Unravelling Cross Infectivity, Host Range and Cultural Variability of *Colletotrichum* spp. associated with Mango

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ABSTRACT: Anthracnose, caused by *Colletotrichum* spp., is a major postharvest disease in mango that significantly affects the quality, shelf life and marketability of fruits in both domestic and export markets. The present study evaluated the growth of two *Colletotrichum* species*viz.*,*C. asianum* and *C. siamense*, on ten different culture media. Among these, potato dextrose agar (88.17 mm), oat meal agar (82.00 mm), malt extract agar (81.00 mm) and Sabouraud's dextrose agar (80.33 mm) were found to be most suitable for the growth of *C. asianum*, while Richards synthetic agar (89.99 mm) supported maximum growth of *C. siamense*. Cross-infectivity studies revealed a broad host range for both species across multiple fruit crops. *C. asianum* was more aggressive on mango (1.61 cm), while *C. siamense* exhibited higher virulence on grapes (2.25 cm) and mango (2.18 cm).The study emphasizes the significance of using appropriate culture media for efficient isolation of *Colletotrichum* spp. and highlight the necessity for species and crop-specific disease management strategies based on their differential virulence and host preferences.

Keywords: Anthracnose, *Colletotrichum* spp., culture media, host range, mango

INTRODUCTION

Mango (*Mangifera indica* L.) popularly known as the 'King of fruits' belongs to *Anacardiaceae* family and is cultivated extensively in tropical and sub-tropical regions (Berardini *et al*., 2005). In India, mango cultivation spans in an area of approximately 2.39 million hectares, with a production of about 20.33 million tonnes and a productivity of 8.50 MT/ha (http://agricoop.nic.in). Anthracnose, caused by *Colletotrichum* spp. is an important post-harvest disease, resulting in yield losses ranging from 30 to 60 per cent, and sometimes extends up to 100 per centwhen grown under wet or highly humid conditions (Prakash *et al*., 1996; Ploetz and Freeman, 2009). It primarily affects leaves, flowers, young fruits, and twigs. Although the infection starts during fruit development as a latent or quiescent phase, the symptoms become prominent and economically damaging after harvest when fruit ripens (Prusky *et al*., 2009).

The growth and development of *Colletotrichum* spp. are influenced by the composition of the culture medium. While potato dextrose agar (PDA) is commonly used, variations in media can significantly affect mycelial growth, sporulation and colony morphology. Studying its cultural characteristics on different media under controlled conditions helps identify optimal growth requirements and suitable media for isolation, identification and long-term storage. It also reveals differences in pigment production and other morphological traits. Therefore, a comparative study was undertaken to determine the most suitable medium for rapid and consistent mycelial growth of *Colletotrichum* spp.

A single species can infect multiple hosts, while several species may infect the same host genus (Fuentes-Aragón *et al*., 2020). Cross-infection studies reveal alternative hosts and survival strategies essential for epidemic development. *Colletotrichum gloeosporioides* is a widespread and economically significant pathogen that affects a variety of hosts in tropical and subtropical regions, including banana, avocado, guava, apple, papaya, and dragon fruit (Nelson, 2008; Maharaj and Rampersad, 2012; Moraes *et al*., 2013; Munir *et al*., 2016; Bordoh *et al*., 2020). Lima *et al*. (2015) reported that fruit crops such as papaya, banana, guava, and bell pepper are susceptible to *C. asianum*, *C. dianesei*, *C. fructicola*, *C. karstii*, and *C. tropicale*. Additionally, various strains of the pathogen can infect non-mango hosts, including ornamental and marsh lupines, as well as herbs like angelica, thyme, caraway, and elder (Paulitz, 1995). In mangoes, larger lesions typically occur at temperatures between 25°C and 30°C, although optimal pathogenicity varies across species (Lima *et al*., 2015).

Identifying species and their cross-infection potential is vital for developing effective disease management practices.

The study aimed to identify the optimal culture medium for the growth of *Colletotrichum* spp. and assess their cross-infectivity on different fruit crops. Understanding media influence and host range helps determine pathogen adaptability, aiding in accurate diagnosis and targeted disease management strategies.

