels, fresh weight of corm, and dry weight of corm compared with *Fusarium* wilt and the control.

The treatment with the antagonist *Trichoderma* T2 significantly increased chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid compared to the control treatment. In addition, treatment with *Trichoderma* strains, both individually and in the presence of the *Fusarium* wilt (T2Fo), showed higher values of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid compared to the treatment with *Fusarium* wilt isolates alone and in the control treatment (Table 7).

Table 7. Mean values of chemical indices in *Freesia* plants.

Treatment	Chlorophyll a (mg g^{-1} fw)	Chlorophyll b (mg g^{-1} fw)	Total chlorophyll ab (mg g^{-1} fw)	Carotenoid (mg g^{-1} fw)	Leaf carbohydrate (mg g^{-1} fw)	Total proline (µg g^{-1} fw)	Peroxidase (µM g^{-1} fw)	PAL (µM g^{-1} fw)	MDA (µM g^{-1} fw)	SOD (µM g^{-1} fw)
C	4.99 ± 0.47^{d}	1.70 ± 0.47^{cd}	6.68 ± 0.58^{f}	2.70 ± 0.16^{e}	5.41 ± 0.09^{c}	3.20 ± 0.02^{f}	3.53 ± 0.03^{g}	4.61 ± 0.04^{g}	3.45 ± 0.19^{g}	4.49 ± 0.02^{f}
Fo	3.19 ± 0.61^{e}	1.36 ± 0.09^{d}	4.52 ± 0.27^{g}	1.86 ± 0.09^{f}	9.94 ± 1.16^{a}	2.37 ± 0.03^{g}	12.54 ± 0.03^{a}	3.67 ± 0.04^{h}	14.14 ± 0.36^{a}	3.41 ± 0.02^{g}
T1	8.59 ± 0.87^{ab}	3.16 ± 0.16^{b}	11.76 ± 0.60^{b}	4.66 ± 0.20^{b}	7.80 ± 0.04^{b}	7.24 ± 0.08^{e}	4.52 ± 0.02^{f}	5.59 ± 0.02^{e}	3.91 ± 0.04^{ef}	6.34 ± 0.09^{de}
T2	9.34 ± 0.35^{a}	3.83 ± 0.06^{a}	13.17 ± 0.40^{a}	5.38 ± 0.75^{a}	7.39 ± 0.03^{b}	7.39 ± 0.11^{d}	4.93 ± 0.09^{e}	6.82 ± 0.11^{d}	3.56 ± 0.18^{fg}	6.43 ± 0.10^{d}
T1T2	8.24 ± 0.71^{b}	2.40 ± 0.34^{c}	10.42 ± 0.59^{c}	3.76 ± 0.13^{c}	7.29 ± 0.27^{b}	7.30 ± 0.19^{de}	4.43 ± 0.02^{f}	5.34 ± 0.03^{f}	4.01 ± 0.03^{e}	5.96 ± 0.02^{e}
T1Fo	4.88 ± 0.03^{d}	2.09 ± 0.13^{c}	6.97 ± 0.22^{ef}	2.83 ± 0.06^{d}	8.32 ± 0.07^{b}	9.15 ± 0.11^{b}	5.63 ± 0.03^{c}	8.53 ± 0.11^{b}	7.37 ± 0.06^{c}	8.40 ± 0.10^{b}
T2Fo	5.11 ± 0.08^{d}	2.87 ± 0.09^{b}	7.90 ± 0.14^{e}	3.45 ± 0.06^{c}	8.12 ± 0.42^{b}	11.25 ± 0.05^{a}	6.75 ± 0.04^{b}	10.19 ± 0.05^{a}	5.94 ± 0.39^{d}	9.46 ± 0.04^{a}
T1T2Fo	6.84 ± 0.02^{c}	2.18 ± 0.02^{c}	9.23 ± 0.28^{d}	2.70 ± 0.11^{de}	8.58 ± 0.19^{ab}	8.24 ± 0.17^{c}	4.48 ± 0.19^{d}	7.92 ± 0.25^{b}	8.85 ± 0.03^{b}	7.90 ± 0.20^{c}

This research showed that plant treatment with *Trichoderma*, both individually and combined in the presence of *Fusarium* wilt fungus, significantly increased peroxidase, catalase, and superoxide dismutase activities compared to the treatment group of *Fusarium* wilt fungus alone.

Discussion

The results of this study showed that different *Trichoderma* treatments can inhibit the growth of *Fusarium* mycelium. Various strains of *Trichoderma* exhibited the capacity to prevent the growth and development of *Trichoderma* fungal hyphae compared to the growth and development of *Fusarium* pathogenic fungal hyphae, as determined through the dual culture test. These findings are consistent with the results reported by Langa-Lomba et al. (2022) and Sundaramoothy and Balabaskar (2013). The results of the dual culture test indicated that the growth rate of *Trichoderma* fungus Th (T1) and Ta6022 (T2) after 96 h is higher, leading to the prevention of pathogenic fungal hyphae growth. This outcome can be attributed to the direct relationship between the growth rate of *Trichoderma* fungus and the production rate of volatile compounds. The 96 h cultures of antagonistic *Trichoderma* strains showed a more inhibitory effect on the growth of pathogenic fungi than cultures 24 and 48 h. These findings confirm previous results reported by Mudawi and Idris (2014) and Sundaramoothy and Balabaskar (2013).

