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Screening of Mango Varieties against Anthracnose Diseases Caused by *Colletotrichum gloeosporioides* and Its *In vitro* Management through Biocontrol Agents and Fungicides

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mango (*Mangifera indica* L.), known as the "King of Fruits," is a major fruit crop cultivated in India and worldwide. Anthracnose, caused by *Colletotrichum gloeosporioides*, is a primary biotic stress affecting mango production, yield, and export quality in all mango-growing regions. This study evaluated the response of mango varieties to anthracnose and its management using effective

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fungicides and biocontrol agents. Ten *C. gloeosporioides* isolates were isolated from samples collected from the Kollam and Thiruvananthapuram districts in Kerala. Among them, isolate C10 from Thiruvananthapuram was the most virulent. Among five local mango varieties (Kottukonam, Priyoor, Neelam, Rumani, and Totapuri) screened through artificial inoculation, Totapuri displayed the lowest disease severity (45.4%), followed by Neelam and Rumani, while Kottukonam exhibited the highest (72.33%). *In vitro*, the study of different biocontrol agents showed that Bacillus amyloliquefaciens VLY24 showed 37.08% inhibition against the pathogen, followed by 32.87% inhibition by *Bacillus velezensis* PSCE-10. Carbendazim 50% WP at 0.1% and 0.2% exhibited 100% mycelial growth suppression.

Keywords: Mango; anthracnose; Colletotrichum; post-harvest; biocontrol agents; fungicides.

1. INTRODUCTION

Mango is known to be the "King of fruits" and belongs to the Anacardiaceae family, and it is an important crop in tropical and subtropical regions worldwide (Grice et al., 2023). Known for its exquisite flavour, mango is a dietary staple in numerous countries and is valued for its rich nutritional and medicinal properties; it is a good source of vitamins like carotene, thiamine, riboflavin, and niacin (Archibald et al., 2003) However, mango cultivation faces significant challenges due to various diseases, which significantly reduce yield. These diseases infect all stages of mango growth, from nursery plants to harvested fruits, and include economically detrimental fungal diseases such as anthracnose, root rot, stem rot, *Penicillium* rot, mucor rot, macrophoma rot, and powdery mildew (Jeevanantham et al., 2024).

Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. is the primary biotic stress affecting mango production, significantly impacting yield and export quality across all mango-producing regions. This disease was first identified in India by Mc Rae in 1924; anthracnose results in various symptoms on mango trees, such as black spots on leaves and fruits, blossom blight, and total unproductiveness (Sudha, 2014). As the fruit ripens, anthracnose appears as black spots in various shapes, which may be slightly recessed or show cracks. Over time, these spots expand and eventually cover the entire fruit, resulting in fruit rot. The disease progresses rapidly post-harvest, especially as fruits ripen and lose natural resistance, making them highly vulnerable during storage and transport (Paudel et al.,2022), Paudel, (2022) reports indicate that approximately 17.7% of mangoes suffer spoilage due to fungal infections during transit, storage, and marketing; Colón et al., (2002) reported the loss to be as high as 75% due to anthracnose.

Chemical fungicides are primarily used for disease control. Successful anthracnose control may be achieved by utilising pre-harvest and post-harvest fungicides (Bally et al.,2013) Postharvest fungicide treatment of fruit is required to reduce the disease's impact on the shelf life of fruit under challenging environmental and storage conditions (Prabakar et al.,2008). Biological control has recently gained attention as an alternative for managing diseases, using microbial antagonists to target pathogens affecting fruits and vegetables. Antagonists combat pathogens through nutrient and space competition, antibiotic production, siderophore release, and by triggering Induced Systemic Resistance (ISR) (Paudel et al., 2022).

The current study aims to assess the response of the mango variety to anthracnose and the management of anthracnose in mango using effective chemical fungicides and bio-control agents.

2. MATERIALS AND METHODS

2.1 Collection of Biocontrol Antagonists

Two bacterial antagonists of *Bacillus amyloliquefaciens* VLY24 and *B. velezensis* PCSE-10 were collected from the Department of Microbiology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala Agricultural University, Kerala for *in vitro* evaluation of bacterial antagonists against anthracnose pathogen.

