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**STUDY ON MORPHOLOGY AND PHYSIOLOGY OF
Colletotrichum spp. COLLECTED FROM SELECTED FRUIT
SEEDLINGS AND THEIR BIOASSAY AGAINST FUNGICIDES**

AND *Trichoderma harzianum*

BY

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SHER-E-BANGLA AGRICULTURAL UNIVERSITY
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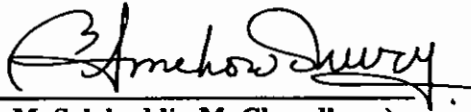
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Submitted to the Faculty of Agriculture,
Sher-e-Bangla Agricultural University, Dhaka,
in partial fulfilment of the requirements
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MASTER OF SCIENCE

**IN
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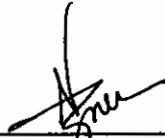
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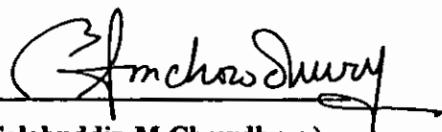
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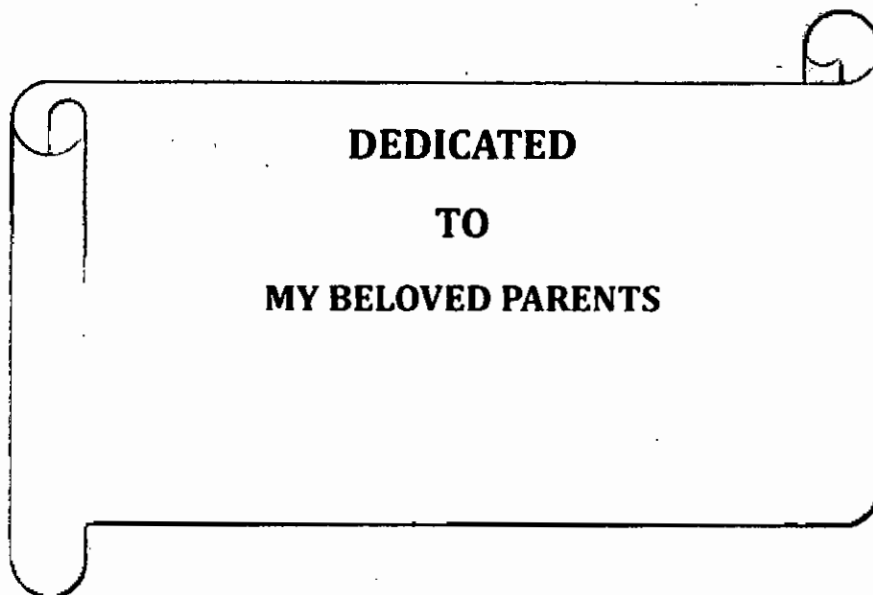
CERTIFICATE

This is to certify that thesis entitled, **'STUDY ON MORPHOLOGY AND PHYSIOLOGY OF Colletotrichum spp. COLLECTED FROM SELECTED FRUIT SEEDLINGS AND THEIR BIOASSAY AGAINST FUNGICIDES AND Trichoderma harzianum'** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY**, embodies the result of a piece of bona fide research work carried out by **MISHAD ISRAAT KHAN, REG NO. 05-01569** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: 22-07-2012
Dhaka, Bangladesh


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**DEDICATED
TO
MY BELOVED PARENTS**



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The Author

STUDY ON MORPHOLOGY AND PHYSIOLOGY OF
***Colletotrichum* spp. COLLECTED FROM SELECTED FRUIT**
SEEDLINGS AND THEIR BIOASSAY AGAINST FUNGICIDES
AND *Trichoderma harzianum*

ABSTRACT

Fifty seven diseased (anthracnose) leaf samples of mango, guava, litchi, lemon and jamun from six nurseries of Dhaka namely Krishibid Upakaran Nursery, Khamarbari Nursery, Nupur Nursery, Yamin Nursery, Tanjila Nursery and Poribesh Sohayok Nursery have been collected. A total of Eighteen isolates of *Colletotrichum gloeosporioides* and one isolate of *Gloeosporium* spp. were isolated and studied for morphological and physiological variability. Bioassay of the isolates was conducted against three fungicides and one bio controlling agent. Highest (17.6 μm) and lowest (7.1 μm) length of conidia were observed for lemon (Dakjamil) and guava (Apel) isolates respectively where highest (4.8 μm) and lowest (2 μm) breadth of conidia were observed for litchi (Bombai) and guava (Apel) isolates respectively. Effect of temperature on the mycelial growth of *Colletotrichum* spp. was studied at 18^oC, 24^oC, 28^oC and 32^oC. The lowest mycelial growth was observed at 18^oC, then it increased at 24^oC and the highest growth was observed at 28^oC while the growth decreased at 32^oC temperature. The highest and the lowest 56.66 mm and 14.16 mm mycelial growth were recorded at 28^oC and 18^oC respectively after 7 days of incubation. Tolerance of 19 isolates of *Colletotrichum* spp. was tested against three fungicides, namely Bavistin 50 WP, Cupravit 50 WP and Indofil M- 45 at the rate of 500, 1000, 1500 and 2000 $\mu\text{g/ml}$ and was incubated for 7 days. Among the three fungicides Bavistin 50WP showed best performance in controlling *Colletotrichum* spp. isolates. *Colletotrichum* spp. isolates could not grow at all in 1500 $\mu\text{g/ml}$ where Cupravit 50WP and Mancozeb 80WP stopped isolates growth at 2000 $\mu\text{g/ml}$. Interaction study between *Colletotrichum* spp. isolates and *Trichoderma* were done by using dual culture method. It was observed that in all the cases *Trichoderma* inhibited growth of *Colletotrichum* spp. after 7 days of incubation *Trichoderma* grew over *Colletotrichum* spp. after 15 days and lysed zone was formed at the point of meeting. *Trichoderma* based bio fungicide could be an effective option to control *Colletotrichum gloeosporioides*.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	=	Percentage
<i>et al.</i>	=	and others
Spp.	=	Species
No.	=	Number
viz.	=	Namely
df.	=	Degrees of freedom
@	=	At the rate of
etc	=	Etcetera
PDA	=	Potato Dextrose Agar media
°C	=	Degree Celsius
Cm	=	Centimeter
µm	=	Micrometer
J.	=	Journal
BBS	=	Bangladesh Bureau of Statistics
RH	=	Relative Humidity
ANOVA	=	Analysis of variances
CV%	=	Percentages of Co-efficient of Variance
LSD	=	Least Significant Difference
Sci.	=	Science
BBS	=	Bangladesh Bureau of Statistics
HKI	=	Helen Keller International



INTRODUCTION

CHAPTER 1

INTRODUCTION

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Seedlings of different fruit species are frequently affected by physical and physiological disorders as well as diseases caused by fungi, bacteria, and viruses (Mittal and Mathur, 1990). Seed borne pathogens affect nursery seedlings and reduce seedling vigour (Abdelmonem and Rasmi, 2003). The climate of Bangladesh harbours plant pathogens and provide luxuriant environment for the growth and reproduction of pathogens, which cause hundreds of different diseases of crops (Fakir, 2001). Mango, guava, litchi, lemon and jamun are the most important fruit crops of Bangladesh. The diseases contribute to great losses of crops. Plant diseases play a major role in reducing yields of horticultural crops in the tropics (Pathak, 1980; Rawal, 1990). It has been estimated that the world production could be increased at least by 28% if the crop could be protected from various disease (Rawal, 1990).

Anthracnose disease caused by *Colletotrichum* spp. is a major factor for death of many seedlings like mango, jamun, lemon, litchi, guava etc. Alahakoon and Brown (1994) isolated *Colletotrichum gloeosporioides* from seedlings of mango which caused up to 40% loss of planting, which were previously believed to be a physiological disorder. Jiger *et al.*, (1998) reported that anthracnose caused by *Colletotrichum gloeosporioides* is a major constrain on the expansion of export trade in fruits such as mango. Anthracnose was recognized as the second most important disease of guava (Rahman *et al.*, 2003). In the investigation *Colletotrichum gloeosporioides* were isolated from anthracnose diseased

leaf. Association of *Colletotrichum gloeosporioides* causing anthracnose of leaf was reported by Pathak (1986). Anthracnose presents great challenges for those who are involved in the international commerce of this fruit.

Colletotrichum is a taxonomically confused genus in urgent need of revision, especially as its members are important plant pathogens (Cannon *et al.*, 2000; Johnston, 2000). The ubiquitous fungus *Colletotrichum gloeosporioides* Penz and Sacc. is the anamorph stage (asexual stage of the pathogenic fungus). *C. gloeosporioides* is responsible for many diseases, also referred to as “anthracnose,” on many tropical fruits including mango, guava, litchi, banana, avocado, papaya, coffee, passion fruit, and others. Mango anthracnose disease caused by *Colletotrichum gloeosporioides* (*Glomerella cingulata*) was studied and reported by many workers (Yamashita, 2000; Akem, 2006; Awasthi *et al.*, 2005). Cooke *et al.*, (2002) reported that Pepper spot of lychee (litchi) was found to be caused by *Colletotrichum gloeosporioides* (*Glomerella cingulata*). *Citrus sinensis* L. is attacked by several pre-and/or postharvest pathogens that effect fruit quality. Anthracnose causes by *Colletotrichum gloeosporioides* (Whiteside *et al.*, 1988). Leaf spot of Jamun caused by *Glomerella cingulata* is an important disease and studied by many workers (Pathak, 1986).

Colletotrichum Corda is one of the economically most important genera of fungi being responsible for anthracnose and other disease of a wide range of plant species (Sutton, 1980). The growth characters of different isolates of *Colletotrichum* spp. varied on different solid media. PDA supported the maximum growth of *C. gloeosporioides*

(Amarjit et al, 2006; Nandinidevi, 2008). Temperature affects the physiological function of the fungi, which in turn affect the phenotypic expression. For each fungus, there is a particular temperature below which it will not grow. Likewise, there is a particular temperature above which the growth ceases. A temperature of 25°C was reported to be the optimum for the growth of *C. gloeosporioides* on mango, almond and avocado (Moriwaki *et al.*, 2003; Gonzalez, 2003). Manjunath, 2009, reported that the optimum growth of C 1 isolate of *C. gloeosporioides* isolated from noni was at 25-28°C.

Considering the above facts, the present investigation was conducted with following objectives:

- Isolation and identification of *Colletotrichum* spp. from collected samples.
- To study the morphological and physiological variations of isolates of *Colletotrichum* spp. obtained from selected seedlings,.
- Bioassay of *Colletotrichum* spp isolates against fungicides and *Trichoderma harzianum* to select suitable option for management.





REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

2.1 General

2.1.1 Distribution of *Colletotrichum* spp.

Colletotrichum spp. is the ethyologic agent of anthracnose disease and plays an important role on agricultural subsistence economies worldwide. These pathogens infect different crops, from monocotyledons plants, such as papaya and turf grass to higher dicotyledons, such as cashew trees. Nevertheless, despite the fact that *Colletotrichum* affect a wide spread number of crops, its pathogenic range increases caused by a rising number of species identified under these Genera that were classified as anthracnose's agent (Peres, 2002).

2.1.2 Economic importance of *Colletotrichum* spp.

Alahakoon and brown (1994) isolated *Colletotrichum gloeosporioides* from seedlings of mango which caused up to 40% loss of planting were previously believed to be a physiological disorder.

Colon-Garay *et al.*, (2002) reported that *Colletotrichum gloeosporioides* cause of anthracnose in various tropical crops, including mango. In Puerto Rico, estimated losses in mango fruits were as high as 75%.

High prevalence of the guava anthracnose disease has been reported in the orchards (Meah and Khan, 1987; Anonymous, 1990). Anthracnose was recognized as the second most important disease of guava (Rahman *et al.*, 2003). In the investigation *Colletotrichum gloeosporioides* were isolated from anthracnose diseased leaf. Association of *Colletotrichum gloeosporioides* causing anthracnose of leaf was reported by Pathak (1986).

2.1.3 Symptoms of *Colletotrichum* spp.

Xie and Xie (1999) described the symptoms of anthracnose disease *Colletotrichum gloeosporioides* (teleomorph: *Glomerella cingulata*) on affected leaves and new shoots of mango trees. When young leaves were attacked, many small brown round spots with faint yellow margins appeared and the badly infected leaves then curled. Infected shoots withered and dried.

In guava anthracnose, infected leaves developed irregular shaped black necrotic spots that coalesced and formed large necrotic areas. The margin of attacked leaves gradually turned dark brown and the black necrotic areas extended backward causing die back. The affected seedlings began to die back from the tip of the branch (Pathak, 1980; Singh, 1998 and Ploetz *et al.*, 1998).

Rawal and Saxana (1997) reported that lemon anthracnose attacks the young leaves, shoots, blossoms and fruits of small acid limes. Young foliage and blossoms are blighted

and distinct lesions formed on leaves and fruits. On leaves, the necrotic spots show acervuli arranged in concentric rings.

2.1.4 The causal organism

The ubiquitous fungus *Colletotrichum gloeosporioides* Penz and Sacc. is the anamorph stage (asexual stage of the pathogenic fungus). *C. gloeosporioides* is responsible for many diseases, also referred to as “anthracnose,” on many tropical fruits including mango, guava, litchi, banana, avocado, papaya, coffee, passion fruit, and others.

Anthracnose is caused by two related species of fungi. *Colletotrichum gloeosporioides* (teleomorph: *Glomerella cingulata*) is responsible in most situations (Dodd *et al.*, 1997).

Mango anthracnose disease caused by *Colletotrichum gloeosporioides* (*Glomerella cingulata*) was studied and reported by many workers (Yamashita, 2000; Akem, 2006; Awasthi *et al.*, 2005).

Cooke *et al.*, (2002) reported that Pepper spot of lychee (litchi) was found to be caused by *Colletotrichum gloeosporioides* (*Glomerella cingulata*).

Leaf spot of Jamun caused by *Glomerella cingulata* is an important disease and studied by many workers (Pathak, 1986).

2.1.5 Taxonomy of *Colletotrichum* spp.

Arx (1957) made a detailed study on the species of the genus *Colletotrichum* and assigned the ascogenous state of *Colletotrichum gloeosporioides* as *Glomerella*

cingulata. Saccardo (1884) placed *Colletotrichum* in Melanconiales and Acervulales, respectively.

Colletotrichum gloeosporioides has been associated with 470 genera of plants (Sutton, 1980).

2.2. Morphology of *Colletotrichum* spp.

Colletotrichum gloeosporioides is a ubiquitous pathogen that has been associated with quiescent infections and post-harvest diseases on several fruits such as avocado, mango, papaya, guava, passion fruit, citrus, apple, grapes and cashew (Simmonds, 1965 and Alahakoon *et al.*, 1994). Morphology of *C. gloeosporioides* was described by several workers (Simmonds, 1965; Mordue, 1971; Irwin and Cameron, 1978; Louis and Cooke, 1985; Davis *et al.*, 1992; Sudhakar, 2000 and Prasanna Kumar, 2001)

McRae (1934) studied the morphological characters of *Glomerella cingulata* on artificial media. The acervuli were round or oblong, black or pink with or without setae. The conidiophores measured about 20-30 μm . The conidia were hyaline or pink in mass, straight rounded at the end and measured 11-32 x 4-55 μm .

Sattar and Malik (1939) observed that the acervuli of the *C. gloeosporioides* developed profusely on diseased parts of the plant (petioles, leaves and fruits). They were irregular and appeared as brown to black dots on the leaves and occurred on both the surface of leaf. Setae were common on twigs but not on fruits. The acervuli when mature exuded

pink masses of conidia under moist conditions. The acervuli were reported to measure 80-250 μm .

Colletotrichum gloeosporioides was grayish to dark gray on Potato Dextrose Agar (PDA) and produced aerial mycelium ranging from a thick mat to sparse or tufts of mycelium. Conidia were hyaline, unicellular and either cylindrical with obtuse ends or ellipsoidal with a rounded apex and narrow, truncate base. They measured 7-20 μm x 2.50-5.00 μm in diameter. Setae were 4-8 x 200 μm , 1 to 47 septate, brown and slightly swollen at the base and tapered at the apex. Orange slimy conidial masses can be formed as the acervuli matured (Litz, 1997).

The fungus was grayish white to dark grey on potato dextrose agar and produce aerial septate mycelia (Prasanna Kumar, 2001).

Rana, (2001) reported that the mycelium is immersed, branched and consists of rather narrow, sparsely septate, hyaline hyphae which turn slightly darken with age. Acervuli are formed on dark stroma .

Ekbote (1994) reported that the fungus *Colletotrichum gloeosporioides* causing anthracnose of mango reached the maximum growth after 12 days of inoculation and least growth was observed on 2 day after inoculation.



