

Article

Antagonistic and Plant Growth-Promoting Potential of Endophytic *Trichoderma* Against *Fusarium* Root Rot in Loquat (*Eriobotrya japonica*)

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Abstract

Loquat (*Eriobotrya japonica*) is highly susceptible to destructive soil-borne *Fusarium* pathogens, which cause severe vascular wilt and root rot. While *Trichoderma* species are widely applied as biocontrol agents, the potential of endophytic *Trichoderma* strains from loquat remains largely unexplored. This study aimed to isolate and evaluate loquat endophytic *Trichoderma* strains for their antifungal and plant growth-promoting capabilities. Ten isolates were identified through morphological and phylogenetic analyses as belonging to *T. asperellum*, *T. virens* and *T. hamatum*. Antagonistic activity was initially screened in vitro via dual-culture assays, followed by in vivo greenhouse pot experiments. In vitro results revealed that *T. asperellum* strains (B077R1 and B077B3) and *T. virens* GFR9 strongly inhibited the mycelial growth of both *F. oxysporum* and *F. solani* (inhibition rates > 50.65%), whereas *T. hamatum* isolates exhibited weaker antagonism (<35.18%). Moreover, *T. asperellum* B077R1 demonstrated the highest biocontrol efficacy in the greenhouse pot assay, significantly reducing disease severity caused by *F. oxysporum* and *F. solani* by 45.99% and 55.50%, respectively. This strain also significantly enhanced plant height, total biomass (fresh and dry weight) and photosynthetic pigment content ($p < 0.05$). These findings suggest that newly isolated endophytic *Trichoderma* strains from loquat exhibit robust host compatibility, offering a promising sustainable strategy for managing root rot in perennial fruit crops.

Keywords: *Trichoderma*; biocontrol; antifungal potential; loquat root rot; *Fusarium*; plant growth attributes



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1. Introduction

Loquat (*Eriobotrya japonica*), a member of the *Rosaceae* family, is a commercially vital perennial fruit-bearing tree indigenous to southern China. Unlike most stone fruit trees, loquat blooms in winter and its fruit matures in summer, filling a critical gap in the fresh fruit market and commanding high economic returns [1]. It has been extensively introduced to subtropical regions worldwide, including Japan, Spain, Turkey and Italy [1,2]. China is the predominant producer, with cultivation spanning over 170,000 hectares and an annual yield of around 400,000 tons [3]. Beyond its agronomic value, the loquat is increasingly

recognized as a functional food. The leaves, flowers and fruit are rich in bioactive compounds such as ursolic acid, carotenoids, and phenolics, which contribute to its significant antioxidant and therapeutic properties in traditional medicine [4]. Consequently, the loquat industry plays a crucial role in increasing farmers' income and rural revitalization.

Despite its economic potential, the sustainable development of the loquat industry is severely threatened by soil-borne diseases. The plant is highly susceptible to vascular wilt pathogens, particularly *Fusarium* species, which colonize the xylem vessels and obstruct water and nutrient transport [5]. Among these, *Fusarium*-induced root rot represents the most destructive constraint [6]. *Fusarium solani* and *Fusarium oxysporum*, ubiquitous soil inhabitants notorious for infecting a wide range of crops, have been identified as the primary etiological agents driving this decline in loquat orchards due to root and fruit rot [7,8]. In several Chinese production regions, such as Chongqing, Yunnan, and Fujian province, the *F. solani* species complex (FSSC) and the *F. oxysporum* species complex (FOSC) have infected approximately 5–10% of plantations. According to Wu [8], the symptoms of the disease include foliar chlorosis (yellowing), wilting, decay of the main root, and cracking of lateral roots, eventually causing the plant to defoliate and die. Given the severity of these symptoms and the rapid spread of the pathogens, effective management of *Fusarium* root rot has become an urgent priority for the loquat industry.

Conventionally, chemical fungicides are employed for disease management. However, their excessive application or misuse poses environmental risks and accelerates the selection pressure for pathogen resistance. Consequently, biological control has emerged as a sustainable alternative for managing *Fusarium* wilt, offering benefits such as environmental compatibility and cost-effectiveness [9]. Among biological control agents (BCAs), *Trichoderma* spp. are one of the most widely studied and commercially utilized species [10]. Substantial evidence underscores their efficacy against *Fusarium*-induced disease. For instance, in vitro assays have demonstrated the potent antifungal activities of *T. viride* and *T. harzianum* against *F. solani* [11]. In practical applications, *T. asperellum* (e.g., T-34 and TM11) has exhibited remarkable biocontrol potential against *F. oxysporum* and *F. commune*, significantly reducing the incidence of root rot disease in tomato and blueberry [12–14]. Similarly, *T. gamsii* 6085 has shown strong antagonism against rice pathogens [15], while field applications of *T. harzianum* SQR-T037 have been reported to enhance tomato biomass and optimize soil nutrient balance [16].

This biocontrol success relies on a multi-faceted defense strategy involving direct pathogen suppression through mycoparasitism and the secretion of hydrolytic enzymes (e.g., chitinases, glucanases, and proteases) [17]. Additionally, these fungi inhibit pathogen growth by synthesizing volatile antimicrobial compounds and prime the plant defense system by inducing systemic resistance [18]. Furthermore, *Trichoderma* spp. can enhance nutrient uptake and secrete phytohormones (e.g., auxins, gibberellins, salicylic acid and abscisic acid), thereby boosting plant vigor and biomass accumulation even under biotic or abiotic stress [19].

Despite this proven efficacy, the field application of commercial *Trichoderma* formulations often yields inconsistent results. Current perspectives emphasize that introduced strains frequently fail to bridge the gap between laboratory and field efficacy due to the inability to align with specific endophyte behaviors and ecological constraints [20–22]. In contrast, indigenous endophytic strains possess co-evolved advantages derived from long-term host interactions, granting them superior biotransformation abilities and environmental fitness [23,24]. Moreover, effective biocontrol is increasingly linked to host-specific transcriptional regulation, suggesting that intimate host-microbe compatibility is a prerequisite for optimal efficacy [25]. However, despite their known potential, the specific

application of indigenous endophytic *Trichoderma* in loquat against *Fusarium*-induced vascular wilt remains largely unexplored.