2.2 Survey and Isolation of the Pathogen

A total of 10 infected mango fruit samples showing anthracnose symptoms were collected from local markets in the Thiruvananthapuram and Kollam districts of Kerala during January and February of 2024. Using the tissue segment method, the pathogens associated with the disease were isolated. The infected fruits were

surface sterilised with 70% ethanol, and small sections were cut from the lesion margins. They were surface sterilised with 1% sodium hypochlorite to eliminate the saprophytes for 45 sec, followed by rinsing in sterile water. The sections were plated onto Potato Dextrose Agar Medium (PDA) and incubated at 25 °C for seven days. The fungal growth was examined daily for up to 7 days. Isolates were subcultured onto fresh PDA slants and stored at 4°C for further studies.

2.3 Cultural and Morphological Characterisation

Fungal Isolates were cultured on PDA plates at 25 °C for 7-10 days. Plugs from colony margins were placed in the centre of each 90 mm diameter plate. Fungal mycelial Character, colony colour and colony diameter were recorded after 7 days at 25 °C. Colony diameters were used to calculate the hyphal growth (cm/day). Different fungal isolates conidial shape, size and colour were measured under 400X Magnification (LAS EZ version 3.4.0).

2.4 Pathogenicity Test and Virulence Rating

Collected isolates were used for pathogenicity and virulence tests on mature and healthy mango fruits. Fungal isolates were incubated on PDA plates for 7 days at 25-28 ℃. The fruits were washed thoroughly by running tap water, surface sterilised with 0.2% sodium hypochlorite and rinsed with sterilised water. After washing and drying, the mangoes are wounded, forming a circle with a 5mm diameter by pinprick using a sterilised needle. Mycelial bits of 6 mm diameter from PDA cultures were placed on the wounded areas and covered with moist cotton. In the control treatment, the wounded areas were covered with moist cotton without mycelial discs. Inoculated fruits were placed in a plastic bag with damp cotton to maintain humidity. The fruits were incubated in a humid chamber. Fruits were checked for the development of symptoms for up to 5 days. Virulence was evaluated by measuring the lesion size at 3 and 5 DAI (Days after inoculation) and the rate of lesion development per day.

2.5 Varietal Screening of Mango against Anthracnose

Mature mango fruits of five different cultivars were collected from the local market for screening against anthracnose disease. Collected mangoes were thoroughly washed under running tap water, then surface-sterilized using 0.2% sodium hypochlorite and rinsed with sterile water. After drying, the mangoes were wounded by creating a 5mm diameter circle with a sterilised needle. Mycelial bits, each 6mm in diameter, were taken from PDA cultures of virulent isolate and placed on the wounded areas, which were then covered with moist cotton soaked in sterile water. For the control, the wounded areas were covered only with damp cotton. The inoculated fruits were kept in plastic bags with moist cotton to maintain humidity, and the bags were secured with rubber bands. The fruits were incubated in a humid chamber, and symptoms were monitored for 7 days. Disease severity of fruits 3, 5 and 7 DAI were recorded using a 0-5 rating scale (Table. 1) as suggested by Prabhakar *et al.* (2008).

Table 1. Disease scale (0-5) for scoring per cent fruit infection of anthracnose disease on mango fruits

Grade	Description
0	No infection
1	<1 %fruit surface infected
2	1-5% fruit surface infected
3	6-25% fruit surface infected
4	26-50% fruit surface infected
5	>50 % fruit surface infected

The per cent disease index (PDI) was calculated by adopting the following formula devised by McKinney (1923).

$$PDI = \frac{sum\ of\ all\ numerical\ rating}{total\ no\ of\ observation\ X\ maximum\ rating} \times 100$$

Based on the calculated PDI, the cultivars were categorised for their reaction against the virulent isolate. As 0 = Immune, 1-10 = resistant, 11-20 = moderately resistant, 21-30 = moderately susceptible, 31-40 = susceptible, 41-100 = highly susceptible.

