CATALOGUING, DOCUMENTATION AND MANAGEMENT OF FUNGAL DISEASES OF STRAWBERRY (*Fragaria x ananassa* Duch.)

by
P. AMRUTHA
(2015-11-009)

THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Agriculture
(PLANT PATHOLOGY)

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR – 680 656
KERALA, INDIA
2017

DECLARATION

I, hereby declare that the thesis entitled **"CATALOGUING, DOCUMENTATION AND MANAGEMENT OF FUNGAL DISEASES OF STRAWBERRY (*Fragaria* x *ananassa* Duch.)"** is a bonafide record of research work done by me during the course of research and that this thesis has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara,
Date: 28/7/17

P. AMRUTHA
(2015-11-009)

CERTIFICATE

Certified that this thesis entitled **"CATALOGUING, DOCUMENTATION AND MANAGEMENT OF FUNGAL DISEASES OF STRAWBERRY (*Fragaria* x *ananassa* Duch.)"** is a bonafide record of research work done independently by Ms. P. Amrutha under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

Vellanikkara,
Date: 27.7.17

Dr. Reshmy Vijayaraghavan
(Major Advisor, Advisory Committee)
Assistant Professor (Plant Pathology)
College of Horticulture
Vellanikkara

CERTIFICATE

We, the undersigned members of the Advisory Committee of Ms. P. Amrutha, a candidate for the degree of **Master of Science in Agriculture** with major field in Plant Pathology, agree that the thesis entitled **"CATALOGUING, DOCUMENTATION AND MANAGEMENT OF FUNGAL DISEASES OF STRAWBERRY (*Fragaria* x *ananassa* Duch.)"** may be submitted by Ms. P. Amrutha in partial fulfillment of the requirement for the degree.

27/7/17

Dr. Reshmy Vijayaraghavan
(Chairperson, Advisory Committee)
Assistant Professor
Department of Plant Pathology
College of Horticulture, Vellanikkara

27/07/2017

Dr. Anita Cherian K.
(Member, Advisory Committee)
Professor & Head
Department of Plant Pathology
College of Horticulture, Vellanikkara

27/7/17

Dr. S. Beena
(Member, Advisory Committee)
Professor
Department of Plant Pathology
College of Horticulture. Vellanikkara

27/07/2017

Dr. Mini Shankar
(Member, Advisory Committee)
Assistant Professor
Department of Floriculture & Landscaping
College of Horticulture, Vellanikkara

EXTERNAL EXAMINER
(G. KARTHIKEYAN)
Professor (Pl. Pathology)
Centre for Plant Protection Studies
TNAU, Coimbatore

ACKNOWLEDGEMENT

First and foremost I bow my head before the ***Almighty God*** *for enlightening and making me confident and optimistic throughout my life and enabled me to successfully complete the thesis work in time.*

It is with immense pleasure I avail this opportunity to express my deep sense of whole hearted gratitude and indebtedness to my major advisor ***Dr. Reshmy Vijayaraghavan*** *for her expert advice, inspiring guidance, valuable suggestions, constructive criticisms, constant encouragement, affectionate advice and above all, the extreme patience, understanding and wholehearted co-operation rendered throughout the course of my study. I really consider it my greatest fortune in having her guidance for my research work.*

I consider it as my privilege to express my deep-felt gratitude to ***Dr. Koshy Abraham, Dr. Sheela T. Paul, Dr. Sally. K. Mathew and Dr. Rehumath Niza, T. J*** *retired professors of Plant Pathology, CoH, Vellanikkara for their constant support, valuable suggestions and critical scrutiny of the manuscript.*

I express my gratitude to ***Dr. Anita Cherian K.*** *Professor and Head, Department of Plant pathology for her immense help and assistance provided for constituting the manuscript.*

I sincerely thank ***Dr. S. Beena*** *Professor for her expert advice, constant inspiration, precious suggestions, generous support and constructive criticisms during my entire study which helped in successful completion of this work.*

I express my sincere thanks to ***Dr. Mini Sankar,*** *Assistant Professor of Floriculture and Landscaping for her constructive criticism and critical scrutiny of manuscript.*

I am deeply obliged to ***Dr. Vimi Louis, Dr. P. Sainamole Kurian*** *and* ***Dr. Deepa James*** *for their invaluable help, guidance and critical assessment throughout the period of work. I thank them for all the help and cooperation they have extended to me.*

I express my gratitude to ***Dr. S. Krishnan,*** *Associate Professor and Head, Dept. of Agricultural Statistics, College of Horticulture, for his valuable assistance, immense help and guidance during the statistical analysis of the data.*

I duly acknowledge the encouragement, moral support, precious suggestions and timely persuasions by my dear seniors, ***Dr. Remya, Dr. Hima, Mr. Ahamed Mujtaba, Ms. Milsha, Mrs. Sumbula, Mrs. Sanju, Mrs. Aarathi and Ms. Darsana*** *not only in my research work but also throughout my PG programme. I express my sincere thanks to my classmates* ***Ms. Femi Jose, Ms. Nusrath Beegum Ms. Deepa, Mr. Kiran and Mr. Debashish*** *for their affection and kind help offered during my thesis work.*

I have infinite pleasure to express whole hearted thanks to my loving juniors ***Mrs. Rahila, Ms. Laya, Ms. Atheena, Ms. Aswathy, Ms. Stella and Ms. Divya*** *for their love, innumerable help and support especially. My endless obligation to* ***Ms. Dilna, Ms. Anjaly, Ms. Ranjini*** *and* ***Ms. Fridin*** *who soleheartedly stood as helping hand all through the research work. Besides, I express my compassion to* ***Mrs. Prassanna, Mrs. Mamtha, Mrs. Sumathi, Mrs. Jismy, Mr. Rahul and Mrs. Santha*** *for their untold help and caution towards me during the research work.*

I thank my dear friends ***Alka Sherief, Anusree Padmanabhan and Anjana Devaraj*** *for the unconditional support, help, timely valuable suggestions and encouragement which gave me enough mental strength and perseverance to get*

through all odds and tedious circumstances and immense thanks to all M.Sc. classmates for their moral support and encouragement.

I am in dearth of words to express my love towards ***my beloved*** *parents and my lovely brother for their boundless affection, moral support, eternal love, deep concern, prayers and personal sacrifices which sustains peace in my life.*

I owe special thanks to Librarian, College of Horticulture, ***Dr. A.T. Francis*** *and all other staff members of Library, who guided me in several ways, which immensely helped for collection of literature for writing my thesis. I am thankful for the service rendered by* ***Mr. Aravind*** *all along the academic period.*

I express my deep sense of gratitude to Kerala Agricultural University for financial and technical support for persuasion of my study and research work. It would be impossible to list out all those who have helped me in one way or another in the successful completion of this work. I once again express my heartful thanks to all those who helped me in completing this venture.

P. Amrutha

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction

1. INTRODUCTION

Strawberry (*Fragaria* x *ananassa* Duch.), an extensively grown hybrid species of genus *Fragaria* is one of the world's most delicious, attractive and refreshing fruits (FAO, 2000). It is a perennial stoloniferous herb belonging to the family Rosaceae. Cherished for its characteristic flavor, colour and tentalizing aroma, strawberry is an important table fruit of millions of people around the world. The crop is grown extensively under temperate, sub-tropical and tropical climate throughout the year suiting to a wide range of agro-climatic conditions. Compared to other fruit crops, strawberry provides quick and higher returns within six months of planting (Sharma and Sharma, 2004). A study carried out by Wolfe *et al.* (2008) suggested that this fruit possesses the largest source of cellular antioxidant activity among 25 different fruits and vegetables consumed by the American population.

United States of America is considered to be the largest producer of strawberries in the world followed by China and Spain. Total world production was estimated as 3.9 million tons, in which almost 7.22 lakh tons is contributed by Asia alone (FAO, 2007). US recorded a total strawberry production of 27.7 million tons in 2012 with a maximum export to Canada earning about 250 million pounds (Wu *et al.*, 2012). According to Tabatabaie and Murthy (2016), California and Florida together shared 99 per cent of strawberry production in USA. Other major strawberry producing countries are Turkey, Spain, Egypt, Korea, Mexico and Poland. In India, the crop is commercially grown in Jammu & Kashmir, Himachal Pradesh, Uttar Pradesh, Maharashtra, West Bengal, Delhi, Punjab, Haryana, Uttarakhand and Rajasthan where it occupies an area of 0.83 thousand hectares with an annual production of 8.42 MT. Panchgani-Mahabaleshwar in Maharashtra is the leading producer of strawberry in India which contributes 85 per cent of the production followed by Jammu and Kashmir. According to APEDA, the export destinations of Indian strawberries are Jordan, Austria, US, Germany and Bangladesh. According to Varma (2014), Kerala has made its debute in the country's strawberry export basket where the farmers in Munnar, Idukki district cultivate the crop in 300 ha

with a production of 4-5 tonnes per hectare and the major domestic market for the fruit are Delhi, Pune, Bangalore, Hyderabad and Kochi.

Though, strawberry represents a very profitable crop for the fresh market and also for the food industry, the occurrence of diseases has played a significant role in reducing the production and productivity. The crop is affected by various fungal, viral and bacterial diseases. Kapytowski and Bojarska (2005) reported that strawberry plantation faces severe yield loss of 15 to 92 per cent when infected by fungal diseases. Globally, Garrido *et al.* (2011) noticed 50 genera of fungi infecting the crop under varied climatic conditions. Even though, many fungal diseases of strawberry has been studied and reported nationally and internationally, no relevant studies pertaining to the crop and its effective disease management have been carried out in Kerala.

Reviewing the vital role possessed by strawberries in fruit industry and its economic importance, an investigation was carried out to record the fungal diseases affecting strawberry in Kerala, considering the due damage caused by these diseases in different seasons followed by cataloguing, documenting and managing the same. Thus, the study was formulated with the following objectives:

- Survey and collection of diseased samples of strawberry.
- Isolation of pathogens and pathogenicity.
- Study of symptomatology of various diseases under natural and artificial conditions.
- Characterisation of pathogens and its identification.
- *In vitro* evaluation of fungicides, organic formulation and biocontrol agents.
- *In vivo* management of major diseases of strawberry.

Review of literature

2. REVIEW OF LITERATURE

Strawberry (*Fragaria* x *ananassa* Duch.), a popular temperate fruit crop, cultivated worldwide, is valued for its sweetness, colour and delicate flavor. Anglo-saxons guessed that the fruit got its name from the way in which the berries were strung on straws or hay cushion during sale. It was Apulius who pictured strawberry in his paintings indicating the medicinal properties in ancient Roman literature. First reference of strawberry cultivation dates back to 1368, when Jean Dudoy, gardener of King Charles V, planted 1200 strawberries in the royal gardens of Louvre in Paris (Sharma and Sharma, 2004). Cultivation of strawberries got popularized during 16th century by the introduction of *Fragaria virginiana* to Europe from eastern North America. But the present day cultivated strawberries are the hybrids of *Fragaria* x *ananassa*. Europe and North America are considered to be the large scale commercial producers of strawberry whereas, USA accounts for the quarter of world strawberry production (FAO, 2015). In India, strawberry performs equally well in climate that are characteristically temperate in the northern latitudes, sub-tropical in the plains, or tropical at high altitude (Pramanick *et al.*, 2013). To date, several important pests and diseases have gained importance over the past few years that pose serious threat to strawberry cultivation in India. With the advent of many tropical varieties, cultivation of strawberry flourished in Kerala. However, the crop is found to be susceptible to several destructive and economically important fungal pathogens which can thrive well under the humid tropical conditions of Kerala.

2.1 FUNGAL DISEASES AND ASSOCIATED PATHOGENS

A glance through the literature revealed that studies on foliar, crown, root and fruit fungal diseases have been reported globally from strawberry growing tracts of USA, Iran, New Zealand, Morocco, China, Israel, Egypt, Canada, Australia and India. Brooks (1920) first described a disease infecting strawberry runners from Florida grown during July and he called it as anthracnose incited by *Colletotrichum* sp. Likewise, Stone

(1922) reported a leaf scorch disease in strawberry prevalent in Ontorio and the pathogenic agent responsible was confirmed as *Diplocarpon earlianum.*

Schuh and Zeller (1944) enlisted seven devastating diseases of strawberry from Oregon district of United States. These were leaf spot by *Mycosphaerella fragariae*, leaf scorch by *Diplocarpon earliana*, leaf blight by *Dendrophoma obscurans,* powdery mildew by *Sphaerotheca humuli*, *Armillaria* crown rot by *Armillaria mellea* red stele by *Phytophthora fragariae* and *Botrytis* grey mold by *Botrytis cinerea.* Miller (1947) reported that apart from *Phytophthora fragariae,* several pathogens like *Ramularia, Rhizoctonia, Fusarium, Chaetomium, Penicillium, Phytophthora, Pythium, Verticillium, Oothe-cium*, and *Pestalotia* spp. also cause root and crown rot of strawberry.

Wilhelm (1961) while working on strawberry diseases in Berkeley revealed the incidence of crown, root, leaf and fruit diseases. According to him, most serious diseases noticed in strawberry were *Verticillium alboatrum* wilt, red stele by *Phytophthora fragariae* and *Botrytis cinerea* rot, whereas the black root rot causing fungal pathogens identified were *Idriella Iunata* as well as *Pythium ultimum, Rhizophagus* and *Rhizoctonia* (*Corticium*) *solani.* Bud and crown rot by *Armillaria mellea* and *Rosellinia necatrix* were also noticed.

Bose (1970) described seven major leaf spot diseases of strawberry under Indian climatic conditions *viz.*, *Pestalotia jeolikotensis* (sp. nov.), *Pilidium concavum*, *Hainesia lythri*, *Dendrophoma obscurans*, *Marsonina fragariae* and *Mycosphaerella fragariae*. Wassenaar and Scheer (1989) opined that *Alternaria* leaf spot caused by *Alternaria alternata* (Fr.) Keissler f. sp. *fragariae* Dingley was the important disease affecting Sivetta cultivar of strawberry in Netherlands.

A recent epidemic of anthracnose in Central Brazil resulted in huge losses ranging from 30 to 68 per cent (Henz *et al.,* 1992). Takeuchi and Horie (1997) isolated a leaf spot pathogen from Japan in 1996 and based on morphological characteristics it was identified as *Phoma exigua*. Bhardwaj and Gupta (2002) was the first to report the

incidence of red stele caused by *Phytophthora fragariae* in strawberry growing tracts of Solan, Himachal Pradesh.

2.1.1 Foliage diseases

Several studies have been carried out on various diseases affecting foliage of strawberry plants. Wormald and Montgomery (1941) reported the incidence the leaf blotch infecting strawberries in Great Britain and the pathogen was identified as *Phyllosticta grandimaculans* Bubak & Krieger. Kerling *et al.* (1964) isolated several fungal species from phyllosphere like *Cladosporium herbarum*, *Botrytis cinerea* and *Gnomonia fructicola.*

A severe pathogen confined to the leaves and stalks was reported by Lele and Phatak (1965) from India caused by *Rhizoctonia bataticola* (*Macrophomina phaseolina).* Peries (1962) and Jhooty and Mckeen (1965) observed the presence of the perithecia of *Sphaerotheca macularis* on infected leaves of strawberry causing powdery mildew. Several authors also reported and studied the incidence of powdery mildew disease of strawberry (Yarwood, 1957; Paulus, 1990; Kanto *et al.*, 2004 and Willocquet *et al.*, 2008).

Tanaka *et al.* (1996) detected typical black to greyish circular leaf spots on leaves of strawberry and the pathogen responsible was confirmed *as Colletotrichum fragariae* (*Glomerella cingulata*). Rao *et al.* (1998) recorded two new pathogenic fungi, *Mycosphaerella fragariae* and *C. fragariae* (*Glomerella cingulata*) causing leaf spot diseases of strawberry from Pune. Likewise, Bhardwaj *et al.* (2003) reported a leaf blight caused by *Rhizoctonia solani* which is considered as the most damaging disease causing heavy crop loss in Himachal Pradesh.

A new leaf rust disease was recorded and described by Agarwal (2001) for the first time from Uttar Pradesh, which is caused by *Phragmidium fragariae-vestitae* sp. nov. on *Fragaria vestita*. Milicevic and Cvjetkovic (2008) published the details of

Phomopsis obscurans, *Gnomonia comari* and *Diplocarpon earliana* (*Diplocarpon earlianum*) causing leaf scorch and red leaf blotch in strawberry

Major fungal pathogens noticed frequently in tropics of strawberry cultivated regions by Murthy and Pramanick (2012) were *Botrytis cinerea*, *Colletotrichum acutatum*, *Phytophthora cactorum*, *Phytophthora fragariae* var. *fragariae*, *Verticillium dahliae* and *Sphaerotheca macularis*. A recent study by Zhao *et al.* (2016) for the first time in China recorded the incidence of *Pestalotiopsis clavispora* causing leaf spot of strawberry.

2.1.2 Fruit diseases

Dodge and Stevens (1924) reported rotting of fruits adhered to black sandy soil in Florida incited by *Rhizoctonia solani*. According to Nicholas (1960), strawberry leaf spot fungus *Mycosphaerella fragariae* occasionally infected the achene of fruits making it unattractive and unmarketable and this black seed disease potentially infected fruits wherever leaf spot was found.

Powelson (1960) showed the presence of *Botrytis* mycelium on senescent petals, stamens and calyces of marketable fruit. Howard and Albertgs (1973) isolated *Pestalotia* sp. causing fruit rot in Florida. Several workers reported the severe incidence of fruit rot caused by *Colletotrichum* spp. in strawberry growing areas (Howard, 1970; Sterne and Fulton, 1983; Lamondia, 1991; Wilson *et al.*, 1992, Turechek *et al.*, 2002). Antoniacci *et al.* (2008) observed *Gnomonia comari* P. Karst infecting fruits, stem and calyx characterized by brown discoloration.

Geotrichum candidum causing waxy fruit rot was recorded from Pune by Rao *et al.* (1998) during the month of December. Sharma and Bhardwaj (2001) investigated strawberry fruit rots in Himachal Pradesh, India and reported the incidence of leather rot (*Phytophthora cactorum*) showing greatest incidence of 17.38 per cent followed by grey mold (*Botrytis cinerea*), hard rot (*Rhizoctonia solani*) and *Phytophthora* rot (*P. nicotianae*).

Naturally rotten fruits were collected and isolated from different fields in Egypt by Timudo-Torrevilla *et al.* (2005) and the prominent fungi noticed were *Alternaria alternata*, *Botrytis cinerea*, *Fusarium solani*, *Pythium ultimum*, *Rhizoctonia fragariae*, *Rhizoctonia solani*, *Phytophthora cactorum*, *Rhizopus nigricans*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, *Aspergillus niger*, *Mucor* sp., *Penicillium* sp. and *Trichoderma* sp. Ayoubi *et al.* (2016) recently noticed Tan brown rot on fruits of strawberry caused by *Pilidium concavum* apart from its infection on leaves.

2.1.3 Crown and Root diseases

Attempts have been made by several workers to study the fungal diseases by soil borne pathogens. Kronenberg *et al.* (1949) conducted a survey on the occurrence of black root rot, a complex infection by *Coniothyrium fuckelii, Cylindrocarpon radicicola, Hainesia (Pezizella) lythri, Pachybasium candi-dum, Ramularia* sp., *Rhizoctonia* sp., and *Verticillium* sp. Wicks and Lee (1982) isolated and confirmed the presence of *Phytophthora fragariae* from the collapsed and wilted roots of strawberry plants in South Australia. According to Ellis *et al.* (1998) *Phytophthora cactorum* is a sporadic disease causing damage upto an extent of 50 per cent.

Verma and Gupta (2010) for the first time noticed heavy mortality of strawberries grown during the month of November-May due to root rot caused by *Curvularia lunata* (*Cochliobolus lunatus*) in fields of Jammu and Kashmir. Golzar *et al.* (2007) opined that a high rate of root and crown decay of strawberries was seen in the Swan Coastal Plain north of Perth and he noticed that *Fusarium oxysporum* f. sp. *fragariae* was reliably separated from crowns and *Phytophthora cactorum* was isolated from roots, crowns and soil and hence considered as a noteworthy pathogen. Apart from that, *Pythium* spp., *Phoma* spp., *Rhizoctonia* spp., *Colletotrichum* spp. and *Macrophomina* spp. were also related with the root and crown decay of strawberries.

A serious outbreak of wilting and root necrosis was noticed in North Carolina, recording a disease severity of 20-30 per cent by Adhikari *et al.* (2013) and the causal agent was recognized as *Cylindrocarpon* sp. Yildiz *et al.* (2014) made an extensive

research on the wilted and blackened necrotic roots of strawberry in Turkey and the causal agent was recognized as *Lasiodiplodia theobromae*. This was thought to be the principle report of *Lasiodiplodia theobromae* causing dieback on strawberry plants. Hajlaoui *et al.* (2015) reported a devastating and emerging pathogen causing charcoal rot of plants and the pathogen responsible was observed as *Macrophomina phaseolina*.

2.2 SEASONAL INFLUENCE OF OCCURRENCE OF DISEASES

Climate change is considered as a serious threat to plants as well as to mankind. According to Gautam *et al.* (2013) a global rise in temperature of 0.74°C has a serious impact on agriculture over last 100 years. Chakraborty *et al.* (1998), Chakraborty and Datta (2003) and Mina and Sinha. (2008) suggested that a change in temperature can lead to a shift in geographical distribution of plant pathogens, can alter the growth stage of pathogens, its development rate and their pathogenicity. Moreover, changing rainfall pattern, relative humidity and increase in the amount of atmospheric gases such as carbon dioxide has also lead to a serious impact of plant ecosystem.

Smith (1952) opined that red core by *Phytophthora fragariae* is the most severe disease in poorly drained areas on heavy soils during winter and black root rot by *Fusarium culmorum, Pachybasium hamatum* var. *candidum* and *Pythium* spp., is most severe in summer on heavy, wet soils in New Zealand in strawberry. According to Nemec (1972), most lesions induced by *Mycosphaerella fragariae* on strawberry leaves occurred when plants were grown during warm days and nights or warm days and cool nights and the optimum fungal growth occurred at 65-75 °F.

Wada *et al.* (1996) reported that leaf spot of strawberry caused by *Alternaria alternata* turned epidemic in late summer, when high relative humidity, heavy rainfall and daily mean temperature of 20-25°C existed. Bhardwaj *et al.* (2003) investigated the epidemiological parameters associated with the development of *Rhizoctonia* leaf blight in strawberry, where he noticed that high rainfall coupled with high soil moisture, high relative humidity and soil temperature ranging between 23 to 25°C prevailing during

July-August were found conducive for the development and spread of web blight disease.

Effect of different environmental factors on disease development of powdery mildew was studied by Amsalem *et al.* (2006) in strawberry. According to him, optimum temperature between 15 and 25°C with relative humidity (RH) higher than 75 per cent, but less than 98 per cent were found to be congenial for conidial germination, whereas high light intensity reduced germination and hyphal growth. Sombardier *et al.* (2009) observed that the spores of *Podosphaera aphanis*, causing powdery mildew of strawberry germinated more on the lower (abaxial) leaf surface than on the upper (adaxial) surface and the optimum temperature for germination was noticed as 22°C, whereas, no infection occurred at 32°C. Gupta and Bhardwaj (2009) noticed that low temperature (15°C), high soil moisture (>75%) and soils with slightly acidic pH (5.5) with sandy loam texture were found highly conducive for the development and spread of red stele disease (*Phytophthora fragariae*) causing high per cent mortality of the runners.

Recent studies conducted by Fang *et al.* (2011) revealed that *Fusarium oxysporum* and *Rhizoctonia* were most virulent at a temperature of 27°C and *Mycosphaerella phaseolina* at 32°C which demonstrates the dominance of these pathogens in the warmer months of the year. Sharma *et al.* (2013) assessed the factors responsible for grey mold rot (*Botrytis cinerea* Pers. ex Fr.) in strawberry by correlation studies and reported that disease incidence was positively correlated with RH and rainfall, and negatively with mean temperature during the crop seasons.

2.3 PATHOGENICITY OF ISOLATES

Robert Koch and Friedrich Loeffler in 1884 developed a technique to prove whether a particular pathogen is responsible for disease development, which is collectively referred to as Koch's postulates. It is considered as a preliminary confirmation method of plant pathogens. Different techniques have been reported by

various researchers for testing the pathogenicity of isolates infecting foliar, fruit and root in various crop plants.

For testing the pathogenicity of *Phytophthora capsici* on black pepper, Tompkins and Tucker (1941) mass multiplied pure fungal cultures on moistened cracked wheat in autoclaved soil in a pot. Likewise, Montgomere and Kennedy (1980) confirmed the infection of *Phytophthora* sp on raspberries by applying compost mixed with 100ml of spore suspension and six agar discs of sporulating mycelium. Eastburn and Gubler (1990) proved the ability of soilborne propagules of *Colletotrichum acutatum* to infect strawberry plants by mixing naturally infected field soil with sterile water with a population density of 10 cfu g^{-1}. Asad-Uz-Zaman *et al.* (2015) also proved the pathogenicity of *Rhizoctonia solani* Kuhn causing black root rot in strawberry by similar method.

Pathogenicity of *Alternaria* leaf spot was proved by Wada *et al.* (1996) by spraying conidial suspension (10^6 conidia/ml) using atomizer and then incubating the inoculated leaves in polyurethane foam mats in moist chamber. Takahashi *et al.* (1997) confirmed the pathogenicity of *Alternaria alternata* causing black leaf spot of strawberry by inoculating one drop (0.05ml) of spore suspension (5×10^5 spores/ml) on the undersurface of the leaf blade. According to Embaby, (2007), pathogenicity of *Pestalotia longisetula* causing fruit rot of strawberries was tested by surface sterilizing the fruit and inoculating conidial suspension of 10^4 cfu ml^{-1} using a sterile syringe. Fernandez-Ortuno *et al.* (2012) pointed out same test for proving pathogenicity of *Botrytis cinerea* on strawberry fruits.

Kanto *et al.* (2007) carried out the pathogenicity test of *Sphaerotheca aphanis* var. *aphanis* causing powdery mildew by excising the youngest leaf of strawberry and applying conidia to abaxial leaf surface with a paint brush. Rodrigues *et al.* (2014) inoculated leaves of strawberry seedlings with conidial suspension of *Pestalotiopsis longisetula* of concentration 2×10^5 conidia ml^{-1}. Similarly, Mouden *et al.* (2014) recorded a technique to test the pathogenicity of *Pestalotiopsis longisetula* Guba on

strawberry leaves by placing a 5mm mycelial disc on the middle of the leaves and secondly by inoculating conidial suspension of concentration 10^5 conidia ml^{-1}.

Dinler *et al.* (2016) and Henry *et al.* (2017) noticed wilting and decline symptoms and vascular discoloration of crown in strawberry while testing the pathogenicity of *Fusarium oxysporum* by dipping the plants in aqueous spore suspension (5 x 10^6 ml spores/ml). Juber *et al.* (2014) tested the pathogenicity of *Fusarium* isolates by mixing the fungal culture with millet seeds and planting them along with healthy strawberry plants. Likewise, Patra and Biswas (2017) detailed the pathogenicity test of *Fusarium oxysporum* of chickpea by planting the crop in pots mass multiplied with the isolates on sand maize meal.

2.4 SYMPTOMATOLOGY

Many studies on symptomatology of various fungal diseases affecting strawberry plants were carried out by several researchers.

2.4.1 Leaf spot

Dingley (1970) observed *Alternaria alternata* (Fr.) Keissler f. sp. *fragariae* f. sp. infecting leaves of strawberry causing brown leaves with dark reddish purple margins of 2-5 mm diameter with greyish brown on under surface of the leaves. However, in the case of *Diplocarpon* sp., margins of lesions are reddish brown, not sharply differentiated, with white centered lesions which were found absent in case of symptoms caused by *Mycosphaerella* sp.

Singh *et al.* (1975) described *Pestalotiopsis* leaf spot as circular, dark brown to chocolate coloured spots bordered by reddish brown or yellowish margins, which later coalesced and extended towards midrib whereby the infected areas turned brittle with dot like fruiting bodies (acervuli) on the necrotic areas which finally got separated from healthy areas. Paulus (1990) and Maas (1998) noticed upper surface of strawberry leaves infected by *Pestalotiopsis* with light brown irregularly distributed spots on the margins.

Wassenaar and Scheer (1988) detailed the infection of brown leaf spot of strawberry caused by *Alternaria alternata* (Fr.) Keissler f. sp. *fragariae* Dingley. According to them, affected leaves turned brown in colour with dark reddish purple lesions on leaflets as well as leaf stalks where severely infected plants appeared weak and leaves die off. Tanaka *et al.* (1996) observed black to greyish circular leaf spots on nursery grown strawberry plants caused by *Colletotrichum fragariae.* Similarly, Mass and Palm (1997) observed V shaped lesions in strawberry leaves caused *by Colletotrichum acutatum* resembling the symptoms of *Phomopsis obscurans*. Tamietti and Matta (2003) noted an unusual disease on apple leaves with irregular spots, turning into brownish black caused by *Diplocarpon mali* where premature leaf fall and severe chlorosis was noticed in extreme cases. According to Bagherabadi *et al.* (2015) leaves infected by *Alternaria tenuissima* were small with yellowish brown spots, later turning brown and finally necrotic.

2.4.2 Powdery mildew

Wilhelm (1961) noticed white powdery growth on leaves, calyces of flowers and fruit surface where the leaves cup upward, turn reddish on the underside and severe infections leads to burned margins. Mass (1998) noticed dense mycelium covering strawberry leaves infected with powdery mildew leading to necrosis and defoliation.

According to De Los Santos *et al.* (2003) powdery mildew infected strawberry leaves appeared reddish or necrotic with fungal mycelium on under surface of the leaf. Bolda and Koike (2003) described *Sphaerotheca macularis* f. sp. *fragariae* causing powdery mildew on leaves and fruits of strawberry. A white fluffy mycelium appeared on the underside of younger leaves whereby the upper surface of leaf turned brown to purple blotchy discoloration resulting in upward leaf curling.

2.4.3 Leaf blight

Anderson (1956) and Bose (1970) documented the symptoms of yellow leaf spot (*Dendrophoma obscurans*) as minute, circular, red to purple spots on the upper leaf surface that gradually coalesced and formed irregular or oblong patches. Spots extended towards the edge of lamina, which become "V" or fan shaped.

Lele and Pathak (1965) noticed symptoms of *Rhizoctonia bataticola* causing leaf blight as circular to spreading spots with purplish dark margins on leaves and ashy grey centre, whereas at advanced stages, leaves showed puckering, curling and defoliation. According to Mass (1998) *Rhizoctonia solani* creates severe leaf blight (web blight) symptoms that completely destroys plants by defoliation with wrinkling and distortion of infected leaves and petioles.

Nita *et al.* (2003) noticed *Phomopsis* leaf blight of strawberry with irregular foliar lesions, with purple halos and they observed three zones in a lesion: a dark-brown center, tan-to-light-brown surrounding rings and reddish or purplish outer zone. According to them, older lesions occurred along veins typically which turned "V" shaped extending towards the leaf margin.

2.4.4 Fruit rot

Fuzzy or velvety appearance of fruits infected by *Botrytis cinerea* Pers. ex *Fragariae* due to profusion of conidiophores bearing conidia was noticed by Powelson (1960). Fungal mycelium was visible on senescent petals, stamens and calyces of marketable fruit. Howard (1970) observed fruits infected by *Colletotrichum fragariae* and noticed circular, dark brown, sunken, firm rot lesions with abundant sporulation. Howard and Albretgs (1973) described *Alternaria tenuissima* causing fruit rot as slightly shrunken and irregular shaped lesions on shoulder of the fruit or under sepals, which appeared dark-green due to abundant sporulation.

Leandro (2002) similarly detailed the symptoms of *Colletotrichum* fruit rot as, water-soaked spots that later developed into firm, sunken, round lesions remaining tanned. Similarly, Embaby (2007) observed strawberry fruits infested by *Pestalotia* sp. developing white aerial mycelium and lesions on rotted fruits. Recently, Capobiango *et al.* (2016) observed strawberry fruits infected with *Colletotrichum siamense* with circular and sunken necrotic lesions producing pink spore masses under humid conditions.

2.4.5 Crown and root rot

Alcock and Howells (1936) reported that *Phytophthora fragariae* infected strawberry roots, produced long primary roots without any laterals and the disease was named as "rat's tail". Bain and Demaree (1945) observed killing of central cylinder of roots and presence of *Phytophthora* mycelium in stele turning into brownish-red colour where the dead lower ends of infected roots turned inky-black colour.

Smith (2008) identified wilted strawberry plants infected by *Colletotrichum fragariae* or *Colletotrichum gloeosporioides* causing anthracnose crown rot. Ishiguro *et al.* (2014) detailed the symptomatology of *Pythium helicoides* infecting strawberry as yellowing and wilting of lower leaves, where roots turned dark and water soaked.

According to Yildiz *et al.* (2014), *Botryodiplodia theobromae* infected plants showed black necrotic discolouration on roots and on crown cross section. Yuan *et al.* (2014), Koike and Gordon (2015) and Dinler *et al.* (2016) observed necrosis on roots and leaves, fruit reduction, deterioration in quality, plant stunting and wilting of strawberry plants affected with *Fusarium oxysporum* f. sp. *fragariae*. According to them, as the disease progressed, vascular and cortical tissues appeared brown to orange brown.

2.5 CHARACTERISATION OF PATHOGENS

Among the various diseases infecting strawberry, pathogens which lead to heavy crop losses are described below along with their important morphological and cultural characteristics.

2.5.1 *Alternaria* spp.

Studies on *Alternaria alternata* (Fr.) Keissler f.sp. *fragariae* was carried out by Dingley (1970) where he noticed conidiophores (17-90 x 3.5-4.5 µm size) arranged singly or in clusters, smooth, septate with few branches, geniculate with many pronounced scars, poroid and with terminal segments slightly swollen. Conidia are borne singly or in chains of upto eight spores, brown or lightly coloured, coarsely echinulate, globose, ovate, or obpyriform, with a short beak multiseptate, muriform, variable in size and septation, usually 20-35 x 8.5-12µm- with 3-6 transverse septa and with 0-4 longitudinal septa. Conidiophores are produced singly as short lateral branches upto 30µm long with 2-6 geniculations freely from the brown superficial floccose mycelium.

Bagherabadi *et al.* (2015) revealed the characteristics of *Alternaria tenuissima* infecting strawberry forming brownish green colony with concentric rings. Conidia ovoid with median constriction, brown with 2-7 transverse and 1-2 longitudinal septa was also observed. Fernandez *et al.* (2015) noted the characteristics of *Alternaria tenuissima* isolated from blueberries with dark olive pigmentation on both sides of plates, light brown, obclavate and muriformly septate conidia born in chains where the spores measured 27 × 13 µm with a beak.

2.5.2 *Botrytis* spp.

Fernandez-Ortuno *et al.* (2012) characterized the colony of grey mold pathogen as initially colourless, later turning into grey brown when conidiphores and conidia develop. Conidia (14 × 9 µm) was ellipsoid to round, with a scar on the point of union to the conidiophore. Sclerotia formed after two weeks appeared hard, dark and irregular

shaped. According to Fernandez-Ortuno *et al.,* (2015) conidia of *Botrytis cinerea* isolated from blueberries was hyaline, smooth-walled, arranged in botryose clusters with a distinctive slightly protuberant hilum.

2.5.3 *Diplocarpon* spp.

Bolton (1963) observed distinctive characteristic features of *Diplocarpon eralianum* infecting strawberry leaves. Conidia uniseptate with a constriction at the septum, dividing the conidia into a hyaline larger upper cell (31-44µ x 5-8µ) with curved apex and lower inflated cell. Dingley (1970) characterized *Diplocarpon eralianum* producing acervuli on subcuticular lesions on upper leaf surface and on poorly developed stroma. Conidiophores are simple, unbranched, upto 12µ long, 3.5-4µ wide, whereas the conidia is (15-20 x 4-5.5µ) hyaline, uniseptate but unevenly divided with a distinctly beaked upper cell.

Mass (1998) noticed oblong-cylindrical, eight-spored asci producing straight or curved ascospores of *Diplocarpon* infecting strawberry leaves. Tamietti and Matta (2003) observed scattered acervuli (95 to 170 µm) erupting through the epidermis of apple leaves infected by *Diplocarpon mali* where the conidia was ampule shaped, uniseptate, constricted at the septum, hyaline, guttulate and 6.1 - 8.4 × 14.6 - 22.0 µm

2.5.4 *Pestalotiopsis* spp.

Studies on *Pestalotiopsis* spp. causing leaf spot of blueberries was carried out by Feng *et al.* (2007) and according to them cultures were black with globular acervuli. Conidiophore were found swollen at the base, globose with phialides at apical end. Mature conidia are straight to fusiform, five-celled with the three middle cells brown and darker than the end cells. Apical cell triangular and hyaline with three simple setulae and base cell terminated in a point 4.0 to 8.6 µm long. Similar morphological and cultural characteristics were noticed by Mouden *et al.* (2014) while working with *Pestalotiopsis longisetula* in strawberry.

2.5.5 *Fusarium* spp.

Cultural characteristics of *Fusarium oxysporum* was studied by Arroyo *et al.* (2009) from strawberry, who noticed white mycelium changing gradually pink on pigment production. Dinler *et al.* (2016) described the presence of two conidia *viz.*, macroconidia with 3 to 5 septa, straight to slightly curved, gently tapered with curved apical end (15.7 to 35.4 × 2.9 to 4.3 µm) and oval to elipsoid microconidia, aseptate and formed abundantly on short monophialides (6.1 to 12.5 x 2.1 to 3.6 µm).

2.5.6 *Colletotrichum* spp.

Gunnell and Gubler (1992) studied the morphological and cultural characteristics of different *Colletotrichum* spp. causing anthracnose in strawberry. Conidia of *Colletotrichum fragariae* were narrowly obovate, straight or occasionally slightly curved. Conidia of *Colletotrichum acutatum* from infected fruit (cv. Yael), were hyaline with elliptic to fusiform conidia, measuring 12.6 µm and that of *Colletotrichum gloeosporioides* isolated from necrotic roots (cv. Tamar), was hyaline with oblong conidia with obtuse ends.

Xie *et al.* (2010) carried out a detailed description of setal characters of *Colletotrichum* sp. Setae of *C. fragariae* were dark brown, uniform in width except for the apical cells which functioned as phialides, and produced conidia in individual isolates. Setae of *C. gloeosporioides* were dark brown, gradually tapered along their entire length to their apices, straight, produced singly and did not produce conidia. Setae of *C. acutatum* were brown to dark brown, tapered, generally aseptate and did not produce conidia, and were shorter than those conidia of *C. fragariae* and *C. gloeosporioides*. The isolates of *C. gloeosporioides* readily produced perithecia containing asci and ascospores, but none of the isolates of *C. fragariae* or *C. acutatum* produced perithecia. On PDA, the colony colour of *C. fragariae* isolates ranged from beige to dark grey. Colonies of *C. gloeosporioides* isolates showed a dense, white mycelial growth turning to dark olive-grey colour. The colony colour of *C. acutatum* isolates was white for 4–5 days but later turned grey-brown.

2.5.7 *Botrydiplodia* spp.

Hong *et al.* (2012) from Korea noticed unicellular or single septate conidia of *Lasiodiplodia theobromae* isolated from mango. Yildiz *et al.* (2014) and Nam *et al.* (2016) described the characteristics of *Lasiodiplodia theobromae* as initially white colonies that turned into dense and black producing dark brown to black colour pycnidia after 30 days. Paraphyses was hyaline, cylindrical, septate, and not branched, round at the apex, upto 55 µm long, and 3-4 µm wide. Conidia was found unicellular when young with single septa, thickwalled, and ellipsoid to obovoid in shape.

2.5.8 *Dendrophoma obscurans*

Fall (1951) observed pycnidia of *Dendrophoma obscurans* on upper leaf surface and noticed oblong spores of size 7µm X 2µm, with pointed ends, vacuoles were present on either end or near the middle. Likewise, characteristics of *Dendrophoma obscurans* was also studied by Sutton (1965). Colony was with sparse white mycelia having epiphyllous or hypophyllous, immersed and black pycnidia, with a papillate protruding circular ostiole with 140-210µm diameter. Conidiophores hyaline, verticillately to irregularly branched with terminal phialides and upto 35µ long. Conidia (5.5-7.5 x I .5-2µ.) hyaline, unicellular, fusiform, 2-3 guttulate, one guttule at each end of the spore.

Zivkonic *et al.* (2007) identified *Phomopsis* sp. infecting plums with woolly to cottony or white to pale brown colony. Conidiomata pycnidial, stromatic, dark brown to black, single or aggregated and uniloculate. Conidia hyaline, aseptate, fusiform to ovate, straight, and frequently biguttulate, with average size 7.3 x 2.5 µm.

Farr *et al.* (2002) similarly described the morphological characteristics of *Phomopsis* where they noticed pycnidial, subcuticular, scattered to confluent, uniloculate, black, broadly spherical to flattened, 200–330 µm high and 440–840 µm wide, beak generally short, less than 60 µm, sometimes longer to 260 µm, uniostiolate conidiomata and conidiophores thin walled, brown, multicellular, cells 3–7 µm wide, elongate, tightly packed, lining pycnidial base and sides upto apex.

2.5.9 *Phytophthora* spp.

Alcock (1929) described the sporangial characters of *Phytophthora fragariae* from diseased red core roots submerged in sterile water as: nonpapillate, proliferous, usually inversely pyriform but variable in shape and size of 50µ x 30µ. Hickman (1940) described ovoid or ellipsoid sporangia arranged terminally on sporophores, 10µ-80µ long, with both sympodial and proliferous branching of conidiophores. Zoospores were irregularly ellipsoidal, biflagellate, 12µ in diameter, occur generally 40 to 50 per sporangium and germinate by germ tube.

Llieva *et al.* (1995) described the characteristics of *Phytophthora* sp. causing root and crown rot of raspberry. According to them, sporangiophores were branched single and loose, with swellings (10.2-13.4 µm) and sporangia 40.0 x 30.4 µm size, papillate, occasionally caducous with pedicels of 10-24 µm long.

According to the studies on *Phytophthora cactorum* conducted by De Los Santos *et al.* (2002) in strawberry, oogonia was spherical with thin walls, antheridia paragynous and attached to the oogonium near the oogonial stalk. Oospores were spherical and double-layered with yellow-brown walls and sporangia borne terminally, colourless and papillate.

2.5.10 *Mycosphaerella* spp.

Plakidas (1941) characterized *Mycosphaerella* as a fungus with groups of erumpent, amphigenous, black, globose with ostiolate perithecia. Asci cylindrical to clavate borne on short stalks, fasciculate, marginal ones curved, with central straight aparaphysates. Each asci contain eight imperfectly biseriate, hyaline, bicellular and obtuse ascospores.

2.6 MANAGEMENT OF FUNGAL DISEASES

Soil and crop harbours several microorganisms in which plant pathogens collectively leads to sizable losses of about 27-42 per cent to farmers, creating a major threat in global food production. Presently farmers rely mainly on chemical practices for disease management especially when the outbreak occurs beyond threshold level. Thus, following preventive and curative measures serve as a better means to minimise the infection and spread of these diseases. Hence an integrated disease management approach with cultural, biological, organic, physical methods, use of resistant varieties and chemicals should be accomplished for better disease management strategy.

2.6.1 Chemical control

According to Saxena *et al.* (2016) and Zaker (2016), as the crops are vulnerable to diseases farmers rely upon chemical fungicides which play an active role in plant disease management. A perusal of the literature revealed that there are only few reports available on the works related to the efficacy of fungicides against diseases of strawberry. Hence, available literature citing the sensitivity of different fungicides against fungal isolates infecting strawberry and other related plants have been documented.

2.6.1.1 *In vitro* evaluation of fungicides

Kataria *et al.* (1989) tested the *in vitro* efficacy of various fungicides against *Rhizoctonia solani* and observed strong inhibition by pencycuron, tolclofos-methyl, carboxin and thiabendazole. Freeman *et al.* (1997) observed good control over anthracnose by *Colletotrichum acutatum* using prochloraz-Zn and prochloraz-Mn alone or in combination with folpet than captan, difenoconazole and propiconazole. Similarly, Munoz (2002) noticed the effectiveness of propiconazole, bitertanol, hexaconazole, imazalil, carbendazim and thiabendazole against *Colletotrichum acutatum* CECT 20240 under *in vitro*. Islam *et al.* (2004) confirmed the efficacy of Bavistin (100, 200 and

300ppm) and Tilt 250 EC (100 and 200ppm) in controlling the radial growth of *Pestalotia palmarum* causing leaf spot of betelnut.

Shelar *et al.* (1997) reported that Benomyl (0.1%), captan (0.2%), carbendazim (0.1%), mancozeb (0.25%) and thiophanate-methyl (0.1%) were highly effective against *Botryodiplodia theobromae*. Similar work conducted by Sahi *et al.* (2012) observed 58.97, 45.08, 30.36 and 25 per cent inhibition with Topsin-M, Daconil, copper oxychloride and carbendazim.

Ponmurugan *et al.* (2006) tested the per cent reduction in mycelial growth of *Phomopsis theae* using contact and systemic fungicides and they observed that carbendzim was more effective followed by dithane M-45, Bordeaux mixture, Calixin, Blitox and Baycor. Singh and Singh (2006) noticed that hexaconazole, mancozeb, copper hydroxide, copper oxychloride, chlorothalonil, propineb and azoxystrobin was 100, 76, 55, 48.7, 48, 46 and 42.7 per cent effective against *Alternaria alternata*. Karande *et al.* (2007) noticed least per cent inhibition of mancozeb and Bordeaux mixture and complete inhibition of Bavistin, copper oxychloride and Tilt over *Colletotrichum gloeosporioides* and complete inhibition by Bavistin and least inhibition by other fungicides over *Fusarium oxysporum*. According to Filoda (2008), Sarfun 500 SC (Carbendazim) and Amistar 250 SC (Azoxystrobin) limited the growth and development of *Colletotrichum gloeosporioides*.

Saju *et al.* (2012) observed the efficacy of carbendazim 50WP at all concentrations followed by Carbendazim 12% + Mancozeb 64% 75 WP against *Pestalotiopsis* sp. infecting large cardamom. Taskeen-Un- Nisa *et al.* (2011) reported greater than 90 per cent inhibition of *Fusarium oxysporum* by hexaconazole and carbendazim and 60-70 per cent by mancozeb, zineb, captan, bitertanol and myclobutanil at 1000ppm.

Studies conducted by Ghazanfar *et al.* (2016) against *Alternaria solani* in tomatoes showed the effectiveness of Dithane M-45 (62.29%) followed by Antracol (56.56%), Topsin-M (47.91%), Blitox (42.59%) and Kavach (39.6%).

2.6.1.2 *In vivo* evaluation of fungicides

a) Foliage diseases

Jordan (1973) determined the effectiveness of benomyl, MBC and thiophante-methyl as pre harvest foliar spray in reducing mildew diseases of strawberry. According to Goszczynski and Cimanowski (1990) eight fungicides *viz*., flusilazole, myclobutanil, fenarimol, triadimefon and bupirimate were found to be most effective against *S. macularis*, followed by thiophanate-methyl, as sulfur was proved to show phytotoxic effects on strawberry seedlings. All the fungicides except bupirimate, effectively controlled *Mycosphaerella fragariae*.

Sharma *et al.* (2005) observed complete protection over *Diplocarpon mali* causing premature leaf fall in apple by protective sprays of copper oxychloride (0.3%), carbendazim (0.05%), zineb (0.3%), dodine (0.075%), Companion (0.25%), dithianon (0.05%), mancozeb (0.35%) and ziram (0.3%). Moreover, Milicevic *et al.* (2004) observed good control over common leaf spot (*Mycosphaerella fragariae* (Tul.) Lindau) and leaf scorch (*Diplocarpon earliana* (El. & Ev.) Wolf) pathogen of strawberry by spraying Folicur Multi 50 (tebuconazole + tolylfluanid), Kidan SC (iprodione) and Quadris KS (azoxystrobin). Akhter *et al.* (2009) reported cent per cent efficiency of Bavistin against *Colletotrichum gloeosporioides* of strawberry.

Carre-Missio *et al.* (2010) described the efficacy of mancozeb in reducing the infection of *Pestalotiopsis longisetula* in strawberry. Ali *et al.* (2013) observed notable results in reduction of *Alternaria* leaf blight and leaf spot (*Hainesia lythri*) with hexaconazole 5 EC (0.03%) followed by carbendazim 50 WP (0.05%) and mancozeb 75 WP (0.3%).

Archana and Jamadar (2014) assessed the effect of systemic and non-systemic fungicides against the pomegranate leaf spot pathogen, *Alternaria alternata* under *in vivo* with and they noticed that systemic fungicides like propiconazole and thiophanate methyl followed by azoxystrobin and hexaconazole showed less disease incidence compared to non-systemic fungicides.

b) Fruit diseases

Washington *et al.* (1999) tested eight fungicides against major fruit rots of strawberry. According to them, Fluzinam gave best control over grey mould (*Botrytis cinerea*), black spot (*Colletotrichum acutatum*) and leather rot (*Phytophthora cactorum*) followed by thiram and dichlofluanid. They also observed that chlorothalonil was as effective as thiram in controlling grey mould, but its efficacy against black spot and leather rot was not determined.

Sallato *et al.* (2007) assessed the efficacy of boscalid, cyprodinil, fenhexamid, fludioxonil, iprodione and pyraclostrobin against *Botrytis cinerea* and *Rhizopus stolonifer* and observed maximum inhibition with fludioxonil, cyprodinil, pyraclostrobin, fenhexamid, bosamid and iprodione. It was also noticed that cyprodinil and fludioxonil showed more than 95 per cent inhibition on *Rhizopus stolonifer* compared to other fungicides and fenhaxamid was the least effective.

Rebollar-Alviter *et al.* (2007) noticed complete control of strawberry leather rot pathogen, *Phytophthora cactorum* when sprayed weekly from bloom till harvest with azoxystrobin, pyraclostrobin and potassium phosphite as well as drenching the soil with mefenoxam twice at early plant growth and fruit set. Valiuskaite *et al.* (2008) observed the effect of cyprodinil + fludioxonil in managing *Botrytis cinerea* infecting strawberry.

c) Crown and Root diseases

According to Montgomerie and Kennedy (1974), during pot culture studies, fungicides like captafol, chloraniformethan, dimethirimol, dinocap, dithianon, drazoxolon, folpet, mancozeb zineb and terrazole were effective against *Phytophthora fragariae* in strawberry. Foliar spraying of fosetyl-Al or metalaxyl at weekly intervals gave 88 to 96 per cent control against strawberry leather rot caused by *Phytophthora cactorum* (Ellis *et al.*, 1998). An attempt was carried out by Asad-us-Zaman *et al.* (2015) to manage black root rot disease caused by *Rhizoctonia solani* Kuhn using Bavistin 50WP, Provax-200 and Ridomil 75EC at 100, 250 and 500 ppm and they observed cent per cent inhibition with Provax-200 at 100ppm.

