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Antagonistic Potential of Trichoderma Isolates from Bamboo and Cardamom Rhizospheres Against Chili Anthracnose Pathogen

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ABSTRACT

Chili production in Indonesia has been severely impacted by anthracnose, a disease caused by *Colletotrichum* species, resulting in significant yield losses. Chemical control methods are generally not recommended due to their limited effectiveness and potential environmental harm. This study explored the use of *Trichoderma* isolates from the rhizospheres of bamboo and cardamom plants as biocontrol agents against *Colletotrichum*. Soil samples were collected, and *Trichoderma* spp. were isolated and identified using both morphological and molecular methods, including ITS region sequencing. Two isolates, BmGr (*Trichoderma virens*, GenBank accession: PQ066098) and KpGr (*Trichoderma hamatum*, GenBank accession: PQ066099), were identified. Dual culture assays revealed that both isolates demonstrated strong antagonistic activity against *Colletotrichum* via competition and mycoparasitism, with inhibition rates were 63.49% for BmGr and 59.84% for KpGr, respectively. These findings highlight the potential of *T. virens* and *T. hamatum* as sustainable alternatives to chemical fungicides, promoting environmentally friendly management strategies for chili anthracnose. Further research, including field trials, is essential to optimize their application and enhance their effectiveness in diverse agricultural systems.

1. INTRODUCTION

Chili is a vital agricultural commodity in Indonesia, essential for domestic consumption and the national economy. The demand for chili continues to grow with the increasing population and expanding industries that use chili as a raw material. Economic analysis indicates that chili farmers can achieve substantial profits, averaging around IDR 73.39 million per growing season after costs. However, chili production has decreased in recent years, from 1.5 million tons in 2020 to 1.39 million tons in 2021, reflecting a concerning decline [1-6].

Chili production in Indonesia faces significant challenges from various diseases, with anthracnose, caused by *Colletotrichum* spp., being one of the most destructive [7-10]. Recent studies have identified new pathogens causing chili anthracnose, such as *Colletotrichum sojae* in China [11] and *Colletotrichum queenslandicum* in Mauritus [12]. These findings highlight the need for continuous monitoring and research to understand the evolving epidemiology of chili anthracnose and to develop innovative control methods to mitigate its impact on chili yield and quality.

Anthracnose leads to substantial yield losses and affects the quality of chili fruits, posing significant challenges for farmers and the agricultural industry. The disease impact varies with environmental conditions, especially during the rainy season when losses can range from 10% to 80% due to favorable humidity and temperature. In contrast, during the dry season, yield losses are typically lower, ranging from 2% to 35%. In extreme cases, particularly in swampy areas during the rainy season and in highly susceptible varieties, yield losses can reach as high as 50% to 100% [3, 13-15].

Chili anthracnose manifests with various symptoms affecting leaves, stems, and fruits. The disease typically begins on fruits with small, slightly sunken, dark yellow spots that darken and enlarge, often merging in high humidity or wet weather. These spots may develop concentric rings resembling a target due to spore mass arrangements. Severe infections lead to extensive necrotic tissue, causing significant marketability issues and post-harvest rots, further diminishing quality. Irregularly shaped brown spots with dark borders can appear on leaves, though less common than fruit symptoms, still indicating severe infection. Infected plants may also experience dieback, where parts of the plant, including stems and branches, start to wilt and die [16-19]. The epidemiology of chili anthracnose is influenced by environmental factors such as rain, which can favor disease development [20].

Adopting sustainable and eco-friendly strategies is crucial for mitigating anthracnose in chili. Utilizing biological control techniques, particularly involving native antagonists like *Trichoderma* species, offers a viable alternative to chemical fungicides. *Trichoderma* species are recognized for their

ability to control pathogens through methods like mycoparasitism, competition, and production of antimicrobial substances. Additionally, they enhance plant growth and boost disease resistance [18, 21-23].

Research indicates that *Trichoderma harzianum* and *Trichoderma asperellum* are particularly effective against *Colletotrichum* species. For example, *T. harzianum* has been shown to significantly reduce disease incidence and enhance plant growth in chili peppers infected with *Colletotrichum* [24, 25]. Similarly, *Trichoderma viride* has been reported to significantly reduce the severity of anthracnose on chili fruits, with culture filtrates demonstrating effective inhibition of mycelial growth and spore germination [26], suggesting that the metabolites produced by *Trichoderma* are directly antagonistic to the pathogen [27, 28]. Field trials have corroborated these findings, revealing that pre-treatment of chili seeds with *Trichoderma* not only reduces the incidence of anthracnose but also improves overall plant health and yield [25, 29].

