

Control Effect and Mechanism of *Trichoderma asperellum* TM11 against Blueberry Root Rot

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Abstract

Fusarium oxysporum is the primary pathogen of blueberry root rot; furthermore, we found that *Fusarium commune* can also cause root rot in blueberries. *Trichoderma* spp. is widely used to control plant diseases. We isolated *Trichoderma asperellum* (TM11) from blueberry rhizosphere soil to explore its control effect and mechanism on *F. oxysporum* and *F. commune*. We found that the inhibitory effects of TM11 volatiles and broth metabolites on *F. oxysporum* were significant, but only *F. commune* volatile metabolites had a significant inhibitory effect on its growth. Twelve known antimicrobial metabolites were detected from the methanol extract of TM11 fermentation broth by HPLC-MS. TM11 lysed and coiled around the hyphae of *F. oxysporum* and *F. commune*. The pot experiment showed that TM11 had significant control effects against *F. oxysporum* and *F. commune*, and inoculation of TM11 prior to that of *F. oxysporum* and *F. commune* was more effective. The TM11, TM11 and *F. oxysporum*, or *F. commune* and distilled water treatments had different effects on the activities of superoxide dismutase, peroxidase and catalase, and the enzyme activity levels exhibited the following order: TM11 > TM11 and *F. oxysporum* or *F. commune* > distilled water. The results showed that TM11 provided effective control of blueberry root rot.

Keywords: *Trichoderma asperellum*, blueberry root rot, biocontrol mechanisms, pot tests

Introduction

Blueberry is a small berry in the *Vaccinium* genus in *Ericaceae* and has among the most antioxidants of any fruit because its fresh fruits contain anthocyanins, superoxide dismutase, and other bioactive antioxidant substances (Faria et al. 2005; Neto 2007; Wang et al. 2015). Blueberry is native to North America, and the United States began wild blueberry selection and commercial blueberry cultivation in 1906 (Bañados 2006). Blueberry root rot is the main disease threatening blueberry cultivation and is generally caused by various fungi. Once infected, the whole blueberry plant will wither and die, seriously restricting the development of the blueberry economy. Most of the pathogens of blueberry root rot are members of the genus *Fusarium*, mainly *Fusarium oxysporum* (Ward 2013; Liu et al. 2014). Fei et al. (2018) reported that *Fusarium commune* could cause blueberry root rot. The conidia of *Fusarium* can be active in the soil for a long time, causing root rot to repeatedly or continuously damage healthy blueberry plants. Dithiocarbamate (mancozeb), inorganic (cop-

per and sulfur formulations), phthalimide (captan), and chloronitrile (chlorothalonil) chemical control of root rot is quick, but pathogens quickly develop resistance (Hahn 2014). Using biological control agents to control root rot is a promising future avenue of disease control (Papavizas and Lumsden 1980; Chet and Inbar 1994). *Trichoderma* spp. are important biocontrol microorganisms that can effectively control various plant diseases. Previous results showed that *Trichoderma harzianum* significantly controlled root rot of *Capsicum annuum* and faba bean (Sid Ahmed et al. 2003; Abdel-Kader et al. 2011). *Trichoderma koningiopsis* can effectively control boxwood blight (Kong and Hong 2017). *Trichoderma asperellum* has been reported to significantly control strawberry disease and rice sheath blight (De França et al. 2015). The biocontrol mechanisms of *Trichoderma* spp. mainly include competition, hyperparasitism, antibiosis, and induction of plant disease resistance (Benítez et al. 2004). Their hyphae can wrap, attach, and penetrate the hyphae of pathogenic fungi (Vinale et al. 2008). The metabolites and volatiles of *Trichoderma* can also effectively inhibit the growth of

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pathogens (Benítez et al. 2004; Meena et al. 2017). *Trichoderma* spp. excrete many antifungal enzymes, such as chitinases and β -glucanases, and chitinase is generally considered an important antifungal enzyme (Harman 2006; Loc et al. 2020). In addition, studies have shown that the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and polyphenol oxidase (PPO) in plants under disease stress increased after inoculation with *Trichoderma* (Behiry et al. 2023; Zhou et al. 2021). Numerous elicitors released by *Trichoderma* may induce different types of signals transmitted within the plant, producing enzymes involved in the suppression of pathogens and enhancing the biochemical and structural barriers in plant organisms, enhancing plant systemic resistance (Nawrocka and Małolepsza 2013). Bigirimana et al. (1997) found that *T. harzianum* can improve the systemic resistance of beans (*Phaseolus vulgaris*) to pathogenic bacteria. Bae et al. (2009) found that *Trichoderma hamatum* colonization of *Theobroma cacao* (cacao) induced an increase in alanine and γ aminobutyric acid concentration in cacao leaves and promoted seedling growth.

In this study, the *Trichoderma asperellum* (TM11) strain was isolated from blueberry rhizosphere soil and identified according to its morphological characteristics and sequencing of the ITS and SSU gene regions, which identified TM11 as *T. asperellum*. The inhibitory effect of the TM11 strain on the blueberry root rot pathogens *F. oxysporum* and *F. commune* was investigated. The biocontrol mechanism of strain TM11 was further analyzed by plate culture and microscopy observations. The control effect of TM11 on blueberry root rot was determined by pot experiments to clarify the biocontrol mechanism of TM11 against blueberry root rot and to evaluate its potential for biological control of blueberry root rot.

Experimental

Materials and Methods

Materials. Plant pathogens: Blueberry root rot (*F. oxysporum* and *F. commune*), blueberry branch blight (*Pestalotiopsis clavispora*), and blueberry brown leaf spot (*Alternaria alternata*) were obtained from the Forestry College, Guizhou University. Sorghum anthracnose (*Colletotrichum sublineola*), corn stalk rot (*Fusarium verticillioides*), pepper anthracnose (*Colletotrichum acutatum*), and wheat head blight (*Fusarium graminearum*) were obtained from the Northwest A&F University College of Plant Protection (China). These fungal species were stored in a refrigerator at -80°C at the Pathology Laboratory, Forestry College, Guizhou University (China).

Soil samples: Rhizosphere soil samples were obtained in April 2019 from organic blueberry fields ($107^{\circ}43'07''\text{E}$, $26^{\circ}25'49''\text{N}$) in Majiang County, Guizhou Province, China. Five sample plots ($5\text{ m} \times 5\text{ m}$) were set along a contour line near the blueberry root rot's incidence area. Eight healthy blueberry plants were randomly selected from each quadrat and dug up. Their roots were obtained, the excess soil particles attached to the roots were removed, the rhizosphere soil was retained, and rhizosphere soil samples were collected by shaking the roots. The soil samples were put into sterile bags, brought back to the laboratory in a portable low-temperature incubator, and stored in a refrigerator at 4°C to isolate potential biocontrol fungi.

Isolation, screening and identification of biocontrol fungi. Biocontrol fungi were isolated by the dilution-plate method (El Komy et al. 2015). Equal amounts of each of the soil from the five plots were mixed, and a 1 g sample was placed in a conical bottle. Then, 20 ml of sterile water was added, and the mixture was shaken for 1 min to disperse the soil into a soil suspension evenly. After standing for 10 min, 200 μl aliquots of the supernatant were spread-plated on the surface of rose bengal agar plates (5 g/l peptone, 20 g/l dextrose, 1 g/l dipotassium hydrogen phosphate, 0.5 g/l magnesium sulfate, 0.01 g/l rose bengal dye, 0.1 g/l chloramphenicol, and 15 g/l agar). Ten replicate plates were established. The plates were cultured at 28°C for 3–5 days in darkness, and a single colony hyphal tip of suspected *Trichoderma* was selected from the culture medium, transferred to PDA (potato broth from 200 g/l, 20 g/l dextrose, and 15 g/l agar), cultured at 28°C and purified 3–5 times. The PDA plate contained only a single colony with the same characteristics. The biocontrol fungi with antibacterial effects against *F. oxysporum* and *F. commune* were screened by the plate antagonism method.

Under sterile conditions, a 5-mm-diameter hyphal plug of biocontrol fungi cultured on PDA and the same-sized hyphal plug of *F. oxysporum* or *F. commune* were placed 2 cm away from the periphery of a Petri plate containing PDA (90 mm diameter) on opposite sides of the plate for both *F. oxysporum* and *F. commune*. Control plates consisted of a mycelial disc from the pathogen placed alone on PDA plates. The experiment included 3 replicates, and the plates were cultured at 28°C for 5 days in darkness. The inhibition of mycelial growth of the pathogen was then calculated as a percentage using the formula below (da Silva et al. 2016):

$$\text{Inhibition (\%)} = 100\% \times \left(1 - \frac{A}{B}\right)$$

where A and B are the colony diameters of plant pathogens in the control and dual culture plates, respectively. Then, the broad-spectrum antimicrobial activity and chitinase enzyme production capacity of the selected

biocontrol fungi were measured by the plate antagonism method and plate transparent circle method (0.01 g $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$, 0.7 g K_2HPO_4 , 0.3 g KH_2PO_4 , 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 20 g agar, 250 ml 1% colloidal chitin, 750 ml distilled water, pH 7.0–7.2).

