

RESEARCH ARTICLE

ASSOCIATION OF *Colletotrichum gloeosporioides* WITH PREMATURE FRUIT DROPPING OF MANGO (*Mangifera indica* L.) AND ITS *IN VITRO* CONTROL USING SELECTED PLANT EXTRACTS, PHYTOHOMONES AND FUNGICIDES

Mst. Tahera Tasmin<sup>1</sup>, Ismail Hossain<sup>2</sup>, Md. Aminul Islam Khan<sup>3</sup>, Mohammad Shahjahan Monjil<sup>1\*</sup>

<sup>1</sup>Bangladesh Agricultural University, Bangladesh

<sup>2</sup>International University of Business Agriculture and Technology, Bangladesh

<sup>3</sup>Major General Mahmudul Hasan Adarsha College, Bangladesh

Received: 09 October 2021, Accepted: 27 May 2022

ABSTRACT

The present study was performed to determine the pathogenic cause of mango fruit drop and its in-vitro control through the application of plant extracts, phytohormones, and chemical fungicides. The prematurely fallen fruits at the pin-head stage, pea stage and marble stage were taken for pathogen isolation. *Colletotrichum gloeosporioides* was identified as the pathogenic cause and several isolates were established. Based on consistent mycelial growth in PDA culture media, one isolate was chosen for in-vitro management study using various treatments. Six plant extracts, two phytohormones viz. NAA, 2,4-D, and six chemical fungicides were tested against *C. gloeosporioides*. With three treatments, namely garlic clove, allamonda leaf and mahogany leaf extracts, mycelial growth of *C. gloeosporioides* was not observed for seven days of study. In comparison to the control treatment, a significantly lower mycelial growth was found with both neem leaf extract and marigold leaf extract. Garlic clove extract, allamonda leaf extract and mahogany leaf extract showed the highest percent growth inhibition (100%) of *C. gloeosporioides* at 7 DAI in compared to the control treatment. In case of NAA and 2,4-D application in PDA, mycelial growth of *C. gloeosporioides* was not detected also. As a result, extracts of garlic clove, neem leaf, allamonda leaf, mahagoni leaf, NAA and 2,4-D as well as chemical fungicides can be introduced in field conditions to assess their effectiveness in controlling premature fruit drop of mango caused by *C. gloeosporioides*.

Keywords: chemical fungicides, *Colletotrichum gloeosporioides*, fruit dropping, mango, phytohormones, plant extracts

INTRODUCTION

The mango (*Mangifera indica* L.), which belongs to the order Sapindales and to the family Anacardiaceae, is the world's most important tropical or subtropical fruit crop (Nelson 2008). Currently, mango is widely regarded as one of the most desirable fruits in the global market. For its exquisite taste, great flavor, appealing color, nutritional value, and superior aroma, mango is often referred to as "The King of Fruits".

Mango is widely grown in Bangladesh and ranks the 7<sup>th</sup> largest mango producer in the world (Ahmad, 2021). Mangoes are the most common crop in almost all of the homestead gardens in Bangladesh, and the total planted area in the year 2018-19 was 95,260 hectares with 1219450 metric tons of mango fruits produced (BBS, 2019). The major mango-growing areas in Bangladesh are Rajshahi, Chapai Nawabgonj, Natore, Kustia, Dinajpur, Thakurgaon, and Rangpur (BBS, 2019).

Corresponding author: smonjil@bau.edu.bd

A great number of little mangoes are seeded on the stem when the mango tree begins to bloom. Mango fruit shedding is a typical fruit thinning process that aims to properly utilize the available nutrients. Many causes contribute to the significant decrease in mango fruit production, including a lack of pollination, fertilization failure, insect infestation, and pathogenic infection. Diseases of mango trees play a major role in fruit set and pre-mature fruit dropping.

Choosing Despite proper flowering and initial fruit development, severe fruit dropping leads to low fruit production in mango orchards and causes considerable economic losses. In Bangladesh, Anthracnose, bacterial leaf spot and leaf blight, powdery mildew on leaves and inflorescences, leaf rust, Fusarium mango deformation, and fruit scab have all been documented (Sarker, 2008). Ploetz (2003) identified anthracnose (*Colletotrichum gloeosporioides*), Gummosis (*Lasiodiplodia theobromae*), sooty mold (*Capnodium mangiferae*), and powdery mildew (*Oidium mangiferae*) are the causes of low fruit setting in the plant. Among these, anthracnose is the most prevalent and severe disease in the most mango-growing areas (Freeman *et al.* 1998). Anthracnose affects all aerial parts of the plant including the stems, branches, twigs, leaves, petioles, flowers and fruits (Rahim *et al.* 2016). Frequent rain and heavy dews enhance infection from the beginning of flowering until the fruits are roughly half-size (Pitkethley and Conde, 2007).

High humidity or rainfall in the early stages of fruit development favor the severity and prevalence of anthracnose (Ploetz, 1998). According to Dodd *et al.* (1991), temperatures of 20 to 30°C and relative humidity of over 95 % favor anthracnose infection on inflorescence, leaves, and fruits. In a severe infection, the entire inflorescence is destroyed so that no fruit set occurs (Dodd *et al.* 1992; Pande *et al.*, 2012; Ploetz, 1998). According to reports from Bangladesh, anthracnose alone accounts for 25 to 30 % of overall mango harvest losses (Reza and Kader, 1995).

As a result, it is critical to identify the reasons for premature fruit drops of mango and adopt appropriate control strategies to reduce fruit dropping and the large quantity of loss. Therefore, the present research was conducted with the following objectives.