We hypothesized that endophytic *Trichoderma* from loquat suppresses *Fusarium* spp. and promotes loquat growth under greenhouse conditions. Therefore, this study was designed to isolate and identify endophytic *Trichoderma* species from loquat. It aimed to assess the antagonistic ability of the isolated *Trichoderma* strains against *Fusarium oxysporum* and *Fusarium solani*, and to investigate their capability to promote loquat growth and enhance physiological resilience under greenhouse conditions.

2. Materials and Methods

2.1. Isolation and Culture Conditions

Root samples exhibiting root rot symptoms were collected from loquat orchards at Yangdu Base, Zhejiang Province. Endophytic fungi were isolated according to [26] with slight modifications. Explants were washed and cut into small segments approximately 0.5 cm wide and 1 cm long using sterilized blades on a clean bench. These segments were surface-sterilized using ethanol (75%) for 4 min and hydrogen peroxide (10%) for 8 min, followed by 3–4 rinses with sterile distilled water, and dried on sterilized filter paper [27]. Moreover, the potato dextrose agar (PDA; AOBOX BIO-TECH, Cat 02-023, Beijing, China) media were augmented with streptomycin sulfate (30 µg mL⁻¹) and incubated at 26 °C with a 12 h light/dark cycle. Plates were regularly flipped and checked for possible contamination, while light exposure helped to promote fungal sporulation [28]. Emerging fungal hyphae were excised with a sterile scalpel and cultured on fresh PDA to obtain pure colonies. Isolates were deposited in the Loquat Germplasm Resources Laboratory, Southwest University, Chongqing, China, assigned unique identification codes (e.g., B077R1, B077R7, and GFR9), and preserved long-term in 30% glycerol at –80 °C.

2.2. Morphological Identification and Characterization

To conduct a comparative analysis of colony growth and morphological traits, plates from fresh monosporic *Trichoderma* cultures were inoculated onto 9 cm Petri dishes filled with 20 mL of PDA, cornmeal dextrose agar (CMA), or synthetic low-nutrient agar (SNA) and incubated at 28 ± 1 °C with 70 ± 5% relative humidity, under a 12:12 h light/dark photoperiod [29]. The examination of morphological characteristics included phialide structure, conidial morphology, and chlamydospore formation, using a Nikon Eclipse Ci microscope (Nikon Corporation, Shinagawa, Japan) paired with a Nikon DS-Ri2 camera. Measurements and image analyses were carried out by using NIS Elements software (v. 4.30.01, Nikon), focusing on 30 individual phialides and conidia for each isolate. The staining and examination of fungal specimens were performed in accordance with the protocols proposed by [30,31].

2.3. Molecular Identification and Phylogenetic Analysis

Trichoderma isolates were cultured on PDA for 3–4 days, and gDNA was isolated by using the CTAB method with minor modifications [32]. The nuclear ribosomal ITS1-5.8S rRNA-ITS2 region (ITS), translation elongation factor 1-α (*tef1-α*), RNA polymerase II second largest subunit (*rpb2*) and ATP citrate lyase large subunit (*ac11*) gene regions were amplified by PCR [33]. PCR was carried out in a 40 µL reaction mixture containing 1 µL gDNA, 20 µL of 2 × Taq PCR Master Mix, 2 µL of each primer (10 µM) and 15 µL of ddH₂O. Each 50 µL reaction contained 1 ng of gDNA, 20 µL of 2 × Taq PCR Master Mix, 1 µM of each primer, and 15 µL of ddH₂O. Primer sequences are provided in Supplementary Table S1. The sequences were deposited in GenBank, and the corresponding accession numbers are listed in Supplementary Table S2.

Phylogenetic analyses were performed based on a combined dataset of ITS, *tef1- α* , *rpb2* and *acl1* to advance taxonomic resolution [33,34]. Maximum likelihood (ML) analysis was conducted using RAxML-HPC2 (v8.2.10) through the CIPRES Science Gateway (<http://www.phylo.org/>) under the GTRGAMMA model with 1000 bootstrap replicates. Maximum parsimony (MP) analysis was carried out in PAUP v4.0a169 with 1000 heuristic replicates. Bayesian inference (BI) was carried out in MrBayes v3.2.6 implemented in PhyloSuite v1.2.3. Phylogenetic trees were visualized using FigTree v1.4.2.

2.4. Antagonistic Activity of *Trichoderma* In Vitro

In vitro antagonistic activity of *Trichoderma* spp. against *F. solani* (FSSC) and *F. oxysporum* (FOSC) was evaluated using a direct confrontation assay on PDA [35]. The pathogenic FSSC used in this study was previously recovered from infected loquat roots [8]. While the FOSC was identified via multi-locus sequence analysis (ITS, *tef1- α* and *rpb2*) using BLASTn and Fusarium-ID (GenBank accession no. MT974325, MT992555, MW072791; unpublished). Mycelial plugs (5 mm³) from the actively growing margins of 7-day-old *Fusarium* cultures and 10 *Trichoderma* isolates derived from loquat explants were co-inoculated onto 9 cm PDA plates to assess interspecific interactions and inhibition [36]. Plugs were placed 3 cm apart and 3 cm from the plate edge. For each *Fusarium*-*Trichoderma* combination, three plates were used as biological replicates, with *Fusarium* monocultures serving as controls (three replicates each). Cultures were incubated in the dark at 28 ± 1 °C for 8 days. Antagonistic effects were expressed as percentage growth inhibition (PGI), calculated according to [37].

$$\text{PGI}(\%) = [(C - T) / C] \times 100$$

where C = Radial growth of the pathogen (control), and T = Radial growth of the pathogen (treated).

2.5. Soil and Plant Material for Bioagents Efficacy Assessment

Commercial nutrient soil (Klasmann, Geeste, Germany) was mixed with perlite and vermiculite (7:2:1, v/v/v), moistened, and autoclaved at 121 °C for 15 min. Disinfected pots (12.7 cm × 11.4 cm) were filled to three-quarters capacity with the sterile substrate and adjusted to 60% field capacity before transplanting. The susceptible loquat cultivar ‘Jinhua No. 1’ was used for greenhouse assays. Loquat seeds were surface-sterilized with 50% carbendazim diluted at 1:500 for 1 h. The seedlings were then cultivated in sterile substrate for 6 months until they reached 5–6 true leaves. Uniform and healthy seedlings were selected for the experiment. The roots were washed gently, surface sterilized with 3% sodium hypochlorite for 1 min, washed three times with sterile distilled water and transplanted into the prepared pots.

