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Exploring the Potential of Epiphytic and Endophytic Microorganisms for the Biological Management of Mango Anthracnose

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Abstract

Anthracnose is one of the most severe post-harvest diseases affecting mango fruits. It impairs the quality of fruits and consequently, their acceptance. Two species *viz*., *Colletotrichum asianum* and *C. siamense* were found to cause anthracnose in mango. Currently, the disease is managed by spraying chemical fungicides. However, their extensive and intensive use has disturbed the ecological balance of microorganisms present in the habitat *i.e.,* the microbiome/ phytobiome. Further, it has resulted in the development of fungicide resistance in pathogens, environmental pollution and risks to human health. Biocontrol agents (BCAs) are used as alternatives to fungicides. The microbes (epiphyte, endophyte and yeast) obtained from mango, pomegranate, grapes and guava were tested against *C. asianum* and *C. siamense* isolated from mango for their antifungal activity. The epiphytic bacteria obtained from mango were effective against both the species of pathogen. *Bacillus subtilis*, *B. siamense* and *B. vezelensis* exhibited the highest mycelial growth inhibition of 61.76, 61.32 and 61.12 per cent, respectively against *C. asianum*. Whereas, against *C. siamense*, *B. siamense* (epiphyte) and *Paenibacillus polymixa* (endophyte) were found effective with 52.52 and 47.02 per cent inhibition, respectively. The study revealed that BCAs can be a good alternative to manage the mango anthracnose and in reducing the chemical residues that impact agricultural commodities.

Keywords : Anthracnose, Antifungal activity, Epiphytes, Endophytes, Mango

Mango (*Mangifera indica* L.) commonly referred to as the 'King of fruits' or the 'apple of tropics' belongs to *Anacardiaceae* family and is grown in tropical and sub-tropical regions (Berardini *et al*., 2005). In India, mango cultivation spans in an area of approximately 2.39 m/ha with a production of about 20.33 MT and a productivity of 8.50 MT/ha (http://agricoop.nic.in). Unfortunately, mango production faces challenges *viz*., inconsistent fruit maturity and ripening and substantial pre and post-harvest economic losses. Post-harvest diseases not only diminish fruit quality but also lead to substantial losses, resulting in the production of unmarketable or blemished fruits that fail to meet the standards of major import markets (Cappellini *et al*., 1988).

Mango anthracnose, caused by *Glomerella cingulata* (Stoneman) Spauld. and H. Schrenk (anamorph: *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz) is an important post-harvest disease, resulting in yield losses ranging from 30 to 60 per cent and sometimes extends up to 100 per cent in fruits grown under wet or highly humid conditions (Prakash *et al*., 1996). It primarily affects leaves, flowers, young fruits and twigs. Recent taxonomic revisions led to the identification of several new species *viz*., *C. asianum*, *C. dianesei*, *C. fructicola*, *C. siamense*,

C. tropicale and *C. karstii* that are pathogenic to mango (Weir *et al*., 2012). Among these species, *C. asianum* stands out as one of the most prevalent, causing mango anthracnose in various regions (Vittale *et al*., 2020).

Mango anthracnose control primarily relies on chemical fungicides, but their continuous use poses risks due to the accumulation of toxic substances harmful to both human and the environment (Saeed *et al*., 2016). Recognizing these impacts, there is a pressing need to explore alternative, effective methods for plant disease management (Ma and Michailides, 2005). Cultural practices, bio-control agents (BCAs), and natural compounds offer promising alternatives to chemical fungicides. Utilizing microorganisms native to the environment presents an effective strategy for controlling pathogen growth and reducing their populations (Syed *et al*., 2018).

Endophytes and epiphytes form mutualistic relationships with their host plants, resulting in beneficial effects such as improved growth, enhanced disease resistance and increased tolerance to environmental stresses. These microorganisms are actively employed as microbial inoculants, contributing to the overall health and well-being of the host plants (Newton *et al*., 2010). They enhance systemic resistance in plants by inducing the production of antifungal bacteriocins, metabolites, antibiotics, iron-chelating siderophores, lytic enzymes and even promote plant growth activities (Saharan & Nehra, 2011 and Saraf *et al*., 2014). This study was conducted to assess the potential of epiphytic/ endophytic bacterial and yeast antagonists for the effective management of mango anthracnose.