Finally, a biocontrol fungus with broad-spectrum antimicrobial activity and chitinase enzyme production capacity was identified by morphological and molecular biological methods. The colony characteristics (colony color, colony shape, and edge characteristics) were observed and photographed with a camera. The morphology of hyphae and spores of the biocontrol fungus were observed using a microscope, and morphological identification was completed with reference to Nguyen et al. (2018). The DNA of biocontrol fungi was extracted from mycelium using a Fungal DNA Mini Kit (Soleibao Biotechnology Co., Ltd., China). The ITS region of the biocontrol fungal strain was amplified using the universal primers ITS (5'-TCCGTAGGTGAACCTGCGG-3', 5'-TCCTCCGCTTATTGATATGC-3') and SSU (5'-GTAGTCATATGCTTGTCTC-3', 5'-CTTC-CGTC AATTCCTTTAAG-3'), and polymerase chain reaction (PCR) was performed using the following cycle conditions: initial denaturation at 98°C for 3 min; followed by 36 cycles of denaturation at 98°C for 10 s, annealing at 56°C for 10 s, extension at 72°C for 10 s and a final extension for 5 min at 72°C; and storage at 12°C. The resulting 587 bp PCR products were assayed by 1.2% agarose gel electrophoresis. The PCR product of the biocontrol fungus was sent to Qingke Xingye Biotechnology Co. Ltd. (China) for sequencing in both the forward and reverse directions, and these were then used to produce a consensus sequence. The sequence of the obtained biocontrol fungus was analyzed by the BLAST program of the National Center for Biotechnology Information (NCBI; <http://www.ncbi.nlm.nih.gov>). The ITS and SSU sequences were compared using nucleotide BLAST with default settings and megablast (highly similar sequences) as the selected program.

Observation of *T. asperellum* TM11 hyperparasitism of blueberry root rot pathogens. The hyperparasitism effect of the TM11 strain on the hyphae of *F. commune* or *F. oxysporum* was determined by confrontation culture on a glass slide. First, the slide was soaked in absolute ethanol for 10 min, the ethanol was removed, and the slide was dried naturally. Then, the slide was placed in an empty Petri dish, and a thin layer of PDA was poured on the slide. After allowing to set, the TM11 and *F. oxysporum* or *F. commune* mycelia were 3 cm apart of the slide to form a two-point confrontation and cultured at 28°C for 3 days. Once the two strains intersected, they were observed and photographed under a light microscope (Haran et al. 1996).

Inhibitory effect of *T. asperellum* TM11 metabolites on blueberry root rot pathogens. The inhibition

of *F. oxysporum* and *F. commune* by volatile metabolites produced by strain TM11 was determined by sandwich plate. Under sterile conditions, strain TM11 and *F. oxysporum* and *F. commune* plugs of the same size (diameter = 5 mm) were obtained and inoculated in the center of two PDA plates. The *F. oxysporum* and *F. commune* inoculated plate were placed inversely over the TM11 plate to form a double dish set and cultured for 5 days at 28°C. Controls consisted of a PDA plate inoculated with *F. oxysporum* or *F. commune* and the uninoculated PDA. Three replicates were set up for each treatment, arranged in a completely randomized design, and cultured for 5 days at 28°C. The inhibition of mycelial growth of the pathogen was then calculated as a percentage using the formula previously described.

Inhibitory effects of the fermentation broth of strain TM11: A 5-mm-diameter hyphal plug of strain TM11 was inoculated into a 250 ml triangular bottle containing 100 ml potato dextrose broth (PDB; potato broth from 200 g/l and 20 g/l dextrose) and cultured for 7 days at 28°C and 160 r/min in the dark. The fermentation broth was filtered through four layers of sterile gauze, and a nylon water system 0.22 μm ($\varnothing$ 25 mm) disposable filter was used to remove the mycelium and spores. Then, the resulting filtrate was mixed with PDA (adding more agar to the PDA to ensure it was set) at a ratio of 1/2 to make an inhibition plate. Mycelial colonized agar plugs of *F. oxysporum* or *F. commune* with a diameter of 7 mm were cut and inoculated in the center of a TM11 broth-amended PDA plate. Control plates consisted of *F. oxysporum* or *F. commune* inoculated on unamended PDA plates. Three replicates were set up for each treatment, and the plates were arranged in a randomized design and incubated for 5 days in a constant temperature environment of 28°C. The colony diameter was measured, and inhibition was calculated (El Komy et al. 2015). The data were statistically analyzed using the equation previously described.

Determination of the antimicrobial metabolites in *T. asperellum* TM11 fermentation broth. A 5-mm-diameter hyphal plug of strain TM11 cultured on PDA was used to inoculate a 250 ml triangular bottle containing 100 ml PDB and cultured for 7 days at 28°C and 160 r/min. The resulting culture was then centrifuged at 12,000 $\times g$ and 4°C to obtain the supernatant, which was subjected to metabolite extraction with 50% methanol buffer. High-performance liquid chromatography-tandem mass spectrometry (HPLC-MS) was used to detect the fermentation extract metabolites and their relative concentrations. Detection conditions of HPLC-MS: TripleTOF[®] 5600 (SCIEX, UK); chromatographic analysis column (Acquity UPLC HSS T3, 100 mm $\times$ 2.1 mm, 1.8 μm); chromatographic separation conditions: the column temperature was 35°C; the mobile phase was solvent A (water, 0.1% formic acid) and solvent B

(acetonitrile, 0.1% formic acid); the flow rate was 0.4 ml/min; the sample injection volume was 4 µl; liquid elution gradient setting: 0–0.5 min, 5% B; 0.5–7 min, 5–100% B; 7–8 min, 100% B; 8–8.1 min, 100–5% B; 8.1–10 min, 5% B. Mass spectrometry conditions: ESI source, collected using ESI positive and negative modes, curtain gas (CUR) pressure was 30 psi, nebulizer gas (GS1) pressure was 60 psi, desolvation gas (GS2) pressure was 60 psi, and desolvation gas temperature was 650°C; in ESI positive mode, the atomization voltage (ISVF) was 5,000 V, and the declustering voltage was 100 V; in ESI negative mode, the atomization voltage (ISVF) was –4,500 V, and the declustering voltage was –100 V. Mass spectrometry data was collected in IDA mode; the TOF MS scanning range was 60–1,200 m/z ; metabolite precursor ions were selected, and after collision-induced dissociation, two-dimensional mass spectrometry analysis was performed to detect their fragment ions.

Control effect of *T. asperellum* TM11 against blueberry root rot pathogens in pot experiments. Control effect of TM11 on blueberry potted plants: *F. oxysporum* and *F. commune* and strain TM11 were inoculated into separate 250 ml triangular bottles containing 100 ml PDB and shake-cultured for 7 days at 28°C and 160 r/min. Four layers of sterile gauze were used for filtration to remove mycelia, and the resulting spore suspensions were diluted with distilled water to dilute the broth containing the spores with a concentration of 1×10^5 spores/ml. One-year-old healthy blueberry seedlings ('Legacy') were selected for testing. Four treatment groups were set up: 1) pathogen only control inoculated with 50 ml spore suspension of *F. oxysporum* or *F. commune* alone; 2) TM11 alone, 50 ml spore suspension of strain TM11; 3) TM11 prior to pathogen, 50 ml spore suspension of strain TM11 48 h prior to inoculation with 50 ml spore suspension of *F. oxysporum* or *F. commune*; 4) TM11 48 h after pathogen, 50 ml spore suspension of *F. oxysporum* or *F. commune* 48 h prior to inoculation with 50 ml spore suspension of TM11; 5) negative control, water. The growth and disease incidence based on leaf drop and root rot of blueberries were observed, the disease incidence and disease index were determined, and the incidence and relative control effects were calculated according to the following formulas (Elshahawy and El-Mohamedy 2019):

$$A (\%) = \left(\frac{B}{C} \right) \times 100\%$$

$$D = \left(\sum \frac{E \times F}{C \times G} \right) \times 100\%$$

$$H (\%) = \left(\frac{D1 - D2}{D1} \right) \times 100\%$$

where A is disease incidence; B is the total number of diseased plants in each treatment; C is the total num-

ber of replicate plants in each treatment; D is disease index; E is the number of diseased plants at all levels; F is the number of corresponding grades; G is the value of the highest disease level; H is a percentage disease control effect; D1 is the control disease index, and D2 is the treatment disease index. Classification criteria of the disease index, which is divided into six levels according to the degree of disease occurrence:

Grade 0: no disease;

Grade 1: diseased spots on the root of blueberry seedlings less than 1.0 cm in size, but the plants were healthy;

Grade 2: blueberry seedling root lesions of 1.0–2.0 cm, seedling leaves slightly withered, and lower leaves rarely shed;

Grade 3: root lesions of blueberry seedlings were more than 2.0 cm, and seedling leaves were withered or fell off;

Grade 4: blueberry seedling showed brown root rot, or the whole plant withered;

Grade 5: blueberry seedling was dead.