2.6. Preparation of Spore Suspension and Pot Experiment

Fungal strains were sub-cultured on PDA, and conidia were harvested from 7-day-old cultures into sterile distilled water. *Trichoderma* and *Fusarium* were incubated under 12 h light/dark cycles to promote sporulation [38]. A hemocytometer was used to adjust the required concentration of the microconidia suspension (1 × 10⁶ spores/mL) to inoculate selected roots of loquat seedlings for the pot experiments to evaluate disease resistance and plant growth, following previously described inoculation methods [8,39]. Prior to inoculation, small wounds were made on the fibrous roots with a sterile scalpel, and the taproots were gently punctured using a sterile needle. This approach simulated natural root injuries under controlled greenhouse conditions and facilitated subsequent *Fusarium* infection. The positive controls (pathogen-only) and *Trichoderma* co-inoculated with *Fusarium* (treated groups) were included in the treatments. The following 4 treatments

were developed: (1) FOSC + H₂O, which received 20 mL of *F. oxysporum* suspension and 20 mL of sterile distilled water; (2) FSSC + H₂O, which received 20 mL of *F. solani* and 20 mL of sterile distilled water; (3) co-inoculation with *F. oxysporum* (20 mL) and 20 mL of *Trichoderma* suspension (*T. asperellum* B077R1, *T. hamatum* B077R7, or *T. virens* GFR9); and (4) co-inoculation with *F. solani* (20 mL) and 20 mL of the corresponding *Trichoderma* spore suspension. The greenhouse experiments were conducted for 60 days, with a second identical inoculation performed at 30 days post-inoculation (dpi). The disease symptoms were monitored repeatedly on the same plants at different intervals, whereas final growth and physiological parameters were recorded at 60 dpi. To ensure successful root colonization, the *Trichoderma* and *Fusarium* suspension were applied directly to the root zone of each pot/plant as a soil drench using wide-orifice pipette tips (5 mL). The suspension was applied slowly around the base of the stem and root zone to allow infiltration into the rhizosphere and to avoid surface runoff. All inoculated plants were maintained in a controlled growth room at 25 ± 1 °C temperature, 80% relative humidity, and a 16/8 h light/dark photoperiod. The pot assay was arranged in a completely randomized design, and the pots were randomly positioned in the growth room to minimize positional effects. All treatments were conducted with three biological replicates.

2.7. Assessment of Plant Growth and Biomass Production

To assess the plant growth in response to the different treatment combinations, several morphological and physiological parameters were measured [40,41]. These parameters included plant height (cm), plant fresh weight (g), plant dry weight (g), root fresh weight (g), and root dry weight (g). The disease severity index (DSI) was evaluated at 60 dpi by assessing the extent of root rot and root discoloration using a 0–5 rating scale. The scale was defined as follows: 0 = roots well developed with no discoloration; 1 = slight browning or less than 25% root discoloration; 2 = 25–50% discoloration; 3 = 50–75% root discoloration; 4 = severe browning of the hypocotyl or >75% discoloration of root; and 5 = completely discolored roots, leading to plant death [42,43]. Since root discoloration affects both external and internal tissues, visual assessments of the proportion of the discolored root area (0–100%) were employed to standardize the disease ratings. The DSI was calculated using the following formula:

$$DSI(\%) = \frac{\sum(\text{Degree of infection} \times \text{Number of plants with same degree}) \times 100}{(\text{Total number of plants} \times \text{Highest degree of infection})}$$

Additionally, plant vigor was evaluated based on a wilting severity scale ranging from 0 to 4: 4 = completely healthy plant; 3 = thin stem and reduced height; 2 = presence of brown lesions on the stem and yellowing leaf tips; 1 = wilting of stems and leaves; and 0 = completely dead plant. Root vigor was quantified using the triphenyl tetrazolium chloride (TTC) reduction assay [44].

2.8. Photosynthetic Pigment Content Measurements

Total chlorophyll content of loquat leaves was determined using a UV/VIS spectrophotometer (AOELAB) following the method of [45]. Fresh leaves (0.1 g) were cut into fine threads and submerged for 72 h in 10 mL of an extraction solvent containing 95% ethanol and 80% acetone (1:2) in the dark at 4 °C till complete tissue decolorization. The absorbance of the resulting extract was measured at 470 nm, 645 nm and 663 nm [46]. Contents of chlorophyll a (C_a), chlorophyll b (C_b), total carotenoids (C_{x+c}) and the total chlorophyll content were converted to mg·g^{−1} fresh weight (FW). The concentrations (μg·mL^{−1}) for

chlorophyll a, chlorophyll b and the sum of leaf carotenoids were calculated using the following equations [47].

$$C_a = 12.25 A_{663.2} - 2.79 A_{646.8}$$

$$C_b = 21.50 A_{646.8} - 5.10 A_{663.2}$$

$$C_{x+c} = (1000 A_{470} - 1.82 \cdot C_a - 85.02 \cdot C_b) / 198$$

where $A_{663.2}$, $A_{646.8}$ and A_{470} represent the absorbance values at 663.2 nm, 646.8 nm and 470 nm, respectively.

The leaf photosynthetic pigment content per unit mass (LPC) and per unit leaf area (LPA) were calculated as follows:

$$LPC \left(\text{mg} \cdot \text{g}^{-1} \right) = \frac{(C \text{ mgL}^{-1} \times V_t \text{ mL})}{(FW_1 \text{ g} \times 1000)} \times n$$

$$LPA \left(\text{mg} \cdot \text{cm}^{-2} \right) = \frac{(C \text{ mgL}^{-1} \times V_t \text{ mL})}{(FW_0 \text{ g} \times 1000)} \times n$$

where C is the total leaf photosynthetic pigment content ($C_a + C_b + C_{x+c}$, converted to $\text{mg} \cdot \text{L}^{-1}$), FW_1 is the fresh weight of the extracted leaf (g), FW_0 is the area of the extracted leaf (cm^2), V_t is the volume of extraction solution ($V_t = 10 \text{ mL}$), and n is the dilution ratio ($n = 1$).

