Mysore J. Agric. Sci., 56 (4) : 277-288 (2022)

Evaluation of Botanicals and Bio-agents against *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. Causing Anthracnose of Mango

MIRWAIS NIAZI, K. KARUNA, Y. M. SOMASHEKAR AND H. S. PADMASHRI
Department of Plant Pathology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
e-Mail : mirwaisniazi797@gmail.com

AUTHORS CONTRIBUTION

MIRWAIS NIAZI : Conceptualization, original draft preparation & analysis;
K. KARUNA : Conceptualization, supervision and draft correction;
Y. M. SOMASHEKAR : Supervision, draft correction;
H. S. PADMASHRI : Data analysis.

Corresponding Author :
K. KARUNA
Directorate of Research
UAS, GKVK, Bengaluru

Received : August 2022
Accepted : October 2022

ABSTRACT

Mango (*Mangifera indica* L.) crop suffers from many diseases, among them anthracnose caused by *Colletotrichum gloeosporioides (*Penz.) Penz. and Sacc. is a major threat to mango production. Antagonistic microorganisms and plant derivatives as postharvest treatments are gaining worldwide interest as a supplement to chemical control. An effort made to evaluate different isolates of biocontrol agents *viz., Pseudomonas fluorescens*, *Bacillus subtilis, Bacillus megatherium, Trichoderma harzianum* and *Trichoderma viride,* botanicals and endophytes against *C. gloeosporioides* causing anthracnose of mango, revealed that among the bacterial bioagents evaluated, *P. fluorescens* [D] isolate from Dharwad was found to be effective in inhibiting the mycelial growth of *C. gloeosporioides* to the maximum extent, as the mycelial growth recorded was least (16.67mm, 77.59 per cent inhibition). Among the different *Trichoderma* isolates, *T. harzianum* Th-55 was found to be highly effective with least mycelial growth (19.17mm, 72.11 per cent inhibition). The mycelial growth was least (8.00 mm) in garlic extract at 20 per cent which was statistically significant over all other botanicals tested. Among the endophytes, the pepper grey color isolate of endophytic bacteria exhibited maximum inhibition (56.42 per cent) and restricted the radial growth of mycelium to 33.50mm.

Keywords : Mango, *Anthracnose,* Post- harvest management, Botanicals, Bacterial bio-agents, *Trichoderma* sp., Endophytes

MANGO (*Mangifera indica* L.) is an important fruit crop of India and other tropical and subtropical countries of the world. Mango is affected by many biotic and abiotic stresses which affect its productivity greatly at all the stages of its development right from plant in nursery to the fruit in storage or transit. The fruit yield of mango is very low in many countries due to various reasons, such as pre-harvest diseases caused by fungi, viruses, bacteria and nematodes (Chowdhury and Rahim, 2009). Mango is prone to fungal diseases like anthracnose, *Rhizopus* rot, stem end rot, *Penicillum* rot, black mould rot, *Mucor* rot, *Pestalotia* rot and powdery mildew, leading to heavy losses in yield (Ploetz, 2002). Among these diseases, anthracnose is the major disease of mango as it occurs on all the growing parts including leaves, twigs, flowers, fruits except root and trunk throughout the year. Anthracnose caused by the fungus *Colletotrichum gloeosporioides* is also known by the name of its prefect stage as *Glomerella cingulata* is the most important disease of mango worldwide (Akem, 2006). It is the major pre and post-harvest disease of the fruit in all mango producing areas of the world (Ploetz and Prakash, 1997).

The symptoms of anthracnose on leaves emerge as irregular shaped black necrotic spots on both the sides. Lesions repeatedly coalesce and form large necrotic areas, commonly along the leaf margins. Blossom blight or panicle anthracnose can affect both the individual flowers and inflorescence stalk. Elongated dark grey to black lesions appear on the stalk. Blighted

The Mysore Journal of Agricultural Sciences

flowers dry and fruits smaller than pea-size can be infected and aborted. Fruit stalks also get infected leading to the fruit drop at various stages. Post-harvest anthracnose appears as rounded brown to black lesions with an unclear border on the fruit surface. Lesions of different size coalesce and cover broad areas of the fruit. Lesions are usually limited to the peel, but in severe cases the fungus attacks the pulp also. In advanced stages of the disease, the fungus produces acervuli and abundant orange to salmon pink masses of conidia on the lesions (Arauz, 2000).

Use of antagonistic microorganisms and plant derivatives as postharvest treatments are gaining worldwide interest as an alternative or as supplement for the existing pesticides. These products will help in reducing the cost, environmental hazards and development of resistance by the pathogens.

Considering the importance of the study of postharvest anthracnose, problems associated with chemical control and advantages of biological control methods, an effort was made to evaluate different biocontrol agents, botanicals and endophytes against *C. gloeosporioides*.

Material and Methods

Collection of Diseased Samples

The diseased samples of mango fruit showing typical symptoms were collected from mango orchard of Department of Horticulture, College of Agriculture, University of Agricultural Sciences, GKVK, Bengaluru.

Isolation Purification and Identification of the Pathogen

The pathogen causing anthracnose disease in mango was isolated from diseased fruit samples. The infected tissue bits were separated with a sterile blade and surface sterilized with –one per cent sodium hypochlorite solution for one min. and subsequently washed three times with sterile distilled water. Then they were transferred into a sterile petri dish containing Potato Dextrose Agar (PDA) medium (Ainsworth, 1971) amended with streptomycin under laminar air flow. The plates were then incubated at room temperature (28 ± 2°C). The emerging colonies were sub cultured on to PDA slants. Single hyphal tip method was followed for making pure culture and maintained on PDA slants (Aneja, 2003). The identity of isolate was confirmed by microscopic observations based on morphological characteristics as per the key suggested by Barnett *et al.* (1972).