The pot experiment was carried out in a greenhouse. Several nutrient bowls with One-year-old blueberry seedlings that were healthy, disease-free, and consistent in growth were selected, and 30 cm diameter pots were potted in sterilized humus soil and inoculated by root injury irrigation. A sterilized scalpel was inserted into the soil near the plant, causing root wounds. Each blueberry seedling was infused with 50 ml of spore suspension or, in the control group, an equal amount of sterile distilled water. No fertilization was applied during the growth period, watering was performed every three days, and the plants were harvested 30 days after the last inoculation and management. Growth medium prepared from organic humus, crushed pine needles, leaves, high-quality sawdust, imported mix of coconut coir, and rice bran and fermented humus soil was purchased from Luyuan Meijia Agricultural Technology Co. Ltd. and autoclaved at 121°C for 2 h before use to remove microorganisms from the substrate.

The plants in the experiment were used to assess the effects of strain TM11 on defense enzyme activity. SOD, POD and CAT were determined by the nitrogen blue tetrazole (NBT) reduction method, visible spectrophotometry, and UV spectrophotometry (Sundar et al. 2004; Guo et al. 2020). Tissue samples were harvested from each of three replicate plants.

Statistical analysis. A phylogenetic tree was constructed using the neighbor-joining method with MEGA7 (Kumar et al. 2016). The data were statistically analyzed by one-way analysis of variance (ANOVA) using IBM® SPSS® Statistics 25. Comparisons among means were performed using Duncan's multiple-range tests ($p < 0.05$).

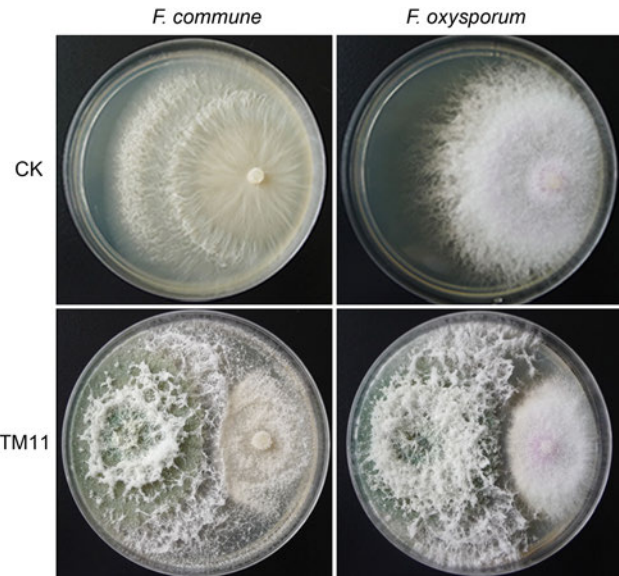


Fig. 1. Inhibitory activity of strain TM11 against *Fusarium commune* and *Fusarium oxysporum*. CK is *F. commune* and *F. oxysporum* alone; on the left side of each plate is the TM11 colony, and on the right side is the colony of *F. commune* or *F. oxysporum*.

Results

Isolation, screening, and identification of bio-control fungi. As shown in Fig. 1, ten strains with the same colony characteristics were isolated from the blueberry rhizosphere soil samples, and we found that the growth rate of TM11 mycelia was faster than that of *F. oxysporum* and *F. commune* mycelia. TM11 quickly

Table I
Percentage inhibition of the mycelial colonies of the blueberry root rot pathogens *Fusarium commune* and *Fusarium oxysporum* by TM11 compared with the untreated control (CK) assessed in dual culture plates after 5 days of incubation.

Strain	<i>Fusarium commune</i>		<i>Fusarium oxysporum</i>	
	Treatment colony radius (cm)	Percentage inhibition (%)	Treatment colony radius (cm)	Percentage inhibition (%)
CK	5.42 ± 0.08 ^a	n.d.	5.53 ± 0.05 ^a	n.d.
TM11	1.73 ± 0.09 ^b	68.01 ± 1.45	1.90 ± 0.06 ^b	65.65 ± 1.22

Data are presented as the mean ± SE. Data with different lowercase letters are significantly different at the 0.05 level.

colonized the PDA plate and sporulated on the surface of the *Fusarium* colony, covering the hyphae and surrounding the pathogen colony in different forms (Fig. 1). TM11 inhibited *F. oxysporum* and *F. commune* by 65.7% and 68.0%, respectively (Table I). In addition, we also found that TM11 is resistant to *P. clavispora*, *F. graminearum*, *C. sublineola*, *F. verticillioides*, *C. acutatum*, and *A. alternata* (Fig. S1), and produces chitinase (Fig. S2). So TM11 was selected for subsequent experiments. The morphology of TM11 inoculated into PDA, colonies on PDA, sporulation structure, and spore morphology were observed. TM11 grew rapidly and filled the dish within three days of incubation. The hyphae grew radially, the colony edges were neat, nearly round, white to green in color, and fluffy, and the aerial hyphae were cotton-wool-like (Fig. 2A and 2B). The conidiophores were produced in paired branches

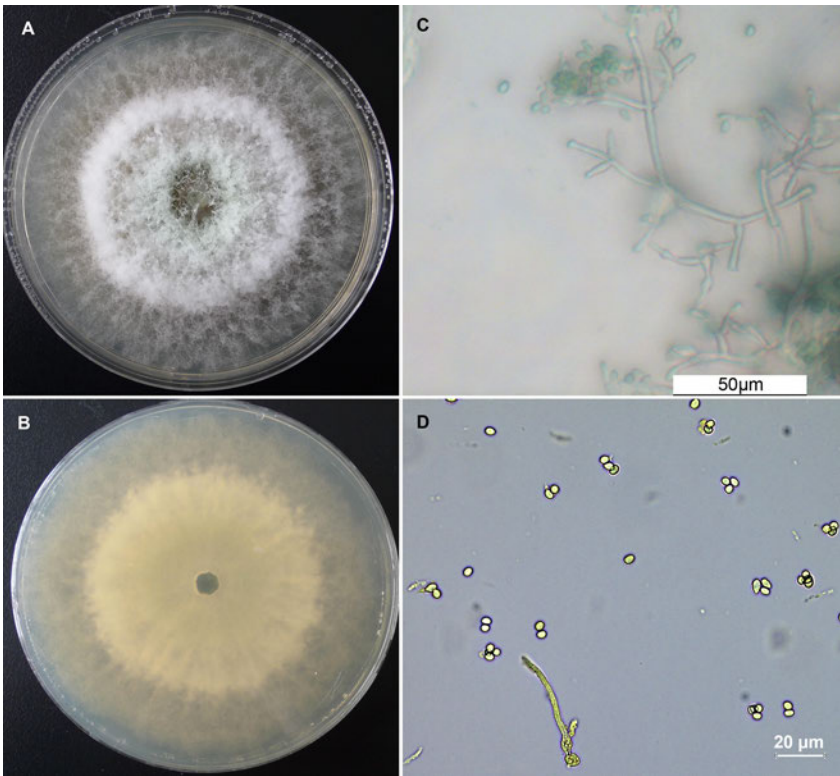


Fig. 2. Morphological characteristics of strain TM11. A–B) colony of TM11 surface and base, C) conidiophores, D) conidia (scale 20 microns).

on either side, often branched 2–3 times (Fig. 2C). The conidia were numerous and oval or round (Fig. 2D). The ITS and SSU sequences for TM11 obtained through BLAST revealed that TM11 was 99–100% identical to *T. asperellum* (GenBank accession No. MW766992 for ITS and OQ592847 for SSU). Neighbor-joining (NJ)

phylogenetic trees were constructed based on ITS and SSU sequences (Fig. 3). The results showed that TM11 and sequences of *T. asperellum* clustered in the same clade and had the closest relationship, and TM11 was identified as *T. asperellum* based on combined morphological and molecular identification.

