Biocontrol Mechanisms of *Trichoderma koningiopsis* PSU3-2 against Postharvest Anthracnose of Chili Pepper

On-Uma Ruangwong 1,2, Chaninun Pornsuriya 3, Kitsada Pitija 4 and Anurag Sunpapao 3,*

1 Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Mueang, Chiang Mai 50200, Thailand; on-uma.ra@cmu.ac.th
2 Innovative Agriculture Research Center, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
3 Agricultural Innovation and Management Division (Pest Management), Faculty of Natural Resources, Prince of Songkla University, Hatyai, Songkhla 90110, Thailand; chaninun.p@psu.ac.th
4 Perkin Elmer Co. Ltd., 290 Soi 17, Rama 9 Rd., Bangkapi, Huay Kwang, Bangkok 10310, Thailand; Kitsada.pitija@perkinelmer.com
* Correspondence: anurag.su@psu.ac.th; Tel.: +66-74-28-6103

**Abstract:** Several mechanisms are involved in the biological control of plant pathogens by the soil-borne *Trichoderma* spp. fungi. The aim of this study was to characterize a new strain of *Trichoderma* as a potential biological control agent to control the postharvest anthracnose of chili pepper caused by *Colletotrichum gloeosporioides*. A total of nine strains of *Trichoderma* spp. were screened for their antifungal activity using a dual culture assay against *C. gloeosporioides*. *Trichoderma koningiopsis* PSU3-2 was shown to be the most effective strain, with a percentage inhibition of 79.57%, which was significantly higher than that of other strains (*p* < 0.05). In the sealed plate method, *T. koningiopsis* PSU3-2 suppressed the growth of *C. gloeosporioides* by 38.33%. Solid-phase microextraction (SPME) was applied to trap volatiles emitted by *T. koningiopsis* PSU3-2, and the GC/MS profiling revealed the presence of antifungal compounds including azetidine, 2-phenylethanol, and ethyl hexadecanoate. The production of cell-wall-degrading enzymes (CWDEs) was assayed through cell-free culture filtrate (CF) of PSU3-2, and the enzyme activity of chitinase and β-1,3-glucanase was 0.06 and 0.23 U/mL, respectively, significantly higher than that in the control (*p* < 0.05). Scanning electron microscopy of the mycelium incubated in cell-free CF of *T. koningiopsis* PSU3-2 showed the abnormal shape of *C. gloeosporioides* hyphae. Application of *T. koningiopsis* PSU3-2 by the dipping method significantly reduced the lesion size (*p* < 0.05) after inoculation with *C. gloeosporioides* compared to the control, and there was no disease symptom development in *T. koningiopsis* PSU3-2-treated chili pepper. This study demonstrates that *T. koningiopsis* PSU3-2 is an effective antagonistic microorganism and a promising biocontrol agent against postharvest anthracnose of chili pepper, acting with multiple mechanisms.

**Keywords:** in vitro tests; β-1,3-glucanase; chitinase; electron microscopy; GC/MS profiling

1. **Introduction**

Rhizosphere soil has long been considered as the main source of isolation of useful beneficial microorganisms [1,2]. At present, numerous soil fungi isolated from soil are employed as biological control agents, especially fungi in the genus *Trichoderma*. *Trichoderma* species are widely used to control numerous plant pathogens and reduce disease severity [3,4], due to their capacity for nutrient and space competition [5,6], parasitism [7], secretion of antimicrobial metabolites [7–10], activation of defense responses [11,12], and promotion of plant growth [8,9,13]. Moreover, metabolites, such as volatile organic compounds (VOCs), secreted from the *Trichoderma* species have been applied to promote plant growth [8,9,14]. Application of the *Trichoderma* species has been used to reduce the disease severity of leaf spots on lettuce [12] and sugar beet [15], as well as brown spots on rice [16]. Biological control presents low human health risks, as well as an environmentally friendly method without the excessive use of chemical fungicides in various crops.
Anthracnose is a common plant disease characterized by dark, sunken lesions on fruits, leaves, and stems containing conidia [17]. The causal agents of this disease, identified as *Colletotrichum* spp., reduce both the quality and the quantity of a harvest yield. Disease severity increases during the rainy season, as conidia of *Colletotrichum* are splashed and dispersed onto fresh fruit, resulting in secondary infection [18]. Anthracnose disease caused by *Colletotrichum* spp. has been reported to negatively impact the cultivation and production of mangoes [19,20], bananas [21], tomatoes [22], and chili peppers [23].

