



Research article

Evaluating compatibility of *Trichoderma asperellum* with dimethomorph and fluazinam for integrated disease management in durian

Jakkaris Cheamchit^a, Wiphawee Leesutthiphonchai^{a,†}, Netnapis Khewkhom^{a,†}, Rawat Piasai^{b,†}, Onuma Piasai^{a,*}

^a Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand

^b ADAMA (Thailand) Ltd., 127/33 Panjathani Tower 28th floor, Nonsi Rd, Chongnonsi, Yannawa, Bangkok 10120, Thailand

Article Info

Article history:

Received 4 September 2026

Revised 11 May 2026

Accepted 15 May 2026

Available online 10 June 2026

Keywords:

Dimethomorph,
Durian disease,
Fluazinam,
Integrated disease management,
Trichoderma asperellum

Abstract

Importance of the work: Fungicide-biocontrol compatibility for sustainable durian disease management remains underexplored.

Objectives: To evaluate the compatibility of *Trichoderma asperellum* with dimethomorph and fluazinam for sustainable durian disease management.

Materials and Methods: Pathogens from diseased durian tissues were identified morphologically and molecularly. Five *Trichoderma* isolates were screened; Tri-1 was confirmed as *T. asperellum*. Dual culture, poisoned food and soil assays were used to assess antagonistic activity and survival under fungicide treatments.

Results: Four pathogens (*Phytophthora palmivora*, *Fusarium solani*, *Lasioidiplodia theobromae*, *Phomopsis* sp.) were isolated. Tri-1 achieved the highest inhibition (up to 86.17% against *Phomopsis* sp.). Dimethomorph had no inhibitory effect on *T. asperellum*, while fluazinam suppressed growth by up to 55.13%. In dual culture, dimethomorph + *Trichoderma* achieved 90–100% pathogen inhibition, whereas fluazinam reduced *Trichoderma* viability. Based on the soil assay results, the microbial population was stable when dimethomorph was used (2.93×10^3 colony-forming units (CFU)/g soil– 3.67×10^3 CFU/g soil at 60 d), while it dropped sharply when fluazinam was used (0.12×10^3 CFU/g soil).

Main finding: *Trichoderma asperellum* was compatible with dimethomorph but was inhibited by fluazinam, which should be useful information in guiding fungicide selection for integrated durian disease management.

† Equal contribution.

* Corresponding author.

E-mail address: agromj@ku.ac.th (O. Piasai)

online 2452-316X print 2468-1458/Copyright © 2026. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), production and hosting by Kasetsart University Research and Development Institute on behalf of Kasetsart University.

<https://doi.org/10.34044/j.anres.2026.60.3.10>

Introduction

Durian (*Durio zibethinus* L.) is a high-value tropical fruit crop, with Thailand ranking among the world's leading producers and exporters (Ek-Amnuay, 2023). However, durian production is constrained increasingly by fungal pathogens that reduce tree vigor, yield and fruit quality; among these, *Phytophthora* spp. are particularly destructive, causing root and stem rot under wet conditions and leading to severe production losses (Suksiri et al., 2018; Chantarasiri and Boontanom, 2021; Singh et al., 2025). Additional pathogens of economic concern include *Colletotrichum* spp. (causing anthracnose), *Lasiodiplodia* spp. and *Phomopsis* spp. (causing fruit rot) and *Fusarium* spp. (causing vascular wilt and dieback) (Chantarasiri and Boontanom, 2021; Piasai et al., 2021; Pongpisutta et al., 2023).

Current disease management in durian orchards relies heavily on synthetic fungicides, particularly dimethomorph and fluazinam, which are routinely applied for their efficacy against key pathogens (Kongtragoul et al., 2021; Phetkhajone et al., 2021). However, intensive fungicide use has raised concerns about the emergence of resistant pathogen populations and unintended negative impacts on beneficial soil microorganisms, including biocontrol agents (BCAs) such as *Trichoderma* spp. (Monte, 2001; Drenth and Guest, 2016; Mukhopadhyay and Kumar, 2020). These limitations have underscored the importance of developing sustainable disease management approaches that minimize ecological disruption. Integrated disease management (IDM) strategies, which combine cultural practices, biological control and selective fungicide application, represent a potential pathway (Sabarre et al., 2021; Singh et al., 2025).

Within this framework, *Trichoderma asperellum* Samuels, Lieckf. & Nirenberg has gained attention as an effective BCA due to its broad-spectrum antifungal mechanisms, including mycoparasitism, competitive exclusion and systemic resistance induction in host plants (Daud et al., 2023). However, the integration of *T. asperellum* into an IDM system requires a clear understanding of its compatibility with the fungicides commonly applied in durian cultivation. Other research has suggested that certain *Trichoderma* strains tolerate specific fungicides, enabling potential combined applications (Drenth and Guest, 2016). Yet, information remains limited regarding the fungicide (particularly fluazinam, which disrupts mitochondrial respiration) on the growth and biocontrol efficacy of *T. asperellum* (Azni et al., 2019).

The present study addresses this knowledge gap by evaluating the compatibility of *T. asperellum* with dimethomorph and fluazinam, focusing on their effects on fungal growth and antagonistic activity against major durian pathogens. The aim was to guide IDM strategies fostering sustainable cultivation practices to safeguard long-term productivity and agroecosystem resilience.

Materials and Methods

Isolation, identification and pathogenicity of durian pathogens

Samples of 'Mon Thong' durian (*Durio zibethinus* L.) exhibiting disease symptoms were collected from orchards in Na Yai Am district, Chanthaburi province, Thailand, for pathogen isolation using the tissue transplanting method (Agrios, 2005). Under aseptic conditions, approximately 5 mm lesion margin tissues were excised with a flame-sterilized knife, surface-sterilized in 0.5% sodium hypochlorite for 3 min, rinsed with sterile water, blotted dry and placed on potato dextrose agar (PDA; Difco; Oxford, UK). Plates were incubated at 25°C for 3 d, after which hyphal tips were transferred to the PDA for purification. For the *Fusarium* isolates, subcultures were made on carnation leaf agar to promote sporulation and facilitate morphological characterization. The isolated fungal strains were maintained in the Kasetsart University Culture Collection (KUFC), Bangkok, Thailand under their respective KUFC accession numbers. Pathogen identification was conducted based on morphological features and molecular analysis.

Pathogenicity was assessed using fresh, physiologically mature durian leaves and fruit rinds. Samples were washed with tap water, surface-sterilized with 70% ethanol for 1 min, rinsed three times in sterile water and air-dried. Each sample was wounded with a sterile needle and mycelial agar plugs of each fungal isolate at age 7 d were inoculated onto the wound sites. Inoculated tissues were incubated in moist chambers (plastic boxes lined with moistened tissue paper) at 25°C. Symptom development was monitored daily and re-isolation of the fungi from symptomatic tissues was performed to fulfill Koch's postulates and confirm pathogen identity (Piasai et al., 2021).

Isolation, identification and screening of antagonistic *Trichoderma* species

Soil samples were collected from a ‘Mon Thong’ durian orchard in Thung Bencha subdistrict, Tha Mai district, Chanthaburi province, Thailand and *Trichoderma* spp. were isolated using the soil plate method (Warcup, 1950). Approximately 0.005–0.015 g of each soil sample was placed in a sterilized Petri dish containing Gochenaure’s glucose ammonium nitrate agar and incubated in darkness at 25°C for 2 d. Emerging colonies were transferred to PDA for purification and morphological observation.

