

Evaluation of mango varieties against *Colletotrichum gloeosporioides* and its management through biocontrol agents and fungicides

Abstract

Mango (*Mangifera indica* L.), known as the "King of Fruits," is a major crop 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 fungicides and biocontrol agents. Ten *C. gloeosporioides* isolates were collected from the Kollam and Thiruvananthapuram districts in Kerala, with isolate C10 from Thiruvananthapuram being the most virulent. Among five local mango varieties (Kottukonam, Priyoor, Neelam, Rumani, and Totapuri) when screened by artificial inoculation, Totapuri exhibited the lowest disease severity (45.4%), followed by Neelam and Rumani and at the same time, Kottukonam showed the highest (72.33%). *In vitro*, *Bacillus amyloliquefaciens* VLY24 showed 37.08% inhibition against the pathogen compared to 32.87% inhibition by *Bacillus velezensis* PSCE-10. Carbendazim 50% WP at 0.1% and 0.2% achieved 100% mycelial growth suppression.

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

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 (1). 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 (2). 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 (3).

Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. is primary biotic stress affecting mango production, significantly impacting yield and export quality across all mango-producing regions. First identified in India by Mc Rae in 1924 (4),

Comment [a1]: COMPLETE THE SENTENCE

anthracnose results in various symptoms on mango trees, such as black spots on leaves and fruits, blossom blight, and total unproductiveness (5). 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 (6). Paudel (2022) (7) reports indicate that approximately 17.7% of mangoes suffer spoilage due to fungal infections during transit, storage, and marketing; Colón (2002) (8) reported the loss to be as high as 75%.

Comment [a2]: MENTION THE DISEASE RESPONSIBLE FOR LOSS

Chemical fungicides are primarily used for disease control. Successful anthracnose control may be achieved by utilising pre-harvest and post-harvest fungicides(9). 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 (10). Biological control has recently gained attention as an alternative for managing diseases, using microbial antagonists to target pathogens under controlled conditions. Antagonists combat pathogens through nutrient and space competition, antibiotic production, siderophore release, and by triggering Induced Systemic Resistance (ISR) (6).

Comment [a3]: PLEASE BE SPECIFIC FOR MANGO

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.

Materials and method

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.

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 rinses 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.

Comment [a4]: PLEASE BE SPECIFIC WITH THE PROTOCOL

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).

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 °C. 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 plugs of 6mm diameter from PDA cultures were placed on the wounded parts covered with moist cotton with sterile water. For control treatment, moist cotton was covered in the wounded part. Inoculated fruits were placed in a plastic bag with damp cotton to maintain humidity. The fruits are 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).

Comment [a5]: REVISE IT

Comment [a6]: THE MEANING IS NOT CLEAR, PLEASE REVISE THE SENTENCE

Comment [a7]: INOCULATED

Comment [a8]: MAINTAIN UNIFORMITY IN TENSE

Varietal screening of mango against anthracnose

Mature mango fruits of 5 different cultivars were collected from the local market for screening against anthracnose disease. 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 plugs, each 6mm in diameter, were taken from PDA cultures of virulent isolate and placed on the wounded areas, which were then covered with moist

Comment [a9]: How many mangoes were taken in one replication? Why the number of replications differs for different evaluations

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) (11).

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) (12).

$$PDI = \frac{\text{sum of all numerical ratings}}{\text{total no of observation} \times \text{maximum rating observed}} \times 100$$
The cultivars were categorised for their reaction against the virulent isolate based on the calculated PDI. As 0 = Immune, 1-10 = resistant, 11-20 = moderately resistant, 21-30 = moderately susceptible, 31-40 = susceptible, 41-100 = highly susceptible.

Comment [a10]: PLEASE WRITE THE FORMULA PROPERLY

***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 (13) on potato dextrose agar medium. A mycelial plug 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 ($28 \pm 2^\circ\text{C}$) for seven days. After the incubation period, the per cent radial growth inhibition was recorded using the following formula (14).