Chili anthracnose is a major constraint in chili production leading to huge losses, especially postharvest anthracnose, which causes the decay of chili pepper in tropical and subtropical regions [24,25]. Developing biological management strategies to control chili anthracnose may benefit disease management in chili peppers. This study, therefore, aimed to explore the potential of *Trichoderma* spp. isolated from soil as a biocontrol agent through dipping application. Multiple mechanisms of *Trichoderma* strains were tested for antifungal activity against *Colletotrichum gloeosporioides*.

2. Materials and Methods

2.1. Source of Trichoderma Species and Colletotrichum gloeosporioides

A total of nine *Trichoderma* strains, namely, *Trichoderma asperelloides* PSA-P1 [9], TSU1 [26], *Trichoderma asperellum* T76-14 [10], *T. koningiiopsis* PSU3-2 (GenBank accession no. LC600711 and LC600712), and *Trichoderma* sp. PSU1-1, Tri1-1, Tri1-2, Tri2-1, and Tri2-2, were obtained from the Culture Collection of Pest Management (CCPM), Faculty of Natural Resources, Prince of Songkla University, whereas *Colletotrichum gloeosporioides* causing postharvest anthracnose of chili pepper was obtained from the Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. *Trichoderma* and *C. gloeosporioides* were cultured on potato dextrose agar (PDA) (Himedia, Mumbai, India) at 28 ± 2 °C for 3 days before bioassays.

2.2. Dual Culture Assay

Nine strains of *Trichoderma* spp. were screened for antifungal activities on the mycelial growth of *C. gloeosporioides* through a dual culture assay on PDA plates [27]. An agar plug of a 5-day-old *C. gloeosporioides* colony was placed on the side of 9 cm Petri dishes, with an agar plug of each *Trichoderma* sp. placed on the opposite side 5 cm from the pathogen. PDA plates with pathogen alone served as the control. The experiment was designed according to a complete randomized block (CRD) with five replicates and repeated twice. The tested plates were incubated at ambient temperature (28 ± 2 °C) for 7 days. Colony radii of *C. gloeosporioides* were measured, and the percentage inhibition was calculated using the method of Rahman et al. [28], as given in Equation (1).

\[
\text{Percentage inhibition (\%) = } \frac{R_1 - R_2}{R_1} \times 100,
\]

where R1 is the radial growth of *C. gloeosporioides* in control, and R2 is the radial growth of *C. gloeosporioides* with treatment.

2.3. Volatile Antifungal Bioassay and Solid-Phase Microextraction GC/MS Analysis

The effect of volatiles emitted by *Trichoderma* spp. was examined using the sealed plate method [10,29]. The most effective *Trichoderma* isolate was cultured in a 20 mL chromatography vial, 20 mm in diameter (PerkinElmer, Waltham, MA, USA), and incubated at 28 ± 2 °C for 10 days. Volatiles emitted by *Trichoderma* were trapped by solid-phase microextraction (SPME) fibers and inserted into the injection port of an SQ8 gas chromatograph (PerkinElmer, Waltham, MA, USA). GC/MS conditions adhered to the method previously described by Phoka et al. [9] and Intana et al. [10]. Total volatiles released from *Trichoderma* were tentatively identified by a computer search of the National Institute of Standards and Technology (NIST) Mass Spectral Library Search Chromatogram.
2.4. Liquid-Phase Cultivation and Enzyme Assay

The effective Trichoderma spp. were cultivated in potato dextrose broth (PDB) and incubated at 28 ± 2 °C for 5 days according to the method of Wonglom et al. [6]. The PDB-cultured Trichoderma spp. were filtrated with a 0.45 µm Minisart® Syringe Filter (Sigma-Aldrich, St. Louis, MO, USA) and used as cell-free culture filtrate (CF). An enzyme assay was conducted to confirm that the cell-free CF of Trichoderma spp. contained cell-wall-degrading enzymes (CWDEs) responsible for the fungal cell-wall degradation, while chitinase and β-1,3-glucanase activities were assayed with 3,5-dinitrosalicylic acid (DNS), as suggested by Miller [30]. Reaction mixtures containing colloidal chitin were used as the substrate in the chitinase assay, whereas mixtures containing laminarin (Sigma-Aldrich, St. Louis, MO, USA) were used as the substrate in the β-1,3-glucanase assay. An assay with PDB alone served as the control. Reducing sugar released in the test reaction mixtures was measured using an ultraviolet/visible light (UV/Vis) spectrophotometer UV5300 (METASH, Shanghai, China) at 550 and 575 nm for β-1,3-glucanase and chitinase, respectively. Enzymes were assayed in three replicates, and the experiments were repeated twice.