MATERIAL AND METHODS

Collection and isolation of the pathogen

Anthracnoseinfected mango fruits were collected from Karnataka (ICAR-IIHR, Bengaluru) and Maharashtra (Nagpur). Small tissue sections from the infected regions were surface sterilized using 1% (w/v) sodium hypochlorite solution for 1 minute, followed by three rinses in sterile distilled water. The sterilized tissues were placed on potato dextrose agar (PDA) and incubated at $28 \pm 2°C$ for 7 days. Fungal colonies emerging from the tissues were subcultured onto fresh PDA plates and purified using the single-spore isolation method (Li *et al.*, 2019). Molecular characterization revealed that the isolate from Karnataka belonged to *C.asianum* (IIHR_CA23; GAPDH accession no. PQ586487), while the isolate from Maharashtra was identified as *C. siamense* (IIHR_CS03; GAPDH accession no. PQ586492) (Supriya, 2025). Pathogenicity of both isolates was confirmed through inoculation on mango leaves and fruits (Mo *et al.*, 2018).

Media preparation and inoculation

Ten different media *viz.*, Potato dextrose agar (PDA), malt extract agar (MEA), Czapeksdox agar (CDA), corn meal extract (CMA), Sabouraud's dextrose agar (SDA), V8 juice agar, water agar (WA), potato carrot agar (PCA), oat meal agar (OMA) and Richards synthetic agar (RSA) were used for the evaluation of cultural and morphological characterization of *Colletotrichum* spp. All these media were autoclaved at 121 °C under 15 psi for 20 mins. Twenty ml of each medium was poured to sterilised Petriplate (90 mm). After solidification, five mm diameter of mycelial plugs from seven days old culture of *C. asianum* and *C. siamense* was placed at centre of the each mediumand incubated at 25±1 °C. The experiment was conducted separately for both the pathogens, with three replications foreach treatment. Colony diameter was measured daily until the colonies reached the edges of the Petri plates. The Cultural and morphological characters such asradial mycelial growth, colony color, texture, margin, surface topography, pigmentation, sectoring, concentric rings and acervuli production were recorded on 9th day to identify the suitable media for the growth of the pathogen.

Cross infection potential of *C. asianum* and *C. siamense* on fruit crops

Eight economically important fruit crops *viz.*, strawberry (*Fragaria × ananassa*), grape (*Vitis vinifera* L.), guava (*Psidium guajava* L.), mango (*Mangifera indica* L.), sweet orange (*Citrus sinensis*), pomegranate (*Punica granatum* L.) and papaya (*Carica papaya* L.) were selected to evaluate the cross-infection potential of *Colletotrichum* spp. These crops represent major commercial hosts across diverse plant families, are epidemiologically relevant due to overlapping cultivation systems, and are well-documented hosts of species within the *C. gloeosporioides* species complex. The selection was deliberately restricted to ensure experimental uniformity, adequate replication, and a biologically realistic assessment of cross-infectivity.

Healthy leaves/ fruits were washed with running water, surface sterilized in 70 per cent ethanol for 30 sec followed by one per centsodium hypochlorite solution for one minute and then finally rinsed with sterile distilled water. After air-drying, detached young healthy leaves and matured fruits were placed into plastic containers (252 × 174 × 93 mm) with moist absorbent cotton to maintain humidity.Two to four stab wounds were made forming a circle of a 5 mm diameter using a sterilized needle. Hyphal plugs from actively growing margins of PDA cultures were placed on each wound spot on the leaves and fruits. Fruits/ leavestreated with sterilised water served as control. The experiment was completely randomized with three replicates per isolate each containing five leaves/ fruits per replicate. The containers were partially sealed in plastic box and incubated at 25°C. Symptom development was observed and recorded on seven days after inoculation (Mo *et al.*, 2018).

Statistical analysis

All experiments were conducted in a completely randomized design at least three times. Statistical analysis was carried out using SPSS 16.0 using ANOVA (P<0.05) and Duncan's multiple-range test (DMRT) was used to find differences among treatments (P < 0.05).

RESULTS AND DISCUSSION

Suitability of different culture media for the growth of *Colletotrichum* spp.