2.2.1. Conidial characters of *Colletotrichum* spp.

Bose *et al.* (1953) reported that the acervuli measured 115-467 x 15-22 μm and the size of conidia varied from 11-16 x 4-6 μm . The causal agent was characterized by immersed, branched, septate and hyaline to brown mycelium and separate to confluent acervuli, which may produce brown smooth, septate, tapered setae. Conidiophores were hyaline, determinate conidiogenous cells. Conidia of *C. gloeosporioides* were hyaline, aseptate prior to germination, smooth and thin walled, cylindrical or oval, straight and size of the conidia varied from 9-24 x 4-12 μm (Sutton, 1980, 1993).

Holiday (1980) and Jefferies *et al.* (1990) reported that the conidia were hyaline, unicellular and highly cylindrical with obtuse ends or ellipsoidal with a round apex and a narrow, truncate base. The conidial size was 7-20 x 2.5-5 μm and formed on hyaline to faintly brown conidiophores in acervuli that are irregular in shape and about 500 μm in diameter.

Naik (1985) recorded that, the conidia of *C. gloeosporioides* isolates of *Colletotrichum* from peach and apple produced rounded ends conidia and fusiform conidia on PDA respectively.

Colletotrichum gloeosporioides conidia were hyaline, unicellular and either cylindrical with obtuse ends or ellipsoidal with a rounded apex and narrow, truncate base. They measured 7-20 μm x 2.50-5.00 μm in diameter (Litz, 1997).

Sattar and Malik (1939) observed that the conidia of the *C. gloeosporioides* were borne on distinct, well-developed hyaline conidiophores. The conidia were straight, cylindrical or oval and the size of the conidia varied from 8-20 x 5-7 μm , hyaline usually with two, and rarely one oil globules.

Ekbote (1994) reported that conidia of *Colletotrichum gloeosporioides* were oblong or cylindrical, hyaline, non-septate with round ends, thin walled, having oil globules in the centre. Conidia on the culture media were found to be in reddish mass. They were usually found in aggregates. The conidia on potato dextrose agar measured 12.12–20.78 μm x 4.33–9.52 μm and average being 17.09 x 7.74 μm .

The fungus was grayish white to dark grey on potato dextrose agar and produce aerial septate mycelia. Conidia were hyaline, unicellular and either cylindrical with obtuse ends or ellipsoidal with a rounded apex and narrow, truncate base. Orange slimy conidial mass formed as the acervuli matured. Conidia measured 4.98-15.69 x 2.76-5.52 μm (Prasanna Kumar, 2001).

Rana (2001) reported that the conidia are sub-hyaline, pinkish in mass, variable in shape, mostly oblong to cylindrical, straight, with obtuse ends, sometime slightly curved, 1-celled but may become 2-celled at the time of germination, contain 1 or 2 oil globules and measure 10- 25 x 3.5-7.0 μm mostly 12-16 x 4-6 μm .

2.3. Effect of temperature on growth of *Colletotrichum* spp.

Abe and Kono (1956) observed the optimum temperature range for *Glomerella cingulata* as 24- 28°C and thermal death point at 55°C for five minutes (moist) and 10 minutes (dry).

Sattar and Malik (1939) found the growth of *Colletotrichum gloeosporioides* at 25 to 30°C and the minimum and maximum range of temperature were 10 to 15°C and 35 to 40°C respectively.

Verma (1969) and Mancini *et al.* (1973) noted the temperature of 25°C and 27°C as optimum for *Glomerella cingulata* respectively. Rajak (1983) reported a temperature of 25°C as the optimum for the growth of *Colletotrichum gloeosporioides*. Naik (1985) reported that 20-30°C as optimum temperature range for *Colletotrichum gloeosporioides*.

Hegde and Hegde (1986) observed maximum growth of the *Colletotrichum gloeosporioides* at 30°C and temperature range for the good growth was 20-35°C.

The fungus *Colletotrichum gloeosporioides* isolated from preivet (*Lingustrum vulgare* L.) showed good growth at temperature range between 20 to 28°C (Orlikowski and Wojdyla, 1991).

Ekbote (1994) reported that maximum growth of fungus was noticed at 26°C. This was followed by 29°C, 23°C, 20°C, 35°C and 15°C which was decreasing order and differed significantly. The least growth recorded at 15°C.

Chakraborty (1997) reported that temperature between 20 to 30°C was necessary for infection in *Stylosanthes* anthracnose caused by *Colletotrichum gloeosporioides*.

Sudhakar (2000) observed that the maximum growth of the *Colletotrichum gloeosporioides* was noticed at 30°C followed by 25°C, 20°C and 35°C.

Ashoka (2005) reported that maximum dry mycelial weight of fungus was observed at a temperature of 30°C (454.00 mg) which was significantly over 25 °C (354.00 mg).

Similarly, Quimio and Quimio (1975) and Ahmed (1985) recorded good growth of *C. gloeosporioides* at a temperature range of 20-30°C whereas Saxena (2002) noticed good growth of *C. gloeosporioides* on pomegranate between 15-35°C with optimum at 28°C. Quesada and Lopez (1980) and Banik *et al.*(1998) reported good growth of *C. gloeosporioides* at 28°C, whereas Rajak (1983) and Ekbote (1994) recorded maximum growth of *C. gloeosporioides* at 28°C.

2.4. Boassay of fungicides

Ali *et al.* (1993) reported that complete inhibition of mycelial growth of *Colletotrichum gloeosporioides* of tea was found in case of bavistin and tilt at the concentration of 100 and 200 ppm respectively followed by folicar 400 ppm and calixin 1500 ppm. Koelsch *et al.* (1995) tested fungicides against *Colletotrichum gloeosporioides* of periwinkle,

propiconazole inhibited maximum growth (96%) and thiophenate methyl with mancozeb partially growth (50%).

Anthracnose was effectively controlled by spraying with carbendazim (0.1%) or Topsin-M (0.1%) or chlorothalonil (0.2%) at 14 days intervals until harvest. Benlate (0.2%) and Dithane Z-78 (0.2%) are extremely toxic to fungus in culture. However, these have not been tested in the field (Bose *et al.*, 1953).

Under *in vitro* studies, zineb, thiram, captan, benomyl, phenyl mercuric chlorides, DDB, prochlora 2 and chlorothalonil were found effective in inhibiting the mycelial growth of *Colletotrichum gloeosporioides* (Eikelen boom, 1964; Saraswathy *et al.*, 1975 and Martinelli and Reis, 1984).

Tomy (1997) reported that among six fungicides tested against *Colletotrichum gloeosporioides* causing black leaf spot in mulberry. Mancozeb, carbendazim and copper oxychloride proved effective in inhibiting radial growth of the pathogen.

Srinivasan and Gunasekaran (1998) reported that contaf (Hexaconazole) at 0.1, 0.15, 0.2 and 0.4 per cent concentration completely inhibited mycelial growth; Indofil M-45 inhibited only upto 88 per cent at 0.5 per cent.

Deshmukh *et al.* (1999) conducted *in vitro* studies with ten fungicides and reported that Bordeaux mixture (1 and 2 per cent) copper oxychloride (0.2 and 0.3 percent), carbendazim (0.1 and 2%), thiophenate methyl (0.1 and 0.2 %) and fosetyl-AL (0.1 and 0.2%) were very effective in inhibiting the growth and sporulation of *Colletotrichum gloeosporioides* causing anthracnose on anthurium.

Washathi and Bhargava (2000) reported fungicides *viz.*, carbendazim (0.05-0.1%), mancozeb (0.2-0.25%), tetramethyl thiuram disulphate (0.25%) and benomyl (0.15%) showed complete inhibition of growth of *Colletotrichum dematium*.

Patel and Joshi (2002) observed that carbendazim (Bavistin 50% WP), thiophanate methyl (Topsin-M 75% WP), propiconazole (Tilt 25% EC) at 250, 500 and 1000 ppm, hexaconazole (Contaf 5% EC) at 750, 1000 and 1500 ppm and tricyclazole (beam 75%EC) at 500 and 1000 ppm observed cent per cent inhibition of *Colletotrichum gloeosporioides* causing leaf spot of turmeric.

In vitro studies using different fungicides against *Colletotrichum* spp. responsible for premature yellowing and bean shedding in vanilla showed that thiophanate methyl even at very low concentration i.e. 100 ppm is highly inhibitory to the fungus followed by carbendazim (250 ppm) or carbendazim + mancozeb mixture 2000 ppm (Suseela Bhai *et al.*, 2003).



Ashoka (2005) tested seven systemic and four non-systemic fungicides at three concentrations under *in vitro* condition against *Colletotrichum gloeosporioides*. Systemic fungicide viz. bayleton, benomyl, prochloraz and Saaf were successful in completely (100%) in inhibiting the growth of *Colletotrichum gloeosporioides* at all three concentrations (0.025, 0.005 and 0.1%) whereas, non-systemic fungicide mancozeb (77.65%) found to be effective in inhibiting the growth of fungus.

2.5. Interaction between *Colletotrichum* and *Trichoderma*

Deshmukh and Raut (1992) reported that *Trichoderma harzianum* Rifai and *T. viride* Pers. overgrew colonies of *Colletotrichum gloeosporioides* and *T. harzianum* was more aggressive than *T. viride*. Narendra Singh (1992) revealed that *T. harzianum* was a strong inhibitor of *C. falcatum* under *in vitro* condition.

Arras (1993) reported that, *Bacillus subtilis* strains isolates from cold stored citrus fruits significantly inhibited (60-92%) the growth of *Colletotrichum gloeosporioides*. Whereas, Ashoka (2005) tested six biocontrol agents and noticed that maximum reduction in colony growth was observed in *T. harzianum* (64.65%) followed by *T. viride* (55.38%) and *T. virens* (54.50%) which were on par with each other. These were followed by *Pseudomonas fluorescens* (50.41%), *T. koningii* (46.72%) and *B. subtilis* (42.48%).

Mederios and Menezes (1994) reported that a paired culture method in Petriplates *C. gloeosporioides* showed a high degree of sensitivity to *Trichoderma harzianum*, *Trichoderma polysporum* (Linkex Pers.) Rifai and *Trichoderma pseudokoningi* Rifai.

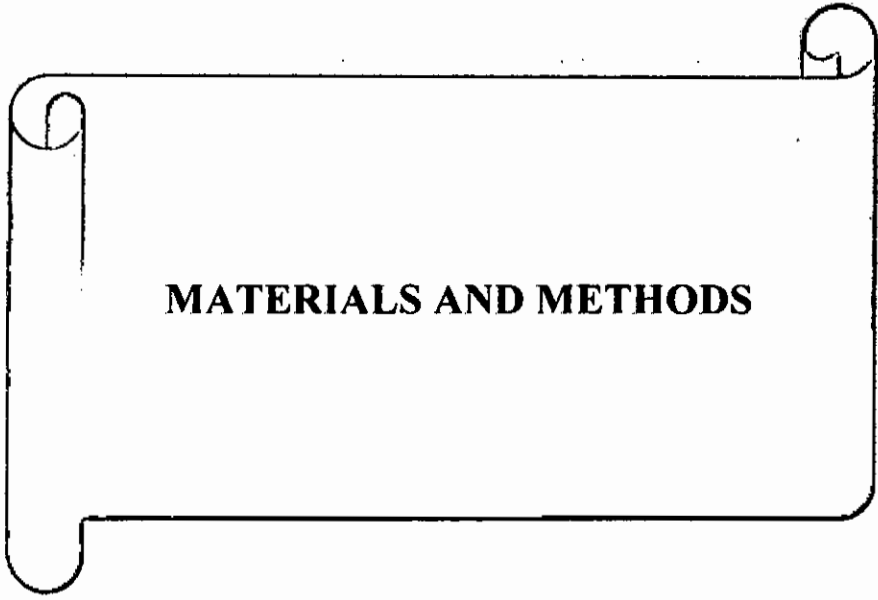
Trichoderma spp. were reported to be effective against guava fruit rot pathogens like *Lasiodiplodia theobromae*, *C. gloeosporioides*, *Pestalotiopsis versicolor*, *Phomopsis psidii* and *Rhizopus arrhizus* (Majumdar and Pathak, 1995).

Rocha *et al.* (1998) reported that *Trichoderma koningii* Oudemans and *T. harzianum* with antagonistic potential against *C. gloeosporioides*. Jeyalakshmi *et al.* (1998) observed that the maximum growth inhibition of the pathogen (*C. capsici*) was exerted by *Sccharomyces cerevisiae* followed by *T. viride*, *T. harzianum*, *T. pileatus* and *T. harzianum*.

Bhuvaneswari (1999) evaluated *Trichoderma viride* under *in vitro* condition against postharvest pathogens of mango and revealed that the growth of *Pestalotia* spp., *A. flavus* and *C. gloeosporioides* were inhibited 72.88, 70.74, 62.41 and 56.83 per cent respectively. In dual culture technique, significantly maximum inhibition was recorded in *T. viride* (66.40%) (Patel and Joshi, 2001).

Shirshikar (2002) reported that, culture and culture filtrate of *Trichoderma viride* was more effective than *T. harzianum* in inhibiting the mycelial growth of *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides*.

Santha Kumari (2002) observed that the isolates of T1 and T2 of *T. harzianum* and the isolates of A1 and A2 of *Aspergillus niger* were found effective in inhibiting the growth of *Colletotrichum gloeosporioides* causing anthracnose of black pepper under *in vitro* condition.



MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1. Experimental site

Experiments were conducted in the M. S. Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207.

3.2. Experimental period

The experiments were conducted from April 2011 to February 2012.

3.3. Collection of diseased samples

Diseased leaf samples of mango (*Mangifera indica* L.), guava (*Psidium guajava*), litchi (*Litchi chinensis*), and lemon (*Citrus limon*) and jamun (*Syzygium cumini*) seedlings were collected from six nurseries of Dhaka namely Krishibid Upakaran Nursery, Khamarbari Nursery, Nupur Nursery, Yamin Nursery, Tanjila Nursery and Poribesh Sohayok Nursery. Diseased leaf was taken in a paper bag and tagged with fruit and variety name, location and date. Then the samples were preserved at 4⁰C in refrigerator for isolation of *Colletotrichum* spp.



Infected Mango Leaf (Trifolia)



Infected Guava Leaf (Kazi)



Infected Litchi Leaf (China- 3)



Infected Lemon Leaf (Alachi)



Infected Litchi Leaf (Deshi)

Plate 1. Anthracnose Diseased Seedling Leaves.

3.4. Sterilization of materials and equipment's

Liquid materials, such as media and distilled water were sterilized in an autoclave following the method (Hazra, 1998) at 121°C and 15 pound per square inch (p.s.i.) for 20 min for surface sterilization 0.1% sodium hypochlorite (NaOCl) was used for plant materials such as leaf, seed etc., and rectified spirit used for other equipment's like inoculation-needles, forceps, inoculation chamber, hands etc.

3.5. Isolation and pure culture

3.5.1. Isolation

Leaves with identical lesions were selected for isolation of the pathogen following the method (Tasiwal, 2008). Leaves having typical symptoms of anthracnose disease were collected from the nurseries. Infected portion was cut into small pieces along with some healthy portion; they were surface sterilized with sodium hypo chlorite solution for 30 seconds. To remove all the traces of sodium hypo chlorite on the surface, the surface bits were washed for two to three times in sterile distilled water and such bits were then transferred to potato dextrose agar (PDA) slants. The slants were incubated at $25 \pm 1^{\circ}\text{C}$ and the growth of the fungus was observed periodically. Pure colonies which developed from the tissue bits were transferred aseptically to obtain pure culture of isolates.

PDA media was prepared by using following method used by Mac Faddin (1985). 4g potato extract (4.0 g of potato extract is equivalent to 200 g of infusion from peeled potatoes), 20g Dextrose and 15g Agar was suspended in one liter of purified water. Then it was heated with frequent agitation and boiled for one minute to completely dissolve the medium. Then the medium autoclaved at 121°C for 15 minutes

3.6. Morphological studies

3.6.1. Preparation of slides

A small amount of pure culture was taken using a sterile needle and transferred to a clean slide. The culture was taken from four positions of the culture plate, two at right angle to each other, one from very close to the inoculation point and another from the midpoint of the radius. Total 3 culture plates of each isolate were used for the morphological studies after 10 days of incubation at 25± 1. The permanent slide prepared with Canada balsam and then heated. The semi- permanent slide prepared with glycerin and nail polish coat on the outer lines of cover slip. Then the slides were observed under the compound microscope.

3.6.2. Measurement of length and breadth of conidia

The shape and size of conidia were recorded in 25 randomly selected conidia using 40X magnification. Length and breadth of conidia measured with the help of Motic Image Plus software.

The following procedure was followed to measure conidial length and breadth using Motic Image Plus software:

- File- Open- Selected file opened by browsing.
- Measure- Calibration Wizard- Calibrated with calibration circle. Then, first, an image was loaded with calibration circle, and objective lens confirmed used to capture the selected image. Then the diameter of this calibration circle was inputted. Finally, the "Calibration" button was clicked to calibrate.
- Magnification of objective lens was also selected by clicking "Measurement" button from the status bar.
- Measure- Line- Expected points were selected by dragging the cursor.

3.6.3. Physiological characterization

The effect of temperature was evaluated for physiological characterization of *Colletotrichum* spp. isolates. The mycelial growth was observed and measured under 18°C, 24°C, 28°C and 32°C temperatures was tested for these 19 isolates.

