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Isolation and characterization of *Colletotrichum gloeosporioides* species complex causing chili anthracnose in Lao Cai province, Viet Nam and evaluation of antifungal activity of selected local plant extracts

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ABSTRACT

This study aimed to investigate the occurrence of chili anthracnose, characterize the associated pathogen, and evaluate the antifungal potential of plant-derived products as sustainable management options in the Muong Khuong area, Lao Cai province. Field investigations showed that anthracnose was prevalent on both hot chili and sweet chili, with disease incidences of 50.50% and 70.48%, respectively, during the July survey. Anthracnose symptoms were observed on leaves and fruits of chili types. The pathogen was isolated from symptomatic tissues and characterized based on colony morphology, microscopic features, pathogenicity tests, and ITS sequence analysis. Re-inoculation on detached chili leaves and fruits reproduced anthracnose symptoms, confirming the pathogenicity of the isolate. BLASTn analysis of the ITS region revealed 100% sequence identity with species within the Colletotrichum gloeosporioides species complex. In vitro antifungal assays demonstrated that extracts from Piper betle, Elsholtzia ciliata, and Ocimum basilicum inhibited the pathogen's mycelial growth. Among them, Piper betle showed the strongest inhibitory effect (62.16% inhibition at 5 days after treatment), followed by Elsholtzia ciliata (23.91% inhibition at 5 days after treatment) and Ocimum basilicum (15.68% inhibition at 3 days after treatment). These findings support the potential use of plant-derived products for sustainable management of chili anthracnose.

1. INTRODUCTION

Originating in the American tropics, chili peppers (*Capsicum* spp.) are today grown all over the world for their fresh, dried, and processed uses (Tripodi & Kumar, 2019). The genus has more than 30 species, 5 of which are domesticated and farmed primarily for food. It is remarkably adaptive to warm, humid climates with temperatures between 18 and 30°C (Khaitov et al., 2019). Chili peppers have global value beyond culinary use because they are high in micronutrients and phytochemicals, which are commonly reported to include vitamins (particularly A and C), minerals, pigments, phenolics, and other

antioxidants, as well as pungent bioactives such as capsaicinoids (Alonso-Villegas et al., 2023). Furthermore, capsaicin and related compounds have been extensively studied for pharmaceutical and cosmetic applications, and are increasingly being incorporated into industrial/technological applications (Maharjan et al., 2024). Anthracnose is one of the most destructive diseases of chili worldwide and can cause substantial yield and quality losses, particularly during periods favorable for infection and epidemic development (Than et al., 2008; Ly et al., 2020). Chili anthracnose is primarily caused by species of *Colletotrichum*, a genus comprising multiple species complexes with high morphological overlap (Than et al., 2008). In particular, members of the *Colletotrichum gloeosporioides* species complex are frequently associated with anthracnose symptoms on diverse hosts, and species delimitation within this complex remains challenging without multilocus approaches (Weir et al., 2012). Although the internal transcribed spacer (ITS) is widely used as a fungal barcode, it often lacks sufficient resolution to distinguish closely related taxa within *Colletotrichum* species complexes, including the *C. gloeosporioides* complex (Weir et al., 2012). The mountainous Muong Khuong region of Lao Cai province is well known for producing "Muong Khuong chili peppers." At present, more than 400 hectares of chili peppers are cultivated in the region, where the crop represents a major source of income and supports rural development in the hilly areas of Lao Cai province. Anthracnose disease poses a serious constraint on chili production, particularly under the humid and wet climatic conditions characteristic of the region. The sustainability of chili farming in Muong Khuong is being threatened by this disease, which has led to significant productivity decreases and fruit quality degradation.

To date, scientific reports that document chili anthracnose pathogens from Lao Cai province, including the Muong Khuong area, through an integrated approach encompassing field symptom observation, pathogen isolation, morphological characterization, and ITS-based taxonomic placement remain scarce. At the same time, disease management in many chili-producing regions depends largely on chemical fungicides, which underscores the need to investigate locally available plant-derived products as complementary management options (Than et al., 2008).

Essential oils and plant extracts contain bioactive compounds with antifungal properties and have been reported as promising components of integrated disease management programs (Isman, 2000; da Cruz Cabral et al., 2013). To develop chili pepper products sustainably and safely for consumers and the ecological environment, numerous studies are currently seeking plant-based solutions for managing chili anthracnose disease. Essential oils and plant extracts are known to contain bioactive compounds with antifungal properties and have been reported as promising components in integrated disease management programs (da Cruz Cabral et al., 2013).

Essential oils and extracts derived from *Piper betle*, *Elsholtzia ciliata*, and *Ocimum basilicum* (basil) were selected based on their traditional use, local availability, and reported antimicrobial activity. Previous studies have demonstrated that essential oils from *Ocimum* species and extracts from *Piper* species exhibit inhibitory effects against a range of phytopathogenic fungi, including *Colletotrichum* spp. (Prakash et al., 2015)(Christopher et al., 2023). However, information on their efficacy against chili anthracnose under local production conditions remains limited. Therefore, evaluating these plant-derived products may contribute to the development of environmentally friendly and locally adapted strategies for sustainable anthracnose management.