A total of ten different media were evaluated for the growth of the pathogen. In *C. asianum*, the growth and appearance of the colony differ across various types of media.The mycelial growth of the pathogen varied from 55.67 mm to 88.17 mm across different culture media. Among the media tested, the highest mycelial growth was observed on PDA (88.17 mm), which was statistically on par with OMA (82.00 mm), MEA (81.00 mm) and SDA(80.33 mm). The least mycelial growth was recorded on V8 juice agar (60.00 mm) and WA (55.67 mm) (Table 1 and Fig. 1).

**Table 1. Effect of different media on the cultural characteristics of *C. asianum* (IIHR_CA23)**

Media	Mycelial growth (mm)*	Colony colour	Colony texture	Surface topography	Colony margin	Growth nature	Pigmentation	Sectoring	Concentric rings	Acervuli production
PDA	88.17^a	Greyish white	Cottony	Flat at centre raised at periphery	Irregular	Aerial	+	+	-	+
RSA	69.25^{bc}	Greyish white	Velvety	Raised mycelium	Irregular	Aerial	-	-	+	+
CDA	77.83^{ab}	Greyish white	Cottony	Flat at centre raised at periphery	Irregular	Aerial	+	-	-	+
MEA	81.00^a	Greyish white	Cottony	Raised at centre flat at periphery	Irregular	Aerial	+	+	-	+
V8	60.00^{cd}	Creamy white	Cottony	Flat mycelium	Irregular	Aerial	-	-	-	-
PCA	62.00^{cd}	White	Cottony	Raised mycelium	Irregular	Aerial	+	+	-	-
SDA	80.83^a	Greyish white	Velvety	Raised at centre flat at periphery	Regular	Aerial	+	-	-	+
CMA	70.17^{bc}	White	Cottony	Raised at centre flat at periphery	Regular	Aerial	-	-	-	-
OMA	82.00^a	White	Cottony	Flat mycelium	Regular	Aerial	+	-	-	+
WA	55.67^d	White	Cottony	Flat mycelium	Regular	Immersed	-	-	-	-

Note :+ : Present, - : Absent; Media: Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Czapeks Dox Agar (CDA), Corn Meal Extract (CMA), Sabouraud's Dextrose Agar (SDA), V8 Juice Agar, Water Agar (WA), Potato Carrot Agar (PCA), Oat Meal Agar (OMA) and Richards Synthetic Agar (RSA).

*Different lower-case letters (a, b, c, d) within the same column indicate significant differences at $p<0.05$ (DMRT).

Colony colour varied from greyish white to white, with textures ranging from cottony to velvety and margins from regular to irregular.On PDA, the colony appeared white with a cottony texture, irregular margins and exhibited flat mycelial growth at the centre with a raised periphery. Sporulation was observed on the 10th day in all media except V8 juice agar, PCA, CMA and WA.
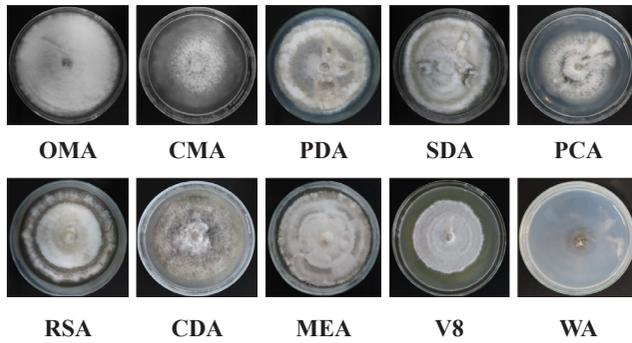
Fig. 1. Mycelial growth of *C. asianum* on different media

Among the different media tested on *C. siamense*, the highest mycelial growth of the pathogen was recorded on RSA (89.99 mm) followed by MEA (86.67 mm) and OMA (80.83 mm).The least mycelial growth was recorded on SDA (58.83 mm) and V8 juice agar (63.75 mm) (Table 2 and Fig. 2). On RSA, the colony appeared white with a velvety texture, regular margin and raised mycelium. Colony colour was white on all media except MEA and PCA, where it appeared as creamy white and light grey, respectively. The texture of the colony was velvety in RSA and SDA, while in rest of media it was cottony. The margin of the colony varied from regular to irregular with flat to raised mycelium. On 7th day,

the sporulation was observed only in PCA along with pigmentation and concentric rings. No sectoring was observed on any of the media tested.

