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Study on Gummosis and Sudden Decline Disease of Mango Plants in Rajshahi Region of Bangladesh

Hossain, Md. Mosharraf

University of Rajshahi, Rajshahi

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STUDY ON GUMMOSIS AND SUDDEN DECLINE DISEASE OF MANGO PLANTS IN RAJSHAHI REGION OF BANGLADESH

A thesis submitted for the degree of

Doctor of Philosophy

Department of Crop Science and Technology
University of Rajshahi

By
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DEPARTMENT OF CROP SCIENCE AND TECHNOLOGY
UNIVERSITY OF RAJSHAHI,
RAJSHAHI-6205, BANGLADESH

DECEMBER, 2020

Dedicated To
My Beloved
Parents

DECLARATION

I do hereby declare that the entire work submitted as a thesis entitled **STUDY ON GUMMOSIS AND SUDDEN DECLINE DISEASE OF MANGO PLANTS IN RAJSHAHI REGION OF BANGLADESH** in the Department of Crop Science and Technology, University of Rajshahi for the degree of Doctor of Philosophy is the result of my own investigation. The thesis contains no materials which has been accepted for the award of any other degree or diploma elsewhere and to the best of my knowledge, the thesis contains no material previously published or written by another person, except where due reference is made in the text.

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CERTIFICATE

I have the pleasure in certifying the thesis entitled **"STUDY ON GUMMOSIS AND SUDDEN DECLINE DISEASE OF MANGO PLANTS IN RAJSHAHI REGION OF BANGLADESH"** submitted to the Department of Crop Science and Technology, Faculty of Agriculture, University of Rajshahi for the degree of Doctor of Philosophy. I do hereby certify that the candidate has fulfilled the requirements and the research work embodied in the thesis was carried out by the candidate. To the best of my knowledge, all the data and materials are genuine and original. No part of the research work has been submitted for any other degree.

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ABSTRACT

Mango is one of the most important fruit in Bangladesh suffering from many diseases. Among the diseases gummosis and sudden decline disease of mango plant is a new disease in Bangladesh. Day by day it is becoming a serious threat to the mango growers of the country. In order to study about the disease a set of four experiments were conducted. A field survey was conducted at five upazilas of Chapainawabganj district and three upazilas of Rajshahi district of Bangladesh during January 2016 to October 2017 to study the incidence and severity of the disease. It was observed that the disease was found in all the surveyed area under investigation. Among the eight upazilas the highest incidence (3.47%) and severity index (10.70%) were recorded in Shibganj upazila of Chapainawabganj district where as the lowest incidence (1.77 %) and severity index (6.00 %) were recorded in putia upazila of Rajshahi district. In case of varietal reaction the highest incidence (3.55 %) and severity index (14.87 %) were recorded in Fazli variety whereas the lowest incidence (0.26 %) and severity index (2.16 %) were recorded in BARI Aam-4. From the experiment it was observed that among the varieties surveyed fazli was very much susceptible to gummosis and sudden decline disease. These studies indicate that older plants might be more susceptible to the disease compared to younger plants. From the results it was observed that the maximum disease incidence was noted in the month of April and the minimum in July. During the survey samples of diseased plant parts were collected and the causal agent was isolated on potato dextrose agar media and identified. The isolated fungus was identified as *Lasiodiplodia theobromae* that was confirmed by pathogenicity test using mango seedlings as test crops following stem inoculation method. *Lasiodiplodia theobromae,* isolated from the diseased plant parts which was able to cause the same

symptom on inoculated mango seedlings. In this study molecular identification and characterization of isolated fungus was conducted using genome sequencing of its region from northern parts of Bangladesh. The BLASTn search revealed that the fungus derived from gummosis and sudden decline disease of mango is *Lasiodiplodia theobromae*. *In vitro* fungi toxicity of eight fungicides from different groups were tested for their efficacy against *L. theobromae*, by following poisoned food technique, proved that Carbendazim was the most effective fungicide followed by Copper hydroxide, Propiconazole and mixture of Pyraclostrobin with Metiram. Six treatments were applied at field condition to manage the disease and it was observed that among the treatments the lowest severity (2.96%) as well as the highest disease reduction (95.56%) after the application of Bordeaux paste along with spraying Carbendazim @ 2gm/L.

CHAPTER –1

INTRODUCTION

CHAPTER 1

INTRODUCTION

Mango (*Mangifera indica* L.) is a member of the Ancaradiaceae that includes 73 genera (Bompard, 2009). It is the most important and popular fruits grown in many tropical and subtropical regions of the world (Pradhan and Wadhi, 1962; Chadha and Pal, 1993) including Bangladesh (Hossain, 1989). Southeast Asia or the Malay Archipelago are believed to represent the center of origin of mango (Mukherjee & Litz, 2009). The tree has been cultivated in India for an estimated 4000 years (Mukherjee & Litz 2009) and is evergreen and can live for more than a hundred years. The height of fully mature trees ranges between 3 - 10 meters and in some situations they can reach over 40 m (Mukherjee & Litz 2009; Bally, 2006). By virtue of wide range, delicious taste, superb flavor, very high nutritive and medicinal value as well as great religio-historical significance, it is being called the "King of fruits" (Hayes, 1953).

Among 3,000 tropical and subtropical fruits species, mango is cultivated in more than 85 countries of the world from them 63 countries produce more than 1000 mt mangoes a year (Galan, 1997, 2008). According to FAO (FAOSTAT, 2008), mango ranked fifth in total production amongst major fruit crops worldwide. On the other hand among the top ten mango producing countries of the world India is one of the largest producers with an annual production 1,51,88,000 mt, representing 40% of the world's production. Other nine major mango producers include China (43,50,000 mt), Thailand (26,00,000 mt), Indonesia (21,31,139 mt),

Pakistan (18,88,449 mt), Mexico (18,27,314 mt) Brazil (12,49,521 mt), Bangladesh (8,89,176 mt), Nigeria (8,50,000 mt), Philippines (8,00,55mt) (FAOSTAT, 2014)

Mango is the most important fruit of Bangladesh and it ranks 1st in terms of area and 3rd in production. Because of its great utility, it occupies a pre-dominant place amongst the fruit crop grown in our country. The total area under mango cultivation is about 1,09,584 acres (inside garden and outside garden) and annual production 1165804 metric tons (inside garden) (BBS, 2019). Mango grows well in all over the country but the commercial cultivation is mainly concentrated in North-Western region of the country such as Rajshahi and Chapainawabganj districts (Uddin *et al*., 2007).

The fruit is very popular among people due to its wide range of adaptability, high nutritive value, richness in variety, delicious taste and excellent flavor. It is a rich source of vitamin A and C. The fruit is consumed raw or ripe. Good mango varieties contain 20% of total soluble sugars. The acid content of ripe desert fruit varies from 0.2 to 0.5 % and protein content is about 1%. Presently, the fruit of local varieties of mango are used for preparing pickle and raw slices. The wood is used as timber, and dried twigs are used for religious purposes. The mango kernel also contains about 8-10% good quality fat which can be used for saponification. Its starch is used in confectionery industry. Mango also has medicinal uses. The ripe fruit has fattening, diuretic and laxative properties. It helps to increase digestive capacity (Saleem and Akhtar, 1989). Average mango seedling trees live more than 100 years whereas grafted ones live only 80 years with an annual production of about 16,000 fruits in peak years at the age more than 100 years old (Singh, 1960).

Mango is affected by more than 140 pathogens during all the stages of its life cycle, causing different levels of damage from the seedlings in the nursery to the fruit in storage (Prakash, 2004). The majority of plant organs, such as the trunk, branches, twigs, leaves, panicles are affected by different pathogens. Among the disease causing agents fungi causes the largest number of diseases while bacteria, algae, angiospermic parasites, gases and nutritional unbalances are the causal agents of many other maladies. According to Pernezy and Simone (2000) mango is infected by at least 100 fungal pathogens, 4 bacterial pathogens, 4 nematode pathogens, 1 algal species, 1 lichen, and 12 disorders caused by abiotic factors that are the causal agents of the maladies.

These pathogens can express different kinds of diseases symptoms (Haggag, 2010). The changes in the environmental conditions however, often can play an important role either in reducing the tree's ability to elicit an active defense response to pathogen infection and invasion (Schoeneweiss, 1984), or in reducing the productivity of the tree in association with the diseases and disorder problems (Prakash, 2004). Mango trees, therefore, can face different levels of stress in different environments, which together with varying levels of pathogen's inoculum pressure, can trigger symptoms development and result in disease expression (Finnemore, 2000).

Among the disease, mango die back and decline are observed to be quite destructive in India and also in many mango growing countries (Pathak, 1980). In Gujarat, the incidence of leaf infecting fungi continues to increase year after year resulting into decline accompanied by exudation of gum and die-back of mango orchards.

Therefore, it can be considered as a destructive disease and limiting factor for mango cultivation in Gujarat. The increase in inoculum potential may lead to destruction of the orchards. In Saurashtra region, many orchards are badly damaged and killed. Farmers have uprooted affected grown up trees in Vanthali and Talala taluka and changed the pattern of cultivation due to die back disease. Twig blight appears to be the initial phase of die-back and decline accompanied by exudation of gum which are the major symptoms with which *Lasiodiploidia sp*. always found associated. Among all diseases infecting mango, disease complex known as "mango sudden death syndrome" (MSDS) or the mango quick decline is the most recent severe threat to the Pakistan mango industry. Recently, incidence of this menace was found 20 and more than 60 percent in Punjab and Sindh Provinces of Pakistan respectively and 60 percent in Al Batinah region of Oman (Al- Adawi *et al.*, 2006; Saeed *et al*., 2006).

Initial symptoms were gummosis from the bark and branch death on affected trees, other symptoms, including vascular discoloration beneath the gummosis and tree death usually occurred within six months of first symptom appearance which were observed during the mango survey in Pakistan (Masood *et al*., 2008). According to a report, prepared by the Ministry of Food, Livestock and Agriculture (Minfal) Pakistan, the disease, which is of "the great economic importance as the adult mango plant dies off within small span of time" is spreading in large areas in the two provinces first in the Punjab and recently in the Sindh (Anonymous, 2005). Similarly, in Oman, mango sudden decline is an economically serious disease characterized by wilting symptoms that usually begin on one side and later spread to involve the entire tree, gum exude from the bark of their trunks or

branches and vascular tissues are discoloured. Other symptoms include terminal and marginal necrosis of leaves, which ultimately lead to the death of leaf blade (Al Adawi *et al*., 2006).

The disease is characterized by the presence of profuse oozing of gum on the surface of the affected wood and bark of the trunk and also on the larger branches but more common on the cracked branches. Under severe infection in susceptible varieties, droplets of gum trickle down on stem and bark turns dark brown with longitudinal cracks and the tree dries up because of cracking, rotting and girdling of stem (Narasimhudu and Reddy, 1992; Khanzada *et al*., 2004a). Severely infected mango trees. Usually mango trees live on average of 80 to 100 years but when it is infected with gummosis the tree is killed and hence disease control of gummosis is very important.

There are different opinions about this disease and some plant disease specialist suggest that it is complicated case emerged mostly due to the combine attack of anthracnose, stem blight, root rot, tip dieback, bacterial leaf spot and malformation disease, which are responsible for the decline. The attack of this disease in the province of Punjab started in 1995 from the district Muzafargarh and now it prevails in every orchard of the mango. When different orchards of mango were surveyed it is observed that 20 percent of the trees had been dead due to this disease. Most likely 60 to 85 percent trees are suffering from this type of disease, in various mango orchards of Sindh (Anonymous, 2005). Similarly, Al-Adawi *et al.* (2003) studied that 60 % of the trees of mango were infected in the parts of Al Batinah region of Oman.

Mostly infection by all fungi on mango are facilitated by beetles as a wounding agent but other factors are also responsible which speed up the quick decline including water stress, extreme summer and winter temperature, sun scorch, high humidity in propagation nurseries, hardpan soils, nutritional deficiencies and improper cultural practices (Das Gupta and Zacchariah, 1945).

Gummosis incited by *Lasiodiplodia theobromae* (Pat.) Griffon & Moube [synonym: *Botryodiplodia theobromae*] is becoming a serious problem in India on many popular varieties of mango particularly during monsoon and post-monsoon periods. In, Brazil, Oman and Pakistan, fungal species isolated from disease mango tree were *Ceratocysis fimbriata*, *C. omanensis* and *Lasiodiplodia theobromae* (Ribeiro, 1980; Al-Adawi *et al*., 2006).

More recently, gummosis has emerged as a serious disease of mango. This disease is also known as decline disease, athajhora rogue, sudden decline disease, stems bleeding and quick decline in different regions in the world. Mango decline was reported from different part of the world. Sudden death of mango plant is appeared in Pakistan during 1998-1999, which is aggravating day by day (Shahbaz *et al.*, 2005) and mango plantations in different areas of Sindh were found suffer from a decline disease showing symptoms of drying of branches from the tip accompanied with a heavy exudation of yellowish-brown gum from stem and branches and browning of vascular tissues (Khanzada *et al.*, 2004). In Oman, sudden decline as a new disease of mango, has affected 60% of the trees in Al Batinah region (Al- Adawi *et al.,* 2003). Most of the researchers have reported *Lasiodiplodia theobromae* (Sunonyms: *Botryodiplodia theobromae, Diplodia theobromae) as* cause of the

decline disorder in Pakistan (Mahmood and Gill, 2002; Khanzada *et al*., 2004; Malik *et al.,* 2005). Gummosis disease occurrence was reported from Niger since the early 1980s (Reckhaus and Adamou, 1987). This disease has been recognized as a significant disease since 1940 and reported as the most serious disease in Jaipur district in India (Verma and Singh., 1970) and also affected 30-40% of the plantations in the Moradabad region of Uttar Pradesh (Prakash and Srivastava, 1987). In India, Narasimhudu and Reddy (1992) were isolated *Botryodiplodia theobromae* producing sign of gummosis on diseased mango trees and also confirmed its pathogenicity. Gummosis disease caused by *Botryodiplodia* sp. was reported from India and China (Rawal, 1998; Wen, 1996). The fungus (*Lasiodiplodia* sp.) was also associated with the decline of mango trees (Gonzalez *et al*., 1999) and the maximum infection of *Lasiodiplodia* sp. in mangoes were affected showing decline symptoms (Jedeja, 2000; Mahmood *et al.,* 2002). This disease occurs throughout the year. At the initial stage of infection gum exudates from the bark of the trunk, big branches, young branches or shoots. All leaves of the infected plant drop and finally, the plant dies (Uddin *et al.,* 2012). The initial symptoms of this disease include gummosis from the bark, bark splitting, yellowing and withering of leaves (Masood *et al.,* 2010). The tree may die within three to six months from the first appearance of gummosis disease symptoms (Hossain *et al*., 2010; Al Adawi *et al*., 2002).

Mmango is subjected to a number of diseases at all stages of development, right from the plants in the nursery to the consumption of fruits (Rawal, 1998). Among these diseases, the proportion of gummosis increased dramatically every year by which mango plants died suddenly (Masood *et al*., 2011). Now, gummosis and sudden decline of mango trees is a serious threat which becoming epidemic in different areas

since last few years in different mango growing region of Bangladesh. The disease is becoming a threat to the mango industry particularly in Rajshahi and Chapainawabganj district. But so far a little works have been done on the issue. Therefore, a comprehensive study is necessary to know the detail informations of the disease. Keeping in view of the above fact, the present study has been designed with the following objectives:

i. To know the prevalence of gummosis and sudden decline disease.
ii. Isolation, purification and identification of the causal agent of the disease.
iii. Molecular characterization of the causal agent.
iv. To find out a suitable management practice of the disease.

CHAPTER –2

REVIEW OF LITERATURE

CHAPTER 2
REVIEW OF LITERATURE

Mango gummosis and sudden decline disease caused by *Lasiodiplodia theobromae* is an economically important and widely distributed disease. *Lasiodiplodia theobromae* is found to infect many horticultural and fruit crops but the literature on mango is meagre. The literature pertaining to the present investigation have been reviewed and presented here under with the following headings.

2.1. Occurrence

Mango decline is the most destructive disease of mango. It is considered to be of complex nature. Till now the disease has been reported from various mango growing countries of the world such as Pakistan, India, USA, Mauritius, Puerto Rico, El–Salvador, Niger, Oman, Peru, Brazil, Western Australia, Egypt and China by various workers viz., Gupta and Zachariah (1945), Smith and Schudder (1951), Anonymous (1962), Alvarez (1967), Verma and Singh (1970), Alvarez and Lopez (1971), Ragab *et al*. (1971), Ovies and Waite (1977), Ribeiro (1980), Reckhaus (1987), Ramos *et al*. (1991), Simone (1999), Al- Adawi (2003), Javier *et al*. (2009), Shahbaz *et al*. (2009), Morales and Rodriguez (2009), Malik *et al*. (2010), Masood *et al*. (2011), Sakalidis *et al*. (2011), Ismail *et al*. (2012), Al- Adawi *et al*. (2013), Hisang (2013) respectively. Rodriguez and Mathos (1988) reported dieback, floral necrosis and gummosis on ten year old mango trees at Haur. Die back disease or decline disorder has been reported in nearly all mango growing regions of the world. The following table shows the literature available from parts of the world (Table 2.1).

Table 2.1. Distribution of mango gummosis/die back disease reported from different parts of the world

Sl.No.	Country	Reference
1	Dutch East Indies	Ciferri and Gonazales (1927)
2	India	Gupta and Zacchariah (1945)
3	South Africa	Spencer and Kennard (1955)
4	Mexico	Alvarez and Lopeez (1971)
5	Nigeria	Reckhaus and Adamou (1987)
6	USA	Ploetz *et al.* (1996)
7	Oman	Al-Adawi *et al*. (2006)
8	Pakistan	Fateh *et al*. (2006)

Mango trees infected by *Diplodia cacaoicola* was reported from Barbados (Bourne, 1921). *Diplodia mangiferae* on branches of mango trees from Dominican Republic (Ciferri and Gonazales, 1927). Mullar (1940) from Dutch East Indies reported *B. theobromae* occurring as a wound parasite on mango trees damaged by sun-scorch. Bitancourt and Jenkins (1943) reported *Botryodiplodia theobromae* (Syn: *L. theobromae*) as a pathogen.

The disease has been reported from Uttar Pradesh, Mumbai, Andhra Pradesh, Rajasthan, Orissa, Delhi and Himachal Pradesh In India by Hayes (1953), Vaheeduddin (1954), Verma and Singh (1970), Rath *et al*. (1978), Pathak (1980), Narasimhudu and Reddy (1992), Sharma (1993), etc.

In Himachal Pradesh, the disease has been encountered in district Kangra, Hamirpur, Una, and parts of Mandi, Bilaspur, Solan and Sirmour. The disease incidence was most prominent in district Kangra (Sharma, 1993).

Rodriguez and Mathos (1988) reported that dieback, floral necrosis and gummosis on 10-year-old mango trees at Huar and found that *L. theobromae was* constantly associated with all the disorders.

Sanchez *et al.* (2013) studied stem-end rot of fruits and dieback of branches in mango (*Mangifera indica* L.) in Mexico and found that *L. theobromae* and *Neofusicoccum parvum*, *Neofusicoccum* spp. and *L. pseudotheobromae* were associated with the disease.

2.2. Incidence and losses

Das Gupta and Zachariah (1945) for the first time reported mango dieback from Uttar Pradesh in India and were to emphasize the importance of die back of mango caused by *B. theobromae.* Edward (1954) from Allahabad isolated *B. theobromae* from dead roots of mango seedlings.

According to Batista (1947) about 27 percent of the mango trees representing one sixth of the total mango population of the area 'Pernambuco' were found severely affected. Rodriguez and Mathos (1988) observed the symptoms like dieback, floral necrosis and gummosis on 10-year-old trees of mango at Huar and found that *L. theobromae* was associated with all the disorders.

Verma and Singh (1970) identified mango gummosis as a serious disease in Jaipur district, which affected 30-40 percent of the plantations in the Morabad region of Uttar Pradesh (Prakash and Srivastava, 1987). Rath and Monahan (1977) reported that B. *theobromae, C. gloeosporioidea* and *Aspergillus spp*. were the primary cause of the blossom blight of mango. Among these fungi *B. theobromae* was reported to incite twig blight.

Sharma (1993) recorded *L. theobromae, C. gloeosporiodes, Rhizoctonia solani, and P. mangifera, Phomopsis, Sclerotium rolfsii and Fusarium solani* pathogens associated with mango decline. He also found that a mixed infection was common and *L. theobromae* was the primary cause of the disease.

Quick decline of mango caused by *B. theobromae* was the most holistic one to cause heavy losses up to 50 percent in-mango grooves of Punjab and Sindh (Mahmood *et al*., 2002)

Al-Adawi *et al.* (2003) reported quick decline as a new disease of mango affecting 60 per cent of the trees in Al Batinah region in Oman. In most cases, the disease has been characterized by the exudation of gum, wilting, dieback, vascular browning and death of the whole tree. They concluded that *L. theobromae* is a causative fungus of this disease.

Leghari (2005) studied about mango gummosis disease and found 20-83.3 percent incidence with a severity of 62.5-85 percent. He also isolated 12 species of fungi belonging to 10 different genera in addition to *L. theobromae* from infected mango trees exhibiting gummosis symptoms.

Mahmood (2008) reported that quick decline showed 100 percent prevalence in surveyed orchards in 7 districts of the Pakistan province. Saeed *et al*. (2011) has reported disease incidence of about 20 and 60 percent in Punjab and Sindh province of Pakistan.

The mango gummosis was reported to be 20 and 60 percent in Punjab and Sindh Provinces of Pakistan, respectively and 60 percent in Al Batinah region of Oman (Saeed *et al.*, 2011; Al-Adawi *et al*., 2006).

Iqbal *et al.* (2007) conducted a survey for mango diseases in 16 locations of Punjab area in Pakistan and they reported different phases of diseases viz., twig blight, tip dieback, gummosis and bark cracking/ splitting. The prevalence of the symptoms was 55, 50, 25 and 25 percent respectively. Maximum intensity of 5.17 percent for quick decline was noted in Sahiwal district.

Hui Fang *et al.* (2012) reported that mango fruit rot disease was found frequently on harvested mango fruits in the major mango-producing areas of southern Taiwan, including Guntain, Fanshan and Yujing during 2009-2011. They revealed that disease incidence ranged from 18.7 percent to 58.1 percent and the incidence was significantly high in Guntain than in Yujing and Fanshan. They identified four *Botryosphaeriaceae* species, among which *L. theobromae* was the most aggressive pathogen to cause disease. Tovar *et al.* (2012) observed 70 percent die back of sapotemamey grafts in Mexico and they identified *L. theobromae* as the causal agent of this disease.

Rashid *et al.* (2013) isolated different fungi *viz., Alternaria alternata, Cladosporium* spp., *Colletotrichum gloeosporioides (P.S. Glomerella cingulata), Dothiorella dominicana, Fusarium* spp., *L. theobromae, Penicillium* spp., *Pestalotiopsis* spp. and *Phomopsis* spp. from samples of mango sudden death disease.

2.3. Symptomatology

Mango Decline or Mango Sudden Decline Syndrome (MSDS) or Mango Sudden Death Syndrome (MSDS) or Mango Tree Mortality (MTM) was the most destructive disease. Prakash and Singh (1976) described gummosis symptoms as severe dieback, twig- blight, bark splitting / cracks and exudation of gum was severe in advanced conditions.

Prakash and Srivastava (1987) detailed the symptoms of gummosis as gum secretion and longitudinal crack of infected stem. In severe cases, the mango trees die due to cracking, rotting and girdling.

Reckhaus (1987) found that water stress was the primary predisposing factor for onset of disease. The presence of the symptoms was noticed in trees which were deficient in manganese, iron or both elements (Schaffer *et al.*, 1988).

Ahmed *et al*. (1995) reported that the onset of die back becomes evident by discoloration and darkening of twigs from tip to downward due to *Diplodia natalensis***.** The symptoms that were associated with mango decline were diverse and included the following symptoms: dieback of terminal shoots with or without accompanying defoliation, gummosis on branches and scaffold limbs, vascular

discolouration, marginal chlorosis and necrosis of leaves, foliar nutritional deficiencies and root degeneration (Ploetz *et al*., 1996).

Ploetz *et al*. (1997) observed the symptoms of decline, tip dieback and gummosis from mango nurseries artificially inoculated with *Alternaria alternata*, *Glomerella cingulata*, *Dothiorella dominicana*, *B. theobromae* and *Phomopsis* sp. The description of symptoms of dieback and gummosis has also been given by various workers viz., Ragab *et al*. (1971), Saleem and Akhtar (1989), Ploetz and Pernezny (2000), Shahbaz *et al*. (2005), Saleem *et al*. (2006), Jiskani *et al*. (2007), etc.

Drying of tip, discoloration and darkening of bark some distance from the tip were common symptoms. Later, it moved downward involving bigger branches as well. As a result, the leaves shed followed by exudation of gum from the diseased portions. In severe cases, bark splitting or cracking was also noticed. Such symptoms may be found alone or in combination of two or more symptoms in different mango orchards of the world (Ploetz, 1999).

Mango decline had gained great attention due to slow or sudden collapse of the plants (Sial, 2002). Jiskani (2002) stated it as disease complex that was caused by combined attack of several different fungi and abiotic factors. It was considered by some scientist as a disorder and by others as a disease (Khanzada *et al*., 2004). The declining plants of mango already present in the orchards were inoculum sources for this malady (Malik *et al*., 2004).

Khanzada *et al.* (2004a) recorded several gummosis symptoms on mango. The affected plants exhibit dieback, gummosis and vascular discoloration. In dieback,

infected twigs die from the tips to back into old wood, which gives a scorched appearance to limb. The affected leaves turn brown and rolls upward. In severe cases, the entire plants is killed. Gummosis: The infected plants show abundant gum secretion from branches, stem and main trunk. Vascular discoloration: Infected twigs, plants and branches showed internal discolouration. Brown streaks visible in vascular region and these were severe in water stress conditions.

Shahbaz *et al.* (2005) explained the disorders like twig blight, gummosis, bark splitting/cracking and wilting as mango gummosis disease. Mango decline complex is observed in the form of twig blight, tip dieback, gummosis and bark splitting (Malik *et al*., 2005). Exudation of gum was found to be the most common symptom of die back affected plants due to the involvement of *B. theobromae* (Khanzada *et al*., 2005; Saleem *et al*., 2006; Fateh *et al*., 2006).

Mango decline is a general term which is used for several diseases and these are generally referred as decline disorders. It included blight, canker, gummosis, twig blight, tip die-back and stem bleeding. Drying of the mango plants were the main characteristic symptoms of all these disorders at initial or advance stage (Malik *et al*., 2005a).

Al-adawi *et al.* (2006) found that mango sudden decline affected trees in Oman which showed wilting symptoms that usually begin on one side and later spread to involve the entire tree. Trees exude amber-coloured gum from the bark of their trunks or branches and vascular tissues were discolored. Tree death occurred approximately 6 months after the first appearance of the symptoms (Al-Adawi *et al*., 2003).

Mango decline disorders showed characteristic symptoms like twig blight, gummosis, bark splitting/cracking and wilting as mango gummosis disease (Iqbal *et al.,* 2007). As preliminary symptoms, drying of tip, discoloration and darkening of the bark from the tip become visible. Later, it progressed downward involving bigger branches as well. As a result, the leaves shed followed by exudation of gum from the diseased portions. Bark splitting or cracking was also be noticed in severe cases. These symptoms were found alone or in combination with other symptoms in different mango orchards (Iqbal *et al*., 2007).

On removal of the affected bark, a thick liquid with foul smell oozed out. As the infection progressed, tree death occurred, perhaps six months from first appearance of symptoms. The injuries caused by insect or physical damage provided avenues for penetration and then further propagation of this fungus in the host plant. The plants affected with these problems were not properly managed and resultantly, the inoculums of this fungus increased day by day and became very aggressive (Shahbaz *et al*., 2009). Initially, a portion of the bark was affected and gradually the whole tree trunk rotted causing collapse of the tree. The wood was stained dark brown, spreading from a point of infection.

In India, the gummosis was accompanied by damage caused by trunk borers resembling *Hypocryphalus mangiferae* from February to October. The grubs caused severe damage by feeding on the bark inside the trunk, boring upward making tunnels, thus weakening and causing hindrance to the transport of water and nutrients from roots to shoots resulting in wilting and drying of the shoots (Masood *et al*., 2011).

2.4. Etiology

Smith and Schudder (1951) reported that the death of mango trees in 1949 in Florida was attributable to higher incidence of *Diplodia sp*. rather than to the poor nutritional condition of the trees. Verma and Singh (1970) reported that in India, die back disease has been recognized as a significant disease since 1940. It was reported as the serious most disease in Jaipur and was caused by *B. theobromae.* Alvarez and Lopez (1971) reported that in Puerto Rico, die back of mango was associated with the conidial stage of *Physalospora rhodina*.

Ribeiro (1980) reported that in Brazil the dieback with symptomatology resembling tip dieback in Florida, was caused by *C. fimbriata* Ellis & Halst. Reckhaus (1987) reported its occurrence from Niger since early 1980' s . The fungus most often observed was *Hendersonula toruloidea* Nattras. He also considered that stress factors enhanced the severity of the disease.

Mango decline was the most destructive disease. Many abiotic (environmental factors, nutritional factors and cultural operations) and biotic (insects and fungi) factors were responsible for development of disease. Among abiotic factors water stress was the primary disposing factor for onset of disease (Reckhaus, 1987). The presence of symptoms was noticed in trees which were deficient in manganese, iron or both elements (Schaffer *et al*., 1988).

Rawal and Ullasa (1988) reported that mango decline was enhanced at relative humidity above 80 percent and temperature of 25 to 31ºC and rains. Masood *et al*. (2008) reported that improper irrigation and severe injuries to plants enhanced the

opportunity for disease to be established. Wounds caused by mechanical pruning may provide an entry point for infection, whilst severe pruning may increase plant stress.

Rawal (1998) reported that mango decline was enhanced by beetle (*Xyleborus affinis*). Masood *et al*. (2011) reported that *Hypocryphalus mangiferae* may be involved in dissemination and as a facilitating agent for the entry of the pathogens.

Ramos *et al*. (1991) reported that mango decline has appeared in Southern Florida since late 1970's. The etiology of disease has remained unknown since its first appearance in the late 1970's. They also provided the first report of *Botryosphaeria ribis* associated with mango dieback and its symptomatology was developed at Florida. Previously, it was reported in the Hawaiian Island in 1929. *B. ribis* induced severe die back in up to 83 percent of the inoculated plants. It invaded the vascular system of mango plants which lead to the production of tyloses in xylem vessels, formation of dark inclusions and presence of fungal hyphae which resulted in the restriction of water mineral transport and ultimately lead to die back.