2.6 In vitro Efficacy of Bacterial Antagonists against Mango Anthracnose

Collected bacterial antagonists of *B. amyloliquifaciens* VLY24 and *B. velezensis* PCSE-10 were studied by dual culture technique (Sivakumar et al., 2002) on potato dextrose agar medium. A mycelial bit of 6 mm diameter was taken from a 7-day-old culture of virulent

pathogen and placed on the centre of a 9 cm diameter Petri dish containing PDA. A loop of bacterial isolates from 24-hour culture was then streaked on PDA 1.5 cm from the plate's edge from both sides. As a control, agar discs of the same fungus were placed on a PDA culture plate without the bacteria. Plates were then incubated at room temperature (28 ± 2°C) for seven days. After the incubation period, the per cent radial growth inhibition was recorded using the following formula (Nene and Thapliyal, 1979).

Per cent Inhibition = Radial growth in control plate- radial growth of treatment plate / Radial growth in control plate * 100

2.7 In vitro Efficacy of Fungicides against Mango Anthracnose

Different concentrations (0.05%, 0.1% and 0.2%) of carbendazim 50 WP (Bavistin) were tested under *in vitro* conditions against *C. gloeosporioides* by poisoned food technique (Vincent, 1947). The required concentration of the fungicides was mixed with 50 ml of sterile water, then mixed with 100 ml double-strength PDA medium, and the poisoned medium was poured into Petri dishes (90 mm diameter) under aseptic conditions. Circular bits of 5mm of the 7-day-old fungus culture were placed at the centre of the petri dish, and each concentration was replicated three times. The petri dish, which had a PDA medium without fungicide, was served as a control. After inoculation, the petri dishes were incubated at 25±1°C. The radial colony growth of the pathogen was recorded when the growth in an untreated petri dish (control) was complete (*i.e.* 90 mm). Per cent inhibition in colony growth was calculated using a formula Vincent devised (1947).

$$I = [(C-T)/C] \times 100$$

Where I = Percent inhibition of mycelial growth (diameter in cm of *C.gloeosporioides*)

C = Mycelial growth (diameter in cm) of *C.gloeosporioides* in control

T = Mycelial growth (diameter in cm) of *C.gloeosporioides* in treatments

3. RESULTS

3.1 Survey and Isolation of the Pathogen

A survey was conducted during January and February of 2024, and infected mango fruit samples showing anthracnose symptoms were collected from local markets in the Thiruvananthapuram and Kollam districts of Kerala. Ten samples of infected mango fruits were collected from surveyed locations. The collected samples are of different varieties. The location of the mango anthracnose sample collection, the variety of the samples, and their GPS coordinates are presented in Table 2. Symptoms are generally the infected fruits have irregular brown, black, and sunken lesions that develop on affected parts. As the disease develops, lesions become soft and sunken, with a pink to orange-coloured conidial mass**.** The fungal pathogens from each sample collected from different locations were isolated under aseptic conditions on a PDA medium and incubated for mycelial growth. The pure culture of the isolates was maintained for further studies.

Table 2. Details of the survey area and varieties collected

District	Location	GPS coordinates	Varieties
Kollam	East Kallada	9.0077ºN,76.6499ºE	Neelum
	Perayam	8.7095ºN,77.0010ºE	Banganapalli
	Chattannur	8.8623ºN,76.7234ºE	Totapuri
Thiruvananthapuram	Mangalapuram	8.6242ºN,76.8485ºE	Vellari
	Kazhakkuttam	8.5686ºN,76.8731ºE	Moovandan
	Neyyattinkara	8.4027ºN, 77.0861ºE	Totapuri
	Veganoor	8.4051ºN, 77.0056ºE	Kottukonam
	Balaramapuram	8.4321ºN,77.0503ºE	Banganapalli
	Kaliyoor	8.4325ºN,77.0167ºE	Banganapalli
	Manacaud	8.4719ºN,76.9518ºE	Neelum