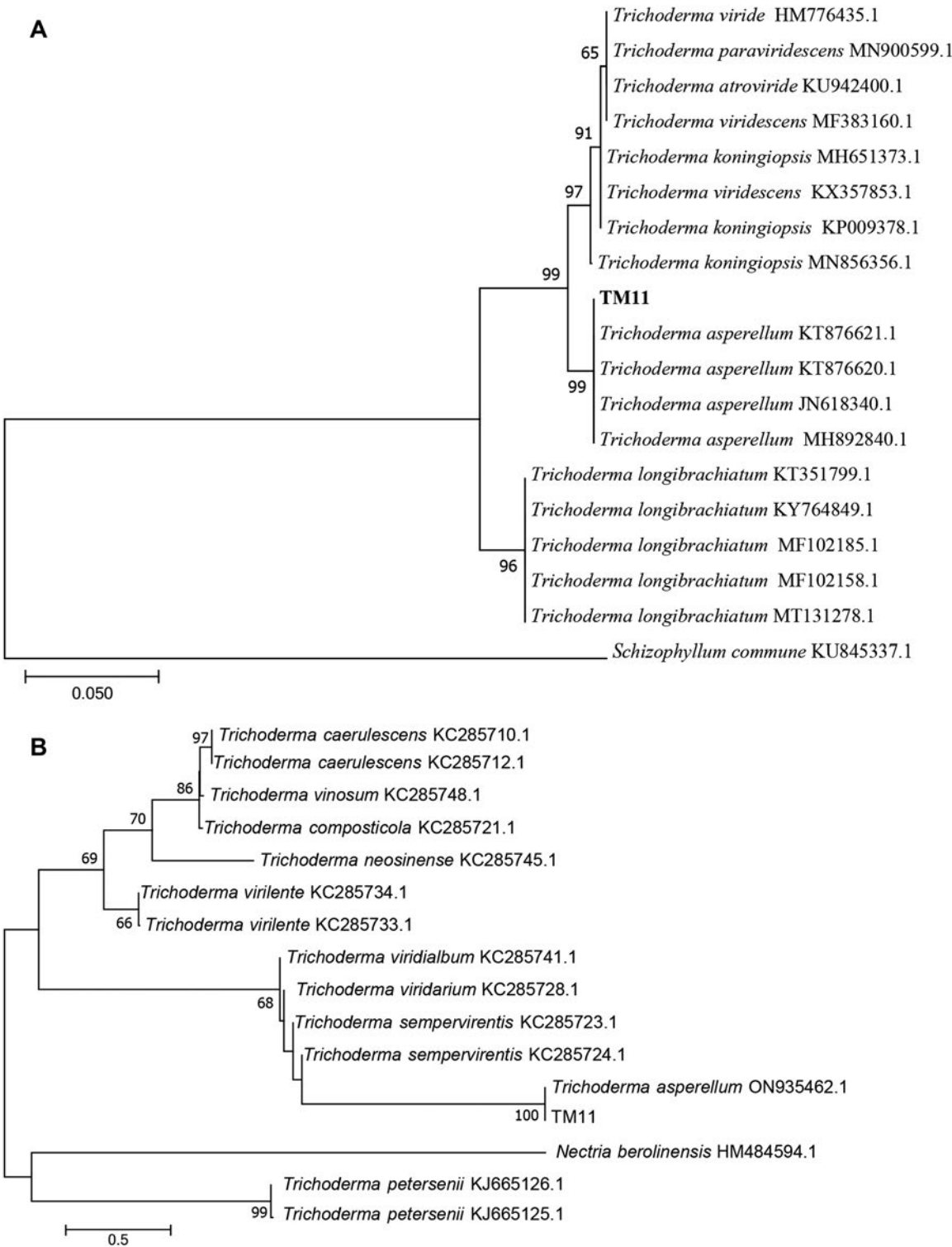


Fig. 3. Phylogenetic trees based on ITS (A) and SSU (B) sequences using the neighbor-joining method. The outgroup was *Schizophyllum commune* and *Nectria berolinensis*; numerical values above the branches are bootstrap percentiles from 1,000 replicates. Parsimony bootstrap values of more than 50% are shown at the nodes.

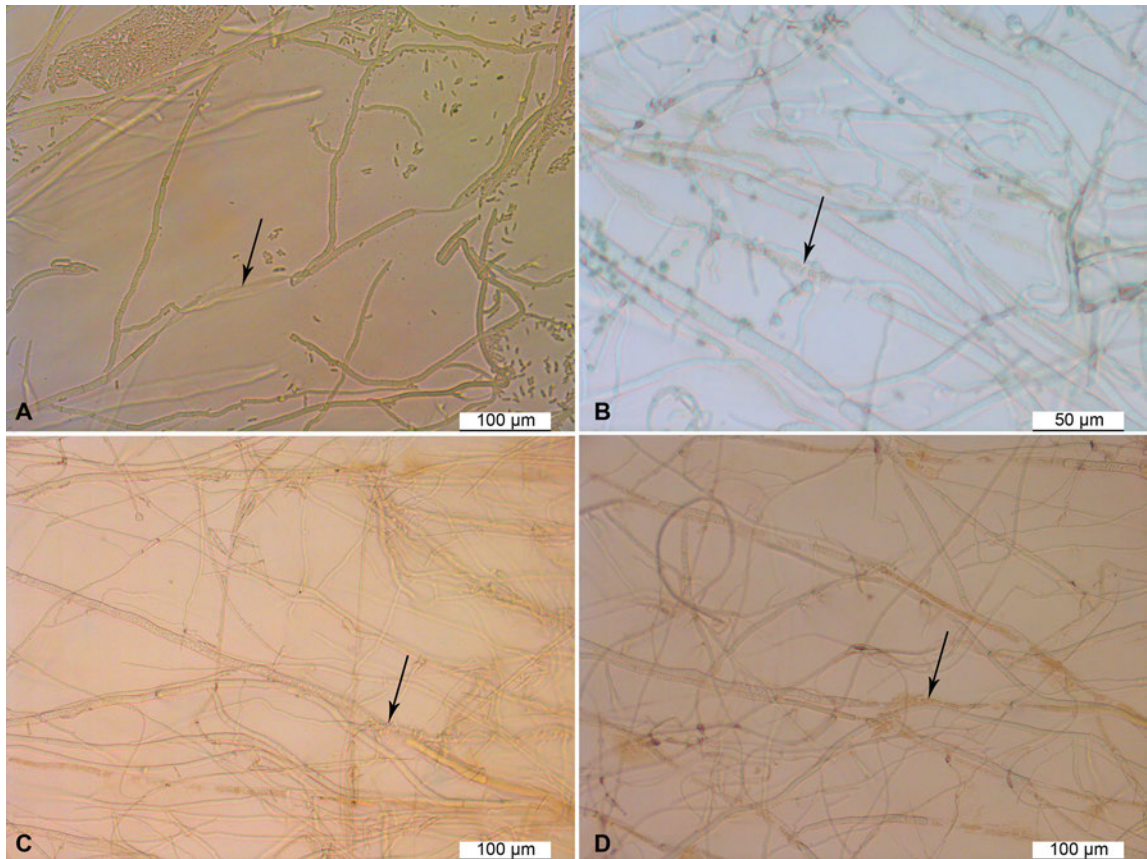


Fig. 4. Inhibition of *Fusarium oxysporum* and *Fusarium commune* hyphal growth by *Trichoderma asperellum* strain TM11 under a microscope.

A) Strain TM11 causes lysis of *F. oxysporum* hyphae (as shown by arrow), B) strain TM11 causes *F. oxysporum* lysis of the hyphae, and the cytoplasm has leaked out of that hyphal (as shown by arrow), C) strain TM11 to coil around the hyphae of *F. commune* (as shown by arrow), D) strain TM11 causes *F. commune* lysis of the hyphae, and the cytoplasm has leaked out of that hyphal (as shown by arrow).

Observation of *T. asperellum* TM11 hyperparasitism of blueberry root rot pathogens. As shown in Fig.4, after TM11 hyphae were in contact with *F. oxysporum* hyphae, the *F. oxysporum* hyphae showed obvious lysis (Fig. 4A and 4B), and *F. commune* hyphae were reduced and slowly disintegrated (Fig. 4C and 4D).

Inhibitory effect of *T. asperellum* TM11 metabolites on blueberry root rot pathogens. As shown in Fig. 5, both the volatile and fermentation metabolites

of TM11 had different inhibitory effects on the growth of both *F. oxysporum* and *F. commune*. For *F. oxysporum*, the percentage inhibition was the same between volatiles and broth metabolites. However, there were differences for *F. commune*: volatile metabolites had a significant inhibitory effect on colony growth. In contrast, the fermentation metabolites had no significant inhibitory effect on colony growth, as shown in Table II. The volatile metabolites of strain TM11 inhibited *F. commune*

Table II
Inhibitory effects of volatile and fermentation metabolites of *Trichoderma asperellum* strain TM11 on the growth of *Fusarium commune* and *Fusarium oxysporum*.

Treatment	<i>Fusarium commune</i>		<i>Fusarium oxysporum</i>	
	Diameter of pathogen colony (cm)	Percentage inhibition (%)	Diameter of pathogen colony (cm)	Percentage inhibition (%)
CK1	5.77 ± 0.05 ^a	n.d.	5.97 ± 0.05 ^a	n.d.
Volatile metabolites	3.83 ± 0.04 ^b	33.53 ± 0.72 ^a	3.77 ± 0.17 ^b	36.87 ± 2.74 ^a
CK2	7.00 ± 0.22 ^a	n.d.	8.87 ± 0.05 ^a	n.d.
Fermentation metabolites	5.83 ± 0.05 ^b	16.67 ± 2.81 ^b	5.60 ± 0.08 ^b	36.84 ± 0.98 ^a

Data are presented as the mean ± SE. Data with different lowercase letters are significantly different at the 0.05 level.

