Journal of Plant Pathology
https://doi.org/10.1007/s42161-024-01813-x

ORIGINAL ARTICLE

First report of *Colletotrichum siamense* and *Colletotrichum endophyticum* associated with anthracnose on avocado (*Persea americana* Mill.) in Vietnam

Le Thi Ha Thanh[1] · Nguyen Thi Thuy Tien[2] · Nguyen Thi Thuy Trang[1] · Tran Quoc Trieu[1] · Nguyen Quang Duc Tien[1] · Nguyen Hoang Loc[1]

Received: 7 February 2024 / Accepted: 17 November 2024
© The Author(s) under exclusive licence to Società Italiana di Patologia Vegetale (S.I.Pa.V.) 2024

Abstract

The avocado anthracnose disease is mostly caused by *Colletotrichum* species, which accounts for the understandable decline in avocado production. This study is conducted to reveal *Colletotrichum* species causing avocado anthracnose using combined morphological characterization and multi-locus phylogenetic analysis based on the data sets of ITS region, ACT, CAL, CHS-1, GAPDH and TUB2 genes. Four isolates were obtained from avocados have anthracnose symptoms and identified as two species, including *Colletotrichum siamense* and *Colletotrichum endophyticum*. These *Colletotrichum* species were classified into the same group as the *C. gloeosporioides* species complex, which was the predominant pathogen involved in the anthracnose of avocados worldwide. This is the first detection of these two *Colletotrichum* species as the cause of anthracnose on avocados in Vietnam. The artificial inoculation using the wounded method demonstrated that four representative isolates were able to infect not only avocado but also mango and banana with different levels of aggressiveness. Most isolates exhibited higher pathogenicity on their original host than other hosts. *C. endophyticum* AD24.3 was the most virulent pathogen among the tested isolates, with the highest disease incidence and lesion diameters on three investigated fruit types. Our findings provide a preliminary understanding of *Colletotrichum* species associated with avocado anthracnose, which will be useful in developing biocontrol methods and the management of avocado post-harvest disease in Vietnam.

Keywords *Colletotrichum* · Anthracnose · Avocado · *Persea americana*

Introduction

Avocado (*Persea americana* Miller), which originated in Mexico and Central America, is an economically important nutritious fruit widely grown in both tropical and sub-tropical regions of the world (Fischer et al. 2023). In Vietnam, avocados are cultivated mainly in the central highland areas, which have favorable conditions such as dry and cool weather. The production and demand for avocados have been increasing in recent years. In 2021, the global production volume of avocados was approximately 8,98 million tons, and the total production of these fruits in Vietnam reached around 213 thousand tons, ranking the second highest in Asia according to the Food and Agriculture Organization (FAO) (http://faostat.fao.org). Anthracnose is one of the most serious diseases that causes heavy post-harvest avocado fruit losses and limits their shelf life (Dissanayake et al. 2021). It caused damage to up to 60% of preharvest losses of avocado fruits in the field and post-harvest rots in Kenya (Kimaru et al. 2020). In South Africa, the disease rate of ripe avocado fruits was recorded at up to 80% (Perez-Jimenez 2008). The initial anthracnose symptom is small light-brown spots on the surface of the fruits; as the fungus develops, lesions rapidly enlarge, become darker, and completely necrotize the fruits' flush (Fischer et al. 2023).

✉ Nguyen Hoang Loc
nhloc@hueuni.edu.vn

[1] Institute of Bioactive Compounds and Department of Biotechnology, University of Sciences, Hue University, Hue, Vietnam

[2] Department of Engineering and Food Technology, University of Agriculture and Forestry, Hue University, Hue, Vietnam

Anthracnose may also appear suddenly, within 1–2 days, on fruits with no symptoms at harvest time (Nelson 2008).

Colletrotrichum species were considered to be associated with the post-harvest anthracnose disease of avocados in many countries, and diverse taxa were found. Previous studies suggested that *C. gloeosporioides* and *C. acutatum* are common pathogens related to anthracnose present in avocados (Nelson 2008). However, recent phylogenetic analyses based on multiple genes, such as ITS, actin (ACT), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), β-tubulin (TUB2), ApMat, calmodulin (CAL), and histon-3 (HIS3), have been used to define a variety species of *Colletotrichum* due to the overlapping morphological characters (Hunupolagama et al. 2015; Zakaria 2021; Sharma et al. 2022; Fuentes-Aragon et al. 2020). Recent studies have revealed over 20 species of *Colletotrichum* genera in association with avocado anthracnose (Zakaria 2021). The most species of *Colletotrichum* recorded in a study were nine species isolated from avocado in Isreal, including *C. aenigma* and *C. alienum*, *C. fructicola*, *C. gloeosporioides sensu strico*, *C. karstii*, *C. nupharicola*, *C. siamense*, *C. theobromicola*, and *C. perseae* (Sharma et al. 2017). Several novel species responsible for avocado anthracnose have been reported recently from many countries: *C. karstii* in Australia (Giblin et al. 2018); C. *kahawae* in South Korea (Kwon et al. 2019); C. *godetiae*, *C. fioriniae*, *C. cigarro*, *C. chrysophilum*, *C. jiangxiense*, and *C. nymphaeae* in Mexico (Fuentes-Aragon et al. 2020); C. *endophytica* in Sri Lanka (Dissanayake et al. 2021) and *C. anthrisci* in Chile (Bustamante et al. 2022).

It is important to accurately identify the *Colletotrichum* species causing anthracnose lesions in avocados for correct diagnosis and effective disease control. However, there is a lack of information about the *Colletotrichum* species causing avocado anthracnose in major avocado-producing countries in Asia. To our knowledge, this is the first report of *Colletotrichum* species causing anthracnose on avocado fruits in Vietnam. In this study, the isolated species were morphologically and molecularly identified, their pathogenicity and cross-infection were also evaluated. These results may facilitate studies on some biological methods to control post-harvest anthracnose diseases in avocados in Vietnam.

Materials and methods

Sample collection

Infected avocado fruits samples of different varieties were collected from Cu Mgar district, Krong Buk district, and Buon Ma Thuot city in Dak Lak province, which leads in the production of avocado in Vietnam. The samples were placed in sealed plastic bags and transferred to the laboratory for fungal isolation.

Fungal isolation

Avocado fruits were washed with tap water to remove dust, surface sterilized in 70% ethanol for 2–3 min, rinsed twice in distilled water, and the excess liquid was removed by using sterile tissue paper. Subsequently, the fruits were incubated in the disinfected plastic box with moistened paper towels at room temperature (25–30 °C) to allow the development of anthracnose symptoms. Small pieces (5 mm^2) of necrotic exocarp tissues were aseptically taken from the margins of lesions and placed at the center of a 9-cm-diameter Petri dish containing potato dextrose agar (PDA) medium (Himedia Laboratories Pvt. Ltd., Mumbai, India). The cultures were incubated at 25 °C for a 7- to 10-day period in the dark until typical mycelium and conidia were observed. Pure cultures were obtained by transferring mycelium discs (5 mm in diameter) from the actively growing colony margin onto fresh PDA plates and allowing them to grow at 25 °C for 7–10 days. The conidia suspensions were prepared by scraping 10-day-old mycelium in 1 mL of sterile distilled water with a sterilized wire loop and filtering through a sterile double-layer muslin cloth to remove mycelia. The conidial concentration was measured and adjusted to 10^5 conidia/mL by use of a hemocytometer (Soares et al. 2020; Wanjiku et al. 2020). The single-conidium-derived cultures of isolates were further characterized and their pathogenicity tested. Pure cultures of fungi were preserved on PDA slant in a test tube and stored at 4 °C for later use. Conidial suspensions were stored in cryogenic vials with 40% glycerol at -80 °C for further studies.

Morphological characterization

The representative fungal isolates obtained from single conidia or hyphal tips of pure cultures were morphologically identified by recording the following features: color, margins, texture, shape of colony, mycelium appearance on the upper and reverse sides, the presence of concentric rings, and conidial shape. Mycelia plugs (5 mm^2) from the growing edge of a 7-day-old culture were transferred onto PDA plates and incubated at 25 °C in the dark. The growth rate of isolates was calculated by the daily increment of the mycelia diameter (in milimeters) for 10 days. Mycelia and conidia were fixed on slides and covered with cover slips. The conidia of each isolate were randomly chosen and measured in terms of length and width at 400× magnification using an Labomed CxL optical microscope (Labo America, Inc, Fremont, USA) (Hunupolagama et al. 2015; Honger et al. 2016).

