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Antagonistic activities of *Trichoderma* spp. isolates against *Neoscytalidium dimidiatum* causing brown spot disease on dragon fruit *Hylocereus undatus*

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ABSTRACT

Dragon fruit cultivation encounters significant challenges due to the brown spot disease caused by *Neoscytalidium dimidiatum*, resulting in reduced yield and quality. In this study, we successfully isolated and identified *N. dimidiatum* as the causal agent of this disease in Long An, Vietnam. Concerns about the potential adverse effects of fungicide administration on the ecosystem and human well-being have prompted the exploration of alternative approaches. *Trichoderma* spp., a group of filamentous fungi, have emerged as promising biocontrol agents due to their competitive nature and ability to induce systemic resistance. Fifteen isolates of *Trichoderma* spp. were evaluated through a dual culture assay and indicated that *Trichoderma viride* (TDF2), *Trichoderma asperellum* (TDF5), and *Trichoderma harzianum* (TC1) exhibited significant antagonistic properties against *N. dimidiatum*. These isolates effectively inhibited the growth of *N. dimidiatum in vitro*, with efficacy ranging from 85.05% to 88.35%. Potted experiments further demonstrated that pre-treatment with *Trichoderma* spp. exhibited the highest level of effectiveness. This approach provided a competitive advantage over the soil-borne pathogen, resulting in a remarkable reduction in disease index by 70.25–75.71%, thus achieving effective disease management. While co-treatment of *Trichoderma* spp. and *N. dimidiatum* showed some potential in reducing disease severity, its efficacy was found to be suboptimal compared to pre-treatment. In summary, the *Trichoderma* spp. isolates investigated in this study have demonstrated their potential in managing brown spot disease, indicating their potential utility as biocontrol agents against *N. dimidiatum* infection in dragon fruit cultivation.

1. INTRODUCTION

Dragon fruit is a lucrative crop in Vietnam, occupying 40,000 hectares of land and generating approximately $895 million annually [1]. However, the expansion of planting areas and the increasing demand for dragon fruit make these crops more vulnerable to pests and diseases [2,3]. The reduction in dragon fruit yields due to various diseases, particularly those caused by fungi, is a significant concern for dragon fruit plantations globally, as reported in previous studies [3,4]. It has been indicated that anthracnose on dragon fruit in Malaysia is attributed to *Colletotrichum gloeosporioides* [4]. In addition, it has been reported that *Gilbertella persicaria* has been identified as the etiological agent responsible for the occurrence of bloom rot in red-fleshed dragon fruit in Thailand [5].

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Neoscytalidium dimidiatum is well-known for causing dragon fruit brown spot disease, which affects the plant's stems, fruits, and flowers, generating brown patches and lesions that can lead to fruit rot and ultimately plant death [6]. On the stems, fruits, and flowers, dragon fruit brown spot disease manifests as small, irregularly shaped brown patches or lesions [7]. The blemishes can expand and combine, causing the damaged tissue to turn dark and finally rot. Fruits with a severe infection may develop cracks and become mushy and watery [8,9]. In Vietnam, brown spot disease is often observed in the rainy season when the weather is warm and humid [3]. The disease can cause significant yield losses and reduce fruit quality, resulting in economic losses for farmers [3]. The disease can be spread by contaminated planting materials, pruning tools, and wind-blown spores, and can be favored by poor plant hygiene practices [3]. However, the identification and management of pathogenic fungal strains on dragon fruit in Vietnam are still rarely documented.

Currently, the management of stem brown spot disease is predominantly dependent on the application of synthetic chemical fungicides. The frequent application of fungicides, which exhibit efficacy against fungicide-resistant strains of the pathogen, may pose

a considerable hazard to human health and the environment, owing to their elevated levels of toxicity [10–12]. The potential of *Trichoderma* spp. as biocontrol agent against various plant fungal pathogens is widely acknowledged [13]. *Trichoderma* spp. inhibit fungal pathogens by competing for space, nutrition, and other resources, hence lowering the growth and survival of pathogens [14]. In addition, *Trichoderma* spp. are able to generate secondary metabolites, such as antibiotics and enzymes, that suppress the growth of pathogen fungus [15–17]. In addition, *Trichoderma* spp. might parasitize other fungi by growing on and penetrating their hyphae, resulting in inhibiting their growing [18]. Numerous investigations have exhibited the effectiveness of *Trichoderma* spp. in combating an extensive broad range of plant-associated fungi, encompassing *Fusarium* sp. [19], *Rhizoctonia* sp. [20], *Phytophthora* sp. [21], and *Sclerotia* sp. [22]. The utilization of *Trichoderma spp.* as biological control agent presents a viable and ecologically sound substitute to chemical pesticides [23]. The utilization of *Trichoderma* spp. in integrated pest management strategies offers cultivators the opportunity to reduce their reliance on chemical fungicides while minimizing the risk of developing fungal resistance strains [24].

