INTEGRATED MANAGEMENT OF ANTHRACNOSE OF SNAKE GOURD (*Trichosanthes cucumerina* L.)

by

ASWANI DEVI

(2014 - 11 - 140)

THESIS

Submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM – 695522

KERALA, INDIA

2016

DECLARATION

I, hereby declare that this thesis entitled **"Integrated management of anthracnose of snake gourd (*Trichosanthes cucumerina* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani

Date: 25/11/2016

ASWANI DEVI

(2014-11-140)

CERTIFICATE

Certified that this thesis entitled "**Integrated management of anthracnose of snake gourd (*Trichosanthes cucumerina* L.)**" is a record of research work done independently by Ms. Aswani Devi (2014-11-140) under my guidance and supervision that has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Vellayani

25 -11- 2016

Dr. Kamala Nayar

(Chairman, Advisory Committee)

Professor (Plant Pathology)

Instructional Farm

College of Agriculture- Vellayani

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Aswani Devi, a candidate for the degree of Masters of Science in Agriculture with major in Plant Pathology, agree that the thesis entitled **" Integrated management of anthracnose of snake gourd (*Trichosanthes cucumerina* L.) "** may be submitted by Ms. Aswani Devi (2104-11-140) , in partial fulfillment of the requirement of the degree.

Dr. Kamala Nayar	Dr. V. K. Girija
Chairman, Advisory Committee	(Member, Advisory Committee)
Professor (Plant Pathology)	Professor and Head
Instructional Farm	Department of Plant Pathology
College of Agriculture- Vellayani	College of Agriculture-Vellayani

Dr. Joy. M.	Dr. Biju Joseph
(Member, Advisory Committee)	(Member, Advisory Committee)
Associate Professor	Assistant Professor
Department of Plant Pathology	Department of Soil science & Ag.chemistry
College of Agriculture-Vellayani	College of Agriculture-Vellayani

EXTERNAL EXAMINER

ACKNOWLEDGEMENT

First and foremost I bow my head before the Almighty who have given me the strength, courage and confidence to complete the thesis work successfully on time.

I am pleased to place my profound etiquette to ***Dr. Kamala Nayar****, Professor (Plant Pathology), Instructional farm, College of Agriculture Vellayani and esteemed Chairman of my Advisory Committee for her learned counsel, unstinted attention, arduous and meticulous guidance, kind treatment during the course of investigation and constructive criticism have installed in me the spirit of confidence and deep sense of respect and gratitude. I value her knowledge and wisdom which nurtured this research in right direction without which fulfillment of this endeavor would not have been possible. The keen interest, abundant encouragement, she has imparted to me throughout my research work are invaluable and because of which I have been able to successfully complete the work assigned to me.*

With ineffable gratitude, I thank ***Dr.V. K Girija.,*** *Professor and Head, Department of Plant Pathology, College of Agriculture, Vellayani and Member of Advisory Committee, for her technical guidance rendered at every stage of work, periodical review of the progress and meticulous personal care.*

It gives me great pleasure to express my deep sense of gratitude to ***Dr. Joy M.,*** *Associate Professor,Department of Plant Pathology, College of Agriculture, Vellayani and Member of Advisory Committee for his crisp criticism and scholarly guidance during the course of this investigation.*

I wish to express my sincere gratitude to ***Dr. Biju Josep,*** *Assistant Professor, Department of Soil Science amd Agricultural Chemistry, College of Agriculture, Vellayani and Member of Advisory Committee for his crisp criticism, for his valuable suggestions and guidance throughout the research work and course of study.*

I wish to express my sincere gratitude to ***Dr. Umamaheswaran*** *(Professor and ADR, NARP) (Department of Plant Pathology, College of Agriculture, Vellayani for*

his valuable suggestions and guidance throughout the research work and course of study.

I am very much thankful to ***Dr. C. Gokulapalan and Dr. K.K Sulochana*** *(Retd. Professors) Department of Plant Pathology, College of Agriculture, Vellayani for their valuble suggestions, encouragement, kind help and co-operation rendered throughout the study*

I acknowledge with sense of gratitude to ***Dr. Vijayaraghava Kumar,*** *Professor and Head, Department of Agricultural Statistics for his valuable advice in suggesting the statistical analysis of data and for his heartful cooperation to accomplish this stupendous task.*

I wish to express my sincere gratitude to each and every teaching and non-teaching staff of Plant Pathology, for their suggestions and guidance throughout the research work.

I sincerely acknowledge the Kerala Agricultural University for financial support during my studies.I express gratitude to my seniors Ms. Shilpa treasa chacko, my friends Neethu and Irshana, my juniors Chinnu, Aparna, Ananthu, Karthika, Preethu and Mamatha for their support and heartful help

I allocate my highest respect and heartful regards from my inner core of hearts to my ever loved parents, ***Sri. Ashok kumar P.*** *and* ***Smt. Padma,*** *who have taught me the concepts of life and their dedicated efforts to educate me to this level and without whose valuable moral support, prayers, blessings and sacrifices, the thesis would not have seen the light of the day. I have been highly fortunate and lucky to express my heartfelt respect to my loving sister* ***Aishwarya****, who has always given encouragement and support with love and care during my struggled period. I wish to thank my uncle, aunty and family friends for their kind blessings and support.*

ASWANI DEVI

CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS USED

µm	Micro meter
µl	Micro litre
°C	Degree Celsius
CD	Critical Difference
cm	Centimeter
DI	Disease incidence
PDI	Percent disease index
et al.	And other co workers
Fig.	Figure
g	Gram
h	Hours
l	Litre
mm	Milli meter
ml	Milli litre
min.	Minutes
sp. or spp.	Species (Singular and plural)
viz.,	Namely
dia.	Diameter
PDA	Potato dextrose agar
PDB	Potato dextrose broth

Introduction

1. INTRODUCTION

Snake gourd (*Trichosanthes cucumerina* L.) is presently cultivated widely in Kerala as an annual vegetable crop due to the growing awareness of its myriads of nutritional benefits comprising of proteins, fats, carbohydrate, soluble fibre as well as nutrients and vitamins like calcium, phosphorous riboflavin, thiamine, niacin and carotene. Above all, this vegetable is rich in soluble fibres and contains low amount of carbohydrate.

This economically important vegetable of India is commonly grown in Southeast Asia, Indo-Malaysia, China, Japan, and Northern Australia (Paris and Maynard, 2008). It grows well in the warm humid conditions of Kerala state. However, frequent incidence of pests and diseases during the different crop stage were considered as the major threat to snake gourd cultivation in Kerala. Among the diseases, the anthracnose caused by *Colletotrichum* sp. is highly destructive causing considerable yield loss (Damm *et al.*, 2013).

A serious outbreak of anthracnose disease of snake gourd, caused by *Colletotrichum lagenarium* (Pass.) Ell and Halst, occurred in Banglore during months of July to October (Prakash *et al.*, 1974). More than 60 per cent field loss was noticed in United States of America due to the infection caused by *Colletotrichum orbiculare* (Thompson and Jenkins, 1985). *C. lagenarium* (synonym of *C. orbiculare*) is universally accepted as the name of the pathogen causing anthracnose of cucurbitaceous crops (Von Arx, 1957). It is reported to have more than 40 plant host species, worldwide, especially cucumber (*Cucumis sativus*), melons (*Cucumis melo*), watermelon (*Citrullus lanatus*), snakegourd (*Trichosanthes cucumerina*), pumpkin (*Cucurbita pepo*) and squash (*Cucurbita maxima*) (Farr and Rossman, 2013). *Colletotrichum* sp. caused anthracnose in a wide range of host plants which are distributed primarily in tropical and subtropical regions of the world (Sutton 1992 and Hyde *et al.*, 2009). According to Pring *et al.*, (1995), this fungus *Colletotrichum* has

the ability to overwinter on alternative hosts such as solanaceous or legume crops, plant debris and rotten fruits in the field.