DNA extraction, PCR amplification and sequencing

The mycelium of each isolate after 10 days of culture was scraped from the surface of the plate, and fungal genomic DNA was extracted using the Qiagen DNA Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The DNA was quantified using a UV spectrophotometer and verified by electrophoresis on 1% agarose gel. To conduct multi-locus identification analysis, six gene sequences of the internal transcribed spacer (ITS) region (ITS1-5.8 S-ITS2 region), ACT, CAL, chitin synthase (CHS-1), GAPDH and TUB2 were amplified using the primer pairs shown in Table 1, including ITS1/ITS4 (White 1990), ACT-512 F/ACT-783R (Carbone and Kohn 1999), CL1C/CL2C (Weir et al. 2012), CHS-79 F/CHS-345R (Carbone and Kohn 1999), GDF/GDR (Templeton et al. 1992) and T1F/T1R (O'Donnell and Cigelnik 1997). The PCR amplification was done with a program as follows: genomic DNA denaturation at 98 ºC for 5 min; followed by 35 cycles: denaturation at 98 ºC for 30 s, annealing at appropriate temperature for each primer pair for 30 s, extention at 72 ºC for 30 s; and a final extension at 72 ºC for 5 min. The PCR products were sequenced using Sanger's method by First BASE Laboratories Sdn Bhd (Seri Kembangan, Malaysia).

Phylogenetic analysis

The comparison of obtained DNA sequences was performed using the Basic Local Alignmnet Search Tool (BLASTn) against other deposited sequences in GenBank (NCBI). The reference sequences of related species were retrieved from GenBank database for multiple sequence alignment (Table 2). The isolated genes along with the reference sequences were aligned using the robust MUSCLE program (https://www.drive5.com/muscle/) with default parameters. The alignment of multiple concatenated genes was then carefully adjusted using the MegaX (https://www.megasoftware.net/) software to eliminate potential errors (Kumar et al. 2018). To select the most appropriate evolutionary model for phylogenetic analysis, the jModelTest v2.1.10 program (https://github.com/ddarriba/jmodeltest2) was employed, incorporating sophisticated methods to identify the best-fit substitution model based on the aligned sequence data. Robust maximum likelihood (ML) phylogenetic trees were subsequently constructed using the efficient IQ-Tree v2.1.3 software (http://www.iqtree.org/) (Minh et al. 2020). Comp rehensive branch support values were calculated using the approximate likelihood ratio test (SH-aLRT) with 10,000 replicates to evaluate branch reliability, aBayesian posterior probabilities to estimate the statistical confidence and an ultrafast bootstrap analysis with 10,000 replicates to assess the sampling robustness. The resulting phylogenetic trees were visualized and annotated using the user-friendly FigTree v1.4.2 program (http://tree.bio.ed.ac.uk/software/figtree/). Final adjustments were made to enhance visual clarity and publication quality using Adobe Illustrator CC 2021 (Adobe Systems, USA) for precise graphical editing.

Pathogenicity and cross-infection assay

Four *Colletotrichum* isolates obtained in this study were tested for their pathogenicity on avocado fruits of cultivar 'Booth' and cross-infection on mango and banana following Koch's postulates. Healthy, unripe fruits of similar size and color, devoid of blemishes or disease symptoms, were collected from grocery stores, then washed with running tap water to remove dust and fungicide residues that remained on the surface. The fruit samples were surface disinfected with 2% sodium hypochlorite for 5 min and rinsed three times with sterile distilled water; the excess water was bloated. The artificial inoculation was performed by wound methods using conidia (Wanjiku et al. 2020). The conidial suspension was prepared with the method described above from the 10-day-old culture of each isolate. Afterwards, the fruits were pinpricked at equally distant points along the fruit surface using a sterile needle of 1 mm diameter at

Table 1 Primer pairs used in this study to amplify genes for multi-locus analysis

Genes	Primer	Sequence (5'-3')	References
The internal transcribed spacer region (ITS)	ITS1	TCCGTAGGTGAACCTGCCG	White et al. (1990)
	ITS4	TCCTCCGCTTATTGATATGC	
Actin gene (ACT)	ACT-512 F	ATGTGCAAGGCCGGTTTCGC	Carbone and Kohn (1999)
	ACT-783R	TACGAGTCCTTCTGGCCCAT	
Calmodulin gene (CAL)	CL1C	GAATTCAAGGAGGCCTTCTC	Weir et al. (2012)
	CL2C	CTTCTGCATCATGAGCTGGAC	
Chitin synthase gene (CHS-1)	CHS-79 F	TGGGGCAAGGATGCTTGGAAGAAG	Carbone and Kohn (1999)
	CHS-345R	TGGAAGAACCATCTGTGAGAGTTG	
Glyceraldehyde 3-phosphate dehydrogenase gene (GAPDH)	GDF	GCCGTCAACGACCCCTTCATTGA	Templeton et al. (1992)
	GDR	GGGTGGAGTCGTACTTGAGCATGT	
β-tubulin gene (TUB2)	T1F	AACATGCGTGAGATTGTAAGT	O'Donnell and Cigelnik (1997)
	T1R	TAGTGACCCTTGGCCCAGTTG	

Table 2 *Colletotrichum* isolates used for phylogenetic analysis with their GenBank accession numbers

Species	Strain	Host	Country	GenBank accession numbers					
				ITS	ACT	CHS-1	CAL	GAPDH	TUB2
Colletotrichum aenigma	ICMP 18608*	*Persea americana*	Israel	JX010244	JX009443	JX009774	JX009683	JX010044	JX010389
	ICMP 18686	*Pyrus pyrifolia*	Japan	JX010243	JX009519	JX009789	JX009684	JX009913	JX010390
Colletotrichum alatae	ICMP 17919*	*Dioscorea alata*	India	JX010190	JX009471	JX009837	JX009738	JX009990	JX010383
Colletotrichum alienum	ICMP 18691	*Persea americana*	Australia	JX010217	JX009580	JX009754	JX009664	JX010018	JX010385
	ICMP 12071*	*Malus domestica*	NZ	JX010251	JX009572	JX009882	JX009654	JX010028	JX010411
Colletotrichum aotearoa	ICMP 18537*	*Coprosma* sp.	NZ	JX010205	JX009564	JX009853	JX009611	JX010005	JX010420
Colletotrichum asianum	ICMP 18580*	*Coffea arabica*	Thailand	JX010196	JX009584	JX009867	JX009727	JX010053	JX010406
Colletotrichum camelliae	CGMCC 3.14925, LC1364*	*Camellia sinensis*	China	KJ955081	KJ954363	MZ799255	KJ954634	KJ954782	KJ955230
Colletotrichum cigarro	ICMP 18534	*Kunzea ericoides*	NZ	JX010227	JX009473	JX009765	JX009634	JX009904	JX010427
	ICMP 18539*	*Olea europaea*	Australia	JX010230	JX009523	JX009800	JX009635	JX009966	JX010434
Colletotrichum cobbittiense	BRIP 66219*	*Cordyline stricta* × *Cordyline australis*	Australia	MH087016	MH094134	MH094135		MH094133	MH094137
Colletotrichum cordylinicola	ICMP 18579*	*Cordyline fruticosa*	Thailand	JX010226	JX009586	JX009864	JX009651	JX009975	JX010440
Colletotrichum chrysophilum	URM 7368, CMM 4268*	*Musa* sp.	Brazil	KX094252	KX093982	KX094083	KX094063	KX094183	KX094285
Colletotrichum clidemiae	ICMP 18658*	*Clidemia hirta*	USA	JX010265	JX009537	JX009877	JX009645	JX009989	JX010438
Colletotrichum endophyticum	CAUG28	Chilli pepper	China	KP145441	KP145329	KP145385	KP145357	KP145413	KP145469
	EIPP 33	*Unknowm*	China	MK330020	MK344284	MK344261	-	MK344238	MK344215
	GBZ8-2	*Thaumatophyllum bipinnatifidum*	China	MZ962375	OK040202	OK040212	OK040207	OK040217	OK040222
	ID45	*Axonopus compressus*	Malaysia	-	ON755314	-	-	ON755300	ON755288
	MFLUCC 13–0418*	*Pennisetum purpureum*	Thailand	KC633854	KF306258	MZ799261	-	KC832854	MZ673954
	OBP5	*Piper nigrum*	India	KJ947310	KJ947187	KJ947233	-	KJ947279	KJ947210
	YN1A3	*Camellia sinensis*	China	KU251559	KU251640	KU251907	KU251802	KU252013	KU252167
	YTJH1	*Bauhinia purpurea*	China	MH291220	MH292794	MH291241	MH292807	MH291264	MH458025
	AD24.3	***Persea americana***	**Vietnam**	**PP177474**	**PP888063**	**PP888064**	**PP888065**	**PP888066**	**PP888067**
	AD24.5	***Persea americana***	**Vietnam**	**PP177475**	**PP888068**	**PP888069**	**PP888070**	**PP888071**	**PP888072**
Colletotrichum fructicola	ICMP 12568	*Persea americana*	Australia	JX010166	JX009529	JX009762	JX009680	JX009946	-
	ICMP 18581, CBS 130416*	*Coffea arabica*	Thailand	JX010165	FJ907426	JX009866	FJ917508	JX010033	JX010405
	ICMP 18727	*Fragaria×ananassa*	USA	JX010179	JX009565	JX009812	JX009682	JX010035	JX010394