Narasimhudu and Reddy (1992) found that mango trees in Andhra Pradesh were severely affected by gummosis. Darvas (1993) reported fungi associated with mango die back were *Alternaria alternata* and *Dothiorella dominicana.* Sharma (1993) reported *B. theobromae* as the primary cause of die back of mango and *C. gloeosporioides* (*Glomerella cingulata*), *Pestalotia mangiferae, Phoma sp*., *Sclerotium (Corticium) rolfsii, Rhizoctonia solani, Diplodia sp. and F. solani* were

also reported pathogenic in Himachal Pradesh. Johnson and Blazquez. (1992) reported that several fungi caused this disease, and the host and environmental factors influenced the prevalence of different species in different situations.

Ploetz *et al*. (1996) reported the fungi associated with mango die back were *A. alternata*, *D. dominicana* and *Phomopsis sp*. Ramos *et al*. (1997) reported fungi associated with mango die back disease were *B. ribis, Diplodia* sp., *Fusarium* sp., *C. gloeosporioides, Curvularia* sp., *Oidium* sp. They demonstrated that *B. ribis* and, less commonly, *Diplodia* sp. *a*s the primary causal organism of tip die back.

Simone (1999) reported the mango decline from Florida showing the similar symptoms and causative agents as described by Ploetz *et al*. (1996). Savant and Raut (2000) observed that die back of mango stone grafts was incited by *C. gloeosporioides* and *Lasiodiplodia theobromae* either alone or in combination. Al Adawi (2003) reported that in Oman, since 1999, up to 60 percent of trees were found affected in parts of the Al Batinah region. He stated that *D. theobromae* (*L. theobromae*) was associated with sudden decline of mango in the Sultanate of Oman. Ploetz (2003) had reported that establishment of *L. theobromae* in the soil had occurred due to the die back of shesham which was also caused by the same species of the fungi.

Khanzada *et al*. (2004b) reported that the decline disease was caused by *L. theobromae* and *Fusarium solani* played no significant role in the disease development. Malik *et al*. (2005a) reported that among other associated fungi, *B. theobromae* was the most common and frequent isolated fungus from entire diseased

samples collected from the plants infected with these disorders individually.

Mahmood *et al*. (2007) carried out the physiological studies of *L. theobromae* the cause of quick decline/sudden death of mango. *L. theobromae* causing sudden death of mango was air borne as well as soil borne pathogen. For its vegetative mycelial growth PDA adjusted at pH-7 was found to be the most suitable. This medium yielded maximum fungal mycelial growth when incubated at 25 °C under alternate light and dark conditions.

Shahbaz *et al*. (2009) reported *L. theobromae* was most frequently isolated fungus from mango plants showing the typical symptoms of quick decline in Pakistan. Javier *et al*. (2009) gave the first report of *Neofusicoccum parvum* associated with dieback of mango trees in Peru. Morales and Rodriquez (2009) reported endophytic fungi in mango 'Haden' grown at Venezuela eastern. The fungi were recorded by employing triple sterilization specific method for endophytous fungi. All the isolated fungi were recognized pathogens of mango. The following species were identified *Fusarium decemcellulare*, *L. theobromae*, *Pestalotiopsis* sp., *Cladosporium* sp. and *C. gloeosporioides*. The distribution of *L. theobromae* and *Cladosporium* sp. was continuous and systematic, without registering variations interphases as much detecting on vegetative as reproductive organs. *F. decemcellulare* and *Pestalotiopsis* sp. were recovered in vegetative organs. *C. gloeosporioides* was isolated in fruits pedicels. *L. theobromae* and *Cladosporium* sp. conidia were even detected within anthers along with the pollen grains. From these results it had come off that the endophytic colonization was an important route for the development of diseases in the mango 'Haden' as dieback or decline, anthracnose and floral malformation caused by *L. theobromae*, *C. gloeosporioides*, *F.*

decemcellulare, respectively.

Malik *et al*. (2010) reported confirmation of the capability of *C. fimbriata* to cause mango sudden death syndrome in Pakistan. The results of this study showed that *L. theobromae* and *N. mangiferae* in mango sudden death might act as secondary colonizers or weaker pathogens as compared to *C. fimbriata*. *L. theobromae* was also nonetheless capable of producing symptoms but with low severity. It needed further investigation as this botryosphaeriaceae fungus mostly lived endophytically and its true parasitic status was still unresolved.

Sakalidis *et al*. (2011) reported that pathogenic botryosphaeriaceae were associated with *Mangifera indica in* the Kimberley Region of Western Australia. The members of the botryosphaeriaceae, in particular *L. theobromae, N. parvum, N. mangiferum* and *B. dothidea*, commonly caused stem cankers, dieback and stem end rot of mangoes worldwide tested. All were pathogenic to mango in comparison to the *Lasiodiplodia* spp. being the most pathogenic. It appeared that either geographic isolation or the unique growing conditions in the Kimberley may have provided an effective barrier to the acquisition or establishment of known botryosphaeriaceous pathogens.

Masood *et al*. (2011) reported that *C. fimbriata* and *L. theobromae* were both pathogenic to mango causing mango quick decline in Pakistan. Ismail *et al*. (2012) reported that *Lasiodiplodia* species was associated with dieback disease of mango in Egypt. In addition to *L. theobromae*, a new species, namely *L. egyptiacae*, was identified. Furthermore, *L. pseudotheobromae* was also newly recorded on mango in Egypt.

Anwar *et al*. (2012) reported that fungi such as *B. theobromae, Alternaria, Acremonium, Scytalidium, Fusarium* and *Ceratocystis* stained both the surface and the deeper wood of mango. Hisang (2013) reported that the causal pathogen was identified as *Botryosphaeria dothidea* based on morphological, genetic (ITS) and pathogenicity analysis. This was thought to be the first report of *B. dothidea* causing mango gummosis in China.

Marques *et al*. (2013) reported that *Botryosphaeria, Neofusicoccum, Neoscytalidium* and *Pseudofusicoccum* species associated with mango in Brazil. *B. mamane* was reported for the first time in association with mango diseases worldwide. All species were pathogenic on mango fruit. There were significant differences in virulence among the species, with *N. dimidiatum* and *N. parvum* being the most virulent species, while *P. stromaticum* was the least virulent.

Marques *et al*. (2013) reported different species of *Lasiodiplodia* associated with mango die back in Brazil. The following species were identified: *L. crassispora, L. egyptiacae, L. hormozganensis, L. iraniensis, L. pseudotheobromae, L. theobromae* and *Lasiodiplodia* sp. *L. theobromae* was the most frequently isolated species. *L. crassispora* was reported for the first time associated with mango diseases worldwide. There were significant differences in mycelial growth rates among the *Lasiodiplodia* species and also in the optimum temperature for growth. All species of *Lasiodiplodia* were pathogenic on mango fruits.

Al-Adawi *et al.* (2013) reported that the mango sudden decline pathogen, *Ceratocystis manginecans*, was vectored by *H. mangiferae* in Oman. The results showed that *H. mangiferae* vectors *C. manginecans* in Oman and were, therefore, an important factor in the epidemiology of this disease.

2.5. Pathogenicity

Khanzada *et al.* (2004b) confirmed the pathogenicity of *L. theobromae* on mango by using stem inoculation method. A 1-2-cm inoculum block from 5 day old culture on potato sucrose agar (PSA) was placed in the cut portion of mango plant and the inoculated portion was wrapped with parafilm. 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 the pathogen was re-isolated from roots, stem, and branches of the test plants to confirm the pathogenicity.

Shahbaz *et al.* (2009) studied pathogenicity of *L. theobromae* by stem inoculation method. The artificial inoculations were done by cutting a small flap on the basal portion of the mango stem and inserting a 5 mm potato dextrose agar piece containing viable culture of the fungus (*L. theobromae*). After 30 days, lesion development was measured distal to the point of inoculation. Re-isolations were made from diseased tissue to confirm pathogenicity of the fungi.

Masood *et al.* (2011) conducted pathogenicity of two pathogens *Ceratocystis fimbriata* and *L. theobromae* which were frequently isolated from diseased tree as well as from the bark beetles. Inoculation of fungi was made by placing a piece of

fungal colony (5 mm, obtained from leading edges of actively growing fungal culture on PDA) in slating cuts under the bark with sterilized scalpel and then covered with para film. In healthy control, only agar slab without fungus was placed in slanting cuts under the bark. For re- isolation of fungi, total 12 to 15 stem pieces were excised from the above and below the point of inoculation sites and plated on PDA. The fungal growth obtained was compared with the representative isolates for confirmation.

Li *et al.* (2013) studied pathogenicity of five isolates of *L. theobromae* under field conditions on 3 year old mango trees. Mycelial plugs of the pathogen were inoculated in wounds on branches with sterile needles and covered by parafilm. Un-colonized PDA plugs were inoculated on control treatments. After two weeks, typical brown lesions with exudation of gum were noticed in branches inoculated with fungus colonized plugs while the control did not produce any symptoms. Koch's postules were fulfilled by reisolation of *L. theobromae* from diseased branch and confirmed as a pathogen of mango. *L. theobromae* is also found pathogenic on several hosts (Table 2.2)

Table 2.2. Host range of *Lasiodiplodia theobromae*

Sl.No.	Host	Disease	Scientific name	Scientist
1	Papaya	Fruit rot	*Carica papaya*	Hunter *et al*. (1969)
2	Horsegram	Seed rot	*Dolichous biflorus*	Maholay and Sohi (1977)
3	Dates	Decaying	*Delonix regia*	Omamor (1988)
4	Dogwoods	Canker	*Cornus florida*	Mullen *et al.*(1991)
5	Lemon	Fruit rot	*Citrus aurantifolia*	Cedeno and Palcios-pru (1992)
6	Guava	Fruit rot	*Psidium guajava*	Patel and Pathak(1993)
7	Pea nut	Collar rot	*Arachis hypogaea*	Phipps and Porter (1998)
8	Mango	Dieback	*Mangifera indica*	Simone (1999)
9	Coconut	Fruit rot	*Cocus nusifera*	Gunasekaran and Srinivasan (2000)
10	Parthenium	Foliar pathogen	*Parthenium hysterophorus*	Kumar and Singh (2000)
11	Yellow passion fruits	Black rot	*Passiflora edulies* f. sp. *flavicarpa*	Viana *et al.* (2000)
12	Sweet potato	Java black rot	*Ipomoea batatas*	Pati *et al.*(2001)
13	Shisham	Decline	*Dalbergia sissoo*	Khan *et al.* (2004)
14	Kumquat	Decline	*Fortunella margarita*	Ko *et al.* (2004)
15	Jackfruit	Leaf blight	*Artocarpus heterophyllus*	Haquet *et al*. (2005)
16	Guava	Wilt	*Psidium guava*	Pandit and Samajpati (2005)
17	Aubergine	Fruit rot	*Solanum melongena*	Woodward *et al.* (2005)
18	Cashew	Gummosis	*Anacardium*	Cardoso *et al.* (2006)
19	Banana	Crown rot	*Musa paradisiaca*	Alvindaa and Natsuaki (2007)
20	Jatropha	Gummosis	*Jatropha podagrica*	Fu *et al*. (2007)
21	Pawpaw	Stem-end rot	*Asimina tribola*	Wang *et al.*(2007)
22	Grapevine	Dieback	*Vitis vinifera*	Burrano *et al.* (2008)
23	Cattleya	Necrotic spots on stem	*Cattleya labiata*	Cabrera and Cudom (2013)

24	Ballon	Dark necrosis	*Asclepias physocarpa*	Fisher *et al.* (2008)
25	Jute	Stem end rot	*Corchorus olitorus*	Sato *et al.* (2008)
26	Cocoa	Dieback	*Theobromae cocoa*	Kannan *et al.* (2009)
27	Mamey trees	Dieback	*Pouteria sapota*	Lopez *et al.*(2009)
28	Nutmeg	Fruit rot	*Myristica fragrans*	Attah and Ahiatsi (2010)
29	Eucalyptus	Gummosis	*Eucalyptus citriodora*	Khalil (2010)
30	Peach	Gummosis	*Prunas percisa*	Simas-Tosin *et al.* (2010)
31	Bottle gourd	Seed rot	*Lagenaria siceraria*	Sultana and Ghaffer (2010)
32	Cycas	Dieback	*Cycas circinalis*	Chakraborty *et al.* (2011)
33	Cassava	rot	*Manihot esculenta*	Bua and Okello (2011)
34	Mulberry	Stemcanker	*Morus alba*	Kumari and Sukumar
35	Pummelo	Fruit rot	*Citrus maxima*	Luo *et al.*(2011)
36	Euphorbia	Decline	*Euphorbia ingens*	Linde *et al.* (2012)
37	Kinnow fruits	Stem end rot	*Citrus reticulata*	Sharma *et al.* (2011)
38	Avacado	Fruit rot	*Persea americana*	Bertetti *et al.*(2012)
39	Mangosteen	Decline	*Garcinia mangostana*	Paim *et al.* (2012)
40	Tuberose	Peduncle blight	*Polianthes tuberosa*	Durgadevi and Sankaralingam (2012)
41	Sapota	Dieback	*Achras sapota*	Tovar *et al.* (2012)
42	Ficus	Dieback	*Ficus carica*	Rehab *et al.* (2014)
43	Longan	Infloresence blight and fruit	*Dimocarpus longan*	Diaz *et al.* (2014)
44	Elephant tree	Canker	*Boswellia papyrifera*	Gezahgne *et al.*(2014)

2.6. Survey

According to Central Mango Research Station (1974-85) report, 30 to 40 percent disease incidence of dieback was reported from Moradabad region, in Uttar Pradesh, varieties Langra, Chausa, Dashehari and Fajli were susceptible (40.6-85.7 percent) to mango die back in Durgapura region of Jaipur, Rajastan.

Maduleti (1989) conducted a survey for mango dieback disease incidence in different varieties of mango growing orchards of Chittoor and Cuddapah districts of Andhra Pradesh and reported 0 to 40 per cent disease incidence in different orchards.

Iqbal *et al.* (2007) investigated on mango gummosis and outlined a survey to assess the prevalence, incidence and intensity of different decline disorders prevailing in mango- growing areas of Punjab (Pakistan). Sixteen locations were visited in 4 districts of Punjab to evaluate the incidence. Four disorders, *i.e*., twig blight, tip dieback, gummosis and bark cracking/splitting, were recorded with 55, 50, 25 and 25 percent prevalence, respectively. The incidence percentage in the same order was 3.17, 4.43, 0.62 and 1.25 percent while the intensity ranged from 16 to 50 percent. The maximum intensity of 5.17 percent for quick decline was noted in Sahiwal district. Chaunsa proved to be the most susceptible cultivar with 6.95 and 3.14 percent incidence and intensity, respectively.

Panhwar *et al.* (2007) conducted a survey on mango sudden decline disease in major mango growing districts of Sindh, Pakistan and maximum disease severity was observed in Hyderabad (4.76 %) followed by Tandu Allahyar (4.18%) and minimum disease severity in Naushahro feroz (1.32 %) and Khirpur (1.46 %).

Rehman *et al*. (2011) surveyed Mango Sudden Death Syndrome disease incidence in four mandals of Muzaffargarh district and found that Kotaddu was the most affected tehsil followed by Alipur and Jatoi, while least affected was Muzaffargarh. Maximum incidence was recorded on variety Chaunsa, followed by Sindhri, Anwar Ratol and Malda. The infected plants showed abundant gum secretion, bark splitting, rotting and twig blight. The fungus *Ceratocystis manginecans* was isolated in maximum percentage, followed by *L. theobromae*. The isolation of these two fungi from all diseased samples suggests that both of them are responsible for Mango Sudden Death Syndrome.

Hui Fang *et al.* (2012) studied mango fruit rot incidence in major mango-producing areas of southern Taiwan, including Guntain, Fanshan, and Yujing during 2009-2011. A disease incidence ranging from 18.7 percent to 58.1 percent, with those of Guntain significantly higher than the incidence found in Yujing and Fanshan. Li *et al.* (2013) carried out a field survey in southern provinces of China during 2012. An outbreak of gummosis was observed in the southern province involving over 30,000 ha with an average of 50 percent disease incidence (PDI) and a maximum of 70 percent in some orchards.

Meer *et al.* (2013) conducted a systematic survey during October, 2011 to assess the status of major post-harvest diseases of mango fruit and the losses due to these diseases in the local markets of Punjab. The study revealed that anthracnose and stem end rot diseases were prevalent 100 percent in the markets of Punjab and *C. gloeosporioides*, *L. theobromae*, *Alternaria alternata* and *Aspergillus niger* were the fungal pathogens associated with anthracnose, stem end rot, Alternaria rot and

Aspergillus rot diseases and these were major post-harvest diseases that damaged the mango fruit.

Diaz *et al.* (2014) surveyed in Puerto Rico and they observed fruit rot and inflorescence blight (rotting of the rachis, rachilla, and flowers) in fields of Longan.

2.7. Morphological and cultural characteristics of the *L. theobromae*

2.7.1. Morphological characters

Sabalpara *et al.* (1991) described morphological variation among *B. theobromae* isolates causing mango twig-blight/die-back. They reported that size of the immature and mature pycnidia varied greatly with the substrate. The pycnidia were smallest in naturally infected twigs and biggest in nutritionally rich medium such as oatmeal agar. Distinct variation was not observed in the size of immature and mature conidia. It is suggested from this study that measurement range of mature pycnidia (189-886 x 154-704 µm) should be taken into account for identification of a species (Punithalingam, 1976).

Morphology of *L. theobromae* on mango was described by Mirzaee *et al.* (2002). According to them the pycnidia are mostly aggregated, spherical and dark brown in colour with thick walls; the conidia are two celled, oval and dark brown in colour produced on potato dextrose agar (PDA). Morphology of *L. theobromae* on poplar twigs was studied by Alves *et al.* (2008), they reported that the pycnidia are uniloculate, dark brown to black, immersed in the host becoming erumpent when mature. Paraphyses were hyaline, cylindrical, and septate, occasionally branched, ends rounded, up to 55 µm long, 3-4 µm wide. Conidia sub ovoid to ellipsoid-

ovoid, apex broadly rounded, tapering to truncate base, widest in middle to upper third, thick-walled, contents granular, initially hyaline and aseptate, remaining hyaline for a long time, finally became dark brown and one-septate but only after discharge from the pycnidia, with melanin deposits on the inner surface of the wall arranged longitudinally giving a striate appearance to the conidia.

Shahbaz *et al.* (2009) detailed the growth of *L. theobromae* on PDA. Cultures were found to be initially white to smoke grey with fluffy, aerial mycelium on PDA. Colonies soon became gray or black and fast spreading with immersed, superficial and branched septate mycelia. The upper surface gradually developed prominent fruiting bodies. Shiny black pycnidia were produced on the surface. Conidia were initially hyaline, unicellular and sub-ovoid to ellipsoid, with a granular content. Mature conidia were bi-celled, cinnamon to dark brown, thick walled, ellipsoidal. Concisely, this study identified black coloured mycelia with few medium greys, not grouped, sub globose pycnidia, fawn coloured and ellipsoid conidia as main morphological and physical features of most of the isolates of *L. theobromae.*

Shah *et al.* (2010) reported that thirteen isolates of *B. theobromae* collected from pear varieties grown in various regions of Punjab were studied for morphological, pathological and molecular characterization. The mycelial growth of *B. theobromae* isolates was classified as fluffy or depressed, uniform to irregular and cottony white turning to black. Colony growth rate varied from 19.1 to 24.9 mm per day. Pycnidia were produced either on the edge, centered or scattered on Petri dishes after 20 to 34 days of incubation. Pycnidia and pycnidiospores ranged in size from 118 to 240 µm and 14.5-35.5 x 6.5-14.5 µm, respectively. Lesion length of different isolates

ranged from 1.9-7.2 x 0.8-3.3 cm with 49.4-90.9 percent infection.

L. theobromae colonies had copious, white, aerial mycelia that turned grey to black with age and formed black pycnidia, Pycnidiospores were oval, greenish brown, with one septum in the middle with a dimension were 20.9-27.5 x 11.0-15.4 µm (Celiker and Michailides, 2012).

Twumasi *et al.* (2014) concluded that the colour of the *L. theobromae* colony was white at the beginning and it gradually turned dark gray. Similarly, the hyphae were also hyaline initially, later turning to dark brown. After 5 days of growth at room temperature, abundant hyphal aggregates were observed on the surface of the colonies. These later developed into stromatic structures containing pycnidial locules. Pycnidiospores (conidia) grow within the locules and were initially hyaline, oval-shaped, one-celled and thick- walled, but later became dark brown, two-celled and longitudinally striated on their surface.

2.7.2. Cultural characters

Alam *et al.* (2001) identified *B. theobromae* as the causal agent of crown rot disease of banana. In their studies they found that 25-30°C temperature optimum for the growth of the pathogen and highest sporulation occurred at 30°C. Similar observations were recorded by Eng *et al*. (2003) when they studied the effect of temperature on growth characteristics of *B. theobromae*. They reported that mycelium growth was higher in glucose and sucrose containing media because of containing presence of ‘Carbon’ sources. Ray (2004) also observed in his studies

that lactose and glucose had similar effect on growth of *B. theobromae.*

Fu *et al.* (2007) described that optimum temperature of *L. theobromae* was 28°C which was responsible for *Jatropha podagrica* gummosis. They also reported PDA and PSA media were most suitable for vegetative growth. Effects of culture media, temperature and light on mycelial growth and pycnidial production of *L. theobromae* was studied by Khanzada *et al.* (2006). They reported Potato sucrose agar (PSA), Corn meal dextrose agar (CMDA) and Yeast extract manitol agar (YEMA) were most suitable for mycelial growth but Potato carrot agar (PCA) was not suitable for either mycelial growth or pycnidia production. YEMA medium was found best medium for pycnidial formation. The fungus grew from 20 to 45ºC, with optimum growth at 30-40ºC with no growth below 15ºC. Maximum number of pycnidia was produced at 35-40ºC. Different light regimes had no impact on myceliumal growth and pycnidia production.

Effects of culture media, carbon source, nitrogen source, temperature, pH and light on mycelial growth and sporulation of *L. theobromae* were studied by Saha *et al.* (2008). Among several carbon sources tested, glucose and sucrose were found superior for growth. Potassium nitrate supplemented media showed maximum growth amongst the tested inorganic nitrogen sources while peptone produced maximum growth among the tested organic nitrogen sources. Tea root extract supplemented potato dextrose agar medium was found to be the most suitable for mycelial growth and sporulation of *L. theobromae*. The fungus grow at temperatures ranging from 4° to 36°C, with optimum growth at 28°C and such growth was lacking at 40°C. There was no significant effect of different light period on growth

of *L. theobromae*, but light enhanced sporulation. The fungus grew well at a pH 3.0 to 8.0 but optimum growth was observed at pH 6.0.

Morphologically and phylogenetically different strains of *Lasiodiplodia* spp. were studied by Sanchez *et al.* (2013). According to them most of the isolates initially developed (1-3 days) grayish-white cottony mycelium with fast and abundant growth, and then switched to olive-gray. Pycnidia were black, pear shaped and ostiolated. *L. theobromae* produced hyaline immature aseptate conidia (amerosporae) which are ellipsoid to subovoid, thick walled with granulated cytoplasm; 20 to 31.02 x 11.36 to 16.36 µm. Mature conidia showed a septum (Didimosporae), dark brown, ellipsoid to ovoid, with irregular longitudinal striations.

Latha *et al.* (2013) investigated on collar and root rot of Physic nut (*Jatropha curcas*) caused by *L. theobromae*, which is an important soil borne disease that caused considerable yield loss. They studied the effects of culture media, temperature, photoperiod, carbon and nitrogen sources and pH on mycelial growth and pycnidial production of *L. theobromae*. Among the growth media tested, potato dextrose agar (PDA) supported the highest growth followed by potato sucrose agar (PSA) and corn meal agar (CMA). Among several carbon sources tested, carboxy methyl cellulose and sucrose were found superior for growth and pycnidial production. The nitrogen sources, viz., ammonium oxalate and ammonium dihydrogen phosphate supported maximum mycelial growth and pycnidial production. The fungus grows at pH 5.0-9.0 and optimum growth was observed at pH 7.0.Venugopal (2013) also categorized 373 isolates of *L. theobromae* isolated

from rotting nuts of coconut into three major groups, viz., dark gray, greyish black and white type. They observed that isolates of dark gray and greyish black group did not exhibit much variation in response to temperature and have shown highest growth at 30°C on PDA whereas the selected isolate of white colony group showed the highest growth at 25°C. Growth of selected isolates of all three groups was found lowest at 10°C.

2.8. Disease management

Mango production remains hampered due to number of diseases at all stages of development i.e. from nursery to the consumption of fruits and it is estimated that production could be increased by 28 per cent if the crop is protected against various diseases (Rawal, 1998). Among infectious diseases, a new disease commonly known as quick decline, has proved to be the most destructive one causing sudden death of plants within few days (Mahmood and Gill, 2002; Sial, 2002). Growers are perturbed over this fatal disease as its incidence in some areas of Pakistan was 75.1 percent (Iqbal *et al*., 2007) which was highly alarming for future of this precious fruit. Due to the complexity of this emerging threat, it has become important to undertake investigation in all possible ways on management of this disease.

Earlier reports in literature on disease control have been limited either to the adoption of the cultural methods like pruning and destruction of infected twigs which was the foremost practice and effective control of this disease. Pasting of pruned trees with a mixture of oil and 5 percent phenol was found effective (Anonymous, 1987), Selection of scion from healthy trees, sterilization of budding

knife, placement of budded tree in dry environment and gradual exposure of it towards sun light were found effective to check the disease under Puerto Rico conditions (Pathak, 1980). Pruning of diseased branches and spraying with 1 per cent Bordeaux mixture was also found effective in controlling the disease (Anonymous, 1987). However in the recent years, efforts have been made to manage the disease through identification of sources of resistance, use of bio-control agents and use of wide array of fungicide spray application besides the adoption of cultural methods.

2.8.1. Use of fungicides against *L. theobromae*

2.8.1.1. *In vitro* screening of fungicides

Sharma and Badiyala (1994) studied the effectiveness of eight fungicides (carbendazim, thiabendazole, thiophanate methyl, mancozeb, copper oxychloride, aeurofungin, Bordeaux mixture and benomyl) against mango gummosis and dieback. Carbendazim was the most effective treatment followed by aeurofungin.

Rawal and Ullasa (1988) reported that mango gummosis was effectively controlled by pruning the infected twigs followed by spraying of carbendazim (0.1%) or Topsin M (0.1 %) or chlorothalonil (0.2 %). Mahmood and Gill (2002) tested the efficacy of fungicides against *L. theobromae* under *in vitro* conditions and reported that Topsin-M and benlate are effective even at 20 ppm and 100 ppm.

Banik *et al*. (1998a) conducted *in vitro* testing of seven fungicides (viz. carbendazim, thiophanate methyl, fosetyl- aluminium, benomyl, mancozeb, metalaxyl and carboxin) against *L. theobromae*. The results stated that carbendazim

completely inhibited the growth of *L. theobromae* at 400 ppm followed by thiophanate methyl at 450 ppm.

Mahmood and Gill (2002) has reported that under *in vitro* conditions thiophanate methyl and benomyl were effective even at 20 ppm and 100 ppm, whereas copper oxychloride was least effective fungicide against *L. theobromae*.

Khan *et al*. (2004) conducted an experiment *in vitro* to determine the efficacy of different fungicides, *i.e.*, Topsin M 70 WP (thiophanate-methyl), Score 25 EC (difenoconazole), Tri-miltox forte (copper carbonate (basic)+copper oxychloride + copper sulfate + mancozeb) and Dithane M-45 (mancozeb) at 10, 20, 50 and 100 ppm, against *B. theobromae*, the causal agent of shisham decline. The data revealed that Topsin M and Score to be the most effective fungicides at 100 ppm concentration while Tri-miltox forte was the least effective against the fungus at all concentrations.

Khanzada *et al.* (2005) reported that mycelial growth of *L. theobromae* was significantly inhibited by carbendazim and thiophanate-methyl when used @ 1 ppm a.i. or more. Alliete was effective at relatively high concentrations i.e., @ 1000 and 10000 ppm a.i., whereas, Copxykil, Cuprocaffaro and Thiovit failed to inhibit the mycelial growth of *L. theobromae*.

Malik *et al*. (2005b) reported the *in vitro* sensitivity of fungus *B. theobromae* to three fungicides viz. thiophanate methyl, copper oxychloride and captan when tested by poisoned food technique.

Fu *et al.* (2007) tested efficacy of fungicides against *Jatropha podagrica*, gummosis caused by *B. theobromae*. The results indicated that the pathogen was highly sensitive to prochloraz (333.33 mg/kg), carbendazim (800 mg/kg), thiram (1000 mg/kg), flusilazole (44.44 mg/kg), propiconazole (375 mg/kg), thiophanate-methyl (700 mg/kg) and mancozeb (1142.86 mg/kg) in which the growth inhibition rates were higher than 90 per cent, and low to chlorothalonil (1400 mg/kg), dimethomorph (538.46 mg/kg), azoxystrobin (250 mg/kg), cupric hydroxide (538 mg/kg), triadimefon (1000 mg/kg) and cuprous oxide (492.57 mg/kg), in which the growth inhibition rates were lower than 75 percent.

Javaid *et al.* (2008) evaluated four fungicides, viz., Acrobat MZ, Dithane M-45, mancozeb and metalaxyl plus mancozeb (recommended doses (R), 0.75R, 0.50R and 0.25R) *in vitro* against *B. theobromae* causing mango dieback and fungicides significantly even at low concentrations reduced the biomass of the test fungus these fungicides are highly effective even at an against the pathogen.

MeiJiao *et al.* (2009) tested the efficacy of 23 fungicides against *L. theobromae*. The results revealed that spergon, propiconazole, flusilazole, prochloraz, iprodione, difenoconazole, tebuconazole, myclobutanil, pyraclostrobin, validamycin, carbendazim, chlorothalonil and mancozeb are effective for the management of *L. theobromae*.

Sales *et al.* (2009) evaluated different fungicides for the control of stem-end rot in mango cv. 'Tommy Atkins'. Among the fungicides evaluated, difenconazole was found to be the best fungicide in controlling mango stem-end rot compared to azoxystrobin, chlorothalonil, nonylphenolethoxylate and propiconazole

Shahbaz *et al.* (2009) evaluated five fungicides, *viz*., thiophanate-methyl, carbendazim, precurecombi (thiophanate-methyl + diethofencarb), copper oxychloride and captan) against *L. theobromae* at two concentrations, 50 and 100 ppm respectively. Thiophanate-methyl, carbendazim and precurecombi showed 100 per cent inhibition over control at 50 and 100 ppm.

Attah and Ahiatsi (2010) revealed that topsin-M (thiophanate-methyl) and carbendazim were the most effective fungicides against *L. theobromae,* the causal agent of nutmeg fruit rot in Ghana. These fungicides suppressed mycelial growth of the fungus completely at all the concentrations (25 to 200 ppm a.i.) tested. Maneb was also found effective against the fungus but at a relatively higher concentration. Funguran-OH (Copper hydroxide) was the least effective against *L. theobromae*.

