“STUDIES ON MANGO GUMMOSIS WITH SPECIAL REFERENCE TO *Lasiodiplodia theobromae* (Pat.) Griffon & Moube”

VEERA SURESH

B. Sc. (Ag.)

MASTER OF SCIENCE IN AGRICULTURE

(PLANT PATHOLOGY)

2014

"STUDIES ON MANGO GUMMOSIS WITH SPECIAL REFERENCE TO *Lasiodiplodia theobromae* (Pat.) Griffon & Moube"

BY

VEERA SURESH
B. Sc. (Ag.)

THESIS SUBMITTED TO THE
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE
(PLANT PATHOLOGY)

CHAIRPERSON: Dr. B. VIDYA SAGAR

DEPARTMENT PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
RAJENDRANAGAR, HYDERABAD - 500 030
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
2014

CERTIFICATE

Mr. **VEERA SURESH** has satisfactorily prosecuted the course of research and that thesis entitled **"STUDIES ON MANGO GUMMOSIS WITH SPECIAL REFERENCE TO *Lasiodiplodia theobromae* (Pat.) Griffon & Moube"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by him for a degree of any University.

Date: **(Dr. B. VIDYA SAGAR)**

Place: Hyderabad Chairperson

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON MANGO GUMMOSIS WITH SPECIAL REFERENCE TO *Lasiodiplodia theobromae* (Pat.) Griffon & Moube"** submitted in partial fulfilment of the requirements for the degree of **Master of Science** in Agriculture of the Acharya N. G. Ranga Agricultural University, Hyderabad is a record of the bonafide original research work carried out by **Mr. VEERA SURESH** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

Thesis approved by the Student Advisory Committee

Chairperson	**Dr. B. VIDYA SAGAR** Associate Professor Department of Plant Pathology College of Agriculture, ANGRAU Rajendranagar, Hyderabad-500 030.	_____
Member	**Dr. P. KISHORE VARMA** Assistant Professor Department of Plant Pathology College of Agriculture, ANGRAU Rajendranagar, Hyderabad-500 030.	_____
Member	**Dr. S. R. KOTESWARA RAO** Professor Department of Entomology College of Agriculture, ANGRAU Rajendranagar, Hyderabad-500 030.	_____

Date of final viva-voce:

LIST OF CONTENTS

DECLARATION

I**, VEERA SURESH,** hereby declare that the thesis entitled **"STUDIES ON MANGO GUMMOSIS WITH SPECIAL REFERENCE TO *Lasiodiplodia theobromae* (Pat.) Griffon & Moube"** submitted to the **Acharya N. G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** is the result of original research work done by me. I also declare that any material contained in the thesis has not been published earlier in any manner.

Place: Hyderabad **(V. SURESH)**

Date: **I. D. No. RAM/12-70**

LIST OF TABLES

LIST OF ILLUSTRATIONS

LIST OF PLATES

3. 1	Pictorial representation of survey area for gummosis	
3. 2	Stem inoculation of *Lasiodiplodia theobromae* on mango seedling	
4.1	Symptoms of mango gummosis	
4.2	Pure culture of *Lasiodiplodia theobromae*	
4.3	Mature and immature conidia of *Lasiodiplodia theobromae* observed under compound microscope (1000 X)	
4.4	Effect of different temperatures and media on the growth of *Lasiodiplodia theobromae* under *in vitro* condition	
4.5	Pycnidial production of *Lasiodiplodia theobromae* on Oat Meal Agar	
4.6	Evaluation of different fungicides against *Lasiodiplodia theobromae* at 500 ppm under *in vitro* condition	
4.7	Evaluation of different fungicides against *Lasiodiplodia theobromae* at 250 ppm under *in vitro* condition	
4.8	Inhibition of mycelial growth of *Lasiodiplodia theobromae* by garlic extract at 5% and 10% concentration under *in vitro* condition	
4.9	Inhibition of mycelial growth of *Lasiodiplodia theobromae* by Trichoderma isolates.	
4.10	Symptoms observed on mango seedlings after one month of inoculation	
4.11	Disease of *Lasiodiplodia theobromae* in Suvernarekha cultivar	
4.12	Susceptible cultivars to mango gummosis	
4.13	Moderately resistant cultivars to mango gummosis	

ACKNOWLEDGEMENTS

I thank the almighty GOD for his love and blessings, without which I would not have

been able to complete my studies hitherto and present this piece of work.

No creation in this world is a solo effort, neither is this thesis. I would like to take this opportunity to acknowledge all, who supported me directly or indirectly, bringing in this thesis to a really good shape.

I feel a great pleasure in expressing my whole hearted sense of gratitude to ***Dr. B. Vidya Sagar,*** *Chairman of Advisory Committee, Associate Professor, Department Of Plant Pathology, College Of Agriculture, Rajendranagar for his Scholastic guidance, constructive criticism, unceasing interest, valuable knowledge, technical advice, patient audience, scientific view, amiable dealing, providing each and every facility, warmer affection and mental support, without whom it was impossible to complete the research project.*

I deem it my privilege in expressing my deep sense of reverence and gratitude and indebtedness to ***Dr. P. Kishore varma,*** *Assistant professor, Department Of Plant Pathology, College Of Agriculture, Rajendranagar and member of my advisory committee for his talented guidance, valuable suggestions, careful and reasoned criticism and meticulous attention to the details and also for his constant encouragement which has led to the present investigation to the final shape.*

I owe my flow of thanks to ***Dr. S. R. Koteswara Rao,*** *Professor, Department of Entomology, College of Agriculture, Rajendranagar and member of Advisory Committee for his worthful suggestions and encouragement during the investigation.*

I offer my profuse regards and thanks to ***Dr. G. Uma Devi****, Professor and Head, Department of Plant pathology for her caring encouragement and indefatigable interest as well as providing equipped laboratory facilities during my research work.*

I am very much thankful to and feel it a great privilege to place on my record with sincere regards and thanks to ***Dr. P. Narayan Reddy,*** *Professor,* ***Dr. B. Pushpavathi,*** *Assosiate Professors,* ***Dr. V. Vijay Krishna,*** *Assistant Professors,* ***Dr. B. Rajeswari,*** *Senior Scientist in Mushroom Cultivation Scheme, Department of Plant Pathology, College of Agriculture, Rajendranagar, Hyderabad for their inspiring, insightful, scholarly guidance and constructive suggestions from time to time in the preparation of this dissertation.*

I would like to express my heartfelt thanks to ***Dr. Ch. Srilatha vani,*** *Editor, Angrau press, ARI, for her cooperation and encouragement during the course of my research work.*

It is time to surface out genuflect love and affectionate gratitude to my dearest parents, Sri.

Veera. Laxmaiah *and* ***Smt. Sharadha*** *for their blessings, unparalleled love and affection, inspiration, persistent encouragement and moral support throughout my educational career. I feel it a rare opportunity to express my bountiful regards, affection and gratitude to my peddamma* ***Smt. Sarojana*** *who constantly inspired me to study.*

With boundless affection, I would hearty acknowledge the constant encouragement, assistance and inspiration given to me by beloved brother - sister-in-law ***Mr. Nagesh- Rama devi,*** *their childrens* ***mittu and lucky*** *and my Grand Parents* ***Smt. Kanthamma – Ramchandraiah.***

Words are not enough to express my heartfelt thanks to my friends, ***Sriram Bhai, Gmc, Venu, sathish, Sai kiran, Jittu, RP, Guna, Vasam Vinay, Bojjam, Dinesh, Asif, Tirupathi, Deva Anil, Rambabu, Sandeep, Vinay, Srinu, Surender, Satthi (Aswaraopet), Rahul (Photo Studio)*** *for their unparallel affection, encouragement, which instilled in me confidence to proceed forward..*

I would like to acknowledge the constant assistance provided by my colleagues, ***Rp, Jalender, Shyam, Afsha.*** *I extended warmest thanks to my seniors* ***Shalini , Padmaja, Swetha singh, Rajesh khanna, Sumalatha, Pramila madam, Yellagoud, Divya rani, Jadesha, Rajender, Divya, Rajesh, Om Prakash, Nagarjuna, Bindu Madhavi, Noorulla, Chandramani, Ramesh, Srinivas*** *and juniors* ***Chandrakala, Raviteja, Yamuna, Yashaswani and Suman.***

I am thankful to non-teaching staff of Department of Plant Pathology ***Andalu, Chandrakala, Ramkoti, Ashabi, Yeshob and Manemma*** *for their help and care during my research work*

I convey my wholehearted thanks to all my well wishers who were far too numerous to have been mentioned here.

The financial assistance rendered by ANGR Agricultural University in the form of stipend is greatly acknowledged.

Place: Hyderabad

Date: ***(V. SURESH)***

LIST OF ABBREVATIONS

%	:	per cent
@	:	at the rate of
BOD	:	Biological Oxygen Demand
CD	:	Critical Difference
Cm	:	Centimeter
CMI	:	Commonwealth Mycological Institute
CMRS	:	Central Mango Research Station
CV	:	Coefficient of Variation
cv.	:	Cultivar
e.g.	:	example
et al.	:	and others
etc.	:	and so on
Fig.	:	Figure
ha	:	Hectare
i.e.,	:	that is
MEA	:	Malt Extract Agar
Mg/l	:	Milligram per litre
ml	:	Milliliter
mm	:	Millimeter
No.	:	Number
NHB	:	National Horticulture Board
OMA	:	Oat Meal Agar
^{o}C	:	Degree Centigrade

PDA	:	Potato Dextrose Agar
PDI	:	Per cent Disease Incidence
Ppm	:	Parts per million
PSA	:	Potato Sucrose Agar
pH	:	Hydrogen ion concentration
psi	:	pounds per square inch
SEm	:	Standard error of mean
Spp	:	Species
t	:	Tonne
viz.,	:	namely

Name of the author :**V. SURESH**

Title of the thesis: "**STUDIES ON MANGO GUMMOSIS WITH SPECIAL REFERENCE TO *Lasiodiplodia theobromae* (Pat.) Griffon & Moube**

Degree : **MASTER OF SCIENCE IN AGRICULTURE**

Faculty : **AGRICULTURE**

Major Field : **PLANT PATHOLOGY**

Chairperson : **Dr. B. VIDYA SAGAR**

University : **ACHARYA N. G. RANGA AGRICULTURAL UNIVERSITY**

Year of Submission :**2014**

ABSTRACT

Mango (*Mangifera indica* L.) is one of the world's most important and esteemed fruit of the tropical and subtropical world and is cultivated extensively as a commercial fruit crop in India.

Mango 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.

Survey was conducted to assess the incidence of gummosis in the major mango growing areas of Andhra Pradesh,*viz.,* Krishna, Khammam, Rangareddy andMedak during June to October,2013.During the survey gummosis incidence was assessed and symptoms characteristic of the disease,*viz.,*gummosis, dieback, and vascular discoloration were noticed in the orchards surveyed. Among the four districts surveyed, maximum disease incidence (13.3 per cent) was recorded in the cultivar, Chinnarasamand least incidence was recorded in Baneshan (2.0 per cent) and kobbarimamidi(2.0 per cent) cultivars at Rekunta village of Krishna district.

Growth rate of *L. theobromae* on different solid media at different temperatures was studied. The radial growth of the mycelium was maximum (8.89 and 8.83) on PSA medium at 30 and 35^{o}C followed by PDA (8.46) at 35^{o}C. Least mycelial growth (6.3) was observed in MEA at 25^{o}C. The maximum pycnidial production was observed on OMA followed by PDA at temperature above 30^{0}C. Least pycnidial production was observed on MEA at all the temperatures tested. The fungal growth on various media was categorized as circular with sparse

aerial mycelium, circular with moderate aerial mycelium and circular with abundant mycelium. The color of colony ranged from whitish grey to blackish grey.

The pathogen was isolated from infected host plant, purified and identified as *L.theobromae* and pathogenicity was proved by stem inoculation method. Morphological characters of *L. theobromae* were studied and following observations were made. Colonies were grey-brown to black with dense aerial mycelia on the PDA medium. Pycnidia were separate or aggregated, dark brown, thick or thin-walled. Conidiophores were hyaline, cylindrical to sub-obpyriform, with oblong, straight and hyaline single celled conidiaandinitially. Gradually the conidiabecame dark brown and produced one septum with longitudinal striations; the size of conidia measured 22-29×11–15 µm.

Efficacy of nine fungicides was tested *in vitro* against *L. theobromae,* of which carbendazim, carbendazim + mancozeb and propiconazole were found superior at both 250 and 500 ppm concentrations with 100 per cent inhibition of the test pathogen. However, mancozeb and propineb were found effective only at 500ppm concentration. Among the botanicals and *Trichoderma* isolates tested *in vitro*, Garlic bulb extract and *Trichoderma* isolates T9, T6, T3 and T2 were found effective against *L. theobromae*.

Screening of ten different mango cultivars against *L. theobromae* by stem inoculation method revealed that Chinnarasalu, Manjeera, Tellagulabi and Suvernarekha were susceptible to gummosis, while Baneshan, Alphonso, Imam Pasand and Pandurivarimamidi were moderately resistant. Among the cultivars screened, Suvernarekha wasfound highly susceptible to mango gummosis.

Chapter I

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the world's most important and esteemed fruit of the tropical and subtropical world and is cultivated extensively as a commercial fruit crop in India. It probably originated in Indo-Burma region and has been cultivated for the last 4000 years with the existence of more than 1000 varieties in Indian subcontinent. 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).

Mango is most important fruit crop in India, and occupies top position among mango growing countries of the world with an area of 25 lakh ha and annual production of 18002.4 MT, and productivity of 7.2 MT per ha.

In India, mango is grown mainly in Andhra Pradesh (4.89 lakh ha), Maharashtra (4.82 lakh ha), Uttar Pradesh (2.74 lakh ha), Karnataka (1.78 lakh ha), Tamil Nadu (1.52 lakh ha), Bihar (1.42 lakh ha) and Gujarat (1.41 lakh ha). Andhra Pradesh recorded highest production of 4,406 thousand MT and 9.0 MT per ha productivity (National Horticulture Board, 2013-14).

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 raw 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 16000 fruits in peak years at the age more than 100 years old (Singh, 1960).

Lasiodiplodia theobromae infects more than 500 plant species and is associated with various diseases like damping-off, wilt, blight, die back, root rot, collar rot, stem necrosis, leaf spot, witches broom, fruit blight, fruit rot, pod rot, boll rot and seed rot in different crops causing extensive losses.

The mango crop is susceptible to various diseases like powdery mildew, anthracnose, die back, blight, red rust, gummosis and sooty mould etc. 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. The incidence of gummosis was reported to be 20 and 60 per cent in Punjab and Sindh Provinces of Pakistan, respectively and 60 percent in Al Batinah region of Oman (Al-Adawi *et al*., 2006**)**.

In Andhra Pradesh, mango gummosis is reported from major mango growing areas and is gaining importance due to the death of the trees with high disease severity.

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 also dies. Commonly mango trees live on average of 80 to 100 years but when it is infect with gummosis the tree is killed and hence disease control of gummosis is very important.

The present management practices include the use of fungicides like carbendazim, thiophanate methyl, benomyl, application of Bordeaux paste and pruning of infected plant parts. Though the disease can be managed with the use of fungicides like carbendazim, the utility of these chemicals and fungicides is limited. An attempt was made to study the impact of different fungicides and chemicals to manage the gummosis disease of mango.

Continuous use of these agrochemicals for controlling the disease may pose several problems like toxicity to non-target organisms, development of resistance among the population of pathogen and environmental pollution. Therefore, an alternative to fungicide application desirable to exploit other environmentally safe means, *viz.*, plant extracts and

bio control agents. These are considered as new rays of hope because they are ecofriendly and can be used as an alternative measure to control plant diseases.

Information on sources of resistance to mango gummosis is not available in India though the crop is an important commercial crop in Andhra Pradesh.

Keeping in view of the above, the following objectives were formulated to elucidate information on incidence, distribution of *Lasiodiplodia theobromae* on different mango growing areas of Andhra Pradesh and integrated management of Gummosis disease.

Objectives

1. To conduct survey for incidence of gummosis in major mango growing areas of Andhra Pradesh.
2. To study morphological and cultural characteristics of *L. theobromae.*
3. *In vitro* evaluation of certain fungicides, botanicals and antagonists against *L. theobromae.*
4. To evaluate different varieties of mango for their reaction to gummosis.

CHAPPER-II

REVIEW OF LITERATURE

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

2.1 Distribution

2.2 Incidence and loss

2.3 Symptomatology

2.4 Pathogenicity

2.5 Survey

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

2.7 *In vitro* evaluation of certain fungicides, botanicals and biological control agents against *L. theobromae*

2.8 Screening of varieties against *L. theobromae*

2.1 Distribution

Mango gummosis/ dieback / mango sudden decline caused by *L. theobromae* is a wide spread disease in major mango growing areas limiting mango production and has become a major threat to the world mango industry.

