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Competitive Research Grant

Sub-Project Completion Report

on

Studies on Gummosis of Shade Trees in Tea Plantation and its Management

Duration of the sub-project
April 2017 to September 2018



Forest Protection Division
Bangladesh Forest Research Institute
Chattogram, Bangladesh



Submitted to
Project Implementation Unit (PIU)-BARC
National Agricultural Technology Program: Phase II
Bangladesh Agricultural Research Council
Farmgate, Dhaka 1215

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Citation

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Acronyms

USSBT	:	UdaliaSamaneasamanBotryodiplodiatheobromae
USSLC	:	UdaliaSamaneasamanLasiodiplodiacrassipes
DSSCL	:	DatmaraSamaneasamanCeriporia lacerate
DSSBN	:	DatmaraSamaneasamanByssochlamysnivea
FAO	:	Food and Agricultural Organization
CHTs	:	Chattogram Hill Tracts
DI	:	Disease Incidence
DSI	:	Disease Severity Index
CG	:	Conidial Germination
MG	:	Mycelial Growth
PDA	:	Potato Dextrose Agar
MEA	:	Malt Extract Agar
PIMG	:	Percent Inhibition of Mecelial Growth
PIRG	:	Percent Inhibition of Radial Growth
PICG	:	Percent Inhibition of Conidial Germination
DB	:	Die Back
GE	:	Gum exudation
VD	:	Vascular discoloration
DMRT	:	Duncan's Multiple Range Test
RH	:	Relative Humidity
CG	:	Conidial Growth
CGG	:	Conidial Growth in Glucose
CGS	:	Conidial Growth in Sucrose
YEA	:	Yeast Extract Agar

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Executive Summary

The gummosis disease of shade trees is becoming a serious problem in tea cultivation areas of Bangladesh. Gummosis is the oozing of fluid/latex from wounds or cankers on planted trees. Infected wood and the defoliation that may occur weakness of the trees but if the disease infect the trunk, the tree may die. A survey was conducted for documentation of shade tree species and assess the gummosis disease incidence in fifteen tea gardens of Chattogram and Moulvibazar Districts of Bangladesh during 2017 and 2018. In the present assessment, a total of 44 species representing 31 genera of 9 families of angiosperms were enlisted. Among the collected species Fabaceae shows highest percentage with 31 species comprising 19 genera (61.29 %) followed by 5 species of Meliaceae comprising 5 genera (16.12 %) and 2 species of Myrtaceae comprising 1 genera (3.22 %). During the survey the highest disease incidence (%) and disease severity index (DSI) were recorded in *A. procera* (40 and 76.67%) at Udalia tea garden, Fatikchari, Chattogram and the lowest was recorded (2.12 and 4.97 %) in *Melia azedarach* at Panchabati tea garden, Bhojpur, Fatikchari and Bhojpur tea garden, Fatikchari, Chittagong. A total of 629 fungi were isolated from 130 gummosis samples in fifteen tea gardens. Among the isolates, *L. crassispora* and *B. theobromae* was the most frequently isolated species. The pathogenicity results showed that, *A. procera* and *A. lebeck* plants inoculated with *L. crassispora* USSLC and *Samanea saman* plant inoculated with *B. theobromae* USSBT showed typical symptoms of the gummosis disease after one month of inoculation. The highest CG and MG of *L. crassispora* USSLC and *B. theobromae* USSBT was observed at 6-8 pH, 90-95 RH and 25-30 °C temperature. Concentration of 2-3% glucose and sucrose were the best for CG and MG and sucrose was better than glucose. Among 7 solid media, the highest MG was found at PDA medium. In *in vitro* condition, chemical fungicide Autostin, ARBA and Knowing fully inhibited mycelial growth and spore germination inhibition of *L. crassispora* USSLC and *B. theobromae* USSBT. Likewise, *T. harzianum* IMI-392433 strain was also inhibited mycelial growth and conidial germination of the both pathogens. Supplementary, in field condition, the lowest percent development of the gummosis lesion was observed when 2% Knowing, Arba, Autostin and Bordeaux mixture was sprayed. Equally, spore suspension of *Trichoderma harzianum* IMI-392433 showed the lowest percent development of gummosis lesion in the field. Based on the results it may be concluded that chemical fungicide Knowing, Arba, Autostin and Bordeaux mixture 2% is the most effective to control gummosis disease of *S. saman*, *A. lebeck*, and *A. procera*. Bio-control fungus *T. harzianum* IMI-392433 can also be used for control of gummosis disease.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. Title of the sub project: Studies on gummosis of shade trees in tea plantation and its management

2. Implementation organization: Forest Protection Division, Bangladesh Forest Research Institute, Chattogram, Bangladesh.

3. Name and full address with phone, cell and E-mail of PI/Co-PI (s):

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4. Sub-project budget (Tk):

4.1. Total: 25,00000/-

4.2. Revised (If any):

5. Duration of the sub-project:

5.1. Start date (based on LoA signed): 8 March 2017

5.2. End date : 30 September 2018

6. Justification of undertaking the sub-project: Tea (*Camellia sinensis* L.) is one of the oldest and most popular and favorite beverages (nonalcoholic) in the world. It plays an important role in Bangladesh's economy. Bangladesh is the 10th in tea production (64 million in 2012) in the world (BTB, 2012). Tea is a shade loving plant. For better tea production 50-70% diffused sunlight is needed (Sana, 1989). If the intensity of sunlight is reduced, production increases to 40% (Redowan, 2003). Tea is grown under a canopy of trees which provide partial shade that is quite essential for good tea leaf production. The popular shade tree species in Bangladesh tea gardens are: Silkoroi (*Albizia procera*); Sirish (*A. lebek*), Kali sirish (*A. odoratissima*), Sau (*A. moluccana*), Chakua koroi (*A. chinensis*), Potka Siris (*A. lucida*), Jamurja/Miringa (*Derris robusta*), Shisu (*Dalbergia sissoo*), Ghora neem (*Melia azedarach*), Rain tree (*S. saman*) etc. (Redowan, 2003). These tree species, conserve soil from erosion and the impact of rainfall drops, enrich soil fertility and organic matter, content through leaf litter and support diverse flora and fauna, especially many bird species. Shade trees help by reducing the rate of evaporation of the

soil moisture and thus maintain a suitable humid atmospheric condition. Shade trees not only used as shade provider, but it also produces fuel wood timber, foods (leaves, pods or flowers) for people fodder, production of tannins, gums, medicines and services like living fences, ornamentals, environmental protection (FAO, 1987). In Bangladesh, there are 166 tea estates and 746 small growers of tea all over the country (Rahman, 2016). Thus to increase tea yields, a large number of shade tree species are planted in various tea garden of Bangladesh (Kalita *et al.*, 2014). But these plants are frequently suffered from gummosis in different ages. Gummosis is the oozing of fluid/latex from wounds or cankers on plantation trees. Gummosis can result from environmental stress, mechanical injury or disease and insect infestation. During the disease progress sunken lesion develops on the bark. These lesions enlarge and gluey, amber-colored fluid oozes from the bark. Infected wood and the defoliation that may occur weakness the trees but if the disease infect the trunk, the tree may die. Recently, 25-30% shade tree mortality has recorded due to gummosis in major tea plantation areas of Bangladesh. There is no information and extensive work has done regarding this problem. Therefore, proper management technology of gummosis is urgent needed to overcome this serious problem.

7. Sub project goal:To find out the suitable management techniques of gummosis disease of shade trees.

8. Sub-project objectives:

- i) To survey for the incidence of gummosis of shade trees in major tea plantation areas of Bangladesh
- ii) To identify the causal agents of gummosis
- iii) To determine effective control measures of gummosis.

9. Implementation location: Fifteen tea gardens of Chattogram and Moulvibazar region (Table-1 & Figure 1) and Bangladesh Forest Research Institute, Chattogram, Bangladesh.

10. Methodology:

10.1. Inventory of shade trees in fifteen tea gardens of Bangladesh: An extensive surveys were conducted for inventory of shade trees in 15 tea gardens of Chattogram and Moulvibazar district (Table 1). This work was conducted several tea estates during 2017 to 2018. During the field survey a large number of fertile specimens of shade trees have been collected for genuine identification. Collected specimens were processed and herbarium specimens were prepared following standard herbarium techniques by Jain and Rao (1977). Identification has been made by matching with herbarium sheets and also using different published literatures and books (Prain, 1903; Bose *et al.*, 1998).

Table 1: Survey tea garden at Chattogram and Maulvibazar district of Bangladesh.

SL no.	Name of tea garden	Location	District	Division
1.	Ramgarh tea estate	Fatikchari	Chattogram	Chattogram
2.	Dantmara tea estate	Bhojpur, Fatikchari	Chattogram	Chattogram
3.	BTRI substation tea estate	Bhojpur, Fatikchari	Chattogram	Chattogram
4.	Udalia tea estate	Katirhat, Fatikchari	Chattogram	Chattogram
5.	Bhojpur tea estate	Bhojpur, Fatikchari	Chattogram	Chattogram
6.	Naseha tea estate	Dantmara, Fatikchari	Chattogram	Chattogram
7.	Haldavalley tea estate	Najirhat, Fatikchari	Chattogram	Chattogram
8.	Neapchun tea estate	Narayanhat, Fatikchari	Chattogram	Chattogram
9.	Panchabati tea estate	Bhojpur, Fatikchari	Chattogram	Chattogram
10.	Aasia tea estate	Bhojpur, Fatikchari	Chattogram	Chattogram
11.	Majan tea estate	Datmara, Fatikchari	Chattogram	Chattogram
12.	Andhar manik tea estate	Chikancherra, Fatikchari	Chattogram	Chattogram
13.	BTRI campus tea estate	Srimangal	Maulvibazar	Sylhet
14.	BTRI substation tea estate	Fultoli, Srimangal	Maulvibazar	Sylhet
15.	Finlay tea estate	Srimangal	Maulvibazar	Sylhet

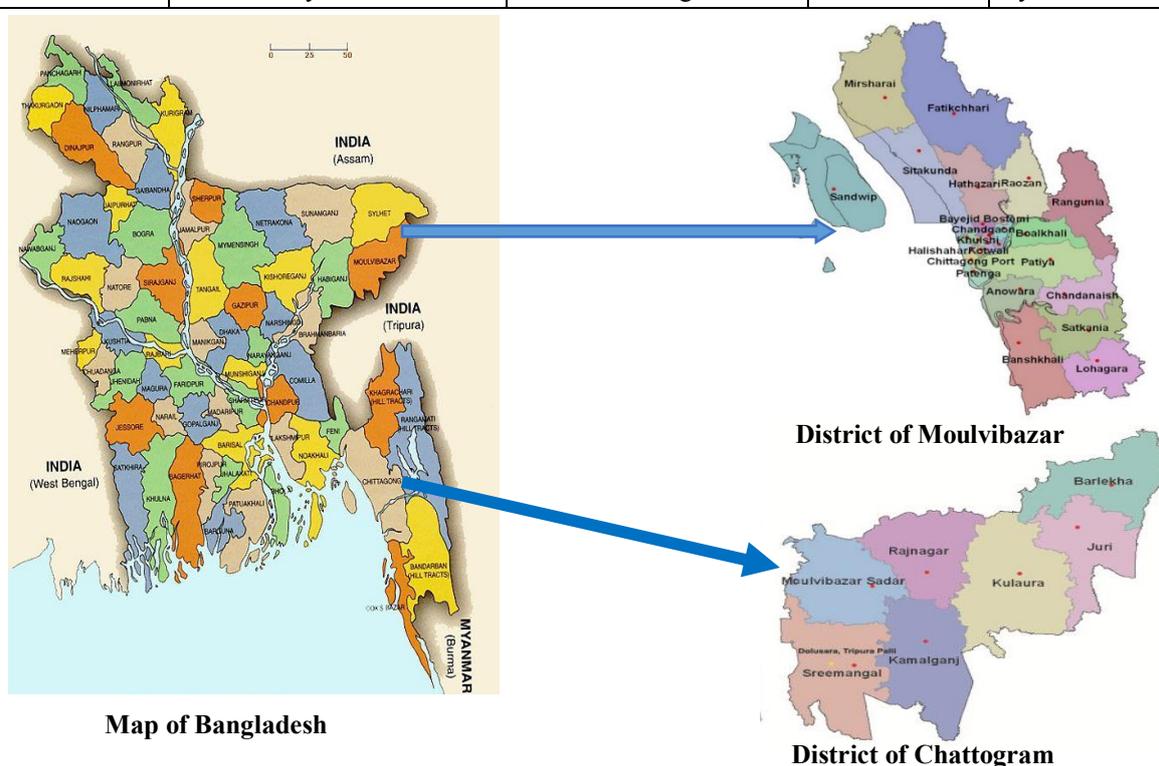


Figure 1: Location Map of Survey garden, Chattogram and Maulvibazar District of Bangladesh.

10.2. Survey for the incidence of gummosis of shade trees in major tea plantation areas of Bangladesh: A survey was conducted for the incidence of gummosis of shade trees at fifteen tea gardens in 2017-18 (Table 1 and Figure 1). In each tea garden, 30 individual shade tree species were selected and a gummosis incidence was assessed. Gummosis incidence was determined as the proportion of plants showing gummosis symptoms and expressed as a

percentage of the total number of plants assessed (Teng and James, 2002). A tree was defined and recorded as cankered when it had any of the following symptoms: discoloration of the bark surface, discoloration of the underlying tissues, dried the whole part of the plant and the exudation of the gum from infected tissues.

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

Observations was recorded

1- Shade tree species; 2- Age of plant; 3- Type of symptoms observed: Die back, Gum exudation.

Disease severity index was calculated as:

Disease severity index

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

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 dead tree.

10.3. Isolation, identification and characterization of pathogen: Isolations were carried out from bark samples, which were collected during the survey. In all, 130 gummosis samples were collected from different species of shade trees and brought back to the laboratory. After surface disinfection of lesions on woody tissue with 0.1% mercuric chloride for 40 second or 16 5% sodium hypochlorite for 1 min, followed by autoclaved water wash three times, small blocks (9 mm²) of diseased bark were aseptically transferred to 2% potato dextrose agar (PDA) plates. Cultures were incubated at 25°C until fungal growth was observed. Fungi isolates were purified by single spore culturing prior to use in experiments. Identification of fungi was based on morphological and microscopic characteristics.

10.4. Pathogenicity test of causal organism: For pathogenicity test, the most frequent fungi viz., *Lasiodiplodia crassispora* USSLC, *Botryodiplodia theobromae* USSBT, *Ceriporia lacerate* DSSCL and *Byssoschlamys nivea* DSSBN were carried out.

10.4.1. Pathogenicity test for *Samanea saman*:The pathogenicity test was conducted at Forest Protection Division, Nursery at BFRI Campus, Chattogram. One year old seedlings of *S. samanea* plant was selected as a host for conducting pathogenicity tests. A 1x2 cm inoculum block was made using a sterilized knife in the stem of *S. samanea* plant. 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 Para film. A 5 mm PDA block without fungus was placed in the control plants. Plants were irrigated after inoculation and the wrapping material was removed from the stems after 2 weeks of inoculation. Plants 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 randomized complete block design with four replications.

10.4.2. Pathogenicity test for *Albizia lebbek* and *Albizia procera*:The experiment was conducted at BFRI Campus, Chattogram. In each experiment, apparently healthy looking plants of *A. lebbek* and *A. procer* were selected. A cut in the stem was made using a sterilized chisel. A 1 x 2 cm inoculum block was made in the trunk of the tree. After that, 5 days old culture of a test fungus on PDA was placed in the gap of the inoculated portion and finally wrapped with Para film. A 1x 2 cm PDA block without fungus was placed in the control plants. Plants were wrapped and the wrapped material was removed from the stems after 2 weeks of inoculation. Plants were monitored for the development of disease symptoms and isolations were made of the test plants to confirm the pathogenicity. The experiments were carried out in randomized complete block design with four replications.

10.5. Influence of different nutrition, physical and environmental parameters on growth and conidial germination of *Lasiodiplodia crassispora* USSLC and *Botryodiplodia theobromae* USSBT

10.5.1. Environmental and nutritional factors

10.5.1.1. 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 35° C), 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 of *L. crassispora* USSLC and *B. theobromae* USSBT. Conidia from the 10 days old culture of PDA plate were taken and conidial suspension (10^3 /ml) were made with sterilized distilled water (for RH, pH and temperature)/different concentrations of glucose and sucrose solution separately. The conidial suspension was taken in sterilized watch glass after that a drop of conidial suspension was taken on separate grove slide and kept at 25° C in a moisture chamber for 24 hours. After incubation period a drop of lactophenol cotton

blue was placed cover conidial suspension on the slide and examined under ($\times 400$) power microscope for reading the percentage of CG. Three replications were used for each particular treatments.

10.5.1.2. Mycelial Growth (MG): 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 used to get favorable MG of *L. crassispota* USSLC and *B. theobromae* USSBT. Effect of RH, pH, temperature, glucose and sucrose on MG of *L. crassispota* USSLC and *B. theobromae* USSBT were done using PDA medium. Different media were autoclaved at $121^{\circ}\text{C}/15\text{lbs}/\text{inch}^2$ pressure, these were poured in sterilized Petri dishes. Agar disc (5 mm) were taken from 10 days old culture of *L. crassispota* USSLC and *B. theobromae* USSBT and placed separately in respect to each treatment in the center of each petri dish and incubated respective condition. After 7 days of incubation radial growth of mycelium was measured by following Brown (1923) methods. Three replications were used for each particular treatments.

10.6. In vitro evaluation of different chemical fungicides, bio agents and microorganism against the pathogen

10.6.1. In-vitro evaluation of fungicides against mycelial growth and conidial germination inhibition of *L. crassispota* USSLC and *B. theobromae* USSBT

10.6.1.1. Mycelial growth inhibition: The efficacy of fungicides against the mycelial growth inhibition of pathogen in laboratory (*in vitro*) by poisoned food technique. Fourteen fungicides (Indofil, Sunvit, Diathene M45, Oxyvit, Rovral, Aimcozim, Thiovit, Ridomil, Amivit, Cupravit, Champion, Knowing, Arba and Autostin) were tested *in vitro* for their effects on spore germination and mycelial growth of *L. crassispota* USSLC and *B. theobromae* USSBT. The radial growth of the colony were recorded at 7th days, 9th days and 11th days when maximum growth was observed in control and per cent inhibition was calculated using the formula given by Vincent's (1947).

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

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 the following table.

SL. No.	Trade Name	Common Name	Chemical Name
1.	Indofil	Indofil	Mencozeb
2.	Knowing	Knowing	Carbendazim
3.	Ridomil	Ridomil gold	Mancozeb
4.	Oxyvit	Oxyvit 50 WP	Copper oxychloride
5.	Cupravit	Cupravit 50 WP	Copper oxychloride
6.	Aimcozim	Aimcozim	Carbandazim
7.	Champion	Champion	Copper hydroxide
8.	Sunvit	Sunvit	Copper oxichloride
9.	Diathane M 45	Diathane M 45	Mancozeb
10.	Thiovit	Thiovit 80 WG	Sulpher
11.	Autostin	Autostin	Carbendazim
12.	Amivit	Amivit	Copper oxychloride
13.	Rovral	Rovral	Eprodion
14.	ABRA	ABRA	Carbendazim

10.6.1.2. Conidial germination inhibition: Conidia of *L. crassispota* USSLC and *B. theobromae* USSBT cultured on PDA plates were taken and conidial suspensions (10^5 /ml) were made separately with different concentrations of different fungicides. These suspensions (1.25 ml) were taken in small sterilized Petri dishes (65 mm) and were kept at $28\pm 2^\circ\text{C}$ for 5-30 minutes. A drop of treated conidial suspension (from different concentration of fungicide) was taken on separate slides continue 5 min. interval and were kept at $28\pm 2^\circ\text{C}$ in a moisture 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 high power microscope ($\times 400$) for recording the percentage of conidial germination. Percentages inhibition of conidial germination (PICG) using the formula by Skidmore and Dickinson (1976). Where, $\text{PICG} = \frac{C_1 - C_2}{C_1} \times 100$.

C_1 = Total number of spore in control treatment.

C_2 = Germination of spore in fungicidal treatment.

10.6.2. In-vitro evaluation of Trichoderma strains against mycelial growth and conidial germination inhibition of *L. crassispota* USSLC and *B. theobromae* USSBT

10.6.2.1. Sources of Trichoderma strains: Six *Trichoderma* strains viz. *T. virens* Miller et al. (IMI-392430), *T. pseudokoningii* (Rifai) (IMI-392431), *T. harzianum* (Rifai) (IMI-392432, 392433 & 392434) and *T. viride* were screened on mycelial growth inhibition of *L. theobromae* USSLC and *B. theobromae* USSBT under *in vitro* condition. These *Trichoderma* strains were previously isolated and identified from soil and garbage samples

and verified by CABI bioscience, Surrey, U.K. The culture of *Trichoderma viride* FPDTV were procured from Forest pathology laboratory, Forest Protection Division (FPD), BFRI, Bangladesh.

10.6.2.2. Mycelial growth inhibition and over-growth test:For mycelial growth inhibition test, dual culture test was performed. An agar disc (6 mm) was taken from 4-day old PDA culture plates of each *Trichoderma* strains and placed at the periphery of the PDA plates (9 mm). Another agar disc of the same size of *L. crassispora* USSLC/*B. theobromae*USSBT was also placed at the periphery but on the opposing end of the same Petri dish (Figure 2). As a control, *L. crassispora* USSLC/*B. theobromae*USSBT was placed in a similar manner on a fresh PDA plate (Figure 2). All pairings were carried out in quadruplicate and incubated at 28°C. Antagonistic activity was tested 4 days after incubation by measuring the radius of the *L. crassispora* USSLC /*B. theobromae*USSBT colony in the direction of the antagonist colony (R_2) and the radius of the *L. crassispora* USSLC /*B. theobromae*USSBT colony in the control plate (R_1). The two readings were transformed into percentage inhibition of radial growth (PIRG) using the formula developed by Skidmore and Dickinson (1976). The inhibition levels were calculated by using the formula; $[(R_1-R_2)/R_1] \times 100$, when R_1 was the mean of colony radius of pathogen in the control dish and R_2 was the mean of colony radius of pathogen in Petri-dish of dual culture test.

Where, $PIRG = \frac{R_1 - R_2}{R_1} \times 100$

Observations were continued on the dual culture plates after 4 days of incubation and PIRG was calculated. Each treatment was performed with four replicates.

