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IDENTIFICATION OF *Lasiodiplodia theobromae* [(Pat.) Griff. & Maubl] AS A CAUSAL PATHOGEN OF RAIN TREE GUMMOSIS [*Samanea saman* (Jacq.) Merr.] AND ITS CONTROL MANAGEMENT

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Abstract

Gummosis in a Rain tree [*Samanea saman* (Jacq.) Merr.] is a new disease in Bangladesh. The prevalence of this disease is increasing over time. An exploratory survey was made to find out the extent of this disease in different areas of Bangladesh during 2017 and 2018. Infested samples of wood were collected to find out associated pathogens. After the isolation of the fungi in the Forest Pathology Laboratory of the Bangladesh Forest Research Institute, the optimal conditions for the growth of the pathogen was determined. Subsequently, the suitable control method was developed. It was found that the roadside plantation of Mongla Sadar Thana, Bagerhat district had the highest disease incidence and severity (42.93 and 54.38 %), and the lowest (12.63 and 18.34 %) was recorded at Satkhira Sadar Thana, Satkhira district, respectively. *Lasiodiplodia theobromae* [(Pat.) Griff. & Maubl)] was found associated with gummosis in the affected trees. The result of the pathogenicity test revealed that there was a similarity in symptoms that arise between artificial inoculation and natural symptoms in the field. The optimal condition of conidial germination, mycelial growth, and sporulation of *L. theobromae* was observed at pH 6-8, 90-95 RH, and 25-30°C temperature. The concentration of 2.5 % glucose and sucrose was the best for conidial germination, mycelial growth, and sporulation, and sucrose was better than glucose. PDA medium had the maximum mycelial growth (72.18 mm) and excellent sporulation, while the YEA medium had the lowest mycelial growth (59.19 mm) and poor sporulation. The fungicides Knowin (Carbendazim), ARBA (Carbendazim), and Autostin (Carbendazim) were found to completely inhibit pathogen mycelial growth and sporulation (100%) at 50, 100, and 150 mg/L concentrations. Furthermore, spraying these fungicides and the Bordeaux mixture at a rate of 2 % in the field resulted in the development of the smallest gummosis lesions. *L. theobromae* was a pathogen causing gummosis disease of rain tree, proven by morphology and Koch's postulates. Different environmental and nutritional factors affect the growth and sporulation of the pathogen and the application of Knowin, ARBA, Autostin, and Bordeaux mixture at 2 % can help control the disease at the field level.

Keywords: Bordeaux mixture, Disease incidence, *Lasiodiplodia theobromae*, Pathogenicity test

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Introduction

Rain tree [*Samanea saman* (Jacq.) Merr.] is a fast-growing multipurpose exotic tree species in Bangladesh that have been widely planted in village woodlots, dams, roadside, community forests, private forests, and homesteads in many districts of the country (Zabala, 1991). The tree is used for various purposes including shade, ornamental, furniture, animal feed, and medicine (Ferdous *et al*., 2010). This tree species has a hugely beneficial contribution to the socio-economic development of the country. Bangladesh is a densely populated country having more than 1000 people per square kilometer with only 12.8% forest cover is not enough to meet the demand for forest products (GoB, 2020). Government and private organizations have taken the initiative in the last 10 to 15 years to plant a variety of tree species on the roadside, fallow lands, and marginal lands to expand the number of forested areas in the country. Due to this initiative of the government, a large number of rain trees are being planted in almost all parts of the country. This is one of the most planted tree species in the Barisal and Khulna districts, contributing approximately 19 % of the total area (Mondal, 2016).

Rain tree is susceptible to various pests and diseases in both nursery and plantation stages. Dieback and gummosis disease are very significant in the Indian sub-continent among plantation diseases (Mondal, 2016). In recent years, rain tree plantations in different areas of Bangladesh suffered from a declining disease showing symptoms of drying branches from the tip accompanied by a heavy exudation of yellowish-brown gum from the stem and its branches and browning of vascular tissues. Infected wood and the defoliation that may occur weaken the trees but if the disease infects the trunk, the tree may die. Recently, 25-30 % rain tree mortality has been recorded due to gummosis in major rain tree plantation areas of Bangladesh (Papia, 2018).

L. theobromae [(Pat.) Griff. & Maubl] has been known as a fungus with a wide host range, estimated at more than 280 plant species, and with varied pathological effects on its hosts (Domsch *et al*., 2007; Khanzada *et al*., 2004). In tropical areas, *L. theobromae* is known to cause major losses to mango, cocoa, banana, and sweet potato farmers (Rieger, 2006; Amusa *et al*., 2003). It is the causal agent of gummosis of branches and trunks of citrus, mango, cashew, and neem (Hasan *et al*., 2020; Khanzada *et al*., 2018; Twumasi, 2014; Cardoso *et al*., 2006; Khalil, 2012). In Bangladesh, there has been no information about this fungus as the disease agent in rain trees and whether *L. theobromae* from various other hosts can infect rain trees. Thus, the main objective of this study was to find out the causal organism associated with the gummosis disease of rain trees and to develop a suitable control method for rain tree gummosis.

