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Antifungal potential of *Enydra fluctuans* extract for postharvest control of *Colletotrichum siamense* in dragon fruit

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Abstract. *Truc NT, Thi QVC, Nhung DTC. 2026. Antifungal potential of* Enydra fluctuans *extract for postharvest control of* Colletotrichum siamense *in dragon fruit. Asian J Agric 10 (1): g100127. https://doi.org/10.13057/asianjagric/g100127. Pitaya*, commonly known as red-fleshed dragon fruit, is rich in bioactive and nutritional compounds; however, its postharvest shelf life is limited due to high susceptibility to fungal diseases, particularly anthracnose caused by *Colletotrichum* spp. These infections significantly reduce fruit quality, shorten storage duration, and decrease economic value. This study investigated the antifungal efficacy of Whole *Enydra fluctuans* Phenolic Extract (WEPE) against postharvest pathogens of dragon fruit under in vitro and in vivo conditions. Fifteen fungal isolates were recovered from infected fruits, among which strain TL12 was identified as *Colletotrichum siamense* based on morphological characteristics and ITS rDNA sequencing. Pathogenicity tests confirmed Koch's postulates, producing disease symptoms comparable to those observed in naturally infected fruits. To the best of our knowledge, this is the first report of *C. siamense* associated with postharvest anthracnose of dragon fruit in the studied region. WEPE obtained using ultrasound-assisted extraction with 45% ethanol exhibited the highest total phenolic content (18.26±1.49 mg GAE/g). In vitro assays demonstrated strong antifungal activity, with minimum inhibitory concentrations of 700 µg/mL for complete mycelial growth inhibition and 5000 µg/mL for spore germination inhibition. In vivo application of WEPE significantly suppressed anthracnose development on dragon fruit in a concentration-dependent manner. After 7 days of incubation, disease severity was reduced by approximately 30.4% and 55% at 700 and 5000 µg/mL, respectively, compared to the control. The results indicate that WEPE possesses strong antifungal potential against *C. siamense* and may be considered a promising natural agent for postharvest disease management in dragon fruit. However, these findings are based on short-term storage experiments under controlled laboratory conditions. Further research involving formulation optimization, extended storage evaluation, and field-scale validation is necessary before practical application in commercial postharvest systems.

Keywords: *Colletotrichum siamense*, dragon fruit, *Enydra fluctuans*, postharvest disease

INTRODUCTION

Pitaya (*Hylocereus costaricensis*), commonly known as red-fleshed dragon fruit, is a tropical fruit widely cultivated and consumed due to its attractive appearance, pleasant flavor, and increasing economic importance. The fruit is recognized for its nutritional value and the presence of various bioactive compounds; however, from a postharvest perspective, its commercial potential is severely constrained by rapid deterioration and high susceptibility to fungal diseases. Owing to its soft tissue structure and high moisture content, dragon fruit has a relatively short postharvest shelf life and is particularly vulnerable to microbial infections during storage and transportation. Previous studies have reported more than 20 fungal species associated with diseases of dragon fruit, among which anthracnose caused by *Colletotrichum* spp. is considered one of the most destructive, leading to severe fruit decay and substantial economic losses. Other important postharvest pathogens include *Bipolaris cactivora* and *Fusarium* spp. (Balendres and Mendoza 2019).

At present, the management of postharvest fungal diseases in dragon fruit relies mainly on the application of synthetic fungicides such as benomyl, carbendazim, propineb, difenoconazole, mancozeb, prochloraz, and Tecto 60 (Ali et al. 2013; Shi et al. 2021). Although these fungicides are effective in suppressing fungal growth, their prolonged and repeated use has raised serious concerns, including the development of fungicide-resistant strains, potential risks to human health due to chemical residues on fresh fruit, and adverse environmental impacts (Shi et al. 2021). In addition to chemical control, physical methods such as heat treatments have been investigated as alternative approaches for postharvest disease management. However, these methods may negatively affect fruit quality, and their practical application is limited by difficulties in achieving uniform heat distribution (Feliziani and Romanazzi 2013; Bordoh et al. 2020).

In response to these limitations, there is growing interest in the development of safer, sustainable, and eco-friendly strategies for postharvest disease control. Among these approaches, plant-derived extracts have attracted

considerable attention due to their natural origin, biodegradability, relatively low toxicity, and broad-spectrum antimicrobial activity (Khetabi et al. 2022). Numerous studies have demonstrated that phenolic-rich plant extracts can effectively inhibit fungal pathogens responsible for postharvest diseases of fruits and vegetables. For instance, methanolic extracts of *Annona muricata* seeds were reported to inhibit up to 90% of mycelial growth of *Alternaria alternata*, the causal agent of black spot disease in tomato fruit (Rizwana et al. 2021). Similarly, extracts derived from marine algae such as *Ulva lactuca* and *Cystoseira myrica* have shown strong antifungal activity against postharvest pathogens of banana, including *Colletotrichum* and *Fusarium* species (Elshikh and Farraj 2024). Despite these advances, much of the existing literature remains broad and fragmented, with limited focus on specific edible plants traditionally used in Southeast Asia and their potential application in postharvest disease management.

Enydra fluctuans, an edible leafy vegetable commonly consumed in Southeast Asia, has long been valued for its nutritional and medicinal properties. Previous studies have shown that Whole *E. fluctuans* Phenolic Extract (WEPE) possess high phenolic content and strong antioxidant capacity (Gatto et al. 2016). In addition, the antibacterial activity of WEPE against several pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Bacillus cereus*, has been well documented (Amin et al. 2012; Ahmad et al. 2016). However, despite these promising biological activities, information regarding the antifungal potential of WEPE, particularly against postharvest fungal pathogens affecting fruits, remains very limited. Notably, to the best of our knowledge, no previous studies have systematically evaluated the in vitro and in vivo antifungal efficacy of WEPE against *Colletotrichum siamense*, a major causal agent of anthracnose in postharvest dragon fruit. This lack of information represents a clear research gap, especially in the context of developing natural and eco-friendly alternatives to synthetic fungicides for postharvest disease control. Accordingly, the present study aimed to isolate and identify the anthracnose pathogen associated with postharvest dragon fruit and evaluate the antifungal activity of WEPE against *C. siamense* under both in vitro and in vivo conditions. The findings of this study are expected to contribute to the understanding of plant-derived phenolic extracts as potential sustainable agents for postharvest disease management and to support the development of environmentally friendly preservation strategies for dragon fruit. This study hypothesizes that the WEPE exhibits antifungal activity against *C. siamense* and can suppress anthracnose development in dragon fruit under postharvest conditions. Specifically, it is proposed that increasing concentrations of WEPE will inhibit fungal mycelial growth in vitro and reduce lesion development in vivo, with efficacy associated with the phenolic content of the extract. Accordingly, this study addresses the following research questions: (i) how ultrasound frequency and extraction solvent influence the TPC of *E. fluctuans*; (ii) which *Colletotrichum* species is associated with postharvest anthracnose of dragon fruit, and how it can be accurately isolated and identified; (iii) to what extent WEPE inhibits the mycelial growth and spore germination of the isolated *Colletotrichum* in vitro; and (iv) how effective WEPE is in suppressing anthracnose lesion development on inoculated dragon fruit under controlled conditions.