Table 2 (continued)

Species	Strain	Host	Country	GenBank accession numbers					
				ITS	ACT	CHS-1	CAL	GAPDH	TUB2
Colletotrichum gloeosporioides	MI 356878, ICMP 17821*	*Citrus sinensis*	Italy	JX010152	JX009531	JX009818	JX009731	JX010056	JX010445
	ICMP 18697	*Vitis vinifera*	USA	JX010154	JX009557	JX009780	JX009736	JX009987	-
Colletotrichum grossum	CAUG7*	Chilli peper	China	KP890165	KP890141	KP890153	KP890147	KP890159	KP890171
Colletotrichum helleniense	CPC 26844, CBS 142418*	*Poncirus trifoliata*	Greece	KY856446	KY856019	KY856186	KY856099	KY856270	KY856528
Colletotrichum henanense	CGMCC 3.17354*	*Camellia sinensis*	China	KJ955109	KM023257	MZ799256	KJ954662	KJ954810	KJ955257
Colletotrichum horii	ICMP 10492, NBRC 7478*	*Diospyros kaki*	Japan	GQ329690	JX009438	JX009752	JX009604	JX009964	JX010450
Colletotrichum jiangxiense	CGMCC 3.17363*	*Camellia sinensis*	China	KJ955201	KJ954471		KJ954752	KJ954902	KJ955348
Colletotrichum kahawae	ICMP 17816*	*Coffea arabica*	Kenya	JX010231	JX009452	JX009813	JX009642	JX010012	JX010444
Colletotrichum makassarense	CPC 28612*	*Capsicum annum*	Indonesia	MH728812	MH781480	MH805850		MH728820	MH846563
Colletotrichum musae	ICMP 17817	*Musa* sp.	Kenya	JX010142	JX009432	JX009815	JX009689	JX010015	JX010395
	CBS 116870*	*Musa* sp.	USA	JX010146	JX009433	JX009896	JX009742	JX010050	HQ596280
Colletotrichum nupharicola	CBS 470.96*	*Nuphar polysepala*	USA	JX010187	JX009437	JX009835	JX009663	JX009972	JX010398
Colletotrichum perseae	CBS 141365*	*Persea americana*	Israel	KX620308	KX620145	MZ799260	KX620206	KX620242	KX620341
Colletotrichum psidii	CBS 145.29*	*Psidium* sp.	Italy	JX010219	JX009515	JX009901	JX009743	JX009967	JX010443
Colletotrichum queenslandicum	ICMP 1778*	*Carica papaya*	Australia	JX010276	JX009447	JX009899	JX009691	JX009934	JX010414
Colletotrichum rhexiae	CBS 133134*	*Rhexia virginica*	Sussex	JX145128	MZ664127	MZ799258		MZ664046	JX145179
Colletotrichum salsolae	ICMP 19051*	*Salsola tragus*	Hungary	JX010242	JX009562	JX009865	JX009696	JX009916	JX010403
Colletotrichum siamense	ICMP 12567	*Persea americana*	Australia	JX010250	JX009541	JX009761	JX009697	JX009940	JX010387
	CBS 125379, ICMP 18643	*Hymenocallis americana*	China	JX010258	GQ856776	GQ856729	GQ849451	JX010060	-
	ICMP 18121	*Dioscorea rotundata*	Nigeria	JX010245	JX009460	JX009845	JX009715	JX009942	JX010402
	ICMP 18739	*Carica papaya*	South Africa	JX010161	JX009484	JX009794	JX009716	JX009921	-
	ICMP 18578, CBS 130417*	*Coffea arabica*	Thailand	JX010171	FJ907423	JX009865	FJ917505	JX009924	JX010404
	ICMP 17795	*Malus domestica*	USA	JX010162	JX009506	JX009805	JX009703	JX010051	JX010393
	AD1.2	***Persea americana***	**Vietnam**	**PP177472**	**PP888053**	**PP888054**	**PP888055**	**PP888056**	**PP888057**
	AD24.7	***Persea americana***	**Vietnam**	**PP177476**	**PP888058**	**PP888059**	**PP888060**	**PP888061**	**PP888062**

Table 2 (continued)

Species	Strain	Host	Country	GenBank accession numbers					
				ITS	ACT	CHS-1	CAL	GAPDH	TUB2
Colletotrichum tainanense	CBS 143666*	*Capsicum annuum*	Taiwan	MH728818	MH781475	MH805845		MH728823	MH846558
Colletotrichum temperatum	CBS 133122*	*Vaccinium macrocarpon*	USA	JX145159	MZ664125	MZ799254		MZ664045	JX145211
Colletotrichum theobromicola	CBS 124945*	*Theobroma cacao*	Panama	JX010294	JX009444	JX009869	JX009591	JX010006	JX010447
Colletotrichum ti	ICMP 4832*	*Cordyline* sp.	New Zealand	JX010269	JX009520	JX009898	JX009649	JX009952	JX010442
Colletotrichum tropicale	CBS 124949, ICMP 18653*	*Theobroma cacao*	Panama	JX010264	JX009489	JX009870		JX010007	JX010407
Colletotrichum viniferum	GZAAS 5.08601*	*Vitis vinifera*	China	JN412804	JN412795			JN412798	JN412813
Colletotrichum xanthorrhoeae	BRIP 45094*	*Xanthorrhoea preissii*	Australia	JX010261	JX009478	JX009823	JX009653	JX009927	JX010448
Colletotrichum yulongense	CFCC 50818*	*Vaccinium dunalianum* var. *urophyllum*	China	MH751507	MH777394	MH793605	MH793604	MK108986	MK108987
Colletotrichum boninense (outgroup)	ICMP 17904, MAFF 305972*	*Crinum asiaticum* var. *sinicum*	Japan	JX010292	JX009583	JX009827	HM582004	JX009905	HM585421
Colletotrichum hippeastri (outgroup)	CBS 241.78, ICMP 17920 IMI 304052	*Hippeastrum* sp.	Netherlands	JX010293	JX009485	JX009838	JX009740	JX009932	JQ005666

Abbreviations of culture collections: NZ: New Zealand; BRIP: Plant Pathology Herbarium, Department of Employment, Economic, Development and Innovation, Queensland, Australia; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CFCC: China Forestry Culture Collection Center, Beijing, China; CGMCC: China General Microbiological Culture Collection Center, Beijing, China; CMM: Culture Collection of Phytopathogenic Fungi Prof. Maria Menezes, Federal Rural University of Pernambuco, Brazil; CPC: Culture collection of Pedro Crous, housed at CBS; GZAAS: Guizhou Academy of Agriculture Sciences Herbarium, China; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; MAFF: MAFF Genebank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; NBRC: NITE Biological Resource Center, Chiba, Japan. Strains isolated in this study are in boldface, -: not sequenced, *: ex-type strains

1 mm of depth. Inoculations were carried out by applying 10 µL of conidial suspension (10^5 conidia/mL) of each isolate to the wounds. Control samples were inoculated with 10 µL of sterile distilled water (Fuentes-Aragon et al. 2020; Kwon et al. 2019). Following inoculation, the infected fruits were incubated at 25 °C in a plastic box with moistened paper towels, covered with cling film to maintain the humidity, and ispected regularly for the appearance of anthracnose symptoms (3–6 days). When symptoms were observed, the lesion diameter of each wound was measured. The disease incidence was calculated based on the percentage of wounds showing anthracnose symptoms. At the end of the pathogenicity assay, the fruits were cut to observe the typical lesions in the mesocarp (Silva-Rojas et al. 2011). The pathogen fungi from the diseased fruits were reisolated and compared with those of the original isolates.

Statistical analysis

All experiments were performed in triplicate, and the results were presented as the mean ± standard error. Data were analyzed by one-way ANOVA (Duncan's test) using SPSS software (ver. 27); p-values < 0.05 were considered significant.