Mycelial discs of 5 mm diameter, collected on the edges of colonies with about a week, were transferred to the center of plates containing PDA medium and incubated under dark, at temperatures of 18°C, 24°C, 28°C and 32°C for 7 days. Every day is measured two perpendicular diameters of the colony of each isolate and recorded.

4. Bio assay of fungicides

Studies on the tolerance of 19 isolates of *Colletotrichum* spp. to different fungicides were tested following poisoned food technique of Nene and Thapliyal (1979).

Trade Name	Active ingredient	Chemical name	Concentrations used (µg/ml)
Bavistin 50 WP	Carbendazim	Methyl benzimidazol-2-yl carbamate	0, 500, 1000, 1500 and 2000
Cupravit 50 WP	Copper oxychloride	Copper oxychloride	0, 500, 1000, 1500 and 2000
Indofil M-45	Mancozeb	N-(2,6 dimethyl phenyl)-N-(methoxyacetyl)-alanine methyl ester (C ₁₄ H ₂₁ NO ₄)	0, 500, 1000, 1500 and 2000

The linear mycelial growth (mm) and percent growth inhibition of mycelium of isolates were observed in-vitro by poisoned food technique. Fungicidal suspension of each concentration was prepared and mixed thoroughly with melted PDA. PDA (100 ml) was taken in each conical flask and were labeled properly as 0, 500, 1000, 1500 and 2000 µg/ml. The mouths of the conical flasks were then plugged with cotton and covered by brown paper and tied up with threads. The medium in flasks was then sterilized in autoclave at 121°C and 15 pound per square inch (p.s.i.) for 20 minutes. After autoclaving, the conical flasks were then allowed to cool at 50°C. Then the specific amount of test chemical was added into the medium to get different concentrations of test

chemicals viz. 0, 500, 1000, 1500 and 2000 µg/ml. The flasks containing medium were shaken with the help of hand to mix the test chemical thoroughly. After those five drops of lactic acid per flasks was added and shaken well. Thus 100 ml of poisoned PDA for concentration was prepared. The poisoned food thus prepared was plated @ 20 ml in each five replicated plates which were previously marked 0, 500, 1000, 1500 and 2000 µg/ml as per treatment.

Mycelial blocks of 5 mm diameter were cut from 7 days old culture of the test pathogens and were placed aseptically at the center of each plate. The plates then incubated at 25±1°C for a period of 7 days.

Radial mycelial growth of the test fungi was determined at every 24 hr. till 7 days. Percent growth inhibition was also calculated. Inhibition of radial growth was computed based on colony diameter on control plate using the following formula as stated by Sundar *et al.* (1995).

$$\% \text{ Inhibition} = \frac{X - Y}{X} \times 100$$

Where,

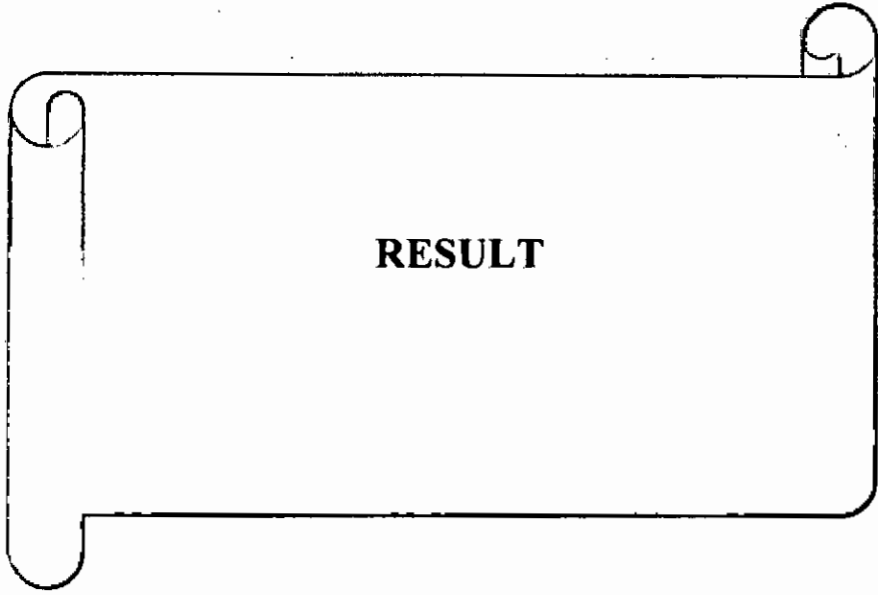
X= Growth of control mycelia

Y= Growth of fungicide treated mycelia



5. Antagonistic activity of *Trichoderma* against isolates of *Colletotrichum* spp.

This study was carried out by using PDA following dual culture methods used by Sultana and Hossain (1999). In case of *Trichoderma harzianum*, a 5 mm block of pure culture was transferred to plate containing PDA at one side, another block of same size of *Colletotrichum* isolate was placed on the other side of the plate in three replicates. The plates were then incubated at 25 ± 1 °C for 7 days, and notes on growth of *Colletotrichum* by *Trichoderma harzianum* and lysed zone formation were recorded. Another set of the same study was done for a period of 15 days and observation on interaction between antagonists and *Colletotrichum* spp. was made, over growth of antagonists and formation of lysed zone between *Colletotrichum* and tested antagonists had also been noted. Moreover, mycelial mat along with culture from the lysed zone was transferred to freshly prepared PDA plates and incubated at room temperature for a week for observing the ability of *Colletotrichum* to grow further.



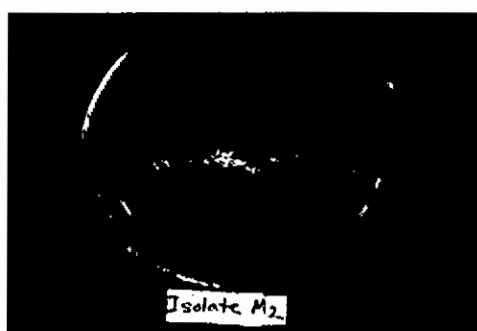
RESULT

CHAPTER 4

RESULT

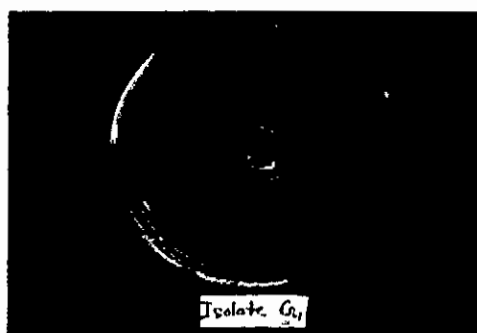
4.1. Isolates of *Colletotrichum* spp.

Eighteen isolates of *Colletotrichum gloeosporioides* and one isolate of *Gloeosporium* spp. were isolated from mango, guava, litchi, lemon and jamun seedlings collected from six nurseries of Dhaka namely Krishibid Upakaran Nursery, Khamarbari Nursery, Nupur Nursery, Yamin Nursery, Tanjila Nursery and Poribesh Sohayok Nursery.



Isolate M2

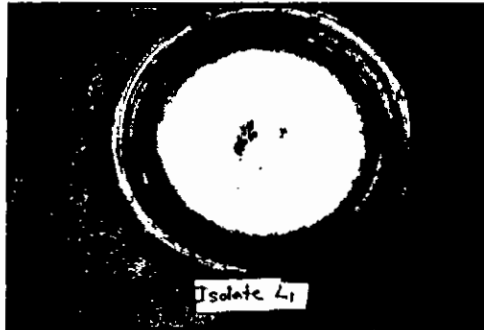
Plate 2. *Colletotrichum gloeosporioides* isolate (M2) collected from mango seedlings.



Isolate G1

Plate 3. *Colletotrichum gloeosporioides* isolate (G1) collected from guava seedlings.





Isolate L1

Plate 4. *Colletotrichum gloeosporioides* isolate (L1) collected from litchi seedlings.



Isolate Le2

Plate 5. *Colletotrichum gloeosporioides* isolate (Le2) collected from litchi seedlings.



Isolate Jm1

Plate 6. *Gloeosporium* spp. isolate (Jm1) collected from jamun seedlings.



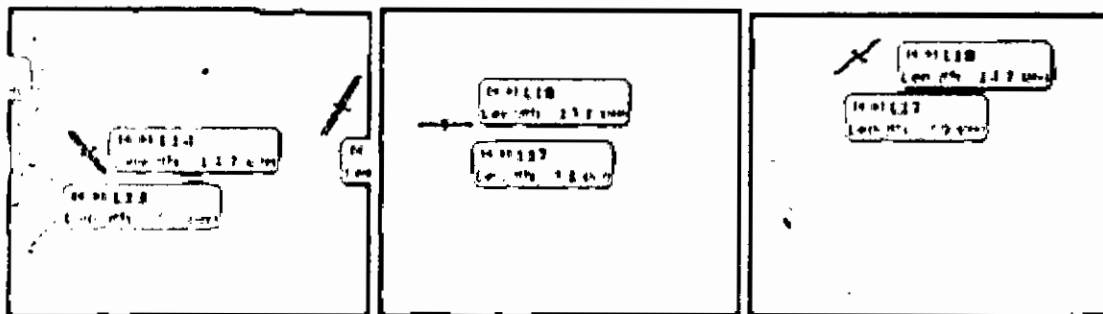
Plate 7. Conidia of *Colletotrichum gloeosporioides* (M1 isolate) observed under compound microscope (X1000).

4.2. Morphology of conidia of *Colletotrichum* spp.

Colony color and length and breadth of the conidia of eighteen isolates of *Colletotrichum gloeosporioides* and one isolate of *Gloeosporium* spp. are presented in Table 1. Highest and lowest length of conidia 17.6 and 7.1 μm measured for Le3 and G3 isolates respectively. Highest and lowest breadth of conidia 4.8 and 2 μm measured for L3 and G3 respectively.

Table 1: Source of isolation morphology and conidial characters of isolates of *Colletotrichum* spp.

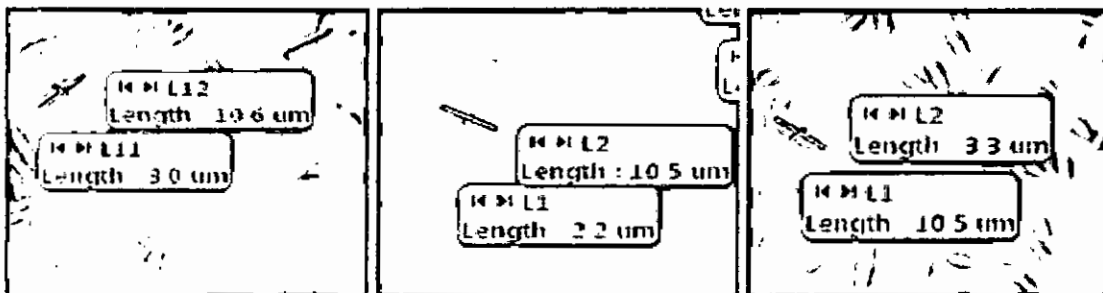
Source of isolation		Name of isolate	Organism	Length of conidia (μm)	Breadth of conidia (μm)	Colony color
Mango	Aam Rupali	M1	<i>Colletotrichum gloeosporioides</i>	15.2 \pm 2.9	3.1 \pm 0.5	Whitish
Mango	Trifola	M2	<i>Colletotrichum gloeosporioides</i>	12.6 \pm 3.1	2.8 \pm 0.3	White
Mango	Him sagar	M3	<i>Colletotrichum gloeosporioides</i>	14.1 \pm 2.3	3.1 \pm 0.7	Whitish
Mango	Langra	M4	<i>Colletotrichum gloeosporioides</i>	11.2 \pm 2.4	2.5 \pm 0.6	White
Mango	Mollika	M5	<i>Colletotrichum gloeosporioides</i>	8.4 \pm 2.4	2.4 \pm 0.0	White
Mango	Naltofala	M6	<i>Colletotrichum gloeosporioides</i>	10.8 \pm 2.7	2.6 \pm 0.4	White
Guava	Madhuri	G1	<i>Colletotrichum gloeosporioides</i>	13.1 \pm 2.4	4.5 \pm 0.3	Grayish
Guava	Kazi	G2	<i>Colletotrichum gloeosporioides</i>	8.7 \pm 3.2	2.1 \pm 0.1	White
Guava	Aapel	G3	<i>Colletotrichum gloeosporioides</i>	7.1 \pm 1.3	2.0 \pm 0.1	Grayish
Guava	Angur	G4	<i>Colletotrichum gloeosporioides</i>	7.8 \pm 1.1	2.2 \pm 0.2	Whitish
Guava	Deshi	G5	<i>Colletotrichum gloeosporioides</i>	12.3 \pm 3.8	3.1 \pm 0.3	Grayish
Litchi	Bedana	L1	<i>Colletotrichum gloeosporioides</i>	14.3 \pm 3.7	4.4 \pm 0.1	White
Litchi	China- 3	L2	<i>Colletotrichum gloeosporioides</i>	9.5 \pm 3.2	3.9 \pm 1.0	Whitish
Litchi	Bombai	L3	<i>Colletotrichum gloeosporioides</i>	15.7 \pm 2.9	4.7 \pm 0.1	White
Lemon	Kajol	Le1	<i>Colletotrichum gloeosporioides</i>	11.8 \pm 3.1	3.6 \pm 0.3	Whitish
Lemon	Alachi	Le2	<i>Colletotrichum gloeosporioides</i>	12.4 \pm 1.2	3.8 \pm 0.8	Whitish
Lemon	Dakjamil	Le3	<i>Colletotrichum gloeosporioides</i>	17.3 \pm 1.1	4.1 \pm 0.2	Orange white
Lemon	Kacha mitha	Le4	<i>Colletotrichum gloeosporioides</i>	16.4 \pm 3.2	4.6 \pm 0.2	Whitish
Jamun	Deshi	Jm1	<i>Gloeosporium (Colletotrichum)</i>	13.6 \pm 1.7	3.9 \pm 0.5	Light pink



Conidia of isolate M1

Conidia of isolate M2

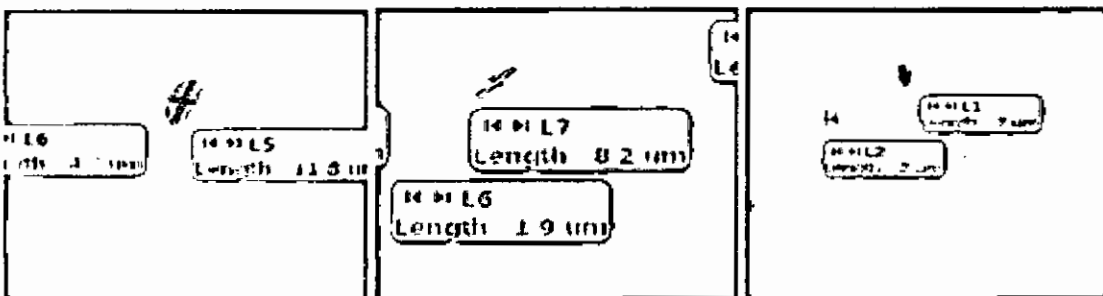
Conidia of isolate M3



Conidia of isolate M4

Conidia of isolate M5

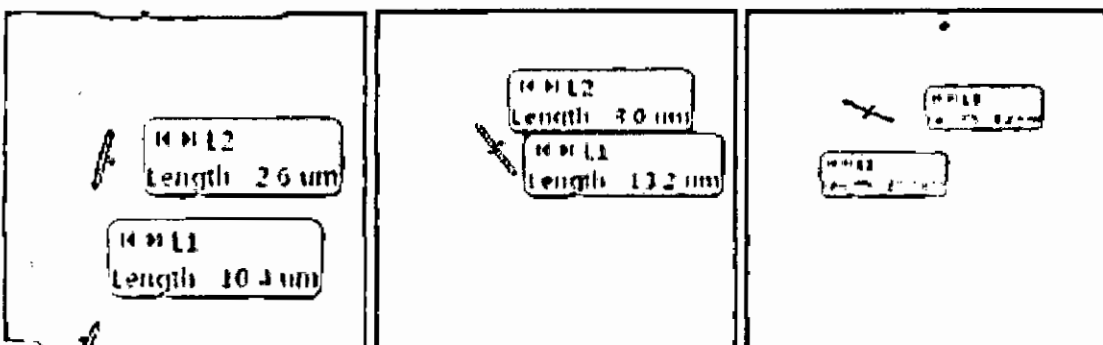
Conidia of isolate M6



Conidia of isolate G1

Conidia of isolate G2

Conidia of isolate G3



Conidia of isolate G4

Conidia of isolate G5

Conidia of isolate L1

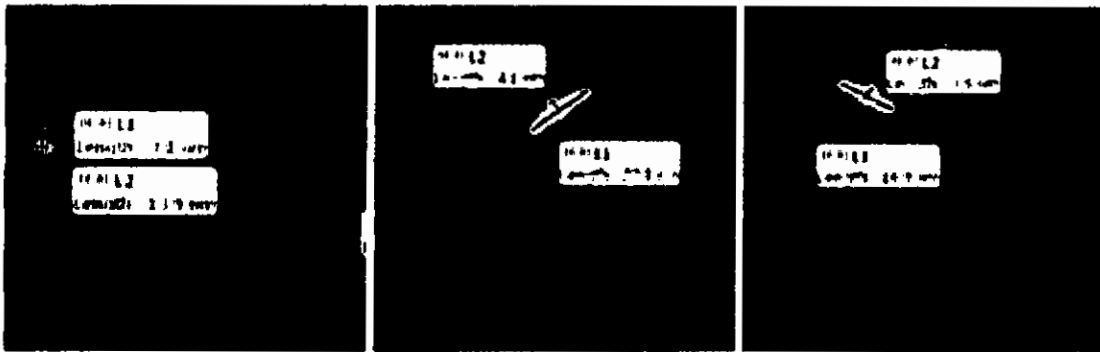
Plate 8: *Colletotrichum* spp. isolates showing length and breadth observed under 400X objective (continued).