- I. To identify and describe the pathogenic agent that is associated with mango premature fruit drop.
- II. To evaluate the effectiveness of selected plant extracts, phytohormones, and chemical fungicides to suppress the *in vitro* growth of the pathogen.

## MATERIALS AND METHODS

### Experimental site

The experiment was performed in two laboratories at Bangladesh Agricultural University (BAU), Mymensingh namely 'Microbiology and Bio-control laboratory' and 'Eco-friendly Plant Disease Management Laboratory'.

### Collection of diseased samples

Diseased fallen premature mango fruits of three different stages, such as pin-head stage, pea stage, and marble stage were collected from the local mango orchard, BAU Germplasm Centre, and several commercial mango gardens. Collected fruit samples were stored in a refrigerator and a total of 30 samples were taken to isolate the pathogen.

### Isolation of causal agents/ pathogen

Isolation of the causative fungus was performed from the collected prematurely dropped fruits by the tissue planting method using PDA media (200 g clean sliced potato tubers, 17 g dextrose, 17 g agar powder, and deionized water up to 1000 ml of volume). Collected fruit samples were surface-sterilized with 10% Chlorox before fungal pathogens were isolated in PDA media. The aseptically cut pieces of mango samples were then placed on plates containing sterilized PDA (approximately 18-20ml/plate) and cultured for mycelial growth at room temperature (25° C±1).

### Purification of pathogens

For the establishment of pure culture, hyphal tip of isolated fungus was placed aseptically

onto a PDA plate with the help of the sterilized tip of an inoculation needle and inoculated plates were incubated in an incubator at 25°C. The method was repeated until the pure culture was obtained. Finally, nine isolates were selected for further research.

### Identification of the pathogen and pathogenicity study

The isolated fungus was identified as *Colletotrichum gloeosporioides* and reported in the result section based on morpho-physiological features (Ilis, 2009) and various literature (Iram and Ahmad, 2013; Pandey *et al.* 2012). Pathogenic structures were observed under the compound microscope by slide preparation from a pure culture of *C. gloeosporioides*. Pathogenicity tests were performed in sterilized plastic boxes with three layers of sterilized kitchen tissues soaked in sterilized distilled water. Freshly harvested marble shaped mango fruits were placed in the prepared boxes and inoculation of *C. gloeosporioides* (Isolate 4) was done using a cotton swab, and incubated at room temperature (25±1°C). For maintaining humid condition sterilized distilled water was sprayed on the inoculated fruits when necessary.

### Growth study of the pathogen

The growth study of *C. gloeosporioides* was carried out using a PDA plate inoculated with a block of mycelium (6 mm diameter) in the center of the PDA plates. After the

inoculation, PDA culture plates were incubated in an incubator at 25°C. Each experiment was repeated twice, with three replications in each case. The mycelial growth, shape, colour and compactness of the isolates were then examined. The average linear growth rate was measured by the by the formula described by Elad *et al.* (1981). The fungal isolates were re-cultured in the test-tube PDA slants under the same temperature condition for 7 days and then preserved in a refrigerator for further studies.

### Evaluation of plant extracts, plant hormones and fungicides against *C. gloeosporioides*

One isolate was chosen from the nine isolates used for the growth inhibition study using plant extracts, plant hormones, and fungicides performing modified food poison technique (Nene and Thapliyal, 1979). Six plant extracts, six fungicides, and two phytohormones were employed in the sensitivity test. Name of the treatments along with their active ingredients/ materials is given in Table 1.

### Treatment Preparation

The plant parts used in the extraction were obtained from the Botanical Garden, Bangladesh Agricultural University (BAU), Mymensingh. Garlic was collected from the local market, and hormones and fungicides were collected from fungicide dealers. The plant materials were cleaned and weighed in a balance before being blended with deionized

**Table 1: Details of the treatments used in the sensitivity test**

Treatments	Trade Name/ Common Name	Active Ingredient/ Material
T1	Neem ( <i>Azadirachta indica</i> )	Leaf extract
T2	Garlic ( <i>Allium sativum</i> )	Clove extract
T3	Allamonda ( <i>Allamanda cathartica</i> )	Leaf extract
T4	Bashok ( <i>Adhatoda vasica</i> )	Leaf extract
T5	Marigold ( <i>Calendula officinalis</i> )	Leaf extract
T6	Mahagoni ( <i>Swietenia macrophyllai</i> L.)	Leaf extract
T7	Bavistin DF	Carbendazim
T8	Tilt 250 EC	Propiconazole
T9	Indofil M-45	Mancozeb
T10	Ridomil Gold	Mefenoxam
T11	Pepertox	Copper oxychloride
T12	Thiovit	Sulphur
T13	NAA	1-Naphthaleneacetic acid
T14	2,4-D	2,4-Dichlorophenoxyacetic acid
T15	Control	-

distilled water (DIW) for extract preparation (Hossain *et al.* 1997). PDA containing plant extracts were made in such a way that plant extract was present in a 1:100 ratio. In the case of fungicide, 0.2% concentration was used. The NAA and 2,4-D, were prepared at a concentration of 0.1%. During hormones preparation, 0.04g NAA and 0.012g 2,4-D were weighed and stored in a beaker with a little water to dissolve. After 20 minutes, water was added to 1L to make 40 ppm and 12 ppm solution of NAA and 2,4-D, respectively.