The integration of *Trichoderma* spp. into agricultural practices can thus be seen as a sustainable approach to managing anthracnose in chili peppers, aligning with the growing emphasis on environmentally friendly disease control methods [30, 31]. Previous research has shown promising results when using *Trichoderma* spp. From agricultural soils in managing other *Colletotrichum*-related diseases, but the unique microbiomes of bamboo and cardamom rhizospheres may provide novel strains with improved efficacy against *Colletotrichum* spp. According to studies [32, 33], bamboo and cardamom plants are known to support diverse microbial communities within their rhizospheres, which may contain powerful biocontrol agents, harbor unique *Trichoderma* isolates with enhanced biocontrol potential. Furthermore, this study aims to address a critical gap in current knowledge by investigating whether isolates from these unique rhizospheres exhibit distinct or enhanced biocontrol properties compared to existing agents.

This research aims to identify and evaluate *Trichoderma* isolates from the rhizospheres of bamboo and cardamom plants. These isolates are being studied for their ability to counteract *Colletotrichum* species, which are the pathogens responsible for chili anthracnose. This study seeks to isolate and characterize these *Trichoderma* isolates to find effective biocontrol agents that can be utilized in sustainable management practices for chili anthracnose.

The objectives of this research are twofold: (1) to isolate and identify *Trichoderma* spp. from the rhizospheres of bamboo and cardamom, and (2) to evaluate the antagonistic potential of these isolates against *Colletotrichum* sp. under in vitro conditions. Through this approach, we aim to contribute to developing integrated disease management strategies that are environmentally friendly and effective in reducing the impact of chili anthracnose.

2. MATERIALS AND METHODS

2.1 Isolation of *Trichoderma* spp.

Rhizospheric soil samples were collected from healthy bamboo and cardamom plants in Sindanggalih, Karangtengah, Garut District (latitude: -7.1762367, longitude: 108.0425615, altitude: 1157 m asl), at a depth of 30 cm and placed into sterile plastic bags. The soil samples were air-dried and sieved to remove debris. Subsequently, 10 g of soil was suspended in 90 mL of sterile distilled water and serially diluted up to 10^{-5} [34]. From each dilution, 0.1 mL was spread on Potato Dextrose Agar plates supplemented with 1.4 mL of 50 mL/L lactic acid to inhibit bacterial growth. The plates were incubated at 28°C for 5-7 days, after which emerging *Trichoderma* colonies were sub-cultured onto fresh PDA plates for purification.

2.2 Morphological characterization of *Trichoderma* spp.

The morphological characterization of *Trichoderma* spp. involved both macroscopic and microscopic observations. For macroscopic observation, the pure isolates of *Trichoderma* spp. were sub-cultured on PDA plates in triplicate and incubated at 28 ± 2°C for 5-7 days. During this period, colony morphology, including color, texture, and growth rate, was recorded from three separate plates to ensure consistency. For microscopic observation, a small portion of the colony from each replicate was mounted on a clean glass slide, covered with a clean coverslip, and observed under an Olympus CX33 microscope to examine the conidiophore structure, phialide arrangement, and conidia shape and size. Measurements and observations were averaged across replicates. Photographs were taken for record and comparison with studies [35, 36], and the images were scaled using ImageRaster software.

2.3 Molecular identification of *Trichoderma* spp.

DNA extraction was performed by harvesting and grinding fungal mycelium of each individual isolate in liquid nitrogen, followed by extracting fungal DNA using Genomic DNA Mini Kit (Plant). PCR amplification targeted the ITS region of the ribosomal DNA using the primers ITS1-F (5'-TCCGTAGGTGAACCTGCGG-3') and ITS2-R (5'-GCTGCGTTCTTCATCGATGC-3') according to the research [37], with PCR conditions consisting of an initial denaturation at 95°C for 2 minutes, followed by 35 cycles of 95°C for 30 seconds, 55°C for 40 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 5 minutes. The PCR product was confirmed through 1% (w/v) agarose gel electrophoresis and visualized under UV transilluminator. Sequencing was conducted for the purified PCR products, which were sent to PT. Widya Teknologi Hayati (Yogyakarta, Indonesia). A phylogenetic tree was constructed using MEGA XI with the Maximum Likelihood method, 1000x bootstrap, and species sequence data from GenBank NCBI (Table 1). *Colletotrichum asianum* was used as the outgroup. The *Trichoderma* spp. sequences that had been analyzed were deposited in GenBank to obtain accession numbers.

