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Studies on Leaf Spot Disease of *Bombax ceiba* Caused by *Colletotrichum gloeosporioides* and Its Management in *In vitro*

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Abstract This study followed the rising occurrence of *Bombax ceiba* leaf spot disease across forests alongside gardens and plantations because the disease seriously endangers tree health. The existing control measures proved ineffective while *Bombax ceiba* leaf spot disease exclusively impacts farmers who use these trees to support their livelihoods. The Bangladesh Forest Research Institute (BFRI) performed a field study to confirm the leaf spot disease causative agent and assess copper fungicide effectiveness in disease control. The research found that *Colletotrichum gloeosporioides* functions as the main cause of the disease. This research evaluated disease severity through studies of pH levels, temperatures (T), humidity (H) and together with media effects. The pathogen showed its highest growth rate on potato dextrose agar (PDA), pH 7, 15 ℃ temperatures and 90% humidity requirements. Carbendazim provided the most effective treatment by completely eliminating fungal spores from the environment thus minimizing disease occurrences. This study showed *Trichoderma* spp. worked successfully as a biological control agent which proved highly effective at lowering disease intensity while providing environmentally responsible methods for disease control.

Keywords *In vitro*, Leaf Spot Disease, *Colletotrichum gloeosporioides*, Bio Control, *Bombax ceiba*

1. Introduction

The *Bombax ceiba* acts as a vital medicinal plant within Bombacaceae family which grows naturally across tropical Asia and some regions of Africa, Australia and Bangladesh, India and Sri Lanka [1]. The flowers of Ceiba differ from those of the genus Ceiba because they appear white in color. The tree receives various local names including Semal in Hindi and Shimool in Bengali and Indian kapok tree in English and maintains cultural and medicinal value in its growing regions [2] [3].

The *Bombax ceiba* tree stands as a deciduous species with 60-75 foot heights that grows to reach 150 feet under suitable environmental conditions. India, Pakistan, Australia and Myanmar are among the diverse tropical to subtropical environments that support the native *Bombax ceiba* tree which requires temperatures from 35- 49 ℃ and rainfall between 75- 460 cm [4].

The *Bombax ceiba* tree plays a significant role in Asian cultural traditions together with historical significance across different parts of Asia. The Bangladeshi people refer to the tree as Shimul and traditional Indian celebrations including Holi use its wood as an important symbol. The conservation efforts might damage the environment in detrimental ways which creates supplementary threats to protect the species [5].

Bombax ceiba supports economic and cultural growth in Natore Sadar Upazila, Bangladesh because local small-holder farmers use the target species as a source of wood and roots while extracting cotton. The existence of *Bombax ceiba* faces danger because *Colletotrichum gloeosporioides* causes leaf spot disease that harms productivity and disrupts farmer profits [1]. A study was conducted at the Bangladesh Forest Research Institute (BFRI) between 2022 and 2023 to investigate pathogen identification and evaluated fungicide-based management to find efficient remedies against the disease.

Biological control agents from *Trichoderma* spp. represent a promising method to handle leaf spot disease on *Bombax ceiba* because they demonstrate environmentally friendly control of plant pathogens. The research demonstrates a dual approach of biological and chemical methods for sustainable disease control because this dual approach helps protect both immediate threats and improves ecosystem vitality while decreasing chemical fungicide dependence. Tree vitality upgrades and biodiversity promotion through these strategies create better conditions for smallholders' income.

These results establish a strategy which helps agricultural practitioners control medicinal plants under changing climate conditions in support of sustainable agricultural practices over the long run.

2. Materials and Methods

2.1. Isolation and Identification of Pathogen

2.1.1. Isolation of Pathogen

8-10 affected leaves were collected from the Forest Protection Division (FPD) nursery. The affected leaf samples were cut into tiny pieces and then soaked in Tween 20 for a minute before being cleaned under running tap water. The samples were thoroughly cleaned in distilled water and then immersed in a 0.1% mercuric chloride ($HgCl_2$) solution for a minute. After being gently washed two or three times in double distilled water, the pieces were aseptically transferred to a Petri plate with solidified Potato Dextrose Agar (PDA- Potato-200g/L, Dextrose-20g/L, Agar-20g/L) under a laminar flow hood. For seven days, the plates were incubated at 25 ± 2 ℃.

The fungal colonies that were growing around the tainted leaf tissues were then relocated for further study.

2.1.2. Pathogenicity Test of the Causal Organism

Koch's postulate method was used to conduct the pathogenicity test. Initially, 200 ml suspension was obtained by inoculating the fungus for 7 days to prepare the fungal suspension. Then the suspension was evenly sprayed onto the leaves of 5 disease-free plants. The sprayed leaves were covered with a polybag to address controlled conditions, left for a whole day, and then removed. The leaves that were inoculated were monitored daily. Disease symptoms developed one month later, the leaf samples were re-isolated from infected plants and the pathogen was confirmed based on the morphological characters.

2.2. Isolation and Identification of *Trichoderma* spp.

2.2.1. Soil Collection and *Trichoderma* spp. Identification

Trichoderma strains were isolated from soil samples collected during March to April 2023 at FPD Nursery (TFPD-T1), BFRI forest (TBFRI-T2), Agrabad (TAGR-T3), Muradpur (TMP-T4), East Madarbari (TEM-T5), and Mirsorai (TMS-T6). Using the soil dilution method, five samples from a depth of 1.5 cm were combined in the amount of 100 grams to isolate the *Trichoderma* strains according to their morphological features and then culturing on a PDA medium.

2.3. Effects of Environmental Factors on Mycelial growth and sporulation of pathogen

2.3.1. Effects of Different Mediums on Mycelial Growth and Sporulation of the Pathogen

Seven growth media were used to observe mycelial growth and sporulation of the pathogen. PDA, SDA (Sabouraud Dextrose Agar), MEA (Malt Extract Agar), OAT (Oatmeal Agar), RSA (Rechard's Synthetic Agar), CZA (Czapek-Dox Agar), and YEA (Yeast Extract Agar) were used to determine the mycelial growth of the pathogen. All the mediums were added separately to 1L distilled water and autoclaved. After cooling down, they were poured into a petri dish for further procedure.