Conidia of isolate L2

Conidia of isolate L3

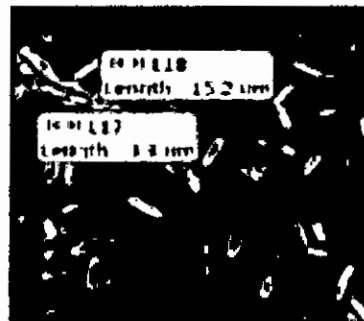
Conidia of isolate Le1



Conidia of isolate Le2

Conidia of isolate Le3

Conidia of isolate Le4



Conidia of isolate Jm1

Plate 8. *Colletotrichum* spp. isolates showing length and breadth observed under 400X objective.

4.3. Effect of temperature on radial growth of *Colletotrichum* spp.

Effect of temperature on the mycelial growth of *Colletotrichum* spp. at 18, 24, 28 and 32°C was studied. All the tested isolates showed higher mycelial growth at 28°C and lower at 18°C. Mycelial growth was increased at 24°C than 18°C and highest growth was observed at 28°C while the growth was decreased at 32°C temperature. The highest (56.66 mm) and the lowest (14.16 mm) mycelial growth were recorded on Jm1 and G3 isolate at 28°C and 18°C respectively after 7 days of incubation. At 18°C temperature highest and lowest 24.66 and 14.16 mm mycelial growth recorded on M4 and G3 isolate (Table- 2). In case of 24°C temperature highest and lowest 37.83 and 25.66 mm mycelial growth recorded on Jm1 and M4 isolate (Table- 3). At 28°C temperature highest and lowest 56.66 and 43.16 mm mycelial growth recorded on Jm1 and M3 isolate (Table- 4). And at 32°C temperature highest and lowest 32.33 and 21.33 mm mycelial growth recorded on L2 and Le4 isolate (Table- 5).

4.3. Effect of temperature on radial growth of *Colletotrichum* spp.

Effect of temperature on the mycelial growth of *Colletotrichum* spp. at 18, 24, 28 and 32°C was studied. All the tested isolates showed higher mycelial growth at 28°C and lower at 18°C. Mycelial growth was increased at 24°C than 18°C and highest growth was observed at 28°C while the growth was decreased at 32°C temperature. The highest (26.66 mm) and the lowest (14.16 mm) mycelial growth were recorded on Inid and Q3 isolate at 28°C and 18°C respectively after 7 days of incubation. At 18°C temperature highest and lowest 24.66 and 14.16 mm mycelial growth recorded on M4 and Q3 isolate (Table-2). Increase of 24°C temperature highest and lowest 37.87 and 25.66 mm mycelial growth recorded on Jm1 and M4 isolate (Table-3). At 28°C temperature highest and lowest 26.66 and 13.16 mm mycelial growth recorded on Jm1 and M3 isolate (Table-4). And at 32°C temperature highest and lowest 35.33 and 21.33 mm mycelial growth recorded on L2 and L4 isolate (Table-5).

Table 2. Radial mycelial growth of *Colletotrichum* spp. isolates at 18⁰ C temperature.

Isolate	Radial mycelial growth(mm)						
	D1	D2	D3	D4	D5	D6	D7
M1	0.66	3.33	10.50	13.66	17.83	19.50	20.66
M2	0.16	3.16	4.33	7.50	13.50	20.66	22.50
M3	1.33	2.50	5.50	7.66	11.33	15.83	20.50
M4	0.66	5.66	11.83	14.83	17.50	20.66	24.66
M5	1.00	0.83	1.83	4.50	6.16	11.50	16.33
M6	1.83	4.00	6.66	9.50	13.66	17.50	22.16
G1	1.66	3.50	6.33	10.33	11.83	15.50	17.83
G2	2.50	5.50	8.66	13.50	16.83	19.33	23.50
G3	0.83	2.50	3.33	5.16	6.16	10.50	14.16
G4	1.33	2.00	4.50	8.50	11.33	15.16	19.33
G5	2.00	4.50	7.66	11.16	13.66	14.16	16.83
L1	0.50	1.83	3.83	6.66	9.00	12.66	15.50
L2	0.66	2.33	4.50	6.33	11.50	19.16	23.66
L3	0.50	0.83	2.66	9.66	14.50	20.66	15.83
Le1	0.00	3.33	4.66	5.00	9.50	14.50	16.16
Le2	0.16	0.83	1.66	3.50	5.50	10.83	14.50
Le3	0.33	3.16	7.50	12.50	18.66	20.16	20.00
Le4	0.66	1.50	2.66	4.16	8.66	12.33	16.50
Jm1	1.66	2.50	4.66	7.66	11.33	18.33	20.83

Each data presents mean of three replications; D represents Day

Table 3: Radial mycelial growth of *Colletotrichum* spp. isolates at 24° C temperature.

Isolate	Radial mycelial growth(mm)						
	D1	D2	D3	D4	D5	D6	D7
M1	1.50	7.83	12.50	16.83	21.50	25.83	31.33
M2	2.33	9.16	13.00	16.83	24.83	29.50	35.16
M3	1.83	7.00	12.83	18.33	21.66	24.50	31.33
M4	2.83	4.50	4.50	10.00	14.33	21.83	25.66
M5	3.16	6.66	12.83	17.83	22.16	27.66	30.83
M6	0.83	3.00	5.50	9.50	16.66	23.16	32.16
G1	2.50	5.83	12.66	18.16	23.50	28.16	34.50
G2	4.00	8.66	12.16	21.00	28.00	29.66	36.00
G3	3.33	9.33	14.50	18.50	22.50	27.50	35.83
G4	1.50	3.00	8.33	15.16	21.83	29.66	36.16
G5	2.66	7.50	13.16	17.16	22.66	27.66	34.50
L1	4.16	7.83	12.16	18.33	23.00	29.83	36.66
L2	3.81	5.50	11.83	18.50	26.16	29.16	33.33
L3	3.50	6.16	13.66	20.33	25.50	27.33	31.16
Le1	1.00	4.50	7.50	12.83	17.50	21.83	26.50
Le2	1.50	3.33	6.00	12.50	18.50	23.50	28.66
Le3	3.50	7.83	10.50	14.66	20.66	25.30	30.00
Le4	2.83	5.16	9.66	12.66	17.16	23.33	28.83
Jm1	3.16	8.16	15.83	22.16	26.50	33.16	37.83

Each data presents mean of three replications; D represents Day

Table 4: Radial mycelial growth of *Colletotrichum* spp. isolates at 28⁰ C temperature.

Isolate	Radial mycelial growth(mm)						
	D1	D2	D3	D4	D5	D6	D7
M1	3.00	10.50	16.66	22.16	28.33	35.66	44.50
M2	2.50	14.83	15.83	23.16	31.16	36.83	46.33
M3	1.16	5.16	12.50	19.83	24.50	29.66	43.16
M4	1.66	2.66	8.50	14.50	20.83	29.50	36.66
M5	2.33	4.50	11.00	16.66	24.83	33.50	42.33
M6	3.83	8.00	17.83	23.50	29.66	41.16	48.83
G1	5.50	3.83	19.83	28.33	37.50	42.83	47.50
G2	8.16	15.33	15.50	34.83	40.16	46.50	53.00
G3	4.16	11.33	20.50	25.50	33.16	41.50	48.83
G4	6.83	15.83	24.83	32.83	38.66	44.83	50.33
G5	4.50	8.16	16.66	24.83	31.50	27.66	46.16
L1	3.83	13.66	21.16	30.16	38.83	47.33	55.50
L2	6.16	13.50	22.50	29.83	37.66	42.33	52.83
L3	6.50	15.50	25.16	32.00	41.16	46.83	54.16
Le1	1.00	5.83	9.83	15.83	24.50	33.33	44.66
Le2	4.83	10.33	16.83	23.16	29.00	38.66	46.66
Le3	4.66	7.66	12.50	21.00	26.83	33.50	41.83
Le4	3.16	8.50	14.16	22.50	26.50	32.83	40.50
Jm1	2.66	3.33	15.83	24.83	36.83	45.16	56.66

Each data presents mean of three replications; D represents Day

Table 4: Radial mycelial growth of *Colletotrichum* spp. isolates at 28⁰ C temperature.

Isolate	Radial mycelial growth(mm)						
	D1	D2	D3	D4	D5	D6	D7
M1	3.00	10.50	16.66	22.16	28.33	35.66	44.50
M2	2.50	14.83	15.83	23.16	31.16	36.83	46.33
M3	1.16	5.16	12.50	19.83	24.50	29.66	43.16
M4	1.66	2.66	8.50	14.50	20.83	29.50	36.66
M5	2.33	4.50	11.00	16.66	24.83	33.50	42.33
M6	3.83	8.00	17.83	23.50	29.66	41.16	48.83
G1	5.50	3.83	19.83	28.33	37.50	42.83	47.50
G2	8.16	15.33	15.50	34.83	40.16	46.50	53.00
G3	4.16	11.33	20.50	25.50	33.16	41.50	48.83
G4	6.83	15.83	24.83	32.83	38.66	44.83	50.33
G5	4.50	8.16	16.66	24.83	31.50	27.66	46.16
L1	3.83	13.66	21.16	30.16	38.83	47.33	55.50
L2	6.16	13.50	22.50	29.83	37.66	42.33	52.83
L3	6.50	15.50	25.16	32.00	41.16	46.83	54.16
Le1	1.00	5.83	9.83	15.83	24.50	33.33	44.66
Le2	4.83	10.33	16.83	23.16	29.00	38.66	46.66
Le3	4.66	7.66	12.50	21.00	26.83	33.50	41.83
Le4	3.16	8.50	14.16	22.50	26.50	32.83	40.50
Jm1	2.66	3.33	15.83	24.83	36.83	45.16	56.66

Each data presents mean of three replications; D represents Day

Table 5: Radial mycelial growth of *Colletotrichum* spp. isolates at 32^o C temperature.

Isolate	Radial mycelial growth(mm)						
	D1	D2	D3	D4	D5	D6	D7
M1	2.66	5.50	9.83	13.00	18.50	22.83	27.50
M2	0.00	4.66	7.50	14.66	21.83	24.50	29.83
M3	2.00	4.83	8.66	12.16	17.66	24.16	31.33
M4	1.16	5.83	11.00	16.66	19.66	23.83	29.66
M5	0.00	0.00	3.83	7.50	13.00	18.33	24.33
M6	2.83	4.50	7.50	12.83	16.50	20.33	25.16
G1	2.50	5.83	9.66	14.50	17.16	22.66	27.50
G2	3.33	6.50	9.33	14.16	19.33	24.50	28.66
G3	1.66	3.16	6.50	10.16	15.50	21.66	25.83
G4	2.83	2.83	7.66	11.00	14.16	20.16	27.50
G5	2.00	6.50	9.16	12.50	14.66	19.33	26.50
L1	0.00	4.66	7.33	9.83	13.83	19.00	24.16
L2	3.16	5.16	8.16	13.16	21.50	25.50	32.33
L3	0.00	1.00	4.66	11.83	18.33	21.33	25.83
Le1	0.00	3.50	6.83	8.66	12.50	17.83	22.50
Le2	0.00	2.66	4.66	6.33	13.66	19.33	25.33
Le3	0.00	3.50	7.16	11.33	15.16	20.00	23.66
Le4	0.83	2.66	8.83	11.83	14.83	17.33	21.33
Jm1	3.16	5.83	8.50	12.50	18.50	23.66	31.83

Each data presents mean of three replications; D represents Day

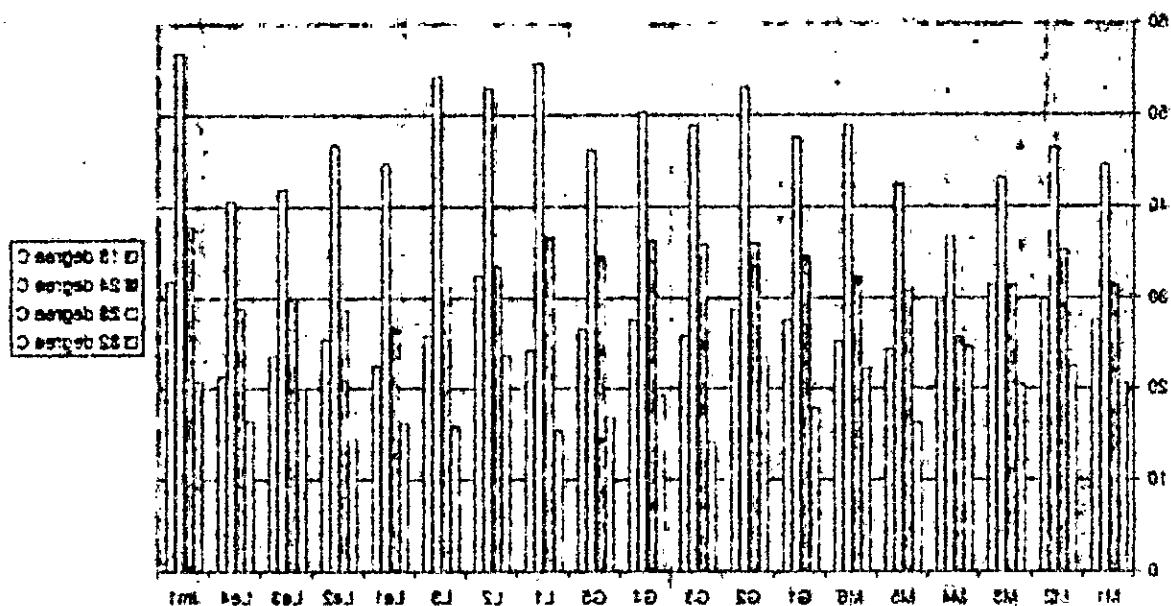


Figure 1. Comparison of growth of *Colletotrichum* spp. isolates at different temperature, X and Y axis represents isolates and different temperature respectively (after 7 days of incubation).



Growth of M1 isolate at different temperature



Growth of G3 isolate at different temperature



Growth of L1 isolate at different temperature



Growth of Le2 isolate at different temperature



Growth of Jm1 isolate at different temperature

Plate 9. Growth of *Colletotrichum* spp. isolates at different temperature.

4.4. Tolerance of *Colletotrichum* spp. isolates to fungicides

Tolerance of 19 isolates of *Colletotrichum* spp. was tested against three fungicides, namely Bavistin 50 WP, Cupravit 50 WP and Indofil M- 45 at the rate of 500, 1000, 1500 and 2000 µg/ml and was incubated for 7 days. Responses of *Colletotrichum* spp. to these three fungicides are given below:

4.4.1. Fungicide tolerance of *Colletotrichum* spp. isolates collected from mango

4.4.1.1. Tolerance of isolate M1 to fungicides

It has been observed that Indofil M- 45 showed significant effect in inhibition of radial mycelial growth of isolate M1 over untreated control by 83%, 84% and 60% at 1500, 1000 and 500 µg/ml respectively. No growth was observed in case of Isolate M1 to Indofil M- 45 at 2000 µg/ml up to 7 days incubation period. The concentrations 1000 µg/ml and 1500 µg/ml resulted statistically similar effect on radial mycelial growth but differed significantly from other treatments. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate M1. It was observed that Cupravit 50WP reduced the mycelial growth of isolate M1 over untreated control by 42% and 32% at 1000 and 500 µg/ml respectively. Isolate M1 failed to grow in Cupravit 50WP at 1500 µg/ml up to 7 days incubation period. Bavistin 50WP reduced the mycelial growth of M1 isolate over untreated control by 96.67% at 500 µg/ml. Isolate M1 could not grow in Bavistin 50WP at 1000 µg/ml up to 7 days incubation period (Table- 6).