Results

Isolation of *Colletotrichum* species

After 2–4 days of incubation, anthracnose symptoms initially appeared as small light-brown spots on the surface with one to several lesions on fruits. As the fruits ripened and the lesions enlarged, they became concave in the center and

turned dark brown or dark sunken, causing the fruits' flesh to rot and forming large necrotic areas (Fig. 1A). The fungal isolates were obtained from infected avocado fruits showing anthracnose symptoms collected from Dak Lak. The colony morphologies and conidial characteristics of the isolates cultured on PDA medium were preliminary identified based on morphological descriptions of *Colletotrichum* species shown by Weir et al. (2012), Liu et al. (2022) and compared to those of previous reported *Colletotrichum* species (Than et al. 2008; Crous et al. 2018; Silva et al. 2019a, b). Four isolates with distinct morphological types that fit the features of *Colletotrichum* species were described in detail, further analyzed for phylogenetic analysis and pathogenicity.

Morphological characterization

Different morphological types of *Colletotrichum* isolates causing avocado anthracnose were observed after 10 days

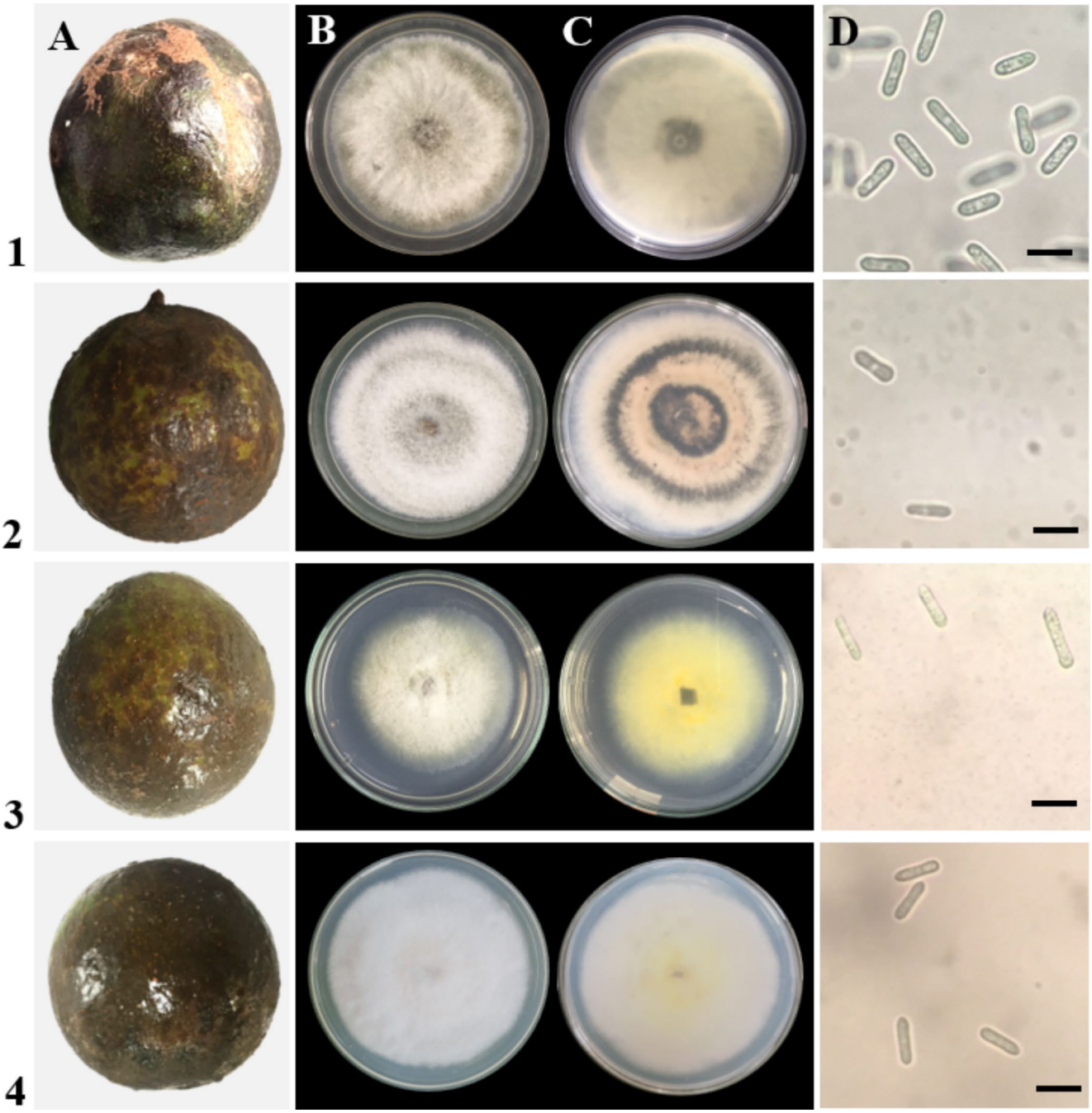

Fig. 1 Morphology of *Colletotrichum* isolates found on infected avocado. **A**: natural anthracnose symptoms on avocado fruits; **B**: upper surface; **C**: lower surface; **D**: conidia. Scale bar = 15 µm. 1: AD1.2, 2: AD24.3, 3: AD24.5, and 4: AD24.7

Table 3 Morphological characteristics of *Colletotrichum* isolates associated with avocado anthracnose

Strains	Colony morphology	Colony diameter (mm)	Growth rate (mm/day)	Conidia shape	Length (L) of conidium (µm)	Width (W) of conidium (µm)	Rate of L/W
Colletotrichum endophyticum AD24.3	White to pale grey zonated colonies, reverse greyish orange with grey concentric rings	$78.94^{a} \pm 0.73$	$7.89^{a} \pm 0.07$	Cylindral, slightly flexous in the middle, obtuse ends	$14.28^{c} \pm 0.59$	$4.96^{a} \pm 0.6$	2.88
Colletotrichum endophyticum AD24.5	White to yellowish, reverse yellow	$54.94^{d} \pm 1.4$	$5.49^{d} \pm 0.14$	Straight, cylindral, rounded or obtuse at the apex	$15.30^{bc} \pm 1.90$	$4.47^{b} \pm 0.46$	3.36
Colletotrichum siamense AD1.2	Whitish grey, reverse pale grey	$77.76^{b} \pm 1.66$	$7.78^{b} \pm 0.17$	Straight, cylindral, rounded at both ends	$16.74^{a} \pm 1.57$	$4.96^{a} \pm 0.38$	3.38
Colletotrichum siamense AD24.7	White, reverse pale yellowish	$76.06^{c} \pm 0.73$	$7.61^{c} \pm 0.07$	Cylindral, rounded or tapered ends	$15.1^{c} \pm 1.46$	$4.09^{b} \pm 0.73$	3.69

Different letters in the same column expressed statistically significant differences with $p < 0.05$ (Duncan's test)

of culture on PDA (Fig. 1B and C). Table 3 shows the morphological characteristics of these isolates. Isolate AD1.2 formed spongy, whitish gray aerial mycelium with the small gray zone in the center and turning gray towards the edges. The reverse side showed a similar pattern with a lighter color. Isolate AD24.3 produced cottony, white to pale gray zonated colonies with an orange conidial mass in the center. The reverse was greyish orange with various concentric rings in light grey, dark grey, and light orange, and different in ring thickness. The surface of isolate 24.5 was cottony and produced colonies with pale yellow moderate aerial mycelium, surrounded by a thin grey hyphae filament margin, yellow on the reverse side, and no visible conidial mass in the centre was observed. The colonies of isolate 24.7 had spongy, velvety white floccular mycelia and cream to yellow in reverse.

The *Colletotrichum* isolates obtained in this study showed rapid growth on PDA medium; the colonies almost covered the whole surface of the dish after 10 days of incubation at 25 °C and indicated statistically significant differences ($p < 0.05$). The isolate AD24.3 reached the highest conlony size of 78.94 mm, with a growth rate of 7.89 mm/day. The average growth rate of the three isolates (AD1.2 and AD24.7) was 7.78 and 7.61 mm/day, respectively. Meanwhile, isolate AD24.5 showed the slowest growth, with only 54.94 mm in colony diameter after 10 days of incubation, corresponding to 5.49 mm of mycelia growing per day.

There was no significant difference in conidial shape and dimension of these isolates. Most of them developed straight, smooth-walled, cylindrical conidia with both round ends, some isolates' conidia had obtuse or slightly tapered apices. The conidia of isolate AD1.2 were more abundant than those of others. The size of conidia varied from 14.65 to 16.74 µm in length and 4.09 to 5.01 µm in width. The ratios between length and width of conidia were in the range of 2.88–3.69. The morphological characteristics of four *Colletotrichum* isolates obtained in this study were similar with the description of *C. gloeosporiodes* species complex by previous studies (Weir et al. 2012; Siddiqui et al. 2014; Manova et al. 2022).