Effect of Antagonistic Bacteria on the Radial Growth of *C. gloeosporioides in vitro*

Over the past few decades, biological control has emerged as an effective strategy to combat decay of fruits. Plant growth promoting rhizobacteria (PGPR) especially *Pseudomonas fluorescens* and *Bacillus subtilis* are promising candidates as bio protectants. The present study was undertaken to evaluate the bacterial bio agents as alternative method to suppress the anthracnose pathogen *in vitro.*

The antagonistic activity of bacterial biocontrol agents *(P. fluorescens*-4 isolates and *B. subtilis*-2 isolates) obtained from Department of Plant Pathology, against *C. gloeosporioides* was tested by dual culture technique (Dennis and Webster, 1971) using PDA medium. In order to get fresh and active growing bacterial bio agents as well as test organism, they were cultured on potato dextrose agar medium. Twenty ml of sterilized and cooled potato dextrose agar medium was poured into sterilized petri plates. After solidification, 5 mm mycelial disc obtained from seven days old culture of *C. gloeosporioides* was placed at one end of the Petri plate containing PDA medium under aseptic conditions. Similarly, at the opposite end approximately 75 mm away from the pathogen culture, one cm long streak of the bacterial bioagent was gently made on to the medium using 2 days old culture of bacterial antagonists. The plates were incubated at room temperature (28±2°C). Three replications were maintained for each treatment. The radial growth (in mm) of the pathogen was measured at ten days after incubation. A control was maintained by inoculating *C. gloeosporioides* alone at one end of the Petri plate. The effective antagonists were selected based on the inhibition of the growth of pathogen.

The per cent inhibition of mycelial growth was calculated according to Vincent (1947).

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

Where,

I = Percent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

Further, angular transformation was made for the data and analyzed statistically.

Bacterial bio-agents evaluated against *C. gloeosporioides*

Bacterial Bio-agents	Source
Pseudomonas fluorescens- D	Department of Plant Pathology, Dharwad
Pseudomonas fluorescens- C-1	Department of Plant Pathology, Chintamani
Pseudomonas fluorescens- C	Department of Plant Pathology, Chintamani
Pseudomonas fluorescens- C-2	Department of Plant Pathology, Chintamani
Bacillus subtilis	Department of Plant Pathology, Dharwad
Bacillus megatherium	Department of Plant Pathology, Dharwad

Effect of Isolates of *Trichoderma* sp. on the Radial Growth of *C. gloeosporioides in vitro*

Antagonistic activities of five isolates of two *Trichoderma* sp. *viz., Trichoderma harzianum* -56, *Trichoderma harzianum* -14 , *Trichoderma viride* -1, *Trichoderma viride* -3 and *Trichoderma harzianum* - 55 collected from IIHR, Bengaluru were evaluated against the mycelial growth of *C. gloeosporioides* by adopting dual culture method on PDA medium *in vitro*.

The antagonistic activity of *Trichoderma* sp. against *C. gloeosporioides* was tested by dual culture technique using PDA medium. In order to get fresh and active growing *Trichoderma* sp. as well as test organism, they were cultured on potato dextrose agar medium. Twenty ml of sterilized and cooled potato dextrose agar medium was poured into sterilized petri plates. After solidification, 5 mm mycelial disc obtained from seven days old culture of *C. gloeosporioides* was placed one cm away from the periphery of petri dish containing PDA medium under aseptic conditions and a same sized agar disc of seven days old *Trichoderma* cultures were placed one cm away from the edge of the same petri plate containing PDA on the opposite side of *C. gloeosporioides*. The plates were incubated at room temperature (28 ± 2°C). Three replications were maintained for each treatment. A control was maintained by inoculating *C. gloeosporioides* alone at one end of the petri plate. The radial growth (in mm) of the pathogen was measured at ten days after incubation. The effective antagonists were selected based on the inhibition of the growth of pathogen.

The per cent inhibition of mycelial growth was calculated according to Vincent (1947).

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

Where,

I = Percent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

Trichoderma* sp. evaluated against *C. gloeosporioides

Fungal Bio- agents	Code number	Source
Trichoderma harzianum -56	Th-56	IIHR, Bengaluru
Trichoderma harzianum-14	Th-14	IIHR, Bengaluru
Trichoderma viride - 1	Tv-1	IIHR, Bengaluru
Trichoderma viride - 3	Tv-3	IIHR, Bengaluru
Trichoderma harzianum -55	Th -55	IIHR, Bengaluru

Effect of Botanicals on the Mycelial Growth of *C. gloeosporioides* under *in vitro*

Botanicals which are relatively economical, safe and non-hazardous can be used successfully against the plant pathogenic fungi. The present investigation was aimed to know the antifungal activity of some aqueous plant extracts against *C. gloeosporioides* using poisoned food technique.

Fresh plants parts (leaves and bulbs) of 100 g each as mentioned in the table were collected and washed in distilled water and leaves/bulbs were grinded into fine paste and mixed in sterile distilled water in the ratio 1:100 w/v. The suspensions were left to stand for 24 hours at room temperature and then filtered through double layer of cheese cloth, centrifuge at 5000 rpm for 10 min. The filtered solution gave 100 per cent, which was used as a stock solution. Ten, fifteen, and twenty ml of stock solution was mixed with 90, 85 and 80 ml of PDA medium and then it was shaken for uniform mixing of plant extracts. Later, the media was sterilized and allowed to cool. Twenty ml of medium was poured into sterilized Petriplates and allowed to solidify. At the center of the Petriplate 5 mm fungal disc of *C. gloeosporioides* was placed using cork borer and such plates were incubated at 28±2°C. Three replications were maintained for each treatment. The control plate was maintained on PDA medium without any plant extract. The radial growth of fungus was recorded at ten days after incubation. The per cent inhibition of mycelial growth of test fungus was calculated by using the formula suggested by Vincent (1947).