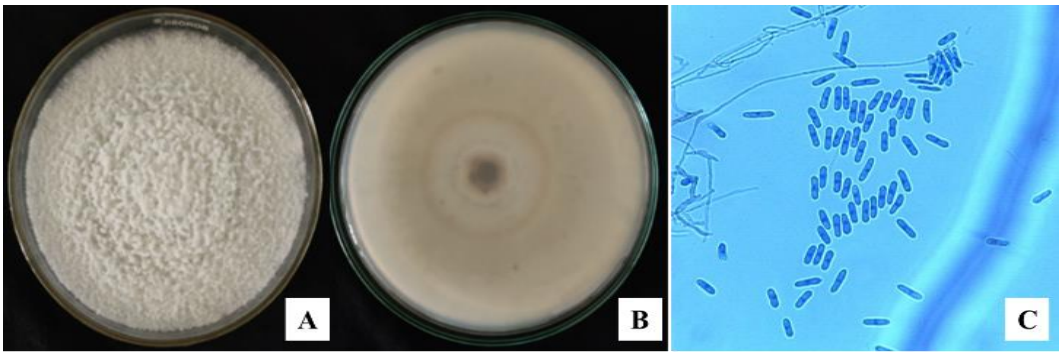

Fig. 1. A- Front view of virulent isolate (C10), B- Rear view of virulent isolate (C10), C- Conidia microscopic image at 400X magnification

Table 3. Mycelial characters of different isolates

Isolates	Nature of mycelial growth	Pigmentation		Radial growth at 7th day
		Front view	Rearview	
C1	Sparse	Whitish with regular margins	Whitish	7.05
C2	Sparse	Off-white with a greyish centre	Greyish with dark grey centre	7.87
C3	Sparse	Yellowish centre with a white zone	Whitish with yellow centre	7.97
C4	Fluffy	Whitish with regular margins	Whitish with a yellowish centre	8.37
C5	Sparse	Greyish centre and margins	Dark grey	8.03
C6	Fluffy	Whitish centre with grey margins	Dark grey centre	8.38
C7	Sparse	Whitish with regular margins	Whitish	8.10
C8	Fluffy	Whitish	Whitish	8.20
C9	Fluffy	Whitish with regular margins	Whitish	8.41
C10	Fluffy	Whitish with regular margins	Whitish with grey centre	8.83

Table. 4 Conidial characteristics of different isolates

Isolates	Conidial characters		
	Shape	Size (µm*)	Colour
C1	Oblong	11.4 x 3.3	Hyaline
C2	Dumbbell	9.3 x 3.5	Hyaline
C3	Oblong	11.2 x 3.5	Hyaline
C4	Dumbbell	9.5 x 3.4	Hyaline
C5	Dumbbell	9.4 x 3.7	Hyaline
C6	Dumbbell	9.7 x 3.8	Hyaline
C7	Cylindrical	10.2 x 3.6	Hyaline
C8	Oblong	11.4 x 3.6	Hyaline
C9	Dumbbell	9.6 x 3.3	Hyaline
C10	Cylindrical	11.3 x 3.6	Hyaline

3.2 Cultural and Morphological Characterisation

All ten isolates were grown in a PDA medium, and their cultural and morphological characters were studied by observing the mycelial growth. Each isolate exhibited variations in the mycelial growth and pattern. Five isolates (C1, C2, C4, C6, C8) appeared to be sparse in mycelial nature, and five isolates (C3, C5, C7, C9, C10) seemed to be fluffy mycelial growth. Most of the isolates appeared to be whitish to grey mycelia

colour with regular margins in the front view and whitish to grey and dark grey at the centre at the rearview when observing the growth on the PDA plate (Table 3). Three isolates (C1, C3, C8) conidia appeared to be oblong, five isolates (C2, C4, C5, C6, C9) conidia appeared to be dumbbell in shape, and two isolates (C7, C10) seemed to be cylindrical. All the isolates' conidia appeared to be hyaline in colour when observed in 400X magnification. The size of the conidia ranges from 9.3 X 3.3 to 11.4 X 3.8µm (Table 4).