Phylogenetic analysis

The molecular identification of obtained *Colletotrichum* strains was conducted by mutilocus phylogenetic analysis based on combined data sets using six genes (ITS, ACT, CAL, CHS-1, GAPDH and TUB2) comprised our 4 isolates and 57 reference sequences of related *Colletotrichum* species from different host deposited on GenBank, with *Colletotrichum boninense* and *Colletotrichum hippeastri* serving as the outgroup taxa. The phylogenetic analysis was constructed using maximum likelihood method by IQ-Tree, the best-scoring ML tree was given in Fig. 2 with approximate likelihood ratio (SH-aLRT), Bayesian posterior probabilities (BYPP) and ultrafast bootstrap (BS) values plotted on the branches. The sequence alignment revealed that from the 61 sequences with 4595 characters (nucleotide sites), there were 3551 constant characters, 612 parsimony informative characters, while 1346 sites were distinct. The best-fit substitution model was TN + F + I + G4 with rate parameters of AC = 1.0000, AG = 3.4943, AT = 1.0000, CG = 1.0000, CT = 5.3658 and GT = 1.0000. The base frequencies were 0.2278, 0.2975, 0.2489 and 0.2258 for base A, C, G and T, respectively. Corrected Alkaline information criterion (AICc) score was 33305.2051 and Bayesian information criterion (BIC) score was 34108.5654.

According to the phylogenetic analysis, 4 isolates obtained in this study were assigned to two distinct clades of the gloeosporioides species complex. For instance, isolates AD1.2 and AD24.7 clustered with reference *C. siamense* strains (ICMP 12567, ICMP 18121, CBS 125379, ICMP 17795, ICMP 18578, ICMP 18739). Of these, two isolates grouped with *C. siamense* ICMP 18578 (ex-type strain) showing high boostrap support (SH-aLRT/BYPP/

Fig. 2 The phylogenetic tree of the *Colletotrichum* species based on ITS, ACT, CAL, CHS-1, GAPDH and TUB2 sequences constructed by the maximum likelihood algorithm. The approximate likelihood ratio support values (SH-aLRT > 70%), Bayesian posterior probabilities (BYPP > 0.8) and ultrafast bootstrap values (BS > 80%) are displayed at the nodes. *Colletotrichum* isolates from avocados in Vietnam are in boldface, strains with * mark are ex-type strains

BS = 91.3/1/97). Isolate ICMP 18578 was found to be a causal agent of *Coffea arabica* in Thailand. While AD24.3 and 24.5 were well clusted with *C. endophyticum* strains, supported by 90–100% ML bootstrap values. In this clade, two isolates were constituted a close relationship to *C. endophyticum* OBP5 isolated from *Piper nigrum* in India with 0.95 posterior probability and a boostrap value of 96%.

Taken together from the morphological comparisons and phylogenetic analysis, our *Colletotrichum* collections causing avocado anthracnose in Vietnam composed two different species, which were *C. siamense* and *C. endophyticum*. The obtained isolates sequences of ITS, ACT, CHS-1, CAL, GAPDH and TUB2 were deposited in GenBank (NCBI) and were provided temporary accession numbers as given in Table 2.

Pathogenicity and cross-infection assay

The pathogenicity assays were carried out using 4 isolates: AD1.2, AD24.7, AD24.3 and AD24.5, which represented 2 species: *C. siamense* and *C. endophyticum*, respectively. The tests were performed using wound methods on host avocado fruits and other tropical fruits such as mango and banana. The disease incidence (DI) and lesion diameter were monitored on infected fruits after 3, 5, and 7 days of inoculation; the results are presented in Table 4; Fig. 3. In general, all the isolates produced disease symptoms on detached avocados and other fruits; however, their aggressiveness was divergent.

In the host avocado fruits, inoculated samples developed anthracnose symptoms after 5 or 7 days; the DI ranged from 66.7 to 100%, while the controls showed symptomlessness. Of the four tested for pathogenicity, isolate *C. endophyticum* AD24.3 attacked avocado fruits more quickly than other strains, with the highest DI (100%) after 5 days. While, isolates *C. endophyticum* 24.5 showed a slower infection as symptoms appeared on the 7th day. The significant differences in lesion diameter between species ($p < 0.05$) were obtained 7 days post-inoculation (Table 4). Particularly, isolate *C. endophyticum* 24.3 was the most virulent strain, causing the largest lesions with 23.17 mm in diameter, followed by those inoculated with isolates *C. siamense* AD24.7 and AD1.2 (mean lesions were 18.25 and 16 mm, respectively). *C. endophyticum* 24.5 might be considered the least virulent among all the isolates tested since it caused a lesion only 10.50 mm in diameter after 7 days. Lesion formation on ripe, mature avocado fruits also differed across the different fungal species (Fig. 3). The lesions produced by isolate *C. endophyticum* 24.3 were dark brown, round, and had even edges. The distinct feature of the lesion on avocado inoculated by this isolate was numerous orange spore masses with a waxy aspect developed from the center of the lesions.

Cross sections of infected avocado fruits exhibited large, dark brown and soft rotten flesh. Necrotic lesions caused by isolate *C. siamense* AD1.2 were brown and round, with white fungal hyphea developing in the wound. Meanwhile, the lesions on fruits infected with the isolate *C. siamense* AD24.7 appeared as small brown spots after 6 days, then rapidly extended and formed dark brown spots on the epicarp of the fruits at 7 days. However, underneath the lesions, the necrotic pulps were smaller, with a round shape toward the center of the fruit, and separated easily from the healthy tissue.

The cross-infection of fungal strains isolated from the true host to the other two fruit types, mango and banana, indicated different degrees of pathogenicity (Table 4). Isolate *C. endophyticum* AD24.3 exhibited its strong cross-pathogenicity on both mango and banana, with a DI of 100% after 3 and 5 days, respectively. Lesion diameters measured on these two fruit types showed no statistical differences,

Table 4 Pathogenicity of *Colletotrichum* species on different fruit types

Isolates	Inoculated fruits	Percentage disease incidence (%)			Lesion diameter (mm)		
		3 days	5 days	7 days	3 days	5 days	7 days
Colletotrichum endophyticum AD24.3	Avocado	-	100	100	-	$19.50^{a} \pm 3.99$	$23.17^{a} \pm 3.13$
	Mango	100	100	100	$5.29^{a} \pm 2.20$	$10.21^{b} \pm 0.96$	$13.54^{c} \pm 2.20$
	Banana	-	100	100	-	$7.61^{c} \pm 1.19$	$13.28^{c} \pm 1.64$
Colletotrichum endophyticum AD24.5	Avocado	-	-	100	-	-	$10.50^{d} \pm 1.87$
	Mango	-	-	83	-	-	$14.80^{c} \pm 4.25$
	Banana	-	-	100	-	-	$5.78^{f} \pm 1.37$
Colletotrichum siamense AD1.2	Avocado	-	66.7	94.5	-	$8.00^{c} \pm 2.19$	$16.00^{bc} \pm 3.35$
	Mango	-	100	100	-	$4.87^{d} \pm 1.07$	$8.03^{de} \pm 2.7$
	Banana	-	-	100	-	-	$2.2^{g} \pm 0.26$
Colletotrichum siamense AD24.7	Avocado	-	-	100	-	-	$18.25^{b} \pm 2.08$
	Mango	-	-	67	-	-	$7.25^{ef} \pm 2.51$
	Banana	-	44.4	100	-	$8.13^{c} \pm 0.25$	$13.89^{c} \pm 1.31$

Different letters in the same column expressed statistically significant differences with $p < 0.05$ (Duncan's test)