Materials and Methods

Survey for the incidence of gummosis disease of rain trees in different parts of Bangladesh

In this present study, a survey was carried out in three districts of Bangladesh namely; Chattogram, Satkhira, and Bagerhat with the view to documenting quantitatively the incidence and severity of the gummosis disease of rain trees from July 2017 to December 2018. From each district, samples from 50 rain trees were collected and a

gummosis incidence was assessed. The age of the trees was obtained from the local forest office and validated by the people living around the spots. The disease incidence and severity were assessed in different planting type's viz. nursery plantation, roadside plantation, orchard plantation, and individual tree planting. Gummosis incidence was determined as the proportion of plants showing gummosis symptoms and expressed as a percentage of the total number of plants assessed (Jagtap *et al.,* 2012). A tree was defined and recorded as having gummosis when it had any of the following symptoms: discoloration of the bark surface, discoloration of the underlying tissues, the dried whole part of the plant, and the exudation of the gum from infected tissues.

Calculating the disease incidence (Jagtap *et al.,* 2012)

$$\text{Percent disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plant } examine} \times 100$$

Disease severity index was calculated as:

$$\text{(DSI)} = \frac{Sum\ of\ all\ disease\ rating}{Total\ number\ of\ assed\ plants \times maximum\ rating\ value} \times 100$$

Disease scale description of disease status

0 = Tree with no symptom associated with gummosis; 1 = Decline symptom associated with gummosis up to 25 % of the branch affected; 2 = widespread decline of the branch associated with gummosis up to 25-50 % of the branch affected; 3 = Decline and death of the branch associated with gummosis up to 50-75 % of the branch affected; 4 = Decline of 75-100 % tree, including the dead tree.

Collection of samples

Stem and branches of rain trees that were showing gummosis were collected from the survey areas and used for pathogen isolation. Both the aerial as well as underground portions of the trees were carefully studied for any pathogenic infection. Disease symptoms were found at the junction of dead and healthy portions of the stems and branches. Diseased specimens were collected in polythene bags and brought to the forest pathology laboratory of Bangladesh Forest Research Institute (BFRI).

Isolation and identification of the pathogen responsible for the disease

In the laboratory, infected tissues were excised with a sterilized scalpel at the point of disease symptom progression, then surface sterilized for 2 minutes with a 70 % ethanol solution. The tissues were then washed three times with sterilized water and then dried on sterile paper towels followed by incubation on PDA (Hi-Media, India) medium at 35°C in the darkroom for 3 days. Lactic acid (1 mL) and streptomycin sulphate (0.5 g/L, Sigma-Aldrich, USA) was incorporated into the medium as supplements. Using a single spore technique, the mycelium from the diseased sample was re-isolated and transferred to a petridish containing fresh PDA media. The Petri dishes were kept in a dark room at 25ºC for 7 days. For pure culture, the experiments were carried out at different times. To grow, the isolated fungus was sub cultured on PDA media. For further experiments, the sub-cultured plates were kept in a refrigerator at 4ºC. Identification of fungi was based on morphological and microscopic characteristics.

Pathogenicity test of the causal organism

The pathogenicity test was conducted at Forest Pathology Laboratory and Nursery at BFRI Campus, Chattogram. One-year-old seedlings of the rain trees were selected as a host for conducting pathogenicity tests. Using a sterile knife, a 1 x 2 cm inoculum block was made into the stem of the rain tree seedlings. A 5 mm inoculum disc from 5- day-old culture of a test fungus on PDA was placed in the gap and the inoculated portion and wrapped with Parafilm. In the control plants, a 5 mm PDA block without fungus was placed. Seedlings were irrigated after inoculation and the wrapping material was removed from the stems after 2 weeks of inoculation. Seedlings were monitored for the development of disease symptoms and isolations were made from the stem of the test plants to confirm the pathogenicity. The experiments were carried out in a randomized complete block design with three replications. Ten plants were used in each replication.

Influence of different nutrition, physical and environmental parameters on the growth and development of pathogen

Environmental and nutritional factors

Conidial germination (CG)

Relative humidity (70, 75, 80, 85, 90, 95 and 100), pH (4, 5, 6, 7, 8, 9 and 10), temperatures (5, 10, 15, 20, 25, 30 and 35ºC), Glucose and Sucrose solutions (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 % concentrations) were used to get suitable condition for CG. Conidia were collected from a 10-day-old PDA plate culture, and suspension (10^3/ml) was prepared using sterilized distilled water (for RH, pH, and temperature)/different concentrations of glucose and sucrose solution separately. A drop of the conidial suspension was placed on a separate groove slide and stored at 25ºC in a moisture chamber for 24 hours after being taken in a sterilized watch glass. After the incubation period, a drop of lactophenol cotton blue was used to cover the conidial suspension on the slide, and the percentage of CG was calculated using (x 40) power microscopes. Three replications were used for each particular treatment.

Mycelial growth and sporulation

Relative humidity, pH, temperature, glucose, sucrose (same range as CG), and seven solid media (Yeast extract Agar, Potato Dextrose Agar, Malt extract agar, Oatmeal, Richards, Sabourauds, and Czapeks) were evaluated to get favourable MG and sporulation of the pathogen. Effect of RH, pH, temperature, glucose, and, sucrose on MG of the pathogen was done using a PDA medium. Different media were autoclaved at 121°C/15lbs/inch^2 pressure; these were poured into sterilized Petri dishes. Agar discs (5 mm) were taken from 10-d10-day-oldture of pathogen and placed separately with respect to each treatment in the centre of each petridish and incubated in the respective conditions. After seven days of incubation, radial mycelium growth was measured by following Brown (1923) methods. Three replications were used for each particular treatment. After seven days, two discs (5 mm in size) were cut selectively and shaken vigorously in a test tube containing 5 ml of distilled sterile water to investigate the sporulation of the pathogen. To facilitate conidia counting easier, a forty- µl cotton blue

solution was poured into it. The ten-µl conidial suspension was put on slides from each treatment with the help of a micropipette. The conidia were (conidia/microscopic field under 40 X) counted for each treatment under a compound microscope.