2.6.2 BIOLOGICAL CONTROL

Biological control is considered as an alternative method to chemical control for plant disease management. A number of strategies are being followed by farmers to control plant diseases as the latter has been proved harmful to the environment. According to Agrios (1988) and Cook (1993), apart from practicing good agronomic and cultural practices, spread of plant diseases has led farmers to rely on these chemicals to manage diseases. However, frequent application of these agrochemicals has led to hazardous changes and effects in surrounding environment and even to human health. As complete elimination of chemical fungicides are not possible and keeping in mind the public concern about harmful effects of chemicals on environment, the concept of biological control has been evolved. Biocontrol agents are a group of beneficial microorganisms which improves plant growth by restricting the negative effects of plant pathogens and alleviation of abiotic stresses (Shoresh *et al.*, 2010). Microbes, including fungi and bacteria are considered as a source of potential biocontrol agents effective against various crop diseases.

According to Harman *et al.* (2004) and Weller, (2007), *Trichoderma* and *Pseudomonas* are considered as common free-living potential biocontrol agents present in the soil ecosystem which creates a long lasting localized and systemic response in plants. Many scientists suggested the mode of action of *Trichoderma* (Chet, 1987; Benitez *et al.*, 2004; Harman and Shoresh, 2007) against various pathogens, which includes phytopathogenic activity through competition, by producing antibiotics, predation and induction of defense response in plants and mycoparasitism. Chet and Inbar (1994) described the activity of *Trichoderma* including secretion of extracellular lytic enzymes *viz.*, β-l,3-glucanase, chitinase, protease, and lipase. Both *Trichoderma* and *Pseudomonas* are excellent rhizosphere colonizers and produce diverse array of metabolites harmful to plant pathogens.

Several researchers proved the effectiveness of *Pseudomonas* as a potential bacterial bioagent that suppress the plant pathogens by competing with nutrients and space available in the rhizosphere (Elad and Baker, 1985 and Elad and Chet, 1987) and

40

production of antibiotics *viz.*, pyocyanine, pyrrolnitrin, 2,4-diacetyl phloroglucinol and siderophores (Pierson and Thomashow, 1992 and Lemanceau *et al.*, 1992). It degrades the chitin and glucan present in the fungal cell wall by releasing various lytic enzymes such as chitinases and β-1,3-glucanases (Velazhahan *et al.*, 1999).

2.6.2.2 *In vitro* evaluation of organic preparations

Sumangala and Patil (2009) observed the sensitiveness of panchagavya against *Curvularia lunata* affecting rice resulting in 86.30 per cent inhibition of mycelial growth under *in vitro* conditions. Srinivasa *et al.* (2011) tested the antifungal activity of essential oils extracted from clove, cedar wood, *Cymbopogon* species, peppermint, *Eucalyptus* and neem against nine *Fusarium* species, including, *F. oxysporum* isolated from maize and sorghum and citronella oil showed the highest inhibitory effect at various concentrations.

Chadha *et al.* (2012) tested per cent reduction in mycelial growth of *Fusarium oxysporum, Colletotrichum capsici* and *Rhizoctonia solani* with cow urine, panchagavya and vermiwash at 2 per cent and recorded 74.77 to 83.37 per cent, 81 to 91 per cent and 40.35 to 79.25 per cent efficacy over control respectively.

According to Muthukumar and Ranganathan (2012), cent per cent control at 0.1 per cent of neem oil was observed against *Colletotrichum musae* isolated from banana. Muthukumar and Ranganathan (2012) noticed cent per cent control with 0.1 per cent of neem oil against *Botryodiplodia theobromae* whereas Sagoua *et al.* (2008) and Adepoju *et al.* (2014) recorded the antifungal activity of neem oil against *Lasiodiplodia* sp. and *Fusarium* spp. infecting banana.

2.6.2.3 *In vitro* evaluation of bioagents

Elad *et al.* (1981) stated that application of *Trichoderma harzianum* in strawberry growing fields and nurseries restricted the disease caused by *Rhizoctonia solani* by 18-46 per cent. Effective strategies to control *Phytophthora* spp. in strawberry using

Serratia plymuthica (Kurze *et al.*, 2001), *Gliocladium* and *Trichoderma* (Vestberg *et al.*, 2004), *Trichoderma* spp. (Porras *et al.*, 2007a, 2007b) or *Pseudomonas fluorescens* strains EPS817 and EPS8 (Augusti *et al.*, 2011) have also been reported.

Kim *et al.* (2007) evaluated the efficacy of various *Bacillus licheniformis* N1 against *Botrytis cinerea* and observed 81 per cent control over chemical fungicides. Sobowale *et al.* (2010) reported the efficiency of *Trichoderma longibrachiatum* against *Botryodiplodia theobromae*. Nath *et al.* (2014) reported that all the antagonists *viz.*, *Trichoderma harzianum, Trichoderma viride, Trichoderma longibrachiatum, Bacillus subtilis* and *Pseudomonas fluorescens* were proved to be efficient in reducing *Lasiodipodia theobromae* infection in banana.

Donmez *et al.* (2011) tested a total of 186 bacterial strains against *Botrytis cinerea* and 36 strains *viz.*, *Bacillus lentimorbus, B. megaterium, B. pumilis, B. subtilis, Enterobacter intermedius, Kurthia sibirica, Paenibacillus polymyxa* and *Pantoea agglomerans* were found to be effective in inhibiting the pathogen under *in vitro* conditions. Zegeye *et al.* (2011) recorded 36.7 per cent inhibition of *Phytophthora infestans* with *Trichoderma viride* and 88 per cent with *Pseudomonas fluorescens* in potato.

Adeniyi *et al.* (2013) reported 90.48 and 80. 12 per cent inhibition of *Lasiodiplodia theobromae* with *Trichoderma viri*de and *Aspergillus niger* in cashew. Chen *et al.* (2014) isolated a strain of biocontrol agent *Bacillus* TS02 from soil and tested it efficacy against strawberry powdery mildew (S*phaerotheca macularis*), where they found 24-49 per cent efficiency over control.

El-ghanam *et al.* (2015) recorded 75-98 per cent inhibition of *Botrytis cinerea* with *Chlorella vulgris, Spirulina platensis, Azotobacter chroococcum, Trichoderma harizianum* and their combinations in strawberry. An attempt was carried out by Asad-us Zaman *et al.* (2015) to manage black root rot disease caused by *Rhizoctonia solani* Kuhn and reported that *Trichoderma harzianum* isolate STA7 showed maximum inhibition of 71.97 per cent compared to other isolates. Pastrana *et al.* (2016) reported

the efficacy of *Trichoderma asperellum* and *Bacillus* spp. against soil borne pathogens, *Macrophomina phaseolina* and *Fusarium solani*.

2.6.3 Integrated disease management of fungal pathogens

Stretch (1989) noticed the potential effects of *Pseudomonas cepacia* and *Aureobasidium pullulans* against blueberry and cranberry fruit rots. Freeman *et al.* (2004) tested the efficacy of biofungicide product, TRICHODEX (*Trichoderma harzianum* strain T-38) against *Colletotrichum acutatum* and *Botrytis cinerea* in strawberry. Porras *et al.* (2007) documented a reduction in soil population of *Phytophthora cactorum* from 88.9 to 99.0 per cent when soil solarization was combined with application of *Trichoderma* spp.

Pertot *et al.* (2008) studied the potential effects of chemicals and biocontrol agents against strawberry mildew (*Podosphaera aphanis* (Wallr.) U. Braun & S. Takam). *Ampelomyces quisqualis, Bacillus subtilis* and *Trichoderma harzianum* T39, could manage the disease, but to a lesser extent than chemical fungicides like azoxystrobin and penconazole. According to De Cal *et al.* (2008), *Penicillium oxalicum* treated cultivars showed significant reduction in powdery mildew infection.

Cota *et al.* (2009) studied the combination effect of *Clonostachys rosea* with fungicide procymidon against *Botrytis cinerea* and noticed 96.62 per cent reduction in disease. Augusti *et al.* (2011) studied the potential combination effects of two Pseudomonads strains EPS817 and EPS894 in protecting strawberry plants from *Phytophthora cactorum* root rot and noticed 76 to 84 per cent efficiency. Sylla *et al.* (2013) noticed 80.7 per cent reduction in conidiation of *Podosphaera aphanis* with multiple strain treatments of *Bacillus subtilis* FZB24 and *Metarhizium anisopliae*. Prastana *et al.* (2016) proved the effectiveness of two commercial formulations *viz.*, *Trichoderma asperellum* T18 strain (Prodigy®) and *Bacillus megaterium* and *B. laterosporus* (Fusbact®) under controlled environment and field conditions in South-Western Spain against *Macrophomina phaseolina* and *Fusarium solani*.

43

Materials & Methods

3. MATERIALS AND METHODS

The study on "Cataloguing, documentation and management of fungal diseases of strawberry (*Fragaria* X *ananassa* Duch.)" was conducted in the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2015-2017. The details of the methodologies followed during the course of the experiment are given below.

3.1 SURVEY AND COLLECTION OF FUNGAL DISEASES OF STRAWBERRY FROM DIFFERENT LOCATIONS

An intensive sampling surveys were conducted in strawberry growing tracts of Kerala to assess the fungal diseases prevailing under polyhouse and open field conditions. Three districts *viz*., Wayanad, Malappuram and Idukki were selected and locations of survey are detailed in Table 3.1. From each location, samples of strawberry plants showing typical symptoms of fungal diseases were collected. The surveys were programmed and conducted to observe the occurrence of diseases prevailing in all the strawberry growing seasons in respective areas.

Table 3.1. Locations of survey for collection of diseased sample

Sl. No.	District	Location	Seasons
1.	Wayanad	Ambalavayal	Dec-Jan, March-April, July-August
2.	Malappuram	Anakkayam	Dec-Jan, March-April, July-August
3.	Idukki	Vattavada	Dec-Jan and March-April
		Koviloor	

3.1.1 Assessment of disease incidence and disease severity

During the survey, the disease incidence and severity were recorded on infected plants showing varied type of symptoms. In each field, 100 plants were randomly

selected and per cent disease incidence (PDI) was assessed for all root rots and foliage diseases by counting the number of affected plants out of total number of plants observed from each plot. The PDI was calculated using the formula given by Wheeler (1969).

$$\text{Per cent disease incidence (PDI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

The per cent disease severity (PDS) was assessed in case of all foliage diseases following a standard score chart of 0-5 scale as depicted in Table 3.2.

Table 3.2. Score chart for assessing the severity of foliage diseases

Grade	Description
0	No symptom
1	< / =1 per cent leaf area infected
2	>1-10 per cent leaf area infected
3	>10-25 per cent leaf area infected
4	>25-50 per cent leaf area infected
5	>50 per cent leaf area infected

Per cent disease incidence was calculated using the formula:

$$\text{Per cent disease severity (PDS)} = \frac{\text{Sum of all numerical ratings}}{\text{Total no. of leaves observed x Maximum disease grade}} \times 100$$

3.1.2 Collection of meteorological data

Weather data *viz.*, maximum and minimum temperature, relative humidity and rainfall prevailing in open field conditions were collected and recorded during the survey period from the observatory of respective areas of Wayanad, Idukki and Malappuram

district and the mean of all the weather parameters were correlated with mean disease severity in order to get a complete profile of the occurrence of diseases.

3.2 ISOLATION OF FUNGAL PATHOGENS

Naturally infected plant parts of strawberry *viz.*, leaves, fruits, crown and roots collected from the surveyed locations were brought to the laboratory and washed thoroughly under running tap water to remove dust and saprophytes adhering on to it. The pathogen was isolated from the diseased tissues of strawberry by tissue segmentation method (Rangaswamy, 1958). Infected portions along with the healthy portion of diseased plants were cut into small bits of size 1 to 1.5cm using a sterile blade and disinfected with sodium hypochlorite (1%) solution for one minute. These bits were subsequently washed thrice in sterile distilled water to remove the traces of sodium hypochlorite. Excess moisture in the sample bit was dried out using a sterilized blotting paper and three bits were placed aseptically on sterile Petri dishes containing solidified Potato Dextrose Agar (PDA) medium. Streptomycin was added to PDA to prevent bacterial contamination. All the inoculated plates were then incubated at room temperature (26 ± 2°C) for five days. The growth of the pathogen was observed and the hyphal tips of the fungi grown were transferred aseptically to solidified PDA in sterile Petri dishes and thereafter, the isolates were purified by single hyphal tip method/single spore isolation to PDA slants for maintenance of the cultures for further studies.

3.3 PATHOGENICITY

Koch's postulates was followed to prove the pathogenicity of the fungal isolates. The ability of the isolates in developing typical symptoms under artificial conditions was studied by artificial inoculation of cultures either by inoculating spore suspension or by placing the mycelial disc on healthy plants or plant parts on which the symptoms usually appears.

3.3.1 Pathogenicity test for foliage and fruit diseases

Pathogenicity tests of each fungus was conducted by inoculating mycelial discs on detached leaves and fruits as well as on live plants by following the method of Rocha *et al.* (1998) called Mycelial Bit Inoculation Method (MBIM). Mycelial plugs of 8mm diameter were cut with a cork borer from the respective fungal culture and inoculated in an inverted position on adaxial surface of leaf lamina and on either sides of fruits following the pin prick method (Jadesha *et al.*, 2012). Humidity was maintained by placing moist cotton swab over the culture bit. The inoculated leaves or fruits were kept at ambient room temperature (25°C) and observed daily for symptom appearance.

Spore suspension method, also called as Mycelial Droplet Inoculation Technique (MDIT) was also used to test the pathogenicity of the isolates as suggested by Denoyes and Baudry (1995). Two month old strawberry plants were selected for the experiment. The plants were watered and kept in moist chamber for 48h prior to inoculation for getting maximum infection and disease development. Spore suspensions of each pathogen was prepared by gently scraping the pure culture of each fungi grown on PDA and mixed with 100ml sterile water, which was then filtered through a muslin cloth to get a final spore count of 2×10^6 spores ml^{-1}. The spore suspension was inoculated by spraying using a hand atomizer after making injuries by pinpricks using sterile needles on healthy leaves and fruits and the inoculated plants were incubated in a moist humid chamber. Control plants were maintained by treating with sterile water. The potted plants after 48 h of inoculation were transferred to a shaded area and sprayed daily with water and symptoms were recorded.

3.3.2 Pathogenicity test for root disease

3.3.2.1 *Spore suspension method*

Spore suspension of 1×10^6 cfu ml^{-1} concentration was prepared from 10 day old culture and this suspension was poured in the rhizosphere region near the lower leaf axil

of each crown after giving an injury to the root using a sterile needle (Urena-Padilla *et al.*, 2002).

The pathogens were reisolated from artificially inoculated plants after symptom development in order to prove Koch's postulates and cultural and morphological characteristics of the isolates were studied and compared with that of the original cultures. The isolates thus obtained were maintained on PDA slants as pure cultures for further studies.

3.4 SYMPTOMATOLOGY OF DISEASES

Symptoms produced by fungal pathogens on foliage, fruits, crown and root were studied under natural conditions during the survey conducted in various locations. In order to study the symptomatology of fungal diseases under artificial conditions, the isolates were artificially inoculated on respective plant parts of strawberry following the standard protocol mentioned in 3.3.1 and 3.3.2. Observations on variations of specific symptom development *viz.*, colour and size of lesion and time taken for symptom development were recorded.

3.5 CHARACTERISATION AND IDENTIFICATION OF PATHOGENS

The isolates of fungal pathogens were studied for their cultural and morphological characteristics for identification.

3.5.1 Cultural characters

Cultural characteristics exhibited by different fungal isolates were studied by visual observation. Observations on colony colour, texture, growth rate and pattern, sporulation and pigmentations, colour on reverse side of culture plates, presence of any fruiting bodies were taken at regular intervals and recorded. For this the pathogen was grown on solidified PDA medium and plates were kept for incubation at 26±2°C.

3.5.2 Morphological characters

Slide culture technique of Riddle (1950) was employed for studying the morphological characteristics of different fungal pathogens isolated from different locations. Microscopic observations on colour of hyphae, branching pattern, presence of septation on hyphae or on conidia, type of spores, its shape, size, L/B ratio and presence of sexual structures were recorded. Camera lucida drawings were made and microphotographs as well as measurements of fungal structures were taken assisted by the Image analyser.

The isolates were then send to National Center for Fungal Taxonomy (NCFT), New Delhi for further confirmation where the cultures were deposited under different accession numbers.

3.6 *In vitro* EVALUATION OF FUNGICIDES, ORGANIC PREPARATIONS AND BIOAGENTS AGAINST PATHOGENS

The efficacy of different fungicides, organic preparations and bioagents against the isolates of fungal pathogens were evaluated under aseptic conditions. The fungitoxic effect of fungicide and organic preparations was tested *in vitro* against the isolated pathogens by employing poison food technique (Zentmeyer, 1955). The experiment was conducted in completely randomized design (CRD) with three replications for each treatment. Dual culture method suggested by Skidmore and Dickinson (1976) was used for testing the efficacy of the reference bio control agent *viz.*, *Trichoderma asperellum* and *Pseudomonas fluorescens* (KAU isolates) against isolated fungal pathogens.

3.6.1 *In vitro* evaluation of fungicides against pathogens

A total of nine fungicides each at three different concentrations were evaluated under *in vitro* against the fungal isolates by poison food technique (Zentmeyer, 1955) (Table 3.3). For this, 100 ml of PDA medium was sterilized in 250 ml conical flasks. After melting the media required quantity of each fungicide to be tested was added to

the medium in separate conical flasks and mixed thoroughly to make up the required final concentration and 20 ml was plated on each 90 mm Petri plate. Using a sterile cork borer, 8mm mycelial disc was cut out from the actively growing culture of the respective test pathogen and placed at the centre of each Petri plate. The plates were kept for incubation at room temperature (26 ±1°C). Media without the fungicide served as control. Observations on growth rate of each fungal pathogen were recorded daily until the cultures on control plates showed full growth. The per cent inhibition of mycelial growth in each treatment was calculated using the formula suggested by Vincent (1947).

$$\text{Per cent inhibition of growth} = \frac{C-T}{C} \times 100$$

C= Growth of fungus in control (mm)

T= Growth of fungus in treatment (mm)

Table 3.3 Fungicides used for *in vitro* evaluation against pathogens

Sl. No.	Chemical name	Trade name	Concentration (Per cent)
1.	Bordeaux mixture	-	0.5, 1, 1.5
2.	Copper oxychloride 50 WP	Fytolan	0.2, 0.25, 0.3
3.	Copper hydroxide 75WP	Kocide	0.1, 0.15, 0.2
4.	Propineb 70 WP	Antracol	0.25, 0.3, 0.35
5.	Difenoconazole 25EC	Score	0.05, 0.1, 0.15
6.	Carbendazim 50 WP	Bavistin	0.05, 0.1, 0.15
7.	Potassium phosphonate	Akomin-40	0.25, 0.3, 0.35
8.	Carbendazim 12% + Mancozeb 63%	Saaf	0.15, 0.2, 0.25
9.	Cymoxanil 8% + Mancozeb 64 %	Curzate M8	0.15, 0.2, 0.25

3.6.2 *In vitro* evaluation of organic preparations against pathogens

An *in vitro* evaluation was carried out to find the inhibitory effects of different organic formulations *viz.*, neem oil, panchagavya, Calphomil and baking powder + vegetable oil mixture using poison food technique against different fungal isolates. The details of treatments are given in Table 3.4:

Table 3.4 Organic preparations used for *in vitro* evaluation against pathogens

Sl. No.	Organic preparations	Concentration (Per cent)
1.	Neem oil	0.15, 0.2, 0.2
2.	Baking powder + Vegetable oil	0.15, 0.2, 0.25
3.	Panchagavya	2.5, 3.0, 3.5
4.	Calphomil	0.2, 0.25, 0.3

Organic formulations as depicted above were disinfected under UV light for one hour before mixing with PDA medium to avoid contamination. Then required quantity of each formulation was mixed with the medium and 20ml was plated on each Petri plate. Tween 20 a non-ionic surfactant, @ 0.2 per cent, was added to the mixture of PDA and neem oil to ensure perfect mixing of neem oil with the medium (Neves *et al.*, 2001). Thereafter, mycelial disc of 8mm diameter of the fungal pathogen was placed aseptically in the centre of the plate. Petri plates without poisoned media served as a check. Plates were kept for incubation at ambient temperature (26 ±1°C). Observations on mycelial growth of each fungal pathogen was taken daily and per cent Inhibition (PI) was calculated using the formula given by Vincent (1947).

3.6.3 *In vitro* evaluation of bio agents by dual culture technique

Efficacy of reference cultures of fungal and bacterial biocontrol agents from KAU *viz.*, *Trichoderma asperellum* and *Pseudomonas fluorescens* were tested against each fungal pathogen of strawberry by following dual culture technique (Dennis and Webstar, 1971).

3.6.3.1 Fungal antagonist

Inoculation of fungal antagonist, *T. asperellum* and the pathogen was carried out with due consideration of the growth rate of both organisms. Mycelial plug of 8mm diameter, cut from seven day old culture of the pathogen and antagonist grown on PDA was inoculated aseptically 2cm away from the periphery of the sterilized Petri plate on opposite sides. Plates inoculated with pathogen alone served as control. Three replications were maintained for each isolate and these plates were then incubated at room temperature (26±1°C). The growth of the pathogen and fungal antagonist was monitored at regular intervals and final observations were taken when the control plates attained full growth. Per cent inhibition of mycelial growth of the pathogen over control was calculated using the formula as mentioned in 3.6.1. The nature of reaction of the antagonist, *T. asperellum* against the pathogen was assessed by following the method of Purkayastha and Bhattacharya (1982).

Types of reactions

- Homogenous (H) - Free intermingling of hyphae
- Overgrowth (O) - Pathogen overgrown by test pathogen
- Cessation of growth (C) - Cessation of growth at line of contact
- Aversion (A) -Development of clear zone of inhibition

3.6.2.2 Bacterial antagonist

The bacterial antagonist *Pseudomonas fluorescens*, was evaluated against each fungal pathogen by simultaneous antagonism by following the method of Utkhede and Rahe (1983). For this, mycelial disc of 8mm diameter from the centre of the pathogen was placed in the centre of the Petri plate, plated with PDA medium and a loopful of the bacterial culture was streaked at both ends of Petri plate 2cm away from the periphery of the plate. Monoculture of the pathogen served as control and the plates were incubated at room temperature (26±1°C). Three replications were maintained for each isolate. Observations on the mycelial growth of the pathogen was recorded when full growth of the pathogen was attained in control plates. Per cent inhibition of the pathogen by the antagonist was calculated as mentioned in 3.6.1.

3.7 MOLECULAR CHARACTERISATION OF MAJOR FUNGAL PATHOGENS OF STRAWBERRY

Four fungal pathogens which showed constant occurrence in all the locations of survey and also which recorded high per cent disease incidence (PDI) and per cent disease severity (PDS) were selected as most virulent pathogens. The molecular characterization of these fungal isolates was carried out for final confirmation regarding its identity prior to *in vivo* experiment. The cultures were sent to Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram to confirm the identity of the pathogen at species level by ITS sequencing. The ITS primers used for DNA barcoading are given below:

Target	Primer Name	Direction	Sequence (5' → 3')
ITS	ITS-1F	Forward	TCCGTAGGTGAACCTGCGG
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC

Sequence analysis and nucleotide homology of each pathogen were analysed through the BLASTn programme of NCBI (http://ncbi.nlm.nhm.gov/blast).

3.8 *In vivo* EVALUATION OF FUNGICIDES AND BIOCONTROL AGENTS ON MAJOR FUNGAL DISEASES OF STRAWBERRY

For *in vivo* evaluation promising treatments were selected based on the results of the previous *in vitro* studies on the efficacy of fungicides and biocontrol agents against the fungal pathogens. A pot culture experiment was laid out to study the management of the predominant fungal pathogens observed during the survey period based on the intensity of the disease. The experiment was carried out during December 2016 - January 2017 at College of Horticulture, Vellanikkara. The details of the experiment is as follows:

Design	: CRD
Number of treatments	: 6
Replications	: 8
Variety	: Winter Dawn

3.8.1 Preparation of potting mixture for planting

Tissue culture plants of strawberry variety, Winter Dawn were raised in growbags of size 35 x 20 x 20 cm. The bags were filled with potting mixture consisting of soil, sand and cowdung in the ratio 1:1:1 and were placed in the net house and irrigated regularly. The cultural operations were carried out as suggested by Singh *et al.*, 2009.

3.8.2 Treatment application

The plants were challenge inoculated with the pathogen by spraying with spore suspension of 2 x 10^6 spores ml^{-1} concentration using a hand sprayer or by root inoculation as described in 3.3. The inoculated plants were kept in humid chamber for 24-48 h in the net house and high relative humidity was maintained throughout the experiment by frequent irrigation. Formulation of *Trichoderma asperellum* was applied

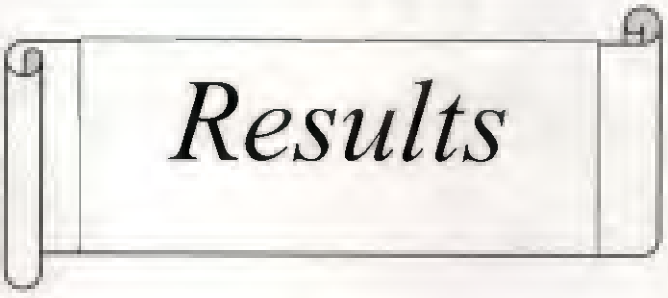
as a prophylactic treatment 10 days prior to challenge inoculation of the pathogen. On symptom appearance, treatments were given as foliar spray or soil drench based on the nature of symptoms developed by the pathogen. Thereafter, two subsequent sprays were given at ten days interval after each treatment. Treatment details are represented in Table 3.5. After the disease development and at ten days intervals of treatment applications, observations were taken and PDI as well as PDS were calculated.

Table 3.5 Details of experiment with the selected fungal pathogens

Sl. No.	Treatments (soil drench and foliar spray)		Concentration (Per cent)
	Common name	Trade name	
1.	T_1-Control	-	-
2.	T_2 - Cymoxanil 8% + Mancozeb 64 % WP	Curzate M8	0.2
3.	T_3- Carbendazim 12% + Mancozeb 63% WP	Saaf	0.2
4.	T_4- Copper hydroxide 77 WP	Kocide	0.15
5.	T_5-Propineb 70 WP	Antracol	0.3
6.	T_6-*Trichoderma asperellum*	-	2.0

3.9 Statistical analysis

Analysis of variance (ANOVA) was performed for the data obtained from various experiments using Web Agri Stat Package (WASP 2.0). Appropriate transformation of data was carried out as required (Gomez and Gomez, 1984). Multiple comparison between the treatment means was done with Duncan's Multiple Range Test (DMRT).


Results

4. RESULTS

The research on "Cataloguing, documentation and management of fungal diseases of strawberry (*Fragariae* x *ananassa* Duch.)" was conducted to detect various fungal pathogens affecting strawberry and to study its symptomatology, seasonal occurrence as well as *in vitro* and *in vivo* management of the disease. The results of the investigations carried out during 2015-17 are presented below:

4.1 SURVEY AND COLLECTION OF DISEASED SAMPLES

Intensive surveys were conducted during 2015 to 2017 in December-January, March-April and July- August from in different strawberry growing tracts of Wayanad, Idukki and Malappuram district. The primary objective of the survey was to collect various diseased samples of infected leaves, fruits, root and crown of strawberries and to assess the disease incidence and severity of all the pathogens during these periods. Diseases noticed in each location of survey in each district under open field and polyhouse conditions are tabulated and given in Table 4.1 and Table 4.2.

Depending on the symptoms produced by different pathogens, the diseases *viz*., leaf spots, leaf blights, crown rots and fruit rots were abbreviated as LS, LB, CR and FR respectively as these were the most common diseases noticed during the survey period. The isolates were designated along with the name of location, *ie*, LSW-1 for leaf spot pathogen isolated from Wayanad, LSI-1 from Idukki, LSM-1 from Malappuram and so on.

4.1.1 Assessment of disease incidence and disease severity

During the survey conducted in different strawberry growing tracts, plant parts showing distinct symptoms were noticed separately and per cent disease incidence (PDI) and per cent disease severity (PDS) were recorded for each of them. The PDI and PDS of each pathogen from three districts are detailed in Table 4.3, 4.4 and 4.5 respectively.

Plate 4.1 Survey in strawberry nursery in Wayanad district

Plate 4.2 Survey under polyhouse condition in Malappuram district

From the survey conducted during three seasons in Wayanad district, the incidence of LBW-1 and fruit rot diseases were noticed only under open field conditions, whereas LSW-1 was observed both under open field and polyhouse conditions. LSW-1 recorded a PDI ranging from 32.3 to 52.9 per cent with maximum during the months of December-January and minimum during July-August. However, the PDS ranged from 15.37 to 22.8 per cent with minimum severity during July-August and maximum during December-January. Fruit rot was noticed only during December-January infecting 20 per cent of the ripened fruits. Among all the diseases surveyed, LSW-1 showed maximum PDI of 52.9 per cent and PDS of 22.8 per cent during December-January and was found to be less severe (15.37%) during July-August.

In Idukki district, survey was conducted in two locations *viz*., Vattavada and Koviloor during March-April and December-January of 2015-2017 where diseases like LSI-1, LBI-2, LBI-3, LSI-2 and CRI-1 were observed. Crown rot pathogen caused complete wilting and death of 30 and 70 per cent plants in the field during December-January and March-April respectively. In case of LSI-1, maximum PDI and PDS of 49.1 and 23.4 per cent were recorded in Vattavada and 40.2 and 19.9 per cent in open fields of Koviloor during March-April while minimum severity of 14.9 and 10.4 per cent was observed during December-January. Likewise, LSI-2 was noticed in both locations with a PDI of 42.6 and 49.2 per cent and PDS of 17.1 and 22.3 per cent in Koviloor and Vattavada respectively during March-April. Leaf blight, LBI-1 was observed both during December-January and March-April with a severity of 13.8 and 19.7 per cent respectively. However, LBI-2 was observed only in Vattavada during March-April showing a PDS and PDI of 6.9 and 18.6 per cent respectively.

In Malappuram district, one leaf blight, two leaf spots and a crown rot disease designated as LBM-1, LSM-1, LSM-2 and CRM-1 were noticed from Anakkayam. LBM-1 was detected throughout the survey period with a PDS of 17.4, 21.7 and 19.2 per cent during December-January, March-April and July-August respectively, whereas PDI ranged from 23.2 to 58.2 per cent in the three seasons. Crown and root rot was observed only in two seasons *viz*., March-April and July-August recording a PDI of 82

and 75 per cent respectively. Another leaf spot, LSM-1, recorded a PDI and PDS of 32.3, 52.4 and 49.6 per cent and 21.4, 23.4 and 20.9 per cent respectively during December-January, March-April and July-August. Moreover, the disease LSM-2 was recorded infecting leaves with a maximum severity of 22.8 per cent during July-August and minimum of 13.4 per cent during December-January.

Table 4.1 Diseases of strawberry observed during the survey period

Sl. No.	District	Location	Disease*
1.	Wayanad	Ambalavayal	Leaf spot 1 (LSW -1)
			Leaf blight 1 (LBW-1)
			Fruit rot 1 (FRW-1)
2.	Idukki	Vattavada	Leaf spot 1 (LSI-1)
			Leaf blight 1 (LBI- 1)
			Leaf blight 2 (LBI-2)
			Leaf spot 2 (LSI-2)
		Koviloor	Leaf spot 1 (LSI-1)
			Leaf spot 2 (LSI-2)
			Crown and root rot 1(CRI-1)
3.	Malappuram	Anakkayam	Leaf spot 1 (LSM-1)
			Crown and root rot 1 (CRM-1)
			Leaf blight 1(LBM-1)
			Leaf spot 2 (LSM-2)

*- abbreviation given in parenthesis

Table 4.2 Diseases of strawberry observed under open field conditions and polyhouse

Sl. No.	District	Diseases	
		Open field	Polyhouse
1.	Wayanad	LSW-1, LBW-1, FRW-1	LSW-1
2.	Idukki	LSI-1, LBI-1, LBI-2, LSI-2, CRI-1	-
3.	Malappuram	CRM-1, LSM-1, LBM-1	LBM-1, LSM-1

Table 4.3 Per cent disease incidence and severity of fungal diseases of strawberry in Wayanad district

Sl. No	Location	Disease	Period					
			Dec- Jan		March-April		July- August	
			PDI	PDS	PDI	PDS	PDI	PDS
1.	Ambalavayal	LSW-1	52.90	22.80	43.30	21.78	32.30	15.37
		LBW-1	46.70	25.20	35.70	19.80	17.34	9.90
		FRW-1	20.00	-	-	-	-	-

PDS-Per cent disease severity PDI-Per cent disease incidence

62

Table 4.4 Per cent disease incidence and severity of fungal diseases of strawberry in Idukki district

Sl. No	Location	Disease	Period			
			Dec-Jan		March April	
			PDI	PDS	PDI	PDS
1.	Vattavada	LSI-1	32.80	14.90	49.10	23.40
		LBI-1	22.20	13.80	25.90	19.70
		LBI-2	-	-	18.60	6.90
		LSI-2	32.30	18.70	49.20	22.30
2.	Koviloor	LSI-1	29.60	10.40	40.20	19.90
		LSI-2	36.20	21.00	42.60	17.10
		CRI-1	30.00	-	70.00	-

PDS-Per cent disease severity PDI-Per cent disease incidence

Table 4.5 Per cent disease incidence and severity of fungal diseases of strawberry in Malappuram district

Sl.No	Location	Disease	Period					
			Dec- Jan		March-April		July- August	
			PDI	PDS	PDI	PDS	PDI	PDS
1.	Anakkayam	LBM-1	23.20	17.40	58.20	21.70	56.10	19.20
		CRM-1	-	-	82.00	-	75.00	-
		LSM- 1	32.30	21.40	52.40	23.40	49.60	20.90
		LSM-2	23.10	13.40	39.20	21.30	38.10	22.80

PDS-Per cent disease severity PDI-Per cent disease incidence

4.1.2 Correlation of weather parameters with fungal diseases

Weather parameters *viz*., temperature, relative humidity and rainfall collected from different locations during the survey were correlated with intensity and severity of each disease, so as to know the influence of these weather factors on disease development. Weather data collected during November 2015 to April 2017 from various districts are furnished in Table 4.6, Table 4. 7 and Table 4.8 and the details regarding correlation studies are presented in Table 4.9 and 4.10.

4.1.2.1 Leaf spots 1 (LSW-1, LSI-1& LSM-1)

The results of the survey revealed that leaf spots *viz*., LSW-1, LSI-1, LSM-1 were prevalent in Wayanad, Idukki and Malappuram district. Among the three meteorological parameters, it was observed that LSW-1 and LSM-1 from Wayanad and Malappuram district showed no significant correlation with temperature. Moreover, among the three meteorological parameters it was noticed that LSW-1 showed a negative correlation with relative humidity and rainfall. A maximum severity of 22.8 per cent was recorded in case of LSW-1 during December-January, when the temperature was 21.92°C, RH of 74.02 per cent and rainfall of 59.6 mm. It was noticed that from December-January to July-August, RH and rainfall increased from 74.02 to 88.81 per cent and 59.6 to 466.5 mm respectively. This showed that an increase in RH coupled with rainfall can reduce the intensity of LSW-1.

Correlation studies of LSI-1 revealed a significant positive influence of temperature and RH with the disease but had no significant correlation with rainfall. Maximum severity of 19.9 per cent was recorded at a higher temperature of 24.04°C during March-April and a lower PDS of 10.4 per cent at 19.74°C in December-January. Thus we can infer that a slight reduction in temperature can reduce the incidence of LSI-1. Similarly, a positive significant relationship existed between RH and rainfall in case of LSM-1, recording a maximum severity of 23.4 per cent during March-April at a higher temperature of 29.2°C and lower RH and rainfall of 48.58 per cent and 4.6 mm respectively. But a higher RH and rainfall of 66.96 per cent and 504 mm resulted in

lower severity of 20.9 per cent during July-August which was most congenial for the disease development.

4.1.2.2 Leaf spot (LSI-2, LSM-2)

In the present study, the pathogen causing LSI-2 was observed in Idukki, where the pathogen depicted no significant relationship with any of the agrometeorological parameters. The per cent disease severity in two locations *viz*., Vattavada and Koviloor was noticed as 18.7 and 21 per cent during December-January and 22.3 and 17.1 per cent during March-April. Higher severity of 22.3 per cent was noticed under high temperature (24.04°C), RH (95.48%) and lower rainfall (45.4mm) conditions. However, a positive correlation with temperature existed in Anakkayam with no influence on other two parameters. The disease recorded a severity of 22.8 per cent during July-August. But decrease in temperature from 29.2 to 24.23°C and increase in rainfall from 3.2 to 504 mm showed a slight increase in severity from 13.4 to 22.8 per cent in Anakkayam. This showed that a slight reduction in temperature coupled with an increase in rainfall favours the occurrence of disease.

4.1.2.3 Leaf blight 1 (LBW-1)

The disease LBW-1 was noticed only in Ambalavayal of Wayanad district and was found to exhibit a negative correlation with relative humidity and rainfall in the disease development which indicated that the severity of disease reduced as RH and rainfall increased. Maximum severity of 25.2 per cent was recorded during December-January at a temperature of 21.92°C, RH of 74.02 per cent and rainfall of 59.6mm with a subsequent reduction in PDS in other seasons. Relative humidity gradually increased from 80.65 to 88.81 per cent and rainfall from 81.0 to 466.5 mm, thereby, showing less severity of 19.8 and 9.9 per cent respectively. Hence, it is to be noted that low RH and rainfall resulted in a higher disease incidence.

4.1.2.4 Leaf blight 1 (LBI-1)

Plants infected with LBI-1 in Idukki district recorded a positive correlation with temperature and negative correlation with rainfall. However, no considerable variation was noticed with relative humidity and therefore had no influence in disease development. Maximum disease severity of 19.7 per cent was observed at an average temperature of 24.04°C at a low rainfall of 45.4 mm in March-April and the minimum severity of 13.8 per cent was recorded during December-January, where the temperature was 19.74°C and rainfall of 82.6 mm.

4.1.2.5 Leaf blight 1 (LBM-1)

The leaf blight pathogen (LBM-1) was observed in Anakkayam where no significant correlation existed between the weather parameters in disease development, indicating that the disease is least dependent on climatological parameters. But a significantly higher severity and incidence of 21.7 and 58.2 per cent was noticed during March-April when temperature was recorded at its peak of 29.2°C along with a lower RH and rainfall of 48.48 per cent and 4.6mm respectively. The severity and incidence of the disease was found very low during December-January.

4.1.2.6 Crown and root rot 1 (CRI-1)

Pathogen infecting crown and root rot of strawberry (CRI-1) was observed only in Koviloor of Idukki district where a low disease incidence of 30 per cent was noticed in December-January and a higher incidence of 70 per cent in March-April. However, the disease was found unaffected by weather parameters as these showed no significant correlation with the variables during the two seasons.

4.1.2.7 Crown and root rot 1 (CRM-1)

In Anakkayam CRM-1 showed a positive correlation with temperature and a non-significant relation existed between RH and rainfall in disease development which

infers that the disease was least navigated by RH and rainfall. It was observed that the incidence decreased from 82 per cent to 75 per cent when RH and rainfall increased from 48.58 to 66.96 per cent and 4.6 to 504 mm. Thus the weather data clearly depicts that, though there was no significant correlation between rainfall and relative humidity, the incidence of CRM-1 was observed at high rainfall of 504mm and relative humidity of 66.96 per cent compared to the incidence in March-April.

Table 4.6 Weather parameters of Wayanad district

Month	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)	Rainfall (mm)
2015				
November	24.24	18.53	83.23	35.9
December	25.00	19.20	76.04	59.6
2016				
January	27.50	16.00	72.00	0.00
February	30.40	17.96	73.24	0.00
March	32.00	19.80	76.95	16.40
April	31.90	21.00	78.06	64.60
May	30.10	20.10	83.25	219.8
June	25.70	19.30	90.95	334.00
July	25.20	18.80	88.98	285.20
August	25.50	18.60	88.64	181.40
September	26.20	17.90	89.14	52.80
October	27.60	17.90	86.32	27.00
November	27.80	16.90	79.81	20.80
December	27.80	16.00	82.38	20.00
2017				
January	28.50	16.20	68.79	28.80
February	30.60	16.80	62.50	0.00
March	30.20	18.50	73.21	77.20

Table 4.7 Weather parameters of Idukki district

Month	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)	Rainfall (mm)
2015				
November	21.93	18.00	94.4	277.9
December	20.82	17.23	95.3	62.00
2016				
January	20.27	16.98	95.01	0.00
February	21.88	18.90	95.73	0.00
March	25.03	21.35	96.18	16.00
April	26.67	23.13	94.79	29.4
May	25.02	21.25	94.77	117.8
June	21.32	17.03	95.58	321.8
July	20.97	17.06	95.28	241.4
August	21.13	17.55	95.27	139.6
September	21.75	17.15	93.93	61.9
October	22.84	19.24	95.34	131.4
November	22.18	19.15	95.95	82.80
December	21.44	18.84	96.82	26.20
2017				
January	20.74	17.97	95.19	56.40
February	25.89	21.55	95.09	57.40
March	23.48	21.05	95.24	87.40

Table 4.8 Weather parameters of Malappuram district

Month	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)	Rainfall (mm)
2016				
January	27.79	16.16	55.00	0.00
February	33.15	18.31	74.20	0.00
March	36.70	20.51	49.38	4.60
April	38.03	21.58	47.79	0.00
May	35.04	20.35	53.70	65.40
June	27.26	18.21	78.36	494.60
July	27.75	19.20	67.93	342.30
August	29.00	21.00	66.00	161.70
September	29.21	20.16	66.63	62.60
October	29.24	20.00	63.70	44.20
November	29.18	18.10	56.66	24.80
December	27.88	17.00	52.12	03.20
2017				
January	34.75	18.83	55.00	0.00
February	36.00	18.44	54.20	0.00
March	35.24	20.06	52.00	6.60

Table 4.9 Correlation of weather parameters with development of fungal diseases of strawberry

Sl. No.	District	Location	Disease	Correlation coefficient		
				Temperature (°C)	Relative humidity (%)	Rainfall (mm)
1.	Wayanad	Ambalavayal	LSW-1	NS	-0.868***	-0.919***
			LBW-1	NS	-0.975***	-0.904***
2.	Idukki	Vattavada	CRI-1	NS	NS	NS
			LSI-1	0.872***	0.501*	NS
			LSI-2	NS	NS	NS
			LBI-1	0.84	NS	-0.590*
3.	Malappuram	Anakkayam	LBM-1	NS	NS	NS
			CRM-1	0.579*	NS	NS
			LSM-2	0.620**	NS	NS
			LSM-1	NS	0.501*	0.541*

†- Significant at 10% level* - Significant at 5% level** - Significant at 1% level ***

NS- Non significant

Table 4.10 Range statistics of the weather factors of the three locations and three seasons

Sl. No.	District	Season	Disease	Temperature (°C)	Relative Humidity (%)	Rainfall (mm)
1.	Wayanad-Ambalavayal	S1	LSW-1, LBW-1	21.92	74.02	59.6
		S2		26.17	80.65	81
		S3		22.02	88.81	466.5
2.	Idukki-Koviloor, Vattavada	S1	LSI-1, CRI-1 LSI-2, LBI-1	19.74	96.05	82.6
		S2		24.04	95.48	45.4
		S3		19.17	95.27	381
3.	Malappuram-Anakkayam	S1	CRM-2, LSM-2, LSM-1, LBM-4	24.61	53.56	3.2
		S2		29.20	48.58	4.6
		S3		24.23	66.96	504

S1- December-January, S2- March-April, S3- July-August

4.2 ISOLATION OF FUNGAL PATHOGENS

Naturally infected samples of strawberry *viz*., leaves, roots and fruits collected during the field survey from different locations were brought to the laboratory. The pathogens were isolated as per the protocol described in 3.2 and then maintained on Potato Dextrose Agar (PDA) slants by periodic sub culturing.

4.3 PATHOGENICITY TEST OF DIFFERENT ISOLATES

The pathogens isolated from strawberry were tested for pathogenicity by artificial inoculation both under *in vitro* and *in vivo* conditions following Koch's postulates. Symptoms observed under natural conditions and part of the plants infected were given due consideration before inoculating the pathogens. Different methods were followed for inoculating pathogens which varied with different types of diseases *viz*., foliage, fruit and root diseases. The descriptions of symptoms developed after inoculation of each pathogen at each stage are given below.

4.3.1 Foliage diseases

4.3.1.1 *Leaf spots (LSW-1, LSI-1, LSM-1, LSI-2)*

The pathogenicity test of LSW-1 was carried out by Mycelial Bit Inoculation Method (MBIM) and spore suspension method as described in 3.3.1. Both methods proved efficient in establishing the pathogenicity. The results showed that the fungus could infect strawberry leaves within four days after spraying and two days after inoculation with mycelial plugs. Symptoms generally appeared as black watery blotches surrounded by a yellow halo, which later enlarged covering the entire leaf lamina.

Leaf spot pathogen (LSI-1) from Vattavada and Koviloor region of Idukki district were also subjected to pathogenicity test. Symptoms developed successfully by both methods. The lesions developed were black and minute which later expanded to an area of 2.9cm^2 within four days and finally spread all over the leaf lamina. Tissues began to rot or blight as lesions enlarged.

Pathogen causing LSM-1 isolated from Anakkayam developed symptoms successfully by both methods. It was observed that a black lesion was developed on third day of inoculation in case of MBIM and on fifth day with spore suspension method, where the lesions were surrounded by a yellow halo especially when spore suspension was sprayed.

Confirmation of pathogenicity of the isolate causing Leaf spot 2 (LSI-2) was done effectively by both methods. Inoculation of spore suspension of 10^6 cfu ml^{-1} of the pathogen developed typical symptoms like brown to black water soaked lesions with small concentric zonations. Lesions were confined towards leaf margins. Size of the lesion slowly enlarged coalescing the surrounding ones.

4.3.1.2 ***Leaf blights (LBW-1, LBI-1, LBI-2, LBM-1)***

Pathogenicity of LBW-1 pathogen by MBIM produced symptoms within three days of inoculation as small brownish black spots with pale brown centre. Size of the lesion covering an area of 3.75 cm^2 developed five days after inoculation, where the lesions turned water soaked and yellow with tan brown spots, leading to complete blightening of leaves.

Pathogenicity of the isolate causing LBI-1 was proved by both MBIM and spore suspension method. Symptoms started to develop on third day after inoculation which later spread to an area of about 1.5cm^2 on 4^{th} day and 4.5cm^2 on 8^{th} day after inoculation. Pathogenicity established by both the above methods developed initial symptoms as black lesions which appeared within two days after inoculation in case of LBI-2. The lesion spread all over the leaf lamina covering an area of upto 5.9cm^2 of the leaf surface. In case of LBM-1, both MBIM and spore suspension method was proved effective in developing symptoms similar to natural infections. Symptoms appeared within three days after inoculation in case of MBIM and four days with spore suspension method. Severe marginal blightening covering an area of 6.6 cm^2 with brownish black pin head like acervuli was observed on infected leaves.

4.3.2 *Fruit rots (FRW-1)*

Mycelial Bit Inoculation Method was employed for testing the pathogenicity of the isolate on detached strawberry fruits. Symptoms developed as brownish sunken lesions two days after inoculation which later turned water soaked spreading to an area of $4cm^2$.

4.3.3 *Crown and root diseases (CRI-1, CRM-1)*

Fungal spore suspension of 10ml with spore concentration of 10^6 cfu ml^{-1} was prepared and using a sterile blade, a small wound was made at the crown region near the roots where the spore suspension was poured into the rhizosphere region of three month old plants. Initial symptoms appeared fifteen days after inoculation with wilting and yellowing of plants whereby the Roots turned stiff and black with a reddish discolouration of the crown region.

Pathogenicity of the isolate CRM-1 was carried out as that of CRI-1. Here, symptoms initiated as wilting and drooping of plants, 13 days after inoculation where the roots became black and crown tissue appeared brown in colour.

Reisolation of the pathogens artificially inoculated on strawberry plants were carried out after symptom expression following Koch's postulates. The pathogen isolated were periodically sub cultured on PDA slants after purification and their cultural and morphological characteristics were studied and compared with the original isolates. Thus the pathogenicity was confirmed for each isolate.

4.4 SYMPTOMATOLOGY OF DISEASES

Symptoms of various diseases caused by fungal pathogens in strawberry were studied both under natural as well as artificial conditions.

4.4.1 Symptomatology under natural condition

Various distinct symptoms developed on strawberry plants caused by different fungal pathogens were recorded during the survey period which included foliage symptoms, fruit rots, crown and root rots.

4.4.1.1 *Foliage symptoms*

The major foliage diseases observed during the study were leaf spots and leaf blights. Among the leaf blights, four distinct types of symptoms were noticed and two types of spots were recorded in case of leaf spots.

4.4.1.1.1 Leaf spots (LSW-1, LSI-1, LSM-1, LSI-2)

In case of the pathogen causing LSW-1 isolated from Ambalavayal, numerous round black spots appeared all over the leaflets which gradually expanded and coalesced covering entire leaves, where the leaves blightened gradually [plate 4.1(i) a]. Leaf spot (LSI-1) isolated from Vattavada and Koviloor regions of Idukki developed black spots on young as well mature leaves which later coalesced and developed bigger lesions. Some spots were surrounded by a yellow halo [plate 4.1(i) b]. These symptoms were similar to that observed with LSW-1.

Symptoms of leaf spot disease, LSM-1, were characterised with an irregular leaf spot converted into blightened areas along the margins and tip of leaves and extending inward into the leaf veins. Petioles were also found infected causing the leaves to bend down. White mycelial growth was seen along the leaves and petiole [plate 4.1(i) c].

Symptoms of LSI-2 initially appeared as brown circular spots of 2 cm size with concentric zonations. The centre of the lesion was light in colour mostly confined towards the leaf margin in a V shaped pattern surrounded by a yellow or dark reddish purple margin. Severely infected foliage turned necrotic and the plant ultimately appeared weak [plate 4.1(i) d].