2.5. Scanning Electron Microscopy

To test the effect of cell-free CF on fungal mycelia morphology, a scanning electron microscope (SEM) was utilized according to the method of Baiyee et al. [12]. A mycelial plug (0.5 cm) of a 7-day-old colony of C. gloeosporioides was incubated in the cell-free CF of effective Trichoderma strains at 37 °C for 1 h, whereas the control was incubated with PDB only. The mycelial plugs were fixed in 3% glutaraldehyde at 4 °C for 24 h and then dehydrated in a 30%, 50%, 60%, 70%, 80%, 90%, and 100% alcohol series, three times each. The samples were coated with gold and observed using a JSM-580 LV SEM (JEOL, Peabody, MA, USA) at the Science Equipment Center, Prince of Songkla University, Songkhla, Thailand.

2.6. In Vivo Test

A spore suspension of effective Trichoderma was prepared, and the concentration was adjusted with sterile distilled water (DW) to 1 × 10⁶ conidia/mL. A spore suspension of the Colletotrichum sp. was prepared in the same manner. Chili peppers were surface-disinfected with 70% ethanol, dipped in the spore suspension of Trichoderma spp., and air-dried in a laminar airflow cabinet. Chili peppers dipped in DW alone and the spore suspension of the Colletotrichum sp. served as the negative and positive controls, respectively. Then, 20 mL spore suspensions of C. gloeosporioides were sprayed onto the chili peppers after being dipped in the spore suspension of Trichoderma for 24 h and incubated in a moist box for 5 days, at which time the lesion development of all treated chili peppers was measured. Each treatment included five chili peppers (five replicates), and each experiment was repeated three times.

2.7. Statistical Analysis

The results regarding fungal inhibition, the enzyme assay, and lesion development were subjected to one-way analysis of variance (ANOVA). Statistically significant differences among treated samples were determined by Tukey’s test.

3. Results

3.1. Antifungal Activity of Trichoderma spp.

After incubation for 7 days, a smaller growth of C. gloeosporioides was observed in the dual culture plate than in the control plate. Nine strains of Trichoderma spp. inhibited the fungal growth of C. gloeosporioides in dual culture plates with inhibition percentages ranging from 60.84 to 79.57% (Figure 1). T. koningiopsis PSU3-2 was shown to be the most effective strain, with a percentage inhibition of 79.57%, statistically higher than that of other strains (p < 0.05) in this assay (Figure 1); therefore, the T. koningiopsis PSU3-2 strain was selected for further bioassays.
3. Results

3.1. Antifungal Activity of Trichoderma spp.

After incubation for 7 days, a smaller growth of \textit{T. koningiopsis} PSU3-2 was observed compared to \textit{T. koningiopsis} PSU1-1, PSU-P1, PSU3-2, T76-14, TSU1, Tri1-1, Tri1-2, Tri2-1, and Tri2-2 (Figure 1). The sealed plate method showed that \textit{T. koningiopsis} PSU3-2 inhibited the fungal growth of \textit{C. gloeosporioides}, with a percentage inhibition of 38.33%. This result reveals that \textit{T. koningiopsis} PSU3-2 produced volatile organic compounds which were responsible for suppressing the mycelial growth of \textit{C. gloeosporioides} in vitro. A total of 16 volatile compounds were detected in \textit{T. koningiopsis} PSU3-2 through GC/MS analysis. The volatile compounds contained carbon numbers ranging from C1 (fluoro(trinitro)methane) to C20 (ethyl (E)-octadec-9-enoate). The major compounds found in this study were 2-phenylethanol, fluoroethane, and 1-oxacyclotetradeca-4,11-diyne, with percentage peak areas of 14.94, 12.85, and 11.588%, respectively (Table 1). According to previous literature reviews, only three compounds were reported as volatile antifungal compounds (VOCs), namely, azetidine (1.507% peak area), 2-phenylethanol (14.941%), and ethyl hexadecanoate (9.036%). Figure 2 shows the mass spectrum of volatile antifungal compounds and their structures. No major peaks were observed in PDA alone, which served as the control group.

3.2. Production of Volatile Antifungal Compounds

The sealed plate method showed that \textit{T. koningiopsis} PSU3-2 inhibited the fungal growth of \textit{C. gloeosporioides}, with a percentage inhibition of 38.33%. This result reveals that \textit{T. koningiopsis} PSU3-2 produced volatile organic compounds which were responsible for suppressing the mycelial growth of \textit{C. gloeosporioides} in vitro. A total of 16 volatile compounds were detected in \textit{T. koningiopsis} PSU3-2 through GC/MS analysis. The volatile compounds contained carbon numbers ranging from C1 (fluoro(trinitro)methane) to C20 (ethyl (E)-octadec-9-enoate). The major compounds found in this study were 2-phenylethanol, fluoroethane, and 1-oxacyclotetradeca-4,11-diyne, with percentage peak areas of 14.94, 12.85, and 11.588%, respectively (Table 1). According to previous literature reviews, only three compounds were reported as volatile antifungal compounds (VOCs), namely, azetidine (1.507% peak area), 2-phenylethanol (14.941%), and ethyl hexadecanoate (9.036%). Figure 2 shows the mass spectrum of volatile antifungal compounds and their structures. No major peaks were observed in PDA alone, which served as the control group.