MATERIALS AND METHODS

Isolation and identification of *Colletotrichum*

Dragon fruits exhibiting typical anthracnose symptoms, including nearly circular or irregular lesions with dark brown concentric rings, a reddish-brown water-soaked center, and visible signs of decay, were selected for fungal isolation. A total of twelve diseased dragon fruits were collected from commercial orchards in Dong Thap province, Mekong Delta, Vietnam, during the main harvest season. Fungal isolation was performed following the method described by Balendres and Mendoza (2019) with minor modifications. Briefly, infected fruits were washed thoroughly under running tap water and rinsed with distilled water. Symptomatic tissues were excised into segments of approximately 1×1 cm, surface-sterilized with 70% (v/v) ethanol for 90 s, and rinsed three times with sterile distilled water to remove residual ethanol. The sterilized tissue segments were aseptically placed onto Potato Dextrose Agar (PDA; Himedia, India) plates and incubated at 30°C for 5-7 days. Emerging fungal colonies were sub-cultured repeatedly to obtain pure isolates, which were subsequently maintained on PDA at 25±2°C for further morphological observation and downstream experiments. A total of 12 *Colletotrichum* isolates were obtained. Among these, two isolates (TL9 and TL12) were selected for pathogenicity comparison because they exhibited the most vigorous growth and represented the dominant morphological groups. Pathogenicity assays showed that isolate TL12 produced the largest lesions and was therefore considered the most virulent. Consequently, TL12 was selected as the representative isolate for all subsequent experiments. Only TL12 was subjected to molecular identification and used for in vitro and in vivo antifungal assays. The pathogenicity of the representative *Colletotrichum* isolate was evaluated on healthy dragon fruits following a modified protocol described by Huang et al. (2021). Uniform, disease-free fruits were washed under running tap water, surface-sterilized with 70% ethanol, and rinsed three times with sterile distilled water. The fruits were inoculated with actively growing mycelial plugs of *Colletotrichum* spp. (5 mm in diameter) excised from the margins of 5-day-old PDA cultures, while control fruits were inoculated with sterile PDA plugs. Inoculated fruits were incubated at 25±2°C in the dark for 7 days under high-humidity conditions maintained by moistened sterile tissue paper. Disease development was monitored daily, and anthracnose symptoms were evaluated based on lesion appearance and expansion. To fulfill Koch's postulates, the pathogen was re-isolated from symptomatic fruit tissues and re-cultured on PDA. The re-isolated fungus exhibited

morphological characteristics identical to those of the original isolate, thereby confirming its identity as the causal agent of anthracnose in dragon fruit.

Preliminary identification of *Colletotrichum* isolates was performed based on morphological characteristics, including colony appearance, mycelial color, conidial shape, and hyphal structure, following the descriptions provided by Santos et al. (2025). For molecular identification, genomic DNA was extracted from actively growing fungal cultures using a Plant/Fungi DNA Isolation Kit (Norgen Biotek, Canada) according to the manufacturer's instructions.

Polymerase Chain Reaction (PCR) amplification was performed using the universal fungal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′ TCCTCCGCTTATTGATATGC-3′) (White et al. 1990; Singh et al. 2023). The PCR reaction was carried out in a 25 µL volume containing 2 µL of DNA template, 8.5 µL of nuclease-free water, 1 µL each of ITS1 and ITS4 primers (20 pmol), and 12.5 µL of PCR Master Mix (Promega, USA). Thermal cycling conditions included an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 50 seconds, annealing at 54°C for 45 seconds, and extension at 72°C for 70 seconds. A final extension step was conducted at 72°C for 5 minutes. PCR products were resolved by electrophoresis on a 2% agarose gel and visualized using a gel documentation system (Analytik Jena). Amplicons of approximately 600 bp were purified and subsequently submitted to a commercial sequencing service (Vietnam) for DNA sequencing. The BLASTn algorithm was employed to compare the obtained fungal sequences against those in the NCBI GenBank database. A phylogenetic tree was constructed using the neighbor-joining method with the Kimura 2-parameter model in MEGA version 5.1, with bootstrap analysis based on 1,000 replicates to evaluate the robustness of the branching.

Preparation of WEPE and determination of Total Phenolic Content (TPC)

Enydra fluctuans was collected from Vinh Long province, Vietnam, at the vegetative growth stage. Only healthy plants free from pests, diseases, and mechanical damage were selected, and the aboveground parts (stems and leaves) were used. The plant material was washed, drained, and dried by hot air at 60°C for 24 h, then ground and sieved through a 0.2 cm mesh. The sieved powder was used for extraction. Ultrasound-Assisted Extraction (UAE) was performed following Gholamnezhad (2019) with modifications. Briefly, 0.5 g (±0.01 g) of plant powder was mixed with 10 mL of solvent (distilled water, 45% ethanol, or 90% ethanol) in a 20 mL test tube. The tubes were placed in an ultrasonic bath connected to a UAE generator, with continuous cold-water circulation to maintain the extraction temperature at 30°C. Different ultrasound frequencies (0, 58, 132, and 192 kHz) and extraction times (10, 20, 30, and 40 min) were evaluated to examine their effects on cavitation intensity, mass transfer efficiency, and phenolic compound release, based on previous studies and preliminary trials. The extracts were sequentially filtered through muslin cloth and qualitative filter paper, diluted tenfold, and used for further analysis. The experiment was arranged in a completely randomized design with three replicates**.** A full 3×4×4 factorial structure was applied, consisting of three solvent types, four ultrasound frequencies, and four extraction times, resulting in 48 treatment combinations. Subsequently, the solvent was removed from the extracts using a rotary evaporator under vacuum at 45°C and a rotation speed of 100 rpm. The resulting WEPE was stored at 4°C until further use in the study.