2.4 Dual culture test

The antagonism test was conducted using the dual culture method [38, 39]. *Colletotrichum* sp. isolate BCG1 obtained from Crop Protection Laboratory Universitas Siliwangi collection was recultured on PDA medium and used for antagonism tests at 5 days after incubation at 28°C. *Trichoderma* spp. and *Colletotrichum* were cultured side by side in a 9 cm petri dish containing sterile PDA. Each isolate was cultured 3 cm from the edge of the petri dish. The antagonistic ability of the rhizosphere fungi was determined based on the percentage inhibition and by comparing the growth rates of each isolate. The degree of antagonism was measured qualitatively. This approach classifies the

effectiveness into terms such as strong, moderate, weak, or no antagonism. Strong antagonism indicates significant inhibition (>50%) of pathogen growth, often with clear and extensive inhibition zones. Moderate antagonism shows noticeable but less pronounced inhibition (20-50%), while weak antagonism presents minimal effects (1-19%). No antagonism means no observable inhibition of the pathogen (0%). In addition, the presence of inhibition zones and parasitism between the rhizosphere fungi and *Colletotrichum* was observed. The percentage of inhibition was calculated after incubation for three days at 28℃ [40, 41]. The formula for the percentage of inhibition according to study [42] is PP = (R1-R2)/R1 × 100%. where, PP = Percentage of radial growth inhibition (%), R1 = Radius of the *Colletotrichum* colony in the control, R2 = Radius of the *Colletotrichum* colony with *Trichoderma* treatment. Each treatment was repeated three times, and each test was repeated twice.

Table 1. Isolates used for phylogenetic tree construction

Species	Isolate/Strain	Isolation Source	Country Origin	Accession Number
Trichoderma virens	F1d5c1	Soil	Malaysia	KP056781
Trichoderma virens	OTU51	Root	China	KY965443
Trichoderma virens	QT22122	Soil	China	KY225664
Trichoderma virens	KY22016	Soil	China	KY225609
Trichoderma virens	TR039	Ant nest	Texas	HQ608079
Trichoderma hamatum	CEN1350	Soil	Brazil	OM515061
Trichoderma hamatum	CEN1334	Soil	Brazil	OM515060
Trichoderma hamatum	CC13A	Root	California	KF856960
Trichoderma hamatum	TR090	Ant nest	Texas	HQ608116
Trichoderma hamatum	EEK_D8	Soil	Netherland	OP346774
Trichoderma asperellum	Tasum66	Soil	China	MT102403
Trichoderma asperellum	Tasp47	Rhizosphere	India	MT065826
Trichoderma asperellum	Tasp5	Soil	Mexico	MN639282
Trichoderma asperellum	Tasp46	Soil	India	MT065825
Trichoderma asperellum	TA1	Rhizosphere	India	OR649253
Colletotrichum asianum	GY20	Mango fruit	Indonesia	OQ179693

Table 2. Macroscopic characteristics of *Trichoderma* spp. isolates from bamboo and cardamom rhizosphere

Isolate	Soil Source	Macroscopic			
		Colony		Growth Rate (mm/day)	Growth Speed
		Front	Reverse		
BmGr	Bamboo rhizosphere	Olive green to dark green, with a cottony or powdery texture and an irregular or fringed margin.	Pale yellow to dark green concentric ring.	8.98	Fast
KpGr	Cardamom rhizosphere	Bright green to dark green, with a fluffy or woolly texture and an irregular or fringed margin.	Pale yellow to light green concentric ring with dark green in the middle.	8.35	Fast

3. RESULTS AND DISCUSSION

3.1 Morphological characterization of *Trichoderma* spp.

The results of the isolation obtained two *Trichoderma* isolates, BmMG and KpGR. All two isolates demonstrate fast growth rates and exhibit green pigmentation with various textures and margins, the differences in their macroscopic characteristics and growth speeds highlight their unique adaptations to their respective rhizosphere environments. Understanding these traits is essential for distinguishing between the isolates and for exploring their ecological roles. Different *Trichoderma* isolates exhibit variations in growth rates, pigmentation, textures, and margins, reflecting their adaptations to specific rhizosphere environments [43]. For instance, the morpho-cultural and molecular characterization of *Trichoderma* species from various regions has allowed for the identification of distinct isolates like *T. koningiopsis*, *T. viride*, *T. asperellum*, *T. asperelloides*, *T. hamatum*, and *T. harzianum* [44].

As seen in Table 2 and Figure 1(a, b, e, f), BmGr, isolated from the bamboo rhizosphere, exhibited colonies that were olive green to dark green in color with a cottony or powdery texture and an irregular or fringed margin. On the reverse side, the colonies showed a pale yellow to dark green concentric ring. The growth rate of BmGr was approximately 8.98 mm per day, indicating a fast growth speed. In contrast, KpGr, originating from the cardamom rhizosphere, showed colonies that were bright green to dark green, with a fluffy or woolly texture and an irregular or fringed margin. The reverse side displayed a pale yellow to light green concentric ring with dark green in the middle. KpGr grew at a rate of 8.35 mm per day, which was slightly slower compared to BmGr and CbGr but still within the fast growth category. The growth rate of *Trichoderma* is a key factor influenced by environmental conditions such as pH, nutrient availability, and interactions with other microorganisms in the rhizosphere. *Trichoderma* isolates are characterized by rapid growth rates and the production of secondary metabolites, which play a crucial role in their antagonistic activity against pathogens. These secondary metabolites not only act as antagonistic agents against pathogens but also serve as sporogenic factors and growth promoters affecting morphological differentiation in *Trichoderma* [45, 46].