The antagonistic activity of all isolates against durian pathogens was assessed using dual culture assays. The percentage of growth inhibition (PGI) was calculated at 7 d using Equation 1:

$$\text{PGI (\%)} = (C - T) / C \times 100 \quad (1)$$

where C is the colony diameter of the control and T is the colony diameter of the treatment, both measured in millimeters (Piasai et al., 2021). Overgrowth levels (OGLs) were scored in four replicates using : +++ (>50% coverage), ++ (21–50%), + (1–20%) and – (none). The isolate with the highest competitive ability was selected for species identification.

Macroscopic and microscopic features were examined using stereo (Olympus; Tokyo, Japan) and compound (Carl Zeiss; Jena, Germany) microscopes and compared with standard identification keys (Cai et al., 2022).

Molecular identification was performed by extracting DNA from PDA-grown mycelia using a lysis buffer (10 mM Tris-HCl, 50 mM ethylenediaminetetraacetic acid, 1% SDS), followed by extraction using a chloroform-to-isoamyl alcohol ratio of 24:1 and ethanol precipitation. The DNA purity and concentration were determined using a NanoDrop spectrophotometer (Thermo Scientific; Wilmington, DE, USA). Polymerase chain reaction (PCR) amplification targeted the internal transcribed spacer (ITS) region with primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATT GATATGC-3') (White et al., 1990) and the translation elongation factor 1-alpha (*tef1*) region with primers EF1-728F (5'-CATCGAGAAGTTCGAGAAGG-3') and TEF1LLRev (5'-AACTTGCAGGCAATGTGG-3') (Cai et al., 2022). Amplicons were sequenced by Macrogen Inc. (Seoul, Republic of Korea) using the Sanger method. Sequences were compared against GenBank using BLAST (National Center for Biotechnology Information, 2024) for species identification. Phylogenetic analyses were

conducted on concatenated ITS and *tef1* sequences. Alignments were generated using MUSCLE (Edgar, 2004) and trees were constructed with both maximum likelihood in the MEGA11 software (Tamura et al., 2021) using 1,000 bootstrap replicates and Bayesian inference in the BEAST v2.6.6 software (Bouckaert et al., 2019) applying a Markov Chain Monte Carlo approach until convergence was achieved (Bouckaert et al., 2019).

Competitive ability of *Trichoderma* in combination with dimethomorph and fluazinam to inhibit mycelial growth of durian pathogens under poisoned food conditions

The antagonistic activity of *Trichoderma* against durian pathogens was assessed using a dual culture assay combined with the poisoned food technique. Mycelial plugs (aged 7 d) of pathogens and *Trichoderma* were excised using a sterile cork borer and transferred onto PDA plates amended with different fungicide concentrations: dimethomorph 50% weight per volume suspension concentrate (w/v SC; 0.01 mL/L, 0.1 mL/L, 1 mL/L, 10 mL/L), fluazinam 50% w/v SC (0.01 mL/L, 0.1 mL/L, 1 mL/L, 10 mL/L) and their combination as dimethomorph 20% + fluazinam 20% w/v SC (0.02 mL/L, 0.2 mL/L, 2 mL/L, 20 mL/L). In dual culture, pathogen and *Trichoderma* plugs were placed opposite each other at 2 cm from the plate edge. Each treatment was performed in four replicates. Colony diameters were measured after 10 d of incubation period and the percentage of mycelial growth inhibition was calculated. The concentration of activator needed to produce 50% of the maximum response (EC_{50}) values were calculated following the method of Alexander et al. (1999).

Evaluation of survival of *Trichoderma asperellum* under co-application with fungicides for management of seedling rot in durian

A completely randomized design was used to evaluate the survival of *T. asperellum* under co-application with fungicides for the management of durian seedling rot. ‘Mon Thong’ durian seedlings (approximately 80 cm tall) were transplanted into 30 cm diameter pots containing autoclaved sandy loam soil (pH 6.5–6.8). The experiment was conducted under greenhouse conditions with an average temperature of 32–34°C. A spore suspension of *T. asperellum* (6.47×10^6 spores/mL) was applied as a 500 mL soil drench per plant. At 1 d after transplantation, seedlings were inoculated with

Phytophthora palmivora (Butler) Butler using a homogenized culture aged 14 d suspended in 10 mL of sterile water. After 24 hr of pathogen establishment, fungicides were applied as soil drenches at 500 mL per plant: dimethomorph 50% w/v SC (1.0 mL/L or 1.5 mL/L), fluazinam 50% w/v SC (0.5 mL/L or 0.75 mL/L), or dimethomorph 20% + fluazinam 20% w/v SC (1.0 mL/L or 1.5 mL/L). A non-treated control was included. Fungicide treatments were repeated 15 d after the initial application.

Soil samples (1 g) were collected at 15 d, 30 d, 45 d and 60 d after the second fungicide application. Populations of *Trichoderma* were determined using the soil dilution plate method on GAN medium, with three replications per treatment. The plates were incubated at room temperature in the dark for 48 hr and *Trichoderma* survival was assessed by counting all the *Trichoderma* colonies that had developed on the plates. The results were expressed as colony-forming units per gram of soil (CFU/g soil).

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA), in which each treatment combination was considered an independent treatment. Prior to analysis, the assumptions of ANOVA were evaluated by testing the normality of residuals using the Shapiro-Wilk test and the homogeneity of variances using Levene's test. All datasets satisfied these assumptions ($p \geq 0.05$); subsequently, they were subjected to one-way ANOVA. When significant differences were detected, treatment means were separated using Duncan's multiple range

test at the 5% significance level ($p < 0.05$). Statistical analyses were performed using the GraphPad Prism software (version 7.0; GraphPad Software, Inc.; Boston, MA, USA).

Results

Isolation, identification and pathogenicity of durian pathogens

Four fungal isolates were obtained from the soil, bark, stems, branches and leaves of the durian samples. Based on morphological characteristics and molecular analyses, the 4 isolates were identified as belonging to four genera: *P. palmivora* (KUFC 0091), *Fusarium solani* (Mart.) Sacc. (KUFC 0092), *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (KUFC 0093) and *Phomopsis* sp. (KUFC 0094). The pathogenicity of these four durian fungal pathogens was tested on durian 'Mon Thong' leaves. At 5 d after inoculation, symptoms of the disease were observed based on the detached leaf inoculation method. Initially, the leaves had a pale-yellow discoloration, which later progressed to dark brown tissue and the lesions expanded to cover almost the entire leaf by day 7 (Fig. 1). The pathogenicity of these same four fungal pathogens was also tested on the rind of durian 'Mon Thong'. At 3 d after inoculation, the durian rind showed signs of decay, particularly on the inner rind surface. White and dark gray hyphae were observed growing from the lesions and the rind tissue became soft and brown, eventually rotting by day 5 after inoculation (Fig. 1).

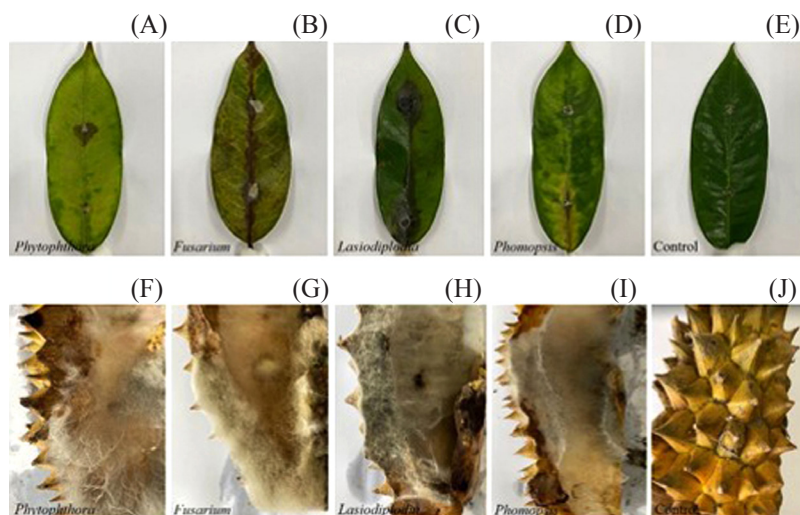


Fig. 1 Symptoms of durian (*Durio zibethinus* cv. 'Mon Thong') leaves (A–E) and fruit rind (F–J) 5 d after inoculation with: (A, F) *Phytophthora palmivora*; (B, G) *Fusarium solani*; (C, H) *Lasiodiplodia theobromae*; (D, I) *Phomopsis* sp.; (E, J) control, with infected tissues exhibiting necrosis and mycelial growth, whereas controls remained asymptomatic.