TPC was determined using the Folin-Ciocalteu method (Singleton et al. 1999) with minor modifications. The reaction mixture contained 50 µL of extract, 250 µL of Folin-Ciocalteu reagent, 750 µL of 7.5% sodium carbonate, and 2 mL of distilled water. After incubation at 40°C for 30 min, absorbance was measured at 750 nm using a UV-Vis spectrophotometer. Total phenolic content was calculated from a gallic acid standard curve and expressed as Gallic Acid Equivalents (GAE).

Antifungal activity of WEPE on *Colletotrichum* sp. in vitro

The antifungal activity of the WEPE was evaluated using the mycelial and spore inhibition methods as described by Tyagi and Malik (2010). For the mycelial growth inhibition assay, 7-mm mycelial plugs were taken from 5-day-old pure cultures of *Colletotrichum* sp. and placed at the center of PDA plates supplemented with different concentrations of WEPE, ranging from 300 to 700 µg WEPE/mL (dissolved in 45% ethanol). Control plates received 45% ethanol instead of the WEPE. Each treatment was conducted in triplicate. The plates were incubated at 28±2°C for 6 days. The growth of the fungal colony was measured on days 1 to 6 of incubation. The percentage of Mycelial Growth Inhibition (MGI) was calculated using the formula:

$$MGI\ (\%) = [(R - r) / R] \times 100$$

Where, R is the colony diameter in the control plate, and r is the colony diameter in the treatment plate. For the spore germination inhibition assay, a spore suspension (10^5 spores/mL) was evenly spread on PDA medium supplemented with the WEPE at concentrations of 1,000, 2,000, 3,000, 4,000, and 5,000 µg WEPE/mL. Control plates contained 45% ethanol instead of the WEPE. Each treatment was replicated three times. The inoculated plates were incubated at 28±2°C for 3 days until the spores developed into fungal hyphae. This assay was conducted as a qualitative observation, aiming only to assess the presence or absence of spore germination and hyphal development rather than to measure inhibition quantitatively.

Antifungal activity of WEPE on *Colletotrichum* sp. in vivo

The in vivo antifungal activity of WEPE against *Colletotrichum* sp. was evaluated according to the method of Šernaite et al. (2020) with minor modifications. Dragon fruits at the same ripening stage and free from visible disease symptoms were selected for the assay. The fruit

surface was sterilized with 70% ethanol and air-dried under aseptic conditions. Each fruit was wounded at one site on the equatorial region using a sterile instrument, creating a wound approximately 7 mm in diameter and 5 mm in depth. WEPE was applied at two biologically justified concentrations derived from the in vitro assays: 700 µg/mL, identified as the Minimum Inhibitory Concentration (MIC) for suppressing mycelial growth, and 5,000 µg/mL, identified as the MIC required to inhibit spore germination of *C. siamense*. These concentrations were selected to capture the minimum effective doses for both key infection stages of the pathogen. A volume of 5 mL of the treatment solution was sprayed onto each fruit using a sterile hand sprayer and allowed to air-dry for 30 min in a laminar flow cabinet. Control fruits were sprayed with sterile distilled water only. After treatment, a 7 mm diameter mycelial plug of *Colletotrichum* sp., previously isolated from diseased dragon fruit, was placed into each wound. Each treatment consisted of six fruits and was conducted in triplicate following a completely randomized design. All fruits were placed individually in plastic bags and incubated at 25±2°C in the dark for 7 days under high-humidity conditions (>90% RH).

Statistical analysis

Experimental data were subjected to statistical analysis using SAS software (version 9.0; SAS Institute Inc., Cary, NC, USA) based on a completely randomized design. One-way Analysis of Variance (ANOVA) was performed, and treatment means were compared using the Least Significant Difference (LSD) test at a 95% confidence level. Results are expressed as mean values ± standard deviation.

RESULTS AND DISCUSSION

Effect of ultrasound frequency and extraction solvent on TPC of WEPE

The effect of ultrasound treatment at different frequencies (58, 132, and 192 kHz) and durations (10-40 minutes) by using three different solvents: distilled water, 45% ethanol, and 90% ethanol on TPC was recorded. Generally, ultrasound significantly enhanced the extraction of TPC compared to the control (0 kHz), with the most notable effect observed at 132 kHz in all solvents. In distilled water, TPC increased significantly at 58 and 132 kHz compared to the untreated sample, with the highest value (12.22 mg GAE/g) recorded at 40 minutes and 58 kHz. Treatment of 45% ethanol resulted in a markedly higher TPC, reaching a maximum of 18.26 mg GAE/g at 30 minutes and 132 kHz. In contrast, 90% ethanol showed significantly lower extraction efficiency in all frequencies and durations, with the highest value being only 8.90 mg GAE/g at 30 minutes and 132 kHz (Table 1). These results suggest that both solvent polarity and ultrasound frequency strongly affect TPC, with 45% ethanol and 132 kHz being the optimal conditions among those tested.

Morphological characteristics of the isolate

Anthracnose caused by *Colletotrichum* is a major postharvest disease of dragon fruit, leading to severe fruit decay and significant reduction in market quality. The morphological characteristics and conidial features of the *Colletotrichum* isolate obtained from postharvest dragon fruit are presented in Figure 1. The symptoms of anthracnose observed in appeared as reddish-orange spots, circular, sunken lesions on the fruit surface, dark brown to black with a slightly depressed center gray-white with a brown border (Figure 1.A). The colony training of TL12 *Colletotrichum* on PDA medium showed an ice-white conidial mass in the front (Figure 1.B), and a dark-gray color was observed in the reverse of the plate (Figure 1.C) after 7 days of incubation. Early, the fungal colony of strain TL12 development reached approximately 30 mm in diameter after 2 days. In the central area, the mycelium was dense, grayish-white, and cottony on the surface of the culture medium. After 7 days of cultivation, the colony reached about 65 mm in diameter, with thick, fluffy, and porous mycelium that turned dark gray in color (Figure 1.B). Under microscopic observation at 40X magnification, cylindrical spores were observed, featuring a constriction in the middle and slightly swollen at both ends (Figure 1.D).