3.3 Pathogenicity and Virulence Rating

The pathogenicity test revealed the same symptoms in fruits inoculated with the pathogen as observed in samples collected during the survey. When the ten isolates were compared for virulence, the most virulent isolates were identified based on the time taken for symptom development and the rate of lesion formation. Isolates C2, C3, and C5 took 4 days to produce symptoms, while isolates C1, C6, C8, and C9 took 3 days. In contrast, isolates C4 and C10 produced symptoms within 2 days of inoculation with *C. gloeosporioides*. The highest rate of lesion development was recorded in isolate C10, with a lesion growth rate of 3.11 cm/day, and the lowest was observed in isolate C5, with a lesion growth rate of 0.54 cm/day (Table 5). Thus, C10 (Fig. 1) was identified as the most virulent isolate among the ten and was selected for further studies.

3.4 Varietal Screening of Mango Against Anthracnose

Five mango varieties grown in Kerala were screened for resistance to anthracnose (*C. gloeosporioides*) through artificial inoculation. The varieties were classified into different resistance levels based on a per cent disease index (PDI) using a 0–5 scale. The lowest disease severity was observed in Totapuri (45.40%), followed by Neelum (60.80%) and Rumani (55.13%). In contrast, the highest severity was recorded in Kottukonam (72.33%) and Priyoor (63.93%), indicating high susceptibility to anthracnose (Table 6). The screening was conducted based on the days until symptom development, lesion size, and PDI. Varieties Kottukonam and Rumani developed symptoms within two days of inoculation, while Neelum, Priyoor, and Totapuri exhibited symptoms three days post-inoculation. Kottukonam had the largest lesion size of 17.33 cm^2 seven days after inoculation (7DAI), whereas Totapuri had the smallest lesion size of 14.27 cm^2 at 7DAI. The highest PDI was observed in Kottukonam (72.33%) and the lowest in Totapuri (45.40%). Based on PDI grading, all varieties tested were highly susceptible to anthracnose (*C. gloeosporioides*) under artificial inoculation conditions.

Table 5. Virulence rating of different isolates

Isolates	DTSD	*Lesion size (l×b) cm^2		*Rate of lesion development (cm day^{-1})
		3DAI	5DAI	
C1	3	0.16 ± 0.02^{de}	1.31 ± 0.22^{de}	1.23
C2	4	0.00 ± 0.00^{e}	0.67 ± 0.23^{ef}	0.67
C3	4	0.00 ± 0.00^{e}	1.83 ± 0.15^{cd}	1.83
C4	2	0.15 ± 0.02^{de}	2.37 ± 0.55^{bc}	2.29
C5	4	0.13 ± 0.02^{de}	0.61 ± 0.20^{f}	0.54
C6	3	0.18 ± 0.04^{d}	0.57 ± 0.15^{g}	0.48
C7	2	1.40 ± 0.20^{b}	2.93 ± 0.70^{b}	2.23
C8	3	0.84 ± 0.05^{c}	1.19 ± 0.21^{def}	0.77
C9	3	0.89 ± 0.08^{c}	1.31 ± 0.12^{de}	0.86
C10	2	1.73 ± 0.21^{a}	3.97 ± 0.79^{a}	3.11
SE(m)±		**0.056**	**0.236**	**0.233**
CD (0.05)		**0.08**	**0.334**	**0.33**

**Value is the mean of three replications; values with the same letters are not significantly different at P<0.05*

Fig. 2. Varietal screening of mango against anthracnose at 7DAI

Table 6. Varietal response of mango fruits upon artificial inoculation with pathogen

Variety	DTSD	*Lesion size (l×b) cm^2			*PDI	Reaction
		3 DAI	5DAI	7DAI		
Kottukonam	2	1.80±0.20^{a}	6.27±0.31^{a}	17.33±0.61^{a}	72.33±2.52^{a}	HS
Neelum	3	1.13±0.47^{b}	4.80±0.20^{b}	15.90±0.56^{b}	60.80±1.06^{c}	HS
Priyoor	3	0.46±0.05^{c}	5.77±0.87^{a}	16.33±1.15^{ab}	63.93±1.68^{b}	HS
Rumani	2	1.21±0.18^{b}	6.20±0.17^{a}	14.27±0.31^{c}	55.13±1.01^{d}	HS
Totapuri	3	0.23±0.15^{c}	3.53±0.50^{c}	8.20±0.20^{d}	45.40±0.53^{e}	HS
SE(m)±		**0.147**	**0.280**	**0.397**	**0.878**	
CD (0.05)		**0.207**	**0.397**	**0.534**	**1.242**	