Antagonistic activity of *Trichoderma* isolates against durian pathogens

Five *Trichoderma* isolates—Tri-1 (KUFC 0101), Tri-2 (KUFC 0102), Tri-3 (KUFC 0103), Tri-4 (KUFC 0104) and Tri-5 (KUFC 0105)—were obtained from soil samples, with each having distinct morphological characteristics. The antagonistic activity of these isolates was evaluated against four fungal pathogens of durian using the dual culture technique. The effectiveness of these isolates in mycelial growth inhibition of the major durian pathogens was assessed based on the PGI and OGL in dual culture assays (Table 1). Tri-1 had the highest PGI against *Phomopsis* sp. and strong inhibition of *P. palmivora*, *F. solani* and *L. theobromae*. In addition, Tri-5 effectively suppressed *P. palmivora*, *F. solani* and *Phomopsis* sp. Both isolates demonstrated broad-spectrum activity, particularly against *P. palmivora* and *Phomopsis* sp. The OGL analysis showed that Tri-1 had the highest mycelial overgrowth

(+++), distinctly against *P. palmivora* and *Phomopsis* sp., while Tri-2 and Tri-5 displayed moderate to low coverage (+ to +++), respectively (Table 1). Mycoparasitism is a specialized antagonistic interaction in which one fungus parasitizes another, leading to the suppression or destruction of the host fungus. In the current study, *Trichoderma* isolate Tri-1 exhibited mycoparasitic activity against the durian fungal pathogens, based on the dual culture assays. Microscopic analysis revealed key mycoparasitic mechanisms, including hyphal coiling and direct penetration. Hyphal coiling, in which *Trichoderma* hyphae wrap around those of the target pathogen, likely restricts growth and induces cellular disruption. Additionally, hyphal penetration was observed, suggesting enzymatic degradation of the pathogen's cell wall and subsequent nutrient extraction (Fig. 2). These findings underscored the potential of the *Trichoderma* isolates, particularly Tri-1, as effective biocontrol agents against fungal pathogens associated with durian diseases.

Table 1 Percentage inhibition of mycelial growth of durian pathogens and overgrowth levels of *Trichoderma* isolates (Tri-1–Tri-5) in dual culture.

Trichoderma isolate	Durian pathogen							
	<i>P. palmivora</i>		<i>F. solani</i>		<i>L. theobromae</i>		<i>Phomopsis</i> sp.	
	PGI	OGL	PGI	OGL	PGI	OGL	PGI	OGL
Tri-1	67.92±0.18 ^a	+++	64.19±0.12 ^a	++	56.94±0.9 ^a	++	86.17±0.14 ^a	+++
Tri-2	64.68±0.17 ^a	+++	60.95±0.13 ^{ab}	-	46.25±0.43 ^b	-	73.14±0.21 ^c	+
Tri-3	62.75±0.13 ^a	++	56.17±0.31 ^b ^c	+	30.97±0.3 ^d	-	62.57±0.44 ^d	+
Tri-4	50.88±0.13 ^b	+++	51.57±0.35 ^c	-	36.25±0.1 ^d	-	60.45±0.28 ^d	+
Tri-5	61.99±0.27 ^a	++	63.85±0.20 ^a	-	43.33±0.30 ^c	-	80.63±0.12 ^b	+
%CV	6.51		5.46		12.65		4.74	

PGI = percentage of growth inhibition; OGL = overgrowth level (+++ >50% coverage, ++ = 21–50%, + = 1–20%, – = none); CV = coefficient of variation. Values (mean ± SD, *n* = 4) within the same column followed by different lowercase superscript letters are significantly (*p* < 0.05) different.

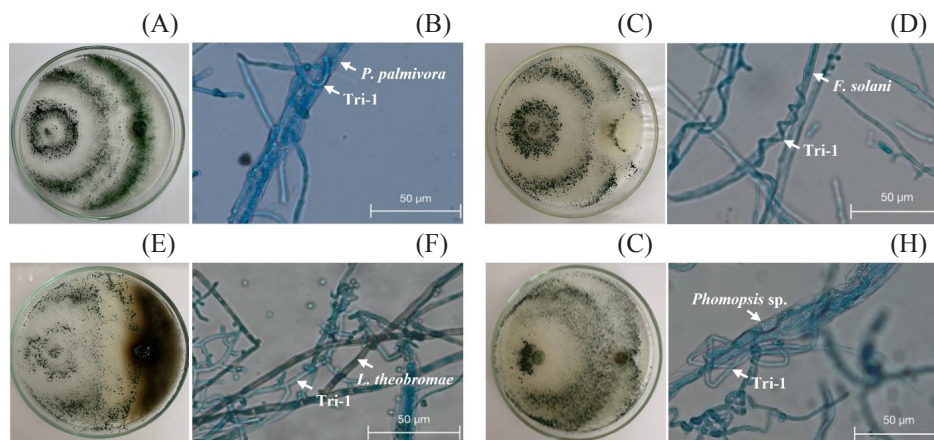


Fig. 2 Antagonistic activity of *Trichoderma asperellum* Tri-1 against durian pathogens in dual culture on potato dextrose agar after 7 d: (A, B) *Phytophthora palmivora*; (C, D) *Fusarium solani*; (E, F) *Lasiodiplodia theobromae*; (G, H) *Phomopsis* sp.

Identification of *Trichoderma* isolate Tri-1

The molecular identification of *Trichoderma* isolate Tri-1 was performed using the ITS and *tefl* regions. PCR products were sequenced using the Sanger method. Nucleotide sequences were analyzed and compared against the GenBank database using Nucleotide BLAST. The analysis indicated that the genetic sequence of isolate Tri-1 had 99.81% identity to *T. asperellum* based on the ITS region and 100% identity based on the *tefl* gene. These findings confirmed that isolate Tri-1 was *T. asperellum*. The phylogenetic analysis of Tri-1, along with the reported sequences of *T. asperellum* and other *Trichoderma* strains in the National Center for Biotechnology Information database, are illustrated in Fig. 3. The accession numbers of sequences used to generate the phylogenetic tree are displayed in Table 2.

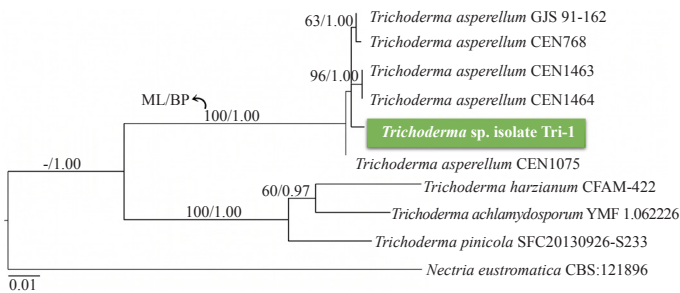


Fig. 3 Phylogenetic tree of *Trichoderma asperellum* Tri-1 (in green box) based on concatenated ITS and *tefl* sequences, including reference *Trichoderma* strains and the outgroup *Nectria eustromatica* CBS121896. The tree was inferred using maximum likelihood and Bayesian inference, with node value representing bootstrap support (%) / posterior probability.