This study aimed to investigate chili anthracnose in Lao Cai province by integrating field symptom assessment, pathogen isolation, morphological characterization, and ITS sequence analysis to determine the taxonomic placement of the causal agent. In addition, the study evaluated the antifungal activity of selected locally available plant-derived products against the identified pathogen *in vitro*, to identify potential alternatives to chemical fungicides for sustainable management of chili anthracnose.

2. MATERIALS AND METHOD

2.1. Materials

Leaf and chili fruit samples showing typical anthracnose symptoms were collected from six different chili fields in Muong Khuong commune, Lao Cai province. Eighteen representative diseased samples were collected from two commonly cultivated chili varieties in Muong Khuong, namely local hot chili (*Capsicum annuum* var. *annuum*) and sweet pepper (*Capsicum annuum* var. *grossum*). Anthracnose fungi were isolated and purified on PDA (Potato Dextrose Agar) medium.

2.2. Methods

2.2.1. Collection of diseased samples and symptom observation

Both sweet chili (*C. annuum* var. *grossum*) and hot chili (*C. annuum* var. *annuum*) were included in the survey. Disease symptoms were recorded on leaves and fruits of both chili types. Anthracnose symptoms initially appeared as small, brown lesions on leaves or fruits, which gradually enlarged and developed into sunken, dark brown to black necrotic spots. Symptomatic leaves and fruits were excised from infected plants, placed in sterile plastic bags, stored in a cooler box, and transported to the laboratory for further analysis.

2.2.2. Fungal isolation

Small tissue fragments (approximately 3-5 mm) were excised from the margins of active lesions on diseased leaves and fruits. The samples were surface-sterilized by immersion in 75% (v/v) ethanol for 30 s, followed by 5% (v/v) sodium hypochlorite for 1 min, rinsed three times with sterile distilled water, and dried on sterile filter paper. The sterilized infected tissue was placed onto potato dextrose agar (PDA) plates and incubated at 25-28°C. Emerging fungal colonies were subcultured aseptically on fresh PDA to obtain pure isolates. One pure isolate from each diseased sample was selected for morphological identification.

2.2.3. Colony and morphological characterization

Colony morphology of the fungal isolate was examined on PDA plates incubated at 25-28°C. Colony characteristics, including growth pattern, texture, color, margin shape, and pigmentation on both the upper and reverse surfaces, were recorded at 3 and 9 5-7 days after incubation. Colony diameter was measured daily to assess radial growth. Microscopic features of the isolate were examined using a light microscope. Fungal structures were mounted in sterile distilled water and observed at 40× magnification. The following morphological characteristics were observed: characteristics of hyphae (septation, branching, and wall texture) and conidia (shape, size, color, and surface ornamentation) were documented. The presence or absence of setae and appressoria was also recorded under the examined conditions. Based on characteristic morphological features, one representative fungal isolate was selected for molecular identification to determine its scientific name.

2.2.4. DNA extraction, PCR amplification and ITS sequencing

Genomic DNA was extracted from fresh fungal mycelia using the cetyltrimethylammonium bromide method (Sambrook & Russell, 2001). The ITS region was amplified using the universal primers ITS1 and ITS4 (White et al., 1990). PCR amplification was performed in a thermal cycler under standard conditions. The amplified products were purified and sequenced by a commercial sequencing service. The resulting ITS sequences were used for molecular identification of the fungal isolates.

2.2.5. Molecular analysis

The ITS sequences obtained in this study were edited and aligned using BioEdit software (Alzohairy, 2011). Sequence similarity was assessed using the BLASTn algorithm against the NCBI GenBank database to determine the closest matching taxa. The taxonomic placement of the isolate was inferred from percentage identity, query coverage, and E-value values obtained from BLASTn searches. Representative top BLASTn hits were summarized in a table for comparison.

2.2.6. Pathogenicity test

Pathogenicity of the representative *Colletotrichum* isolate was evaluated on detached leaves of chili plants under controlled conditions. The isolate was cultured on PDA plates at 25-28°C for 7 days to produce conidia. A conidial suspension was prepared in sterile distilled water and adjusted to a concentration of 2.0×10^6 conidia mL^{-1}. Healthy, young leaves of both sweet chili and hot chili, free from visible disease symptoms, were washed under running tap water, surface-sterilized with 75% (v/v) ethanol, and rinsed with sterile distilled water. Detached leaves were placed in plastic containers lined with moist sterile tissue paper to maintain high humidity. Small wounds were made on the leaf surface using a sterile needle, and each wound was inoculated with 100 µL of the conidial suspension. Sterile distilled water was used as the negative control. The containers were sealed with plastic film and incubated at 25-28°C. Disease development was assessed by measuring disease incidence (DI) rate (%) and lesion size in two perpendicular directions on each leaf at 24, 48, 72, 96, 120, 144, and 168 hours post-inoculation (hpi). The Area Under the Disease Progress Curve (AUDPC) was calculated using the following formula: $AUDPC=i=1\sum n-1 [2Yi+Yi+1\times(Xi+1-Xi)$ (Campbell & Madden, 1990).