**Value is the mean of four replications, DTSD- Days taken for symptoms development, values with the same letters are not significantly different at P<0.05*

Table 7. *In vitro* efficacy of bacterial antagonists against mango anthracnose

Bacterial antagonist	*Inhibition zone (mm)	% inhibition*
Bacillus amyloliquifaciens VLY24	13.86±0.96^{a}	37.08 ± 3.35^{a}
Bacillus velezensis PCSE-10	13.01±0.60^{b}	32.87 ± 4.74^{b}
SE(m)±	**0.267**	**1.367**
CD (0.05)	**0.377**	**1.934**

**Value is the mean of nine replications; values with the same letters are not significantly different at P<0.05*

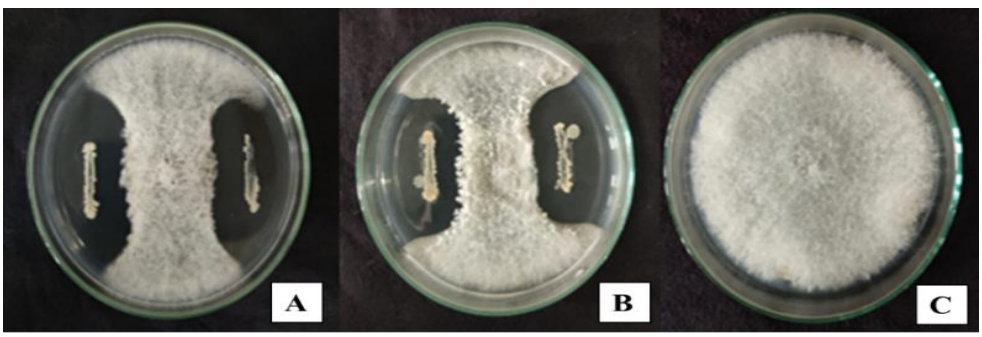

Fig. 3. *In vitro* efficacy of bacterial antagonists against mango anthracnose A- *B. amyloliquifaciens* VLY24, B *B.velezensis* PCSE-10, C- Control

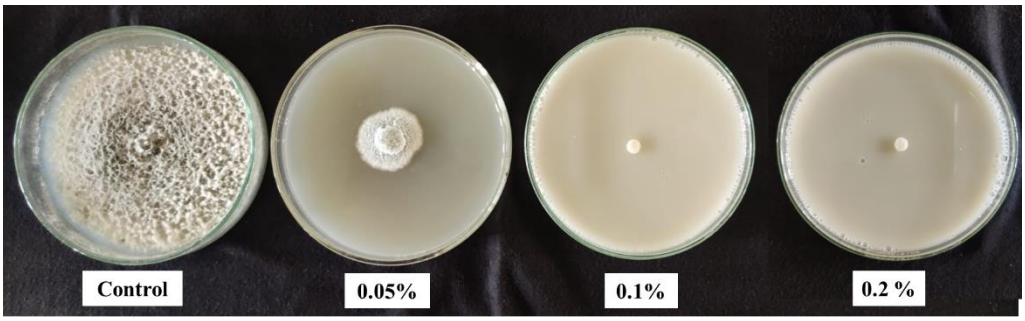

Fig. 4. *In vitro* efficacy of fungicides against mango anthracnose at different concentrations

3.5 *In vitro* Efficacy of Bacterial Antagonists against Mango Anthracnose

Two bacterial antagonists, *B. amyloliquefaciens* VLY24 and *B. velezensis* PSCE-10, were obtained from the Department of Agricultural Microbiology, College of Agriculture, Vellayani, Kerala Agricultural University. *In vitro* evaluation was conducted against the virulent isolate (C10). *B. amyloliquefaciens* VLY24 exhibited significantly higher inhibition (37.08%) compared to *B. velezensis* PSCE-10 (32.87%) (Fig. 2) against the anthracnose pathogen (Table 7).