In-vitro evaluation of fungicides against mycelial growth, sporulation, and conidial germination inhibition of pathogen

Mycelial growth inhibition

Fourteen commercial fungicides were tested *in vitro* for their effects on conidial germination and mycelial growth of the pathogen followed by the poisoned food technique. The radial growth of the colony was recorded on the 7th day when maximum growth was observed, and percent inhibition was calculated using the formula given by Vincent (1927).

I = $\frac{C-T}{C}\times$ 100; Where I = Percent Inhibition; C = Radial growth of fungus in control; T= Radial growth of fungus in treatment. The details of the fungicides used against the pathogen are given in Table 1.

Table 1. The details of the fungicides used against the pathogen

Sl. No.	Trade name	Chemical name
1.	Indofil	Mencozeb
2.	Knowin	Carbendazim
3.	Ridomil	Manocozeb
4.	Oxyvit	Copper oxychloride
5.	Cupravit	Copper oxychloride
6.	Aimcozim	Carbandazim
7.	Champion	Copper hydroxide
8.	Sunvit	Copper oxychloride
9.	Diathane M 45	Mancozeb
10.	Thiovit	Sulpher
11.	Autostin	Carbendazim
12.	Amivit	Copper oxychloride
13.	Rovral	Eprodion
14.	ABRA	Carbendazim

Conidial germination inhibition

Conidia of *L. theobromae* cultured on PDA plates were taken and suspensions (10^5/ml) were made separately with different concentrations of different fungicides. These suspensions (1.25 ml) were taken in small sterilized Petridishes (65 mm) and were kept at 28±2^{o}C for 5-30 minutes. A drop of treated conidial suspension (from different concentrations of fungicide) was taken on separate slides to continue for 5 min. interval and was kept at 28±2^{o}C in a humidity chamber for 24 hrs of incubation. Then a drop of

lactophenol cotton blue was placed on the conidial suspension on the slides. The slides were examined under the high-power microscope (×40) for recording the percentage of conidial germination. Three replications were used for each particular treatment. Percentage inhibition of conidial germination (PICG) using the formula by Skidmore and Dickinson (1976). Where PICG = C_1-C_2/C_1 × 100.

C_1 = Total number of conidia in the control treatment.

C_2 = Germination of conidia in fungicidal treatment.

Inhibition of the sporulation

Once the control had reached maximum growth, circular portions of 1 cm in diameter were taken from the active growth site of each treatment and the corresponding repetitions and placed on Petri dishes containing 5 mL sterile distilled water; the mycelium was gently taken with the help of a sterilized glass handle, and the conidia were counted with a hemocytometer by-product, treatment, and repetition. The control proceeded through such a process. Three replications were used for each particular treatment.

Efficacy of chemical fungicides to control rain tree gummosis under field conditions

The experiments were carried out in the Forest Protection Division Nursery (FPD) at BFRI. Six-month-old rain tree seedlings were used in this study. Seedlings were previously inoculated with agar culture discs containing the mycelium of *L. theobromae* at the stem as described before (Pathogenicity test). After 30 days of inoculation when the disease is in progress, plants were then either sprayed with the fungicide (2 %; treatment) or with sterilized distilled water (control). The application of fungicide on the stem started in May to August 2018. Fourteen commercial fungicides (Indofil, Sunvit, Diathene M45, Oxyvit, Rovral, Aimcozim, Thiovit, Ridomil, Amivit, Cupravit, Champion, Knowing, Arba, and Autostin) and Bordeaux mixture (2 %) were sprayed for control of gummosis disease of rain tree under field condition. A total of three replications were at individual treatment and lesion size was recorded after 30 days interval. Ten plants were used in each replication.

Treatments

There were the following 14 treatments. T_0 = Control (Without fungicide), T_1 = Indofil, T_2 = Sunvit, T_3 = Diathene M45, T_4 = Oxyvit, T_5 = Rovral, T_6 = Aimcozim, T_7 = Thiovit, T_8 = Ridomil, T_9 = Amivit, T_{10} = Cupravit, T_{11} = Champion, T_{12} = Knowing, T_{13} = Arba, T_{14} = Autostin, T_{15} = Bordeaux mixture.

Statistical analysis

All data were analyzed by DMRT using the help of the computer package program SPSS (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Survey of disease incidence in different rain tree growing areas of Bangladesh

The incidence and severity percentages of rain tree gummosis in different planting types were shown in Table 2. The highest incidence and severity percentage assessed in roadside plantation were at Mongla Sadar Thana, Bagerhat district (42.93 and 54.38 %), and the lowest incidence and severity percentages (12.63 and 18.34 %) were recorded at Satkhira Sadar thana, Satkhira district, respectively.

Table 2. Incidence and severity of rain tree gummosis in a different part of Bangladesh.