The variation in colony characteristics and growth of *C. gloeosporioides* on different solid media can be attributed to differences in nutrient availability. Since fungi absorb nutrients and energy from the medium they grow on, it is essential to provide the necessary elements and compounds in the culture media to support their growth and metabolic functions. Not all media are equally suitable for every fungus and there is no universal substrate or artificial medium that supports the growth of all fungal species (Jagana *et al*., 2017).

Colletotrichum spp. exhibited growth on all the media tested. The PDA has recorded the highest mycelial growth for *C. asianum*, whereas RSA was most suitable for *C. siamense*. Similar observations were made by Akhtar *et al*. (2018), who reported that PDA was found to be highly suitable for mycelial growth of *C. capsici*. Rathod *et al*. (2022) also recorded maximum growth of *C. gloeosporioides* from ginger on RSA and PDA at 27 ± 1 °C.

Table 2: Effect of different media on cultural characteristics of *C. siamense* (IIHR_CS03)

Media	Mycelial growth (mm)*	Mycelial growth rate (mm/day)*	Colony colour	Colony texture	Surface topography	Colony margin	Growth nature	Pigmentation	Concentric rings	Acervuli production
PDA	77.25[cd]	8.58[cd]	White	Cottony	Raised mycelium	Irregular	Aerial	-	-	-
RSA	89.99[a]	9.96[a]	White	Velvety	Raised mycelium	Regular	Aerial	-	-	-
CDA	67.50[e]	7.50[e]	White	Cottony	Raised mycelium	Irregular	Aerial	+	-	-
MEA	86.67[ab]	9.63[ab]	Creamy white	Cottony	Flat mycelium	Regular	Aerial	-	+	-
V8	63.75[ef]	7.08[ef]	White	Cottony	Flat mycelium	Irregular	Aerial	-	-	-
PCA	75.67[cd]	8.41[cd]	Light grey	Cottony	Flat mycelium	Regular	Aerial	+	+	+
SDA	58.83[f]	6.54[f]	White	Velvety	Raised at centre flat at periphery	Regular	Aerial	-	-	-

Media	Growth	Value	Colour	Texture	Margin	Type	Mycelium	+	+	-
CMA	77.00ᶜ	8.56ᵉ	White	Cottony	Flat mycelium	Regular	Aerial	+	+	-
OMA	80.83ᵇᶜ	8.98ᵇᶜ	White	Cottony	Raised at centre flat at periphery	Irregular	Aerial	+	-	-
WA	69.00ᵈᵉ	7.67ᵈᵉ	White	Cottony	Flat mycelium	Irregular	Immersed	-	-	-

Note :+ : Present, - : Absent; *Different lower-case letters (a, b, c, d, e, f) within the same column indicate significant differences at p<0.05 (DMRT); Media: Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Czapeks Dox Agar (CDA), Corn Meal Extract (CMA), Sabouraud's Dextrose Agar (SAM), V8 Juice Agar, Water Agar (WA), Potato Carrot Agar (PCA), Oat Meal Agar (OMA) and Richards Synthetic Agar (RSA).
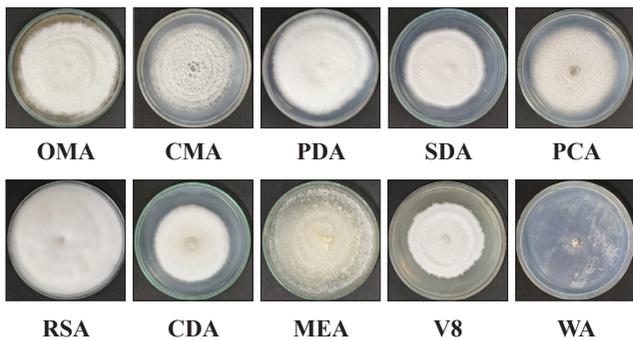

Fig. 2. Mycelial growth of *C. siamense* on different media

Likewise, Lokare *et al*. (2021) observed that CDA and PDA were the most suitable media for culturing *C. gloeosporioides* from mango, while the least growth was recorded on water agar.