3.6 *In vitro* Efficacy of Fungicides against Mango Anthracnose

For *in vitro* evaluation, carbendazim 50% WP was used against the most virulent isolate (C10). The mycelial growth of the pathogen varied in its sensitivity to different concentrations of the fungicide. Compared to the control, the fungicide suppressed the growth of the pathogen. At 0.1% and 0.2% carbendazim concentrations, 100% mycelial suppression was observed, while at a 0.05% concentration, 76.67% mycelial suppression was recorded.

4. DISCUSSION

The present study evaluated the pathogenic variability and management of anthracnose disease in mango caused by *Colletotrichum gloeosporioides*, focusing on isolates from Kerala, India. Anthracnose, a major fungal disease affecting mangoes, significantly impacts fruit yield and quality. Symptoms, including dark brown to black lesions with sunken areas and pink to orange conidial masses, were observed and aligned with findings by Onyeani *et al.* (2012) and Pandey *et al.* (2012) on anthracnose symptoms in mangoes. Characterisation of ten isolates revealed differences in mycelial and conidial traits, with some isolates (C1, C2, C3) displaying sparse mycelial growth and distinct pigmentation. Morphological diversity in conidial shapes (primarily oblong and dumbbell-shaped) and colony colours was observed, resembling reports from Bangladesh (Nguyen et al., 2009, Sharma and Badiyala, 1998). Varietal screening indicated that all tested mango varieties were susceptible to anthracnose, with Totapuri exhibiting comparatively lower disease severity. The highest infection severity was found in Kottukonam (72.33% PDI) and Priyoor (63.93% PDI) varieties, highlighting the susceptibility of local cultivars. Similarly, Sharma and Badiyala (1998) found no mango cultivars resistant to anthracnose.

In *in vitro* efficacy trials, bacterial antagonists *Bacillus amyloliquefaciens* VLY24 and *Bacillus velezensis* PCSE-10 demonstrated notable pathogen inhibition, with *B. amyloliquefaciens* VLY24 showing a higher inhibition rate of 37.80% and *B. velezensis* PCSE-10 showing 32.87%. These findings align with previous studies on the effectiveness of biocontrol agents in managing postharvest diseases (Paudel et al., 2022, Russi et al., 2024 reported that *B. velezensis* S26 effectively controlled *Colletotrichum* spp. and *Botrytis cinerea* isolates *in vitro* Choub et al., 2021 while Choub et al., (2021) showed that *B. velezensis* CE 100 produces antifungal lytic enzymes inhibiting spore germination and mycelial growth of *C. gloeosporioides* Mochizuki (2012) also found that *B. amyloliquefaciens* S13-3 inhibits *C. gloeosporioides in vitro* Liang et al., 2022, Jiang *et al., 2020* also reported the efficacy of *B. amyloliquefaciens* PMB04 in controlling mango anthracnose. Carbendazim at concentrations of 0.1% and 0.2% showed 100% inhibition of *C. gloeosporioides* mycelial growth; these results are in agreement with Prabakar *et al*., 1923, who found that carbendazim (0.1%) effectively inhibited mycelial growth and conidial germination of *C. gloeosporioides in vitro* studies.

Singh *et al.,2020* reported that carbendazim inhibited the growth of pathogens up to 98.23 and 96.07% at 100 µg/ml concentration.

5. CONCLUSION

The study highlighted significant variability amongst pathogen *C. gloeosporioides* isolates from Kerala affecting mango varieties, with isolate C10 being the most virulent. The varietal screening revealed that all tested mango varieties were susceptible to anthracnose, with Totapuri showing the lowest susceptibility at 45%. The efficacy of biocontrol agents and chemical treatments was demonstrated, with *B. amyloliquefaciens* VLY24 exhibiting higher pathogen inhibition than *B. velezensis* PCSE-10. Carbendazim fungicide at 0.1% and 0.2% achieved complete pathogen suppression. These results highlight the potential of integrating biocontrol agents with fungicides to manage mango anthracnose effectively.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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