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

Where,

I = Percent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

Effect of Endophytes on the Radial Growth of *C. gloeosporioides in vitro*

Endophytic microbes are microorganisms that live in the intercellular spaces of plant for most if not all their life cycles with no pathogenic effects on their hosts. Endophytes may share the same habitat of phytopathogens and develop a key role in plants defense.

Isolation of Endophytic Bacteria

Eleven sub-epidermis micro-organisms were isolated from various horticultural crops (cucumber, eggplant, pepper, tomato, zucchini, orange, lemon and plum) on nutrient agar. The sample part fruit from different plant were cut separately and washed with water. The samples were then sterilized with 70 per cent alcohol for one minute, soaked in 1.25 per cent NaOCl for 3 minutes, washed with 70 per cent alcohol three times, and rinsed with sterile water for three times, then dried on sterile tissue. Sterile fruits were crushed separately with mortar and dissolved with sterile water until dissolved. One ml of sample solution was diluted by adding 9 ml of physiological solution (0.85% NaCl) until 10^4 dilutions. A total of 0.1 ml of extract solution from 10^3 and 10^4 dilutions were spread on Nutrient Agar (NA) media and incubated at room temperature @ 27^0C for 48 hours. The colonies were identified based on colony characters.

Plant extracts used for *in vitro* evaluation of *C. gloeosporioides*

Scientific name	Scientific name	Family	Part used
Allium sativum L.	Garlic	Amaryllidaceae	Rhizome
Allium cepa L.	Onion	Amaryllidaceae	Rhizome
Zingiber officinale	Ginger	Zingiberaceae	Rhizome
Curcuma longa	Turmeric	Zingiberaceae	Rhizome
Mentha spicata	Mint	Lamiaceae	Leaf extract
Agave Americana	Agave	Asparagaceae	Leaf extract
Calotropis procera	Calotropis	Apocynaceae	Leaf extract
Gliricidia sepium	Gliricidia	Fabaceae	Leaf extract
Simarouba glauca	Simarouba	Simaroubaceae	Leaf extract
Pongamia pinnata L.	Pongamia	Fabaceae	Leaf extract

In vitro screening of endophytic bacteria against *C. gloeosporioides*

In vitro testing of the inhibitory ability of endophytic bacteria against *C. gloeosporioides* was carried out through dual culture technique. Five mm mycelial disc obtained from seven days old culture of *C. gloeosporioides* was placed one centimeter away from the periphery of petri dish containing PDA medium under aseptic conditions and a same sized agar disc of seven days old endophyte cultures was placed one centimeter away from the edge of the same petri plate containing PDA on the opposite side of *C. gloeosporioides*. The plates were incubated at room temperature (28±2°C). Three replications were maintained for each treatment. A control was maintained by inoculating *C. gloeosporioides* alone at one end of the petri plate. The radial growth (in mm) of the pathogen was measured at ten days after incubation. The effective endophytes were selected based on the inhibition of the growth of pathogen. The percent inhibition of mycelial growth of test fungus was calculated by using the formula suggested by Vincent (1947).

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

Where,

I = Per cent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

The per cent inhibition data in all the experiments were normalized through Arc sign data transformation technique. Completely randomized design was used in the assay of bacterial, *Trichoderma*, isolates, botanicals and endophytic bacteria against *C. gloeosporioides*. Each treatment was repeated three times. Data were analyzed using the R I 386, 4.0.3 version statistical software. The different antagonists and botanicals were ranked based Fischer's least significant difference test at one per cent level.

Results and Discussion

Collection of Disease Samples

Diseased mango fruits showing typical anthracnose symptoms (Plate 1) were collected from mango orchard at Department of Horticulture, University of Agriculture Sciences, GKVK, Bengaluru. Symptoms initially started as small, round and depressed spots (Plate 1). Later on these spots coalesced to cover large area and looked like rotten patches. Under humid conditions, pinkish spore masses were noticed which are acervuli of the fungus. Similar symptoms were reported by various workers (Bhuvaneshwari, 1999; Shirshikar, 2002 and Hasbnis, 1984).

Plate 1 : Typical Anthracnose symptoms on mango fruits

Isolation Purification and Identification of the Pathogen

The pathogen *C. gloeosporioides* was successfully isolated on PDA culture medium from the infected mango fruits showing typical anthracnose symptoms. Isolation was carried out at the Department of Plant Pathology, College of Agriculture, UAS, GKVK, Bengaluru. The pathogen produced colonies with abundant whitish (Plate 2), septate and hyaline aerial mycelium, slimy pinkish spore masses, acervulate (Plate 3).

Plate 2: Pure culture of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. on PDA

The fungal culture of the pathogen was purified and the pure culture of *C. gloeosporioides* was maintained for carrying out the investigation. The hyphal tip of isolate fungus growing on PDA was cut and shifted to a slant containing PDA as basal medium and kept

The Mysore Journal of Agricultural Sciences

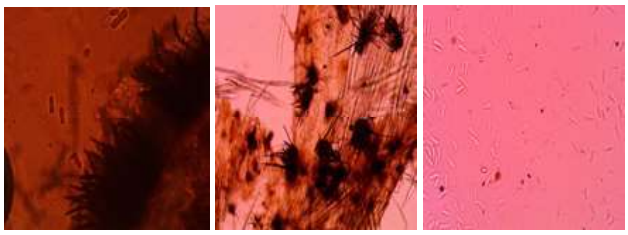

Plate 3: Photographs showing Mycelia, Conidiophore and Conidia (10X)

for incubation. After incubation the slant was transferred to refrigerator and sub-cultured at regular intervals of time.