Material and Methods

Collection and Isolation of the Pathogen

The pathogen was isolated from mango samples collected from Karnataka (ICAR-IIHR) and Maharashtra (Nagpur). A small sections of anthracnose-infected fruit was placed in one per cent (w/v) sodium hypochlorite solution for one minute followed by washing in sterile distilled water three times. It was then placed on potato dextrose agar (PDA) medium and incubated at 28 ± 2°C for 7 days. The isolates were then subcultured onto fresh PDA and purified by single-spore culture (Li *et al*., 2019).

Pathogenicity Test

The two isolates were used for pathogenicity tests on detached leaves and fruits of mango in controlled conditions. Isolates were incubated on PDA plates for 7 to 10 days at 25°C in the laboratory. Fresh young leaves and fresh harvested mango fruits without visible disease symptoms from ICAR-IIHR, were used for inoculation. The leaves and fruits were washed with running water, surface sterilized in 70 per cent ethanol for 30 sec and 1 per cent NaClO for 1 min and finally rinsed with sterile distilled water.

After air-drying, detached young healthy leaves (12 to 15 cm) and matured fruits were placed into plastic containers (252 × 174 × 93 mm) with moist absorbent cotton to maintain humidity and four to five stab wounds were made forming a circle with 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. The experiment was completely randomized with three replicates per isolate involving five leaves/ fruits per replicate. Fifteen leaves/ fruits were inoculated for each isolate and in controls, they were treated with sterile agar plugs. The containers were partially sealed in plastic box and incubated at 25°C in the dark in a growth chamber. The symptom development was noticed up to 7 days (Mo *et al*., 2018).

Evaluation of Antagonistic Activity of Bacterial and Yeast Epiphytes/ Endophytes

A total of 19 isolates (epiphyte/ endophyte/ yeast), which were already characterized were used in the present study for their efficacy against post-harvest disease in mango caused by *Colletotrichum* spp. Out of 19 isolates, 11 isolates were epiphytes obtained from grapes and mango while the rest were endophytes. Among endophytes, six isolates were obtained from pomegranate and guava. The two isolates were yeast cultures obtained from grapes and

Table 1

List of epiphytes/ endophytes evaluated against *Colletotrichum* spp. causing mango anthracnose

Isolates	Species	Source	Remarks
IIHR_PCRB05	*Bacillus subtilis*	Pomegranate	Endophyte
IIHR_GGRB08	*Paenibacillus polymixa*	Guava	Endophyte
IIHR_GBRB09	*Bacillus paralicheniformis*	Guava	Endophyte
IIHR_GKRB07	*Bacillus subtilis*	Guava	Endophyte
IIHR_PIRB01	*Lysinbacillus fusiformis*	Pomegranate	Endophyte
IIHR_GGRB12	*Paenibacillus polymixa*	Guava	Endophyte
IIHR_GIFYO1	*Hanseniaspora opuntiae*	Grapes	Yeast (Epiphyte)
IIHR_GAPB02	*Paenibacillus polymyxa*	Grapes	Epiphyte
IIHR_GIPB03	*Lysinibacillus fusiformis*	Grapes	Epiphyte
IIHR_GSTB02	*Bacillus* sp. / *australimaris*	Grapes	Epiphyte
IIHR_MIFB05	*Bacillus subtilis*	Mango	Epiphyte
IIHR_MIFB02	*Bacillus velezensis*	Mango	Epiphyte
IIHR_MIFB03	*Bacillus siamense*	Mango	Epiphyte
IIHR_MIFB04	*Bacillus amyloliquefaciens*	Mango	Epiphyte
IIHR_MIFB07	*Bacillus subtilis*	Mango	Epiphyte
IIHR_MIFB06	*Bacillus subtilis*	Mango	Epiphyte
IIHR_MIFB01	*Bacillus subtilis*	Mango	Epiphyte
IIHR_MIFY01	*Hanseniaspora opuntiae*	Mango	Yeast (Epiphyte)
IIHR_GSPB03	*Bacillus velezensis*	Grapes	Epiphyte

mango (Patil *et al*., 2022 and Maruti *et al*., 2021). The list of epiphyte/ endophyte/ yeast isolates is presented in Table 1.