Results on *Trichoderma* treatments, both separately and simultaneously, and in the presence of the *Fusarium* wilt fungus isolate, revealed the dominance of the antagonist over the pathogenic isolate. Previous research demonstrated that *Trichoderma* species secrete secondary metabolites, influencing the inhibition of pathogenic microorganisms and stimulating plant growth (Carvalhais et al., 2015; Chaverri et al., 2015). In addition, studies such as Cia et al. (2013) and Dessbresses and Stougaard (2011) on the effect of antagonistic fungi on the interaction between the plants and *Trichoderma* species have shown that successful regulation of the root system results in increased length of the lateral and primary roots, leading to improved nutrient absorption by the plant.

The decrease in *Freesia* plant death by *Trichoderma* species was associated with increased plant growth. Meanwhile, the effect of *Trichoderma* antagonist fungi against *Fusarium* wilt of *Freesia* plants in greenhouse conditions indicated that *Trichoderma* reduces the occurrence of the disease and promotes plant growth. These results are consistent with other research (Inayati et al., 2021; Solis-Palacios et al., 2021).

One of the antagonistic mechanisms of *Trichoderma* in the soil environment is competition to occupy an ecological niche in the rhizosphere or in the root to obtain food resources. Therefore, some research has determined the ability of some strains of *Trichoderma* to strongly colonize the root as an important mechanism in the biocontrol process (Ali-Khan et al., 2020). *Trichoderma* from different species can quickly spread in the root system of plants, and this phenomenon varies among *Trichoderma* species, due to their different rates of growth (Abdul-Rauf et al., 2015; Nusaibah and Musa, 2018).

In this study, *Freesia* plant growth indices were influenced by *Trichoderma*, with the treatment related to *Trichoderma* T2 having the greatest effect on the growth of *Freesia* plant in the indices of plant height, number of leaves, Leaf width, leaf surface, and fresh weight and dry weight of *Freesia* plant. The production of ACC by *Trichoderma* inhibits the formation of ethylene in the plant, leading to an increase in root growth. Additionally, it activates the indole acetic acid (IAA) reaction in the plant by releasing nitrilase enzyme (Desbrosses and Stougaard, 2011; Nzanza et al., 2012). On the other hand, the presence of sucrose in the root of the plant enhances the growth of *Trichoderma* fungus (Nieto-Jacobo et al., 2017).

At the same time, the interaction effects of probiotic treatment in the presence of two strains of *Trichoderma* (T1 and T2) were reflected in the average flower growth indices of *Freesia* plants. These treatments reached the stage of floral bud initiation and flowering earlier. However, the treatment infected with *Fusarium* disease fungus alone needed the longest time to reach the flower bud formation and flowering compared to *Trichoderma* T1Fo and T2Fo treatments separately. the effectiveness of these treatments was evident in reducing the severity of *Fusarium* vascular wilt disease and the effects of antagonists on the growth of *Freesia* plants. In addition, *Trichoderma* T2, being the most effective, increased the flower growth indicators of *Freesia* plants, including the number of flowers on the main spike, the diameter of the first flower, the length of the main flower raceme, the fresh weight of the flower raceme, and the dry weight of the flower stem compared to the control treatment. This is attributed to the probiotic treatment of *Trichoderma atroviride*, which produces indole acetic acid (IAA) compounds with a stimulating effect on plant growth (Medina et al., 2013; Motozu, 2016). It has been demonstrated that several species of *Trichoderma* produce auxin phytohormones, especially indole-3-acetic acid (IAA), which is essential for most processes responsible for proper plant growth and development (Nieto-Jacobo et al., 2017). Furthermore, the gibberellic acid (GA) production by *Trichoderma* strains is positively correlated with the synthesis of the IAA and the plant regulator ACC-deaminase. Moreover, IAA plays a crucial role in determining the competition between fungal species coexisting in a given environment (Vinalea et al., 2012).

Antagonistic factors with different degrees were able to inhibit the growth of the disease-causing fungus. *Trichoderma* treatments, both individually and in combination with the disease-causing *Fusarium* isolate, especially T2Fo treatment, were more effective in reducing the severity of the disease and improving vegetative and reproductive growth. As mentioned, *Trecoderma* can increase auxin production. It has been reported that auxin increases the growth rate, leading to the conversion of ADP in the leaf to ATP, resulting in increased cell development and growth (Naeem and Aftab, 2021).