Combined in vitro effects of dimethomorph, fluazinam and *Trichoderma asperellum* isolate Tri-1 against pathogenic fungi of durian

The efficacy of the *T. asperellum* isolate Tri-1 and fungicides (dimethomorph 50% w/v SC, fluazinam 50% w/v SC and their combination) against four fungal pathogens associated with durian diseases was evaluated across different fungicide concentrations (Fig. 4).

The combination of dimethomorph 20% + fluazinam 20% w/v SC had the highest efficiency regarding mycelial growth inhibition, achieving 100% inhibition of *P. palmivora* and *Phomopsis* sp. at 2 mL/L and above, while also having strong activity against *L. theobromae* (97.21% at 20 mL/L). Fluazinam 50% w/v SC alone was highly effective against *P. palmivora* and *Phomopsis* sp., reaching 100% inhibition at 10 mL/L, with dimethomorph 50% w/v SC also at 10 mL/L for

Table 2 Accession numbers used for phylogenetic analysis.

Species	Accession number	
	ITS	<i>tefl</i>
<i>Trichoderma asperellum</i> Tri-1 (present study)	PX495657	PX584305
<i>Trichoderma asperellum</i> CEN1463	MK714888	MK696646
<i>Trichoderma asperellum</i> CEN768	KC576729	ON101438
<i>Trichoderma asperellum</i> CEN1075	OM515033	ON101435
<i>Trichoderma asperellum</i> CEN1464	MK714908	MK696666
<i>Trichoderma asperellum</i> GJS 91-162	FJ442224	FJ463285
<i>Trichoderma achlamyosporum</i> YMF 1.06226	MN977791	MT070156
<i>Trichoderma pinicola</i> SFC20130926-S233	MH050354	MH025981
<i>Trichoderma afarasin</i> CEN141	OM515005	ON101407
<i>Trichoderma kunigamense</i> TAMA 193	AB807633	AB807645
<i>Nectria eustromatica</i> CBS:121896	MH863160	HM534875

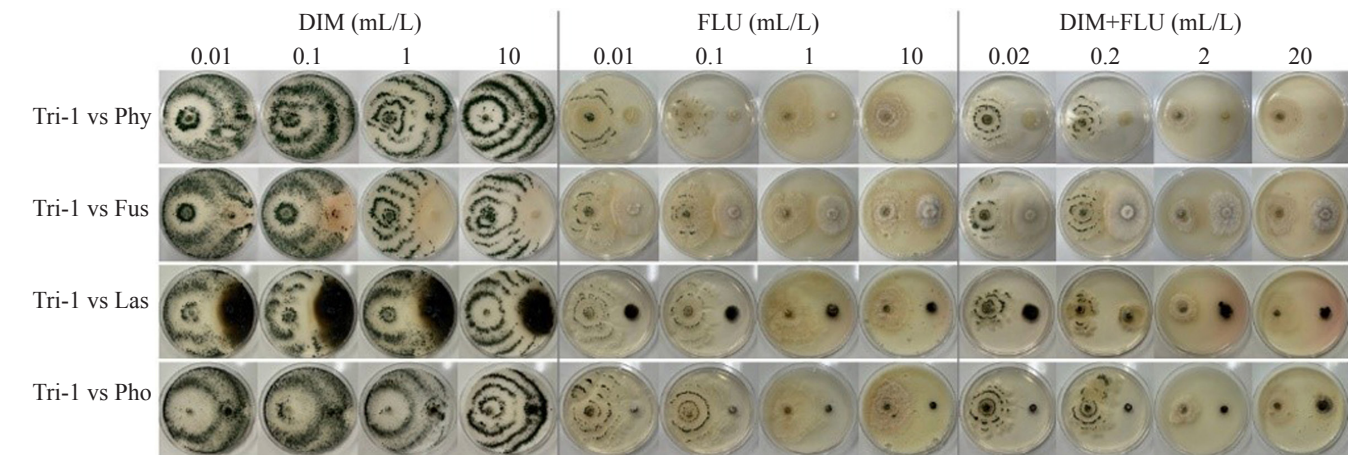


Fig. 4 Colony growth of *Trichoderma asperellum* Tri-1 versus (vs) that of four durian pathogens—*Phytophthora palmivora* (Phy), *Fusarium solani* (Fus), *Lasiodiplodia theobromae* (Las) and *Phomopsis* sp. (Pho)—in dual culture on potato dextrose agar amended with dimethomorph (DIM), fluazinam (FLU), or DIM + FLU combinations at different concentrations.

the complete inhibition of *P. palmivora*. Among the tested fungi, *F. solani* had the highest resistance, with the greatest inhibition (74.66%) observed at a concentration 0.01 mL/L dimethomorph 50% w/v SC, while lower efficacy was observed across other treatments. These findings indicated that the dimethomorph 20% + fluazinam 20% w/v SC combination at concentrations ≥ 2 mL/L was highly effective for broad-spectrum fungal disease management in durian, particularly against *P. palmivora* and *Phomopsis* sp. (Fig. 5, Table 3). The fungicidal efficacy of dimethomorph, fluazinam and their combination differed substantially among the tested pathogens (Table 4). For *P. palmivora*, dimethomorph and fluazinam had EC_{50} values of 419.41 mL/L and 527.50 mL/L, respectively, whereas the mixture markedly improved activity, reducing the EC_{50} to 153.49 mL/L. Against *F. solani*, dimethomorph showed very weak inhibition ($EC_{50} > 10,000$ mL/L), while fluazinam was more effective (148.61 mL/L); however, the mixture displayed only moderate inhibition (1,082.34 mL/L). For *L. theobromae*, fluazinam demonstrated greater activity (94.22 mL/L) than dimethomorph (1,119.21 mL/L), with the mixture resulting in the strongest inhibition, yielding the lowest EC_{50} value (49.16 mL/L). Against *Phomopsis* sp., only dimethomorph had measurable inhibition (1,778.28 mL/L), while fluazinam and the mixture produced similar inhibition

levels across all the tested concentrations; thus, EC_{50} values could not be determined for these two treatments.

The four durian pathogenic fungi were co-cultured with *T. asperellum* isolate Tri-1 on PDA medium supplemented with fungicides. Based on the experimental results, all the treatments had significantly different inhibition percentages and EC_{50} values compared to the control, suggesting that the application of *T. asperellum* in combination with fungicides could enhance disease control in durian. However, when evaluating the inhibition of *T. asperellum* growth on PDA medium containing fungicides, it was observed that dimethomorph 50% w/v SC had no effect on the growth of *T. asperellum*, showing no inhibition. In contrast, fluazinam 50% w/v SC produced varying degrees of inhibition on *T. asperellum* growth. The inhibition of *T. asperellum* at a given fungicide dose varied depending on the co-cultured pathogen. For example, at 10 mL/L fluazinam, the inhibition ranged from 47.50% with *P. palmivora* to 55.13% with *L. theobromae* (Fig. 5, Table 5). This variation suggests that interactions among the fungicide, *Trichoderma* and specific pathogens may influence the growth response, possibly through differences in metabolic activity or fungicide degradation in the dual culture. Further investigation is warranted to clarify these pathogen-dependent effects.