2.2.7. Preparation of plant extracts and essential oils

Crude extracts of *Piper betle* and *Elsholtzia ciliata* were prepared using the same extraction procedure with slight modifications from previously reported methods (Zhang et al., 2018). Briefly, 100 g of dried plant material (leaves for *P. betle*; stems and leaves for *E. ciliata*) were soaked in 1 L of distilled water (1:10, w/v) and subjected to ultrasonic-assisted extraction for 2 h at 80 °C. The extracts were filtered to remove plant residues and subsequently concentrated under reduced pressure using a rotary vacuum evaporator at 80°C to obtain 50 mL of crude extract. Essential oil of *O. basilicum* was obtained by hydrodistillation following a standard procedure (Soković et al., 2010). Fresh basil material (1.5 kg of stems and leaves) was distilled with 5 L of water for 3 h, yielding 1.8 mL of essential oil. The oil was prepared as a 1% (v/v) stock solution in distilled water with Tween 80 as an emulsifier before use in antifungal experiments. The obtained extracts were used for antifungal assays through 1% stock solutions.

2.2.8. In vitro antifungal activity assay

The antifungal activity of plant-derived products against the chili anthracnose pathogen was evaluated using the poisoned food technique, with minor modifications based on standard protocols (Grover & Moore, 1962). Appropriate volumes of each plant extract or essential oil were incorporated into molten PDA medium at approximately 50°C to obtain the desired final concentrations. The extracts were prepared as 1% stock solutions, and a 0.1% solution was prepared from the stock for the experiments in this study. A total of 0.5 mL of the 0.1% crude extract solution was added to each Petri dish containing PDA medium. The amended PDA was poured into sterile Petri dishes and allowed to solidify. PDA medium without plant-derived products served as the untreated control. A 5-mm-diameter mycelial plug was excised from the actively growing margin of a 7-day-old fungal culture and placed at the center of each Petri dish. The plates were incubated at 25-28°C in the dark. Colony diameter was measured along two perpendicular axes at 1, 3, and 5 days post-inoculation (dpi), and the mean value was calculated for each plate. Each treatment was conducted in triplicate, and the experiment was repeated independently. Antifungal activity was assessed by comparing colony diameters on treated plates with those on the control plate. The inhibitory effect of plant extracts on fungal mycelial growth was calculated as a percentage using the following formula (Pheaktra et al., 2023): Inhibitory effect (%) = (Colony diameter of control - Colony diameter of treatment)/ Colony diameter of control x 100.

2.2.9. Statistical analysis

All experiments were conducted using a completely randomized design. Data obtained from pathogenicity tests and *in vitro* antifungal assays were expressed as mean ± standard deviation. Colony diameter and lesion area measurements were calculated from three independent replicates for each treatment. Statistical analyses were performed using analysis of variance to evaluate differences among treatments. When significant differences were detected, mean values were compared at a significance level of p-value< 0.05. Statistical results were used to support the interpretation of treatment effects, while numerical data are presented in tables and figures.

3. RESULTS AND DISCUSSION

3.1. Field investigation of chili anthracnose incidence

A field investigation was conducted in chili-growing areas of Muong Khuong commune, Lao Cai province, to assess the incidence of anthracnose under natural conditions. DI rates were recorded for both hot and sweet chili during two survey periods in July and November 2025. Detailed DI data for each chili type and survey period are presented in Table 1.

Table 1. Incidence of chili anthracnose under field conditions in Muong Khuong commune, Lao Cai province

Survey time	Chili type	DI(%)
July 2025	Hot chili	50.50
	Sweet chili	70.48
November 2025	Hot chili	55.30
	Sweet chili	28.69

The results indicated a high incidence of anthracnose during the July survey, with disease rates of 50.50% in hot chili plants and 70.48% in sweet chili plants. During the November survey, anthracnose incidence declined, with recorded values of 55.30% in hot chili and 28.69% in sweet chili. In Viet Nam, hot chili (*C. frutescens*) constitutes one of the most important and widely cultivated spice crops, reflecting the country's substantial role in global chili production (Tam et

al., 2015). The commercial importance of chili peppers continues to expand worldwide, with sweet chili (*C. annuum*) recognized as the most economically significant species at the global scale. This crop holds substantial economic value and plays a key role in both local food systems and international trade (Hernandez-Perez et al., 2020).

3.2. Disease symptoms and isolation of the pathogen

Following the field investigation of DI (Figure 1), typical anthracnose symptoms were documented on chili plants in Muong Khuong commune, Lao Cai province, at different growth stages.