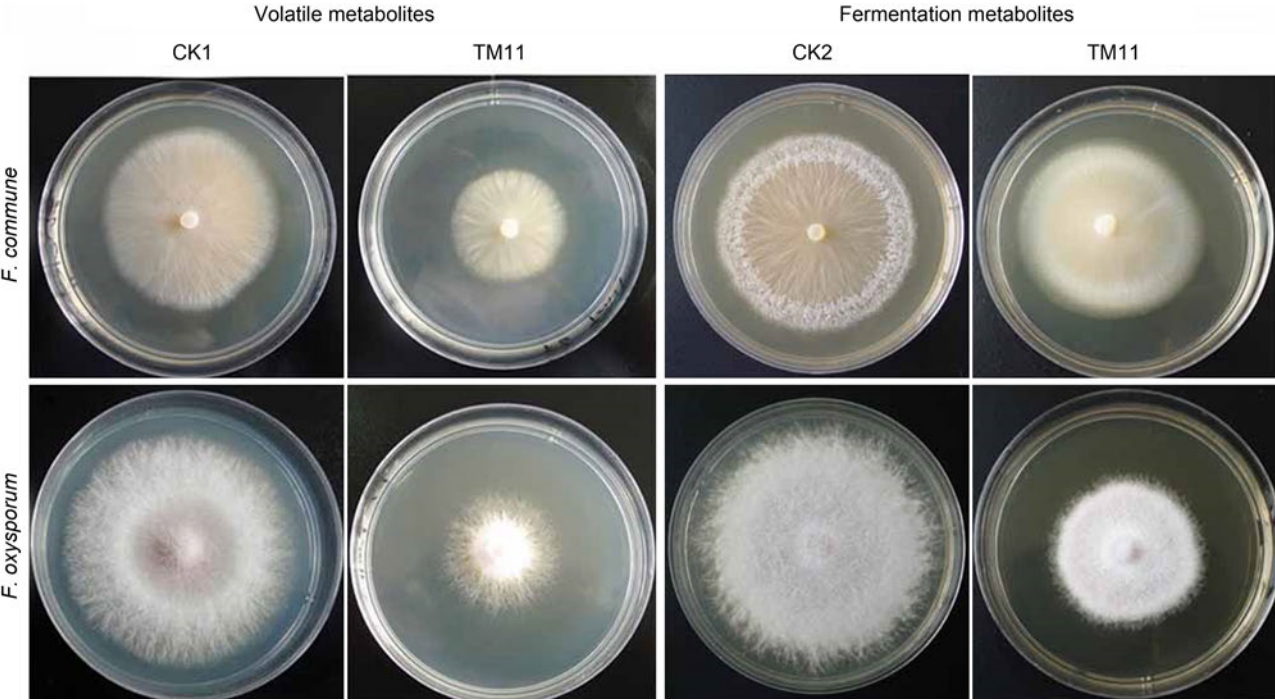


Fig. 5. Inhibitory effects of volatile metabolites and fermentation metabolites of *Trichoderma asperellum* strain TM11 on the growth of *Fusarium commune* and *Fusarium oxysporum*.
CK1 and CK2 represent the growth of *F. commune* and *F. oxysporum* on PDA; volatile metabolites mean volatile organic compounds (VOCs) and fermentation metabolites mean secondary metabolites, including alkaloids, ketones, esters, phenols and organic acids, such as erucamide, dibutyl phthalate and benzophenone.

and *F. oxysporum* by 33.5% and 36.9%, respectively; TM11 fermentation metabolites had the weakest inhibitory effect, with inhibition rates on *F. commune* and *F. oxysporum* of 16.7% and 36.8%, respectively (Table II).

Determination of the antimicrobial metabolites in *T. asperellum* TM11 fermentation broth. Compounds in methanol extracts of the fermentation broth of *T. asperellum* strain TM11 were analyzed by HPLC-MS. The results showed that under positive ion mode, the total ion current method was conducive to providing information about the compounds in the extract, so positive ion mode was used to analyze the antagonistic substances in the methanol extract (Fig. S3). Strain TM11 was shown to secrete a variety of antagonistic metabolites. The metabolite analysis results are shown in Table III. There were 12 chemical compounds identified, and their relative abundances (peak area) were as follows: erucamide>dibutyl phthalate>benzophenone>benzothiazole>citric acid>betainel>dipropyl phthalate > alpha-curcumene > phenylalanine > 3-hydroxycinnamic acid>dioctyl phthalate>chlorogenic acid > 4-hydroxybenzoic acid.

Control effect of *T. asperellum* TM11 on blueberry root rot pathogens in potted plants. As shown in Fig. 6, in the negative control, inoculation with TM11 and *F. commune* or *F. oxysporum* significantly reduced disease compared with inoculation with only *F. commune* or *F. oxysporum* (Table IV) (Fig. 6B, 6C, 6E and

6F). The effect of the TM11 inoculation sequence on disease caused by *F. commune* or *F. oxysporum* significantly differed, with strain TM11 significantly reducing blueberry root rot when inoculated 48 h before inoculation with *F. commune* or *F. oxysporum* (treatment 1) (Fig. 6C and 6F) compared with inoculation with the

Table III
Types and relative abundances of antimicrobial metabolites produced in culture filtrates by *Trichoderma asperellum* strain TM11 based on HPLC-MS analysis of methanol extracts of fermentation broth.

Chemical compound	Peak area	Retention time (min)	m/z
Erucamide	221862.78	7.22	338
Dibutyl phthalate	158594.53	6.73	279
Benzophenone	24466.55	6.00	183
Benzothiazole	49711.09	7.09	136
Citric acid	49252.02	1.11	193
Betainel	6165.18	0.89	118
Dipropyl phthalate	5286.64	6.07	251
alpha-Curcumene	3619.38	5.43	203
Phenylalanine	2535.44	2.52	166
3-Hydroxycinnamic acid	2500.47	2.90	165
Chlorogenic acid	1903.63	3.02	355
4-Hydroxybenzoic acid	517.80	3.16	139

Peak area indicates relative abundance.



Fig. 6. Effect of blueberry seedling growth inoculated with *Fusarium oxysporum* or *Fusarium commune*.

A) Control group: inoculated with *F. commune* alone, B) treatment 1: *F. commune* inoculated first, and TM11 inoculated 48 hours later, C) treatment 2: TM11 inoculated first, then *F. commune* inoculated 48 hours later, D) control group: inoculated with *F. oxysporum* alone, E) treatment 1: *F. oxysporum* inoculated first, and TM11 inoculated 48 hours later, F) treatment 2: TM11 inoculated first, then *F. oxysporum* inoculated 48 hours later.

pathogen 48 h before inoculation with TM11 (treatment 2) (Fig. 6B and 6E). As shown in Table IV, with the disease index in plants inoculated only with *F. commune* being 96.00, the disease index of treatment 1 was 6.67, which was 89.33 less than that of the pathogen control treatment, and the relative control effect was 93.05%. The control group disease index decreased by 68.00, and the relative control effect was 70.92%. The disease index in plants inoculated only with *F. oxysporum* was 97.33%. Treatment 1 inoculated with TM11 48 h before inoculation with *F. oxysporum* reduced the disease by 84.76%; treatment 2 inoculated with *F. oxysporum* first and inoculated with TM11 after 48 h reduced the disease by 62.46%. This indicated that TM11 had a strong inhibitory effect on blueberry root rot.

Effects of *T. asperellum* TM11 on the activity of antioxidant enzymes in blueberry seedlings. As shown in Table V, the CAT, SOD, and POD enzyme activities in the roots and leaves of blueberry seedlings inoculated

with TM11 were significantly higher than those in plants inoculated with the water-negative control, and the root enzyme activity was higher than the leaf enzyme activity. Compared with the negative control, the activities of CAT, POD, and SOD in the roots inoculated with TM11 were increased by 1.01 times, 1.41 times and 0.21 times, and the activities of CAT and POD in the leaves were increased by 0.85 times, 1.49 times, and 1.15 times, respectively. For leaves, TM11 inoculation and inoculation of TM11 and *F. commune* or *F. oxysporum* on CAT enzyme activity. The activities of SOD and POD were the highest after inoculation with TM11 alone, which were 447.0 U/g and 353.2 U/g, respectively. There was no significant difference between TM11 inoculation and mixed inoculation of TM11 and *F. commune* or *F. oxysporum* on SOD activity for roots. The enzyme activities of CAT and POD were significantly higher in plants inoculated with TM11 alone, which were 279.2 U/g and 363.6 U/g, respectively.