Isolation and identification of *Colletotrichum* spp

Twelve isolates were isolated from collected samples of the diseased dragon fruit from Dong Thap province, and primary classified according to the morphological characteristics, with orders following 1-12. Among the 12 isolates, 9 and 12 isolates were identified as TL9 and TL12, respectively. Figure 2 shows disease progression in virulence between strains TL9 and TL12 on dragon fruit. TL9 caused small, dry, ivory-white lesions that expanded slowly and remained mostly superficial. On day 6, slight enlargement and tissue depression were observed. In contrast, TL12 induced rapidly expanding, soft, and water-soaked lesions. On day 3, TL12 lesions were larger and showed tissue maceration, and on day 6, dense white mycelia spread extensively, causing severe soft rot beyond the initial wound. These results indicate that TL12 is significantly more virulent than TL9, and the morphology of the re-isolates from the infected fruit was consistent with the inoculated isolate. Thus, the representative isolate of TL12 type was identified as the pathogenic isolate that causes dragon fruit anthracnose according to Koch's postulates; therefore, it was used in subsequent experiments.

Molecular identification of the isolate of *Colletotrichum*

Figure 3.B shows that the ITS sequence of isolate TL12 clustered together with *C. siamense* (GenBank accession no. LC052316.1) and *C. populi* isolate HZZ-32 (ON964996.1), exhibiting 100% sequence identity with previously deposited *Colletotrichum* spp. sequences in the NCBI database. In contrast, other closely related species within the *C. gloeosporioides* species complex, including *C. fructicola* isolate YJSG024 (OR248351.1), *C. gloeosporioides* isolate ISE017 (MG661732.1), and *C. aenigma* isolate OGCLZ1 (LC684904.1), formed distinct

clades with moderate bootstrap support (50-79%), indicating clear genetic divergence from isolate TL12. *Verticillium alfalfae* isolate LM33 (MH628645.1) was used as an outgroup and was clearly separated from the *Colletotrichum* clade, confirming the reliability of the phylogenetic reconstruction. Based on ITS sequence similarity and phylogenetic clustering, isolate TL12 was identified as *C. siamense*, a member of the *C. gloeosporioides* sensu lato species complex.

Effect of WEPE on *Colletotrichum isolate* of dragon fruit in vitro

Figure 4 shows the WEPE at different concentrations inhibiting the in vitro growth of *Colletotrichum* sp. over 1, 3, 5, and 7 days of incubation. Results indicated that the application of WEPE significantly inhibited the mycelial growth of *Colletotrichum*. The percentage of fungal inhibition increased with higher concentrations of the extract, showing a dose-dependent response. At low concentrations, 300 µg WEPE/mL, the WEPE inhibited fungal growth by 30%, while higher concentrations (400 µg WEPE/mL and 500 µg WEPE/mL), moderate inhibition was observed, with percentages ranging from 60% and 68%, respectively, over the seven days. Increasing the concentration to 600 µg WEPE/mL led to 85% inhibition. Remarkably, the highest concentration of 700 µg WEPE/mL completely inhibited fungal growth, reaching 100% inhibition throughout 7 days of observation, suggesting that WEPE possesses strong antifungal properties (Figures 5.A-F).

Colony developments of *Colletotrichum* were observed with differences concentration of WEPE with 45% ethanol plates serving as controls (Figure 5). The fungal colony fully covered the entire surface of the PDA plate, which was observed in the control treatment after 4 days of incubation. However, WEPE noticeably suppressed the radial expansion of the fungal colony in a concentration-dependent manner. At 300 and 400 µg WEPE/mL, the extract produced moderate to strong inhibition of colony growth, and at 500 µg WEPE/mL, fungal development was almost completely suppressed. Further inhibition was observed at 600 µg WEPE/mL, where fungal growth was restricted to only 10% of the PDA plate. Notably, complete inhibition was recorded at 700 µg WEPE/mL, with no visible mycelial growth. These findings indicate that WEPE significantly suppresses the mycelial growth of *Colletotrichum* in a dose-dependent manner under *in vitro* conditions.

Table 1. Effect of ultrasound frequency and extraction solvent on TPC of WEPE

Treatment time (min)	Ultrasound frequency (kHz)			
	0	58	132	192
Distilled Water				
10	3.86±1.42^{ba}	9.16±0.26^{aa}	10.48±1.12^{aa}	5.82±2.70^{ba}
20	4.66±0.55^{ba}	11.28±1.45^{aa}	9.73±4.10^{aa}	5.59±0.42^{ba}
30	4.11±0.25^{ba}	10.22±1.46^{aa}	7.47±3.24^{aa}	5.99±2.01^{ba}
40	4.49±0.94^{ba}	12.22±2.62^{aa}	10.54±2.60^{aa}	5.67±2.21^{ba}
Ethanol 45%				
10	12.16±0.97^{ba}	16.11±3.15^{aa}	17.37±1.04^{aa}	13.56±0.47^{ba}
20	12.26±0.85^{ba}	18.23±2.28^{aa}	17.94±1.82^{aa}	13.80±1.40^{ba}
30	11.23±0.81^{ba}	17.49±0.97^{aa}	18.26±1.49^{aa}	13.08±1.29^{ba}
40	11.67±1.20^{ba}	15.97±1.54^{aa}	17.83±2.34^{aa}	12.42±0.91^{ba}
Ethanol 90%				
10	2.79±0.32^{ca}	6.64±2.06^{ba}	8.73±0.23^{aa}	6.62±1.33^{ba}
20	2.64±0.03^{ca}	6.87±2.80^{ba}	8.39±0.45^{aa}	7.05±0.57^{ba}
30	3.02±0.12^{ca}	6.96±3.99^{ba}	8.90±0.85^{aa}	7.15±0.44^{ba}
40	2.98±0.22^{ca}	7.27±3.70^{ba}	8.62±1.01^{aa}	7.22±0.36^{ba}

Note: Values are expressed as the mean of three replicates. Means within the same column followed by different superscript letters are significantly different (p<0.05) according to the LSD test. Lowercase letters (a-c) indicate significant differences among ultrasound frequencies, whereas uppercase letters indicate significant differences among extraction times