3.3. Cell-Wall-Degrading Enzyme Activities

The activity of CWDEs, including chitinase and $\beta$-1,3-glucanase, was assayed through the cell-free CF of \textit{T. koningiopsis} PSU3-2. The enzyme activity of chitinase and $\beta$-1,3-glucanase in the cell-free CF of \textit{T. koningiopsis} PSU3-2 was 0.061 and 0.227 U/mL (Figure 3), respectively, significantly higher (p < 0.05) than that in the control (PDB alone).

3.4. Effect of Cell-Free CF on Fungal Mycelia

SEM analysis was conducted to confirm the nature of the cell-free CF of \textit{T. koningiopsis} PSU3-2 containing CWDEs or antifungal compounds responsible for inhibiting the fungal growth of \textit{C. gloeosporioides}. The SEM micrograph of the control (PDB alone) exhibited no morphological change in the fungal mycelia of the \textit{Colletotrichum} sp. (Figure 4), whereas the fungal mycelia incubated in the cell-free CF of \textit{T. koningiopsis} PSU3-2 displayed abnormal shapes and mycelial distortions (Figure 4).

3.5. Effect of Trichoderma on Lesion Development

Treatment of \textit{T. koningiopsis} PSU3-2 using the dipping method prior to inoculation with \textit{Colletotrichum} sp. significantly reduced the size of anthracnose lesions (p < 0.05) analyzed for all chili peppers in all treatments. The lesion sizes developed on the chili pepper of the untreated control group, the \textit{Trichoderma} PSU3-2-treated chili pepper, and \textit{C. gloeosporioides} inoculation alone (control) were 0, 0, and 1.28 cm in diameter, respectively (Figure 5). There was no disease development in the \textit{T. koningiopsis} PSU3-2-treated chili pepper fruit after incubation for 5 days.

---

Figure 1. Percentage inhibition of \textit{Trichoderma} spp. against \textit{Colletotrichum} \textit{gloeosporioides}. Different letters indicate statistically significant differences among treatments (p < 0.05) using Tukey’s test.

Figure 2 shows the mass spectrum of volatile antifungal compounds and their structures. No major peaks were observed in PDA alone, which served as the control group.
Table 1. International Union of Pure and Applied Chemistry (IUPAC) names of volatile compounds produced by *T. koningiopsis* PSU3-2 identified through solid-phase microextraction (SPME)/GC/MS analysis.

| Retention Time | IUPAC Name                          | Percentage Match | Percentage Area | Formula   |
|----------------|-------------------------------------|------------------|-----------------|-----------|
| 1.463          | fluoro(trinitro)methane             | 95               | 4.2             | CFN₃O₆    |
| 1.528          | fluoroethane                        | 78.9             | 12.851          | C₂H₂F     |
| 2.274          | azetidine                           | 89.9             | 1.507           | C₃H₇N     |
| 5.824          | 3-isopropyl-5-methylhexan-2-one     | 71.8             | 1.581           | C₁₀H₂₀O   |
| 6.534          | 2-phenylethanol                      | 91.8             | 14.941          | C₆H₁₀O₇   |
| 6.71           | (4-nitrophenyl) heptanoate         | 79.2             | 3.181           | C₁₅H₁₇N₄O₄|
| 7.65           | 3-methylidene-1,2-dihydroindene     | 88.2             | 0.541           | C₁₀H₁₀    |
| 9.389          | (E)-2,5,6-trimethylhept-4-en-3-one  | 74.9             | 1.096           | C₁₀H₁₇O   |
| 10.95          | 1-oxacyclotetradeca-4,11-diyne     | 75.2             | 0.976           | C₁₃H₁₈O   |
| 11.09          | 1-oxacyclotetradeca-4,11-diyne     | 76.7             | 11.588          | C₁₃H₁₈O   |
| 11.75          | 2,4-di-tert-butylphenol            | 77.4             | 0.41            | C₁₄H₂₅O   |
| 13.03          | cyclohex-2-en-1-ylmethylbenzene     | 70.5             | 0.809           | C₁₃H₁₆    |
| 13.81          | 2,2-dimethyl-3-(3-methylpenta-2,4-dienyl)oxirane | 80       | 0.53            | C₁₀H₁₆O   |
| 14.59          | (9E,12E)-octadeca-9,12-dienoic acid | 80.2         | 1.131           | C₁₈H₃₅O₂  |
| 14.82          | ethyl (E)-octadec-9-enolate         | 81.5             | 3.631           | C₂₀H₃₈O₂  |
| 16.02          | ethyl pentadecanoate                | 83.2             | 1.452           | C₁₇H₃₉O₂  |
| 17.01          | ethyl hexadecanoate                 | 85.9             | 9.036           | C₁₈H₃₉O₂  |