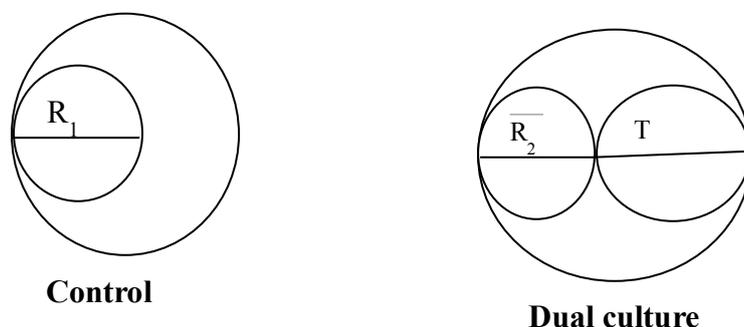


Figure 2:Method of dual culture. R_1/R_2 : Mycelial growth of Pathogen and T: *Trichoderma*

10.6.2.3. Determination of inhibitory activity of secondary metabolites of *Trichoderma* strains using normal poison agar technique

10.6.2.3.1. Screening by Poison Agar Technique Using Crude Metabolites

10.6.2.3.1.1. Preparation of culture filtrates of *Trichoderma*: Two hundred milliliters of Richard's solution (KNO₃: 1.0 g, KH₂ PO₄: 0.5 g, MgSO₄·7H₂O: 0.25 g, glucose: 34 g, and trace amounts of FeCl₃ in 1 liter distilled water, pH 6.5) was prepared and poured into 500 ml conical flasks and autoclaved for 15 minute at 121° C/1.05 kg/cm² pressure. Six pieces of agar discs (6 mm) were kept in a flask (with media) for each strain of *Trichoderma* with four replicates. The flasks were incubated on a Gallenkamp orbital incubator at 100 rpm at 28°C (Dennis and Webster, 1971). The culture filtrates were collected after 30 days of incubation. These were then concentrated to about 50% using a vacuum evaporator at 38~40°C and filtered by sterilized membrane filter.

10.6.2.3.1.2. Screening technique: For normal poison agar method, seven-day-old culture discs (6 mm) of *L. crassispora* USSLC/*B. theobromae* USSBT were inoculated at the center of previously prepared poison agar plates and incubated at room temperature (28 ± 2° C) for 10 days. Observation was made on radial extension of the mycelia on culture plates for the experimental treatment and control. Data were recorded on the mycelial extension of colony diameter after 4 to 10 days of inoculation. The readings were calculated for the percentage inhibition of radial growth (PIRG) based on the formula by Skidmore and Dickinson (1976), the same as for the dual culture experiment. Each treatment was performed with four replicates.

10.6.2.3.1.3. Determination of inhibitory activity of secondary metabolites of *Trichoderma* strains against spore germination inhibition of *L. crassispora* USSLC/*B. theobromae* USSBT: Conidia obtained from 10-day-old PDA cultures of *L. crassispora* USSLC/*B. theobromae* USSBT were separately suspended in full strength (undiluted) filtrate of *Trichoderma* strains and also in 25, 50 and 75% dilutions of the filtrates in PDB. Each of the spore suspensions were readjusted to a concentration of 20,000 spores/ml and four replicate drops (1 ml) of the suspension incubated on microscope slides in humidified Petri dishes for up to 9 h. The percentage spore germination was determined hourly. Spore suspension of each of the fungi incubated in PDB served for comparison.

Percentages inhibition of conidial germination (PICG) using the formula by Skidmore and Dickinson (1976). Where, $PICG = \frac{C_1 - C_2}{C_1} \times 100$.

10.7. Efficacy of chemical fungicides for control of gummosis under field condition: The experiments were carried out in the Forest Protection Division Nursery (FPD) at Bangladesh Forest Research Institute, Chattogram, Bangladesh. Six-month-old *S. saman*, *A. lebbeck* and *A. Procerase* seedlings were used in this study. Seedlings were previously inoculated with agar culture discs containing mycelium of *L. crassispora* USSLC/*B.*

theobromae USSBT at the stem as described before (Pathogenicity test). After 30 days of inoculation when disease is in progress, plants were then either sprayed with the fungicide (2 %; treatment) or with sterilized distilled water (control). Application of fungicide on the stem started from May of August 2018. Fourteen commercially 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 *S. saman*, *A. lebbeck* and *A. procera* under field condition. Total ten replications were at individual treatment and lesion size was recorded after 30 days interval.

10.7.1. Treatments: There were following 14 treatments

T₀=Control (Without fungicide), T₁=Indofil, T₂= Sunvit, T₃=Diathene M45, T₄=Oxyvit, T₅=Rovral, T₆=Aimcozim, T₇=Thiovit, T₈=Ridomil, T₉=Amivit, T₁₀=Cupravit, T₁₁=Champion, T₁₂=Knowing, T₁₃=Arba, T₁₄=Autostin, T₁₅= Bordeaux mixture.

10.8. Efficacy of *Trichoderma* strains for control gummosis of *S. saman*, *A. lebbeck* and *A. procera* under field condition: For disease assays on *S. saman*, *A. lebbeck* and *A. procera* seedlings under field conditions a pot trial experiment was conducted during April-September, 2018. The growing tip region of the stem of 6-month-old *S. saman*, *A. lebbeck* and *A. procera* seedlings were surface-sterilized with 70% ethanol, mechanically wounded, and inoculated with 5mm PDA plugs (5 days old) colonized with *L. crassispota* USSLC/B. *theobromae* USSBT culture or non-colonized plugs (controls) (described before as pathogenicity test). The area of inoculation was covered with Parafilm. After 30 days of inoculation when disease is in progress plants the lesion were then either sprayed with the spore suspension @ 5 mL of *Trichoderma* strains (5×10^5 spores/mL). After 24 h, the wounded portion were further sprayed with the spore suspension of *L. crassispota* USSLC/B. *theobromae* USSBT @ 5 mL of conidial suspension (3×10^5 spores/mL) obtained from 7-day-old culture plates. The lesions on the surface of the exposed wood were measured (length x mean breadth) to estimate their approximate area. Total ten replications were at individual treatment and lesion size was recorded after 30 days interval.

10.8.1. Treatments: Six *Trichoderma* strains viz. *T. virens* IMI-392430, *T. pseudokoningii*, *T. harzianum* (IMI-392432, 392433 & 392434) and *T. viride* were used in this study. The treatments were as follows: T₀= Control (*L. crassispota* USSLC/B. *theobromae* USSBT), T₂=*T. virens* IMI-392430 + *L. crassispota* USSLC/B. *theobromae* USSBT, T₃=*T. pseudokoningii* + *L. crassispota* USSLC/B. *theobromae* USSBT, T₄ =*T. harzianum* IMI-392432 + *L. crassispota* USSLC/B. *theobromae* USSBT, T₅ = *T. harzianum* IMI-392433 + *L. crassispota* USSLC/B. *theobromae*

USSBT, T₆ = *T. harzianum* IMI-392434 + *L. crassispora* USSLC/B. *theobromae* USSBT, T₇ = *T. viride* + *L. crassispora* USSLC/B. *theobromae* USSBT, T₈ = *T. virens* IMI-392430, T₉ = *T. pseudokoningii*, T₁₀ = *T. pseudokoningii*, T₁₁ = *T. harzianum* IMI-392432, T₁₂ = *T. harzianum* IMI-392433, T₁₃ = *T. harzianum* IMI-392434, T₁₄ = *T. viride*.

11. Results and Discussion

11.1. Inventory of shade trees in major tea gardens of Bangladesh: During the present investigation, mainly two types of shade trees viz., permanent shade trees and temporary shade trees were found in all surveyed tea garden of Bangladesh. Permanent shade trees are planted for a longer rotation (about 40 years). It takes a long period to be established. That's why at the initial stage of plantation temporary shade trees along with the permanent shade trees are planted to protect the tea plants from direct sun light. When the permanent shade trees become established after 5/6 years, the temporary shade trees are removed. In the present assessment, a total of 44 species representing 31 genera of 9 families of angiosperms were enlisted (Table 2). Among the collected species Fabaceae shows highest percentage with 31 species comprising 19 genera (61.29 %) followed by 5 species of Meliaceae comprising 5 genera (16.12 %) and 2 species of Myrtaceae comprising 1 genera (3.22 %) (Figure 3 and 4). Apocynaceae, Moraceae, Protaceae, Lythraceae, Anacardiaceae and Phyllanthaceae each family show the one species of one genera (Figure 3). Apocynaceae, Moraceae, Protaceae, Lythraceae, Anacardiaceae and Phyllanthaceae shows 3.22 % generic components of the entire tree flora of the tea plantations areas (Figure 4). The popular permanent shade trees among the tea gardeners include *A. odoratissima*, *A. chinensis*, *A. lebeck*, *A. lucidior*, *A. procera* and *D. robusta* (Figure 5, 6, 7). The Fabaceae is the dominant family. These type of shade trees not only provides shade to tea plants but also helps in replenishing nitrogen loss and controls insect pest due to bio pesticide properties of the tree (Pangging and Mandal, 2017).

11.2. Survey for the incidence of gummosis of shade trees in major tea plantation areas of Bangladesh: The survey results have shown that the disease was prevalent in all the surveyed gardens with different magnitude of infection. During the survey conducted in different tea gardens gummosis symptoms were observed. Dieback and vascular discoloration mostly were not observed. The gumming and cracking symptoms were observed on trunks and branches which produced a light yellow and brown gum across the sampling gardens (Table 4 & Figure 8, 9, 10, 11, 12 & 13). The overall result of disease incidence (%) and disease severity index (DSI) showed that *Albizzia procera* were highly

affected by the gummosis disease at survey areas. The highest disease incidence (%) and disease severity index (DSI) were recorded in *Albizzia procera*(40% and 76.67%)at Udalia tea garden, Fatikchari, Chattogram. The lower disease incidence (%) and disease severity index (DSI) was recorded (2.12 and 4.97 %)in *Melia azedarach* at Panchabati tea garden, Fatikchari and Bhojpur tea garden, Fatikchari, Chattogram.(Table 3 and 5).

Table 2: Types and abundance of shade trees at different tea gardens

Sl. No.	Scientific Name	Tea garden name	Habits	Vernacular Name	Family	Status	Common uses
Permanent shade trees							
1.	<i>Acacia auriculiformis</i>	RMG, DM, BP, HV, NPC,AS, ADM	Tree	Akashmoni	Fabaceae	Less common	Ornamental, shade, making paper, timber, fuel wood etc.
2.	<i>Acacia hybrid</i>	BP, MJ, DM	Tree	Acacia hybrid	Fabaceae	Rare	Ornamental, timber, fire-breaks, wind breaks, soil protections, shade, fodder, medicine etc.
3.	<i>Albizia chinensis</i>	RMG, DM, BTRISS, UDL, BP, NDFC, HV, NPC, PACB, AS, MJ, ADM, BTRICS, BTRISSF, FLA	Tree	Chakuakoroi	Fabaceae	Most common	Ornamental, shade, nitrogen fixation, fuel, timber etc.
4.	<i>Albizia lebeck</i>	RMG, DM, BTRISS, UDL, BP, NDFC, HV, NPC, PACB, AS, MJ, ADM, BTRICS, BTRISSF, FLA	Tree	Sirish	Fabaceae	Most common	Environmental management, shade, timber, forage, medicine etc.
5.	<i>Albizia lucida</i>	RMG, DM, BTRISS, UDL, BP, NDFC, HV, NPC, PACB, AS, MJ, ADM, BTRICS, BTRISSF, FLA	Tree	PotkaSirish	Fabaceae	Most common	Ornamental, shade, nitrogen fixation, timber, fuel wood, etc.
6.	<i>Acacia mangium</i>	BP, HV, NPC, PACB	Tree	Mangium	Fabaceae	Rare	Ornamental, nitrogen fixation, intercropping, shade, fuel, timber, soil erosion and ecological restoration.
7.	<i>Albizia moluccana</i>	RMG, BP, NDFC, HV, NPC, PACB, AS, MJ, ADM	Tree	Sau/Moluccan sau	Fabaceae	Less Common	Ornamental, shade, fuel wood, timber, nitrogen fixation, agroforestry etc.
8.	<i>Albizia procera</i>	RMG, DM, BTRISS, UDL, BP, NDFC, HV, NPC, PACB, AS, MJ, ADM, BTRICS, BTRISSF, FLA	Tree	Silkoroi	Fabaceae	Most common	Ornamental, shade, fuel wood, timber, nitrogen fixation, etc.
9.	<i>Albizia richardiana</i>	UDL, NPC, AS, MJ	Tree	Rajkoroi/Gaganshiris	Fabaceae	Rare	Ornamental, medicine, shade, nitrogen fixation, timber, fuel wood etc.
10.	<i>Albizia falcataria</i>	RMG, DM, UDL, BP, NDFC, HV, NPC, AS, ADM, BTRISSF, FLA	Tree	MalakanaKoro i	Fabaceae	Less Common	Medicine, shade, fire wood, timber, pulp and paper, making match box, nitrogen fixation etc.
11.	<i>Adenanthera pavonina</i>	RMG, UDL, BP, HV, NPC, AS, MJ, ADM	Tree	RaktaKambal	Fabaceae	Less common	Ornamental, shade, nitrogen fixation, medicinal, timber, fuel wood etc.
12.	<i>Albizia odoratissima</i>	RMG, DM, BTRISS, UDL, BP, NDFC, HV, NPC, PACB, AS, MJ, ADM, BTRICS, BTRISSF, FLA	Tree	Kali Sirish	Fabaceae	Most common	Ornamental, shade, agroforestry, nitrogen fixation, timber and fuel wood.
13.	<i>Alstonia scholaris</i>	RMG, DM, UDL, BP, HV, PACB, AS, MJ, ADM	Tree	Chatim	Apocynaceae	Less common	Medicine, ornamental, making pencil, timber and fuel wood.

Table to be continued

14.	<i>Artocarpuschaplasha</i>	DM, UDL	Tree	Chapalish	Moraceae	Rare	Timber, shade, fuel wood.
15.	<i>Azadirachta indica</i>	RMG, DM, UDL, BP, NDFC, HV, NPC, PACB, AS, MJ, ADM	Tree	Neem	Meliaceae	Less common	Vegetable, medicine, pest and disease control, fuel wood etc.
16.	<i>Bauhinia purpurea</i>	DM, UDL, BP, AS, MJ	Tree	Raktakanchan	Fabaceae	Rare	Ornamental, vegetables, medicine, fuel wood etc.
17.	<i>Butea monosperma</i>	DM, UDL, AS	Tree	Palash	Fabaceae	Rare	Ornamental, medicine, fuel wood etc.
18.	<i>Cassia fistula</i>	RMG, BP, NDFC, HV, NPC, AS, MJ, ADM	Tree	Badarlathi	Fabaceae	Less common	Cattle feed, medicine, ornament etc.
19.	<i>Cassia siamea</i>	BP, NDFC, HV, AS	Tree	Minjiri	Fabaceae	Rare	Shade, nitrogen fixation, ornamental, timber, soil erosion, shelter belts, wind break, fuel wood, medicine etc.
20.	<i>Chukrasia tabularis</i>	DM, BP, AS	Tree	Chikrassi	Meliaceae	Rare	Timber, fuel wood, gum, resin and medicine.
21.	<i>Dalbergia assamica</i>	RMG, DM, NPC	Tree	Mouhita/Medeluwa	Fabaceae	Rare	Shade, timber, fuel wood, shelter belt, wind break etc.
22.	<i>Dalbergia sericea</i>	RMG, DM, UDL, BP, HV, NPC, PACB, AS, MJ, ADM	Tree	Silky Dalbergia	Fabaceae	Less common	Timber, shade, nitrogen fixation, fuel wood etc.
23.	<i>Dalbergia sissoo</i>	RMG, UDL, BP, , NPC, PACB, AS, MJ, ADM	Tree	Shisu	Fabaceae	Less common	Timber, fuel wood, pesticide, construction etc.
24.	<i>Delonix regia</i>	DM, BP, AS, MJ	Tree	Gulmohar	Fabaceae	Rare	Ornamental, shade, medicine, food, timber, fuel etc
25.	<i>Derris robusta</i>	RMG, DM, BTRISS, UDL, BP, NDFC, HV, NPC, PACB, AS, MJ, ADM, BTRICS, BTRISSF, FLA	Tree	Jamurja/Mirinda	Fabaceae	Most common	Shade, timber, fuel wood, insecticide, nitrogen fixation etc.
26.	<i>Erythrina variegata</i>	RMG, UDL, HV	Tree	Madar	Fabaceae	Rare	Ornament, shade, soil conservation, medicine, agroforestry crop, fuel wood etc.
27.	<i>Eucalyptus robusta</i>	DM, UDL, AS	Tree	Eucalyptus	Myrtaceae	Rare	Ornamental, fuel, fiber, timber, essential oil, medicine, shade etc.
28.	<i>Eucalyptus camaldulensis</i>	DM, UDL	Tree	Red gum	Myrtaceae	Rare	Timber, pulp, fire wood, dying, ornamental, agroforestry, essential oil, medicine, fuel, shade etc.
29.	<i>Gravillea robusta</i>	RMG, DM, UDL, BP	Tree	Silky oak	<u>Proteaceae</u>	Rare	Ornamental, street tree, agroforestry, gum resin, firewood etc.
30.	<i>Lagerstroemia</i>	RMG, BP, AS, MJ	Tree	Jarul	Lythraceae	Rare	Food, medic firewood etc. <i>Table to be continued</i>

	<i>speciosa</i>						
31.	<i>Leucaenaleucocephalla</i>	DM, UDL, NDFC, HV, NPC, AS, MJ, ADM	Tree	Ipilipil	Fabaceae	Less common	Apiculture, fuel wood, fiber, timber, Nitrogen fixation, soil improver, shade provider etc.
32.	<i>Mangifera indica</i> L.	RMG, BP, AS	Tree	Aam	Anacardiaceae	Rare	Ayurveda medicine, food, timber, fuel wood etc.
33.	<i>Melia azedarach</i>	DM, UDL, BP, AS	Tree	Ghora neem	Meliaceae	Rare	Medicine, agroforestry, insecticide, fire wood etc.
34.	<i>Phyllanthusemblica</i>	DM, UDL	Tree	Amlaki	Phyllanthaceae	Rare	Traditional medicine, ink, shampoo, tannin, fire wood etc.
35.	<i>Samanea saman</i>	DM, UDL	Tree	Shirish/Rain tree	Fabaceae	Rare	Shade, medicinal, agroforestry, timber, ornamental etc.
36.	<i>Cassia siamea</i>	RMG, HV, AS	Tree	Minjiri	Fabaceae	Rare	Shade, medicine, timber, fuel wood etc.
37.	<i>Swieteniamacrophylla</i>	RMG, BTRISS, UDL, BP, MJ, ADM	Tree	Mahagoni	Meliaceae	Rare	Medicinal, timber, agroforestry, fuel wood etc.
38.	<i>Toonaciliata</i>	AS, MJ	Tree	Tun	Meliaceae	Rare	Shade, timber, fuel wood etc.
Temporary shade trees							
39.	<i>Indigoferateysmannii</i>	RMG, DM, BTRISS, UDL, BP, NDFC, HV, NPC, PACB, AS, MJ, ADM, BTRICS, BTRISSF, FLA	Shrubs	Indigofera	Fabaceae	Most common	Indigo dye, medicine etc.
40.	<i>Cajanuscajan</i>	RMG, DM, UDL, BP, NDFC, HV, NPC, PACB, AS, MJ, ADM,	Shrubs	Arhor	Fabaceae	Common	Food, fuel, nitrogen fixation.
41.	<i>Tephrosia candida</i>	AS, MJ	Shrubs	White tephrosia	Fabaceae	Rare	Food, fuel, nitrogen fixation, soil improvement etc.
42.	<i>Gliricidiasepium</i>	RMG, DM, UDL, BP, NDFC, HV, NPC, AS, MJ, ADM	Shrubs	Gliricidia	Fabaceae	Less common	Shade, honey, rodenticide, medicinal, firewood etc.
43.	<i>Erythralithosperma</i>	RMG, UDL	Small tree	Dadap	Fabaceae	Rare	Shade, ornamental, nitrogen fixation, medicine, firewood etc.
44.	<i>Desmodiumgyroides</i>	RMG, BP, NPC	Medium size tree	Tick clover	Fabaceae	Rare	Shade, ornamental, green manure, nitrogen fixation, medicine, etc.

RMG- Ramgarh; DM-Dantmara; BTRISS- BTRI substation, Fatikchari; UDL- Udalia; BP- Bhojpur; NDFC- Naseha; HV- Haldavalley; NPC- Neapchun; PACB- Panchabati; AS- Aasia; MJ- Majan; ADM-Andhar manik; BTRICS- BTRI campus, Srimangal; BTRISSF-BTRI substation, Fultoli, Srimangal; FLA- Finlay.

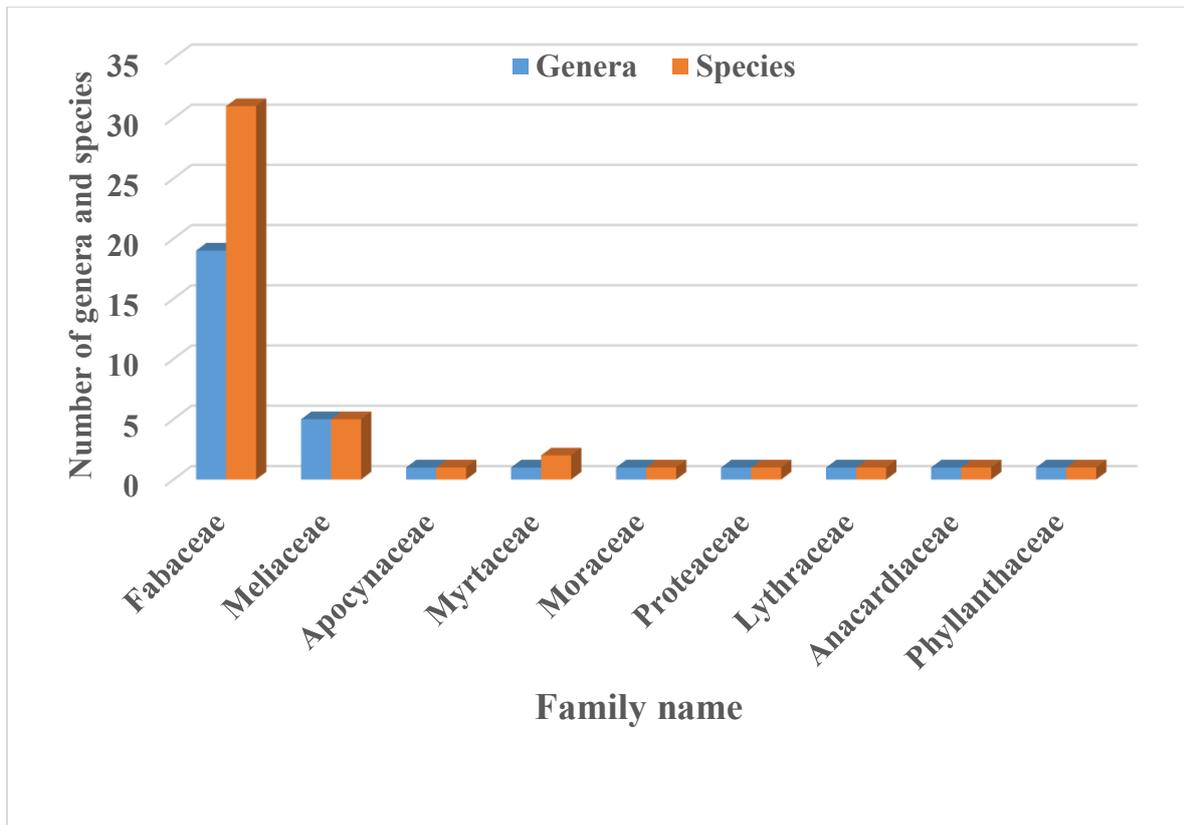


Figure 3: Family wise showing the number of genera and species of shade trees at 15 tea garden of Bangladesh.