Twenty milliliter of the liquid medium was poured into the sterilized Petri plates and eight days old culture was cut into five mm discs with the help of sterilized cork borer and transferred under the aseptic condition. Each treatment was replicated thrice, Petri plates were inoculated at 28±1°C for seven days and growth of the colony was recorded in each treatment. (Naik *et al*., 1988).

The fungal culture isolated from diseased mango fruit was identified as *C. gloeosporioides* causing anthracnose disease on the basis of microscopic observation for morphological and conidial characteristics. Conidia were oblong or oval or cylindrical, straight, hyaline, non-septate with rounded ends, thin walled having oil globules in the center.

Isolation and identification of the pathogen during the present investigation are coordinated with view of following reports *viz*., Munjul and Gupta (1965), Agostini *et al*., (1992), Sharma and Verma (2007), Mukherjee *et al*. (2011) who have successfully isolated pathogen *C. gloeosporioides* from mango fruit responsible for anthracnose disease.

Effect of Antagonistic Bacteria on the Radial Growth of *C. gloeosporioides in vitro*

The results of the evaluation of antagonistic bacteria against *C. gloeosporioides* revealed that (Table 1, Fig. 1 and Plate 4) all the bacterial bio-agents evaluated significantly inhibited the mycelial growth of *C. gloeosporioides*.

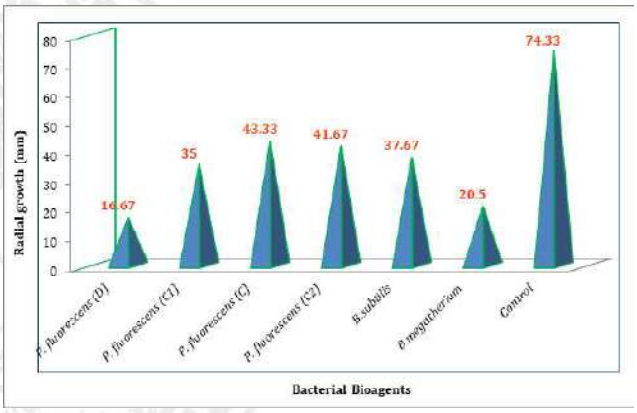

Fig. 1: Effect of Antagonistic Bacteria on the Radial Growth of *C. gloeosporioides in vitro*

TABLE 1

Effect of antagonistic bacteria on the radial growth of *C. gloeosporioides in vitro*

Tr. No.	Bacterial Bio-agents	Isolate and code number	Radial growth (mm)	Inhibition over control (%)	Rank as per CD value
T1	*Pseudomonas fluorescens*	Dharwad - D	16.67	77.59 (61.75)	a
T2	*Pseudomonas fluorescens*	GKVK - C-1	35.00	52.55 (46.46)	b
T3	*Pseudomonas fluorescens*	GKVK- C	43.33	41.79 (40.28)	b
T4	*Pseudomonas fluorescens*	Chintamani - C-2	41.67	43.97 (41.53)	b
T5	*Bacillus subtilis*	GKVK	37.67	49.32 (44.61)	b
T6	*Bacillus megatherium*	Dharwad	20.50	72.52 (58.38)	a
T7	Control		74.33		
	SEm ±			3.13	
	CD @ p= 0.01			15.19	

Figure in Parenthesis indicate Arcsine values

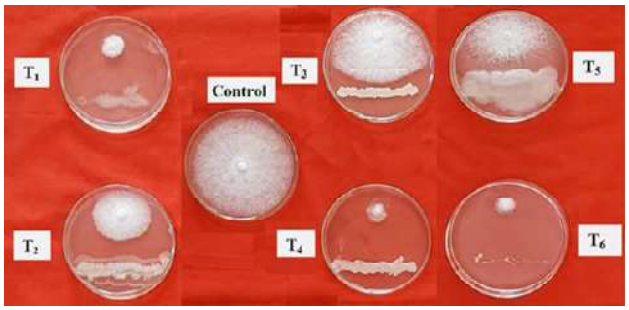

Plate 4: Effect of Antagonistic Bacteria on the Radial Growth of *C. gloeosporioides in vitro*

T1: *Pseudomonas fluorescens*[D] T2: *P. fluorescens*[C-1]
T3: *P. fluorescens*[C] T4: *P. fluorescens* [C-2]
T5: *Bacillus subtilis* [GKVK] T6: *B. megatherium* [D]

Among the bacterial bio-agents evaluated, *P. fluorescens* [D] isolate from Dharwad was found to be effective in inhibiting the mycelial growth of *C. gloeosporioides* to the maximum extent, as the mycelial growth recorded was least (16.67 mm, 77.59 per cent inhibition) and this isolate, which was on par with *B. megatherium* Dharwad isolate in which 20.20mm mycelial growth (75.52 per cent inhibition) was recorded. The mycelial growth with inhibition (%) in *P. fluorescens: C1* (35 mm, 52.55 per cent), *B. subtilis* (37.67 mm, 49.32 per cent), *P. fluorescens*: C2 (41.67 mm, 43.39 per cent) and *P. fluorescens*: C *(*43.33 per cent) isolates were found to be on par with each other, but their efficacy was significantly lower than *P. fluorescens* D and *B. megatherium*. Koomen and Jeffries (1993) reported that *Bacillus cereus* and *P. fluorescens* inhibited *C. gloeosporioides.* Variation in antagonistic efficacy between isolates was reported by several workers (Viswanathan and Samiyappan, 2006; Prabakar *et al.,* 2008; Bardas *et al.,* 2009 and Muhammad *et al.,* 2010). The present findings were also in consonance with those results reported by the Udhayakumar *et al.* (2019) who demonstrated that increase in the concentration of culture filtrate of *P. fluorescens* resulted in enhanced inhibition of the test fungus. Vivekananthan *et al.* (2006) suggested the lytic enzyme induced by *P. fluorescens* and other biocontrol organism mediate defense against the *C. gloeosporioides.*

Effect of Isolates of *Trichoderma* sp. on the Radial Growth of *C. gloeosporioides in vitro*

The effect of *Trichoderma* sp. against *C. gloeosporioides* was evaluated *in vitro* by dual culture technique. Based on the radial growth of the fungus, the per cent inhibition was calculated. The results are presented in the Table 2, Fig. 2 and Plate 5.