Bhatt and Jadeja (2010) tested the efficacy of 13 fungicides (carbendazim, propiconazole, triadimefon, thiophanate methyl, triadimorph, hexaconazole, difenzole, fosetyl AL, carbandazim + copperoxychloride, iprodione + carbendazim, mancozeb + carbendazim, cyamoxanil + mancozeb, mancozeb + metalaxyl at 250, 500, 750, 1000 ppm) against *L. theobromae* causal agent of die back and post-harvest diseases. They reported that carbendazim was completely inhibiting the pathogen at all concentrations and alsofound that carbandazim + copper oxychloride, iprodione + carbendazim, mancozeb + carbendazim also completely inhibit the growth of *L. theobromae*.

Studies conducted by Sahi *et al.* (2012) revealed the effectiveness of Topsin M and Daconil against the mycelial growth of *B. theobromae*, the causal organism of

quick decline of mango (*Mangifera indica* L.) Mancozeb was found least effective in inhibiting the mycelial growth of *B. theobromae*.

Sahi *et al*. (2012) reported the *in vitro* sensitivity of fungus *B. theobromae* to four fungicides viz., thiophanate methyl, chlorothalonil, copper oxychloride and mancozeb when tested by poisoned food technique (Falck, 1907). Three concentrations (10, 20, 40 ppm) of each fungicide were added to PDA medium at the time of pouring into 9 cm glass Petri plates. After solidification, 5 mm discs of seven days old culture of *B. theobromae* were placed in the centre of the test plates and incubated at temperature of 25±1°C. The data on mean colony growth of the fungus was recorded after 2 and 4 days of inoculation. It revealed from analysis that thiophanate methyl and chlorothalonil proved to be best fungicides as compared to others.

Bhadra *et al*. (2014) studied the effectiveness of carbendazim and mancozeb against mango gummosis and dieback. They reported carbendazim effective to control *L. theobromae* at 70 ppm whereas mancozeb showed no significant effect at any concentration.

2.8.1.2. *In vivo* screening of fungicides

For effective control of the gummosis disease, pruning and destruction of infected twigs is the foremost practice. Spraying the trees periodically with copper oxychloride is recommended by Alvarez and Lopez (1971). Pasting of trees with a mixture of oil and 5 per cent phenol was found effective (Batista, 1947). Sprays of carbendazim (0.1%) or methyl thiophanate (0.1%) or chlorothalonil (0.2%) at

fortnightly interval during rainy season is important. Sometimes shot hole borers also predispose the trees to infection and hence proper insecticides are also to be sprayed. Healthy twigs should be selected for grafting of seedling during propagation. Several authors have evaluated various fungicides against mango gummosis.

Rawal *et al*. (1983) reported that spraying of infected orchards with 0.1 percent carbendazim or 0.2 percent daconil at 15 days intervals has been found effective to control the fruit rot of papaya caused by *L. theobromae*.

Rawal and Ullasa (1988) also reported that mango gummosis and dieback was effectively controlled by pruning the infected twigs followed by spraying of carbendazim (0.1%) or thiophanate methyl (0.1%) or chlorothalonil (0.2%).

Khanzada *et al*. (2005) evaluated the effect of three fungicides under field conditions viz., Carbendazim @ 2 g/l, thiophanate methyl @ 1.43 g/l and fosetyl-aluminium @ 1.25 g/l. In field experiment, carbendazim was found to be more effective than thiophanate methyl and fosetyl-aluminium in reducing the fungal infection in mango plants, suppressing the gum exudation, dieback and wilting resulting in significant enhancement in vegetative growth of plants.

Sultana and Ghaffar (2010) reported thiophanate methyl and carbendazim as most effective fungicide in reducing root infection of bottle gourd (caused by *L. theobromae*) followed by fosetyl-aluminium, benomyl, carboxin. Maximum reduction in seedling mortality was exhibited by benomyl, fosetyl-aluminium,

carbendazim and thiophanate methyl. Similarly, the maximum seed germination was exhibited by fungicides benomyl and thiophanate methyl followed by carbendazim, fosetyl-aluminium, carboxin, mancozeb and metalaxyl.

Ojha *et al.* (2010) evaluated five fungicides (carbendazim, captan, mancozeb, thiophanate methyl and tridemorph) against *L. theobromae*, the causal agent of die-back in *Dalbergia sissoo* in different regions of Burdwan district, West Bengal. Among them carbendazim was found to be the most effective followed by thiophanate methyl.

2.8.2. Use of botanicals

Sardsud *et al.* (1994) reported the antifungal activity of *Acorus calamus*, garlic, *Centellaa siatica*, *Cyperus rotundus, Languas galangal* and *Rhinacanthus nasutus* plant extracts against *L.theobromae* at 0.001, 0.01, 0.1 and 1.0 percent concentration. Among them *A. calamus* extract at 1.0 percent completely inhibited the growth of *L. theobromae*.

Bankole and Adebanjo (1995) tested the *in vitro* and *in vivo* efficacy of leaf extracts from 5 plants (*Cymbopogon citratus, Azadirachta indica, Morinda lucida, Chromolaena odorata* and *Delonix regia*) in inhibiting the growth of *B. theobromae* in Nigeria. Aqueous extracts of *C. citratus* completely inhibited the growth of *B. theobromae* followed by extracts from *A. indica*. Extracts from *C. odorata* and *D. regia* were ineffective.

Studies conducted by Lima *et al.* (1996) on aqueous extracts from garlic (0.1; 1.0; 10.0; 30.0; 50.0 g L^{-1}) and their volatiles against *L. theobromae* under *in vitro*

conditions. They revealed that, 30.0 and 50.0 g/litre completely inhibited the mycelial growth of the pathogen and the extract volatile components (10.0 to 50.0 g/litre) was acted as inhibitory to the germination of the pathogen.

Nwachukwu *et al.* (2001) studied the efficacy of leaf extracts of basil (*Ocimum basilicum*), bitter leaf (*Vernonia amygdalina*), lemon grass (*Cymbopogon citratus*), neem (*A. indica*) and papaya (*Carica papaya*) against major seed borne fungus of African yam bean seeds, *B. theobromae*. Neem extract proved most effective, while lemon grass extract was the least. Leaf extracts of neem, basil, bitter leaf and papaya, which are cheap and environmentally safe are promising for protecting African yam bean seeds against major seed-borne fungi.

Dubey *et al.* (2008) evaluated essential oil extracted from the leaves of *Amomum subulatum* (Zingiberaceae) for mango fruit rot control. The essential oil of *A. subulatum* exhibited absolute antifungal activity against two mango rotting fungi, *viz*., *B. theobromae* and *C. gloeosporioides*, the common storage fungi causing stem end rot and anthracnose disease of mango fruits. The oil showed its absolute fungitoxicity at the minimum inhibitory concentration of 500 µL/L.

Okigbo *et al*. (2009) investigated *in vitro* fungitoxic effects of *Allium sativum* (L.) and *Ocimum gratissimum* (L.) against cassava root rot fungi, *viz*., *F. oxysporum, F. solani, B. theobromae, Macrophomina phaseolina, Penicillium oxalicum* on mycelial growth of all tested fungi, extracts of *O. gratissimum* showed slight to moderately effective inhibition on mycelial growth of all fungi, with the exception of *B. theobromae* and *M. phaseolina*, which showed the lowest percentage of inhibition with both plant extracts.

Sharma *et al.* (2011) tested aqueous extract of nine indigenous medicinal and aromatic plants on *B. theobromae* in which *A. sativum* inhibited 100 percent mycelium growth of the pathogen followed by *Curcuma longa* (77.35 percent).

Sahi *et al.* (2012) studied the effectiveness of neem (*A. indica*), garlic (*A. sativum*), onion (*Allium cepa*) and safeda (*Eucalyptus camaldulensis*) extracts against the mycelial growth of *B. theobromae.* Safeda and neem extracts were found most effective while garlic and onion extracts were comparatively and statistically less effective in inhibiting the vegetative growth of the fungus.

Khewkhom *et al.* (2013) conducted bioassay of rhizome crude extracts of the Zingiberaceae family, *Alpinia galanga, Zingiber montanum, Curcuma longa and C. zedoaria* against *L. theobromae*, C. *gloeosporioides, Pestalotiopsis* spp., and *Phomopsis* spp., the causal agents of fruit rot of mangosteen. They found that *A. galangal* extracts was most effective than other extracts.

Kumah *et al.* (2013) evaluated different plant extracts, viz., *Moringa oleifera* (leaf extract), *A. indica* (seed extract) and *Cassia alata* (leaf extract) and *Zingiber officinale* (rhizome extract) against *B. theobromae* the main causative agent of crown rot disease in the eastern region of Ghana. *Z. officinale (*rhizome extract) at a concentration of 66.67 percent w/v was the most effective of the botanicals tested against crown rot disease.

Further, the leaf extract of Zimmu (an interspecific hybrid of *Allium cepa L. X Allium sativum* L.) and tuber extract of *Zehneria scabra* also inhibited mycelial growth and spore germination of *L. theobromae* and *Colletotrichum musae*, the causal agents of crown rot disease of banana. The efficacy of these plant extracts was attributed to direct fungitoxic property against the test pathogens and elicitation of defense related compounds in banana fruits (Sangeetha *et al*., 2013).

2.8.3. Use of bio agents against *L. theobromae*

Biological control of plant pathogens by micro-organisms has been considered a more naturally and environmentally acceptable alternative to the existing chemical treatment methods. The control of plant pathogens by biocontrol agents though has been found to be less effective and more variable than competitive chemical pesticides yet it has become one of the important control measures in IDM strategy against many soil borne diseases, being an eco-friendly nature.

Among fungal genera investigated as biocontrol agents against *B. theobromae*, the genus *Trichoderma* received great attention. *Trichoderma* spp. are now the most common fungal biological control agents that have been comprehensively researched and deployed throughout the world. The earlier workers Mortuza and Ilag (1999), Yadav and Majumdar (2005), Kunz (2007) have reported antagonistic potentiality of *Trichoderma* spp. against *L. theobromae*. Mortuza and Ilag (1999) reported that *T. harzianum* exhibited the greatest inhibition in dual culture whereas Yadav and Majumdar (2005) reported that *T. viride* was more effective than *T. harzianum*. *T. harzianum* had antagonist effect against *B. theobromae*, *Colletotrichum gloeosporioides* and *Fusarium oxysporum* f.sp. *pini* (Sivakumar *et al*., 2000; Dar *et al*., 2013).

Thangavelu *et al.* (2007) evaluated various species of *Trichoderma* against the banana crown rot pathogen, *L. theobromae*. They revealed that the *Trichoderma* species, *viz., T. pseudokoningii* and *T. viride* (Isolates, S7, RT1, and S17), and two *Pseudomonas* species, *P. aeruginosa* and *P. viridiflava*, were found to inhibit the mycelial growth and conidial germination of the pathogen. Further, Prasad *et al*. (2014) found *T. harzianum* and *T. koningii* to be effective against *L. theobromae* responsible for die-back of sissoo. Similarly, *T. harzianum* and *T. koningii* were effective against banana crown rot pathogen *L. theobromae* (Sangeetha *et al.*, 2009).

Priya and Nagaveni (2009) tested efficacy of six *Trichoderma* species (*T. viride, T. harzianum, T. pseudokoningii, T. koningii, T. virens* and *T. hamatum*) against *L. theobromae*, pathogen of fruit rot of *Elaeocarpus munronii*. *T. virens* and *T. hamatum* were found effective in inhibiting the pathogen by producing volatile metabolites and *T. pseudokoningii* was the effective inhibitor of pathogen through non-volatile metabolite production.

Sangeetha *et al*. (2009) tested the effect of twelve isolates of *Trichoderma* spp. from the soil of organic banana orchards ('native isolates') and eight isolates of *Trichoderma* spp. from culture collections ('introduced isolates') on *L. theobromae* and *C. gloeosporioides*. Eight isolates of *Trichoderma* spp. as single antagonists, did not satisfactorily controlled crown rot on the fruits as compared with carbendazim. However, two isolates of *T. viride*, one of *T. harzianum* and one of *T. koeningii* performed well when applied singly and these were selected for evaluation in isolate mixtures. Out of the eleven two- way and four- way mixtures of these isolates, the four- way and three way mixtures reduced crown rot incidence, both at room temperature and in cold storage.

Sultana and Ghaffar (2010) evaluated effectiveness of biocontrol agent's viz. *T. harzianum*, *T. viride*, *Gliocladium virens*, *Stachybotrys atra* and *Bacillus subtilis* against *L. theobromae*. The results revealed *B. subtilis* best in reducing the seed and seedling infection in bottle gourd caused by *L. theobromae* followed by treatments with *T. harzianum*, *T. viride*, *G. virens* and *S. atra*. Microscopic investigation demonstrated direct parasitism and coiling of *T. harzianum* and *T. viride* around hyphae of *L. theobromae*, causing swollen, deformed shortened, or round cells of the pathogen. Granulation of cytoplasm and disintegration of the hyphal walls of *L. theobromae* were also noticed.

Buensanteai and Athinuwat (2012) investigated the antagonistic mode of action of *T. virens* isolated from cassava field against *L. theobromae* the causal agent of cassava stem rot in Thialand. *In vitro* screening of fifteen isolates of *T. virens* was carried out by using dual culture technique. Among these fifteen isolates the *T. virens* isolate TvSUT10, was the most effective isolate and inhibited *L. theobromae* mycelial growth by 84.12 percent due to antagonistic mechanism, moreover, highest activity of Trichodermaβ-1,3-glucanase activity was in strain of *T. virens* TvSUT10 (25-7U/ML).

Suhannaa *et al*. (2013) demonstrated the 47 isolates of *Trichoderma* spp. against mango stem end rot caused by *L. theobromae.* They revealed that the isolates T46 and T9 exhibited maximum inhibition of growth of the pathogen at day 6 (77.65%) and day (87.45%) respectively.

2.9 Screening of varieties against *L. theobromae*

Ramos *et al*. (1997) evaluated 122 mango cultivars against *Botryosphaeria ribis* causing tip dieback disease in Florida and concluded that no significant differences in mean disease severity were present in different groups of mango cultivars and field resistance to tip dieback may be present in some mango cultivars. Presence of resistance in Banganpalli, Saroli and Saleh Bhai varieties of mango could be utilized to transfer resistance to otherwise susceptible varieties in future.

Reddy *et al.* (2005) screened 10 cultivars of mango (Neeleshan, Dasheri Mahmooda, AU Rumani, Totapuri, Suwarnarekha, Vikarabadmahmooda, Baneshan, Cherukurasam, Dasheri and Manjeera) in Andhra Pradesh, India, to determine the source of resistance to stem end rot disease (*B. theobromae*). The Percent Disease Index (PDI) was lowest in Dasheri, Mahmooda (12.3%), Neeleshan (24.4%), Baneshan (29.4%) and Totapuri (30.0%). AU Rumani, Cherukurasam and Vikarabad Mahmooda had the highest mean PDI for stem end rot.

Malik *et al*. (2005a) reported that all the traditional and commercial mango cultivars were found more or less affected with all the decline disorders. Chausa cultivar proved to be more susceptible to all the disorders but was highly prone to tip die back, twig blight and stem bleeding or cankers showing 1.125, 0.8 and 0.2 percent disease incidence, respectively. Similarly, cultivar Sindhri was found more susceptible to gummosis, bark splitting and wilting with 0.3, 0.475 and 0.775 percent disease incidence, respectively. Among desi varieties only two disorders viz. tip die back (0.025%) and bark splitting (0.15%) were observed. Similar

investigation were carried was out by Mahmood *et al.* (2007) in Punjab province of Pakistan, in which they screened nine mango varieties against *L. theobromae* and found that among these, Sindhri appeared as highly susceptible whereas Langra and Desi were comparatively less susceptible to the *L. theobromae*. The studies however revealed that Langra was among the highly susceptible varieties. Similarly,

Mahmood *et al.* (2007) also reported Sindheri, shows high level of susceptibility against *L. theobromae*. The cultivars Malda and Chainsaw also showed significant proneness to the pathogen, while Langra and Desi were found comparatively less susceptible to the disease.

Khan and Masood (2011) raised mango seedlings established in earthen pits under complete randomized design (CRD) for three months and transplanted in the main field. The transplanted mango cultivars, *viz.*, Ratol-12, black chaonsa, white chaonsa, Fajri, Dosehri, Langra, Sindhri and Summer Bahisht were inoculated with *B. theobromae*, one of the causal agents of mango decline. Among the varieties evaluated for resistance, Dosehri was found to be comparatively tolerant to the disease as compared to others. Regarding the appearance of percent disease symptoms, Ratol-12 showed the highest disease symptoms followed by Langra, Fajri and then black Chounsa.

Saeed *et al*. (2011) also evaluated eight mango varieties against *L. theobromae* and reported that Dashehari was comparatively tolerant than other varieties, whereas, Anwar Ratol was highly susceptible followed by Langra, Fajri and Black Chausa.

Langra was the most susceptible variety followed by Anwar Ratol and Dashehari. This variation in the resistance and susceptibility might be due to the genetic variation in different isolates of *L. theobromae.*

Karunanayake *et al.* (2014) investigated fruits of eleven mango cultivars for anthracnose and SER (stem-end rot) development during ripening. The cultivars, 'Gira' and 'Karutha Colomban', were among the most resistant to anthracnose, but were susceptible to SER. The cultivar 'Willard' was found most susceptible to anthracnose but resistant to SER. 'Willard, 'Rata' and 'Kohu' cultivars are resistant to stem end rot the cultivars 'Gira' and 'Karutha Colomban' are susceptible to stem end rot.

Khanzada *et al*. (2015) reported the resistance of mango cultivars to mango decline, *L. theobromae*. The data collected revealed that most of the mango varieties were susceptible to *L. theobromae* except Banganpalli, Saroli and Saleh Bhai. Typical symptoms of mango decline like gum exudation and browning of vascular tissues after inoculation of pathogen was not produced in Banganpalli and SalehBhai. All other cultivars showed moderate to severe disease symptoms. Consequently, significantly least pathogen infection was recorded in cultivar Saleh Bhai followed by Saroli and Banganpalli, whereas maximum *L. theobromae* infection was observed in cultivar Langra followed by Sindhri and remaining cultivars. *L. theobromae* was highly virulent to mango varieties Langra, Sindhri and Almas, where it causes 90 to 100 percent plant mortality (high susceptibility). However, it failed to cause any impact on mango varieties Banganpalli and SalehBhai in term of plant mortality. Other varieties viz., Chausa, Neelum, Jagidar, Anwar Ratol, Gulab

Khasa, Zafran, Anmol, Sawarnica, Dashehari, Bombay, Mehran, Rampuri, Lal Badshah and Tota Puri showed moderate susceptibility against the test pathogens and slight to medium dieback, gummosis and internal browning of vascular tissues observed.

CHAPTER –3

MATERIALS AND METHODS

CHAPTER-3
MATERIALS AND METHODS

3.1. EXPERIMENT-I: SURVEY ON GUMMOSIS AND SUDDEN DECLINE (GASD) DISEASE OF MANGO PLANTS AT RAJSHAHI AND CHAPAINAWABGANJ DISTRICTS

3.1.1. Survey area

An extensive random field survey was conducted during January 2016 to October 2017 at different upazilas of Rajshahi and Chapainawabganj districts. In Rajshahi, Bagha, Charghat and putia upazila were in consideration. On the other hand five upazilas of Chapainawabganj district i.e Bholahat, Nachole, Gomostapur, Shibganj and Sadar upazila were surveyed.

3.1.2. No. of orchard and variety

A total of ten mango orchards comprising more than hundred mango trees of different popular mango varieties from each upazila were selected randomly for the survey programme. During survey eleven mango varieties were observed in different locations. The varieties are BARI Aam (mango)-1, BARI Aam-2, BARI Aam-3, BARI Aam-4, Ashiwina, Fazli, Bomby, Gopalbhog, Khirsapat, Langra and Gootee (local).

3.1.3. Time of survey

The survey was started in the month of January 16 and continued up to October 17. The selected orchards were visited after every three months. Data were collected from

the orchard owner through an interview. Trees died recently by the disease and trees infected during the survey period were noted down during the survey period.

3.1.4. Disease evaluation parameters:

3.1.4.1. Disease incidence

The infection was identified on the basis of symptoms i.e., oozing of gum, rotting, bark splitting, cankers, drying of twigs or branches, wilting and blackening specifically on different portions of mango tree. Therefore, disease incidence was calculated as number of infested plants showing above mentioned any single symptoms out of total numbers of mango plants observed. All the collected data were calculated and expressed in percentage using following formula:

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

3.1.4.2. Severity index of the disease on different mango varieties

Survey of mango orchards were carried out to record symptoms and severity to calculate severity index of the gummosis and sudden decline disease on trees of different mango varieties. The most characteristic symptoms of the gummosis and sudden decline disease on different parts of mango trees were recorded. For calculating severity index the raw data were recorded from three orchards located at three locality of each upazila. Each locality was considered as a replicate. There were 11 mango varieties grown in the survey area. All the infected and healthy mango trees standing on the farms were observed. Trees showing somewhat different to the normal including freshly infected, partially dead and totally dead trees were considered as infected.

Severity of mango gummosis and sudden decline disease within individual diseased trees was assessed following 0-5 scale described by Panhwar, *et al.* (2007). The disease rating scale are described in table 3.1.

Table 3.1. Description of 0-5 scale developed by Panhwar, *et al.* (2007).

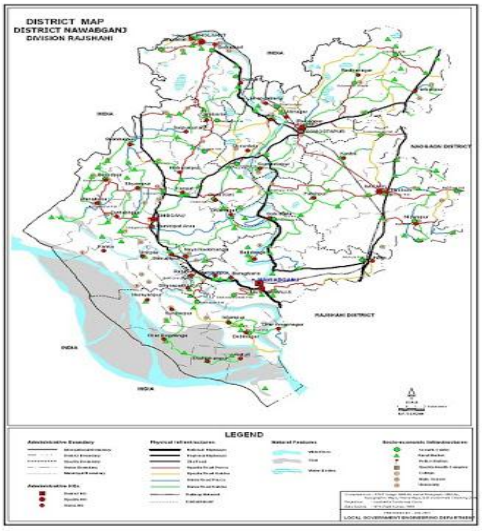
Scale	Disease rating	Description of symptoms	Severity (%)
0	No disease	No signs of disease	0.0
1	Very mildly diseased	Gum traces oozed out/ few smaller branches dried	10.0
2	Mildly diseased	Oozing of gum started/ few branches become dry	25.0
3	Moderately diseased	Up to 35% of the tree become dead	50.0
4	Highly diseased	More than 35% of the tree become dead	75.0
5	Dead	Foliage of whole tree wilted to decline the tree	100.0

From the raw severity data the severity index of gummosis and sudden decline disease was calculated using the following formula (Panhwar, *et al.,* 2007).

$$\text{Disease severity index (\%)} = \frac{\text{Some of all disease ratings}}{\text{No of trees observed} \times \text{Maximum disease grade}} \times 100$$

3.1.4.3. Age response

Assuming the possible variation in age response of mango plants four particular age levels viz. 20 ± 5, 30 ± 5, 40 ± 5 and 50 ± 5 year old plants were also observed to determine the occurrence of disease.

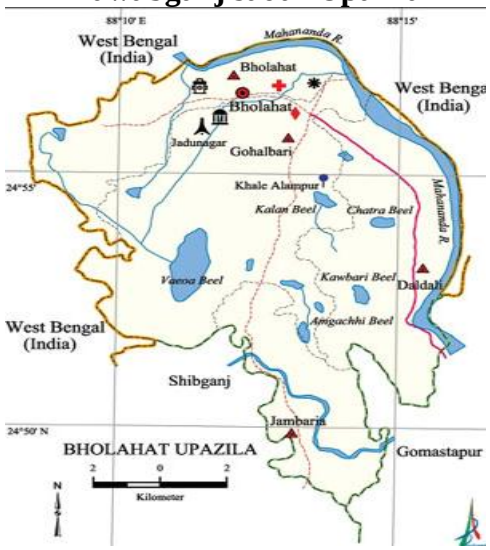


Chapainawabganj District

Shibganj upazlia

Nawabganj sadar Upazila

Gomastapur Upazila



Bholahat Upazila

Nachole Upazila

Fig. 3.1: Survey area of Chapainawabganj District

Rajshahi District

Bagha Upazila

Charghat Upazila

Putia Upazila

Fig. 3.1: Survey area of Rajshahi District

3.2. EXPERIMENT-II: ISOLATION, PURIFICATION, IDENTIFICATION AND CHARACTERIZATION OF THE CAUSAL AGENT OF GUMMOSIS AND SUDDEN DECLINE DISEASE OF MANGO PLANTS

3.2.1. Sample collection

Diseased sample were collected from the infected mango trees for isolation and identification of fungi causing gummosis and sudden decline disease in Bangladesh. The samples were taken as infected along with living portions from mango trees of different varieties as live position, not dead. The samples were put in sterilized polythene bags and brought into the laboratory for isolation and identification of the organism (plate 3.1). The samples were collected from different orchards located at different locations of chapainawabganj and Rajshahi districts.

3.2.2. Laboratory for isolation

The isolation activities of the suspected causal pathogens were done in the Plant pathology laboratory of Regional Horticulture Research Station (RHRS), Chapainawabganj and Fruit Research Station (FRS), Rajshahi.

3.2.3. Method of isolation

The suspected causal pathogens were isolated through tissue plating methods on potato dextrose agar (PDA) as the standard medium of isolation (Al-Adawi *et al*., 2006).

3.2.4. Procedure of isolation

For isolation, all the diseased samples were cut into small pieces (1-2 cm) containing disease portion along with healthy portion with a sterilized scissors and a knife. The small pieces of samples were first washed in running tap water to wash out dust and

other unnecessary material. These pieces were dipped in 0.1% mercuric chloride ($HgCl_2$) solution contained in a petridish for 2-3 minutes for surface sterilization, then washed thoroughly in two changes of distilled sterile water for 3-4 minutes each to remove toxic effect of mercuric chloride and then dried completely on double layer of sterilized tissue papers. Five treated pieces of sample were transferred in each petridish containing freshly prepared sterilized potato dextrose agar (PDA) medium. The petriplates were then incubated at 25± 2^0C with 12 hours alternate periods of light and darkness till the initial fungal growth appeared. The grown fungi then were sub-cultured for purification.

3.2.5. Purification of pathogen

Purification was carried out using single spore isolation technique (Toussoun and Nelson, 1976). Spore suspension of 10 days old culture was prepared in the test tube (15×1cm) containing 5 ml of sterilized water. The spore density in suspension was observed under compound microscope taking a drop of suspension on a glass slide. The spore density was adjusted with the addition on sterile water to get 1-5 spore per microscopic field (10x). One drop of the suspension was then poured on water agar medium (2%) in petridishes and spread over the medium thoroughly with a sterilized needle and the petridishes were then incubated for 16-20 hr at 25 ± 1^0C and allowed the spores to germinate. A germinated spore was then picked up under a microscope and transferred to a slant culture in the tube and incubated for 10 days at 25 ± 1^0C under 12hr darkness alternate with florescence light or near ultra-violate light (NUV light) for 10 days for growth and sporulation. The pure culture of the pathogen thus obtained was then preserved at 4^0C in the refrigerator. For identification, conidia were taken from mature colonies and examined for size, shape and colour. Details of the

cultural characteristics and microscopic details were noted and the fungus was identified following Ellis (1971).

3.2.6. Identification of pathogen

After incubation of pure fungal culture, the growth of isolated fungi was identified on the basis of their mycelial and conidial characters through standared mycological descriptions by CMI keys (Punithalingam, 1976). The electrical compound microscope was used for observation of the pathogen. Photographs of fungal morphology were taken using digital camera connected with compound microscope and computer to confirm the identification of isolated fungi.

3.2.7. Pathogenicity test

The pathogenicity test was conducted in pots at RHRS and FRS. 3 years old seedlings of susceptible mango cultivar fazli were selected as a host for conducting pathogenicity tests. A slant cut in the stem was made using a sterilized knife (plate 4.7a). A 5 mm inoculum disc from 5 day old culture of a test fungus on PDA was placed in the gap (plate 4.7b) and the inoculated portion was wrapped with Para film (plate 4.7c). Same type of slant cut in the stem was made using a sterilized knife. A 5 mm PDA block without fungus was placed in the gap of control plants and was wrapped with Para film (plate 4.7e). 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. (Khanzada *et al*., 2004)

3.3. EXPERIMENT-III: MOLECUAR CHARACTERIZATIONS OF *LASIODIPLODIA THEOBROMAE*

3.3.1. Preparation of culture

Culture of 11 isolates of *L. theobromae* was grown on PDA. Hyphal tip of 4 day old pure culture was transferred into 250 ml conical flask containing 100 ml potato dextrose (PD) broth and placed on electric shaker for four days at 120 rpm at 25°C. Four days old mycelia of *L. theobromae* from potato dextrose broth were filtered with filter paper to remove water. The filtered mylcelial mat was blotted, dry to remove moisture as much as possible. The blotted; flattened mylcelium of *L. theobromae* was folded into an aluminium foil paper and frozen at -20ºC.

3.3.2. Fungal genomic DNA extraction

DNA was extracted from 11 isolates of *L. theobromae* following the method of Reader and Broda (1985). Frozen mycelia were grounded in to fine powder using mortar and pestle by liquid Nitrogen. An amount of 0.5gm powdered mycelia were poured into 1.5 µl Centrifuge tube and homogenized with 600 µl of extraction buffer (250 mM NaCl, 100 mM Tris-HCl,100m MEDTA, Sigma Aldrich) by stirring on vortex for lysis of the cells. 100 µl of 10% sodium dodecyle sulphate (SDS) was added in to centrifuge tube containing powdered mycelia and extraction buffer and then mixed by inverting the tube 5-6 times and incubated for 30 minutes at 65°C in water bath. After incubation, 264 of 3M sodium acetate (pH.5.2) was added into the centrifuge tube, mixed gently and kept in ice for 15 minutes. The mixture was then centrifuged (Hawkd15/05) at 13000 rpm (4°C) at for 10 minutes and supernatant was transferred in to a new tube. After that, (500 µl) of Chloroform was added in to the collected supernatant and mixed thoroughly by vortex. The mixer was centrifuged at 12500 rpm at 4°C for 30 minutes to precipitate the protein and long chain

polysaccharides from the DNA. The upper aqueous phase was collected into new tubes very carefully without disturbing the interface.