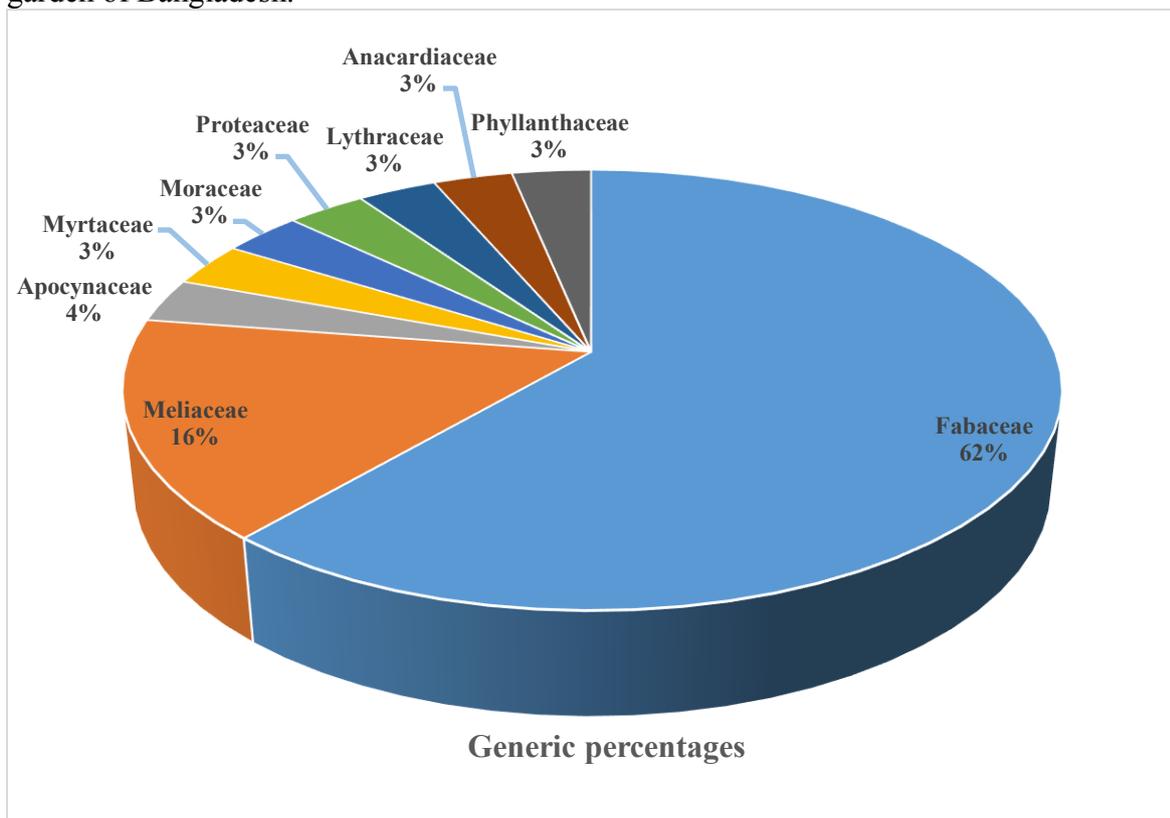


Figure 4: Family wise generic percentage of available shade trees of the study areas.



Albizia lebbeck



Derris robusta



Albizia procera



Albizia odoratissima



Melia azedarach



Acacia auriculiformis

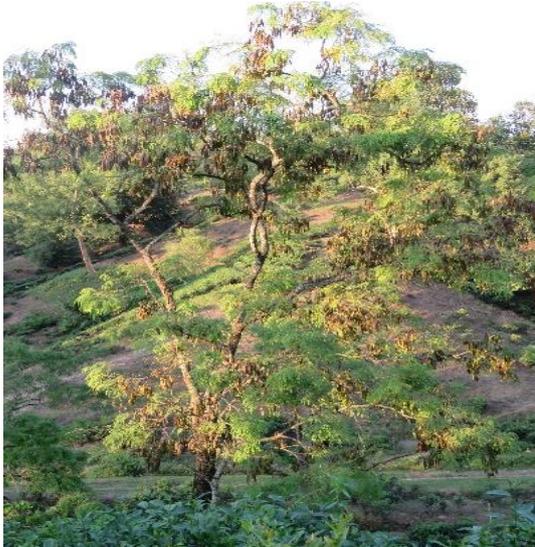
Figure 5: Different types of shade trees at different tea gardens of Bangladesh



Cassia siamea



Mangifera indica



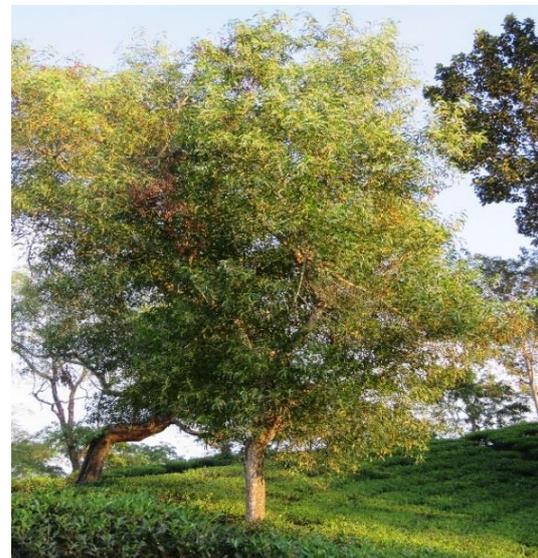
Albizia chinensis



Artocarpus chaplasha



Albizia lucidior



Acacia hybrid

Figure 6: Different types of shade trees at different tea gardens of Bangladesh



Albizia moluccana



Albizia richardiana



Swintonia microphylla



Azadirachta indica



Indigofera teysmannii



Gliricidia sepium

Figure 7: Different types of shade trees at different tea gardens of Bangladesh

Table 3: Incidence of shade tree gummosis in fifteen tea garden of Bangladesh

Locations	Garden name	Average age of trees (Yrs)	Incidence (%)									
			Shade tree species									
			<i>A. procera</i>	<i>A. lebek</i>	<i>A. odoratissima</i>	<i>A. moluccana</i>	<i>A. chinensis</i>	<i>A. lucida</i>	<i>Derris robusta</i>	<i>Dalbergia sissoo</i>	<i>Melia azedarach</i>	<i>S. saman</i>
Fatikchari	Ramgarh	10-25	20.28 ef	36.67a	10.87 a	6.67 cd	3.34 d	6.67 bc	6.67 b	3.33 d	3.33 b	-
Bhojpur, Fatikchari	Dantmara	12-30	23.33 d	30.86b	6.67 bc	3.34 f	10 a	3.33 d	3.33 d	3.33 d	6.66 a	28.85 a
Bhojpur, Fatikchari	BTRI substation	7-25	13.34 h	23.34d	3.34 f	6.67 cd	3.34 d	3.33 d	3.33 d	3.33 d	6.66 a	-
Katirhat, Fatikchari	Udalia	10-40	40 a	36.66 a	10 a	10 a	6.68 b	10 a	6.68 b	13.34 a	3.33 b	24.85 b
Bhojpur, Fatikchari	Bhojpur	7-30	10.21 i	23.36d	6.67 bc	3.34 f	3.33 d	6.67 bc	10 a	3.33 d	3.33 b	22.85 c
Dantmara, Fatikchari	Naseha	10-30	25.32 c	12.27f	6.84 bc	8.64 ab	5.75 bc	6.43 bc	8.63 a	5.63 bc	2.34 b	-
Najirhat, Fatikchari	Haldavalley	7-25	21.87 de	16.74e	5.42 cde	6.74 cd	5.39 bc	7.96 b	6.43 b	6.42 b	2.76 b	-
Narayanhat, Fatikchari	Neapchun	10-30	15.63 g	12.85f	7.97 b	6.31 cde	5.49 bc	5.74 c	5.73 bc	5.42 bc	3.42 b	-
Bhojpur, Fatikchari	Panchabati	7-30	12.86 h	10.53g	4.73 def	5.84 de	4.63 cd	5.97 c	4.86 bcd	4.36 cd	2.12 b	-
Bhojpur, Fatikchari	Aasia	10-35	18.98 f	9.64g	3.86 ef	4.76 ef	4.21 cd	6.12 bc	4.32 cd	4.18 cd	3.54 b	-
Datmara, Fatikchari	Majan	7-30	27.75 b	8.95g	5.78 cd	7.85 bc	5.76 bc	4.76 cd	5.23 bcd	3.97 cd	2.85 b	-
Chikancherra, Fatikchari	Andhar manik	7-35	19.63 f	15.97e	4.98 cdef	5.62 de	4.89 bcd	5.85 c	4.31 cd	3.42 d	2.18 b	-
Srimangal	BTRI campus	7-35	13.34 h	10 g	10 a	6.67 cd	6.68 b	6.67 bc	3.33 d	-	-	-
Fultoli, Srimangal	BTRI substation	10-35	16.67 g	20 e	10 a	6.68 cd	3.33 d	3.33 d	3.33 d	-	-	-
Srimangal	Finlay	7-35	6.67 j	13.34 f	3.34 f	3.33 f	3.33 d	6.67 bc	3.33 d	-	-	-
SD		-	8.17	9.53	2.55	1.92	1.82	1.78	2.06	2.83	1.53	3.05
CV (%)		-	42.86	50.87	38.21	31.09	35.99	29.99	39.04	56.49	43.39	11.97

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

Table 4: Symptoms observed in shade trees at fifteen tea gardens of Bangladesh

Locations	Garden name	Type of symptoms																													
		Shade tree species																													
		<i>Albizia procera</i>			<i>A. lebek</i>			<i>A. odoratissima</i>			<i>A. moluccana</i>			<i>A. chinensis</i>			<i>A. lucida</i>			<i>Derris robusta</i>			<i>Dalbergia sissoo</i>			<i>Melia azedarach</i>			<i>S. saman</i>		
D B	G E	VD	DB	G E	V D	DB	G E	V D	DB	GE	VD	DB	G E	V D	D B	G E	V D	D B	G E	V D	D B	G E	V D	D B	G E	V D	D B	G E	VD		
Fatikchari	Ramgarh	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	-	-	-
Bhojpur, Fatikchari	Dantmara	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N
Bhojpur, Fatikchari	BTRI substation	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	-	-	-
Katirhat, Fatikchari	Udalia	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N
Bhojpur, Fatikchari	Bhojpur	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N
Dantmara, Fatikchari	Naseha	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	-	-	-
Najirhat, Fatikchari	Haldavalley	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	-	-	-
Narayanhat, Fatikchari	Neapchun	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	-	-	-
Bhojpur, Fatikchari	Panchabati	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	-	-	-
Bhojpur, Fatikchari	Aasia	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	-	-	-
Datmara, Fatikchari	Majan	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	-	-	-
Chikancherr a, Fatikchari	Andhar manik	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	-	-	-
Srimangal	BTRI campus	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	-	-	-
Fultoli, Srimangal	BTRI substation	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	-	-	-
Srimangal	Finlay tea	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	-	-	-

DB= Die back; GE= Gum exudation; VD = Vascular discoloration; N= No; Y= Yes.

Table 5: Disease severity index (DSI) of shade tree gummosis in fifteen tea garden of Bangladesh.

Locations	Garden name	Disease severity (%)									
		Shade tree species									
		<i>A. procera</i>	<i>A. lebek</i>	<i>A. odoratissima</i>	<i>A. moluccana</i>	<i>A. chinensis</i>	<i>A. lucida</i>	<i>Derris robusta</i>	<i>Dalbergia sissoo</i>	<i>Melia azedarach</i>	<i>S. saman</i>
Fatikchari	Ramgarh	46.38 g	26.67 j	23.34 d	6.67 h	13.33 d	10 h	20 ef	10.69 e	12.68 d	-
Bhojpur, Fatikchari	Dantmara	40.29 i	40 d	20 e	13.34 f	16.67 c	20 d	40 a	16.67 c	16.67 c	32.65 b
Bhojpur, Fatikchari	BTRI substation	53.33 d	36.67 e	30 b	10 g	20 b	30 a	36.68 b	6.67 g	10.12 ef	-
Katirhat, Fatikchari	Udalia	76.67 a	50 a	33.34 a	33.34 a	23.34 a	26.67 b	26.68 c	20.24 b	23.34 a	38.86 a
Bhojpur, Fatikchari	Bhojpur	50 e	36.67 e	26.67 c	23.34 c	10 e	13.34 fg	23.34 d	13.34 d	4.97g	28.13 c
Dantmara, Fatikchari	Naseha	63.34 b	33.34 gh	26.67 c	20 d	6.67 g	16.68 e	16.67 h	6.68 g	10 ef	-
Najirhat, Fatikchari	Haldavalley	60.14 c	36.67 e	16.68 f	16.67 e	16.68 c	23.34 c	13.34 i	40 a	20 b	-
Narayanhat, Fatikchari	Neapchun	58.98 c	30 i	20 e	6.67 h	10 ef	10 h	26.67 c	6.86 g	13.35 d	-
Bhojpur, Fatikchari	Panchabati	63.76 b	46.84 b	23.74 d	12.74 f	9.87 ef	18.74 d	25.74 c	8.85 f	10.97 e	-
Bhojpur, Fatikchari	Aasia	59.63 c	42.76c	21.64 ef	16.85 e	16.75 c	16.63 e	22.74 d	7.56 fg	8.76 f	-
Datmara, Fatikchari	Majan	48.63 ef	38.75 d	18.74 f	18.54 d	12.95 d	12.39 g	20.97 e	6.53 g	10.74 e	-
Chikancherra, Fatikchari	Andhar manik	43.74 h	35.42 ef	23.64 d	12.53 f	18.95 b	16.85 e	18.65 fg	8.95 f	13.64 d	-
Srimangal	BTRI campus	42.65 h	28.53 i	29.74 bc	23.84 c	13.63 d	14.32 f	19.74 ef	-	-	-
Fultoli, Srimangal	BTRI substation	49.74 e	31.74 h	24.63 cd	28.64 b	8.63 f	12.53 g	17.52 gh	-	-	-
Srimangal	Finlay	47.63 fg	34.63 fg	26.58 c	24.63 c	10.53 e	14.68 f	12.84 i	-	-	-
SD		9.91	6.47	4.59	7.84	4.74	5.85	7.65	9.64	5.03	5.38
CV (%)		18.46	17.68	18.84	43.95	34.19	34.29	33.59	75.59	38.93	16.22

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

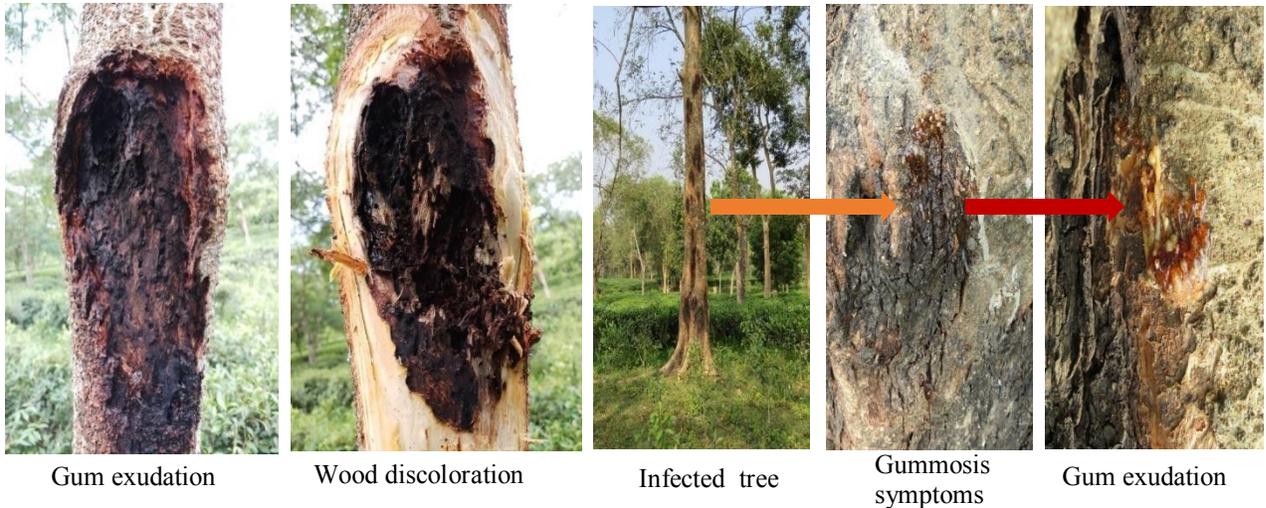


Figure 8: Gummosis symptoms of *Albizia lebek* at Bangladesh Tea Research Station Sub Station garden, Uddalia, Najirhat, Fatikchari, Chattogram

Figure 9: Gummosis symptoms of *A. procera* at Uddalia tea garden, Najirhat, Fatikchari, Chattogram

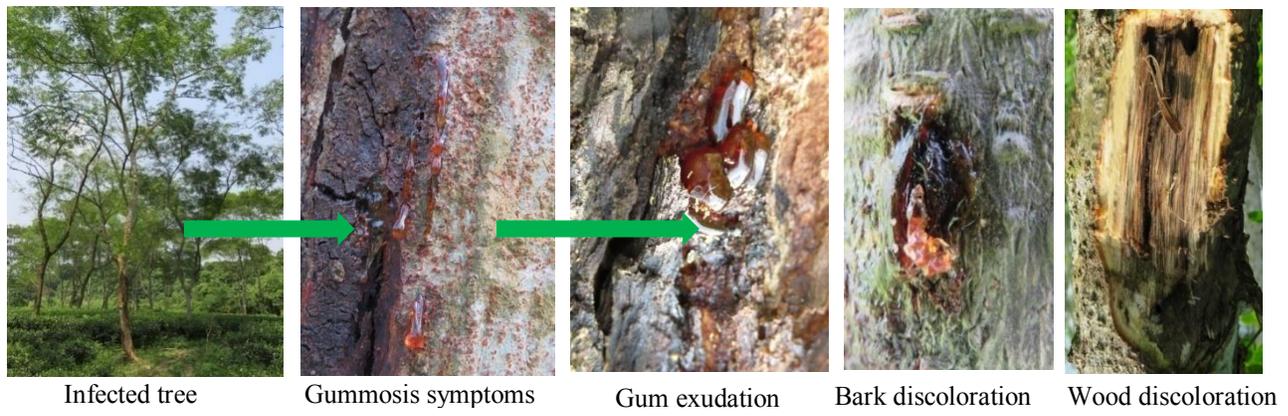


Figure 10: Gummosis symptoms of *Derris robusta* at Uddalia tea garden Fatikchari, Chattogram.



Figure 11: Gummosis symptoms of *A. procera* at BTRI substation, Fultoli tea garden Moulvibazar.

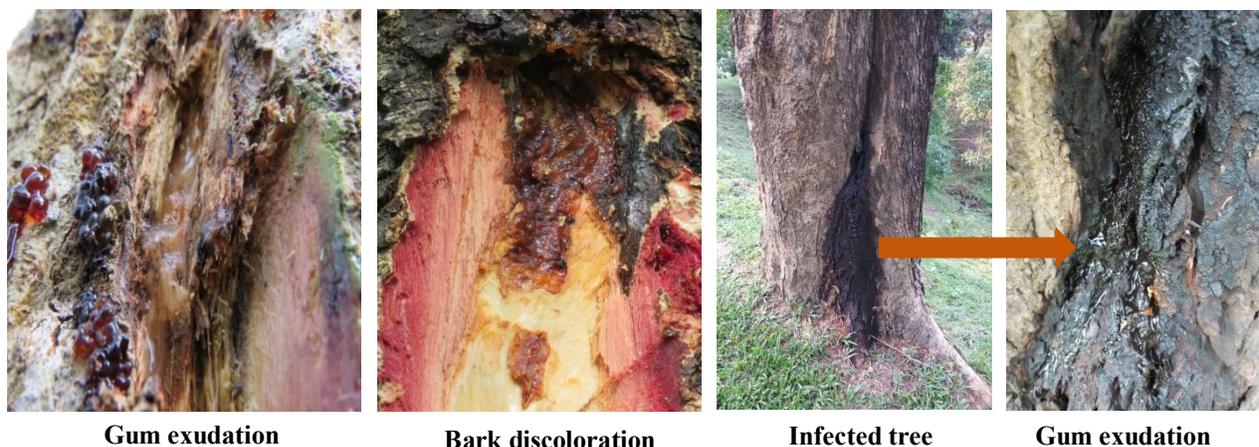


Figure 12: Gummosis symptoms of *A. procera* at BTRI tea garden, Srimangal, Moulovibazar.

Figure 13: Gummosis symptoms of *Samanea saman* (Rain tree)

11.3. Isolation, identification and characterization of pathogen: In this study, a total of 629 isolates of fungi were isolated from 130 gummosis samples (Table 5 and Figure 15). On the basis of morphological characteristics, these 629 isolates of fungi were divided into 13 aggregate groups (Table 7). The number of fungus groups was reduced to 13 species. These species of fungus were identified as *Mucor* sp. (13 isolates), *Rhizopus* sp. (25 isolates), *Fusarium oxysporum* (36 isolates), *Aspergillus niger* (57 isolates), *Aspergillus fumigatus* (40 isolates), *Aspergillus flavus* (39 isolates), *Aspergillus terreus* (24 isolates), *Penicillium* sp. (25 isolates), *Fusarium solani* (20 isolates), *Lasiodiplodia crassispora* (130 isolates), *Botryodiplodia theobromae* (109 isolates), *Ceriporia lacerate* (58 isolates) and *Byssochlamys nivea* (53 isolates). The identified fungal species are characterized in Table 7. *Lasiodiplodia crassispora* (130 isolates) was the most frequently isolated species followed by *Botryodiplodia theobromae* (109 isolates), *Ceriporia lacerate* (58 isolates) and *Byssochlamys nivea* (53 isolates) was recovered in all the samples.

11.4. Pathogenicity test: After one month of inoculation in *S. saman* plants with *B. theobromae* USSBT showed typical symptoms of the disease. Whereas, no such symptoms were observed on plants inoculated with *L. crassispora* USSL, *Ceriporia lacerate* DSSCL and *Byssochlamys nivea* DSSBN. Un-inoculated plants showed no gummosis (Figure 16 and Table 8). In control plants, no vascular browning was observed whereas in plants inoculated with *B. theobromae* USSBT. Similarly, wilting and death of leaves at the branch apices was only observed where plants were inoculated with *B. theobromae* USSBT. It clearly indicated that the gummosis disease is caused by *B. theobromae* USSBT and it plays a significant role in disease

development. Re-isolation from the dead and green branches of *Botryodiplodia theobromae* inoculated plants showed up to 95% recovery of the fungus.