Mango trees attacked 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.

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 Die back disease/ Mango gummosis reported in different parts of the world

S.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)

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

2.2.1 World Scenario:

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.

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 per cent incidence with a severity of 62.5-85 per cent. He also isolated 12 species of fungi

belonging to 10 different genera in addition to *L. theobromae* from infected mango trees exhibiting gummosis symptoms.

The mango gummosis was reported to be 20 and 60 per cent in Punjab and Sindh Provinces of Pakistan, respectively and 60 per cent in Al Batinah region of Oman (Saeed, 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 55.0, 50.0, 25.0 and 25.0 per cent respectively. Maximum intensity of 5.17 per cent 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 per cent to 58.1 per cent 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 per cent 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.

2.2.2 Indian Scenario

In India, mango dieback disease was first reported by Das Gupta and Zachariah in 1945 from Uttar Pradesh and were first 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.

Verma and Singh (1970) identified mango gummosis as a serious disease in Jaipur district, which affected 30-40 per cent of the plantations in the Morabad region of Uttar Pradesh (Prakash and Srivastava, 1987).

Rath and Mohanan (1977) reported that *B. theobromae*, *C. gloeosporioidea* and *Aspergillus spp*. were the primary incitants 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, 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.

2.3 Symptomatology

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.

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).

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).

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).

2.4 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^2, obtained from leading edges of actively growing fungal culture on PDA) in slating cuts under the bark with sterilized scalpel and then covered with parafilm. 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*

S.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 disease	*Delonix regia*	Omamor (1988)
4	Dogwoods	Canker	*Cornus florida*	Mullen *et al.*(1991)
5	Lemon	Fruit rot	*Citrus aurantifolia*	Cedeno and Palcios-pru *et al.*(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*	Haqueet *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 occidentale*	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 plants	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 (2011)

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 rot	*Dimocarpus longan*	Diaz *et al.* (2014)
44	Elephant tree	Canker	*Boswellia papyrifera*	Gezahgne *et al.*(2014)

2.5 Survey

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

Maduleti (1989) conducted a survey for mango dieback disease incidence 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 55.0, 50.0, 25.0 and 25.0 per cent prevalence, respectively. The incidence percentage in the same order was 3.17, 4.43, 0.62 and 1.25 per cent while the intensity ranged from 16.0 to 50.0 per cent. The maximum intensity of 5.17

per cent for quick decline was noted in Sahiwal district. Chaunsa proved to be the most susceptible cultivar with 6.95 and 3.14 per cent 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 per cent) followed by Tandu Allahyar (4.18) and minimum disease severity in Naushahro feroz (1.32 per cent) and Khirpur (1.46 per cent).

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 per cent to 58.1 per cent, 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 per cent disease incidence (PDI) and a maximum of 70 per cent 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 per cent 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.

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

2.6.1 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 4m) 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 118.0 to 240.0 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 per cent 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.

2.6.2 Cultural characters

Alam *et al.* (2001) identified *B. theobromae* was the causal agent of crown rot disease of banana, in their studies they found that 25-30^{o}C temperature optimum for the growth of the pathogen and highest sporulation occurred at 30^{o}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^{o}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 mycelium 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 isolates. 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.s

Jash *et al.* (2003) reported that sucrose was the best carbon source for growth of *Alternaria zinniae* followed by starch and maltose.

2.7 Evaluation of certain fungicides, botanicals and biological control agents sps. against *L. theobromae*

2.7.1 Effect of fungicides on *L. theobromae*

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 per cent) or methyl thiophanate (0.1 per cent) or chlorothalonil (0.2 per cent) 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.

Sharma and Badiyala (1994) studied the effectiveness of 8 fungicides (carbendazim, thiabendazole, thiophanate-methyl, mancozeb, copper oxychloride, aureofungin, Bordeaux mixture and benomyl) against mango gummosis. Carbendazim was the most effective treatment followed by Bordeaux mixture and aureofungin.

Rawal and Ullasa (1988) reported that mango gummosis was effectively controlled by pruning the infected twigs followed by spraying of carbendazim (0.1 per cent) or

Topsin M (0.1 per cent) or chlorothalonil (0.2 per cent). 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.

Khan and Asad Masood (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.

Meah *et al.* (1991) studied the efficacy of mancozeb and iprodione on mango stem-end rot caused by *B. theobromae*. Mature green hard mangoes when dipped for three minutes in heated (50^{o} C) aqueous solutions of mancozeb (3g a.i./l) and iprodione (0.5 and 0.75g (a.i./l)) gave excellent control of stem-end rot infections. Symptoms or signs didn't developed either in storage or during ripening and afterwards.

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*. In field experiment, carbendazim was found to be more effective than thiophanate-methyl and Alliete in reducing the fungal infection in mango plants, suppressing the gum exudation, dieback and wilting resulting in significant enhancement in vegetative growth of plants.

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 per cent.

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 effectiveeven 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 also

found that carbandazim+copperoxychloride, iprodione+carbendazim, mancozeb+ carbendazim also completely inhibit the growth of *L. theobromae*.

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.

Sultana and Ghaffar (2010) evaluated different fungicides against *L. theobromae*, causing seed rot and seedling rot and root infection on bottle gourd under *in vitro* and *in vivo* conditions. Carbendazim and Topsin-M completely inhibited the growth of *L. theobromae in vitro* at 50 ppm whereas Aliette, benlate, mancozeb, ridomil and vitavax showed complete inhibition of colony growth at 100 ppm. Invariably all fungicides at three different concentrations, *viz*., 1, 2, 3 g (a.i.) kg-1 seeds reduced the recovery of the seed borne fungi. The most effective seed treatments were benlate, topsin-M, carbendazim and Aliette @ 3 g kg-1 seeds which enhanced seed germination and reduced seed infection in bottle gourd.

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*.

.

2.7.2 Effect of botanicals on *L. theobromae*

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 per cent concentration. Among them *A. calamus* extract at 1.0 per cent 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 pawpaw (*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 pawpaw, 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 per cent mycelium growth of the pathogen followed by *Curcuma longa* (77.35 per cent).

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 per cent 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).

2.7.3 Effect of bioagents on *L. theobromae*

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.

Sultana and Ghaffar (2010) studied the effect of five microbial antagonists (*Bacillus subtilis, T. harzianum, T. viride, Gliocladium virens* and *Stachybotrys atra*) in the control of *L. theobromae*, the cause of seed rot, seedling and root infection on bottle gourd under laboratory and field conditions. The bioagent *Bacillus subtilis* was recorded as a potential antagonist in reducing the infection followed by *T. harzianum* and *T. viride.*

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.

2.8 Screening of varieties against *L. theobromae*

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 Per cent Disease Index (PDI) was lowest in Dasheri, Mahmooda (12.3 per cent), Neeleshan (24.4 per cent), Baneshan (29.4 per cent) and Totapuri (30.0 per cent). AU Rumani, Cherukurasam and Vikarabad Mahmooda had the highest mean PDI for stem end rot.

Mahmood *et al.* (2007) also reported Sindheri, shows high level of susceptibility against *L. theobromae*. The cultivars Malda and Chaunsa 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 per cent disease symptoms, Ratol-12 showed the highest disease symptoms followed by Langra, Fajri and then black Chounsa.

Saeed *et al*. (2011) screened various genotypes, *viz*., Ratol-12, black chaonsa, white chaonsa, Fajri, Dosehri, Langra, Sindhri and Summer Bahisht against *B. theobromae* through artificial inoculation. They found that the cultivar Dosehri was comparatively tolerant to the disease and the cultivar Ratol-12 was highly susceptible to the test pathogen.

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.

Uma and Thirupathaiah (2009) screened three mulberry genotypes, *viz*., S13, M5 and Berhampur local against leaf spot disease caused by *B. theobromae* in Khammam, Andhra Pradesh. They reported that S13 variety was resistant, M5 variety was moderately resistant and Berhampur local was highly susceptible to the disease. Leaf-spot disease of mulberry was found to be more prevalent in rainy season than in summer and winter ones.

Chapter III

MATERIAL AND METHODS

The materials used and methods employed in conducting the experiments are described hereunder.

3.1 Location of Work

The present experiments were carried out in the Department of Plant Pathology, College of Agriculture, Rajendranagar, Hyderabad and Agricultural College, Aswaraopet, Acharya N.G. Ranga Agricultural University, Andhra Pradesh.

3.2 Materials and Methods

3.2.1 Glassware

Glassware of Borosil was used throughout the present investigation. The glassware used in present study were Petri plates (90 mm diameter), conical flasks (250, 500, 1000 ml), measuring cylinders (25, 250 and 500 ml), test tubes, pipettes (0.1, 1.0, 2.0, 5.0 ml and 10 ml) etc.

3.2.1.1 Cleaning of Glassware: The glassware was first cleaned with a detergent followed by thorough cleaning with tap water. The cleaned glassware were placed in potassium dichromate solution for 24 h and finally rinsed with distilled water for 3-4 times. Then they were air dried before use.

Potassium dichromate ($K_2Cr_2O_7$)	:	60 g
Concentrated sulphuric acid (H_2SO_4)	:	60 ml
Distilled water	:	1000 ml

3.2.2 Chemicals

Chemicals of Analytical Reagent (AR) and Guaranteed Reagent (GR) grades of standard make were used. The pH of the media was adjusted using either 0.1 N HCl or 0.1 N NaOH.

3.2.3 Equipment

Compound microscope (10x, 40x magnifications) was used for observing and describing the fungi. Hot air oven and autoclave were used for sterilization of glassware and media, respectively. Incubators were used for incubating test materials at different temperatures. The cultures were stored in a refrigerator. Weighments were done on a single pan electronic balance with a sensitivity of 0.001 g. Other tools which were used in the present investigation for various purposes included camel brush, inoculation needle, pots etc.

3.2.4 Culture Media

The following Potato Dextrose Agar (PDA) media were used for isolation, culturing and maintenance of fungi in the laboratory.

3.2.4.1 Potato Dextrose Agar (PDA) Medium:

PDA medium was prepared using the following components for culturing of fungi in the laboratory.

Peeled Potatoes	: 200 g
Dextrose	: 20 g
Agar	: 20 g
Distilled water	: 1000 ml

Peeled potato pieces were boiled in 500 ml of distilled water in a 1000 ml beaker till the pieces got softened. The extract was filtered through a double layered muslin cloth. To another 500 ml of distilled water in another 1000 ml beaker, 20g of agar was added and melted till it got dissolved. Both the solutions were mixed in another 1000 ml beaker into which 20 g of dextrose was added. The final volume of the medium was made up to 1000 ml by addition of distilled water. The p^H of the medium was adjusted to 6.8 with 1 N NaOH or 1 N HC1 as the case may be with the pH meter. The medium was distributed to culture tubes and conical flasks at 8.0 ml and 100 ml each, respectively. The medium was sterilized in an autoclave at 15 psi (121^oC) for 15 minutes.

3.2.5 Preparation of PDA Slants

PDA slants were prepared by transferring 8.0 ml of the medium to culture tubes. The tubes were plugged with non-absorbent cotton and sterilized in an autoclave. After sterilization, the tubes were removed from the autoclave when they were still in hot condition (*i.e*. approximately 40°C) and kept in a slanting position for the medium to solidify. After solidification, the slants were kept in refrigerator for further use.

3.2.6 Plating of Medium

The sterilized medium was melted and distributed in petriplates (9 cm diameter) at the rate of 20 ml per plate aseptically in the laminar air flow chamber and allowed to solidify. The plates containing the medium were used for culturing and maintenance of fungus in the laboratory.

3.2.7 Sterilization

Glassware used for present investigation were kept in sterilization tins or wrapped in brown paper and were sterilized in hot air oven at 160°C for 90 minutes.

Surface of Laminar Air Flow chamber (LAF) was sterilized by wiping with cotton swab dipped in alcohol. Inoculation loop, cork borer and scalpel were sterilized by dipping in alcohol and heated to red hot using a spirit lamp.

Culture media and distilled water were sterilized in an autoclave at 15 psi for 20 minutes.

3.3 Isolation of the pathogen

Mango twigs infected with gummosis were collected from different mango growing areas of Andhra Pradesh (Nuziveedu, Sangareddy, Aswaraopet and Chevella). These twigs were put in sterilized polythene bags and brought to the laboratory for isolation and identification of the organism.

3.3.1 Procedure for isolation

Mango twigs infected with gummosis were collected from severely infected Navaneetham variety from Horticulture Research Station, Aswaraopet. Infected plant material was cut into 1 -2 cm long pieces containing disease portion along with healthy portion; surface sterilized with 0.1% Mercuric chloride for two minutes and were placed in Petri plates containing Potato Dextrose Agar at 25$\pm$ 1^0C with 12 hours alternate periods of light and darkness. After 3 days of incubation mycelial growth was observed along the disinfected diseased twigs. Hyphal tips from the advancing mycelia were transferred to the Potato dextrose agar slants.

3.3.2 Identification of pathogen

The isolated pathogen was identified as *Lasiodiplodia theobromae* based on its mycelial and conidial characters through standard mycological descriptions by CMI keys (Punithalingam, 1976).

3.3.3 Pathogenicity test

The pathogenicity test was conducted in pots at Agricultural College, Aswaraopet. One year old seedlings of susceptible mango cultivar Suvernarekha was selected as a host for conducting pathogenicity tests. A slant cut in the stem was made using a sterilized knife. A 5 mm inoculum disc from 5- day- old culture of a test fungus on PDA was placed in the gap and the inoculated portion was wrapped with Para film. A 5 mm PDA block without fungus was placed in the control plants. Plants were irrigated after inoculation and the wrapping material was removed from the stems after

2 weeks of inoculation. Plants were monitored for the development of disease symptoms and isolations were made from the stem of the test plants to confirm the pathogenicity. (Khanzada *et al*., 2004)

3.4 To survey for disease incidence in major mango growing areas of Andhra Pradesh.

A roving survey was conducted in major mango growing areas of Andhra Pradesh, *i.e*., Krishna, Khammam, Medak and Rangareddy districts during 2012-13 rainy season (June to October) to know the prevalence of mango gummosis. The disease incidence in each cultivar was recorded by counting the number of infected plants out of the total number of plants assessed per cultivar and expressed in percentage.

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total no. of plants assessed}} \times 100$$

Observations recorded:

1. Variety
2. Age of the plant
3. Type of symptoms observed: Twig blight, gum exudation and blackening

3.5 To study morphological and cultural characteristics of the pathogen

Lasiodiplodia culture identified as stated earlier and maintained on PDA was utilized to study morphological and cultural characters of the pathogen on various growth media *viz*., Potato dextrose agar, Potato sucrose agar, oat meal agar and malt extract agar and evaluated at three different temperatures (25, 30 and 35^0C).

3.5.1 Morphological characteristics

The characters of mycelium, conidia and pycnidial formation of *L. theobromae* was recorded.

3.5.2 Cultural characteristics

The cultural characteristics *viz*., type of growth, colour, type of margin and topography of the colony of *L. theobromae* was recorded when the growth of the

pathogen reached the edges of petriplates in one of the culture media tested. The morphological characters of the pathogen were studied on the following solid media.

i) Potato dextrose agar (PDA)
ii) Potato sucrose agar (PSA)
iii) Oat meal agar (OMA)
iv) Malt extract agar (MEA)

The composition of above mentioned media are given below.

i) Potato Dextrose Agar (PDA)

Peeled potato slices : 200 g
Dextrose : 20 g
Agar : 20 g
Distilled water : 1000 ml
pH : 6.0

ii) Potato Sucrose Agar (PSA)

Peeled potato slices : 200 g
Sucrose : 20 g
Distilled water : 1000 ml
Agar : 20 g
pH : 5.6

iii) Malt Extract Agar

Malt extract : 20 g
Glucose : 20 g
Peptone : 1 g
Distilled water : 1000 ml
Agar : 20 g
pH : 6.0

iv) Oat Meal Agar

Oatmeal : 50 g
Distilled water : 1000 ml
Agar : 20 g
pH : unadjusted

3.6 Evaluation of different fungicides, botanicals and antagonists agents against *L. theobromae in vitro*

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

Nine fungicides were evaluated against *L.theobromae* by poisoned food technique (Nene and Thapliyal, 1993) at two concentrations *i.e.,* at 250 and 500 mgl^{-1}. The list of fungicides evaluated is furnished in Table 3.1.