2.3.2. Effects of pH on Mycelial Growth and Sporulation of the Pathogen

In this experiment, a total of nine conical flasks were filled with PDA medium with different pH values. The pH values ranged from 4 to 8, with intervals of 0.5 pH units. Then, the growth pattern and sporulation of fungal species were observed under different acidic or alkaline conditions.

2.3.3. Effects of Temperature on Mycelial Growth and Sporulation of the Pathogen

The experiment involved calculating mycelial growth and sporulation at various temperatures ranging from 10 ℃

to 40 ℃. Pathogen was grown on PDA media and then inoculated onto sterile PDA plates. After seven days of incubation, the pathogen's progress was carefully observed and recorded as data.

2.3.4. Effects of Humidity on Mycelial Growth and Sporulation of the Pathogen

Fungal cultures were inoculated on PDA plates and incubated in a humidity chamber. The humidity was maintained at 60% to 90% and was monitored for signs of fungal development for seven days, as close observation was maintained during the incubation period, for any changes in the colony.

2.4. *In vitro* Screening of Different Fungicides and *Trichoderma* spp. against Pathogen

2.4.1. Screening of Fungicide against Pathogen

To suppress the causative agent, 6 fungicides were used **(Table 1).** A ratio combination of 0.025, 0.050, and 0.075 g/l of each fungicide was added to 500 milliliters of PDA solution, four replication plates for each, to form a solution.

2.4.2. Screening of *Trichoderma* Strain against Pathogen

This experiment used the dual-culture strategy. Six different species of *Trichoderma* were first collected and their fresh cultures were made. A 6 mm agar disc was included with each 4-day-old PDA culture plate of the *Trichoderma* isolate and was positioned near the PDA plates' edge (9 mm). At the other edge of the same Petri plate was another agar disc the same size as the pathogenic fungus (Fig. 1). The pathogenic fungus was equally placed on a fresh PDA plate as a control. Every pairing was carried out in four sets and kept at room temperature for development. To identify the antagonistic activity, the pathogenic fungal colony's radius was measured four days after incubation. To spot changes in the growth patterns of the fungus, regular observations were conducted.

Table 1. Fungicide name and Chemical name

Serial no.	Trade name	Chemical name	Group
1	Knowin	Methyl benzimidazole 2-ylcarbamate	Carbendazim
2	Sunvit	Copper oxychloride	Copper Oxychloride
3	Champion	Copper Hydroxide	Copper Hydroxide
4	L M- 45	$C_{40}H_{60}Mn_9N_{20}S_{40}Zn$	Mancozeb
5	Cuproxat	Copper Sulphate	Copper + Sulphate
6	Powder Sulfur	Sulfur	Sulfur

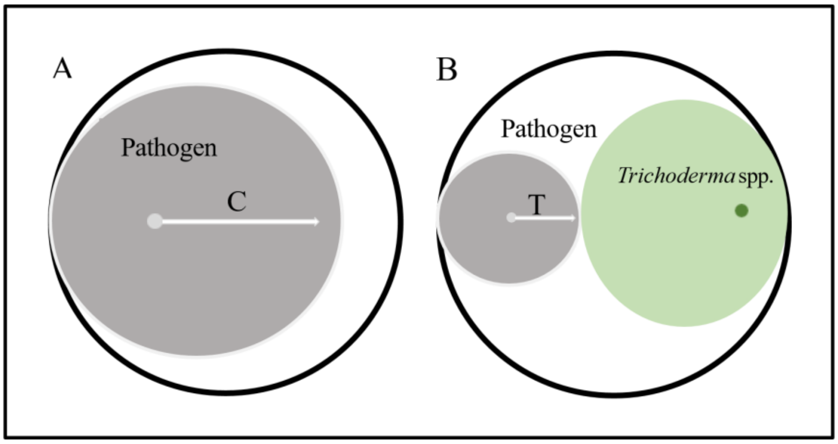

Figure 1. Dual culture method. A- Control plate; B-Dual culture plate. Gray color circle -Pathogen, Green color circle- *Trichoderma* spp.; C- Mean distance of the causal organism growth (measured in cm) from the point of inoculation to the margins in the control dishes; T- Mean distance of the causal organism growth (measured in cm) from the point of inoculation to the colony margins in treated dishes in the direction of the *Trichoderma* spp.

3. Result

3.1. Isolation and Identification of Pathogen

3.1.1. Isolation and Identification of Pathogen

The isolated pathogen was identified as *Colletotrichum gloeosporioides*, the causal agent of the disease based on morphological characteristics. Initially, the colony appeared white, but with time, it turned olivaceous grey, with orange conidial pustules at the center. The aerial mycelium was somewhat flocculent, and the reverse dark grey. The conidia were hyaline, aseptate, uninucleate cylindrical shapes and the acervuli exhibited shapes, colors and curvatures of 1 - 4 septas (Fig. 2) [6].

3.1.2. Pathogenicity Test of Pathogen

Bombax ceiba leaf spot disease was found to be caused by *Colletotrichum gloeosporioides* after the fungus had been incubated for seven days (Fig. 3).

3.2. Isolation & Identification of *Trichoderma* spp.

3.2.1. Collection of Soil

Collected soils were tested for identifying *Trichoderma* spp.

3.2.2. *Trichoderma* spp. Identification

After seven days morphological characters were observed to separate the *Trichoderma* spp.

3.2.3. Morphological Characteristics of *Trichoderma* spp.

After three days morphological characters were observed to separate the *Trichoderma* spp. **(Table 2)**.