### Inhibition study of mycelia growth of *C. gloeosporioides*

The poisoned food technique with a slight modification (Monjil *et al.* 2013) was pursued to study the inhibition of fungal growth. After the autoclaving procedure, plant extract from each plant sample was added to the PDA medium just before plating in petri dishes. Using a block cutter (6 mm in diameter), the mycelial block was cut and transferred into the central position of the PDA plate containing each ingredient.. For each treatment 3 replications were performed using Completely Randomized Design (CRD). Only PDA was used to make the control plates without other tested ingredients. On daily basis, radial mycelial growth (mm) of *C. gloeosporioides* was recorded (Mukherjee *et al.* 2011). The effect of plant extracts or fungicides or phytohormones was calculated as percent growth inhibition using the

following formulae as described by Onyeani and Amusa (2015).

Percent growth inhibition =  $100 \times (T_1 - T_2) / T_1$   
where,

T<sub>1</sub>= Mycelial growth in the control,

T<sub>2</sub>= Mycelial growth in the treated plate

### Statistical Analysis

Experimental data were analyzed for significance and the treatments were compared with Duncan's multiple range test (DMRT) with a probability of 5%.

## RESULTS

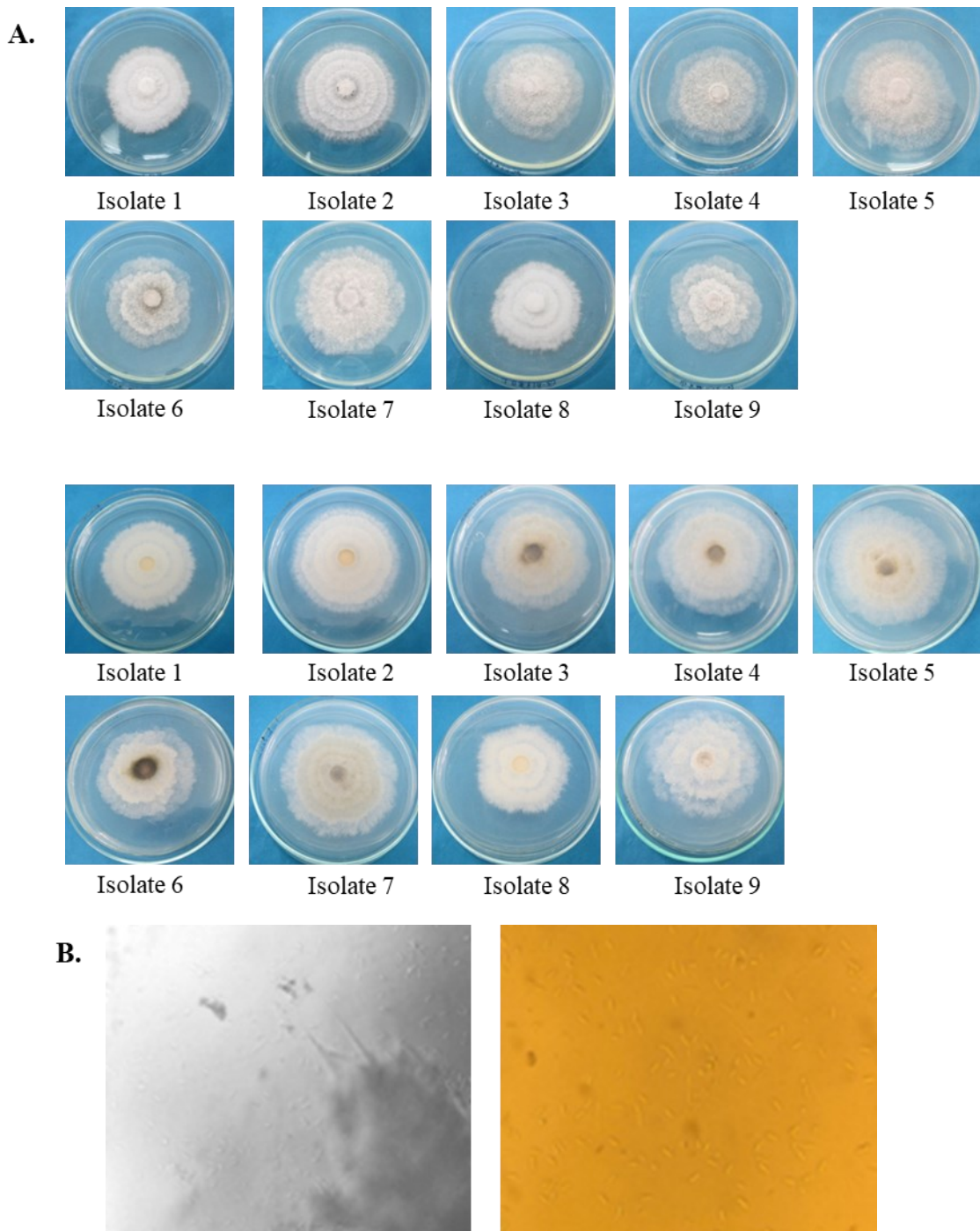
### Identification of the pathogen

The pathogen was identified by observing different morphological characters and through microscopic features by slide preparation. The pathogen was identified as *Colletotrichum gloeosporioides* (Table 1 and Fig. 1). Isolates were morphologically described on the basis of colony color, shape, and compactness prior to the pathogenicity test. The morphological characters of the selected 9 isolates grown on PDA at 25±1°C were studied on the basis of colony color, shape and compactness and their colony characteristics are presented in Table 2.

The colour of the upper side appeared as white, white with brownish speck during maturity, and creamy white while the below side of the cultures vary from creamy white, brownish-white, to blackish white.