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 Petri dishes so as to get the desired concentration of active ingredient of each fungicide separately *i.e*., 250 and 500 mgl^{-1} doses. Twenty ml of amended medium was poured in 90 mm sterilized Petri dishes and allowed to solidify. Mycelial discs of 5 mm diameter from 3 day old culture was inoculated at the center of the Petri plate and then incubated at 18± 2^0C for 3-4 days. Control was maintained without fungicide. Three replications were maintained for each treatment. Per cent inhibition of mycelial growth was calculated using the formula (Vincent, 1927).

$$I = (C-T/C) X 100$$

Where,

I = Per cent inhibition of mycelial growth

C = Colony diameter in control (cm)

T = Colony diameter in treatment (cm)

Table 3.1: List of fungicides tested against *L. theobromae* by poisoned food technique under *in vitro* conditions.

S.No	Common name	Trade name	Source of supply
1	Carbendazim	Bavistin	BASF India Ltd.
2	Mancozeb	Dithane M-45	Dow Agro Sciences
3	Carbendazim+Mancozeb	SAAF	United Phosphorus Limited
4	Propiconazole	Tilt	Syngenta India Ltd.
5	Pyraclostrobin	Headline	BASF India Ltd.
6	Pyraclostrobin+ Metiram	Cabriotop	BASF India Ltd.
7	Azoxystrobin	Amistar	Syngenta India Ltd.
8	Propineb	Antracol	Bayer India Ltd.
9	Tebuconazole +Trifloxystrobin	Nativo	Bayer India Ltd.

3.6.2 Evaluation of different botanicals against *L. theobromae in vitro*

3.6.2.1 Botanicals

The present investigation was carried out by eight botanicals to evaluate different plant species for their fungitoxicant properties against *L.theobromae.*

Table 3.2: List of plant extracts tested against *L. theobromae* by poisoned food technique under *in vitro* conditions.

S.No.	Common name	Botanical name	Family	Plant part used
1	Neem	*Azadirachta indica*	Meliaceae	Leaf
2	Wild Tulasi	*Ocimum sanctum*	Lamiaceae	Leaf
3	Glory flower	*Clerodendron infortunatum*	Verbenaceae	Leaf
4	Datura	*Datura metal*	Solanaceae	Leaf

5	Karanj	*Pongamia pinnata*	Leguminoceae	Leaf
6	Bitter oleander	*Holarrhena pubescens*	Apocynaceae	Leaf
7	Duranta	*Duranta erecta*	Verbenaceae	Leaf
8	Garlic	*Allium cepa*	Liliaceae	Bulb

3.6.2.2 Preparation of cold aqueous extract:

Fresh leaves or bulbs of the test plant were collected and washed first in tap water and then in distilled water. Then 100 grams of fresh sample was crushed in a surface sterilized mortar and pestle by adding 100 ml sterile distilled water (1:1 W/V). The extract was filtered through two layers of muslin cloth. The extract was strained through Millipore bacterial filter and finally filtrate thus obtained was used as stock solution for *in vitro* studies.

3.6.2.3 *In vitro* evaluation of plant-extracts against *L. theobromae*

To study the antifungal activity of plant extracts on the growth of *L. theobromae*, the poisoned food technique was followed (Nene and Thapliyal, 1993) Ten ml and five ml of stock solution was mixed with 90 and 95 ml of sterilized molten PDA medium respectively, so as to get 10 and 5 percent concentration. The medium was shaken thoroughly for uniform, mixing of test extract.

Twenty ml of medium was poured into each of the 90 mm sterilized petriplates. Each plate was seeded with 5 mm mycelial disc taken from the periphery of 3 day-old fungal culture and incubated at 18 ± 2^0C till the growth of colony touches the periphery in the control plate. Three replications were maintained for each treatment. Suitable control plates were maintained by growing the cultures on PDA without the botanicals. The radial growth of the colony was recorded when the maximum growth was observed in control and per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1927).

$$I = (C-T/C) X 100$$

Where

I = Per cent inhibition of mycelial growth

C = Colony diameter in control (cm)

T = Colony diameter in treatment (cm)

3.6.3 *In vitro* evaluation of antagonists against *L. theobromae*

Eleven *Trichoderma* isolates were obtained from department of plant pathology, College of Agriculture, Rajendranagar, Hyderabad.

These *Trichoderma* isolates were screened for their antagonism against the *Lasiodiplodia theobromae* by using dual culture technique (Dennis and Webster, 1971). About 20 ml PDA was poured into sterile petriplates and allowed to solidify, from previously grown young cultures of antagonists and test pathogen, a 0.5 cm fungal disc of test fungus and respective bioagent were transferred aseptically to petriplates simultaneously by leaving suffient space in between two discs. Three replications were maintained for each treatment. The petriplates were incubated at 18 ± 2^{0}C till the growth of culture in control covered entire petriplates. Colony diameter of both the test fungus and bioagent were measured and per cent inhibition was calculated and the data was analyzed statistically.

$$I = (C-T/C) X 100$$

where

I = Per cent inhibition of mycelial growth

C = Colony diameter in control (cm)

T = Colony diameter in treatment (cm)

The effective antagonistic bio agent screened by dual culture method against *L. theobromae* were tested and further used for the management of the disease.

3.7 To evaluate different varieties of mango for resistance to gummosis

One year old seedlings of ten different varieties of mango were collected from Horticultural Research Station, Aswaraopet, Khammam district. The popular mango varieties, Chinna rasalu, Manjeera, Tellagulabi, Totapuri, Baneshan, Alphonso, Amrapali, Imampasand, Suvernarekha and Pandurivari mamidi were selected for resistance screening to *L. theobromae* laid out in a Randomized Block Design. Five

replications were maintained for each cultivar. The observations on disease severity were recorded after one month of inoculation.

The severity of disease symptoms in twigs, branches, leaves and stem of individual plant was rated using a 1-5 scale (Modified from Ramos *et al*., 1997) corresponding to per cent disease severity from 0 to 100 % which has been described as under

Scale	**Description**	**Percent disease severity (%)**	**Reaction**
1	Seedlings free of disease	0	Resistant
2	An early stage of infection characterized by browning of leaf petioles and mid-veins and presence of marginal leaf blade necrosis	1-25	Moderately Resistant
3	The presence of dead leaves, which may remain attached at the tip of main stem, vascular browning, and evidence of gummosis from the stem	26-50	Moderately Susceptible
4	Dead leaves with progressive browning and extensive gummosis from the stem portions	51-75	Susceptible
5	Severe dieback that extended to major portions of the plant with profuse gummosis	76-100	Highly Susceptible

Percent Disease index (PDI) was calculated as per the formula of Wheeler (1969).

$$PDI = \frac{\text{Sum of individual ratings}}{\text{No. of seedlings assessed}} \times \frac{100}{\text{Max. Disease grade}}$$

3.8 Statistical Analysis

The data recorded was analyzed following the analysis of variance, as suggested by Panse and Sukhatme (1978) and the data was transformed using Angular transformations wherever necessary and statistically analyzed using Completely Randomized Design or Randomized Block Design as per procedures given by Snedecor and Cochran (1967).

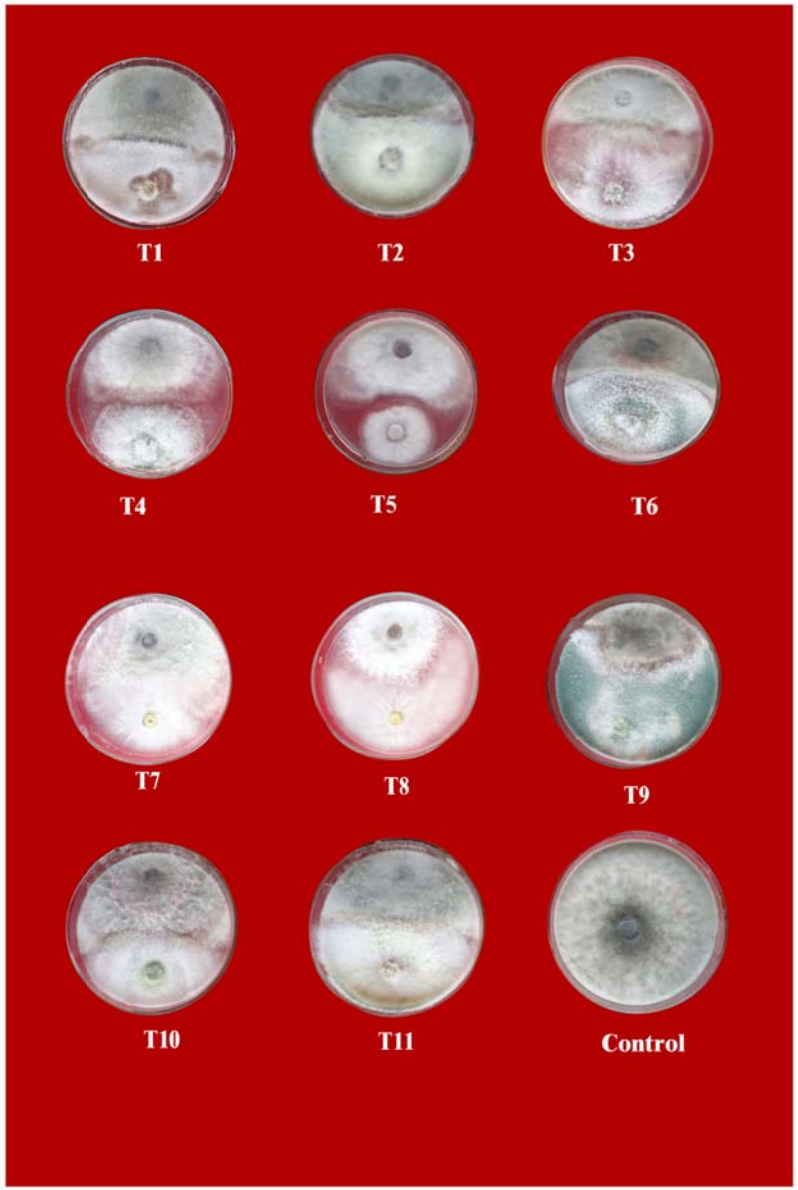


Plate 4.9 : Inhibition of mycelial growth of *Lasiodiplodia theobromae* by Trichoderma isolates

Plate No. 4.13: Moderate resistant cultivars to mango gummosis

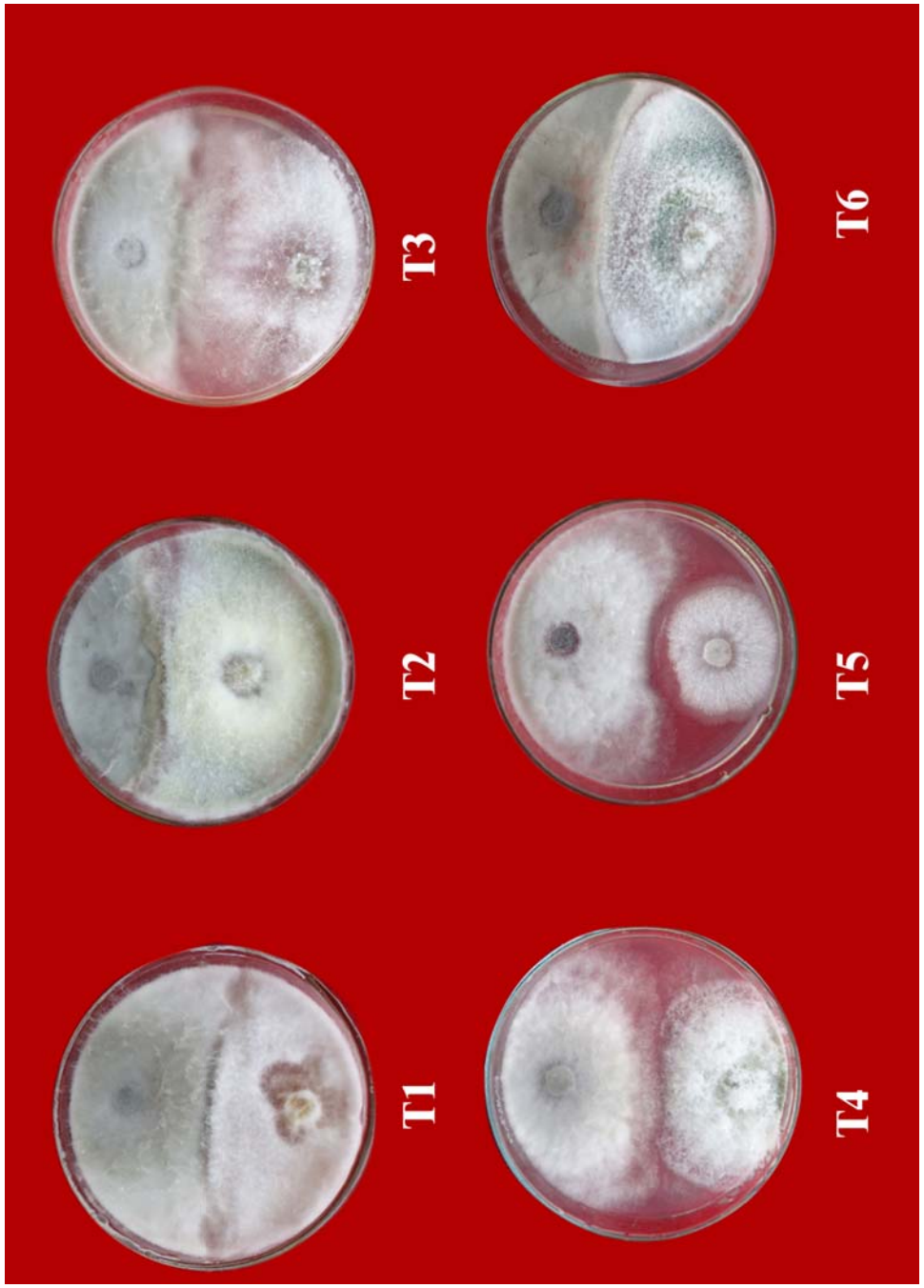


Plate No. 4.2: Pure culture of *Lasiodiplodia theobromae*

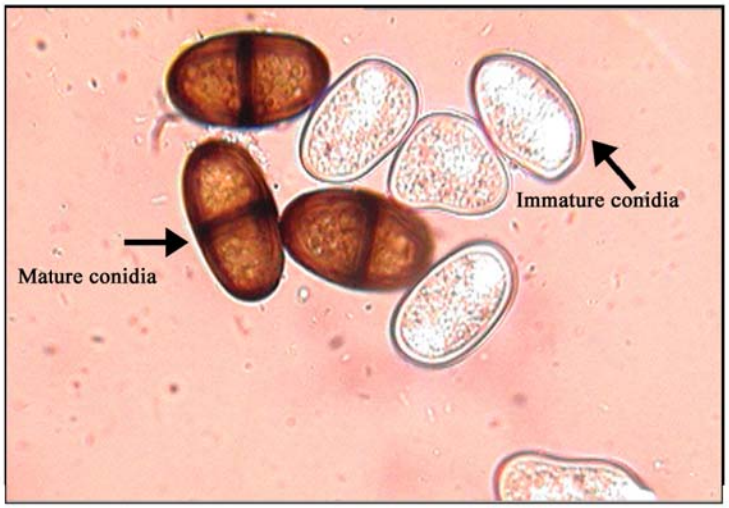


Plate No. 4.3: Mature and Immature conidia of *Lasiodiplodia theobromae* observed under compound microscope 1000 X

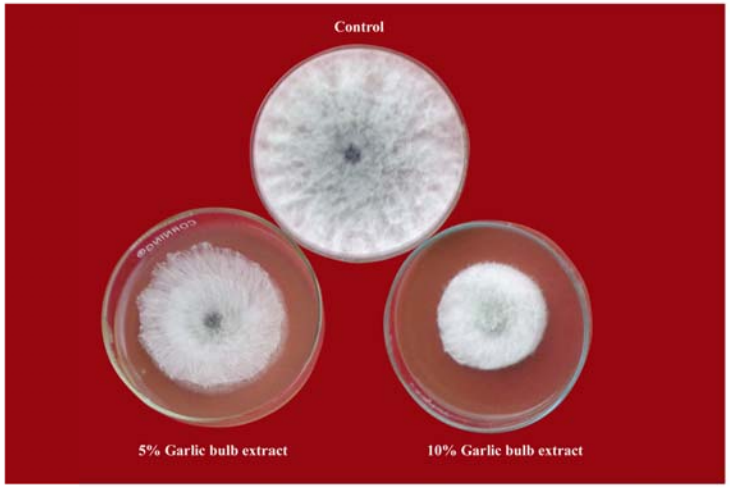
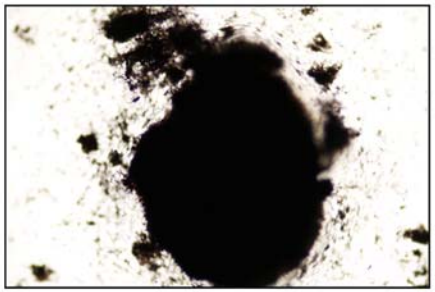


Plate No. 4.8: Inhibition of mycelial growth of *Lasiodiplodia theobromae* by using garlic extract at 5% and 10% concentration under *invitro* condition