Table IV
Relative control effect of *Trichoderma asperellum* strain TM11 against *Fusarium commune* and *Fusarium oxysporum* disease in blueberry plants in a pot test after four weeks.

Treatment	<i>Fusarium commune</i>		<i>Fusarium oxysporum</i>	
	Disease index	Percentage disease (%)	Disease index	Percentage disease (%)
Control	96.00 ± 3.27 ^a	n.d.	97.33 ± 1.88 ^a	n.d.
Inoculation with TM11 first	6.67 ± 1.89 ^c	93.05 ± 1.99 ^a	14.67 ± 1.89 ^c	84.76 ± 1.57 ^a
Inoculation with pathogens first	28.00 ± 3.27 ^b	70.92 ± 2.41 ^b	36.00 ± 3.27 ^b	62.46 ± 1.28 ^b

Data are presented as the mean ± SE. Data with different lowercase letters are significantly different at the 0.05 level.

Table V
Effects of inoculation with *Trichoderma asperellum* strain TM11 and *Fusarium commune* or *Fusarium oxysporum* on SOD, POD, and CAT activities in leaf and root tissue of blueberry seedlings.

Treatment	Leaf			Root		
	CAT (U/g fresh weight)	SOD (U/g fresh weight)	POD (U/g fresh weight)	CAT (U/g fresh weight)	SOD (U/g fresh weight)	POD (U/g fresh weight)
CK	128.14 ± 5.86 ^b	208.01 ± 4.05 ^c	142.08 ± 4.05 ^d	140.96 ± 3.30 ^c	406.44 ± 2.02 ^b	150.86 ± 3.60 ^d
TM11	224.59 ± 12.19 ^a	446.95 ± 3.21 ^a	353.22 ± 3.37 ^a	279.22 ± 1.36 ^a	490.13 ± 1.77 ^a	363.61 ± 11.14 ^a
TM11 + <i>F. commune</i>	217.09 ± 7.41 ^a	352.75 ± 4.79 ^b	309.32 ± 6.20 ^b	228.79 ± 3.43 ^b	469.36 ± 4.60 ^a	313.66 ± 4.75 ^b
TM11 + <i>F. oxysporum</i>	213.37 ± 9.78 ^a	349.54 ± 4.29 ^b	205.23 ± 0.30 ^c	274.70 ± 1.65 ^a	490.39 ± 12.74 ^a	306.37 ± 9.38 ^b

Data are presented as the mean ± SE. Data with different lowercase letters are significantly different at the 0.05 level.

Discussion

In this study, we isolated and identified the fungus *T. asperellum* TM11 from healthy blueberry rhizosphere soil. The morphological characteristics and physiological metabolism of TM11 are consistent with those of other *T. asperellum* strains, but it is worth noting that the 12 known chemical compounds (Sahebani and Hadavi 2008; Chong et al. 2009; Dihazi et al. 2012; Herrera et al. 2015; Bhutia et al. 2016; Ahsan et al. 2017; Mu et al. 2017; Abdelhai et al.2019; Zhang et al. 2020; Al-Askar et al. 2021; Xie et al. 2021) in its fermentation fluid are different from those of other *T. asperellum* fermentation fluids (Wu et al. 2017). *Trichoderma* species have been shown to have good antagonistic activity and can control a variety of plant pathogens (de los Santos-Villalobos et al. 2013; Muniroh et al. 2019; Ruangwong et al. 2021). Our research results are consistent with those of other research; *T. asperellum* has an inhibitory effect on the growth of *F. oxysporum* and *F. commune* but also has a good inhibitory effect on the other six pathogen species. The effective antibiosis mechanism of *Trichoderma* species is reported to be related to the ability of *Trichoderma* species to produce volatile organic compounds (VOCs) and secondary metabolites (Takahashi et al. 2011; Arai et al. 2015; Ruangwong et al. 2021). In this study, we found that *T. asperellum* volatile metabolites significantly inhibited *F. commune* and *F. oxysporum*, with inhibition percentages of 33.5% and 36.9%, respectively. *T. asperellum* was shown to produce 13 secondary metabolites, with erucamide, dibutyl phthalate, benzothiazole, citric acid, betainel, dipropyl phthalate, and phenylalanine reported to have the most inhibitory activity against pathogens (Podile and Laxmi 1998; Bhutia et al. 2016; Bae et al. 2017; Nakkeeran et al. 2020). Moreover, secondary metabolites lead to the death of pathogens by inhibiting spore production and hyphal growth (Keswani et al. 2017). In the current study, the fermentation metabolites in the broth to be observed had less activity against *F. commune*, which

may be related to the incubation temperature, light conditions, and pH of the broth affecting the production of metabolites specifically inhibitory to *F. commune* (Mathivanan et al. 2008). Mukherjee and Raghu (1997) observed that *Trichoderma* fermentation metabolites from cultures incubated at temperatures above 30°C were ineffective in inhibiting pathogens. Furthermore, Mischke (1997) found that *Trichoderma* extracts produced under light and low pH showed increased inhibitory activity against pathogens.

In addition to the production of inhibitory metabolites, we found that *T. asperellum* strain TM11 had a significant parasitic effect on *F. oxysporum* and *F. commune*, coiling around the hyphae of the pathogenic fungi and causing lysis of the hyphae and the cytoplasm to leak out of the hypha. Hyperparasitism is one of the important biocontrol mechanisms by which *Trichoderma* inhibits pathogens. When *Trichoderma* and pathogens are subjected to confrontation assays, *Trichoderma* recognizes the pathogen hyphae through lectins and produces a large amount of chitinase and glucanase to dissolve the pathogen cell walls (Calonje et al. 2000). The biocontrol hyphae enter pathogen hyphae, parasitizing and simultaneously wrapping around and finally penetrating and causing the disintegration of the pathogen hyphae (Yi and Chi 2011). Similar to the results of the current study, Jiang et al. (2016) showed that *T. asperellum* could collapse the mycelium of pathogenic *Phytophthora capsici* colonies by lysis of the hyphae.

The results of the study indicated that CAT, SOD, and POD enzyme activities of blueberry plant tissue were induced by inoculation with *T. asperellum* strain TM11 or TM11 and *F. oxysporum* or *F. commune* and they were significantly higher than the water control. The TM11 enzyme activity was the highest after inoculation. Li et al. (2019) reported that CAT, SOD, and POD enzyme activities increased significantly in ‘Legacy’ inoculated with *T. asperellum* and *T. asperellum* and *F. oxysporum*. This is due to *Trichoderma* stimulating

systemic defense responses in plants grown by activating defense enzymes, including peroxidase, polyphenol oxidase, and chitinase (Elshahawy and El-Mohamedy 2019), combined with the inhibitory effect of *Trichoderma* on pathogenic fungi to enhance plant disease resistance (Saravanakumar et al. 2016). In this study, inoculation TM11 significantly reduced disease caused by inoculation with *F. commune* or *F. oxysporum*, but inoculation with *T. asperellum* strain TM11 prior to the pathogen was more effective at controlling disease than when inoculated after *F. commune* or *F. oxysporum*. It is probably because this gives *T. asperellum* strain TM11 time to establish and colonize the root region before pathogen inoculation, allowing it to preferentially occupy the limited niche and compete for nutrients, thereby reducing the space and nutrients available for the pathogens (He et al. 2020). However, further studies are needed to determine the colonization level of *Trichoderma* in the rhizosphere.

F. oxysporum is the main pathogen of blueberry root rot; furthermore, *F. commune* has also been reported to cause blueberry root rot (Li et al. 2023). Although *T. asperellum* has been reported for the biological control of plant root rot, there have been few reports on the biocontrol control of blueberry root rot. The only studies have been on the control of blueberry root rot caused by *F. oxysporum*, while there have been few reports on the control of blueberry root rot caused by *F. commune*. We found that *T. asperellum* TM11 has a good control effect on both pathogens (*F. commune* and *F. oxysporum*) and a high potential for preventing and controlling blueberry root rot.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Abdelhai MH, Awad FN, Yang Q, Mahunu GK, Godana EA, Zhang H. Enhancement the biocontrol efficacy of *Sporidiobolus parosaeus* Y16 against apple blue mold decay by glycine betaine and its mechanism. *Biol Control*. 2019 Dec;139:104079. <https://doi.org/10.1016/j.biocontrol.2019.104079>

Abdel-Kader M, El-Mougy N, Lashin S. Essential oils and *Trichoderma harzianum* as an integrated control measure against faba bean root rot pathogens. *J Plant Prot Res*. 2011 Jul;51(3):306–313. <https://doi.org/10.2478/v10045-011-0050-8>

Ahsan T, Chen J, Zhao X, Irfan M, Wu Y. Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by *Streptomyces* strain KX852460 for the biological control of *Rhizoctonia solani* AG-3 strain KX852461 to control target spot disease in tobacco leaf. *AMB Express*. 2017 Dec;7(1):54. <https://doi.org/10.1186/s13568-017-0351-z>