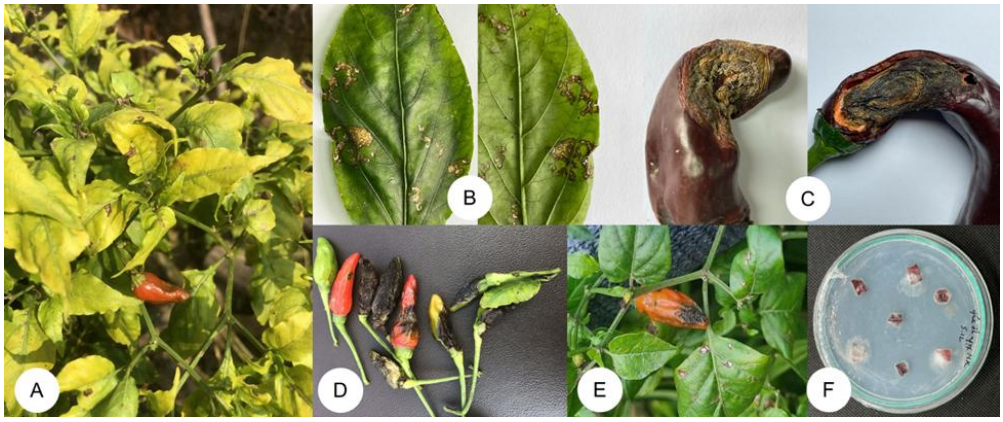

Figure 1. Anthracnose symptoms on chili plants and isolation of the pathogen in Muong Khuong commune, Lao Cai province

(A) Chili plant showing anthracnose symptoms under field conditions. (B) Upper and lower leaf surfaces of sweet chili exhibiting necrotic lesions. (C) Sweet chili fruit with typical anthracnose symptoms. (D) Hot chili fruits showing advanced anthracnose lesions. (E) Hot chili leaf with necrotic spots caused by anthracnose. (F) Isolation of the pathogen from diseased tissues on PDA medium.

Under field conditions, infected chili plants showed a general decline in vigor, characterized by leaf yellowing and premature defoliation in severely affected plants (Figure 1A). On chili leaves, initial symptoms appeared as small, irregular to circular brown lesions on both the upper and lower leaf surfaces. As the disease progressed, these lesions enlarged, coalesced, and became necrotic, often exhibiting dark margins with lighter centers (Figures 1B, 1E). In advanced cases, extensive necrosis led to tissue collapse and partial leaf blight.

Anthracnose symptoms were observed on the fruits of both chili types. Infected fruits exhibited sunken necrotic lesions that ranged from dark brown to black and often originated at the fruit tip or along the lateral surface. With disease progression, lesions increased in size and led to fruit deformation, shriveling, and tissue decay (Figures 1C, 1D). Under field conditions, severely affected fruits became dry and mummified. Symptomatic fruit tissues were collected for pathogen isolation. Tissue fragments excised from the margins of active lesions were surface-sterilized and cultured on potato dextrose agar. Following incubation, fungal colonies consistently emerged from the plated tissues, which confirmed successful isolation of the pathogen associated with the observed anthracnose symptoms (Figure 1F).

3.3. Morphological characteristics of the isolate

The fungal isolate obtained from anthracnose lesions on chili fruits exhibited characteristic colony morphology when cultured on PDA (Figure 2).

After 3 days of incubation, the colony appeared circular with a compact growth pattern and a dense, whitish mycelial mass at the center, surrounded by a translucent to pale-gray peripheral zone (Figure 2A). The colony margin appeared smooth to slightly irregular, which reflected rapid radial growth at the early stage of development. After 9 days of incubation, the colony reached full expansion and displayed distinct morphological features on both surfaces. On the upper surface, the colony exhibited a cottony to floccose texture and a white to light gray coloration, with a dense central region and a loosely structured peripheral zone (Figure 2B). The reverse surface showed gray to dark gray pigmentation that was concentrated at the center and decreased toward the margins (Figure 2C). Microscopic examination showed well-developed,

hyaline, septate hyphae with smooth walls and frequent branching that formed an extensive mycelial network. The hyphae were elongated, slightly tapered toward the apex, with a clavate structure at the tip (Figure 2D). Conidia were abundant, hyaline, smooth-walled, and predominantly cylindrical to oblong with rounded ends (Figures 2E, 2F), with dimensions of approximately 12-18 µm in length and 4-6 µm in width, as estimated from the scale bars. Setae and appressoria were not observed under the examined conditions. Based on colony morphology on PDA medium and microscopic characteristics of hyphae and spores, the isolate exhibits typical characteristics of a species belonging to the *Colletotrichum gloeosporioides* complex, a common anthracnose pathogen in chili peppers.