A) Pycnidial production on OMA

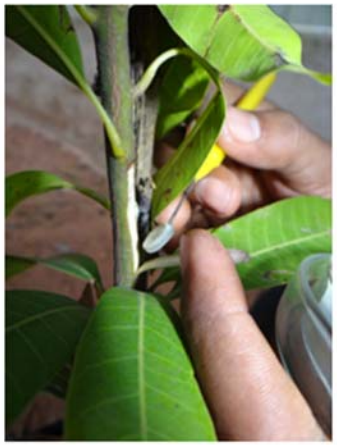
B) Conidia



c) Pycnidium

Plate No. 4.5: Pycnidial production of *Lasiodiplodia theobromae* on OMA

A) Slant cut by sterilized knife



B) Inoculation of fungus disc into cut portion

C) Wrapped by polythene

Plate No. 3.1: Stem inoculation of *Lasiodiplodia theobromae* on mango seedling

A) Gum oozing from the inoculated mango seedling

B) Twig blight

C) Marginal necrosis of foliage in inoculated seedling

Plate No. 4.10: Symptoms observer on inoculated mango seedlings after one month of inoculation

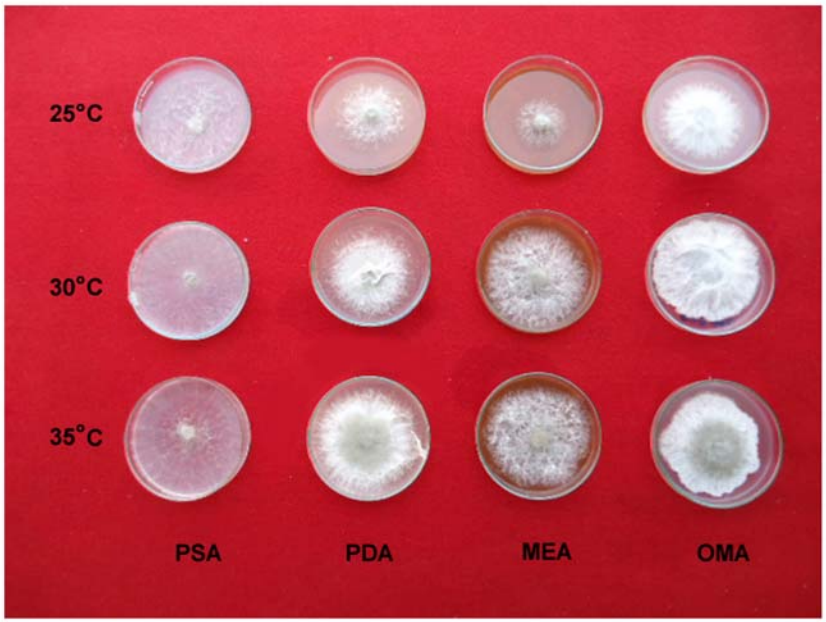


Plate No. 4.4: Effect of different temperature and media on the growth of *Lasiodiplodia theobromae* under *invitro* condition

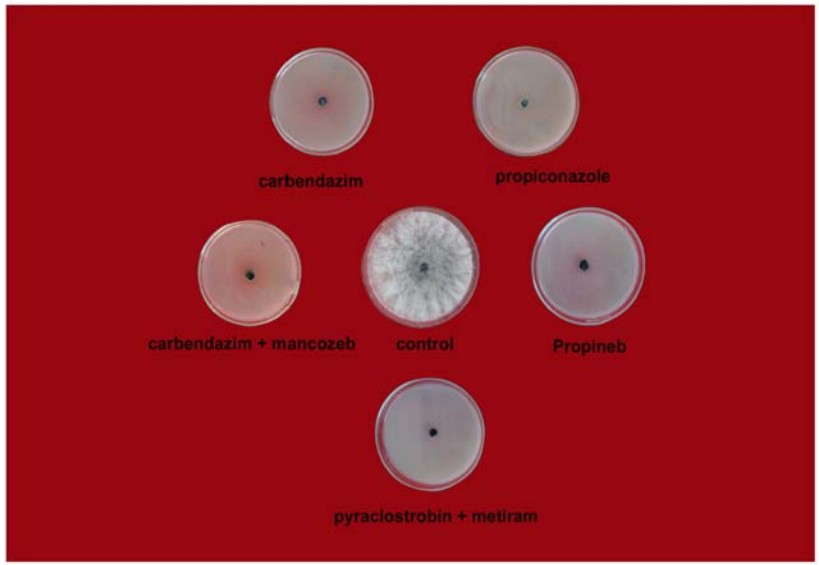


Plate No. 4.6: Evaluation of different fungicides against *Lasiodiplodia theobromae* at 500 ppm under *invitro* condition

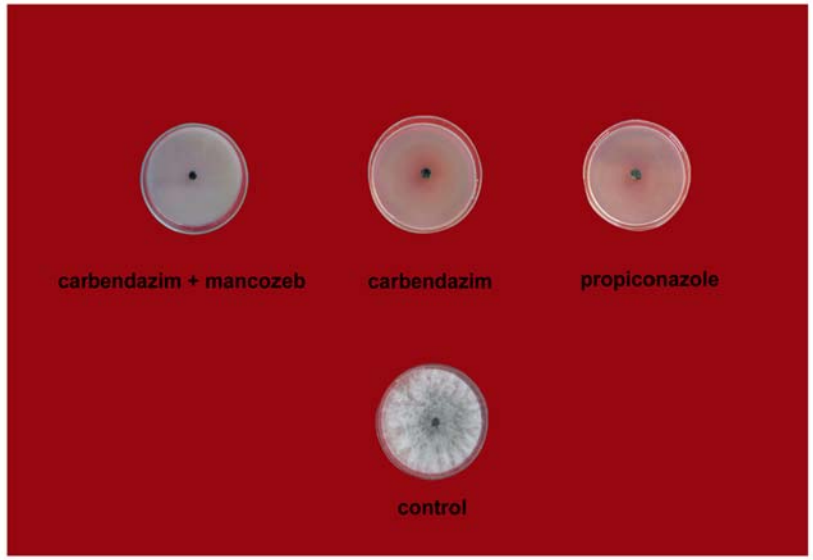


Plate No. 4.7: Evaluation of different fungicides against *Lasiodiplodia theobromae*

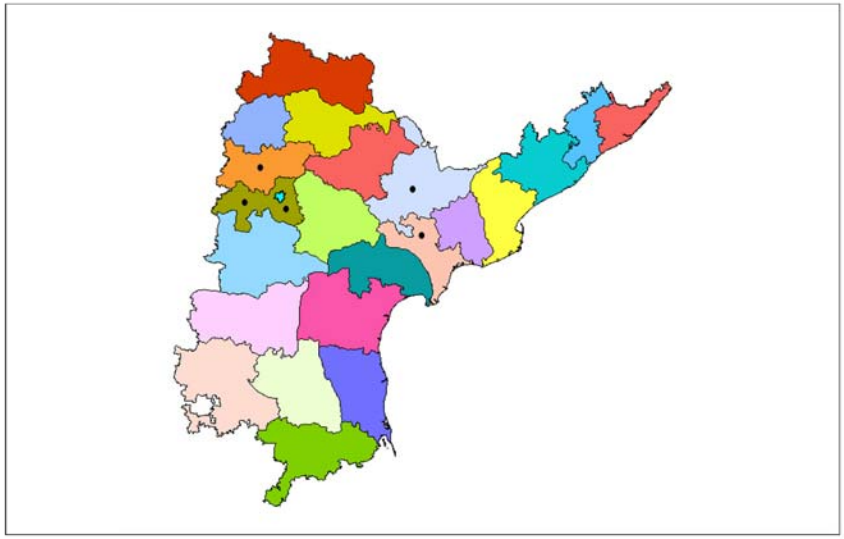


Plate No. 3.2: Pictorial representation of survey area for mango gummosis

A) Die back

B) Gummosis

C) Bark splitting

D) Vascular discolouration

E) Partial wilting

Plate No. 4.1: Symptoms of mango gummosis

Plate No. 4.11: Disease of *Lasiodiplodia theobromae* in suvernarekha cultivar

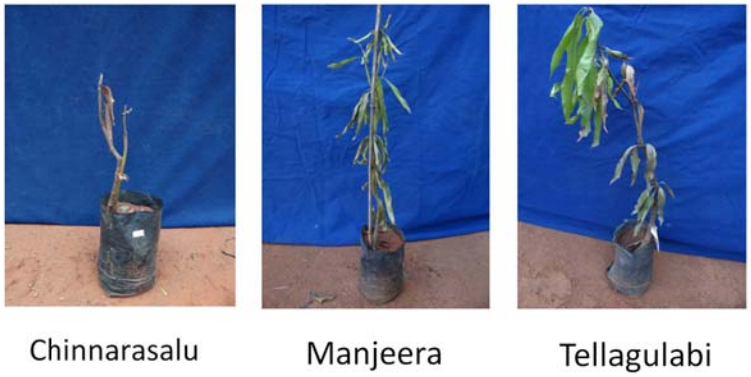


Plate No. 4.12: Susceptible cultivars to mango gummosis

Chapter IV

RESULTS AND DISCUSSION

The results of the experiments pertaining to the present investigation are presented here under the following heading.

4.1 Survey ofmango gummosis incidence

Survey was conducted to assess the incidence of gummosis in the major mango growingareas of Andra Pradesh *viz.,* Krishna, Khammam, Rangareddy, Medakduring June to October in 2013. The information pertaining to disease incidence is furnished in Table. 4.1. During the survey conducted in different mango orchards symptoms like gummosis, dieback and vascular discoloration were mostly observed(Plate. 4.1).

Gummosis**:** The infected plant parts show abundant gum secretion from branches, stem, and main trunk. Initially the gum appears as a small droplet. However, as the disease progresses, it increases and covers most of the branch and trunk. Under severe conditions, the outer wood of a branch cracks and splits and exudes a yellow to brown, gum-like substance.

Dieback: In affected plants, twigs die from the tips to back into old wood, giving a scorched appearance to the limb. The young green twigs start withering first at the base and then extending outwards along the veins of leaf edges. The affected leaf turns brown and its margins roll upwards. Leaves scorch and fall, leaving a dead branch. In severe conditions, branches start drying one after another in a sequence resulting in death of the whole tree.

Vascular discoloration: In infected plants, the twigs and branches showed internal discoloration. Brown streaks in vascular regions are visible upon splitting the twigs lengthwise.

Among the orchards surveyed in four districts, maximum per cent disease incidence was recorded 13.3 per cent in Chinnarasam at Rekunta village of Krishna district, while the varieties, Suvernarekha (Rekunta), Navaneetham (Aswaraopet), Manjeera, Khader pasand (Sangareddy) recorded 10.0 per cent of gummosis incidence. The cultivars Suvernarekha, Lalmuni, Mala-1 from Sangareddy and

Manjeera (Aswaraopet) showed 6.66 per cent while Totapuri (Aswaraopet), Bobbilipunasa, Navaneetham (Sangareddy)varieties showed 3.33 per cent disease incidence the varieties kobbarimamidi, Baneshan(Rekunta)recorded less incidence (2.00 per cent).

The cultivar Suvernarekha recorded highest disease incidence in Rekunta (10 per cent), while minimum was recorded in Sangareddy (6.66 per cent).

Among orchardssurveyed the varieties Chinnarasalu, Suvernarekha, Navaneetham, Manjeera, Khader pasand varietieswere exhibiting profuse gum oozing when compared to Baneshan and Totapuri.In the present study, the per cent disease incidence was ranged from 2.0 to 13.3.

Similarly Maduleti (1989) recorded 0 to 40 per cent of mango dieback (*B. theobromae*)disease incidence in different orchards in Andhra Pradesh.

According to C.M.S.R., (1974-85) report, 30 to 40 per cent disease incidence of dieback was reported from Moradabad region, in Uttar Pradesh and they also reported the cultivars Langra, Chausa, Dashehari and Fajli were susceptible (40.6-85.7 per cent) to mango die back in Durgapura region of Jaipur, Rajastan.

The high incidence of dieback was recorded in Jaipur district of Rajastan from india(Verma and Singh, 1970). Severe incidence of mango dieback recorded in Uttar Pradesh by Prakash and singh(1976).

Panhwar *et al.* (2007) also reported that maximum disease severity in Hyderabad (4.76%) followed by Tandu allahyar (4.18) and minimum disease severity was record in naushahro feroz (1.32%) andKhirpur (1.46 %) respectively inmajor mango growing districts of Sindh, Pakistan. Similarly Iqbal*et al. (*2007) also reported maximum disease incidence in Sahiwal districtand observed Chaunsa variety was most susceptible cultivar with 6.95 per cent disease incidence Punjab, Pakistan. Khan *et al.* (2004)and Rehman*et al.* (2011) also reported varied disease incidence in different mango growing areas around the world.

4.2 To study morphological and cultural characteristics

4.2.1 Isolation and pathogenicity

Mango twigs with typical gummosis symptoms were collected from mango orchards from Aswaraopet. The fungus was isolated and brought into culture on PDA following tissue isolation technique. Finally, pure culture of fungus isolated by single spore isolation and maintained on PDA. All isolations made from the diseased twigs yielded *L. theobromae.*

Pathogenicity test was carried out by stem inoculation method, by inoculating homogenized mycelial discs of *L. theobromae* on one year old mango seedlings. The typical symptoms of the disease developed in one month seedlings after inoculation. The pathogen was re-isolated from the infected tissues and the morphology of the fungus compared with original.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 by stem inoculation mango gummosis by this method.

4.2.2Morphological Characteristics:

Colonies were grey-brown to black with dense aerial mycelia on the media. Pycnidia were separate or aggregated, dark brown, thick or thin-walled. Conidiophores were hyaline, cylindrical to subobpyriform, Conidia were oblong, straight, hyalineat first, aseptate and then they became dark brown, produced one septum with longitudinalstriations, the size if conidia measured 22-29 µm×11–15 µm.(Plate no. 4.4 and 4.5)

Celiker *et al.* (2012) reported that *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 and measured were 20.9-27.5 µm x 11.0-15.4 µm.

Twumasi*et al.* (2014) reported 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.

These results are in agreement with the Punithalingam (1976), Alves*et al.* (2008), Shahbaz *et al*.(2009) and Li *et al.*(2013).

4.2.3 Cultural characteristics

4.2.3.1 Effect of different temperatures on the growth of *L. theobromae* on different solid media

The cultural characters of *L. theobromae* was studied on four different media *viz.,* Potato Dextrose Agar (PDA), Potato Sucrose Agar (PSA), Oat Meal Agar (OMA) and Malt Extract Agar (MEA) at three different temperatures (25^{o}C, 30^{o}C and 35^{o}C) in Table 4.2 and fig. 4.1.

L. theobromae varied in their growth rate on different media at different temperatures. The radial growth of the mycelium was maximum (8.89 cm and 8.83 cm) on PSA medium at 30 and 35^{o}C followed by PDA (8.46 cm) at 35^{o}C. Least mycelial growth was observed in MEA at 25^{o}C (Plate no. 4.2.). The results are in accordance with that of Saha *et al*. (2008) who reported that glucose and sucrose are the best carbon sources for the mycelial growth of *L. theobromae* and PDA was found to be one of the best medium for its growth. Alam *et al.* (2001) and Eng*et al*. (2003) also found that mycelium growth was higher in glucose and sucrose containing media.

Similarly Fu *et al.* (2007) reported that PDA and PSA were most suitable for vegetative growth of *L. theobromae*. Several carbon sources including dextrose, sucrose and D-mannose were found to be utilized by the fungus for mycelium growth. Moreover, the growth on different media was found to be dependent on temperature of incubation. The temperature for mycelial growth of *L. theobromae* was found to be in the range of 4 to 36^{0}C (Saha *et al*., 2008) with optimum being 25-30 ^{0}C (Alam *et al*., 2001).

In the present investigation, a significant interaction was found between the type of medium and temperature of incubation on pycnidial production. The maximum pycnidial production was observed on OMA followed by PDA at temperature above 30^{0}C. The pycnidial production was more and rapid when incubated at high temperatures (30 and 35^{0}C) compared to low temperature (25^{0}C).

Least pycnidial production was observed on MEA at all the temperatures tested. (Plate no. 4.3)

The fungal growth on various media was categorized as circular with sparse aerial mycelium, circular with moderate aerial mycelium and circular with abundant mycelium. The color of colony ranged from whitish grey to blackish grey. 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 isolates. 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.

4.3*In vitro* evaluation of various fungicides,botanicals and antagonistsagainst *Lasiodiplodia theobromae*

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

The sensitivity of different fungicides *viz.,* carbendazim, mancozeb, carbendazim + mancozeb, propiconazole, pyraclostrobin, pyraclostrobin + metiram, azoxystrobin, propineb and tebuconazole + trifloxystrobinagainst*L. theobromae*was tested *in vitro* by poisoned food technique at 500 ppm and 250 ppm.