**Table 2: Comparison of the different isolates of *Colletotrichum gloeosporioides* on the basis of colony characters**

Isolate	Colony characters			Shape	Compactness
	Color				
	Upper side	Below side			
Isolate 1	White	Creamy white	Regular	Loose	
Isolate 2	White	Creamy white	Regular	Loose	
Isolate 3	Creamy white	Brownish white with black	Irregular	Loose	
Isolate 4	Creamy white	Brownish white with black	Regular	Loose	
Isolate 5	Creamy white	Brownish white with black	Regular	Loose	
Isolate 6	White with brown	Brownish white with black	Irregular	Loose	
Isolate 7	White with brown	Brownish white with black	Regular	Loose	
Isolate 8	White	Creamy white	Regular	Loose	
Isolate 9	Creamy white	Brownish white	Irregular	Loose	



**Figure 1. Isolation and identification of *Colletotrichum gloeosporioides*. A. Culturing different isolates of *C. gloeosporioides* and their growth study, and B. Acervulus and conidia of *C. gloeosporioides***

Tested isolates exhibited a range of mycelial growth patterns, ranging from a regular to an irregular form, with compact or loose growth. Among the 9 isolates, isolate 1, 2 and 8 had similar characteristics with white color, regular shape, and loose consistency. White,

irregular, and loose colonies were found in the 'Isolate 3' whereas 'Isolate 4' and 'Isolate 5' had creamy white, regular, and loose colonies. Colony of 'Isolate 9' was creamy white, irregular, and loose while 'Isolate 7' was blackish white, regular, and loose. The

'Isolate 6' showed blackish white, irregularly shaped, and loose in appearance.

### Comparative growth of the different Isolates of *C. gloeosporioides*

The radial mycelial growth (cm) of the nine isolates of *C. gloeosporioides* was measured from 1 to 7 days after inoculation (Table 2). On the first day after inoculation, the highest radial mycelial growth was observed in 'Isolate 7' followed by Isolate 4 and Isolate 6. From the first DAI (days after inoculation) to 7 DAI, steady mycelial growth was observed in all isolates. At 7 DAI, the highest mycelial growth of *C. gloeosporioides* was observed in 'Isolate 7' (8.6 cm) followed by 'Isolate 4' (8.55 cm) and 'Isolate 6' (8.55 cm). At the same time, the lowest radial growth (6.90 cm) of *C. gloeosporioides* was observed in 'Isolate 8'. At seven DAI, the highest mycelial radial growth rate (cm/day) (1.23cm/day) was observed in 'Isolate 7' closely followed by 'Isolates 4 and 6 (1.22 cm/day), and 'Isolates 2, 3 and 9 (1.21cm/day). The lowest mycelial growth rate (0.99cm/day) was observed in 'Isolate 8' followed by 'Isolate 1' and 5' (1.00cm/day).

### Mycelial growth inhibition of *C. gloeosporioides*

Among the nine isolates, 'Isolate 4' was taken for the growth inhibition study because of

consistent results (similar linear growth among different replications) and stable growth characters in PDA media. 'Isolate 4' was then evaluated against different plant extracts, fungicides, and phytohormones (Table 3 and Fig. 2). Three treatments, namely garlic clove extract (T2), allamonda leaf extract (T3), and mahagoni leaf extract (T6), were found to have the greatest inhibition of mycelial growth (zero growth) of *C. gloeosporioides* (Isolate 4) compared to the other treatments (T6) over the seven-day period.

The second highest inhibition of mycelial growth over control (78.49%) was observed in T4 (Bashok leaf extract) where up to 4 DAI, no mycelial development was seen but afterwards, the fungus started growing slowly. Compared to the control treatment, significantly lower mycelial growth was observed by both neem leaf extract (T1) and marigold leaf extract (T5).

The minimum inhibition of mycelial growth (13.37%) over the control was observed in T5 (Marigold leaf extract). All fungicides (T7 to T12) except Pepertox (T 11) could inhibit the mycelial growth of *C. gloeosporioides* completely (Table 3). Pepertox significantly inhibited mycelial growth of the fungus when