The growth patterns and colony appearance of *Trichoderma* are influenced by various factors, including the nutrient media

used for cultivation. Studies have shown that *Trichoderma* isolates display early sporulation and maximum mycelial development on specific solid media such as corn meal agar and oat meal agar [47]. Additionally, the differentiation of *Trichoderma* isolates based on mycelia growth rate, colony appearance, and microscopic morphological features like phialides and phialospores contribute to understanding the adaptability and diversity within the genus [48]. Furthermore, the morphological identification of *Trichoderma* species relies on observations of colony appearance, growth rate, and microscopic characteristics such as conidiophore branching patterns, phialide organization, and conidia shapes and sizes [49-52].

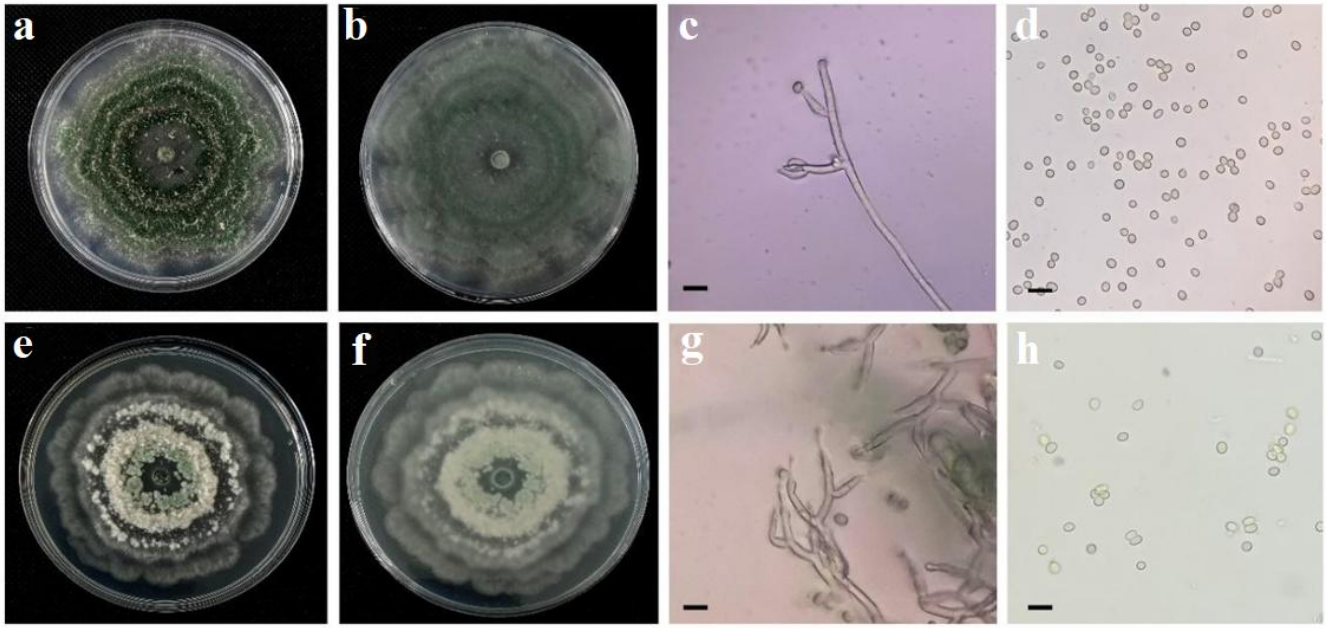

Figure 1. Macroscopic and microscopic characteristics of *Trichoderma* spp. isolates
Note: BmGr isolate (a-d); KpGr isolate (e-h); front colony (a,e); reverse colony (b, f); conidiophore and phialide (c, g); conidia (d, h, i). The black scale bar is 10 µm.

Table 3. Microscopic characteristics of *Trichoderma* spp. isolates from bamboo and cardamom rhizosphere

Isolate	Microscopic Features								
	Conidia				Phialid				Conidiophor
	Shape and Colour	Range Size L × W (µm)	Length ± S.D. (µm)	Width ± S.D. (µm)	Shape and Colour	Range Size L × W (µm)	Length ± S.D. (µm)	Width ± S.D. (µm)	
BmGr	Flask-shaped structures, typically ellipsoidal to cylindrical, smooth-walled conidia in slimy heads, hyaline to bright green	3.49-5.17 × 2.33-3.94	4.23 ± 0.55	2.98 ± 0.52	Flask-shaped and produce conidia, hyaline	14.33-20.76 × 4.02 – 7.88	18.262 ± 2.62	5.598 ± 1.63	Branched, colorless to pale yellow structures that produce conidia in whorls or clusters on flask-shaped phialides.
KpGr	Ellipsoidal to cylindrical, smooth-walled, and green, produced in dense clusters	3.27-6.14 × 2.61 – 4.52	5.155 ± 0.79	3.292 ± 0.51	Slender, flask-shaped structures, produce conidia, hyaline	16.91 – 20.75 × 2.2 – 3.63	18.33 ± 1.55	3.06 ± 0.53	Erect, branched, and hyaline to pale green, producing conidia in dense clusters on slender, flask-shaped phialides.