Per cent Inhibition= $\frac{\text{Radial growth in control plate} - \text{radial growth of treatment plate}}{\text{Radial growth in control plate}} \times 100$

***In vitro* efficacy of fungicides against mango anthracnose**

Carbendazim 50 WP (Bavistin) were evaluated under *in vitro* conditions against the anthracnose pathogen at different concentrations of 0.05%, 0.1% and 0.2% by poisoned food technique (15). 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 $25 \pm 1^\circ\text{C}$. 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) (16).

$$I = \frac{(C - T)}{C} \times 100$$

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

Results

Survey and Isolation of the pathogen

A survey was conducted during January and February of 2024, where infected mango fruit samples showing anthracnose symptoms were collected from local markets of 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 mango anthracnose sample collection, variety 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 pathogen from each sample collected from different locations was 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	9.0077°N,76.6499°E	Neelum
	Perayam	8.7095°N,77.0010°E	Banganapalli
	Chattannur	8.8623°N,76.7234°E	Totapuri
Thiruvananthapuram	Mangalapuram	8.6242°N,76.8485°E	Vellari
	Kazhakkuttam	8.5686°N,76.8731°E	Moovandan
	Neyyattinkara	8.4027°N, 77.0861°E	Totapuri
	Veganoor	8.4051°N, 77.0056°E	Kottukonam
	Balaramapuram	8.4321°N,77.0503°E	Banganapalli
	Kaliyoor	8.4325°N,77.0167°E	Banganapalli
	Manacaud	8.4719°N,76.9518°E	Neelum

Cultural and Morphological Characterisation

All the 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).

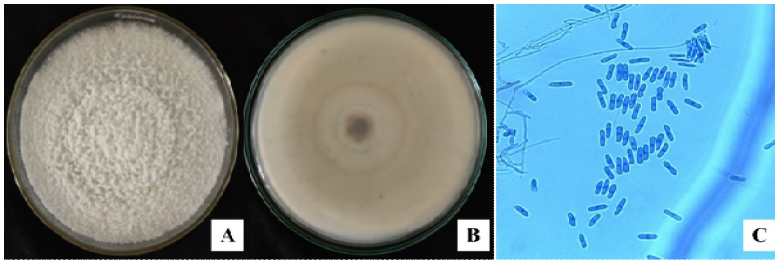


Figure 1: A- Front view of virulent isolate (C10), B- Rear view of virulent isolate (C10), C- Conidia microscopic image at 400X magnification

Table. 3 Mycelial characters of different isolates

Isolates	Nature of mycelial growth	Pigmentation		Radial growth at 7 th 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

Pathogenicity and virulence rating

The pathogenicity test revealed that the same symptoms, which were found to be the same as the sample collected during the survey. When compared for their virulence, the ten isolates were used to identify the most virulent isolates for further study based on the days taken for symptoms development and rate of lesion development. Isolates C2, C3 and C5 took 4 days to produce symptoms, whereas C1, C6, C8 and C9 were 3 days where, as 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 3.11 cm/day lesion, and the lowest in isolate C5, with a 0.54 cm/day lesion (Table. 5). Hence, C10 (Figure 1) was identified as the most virulent isolate among the ten isolates and was used for further studies.

Comment [a11]: REVISE THE SENTENCE

Comment [a12]: How was the rate of lesion development calculated?

Table. 5 Virulence rating of different isolates

Isolates	DTSD	Lesion size cm ²		Rate of lesion development (cm day ⁻¹)
		3DAI	5DAI	
C1	3	0.160 ± 0.020 ^{de}	1.310 ± 0.217 ^{de}	1.23
C2	4	0.000 ± 0.000 ^e	0.670 ± 0.234 ^{ef}	0.67
C3	4	0.000 ± 0.000 ^e	1.833 ± 0.153 ^{cd}	1.83
C4	2	0.153 ± 0.021 ^{de}	2.367 ± 0.551 ^{bc}	2.29
C5	4	0.130 ± 0.017 ^{de}	0.610 ± 0.201 ^f	0.54
C6	3	0.177 ± 0.038 ^d	0.567 ± 0.153 ^g	0.48
C7	2	1.400 ± 0.200 ^b	2.933 ± 0.702 ^b	2.23
C8	3	0.837 ± 0.047 ^c	1.190 ± 0.210 ^{def}	0.77
C9	3	0.893 ± 0.083 ^c	1.313 ± 0.121 ^{de}	0.86
C10	2	1.733 ± 0.208 ^a	3.973 ± 0.793 ^a	3.11
SE(m)±		0.056	0.236	0.233
CD (0.05)		0.08	0.334	0.33