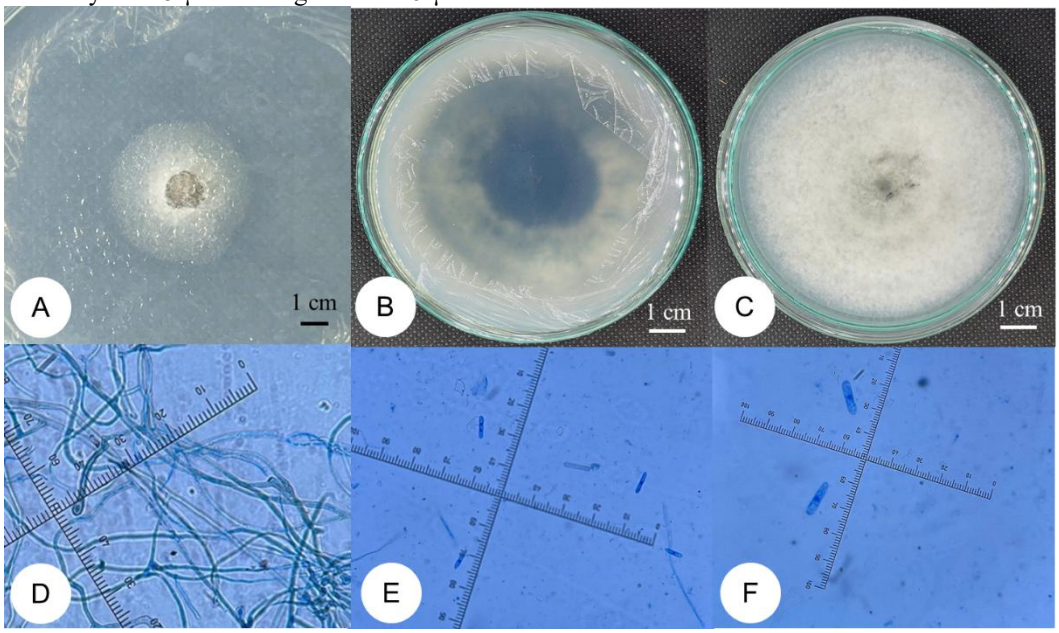

Figure 2. Colony morphology and microscopic features of the *Colletotrichum* isolate obtained from chili anthracnose in Lao Cai province

(A) Colony morphology after 3 days of incubation on PDA. (B) Upper surface of the colony after 9 days of incubation. (C) Reverse side of the colony after 9 days of incubation. (D) Septate hyphae observed under light microscopy. (E-F) Hyaline, cylindrical conidia were observed under light microscopy 40X.

3.4. Pathogenicity test

Following artificial inoculation, typical anthracnose symptoms developed on both chili leaves and fruits, closely resembling those observed under natural field conditions (Section 3.1), whereas no symptoms were observed on the control treatments throughout the experimental period (Table 2, Figure 3). On inoculated leaves, symptoms initially appeared as small necrotic spots that progressively expanded and coalesced over time. In contrast, inoculated fruits developed characteristic anthracnose lesions, manifested as progressively enlarging, sunken, dark necrotic areas.

Table 2. Time of symptom onset and disease incidence rate (%) on chili leaves and fruits after artificial inoculation of the Muong Khuong hot chili cultivar

Hpi (hours)	24	48	72	96	120	144	168	AUDPC
Leaf (C)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Leaf (T)	63.0	70.0	77.0	77.0	77.0	77.0	77.01	10.75
Fruit (C)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Fruit (T)	60.0	67.0	70	73.0	73.0	73.0	73.0	10.14

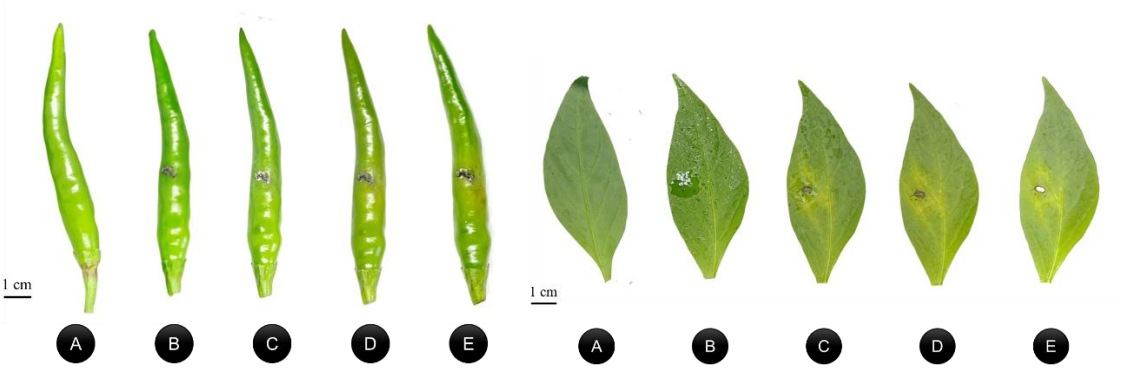

Figure 3. Development of anthracnose symptoms on chili leaves and fruits after re-inoculation

(A) control; (B) 24 hpi; (C) 72 hpi; (D) 120 hpi; (E) 168 hpi.

DI on inoculated leaves was first recorded at 24 hpi, reaching 63.0%, and increased to 77.0% by 72 hpi, remaining stable thereafter until 168 hpi. In contrast, control leaves showed 0.0% DI at all observation times (Table 2). Similarly, inoculated chili fruits exhibited disease symptoms from 24 hpi, with a DI of 60.0%, which increased to 73.0% by 96 hpi and remained unchanged up to 168 hpi. No disease symptoms were observed on control fruits during the experiment (Table 2).

Quantitative assessment of lesion development further confirmed the isolate's pathogenicity. On inoculated leaves, lesion area increased progressively from 0.21 ± 0.01 cm^2 at 24 hpi to 3.33 ± 0.15 cm^2 at 168 hpi. A similar trend was observed on inoculated fruits, where lesion area expanded from 0.19 ± 0.01 cm^2 at 24 hpi to 2.70 ± 0.03 cm^2 at 168 hpi (Table 3).