The fungus produces symptoms on leaves, stems and fruits of cucumber plants and severely hinders the cucumber crop production (Thompson and Jenkins, 1985 and Wasilwa *et al.*, 1993). On cucumber fruits, circular sunken water-soaked lesions were formed that expanded and turned black in moist weather, eventually covered with pink spore masses. On the leaves, lesions were pale brown to reddish, and centres got cracked and formed shot holes (Sitterly and Keinath, 1996).

Anthracnose develops in all the above ground parts of snake gourd plant causing leaf spot, blight and fruit rot during growing period. The disease becomes more severe during warm and wet summer which may result in early defoliation, yield loss and lower quality of fruits. Symptoms of anthracnose invasion were characterised by development of sunken necrotic lesions on foliage, twigs, flowers and fruits, as well as causing crown rot, stem rot and seedling blight (Waller *et al.*, 2002).

Although the disease is widespread in snake gourd fields of Kerala the vegetable growers are not aware of the mode of spread and consequently, the control measures to be adopted for this disease. Chemical control of anthracnose in cucurbits involves frequent application of fungicides such as mancozeb, carbendazim, difenoconazole and benomyl (Damm *et al.*, 2013). Continuous fungicide applications cause negative effects on farmers' income, environment and health, particularly in developing countries (Voorrips *et al.*, 2004). Moreover the constant use of fungicides like mancozeb is not encouraged on the account of its toxic residues which may cause much harm to the environment, in due course. So the scope of newer fungicides like strobilurins is being tested against various crop diseases of Kerala. Silicon amendments proved to be effective in controlling both soil borne and foliar fungal diseases in cucumber, rice, sugarcane, turf and several other plant species (Datnoff *et al.*, 2001). Sun *et al.*, (2002) reported the reduction in incidence of anthracnose

disease in cucumber caused by *C. orbiculare,* by the application of silicon. The use of bio-control agents like *Pseudomonas fluorescens* and *Trichoderma viride* are harmless and effective for controlling plant diseases (Ashraf and Zuhaib, 2013). Effective control of *Colletotrichum* diseases usually involves the use of a combination of control measures *viz.*, cultural control, biological control, chemical control and intrinsic resistance (Wharton and Dieguez-Uribeondo, 2004).

So far, in Kerala, the anthracnose in snake gourd was not studied in detail and hence the pathogen causing the disease was not confirmed up to species level. In the light of above aspects, the present study was undertaken with an objective of making a comparative evaluation of the efficacy of foliar application of the nutrient potassium silicate, bio-control agents and newer fungicide molecule for the management of anthracnose disease of snake gourd. A preliminary survey for assessing and studying the symptom development of anthracnoe disease affecting the snake gourd plants in different locations near College of Agriculture, Vellayani were also envisaged following which the pathogenic isolates obtained from surveyed locations were identified and tested both under *in vitro* and *in vivo* conditions against different chemical and non-chemical treatments which were proposed to be included in the integrated management of the disease.

Review of literature

2. REVIEW OF LITERATURE

Cucurbitaceae is a large group of summer vegetable crops, which consist of 118 genera and 825 species. It is mainly distributed in tropical and subtropical regions although a few of them are grown in temperate regions also (Jeffrey, 1990). India is the second largest producer of vegetables with 2.8 per cent of total cropped area and 13.38 per cent of total vegetable production (Damm *et al.,* 2013). Being the largest cash crop, about 4,929,400 million tonnes of cucurbits were produced in India (FAOSTAT, 2010). *Fevilleae*, *Melothrieae*, *Cucurbitaceae*, *Sicyoideae* and *Cyclanthereae* are the five subfamilies included under this family (Whitaker and Davis, 1962). *Cucumis sativus* L. (cucumber), *Momordica charantia* L. (bitter gourd), *Luffa actangua* L. Roxb (ridge gourd), *Cucumis melo* L. (muskmelon), *Cucurbita pepo* L. (pumpkin), *Cucurbita moschata* Duch and Poir. (squash) and *Trichosanthes cucumerina* L.(snake gourd) are the main species included under the group (Jeffrey, 1990).

Anthracnose, derived from the Greek word meaning 'coal', is the common term used for designating plant diseases characterized by very dark, sunken lesions containing spores (Issac, 1992). Anthracnose is one of the most common diseases affecting cucurbitaceous crops. In addition to snake gourd, anthracnose can affect cucumber, cantaloupe, chayote, citron, gherkin, gourd, honeydew melon, muskmelon, watermelon, and many non-cucurbit species. The disease causes serious economic losses to several economically important vegetable crops worldwide. However pumpkin and squash are rarely affected by the disease (Wasilwa *et al.,* 1993). Field losses caused by *Colletotrichum* species have been reported to be more than 60 per cent in cucumber crop in the United States (Thompson and Jenkins, 1985).

The major threat to snake gourd cultivation in Kerala is the frequent incidence of pests and diseases during the different crop stages. One of the most destructive diseases among these is the anthracnose caused by *Colletotrichum* species resulting up to 70 per cent yield loss (Zitter *et al.,* 1996 and Koike *et al.,* 2007). A

serious outbreak of anthracnose disease of snake gourd caused by *C. lagenarium* (Pass.). Ell and Halst occurred in Bangalore during the months of July to October (Prakash *et al.,* 1974). Sixty per cent of field loss was caused by *C. orbiculare* in United States of America (Thompson and Jenkins, 1985).

The genus *Colletotrichum* belongs to the sub division Deuteromycotina (Fungi imperfecti), Class Coelomycetes, Order Melanconiales and Family Melanconiaceae. The genus *Colletotrichum* was established by Corda (1831), for fungi characterized by hyaline, curved fusiform conidia and setose acervuli. *C. orbiculare* is the universally accepted pathogen causing anthracnose in cucurbits and is synonymously known as *C. lagenarium* as reported by Von Arx, 1957.

Damm *et al.,* 2013 reported that the temperature of 70 to 80°F was optimum for disease development. High relative humidity in canopy for a prolonged period and film of water on the leaf surface favored spore production, germination and infection. Hence, anthracnose was more severe in mid-season with rainy and warm weather conditions (Li and Zang, 2014).

The yield losses due to anthracnose in different crops varied from 10 per cent to 60 per cent in different parts of India (Pandey *et al.,* 2006). Sundaravadana *et al.,* (2007) recorded a higher disease incidenc of 27.31 per cent in panicle anthracnose or blossom blight in mango which was caused by *C. gloeosporoides*. Disease incidence of anthracnose in bottle gourd was 14 per cent in Chittagong regions of Bangladesh as reported by Hossain *et al.,* 2010. Anamika *et al.,* (2014) conducted a survey to assess the incidence of anthracnose in chilli and reported that incidence ranged from 55.33 per cent to 71.10 per cent.

2. SYMPTOM DEVELOPMENT OF ANTHRACNOSE LEAF SPOT OF CUCURBITS IN THE FIELD.

The disease develops in all the aerial parts of the snake gourd plants causing leaf spot, blight and fruit rot during growing season. Severe epidemics of the disease

occurred during warm and wet summer which resulted in early defoliation, yield loss and lower quality of fruits. The fungus produced symptoms on leaves, stems and fruits of cucumber plants and severely hindered the cucumber crop production (Thompson and Jenkins, 1985 and Wasilwa *et al.*, 1993). On cucumber fruits, circular sunken water-soaked lesions were formed that expanded and turn black in moist weather, eventually covered with pink spore masses on leaves, the lesions were pale brown to reddish, and centers got cracked and formed shot holes (Sitterly and Keinath, 1996).