3.3.3. Precipitation and re-suspension of DNA

Precipitation of DNA was done following the procedure of Wilson (2001). Two volumes of pure ethanol (99%) were added into the DNA suspension, kept on ice for 10 minutes to allow the DNA molecules to aggregate. The DNA was precipitated down to the bottom of the centrifuge tube as a pellet by centrifuging at 13000 rpm at 4°C for five minutes. Then the supernatant was discarded using a wide bore pipette and preserved the centrifuge tube with the DNA pellet. An amount of 800 µl of 70% ethanol was added into the centrifuge tube, stirred on vortex for washing the DNA pellet and centrifuge at 13000 rpm at 4°C for two minutes, ethanol was discarded using a micropipette. The centrifuged tube containing DNA pellet was dried into vacuum desiccators for two minutes. The DNA pellet was re- suspended by 50 µl TE buffer (10mM Tris, pH-8.0, 1M EDTA, Sigma Aldrich). The DNA solution was preserved at -20°C for further studies.

3.3.4. Quantification of genomic DNA

Prior to set of PCR reaction the extracted fungal genomic DNA was check by run on 0.7% agarose gel and then quantify by spectrophotometer (Nanodrop-1000).

3.3.5. Preparation of 0.7% agarose gel

For preparation of 0.7% agarose gel 0.7g agarose was weighed in a conical flask. An amount of 100 ml of 1×TAE buffer was poured into the conical flask having 0.7g agarose and melted by heating in a microwave oven until dissolved fully. The melted

agarose gel was cooled down under flowing tap water. The plate of the gel tank was placed flat on the table. The gel was poured smoothly and continuously starting from one corner until reached to the other corner. Then comb was inserted halfway into the gel immediately for making well, allowing an hour for polymerization before removing the comb gently. The casted plate was assembled in the tank. The gel was submerged into in to the 1×TAE running buffers in gel tank.

3.3.6. Gel loading and running

PCR product (10µl) stained with 2µl of 6× loading dye was loaded in to the well of the agarose gel and electrophoresed for 25 minutes at 100 volts to move the negatively charged DNA towards the anode.

3.3.7. Visualization of the PCR product in 2% Agarose gel

The gel was taken out of the gel tank and stained with ethidium bromide solution (0.5 µl) for an hour. The stained gel was rinsed with water for distaining, illuminated on UV trans-illuminator and photographed by gel documentation (Bio-Rad) for measuring the bands of amplified DNA fragments. The selected RAPD primer was used for the rest of the studies with 11 selected isolates for amplification of DNA. The gel used to resolve the amplified DNA was stained, illuminated under UV light and the fingerprints photographed by gel documentation.

3.3.8. Amplification of ITS region of fungal genomic DNA using ITS 4 and ITS 5 primers and sequencing

PCR was performed using BIORAD Thermo-cycler (USA). The universal primers for PCR were synthesized from Invent Technology, Bangladesh. The primer pairs and

ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) (White *et al*., 1990) were used for rDNA amplification. The PCR amplification was carried out in 25µl reaction mixture containing 1ng of DNA sample, 5µl of 5× PCR buffer, 2.5mM $MgCl_2$, 2.0µl of 2mM dNTPs (Promega, USA), 20pmol of each forward and reverse primer (1.0µl) and 0.2µl of Taq DNA Polymerase and made up to 25µl with nuclease free water. The PCR conditions include initial denaturation at 94ºC for 3min, 30 cycles of denaturation at 94ºC for 30 s, primer annealing at 55ºC for 30 s, followed by primer extension for 30 s at 72ºC and final extension at 72ºC for 10min. The amplicon was gel purified by PCR clean up kits (Promega, USA) and sending for sequencing to Invent Technology, Bangladesh. Based on the morphological characteristics of the fungi I have sequenced eleven isolates namely MGSD001, MGSD002, MGSD003, MGSD004, MGSD005, MGSD006, MGSD017, MGSD028, MGSD045, MGSD071, MGSD091 respectively.

3.3.9. Sequence analysis

The assembly of fungal genome was performed using the web service of CAP3 program (http://doua.prabi.fr/software/cap3) and Bio-Edit V5. ITS-rDNA sequences were aligned using the CLUSTALW2 program (Larkin et al. 2007). The assembled sequenced contain partial sequence of 18s ribosomal RNA gene, ITS-1, 5.8s ribosomal RNA gene, ITS2 complete sequence and partial sequence of 28S ribosomal RNA partial sequence were submitted to NCBI (https://www.ncbi.nlm.nih.gov/) with the accession number MW138048-MW138058 respectively. The submitted sequence information of all 11 isolates that were responsible for mango gummosis disease in Bangladesh were presented in the Appendix 3. Phylogenetic tree was constructed

based on the Neighbor-Joining [NJ] Method in MEGA version 10 (Kumar *et al*. 2018) using Kimura-two parameter model with 1000 bootstrap replications.

3.4. EXPERIMENT-IV: MANAGEMENT OF GUMMOSIS AND SUDDEN DECLINE DISEASE OF MANGO PLANTS.

3.4.1. Evaluation of different fungicides against *L. theobromae in vitro*

Eight fungicides were evaluated against *L. theobromae* by poisoned food technique (Nene and Thapliyal, 1993) at 25 ppm, 50 ppm, 75ppm and 100 ppm. The list of fungicides evaluated is furnished in Table 3.2.

The required quantities of fungicides were weighed and mixed in the potato dextrose agar medium by thorough shaking for uniform mixing of the fungicide before pouring into petridishes so as to get the desired concentration of active ingredient of each fungicide separately. 20 ml of amended medium was poured in 90 mm sterilized petridishes and allowed to solidify. Mycelial discs of 5 mm diameter from 3 day old culture was inoculated at the center of the petriplate and then incubated at room temperature for 3-4 days. Control was maintained without fungicide. Three replications were maintained for each treatment. Percent inhibition of mycelial growth was calculated using the formula (Vincent, 1927).

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

Where,

I = Percent inhibition of mycelial growth
C = Colony diameter in control (cm)
T = Colony diameter in treatment (cm)

Table 3.2: List of fungicides tested against *L. theobromae by* poisoned food technique under *in vitro* condition.

Sl. No	Common name	Trade name	Source of supply
1	Carbendazim	Arba	Intefa Bangladesh Ltd.
2	Mancozeb	Indofil M -45	BASF Bangladesh Ltd.
3	Copper hydroxide	Zibal	Intefa Bangladesh Ltd.
4	Propiconazole	Tilt	Syngenta Bangladesh Ltd.
5	Pyraclostrobin+ Metiram	Cabriotop	BASF Bangladesh Ltd.
6	Azoxystrobin	Amistar top	Syngenta Bangladesh Ltd.
7	Propineb	Antracol	Bayer Bangladesh Ltd.
8	Tebuconazole+Trifloxystrobin	Nativo	Bayer Bangladesh Ltd.

3.4.2. Integrated management at field condition

In order to establish an integrated management strategy an experiment was carried out to control mango gummosis and sudden decline disease. In this study, the effect of chemical and cultural control alone, as well as in combination were observed.

3.4.2.1. Experimental site

The experiment was conducted at the research field of plant pathology division, Regional Horticulture Research Station (RHRS), Bangladesh Agricultural Research Institute (BARI), Chapainawabganj.

3.4.2.2. Variety of mango

For field experimentation all the treatments were applied on naturally infected mango tree of fazli variety in this investigation. Fully grown mature mango trees with mildly diseased severity showing typical disease symptoms were selected for this experiment.

3.4.2.3. Treatments

In general the experiment comprised six treatments: The details of the treatments are shown in table 3.3.

Table 3.3. Application of different treatments in field condition

Treatments	Descriptions of treatments
T1	Spraying with the best fungicide selected from *in vitro* test
T2	Injection with the best fungicide from *in vitro* test
T3	Application of Bordeaux paste
T4	Bordeaux paste + T 1
T5	Bordeaux paste + T 2
T0	Control

3.4.2.4. Procedure of treatments application

3.4.2.4.1. Fungicides application

Spraying method: The best fungicide(s) selected from the *in vitro* test were sprayed maintaining standard dose to the infected mango plants. The test plants were thoroughly sprayed with fungicide solution six times with 10 days interval with the help of power sprayer.

Injection method: The best fungicide selected from *in vitro* test were applied by injection method.

3.4.2.4.2. Application of Bordeaux paste

In case of T3 (Application of Bordeaux paste) the points of gum exudation were cleaned with a sharp knife and Bordeaux paste was applied on the cleaned area with a brush (plate 3.2 & 3.3). The paste was applied 3-4 times at an interval of 10 days. But in case of T4 first applied Bordeaux paste and then sprayed fungicide. On the other hand in case of T5 first applied Bordeaux paste and then injected fungicides.

3.4.2.4.3. Control plants

In case of control treatments plants received no control measure either chemical or cultural and therefore served as control plants.

3.4.3. Design of experiment

The experiment was laid out in a Completely Randomized Design (CRD) for lab experiment and Randomized Complete Block Design (RCBD) with three replications for field experiment.

3.4.4 Data recording

To compare the effect of different treatments the data on disease severity was recorded after 4, 8 and 12 month of treatment application (MATA) and calculated following same procedure described in experiment -I

3.4.5 Statistical analysis

All the collected data were analyzed using MSTAT-C programme. Means were compared by Duncan's Multiple Range Test (DMRT) at 1% level of significance.

Plate 3.1. Sample collection from gummosis and sudden decline disease infected mango plants

Plate 3.2 Application of Bordeaux paste: (a) removing of bark from the gumming point, (b) cleaned portion (c&d) pasting of Bordeaux paste by hand brush

Plate 3.3. Steps of injection method of fungicide application: (a) prepared sponge for inserting into the hole (b) making a hole on the trunk on infected mango tree by handrail and (c) motorized drill (d) insertion of sponge into the hole (e) completely inserted sponge (f) inject fungicide solution by syringe.

CHAPTER –4

RESULTS AND DISCUSSION

CHAPTER 4

RESULTS AND DISCUSSION

A total of four experiments were conducted during 2016 to 2018. The results of the experiments obtained during the course of present investigation are presented and described here accordingly.

4.1. EXPERIMENT- I: SURVEY ON GUMMOSIS AND SUDDEN DECLINE (GASD) DISEASE OF MANGO PLANTS AT CHAPAINAWABGANJ AND RAJSHAHI DISTRICTS

A Survey was conducted to assess the incidence and severity of gummosis and sudden decline disease of mango in the major mango growing areas of Chapainawabganj and Rajshahi districts, Bangladesh during January 2016 to October 2017.

4.1.1. Field symptoms of the disease observed during field survey

During the survey conducted in different mango orchards, symptoms like gummosis, dieback and vascular discoloration were observed (Plate 4.1- 4.3). The infected plant parts showed abundant gum secretions from stem or main trunk and branches. Initially the gum appeared in the form of small droplets (Plate 4.1a-c). However, as the disease progressed, it increased and covered most of the branches and trunk (Plate 4.1d-f). Under severe conditions, the outer wood of a branch splits and exudes a yellow to brown or black gum-like substance (Plate 4.1d-i). The infected plants also showed internal discolouration. Brown to blackish streaks in vascular regions are visible upon removing the bark of gum exuding point. (Plate 4.2a-f).

In some cases it was observed that the affected plants, twigs die from the tips to back into old wood, giving a scorched appearance to the limb (Plate 4.3d). The affected leaf turns brown and its margins roll upwards (Plate 4.3.e). Leaves scorch and fall, leaving a dead branch (Plate 4.3d). In severe conditions, branches start drying one after another in a sequence (Plate 4.3 a-f) resulting in death of the whole tree (Plate 4.4 a-c) .The peculiarity of the disease is that the affected trees showed wilting symptoms that usually begin one side and sequentially spread to another side (Plate 4.3. a-f) and finally the whole plant wilted with in very short time (Plate 4.4. a-c). During the survey period it was observed that an affected plant died or wilted within three to six months. Different workers also reported the same symptoms. Khanzada *et al.* (2004a) recorded several gummosis symptoms on mango. The affected plants exhibit dieback, gummosis and vascular discoloration. In dieback, infected twigs die from the tips to back into old wood, which gives a scorched appearance to limb. The affected leaves turn brown and rolls upward. In severe cases, the entire plants were killed. In gummosis, the infected plants showed abundant gum secretion from branches, stem and main trunk. In vascular discoloration, infected twigs, plants and branches showed internal discolouration. Brown streaks visible in vascular region and these were severe in water stress conditions. Shahbaz *et al.* (2005) explained the disorders like twig blight, gummosis, bark splitting/cracking and wilting as mango gummosis disease. Mango decline complex is observed in the form of twig blight, tip dieback, gummosis and bark splitting (Malik *et al*., 2005). Exudation of gum was found to be the most common symptom of die back affected plants due to the involvement of *B. theobromae* (Khanzada *et al*., 2005; Saleem *et al*., 2006; Fateh *et al*., 2006). Al-adawi *et al*. (2006) found that mango sudden decline affected trees in Oman which showed

Plate 4.1. Symptoms of gummosis on mango tree: (a) small droplet of gum exuding from trunk of mango tree (b&c) small droplet of gum exuding from branch of mango tree (d-f) heavy gummosis on stem and branches (g-i) splitting of bark and gum oozing out from trunk.

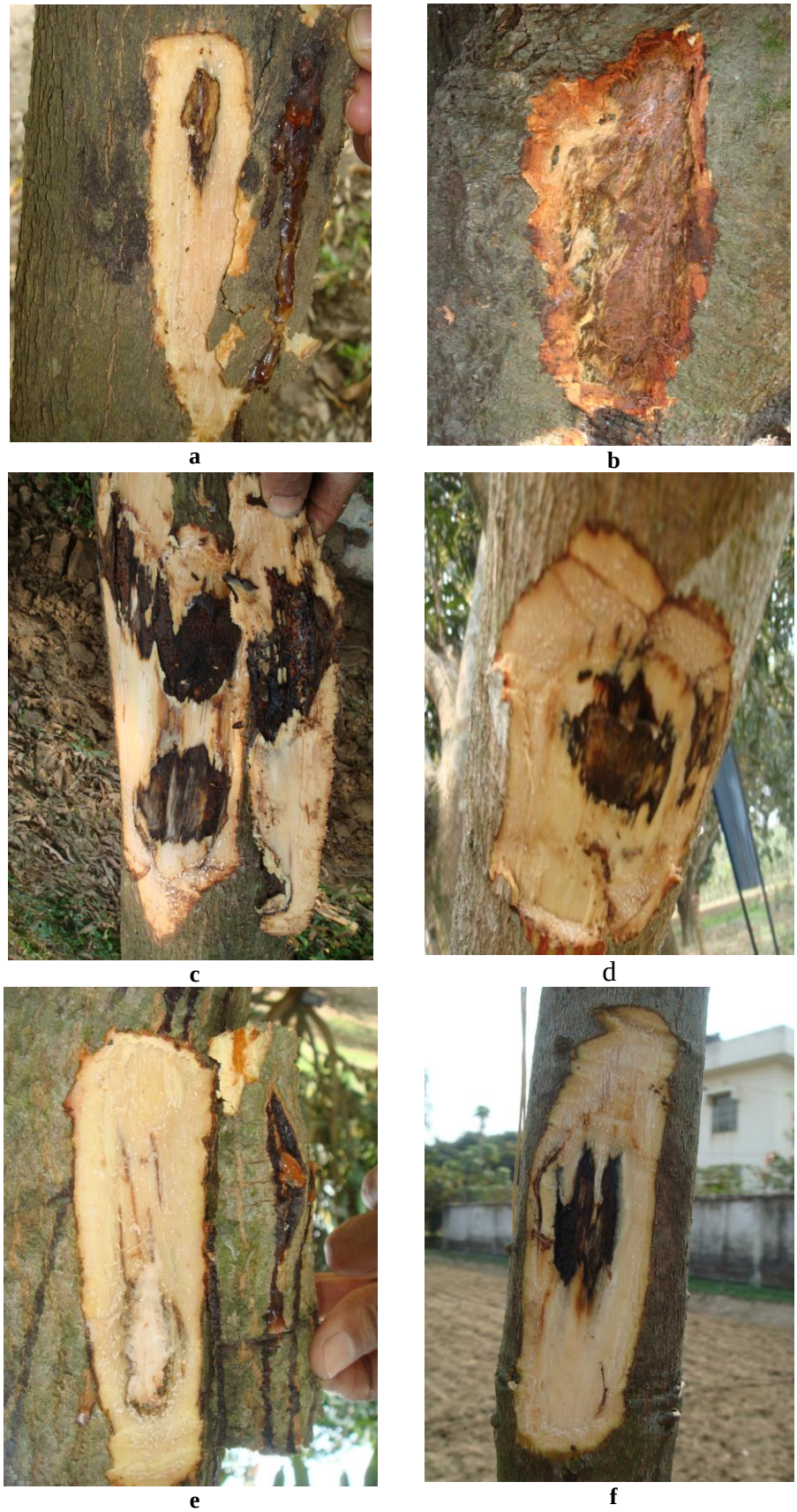
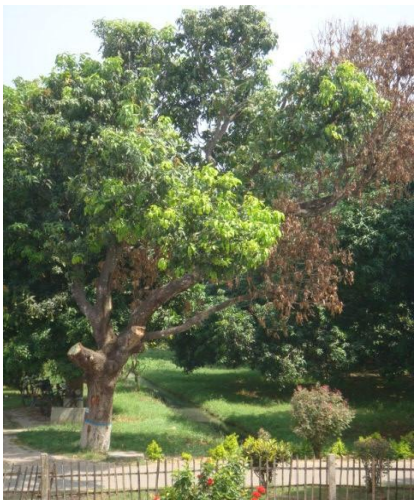


Plate 4.2. Vascular discoloration (a-f) of mango plants due to attack of gummosis and sudden decline disease.

a



b

c

d

e

f

Plate 4.3. Sequential death of branches (a-f) of mango plants due to attack of gummosis and sudden decline disease.

a **b**

c

Plate 4.4. Complete death (a-c) of mango plants due to attack of gummosis and sudden decline disease.

wilting symptoms that usually begin on one side and later spread to involve the entire tree. Trees exude amber-coloured gum from the bark of their trunks or branches and vascular tissues were discolored. Tree death occurred approximately 6 months after the first appearance of the symptoms (Al-Adawi *et al*., 2003).

4.1.2. Incidence of gummosis and sudden decline disease in different locations

The information pertaining to the disease incidence were calculated and furnished in Table 4.1 and 4.2. Among the orchards surveyed in eight upazilas under investigation the disease incidence varied from 1.75% to 3.33% in 2016. The highest incidence of the disease (3.33%) was monitored in Shibganj upazila of Chapainawabganj district followed by Gomostapur (3.05%) and Sadar (2.97%) upazila of the same district while the lowest incidence (1.75%) was observed in Charghat upazila of Rajshahi district which was followed by Putia (1.76%) and Bagha (1.96%) upazila of the same district in 2016 (Table 4.1).

In the year 2017, the disease incidence varied from 1.78% to 3.61% where the highest incidence (3.61%) was recorded in Shibganj upazila and the lowest incidence (1.78%) was observed in Putia upazila (Table 4.2). From the pooled data on disease incidence (Fig 4.1) it was observed that the highest disease incidence (3.47%) was recorded in Shibganj followed by Sadar (3.14%) and Gomostapur (3.06%) upazila of Chapainawabganj district and the lowest disease incidence (1.77%) was observed in Putia upazila of Rajshahi district. Other area showed intermediate results on disease incidence. The mango orchards in Shibganj upazila are closer and older as compared to the others areas. This might be the probable cause of higher incidence of the disease. On the other hand, the mango orchards in Putia upazila are scattered and younger than Shibganj which might be the cause of less disease incidence. The mango gummosis was reported to be 20 and 60 percent in Punjab and Sindh Provinces of Pakistan, respectively and 60 percent in Al Batinah region of Oman (Saeed, 2011; Al-Adawi *et al*., 2006). Verma and Singh (1970) identified mango gummosis as a serious disease in Jaipur district, which affected 30-40 percent of the

plantations in the Morabad region of Uttar Pradesh. Quick decline of mango caused by *B. theobromae* was the most holistic one to cause heavy losses up to 50 percent in-mango grooves of Punjab and Sindh (Mahmood *et al*., 2002). Leghari (2005) studied about mango gummosis disease and found 20-83 percent incidence.

From the results of incidence of gummosis and sudden decline in different months it was observed that the maximum disease incidence was noted in the month of April and the minimum in July in both 2016 and 2017 (Fig 4.2). The temperature is high in March to April in Bangladesh and at that time weather is dry that might be the cause of maximum diseases incidence in April. On the other hand after June rainy season starts which helps the plant to overcome the infection of the disease to some extant. That might be the cause of low disease incidence compared to other months. Hossain *et al.* (2010) reported gummosis disease infections have been found throughout the year, but November to March shows most severe infection on mango trees in Bangladesh. Khanzada *et al.* (2005) reported severe symptoms of mango gummosis and decline was found in areas with water stress. According to Khanzada *et al*. (2004) the gummosis disease symptoms can be found at mango orchard throughout the year. Mango trees growing under water stress show more severe symptoms compared to regularly watered plants, indicating that water stress apparently predisposes the mango tree and enhances the severity of diseases. Shahbaz *et al.* (2009) claimed that the fungus responsible for mango gummosis and sudden decline disease requires moist conditions for initial establishment and symptoms development. For that reason the disease is more severe in the periods from February to March and from August to September (due to favorable temperature and high humidity). Once a fungus

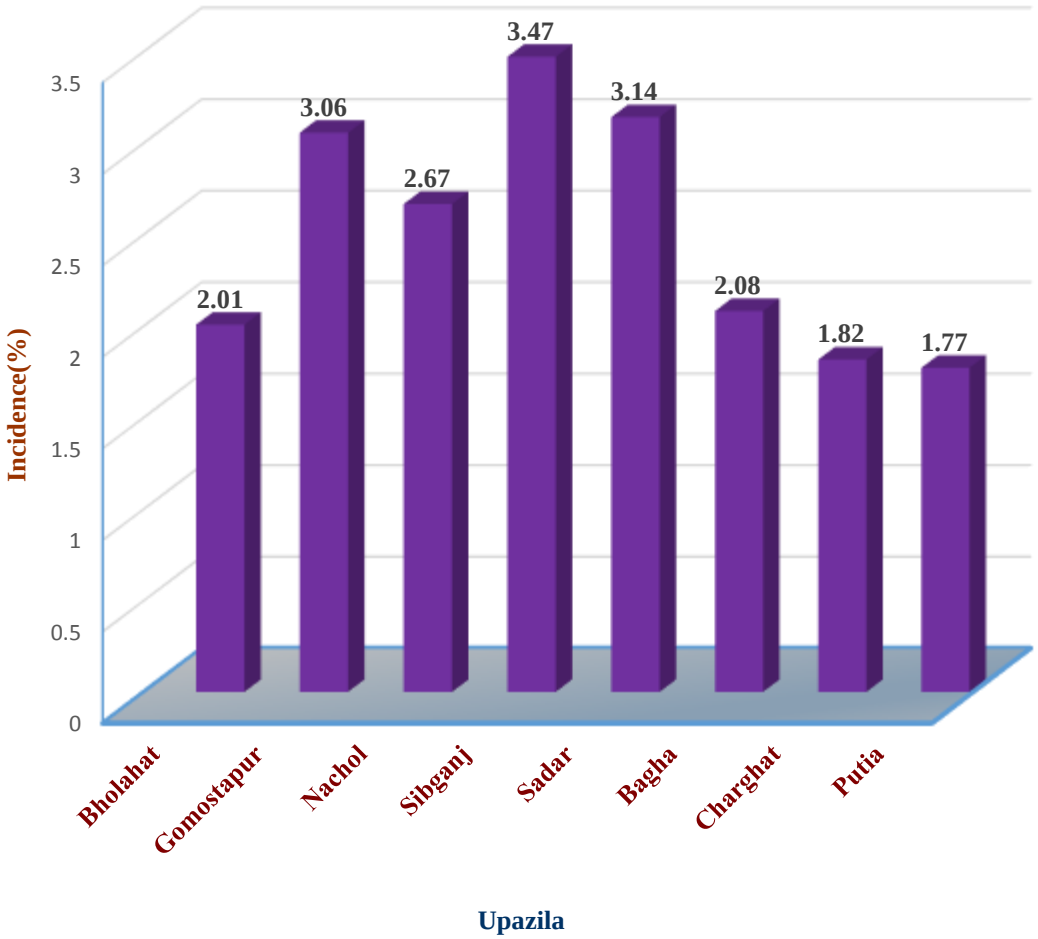
establishes on mango trees, it may survive even in dry months as observed in some mango orchards of Pakistan (Schaffer *et al.*, 1988). Rawal (1998) noted that relative humidity above 80%, temperature from 25.0^0 to 31.5^0 and rains are the favorable conditions for the development of gummosis disease in mango plants.

Table 4.1. Incidence of gummosis and sudden decline disease in different upazilas of Chapainawabganj and Rajshahi districts in 2016

District	**Upazila**	**Incidence (%) in different months of 2016**				
		January	**April**	**July**	**October**	**Mean**
Chapainawabganj	Bholahat	1.78	2.17	1.82	2.05	1.96
	Gomostapur	2.73	3.81	2.10	3.55	3.05
	Nachol	2.54	3.22	2.23	2.41	2.60
	Shibganj	2.87	3.70	3.13	3.60	3.33
	Sadar	2.32	3.82	2.22	3.51	2.97
Rajshahi	Bagha	1.92	2.44	1.83	1.65	1.96
	Charghat	1.62	2.63	1.12	1.62	1.75
	Putia	1.54	2.43	1.42	1.64	1.76

Table 4.2. Incidence of gummosis and sudden decline disease in different upazilas of Chapainawabganj and Rajshahi districts in 2017

District	Upazila	Incidence (%) in different months of 2017				
		January	**April**	**July**	**October**	**Mean**
Chapainawabganj	Bholahat	1.81	2.38	1.75	2.26	2.05
	Gomostapur	2.7	3.89	2.15	3.5	3.06
	Nachol	2.44	3.36	2.14	2.98	2.73
	Shibganj	3.17	4.52	3.02	3.73	3.61
	Sadar	3.21	3.98	2.34	3.71	3.31
Rajshahi	Bagha	1.71	2.91	1.65	2.53	2.20
	Charghat	1.55	2.49	1.35	2.13	1.88
	Putia	1.35	2.41	1.27	2.09	1.78



Upazila

Fig. 4.1. Incidence of gummosis and sudden decline disease of mango plants in different upazilas of Chapainawabganj and Rajshahi districts during 2016 and 2017

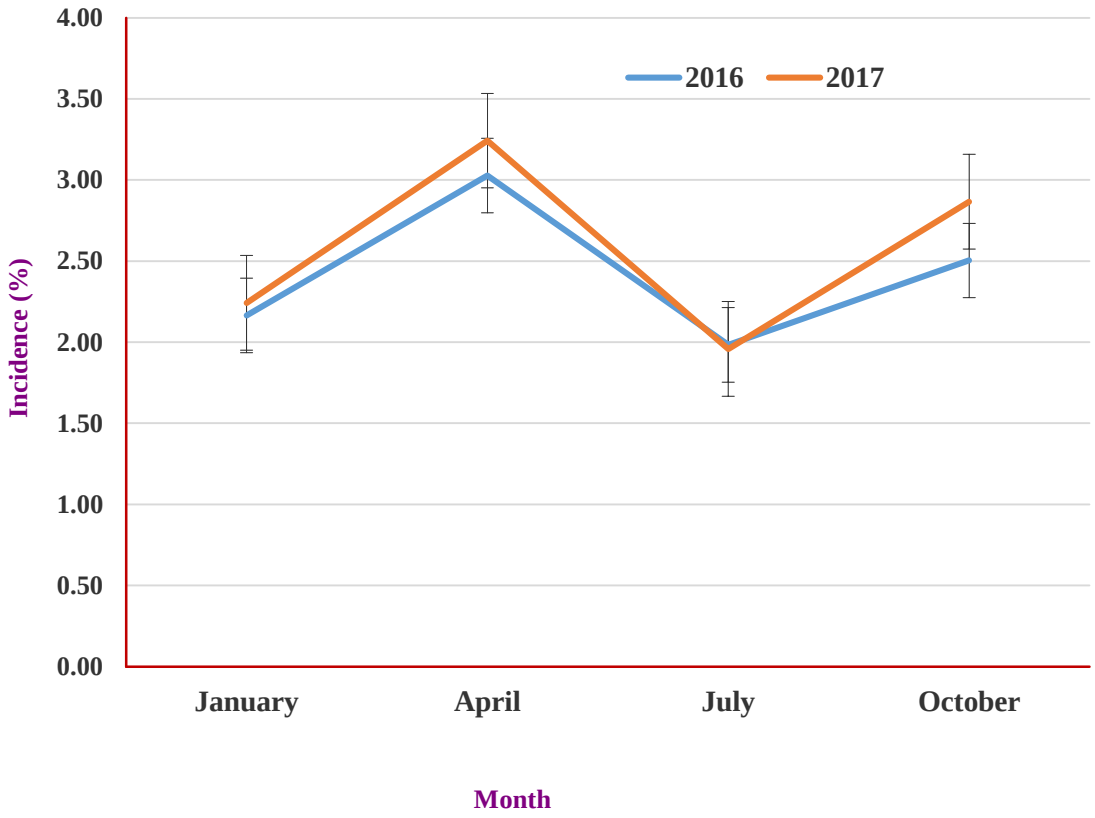


Fig. 4.2. Incidence of gummosis and sudden decline disease of mango plants in different months during 2016 and 2017.

4.1.3. Varietal reaction on incidence of gummosis and sudden decline disease

Mango varieties under investigation showed differential reaction to the disease incidence. The incidence varied from 0.06% to 3.41% in all areas under investigation during 2016 (Table 4.3). Among the varieties the highest incidence (3.41%) was recorded in Fazli followed by Gootee (2.52%), Langra (1.58%) and Ashwina (1.47%) variety while the lowest diseases incidence (0.06 %) was recorded in BARI Aam-4 followed by BARI Aam-1 (0.44%), Bomby (0.70%), BARI Aam-2 (0.74%) and Gopalbhog (0.74%) varieties during 2016.