Table 6. Tolerance of Isolate M1 to fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	% growth inhibition
Indofil M-45	Control	0.50a	3.33a	8.16a	13.67a	18.83a	19.17a	23.17a	
	500	0.00b	1.83b	2.67b	4.83b	7.16b	8.00b	9.16b	60
	1000	0.00b	0.00c	0.67c	1.00c	1.83c	3.00c	3.67c	84
	1500	0.00b	0.00c	0.33c	0.50cd	0.83cd	2.50c	4.00c	83
	2000	0.00b	0.00c	0.00c	0.00d	0.00d	0.00d	0.00d	100
	LSD(p=0.05)	0.4210	0.8142	1.287	0.8250	1.120	1.369	1.095	
Cupravit 50WP	Control	1.17a	13.33a	15.33a	22.00a	26.67a	36.33a	50.83a	
	500	0.00b	12.33b	14.33b	17.50b	21.83b	26.00b	34.67b	32
	1000	0.00b	0.00c	0.00c	4.33c	7.00c	23.00c	29.33c	42
	1500	0.00b	0.00c	0.00c	0.00d	0.00d	0.00d	0.00d	100
	2000	0.00b	0.00c	0.00c	0.00d	0.00d	0.00d	0.00d	100
	LSD(p=0.05)	0.2455	0.5954	0.5954	1.179	1.197	1.252	1.639	
Bavistin 50WP	Control	2.50a	13.67a	17.50a	23.83a	30.00	37.33a	45.00a	
	500	0.00b	0.00b	0.00b	0.00b	0.00b	0.333 b	1.50b	96
	1000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00c	100
	1500	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00c	100
	2000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00c	100
	LSD(p=0.05)	0.4210	0.2455	0.7292	0.2455	0.4210	0.5954	0.4210	

Each data presents mean of three replications; D represents Day

4.4.1.2. Tolerance of isolate M2 to fungicides

Indofil M- 45 showed significant effect on inhibition of radial mycelial growth of isolate M2 over untreated control by 87%, 65% and 60% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate M2 failed to grow in Indofil M- 45 at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate M2. It was observed that Cupravit 50WP reduced the mycelial growth of isolate M2 over untreated control by 34% and 24% at 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate M2 could not grow as Cupravit 50WP amended media at 1500 $\mu\text{g/ml}$ up to 7 days incubation period. Bavistin 50WP reduced the mycelial growth of M2 isolate over untreated control by 99.06% at 500 $\mu\text{g/ml}$ which one is statistically similar with 1000, 1500 and 2000 $\mu\text{g/ml}$ treatment where 100% growth inhibition is done. No growth of Isolate M2 has been observed in Bavistin 50WP at 1000 $\mu\text{g/ml}$ up to 7 days incubation period (Table- 7).

Table 7. Tolerance of Isolate M2 to fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							% growth inhibition
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
Indofil M-45	Control	2.00a	4.33a	8.00a	11.33a	12.83a	16.00a	20.33a	
	500	0.17b	1.16b	2.83b	3.83b	6.00b	7.16b	8.16b	60
	1000	0.00b	0.50b	0.83c	1.83c	3.67c	4.50c	5.16c	75
	1500	0.00b	0.00b	0.50c	0.83c	1.50cd	2.16cd	2.67d	87
	2000	0.00b	0.00b	0.00c	0.00c	0.00d	0.00d	0.00e	100
	LSD(p=0.05)	0.8771	2.023	1.617	1.990	2.309	2.654	2.034	
	Cupravit 50WP	Control	2.67a	14.33a	21.17a	25.67a	36.67a	43.67a	52.67a
500		0.00b	7.33b	14.00b	18.67b	25.00b	31.33b	40.00b	24
1000		0.00b	0.00c	2.17c	9.83c	15.33c	28.00c	34.67c	34
1500		0.00b	0.00c	0.00d	0.00d	0.00d	0.00d	0.00d	100
2000		0.00b	0.00c	0.00d	0.00d	0.00d	0.00d	0.00d	100
LSD(p=0.05)		0.4874	0.5954	0.8669	0.9339	1.252	1.566	0.7292	
Bavistin 50WP	Control	3.33a	12.67a	18.50a	24.33a	34.17a	44.50a	53.33a	
	500	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.50b	99
	1000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	1500	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	2000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	LSD(p=0.05)	0.4874	0.6440	0.4210	0.4874	0.2455	0.4210	0.6440	

Each data presents mean of three replications; D represents Day

4.4.1.3. Tolerance of isolate M3 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on isolate M3 over untreated control by 87% and 58% at 1000 and 500 µg/ml respectively. Isolate M3 could not grow in Indofil M- 45 at 1500 µg/ml up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate M3. It was observed that Cupravit 50WP reduced the mycelial growth of isolate M3 over untreated control by 26% at 500 µg/ml. Isolate M3 failed to Cupravit 50WP at 1000 µg/ml up to 7 days incubation period. Bavistin 50WP reduced the mycelial growth of M3 isolate over untreated control by 95.97% at 500 µg/ml. No growth of Isolate M3 has been observed in Bavistin 50WP at 1000 µg/ml up to 7 days incubation period (Table- 8).

Table 8. Tolerance of Isolate M3 to fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							% growth inhibition
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
Indofil M-45	Control	0.50a	4.50a	9.66a	13.83a	16.33a	22.33a	26.33a	
	500	0.33a	1.00b	2.83b	6.00b	7.16b	9.16b	11.00b	58
	1000	0.00a	0.00c	0.00c	0.33c	0.67c	1.50c	3.33c	87
	1500	0.00a	0.00c	0.00c	0.00c	0.00c	0.00d	0.00d	100
	2000	0.00a	0.00c	0.00c	0.00c	0.00c	0.00d	0.00d	100
	LSD($p=0.05$)	0.5711	0.5554	0.7095	1.031	1.391	0.797	1.114	
Cupravit 50WP	Control	1.50a	9.17a	16.50a	22.33a	32.33a	36.50a	44.33a	
	500	1.00a	6.83b	13.00b	17.50b	25.17b	30.00b	33.00b	26
	1000	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	1500	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	2000	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	LSD($p=0.05$)	0.5156	0.3206	0.4210	0.6440	0.8250	0.9847	0.9729	
Bavistin 50WP	Control	1.00a	5.33a	12.00a	20.00a	28.17a	36.50a	45.50a	
	500	0.00b	0.00b	0.00b	0.00b	0.50b	1.50b	1.83b	95
	1000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00c	0.00c	100
	1500	0.00b	0.00b	0.00b	0.00b	0.00b	0.00c	0.00c	100
	2000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00c	0.00c	100
	LSD($p=0.05$)	0.4210	0.4874	0.8420	0.8420	0.5292	0.6657	0.7095	

Each data presents mean of three replications; D represents Day



4.4.1.4. Tolerance of isolate M4 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on isolate M4 over untreated control by 84%, 53% and 60% at 1000 and 500 $\mu\text{g/ml}$ respectively. No growth of Isolate M4 has been observed in Indofil M- 45 at 1500 $\mu\text{g/ml}$ up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate M4. It was observed that Cupravit 50WP reduced the mycelial growth of isolate M4 over untreated control by 51% and 16% at 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate M4 could not grow in Cupravit 50WP at 1500 $\mu\text{g/ml}$ up to 7 days incubation period. Bavistin 50WP reduced the mycelial growth of M4 isolate over untreated control by 39.47% at 500 $\mu\text{g/ml}$. Isolate M4 failed to grow in Bavistin 50WP at 1000 $\mu\text{g/ml}$ up to 7 days incubation period (Table- 9).

Table 9. Tolerance of Isolate M4 fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							% growth inhibition
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
Indofil M-45	Control	3.83a	6.83a	13.83a	20.00a	25.33a	30.33a	33.17a	
	500	0.00b	0.33b	1.167b	1.83b	6.16b	10.00b	15.50b	53
	1000	0.00b	0.00b	0.00c	0.00c	0.67c	4.00c	5.16c	84
	1500	0.00b	0.00b	0.00c	0.00c	0.00c	0.00d	0.00d	100
	2000	0.00b	0.00b	0.00c	0.00c	0.00c	0.00d	0.00d	100
	LSD(p=0.05)	0.2455	0.7877	0.6440	0.8771	0.9564	1.216	0.7877	
Cupravit 50WP	Control	1.33a	2.33a	4.50a	12.67a	20.17a	27.50a	35.33a	
	500	0.00b	0.67b	1.33b	4.67b	11.67b	21.50b	29.67b	16
	1000	0.00b	0.00c	0.00c	0.00c	1.67c	12.67c	17.33c	51
	1500	0.00b	0.00c	0.00c	0.00c	0.00d	0.00d	0.00d	100
	2000	0.00b	0.00c	0.00c	0.00c	0.00d	0.00d	0.00d	100
	LSD(p=0.05)	0.4874	0.5711	0.9262	0.2977	0.6994	0.7966	1.165	
Bavistin 50WP	Control	0.50a	2.50a	8.50a	12.17a	19.83a	28.67a	35.67	
	500	0.00a	0.00b	0.00b	0.00b	0.00b	1.50a	2.33b	93
	1000	0.00a	0.00b	0.00b	0.00b	0.00b	0.00c	0.00c	100
	1500	0.00a	0.00b	0.00b	0.00b	0.00b	0.00c	0.00c	100
	2000	0.00a	0.00b	0.00b	0.00b	0.00b	0.00c	0.00c	100
	LSD(p=0.05)	0.7292	0.4210	0.4210	0.6440	0.7389	0.7095	0.4874	

Each data presents mean of three replications; D represents Day

4.4.1.5. Tolerance of isolate M5 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on isolate M5 over untreated control by 92%, 86% and 50% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate M5 failed to grow in Indofil M- 45 at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate M5. It was observed that Cupravit 50WP reduced the mycelial growth of isolate M5 over untreated control by 74% and 18% at 1000 and 500 $\mu\text{g/ml}$ respectively. No growth of Isolate M5 has been observed in Cupravit 50WP at 1500 $\mu\text{g/ml}$ up to 7 days incubation period. Bavistin 50WP reduced the mycelial growth of M5 isolate over untreated control by 99.13% and 97.82% at 500 and 1000 $\mu\text{g/ml}$ respectively. Isolate M5 could not grow in Bavistin 50WP at 1500 $\mu\text{g/ml}$ up to 7 days incubation period (Table- 10). 500, 1000 $\mu\text{g/ml}$, 1500 and 2000 $\mu\text{g/ml}$ resulted statistically similar effect on radial mycelial growth but differed significantly from control.

Table 10. Tolerance of Isolate M5 fungicides.

	Concentration (µg/ml)	Radial mycelial growth(mm)							% growth inhibition
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
Indofil M-45	Control	2.66a	9.17a	14.00a	23.00a	32.50a	39.00a	46.00a	
	500	1.83b	5.17b	7.17b	11.50b	15.33b	18.17b	22.83b	50
	1000	0.00c	0.00c	0.33c	2.167c	5.17c	6.00c	6.53c	85
	1500	0.00c	0.00c	0.00c	0.00d	0.50d	1.83d	3.67d	92
	2000	0.00c	0.00c	0.00c	0.00d	0.00d	0.00e	0.00e	100
	LSD(p=0.05)	0.7095	0.7877	0.8771	0.8504	1.101	1.728	0.8207	
Cupravit 50WP	Control	1.50a	5.66a	13.67a	21.00a	25.67a	31.50a	36.33a	
	500	0.17b	1.83b	3.50b	13.83b	21.83b	27.67b	29.83b	17
	1000	0.00b	0.00c	0.00c	0.00c	0.83c	2.50c	9.50c	73
	1500	0.00b	0.00c	0.00c	0.00c	0.00c	0.00d	0.00d	100
	2000	0.00b	0.00c	0.00c	0.00c	0.00c	0.00d	0.00d	100
	LSD(p=0.05)	0.5292	0.8771	0.7095	0.8250	0.9564	1.060	1.603	
Bavistin 50WP	Control	2.00a	5.00a	9.00a	12.00	20.50a	31.17a	38.00a	
	500	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.83b	97
	1000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.33b	99
	1500	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	2000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	LSD(p=0.05)	0.4210	0.4210	0.8420	0.8420	0.4210	0.6440	0.9186	

Each data presents mean of three replications; D represents Day

4.4.1.6. Tolerance of isolate M6 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on isolate M6 over untreated control by 93%, 90% and 77% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. No growth of Isolate M6 has been observed in Indofil M- 45 at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. 1000 $\mu\text{g/ml}$ and 1500 $\mu\text{g/ml}$ resulted statistically similar effect on radial mycelial growth but differed significantly from other treatments. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate M6. It was observed that Cupravit 50WP reduced the mycelial growth of isolate M6 over untreated control by 66% and 6% at 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate M6 could not grow in Cupravit 50WP at 1500 $\mu\text{g/ml}$ up to 7 days incubation period. Bavistin 50WP reduced the mycelial growth of M6 isolate over untreated control by 99.25%, 92.05% at 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate M6 failed to grow in Bavistin 50WP at 1500 $\mu\text{g/ml}$ up to 7 days incubation period. 1000 $\mu\text{g/ml}$, 1500 and 2000 $\mu\text{g/ml}$ resulted statistically similar effect on radial mycelial growth but differed significantly from other two treatments (Table- 11).

Table 11. Tolerance of Isolate M6 fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							% growth inhibition
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
Indofil M-45	Control	1.00a	4.17a	7.17a	11.17a	15.00a	20.17a	22.67a	
	500	0.00b	0.00b	0.00b	0.33b	1.17b	3.167b	5.17b	77
	1000	0.00b	0.00b	0.00b	0.00b	0.67bc	1.50c	2.17c	90
	1500	0.00b	0.00b	0.00b	0.00b	0.33bc	0.50d	1.50c	93
	2000	0.00b	0.00b	0.00b	0.00b	0.00c	0.00d	0.00d	100
	LSD(p=0.05)	0.8420	0.8771	0.6440	0.9262	0.9564	0.8931	0.8420	
Cupravit 50WP	Control	1.33a	2.33a	5.33a	12.67a	18.67a	25.33a	36.33a	
	500	0.67b	1.00b	4.17b	9.83b	16.67b	24.17b	33.33b	8
	1000	0.00c	0.00c	0.67c	1.00c	2.33c	6.67c	12.33c	66
	1500	0.00c	0.00c	0.00d	0.00d	0.00d	0.00d	0.00d	100
	2000	0.00c	0.00c	0.00d	0.00d	0.00d	0.00d	0.00d	100
	LSD(p=0.05)	0.3206	0.4874	0.4534	0.6763	0.6188	0.8055	0.5954	
Bavistin 50WP	Control	3.00a	8.00a	15.00a	20.00a	29.50a	34.00a	44.00a	
	500	0.00b	0.00b	0.00b	0.00b	1.33b	2.50b	3.50b	92
	1000	0.00b	0.00b	0.00b	0.00b	0.00b	0.83c	0.33c	99
	1500	0.00b	0.00b	0.00b	0.00b	0.00b	0.00c	0.00c	100
	2000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00c	0.00c	100
	LSD(p=0.05)	0.4210	0.4210	0.8420	1.031	4.723	1.080	0.9489	

Each data presents mean of three replications; D represents Day

4.4.2. Fungicide tolerance of *Colletotrichum* spp. isolates collected from guava

4.4.2.1. Tolerance of isolate G1 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on isolate G1 over untreated control by 60%, 40% and 32% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate G1 could not grow in Indofil M- 45 at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate G1. It was observed that Cupravit 50WP reduced the mycelial growth of isolate G1 over untreated control by 65.51% and 15.17% at 1000 and 500 $\mu\text{g/ml}$ respectively. No growth of Isolate G1 has been observed in Cupravit 50WP at 1500 $\mu\text{g/ml}$ up to 7 days incubation period. Bavistin 50WP reduced the mycelial growth of G1 isolate over untreated control by 87.76% at 500 $\mu\text{g/ml}$. Isolate G1 failed to grow in Bavistin 50WP at 1000 $\mu\text{g/ml}$ up to 7 days incubation period (Table- 12).

Table 12. Tolerance of Isolate G1 to fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							% growth inhibition
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
Indofil M-45	Control	5.00a	13.17a	19.00a	27.67a	36.83a	42.00a	48.17a	
	500	2.50b	5.66b	10.83b	18.83b	26.00b	30.17b	32.83b	32
	1000	1.67b	5.00b	8.83c	14.33c	21.17c	26.00c	28.83c	40
	1500	1.17bc	3.50c	6.50d	8.83d	13.17d	17.17d	19.33d	60
	2000	0.00c	0.00d	0.00e	0.00	0.00e	0.00e	0.00e	100
	LSD(p=0.05)	1.347	1.443	1.518	1.269	1.045	1.134	1.566	
Cupravit 50WP	Control	2.83a	6.33a	11.33a	16.33a	22.83a	30.83b	48.33a	
	500	0.83b	2.00b	2.67b	8.67b	20.67b	35.67a	41.00b	15
	1000	0.00c	0.00c	0.00c	0.00c	1.00c	6.50c	16.67c	65
	1500	0.00c	0.00c	0.00c	0.00c	0.00c	0.00d	0.00d	100
	2000	0.00c	0.00c	0.00c	0.00c	0.00c	0.00d	0.00d	100
	LSD(p=0.05)	0.8142	0.5711	0.6440	0.6440	1.509	0.9262	1.101	
Bavistin 50WP	Control	2.16a	11.33a	16.00a	29.17a	40.00a	49.00a	57.17a	
	500	0.00b	1.00b	1.50b	2.00b	3.16b	4.00b	7.00b	87
	1000	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	1500	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	2000	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	LSD(p=0.05)	0.2455	0.6440	0.8931	0.9262	0.9262	1.031	0.9262	

Each data presents mean of three replications; D represents Day



4.4.2.2. Tolerance of isolate G2 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on isolate G2 over untreated control by 55%, 48% and 24% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. No growth of Isolate G2 has been observed in Indofil M- 45 at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate G2. It was observed that Cupravit 50WP reduced the mycelial growth of isolate G2 over untreated control by 94% , 84% and 66% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate G2 failed to grow in Cupravit 50WP at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. Bavistin 50WP reduced the mycelial growth of G2 isolate over untreated control by 80.79% at 500 $\mu\text{g/ml}$. Isolate G2 could not grow in Bavistin 50WP at 1000 $\mu\text{g/ml}$ up to 7 days incubation period (Table- 13).