Lesions were observed as dark brown which were more or less circular and became concentric in nature as the lesion progressed (Damm *et al.,* 2013). On leaves the disease appeared as small circular spots that coalesced to form large elliptical spots and under severe conditions, defoliation of affected plant occurred (Mc Govern, 1995). Symptoms of anthracnose in cucumber, were sunken necrotic lesions on leaves, stems, flowers and fruit, as well as crown rot, stem rots and seedling blight (Waller *et al.,* 2002). Anthracnose was a common fungal disease of cucurbits observed in fields and green houses and the symptoms developed on all above ground parts of cucurbits and caused leaf spot, blight, stem canker and fruit rot during growing seasons as reported by Li and Zang, (2014).

2.1. Isolation of pathogen

Colletotrichum species that caused serious disease on plants had also been commonly isolated as endophytes from healthy plants, and were identified as saprobes on dead plant material (Michereff *et al.,* 1993; Photita *et al.*, 2004, 2005; Promputtha *et al.*, 2002; Toofanee and Dulymamode, 2002; Lin *et al.,* 2002 and Hyde, 2009).

Than *et al.,* (2008) isolated *C. coffeanum* from coffee berries and Bharathi *et al.,* (2004) isolated *C. capsici* from infected chilly seed. *Colletotrichum* sp were isolated from anthracnose infected fruits of chilli, papaya, coffee, mango and rose apple and

cultured in potato dextrose agar medium as reported by Pratibha *et al.,* (2004). Ashutosh *et al.,* (2012) isolated *C. gloeosporioides* from anthracnose infected samples of mango and Avinash *et al.,* (2013) isolated *Colletotrichum* sp. from infected samples of cucurbits using standard blotter method.

2.2 Studies on pathogenicity

Earlier findings revealed that *C. lagenarium* is the causal pathogen of leaf, stem and fruit anthracnose of snake gourd. The fungus was reported as the causal agent of anthracnose diseases on infected leaves of snake gourd by Prakash *et al.* (1974); Timchenko (1977) and Peregrine *et al.,* (1984). Symptom expression of *C. gloeosporiodes* was observed five days after artificial inoculation in chilli (Sanders and Korsten, 2003; Pal *et al.,* 2006; Intana *et al.*, 2007; Mesta *et al.*, 2009; Ratanacherdchai *et al.*, 2010; Rahman *et al.*, 2013 and Azad and Azad, 2015).

Artificial inoculation done on ten days old detatched leaves of bottle gourd with 100 to 200 conidia / ml of *C. lagenarium,* kept on moist blotter in glass Petri dish at 25 to 30° C maintaining 100 per cent relative humidity for 24 h, gave the best result of pathogenicity of the fungus (Suhag and Duhan, 1984). Daykin and Milholland (1984) studied the pathogenicity of *C. gloeosporioides* that caused ripe rot of mascudine grape and Gubler *et al.,* 1988 tested the pathogenicity of anthracnose of strawberry caused by *C. acutatum.* Artificial inoculation methods *in vitro* were commonly used to test the pathogenicity of a fungal species, as it was easy to control environmental conditions (Photita *et al.,* 2004). Common inoculation methods for pathogenicity testing included drop inoculation and wound /drop inoculation (Kanchana-udomkan *et al.,* 2004 and Jeun *et al.,* 2008); micro-injection and spraying with high pressure guns (Freeman, 1996; Lin *et al.*, 2002; AVRDC, 2002; Than *et al.* 2008 and Cai *et al.* 2009).

In pathogenicity test, six plants (cv. Jamaican squash) for each of the five isolates were spray inoculated with a conidial suspension (1.0×10^6 conidia/ml).

Negative controls were sprayed with sterile distilled water. In repeated tests, plants were symptomatic of infection seven days post inoculation. There were no symptoms on control plants and Koch's postulates were fulfilled with the re-isolation of the pathogen from symptomatic leaf tissues (Rampersad, 2010).

2.2.1 Symptom development in pathogenicity test

TeBeest *et al.,* (1977) defined the Incubation period (IP) as the time measured in days between inoculation and the appearance of initial symptoms and the Disease Development Period (DDP) as the time measured in days between inoculation and appearance of mature lesions.

Palenchar (2009) reported that initial symptoms of anthracnose appeared on cucumber leaves six days after artificial inoculation with *C. orbiculare*. In coffee plants, lesion areas were evaluated by measuring length and width of typical anthracnose lesions developed on coffee berries and leaves from 1-15 days after inoculation (Prihastuti *et al.,* 2009). In general, the first anthracnose lesion appeared on the inoculated leaf 3 days after inoculation on cucumber leaves. The brown lesions enlarged with time, up to 5 days after the appearance of initial symptom and coalesced with each other to form large diseased area on the leaves (Negishi, 2011). Symtom expression of anthracnose in noni took 8 days as reported by Manjunath *et al.,* (2010) and that of chilli was 5 days, according to Linu *et al.,* (2013).

2.2.2 Cultural and morphological characters of the pathogenic isolates

Differentiation among the *Colletotrichum* morpho-groups based on traditional methods such as conidial shape and size appeared to be reliable as the differences of conidial size, both in length and width of conidia, were statistically significant (Simmonds. 1965., Sutton, 1962, 1965, 1966, 1968, 1980 and von Arx. 1981). The identification of species of *Colletotrichum* has relied primarily on morphological differences such as colony color, size, and shape of conidia, optimal temperature, presence or absence of setae, and existence of the teleomorph (Freeman *et al.*, 1998).

Morpho-taxonomic criteria such as conidial shape and size, morphology and size of acervuli, morphology of setae, temperature response on potato dextrose agar medium (PDA) and host specificity, as well as molecular identification techniques, were currently in use for identification of *Colletotrichum* sp. (Sutton, 1992; Freeman *et al.,* 1998). Bose *et al.*, (1973), observed that the size of conidia of *Colletotrichum gloeosporides* varied from 11 µm to 16 µm x 4 µm to 6 µm and 13.8 µm x 4.8 µm with ellipsoidal shape. The conidial size varied from 10.5 µm to 15 µm x 6 µm to 7 µm with cylindrical to ellipsoidal in shape (Chowdappa *et al.,* 2012). *C. gloeosporoides* produce acervuli bearing 15 to 30 number of setose (Shilpa, 2015). The species *C. coffeanum* (Noack, 1993) and *G. coffeanum* (Delacroix. 1897) under which *Colletotrichum* from coffee was originally described are apparently both synonymous with *C. gloeosporioides*. Firman and Waller (1977), Sutton (1980), von Arx (1981) and Holiday (1989) reported that the conidial size of *C. coffeanum* ranged from 10 µm to19 µm x 4 µm to 5.5µm. Conidial size of the pathogen *C. coffeanum* was 10 µm to 20 µm x 4 µm - 5µm as reported by Prihastui *et al.,* (2009). Acervuli were medium to dark brown bearing less number of setae in case of anthracnose in coffee. Jinyoung *et al.,* (2002) reported that conidia of *C. musae* were aseptate, hyaline, mostly ellipsoidal, ranging from 10 µm to 18 µm and 4 µm to 9 µm. Thangamani *et al.,* (2011) recorded conidial size of 10 µm to 18 µm x 4 µm to 9µm and coloured acervuli in case of anthracnose in banana. The name *C. kahawae* was given to the pathogen *C. coffeanum* by Waller *et al.,* 1993 which caused infection specifically on coffee plants. Sreenivasaprasad *et al.,* (1993) showed that *C. kahawae* was very close to *C. gloeosporioides* on the basis of rDNA sequences. However this was based on a single base difference in rDNA sequences data used to discriminate related taxa. Due to limited number of informative sites identified, the underlying difference was very small. Cannon *et al.,* (2008) also showed that *C.kahwae* was closely related to *C. gloeosporioides* based on rDNA-ITS sequence analysis. In many of the earlier reports, coffee plant was exclusively indicated as the host plant of *C*

.coffeanum. Recently the pathogen has been reported to infect other host plants like gerbera (Yaling *et al.*, 2015).