On the other hand, disease incidence varied from 0.45% to 3.69 % in all areas investigated during 2017 (Table 4.4). The highest incidence (3.69%) was recorded in the variety Fazli followed by Gootee (2.74%), Langra (1.71%), Ashwina (1.70%) and Khirsapat (1.42%) variety while the lowest diseases incidence (0.45%) was recorded in BARI Aam-4 followed by BARI Aam-1 (0.50%). From the pooled data of 2016 and 2017 it was observed that among the variety observed the highest disease incidence (3.55%) was recorded in the varieties Fazli followed by Gootee (2.63%), Langra (1.65%), Ashwina (1.59%) and Khirsapat (1.35%) while the lowest diseases incidence (0.26 %) was recorded in BARI Aam-4 followed by BARI Aam-1 (0.47%) and BARI Aam-2 (0.90%) (Fig 4.3). From the results it is that the trend of disease incidence is almost similar in both the surveyed year depending on mango variety and there was no variety found free from the disease infestation during the study period. In all locations, it was observed that the variety Fazli was very much susceptible to mango gummosis and sudden decline disease compared to other varieties while BARI Aam-4 was less susceptible. Begum *et al.* (2003) reported that

Table 4.3 Varietal reaction in incidence of gummosis and sudden decline disease in different upazilas of Chapainawabganj and Rajshahi districts in 2016

Variety name	**Incidence (%)**								
	Bholahat	Gomostapur	Nachol	Shibganj	Sadar	Bagha	Charghat	Putia	Mean
BARI Aam-1	-	-	-	-	3.50	-	-	-	0.44
BARI Aam-2	0.55	1.94	0	0	0.56	1.18	1.68	-	0.74
BARI Aam-3	0.45	1.85	1.45	1.12	0.95	0.85	1.20	1.21	1.14
BARI Aam-4	-	-	0.25	-	0.25	-	-	-	0.06
Ashiwina	1.80	1.72	1.94	1.54	1.14	1.57	0.95	1.12	1.47
Fazli	2.24	4.91	3.37	4.87	4.70	2.57	2.50	2.15	3.41
Bomby	-	1.52	1.12	1.14	1.84	-	-	-	0.70
Gopalbhog	0.25	0.25	1.16	1.48	0.94	0	0.97	0.85	0.74
Khirsapat	1.23	0.78	1.69	1.26	1.45	1.51	1.12	1.12	1.27
Langra	1.85	1.15	1.87	1.42	2.73	1.26	1.25	1.13	1.58
Gootee	1.85	2.88	1.92	4.45	3.18	2.63	1.75	1.5	2.52

Table 4.4 Varietal reaction in incidence of gummosis and sudden decline disease in different upazilas of Chapainawabganj and Rajshahi districts in 2017

Variety name	Incidence (%)								
	Bholahat	Gomostapur	Nachol	Shibganj	Sadar	Bagha	Charghat	Putia	Mean
BARI Aam-1	-	-	-	-	3.98	-	-	-	0.50
BARI Aam-2	1	2.05	0.28	0	1.1	1.1	1.85	-	1.05
BARI Aam-3	0.65	1.98	1.45	1.45	1.15	1.15	1.5	1.24	1.32
BARI Aam-4	-	-	0.25	-	0.65	-	-	-	0.45
Ashiwina	1.96	1.88	1.94	1.74	2.22	1.55	1.15	1.18	1.70
Fazli	2.35	4.98	3.37	4.98	5.12	3.45	2.85	2.45	3.69
Bomby	-	1.78	1.12	1.36	1.21	-	-	-	1.37
Gopalbhog	0.45	1.1	1.16	1.74	0.86	0.85	1.1	0.98	1.03
Khirsapat	1.53	1.15	1.69	1.35	1.25	1.84	1.25	1.27	1.42
Langra	1.87	1.56	1.87	1.54	2.85	1.16	1.46	1.34	1.71
Gootee	1.99	3.12	1.92	4.65	3.1	3.15	2.15	1.86	2.74

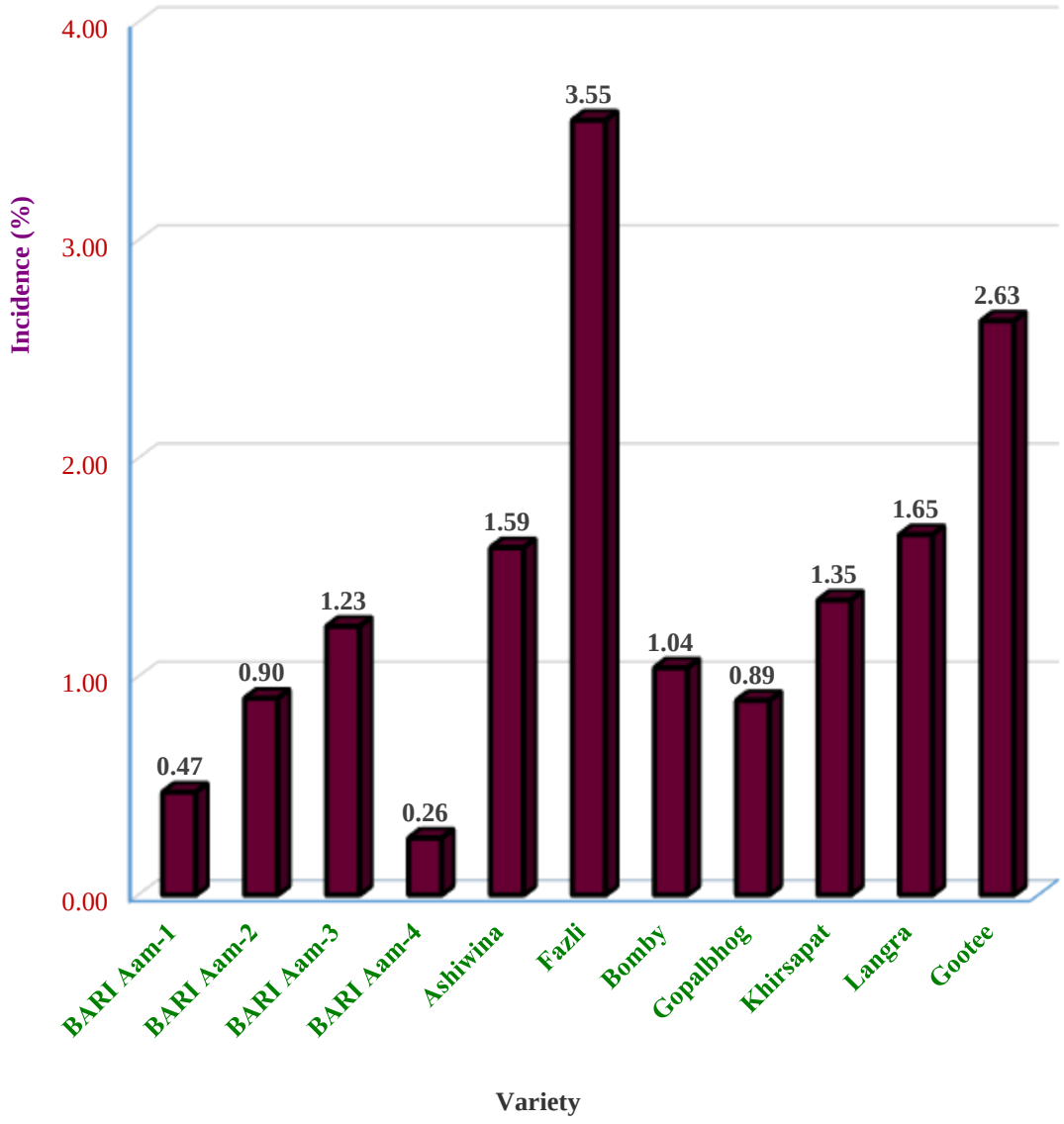


Fig.4.3. Varietal reaction in incidence of gummosis and sudden decline disease in different upazilas of Chapainawabganj and Rajshahi districts in 2016 and 2017.

all exotic cultivars, the commercial variety Fazli and Langra were susceptible to mango gummosis. Nizamani *et al.* (2005) reported that tip dieback disease suddenly dried mango trees completely as scorched by fire after oozing out of gummy substance on the main trunk. Maximum incidence and severity of tips dieback disease was on Langra, Chaunsa, Sindhri and Siroli respectively.

4.1.4. Incidence of gummosis and sudden decline disease on different aged plants in different locations

4.1.4.1. Incidence on 20 ± 5 years old plants

Disease incidence on 20 ± 5 years old plants on surveyed locations during 2016 and 2017 is presented in Fig 4.4 and Appendix 1&2. Depending on locations it was observed that disease incidence varied from 1.10% to 2.75%. The highest incidence (2.75%) was monitored in Shibganj upazila of Chapainawabganj district which was statistically identical with Gomostapur upazila but significantly differed with others in 2016. On the other hand the lowest incidence (1.10%) was recorded in Charghat upazila of Rajshahi district which was statistically identical compared to other upazila. In the year 2017, the disease incidence varied from 1.15% to 2.75% where the highest incidence (2.75%) was recorded in Gomostapur upazila of Chapainawabganj district which was statistically identical with Shibganj, Sadar and Nachol upazila of the same district but significantly differed with others. Among the locations the lowest incidence (1.15%) in Charghat upazila of Rajshahi district which was statistically similar with Putia upazila of the same district.

4.1.4.2. Incidence on 30 ± 5 years old plants

Disease incidence of 30 ± 5 years old plants on surveyed locations during 2016 and 2017 is presented in Fig 4.5 and Appendix 1&2. Depending on locations it was observed that the disease incidence varied from 1.65% to 3.25% in 2016 and 1.54% to 4.25% in 2017. Among the locations statistically the highest incidence of the disease (3.25% & 4.25%) was monitored in Shibganj upazila of Chapainawabganj district in both the year. In 2016 the lowest incidence (1.65%) was recorded in Charghat upazila of Rajshahi district which is statistically similar with Bagha and Putia upazila of the same district but, significantly differed with others. In the year 2017, the lowest incidence (1.54 %) was observed in Putia upazila which was similar to Bagha and Charghat upazila but differed with other locations.

4.1.4.3. Incidence on 40 ± 5 years old plants

Disease incidence of 40 ± 5 years old plants on surveyed locations during 2016 and 2017 is presented in Fig 4.6 and Appendix 1&2. Depending on locations it was observed disease incidence varied from 1.50% to 3.60% in 2016. Among the locations the highest incidence of the disease (3.60%) was monitored in sadar upazila of Chapainawabganj which was similar with Shibganj and Gomostapur upazila of Chapainawabganj district but significantly differed with others. On the other and the lowest incidence (1.50%) was recorded in Putia upazila of Rajshahi district which was statistically similar with other two upazila of the same district but significantly differed with others. In the year 2017, the disease incidence varied from 1.56% to 3.65% where the highest incidence (3.65%) was recorded in Shibganj upazila of Chapainawabganj that is identical with Sadar upazila of the same district but significantly differed with other locations. On the other hand the lowest incidence

(1.56%) in Putia upazila of Rajshahi district which was statistically similar with Bagha and Charghat upazila.

4.1.4.4. Incidence on 50 ± 5 years old plants

Disease incidence of 50 ± 5 years old plants on surveyed locations during 2016 and 2017 is presented in Fig 4.7 and Appendix 1&2. Depending on locations it was observed that the disease incidence varied from 2.00% to 3.96% in 2016 and 2.45% to 4.96% in 2017 (Fig 4.7 and Appendix 1&2). In 2016, the highest incidence of the disease (3.96%) was monitored in Gomostapur upazila which was statistically similar with Sibganj and Sadar upazila of Chapainawabganj district but significantly differed with other locations. Among the locations the lowest incidence (2.00%) was recorded in Putia upazila which was statistically similar to Bagha and Charghat upazila of Rajshahi district and Bholahat upazila of Chapainawabganj district. In the year 2017, statistically the highest incidence (4.96%) was recorded in sadar upazila of Chapainawabganj district and the lowest incidence (2.45%) in Bholahat upazila of the same district. From the results presented in Fig.4.4 to Fig.4.7 it is observed that with some exception the diseases incidence was higher in 2017 than 2016. It indicate that the disease incidence is increasing day by day in Rajshahi region although statistically there was no significant difference on gummosis and sudden decline disease infection between these two years depending on locations.

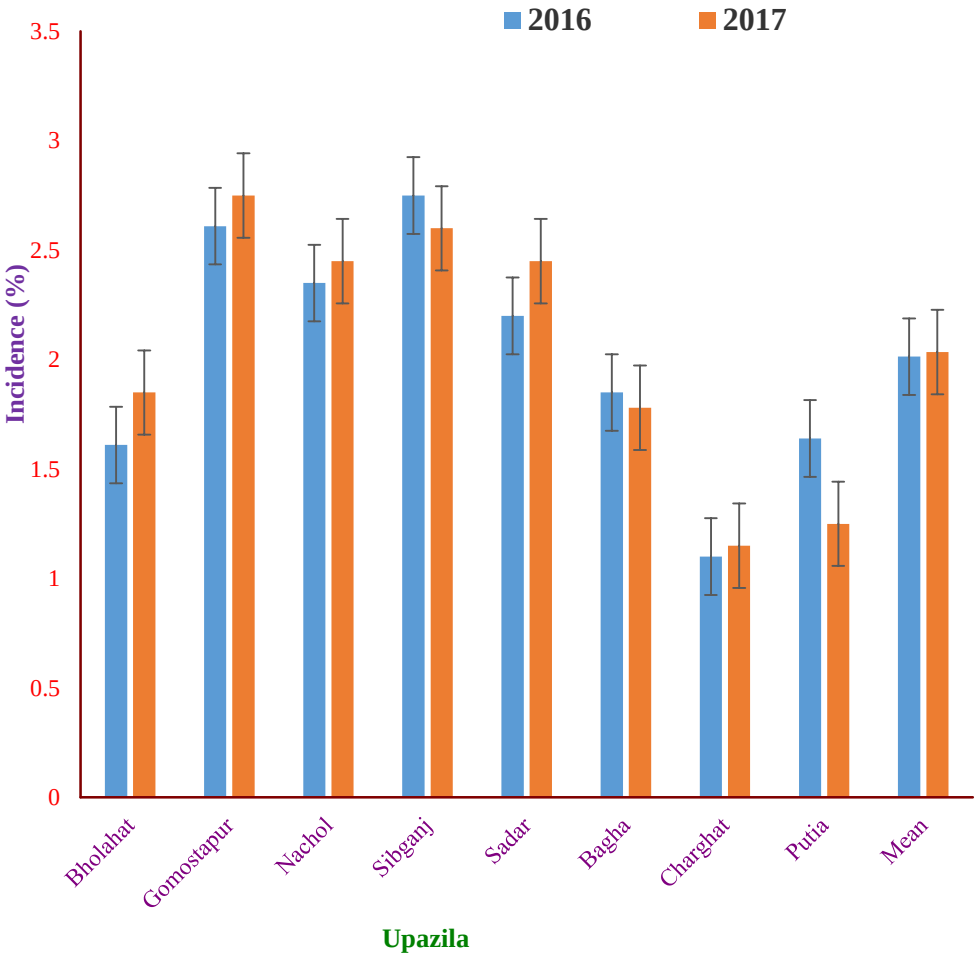


Fig. 4.4. Incidence of gummosis and sudden decline on 20 ± 5 years old plants on surveyed locations during 2016 and 2017

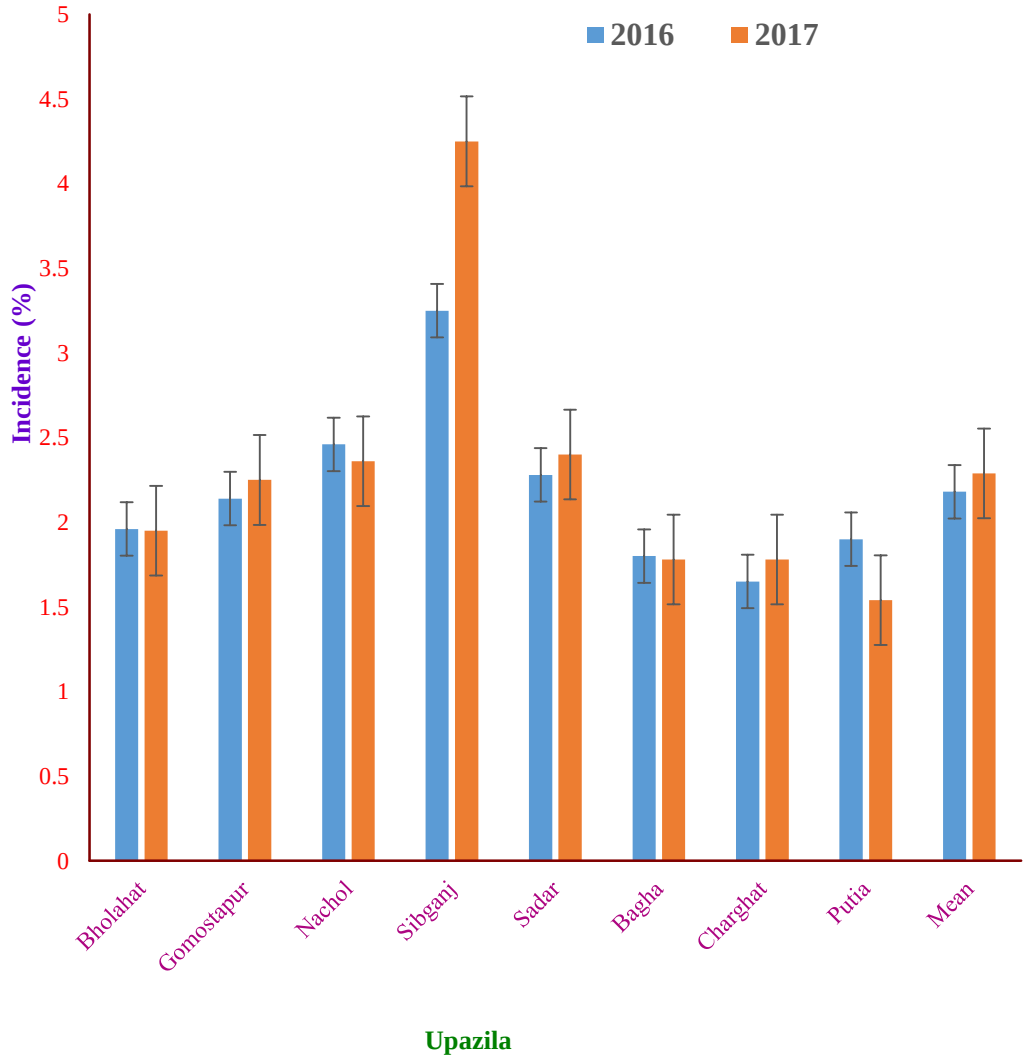


Fig.4.5. Disease incidence on 30 ± 5 years old plants on surveyed locations during 2016 and 2017

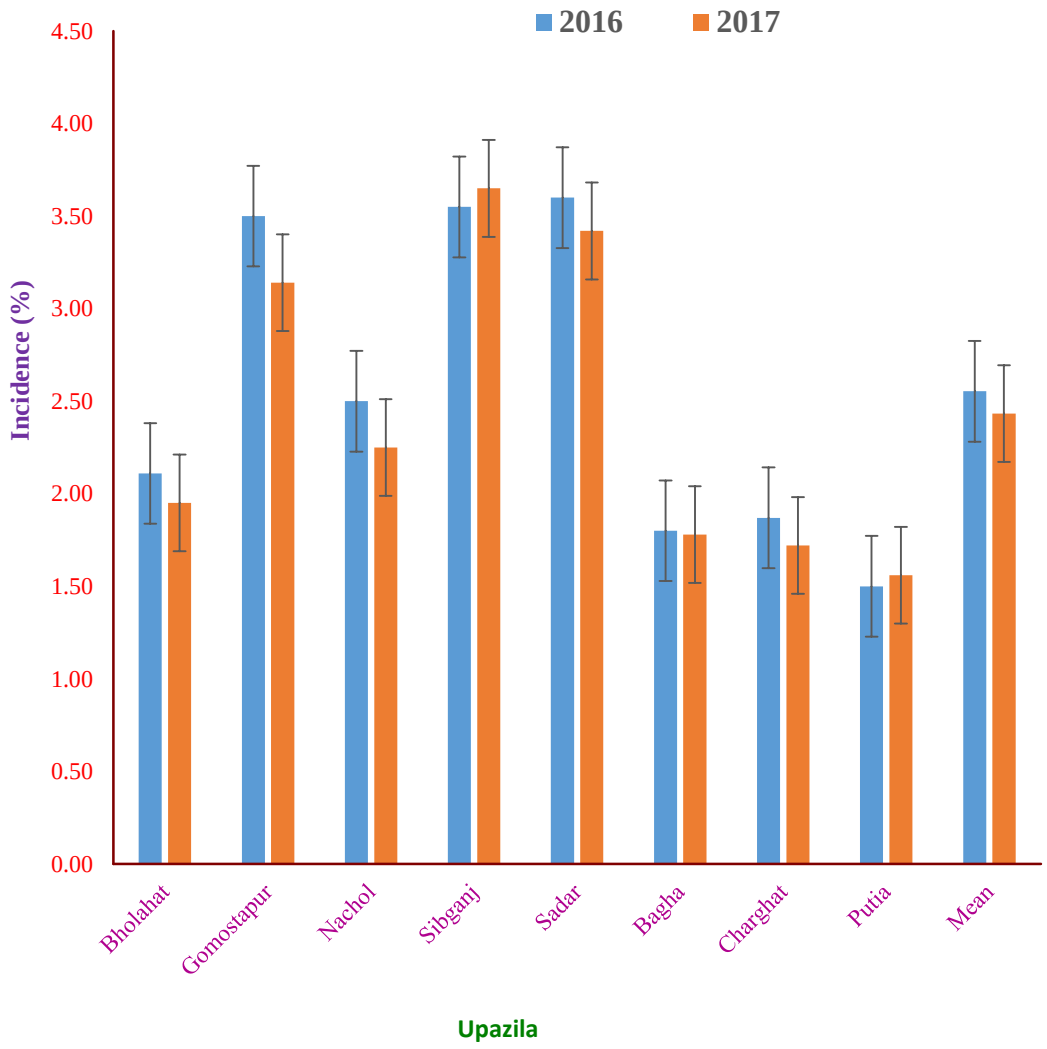


Fig.4.6. Disease incidence on 40 ± 5 years old plants on surveyed locations during 2016 and 2017

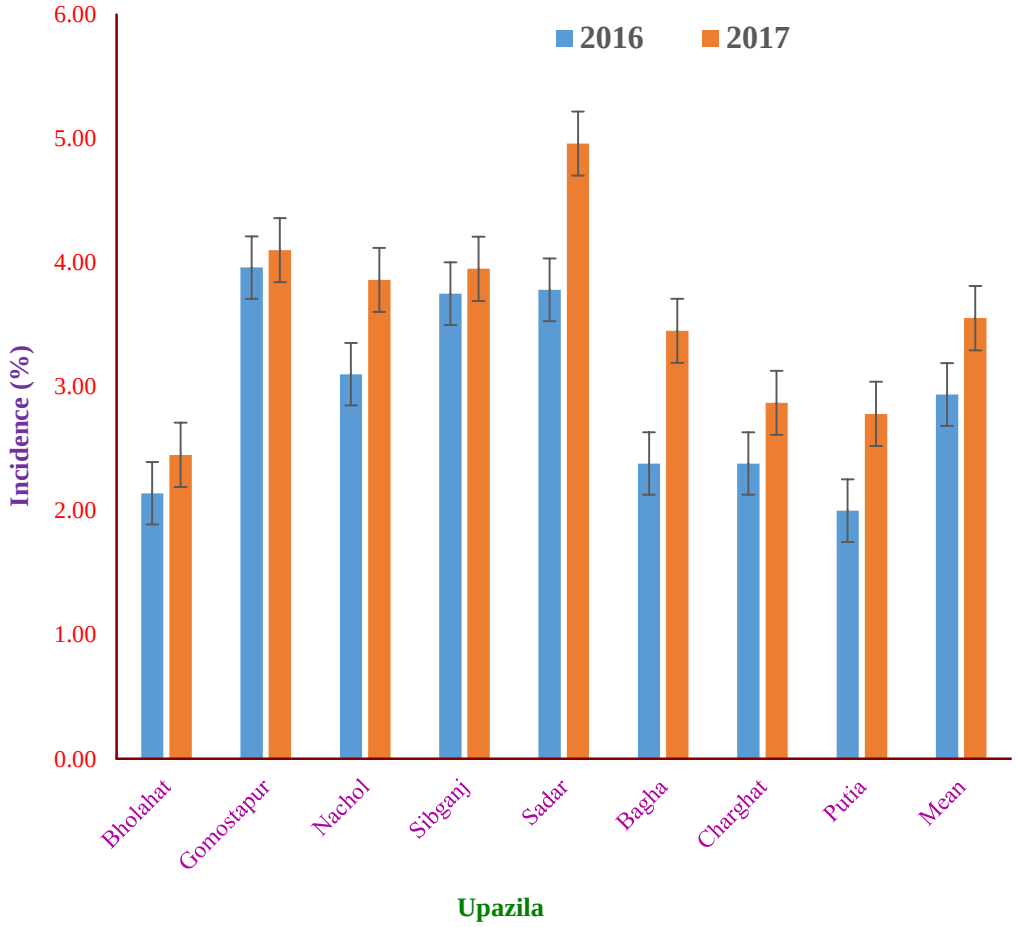


Fig.4.7. Disease incidence on 50 ± 5 years old plants on surveyed locations during 2016 and 2017

4.1.4.5. Relation of plant age with gummosis and sudden decline disease infection

Results on relation between age of the plant and gummosis infection are presented in Fig. 4.8. Result have shown that with the increase of age of plants, gummosis infection also increased in both the year. It indicate that older plants might be more susceptible to gummosis and sudden decline disease compared to younger plants.

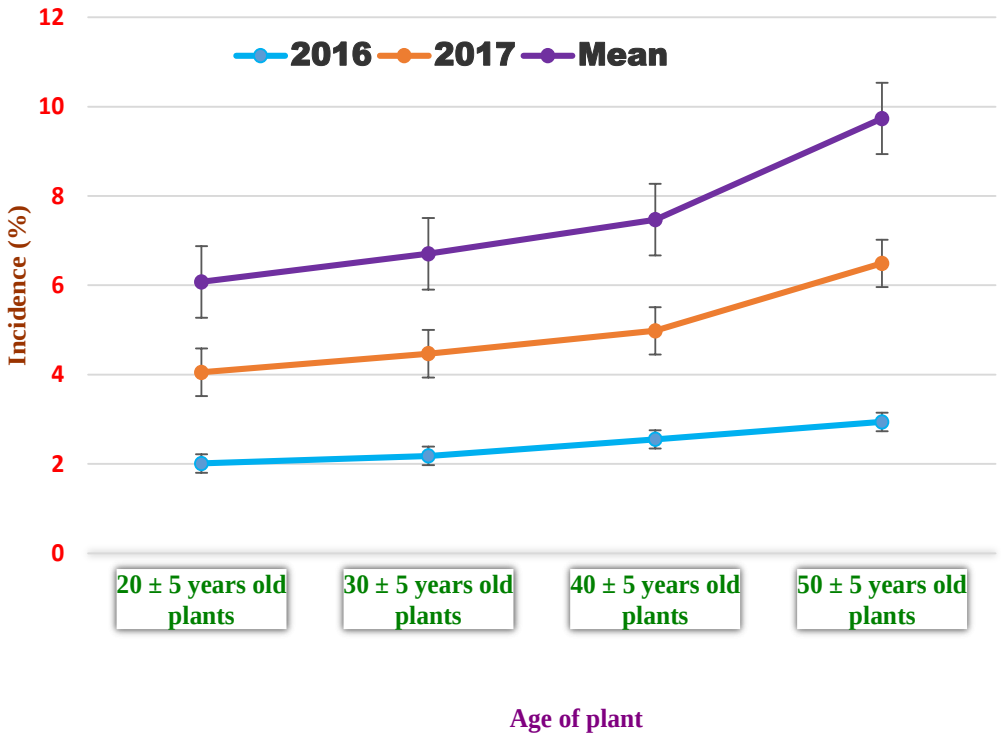


Fig 4.8. Relation of plant age with gummosis and sudden decline disease infection

4.1.5. Disease severity index

4.1.5.1. Severity index of gummosis and sudden decline disease in different locations

Among the orchards surveyed in eight locations disease severity index of gummosis and sudden decline were varied from 5.33 % to 9.73 % during 2016 (Table 4.5). The highest disease severity index (9.73%) was recorded in Shibganj upazila of Chapainawabganj district followed by sadar (8.40%) and Bholahat upazila (7.47%) of the same district. Among the locations the lowest disease severity index (5.33%) was recorded in Putia upazila of Rajshahi district followed by Charghat (5.60%) upazila of the same district.

On the other hand, disease severity index varied from 6.40% to 11.67% during 2017 (Table 4.5). In 2017 the highest disease severity index (11.67%) was recorded in Shibganj upazila of Chapainawabganj district followed by Bholahat (8.67%) and Sadar upazila (8.07%) of the same district. But, the lowest disease severity index (6.40%) was recorded in Gomostapur upazila of Chapainawabganj district followed by Putia (6.67%) upazila of Rajshahi district.

Considering both the year the pooled date on disease severity index are shown in Fig. 4.9. Among the locations the highest severity index (10.70 %) was recorded in Shibganj uapzila of Chapainawabganj district followed by sadar (8.23%) and Bholahat upazila (8.07%) of the same district. But, the lowest disease severity index (6.00%) was recorded in Putia upazila of Rajshahi district followed by Charghat (6.43%) upazila of the same district. The mango orchards of Shibganj uapzila is very closer and the maximum trees of that orchards are older than that of other surveyed

locations. On the other hand the mango orchards of Putia uapzila is scatteredly developed and the maximum trees are younger compared to other surveyed locations that's might be the cause of the difference of disease severity in different locations.