Al-Askar AA, Saber WIA, Ghoneem KM, Hafez EE, Ibrahim AA. Crude citric acid of *Trichoderma asperellum*: tomato growth promoter and suppressor of *Fusarium oxysporum* f. sp. *lycopersici*. *Plants*. 2021 Jan;10(2):222. <https://doi.org/10.3390/plants10020222>

Arai M, Han C, Yamano Y, Setiawan A, Kobayashi M. Aaptamines, marine spongean alkaloids, as anti-dormant mycobacterial substances. *J Nat Med*. 2014 Apr;68(2):372–376. <https://doi.org/10.1007/s11418-013-0811-y>

Bae H, Sicher RC, Kim MS, Kim SH, Strem MD, Melnick RL, Bailey BA. The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. *J Exp Bot*. 2009 Jul;60(11):3279–3295. <https://doi.org/10.1093/jxb/erp165>

Bae SJ, Park YH, Bae HJ, Jeon J, Bae H. Molecular identification, enzyme assay, and metabolic profiling of *Trichoderma* spp. *J Microbiol Biotechnol*. 2017 Jun;27(6):1157–1162. <https://doi.org/10.4014/jmb.1702.02063>

Bañados MP. Blueberry production in South America. *Acta Hortic*. 2006;715:165–172. <https://doi.org/10.17660/ActaHortic.2006.715.24>

Behiry S, Soliman SA, Massoud MA, Abdelbary M, Kordy AM, Abdelkhalek A, Heflish A. *Trichoderma pubescens* elicited induced systemic resistance in tomato challenged by *Rhizoctonia solani*. *J Fungi (Basel)*. 2023 Jan 27;9(2):167. <https://doi.org/10.3390/jof9020167>

Benítez T, Rincón AM, Limón MC, Codón AC. Biocontrol mechanisms of *Trichoderma* strains. *Int Microbiol*. 2004 Dec;7(4):249–260.

Bhutia DD, Zhimo Y, Kole R, Saha J. Antifungal activity of plant extracts against *Colletotrichum musae*, the post harvest anthracnose pathogen of banana cv. Martaman. *Nutr Food Sci*. 2016 Feb;46(1):2–15. <https://doi.org/10.1108/NFS-06-2015-0068>

Bigirimana J, De Meyer G, Poppe J, Elad Y, Höfte M. Induction of systemic resistance on bean (*Phaseolus vulgaris*) by *Trichoderma harzianum*. *Meded – Fac Landbouwkde Toegepaste Biol Wet (Univ Gent)*. 1997;62:1001–1007.

Calonje M, Novaes-Ledieu M, Bernardo D, Ahrazem O, Mendoza CG. Chemical components and their locations in the *Verticillium fungicola* cell wall. *Can J Microbiol*. 2000 Feb;46(2):101–109. <https://doi.org/10.1139/w99-120>

Chet I, Inbar J. Biological control of fungal pathogens. *Appl Biochem Biotechnol*. 1994 Jul;48(1):37–43. <https://doi.org/10.1007/BF02825358>

Chong KP, Rossall S, Atong M. *In vitro* antimicrobial activity and fungitoxicity of syringic acid, caffeic acid and 4-hydroxybenzoic acid against *Ganoderma boninense*. *J Agric Sci*. 2009;1(2):15. <https://doi.org/10.5539/jas.v1n2p15>

da Silva JAT, de Medeiros EV, da Silva JM, Tenório DA, Moreira KA, Nascimento TCES, Souza-Motta C. *Trichoderma aureoviride* URM 5158 and *Trichoderma hamatum* URM 6656 are Biocontrol agents that act against cassava root rot through different Mechanisms. *J Phytopathol*. 2016 Dec;164(11–12):1003–1011. <https://doi.org/10.1111/jph.12521>

de França SKS, Cardoso AF, Lustosa DC, Ramos EMLS, de Filippi MCC, da Silva GB. Biocontrol of sheath blight by *Trichoderma asperellum* in tropical lowland rice. *Agron Sustain Dev*. 2015 Jan;35(1):317–324. <https://doi.org/10.1007/s13593-014-0244-3>

- de los Santos-Villalobos S, Guzmán-Ortiz DA, Gómez-Lim MA, Délano-Frier JP, de-Folter S, Sánchez-García P, Peña-Cabralles JJ. Potential use of *Trichoderma asperellum* (Samuels, Liechfeldt et Nirenberg) T8a as a biological control agent against anthracnose in mango (*Mangifera indica* L.). *Biol Control*. 2013 Jan;64(1):37–44. <https://doi.org/10.1016/j.biocontrol.2012.10.006>
- Dihazi A, Jaiti F, WafaTaktak, kilani-Feki O, Jaoua S, Driouich A, Baaziz M, Daayf F, Serghini MA. Use of two bacteria for biological control of bayoud disease caused by *Fusarium oxysporum* in date palm (*Phoenix dactylifera* L.) seedlings. *Plant Physiol Biochem*. 2012 Jun; 55:7–15. <https://doi.org/10.1016/j.plaphy.2012.03.003>
- El Komy MH, Saleh AA, Eranthodi A, Molan YY. Characterization of novel *Trichoderma asperellum* isolates to select effective biocontrol agents against tomato *Fusarium* wilt. *Plant Pathol J*. 2015 Mar; 31(1):50–60. <https://doi.org/10.5423/PPJ.OA.09.2014.0087>
- Elshahawy IE, El-Mohamedy RS. Biological control of *Pythium* damping-off and root-rot diseases of tomato using *Trichoderma* isolates employed alone or in combination. *J Plant Pathol*. 2019 Aug; 101(3):597–608. <https://doi.org/10.1007/s42161-019-00248-z>
- Faria A, Oliveira J, Neves P, Gameiro P, Santos-Buelga C, de Freitas V, Mateus N. Antioxidant properties of prepared blueberry (*Vaccinium myrtillus*) extracts. *J Agric Food Chem*. 2005 Aug; 53(17):6896–6902. <https://doi.org/10.1021/jf0511300>
- Fei NY, Qi YB, Meng TT, Fu JF, Yan XR. First report of root rot caused by *Calonectria ilicicola* on blueberry in Yunnan Province, China. *Plant Dis*. 2018 May;102(5):1036–1036. <https://doi.org/10.1094/PDIS-09-17-1337-PDN>
- Guo R, Wang Z, Zhou C, Huang Y, Fan H, Wang Y, Liu Z. Biocontrol potential of *Trichoderma asperellum* mutants T39 and T45 and their growth promotion of poplar seedlings. *J For Res*. 2020 Jun; 31(3):1035–1043. <https://doi.org/10.1007/s11676-018-0797-0>
- Hahn M. The rising threat of fungicide resistance in plant pathogenic fungi: *Botrytis* as a case study. *J Chem Biol*. 2014 Oct;7(4): 133–141. <https://doi.org/10.1007/s12154-014-0113-1>
- Haran S, Schickler H, Chet I. Molecular mechanisms of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum*. *Microbiology*. 1996 Sep;142(9):2321–2331. <https://doi.org/10.1099/00221287-142-9-2321>
- Harman GE. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology*. 2006 Feb;96(2):190–194. <https://doi.org/10.1094/PHYTO-96-0190>
- He W, Megharaj M, Wu CY, Subashchandrabose SR, Dai CC. Endophyte-assisted phytoremediation: Mechanisms and current application strategies for soil mixed pollutants. *Crit Rev Biotechnol*. 2020 Jan;40(1):31–45. <https://doi.org/10.1080/07388551.2019.1675582>
- Herrera Cano N, Ballari MS, López AG, Santiago AN. New synthesis and biological evaluation of benzothiazole derivatives as antifungal agents. *J Agric Food Chem*. 2015 Apr;63(14):3681–3686. <https://doi.org/10.1021/acs.jafc.5b00150>
- Jiang H, Zhang L, Zhang J, Ojaghian MR, Hyde KD. Antagonistic interaction between *Trichoderma asperellum* and *Phytophthora capsici* in vitro. *J Zhejiang Univ Sci B*. 2016 Apr;17(4):271–281. <https://doi.org/10.1631/jzus.B1500243>
- Keswani C, Bisen K, Chitara MK, Sarma BK, Singh HB. Exploring the role of secondary metabolites of *Trichoderma* in tripartite interaction with plant and pathogens. In Singh J, Seneviratne G, editors. *Agro-environmental sustainability*. Cham (Germany): Springer; 2017. p. 63–79. https://doi.org/10.1007/978-3-319-49724-2_4
- Kong P, Hong C. Biocontrol of boxwood blight by *Trichoderma koningiopsis* Mb2. *Crop Prot*. 2017 Aug;98:124–127. <https://doi.org/10.1016/j.cropro.2017.03.015>
- Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Mol Biol Evol*. 2016 Jul; 33(7):1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Li M, Ma G, Lian H, Su X, Tian Y, Huang W, Mei J, Jiang X. The effects of *Trichoderma* on preventing cucumber *fusarium* wilt and regulating cucumber physiology. *J Integr Agric*. 2019 Mar;18(3):607–617. [https://doi.org/10.1016/S2095-3119\(18\)62057-X](https://doi.org/10.1016/S2095-3119(18)62057-X)
- Li S, Hou R, Zhang F, Shang X. First report of *Fusarium commune* causing root rot of blueberry plants in Guizhou Province, China. *Plant Dis*. 2023 Apr;107(4):1227. <https://doi.org/10.1094/PDIS-06-22-1305-PDN>
- Liu YH, Lin T, Ye CS, Zhang CQ. First report of *Fusarium* wilt in blueberry (*Vaccinium corymbosum*) caused by *Fusarium oxysporum* in China. *Plant Dis*. 2014 Aug;98(8):1158–1158. <https://doi.org/10.1094/PDIS-02-14-0167-PDN>
- Loc NH, Huy ND, Quang HT, Lan TT, Thu Ha TT. Characterisation and antifungal activity of extracellular chitinase from a biocontrol fungus, *Trichoderma asperellum* PQ34. *Mycology*. 2020 Jan 02;11(1):38–48. <https://doi.org/10.1080/21501203.2019.1703839>
- Mathivanan N, Prabavathy VR, Vijayanandraj VR. The effect of fungal secondary metabolites on bacterial and fungal pathogens. In Karlovsky P, editor. *Secondary metabolites. Soil ecology*. Berlin, Heidelberg (Germany): Springer; 2008. p. 129–140. https://doi.org/10.1007/978-3-540-74543-3_7
- Meena M, Swapnil P, Zehra A, Dubey MK, Upadhyay RS. Antagonistic assessment of *Trichoderma* spp. by producing volatile and non-volatile compounds against different fungal pathogens. *Arch Phytopathol Pflanzenschutz*. 2017 Aug;50(13–14):629–648. <https://doi.org/10.1080/03235408.2017.1357360>
- Mischke S. A quantitative bioassay for extracellular metabolites that antagonize growth of filamentous fungi, and its use with biocontrol fungi. *Mycopathologia*. 1997;137(1):45–52. <https://doi.org/10.1023/A:1006814521872>
- Mu J, Li X, Jiao J, Ji G, Wu J, Hu F, Li H. Biocontrol potential of vermicompost through antifungal volatiles produced by indigenous bacteria. *Biol Control*. 2017 Sep;112:49–54. <https://doi.org/10.1016/j.biocontrol.2017.05.013>
- Mukherjee PK, Raghu K. Effect of temperature on antagonistic and biocontrol potential of *Trichoderma* sp. on *Sclerotium rolfsii*. *Mycopathologia*. 1997;139(3):151–155. <https://doi.org/10.1023/A:1006868009184>
- Muniroh MS, Nusaibah SA, Vadmalai G, Siddique Y. Proficiency of biocontrol agents as plant growth promoters and hydrolytic enzyme producers in *Ganoderma boninense* infected oil palm seedlings. *Curr Plant Biol*. 2019 Dec;20:100116. <https://doi.org/10.1016/j.cpb.2019.100116>
- Nakkeeran S, Priyanka R, Rajamanickam S, Sivakumar U. *Bacillus amyloliquefaciens* alters the diversity of volatile and non-volatile metabolites and induces the expression of defence genes for the management of *Botrytis* leaf blight of *Lilium* under protected conditions. *J Plant Pathol*. 2020 Nov;102(4):1179–1189. <https://doi.org/10.1007/s42161-020-00602-6>
- Nawrocka J, Małolepsza U. Diversity in plant systemic resistance induced by *Trichoderma*. *Biol Control*. 2013 Nov;67(2):149–156. <https://doi.org/10.1016/j.biocontrol.2013.07.005>
- Neto CC. Cranberry and blueberry: evidence for protective effects against cancer and vascular diseases. *Mol Nutr Food Res*. 2007 Jun; 51(6):652–664. <https://doi.org/10.1002/mnfr.200600279>
- Nguyen TTT, Lee HB. Isolation and characterization of three Zygomycetous fungi in Korea: *Backusella circina*, *Circinella muscae*, and *Mucor ramosissimus*. *Mycobiology*. 2018 Dec 21;46(4):317–327. <https://doi.org/10.1080/12298093.2018.1538071>
- Papavizas GC, Lumsden RD. Biological control of soilborne fungal propagules. *Annu Rev Phytopathol*. 1980 Sep;18(1):389–413. <https://doi.org/10.1146/annurev.py.18.090180.002133>
- Podile AR, Laxmi VDV. Seed Bacterization with *Bacillus subtilis* AF 1 increases phenylalanine ammonia-lyase and reduces the incidence of