2.9. Statistical Analyses

Prior to the ANOVA, the data were tested for normality and homogeneity of variances using the Shapiro–Wilk and Levene’s tests, respectively. All data shown represent the mean $\pm$ standard error (S.E.) derived from three biological replicates. Statistical analyses were conducted using a one-way analysis of variance (ANOVA) in Statistix 10.0 (Analytical Software, Tallahassee, FL, USA). The significance of differences between treatments was assessed using Tukey’s honestly significant difference (HSD) test at $p < 0.05$. Further statistical details are provided in the results section and figure captions.

3. Results

3.1. Isolation and Morphological Characterization of Endophytic *Trichoderma* from Loquat

A total of 100 endophytic fungal isolates were recovered from loquat tissues, among which 39 isolates were obtained from roots. Molecular identification based on the ITS region revealed that *Fusarium* (35.90%) was the dominant genus, followed by *Trichoderma* (23.07%), *Lasiodiplodia* (12.82%), *Meyerozyma* (10.26%), *Calonectria* (5.12%), *Phomopsis* (2.56%), *Xylaria* (2.56%), and *Macrophomina* (2.56%). Although 23 isolates were initially identified as *Trichoderma*, 10 representative isolates were selected for detailed characterization based on preliminary screening for vigorous growth and distinct morphotypes. Morphological analysis of the selected *Trichoderma* isolates showed rapid mycelial proliferation, typically occupying an entire 9 cm Petri plate within 4 days. Conidiophore formation notably increased on the third day of hyphal growth. Sporulation proceeded via the emergence of clustered, spherical conidia at the branch tips, developing into dense spore masses after 7 days. Based on macro- and micro-morphological characteristics, these 10 *Trichoderma* isolates were classified into 3 different groups. Group 1 isolates (B077R1, B077R2, B077B3, B077R5, B077R6, and B077R9) exhibited rapid growth, completely filling the plates in 3–4 days. Their aerial hyphae developed compact, short villi closely appressed to the medium surface. Colonies initially appeared white, progressively turning green and ultimately forming dark green concentric

rings during advanced sporulation. Spore clusters displayed an uneven distribution, and the conidiophores were primarily pyramidal with ampulliform phialides. The conidia were round to oval, measuring $3.53\text{--}3.81 \times 2.90\text{--}3.00 \mu\text{m}$. Conversely, Group 2 isolates (B077R7, B256R1, and B256R2) displayed restricted growth and an absence of sporulation on PDA. However, when cultivated on CMA, sporulation occurred after 4–5 days, yielding white, low-density, cotton-like colonies without a distinct odor. Hyphal branches typically showed an opposite arrangement with conical morphology. Generally, one or more ampule peduncles were connected to the apex of the conidiophores, and the conidia produced on CMA were oval-shaped with a smooth texture, measuring $3.81\text{--}4.10 \times 3.22\text{--}3.35 \mu\text{m}$. Isolate GFR9, categorized as Group 3, established rapid growth on PDA, covering the medium surface within 3–4 days. Colonies formed cotton-like masses or compact clusters with irregularly distributed conidiophores, lacking distinct conical or pyramidal structures. The conidia were spherical, pale green, and measured $4.00\text{--}4.51 \times 3.33\text{--}3.48 \mu\text{m}$. Numerous chlamydospores were observed, which were generally spherical and light brown (Figure 1).

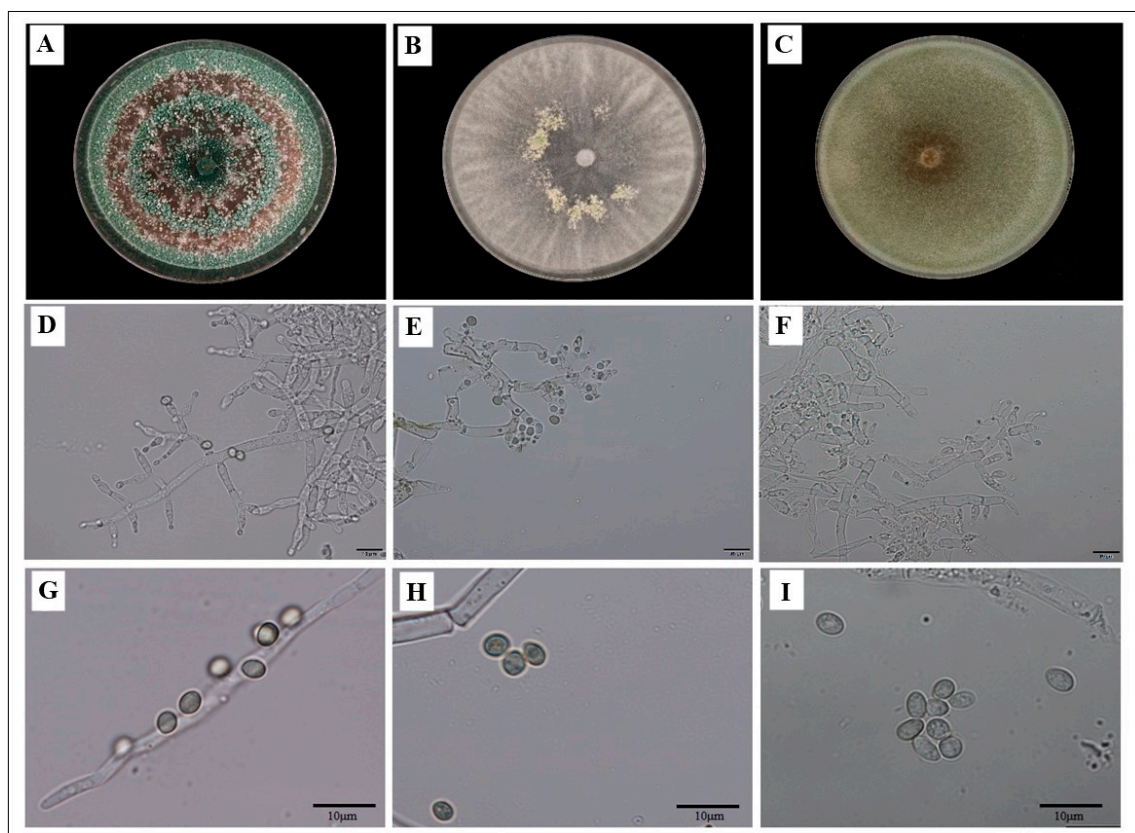


Figure 1. Macro- and micro- morphological characteristics of representative endophytic *Trichoderma* isolates. (A–C) Colony morphologies of *T. asperellum* on PDA (A), *T. hamatum* on CMA (B), and *T. virens* on PDA (C). (D–F) Microscopic features showing conidiophores and phialides of *T. asperellum* (D), *T. hamatum* (E), and *T. virens* (F). (G–I) Conidia and chlamydospores of *T. asperellum* (G), *T. hamatum* (H), and *T. virens* (I). Scale bars = 10 μm.