It is evident from the data presented in Table 4.3 and Fig 4.2 that all the fungicides tested were significantly superior over control in inhibiting the growth of *L. theobromae*. Significant differences among the treatments were also observed. Hundred per cent reduction in the radial growth of *L. theobromae* was recorded by the fungicides carbendazim, mancozeb, carbendazim + mancozeb, propiconazole and propineb followed by pyraclostrobin + metiram (86.11 per cent), tebuconazole + trifloxystrobin(79.81 per cent), azoxystrobin (46.85 per cent) while minimum per cent inhibition of the pathogen was recorded in pyraclostrobin (26.11) (plate 4.6)

The most effective fungicide in descending order at 500 ppm, Carbendazim = mancozeb = carbendazim + mancozeb =propiconazole = propineb>pyraclostrobin + metiram>tebuconazole + trifloxystrobin> azoxystrobin >pyraclostrobin

Similar observations were also made at 250 ppmconcentration of the fungicide. All the fungicides were significant in inhibiting the pathogen *L.theobromae.*The differences among the treatments were also significant except in

case of tebuconazole + trifloxystrobin and propineb which were on par with one another. Complete inhibition in growth of pathogen was recorded by carbendazim, carbendazim + mancozeb and propiconazole, followed by pyraclostrobin + metiram which inhibit 83.70 per cent mycelium growth.Tebuconazole + trifloxystrobin and propinebinhibited the test fungus by 75.00 and 72.59 per cent respectively. The minimum per cent inhibition was observed in pyraclostobin20.00 followed by azoxystrobin 35.37. (Plate 4.7)

The most effective fungicide in descending order at 250 ppm, Carbendazim = carbendazim + mancozeb = propiconazole >pyraclostrobin + metiram>tebuconazole + trifloxystrobin>propineb> mancozeb > azoxystrobin >pyraclostrobin

The fungicides mancozeb and Propineb recorded 66.30 and 72.59 per cent inhibition respectively at 250 ppm but these exhibited maximum (100) inhibition at 500 ppm.

The effectiveness of carbendazim in reducing of *L. theobromae*growth was reported by Sharma and Badiyala (1994), Rawal and Ullasa (1988) who described carbendazim superiority in inhabiting *L. theobromae* comparedto other fungicides.

Khan *et al.* (2004),Sulthana and Ghaffar (2010) reported thatmancozebwas effective ininhibiting*L. theobromae* at 100 ppm concentration. In the present study mancozeb at 250 ppm, inhibited 66.3 per cent only whereas at 500 ppm recorded 100 per cent inhibition observed. The variation in inhibiting the pathogen may be due to variation in isolates and environmental factors affect the survival of the pathogen. The results are agreement with Sahi *et al.* (2012) who reported that mancozeb was the least effective fungicide in inhibiting the mycelial growth of *L. theobromae.*

Meijiao*et al.* (2009) reported that propiconazole, carbendazim and mancozeb effectively control the pathogen.The effectiveness of carbendazim+mancozeb and propiconazolewas also recorded byBhatt and Jadeja(2010).

In the present investigation, the fungicides *viz.,* carbendazim, carbendazim + mancozeb, propiconazole were also found effective. The results in agreement with the Attah and Ahiasti (2010), Meijiao *et al.* (2009) and Sahi *et al.* (2012).

4.3.2 *In vitro* evaluation of botanicals against *L.theobromae*

Eight plant extracts was assayed at 5 % and 10 % concentrations usingpoisoned food technique, andthe results are presented in Table 4.4 and fig. 4.3.

At 5 % concentration the mycelial growth inhibition varied in different treatments irrespective of concentration of the botanicals used. Among the plant extracts tested, maximum per cent inhibition was recorded in garlic with (25.56), followed by neem (4.81 per cent), glory flower (4.07 per cent),datura (2.59), karanj (2.04), bitter oleander (2.22) and wild Tulasi (2.22) while minimum per cent inhibition was recorded in duranta with 1.85 per cent at 5 % concentration. However garlic was found to be significant over other treatments whereas all the other treatments were non-significant.

Similarly at 10 % concentration also garlic showed highest percent inhibition with 35.93 per cent followed by neem (8.15), glory flower (7.41), karanj(5.93), Datura (3.52)and wild Tulasi(2.59) and the least inhibition was observed in Bitter oleander and Duranta at 2.22 and 2.22 per cents respectively. However garlic was found to be significant overother treatments, whereas all the treatments were non-significant except datura was significant over karanj.

Garlic was found to be significant in inhibiting the growth of *L. theobromae* among botanicals tested (Plate No. 4.8).

Sahi *et al.* (2012) reported garlic and neem shows inhibitory effect on *L. theobromae.* Lima *et al.* (1996) and Sharma *et al.* (2011) also reported aqueous garlic extracts inhibit the *L. theobromae.*

The results of the present study are in agreement with the Okigbo*et al*. (2009) reported that *A. sativum* extracts effective inhibition (25.2-86.9%) on mycelial growth of the *L. theobromae*. The most toxic effect of the extracts was observed with *A. sativum* at 10%, with significant ($P<0.01$) inhibition on the fungi. However in the present investigation neem was recorded 8.15 per cent inhibition by pathogen. This may be due to variation in isolate or due to different environmental conditions effecting the growth of the pathogen.

4.3.3*In vitro* evaluation of *Trichoderma* isolates against *Lasiodiplodia theobromae*

The antagonistic effect of eleven*Trichoderma* isolates was assayed by dual culture method as mentioned in materials and methods. *Trichoderma* isolates showed significant difference in inhibiting the mycelial growth of *L. theobromae* (Table 4.5 and fig. 4.4). Differences among the treatments were observed with per cent inhibition in mycelial growth ranging from 61.1 (T 4) to 81.85 (T 9). Among the treatments tested, the *Trichoderma* isolates 9, 6, 3 and 2 recorded maximum inhibition against *L.*

theobromae and are statistically on par with each other and superior over the treatment, T10. The *Trichoderma* isolates, T1, T7, T11, T5, T8 and T4 recorded 71.11, 70, 70, 69.63, 65.19 and 61.11 per cent inhibition against *L. theobromae*, respectively. (Plate no. 4.9)

The results of the present investigation revealed that *Trichoderma* isolates effectively inhibited the growth of *L. theobromae*. Suhannaa*et al*. (2013) also reported the potential use of *Trichoderma* sp. against *B. theobromae,* the causal agent of mango stem end rot. *T. virens* and *T. hamatum* were also found effective in inhibiting the growth of *L. theobromae* by producing volatile metabolites. Similarly, *T. pseudokoningii* was also effective against the pathogen through non-volatile metabolite production (Priya and Nagaveni, 2009; Sangeetha *et al.* 2009). Moreover, the variation in the growth inhibition of *L. theobromae* by the same species of *Trichoderma* was also reported. Among various *Trichoderma viride* isolates tested by Thangavelu*et al*. (2007) against the crown rot pathogen, *T. viride* isolates, S7, RT1 and S17 were found effective. Prasad *et al*. (2014) also reported the efficacy of *T.harzianum*and *T. koningii* and *Aspergillusniger*in controlling *L. theobromae.*

4.4Evaluation of different varieties of mango for resistance to gummosis

One year old seedlings of ten different varieties of mango *viz*.,Chinnarasalu, Manjeera, Tellagulabi, Totapuri, Baneshan, Alphonso, Amrapali, Imampasand, Suvernarekha, Pandurivari mamidi were screened for resistance against *L. theobromae*and the results are presented in table4.6.

Marginal necrosis, twig blight, gum oozing and vascular discoloration were the most frequent symptoms of mango gummosis disease (plate no.4.10). Perusal of the data revealed that all the symptoms characteristic of the disease were observed in the cultivars, Suvernarekha, Chinnarasalu, Manjeera and Tellagulabi. Marginal necrosis was observed in all the cultivars inoculated and is the only symptom observed in the cultivars, Totapuri, Alphonso, Imampasand and Pandurivari mamidi.Profuse gummosis was observed in suvernarekha and Chinnarasalu followed by Manjeera and Tellagulabi. However, gummosis symptoms were not observed in Totapuri, Baneshan, Amrapali, Alphonso, Imampasand and Pandurivari mamidi.

Data on per cent disease severity showed that the cultivar Suvernarekha is highly susceptible to mango gummosis with disease severity of 84 per cent.Chinnarasalu, Manjeera and Tellagulabiwere also found susceptible to mango gummosis with more than 50 per cent disease severity. The cultivars, Totapuri and Amrapali were found moderately susceptible with 32 and 36 per cent disease severity, respectively. Baneshan, Alphonso, Imampasand andPandurivarimamidihave shownmoderatelyresistant reaction(Plate no.4.11,4.12,4.13).

Reddy *et al.* (2005) screened 10 cultivars of mango (Neeleshan, Dasheri mahmooda, AU Rumani, Totapuri, Swarnarekha, Vikarabadmahmooda, Baneshan, Cherukurasam, Dasheri and Manjeera) and reported low PDI of stem end rot disease (*B. theobromae*) in Dasheri mahmooda (12.3%), Neeleshan (24.4%), Baneshan (29.4%) and Totapuri (30.0%). The varieties AU Rumani, Cherukurasam and Vikarabadmahmoodarecorded high mean PDI for stem end rot.

Karunanayake*et al.* (2014) screened fruits ofmango cultivars for stem end rot. The cultivars Willard, Rata and Kohu cultivars as resistant to stem end rot caused by *L. theobromae* while Gira and KaruthaColomban were susceptible to stem end rot.

Saeed *et al*. (2011) reported that the cultivar Dosehri was comparatively tolerant to the disease and the cultivar Ratol-12 was highly susceptible to the *L. theobromae*.Khan *et al.* (2011) also reported Dosehri variety was comparatively tolerant to the mango disease as compared to others. Ratol-12 showed the highest disease symptoms followed by Langra, Fajri and then black Chounsa similarly Mahmood *et al.* (2007) also reported that sindheri, showed high level of susceptibility against *L. theobromae*. Malda and Chaunsa also showed significant proneness to the pathogen, while Langra and Desi were found comparatively less susceptible to the disease.

The present studyrevealed that mango varieties,*viz.,* Baneshan, Alphonso, Imampasand and Pandurivarimamidi are found moderately resistant to gummosis.

Table No. 4.5: Antagonistic activity of *Trichoderma* isolates against *Lasiodiplodia theobromae*

S.no	Isolate	*Per cent inhibition
1	*Trichoderma* isolate1 (T1)	71.11 (57.47)**
2	*Trichoderma* isolate 2 (T2)	80.74 (64.50)
3	*Trichoderma* isolate 3 (T3)	80.37 (63.69)
4	*Trichoderma* isolate 4 (T4)	61.11 (51.40)
5	*Trichoderma* isolate 5 (T5)	69.63 (56.77)
6	*Trichoderma* isolate 6 (T6)	81.48 (64.53)
7	*Trichoderma* isolate 7 (T7)	70.00 (57.47)
8	*Trichoderma* isolate 8 (T8)	65.19 (53.82)
9	*Trichoderma* isolate 9 (T9)	81.85 (64.77)
10	*Trichoderma* isolate 10 (T10)	74.44 (59.61)
11	*Trichoderma* isolate 11 (T11)	70.00 (56.77)
12	Control	0.00 (2.87)
CD at 5% SEm±		1.460 0.497

***** Mean of three replications

**Figures in parentheses are angular transformed values

Table No. 4.4: *In vitro* evaluation of botanicals on radial growth of *L. theobromae*

S. No.	Botanicals	*Per cent inhibition at 5 % concentration	*Per cent inhibition at 10 % concentration
1	Neem	4.81 (12.65)**	8.15 (16.57)
2	Wild Tulasi	2.22 (8.37)	2.59 (9.21)
3	Glory flower	4.07 (11.45)	7.41 (15.78)
4	Datura	2.59 (9.21)	3.52 (10.70)
5	Karanj	2.04 (8.18)	5.93 (14.08)
6	Bitter oleander	2.22 (8.37)	2.22 (8.37)
7	Duranta	1.85 (7.73)	2.22 (8.37)
8	Garlic	25.56 (30.35)	35.93 (36.81)
9	Control	0.00 (2.87)	0.00 (2.87)
CD at 5% SEm±		2.64 0.88	2.15 0.72

***** Mean of three replications

**Figures in parentheses are angular transformed values

Table No. 4.3: *In vitro* evaluation of fungicides on radial growth of *Lasiodiplodia theobromae* at 250 ppm and 500 ppm

S. No.	Fungicides	250 ppm *Per cent inhibition	500 ppm *Per cent inhibition
1	Carbendazim	100 (87.10)**	100 (87.10)
2	Mancozeb	66.30 (54.52)	100 (87.10)
3	Carbendazim+ Mancozeb	100.00 (87.10)	100 (87.10)
4	Propiconazole	100.00 (87.10)	100 (87.10)
5	Pyraclostrobin	20.00 (26.55)	26.11 (30.71)
6	Pyraclostrobin + Metiram	83.70 (66.18)	86.11 (68.12)
7	Azoxystrobin	35.37 (36.48)	46.85 (43.18)
8	Propineb	72.59 (58.58)	100.00 (87.10)
9	Trifloxystrobin+ Tebuconazole	75.00 (59.98)	79.81 (63.29)
10	Control	0.00 (2.87)	0.00 (2.87)
	CD at 5% SEm±	3.33 1.12	1.19 0.40

* Mean of three replications

**Figures in parentheses are angular transformed values

Table No. 4.2: Effect of different temperatures and media on the growth of *Lasiodiplodia theobromae* under *in vitro* condition

Temperature (C^o)	Media	Diameter (cm)	Colony colour	Type of growth	Pycnidia Production
35	PDA	8.46	Whitish grey	CA	+ +
	PSA	8.89	Black grey	CS	+
	OMA	7.84	Black grey	CA	+ + +
	MEA	7.79	Black grey	CA	+
30	PDA	7.91	Whitish grey	CA	+ +
	PSA	8.83	Whitish grey	CS	+
	OMA	7.82	Whitish grey	CA	+ + +
	MEA	7.74	Black grey	CA	+
25	PDA	6.71	Whitish grey	CA	+
	PSA	7.71	Whitish grey	CS	+
	OMA	6.86	Whitish grey	CA	++
	MEA	6.3	Black grey	CA	+

	Temperature (T)	Media (M)	MxT
SEm±	0.0375	0.0433	0.0750
CD at 5 %	0.1067	0.1232	0.213

PDA – Potato Dextrose Agar
PSA- Potato Sucrose Agar
OMA- Oat Meal Agar
MEA- Malt Extract Agar

CA-Circular with abundant aerial mycelium
CS-Circular with sparse aerial mycelium

+ : Sparse

+ + : Moderate

+ + + : High

Table No. 4.6: Symptoms observed in mango seedlings inoculated with *Lasiodiplodia theobromae*

S.no	Genotypes	Reaction	Marginal necrosis	Twig blight	Gum oozing	Vascular browning
1	Chinnarasalu	Susceptible	Yes	Yes	+++	++
2	Manjeera	Susceptible	Yes	Yes	+++	+
3	Tellagulabi	Susceptible	Yes	Yes	++	+
4	Totapuri	Moderately Susceptible	Yes	Yes	+	No
5	Baneshan	Moderately Resistant	Yes	No	No	No
6	Amrapali	Moderately Susceptible	Yes	Yes	+	No
7	Alphonso	Moderately Resistant	Yes	No	No	No
8	Imam pasand	Moderately Resistant	Yes	No	No	No
9	Suvernarekha	Highly Susceptible	Yes	Yes	+++	+++
10	Pandurivari mamidi	Moderately Resistant	Yes	No	No	No