4.4.1.1.2 Leaf blights (LBW-1, LSI-1, LSI-2, LBM-1)

Leaf blight (LBW-1) was observed on mature leaves of nursery plants where the symptoms initiated as small reddish black spots all over the leaf lamina which later coalesced to develop a purplish discoloration which were observed on veins also [plate 4.1(i) e]. Symptoms of LBI-1 initiated with small circular brown spots on leaves which later spread all over the leaf lamina forming a 'V' shaped enlarged lesion from the margin progressing inward. Purple lesions with a yellow halo expanded through major veins and the whole leaf turned brown. Infections were confined to the plants grown in open field conditions under Vattavada regions of Idukki district [plate 4.1(i) f].

The pathogen causing LBI-2 isolated from Idukki district produced symptoms as small brown spots on leaf lamina which enlarged later caused blightening of the margins. In some cases, lesions spread all over the leaves, where the whole leaf turned brown [plate 4.1(ii) g]. Organism associated with leaf blight (LBM-1) produced light brown spots on both young and mature leaves with black pin head like acervuli on upper leaf surface. Spots enlarged and spread irregularly all over the leaf surface starting from margin giving a blightened appearance which ultimately led to drying up of leaves [plate 4.1(ii) h].

4.4.1.2 Fruit rot (FRW-1)

Rotten fruits were collected from open fields of Ambalavayal where black and hard encrustations were formed on either side of the fruits which ultimately led to fruit rot [plate 4.1(ii) i].

4.4.1.3 Crown and root rots (CRI-1, CRM-1)

In field, plants infected with CRI-1 showed poor growth with wilting, stunted and withering due to root and crown rot infection, thereby resulting in complete death and collapse of plants. All the main roots nearing the crown region turned dark brown to black. Moreover, the vascular and cortical tissues developed a reddish-orange

Plate 4.3 (i) Symptomatology of diseases

a) Leaf spot 1 (LSW-1)

b) Leaf spot 1 (LSI-1)

c) Leaf spot 1 (LSM-1)

d) Leaf spot 2 (LSI-2)

e) Leaf blight 1 (LBW-1)

f) Leaf blight 1 (LBI-1)

discolouration and also the roots appeared black in colour with white fluffy mycelial growth [plate 4.1(ii) j].

The pathogen CRM-1 was isolated from Anakkayam under polyhouse conditions. Symptoms appeared as complete wilting and withering of plants. Leaf petiole near the crown region were found infected showing a slight rotting. The infected crowns when cut open showed an internal reddish discoloration [plate 4.1(ii) k].

4.4.2 Symptomatology under artificial condition

To study the symptomatology of different pathogens under artificial conditions, the pathogens isolated were artificially inoculated as per the protocol mentioned in 3.3.1 and 3.3.2. Symptom development under artificial inoculation was similar to that under natural conditions. Lesion size and time for symptom development after pathogen inoculation are presented in Table 4.11.

On leaves, the pathogen causing LSW-1 produced initial symptoms within 48h with a lesion size of 1.3 cm^2 and the symptoms spread completely within 6 to 8 days with a lesion size ranging from 4.7 to 5.5 cm^2. Infected leaves appeared yellow and started to defoliate on live plants. In case of LSI-1, symptoms initiated on second day of inoculation of size 1.2 cm^2 and further spreading to 2.9 to 5.7 cm^2 within 8 days. Likewise, in LSM-1, symptom developed on third day and continued to spread upto an area of about 5.2 cm^2 of leaf lamina on the 8^{th} day. Black lesions spread from margin of the leaf towards the bottom were seen in case of LSI-2. Lesions covered 1.3 cm^2 of leaf surface on second day and progressed to expand upto 7.2 cm^2. Minute lesion of size 1.9 cm^2 were produced by LBW-1 on 4^{th} day of inoculation which started to enlarge its size by reaching upto 5.4 cm^2. Similarly, LBI-1 started expanding all over the inoculated leaf surface by growing upto 4.5cm^2 within 8 days. Moreover, leaf blight-2 (LBI-2) also showed early symptoms on leaf surface on second day of inoculation where size of lesions ranged from 1.2 to 5.9 cm^2. Black lesions covering an area 1.2 cm^2 on the second day of inoculation was recorded in case of LBM-1 subsequently expanding to 6.3 cm^2 on 8^{th} day after inoculation.

Plate 4.3 (ii) Symptomatology of diseases

g) Leaf blight 2 (LBI-2)

h) Leaf blight 1 (LBM-1)

h) Fruit rot (FRW-1)

j) Crown and root rot (CRI-1)

k) Crown and root rot (CRM-1)

Table 4.11 Differential response of artificial inoculation of fungal pathogens on strawberry plants

Sl. No.	Symptom	Lesion size (cm^2)				Days of first symptom development
		Days after inoculation				
		2	4	6	8	
1.	LSW-1	1.3	2.5	4.7	5.5	2
2.	LSI-1	1.2	2.9	4.6	5.7	2
3.	LSM-1	0	1.9	3.2	5.2	3
4.	LSI-2	1.3	3.4	6.1	7.2	2
5.	LBW-1	0	1.9	3.1	5.4	3
6.	LBI-1	0	1.5	2.9	4.5	3
7.	LBI-2	1.2	2.6	3.9	5.9	2
8.	LBM-1	1.2	3.00	5.5	6.3	2
9.	FRW-1	1.4	2.9	-	-	2

Pathogen causing fruit rot (FRW-1) produced typical sunken and water soaking on inoculated areas of the fruit. Size of lesions expanded from 1.4 to 2.9 cm^2 within 4 days of inoculation. Pathogen causing crown and root rot were inoculated on live plants by adding spore suspension in the rhizosphere region. In case of CRI-1, symptoms like wilting and discolouration initiated on 15th day of inoculation. Similar symptoms were observed with CRM-1 too, where it developed on 16th day after inoculation.

4.5 CHARACTERISATION AND IDENTIFICATION OF PATHOGEN

Cultural and morphological characterisation of the isolated fungal pathogens were carried out for identification of the pathogens involved in disease development. The detailed description of each fungal isolate are presented below.

4.5.1 Cultural and morphological characters

4.5.1.1 Leaf spots

a) LSW-1, LSI-1, LSM-1

Three leaf spot pathogens LSW-1, LSI-1 and LSM-1 were recorded, one each from three different districts during the survey. The cultural and morphological scharacteristics of each of them were studied in detail. The pathogen causing LSW-1 was characterised with white aerial mycelia gradually turning grey. Reverse side of the plate appeared dark grey. Pathogen attained full growth in Petri dish in ten days at room temperature. Hyphae hyaline, septate, turned to greyish white, conidia hyaline, single celled, straight and cylindrical with round or obtuse ends of size 8.88-15.06 x 2.34-5.01µm [plate 4.2(i) a].

Isolate LSI-1 from Idukki developed dark grey colonies with subsurface growth. Reverse side of the colony appeared grey to greenish black. The isolate produced pink spore mass in the medium after 23 days after inoculation. Colony on reverse appeared greyish black in colour. Hyphae septate, hyaline initially, turning to greyish black, conidia hyaline, one celled, oblong with round ends measuring 7.60-14.30 x 3.43-6.12 µm [plate 4.2 (i) b].

The isolate LSM-1 in culture appeared pale white to grey forming a uniform growth with regular margins. Colony on reverse side of the plate appeared grey and attained 90 mm in ten days. Hyphae hyaline, turned grey on ageing and septate. Conidia cylindrical, sides straight with round ends. Conidial size ranged from 6.98-13.54 x 3.12-

6.7 µm. Based on these characteristics, the pathogen causing LSW-1, LSI-1 and LSM-1 were tentatively identified as *Colletotrichum* sp. [plate 4.2 (i) c].

b) LSI-2

The pathogen causing LSI-2 prevalent in Vattavada and Koviloor regions of Idukki district and Anakkayam of Malappuram district produced submerged mycelium with profuse growth which attained 90 mm within 11 days. Colony initially creamy to ashy white, gradually turned grey with margins entire and circular. Plates on reverse side appeared greyish black. Hyphae thin, septate, brown in colour. Conidia abundant, spindle to ellipsoidal, obclavate, formed in chains, dark brown, transversely (1-3) and longitudinally (2-6) septate (muriform) with size ranging from 24.96 to 46.89 µm long to 11.40 to 15.69 µm wide with short beak of 1.26-1.53 µm. Based on the above characters the fungus was tentatively identified as *Alternaria* sp. [plate 4.2 (i) d].

4.5.1.2 Leaf blights

a) LBW-1

Thin, pale white mycelia gradually turning to brown of LBW-1 was observed on PDA when cultured under artificial conditions. Margin of the colony was smooth and circular. Colony diameter reached 90 mm within three days indicating its fast growth. Hyphae septate, initially appeared hyaline where the colour turned light brown after seven days and branches arised at right angles below the septa with distinct constrictions.

Hyphal length and width ranged from 88.23 to 98.17µm and 7.45-8.47 µm and branches arose 27.93 µm down the septa of the hyphae. Sporulation found absent. Based on these cultural and morphological characteristics, the isolate was identified tentatively as *Rhizoctonia* sp. [plate 4.2 (i) e]

b) LBI-1

Pathogen causing LBI-1 produced pale white to grey colonies in culture expanding in a zonate manner with irregular margins. Culture on the reverse side of the

Plate 4.4 (i) Cultural characters of pathogens

a) *C. gloeosporioides* (LSW-1)

b) *C. gloeosporioides* (LSI-1)

c) *C. gloeosporioides* (LSM-1)

d) *A. alternata* (LSI-2)

e) *R. solani* (LBW-1)

f) *P. exigua* (LBI-1)

Plate 4.5 (i) Morphological characters of pathogens

a) *C. gloeosporioides* (LSW-1)

b) *C. gloeosporioides* (LSI-1)

c) *C. gloeosporioides* (LSM-1)

d) *A. alternata* (LSI-2)

e) *R. solani* (LBW-1)

f) *P. exigua* (LBI-1)

plate appeared greyish. Growth of the pathogen in 90 mm plates was completed within 12 days. Hyphae initially hyaline, turned brown and septate. Conidia hyaline, aseptate, ellipsoidal to oblong, 6.18 to 7.62 × 2.29 to 3.27 µm dimension. Copious amount of globoid, olivaceous black pycnidia developed in 30 days were seen scattered and submerged in agar. Based on these characteristics, pathogen is tentatively identified as *Phoma* sp. [plate 4.2 (i) f].

c) LBI-2

Pathogen causing LBI-3 observed in Vattavada of Idukki district produced raised, fluffy, velvety greyish black mycelium on PDA which later turned flat dark black on maturity and completed its growth in Petri plate within five days of inoculation. Mycelium septate, dark brown to black, branched and showed excellent sporulation after 16 days of inoculation. Conidiophore sympodial, conidia dark brown, four celled with three to four septa, two cells at centre larger and darker than terminal cells. Cells at both ends light brown with rounded tips. Third cell from base is slightly curved and the conidia assumes the shape of a boat or half-moon. Spores 16.65- 18.98 µm long and 6.68- 9.96 µm wide. The above characters thus tentatively confirms the identity of the isolate as *Curvularia* sp. [plate 4.2 (ii) g].

d) LBM-1

The leaf blight LBM-1 observed from Anakkayam district during March-April and July-August produced white fluffy aerial mycelium with black ink mass which are the acervuli bearing conidia after 15 days of inoculation. Pathogen attained full growth in plate in 10 days. Reverse side of colony appeared light golden yellow. Conidia spindle shaped, straight or slightly curved with five cells. Size varied from 22.02 to 36.95 µm long and 8.85 to 11.98µm wide. Three median cells at the centre appeared darker and thicker than the end cells which were hyaline and thinner. Apical cells were observed to be slightly pointed. Based on these characters the identity of the pathogen was tentatively confirmed as *Pestalotiopsis* sp. [plate 4.2 (ii) h].

4.5.1.3 Fruit rot

a) FRW-1

Mycelia in culture white, later turning light brown producing long thread like hyphae showing fast abundant growth which attained 90 mm within three days. Margin of colony circular, smooth and hyphae hyaline gradually turned brown and branches arise at 90° below the septa with distinct constrictions. Sporulation absent and hyphal length ranged from 121.23 to 150.98 µm with new hyphae arising 24.01 µm at right angles. Based on these cultural and morphological characteristics, the isolate was identified as *Rhizoctonia* sp. [plate 4.2 (ii) i].

4.5.1.4 Crown and root rots

a) CRI-1

The disease was observed in open fields of Koviloor region of Idukki district. Pathogen upon culturing on PDA produced white fluffy mycelium covering the Petri plates in six days. Subsequently, the colour changed and turned light pinkish to purple after incubating at 26 ±2°C for 11 days. Pathogen produced aerial mycelium with regular margins. Hyphae hyaline, septate and branched. Colonies produced both macroconidia and microconidia with phialides. Macroconidia hyaline with 3 to 4 septae, slightly curved apical cells and foot shaped basal cells measuring 8.50 to 18.71µm long and 2.1 to 4.9 µm wide. Microconidia borne abundantly and are one celled, oval to ellipsoid with size of 1.64 to 3.92 x 4.12 to 9.34 µm. In accordance with these characters, the pathogen was identified as *Fusarium* sp. [plate 4.2 (ii) j].

b) CRM-1

Crown and root rot pathogen CRM-1 isolated from Anakkayam developed colonies on PDA initially white to smoky grey, mycelium dense mat like, attained full plate growth within three days when incubated at room temperature. Subsequently, colour changed to greyish black to deep black and the colony appeared as fluffy aerial

growth with deep black color on the reverse side. Dark black pycnidia developed in twenty days which aggregated to form tough and round structures. Hyphae septate with brown to black color. Conidia initially single celled, hyaline, smooth, thick walled, with tapered apex and brown, ellipsoid or obovate, thick walled, with round apex and longitudinal striations when mature. A single septa divided the conidia into two with both ends round with 23.54 - 32.17 x 12.75 -16.05 μm size. These characteristics tentatively confirms the pathogen as *Lasiodiplodia* sp. [plate 4.2 (ii) k].

On comparing the cultural and morphological characteristics given in CMI description of fungi and bacteria and in the book 'Hyphomycetes: Taxonomy and Biology' (Subramanian, 1983), all the fungal isolates were tentatively identified. For further confirmation of the isolates upto the species level, the isolates were send to NCFT, New Delhi where the cultures were identified and thereafter maintained in the repository with proper identification numbers (ID. No.). The details are presented in the Table 4.12.

Plate 4.4 (ii) Cultural characters of pathogens

g) *C. lunata* (LBI-2)

h) *P. longisetula* (LBM-1)

i) *R. solani* (FRW-1)

j) *F. oxysporum* (CRI-1)

k) *L. theobromae* (CRM-1)

Plate 4.5 (i) Morphological characters of pathogens

g) *C. lunata* (LBI-2)

h) *P. longisetula* (LBM-1)

i) R. solani (FRW-1)

j) *F. oxysporum* (CRI-1)

k) *L. theobromae* (CRM-1)

Table 4.12 Identification of fungal pathogens

Sl. No.	Symptom	Pathogen	ID. No.
1.	Leaf spot1 (LSW-1)	*Colletotrichum gloeosporioides*	8227.16
2.	Leaf spot 1(LSI-1)	*Colletotrichum gloeosporioides*	8228.16
3.	Leaf spot 1(LSM-1)	*Colletotrichum gloeosporioides*	8225.16
4.	Leaf spot 2(LSI-2)	*Alternaria alternata*	8230.16
5.	Leaf blight 1(LBW-1)	*Rhizoctonia solani*	8231.16
6.	Leaf blight 1(LBI-1)	*Phoma exigua*	8229.16
7.	Leaf blight 2(LBI-2)	*Curvularia lunata*	8223.16
8.	Leaf blight 1(LBM-1)	*Pestalotiopsis longisetula*	8233.16
9.	Fruit rot 1(FRW-1)	*Rhizoctonia solani*	8232.16
10.	Crown and root rot 1(CRI-1)	*Fusarium oxysporum*	8225.16
11.	Crown and root rot 1(CRM-1)	*Lasiodiplodia theobromae*	8224.16

4.6 *In vitro* EVALUATION OF FUNGICIDES, ORGANIC PREPARATIONS AND BIOAGENTS AGAINST PATHOGENS

Efficacy of different fungicides, organic preparations and biocontrol agents were evaluated against fungal pathogens of strawberry under *in vitro* conditions. Poison food technique was used to evaluate fungicides and organic preparations and those with biocontrol agents with dual culture technique respectively as described in 3.6.1 and 3.6.2. Details of the experiment are presented in Tables 4.13 to 4.22.

4.6.1 *In vitro* evaluation of fungicides

The pathogens *viz.*, *Colletotrichum gloeosporioides* (LSW-1, LSI-1, LSM-1), *Rhizoctonia solani* (LBW-1, FRW-1), *Pestalotiopsis longisetula, Alternaria alternata, Phoma exigua, Fusarium oxysporum, Curvularia lunata* and *Botyrodiplodia theobromae* were tested against three doses of nine fungicides. A significant difference was observed in the mycelial growth of different pathogens when tested with various concentrations of different fungicides.

4.6.1.1 *Colletotrichum gloeosporioides* (LSW-1, LSI-1, LSM-1)

In vitro evaluation of LSW-1 pathogen with six fungicides *viz.*, carbendazim 12% + mancozeb 63% (Saaf), copper hydroxide 77WP (Kocide), copper oxychloride 50WP (Fytolan), propineb 70WP (Antracol), difenoconazole 25 EC (Score) and carbendazim 50WP (Bavistin) revealed cent per cent inhibition at all concentrations. From the Table 4.13, it was observed that both cymoxanil 8% + mancozeb 64% (Curzate M8) and Bordeaux mixture at its lower concentration of 0.15 and 0.5 per cent inhibited the mycelial growth by 97.67 and 88.88 per cent whereas cent per cent inhibition was noticed at two higher concentrations (Plate 4.4). However, among various fungicides tested, potassium phosphonate (Akomin 40) showed the least inhibition of 42.67, 43.33 and 43.88 per cent at 0.25, 0.3 and 0.35 per cent concentration respectively.

In case of LSI-1, fungicides *viz.*, carbendazim 12% + mancozeb 63% (Saaf) and carbendazim 50WP (Bavistin) recorded cent per cent inhibition of the pathogen. Also, propineb 70 WP (Antracol) at higher two concentrations of 0.3 and 0.35 per cent

inhibited the mycelial growth by cent per cent. These observations were closely followed by cymoxanil 8% + mancozeb 64% (Curzate M8) where concentrations of 0.15, 0.2 and 0.25 per cent restricted the fungal growth by 93.44, 95.44 and 97.67 per cent respectively. It was also noticed that difenoconazole 25 EC (Score), copper oxychloride 50WP (Fytolan) and Bordeaux mixture exhibited 70 to 80 per cent inhibition in the growth of the pathogen (Plate 4.5). However, fungicides like copper hydroxide 77WP (Kocide) and potassium phosphonate (Akomin 40) were found comparatively less effective against the pathogen.

Studies conducted on LSM-1 pathogen revealed that fungicides *viz.*, carbendazim 12% + mancozeb 63% (Saaf), cymoxanil 8% + mancozeb 64% (Curzate M8), copper hydroxide 77WP (Kocide), carbendazim 50WP (Bavistin) and Bordeaux mixture at all the three concentrations and highest two concentrations of Propineb 70WP (Antracol) and difenoconazole 25 EC (Score) showed cent per cent inhibition against the pathogen. However, copper oxychloride 50WP (Fytolan) at 0.2, 0.25 and 0.3 per cent showed an inhibition of 77.22, 80.33 and 83.77 per cent respectively. Potassium phosphonate (Akomin 40) showed the least inhibition on the pathogen at all its concentrations (Plate 4.6).

4.6.1.2 *Alternaria alternata* (LSI-1)

Among the various fungicides tested, cymoxanil 8% + mancozeb 64% (Curzate M8), copper hydroxide 77WP (Kocide), copper oxychloride 50 WP (Fytolan) and propineb 70WP (Antracol) were found cent per cent effective against the leaf blight pathogen at all the three concentration. Highest concentration of carbendazim 12% + mancozeb 63% (Saaf) and Bordeaux mixture at 1 and 1.5 per cent were also found cent per cent effective. Difenoconazole 25 EC (Score) at 0.05, 0.2 and 0.15 per cent recorded 76.44, 78.32 and 89.0 per cent inhibition respectively. Carbendazim 50WP (Bavistin) showed an inhibition of 33.88 to 55.55 per cent at varying concentrations. Potassium phosphonate (Akomin 40) was the least effective among all the fungicides tested (Plate 4.7).

Table 4.13 *In vitro* evaluation of fungicides against *Colletotrichum gloeosporioides*

Sl No.	Fungicide	Conc (%)	Per cent Inhibition over control		
			Colletotrichum gloeosporioides (LSW-1)	*Colletotrichum gloeosporioides* (LSI-1)	*Colletotrichum gloeosporioides* (LSM-1)
1.	Carbendazim 12% + Mancozeb 63% (Saaf)	0.15	100(10) [a]	100(10) [a]	100(10) [a]
		0.20	100(10) [a]	100(10) [a]	100(10) [a]
		0.25	100(10) [a]	100(10) [a]	100(10) [a]
2.	Cymoxanil 8% + Mancozeb 64% (Curzate M8)	0.15	97.67(9.86) [b]	93.44(9.66) [d]	100(10) [a]
		0.20	100(10) [a]	95.44(9.76) [c]	100(10) [a]
		0.25	100(10) [a]	97.67(9.89) [b]	100(10) [a]
3.	Copper hydroxide 77WP (Kocide)	0.10	100(10) [a]	46.67(6.84) [m]	100(10) [a]
		0.15	100(10) [a]	47.22(6.88) [m]	100(10) [a]
		0.20	100(10) [a]	50(7.1) [l]	100(10) [a]
4.	Copper oxychloride 50WP (Fytolan)	0.20	100(10) [a]	83.33(8.7) [i]	77.22(8.81) [e]
		0.25	100(10) [a]	86.67(8.9) [f]	80.33(8.98) [c]
		0.30	100(10) [a]	100(10) [a]	83.77(9.19) [b]
5.	Propineb 70WP (Antracol)	0.25	100(10) [a]	93.33(9.68) [d]	84.44(9.22) [b]
		0.30	100(10) [a]	100(10) [a]	100(10) [a]
		0.35	100(10) [a]	100(10) [a]	100(10) [a]
6.	Carbendazim 50WP (Bavistin)	0.05	100(10) [a]	100(10) [a]	100(10) [a]
		0.10	100(10) [a]	100(10) [a]	100(10) [a]
		0.15	100(10) [a]	100(10) [a]	100(10) [a]
7.	Difenoconazole 25EC (score)	0.05	100(10) [a]	70.77(8.39) [k]	79.44(8.93) [d]
		0.10	100(10) [a]	72.77(8.54) [j]	100(10) [a]
		0.15	100(10) [a]	82.44(9.1) [e]	100(10) [a]
8.	Potassium phosphonate (Akomin 40)	0.25	42.67(6.52) [f]	11.11(3.35) [p]	19.44(4.49) [h]
		0.30	43.33(6.61) [e]	12.44(3.51) [o]	26.67(5.1) [g]
		0.35	43.88(6.73) [d]	13.67(3.72) [n]	31.67(5.65) [f]
9.	Bordeaux Mixture	0.50	88.88(9.38) [c]	77.77(8.8) [h]	100(10) [a]
		1.0	100(10) [a]	78.88(8.86) [g]	100(10) [a]
		1.50	100(10) [a]	79.44(8.93) [f]	100(10) [a]
	CD (0.05)		0.044	0.042	0.036

*Mean of the three replications

In each column figure followed by same letter do not differ significantly according to DMRT.

$\sqrt{x+0.5}$ transformed values are given in parantheses

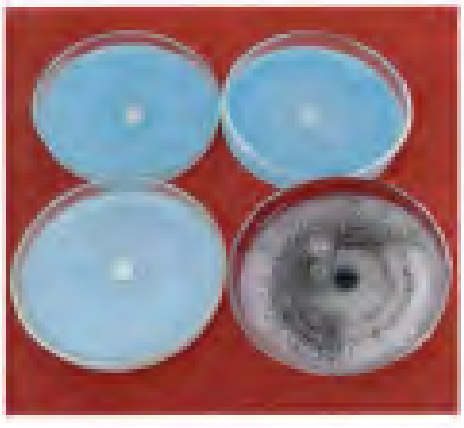
Plate 4.6 *In vitro* evaluation of fungicides against *Colletotrichum gloeosporioides* (LSW-1)

a) Carbendazim 12%+mancozeb 63% b) Copper hydroxide 77WP

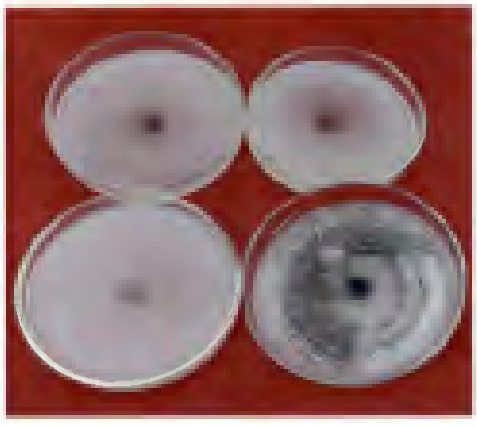
c) Copper oxychloride 50WP d) Propineb 70WP

e) Difenoconazole 25EC f) Carbendazim 50WP

Plate 4.7 *In vitro* evaluation of fungicides against *Colletotrichum gloeosporioides* (LSI-1)

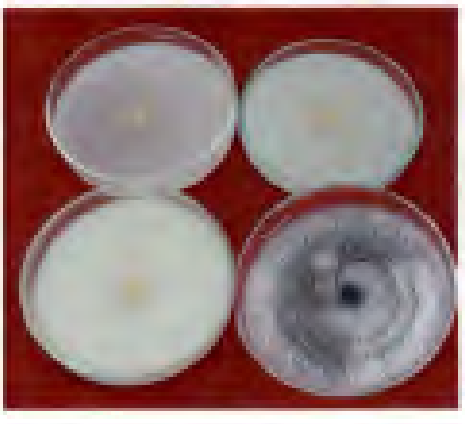


a) Carbendazim 12% + mancozeb 63%



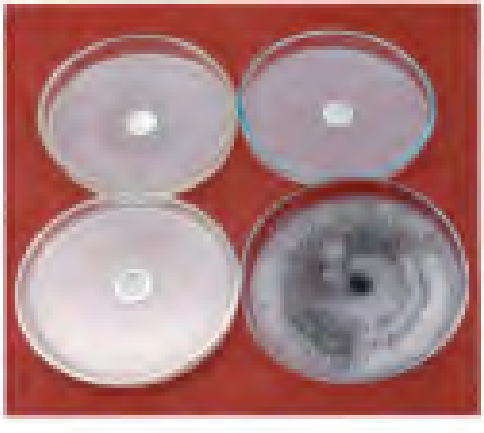
b) Carbendazim 50WP

c) Propineb 70WP



d) Cymoxanil 8%+mancozeb 64%

e) Copper oxychloride 50WP



f) Difenoconazole 25EC

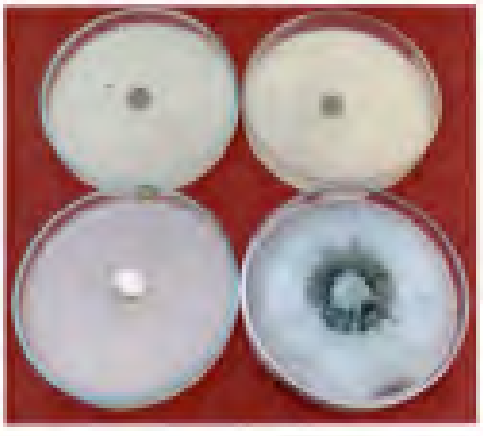
Plate 4.8 *In vitro* evaluation of fungicides against *Colletotrichum gloeosporioides* (LSM-1)

a) Carbendazim 12% + mancozeb 63%

b) Copper hydroxide 77WP

c) Carbendazim 50WP

d) Bordeaux mixture



e) Cymoxanil 8%+mancozeb 64%

f) Propineb 70WP

Table 4.14 *In vitro* evaluation of fungicides against *Alternaria alternata*, *Rhizoctonia solani* and *Phoma exigua*

Sl No.	Fungicide	Conc (%)	Per cent Inhibition over control		
			Alternaria alternata (LSI-2)	*Rhizoctonia solani* (LSW-1)	*Phoma exigua* (LSI-1)
1.	Carbendazim 12% + Mancozeb 63% (Saaf)	0.15	55(7.47)^{f}	100(10)^{a}	100(10)^{a}
		0.20	72.22(8.52)^{e}	100(10)^{a}	100(10)^{a}
		0.25	100(10)^{a}	100(10)^{a}	100(10)^{a}
2.	Cymoxanil 8% + Mancozeb 64% (Curzate M8)	0.15	100(10)^{a}	77.77(8.79)^{d}	100(10)^{a}
		0.20	100(10)^{a}	78.88(8.88)^{c}	100(10)^{a}
		0.25	100(10)^{a}	80(8.94)^{b}	100(10)^{a}
3.	Copper hydroxide 77WP (Kocide)	0.10	100(10)^{a}	65(8.1)^{h}	58.33(7.56)^{c}
		0.15	100(10)^{a}	69.44(8.38)^{g}	58.88(7.62)^{c}
		0.20	100(10)^{a}	72(8.52)^{f}	68.33(8.26)^{b}
4.	Copper oxychloride 50WP (Fytolan)	0.20	100(10)^{a}	25.55(5.12)^{l}	100(10)^{a}
		0.25	100(10)^{a}	36.67(5.87)^{k}	100(10)^{a}
		0.30	100(10)^{a}	39(6.07)^{g}	100(10)^{a}
5.	Propineb 70WP (Antracol)	0.25	100(10)^{a}	100(10)^{a}	100(10)^{a}
		0.30	100(10)^{a}	100(10)^{a}	100(10)^{a}
		0.35	100(10)^{a}	100(10)^{a}	100(10)^{a}
6.	Carbendazim 50WP (Bavistin)	0.05	33.88(5.82)^{h}	100(10)^{a}	100(10)^{a}
		0.10	40(6.32)^{g}	100(10)^{a}	100(10)^{a}
		0.15	55.55(7.46)^{f}	100(10)^{a}	100(10)^{a}
7.	Difenoconazole 25EC (score)	0.05	76.44(8.75)^{d}	57.77(7.64)^{i}	100(10)^{a}
		0.10	78.32(8.88)^{c}	72.22(8.56)^{f}	100(10)^{a}
		0.15	89(9.46)^{b}	75(8.66)^{e}	100(10)^{a}
8.	Potassium phosphonate (Akomin 40)	0.25	16.67(4.11)^{k}	0	23.33(4.97)^{f}
		0.30	24.44(4.96)^{j}	0	25.67(5.3)^{e}
		0.35	27.77(5.27)^{i}	0	29.44(5.97)^{d}
9.	Bordeaux Mixture	0.50	78.88(8.89)^{c}	100(10)^{a}	100(10)^{a}
		1.0	100(10)^{a}	100(10)^{a}	100(10)^{a}
		1.50	100(10)^{a}	100(10)^{a}	100(10)^{a}
	CD (0.05)		0.029	0.047	0.039

*Mean of the three replications

In each column figure followed by same letter do not differ significantly according to DMRT.

$\sqrt{x+0.5}$ transformed values are given in parantheses

97

Plate 4.9 *In vitro* evaluation of fungicides against *Alternaria alternata* (LSI-2)

a) Cymoxanil 8%+mancozeb 64%

b) Copper hydroxide 77WP

c) Propineb 70WP

d) Copper oxychloride 50WP

e) Bordeaux mixture

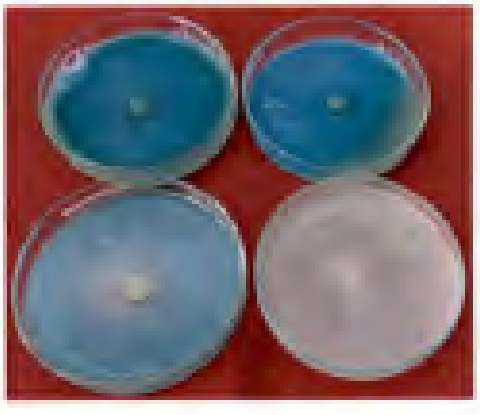
f) Difenoconazole 25EC

Plate 4.10 *In vitro* evaluation of fungicides against *Rhizoctonia solani* (LBW-1)

a) Carbendazim 12%+mancozeb 63%

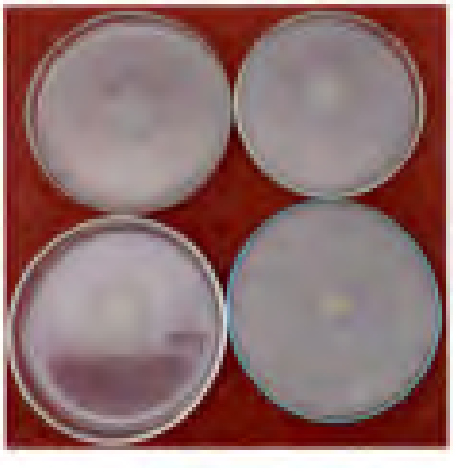
b) Propineb 70WP

c) Carbendazim 50WP



d) Bordeaux mixture

e) Cymoxanil 8%+mancozeb 64%



f) Difenoconazole 25EC

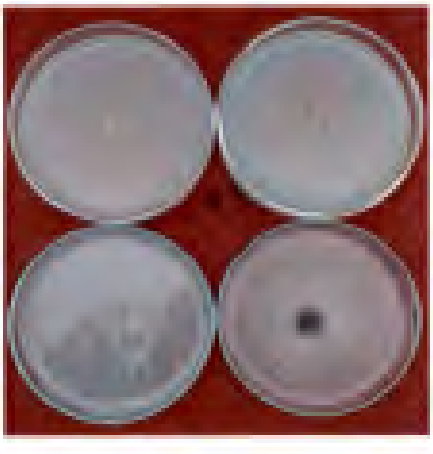
Plate 4.11 *In vitro* evaluation of fungicides against *Phoma exigua* (LBI-1)

a) Copper oxychloride 50WP

b) Carbendazim 50WP

e) Cymoxanil 8%+mancozeb 64%

d) Propineb 70WP



e) Carbendazim 12%+mancozeb 63%

f) Difenoconazole 25EC

4.6.1.3 *Rhizoctonia solani* (LBW-1)

Four fungicides *viz.*, carbendazim 12% + mancozeb 63% (Saaf), carbendazim 50WP (Bavistin), propineb 70WP (Antracol) and Bordeaux mixture inhibited the pathogen by cent per cent at all the concentrations tested. The fungicides cymoxanil 8% + mancozeb 64% (Curzate M8) at all concentrations recorded a per cent inhibition of 77 to 80. Copper hydroxide 77WP (Kocide) and difenoconazole 25 EC (Score) at the highest concentrations of 0.15 and 0.2 per cent was equally effective against the pathogen by 72 to 75 per cent respectively (Plate 4.8). Copper oxychloride 50 WP (Fytolan) at all concentrations showed comparatively less inhibition of below 40 per cent, whereas, potassium phosphonate (Akomin 40) showed the least per cent inhibition of the pathogen at all the concentrations.

4.6.1.4 *Phoma exigua* (LBI-1)

All the six fungicides *viz.*, carbendazim 12% + mancozeb 63% (Saaf), propineb 70 WP (Antracol), difenoconazole 25 EC (Score), cymoxanil 8% + mancozeb 64% (Curzate M8), carbendazim 50WP (Bavistin), copper oxychloride 50WP (Fytolan) and Bordeaux mixture at all concentrations showed cent per cent inhibition against the pathogen (Plate 4.9). However, copper hydroxide 77WP (Kocide) could exhibit only more than 50 per cent inhibition of the pathogen and potassium phosphonate (Akomin 40) recorded the lowest level of inhibition at all concentration.

4.6.1.5 *Curvularia lunata* (LSI-2)

Studies pertaining to the inhibition of fungicides against *Curvularia lunata* revealed that four fungicides *viz.*, cymoxanil 8% + mancozeb 64% (Curzate M8), propineb 70WP (Antracol), difenoconazole 25 EC (Score) and Bordeaux mixture exhibited 100 per cent inhibition at all the three concentrations. An inhibition greater than 70 per cent was recorded with copper hydroxide 77 WP (Kocide) at all concentrations. Moreover, carbendazim 12% + mancozeb 63% (Saaf) and copper oxychloride 77 WP (Fytolan) inhibited the pathogen above 65 and 80 per cent

respectively at various concentrations tested (Plate 4.10). Other chemicals like carbendazim 50WP (Bavistin) and potassium phosphonate (Akomin 40) showed very poor inhibition of the pathogen.

4.6.1.6 *Pestalotia longisetula* (LBM-1)

From the Table 4.15 it was observed that except for carbendazim, potassium phosphonate and difenoconazole, all the fungicides exhibited cent per cent inhibition of the pathogen. Difenoconazole 25 EC (Score) at all concentrations were also effective against the pathogen as it showed an inhibition of above 80 per cent (Plate 4.11). However, carbendazim 50WP (Bavistin) at 0.05, 0.1 and 0.15 per cent recorded only 57.22, 66.11 and 75 per cent inhibition. Potassium phosphonate (Akomin 40) showed the least inhibition of the pathogen at all concentration.

4.6.1.7 *Rhizoctonia solani* (FRW-1)

Out of the total nine fungicides tested, four fungicides *viz.*, carbendazim 12% + mancozeb 63% (Saaf), cymoxanil 8% + mancozeb 64% (Curzate M8), propineb 70WP (Antracol) and Bordeaux mixture at all concentrations exhibited higher efficacy in inhibiting the pathogen by cent per cent. However, copper hydroxide 77WP (Kocide) and difenoconazole 25 EC (Score) inhibited the pathogen only by 54.44 to 75 per cent and 58.55 to 70.44 per cent at three concentrations tested (Plate 4.12). Likewise, copper oxychloride 50 WP (Fytolan) recorded only less than 45 per cent in inhibiting the pathogen. Carbendazim 50WP (Bavistin) and potassium phosphonate (Akomin 40) were found to be the least effective.

4.6.1.8 *Fusarium oxysporum* (CRI-1)

Cent per cent inhibition of the pathogen was achieved with fungicides *viz.*, carbendazim 12% + mancozeb 63% (Saaf), cymoxanil 8% + mancozeb 64% (Curzate M8), copper hydroxide 77WP (Kocide) and carbendazim 50WP (Bavistin) at all the three concentrations. Complete inhibition of the pathogen by Bordeaux mixture at highest concentration of 1.5 per cent was noticed and an inhibition greater than 85 per

cent was noticed at the lower and recommended dose of 0.5 and 1.0 per cent. Difenoconazole 25 EC (Score) showed 83.50 to 88.30 per cent efficacy in inhibiting the pathogen at various concentrations tested (Plate 4.13). Likewise, propineb 70WP (Antracol) inhibited the pathogen above 70 per cent at all concentrations, whereas, copper oxychloride 50 WP (Fytolan) could inhibit the pathogen only by 36.10 to 52.77 per cent inhibition at the three concentrations tested. Potassium phosphonate (Akomin 40) showed comparatively lesser inhibition of below 35 per cent at all concentrations.

4.6.1.9 *Lasiodiplodia theobromae* (CRM-1)

Data given in Table 4.16 revealed that carbendazim 12% + mancozeb 63% (Saaf), copper hydroxide 77 WP (Kocide) at all concentrations and the highest concentration of cymoxanil 8% + mancozeb 64% (Curzate M8) (0.2%) showed 100 per cent inhibition of the pathogen. Bordeaux mixture recorded an inhibition of 69.44, 81.11 and 83.55 per cent efficacy at 0.5, 1 and 1.5 per cent concentrations. Difenoconazole 25 EC (Score) showed 62.22 to 76.67 per cent inhibition for the lowest concentration of 0.05 and the highest concentration of 0.15 per cent. Similarly, propineb 70 WP (Antracol) at all concentrations inhibited below 70 per cent of mycelial growth of the pathogen, whereas, carbendazim 50WP (Bavistin) also showed least per cent inhibition of below 35 per cent against the pathogen (Plate 4.14). Copper oxychloride 77 WP (Fytolan) and potassium phosphonate (Akomin 40) could not inhibit the pathogens at all three concentrations tested.

Plate 4.12 *In vitro* evaluation of fungicides against *Curvularia lunata* (LBI-2)

a) Cymoxanil 8%+mancozeb 64%

b) Difenoconazole 25EC

c) Propineb 70WP

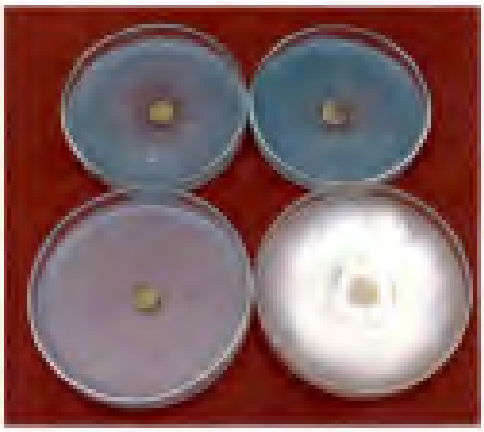
d) Bordeaux mixture

e)Copper oxychloride 50WP

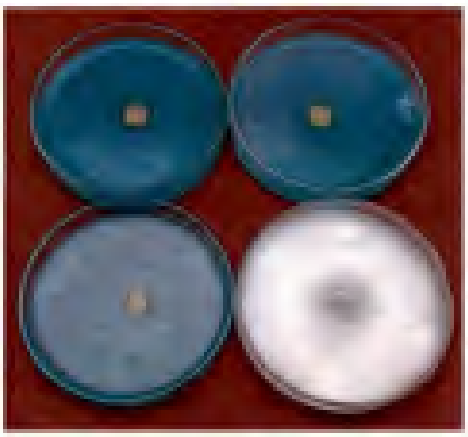
f) Carbendazim 12% + Mancozeb 63%

Plate 4.13 *In vitro* evaluation of fungicides against *Pestalotiopsis longisetula* (LBM-1)

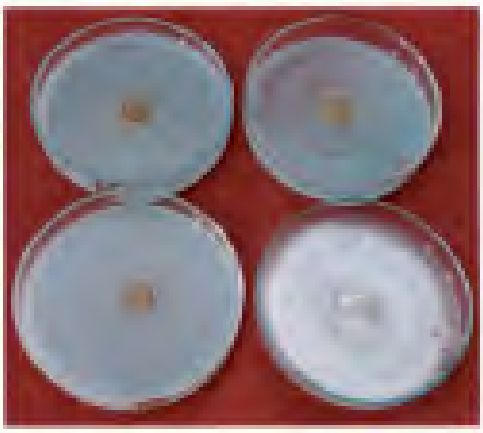
a) Propineb 70WP



b) Copper hydroxide 77WP



b) Bordeaux mixture



d) Copper oxychloride 50WP

e) Cymoxanil 8%+ Mancozeb 64%

f) Carbendazim 12%+ Mancozeb 63%

Table 4.15 *In vitro* evaluation of fungicides against *Curvularia lunata*, *Pestalotiopsis longisetula* and *Rhizoctonia solani*

Sl No.	Fungicide	Conc (%)	Per cent Inhibition over control		
			Curvularia lunata (LSI-2)	*Pestalotiopsis longisetula* (LBM-1)	*Rhizoctonia solani* (FRW-1)
1.	Carbendazim 12% + Mancozeb 63% (Saaf)	0.15	66.67(8.17)^{i}	100(10)^{a}	100(10)^{a}
		0.20	68.88(8.3)^{h}	100(10)^{a}	100(10)^{a}
		0.25	73.33(8.6)^{e}	100(10)^{a}	100(10)^{a}
2.	Cymoxanil 8% + Mancozeb 64% (Curzate M8)	0.15	100(10)^{a}	100(10)^{a}	100(10)^{a}
		0.20	100(10)^{a}	100(10)^{a}	100(10)^{a}
		0.25	100(10)^{a}	100(10)^{a}	100(10)^{a}
3.	Copper hydroxide 77WP (Kocide)	0.10	71.11(8.4)^{g}	100(10)^{a}	54.4(7.45)^{f}
		0.15	73.88(8.57)^{f}	100(10)^{a}	70.87(8.38)^{c}
		0.20	74.44(8.65)^{e}	100(10)^{a}	75(8.7)^{b}
4.	Copper oxychloride 50WP (Fytolan)	0.20	82.67(9.07)^{d}	100(10)^{a}	38.88(6.29)^{i}
		0.25	84.33(9.18)^{c}	100(10)^{a}	41.66(6.51)^{h}
		0.30	85.67(9.28)^{b}	100(10)^{a}	43.55(6.67)^{g}
5.	Propineb 70WP (Antracol)	0.25	100(10)^{a}	100(10)^{a}	100(10)^{a}
		0.30	100(10)^{a}	100(10)^{a}	100(10)^{a}
		0.35	100(10)^{a}	100(10)^{a}	100(10)^{a}
6.	Carbendazim 50WP (Bavistin)	0.05	15.55(3.93)^{m}	57.22(7.59)^{g}	0(.7)
		0.10	21.67(4.06)^{l}	66.11(8.14)^{f}	0(.7)
		0.15	16.11(4.69)^{k}	75(8.83)^{e}	0(.7)
7.	Difenoconazole 25EC (score)	0.05	100(10)^{a}	81.11(9.04)^{d}	58.55(7.69)^{e}
		0.10	100(10)^{a}	83.55(9.12)^{c}	67.31(8.19)^{d}
		0.15	100(10)^{a}	85.55(9.27)^{b}	70.44(8.4)^{c}
8.	Potassium phosphonate (Akomin 40)	0.25	11(3.4)^{o}	27.77(5.27)^{j}	0(.7)^{j}
		0.30	13.2(3.6)^{n}	30.55(5.57)^{i}	0(.7)^{j}
		0.35	22(5.1)^{j}	36.67(6.08)^{h}	0(.7)^{j}
9.	Bordeaux Mixture	0.50	100(10)^{a}	100(10)^{a}	100(10)^{a}
		1.0	100(10)^{a}	100(10)^{a}	100(10)^{a}
		1.50	100(10)^{a}	100(10)^{a}	100(10)^{a}
	CD (0.05)		0.043	0.028	0.031

*Mean of the three replications

In each column figure followed by same letter do not differ significantly according to DMRT.

$\sqrt{x+0.5}$ transformed values are given in parantheses

106

Plate 4.14 *In vitro* evaluation of fungicides against *Rhizoctonia solani* (FRW-1)

a) Bordeaux mixture

b) Cymoxanil 8%+ Mancozeb 64%

c) Carbendazim 12%+ Mancozeb 63%

d) Difenoconazole 25EC

e) Copper hydroxide 77WP

Copper oxychloride 50WP

Table 4.16 *In vitro* evaluation of fungicides against *Fusarium oxysporum* and *Lasiodiplodia theobromae*

Sl No.	Fungicide	Conc (%)	Per cent Inhibition over control	
			Fusarium oxysporum (CRI-1)	*Lasiodiplodia theobromae* (CRM-2)
1.	Carbendazim 12% + Mancozeb 63% (Saaf)	0.15	100(10)^{a}	100(10)^{a}
		0.20	100(10)^{a}	100(10)^{a}
		0.25	100(10)^{a}	100(10)^{a}
2.	Cymoxanil 8% + Mancozeb 64% (Curzate M8)	0.15	100(10)^{a}	93.2(9.76)^{b}
		0.20	100(10)^{a}	96.3(9.77)^{b}
		0.25	100(10)^{a}	100(10)^{a}
3.	Copper hydroxide 77WP (Kocide)	0.10	100(10)^{a}	100(10)^{a}
		0.15	100(10)^{a}	100(10)^{a}
		0.20	100(10)^{a}	100(10)^{a}
4.	Copper oxychloride 50WP (Fytolan)	0.20	36.1(6.05)^{k}	0
		0.25	42.7(6.55)^{j}	0
		0.30	52.7(7.27)^{i}	0
5.	Propineb 70WP (Antracol)	0.25	86.3(8.56)^{g}	55.55(7.52)^{j}
		0.30	88.5(8.70)^{f}	64.11(8.04)^{h}
		0.35	90.1(8.75)^{e}	69.44(8.37)^{g}
6.	Carbendazim 50WP (Bavistin)	0.05	100(10)^{a}	23.33(4.97)^{m}
		0.10	100(10)^{a}	31.67(5.68)^{l}
		0.15	100(10)^{a}	33.23(5.84)^{k}
7.	Difenoconazole 25EC (score)	0.05	83.5(8.4)^{h}	62.22(7.95)^{i}
		0.10	86.2(8.67)^{f}	75(8.68)^{f}
		0.15	88.3(8.97)^{d}	76.67(8.79)^{e}
8.	Potassium phosphonate 40% (Akomin 40)	0.25	28.3(5.33)^{n}	0
		0.30	28.8(5.39)^{m}	0
		0.35	31.6(5.62)^{l}	0
9.	Bordeaux Mixture	0.50	86.1(9.28)^{c}	69.44(8.4)^{g}
		1.0	93.3(9.66)^{b}	81.11(8.99)^{d}
		1.50	100(10)^{a}	83.55(9.09)^{c}
	CD (0.05)		0.029	0.038

*Mean of the three replications

In each column figure followed by same letter do not differ significantly according to DMRT.