- Fusarial Wilt in Pigeonpea*. J Phytopathol. 1998 Jul;146(5–6):255–259. <https://doi.org/10.1111/j.1439-0434.1998.tb04687.x>
- Ruangwong OU, Wonglom P, Suwannarach N, Kumla J, Thaochan N, Chomnunti P, Pitija K, Sunpapao A. Volatile organic compound from *Trichoderma asperelloides* TSU1: impact on plant pathogenic fungi. J Fungi. 2021 Mar;7(3):187. <https://doi.org/10.3390/jof7030187>
- Sahebani N, Hadavi N. Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Soil Biol Biochem. 2008 Aug;40(8):2016–2020. <https://doi.org/10.1016/j.soilbio.2008.03.011>
- Saravanakumar K, Yu C, Dou K, Wang M, Li Y, Chen J. Synergistic effect of *Trichoderma*-derived antifungal metabolites and cell wall degrading enzymes on enhanced biocontrol of *Fusarium oxysporum* f. sp. *cucumerinum*. Biol Control. 2016 Mar;94:37–46. <https://doi.org/10.1016/j.biocontrol.2015.12.001>
- Sid Ahmed A, Ezziyyani M, Pérez Sánchez C, Candela ME. Effect of chitin on biological control activity of *Bacillus* spp. and *Trichoderma harzianum* against root rot disease in pepper (*Capsicum annuum*) plants. Eur J Plant Pathol. 2003;109(6):633–637. <https://doi.org/10.1023/A:1024734216814>
- Sundar D, Perianayaguy B, Reddy AR. Localization of antioxidant enzymes in the cellular compartments of sorghum leaves. Plant Growth Regul. 2004;44(2):157–163. <https://doi.org/10.1023/B:GROW.0000049418.92833.d6>
- Takahashi Y, Kubota T, Shibasaki A, Gonoi T, Fromont J, Kobayashi J. Nakijinamines C-E, new heteroaromatic alkaloids from the sponge *Suberites* species. Org Lett. 2011 Jun;13(12):3016–3019. <https://doi.org/10.1021/ol2008473>
- Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M. *Trichoderma*-plant-pathogen interactions. Soil Biol Biochem. 2008 Jan;40(1):1–10. <https://doi.org/10.1016/j.soilbio.2007.07.002>
- Wang LJ, Wu J, Wang HX, Li SS, Zheng XC, Du H, Xu YJ, Wang LS. Composition of phenolic compounds and antioxidant activity in the leaves of blueberry cultivars. J Funct Foods. 2015 Jun;16:295–304. <https://doi.org/10.1016/j.jff.2015.04.027>
- Ward NA. Blueberry root rot. Extension Plant Pathologist. 2013; PPFS-FR-S-19.
- Wu Q, Sun R, Ni M, Yu J, Li Y, Yu C, Dou K, Ren J, Chen J. Identification of a novel fungus, *Trichoderma asperellum* GDFS1009, and comprehensive evaluation of its biocontrol efficacy. PLoS One. 2017 Jun;12(6):e0179957. <https://doi.org/10.1371/journal.pone.0179957>
- Xie Y, Peng Q, Ji Y, Xie A, Yang L, Mu S, Li Z, He T, Xiao Y, Zhao J, et al. Isolation and identification of antibacterial bioactive compounds from *Bacillus megaterium* L2. Front Microbiol. 2021 Mar;12:645484. <https://doi.org/10.3389/fmicb.2021.645484>
- Yi HW, Chi YJ. Biocontrol of Cytospora canker of poplar in north-east China with *Trichoderma longibrachiatum*. For Pathol. 2011 Aug;41(4):299–307. <https://doi.org/10.1111/j.1439-0329.2010.00704.x>
- Zhang D, Bi W, Kai K, Ye Y, Liu J. Effect of chlorogenic acid on controlling kiwifruit postharvest decay caused by *Diaporthe* sp. LWT. 2020 Oct;132:109805. <https://doi.org/10.1016/j.lwt.2020.109805>
- Zhou Y, Yang L, Wang J, Guo L, Huang J. Synergistic effect between *Trichoderma virens* and *Bacillus velezensis* on the control of tomato bacterial wilt disease. Hortic. 2021 Nov 01;7(11):439. <https://doi.org/10.3390/horticulturae7110439>

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