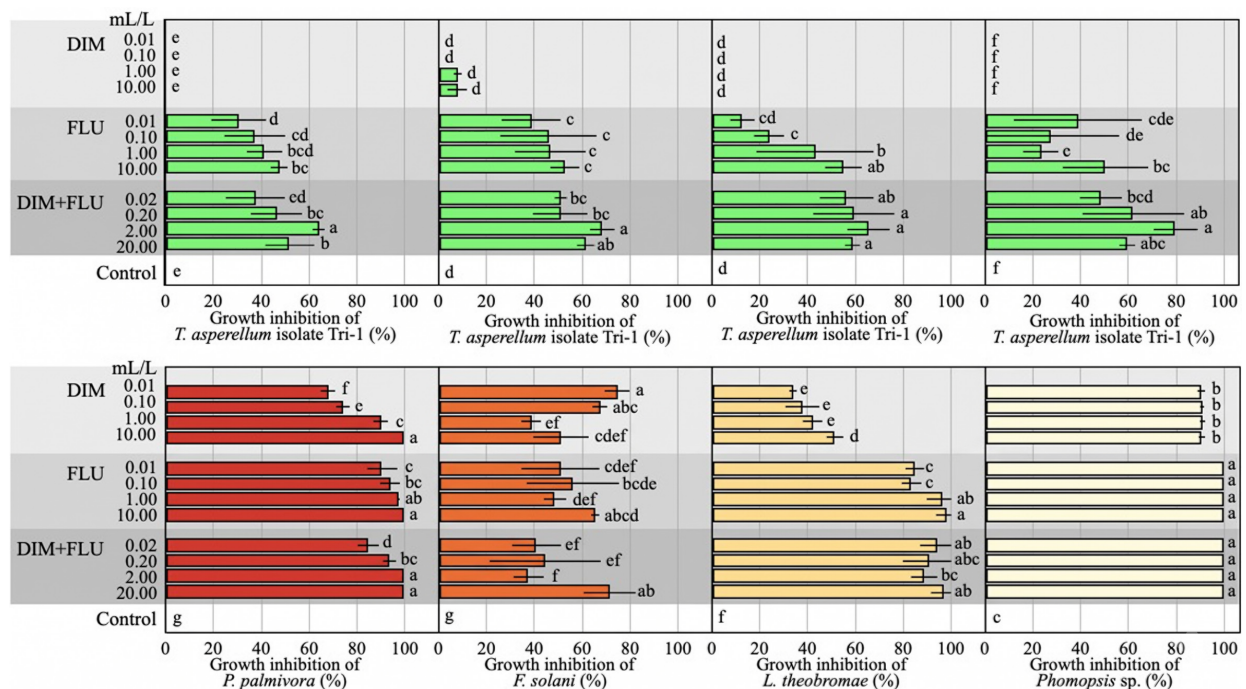


Fig. 5 Effects of fungicides on mycelial growth of *Trichoderma asperellum* Tri-1 (top) and four durian pathogens (bottom) in dual culture on PDA under different treatments and concentrations after 10 d, where DIM = dimethomorph 50% w/v SC, FLU = fluazinam 50% w/v suspension concentrate (SC) and DIM+FLU = combination of dimethomorph 20% + fluazinam 20% w/v SC. Different lowercase letters on horizontal columns denote significant differences ($p < 0.05$) among means.

Table 3 Inhibition of mycelial growth of durian pathogens by *Trichoderma asperellum* Tri-1 under fungicide treatments on potato dextrose agar after 10 d.

Fungicide	Dose		Mycelial growth inhibition of durian pathogen (%)			
	mL/L	mg a.i./L	<i>P. palmivora</i>	<i>F. solani</i>	<i>L. theobromae</i>	<i>Phomopsis</i> sp.
DIM	0.01	0.005	68.09±2.99 ^f	74.66±5.21 ^a	33.90±1.68 ^e	90.54±1.60 ^b
	0.1	0.05	74.14±2.53 ^e	67.47±3.03 ^{abc}	37.83±7.03 ^e	90.87±0.80 ^b
	1	0.5	90.00±3.17 ^c	38.70±3.93 ^{ef}	42.22±4.02 ^e	90.87±1.09 ^b
	10	5	100 ^a	51.10±11.60 ^{cdef}	51.40±3.48 ^d	90.71±1.33 ^b
FLU	0.01	0.005	90.47±6.44 ^c	50.86±16.21 ^{cdef}	85.06±3.77 ^c	100 ^a
	0.1	0.05	93.95±4.07 ^{bc}	56.16±19.28 ^{bcd}	83.47±4.28 ^c	100 ^a
	1	0.5	97.56±0.63 ^{ab}	48.46±4.75 ^{def}	96.62±6.76 ^{ab}	100 ^a
	10	5	100 ^a	65.41±1.72 ^{abcd}	97.94±4.11 ^a	100 ^a
DIM+FLU	0.02	0.004+0.004	84.66±4.39 ^d	40.75±10.31 ^{ef}	94.26±7.14 ^{ab}	100 ^a
	0.2	0.04+0.04	93.34±2.72 ^{bc}	44.52±23.37 ^{ef}	91.00±10.99 ^{abc}	100 ^a
	2	0.4+0.4	100 ^a	37.33±6.33 ^f	88.72±5.55 ^{bc}	100 ^a
	20	4+4	100 ^a	71.58±10.90 ^{ab}	97.21±5.58 ^{ab}	100 ^a
Control			0 ^g	0 ^g	0 ^f	0 ^c
%CV			3.47	22.69	8.11	0.77

a.i. = active ingredient; DIM = dimethomorph 50% w/v SC, FLU = fluazinam 50% w/v suspension concentrate (SC) and DIM+FLU = combination of dimethomorph 20% + fluazinam 20% w/v SC; CV = coefficient of variation.

Values (mean ± SD, $n = 4$) within the same column followed by different lowercase superscript letters are significantly ($p < 0.05$) different.

Table 4 EC₅₀ of dimethomorph, fluazinam and mixture of dimethomorph and fluazinam for *in vitro* inhibition of mycelial growth of four fungal pathogens.

Fungicide	EC ₅₀ of fungal pathogens (mL/L)			
	<i>P. palmivora</i>	<i>F. solani</i>	<i>L. theobromae</i>	<i>Phomopsis</i> sp.
DIM	419.41	>10,000.00	1,119.21	1,778.28
FLU	527.50	148.61	94.22	-
DIM+FLU	153.49	1,082.34	49.16	-

EC₅₀ = concentration of activator needed to produce 50% of the maximum response; DIM = dimethomorph 50% w/v SC, FLU = fluazinam 50% w/v suspension concentrate (SC) and DIM+FLU = combination of dimethomorph 20% + fluazinam 20% w/v SC.

Table 5 Mycelial growth inhibition of *Trichoderma asperellum* Tri-1 following co-cultivation with durian pathogens under fungicide treatments on potato dextrose agar after 10 d.