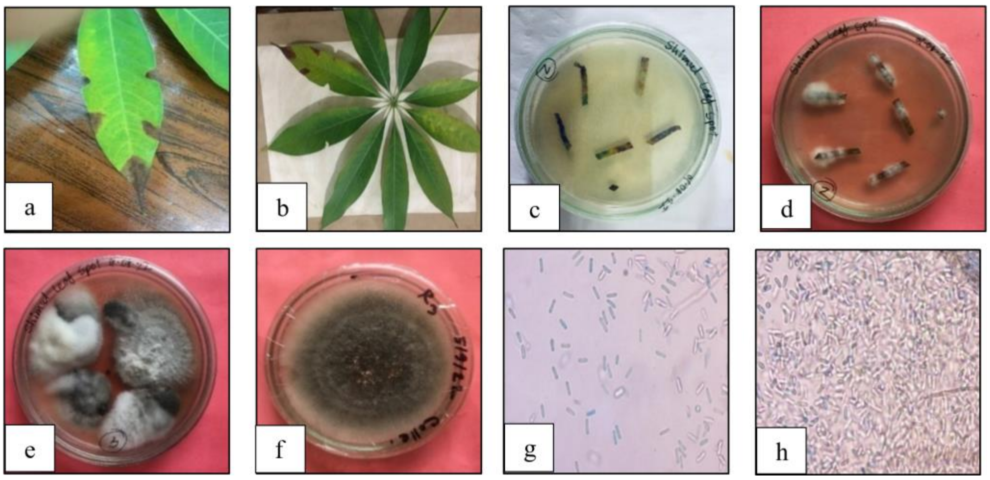

Figure 2. Leaf spot diseases of *Bombax ceiba* and its pathogen *Colletotrichum gloeosporioides.* (a, b- infected leaves; c, d, e- culture of leaf spot disease; f- fresh culture; g, h- Spore of pathogen)

Figure 3. Pathogenicity test (a, b- Suspension of pathogen; c- Disease free plant; d- Spray pathogen suspension in disease free plant; e, f- Cover the whole plant with polybag; g- Control plant; h- Infected plant, i, j- Infected plant leaf; k, l, m- Re-culture of pathogen; n, o- Spore of *Colletotrichum gloeosporioides*)

Table 2. Characterization and identification of *Trichoderma* species isolated from different habitats

Group Of Strain	Source Of Sample	Macro and Microscopic Characteristics
Group-1 TFPD-T1	Rhizospheric soil, FPD nursery, BFRI	The colony was white at first, then whitish green. It generated round to almost round conidia, and it carried coupled conidiophores with between three and four extended phialides (Fig. 4).
Group-2 TBFRI-T2	Soil, BFRI	The colony previously had a watery, white bright greenish color in the center. After that, under a microscope, it exhibited certain characteristics then took on a soft, velvety texture before developing a dark color of green (Fig. 4).
Group-3 TAGR-T3	Soil, Agrabad	There were white to slightly greenish threads at first. They eventually turned a light green color after developing a deep green color in the center and moving outside. In addition, round spores emerged on tiny stems and pointed in different directions at every spot (Fig. 4).
Group-4 TMP-T4	Nursery soil, Muradpure	The colonies commonly have a dark green to greenish-blue color and expand quickly. Spores on fungi typically occur in pairs or in an irregular placement, appearing narrow ellipsoidal or cylinders (Fig. 4).
Group-5 TEM-T5	Humas, East Madarbari	A clear, crystalline colony with typical upward-growing filaments is observed in the sample. It develops from white to dark green after seven days. The spores are smooth, oval-shaped, and light green in color, and the spore-producing structures are grouped in a predictable way (Fig. 4).
Group-6 TMS-6	Cultivated land soil, Mirsorai	The colony looked soft and cottony, with a dark green color. Conidiophores on it extended off. Phialides are cylinder-shaped or somewhat expanded. Conidia are cylindrical and light green in color (Fig. 4).

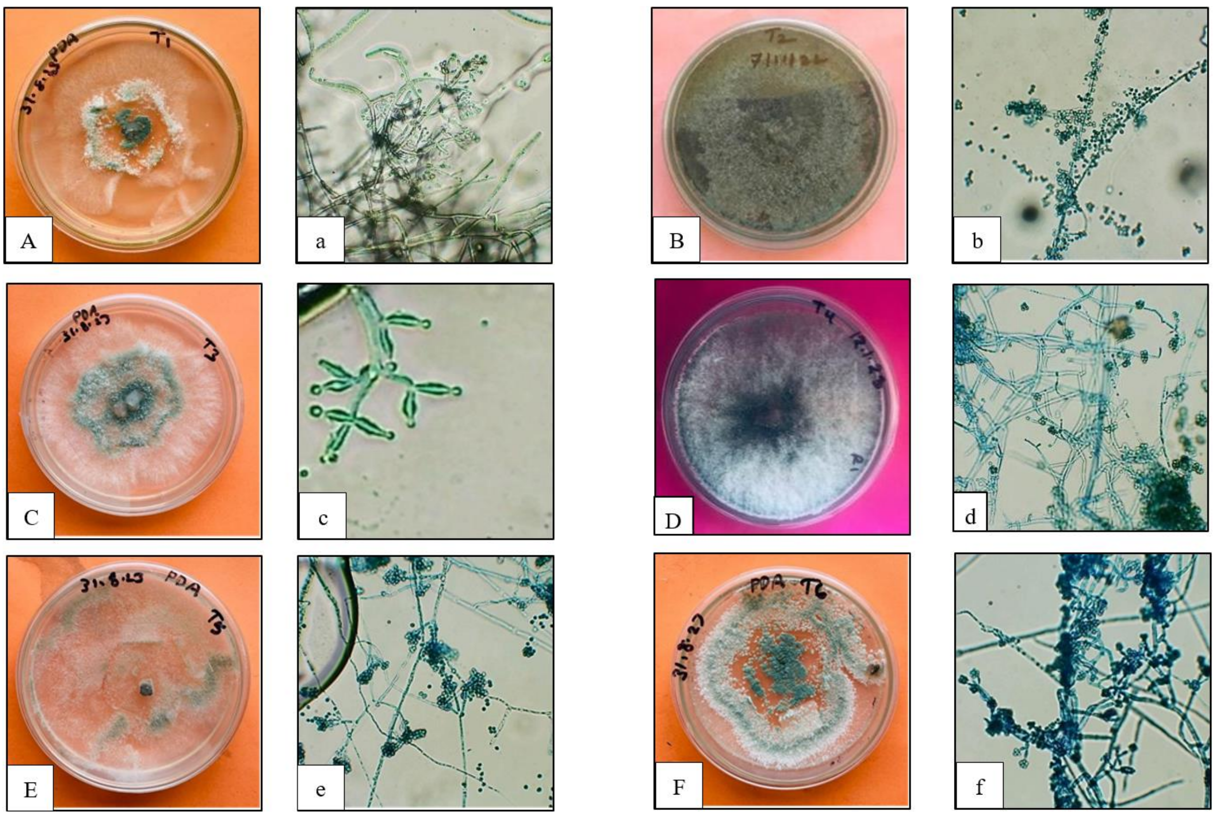

Figure 4. Different colony of *Trichoderma* A- TFPD, a- Microscopic view; B-TBFRI, b- Microscopic view; C-TAGR, c- Microscopic view; D- TMP, d- Microscopic view; E-TEM, e- Microscopic view; F-TMS, f- Microscopic view