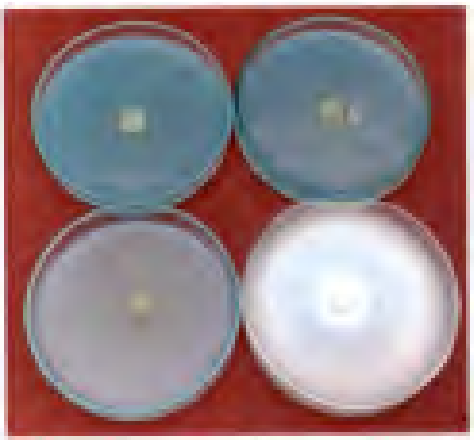
$\sqrt{x+0.5}$ transformed values are given in parantheses

Plate 4.15 *In vitro* evaluation of fungicides against *Fusarium oxysporum* (CRI-1)

a) Carbendazim 50WP

b) Cymoxanil 8% + Mancozeb 64%

c) Carbendazim 12% + Mancozeb 63%



d) Copper hydroxide 77WP

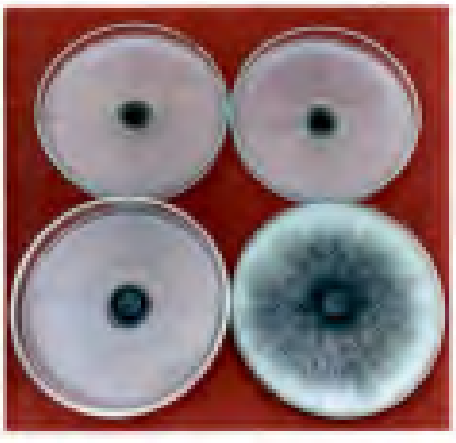
e) Difenoconazole 25EC

f) Bordeaux mixture

Plate 4.16 *In vitro* evaluation of fungicides against *Lasiodiplodia theobromae* (CRM-1)

a) Carbendazim 12% + mancozeb 63%

b) Cymoxanil 8%+mancozeb 64%



c) Difenoconazole 25EC

d) Bordeaux mixture

e) Propineb 70WP

f) Copper hydroxide 77WP

Fig 4.1 Efficacy of carbendazim 12% + mancozeb 63% against crown rots and leaf spots

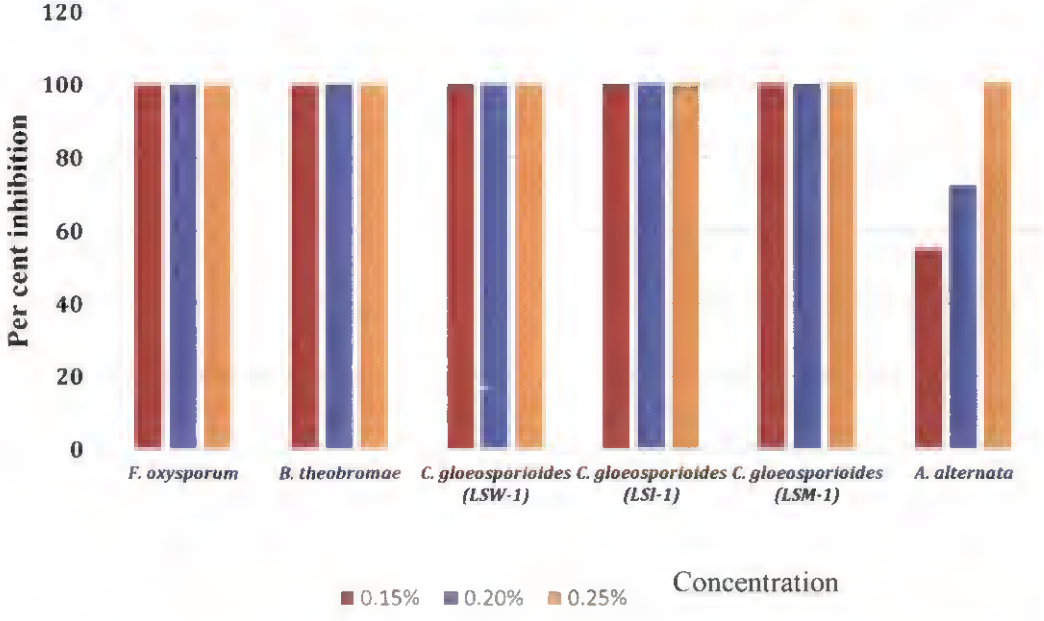


Fig 4.2 Efficacy of cymoxanil 8% + mancozeb 64% against leaf blights and fruit rot

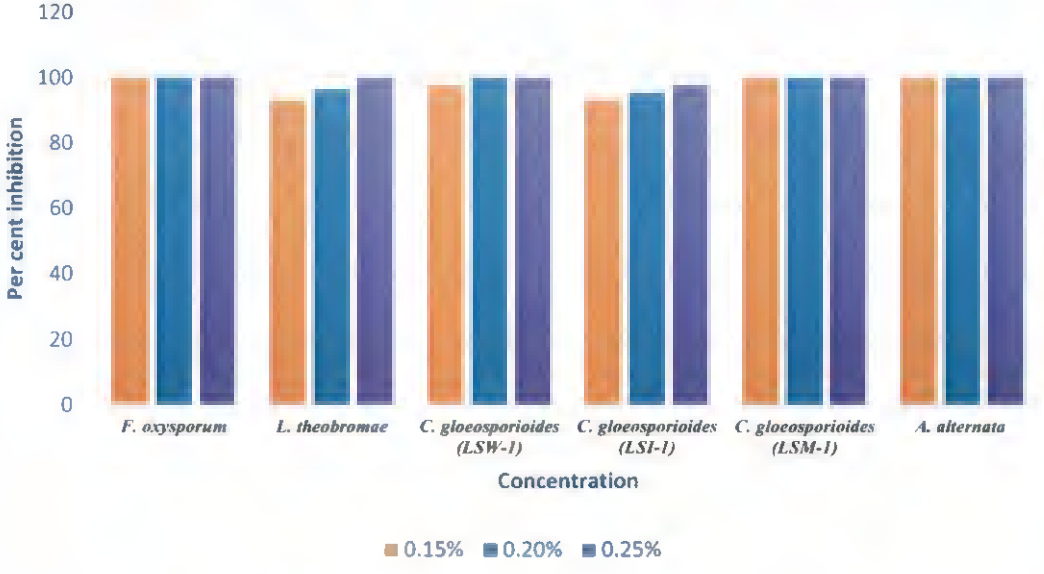


Fig 4.3 Efficacy of carbendazim 50WP against crown rots and leaf spots

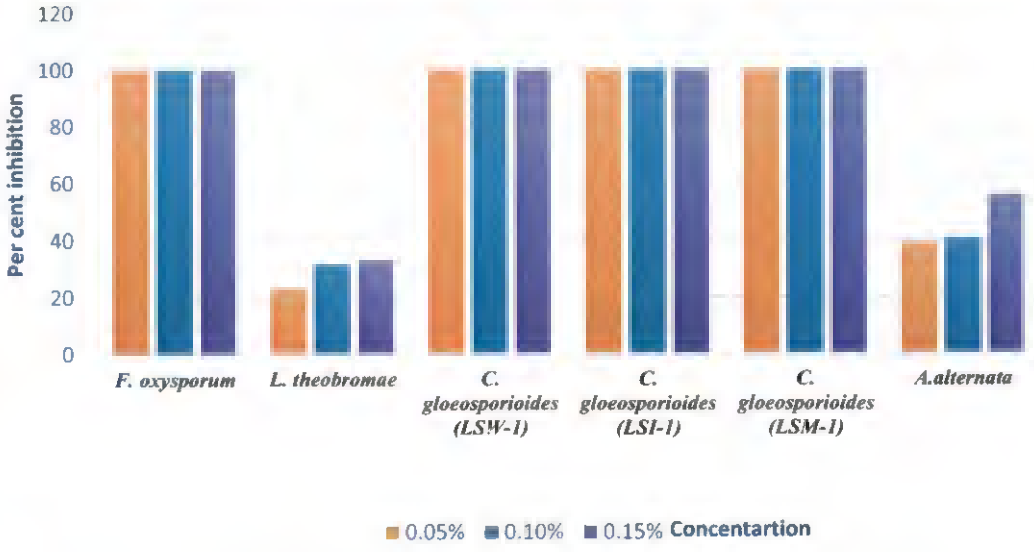


Fig 4.4 Efficacy of copper oxychloride 50WP against crown rots and leaf spots

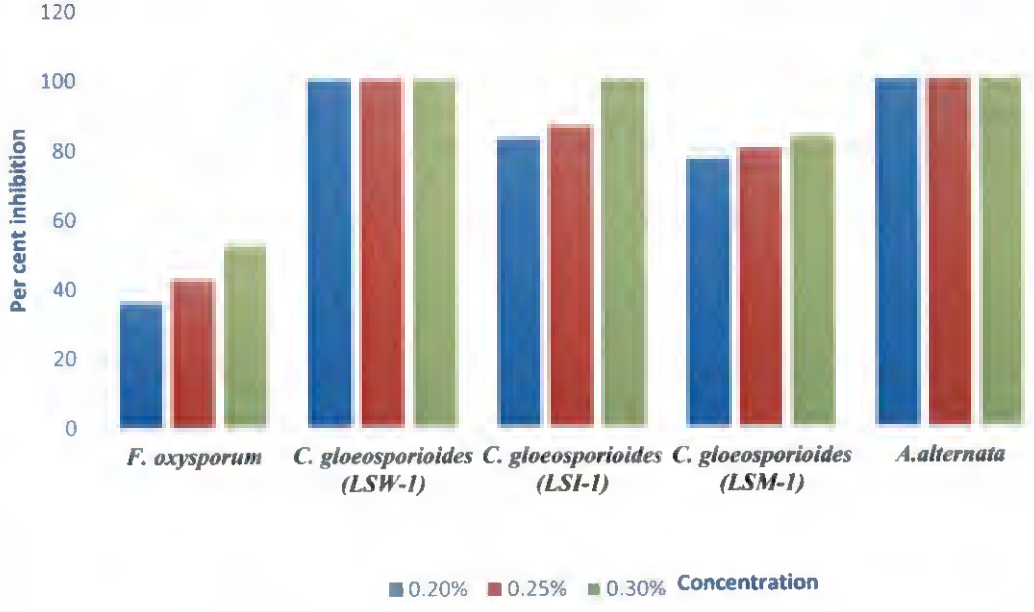


Fig 4.5 Efficacy of copper hydroxide 77WP against crown rots and leaf spots

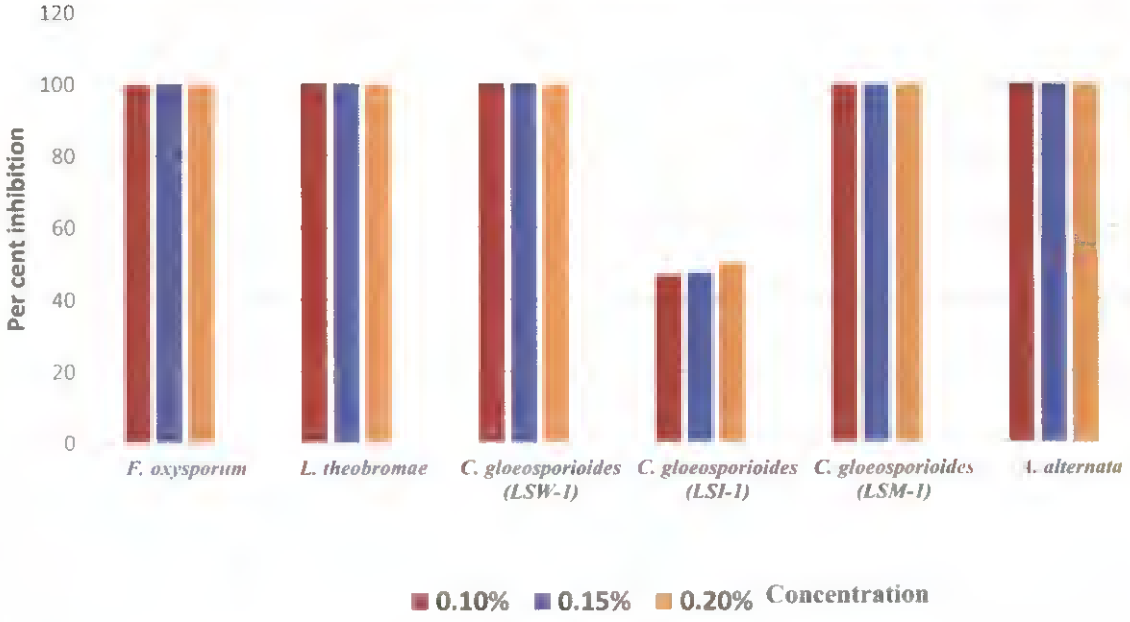


Fig 4.6 Efficacy of propineb 70WP against crown rots and leaf spots

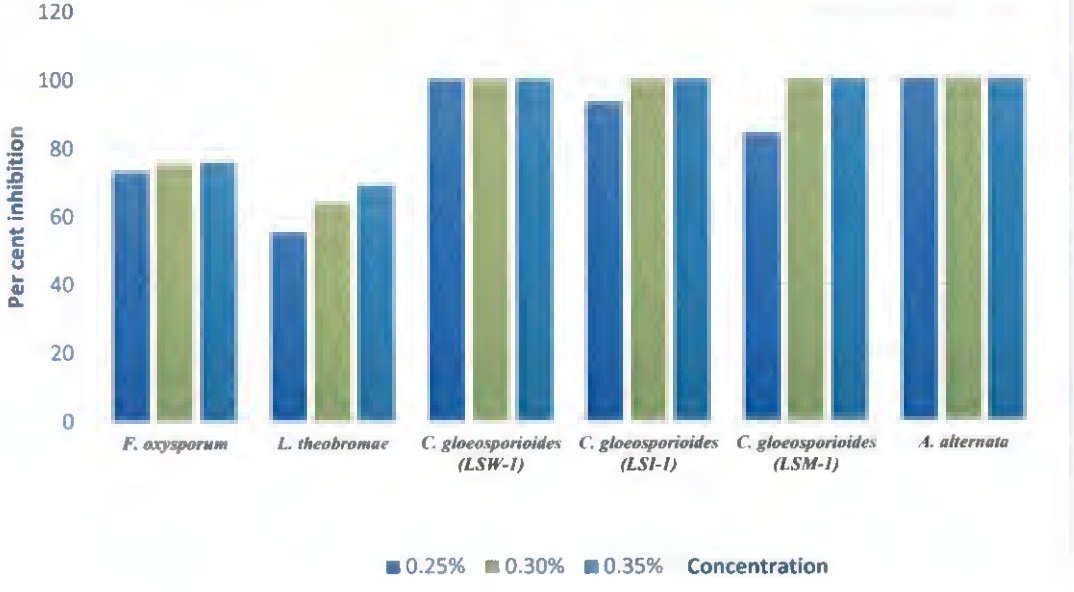


Fig 4.7 Effect of Bordeaux mixture against crown rots and leaf spots

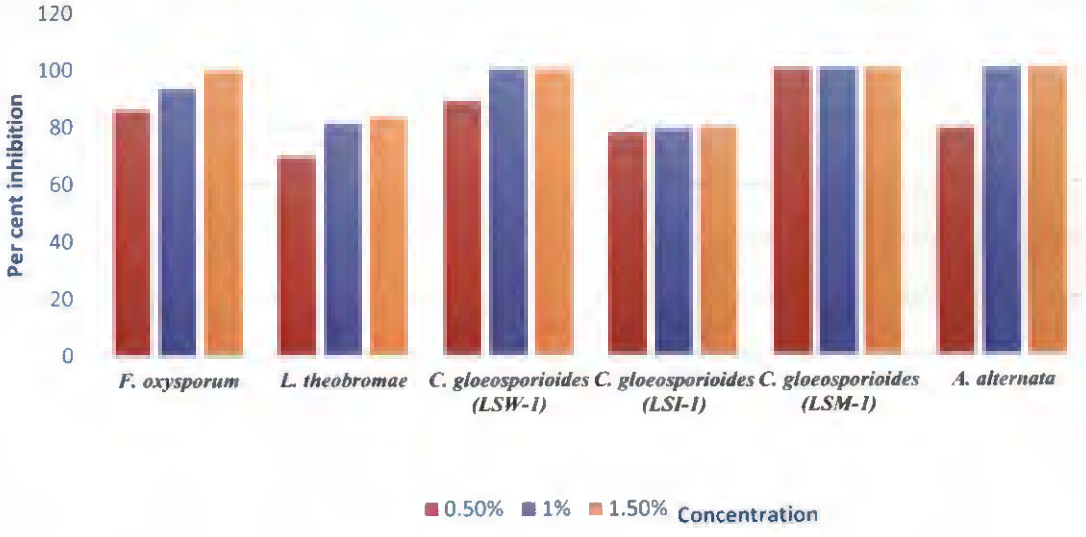


Fig 4.8 Effect of Potassium phosphonate against crown rots and leaf spots

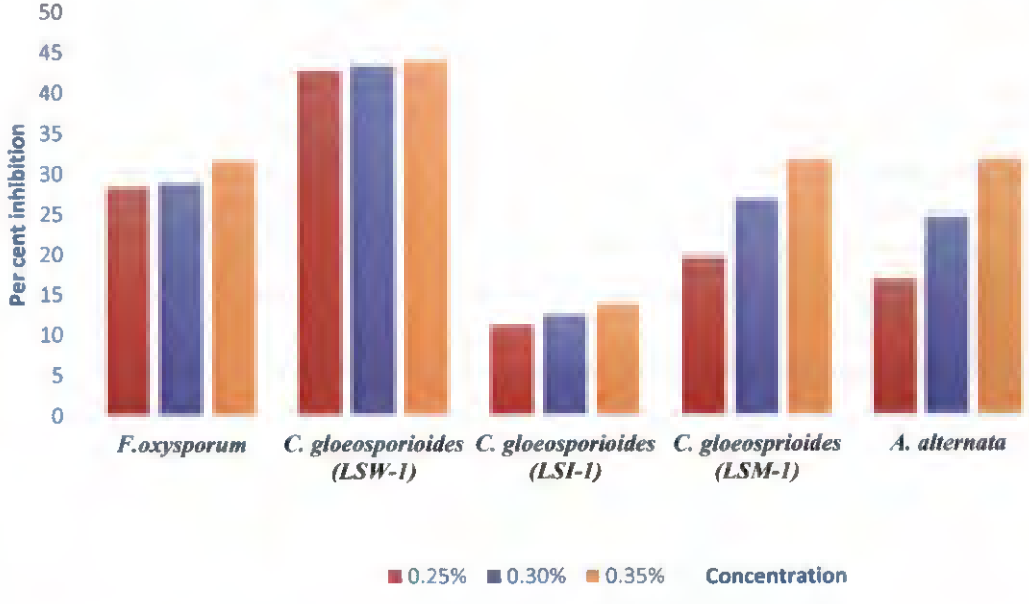
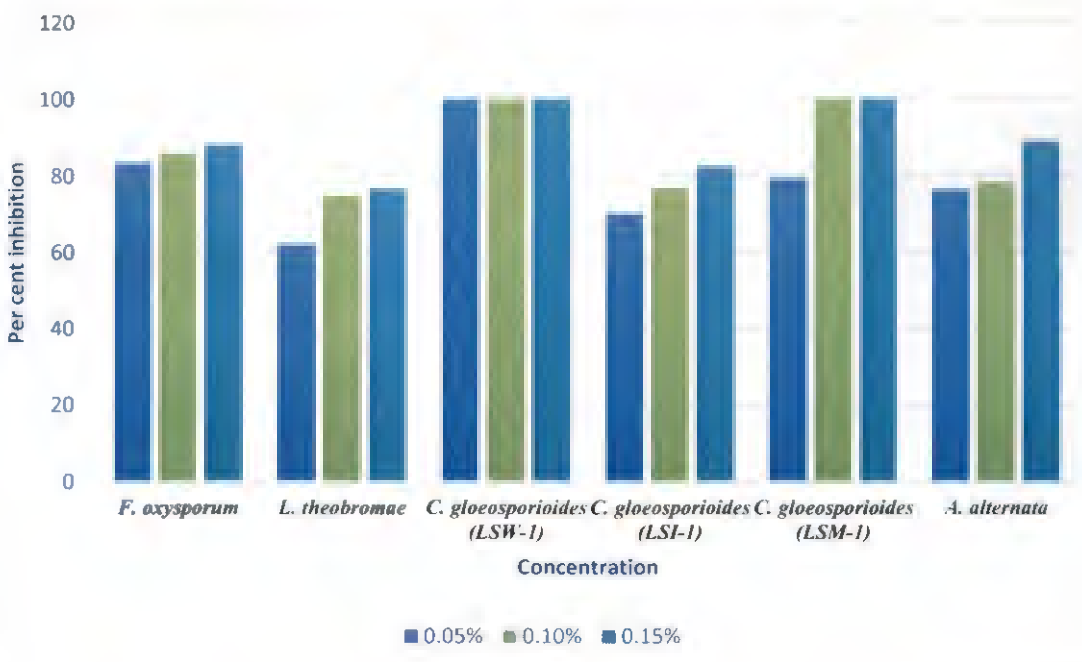


Fig 4.9 Effect of difenoconazole 25EC against crown rots and leaf spots



4.6.2 *In vitro* evaluation of organic preparations

Various organic preparations and formulations *viz*., Calphomil, panchagavya, neem oil and baking powder + vegetable oil mixture were tested for its efficacy against different pathogens. Evaluation was carried out by poison food technique as followed in case of fungicides and per cent inhibition in mycelial growth was recorded as shown in Table 4.17, 4.18, 4.19 and 4.20 and in plate 4.15, 4.16, 4.17 and 4.18.

4.6.2.1 *Colletotrichum gloeosporioides* (LSW-1, LSI-1 and LSM-1)

Pathogen causing LSW-1 when subjected to various organic preparations, it was observed that Calphomil at 0.2, 0.25 and 0.3 per cent were found to inhibit the pathogen growth by 13.33, 18.33 and 20.67 per cent respectively. However, neem oil could restrict the mycelial growth of the pathogen below 25.2 per cent. Likewise, panchagavya could inhibit pathogen spread only by 36.3 per cent at 4 per cent concentration and baking powder + vegetable oil exhibited 27.22 and 30.20 per cent inhibition at 0.2 and 0.3 per cent respectively.

In the case of LSI-1, Calphomil at different concentrations recorded a zero per cent inhibition. Neem oil, baking powder + vegetable oil and panchagavya at all concentrations could inhibit the growth of the pathogen below 30 per cent only.

Leaf spot pathogen LSM-1 recorded 56.67, 69.11 and 75.33 per cent inhibition with Calphomil at 0.2, 0.25 and 0.3 per cent. Neem oil showed an inhibition of less than 20.11 per cent at all concentrations. Panchagavya and baking powder + vegetable oil showed below 30 per cent inhibition at various concentrations tested.

Plate 4.17 (i) *In vitro* evaluation of Calphomil against fungal pathogens

a) *C. gloeosporioides* (LSW-1)

b) *C. gloeosporioides* (LSI-1)

c) *C. gloeosporioides* (LSM-1)

d) *A. alternata* (LSI-2)

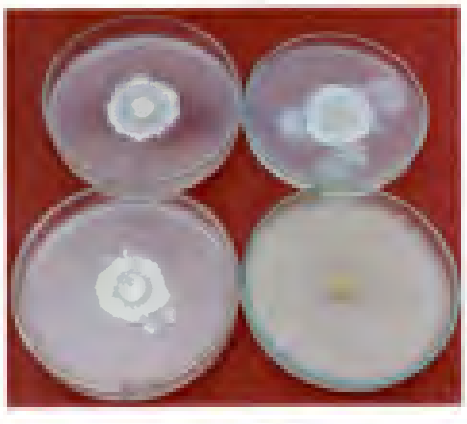
e) *R. solani* (LBW-1)

f) *P. exigua* (LBI-1)

Plate 4.17 (ii) *In vitro* evaluation of Calphomil against fungal pathogens

g) *C. lunata* (LBI-2)

h) *P. longisetula* (LBM-1)



i) R. solani (FRW-1)

j) *F. oxysporum* (CRI-1)

k) *L. theobromae* (CRM-1)

Table 4.17 Per cent inhibition of fungal pathogens by organic preparations against *Colletotrichum gloeosporioides* (LSW-. LSI-1 & LSM-1)

Sl. No	Formulation	Conc (%)	Per cent inhibition		
			Colletotrichum gloeosporioides (LSW-1)	*Colletotrichum gloeosporioides* (LSI-1)	*Colletotrichum gloeosporioides* (LSM-1)
1.	Calphomil	0.2	13.33(3.66)^{l}	0	56.67(7.53)
		0.25	18.33(4.28)^{k}	0	69.11(8.32)
		0.3	20.67(4.55)^{i}	0	75.33(8.66)
2.	Neem oil	0.15	20.33(4.52)^{j}	16.67(4.15)^{g}	16.67(4.09)
		0.2	23.44(4.84)^{h}	18.16(4.33)^{f}	18.48(4.31)
		0.25	25.2(5.02)^{g}	20.1(4.5)^{e}	20.0(4.4)
3.	Panchagavya	2.0	32.11(5.67)^{c}	23.67(4.8)^{d}	22.22(4.7)
		3.0	35.22(5.97)^{b}	26.33(5.2)^{ab}	23.33(4.83)
		4.0	36.30(6.03)^{a}	27.10(5.26)^{a}	25.10(5.02)
4.	Baking powder + Vegetable oil	0.2	27.22(5.22)^{f}	20.10(4.55)^{e}	26.60(5.16)
		0.25	28.10(5.32)^{e}	24.20(4.97)^{c}	27.90(5.26)
		0.3	30.20(5.5)^{d}	26.11(5.16)^{b}	30.00(5.48)
	CD (0.05)		0.027	0.088	0.019

*Mean of the three replications

In each column figure followed by same letter do not differ significantly according to DMRT.

$\sqrt{x+0.5}$ transformed values are given in parantheses

4.6.2.2 *Alternaria alternata* (LSI-2)

Calphomil showed 58.33, 66.11 and 67.77 per cent efficiency against *Alternaria alternata* at 0.2, 0.25 and 0.3 per cent respectively. Neem oil exhibited an inhibition of below 31 per cent efficacy against the pathogen. Panchagavya and baking powder + vegetable oil mixture showed comparatively less inhibition of below 33 per cent (Table 4.18).

4.6.2.3 *Rhizoctonia solani* (LBW-1)

Calphomil and neem oil at all concentrations were found least effective against the LBW-1 pathogen. Similarly, panchagavya and baking powder + vegetable oil mixture showed an inhibition of below 30 per cent only.

4.6.2.4 *Phoma exigua* (LBI-1)

In vitro evaluation of neem oil against the pathogen recorded below 10 per cent inhibition. However, it was noticed that Calphomil at various concentrations recorded a per cent inhibition of above 40 per cent. Moreover, baking powder + vegetable oil mixture and panchagavya could restrict the growth of the pathogen below 35.4 per cent only.

4.6.2.5 *Curvularia lunata* (LBI-2)

When pathogen was tested against neem oil (0.2% and 0.25%), 5.55, 7.23 and 9.30 per cent restriction in mycelial growth was recorded. Calphomil at 0.2, 0.25 and 0.3 per cent inhibited the pathogen by 15.44, 20.77 and 23.33 per cent. Panchagavya recorded upto 25 per cent reduction in mycelial growth at various concentrations. Baking powder + vegetable oil could restrict the growth of the fungus by 24.44 per cent only.

Plate 4.18 (i) *In vitro* evaluation of neem oil against fungal pathogens

a) *C. gloeosporioides* (LSW-1)

b) *C. gloeosporioides* (LSI-1)

c) *C. gloeosporioides* (LSM-1)

d) *A. alternata* (LSI-2)

e) *R. solani* (LBW-1)

f) *P. exigua* (LBI-1)

Plate 4.18 (ii) *In vitro* evaluation of neem oil against fungal pathogens

g) *C. lunata* (LBI-2)

h) *P. longisetula* (LBM-1)

ii) R. solani (FRW-1)

j) *F. oxysporum* (CRI-1)

k) *L. theobromae* (CRM-1)

Table 4.18 Per cent inhibition of fungal pathogens by organic preparations against *Alternaria alternata*, *Rhizoctonia solani* and *Phoma exigua*

Sl. No	Formulation	Conc (%)	Per cent inhibition		
			Alternaria alternata (LSI-2)	*Rhizoctonia solani* (LBW-1)	*Phoma exigua* (LBI-1)
1.	Calphomil	0.2	58.33(7.64)^{c}	0	44.44(6.66)^{c}
		0.25	66.11(8.13)^{b}	0	47.44(6.88)^{b}
		0.3	67.77(8.22)^{a}	0	53.88(7.3)^{a}
2.	Neem oil	0.15	28.80(5.36)^{h}	0	5.55(2.3)^{k}
		0.2	29.30(5.42)^{g}	0	6.67(2.6)^{j}
		0.25	31.0(5.59)^{e}	0	9.93(3.14)^{i}
3.	Panchagavya	2.0	28.50(5.33)^{hi}	23.20(4.88)^{f}	31.30(5.6)^{f}
		3.0	30.10(5.49)^{f}	27.55(5.3)^{d}	33.90(5.8)^{e}
		4.0	32.40(5.72)^{d}	29.43(5.47)^{a}	35.40(5.98)^{d}
4.	Baking powder + Vegetable oil	0.2	27.20(5.22)^{j}	25.50(5.11)^{e}	21.0(4.59)^{h}
		0.25	28.30(5.32)^{i}	27.80(5.32)^{c}	26.10(5.12)^{g}
		0.3	30.20(5.50)^{f}	29.20(5.46)^{b}	33.80(5.8)^{e}
	CD (0.05)		0.029	0.014	0.021

*Mean of the three replications

In each column figure followed by same letter do not differ significantly according to DMRT.

$\sqrt{x+0.5}$ transformed values are given in parantheses

4.6.2.6 *Pestalotiopsis longisetula* (LBM-1)

Among the different formulations tested, Calphomil at all concentrations showed an inhibition above 80 per cent. Neem oil, baking powder + vegetable oil mixture and panchagavya inhibited the mycelial growth only below 30 per cent.

4.6.2.7 *Rhizoctonia solani* (FRW-1)

Among the various organic preparations used against FRW-1, Calphomil recorded the highest inhibition of 55.33 to 63.88 per cent at different concentrations tested. Neem oil was not found effective against the pathogen. Likewise, panchagavya and baking powder + vegetable oil mixture could inhibit the mycelial growth below 30 per cent only.

4.6.2.8 *Fusarium oxysporum* (CRI-1)

The pathogen when tested against the organic preparations, the results revealed that Calphomil at 0.2, 0.25 and 0.3 per cent recorded 22.77, 44.44 and 52.77 per cent inhibition respectively. Neem oil, panchagavya and baking powder + vegetable oil mixture at different concentrations recorded an inhibition of below 18 per cent only.

4.6.2.9 *Lasiodiplodia theobromae* (CRM-1)

Observations from the table revealed that neem oil, panchagavya, and baking powder + vegetable oil mixture and Calphomil at all concentrations was found the least effective as no inhibition of the pathogen was noticed.

Plate 4.19 (i) *In vitro* evaluation of panchagvaya against fungal pathogens

a) *C. gloeosporioides* (LSW-1)

b) *C. gloeosporioides* (LSI-1)

c) *C. gloeosporioides* (LSM-1)

d) *A. alternata* (LSI-2)

e) *R. solani* (LBW-1)

f) *C. lunata* (LBI-2)

Plate 4.19 (ii) *In vitro* evaluation of panchagvaya against fungal pathogens

g) *P. exigua* (LBI-1)

h) *P. longisetula* (LBM-1)

iii) *R. solani* (FRW-1)

j) *F. oxysporum* (CRI-1)

k) *L. theobromae* (CRM-1)

Table 4.19 Per cent inhibition of fungal pathogens by organic preparations against *Curvularia lunata*, *Pestalotiopsis longisetula* and *Rhizoctonia solani*

Sl. No	Formulation	Conc (%)	Per cent inhibition		
			Curvularia lunata (LBI-2)	*Pestalotiopsis longisetula* (LBM-1)	*Rhizoctonia solani* (FRW-1)
1.	Calphomil	0.2	15.44(3.94)^{f}	83.33(9.13)^{c}	55.33(7.47)^{c}
		0.25	20.77(4.54)^{e}	84.44(9.2)^{b}	58.33(7.67)^{b}
		0.3	23.33(4.8)^{b}	87.77(9.36)^{a}	63.88(8.01)^{a}
2.	Neem oil	0.15	5.55(2.3)^{i}	15.0(3.9)^{k}	0
		0.2	7.23(2.7)^{h}	17.60(4.17)^{j}	0
		0.25	9.30(3.06)^{g}	18.20(4.27)^{i}	0
3.	Panchagavya	2.0	21.33(4.61)^{d}	26.77(5.17)^{g}	23.33(4.88)^{g}
		3.0	22.11(4.7)^{c}	27.67(5.2)^{e}	25.38(5.02)^{f}
		4.0	23.40(4.8)^{b}	28.40(5.33)^{d}	25.50(5.11)^{e}
4.	Baking powder + Vegetable oil	0.2	20.55(4.53)^{e}	25.11(5.03)^{h}	22.22(4.76)^{h}
		0.25	23.10(4.82)^{b}	27.30(5.22)^{f}	23.40(4.8)^{g}
		0.3	24.40(4.90)^{a}	28.30(5.33)^{d}	26.0(5.16)^{d}
	CD (0.05)		0.046	0.30	0.019

*Mean of the three replications

In each column figure followed by same letter do not differ significantly according to DMRT.

$\sqrt{x+0.5}$ transformed values are given in parantheses

Table 4.20 Per cent inhibition of fungal pathogens by organic preparations against *Lasiodiplodia theobromae* and *Fusarium oxysporum*

Sl. No	Formulation	Conc (%)	Per cent inhibition	
			Fusarium oxysporum (CRI-1)	*Lasiodiplodia theobromae* (CRM-1)
1.	Calphomil	0.2	22.77(4.7)^{c}	0
		0.25	44.40(6.63)^{b}	0
		0.3	52.77(7.26)^{a}	0
2.	Neem oil	0.15	7.60(2.75)^{k}	0
		0.2	9.30(3.06)^{j}	0
		0.25	11.10(3.35)^{i}	0
3.	Panchagavya	2.0	11.20(3.35)^{i}	0
		3.0	13.40(3.68)^{h}	0
		4.0	15.10(3.89)^{g}	0
4.	Baking powder + Vegetable oil	0.2	15.30(3.92)^{f}	0
		0.25	16.20(4.02)^{e}	0
		0.3	18.0(4.25)^{d}	0
	CD(0.05)		0.023	-

*Mean of the three replications

In each column figure followed by same letter do not differ significantly according to DMRT.

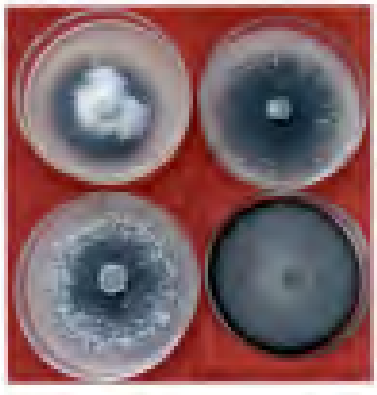
$\sqrt{x+0.5}$ transformed values are given in parantheses

Plate 4.20 (i) *In vitro* evaluation of baking powder + vegetable oil against fungal pathogens

a) *C. gloeosporioides* (LSW-1)

b) *C. gloeosporioides* (LSI-1)

c) *C. gloeosporioides* (LSM-1)

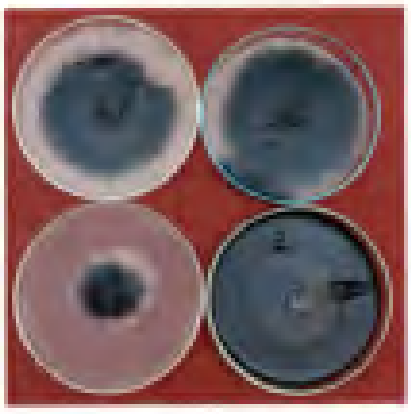


d) *A. alternata* (LSI-2)

e) *R. solani* (LBW-1)

f) *P. exigua* (LBI-1)

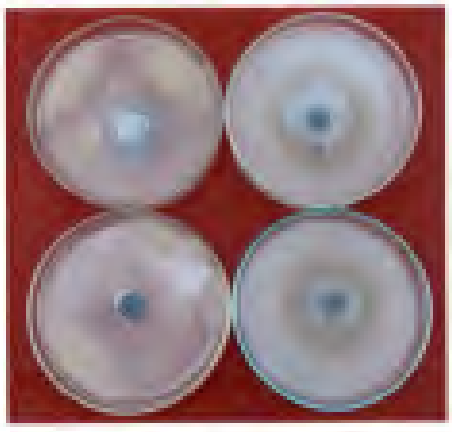
Plate 4.20 (i) *In vitro* evaluation of baking powder + vegetable oil against fungal pathogens



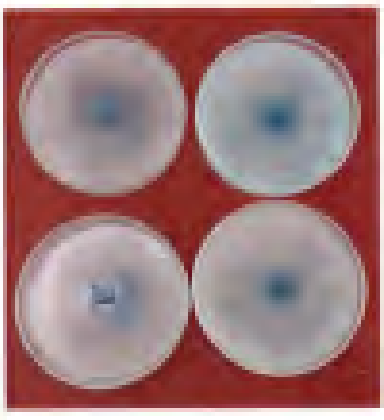
g) *C. lunata* (LBI-2)

h) *P. longisetula* (LBM-1)

i) *R. solani* (FRW-1)



j) *F. oxysporum* (CRI-1)



k) *L. theobromae* (CRM-1)

4.6.3 *In vitro* evaluation of bio agents

Trichoderma asperellum and *Pseudomonas fluorescens*, the reference cultures from KAU were tested against eleven fungal pathogens of strawberry and the results are given in Table 4.21 and Table 4.22 respectively.

4.6.3.1 *Colletotrichum gloeosporioides* (LBW-1, LBI-1 and LBM-1)

The isolates of *Colletotrichum gloeosporioides* (LBW-1, LBI-1 and LBM-1) from Wayanad, Idukki and Anakkayam were evaluated against *Trichoderma asperellum* by dual culture assay and it was noticed that *Trichoderma asperellum* exhibited better control by overgrowing on the pathogen and restricting its growth by cent per cent [Plate 4.19 (i) a, b, c]. However, the bacterial antagonist, *Pseudomonas fluorescens* could restrict the mycelial growth of the pathogen ranging from 65-70 per cent only.

4.6.3.2 *Alternaria alternata* (LSI-2)

Response of *Trichoderma asperellum* against the fungal pathogen, *Alternaria alternata* showed better control by overgrowth mechanism of inhibition. *T. asperellum* inhibited the pathogen by 100 per cent [Plate 4.19 (i) d]. Contrary to this, the bacterial bioagent could inhibit the pathogen only by 56.67 per cent.

4.6.3.3 *Rhizoctonia solani* (LBW-1)

The growth of the pathogen causing leaf blight isolated from Ambalavayal was inhibited completely by overgrowth mechanism of antagonism by *T.asperellum*. But the bacterial antagonist, *P. fluorescens* could restrict the growth only by 55.56 per cent [Plate 4.19 (i) e].

4.6.3.4 *Phoma exigua* (LBI-1)

Trichoderma asperellum and *Pseudomonas fluorescens* when tested against the fungal pathogen, *Phoma exigua,* it was noticed that *T. asperellum* was a better antagonist restricting the mycelial growth by 100 per cent where *T. asperellum* overgrew the

pathogen [Plate 4.19 (i) f]. However, the pathogen was inhibited by *P. fluorescens* only by 45.61 per cent.

4.6.3.5 *Curvularia lunata* (LBI-2)

In case of *Curvularia lunata*, growth of the pathogen was restricted upto 100 per cent by the fungal antagonist, *T. asperellum* whereas the bacterial antagonist *P. fluorescens* was not at all effective against the pathogen isolated from Idukki [Plate 4.19 (i) g].

4.6.3.6 *Pestalotiopsis longisetula* (LBM-1)

Trichoderma asperellum and the fungal pathogen *Pestalotiopsis longisetula* showed a homogeneous growth when they were subjected to dual culture technique [Plate 4.19 (i) h]. Pathogen exhibited an inhibition of 66.67 per cent by the fungal antagonist, while the bacterial antagonist *P. fluorescens* inhibited the pathogen by 56.67 per cent, showing poor antagonistic action when compared with *T. asperellum.*

4.6.3.7 *Rhizoctonia solani* (FRW-1)

The pathogen causing fruit rot showed an aversion towards the fungal biocontrol agent *Trichoderma asperellum* when cultured under dual plate assay [Plate 4.19 (ii) i]. The bioagent restricted the mycelial growth by 66.67 per cent, whereas, the bacterial biocontrol agent, *P. fluorescens* was not found effective as it inhibited the mycelial growth by 33.33 per cent only.

4.6.3.8 *Fusarium oxysporum* (CRI-1)

Fungal pathogen *Fusarium oxysporum*, when inoculated separately with the fungal and bacterial biocontrol agent by dual culture technique, the pathogen showed an aversion against *Trichoderma asperellum* in growth and inhibited the pathogen by 68 per cent. *P. fluorescens* indicated less efficacy compared to *T. asperellum* as they could restrict the pathogen growth only upto 57.67 per cent [Plate 4.19 (ii) j].

4.6.3.9 *Lasiodiplodia theobromae* (CRM-1)

The bioagent, *T. asperellum* recorded a maximum of 74.10 per cent efficacy in inhibiting the mycelial growth of the pathogen with overgrowth mechanism of antagonism. However, *P. fluorescens* could not restrict the growth of the pathogen and hence found to be least effective [Plate 4.19 (ii) k].

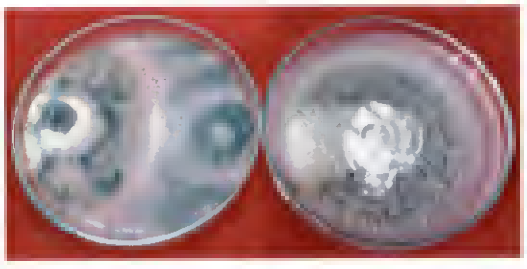
Table 4.21 Per cent inhibition of fungal pathogens by *Trichoderma asperellum*

Sl. No.	Pathogen	Per cent inhibition of pathogen	Antagonistic reaction
1.	*Colletotrichum gloeosporioides* (LSW-1)	100(10)[a]	O
2.	*Colletotrichum gloeosporioides* (LSI-1)	100(10)[a]	O
3.	*Colletotrichum gloeosporioides* (LSM-1)	100(10)[a]	O
4.	*Alternaria alternata* (LSI-2)	100(10)[a]	O
5.	*Rhizoctonia solani* (LBW-1)	100(10)[a]	O
6.	*Phoma exigua* (LBI-1)	100(10)[a]	O
7.	*Curvularia lunata* (LBI-2)	100(10)[a]	O
8.	*Pestalotiopsis longisetula* (LBM-1)	66.67(8.17)[d]	H
9.	*Rhizoctonia solani* (FRW-1)	66.67(8.17)[d]	A
10.	*Fusarium oxysporum* (CRI-1)	68.00(8.24)[c]	C
11.	*Lasiodiplodia theobromae* (CRM-1)	74.10(8.61)[b]	O
	CD (0.05)	0.003	

H – Homogenous
O – Overgrowth
C – Cessation of growth
A – Aversion

*Mean of the three replications
In each column figure followed by same letter do not differ significantly according to DMRT.
$\sqrt{x+0.5}$ transformed values are given in parantheses

Plate 4.21 (i) *In vitro* evaluation of *Trichoderma asperellum* against fungal pathogens



a)*C. gloeosporioides* (LSW-1)

b) *C. gloeosporioides* (LSI-1)

c) *C. gloeosporioides* (LSM-1)

d) *A. alternata* (LSI-2)

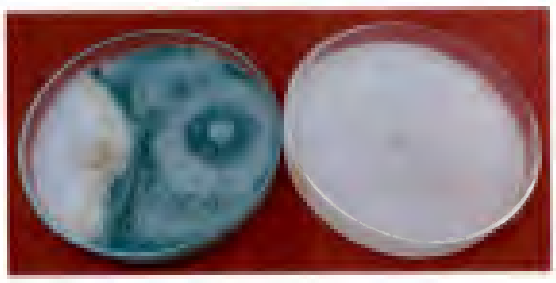
e) *R. solani* (LBW-1)

f) *P. exigua* (LBI-1)

g) *C. lunata* (LBI-2)

h) *P. longisetula* (LBM-1)

Plate 4.21 (ii) *In vitro* evaluation of *Trichoderma asperellum* against fungal pathogens



i) *R. solani* (FRW-1)

j) *F. oxysporum* (CRI-1)

k) *L. theobromae* (CRM-1)

Plate 4.22 (i) *In vitro* evaluation of *Pseudomonas fluorescens* against fungal pathogens

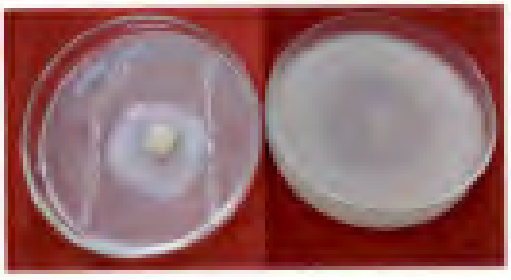
a) *C. gloeosporioides* (LSW-1)

b) *C. gloeosporioides* (LSI-1)

c) *C. gloeosporioides* (LSM-1)

d) *A. alternata* (LSI-2)

Plate 4.22 (ii) *In vitro* evaluation of *Pseudomonas fluorescens* against fungal pathogens

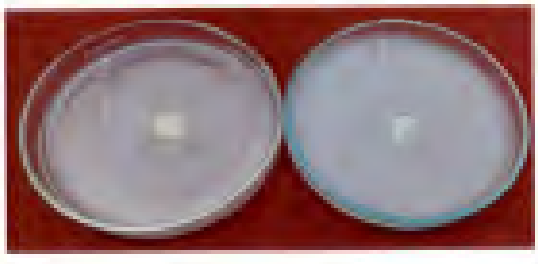


e) *R. solani* (LBW-1)

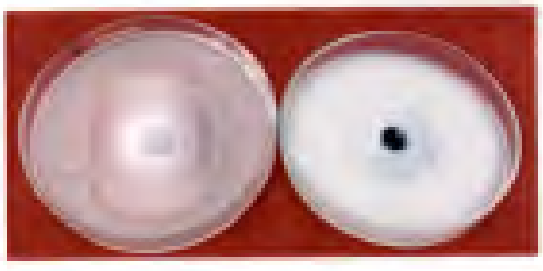
f) *P. exigua* (LBI-1)

g) *C. lunata* (LBI-2)

h) *P. longisetula* (LBM-1)



i) *R. solani* (FRW-1)



j) *F. oxysporum* (CRI-1)

k) *L. theobromae* (CRM-1)

Table 4.22 Per cent inhibition of fungal pathogens by *Pseudomonas fluorescens*

Sl. No.	Pathogen	Per cent inhibition of pathogen
1.	*Colletotrichum gloeosporioides* (LSW-1)	67.67(8.26)[b]
2.	*Colletotrichum gloeosporioides* (LSI-1)	70.55(8.41)[a]
3.	*Colletotrichum gloeosporioides* (LSM-1)	66.67(8.19)[c]
4.	*Alternaria alternata* (LSI-2)	56.67(7.56)[e]
5.	*Rhizoctonia solani* (LBW-1)	55.55(7.49)[f]
6.	*Phoma exigua* (LBI-1)	45.61(6.78)[g]
7.	*Curvularia lunata* (LBI-2)	0.00
8.	*Pestalotiopsis longisetula* (LBM-1)	56.67(7.56)[e]
9	*Rhizoctonia solani* (FRW-1)	33.33(5.82)[h]
10.	*Fusarium oxysporum* (CRI-1)	57.67(7.63)[d]
11.	*Lasiodiplodia theobromae* (CRM-1)	0.00
	CD (0.05)	0.012

*Mean of the three replications
In each column figure followed by same letter do not differ significantly according to DMRT.
$\sqrt{x+0.5}$ transformed values are given in parantheses

4.7 MOLECULAR CHARACTERISATION OF MAJOR PATHOGENS OF STRAWBERRY

The pathogens showing highest per cent disease incidence (PDI) and per cent disease severity (PDS) from different locations surveyed were selected *viz.*, *Pestalotiopsis longisetula, Colletotrichum gloeosporioides, Fusarium oxysporum* and *Lasiodiplodia theobromae* for *in vitro* studies. The confirmation of identity of pathogens upto species level was done by molecular characterisation. The fungal cultures were sent to Rajiv Gandhi Centre for biotechnology (RGCB), Thiruvananthapuram, where sequencing of ITS region was carried out. The sequences retrieved were blasted in the online BLASTn programme of NCBI to analyze and to find the nucleotide homology of each of these pathogens. Details of the result of the sequence comparison of four isolates are presented in Table 4.23, 4.24, 4.25 and 4.26.

4.7.1 Sequence comparison of *Colletotrichum gloeosporioides* isolate

Out of the several hits obtained during the sequence comparison of *Colletotrichum* with other sequences NCBI, all of them showed 100 per cent identity with 100 per cent query coverage. Similarity of the isolate was exhibited with different strains of *Colletotrichum gloeosporioides viz.*, strain F210042 (Accession KX197386.1), strain HC73 (Accession KU304499.1), strain CG45 (Accession KJ632417.1), strain CCGHN01 (Accession GQ865569.1) and strain SL37 (Accession KY302642.1). Thus, the cultural and morphological characteristics observed were found in line with the results shown in Table 4.1 and thus the identity of the isolate was certified to be *Colletotrichum gloeosporioides*.

4.7.2 Sequence comparison of *Pestalotiopsis longisetula* isolate

Nucleotide sequence of ITS of *Pestalotiopsis* was compared with other sequences and the search revealed that all of the strains indicated 100 per cent identity. The sequence also showed 100 per cent query coverage with *Neopestalotiopsis*

137

*clavispor*a isolate PG7 (Accession KY606543.1), *Neopestalotiopsis clavispora* isolate P3 (Accession KY810809.1), *Neopestalotiopsis clavispora* isolate P1 (Accession KY810807.1), *Neopestalotiopsis clavispora* isolate P01 (Accession KR052094.1), *Neopestalotiopsis clavispora* isolate H4405 (Accession GU595048.1), *Neopestalotiopsis clavispora* isolate P07-07 (Accession EU342214.1) and *Neopestalotiopsis clavispora* isolate FG13 (Accession EU030329.1). Thus based on the observations mentioned above, the isolate was confirmed as *Neopestalotiopsis clavispora*.

4.7.3 Sequence comparison of *Fusarium oxysporum* isolate

Comparison of nucleotide sequence of the isolate *Fusarium* with other sequences showed cent per cent identity and query coverage with a maximum score of 861 with different isolates of *Fusarium oxysporum*. *Fusarium oxysporum* f. sp. *lycopersici* (Accession KY587331.1), *Fusarium oxysporum* isolate F2 (Accession KY810793.1), *Fusarium oxysporum* isolate F1 (Accession KY810792.1), *Fusarium oxysporum* culture collection MUT<ITA>:2205 (Accession KX551959.1), *Fusarium oxysporum* isolate ELRF 8 (Accession KX786247.1), *Fusarium oxysporum* isolate A549 (Accession KX463005.1) and *Fusarium oxysporum* isolate F. ox4bal (Accession KX058546.1) are some of strains of that showed similarity with the isolate from strawberry. Hence, the result confirmed the identity of the fungus as *Fusarium oxysporum* based on the cultural and morphological characters of the isolate.