The aim of this investigation was to achieve the following: (1) determination of the etiological agent accountable for brown spot disease on dragon fruit in Long An, Vietnam, and (2) assessment of *Trichoderma* spp. for their antagonistic attributes against the aforementioned pathogen responsible for the brown spot disease on dragon fruit.

2. MATERIALS AND METHODS

2.1. Isolation and Identification of the Pathogen Causing Brown Spot Disease in Dragon Fruit

A total of ten stem canker samples displaying symptoms of brown spot disease were gathered from a dragon fruit plantation located in the province of Long An, Vietnam. The specimens were preserved in a plastic container with ice during transit to the laboratory. The identification of fungal pathogens was conducted through tissue transplanting, following the methodology outlined by Pornsuriya *et al.* [25]. Tissue fragments measuring 2–3 mm, which was found to be infected, underwent a process of disinfection using a 0.5% solution of sodium hypochlorite. Following this, the fragments were rinsed and subsequently placed on water agar, then incubated at a temperature of 28°C for a period of 72 h [26]. Subsequently, the hyphal tips were transplanted onto a potato dextrose agar (PDA) medium to facilitate additional isolation. The utilization of Koch›s postulate was implemented in the experimentation process, wherein the inoculation of healthy dragon fruit leaves with isolated fungi was conducted to examine the potential causal relationship between the fungi and the manifestation of brown spot disease symptoms [27].

2.2. Morphology Study

The fungal isolate obtained from the second isolation purification was subjected to further analysis. A 5 mm mycelial disk was inoculated on PDA and kept under observation for daily monitoring. The cultivation took place at a temperature of 28°C and a relative humidity of 50–60% for 7 days. During this time, the colony color, radial expansion, and presence of conidia were recorded. To conduct a microscopic examination of the fungal isolate, mycelia were aseptically retrieved utilizing a sterile needle and subsequently deposited into a droplet of lactophenol cotton blue. The hyphal texture, phialides, and conidial shape were examined under a Nikon Eclipse E100 microscope at a magnification of ×1000. The morphological characteristics were identified through the utilization of descriptions provided by Watanabe (2010) and Humber (2012) [28,29].

2.3. Molecular Identification of Fungal Isolate

The fungal specimen underwent molecular identification through the utilization of universal internal transcribed spacer (ITS) primers and the “ITS” region. The ITS primers used were: ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [30], nuclear large subunit (LSU) primers were: LROR (5′-ACCCGCTGAACTTAAGC-3′) and LR5 (5′-TCCTGAGGGAAACTTCG-3′) [31], β-tubulin (*tub*) primers were: Bt1a (5′-TTCCC CGTCTCCACTTCTTCATG-3′) and Bt1b (5′-GACGAGATCGTTCATGTTGAACTC-3′) [32], translation elongation factor 1-α gene (TEF1α) primers were EF1F (5′-ATGGGTAAGGARGACAAGAC-3′) and EF1R (5′-GGARGTACCAGTSATCATGTT-3′) [33]. The T100 thermal cycler (Bio-Rad, Irvine, CA, USA) was utilized to conduct the polymerase chain reaction (PCR) reaction under the following cycling conditions: an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR products were visualized by means of agarose gel electrophoresis, utilizing a 1% agarose gel that was stained with GelRed Loading Buffer (TBR, Viet Nam) and subsequently examined under UV light. The PCR amplicons were sequenced by Nam Khoa Biotek, Viet Nam, and the resulting sequences were analyzed for similarity using the Nucleotide Basic Local Alignment Search Tool (BLASTn) algorithm provided by the National Center for Biotechnology Information by comparing them to the GenBank database. The ITS-5.8S rDNA sequence was utilized for phylogenetic analysis through the implementation of the Clustal-W function and neighbor-joining method using MEGA 6.0 [34].

2.4. *In Vitro* Assessment of the Antagonistic Potential of *Trichoderma* spp. against *N. dimidiatum*

A dual culture assay was proposed by Sivan and Chet to assess the inhibitory effect of *Trichoderma* spp. isolates on the mycelial growth of *N. dimidiatum* [35]. A 5 mm disc derived from a 7-day-old culture of *N. dimidiatum* was positioned on the left side of a Petri dish with a diameter of 90 mm. A 5 mm disc derived from each isolate of *Trichoderma* sp. was placed at a distance of 3 cm on the opposite side of the dish, then incubated at 28°C for 7 days. The group consisting only of *N. dimidiatum* under cultivation was designated as the control group. The assessment of pathogen growth inhibition was conducted for 7 days and quantified as a percentage utilizing the prescribed formula:

$$GI\ (\%) = (C - T)/C \times 100$$

In this formula, GI represents the percentage of growth inhibition, C denotes the radial growth of the pathogen in the control, and T represents the radial growth of the pathogen in dual culture with the antagonist.