Districts	Thana/union	Planting type	Age (Year/Month)	Incidence (%)	Disease severity (%)
Chattogram	Ramgarh, Fatikchari	Orchard plantation	15-38 years	27.75 c	29.38 d
	Udalia, Katirhat, Fatikchari	Orchard plantation	25-45 years	24.85 de	26.52 f
	Dantmara, Bhojpur, Fatikchari	Orchard plantation	25-40 years	28.85 bc	32.65 g
	Chattogram sadar, BFRI, campus	Nursery	6-12 month	14.79 h	19.18 h
Satkhira	Shyamnagar	Road-side plantation	10-45 years	25.72 d	28.47 e
	Kalaroa	Road-side plantation	15-50 years	23.28 ef	26.79 ef
	Satkhira Sadar	Road-side plantation	15-45 years	12.63 i	18.34 i
Bagerhat	Chila, Mongla	Single plantation	15-50 years	18.32 g	22.71 g
	Chandpai, Mongla	Single plantation	15-60 years	22.46 h	29.18 d
	Sundarban 89	Road-side plantation	7-45 years	29.61 b	35.89 b
	Mongla sadar	Road-side plantation	15-65 years	42.93 a	54.38 a

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

Symptoms of gummosis disease on rain tree

The affected trees initially had sunken lesions on the trunks, twigs, and branches. These recessed lesions develop darker in color over time, and exudation of yellowish, white, or transparent gum through them becomes more noticeable, it spreads throughout the body within three to six months and then the tree dies. Diseased trees suffer from

defoliation, but if the disease spreads to the trunk, the tree may die. Infected trees frequently had additional symptoms, such as vascular discoloration beneath the gummosis. Canker develops on the stem and branches of plants as they grow older. Infected trees show signs of different levels of dieback (Fig. 1 A-D).

Isolation and identification of the pathogen

On PDA, *L. theobromae* (Pat.) Griff. & Maubl, synonym *Botryodiplodia theobromae* colonies had white aerial mycelia that eventually turned dark olivaceous mycelium (Fig.1E). Conidia ranging in color from dark brown to black were produced by mycelium (Fig. 1G).

Fig. 1. Gummosis disease symptoms and causal organism of rain tree gummosis. A: An affected mature rain tree showing dead branch at a road site plantation in Shyamnagar, Satkhira; B: Gum exudation in the main trunk; C: bark cracking symptoms in a stem in a younger rain tree; D: Xylem necrosis of the gum-secreting incision; E: *L. theobromae* cultured on PDA medium after 7 days; F & G: Mycelium and conidia of *L. theobromae.*

Mycelial growth and production of immature and mature conidia have also been observed. Conidia were sub-ovoid or ellipsoid, thick-walled, hyaline, and one-celled when immature, but matured to dark brown, two-celled, and with irregular longitudinal striations. The size of mature conidia averaged 24.6 ± 0.24 µm long and 13.9 ± 0.16 µm wide (Fig.1G). Pycnidia contained septate paraphyses. Based on the morphological and microscopic characters observed and by comparing with those previously reported (Auger *et al*., 2004; Larignon *et al.*, 2001; Phillips, 2002; Punitthalingam and Waller, 1976; Taylor *et al.*, 2005; Úrbez-Torres *et al.*, 2006; Pavlic *et al.*, 2007), the fungus was confirmed as *L. theobromae*.

Pathogenicity test of the causal organism

L. theobromae was found associated with the gummosis disease of rain tree. After 15 days of inoculation in rain tree plants, *L. theobromae* showed typical symptoms of the disease. No vascular browning was observed in control plants. Likewise, dyeing of

tips, internal browning, death of leaves at the branch apices, and gum exudation were observed where plants were inoculated with pathogen after 15 days (Fig. 2 A-I and Table 3). Previous studies have noted that *L. theobromae* is a wound parasite of plants (Punithalingam, 1976), although it is a rare source of human infection (VSlez and Diaz, 1985). This fungus attacks more than 500 species of plants in different parts of the world (Punithalingam, 1980). It is a common tropical and subtropical plant pathogen with a wide host range associated with different decline syndromes, including *Acasia confusa*, *Albizia falcataria (Paraserianthus falcataria)*, *Eucalyptus* sp., *Mangifera sylvatica*, *M. indica*, *Mangnolia candolii*, *Paulownia fortune*, *Vitis vinifera*, *Prunus domestica*, *Citrus lemon*, and *Vitis vinifera*, among others (Alves *et al.*, 2008; Abdollahzadeh *et al.*, 2010; Trakunyingcharoen *et al.*, 2018; Pipattanapuckdee *et al.*, 2019; Pillay *et al.*, 2013; Slippers and Wingfield, 2007; Shahbaz *et al.*, 2009; de Silva *et al.*, 2019). This pathogen has become important because it causes numerous diseases, including seed rot (Gure *et al.*, 2005), and stem canker, dieback, root rot, fruit rot, leaf spot, and witches' broom (Punithalingam, 1980).

Pathogen re-isolation and confirmation by microscopic observation

At the end of the experiment of pathogenicity, re-isolation of the pathogen was carried out for identification. The mycelia and conidia of the re-isolated pathogen were observed under a compound microscope. Based on Koch's postulate examination, it was possible to reproduce the disease by artificially inoculating the rain tree plant with pathogens to cause the recurrence of the disease (Khalil, 2012). It indicated that the gummosis disease is caused by *L. theobromae* and it plays a significant role in disease development. Re-isolation from the dead and green branches of *L. theobromae* inoculated plants showed up to 95 % recovery of the fungus (Data not shown).