3.3. Effects of Environmental Factors on Mycelial Growth and Sporulation of Pathogen

3.3.1. Effects of Different Mediums on Mycelial Growth and Sporulation of Pathogen

To calculate the fungal mycelial growth and sporulation, the effect of seven mediums such as PDA, SDA, MEA, Oatmeal Agar, RSA, Czapeks and YEA has been applied on *Colletotrichum gloeosporioides*. The results showed that the Potato Dextrose agar (PDA) medium shows the best growth and an excellent sporulation rate while the SDA medium shows the lowest growth and a fair sporulation rate, followed by Oatmeal, Czapeks, YEA, MEA and RSA media **(Table 3, Fig. 5).**

Table 3. Effect of different Mediums on mycelial growth and sporulation of *C. gloeosporiodes*

Medium	Mycelial growth (M ±SE)* mm	Mycelial weight (M ±SE)*gm	Sporulation
PDA	**88.22 ±0.58^{a}**	**0.326**	**++++**
RSA	59.00 ±2.19^{bc}	0.221	+++
Oatmeal	87.33 ±0.58^{a}	0.316	++++
MEA	62.22 ±2.00^{bc}	0.206	+++
SDA	58.33 ±2.08^{c}	0.154	++
Czapeks	68.00 ±2.01^{b}	0.291	+++
YEA	67.44 ±0.58^{b}	0.258	+

*Excellent (++++), Good (+++), and Fair (++) * SE (Standard error) = σ / √n; M= Mean of three replications. *In the column same letters are not significantly different by DMRT at the 5% level.

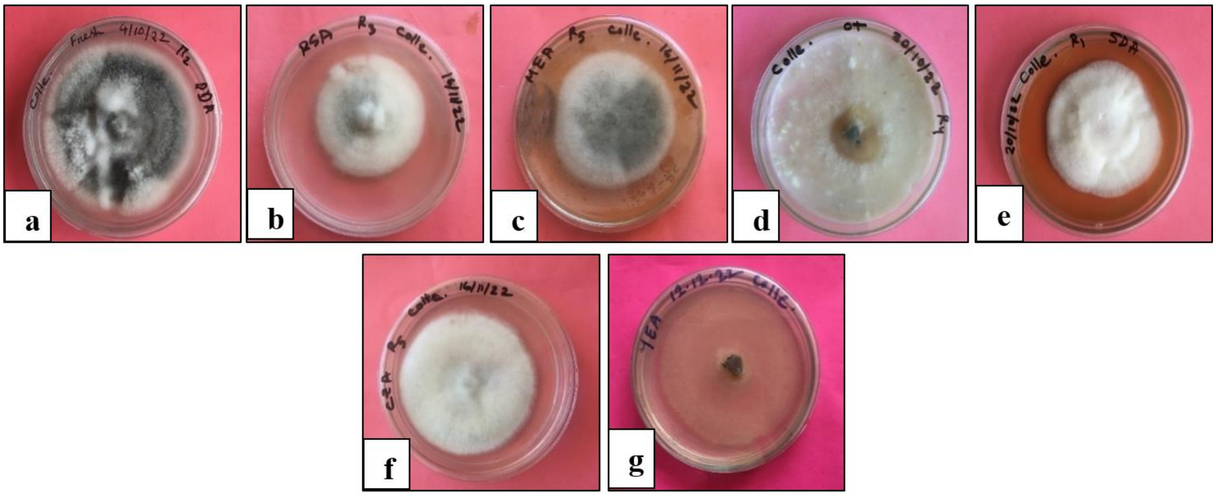

Figure 5. Medium effect on *Bombax ceiba* leaf spot disease caused by *C. gloeosporioides* (a- PDA medium; b- RSA medium; c- Oatmeal medium; d- MEA medium; e -SDA medium; f- CZA medium; g- YEA medium)

3.3.2. Effects of Different pH on Mycelial Growth and Sporulation of the Pathogen

To calculate the fungal mycelial growth and sporulation, the effect of nine pH ranging 4 to 8 has been applied on *C. gloeosporiodes*. The results showed that pH 7 shows the best growth with 87 mm and an excellent sporulation rate while pH 4 shows the lowest growth with 71.33 mm and a fair sporulation rate, followed by pH 5, 8, 6, 5.5, 6.5, 7.5 and 4.5 **(Table 4, Fig. 6).**

3.3.3. Effects of Different Temperatures on Mycelial Growth and Sporulation of Pathogen

To calculate the fungal mycelial growth and sporulation, the effect of seven temperatures ranging from 10 ℃ to 40 ℃ has been applied on *Colletotrichum gloeosporioides*. The results showed that 15 ℃ temperature shows the best growth with 80.42 mm and an excellent sporulation rate while 40°C doesn't show any significant growth, followed by temperature 10, 20, 25, 30 and 35 **(Table 5, Fig.7).**

3.3.4. Effects of Different Humidity on Mycelial Growth and Sporulation of Pathogen

To calculate the fungal mycelial growth and sporulation, the effect of four humidity ranging 60 to 90 has been applied on *Colletotrichum gloeosporioides*. The results showed that Humidity 90 shows the best growth with 50.66 mm and an excellent sporulation rate while Humidity 60 is the lowest with 26.88 mm and a fair sporulation rate, followed by Humidity 70 and 80 **(Table 6, Fig. 8).**

3.4. *In vitro* Screening of Different Fungicides and *Trichoderma* spp.against the Causal Organism

Inhibition of mycelial growth

Measurement of fungal mycelial growth of *C. gloeosporioides* was taken after 7th days. The inhibition percentage of mycelial growth was calculated by the following formula

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

Where,
I = Percentage of inhibition,
C = The diameter of the fungal colony in control plates
T= The diameter of the fungal colony in treated plates

- Three bio-control agents were taken for six treatments (T1-T6) and three replications against *C. gloeosporioides* on PDA medium.
- The mycelial growth inhibition was observed and inhibition percentage was calculated.