Same results were also observed in *A. lebbeck* and *A. procera* plant. *A. lebbeck* and *A. procera* plant inoculation with *L. crassispora* USSLC showed typical symptoms of the disease. Whereas, no such symptoms were observed on plants inoculated with *B. theobromae* USSBT, *C. lacerate* DSSCL and *B. nivea* DSSBN. Un-inoculated plants showed no gummosis (Figure 17 and 18; Table 9 and 10).

Table 6: Frequency of isolation and identification of fungal pathogen from gummosis samples

Garden name	Shade tree species	Fungal pathogens													Total
		MS	RS	FO	AN	AF	AFL	AT	PS	FS	LC	BT	CL	BN	
Ramgarh	<i>Albizzia procera</i>	-	01	01	01	-	01	01	-	01	01	01	01	01	10
	<i>A. lebek</i>	01	-	01	01	-	-	-	01	-	01	01	01	01	08
	<i>A. odoratissima</i>	-	-	01	-	-	-	-	-	-	01	-	01	-	03
	<i>A. moluccana</i>	01	-	-	-	01	01	-	-	-	01	-	01	01	06
	<i>A. chinensis</i>	-	-	-	-	01	-	-	01	-	01	01	01	-	05
	<i>A. lucida</i>	-	01	01	01	-	01	-	-	-	01	-	01	-	06
	<i>Derris robusta</i>	01	-	-	01	01	-	-	-	-	01	01	-	01	06
	<i>Dalbergia sissoo</i>	-	01	01	-	01	01	-	-	-	01	-	01	-	06
	<i>Melia azedarach</i>	-	-	-	01	01	-	-	01	-	01	-	01	-	05
<i>S. saman</i>	-	-	-	01	01	-	-	01	-	01	01	01	01	07	
Dantmara	<i>Albizzia procera</i>	-	-	-	01	01	-	-	-	01	01	-	01	05	
	<i>A. lebek</i>	-	-	-	-	01	-	-	01	-	01	01	-	04	
	<i>A. odoratissima</i>	01	-	-	01	-	-	-	-	01	-	-	-	03	
	<i>A. moluccana</i>	-	-	-	01	-	01	-	-	01	01	01	-	05	
	<i>A. chinensis</i>	-	-	-	01	-	-	01	-	-	01	-	01	05	
	<i>A. lucida</i>	-	-	01	01	-	-	-	01	-	01	01	01	07	
	<i>Derris robusta</i>	-	-	-	01	01	-	-	-	-	01	-	01	-	04
BTRI substation, Bhojpur, Fatikchari	<i>Albizzia procera</i>	-	-	01	-	-	01	-	-	01	-	01	-	04	
	<i>A. lebek</i>	-	-	-	01	01	01	-	-	01	01	01	01	08	
	<i>A. odoratissima</i>	-	-	-	-	-	-	01	-	-	01	01	-	04	
	<i>A. moluccana</i>	-	-	-	-	01	01	-	-	01	-	01	-	04	
	<i>A. chinensis</i>	01	-	01	01	-	-	-	01	-	01	01	-	07	
	<i>A. lucida</i>	-	-	-	-	-	-	-	-	01	-	01	01	03	
	<i>Derris robusta</i>	-	01	01	-	-	-	-	-	01	01	01	01	07	
	<i>Dalbergia sissoo</i>	-	-	-	01	01	-	-	-	-	01	01	-	05	
	<i>Melia azedarach</i>	-	-	-	01	-	01	-	-	-	01	01	-	05	
<i>S. saman</i>	-	-	01	01	01	01	-	-	-	01	01	-	06		
Udalia	<i>Albizzia procera</i>	-	-	01	-	-	-	-	01	-	01	-	01	05	
	<i>A. lebek</i>	01	-	-	-	01	-	01	-	-	01	01	-	06	
	<i>A. odoratissima</i>	-	-	01	-	-	01	-	-	01	-	01	-	05	
	<i>A. moluccana</i>	-	-	-	-	01	-	-	01	-	01	01	-	05	
	<i>A. chinensis</i>	-	-	01	-	-	-	-	-	01	01	01	-	04	
	<i>A. lucida</i>	-	-	-	01	-	-	-	-	01	01	-	01	04	
	<i>Derris robusta</i>	01	-	-	01	01	-	01	-	-	01	01	-	07	
	<i>Dalbergia sissoo</i>	-	01	-	01	-	01	-	01	-	01	-	-	06	
<i>Melia azedarach</i>	-	01	-	01	01	-	01	-	-	01	01	01	07		
<i>S. saman</i>	-	-	01	-	01	-	-	01	-	01	01	-	05		
Bhojpur	<i>Albizzia procera</i>	01	01	-	01	-	01	-	-	-	01	01	01	08	

Table to be continued

	<i>A. lebek</i>	-	-	01	-	-	-	-	-	01	01	01	01	01	06
	<i>A. odoratissima</i>	-	-	-	-	01	-	01	-	-	01	01	-	01	05
	<i>A. moluccana</i>	01	-	-	01	01	-	-	-	-	01	01	-	01	06
	<i>A. chinensis</i>	-	-	01	01	01	01	01	-	-	01	01	-	01	08
	<i>A. lucida</i>	-	-	01	01	-	-	-	-	-	01	-	01	01	05
	<i>Derris robusta</i>	-	-	01	01	-	-	-	-	-	01	01	-	-	04
Naseha	<i>Albizzia procera</i>	-	-	-	-	-	01	01	-	01	01	-	01	-	05
	<i>A. lebek</i>	-	-	01	-	01	-	-	-	-	01	01	01	-	05
	<i>A. odoratissima</i>	-	-	-	-	-	01	01	-	-	01	-	01	-	04
	<i>A. moluccana</i>	-	-	01	01	-	01	-	-	-	01	01	01	01	07
	<i>A. chinensis</i>	01	-	-	-	-	-	-	-	-	01	01	01	-	04
	<i>A. lucida</i>	-	-	01	-	01	01	-	01	-	01	-	01	-	06
	<i>Derris robusta</i>	-	-	-	01	-	-	-	-	-	01	01	-	01	04
Haldavalley	<i>Albizzia procera</i>	01	-	-	01	01	01	-	-	-	01	01	-	01	07
	<i>A. lebek</i>	-	-	-	-	-	-	01	-	01	01	01	-	-	04
	<i>A. odoratissima</i>	-	-	01	01	-	01	-	-	-	01	01	-	01	06
	<i>A. moluccana</i>	-	-	-	-	-	-	-	01	-	01	01	-	-	03
	<i>A. chinensis</i>	-	-	01	-	-	-	-	-	-	01	01	01	-	04
	<i>A. lucida</i>	-	-	-	-	-	01	-	-	-	01	01	-	01	04
Neapchun	<i>Derris robusta</i>	-	-	-	01	-	-	-	-	01	01	01	-	-	04
	<i>Albizzia procera</i>	-	-	01	01	01	-	-	-	-	01	01	01	01	07
	<i>A. lebek</i>	01	-	-	-	-	01	01	-	-	01	01	01	-	06
	<i>A. odoratissima</i>	-	01	-	-	-	-	-	-	-	01	01	-	01	04
	<i>A. moluccana</i>	-	-	01	01	01	01	01	-	01	01	01	01	-	09
	<i>A. chinensis</i>	-	-	-	01	01	-	-	-	-	01	01	01	-	05
	<i>A. lucida</i>	-	-	-	01	-	-	-	01	-	01	01	01	-	05
	<i>Derris robusta</i>	01	01	-	-	-	-	-	-	-	01	01	-	01	05
<i>Dalbergia sissoo</i>	-	-	-	01	-	-	01	-	01	01	01	01	-	06	
<i>Melia azedarach</i>	-	-	-	01	01	01	-	-	-	01	01	-	-	05	
Panchabati	<i>Albizzia procera</i>	-	-	01	-	-	01	-	-	-	01	01	01	01	06
	<i>A. lebek</i>	-	-	-	01	01	-	-	01	-	01	01	-	01	06
	<i>A. odoratissima</i>	-	-	01	01	01	-	01	-	-	01	01	01	-	07
	<i>A. moluccana</i>	-	-	-	-	-	-	-	-	-	01	01	01	-	03
	<i>A. chinensis</i>	-	-	-	-	-	01	-	-	01	01	01	01	-	05
	<i>A. lucida</i>	-	-	-	01	-	-	-	-	-	01	01	-	01	04
	<i>Derris robusta</i>	-	-	-	01	-	01	-	-	-	01	01	-	01	05
	<i>Dalbergia sissoo</i>	-	-	-	01	-	-	-	-	01	01	01	-	-	04
<i>Melia azedarach</i>	-	01	-	01	-	-	-	01	-	01	-	01	01	06	
Aasia	<i>Albizzia procera</i>	-	-	-	-	-	01	-	-	01	01	01	-	-	04
	<i>A. lebek</i>	-	-	-	-	-	-	01	-	-	01	01	-	-	03

	<i>A. odoratissima</i>	-	-	-	-	-	-	-	01	-	01	01	-	01	04
	<i>A. moluccana</i>	-	-	-	-	-	-	-	-	-	01	01	-	-	02
	<i>A. chinensis</i>	-	01	-	-	-	-	-	-	-	01	-	-	-	02
	<i>A. lucida</i>	-	-	-	-	-	-	-	-	-	01	-	-	-	01
	<i>Derris robusta</i>	--	-	-	-	-	-	-	-	-	01	01	01	01	04
	<i>Dalbergia sissoo</i>	-	-	-	-	-	-	-	-	-	01	01	-	-	02
	<i>Melia azedarach</i>	-	-	01	-	01	-	-	-	-	01	01	-	-	04
Majan	<i>Albizzia procera</i>	-	-	-	-	-	-	-	-	-	01	01	-	-	02
	<i>A. lebek</i>	-	-	-	-	-	-	-	-	-	01	01	-	-	02
	<i>A. odoratissima</i>	-	01	-	01	-	-	-	-	-	01	01	-	-	04
	<i>A. moluccana</i>	-	-	-	-	-	-	-	01	-	01	01	-	-	03
	<i>A. chinensis</i>	-	-	-	-	-	01	-	-	-	01	01	-	-	03
	<i>A. lucida</i>	-	01	-	-	01	-	-	-	-	01	01	-	-	04
	<i>Derris robusta</i>	-	-	-	-	-	-	01	-	-	01	01	-	-	03
	<i>Dalbergia sissoo</i>	-	01	-	-	-	01	-	-	-	01	01	-	-	04
	<i>Melia azedarach</i>	-	-	-	-	-	-	-	01	-	01	01	-	-	03
Andhar manik	<i>Albizzia procera</i>	-	01	-	-	-	-	-	-	01	01	01	01	-	05
	<i>A. lebek</i>	-	-	-	01	-	01	-	-	-	01	01	-	-	04
	<i>A. odoratissima</i>	-	-	-	-	01	-	01	-	-	01	01	-	-	04
	<i>A. moluccana</i>	-	-	-	01	-	01	-	-	-	01	01	-	-	04
	<i>A. chinensis</i>	-	-	-	-	01	-	-	01	-	01	01	-	-	04
	<i>A. lucida</i>	-	-	-	01	-	-	-	-	-	01	01	-	-	03
	<i>Derris robusta</i>	-	-	-	-	01	-	-	-	-	01	01	-	-	03
	<i>Dalbergia sissoo</i>	-	-	-	-	-	01	-	-	-	01	01	-	-	03
	<i>Melia azedarach</i>	-	01	-	01	-	-	-	-	-	01	01	-	-	04
BTRI campus, Srimangal	<i>Albizzia procera</i>	-	01	01	-	-	01	-	-	-	01	01	01	01	07
	<i>A. lebek</i>	-	-	-	01	-	01	-	-	-	01	01	01	01	06
	<i>A. odoratissima</i>	-	01	-	01	-	-	01	-	-	01	01	01	-	06
	<i>A. moluccana</i>	-	-	01	-	01	-	-	-	-	01	01	01	01	06
	<i>A. chinensis</i>	-	01	-	01	-	-	-	-	-	01	01	-	-	04
	<i>A. lucida</i>	-	-	01	-	-	-	-	-	-	01	01	-	-	03
	<i>Derris robusta</i>	-	-	01	-	-	-	-	-	-	01	01	-	-	03
	<i>Dalbergia sissoo</i>	-	-	-	-	01	-	01	-	-	01	01	-	-	04
	<i>Melia azedarach</i>	-	-	01	-	-	-	01	-	-	01	01	-	-	04
BTRI substation, Fultoli, Srimangal	<i>Albizzia procera</i>	-	01	-	-	-	01	-	-	-	01	01	01	01	06
	<i>A. lebek</i>	-	-	-	01	-	-	-	01	-	01	01	01	01	06
	<i>A. odoratissima</i>	-	01	-	-	-	-	-	-	01	01	01	01	01	06
	<i>A. moluccana</i>	-	-	01	01	-	01	-	-	-	01	01	01	01	07
	<i>A. chinensis</i>	-	-	01	-	-	-	01	-	-	01	01	01	01	06
	<i>A. lucida</i>	-	-	-	01	-	-	-	01	-	01	01	-	-	04
	<i>Derris robusta</i>	-	-	-	-	01	01	-	-	-	01	01	-	-	04

Table to be continued

	<i>Dalbergia sissoo</i>	-	-	-	-	-	-	-	-	01	01	01	-	-	03
	<i>Melia azedarach</i>	-	-	-	-	-	01	-	-	-	01	01	-	-	03
Finlay, Srimangal	<i>Albizia procera</i>	-	01	-	-	-	-	-	01	01	01	01	01	01	07
	<i>A. lebek</i>	-	-	01	-	-	-	-	-	-	01	01	-	01	04
	<i>A. odoratissima</i>	-	-	-	01	-	-	01	01	-	01	01	-	-	05
	<i>A. moluccana</i>	-	01	-	-	-	-	-	-	01	01	01	01	-	05
	<i>A. chinensis</i>	-	-	-	-	01	-	-	-	-	01	01	-	-	03
	<i>A. lucida</i>	-	-	-	01	-	-	-	-	-	01	01	01	01	05
	<i>Derris robusta</i>	-	01	-	-	-	-	01	-	-	01	01	01	01	06
	<i>Dalbergia sissoo</i>	-	01	-	01	-	-	-	-	01	01	01	-	-	05
	<i>Melia azedarach</i>	-	-	-	-	01	-	-	01	-	01	01	-	-	04
Total		13	25	36	57	40	39	24	25	20	130	109	58	53	629
Total isolates	629														

MS=*Mucor* sp; RS = *Rhizopus* sp.; FO = *Fusarium oxysporum*; AN = *Aspergillus niger*; AF = *Aspergillus fumigatus*; AFL = *Aspergillus flavus*; AT = *Aspergillus terreus*; PS = *Penicillium* sp.; FS = *Fusarium solani*; LC = *Lasiodiplodia crassisporea*; BT = *Botryodiplodia theobromae*. CL = *Ceriporia lacerate*; BN = *Byssochlamys nivea*.

Table 7. List of 13 isolated fungi isolated and identification from nine gummosis infected plants (*A. procera*, *A. lebek*, *A. odoratissima*, *A. moluccana*, *Samanea saman*, *A. lucida*, *A. chinensis*, *Derris robusta*, *Dalbergia sissoo* and *Melia azedarach*).

Isolates code name	Macro-microscopic characteristics	Identification Species (Tentative)	Group name	Number of isolates
DALMS, DAMMS, DDRMS, BTRISFAOMS, UACMS, VALMS, VDRMS, BTRIAPMS, BTRIAMMS, BTRISFACMS, FMAPMS, RALMS, RDRMS	Fast-growing fungus able to cover whole PDA plate within 2-4 days. Mycelia was soft cottony and sticky. It becomes light yellow to deep yellow color. Round shaped fruiting bodies (sporangia) were densely produced in a small path of culture. Sporangioophores were long and terminate in round spore-filled sporangia (50µm-300µm diameter). The sporangia had a thin wall which when mature dissolves (or is disrupted) to release round or somewhat ellipsoidal sporangiospores (4µm-8µm diameter) (Figure 15.a & b).	<i>Mucor</i> sp.	Group-1	13
DAPRS, DALRS, DDSRS, UDRRS, VDSRS, VMARS, BTRIAPRS, RAORS, RDRRS, AMARS, FACRS, NAORS, NALRS, NDSRS, PAPRS, PMARS, AAPRS, AAORS, AACRS, MAPRS, MAORS, AAPRS, AAMRS, ADRRS, ADSRS	Rapid growth rate; maturing within 2-3 days. It quickly fills a Petri dish (agar surface) with a typically cotton candy like colony, initially white that turns grey to gray-pigmented. The reverse is white to pale. Stolons run the mycelia, connecting groups of long (up to 4 mm) usually unbranched sporangioophores. Rhizoids present (Figure 15. c& d).	<i>Rhizopus</i> sp.	Group-2	25
DAPFO, DALFO, DAOFO, DALFO, DDSFO, BTRIALFO, UAPFO, UACFO, UDRFO, USSFO, VAPFO, VAOFO, VASFO, VSSFO, BTRIALFO, BTRIFACFO, BTRIFALFO, BTRIDRFO, BTRIALFO, BTRIAMFO, BTRIALFO, FAOFO, FACFO, RAPFO, RAMFO, AAPFO, AAOFO, HMAFO, AAPFO, AAMFO, AALFO, ADRFO, AMAFO, MAMFO, MACFO, AALFO	Colonies growing rapidly, 4.5 cm in four days, aerial mycelium white, becoming purple, with discrete orange sporodochia present in some strains. Conidiophores are short, single, lateral monophialides in the aerial mycelium, later arranged in densely branched clusters. Macroconidia are fusiform, slightly curved, pointed at the tip, mostly three septate, basal cells pedicellate, 23-54 x 3-4.5 µm. Microconidia are abundant, never in chains, mostly non-septate, ellipsoidal to cylindrical, straight or often curved, 5-12 x 2.3-3.5 µm. Chlamyospores are terminal or intercalary, hyaline, smooth or rough walled, 5-13 µm (Figure 15. o&p).	<i>Fusarium oxysporum</i>	Group-3	36
DAPAN, DALAN, DALAN, DDRAN, DMAAN, DSSAN, BTRIFAPAN, BTRIFAOAN, BTRIFAMAN, BTRIFACAN, BTRIFALAN, BTRIFDRAN, UALAN, UACAN, UDSAN, UMAAN, USSAN, USSAN, VDRAN, VDSAN, VMAAN, BTRIAPAN, BTRIAMAN, BTRIACAN, BTRIALAN, BTRIDRAN, BTRIFAMAN, BTRIFALAN, FAPAN, FAOAN, FDRAN, RAPAN, RAMAN, RACAN, RALAN, RDSAN, RMAAN, AALAN, AAOAN, AALAN, ADRAN, ADSAN, AMAAN, NAOAN, PALAN, PAMAN, PALAN, PMAAN, AALAN, AAOAN, AACAN, MALAN, MAMAN, MALAN, AAOAN, AALAN, ADSAN	Moderate-growing fungus (0.83% radial growth rate h ⁻¹) was exhibited with dark black mycelia/spores mass in PDA culture. Additional symptom was absent at the edge of periphery. Long conidiophores produced a round cluster structured fruiting body at its swollen apex (Figure 15. e& f).	<i>Aspergillus niger</i>	Group-4	57
DAMAF, DACAF, DACAF, DDRAF, DDSAF, DMAAF, DSSAF, BTRIFAPAF, BTRIFALAF, BTRIFDRAF, UAMAF, UDSAF, USSAF, VALAF, VAMAF, VDRAF, VMAAF, VSSAF, VAOAF, VAMAF,	Fast-growing fungus able to cover whole PDA plate within 2-4 days. Conidia were 2.5 to 3 µm in diameter and produced in chains basipetally from	<i>Aspergillus fumigatus</i>	Group-5	40

Table to be continued

VACAF, BTRIALAF, BTRIALAF, FALAF, RAPAF, RAMAF, RACAF, RMAAF, AALAF, AAOAF, HMAAF, NALAF, PAOAF, PACAF, PDRAF, AAMAF, ADRAF, MDRAN, AACAF, AMAAF	greenish phialides. Mycelia were soft cottony sticky and it becomes light gray to green color (Figure 15 i & j).			
DAPAF, DAMAF, DALAF, DDSAF, BTRIAMAF, UAPAF, UALAF, UAMAF, UMAAF, USSAF, VAOAF, VDSAF, BTRIFAPAF, BTRIFACAF, BTRIAFAPAF, BTRIAOAF, BTRIAMAF, BTRIALAF, FAPAF, FAOAF, FALAF, RALAF, RAMAF, RMAAF, AAPAF, AACAF, ADRAF, HAPAF, NACAF, NDSAF, PALAF, PAMAF, PDSAF, AAPAF, AALAF, MAPAF, MAMAF, MDRAF, MMAAF	Fast-growing fungus. Colonies on PDA medium attaining 3.5-5.5 cm diam in 7 days at 25° C. Colony surface velvety to occasionally floccose. Colonies yellowish-green, consisting of a dense felt of conidiophores. Conidial heads en masse grayish green and radiate, splitting into several poorly defined columns, conidiogenous cells uni- and biseriate. Conidiophore stipes rough-walled, hyaline. Vesicles spherical, 25-45 mm diam. Conidia echinulate, (sub) spherical, 3.5 mm diam (Figure 15 g & h).	<i>Aspergillus flavus</i>	Group-6	39
DAPAT, BTRIFACAT, UAOAT, VALAT, VDRAT, VMAAT, VAOAT, VACAT, BTRIPAT, BTRIAOAT, FALAT, RALAT, RAMAT, RDSAT, AAOAT, HALAT, NDRAT, PAOAT, AAOAT, ADSAT, AMAAT, MACAT, AAOAT, ADRAT	Colonies on PDA agar media appeared as rapidly growing powdery colonies with a characteristic buff or cinnamon-brown colour on the surface and a yellow to beige-brown colour on the reverse. Hyphae are septate and hyaline. Conidiophores are smooth walled and hyaline. Biseriate phialides, extending from the upper portion of the vesicle. Conidia form in long chains is round, smooth walled (Figure 15 k & l).	<i>Aspergillus terreus</i>	Group-7	24
DALPS, DACPS, DMAPS, DSSPS, BTRIFALPS, BTRIFALUPS, UACPS, VAPPS, VAMPS, VDSPS, VSSPS, BTRIALUPS, FAMPSP, RALUPS, AALPS, AMAPS, FAOPSP, NAMPS, NMAPS, PACPS, MALEPS, MALUPS, AAPPS, AAOPS, AAMPSP	Slow-growing dark grey smooth mycelial mat was exhibited on PDA culture. Fine folded scars appeared along radius from center making furrows and ridges alternatively. Sometimes serrated margin was also noticed. Both single/branched conidiophores produced short-to-intermediate length conidial chain on phialides (Figure 15 m & n).	<i>Penicillium</i> sp.	Group-8	25
DAPFS, BTRIAMFS, UALFS, UDRFS, VAOF, BTRIAOFS, BTRIPAFS, FALFS, FDRFS, RAMFS, RDSFS, AACFS, ADSFS, HAPFS, PAPFS, MAOFS, MDSFS, AAPFS, AAMFS, ADSFS	Colonies are usually fast growing, brightly colored and have a cottony aerial mycelium and produce both macro- and microconidia from slender phialides. Macroconidia are hyaline, two- to several-celled, fusiform- to sickle-shaped, mostly with an elongated apical cell and pedicellate basal cell. Microconidia are 1- to 2-celled, hyaline, pyriform, fusiform to ovoid, straight or curved (Figure 15 y & z).	<i>Fusarium solani</i>	Group-9	20
DAPLC, DALLC, DAOLC, DAMLC, DACLC, DALLC, DDRLC, DDSLC, DMALC, DSSLC, BTRIFAPLC, BTRIFALLC, BTRIFAOLC, BTRIFAMLC, BTRIFACLC, BTRIFALLC, BTRIFDRLC, UAPLC, UALLC, UAOLC, UAMLC, UACLC, UALULC, UDRLC, UDSL, UMALC, USSLC, VAPLC, VALLC, VAOLC, VAMLC, VACLC, VLCLC, VDRLC, VDSL, VMALC, VSSLC, BTRIFLC, BTRIFALLC, BTRIAOLC, BTRIFAMLC, BTRIAOLC, BTRIFALULC, BTRIFDRLC, BTRIPALC, BTRIALLC, BTRIAOLC, BTRIAMLC, BTRIAOLC, BTRIALLC, BTRIDRLC, FAPLC, FALLC, FAOLC, FAPLC, FACLC, FALLC, FDRLC, RAPLC, RALLC, RAOLC, RAMLC, RALC, RALLC, RDRLC, RDSL, RMALC, AAPLC, AALLC, AAOLC, AAMLC, AACLC, AALLC, ADRLC, ADSLC, AMALC, HAPLC, HALLC, HAOLC, HAMLC, HACLC, HALLC, HDRLC, HDSL, HMALC, NAPLC, NAPLC, NAOLC, NAMLC, NALC, NALLC, NDRLC, NDSL, NMALC, PAPLC, PALLC, PAOLC, PAMLC,	Fast-growing fungus able to cover whole PDA plate within 2-4 days. Conidia were double layered, hyaline and unicellular at initial stage but on maturity it became light to dark-brown colour with typical striate formation, equally 2-celled, oblong, bilaminate with the size of 14.5 -28.8µ(av. 26.9µ) x 8.0- 11.0 µ (av. 15.9µ) (Figure 15 s & t).	<i>Lasiodiplodia crassispora</i>	Group-10	130