Fungicide	Dose		Mycelial growth inhibition of <i>Trichoderma asperellum</i> isolate Tri-1 (%)			
	mL/L	mg a.i./L	<i>P. palmivora</i>	<i>F. solani</i>	<i>L. theobromae</i>	<i>Phomopsis</i> sp.
DIM	0.01	0.005	0 ^e	0 ^d	0 ^d	0 ^f
	0.1	0.05	0 ^e	0 ^d	0 ^d	0 ^f
	1	0.5	0 ^e	8.01±1.85 ^d	0 ^d	0 ^f
	10	5	0 ^e	8.01±4.13 ^d	0 ^d	0 ^f
FLU	0.01	0.005	30.45±11.48 ^d	38.78±12.39 ^c	12.82±5.20 ^{cd}	38.94±26.67 ^{cde}
	0.1	0.05	37.34±12.73 ^{cd}	45.99±20.19 ^c	23.97±6.47 ^c	27.88±28.46 ^{de}
	1	0.5	41.35±7.44 ^{bcd}	46.63±14.78 ^c	43.27±24.58 ^b	23.56±7.46 ^e
	10	5	47.50±3.64 ^{bc}	52.72±5.88 ^{bc}	55.13±7.71 ^{ab}	50.48±17.82 ^{bc}
DIM+FLU	0.02	0.004+0.004	37.66±12.36 ^{cd}	51.12±2.68 ^{bc}	56.25±11.30 ^{ab}	48.30±8.84 ^{bcd}
	0.2	0.04+0.04	46.47±10.74 ^{bc}	50.80±11.54 ^{bc}	59.46±16.93 ^a	62.02±21.15 ^{ab}
	2	0.4+0.4	64.10±2.39 ^a	68.27±5.24 ^a	65.71±8.82 ^a	79.65±9.16 ^a
	20	4+4	51.76±10.38 ^b	61.54±3.73 ^{ab}	58.97±3.35 ^a	59.62±3.18 ^{abc}
Control			0 ^e	0 ^d	0 ^d	0 ^f
%CV			27.58	26.62	33.75	46.29

a.i. = active ingredient; DIM = dimethomorph 50% w/v SC, FLU = fluazinam 50% w/v suspension concentrate (SC) and DIM+FLU = combination of dimethomorph 20% + fluazinam 20% w/v SC; CV = coefficient of variation.

Values (mean ± SD, $n = 4$) within the same column followed by different lowercase superscript letters are significantly ($p < 0.05$) different.

Population dynamics of Trichoderma asperellum isolate Tri-1 in soil infested with Phytophthora palmivora under different fungicide treatments

The population dynamics of *T. asperellum* isolate Tri-1 in soil infested with *P. palmivora* under varying concentrations of dimethomorph and fluazinam over time are presented in Table 6 and Fig. 6. Across all time points, the control treatment consistently produced the highest *T. asperellum* population levels, with colony counts in the range 7.60×10^3 CFU/g soil– 8.00×10^3 CFU/g soil, indicating no adverse effects on fungal viability in the absence of fungicide application. Among the fungicide treatments, dimethomorph 50% w/v SC applied alone at concentrations of 1 mL/L or 1.5 mL/L maintained relatively high *T. asperellum* populations throughout the 60 d observation period. By day 60, colony counts remained above 2.93×10^3 CFU/g soil—significantly lower than the control,

yet significantly higher than those recorded for fluazinam or combination treatments—indicating a comparatively greater compatibility of *T. asperellum* with dimethomorph. Conversely, fluazinam 50% w/v SC applied at 0.5 mL/L or 0.75 mL/L had a prominent inhibitory effect on *T. asperellum* survival, with fungal populations declining sharply over time, falling as low as 0.12×10^3 CFU/g soil at 60 d. The combined application of dimethomorph 20% + fluazinam 20% w/v SC produced intermediate inhibitory effects, with colony counts in the range 0.14×10^3 CFU/g soil– 1.53×10^3 CFU/g soil at 60 d, depending on the concentration used. The resulting values were significantly depressed compared to the dimethomorph-only treatment, suggesting that the inclusion of fluazinam substantially compromised fungal viability, even when paired with a more favorable fungicide. These findings underscored the critical importance of fungicide selection in IDM strategies incorporating *Trichoderma*-based biocontrol.

Table 6 Population dynamics of *Trichoderma asperellum* Tri-1 in soil infested with *Phytophthora palmivora* under different fungicide treatments over time.

Fungicide	Dose		<i>T. asperellum</i> colony count (CFU $\times 10^3$ /g soil)			
	mL/L	mg a.i./L	15 d	30 d	45 d	60 d
DIM	1	0.5	6.13 $\pm$ 0.31 ^a	7.47 $\pm$ 1.01 ^a	4.33 $\pm$ 0.7 ^b	3.67 $\pm$ 0.58 ^b
	1.5	0.75	6.60 $\pm$ 0.92 ^a	8.07 $\pm$ 0.31 ^a	4.53 $\pm$ 0.76 ^b	2.93 $\pm$ 0.23 ^b
FLU	0.5	0.25	1.33 $\pm$ 0.15 ^b	1.93 $\pm$ 0.15 ^c	1.62 $\pm$ 0.21 ^d	0.21 $\pm$ 0.15 ^d
	0.75	0.375	1.00 $\pm$ 0.06 ^b	1.85 $\pm$ 0.15 ^c	1.53 $\pm$ 0.15 ^d	0.12 $\pm$ 0.1 ^d
DIM+FLU	1	0.2+0.2	1.73 $\pm$ 0.12 ^b	4.00 $\pm$ 0.4 ^b	3.00 $\pm$ 0.40 ^c	1.53 $\pm$ 0.31 ^c
	1.5	0.3+0.3	1.53 $\pm$ 0.12 ^b	1.87 $\pm$ 0.12 ^c	0.67 $\pm$ 0.42 ^d	0.14 $\pm$ 0.12 ^d
Control			7.60 $\pm$ 4.89 ^a	7.87 $\pm$ 0.81 ^a	8.00 $\pm$ 0.8 ^a	8.00 $\pm$ 1.79 ^a
%CV			50.74	11.25	16.18	30.69

a.i. = active ingredient; CFU = colony-forming units; DIM = dimethomorph 50% w/v SC, FLU = fluazinam 50% w/v suspension concentrate (SC) and DIM+FLU = combination of dimethomorph 20% + fluazinam 20% w/v SC; CV = coefficient of variation.

Values (mean $\pm$ SD, $n = 3$) within the same column followed by different lowercase superscript letters are significantly ($p < 0.05$) different.

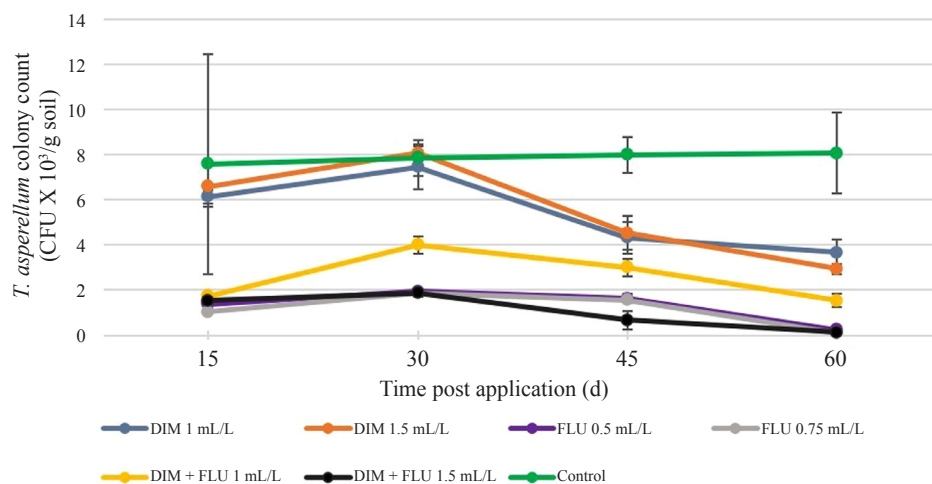


Fig. 6 Effects of dimethomorph (DIM), fluazinam (FLU) and DIM+FLU combinations at different concentrations on soil populations of *Trichoderma asperellum* Tri-1 during 60 d, where colony-forming units were measured at 15 d, 30 d, 45 d and 60 d post application, indicating that controls maintained stable populations, whereas fungicide treatments reduced populations, particularly at higher concentrations.