Table 4.5 Severity index of gummosis and sudden decline disease in different locations in 2016 and 2017

Name of District	Name of Upazila	**Disease severity index (%)**	
		2016	**2017**
Chapainawabganj	Bholahat	7.47	8.67
	Gomostapur	6.80	6.40
	Nachol	6.40	7.33
	Shibganj	9.73	11.67
	Sadar	8.40	8.07
Rajshahi	Bagha	7.20	7.53
	Charghat	5.60	7.27
	Putia	5.33	6.67

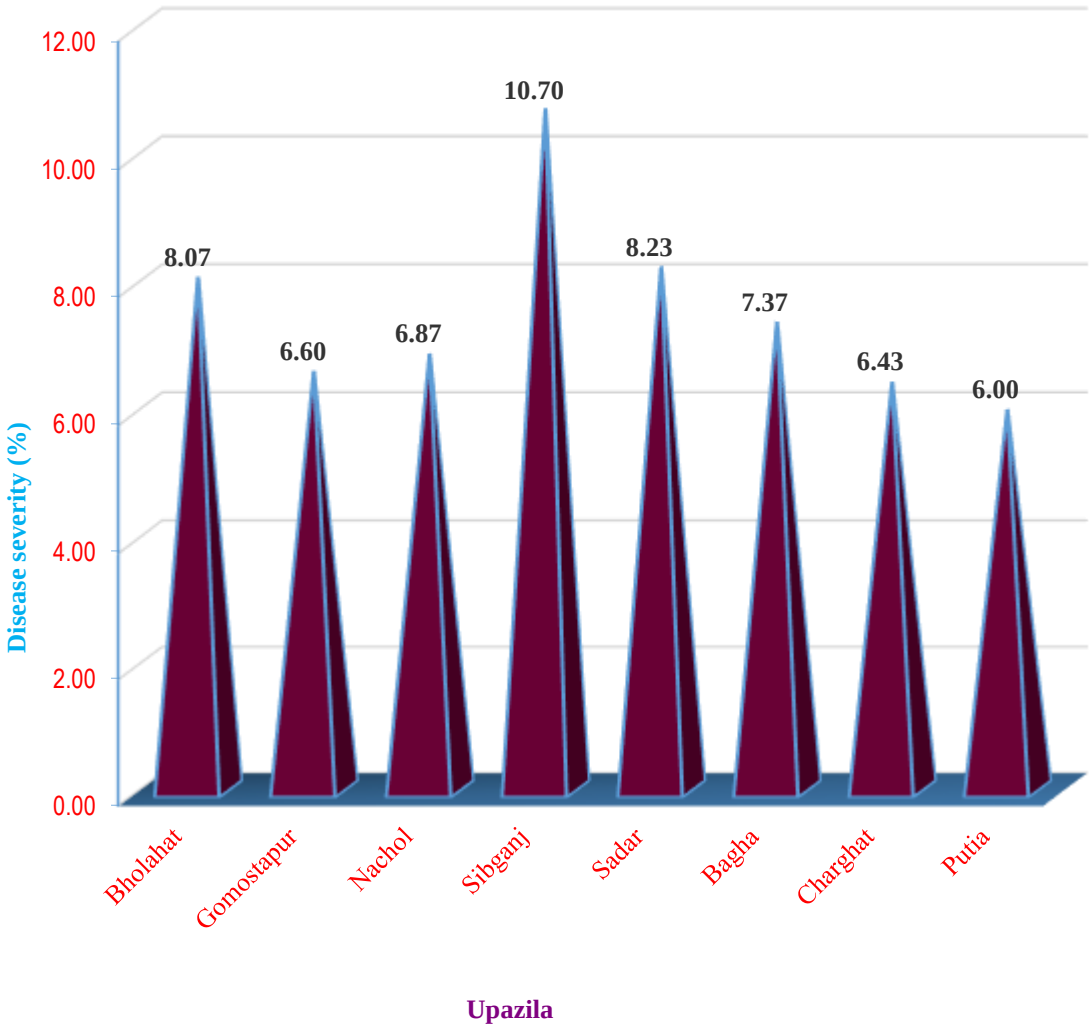


Fig.4.9. Mean severity index of gummosis and sudden decline disease on 2016 and 2017 in different locations.

4.1.5.2. Severity index of gummosis and sudden decline disease in different varieties

Mango varieties under investigation also showed differential reaction to the disease severity. The severity varied from 1.77 % to 13.77% depending on the variety under investigation during 2016 (Table 4.6). The highest severity (13.77 %) was recorded in the variety Fazli followed by Gootee (10.22%) while the lowest severity (1.77 %) was recorded in BARI Aam-4 during 2016. On the other hand, disease severity varied from 2.22% to 15.98 % in all varieties investigated during 2017 (Table 4.6). Here also the highest severity (15.98%) was recorded in the variety Fazli followed by Gootee (11.55%) variety but the lowest severity (2.22%) was recorded in BARI Aam-1. Form the pooled data of 2016 and 2017 it was observed that among the varieties observed, the highest disease severity (14.87 %) was recorded in the variety Fazli followed by Gootee (10.88%) and Ashwinia (10.22%) variety. The lowest severity (2.16 %) was recorded in BARI Mango-4 which was almost similar to other four BARI released varietes i.e BARI Aam-1 (2.22%), BARI Aam-2 (2.38%), BARI Mango-3(3.05%) and other two popular commercial varieties i.e gopalbhog (3.10%) and Bomby (3.21%) (Fig 4.10). From the results presented in Table 4.6 and Fig 4.10 it is clear that there was no variety found free from the disease infestation during the study period although their infection level showed considerable variation. Considering variety, it was observed that Fazli was very much susceptible to gummosis and sudden decline disease followed by Gootee variety with some exception during the survey period. Similar results were observed by Begum *et al.* (2003). Panhwar *et al.* (2005) reported that maximum mean disease severity index was recorded on indigenous (local) variety followed by Sindhri, Langara, Chaunsa, Dusheri,

Siroli, Gulab Khasa, Collector, Neelam, Fajri, Beganpali, Anwar Ratole, Almas and Swarnarika.

Table 4.6 Severity index of gummosis and sudden decline disease in different varieties in 2016 and 2017

Name of Variety	**Disease severity index (%)**	
	2016	**2017**
BARI Aam-1	2.22	2.22
BARI Aam-2	2.22	2.55
BARI Aam-3	2.66	3.44
BARI Aam-4	1.77	2.55
Ashiwina	9.78	10.66
Fazli	13.77	15.98
Bomby	3.11	3.32
Gopalbhog	2.66	3.55
Khirsapat	7.11	8.11
Langra	5.11	6.55
Gootee	10.22	11.55

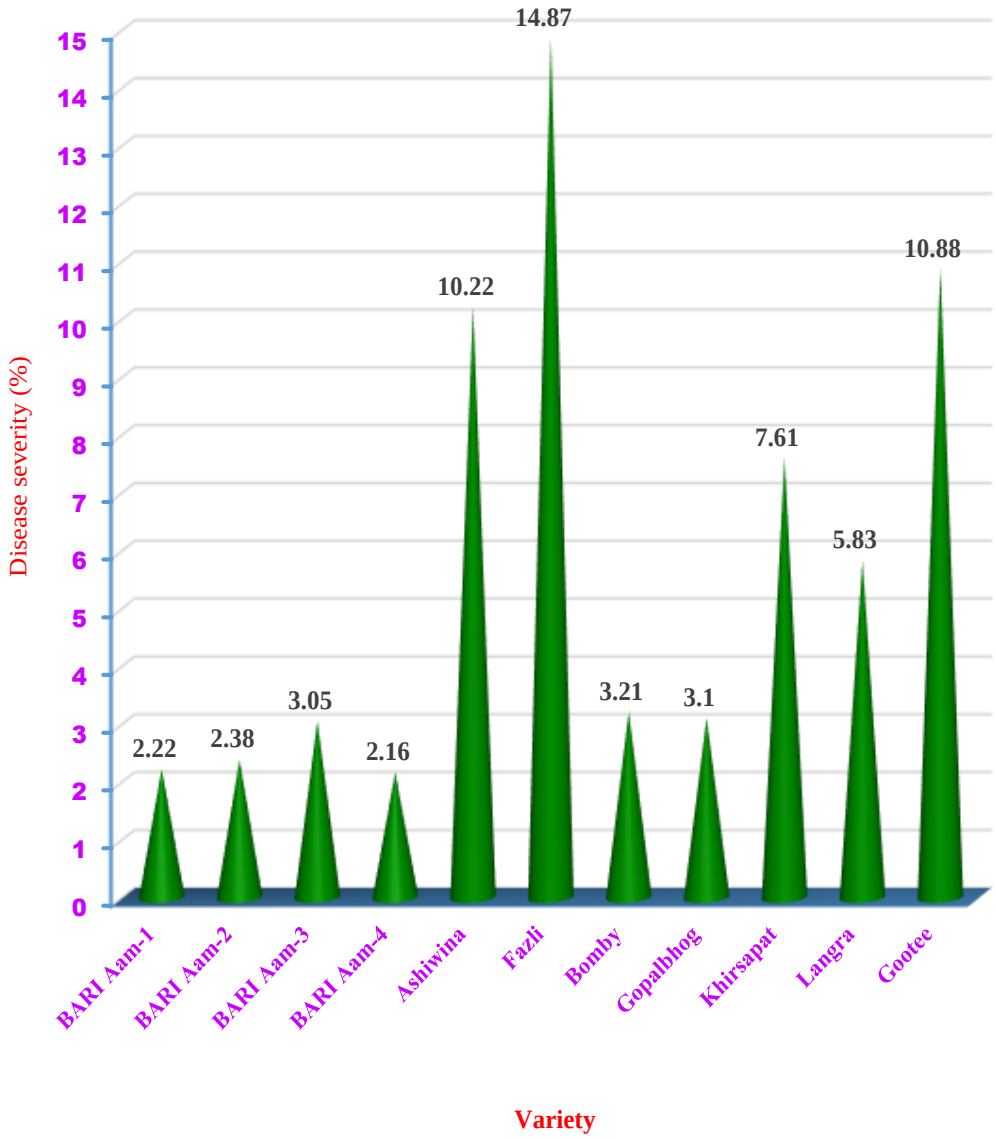


Fig.4.10. Mean severity index of gummosis and sudden decline disease on 2016 and 2017 in different mango varieties.

4.1.6. Death percentage of mango plants

4.1.6.1. Death percentage of mango plants in different locations

Among the orchards surveyed in eight locations dead plants due to gummosis and sudden decline disease varied from 1.32 % to 4.94 % during 2016 (Table 4.7). The highest death (4.94%) was recorded in Shibganj upazila of Chapainawabganj district followed by Gomostapur (2.04%) and Sadar upazila (1.87%) of the same district. On the other hand the lowest death (1.32%) was recorded in Putia upazila of Rajshahi district followed by Bagha (1.39%) upazila of the same district.

During 2017, the percentage of dead plant varied from 1.50% to 5.05% (Table 4.8). Among the surveyed locations the highest death (5.05 %) was recorded in Shibganj upazila of Chapainawabganj district followed by Gomostapur (2.24%) and Sadar upazila (2.00%) of the same district. But, the lowest death (1.50 %) was recorded in both Putia upazila of Rajshahi and Bholahat upazila of Chapainawabganj district.

Considering both the year the pooled date on percentage of dead plants are shown in Fig. 4.11. Among the surveyed upazila the highest death (5.00%) was recorded in Shibganj upazila of Chapainawabganj district followed by Gomostapur (2.14%) and Sadar upazila (1.94%) of the same district. On the other hand the lowest death (1.41%) due to gummosis and sudden decline was recorded in Putia upazila of Rajshahi district which was followed by Bholahat upazila (1.46%) of Chapainawabganj district. The incidence and severity of gummosis and sudden decline is higher in Shibganj upazila of Chapainawabganj district and the lower in Putia upazila of Rajshahi district compared to the other surveyed locations. Similar trend was found in case of dead plants. The exact cause of death is unknown.

Table 4.7. Percent death of mango plants due to gummosis and sudden decline disease in different upazilas of Chapainawabganj and Rajshahi districts in 2016

Name of District	Name of Upazila	Distribution of dead plants (%)
Chapainawabganj	Bholahat	1.42
	Gomostapur	2.04
	Nachol	1.60
	Shibganj	4.94
	Sadar	1.87
Rajshahi	Bagha	1.39
	Charghat	1.48
	Putia	1.32

Table 4.8 Death of mango plants due to gummosis and sudden decline disease in different upazilas of Chapainawabganj and Rajshahi districts in 2017

Name of District	Name of Upazila	Distribution of dead plants (%)
Chapainawabganj	Bholahat	1.50
	Gomostapur	2.24
	Nachol	1.68
	Shibganj	5.05
	Sadar	2.00
Rajshahi	Bagha	1.56
	Charghat	1.73
	Putia	1.50

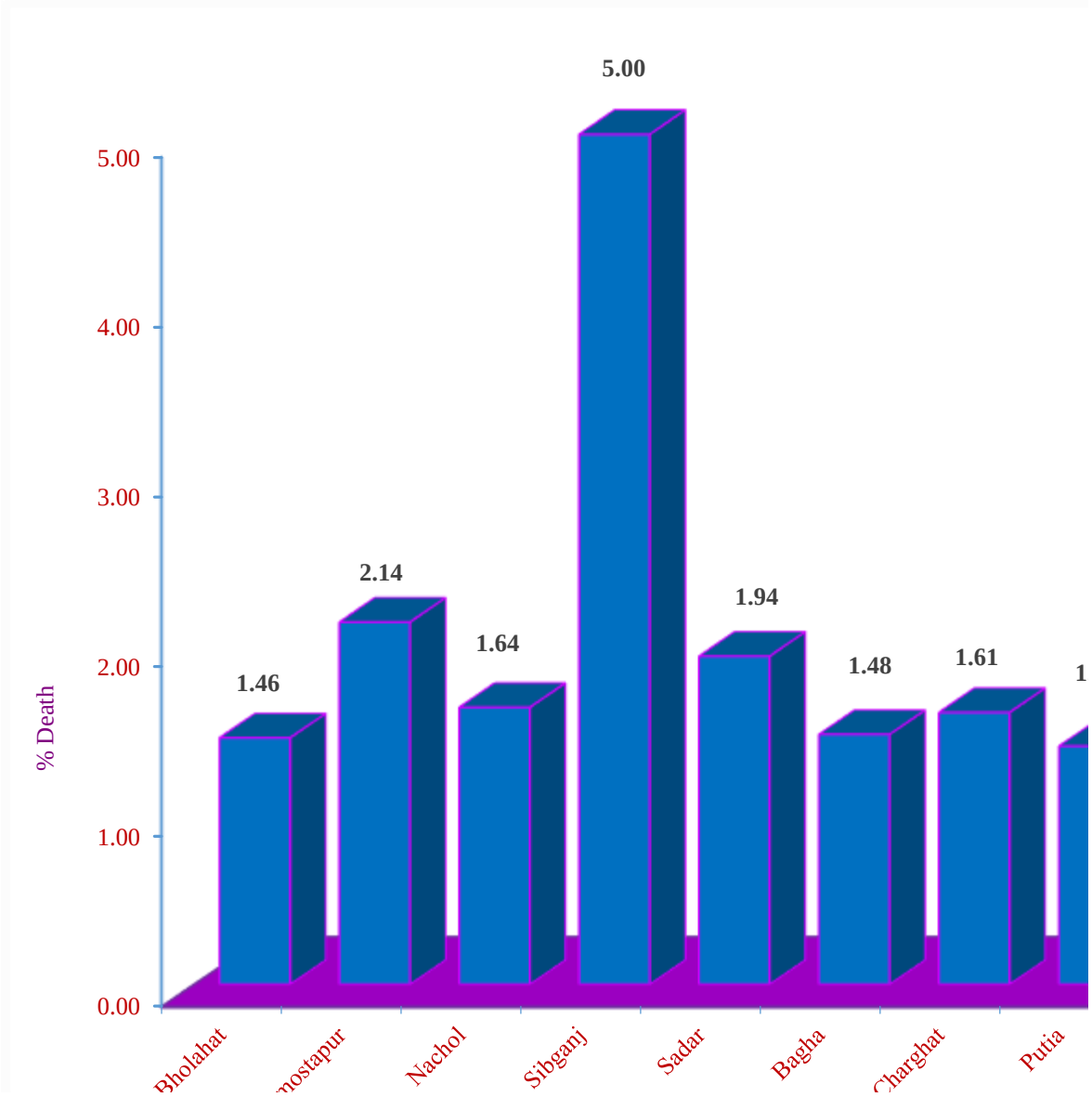


Fig 4.11. Dead of mango plants due to gummosis and sudden decline disease during 2016 and 2017

4.1.6.2. Dead of mango plants in different varieties

Death percentage of plants due to gummosis and sudden decline disease varied from 0% to 2.36% depending on mango variety in 2016 (Table 4.9). The highest death (2.36%) was recorded in Fazli variety followed by Langra (1.10%), Gopalbhog (1.06%) and Gootee variety (1.00%) while the lowest death (0.27%) was recorded in Bombay variety followed by Khirsapat (0.57%) and Ashiwinia variety (0.76%) during 2016 (Table 4.9).

In 2017, the death percentage of mango plants due to the attack of gummosis and sudden decline disease varied from 0 to 2.84% (Table 4.10). Among the varieties the highest death (2.84 %) was recorded in Fazli variety followed by Gootee (1.64%), Bombay (1.42%) and Langra variety (1.28%) while the lowest death (0.98%) was recorded in Ashiwina variety followed by Khirsapat (1.01%), BARI Aam -2 (1.02%) (Table 4.10). But, no dead plants were found in the varieties of BARI Aam-1, BARI Aam -3 and BARI Aam -4 in both the surveyed years in all locations surveyed.

Fig. 4.12 represents the results of the pooled data of 2016 and 2017. From this figure. it is clearly observed that among the varieties the highest death (2.60%) was recorded in Fazli variety followed by Gootee (1.32%), Langra (1.19%) and Gopalbhog (1.10%) while the lowest death (0.79%) was recorded in Khirsapat followed by Bombay (0.85%), Ashiwina (0.87%), and BARI Aam -2 (0.97%). But, no dead plants were found in the varieties of BARI Aam-1, BARI Aam -3 and BARI Aam -4 all the locations surveyed. These varieties may be tolerant to the disease.

Table 4.9. Varietal reaction of dead mango plants due to gummosis and sudden decline disease in different upazilas of Chapainawabganj and Rajshahi districts in 2016

Variety Name	Plant died (%)in different upazila								
	Bholahat	Gomostapur	Nachol	Sibganj	Sadar	Bagha	Charghat	Putia	Mean
BARI Aam-1	-	-	-	-	0	-	-	-	0.00
BARI Aam-2	0.85	3.45	0	0	0	1.12	1.12	0.85	0.92
BARI Aam-3	0	0	-	-	0	0	0	0	0.00
BARI Aam-4	-	-	0	-	0	-	-	-	0.00
Ashiwina	0.83	1.42	0.43	0.51	0	1.33	0.56	1.02	0.76
Fazli	1.59	1.71	3.21	4.31	3.44	0.17	2.65	1.78	2.36
Bomby	-	-	0	2.12	0	-	-	-	0.27
Gopalbhog	1.66	0	1.11	1.26	1.13	1.00	1.23	1.11	1.06
Khirsapat	1.10	0	0	2.08	0	1.35	0	0	0.57
Langra	1.5	1.05	0	3.63	1.54	0	1.1	0	1.10
Gootee	0.32	1.12	0	4	0.87	0.46	1.22	0	1.00

Table 4.10. Varietal reaction in percent death of mango plants due to gummosis and sudden decline diseases in different upazilas of Chapainawabganj and Rajshahi districts in 2017

Variety Name	Plant died (%)in different upazila								
	Bholahat	Gomostapur	Nachol	Sibganj	Sadar	Bagha	Charghat	Putia	Mean
BARI Aam-1	-	-	-	-	0	-	-	-	0.00
BARI Aam-2	0.95	2.95	0	0	0	1.5	1.5	1.25	1.02
BARI Aam-3	0	0	-	-	0	0	0	0	0.00
BARI Aam-4	-	-	0	-	0	-	-	-	0.00
Ashiwina	0.93	1.3	0.65	1.1	0	1.56	1	1.26	0.98
Fazli	2.1	1.8	2.98	4.75	4.45	2.14	2.55	1.98	2.84
Bombay	-	-	1.45	1.85	0.95	-	-	-	1.42
Gopalbhog	1.66	0	0.95	1.35	1.56	0.75	1.45	1.35	1.13
Khirsapat	1.78	0	0	2.15	1.1	1.58	0.98	0.5	1.01
Langra	1.65	0.98	0	2.85	1.85	0.85	1.22	0.8	1.28
Gootee	1.36	1.3	0	4.45	1.84	1.45	1.45	1.25	1.64

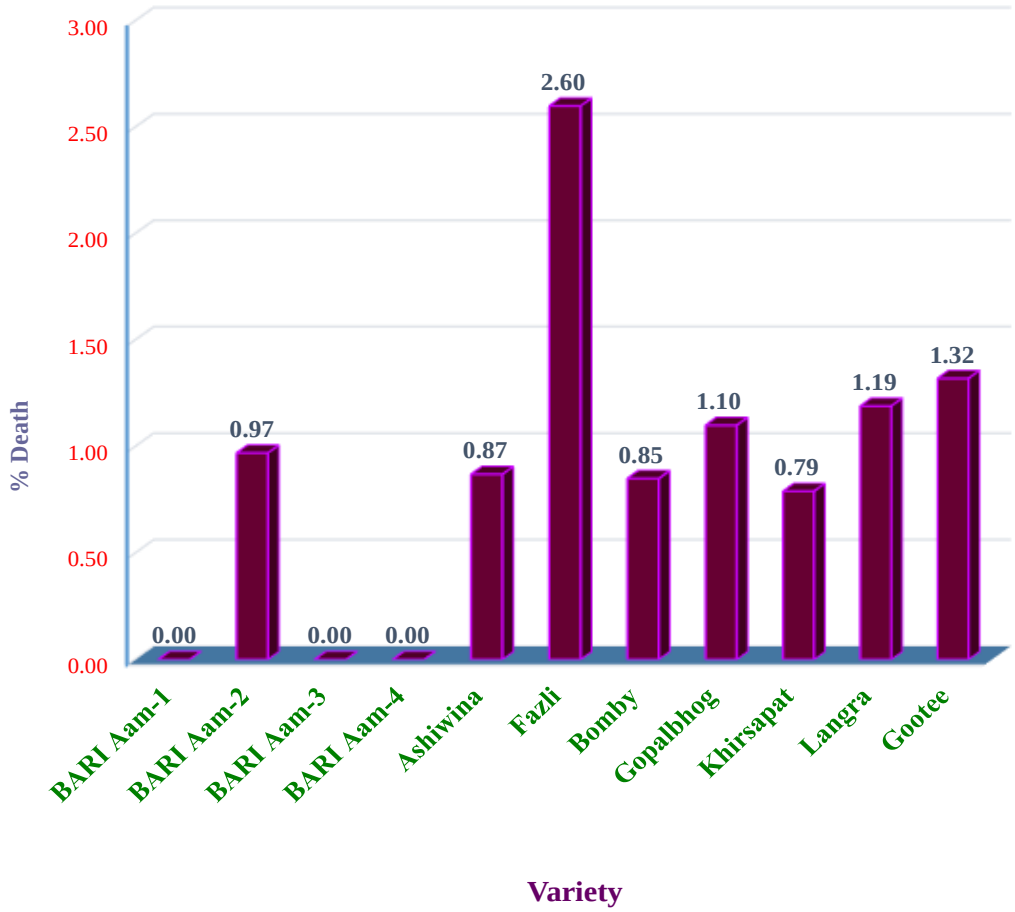


Fig 4.12. Varietal reaction of dead mango plants due to gummosis and sudden decline disease in different upazilas of Chapainawabganj and Rajshahi districts during 2016 and 2017

4.2. EXPERIMENT-II: ISOLATION, PURIFICATION, IDENTIFICATION AND CHARACTERIZATION OF THE CAUSAL AGENT OF GUMMOSIS AND SUDDEN DECLINE DISEASE OF MANGO PLANTS

During survey, diseased sample with typical gummosis symptoms were collected from different mango orchards from Rajshahi and Chapainawabganj districts of Bangladesh. The isolations of the associated micro-organisms were done from infected twigs and gummy exudates through standard tissue isolation method under aseptic conditions. The associated micro-organisms were then purified through hyphal tip technique and maintained on PDA and identified on the basis of morphological characters documented in standard authentic description (Barnett, 1960; Von Arx, 1981). All isolations made from the diseased samples were found to be *L. theobromae*.

4.2.1. Characterization and identification of micro organisms

The mycelium and pycnidiospore of the isolated fungus was examined under compound microscope. The mycelium was initially white, soon become black and fast spreading with immersed and superficial, branched, septate mycelium. Shiny black pycnidia were produced on the surface (Plate.4 5). Conidia were initially hyaline, unicellular, sub ovoid to ellipsoidal, with a granular content. Mature conidia were two celled, cinnamon to dark brown, thick walled, ellipsoidal, often with longitudinal striations (Plate. 4.6a-f). According to Sutton (1980) and Ellias (1980) the isolated fungus was identified as *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. The same fungus was isolated from the gummosis infected mango plants and was described as the causal agent of the disease by Khanzada *et al*., 2004, Mahmood and Gill 2002 and Malik *et al*., 2005.

L. theobromae colonies on PDA was moderately dense, raised mycelium mat, initially white to smoky-grey, turning greenish grey on the surface and greenish grey in reverse, becoming dark slate blue with age (Plate 4.5). Cardinal temperature requirements for growth was minimum at 15°C, maximum at 35°C and optimum 25°C. Conidia oozing from pycnidia were initially hyaline, smooth, thick-walled, aseptate, obovoid to ellipsoid, granular, mostly somewhat tapered at apex and rounded at base, becoming brown, single septate, with longitudinal striations on the inner surface of the conidial wall due to the melanin deposits (Plate 4.6).The mycelium of the isolated fungus grows vigorously on PDA. The aerial mycelium grows uniformly in all direction and fully covered the surface of media within three to four days. Initially when the culture is three days old it is white in appearance (Plate 4.5). Gradually, the colour of the colony changes from white to light grey in four to seven days of incubation (Plate 4.5). After two weeks of incubation the colony colour changes from light grey to black colour (Plate 4.5). The bottom of fungus became dark black after three weeks of incubation (Plate 4.5). As the culture becomes dark black in colour, leads to production of pycnidia after three weeks of incubation (Plate 4.5). The pycnidia produced are initially soft but gets harder at maturity (after three to four weeks). Pycnidia were produced at the centre, middle or edges of the culture plate. The pycnidia matures after four weeks of incubation and leads to sporulation (Plate 4.5). During sporulation two main types of spores were produced. Immature spores were hyaline, non-septate, thick-walled, oval to oblong in shape (Plate 4.6) whereas mature spores were cinnamon brown to dark brown

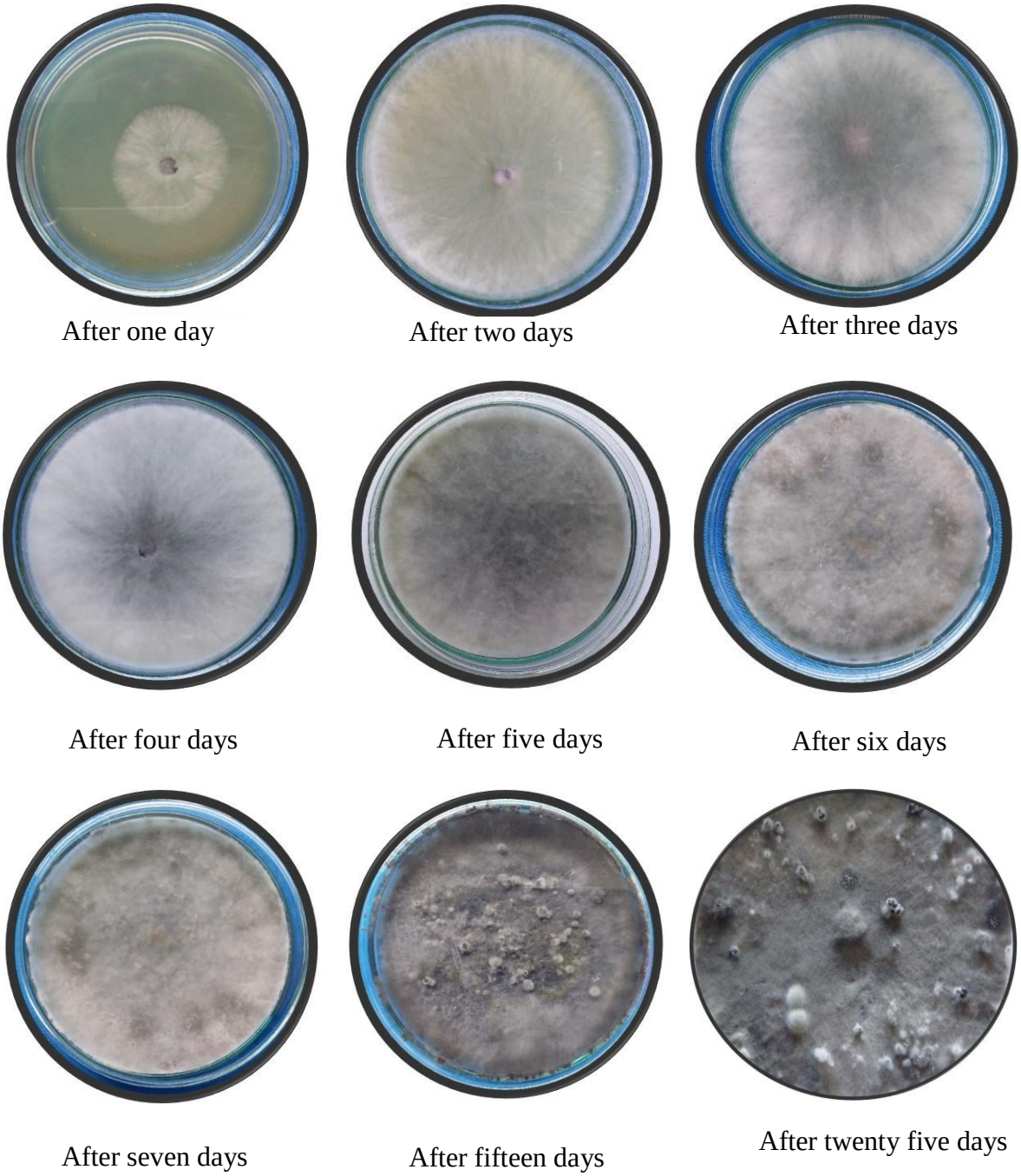
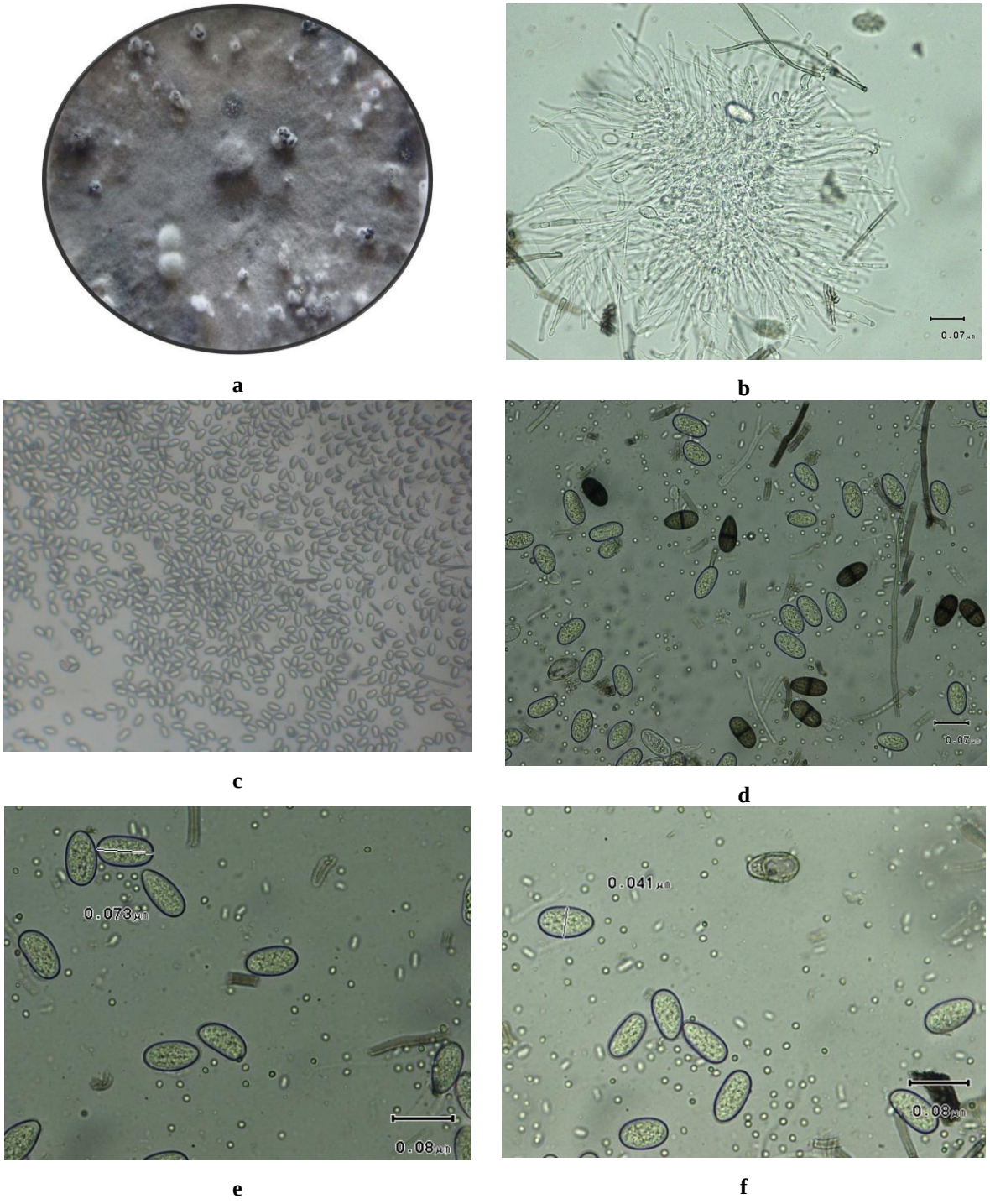


Plate-4.5. Colony of *L. theobromae* on PDA at different days after incubation



a **b** **c** **d** **e** **f**

Plate 4.6. Cultural and morphological characteristics of *Lasiodiplodia theobromae* (a) mycelium growth on PDA showing pycnidia (b) hyphae (c-f) immature and mature conidia.

in colour with one septum, oval to oblong in shape (Plate 4.6d) which develops irregular longitudinal striations at later stages (Plate 4.6). Similar morphological characteristics was also observed by different authors Punithalingam (1976); Denman *et al*. (2000); Burgess *et al*. (2006); Pavlic *et al*. (2007); Ismail *et al*.(2012); Shahbaz *et al*. (2009) and Norhayati *et al*. (2016).