Figure 2. Total ion chromatogram of volatile compounds identified from *T. koningiopsis* PSU3-2 through GC/MS analysis. Peaks at 2.27, 6.53, and 17.01 min were tentatively identified as azetidine, 2-phenylethanol, and ethyl hexadecanoate, the structures of which are shown. Numbers in parentheses indicate the percentage of peak areas.
Figure 3. Cell-wall-degrading enzyme activities of cell-free culture filtrate (CF) of T. koningiopsis PSU3-2: (A) enzyme activity of β-1,3-glucanase; (B) enzyme activity of chitinase. Different letters indicate statistically significant differences among treatments (p < 0.05) using Tukey’s test.

Figure 4. Effects of cell-wall-degrading enzymes on the fungal morphology of C. gloeosporioides (A) hypha of C. gloeosporioides incubated in potato dextrose broth alone; (B) hypha of C. gloeosporioides incubated in cell-free culture filtrate (CF) of T. koningiopsis PSU3-2.
and the quantity of chili pepper production emitted from species with a diversity of volatile compounds [31]. The VOCs emitted by T. koningiopsis [3,4,6,31] were documented as being capable of emitting VOCs to restrict the mycelial growth of S. sclerotiorum [3,4,6,31]. In vitro studies revealed the competition mechanism of T. koningiopsis against postharvest anthracnose of chili pepper fruit (Figure 4). Furthermore, treatment with T. koningiopsis PSU3-2 effectively suppressed the fungal growth of the C. gloeosporioides (Figure 3), along with overproduction of CWDEs leading to a morphological change in the C. gloeosporioides (Figure 4). Furthermore, treatment with T. koningiopsis PSU3-2 protected chili peppers from postharvest anthracnose decay (Figure 5).

The ability to compete for nutrients and space is commonly found in several Trichoderma spp. to overcome the growth of fungal pathogens through a dual culture assay [3,4,6,31]. In vitro studies revealed the competition mechanism of Trichoderma spp. against Sclerotium sclerotiorum [32], Rhizoctonia solani, Macrophomina phaseolina [33], and Curvularia oryzae [3]. Our findings in this study are in agreement with previous publications that found that T. koningiopsis PSU3-2 grew faster than the C. gloeosporioides, effectively inhibiting the growth of the C. gloeosporioides in PDA-assayed plates, thereby suggesting a competition mechanism involved in biocontrol activity (Figure 1).

VOCs have been reported as being produced and released by several Trichoderma species with a diversity of volatile compounds [31]. The VOCs emitted by Trichoderma species display multiple functions; they have antifungal properties, induce a defense response, and promote plant growth [8,9]. Among the 16 VOCs produced by T. koningiopsis PSU3-2, three compounds, namely, azetidine, 2-phenylethanol, and ethyl hexadecanoate, have been reported to have antimicrobial activity [34–36]. For instance, 2-phenylethanol emitted from T. asperellum T76-14 was reported to control the postharvest fruit rot of muskmelon [10]. Therefore, the VOCs of T. koningiopsis PSU3-2 containing azetidine, 2-
phenylethanol, and ethyl hexadecanoate may be associated with the suppression of the mycelial growth of the *C. gloeosporioides*, suggesting the antibiosis mechanism of *T. koningiopsis* PSU3-2. Several *Trichoderma* species produce and secrete hydrolytic enzymes responsible for degrading the fungal cell wall. The main CWDEs produced by *Trichoderma* species are chitinase and β-1,3-glucanase [37]. Chitinase restricts fungal growth by degrading chitin, the major component within the fungal cell wall [38], whereas β-1,3-glucanase hydrolyzes β-glucan to oligosaccharide and glucose [39]. A combination of both enzyme activities strongly suppresses the growth of several plant fungal pathogens [4]. Our results demonstrate a high activity of CWDEs in the cell-free CF of *T. koningiopsis* PSU3-2 (Figure 3), possibly related to the inhibition of fungal growth. We confirmed through SEM analysis that the cell-free CF of *T. koningiopsis* PSU3 contained CWDEs, which caused lysis and distortion of the *C. gloeosporioides* hyphae (Figure 4). The ability to produce CWDEs capable of creating mycelial lysis (holes), further resulting in fungal penetration in the host fungi, suggests mycoparasitism [40]. Baiyee et al. [4] similarly observed high activities of chitinase and β-1,3-glucanase, which caused abnormal changes in the fungal mycelia. These findings may be the result of CWDEs or some type of antifungal compound released by *T. koningiopsis* PSU3-2. However, we only studied the effects of cell-free CF, and we did not observe other metabolites in this study.