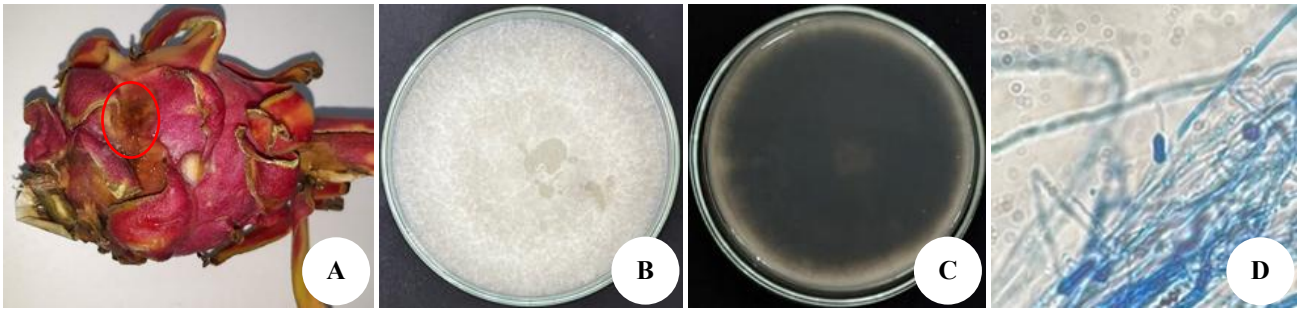

Figure 1. Morphological characteristics of *Colletotrichum* sp. isolated from dragon fruit. A. Anthracnose lesions on dragon fruit (red circles), B. Colony morphology on the upper surface, C. Reverse side of the colony, D. Hyphae and conidia under light microscopy

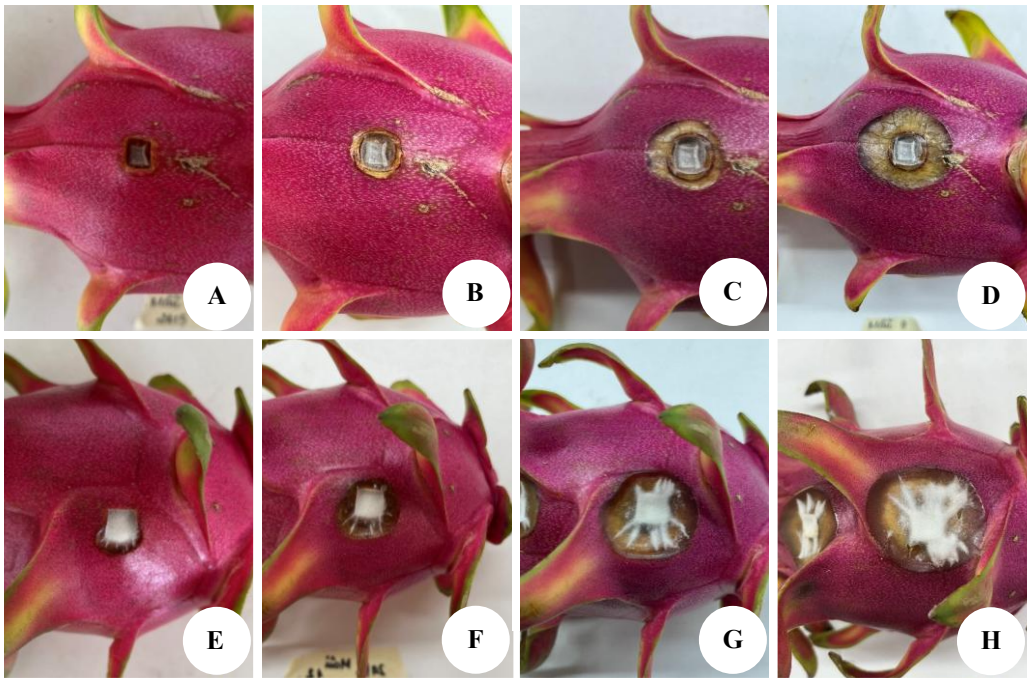

Figure 2. The pathogenicity test of the representative isolate of A-D. TL9 and E-H. TL12 (E-H) on dragon fruit with the mycelial discs from days 3-6 inoculation, respectively