Comment [a13]: If it's the rate of growth per day than the lesion on the fifth day must be larger than 3

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

Varietal screening of mango against anthracnose

Five mango varieties grown in Kerala were screened by artificial inoculation against anthracnose (*C. gloeosporioides*). The varieties were grouped under different degrees of resistance based on a per cent disease index using a 0-5 scale. The lowest disease severity was recorded in Totapuri (45.40%), followed by Neelum (60.80 %) and Rumani (55.13 %). The highest was recorded in Kottukonam (72.33 %) and Priyoor (63.93 %), which exhibit high anthracnose intensity (Table.6). The varietal screening was done based on days taken for symptoms development, lesion size produced and per cent disease index. Variety Kottukonam and Rumani produced symptoms within 2 days of inoculation with anthracnose pathogen. Neelum, Priyoor and Totapuri expressed symptoms at 3 days after inoculation. Kottukonam produced the maximum lesion size of 17.33 cm² (7DAI), and Totapuri produced the minimum lesion size of 8.20 cm² (7DAI) compared with other varieties. The highest PDI was observed in Kottukonam 72.33%, and the lowest in Totapuri 45.40%. Based on PDI grading, all the varieties are highly susceptible to anthracnose (*C. gloeosporioides*) when artificially inoculated (Figure 6).

Comment [a14]: PAST TENSE

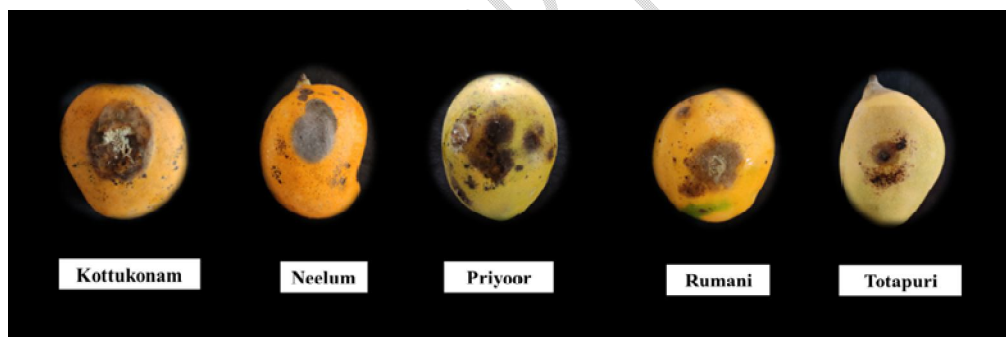


Figure 2: Varietal screening of mango against anthracnose at 7DAI

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 ^c	HS
SE(m)±		0.147	0.28	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

In vitro efficacy of bacterial antagonists against mango anthracnose

Two bacterial antagonists, *B. amyloliquifaciens* VLY24 and *B. velezensis* PSCE-10, were obtained from the Department of Agricultural Microbiology of College of Agriculture, Vellayani, Kerala Agricultural University and **invitro** evaluation was done against virulent isolates (C10). *B. amyloliquifaciens* VLY24 showed significantly higher inhibition (37.08%) than *B. velezensis* PSCE-10 (32.87%) (figure 2) against anthracnose pathogen (Table 7).

Comment [a15]: ITALICISE

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

Bacterial antagonist	Inhibition zone (mm)	% inhibition
<i>Bacillus amyloliquifaciens</i> VLY24	13.86±0.96 ^a	37.08 ± 3.35 ^a
<i>Bacillus velezensis</i> 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

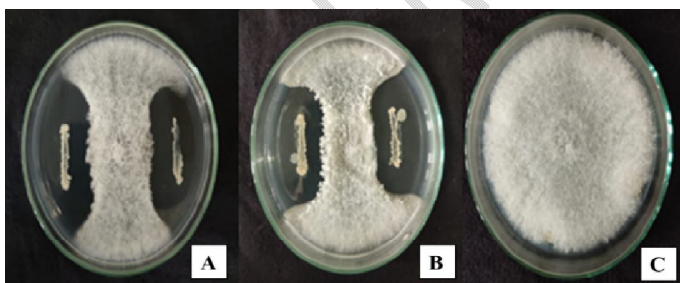


Figure 3: In vitro efficacy of bacterial antagonists against mango anthracnose A- *B. amyloliquifaciens* VLY24, B. *B. velezensis* PCSE-10, C- Control