The microscopic examination of the isolates BmGr and KpGr, as is shown in Table 3 and Figure 1(c, d, g, h) revealed distinct morphological characteristics that were important for their identification and differentiation. Both isolates produced smooth-walled, green conidia and exhibited variability in conidial shape and size, phialid dimensions, and conidiophore characteristics. BmGr conidia were ellipsoidal to cylindrical, smooth-walled, and varied from hyaline to bright green in color. They measured between 3.49-5.17 µm in length and 2.33-3.94 µm in width, with average dimensions of 4.23 µm in length and 2.98 µm in width. The phialides of BmGr were flask-shaped, hyaline, and ranged in length from 14.33-20.76 µm and in width from 4.02-7.88 µm, with mean values of 18.26 µm and 5.60 µm, respectively. The conidiophores were branched and colorless to pale yellow, producing conidia in whorls or clusters on the flask-shaped phialides. Compared to BmGr, KpGr conidia were ellipsoidal to cylindrical, smooth-walled, and green, with sizes ranging from 3.27-6.14 µm in length and 2.61-4.52 µm in width, and average dimensions of 5.16 µm in length and 3.29 µm in width. The phialides were slender, flask-shaped, and hyaline, ranging from 16.91-20.75 µm in length and 2.2-3.63 µm in width, with mean dimensions

of 18.33 µm in length and 3.06 µm in width. The conidiophores of KpGr were erect, branched, and hyaline to pale green, producing conidia in dense clusters on slender, flask-shaped phialides. Although KpGr's conidial shape and color were similar to those of BmGr, the differences in size and density of the clusters suggested unique adaptations among these isolates. These differences are crucial for accurate identification and understanding the biological behavior of the fungi, which have implications for disease management and ecological studies.

The dimensions of conidia, including their length and diameter, vary significantly among different *Trichoderma* species, and these morphological traits are often used to classify and identify isolates. A study conducted by López-Quintero et al. [53] has demonstrated that conidial dimensions can be measured accurately, allowing for differentiation among closely related species based on these metrics. Additionally, the density of conidial clusters plays a significant role in species identification and ecological adaptation. Higher conidial densities can indicate particular species' adaptations to environmental conditions, influencing their growth and reproductive strategies. For instance, some *Trichoderma* isolates exhibit a strong ability to respond to diverse environmental signals, which correlates with their growth rates and conidial production [54].

Environmental factors such as temperature, chemical pressure, ultraviolet radiation, and cold stress influence conidial density and germination [55]. The regulation of gene expression networks also affects conidial production and density, impacting both asexual lifecycles [56]. Furthermore, factors like incubation temperature can alter conidial density, with higher temperatures leading to a decrease in conidial length [57]. This intricate interplay of morphological traits and environmental conditions underscores the complexity of *Trichoderma* species' adaptations and their classification.

3.2 Molecular identification of *Trichoderma* spp.

The provided phylogenetic tree (Figure 2), supported with BLAST results (Table 4 and Table 5), illustrates the evolutionary relationships among different isolates of *Trichoderma*, including *Trichoderma virens* and *Trichoderma hamatum*, with *Colletotrichum asianum* GY20 as an outgroup. The tree shows a well-supported clade (bootstrap value of 100) that includes all the *T. virens* strains: TR039, QT22122, OTU51, KY22016, F1d5c1, and BmGr. Another distinct clade, with strong bootstrap support (99), includes *T. hamatum* strains: CEN1334, CC13A, CEN1350, EEK D8, KpGr, and TR090. These results indicated that the BmGr isolate was closely related to *Trichoderma virens*, while the KpGr isolates was *T. hamatum.* The *T. virens* BmGr and *T. hamatum* KpGr sequences were then submitted to GenBank with accession numbers PQ066098 and PQ066099, respectively. The tree also reveals that *T. virens* clade was more distantly related to *T. hamatum* and *T. asperellum* compared to the relationship between *T. hamatum* and *T. asperellum* clade. The tight clustering and high bootstrap values within the *T. virens* and *T. hamatum* clades indicate strong intraspecies conservation. This suggests that these isolates are either under similar selective pressures or have not had enough time to accumulate significant genetic differences. The larger genetic distances and distinct clades between *T. virens* and the other two species imply a greater evolutionary divergence. These findings are crucial for understanding the evolutionary biology of these fungi and their potential applications in biological control and agriculture.