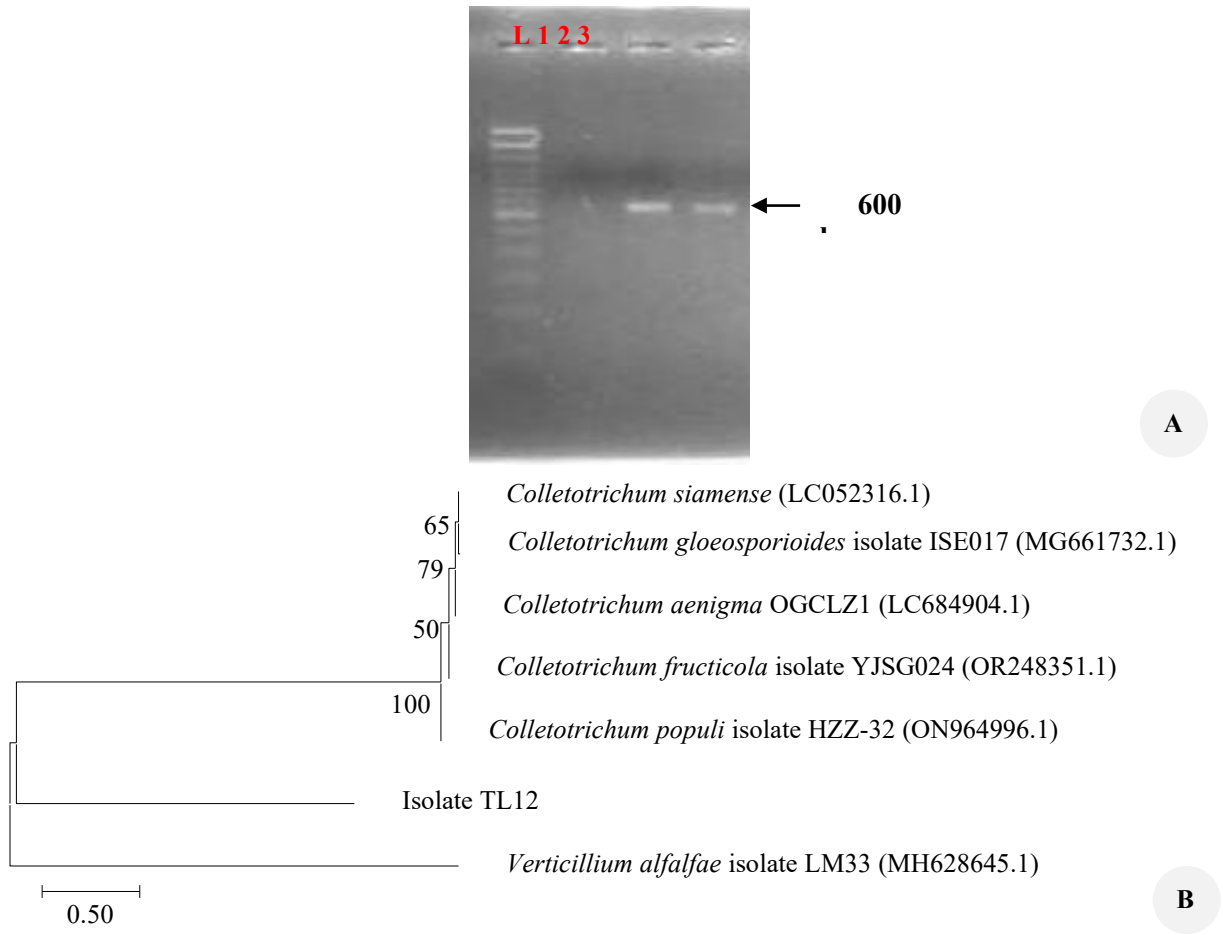

Figure 3. *Colletotrichum* identification. A. Amplification results of the ITS gene segment of *Colletotrichum* isolate TL12 strains (L: 100 bp plus standard ladder; Lane 1: Negative control; Lanes 2-3: fungal isolates; B. The phylogenetic tree is shown based on the ITS sections of ribosomal DNA sequences

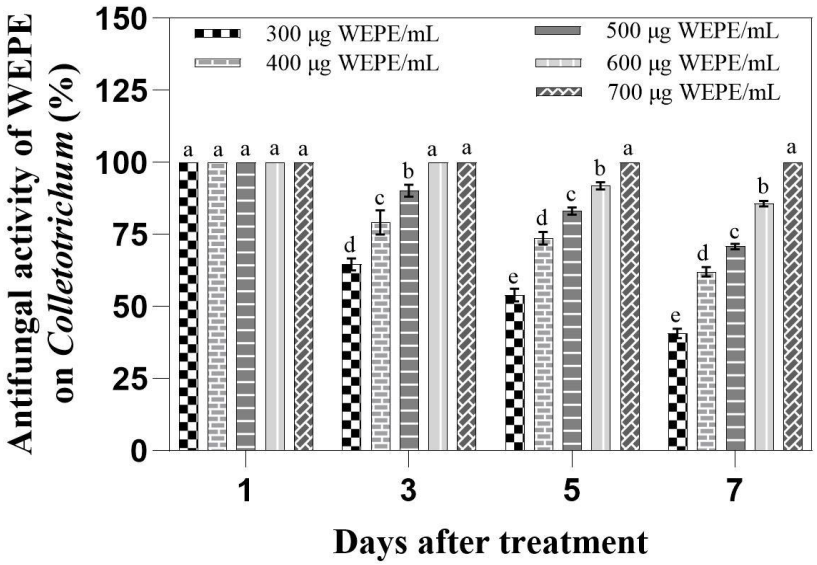

Figure 4. Antifungal activity of WEPE on Colletotrichum after 7 days of treatment. Different letters within the same group indicate a significant difference at the 5% level according to the LSD test. The values are expressed as mean ± standard deviation

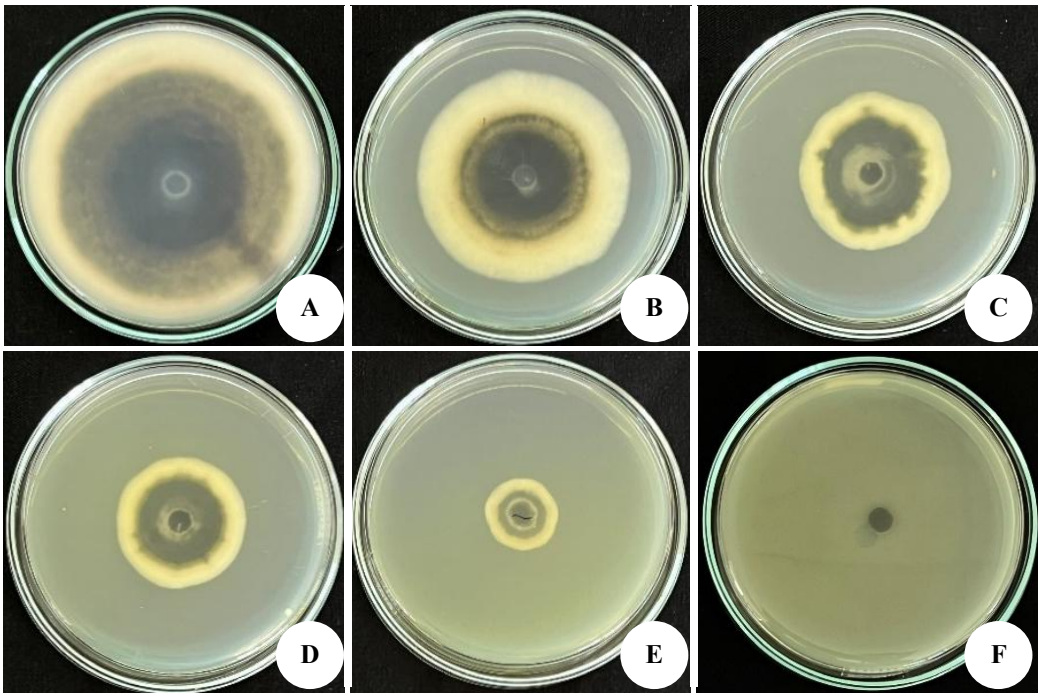

Figure 5. Antifungal activity of WEPE on the mycelium of *Colletotrichum* after 4 days of treatment. A. Control; B. 300; C. 400; D. 500; E. 600, and F. 700 µg WEPE/mL