In the present study, the mycelial growth of both pathogens ranged between 55.0 mm and 89.99 mm. Similarly, Bhagat *et al.* (2022) reported that *C. lindemuthianum* showed growth ranging from 62.33 mm to 90.00 mm across different media, with PDA supporting the highest growth. The fungal colonies were typically cottony to fluffy in texture, with margins varying from regular to irregular and colony coloration ranging from white to whitish grey. Kumar *et al.* (2012) also observed similar cultural characteristics for *C. capsici* on PDA and OMA. The better growth of *Colletotrichum* spp. on PDA and RSA may be due to their rich and balanced nutrient composition.

Cross infection potential of *C. asianum* and *C. siamense* on fruit crops

Detached leaves were used for strawberry, grape, guava, and mango, while mature fruits were used for pomegranate, amla, sweet orange, and papaya. The selection was based on the typical infection site of *Colletotrichum* spp. in each host. This ensured the use of the most susceptible and naturally targeted tissues. It also allowed for uniformity and biological relevance in cross-infection studies.

The *Colletotrichum* spp. was found to infect all the fruit crops with varying degrees of infection. For *C. asianum*, the lesion diameter has varied from 0.50 to 1.61 cm. The least lesion diameter was recorded in sweet orange and Papaya (0.50 cm) whereas, highest in mango (1.61 cm). For *C. siamense*, the lesion diameter has been varied from 0.50 to 2.25 cm, the maximum lesion diameter was recorded in grapes (2.25 cm) and mango (2.18 cm) whereas, minimum was recorded in sweet orange (0.50 cm) (Table 3 and Fig 3).

Table 3: Cross-infectivity of two *Colletotrichum* spp. onfruit crops

Fruits/ Leaves	Variety / Cultivar	Lesion diameter (7ᵗʰ day)	
		C. asianum	*C. siamense*
Strawberry	Winter dawn	1.29ᵇ	2.10ᵇ
Grapes	Dilkhush	0.95ᶜ	2.25ᵃ
Guava	Allahabad Safeda	0.82ᵈ	1.35ᵈ
Mango	Totapuri	1.61ᵃ	2.18ᵃᵇ
Amla	Hybrid 10	0.79ᵈ	1.57ᶜ
Sweet Orange	Mosambi	0.50ᶠ	0.50ᶠ
Pomegranate	Bhagwa	0.50ᶠ	1.56ᶜ
Papaya	Red lady	0.68ᵉ	0.73ᵉ