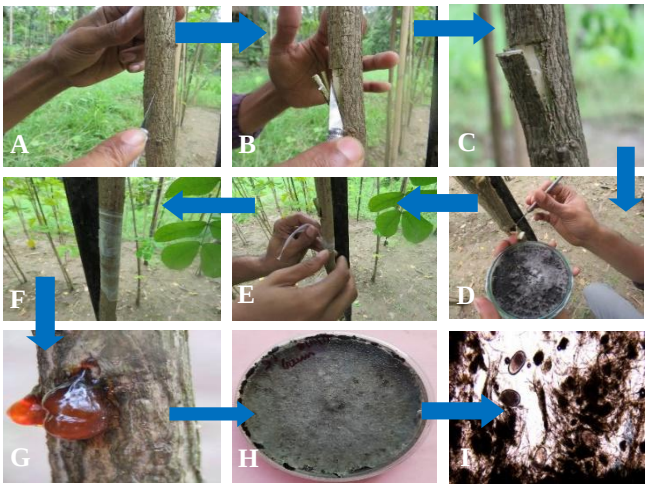

Fig. 2. Pathogenicity tests of *L. theobromae*, the causative organism rain tree gummosis at different stages. A: block was made using a sterilized knife (1 x 2 cm size); (B & C) Remove the bark from the cut portion; (D) Inoculation of fungus disc in the cut portion; (E & F) Wrapped by parafilm; (G) Gum oozing from inoculated portion after 15 days of inoculation; (H) Re-isolated of *L. theobromae* from infected portion; I: Microscopic view of *L. theobromae.*

Influence of different nutrition, physical and environmental parameters on conidial germination, mycelial growth, and sporulation of *L. theobromae*

According to the results, pH has a significant impact on mycelial growth, conidia germination, and sporulation of *L. theobroma*. The highest CG, MG, and sporulation of *L. theobromae* were observed at 6-8 pH. Relatively less growth was obtained at the 4 and 10 pH levels (Table 4 & 5). Filamentous fungi are known to be acid-tolerant, with most of them preferring a pH of 5.0 to 6.0 for cellular development and various metabolic functions (Rosfarizan *et al*., 2000). This studies have similarities with the records of Baloch *et al.*, (2018) who recorded the maximum mycelial growth of *L. theobromae* on media in which pH levels were adjusted at 7 and 8. The results of *L. theobromae* conidia formation at various pH levels were similar to those reported by Zhao *et al*., (2010).

Table 3. The severity of symptoms on rain tree plants inoculated with *L. theobromae*

Treatments	Symptoms produced on rain tree plant			
	Dyeing of tips	Gum exudation	Internal browning	Death of leaves at the branch apices
L. theobromae	1	3	2	2
Control	0	0	0	0

0 = No symptoms, 1 = Very light, 2 = Moderate, 3 = Severe symptoms.

Relative humidity is one of the major limiting factors determining pathogens' growth, conidial germination, and disease development. In the present study, *L. theobromae* showed variation in its mycelial growth, conidial germination, and sporulation at different relative humidity levels. Among the seven relative humidity levels tested, the maximum mycelial growth, conidial germination, and excellent sporulation were recorded at 95 % relative humidity, followed by a 90 % relative humidity level (Table 4 & 5). Similarly, the earlier reports of Udhayakumar (2018) observed the highest percentage of conidial germination, and mycelial growth of *Colletotrichum falcatum* at 90 to 100 % relative humidity. In another study, Gadgile *et al*., (2009) stated that the development of *B. theobromae* rot is dependent on relative humidity.

On the PDA medium, the colony growth of *L. theobromae* varied in response to temperature changes. The temperature ranges of 25°C and 30°C were shown to be optimal for the fungus's fastest mycelial growth, conidial germination, and sporulation. The influence of other temperature ranges was moderate (Tables 4 and 5). These findings are in full agreement with Rehman *et al*., (2011) who observed the highest growth of *L. theobromae* when it was incubated at 30ºC and 25ºC, and the minimum growth was obtained when *L. theobromae* was incubated at 15ºC. Fernández *et al*., (2014) found that temperature highly affected the mycelial growth of *B. cinerea* isolates and discriminate isolates based on their temperature optima.

Different media had a significant impact on the mycelial radial growth rate and sporulation of *L. theobromae*. The highest mycelial colony growth and sporulation of the test fungus were observed on the PDA medium, whereas the minimum mycelial colony

growth and poor sporulation were seen on the YEA medium (Table 5). The presented results were consistent with those of Alam *et al.,* (2001), who found that *L. theobromae* mycelium growth was highest on Potato Dextrose Agar and Czapek Dox agar media. Likewise, on Potato Dextrose Agar, Baloch *et al.,* (2018) observed the quickest mycelial development of this fungus. Several other workers also stated that PDA was the best media for the mycelial growth of *L. theobromae* (Maheshwari *et al.*, 1999).

Different concentrations of glucose and sucrose significantly inhibited MG, CG, and sporulation in *L. theobromae*. The highest MG, CG, and excellent sporulation, were in 2.5 of glucose and sucrose solution. Sucrose has shown better results than glucose (Tables 4 and 5). Jash *et al*., (2003) observed that sucrose is the best carbon source for the growth of *Alternaria zinniae* followed by starch and maltose. In another study, Ray (2004) showed that lactose and glucose had a similar effect on the growth of *L. theobromae*.