In vitro Antifungal Activity of Bacterial and Yeast Epiphytes/ Endophytes

All the bacterial and yeast isolates were screened for their antagonistic activity against *Colletotrichum* spp. causing anthracnose disease in mango by dual culture assay (Dennis and Webstar, 1971) on PDA medium. A fully grown 7mm fungal disc was placed in the centre of the PDA plate while bacterial isolate was streaked on both sides of the fungal disc at equidistance. PDA plate inoculated only with the pathogen culture served as control. After 3-5 days of incubation, plates were observed for the antagonism by endophytic/epiphytic bacteria. Percentage growth inhibition was calculated on the 3rd, 6th, 9th and 12th day after inoculation (DAI). The percentage of inhibition was calculated using the formula:

$$I = (R1-R2) / R1 \times 100$$

Where, I = Per cent inhibition

R1 = Colony diameter (cm) of pathogen in control

R2 = Colony diameter (cm) of pathogen in treatment

Per cent growth inhibition was categorized on a scale given by Korsten *et al.* (1995) from 0 to 4 *i.e.,* 0 per cent = 0, 1 to 25 per cent = 1, 26-50 per cent = 2, 51-75 per cent = 3 and 76-100 per cent = 4. Isolates that reduced pathogen development by producing a demarcation zone or greater growth inhibition were selected for subsequent evaluation of antagonism on grapes.

Experimental Design and 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 was used to find differences among treatments (P < 0.05).

Results and Discussion

Collection and Isolation of the Pathogen

The samples were collected from ICAR-IIHR, Bengaluru, Karnataka and Nagpur, Maharashtra. Based on morphological and molecular characterization using ITS, ACT, GAPDH, TUB2 and CHS-1, two different pathogens were identified *viz*., *C. asianum* and *C. siamense* (unpublished data).

***In vitro* Antifungal Activity of Yeast and Bacterial Epiphytes/ Endophytes**

A total of 19 isolates of epiphytes/endophytes/yeast were screened against *Colletotrichum* spp. in mango.

Colletotrichum asianum : The inhibition percentage varied from 1.88 to 61.76 per cent. The maximum growth inhibition recorded in IIHR_MIFB05 (*Bacillus subtilis*, 61.76%) was on par with IIHR_MIFB03 (*B. siamense*, 61.32%) and IIHR_MIFB02 (*B. velezensis*, 61.12%) followed by IIHR_GGRB08 (*Paenibacillus polymixa*, 59.78%) and IIHR_MIFB07 (*B. subtilis*, 57.13%). The least inhibition was recorded in IIHR_MIFB01 (*B. subtilis*) and IIHR_GIPB03 (*Lysinibacillus fusiformis*) with 1.88 and 2.46 per cent, respectively. Among two yeast isolates, the IIHR_GIFY01 (*Hanseniaspora opuntiae*) and IIHR_MIFY01 (*H. opuntiae*) demonstrated growth inhibition of 21.82 and 6.40 per cent respectively (Table 2 and Fig. 1).

Among the tested isolates, seven were categorized in growth inhibition scale 3 (50 to 75%), four isolates were classified into scale 2 (25 to 50%) and the remaining eight isolates were classified as scale 1 (1 to 25%) (Table 4).

Colletotrichum siamense : IIHR_MIFB03 (*B. siamense*) exhibited the highest growth inhibition

Table 2

Inhibition of *C. asianum* mycelial growth by endophytes / epiphytes

Endophyte / Epiphyte	Per cent inhibition of *C. asianum*			
	3 DAI	6 DAI	9 DAI	12 DAI
IIHR_PCRB05	25.95 defg (30.61)	48.85 bcd (44.33)	52.70 ab (46.53)	50.75 ab (45.41)
IIHR_GGRB08	43.22 a (41.09)	54.37 abc (47.49)	58.55 ab (49.90)	59.78 a (50.62)
IIHR_GBRB09	12.35 hi (20.57)	28.78 fg (32.43)	29.34 de (32.79)	27.51 c (31.62)
IIHR_GKRB07	29.18 cde (32.68)	50.50 abc (45.27)	43.81 bcd (41.43)	41.38 b (40.02)
IIHR_PIRB01	26.30 defg (30.84)	30.16 efg (33.30)	24.09 e (29.38)	26.53 c (30.99)
IIHR_GGRB12	21.56 efgh (27.65)	52.64 abc (46.49)	55.07 ab (47.89)	55.97 a (48.41)
IIHR_GIFYO1	1.24 i (6.38)	26.00 g (30.65)	25.25 e (30.15)	21.82 c (27.83)
IIHR_GAPB02	17.65 fgh (24.83)	41.74 cdef (40.23)	47.69 abc (43.66)	47.93 ab (43.80)