The application of a *Trichoderma* spore suspension has been shown to successfully control several plant diseases [3,16,41]. Treatment with a spore suspension of *Trichoderma spirale* T76-1 reduced the disease severity of lettuce leaf spots caused by *Corynespora cassiicola* and *Curvularia aeria* [4]. Root dipping with a *T. asperellum* T1 spore suspension was reported to activate defense responses in lettuce against leaf spot disease [12]. Treatment with *Trichoderma* protected tomato plants from infection by *Phytophthora nicotianae* [42]. Jogaiah et al. [43] demonstrated that the application of a *Trichoderma virens* spore suspension mediated resistance in tomatoes against *Fusarium* wilt by activating the jasmonic and salicylic pathways. Our study showed that chili peppers dipped in a spore suspension of *T. koningiopsis* PSU3-2 displayed no anthracnose lesions (Figure 5). Therefore, the biological activity of *T. koningiopsis* PSU3-2 is able to limit fungal infections, thereby controlling postharvest anthracnose of chili pepper fruit.

5. Conclusions

This study revealed the potential of a new strain of *T. koningiopsis* PSU3-2 isolated from soil as a biocontrol agent against anthracnose of chili pepper fruit caused by a *C. gloeosporioides*. The ability to compete for nutrients and space (competition), the production of VOCs (antibiosis), and the production of CWDEs (mycoparasitism) were the main factors contributing to its success in controlling the postharvest anthracnose of chili pepper fruit. The potential to develop a biopesticide to control chili anthracnose using *T. koningiopsis* PSU3-2 needs to be verified in the near future.

**Author Contributions:** Conceptualization, O.-U.R. and A.S.; methodology, O.-U.R., C.P., and K.P.; software, K.P.; validation, O.-U.R. and A.S.; formal analysis, K.P.; investigation, O.-U.R., C.P., and A.S.; resources, O.-U.R., and A.S.; data curation, K.P.; writing—original draft preparation, O.-U.R., C.P., and K.P.; writing—review and editing, A.S.; supervision and project administration, A.S.; funding acquisition, O.-U.R. and A.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Prince of Songkla University annual government statement of expenditure under the Plant Genetic Conservation Project under the Royal initiative of Her Royal Highness Princess Maha Chakri Sirindhorn, Year 2019, grant number NAT620297S.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** The authors would like to specially thank the Prince of Songkla University and the Center of Excellence in Agricultural and Natural Resources Biotechnology (CoE-ANRB) phase 3
for the facilities, PerkinElmer Co. Ltd., Bangkok, Thailand for the GC/MS analysis, the Innovative Agriculture Research Center, Faculty of Agriculture, Chiang Mai University for partial support, and MDPI’s English editing service for English editing.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Mendes, R.; Garbeva, P.; Raaijmakers, J.M. The rhizosphere microbiome: Significance of plant beneficial pathogenic, and human pathogenic microorganisms. *FEMS Microbiol. Rev.* 2013, 37, 634–663. [CrossRef] [PubMed]

2. Abdelmoteleb, A.; González-Mendoza, D. A novel *Streptomyces* rhizobacteria from desert soil with diverse anti-fungal properties. *Rhizosphere* 2020, 16, 100243. [CrossRef]

3. Sunpapao, A.; Chairin, T.; Ito, S. The biocontrol by *Streptomyces* and *Trichoderma* of leaf spot disease caused by *Curvularia oryzae* in oil palm seedlings. *Biol. Control.* 2018, 123, 36–42. [CrossRef]

4. Baiyee, B.; Pornsuriya, C.; Ito, S.; Sunpapao, A. The biocontrol by *Streptomyces* and *Trichoderma* of leaf spot disease caused by *Curvularia oryzae* in oil palm seedlings. *Biol. Control.* 2018, 123, 36–42. [CrossRef]