Table 13. Tolerance of Isolate G2 to fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	% growth inhibition
Indofil M-45	Control	3.50a	6.17a	11.33a	15.17a	23.00a	34.00a	45.67a	
	500	0.00b	1.00b	2.33b	3.00b	13.33b	24.67b	34.67b	24
	1000	0.00b	0.00b	1.33bc	1.83c	7.33c	15.17c	23.67c	48
	1500	0.00b	0.00b	0.67c	0.83d	5.17d	11.17d	20.67d	55
	2000	0.00b	0.00b	0.00c	0.00d	0.00e	0.00e	0.00e	100
	LSD($p=0.05$)	0.4210	1.449	1.418	0.9089	1.147	1.458	0.9728	98
Cupravit 50WP	Control	9.83a	13.67a	25.83a	30.67a	35.50a	41.33a	45.00a	
	500	6.67b	11.67b	13.33b	13.67b	15.17b	15.00b	15.50b	66
	1000	0.00c	0.00c	4.667c	5.00c	5.83c	6.50c	7.33c	84
	1500	0.00c	0.00c	0.00d	0.00d	0.00d	0.83d	2.50d	94
	2000	0.00c	0.00c	0.00d	0.00d	0.00d	0.00e	0.00e	100
	LSD($p=0.05$)	0.5711	0.8931	1.002	0.6969	0.5711	0.6994	1.120	
Bavistin 50WP	Control	4.16a	9.00a	13.00a	21.00 a	27.33a	30.33a	33.83a	
	500	0.00b	0.00b	0.00b	0.83b	1.83b	4.50b	6.50b	80
	1000	0.00b	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	100
	1500	0.00b	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	100
	2000	0.00b	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	100
	LSD($p=0.05$)	0.6440	0.8420	0.8420	0.8250	0.5156	0.5711	0.4375	

Each data presents mean of three replications; D represents Day

4.4.2.3. Tolerance of isolate G3 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth of isolate G3 over untreated control by 40%, 18% and 10% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate G3 could not grow in Indofil M- 45 at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate G3. It was observed that Cupravit 50WP reduced the mycelial growth of isolate G3 over untreated control by 87% and 9% at 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate G3 failed to grow in Cupravit 50WP at 1500 $\mu\text{g/ml}$ up to 7 days incubation period. Bavistin 50WP reduced the mycelial growth of G3 isolate over untreated control by 94.54% and 86.60% at 1000 and 500 $\mu\text{g/ml}$. No growth of Isolate G3 has been observed in Bavistin 50WP at 1500 $\mu\text{g/ml}$ up to 7 days incubation period (Table- 14).

Table 14. Tolerance of Isolate G3 to fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	% growth inhibition
Indofil M-45	Control	3.50a	11.67a	18.67a	22.50a	28.17a	34.33a	38.67a	
	500	0.33c	5.00b	10.17b	17.33b	25.50b	29.67b	34.50b	10
	1000	2.17b	5.17b	10.17b	13.00c	16.17c	28.17c	31.33c	18
	1500	0.67c	3.67c	8.833c	9.83d	13.00d	17.83d	23.00d	40
	2000	0.00c	0.00d	0.00d	0.00e	0.00e	0.00e	0.00e	100
	LSD(p=0.05)	0.8851	0.8931	0.8771	1.191	1.191	1.147	1.252	
Cupravit 50WP	Control	3.33a	13.17a	13.50a	16.50a	20.67a	25.67a	34.67a	
	500	0.00b	0.83b	5.50b	13.33b	18.67b	21.83b	31.50b	9
	1000	0.00b	0.00c	0.00c	0.00c	0.00c	0.83c	4.67c	86
	1500	0.00b	0.00c	0.00c	0.00c	0.00c	0.00d	0.00d	100
	2000	0.00b	0.00c	0.00c	0.00c	0.00c	0.00d	0.00d	100
	LSD(p=0.05)	0.2455	0.6763	0.5156	0.5711	0.5156	0.4534	0.5954	
Bavistin 50WP	Control	1.00a	2.33a	7.00a	15.50a	22.33a	30.33a	27.33a	
	500	0.00b	0.00b	0.33b	3.50b	5.50b	7.16b	8.00b	70.73
	1000	0.00b	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	100
	1500	0.00b	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	100
	2000	0.00b	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	100
	LSD(p=0.05)	0.7292	0.8771	0.8771	0.5554	0.5711	0.7095	0.8771	

Each data presents mean of three replications; D represents Day

4.4.2.4. Tolerance of isolate G4 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on isolate G4 over untreated control by 82%, 82% and 63% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate G4 could not grow in Indofil M- 45 at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. 1000 $\mu\text{g/ml}$ and 1500 $\mu\text{g/ml}$ resulted statistically similar effect on radial mycelial growth but differed significantly from other treatments. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate G4. It was observed that Cupravit 50WP reduced the mycelial growth of isolate G4 over untreated control by 93% and 18% at 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate G4 failed to grow in Cupravit 50WP at 1500 $\mu\text{g/ml}$ up to 7 days incubation period. 1000, 1500 and 2000 $\mu\text{g/ml}$ resulted statistically similar effect on radial mycelial growth but differed significantly from two treatments. Bavistin 50WP reduced the mycelial growth of G4 isolate over untreated control by 70.73% at 500 $\mu\text{g/ml}$. No growth of Isolate G4 has been observed in Bavistin 50WP at 1000 $\mu\text{g/ml}$ up to 7 days incubation period (Table- 15).

Table 15. Tolerance of Isolate G4 to fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							% growth inhibition
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
Indofil M- 45	Control	1.33a	2.167a	3.83a	6.00a	10.17a	14.00a	19.17a	
	500	0.00b	0.00b	0.83b	3.00b	4.17b	5.33b	7.00b	63
	1000	0.00b	0.00b	0.00c	0.67c	2.17c	3.00c	3.50c	82
	1500	0.00b	0.00b	0.00c	0.83c	1.83c	3.00c	3.50b	82
	2000	0.00b	0.00b	0.00c	0.00c	0.00c	0.00d	0.00d	100
	LSD(p=0.05)	0.4874	0.6440	0.7877	1.418	1.753	1.178	1.649	
Cupravit 50WP	Control	4.17a	10.33a	18.00a	24.17a	30.50a	34.50a	40.67a	
	500	0.00b	2.66b	10.00b	15.67b	21.33b	25.83b	33.33b	18
	1000	0.00b	0.00c	0.00c	0.00c	0.00c	1.00c	2.83c	93
	1500	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	2000	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	LSD(p=0.05)	0.2455	0.6440	0.8931	0.7877	0.5292	1.233	4.080	
Bavistin 50WP	Control	0.67a	5.67a	12.83a	19.50a	23.50a	28.00a	33.50a	
	500	0.00b	0.00b	1.16b	2.00b	2.67b	3.33b	4.00b	88
	1000	0.00b	0.00b	0.00c	0.00c	0.16c	0.83c	1.83c	94
	1500	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	0.00d	100
	2000	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	0.00d	100
	LSD(p=0.05)	0.4874	0.4874	0.7389	3.322	0.9186	0.8690	0.6763	

Each data presents mean of three replications; D represents Day

4.4.2.5. Tolerance of isolate G5 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on isolate G5 over untreated control by 71%, 35% and 22% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. No growth of Isolate G5 has been observed in Indofil M- 45 at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate G5. It was observed that Cupravit 50WP reduced the mycelial growth of isolate G5 over untreated control by 76.88% and 72.98% at 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate G5 could not grow in Cupravit 50WP at 1500 $\mu\text{g/ml}$ up to 7 days incubation period. Tolerance study clearly showed that isolate G5 failed to grow in even 500 μg Bavistin/ml i.e, Bavistin 50WP totally inhibited the mycelial growth of isolate G5 at 500 $\mu\text{g/ml}$ after 7 days of incubation (Table- 16).

Table 16. Tolerance of Isolate G5 fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	% growth inhibition
Indofil M-45	Control	3.50a	9.00a	13.83a	18.67a	23.50a	28.50a	35.67a	
	500	1.67b	6.00b	10.50b	15.33b	21.33b	23.67b	27.67b	22
	1000	1.17bc	5.33b	6.83c	12.00c	15.50c	20.00c	23.17c	35
	1500	0.00c	0.33c	1.17d	3.00d	5.50d	7.67d	10.33d	71
	2000	0.00c	0.00c	0.00d	0.00e	0.00e	0.00e	0.00e	100
	LSD(p=0.05)	1.575	1.684	1.239	1.899	1.433	1.073	1.551	
	Control	1.17a	4.50a	9.67a	12.50a	15.83a	39.33a	51.17a	
Cupravit 50WP	500	0.67a	1.83b	2.17c	3.83c	5.50c	8.67b	11.83c	76
	1000	0.00b	0.50c	3.50b	4.50b	6.67b	8.17b	13.83b	72
	1500	0.00b	0.00c	0.00d	0.00d	0.00d	0.00c	0.00d	100
	2000	0.00b	0.00c	0.00d	0.00d	0.00d	0.00c	0.00d	100
	LSD(p=0.05)	0.5156	0.8771	0.9186	0.6440	0.7966	0.7484	0.8055	
	Control	3.00a	7.00a	15.00a	23.00a	30.67 a	36.33a	45.33a	
Bavistin 50WP	500	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	1000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	1500	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	2000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	LSD(p=0.05)	0.8420	0.8420	0.8420	0.8420	0.4874	0.4874	0.4874	

Each data presents mean of three replications; D represents Day

4.4.3. Fungicide tolerance of *Colletotrichum* isolates collected from litchi

4.4.2.1. Tolerance of isolate L1 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on isolate L1 over untreated control by 62%, 49% and 30% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate L1 failed to grow in Indofil M- 45 at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate L1. It was observed that Cupravit 50WP reduced the mycelial growth of isolate L1 over untreated control by 37.63% 500 $\mu\text{g/ml}$ respectively. No growth of Isolate L1 has been observed in Cupravit 50WP at 1000 $\mu\text{g/ml}$ up to 7 days incubation period. Bavistin 50WP reduced the mycelial growth of L1 isolate over untreated control by 96.74 and 76.10% at 1000 and 500 $\mu\text{g/ml}$. Isolate L1 could not grow in Bavistin 50WP at 1500 $\mu\text{g/ml}$ up to 7 days incubation period (Table-17).

Table 17. Tolerance of Isolate L1 fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							% growth inhibition
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
Indofil M-45	Control	5.17a	14.00a	16.83a	19.83a	28.17a	32.33a	36.83a	
	500	1.33b	2.60b	7.00b	12.17b	19.33b	23.50b	25.67b	30
	1000	1.00bc	2.67b	7.17b	10.00bc	15.17c	15.67c	18.67c	49
	1500	0.33bc	1.67bc	6.00b	7.83c	11.00d	13.00d	14.17d	62
	2000	0.00c	0.00c	0.00c	0.00d	0.00e	0.00e	0.00e	100
	LSD(p=0.05)	1.309	1.812	1.635	2.214	1.769	1.141	1.269	
Cupravit 50WP	Control	4.50a	14.33a	23.67a	33.33a	41.17a	47.50a	63.33a	
	500	0.00b	5.50b	10.83b	22.00b	32.00b	34.33b	39.50b	44
	1000	0.00b	0.50c	0.00c	0.00c	0.00c	0.00c	0.00c	55
	1500	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	83
	2000	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	LSD(p=0.05)	0.4210	0.7389	0.8504	0.8771	5.067	0.5711	0.5711	
Bavistin 50WP	Control	1.00a	4.667a	9.00a	15.33 a	20.17a	25.33a	30.67a	
	500	0.00b	0.00b	0.33b	1.16 b	3.83 b	6.50b	7.33b	76.10
	1000	0.00b	0.00b	0.00c	0.00c	0.00c	0.33c	1.00c	96.74
	1500	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	0.00d	100
	2000	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	0.00d	100
	LSD(p=0.05)	0.8420	1.287	0.8771	0.9262	2.747	0.7877	0.7095	

Each data presents mean of three replications; D represents Day



4.4.3.2. Tolerance of isolate L2 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on isolate L2 over untreated control by 71%, 57% and 46% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. No growth of Isolate L2 has been observed in Indofil M- 45 at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate L2. It was observed that Cupravit 50WP reduced the mycelial growth of isolate L2 over untreated control by 79, 44% and 30% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate L2 failed to grow in Cupravit 50WP at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. Bavistin 50WP reduced the mycelial growth of L2 isolate over untreated control by 99.60% at 500 $\mu\text{g/ml}$. Isolate L2 could not grow in Bavistin 50WP at 1000 $\mu\text{g/ml}$ up to 7 days incubation period. 500, 1000, 1500 and 2000 $\mu\text{g/ml}$ resulted statistically similar effect on radial mycelial growth but differed significantly from control (Table- 18).

Table 18. Tolerance of Isolate L2 fungicides.

	Concentration (µg/ml)	Radial mycelial growth(mm)							% growth inhibition
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
Indofil M-45	Control	2.17a	8.33a	12.00a	27.17a	34.50a	40.33a	47.50a	
	500	0.00b	0.33b	4.50b	13.00b	19.50b	22.67b	25.83b	46
	1000	0.00b	0.50b	4.00b	8.17c	11.17c	14.33c	20.50c	57
	1500	0.00b	0.00b	1.17b	2.83d	6.00d	10.17d	13.67d	71
	2000	0.00b	0.00b	0.00b	0.00e	0.00e	0.00e	0.00e	100
	LSD(p=0.05)	0.6440	0.5954	4.807	1.045	0.8250	0.6867	0.7095	
Cupravit 50WP	Control	5.50a	12.33a	21.67a	28.67a	36.00a	42.17a	50.83a	
	500	2.67b	8.50b	14.33b	15.50b	21.00b	25.50b	35.33b	30
	1000	0.83c	3.67c	4.33c	8.33c	14.17c	22.67c	28.33c	44
	1500	0.00c	0.00d	0.00d	0.83d	2.67d	5.50d	10.67d	79
	2000	0.00c	0.00d	0.00d	0.00d	0.00e	0.00e	0.00e	100
	LSD(p=0.05)	0.9729	0.6440	1.002	0.8851	1.114	0.5954	0.8250	
Bavistin 50WP	Control	5.167a	7.167a	14.50a	21.67a	28.67a	35.00a	40.33a	
	500	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.16b	99
	1000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	1500	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	2000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	LSD(p=0.05)	0.8771	0.6440	0.4210	0.4874	0.4874	0.8420	0.5711	

Each data presents mean of three replications; D represents Day

4.4.3.3. Tolerance of isolate L3 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on isolate L3 over untreated control by 62%, 38% and 31% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate L3 failed to grow in Indofil M- 45 at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate L3. It was observed that Cupravit 50WP reduced the mycelial growth of isolate L3 over untreated control by 61% at 500 $\mu\text{g/ml}$. Isolate L3 could not grow in Cupravit 50WP at 1000 $\mu\text{g/ml}$ up to 7 days incubation period. Bavistin 50WP reduced the mycelial growth of L3 isolate over untreated control by 68.05% at 500 $\mu\text{g/ml}$. No growth of Isolate L3 has been observed in Bavistin 50WP at 1000 $\mu\text{g/ml}$ up to 7 days incubation period (Table- 19).

Table 19. Tolerance of Isolate L3 fungicides.