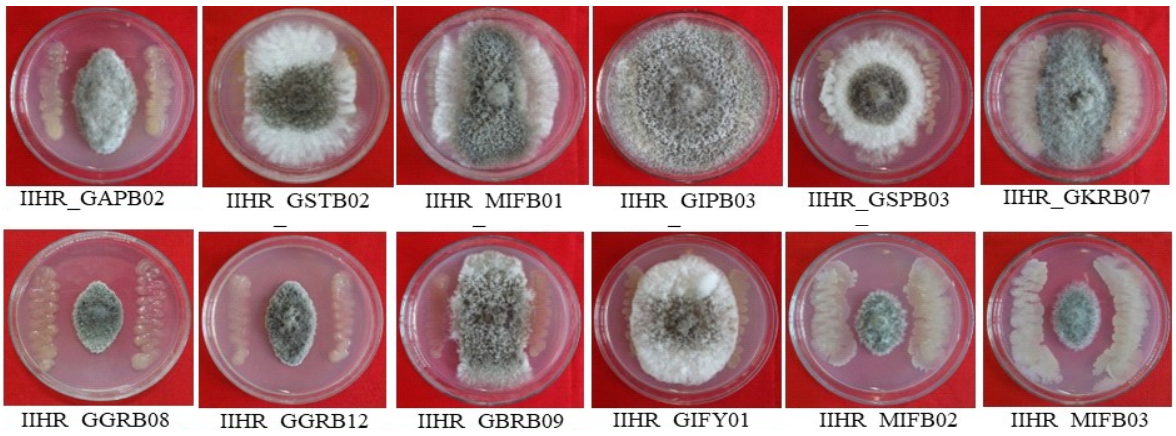
Continued....

Table 2 Continued....

Endophyte / Epiphyte	Per cent inhibition of *C. asianum*			
	3 DAI	6 DAI	9 DAI	12 DAI
IIHR_GIPB03	3.25 ^{i} (10.39)	6.48 ^{h} (17.74)	5.92 ^{f} (14.08)	2.46 ^{d} (9.01)
IIHR_GSTB02	28.80 ^{cdef} (32.44)	31.29 ^{efd} (34.00)	23.68 ^{e} (29.10)	22.43 ^{c} (28.25)
IIHR_MIFB05	32.45 ^{abcde} (34.71)	62.24 ^{ab} (52.07)	59.38 ^{ab} (50.38)	61.76 ^{a} (51.78)
IIHR_MIFB02	40.73 ^{ab} (39.64)	63.35 ^{a} (52.72)	60.99 ^{a} (51.33)	61.12 ^{a} (51.40)
IIHR_MIFB03	38.71 ^{abc} (38.46)	58.74 ^{ab} (50.01)	61.72 ^{a} (51.76)	61.32 ^{a} (51.52)
IIHR_MIFB04	36.36 ^{abcd} (37.07)	43.06 ^{cde} (40.99)	23.41 ^{e} (28.92)	23.82 ^{c} (29.20)
IIHR_MIFB07	31.27 ^{bcde} (33.99)	54.18 ^{abc} (47.28)	57.48 ^{ab} (49.28)	57.13 ^{a} (49.08)
IIHR_MIFB06	27.14 ^{defg} (31.39)	43.35 ^{cde} (41.16)	34.50 ^{cde} (35.96)	23.20 ^{c} (28.78)
IIHR_MIFB01	16.10 ^{gh} (23.65)	28.38 ^{fg} (32.18)	21.06 ^{e} (27.31)	1.88 ^{d} (7.88)
IIHR_MIFY01	2.06 ^{i} (8.25)	11.17 ^{h} (19.51)	4.01 ^{f} (11.55)	6.40 ^{d} (14.65)
IIHR_GSPB03	4.52 ^{i} (12.27)	36.83 ^{defg} (37.35)	28.11 ^{e} (32.01)	24.33 ^{c} (29.54)

	Endophyte(E)	Days(D)	E×D
SEm±	2.17	0.99	4.35
CD@ 1%	6.08	2.72	12.16

Values are the mean of three replications, Figures in parenthesis are arc-sine transformed.
Values followed by the same alphabets within the column do not differ significantly at P < 0.05