PACLC, PALLC, PDRLC, PDSLCL, PMALC, AAPLC, AALLC, AAOLC, AAMLC, AACLC, AALLC, ADRLC, ADSLC, AMALC, MAPLC, MALLC, MAOLC, MAMLC, MACLC, MALLC, MDRLC, MDSLCL, MMALC, AAPLC, AALLC, AAOLC, AAMLC, AACLC, AALLC, ADRLC, ADSLC, AMALC				
DAPBT, DALBT, DACBT, DDRBT, DSSBT, BTRIFAPBT, BTRIFALBT, BTRIFAMBT, BTRIALBT, UALBT, UAOBT, UACBT, UDRBT, UDSBT, UMABT, USSBT, VALBT, VAMBT, VACBT, VALBT, VDRBT, VMABT, VSSBT, BTRIFAPBT, BTRIFALBT, BTRIFAObT, BTRIFAMBT, BTRIFACBT, BTRIFDRBT, BTRIALBT, BTRIAMBt, BTRIACBT, BTRIDRBT, FAPBT, FALBT, FAObT, FAMBt, FACBT, FALBT, FDRBT, RAPBT, RALBT, RAOBT, RAMBT, RACBT, RALBT, RDRBT, RDSBT, RMABT, AAPBT, AALBT, AAOBT, AAMBT, AACBT, AALBT, ADRBT, ADSBT, HAPBT, HALBT, HAOBT, HAMBt, HDRBT, HDSBT, HMAbT, NAPBT, NALBT, NAOBT, NAMBT, NACBT, NALBT, NDRBT, NDSBT, NMABT, PAPBT, PALBT, PAObT, PAMBt, PACBT, PALBT, PDRBT, PDSBT, PMABT, AAPBT, AALBT, AAOBT, AAMBT, AACBT, AALUBT, ADRBT, ADSBT, AMABT, MAPBT, MALBT, MAObT, MAMBT, MACBT, MALBT, MDRBT, MDSBT, MMZBT, AAPBT, AALBT, AAOBT, AAMBT, AACBT, AALUBT, ADRBT, ADSBT, AMABT	Colonies greyish sepia to mouse grey to black, fluffy with abundant aerial mycelium; reverse fuscous black to black. Conidia subovoid to ellipsoid-ovoid, apex broadly rounded, tapering to a truncate base, widest in middle to upper third, thick-walled, contents granular, initially hyaline and aseptate, becoming dark brown and one-septate only after discharge from the pycnidia. Conidia size was 13–15.5(–18.5) µm (Figure 15 w & x).	<i>Botryodiplodia theobromae</i>	Group-11	109
DAPCL, DALCL, DAOCL, DAMCL, DACCL, DALCL, DDSCL, DMACL, DSSCL, BTRIFACCL, BTRIFALCL, BTRIFDRCL, UAPCL, UALCL, UAMCL, UALCL, UDRCL, VAPCL, VAObT, VAMCL, VACCL, VMACCL, BTRIFAPCL, BTRIFALCL, BTRIFALCL, BTRIApCL, BTRIALCL, BTRIAOCL, BTRIAMCL, BTRIAcCL, BTRIALCL, FACCL, RAPCL, RALCL, RAMCL, RACCL, RALCL, RDSCL, AAPCL, AAOCL, AAMCL, AACCL, AMCCL, HDRCL, PAPCL, AAPCL, AALCL, AAOCL, AAMCL, MAPCL, MALCL, MAOCL, MAMCL, MACCL, AAPCL, AAMCL, AALCL, ADRLC	Basidiocarp resupinate, effused, confluent, soft when fresh, then fragile, firmly attached to the substrate; hymenophore poroid, white, buff to ochreous; dissepiments entire to lacerate; pores angular, 2–5 per mm; margin white. Hyphal system monomitic; contextual hyphae 3–5.7 µm in diameter, smooth, thin- to slightly thick-walled, simple-septate; tramal hyphae 2.3–4.6 µm in diameter, smooth, thin- to slightly thick-walled, simple-septate, cystidia lacking; basidia clavate, 11–16.5 × 3.5–5.5 µm, with 4 sterigmata; basidiospores oblong-ellipsoid to ellipsoid, 3.5–5 × 2–3 µm (ave. 3.9 ± 0.2 × 2.5 ± 0.4 µm, n = 25), smooth, thin-walled (Figure 15 u & v).	<i>Ceriporia lacerata</i>	Group-12	58
DAPBN, DALBN, DAOBN, DAMBN, DDRBN, DSSBN, BTRIACBN, BTRIALBN, UALBN, UAOBN, UACBN, UALBN, UDRBN, UDSBN, UMABN, VAPBN, VALBN, VALBN, VDRBN, VDSBN, BTRIFAPBN, BTRIFALBN, BTRIFAObN, BTRIFAMBN, BTRIFACBN, BTRIFALBN, BTRIAMBN, BTRIDRBN, FAPBN, FAObN, FALBN, RAPBN, RAOBN, RDRBN, AAPBN, AALBN, AALBN, ADRBN, AMABN, HAOBN, HDRBN, ASAPBN, ASALBN, ASAMBN, MAPBN, MALBN, MAObN, MAMBN, MACBN, ADAPBN, ADALBN, ADALBN, ADDRBN	The colour of the mycelia in culture on PDA was highly variable, ranging from olive to brown, with tufts of white mycelia dispersed across most cultures. Chlamydo spores and conidia were frequently observed. Chlamydo spores was 2.5–5 µm, and conidia as 2.2 µm (Figure 15 q & r).	<i>Byssoschlamys nivea</i>	Group-13	53



Figure 14: Photograph shows infected bark samples and various types of fungus identified from infected samples cultured on PDA medium.

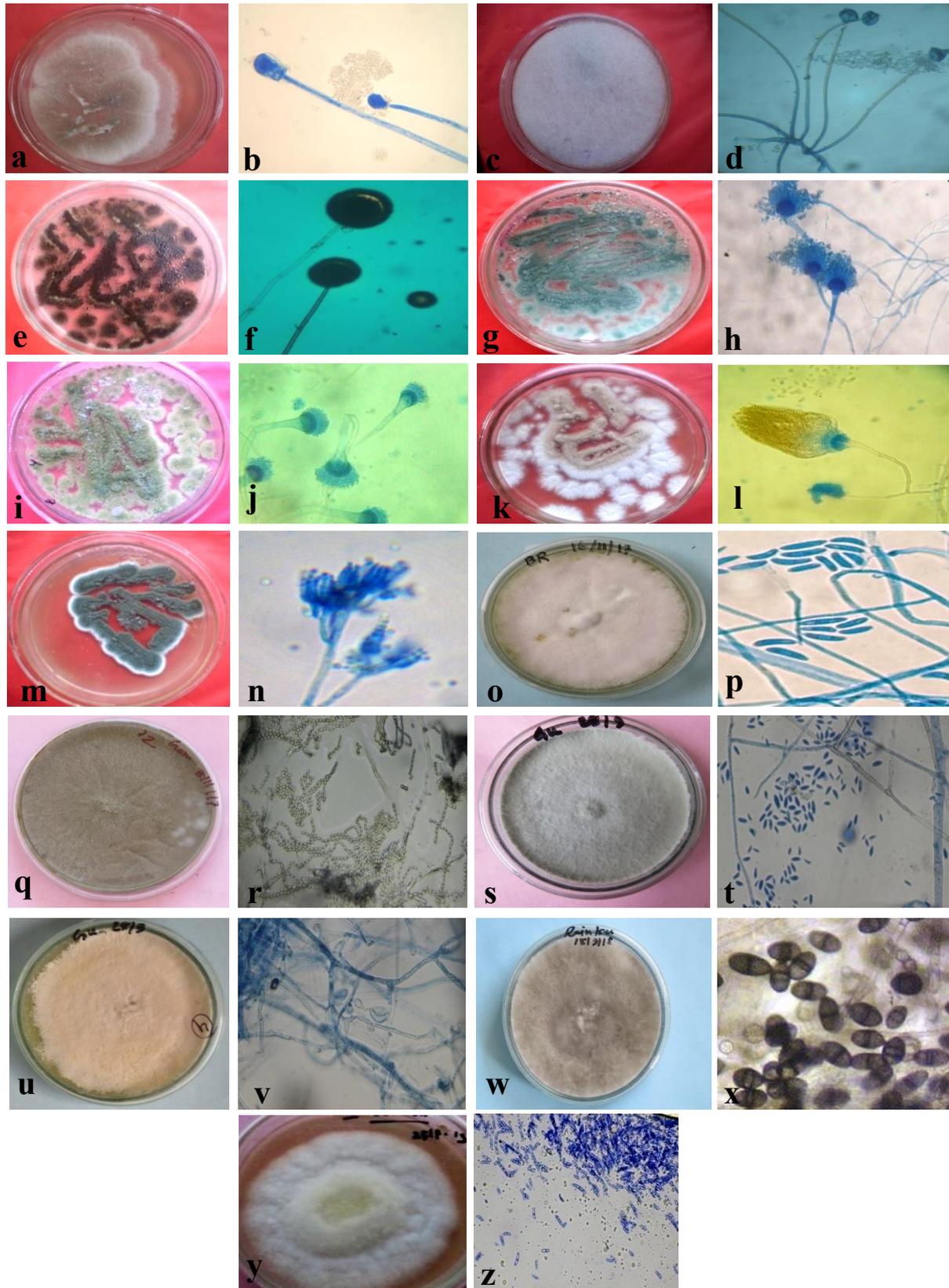


Figure 15: Photograph shows colonies of different fungal strains isolated from gummosis samples cultured on PDA plate (dorsal view) and conidiophore with conidia. a & b *Mucor* sp.; c & d *Rhizopus* sp.; e & f: *Aspergillus niger*; g & h: *Aspergillus flavus*; i & j: *Aspergillus fumigatus*; k & l: *Aspergillus terreus*; m & n: *Penicillium* sp.; o & p: *Fusarium oxysporium*; q & r: *Byssosclamyces nivea*; s & t: *Lasiodiplodia crassispora*, u & v: *Ceriporia lacerate*; w & x: *Botryodiplodia theobromae*; y & z: *Fusarium solani*.

Table 8. Severity of symptoms on *Samanea saman* plants inoculated with *Lasiodiplodia crassispora* USSLC, *Botryodiplodia theobromae* USSBT, *Ceriporia lacerate* DSSCL and *Byssochlamys nivea* DSSBN.

Pathogens	Symptoms produced on <i>Samanea saman</i> plant		
	Drying of tips	Gum exudation	Internal browning
<i>L. crassispora</i> USSLC	2	2	3
<i>B. theobromae</i> USSBT	3	3	1
<i>C. lacerate</i> DSSCL	0	0	0
<i>B. nivea</i> DSSBN	1	0	0

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

Table 9. Severity of symptoms on *A. lebbeck* plants inoculated with *L. crassispora* USSLC, *B. theobromae* USSBT, *C. lacerate* DSSCL and *B. nivea* DSSBN.

Pathogens	Symptoms produced on <i>Samanea saman</i> plant		
	Drying of tips	Gum exudation	Internal browning
<i>L. crassispora</i> USSLC	0	3	3
<i>B. theobromae</i> USSBT	0	0	0
<i>C. lacerate</i> DSSCL	0	0	0
<i>B. nivea</i> DSSBN	0	0	0

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

Table 10. Severity of symptoms on *A. procer* plants inoculated with *L. crassispora* USSLC, *B. theobromae* USSBT, *C. lacerate* DSSCL and *B. nivea* DSSBN.

Pathogens	Symptoms produced on <i>Samanea saman</i> plant		
	Drying of tips	Gum exudation	Internal browning
<i>L. crassispora</i> USSLC	0	3	3
<i>B. theobromae</i> USSBT	0	0	0
<i>C. lacerate</i> DSSCL	0	0	0
<i>B. nivea</i> DSSBN	0	0	0

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

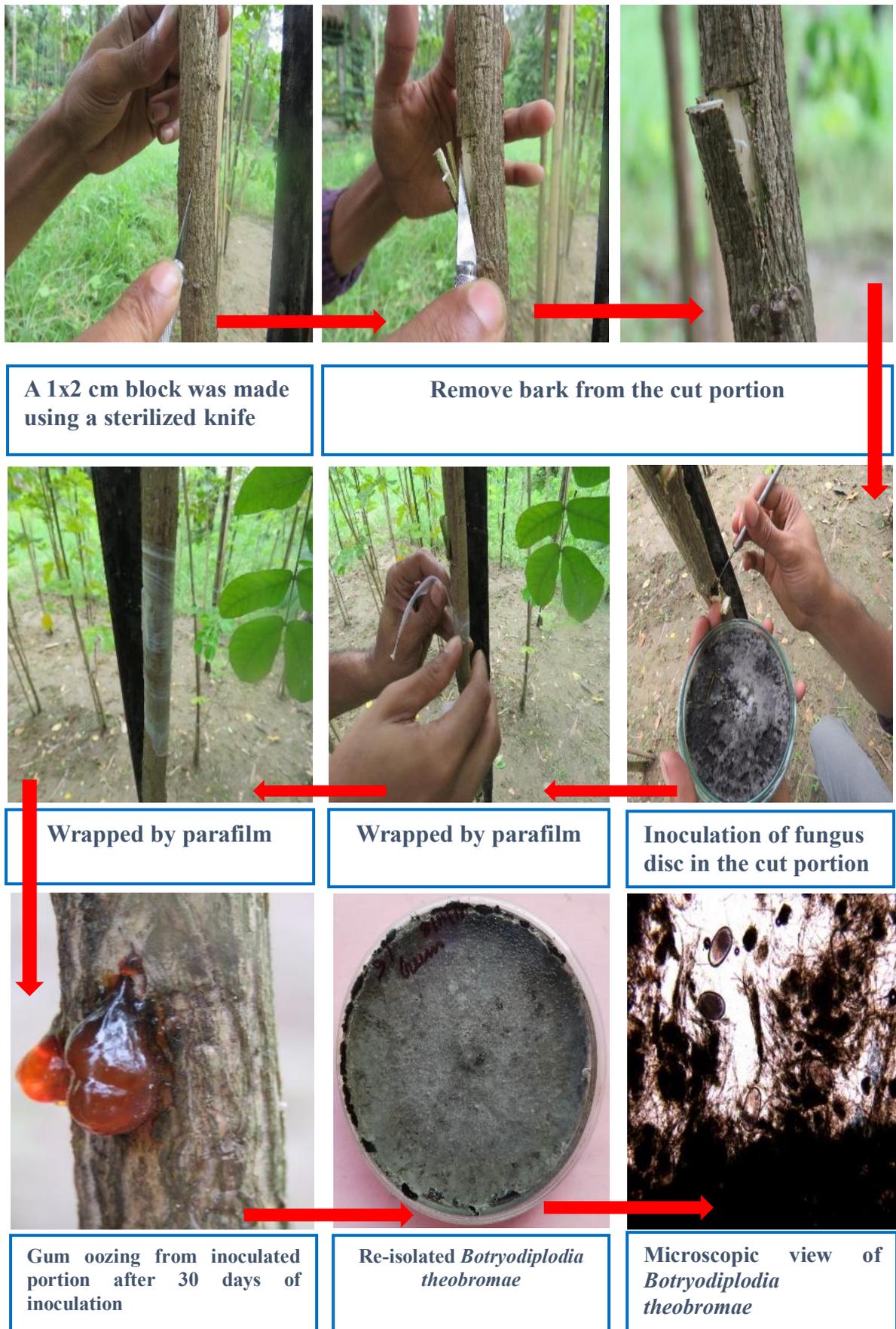


Figure 16: Pathogenicity test of *B. theobromae*USSBT inoculated in *S saman*

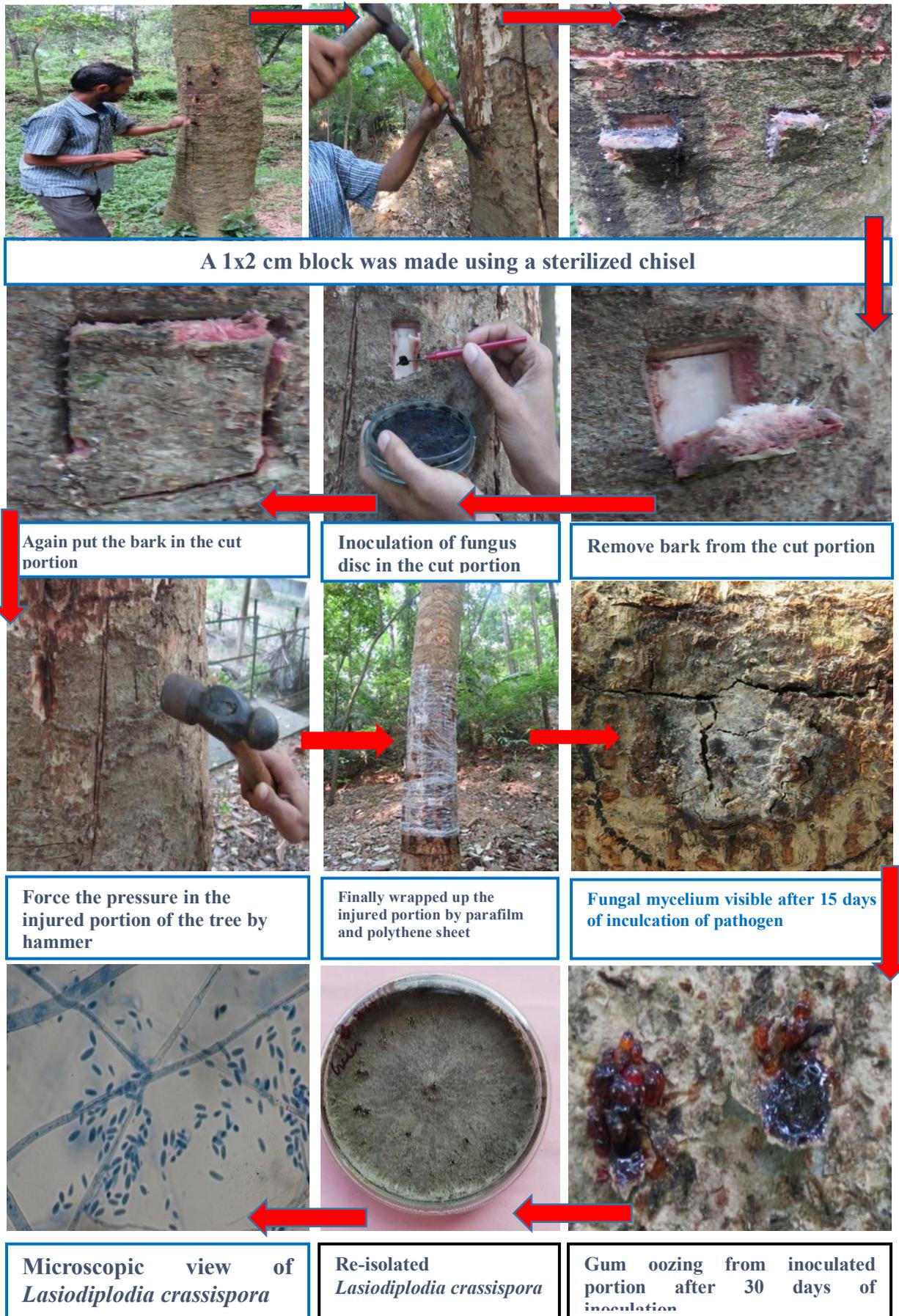


Figure 17: Pathogenicity test of *L. crassispora* USSLC inoculated in *A. procera*



Figure 18: Pathogenicity test of *L. crassispora* USSLC inoculated in *A. leebeck*

11.5. Influence of different nutrition, physical and environmental parameters on growth and conidial germination of *L. crassispota* USSLC and *B. theobromae* USSBT:

Environmental and nutritional factors affecting CG and MG of *L. crassispota* USSLC and *B. theobromae* USSBT were observed. The highest CG (Conidial germination) and MG (Mycelial Growth) of *L. crassispota* USSLC and *B. theobromae* USSBT was observed at 6-8 pH, 90-95 RH and 25-30 °C concentration of 2-3% glucose and sucrose were the best for CG and MG of *L. crassispota* USSLC and *B. theobromae* USSBT and sucrose was better than glucose. Among 7 solid media, the highest MG was found at PDA medium (Table 11, 12, 13 & 14).