Antifungal activity of WEPE on spore of *Colletotrichum*

Figure 6 illustrates the inhibitory effects of WEPE at different concentrations on *Colletotrichum* spore development after 4 days of incubation. In the control treatment (45% ethanol), spores densely covered the entire surface of the PDA plate, forming a smooth and compact fungal mat. In contrast, the antifungal effectiveness of WEPE varied depending on its concentration. At 1,000 µg/mL, spores colonized the entire plate surface, producing a loose mycelial mass that appeared white to cream in color and cotton-like in texture. At a concentration of 2,000 µg/mL, the spores formed tightly packed, cream-colored structures resembling clustered pebbles. At 3,000 µg/mL, fine cream-colored spore growth was observed only around the periphery of the plate. A similar growth pattern was observed at 4,000 µg/mL; however, the colony diameter was noticeably reduced. Complete inhibition of spore

development was recorded at 5,000 µg/mL, where no fungal growth was detected.

Effect of WEPE on *Colletotrichum* isolate of dragon fruit in vivo

The *in vivo* efficacy of WEPE against *Colletotrichum* infection in dragon fruit was evaluated at concentrations of 700 and 5,000 µg/mL, with sterile distilled water used as the control (Table 2 and Figure 7). On the first day of incubation, no significant differences in lesion diameter were observed among treatments, with all lesions measuring approximately 0.78 cm. After 4 days, the lesion diameter in the control fruit increased to 2.20 cm, whereas significantly smaller lesions were observed in fruits treated with WEPE at 700 µg/mL (1.70 cm) and 5,000 µg/mL (1.36 cm). This inhibitory effect became more pronounced over time. After 7 days of incubation, lesion diameters reached 4.46 cm in the control, compared with 3.30 cm and 2.40 cm in fruits treated with 700 and 5,000 µg/mL WEPE, respectively (Figure 7). These results demonstrate that WEPE significantly suppresses the development of anthracnose lesions caused by *Colletotrichum*, with higher concentrations exhibiting greater antifungal efficacy.

Table 2. Effect of WEPE on the lesion diameter caused by *Colletotrichum* isolate TL12 of dragon fruit

Concentration of WEPE (µg/mL)	Lesion diameter caused by *Colletotrichum* isolate TL12 (cm)		
	Days after inoculation		
	1	4	7
Control	0.78±0.05^{a}	2.20±0.05^{b}	4.46±0.05^{c}
700	0.74±0.05^{a}	1.70±0.05^{a}	3.30±0.05^{b}
5,000	0.74±0.05^{a}	1.36±0.05^{a}	2.40±0.05^{a}

Note: Different letters within the same column indicate a significant difference at the 5% level according to the LSD test. The values are expressed as mean ± standard deviation

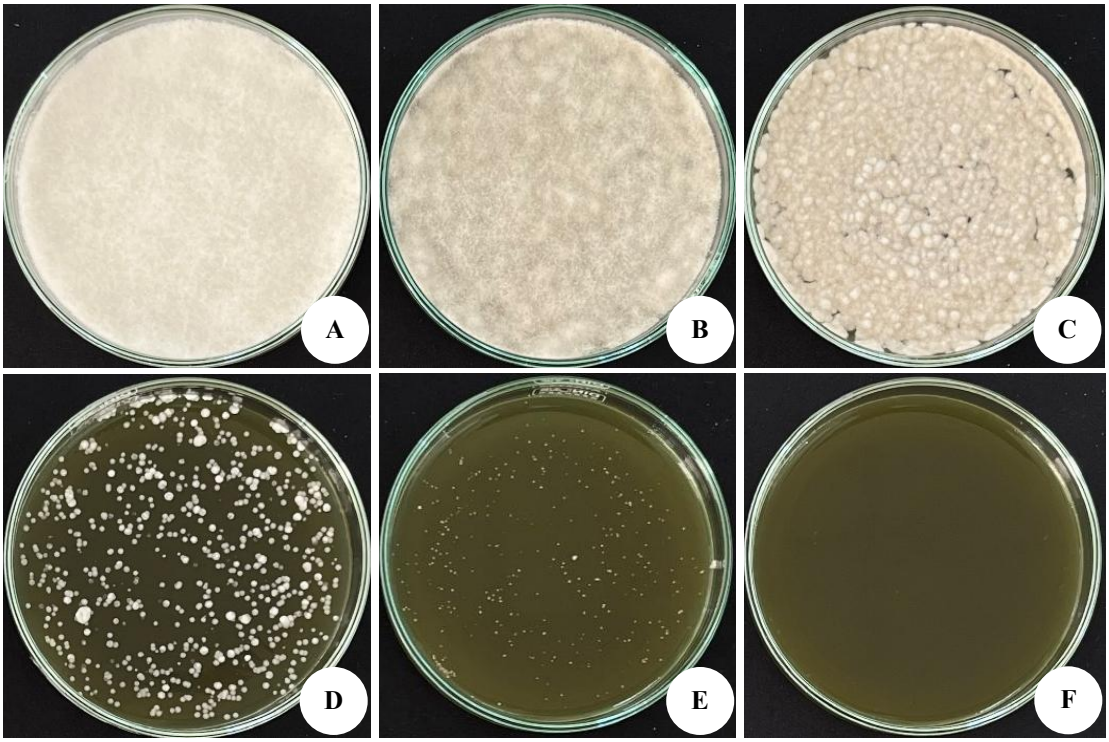

Figure 6. Antifungal activity of WEPE on spore of *Colletotrichum* after 4 days of treatment. A. Control; B. 1,000; C. 2,000; D. 3,000; E. 4,000; F. 5,000 µg WEPE/mL

Figure 7. Disease incidence of dragon fruit after 7 days of *Colletotrichum* inoculation. A. Control, B. 700 µg WEPE/mL, C. 5,000 µg WEPE/mL