Following a 7-day of incubation, the region where isolates of *Trichoderma* spp. and *N. dimidiatum* interacted was carefully collected from the Petri dishes. The collected samples were then stained with lacto phenol cotton blue and observed under a light microscope at a magnification of ×1000 to investigate the interaction between the *Trichoderma* spp. and *N. dimidiatum*, with a particular focus on determining whether *Trichoderma* spp. came into contact with the hyphae of *N. dimidiatum* and formed coilings around them.

2.5. Effectiveness of *Trichoderma* spp. in Lowering Dragon Fruit Brown Spot Disease in the Pot Experiment

The study utilized a randomized complete block design that consisted of three treatments: pre-treatment, co-treatment, and post-treatment, each replicated three times, and each replication containing 10 dragon fruit plants [36,37]. For the pre-treatment, fungicides propineb 2.5% (according to the recommendation from the manufacture) or *Trichoderma* spp. was applied to the soil at a rate of 1×10^6 conidia/g for 3 days before *N. dimidiatum* inoculation at a rate of 1×10^4 conidia/g per pot. The co-treatment involved the simultaneous addition of propineb 2.5% or *Trichoderma* spp. to the soil at a rate of 1×10^6 conidia/g and *N. dimidiatum* inoculation at a rate of 1×10^4 conidia/g soil. In the post-treatment, the dragon fruit plants were first infected with *N. dimidiatum* at a rate of 1×10^4 conidia/g soil for 3 days before the addition of propineb 2.5% or *Trichoderma* spp. at a rate of 1×10^6 conidia/g per pot. The control group consisted of dragon fruit plants infected with *N. dimidiatum* at a rate of 1×10^4 conidia/g soil without any treatment with *Trichoderma* spp. The assessment of disease severity was conducted through the computation of the percentage of brown spot disease present on the dragon fruit plants, followed by the assignment of a severity rating on a scale ranging from 1 to 5, after a period of 7 and 14 days. The disease severity was assessed on a scale of 1–5, where a score of 1 represented the absence of symptoms and a score of 5 represented the presence of severe symptoms. The categorization of disease severity was established based on the percentage of disease symptoms observed. The levels were classified as no disease (<1%), level 1 (1–10%), level 2 (11–20%), level 3 (21–30%), level 4 (31–40%), and level 5 (>41%) [37].

The disease index and its corresponding control efficacy were documented using the formula [38]:

$$\text{Disease index} = \frac{\sum(\text{Number of diseased plants or leaves at each level} \times \text{The disease grade value})}{\text{The total number of investigated plants or leaves} \times \text{The highest value}}$$

$$\text{Relative control effect}(\%) = \frac{(\text{Disease index of control} - \text{Disease index of treatment})}{\text{Disease index of control}} \times 100$$

3. RESULTS

3.1. Isolation and Identification of the Fungus Responsible for Dragon Fruit Brown Spot Disease

Fungal strains were obtained from branches of dragon fruit affected by brown spot disease in Duc Hoa district, Long An province, Viet Nam. The stems of the dragon fruit exhibited sunken brown spots, which indicated the presence of the brown spot disease [Figure 1a]. Tissue samples with spots were collected and isolated on PDA medium for further investigation. After 10 days of cultivation, the colonies changed in color from white and cottony to yellowish-brown or dark brown, and the colony surface became rough or woolly [Figure 1b]. The conidia observed were unicellular, cylindrical, slightly curved, egg-shaped, or elliptical [Figure 1c]. The results of the BLAST analysis indicated that the fungal strain in question exhibited complete homology with *N. dimidiatum*. The phylogenetic tree constructed using ITS rDNA indicated that the fungal strain under investigation and *N. dimidiatum* are closely related, as they were found to be on the same branch. In contrast, the other Neoscytalidium species displayed a greater genetic distance from them, as depicted in Figure 1d. Moreover, the DNA sequence of the fungal strain was utilized to construct a maximum likelihood (ML) tree, employing the *tub* [Figure 1e] and LSU [Figure 1f] regions. The results demonstrated that the fungal strain clustered within the same clade as *N. dimidiatum*, aligning with the phylogenetic tree based on the ITS region. The pathogenicity of the fungal strain was confirmed using Koch's postulates, where incubating healthy dragon fruit leaves with *N. dimidiatum* conidia resulted in brown spot disease. The re-isolated fungus has a comparable morphology to the previously isolated *N. dimidiatum*.

3.2. Screening of Antagonist *Trichoderma* spp. Strain against *N. dimidiatum in Vitro*

Fifteen isolates of *Trichoderma* spp. were procured from diverse plantations, comprising 5 isolates from soil samples of dragon fruit (TDF), 5 from pepper plantations (TP), and 5 from cocoa soils (TC). *In vitro*, the dual culture assay was employed to evaluate the antagonistic activity of various strains of *Trichoderma* spp. against *N. dimidiatum*, as presented in Table 1. Our findings indicated that the *Trichoderma* spp. exhibited discernible antagonistic properties against *N. dimidiatum*, as manifested by the expansion and colonization of the pathogen's mycelium.