5. Sharma, V.; Salwan, R.; Sharma, P.N. The comparative mechanistic aspects of *Trichoderma* and probiotic: Scope for future research. *Physiol. Mol. Plant Pathol.* 2019, 129, 195–200. [CrossRef]

6. Wonglom, P.; Daengsuwan, W.; Ito, S.; Sunpapao, A. Biological control of *Sclerotium* fruit rot of snake fruit and stem rot of lettuce by *Trichoderma* sp. T76-12/2 and the mechanism involved. *Physiol. Mol. Plant Pathol.* 2019, 107, 1–7. [CrossRef]

7. Bailey, B.A.; Bae, H.; Strem, M.D.; Crozier, J.; Thomas, S.E.; Samuels, G.J.; Vinyard, B.T.; Holmes, K.A. Antibiogenesis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. *Biocontrol Sci. Technol.* 2008, 18, 24–35. [CrossRef]

8. Wonglom, P.; Ito, S.; Sunpapao, A. Volatile organic compounds emitted from endophytic fungus *Trichoderma asperellum* T1 mediated antifungal activity, defense response and promote plant growth in lettuce (*Lactuca sativa*) caused by *Fusarium incarnatum*. *J. Fungi* 2020, 6, 341. [CrossRef] [PubMed]

9. Intana, W.; Kheawleng, S.; Sunpapao, A. *Trichoderma asperellum* T76-14 Released Volatile Organic Compounds against Postharvest Fruit Rot in Muskmelons (*Cucumis melo*) Caused by *Fusarium oxysporum* f. sp. *lycopersici*. *Fungal Ecol.* 2020, 13, 100867. [CrossRef]

10. Vinodkumar, S.; Indumathi, T.; Nakkeeran, S. *Trichoderma asperellum* (NVTA2) as a potential antagonist for the management of stem rot in carnation under protected cultivation. *Biol. Control.* 2017, 113, 58–64. [CrossRef]

11. Aamir, M.; Kashyap, S.P.; Zehra, A.; Dubey, M.K.; Singh, V.K.; Ansari, W.A.; Upadhyay, R.S.; Singh, S. *Trichoderma conicum* bio-priming modulates the WRKYs defense programming in tomato against the *Fusarium oxysporum* f. sp. *lycopersici* (Fol) challenged condition. *Front. Plant Sci.* 2019, 10, 911. [CrossRef]

12. Zhang, S.; Gan, Y.; Xu, B. Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. *Front. Plant Sci.* 2016, 7, 1045. [CrossRef] [PubMed]

13. Vincenzi, V.B.; Aimone, P.; Chen, C.Q.; Yang, L.N.; Li, J.; Wang, X.; et al. First report of postharvest anthracnose on mango (*Mangifera indica*) caused by *Colletotrichum truncatum* in China. *Plant Dis.* 2017, 101, 833. [CrossRef]

14. Li, Q.; Shu, J.; Zhang, L.; Huang, S.; Guo, T.; Mo, J.; Ning, P.; Hsiang, T. First report of mango leaf anthracnose caused by *Colletotrichum truncatum* in Vietnam. *Plant Dis.* 2020, 104, 1558. [CrossRef]

15. Zakaria, L.; Mahak, S.; Zakaria, M.; Salleh, B. Characterisation of *Colletotrichum* species associated with anthracnose of banana. *Trop. Life Sci. Res.* 2009, 20, 119–125. [CrossRef]

16. Zhao, Y.; Zhao, C.; Liu, X. First report of anthracnose of Capsicum spp. caused by *Colletotrichum truncatum* in China. *Plant Dis.* 2014, 98, 678. [CrossRef]

17. Ali, A.; Bordoh, P.K.; Singh, A.; Siddiqui, Y.; Droby, S. Post-harvest development of anthracnose in pepper (*Capsicum* spp): Etiology and management strategies. *Crop Prot.* 2016, 90, 132–141. [CrossRef]

18. Yao, Y.Z.; Zhang, C.; Liu, F.; Wang, W.; Lai, L.; Cai, L.; Liu, X.-L. *Colletotrichum* species causing anthracnose disease of chili in China. *Persoonia* 2017, 38, 20–37. [CrossRef] [PubMed]
26. Ruangwong, O.-U.; 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, 7, 187. [CrossRef] [PubMed]

27. Castillo, F.D.H.; Padilla, A.M.B.; Morales, G.G.; Siller, M.C.; Herrera, R.R.; Gonzales, C.A.N.; Reyes, F.C. In vitro antagonistic action of *Trichoderma* strains against *Sclerotinia sclerotiorum* and *Sclerotium cepivorum*. *J. Fungi* 2021, 7, 187. [CrossRef]

28. Rahman, M.A.; Begum, M.F.; Alam, M.F. Screening of *Trichoderma* isolates as a biological control agent against *Ceratocystis paradoxa* causing pineapple disease of sugarcane. *Microbiology* 2009, 37, 277–285.