In-vitro evaluation of fungicides against mycelial growth, conidial germination and inhibition of sporulation of *L. theobromae*

The percent inhibition of mycelial growth, conidial germination, and sporulation of *L. theobromae* by different fungicides (Indofil, Sunvit, Diathene M45, Oxyvit, Rovral, Aimcozim, Thiovit, Ridomil, Amivit, Cupravit, Champion, Knowin, Arba, and Autostin) varied significantly ($p \leq 0.05$) affected at different concentrations *in vitro*. The highest percent inhibition of mycelial growth, conidial germination, and sporulation (100 %) were observed with Knowin, ARBA, and Autostin at 50, 100, and 150 mg/L concentrations (Fig. 3, 4 and 5). These results agree with those of Pitt *et al.*, (2010) who also reported that mycelial growth of *Diplodia seriata*, *Neofusicoccum parvum, Lasiodiplodia theobromae,* and *Botryosphaeria dothidea* was significantly inhibited by carbendazim, tebuconazole, procymidone, iprodione, and fluconazole. Khanzada *et al.,* (2004) also found that Carbendazim and Thiophanate-methyl were highly effective in inhibiting the growth of the *Lasiodiplodia theobromae*. Saeed *et al*., (2017) found that the systemic chemical fungicides, Score, Cidely Top, and Penthiopyrad, significantly inhibited the mycelial growth of *L. theobromae* in *in vitro*.

Efficacy of chemical fungicides for control of rain tree gummosis under field condition

Results presented in Table 6 indicates that fungicide treatment significantly reduced the percent development of the lesion of gummosis with the untreated control. The lowest percent development of the lesion was observed when 2 % Knowin, Arba, Autostin, and Bordeaux mixture were sprayed (Fig. 6). The efficacy of the benzimidazole fungicides against a broad group of wood pathogens was demonstrated by Luque *et al.*, (2008), who reported that carbendazim and thiophanate methyl were the most effective in reducing mycelial growth of *Diplodia corticola* isolated from oak trees. In their subsequent field experiments, they also observed that carbendazim was the most effective fungicide and that thiophanate methyl was the next most effective at reducing numbers of surface lesions caused by *D. corticola* on oak trees in Spain. Similarly, Carbendazim, Sodium orthophenylphenate, Potassium metabisulfite, Mancozeb, Carboxin, Dodine, Iprodione, and Thiabendazole were evaluated for control of *B. theobromae*on mango cv.

Dashehari by Sharma *et al.*, (1994). They found that 0.1 percent Carbendazim (dip treatment) was the most effective fungicide for control of *B. theobromae.* Assuah, (1997) worked on the etiology and control of citrus gummosis disease at the University Agriculture Station, Kade, Ghana, and observed that Bordeaux mixture (1:4) and Bavistin (50% carbendazim) at 2 gm /L were effective against the disease.

Table 4. Effect of different environmental and nutritional factors on conidial germination of *L. theobromae*

pH		RH (%)		Temperature (°C)		Glucose/Sucrose (%)		
Rate	CG (%)*	Rate	CG (%)*	Rate	CG (%)*	Rate	CGG (%)*	CGS (%)*
4	45.12 f	70	20.12 g	05	45.32 f	0.5	20.26 f	48.14 f
5	52.38 d	75	45.72 f	10	53.86 e	1.0	25.42 e	52.28 e
6	74.95 b	80	69.53 d	15	69.28 d	1.5	47.78 c	57.31 c
7	85.15 a	85	73.28 c	20	73.92 c	2.0	52.31 b	61.28 b
8	69.53 c	90	75.42 b	25	85.29 a	2.5	56.16 a	63.14 a
9	48.85 e	95	85.29 a	30	82.14 b	3.0	51.38 b	55.23 d
10	25.34 g	100	58.24 e	35	45.12 f	3.5	45.92 d	51.18

*Mean of three replications

RH: Relative Humidity, **CG:** Conidial Germination, **CGG**: Conidial Growth in Glucose, **CGS:** Conidial Growth in Sucrose. All treatments were observed (except temperature effect) at 25°C. In a column, the same letters are not significantly different by DMRT at the 5 % level.

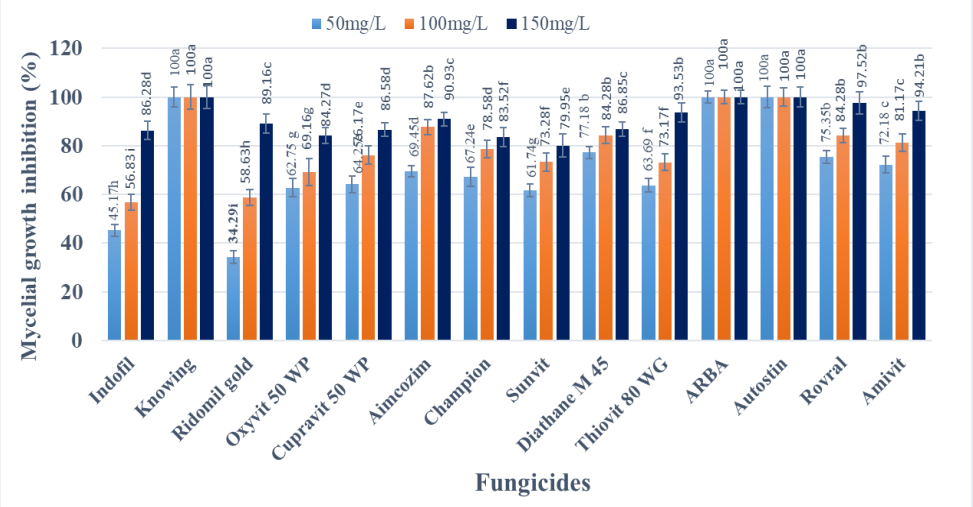

Fig. 3. Effect of different concentrations of the fungicides on the mycelial growth inhibition of L. theobromae. Bars marked by the same letters are not significantly different ($p < 0.05$) by DMRT analysis.