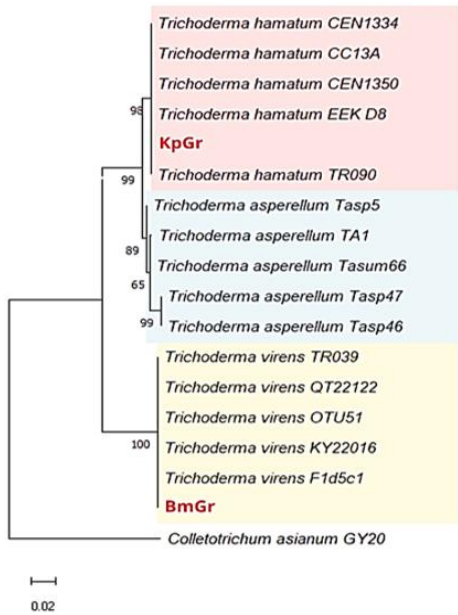

Figure 2. The maximum likelihood phylogenetic tree of *Trichoderma* spp. bootstrap values (1000 replications) above 50% shown at the nodes

Colletotrichum asianum serves as the outgroup. Isolates from this study are marked in red. The scale bar represents the number of substitutions per nucleotide position

Table 4. BLAST result of *Trichoderma* isolate KpGr ITS sequence

Species	Strain/Isolate	Max Score	Query Cover (%)	Percent Identity (%)	Accession Number
Trichoderma hamatum	EEK_D8	1146	99	99.68	PQ066099
Trichoderma hamatum	CEN1350	1146	99	99.68	OM515061
Trichoderma hamatum	CEN3413	1146	99	99.68	OM515060
Trichoderma hamatum	CC13A	1146	99	99.68	KF856960
Trichoderma hamatum	TR090	1144	99	99.68	HQ608116

Table 5. BLAST result of *Trichoderma* isolate BmGr ITS sequence

Species	Strain/Isolate	Max Score	Query Cover (%)	Percent Identity (%)	Accession Number
Trichoderma virens	F1d5c1	1170	100	99.53	KP056781
Trichoderma virens	OTU51	1155	99	99.22	KY965443
Trichoderma virens	QT22122	1155	99	99.22	KY225664
Trichoderma virens	KY22016	1155	99	99.22	KY225609
Trichoderma virens	TR039	1155	99	99.22	HQ608079

As can be seen in Table 6, among the *Trichoderma virens* isolates, which included TR039, QT22122, OTU51, KY22016, F1d5c1, and BmGr, the pairwise distance was consistently 100%. This indicated that these strains were genetically identical or extremely similar, suggesting a high level of genetic conservation within this group. Such genetic similarity might have reflected their adaptation to similar ecological niches or their recent divergence from a common ancestor. In contrast, the *Trichoderma hamatum* isolates, which consisted of TR090, KpGr, EEK_D8, CEN1350, CEN1334, and CC13A, also showed high similarity among themselves, with pairwise distances around 100%. When compared to *T. virens* isolates, the *T. hamatum* isolates exhibited a pairwise distance of approximately 83%, indicating a moderate genetic difference between these two species. This moderate difference suggested that *T. hamatum* and *T. virens* might have diverged from a common ancestor at a more distant point in time than the divergence within each species.

The pairwise distances indicated minimal variation within the *T. virens* and *T. hamatum* groups, suggesting that these groups were highly conserved. The interspecies variation indicated moderate genetic differences between *T. virens* and *T. hamatum*. The pairwise distance matrix provided valuable insights into the genetic relationships and evolutionary distances among different isolates of *Trichoderma.* This information was crucial for understanding their taxonomy, evolutionary biology, and potential applications in biological control and agriculture [58]. The observed genetic distances could help guide future research on the evolutionary history of these fungi and their potential use in biocontrol strategies.

Table 6. Pairwise distance between *Trichoderma* spp. isolates from bamboo and cardamom rhizosphere

Isolates	Homology (%)											
	1	2	3	4	5	6	7	8	9	10	11	12
1	100											
2	100	100										
3	100	100	100									
4	100	100	100	100								
5	100	100	100	100	100							
6	100	100	100	100	100	100						
7	83	83	83	83	83	83	100					
8	83	83	83	83	83	83	100	100				
9	83.1	83	83	83	83	83	100	100	100			
10	83.1	83	83	83	83	83	100	100	100	100		
11	83.1	83	83	83	83	83	100	100	100	100	100	
12	83.1	83	83	83	83	83	100	100	100	100	100	100

Note: 1= *Trichoderma hamatum* CEN1334; 2 = *Trichoderma hamatum* CC13A; 3 = *Trichoderma hamatum* CEN1350; 4 = *Trichoderma hamatum* EEK_D8; 5 = *Trichoderma hamatum* KpGr; 6 = *Trichoderma hamatum* TR090; 7 = *Trichoderma virens* TR039; 8 = *Trichoderma virens* QT22122; 9 = *Trichoderma virens* OTU51; 10 = *Trichoderma virens* KY22016; 11 = *Trichoderma virens* F1d5c1; 12 = *Trichoderma virens* BmGr.