Discussion

Species of the genus *Colletotrichum* are widely recognized as major postharvest pathogens causing anthracnose in a broad range of tropical fruits. Traditionally, *Colletotrichum gloeosporioides* has been regarded as the predominant causal agent of anthracnose in dragon fruit (*Hylocereus* spp.) worldwide (Takahashi et al. 2008). However, increasing evidence suggests that anthracnose etiology is geographically dependent and taxonomically complex, often involving multiple *Colletotrichum* species, such as *C. truncatum*, *C. siamense*, and *C. aenigma* (Vijaya et al. 2015; Zhang et al. 2024). In the present study, the pathogen isolated from postharvest anthracnose-infected dragon fruit collected in Dong Thap Province, Vietnam, was identified as *C. siamense* based on morphological characteristics and ITS sequence analysis. Although ITS sequencing alone may not fully resolve species boundaries within the *C. siamense* species complex, the congruence between morphological traits and molecular data supports reliable species-level identification in this case. To our knowledge, this is the first report of *C. siamense* causing postharvest anthracnose of dragon fruit in this region. This finding reinforces growing evidence that *C. siamense* is an emerging; ecologically versatile pathogen with a broad host range, previously reported on strawberry, mango, and dragon fruit in other countries (Luo et al. 2021; Rattanakreetakul et al. 2023). The detection of *C. siamense* in Vietnam highlights the need for region-specific pathogen surveillance and underscores the risk of misidentification when relying solely on classical taxonomy. Following pathogen identification, this study focused on optimizing the extraction of bioactive phenolic compounds from *E. fluctuans* using UAE. UAE significantly enhanced phenolic recovery, consistent with previous reports attributing this effect to acoustic cavitation, which disrupts plant cell walls and facilitates solvent penetration (Mason et al. 2011). The highest TPC was obtained using 45% ethanol combined with an ultrasound frequency of 132 kHz, suggesting an optimal balance between solvent polarity and ultrasonic energy. Aqueous ethanol likely improves phenolic solubility while enhancing mass transfer through reduced surface tension, whereas excessive ethanol concentrations decrease solvent polarity and limit the extraction of polar phenolics. Moreover, solvents with high vapor pressure may dampen cavitation intensity, thereby reducing extraction efficiency (Wang and Weller 2006; Chemat et al. 2017). Although the UAE effectively increased phenolic recovery, extending the extraction time beyond 20-30 min did not result in a significant increase in TPC. Prolonged sonication may promote thermal and oxidative degradation of phenolic compounds, offsetting the benefits of extended extraction. This observation underscores that extraction efficiency depends not only on maximizing cell disruption but also on preserving compound stability through careful optimization of extraction parameters (O'Donnell et al. 2010).

Plant-derived phenolic compounds have attracted increasing interest as natural antifungal agents against postharvest pathogens, including *Colletotrichum* spp. In the present study, the WEPE exhibited strong antifungal activity against *C. siamense*, as demonstrated by a marked reduction in lesion development on dragon fruit. The antifungal effect of WEPE was clearly concentration-dependent, with the highest level of disease suppression observed at 5,000 µg/mL after 7 days of incubation. This dose-response relationship suggests that increasing phenolic concentration enhances the probability of interactions between bioactive compounds and fungal cellular targets, leading to cumulative inhibitory effects. Differences in antifungal efficacy among plant extracts are likely attributable to variations in phenolic composition, molecular structure, and the number and position of hydroxyl (-OH) groups on phenolic rings, which influence membrane affinity and binding strength to fungal proteins and lipids (Konuk and Ergüden 2020; Ultee et al. 2020). Phenolic compounds exert antifungal activity through multiple, often synergistic mechanisms. These include disruption of fungal cell membrane integrity via interactions with membrane proteins and lipids, resulting in increased permeability, leakage of intracellular components, and inhibition of spore germination (Díaz-García et al. 2024). Additionally, phenolics may interfere with fungal cell wall biosynthesis, inhibit key metabolic enzymes, and chelate essential metal ions, collectively impairing fungal growth and pathogenicity (Teshome et al. 2022). At higher concentrations, these mechanisms act in concert, leading to pronounced reductions in fungal viability. The antifungal activity observed in this study is consistent with previous reports on phenolic-rich plant extracts against *Colletotrichum* spp. For example, sweet orange peel decoction reduced conidial production by 94.58% and significantly inhibited spore germination of *C. gloeosporioides* infecting dragon fruit (Sulyanti et al. 2019). Ginger crude extract at 10 g/L effectively suppressed mycelial growth and conidial germination of *C. gloeosporioides* (Bordoh et al. 2020), while mangosteen pericarp extract significantly reduced anthracnose incidence in banana fruit during cold storage and subsequent shelf life (Montri et al. 2020). Beyond direct antifungal activity, the antioxidant properties of phenolic compounds may indirectly contribute to disease suppression by mitigating oxidative stress and enhancing host defense responses in fruit tissues. Such dual functionality, combining antimicrobial and antioxidant effects, positions phenolic-rich extracts as promising alternatives to synthetic fungicides in sustainable postharvest disease management (Hajji-Hedfi et al. 2024).

In conclusion, this study demonstrated that 45% ethanol combined with ultrasound extraction at 132 kHz for 30 min yielded the highest total phenolic content of WEPE. A single pathogenic species, *C. siamense*, was isolated and identified as the causal agent of postharvest anthracnose in dragon fruit based on morphology and ITS sequencing. WEPE showed strong antifungal efficacy, inhibiting mycelial growth at 700 µg/mL and fully suppressing spore development at 5,000 µg/mL, and effectively reduced lesion development in vivo. These results indicate that WEPE can significantly suppress disease progression during short-term postharvest storage, highlight the potential role of phenolic-rich plant extracts in fungal

growth inhibition, a natural antifungal agent for managing postharvest anthracnose in dragon fruit, and support its application as an eco-friendly alternative to synthetic fungicides.

Despite these promising findings, several limitations should be considered. The experiments were conducted under controlled laboratory conditions with short storage durations, and antifungal efficacy was evaluated against a single *C. siamense* isolate. In addition, pathogen identification relied on ITS rDNA sequencing, which may have limited resolution within the *Colletotrichum* species complex. The study also did not assess extract stability, residue persistence, or effects on fruit sensory quality. Future research should focus on extended storage trials, multi-isolate or multi-species evaluations, and the development of stable WEPE formulations suitable for commercial application. Integrating advanced molecular identification, assessing modes of antifungal action, and validating efficacy under field or supply-chain conditions will be essential to confirm the practical potential of WEPE as a sustainable alternative for postharvest disease management in dragon fruit.

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