+: Low; + + : Moderate; + + + : High

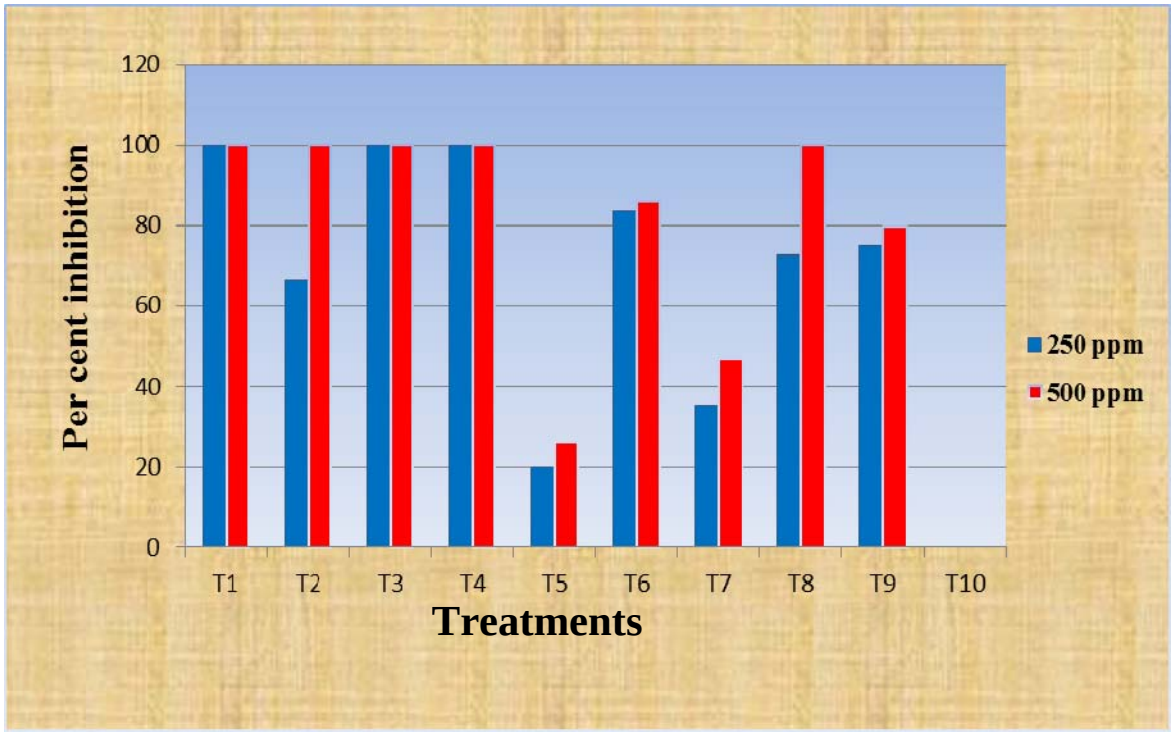
Table No. 4.7: Reaction of different varieties of mango seedlings to *L. theobromae*

Sl. No.	Genotype	Per cent Disease Index (PDI)	Disease Reaction
1	Chinnarasalu	72	Susceptible
2	Manjeera	68	Susceptible
3	Tellagulabi	64	Susceptible
4	Totapuri	32	Moderately Susceptible
5	Baneshan	24	Moderately Resistant
6	Amrapali	36	Moderately Susceptible
7	Alphonso	24	Moderately Resistant
8	Imam pasand	24	Moderately Resistant
9	Suvernarekha	84	Highly Susceptible
10	Pandurivari mamidi	24	Moderately Resistant

Table 4.1 Gummosis disease incidence in major mango growing areas of Andhra Pradesh

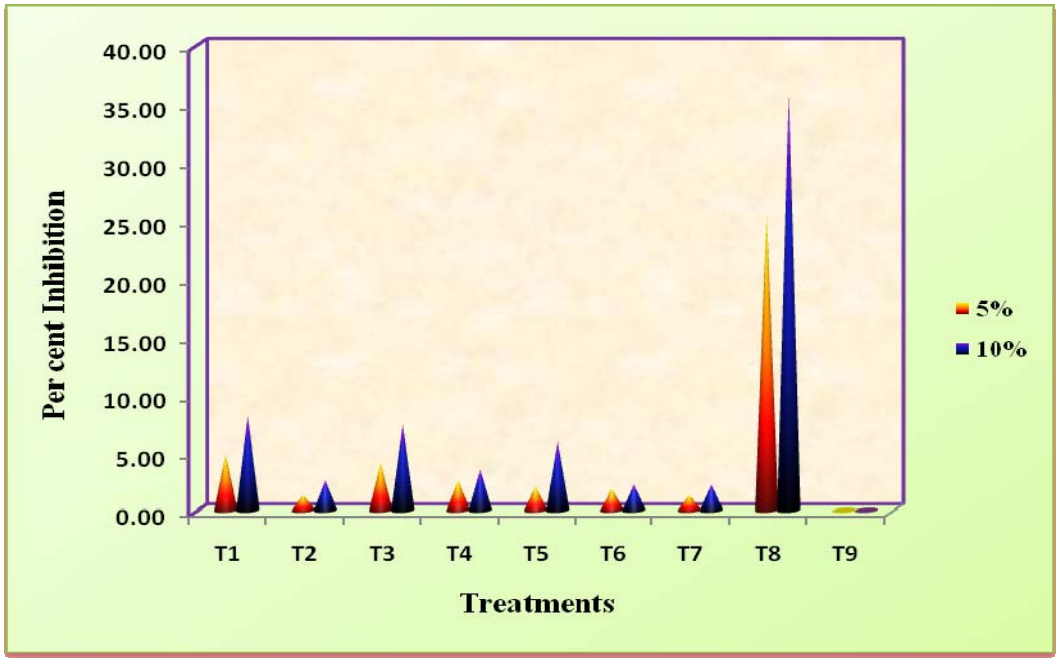
Sample No.	District	Mandal	Village	Variety	Age of trees (Years)	Type of symptoms			Disease incidence (%)
						Dieback	Gum exudation	Vascular discoloration	
1	Krishna	Nuziveedu	Nuziveedu	Baneshan	40	No	No	No	-
		"	"	Totapuri	40	No	No	No	-
		"	Rekunta	Chinnarasam	30	Yes	Yes	Yes	13.3
		"	"	Suvarnarekha	15	Yes	Yes	Yes	10.0
		"	"	Totapuri	15	Yes	No	No	3.3
		"	"	Baneshan	15	No	No	No	2.0
		"	"	Kobbarimamidi	15	Yes	No	No	2.0
		"	"	Cherukurasam	15	No	No	No	Nil
2	Khammam	Aswraopet	Aswraopet	Navanetham	30	Yes	Yes	Yes	10.0
		"	"	Manjeera	30	Yes	Yes	Yes	6.66
3	Rangareddy	Chevella	Chevella	Baneshan	45	No	No	No	Nil
		"	Parigi	Baneshan	45	No	No	No	Nil
4	Medak	Sangareddy	Sangareddy	Manjeera	40	Yes	Yes	Yes	10.0
		"	"	Lalmuni	57	Yes	Yes	Yes	6.66
		"	"	Mala-1	57	Yes	Yes	Yes	6.66
		"	"	Bobbilipunsa	57	Yes	Yes	Yes	3.3
		"	"	Khader pasand	57	Yes	Yes	Yes	10.0
		"	"	Navaneetham	50	Yes	Yes	Yes	3.33
		"	"	Suvernarekha	50	Yes	Yes	Yes	6.66

Figure 4.2: Effect of different fungicides on the radial growth of *Lasiodiplodia theobromae*



T 1	Carbendazim
T 2	Mancozeb
T 3	Carbendazim+ Mancozeb
T 4	Propiconazole
T 5	Pyraclostrobin
T 6	Pyraclostrobin + Metiram
T 7	Azoxystrobin
T 8	Propineb
T 9	Trifloxystrobin + Tebuconazole
T10	Control

Figure 4.3: Effect of botanicals on the radial growth of *Lasiodiplodia theobromae* at 5% and 10% concentration



T 1	Neem
T 2	Wild Tulasi
T 3	Glory flower
T 4	Datura
T 5	Karanj
T 6	Bitter oleander
T 7	Duranta
T 8	Garlic
T 9	Control

Figure 4.1: Effect of different temperatures and media on the growth of *L. theobromae* under *in vitro* condition

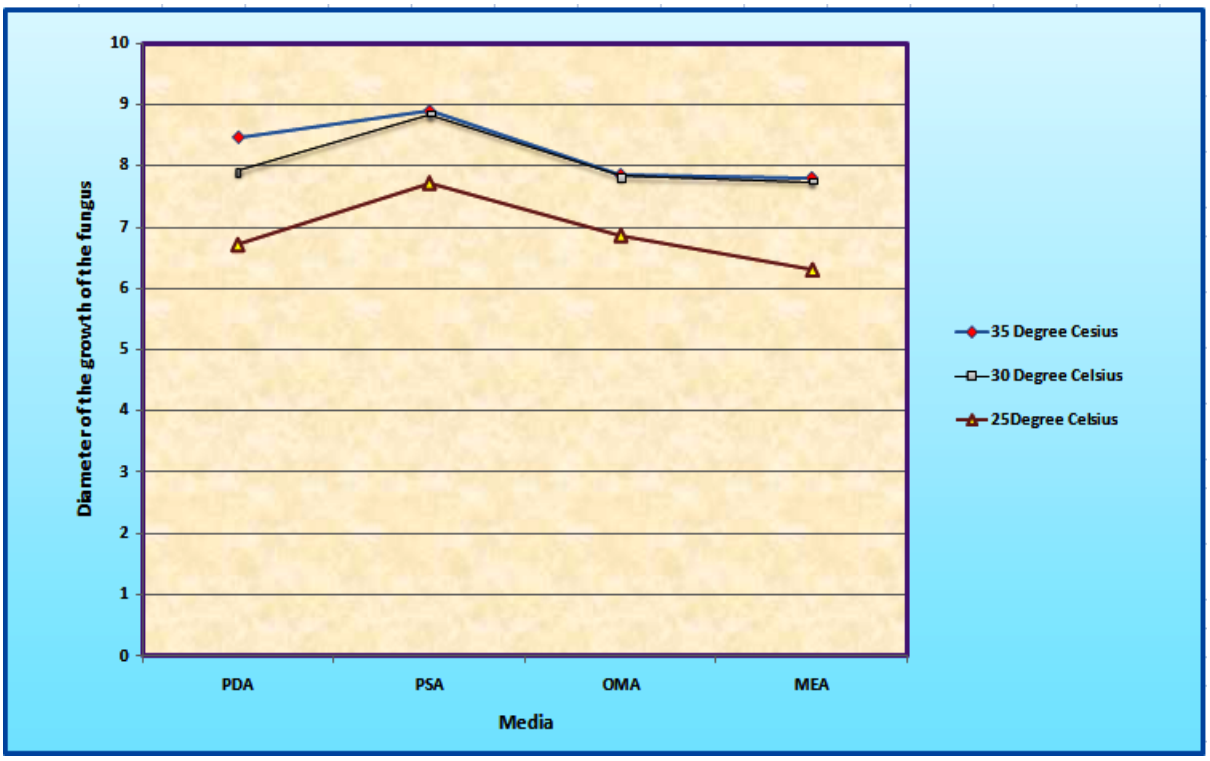
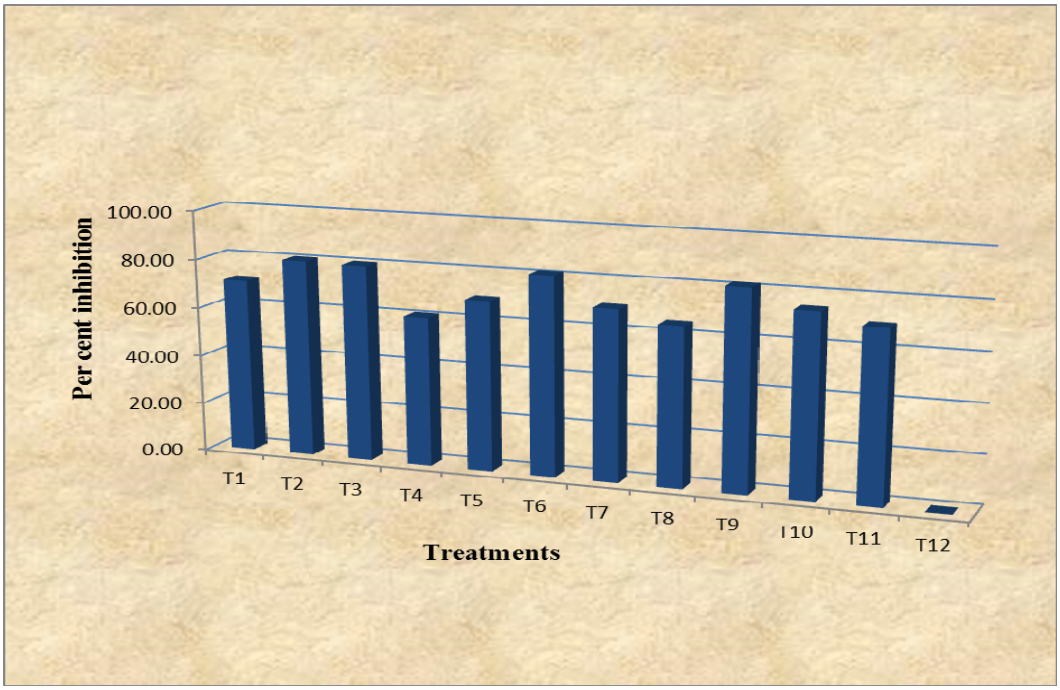


Figure 4.4: Effect of different antagonists on the radial growth of *L. theobromae*



T1-*Trichoderma* isolate 1

T2-*Trichoderma* isolate 2

T3-*Trichoderma* isolate 3

T4-*Trichoderma* isolate 4

T5-*Trichoderma* isolate 5

T6-*Trichoderma* isolate 6

T7-*Trichoderma* isolate 7

T8- *Trichoderma* isolate8

T9- *Trichoderma* isolate 9

T10- *Trichoderma* isolate 10

T11- *Trichoderma* isolate 11

T12- Control

Chapter V

SUMMARY AND CONCLUSIONS

Mango (*Mangifera indica* L.) is one of the world's most important fruit crop in tropical and subtropical world and is cultivated extensively as a commercial crop in India. Mango 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 Andhra Pradesh, mango gummosis is reported from major mango growing areas and is gaining importance due to the death of the trees with high disease severity. Keeping in view the importance of crop and losses caused due to this disease, investigation was carried out to elucidate information on incidence and distribution of *L. theobromae* in different mango growing areas of Andhra Pradesh, to study cultural and morphological characteristics of *L. theobromae*, preliminary evaluation of various management options for gummosis under laboratory conditions and screening of ten mango varieties against the pathogen for disease resistance. The results obtained in these investigations are summarized below.

Survey was conducted to assess the incidence of gummosis in the major mango growing areas of Andhra Pradesh, *viz.,* Krishna, Khammam, Rangareddy and Medak districts during June to October, 2013. During the survey of different orchards gummosis incidence was assessed and different symptoms of the disease, *viz.,* twig blight, dieback and gummosis were observed in the orchards.

Among the four districts surveyed, maximum disease incidence was recorded in Rekunta village of Krishna district. Further, incidence of the disease was found to be higher in the cultivar Chinnarasam followed by Suvernarekha. However, in Aswaraopet mandal of Khammam district, the cultivar Navaneetham was found highly susceptible with profuse oozing of gum from the main stem. Manjeera, Khader pasand cultivars from Sangareddy, also recorded high incidence of gummosis. The disease incidence was low in Baneshan and Totapuri cultivars compared to all other cultivars surveyed. The disease incidence in the mango cultivars was found influenced by various factors like type of soil, prevailing weather conditions, age of the orchard, etc. For example, the cultivar

Navaneetham recorded 10 per cent disease incidence in Aswaraopet mandal of Khammam district and 3.33 per cent incidence in Sangareddy.

Repeated isolations from diseased twigs collected from various locations yielded *L. theobromae* on PDA confirming it as the major pathogen associated with mango gummosis. Pathogenicity test carried out by stem inoculation method, by inoculating mycelial discs of *L. theobromae* on one year old mango seedlings, produced typical symptoms of the disease one month after inoculation.

The experiment conducted on the influence of different media and temperatures on the growth of *L. theobromae* revealed that the pathogen can grow at temperatures ranging from 25 to 35^0C with maximum growth and pycnidial production at 35^0C. The growth and pycnidial production varied with the type of medium used and the temperature of incubation. Potato sucrose agar supported maximum radial growth; however, Oat meal agar supported maximum pycnidial production at 35^0C. The pycnidial production was more and rapid when incubated at high temperatures (30 and 35^0C) compared to low temperature (25^0C). Least mycelial growth and pycnidial production was observed on MEA at all the temperatures tested. The fungal growth on various media was categorized as circular with sparse aerial mycelium, circular with abundant mycelium. The color of colony ranged from whitish grey to blackish grey.

Colonies were grey-brown to black with dense aerial mycelia on the media. Pycnidia were separate or aggregated, dark brown, thick or thin-walled. Conidiophores were hyaline, cylindrical to subobpyriform, Conidia were oblong, straight, hyaline and, at first, aseptate. Then they became dark brown, produced one septum with longitudinal striations, the size of conidia measured 22-29 µm ×11–15 µm.

Among the fungicides tested for their efficacy against *L. theobromae* under *in vitro* conditions, carbendazim, mancozeb, carbendazim + mancozeb, propiconazole and propineb were found to be superior in inhibiting the growth of *L. theobromae* at 500 ppm, while the strobilurin fungicide, pyraclostrobin was found least effective. The fungicides, carbendazim, carbendazim + mancozeb and propiconazole were found effective even at low concentrations (250ppm).