Von Arx (1981) included the pathogen *C.musae* under *C. gloeosporioides* as specific to *Musa*, while Sutton (1980; 1982 and 1992) accepted this as distinct species which was supported by recent molecular work (L. Cai, pers.comm). This taxon has been reported as the major causal organism of anthracnose and also responsible for causing crown rot, blossom end rot and tip rot of banana (Nazriya *et al.*, 2007). *C. musae* has several host plants other than banana (Musa spp.). In addition to *Musa* spp., *C. musae* has been reported to be pathogenic to apple (*Malus pumila*), mango (*Mangifera indica*), avocado (*Persea americana*), guava (*Psidium guajava*) and *Vigna* sp. (Sutton and Waterston, 1970). y Recently, Mahadtanapuk *et al.*, (2007) found *C.musae* was a pathogen causing anthracnose on curcuma flowers (*Curcuma alismatifolia* Gagnep). Daundasekera *et al.*, (2008), Da Silva *et al.*, (2008) and Niroshini and Karunaratnte, (2009) also reported diversity in the host range of the pathogen *C. musae*.

Sporulation, pigmentation and colony characters of anthracnose pathogens were reported by Denobys and Baurdy, (1995) in strawberry, Kuramae *et al.*, (1997) in citrus, Schwarts *et al.*, (2007) in cucurbits and Manjunath, (2011) in noni. Potato dextrose agar, Richards' agar and Czapek's (Dox) agar were used to detect the effect of mycelial growth and sporulation of *Colletotrichum* sp. from water hyacinth (Ding *et al.*, 2007). Anand *et al.*, (2009) reported that the isolates of *Colletotrichum* produced white colonies on Richards' agar, oat meal agar and on PDA and greyish white, whitish black and black coloured colonies on Czapek's (Dox) agar, chilli leaf extract agar and malt extract agar respectively. *Colletotrichum* isolates produced cottony growth on PDA with a colour of grayish white to dark grey on ventral surface whereas reverse of the colonies were grey to black. The colonies produced pink saffron or creamy white conidial mass (Manjunath *et al.*, 2010). Earlier studies of Mc

Donald, (1926) and Rayner, (1952) in Kenya indicated that *C. coffeanum* causing coffee berry disease (CBD) producing slow growing, cottony, dark, greenish-grey colonies. Hindorf, (1970) reported that these pathogenic isolates were *C. gloeosporioides* which were referred to as *C. coffeanum* by Noack in 1903. Prihausthi *et al.,* (2009) reported that the pathogen *C. coffeanum* produced on PDA, creamy white to olivaceoue green mycelium with bright orange coloured spore mass. Thangamani *et al.,* (2011) observed that *C. musae* produced blackish white aerial mycelium with light orange coloured spore mass on PDA. The colony colour of *C. gloeosporiodes* in different solid media was studied by Hubbali *et al.,* (2011). *Colletotrichum* sp. produced white to grayish white colonies on Richards' agar and PDA as reported by (Chowdappa *et al.,* 2012).

Hiremath *et al.* (1993) and Ekbote *et al.* (1997) reported that, maximum dry weight of mycelia growth of *C. gloeosporioides* was in Richards' broth. The fungus attained maximum dry mycelial weight when it was incubated for 10 days, thereafter it showed decreased dry mycelial weight which indicated that the occurrance of autolysis. Lilly and Barnett, (1951) also reported autolysis in fungi after attaining maximum growth when cellular enzymes begin to digest the various cell constituents. Ekbote, (1994) and Hiremath *et al.* (1993) also recorded maximum mycelial dry weight of *C.gloeosporioides* in oat meal broth, potato dextrose broth and Richards' broth. Mesta, (1996) and Chidanandaswamy, (2001) reported good growth of *C.gloeosporiodes* on Richards' broth and Mello *et al.*, (2004) found that oat meal and potato dextrose media were the best for growth of *C. gloeosporioides.*

2.2.2.1. Effect of different light sources on growth of pathogenic isolates

Light showed pronounced effect on growth of fungi. Chowdhuary (1936) observed that continuous light or darkness inhibited sporulation of *Colletotrichum graminicola*, while the cultures exposed to alternate light and darkness sporulated earlier. Kamanna, (1996); Sudhakar, (2000); Alexander *et al.,* (2004); Ashoka, (2005); Narendra Kumar, (2006) and Soltani *et al.,*(2014) observed that exposure of

C. gloeosporioides to alternate light and darkness for a consecutive period of 12 h resulted maximum growth and sporulation. Bokhari *et al.,* (2013) reported that exposure of *C. musae* to UV light for a period of 45 mins inhibited the mycelial growth. Yaling *et al.,* (2015) reported that the cultures of *C. coffeanum* exposed to fluorescent light for a consecutive period of 12 h of light and darkness gave more mycelial growth and sporulation compared to other treatments.

2.3 *In vitro* evaluation of chemicals and non-chemical methods for inhibition of anthracnose pathogen.

2.3.1 a) Assay of chemicals and bio-control agents on growth inhibition of the pathogen by poison food technique.

Leinhos *et al.*, 1997 and Bartlett *et al.*, 2002 observed that when azoxystrobin was used, the inhibition of spore germination and zoospore motility, which are the developmental stages of fungi were greater than the inhibition of the fungal mycelial growth. Sundaravadana *et al.,* (2006) reported mycelial growth inhibition over the control was more than 60 per cent when three concentrations (0.05 per cent, 0.10 per cent and 1 per cent) of azoxystrobin were studied against pathogen. Sundaravadana *et al.,* (2007) studied the *in vitro* efficacy of azoxystrobin on mango leaf and panicle anthracnose and reported that azoxystrobin at 1.0, 2.0 and 4.0 ml/l showed 65.39 per cent, 68.29 per cent and 69.62 per cent mycelial inhibition respectively. Anand *et al.*, (2010) reported that azoxystrobin treatment resulted in minimum incidence of fruit anthracnose in chilli. Adhikari *et al.,* (2013) reported that azoxystrobin at (0.25, 0.50 and 1 per cent) slightly inhibited the mycelial growth of *C. gloeosporiodes* in mango.

Srivastava and Soni, (1993) reported that mancozeb (0.25 per cent) was effective fungicide for control of anthracnose under laboratory and fiel conditions. The lower dose of 100 mg mancozeb/l PDA reduced the growth of the pathogen *C. gloeosporiodes* by 49.21 per cent in cucurbits after seven days, which remained the same after fourteen days (Hussain *et al.,* 2008). He also reported that the fungicide

mancozeb which is a derivative of dithiocarbamic acid is toxic to fungi because they are metabolized to isothiocyanate radicals inside the pathogen cells, which inactivated the –SH group of aminoacids and enzymes. Shinde *et al.,* (2012) reported that mancozeb @ 300 ppm concentration showed highest inhibitory effect on *C. capsici* and recorded maximum inhibition of 100 per cent and least mycelia dry weight. Sileshi *et al.,* 2014 evaluated three synthetic fungicide at different concentration by the poisoned food technique against the bean anthracnose pathogen *C. lindemuthianum,* and observed that, there was least mycelia growth of the pathogen in medium amended with mancozeb at 250 ppm and there was no growth at all, of the mycelium in media amended with mancozeb at 500 ppm, when compared to the other two fungicides *viz.,* mancolaxyl and folpan.