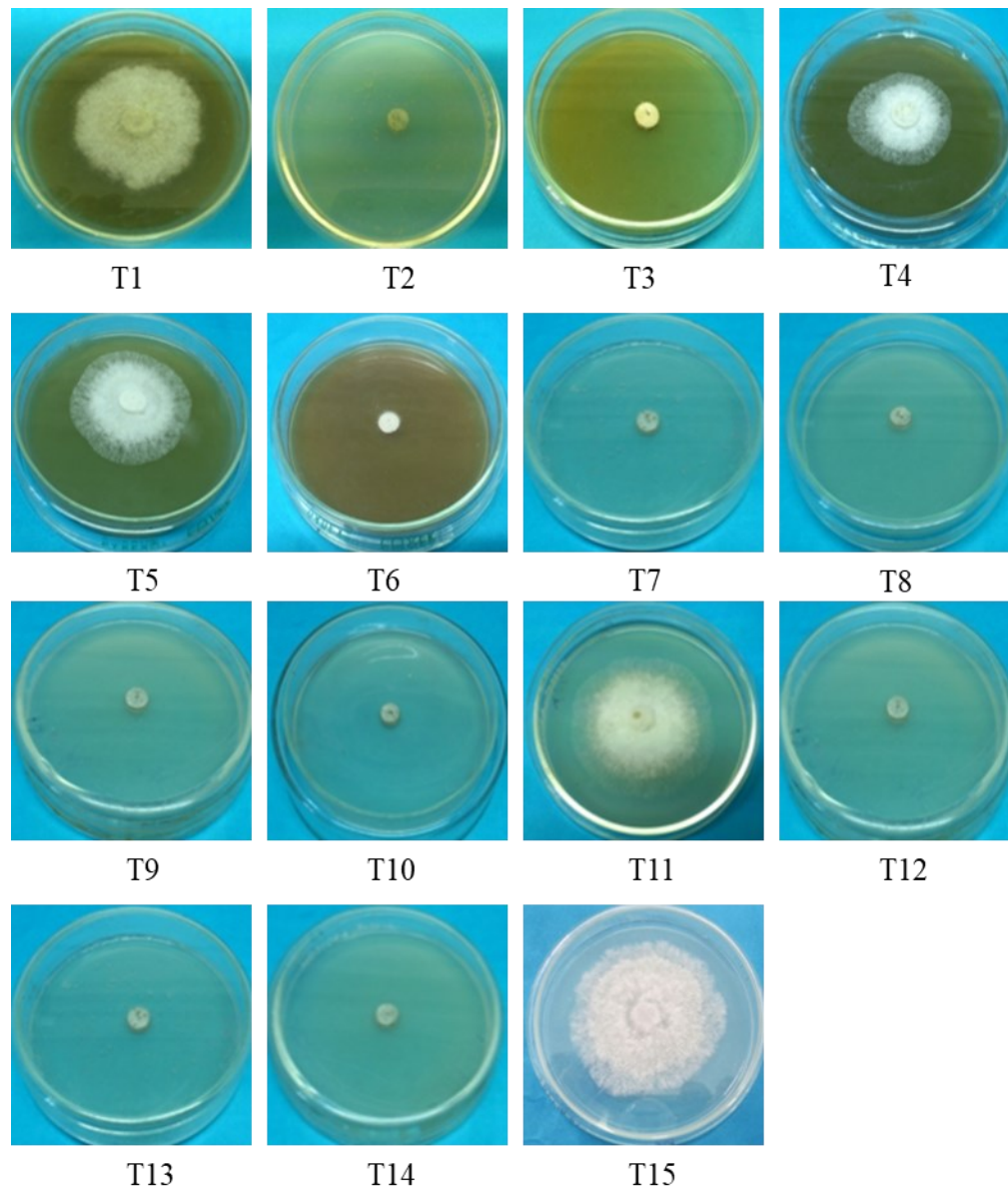
**Table 3: Comparative radial mycelial growth (cm) of *Colletotrichum gloeosporioides* for different isolates**

Isolates	Average radial mycelial growth (cm)							Average growth rate after 7 DAI (cm/day)
	1 DAI	2 DAI	3 DAI	4 DAI	5 DAI	6 DAI	7 DAI	
Isolate 1	1.95	3.49	4.61	5.65	6.05	6.475	7.00	1.00 <sup>b</sup>
Isolate 2	2.21	4.18	5.47	6.60	7.20	7.85	8.50	1.21 <sup>a</sup>
Isolate 3	2.10	4.00	5.33	6.35	7.00	7.75	8.50	1.21 <sup>a</sup>
Isolate 4	2.38	4.56	5.58	6.80	7.30	7.90	8.55	1.22 <sup>a</sup>
Isolate 5	2.00	3.60	4.73	5.60	6.05	6.50	7.00	1.00 <sup>b</sup>
Isolate 6	2.25	4.10	5.33	6.55	7.20	7.875	8.55	1.22 <sup>a</sup>
Isolate 7	2.42	4.49	5.72	6.95	7.45	8.05	8.60	1.23 <sup>a</sup>
Isolate 8	2.08	3.50	4.60	5.50	5.90	6.40	6.90	0.99 <sup>b</sup>
Isolate 9	2.30	4.49	5.77	6.40	7.05	7.70	8.45	1.21 <sup>a</sup>
Level of significance	-	-	-	-	-	-	-	*

Here, DAI= Days after incubation

Data of average growth rate after 7 DAI were subjected to Duncan's Multiple Range Test using a statistical computer package (MSTAT C). Each value represents the mean of three replications. In the column, figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT). \*P < 0.05 versus control treatment (Significant at 5% level of probability).





**Figure 2: Effect of plant extracts on mycelial growth of *C. gloeosporioides* at fifth days of inoculation.**

T1= Neem Leaf Extract, T2= Garlic Clove extract, T3= Allamonda Leaf Extract, T4= Bashok Leaf Extract, T5= Marigold Leaf Extract, T6= Mahagoni Leaf Extract, T7= Bavistin DF, T8= Tilt 250 EC, T9= Indofil M-45, T10= Ridomil, T11= Pepertox, T12= Thiovit, T13= NAA , T14= 2,4- D, and T15= Control

compared to control at the 7 DAI with a percentage of 23. Statistically similar mycelial growth (no mycelial growth) observed by NAA (T13) and 2,4-D (T14).

## DISCUSSION

The experiment was carried out to identify the causal agent of fruit dropping of mango as well as to understand the efficacy of selected plant extracts, phytohormones and fungicides

through an *in vitro* study. *Colletotrichum gloeosporioides* was identified from dropped mango fruits and found infective to fresh mangoes leading to anthracnose symptoms. According to Onyeani *et al.* (2012), *C. gloeosporioides* was the major cause of anthracnose in mango while Jeffries *et al.* (1990) reported *C. gloeosporioides* as the primary cause of anthracnose in disease.