Table 4. Effect of pH on mycelial growth and sporulation of *C. gloeosporiodes*

pH	Mycelial growth (M±SE) mm	Mycelial weight (M±SE)gm	Sporulation
4.0	71.33 ±0.68^{c}	0.152	++
4.5	80.00 ±1.53^{b}	0.438	+++
5.0	83.66 ±1.33^{ab}	0.444	+++
5.5	80.66 ±0.67^{b}	0.366	+++
6.0	81.00 ±0.33^{b}	0.334	+++
6.5	80.66 ±1.33^{b}	0.355	++
7.0	**87.00 ±0.58^{a}**	**0.483**	**++++**
7.5	80.66 ±2.28^{b}	0.265	++++
8.0	81.33 ±0.88^{b}	0.299	+++

Excellent (++++), Good (+++) and Fair (++), SE (Standard error) = σ / √n; M= Mean of three replications *In the column same letters are not significantly different by DMRT at 5% level.

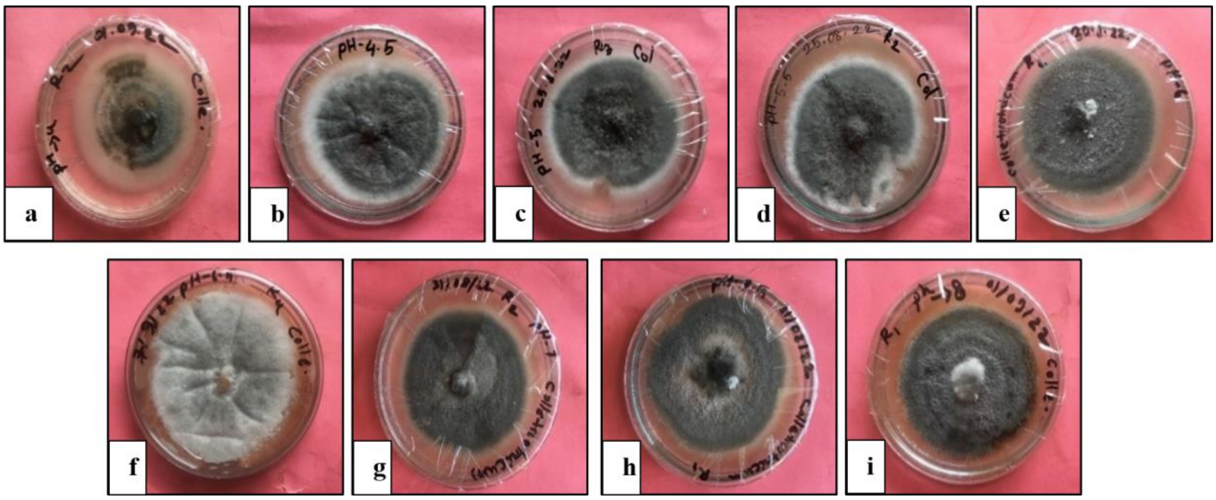

Figure 6. pH effect on *Bombax ceiba* leaf spot disease caused by *C. gloeosporioides* (a- pH 4; b- pH 4.5; c- pH 5; d- pH 5.5; e- pH 6; f- pH 6.5; g- pH 7; h- pH 7.5; i- pH 8)

Table 5. Effect of Temperature on mycelial growth and sporulation of *C. gloeosporioides*

Temperature	Mycelial growth (M±SE) mm	Mycelial weight (M±SE)gm	Sporulation
10 ℃	74.00 ±2.150^{a}	0.219	+++
15 ℃	**80.42 ±2.887^{a}**	**0.366**	**++++**
20 ℃	51.66 ±0.667^{b}	0.276	+++
25 ℃	47.67 ±0.882^{b}	0.173	+++
30 ℃	45.55 ±0.333^{b}	0.141	+++
35 ℃	29.55 ±0.577^{c}	0.095	++
40 ℃	0^{d}	0	-

Excellent (++++), Good (+++), Fair (++) and Neel (-); SE (Standard error) = σ / √n; M= Mean of three replications. *In the column same letters are not significantly different by DMRT at the 5% level.

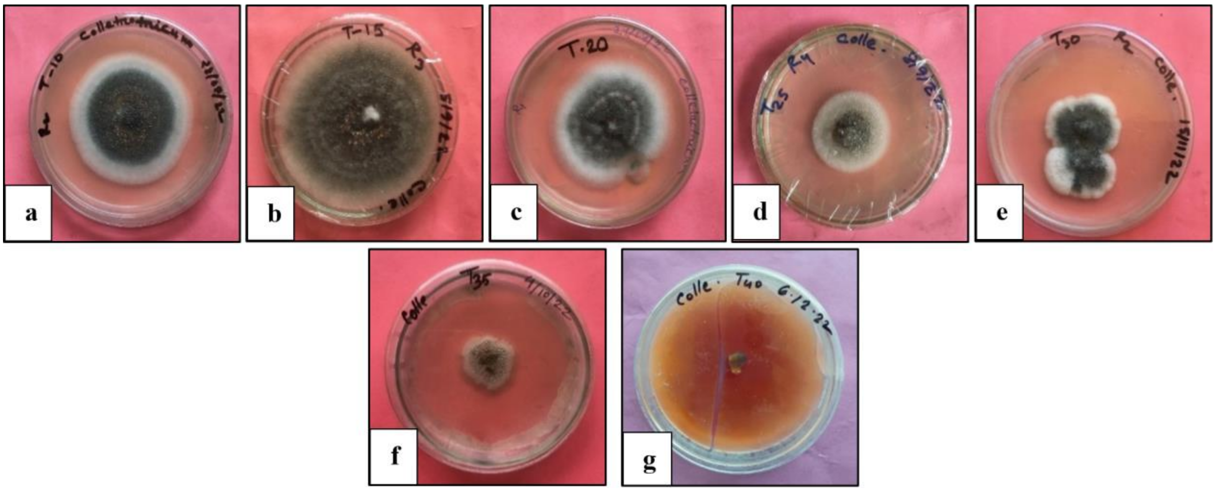

Figure 7. Temperature effect on *Bombax ceiba* leaf spot disease caused by *C. gloeosporioides* (a- Tmp 10 ℃; b- Tmp 15 ℃; c- Tmp 20 ℃; d- Tmp 25 ℃; e- Tmp 30 ℃; f- Tmp 35 ℃; g- Tmp 40 ℃)

Table 6. Effect of Humidity on mycelial growth and sporulation of *C. gloeosporioides.*

Humidity	Mycelial growth (M±SE) mm	Mycelial weight (M±SE)gm	Sporulation
60	26.88±1.856[b]	0.060	++
70	45.55±2.88[a]	0.085	++
80	28.44±0.33[b]	0.075	+++
90	**50.66±1.67[a]**	**0.0951**	**+++**

Good (+++) and Fair (++); SE (Standard error) = σ / √n; M= Mean of three replications; *In the column same letters are not significantly different by DMRT at 5% level.