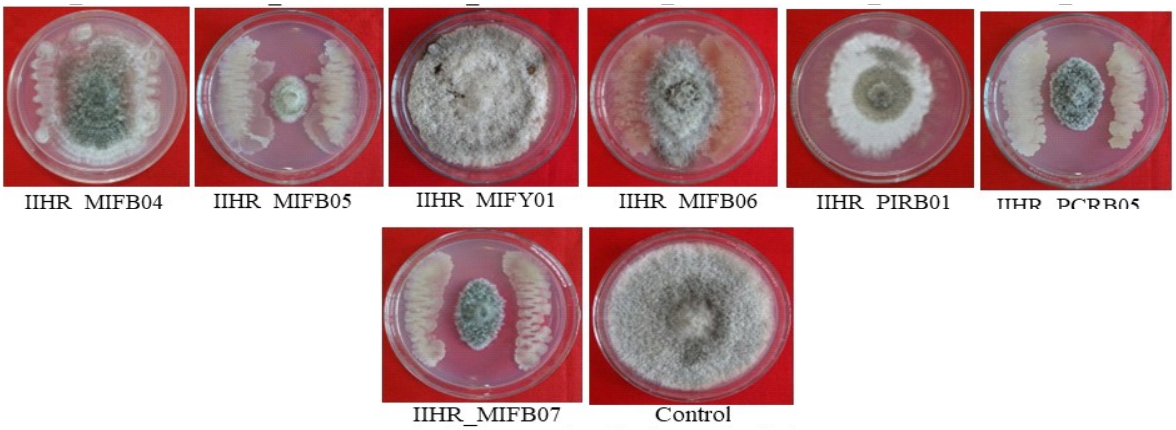


Fig. 1 : *In vitro* antifungal activity of epiphyte/ endophytes against *C. asianum* after 12 days of incubation by dual culture assay on PDA

at 52.52 per cent, followed by IIHR_GSPB03 (*B. velezensis*, 50.13%) and IIHR_GGRB08 (*P. polymixa*, 47.02%). Conversely, IIHR_GIPB03 (*L. fusiformis*, 6.03%) and IIHR_MIFB04 (*B. amyloliquefaciens*, 6.05%) showed the least growth inhibition. The overall inhibition percentage ranged from 6.03 to 52.52 per cent. Among the yeast isolates, IIHR_MIFY01 (*H. opuntiae*) and IIHR_GIFY01 (*H. opuntiae*) recorded inhibition percentages of 16.72 and 18.99 per cent, respectively (Table 3, Fig. 2).

Among the isolates tested, only one isolate (IIHR_MIFB03, *B. siamense*) was categorized on scale 3 (50 to 75%), while nine isolates each were placed on scale 2 (25 to 50%) and scale 1 (1 to 25%), respectively (Table 4).

The living organisms can be used as biocontrol agents to suppress or inhibit the growth, infection or reproduction of another organism. Disease control by exploiting antagonist organisms has become a viable disease management strategy. (Pertot *et al*., 2017).

TABLE 3

Percentage inhibition of bacterial and yeast isolates against *C. siamense* using dual culture assay

Endophyte / Epiphyte	Per cent inhibition of *C. siamense*			
	3 DAI	6 DAI	9 DAI	12 DAI
IIHR_PCRB05	31.03 ^{bcd}	31.15 ^{bcd}	29.31 ^{ABC}	29.75 ^{cd}
	(33.84)	(33.92)	(32.77)	(33.04)
IIHR_GGRB08	31.77 ^{bc}	31.94 ^{bcd}	39.48 ^{a}	47.02 ^{ab}
	(34.29)	(34.40)	(38.91)	(43.27)
IIHR_GBRB09	19.80 ^{defg}	27.54 ^{cd}	22.16 ^{cde}	27.66 ^{cd}
	(26.41)	(31.64)	(28.07)	(31.71)
IIHR_GKRB07	18.49 ^{efgh}	13.04 ^{efg}	13.56 ^{ef}	23.03 ^{def}
	(25.45)	(21.16)	(21.60)	(26.66)
IIHR_PIRB01	7.63 ^{hi}	17.91 ^{e}	24.30 ^{cd}	22.84 ^{def}
	(16.03)	(25.02)	(29.52)	(28.53)
IIHR_GGRB12	25.55 ^{cdefg}	30.87 ^{bed}	36.57 ^{a}	43.30 ^{b}
	(30.05)	(33.74)	(37.20)	(41.13)
IIHR_GIFYO1	15.41 ^{fghi}	12.16 ^{efg}	14.45 ^{ef}	18.99 ^{ef}
	(23.11)	(20.40)	(22.34)	(25.82)