Table 3. Lesion area (cm^2) on chili leaves and fruits at different times after inoculation

hpi (hours)	24	48	72	96	120	144	168	AUPDC
Leaf (T)	0.21±0.01^a	0.43±0.01^a	0.83±0.03^a	1.43±0.01^a	1.94±0.02^a	2.62±0.08^a	3.33±0.15^a	216.48
Fruit (T)	0.19±0.01^a	0.37±0.01^b	0.66±0.02^b	1.05±0.03^b	1.45±0.01^b	2.13±0.03^b	2.70±0.03^b	170.52

Different letters within a column indicate statistically significant differences between leaf and fruit lesions at the same observation time ($p < 0.05$).

Visual observation showed a clear progression of anthracnose symptoms over time, from small necrotic spots at early stages to large, sunken lesions at later stages on both leaves and fruits (Figure 3). The absence of symptoms in the control and the consistent development of typical anthracnose lesions on inoculated tissues confirm that the isolated fungus is pathogenic to chili and capable of reproducing disease symptoms observed under field conditions.

The Area Under the Disease Progress Curve (AUDPC) clearly describes disease development over time. In the control treatment, both leaves and fruits remained symptomless throughout the experimental period (AUDPC = 0), indicating that no cross-contamination or external infection occurred. Therefore, disease development resulted solely from inoculation with the isolated fungal strain.

Based on the AUDPC values, *C. gloeosporioides* exhibited high pathogenicity to both leaves and fruits of chili plants within only 24 hours after inoculation. Disease development was most rapid between 24-72 hpi and entered a relatively stable phase from 96 hpi onward. The AUDPC values further indicated that the cumulative disease severity on leaves (10.752) was higher than that on fruits (10.140), suggesting that leaf tissues were more susceptible than fruit tissues to the investigated fungal isolate.

The progression dynamics of anthracnose lesions caused by the isolated strain showed that lesions on both chili leaves and fruits continuously expanded from 24 to 168 hpi. At 24 hpi, lesion areas on leaves (0.21 cm^2) and fruits (0.19 cm^2) were not significantly different. However, from 48 hpi onward, lesions on leaves developed more rapidly and were consistently larger than those on fruits, with statistically significant differences at the 0.05

level. These results were fully consistent with the AUDPC values, with the cumulative disease pressure on leaf tissues (AUDPC = 216.48) significantly higher than that on fruit tissues (AUDPC = 170.52).

3.5. ITS sequence analysis and taxonomic placement of the pathogen

The ITS region of the representative fungal isolate obtained from chili anthracnose lesions was successfully amplified and sequenced. After trimming low-quality regions at both ends, the edited ITS sequence was 466 bp in length, with a GC content of 53.65%, which falls within the typical range reported for species of the genus *Colletotrichum*. BLASTn analysis against the NCBI GenBank database revealed that the ITS sequence shared 100% sequence identity and 100% query coverage (E-value = 0.0) with numerous reference sequences deposited under different species names, including *Colletotrichum theobromicola*, *C. siamense*, *C.* gloeosporioides, *C. tropicale*, as well as several unidentified *Colletotrichum* spp. and *Glomerella* spp. Representative top BLASTn hits and their corresponding similarity parameters are summarized in Table 4. All of these taxa are currently recognized as members of the *C. gloeosporioides* species complex.

Comprehensive taxonomic revisions have demonstrated that many species within the *C. gloeosporioides* species complex share identical or nearly identical ITS sequences, despite being clearly distinguishable using multilocus phylogenetic analyses (Weir et al., 2012).

Table 4. BLASTn results of the ITS sequence of the chili anthracnose isolate

Accession number	Species	Identity (%)	Query coverage (%)	E-value
MH863488.1	*Colletotrichum theobromicola*	100	100	0.0
MT434612.1	*C. siamense*	100	100	0.0
PV436897.1	*C. gloeosporioides*	100	100	0.0
KR445674.1	*C. tropicale*	100	100	0.0
JX040843.1	*Glomerella* sp.	100	100	0.0

In the present study, species-level identification was therefore not attempted based solely on ITS sequence data. Instead, ITS analysis was used as a supportive molecular tool to confirm the placement of the chili anthracnose isolate within the *C. gloeosporioides* species complex. This conservative taxonomic interpretation avoids potential misidentification and is consistent with current recommendations for *Colletotrichum* systematics (Cai et al., 2009). When considered together with characteristic disease symptoms observed in the field, colony morphology on PDA, and microscopic features of hyphae and conidia, the ITS-based results provide reliable evidence that the pathogen associated with chili anthracnose in Lao Cai province belongs to the *C. gloeosporioides* species complex. This level of identification is appropriate for regional disease investigations and conference-oriented studies, and it provides a reliable taxonomic framework for subsequent evaluation of antifungal activity of plant-derived products.

3.6. Antifungal activity of plant-derived products

The antifungal activity of selected plant-derived products against the *Colletotrichum gloeosporioides* species complex was evaluated based on mycelial growth inhibition *in vitro*. Colony diameter was measured at 1, 3, and 5 dpi on PDA media amended with different plant extracts, including *Piper betle* (PbT), *Elsholtzia ciliata* (EcT), and *Ocimum basilicum* (ObT), and compared with the untreated control. Fungal growth on extract-amended media was consistently lower than the control at 1 and 3 dpi, and by 5 dpi (Figure 4A), growth remained strongly suppressed in the PbT treatment (1.82 mm), while larger colonies were observed for EcT (3.66 mm) and ObT (4.34 mm); the untreated control showed the greatest colony diameter (4.81 mm) (Figure 4B).