**Table 4: Effect of different plant extracts, fungicides and phytohormones on radial mycelial growth of the *Colletotrichum gloeosporioides***

Treatment	1 DAI	2 DAI	3 DAI	4 DAI	5 DAI	6 DAI	7 DAI
T1	0.32 <sup>c</sup>	0.91 <sup>d</sup>	1.60 <sup>b</sup>	2.425 <sup>e</sup>	3.3 <sup>c</sup>	4.275 <sup>c</sup>	5.25 <sup>c</sup>
T2	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T3	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T4	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	1.3 <sup>d</sup>	1.55 <sup>d</sup>	1.85 <sup>d</sup>
T5	0.49 <sup>c</sup>	1.27 <sup>c</sup>	2.42 <sup>bc</sup>	3.05 <sup>b</sup>	4.1 <sup>b</sup>	6.45 <sup>b</sup>	7.45 <sup>b</sup>
T6	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T7	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T8	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T9	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T10	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T11	1.10 <sup>b</sup>	1.45 <sup>b</sup>	2.18 <sup>c</sup>	3.03 <sup>b</sup>	4.53 <sup>b</sup>	5.40 <sup>bc</sup>	6.63 <sup>bc</sup>
T12	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T13	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T14	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T15	1.20 <sup>a</sup>	3.20 <sup>a</sup>	5.00 <sup>a</sup>	6.4 <sup>a</sup>	7.3 <sup>a</sup>	8.0 <sup>a</sup>	8.6 <sup>a</sup>
Level of significance	**	**	**	**	**	**	**

Here, T1= Neem Leaf Extract, T2= Garlic Clove extract, T3= Allamonda Leaf Extract, T4= Bashok Leaf Extract, T5= Marigold Leaf Extract, T6= Mahagoni Leaf Extract, T7= Bavistin DF, T8= Tilt 250 EC, T9= Indofil M-45, T10= Rido-mil, T11= Pepertox, T12= Thiovit, T13= NAA, T14= 2,4- D, and T15= Control

Data were subjected to Duncan's Multiple Range Test using a statistical computer package (MSTAT C). Each value represents the mean of three replications. In a column, figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT). \*\*P < 0.01 versus control treatment (Significant at 1% level of probability). \*P < 0.05 versus control treatment (Significant at 5% level of probability).

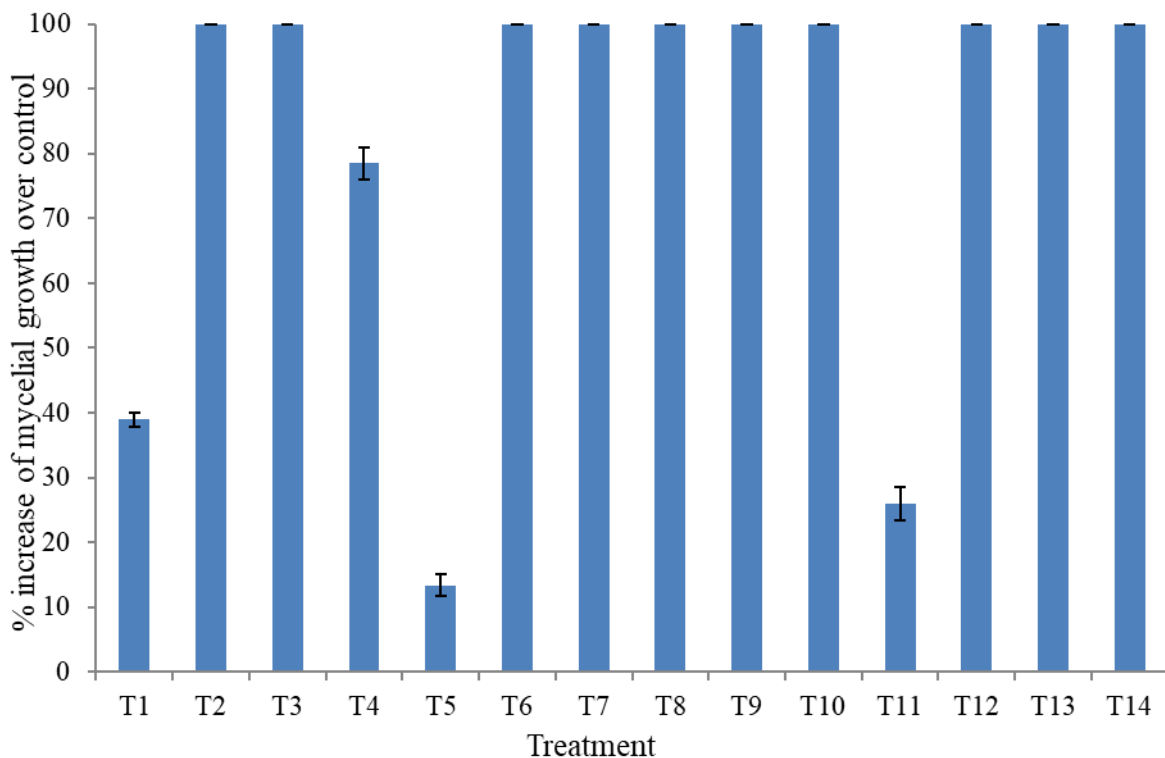
In the PDA culture media, *C. gloeosporioides* was observed as white or creamy white and later on blackish white mycelial growth. The mycelial culture was regular or irregular in shape, and the compactness was loose and these results are in conformity with the others. Holliday (1980) reported that *C. gloeosporioides* grows well on PDA media with a greyish white to dark grey mycelial mat varying from a thick mat to sparse tufts. Vithanage *et al.* (2014) found that *C. gloeosporioides* cultures on PDA were white to grey, cottony and consisted of concentric markings. A similar trend was also observed by Adhikary *et al.* (2013) who reported that *C. gloeosporioides* appeared white with smooth margins on PDA media. A comparative growth study was conducted for the nine isolates of *C. gloeosporioides*. The mean growth of the mycelial colony of the isolates at 7 DAI (days after incubation) ranges from 6.9 cm to 8.55 cm. and the average mycelial radial growth ranges from 0.99-1.23 cm/day. Vithanage *et al.* (2014)

found that the growth rate of *C. gloeosporioides* was 6.7 - 12.4 mm/day.