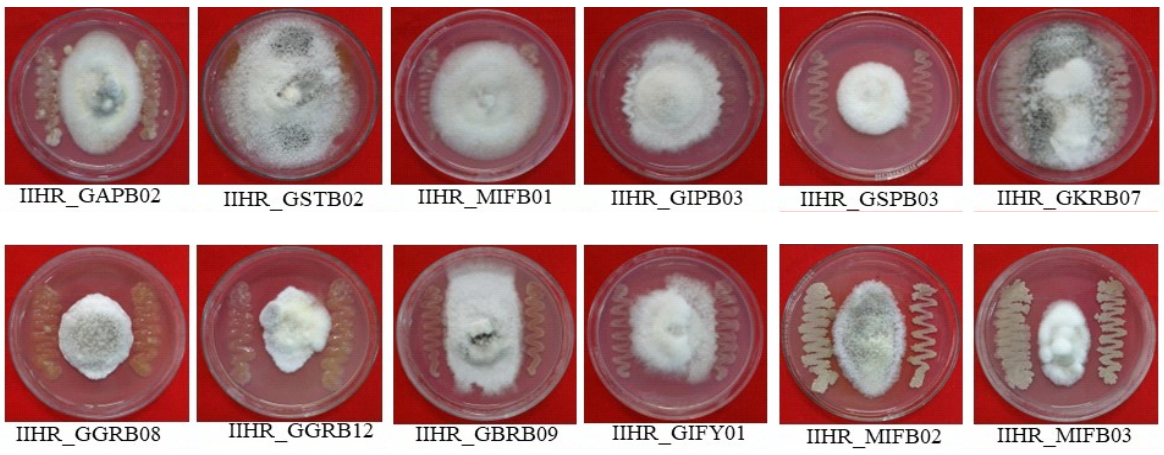
Continued....

TABLE 3 Continued....

Endophyte / Epiphyte	Per cent inhibition of *C. siamense*			
	3 DAI	6 DAI	9 DAI	12 DAI
IIHR_GAPB02	31.10 bcd	27.02 d	27.81 bc	31.26 cd
	(33.88)	(31.31)	(31.81)	(33.98)
IIHR_GIPB03	13.90 ghi	6.16 fg	2.94 g	6.03 g
	(21.89)	(14.36)	(9.86)	(14.21)
IIHR_GSTB02	27.23 bcde	14.54 ef	8.12 fg	18.05 ef
	(31.44)	(22.40)	(16.55)	(25.13)
IIHR_MIFB05	37.25 ab	42.89 a	36.20 ab	41.24 b
	(37.60)	(40.90)	(36.97)	(39.93)
IIHR_MIFB02	37.90 ab	36.91 abc	38.90 a	43.44 b
	(37.98)	(37.40)	(38.57)	(41.21)
IIHR_MIFB03	22.14 cdefg	44.10 a	44.38 a	52.52 a
	(38.06)	(41.60)	(41.75)	(46.42)
IIHR_MIFB04	5.88 i	4.99 g	3.16 g	6.05 g
	(14.02)	(12.91)	(10.24)	(14.23)
IIHR_MIFB07	18.02 efgh	17.77 e	16.76 def	16.49 f
	(25.11)	(24.93)	(24.16)	(23.95)
IIHR_MIFB06	46.40 a	34.76 abcd	28.97 abc	28.82 cd
	(45.92)	(36.11)	(32.55)	(32.45)
IIHR_MIFB01	30.39 bcd	15.93 e	17.89 ed	23.69 cd
	(33.44)	(23.52)	(25.01)	(29.11)
IIHR_MIFY01	12.99 ghi	10.11 efg	9.04 fg	16.72 f
	(21.11)	(18.53)	(17.49)	(24.12)
IIHR_GSPB03	22.86 cdefg	37.75 ab	43.19 a	50.13 a
	(28.54)	(37.89)	(41.07)	(45.05)