Trichoderma isolates were identified based on the morphological characteristics of the fungus such as mycelial growth, colour, shape of conidia, conidiophores (Sudha and Narendrappa, 2015).

Among the *Trichoderma* sp. evaluated against *C. gloeosporioides in vitro, Trichoderma harzianum viz*., Th 55, Th-56 and Th-14 significantly inhibited the growth. The radial growth mycelium of

TABLE 2

Effect of isolates of *Trichoderam* sp. on the radial growth of *C. gloeosporioides in vitro*

Tr. No.	Bacterial Bio-agents	Code number	Radial growth (mm)	Inhibition over control (%)	Rank as per CD value
T1	*Trichoderma harzianum*	Th-56	21.00	70.08 (56.84)	a
T2	*Trichoderma harzianum*	Th-14	20.33	70.32 (56.99)	a
T3	*Trichoderma viride*	Tv-1	35.00	50.67 (45.38)	b
T4	*Trichoderma viride*	Tv-3	38.33	45.33 (42.32)	b
T5	*Trichoderma harzianum*	Th-55	19.17	72.11 (58.13)	a
T6	Control		70.00		
	SEm ±		3.58		
	CD @ p= 0.01		17.83		

Figure in Parenthesis indicate Arcsine values

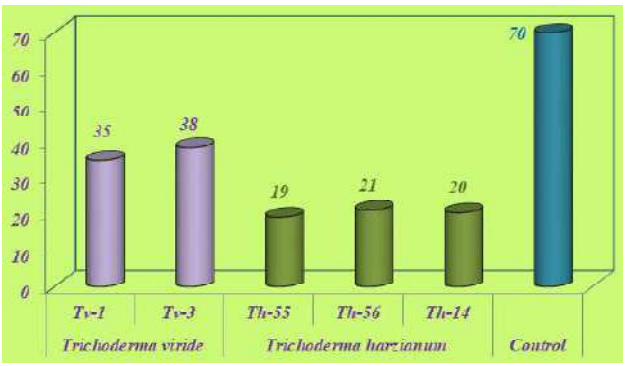

Fig. 2: Effect of isolates of *Trichoderam* sp. on the radial growth of *C. gloeosporioides in vitro*

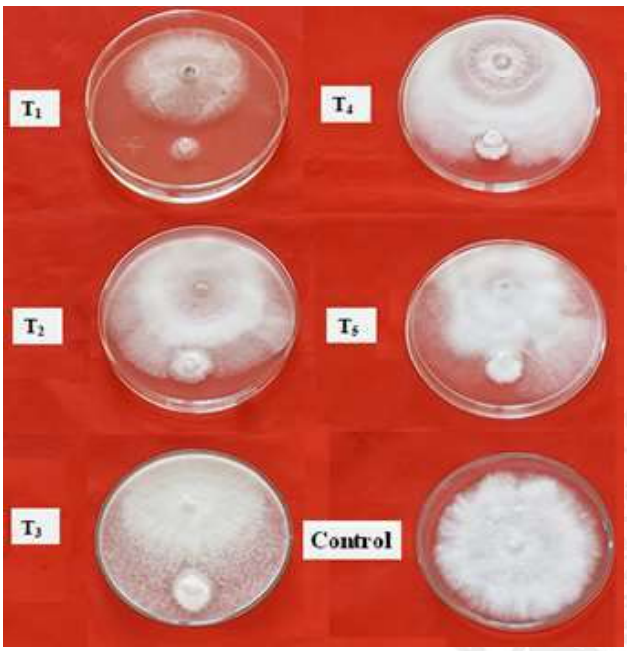

Plate 5: Effect of isolates of *Trichoderma* sp. on the radial growth of *C. gloeosporioides in vitro*

T1: *Trichoderma harzianum* Th-56
T2: *T. harzianum* Th-14
T3: *T. viride* Tv-1
T4: *T. viride* Tv-3
T5: *T. harzianum* Th-55
T6: Control

C. gloeosporioides in the different isolates ranged from 19.17 mm to 38.33 mm, while in control it was 70 mm. Among the different isolates, *T. harzianum* Th-55 was found to be highly effective with least mycelial growth (19.17 mm, 72.11 per cent inhibition) which was on par with *T. harzianum* –Th- 14 (20.33 mm, 70.32 per cent) and *T. harzianum* -Th-56 (21 mm, 70.08 per cent inhibition). While in *T. viride*-Tv-3, 38.33 mm (45.33 per cent inhibition) mycelial growth was observed which was on par with *T. viride*-Tv-1 with 35 mm mycelial growth and 50.67 per cent inhibition. *T. harzianum* -Th-55, *T. harzianum* –Th-14 and *T. harzianum* -Th-56 exhibited significantly higher inhibition of mycelial growth over *T. viride*-Tv-1 and *T.viride*- Tv-3 isolates. Kaur *et al.* (2006) suggested that volatile metabolites produced by *Trichoderma* sp. suppressed the growth of *C. capsici in vitro*. Deshmukh and Raut (1992) reported that *T. harzianum* and *T. viride* overgrew the colonies of *C. gloeosporioides*.

Effect of Botanicals on the Mycelial Growth of *C. gloeosporioides* Under *in vitro*

The antagonistic effect of selected ten botanicals against *C. gloeosporioides* was evaluated *in vitro* by poison food technique. Based on the radial growth of the fungus, the per cent inhibition was calculated. The results are presented in the Table 3, Fig. 3 and Plate 6.