Table 11: Effect of different environmental and nutritional factors on conidial germination of *L. crassispota* USSLC

Percentages of conidial germination after 24 h								
pH	CG	RH (%)	CG	Temperature (°C)	CG	Glucose/Sucrose (%)	CGG	CGS
4	45 f	70	20 g	05	45 f	0.5	20 f	48 f
5	52 d	75	45 f	10	53 e	1.0	25 e	52 e
6	74 b	80	69 d	15	69 d	1.5	47 c	57 c
7	85 a	85	73 c	20	73 c	2.0	52 b	61 b
8	69 c	90	75 b	25	85 a	2.5	56 a	63 a
9	48 e	95	85 a	30	82 b	3.0	51 b	55 d
10	25 g	100	58 e	35	45 f	3.5	45 d	51 e

RH: Relative Humidity, CG: Conidial Growth, CGG: Conidial Growth in Glucose, CGS: Conidial Growth in Sucrose. All treatments were observed (except temperature effect) at 25°C.

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

Table 12: Effect of different environmental and nutritional factors on mycelial growth of *L. crassispota* USSLC

Mycelial growth (mm) after 3 days										
pH	MG	RH (%)	MG	Temperature (°C)	MG	Media	MG	Glucose/Sucrose (%)	MGG	MGS
4	68 e	70	49 f	05	00 g	Richards	65 c	0.5	58 f	62 g
5	72 d	75	52 g	10	25 f	MEA	69 b	1.0	65 e	69 f
6	85 b	80	58 e	15	35 e	Oatmeal	63 d	1.5	78 d	74 e
7	89 a	85	69 c	20	69 d	PDA	72 a	2.0	85 c	89 d
8	78 c	90	72 b	25	82 a	Sabourauds	62 d	2.5	92 a	96 a
9	65 f	95	79 a	30	79 b	Czapeks	58 e	3.0	88 b	94 b
10	58 g	100	63 d	35	72 c	YEA	59 f	3.5	85 c	92 c

YEA: Yeast extract Agar, **PDA:** Potato Dextrose Agar, **MEA:** malt extract agar; **RH:** Relative Humidity, **MG:** Mycelial Growth, **MGG:** Mycelial Growth in Glucose, **MGS:** Mycelial Growth in Sucrose. All treatments were observed (except temperature effect) at 25°C.

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

Table 13: Effect of different environmental and nutritional factors on conidial germination of *B. theobromae* USSBT

Percentages of conidial germination after 24 h								
pH	CG	RH (%)	CG	Temperature (°C)	CG	Glucose/Sucrose (%)	CGG	CGS
4	73 c	70	25 f	05	48 e	0.5	26 e	51 d
5	57 e	75	44 e	10	58 d	1.0	29 d	54 c
6	84 b	80	68 c	15	72 c	1.5	49 c	59 b
7	87 a	85	75 b	20	74 c	2.0	54 b	64 a
8	71 d	90	76 b	25	88 a	2.5	58 a	65 a
9	52 f	95	87 a	30	85 b	3.0	55 b	58 b
10	28 g	100	59 d	35	48 e	3.5	48 c	53 c

RH: Relative Humidity, **CG:** Conidial Growth, **CGG:** Conidial Growth in Glucose, **CGS:** Conidial Growth in Sucrose. All treatments were observed (except temperature effect) at 25°C.

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

Table 14: Effect of different environmental and nutritional factors on mycelial growth of *B. theobromae* USSBT

Mycelial growth (mm) after 3 days										
pH	MG	RH (%)	MG	Temp eratur e (°C)	MG	Media	MG	Glucos e/Sucro se (%)	MGG	MG S
4	72 e	70	52 g	05	00 g	Richards	66 d	0.5	61 g	64 g
5	74 d	75	54 f	10	28 f	MEA	72 b	1.0	68 f	71 f
6	88 b	80	59 e	15	38 e	Oatmeal	64 e	1.5	78 e	74 e
7	92 a	85	72 b	20	72 d	PDA	76 a	2.0	85 d	89 d
8	82 c	90	69 c	25	87 a	Sabourauds	68 c	2.5	95 a	98 a
9	68 f	95	82 a	30	82 b	Czapeks	61 f	3.0	92 b	96 b
10	61 g	100	66 d	35	74 c	YEA	59 g	3.5	88 c	94 c

YEA: Yeast extract Agar, **PDA:** Potato Dextrose Agar, **MEA:** malt extract agar; **RH:** Relative Humidity, **MG:** Mycelial Growth, **MGG:** Mycelial Growth in Glucose, **MGS:** Mycelial Growth in Sucrose. All treatments were observed (except temperature effect) at 25°C.

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

11.6. *In vitro* evaluation of different chemical fungicide, bio agents and microorganism against the pathogen

11.6.1. In-vitro evaluation of fungicides against mycelial growth and conidial germination inhibition of *L. crassispora* USSLC and *B. theobromae* USSBT: Fourteen fungicides (Indofil, Sunvit, Diathene M45, Oxyvit, Rovral, Aimcozim, Thiovit, Ridomil, Amivit, Cupravit, Champion, Knowing, Arba and Autostin) were tested at 50, 100 and 150 mg/L

concentration in *in vitro* for their effects on mycelial growth and spore germination inhibition of *L. crassispota*USSLC and *B. theobromae* USSBT. The percent inhibition of mycelial growth and spore germination inhibition by different fungicides varied significantly ($p \leq 0.05$) different at different concentrations. The highest percent inhibition of mycelial growth and conidial germination inhibition (100 %) were observed with Knowing, ARBA and Autostin at 50, 100 and 150 mg/L concentration for *L. crassispota* USSLC and *B. theobromae*USSBT. For both pathogen mycelial growth and spore germination was fully inhibited by Autostin, ARBA and Knowing (Table 15, 16, 17 & 18; Figure 19 & 20).

11.6.2. In-vitro evaluation of *Trichoderma* strains against mycelial growth and conidial germination inhibition of *L. crassispota* USSLC and *B. theobromae* USSBT

11.5.2.1. Screening by dual culture technique: Each *Trichoderma* strains inhibited the radial mycelial growth of *L. crassispota* USSLC and *B. theobromae* USSBT. The percentage inhibition of radial growth (PIRG) values ranged from 58.79 to 79.84% for *L. crassispota* USSLC and 61.63 to 80.78% for *B. theobromae*USSBT (Table 19 & 22; Figure 21, 22 and 23). The highest PIRG values (79.84 % and 80.78 %) were observed with *T. harzianum* IMI-392433 for *L. crassispota* USSLC and *B. theobromae* USSBT, respectively. The lowest percent inhibition of radial growth were observed with *T. pseudokoningii* IMI-392431 for *B. theobromae*USSBT (58.79 %) and *L. crassispota*USSLC (61.63 %), respectively.

11.5.2.2. Screening by poison agar technique: The PIRG values by metabolites of *Trichoderma* strains varied significantly different ($p \leq 0.05$) at different concentrations. With the normal poison agar method, the highest PIRG values were achieved at 75% concentration metabolites of *T. harzianum* IMI-392433 for *L. crassispota* USSLC (81.57%) and *B. theobromae*USSBT (83.28 %)(Table 20 & 23).

11.5.2.3. Inhibition of conidial germination: The percent inhibition of conidial germination values by metabolites of *Trichoderma* strains varied significantly different ($p \leq 0.05$) at different concentrations. The highest percent inhibition of conidial germination values (88.27 and 89.46%) were achieved at 75% concentration metabolites of *T. harzianum* IMI-392433 for *L. crassispota*USSLC and *B. theobromae*USSBT(Table 21 & 24). The lowest percent inhibition of conidial germination were observed with *T. pseudokoningii* IMI-392431 for *L. crassispota*USSLC (75.47 %) and *B. theobromae*USSBT (74.29 %), respectively.

Table 15: Effect of different fungicides on mycelia growth inhibition of *L. crassispora* USSLC

Fungicides name	Concentrations (mg/L)		
	50	100	150
Indofil	47.28 h	58.24 i	88.54 d
Knowing	100 a	100 a	100 a
Ridomil gold	37.14 I	60.73 h	88.98 d
Oxyvit 50 WP	63.81 g	71.24 g	81.48 e
Cupravit 50 WP	68.57 e	78.42 e	88.26 d
Aimcozim	71.85 d	89.87 b	89.31 d
Champion	68.57 e	80.32 d	79.67 f
Sunvit	63.81 g	75.37 f	82.83 e
Diathane M 45	79.24 b	86.34 c	88.67 d
Thiovit 80 WG	65.72 f	75.76 f	89.31 d
ARBA	100 a	100 a	100 a
Autostin	100 a	100 a	100 a
Rovral	78.71 b	88.71 b	98.48 b
Amivit	75.43 c	85.43 c	96.57 b

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

Table 16: Effect of different fungicides on conidial germination inhibition % of *L. crassispora* USSLC

Fungicides name	Concentrations (mg/L)		
	50	100	150
Indofil	60.28 h	68.24 g	78.54 e
Knowing	100 a	100 a	100 a
Ridomil gold	57.14 i	68.73 g	78.98 e
Oxyvit 50 WP	73.81 g	81.24 f	91.48 d
Cupravit 50 WP	78.57 e	88.42 d	98.26 b
Aimcozim	81.85 d	92.87 c	98.31 b
Champion	78.57 e	88.32 d	95.67 c
Sunvit	73.81 g	85.37 e	94.83 c
Diathane M 45	89.24 b	96.34 b	98.67 ab
Thiovit 80 WG	75.72 f	85.76 e	95.31 c
ARBA	100 a	100 a	100 a
Autostin	100 a	100 a	100 a
Rovral	88.71 b	96.71 b	97.75 ab
Amivit	85.43 c	95.43 b	96.57 ab

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

Table 17: Effect of different fungicides on mycelia growth of *B. theobromae* USSBT

Fungicides name	Concentrations (mg/L)		
	50	100	150
Indofil	45.17 h	56.83 i	86.28 d
Knowing	100 a	100 a	100 a
Ridomil gold	34.29 I	58.63 h	89.16 c
Oxyvit 50 WP	62.75 g	69.16 g	84.27 d
Cupravit 50 WP	64.25 e	76.17 e	86.58 d
Aimcozim	69.45 d	87.62 b	90.93 c
Champion	67.24 e	78.58 d	83.52 f
Sunvit	61.74 g	73.28 f	79.95 e
Diathane M 45	77.18 b	84.28 b	86.85 c
Thiovit 80 WG	63.69 f	73.17 f	93.53 b
ARBA	100 a	100 a	100 a
Autostin	100 a	100 a	100 a
Rovral	75.35 b	84.28 b	97.52 b
Amivit	72.18 c	81.17 c	94.21 b

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

Table 18: Effect of different fungicides on conidial germination inhibition % of *B. theobromae* USSBT

Fungicides name	Concentrations (mg/L)		
	50	100	150
Indofil	62.18 g	66.16 g	75.37 e
Knowing	100 a	100 a	100 a
Ridomil gold	59.39 f	65.62 g	76.73 e
Oxyvit 50 WP	75.87 e	79.43 f	87.52 d
Cupravit 50 WP	79.62 d	86.32 d	96.15 ab
Aimcozim	85.74 c	90.62 c	97.26 ab
Champion	80.83 e	86.26 d	93.58 c
Sunvit	75.95 g	83.63 e	92.73 c
Diathane M 45	91.17 b	94.12 b	96.18 ab
Thiovit 80 WG	76.84 f	83.62 e	93.26 c
ARBA	100 a	100 a	100 a
Autostin	100 a	100 a	100 a
Rovral	90.72 b	94.59 b	95.14 ab
Amivit	83.32 c	91.12 b	94.32 ab

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

Table 19: Efficacy of *Trichoderma* strains on mycelia growth inhibition of *L. crassispora* USSLC by dual culture technique.

Strains	Percentages inhibition of radial growth (PIRG)
<i>T. viride</i>	76.25 b
<i>T. virens</i> IMI-392430	65.87 d
<i>T. pseudokoningii</i> IMI-392431	58.79 e
<i>T. harzianum</i> IMI-392432	75.98 b
<i>T. harzianum</i> IMI-392433	79.84 a
<i>T. harzianum</i> IMI- 392434	73.57 c

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

Table 20: Efficacy of secondary metabolites of *Trichoderma* strains on the percentage inhibition of mycelia growth (PIMG) of *L. crassispota* USSLC after 10 days of incubation.

Strains	Percent inhibition of mycelia growth (PIMG) ¹		
	Secondary metabolites of <i>Trichoderma</i> (%) ²		
	25	50	75
<i>T. virens</i> IMI-392430	50.28 c	60.38 b	62.83 c
<i>T. pseudokoningii</i> IMI-392431	48.37 d	57.34 c	59.86 d
<i>T. harzianum</i> IMI-392432	62.87 b	69.86 a	78.93 b
<i>T. harzianum</i> IMI-392433	66.58 a	71.58 a	81.57 a
<i>T. harzianum</i> IMI- 392434	63.52 b	70.39 a	80.34 ab
<i>T. viride</i>	62.37 b	69.74 a	78.62 ab

¹ Mean in a column same letters are not significantly different by DMRT at 5% level

² PIMG values of *L. crassispota* USSLC by secondary metabolites of *Trichoderma* strains on food poisoning agar technique calculated from four replications.

Table 21: Efficacy of secondary metabolites of *Trichoderma* strains on the percentage inhibition of conidial germination (PICG) of *L. crassispota* USSLC after 12 hours of incubation

Strains	Percent inhibition of Conidial Germination (PICG) ¹		
	Secondary metabolites of <i>Trichoderma</i> (%) ²		
	25	50	75
<i>T. virens</i> IMI-392430	52.87 c	70.28 c	81.21 b
<i>T. pseudokoningii</i> IMI-392431	49.67 d	65.48 d	75.47 c
<i>T. harzianum</i> IMI-392432	62.57 b	75.48 b	87.28 a
<i>T. harzianum</i> IMI-392433	68.73 a	78.91 a	88.27 a
<i>T. harzianum</i> IMI- 392434	62.87 b	74.27 b	86.47 a
<i>T. viride</i>	61.96 b	71.94 b	84.73 a

¹ Means in a column same letters are not significantly different by DMRT at 5% level

² PICG values of *L. crassispota* USSLC by secondary metabolites of *Trichoderma* strains calculated from four replications.

Table 22: Efficacy of *Trichoderma* strains on mycelia growth inhibition of *B. theobromae* USSBT by dual culture technique

Strains	Percentages inhibition of radial growth (PIRG) ¹
<i>T. viride</i>	77.49 b

<i>T. virens</i> (Miller) IMI-392430	66.98 d
<i>T. pseudokoningii</i> IMI-392431	61.63 e
<i>T. harzianum</i> (Rifai) IMI-392432	78.98 b
<i>T. harzianum</i> (Rifai) IMI-392433	80.78 a
<i>T. harzianum</i> (Rifai) IMI- 392434	71.68 c

¹ In a column same letters are not significantly different by DMRT at 5% level

¹PIRG values of *B. theobromae*USSBT by *Trichoderma* strains on PDA by dual culture technique calculated from four replications.

Table 23: Efficacy of secondary metabolites of *Trichoderma* strains on the percentage inhibition of mycelia growth (PIMG) of *B. theobromae* USSBT after 10 days of incubation

Strains	Percent inhibition of mycelia growth (PIMG) ¹		
	Secondary metabolites of <i>Trichoderma</i> (%) ²		
	25	50	75
<i>T. virens</i> IMI-392430	50.28 c	60.38 b	62.83 c
<i>T. pseudokoningii</i> IMI-392431	48.37 d	57.34 c	59.86 d
<i>T. harzianum</i> IMI-392432	62.87 b	69.86 a	78.93 b
<i>T. harzianum</i> IMI-392433	66.58 a	71.58 a	83.28 a
<i>T. harzianum</i> IMI- 392434	63.52 b	70.39 a	80.34 ab
<i>T. viride</i>	62.96 b	68.53 b	78.54 ab

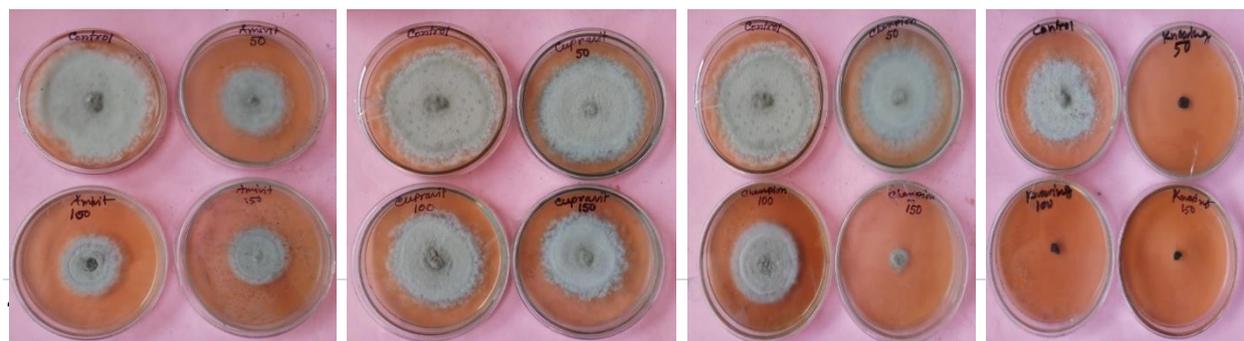
In a column same letters are not significantly different by DMRT at 5% level

² PIMG values of *B. theobromae* USSBTby secondary metabolites of *Trichoderma* strains on food poisoning agar technique calculated from four replications.

Table 24: Efficacy of secondary metabolites of *Trichoderma* strains on the percentage inhibition of conidial germination (PICG) of *B. theobromae* USSBT after 12 hours of incubation

Strains	Percent inhibition of Conidial Germination (PICG) ¹		
	Secondary metabolites of <i>Trichoderma</i> (%) ²		
	25	50	75
<i>T. virens</i> (Miller) IMI-392430	54.75 c	69.89 c	80.41 b
<i>T. pseudokoningii</i> IMI-392431	50.42 d	63.27 d	74.29 c
<i>T. harzianum</i> (Rifai) IMI-392432	64.74 b	74.28 b	85.38 a
<i>T. harzianum</i> (Rifai) IMI-392433	70.28 a	79.85 a	89.46 a
<i>T. harzianum</i> (Rifai) IMI- 392434	61.26 b	76.38 b	87.25 a
<i>T. viride</i>	60.83 b	74.42 b	84.93 a

¹In a column same letters are not significantly different by DMRT at 5% level; ² PICG values of *B. theobromae* USSBTby secondary metabolites of *Trichoderma* strains calculated from four replications.



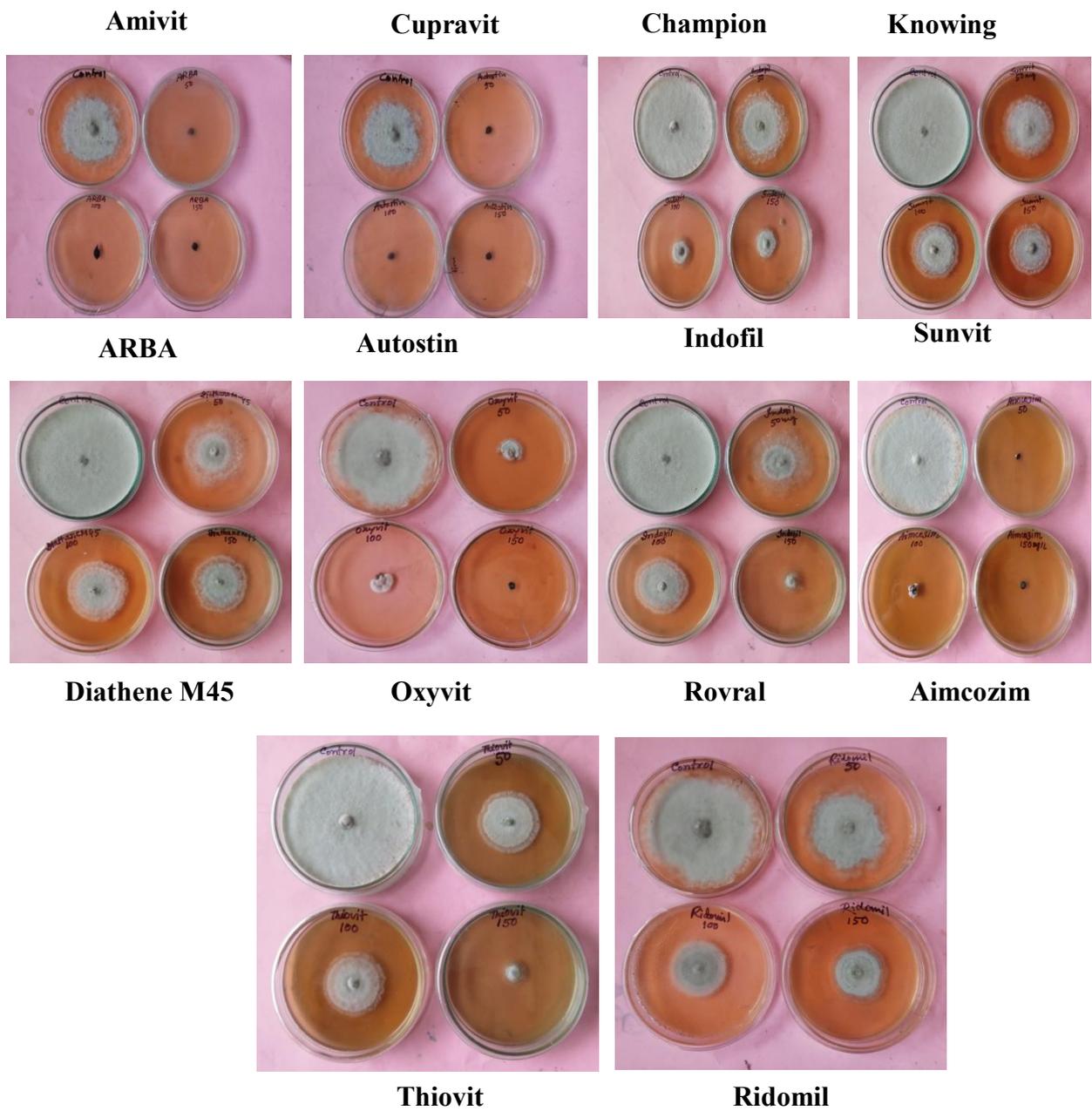
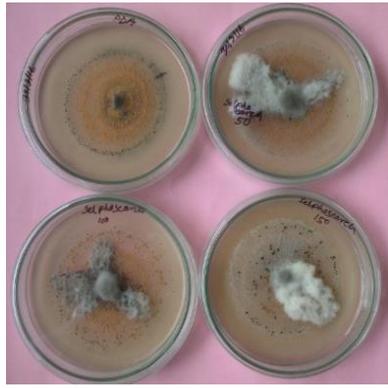
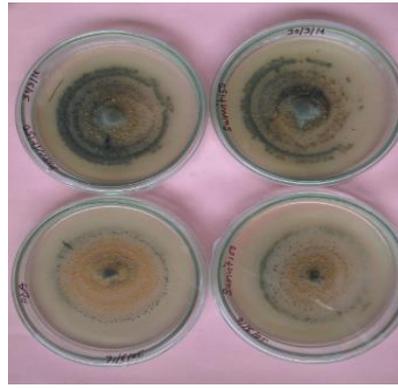