Table 7. Antagonism of *Trichoderma* spp. against *Colletotrichum* sp. in the dual culture test

Species	Inhibition (%)				Inhibition Degree	Antagonistic Mechanism		
	R1	R2	R3	Mean		Competition	Antibiosis	Micoparasitsm
T. virens isolate BmGr	71.42	33.33	85.71	63.49	Strong	+	-	+
T. hamatum isolate KpGr	54.28	66.66	58.57	59.84	Strong	+	-	+

Note: R = Replication.

3.3 Antagonistic potential of *Trichoderma* spp.

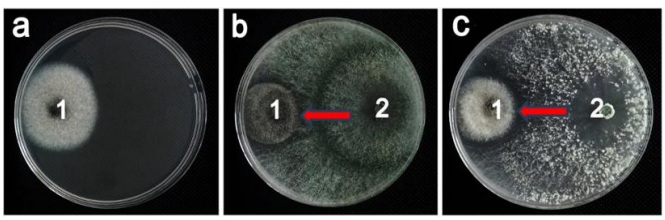

Figure 3. Dual culture test of *Trichoderma* spp. against *Colletotrichum* sp.
Description: a = control; 1 = *Colletotrichum* sp. isolate BCG1; 2 = *Trichoderma* spp. isolates; b = *Trichoderma virens* isolate KpGr; c =*Trichoderma hamatum* isolate BmGr. Red arrow is competition zone between *Trichoderma* spp. and *Colletotrichum* sp.

The results presented in Table 7 and Figure 3 illustrated the antagonistic effects of two *Trichoderma virens* isolate BmGr and *Trichoderma hamatum* isolate KpGr against *Colletotrichum* sp. in a dual culture test. Isolate BmGr showed inhibition percentages of 71.42%, 33.33%, and 85.71% for the three replicates (R1, R2, R3), respectively, with a mean inhibition percentage of 63.49% and a strong degree of inhibition. Isolate KpGr exhibited inhibition percentages of 54.28%, 66.66%, and 58.57%, with a mean inhibition percentage of 59.84%, also indicating a strong degree of inhibition. Both isolates employed competition and mycoparasitism as antagonistic mechanisms, with no evidence of antibiosis. BmGr demonstrated higher variability in inhibition percentages across replicates, while KpGr displayed more consistent performance.

Mycoparasitism was confirmed by direct microscopic examination, which revealed physical interactions between *Trichoderma* hyphae and *Colletotrichum* sp. during these observations, *Trichoderma* hyphae were seen coiling around the pathogen's hyphae, consistent with known mechanisms of mycoparasitism. Additionally, the absence of antibiosis was supported by dual culture assays, where no clear inhibition zones were observed between *Trichoderma* spp. and *Colletotrichum* sp. on the agar medium. This suggested that

the isolates did not produce diffusible antimicrobial compounds under the experimental conditions. Instead, the reduced pathogen growth in dual cultures was attributed to nutrient competition and direct mycoparasitic interaction, as evidenced by the reduction in pathogen biomass near *Trichoderm*a. These findings highlighted the multifaceted mechanisms of *Trichoderma* spp. in pathogen control, emphasizing the role of physical interaction and resource competition rather than antibiosis.

Trichoderma are saprophytic and thrive in nutrient-rich environments, allowing them to outcompete pathogenic fungi for essential resources such as nutrients and space. By occupying the ecological niche that pathogens would otherwise exploit, *Trichoderma* spp. effectively inhibit pathogen growth [59, 60]. One of the primary mechanisms by which *Trichoderma* spp. exert their biocontrol effects is through competition for nutrients and space. This competitive advantage is crucial, as it allows *Trichoderma* to establish itself in the rhizosphere, thereby limiting the resources available to pathogenic fungi. For instance, Manandhar et al. [61] highlighted that *Trichoderma*'s ability to bind to pathogens plays a significant role in this competitive dynamic. Additionally, Bhandari [62] noted that *Trichoderma* species demonstrate strong biocontrol activities under both in vitro and field conditions, emphasizing their capacity to outcompete pathogens for essential nutrients.

Despite this variability, *T. virense* isolate BmGr and *T. hamatum* isolate KpGr consistently exhibited strong inhibition, primarily through competition and mycoparasitism. These findings suggest that both isolates could serve as potent biocontrol agents against *Colletotrichum* sp., providing an eco-friendly alternative to chemical fungicides. The dual culture test results affirmed the antagonistic potential of *Trichoderma* spp. against *Colletotrichum* sp., supporting their use as biological control agents. Further research and field trials could help optimize their application and effectiveness in various agricultural contexts.