	Endophyte(E)	Days(D)	E×D
SEm±	1.42	0.65	2.84
CD@ 1%	3.98	1.82	7.96

Values are the mean of three replications, Figures in parenthesis are arc-sine transformed.
Values followed by the same alphabets within the column do not differ significantly at P < 0.05



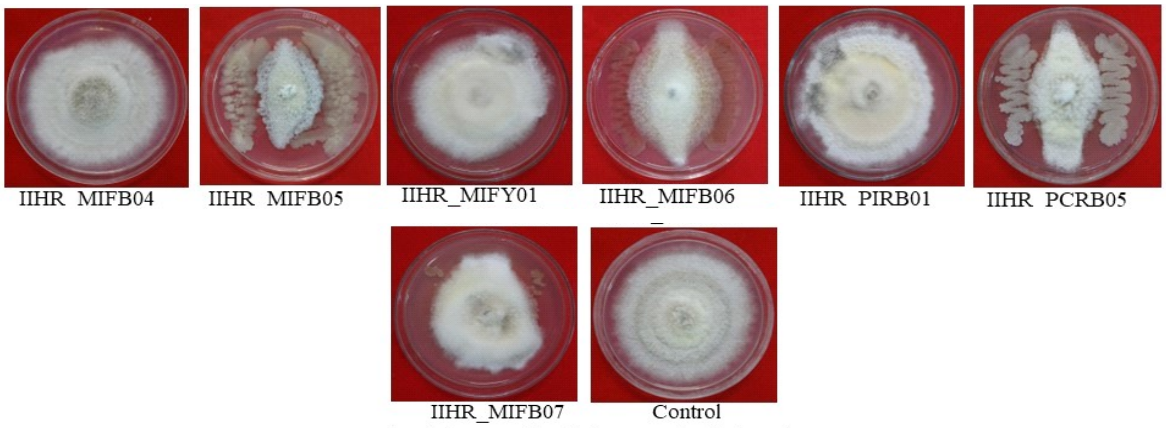


Fig 2. *In vitro* antifungal activity of epiphyte/ endophytes against *C. siamense* after 12 days of incubation by dual culture assay on PDA

TABLE 4

Growth inhibition category upon screening of Epiphyte/ endophytes against mango anthracnose

Isolates	Growth inhibition category	
	C. asianum	*C. siamense*
IIHR_PCRB05	3	2
IIHR_GGRB08	3	2
IIHR_GBRB09	2	2
IIHR_GKRB07	2	1
IIHR_PIRB01	2	1
IIHR_GGRB12	3	2
IIHR_GIFYO1	1	1
IIHR_GAPB02	2	2
IIHR_GIPB03	1	1
IIHR_GSTB02	1	1
IIHR_MIFB05	3	2
IIHR_MIFB02	3	2
IIHR_MIFB03	3	3
IIHR_MIFB04	1	1
IIHR_MIFB07	3	1
IIHR_MIFB06	1	2
IIHR_MIFB01	1	1
IIHR_MIFY01	1	1
IIHR_GSPB03	1	2

Values were categorized on a scale from 0 to 4, where 0 = No growth inhibition 1 = 1 to 25 %, 2 = 26 to 50 %, 3 = 51 to 75 % and 4 =76 to 100%

The report on the use of epiphytes against mango anthracnose (*C. asianum* and *C. siamense*) is very sparse. Epiphytic *Bacillus* spp. such as *B. subtilis*, *B. siamense and B. velezensis* obtained from mango were found effective against tested pathogens compared to other epiphytes, endophytes and yeast isolated from pomegranate, guava and grapes. Introducing unfamiliar antagonist microorganisms to a host can lead to unwanted consequences, including complications arising from the compatibility between biocontrol agents and the host. Therefore, utilizing native antagonist microorganisms as the primary source of biocontrol agents is a safer and more dependable approach.