3.2. Multi-Locus Phylogenetic Analysis of the *Trichoderma* Isolates

To elucidate the robust taxonomic affinities of the selected isolates, a multi-locus phylogeny was inferred from a concatenated sequence dataset of four genetic loci including ITS, *tef1-α*, *rpb2* and *acl1*. The tree topology derived from the BI analysis was consistent with that obtained in an ML analysis. However, the resulting phylogenetic tree successfully resolved the ten isolates into three well-supported clades, aligning them with recognized *Trichoderma* species (Figure 2). Specifically, six isolates (B077R1, B077R2,

[illegible]

<https://doi.org/10.3390/horticulturae12070859>

a growth suppression rate of 69.42%, followed by B077B3 (66.15%) and B077R2 (55.94%). In contrast, *T. hamatum* B256R1 showed the lowest inhibition at 19.20%. These suppression rates were consistent with the significant reductions observed in pathogen colony diameters (Figure 3D,F). A similar inhibitory trend was observed against *F. solani*. *T. asperellum* B077R1 yielded the maximum inhibition (56.67%), followed by B077B3 (54.85%) and *T. virens* GFR9 (54.56%), whereas B256R2 showed the lowest efficacy (26.88%) (Figure 3E,G). Overall, *T. asperellum* B077R1 and *T. virens* GFR9 consistently exhibited superior and broad-spectrum antagonistic potential against both *Fusarium* species.

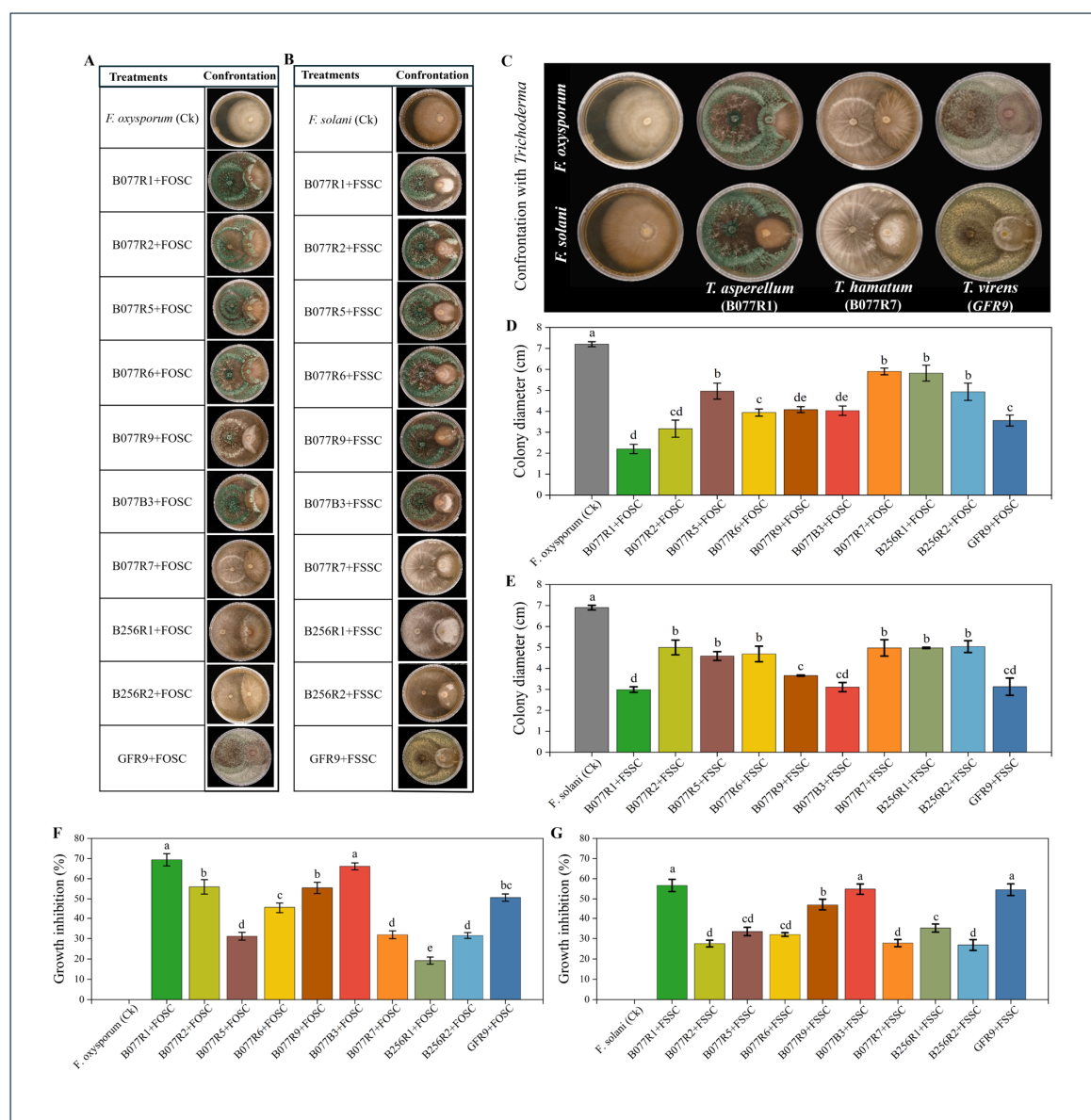


Figure 3. In vitro antagonistic activity of endophytic *Trichoderma* isolates against *Fusarium* pathogens in dual culture assays. (A,B) Representative confrontation images of *Trichoderma* against *F. oxysporum* (A) and *F. solani* (B). (C) Macroscopic observations of spatial competition and overgrowth by representative *T. asperellum* (B077R1), *T. hamatum* (B077R7), and *T. virens* (GFR9) against both pathogens. (D,E) Colony diameters of *F. oxysporum* (D) and *F. solani* (E) co-cultured with different *Trichoderma* isolates. (F,G) Percent growth inhibition of *F. oxysporum* (F) and *F. solani* (G) co-cultured with different *Trichoderma* isolates. Data are presented as the mean $\pm$ standard error (SE) of three independent replicates ($n = 3$). Statistical significance was determined using one-way ANOVA, and different lowercase letters above the bars indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$).