Under the study of *C. gloeosporioides* growth suppression, all treatments showed a significant effect in inhibiting mycelial growth. Plant extracts of garlic clove, allamonda leaf and mahagoni leaf showed the lowest mycelial growth in PDA media. Pandey *et al.* (2012) observed that leaf extract of *Azadirachta indica* (Neem) effectively inhibited the radial mycelial growth of *C. gloeosporioides*. In another study, it was reported that alcoholic extract of neem leaf significantly inhibited the mycelial growth of *C. gloeosporioides* (Onyeani and Amusa, 2015).

Plant extracts were found to have a considerable inhibitory effect on mycelial growth. In comparison to the control treatment, Garlic clove, Allamonda, and Mahagoni leaf extracts resulted in the greatest growth inhibition (100%) of *C. gloeosporioides* over the control treatment. In





**Figure 3. Percent growth inhibition of *Colletotrichum gloeosporioides* at 7 DAI over control treatment. Each value represents the mean and standard deviation of three replications**

T1= Neem Leaf Extract, T2= Garlic Clove extract, T3= Allamonda Leaf Extract, T4= Bashok Leaf Extract, T5= Mari-gold Leaf Extract, T6= Mahagony Leaf Extract, T7= Bavistin DF, T8= Tilt 250 EC, T9= Indofil M-45, T10= Ridomil, T11= Pepertox, T12= Thiovit, T13= NAA, T14= 2,4- D, and T15= Control

an *in-vitro* investigation, Mukherjee *et al.* (2011) observed that mycelial growth of *C. gloeosporioides* was effectively reduced by garlic extract. The growth of *Penicillium digitatum*, *Aspergillus niger*, and *Fusarium* sp. isolated from naturally infected citrus fruit was suppressed by the *in-vitro* antifungal activity of Mahagony (Singh *et al.* 2012).

Except for Pepertox, all of the chemical fungicides completely suppressed *C. gloeosporioides* and its mycelial growth in PDA media. In the case of Pepertox, the petri plates were contaminated severely. Meah and Khan (1986) reported that Bavistin, Roval, and Topsin M effectively suppressed the mycelial growth of *C. gloeosporioides*. Imtiaj *et al.* (2005) reported Bavistin DF, Dithane M-45, and Tilt 250 EC are the most effective fungicides against the other fungal pathogen, *Bipolaris sorokiniana*. The complete reduction of *C. gloeosporioides* radial growth

was observed by Bavistin and Dithane M-45 (Akhter *et al.* 2009).

### CONCLUSIONS

Based on the findings of the study, aqueous extract of garlic clove, Neem leaf, Allamonda leaf, Mahagony leaf and chemical fungicides such as Bavistin DF, Tilt 250 EC, Ridomil Gold and Thiovit have a considerable inhibitory effect against *Colletotrichum gloeosporioides*. Among the plant extracts, Garlic clove extract was found effective in controlling fruit dropping of mango followed by Neem leaf extract. Phytohormone 2,4-D was found to reduce fruit dropping of mango. However, this study needs to be conducted in field conditions before recommending to farmers the management of premature fruit drops of mango caused by *C. gloeosporioides*.

**AUTHOR CONTRIBUTION**

MTT created the research plan, did the research, analyzed data, and wrote the original draft. IH, and MSM contributed by supervision, writing- reviewing, and editing. All authors have read and consented to the publication of the manuscript.