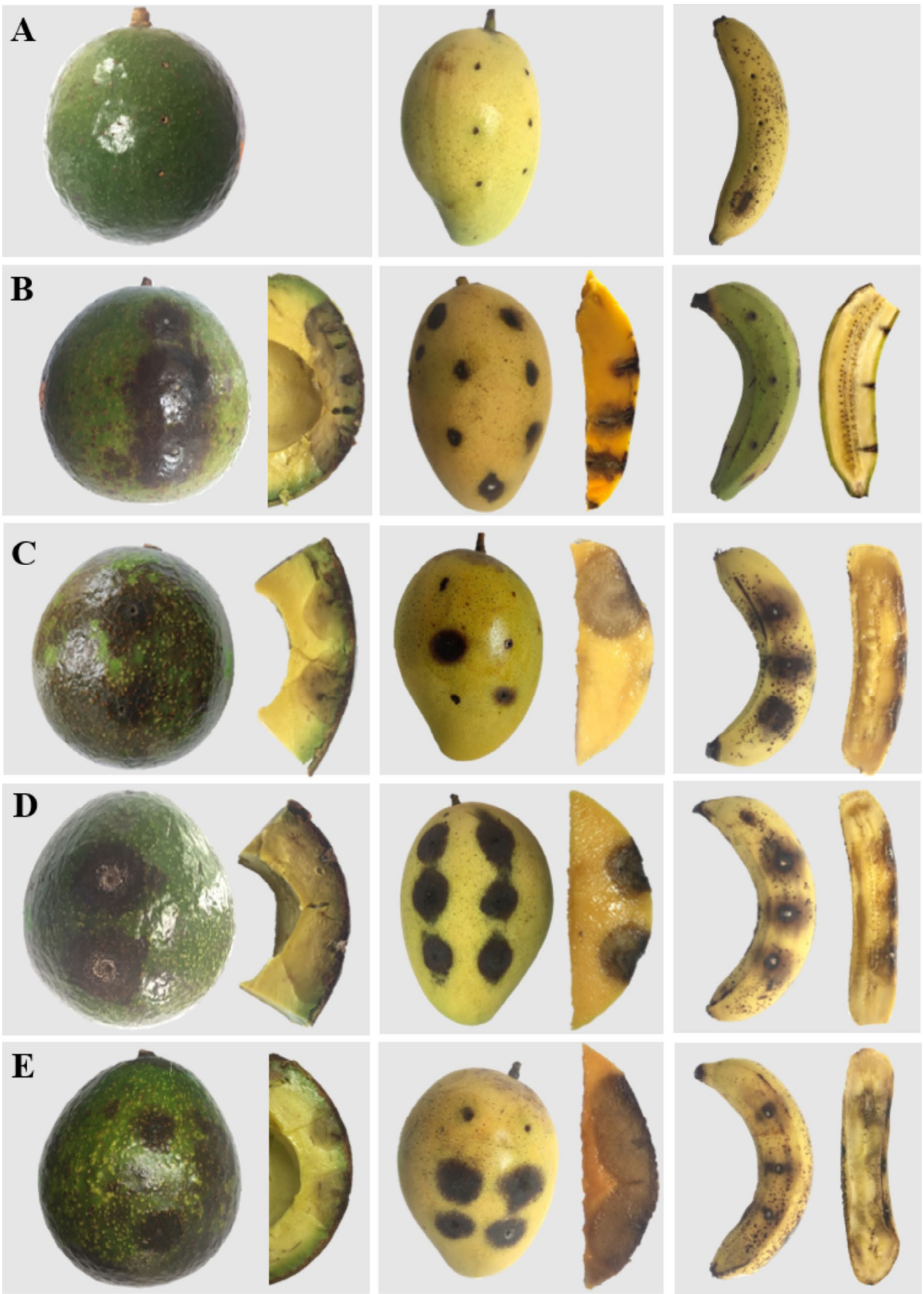

Fig. 3 Pathogenicity assay of *Colletotrichum* isolates on wounded fruits after 7 days of inoculation. **A**: controls, **B**: *Colletotrichum siamense* AD1.2, **C**: *Colletotrichum siamense* AD24.7, **D**: *Colletotrichum endophyticum* AD24.3, **E**: *Colletotrichum endophyticum* AD24.5

with approximately 13 mm at 7 days after inoculation. While isolate *C. siamense* AD1.2 was weakly pathogenic when tested on mango and banana. In contrast, the cross-infections of two isolates, *C. endophyticum* AD24.5 and *C. siamense* AD24.7, varied for different fruit types. Isolate *C. endophyticum* AD24.5 was more aggressive on mango than banana based on the observation of lesion diameter expansion. On the other hand, strain *C. siamense* AD24.7 showed weak invading capacity on mango (7.25 mm in diameter) compared to banana (13.89 mm). The anthracnose symptoms on two tested fruits formed initially round brown lesions, then turned black to dark; the flesh was sunken and soft rot. Notably, the isolate *C. endophyticum* AD24.5 indicated larger necrotic lesions in the mesocarp of mango than those caused by *C. endophyticum* AD24.3, though the lesion diameters were not statistically different (Fig. 3). The morphological features of colony and conidia of these four re-isolated fungi were similar to the original isolates.

Dicussions

Anthracnose is the most severe postharvest disease of avocados, which causes significant losses during storage and transport of fruits (Nelson 2008; Fischer et al. 2023). *Colletotrichum* spp. are well-known as main agent causing avocado anthracnose with their high pathogenicity. Therefore, the accurate species delimitation is necessary to address the specific control methods for avocado diseases management. Up to now, there has been no study providing information about the fungal agents responsible for avocado anthracnose in Vietnam. In the present study, four *Colletotrichum* isolates from avocado with anthracnose symptoms collected in Vietnam were characterized for the first time using morphological characterization, multi-gene analyses, and an artificial infection assay.

The preliminary identification by colony and conidia morphology revealed that all four isolates obtained from anthracnose lesions produced similar morphologies to species of the *C. gloeosporioides* complex. The colony morphologies of isolates were cottony, whitish gray, or creamish color mycellium with a darker center on the upper side and creamish grey with circular on the reverse side; some isolates produced salmon conidial masses. The similar features of species that belonged to the *Gloeosporioides* complex which affected avocados were also described in several studies (Kimaru et al. 2018; Ayvar-Serna et al. 2020; Dissanayake et al. 2021; Sharma et al. 2015b; Soares et al. 2020; Manova et al. 2022). Additionally, the *C. gloeosporioides* complex includes species that produce hyaline, straight, cylindrical conidia with rounded or obtuse ends; the conidial size ranges from 11 to 16×4–6 µm (Sharma et al. 2015b; Siddiqui et al. 2014). The conidial features of four isolates in this study matched these characteristics and showed no significant difference among isolates. Our findings indicated that these isolates might belong to the same *C. gloeosporioies* species complex. Within 16 species complexes of *Colletotrichum* species, *C. gloeosporioides* is one of the complexes have high species diversity with a wide host range (Liu et al. 2022). In addition, *C. gloeosporioies* species complex shows a broad global distribution and is commonly found in warm environment (Talhinhas et al. 2021; Salotti et al. 2022; Hsieh et al. 2023). To date, 57 species have been described in the *Gloeosporioides* species complex, which is classified into three major clades: theobromicola, kahawae, and gloeosporioides (Talhinhas et al. 2021).

However, previous reports revealed that the morphological traits of different *Colletotrichum* species were dependent on the environment; thus, the identification of members of the genus *Colletotrichum* based on morphological characteristics might be misleading (Phoulivong et al. 2010; Weir et al. 2012). Several studies have elucidated issues related to species boundaries and *Colletotrichum* species identification based on DNA barcodes (Weir et al. 2012; Zhafarina et al. 2021; Oliveira et al. 2022). Hence, the multi-locus phylogenetic analysis was widely conducted not only for identify the taxa relationship but also for species delineation. Molecular analyses relied on six genes, including ITS, ACT, CAL, CHS-1, GAPDH and TUB2, suggested that four isolates in this study were classified as *C. siamense* and *C. endophyticum*, which belong to the *C. gloeosporioides* complex. Previous studies reported that the predominant species related to avocado anthracnose worldwide belonged to the gloeosporioides species complex, followed by the acutatum and boninense complexes (Giblin et al. 2018; Fuentes-Aragon et al. 2020; Hofer et al. 2021). Some common species in this complex that affect avocados are *C. aenigma*, *C. alienum*, *C. fructicola*, *C. kahawae*, *C. cigarro*, and *C. siamens*e (Weir et al. 2012; Sharma et al. 2017; Zakaria 2021). *C. siamense* was recorded on many hosts without host specificity and commonly found in tropical and subtropical countries. It was prominent in causing anthracnose on chili in Indonesia, Sri Lanka and Thailand (de Silva et al. 2019a; Silva et al. 2019b), on *Theobroma cacao* fruits in Venezuela (Mohali-Castillo et al. 2022), on mango in Mexico (Tovar-Pedraza et al. 2020) and Thailand (Rattanakreetakul et al. 2023). *C. siamense* was also identified as a main agent of avocado anthracnose worldwide, as in Ghana (Honger et al. 2016), Mexico (Fuentes-Aragon et al. 2020), Australia (Giblin et al. 2018), Brazil (Soares et al. 2020), China (Li et al. 2022). The identification of *C. siamense* remained controversial, some studies supported it as species complex comprised one to seven species based on multi-genes analysis and

different morphological characteristics of *C. siamense* isolates (Sharma et al. 2015a; Weir et al. 2012). Meanwhile, Liu et al. (2016) concluded it was a single species relied on genealogical concordance phylogenetic species recognition and coalescent methods. The phylogenetic analysis with multiple DNA barcodes in this study showed that our two isolates assigned to a subclade within *C. siamense* clade which was close to isolate *C. siamense* ICMP 18578 with 97% support values. Hence, our isolates were reffered as *C. siamense*.