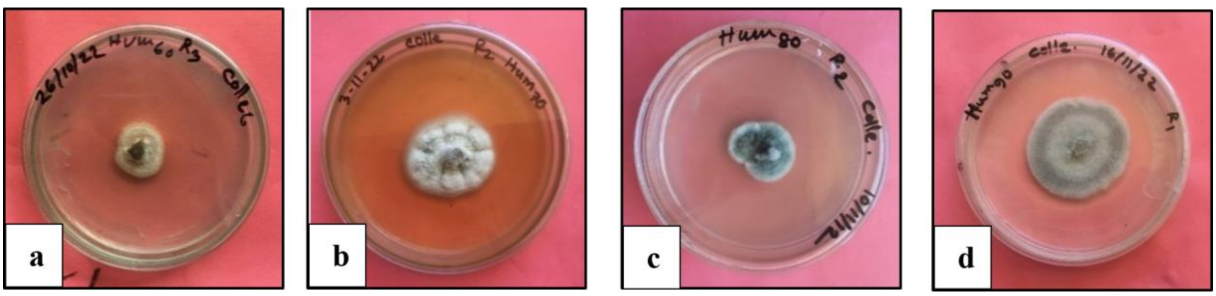

Figure 8. Humidity effect on *Bombax ceiba* leaf spot disease Caused by *C. gloeosporioides* (a- Hum 60; b- Hum 70; c- Hum 80; d- Hum 90)

3.4.1. Chemical Fungicide

Fungicides such as L M-45, Knowin, Sulphur, Sunvit, Copper Oxychloride, and Copper Sulphate have been applied to control the root rot disease. The results showed that Knowin is the best fungicide compared to other fungicides to control the disease with 100% inhibition rate. This inhibition rate is higher than any other fungicide **(Fig. 9, Table 7).**

3.4.2. *Trichoderma* Strain

Dual Culture method

CT1= *Colletotrichum* sp. + T1

CT2= *Colletotrichum* sp. + T2

CT3= *Colletotrichum* sp. + T3

CT4= *Colletotrichum* sp. + T4

CT5= *Colletotrichum* sp. + T5

CT6= *Colletotrichum* sp. + T6

Six *Trichoderma* spp. had been applied to control the disease. The results showed that T6 worksbest to inhibit the pathogen with 34.55% inhibition rate (Fig.10). The inhibition rate is higher than any other *Trichoderma* spp. strain.