	Concentration (µg/ml)	Radial mycelial growth(mm)							% growth inhibition
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
Indofil M- 45	Control	2.17a	5.17a	12.17a	19.00a	24.17a	29.33a	33.67a	
	500	1.00b	2.50b	5.33b	11.50b	15.67b	17.83b	23.33b	31
	1000	0.50bc	1.33c	3.17c	7.00c	10.50c	13.67c	21.00c	38
	1500	0.00c	0.33cd	1.67d	3.00d	6.17d	9.33d	12.67d	62
	2000	0.00c	0.00d	0.00e	0.00e	0.00e	0.00e	0.00e	100
	LSD(p=0.05)	0.7095	1.024	1.101	1.114	1.073	1.321	0.6867	
Cupravit 50WP	Control	5.83a	12.00a	21.67a	28.83a	36.67a	42.33a	45.50a	
	500	0.83b	8.17b	14.50b	15.67b	16.17b	16.67b	17.67b	61
	1000	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	1500	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	2000	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	LSD(p=0.05)	0.6301	0.9729	0.5711	0.7095	0.6329	0.7095	0.5711	
Bavistin 50WP	Control	0.67a	2.50a	6.00a	12.83a	19.67a	25.00a	31.83a	
	500	0.00b	2.16a	3.00b	4.16b	6.16b	8.16b	10.17b	68.05
	1000	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	100
	1500	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	100
	2000	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	100
	LSD(p=0.05)	0.4874	0.8250	0.9874	0.7389	1.263	1.141	1.956	

Each data presents mean of three replications; D represents Day

4.4.4. Fungicide tolerance of *Colletotrichum* isolates collected from Lemon

4.4.4.1. Tolerance of isolate Le1 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on isolate Le1 over untreated control by 85%, 79% and 53% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. 1000 $\mu\text{g/ml}$ and 1500 $\mu\text{g/ml}$ resulted statistically similar effect on radial mycelial growth but differed significantly from other treatments. No growth of Isolate Le1 has been observed in Indofil M- 45 at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate Le1. It was observed that Cupravit 50WP reduced the mycelial growth of isolate Le1 over untreated control by 67% at 500 $\mu\text{g/ml}$. Isolate Le1 failed to grow in Cupravit 50WP at 1000 $\mu\text{g/ml}$ up to 7 days incubation period. Bavistin 50WP reduced the mycelial growth of Le1 isolate over untreated control by 97.57% and 93.18 at 1000 and 500 $\mu\text{g/ml}$. Isolate Le1 could not grow in Bavistin 50WP at 1500 $\mu\text{g/ml}$ up to 7 days incubation period (Table- 20).



Table 20. Tolerance of Isolate Le1 fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							% growth inhibition
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
Indofil M-45	Control	2.17a	5.17a	12.17a	19.00a	24.17a	29.33a	33.67a	
	500	1.00b	2.50b	5.33b	11.50b	15.67b	17.83b	23.33b	31
	1000	0.50bc	1.33c	3.17c	7.00c	10.50c	13.67c	21.00c	38
	1500	0.00c	0.33cd	1.67d	3.00d	6.17d	9.33d	12.67d	62
	2000	0.00c	0.00d	0.00e	0.00e	0.00e	0.00e	0.00e	100
	LSD(p=0.05)	0.7095	1.024	1.101	1.114	1.073	1.321	0.6867	
Cupravit 50WP	Control	5.83a	12.00a	21.67a	28.83a	36.67a	42.33a	45.50a	
	500	0.83b	8.17b	14.50b	15.67b	16.17b	16.67b	17.67b	61
	1000	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	1500	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	2000	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	LSD(p=0.05)	0.6301	0.9729	0.5711	0.7095	0.6329	0.7095	0.5711	
Bavistin 50WP	Control	0.67a	2.50a	6.00a	12.83a	19.67a	25.00a	31.83a	
	500	0.00b	2.16a	3.00b	4.16b	6.16b	8.16b	10.17b	68.05
	1000	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	100
	1500	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	100
	2000	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	100
	LSD(p=0.05)	0.4874	0.8250	0.9874	0.7389	1.263	1.141	1.956	

Each data presents mean of three replications; D represents Day

4.4.4.2. Tolerance of isolate Le2 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on isolate Le2 over untreated control by 92%, 86% and 14% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate Le2 failed to grow in Indofil M- 45 at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. Performance of 1500 $\mu\text{g/ml}$ treatment dose not significantly differs with 1000 and 2000 $\mu\text{g/ml}$. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate Le2. It was observed that Cupravit 50WP reduced the mycelial growth of isolate Le2 over untreated control by 89% and 71% at 1000 and 500 $\mu\text{g/ml}$ respectively. Isolate Le2 could not grow in Cupravit 50WP at 1500 $\mu\text{g/ml}$ up to 7 days incubation period. Bavistin 50WP reduced the mycelial growth of Le2 isolate over untreated control by 92.39%, 81.62% at 1000 and 500 $\mu\text{g/ml}$. No growth of Isolate Le2 has been observed in Bavistin 50WP at 1500 $\mu\text{g/ml}$ up to 7 days incubation period (Table- 21).

Table 21. Tolerance of Isolate Le2 fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							% growth inhibition
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
Indofil M- 45	Control	0.00	2.17a	5.00a	7.33a	9.00a	12.00a	13.83a	
	500	0.00	0.17b	0.67b	4.50b	6.67b	11.50a	11.83b	14
	1000	0.00	0.00b	0.00b	0.00c	0.67c	1.00b	2.00c	86
	1500	0.00	0.00b	0.00b	0.00c	0.50c	0.83b	1.17cd	92
	2000	0.00	0.00b	0.00b	0.00c	0.00c	0.00b	0.00d	100
	LSD($p=0.05$)	0.00	0.3670	1.060	1.038	1.227	1.354	1.309	
Cupravit 50WP	Control	4.00a	9.50a	16.00a	22.33a	30.50a	36.33a	51.67a	
	500	1.00b	2.50b	4.33b	5.00b	8.50b	13.83b	14.83b	71
	1000	0.00c	0.00c	0.00c	0.00c	1.17c	3.17c	5.50c	89
	1500	0.00c	0.00c	0.00c	0.00c	0.00d	0.00d	0.00d	100
	2000	0.00c	0.00c	0.00c	0.00c	0.00d	0.00d	0.00d	100
	LSD($p=0.05$)	0.5554	0.5585	0.6440	0.9262	0.7095	0.6549	0.9945	
Bavistin 50WP	Control	2.16a	7.16a	10.67a	17.17a	24.27a	29.00a	37.17a	
	500	0.00b	0.33b	1.33b	1.83b	2.83b	5.83b	6.83b	81
	1000	0.00b	0.00b	0.00c	0.00c	1.33c	2.00c	2.83c	92
	1500	0.00b	0.00b	0.00c	0.00c	0.00d	0.00d	0.00d	100
	2000	0.00b	0.00b	0.00c	0.00c	0.00d	0.00d	0.00d	100
	LSD($p=0.05$)	0.6440	0.9729	1.252	0.8504	1.024	1.197	1.342	

Each data presents mean of three replications; D represents Day

4.4.4.3. Tolerance of isolate Le3 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on isolate Le3 over untreated control by 92%, 86% and 14% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. No growth of Isolate Le3 has been observed in Indofil M- 45 at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate Le3. It was observed that Cupravit 50WP reduced the mycelial growth of isolate Le3 over untreated control by 77% at 500 $\mu\text{g/ml}$. Isolate Le3 could not grow in Cupravit 50WP at 1000 $\mu\text{g/ml}$ up to 7 days incubation period. Tolerance study clearly showed that isolate Le3 failed to grow in even 500 μg Bavistin/ml i.e, Bavistin 50WP totally inhibited the mycelial growth of isolate Le3 at 500 $\mu\text{g/ml}$ after 7 days of incubation (Table- 22).



Table 22. Tolerance of Isolate Le3 fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							% growth inhibition
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
Indofil M-45	Control	0.00	2.00a	7.67a	10.00a	13.83a	14.17a	15.17a	
	500	0.00	0.00b	0.33b	1.50b	3.67b	5.00b	6.50b	57
	1000	0.00	0.00b	0.17b	1.50b	3.67b	4.83b	6.33b	58
	1500	0.00	0.00b	0.00b	0.67b	2.67b	3.33b	4.17c	73
	2000	0.00	0.00b	0.00b	0.00b	0.00c	0.00c	0.00d	100
	LSD(p=0.05)	0.00	0.4210	1.342	2.044	1.509	1.744	1.556	
Cupravit 50WP	Control	2.67a	7.50a	10.67a	14.33a	21.17a	25.83a	32.67a	
	500	0.83b	2.83b	5.66b	6.00b	6.67b	7.17b	7.67b	77
	1000	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	1500	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	2000	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	100
	LSD(p=0.05)	0.5711	0.4874	0.5954	0.9489	0.5156	0.4874	0.5954	
Bavistin 50WP	Control	3.83a	7.16a	12.33a	21.00a	26.17a	33.17a	41.50a	
	500	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	1000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	1500	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	2000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	100
	LSD(p=0.05)	0.6440	0.6440	0.8771	0.8420	0.6440	0.6440	1.518	

Each data presents mean of three replications; D represents Day

4.4.4.4. Tolerance of isolate Le4 to fungicides

It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on isolate Le4 over untreated control by 70%, 32% and 27% at 1500, 1000 and 500 $\mu\text{g/ml}$ respectively. No growth of Isolate Le4 has been observed in Indofil M- 45 at 2000 $\mu\text{g/ml}$ up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of isolate Le4. It was observed that Cupravit 50WP reduced the mycelial growth of isolate Le4 over untreated control by 85% at 500 $\mu\text{g/ml}$. Isolate Le4 could not grow in Cupravit 50WP at 1000 $\mu\text{g/ml}$ up to 7 days incubation period . Bavistin 50WP reduced the mycelial growth of Le4 isolate over untreated control by 97.47% at 500 $\mu\text{g/ml}$. Isolate Le4 failed to grow in Bavistin 50WP at 1000 $\mu\text{g/ml}$ up to 7 days incubation period (Table- 23).

Table 23. Tolerance of Isolate Le4 to fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							% growth inhibition
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
Indofil M-45	Control	1.50a	5.33a	11.00a	16.17a	23.17a	28.00a	30.33a	
	500	0.67b	2.50b	3.50b	7.00b	11.17b	16.17b	22.00b	27
	1000	0.33b	1.50c	2.67b	5.83c	10.17c	14.33c	20.50c	32
	1500	0.00b	0.00d	1.50c	2.83d	5.00d	7.17d	9.00d	70
	2000	0.00b	0.00d	0.00d	0.00e	0.00e	0.00e	0.00e	100
	LSD($p=0.05$)	0.8250	0.9262	0.9945	0.7484	0.8931	1.406	1.449	
Cupravit 50WP	Control	1.67a	6.17a	10.83a	13.83a	19.50a	25.17a	31.33a	
	500	0.00b	0.00b	0.00b	1.67b	2.00b	2.83b	4.83b	85
	1000	0.00b	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	100
	1500	0.00b	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	100
	2000	0.00b	0.00b	0.00b	0.00c	0.00c	0.00c	0.00c	100
	LSD($p=0.05$)	0.2455	0.2455	0.2455	0.7389	0.6329	0.7389	0.5156	
Bavistin 50WP	Control	2.00a	3.83a	7.50a	13.50a	20.83a	25.67a	32.83a	
	500	0.00b	0.00b	0.00b	0.00b	0.00b	0.33b	0.83b	97
	1000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00c	100
	1500	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00c	100
	2000	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00c	100
	LSD($p=0.05$)	0.4210	0.6440	0.4210	0.4210	0.2455	0.6440	0.3670	

Each data presents mean of three replications; D represents Day

Table 24. Tolerance of Isolate Jm1 to fungicides.

	Concentration ($\mu\text{g/ml}$)	Radial mycelial growth(mm)							
		1 st day	2nd day	3rd day	4th day	5th day	6th day	7th day	% growth inhibition
Indofil M-45	Control	3.00a	7.83a	16.17a	20.50a	23.83a	35.33a	48.67a	
	500	0.00b	0.83b	6.00b	9.00b	13.83b	23.83b	34.33b	29
	1000	0.00b	0.33b	2.67c	4.00c	7.67c	17.83c	24.83c	49
	1500	0.00b	0.17b	1.50cd	2.67c	7.00c	12.83d	21.67	55
	2000	0.00b	0.00b	0.00d	0.00d	0.00d	0.00e	0.00e	100
	LSD($p=0.05$)	0.8420	1.354	1.603	1.852	1.203	1.287	2.194	
Cupravit 50WP	Control	2.00a	9.67a	14.50a	24.17a	36.33a	45.33a	55.50a	
	500	1.33b	5.50b	11.67b	16.50b	24.67b	34.00b	39.83b	28
	1000	0.83b	3.67c	9.17c	14.17c	14.67c	15.00c	15.67c	72
	1500	0.00c	0.00d	1.17d	0.00d	1.83d	4.50d	5.33d	90
	2000	0.00c	0.00d	0.00e	0.00d	0.00e	0.00e	0.00e	100
	LSD($p=0.05$)	0.5954	0.6994	0.7292	1.759	1.127	1.233	1.292	
Bavistin 50WP	Control	3.83a	9.16a	16.00a	23.17a	27.17a	34.00a	36.17a	
	500	0.00b	0.00b	0.00b	0.50b	2.16b	4.00b	7.50b	79.26
	1000	0.00b	0.00b	0.00b	0.00b	0.00c	0.00c	0.00c	100
	1500	0.00b	0.00b	0.00b	0.00b	0.00c	0.00c	0.00c	100
	2000	0.00b	0.00b	0.00b	0.00b	0.00c	0.00c	0.00c	100
	LSD($p=0.05$)	0.6440	0.6440	0.8420	1.101	1.209	1.114	0.7389	

Each data presents mean of three replications; D represents Day

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4.5. Interaction study

4.5.1. Antagonistic effect of *Trichoderma harzianum* with *Colletotrichum* spp. (7 days of incubation period)

Trichoderma inhibited the growth of all 19 isolates of *Colletotrichum* spp. (Table-25). In case of control the fungus *Colletotrichum* spp. grew well and produced luxuriant mycelia.

4.5.2. Antagonistic effect of *Trichoderma harzianum* with *Colletotrichum* spp. (15 days of incubation period)

It was observed that in all the cases *Trichoderma harzianum* grew over the *Colletotrichum* spp. and lysed zone was formed at the point of meeting (Table- 25). In case of control, luxuriant mycelial growths were observed.

Table-25: Interaction of *Colletotrichum* isolates and *Trichoderma harzianum* (Dual culture method).

Name of isolates	Interaction after	
	7 days	15 days
M1	TIS	TGS + Lysed
M2	TIS	TGS + Lysed
G1	TIS	TGS + Lysed
G2	TIS	TGS + Lysed
L1	TIS	TGS + Lysed
L2	TIS	TGS + Lysed
Le2	TIS	TGS + Lysed
Le3	TIS	TGS + Lysed

TIS= *Trichoderma* inhibited growth of *Colletotrichum* spp.

TGS= *Trichoderma* grew over *Colletotrichum* spp.



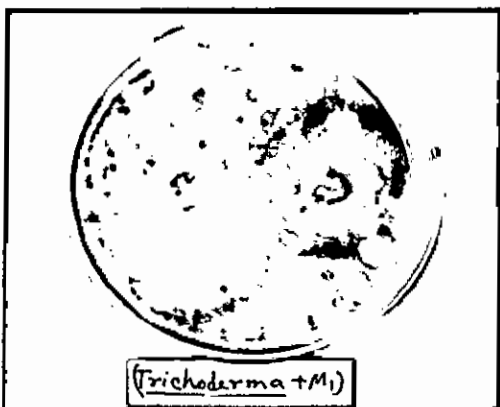


Plate 10. Dual culture of *Trichoderma*+M1 Isolate

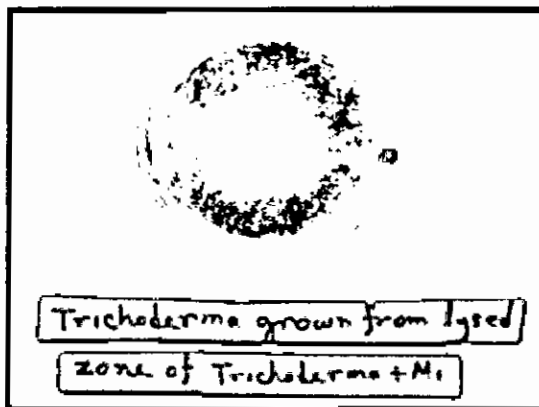
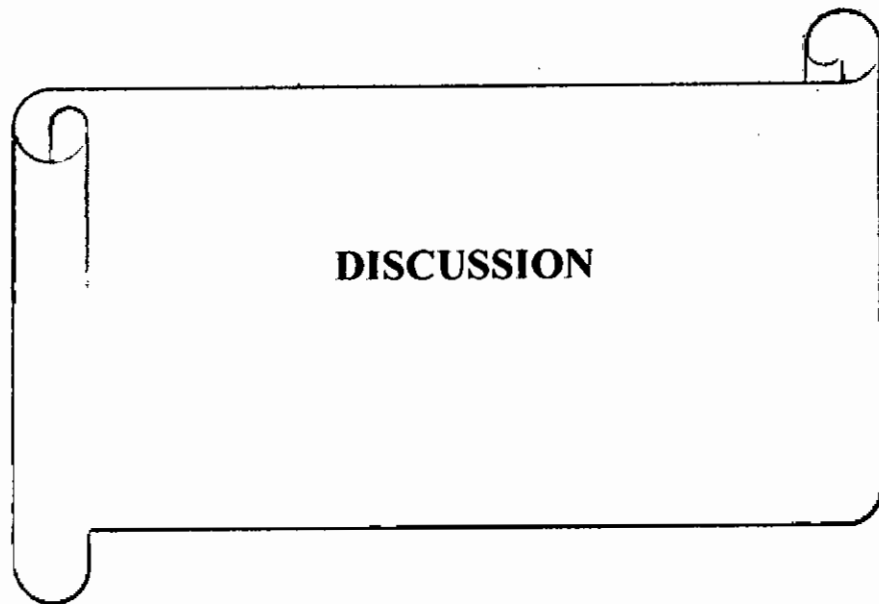


Plate 11. growth of *Trichoderma* from lysed zone



DISCUSSION

CHAPTER 5

DISCUSSION

The present study was undertaken to analyze nineteen isolates of *Colletotrichum* spp. morphologically, physiologically and on the basis of bioassay against three fungicides and one bio controlling agent. Isolates were collected from anthracnose diseased leaf samples of mango, guava, litchi, lemon and jamun seedling showing typical anthracnose symptoms. Morphological study was done on the basis of colony color and measurement of conidial length and breadth of isolates to find out differences on isolates conidial length and breadth. Physiological study was done on the basis of mycelial growth of isolates at different temperature to find out best suited and inhibited growth temperature for the fungus. Bioassay was done on the basis of response of isolates to three fungicides namely Bavistin 50 WP, Cupravit 50 WP and Indofil M- 45 and bio control agent *Trichoderma harzianum*.