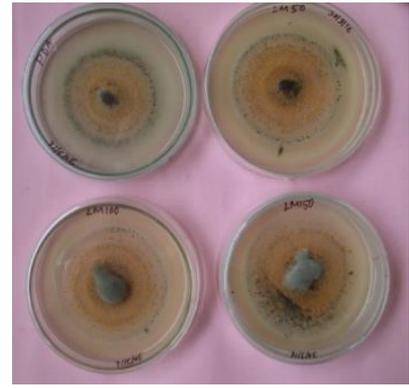
Figure 19: Effect of fungicides on mycelial growth of *L. crassispota* USSLC



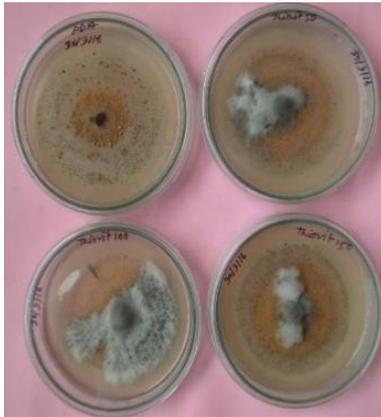
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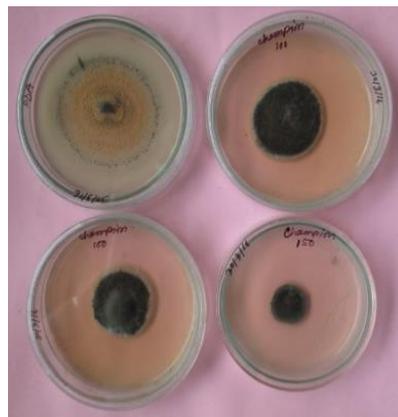
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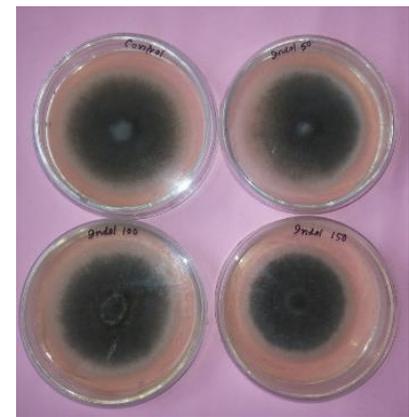
Champion



Thiovit



ARBA



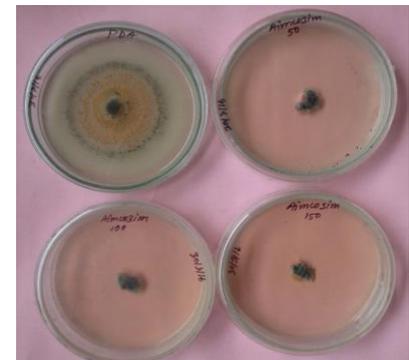
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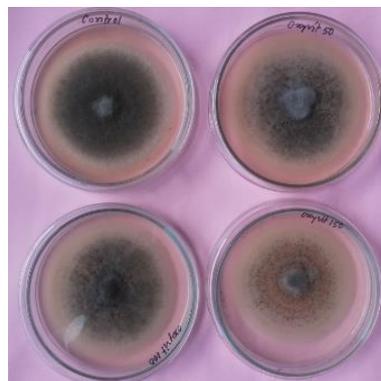
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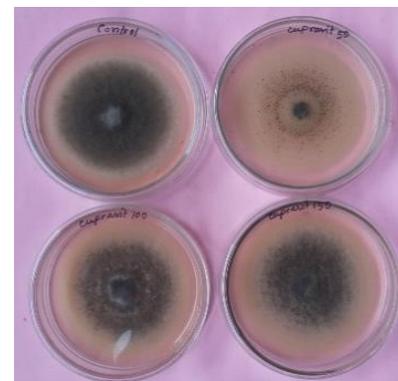
Rovral



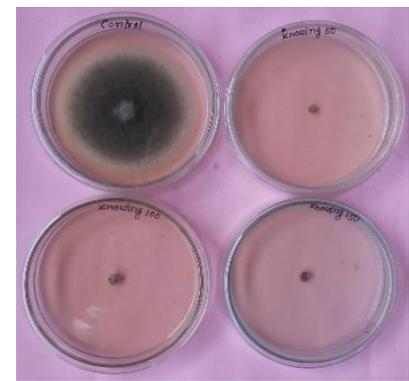
Aimcozim



Ridomil



Cupravit



Knowing

Figure 20: Effect of fungicides on mycelial growth of *B. theobromae* USSBT



Fig 21: Antagonistic effects of *Trichoderma* strains against *L. crassispora* USSLC in dual culture technique after 7 days. L, T₁, T₂, T₃, T₄, T₅ and T₆ indicates *Lasiodiplodia crassispora*, *T. viride*, *T. virens* IMI-392430, *T. pseudokoningii* IMI-392431, *T. harzianum* IMI-392432, *T. harzianum* IMI-392433, and *T. harzianum* IMI-392434, respectively.

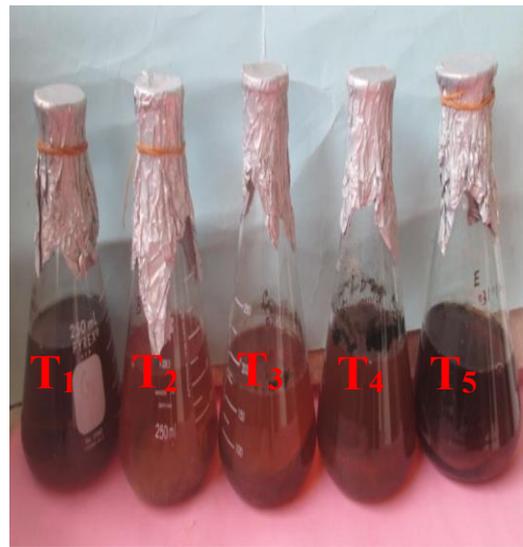


Fig 22: Secondary metabolites of *Trichoderma* strains. T₁, T₂, T₃, T₄, and T₅ indicates *T. virens* IMI-392430, *T. pseudokoningii* IMI-392431, *T. harzianum* IMI-392432 and *T. harzianum* IMI-392433.

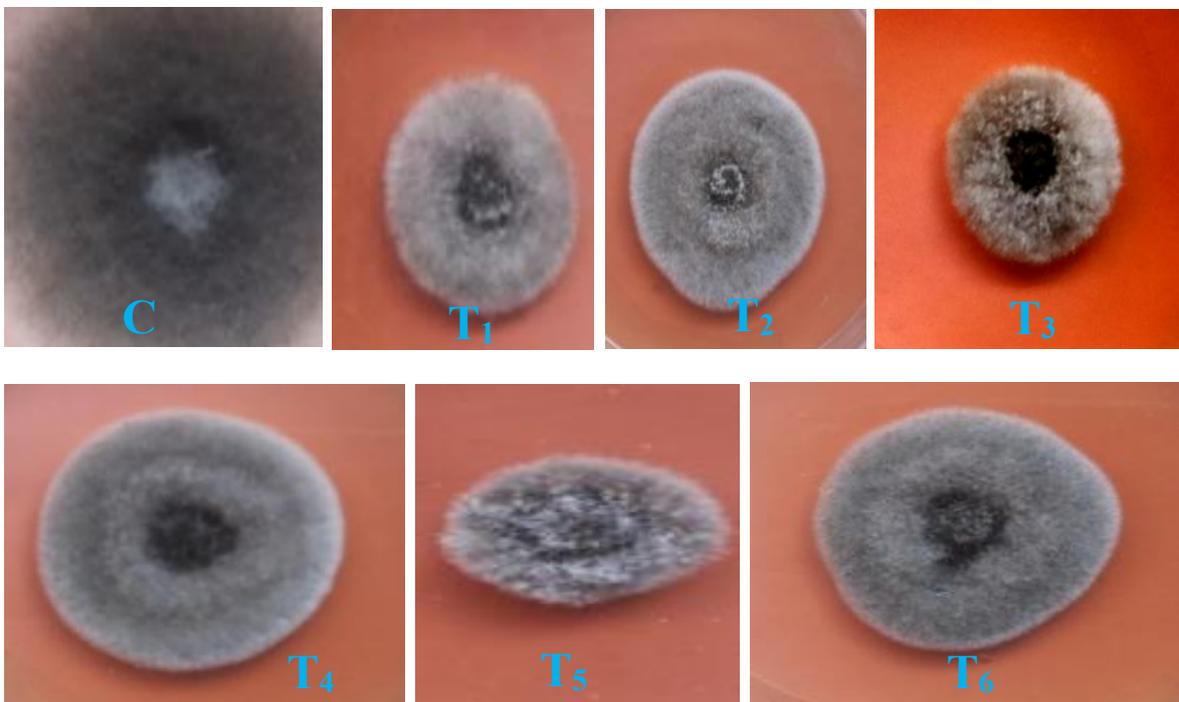


Figure 23: Effects of secondary metabolites of *Trichoderma* strains in PDA on mycelial growth inhibition of *B. theobromae* USSBT by normal poison agar technique at 80 % concentration after 10 days of incubation. C = control, T₁ = *T. virens* IMI-392430, T₂ = *T. pseudokoningii* IMI-392431, T₃ = *T. viride*; T₄ = *T. harzianum* IMI-392432, T₅ = *T. harzianum* IMI-392433, T₆ = *T. harzianum* IMI-392434.

11.7. Efficacy of chemical fungicides for control gummosis of *S. saman* under field condition: Results presented in Table 24 indicates that fungicide treatment significantly reduced percent development of the lesion of gummosis with the untreated control. The lowest percent development of the lesion was observed when 2% Knowing, Arba, Autostin and Bordeaux mixture were sprayed. The present study indicates that Knowing, Arba, Autostin and Bordeaux mixture (2%) was most effective to control gummosis disease of *Samanea saman* under field condition.

Table 25. 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
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

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

T₀=Control (Without fungicide), T₁=Indofil, T₂= Sunvit, T₃=Diathene M45, T₄=Oxyvit, T₅=Rovral, T₆=Aimcozim, T₇=Thiovit, T₈=Ridomil, T₉=Amivit, T₁₀=Cupravit, T₁₁=Champion, T₁₂=Knowing, T₁₃=Arba, T₁₄=Autostin, T₁₅= Bordeaux mixture

11.8. Efficacy of *Trichoderma* strains for control gummosis of *S. saman* under field condition: The response of these selected *Trichoderma* strains to control gummosis disease under field condition clearly indicated that *Trichoderma* to be effective against gummosis disease. Results presented in Table 26 indicate that *Trichoderma* significantly reduced percent development of the lesion of gummosis with the untreated control. The lowest percent development of the lesion was observed when spore suspension of *T. harzianum* IMI-3924332 (T₁₁) was sprayed. The present study indicates that *T. harzianum* IMI-3924333 (T₁₁) was most effective to control gummosis disease of Rain tree (*Samanea saman*) under field condition.

Table 26. Influence of the *Trichoderma* strains on the development of the lesion size on the stem of Rain tree (*Samanea saman*) at @ 5 ml (5 × 10⁵ conidia/ml)

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	6.13	8.32	8.76	8.98	9.87	61.01 a
T ₁	6.9	7.83	8.98	9.34	9.48	37.39d
T ₂	6.2	6.85	7.94	8.43	8.87	43.06c
T ₃	6.4	6.87	7.65	8.64	8.79	37.34d
T ₄	6.8	8.34	8.98	9.43	9.86	45b
T ₅	7.1	7.9	8.32	8.97	8.98	26.47 g
T ₆	6.8	7.28	7.98	8.43	8.58	26.17g
T ₇	7.1	7.96	8.43	9.32	9.64	35.78d
T ₈	6.8	7.64	7.98	8.75	8.83	29.85f
T ₉	6.8	7.19	7.89	8.42	8.59	26.33g
T ₁₀	7.1	7.86	8.12	8.64	8.75	23.24h
T ₁₁	7.6	7.43	8.32	8.67	8.98	18.15i
T ₁₂	7.8	8.73	9.89	9.92	9.98	27.94g
T ₁₃	7.4	7.98	8.25	9.75	9.83	32.83e
SD	0.76	0.81	0.91	0.97	1.03	13.61
CV (%)	10.89	10.61	11.01	11.08	11.56	44.92

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

T₀ = Control (*B. theobromae*), T₂ = *T. virens* IMI-392430 + *B. theobromae*, T₃ = *T. pseudokoningii* IMI-392431 + *B. theobromae*, T₄ = *T. harzianum* IMI-392432 + *B. theobromae*, T₅ = *T. harzianum* IMI-392433 + *B. theobromae*, T₆ = *T. harzianum* IMI-392434 + *B. theobromae*, T₇ = *T. viride* + *B. theobromae*, T₈ = *T. virens* IMI-392430, T₉ = *T. pseudokoningii* IMI-392431', T₁₀ = *T. harzianum* IMI-392432, T₁₁ = *T. harzianum* IMI-392433, T₁₂ = *T. harzianum* IMI-392434, T₁₃ = *T. viride*.

11.9. Efficacy of chemical fungicides for control gummosis of *A. procera* under field condition: Fungicide treatment significantly reduced percent development of the lesion of gummosis of *A. proceraw* with the untreated control (Table 27). The lowest percent development of the lesion was observed when 2% Knowing, Arba, Autostin and Bordeaux mixture were sprayed. The present study indicates that Knowing, Arba, Autostin and Bordeaux mixture 2% was most effective to control gummosis disease of *A. procera* under field condition.

Table 27. Influence of the fungicidal treatment on the development of the lesion size on the stem of *A. procera* 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	6.84	7.54	7.86	7.93	8.53	91.47 a
T ₁	7.96	8.43	8.64	8.95	9.87	23.99 f
T ₂	8.76	8.98	9.21	9.53	10.64	89.36 b
T ₃	9.38	9.78	9.87	9.96	10.42	11.09 i
T ₄	8.96	9.43	9.59	9.64	9.89	10.37 i
T ₅	7.36	7.89	7.93	8.32	9.23	25.40 e
T ₆	8.74	9.76	9.89	9.95	10.38	18.76 g
T ₇	7.96	8.63	8.75	8.96	9.75	22.48 f
T ₈	6.84	7.86	8.32	8.75	9.64	40.94 c
T ₉	8.63	8.97	9.25	9.38	9.87	14.36 h
T ₁₀	7.59	7.95	8.12	8.59	9.78	28.85 d
T ₁₁	7.94	8.32	8.76	8.97	9.13	14.98 h
T ₁₂	8.95	9.21	9.54	9.31	9.75	8.93 j
T ₁₃	8.64	8.87	8.98	9.26	9.32	7.87 k
T ₁₄	9.53	9.75	9.87	9.95	9.97	4.42 l
T ₁₅	8.64	8.89	8.96	9.23	9.41	8.91 j
SD	0.83	0.73	0.69	0.59	0.52	26.78
CV (%)	9.98	8.24	7.59	6.41	5.43	101.23

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

T₀=Control (Without fungicide), T₁=Indofil, T₂= Sunvit, T₃=Diathene M45, T₄=Oxyvit, T₅=Rovral, T₆=Aimcozim, T₇=Thiovit, T₈=Ridomil, T₉=Amivit, T₁₀=Cupravit, T₁₁=Champion, T₁₂=Knowing, T₁₃=Arba, T₁₄=Autostin, T₁₅= Bordeaux mixture.

11.10. Efficacy of *Trichoderma* strains for control gummosis of *A. procera* under field condition:

The response of these selected *Trichoderma* strains to control gummosis disease under field condition clearly indicated that *Trichoderma* to be effective against gummosis disease. Results presented in Table 28 indicate that *Trichoderma* significantly reduced percent development of the lesion of gummosis with the untreated control. The lowest percent development of the lesion was observed when spore suspension of *T. harzianum* IMI-3924332 (T₁₁) was sprayed. The present study indicates that *T. harzianum* IMI-3924333 (T₁₁) was most effective to control gummosis disease of *A. procera* under field condition.

Table 28. Influence of the *Trichoderma* strains on the development of the lesion size on the stem of Rain tree *Albizia procera* @ 5 ml (5 × 10⁵ conidia/ml).

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	6.18	8.32	8.76	8.97	10.87	75.88 a
T ₁	6.12	7.65	8.65	8.86	8.98	46.73 b
T ₂	6.86	8.13	8.79	8.96	10.12	47.52 b
T ₃	6.64	8.28	8.96	9.32	9.76	46.98 b
T ₄	6.76	7.43	8.75	9.34	9.98	47.64 b
T ₅	6.98	7.95	8.65	9.86	9.89	41.69 d
T ₆	6.43	7.19	7.76	8.12	9.23	43.54 c
T ₇	6.97	7.15	8.54	9.43	10.21	46.48 b
T ₈	7.49	8.24	8.75	8.95	9.97	33.11 e
T ₉	7.42	7.87	8.53	8.95	9.75	31.40 f
T ₁₀	7.37	7.65	7.97	8.12	8.53	15.73 i
T ₁₁	6.98	7.48	7.68	7.76	8.12	16.34 i
T ₁₂	7.12	7.34	7.54	7.75	8.43	18.39 h
T ₁₃	7.15	8.24	8.16	8.29	8.69	21.53 g
SD	0.42	0.43	0.48	0.65	0.81	16.59
CV (%)	6.24	5.42	5.64	7.45	8.53	43.59

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

T₀ = Control (*B. theobromae*), T₂ = *T. virens* IMI-392430 + *B. theobromae*, T₃ = *T. pseudokoningii* IMI-392431 + *B. theobromae*, T₄ = *T. harzianum* IMI-392432 + *B. theobromae*, T₅ = *T. harzianum* IMI-392433 + *B. theobromae*, T₆ = *T. harzianum* IMI-392434 + *B. theobromae*, T₇ = *T. viride* + *B. theobromae*, T₈ = *T. virens* IMI-392430, T₉ = *T. pseudokoningii* IMI-392431, T₁₀ = *T. harzianum* IMI-392432, T₁₁ = *T. harzianum* IMI-392433, T₁₂ = *T. harzianum* IMI-392434, T₁₃ = *T. viride*.

11.11. Efficacy of chemical fungicides for control gummosis of *A. lebek* under field condition: Fungicide treatment significantly reduced percent development of the lesion of gummosis of *A. lebek* with the untreated control (Table 29). The lowest percent development of the lesion was observed when 2% Knowing, Arba, Autostin and Bordeaux mixture were sprayed. The present study indicates that Knowing, Arba, Autostin and Bordeaux mixture 2% was most effective to control gummosis disease of *A. lebek* under field condition.

Table 29. Influence of the fungicidal treatment on the development of the lesion size on the stem of *A. lebek* 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	6.26	6.38	7.95	8.25	9.48	51.43 a
T ₁	6.85	7.12	7.64	7.89	7.97	16.35 g
T ₂	7.12	7.47	7.58	7.69	8.35	17.27 g
T ₃	6.94	7.54	7.86	7.98	8.36	20.46 f
T ₄	6.31	7.53	7.98	8.47	8.98	42.31 b
T ₅	6.75	7.17	7.69	7.96	9.12	35.11 d
T ₆	7.12	7.86	7.98	8.75	9.87	38.62 c
T ₇	6.83	7.54	7.94	8.15	9.52	39.38 c
T ₈	7.36	7.86	7.89	8.75	9.85	33.83 d
T ₉	6.74	6.86	6.98	7.64	8.79	30.42 e
T ₁₀	6.85	6.97	7.68	8.79	9.85	43.79 b
T ₁₁	7.18	7.86	7.98	8.75	9.42	31.19 e
T ₁₂	6.76	6.97	7.35	7.48	7.52	11.24h
T ₁₃	6.95	7.12	7.43	7.56	7.64	9.92 h
T ₁₄	7.42	7.53	7.64	7.69	7.72	4.04 i
T ₁₅	7.28	7.37	7.43	7.49	7.54	3.57 i
SD	0.33	0.41	0.28	0.48	0.88	15.07
CV (%)	4.78	5.62	3.72	6.04	10.07	56.24

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

T₀=Control (Without fungicide), T₁=Indofil, T₂= Sunvit, T₃=Diathene M45, T₄=Oxyvit, T₅=Rovral, T₆=Aimcozim, T₇=Thiovit, T₈=Ridomil, T₉=Amivit, T₁₀=Cupravit, T₁₁=Champion, T₁₂=Knowing, T₁₃=Arba, T₁₄=Autostin, T₁₅= Bordeaux mixture.

11.12. Efficacy of *Trichoderma* strains for control gummosis of *A. lebekunder* field condition: Results presented in Table 30 indicate that *Trichoderma* significantly reduced percent development of the lesion of gummosis comparewith the control(T₀).The lowest percent development of the lesion was observed when spore suspension of *T. harzianum* IMI-3924332 (T₁₁) was sprayed and the highest percent development of the lesion was observed in control (T₀). The present study indicates that *T. harzianum* IMI-3924333 (T₁₁) was effective to control gummosis disease of *A. lebekunder* field condition.

Table 30. Influence of the *Trichoderma* strains on the development of the lesion size on the stem of *A. lebekat* @ 5 ml (5 × 10⁵ conidia/ml).

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	6.75	7.86	8.43	8.86	10.32	52.88 a
T ₁	7.43	7.89	8.35	8.98	9.78	23.95d
T ₂	6.43	6.96	8.75	8.86	8.98	39.66 b
T ₃	6.28	6.78	7.47	7.85	8.12	29.29 c
T ₄	6.76	6.98	7.32	7.86	8.64	27.81 c
T ₅	7.32	7.38	7.87	7.89	8.56	16.93 gh
T ₆	6.84	6.95	7.34	7.97	8.34	21.92 ef
T ₇	6.69	6.76	6.85	7.53	8.23	23.02 de
T ₈	7.58	7.68	7.89	7.96	8.98	18.46 g
T ₉	6.95	7.42	7.57	7.85	8.42	21.15 f
T ₁₀	7.53	7.65	7.98	8.13	8.26	9.69 j
T ₁₁	6.39	6.54	6.89	7.46	7.23	13.14 i
T ₁₂	7.35	7.47	7.68	7.96	8.38	14.01 i
T ₁₃	6.53	6.87	6.98	7.45	7.61	16.54 h
SD	0.44	0.45	0.58	0.51	0.78	11.41
CV (%)	6.48	6.15	7.62	6.24	9.23	48.59

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

T₀ = Control (*Lasioidiplodia crassispora*), T₂ = *T. virens* IMI-392430 + *L. crassispora*, T₃ = *T. pseudokoningii*IMI-392431 + *L. crassispora*, T₄ = *T. harzianum* IMI-392432 + *L. crassispora*, T₅ = *T. harzianum* IMI-392433 + *L. crassispora*, T₆ = *T. harzianum* IMI-392434 + *L. crassispora*, T₇ = *T. viride* + *L. crassispora*, T₈ = *T. virens* IMI-392430, T₉ = *T. pseudokoningii*IMI-392431', T₁₀ = *T. harzianum* IMI-392432, T₁₁ = *T. harzianum* IMI-392433, T₁₂ = *T. harzianum* IMI-392434, T₁₃ = *T. viride*.