C. endophyticum (or *C. endophytica*) was not a common agent causing anthracnose on avocado compared to other *Colletotrichum* species. This species has been documented in Thailand as an endophytic fungal from tropical grasses (Manamgoda et al. 2013), in China as a pathogen of *Bauhinia blakeana* (Liang et al. 2022), in China as leaf spot causing agent of ornamental plants (Zhang et al. 2023), and was found from anthracnose on citrus (Shidiq et al. 2024) and chili (de Silva et al. 2019a; Silva et al. 2019b). Recently, two reports indicated *C. endophyticum* caused anthracnose on avocado in Sri Lanka (Dissanayake et al. 2021) and Thailand (Armand et al. 2023). The molecular identification indicated that our two isolates clustered in a distinct clade containing the ex-type strain MFLUCC 13–0418 and other reference isolates of *C. endophyticum*. Our new findings of *C. endophyticum* on avocado in Vietnam will expand understanding of this recently recorded pathogen of avocado for effective control methods in addition to other well-known agent of avocado anthracnose.

To understand the pathogenicity and host-specificity of species identified in this study, the artificial infection of four isolates delineated to specific species were performed using wounded method on Booth avocado cultivars, mango and banana fruits. Generally, pathogenicity assays indicated that four isolates were pathogenic to their true host avocado and able to cross-infect other fruits. Numerous studies reported that *C. gloeosporioides* species complex had a broad host range and caused anthracnose diseases on various tropical fruits worldwide (Zakaria 2021; Sharma et al. 2015b). Most species within this complex are polyphagous, while others show strong specialisation towards one host (Talhinhas et al. 2021). Among these isolates, *C. endophyticum* AD24.3 was considered the strongest strain with large lesions on three fruit types. While, *C. endophyticum* 24.5 was weakly pathogenic to avocado comparing to other isolates. In the study of Dissanayake et al. (2021), C. *endophyticum* was proven its pathogenicity on avocado after 6–7 days of incubation and there was no information about lesion diameter on avocado fruits of this species. Shidiq et al. (2024) recorded *C. endophyticum* showing smaller lesion diameter on citrus compared to other prevalent *Colletotrichum* species; similar context was described in pathogenicity of *C. edophyticum* on chili in study of de Silva et al. (2019a, b). Two isolates *C. siamense* AD1.2 and AD24.7 showed similar agressiveness on avocado in artificial assays with no differences in lesion diameter, but exposed different infection on mango and banana fruits. *C. siamense* proved to be virulent pathogen of avocado with 85.9% of disease severity percentage on avocado in Isreal (Sharma et al. 2017). In the study of Giblin et al. (2018), C. *siamenese* was pathogenic on avocado fruit and exhibited different pathogenicity on mango fruit with 4/24 isolates were pathogenic and 11/24 isolates were weakly pathogenic. Our results revealed that the morphological features and pathogenicity of obtained isolates within same species would be signifficant different, which reinforced the important of using multi-gene analysis for species delimitation. In this study, *C. siamense* and *C. endophyticum* were illustrated as new geographical records on avocado in Vietnam.

Conclusion

This work has expanded the information of four species from *C. gloeosporioides* species complex associates with anthracnose of avocado in Vietnam. The identification of these isolates provides the basis for studies of biocontrol methods development and disease management of avocado in Vietnam.

Acknowledgements The authors thank the partial support of Hue University under the Core Research Program, Grant No. NCTB. DHH.2025.04.

Funding This work was supported by Vingroup Innovation Foundation under Grant (Code: VINIF.2022.DA00117).

Data availability The data presented in the study are included in the manuscript.

Declarations

Ethical approval The study did not involve human and/or animal participation.

Competing interest The authors declare that they have no conflict of interest exists.

References

Armand A, Jaywardena RS (2023) Morphomolecular identification and pathogenicity of *Colletotrichum* species associated with avocado anthracnose in northern Thailand. Plant Pathol 73:186–197. https://doi.org/10.1111/ppa.13792

Ayvar-Serna S, Díaz-Nájera JF, Mateo VH, Camacho-Tapia M, Valencia-Rojas GA, Lima NB, Tovar-Pedraza JM (2020) First report of *Colletotrichum jiangxiense* causing avocado anthracnose in

Mexico. Plant Dis 105:502. https://doi.org/10.1094/PDIS-03-20-0459-PDN

Bustamante MI, Osorio-Navarro C, Fernandez Y, Bourret TB, Zamorano A, Henrique-Saez JL (2022) First record of *Colletotrichum anthrisci* causing anthracnose on avocado fruits in Chile. Pathogens 11:1204. https://doi.org/10.3390/pathogens11101204

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556. https://doi.org/10.1080/00275514.1999.12061051

Crous PW, Wingfield MJ, Burgess TI, Hardy GESJ, Gene J, Guarro J, Baseia I, Garcia D, Gusmao LFP, Souza-Motta CM et al (2018) Fungal planet description sheets: 716–784. Persoonia 40:240–393 https://doi.org/10.3767/persoonia.2018.40.10

de Silva DD, Groenewald JZ, Crous PW, Ades PK, Nasruddin A, Mongkolporn O, Taylor PWJ (2019a) Identification, prevalence and pathogenicity of *Colletotrichum* species causing anthracnose of *Capsicum annuum* in Asia. IMA Fungus 10:8. https://doi.org/10.1186/s43008-019-0001-y

Dissanayake DMS, Adikaram NKB, Yakandawala DMD, Jayasinghe L (2021) Molecular phylogeny-based identification of *Colletotrichum Endophytica* and *C. siamense* as causal agents of avocado anthracnose in Sri Lanka. Ceylon J Sci 50:449–458. https://doi.org/10.4038/cjs.v50i4.7943

Fischer IH, Firmino AC (2023) Main disease of avocado in Brazil. Revisão Anual De Patologia De Plantas 29:106–130. https://doi.org/10.31976/0104-038321v290005

Fuentes-Aragon D, Silva-Rojas HV, Guarnaccia V, Mora-Aguilera JA, Aranda-Ocampo S, Bautista-Martínez N (2020) *Colletotrichum* species causing anthracnose on avocado fruit in Mecurrenturrent status. Plant Pathol 69:1513–1528. https://doi.org/10.1111/ppa.13234

Giblin FR, Tan YP, Mitchell R, Coates LM, Irwin JAG, Shivas RG (2018) *Colletotrichum* species associated with pre- and post-harvest diseases of avocado and mango in eastern Australia. Australas Plant Pathol 47:269–276. https://doi.org/10.1007/s13313-018-0553-0

Hofer KM, Braithwaite M, Braithwaite LJ, Sorensen S, Siebert B, Pather V, Goudie L, Williamson L, Alexander BJR, Toome-Heller M (2021) First report of Colletotrichum Fructicola, C. Perseae, and C. siamense causing anthracnose disease of avocado (Persea americana) in New Zealand. Plant Dis 105:1564. https://doi.org/10.1094/pdis-06-20-1313-PDN

Honger JO, Offei SK, Oduro KA, Odamtten GT, Nyaku ST (2016) Identification and molecular characterisation of *Colletotrichum* species from avocado, citrus and pawpaw in Ghana. S Afr J Plant Soil 33:177–185. https://doi.org/10.1080/02571862.2015.1125958

Hsieh TF, Shen YM, Huang JH, Tsai JN, Lu MT, Lin CP (2023) Insights into grape ripe rot: a focus on the *Colletotrichum gloeosporioides* species complex and its management strategies. Plants 12:2873. https://doi.org/10.3390/plants12152873

Hunupolagama DM, Wijessundera RLC, Chandrasekharan NV, Wijesundera WSS, Kathriarachchi HS, Fernando THPS (2015) Characterization of *Colletotrichum* isolates causing avocado anthracnose and first report of *C. Gigasporum* infecting avocado in Sri Lanka. Plant Pathol Quar J Fungal Biolog 5:132–143. https://doi.org/10.5943/ppq/5/2/10

Kimaru SK, Monda E, Cheruiyot RC, Mbaka J, Alakonya A (2018) Morphological and molecular identification of the causal agent of anthracnose disease of avocado in Kenya. Int J Microbiol 2018:4568520. https://doi.org/10.1155/2018/4568520

Kimaru KS, Muchemi KP, Mwangi JW (2020) Effects of anthracnose disease on avocado production in Kenya. Cogent Food Agric 6:1. https://doi.org/10.1080/23311932.2020.1799531

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across comuting platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Kwon JH, Choi O, Lee Y, Kim S, Kang B, Kim J (2019) Anthracnose on postharvest avocado caused by *Colletotrichum kahawae* subsp. *ciggaro* in South Korea. Can J Plant Pathol 42:508–513. https://doi.org/10.1080/07060661.2019.1696891