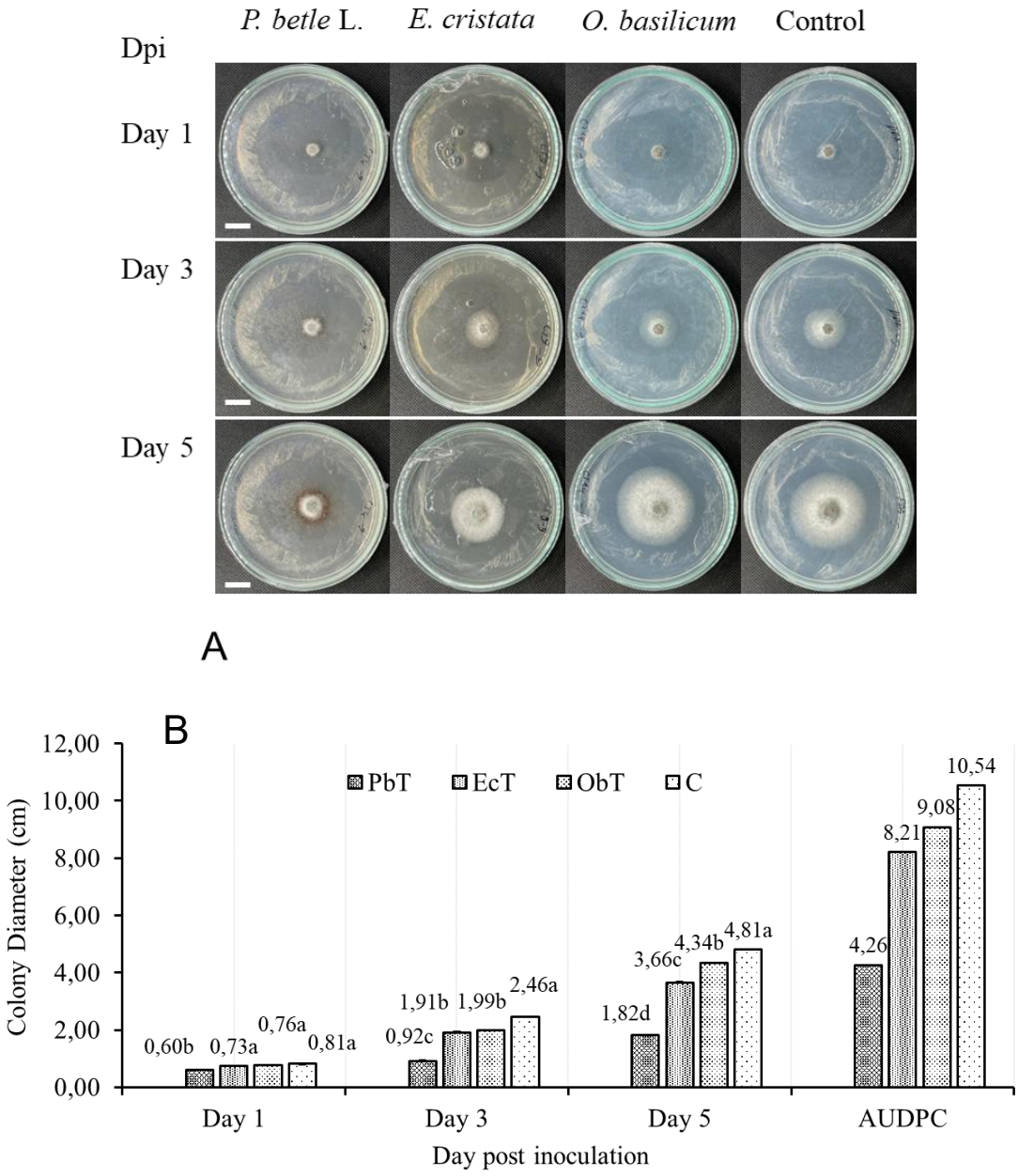

Figure 4. The growth of *C. gloeosporioides* species complex cultured on media supplemented with different plant extracts