4.7.3 Sequence comparison of *Lasiodiplodia theobromae* isolate

Sequence of *Lasiodiplodia* when compared with database retrieved from NCBI revealed that all of the sequences were homologous with *Lasiodiplodia theobromae* showing 100 per cent identity and 99 per cent query coverage. The strains of *Lasiodiplodia theobromae* were CMW25212, CF/UENF439, CF/UENF437, CF/UENF435, CF/UENF432, CF/UENF431 and CF/UENF430. Hence, the isolate was confirmed with the results of molecular characterisation as *Lasiodiplodia theobromae*.

Plate 4.23 Agarose gel electrophoresis picture of *C. gloeosporioides* (A), *P. longisetula* (B), *F. oxysprum* (C) and *L. theobromae* (Z)

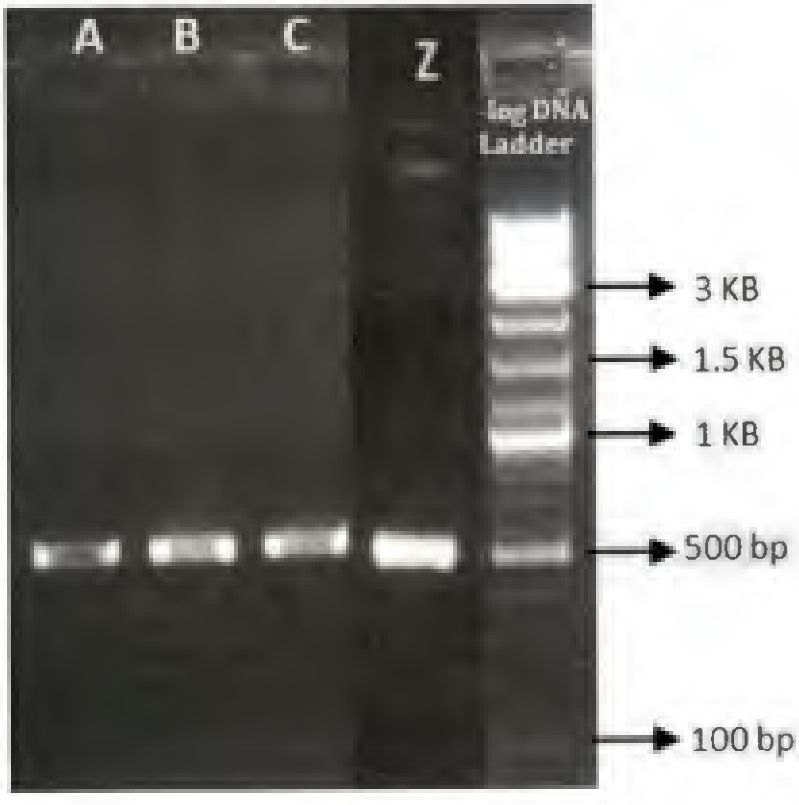


Table 4.23 Sequence homology of *Colletotrichum gloeosporioides* in BLASTn analysis

Sl. No.	Description	Max. score	Query Coverage (%)	E value	Identity (%)	Accession
1.	*Colletotrichum gloeosporioides* strain F210042	907	100	0.0	100	KX197386.1
2.	*Colletotrichum gloeosporioides* strain HC73	907	100	0.0	100	KU304499.1
3.	*Colletotrichum gloeosporioides* strain CG45	907	100	0.0	100	KJ632417.1
4.	*Colletotrichum gloeosporioides* strain CCGHN01	907	100	0.0	100	GQ865569.1
5.	*Colletotrichum gloeosporioides* strain SL37	902	100	0.0	100	KY302642.1

Table 4.24 Sequence homology of *Pestalotiopsis longisetula* in BLASTn analysis

Sl. No.	Description	Max. score	Query Coverage (%)	E value	Identity (%)	Accession
1.	*Neopestalotiopsis clavispora* isolate PG7	946	100	0.0	100	KY606543.1
2.	*Neopestalotiopsis clavispora* isolate P3	946	100	0.0	100	KY810809.1
3.	*Neopestalotiopsis clavispora* isolate P1	946	100	0.0	100	KY810807.1
4.	*Neopestalotiopsis clavispora* isolate P01	946	100	0.0	100	KR052094.1
5.	*Neopestalotiopsis clavispora* isolate H4405	946	100	0.0	100	GU595048.1
6.	*Neopestalotiopsis clavispora* isolate P07-07	946	100	0.0	100	EU342214.1
7.	*Neopestalotiopsis clavispora* isolate FG13	946	100	0.0	100	EU030329.1

140

Table 4.25 Sequence homology of *Fusarium oxysporum* in BLASTn analysis

Sl. No.	Description	Max. score	Query Coverage (%)	E value	Identity (%)	Accession
1.	*Fusarium oxysporum* f. sp. *lycopersici*	861	100	0.0	100	KY587331.1
2.	*Fusarium oxysporum* isolate F2	861	100	0.0	100	KY810793.1
3.	*Fusarium oxysporum* isolate F1	861	100	0.0	100	KY810792.1
4.	*Fusarium oxysporum* culture collection MUT<ITA>:2205	861	100	0.0	100	KX551959.1
5.	*Fusarium oxysporum* isolate ELRF 8	861	100	0.0	100	KX786247.1
6.	*Fusarium oxysporum* isolate A549	861	100	0.0	100	KX463005.1
7.	*Fusarium oxysporum* isolate F. ox4bal	861	100	0.0	100	KX058546.1

Table 4.26 Sequence homology of *Lasiodiplodia theobromae* in BLASTn analysis

Sl. No.	Description	Max. score	Query Coverage (%)	E value	Identity (%)	Accession
1.	*Lasiodiplodia theobromae* isolate CMW25212	929	99	0.0	100	KU997392.1
2.	*Lasiodiplodia theobromae* strain CF/UENF437	929	99	0.0	100	KY655210.1
3.	*Lasiodiplodia theobromae* strain CF/UENF435	929	99	0.0	100	KY655208.1
4.	*Lasiodiplodia theobromae* strain CF/UENF432	929	99	0.0	100	KY655205.1
5.	*Lasiodiplodia theobromae* strain CF/UENF431	929	99	0.0	100	KY655204.1
6.	*Lasiodiplodia theobromae* strain CF/UENF430	929	99	0.0	100	KY655203.1

141

Table 4.27 Genomic sequence of *Colletotrichum gloeosporioides* and *Neopestalotiopsis clavispora*

Pathogen	Sequence (5'-3')
Colletotrichum gloeosporioides	AGGGATCATTACTGAGTTTACGCTCTACAACCCTTTGTGAAC ATACCTATAACTGTTGCTTCGGCGGGTAGGGTCTCCGCGACC CTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGA GGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGT ACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGT TCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC ACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGA GCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCT ACAGCTGATGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCC GGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGAT CCGGAGGGACTCTTGCCGTAAAACCCCCCAATTTT
Neopestalotiopsis clavispora	AGGGATCATTATAGAGTTTTCTAAACTCCCAACCCATGTGA ACTTACCTTTTGTTGCCTCGGCAGAAGTTATAGGTCTTCTTA TAGCTGCTGCCGGTGGACCATTAAACTCTTGTTATTTTATGT AATCTGAGCGTCTTATTTTAATAAGTCAAAACTTTCAACAAC GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGA ATCTTTGAACGCACATTGCGCCCATTAGTATTCTAGTGGGCA TGCCTGTTCGAGCGTCATTTCAACCCTTAAGCCTAGCTTAGT GTTGGGAATCTACTTCTTTTATTAGTTGTAGTTCCTGAAATA CAACGGCGGATTTGTAGTATCCTCTGAGCGTAGTAATTTTTT TTCTCGCTTTTGTTAGGTGCTATAACTCCCAGCCGCTAAACC CCCAATTTTTTGTGGTTGACCTCGGATCAGGTAGGAATACCC GCTGAACTTAA

Table 4.28 Genomic sequence of *Fusarium oxysporum* and *Lasiodiplodia theobromae*

Pathogen	Sequence (5'-3')
Fusarium oxysporum	AGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACAT ACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACG GGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAA CTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGG ATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGA TAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG AACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTC GAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGACTCG CGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCT TCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCG GCCACGCCGTTAAACCCCAACTTCTGAAT
Lasiodiplodia theobromae	AAGGATCATTACCGAGTTTTCGAGCTCCGGCTCGACTCTCCCAC CCTTTGTGAACGTACCTCTGTTGCTTTGGCGGCTCCGGCCGCCA AAGGACCTTCAAACTCCAGTCAGTAAACGCAGACGTCTGATAA ACAAGTTAATAAACTAAAACTTTCAACAACGGATCTCTTGGTTC TGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC GCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTAC AACCCTCAAGCTCTGCTTGGAATTGGGCACCGTCCTCACTGCGG ACGCGCCTCAAAGACCTCGGCGGTGGCTGTTCAGCCCTCAAGC GTAGTAGAATACACCTCGCTTTGGAGCGGTTGGCGTCGCCCGCC GGACGAACCTTCTGAACTTTTCTCAAGGTTGACCTCGGATCAGG TAGGGATACCCGCTGAACTTAA

4.8 *In vivo* EVALUATION OF FUNGICIDES AND BIOCONTROL AGENTS ON MAJOR FUNGAL DISEASES OF STRAWBERRY

Studies conducted initially as part of the survey revealed that four pathogens *viz.*, *Colletotrichum gloeosporioides, Neopestalotiopsis clavispora, Fusarium oxysporum* and *Lasiodiplodia theobromae* recorded the highest per cent disease incidence and severity and therefore selected for *in vivo* experiments. An experiment was laid out in CRD at College of Horticulture, Vellanikkara and the fungicides and bioagents which were found effective under *in vitro* were chosen as treatments so as to find out whether these show similar results under field conditions. Experiment was conducted following the aforesaid mentioned protocol described in materials and methods. Observations on per cent disease incidence and severity were recorded at regular intervals. The results are presented in Table 4.27, 4.28, 4.29 and 4.30.

4.8.1 Management of *Colletotrichum gloeosporioides*

In this study, *Colletotrichum gloeosporioides* was challenge inoculated to the leaves by spraying the spore suspension to two month old strawberry plants maintained under ambient conditions. First, symptoms appeared as water soaked black lesions surrounded by a yellow halo which was noticed eight days after inoculation. Observations on PDS and PDI are tabulated in Table 4.27 along with the reduction in disease after each treatments. Per cent disease incidence in each treatment was recorded after eight days of challenge inoculation which ranged from 7.98 to 38 per cent, where the highest disease severity was noticed in case of T_5 (propineb 70WP) (0.3%) and the least in T_6 (*Trichoderma asperellum*) (2%).

Observations taken 10 days after the first treatment application indicated a significant difference among the various treatments. The treatment T_6 (*Trichoderma asperellum*) (2%) could reduce the disease upto 5.02 per cent from 7.98 per cent showing the maximum per cent reduction of 78.63. These results were on par with T_3 (carbendazim 12% + mancozeb 63%) (0.2%) with 73.78 per cent reduction of disease

over control. However, T_5 (propineb 70WP) (0.3%) exhibited the least per cent reduction of 42.38 per cent disease when compared with other treatments like T_4 (Copper hydroxide 77WP) (0.2%) and T_2 (cymoxanil 8% + Mancozeb 64%) (0.2%) which showed 63.70 and 56.55 per cent reduction over control.

Ten days after the second application of treatments, T_6 (*Trichoderma asperellum*) (2%) could reduce the severity upto 88.07 per cent, which was closely followed by T_3 (Carbendazim 12% + Mancozeb 63%) (0.2%) upto 86.48 per cent and T_4 (Copper hydroxide 77WP) (0.2%) upto 80.81 per cent showing maximum efficiency in disease management. The treatments T_2 (Cymoxanil 8% + Mancozeb 64%) (0.2%) and T_5 (Propineb 70WP) (0.3%) also showed a per cent reduction of disease over control by 77.68 and 66.54 per cent respectively.

4.8.2 Management of *Neopestalotiopsis clavispora*

One of the major leaf blight pathogen, *Neopestalotiopsis clavispora* was challenge inoculated on two month old plants by using spore suspension method and symptoms were observed seven days after inoculation. Observations on disease incidence and disease severity for each treatment are tabulated in Table 4.28. Per cent disease incidence (PDI) and percent disease severity (PDS) was recorded seven days after challenge inoculation where the former ranged from 43.39 to 55.98 per cent and latter from 28.1 to 37.17 per cent with the maximum in T_1 (Control) and minimum in T_6 (*Trichoderma asperellum*). Similar trend was also followed in case per cent disease severity (PDS). The data depicted in Table 4.28 provides the evidence that there is a significant reduction in disease severity after treatment application.

Table 4.29 Effect of treatments on per cent disease incidence and per cent disease severity of *Colletotrichum gloeosporioides*

Treatment No.	Treatments (foliar spray)	Conc (%)	8 days after inoculation		10 days after first spray		10 days after second spray	
			*PDI	*PDS	*PDS	Per cent disease reduction over control	*PDS	Per cent disease reduction over control
T_1	Control	-	38	19.2 (4.35)[a]	23.5 (4.83)[a]	-	27.83	-
T_2	Cymoxanil 8% + mancozeb 64% (Curzate M8)	0.2	18.13	14.41 (3.7)[a]	10.21 (3.12)[a]	56.55	6.21 (5.26)[a]	77.68
T_3	Carbendazim 12% + mancozeb 63% (Saaf)	0.2	14.77	9.29 (3.02)[ab]	6.16 (2.47)[bc]	73.78	3.76 (1.99)[bc]	86.48
T_4	Copper hydroxide 77WP (Kocide)	0.2	14.35	11.38 (3.19)[ab]	08.53 (2.76)[bc]	63.70	5.34 (2.19)[bc]	80.81
T_5	Propineb 70 WP(Antracol)	0.3	25.28	19.16 (3.89)[a]	13.54 (3.34)[b]	42.38	9.31 (2.76)[b]	66.54
T_6	*Trichoderma asperellum*	2	7.98	7.84 (1.92)[ab]	5.02 (1.77)[c]	78.63	3.32 (1.53)[c]	88.07
	CD (0.05)	-	-	1.49	1.31		1.089	
	CV			44.19	42.50		39.89	

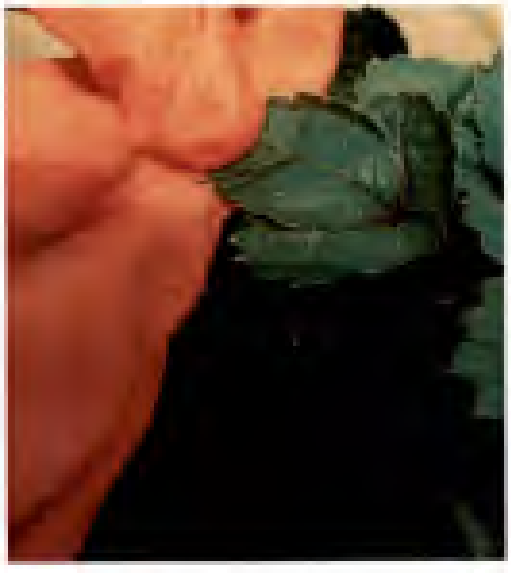
*Mean of the eight replications

In each column figure followed by same letter do not differ significantly according to DMRT.

√x+0.5 transformed values are given in parantheses PDS- Per cent disease severity, PDI- Per cent diseases incidence

Plate 4.24 *In vivo* evaluation of fungicides & biocontrol agents against *Colletotrichum gloeosporioides*

a. Experiment plot



b. Challenge inoculation

c. Symptom appearance

Table 4.30 Effect of treatments on per cent disease incidence and per cent disease severity of *Neopestalotiopsis clavispora*

Treatment No.	Treatments (foliar spray)	Conc (%)	7 days after inoculation		10 days after first spray		10 days after second spray	
			*PDI	*PDS	*PDS	Per cent disease reduction over control	*PDS	Per cent disease reduction over control
T_1	Control	-	55.98	37.17	39.79	-	43.43 (6.56)[a]	-
T_2	Cymoxanil 8% + mancozeb 64% (Curzate M8)	0.2	43.39	31.80	23.27	39.20	13.20 (3.91)[b]	69.60
T_3	Carbendazim 12% + mancozeb 63% (Saaf)	0.2	51.63	28.1	19.43	51.16	11.12 (3.39)[bc]	74.39
T_4	Copper hydroxide 77WP (Kocide)	0.2	43.71	35.17	25.59	35.68	11.37 (3.52)[b]	73.81
T_5	Propineb 70 WP(Antracol)	0.3	47.33	33.58	23.76	40.28	9.54 (3.89)[b]	78.03
T_6	*Trichoderma asperellum*	2	35.45	23.6	18.53	53.43	10.52 (2.28)[c]	75.77
	CD (0.05)			NS	NS		1.139	
	CV			29.08	29.93		28.72	

*Mean of the eight replications

In each column figure followed by same letter do not differ significantly according to DMRT.

√x+0.5 transformed values are given in parantheses PDS- Per cent disease severity, PDI- Per cent diseases incidence.

Plate 4.25 *In vivo* evaluation of fungicides & biocontrol agents against *Neopestalotiopsis clavispora*

a. Experiment plot

b. Challenge inoculation

c. Symptom appearance

Per cent disease severity was recorded after every 10 days of treatment application. Though, the per cent disease severity between the treatments were not significantly different, maximum reduction in disease of 53.43 per cent was recorded with T_6 (*Trichoderma asperellum*) (2%) and T_3 (Carbendazim 12% + Mancozeb 63%) (0.2%) (51.16%) followed by T_5 (Propineb 70WP) (0.3%) (40.28%) and T_2 (Cymoxanil 8% + Mancozeb 64%) (0.2%) (39.2%) and the least per cent of disease reduction with T_4 (Copper hydroxide 77WP) (0.2%) (35.68%).

A significant reduction among the treatments were noticed 10 days after second treatment application. The per cent disease reduction over control was 69.60 to 78.03 per cent with the maximum in T_5 (Propineb 70WP) (0.3%) and a minimum in T_2 (Cymoxanil 8% + Mancozeb 64%) (0.2%).

4.8.3 *Management of Fusarium oxysporum*

One of the most common soil borne pathogen infecting strawberry plants is *Fusarium oxysporum* and hence an experiment was conducted in two month old plants by challenge inoculating the pathogen. Symptoms first appeared 15 days after challenge inoculation and PDI was recorded at the time of symptom expression and twice at 10 days after treatment application. After 15 days of challenge inoculation, the disease incidence ranged from 62.5 to 100 per cent Observations recorded after 10 days of treatment application showed highest incidence of 75 per cent in T_2 (Cymoxanil 8% + Mancozeb 64%) (0.2%) and T_5 (Propineb 70WP) (0.3%) and 50 per cent in T_3 (Carbendazim 12% + Mancozeb 63%) (0.2%), T_4 (Copper hydroxide 77WP) (0.2%) and T_6 (*Trichoderma asperellum*). However, a drastic reduction in disease incidence of 50 per cent was noticed with T_3 (Carbendazim 12% + Mancozeb 63%) (0.2%) and T_4 (Copper hydroxide 77WP) (0.2%) ten days after second treatment application.

4.31 Effect of treatments on per cent incidence of *Fusarium oxysporum*

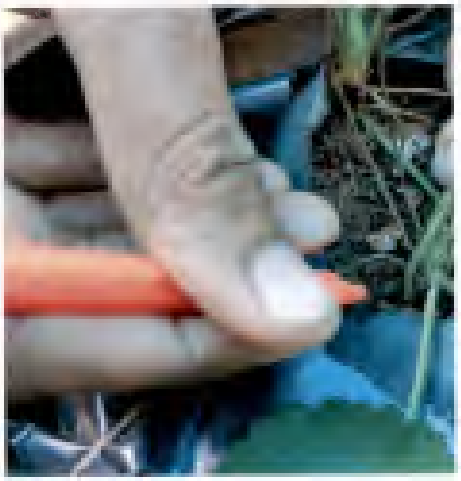
Treatment No.	Treatments (soil drench)	Conc (%)	15 days after inoculation	10 days after first drench		10 days after second drench	
			*PDI	*PDI	Per cent disease reduction over control	*PDI	Per cent disease reduction over control
T_1	Control	-	100	100	-	100^{a}	-
T_2	Cymoxanil 8% + mancozeb 64% (Curzate M8)	0.2	87.5	75	25	62.5^{ab}	37.5
T_3	Carbendazim 12% + mancozeb 63% (Saaf)	0.2	75	50	50	25^{b}	75
T_4	Copper hydroxide 77WP (Kocide)	0.2	75	50	50	25^{b}	75
T_5	Propineb 70 WP(Antracol)	0.3	75	75	25	62.5^{ab}	37.5
T_6	*Trichoderma asperellum*	2	62.5	50	50	50^{ab}	50
	CD(0.05)		NS	NS		45.72	
	CV		49.79	69.43		80.56	

*Mean of the eight replications

PDI-Per cent disease incidence

Plate 4.26 *In vivo* evaluation of fungicides & biocontrol agents against *Fusarium oxysporum*

a. Experiment plot



b. Challenge inoculation

c. Symptom appearance

4.8.4 *Management of Lasiodiplodia theobromae*

Major crown and root rot pathogen, *Lasiodiplodia theobromae* was challenge inoculated into the rhizosphere region by root inoculation with spore suspension on two month old strawberry plants. First symptom developed 16 days after challenge inoculation and fungicides were applied twice at 10 days interval. Observations on PDI were recorded and ten days after each treatment application as depicted in Table 4.30.

The results of the experiment revealed that per cent disease incidence were noticed in the ranged from 87.5 to 100 per cent 16 days after challenge inoculation of the pathogen with the lowest in T_6 (*Trichoderma asperellum*). Cent per cent disease incidence was noticed in T_1 (Control) and T_2 (Cymoxanil 8% + Mancozeb 63%) (0.2%) and lowest disease incidence of 75 per cent in T_6 (*Trichoderma asperellum*). However, it was noticed that there was a drastic reduction in disease incidence after first treatment application from 62.5 to 87.5 per cent with lowest incidence in T_3 (Cymoxanil 8% + Mancozeb 63%) (0.2%) and T_6 (*Trichoderma asperellum*) (2%) and highest in T_5 (Propineb 70WP) (0.3%). Observations on disease incidence 10 days after second treatment application showed 50 per cent disease incidence in T_3 (Cymoxanil 8% + Mancozeb 63%) (0.2%), 62.5 per cent in T_4 (Copper hydroxide 77WP) (0.2%) and T_6 (*Trichoderma asperellum*) (2%) and the highest incidence of 87.5 per cent in T_2 (Cymoxanil 8% + Mancozeb 64%) (0.2%).

Plate 4.27 *In vivo* evaluation of fungicides & biocontrol agents against *Lasiodiplodia theobromae*

a. Experiment plot

b. Challenge inoculation

c. Symptom appearance

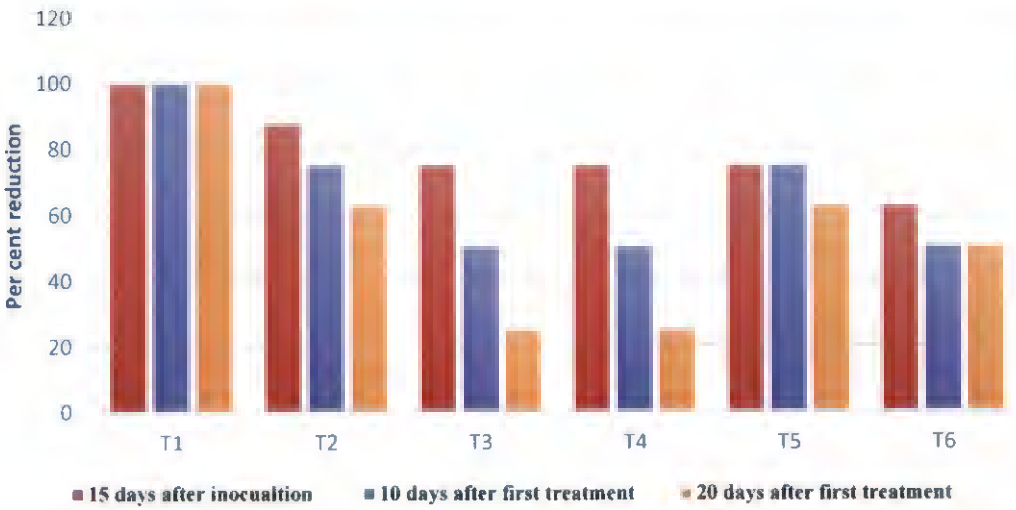
4.32 Effect of treatments on per cent incidence of *Lasiodiplodia theobromae*

Treatment No.	Treatments (soil drench)	Conc (%)	16 days after inoculation	10 days after first drench		10 days after second drench	
			*PDI	*PDI	Per cent disease reduction over control	*PDI	Per cent disease reduction over control
T_1	Control	-	100	100	-	100	-
T_2	Cymoxanil 8% + mancozeb 64% (Curzate M8)	0.2	100	100	0	75	25
T_3	Carbendazim 12% + mancozeb 63% (Saaf)	0.2	87.5	62.5	37.5	50	50
T_4	Copper hydroxide 77WP (Kocide)	0.2	87.5	75	25	50	50
T_5	Propineb 70 WP(Antracol)	0.3	87.5	87.5	12.5	75	25
T_6	*Trichoderma asperellum*	2	75	62.5	37.5	62.5	37.5
	CD(0.05)		NS	NS		NS	
	CV		34.98	47.01		66.86	

*Mean of the eight replications

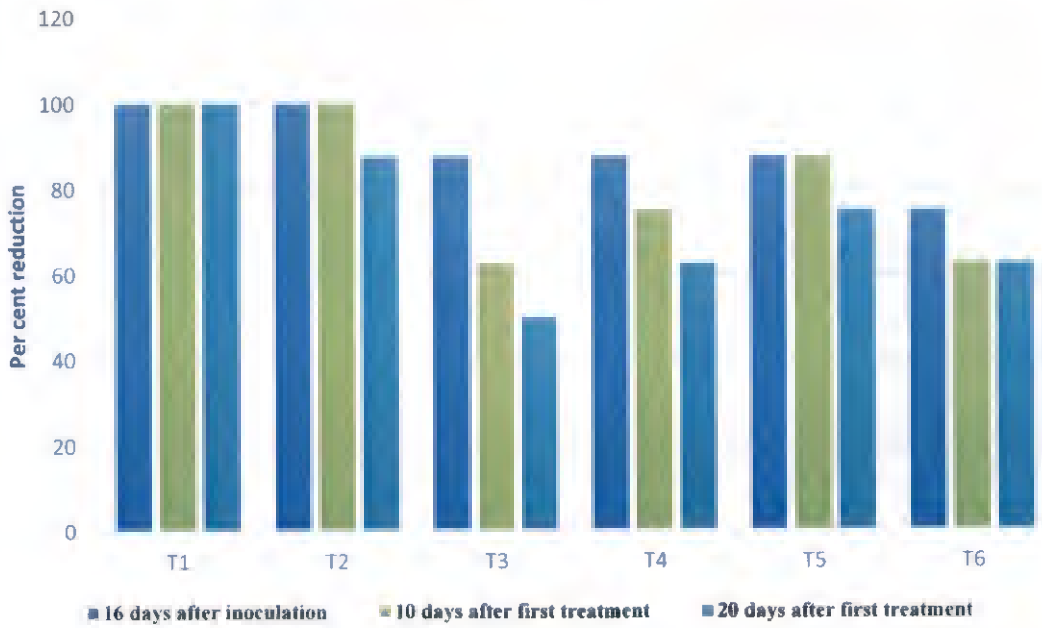
PDI- Per cent disease incidence.

Fig 4.10 Effect of treatments on per cent disease incidence of *Fusarium oxysporum*



T_1 - Control, T_2 - Cymoxanil 8% + Mancozeb 64%, T_3 - Carbendazim 12% + Mancozeb 63%, T_4 - Copper hydroxide 77WP, T_5 - Propineb 70 WP, T_6 - *Trichoderma asperellum*

Fig 4.11 Effect of treatments on per cent disease incidence of *Lasiodiplodia theobromae*



T_1 - Control, T_2 - Cymoxanil 8% + Mancozeb 64%, T_3 - Carbendazim 12% + Mancozeb 63%, T_4 - Copper hydroxide 77WP, T_5 - Propineb 70 WP, T_6 - *Trichoderma asperellum*


Discussion

5. DISCUSSION

Fruits, an indispensable part of human diet are considered as a best source of nutrients and occupies a substantial position in world agricultural market. Among the fruits, strawberry (*Fragariae* x *ananassa* Duch.) is an important attractive, high value, fruit with juicy texture and aroma, cultivated worldwide in recent years. Apart from being consumed as a fresh fruit, it has a great demand in processing as well as in cosmetic industry. The fruit of strawberry being a good source of vitamin C, manganese, dietary fibre and other nutrients is also an excellent source of unsaturated fatty acids and hence leads to less occurrence of cardiovascular diseases. Though strawberry represents a very profitable crop for the fresh market and also for food industry, the crop is being affected by a number of diseases in the field which has created a serious threat in both national as well as international market pausing its demand among the consumers. In Kerala, the crop is being cultivated in tropical and sub-tropical parts where the climate pose a major role in disease development. A perusal of the literature revealed that strawberry plants are infected by a number of fungal diseases *viz*., leaf spot (*Mycosphaerella fragariae*, *Alternaria alternata*), leaf scorch (*Diplocarpon earliana*), leaf blight (*Dendrophoma obscurans*), web blight (*Rhizoctonia solani*), powdery mildew (*Sphaerotheca humuli*), crown rot (*Armillaria mellea, Fusarium oxysporum* and *Colletotrichum acutatum),* red stele (*Phytophthora fragariae*), grey mold (*Botrytis cinerea),* black leaf spot (*Colletotrichum fragariae*) and fruit rot (*Colletotrichum acutatum* and *Rhizoctonia solani*) which resulted in huge crop losses (Schuh and Zeller, 1944; Miller, 1947; Wassenaar and Scheer, 1988; Tanaka *et al*., 1996; Bhardwaj and Gupta, 2002; Bhardwaj *et al*., 2003; Golzar *et al*., 2007 and Kaur *et al*., 2016).

However, no studies have been made in Kerala in order to identify, document and manage the fungal diseases of strawberry. Hence being the first attempt, the present investigation was focused to document various fungal diseases of strawberry and to

study its etiology, symptomatology and management of diseases both under *in vitro* and *in vivo* conditions.

5.1. SURVEY, COLLECTION AND ISOLATION OF FUNGAL DISEASES OF STRAWBERRY

An intensive survey was conducted in three districts *viz.*, Wayanad, Idukki and Malappuram during 2015-2017 to catalogue the different diseases prevailing in strawberry growing tracts. As part of the investigation, disease incidence, severity and symptoms under natural and artificial conditions were recorded. Prevalence of four leaf spots, four leaf blights, two crown and root rots and one fruit rot were recorded during the survey. Leaf spots and leaf blights were of common occurrence throughout the surveyed locations. Similar reports on leaf spots of strawberry were elucidated by Dingley (1970), Howard and Albregts (1983), Wassenaar and Scheer (1988), Tanaka *et al.* (1996), Curry *et al.* (2002) and Bagherabadi *et al.* (2015) and on leaf blights by Kim *et al.* (1992), Nita *et al.* (2003), Dil *et al.* (2013) and Ayoubi *et al.* (2016). Fruit rots of strawberry was noticed by Dodge and Stevens (1924) and crown and root rots by Koike *et al.* (2013), Yildiz *et al.* (2009), Koike and Jordan (2015) and Nam *et al.* (2016).

The diseased samples were collected and brought to the laboratory, where isolation of pathogens was carried out as per standard protocols. Based on morphological and cultural characteristics, the pathogens were identified upto genus level by comparing the characteristics given in CMI Descriptions of Pathogenic Fungi and Bacteria. Thus, the pathogens causing LSM-1, LSI-1 and LSW-1 were identified as *Colletotrichum* sp., LS1-2 as *Alternaria* sp., LBW-1 as *Rhizoctonia* sp., LB1-1 as *Phoma* sp., LBI-2 as *Curvularia* sp., LBM-1 as *Pestalotia* sp., FRW-1 as *Rhizoctonia* sp., CRI-1 as *Fusarium* sp. and CRM-1 as *Lasiodiplodia* sp.

5.1.1 Assessment of per cent disease incidence, per cent disease severity and correlation with weather parameters

Disease development under field conditions depends primarily on factors like host, pathogen and environment which together constitute the disease triangle. Among the three factors, the time of initial appearance of the disease, its spread, build up and its severity is highly influenced by prevailing weather parameters like temperature, relative humidity and rainfall. Hence, the role of agrometeorological parameters in relation to disease development is very essential, as an early forecast can considerably help the farmers to practice better preventive measures thereby leading to higher yields and profits. Thus, the present investigation was carried out to study the periodical progression of the disease and its correlation with prevailing weather parameters *viz*., rainfall, relative humidity and temperature.

The periodical progression of fungal diseases of strawberry showed a variable disease development since November 2015 to March 2017. From various diseases noticed in different seasons, the incidence of *Colletotrichum gloeosporioides* was of common occurrence which prevailed in all three districts *viz*., Wayanad, Idukki and Malappuram. Correlation of the diseases with weather parameters of Ambalavayal and Anakkayam depicted no relation with temperature. At Ambalavayal, a negative correlation existed with relative humidity and rainfall which showed highly significant results. Ambalavayal recorded a higher incidence and severity of 52.9 and 22.8 per cent during respectively December-January and here, increase in RH and rainfall plays an important role in reducing the disease severity over the seasons as it is negatively correlated with the disease. LSI-1 in Idukki recorded a maximum severity of 19.9 per cent during March-April and a minimum of 10.4 per cent in December-January when temperature was 24.04°C and 19.74°C respectively depicting a positive correlation with temperature and rainfall. LSM-1 in Anakkayam recorded a positive correlation with RH and rainfall where maximum severity of 23.4 per cent was observed during March-April at a higher temperature of 29.2°C and lower RH and rainfall of 48.58 per cent and 4.6 mm. It is interesting to note that a relative humidity of 66.96 per cent and a heavy rainfall

of 504 mm resulted in lower severity of 20.9 per cent during July-August. Several studies have shown the occurrence of *Colletotrichum* leaf spot. Smith and Black (1987) observed a high disease severity of *Colletotrichum fragariae* in strawberry at a high temperature of 35°C and cent per cent relative humidity, showing a positive influence of temperature and RH on disease development. However, the above findings were closely supported by Wilson *et al.* (1992), where they reported maximum disease incidence of *Colletotrichum acutatum* during March when the temperature ranged from 11.86 to 23. 9°C, relative humidity of 61-94 per cent and rainfall of 62.9mm. Hang *et al.* (2007) observed that the optimum temperature required for *Colletotrichum gloeosporioides* to cause infection in strawberry is 28-32°C along with a relative humidity of above 90 per cent which was not comparable with the results of present study. According to them the disease is considered as a typical temperature and high humidity disease. Nevertheless, Pan and Mishra (2010) noticed a non-significant positive correlation with maximum relative humidity, temperature and total rainfall and significant positive results with minimum relative humidity, temperature and number of rainy days in case of *Colletotrichum gloeosporioides* infecting guava. While, Ji *et al.* (2012), opined that the pathogen *Colletotrichum fragariae* infecting strawberry spreads at a temperature of 25-30°C in rainy days of midsummer.

The leaf spot diseases, LSI-2 and LSM-2 from Idukki and Malappuram caused by *Alternaria alternata* showed a non-significant correlation with relative humidity and rainfall. In Idukki, there existed no relation with weather parameters for disease development. However at Anakkayam the severity was found positively correlated with temperature. It was noticed that a maximum severity of 22.3 per cent was noticed under high temperature (24.04°C), RH (95.48 %) and rainfall conditions (45.4mm). It is also to be noted that, notable variation was observed in the intensity of the disease during July-August and March-April. A decrease in temperature from 29.2 to 24.23°C slightly intensified the severity from 21.3 to 22.8 per cent. Thus, it may be inferred that decrease in temperature can reduce disease development in Anakkayam. Wada *et al.* (1996) reported that leaf spot caused by *Alternaria alternata* in strawberry turned epidemic in

late summer, where relative humidity and rainfall was high and the daily mean temperature ranged from 20-25°C. Sahu *et al.* (2014) commented that disease severity of *Alternaria solani* causing early blight in tomato was comparatively higher when the temperature ranged from 25.6 to 28.3°C and 13.6-16.4°C with an average relative humidity of 65 per cent. According to them and contrary to the above findings, disease severity indicated a significant negative correlation with temperature and positive correlation with relative humidity. However, the present result is contradictory to the reports detailed by several authors (Sarkar and Sengupta, 1978, Humpherson- Jones and Ainsworth, 1983, Maude *et al.*, 1986, Hiremath *et al.*, 1990, Rajiv Kumar and Singh, 1996, Das *et al.*, 1998 and Kemmitt, 2002) in various crops like mustard and sunflower incited by *Alternaria* sp.

Leaf blight disease (LBW-1) caused by *Rhizoctonia* sp. was observed as the devastating disease at Ambalavayal causing significant economic losses. Correlation study revealed that the disease had a negative influence with relative humidity and rainfall, however it was found non-significant with temperature. Maximum severity of 25.2 per cent was recorded during December-January at a temperature of 21.92°C, RH of 74.24 per cent and rainfall of 59.6mm with a subsequent reduction in other seasons. Rainfall and relative humidity showed an upturn reaching upto 466.5 mm and 88.81 per cent in July-August. De Los Santos *et al.* (2003) studied the conditions favourable for web blight of strawberry and revealed that high levels of soil moisture with high relative humidity and temperature is highly congenial for disease development which is contradictory to the present findings. Similarly, the results presented by Bharadwaj *et al.* (2003) indicated a positive correlation with mean temperature, relative humidity and rainfall in case of web blight caused by *Rhizoctonia solani* in strawberry where they noticed that high relative humidity, soil moisture and temperature along with high rainfall during July-August is most conducive for disease spread which was found not comparable with the present results where the least severity of disease was recorded during July –August.

Foliage disease LBI-1 was recorded during December-January and March-April in Vattavada incited by *Phoma* sp. The disease was found positively correlated with temperature and negatively with rainfall. It was noticed that the severity increased from 13.8 observed in December-January to 19.7 per cent in March-April. The data also clearly depicted that there was no significant relation of the disease with relative humidity. Hence, it may be inferred that the pathogen favours higher temperature for disease build up. Thus, the correlation analysis reveals that comparatively lesser severity occurs at cooler climatic conditions. Different from the above results, Borekar *et al.* (2014) pointed out negative correlation of the leaf spot disease incited by *Phoma* sp with rainfall, temperature and RH during December showing that the disease was intense only during cool weather conditions. Likewise, Toscano-underwood *et al.* (2001) observed maximum lesions formation in case of *Phoma lingam* causing leaf spot in brassica, when the temperature was found between 15 to 20°C.

Leaf blight pathogen (LBM-1) incited by *Pestalotiopsis* sp. in Anakkayam were not found much influenced by climatic factors as no significance existed between the weather parameters in disease development. However, a higher PDS and PDI of 21.7 and 58.2 per cent was recorded during March-April when temperature was at its peak of 29.2°C along with lower RH and rainfall of 48.48 per cent and 4.6mm respectively. While, the severity and incidence was found very low during December-January. The present results are contradictory to the report by Moustafa *et al.* (2016) where they observed 64 per cent of incidence of guava leaf spot caused by *Pestalotia psidii* when correlated with temperature and 60 per cent with RH. Similarly, Rodrigues *et al.* (2014) elucidated the incidence of strawberry leaf spot caused by *Pestalotiopsis longisetula* with temperature lower than 25°C, high RH and intense rainfall which was not in accordance with the present findings.

Fruit rots were recorded during the survey from Ambalavayal only, with the less intensity during December-January where the weather factors which might have intensified the spread of disease were low temperature, high RH and sparse with less intense rainfall.

Crown and root rot (CRI-1) of strawberry plants infected by *Fusarium* sp. was recorded from Idukki. Plants were found infected when grown under open field conditions with an incidence of 70 per cent during March-April in Koviloor. Maximum incidence of 45 per cent was observed in December-January at a temperature of 27.92°C, a lower RH of 61 per cent and rainfall of 52.9 mm and minimum incidence of 15 per cent at 30.4°C temperature, 67 per cent RH and 35.6mm rainfall. Smith (1952) opined that black root rot of strawberry caused by *Fusarium culmorum* is severe in summer on heavy and wet soils, where the plants are grown in succession for several years. Recent evidences of Mina and Dubey (2010) interpreted the role of soil temperature and ambient atmospheric temperature in development of *Fusarium oxysporum* in chickpea. Similar findings on the progression of disease in relation to climatic factors have been elucidated by Fang *et al.* (2011) where he observed optimum temperature of 27°C for disease development in strawberry caused by *Fusarium oxysporum*.

In case of crown and root rot (CRM-1) caused by *Botryodiplodia* sp., the correlation studies revealed a positive influence of temperature on disease development. However, a negative correlation was noticed with RH and rainfall which indicated that the disease upsurge, when RH and rainfall decrease. This was evident from the incidence of the disease which decreased from 85 to 72 per cent when rainfall increased from 4.6 mm to 504 mm and RH from 48.58 per cent to 66.96 per cent. Pan and Mishra (2010) experimented the influence of weather parameters in guava on the occurrence of *Botryodiplodia theobromae* where they observed negative correlation with relative humidity, maximum and minimum temperature and total rainfall.

5.2 PATHOGENICITY OF FUNGAL ISOLATES

Fungal isolates of respective diseases were subjected to pathogenicity testing artificially on live plants as well as on detached leaves. Several techniques were employed for each kind disease depending upon the symptoms expressed. Mycelial plugs (MBIM) were placed over the leaf surface as suggested by Rocha *et al* (1998) for foliar as well as fruit pathogens. Studies on pathogenicity of leaf spot pathogen,

Alternaria sp. is in congruence with Wassenaar and Scheer (1988), Wada *et al.* (1996) and Bagherabadi *et al.* (2015) who effectually confirmed the techniques by spraying the conidial suspension of 2 x 10^5 cfu ml^{-1} on leaves of strawberry. Moreover, methods followed by Sharifi *et al.* (2008), Rodrigues *et al.* (2014) and Mouden *et al.* (2014) were found parallel to the present study as they could prove the pathogenicity of *Pestalotiopsis longisetula* using conidial suspension and mycelial plugs, where the symptom initiated within 5 days after inoculation with small water soaked lesions on leaves of strawberry. Likewise, Dil *et al.* (2013) placed mycelial plugs on the wounded leaves of blueberries to prove the pathogenicity of *Curvularia inaequalis* in a manner similar to that of the present work. Howard and Albregts (1973), Denoyes and Baudry (1995), Curry *et al.* (2002), Mertely and Legard (2004) and Akhter *et al.* (2009) also used conidial suspension of 2 x 10^6 cfu ml^{-1} in deionized sterile water to spray strawberry plants to prove the pathogenicity of *Colletotrichum gloeosporioides* causing black leaf spot. Further, Pathogenicity of *Phoma* leaf spot of grapes was tested by spraying the conidial suspension as suggested by Nita *et al.* (2003). Anees *et al.* (2016) described the pathogenicity testing of *Rhizoctonia solani* leaf blight by placing mycelial bits over the detached leaf surface. For crown and root rot pathogens, Urena-Padilla *et al.* (2002) and Nam *et al.* (2016) successfully pointed out the technique of pouring the conidial suspension of 2 x 10^5 cfu ml^{-1} of *Fusarium oxysporum* and *Botryodiplodia theobromae* in crown region of plants.

5.3 SYMPTOMATOLOGY OF PATHOGENS

Symptoms of different diseases associated with strawberry were studied both under field and artificial conditions. Three leaf spots caused by *Colletotrichum gloeosporioides* were recorded one each from three locations. The pathogen causing LSW-1 produced black circular spots all over the leaf that further coalesced leading to blightening symptoms. Similar symptoms were also noticed in case of LSI-1 where black spots were surrounded by a yellow halo. However, LSM-1produced black irregular spots near the margins and gradually extending towards veins and petioles.

These observations were in line with those reported band Albregts (1973) and Tanaka *et al.* (1996) where they pointed out similar symptoms in anthracnose leaf spot of strawberry along with its infection on petioles. Several workers like Paulus (1990) and Curry *et al.* (2002) also observed circular spots on leaves with water soaked lesions which later dried and turned brown or purple brown apart from infection on petioles of strawberry.

Pathogen responsible for causing LSI-2 disease was *Alternaria* sp., where brown concentric zonations near the leaf margin with yellow or dark reddish purple margin was observed. The descriptions of above symptoms was in uniformity with the findings putforth by Dingley (1970), Wassenar and Scheer (1988) and Bagherabadi *et al.* (2015) in strawberry.

Among the leaf blights, symptoms of LBW-1 caused by *Rhizoctonia* sp. initiated as small reddish black spots on mature leaves that later advanced with a purplish discolouration extending towards veins. Observations of Zeller (1932) and Lele and Pathak (1965) was in accordance with the aforesaid symptoms where they noticed severe purpling of strawberry leaves. Likewise, Kim *et al.* (1992) observed enlarged spots and distorted leaves with severe blightening and distortion of strawberry. Moreover, De Los Santos *et al.* (2003) recorded the severity of *Rhizoctonia* causing web blight that finally lead to complete defoliation of strawberry.

Symptoms of *Phoma* leaf spot (LBI-1) began with a small circular to irregular brown spots, that later coalesced to form 'V' shaped lesions extending from margin towards leaf lamina leading to severe leaf blightening. Many workers have reported the occurrence of similar symptoms showing typical leaf blight caused by *Phoma* sp. in various crops. Nita *et al.* (2003), Saju *et al.* (2012), Shivanna *et al.* (2014), Choi *et al.* (2014), Park *et al.* (2014) and Kumla *et al.* (2016).

Pathogen associated with leaf blight of strawberry (LBI-2) was *Curvularia* sp. which developed small brown coloured spots with irregular borders on leaf lamina that later enlarged casuing severe blight of the margins. Dil *et al.* (2013) and Ayoubi *et al.*

(2017) detailed the pathogenic potential of *Curvularia inaequalis* causing leaf spot in blueberry and strawberry with identical symptoms.

Another leaf blight (LBM-1) caused by *Pestalotiopsis* sp. isolated from Malappuram produced light brown spots on both young as well as mature leaves with black pin head like acervuli on upper leaf surface. The above descriptions were comparable with the symptoms elucidated by Singh *et al.* (1975), Paulus (1990), Maas (1998), Bhardwaj and Sharma (1999) and Rodrigues *et al.* (2014) on strawberry leaves which enlarged giving a blighted appearance. In severe cases, symptoms appeared as 'V' shaped lesions that ultimately led to drying up of leaves. Moustafa *et al.* (2015) also noticed similar symptoms on guava fruits infected by *Pestalotia psidii*.

The fruit rot (FRW-1) caused by *Rhizoctonia solani* was the most destructive disease that reduced the commercial value of the crop in Wayanad district. Symptoms appeared as rotting of fruit with black, hard lesions. According to, Dodge and Stevens (1924) the fruits infected with *Rhizoctonia* appeared hard, dry and leathery and these symptoms were predominant in berries that are in contact with soil. However, De Los Santos *et al.* (2003) observed that *Rhizoctonia fragariae* causes hard rot in green fruits while *Rhizoctonia solani* attacks ripe strawberry fruits.

An attempt was made to study the crown root rot symptoms caused by *Fusarium* pathogen of strawberry where it appeared as reddish-orange discolouration of internal cortical tissues with poor growth and stunting of plants, which gradually lead to complete death and collapse. The description of symptoms detailed above on strawberry plants was in congruence with the findings putforth by Arroyo *et al.* (2009), Koike *et al.* (2009), Fang *et al.* (2011a), Fang *et al.* (2011 b), Fang *et al.* (2013) and Koike and Gordon (2015).

Another crown and root rot pathogen caused by *Botryodiplodia theobromae* in strawberry plants exhibited wilting and die back of runners with black rot on root and crown region. Yildiz *et al.* (2014) and Nam *et al.* (2016) observed black necrotic discolouration on roots and crown of strawberry apart from the infection on leaf petiole.

167

5.4 CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF PATHOGENS

Pathogens after isolation and pathogenicity tests were subjected to cultural and morphological characterisation for identification. The pathogens were thus identified upto the genus level and for further confirmation upto the species level, the cultures were send to National Centre for Fungal Taxonomy (NCFT), New Delhi.

Leaf spot pathogens (LSW-1, LSI-1 and LSM-1) caused by *Colletotrichum* sp. were recorded from three different districts during the survey. Cultural and morphological characteristics of each of them were studied in detail. Anthracnose pathogen causing LSW-1 was characterised as white to grey colonies with aerial mycelia with dark grey on the reverse side. Hyphae hyaline, changing to greyish white, conidia hyaline, single celled, straight and cylindrical with round or obtuse ends of size 8.88-15.06 x 2.34-5.01µm. The characteristics of LSW-1 were consistent with the description as reported by Kim *et al.* (2009) and Xu *et al.* (2013) in blueberry. The pathogen LSI-1 in culture also developed beige to dark grey colonies with subsurface growth, where the conidia is hyaline, single celled with round to oblong ends where the size of the conidia ranged from 7.6-14.3 x 3.43-6.12 µm. Likewise, colony of LSM-1 appeared grey producing unicellular, cylindrical conidia with straight to round ends where the size ranged from 6.98-13.54 x 3.12-6.7 µm. Similar conidial characteristics as LSI-1 and LSM-1 were observed by Bose *et al.* (1973), Gunnell and Gubler (1992), Smith (2008) Embaby *et al.* (2010) Xie *et al.* (2010) and Chowdappa *et al.* (2012) is strawberry and mango incited by *Colletotrichum gloeosporioides*. Hence, the pathogen was confirmed as *Colletotrichum gloeosporioides.*

The pathogen causing leaf spot 2 (LSI-2) (*Alternaria* sp.) produced ashy white colony that gradually turned grey on maturity with submerged mycelium where the hyphae was thin, septate and brown in colour. Conidia appeared dark brown, 24.96 to 46.89 µm to 11.40 to 15.69µm spindle to ellipsoidal shaped with transverse and longitudinal septa arranged in chains. These observations were in congruence with those reported by Wada *et al.* (1996), Takahashi *et al* (1997), Bagherabadi *et al.* (2015) and

Fernandez *et al.* (2015) in strawberry and blueberry and finally the pathogen was confirmed as *Alternaria alternata*.