Li M, Feng W, Yang J, Gao Z, Zhang Z, Zhang W, Wang S, Wang W, Gong D, Hu M (2022) First report of anthracnose caused by *Colletotrichum siamense* on avocado fruits in China. Crop Prot 155:105922. https://doi.org/10.1016/j.cropro.2022.105922

Liang L, Li S, Han S, Qiao T, Li S, Zhu T (2022) First report of *Colletotrichum Endophyticum*, a causal agent of leaf spot of *Bauhinia Blakeana*, in Southwest China. Plant Dis 107:956. https://doi.org/10.1094/PDIS-05-22-1230-PDN

Liu F, Wang M, Damm U, Crous PW et al (2016) Species boundaries in plant pathogenic fungi: a *Colletotrichum* case study. BMC Evol Biol 16:81. https://doi.org/10.1186/s12862-016-0649-5

Liu F, Ma ZY, Hou LW, Diao YZ, Wu WP, Damm U, Song S, Cai L (2022) Updating species diversity of *Colletotrichum*, with a phylogenomic overview. Stud Mycol 101:1–56. https://doi.org/10.3114/sim.2022.101.01

Manamgoda DS, Udayanga D, Cai L, Chukeatirote E, Hyde KD (2013) Endophytic Colletotrichum from tropical grasses with a new species C. Endophytica. Fungal Divers 61:107–115. https://doi.org/10.1007/s13225-013-0256-3

Manova V, Stoyanova Z, Rodeva R, Boycheva I, Korpelainen H, Vesterinen E, Wirta H, Bonchev G (2022) Morphological, pathological and genetic diversity of the *Colletotrichum* species, pathogenic on solanaceous vegetable crops in Bulgaria. J Fungi 8:1123. https://doi.org/10.3390/jof8111123

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol 37:1530–1534. https://doi.org/10.1093/molbev/msaa015

Mohali-Castillo SR, Stewart JE (2022) First report of *Colletotrichum siamense* associated with anthracnose on *Theobroma cacao* fruits in Venezuela. New Dis Rep 46:e12131. https://doi.org/10.1002/ndr2.12131

Nelson S (2008) Anthracnose of avocado. Plant Disease PD-58. College of Tropical Agriculture and Human resources. University of Hawai'I at Mānoa, USA

O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus Fusarium are nonorthologous Mol Phylogenet Evol 7:103–116. https://doi.org/10.1006/mpev.1996.0376

Oliveira MS, Wang NY, Peres NA (2022) Multilocus phylogenetic analyses of *Colletotrichum gloeosporioides* species complex causing crown rot on strawberry in Florida. Phytopathology 112:898–906. https://doi.org/10.1094/PHYTO-04-20-0151-R

Pérez-Jiménez RM (2008) Significant avocado disease caused by fungi and oomycetes. Eur J Plant Sci Biotechnol 2:1–24

Phoulivong S, Cai L, Chen H, McKenzie EH, Abdelsalam K, Chukeatirote E, Hyde KD (2010) *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. Fungal Divers 44:33–43. https://doi.org/10.1007/s13225-010-0046-0

Rattanakreetakul C, Keawmanee P, Bincader S, Mongkolporn O, Phuntumart V, Chiba S, Pongpisutta R (2023) Two newly identified *Colletotrichum* species associated with mango anthracnose in Central Thailand. Plants 12:1130. https://doi.org/10.3390/plants12051130

Salotti I, Ji T, Rossi V (2022) Temperature requirements of *Colletotrichum* spp. belonging to different clades. Front Plant Sci 13:953760. https://doi.org/10.3389/fpls.2022.953760

Sharma M, Kulshrestha S (2015b) *Colletotrichum gloeosporioanes*: An anthracnose causing pathogen of fruits and vegetables. Biosci

Biotechnol Res Asia 12:1233–1246. https://doi.org/10.13005/bbra/1776

Sharma G, Pinnaka AK, Shenoy BD (2015a) Resolving the *Colletotrichum siamense* species complex using ApMat marker. Fungal Divers 71:247–264. https://doi.org/10.1007/s13225-014-0312-7

Sharma G, Maymon M, Freeman S (2017) Epidemiology, pathology and identification of *Colletotrichum* including a novel species associated with avocado (*Persea americana*) anthracnose in Israel. Sci Rep 7:15839. https://doi.org/10.1038/s41598-017-15946-w

Sharma G, Maymon M, Meshram V, Freeman S (2022) Characterization of *Colletotrichum* isolates from strawberry and other hosts with reference to cross-inoculation potential. Plants 11:2373. https://doi.org/10.3390/plants11182373

Shidiq MAA, Widyaningsih S, Wibowo A, Widiastuti A (2024) First report of *Colletotrichum queenslandicum* and *Colletotrichum Endophyticum* causing citrus anthracnose in Indonesia. J Saudi Soc Agric Sci 23:393–403. https://doi.org/10.1016/j.jssas.2024.03.005

Siddiqui Y, Ali A (2014) *Colletotrichum gloeosporioides* (Anthracnose). In: Bautista-Baños S (ed) Postharvest Decay - Control Strategies. Academic, New York, pp 337–371. https://doi.org/10.1016/B978-0-12-411552-1.00011-9

Silva DD, Groenewald JZ, Crous PW, Ades PK, Nasuddin A, Mongkolporn O, Taylor PWJ (2019b) Identification, prevalence and pathogenicity of *Colletotrichum* species causing anthracnose of *Capsicum annuum* in Asia. Fungus 10:8. https://doi.org/10.1186/s43008-019-0001-y

Silva-Rojas HV, Avilia-Quezada GD (2011) Phylogenetic and morphological identification of *Colletotrichum boninense*: a novel causal agent of anthracnose in avocado. Plant Pathol 60:899–908. https://doi.org/10.1111/j.1365-3059.2011.02452.x

Soares MGO, Alves E, Silveira AL, Pereira FD, Guimaraes SSC (2020) *Colletotrichum siamense* is the main aetiological agent of anthracnose of avocado in south-eastern Brazil. hiPlant Pathol 70:154–166. https://doi.org/10.1111/ppa.13262

Talhinhans P, Baroncelli R (2021) *Colletotrichum* species and complexes: geographic distribution, host range and conservation status. Fungal Divers 110:109–198. https://doi.org/10.1007/s13225-021-00491-9

Templeton MD, Rikkerink EHA, Solon SL, Crowhurst RN (1992) Cloning and molecular characterization of the glyceraldehyde-3-phosphate dehydrogenaseencoding gene and cDNA from the plant pathogenic fungus *glomerella cingulata*. Gene 122:225–230. https://doi.org/10.1016/0378-1119(92)90055-t

Than PP, Jeewon R, Hyde KD, Pongsupasamit S, Monkolporn O, Taylor PWJ (2008) Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose on Chilli (*Capsicum* spp.) in Thailand. Plant Pathol 57:562–572. https://doi.org/10.1111/j.1365-3059.2007.01782.x

Tovar-Pedraza JM, Mora-Aguilera JA, Nava C, Lima NB (2020) Distribution and pathogenicity of *Colletotrichum* species associated with mango anthracnose in Mexico. Plant Dis 104:137–146. https://doi.org/10.1094/PDIS-01-19-0178-RE

Wanjiku EK, Waceke JW, Wanjala BW, Mbaka JN (2020) Identification and pathogenicity of fungal pathogens associated with stem end rots of avocado fruits in Kenya. Int J Microbiol 2020:4063697. https://doi.org/10.1155/2020/4063697

Weir BS, Johnston PR, Damm U (2012) The *Colletotrichum gloeosporioides* species complex. Stud Mycol 73:115–180. https://doi.org/10.3114/sim0011

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, New York, pp 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zakaria L (2021) Diversity of *Colletotrichum* species associated with anthracnose disease in tropical fruit crops - a review. Agriculture 11:297. https://doi.org/10.3390/agricultue11040297

Zhafarina S, Wibowo A, Widiastuti A (2021) Multi-genetic analysis of *Colletotrichum* spp. associated with postharvest disease of fruits anthracnose in special region of Yogyakarta, Indonesia. Pak J Bio Sci 24:53–65. https://doi.org/10.3923/pjbs.2021.53.65

Zhang YX, Chen JW, Manawasinghe IS, Lin YH, Jayawardena RS, McKenzie EHC, Hyde KD, Xiang MM (2023) Identification and characterization of *Colletotrichum* species associated with ornamental plants in Southern China. Mycosphere 14:262–302. https://doi.org/10.5943/mycosphere/14/si2/5

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.