The length and breadth of the conidia of eighteen isolates of *Colletotrichum gloeosporioides* and one isolate *Gloeosporium* were measured. Highest and lowest length of conidia 17.6 and 7.1 μ m measured for Le3 and G3 isolates respectively. Highest and lowest Breadth of conidia 4.8 and 2 μ m measured for L3 and G3 respectively. Bose *et al.* (1953) reported that the size of conidia varied from 11-16 x 4-6 μ m. Holiday (1980) reported that the conidia were hyaline, unicellular and highly cylindrical with obtuse ends or ellipsoidal with a round apex and a narrow, truncate base. The conidial size was 7-20 x

2.5-5 μm and formed on hyaline to faintly brown conidiophores in acervuli that are irregular in shape and about 500 μm in diameter.

Effect of temperature on the mycelial growth of *Colletotrichum* spp. at 18, 24, 28 and 32 $^{\circ}\text{C}$ was studied. All the tested isolates showed higher mycelial growth at 28 $^{\circ}\text{C}$ and lower at 18 $^{\circ}\text{C}$. Mycelial growth increases at 24 $^{\circ}\text{C}$ than 18 $^{\circ}\text{C}$ and highest observed at 28 $^{\circ}\text{C}$ while the growth decreases at 32 $^{\circ}\text{C}$ temperature. The highest and the lowest 56.66 mm and 14.16 mm mycelial growth were recorded on Jm1 and G3 isolate at 28 $^{\circ}\text{C}$ and 18 $^{\circ}\text{C}$ respectively after 7 days of incubation. At 18 $^{\circ}\text{C}$ temperature highest and lowest 24.66 and 14.16 mm mycelial growth recorded on M4 and G3 isolate. In case of 24 $^{\circ}\text{C}$ temperature highest and lowest 37.83 and 25.66 mm mycelial growth recorded on Jm1 and M4 isolate. At 28 $^{\circ}\text{C}$ temperature highest and lowest 56.66 and 43.16 mm mycelial growth recorded on Jm1 and M3 isolate. And at 32 $^{\circ}\text{C}$ temperature highest and lowest 32.33 and 21.33 mm mycelial growth recorded on L2 and Le4 isolate. Quimio and Quimio (1975) and Ahmed (1985) recorded good growth of *C. gloeosporioides* at a temperature range of 20-30 $^{\circ}\text{C}$ whereas Saxena (2002) noticed good growth of *C. gloeosporioides* on pomegranate between 15-35 $^{\circ}\text{C}$ with optimum at 28 $^{\circ}\text{C}$. Quesada and Lopez (1980) and Banik *et al.* (1998) reported good growth of *C. gloeosporioides* at 28 $^{\circ}\text{C}$, whereas Rajak (1983) and Ekbote (1994) recorded maximum growth of *C. gloeosporioides* at 28 $^{\circ}\text{C}$.

Nineteen isolates of *Colletotrichum* spp. was tested against three fungicides, namely Bavistin 50 WP, Cupravit 50 WP and Indofil M- 45 at the rate of 500, 1000, 1500 and

2.5-2 mm and formed on hyaline to faintly brown conidiophores in asci which that are

irregular in shape and about 200 nm in diameter.

Effect of temperature on the mycelial growth of *Colletotrichum* spp. at 18, 24, 28 and

32°C was studied. All the tested isolates showed higher mycelial growth at 28°C and

lower at 18°C. Mycelial growth increases at 24°C than 18°C and highest observed at 28°C

while the growth decreases at 32°C temperature. The highest and the lowest 26.66 mm

and 4.16 mm mycelial growth were recorded on Jindal G7 isolate at 28°C and 18°C

respectively after 7 days of incubation. At 18°C temperature highest and lowest 24.00

and 14.16 mm mycelial growth recorded on M4 and G3 isolates. Increase of 24°C

temperature. Highest and lowest 37.83 and 25.00 mm mycelial growth recorded on JMI

and M4 isolates. At 28°C temperature highest and lowest 26.66 and 43.16 mm mycelial

growth recorded on JMI and M3 isolates. At a 32°C temperature highest and lowest

32.33 and 21.33 mm mycelial growth recorded on L3 and L4 isolates. Quaino and

Quaino (1975) and Vined (1982) recorded good growth of *C. gloeosporoides* at a

temperature range of 20-30°C whereas Saxena (2002) noticed good growth of *C.*

gloeosporoides on pomgranate between 15-35°C with optimum at 28°C. Goveas and

Lopez (1980) and Banik et al. (1998) reported good growth of *C. gloeosporoides* at

28°C, whereas Rajak (1983) and Ekpor (1994) recorded maximum growth of *C.*

gloeosporoides at 28°C.

Nineeen isolates of *Colletotrichum* spp. was tested against three fungiicides, namely

Baystatin 50 WP, Cupra 20 WP and Indofil M-45 at the rate of 500, 1000, 1500 and

2000 µg/ml for 7 days. It has been observed that Indofil M- 45 showed significant effect on inhibition of radial mycelial growth on *Colletotrichum gloeosporioides* over untreated control by 1500, 1000 and 500 µg/ml. In all the cases *Colletotrichum gloeosporioides* could not grow in Indofil M- 45 at 2000 µg/ml up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of *Colletotrichum gloeosporioides*. It was observed that in most the cases Cupravit 50WP reduced the mycelial growth over untreated control by 1000, 500 µg/ml. In all the cases *Colletotrichum gloeosporioides* failed to grow in Cupravit 50WP at 2000 µg/ml up to 7 days incubation period. But G2, L2, Jm1 isolates grew on 1500 µg/ml where others could not. Bavistin 50WP showed the best effect on reducing the radial mycelial growth of *Colletotrichum gloeosporioides*. *Colletotrichum gloeosporioides* could not grow over 1000 µg/ml in most of the cases. Bavistin 50WP at the rate of 1500, 2000 g/ml stopped the growth of the isolates at all. Tomy (1997) reported that among tested six fungicides against *Colletotrichum gloeosporioides* causing black leaf spot in mulberry and he reported mancozeb, carbendazim and copper oxychloride proved to be effective in inhibiting radial mycelial growth of the pathogen. Mancozeb, carbendazim and copper oxychloride proved effective in inhibiting radial growth of the pathogen. Srinivasan and Gunasekaran (1998) reported that contaf (Hexaconazole) at 0.1, 0.15, 0.2 and 0.4 per cent concentration completely inhibited mycelial growth; Indofil M-45 inhibited only upto 88 per cent at 0.5 percent.

2000 µg/ml for 7 days. It has been observed that Indofil M-45 showed significant effect on inhibition of radial mycelial growth on *Colletotrichum gloeosporioides* or untreated control by 1500, 1000 and 500 µg/ml. In all the cases *Colletotrichum gloeosporioides* could not grow in Indofil M-45 at 2000 µg/ml up to 7 days incubation period. *Colletotrichum gloeosporioides* showed significant effect in arresting radial mycelial growth of *Colletotrichum gloeosporioides*. It was observed that in most the cases Captan/50WP reduced the mycelial growth over untreated control by 1000-500 µg/ml. In all the cases *Colletotrichum gloeosporioides* failed to grow in Captan/50WP at 2000 µg/ml up to 7 days incubation period. But G2 L2 (2ml solution) on 1500 µg/ml where others could not. Bavistin 50WP showed the best effect on reducing the radial mycelial growth of *Colletotrichum gloeosporioides*. *Colletotrichum gloeosporioides* could not grow over 1000 µg/ml in most of the cases. Bavistin 50WP at the rate of 1500, 2000 g/ml stopped the growth of the isolates at all four (1997) reported that among tested six fungicides against *Colletotrichum gloeosporioides* causing black leaf spot in mulberry and he reported mancozeb, carbendazim and copper oxychloride proved to be effective in inhibiting radial mycelial growth of the pathogen. Mancozeb, carbendazim and copper oxychloride proved effective in inhibiting radial growth of the pathogen. Stinazam and (Janszkanan) (1998) reported that control (Hexaconazole) at 0.1, 0.15, 0.2 and 0.4 per cent concentration completely inhibited mycelial growth Indofil M-45 inhibited only upto 88 per cent at 0.2 percent.

Trichoderma inhibited the growth of selected 9 isolates of *Colletotrichum* spp. In case of control (*Colletotrichum* spp.) the fungus grew well and produced luxuriant mycelia. It was observed that in all the cases *Trichoderma harzianum* grew over the *Colletotrichum* spp. and lysed zone was formed at the point of meeting. In all the cases *Trichoderma* recovered from lysed zone. Deshmukh and Raut (1992) reported that *Trichoderma harzianum* Rifai and *T. viride* Pers. overgrew colonies of *Colletotrichum gloeosporioides* and *T. harzianum* was more aggressive than *T. viride*. Narendra Singh (1992) revealed that *T. harzianum* was a strong inhibitor of *C. falcatum* under *in vitro* condition. Medeiros and Menezes (1994) reported that a paired culture method in petriplates *C. gloeosporioides* showed a high degree of sensitivity to *Trichoderma harzianum*, *Trichoderma polysporum* (Linkex Pers.) Rifai and *Trichoderma pseudokoningi* Rifai.

Trichobryum inhibited the growth of selected 2 colonies of Colletotrichum sp. Inoculated in
control (Colletotrichum sp.) the fungus grew well and produced numerous mycelia. It
was observed that in all the cases Trichobryum was the dominant growth over the Colletotrichum
sp. and that zone was formed in the form of reaction. In all the cases Trichobryum
recovered from leaf zone. Debono and Ryan (1992) reported that Trichobryum
horizontally killed and T. white there were several colonies of Colletotrichum sp. on leaves.
and T. horvathii was more aggressive than T. white. Nandha Singh (1992) revealed
that T. horvathii was a strong inhibitor of T. white even under in vitro condition.
Medeiros and Mendes (1994) reported that a paired culture method in Petri dishes of
glucosamine showed a high degree of sensitivity to Trichobryum pantherinum.
Trichobryum pantherinum (R. & S.) Ryan and Trichobryum pantherinum R. & S.



SUMMARY AND CONCLUSION

CHAPTER 6

SUMMARY AND CONCLUSION

An investigation was conducted in Seed Pathology Laboratory in the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207, to study *Colletotrichum* spp. isolates from mango, guava, litchi, lemon and jamun seedling leaves. Total Eighteen isolates of *Colletotrichum gloeosporioides* and one isolate of *Gloeosporium* spp. were isolated from these fruit seedlings leaves which were collected from six nurseries of Dhaka namely Krishibid Upakaran Nursery, Khamarbari Nursery, Nupur Nursery, Yamin Nursery, Tanjila Nursery and Poribesh Sohayok Nursery.

Isolates conidial length and breadth of isolates was measured with the help of MOTIC Image Plus software. Highest and lowest length of conidia 17.6 and 7.1 μm measured for Le3 and G3 isolates respectively. Highest and lowest Breadth of conidia 4.8 and 2 μm measured for L3 and G3 respectively.

Isolates were tested under different temperatures. Growth rate of isolates was lowest in 18⁰C then it increases at 24⁰C, highest growth observed at 28⁰C and then it decreases at 32⁰C temperature. Among all the data, highest and lowest 56.66 and 14.16 mm mycelial growth recorded on Jm1 and G3 isolate at 28⁰C and 18⁰C respectively after 7 day incubation.

CHAPTER 6

SUMMARY AND CONCLUSION

An investigation was conducted in Seed Pathology Laboratory in the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SABU), Sher-e-Bangla Nagar, Dhaka-1207 to study *Colletotrichum* spp. isolates from mango, guava, litchi, lemon and jaman seedling leaves. Total Eighteen isolates of *Colletotrichum gloeosporioides* and one isolate of *Gloeosporium* spp. were isolated from these fruit seedling leaves which were collected from six nurseries of Dhaka namely: Lakshidip (Dhaka) Nursery, Kismatnagar Nursery, Nupur Nursery, Yamun Nursery, Tanjira Nursery and Poribesh (Sylhet) Nursery.

Isolates conidial length and breadth of isolates was measured with the help of MICR Image Plus software. Highest and lowest length of conidia 17.6 and 7.1 μ m measured for I-3 and G-3 isolates respectively. Highest and lowest Breadth of conidia 4.8 and 2 μ m measured for I-3 and G-3 respectively.

Isolates were tested under different temperatures. Growth rate of isolates was lowest in 18°C then it increases at 24°C, highest growth observed at 28°C and then it decreases at 32°C temperature. Among all the data, highest and lowest 26.66 and 14.16 mm mycelial growth recorded on 7th and 13th isolate at 28°C and 18°C respectively after 7 day

incubation.

In case of bioassay test it has been observed that Indofil M-45 showed significant effect on inhibition of radial mycelial growth on *Colletotrichum gloeosporioides* over untreated control by 1500, 1000 and 500 µg/ml. In all the cases *Colletotrichum gloeosporioides* could not grow in Indofil M-45 at 2000 µg/ml up to 7 days incubation period. Cupravit 50WP showed significant effect in arresting radial mycelial growth of *Colletotrichum*. It was observed that in most of the cases Cupravit 50WP reduced the mycelial growth over untreated control by 1000, 500 µg/ml. In all of the cases *Colletotrichum* failed to grow Cupravit 50WP at 2000 µg/ml up to 7 days incubation period. But G2, L2, Jm1 isolates grew on 1500 µg/ml where others could not. Bavistin 50WP showed the best effect on reducing the radial mycelial growth of *Colletotrichum*. *Colletotrichum* could not grow over 1000 µg/ml in most of the cases. And 1500, 2000 g/ml bavistin stopped the growth at all.

Trichoderma inhibited the growth of selected isolates of *Colletotrichum* spp. In case of control (*Colletotrichum* spp.), the fungus grew well and produced luxuriant mycelia. It was observed that in all of the cases *Trichoderma harzianum* grew over the *Colletotrichum* spp. and lysed zone was formed at the point of meeting. In case of control, luxuriant mycelial growths were observed.

From the foregoing discussion, it is clearly evident that, *Colletotrichum gloeosporioides* can be better controlled in extreme temperature, using Bavistin 50WP as fungicide and *Trichoderma harzianum* as bio control agent.

incases of bioassay test it has been observed that isolates M-42 showed significant effect on inhibition of radial mycelial growth on Colletotrichum gloeosporioides over untreated control by 1500, 1000 and 500 µg/ml. In all the cases Colletotrichum gloeosporioides could not grow in isolates M-42 at 5000 µg/ml up to 7 days incubation period. Cupressi 20W^c showed significant effect in arresting radial mycelial growth of Colletotrichum. It was observed that in most of the cases Cupressi 20W^c reduced the mycelial growth over untreated control by 1000, 500 µg/ml. In 97% of the cases Colletotrichum failed to grow Cupressi 20W^c at 5000 µg/ml up to 7 days incubation period. But G2, I.2, III isolates grew on 1500 µg/ml where others could not. Bavistin 20W^p showed the best effect on reducing the radial mycelial growth of Colletotrichum. Colletotrichum could not grow over 1000 µg/ml in most of the cases. And 1500, 2000 µg/ml Bavistin stopped the growth in all.

Trichoderma inhibited the growth of selected isolates of Colletotrichum spp. In case of control (Colletotrichum spp.) the fungus grew well and produced luxuriant mycelia. It was observed that in all of the cases Trichoderma parvum grew over the Colletotrichum spp. and fused zone was formed at the point of meeting. In case of control luxuriant mycelial growths were observed.

From the foregoing discussion, it is clearly evident that Colletotrichum gloeosporioides can be better controlled in extreme temperature using Bavistin 20W^p as fungicide and

Trichoderma parvum as bio control agent.

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CHAPTER 7

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