Rhizoctonia sp. causing leaf blight (LBW-1) produced thin and pale white mycelia turning brown with smooth and circular margins. Hyphae hyaline with hyphal length 88.23 to 98.17µm with branches arising at 90° below the septa with distinct constrictions and sporulation was found absent. These characteristics are in with the observations of Sneh *et al.* (1991), Nechet and Halfeld-Vieira (2007) and Lal and Kandhari (2009). Hence the pathogen was identified as *Rhizoctonia solani*.

Phoma leaf spot pathogen (LSI-1) upon culturing produced pale white to grey colonies with dark green to black globoid olivaceous pycnidia in culture expanding in a zonate manner with irregular margins. Hyphae initially hyaline, turned brown and septate and conidia were hyaline, aseptate, ellipsoidal to oblong with 6.18 to 7.62 × 2.29 to 3.27 µm dimension. These characteristics were in agreement with the findings of Shivanna *et al.* (2014), Choi *et al.* (2014) Park *et al.* (2014) and Kumla *et al.* (2016). Based on the above characters, the pathogen was identified as *Phoma exigua*.

Isolate of *Curvularia* sp. causing leaf blight (LBI-2) produced velvety greyish black mycelium on PDA which later turned dark black. Conidia boat or half-moon, 6.682- 9.964 µm long and 16.653- 18.984µm wide, dark brown, four celled, two cells at the centre larger and darker than the terminal cells. Verma and Gupta (2010) and Zhong *et al.* (2016) studied similar morphological characters of the pathogen. Apart from this, Dil *et al.* (2013) observed *Curvularia inaequalis* infecting blueberries with similar cultural and morphological characters. Based on the above characters, coupled with symptomatology and pathogenicity, the isolate was identified as *Curvularia lunata*.

During the period of study, the pathogen causing leaf blight (LBM-1) was recorded as *Pestalotiopsis* sp. which produced white fluffy aerial mycelium covered with black ink mass bearing conidia on PDA. Conidia spindle shaped, straight or slightly curved with five cells, where central three cells were darker and thicker and apical cells slightly pointed with end cells hyaline measuring 22.02 to 36.948 µm long and 8.85 to

11.98µm wide. Embaby (2007), Mouden *et al.* (2014) and Ayoubi and Soleimani (2017) observed similar results in strawberry infected by *Pestalotia* sp. These findings were also in corroboration with that observed by Feng *et al.* (2007), Espinoza *et al.* (2008), Luan *et al.* (2008) and Gonzalez *et al.* (2012) in blueberry and Moustafa *et al.* (2015) in guava. The isolate thus obtained was confirmed as *Pestalotiopsis longisetula*.

Mycelia of *Rhizoctonia* sp. causing fruit rot (FRW-1) developed white long thread like hyphae with length ranging from 121.23 to 150.98 µm along with distinct right angle branching. These observations were in line with that of Sneh *et al.* (1991), Nechet and Halfeld-Vieira (2007) and Lal and Kandhari (2009). Thus the isolate was confirmed as *Rhizoctonia solani* based on the above said characters.

Fusarium crown and root rot infecting strawberry plants identified from Idukki district produced white fluffy mycelium which subsequently turned light pinkish. Hyphae were hyaline, septate and branched. Colonies produced macroconidia and microconidia, 2-4 septate with slightly curved apical cells and foot shaped basal cells measuring 8.50 to 18.71µm long and 2.1 to 4.9µm wide, whereas microconidia was one celled, oval to ellipsoid, borne abundantly of size 1.64 to 3.92 x 4.12 to 9.34 µm. The above descriptions were in line with those studied by Cha *et al.* (2007), Holguin-Pena (2005), Arroyo *et al.* (2009), Ignjatov *et al.* (2015), Joshi *et al.* (2013), Liu *et al.* (2014) Wright *et al.* (2014) and Dinler *et al.* (2016). Thus, the pathogen was confirmed as *Fusarium oxysporum*.

Similarly, crown and root rot pathogen isolated from Malappuram district produced white colonies that turned greyish black. These observations were in conformity with the observations of Yildiz *et al* (2014) and Bhadra *et al.* (2014) where hyphae was septate with brown to black colour *and* conidia single celled hyaline and brown when young, ellipsoid or obovate and thick walled, when mature. Dimension of conidia ranged from 23.54 - 32.17 x 12.75 -16.05 µm size. The above descriptions were also pointed out by Xie *et al.* (2014) and Nam *et al.* (2016) in mulberry plants infected by *Lasiodiplodia theobromae*.

170

5.5 DISEASE MANAGEMENT

According to Govorova (1993) and Kapytowski and Bojarska (2005), severe yield loss of 15-92 per cent was recorded in strawberry plantations infected with various fungal pathogens leading to heavy crop losses. Hence, better disease management practices involving cultural, biological and chemical practices along with use of best disease resistant variety should be adopted at every stage of crop production.

5.5.1 *In vitro* evaluation of fungicides against plant pathogens

Fungicides play a major role in the management of various crop diseases. However, these should be used safely according to correct prescriptions and dosage so as to get sufficient results. Hence, an attempt was made to evaluate the fungicides under *in vitro* conditions to get a preliminary information regarding its efficacy before they are being recommended under open field conditions.

The study was formulated to evaluate the *in vitro* efficacy of nine fungicides against eleven pathogens associated with strawberry diseases. Contact fungicides like Bordeaux mixture, copper hydroxide 77WP, propineb 77 WP, copper oxychloride 50WP and systemic fungicides *viz*., difenoconazole 25EC, carbendazim 50WP and potassium phosphonate were used for the study. Two combination fungicides like carbendazim 12% + mancozeb 63% and cymoxanil 12% + mancozeb 64% were also included as treatments against 11 pathogens.

Colletotrichum gloeosporioides causing leaf spot 1 (LSW-1) of strawberry were tested for its sensitivity against nine fungicides under *in vitro* conditions. Two copper fungicides *viz*., copper hydroxide 77WP (Kocide), copper oxychloride 50WP (Fytolan), a combination chemical carbendazim 12% + mancozeb 63% (Saaf) along with other three fungicides *viz*., propineb 70WP (Antracol), difenoconazole 25 EC (Score) and carbendazim 50WP (Bavistin) exhibited cent per cent inhibition. Another combination fungicide, cymoxanil 8% + mancozeb 64% (Curzate M8) and copper fungicide, Bordeaux mixture inhibited the mycelial growth by 97.67 and 88.88 per cent at lower

concentration of 0.15 and 0.5 per cent and cent per cent at the higher two concentrations. The effect of Bavistin, Fytolan, Score and Bordeaux mixture are in agreement with the study conducted by Karande *et al.* (2007), Patil *et al.* (2009) and Singh *et al.* (2008) in pepper, mustard and guava infected with *Colletotrichum gloeosporioides*. Only potassium phosphonate (Akomin-40) showed the least inhibition of 40 per cent and above at all concentrations tested. Since this fungicide is effective for the control of plant diseases caused by Oomycetes (Williams *et al.* 1977)

Colletotrichum gloeosporioides causing black leaf spot (LSI-1) from Idukki tested for its sensitivity against fungicides indicated cent per cent efficacy of carbendazim 12% + mancozeb 63% (Saaf) and carbendazim 50WP (Bavistin). Venkataravanappa (2006) and Prashanth and Sataraddi (2007) made similar observations regarding the efficacy of Saaf in mango and pomegranate affected with the pathogen, *Colletotrichum gloeosporioides*. Similarly, Prasad *et al.* (1998) and Akhter *et al.* (2009) reported cent per cent efficiency of Bavistin against *Colletotrichum gloeosporioides* of mandarin and strawberry. Likewise, Propineb 70 WP (Antracol) at 0.3 and 0.35 per cent showed cent per cent inhibition which was contradictory to the observation made by Tasiwal *et al.* (2009) and Jagtap *et al.* (2015), where they reported 67.46 per cent inhibition of *Colletotrichum gloeosporioides* of papaya and pomegranate. In the present study, cymoxanil 8% + mancozeb (64%) (Curzate M8) could inhibit the growth upto 97 per cent whereas difenoconazole 25 EC (Score), copper fungicide *viz.*, copper oxychloride 50WP (Fytolan) and Bordeaux mixture showed 70 to 80 per cent inhibition. Prashanth *et al.* (2008) and Patil *et al.* (2009) made similar findings regarding the ability of copper oxychloride and difenoconazole to inhibit *Colletotrichum gloeosporioides* infecting pomegranate and long pepper. Copper hydroxide 77WP (Kocide) and potassium phosphonate (Akomin-40) were found comparatively less effective.

In case of *Colletotrichum gloeosporioides* (LSM-1) isolated from Anakkayam, two combination chemicals *viz.*, carbendazim 12% + mancozeb 63% (Saaf), cymoxanil 8% + mancozeb 64% (Curzate M8), two copper fungicides *viz.*, copper hydroxide 77WP

(Kocide) and Bordeaux mixture and carbendazim 50WP (Bavistin) at all three concentrations and highest two concentrations of Propineb 70WP (Antracol) and difenoconazole 25 EC (Score) recorded cent per cent efficiency. Other fungicide, copper oxychloride 50WP (Fytolan) could show 77 to 83 per cent inhibition at concentrations ranging from 0.2 to 0.3 per cent. However, potassium phosphonate (Akomin-40) showed the least inhibition of the pathogen at all concentrations. Ramani *et al.* (2015) reported cent per cent efficacy of *Colletotrichum gloeosporioides* with carbendazim, copper oxychloride and the combination fungicide, carbendazim (12%) + mancozeb (63%), while 56.68 per cent inhibition was reported with Curzate M8 in banana. Saju *et al.* (2013) also noticed complete inhibition of *Colletotrichum gloeosporioides* with copper oxychloride followed by the combined formulation of Saaf in cardamom. Likewise, Patil *et al.* (2009) observed cent per cent efficacy of Saaf and propiconazole, 71.46 per cent inhibition with carbendazim and 36.58 per cent inhibition with copper oxychloride in long pepper against *Colletotrichum gloeosporioides*.

Amongst the different fungicides tested against *Alternaria alternata* (LSI-2), cymoxanil 8% + mancozeb 64% (Curzate M8), copper hydroxide 77WP (Kocide), copper oxychloride 50 WP (Fytolan), Bordeaux mixture (1%, 1.5%) and propineb 70WP (Antracol) were found 100 per cent effective over control. Efficacy of copper oxychloride and propineb is in congruence with the findings of Gohel and Solanki (2011) and Chethana *et al.* (2013) against *Alternaria alternata* in onion and chilli. Mathivanan and Prabhavathy (2007) and Waghe *et al.* (2015) opined that carbendazim 12% + mancozeb 63% (Saaf) showed 90 to 100 per cent inhibition against *Alternaria helianthi* in sunflower which is in line with the present results. Gohel and Solanki (2011) pointed out cent per cent efficacy of difenoconazole 25 EC (Score) against *Alternaria solani* which is contradictory to the present findings in chilli. However, Roopa *et al.* (2014) observed only 77.03 per cent inhibition of *A. alternata* with difenoconazole. In the present study, carbendazim 50WP (Bavistin) showed only 33.88 to 55.55 per cent inhibition which is in agreement with the observations of Gohel and Solanki (2011) against *Alternaria solani* in chilli. But Kumar *et al.* (2013) reported cent per cent efficacy

of *Alternaria alternata* with carbendazim in chilli whereas, Taware *et al.* (2014) noted 94.44 per cent control in safflower incited by *Alternaria carthami*, which is contrary to the above results. Potassium phosphonate (Akomin-40) was the least effective amongst all fungicides tested.

In the case of *Rhizoctonia solani* (LBW-1) causing leaf blight isolated from Wayanad, carbendazim 12% + mancozeb 63% (Saaf), carbendazim 50WP (Bavistin), propineb 70WP (Antracol) and Bordeaux mixture were found cent per cent effective. Dutta and Kalha (2011) recorded cent per cent inhibition of *Rhizoctonia solani* by Saaf (Carbendazim 12% + Mancozeb 63%) and 98.8 per cent efficacy with carbendazim alone in paddy. Seema *et al.* (2010) also pointed out the efficacy of Bavistin (Carbendazim 50% WP) and Companion (Carbendazim 12% + Mancozeb 63% WP) on *Rhizoctonia solani* affecting tobacco. Cymoxanil 8% + mancozeb 64% (Curzate M8) at 0.2 and 0.25 per cent recorded 78.88 to 80 per cent inhibition of the pathogen. Copper hydroxide 77WP (Kocide) and difenoconazole 25 EC (Score) at higher concentrations were equally effective against the pathogen by 72 to 75 per cent, whereas copper oxychloride 50WP (Fytolan) showed comparatively less inhibition of 25.55 to 36.67 per cent efficacy. Contrary to the above observations, Pawar *et al.* (2015) noticed difenoconazole to be cent per cent effective against *Rhizoctonia solani* in rice. The study supports the findings of Ray and Kumar (2008) where they reported 100 per cent sensitivity of pathogen towards carbendazim and propiconazole and 18.82 per cent inhibition with copper oxychloride against *Rhizoctonia solani* in soyabean. However, Srinivas *et al.* (2013) recorded 70.89 per cent inhibition with copper oxychloride against *Rhizoctonia solani* in rice which is not in agreement with the findings of present study. Parallel to the observations made above, Raj *et al.* (2016) pointed out cent per cent efficiency against *Rhizoctonia solani* with Saaf in chilli. Among the fungicides tested Potassium phosphonate (Akomin-40) showed the least per cent inhibition of the pathogen at all concentrations.

Six fungicides, carbendazim 12% + mancozeb 63% (Saaf), propineb 70 WP (Antracol), difenoconazole 25 EC (Score), cymoxanil 8% + mancozeb 64% (Curzate

M8), carbendazim 50WP (Bavistin), copper oxychloride 50WP (Fytolan) and Bordeaux mixture showed remarkably good control of the pathogen, against *Phoma exigua* under *in vitro* conditions when evaluated. Saju *et al.* (2012) recorded 87.18 per cent inhibition with copper oxychloride, carbendazim and carbendazim 12% + mancozeb 63% with a slight variation from the observations presented above against *Phoma hedericola* in cardamom. Behera *et al.* (2013) and El-Deeb *et al.* (2016) recorded above 70 per cent inhibition with carbendazim and with combination of carbendazim 12% + mancozeb 63% in pigeon pea and date palm against *Phoma cajani* and *Phoma* sp. respectively. However, copper hydroxide 77WP (Kocide) was found to exhibit only more than 50 per cent inhibition. Potassium phosphonate (Akomin-40) recorded the lowest level of inhibition among the different fungicides tested.

Studies pertaining to the inhibition of fungicides against *Curvularia lunata* revealed that four fungicides *viz.*, cymoxanil 8% + mancozeb 64% (Curzate M8), propineb 70WP (Antracol), difenoconazole 25 EC (Score) and Bordeaux mixture exhibited 100 per cent inhibition at all the three concentrations. Karmarkar *et al.* (2015) recorded cent per cent sensitivity of *Curvularia lunata* with combination of carbendazim 18% + mancozeb 50% (Sprint) and propiconazole 13.9% + difenoconazole 13.9% against *Curvularia lunata* infecting rice. Other chemicals like carbendazim 50WP (Bavistin) and potassium phosphonate (Akomin-40) showed very poor inhibition. An inhibition greater than 80 per cent was recorded with copper hydroxide 77 WP (Kocide) at all concentrations. Moreover, carbendazim 12% + mancozeb 63% (Saaf) and copper oxychloride 77 WP (Fytolan) inhibited the pathogen by 73 per cent at 0.2 and 0.15 per cent. According to Pawar (2012), more than 60 per cent inhibition was noticed with carbendazim and Bordeaux mixture and 85 per cent with combination product, mancozeb + carbendazim (0.25%) (Companion) against *Curvularia lunata* in gladiolus. Yadav and Ratnoo (2014) also observed a higher inhibition of *Curvularia lunata* of more than 80 per cent with carbendazim against cotton leaf spot, whereas, Kithan and Daiho (2014) obtained more than 90 per cent with carbendazim and copper oxychloride at 0.3 per cent when treated against *Curvularia lunata* of cardamom. Contradictory to the

present study, Tekade *et al.* (2017) when tested against *Curvularia lunata* of coleus, noticed an inhibition of more than 75 per cent with Curzate M8, 83.88 per cent with copper oxychloride, 100 per cent with Saaf and 10.71 per cent with carbendazim.

Findings of the present study showed that *in vitro* evaluation of six fungicides *viz.*, carbendazim 12% + mancozeb 63% (Saaf), cymoxanil 8% + mancozeb 64% (Curzate M8), copper hydroxide 77WP (Kocide), copper oxychloride 50 WP (Fytolan), propineb 70WP (Antracol) at all concentrations were cent per cent effective against *Pestalotiopsis longisetula*. Contradictory to the above results, Kumhar *et al.* (2016) recorded only more than 69 per cent inhibition with Saaf, copper hydroxide and copper oxychloride against *Pestalotiopsis theae* causing grey blight of tea. However, Rahman *et al.* (2013) showed cent per cent efficacy with combination of difenoconazole + propiconazole against *Pestalotia palmarum* in coconut which was in accordance with the present study. Difenoconazole 25 EC (Score) at 0.1 and 0.15 per cent showed cent per cent inhibition. According to Islam *et al.* (2004), Barman *et al.* (2015) and Ray *et al.* (2016), carbendazim was cent per cent effective against the pathogen which was in opposition to the present study that recorded only 57 to 75 per cent inhibition against *Pestalotiopsis* sp. isolated from betel nut, tea and Somtree. Moreover, Saju *et al.* (2012) noticed 88.6 per cent restriction of *Pestalotiopsis* sp. with carbendazim in cardamom and Ray *et al.* (2016) also reported 88.44 per cent with copper oxychloride *against Pestalotiopsis disseminata* in Somtree.

In vitro screening of fungicides against *Rhizoctonia solani* causing fruit rot (FRW-1) in strawberry revealed that carbendazim 12% + mancozeb 63% (Saaf), cymoxanil 8% + mancozeb 64% (Curzate M8), propineb 70WP (Antracol) and Bordeaux mixture at all concentrations were 100 per cent effective. According to Srinivas *et al.* (2013) and Raj *et al.* (2016), Saaf showed cent per cent and propineb 96.27 per cent efficacy against *Rhizoctonia solani* of rice and chilli. However, copper hydroxide 77WP (Kocide) and difenoconazole 25 EC (Score) inhibited the pathogen from 50 to 70 per cent. Apart from other fungicides, copper oxychloride 50 WP (Fytolan) recorded less than 45 per cent in inhibiting pathogen. Conversely, Srinivas *et al.* (2013)

and Raj *et al.* (2016) recorded 70 to 100 per cent inhibition of *Rhizoctonia solani* of rice and chilli with copper oxychloride. Carbendazim 50WP (Bavistin) and potassium phosphonate (Akomin-40) were found to be the least per cent effective. Nevertheless, Seema *et al.* (2010) pointed out higher efficacy of Bavistin (Carbendazim 50% WP) in inhibiting the pathogen *Rhizoctonia solani* of tobacco.

A complete inhibition in radial growth of the pathogen, *Fusarium oxysporum* was observed with chemicals *viz.*, carbendazim 12% + mancozeb 63% (Saaf), cymoxanil 8% + mancozeb 64% (Curzate M8), copper hydroxide 77WP (Kocide) and carbendazim 50WP (Bavistin) at all concentrations. Several workers reviewed cent per cent efficacy with Saaf (Harender *et al.*, 2005, Kumari *et al.*, 2014 and Rajan *et al.*, 2013) and with Curzate M8 and Bavistin (Raju *et al.*, 2008, Dar *et al.*, 2013 and Somu *et al.*, 2014) whereas, Madhavi and Bhattiprolu (2011) observed more than 90 per cent efficacy with Curzate M8 and carbendazim against *Fusarium* sp. isolated from gladiolus, chickpea, banana, fir and chilli which is in accordance with that of the results of present study. Bordeaux mixture at 1.5 per cent recorded 100 per cent inhibition of the pathogen whereas, difenoconazole 25 EC (Score) at all concentrations showed 70 to 80 per cent efficacy over control. Similar results were also reported by Somu *et al.* (2014) against *Fusarium oxysporum* f. sp. *cubense* in banana. Propineb 70WP (Antracol) showed an inhibition of more than 70 per cent at all concentrations tested. However, comparatively less inhibition was noticed by Dar *et al.* (2013) with copper oxychloride 50 WP (Fytolan) which is in congruence with the present findings in *Fusarium oxysporum* isolated from fir.

In the present investigation, *Lasiodiplodia theobromae* was evaluated against several chemicals and cent per cent inhibition was observed with carbendazim 12% + mancozeb 63% (Saaf) and copper hydroxide 77 WP (Kocide). Cymoxanil 8% + mancozeb 64% (Curzate M8) at 0.2 per cent exhibited cent per cent inhibition and upto 96 per cent with the lower two concentrations. On the other hand, Bordeaux mixture recorded only 69 to 81 per cent efficacy at all concentrations tested. The inhibitory action of difenoconazole 25 EC (Score), propineb and Saaf was in accordance with that of

Hedge *et al.* (2013), who noticed 72 to 100 per cent inhibition with the three fungicides tested against *Botryodiplodia theobromae* in Jatropa. Sultana and Ghaffar (2010) recorded cent per cent inhibition of the pathogen in case of bottle gourd incited by *Botryodiplodia theobromae* with carbendazim and Alliete which was contradictory to the present results. Oyedeji and Kareem (2016) also noticed cent per cent sensitivity of *Botryodiplodia theobromae* infecting pineapple with Saaf and 94.25 per cent with carbendazim. Copper oxychloride 77 WP (Fytolan) was found the least effective with *Lasiodiplodia theobromae* which was in line with the findings of Shellar *et al.* (1997), Mahmood and Gill (2002) and Khanzada *et al.* (2004) in mango.

5.5.2 *In vitro* evaluation of organic preparations and biocontrol agents

Although, several chemicals are available for the management of crop diseases, continuous and inappropriate use of toxic chemicals cause undesirable effects such as residual toxicity, development of resistance, environmental pollution and health hazards to human and animals. Hence, attention is now being focused on developing environmentally safe, economic and effective alternatives for the management of plant diseases. Keeping this in view, organic formulations and biocontrol agents, *Trichoderma asperellum* and *Pseudomonas fluorescens*, the reference cultures of KAU were evaluated against the fungal pathogens under *in vitro* conditions.

5.5.2.1 Organic preparations

Various organic preparations and formulations like Calphomil, panchagavya, neem oil and baking powder + vegetable oil mixture were tested for its efficiency against the isolated pathogens. Calphomil, a biofungicide, is found to eradicate fungal diseases and is manufactured from the actinomycetes strains *viz.*, *Streptomyces griseus* CBCC 2786 and *Streptomyces violaceus* CBCC 2788. Likewise, several researchers worldwide have elucidated the antifungal activity of baking soda and vegetable oil in controlling plant pathogenic fungi which is often used as a preventive measure and not as a curative one (Williams *et al.*, 1993; Elmer *et al.*, 1997 and Anon, 1997) where the fungicidal effect of bicarbonate might be due to the bicarbonate compounds of salts present in it

(Hang and Woodams, 2003). Moreover, Govindhachari *et al.* (1998) proved the antifungal activity of neem oil which is due to the presence of triterpenoids. Jarvis *et al.* (1999) reported the presence of limonoids in azadirachtin and various other compounds like salanin, nimbin etc. Similarly, according to Valliyamayi and Shekar (2012), panchagavya acts as a growth promoter and immunity booster and also restricts the growth of many common fungal diseases. Hence an *in vitro* study was carried out to evaluate the aforesaid formulations against the isolated fungal pathogens.

Leaf spot caused by *Colletotrichum gloeosporioides* (LSW-1) when treated against Calphomil at all these concentrations showed less than 21 per cent inhibition over control. However, neem oil could restrict the mycelial growth of LSW-1 and LSM-1 only upto 25 per cent and baking powder + vegetable oil as well as panchagavya exhibited an inhibition upto 35 per cent. Similarly, Calphomil evaluated against LSI-1 and LSM-1 at different concentrations recorded zero per cent and upto 75 per cent inhibition, whereas, neem oil and baking powder + vegetable oil mixture and panchagavya inhibited the pathogen upto 30 per cent. Deviating from the above observations, Muthukumar and Ranganathan (2012) observed cent per cent control at 0.1 per cent of neem oil against *Colletotrichum musae* in banana. Antifungal activity of neem oil was also described by Sagoua *et al.* (2008) in banana infected with *Colletotrichum* sp. Ashlesha and Paul (2014) observed 89.47 per cent inhibition with panchagavya against *Colletotrichum capsici* in chilli. The ineffectiveness of Calphomil may be attributed to the fact that the biofungicide is reported to be effective against the fungi belonging to Oomycetes like *Pythium*, *Phytophthora* and downy mildew. Contrary to the present findings, Anon (1998), reported that the bicarbonate products are found effective against many fungal diseases which includes the anthracnose caused by *Colletotrichum* sp.

Data revealed that Calphomil showed 55 to 70 per cent efficiency against *Alternaria alternata,* whereas, neem oil exhibited only 31 per cent efficacy over the pathogen. Conversely, Chethana *et al.* (2013) reviewed 76.94 per cent control over *Alternaria porri* in onion with neem oil. In the present study, panchagavya and baking

powder + vegetable oil mixture showed comparatively less inhibition ranging upto 32 per cent. According to Joseph and Sankarganesh (2011) and Sahu and Verma (2015) a higher antifungal activity of 53.7 per cent reduction over control was noticed with panchagavya against *Alternaria helianthi* and *Alternaria sesame* in sesamum. The above findings are not in validation with Abd-El-Kareem (2007) and Zaker (2014) where they observed cent per cent efficacy of bicarbonate with *Alternaria alternata* and *Alternaria solani* of tomato under *in vitro* conditions. However, Fagundes *et al.* (2013) reported that inorganic salts of carbonate and biocarbonate were not effective against *Alternaria* black rot of cherry tomato caused by *Alternaria alternata* which is in agreement the present study.

Calphomil and neem oil at recommended concentrations were found the least per cent effective against *Rhizoctonia solani* causing leaf blight, but panchagavya and baking powder + vegetable oil mixture recorded 29 per cent control over the pathogen, which is in agreement with the findings of Dogra (2006) who also observed antifungal activity of panchagavya (4%) against *R. solani*. Similarly, Ashlesha and Paul (2014) obtained 85.56 per cent inhibition with panchagavya (4%) against *Rhizoctonia solani* in bell pepper. However, Jandaik and Sharma (2016) noticed 76.92 per cent inhibition with panchagavya at 15 per cent in capsicum infected with *Rhizoctonia solani*.

In the present study, *Phoma exigua* when subjected to *in vitro* testing with different formulations revealed only nine per cent efficiency when neem oil was used, which is in congruence with the findings of Somda *et al.* (2007) in *Phoma sorghina* in lemongrass. More than 40 per cent control was recorded with Calphomil at various concentrations, while, panchagavya restricted the mycelial growth only upto 35 per cent. Baking powder + vegetable oil mixture was also found the less effective against the pathogen which is found contradictory to the findings of Anon (1998) where they observed the effectiveness of potassium bicarbonate against *Phoma* sp.

Curvularia lunata when subjected to *in vitro* evaluation with organic preparations recorded 5.55 to 7.53 per cent restriction in mycelial growth with neem oil

(0.15, 0.2% and 0.25%). Nevertheless, Yadav and Ratnoo (2014) and Adepoju *et al.* (2014) pointed out 67.07 and 37.76 per cent control over *Curvularia spp.* and *Curvularia lunata* in cotton with neem oil. Calphomil at various concentrations could inhibit the pathogen by 15.44 to 23.33 per cent and panchagavya and baking powder + vegetable oil recorded upto 21 per cent reduction over control. However, Joseph and Sankarganesh (2011) pointed out the antifungal activity of panchagavya against *Curvularia* spp. which is not the in accordance with the results of the present study.

Among the different formulations tested against *Pestalotiopsis longisetula* Calphomil showed 83.33 to 87.77 per cent efficacy in inhibiting the pathogen. Neem oil and baking powder + vegetable oil mixture at different concentration inhibited the mycelial growth upto 18.20 and 28.40 per cent. Contrary to the above results, Barman *et al.* (2015) noticed 94.3 to 100 per cent inhibition of the pathogen with neem oil at 0.05 and 0.1 per cent concentrations against *Pestalotiopsis theae* in tea. However, panchagavya was found only 28 per cent effective when used at various concentrations, when compared to *in vitro* conditions, the effectiveness of panchagavya in the field may be due to the presence of organic matter which favours the growth of beneficial microorganisms (Swaminathan, 2005).

Among all the organic preparations tested against *Rhizoctonia* fruit rot (FRW-1), Calphomil recorded the highest inhibition of 55.33 to 63.88 per cent at different concentrations, while neem oil was found the least effective. However, panchagavya and baking powder + vegetable oil mixture could restrict the mycelial growth upto 26 per cent. Several workers pointed out the antifungal activity of panchagavya against *Rhizoctonia solani* (Dogra, 2006; Ashlesha and Paul, 2014 and Jandaik and Sharma, 2016) in capsicum.

In vitro evaluation of organic preparations against *Fusarium oxysporum* revealed that Calphomil at different concentrations exhibited 20 to 55 per cent control. Neem oil recorded only 11 per cent efficacy over control. Sagoua *et al.* (2008) and Adepoju *et al.* (2014) pointed out the antifungal activity of neem oil in inhibiting the pathogen upto

7.37 per cent against *Fusarium* spp. in banana. Likewise, panchagavya and baking powder + vegetable oil mixture also recorded a per cent inhibition of below 20.3 per cent only. Joseph and Sankarganesh (2011) and Ashlesha and Paul (2014) observed the antimicrobial property of panchagavya which exhibited 67.25 per cent control over *Fusarium solani* and 79.05 per cent against *Fusarium oxysporum* in capsicum when used at 4 per cent. Similarly, Jandaik and Sharma (2016) reported 82.62 per cent control with panchagavya at 15 per cent against *Fusarium oxysporum* in capsicum. According to a study by Hang and Woodams, (2003), baking soda were found capable of significantly reducing the mycelial growth of *Fusarium oxysporum* by greater than 95 per cent under *in vitro* conditions.

The pathogen *Lasiodiplodia theobromae,* when subjected to poison food technique with neem oil, panchagavya, baking powder + vegetable oil mixture and Calphomil, it was found that all formulations recorded zero per cent inhibition at all concentrations. However, Muthukumar and Ranganathan (2012) and Sagoua *et al.* (2008) recorded the antifungal activity of neem oil against *Botryodiplodia theobromae* in banana. Contradictory to the results of the present study, Kurosaki *et al.* (2007) and Badadami *et al.* (2007) observed the antimicrobial activity of panchagavya and was found to be effective against all the pathogens of Capsicum

5.5.2.2 Biocontrol agents

Two reference cultures from KAU were evaluated against the isolated pathogens. It was observed that *Trichoderma asperellum* when evaluated against LSW-1, LSI-1 and LSW-1 pathogen incited by *Colletotrichum gloeosporioides* isolated from Wayanad, Idukki and Malappuram, the isolates exhibited overgrowth mechanism of antagonism showing cent per cent inhibition. However, *Pseudomonas fluorescens* could inhibit the pathogen only by 67.7 to 70 per cent. Conversely, Tasiwal *et al.* (2009), Ramani *et al.* (2015) and Tapwal *et al.* (2015) pointed out only less than 61 per cent control with *Trichoderma viride* and 42.87 per cent using *Pseudomonas fluorescens* with *Colletotrichum gloeosporioides* infecting papaya and banana, whereas, Patil *et al.*

(2009) recorded 70.42 per cent and 20.72 per cent inhibition of *Colletotrichum gloeosporioides* with *Trichoderma viride* and *P. fluorescens* in long pepper. In congruence with the present results, Dev *et al.* (2016) observed 100 per cent efficacy with several isolates of *Trichoderma viride* and 47.6 per cent with *Pseudomonas fluorescens* in pomegranate infected with *Colletotrichum gloeosporioides.*

Trichoderma asperellum was found cent per cent effective against *Alternaria alternata* (LBI-2) and 56.67 per cent with *Pseudomonas fluorescens*. Chethana *et al.* (2013) and Roopa *et al.* (2014) recorded upto 71.53 to 79.5 per cent inhibition with *T. harzianum* and *T. viride* and 36.22 per cent with *P. fluorescens a*gainst *Alternaria* sp. of tomato and onion. Tapwal *et al.* (2015) recorded upto 34 per cent control over *Alternaria alternata* with *T. viride* and *T. harzianum*. Mishra and Gupta (2012) observed *P. fluorescens* recording 19.20 per cent control over *Alternaria porri* in onion. Ngoc *et al.* (2013) tested several isolates of *Trichoderma* with the pathogen recording maximum efficacy over *Alternaria solani*. Rahman *et al.* (2015) noticed maximum control with *T. viride* and *T. harzianum over Alternaria porri* in onion. Taware *et al.* (2014) reported the efficacy of *Trichoderma viride* and *Pseudomonas fluorescens* against *Alternaria carthami* in safflower.

In vitro studies of *T. asperellum* with *Rhizoctonia solani* (LBW-1) recorded cent per cent inhibition of the pathogen, whereas, the bacterial antagonist *Pseudomonas fluorescens* exhibited 55.56 per cent efficacy over control. Amin and Razdan (2010) and Parizi *et al.* (2012) noticed 63.52 and 71.4 per cent control over pathogen with *Trichoderma viride* against *Rhizoctonia solani* infecting tomato and Rosselle. Seema and Devaki (2012) and Srinivas *et al.* (2013) also proved *Trichoderma viride* to be 67 and 72.65 per cent efficacious against *Rhizoctonia solani* in tobacco and rice. Mezeal (2014) in tomato noted a higher inhibition of 81.30 per cent with *P. fluorescens* against *Rhizoctonia solani.* But, Tapwal *et al.* (2015) reported an inhibition of only 1.45 per cent and 5.10 per cent with *T. viride* and *T. harzianum* against *Rhizoctonia solani.*

While studying the antagonistic effect of *Trichoderma asperellum* and *Pseudomonas fluorescens* tested against *Phoma exigua* (LBI-1), the former recorded 100 per cent and latter 45.61 per cent control over the pathogen. Different from the results of the *in vitro* experiment, Parizi *et al.* (2012) and Mokhtar and Dehimat (2015) reported 71.16 and 39.58 per cent inhibition with *Trichoderma viride against Phoma exigua* in rosselle and tomato respectively.

Cent per cent inhibition was recorded with *Curvularia lunata* (LBI-2) by the fungal antagonist, *Trichoderma asperellum,* whereas bacterial antagonist, *Pseudomonas fluorescens* was found the least effective. Contrary to the above results, Pawar *et al.* (2012) and Bisht *et al.* (2013) could record only 86.5 and 83.32 per cent inhibition with *T. viride* against *Curvularia lunata* and *Curvularia pallescens* and *Curvularia* sp. in gladiolus and maize. Kithan and Daiho (2014) pointed out 68.85 per cent efficacy of *T. viride* and 51.36 per cent with *P. fluorescens* against *Curvularia lunata* infecting cardamom. Similarly, Tapwal *et al.* (2015) against *Curvularia lunata* noticed 46.79 and 25.64 per cent inhibition with *T. viride* and *T. harzianum*. Tekade *et al.* (2017) observed 60.81 per cent control with *T. viride* and 33.41 per cent with *Pseudomonas fluorescens* when evaluated against *Curvularia lunata* in coleus.

Fungal antagonist when tested against *Pestalotiopsis longisetula* (LBM-1), it recorded an inhibition of 66.67 per cent while, *Pseudomonas fluorescens* inhibited the pathogen by 56.67 per cent. Saju *et al.* (2012) recorded 50.9 per cent control with *Trichoderma viride* and 41.3 per cent by *Pseudomonas fluorescens* in case of *Pestalotiopsis* sp. isolated from cardamom. Barman *et al.* (2015) recorded a higher inhibition of 72.4 per cent by *T. viride* and 35.4 per cent by *Pseudomonas fluorescens* and Kumhar *et al.* (2016) noticed 62.5 per cent control over pathogen with *T. asperellum* against *Pestalotiopsis theae* in tea.

Observations on the *in vitro* evaluation of *Rhizoctonia solani* (FRW-1) with *Trichoderma asperellum* tested 66.67 per cent inhibition and *Pseudomonas fluorescens* exhibited only 33.33 per cent control. In congruence with above findings, Amin and

Razdan (2010), Parizi *et al.* (2012), Seema and Devaki (2012) and Srinivas *et al.* (2013) noticed upto 63.52 and 71.4 per cent control over *Rhizoctonia solani* with *Trichoderma viride* infecting tomato, rosselle, tobacco and rice. But, Mezeal (2014) noted a higher inhibition of 81.30 per cent with *P. fluorescens* against *Rhizoctonia solani* from tomato whereas, Tapwal *et al.* (2015) reported an inhibition of only 1.45 per cent and 5.10 per cent with *T. viride* and *T. harzianum* when tested against *Rhizoctonia solani.*

Fusarium oxysporum (CRI-1) when evaluated against *Trichoderma asperellum* and *Pseudomonas fluorescens,* indicated relatively less efficacy of the pathogen where antagonist restricted the growth upto 65.67 and 57.67 per cent respectively. Raju *et al.* (2008), Rajan *et al.* (2013), Madhavi and Bhattiprolu (2011), Ragab *et al.* (2012) and Bashar and Chakma (2014) reviewed 63 per cent inhibition with *Trichoderma viride* and 57.14 per cent with *Pseudomonas fluorescens* against *Fusarium oxysporum* and *Fusarium solani* infecting chickpea, chilli and brinjal. Similar findings were reported by Bardia and Rai (2011) and Kumar and Naik (2015) against *Fusarium oxysporum* isolated from cumin and castor. Contrary to the above results, a higher inhibition of 92.5 per cent and 86.5 per cent was noticed by Dar *et al.* (2013) against *Fusarium oxysporum* infecting fir. However, Tapwal *et al.* (2015) could observe only 15.80 and 27.04 per cent inhibition with *T. viride* and *T. harzianum.*

In reviewing the effect of antagonists against *Lasiodiplodia theobromae, Trichoderma asperellum* recorded a maximum of 74.10 per cent efficacy against the pathogen (CRM-1). However, *Pseudomonas fluorescens* was found zero per cent effective. Nonetheless, Adeniyi *et al.* (2013) noted 25.38 per cent control only with *T. viride* in cashew infected with *Botryodiplodia theobromae*.

5.5.3 *In vivo* evaluation of fungicides and bioagents on major fungal diseases of strawberry

Results of the *in vitro* studies with fungicides, organic formulations and bioagents against pathogens may not always be reciprocated under field conditions, since *in vitro* evaluation is considered only as a preliminary step before any disease

management strategy. Hence, efficacy of fungicides and bio control agents tested under laboratory conditions against plant pathogens should be ascertained under natural conditions. Thus, to formulate better management strategies under *in vivo* conditions, field evaluation of plant protection chemicals, organic formulations and bioagents were carried out with the selected pathogens isolated from strawberry plants.

Recalling back the results of the field survey conducted in four districts, four pathogens *viz.*, *Colletotrichum gloeosporioides*, *Pestalotiopsis longisetula*, *Fusarium oxysporum* and *Lasiodiplodia theobromae* recorded higher per cent of disease severity and incidence under field conditions. *Colletotrichum gloeosporioides* (LSW-1, LSI-1 and LSM-1) recorded a per cent disease severity of 14.9 to 23.4 per cent and *Pestalotiopsis longisetula* showed a severity of 17.4 to 21.4 per cent at various locations of survey in different seasons. Similarly, higher disease incidence upto 70 per cent was noticed with *Fusarium oxysporum* (CRI-1) causing wilt in Idukki district, whereas severe infection of crown and root rot caused by *Lasiodiplodia theobromae* (CRM-1) was observed in open fields of Malappuram. Thus, the four pathogens though identified tentatively by cultural and morphological characters were subjected to molecular characterisation at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram to confirm its identity upto species level before the *in vivo* experiment. Cent per cent sequence homology of *Pestalotiopsis longisetula* was observed with *Neopestalotiopsis clavispora,* whereas, the sequence of *Colletotrichum, Fusarium* and *Lasiodiplodia* cultures showed cent per cent identity with *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Lasiodiplodia theobromae*. Thus, the results of cultural and morphological characters except for *Pestalotiopsis longisetula* was found in congruence with molecular characterization of the isolates.

Hence, an *in vivo* experiment was carried out with the selected fungicides and biocontrol agents obtained from *in vitro* experiment against the major pathogens of strawberry *viz.*, *Colletotrichum gloeosporioides*, *Neopestalotiopsis clavispora*, *Fusarium oxysporum* and *Lasiodiplodia theobromae* so as to understand the field efficacy of the results observed under laboratory conditions.

5.5.3.1 Management of *Colletotrichum gloeosporioides*

In the present study, *Colletotrichum gloeosporioides* was challenge inoculated to the leaves by spraying spore suspension to two month old strawberry plants maintained under ambient conditions. It was observed that the per cent disease severity, eight days after inoculation ranged from 7.98 to 25.28 per cent with highest in T_5 (propineb 70WP) (0.3%) and least in T_6 (*Trichoderma asperellum*) (2%). However, after the first treatment application a significant difference was noticed among various treatments, where T_6 (*Trichoderma asperellum*) (2%) showed the maximum per cent reduction of 78.63 per cent, closely followed by T_3 (carbendazim 12% + mancozeb 63%) (0.2%) recording 73.78 per cent reduction over control. The least per cent reduction of 42.38 per cent was noticed with T_5 (propineb 70WP) (0.3%) compared with other treatments.

After two rounds of fungicide application, the treatment T_6 (*Trichoderma asperellum*) (2%) showed the maximum reduction of 88.07 per cent followed by T_3 (carbendazim 12% + mancozeb 63%) (0.2%) (86.48 per cent) and T_4 (copper hydroxide 77WP) (0.2%) (80.81 per cent) which were on par with each other. These treatments were followed by T_2 (cymoxanil 8% + mancozeb 64) (0.2%) and T_5 (propineb 70WP) (0.3%) which depicted comparatively lower efficiency of 77.68 and 66.54 per cent respectively. The efficacy of *Trichoderma* in soil may be attributed to the presence of proper amount of organic matter that lead to the multiplication and activation of spores. Moreover, according to Baker and Cook, (1982), Stindt and Welzein, (1990), Welzein, (1991) and Ma *et al.* (2001), antagonists compete with the pathogen for infection sites, leaving limited space for pathogens to proliferate or they may secrete secondary metabolites on the plant surface, and may parasitize pathogens directly when applied prophylactically. Solanki (2015) recorded comparable results with Saaf and propineb against *Colletotrichum gloeosporioides* in mango showing 79.03 and 69.01 per cent efficacy over control. Contrary to the above observations, Subedi *et al.* (2015), Ingle *et al.* (2014) and Patil *et al.* (2009) observed only 42.19, 56.37 and 33.38 per cent disease control with Saaf against *Colletotrichum gloeosporioides* of soyabean and pepper, while,

Diedhiou *et al.* (2014) recorded 84 percent control with mancozeb and Bhagwat *et al.* (2016) noticed 79.38 per cent efficacy with carbendazim alone in disease reduction of mango. Kowata *et al.* (2010) reported only 35.6 per cent control with propineb against *Colletotrichum* leaf spot of apple. While, Cole *et al.* (2005) observed upto 74 per cent control with copper hydroxide and mancozeb alone against *Colletotrichum gloeosporioides* in winter creeper.

Patil *et al.* (2009) observed 28.36 per cent reduction of *Colletotrichum gloeosporioides* with *Trichoderma viride* in infected pepper plants. Similarly, the efficacy of field application of *Trichoderma* against *Colletotrichum lindemuthianum* in beans was recorded by Padder *et al.* (2010). A perusal of literature revealed that antagonistic potential of different species of *Trichoderma* against *Colletotrichum* spp. Sivakumar *et al.* (2000), Soytong *et al.* (2005), Shovan *et al.* (2008) and Sobowale *et al.* (2010) proved the efficacy of *Trichoderma* in rambutan, grapes, soybean and cassava. Likewise, Sawant *et al.* (2012) recorded a subsequent reduction in *Colletotrichum gloeosporioides* infection of grapevine foliage with *Trichoderma* sp.

5.5.4.2 Management of *Neopestalotiopsis clavispora*

Challenge inoculation of the leaf blight pathogen, *Neopestalotiopsis clavispora,* on two month old strawberry plants revealed that the severity of infection ranged from 35.45 to 55.98 per cent with maximum incidence in T_1 (Control). Treatments were applied twice at 10 days interval. Though there was no significant difference between the treatments seven days after challenge inoculation, the maximum disease reduction was recorded with T_6 (*Trichoderma asperellum*) (2%) (53.43%) and T_3 (carbendazim 12% + mancozeb 63%) (0.2%) (51.16%) followed by T_5 (propineb 70WP) (0.3%) (40.28%) and T_2 (cymoxanil 8% + mancozeb 64) (0.2%) (39.2%) and least per cent disease reduction was noticed with T_4 (copper hydroxide 77WP) (0.2%) (35.68%). However, after the second fungicidal application, a significant difference was recorded among the treatments with a per cent reduction of 78.03 with T_5 (propineb 70WP),

followed by T_6 (*Trichoderma asperellum*) (75.77 per cent) and T_3 (carbendazim 12% + mancozeb 63%) showing 74.39 per cent reduction over control. Minimum disease severity was noticed with the treatment T_2 (cymoxanil 8% + mancozeb 64) (0.2%). Moustafa *et al.* (2015) noticed that propineb and copper hydroxide could manage the disease caused by *Pestalotia psidii* only upto 60 per cent in guava. Antu (2013) conducted an experiment in guava infected with *Pestalotia* where he observed the effectiveness of Saaf and Curzate M8 showing a PDS of less than 18 per cent. According to Shin *et al.* (2010) copper hydroxide and carbendazim are highly insensitive against *Pestalotiopsis longisetula* and *P. theae.* While, Sanjay *et al.* (2008) observed only 22.6 per cent disease incidence when treated with the combination fungicide, Companion and 20.7 per cent each with mancozeb, carbendazim and copper oxychloride against *Pestalotiopsis theae*. Carre-Missio *et al.* (2010) described the efficacy of mancozeb in reducing the infection of *Pestalotiopsis longisetula* in strawberry. The efficacy of the biocontrol agent, *Trichoderma viride* was also reported by Sanjay *et al.* (2008) against *Pestalotiopsis theae* of tea.

5.5.4.3 Management of *Fusarium oxysporum*

An *in vivo* experiment on *Fusarium oxysporum* in strawberry plants were carried out with the best treatments screened under lab study. Soon after challenge inoculation of the pathogen, an incidence of 62.5 to 100 per cent was obtained in varying treatments. However, plants after second treatment application showed a significant reduction in the disease incidence, where, T_3 (carbendazim 12% + mancozeb 63%) (0.2%) and T_4 (copper hydroxide 77WP) (0.2%) recorded only 25 per cent of the disease incidence followed byT_6 (*Trichoderma asperellum*) (2%) with 50 per cent incidence. Highest percentage of disease incidence of 62.5 was noticed with the treatments T_2 (cymoxanil 8% + mancozeb 64) (0.2%) and T_5 (propineb 70WP) (0.3%). The less efficacy of *Trichoderma* under field conditions can be due to the presence of higher potential amount of pathogen in soil and due to the lack of sufficient time for multiplication under the root zone. According to Sivan and Chet (1993), failure of the biocontrol agent to proliferate in field may have been due to biotic or abiotic factors present at the

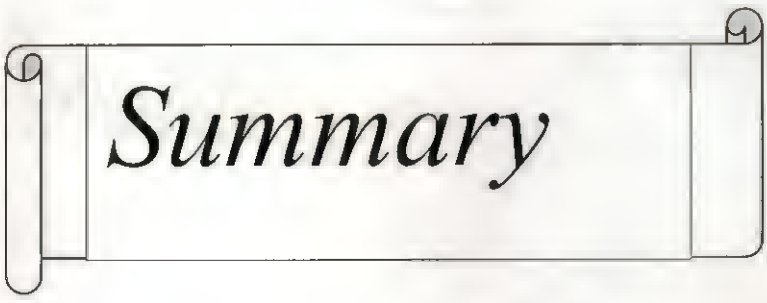
experimental site. Also, the inefficacy of the antagonist in the field may be related to low supply of organic material since it is the organic matter on which antagonist multiplies and becomes more competitive against microorganisms. Sumana *et al.* (2012) and Akhtar *et al.* (2017) observed the efficacy of copper hydroxide and propineb against *Fusarium* in tobacco and tomato. Amini and Sidovich (2010) and Narayanan *et al.* (2015) observed that carbendazim could alone control the disease from 83.9 to 100 per cent in infected tomato plants and 59.6 per cent in blueberry. However, Narayanan *et al.* (2015) observed that the combination fungicide, Saaf could reduce the disease incidence of *Fusarium* only upto 28.8 per cent in blueberry. Akrami and Yousefi (2015) and Narayanan *et al.* (2015) recorded upto 85 to 87 per cent disease control with the combination of *T. harzianum, T. asperellum*, and *T. virens* against *Fusarium solani* and *Fusarium oxysporum* in tomato and upto 57.6 per cent with *T. viride* in blueberry.

5.5.3.4 Management of *Lasiodiplodia theobromae*

An attempt was made to study the effect of fungicides and biocontrol agents against *Lasiodiplodia theobromae* in strawberry. Spore suspension was poured into the root surface for challenge inoculation. About 75 to 100 per cent infection was noticed in various treatments with the maximum infection in T_1 (Control) and T_2 (cymoxanil 8% + mancozeb 63%) (0.2%) and lowest in T_6 (*Trichoderma asperellum*) (2%). Observations taken 10 days after first drenching recorded upto 37.5 per cent control with treatments T_3 (carbendazim 12 % + mancozeb 64%) (0.2%) and T_6 (*Trichoderma asperellum*) (2%), 25 and 12.5 per cent with T_4 (copper hydroxide 77WP) (0.2%) and T_5 (propineb 70WP) (0.3%). However, the treatment T_2 (cymoxanil 8% + mancozeb 63%) (0.2%) was found to be least effective. It is interesting to note that, application of *Trichoderma* was carried out as a prophylactic measure 10 days before the application of fungicides which is in congruence with the study of Martin and Loper (1999) where they pointed out that *Trichoderma* are early colonizers off substrates and reduce the activity of other fungi simply by substrate occupation and depletion when applied prophylactically. Second treatment application recorded 50 per cent reduction in disease with T_3 (carbendazim 12

% + mancozeb 64%) (0.2%) and T_4 (Copper hydroxide 77WP) (0.2%) and least control of only 25 per cent with T_5 (Propineb 70WP) (0.3%) and T_2 (cymoxanil 8% + mancozeb 63%) (0.2%). In consonance with the above results, Rawal (1998) and Khanzada *et al.* (2004) reported the efficacy of carbendazim and Diedhiou *et al.* (2014) reported the 40 per cent field efficacy with mancozeb against *Botryodiplodia theobromae* in mango. Latha *et al.* (2011) recorded the efficacy of carbendazim recording 63.10 per cent disease reduction and 33.4 per cent in case of *Trichoderma viride* treated along with FYM and neem cake in fields of Jatropha.