Discussion

Four fungal pathogens—*P. palmivora*, *F. solani*, *L. theobromae* and *Phomopsis* sp.—were isolated successfully from durian tissues and identified based on morphological and molecular methods. Pathogenicity assays on durian leaves and rinds verified their virulence, consistent with other reports that identified these fungi as major causal agents of durian diseases in Thailand (Leslie and Summerell, 2006; Chantarasiri and Boontanom, 2021; Ek-Amnuay, 2023; Pongpisutta et al., 2023; Sarker et al., 2023). These findings reinforce the importance of developing effective management strategies to mitigate pathogen-associated yield and quality losses.

The antagonistic assays demonstrated that *Trichoderma* isolate Tri-1 had the highest antagonistic potential among the isolates tested, suppressing pathogen growth through competitive exclusion and mycoparasitism. This activity was likely mediated by hydrolytic enzymes, including chitinases, β -glucanases and proteases, which degrade pathogen cell walls (Baek et al., 1999; Baiyee et al., 2019; Wonglom et al., 2019; Nguyen et al., 2020). The role of chitinase is particularly important, given the abundance of chitin in fungal cell walls. Similar antagonistic activity of *Trichoderma* spp. has been reported against a wide range of phytopathogens (Saikour, 2013; Sánchez-Montesinos et al., 2021; Singh et al., 2025), supporting the potential of Tri-1 as a promising biocontrol agent.

Furthermore, the molecular characterization confirmed Tri-1 as *T. asperellum*, with ITS and *tefl* sequences providing reliable resolution. While ITS is a widely used barcode, often it lacks discriminatory power at the species level within *Trichoderma* (Cai et al., 2022). In contrast, *tefl* offers greater phylogenetic resolution, particularly for species in the *T. harzianum* complex (Savitha and Sriram, 2015; Rahimi et al., 2021; Hussein and Saadullah, 2023). Therefore, in the present study, the combined use of ITS and *tefl* provided robust identification, ensuring accuracy in strain selection for biocontrol research and applications.

The fungicide compatibility assays revealed distinct responses of *T. asperellum* Tri-1 to the two fungicides tested. Dimethomorph (Fungicide Resistance Action Committee (FRAC) Group 40), a carboxylic acid amide fungicide that interferes with oomycete cell wall and cellulose deposition, allowed partial persistence of *T. asperellum* Tri-1, although its population gradually declined at higher concentrations (Bailey et al., 2010). This partial tolerance likely reflected

the difference in cell wall composition between *Trichoderma* (chitin-based) and oomycetes (cellulose-based), as the target site of dimethomorph is specific to cellulose synthesis. Hence, dimethomorph showed limited interference with the biocontrol fungus.

In contrast, fluazinam (FRAC Group 29), a fungicide that uncouples mitochondrial respiration, had pronounced inhibitory effects on *T. asperellum* growth, particularly under prolonged exposure (FRAC, 2025). The non-specific disruption of cellular energy metabolism by fluazinam can adversely affect both pathogenic and beneficial fungi, resulting in strong growth suppression. These findings underline that the compatibility between chemical fungicides and biocontrol agents is largely mechanism dependent.

Their combined application produced additive inhibitory effects, consistent with their distinct mechanisms of action (Woo et al., 2014). Although *Trichoderma* spp. have tolerance mechanisms, such as ATP-binding cassette transporters (Widmer, 2019), these defenses were insufficient under sustained fungicide pressure. These findings highlight the need for careful selection of fungicides in IDM to preserve the efficacy of biocontrol agents. Beyond durian systems, the present work provides broader implications for integrating *Trichoderma* spp. with fungicides in perennial fruit crops, offering a pathway toward reducing chemical inputs while improving ecological resilience. These findings should help with implementing broader efforts toward reducing chemical inputs and enhancing the ecological resilience of perennial fruit production systems in Southeast Asia.

The recovery of *Trichoderma* colonies from the soil microcosm indicated that *T. asperellum* could survive under co-application with fungicides. Colony counts represented total *Trichoderma* populations rather than being specific to the Tri-1 strain. However, since the soil used in this experiment was autoclaved prior to planting, it is reasonable to assume that the recovered colonies originated primarily from the introduced Tri-1 strain rather than from indigenous *Trichoderma* species. This assumption is supported by the sterile nature of the soil prior to inoculation. Nevertheless, molecular identification of recovered colonies is recommended for future studies to confirm this inference and to understand better the persistence and competitive ability of the Tri-1 strain under fungicide co-application conditions.

Conclusion

This study demonstrated that *T. asperellum* Tri-1 had strong antagonistic activity against major durian pathogens through competitive and enzymatic mechanisms, highlighting its potential as a reliable biological control agent. Molecular identification using ITS and *tef1* sequences provided robust species confirmation, underscoring the importance of multilocus approaches for accurate strain selection in biocontrol programs. Fungicide compatibility assays revealed that Tri-1 tolerated dimethomorph at moderate concentrations but was strongly inhibited by fluazinam, particularly under prolonged exposure or in combination treatments. These findings emphasize that the successful integration of *T. asperellum* into IDM programs depends on careful fungicide selection and dosage optimization. Field-based validation is recommended to confirm the compatibility of Tri-1 with chemical control approaches. Overall, the present work has provided practical insights for developing sustainable durian disease management strategies and broader implications for chemical dependency reduction while promoting ecological resilience in perennial fruit production systems.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This research was funded by Kasetsart University, Bangkok, Thailand through the Graduate School Fellowship Program. The Kasetsart University Research and Development Institute (KURDI) provided assistance with English editing.