Potassium silicate has direct inhibitory effect on fungal growth of *Colletotrichum* sp. (Bekker *et al.,* 2009). Soluble potassium silicate completely suppressed the mycelial growth of *C. gloeosporioides* at a concentration of 40 ml/l (Polanco *et al.,* 2014). Menzies *et al.,* (1992) also reported the 100 per cent mycelial inhibition of *Erisiphae cichoracearum* in cucumber and melon. Menzies *et al.,* (1992); Epstein *et al.,*(1999); Kaiser *et al.,*(2005) and Bekker *et al.,*(2013) observed that the complete mycelial inhibition of the anthracnose pathogen when it was amended with different concentrations of potassium silicate was due to the effect of higher pH.

The potential for biological control of *C. gloeosporioides* had been suggested as early as in 1976 by Lenne and Parbery. Several plant growth promoting fungal antagonists such as *Trichoderma* spp. (Papavizas, 1980) and rhizhobacteria such as *P. fluorescens* (Kloepper *et al.*, 1992) have been reported to be promising in controlling various plant pathogens.

Chidanandaswamy (2001) reported that *P. fluorescens* inhibited the growth rate of *C. capsici* causing leaf spot of turmeric followed by the fungal antagonists' *viz., T. harzianum* (Rifai) and *T. viride* (Pers) under *in vitro* conditions. Barathi *et al.,*

(2004) reported that *P. fluorescens* was effective against various phytopathogens. Bharathi *et al.*,(2004); Srinivas *et al.*, (2006); Muthukumar *et al.*, (2010) and Anand *et al.*, (2010) reported that *P. s fluorescens* (2 per cent) strongly inhibited the growth of *C. capsici in vitro*. *P. fluorescens* produced compounds like pseudo bactin, HCN, salicylic acid, pyrolnitrine, pyocyanine which induced systemic resistance in host plant or showed specific interference with fungal pathogens (Ongena *et al*, (1999); Velazhahan *et al.*, (1999); Dave and Dube, (2000); Gupta *et al.*, (2001); Pandey *et al.*, (2006); Hofte and Bekker, (2007); Reddy *et al.*, (2008) and Muthukumar *et al.*, (2010). Ngullie *et al.*, (2010) tested seven antagonists against *C. gloeosporioides* and observed that *P. fluorescens* exerted the maximum inhibition (67.42 per cent) of mycelial growth of *C. gloeosporioides* followed by *T. viride* and *Bacillus subtils* which inhibited mycelial growth of *C. gloeosporioides* by (63.34 per cent) and (56.86 per cent) respectively compared to the control. Significant reduction of mycelial growth of *Colletotrichum* sp. in presence of *P. fluorescens* was reported by Ramyasmruthi *et al.*, (2012).

Antagonistic activity of *Trichoderma* for controlling wide range of microbes was well studied, documented and demonstrated for more than seven decades ago (Weindling, 1934). *Trichoderma* strains have the ability to produce several lytic enzymes such as chitinases and 1, 3-β-glucanases (Horvath *et al.*, 1998).These enzymes played a key role in the lysis of cell walls of plant pathogens during the antagonistic action of *Trichoderma* sp. Jeyalakshmi and Seetharaman (1999) reported that *T. viride* reduced the mycelial growth of *Colletotrichum* sp either by growing over the pathogen, causing coiling, lysis and abnormalities on hyphae. Strains of *Trichoderma* produced antifungal metabolites which had the ability to suppress mycelial growth of the phytopathogens (Viterbo *et al.*, 2002). Padder *et al.* (2010) reported that talc based formulation of *T. viride* and *T. harzianum*, recorded 69.21 per cent and 64.20 per cent mycelia growth inhibition against *C. lindemuthianum* respectively. The anti microbial metabolites produced by *T. viride*

were effective against several plant pathogens like *C. lagenarium, C. acutatum* and *C. gloeosporioides.*

According to several studies, the different compounds like HCN, siderophore, chitinase and protease produced by *P. fluorescens* (2 per cent) were known to inhibit mycelial growth in several *Colletotrichum* sp. (Vidyasekaran *et al.,* 1997 and Viswanathan and Samiyappan, 1999). Mixing of culture filtrates of bio-agents with fungicides to control plant pathogens *viz., Colletotrichum* sp. have been reported Fan and Tian, (2001) and Yoshida *et al.*, (2001). Antagonist fungi especially *Trichoderma* spp. and the bacteria *P. fluorescens* have been widely used for the control of a number of plant pathogens (Rini and Sulochana, 2007). Talc based formulation of *T. viride* isolates were also reported to control anthracnose disease of chilli (Intana *et al.*, 2007). Volatile component of *Trichoderma* sp. suppressed the mycelial growth of *Colletotrichum capsici* as reported by Ajith *et al.,* (2010). Sivakumar *et al.,* (2000) studied the effect of *T. koningii, T. harzianum and T. viride* on *C. coccodes* and found *T. koingii* as the most effective antagonist against the pathogen. Inhibition of mycelial growth may be due to mycoparasitism and antagonism of *P. fluorescens* against *C. gloeosporioides* (Sturz *et al.,* 1998). Mandeep *et al.,* (2006) recorded that *T. viride* reduced the mycelial growth of *C. capsici* by 52.5 per cent followed by *T. virens* (38.12 per cent). Two species of *Trichoderma* were tested against *Colletotrichum* sp. Infecting cucumber, mycelial inhibition of 83.00 and 75.50 per cent were recorded by Intana *et al.,* (2007).

2.3.1.1Effect of chemical and non-chemical treatments on germination of conidia of anthracnose pathogen

Mode of action of new generation fungicide azoxystrobin was by blocking the transfer of electrons from cytochrome b to cytochrome c there by creating an energy deficiency which leads to fungal death. Evidence of this effect on fungi was observed in spore mortality and spore inhibition (Harrison and Tedford, 2002). Hsiang *et al.*, (2004) reported that azoxystrobin at 1.0-100 µg a.i/ml prevented

germination of uredospores of *P. hemerocallidis*. Conidial germination and appressorial formation in black rot pathogen of grape (*G. bidwellii*) was inhibited by azoxystrobin as reported by Hoffman and Wilcox, (2003). Archana, (2009) reported that the extent of inhibition of sporangial germination of downy mildew pathogen in grapes increased with the increase in concentration ofazoxystrobin. Complete inhibiton of the sporangial germination of *Plasmopara viticola* was recorded when spore suspension was treated with azoxystrobin from 300 ppm onwards. Ahiladevi *et al.*, (2014) reported that azoxystrobin at 0.12 per cent was very effective in reducing mycelia growth and spore germination of *U. necator* by 83.27 per cent.

Wainwright (1993) reported that the potassium silicate acted as a nutrient source and stimulated spore formation and germination of *C. gloeosporiodes*. Singh *et al.*, (1990) reported that potassium silicate acted as a nutrient source and there by increased the conidial germination of *C. gloeosporioides*. He also observed that fungicide mancozeb recorded a mean spore germination of 2.20 per cent. In a study conducted by Imtiaj *et al.*, (2005) mancozeb completely inhibited conidial germination of *C. gloeosporiodes* when spore suspension of the pathogen was treated at high concentration of 500 ppm.

Govindasamy and Balasubramanian (1989) reported the reduction in germination rate of urediospores of *P. arachidis* when spore suspension was amended with *T. viride*. Iqbal *et al.*, (1994) observed that the antagonistic fungi *T. harzianum* was effective in suppressing the spore germination of *C. falcatum*. Secretion of enzyme such as endochitinase, chitobiosidase, glucan 1,3 β galactosidase by *Trichoderma* spp. strongly inhibited spore germination during plant pathogen attack (Tronsmo and Hjeljord, 1997). Several earlier works suggested the antagonistic ability of *Trichoderma* isolates against *C. capsici* causing anthracnose in chilli Jeyalakshmi *et al.*, (1996), Gorawar, (2004) and Srinivas *et al.*, (2006). Intana *et al.*, (2007) reported that two wild strains of *T .harzianum* inhibited conidial germination of *C. gloeosporiodes* and observed that antifungal metabolites produced by *T.*

harzianum were responsible for the spore inhibition. Similar observations were also made by Jeyalakshmi *et al.,* (1998) in their study on the inhibition of spore germination of *C .capsici* by 92.32 per cent.