Fig. 3: Effect of botanicals on the mycelial growth of *C. gloeosporioides* under *in vitro*

Plate 6: Effect of botanicals on the mycelial growth of *C. gloeosporioides* under *in vitro*

T1: Garlic
T2: Onion
T3: Ginger
T4: Turmeric
T5: Mint
T6: Agave
T7: Calotrophis
T8: Glyricidia
T9:Simarauba
T10: Pongamia

TABLE 3

Effect of botanicals on the mycelial growth of *C. gloeosporioides* under *in vitro*

Botanicals	Mycelial growth					
	10%	Inhibition over control (%)	15%	Inhibition over control (%)	20%	Inhibition over control (%)
Garlic	23.3	73.18 [bc] (58.81)	18.3	79.01 [b] (62.73)	8.0	90.97 [a] (72.52)
Onion	71.6	17.11 (24.43)	74.0	14.51 (22.39)	65.0	24.66 (29.78)
Ginger	41.3	52.31 (46.32)	45.6	47.21 (43.40)	36.0	58.43 (49.86)
Turmeric	37.3	56.93 (48.99)	35.3	59.23 (50.32)	32.6	62.29 (52.12)
Mint	71.0	17.64 (24.84)	67.3	20.78 (27.12)	54.0	37.60 (37.82)
Agave	36.3	58.07 (49.64)	37.6	56.47 (48.72)	33.0	61.90 (51.88)
Calotrophis	46.3	46.47 (42.98)	43.0	50.34 (45.20)	39.6	54.15 (47.38)
Glyricidia	29.0	66.51 [c] (54.64)	28.3	67.33 [c] (55.14)	21.0	75.87 [bc] (60.58)
Simarauba	79.6	7.84 (16.27)	74.3	13.50 (21.56)	69.6	18.35 (25.36)
Pongamia	60.6	29.32 (32.79)	61.6	28.64 (32.36)	52.3	39.35 (38.85)
Control	86.0		86.0		86.0	
SEm ±		2.51		CD @ 1% level		10.31

Figure in Parenthesis indicate Arcsine values

The results revealed that in all the botanicals tested at all concentrations the radial growth of mycelium of *C. gloeosporioides* was significantly inhibited. The mycelial growth in the different botanicals tested ranged from 8.00 mm in Garlic at 20 per cent to 79.60 mm in simarauba at 10 per cent with 90.97 per cent and 7.84 per cent inhibition in mycelial growth, while in control the mycelial growth was 86.00 mm. The mycelial growth was least (8.00 mm) in garlic extract at 20 per cent which was statistically significant over all other botanicals tested. Mycelial growth in garlic extract at 15 per cent was 18.30 mm (79.01 per cent inhibition) which on par with Garlic at 10 per cent (23.30 mm, 73.18 per cent) and glyricidia at 20 per cent (21.00 mm, 75.87 per cent). While, the mycelial growth in Glyricidia at all the concentrations tested (10 per cent -29.00 mm, 15 per cent -28.30 mm and 20 per cent -21.00 mm) were on par with garlic at 10 percent (23.30 mm). Simarauba, mint, pongamia and onion were less effective in inhibiting the mycelial growth when compared to ginger, turmeric and calotrophis. No significant difference in the mycelial growth in different concentration exists among the botanicals *viz.,* simarauba, mint, pongamia, onion, ginger, turmeric and calotrophis tested. The results are in consensus with Kota (2003) who found that garlic bulb extract at 10 per cent concentration was found to be most effective in inhibiting mycelial growth (68.91 per cent), Mukherjee *et al.* (2011) who reported the highest percentage inhibition (74.35 per cent) of *C. gloeosporioides* under *in vitro* in garlic extract at 70 per cent concentration and Gahlot *et al*. (2021) who found that among all the phytoextracts evaluated under *in vitro* against *C. gloeosporioides* garlic clove was spotted most effective at all the three concentrations (5, 10 and 15 per cent) with 100 per cent inhibition in mycelial growth.

Effect of Endophytic Bacteria Isolated from Various Horticultural Sources on the Radial Growth of *C. gloeosporioides in vitro*

Based on the colony characters on nutrient agar medium the isolated microorganisms were identified as bacteria. The results [Table 4, Fig. 4 and Plate 7] of antagonistic effect of the eleven strains of micro-

TABLE 4

Effect of endophytes on the radial growth of *C. gloeosporioides in vitro*

Tr. No.	Endophytes bacteria	Radial growth (mm)	Inhibition over control (%)	Rank as per CD value
T1	Zucchini gray colour	58.67	23.29 (28.85)	d
T2	Zucchini yellow colour	55.00	28.17 (32.06)	d
T3	Cucumber pink colour	53.33	30.38 (33.45)	d
T4	Pepper gray colour	33.50	56.42 (48.69)	a
T5	Cucumber yellow colour	50.00	34.59 (36.03)	d
T6	pepper yellow colour	39.23	48.82 (44.32)	b
T7	Eggplant	40.67	47.01 (43.28)	b, c
T8	Plum	53.17	30.59 (33.58)	d
T9	Tomato	52.50	31.57 (34.12)	d
T10	Lemon	49.17	35.60 (36.63)	d
T11	Orange	45.83	40.12 (39.30)	c
T12	Control	76.67	-	
SEm ±			1.82	
CD @ p=0.01			8.03	