Among eight plant extracts assayed *in vitro*, maximum inhibition (25.56%) of mycelium growth was recorded in garlic bulb extract at 10 per cent concentration. Most of

the plant extracts tested were found ineffective in inhibiting the mycelial growth of *L. theobromae*.

Among eleven *Trichoderma* isolates assayed *in vitro* T9, T6, T3 and T2 recorded maximum inhibition against *L. theobromae.*

One year old seedlings of ten different varieties of mango, *viz.,* Chinna rasalu, Manjeera, Tellagulabi, Totapuri, Baneshan, Alphonso, Amrapali, Imampasand, Suvernarekha and Pandurivari mamidi were screened for resistance.

It was found that the cultivars, Chinnarasalu, Manjeera, Tellagulabi and Suvernarekha were susceptible to mango gummosis, while, Baneshan, Alphonso, Imampasand and Pandurivari mamidi were moderately resistant. Gummosis symptoms were not observed in Baneshan, Totapuri, Alphonso and Pandurivari mamidi. Among the cultivars screened, Suvernarekha was found highly susceptible to *L. theobromae* with disease severity of 84 per cent. Chinna rasalu, Manjeera and Tellagulabi were also found susceptible with 72, 68 and 64 per cent disease severity. None of the cultivars have shown high resistance to the disease.

LITERATURE CITED

Al Adawi, A.O., Deadman, M.L., Al Rawahi, A.K., Khan, A.J and Al Maqbali, Y.M. 2003. *Diplodia theobromae*associated with sudden decline of mango in the Sultanate of Oman. *Plant Pathology*.52: 419.

Al Adawi, A.O., Deadman, M.L., Al Rawahi, A.K., Al Maqbali, Y.M., Al Jahwari, A.A., Al Saadi, B.A., Al Amri, I.S and Wing field, M.J. 2006. Etiology and causal agents of mango sudden decline disease in the Sultanate of Oman. *European Journal of Plant Pathology*. 116: 247–254.

Alam, M.S., Begum, M.F., Sarkar, M.A., Islam, M.R and Alam, M.S. 2001. Effect of temperature, light and media on growth, sporulation, formation of pigments and pycnidia of *Botryodiplodia theobromae*Pat.*Pakistan Jounal of Biological Sciences*. 4: 1224-1227.

Alveraz, G.L.A and Lopez, J.G. 1971. Gummosis die-back and fruit rot of mango (*Mangifera indica* L.) caused by *Physalosporarhodina* (B&C) Cke. in Puerto Rico. *Journal of Agriculture*.55(4): 435-450.

Alves, A., Crous, P.W., Correia, A and Phillips, A.J.L. 2008. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. *Fungal Diversity*. 28: 1-13.

Alvindia, D.G andNatsuaki, K.T. 2007.Control of crown rot-causing fungal pathogens of banana by inorganic salts and a surfactant.*Crop Protection.*26(11): 1667–1673.

Attah, A.I and Ahiatsi, E.N. 2010. Causal agent of premature fruit rot of nutmeg (*Myristicafragrans*Houtt.) and its mycelial growth inhibition with some fungicides. *Ghana Journal of Horticulture*.8: 71-77.

*Bankole, S. A and Adebanjo, A. 1995.Inhibition of growth of some plant pathogenic fungi using from some Nigerian plants.*International Journal of Tropical Plant Diseases*. 13(1): 91-95.

*Batista, A.C. 1947. A serious disease of mango.*Ph.D.Thesis*, Pemambuco College of Agriculture, Pemambuco. 19:212-215.

Bertetti, D., Amatulli, M.T., Cardinale, J., Gullino, M.L and Garibaldi, A. 2012.First report of stem-end rot caused by *Lasiodiplodia theobromae* (Pat.)Griffon &Maubl.on avocado (*Perseaamericana*Mill.) fruits in Italy. *ProtezionedelleColture*. 3: 49-51.

Bhatt, H.R and Jadeja, K.B. 2010.Mango stem end rot management with carbendazim. *Indian Phytopathology*. 63 (1): 103-105.

*Bitancourt, A.A and Jenkins, A.B. 1943. Scab of mango caused by byElsinoe. *Phytopathology*.33:1.

*Bourne, B.A.1921. Fungoid attacks reported or observed. *Reportof the Department Agriculture*, Barbados.10-11.

Bua, B and Okello, C. 2011. Isolation and identification of cassava root rot disease causal pathogens from Lira district, Uganda. *In proceedings of 10th African crop science workshop on cassava rot,* African crop science society Maputo, Mozambique.10: 183-186.

Burruano, S., Mondello, V., Conigliaro, G., Alfonzo, A., Spagnolo, A and Mugnai, L.2008.Grapevine decline in Italy caused by *Lasiodiplodia theobromae*.*PhytopathologiaMediterranea*. 47(2): 132-136.

Cabrera, M.G and Cudom, M.A. 2013. Occurrence of *Lasiodiplodia theobromae* in Cattleya spp. in Corrientes, Argentina.*Summa Phytopathologica*. 39 (2):143.

Cardoso, J.E., Paiva, J.R., Cavalcanti, J.J.V., Santos, A.A and Vidal, J.C. 2006.Evaluation of resistance in dwarf cashew to gummosis in north-eastern Brasil.*Crop Protection.* 25: 855-859.

Cedeno, L and Palacios-Pru, E. 1992.Identificacion of *Botryodiploida theobromae* as the cause of lesions and gummosis in citrus.*RevistaFitopatogiaVenezola*. 5: 10-13.

Celiker, N.M and Michailides, T.J. 2012.First report of *Lasiodiplodia theobromae*causing canker and shoot blight of fig in Turkey.*New Disease Reports.*25: 12.

*Central Mango Research Station. Report for 1974-85. Studies on diseases of mango and their control.

Chakraborty, M.R., Ojha, S and Chatterjee, N.C. 2011. Die-back disease of Cycas - a new record from Burdwan, West Bengal. *Journal of Mycopathological Research.*49(1): 195-196.

*Cifferri, R and Gonzales, P.R. 1927. Parasitic and saprophytic fungi of the Dominican Republic.*Real Sociedad Espanola de Historia Natural.*27:68-81.

Dennis, C and Webster, J. 1971. Antagonistic properties of species groups of *Trichoderma* III hyphal interactions.*Transactions of British Mycological Society*. 57: 363-369.

Diaz, S.L.M., Rivera-Vargas, L.I., Goenaga, R and French-Monar, R.D. 2014. First report of *Lasiodiplodia theobromae* causing inflorescence blight and fruit rot of longan (*Dimocarpus longan* L.) in Puerto Rico. *Plant Disease*. 98(2): 279.

Dubey, R.K., Kumar, R., Jaya.,Chansouria, J.P.N and Dubey, N.K. 2008.Evaluation of *Amomumsubulatum* roxb oil as a source of botanical fungitoxicant for the protection of mango fruits from fungal rotting.*Journal of Food Safety.*28(3): 400-412.

Durgadevi, D and Sankaralingam, A. 2012.First report of peduncle blight of tuberose caused by *Lasiodiplodia theobromae* in India.*New disease reports.*26: 5.

*Edward, J.C. 1954. *Macrophomina* and *Botryodiplodia,* two distinct genera of Aphneropaidaceae.*Allahabad farmer*.28: 3.

Eng, F., Gutierrez-Rojas, M and Favela-Torres, E. 2003.A survey of temperature and pH effect on colonial growth of *Botryodiplodia theobromae* RC1.*RevistaIberoamericana de Micologia*. 20: 172-175.

Fateh, F.S., Kazmi, M.R., Ahmad, I and Ashraf, M. 2006. *Ceratocystis fimbriata* isolated from vascular bundles of declining mango trees in Sindh, Pakistan. *Pakistan Journal of Botany*. 38 (2): 1257-1259.

Fischer, I.H., Almeida, A.M., Garcia, M.J.M., Bertani, R.M.A and Bueno, C.J. 2008.First report of *Lasiodiplodia theobromae* on *Asclepiasphysocarpa* in Brazil.*Australasian Plant Disease Notes*. 3: 116-117.

Fu, G., Huang, S.L., Wei, J.G., Yuan, G.Q., Ren, J.G., Yan, W.H and Cen, Z.L. 2007. First record of *Jatropha podagrica* gummosis caused by *Botryodiplodiatheobromae* in China.*Australasian Plant Disease Notes.*2: 75–76.

Gezahgne, A., Yirgu, A and Kassa, H. 2014. First report of *Lasiodiplodia theobromae* causing canker on tapped *Boswelliapapyrifera*trees in Ethiopia. *New Disease Reports.*29: 11.

Gunasekaran, M and Srinivasan, N. 2000.Involvement of *Lasiodiplodia theobromae* (Pat.)Griffon and Maubl.in fruit rot of coconut in India. *Tropical Agriculture*. 77(2): 123-125.

Gupta, S.N and Zachariah, A.T. 1945. Dieback of mango.A new disease in India.*Indian Journal of Botanical Sciences*. 24(1): 101-108.

Haque, M.M., Mridha, M.A., Bhuiyan, M.K., Huda, S.M.S and Uddin, M.B. 2005. Studies on the occurrence and severity of leaf blight of Jack fruit (*Artocarpusheterophyllus*Lam.) Caused by *Botrydiplodia theobromae* pat.*Journal of Mycopathological Research.* 43(1): 91-94.

Hayes, W.B. 1953. Fruit growing in India, Kitabistan, Allahabad, India.

HuiFang, N., HongRen, N., RueyShyang, C., TingHsuan, H and RueyFen, L. 2012. A nested multiplex PCR for species-specific identification and detection of Botryosphaeriaceae species on mango.*European Journal of Plant Pathology*. 133 (4): 819-828.

Hunter, J.E., Buddenhagen, I.W and Kojima, E.S. 1969.Effeciency of fungicides, hot water and gamma radiation for control of postharvest fruit rots of papaya. *Plant disease reports.*53: 279-284.

Iqbal, Z.,mValeem, E.E., Shahbaz, M., Ahmad, K., Khan, Malik, T and Danish, M. 2007. Determination of different decline disorders in mango orchards of the Punjab.
Pakistan journal of Botany. 39(4): 1313-1318.

Jash, S., Dutta, S, Bandyopadhyay, S and Laha, S.K. 2003. Effect of different culture media, pH and carbon sources on growth and sporulation of *Alternariazinniae*Pape causing leaf and flower blight of marigold.*Environmental Ecology.* 21: 321-325.

Javaid, A., Javaid, A and Akhtar, N. 2008.*In vitro* chemical control of *Botryodiplodia theobromae*, the cause of dying back disease of mango.*Pakistan Journal of Plant Pathology*. 20(2): 195-199.

Kannan, C., Karthik, M and Priya, K. 2009.*Lasiodiplodia theobromae* causes a damaging diebackof cocoa in India.*New Disease Reports*.19: 63.

Karunanayake, K. O. L. C., Sinniah, G. D., Adikaram, N. K. B and Abayasekara, C. L. 2014. Cultivar differences in antifungal activity and the resistance to postharvest anthracnose and stem-end rot in mango (*Mangifera indica* L.). *Australasian Plant Pathology*. 43(2): 151-159.

Khalil, O. 2010. *Lasiodiplodia theobromae* Associated with Gummosis in Eucalyptus spp. in the Sudan. *Journal of Science*. 1(1): 27-34.

Khan, S.H., Idrees, M., Muhammad, F., Mahmood, A and Zaidi, S.H. 2004.Incidence of shisham (*Dalbergiasissoo*Roxb.) decline and *in vitro* response of isolated fungus spp. to various fungicides.*International Journal of Agriculture and Biology*. 6(4): 611-614.

Khan, S.S and Asad Masood, M.I. 2011. Symptom development after artificial inoculation of *Botryodiplodia theobromae*, a possible causal organism to quick decline in mango trees. *Pakistan Journal of Agricultural Sciences*. 48(4): 289-294.

Khanzada, M.A., Lodhi, A.M and Shahzad, S. 2004a. Mango dieback and gummosis in Sindh Pakistan caused by *Lasiodiplodia theobromae*.*Plant Pathology*.57: 381.

Khanzada, M.A., Lodhi, A.M and Shahzad, S. 2004b. Pathogenicity of *Lasiodiplodia theobromae* and *Fusarium* solani on Mango.*Pakistan Journal of Botany*. 36(1):181-189.

Khanzada, M.A., Lodhi, A.M and Shahzad, S. 2005. Chemical control of *L. theobromae*, the causal agent of mango decline in Sindh.*Pakistan Journal of Botany*. 37(4): 1023-1030.

Khanzada, M.A., Rajput, A.Q and Shahzad, S. 2006. Effect of medium, temperature, light and inorganic fertilizers on *in vitro* growth and sporulation of *L. theobromae* isolated from mango.*Pakistan Journal of Botany*. 38(3): 885-889.

Khewkhom, N., Sangchote, S and Sungsiri, T. 2013. Postharvest control of fruit rot of mangosteen by plant extracts from Zingiberaceae family. *ActaHorticulturae*. 973: 119-124.

Ko, W.H., Wang, I.T and Ann, P.J. 2004. *Lasiodiplodia theobromae* as a causal agent of kumquat dieback in Taiwan.*Plant Disease*. 88(12): 1383.

Kumah, P., Ampomah, E.O., Olympio, N.S and Moses, E. 2013.Efficacy of four botanicals and two chemical fungicides in the control of crown rot disease of banana (*Musa* spp. AAA) 'medium cavendish'. *Acta Horticulture*.1007: 385-392.

Kumar, P.S and Singh, S.P. 2000. First report of *Lasiodiplodia theobromae* as a foliar pathogen of *Partheniumhysterophorus.Plant Disease.* 84(12): 1343.

Kumari, G.B.G and GovindaiahSukumar, J. 2011. Incidence of stemcanker (*Lasiodiplodia theobromae*) in mulberry nurseries of Kolar district.*Indian Journal of Sericulture.* 50(1): 78-81.

Latha, P., Prakasham, V., Jonathan, E.J., Samiyappan, R and Natarajan, C. 2013. Effect of culture media & environmental factors on mycelial growth and pycnidial production of *L.theobromae* in Physic nut (*Jatropacurcas*).*Journal of environmental biology*.34 : 683-687.

Leghari, T.N. 2005.Epidemiology and yield losses by sudden death syndrome in mango orchards of Sindh and its possible control.*M. Sc. Thesis*. Sindh Agriculture University, Tandojam, Pakistan.

Lima, G. S.A., de Lima, N.M.F and Lopez, A.M.Q. 1996. Effect of aqueous extracts from garlic bulbs (*Allium sativum)* on germination and mycelial growth of *Botryodiplodia theobromae* Pat. *in vitro*. *Ciencia Agricola*. 4:7-15.

Linde , J.A.V. , Roux, J., Wingfield, M.J and Six, D.L. 2012. Die-off of giant Euphorbia trees in South Africa: Symptoms and relationships to climate. *South African Journal of Botany.* 83: 172–185.

Li, Q.L., Guo, T.X., Pan, Z.B., Huang, S.P., Mo, J.Y., Ning, P and Hsiang, T. 2013. An outbreak of gummosis of mango trees caused by *Lasiodiplodia theobromae* in Guangxi, south China.*Plant Disease*. 97(5): 690.

Lopez, A.F., Aguilera, J.M.A., Cardenas–Soriano and Teliz–Ortiz, E.D. 2009. Etiology and histopathology of dieback disease on mamey trees (*Pouteriasapota* (Jacq.) H. E. Moore and Stearn) in Guerrero, Mexico.*Agrociencia.* 43:717-728.

Luo, M., Dong, Z. Y., Bin, S. Y and Lin, J. T. 2011. First report of fruit rot disease on pomelo caused by *Lasiodiplodia theobromae* in China.*Plant Disease*.95(9): 1190.

Maduleti, M.C. 1989. Studies on die back and death of mango trees (*Mangifera indica* L.)*M.sc.Thesis.*Acharya N.G. Ranga Agricultural University, Hyderabad, India.

Maholay, M.N and Sihi, H.S. 1977. Studies on Botrydiplodia rot of *Dolichusbiflorus*. *Review of Plant Pathology*.57: 1927.

Mahmood, A and M.A. Gill. 2002. Quick decline of mango and *In vitro* response of fungicides against the disease. *International Journal of Agriculture &Biology*.4(1): 39-40.

*Mahmood, A., Khan, S.N and Ali, S. 2007. Screening of genetic source against sudden death / quick decline *Lasiodiplodia theobromae* of mango.November 19-21, *3rd International phytopathology conference*, University of the Punjab,Lahore.125-132.

Malik, A.H., Khan, S. M., Iqbal, Z., Malik, M.T., Saleem, A and Haq, I. 2005. Histological and control studies on *Botryodiplodia theobromae*, the cause of mango decline in the Punjab. *Pakistan Journal of Botany*. 17: 18-21.