3.4. In Vivo Biocontrol Efficacy of *Trichoderma* Isolates Against *Fusarium* Root Rot in Loquat

3.4.1. Effect of *Trichoderma* Isolates on the Disease Severity and Root Viability

The application of different treatments resulted in varying degrees of disease severity and plant physiological responses. Loquat plants inoculated exclusively with either *F. oxysporum* or *F. solani* exhibited typical symptoms, including foliar chlorosis, root browning, and fine root decay (Figure 4A,B). These infected root systems displayed severe structural degradation, leading to a substantial reduction in total biomass compared to the *Trichoderma* co-inoculated treatments. In contrast, co-inoculation with *Trichoderma* isolates significantly mitigated these symptoms, with plants maintaining green foliage and developing robust root systems with fewer necrotic lesions. As shown in Figure 4C, the highest disease severity was recorded in the *F. oxysporum*-infected control, with a disease severity index (DSI) of 66.66%. However, co-inoculation with *T. asperellum* B077R1 significantly reduced the DSI to 45.99%. Similarly, *T. hamatum* B077R7 and *T. virens* GFR9 reduced the DSI to 39.99%. A similar trend was observed against *F. solani*, with the DSI reaching 63.33% in the pathogen-only control and significantly decreasing to 55.50% following *T. asperellum* B077R1 treatment.

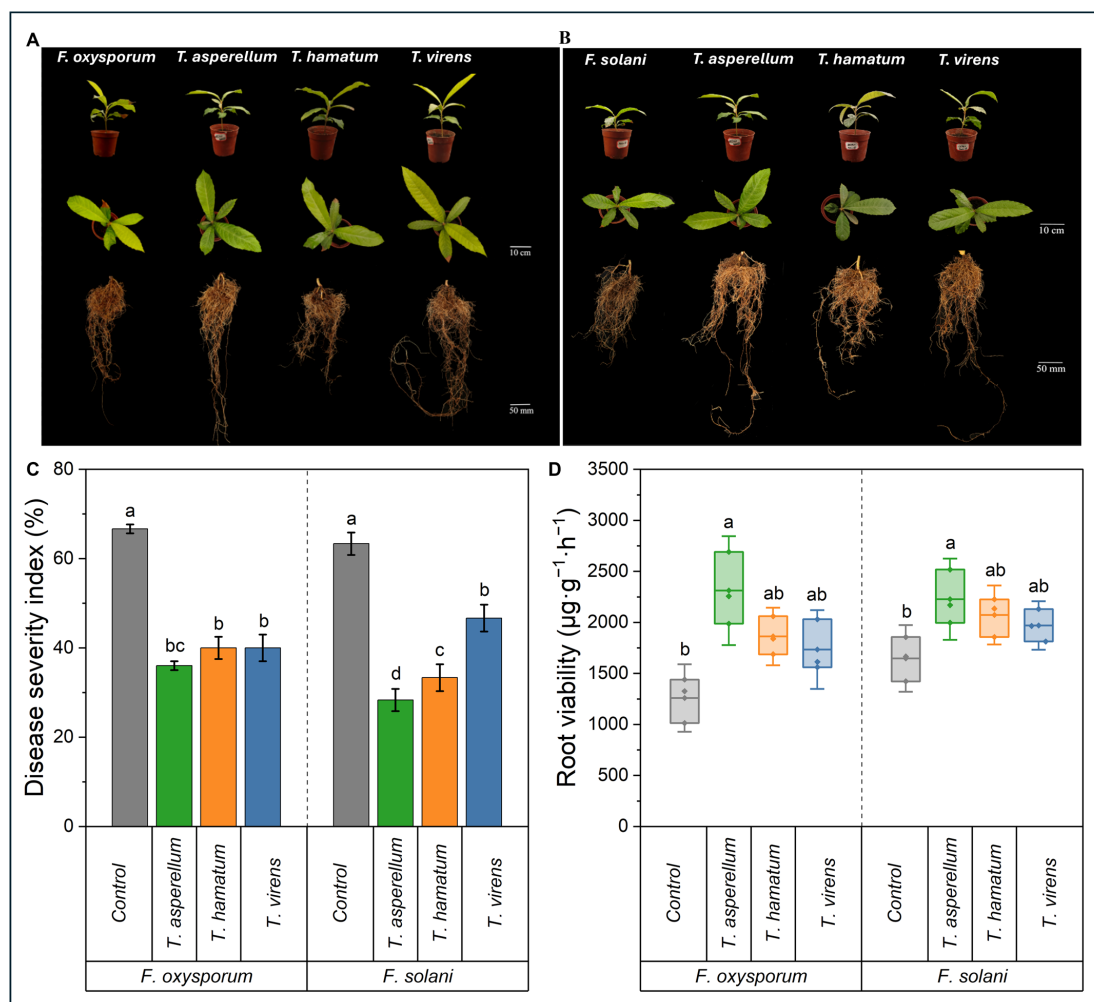


Figure 4. Biocontrol efficacy of *Trichoderma* against *Fusarium* root rot in loquat. (A,B) Morphological observations of plant shoots and roots under *F. oxysporum* (A) and *F. solani* (B) stress, either inoculated alone (Control) or co-inoculated with *T. asperellum* B077R1, *T. hamatum* B077R7, and *T. virens* GFR9. (C) Disease severity index (%). (D) Root viability ($\mu\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$). The bars and box plots represent the means of three biological replicates with standard errors. Different lowercase letters above the bars indicate statistical significance at $p < 0.05$ according to Tukey's test.

Root viability, quantified by the TTC reduction assay, exhibited significant variation among treatments (Figure 4D). Under *F. oxysporum* stress, plants treated with *T. asperellum* B077R1 exhibited the highest root viability ($2311.59 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$), followed by *T. hamatum* B077R7 ($1862.31 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) and *T. virens* GFR9 ($1734.48 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$). All *Trichoderma* treatments resulted in significantly higher viability compared to the *F. oxysporum*-infected control ($1259.42 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$). Under *F. solani* stress, *T. asperellum* B077R1 again provided the maximum protection ($2227.54 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$), followed by *T. hamatum* B077R7 ($2072.46 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) and *T. virens* GFR9 ($1969.57 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$). These values were notably higher than the $1647.82 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ recorded in the *F. solani*-infected control.