Figure in Parenthesis indicate Arcsine values

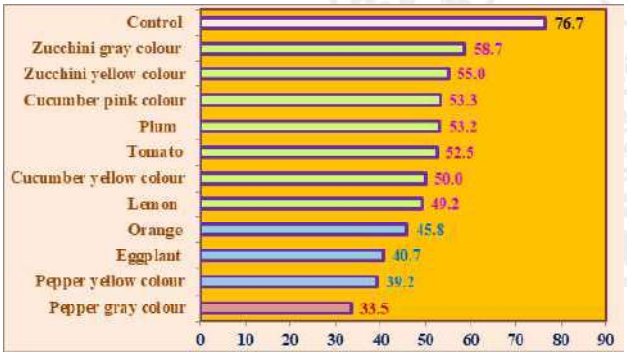

Fig. 4: Effect of endophytes on the radial growth of *C. gloeosporioides in vitro*

organisms isolated from different horticultural sources using dual culture method indicated by a clear zone of inhibition showed that all the isolates evaluated inhibited the growth of *C. gloeosporioides in vitro.* The radial growth of the mycelium in the eleven isolates of micro-organisms ranged from 33.50 to 58.67 mm, while in control it was 76.67 mm. The pepper gray color isolate exhibited maximum inhibition (56.42 per cent) and restricted the radial growth of mycelium to 33.50 mm. The radial growth of mycelium in pepper yellow colour was 39.23 mm (48.82 per cent inhibition) which was par with eggplant (40.67 mm radial growth, 47.01 per cent inhibition). Zucchini gray colour, zucchini yellow colour, cucumber pink colour, cucumber yellow colour, pepper yellow colour, plum, tomato and lemon were on par among themselves with radial growth of mycelium in different isolates ranging between 49.17 mm to 58.67 mm with 23.29 to 35.60 per cent inhibition, but less effective than other orange (45.83 mm, 40.12 per cent inhibition). The results are supported by Khaeruni *et al*. (2019) who reported 8 isolates of endophytic bacteria having very strong antagonistic activity with more than 50 per cent inhibitory effect on *C. gloeosporioides* CDKW01 causing anthracnose disease in cocoa and twelve isolates that could potentially inhibit the growth of fungus *Colletotrichum* sp. Murtado *et al.* (2020) who found that out of a total of 40 isolates endophytic bacterial isolated from healthy and diseased onion plant from different locations and plant parts, Six isolates of endophytic bacteria had the greatest ability

Plate 7: Effect of endophytes on the radial growth of C. *gloeosporioides in vitro*

T1: Zucchini gray colour T2: Zucchini yellow colour
T3: Cucumber pink colour T4: Pepper gray colour
T5: Cucumber yellow colour T6: Pepper yellow colour
T7: Eggplant T8: Plum
T9: Tomato T10:Lemon
T11: Orange

to inhibit the growth of the suspected pathogenic fungi of *Colletotrichum* sp.

Among the bacterial bioagents evaluated, *P. fluorescens* [D] isolate from Dharwad was found to be effective in inhibiting the mycelial growth of *C. gloeosporioides* to the maximum extent, as the mycelial growth recorded was least (16.67 mm, 77.59 per cent inhibition). *T. harzianum* Th-55 was found to be highly effective with least mycelial growth (19.17 mm, 72.11 per cent inhibition). In *Trichoderma viride*-Tv-3, 38.33 mm (45.33 per cent inhibition) mycelial growth was observed which was on par with *Trichoderma viride*- Tv-1 with 35 mm mycelial growth and 50.67 per cent inhibition. The mycelial growth was least (8.00 mm) in garlic extract at 20 per cent which was statistically significant over all other botanicals tested. Among the endophyte, the pepper gray color isolate of endophytic bacteria exhibited maximum inhibition (56.42 per cent) and restricted the radial growth of mycelium to 33.50 mm.

References

AGOSTINI, J. P., TIMMER, L. W. AND MITCHELL, D. J., 1992, Morphological and pathological characteristics of strains of *Colletotrichum gloeosporioides* from citrus. *Phytopathol.*, **82** (11) : 1377 - 1382.

AINSWORTH, G. C., 1971, Dictionary of fungi.VI Edn, Common Wealth *Mycological Institute*, Kew, Survey, England, pp. : 631.

AKEM, C. N., 2006, Mango anthracnose disease: Present status and future research priorities. *Plant Pathol. J.*, **5** (3) : 266 - 273.

ANEJA, K. R., 2003, Experiments in microbiology plant pathology and biotechnology, 4th ed., New Age *International Publishers*, New Delhi.

ARAUZ, L. F., 2000, Mango anthracnose : Economic impact and current options for integrated management. *Plant Dis*., **84** (6) : 600 - 611.

BARDAS, G. A., LAGOPODI, A. L., KODOGLIDOU, K. AND TZAVELLA-KLONARI, K., 2009, Biocontrol of three *Colletotrichum lindemuthianum* traces using *Pseudomonas chlororaphis* PCL 1391 and *Pseudomonas fluorescens* WCS365. *Biol. Contr*., **49** : 139 - 145.

BARNETT, H. L., BARRY, B. AND HUNTER, H., 1972, Illustrated genera of imperfect fungi. *Burgeos Publishing Company*, Minnesota.

BHUVANESWARI, V., 1999, Studies on postharvest diseases of mango. *M.Sc. (Agri.) Thesis*, Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad.

CHOWDHURY, M. N. A. AND RAHIM, M. A., 2009, Integrated crop management to control anthracnose (*Colletotrichum gloeosporioides*) of Mango. *J. Agric. Rural Dev*., **7** (1&2) : 115 - 120.

DENNIS, C. AND WEBSTER, J., 1971, Antagonistic properties of species-groups of *Trichoderma*. I. Production of non-volatile antibiotics. *Trans. Brit. Mycol. Soc.*; **57** : 25 - 39.

DESHMUKH, P. P. AND RAUT, J. G., 1992, Antagonism by *Trichoderma* spp. on five plant pathogenic fungi. *New Agriculturist*, **3** : 127 - 130.