References

- Agrios, G.N. 2005. Plant Pathology, 5th ed. Elsevier Academic Press, Burlington, MA, USA.
- Alexander, B., Browne, D.J., Reading, S.J., Benjamin, I.S. 1999. A simple and accurate mathematical method for calculation of the EC50. *J. Pharmacol. Toxicol. Methods* 41: 55–58. doi.org/10.1016/s1056-8719(98)00038-0
- Azni, I., Sundram, S., Ramachandran, V. 2019. Pathogenicity of Malaysian *Phytophthora palmivora* on cocoa, durian, rubber and oil palm determines the threat of bud rot disease. *For. Pathol.* 49: e12557. doi.org/10.1111/efp.12557
- Baek, J.M., Howell, C.R., Kenerley, C.M. 1999. The role of an extracellular chitinase from *Trichoderma virens* Gv29-8 in the biocontrol of *Rhizoctonia solani*. *Curr. Genet.* 35: 41–50. doi.org/10.1007/s002940050431
- Bailey, K.L., Boyetchko, S.M., Laengle, T. 2010. Social and economic drivers shaping the future of biological control: A Canadian perspective on the factors affecting the development and use of microbial biological control agents. *Biol. Control* 52: 221–229. doi.org/10.1016/j.biocontrol.2009.05.003
- Baiyee, B., Pornsuriya, C., Ito, S.I., Sunpapao, A. 2019. *Trichoderma spirale* T76-1 displays biocontrol activity against leaf spot on lettuce (*Lactuca sativa* L.) caused by *Corynespora cassiicola* or *Curvularia aeria*. *Biol. Control.* 129: 195–200. doi.org/10.1016/j.biocontrol.2018.10.018
- Bouckaert, R., Vaughan, T.G., Barido-Sottani, J., et al. 2019. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* 15: e1006650.
- Cai, F., Dou, K., Wang, P., Chenthamara, K., Chen, J., Druzhinina, I.S. 2022. The current state of *Trichoderma* taxonomy and species identification. In: Amaresan, N., Sankaranarayanan, A., Dwivedi, M.K., Druzhinina, I.S. (Eds.). *Advances in Trichoderma Biology for Agricultural Applications*. Springer, Cham, Switzerland.
- Chantarasiri, A., Boontanom, P. 2021. *Fusarium solani* and *Lasiodiplodia pseudotheobromae*, fungal pathogens causing stem rot disease on durian trees (*Durio zibethinus*) in eastern Thailand. *New Dis. Rep.* 44: e12026. doi.org/10.1002/ndr2.12026
- Daud, M., Abualqumssan, A., Rashid, F., Saad, M. 2023. Durian disease classification using transfer learning for disease management system. *J. Inf. Syst. Technol. Manag.* 8: 67–77. doi.org/10.35631/JISTM.833006
- Drenth, A., Guest, D. 2016. Fungal and oomycete diseases of tropical tree fruit crops. *Annu. Rev. Phytopathol.* 54: 373–395. doi.org/10.1146/annurev-phyto-080615-095944
- Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32: 1792–1797. doi.org/10.1093/nar/gkh340
- Ek-Amnuay, P. 2023. Durian, King of Fruits II. Siam Insect-Zoo & Museum. Chiang Mai, Thailand. [in Thai]
- Fungicide Resistance Action Committee. 2025. FRAC Code List® 2024: Fungal control agents sorted by cross-resistance pattern and mode of action. <https://www.frac.info/media/kufnaceb/frac-code-list-2024.pdf>, 25 April 2025.
- Hussein, L., Saadullah, A. 2023. DNA Marker Identification of *Trichoderma* and *Fusarium* Level Species. *IOP Conf. Ser.: Earth Environ. Sci.* 1252: 012175. doi.org/10.1088/1755-1315/1252/1/012175
- Kongtragoul, P., Ishikawa, K., Ishii, H. 2021. Metalaxyl resistance of *Phytophthora palmivora* causing durian diseases in Thailand. *Horticulturae* 7: 375. doi.org/10.3390/horticulturae7100375
- Leslie, J.F., Summerell, B.A. 2006. The *Fusarium* Laboratory Manual. Blackwell Professional, Ames, IA, USA.
- Monte, E. 2001. *Trichoderma* spp. as biocontrol agents: applications and mechanisms. *Phytopathology* 91: 455–463. doi.org/10.5772/intechopen.1003697
- Mukhopadhyay, R., Kumar, D. 2020. *Trichoderma*: a beneficial antifungal agent and insights into its mechanism of biocontrol potential. *Egypt. J. Biol. Pest Control* 30: 133. doi.org/10.1186/s41938-020-00333-x

- National Center for Biotechnology Information. 2024. BLAST: Basic Local Alignment Search Tool. <https://blast.ncbi.nlm.nih.gov/Blast.cgi>, 27 June 2024.
- Nguyen, H.L., Nguyen, D.H., Hoang, T.Q., Tran, T.L., Tran, T.T.H. 2020. Characterisation and antifungal activity of extracellular chitinase from a biocontrol fungus *Trichoderma asperellum* PQ34. *Mycology* 11: 38e48. doi.org/10.1080/21501203.2019.1703839
- Phetkhajone, S., Pichakum, A., Songnuan, W. 2021. The study of the kinetics of metalaxyl accumulation and dissipation in durian (*Durio zibethinus* L.) leaf using high-performance liquid chromatography (HPLC) technique. *Plants* 10: 708. doi.org/10.3390/plants10040708
- Piasai, R., Chalmers, P., Piasai, O., Khewkhom, N. 2021. Postharvest fungicide dips to control fruit rot of 'Monthong' durian (*Durio zibethinus*). *Eur. J. Plant Pathol.* 160: 325–336. doi.org/10.1007/s10658-021-02246-3
- Pongpisutta, R., Keawmanee, P., Sanguansub, S., Dokchan, P., Bincader, S., Phuntumart, V., Rattanakreetakul, C. 2023. Comprehensive investigation of die-back disease caused by *Fusarium* in durian. *Plants* 12: 3045. doi.org/10.3390/plants12173045
- Rahimi, M.J., Cai, F., Grujic, M., Chenthamara, K., Druzhinina, I.S. 2021. Molecular Identification of *Trichoderma reesei*. In: Mach-Aigner, A.R., Martzy, R. (Eds.). *Trichoderma reesei*. Methods in Molecular Biology. Humana Press. New York, NY, USA, pp. 157–175.
- Sabarre, A., Navidad, A., Torbela, D., Adtoon, J. 2021. Development of durian leaf disease detection on android device. *IJECE* 11: 4962. doi.org/10.11591/ijece.v11i6.pp4962-4971
- Saikeur, A. 2013. Isolation and screening of *Trichoderma* spp. from bamboo soil in Nam Tok Yong National Park, Nakhon Si Thammarat Province for inhibition of *Phytophthora palmivora* (Butler) Butler. *PAST* 3: 1–13. [in Thai]
- Sánchez-Montesinos, B., Santos, M., Moreno-Gavira, A., Marín-Rodulfo, T., Gea, F.J., Diáñez, F. 2021. Biological control of fungal diseases by *Trichoderma aggressivum* f. *europaeum* and its compatibility with fungicides. *J. Fungi* 7: 598. doi.org/10.3390/jof7080598
- Sarker, S.R., Burgess, T.I., Hardy, G.E.S.J., McComb, J. 2023. Closing the gap between the number of *Phytophthora* species isolated through baiting a soil sample and the number revealed through metabarcoding. *Mycol. Prog.* 22: 39. doi.org/10.1007/s11557-023-01892-7
- Savitha, M., Sriram, S. 2015. Morphological and molecular identification of *Trichoderma* isolates with biocontrol potential against *Phytophthora* blight in red pepper. *PMHE* 21: 194–202.
- Singh, A., Chow, C., Nathaniel, K., Vun, Y.L., Javad, S., Jabeen, K. 2025. Management of *Phytophthora* and *Phytophthium* oomycete diseases in durian (*Durio zibethinus*). *Crop Prot.* 190: 107086. doi.org/10.1016/j.cropro.2024.107086
- Suksiri, S., Laipasu, P., Soyong, K., Poeaim, S. 2018. Isolation and identification of *Phytophthora* sp. and *Pythium* sp. from durian orchard in Chumphon Province, Thailand. *Int. J. Agric. Technol.* 14: 389–402.
- Tamura, K., Stecher, G., Kumar, S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* 38: 3022–3027. doi.org/10.1093/molbev/msab120
- Warcup, J. 1950. The soil-plate method for isolation of fungi from soil. *Nature* 166: 117–118.
- White, T.J., Bruns, T.D., Lee, S.B., Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.). *PCR Protocols: A Guide to Methods and Applications*. Academic Press. San Diego, CA, USA, pp. 315–322.
- Widmer, T.L. 2019. Compatibility of *Trichoderma asperellum* isolates to selected soil fungicides. *Crop Prot.* 120: 91–96. doi.org/10.1016/j.cropro.2019.02.017
- Wonglom, P., Daengsuwan, W., Ito, S.I., Sunpapao, A. 2019. Biological control of *Sclerotium* fruit rot of snake fruit and stem rot of lettuce by *Trichoderma* sp. T76-12/2 and the mechanisms involved. *Physiol. Mol. Plant Pathol.* 107: 1–7. doi.org/10.1016/j.pmpp.2019.04.007
- Woo, S.L., Ruocco, M., Vinale, F., et al. 2014. *Trichoderma*-based products and their widespread use in agriculture. *Open Mycol. J.* 8: 71–126. doi.org/10.2174/1874437001408010071