Note: Values followed by different letters within a column are significantly different at p ≤ 0.05 (DMRT).
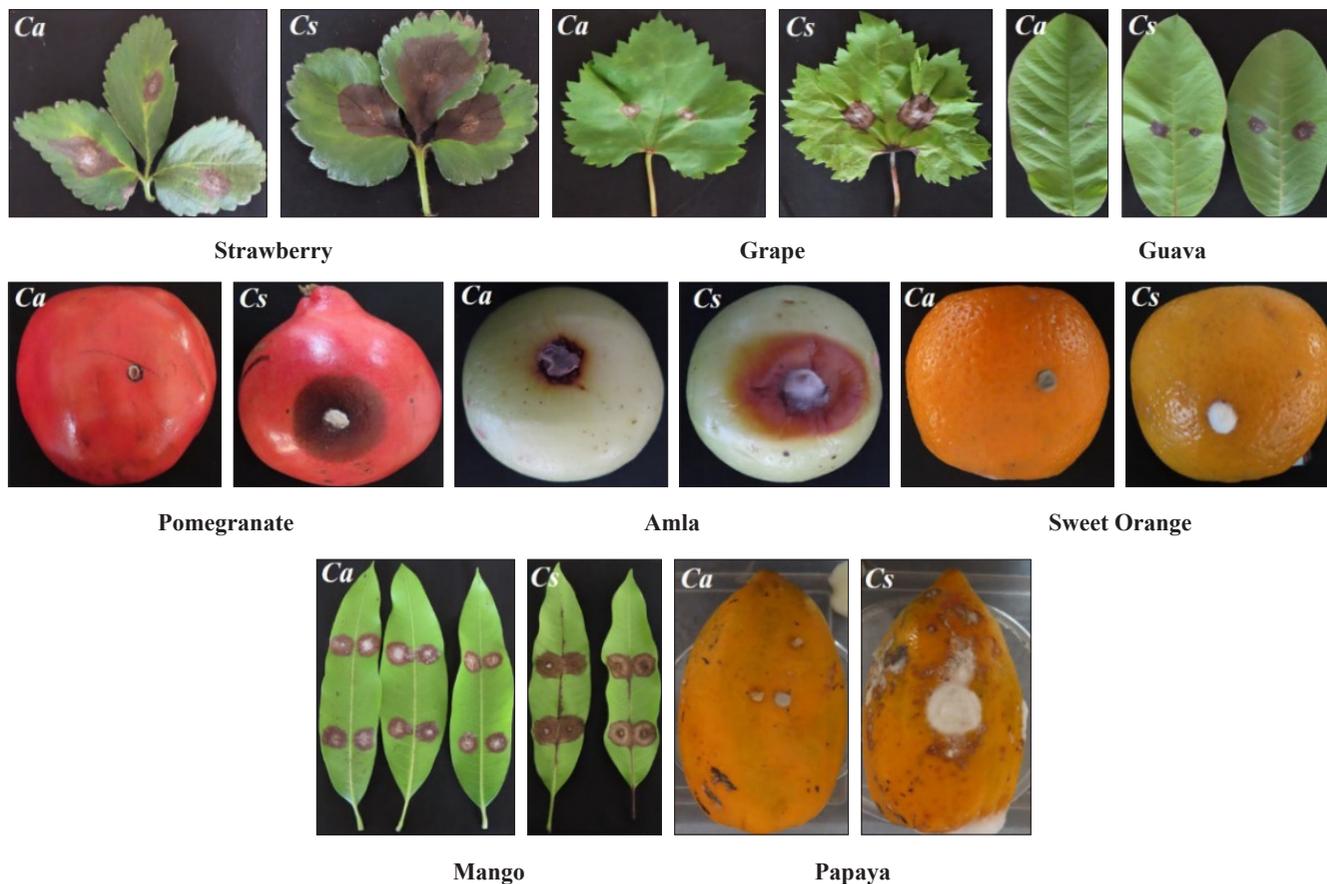
Fig 3. Symptom expression on detached leaves and fruits of various fruit crops after inoculation with *C. asianum* (*Ca*) and *C. siamense* (*Cs*)

Understanding whether a *Colletotrichum* species is host-specific or has a broad host range is essential for accurate classification and effective disease management (Cai *et al*., 2009). In this study, pathogenicity tests on alternative hosts showed that both *Colletotrichum* species lacked strict host specificity, consistent with earlier reports that many *Colletotrichum* species can infect a wide range of hosts (Freeman *et al*., 1998; Hyde *et al*., 2009; Cannon *et al*., 2012). However, both isolates exhibited greater aggressiveness on mango; the host from which they were originally isolated suggesting that despite a broad host range, host-specific adaptation and co-evolution may enhance virulence and infection efficiency on their native host, influenced by specific pathogen-host interactions and the traits of both the fungal isolates and the fruit hosts.

Sanders and Korsten (2003) demonstrated that isolates of *C. gloeosporioides* from mango were capable of infecting guava, chili and papaya, confirming cross-infection potential. Similarly, Lima *et al*. (2015) observed no host specificity during cross-pathogenicity tests involving papaya, banana, guava, and bell pepper, although they noted that some *Colletotrichum* species showed a preference for certain mango cultivars, suggesting possible cultivar-level susceptibility.In contrast, Hayden *et al*. (1994), using detached fruit assays, found that isolates from mango were highly aggressive on mango fruit but showed reduced aggressiveness on other hosts such as banana, pawpaw, and strawberry. This suggested the existence of a mango-specific biotype of *C. gloeosporioides*. Overall, these findings highlight the diverse host interactions of *Colletotrichum* species, ranging from broad host ranges to potential host or cultivar preferences.

ACKNOWLEDGEMENT

AUTHORS CONTRIBUTION

MLS: conceptualization, designing and implementation of the experiments and MS preparation; GYR: implementation of the experiments; SS: planning, supervising and formal analysis and MS finalisation.

CONFLICT OF INTEREST

The authors do not have any conflict of interest with respect to the content of the article.

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