Recollecting the results of survey, on identification of different fungal diseases infecting strawberry conducted in various locations of Kerala *viz.*, Wayanad, Idukki and Malappuram, revealed four leaf spots incited by *Colletotrichum gloeosporioides* (LSW-1, LSI-1, LSM-1) and *Alternaria alternata* (LSI-2), four leaf blights caused by *Rhizoctonia solani* (LBW-1), *Phoma exigua* (LS1), *Curvularia lunata* (LSI-2) and *Neopestalotiopsis clavispora* (LBM-1), a fruit rot by *Rhizoctonia solani* (FRW-1) and two crown and root rots by *Fusarium oxysporum* (CRI-1) and *Lasiodiplodia theobromae* (CRM-1) was recorded. Among the various diseases observed, *Colletotrichum gloeosporioides* was found infecting strawberry in all the three locations, while the other pathogens *Neopestalotiopsis clavispora, Fusarium oxysporum* and *Lasiodiplodia theobromae* were found to be the most destructive, where the former causes severe leaf blight and the latter two are found to cause crown and root rots which results in complete crop failure. Moreover, results of the *in vitro* study with various fungicides and biocontrol agents will not always be in consonance with the observations under *in vitro* conditions. Hence further studies should be focused to carry out multilocational trials in various agroclimatic conditions in different strawberry growing tracts of Kerala so as to prove the efficacy of fungicides, biocontrol agents and organic preparations. Thus, the results of the research has depicted a clear picture on the various fungal diseases affecting strawberry and their management with different plant protection chemicals and bioagents.


Summary

6. SUMMARY

Strawberry (*Fragariae* x *ananassa* Duch.), a fruit of commercial importance is valued all over the world for its varied taste and characteristics. But the international market value of the crop is seriously affected by the incidence of fungal diseases. Hence, the present research on "Cataloguing, documentation and management of fungal diseases of strawberry (*Fragariae* x *ananassa* Duch.)" was conducted in order to identify different fungal pathogens inflicting the crop and to study its symptomatology, seasonal occurrence and management of diseases under *in vitro* and *in vivo* conditions.

1. An intensive survey conducted in three districts of Kerala *viz*., Wayanad, Idukki and Malappuram during three different seasons both under open field and polyhouse conditions indicated the revealed of leaf spots, leaf blights, fruit rots and crown and root rot diseases infecting strawberry.

- Diseases were depicted based on the symptoms expressed during survey and location of collection and it was expressed as leaf spots (LSW-1, LSI-1, LSM-1 and LSI-2), leaf blights (LBW-1, LBI-1, LBI-2, LBM-1), fruit rot (FRW-1) and crown and root rots (CRI-1, CRM-1).
- Survey conducted in Wayanad recorded the incidence of diseases like LSW-1, LBW-1 and FRW-1, where LSW-1 recorded a maximum PDS and PDI of 22.8 and 52.9 per cent followed by LBW-1 with PDS and PDI of 25.2 and 46.7 per cent. The per cent incidence of fruit rot infection was restricted only upto 20 per cent.
- In Idukki, diseases like LSI-1, LBI-1, LBI-2, LSI-2 and CRI-1 was noticed where, CRI-1 was the only disease infecting crown and roots recording an incidence of 70 per cent. LSI-1 and LSI-2 was noticed in both locations *viz*., Vattavada and Koviloor with a PDS and PDI of 17.1 and 42.6 per cent and 23.4 and 49.1 per cent respectively.

- Diseases like LSM-1, LBM-1 and CRM-1 was noticed in strawberry plants at Malappuram district, where LBM-1 recorded a severity of 21.7 per cent and incidence of 58.2 per cent and CRM-1 recorded a highest PDI of 82 per cent.

2. Influence of weather parameters on disease development was recorded based on correlation studies.

- LSW-1 and LSM-1 observed in Wayanad and Malappuram was found to be negatively and positively correlated with relative humidity and rainfall with no significance with temperature, whereas, LSI-1 in Idukki showed a significant positive correlation with temperature and relative humidity only.
- LSI-2 did not show any significant relationship with weather parameters in Idukki.
- LBW-1 showed no significance with temperature, however a negative correlation was noticed with relative humidity and rainfall.
- A positive significant correlation was obtained with temperature in case of LBI-1 and reverse relationship with rainfall. However, the disease showed no influence with relative humidity.
- LBM-1 recorded non-significant results with all the weather parameters under study.
- Crown and root rot pathogens *viz.*, CRI-1 did not shown any correlation with any of the weather parameters whereas, CRM-1 had a positive influence on temperature and negative correlation with relative humidity and rainfall.

3. Eleven different fungal isolates were obtained following standard isolation procedure from different locations of survey and its pathogenicity was proved by Mycelial Bit Inoculation Technique (MBIT) for foliage and fruit diseases and spore suspension method for foliage and even for crown and root rot diseases.

4. Symptomatology studies of different diseases were conducted both under natural and artificial conditions.

- LSW-1 under natural conditions, produced numerous black round spots all over the leaflets that gradually coalesced covering entire leaves.
- LSI-1 showed black spots on leaves covered by yellow halo which later coalesced to form bigger lesions.
- LSM-1 produced irregular spots converting to blightened areas along the margins extending towards the veins and bending down of petioles was noticed with white mycelial growth.
- LSI-2 developed brown circular spots with concentric zonations which was confined towards leaf margin expanding in a V shaped manner surrounded by a yellow or dark reddish purple margins.
- Symptoms of LBW-1 was noticed as small reddish black spots which later coalesced to form a purplish discolouration upto to the veins.
- LBI-1 produced small circular brown spots on leaves which coalesced to form V shaped lesions progressing from margin inwards.
- LBI-2 initiated as small brown spots later enlarging to form blights.
- LBM-1 was noticed as light to dark brown blightened areas with black pin head like acervuli.
- Symptoms of fruit rot (FRW-1) was noticed as black, hard encrustations on either sides of the fruits.
- In case of crown and root rots (CRI-1 and CRM-1), symptoms appeared as wilting, withering, collapse and death of plants with reddish brown discolouration of vascular tissues and crown region when cut open.

5. Cultural and morphological characters of pathogens were studied and the isolates were identified upto the genus level. However, identification of pathogens upto species level was confirmed with the results from National Centre for Fungal Taxonomy (NCFT) and hence cultural and morphological characterisation was recorded.

- Leaf spots *viz.*, (LSW-1, LSI-1 and LSM-1) were found to be caused by the pathogen, *Colletotrichum gloeosporioides*, LSI-2 was identified as *Alternaria*

alternata, LSW-1 as *Rhizoctonia solani*, LSI-1 as *Phoma exigua*, LSI-2 as *Curvularia lunata*, LSM-1 as *Pestalotiopsis longisetula*, fruit rot (FRW-1) pathogen as *Rhizoctonia solani*, crown and root rot pathogens CRI-1 and CRM-1 as *Fusarium oxysporum* and *Lasiodiplodia theobromae*.

6. *In vitro* evaluation of various fungicides, organic preparations and bioagents against fungal pathogens revealed that

- In case of *Colletotrichum gloeosporioides* (LSW-1) from Ambalavayal, all fungicides except Akomin recorded 88 to 100 per cent inhibition.
- Carbendazim 12% + mancozeb 63%, propineb 70 WP and carbendazim 50WP recorded cent per cent inhibition of *Colletotrichum gloeosporioides* (LSI-1) from Idukki district.
- *Colletotrichum gloeosporioides* (LSM-1) from Malappuram recorded a cent per cent sensitivity with carbendazim 12% + mancozeb 63%, cymoxanil 8% + mancozeb 64%, copper hydroxide 77 WP, carbendazim 50WP and Bordeaux mixture.
- Fungicides like cymoxanil 8% + mancozeb 64%, copper hydroxide 77WP, propineb 70WP, difenconazole 25EC and Bordeaux mixture recorded 75 to 100 per cent inhibition against *Alternaria alternata* (LSI-2).
- Carbendazim 12% + mancozeb 63%, propineb 70 WP, carbendazim 50WP and Bordeaux mixture was proved cent per cent efficient against *Rhizoctonia solani* (LSW-1), whereas, cymoxanil 8% + mancozeb 64% and difenconazole 25EC showed upto 80 per cent control.
- All fungicides except copper hydroxide 77WP and potassium phosphonate were found cent per cent effective against *Phoma exigua* (LBI-1).
- Bordeaux mixture, cymoxanil 8% + mancozeb 64%, difenconazole 25EC, propineb 70WP and copper oxychloride 50 WP recorded 82 to 100 per cent control over *Curvularia lunata* (LBI-2).

- *Pestalotiopsis longisetula* (LBM-1) recorded cent per cent inhibition with carbendazim 12% + mancozeb 63%, cymoxanil 8% + mancozeb 64%, copper hydroxide 77WP, copper oxychloride 50WP, propineb 70WP and Bordeaux mixture.
- Fruit rot pathogen *Rhizoctonia solani* (FRW-1) was found to be sensitive against combination fungicides like carbendazim 12% + mancozeb 63% and cymoxanil 8% + mancozeb 64%, copper fungicide Bordeaux mixture and to a systemic fungicide propineb 70WP.
- Carbendazim 12% + mancozeb 63%, cymoxanil 8% + mancozeb 64%, copper hydroxide 77WP and carbendazim 50 WP showed cent per cent control and Bordeaux mixture depicted 86 to 100 per cent inhibition against *Fusarium oxysporum* (CRI-1).
- *Lasiodiplodia theobromae* (CRM-1) showed sensitiveness of 93 to 100 per cent against carbendazim 12% + mancozeb 63%, copper hydroxide 77WP and combination of cymoxanil 8% + mancozeb 64%.
- Among various organic formulations tested, Calphomil recorded upto 75.33 per cent control, neem oil 5.55 to 31 per cent, panchagavya 11.2 to 36.3 per cent and baking powder + vegetable oil mixture showed 0 to 33.80 per cent inhibition over different fungal pathogens tested.
- Dual culture evaluation of *Trichoderma asperellum* and *Pseudomonas fluorescens* inhibited the fungal pathogens by 66.67 to 100 per cent and 0 to 70.55 per cent respectively.

7. Molecular characterisation of four pathogens *viz.*, *Colletotrichum gloeosporioides, Pestalotiopsis longisetula, Fusarium oxysporum* and *Lasiodiplodia theobromae* were carried out at Rajiv Gandhi Centre for Biotechnology (RGCB), Trivandrum, which indicated 100 per cent identity with all the pathogens. But *Pestalotiopsis longisetula* was identified as *Neopestalotiopsis clavispora.*

8. Four pathogens *viz.*, *Colletotrichum gloeosporioides, Neopestalotiopsis clavispora, Fusarium oxysporum* and *Lasiodiplodia theobromae* subjected to molecular identification were selected for evaluation of promising fungicides and bioagents under field conditions.

- *Trichoderma asperellum* followed by carbendazim 12% + mancozeb 63% recorded 88.07 and 86.48 per cent control over *Colletotrichum gloeosporioides*.
- Propineb 70 WP, *Trichoderma asperellum* and carbendazim 12% + mancozeb 63% exhibited highest per cent disease reduction of 78.03, 75.77 and 74.39 per cent respectively against *Neopestalotiopsis* leaf blight.
- Carbendazim 12% + mancozeb 63% (0.2%) and copper hydroxide 77WP (0.2%) showed a disease reduction of 75 per cent in plants infected by *Fusarium oxysporum*.
- A per cent disease reduction of 50 per cent was noticed in plants challenge inoculated with *Lasiodiplodia theobromae* when treated with carbendazim 12% + mancozeb 63% (0.2%) followed by 37.5 per cent by *Trichoderma asperellum* (2%) and copper hydroxide 77WP (0.2%).

References

REFERENCES

Abd-El-Kareem, F. and Abd-El-Latif, F. 2012. Using bicarbonates for controlling late blight disease of potato plants under field conditions. *Life Sci. J.* 9(4):2080-2085.

Adeniyi, D. O., Adedeji, A. R., Oduwaye, O. F. and Kolawole, O. O. 2013. Evaluation of biocontrol agents against *Lasiodiplodia theobromae* causing inflorescence blight of cashew in Nigeria. *IOSR J. Agric. Vet. Sci.* 5(3):46-48.

Adepoju A.O. and Ogunkunle A.T. and Femi-Adepoju, A.G., 2014. Antifungal activities of seed Oil of neem (*Azadirachta indica* A. Juss.). *Global J. Biol. Agric. Health Sci.* 3(1):106-109

Adhikari, T. B., Hodges, C. S. and Louws, F. J. 2013. First Report of *Cylindrocarpon* sp. associated with root rot disease of strawberry in North Carolina. *Plant Dis.* 97(9):1251-1251.

Agarwal, D. K. 2001. *Phragmidium fragariae*-vestitae sp. nov. on *Fragaria vestita* from India. *J. Mycopathol. Res.* 39(2):105-106.

Agrios, N.A., 1988. *Plant Pathology* (3rd Ed.). Academic Press, USA. 952p.

Akhter, M., Alam, S., Islam, M. and Lee, M. W. 2009. Identification of the fungal pathogen that causes strawberry anthracnose in Bangladesh and evaluation of *in vitro* fungicide activity. *Mycobiol.* 37(2):77-81.

Akhtar, T., Shakeel, Q., Sarwar, G., Muhammad, S., Iftikhar, Y., Ullah, M. I. and Hannan, A. 2017. Evaluation of fungicides and biopesticides for the control of *Fusarium* wilt of tomato. *Pak. J. Bot.* 49(2):769-774.

Akrami, M. and Yousefi, Z. 2015. Biological control of *Fusarium* wilt of tomato (*Solanum lycopersicum*) by *Trichoderma* spp. as antagonist fungi. In *Biological Forum. Res. Trend* 7(1):887-888.

Alcock, N. L. 1929. A root disease of strawberry. Gradeners chronicle, 14-15.

Alcock, N. L. and Howells, D. V. 1936. The *Phytophthora* disease of strawberry. *Sci. Hortic.* 4:52-58.

Ali, A., Najar, A. G., Bhat, G. N., Ambardar, V. K., Najeeb, M. M. and Mohd, J. J. 2013. Integrated management of foliage and fruit diseases of strawberry under Kashmir ecology. *Plant Dis. Res.* 28(2):55-59.

Amin, F and Razdan, V. K. 2010. Potential of *Trichoderma* species as biocontrol agents of soil borne fungal propagules. *J. Phytol. 2*(10):38-41.

Amini, J and Sidovich, D. 2010. The effects of fungicides on *Fusarium oxysporum* f. sp. *lycopersici* associated with *Fusarium* wilt of tomato. *J. Plant Prot. Res.* 50(2): 172-178.

Amsalem, L., Freeman, S., Rav-David, D., Nitzani, Y., Sztejnberg, A., Pertot, I. and Elad, Y. 2006. Effect of climatic factors on powdery mildew caused by *Sphaerotheca macularis* f. sp. fragariae on strawberry. *European J. Plant Pathol.* 114(3):283-292.

Anderson, H.W. 1956. Diseases of fruit crops. McGraw Hill Book Company Inc. New York, Toronto, London, 501pp.

Anees, M. M., Rashmi, C. R., Yamini, V. C. K. and Govindan, M. 2016. Report on new foliar blight disease caused by *Rhizoctonia Solani* on Chilli, Brinjal and Okra from India. *Imperial J. Interdisciplinary Res. 2*(4):182-184.

Anon. 1998. Powdery mildew fungicide. The Grower. March. p. 6.

Antoniacci, L., De Paoli, E., Montuschi, C., Ceredi, G. and Gengotti, S. 2008. *Gnomonia comari* on Strawberry: Preliminary Study on Control Strategy in the North of Italy. In *VI International Strawberry Symposium 842*, pp. 251-254.

Antu S. K. 2013. Studies on canker disease of guava *(Psidium guajava)* caused by *Pestalotiopsis psidii Pat.* M.Sc thesis, Mahatma Phule Krishi Vidyapeeth, Rahuri, 87p.

Archana, B. C. and Jamadar, M. M. 2014. Management of leaf spot and fruit spot/rot of pomegranate (*Punica granatum* L.,) caused by *Alternaria alternata* (Fr.) Keissler. *Karnataka J. Agric. Scie.* 27: 247-249.

Arroyo, F. T., Llergo, Y., Aguado, A. and Romero, F. 2009. First report of *Fusarium* wilt caused by *Fusarium oxysporum* on strawberry in Spain. *Plant Dis.* 93(3):323-323.

Agusti, L., Bonaterra, A., Moragrega, C., Camps, J. and Montesinos, E. 2011. Biocontrol of root rot of strawberry caused by *Phytophthora cactorum* with a combination of two *Pseudomonas fluorescens* strains. *J. Plant Pathol.* 363-372.

Ayoubi, N. and Soleimani, M. J. 2016. Strawberry Fruit Rot Caused by *Neopestalotiopsis iranensis* sp. nov., and N. mesopotamica. *Curr. Microbiol.* *72*(3):329-336.

Ayoubi, N., Soleimani, M. J., Zare, R. and Zafari, D. 2017. First report of *Curvularia inaequalis* and *C. spicifera* causing leaf blight and fruit rot of strawberry in Iran. *Nova Hedwigia.* 105(1-2):75-85.

Ayoubi, N., Soleimani, M. J. and Zare, R. 2016. *Pilidium concavum*, causing Tan-brown Rot on Strawberry in Iran. *J. Plant Pathol.* 98(3):667-669.

Asad-Uz-Zaman, M., Bhuiyan, M. R., Khan, M. A. I., Bhuiyan, M. K. A. and Latif, M. A. 2015. Integrated options for the management of black root rot of strawberry caused by *Rhizoctonia solani* Kuhn. *Comptes rendus biologies*, 338(2):112-120.

Ashlesha and Paul, Y.S. 2014. Antifungal Bioefficacy of Organic inputs against fungal pathogens of Bell Pepper. *Indian J. Res.* 3(6):4-6.

Badadani, M. S., Babu, S. V. and Shetty, K. T. 2007. Optimum conditions of autoclaving for hydrolysis of proteins and urinary peptides of prolyl and hydroxyprolyl residues and HPLC analysis. *J. Chromatography Anal. Technol. Biomedical Life Sci.* 847:267-274.

Bain, H. F. and Demaree, J. B. 1945. Red stele root disease of the strawberry caused by *Phytophthora fragariae. J. Agric. Res. 70*(1):11.

Baker, K.F., Cook, R.J., 1982. Biological Control of Plant Pathogens. The American Phytopathological Society, St. Paul, MN, 433pp.

Bardia, P. K. and Rai, P. K. 2011. *In vitro* and field evaluation of biocontrol agents and fungicides against wilt of cumin caused by *Fusarium oxysporum* f. sp. *cumini. J. Spices and Aromat. Crops* 16(2):88-92.

Barman, H., Roy, A. and Das, S. K. 2015. Evaluation of plant products and antagonistic microbes against grey blight (*Pestalotiopsis theae*), a devastating pathogen of tea. *African J. Microbiol. Res.* 9(18):1263-1267.

Bashar, M. A. and Chakma, M. 2014. In vitro control of Fusarium solani and F. oxysporum the causative agent of brinjal wilt. *Dhaka University J. Biol. Sci.* 23(1):53-60.

Behera, S., Singh, R. B. and Balai, L. P. 2013. Efficacy of Fungicides on *in vitro* Growth of Pigeonpea against Stem Canker. *Trends in Biosciences* 6(2):190-191.

Benitez, T., Rincon, A. M., Limon, M. C. and Codon, A. C. 2004. Biocontrol mechanisms of *Trichoderma* strains. *Int. Microbial.* 7(4):249-260.

Bhadra, M., Khair, A., Hossain, M. A. and Sikder, M. M. 2014. Efficacy of *Trichoderma* spp. and fungicides against *Lasiodiplodia theobromae. Bangladesh J. Sci. Ind. Res.* 49(2):125-130

Bhagwat, R. G., Mehta, B. P. and Patil. V. A. 2016. Evaluation of fungicides and biological agents for the management of mango anthracnose. *Int. J. Environ. Agric. Res.* 2(4):49-52

Bagherabadi, S., Zafari, D. and Soleimani, M. J. 2015. First report of leaf spot of strawberry caused by *Alternaria tenuissima* in Iran. *J. Plant Pathol. Microbiol.* 6(3):254-258.

Bhardwaj, L. N. and Sharma, S. K. 1999. Diseases of strawberry, Gooseberry and raspberry. *Diseases of horticultural crops-fruits. Indus Pub. Com., New Delhi*, 316-336.

Bhardwaj, L. N. and Gupta, M. 2002. Occurrence of red stele of strawberry in India. *Plant Disease Res.* 17(2):380

Bhardwaj, L. N., Bala, R. and Gupta, B. 2003. Effect of epidemiological parameters on the development of web blight of strawberry. In *VII International Symposium on Temperate Zone Fruits in the Tropics and Subtropics-Part Two 696* pp. 367-370.

Bisht, S., Kumar, P., Srinivasanraghavan, A. and Purohit, J. 2013. *In vitro* management of *Curvularia* leaf spot of maize using botanicals, essential oils and bio-control agents. *Bioscan Suppl. Med. Plants* 8:731-733.

Bolda, M. and Koike, S. 2003. A Treatise on Powdery Mildew in Strawberry. Strawberries and Caneberries ANR Blogs. 1-7.

Bolton, A. T. 1963. A new species of *Marssonina* on strawberry. *Canadian J. Botany* 41(2):237-241.

Borekar, K. C., Ingle, S. T., Pardhi, S. U. and Kadam, N. A. 2014. Correlation of weather parameters and efficiency of botanicals against *Phoma* Sp. and *Fusarium semitectum* causing leaf spot and basal rot of Korphad (Aloe vera). *J. Plant Disease Scie.* 9(1):82-85.

Bose, S. K. 1970. Diseases of valley fruits in Kumaon (III). Leaf-spot diseases of strawberry. *Prog. Hortic. 2*(2):33-53.

Bose, S.K., Sindhan, G.S. and Pandey, B.H. 1973. Studies on dieback disease of mango in the Tarai region of Kuaon. *Prog. Hort*ic. 70:557-584.

Brooks, A. 1920. Anthracnose of Strawberry (*Colletotrichum* sp.) reported again from Florida. *Plaid Disease Reporter* 11(11).

Capobiango, N. P., Pinho, D. B., Zambolim, L., Pereira, O. L. and Lopes, U. P. 2016. Anthracnose on strawberry fruits caused by *Colletotrichum siamense* in Brazil. *Plant Disease* 100(4):859.

Carrie-Missio, V., Rodrigues, F. A, Schurt, D. A., Rezende, D. C., Ribeiro, N. B. and Zambolim, L. 2010. Foliar application of potassium silicate, acibenzolar-S-

methyl and fungicides in the reduction of Pestalotia strawberry spot. *Trop. Plant Pathol.* 35:182–185.

Cha, S. D., Jeon, Y. J., Ahn, G. R., Han, J. I., Han, K. H. and Kim, S. H. 2007. Characterization of *Fusarium oxysporum* isolated from paprika in Korea. *Mycobiol, 35*(2):91-96.

Chadha, S., Saini, J. P. and Paul, Y. S. 2012. Vedic Krishi: Sustainable livelihood option for small and marginal farmers. *Indian J. Tradit. Knowl.* 11(3):480-486.

Chakraborty, S., Murray, G. M., Magarey, P. A., Yonow, T., O'Brien, R. G., Croft, B. J. and Sutherst, R. W. 1998. Potential impact of climate change on plant diseases of economic significance to Australia. *Australasian Plant Pathol.* 27(1):15-35.

Chakraborty, S. and Datta, S. 2003. How will plant pathogens adapt to host plant resistance at elevated CO_2 under a changing climate? *New Phytol.* 159:733–742.

Chet I. 1987. *Trichoderma*—application, mode of action, and potential as a biocontrol agent of soilborne plant pathogenic fungi. In: *Innovative Approaches to Plant Disease Control* (1st Ed.). Chet, pp. 137–160.

Chet, I. and Inbar, J. 1994. Biological control of fungal pathogens. *Appl. Biochem. Biotechnol.* 48(1):37-43.

Chethana, B. S., Ganeshan, G., Rao, A. S. and Bellishree, K. 2013. *In vitro* evaluation of plant extracts, bioagents and fungicides against *Alternaria* porri (Ellis) Cif. causing purple blotch disease of onion. *Pest Manag. Hortic. Ecosyst.* 18(2):194-198.

Chen, C., Wei, Y., Fan, T., Zhang, W., Zhang, G., Liu, Q. and Zhang, L. 2014. Bio-control *Bacillus* strain TS02 for control of strawberry powdery mildew and its molecular identification. *Acta horticulturae*. 1049:607-611.

Choi, I. Y., Cho, S. E., Park, J. H. and Shin, H. D. 2014. First report of leaf spot caused by a *Phoma* sp. on *Schisandra chinensis* in Korea. *Plant Disease* 98(1):157-157.

Chowdappa, P., Chethana, C. S., Bharghavi, R., Sandhya, H. and Pant, R. P. 2012. Morphological and molecular characterization of *Colletotrichum gloeosporioides* (Penz) Sac. isolates causing anthracnose of orchids in India. *J. Biotechnol. Bioinforma. Bioeng.* 2(1):567-572.

Cota L. V, Maffia L. A, Mizubuti, E. S. C and Macedo, P. E. F. 2009. Biological control by *Clonostachys rosea* as a key component in the integrated management of strawberry gray mold. *Biol Control* 50:222–230.

Cole, J. T., Cole, J. C. and Conway, K. E. 2005. Effectiveness of selected fungicides applied with or without surfactant in controlling anthracnose on three cultivars of Euonymus fortunei. *J. Appl. Hortic.* 7(1):16-19.

Cook, R.J., 1993. Making greater use of introduced microorganisms for biological control of plant pathogens. *Annu. Rev. Phytopathol.* 31: 53-80.

Curry, K. J., Abril, M., Avant, J. B. and Smith, B. J. 2002. Strawberry anthracnose: Histopathology of *Colletotrichum acutatum* and *C. fragariae*. *Phytopathol.* 92(10):1055-1063.

Dar, W. A., Beig, M. A., Ganie, S. A., Bhat, J. A. and Razvi, S. M. 2013. *In vitro* study of fungicides and biocontrol agents against *Fusarium oxysporum* f. sp. pini

causing root rot of Western Himalayan fir (Abies pindrow). *Sci. Res. Essays* 8(30):1407-1412.

Das, N. D., Sankar, G. R. M. and Srivastav, N. N. 1998. Studies on progression of Alternaria blight disease *Alternaria helianthi* (Hansf.) Tubaki and Nishihara of sunflower. *Ann. Plant Prot. Sci.* 6: 209-211.

De Cal, A., Redondo, C., Sztejnberg, A. and Melgarejo, P. 2008. Biocontrol of powdery mildew by *Penicillium oxalicum* in open-field nurseries of strawberries. *Biol. Control* 47(1):103-107.

De Los Santos, B., Barrau, C. and Romero, F. 2003. Strawberry fungal diseases. *J. Food Agric. Environ.* 1:129-132.

De Los Santos, B. D. L., Porras, M., Blanco, C., Barrau, C. and Romero, F. 2002. First report of *Phytophthora cactorum* on strawberry plants in Spain. *Plant Disease* 86(9):1051-1051.

Dennis, C. and Webster, J. 1971. Antagonistic properties of species-groups of Trichoderma: III. Hyphal interaction. *Trans. British Mycol. Soc.* 57(3):363-369.

Denoyes, B. and Baudry, A. 1995. Species identification and pathogenicity study of french *Colletotrichum* strains isolated from strawberry using morphological and cultural characteristics. *Phytopathol.* 85(1):53-57.

Dev, D., Konda, S., Puneeth, M. E., Tanuj, N., Singh, Prachi and Narendrappa. T. 2016. *In vitro* evaluation of bioagents and botanicals against *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc. causing anthracnose of Pomegranate. *Eco. Env. Cons.* 22 (3): 1229-1232.

Dickinson, C. H. and Skidmore, A. M. 1976. Interactions between germinating spores of Septoria nodorum and phylloplane fungi. *Trans. British Mycol. Soc.* 66(1): 45-56.

Diedhiou, P. M., Diallo, Y., Faye, R., Mbengue, A. A. and Sene, A. 2014. Efficacy of different fungicides against mango anthracnose in Senegalese soudanian agroclimate. *American J. Plant Sci.* 5(15):2224.

Dil, T., Karakaya, A. and Oguz, A. C. 2013. Blueberry fungal diseases in rize, Turkey. Proceedings – 24th International Scientific-Expert Conference of Agriculture and Food Industry – Sarajevo. 409-412.

Dinler, H., Benlioglu, S. and Benlioglu, K. 2016. Occurrence of *Fusarium* wilt caused by *Fusarium oxysporum* on strawberry transplants in Aydın Province in Turkey. *Australasian Plant Disease Notes* 11(1):1-3.

Dingley, J. M. 1970. Records of fungi parasitic on plants in New Zealand 1966–68. *New Zealand J. Agric. Res.* 13(2):325-337.

Donmez, M. F., Esitken, A., Yildiz, S. and Ercisli, S. 2011. Biocontrol of *Botrytis cinerea* on strawberry fruit by plant growth promoting bacteria. *J. Anim. Plant Sci*, 21(4):758-763.

Dodge, B. O. and Stevens, N. E. 1924. The *Rhizoctonia* brown rot and other fruit rots of strawberries. *J. Agric. Res.* 28(7):643-648.

Dogra, S. 2006. Antifungal potential of panchgavya against some soil borne pathogens. M.Sc. Thesis, Department of Plant Pathology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India.

Dutta, U. and Kalha, C. S. 2011. *In vitro* evaluation of fungicides, botanicals and bioagents against *Rhizoctonia solani* causing sheath blight of rice. *J. Mycopathological Res.* 49(2):333-336.

Eastburn, D. M. and Gubler, W. D. 1990. Strawberry anthracnose: Detection and survival of Colletotrichum acutatum in soil. *Plant Disease* 74(2):161-163.

Embaby, E. 2007. *Pestalotia* fruit rot on strawberry plants in Egypt. *Egypt J Phytopathol.* 35:99-110.

Embaby, E. M., Ragab, M. E., Doug, D., Al, K. A., Ahmed, R., Zveibil, A. and Freeman, S. 2010. First report of *Colletotrichum acutatum* and *C. gloeosporioides* causing anthracnose diseases on strawberry in Egypt. *Plant Pathol.* 59(4):808-808.

Elad, Y., Chet, I. and Henis, Y. 1981. Biological control of *Rhizoctonia solani* in strawberry fields by *Trichoderma harzianum. Plant Soil* 60(2):245-254.

Elad, Y. and Chet, I. 1987. Possible role of competition for nutrition in biocontrol of *Pythium* damping-off by bacteria. *Phytopathol*, 77:190-195

Elad, Y. and Baker, R. 1985. The role of competition for iron and carbon in suppression of chlamydospore germination of *Fusarium oxysporum. Phytopathol.* 75:190-195.

El-Deeb H. M, Lashin S. M, and Arab Y. A. 2016. Date-palm leaves infection with spotting fungi isolated from some ornamental palms. *Res. J. Pharma. Biol. Chem. Sci.* 7(3): 74-81.

El-ghanam, A., Farfour, A. and Ragab, S. 2015. Bio-Suppression of strawberry fruit rot disease caused by *Botrytis cinerea. Plant Pathol. Microbiol.*

Ellis, M. A., Wilcox, W. F. and Madden, L. V. 1998. Efficacy of metalaxyl, fosetyl-aluminum, and straw mulch for control of strawberry leather rot caused by *Phytophthora cactorum. Plant Disease* 82(3):329-332.

Elmer, W. H. and Farandino, F. J. 1997. Managing powdery mildew of cucurbits. *The Natural Farmer. Summer*, 26.

Espinoza, J. G., Briceno, E. X., Keith, L. M. and Latorre, B. A. 2008. Canker and twig dieback of blueberry caused by *Pestalotiopsis* spp. and a *Truncatella* sp. in Chile. *Plant Disease* 92(10):1407-1414.

Fall, J. 1951. Studies on fungus parasites of strawberry leaves in Ontario. *Canadian J. Botany* 29(4):299-315.

Fang, X., Phillips, D., Li, H., Sivasithamparam, K. and Barbetti, M. J. 2011. Comparisons of virulence of pathogens associated with crown and root diseases of strawberry in Western Australia with special reference to the effect of temperature. *Scientia Horticulturae* 131:39-48.

Fang, X. L., Phillips, D., Li, H., Sivasithamparam, K. and Barbetti, M. J. 2011. Severity of crown and root diseases of strawberry and associated fungal and oomycete pathogens in Western Australia. *Australasian Plant Pathol.* 40(2):109-119.

Fang, X., Finnegan, P. M. And Barbetti, M. J. 2013. Wide variation in virulence and genetic diversity of binucleate *Rhizoctonia* Isolates Associated with Root Rot of strawberry in Western Australia. *PLoS ONE* 8(2): e55877. doi:10.1371/journal.pone.0055877

FAO. 2000. Food and Agricultural organization of United States. Statistical databases. Available online:hhtp://www,fao.org/.

FAO. 2007. Food and Agricultural organization of United States. Statistical databases. Available online:hhtp://www,fao.org/.

FAO. 2015. Food and Agricultural organization of United States. Statistical databases. Available online:hhtp://www,fao.org/.

Fagundes, C., Perez-Gago, M. B., Monteiro, A. R. and Palou, L. 2013. Antifungal activity of food additives *in vitro* and as ingredients of hydroxypropyl

methylcellulose-lipid edible coatings against *Botrytis cinerea* and *Alternaria alternata* on cherry tomato fruit. *Int. J. Food Microbiol.* 166(3):391-398.

Farr, D. F., Castlebury, L. A. and Rossman, A. Y. (2002). Morphological and molecular characterization of *Phomopsis vaccinii* and additional isolates of Phomopsis from blueberry and cranberry in the eastern United States. *Mycologia* 94(3):494-504.

Feng, L., Luan, Y., Fan, Y. and Zhong, H. 2007. Pathogen identification of leaf spot disease on blueberry. *J. Northeast Agric. University*, *5*, 009.

Fernandez, R. L., Rivera, M. C., Varsallona, B. and Wright, E. R. 2015. Disease Prevalence and Symptoms Caused by *Alternaria tenuissima* and *Pestalotiopsis guepinii* on Blueberry in Entre Ríos and Buenos Aires, Argentina. *American J. Plant Sci.* 6(19):3082.

Fernandez-Ortuno, D., Grabke, A., Li, X. and Schnabel, G. 2015. Independent emergence of resistance to seven chemical classes of fungicides in *Botrytis cinerea*. *Phytopathol.* 105(4):424-432.

Fernandez-Ortuno, D., Chen, F. and Schnabel, G. 2012. Resistance to pyraclostrobin and boscalid in *Botrytis cinerea* isolates from strawberry fields in the Carolinas. *Plant Disease* 96(8):1198-1203.

Filoda, G. 2008. Impact of some fungicides on mycelium growth of *Colletotrichum gloeosporioides* [Penz.] Penz. and Sacc. *Pestycydy* 3:109-116.

Freeman, S., Nizani, Y., Dotan, S., Even, S. and Sando, T. 1997. Control of *Colletotrichum acutatum* in strawberry under laboratory, greenhouse, and field conditions. *Plant disease* 81(7):749-752.

Freeman, S., Minz, D., Kolesnik, I., Barbul, O., Zveibil, A., Maymon, M. and Dag, A. 2004. *Trichoderma* biocontrol of *Colletotrichum acutatum* and *Botrytis cinerea* and survival in strawberry. *European J. Plant Pathol.* 110(4):361-370.

Garrido, C., Carbu, M., Fernandez-Acero, F. J., Gonzalez-Rodriguez, V. E. and Cantoral, J. M. 2011. New insights in the study of strawberry fungal pathogens. *Genes Genomes Genomics* 5(1): 24-39.

Gautam, H. R., Bhardwaj, M. L. and Kumar, R. 2013. Climate change and its impact on plant diseases. *Curr. Sci.* 105(12):1685-1691.

Ghazanfar, M. U., Raza, W., Ahmed, K. S., Qamar, J., Haider, N. and Rasheed, M. H. 2016. Evaluation of different fungicides against *Alternaria solani* (Ellis & Martin) Sorauer cause of early blight of tomato under laboratory conditions. *Int. J. Zool. Stud.* 1(5):08-12.

Gohel, N. M. and Solanky, K. U. 2011. *In-vitro* and *In-vivo* evaluation of fungicides against *Alternaria alternata* causing leaf spot and fruit rot disease of chilli *Green Farming* 3(1): 84-86.

Gomez, K. A. and Gomez, A. A. 1984. *Statistical procedures for agricultural research*. John Wiley & Sons.

Gonzalez, P., Alaniz, S., Montelongo, M. J., Rauduviniche, L., Rebellato, J., Silvera-Perez, E. and Mondino, P. 2012. First report of *Pestalotiopsis clavispora* causing dieback on blueberry in Uruguay. *Plant Dis.* 96(6):914

Golzar, H., Phillips, D. and Mack, S. 2007. Occurrence of strawberry root and crown rot in Western Australia. *Australasian Plant Dis. Notes* 2(1):145-147.

Govorova, G. 1993. Methodological base of strawberry breeding in Russia for fungal pathogen resistance. *Acta Hort.* 348:458-462.

Govindachari, T. R., Suresh, G., Gopalakrishnan, G., Banumathy, B. and Masilamani, S. 1998. Identification of antifungal compounds from the seed oil of *Azadirachta indica*. *Phytoparasitica*, 26(2):109-116.

Gupta, M. and Bhardwaj, L. N. 2009. Effect of epidemiological parameters on the development of red stele of strawberry. In *Biological Forum* 1(1):18-21

Gunnell, P.S. and Gubler, W. D. 1992. Taxonomy and morphology of *Colletotrichum* species pathogenic to strawberry. *Mycologia* 84(2):157-165.

Hajlaoui, M. R., Mnari-Hattab, M., Sayeh, M., Zarrouk, I., Jemmali, A. and Koike, S. T. 2015. First report of Macrophomina phaseolina causing charcoal rot of strawberry in Tunisia. *New Disease Reports* 32:14-14.

Hang, H.Y., Zhang, M.H., Liu, Z.H., Yu, H.B., and Wang, D.L. 2007. Identification and biological characteristics of *Colletotrichum gloeosporioides* on strawberry. *J. Shenyang Agr. Uni.* 38 (3):317–321.

Hang, Y. D. and Woodams, E. E. 2003. Control of *Fusarium oxysporum* by baking soda. *LWT-Food Sci. Technol.* 36(8):803-805.

Harender, R., Suneel, A. and Sachin, U. 2005. Evaluation of fungicides for efficacy against *Fusarium* yellows in gladiolus. *J. Ornamental Hortic.* (New Series). 8(4): 320-321.

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I. and Lorito, M. 2004. Trichoderma species—opportunistic, avirulent plant symbionts. *Nat. Rev. Microbial.* 2(1):43-56.

Harman G, and Shoresh M. 2007. The mechanisms and applications of opportunistic plant symbionts. *Novel Biotechnologies for Biocontrol Agent Enhancement and Management*, 131-155.

Hegde, Y. R., Hiremani, N. S., Keshgond, R. S and Chavhane, T.L. 2013. Evaluation of fungicides against *Botryodiplodia theobromae* causing collar rot in *Jatropha curcas. Int. J. Plant Prot.* 6 (1):45- 47.

Henry, P. M., Kirkpatrick, S. C., Islas, C. M., Pastrana, A. M., Yoshisato, J. A., Koike, S. T., and Gordon, T. R. 2017. The Population of *Fusarium oxysporum* f. sp. fragariae, Cause of *Fusarium* Wilt of Strawberry, in California. *Plant Dis.* 101(4), 550-556.

Henz, G. P., Boiteux, L. S. and Lopes, C. A. 1992. Outbreak of strawberry anthracnose caused by *Colletotrichum acutatum* in Central Brazil. *Plant Dis.* 76(2).12

Hiremath, P. C., Kulkarni, M. S. and Lokesh, M. S. 1990. An epiphytotic of *Alternaria* blight of sunflower in Karnataka. *Karnataka J. Agric. Sci.* 3: 277-278.

Hickman, C. J. 1940. The Red Core root disease of the strawberry caused by *Phytophthora fragariae*. *J. Pomol. Hortic. Sci.* 18: 89–119

Holguin-Pena, R. J. 2005. Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* race 3 in Baja California Sur, Mexico. *Plant Dis.* 89(12):1360-1360.

Hong, S. K., Lee, S. Y., Choi, H. W., Lee, Y. K., Joa, J. H. and Shim, H. 2012. Occurrence of stem-end rot on mango fruits caused by *Lasiodiplodia theobromae* in Korea. *The Plant Pathol. J.* 28(4):455-455.

Howard, C. 1970. Strawberry fruit rot caused by *Colletotrichum fragariae*. *Phytopathol.* 60:1296-7.

Howard, C. M., and Albregts, E. E. 1973. Strawberry fruit rot caused by *Pestalotia longisetula*. *Phytopathol.* 63:862-863.

Humpherson-Jones, F. M .and Ainsworth, L. F. 1983. Canker of brassicas. 33rd Annual report for 1982, National Vegetable Research Station, Wellsbourne, Warwick, UK. pp. 63-64.

Ignjatov, M., Milosevic, D., Nikolic, Z., Gvozdanovic-Varga, J., Jovicic, D. and Zdjelar, G. 2015. *Fusarium oxysporum* as causal agent of tomato wilt and fruit rot. *Pestic. Phytomed.* 7(1):25-31.

Ingle, Y. V., Patil, C. U., Thakur, K. D. and Ingle, K. 2014. Effect of fungicides and plant resistance activator on Colletotrichum leaf spot of soybean. *The Bioscan*, 9(3):1187-1190.

Ilieva, E., Arulappan, F. X., & Pieters, R. 1995. Phytophthora root and crown rot of raspberry in Bulgaria. *European J. Plant Pathol.* 101(6):623-626.

Ishiguro, Y., Otsubo, K., Watanabe, H., Suzuki, M., Nakayama, K., Fukuda, T. and Kageyama, K. 2014. Root and crown rot of strawberry caused by *Pythium helicoides* and its distribution in strawberry production areas of Japan. *J. Gen. Plant Pathol.* 80(5):423-429.

Islam, M. R., Hossain, M. K., Bahar, M. H. and Ah, M. R. 2004. Identification of the causal agent of leaf spot of Betelnut and in vitro evaluation of fungicides and plant extracts against it. *Pakistan J. Biol. Sci.* 7(10):1758-1761.

Jadesha, G., Haller, H., Mondhe, M. K., Hubballi, M., Prabakar, K. and Prakasam, V. 2012. Application of plant extracts induced the changes in biochemical composition of banana fruits. *Ann. Biol. Res. 3*:5133-5140.

Jagtap, N. M., Ambadkar, C. V. and Bhalerao, G. A. 2015. *In vitro* evaluation of different fungicides against *Colletotrichum gloeosporioides* causing anthracnose of pomegranate. *Int. J. Agric. Sci. 11*(2), 273-276.

Jandaik, S and Sharma V. 2016. Antifungal potential of panchagavya against soil borne fungal pathogens associated with Capsicum nurseries. *Int. Invention J. Agric. Soil Sci* .4(2): 22-26.

Jarvis, A. P., Morgan, E. D. and Edwards, C. 1999. Rapid separation of triterpenoids from neem seed extracts. *Phytochemical Anal.* 10(1):39-43.

Jhooty, J. S. and McKeen, W. E. 1965. Studies on powdery mildew of strawberry caused by *Sphaerotheca macularis*. *Phytopathol.* 55(3):281.

Ji, M. X., Yang, J. H., Wu, X., Zhu, C. G., Xiao, T., Yao, K. B., and Zhuang, Y. Q. 2012. Biocontrol of strawberry anthracnose caused by *Colletotrichum fragariae*. *Jiangsu J. Agr. Sci.* 28 (6):1498–1500.

Jordan, V. W. L. 1973. The effects of prophylactic spray programmes on the control of pre-and Post-harvest diseases of strawberry. *Plant Pathol.* 22(2):67-70.

Joseph B. and Sankarganesh P. 2011. Antifungal efficacy of panchagavya. *Int. J. Pharm. Tech. Res.* 3(1): 585-588.

Joshi, M., Srivastava, R., Sharma, K. and Prakash, A. 2013. Isolation and characterization of *Fusarium oxysporum*, a wilt causing fungus, for its pathogenic and non-pathogenic nature in tomato (*Solanum lycopersicum*). *J. App. Nat. Sci.* 5:108-117.

Juber, K. S., Al-Juboory, H. H. and Al-Juboory, S. B. 2014. *Fusarium* wilt disease of strawberry caused by *Fusarium oxysporum* f. sp. fragariae in Iraq and its control. *J. Exp. Biol. Agric. Sci.* 2(4), 419-427.

Kanto, T., Maekawa, K. and Aino, M. 2007. Suppression of conidial germination and appressorial formation by silicate treatment in powdery mildew (*Sphaerotheca aphanis*) of strawberry (*Fragaria* x *ananassa*). *J. Gen. Plant Pathol.* 73(1):1-7.

Kanto, T., Miyoshi, A., Ogawa, T., Maekawa, K. and Aino, M. 2004. Suppressive effect of potassium silicate on powdery mildew of strawberry in hydroponics. *J. Gen. Plant Pathol.* 70(4):207-211.

Kapytowski, J. and Bojarska, J. E. 2005. Current status and trends in production of strawberries in Poland. *Belsad fruit–growing* 17(2):310-313.

Karande, M. G., Raut, S. P. and Gawande, A. D. 2007. Efficacy of fungicides, bio-organics and plant extracts against *Colletotrichum gloeosporioides* and *Fusarium oxysporum*. *Ann. Plant Prot. Sci.* 15(1):267-268.

Karmakar, S., Mondal, R., Dasgupta, S., Guha, P. and Mandal, A. K. 2015. Evaluation of some newly evolved fungicides against *Helminthosporium oryzae*, *Alternaria padwickii*, *Fusarium moniliforme*, *Curvularia lunata* and *Sarocladium oryzae* causing grain discoloration disease of rice under *in vitro* condition. *Int. J. Sci. Res.* 5(6):1108-1111

Kataria, H. R., Singh, H. and Gisi, U. 1989. Interactions of fungicide-insecticide combinations against *Rhizoctonia solani* in vitro and in soil. *Crop Prot.* 8(6):399-404.

Kaur, S. J., Thind, S. K. and Arora, A. 2016. Epidemiology of strawberry anthracnose (*Colletotrichum gloeosporioides*) in Punjab: an agrometeorological approach. *Plant Dis. Res.* 31(2):154-157.

Kerling, L. C. P., van der Plaats Niterik, A., Hermanides-Nijhof, E. J. and van den Heuvel, J. 1964. *Fungi in the phyllosphere of leaves of rye and strawberry.*

Khanzada, M. A., Lodhi, A. M. and Shahzad, S. 2004. Pathogenicity of *Lasiodiplodia theobromae* and *Fusarium solani* on mango. *Pakistan J. Botany* 36(1):181-190.

Kemmitt, G. 2002. Early blight of potato and tomato. The Plant Health Instructor.

Kim, C. H. and Chung, H. S. 1992. Differential growth response of *Rhizoctonia solani*, causal organism of ginseng damping-off, to light irradiation. *Research Reports of the Rural Development Administration (Korea Republic).*

Kim, W. G., Hong, S. K., Choi, H. W. and Lee, Y. K. 2009. Occurrence of anthracnose on highbush blueberry caused by *Colletotrichum* species in Korea. *Mycobiol.* 37(4), 310-312.

Kim, H. J., Lee, S. H., Kim, C. S., Lim, E. K., Choi, K. H., Kong, H. G., Kim, D. W., Lee, S-W. and Moon, B. J. 2007. Biological control of strawberry gray mold caused by *Botrytis cinerea* using *Bacillus licheniformis* N1 formulation. *J. Microbiol. Biotechnol.* 17:438-444.

Kithan, C and Daiho, L. 2014. *In Vitro* evaluation of botanicals, bio-agents and fungicides against leaf blight of *Etlingera linguiformis* caused by *Curvularia lunata* Var. Aeria. *J Plant Pathol. Microbiol.* 5:3

Koike, S. T., Kirkpatrick, S. C. and Gordon, T. R. 2009. *Fusarium* wilt of strawberry caused by *Fusarium oxysporum* in California. *Plant Dis.* 93(10), 1077-1077.

Koike, S. T., Gordon, T. R., Daugovish, O., Ajwa, H., Bolda, M., & Subbarao, K. (2013). Recent developments on strawberry plant collapse problems in California caused by Fusarium and Macrophomina. *Int. J.Fruit sc.* 13(1-2), 76-83.

Koike, S. T. and Gordon, T. R. 2015. Management of *Fusarium* wilt of strawberry. *Crop Prot.* 73:67-72.

Kowata, L. S., Strapasson, M., Challiol, M. A., Mio, M. D. and Larissa, L. 2010. Glomerella leaf spot in apple: validation of proposed diagrammatic scale and efficiency of fungicides. *Ciencia Rural* 40(7):1502-1508.

Kronenberg, H. G., Gerritsen, J., Klinkenberg erkelens, M. A. and Zweede, A. 1949. The Strawberry. *The Strawberry.* 327.

Kumar, M., Bhadauria, V., Singh, K., Singh, C. and Yadav, A. K. 2013. Evaluation of fungicide efficacy for the management of *Alternaria* leaf spot disease on chilli. *Plant Pathol. J.* 12(1):32.

Kumar, J. A and Naik, L. K. 2015. *In vitro* evaluation of biocontrol agents against isolated wilt pathogen of Castor (*Ricinus communis* L.). *ecialise Sp* 49(2):221-223.