The Mysore Journal of Agricultural Sciences

GAHLOT, D., LAL, M. N., TRIVEDI, A. AND KUMAR, S., 2021, *in vitro* efficacy of fungicides and phyto-extracts against *Colletotrichum gloeosporioides* causing Anthracnose of Mango. *Ann. Plant Prot. Sci.,* **29** (3) : 217 - 221.

HASABNIS, S. N., 1984, Studies on storage rot of mango (*Mangifera indica* L.) fruits caused by *Colletotrichum gloeosporioides* Penz. and *Botryodiplodia theobromae* Pat. *M.Sc. (Agri.) Thesis,* Konkan Krishi Vidyapeeth, Dapoli.

KAUR, N., SHARMA, O. P. AND SHARMA, P. N., 2006, *In vitro* effect of *Trichoderma* species on *Colletotrichum capsici* causing fruit rot of chilli (*Capsicum annuum* L.). *Indian Phytopathol*, **59** : 243 - 245.

KHAERUNI, A., ZULKIFLI, M., WIJAYANTO, T., SUTARIATI, G. A. K., BANDE, L. O. S. AND ARINI, R., 2019, *In vitro* test of endophytic antagonist activity for *Colletotrichum gloeosporioides*, causal agent of anthracnose in cocoa. In *IOP Conference Series: Earth and Environmental Science*, **260** (1) : 012136.

KOOMEN, I. AND JEFFRIES, P., 1993, Effects of antagonistic microorganisms on the post-harvest development of *Colletotrichum gloeosporioides* on mango. *Plant Pathol.*, **42** (2) : 230 - 237.

KOTA, V. R., 2003, Biological management of postharvest fungal diseases of major fruits. *M.Sc. (Agri.) Thesis*, University of Agricultural Sciences, Dharwad.

MUHAMMAD, H., SHAHID, A. AND FAUZIA, H., 2010, Suppression of red rot caused by *Colletotrichum falcatum* on sugarcane plants using plant growth promoting *rhizobacteria. Biocontrol*, **55** : 531 - 542.

MUKHERJEE, S., KHANDKER, M. R., ISLAM AND SONIA, B. SHAHID, J., 2011, Efficacy of some plant extracts on the mycelial growth of *Colletotrichum gloeosporioides*. *Bangladesh Agril. Univ.,* **9** (1) : 43 - 47.

MUNJAL, R. L. AND GUPTA, J. N., 1965, Anthracnose of *Celosia argentea* var. *cristata* in Delhi. *Indian Phytopathol*., **18** : 218 - 220.

MURTADO, 2020, Isolation and characterization endophytic bacteria as biological control of fungus *Colletotrichum* sp. on onion plants (*Allium cepa* L.). The 3rd IOP Conf. Ser. : *Earth Environ. Sci*., **457** : 012043.

NAIK, M. K., HIREMATH, P. C. AND HEGDE, R. K., 1988, Physiological and nutritional studies on *Colletotrichum gloeosporioides,* a causal agent of anthracnose of beetlevine. *Mysore J. Agric. Sci.,* **22**: 471-474.

PLOETZ, R. C., 2002, The major diseases of mango: strategies and potential for sustainable management. In VII International Mango Symposium, **645**: 137 - 150.

PLOETZ, R. D. AND PRAKASH, O., 1997, Foliar, floral and soil-borne diseases. Litz, R.E. (Ed.), The Mango: Botany, Production and Uses. CAB International, Cambridge. pp. : 281 - 325.

PRABAKAR, K., RAGUCHANDER, T., SARAVANAKUMAR, D., MUTHULAKSHMI, P., PARTHIBAN, V. K. AND PRAKASAM, V., 2008, Management of post-harvest disease of mango anthracnose incited by *Colletotrichum gloeosporioides*. Arch. Phytopathol. *Plant Prot.,* **41**: 333 - 339.

SHARMA, A. AND VERMA, K. S., 2007, *In vitro* Cross pathogenicity and management of *Colletotrichum gloeosporioides* causing Anthracnose of Mango. *Ann. Pl. Prot. Sci.,* **15** (1) : 186 - 188.

SHIRSHIKAR, G. S., 2002, Studies on fruit rots of mango (*Mangifera indica* L.) caused by *Botryodiplodia theobromae* Pat. and *Colletotrichum gloeosporioides* Penz. and their management. *M.Sc. (Agri.) Thesis*, Konkan Krishi Vidyapeeth, Dapoli, Maharashtra.

SUDHA, S. AND NARENDRAPPA, T., 2015, *In vitro* efficacy of bioagents against *Colletotrichum gloeosporioides* causing anthracnose of mango. *Mysore J. Agric. Sci.,* **49** (2): 355 - 358.

UDHAYAKUMAR, R., MUTHUKUMAR, A. AND USHARANI, S., 2019, *In-vitro* evaluation of bacterial antagonists against mango anthracnose pathogen *Colletotrichum gloeosporioides*. *Multilogic in Science*., VIII (XXVIII), pp : 226 - 230.

VINCENT, J. M., 1947, Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, **159** (4051) : 850 - 850.

VISWANATHAN, R. AND SAMIYAPPAN, R., 2006, *Pseudomonas* spp. colonization in sugarcane rhizosphere reduces titer of *Colletotrichum falcatum* Went-causing red rot disease of sugarcane. *Arc. Phytopathol Plant Prot*., **39** : 39 - 44.

VIVEKANATHAN, R., RAVE, M., RAMANATHAN, A. AND SAMIYAPPAN, R., 2006, Lytic enzymes induced by *Pseudomonas fluorescens* and other biocontrol organisms mediate defense against the anthracnose pathogen in mango. *J. Microbiol. Biotechnol.*, **10** : 235 - 244.