B. subtilis inhibited the mycelial growth of *C. asianum* and *C. siamense* by 61.76 and 41.24 per cent, respectively. The results were found to be similar to Xu *et al*. (2020), who reported that *B. subtilis* 1-L-29 from *Camellia oleifera* that inhibited *C. fructicola*, *C. siamense*, *C. asianum*, *Fusarium proliferatum*, *A. camellia* and *Pseudomonas syringae*. *B. subtilis*, isolated from the rhizosphere of chilli, showed high antagonistic activity against *C. gloeosporioides* whereas, the mutant strain lacked both antagonistic and hydrolytic activity (Ashwini and Srividya, 2014). Duangkaew and Monkhung (2021) reported that in mango, *B. subtilis* inhibited the mycelial growth of *Colletotrichum* spp. and *Pestalotiopsis* sp. by 49.31 per cent and 42.55 per cent, respectively. *B. subtilis* GYUN-2311 (GYUN-2311), isolated from the

rhizosphere soil of an apple orchard, exhibited antagonistic activity against eight *Colletotrichum* species. The culture filtrate obtained from *B. subtilis*, also inhibited germination and appressorium formation in *C. siamense* and *C. acutatum.* It also exhibited plant growth-promoting (PGP) activity, lytic enzyme activity, siderophore production and the ability to solubilize insoluble phosphate.

B. siamense, an epiphyte from mango exhibited the highest mycelial growth inhibition of *C. asianum* and *C. siamense* by 61.32 and 52.52 per cent respectively, whereas, *P. polymyxa* exhibited 47.02 per cent mycelial growth inhibition against *C. siamense*. Earlier Suprapta *et al*. (2020), isolated 1040 rhizobacteria isolates, out of which ten isolates inhibited the growth of *C. scovillei* on chilli by more than 80 per cent. Among these isolates, *P. polymxa* and *B. subtilis* possessed inhibitory activity at 94.9 and 94.3 per cent, respectively. Two compounds were detected in the hexane phase of cell-free filtrate *P. polymyxa*, namely, 3-hydroxy-2-butanone and 2, 3-butanediol. These compounds may be responsible for antifungal activity against *C. scovillei*, *P. polymyxa* was also found effective against *Ceratocystis fimbriata* and *F. oxysporum* (Maruti and Sriram, 2021).

Lim *et al*. (2017) reported that *B. velezensis* from roots of Korean ginseng inhibited mycelial growth of *Alternaria panax*, *Botrytis cinerea*, *Colletotrichum coccodes*, *Fusarium oxysporum*, *Magnoporthe oryzae* and *Phytophthora capsici*. Bacillomycin L. and fengycin A, are the two antifungal compounds identified by MS/MS analysis. Similar results were obtained for *C. asianum* and *C. siamense* with 61.12 and 43.44 per cent inhibition, respectively.

H. opuntiae, a yeast obtained from grapes and mango were not effective against *Colletotrichum* spp. It may be because a different mode of action compared to that obtained from the fig. was found effective against *Penicillium expansum*, *B. cinerea*, *Cladosporium cladosporioides* with competition for nutrients and space. (Chen *et al.,* 2020 and Ruiz-Moyano *et al*., 2016). In our earlier study, *H. opuntiae* from mango inhibited post-harvest pathogens of grapes *viz*., *Alternaria alternata*, *C. gloeosporioides* and *P. citrinum* by 60.18, 27.64 and 79.51 per cent, respectively (Patil *et al*., 2022). However, it was not effective against mango anthracnose pathogen because the mode of action against post-harvest pathogens of grapes was through VOC production (unpublished data). The other yeasts such as *Metschnikowia pulcherrima*, *Debaryomyces nepalensis* obtained from mango were found effective against *C. gloeosporioides* with different modes of action such as competition for nutrients and space, mycoparasitism and secretion of defense-related enzymes (Tian *et al*., 2018 and Zhou *et al*., 2018).

Bacillus species, which are considered good sources of novel molecules with anti-microbial activity, produce well-known substances such as bacitracin, bacteriocins and antimicrobial lipopeptides. They also produce volatile organic compounds (VOCs), some of which promote plant growth and/or activate plant defense mechanisms by triggering systemic resistance. (Xu *et al*., 2020). In addition, they also produce indole acetic acid, solubilize phosphate, can grow on N-free media and produce siderophores. This study revealed that antagonistic bacteria can be exploited as a choice to manage anthracnose disease by reducing the chemical residues in agricultural production.

In conclusion, the epiphytic *B. subtilis*, *B. siamense* and *B. vezelensis* from mango and endophyte *P. polymyxa* from guava have shown potential antifungal activity against *C. asianum* and *C. siamense* infecting mango. Further study on their antifungal mechanism and *in vivo* disease suppression could provide more information for commercial application.

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