29. Dennis, C.; Webster, J. Antagonistic properties of species-groups of *Trichoderma*. *Trans. Br. Mycol. Soc.* 1971, 57, 41–48. [CrossRef]

30. Miller, G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 1959, 31, 426–428. [CrossRef]

31. Stracquadanio, C.; Quiles, J.M.; Meca, G.; Cacciola, S.O. Antifungal Activity of Bioactive Metabolites Produced by *Trichoderma asperellum* and *Trichoderma atroviride* in Liquid Medium. *J. Fungi* 2020, 6, 263. [CrossRef]

32. Matroudi, S.; Zamani, M.R.; Motallebi, M. Antagonistic effects of three species of *Trichoderma* sp. on *Sclerotium sclerotiorum*, the causal agent of canola stem rot. *Egypt. J. Biol.* 2009, 11, 37–44.

33. Monteiro, V.; Silva, R.N.; Steindorff, A.; Costa, F.; Noronha, E.; Ricart, C.; de Sousa, M.; Vainstein, M.; Monteiro, V.; Ulhoa, C. New insight in *Trichoderma harzianum* antagonism of fungal plant pathogens by secreted protein analysis. *Curr. Microbiol.* 2010, 61, 298–305. [CrossRef] [PubMed]

34. Choi, G.J.; Jang, K.S.; Choi, Y.H.; Yu, J.H.; Kim, J.-C. Antifungal activity of lower alkyl fatty acid esters against powdery mildews. *Plant Pathol. J.* 2010, 26, 360–366. [CrossRef]

35. Angel, L.P.L.; Yusof, M.T.; Ismail, I.S.; Ping, B.T.Y.; Azni, I.N.A.M.; Kamarudin, N.H.; Sundram, S. An in vitro study of antifungal activity of *Trichoderma virens* 7b and profile of its non-polar antifungal components released against *Ganoderma boninense*. *J. Microbiol.* 2016, 54, 732–744. [CrossRef]

36. Deep, A.; Kumar, P.; Narasimhan, B.; Lim, S.M.; Ramasamy, K.; Mishra, R.K.; Mani, V. 2-Azetidine Derivatives: Synthesis, antimicrobial, anticancer, evaluation and qsar studies. *Acta Pol. Pharm.* 2016, 73, 65–78. [PubMed]

37. Asad, S.A.; Tabassum, A.; Hameed, A.; Hassan, F.; Afzal, A.; Khan, S.A.; Ahmed, R.; Shahzad, M. Determination of lytic enzyme activities of indigenous *Trichoderma* isolates from Pakistan. *Braz. J. Microbiol.* 2015, 46, 1053–1064. [CrossRef]

38. Collinge, D.B.; Kragh, K.M.; Mikkelsen, J.D.; Nielsen, K.K.; Rasmussen, U.; Vad, K. Plant chitinases. *Plant J.* 1993, 3, 31–40. [CrossRef]

39. Pitson, S.M.; Seviour, R.J.; McDougall, B.M. Noncellulolytic fungal β-glucanases: Their physiological and regulation. *Enzym. Microb. Technol.* 1993, 15, 178–192. [CrossRef]

40. Sunpapao, A. *Antagonistic Microorganisms: Current Research and Innovations*; Lambert Academic Publishing: Saarbrücken, Germany, 2020; p. 120.

41. Dawidziuk, A.; Popiel, D.; Kaczmarek, J.; Strakowska, J.; Jedryczka, M. Optimal *Trichoderma* strains for control of stem canker of brassicas: Molecular basis of biocontrol properties and azole resistance. *BioControl* 2016, 61, 755–768. [CrossRef]

42. La Spada, F.; Stracquadanio, C.; Riolto, M.; Pane, A.; Cacciola, S.O. *Trichoderma* counteracts the challenge of *Phytophthora nicotianae* infections on tomato by modulating plant defense mechanisms and the expression of crinkler, necrosis-inducing *Phytophthora* protein 1, and cellulose-binding elicitor lectin pathogenic effectors. *Front. Plant Sci.* 2020, 11, 1–16.

43. Jogaiah, S.; Abdelrahman, M.; Tran, L.-S.P.; Ito, S. Different mechanisms of *Trichoderma virens*-mediated resistance in tomato against Fusarium wilt involve the jasmonic and salicylicacid pathways. *Mol. Plant Pathol.* 2018, 19, 870–882. [CrossRef]