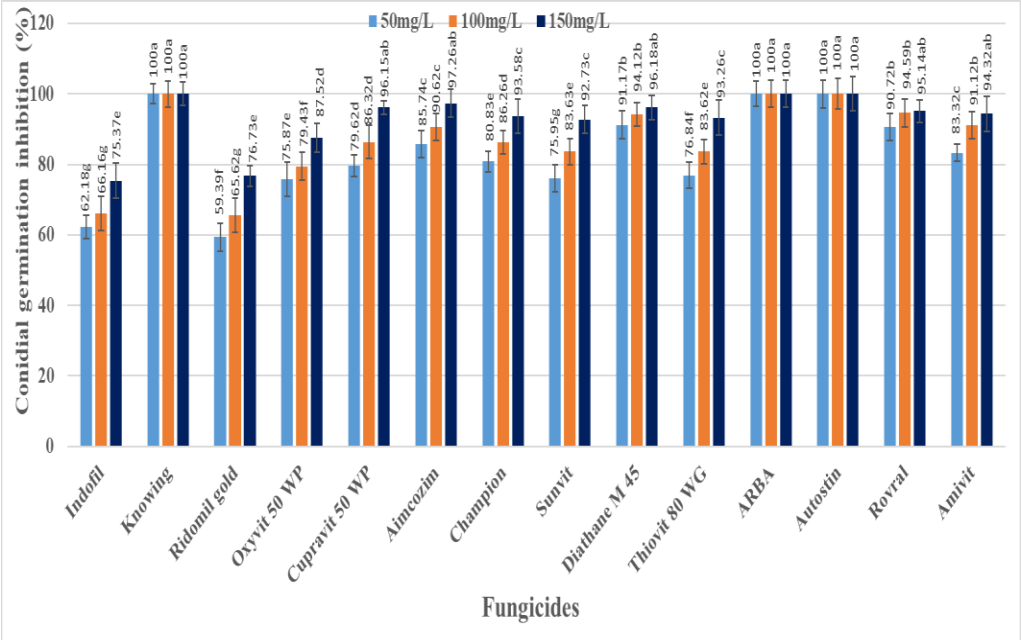

Fig. 4. Effect of different concentrations of the fungicides on the conidial germination inhibition of *L. theobromae.* Bars marked by the same letters are not significantly different ($p < 0.05$) by DMRT analysis.

Table 5. Effect of different environmental and nutritional factors on mycelial growth (mm) and sporulation of *L. theobromae* after 7 days

Mycelial growth (mm) and sporulation of *L. theobromae*																	
	pH			RH (%)			Temperature (°C)			Media			Glucose (%)			Sucrose (%)	
Rate	MG* (mm)	Spor	Rate	MG* (mm)	Spor	Rate	MG* (mm)	Spor	Media name	MG* (mm)	Spor	Rate	MGG* (mm)	Spor	Rate	MGS* (mm)	Spor
4	68.21 e	-	70	49.72 f	-	05	00 g	-	Richards	65.17 c	+++	0.5	58.16 f	+	0.5	62.75 g	+
5	72.31 d	++	75	52.41 g	+	10	25.13 f	+	MEA	69.38 b	+++	1.0	65.27 e	+	1.0	69.42 f	+
6	85.18 b	++++	80	58.16 e	++	15	35.14 e	++	Oatmeal	63.27 d	++	1.5	78.82 d	+	1.5	74.38 e	++
7	**89.82 a**	++++	85	69.18 c	+++	20	69.37 d	+++	PDA	**72.18 a**	++++	2.0	85.98 c	++++	2.0	92.29 c	++++
8	78.12 c	++++	90	72.27 b	++++	25	**82.16 a**	++++	Sabourauds	62.26 d	++	2.5	**92.38 a**	++++	2.5	**96.27 a**	++++
9	65.91 f	+	95	**79.38 a**	++++	30	79.18 b	++++	Czapeks	58.17 e	++	3.0	88.19 b	++++	3.0	94.17 b	++++
10	58.41 g	-	100	63.14 d	+++	35	72.12 c	+++	YEA	59.19 f	++	3.5	83.28 c	+++	3.5	89.28 d	+++

*Mean of three replications
MG = Mycelial growth, MGG = Mycelial growth in glucose, MGS = Mycelial growth in sucrose
Spor = Sporulation, - = Nil, + = Poor, ++ = Fair, +++ = Good, ++++ = excellent
In a column, the same letters are not significantly different by DMRT at the 5 % level.