The discontinuation of *N. dimidiatum* hyphae growth was observed after 5 days of co-culture, whereas the *Trichoderma* spp. exhibited continued growth until complete coverage of the pathogen colony and the entire plate was achieved [Figure 2a]. The efficacy of *Trichoderma* spp isolates fight against *N. dimidiatum* was recorded to as 52.27–88.35% after 7 days of co-incubation, with TDF2, TDF5, and TC1 isolates showing the highest efficacy at 88.35%, 86.65%, and 85.05%, respectively [Table 1]. The antagonistic phenomenon of *Trichoderma* spp. was further studied by microscopy observations, which revealed an interaction between *Trichoderma* spp. and *N. dimidiatum*. *Trichoderma* spp. isolates produced hyphal branches that were directed toward *N. dimidiatum* colonies. The hyphae of *Trichoderma* TDF2, TDF5, and TC1 came into contact with and formed coilings with the hyphae of *N. dimidiatum*, ultimately leading to the inhibition of the growth of *N. dimidiatum* hyphae [Figure 2b-d].

Table 1: Effectiveness of antagonistic *Trichoderma* spp. against *Neoscytalidium dimidiatum* in dual culture.

Sample code	Sampling material	Sampling location	% inhibition		
			2 days	5 days	7 days
TDF1	Dragon fruit rhizosphere	Long An province, Viet Nam	5.41^{de}	35.79^{c}	54.74^{d}
TDF2			19.94^{a}	57.28^{ab}	88.35^{a}
TDF3			15.84^{ab}	37.97^{c}	56.53^{d}
TDF4			7.24^{c-e}	36.51^{c}	58.06^{cd}
TDF5			15.05^{ab}	65.64^{a}	86.65^{ab}
TP1	Pepper rhizosphere	Dak Lak Province, Viet Nam	10.15^{b-d}	36.15^{c}	55.05^{d}
TP2			4.13^{e}	35.79^{c}	52.27^{d}
TP3			19.19^{ab}	41.31^{c}	65.61^{cd}
TP4			5.41^{de}	34.53^{c}	57.93^{d}
TP5			17.58^{ab}	37.23 ^{c}	58.91^{d}
TC1	Cocoa rhizosphere	Dak Lak Province, Viet Nam	11.99^{a-c}	61.13^{ab}	85.05^{a}
TC2			1.65^{e}	35.24^{c}	57.93^{cd}
TC3			14.90^{ab}	50.45^{a-c}	69.45^{b-d}
TC4			13.91^{a-d}	38.70^{bc}	67.05^{cd}
TC5			14.76^{ab}	39.62^{c}	75.46^{a-c}

Statistically significant differences between groups (P<0.05) are denoted by different lowercase letters (a-e).