A. Colony growth of C. gloeosporioides species complex, B. Colony diameter (cm)

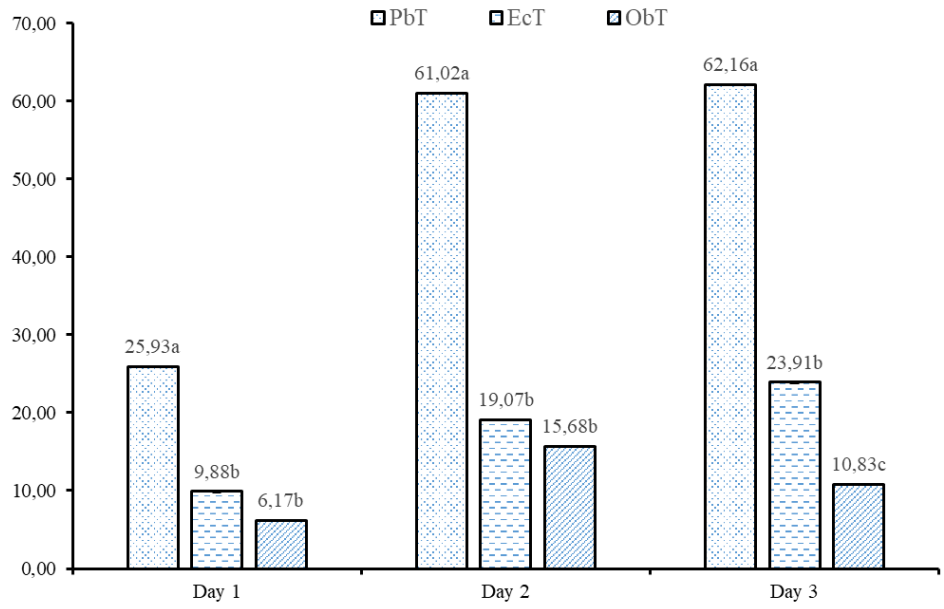

Figure 5. The inhibitory effect of plant extracts on C. *gloeosporioides* species complex (%)

All tested plant-derived products reduced the pathogen's mycelial growth compared with the untreated control throughout the incubation period. Among the treatments, PbT extract consistently showed the strongest inhibitory effect, followed by EcT and ObT, as reflected by smaller colony diameters at successive observation times (Figure 4). All three plant extracts showed inhibitory effects against the fungal pathogen from the first day after treatment (Figure 5). However, the inhibitory effects of the extracts differed significantly at the 0.05 level. On the first day after treatment, the PbT extract exhibited an inhibitory effect of 25.93%, whereas the inhibitory effects of EcT and ObT were statistically similar at 9.88% and 6.17%, respectively. After 3 days, the inhibitory effects of all three extracts increased, with PbT remaining significantly higher, while EcT and ObT remained statistically similar, reaching 61.02% (a), 19.07% (b), and 15.68% (b), respectively. By the fifth day, significant differences were observed among all three extracts: the inhibitory effects of PbT and EcT increased slightly to 62.16% (a) and 23.91% (b), respectively, whereas the efficacy of ObT decreased to 10.83% (c). Thus, at the extract concentrations used in this study, PbT and EcT exhibited the highest inhibitory efficacy at 5 days post-treatment, whereas ObT showed its maximum inhibitory effect at 3 days post-treatment. The growth trends and inhibitory effects shown in Figure 4 and Figure 5 indicate sustained suppression of fungal growth in extract-treated media compared with the control. These findings demonstrate that the evaluated plant-derived products exhibit antifungal activity against the *C. gloeosporioides* species complex, with effectiveness varying by plant source.

This result is consistent with previous studies reporting that PbT contains phenolic compounds such as eugenol, chavicol, and hydroxychavicol, which exhibit strong antifungal effects against phytopathogenic fungi, including *Colletotrichum* species (Christopher et al., 2023; Prakash et al., 2015). The inhibitory effects observed for EcT and ObT also align with earlier reports that essential oils and extracts from these plants exhibit antifungal properties due to the presence of terpenoids and phenylpropanoids, which disrupt fungal cell membranes and metabolic processes (Sokovic et al., 2010; da Cruz Cabral et al., 2013). Although the antifungal efficacy varied among plant sources, all treatments reduced fungal growth compared with the untreated control, which indicates a broad inhibitory potential against the chili anthracnose pathogen. These findings support integrating locally available plant-derived products into sustainable anthracnose management strategies and provide a foundation for further studies under greenhouse and field conditions to evaluate their practical applicability.

4. CONCLUSION

A survey of chili-growing fields in Muong Khuong revealed that anthracnose is a major disease causing significant economic losses to growers. The disease affected both hot chili and sweet pepper cultivars, with very high disease incidence during the humid rainy season, reaching 50.50% in the local hot chili cultivar and 70.48% in the sweet pepper cultivar.

This study successfully isolated and characterized the pathogen associated with chili anthracnose in Lao Cai province, Viet Nam. Based on disease symptoms, characteristic morphological traits, and ITS sequence analysis, the pathogen was identified as a member of the *Colletotrichum gloeosporioides* species complex.

C. gloeosporioides exhibited high pathogenicity to both leaves and fruits of chili plants within only 24 hours after inoculation. The progression of lesions on chili leaves and fruits continuously expanded from 24 to 168 hpi; from 48 hpi onward, lesions on leaves developed more rapidly and were consistently larger than those on fruits.

In vitro assays demonstrated that the tested plant-derived extracts from *Piper betle, Elsholtzia penduliflora* and *Oncimum basilium* exhibited antifungal activity against the pathogen, with varying levels of effectiveness. At a concentration of 0.1% of the stock solution, the extract from *Piper betle* (PbT) showed an inhibitory effect of 62.36% against *C. gloeosporioides*, while the extract from *Elsholtzia penduliflora* (EcT) reached 23.91% after 5 days of treatment, and the extract from *Ocimum basilicum* (ObT) achieved 15.68% after 3 days of treatment. These findings provide a scientific basis for developing sustainable anthracnose management strategies in chili production areas of northern Viet Nam.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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