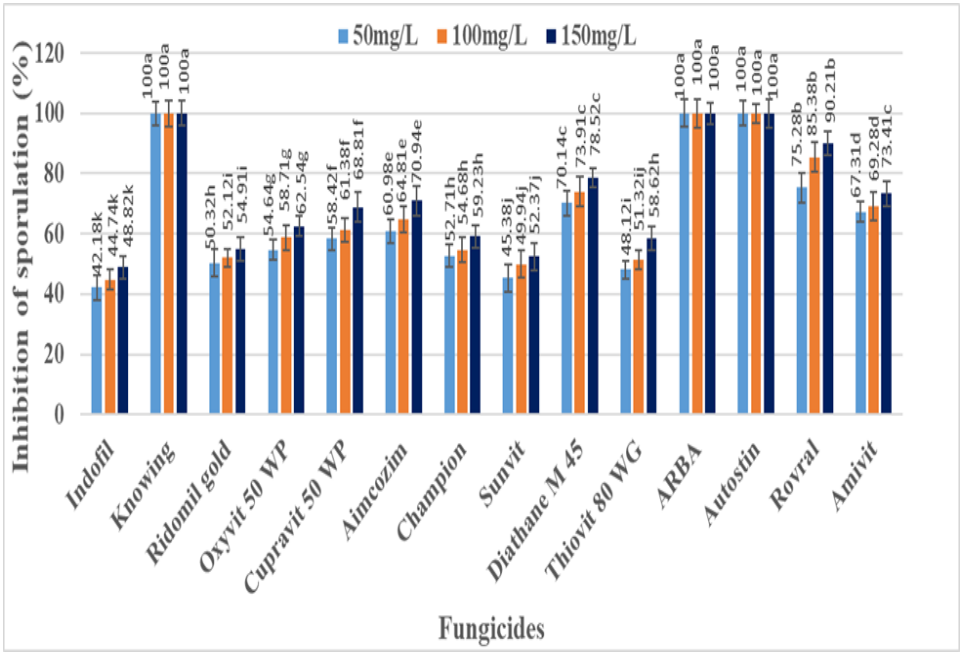

Fig. 5. Effect of different concentrations of the fungicides on the sporulation inhibition of *L. theobromae*. Bars marked by the same letters are not significantly different ($p < 0.05$) by DMRT analysis.

Table 6. Influence of the fungicidal treatment on the development of the lesion size on the stem of *S. saman* at 2 % concentration

Treatments	Lesion size before spray (cm) (April)*	Development and size of the lesion (cm)				Percent development of the lesion*
		May*	June*	July*	August*	
T_0	5.4	5.8	6.2	6.8	7.3	35.18 a
T_1	7.8	7.9	8.3	8.9	9.12	16.92 f
T_2	7.4	7.8	8.2	8.4	8.6	16.21 f
T_3	6.8	7.1	7.5	8.3	7.9	16.18 f
T_4	6.3	6.6	6.9	7.4	7.6	20.63 e
T_5	5.3	5.6	5.8	6.3	6.6	24.53 d
T_6	5.8	6.2	6.4	6.9	7.4	27.59 c
T_7	6.9	7.3	7.4	7.8	8.1	17.39 f
T_8	6.4	7.3	7.6	7.9	8.4	31.25 b
T_9	5.9	6.2	6.5	6.9	7.2	22.04 e
T_{10}	7.9	8.1	8.4	8.6	8.9	12.65 g
T_{11}	7.4	7.6	7.9	8.2	8.7	17.57 f

Table 6. Contd.

Treatments	Lesion size before spray (cm) (April)*	Development and size of the lesion (cm)				Percent development of the lesion*
		May*	June*	July*	August*	
T_{12}	8.4	8.2	7.9	8.1	8.2	2.44 j
T_{13}	8.2	7.8	7.6	7.7	7.8	4.87 i
T_{14}	7.9	7.6	7.4	7.1	7.2	8.86 h
T_{15}	7.2	6.8	6.4	6.6	6.8	5.56 i
SD	0.998	0.828	0.81	0.78	0.75	9.35
CV (%)	14.39	11.64	11.13	10.26	9.61	53.51

*Mean of three replications

In a column, the same letters are not significantly different by DMRT at 5% level

T_0 = Control (Without fungicide), T_1 = Indofil, T_2 = Sunvit, T_3 = Diathene M45, T_4 = Oxyvit, T_5 = Rovral, T_6 = Aimcozim, T_7 = Thiovit, T_8 = Ridomil, T_9 = Amivit, T_{10} = Cupravit, T_{11} = Champion, T_{12} = Knowing, T_{13} = Arba, T_{14} = Autostin, T_{15} = Bordeaux mixture

Fig. 6. Effectiveness of chemical fungicides spraying in 2 % on lesion development of rain tree gummosis under field condition (before and after spray). (A_1& A_2) Control; (B_1& B_2) Indofil; (C_1& C_2) Sunvit; (D_1& D_2) Diathene M45; (E_1 & E_2) Oxyvit; F_1& F_2: Rovral; (G_1& G_2) Aimcozim; (H_1& H_2) Thiovit; (I_1&I_2) Ridomil; (J_1& J_2) Amivit; (K_1& K_2) Cupravit; (L_1& L_2) Champion; (M_1& M_2) Arba; (N_1& N_2) Autostin; (O_1& O_2) Bordeaux mixture; (P_1& P_2) Knowing.

Conclusion

The morphological observation indicated that the pathogen causing gummosis disease on rain trees was *L. theobromae*. The result of the pathogenicity test showed that there was a similarity in symptoms that arise between artificial inoculation and natural symptoms in the field. This test indicated that *L. theobromae* was the causal agent of rain tree gummosis disease. This fungus grows well at a pH of 6-8, relative humidity of 90-95 %, a temperature of 25-30°C, and a glucose and sucrose concentration of 2.5 %. The PDA medium is suitable for the growth and sporulation of this fungus. The fungicides Knowing, ARBA, Autostine, and the Bordeaux mixture have shown good results in controlling gummosis disease. In the future, this preliminary study would help in the development of long-term management strategies to control gummosis disease in rain trees in Bangladesh.

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Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this paper.

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