4.2.2. Pathogenicity test

Pathogenicity test was carried out by stem inoculation method, by inoculating homogenized mycelial discs of *L. theobromae* on 3 year old mango seedlings. Plants inoculated with *Lasiodiplodia theobromae* showed typical symptoms of the disease as observed during the survey programme after one month of inoculation. Control plants did not exhibit any symptom and remained normal and healthy (plate 4.7). Reisolation of the fungus from the infected area always produced the same pathogen. The results clearly indicated that the gummosis is caused by the fungus *Lasiodiplodia theobromae*. Khanzada *et al.* (2004b) also confirmed the pathogenicity of *L. theobromae* on mango by using stem inoculation method. Shahbaz *et al.* (2009), Masood *et al.* (2011) also confirmed the pathogenicity of mango gummosis by this method.

Plate.4.7 : Different steps (a-f) of pathogenicity: (a) slant cut by sterilized knife (b) inserting fungus disc into cut portion (c) wrapped the inoculated portion by polythene (d) gum oozing from the inoculated mango seedling (e) wrapped the control plant by polythene (f) no gum seen in control plant

4.3. EXPERIMENT-III: MOLECUAR CHARACTERIZATIONS OF *LASIODIPLODIA THEOBROMAE*

4.3.1. PCR amplification of ITS region of *L. theobromae* isolates

Results of PCR amplification of ITS region of *L. theobromae* isolates from mango in Bangladesh are shown in Fig. 4.13. The amplicon size 753 bp of the ITS region were amplified by using ITS4 and ITS5 primers which include partial sequence of 18S ribosomal RNA gene, ITS-1, 5.8S ribosomal RNA gene, ITS2 complete sequence and partial sequence of 28S ribosomal RNA sequence (Fig.4.13).

Fig. 4.13. Agarose gel electrophoresis showing the amplification of fungal DNA using ITS1 and ITS4 primers. Lane M= DNA Marker and lane 1-11 fungal genomic DNA of isolate MGSD-001, MGSD-002, MGSD-003, MGSD-004, MGSD-005, MGSD-006, MGSD-017, MGSD-028, MGSD-045, MGSD-071and MGSD-091 respectively.

4.3.2. Phylogenetic placement of *L. theobromae* isolates from mango in Bangladesh

A total of 11 isolates ITS region was sequenced and all sequences showed 96-100% similarity with sequences of *L. theobromae* from GeneBank by nBLAST search analysis. Phylogenetic tree was constructed using MEGA V10 where *Alternaria infectoria* is an out group. For construction of a phylogenetic tree, sequences of *L. theobromae* collected worldwide retrieving from GeneBank, along with *L. brasiliensis*, *L. egyptiacae*, *L. hormozganensis*, L. bruguierae, *L. margaritacea*, *L. parva*, *L. mahajangana*, *L. citricola*, *L. gilanensis*, L. marypalmiae, *L. pseudotheobromae*, *L. euphorbicola*, *L. subglobosa*, *L. chinensis*, *L. hyaline* and *Diplodia cajani* are clustering with the *L. theobromae* isolates from mango in Bangladesh produced a separate subgroup, supporting that the isolates identified in this study is *L. theobromae* (Fig. 4.14). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tajima-Nei method (Tajima F. and Nei. M., 1984) and are in the units of the number of base substitutions per site. The analysis involved 27 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA10 (Kumar *et al*. 2018).

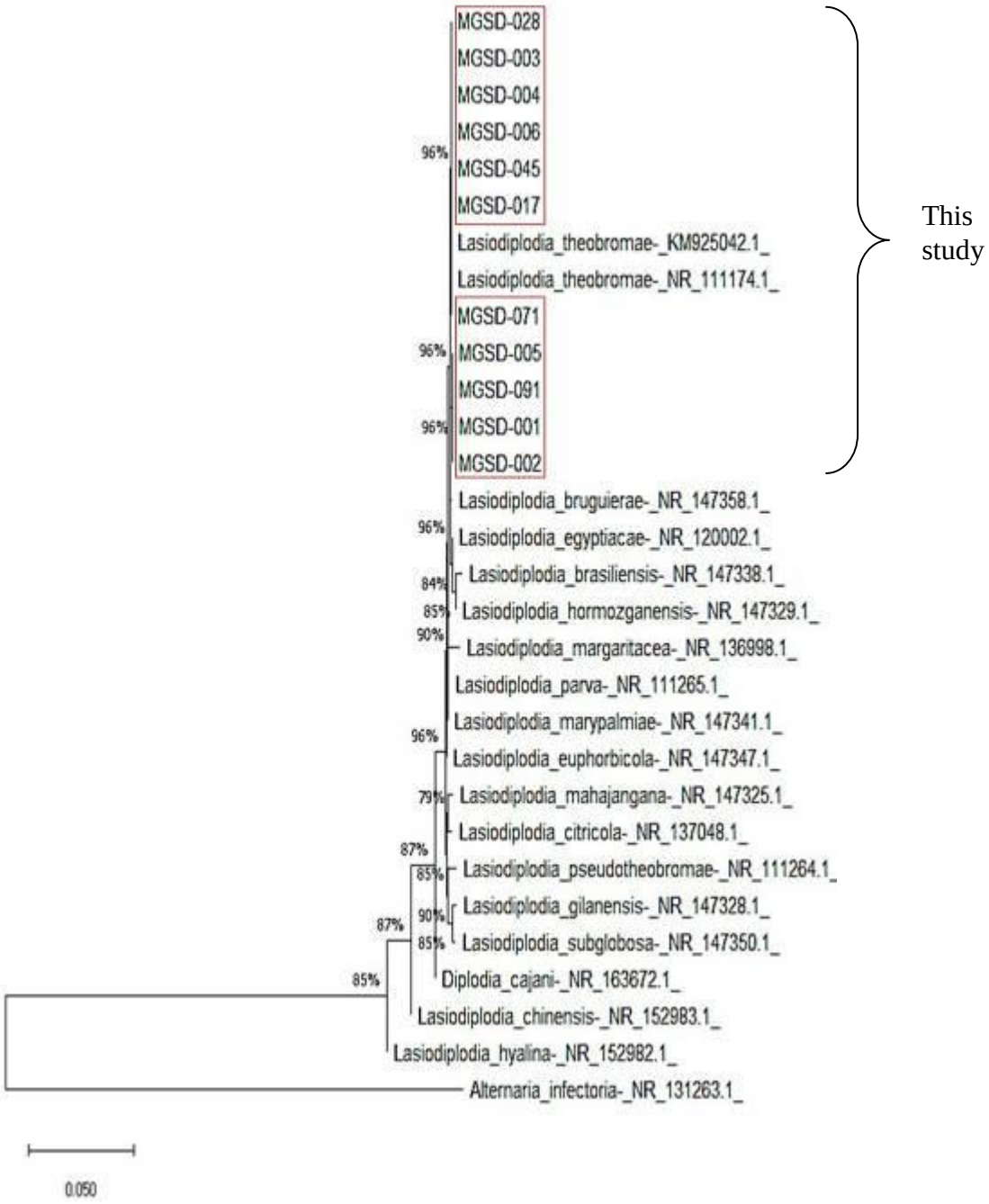


Fig. 4.14. Phylogenitic tree of *L. theobromae* (Saitou and Nei 1987).

4.3.3. Multiple sequence alignment of *L. theobromae* isolates from Bangladesh

The multiple sequence alignment was performed using Bio-edit software V5. The results of the multiple sequenced alignments indicate that the Internal transcribed (ITS) region of *L. theobromae* is conserved (Table 4.11)

Table 4.11 The multiple sequence alignment of 11 isolates of *L. theobromae*

```
                        10        20        30        40        50        60        70        80
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
MGSD001-MW138048  TTTGGAAGTAAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTTTTCGAGCTTCGGCTC
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MGSD045-MW138056  --TGGAAGTAAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTTTTCGAGCTTCGGCTC
MGSD071-MW138057  -TTGGAAGTAAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTTTTCGAGCTTCGGCTC
MGSD091-MW130058  TTTGGAAGTAAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTTTTCGAGCTTCGGCTC

                        90       100       110       120       130       140       150       160
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
MGSD001-MW138048  GACTCTCCCACCCTTTGTGAACGTACCTCTGTTGCTTTGGCGGCTTCGGCCGCCAAAGGACCTTCAAACTCCAGTCAGTA
MGSD002-MW138049  GACTCTCCCACCCTTTGTGAACGTACCTCTGTTGCTTTGGCGGCTTCGGCCGCCAAAGGACCTTCAAACTCCAGTCAGTA
MGSD003-MW138050  GACTCTCCCACCCTTTGTGAACGTACCTCTGTTGCTTTGGCGGCTTCGGCCGCCAAAGGACCTTCAAACTCCAGTCAGTA
MGSD004-MW138051  GACTCTCCCACCCTTTGTGAACGTACCTCTGTTGCTTTGGCGGCTTCGGCCGCCAAAGGACCTTCAAACTCCAGTCAGTA
MGSD005-MW138052  GACTCTCCCACCCTTTGTGAACGTACCTCTGTTGCTTTGGCGGCTTCGGCCGCCAAAGGACCTTCAAACTCCAGTCAGTA
MGSD006-MW138053  GACTCTCCCACCCTTTGTGAACGTACCTCTGTTGCTTTGGCGGCTTCGGCCGCCAAAGGACCTTCAAACTCCAGTCAGTA
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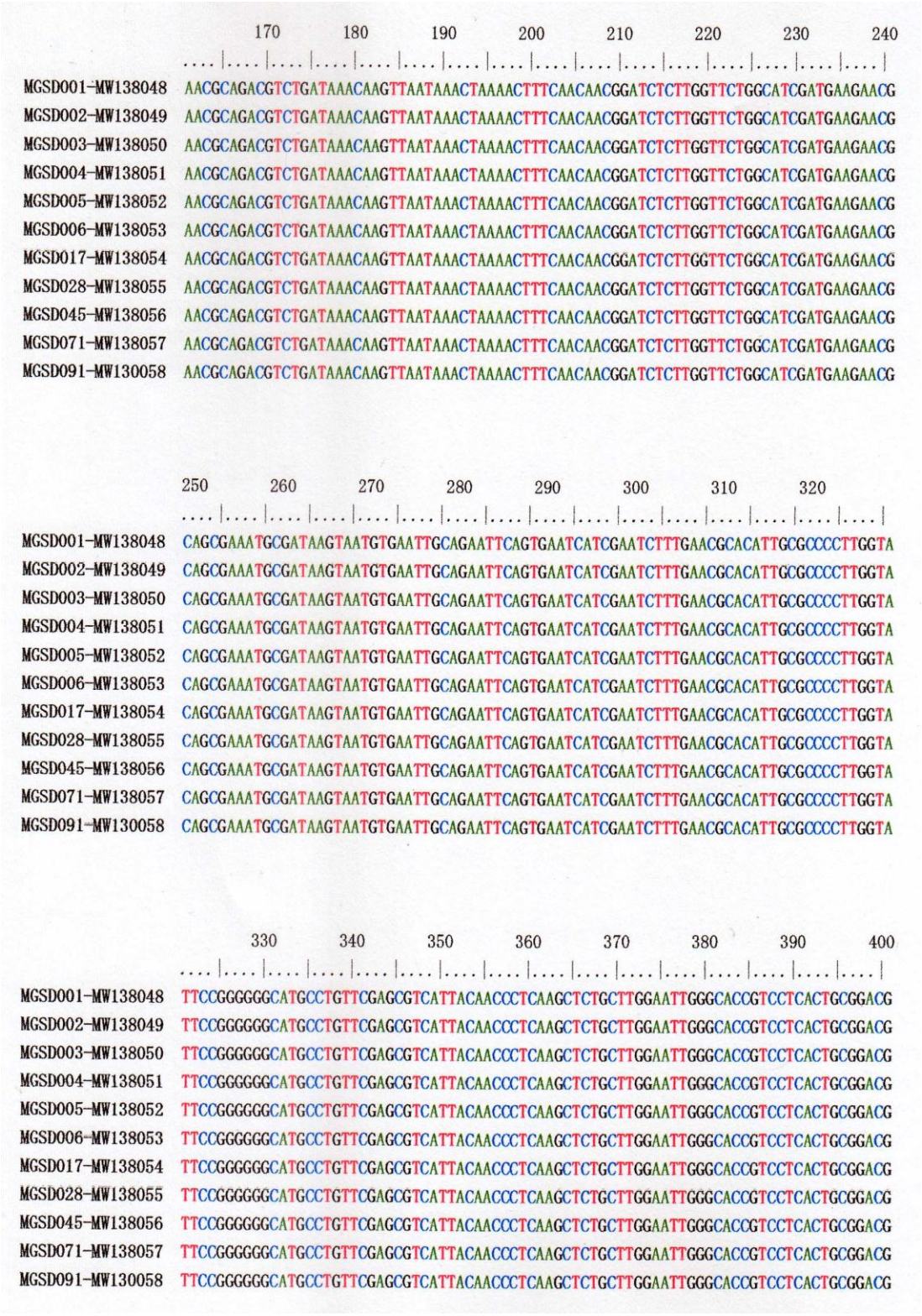
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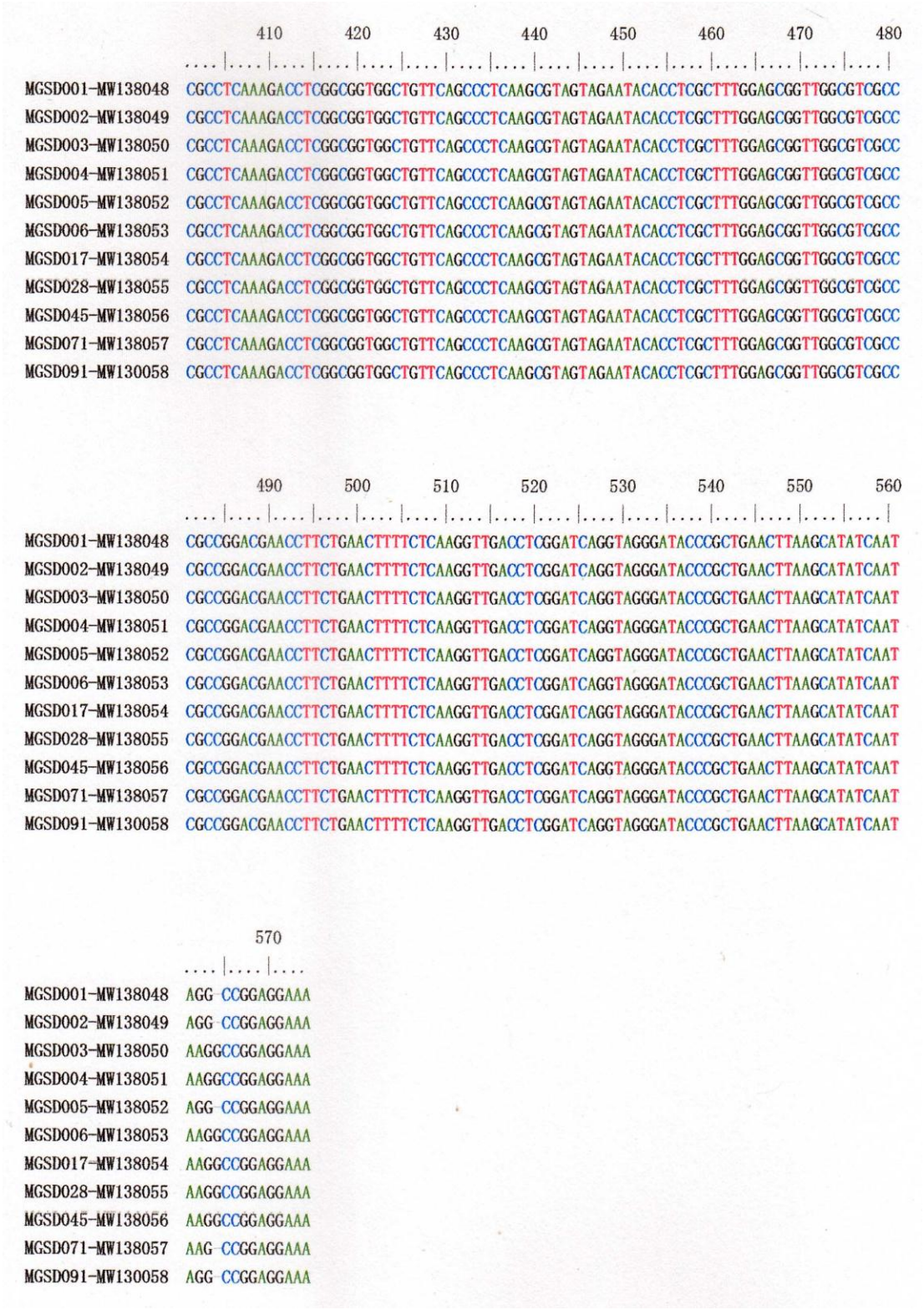
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In present study, molecular identification and characterization of isolated fungi was conducted using genome sequencing of the ITS region from Northern Parts of Bangladesh. The BLASTn search revealed that the fungi derived from gummosis disease of mango is *Lasiodiplodia theobromae*. On the other hand, phylogenetic approaches based on DNA sequence data have played a significant role in distinguishing species in *Lasiodiplodia* (Prakash 2003; Ploetz 2003; Khanzada *et al*. 2015; Naqvi *et al*. 2015). Previous studies have used combined ITS regions to clarify the taxonomy and phylogenetic relationships of species in *Lasiodiplodia* (Prakash 2003; Kazmi *et al*. 2005). The current phylogenetic analyses with combined ITS sequence data gave good resolution of phylogenetic separations among *Lasiodiplodia* species and provide insights in to taxonomic novelties. Saeed *et al.*(2017) have amplified and sequenced the target regions of 5.8S rRNA (ITS) and elongation factor 1- (EF1-a) genes for the detection of *Laseodiplodia* from the infected mango in UAE de Silva *et al*. (2019) have also reported utilization of the phylogenetic relationships to identify the new species of *Lasiodiplodia*.

Based on the phylogenetic relationship it is clear that there is variation exists among the *L. theobromae* isolates. From the phylogentic analysis the isolates MGSD-028, MGSD-003, MGSD-004, MGSD-006, MGSD-045 and MGSD-017 yielded a same group in a same cluster, while other five isolates have clustered differently. These isolates were collected from the same area but showing some differences. So, the microclimate of different regions may influence the pathogen morphology and genetic diversity. So, it is urgent demand to know the genetic variability of *L. theobromae* by sequencing others isolates form different hosts and agro-ecological zones from Bangladesh.

4.4. EXPERIMENT- IV: MANAGEMENT OF GUMMOSIS AND SUDDEN DECLINE DISEASE OF MANGO PLANTS

4.4.1. *In vitro* evaluation of fungicides against *L. theobromae*

In vitro fungi toxicity of eight fungicides from different groups available in the market were tested for their efficacy against *L. theobromae,* by following poisoned food technique at 25 ppm, 50 ppm, 75 ppm and 100 ppm . The radial growth of the mycelium of the test pathogen were recorded after six days of incubation. The radial growth of colony were recorded and results are presented in Table 4.12. On the other hand from the results of colony growth and percent mycelia growth inhibition of *L. theobromae,* were calculated and results are presented in Table 4.13.

From the data presented in table 4.12 and 4.13 it is evident that all the fungicides at all concentration tested were significantly superior over control in inhibiting the growth of *L. theobromae*. Significant differences among the treatments were also observed. With the increase of concentration the efficacy of the fungicides increased in all cases. From both the table it is clearly observed that the lowest mean redial growth (3.24 mm) and highest mean inhibition (92.79%) of radial growth of *L. theobromae* was recorded by the fungicide Arba followed by Zibal (6.14 mm and 86.36%), Tilt (7.79 mm and 82.69%), Cabriotop (8.77 mm and 80.51%) while the highest redial growth (15.91 mm) and lowest inhibition (64.64%) of radial growth of *L. theobromae* was recorded by the fungicide Antracol. Rest of the fungicides showed intermediate results.

Considering all concentration the most effective fungicide in descending order is Arba (Carbendazim) >Zibal(Copper hydroxide) >Tilt (Propiconazole)> Cabriotop (Pyraclostrobin + Metiram)> Amistar top (Azoxystrobin)>Indofil M-45 (Mancozeb)>Nativo (Tebuconazole +Trifloxystrobin)> Antracol (Propineb).

Rawal and Ullasa (1988), Sharma and Badiyala (1994) reported that carbendazim is effective in reducing growth of *L. theobromae*. They described carbendazim superiority in inhabiting *L. theobromae* compared to other fungicides. MeiJiao *et al.* (2009) reported that propiconazole, carbendazim and mancozeb effectively control the pathogen. The effectiveness of carbendazim+mancozeb and propiconazole was also recorded by Bhatt and Jadeja (2010). Sahi *et al.* (2012) reported that mancozeb was the least effective fungicide in inhibiting the mycelial growth of *L. theobromae.*

Banik *et al*. (1998a) reported that under *in vitro* testing of fungicides, carbendazim at 400 ppm completely inhibited the growth of *B. theobromae* followed by thiophanate methyl at 450 ppm. Yadav and Majumdar (2004) reported effectiveness of carbendazim and mancozeb against *L. theobromae*. Muhammad *et al*. (2005) observed significant inhibition of mycelia growth of *L. theobromae* by carbendazim and thiophanate methyl when used @ 1 ppm a.i. or more. Khanzada *et al*. (2005) have also reported that mycelial growth of *L. theobromae* was significantly inhibited by carbendazim and thiophanate methyl when used @ 1 ppm a.i. or more. They also reported that in field experiment also carbendazim was found to be more effective than thiophanate methyl in reducing the fungal infection in mango plants, suppressing the gum exudation, dieback and wilting resulting in significant

enhancement in vegetative growth of the plants. Similar, results were reported by Sultana and Ghaffar (2010). They reported that carbendazim and thiophanate methyl completely inhibited the growth of *B. theobromae* under *in vitro* at 50 ppm. Masood *et al*. (2014) reported that fungicides application as injection was initially demonstrated on few number of infected mango trees and found that response of carbendazim, thiophanate methyl and difenoconazole were at par in suppressing the disease severity in mango.

Table 4.12. Effect of various concentrations of fungicides on colony growth of *L. theobromae*. after six days of incubation

Fungicides	**Radial growth (mm) at different Concentrations**				
	25 ppm	**50 ppm**	**75 ppm**	**100 ppm**	**Mean**
Zibal	10.50	6.30	4.50	3.25	6.14 g
Indofil M -45	15.80	12.75	11.45	13.25	13.31 d
Tilt	9.45	8.35	7.20	6.15	7.79 f
Cabriotop	10.95	9.85	7.78	6.5	8.77 e
Arba	5.12	3.10	3.5	1.25	3.24 h
Amistar top	15.65	13.45	12.20	10.00	12.83 d
Antracol	18.25	17.00	15.95	12.45	15.91 b
Nativo	17.85	15.50	14.45	13.25	15.26 c
Control	45.00	45.00	45.00	45.00	45.00 a

Means followed the same letters do not significantly differ at 1% level by DMRT

Table 4.13. Percent inhibition of colony growth of *L. theobromae* at different fungicides under *in vitro* condition

Fungicides	**Inhibition (%) of colony growth at different Concentrations**				
	25 ppm	**50 ppm**	**75 ppm**	**100 ppm**	**Mean**
Zibal	76.67	86.00	90.00	92.78	86.36 b
Indofil M -45	64.89	71.67	74.56	70.56	70.42 f
Tilt	79.00	81.44	84.00	86.33	82.69 c
Cabriotop	75.67	78.11	82.71	85.56	80.51 d
Arba	88.62	93.11	92.22	97.22	92.79 a
Amistar top	65.22	70.11	72.89	77.78	71.50 e
Antracol	59.44	62.22	64.56	72.33	64.64 h
Nativo	60.33	65.56	67.89	70.56	66.08 g
Control	0.00	0.00	0.00	0.00	0.00 i

Means followed the same letters do not significantly differ at 1% level by DMRT

4.4.2. Effect of different treatments on disease severity and percent disease reduction: Effect of different treatments on disease severity and percent disease reduction over control treatment are shown in fig 4.15. After one year of treatment application it was observed that all the treatments performed better compared to control treatment. Among the treatments the lowest severity (2.96%) as well as the highest disease reduction (95.56%) was recorded from T4 treatment followed by T5 (16.06% & 78.10).

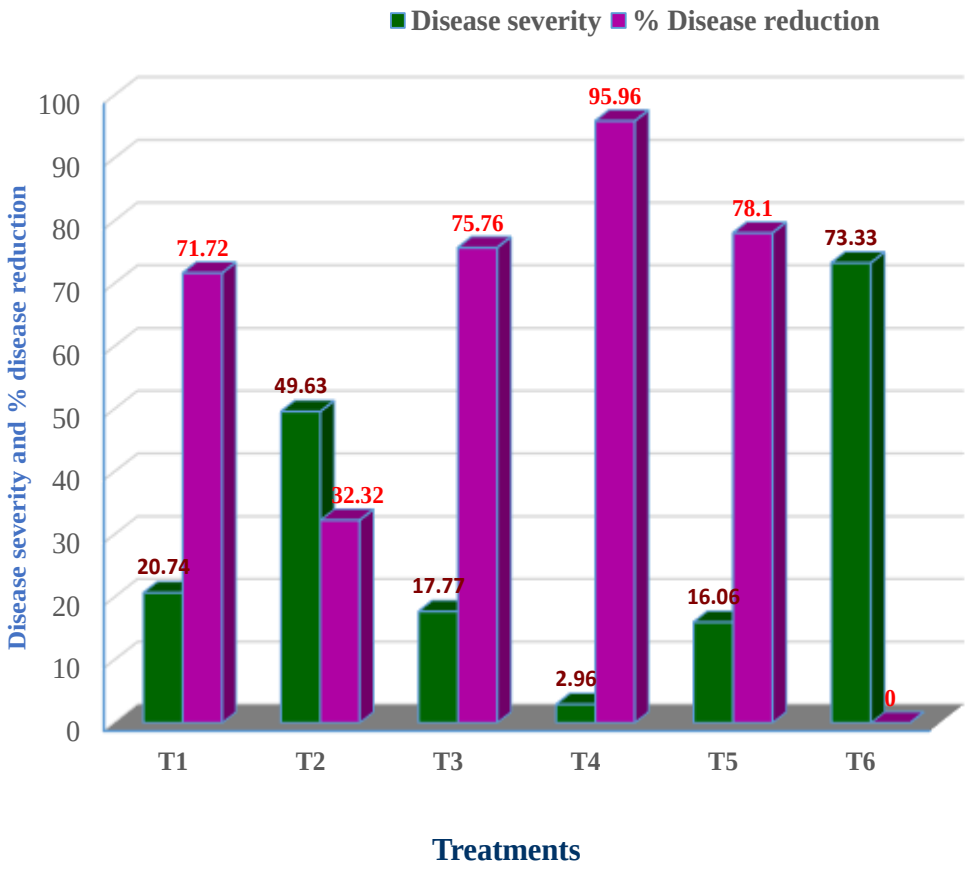

Fig 4.15. Mean Disease severity and percent disease reduction of different treatments over control after one year of treatment application

T1 = Spraying with Arba (Carbendazim)

T2 = Injection of Arba

T3 = Application of Bordeaux paste

T4 = Bordeaux paste + T 1

T5 = Bordeaux paste + T 2

T6 = Control

CHAPTER –5

CONCLUSION

CHAPTER 5
CONCLUSION

From the results obtained in the present study revealed that-

- Gummosis and sudden decline disease of mango plants was found in all the surveyed area under investigation and it is becoming a threat for mango production in Rajshahi and Chapainawabganj region.
- Among the variety surveyed fazli was very much susceptible followed by gootee, langra, ashiwina and khirsapat
- Comparatively older plants are very much susceptible to the disease.
- *Lasiodiplodia theobromae* was the causal agent of Gummosis and sudden decline of mango plants.
- Application of Bordeaux paste only at initial infection of young plant satisfactorily prevented disease development.
- Application of Bordeaux paste along with Arba (carbendazim) @ 2gm/lit spraying completely check the disease development at initially infected both young and old plant but not effective in severely infected plant.
- Further study is necessary to draw a comprehensive conclusion regarding all issues of the disease.

CHAPTER –6

REFERENCES

APPENDICES

Appendix 1. Incidence of gummosis and sudden decline disease on different aged plants on surveyed locations in 2016

Name of District	Name of Upazila	Disease incidence(%) in plants of different age group				
		20 ± 5 years old plants	30 ± 5 years old plants	40 ± 5 years old plants	50 ± 5 years old plants	**mean**
Chapainawabganj	Bholahat	1.61	1.96	2.11	2.14	**1.96**
	Gomostapur	2.61	2.14	3.50	3.96	**3.05**
	Nachol	2.35	2.46	2.50	3.10	**2.60**
	Sibganj	2.75	3.25	3.55	3.75	**3.33**
	Sadar	2.20	2.28	3.60	3.78	**2.97**
Rajshahi	Bagha	1.85	1.80	1.80	2.38	**1.96**
	Charghat	1.10	1.65	1.87	2.38	**1.75**
	Putia	1.64	1.90	1.50	2.00	**1.76**
	Mean	**2.01**	**2.18**	**2.55**	**2.94**	**2.42**

Appendix 2. Incidence of gummosis and sudden decline disease on different aged plants on surveyed locations in 2017

Name of District	Name of Upazila	Disease incidence(%) in plants of different age group				
		20 ± 5 years old plants	30 ± 5 years old plants	40 ± 5 years old plants	50 ± 5 years old plants	**Mean**
Chapainawabganj	Bholahat	1.85	1.95	1.95	2.45	**2.05**
	Gomostapur	2.75	2.25	3.14	4.1	**3.06**
	Nachol	2.45	2.36	2.25	3.86	**2.73**
	Sibganj	2.6	4.25	3.65	3.95	**3.61**
	Sadar	2.45	2.40	3.42	4.96	**3.31**
Rajshahi	Bagha	1.78	1.78	1.78	3.45	**2.20**
	Charghat	1.15	1.78	1.72	2.87	**1.88**
	Putia	1.25	1.54	1.56	2.78	**1.78**
	Mean	**2.04**	**2.29**	**2.43**	**3.55**	**2.58**

APPENDICES

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Appendix 1. Incidence of gummosis and sudden decline disease on different aged plants on surveyed locations in 2016

Name of District	Name of Upazila	Disease incidence(%) in plants of different age group				
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Chapainawabganj	Bholahat	1.61	1.96	2.11	2.14	**1.96**
	Gomostapur	2.61	2.14	3.50	3.96	**3.05**
	Nachol	2.35	2.46	2.50	3.10	**2.60**
	Sibganj	2.75	3.25	3.55	3.75	**3.33**
	Sadar	2.20	2.28	3.60	3.78	**2.97**
Rajshahi	Bagha	1.85	1.80	1.80	2.38	**1.96**
	Charghat	1.10	1.65	1.87	2.38	**1.75**
	Putia	1.64	1.90	1.50	2.00	**1.76**
	Mean	**2.01**	**2.18**	**2.55**	**2.94**	**2.42**

Appendix 2. Incidence of gummosis and sudden decline disease on different aged plants on surveyed locations in 2017

Name of District	Name of Upazila	Disease incidence(%) in plants of different age group				
		20 ± 5 years old plants	30 ± 5 years old plants	40 ± 5 years old plants	50 ± 5 years old plants	**Mean**
Chapainawabganj	Bholahat	1.85	1.95	1.95	2.45	**2.05**
	Gomostapur	2.75	2.25	3.14	4.1	**3.06**
	Nachol	2.45	2.36	2.25	3.86	**2.73**
	Sibganj	2.6	4.25	3.65	3.95	**3.61**
	Sadar	2.45	2.40	3.42	4.96	**3.31**
Rajshahi	Bagha	1.78	1.78	1.78	3.45	**2.20**
	Charghat	1.15	1.78	1.72	2.87	**1.88**
	Putia	1.25	1.54	1.56	2.78	**1.78**
	Mean	**2.04**	**2.29**	**2.43**	**3.55**	**2.58**

Appendix 3.1. The nucleotides sequences of ITS region of *L. theobromae* submitted to NCBI (Isolate MGSD001)

LOCUS MW138048 573 bp DNA linear PLN 20-OCT-2020
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VERSION MW138048
KEYWORDS .
SOURCE Lasiodiplodia theobromae
ORGANISM Lasiodiplodia theobromae
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Dothideomycetes; Dothideomycetes incertae sedis; Botryosphaeriales; Botryosphaeriaceae; Lasiodiplodia.
REFERENCE 1 (bases 1 to 573)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Molecular identification of the fungi associated with gummosis disease of mango in Bangladesh
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 573)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Direct Submission
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ORIGIN
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       61 gagttttcga gcttcggctc gactctccca ccctttgtga acgtacctct gttgctttgg
      121 cggcttcggc cgccaaagga ccttcaaact ccagtcagta aacgcagacg tctgataaac
      181 aagttaataa actaaaactt tcaacaacgg atctcttggt tctggcatcg atgaagaacg
      241 cagcgaaatg cgataagtaa tgtgaattgc agaattcagt gaatcatcga atctttgaac
      301 gcacattgcg ccccttggta ttccgggggg catgcctgtt cgagcgtcat tacaaccctc
      361 aagctctgct tggaattggg caccgtcctc actgcggacg cgcctcaaag acctcggcgg
      421 tggctgttca gccctcaagc gtagtagaat acacctcgct ttggagcggt tggcgtcgcc
      481 cgccggacga accttctgaa cttttctcaa ggttgacctc ggatcaggta gggatacccg
      541 ctgaacttaa gcatatcaat aggccggagg aaa
//
```