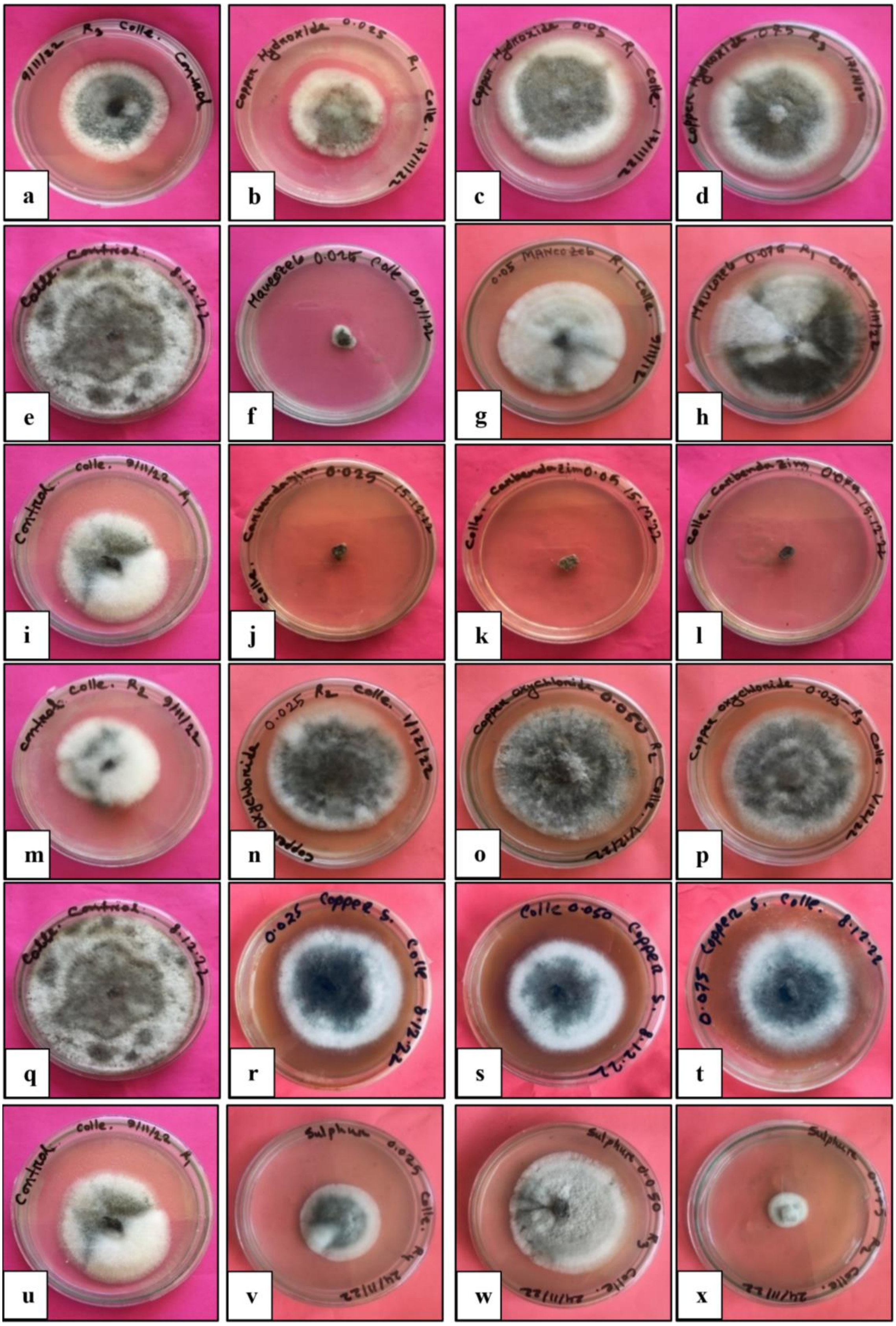

Figure 9. Fungicide effect on mycelial growth of *Bombax ceiba* leaf spot disease caused by *C. gloeosporioides* (a, e, i, m, q, u - control plate; b, c, d- Copper Hydroxide treatment plates of 0.025, 0.050, 0.075 concentration; f, g, h- Mancozeb treatment plates of 0.025, 0.050, 0.075 concentration; j, k, l- Carbendazim treatment plates of 0.025, 0.050, 0.075 concentration; n, o, p- Coper oxychloride treatment plates of 0.025, 0.050, 0.075 concentration: r, s, t- Copper sulphate treatment plates of 0.025, 0.050, 0.075 concentration; v, w, x- Sulphar treatment plates of 0.025, 0.050, 0.075 concentration)

Table 7. Effect of Chemical Fungicide on mycelial growth and sporulation of *C. gloeosporiodes*

Fungicide Name	Inhibition rate of Pathogen (%)		
	0.025	0.050	0.075
L M-45(Mancozeb)	3.22^{ef}	18.80^{de}	77.27^{b}
Knowin (Carbandazim)	**100^{a}**	**100^{a}**	**100^{a}**
Sulphur	45.33^{c}	17.01^{def}	66.90^{b}
Sunvit (Copper Hydroxide)	20.04^{d}	13.49^{ef}	18.69^{de}
Copper Oxychloride	5.22^{def}	3.22^{ef}	3.79^{ef}
Copper Sulphate	2.18^{f}	13.49^{def}	14.19^{def}

*In the column same letters are not significantly different by DMRT at 5% level.

Table 8. Inhibition rate of *Trichoderma* spp. on mycelial growth *C. gloeosporioides*

Treatment	**Inhibition Rate I={(c-t)/c}100**
CT1	11.86%^{d}
CT2	17.81%^{cd}
CT3	24.80%^{bc}
CT4	19.32%^{bcd}
CT5	26.03%^{ab}
CT6	34.55%^{a}

*In the column same letters are not significantly different by DMRT at 5% level.

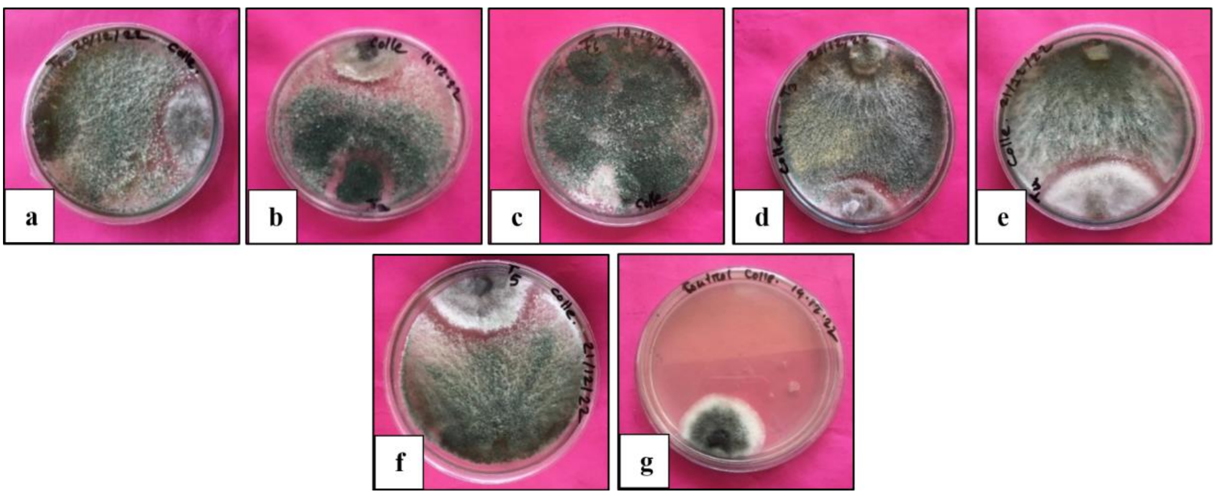

Figure 10. Duel culture (a= *C. gloeosporioides* + T$_1$; b= *C. gloeosporioides* + T$_2$; c= *C. gloeosporioides* + T$_3$; d= *C. gloeosporioides* + T$_4$; e= *C. gloeosporioides* + T$_5$; f= *C. gloeosporioides* + T$_6$; g= Control)

4. Discussion

The multifunctional plant *Bombax ceiba*, which is prized for both its medicinal and silk cotton qualities, has been utilized for ages in Siddha, Unani, and Ayurvedic medicine because of its versatility. *Bombax ceiba* is susceptible to various diseases. These diseases include leaf spots, leaf blight, leaf rust, root rot, sooty mold, and seed disease.

These include *Myrothecium roridium, Chrysomyxa bombacis, Uredo bombacis,* and *Cercospora follicola* [7], *Dematophora necatrix, Hartig, Stachybotrys atra Hansf* and *Alternaria chlamydospora Mouchaccak* [8], *Colletotrichum gloeosporioides* [7], [9]. Leaf blight is caused by *Dicellomyces bombacis* [7] and *Corticium rolfsii* [9]. Leaf rust affects *bombax ceiba* with *Chrysomyxa bombacis* [10] and *Uredo bombacis* [9]. On the other hand, there are diseases like root rot Disease caused by *Ganoderma lucidium* and *Fusarium Semitectum* [10]; dieback disease caused by *Phomopsis salmalica* [7]; canker disease caused by *Botryosphaeria* spp. and *Lasiodiplod pseudotheobromae* [11]. Few more diseases were found named damping off and sooty mold caused by

Fusarium Semitectum and a species of *Capnodium* [10].