The *Trichoderma* isolates in this study exert their antagonistic effects primarily through competition and mycoparasitism, rather than antibiosis. The absence of antibiosis suggests that the inhibition of *Colletotrichum* sp. is largely due to competitive exclusion of the pathogen and direct parasitism, rather than the production of antimicrobial compounds. This finding aligns with Ahmad et al. [63], who demonstrated that an endophyte reduced corm-rot through similar mechanisms, highlighting the importance of competitive exclusion and direct parasitism in pathogen management. Neupane et al. [64] supported this view by noting that antibiosis involves antimicrobial compound production, which was not observed in our study. Additionally, Adhikari [65] emphasized the need to understand various biological control mechanisms, including antibiosis, for effective pathogen management.

The variability in the performance of *T. virens* isolate BmGr may be due to environmental conditions or genetic differences between replicates. In contrast, the consistent inhibitory performance of *T. hamatum* KpGr suggests a more stable interaction with the pathogen, potentially making it more suitable for practical applications. Despite this variability, both isolates offer promise as eco-friendly alternatives to chemical fungicides, supporting the shift towards sustainable agricultural practices.

Genetic diversity within *Trichoderma* isolates might contribute to the variability observed in their antagonistic performance against phytopathogenic fungi. This diversity is primarily attributed to the genetic variability among different *Trichoderma* species and strains, which can arise from mutations, chromosomal rearrangements, and recombination processes within their genomes [66]. For instance, studies have shown that multiple introductions of Trichoderma isolates into various environments can enhance genetic diversity, leading to the formation of distinct genotypes that exhibit varying levels of antagonistic activity [67]. The presence of diverse genetic backgrounds allows for a broader range of biochemical and physiological traits, which are crucial for effective biocontrol [68].

Moreover, the heterogeneity among fungal propagules, such as spores and mycelial fragments, plays a vital role in determining the efficacy of *Trichoderma* as biocontrol agents. Different isolates exhibit variations in morphological and physiological characteristics, which can influence their ability to compete for resources and establish dominance in the rhizosphere [69]. For example, certain isolates may produce higher levels of lytic enzymes, such as chitinases and glucanases, which are essential for degrading the cell walls of pathogenic fungi [70, 71]. This enzymatic activity is often linked to the genetic makeup of the isolates, suggesting that those with a more diverse genetic background may possess enhanced antagonistic capabilities [72].

Research has shown that *T. virens* is an effective biocontrol agent that inhibits plant pathogens through multiple mechanisms. This includes inducing localized or systemic resistance responses in plants, explaining its non-pathogenic nature. Studies [73, 74] have demonstrated its ability to control plant pathogens, particularly fungi, through mycoparasitism and bioactive compound production. Furthermore, *T. virens* enhances plant growth and induces plant defense-related enzymes, contributing to its pathogen resistance capabilities [75].

Similarly, *T. hamatum* is known for its biocontrol efficacy against various plant pathogens. It has been shown to inhibit pathogens such as *Sclerotinia sclerotiorum* [76], *Fusarium oxysporum* [77], *Meloidogyne incognita* [78], *Lasiodiplodia theobromae* [79], and *Rhizoctonia solani* [80]. Studies have also highlighted its effectiveness in reducing disease in crops like strawberries [81] and its ability to induce systemic resistance in plants [82]. Additionally, *T. hamatum* promotes plant growth and produces hydrolytic enzymes that contribute to its antagonistic potential [83].

4. CONCLUSIONS

This study ultimately identified and characterized two *Trichoderma* isolates, *Trichoderma virens* (BmGr) and *Trichoderma hamatum* (KpGr), from bamboo and cardamom rhizospheres, respectively, and demonstrated their strong antagonistic potential against *Colletotrichum* sp., a major pathogen of chili anthracnose. Both isolates effectively inhibited the pathogen's growth through competition and mycoparasitism, with average inhibition rates of 63.49% for BmGr and 59.84% for KpGr. These findings contribute to the academic field by expanding the theoretical understanding of microbial interactions in underexplored rhizospheres and providing a methodological framework for isolating and evaluating biocontrol agents from diverse ecological niches. This approach also highlights the potential of integrating *Trichoderma* spp. into sustainable disease management

practices as an alternative to chemical fungicides.

To advance this research, future studies should prioritize field trials to assess the real-world efficacy of these isolates, with specific attention to designing experiments that evaluate their performance under varying environmental conditions. Investigating the genetic stability of the isolates will be critical to ensure consistent biocontrol activity. Additionally, exploring potential synergies between these isolates and other agronomic practices could further optimize their application. These steps will not only validate the findings but also provide actionable insights for implementing eco-friendly disease management strategies in agricultural systems.

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