Six month old plantlet of *Samanea samnea* (Rain tree) planted at FPD, Nursery, BFRI, Chattogram.



Pathogen inoculation in *Samanea samnea* (Rain tree) plantlet



Data recording from experimental field

Figure 24: Experimental field of *S. saman* at Forest Protection Division nursery, BFRI campus, Chattogram

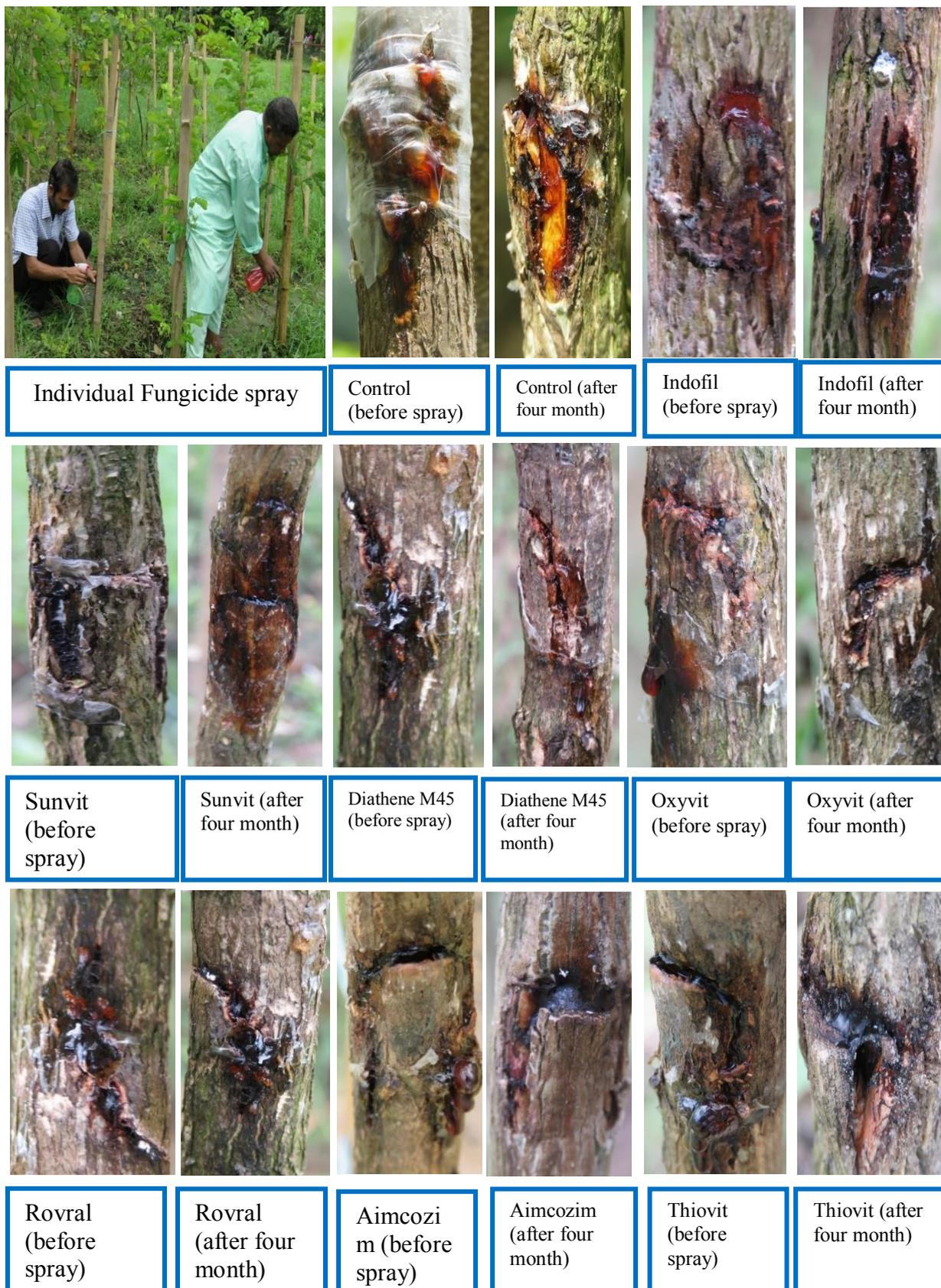


Figure 25: Effect of fungicide treatments on artificially inoculated *S. saman* seedlings with *B. theobromae* USSBT

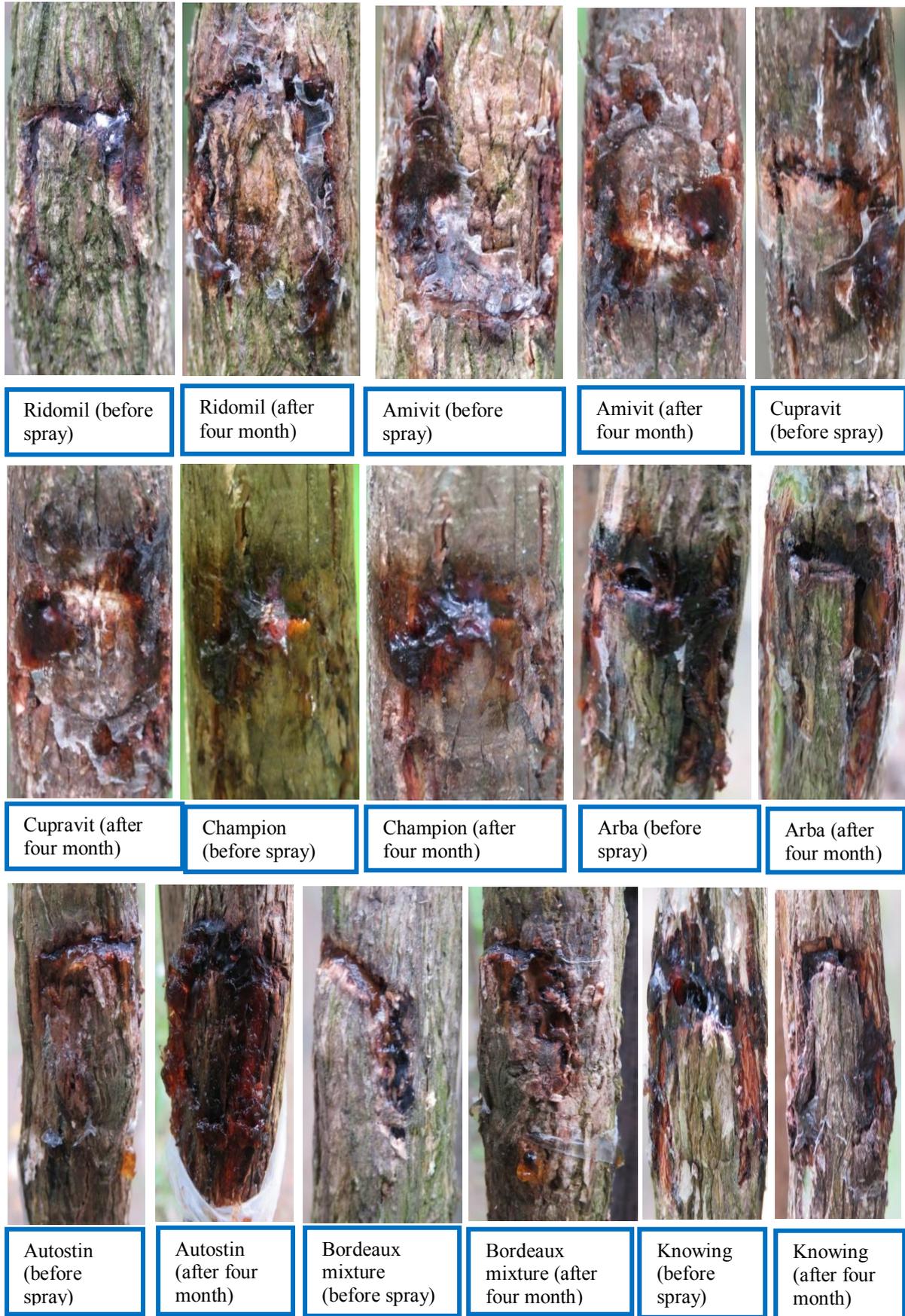


Figure 26: Effect of fungicide treatments on artificially inoculated *S saman* seedlings with *B. theobromae* USSBT

12. Research highlights:

- During the inventory of shade tree in fifteen tea garden of Bangladesh, a total of 44 species of Angiosperm representing 31 genera of 9 families was enlisted. Among the collected species Fabaceae shows highest percentage with 31 species comprising 19 genera (61.29 %) followed by 5 species of Meliaceae comprising 5 genera (16.12 %) and 2 species of Myrtaceae comprising 1 genera (3.22 %). The legume trees are most dominant. The most common shade tree species in tea gardens are: *A. odoratissima*, *A. chinensis*, *A. lebeck*, *A. lucidior*, *A. procera* and *D. robusta*.
- The highest gummosis disease incidence (%) and disease severity index (DSI) were recorded in *Albizzia procera* (40% and 76.67%) at Udalia tea garden, Fatikchari, Chattogram. The lower disease incidence (%) and disease severity index (DSI) was recorded (2.12 and 4.97 %) in *Melia azedarach* at Panchabati tea garden, Mirjapur, Fatikchari and Vugpur tea garden, Fatikchari, Chattogram.
- A total of 629 isolates of fungi were isolated from 130 gummosis samples. The species of fungus were identified as *Mucor* sp. (13 isolates), *Rhizopus* sp. (25 isolates), *Fusarium oxysporum* (36 isolates), *Aspergillus niger* (57 isolates), *Aspergillus fumigatus* (40 isolates), *Aspergillus flavous* (39 isolates), *Aspergillus terreus* (24 isolates), *Penicillium* sp. (25 isolates), *Fusarium solani* (20 isolates), *Lasiodiplodia crassispora* (130 isolates), *Botryodiplodia theobromae* (109 isolates), *Ceriporia lacerate* (58 isolates) and *Byssosclamyces nivea* (53 isolates).
- The pathogenicity test proved that *Lasiodiplodia crassispora* caused *A. lebeck* and *A. procera* gummosis and *Botryodiplodia theobromae* caused *S. saman* gummosis.
- pH (6-8), RH (Relative humidity) 90-95 and Temperature (25-30 °C) is favorable for CG (Conidial germination) and MG (Mycelial Growth) of *L. crassispora* and *B. theobromae*. Concentration of 2-3% glucose and sucrose is the best for CG and MG and sucrose is better than glucose. PDA (Potato dextrose agar) medium is the best for mycelial growth of *Lasiodiplodia crassispora* and *Botryodiplodia theobromae*.
- Chemical fungicides Autostin, ARBA, Knowing and Bordeaux mixture can be used to control gummosis disease of *A. procera*, *A. lebek* and *S. saman*.
- *Trichoderma harzianum* can also be used as a biocontrol agent against gummosis disease of *A. procera*, *A. lebek* and *S. saman*.

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	100	210000	100	210000	
(b) Lab & field equipment	100	390000	100	390000	
(c) Other capital items					

2. Establishment/renovation facilities: Not applicable

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

3. Training/study tour/ seminar/workshop/conference organized: Not applicable.

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training					
(b) Workshop					

C. Financial and physical progress

Line items	Total approved budget			Total fund received till date			Total expenditure till date (up to September-2018)		
	RPA	GoB	Total	RPA	GoB	Total	RPA	GoB	Total
1	2			3			4		
A. Contractual Staff Salary	233920	0	233920	216335	0	216335	216335	0	216335
B. Field Research / Lab expenses and supplies	827110	23970	851080	825892	25023	850915	825892	25023	850915
C. Operating Expenses	295753	4247	300000	228849	1694	230542	228849	1694	230542
D. Vehicle Hire and Fuel, Oil and Maintenance	216750	38250	255000	214124	27769	241893	214124	27769	241893
E. Training/Workshop/ Seminar etc.	110000	0	110000	0	0	0	0	0	0
F. Publications and printing	76500	13500	90000	0	0	0	0	0	0
G. Contingencies	53160	6840	60000	53258	3503	56761	53258	3503	56761
H. Capital Expenses	566752	33248	600000	567297	32703	600000	567297	32703	600000
Total	2379945	120055	2500000	2105755	90692	2196447	2105755	90692	2196447

Fig in Tk:

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output(i.e. product obtained, visible, measurable)	Outcome(short term effect of the research)
Survey for the incidence of gummosis of shade trees in major tea growing areas of Bangladesh	Gummosis disease incidence (%) and disease severity index (DSI) has recorded.	Planters will be get information about gummosis disease.	Researchers and gardeners will be benefited.
Identify the causal organism	The causal organism of gummosis of shade trees has identified.	Information about causal organism of gummosis disease has generated. It will be easy for scientist or researchers for develop suitable control measure of gummosis	Gardeners, researchers and scientist will be benefited.
Development of suitable control measure of gummosis	Suitable control measures has developed.	Disease management technique has developed that will be helpful to reduce the infestation rate.	Gardeners will be benefited.

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.	Not applicable		
Journal publication	On going		i) One article has submitted to SAARC journal of Agriculture. ii) Another two articles are under process for submission.
Information development	Not applicable		
Other publications, if any	Not applicable		

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity)

Description of the technology:

Management practices:

- Monitor tree trunks and branches regularly to detect infestations before they become serious.
- Prune out and destroy badly cankered limbs.
- Practice good orchard sanitation.
- Maintain tree health with good silvicultural practices. Monitor for symptoms routinely and manage the fertilization and irrigation system properly. Manure and fertilizers should be applied according to soil test recommendations. Avoid late summer fertilization.
- Avoid mechanical injuries to trunk and roots.

Management practices:

- Control insect pests, such as borer insect. Spray the bark with 0.1% dieldrin or Malathion. Inject of 5 ml of 0.1 % dichlorvos into larval hole or plugging the larval hole with cotton soaked with kerosene will be found superior. Avoid pruning trees when borer adults are flying, usually late winter through late summer.
- Replace old declining trees.
- Before sowing of seeds in the nursery, the seeds should be treated by recommended chemical fungicide or *Trichoderma*.
- Healthy and pathogen free plant lets should be selected for planting.

Chemical control:

- When the disease is in progress in infected trees, disease portions should be scraped-out with a sharp knife and the cut surface will be disinfected with Mercuric chloride (0.1%) or Potassium permanganate solution (1%) using a swab of cotton. After that, paint in infected portion with Bordeaux paste (2%) for four month.
- When disease becomes clear in the trunk and branches chemical fungicides Bordeaux mixture (2%) or Autostin (Carbendazim) /ARBA (Carbendazim)/ Knowing (Carbendazim) @ 2 g/l should be sprayed after 7 days interval up to four months.

Biological control:

- When the disease is in progress in infected trees, disease portions should be scraped-out with a sharp knife and liquid formulation of *Trichoderma*@ 5 ml (5×10^5 conidia/ml) should be sprayed after 7 days interval in the lesion for four month.

Benefits of technology:

- Healthy shade tree will be ensured followed by proper management techniques of gummosis disease. Which will be influenced the growth and quality of tea.
- Disease will be reduced by application of recommended chemical fungicides. That will be protect the tree from dying.
- *Trichoderma* can be used as potential biocontrol agent to control this disease. The bio-agents might be used for plant disease management that diminish our economic loss through overcoming any environmental risk.

ii. **Generation of new knowledge that help in developing more technology in future**

- The shade trees are an integral part of the tea cultivation. The shade trees provide partial shade to the tea plants, which is important for improving the quality of the tea leaf. Right type of shade trees and their proper management is a prerequisite for successful tea crop growing. For this persists, a floristic exploration was carried out to documentation of shade tree species at fifteen tea gardens in Chattogram and Moulvibazar District of Bangladesh. During the investigation a total of 44 species of Angiosperm representing 31 genera of 9 families was enlisted. In the assessment, Fabaceae family shows the highest number of shade trees comprising 19 genera and 31 species. In future, information about the documentation of shade tree species in tea garden will be very helpful to find out the right type of shade tree species on the growth and yield of tea plants in tea cultivation areas of Bangladesh.
- *Lasiodiplodia crassispora* and *Botryodiplodia theobromae* was isolated and identified consistently from the diseased tissues of affected plants in fifteen tea garden of Bangladesh. Pathogenicity of these fungus were confirmed by artificial inoculations on plants. According to the literature and scientific paper, these fungus has been reported as a pathogen of more than 500 plant species and causes symptoms including canker, dieback, damping-off, wilt, root rot, collar rot and fruit rots. This is the first record of *Lasiodiplodia crassispora* and *B. theobromae* on *A. lebeck*, *A. procera* and *S. saman* gummosis in Bangladesh. Very little information is available on the pathogen and management aspects of this disease. Therefore, different contact, morphological, cultural, environmental, biological and fungicidal effect were evaluated on *Lasiodiplodia crassispora* and *B. theobromae*. In this study, information about these causal organisms and management practice of gummosis disease has developed that will helpful to other researcher and scientist for developing more technology to control this disease in future.

iii. **Technology transferred that help increased agricultural productivity and farmers' income**

- Technology for management of gummosis disease has developed that will be helpful for farmers and gardeners to control this disease.
- Proper shade management will be ensured by adopting proper disease management system of shade trees in tea garden of Bangladesh. As a result, the production of such tea will be increased. On the other hand, other demand like timber wood, fuel wood, medicine etc. will be attained. Ultimately, farmers' income will be increased. That will be lead to economic development of our country.

iv. Policy Support

- In the tea garden of Bangladesh many shade tree species are planted for shade purpose. Nowadays, shade tree gummosis is a new disease in tea garden of Bangladesh causing several damage of trees in the garden. During survey, the gummosis disease was observed in only nine shade tree species like, *A. procera*, *A. lebek*, *A. odoratissima*, *A. moluccana*, *A. chinensis*, *A. lucida*, *Derris robusta*, *Dalbergia sissoo*, *S. saman* and *Melia azedarach*. By this study, it has been possible to control gummosis in only three plant species viz. *A. procera*, *A. lebek* and *S. saman*. Detailed research is needed to find out suitable control measure the gummosis disease of the rest of the species. More time and a lot more budget is needed to detail the research work. In this regard, various government and non-governmental organizations have to take initiative to conduct research on this topics.
- Further research is required regarding the etiology and epidemiology of the gummosis in different shade tree species in major tea cultivation areas of Bangladesh.
- This study also recommends that another survey should be organized to cover as many districts and agroecological zones as possible to assess the disease status and advice on the best ways for management.
- Disease tolerant varieties of shade tree species have to be developed. Research programs should be undertaken in various research institutes for the development of disease resistant varieties.
- There is no such plant breeding institute which can provide disease free nursery stock and varieties to the shade trees growers.
- A strong programme of awareness are recommended to creation and training of farmers and frontline extension workers on the identification, management and control of shade tree gummosis.

G. Information regarding Desk and Field Monitoring

- i) **Desk Monitoring** [description & output of consultation meeting, monitoring workshops/seminars etc.): Not applicable.

Report type	Actual date of submission(s)	Total Number(s)	Remarks(if anything otherwise)
a. Inception report	As per schedule	1	
b. Monthly reports*	As per schedule	14	
c. Statement of expdts.(SoE)*	As per schedule	14	
d. Quarterly report(s)*	As per schedule	3	
e. Six monthly report	As per schedule	3	
f. Procurement plan	As per schedule	1	
g. Field Monitoring Report(s)**	As per schedule	1	

- ii) **Field Monitoring (time& No. of visit, Team visit and output):**One field monitoring has conducted as per time schedule.

Monitoring Date	Monitored by	Monitored places	Attendees name	Responsibility	Comments
22 March 2018 to 24 March 2018	Md. Manzur Hasan Bhuyan Deputy project Director PMU, NATP-2 project and Join Secretary Government of the Peoples Republic of Bangladesh	Forest pathology lab and Nursery, Forest protection Division, Bangladesh Forest Research Institute, Chattogram.	Dr. M. Ahsanur Rahman	Principle investigator of project	Satisfactory
			Md. Rafiqul Islam	Co-principle investigator of project	

I. Lesson Learned/Challenges (if any)

- Workers who have been recruited in the tea gardens are not technical persons in most of the cases they have lack of knowledge on proper management of gummosis disease of shade trees. Proper management techniques of shade trees and tea plantation are even unknown to them.
- In many tea estates proper shade trees and ancillary combination is not maintained. Then layout recommended by tea scientists are not followed rather they are planted in general way.
- Worldwide a large number of leguminous or non-leguminous trees are recommended for using as permanent shade trees to provide shade over tea plantation. But in Bangladesh only a few species like *A. oleratissima*, *A. moluccana*, *A. chinensis*, *A. lucidia*, *A. lebeck*, *A. procera*, *Leucaena leucocephala*, *Derris robusta*, *Dalbergia sissoo* and *Melia azedarach* are used extensively. No trial for other species, which could be used as shade trees. If that could be done biodiversity would be enriched.

J. Challenges (if any)

- Deep-rooted leguminous trees should be selected as shade trees for using in the tea estates. Research recommendations of BTRI scientists regarding various aspects of shade tree and tea management should be properly implemented. Private companies must be liberal towards the innovations recommended by the tea scientists.
- The workers of tea garden should be given training of the proper method of silvicultural operation of shade trees like lopping, uprooting, planting, pruning, thinning, fertilizing and disease management of shade trees that would be increase productivity of tea.
- Shade trees should be leguminous providing higher litter fall and fixes N_2 to the soil. Right species should be selected for right soil and climatic characteristics. Matching of species with site is important for a successful tea plantation. Proper spacing and planting technique should be maintained throughout the plantation.

- Cultural operation such as weeding, gap filing, maturing, pruning, tipping and plucking should be done properly, which influences the growth and quality of tea.

K. Concluding remarks:

The shade tree gummosis has identified for the first time in Bangladesh and confirmed as a disease of importance as it occurred in all the major tea growing areas in the country. The disease was found to retard growth and development of affected trees which eventually impact negatively on the performance of the trees. The ubiquitous fungi, *Lasiodiplodia crassispora* and *Botryodiplodia theobromaeh* has consistently isolated and confirmed as the causal agent of the disease. Proper management techniques has developed to control these pathogen. It is therefore recommended that further comprehensive research is needed regarding the etiology and epidemiology of the gummosis in different shade tree species at major tea cultivation areas of Bangladesh.

Signature of the Principle Investigator
Date:.....
Seal:

Counter signature of the Head of the
organization/authorized representative
Date:.....
Seal:

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