The increase of the mean growth indices of corm diameter, fresh weight, and dry weight in the treatment of *Trichoderma* separately along with the *Fusarium* disease (T2Fo) was attributed to the ability of antagonist T2 in reducing the severity of *Fusarium* disease and its positive effect on the growth of *Freesia* plants. This increased corm diameter, as well as fresh weight and dry weight of corm, compared to the treatment of disease alone.

In this research, the *Trichoderma* strain T2 increased the chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid compared to the other treatments. This increase contributed to the overall height of the plant and flower stem. Some strains of *Trichoderma* can increase plant biomass production and stimulate lateral root growth through auxin-dependent mechanisms, producing indole-3-acetic acid (IAA) or auxin analogs (Wilson et al., 2008).

The results regarding proline content and antioxidant enzyme activity showed that the treatment with *Trichoderma*, in the absence of pathogens, had a significant effect on these indexes.

However, the mentioned treatments, even in the presence of the pathogen, also caused a significant increase in the amount of proline content and activity of antioxidant enzymes. Accumulation of proline plays a very effective role in adapting to stress conditions, contributing to intracellular osmosis and its regulation, stabilizing the protein structure and cell membrane, scavenging radical oxygen species, cellular pH regulation, and revival of oxidation reactions. These processes are directly linked to plant defense systems against pathogens (Illescas et al., 2022; Alwhibi et al., 2017). As the results showed, the treatments that increased the proline content could improve the growth indicators and enzyme activity even in the presence of the disease.

The results showed that *Trichoderma* treatment influenced lipid peroxidation, with the lowest levels recorded in T1 and T2 treatments. Given that lipid peroxidation is a marker for assessing resistance levels and changes in antioxidant enzymes, the use of T1 and T2 was effective in reducing lipid peroxidation. This efficacy is attributed to the important role of antioxidant system activity as well as the high content of proline. The results of this research align with previous studies (Medina et al., 2013; Alwhibi et al., 2017), that highlighted Trichoderma fungus as a suitable stimulus for inducing enzymes, including those involved in the synthesis of peroxidase. This enzyme plays a crucial role in constructing plant defenses, leading to the synthesis of compounds like hydrogen peroxide, phenylalanine ammonia lyase, and lignin in the plant cell wall. Additionally, it promotes the Cross-linking of hydroxyproline in the root cell wall, resulting in increased resistance of root tissues against pathogenic agents.

The results of the present research are consistent with previous findings (Wilson et al., 2008), demonstrating that the use of *Trichoderma* strains resulted in reduced production of small corms and increased fresh weight and dry weight of corm in the treatment of *Trichoderma* T1, Moreover, treatment with *Trichoderma* T2 caused an increase in the diameter of cormels, number of cormels, fresh weight and dry weight of cormels. Notably, a significant decrease in the intensity of *Fusarium* disease was observed under *Trichoderma* treatment separately in the presence of *Fusarium* disease agent compared to the treatment of *Fusarium* infection alone. This decrease in corm diameter, weight of corm, and number of corms is significantly related to the reduction in the number of production corms, consistent with findings from Houssine et al. (2010) and Papavizas (1985).

The use of *Trichoderma* contributes to disease control in the *Freesia* plant by activating the antioxidant system and increasing proteins related to defense against pathogens. The existence of antioxidant systems, especially defense enzymes like peroxidase, phenylalanine ammonia-lyase, ascorbate, and superoxide dismutase, is highlighted for their essential role in strengthening the cell wall and stimulating the plant's defense system. Additionally, these enzymes contribute to non-enzymatic pathways of salicylic acid and jasmonic acid, ultimately reducing peroxidation (Salas-Marina et al., 2011). This multifaceted mechanism is crucial for disease control, as suggested by the results of the current study, aligning with findings from other research (Malolepsza et al., 2017; Medina et al., 2013). Overall, the findings of the current research underscore that *Trichoderma* fungus serves as a favorable stimulus for inducing a series of enzymes, including those involved in peroxidase synthesis and plant defense mechanisms, and affirms the biocontrol ability of the studied *Trichoderma* strains, aligning. This fact highlights the potential of *Trichoderma* as a biocontrol agent, contributing to plant health and disease control.

Conclusion

The results of the present study indicated that *Trichoderma* strain *T. atroviride* plays an important role in increasing plant growth, including plant height, number of leaves, leaf surface, and fresh weight and dry weight of *Freesia* plants. It demonstrates biological control against *Fusarium* wilt fungus. *T. atroviride* strain improved reproductive growth and increased the performance of *Freesia* plants in flowering indices, accelerating between 10-13 days. This stimulation affects the growth of buds and flowers in *Freesia* plants, influencing their size, flower stem height, and flower longevity, ultimately contributing to the production of *Freesia* cut flowers. Overall, the best biological agent for increasing and improving *Freesia* production occurred from the *T. harzianum* strain (T1), suggesting the potential of *Trichoderma* as a biocontrol agent, contributing to *Freesia* health and disease control, thus producing more and better plants.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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