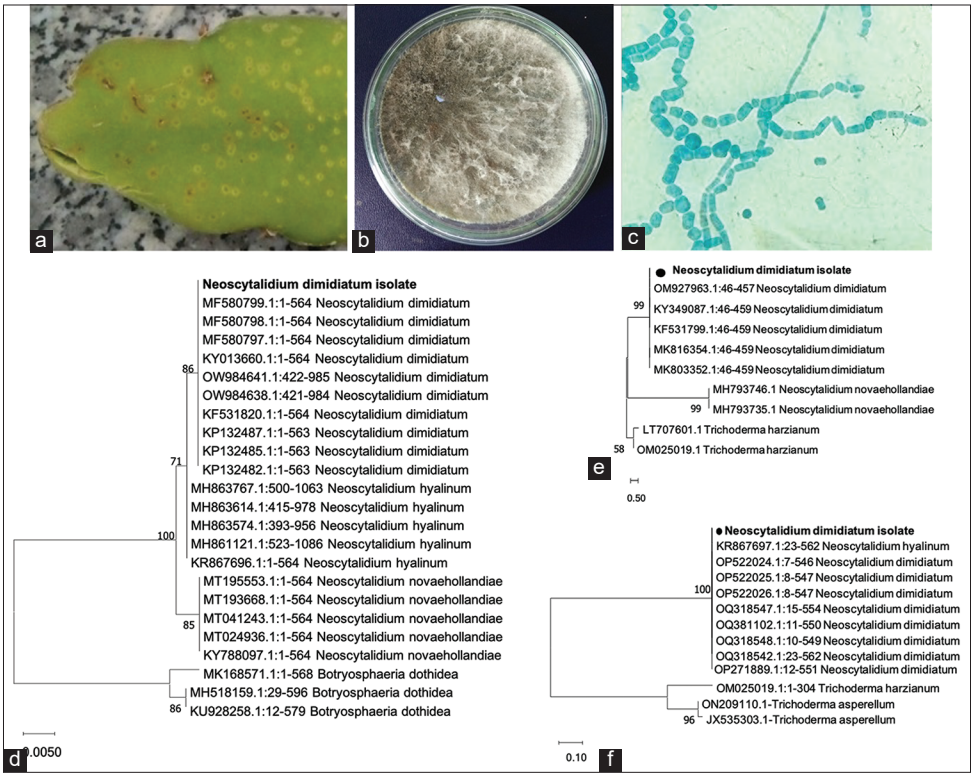

Figure 1: Characterization of *Neoscytalidium dimidiatum* isolated from brown spot disease-infected dragon fruit. (a) Dragon fruit branches with brown spot disease. (b) Macroscopic morphology of *N. dimidiatum* isolates in potato dextrose agar culture medium. (c) *N. dimidiatum* conidia under microscope at ×1000 magnification. (d) Phylogenetic tree constructed from the internal transcribed spacer gene sequences, (e) *tub* gene sequences, and (f) large subunit gene sequences of *N. dimidiatum* in this study and other known *N. dimidiatum* spp. strains from the GenBank database. In this investigation, *N. dimidiatum* was highlighted in bold.

Three *Trichoderma* strains (TDF2, TDF5, and TC1) were chosen for further experimentation based on their high efficacy in antagonism, as indicated by the results of the *in vitro* experiments.

3.3. Molecular Identification of Isolated Fungi

The genomic DNA of three isolates of *Trichoderma* spp., which demonstrated antagonistic activity against the brown spot pathogen *N. dimidiatum*, was amplified using the PCR method with an estimated size of 600 base pairs. Following this, the amplified DNA fragments underwent sequencing and were subsequently subjected to homology comparison using the BLAST. The ITS gene regions of both the isolates and reference sequences sourced from GenBank were utilized to develop phylogenetic trees. The findings obtained from the analysis of sequencing and homology comparison indicate that *Trichoderma* isolates TDF2, TDF5, and TC1 pertain to *Trichoderma viride*, *Trichoderma asperellum*, and *Trichoderma harzianum*, correspondingly. The construction of a phylogenetic tree utilizing both the isolate strains and reference strains provided confirmation of the proximity between the two groups [Figure 3a]. To enhance the reliability of the molecular identification process, additional genetic regions, namely *tub* [Figure 3b] and TEF1α [Figure 3c] regions were examined. The analysis of these regions yielded consistent results that aligned with the initial findings based on the ITS rDNA analysis.

3.4. Antagonistic Activity of *Trichoderma* spp against Brown Spot Disease Causes by *N. dimidiatum* in Pot Experiment

The activity of isolated strains of *Trichoderma* spp. against the brown spot pathogen *N. dimidiatum* was investigated under different incubation conditions. The establishment pre-treatment involved applying *Trichoderma* spp. strains to the base of dragon fruit trees 3 days before *N. dimidiatum* infection, while in the co-treatment groups, dragon fruit plants were co-fertilized with *Trichoderma* spp. and *N. dimidiatum*. Finally, during the post-treatment groups, dragon fruit plants were infected with *N. dimidiatum* for 3 days and then treated with *Trichoderma* spp. The infection group showed a disease index of 4.01–6.41 after 7–14 days, indicating the presence of brown spot disease on dragon fruit branches [Figure 4]. The highest reduction in the disease index was observed in the *Trichoderma* spp. pre-treatment group [Figure 4], with a relative control effect of 69.53–72.47% [Table 2]. This pre-treatment outperformed the propineb pre-treatment group, which had a relative control effect of 66.28% [Table 2]. Under the co-treatment conditions, the disease index reduction was approximately 51.46–82.23% in the *Trichoderma* spp.-treated groups and 83.75% in the propineb-treated group [Table 2]. However, *Trichoderma* spp. co-treatment groups were less effective than the pre-treatment groups [Figure 4 and Table 2]. Post-treatment with *Trichoderma* spp. did not show obvious antifungal activity against *N. dimidiatum*, with a relative control effect of 18.69–34.11%, while propineb showed a relative control effect of 63.13% [Table 2]. These findings suggest that the pre-incubation treatment of *Trichoderma* spp. is a highly effective strategy for controlling brown spot disease in dragon fruit. However, further studies are needed to determine the underlying mechanisms of *Trichoderma* spp. antifungal activity and optimize the treatment protocols for better disease management.