**REFERENCES**

- Adhikary NK, Dey S, Tarafdar J 2013 Studies on morphology of mango anthracnose disease causing fungus *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. and efficacy of azoxystrobin against the fungus under in vitro and in vivo conditions. *The Bioscan*, 8(2): 493-497.
- Ahmad R 2021 Bangladesh 7th largest mango producer in the world. *Dhaka Tribune*. <https://www.dhakatribune.com/business/economy/2021/07/18/bangladesh-is-top-10-in-global-mango-exporters>.
- Akhter MS, Alam S, Islam MS, Lee MW, 2009 Identification of the fungal pathogen that causes strawberry anthracnose in Bangladesh and evaluation of in vitro fungicide activity. *Mycobiology*, 37(2): 77-81.
- Imtiaj A, Rahman SA, Alam S, Parvin R, Farhan, KM, Kim SB, Lee TS 2005 Effect of fungicides and plant extracts on the conidial germination of *Colletotrichum gloeosporioides* causing mango anthracnose. *Mycobiology*, 33(4): 200-205. <https://doi.org/10.4489/MYCO.2005.33.4.200>
- BBS 2020: Statistical Yearbook of Bangladesh 2019 (31<sup>st</sup> Series, May-2020). Bangladesh Bureau of Statistics, Statistics and Informations Division, Ministry of Planning, Government of Peoples Republic of Bangladesh.
- Dodd JC, Estrada AB, Matcham J, Jeffries P, Jeger MJ 1991 The effect of climatic factors on *Colletotrichum gloeosporioides*, the causal agent of mango anthracnose in the Philippines. *Plant Pathology*, 40: 568-575.
- Dodd JC, Estrada AB, Matcham J, Jeger MJ 1992 Epidemiology of *Colletotrichum gloeosporioides* in the tropics. p 308-325 In: JA Bailey and MJ Jeger (eds). *Colletotrichum-Biology, Pathology and Control*. Wallingford, UK; CAB International.
- Ellis D 2009 *Colletotrichum coccodes*. The University of Adelaide, Australia. <http://www.mycology.adelaide.edu.au>.
- Elad YI Chet and Henis Y 1981 A selective medium for improving quantitative isolation of *Trichoderma* spp. From soil. *Phytoparasitica*, 9(1): 59-69
- Falex JL, Zainon MN, Fauziah I 2013 Isolation of *Colletotrichum gloeosporioides* and antagonist bacteria from Harumanis mango and screening for biocontrol activities. *The Open Conference Proceedings Journal*, 4: 254
- Freeman S 2008 Management, survival strategies, and host range of *Colletotrichum acutatum* on Strawberry. *HortScience*, 43(1): 66-68.
- Holliday P 1980 *Fungus Diseases of Tropical Crops*, Cambridge University Press, Cambridge. 336 pp.
- Hossain I, Mahamud M, Ashrafuzzaman H 1997 Effects of plant extracts on fungi (*Bipolaris sorokiniana* and *Rhizoctonia solani*) and okra mosaic disease. *Ecoprint* 4(1): 35-42.
- Iram S, Ahmad HMI 2013 Major post harvest diseases of mango and their management. *International Journal of Agronomy and Plant Production* 4: 3470-3484.
- Jeffries P, Dodd JC, Jeger MJ, Plumbley RA 1990 The biology and control of *Colletotrichum* species on tropical fruit crops. *Plant Pathology*, 39:343-366.
- Meah MB, Khan AA 1986 Survey of diseases of some important fruits and vegetables of Bangladesh. Annual Progress Report (1985-1986) Mymensingh: Department of Plant Pathology. Bangladesh Agricultural University; p. 113.
- Monjil MS, Shibata Y, Takemoto D, Kawakita K 2013 Bis-aryl methanone

- compound is a candidate of nitric oxide producing elicitor and induces resistance in *Nicotiana benthamiana* against *Phytophthora infestans*. *Nitric Oxide* 29: 34-45.
- Mukherjee A, Khandker S, Islam MR, Shahid SB 2011: Efficacy of some plant extracts on the mycelial growth of *Colletotrichum gloeosporioides*. *Journal of Bangladesh Agricultural University* 9(1): 43-47.
- Nelson SC 2008 Mango anthracnose (*Colletotrichum gloeosporioides*). College of Tropical Agriculture and Human Resource. Publication PD-48.
- Nene YL, Thapliyal BW 1979 Fungicides in plant disease control. Oxford & IBH Publisher.
- Onyeani CA, Amusa NA 2015 Incidence and Severity of Anthracnose in Mango Fruits and its Control with Plant Extracts in South West Nigeria. *International Journal of Agricultural Research*,10: 33-43.
- Onyeani CA, Osunlaja SO, Owuru OO, Sosanya OS 2012 Mango fruit anthracnose and the effects on mango yield and market values in Southwestern Nigeria. *Asian Journal of Agricultural Research*,6(4): 171-179.
- Pande A, Kamle M, Chauhan UK, Pande BK 2009: Evaluation of plant extracts against *Colletotrichum gloeosporioides* an incited of mango anthracnose disease. *Plant Archives*,9 (2): 947-949.
- Pandey A, Yadava LP, Mishra RK, Pandey BK, Muthukumar M 2012 Studies on the incident and pathogenesis of *Colletotrichum gloeosporioides* Penz. Causes Anthracnose of Mango. *International Journal of Science*, 3(2): 220-232.
- Pitkethley R, Conde B 2007 Mango anthracnose. *Agnote* No. 123, August 2007. [http://www.nt.gov.au/d/Content/File/p/Plant\\_Pest/604.pdf](http://www.nt.gov.au/d/Content/File/p/Plant_Pest/604.pdf).
- Ploetz RC 1998 Anthracnose. In: RC Ploetz, GA Zetmeyer, WT Nishijima, KG Rohrbach and HD Ohr. (Editors), *Compendium of tropical fruit diseases*. The American Phytopathological Society, Minnesota. p 35-36.
- Ploetz RC 2003 Diseases of mango. In: Ploetz RC (Editor), *Diseases of tropical fruit crops*. University of Florida (UFAS), Florida. p. 329-331.
- Rahim M, Anowar MM, Begum KS, Monjil MS 2016 Management of insect pest and diseases of vegetable crops in Bangladesh (Bengali). Published by-USAID/ Agro-inputs project (AIP) and agro-input retailers networks (AIRN).
- Reza MMA, Kader KA 1995 Efficacy of pre-harvest field sprays of fungicide on the control of anthracnose and stem end rot of mango. *Annual Report, Regional Horticultural Research Institute, Nawabgonj*. pp 55-58.
- Sarker SR 2008 Nursery diseases of mango and their management. *Masters Thesis, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh*. pp. 64-68.
- Singh H, Alsamarai G, Syarhabil M 2012 Performance of botanical pesticides to control post-harvest fungi in citrus. *International Journal of Scientific & Engineering Research* 3(4): 1-4.
- Vithanage IK, Adikaram N, Yakandawala D 2014 Molecular and Morphological Characterization of *Colletotrichum* Causing Mango Anthracnose in Sri Lanka. *Proceedings of the Peradeniya Univ. International Research Sessions, Sri Lanka, Vol. 18, Abstract No: 456 Plant Science and Forestry* 573p.