Masood, A., Saeed, S., Silveira, S.F.D., Akem, C.N., Hussain, N and Farooq, M. 2011. Quick decline of mango in pakistan: survey and pathogenicity of fungi. *Pakistan Journal of Botany*. 43(3): 1793-1798.

Meah M.B., Plumbley, R.A., Jeger and M.J. (1991) Growth and infectivity of *Botryodiplodia theobromae* causing stem end rot of mango. *Mycological Research*. 95: 405-408.

Meer, H., Iram, S., Ahmad, I., Fateh, F.S and Kazmi, M.R. 2013.Identification and characterization of post-harvest fungal pathogens of mango from domestic markets of Punjab.*International Journal of Agronomy and Plant Production*. 4(4): 650-658.

MeiJiao, H., Chao, S., Yong, A., Min, L.,FengZhen, Y and Yin, G.Z. 2009. Resistance of*Botryodiplodia theobromae* to carbendazim and the fungicides screening using stem end rot of mango fruit as a control.*Journal of Fruit Science*.26 (5): 671-677.

Mirzaee, M.R., Azadvar, M and Ershad, D. 2002. The incidence of *Lasiodiplodia theobromae* the cause of fruit and stem-end rot of mango in Iran. *Iranian Journal of Plant Pathology*.38: 148-149.

Mullen, J.M., Gilliam, C.H., Hagan, A.K and Morgan-Jones, G. 1991. Canker of dogwood caused by *Lasiodiplodia theobromae*, a disease influenced by drought stress or cultivar selection. *Plant Disease.*75: 886-889.

*Muller, H.R.A 1940. Survey of the most important mango diseases in the Dutch East Indies.*Review of applied mycology*. 19: 355.

Narasimhudu, Y and Reddy, P.S.N. 1992.A note on gummosis of mango.*Indian Phytopathology*. 45(2): 261-262.

National Horticultural Board.10 February 2013-14.http://nhb.gov.in.html.

Nene, Y.L and Thapliyal, P.N. 1993.*Fungicides in plant disease control* (3rd edition). Oxford and IBH Publishing Company Private Limited. 691.

Nwachukwu, E. O and Umechuruba, C. I. 2001. Antifungal activities of some leaf extracts on seed-borne fungi of African yam bean seeds, seed germination and seedling emergence. *Journal of Applied Science & Environmental Management*. 5(1): 29-32.

Ojha, S., Khatun, S., Chakraborty, M. R and Chatterjee, N. C. 2010. Occurrence of die-back of *Dalbergiasissoo* in West Bengal and evaluation of fungicidal control of its pathogen.*International Journal of Plant Protection*. 3(1):17-19.

Okigbo, R. N., Okorie, R. E and Putheti, R. R. 2009. *In vitro* effects of garlic (*Allium sativum* L.) and African basil (*Ocimum gratissimum* L.) on pathogens isolated from rotted cassava roots. *Interciencia.*34(10): 742-747.

Omamor, I. B. 1988. Black rot of date fruit: a new post-harvest decay caused by *Botryodiplodia theobromae*. *Date Palm Journal*. 6(1):299-305.

Paim, E.C.A., Silveira, A.J., Bezerra, J.L., Newman luz, E.D.M and Sacramento, C.K. 2012. Etiology of the decline of mangosteen in the southern Bahia.*Brazilian Journal of Tropical Fruits*. 34(4): 1074-1083.

Pandit, P. K and Samajpati, N. 2005. Wilt disease of guava caused by *Botryodiplodia theobromae* Pat. *Journal of Mycopathological Research.*43(1): 41-43.

Panhwar, A., Nizamani, S.M., Rustamani, M.A., Khuhro, R.D and Jiskani, M.M. 2007. Occurrence and severity of decline disease complex on different mango varieties in Sindh, Pakistan. *PakistanJournal Agriculture Engineering Veterinary Science*. 23(1): 14-20.

Panse, V.G and Sukhatme, P.V. 1978. *Statistical Methods for Agricultural Workers*.Indian Council of Agricultural Research, New Delhi. 136.

Patel, K. D and Pathak, V. N. 1993.Incidence of guava fruit rots and losses due to Rhizopus and Botryodiplodia in Udaipur and Ahmedabad markets.*Indian Journal of Mycology and Plant Pathology*. 23(3): 273-277.

Pati, S. P., Maheswari, S. K and Ray, R. C. 2001. Effect of culture media, temperature, pH, carbon and nitrogen sources on growth of *Botryodiplodia theobromae* causing Java black rot of sweet potato tubers.*Journal of Mycopathological Research*. 39(1): 15-19.

Phipps, P. M and Porter, D. M. 1998. Collar rot of peanut caused by *Lasiodiplodia theobromae*. *Plant Disease*. 82: 1205-1209.

Ploetz, R.C., David, B and Schaffer, B. 1996.A re-examination of mango decline in Florida.*Plant Disease*. 80(6): 664-668.

Ploetz, R.C. 1999. Malformation: A unique and important disease of Mango, *Mangifera indica* L. In B.A. Summerell (ed.)*Paul E. Nelson Memorial Symposium*. APS Press, St. Paul, 1-8.

Prakash, O and Srivastava, K.C. 1987. *Mango diseases and their management*. A world review (book). Today and tomorrow Printer and Publishers, KaralBagh, New Delhi. 175.

Prakash, O and Singh, U.N. 1976. New disease of mango.In *Proceedings of Fruit Research workshop*.24-28 May, 1976, Nyderabad, India.300-302

Prakash, Om. 1996. Principal diseases of mango, causes and control. In S.J. Singh (ed.)*Advances in diseases of Fruit Crops in India*.Kalyani Publishers, Ludhiana, India.191-256.

Prasad, S. C. R., Harsh, N. S. K., Ahuja, R and Shakuntala.K. 2014. Bark canker and die-back of *Dalbergiasissoo* in Haryana and Punjab caused by *Lasiodiplodia theobromae. Indian Forester*.140(1):76-79.

Priya, K. S. Nagaveni, H. C. 2009. Screening of Trichodermaspp against *Lasiodiplodia theobromae* causing fruit rot of *Elaeocarpusmunronii*.*Indian Journal of Plant Protection*. 37(1/2):166-169

Punithalingam E. 1976. *Botryodiplodia theobromae*. CMI Descriptions of Pathogenic Fungi and Bacteria, No. 519. Commonwealth Mycological Institute, Kew, Surrey, England.

Ramos, L.J., S.P. Lara, R.T. McMillan and J.R. Narayanan. 1997. The resistance of mango (*Mangifera indica*) cultivars to tip dieback disease in Florida. *Plant Disease.* 81: 509-514.

Rashid, A., Iram, S and Ahmad, I. 2013. Study on incidence, molecular characterization and pathogenesis of different fungi associated with sudden death of mango. *International Journal of Agronomy and Plant Production*. 4: 3485-3488.

Rath, G. C and Mohanan, M. K. 1977.Blossom blight of mango in Orissa.*Science and culture.* 43: 234-236.

Rawal, R.D and Ullasa, B.A. 1988. Efficacy of pre-harvest field sprays of fungicides against stem end rot of mango. *Acta Horticulture*. 231: 537-539.

Ray, R.C.2004. Extracellular amylase (production by fungi) *Botryodiplodia theobromae* and *Rhizopusoryzae* grown on cassava starch residue.*Journal of Environmental Biolology*. 25: 489-495.

Reckhaus, P and Adamou, I. 1987. Hendersonula dieback of mango in Niger.*Plant Disease.*71: 1045.

Reddy, T. N., Chaturvedi, A and Babu, J. D. 2005.Screening of mango cultivars against important post-harvest diseases in Andhra Pradesh.*Journal of Research ANGRAU*. 33(3): 71-73.

Rehab, M.E.A.A., Rashed, M.F., Ammar, M.I and El-Morsy, S.A. 2014.Dieback and sooty canker of Ficus trees in Egypt and its control.*Pakistan Journal of Biological Sciences.* 17(3): 364-371.

Rehman,A., Abbas, T., Khan, N.K and Saira, M. 2011.Investigations on mango sudden death syndrome affected plant parts in districtMuzaffargarh. *PakistanJournal of Botany*. 23(2): 125-130.

Rodriguez, G.E and Mathos, L. 1988. Dieback of mango (*Mangifera indica* L.) and behaviour of five varieties with respect to the causal agent.*Fitopathologia*.23: 41-48.

Sabalpara, A.N., Vala, D.G and Solanky, K.U. 1991. Morphological variation in *Botryodiplodia theobromae* pat. Causing twig-blight and die-back of mango.*Acta Horticulture.*291 : 312-316.

Saeed, S., Ijaz Khan, M and Masood, A. 2011.Symptom development after artificial inoculation of *Botryodiplodia theobromae*, a possible causal organism quick decline in mango trees.*Pakistan Journal of Agriculture Science*. 48 (4): 289-294.

Saha, A., Mandal, P., Dasgupta, S and Saha, D. 2008. Influence of culture media and environmental factors on mycelial growth and sporulation of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl.*Journal of Environmental Biology*. 29(3): 407-410.

Sahi, S. T., Habib, A., Ghazanfar, M. U and Badar, A. 2012.*In vitro* evaluation of different fungicides and plant extracts against *Botryodiplodia theobromae*, the causal agent of quick decline of mango. *Pakistan Journal of Phytopathology*.24(2): 137-142.

*Saleem, A and K.M. Akhtar. 1989. Mango diseases and their control. In A. G. Choudhry and M. A. Syed(eds.) *Proceedings of theInternational Mango Workshop*. February 27 to March 01, 1989. Agriculture Extension, Multan.143-154.

Sales J.R., Nunes, G.H.S., Lima, L.L., Guimaraes, I.M., Morais, P.L.D. 2009. Chemical control stem rot Caused by *Lasiodiplodia theobromae* on mangoes fruits. *Brazilian Journal of Tropical Fruits*.31(3): 907-910.

Sanchez, M.S., Angel, D.N., Sandoval-Islas, J.S., Téliz-Ortiz, D., Orozco-Santos, M and Silva-Rojas, V. H. 2013. Fungi Associated to stem-end rot and dieback of Mango (*Mangifera indica* L.) Collar Rot of Peanut Caused by *Lasiodiplodia theobromae.Agrociencia Mexico*.47: 1.

Sangeetha, G., Thangavelu, R., Rani, S. U and Muthukumar, A. 2013. Antimicrobial activity of medicinal plants and induction of defense related compounds in banana fruits cv. Robusta against crown rot pathogens. *Biological Control*. 64(1): 16-25.

Sangeetha, G.,Usharani, S and Muthukumar, M. 2009. Biocontrol with Trichoderma species for the management of postharvest crown rot of banana.*PhytopathologiaMediterranea*. 48: 214–225.

Sardsud, U., Sardsud, V., Sittigul, C and Chaiwangsri, T. 1994. Effects of plant extracts on the *in vitro* and *in vivo* development of fruit pathogens. *In Development of postharvest handling technology for tropical tree fruits*: a workshop held in Bangkok, Thailand. 16-18 July 1992-1994. 60-62.

Sato, T., Iwamoto, Y., Tomioka, K., Taba, S., Ooshiro, A and Takaesu, K. 2008. Black band of Jew's marrow caused by *Lasiodiplodia theobromae*.*Journal of General Plant Pathology*. 74(1): 91-93.

Shahbaz, M., Iqbal, Z and Saleem and Anjum, M.A. 2009.Association of *Lasiodiplodia theobromae* with different decline disorders in mango (*Mangifera indica* L.).*Pakistan Journal of Botany*. 41(1): 359-368.

Shahbaz, M., Khan, S.M., Iqbal, Z., Rehman, A., Muhammad, F and Saleem, A. 2005. Etiological studies to explore the causal agent of mango decline in the Punjab, Pakistan. *Pakistan Journal of Phytopathology*.17: 33-35.

Shah, M.D., Verma, K.S., Singh, K and Kaur, R. 2010.Morphological, pathological and molecular variability in *Botryodiplodia theobromae* (Botryosphaeriaceae) isolates associated with die-back and bark cankerof pear trees in Punjab, India. *Genetics and Molecular Research*. 9 (2): 1217-1228.

Sharma, I. 1993. A note on population dynamics and etiology of die back of mango in Himachal Pradesh.*New Agriculturist*. 2(2): 229-230.

Sharma, I. M and Badiyala, S.D. 1994.Effect of pre-harvest fungicidal sprays against stem end rot of mango fruits in storage caused by *Botryodiplodia theobromae* Pat.in Himachal Pradesh. *Indian Journal of Mycology and Plant Pathology*. 24(2): 141-142.

Sharma, R. N., Maharshi, R. P and Gaur, R. B. 2011.Prevalance of newly recorded pre-harvest stem-end rot of kinnow (*Citrus reticulata*) fruits from Rajasthan and its management.*Indian Phytopathology*. 64(3): 296-298.

Simas-Tosin, F. F., Barrzaz, R. R., Petkowicz, C. L. O., Silveira, J.L. M., Sassaki, G. L and Santos, E.M.R. 2010. Rheological and structural characteristics of peach tree gum exudate. *Food Hydrocolloids*. 24: 486–493.

Simone, G.W. 1999. Diseases control in Mango *(Mangifera indica).*Plant Pathology Department Document *PDMG-V3*-22 document, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

*Singh, L.B. 1960. The mango: Botany, cultivation and utilization. Leonard Hill Ltd, London.

*Snedecor, G.W and Cochron, W.G. 1967. *Statistical Methods*. Oxford and IBH publishing

Co. Pvt. Ltd New Delhi. 593.

*Spencer, J. L and Kennard, W.C. 1955. Out breaks and new records. Puerto Rico.F.A.O. *Plant Protection Bulletin*. 4:43.

Suhannaa, A.Y.,Aifaa, N.H and Shazalwardi, S. 2013. Trichoderma sp. as a Biological control agent in the Postharvest treatment of mango stem end rot.*Journal of Tropical Agriculture and Food Science*. 41(1): 159– 168.

Sultana, N and Ghaffar, A. 2010. Effect of fungicides and microbial antagonists in the control of *Lasiodiplodia theobromae*, the cause of seed rot, seedling and root infection of bottle gourd. *Pakistan Journal of Agricultural Research*. 23(1/2): 46-52.

Thangavelu, R. Sangeetha, G andMustaffa, M. M.2007.Cross-infection potential of crown rot pathogen (*Lasiodiplodia theobromae*) isolates and their management using potential native bioagents in banana.*Australasian Plant Pathology*. 36(6):595-605.

Tovar Pedraza, J. M., Mora Aguilera, J. A., Nava Diaz, C., Teliz Ortiz, D.,Valdovinos, P.G.A., Villegas M.G and Hernandez, M.J. 2012. Identification, pathogenicity, and

histopathology of *Lasiodiplodia theobromae* on mameysapote grafts in Guerrero, Mexico.*Agrociencia*. 46: 146-161.

Twumasi, P., Ohene-Mensah, G and Moses. 2014. The rot fungus *Botryodiplodia theobromae* strains cross infect cocoa, mango, banana and yam with significant tissue damage and economic losses.*African Journal of Agricultural Research.*6(9): 613-619.

Uma, M and Thirupathaiah, V. 2009. Reaction of three genotypes of mulberry (*Morusalba* L.) to *Botrydiplodia theobromae*. *Agricultural Science Digest*. 29(3): 228-229.

Venugopal, S. 2013. Efficacy of extracts of certain pteridophytes on growth, sporulation and spore germination of *Lasiodiplodia theobromae* the incitant of fruit rot in coconut. *LifesciencesLeaflets*.1(1): 91-95.

Verma, O.P and Singh, R.D. 1970. Epidemology of mango dieback caused by *Botrydiplodia theobromae* pat.*Indian journal of Agricultural sciences.* 40: 813-818.

Viana, F. M. P., Santos, A. A., dos Sobrinho, C. A., Freire F. C. O and Cardoso, J. E. 2000. Black rot: a new disease of passion fruit caused by *Lasiodiplodia theobromae* in the northeastern region. *FitopatologiaBrasileira*. 25(4): 671.

Vincent, J. M. 1927: Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*.59: 850.

Wang, H. L., Chen, P. H., Ni, H. F and Chen, R. S. 2007. Physiological characterization and screen of control chemicals for *Lasiodiplodia theobromae* of papaya. *Plant Pathology Bulletin*. 16(2): 71-77.

Wheeler, B.E.S. 1969. *An introduction to plant disease*. John Wiley and Sons Ltd., London, U.K., p. 301.

Woodward, J. E., Langston, D. B., Jr. Brock, J. H., Kemerait, R. C., Jr. Brenneman, T. B and Beard, G. H. 2005. First demonstration of Koch's postulates for *Lasiodiplodia theobromae* fruit spot on eggplant (*Solanummelongena*). *Plant Disease*. 89(6): 687.

*** Originals not seen.**