Kumari, A., Kumar, R., and Kumar, H. 2014. Efficacy of fungicides and *Trichoderma viride* against *Fusarium oxysporum* f. Sp. Cubense *in-vitro*. *The Bioscan*, 9(3):1355-1358.

Kumhar, K.C., Babu, A., Bordoloi, M., Benarjee, P and Rajbongshi, H. 2016. Comparative bioefficacy of fungicides and *Trichoderma* spp. against *Pestalotiopsis theae*, causing grey blight in tea (Camellia sp.): An *in vitro* study. *Int. J. Curr. Res. Biosci. Plant Biol.* 3(4): 20-27.

Kumla, J., Suwannarach, N. and Lumyong, S. 2016. First report of *Phoma* leaf spot disease on cherry palm caused by *Phoma herbarum* in Thailand. *Canadian J. Plant Pathol.* 38(1):103-106.

Kurosaki, N., Yamato, O., Sasamato, Y., Mori, F., Imoto, S., Kojima, T. and Maede, Y. 2007. Clinico-pathological finding in peripartum dairy cows fed anions salts lowering the dietary cation- anion difference: involvement of serum inorganic phosphorus, chloride and plasma estrogen concentration in milk fever. *Japanese J. Vet. Res.* 55:3-12.

Kurze, S., Bahl, H., Dahl, R. and Berg, G. 2001. Biological control of fungal strawberry diseases by *Serratia plymuthica* HRO-C48. *Plant Dis.* 85(5):529-534.

Lal, M. and Kandhari, J. 2009. Cultural and morphological variability in *Rhizoctonia solani* isolates causing sheath blight of rice. *J. Mycol. Plant Pathol.* 39(1):77.

Ilieva, E., Arulappan, F. X. and Pieters, R. 1995. *Phytophthora* root and crown rot of raspberry in Bulgaria. *European J. Plant Pathol.* 101(6), 623-626.

Latha, P., Anand, T., Prakasam, V., Jonathan, E. I., Paramathma, M. and Samiyappan, R. 2011. Combining *Pseudomonas*, *Bacillus* and *Trichoderma* strains with

organic amendments and micronutrient to enhance suppression of collar and root rot disease in physic nut. *Appl. Soil Ecol.* 49:215-223.

Lele, V. C. and Phatak, H. C. 1965. Leaf blight and dry stalk rot of strawberry caused by *Rhizoctonia bataticola*. *Indian Phytopathol*, 18: 38-42.

Leandro, L. F. S., Gleason, M. L., Nutter Jr, F. W., Wegulo, S. N. and Dixon, P. M. 2001. Germination and sporulation of *Colletotrichum acutatum* on symptomless strawberry leaves. *Phytopathol.* 91(7), 659-664.

Lemanceau, P., Bakker, P. A. H. M., Dekogel, W. J., Alabouvette, C. and Schippers, B. 1992. Effect of pseudobactin 358 produced by *Pseudomonas putida* WSC358 on suppression of *Fusarium* wilt of carnations by non pathogenic *Fusarium oxysporum*. *Appl. Environ. Microbiol.* 58:2978-2980

Lamondia, J. A. 1991. First report of strawberry anthracnose caused by *Colletotrichum acutatum* in Connecticut. *Plant Dis.* 75:128.

Liu, Y. H., Lin, T., Ye, C. S. and Zhang, C. Q. 2014. First report of *Fusarium* wilt in blueberry (*Vaccinium corymbosum*) caused by *Fusarium oxysporum* in China. *Plant Dis.* 98(8):1158-1158.

Luan, Y. S., Shang, Z. T., Su, Q., Feng, L. and An, L. J. 2008. First report of a *Pestalotiopsis* sp. causing leaf spot of blueberry in China. *Plant Dis.* 92(1):171-171.

Ma, L.P., Gao, F., Qiao, W and Hao, B.Q., 2001. Control of sweet pepper Fusarium wilt with compost extracts and its mechanism. *Chin. J. Appl. Environ. Biol.* 7: 84–87

Mass, J. L. 1998. *Compendium of Strawberry Diseases*. 3rd Ed. The American Phytopathological Society. 318 pp.

Maas, J. L. and Palm, M. E. 1997. Occurrence of anthracnose irregular leaf spot, caused by *Colletotrichum acutatum*, on strawberry in Maryland. *Adv. Strawberry Res. 16*:68-70.

Madhavi, G. B. and Bhattiprolu, S. L. 2011. Evaluation of fungicides, soil amendment practices and bioagents against *Fusarium solani*-causal agent of wilt disease in chilli. *J. Hortic. Sci. 6*(2):141-144.

Mahmood, A. and M.A. Gill. 2002. Quick decline of mango and *in vitro* response of fungicides against the disease in Pakistan. *Int. J. Agri. Biol.* 4: 39-40.

Martin, F. N. and Loper, J. E. 1999. Soilborne plant diseases caused by Pythium spp.: ecology, epidemiology, and prospects for biological control. *Critical reviews in plant sciences* 18(2):111-181

Mathivanan, N. and Prabavathy, V. R. 2007. Effect of carbendazim and mancozeb combinationon *Alternaria* leaf blight and seed yield in sunflower (*Helianthus annus* L.). *Arch. Phytopathol. Plant Prot.* 40(2):90-96.

Maude, R. B., Bambridge, J. M., Spencer, A., Suett, D. L., Drew, R. L. K., Humpherson-Jones., F. M., O brien, M. J., Crute, I. R. and Gordon, P. L. 1986. Fungus diseases of brassicas-biology, resistance and control. 36th Annual report for 1985, National Vegetable Research Station, Wellsbourne, Warwick, UK. pp. 63-64.

Mertely, J. C. and Legard, D. E. 2004. Detection, isolation, and pathogenicity of *Colletotrichum* spp. from strawberry petioles. *Plant Dis.* 88(4):407-412.

Mezeal, I. A. 2014. Study biocontrol efficacy of *Pseudomonas fluorescens* and *Bacillus subtilis* against *Rhizoctonia solani* and *Fusarium oxysporum* causing disease in tomato (*Lycopersicon Esculentum* L.) *Indian J. Fundam. Appl. Life Sci.* 4(4): 175-183.

Mokhtar, H. and Dehimat, L. 2015. *In vitro* and *In vivo* efficiency of *Trichoderma harzianum* against *Phoma* and *Glocladium* soft rot occurred on tomato fruits (*Lycopersicon esculentum*). *Int. J. Curr. Microbiol. App. Sci.* 4(8):141-147.

Montgomerie, I. G. and Kennedy, D. M. 1980. The pathogenicity of *Phytophthora* species to red raspberry. In *Symposium on Breeding and Machine Harvesting of Rubus* 112 (pp. 167-176).

Montgomerie, I. G. and Kennedy, D. M. 1974. Preliminary evaluation of fungicides to control red core of strawberry. *Plant Pathol.* 23(1):42-45.

Moustafa, M. S. H., El–Dakar, H. A. and Alkolaly, A. M. 2015. *Pestalotia* leaf Spot a New Disease affect Guava Trees in Egypt. *Int. J. Sci. Eng. Res.* 6(10):1306-1312.

Mouden, N., Benkirane, R., Ouazzani Touhami, A. and Douira, A. 2014. Pathogenic capacity of Pestalotia longisetula Guba reported for the first time on strawberry (*Fragaria* x *ananassa* Duch.) in Morocco. *Int. J. Pure App. Biosci. 2*(4):132-141.

Miller, P. W. 1947. Fungi associated with root lesions of the strawberry in Oregon. *Plant Dis. Reporter* 31:90-99.

Milicevic, T. and Cvjetkovic, B. 2008. Strawberry leaf scorch (*Diplocarpon earliana* (Ell. &Ev.) Wolf). *Glasilo biljne zaštite*, 1(1):20-24.

Milicevic, T., Topolovec-Pintaric, S., Cvjetkovic, B. and Duralija, B. 2004. The sensitivity of strawberry cultivars to major fungal foliar diseases in Croatia, and the possibilities for their control. In *V International Strawberry Symposium 708* (pp. 127-130).

Mina, U and Dubey, S. C. 2010. Effect of environmental variables on development of *Fusarium* wilt in chickpea (*Cicer arietinum*) cultivars. *Indian J. Agric. Sci.* 80(3):231.

Mina, U. and Sinha, P. 2008. Effects of climate change on plant pathogens. *Environ. News* 14(4):6–10.

Mishra, R. K. and Gupta, R. P. 2012. *In vitro* evaluation of plant extracts, bio-agents and fungicides against Purple blotch and *Stemphylium* blight of onion. *J. Med. Plants Res.* 6(48):5840-5843.

Munoz, F. R. 2002. Effect of different fungicides in the control of *Colletotrichum acutatum*, causal agent of anthracnose crown rot in strawberry plants. *Crop Prot.* 21(1):11-15.

Murthy, B. N. S. and Pramanick, K. K. 2012. Strawberry cultivation in mild-tropics: prospects and challenges from diseases perspective. In *VII International Strawberry Symposium 1049* (pp. 151-159).

Muthukar, A and Ranganatahan, P. 2012. *In vitro* and *in vivo* evaluation of plant oils against anthracnose pathogen *Colletotrichum musea* and *Botryodiplodia theobromae*. *Indian J. Plant Prot.* 40(2):91-94.

Nam, M. H., Park, M. S., Kim, H. S., Lee, E. M., Park, J. D. and Kim, H. G. 2016. First report of dieback caused by *Lasiodiplodia theobromae* in strawberry plants in Korea. *Mycobiol.* 44(4): 319-324.

Narayanan, P., Vanitha, S., Rajalakshmi, J., Parthasarathy, S., Arunkumar, K., Nagendran, K. and Karthikeyan, G. 2015. Efficacy of bio-control agents and fungicides in management of mulberry wilt caused by *Fusarium solani*. *J. Biol. Control* 29(2):107-114.

Nath, K., Ku, S. and Gl, K. 2014. Effective approaches of potential bioagent, phytoextracts, fungicide and cultural practice for management of banana fruit rot disease. *J. Plant Pathol. Microbiol.*

Nechet, K. D. L. and Halfeld-Vieira, B. A. 2007. First report of *Rhizoctonia solani* causing web blight on pigeonpea in Brazil. *Fitopatologia Brasileira*, 32(4), 358-358.

Nemec, S. 1972. Temperature effects on *Mycosphaerella fragariae* and strawberry leaf spot development. *Plant disease reporter*.

Neves, P. M., Hirose, E., Tchujo, P. T. and Moino Jr, A. 2001. Compatibility of entomopathogenic fungi with neonicotinoid insecticides. *Neotropical Entomol.* 30(2):263-268.

Ngoc, N. K., Narendrappa, T. and Chaudhary, M. 2013. Management of tomato early blight disease (*Alternaria solani* (Elis and Martin) Jones and Grout) through biological and chemical methods. *Mysore J. Agric. Sci.* 47(2):241-245.

Nichols, M. A. 1960. Black seed disease of strawberry fruit. *New Zealand Journal of Agriculture, 101*.

Nita, M., Ellis, M. A., and Madden, L. V. 2003. Reliability and accuracy of visual estimation of *Phomopsis* leaf blight of strawberry. *Phytopathol.* 93: 995-1005.

Oyedeji, E. O., and Kareem, K. T. 2016. In-vitro Evaluation of Some Fungicides against *Botrydiplodia theobromae*: Causal Pathogen of Pineapple Dieback. *American J. Exp. Agric.* 11(5):106-109.

Padder, B. A., Sharma, P. N., Kapil, R., Pathania, A. and Sharma, O. P. 2010. Evaluation of bioagents and biopesticides against *Colletotrichum lindemuthianum* and its integrated management in Common Bean. Notulae Scientia Biologicae. 2:72-76.

Pan, S. and Mishra, N. K. 2010. Epidemiological studies on some diseases of guava (*Psidium guajava* L.). *J. Plant Prot. Sci.* 2(2):49-52.

Park, J. H., Cho, S. E., Lee, C. K., Lee, S. H. and Shin, H. D. 2014. First report of leaf spot caused by *Phoma dictamnicola* on *Dictamnus dasycarpus* in Korea. *Plant Dis.* 98(10):1443-1443.

Parizi, T. E., Ansari, M. and Elaminejad, T. 2012. Evaluation of the potential of *Trichoderma viride* in the control of fungal pathogens of Roselle (*Hibiscus sabdariffa* L.) *in vitro*. *Microbial pathogenesis* 52(4):201-205.

Patil, C. U., Zape, A. S. and Wathore, S. D. 2009. Efficacy of fungicides and bioagents against *Colletotrichum gloeosporioides* causing blight in *Piper longum*. *Int. J Plant Prot.* 2(1):63-66.

Patra, S. and Biswas, M. K. 2017. Studies on cultural, morphological and pathogenic variability among the isolates of *Fusarium oxysporum* f. Sp. *Ciceri* causing wilt of chickpea. *Int. J. Plant Anim. Environ. Scis.* 7(1):11-16

Paulus, A. O. 1990. Fungal diseases of strawberry. *Hort. Sci.* 25(8):885-889

Pastrana, A. M., Basallote-Ureba, M. J., Aguado, A., Akdi, K. and Capote, N. 2016. Biological control of strawberry soil-borne pathogens *Macrophomina phaseolina* and *Fusarium solani*, using *Trichoderma asperellum* and *Bacillus* spp. *Phytopathologia Mediterranea*, 55(1):109-111.

Pawar, D. M. 2012. *In vitro* evaluation of fungicides and organics against *Curvularia lunata* and *Curvularia pallescens* causing leaf blight in gladiolus. *Int. J. Plant Prot.* 5(2):442-443.

Pawar, S. V., Borkar, P. G. Joshi, P. V. and Salvi, P. P. 2015. *In vitro* evaluation of different fungicides and bio-agents against *Rhizoctonia solani* Kuhn incitent of sheath blight of rice. *Trends in Biosciences* 8(14):3622-3626.

Pertot, I., Zasso, R., Amsalem, L., Baldessari, M., Angeli, G. and Elad, Y. 2008. Integrating biocontrol agents in strawberry powdery mildew control strategies in high tunnel growing systems. *Crop Prot.* 27:622-631.

Peries, O. S. 1962. Studies on strawberry mildew, caused by *Sphaerotheca macularis* (Wallr. ex Fries) Jaczewski. *Ann. App. Biol.* 50(2):211-224.

Plakidas, A. 1941. Purple leaf spot of Strawberry. *Phytopathol.* 31(3). 225-240

Pierson, L.S. and Thomashow, L.S. 1992. Cloning and heterologous expression of the phenazine biosynthetic locus from *Pseudomonas aureofaciens*. *Mol. Plant-Microbe Interact.* 5:330-339

Ponmurugan, P., Baby, U. I. and Gopi, C. 2006. Efficacy of certain fungicides against *Phomopsis theae* under *in vitro* conditions. *African J. Biotechnol.* 5(5):434-436.

Porras, M., Barrau, C. and Romero, F. 2007. Effects of soil solarization and *Trichoderma* on strawberry production. *Crop prot.* 26(5):782-787.

Porras, M., Barrau, C., Arroyo, F. T., Santos, B., Blanco, C. and Romero, F. 2007. Reduction of *Phytophthora cactorum* in strawberry fields by *Trichoderma* spp. and soil solarization. *Plant Dis.* 91(2):142-146.

Powelson, R. L. 1960. Initiation of strawberry fruit rot caused by botrytis cinerea. *Phytopathol.* 50:491-494.

Pramanick, K. K., Kishore, D. K., Sharma, S. K., Das, B. K., and Murthy, B. N. S. 2013. Strawberry Cultivation under Diverse Agro-Climatic Conditions of India. *Int. J. Fruit Sci.* 13(1-2):36-51.

Prasad, M. S., Singh, A.K. and M.S. Prasad. M. S. L. 1998. *In vitro* evaluation of fungicides against *Collectorichum gloeosporioides*, the anthracnose of khasi mandarin. *Indian J. Hill Farmg.* 11(1 & 2): 102 – 103.

Prashanth. A. and Sataraddi. 2007. Investigations on anthracnose (*Colletotrichum gloeosporioides* (Penz.) Fenz. and Sacc.) of Pomegranate. *Karnataka J. Agril. Sci.* 20(4): 929.

Prashanth, A., Sataraddi, A. R., Naik, M. K., Patil, M. B. and Patil, R. S. 2008. Evaluation of fungicides, bioagents and botanicals against pomegranate anthracnose. *Indian J. Plant Prot.* 36(2):283-287.

Pastrana, A. M., Basallote-Ureba, M. J., Aguado, A., Akdi, K. and Capote, N. 2016. Biological control of strawberry soil-borne pathogens Macrophomina phaseolina and Fusarium solani, using Trichoderma asperellum and Bacillus spp. *Phytopathologia Mediterranea*, 55(1), 109.

Purkayastha, R. P and Bhattacharya, B. 1982. Antagonsim of microorganisms from jute phyllosphere towards *Colletotrichum cochori*. *Trans. Br. Mycol. Soc.* 78:504-513.

Ragab, M. M., Ashour, A. M. A., Abdel-Kader, M. M., El-Mohamady, R. and Abdel-Aziz, A. 2012. *In vitro* evaluation of some fungicides alternatives against *Fusarium oxysporum* the causal of wilt disease of pepper (*Capsicum annum* L.). *Int. J. Agric. Forestry* 2(2):70-77.

Rahman, S., Adhikary, S. K., Sultana, S. and Jahan, N. 2013. *In vitro* evaluation of some selected fungicides against *Pestalotia palmarum* (Cooke.) causal agent of grey leaf spot of coconut. *J. Plant Pathol. Microbiol.* Abstr.

Rahman, S. M., Muniruzzaman, S. M., Nusrat, S. and Khair, A. 2015. *In vitro* evaluation of botanical extract, bioagents and fungicides against purple blotch diseases of bunch onion in Bangladesh. *Advan Zool Bot*, 3:179-183.

Raj, Lal, A. A., Naik, T. S. and Shivakumar, K. V. 2016. Evaluation of fungicides against *Rhizoctonia solani* the causal agent of seedling rot of chilli. *Advances in Life Sci.* 5(3):729-731.

Rajan, P. V., Saifulla, M. and Pallavi, M. S. 2013. In vitro evaluation of bio-agents, fungicides and herbicides against *Fusarium oxysporum* f. sp. *ciceri* causing wilt of Chickpea. *BIOINFOLET-A Quarterly Journal of Life Sciences*, 10(2a), 403-405.

Rajivkumar and Singh, S. B. 1996. Influence of weather factors on *Alternaria* leaf spot development in sunflower. *Indian J. Mycol. Pl. Pathol.* 26: 196-198.

Raju, G. P., Rao, S. R. and Gopal, K. 2008. *In vitro* evaluation of antagonists and fungicides against the red gram wilt pathogen *Fusarium oxysporum* f. sp. Udam (butler) Snyder and Hansen. *Legume Res.* 31(2), 133-135.

Ramani, V. N., Davara, D. K., Anadani, V. P. and Detroja, A. M. 2015. Evaluation of fungicides, botanicals and biocontrol agents against banana anthracnose disease under *in vitro* condition. *Int. J. Plant Prot.* 8(2):228-233.

Rangaswamy, G. 1958. Diseases of crop plants in India, prentice hall of India Pvt. Ltd. New Delhi, P-504.

Rao, V. G., Patil, P. L. and Sonawane, R. B. 1998. Waxy rot of strawberry fruit. *J. Maharashtra Agric. Univ.* 23(2), 202-203.

Rawal, R.D. 1998. Management of fungal diseases in tropical fruits. In: Tropical. Fruits in Asia:Diversity, Maintenance, Conservation and Use. (Eds.): R.K. Arora and V. Ramanatlia Rao. Proceedings of the IPGRI-ICAR-UTFANET Regional training course on the conservation and use of germplasm of tropical fruits in Asia held at Indian Institute of Horticultural Research, 18-3J May 1997, Bangalore, India.

Ray, A. and Kumar, P. 2008. Evaluation of fungicides against *Rhizoctonia solani* Kuhn, the inicitant of aerial blight of soybean. *Pantnagar J. Res.* 6(1):42-47.

Ray, M. K., Mishra, P. K. and Baruah, P. K. 2016. Control of fungal pathogen *Pestalotiopsis disseminata* causing grey blight disease in Som (*Persea bombycina* Kost.): An *in vitro* study. *Int. J. Pure App. Biosci.* 4(6):180-185.

Rebollar-Alviter, A., Madden, L.V. and Ellis, M. A., 2007. Pre-and post-infection activity of azoxystrobin, pyraclostrobin, mefenoxam, and phosphate against leather rot of strawberry, caused by *Phytophthora cactoram. Plant Dis.* 91:559-564.

Riddle RW (1950). Permanent stained mycological preparation obtained by slide culture. *Mycologia* 42: 265-70.

Rocha, J. de. R-de.S., Oliveira, N.T. de and Menezes, M. de. 1998. Comparison of inoculation methods efficiency for evaluation of *Colletotrichum gloeospoiroides* isolates pathogenicity on passion fruits (*Passiflora edulis*). *Brazilian Archives of Biol.Technol.* 41(1): 145-153.

Rodrigues, F. A., Silva, I. T., Antunes Cruz, M. F. and Carre-Missio, V. 2014. The infection process of *Pestalotiopsis longisetula* leaf spot on strawberry leaves. *J. Phytopathol.* 162(10), 690-692.

Roopa, R. S., Yadahalli, K. B. and Kavyashree, M. C. 2014. Evaluation of natural plant extracts, antagonists and fungicides against early blight caused by *A. solani in vitro*. *The Bioscan*, 9(3):1309-1312.

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. *Pak. J. Phytopath*, 24(2):137-142.

Sahu, D., Khare, C. and Patel, R. 2014. Eco friendly management of early blight of tomato using botanical plant extracts. *I Control Pollution* 30(2):205-208.

Sahu, U. and Verma, K. P. 2015. In vitro efficacy of oil cakes and cow byproducts against leaf spot of Sesame. *Ann. Plant Prot. Sci.* 23(2):409-410.

Sagoua, W., Ducamp, M. N. and Loiseau, G. 2008. *In vitro* antifungal activity of Neem oil against banana pathogens. In *IV International Symposium on Tropical and Subtropical Fruits 975* (pp. 197-207).

Saju, K. A., Deka, T. N., Sudharshan, M. R., Gupta, U. and Biswas, A. K. 2012. Incidence of *Phoma* leaf spot disease of large cardamom (*Amomum subulatum* Roxb.) and *in vitro* evaluation of fungicides against the pathogen. *J. Spices Aromat.Crops* 20(2).

Saju, K. A., Deka, T. N., Gupta, U., Biswas, A. K., Sudharshan, M. R., Vijayan, A. K. and Thomas, J. 2013. Identity of *Colletotrichum* infections in large cardamom (*Amomum subulatum* Roxb.). *J. Spices Aromat.Crops* 22(1):101-103.

Saju, K. A., Mech, S., Deka, T. N. and Biswas, A. K. 2012. In vitro evaluation of biocontrol agents, botanicals and fungicides against *Pestalotiopsis* sp. infecting large cardamom (Amomum subulatum Roxb.). *J. Spices Aromat. Crops*: *20*(2).

Sallato, B. V., Torres, R., Zoffoli, J. P. and Latorre, B. A. 2007. Effect of boscalid on postharvest decay of strawberry caused by *Botrytis cinerea* and *Rhizopus stolonifer*. *Spanish J. Agric. Res.* 5(1):67-78.

Sanjay, R., Ponmurugan, P. and Baby, U. I. 2008. Evaluation of fungicides and biocontrol agents against grey blight disease of tea in the field. *Crop Prot.* 27(3): 689-694.

Sarkar, B. and Sengupta, P. K. 1978. Studies on some aspects of the epidemiology of *Alternaria* leaf blight of mustard (Brassica sp.) Bentrage zur Tropischen landwirt-schaft and Veterinarmedizen. 16(1): 91-96.

Sawant, I. S., Rajguru, Y. R., Salunkhe, V. P. and Wadkar, P. N. 2012. Identification of efficient *Trichoderma* species and isolates from diverse locations in India for biological control of anthracnose disease of grapes. *J. Biol. Control* 26: 144-54.

Saxena, A., Sarma, B. K., & Singh, H. B. (2016). Effect of azoxystrobin based fungicides in management of chilli and tomato diseases. *Proceedings of the National Academy of Sciences, India Section B: Biol. Scie.* 86(2):283-289.

Schuh, J. and Zeller, S. M. 1944. Insect pests and diseases of strawberry in Oregon.

Seema, M and Devaki, N. S. 2012. In vitro evaluation of biological control agents against *Rhizoctonia solani*. *J. Agric. Technol.* 8(1):233-240.

Seema, M., Devaki, N. S. and Sreenivas, S. S. 2010. In vitro evaluation of fungicides against *Rhizoctonia solani* infecting FCV tobacco in Karnataka light soils. *Pestology* 34(11):36-38.

Sharifi, K., JavadI, E. A. and Mahdavi, M. 2008. A new *Pestalotiopsis* species for the mycoflora of Iran. Rostaniha. 9(2):118.

Sharma, A. and Bhardwaj, L. N. 2001. Influence of temperature, relative humidity and nutrient solution on sporangial germination and zoospore liberation in Phytophthora cactorum causing leather rot of strawberry. *Plant Dis. Res.-ludhiana* 16(2):243-246.

Sharma, V. P. and Sharma, R. R. 2004. The strawberry. Indian Council of Agricultural Research, New Delhi. Pp 166.

Sharma, A., Bhardwaj, L. N. and Gupta, M. 2005. Leather rot of strawberry and its management-A Review. Agricultural reviews-agricultural research communications centre India, 26(1), 59.

Sharma, J. N., Sharma, P. K. and Sharma, A. 2005. Studies on epidemiology and management of *Marssonina* blotch, the cause of premature leaf fall in

apple. *Integrated plant disease management. Challenging problems in horticultural and forest pathology, Solan, India, 14 to 15 November 2003*, 1-7.

Shelar, S. A., Padule, D. N., Sawant, D. M. and Konde, B. K. 1997. Physiological studies on *Botryodiplodia theobromae* Pat causing die-back disease of mango. *J. Maharashtra Agric. Univ.* 22(1):202-204.

Shin, G. H., Hur, J. S. and Koh, Y. J. 2000. Chemical control of gray blight of tea in Korea. *Plant Pathol. J.* 16(3):162-165.

Shivanna, M. B., Achar, K. G. S., Vasanthakumari, M. M. and Mahishi, P. 2014. *Phoma* leaf spot disease of *Tinospora cordifolia* and its effect on secondary metabolite production. *J. Phytopathol.* 162(5):302-312.

Shoresh, M., Harman, G. E. and Mastouri, F. 2010 Induced systemic resistance and plant responses to fungal biocontrol agents. *Annu Rev Phytopathol.* (in press)

Shovan, L. R., Bhuiyan, M. K. A., Begum, J. A. and Pervez, Z. 2008. *In vitro* control of *Colletotrichum dematium* causing anthracnose of soybean by fungicides, plant extracts and *Trichoderma harzianum*. *Int J Sustain Crop Prod* 3: 10-7.

Singh, S. J. Porakash, O. and Tewari, R. P. 1975. A leaf spot disease of strawberry from India. *Indian Phytopathol.* 28: 303-304.

Singh, P. C. and Singh, D. 2006. *In vitro* evaluation of fungicides against *Alternaria alternata*. *Ann. Plant Prot. Sci.* 14(2):500-502.

Singh, R., Sharma, R. R., Kumar, A. and Singh, D. B. 2008. Package of practices for strawberry cultivation with modern techniques under north Indian plains. In *VI International Strawberry Symposium 842*:607-610.

Singh, A., Verma, K.S. and Mohan, C. 2008. Evaluation of fungicides against *Colletotrichum gloeosporioides* causing anthracnose of guava. *Pl. Dis. Res.*, 23: 91-992.

Sivakumar D, Wijeratnam RSW, Wijesundera RLC, Marikar FMT, Abeyesekere M. 2000. Antagonistic effect of *Trichoderma harzianum* on postharvest pathogens of Rambutan (Nephelium lappaceum). *Phytoparasitica*, 28: 240-7.

Sivan, A. and Chet, I. (1993). Integrated control of Fusarium crown and root rot of tomato with *Trichoderma harzianum* in combination with methyl bromide or soil solarization. *Crop Protection*, *12*(5), 380-386.Shoresh, M., Harman, G. E. and Mastouri, F. 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. *Ann. Rev. Phytopathol.* 48:21-43.

Skidmore, A. M and Dickinson, C. H. 1976. Colony interaction and hyphal interference between *Septoria nodorum* and phylloplane fungi. *Trans. Br. Mycol. Soc.* 66: 57-64

Smith, B. J. 2008. Epidemiology and pathology of strawberry anthracnose: a North American perspective. *Hort Sci.* 43(1):69-73.

Smith, H. C. 1952. Strawberry fungus disease in New Zealand. *Strawberry fungous disease in New Zealand. Report. Sci. Congr. roy. Soc.* N.Z., 47-48 pp.

Smith, B. J and Black, L. L. 1987. Resistance of strawberry plants to *Colletotrichum fragariae* affected by environmental conditions. *Plant dis.* 71(9):834-837.

Sneh, B., Burpee, L. and Ogoshi, A. 1991. *Identification of Rhizoctonia species*. APS press.

Sobowale, A. A., Jonathan, S. G., Odu, B. O., Ayansina, A. D. V. and Ojikutu, T. K. 2010. *Trichoderma longibrachiatum* as an antagonist of *Botryodiplodia theobromae*. *Archives of Phytopathol. Plant Prot.* 43(5):479-484

Sombardier, A., Savary, S., Blancard, D., Jolivet, J. and Willocquet, L. 2009. Effects of leaf surface and temperature on monocyclic processes in *Podosphaera aphanis*, causing powdery mildew of strawberry. *Canadian J. Plant Pathol.* 31(4):439-448.

Somda, I., Leth, V. and Sereme, P. 2007. Antifungal effect of Cymbopogon citratus, Eucalyptus camaldulensis and Azadirachta indica oil extracts on sorghum seed-borne fungi. *Asian J. Plant Sci.* 6(8):1182-1187.

Somu, R., Thammaiah, N., Swamy, G. S. K., Kulkarni, M. S. and Devappa, V. 2014. *In vitro* evaluation of fungicides against *Fusarium oxysporum* f. sp. *cubense*. *Int. J. Plant Prot.* 7(1):221-224.

Solanki, S. 2015. *Studies on anthracnose of mango* (*Mangifera indica L.) caused by colletotrichum gloeosporioides (Penz.) sacc* (Doctoral dissertation, JNKVV).

Soytong. K., Srinon, W., Rattanacherdchai, K., Kanokmedhakul, S. and Kanokmedhakul, K. 2005. Application of antagonistic fungi to control anthracnose disease of grape. *J. Agric. Biotechnol.* 1: 33-41.

Sreenivasa, M. Y., Dass, R. S., Raj, A. P. C., Prasad, M. N. N., Achar, P. N. and Janardhana, G. R. 2011. Assessment of the growth inhibiting effect of some plant essential oils on different *Fusarium* species isolated from sorghum and maize grains. *J. Plant Dis. Prot.* 208-213.

Srinivas, P., Ratan, V., Patel, A. P. and Madhavi, G. B. 2013. Review on banded leaf and sheath blight of rice caused by *Rhizoctonia solani* Kuhn.

Sterne, R. E. and Fulton, J. P. 1983. Strawberry anthracnose and crown rot in Arkansas [*Colletotrichum fragariae*]. *Arkansas Farm Research*.

Stindt, A. and Weltzien, H.C. 1990. Untersuchungen zur Wirkung und zu den Wirkungs-mechanismsmen von Kompost Extrackten auf Botrytis cinerea Pers. Ex. Nocca & Balb an Erdbeeren, Kopfsalat und Buschboh-nen. Erwerbsobstbau 33, 28–29.

Stone, R. E. 1922. Leaf scorch or mollisiose of strawberry. *Phytopathol*, 12:375-380.

Stretch, A. W. 1989. Biological control of blueberry and cranberry fruit rots (*Vaccinium corymbosu* L. and *Vaccinium macrocarpon* Ait.). *Acta Hort.* 241, 301–306

Subedi, S., Gharti, D. B., Neupane, S and Ghimire, T. 2015. Management of Anthracnose in Soybean using Fungicide. *J. Nepal Agric. Res. Council.* 1*:29-32.*

Subramanian, C. V. 1983. Hyphomycetes. Taxonomy and Biology. *Academic press Science .502 p.*

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 J. Agric. Res. Vol, 23*:1-2.

Sumana, K., Ramakrishnan, S., Sreenivas, S. S. and Devaki, N. S. 2012. Field evaluation of promising fungicides and bioagents against *Fusarium* wilt and root knot complex disease in FCV tobacco crop. *J. Agric. Technol.* 8(3):983-991.

Sumangala, K. and Patil, M. B. 2009. Panchagavya-an organic weapon against plant pathogens. *J. Plant Dis. Scie.* 4(2):147-151.

Sutton, B. C. 1965. Typification of Dendrophoma and a reassessment of *D. obscurans*. *Trans. British Mycol. Society* 48(4):611-616.

Swaminathan, C. 2005, Food production through vrkshayurvedic way. In: Technol. for Natural Farming. Eds. Agriculture College & Research Institute, Madurai, Tamilnadu, India. pp:18-22

Sylla, J., Alsanius, B. W., Krüger, E., Becker, D. and Wohanka, W. 2013. *In vitro* compatibility of microbial agents for simultaneous application to control strawberry powdery mildew (*Podosphaera aphanis*). *Crop Prot.* 51:40-47.

Tabatabaie, S. M. H. and Murthy, G. S. 2016. Cradle to farm gate life cycle assessment of strawberry production in the United States. *J. Cleaner Production* 127:548-554.

Takeuchi, J. and Horie, H. Proceedings of the Kanto-Tosan Plant Protection Society 1997. Occurrence of leaf spot of strawberry saxifraga caused by *Phoma exigua*. 44 pp.179-181.

Takahashi, H., Furuya, H., Takai, T. and Matsumoto, T. 1997. Characteristics of *Alternaria alternata* strawberry pathotype isolated in New Zealand and the resistance of the 'Akita Berry' strawberry to the fungus *J. Japan. Soc. Hort. Sci.* 65 (4): 785-790.

Tamietti, G. and Matta, A. 2003. First report of leaf blotch caused by *Marssonina coronaria* on apple in Italy. *Plant Dis.* 87(8):1005-1005.

Tanaka, M. A. S., Passos, F. A. and Betti, J. A. 1996. Irregular leaf spot of strawberry, caused by *Colletotrichum acutatum*, in Brazil. *Fitopatologia Brasileira (Brazil)*.

Tapwal, A., Thakur, G., Chandra, S. and Tyagi, A. 2015. *In-vitro* evaluation of *Trichoderma* species against seed borne pathogens. *Int. J. Pharma Biosci.* 1(10), 14-19.

Tasiwal, V., Benagi, V. I., Hegde, Y. R., Kamanna, B. C. and Naik, K. R. 2009. *In vitro* evaluation of botanicals, bioagents and fungicides against anthracnose of papaya caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. *Karnataka J. Agric. Sci.* 22(4):803-806.

Taskeen-Un-Nisa, W. A., Bhat, M. Y., Pala, S. A. and Mir, R. A. 2011. *In vitro* inhibitory effect of fungicides and botanicals on mycelial growth and spore germination of *Fusarium oxysporum*. *J. Biopesticides* 4(1):53-56.

Taware, M. R., Gholve, V. M. and Dey, U. 2014. Bio-efficacy of fungicides, bioagents and plant extracts/botanicals against *Alternaria carthami*, the causal agent of *Alternaria* blight of Safflower (*Carthamus tinctorius* L.). *African J. Microbiol. Res.* 8(13), 1400-1412.

Tekade, A., Koche, M. D., Kothikar, R. B. and Surpam, A. N. 2017. Efficacy of fungicides and bioagents against *Curvularia lunata* causing blight of coleus under laboratory conditions. *J. Med. Plants* 5(2):189-191.

Timudo-Torrevilla, O. E., Everett, K. R., Waipara, N. W., Boyd-Wilson, K. S. H., Weeds, P., Langford, G. I. and Walter, M. 2005. Present status of strawberry fruit rot diseases in New Zealand. *New Zealand Plant Prot.* 58:74-79.

Tompkins, C. M. and Tucker, C. M. 1941. Root rot of pepper and pumpkin caused by *Phytophthora capsici*. *J.Agric. Res.* 63(7). 417-426.

Toscano-Underwood, C., West, J. S., Fitt, B. D., Todd, A. D. and Jedryczka, M. 2001. Development of *Phoma* lesions on oilseed rape leaves inoculated with ascospores of A-group or B-group *Leptosphaeria maculans* (stem canker) at different temperatures and wetness durations. *Plant Pathol,* 50(1):28-41.

Turechek, W. W., Heidenreich, C. and Pritts, M. P. 2002. First report of strawberry anthracnose (*Colletotrichum acutatum*) in strawberry fields in New York. *Plant Dis.* 86(8):922-922.

Urena-Padilla, A. R., MacKenzie, S. J., Bowen, B. W. and Legard, D. E. 2002. Etiology and population genetics of *Colletotrichum* spp. causing crown and fruit rot of strawberry. *Phytopathol.* 92(11): 1245-1252.

Utkhede, R. S. and Rahe, J. E. 1983. Effect of Bacillus subtilis on growth and protection of onion against white rot. *J. Phytopathol.* 106(3):199-203.

Valiuskaite, A., Raudonis, L. and Surviliene, E. 2008. Control of grey mold and white leaf spot in strawberry. *Zemdirbyste-Agric.* 95(3):221-226.

Vallimayil, J. and Sekar, R. 2012. Investigation on the effect of panchagavya on sounthern sunnhemp mosaic virus (SSMV) infected plant systems. *Glob. J. Environ. Res.* 6(2):75-79.

Varma, M. S. Kerala gears up for strawberry export debut with Munnar plant, The Financial Express. 25 Feb. 2014, p. 15.

Velazhahan, R., Samiyappan, R. and Vidhyasekaran, P. 1999. Relationship between antagonistic activities of *Pseudomonas fluorescens* isolates against *Rhizoctonia solani* and their production of lytic enzyme. *J. Plant Dis. Prot.* 106:244-250.

Venkataravanappa, V., Nargund, V. B., Kumar, M. K., Prasanna, Reddy, Laxminarayana, C. N. and Basavarajappa, M. P. 2006. Efficacy of different fungicides and botanicals against *Colletotrichum gloeosporioides* an incitant of mango anthracnose. *J. Pl. Dis. Sci.* 1(2): 200-202.

Verma, V. S. and Gupta, V. K. 2010. First Report of *Curvularia lunata* causing root rot of strawberry in India. *Plant dis.* 94(4):477-477.

Vestberg, M., Kukkonen, S., Saari, K., Parikka, P., Huttunen, J., Tainio, L. and Lemoine, M. C. 2004. Microbial inoculation for improving the growth and health of micropropagated strawberry. *Appl. Soil Ecol.* 27(3):243-258.

Vincent, J. M. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* 159(4051):850.

Wada, H., Cavanni, P., Bugiani, R., Kodama, M., Otani, H. and Kohmoto, K. 1996. Occurrence of the strawberry pathotype of *Alternaria alternata* in Italy. *Plant dis.* 80(4):372-374.

Waghe, K. P., Wagh, S. S., Kuldhar, D. P. and Pawar, D. V. 2015. Evaluation of different fungicides, bioagents and botanicals against *Alternaria* blight caused by *Alternaria helianthi* (Hansf) of sunflower. *African J. Agric. Res.* 10(5):351-358.

Washington, W. S., Engleitner, S., Boontjes, G. and Shanmuganathan, N. 1999. Effect of fungicides, seaweed extracts, tea tree oil, and fungal agents on fruit rot and yield in strawberry. *Anim. Prod. Sci.* 39(4):487-494.

Wassenaar, L. M. and van der Scheer, H. T. 1988. *Alternaria* leaf spot in strawberry. In *International Strawberry Symposium 265*, pp. 575-578.

Weller, D. M. 2007. Pseudomonas biocontrol agents of soilborne pathogens: looking back over 30 years. *Phytopathol.* 97(2):250-256.

Weltzien, H.C., 1991. Biocontrol of foliar fungal diseases with compost extracts. In: Andrews, J.H., Hirano, S.B. (Eds.), Microbiology Ecology of Leaves. Springer, New York, NY, pp. 430–450.

Wheeler, B. E. J. 1969. An introduction to plant disease and fungi, John Wiley. *Phytopathol.* 22: 837-845.

Wicks, T. J. and Lee, T. C. 1982. *Phytophthora fragariae* in South Australia. *Australasian Plant Pathol.* 11(4):55-56.

Wilhelm, S. 1961. *Diseases of strawberry: a guide for the commercial grower* (No. 04; USDA, Folleto 1708.).

Wheeler, B. E. J. 1969. An introduction to plant disease and fungi, John Wiley. *Phytopthol.* 22:837-845.

Williams, D. J., Beach, B. G. W., Horriere, D., Marachel, G. 1977. A new systemic fungicide with activity against Phycomycete diseases. Proceedings of the British Crop Protection Conferences 2:565-573

Williams, G. and Williams, P. 1993. Baking soda and horticultural oil vs. powdery mildew. *HortIdeas. May*, 51.

Willocquet, L., Sombardier, A., Blancard, D., Jolivet, J. and Savary, S. 2008. Spore dispersal and disease gradients in strawberry powdery mildew. *Canadian J. Plant pathol.* 30(3):434-441.

Wilson, L. L., Madden, L. V. and Ellis, M. A. 1992. Overwinter survival of *Colletotrichum acutatum* in infected strawberry fruit in Ohio. *Plant dis.* 76(9):948-950.

Wormald, H. and Montgomery, H. B. S. 1941. Leaf blotch of strawberries. A disease new to Britain. Gardeners' Chronicle 110: 180.

Wolfe, K.L., Kang, X., He, X., Dong, M., Zhang, Q. and Liu, R.H. 2008. Cellular antioxidant activity of common fruits. *J. Agric. Food Chem.* 56:8418-8426.

Wright, E. R., Rivera, M. C., Campanella, E. R., Farinon, O. M., Berretta, M. F. and Perez, B. A. 2014. *Fusarium* branch blight on highbush blueberry in Argentina. *African J. Biotechnol.* 13(51):4628-4634.

Wu, F., Guan, Z. and Whidden, A. 2012. Strawberry industry overview and outlook. *Unpublished manuscript, Gulf Coast Research and Education Center, University of Florida, Gainesville, Florida. Retrieved from http://www. fred. ifas. ufl. edu/pdf/webinar/Strawberry. pdf.* 1-12

Xie, L., Zhang, J. Z., Wan, Y. and Hu, D. W. 2010. Identification of Colletotrichum spp. isolated from strawberry in Zhejiang Province and Shanghai City, China. *J. Zhejiang Univ-Science* 11(1):61-70.

Xie, H. H., Wei, J. G., Liu, F., Pan, X. H. and Yang, X. B. 2014. First report of mulberry root rot caused by *Lasiodiplodia theobromae* in China. *Plant Dis.* 98(11):1581-1581.

Xu, C. N., Zhou, Z. S., Wu, Y. X., Chi, F. M., Ji, Z. R. and Zhang, H. J. 2013. First report of stem and leaf anthracnose on blueberry caused by *Colletotrichum gloeosporioides* in China. *Plant dis.* 97(6):845-845.

Yadav, M. L. and Ratnoo, R. S. 2014. Management of leaf spot of cotton caused by *Curvularia lunata*. *J. Plant Disease Sci.* 9(1):105-107.

Yarwood, C. E. 1957. Powdery mildews. *The Botanical Rev.* 23(4):235-301.

Yildiz, A., Benlioglu, K. and Benlioglu, H. S. 2014. First report of strawberry dieback caused by *Lasiodiplodia theobromae*. *Plant Dis.* 98(11):1579-1579.

Yuan, H., Ling, X., Liu, T., Chen, T., Yang, Y., Yao, S. and Zhang, B. 2014. Microscopic observations of strawberry plant colonization by a GFP-labelled strain of Fusarium oxysporum f. sp. fragariae. *Canadian J. Plant Pathol.* 36(4):501-508.

Zaker, M. 2014. Antifungal evaluation of some inorganic salts against three phytopathogenic fungi. *Int. J. Agric. Crop Sci.* 7(14):1352.

Zaker, M. 2016. Natural Plant Products as Eco-friendly Fungicides for Plant Diseases Control-A Review. *The Agriculturists*, 14(1):134-141.

Zegeye, E. D., Santhanam, A., Gorfu, D., Tessera, M. and Kassa, B. 2011. Biocontrol activity of *Trichoderma viride* and *Pseudomonas fluorescens* against *Phytophthora infestans* under greenhouse conditions. *J. Agric. Technol.* 7(6): 1589-1602.

Zeller, S. M. 1932. *A strawberry disease caused by Rhizoctonia*. Corvallis, Or.: Agricultural Experiment Station. Oregon State Agricultural College.1-22

Zentmeyer, G.A. 1955. A laboratory method for testing soil fungicides with *Phytophthora cinnamomii*, a test organism. *Phytopathol.* 45: 398-404.

Zhao, J., Ma, Z., Liu, Z., Shang, Q., Zhao, X. and Wei, Y. 2016. *Pestalotiopsis clavispora* causing leaf spot on strawberry. *Mycosystema* 35(1):114-120.

Zhong, L. C., Ai, Y. J., Chun, R. H. and Yi, Y. D. 2016. Identification of *Curvularia clavata* causing leaf spot on pineapple (*Ananas comosus*) in China. *Canadian J. Plant Pathol.* 38(2):250-253.

Zivkovic, S. T., Stojanovic, S. D., Balaz, J. and Gavrilovic, V. P. 2007. Characteristics of Phomopsis sp. isolates of plum trees origin. *Zbornik Matice srpske za prirodne nauke*, (113):83-91.

CATALOGUING, DOCUMENTATION AND MANAGEMENT OF FUNGAL DISEASES OF STRAWBERRY (*Fragariae* x *ananassa* Duch.)

by

P. AMRUTHA

(2015-11-009)

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Agriculture

(PLANT PATHOLOGY)

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR – 680 656

KERALA, INDIA

2017

ABSTRACT

Strawberry (*Fragaria* x *ananassa* Duch.), hybrid species of genus *Fragaria,* cherished for its characteristic flavour, colour and tentalizing aroma, is becoming an important table fruit of millions of people around the world. However, the crop is inflicted by several fungal diseases that reduce its commercial value. Hence, the present investigation was carried out to identify and catalogue the major fungal diseases of strawberry growing in Kerala.

Purposive sampling surveys were carried out in strawberry growing tracts of Kerala *viz.*, Wayanad, Idukki and Malappuram to collect infected samples and also to assess the incidence of fungal diseases during different periods *viz.*, December-January, March-April and July-August. During the survey, four leaf spots (LSW-1, LSI-1, LSM-1 and LSI-2), four leaf blights (LBW-1, LBI-1, LBI-2 and LBM-1), one fruit rot (FRW-1) and two crown and root rots (CRI-1 and CRM-1) were noticed. Among the crown and root rot diseases, CRM-1 recorded the highest per cent disease incidence (PDI) of 82 per cent. Leaf blight (LBW-1) recorded maximum severity of 25.2 per cent among foliage diseases. Correlation studies were carried out to elucidate the influence of weather parameters on disease development.

Symptomatology of different diseases was studied both under natural and field conditions. Pathogenicity was proved by mycelial bit inoculation and spore suspension method. Cultural and morphological characterisation of the isolates were carried out and for further confirmation of the identity upto species level, the isolates were sent to National Centre for Fungal Taxonomy (NCFT), New Delhi. The pathogens causing LSW-1, LSI-1 and LSM-1 were identified as *Colletotrichum gloeosporioides*, LSI-2 as *Alternaria alternata*, LBW-1 as *Rhizoctonia solani*, LBI-1 as *Phoma exigua*, LBI-2 as *Curvularia lunata*, LBM-1 as *Pestalotiopsis longisetula* and FRW-1 as *Rhizoctonia solani*. The two crown and root rot pathogens, CRI-1 and CRM-1 were confirmed as *Fusarium oxysporum* and *Lasiodiplodia theobromae* respectively.

In order to recommend an appropriate management strategy for the aforesaid diseases, *in vitro* and *in vivo* evaluation were carried out using fungicides, biocontrol agents and organic formulations. Fungicides *viz.*, carbendazim 12% + mancozeb 63%, propineb 70 WP, Bordeaux mixture, cymoxanil 8% + mancozeb 64%, difenoconazole 25EC and carbendazim 50WP were found effective against various foliage diseases. Carbendazim 12% + mancozeb 63%, cymoxanil 8% + mancozeb 64%, copper hydroxide 77WP and carbendazim 50 WP recorded cent per cent reduction in mycelial growth of *Fusarium oxysporum* (CRI-1). Similarly, carbendazim 12% + mancozeb 63%, copper hydroxide 77WP, cymoxanil 8% + mancozeb 64% recorded 93-100 per cent reduction of *Lasiodiplodia theobromae* (CRM-1). Results of dual culture studies with *Trichoderma asperellum* and *Pseudomonas fluorescens* against pathogens revealed 66.67 to 100 and 0 to 70.55 per cent control respectively. Likewise, organic formulations like Calphomil recorded an inhibition ranging from 13.3 to 75.33 per cent, whereas neem oil, panchagavya and baking powder + vegetable oil mixture could restrict the growth of pathogen only upto 34 per cent.

In vivo experiment was conducted to study the efficacy of fungicides and biocontrol agents under natural conditions also. Accordingly, four major selected pathogens *viz.*, *C. gloeosporioides, P. longisetula, F. oxysporum* and *L. theobromae* were subjected to molecular characterisation prior to *in vivo* studies. The sequence homology on molecular studies revealed that the isolates showed similarity to *C. gloeosporioides, Neopestalotiopsis clavispora, F. oxysporum* and *L. theobromae.* Results of the pot culture experiment revealed that *Trichoderma asperellum* showed better control against *C. gloeosporioides* compared to other treatments followed by carbendazim 12% + mancozeb 63%. Propineb 70 WP, *T. asperellum* and carbendazim 12% + mancozeb 63% reduced the severity caused by *Neopestalotiopsis* leaf blight disease. The combination fungicide carbendazim 12% + mancozeb 63% (0.2%) was found equally efficient against *F. oxysporum* and *L. theobromae.*

Thus, the study has enlightened our knowledge on the various fungal diseases inflicting strawberry as well as the role of weather in disease development and the

management practices using plant protection chemicals and bioagents both under *in vitro* and *in vivo* conditions. Hence, further studies should be focused to carry out multilocational trials in strawberry growing tracts of Kerala.