Screening inspection of *Bombax ceiba* leaf spot disease of the present study indicates that *Colletotrichum gloeosporioides* causes the leaf spot disease. Samples were collected from the FPD nursery; Chittagong was tested in *in vitro* conditions at Bangladesh Forest Research Institute. In this this research it was found that the causal organism of *Bombax ceiba* leaf spot disease is *Colletotrichum gloeosporioides* (Fig.2). A pathogenicity test was also conducted followed by the Koch postulates method and the result showed the same (Fig. 3). This finding shows similarity with the previous research works by Soni [7], Abbas et al [8] and Sharma et al [9] which also identified *Colletotrichum* species as pathogens causing similar symptoms in *Bombax ceiba* plants across different regions of the world. In the recent study, Carbendazim has been identified as the most effective fungicide against *Colletotrichum gloeosporioides*, exhibiting a remarkable inhibition rate of 100% (Fig. 9 & Table 7).

It's essential to consider the work of Cao et al. [12], Zapata & Zapata [13], Magarey et al. [14], Filoda [15] and Nakpalo et al. [16] According to their work, Carbendazim, Benomyl, Chlorothalonil, Sarfun, Amistar, Prochloraz, Propiconazole and Azoxystrobin showed the maximum inhibition of mycelial growth at all Mconcentrations. The effect of Carbendazim on *Colletotrichum* sp. is differential; it is more effective for the control of species of the *Colletotrichum gloeosporioides* complex than for *Colletorichum acutatum* [13]. Some of them also found results different from the present study. In their study they found Benomyl was the most efficient fungicide in *in vitro* conditions for the control of *Colletotrichum gloeosporioides* causing diseases in grapefruits inhabiting mycelial growth and the production of spore at twice the commercial dose [12]. Chlorothalonil (chloronitriles group) was the active ingredient inhibiting the mycelial growth of *Colletotrichum gloeosporiodes* and with similar effects on sporulation using commercial dose [14]. The Sarfun 500 SC and Amistar 250 SC can be therefore regarded as a prospective means of limiting growth and development of *Colletotrichum gloeosporioides* [15]. Carbendazim, Prochloraz, Propiconazole, and Azoxystrobin also showed best results against *Colletotrichum gloeosporioide* [16].

Trichoderma spp. is a bio-fungicide that successfully controls leaf spot disease of *Bombax ceiba*. In the recent study, an *in vitro* experiment was also conducted to evaluate the effectiveness of six different biocontrol agents containing *Trichoderma* spp. (T_1-T_6) (Fig. 4 & Table 2) against *Colletotrichum gloeosporioide* using the dual culture method. The results showed that treatment CT_6 (*Colletotrichum gloeosporioide* + T_6) was the most effective in reducing the incidence of leaf spot diseases in *Bombax ceiba* plants. The treatment demonstrated a 34.55% inhibition rate within three days of application (Fig. 10 & Table 8). *Trichoderma* against *Phytophthora infestans* and *Colletotrichum gloeosporioides* showed up to 22.5% inhibition, and *Trichoderma* strains demonstrated the potential for bio-control of pests using unique carbon sources [17]. *Trichoderma koningiopsis* PSU3-2 was isolated from soil as a bio control agent against anthracnose of chili pepper fruit caused by *Colletotrichum gloeosporioides* [18]. *Trichoderma* isolates were considered efficient antagonists by Bell's score criteria. The *T. harzianum*, *T. asperellum* and *T. longibrachiatum* species have greater potential in the biocontrol of *C. gloeosporioides* [19]. *T. longibrachiatum* obtained the highest percentage inhibition of 64.68% compared to *T. harzianum* and *T. asperellum* with 59.16% and 47.73% inhibition respectively observed against *C. gloeosrioides* [20]. Eight isolates of *Trichoderma* were tested *in vitro* against *C. gloeosporioides*. In general, *T. harzianum*-1 and 2 and *T. viride*-2 had shown high antagonism against *C. gloeosporioides* inhibiting their mycelial growth spore production and spore germination [21].

In the recent study, it has explored the impact of various environmental factors on the growth and sporulation of *Colletotrichum gloeosporioides* in *in vitro*. The findings demonstrated that the optimal conditions for growth and sporulation of the fungus are PDA as the growth medium (Fig.5 and Table 3), pH 7.0 (Fig. 6 & Table 4), a temperature of 15 ℃ (Fig.7 & Table 5), and a relative humidity of 90% (Fig. 8 & Table 6).

These findings resonate with previous research such as Ruiz et al. [22] and Khanzada et al. [23]. These research works have investigated the influence of factors like growth medium, pH, temperature, and humidity on fungal and pathogen growth. In these studies, some showed similar results to the present time and some showed different results.

The environmental conditions that favor *Colletotrichum gloeosporioides* caused disease are relative humidity greater than 90% and temperatures between 22 and 32 °C. These conditions are where most symptoms develop. However, during postharvest, tissue damage can occur in drier conditions due to the presence of the fungus and the aging of the fruits. The pathogen can survive in crop residues and grow saprotrophically after being dispersed by rain splashes and air transmission [22]. The minimum, optimum, and maximum temperatures for *C. gloeosporioides* were 20 ℃, 30 ℃, and 40 ℃, respectively. It grows best at pH 5 followed by pH 6 [23].

While chemical fungicides like Carbendazim offer rapid disease suppression, the use of biocontrol agents such as *Trichoderma* spp. presents a sustainable and environmentally friendly approach. Further research is needed to explore the potential of other biocontrol agents and to evaluate the long-term efficacy of different control strategies under field conditions.

5. Conclusions

This study was conducted at BFRI, Chattogram from 2022 to 2023 identified *Colletotrichum gloeosporioides* as the causing pathogen of leaf spot disease in *Bombax ceiba*. The PDA medium is best for fungal growth and ideal conditions were found to be a pH of 7, a temperature of 15 ℃, and 90% humidity. As for controlling, Carbendazim inhibited the fungus with 100% effectiveness. On the other hand, *Trichoderma*-based biocontrol agent CT6 achieved 34.55% inhibition rate. This study highlights *Trichoderma* based bio-fertilizers as eco-friendly alternatives to chemical fungicides. These findings provide long term and cost-effective disease management strategies to improve the *Bombax ceiba*'s health and productivity of in Bangladesh.

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