Appendix 3.2. The nucleotides sequences of ITS region of *L. theobromae* submitted to NCBI (Isolate MGSD002)

LOCUS MW138049 573 bp DNA linear PLN 20-OCT-2020
DEFINITION Lasiodiplodia theobromae isolate MGSD002 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.
ACCESSION MW138049
VERSION MW138049
KEYWORDS .
SOURCE Lasiodiplodia theobromae
ORGANISM Lasiodiplodia theobromae
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Dothideomycetes; Dothideomycetes incertae sedis; Botryosphaeriales; Botryosphaeriaceae; Lasiodiplodia.
REFERENCE 1 (bases 1 to 573)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Molecular identification of the fungi associated with gummosis disease of mango in Bangladesh
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 573)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Direct Submission
JOURNAL Submitted (20-OCT-2020) Plant Pathology, Bangladesh Agricultural Research Institute, Chawrasta road, Joydebpur, Gazipur 1701, Bangladesh
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..573
/organism="Lasiodiplodia theobromae"
/mol_type="genomic DNA"
/isolate="MGSD002"
/host="mango"
/db_xref="taxon:45133"
/country="Bangladesh"
/collection_date="15-Jul-2018"
/collected_by="Md Mosharraf Hossain"
/PCR_primers="fwd_name: ITS4, fwd_seq: tcctccgcttattgatatgc, rev_name: ITS5, rev_seq: ggaagtaaaagtcgtaacaagg"
misc_RNA <1..>573
/note="contains small subunit ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and large subunit ribosomal RNA"

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ORIGIN
        1 tttggaagta aaaagtcgta acaaggtttc cgtaggtgaa cctgcggaag gatcattacc
       61 gagttttcga gcttcggctc gactctccca ccctttgtga acgtacctct gttgctttgg
      121 cggcttcggc cgccaaagga ccttcaaact ccagtcagta aacgcagacg tctgataaac
      181 aagttaataa actaaaactt tcaacaacgg atctcttggt tctggcatcg atgaagaacg
      241 cagcgaaatg cgataagtaa tgtgaattgc agaattcagt gaatcatcga atctttgaac
      301 gcacattgcg ccccttggta ttccgggggg catgcctgtt cgagcgtcat tacaaccctc
      361 aagctctgct tggaattggg caccgtcctc actgcggacg cgcctcaaag acctcggcgg
      421 tggctgttca gccctcaagc gtagtagaat acacctcgct ttggagcggt tggcgtcgcc
      481 cgccggacga accttctgaa cttttctcaa ggttgacctc ggatcaggta gggatacccg
      541 ctgaacttaa gcatatcaat aggccggagg aaa
//
```

Appendix 3.3. The nucleotides sequences of ITS region of *L. theobromae* submitted to NCBI (Isolate MGSD003)

LOCUS MW138050 574 bp DNA linear PLN 20-OCT-2020
DEFINITION Lasiodiplodia theobromae isolate MGSD003 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.
ACCESSION MW138050
VERSION MW138050
KEYWORDS .
SOURCE Lasiodiplodia theobromae
ORGANISM Lasiodiplodia theobromae
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Dothideomycetes; Dothideomycetes incertae sedis; Botryosphaeriales; Botryosphaeriaceae; Lasiodiplodia.
REFERENCE 1 (bases 1 to 574)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Molecular identification of the fungi associated with gummosis disease of mango in Bangladesh
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 574)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Direct Submission
JOURNAL Submitted (20-OCT-2020) Plant Pathology, Bangladesh Agricultural Research Institute, Chawrasta road, Joydebpur, Gazipur 1701, Bangladesh
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..574
/organism="Lasiodiplodia theobromae"
/mol_type="genomic DNA"
/isolate="MGSD003"
/host="mango"
/db_xref="taxon:45133"
/country="Bangladesh"
/collection_date="01-Aug-2018"
/collected_by="Md Mosharraf Hossain"
/PCR_primers="fwd_name: ITS4, fwd_seq: tcctccgcttattgatatgc, rev_name: ITS5, rev_seq: ggaagtaaaagtcgtaacaagg"
misc_RNA <1..>574
/note="contains small subunit ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and large subunit ribosomal RNA"
ORIGIN

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        1 gttggaagta aaaagtcgta acaaggtttc cgtaggtgaa cctgcggaag gatcattacc
       61 gagttttcga gcttcggctc gactctccca ccctttgtga acgtacctct gttgctttgg
      121 cggcttcggc cgccaaagga ccttcaaact ccagtcagta aacgcagacg tctgataaac
      181 aagttaataa actaaaactt tcaacaacgg atctcttggt tctggcatcg atgaagaacg
      241 cagcgaaatg cgataagtaa tgtgaattgc agaattcagt gaatcatcga atctttgaac
      301 gcacattgcg ccccttggta ttccgggggg catgcctgtt cgagcgtcat tacaaccctc
      361 aagctctgct tggaattggg caccgtcctc actgcggacg cgcctcaaag acctcggcgg
      421 tggctgttca gccctcaagc gtagtagaat acacctcgct ttggagcggt tggcgtcgcc
      481 cgccggacga accttctgaa cttttctcaa ggttgacctc ggatcaggta gggatacccg
      541 ctgaacttaa gcatatcaat aaggccggag gaaa
```

//

Appendix 3.4. The nucleotides sequences of ITS region of *L. theobromae* submitted to NCBI (Isolate MGSD004)

LOCUS MW138051 572 bp DNA linear PLN 20-OCT-2020
DEFINITION Lasiodiplodia theobromae isolate MGSD004 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.
ACCESSION MW138051
VERSION MW138051
KEYWORDS .
SOURCE Lasiodiplodia theobromae
ORGANISM Lasiodiplodia theobromae
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Dothideomycetes; Dothideomycetes incertae sedis; Botryosphaeriales; Botryosphaeriaceae; Lasiodiplodia.
REFERENCE 1 (bases 1 to 572)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Molecular identification of the fungi associated with gummosis disease of mango in Bangladesh
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 572)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Direct Submission
JOURNAL Submitted (20-OCT-2020) Plant Pathology, Bangladesh Agricultural Research Institute, Chawrasta road, Joydebpur, Gazipur 1701, Bangladesh
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..572
/organism="Lasiodiplodia theobromae"
/mol_type="genomic DNA"
/isolate="MGSD004"
/host="mango"
/db_xref="taxon:45133"
/country="Bangladesh"
/collection_date="10-Aug-2018"
/collected_by="Md Mosharraf Hossain"
/PCR_primers="fwd_name: ITS4, fwd_seq: tcctccgcttattgatatgc, rev_name: ITS5, rev_seq: ggaagtaaaagtcgtaacaagg"
misc_RNA <1..>572
/note="contains small subunit ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and large subunit ribosomal RNA"
ORIGIN

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       61 gttttcgagc ttcggctcga ctctcccacc ctttgtgaac gtacctctgt tgctttggcg
      121 gcttcggccg ccaaaggacc ttcaaactcc agtcagtaaa cgcagacgtc tgataaacaa
      181 gttaataaac taaaactttc aacaacggat ctcttggttc tggcatcgat gaagaacgca
      241 gcgaaatgcg ataagtaatg tgaattgcag aattcagtga atcatcgaat ctttgaacgc
      301 acattgcgcc ccttggtatt ccgggggggca tgcctgttcg agcgtcatta caaccctcaa
      361 gctctgcttg gaattgggca ccgtcctcac tgcggacgcg cctcaaagac ctcggcggtg
      421 gctgttcagc cctcaagcgt agtagaatac acctcgcttt ggagcggttg gcgtcgcccg
      481 ccggacgaac cttctgaact tttctcaagg ttgacctcgg atcaggtagg gatacccgct
      541 gaacttaagc atatcaataa ggccggagga aa
//
```

Appendix 3.5. The nucleotides sequences of ITS region of *L. theobromae* submitted to NCBI (Isolate MGSD005)

LOCUS MW138052 573 bp DNA linear PLN 20-OCT-2020
DEFINITION Lasiodiplodia theobromae isolate MGSD005 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.
ACCESSION MW138052
VERSION MW138052
KEYWORDS .
SOURCE Lasiodiplodia theobromae
ORGANISM Lasiodiplodia theobromae
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Dothideomycetes; Dothideomycetes incertae sedis; Botryosphaeriales; Botryosphaeriaceae; Lasiodiplodia.
REFERENCE 1 (bases 1 to 573)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Molecular identification of the fungi associated with gummosis disease of mango in Bangladesh
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 573)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Direct Submission
JOURNAL Submitted (20-OCT-2020) Plant Pathology, Bangladesh Agricultural Research Institute, Chawrasta road, Joydebpur, Gazipur 1701, Bangladesh
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..573
/organism="Lasiodiplodia theobromae"
/mol_type="genomic DNA"
/isolate="MGSD005"
/host="mango"
/db_xref="taxon:45133"
/country="Bangladesh"
/collection_date="15-Sep-2018"
/collected_by="Md Mosharraf Hossain"
/PCR_primers="fwd_name: ITS4, fwd_seq: tcctccgcttattgatatgc, rev_name: ITS5, rev_seq: ggaagtaaaagtcgtaacaagg"
misc_RNA <1..>573
/note="contains small subunit ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and large subunit ribosomal RNA"
ORIGIN

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        1 tttggaagta aaaagtcgta acaaggtttc cgtaggtgaa cctgcggaag gatcattacc
       61 gagttttcga gcttcggctc gactctccca ccctttgtga acgtacctct gttgctttgg
      121 cggcttcggc cgccaaagga ccttcaaact ccagtcagta aacgcagacg tctgataaac
      181 aagttaataa actaaaactt tcaacaacgg atctcttggt tctggcatcg atgaagaacg
      241 cagcgaaatg cgataagtaa tgtgaattgc agaattcagt gaatcatcga atctttgaac
      301 gcacattgcg ccccttggta ttccgggggg catgcctgtt cgagcgtcat tacaaccctc
      361 aagctctgct tggaattggg caccgtcctc actgcggacg cgcctcaaag acctcggcgg
      421 tggctgttca gccctcaagc gtagtagaat acacctcgct ttggagcggt tggcgtcgcc
      481 cgccggacga accttctgaa cttttctcaa ggttgacctc ggatcaggta gggatacccg
      541 ctgaacttaa gcatatcaat aggccggagg aaa
```

//

Appendix 3.6. The nucleotides sequences of ITS region of *L. theobromae* submitted to NCBI (Isolate MGSD006)

LOCUS MW138053 572 bp DNA linear PLN 20-OCT-2020
DEFINITION Lasiodiplodia theobromae isolate MGSD006 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.
ACCESSION MW138053
VERSION MW138053
KEYWORDS .
SOURCE Lasiodiplodia theobromae
ORGANISM Lasiodiplodia theobromae
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Dothideomycetes; Dothideomycetes incertae sedis; Botryosphaeriales; Botryosphaeriaceae; Lasiodiplodia.
REFERENCE 1 (bases 1 to 572)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Molecular identification of the fungi associated with gummosis disease of mango in Bangladesh
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 572)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Direct Submission
JOURNAL Submitted (20-OCT-2020) Plant Pathology, Bangladesh Agricultural Research Institute, Chawrasta road, Joydebpur, Gazipur 1701, Bangladesh
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..572
/organism="Lasiodiplodia theobromae"
/mol_type="genomic DNA"
/isolate="MGSD006"
/host="mango"
/db_xref="taxon:45133"
/country="Bangladesh"
/collection_date="26-Sep-2018"
/collected_by="Md Mosharraf Hossain"
/PCR_primers="fwd_name: ITS4, fwd_seq: tcctccgcttattgatatgc, rev_name: ITS5, rev_seq: ggaagtaaaagtcgtaacaagg"
misc_RNA <1..>572
/note="contains small subunit ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and large subunit ribosomal RNA"
ORIGIN

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        1 tggaagtaaa aagtcgtaac aaggtttccg taggtgaacc tgcggaagga tcattaccga
       61 gttttcgagc ttcggctcga ctctcccacc ctttgtgaac gtacctctgt tgctttggcg
      121 gcttcggccg ccaaaggacc ttcaaactcc agtcagtaaa cgcagacgtc tgataaacaa
      181 gttaataaac taaaactttc aacaacggat ctcttggttc tggcatcgat gaagaacgca
      241 gcgaaatgcg ataagtaatg tgaattgcag aattcagtga atcatcgaat ctttgaacgc
      301 acattgcgcc ccttggtatt ccgggggggca tgcctgttcg agcgtcatta caaccctcaa
      361 gctctgcttg gaattgggca ccgtcctcac tgcggacgcg cctcaaagac ctcggcggtg
      421 gctgttcagc cctcaagcgt agtagaatac acctcgcttt ggagcggttg gcgtcgcccg
      481 ccggacgaac cttctgaact tttctcaagg ttgacctcgg atcaggtagg gatacccgct
      541 gaacttaagc atatcaataa ggccggagga aa
//
```

Appendix 3.7. The nucleotides sequences of ITS region of *L. theobromae* submitted to NCBI (Isolate MGSD017)

LOCUS MW138054 572 bp DNA linear PLN 20-OCT-2020
DEFINITION Lasiodiplodia theobromae isolate MGSD017 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.
ACCESSION MW138054
VERSION MW138054
KEYWORDS .
SOURCE Lasiodiplodia theobromae
ORGANISM Lasiodiplodia theobromae
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Dothideomycetes; Dothideomycetes incertae sedis; Botryosphaeriales; Botryosphaeriaceae; Lasiodiplodia.
REFERENCE 1 (bases 1 to 572)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Molecular identification of the fungi associated with gummosis disease of mango in Bangladesh
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 572)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Direct Submission
JOURNAL Submitted (20-OCT-2020) Plant Pathology, Bangladesh Agricultural Research Institute, Chawrasta road, Joydebpur, Gazipur 1701, Bangladesh
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..572
/organism="Lasiodiplodia theobromae"
/mol_type="genomic DNA"
/isolate="MGSD017"
/host="mango"
/db_xref="taxon:45133"
/country="Bangladesh"
/collection_date="16-Oct-2018"
/collected_by="Md Mosharraf Hossain"
/PCR_primers="fwd_name: ITS4, fwd_seq: tcctccgcttattgatatgc, rev_name: ITS5, rev_seq: ggaagtaaaagtcgtaacaagg"
misc_RNA <1..>572
/note="contains small subunit ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and large subunit ribosomal RNA"
ORIGIN

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       61 gttttcgagc ttcggctcga ctctcccacc ctttgtgaac gtacctctgt tgctttggcg
      121 gcttcggccg ccaaaggacc ttcaaactcc agtcagtaaa cgcagacgtc tgataaacaa
      181 gttaataaac taaaactttc aacaacggat ctcttggttc tggcatcgat gaagaacgca
      241 gcgaaatgcg ataagtaatg tgaattgcag aattcagtga atcatcgaat ctttgaacgc
      301 acattgcgcc ccttggtatt ccgggggggca tgcctgttcg agcgtcatta caaccctcaa
      361 gctctgcttg gaattgggca ccgtcctcac tgcggacgcg cctcaaagac ctcggcggtg
      421 gctgttcagc cctcaagcgt agtagaatac acctcgcttt ggagcggttg gcgtcgcccg
      481 ccggacgaac cttctgaact tttctcaagg ttgacctcgg atcaggtagg gatacccgct
      541 gaacttaagc atatcaataa ggccggagga aa
//
```

Appendix 3.8. The nucleotides sequences of ITS region of *L. theobromae* submitted to NCBI (Isolate MGSD028)

LOCUS MW138055 574 bp DNA linear PLN 20-OCT-2020
DEFINITION Lasiodiplodia theobromae isolate MGSD028 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.
ACCESSION MW138055
VERSION MW138055
KEYWORDS .
SOURCE Lasiodiplodia theobromae
ORGANISM Lasiodiplodia theobromae
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Dothideomycetes; Dothideomycetes incertae sedis; Botryosphaeriales; Botryosphaeriaceae; Lasiodiplodia.
REFERENCE 1 (bases 1 to 574)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Molecular identification of the fungi associated with gummosis disease of mango in Bangladesh
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 574)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Direct Submission
JOURNAL Submitted (20-OCT-2020) Plant Pathology, Bangladesh Agricultural Research Institute, Chawrasta road, Joydebpur, Gazipur 1701, Bangladesh
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..574
/organism="Lasiodiplodia theobromae"
/mol_type="genomic DNA"
/isolate="MGSD028"
/host="mango"
/db_xref="taxon:45133"
/country="Bangladesh"
/collection_date="18-Oct-2018"
/collected_by="Md Mosharraf Hossain"
/PCR_primers="fwd_name: ITS4, fwd_seq: tcctccgcttattgatatgc, rev_name: ITS5, rev_seq: ggaagtaaaagtcgtaacaagg"
misc_RNA <1..>574
/note="contains small subunit ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and large subunit ribosomal RNA"
ORIGIN

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        1 gttggaagta aaaagtcgta acaaggtttc cgtaggtgaa cctgcggaag gatcattacc
       61 gagttttcga gcttcggctc gactctccca ccctttgtga acgtacctct gttgctttgg
      121 cggcttcggc cgccaaagga ccttcaaact ccagtcagta aacgcagacg tctgataaac
      181 aagttaataa actaaaactt tcaacaacgg atctcttggt tctggcatcg atgaagaacg
      241 cagcgaaatg cgataagtaa tgtgaattgc agaattcagt gaatcatcga atctttgaac
      301 gcacattgcg ccccttggta ttccgggggg catgcctgtt cgagcgtcat tacaaccctc
      361 aagctctgct tggaattggg caccgtcctc actgcggacg cgcctcaaag acctcggcgg
      421 tggctgttca gccctcaagc gtagtagaat acacctcgct ttggagcggt tggcgtcgcc
      481 cgccggacga accttctgaa cttttctcaa ggttgacctc ggatcaggta gggatacccg
      541 ctgaacttaa gcatatcaat aaggccggag gaaa
```

//

Appendix 3.9. The nucleotides sequences of ITS region of *L. theobromae* submitted to NCBI (Isolate MGSD045)

LOCUS MW138056 572 bp DNA linear PLN 20-OCT-2020
DEFINITION Lasiodiplodia theobromae isolate MGSD045 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.
ACCESSION MW138056
VERSION MW138056
KEYWORDS .
SOURCE Lasiodiplodia theobromae
ORGANISM Lasiodiplodia theobromae
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Dothideomycetes; Dothideomycetes incertae sedis; Botryosphaeriales; Botryosphaeriaceae; Lasiodiplodia.
REFERENCE 1 (bases 1 to 572)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Molecular identification of the fungi associated with gummosis disease of mango in Bangladesh
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 572)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Direct Submission
JOURNAL Submitted (20-OCT-2020) Plant Pathology, Bangladesh Agricultural Research Institute, Chawrasta road, Joydebpur, Gazipur 1701, Bangladesh
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..572
/organism="Lasiodiplodia theobromae"
/mol_type="genomic DNA"
/isolate="MGSD045"
/host="mango"
/db_xref="taxon:45133"
/country="Bangladesh"
/collection_date="22-Oct-2018"
/collected_by="Md Mosharraf Hossain"
/PCR_primers="fwd_name: ITS4, fwd_seq: tcctccgcttattgatatgc, rev_name: ITS5, rev_seq: ggaagtaaaagtcgtaacaagg"
misc_RNA <1..>572
/note="contains small subunit ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and large subunit ribosomal RNA"
ORIGIN

```
        1 tggaagtaaa aagtcgtaac aaggtttccg taggtgaacc tgcggaagga tcattaccga
       61 gttttcgagc ttcggctcga ctctcccacc ctttgtgaac gtacctctgt tgctttggcg
      121 gcttcggccg ccaaaggacc ttcaaactcc agtcagtaaa cgcagacgtc tgataaacaa
      181 gttaataaac taaaactttc aacaacggat ctcttggttc tggcatcgat gaagaacgca
      241 gcgaaatgcg ataagtaatg tgaattgcag aattcagtga atcatcgaat ctttgaacgc
      301 acattgcgcc ccttggtatt ccgggggggca tgcctgttcg agcgtcatta caaccctcaa
      361 gctctgcttg gaattgggca ccgtcctcac tgcggacgcg cctcaaagac ctcggcggtg
      421 gctgttcagc cctcaagcgt agtagaatac acctcgcttt ggagcggttg gcgtcgcccg
      481 ccggacgaac cttctgaact tttctcaagg ttgacctcgg atcaggtagg gatacccgct
      541 gaacttaagc atatcaataa ggccggagga aa
```

//

Appendix 3.10. The nucleotides sequences of ITS region of *L. theobromae* submitted to NCBI (Isolate MGSD071)

LOCUS MW138057 572 bp DNA linear PLN 20-OCT-2020
DEFINITION Lasiodiplodia theobromae isolate MGSD071 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.
ACCESSION MW138057
VERSION MW138057
KEYWORDS .
SOURCE Lasiodiplodia theobromae
ORGANISM Lasiodiplodia theobromae
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Dothideomycetes; Dothideomycetes incertae sedis; Botryosphaeriales; Botryosphaeriaceae; Lasiodiplodia.
REFERENCE 1 (bases 1 to 572)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Molecular identification of the fungi associated with gummosis disease of mango in Bangladesh
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 572)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Direct Submission
JOURNAL Submitted (20-OCT-2020) Plant Pathology, Bangladesh Agricultural Research Institute, Chawrasta road, Joydebpur, Gazipur 1701, Bangladesh
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..572
/organism="Lasiodiplodia theobromae"
/mol_type="genomic DNA"
/isolate="MGSD071"
/host="mango"
/db_xref="taxon:45133"
/country="Bangladesh"
/collection_date="25-Oct-2018"
/collected_by="Md Mosharraf Hossain"
/PCR_primers="fwd_name: ITS4, fwd_seq: tcctccgcttattgatatgc, rev_name: ITS5, rev_seq: ggaagtaaaagtcgtaacaagg"
misc_RNA <1..>572
/note="contains small subunit ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and large subunit ribosomal RNA"
ORIGIN

```
        1 ttggaagtaa aaagtcgtaa caaggtttcc gtaggtgaac ctgcggaagg atcattaccg
       61 agttttcgag cttcggctcg actctcccac cctttgtgaa cgtacctctg ttgctttggc
      121 ggcttcggcc gccaaaggac cttcaaactc cagtcagtaa acgcagacgt ctgataaaca
      181 agttaataaa ctaaaacttt caacaacgga tctcttggtt ctggcatcga tgaagaacgc
      241 agcgaaatgc gataagtaat gtgaattgca gaattcagtg aatcatcgaa tctttgaacg
      301 cacattgcgc cccttggtat tccgggggcc atgcctgttc gagcgtcatt acaaccctca
      361 agctctgctt ggaattgggc accgtcctca ctgcggacgc gcctcaaaga cctcggcggt
      421 ggctgttcag ccctcaagcg tagtagaata cacctcgctt tggagcggtt ggcgtcgccc
      481 gccggacgaa ccttctgaac ttttctcaag gttgacctcg gatcaggtag ggatacccgc
      541 tgaacttaag catatcaata agccggagga aa
```

//

Appendix 3.11. The nucleotides sequences of ITS region of *L. theobromae* submitted to NCBI (Isolate MGSD091)

LOCUS MW138058 573 bp DNA linear PLN 20-OCT-2020
DEFINITION Lasiodiplodia theobromae isolate MGSD091 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.
ACCESSION MW138058
VERSION MW138058
KEYWORDS .
SOURCE Lasiodiplodia theobromae
ORGANISM Lasiodiplodia theobromae
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Dothideomycetes; Dothideomycetes incertae sedis; Botryosphaeriales; Botryosphaeriaceae; Lasiodiplodia.
REFERENCE 1 (bases 1 to 573)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Molecular identification of the fungi associated with gummosis disease of mango in Bangladesh
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 573)
AUTHORS Hossain,M.M., Islam,S., Akhter,M.S.A., Khatun,F., Ali,M.K. and Islam,M.S.
TITLE Direct Submission
JOURNAL Submitted (20-OCT-2020) Plant Pathology, Bangladesh Agricultural Research Institute, Chawrasta road, Joydebpur, Gazipur 1701, Bangladesh
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..573
/organism="Lasiodiplodia theobromae"
/mol_type="genomic DNA"
/isolate="MGSD091"
/host="mango"
/db_xref="taxon:45133"
/country="Bangladesh"
/collection_date="16-Nov-2018"
/collected_by="Md Mosharraf Hossain"
/PCR_primers="fwd_name: ITS4, fwd_seq: tcctccgcttattgatatgc, rev_name: ITS5, rev_seq: ggaagtaaaagtcgtaacaagg"
misc_RNA <1..>573
/note="contains small subunit ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and large subunit ribosomal RNA"
ORIGIN

```
        1 tttggaagta aaaagtcgta acaaggtttc cgtaggtgaa cctgcggaag gatcattacc
       61 gagttttcga gcttcggctc gactctccca ccctttgtga acgtacctct gttgctttgg
      121 cggcttcggc cgccaaagga ccttcaaact ccagtcagta aacgcagacg tctgataaac
      181 aagttaataa actaaaactt tcaacaacgg atctcttggt tctggcatcg atgaagaacg
      241 cagcgaaatg cgataagtaa tgtgaattgc agaattcagt gaatcatcga atctttgaac
      301 gcacattgcg ccccttggta ttccgggggg catgcctgtt cgagcgtcat tacaaccctc
      361 aagctctgct tggaattggg caccgtcctc actgcggacg cgcctcaaag acctcggcgg
      421 tggctgttca gccctcaagc gtagtagaat acacctcgct ttggagcggt tggcgtcgcc
      481 cgccggacga accttctgaa cttttctcaa ggttgacctc ggatcaggta gggatacccg
      541 ctgaacttaa gcatatcaat aggccggagg aaa
```

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