2.3.1.2 Observations on variation in conidial morphology

Recently, Akthar and Singh (2007); Sangdee *et al.,* (2011); Christopher *et al.,* (2013) and Masoodi *et al.,* (2013) had observed morphological variability as well as pathogenicity among *Colletotrichum* spp. causing anthracnose in chilli. Zivkovic *et al.,* 2010 reported variation in conidial morphology *viz.,* shape and size of the conidia of *C. gloeosporioides.*

2.3.1 b) Assay of biocontrol agents on growth inhibition of the pathogen by dual culture method.

Dual culture is a method that developed in the eighties to show the relationship (interactions) between the two organisms involving the stimulation or inhibition of their growth, in which one of the organisms was a bio-control agent, while the second organism was the studied fungal species (Huang and Hoes, 1976).

Michereff *et al.,* (1993) studied the *in vitro* inhibition of *Trichoderma* sp. against *C. gramnicola* in sorghum and reported that there was no interaction in paired culture, but inhibition zones were observed. Benitez *et al.* (2004) isolated chemicals like harzianic acid, tricholin and viridine, which played a vital role in antagonistic behavior of *Trichoderma* sp. *Trichoderma* produced certain anti microbial metabolites which was highly effective against a wide range of fungal plant pathogens such as, *C. lagenarium, C. acutatum, C. gloeosporioides* (Yan *et al.,* 2001 and Svetlana *et al.,* 2010). Significant reduction of mycelial growth of *Colletotrichum* sp. due to the interaction with *P. fluorescens* in dual culture was reported by Pallavi *et al.,* (2012). *P. fluorescens* was found to be effective against *C. capsici* and recorded 75.60 per cent mycelial inhibition (Suthin *et al.*, 2013). *In vitro* results of a study using dual culture technique indicated that *T. harzianum* strain IMI 392433

greatly inhibited the mycelial growth, conidial germination and elongation of germ tube of *C. capsici* (Rahman *et al.*, 2013).

2.4 Management of anthracnose leaf spot of snake gourd in green house study.

Silicon amendments proved to be effective in controlling both soil borne and aerial fungal diseases in cucumber, rice, sugarcane, turf and several other plant species (Datnoff *et al.*, 2007). Sun *et al.*, (2002) reported the reduction in incidence of anthracnose disease in cucumber caused by *C. orbiculare* by the application of silicon. Studies conducted in cucumber leaves investigating the process of infection of plants showed that resistance to infection can be acquired by the expression of protein, rich in proline together with presence of silica at the site of pathogen penetration (Kauss *et al.*, 2003). An average of 63 per cent reduction of angular leaf spot of bean by the application of potassium silicate was reported by Moraes *et al.*, (2005). Polanco *et al.*, (2014) observed that foliar application of potassium silicate resulted in satisfactory control of anthracnose in common bean, which may be due to the formation of physical barrier as a result of deposition of silicon on leaf surface or due to the osmotic effect of silicate sprayed on to the leaf. Anthracnose severity in common bean was reduced by 41 percent by foliar application of potassium silicate.

Fungicides zineb (0.2 per cent) and mancozeb (0.4 per cent) were effective against chilli anthracnose as reported by (Mallaraju and Swami, 1988). Efficacy of mancozeb (0.2 percent) spray in reducing the disease incidence in chilli (Das and Mohanthy, 1988 and Paramasivan *et al.*, 2008). Hussain *et al.*, (2011) reported that the fungicide mancozeb (0.3 per cent) was effectively reduced the anthracnose disease to 100 per cent in tomato. Foliar application of 0.4 per cent mancozeb recorded 65.50 per cent disease suppression in common bean (Amin *et al.*, 2014).

Management of plant diseases by the bacterial bio-control agent *P. fluorescens* as bacterial suspension or through different formulations have been reported many in many crops (Vidhyasekharan *et al.*, (1997) and Viswanathan and Samiyappan,

(1999). Ngullie *et al.*, (2009) evaluated the efficacy of fungal antagonists and reported that application of *T. viride* (2 per cent) resulted a disease reduction of 61.41 per cent in chilli. Ramkumar *et al.,* (2012) reported 12 per cent disease severity and 48.12 per cent disease incidence in case of turmeric anthracnose sprayed with talc based formulation of *P. fluorescens* (2 per cent). This suppression of disease was attributed either due to the impact of antifungal compounds produced by the microbe or due to its hyper parasitism over the pathogen or by induction of systemic resistance in the host plant in which the pathogen caused infection. *Trichoderma* strains significantly reduced on the anthracnose disease in chilli by an average of 69.52 - 81.39 percent (Rahman *et al.*, 2013).

Foliar application of potassium silicate caused significant increase in plant growth, leaf area index (LAI) and leaf area duration (LAD) in common bean according to Polanco *et al.*, (2013). Sole foliar application of *P. fluorescens* (2 per cent) and mancozeb (0.4 per cent) at thirty days after transplanting increased mean plant height, number of leaves and flowers per plant in case of anthracnose of chilli (Suthin *et al.,* 2013).

Growth promoting effects by *Trichoderma* have been observed in cucurbits by Chang *et al.*, (1986) and Windham *et al.,* (1986). Fungal antagonist *Trichoderma* increased average shoot length, root length, shoot weight, root weight and vigour index of crops *viz.,* sweet gourd, snake gourd, cowpea, cucumber and okra (Yeasmin, 2004). Ninteen isolates of *Trichoderma* promoted growth of cucumber up to 100 per cent and extended protection to cucumber from anthracnose disease up to 88.3 per cent by basal application of the fungal antagonist (Veronica *et al.,* 2011).

Materials and Methods

3. MATERIALS AND METHODS

The study on integrated management of anthracnose of snake gourd comprising of laboratory and green house experiments were conducted at the Department of Plant Pathology, College of Agriculture, Vellayani and Instructional Farm, College of Agriculture, Vellayani. Preliminary surveys were lso conducted in snake gourd fields of Thiruvananthapuram district during the onset of the study, in order to meet the comparitive assessment of anthracnose disease prevelant in snake gourd fields and also to obtain the typical pathogenic isolates that were to be tested in laboratary and green house experiment for the management of the diseas as described below:

3.1 ISOLATION OF PATHOGEN FROM DISEASED LEAVES OF SNAKE GOURD PLANTS SHOWING SYMPTOMS OF ANTHRACNOSE CAUSED BY *Colletotrichum* sp.

Leaf samples showing typical symptoms of anthracnose of snake gourd plants were collected from different locations in and around College of Agriculture Vellayani, Thiruvananthapuram district, to isolate the the pathogen *Colletotrichum* sp. For the collection of leaf samples of the disease, surveys was conducted in snake gourd fields of different locations during which, in addition to observation of the symptom development, assessment of incidence and severity of anthracnose disease affecting foliage of the crop were also made.

3.1.1 Symptom development, incidence and severity of anthracnose of snake gourd plants in different field locations.

Surveys were conducted in Thiruvananthapuram district (8.36° N latitude and 77.01°E longitude) during October 2014. Snake gourd fields of five different locations, where the crop was cultivated on large scale, were selected for the survey.

3.1.1.1 Symptom development of inoculated leaf.

Symptom development of anthracnose disease that was prevalent mainly on foliage of snake gourd plants were observed and recorded during the survey. Laboratory studies were conducted with the leaf samples of the disease, collected from the snake gourd fields, in order to record the nature and development of symptoms, starting from initial stage of infection upto maturity of the disease.