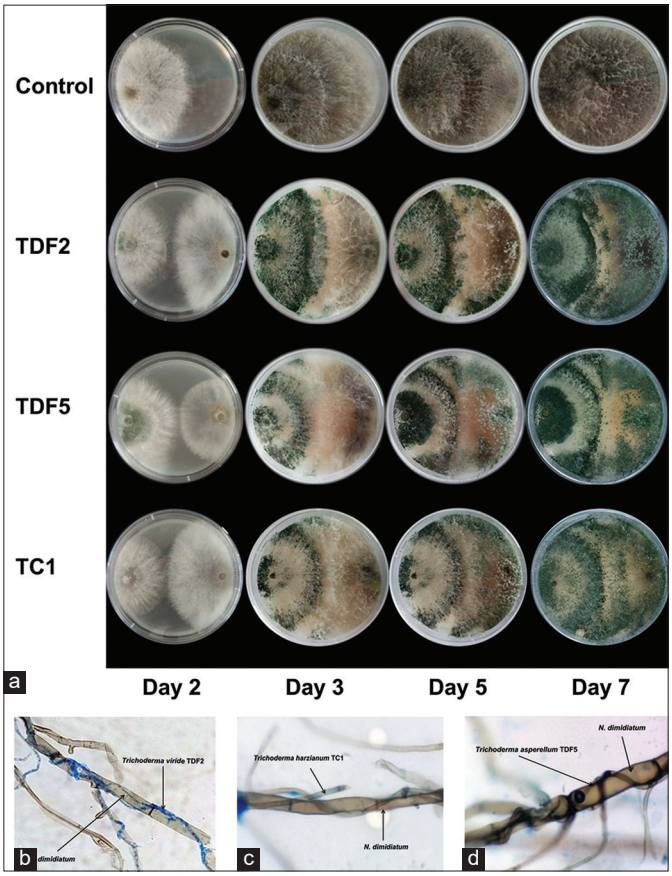

Figure 2: (a) Representative photos of three antagonistic *Trichoderma* spp. against *Neoscytalidium dimidiatum* in dual culture. Representative photos of the interaction between TDF2. (b) TDF5 (c), and TC1 (d), hyphae with *Neoscytalidium dimidiatum.*

Table 2: Relative control effect of antagonistic *Trichoderma* spp. against brown spot disease in dragon fruit caused *Neoscytalidium dimidiatum* infection.

Treatment	Relative control effect	
	7 days	14 days
Pre-treatment		
Propineb	34.21^{b-d}	66.28^{a-c}
TDF2	69.29^{ab}	75.71^{a}
TDF5	56.29^{abc}	71.98^{ab}
TC1	64.39^{abc}	70.25^{a-c}
Co-treatment		
Propineb	77.96^{a}	77.89^{a}
TDF2	50.99^{a-d}	45.23^{de}
TDF5	43.67^{b-e}	49.13^{d}
TC1	38.09^{c-e}	60.99^{cd}
Post-treatment		
Propineb	45.98^{b-d}	63.13^{b-d}
TDF2	19.11^{e}	19.15^{ef}
TDF5	0.67^{e}	4.75^{f}
TC1	15.42^{de}	7.29^{f}

Statistically significant differences between groups (P<0.05) are denoted by different lowercase letters (a-e).

4. DISCUSSION

The cultivation of dragon fruit is significantly challenged by the brown spot disease caused by *N. dimidiatum*, which leads to compromised yield and quality [39]. Our investigation has effectively isolated and identified *N. dimidiatum* as the etiological factor responsible for the brown pot disease of dragon fruit in Long An province, Vietnam. Cultural identification of the fungus was based on its characteristic features, such as dark brown to black colonies with a velvet-like texture on PDA medium [8]. These characteristics are consistent with previous reports of *N. dimidiatum* in dragon fruit [8]. Molecular identification was based on the ITS region of the fungal genome, which is a widely used DNA barcode for fungal identification. The ITS region is highly variable among different fungal species, making it a useful tool for species-level identification [40]. Our results showed that the ITS sequences obtained from the isolated fungal cultures had a high sequence identity with previously reported sequences of *N. dimidiatum*.

Several measures can be implemented to manage the *N. dimidiatum*-related disease in dragon fruit cultivation, such as removing infected leaves and controlling humidity, which has proven to be effective [3]. Whilst fungicides have demonstrated potential in managing brown spot disease, their application can yield detrimental consequences for both the environment and human well-being and may facilitate the emergence of fungus-resistant strains [41]. The filamentous fungi belonging to the *Trichoderma* spp. have been the subject of considerable scientific research due to their potential as a biocontrol agent against various plant pathogens [42]. *Trichoderma* spp. exhibit various mechanisms to inhibit plant pathogens, such as nutrient and space competition, antibiosis synthesis, and induction of systemic resistance in plants [16,43,44]. The perceived relationship between the biocontrol agent and the pathogen is often characterized as antagonistic due to their direct interaction. Consequently, the present investigation employed a dual culture assay to evaluate the isolates of *Trichoderma* spp. with regard to their antagonistic impact on *N. dimidiatum* pathogens. Among 15 isolates of *Trichoderma* spp. tested, *T. viride* (TDF2), *T. asperellum* (TDF5), and *T. harzianum* (TC1) showed significant antagonistic activity against *N. dimidiatum*. Furthermore, our study indicated the *Trichoderma* spp.*'s* mycoparasitic mechanism [45], which involves physically interacting with *N. dimidiatum*. Specifically, the hyphae of *Trichoderma* TDF2, TDF5, and TC1 coiled around the hyphae of *N. dimidiatum*, potentially restricting its growth and limiting its ability to infect plants. The results indicate that *Trichoderma* exhibits significant promise as a biocontrol agent for combating plant pathogens and can serve as a crucial component of sustainable agriculture.