In vitro efficacy of fungicides against mango anthracnose

For **invitro** evaluation, carbendazim 50% WP was used against the most virulent isolates (C10). Mycelial growth of the pathogen varied in its sensitivity to different concentrations of the fungicides. Compared to control, the fungicides suppressed the growth of the pathogen. At 0.1% and 0.2% concentration of carbendazim, 100% of mycelial suppression was exhibited, and at 0.05% concentration, 76.67% of mycelial suppression was observed (Figure 3).

Comment [a16]: ITALICISE

Discussion

The present study evaluated the pathogenic variability and management of anthracnose disease in mango caused by *C. gloeosporioides*, focusing on isolates from Agro-ecological units 1 & 8 in Kerala, India. Anthracnose, a primary fungal disease in mango, severely affects fruit yield and quality. Dark brown to black lesions with sunken areas and pink to orange conidial masses were observed, consistent with findings by Onyeani *et al.* (2012) (17) and Pandey *et al.* (2012) (18) on anthracnose symptoms in mangoes. Characterisation of ten isolates revealed varying mycelial and conidial traits, with some isolates (e.g., C1, C2, C3) showing sparse mycelial growth and distinct pigmentation patterns. Morphological diversity was noted in conidial shapes (mainly oblong and dumbbell) and colony colours, similar to reports from Bangladesh (19)(20). The study's varietal screening showed that all tested mango varieties exhibited susceptibility to anthracnose, and Totapuri showed comparatively lower disease severity. The highest severity of infection in Kottukonam (72.33% PDI) and Priyoor (63.93% PDI) varieties highlights the susceptibility of local cultivars. Sharma and Badiyala (1998) (21) observed that none of the mango cultivars were resistant to anthracnose diseases in their studies.

In vitro efficacy trials of bacterial antagonists of *B. amyloliquifaciens* VLY24 and *B. velezensis* PCSE-10 demonstrated promising levels of pathogen inhibition, with *B. amyloliquifaciens* VLY24 showing higher inhibition rates of 37.80% and *B. velezensis* PCSE-10 32.87%. These findings are similar to prior studies (6)(22) on the effectiveness of biocontrol agents in managing postharvest diseases. Russi (2024) (23) reported that *B. velezensis* S26 effectively controlled *Colletotrichum* spp. and *Botrytis cinerea* isolates *in vitro*. Choub (2021) (24) reported that *B. velezensis* CE 100 could produce antifungal lytic enzymes that inhibited the spore germination and mycelial growth of *C. gloeosporioides*. Mochizuki (2012) (25) studies suggested that *B. amyloliquifaciens* S13-3 inhibit *C. gloeosporioides in vitro*. In this study, chemical fungicides, carbendazim at concentrations of 0.1 and 0.2% achieved 100% inhibition of *C. gloeosporioides* mycelial growth. Prabakar *et al.* (2008) (11) reported through *in vitro* studies that carbendazim (0.1%) inhibited the mycelial growth and conidial germination of *C. gloeosporioides*.

Conclusion

Comment [a17]: REVISE THE SENTENCE

The objective of this study was to identify the pathogen causing anthracnose in mango, which is *C. gloeosporioides*, the varietal response to its artificial inoculation, and finally the *in vitro* efficacy of two bacterial isolates, *B. amyloliquifaciens* VLY24 and *B. velezensis* PCSE-10, and carbendazim fungicide. The morphological and pathogenic variability among the isolates indicated the complexity of the pathogen. The most virulent isolate of the pathogen obtained was C10. Varietal screening highlighted differential susceptibility, all varieties screened were susceptible to the anthracnose pathogen, with the least susceptible being Totapuri (45 %). Both biological and chemical treatments were effective in reducing the pathogen growth. *B. amyloliquifaciens* VLY24 showed higher inhibition rates than *B. velezensis* PCSE-10. Carbendazim at 0.1% and 0.2% showed 100% efficacy for pathogen suppression. These findings suggest that an integrated approach utilizing biocontrol agents and fungicides may be effective for mango anthracnose management.

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