Symptom development, of anthracnose disease was studied in each of selected location consisting of more than two hundred snake gourd plants in the actively growing stage, by selecting 10 plants at random which were pre-tagged in order to collect 5 leaf samples of the disease from each plant, at ten days interval. Nature of symptom development was studied from these leaf samples collected from the pre-tagged plants, starting from the onset of the anthracnose symptoms and continued at 10 days interval, up to the final stage of the disease (Amin *et al.*, 2014).

3.1.1.1.1 Incidence and severity of anthracnose disease in snake gourd plants of different locations.

Disease incidence (DI) is the proportion or percentage of infected plant units in the field (Agrios, 2005). Incidence of anthracnose of snake gourd plants in each of the selected fields was assessed during the survey, by observing infection of the disease in a random sample of 50 plants.

The disease incidence (DI) was recorded according to James, (1974) and Abdel- kader *et al.*, (2012) as follows:-

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

Percentage disease index (PDI) /severity denotes the percentage of relevant host tissue (or) organ covered by symptom (or) lesion, damaged by the disease and it depends on number and size of lesion present on infected part.

Disease index was calculated based on the score chart proposed by (Goncalves *et al.,* 1997) Table 1; Plate 1 as follows;

Disease index (severity) was calculated according to disease index formula (Mc kinney, 1923)

$$\text{Disease Severity /Index} = \frac{\text{Sum of all ratings}}{\text{Total number of leaves examined}} \times \frac{100}{\text{maximum disease grade}}$$

3.1.2 Isolation of pathogen

The pathogen was isolated from the leaf samples collected from different surveyd locations, on potato dextrose agar (PDA) medium (Rangaswami, 1958). Leaves showing characteristic symptoms of anthracnose were cut into small pieces of 1.0 cm to 1.5 cm, surface sterilized with 0.1per cent mercuric chloride for 1 min and washed in sterile distilled water. The surface sterilized leaf bits were transferred to Petri plates containing PDA medium which was amended with streptomycin sulphate. The plates were incubated at 28 ± 2°C for seven days and observed for the fungal growth (Hubbali *et al.*, 2011). Fungal colonies exhibiting typical characters of the anthracnose leaf spot pathogen, that were consistently obtained during the isolation from the disease specimens of anthracnose leaf spot, were transferred to PDA slants and stored at room temperature (28 ± 2°C) for conducting subsequent studies.

3.2 Studies on pathogenicity

Pathogenicity tests of the isolates obtained and maintained in 3.1.2 were conducted for proving Koch's postulates, as follows

a) Artificial inoculation on detatched leaf

Table 1. Score chart used for assessing anthracnose of snake gourd (Goncalves *et al.*, 1997).

Disease scale	Per cent leaf area affected
0	0%
1	1-10 %
3	11-15 %
5	16-25%
7	26-50
9	More than 51 %

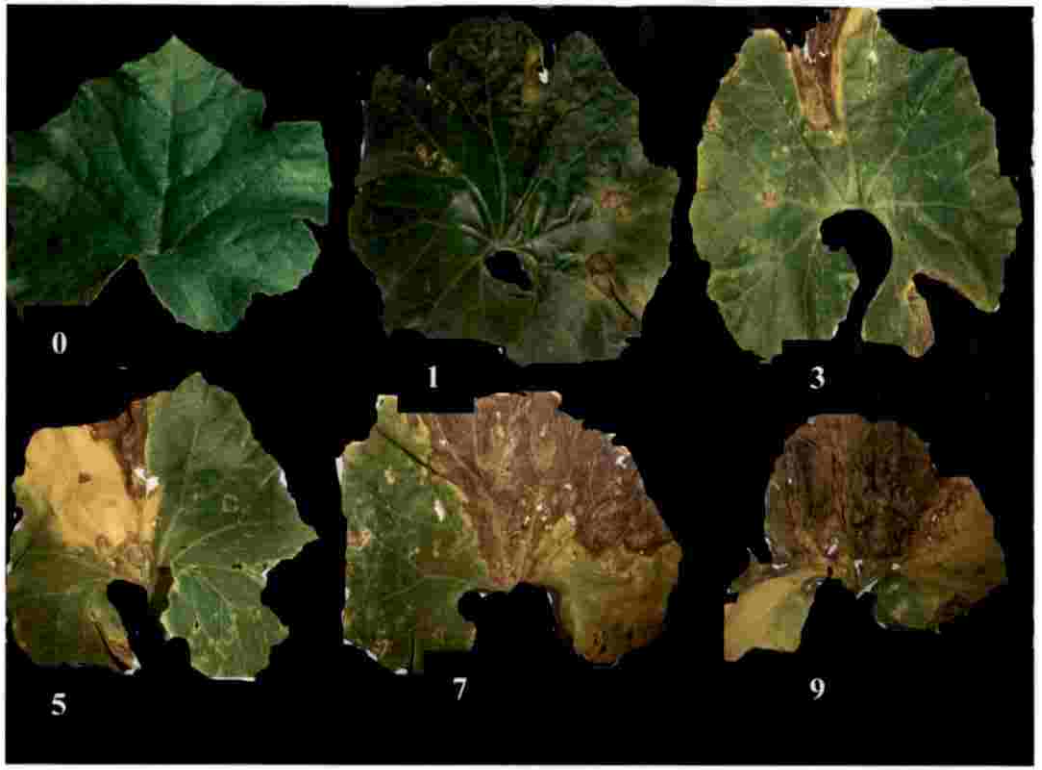


Plate 1. Score chart of anthracnose of snake gourd

b) Artificial inoculation of intact leaves of thirty days old potted plants of *Trichosanthes cucumerina* L. (cultivar- Kaumudi)

3.2.1 a) On detached leaf

Pathogenicity tests were conducted on healthy and detached snake gourd leaves (cultivar- Kaumudi) using small discs of mycelium which were transferred from pure colonies of the isolated and identified fungal pathogen obtained from diseased leaf samples. The pathogen was inoculated after wounding the leaf with pin pricks on adaxial side. The control was maintained by inoculating leaves of healthy plants with plain agar discs devoid of inoculum. The inoculated leaves were kept in moist chamber under laboratory conditions for seven days and observations on the development of disease symptoms were taken 24 h after inoculation of pathogen. After typical symptoms of the disease were expressed on the inoculated leaves, the pathogen was re-isolated and the morphological characters were compared with those of the respective isolate. Lesion size of each isolate was estimated and was expressed in cm^2

b) On leaves of potted plants of Trichosanthes (cultivar - Kaumudi)

Pathogenicity tests were also conducted on thirty days old potted plants of snake gourd (cultivar- Kaumudi). Seedlings of snake gourd were transplanted from nursery in UV stabilized 600 gauge, 150 micron grow bag (40 cm x 24cm x 24 cm) which were filled with potting mixture comprising of river sand, farm yard manure and soil in the proportion of 1: 1: 1. After thirty days of growth, five snake gourd plants with seven to eight leaves, were artificially inoculated by pin prick method on adaxial side with mycelial culture of each isolate obtained from different locations. Five plants inoculated without the pathogen were maintained as control. Second and third fully opened leaves from bottom were selected for inoculating on the upper (adaxial) side of leaf after wounding. A thin layer of moist cotton was placed over the

wounded area. Humidity was maintained over 90 per cent for first 5 days by spraying water.

After typical symptoms of the disease were expressed on the inoculated leaves, the pathogen was re-isolated and the morphological characters were compared with those of the respective isolate. Size of the lesions that appeared on each inoculated leaf was estimated by measuring the area which was expressed in cm^2. Virulence rating was also assessed by a scale formulated visually on the basis of the lesion size observed.