After conducting an *in vitro* screening of *Trichoderma* spp. that showed potential as antagonists against *N. dimidiatum*, a potted evaluation was performed to evaluate the effectiveness of *Trichoderma* spp. in protecting dragon fruit from brown spot disease caused by *N. dimidiatum*. These findings suggest that *Trichoderma* spp. displays significant potential as a biocontrol agent for the mitigation of brown spot disease in dragon fruit plants. The results of the study indicate that pre-treatment with *Trichoderma* spp. proved to be the most efficient method, as it facilitated the establishment of competitive advantage of *Trichoderma* spp. over the pathogen in the soil [46], resulting in the high relative control effect of TDF2, TDF5, and TC1 on the damping-off of brown pot disease in dragon fruit leaves reached 75.71%, 71.98%, and 70.25%, respectively, much higher control efficiency compared to propineb (66.28%). According to

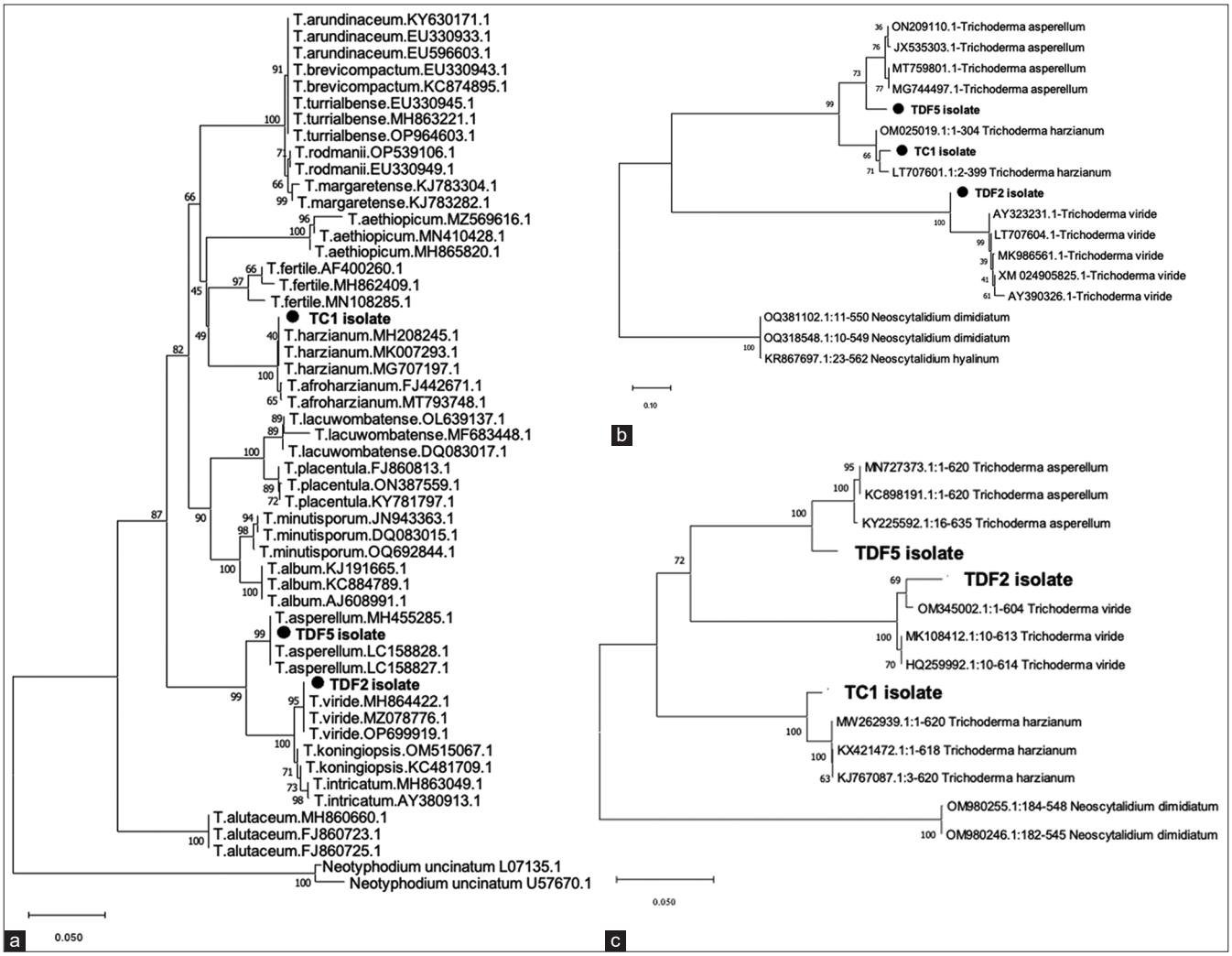

Figure 3: Phylogenetic tree constructed from the internal transcribed spacer gene sequences (a), translation elongation factor 1α gene sequences (b), and *tub* gene sequences (c) of *Trichoderma* spp. in this study and other known *Trichoderma* spp. strains from the GenBank database. In this investigation, *Trichoderma* isolates was highlighted in bold.

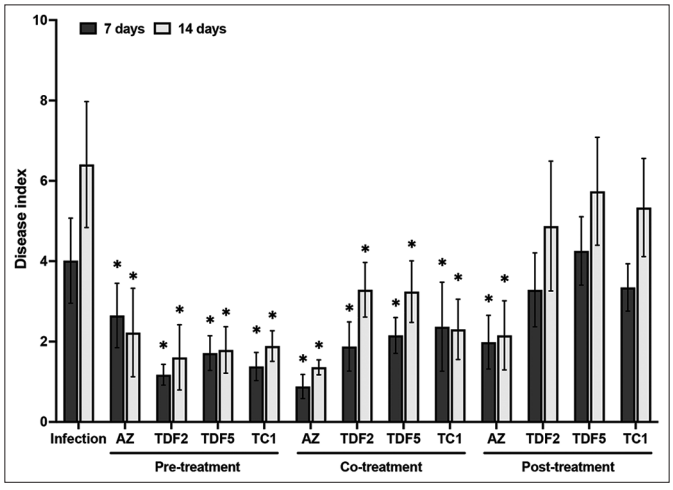

Figure 4: Effectiveness of *Trichoderma* isolates in the management of brown spot disease in dragon fruit caused by *Neoscytalidium dimidiatum* infection in pot experiment. Data are presented as the means of triplicate analysis ± standard deviation. *Indicate significant differences compared with the infection group.

prior research, *T. asperellum*, *T. spirale*, *T. koningiopsis*, and *T. reesei* have been identified as potential agents for combating *Rigidoporus microporus*, the causative agent of white root rot disease (WRD) in *Hevea brasiliensis*, which lower the disease index by 70% [46]. The findings of this study suggest that the isolates of *Trichoderma* spp. that were selected for their antagonistic properties exhibit robust disease control capabilities and hold significant promise for employment in biocontrol measures, particularly in the context of countering *N. dimidiatum* pathogens.