3.4.2. Effect of Trichoderma Isolates on Plant Growth Parameters

Consistent with the reduction in disease severity, *Trichoderma* inoculation effectively alleviated the growth inhibition induced by *Fusarium* stress, particularly in terms of biomass accumulation (Figure 5A–E). Under *F. oxysporum* stress, although plant height showed no statistical difference among the infected treatments (Figure 5A), the application of *T. asperellum* B077R1 significantly mitigated the loss of plant biomass compared to the stunted pathogen-only control. Specifically, B077R1 treatment maximized plant fresh weight (12.77 g) and dry weight (5.95 g), which were significantly higher than those of the infected control. Root development was correspondingly sustained, with fresh and dry weights reaching 6.05 g and 2.48 g, respectively. These growth parameters generally surpassed the values obtained for *T. hamatum* B077R7 and *T. virens* GFR9. A commensurate protective effect was observed against *F. solani*. While plant fresh weight did not differ significantly across treatments (Figure 5B), inoculation with B077R1 effectively counteracted the depletion of dry matter caused by the pathogen, yielding the highest plant dry weight (4.22 g) and root dry weight (2.05 g). In stark contrast, the *F. solani*-only control exhibited severely restricted growth. Collectively, these results indicate that the antagonistic activity of *T. asperellum* B077R1 effectively preserves plant vigor and dry matter accumulation under severe pathogen pressure.

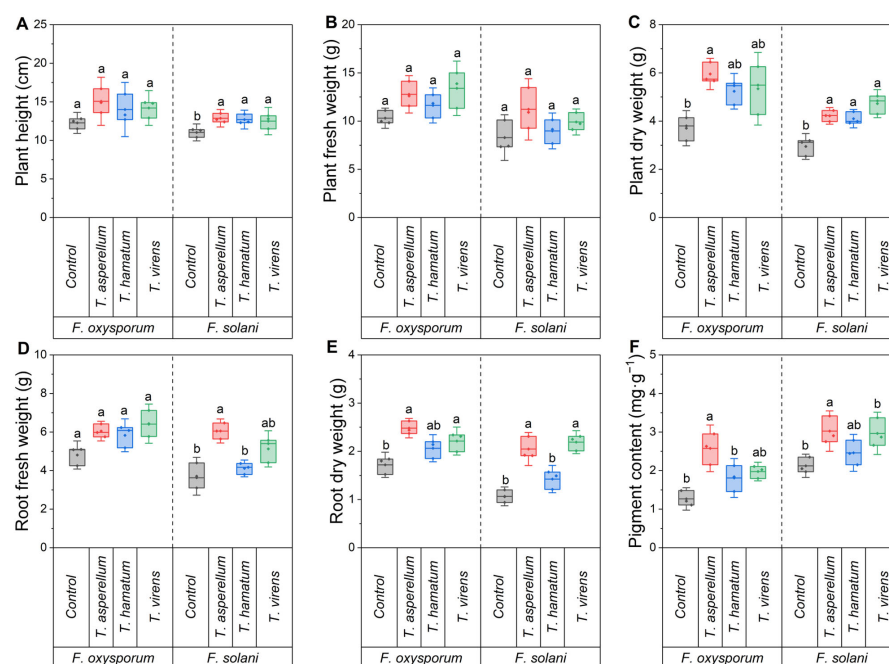


Figure 5. Effect of *Trichoderma* isolates on physiological parameters of loquat under *Fusarium* stress. (A) Plant height, (B) Fresh weight, (C) Dry weight, (D) Root fresh weight, (E) Root dry weight, (F) Photosynthetic pigment content. The bars and box plots represent the means of three biological replicates with standard errors. Different lowercase letters above the bars indicate statistical significance at $p < 0.05$ according to Tukey's test.

3.4.3. Effect of *Trichoderma* Isolates on Leaf Photosynthetic Pigment Content

As foliar chlorosis is a primary symptom of *Fusarium*-induced root rot, the total content of photosynthetic pigments (chlorophyll a, b, and carotenoids) was quantified to evaluate the physiological health of the plants. Inoculation with *F. oxysporum* or *F. solani* alone imposed severe stress, resulting in the lowest pigment accumulation in the infected controls. However, co-inoculation with *Trichoderma* isolates alleviated this chlorosis to varying extents. Among the treatments, *T. asperellum* B077R1 exhibited the most consistent and strongest capability to preserve the photosynthetic machinery (Figure 5F). Under *F. oxysporum* stress, the pigment content in the infected control was severely suppressed to $1.26 \text{ mg} \cdot \text{g}^{-1}$. Treatment with B077R1 effectively counteracted this degradation, increasing the total pigment content to $2.6 \text{ mg} \cdot \text{g}^{-1}$ (a 105% increase), which was the only treatment statistically higher than the control. Conversely, *T. hamatum* B077R7 did not show a significant difference from the infected control. A parallel trend was observed under *F. solani* stress. While the infected control exhibited depleted pigment levels ($2.11 \text{ mg} \cdot \text{g}^{-1}$), the B077R1 treatment maintained a significantly higher concentration at $3.10 \text{ mg} \cdot \text{g}^{-1}$ (an increase of 46.9%). These findings demonstrate that *T. asperellum* B077R1 effectively prevents pathogen-induced chlorosis, thereby sustaining the vital photosynthetic potential required for plant recovery.

4. Discussion

The effectiveness of biological control agents (BCAs) often depends on their ability to adapt to local rhizosphere conditions. In this study, we screened indigenous *Trichoderma* endophytes from loquat roots to identify potential antagonists against *Fusarium*-induced root rot. Although isolates of *T. asperellum*, *T. hamatum*, and *T. virens* were identified, their biocontrol activities varied significantly. This variability indicates that biocontrol efficacy is often strain-specific rather than conserved at the species level, presenting a challenge for consistent field application. For instance, while *T. hamatum* has been reported as effective against *Fusarium* root rot in *Panax notoginseng* [48], our isolate *T. hamatum* B077R7 showed limited activity compared to *T. asperellum* B077R1. This discrepancy suggests that host-genotype compatibility plays a pivotal role in determining BCA performance. Indeed, the effectiveness of *Trichoderma* as a biocontrol agent is primarily driven by its competence in the rhizosphere, persistence, and ability to establish a stable and beneficial relationship with roots that improve plant resistance and promote soil health [49,50]. Consequently, reliance on general commercial strains may yield inconsistent results in loquat orchards. Our findings strongly support the strategy of isolating and utilizing indigenous strains, such as *T. asperellum* B077R1, which are pre-adapted to the specific ecological niche of the target crop.