Co-treatment of *Trichoderma* spp. and *N. dimidiatum* also showed potential in reducing brown spot disease index. However, the protective effect of TDF2 and TDF5 against *N. dimidiatum* was decreased compared to pre-treatment, suggesting that the timing of *Trichoderma* spp. application might be critical for the management of *N. dimidiatum* infection. Additionally, post-treatment with *Trichoderma* was found to be less effective in reducing *N. dimidiatum* infection in dragon fruit. Previous research has demonstrated that the timing of *Trichoderma* spp. application is crucial for protecting grapevine wounds. Specifically, early application within 5–6 h after pruning has been shown to be more effective in preventing pathogen infection, while the effectiveness significantly decreases after 48 h post-pruning [47]. These findings

underscore the importance of early intervention for preventing brown spot disease development cause by *N. dimidiatum* infection.

5. CONCLUSION

To summarize, the cultivation of dragon fruit is considerably impeded by the brown spot disease instigated by *N. dimidiatum*, which results in reduced yield and quality, thereby culminating in economic setbacks for cultivators. The present investigation effectively determined *N. dimidiatum* as the etiological factor responsible for the brown spot ailment on dragon fruit in Long An, Viet Nam. Furthermore, we demonstrated the potential of *Trichoderma* spp. as a biocontrol agent in mitigating the growth of *N. dimidiatum*. Our results indicated that *Trichoderma* spp. displays considerable potential in managing brown spot disease in dragon fruit, thus making a valuable contribution to the progress of sustainable agriculture. The optimal timing of *Trichoderma* spp. application is of paramount importance, and prompt intervention is imperative to avert the onset of disease. The aforementioned discoveries possess significant ramifications for the administration of brown spot ailment in dragon fruit farming and have the potential to aid in the establishment of sustainable and environmentally conscious agricultural methodologies.

6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All data generated or analyzed during this study are included in this manuscript.

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