Loquat root rot is caused by a complex of pathogens, primarily *F. solani* and *F. oxysporum*. Our results demonstrated that *T. asperellum* B077R1 effectively inhibited both pathogens in vitro and in vivo. These results align with previous reports demonstrating the broad-spectrum efficacy of *T. asperellum* across diverse crops. For example, *T. asperellum* has successfully controlled *Fusarium* wilt in cucumber [51] and banana [52], as well as root rot in dry beans [53]. In dual culture assays, the isolate B077R1 exhibited rapid mycelial growth, physically overgrowing and spatially restricting the *Fusarium* colonies (Figure 3). This behavior points to direct mycoparasitism and competition for nutrients as primary modes of action. Direct mycoparasitism involves the secretion of cell-wall-degrading enzymes (e.g., chitinases) and antimicrobial secondary metabolites, which lyse pathogen hyphae [54,55]. For instance, *T. virens* from the roots of healthy apple trees directly suppressed *F. proliferatum* and released secondary metabolites exhibiting both antifungal and plant growth-promoting activities [56]. However, antagonistic activity on agar plates

does not always translate to soil environments. In our pot experiments, the sustained protection, which was characterized by significantly reduced disease severity and high root viability, suggests that B077R1 also possesses strong rhizosphere competence. By successfully colonizing the root surface, B077R1 likely excludes pathogens from infection sites. This ecological exclusion mechanism parallels findings in watermelon, where *T. asperellum* application reduced pathogen abundance by modulating the rhizosphere microbiome and enriching beneficial taxa like *Pseudomonas* [57]. Therefore, based on the combined in vitro and in vivo data, it is likely that B077R1 suppresses loquat root rot through a synergistic mechanism of direct mycoparasitic attack and competitive exclusion in the rhizosphere.

Fusarium infection is known to cause chlorosis and pigment loss, often attributed to the production of toxins such as fusaric acid and the induction of oxidative stress [58]. Previous studies on apple seedlings indicated that such infection leads to increased membrane permeability and reduced leaf water content, resulting in cellular damage that directly contributes to photosynthetic pigment degradation [59]. In this study, infection with *F. oxysporum* and *F. solani* significantly reduced chlorophyll and carotenoid contents, but treatment with B077R1 effectively prevented this degradation. This maintenance of photosynthetic pigments indicates that *Trichoderma* alleviates physiological stress, possibly by scavenging reactive oxygen species (ROS) and maintaining metabolic homeostasis. Previous research in maize seedlings demonstrated that *T. asperellum* enhances antioxidant enzyme activities, thereby protecting cellular structures from oxidative damage [60]. By preserving chloroplast integrity [32], B077R1 ensures the maintenance of photosynthetic capacity, which provides the necessary energy for the biomass recovery observed in treated plants. This physiological defense mechanism complements direct pathogen suppression, ensuring plant survival even under pathogen stress.

In addition to disease suppression, treatment with B077R1 significantly increased plant biomass and root development. This growth promotion is likely mediated by robust root colonization and the subsequent reciprocal exchange of metabolites. *Trichoderma* species are known to solubilize soil nutrients [61] and produce phytohormones, such as auxins and gibberellins, which stimulate root proliferation [19]. Upon successful root colonization, *Trichoderma* develops a mutualistic relationship with the host plant, marked by a reciprocal exchange of metabolites and nutrients. Similar auxin-mediated effects have been reported in *Arabidopsis*, where *T. virens* enhanced lateral root formation [62], and in peppermint, where *T. viride* colonization promoted both vegetative growth and essential oil biosynthesis [63]. The growth improvements observed were primarily due to reduced growth suppression caused by pathogens, as healthy mock-inoculated and *Trichoderma*-alone controls were not included. These controls should be included in future studies to determine if the growth recovery is due to biocontrol or to direct growth promotion of the plants.

Furthermore, the significant reduction in disease symptoms suggests the potential activation of plant defense mechanisms. *Trichoderma* species are widely reported to induce systemic resistance (ISR) via salicylic acid and jasmonic acid signaling pathways [64,65]. While the specific molecular pathways in the loquat-*Trichoderma* interaction remain to be fully elucidated, the robust phenotypic recovery observed in our study strongly implies that *T. asperellum* B077R1 primes the host immune system. Collectively, our findings demonstrate that *T. asperellum* B077R1 provides protection against *Fusarium* root rot through direct pathogen suppression and the effective preservation of host physiological vigor and growth.

In this study, we screened and evaluated individual *Trichoderma* isolates. However, compared to single strains, microbial consortia typically exhibit superior environmental resilience and stronger antagonistic effects in natural ecosystems [66]. Therefore, developing and evaluating a consortium using these promising *Trichoderma* isolates will be a

critical next step to further enhance their practical efficacy in the field. Although this study highlights the biocontrol and growth-promoting abilities of the isolated *Trichoderma* strains, it mainly relies on phenotypic and pathological data. We acknowledge that the lack of physiological and biochemical assays is a limitation, as these are needed to fully explain the plant-microbe interactions. Therefore, our future work will include biochemical and molecular analyses to better understand the specific mechanisms behind these effects.

5. Conclusions

This study demonstrates the significant biocontrol potential of indigenous endophytic *Trichoderma* isolates against *Fusarium*-induced root rot in loquat. Based on the strong *in vitro* antagonistic activity observed against both *F. oxysporum* and *F. solani*, *in vivo* assays further corroborated these findings by demonstrating that inoculation with all the tested isolates, most notably *T. asperellum* B077R1, effectively mitigated disease severity while preserving root viability, plant biomass, and photosynthetic capacity under severe pathogen stress. These findings highlight the ecological advantage of utilizing host-adapted endophytes for disease management. Ultimately, *T. asperellum* B077R1 represents a highly promising candidate for the development of sustainable biocontrol strategies to protect perennial fruit crops against soil-borne pathogens, warranting further investigation in field-scale applications.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae12070859/s1>, Table S1: Primers used in this study; Table S2: GenBank accession numbers of isolated *Trichoderma* strains.

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