Scale for assessing virulence rating.

+: 1-2 cm^2

++: 2.1-4 cm^2

+++: 4.1-6 cm^2

++++: 6.1-8 cm^2

+++++: 8.1-10 cm^2

3.2.2 Observation of symptom development in pathogenicity test

Each inoculated leaf of potted plant of snake gourd (cultivar - Kaumudi) was observed for appearance of symptoms from 24 h after inoculation and continued up to seven days. Nature and development of symptoms of inoculated leaves were carefully studied and classified into different stages.

3.2.3 Estimation of time taken for initial symptom development (incubation period (IP) and disease development period (DDP)

The Incubation period (IP- time calculated as days between inoculation and the appearance of initial symptoms) and the disease development period (DDP- period calculated as days between inoculation and appearance of mature lesions) were observed and recorded according to TeBeest, (1977). Virulence rating was calculated

based on the size of lesion formed on leaves as indicated in 3.2.1 (b). The most virulent isolate of the pathogen was screened based on the shortest IP and DDP and virulence rating recorded in this study

3.2.4 Cultural and Morphological characters of the different isolates

A comparative study of the cultural and morphological characters of the different isolates obtained in 3.1.2 was conducted in detail as follows.

3.2.4.1 Cultural characters

Cultural characters of fungal growth on PDA were recorded under 40 x objective magnification seven days after inoculation, by observing colony colour (both upper and reverse), diameter and texture of mycelial colony, number of days taken for spore mass production, spore mass colour and the time taken for fungal colony of each isolate to completely cover the surface of agar medium in the Petri plate (9 cm dia.).

3.2.4.2 Growth of pathogenic isolates in different natural, semi synthetic and synthetic media

Different natural, semi synthetic and synthetic media on solid state *viz.* potato dextrose agar, carrot agar, oat meal agar, Richards' agar, Czapek's (Dox) agar and Sabouraud's agar (Ainsworth 1961 and Naik, 1985) were screened for assessing the growth of five pathogenic isolates on the above media. Observations on colony diameter, colony colour and texture, were recorded in each medium seven days after inoculation. The media producing maximum growth of each isolate were screened in the experiment.

The broth of the respective media tested above *viz.*, potato dextrose broth, carrot broth, oat meal broth, Richards' broth, Czapek's (Dox) broth and Sabouraud's broth poured were 500 ml conical flask and pathogenic isolates were inoculated into the respective media. After the mycelium attained full growth on the surface of broth,

the broth culture was filtered through Whatman No.4 filter paper and dried up to constant weight in hot air oven at 50°C. Number of day taken for the completion of mycelial growth and dry weight of mycelial mat obtained from each isolate were recorded (Amarjith and Chander, 2006). The broth resulting in maximum dry weight of mycelial mat was screened in the above experiment.

3.2.4.3. Effect of different sources of light on growth of pathogenic isolates

The effects of light sources on the growth of pathogenic isolates were studied by exposing the inoculated cultures to alternate cycles of 12 h of fluorescent light and 12 h of darkness (185 nm - 253.7nm), 12 h of fluorescent light (185 nm- 253.7 nm), 12 h of light emitting diode light (LED -365 nm) and 45 min of ultra violet light (U.V - 10 nm-380 nm) at room temperature (25 ± 2°C). Control was maintained by exposure of pathogenic cultures to normal day light and darkness.

Mycelial disc (5 mm dia.) of each isolate was transferred to natural, semi-synthetic and synthetic agar media screened under 3.2.4.2. Three replications were maintained for each treatment and the inoculated plates were incubated at 27 ± 2° C in the laboratory where the different light sources were provided for required period of exposure. Mycelial growth was recorded by measuring colony diameter expressed in seven days after inoculation (Jalal *et al.*, 2014).

3.2.4.4 Morphological characters

The pathogenic isolates grown on PDA were examined under 40 x magnifications for observing and recording the morphological characters of the culture like colour, width of hyphae and septations if any. The culture was also observed for presence of acervuli and spores which if present were examined for recording their size and shape, presence of acervulus, number of setae per acervuli and size and shape of spores.

The isolates obtained from surveyed locations were tentatively identified based on morphological and cultural characters observed in the above study. The identities

of the isolates were further confirmed by the morphological characterization undertaken at National Fungal Collection Culture India (NFCCI), Pune.

3.3 *In vitro* evaluation of chemicals and biocontrol agents for inhibition of anthracnose pathogen

Different chemicals and bio control agents were evaluated for their efficacies in inhibiting the growth of the most virulent isolate of the anthracnose pathogen screened in 3.2.3

The selected chemicals (two fungicides *viz.* azoxystrobin- 23% SC and mancozeb, 75 % WP and the nutrient potassium silicate 32 % EC) and two KAU talc based formulation (chemically magnesium silicate) of bio-control agents (fungal antagonist *T. viride* (2.8×10^6 cfu/ g) and bacterial antagonist *P. fluorescens* (9×10^9 cfu/ml) were evaluated in the laboratory of Department of Plant Pathology, College of Agriculture, Vellayani by poison food technique and dual culture technique as follows;

3.3.1 a) Assay of chemicals and biocontrol agents on inhibition of growth of the pathogen by poisoned food technique.

The nutrient potassium silicate, KAU talc based formulations of bio-control agents *T. viride*, *P. fluorescens* and the fungicides azoxystrobin and mancozeb were evaluated for inhibition of mycelial growth of the most virulent isolate of pathogen by following the poisoned food technique (Nene and Thapliyal, 1993) as described below:

Details of laboratory experiment

Design : CRD

Replications : 3

Treatments : 7

The efficacy of each fungicide, bio-control agent and nutrient was tested at recommended concentrations. Concentration of chemicals and bio-control agents evaluated in the poison food technique, are presented in (Table 2).

Molten potato dextrose agar (PDA) was separately mixed with the respective concentration of fungicide *viz.,* mancozeb (0.4 per cent) and azoxystrobin (0.1 per cent), chemical nutrient *viz.,* potassium silicate (0.5 per cent), combination of azoxystrobin (0.1 per cent) + KAU talc based formulation of *P. fluorescens* (2 per cent), and KAU talc based formulations of bio-control agents *viz., T. viride* (2 per cent) and *P. fluorescens* (2 per cent), poured into sterilized Petri plates and allowed to solidify. The most virulent isolate of anthracnose pathogen was cultured on PDA contained in Petri plate. A disc of 5 mm diameter was transferred from the culture and placed aseptically at centre of each Petri plate (9 cm.dia) amended with test nutrient, fungicides and bio-control agents and incubated at(25 ± 2°C) for ten days. Culture discs grown under same conditions on PDA without any any amendment were maintained as control. Mycelial growth of the pathogen in each inoculated plate was recorded by measuring the colony diameter.

Per cent inhibition of the pathogen over control was calculated by using the formula (Vincent, 1927)

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

where,

I-Per cent growth of inhibition

C - Mycelial growth of the pathogen in control (colony diameter measured in mm)

Table 2. The treatments and their concentrations tested in poisoned food technique

Treatment	Name	Trade name	Field dose (%)	Nature of fungicide
T1	Azoxystrobin	Amistar	0.1%	Systemic
T2	KAU talc based formulation of *T.viride*	-	2.0%	Bio-control agent
T3	KAU talc based formulation of *P. fluorescens*	-	2.0%	Bio-control agent
T4	Azoxystrobin + KAU talc based formulation of *P. fluorescens*	Amistar + Bio agent	0.1%+2.0%	Combination
T5	Potassium silicate	Greensil plus	0.5%	Foliar nutrient
T6	Mancozeb	Indofil M-45	0.4%	Contact

T- Mycelial growth of the pathogen in treatment (colony diameter measured in mm)

3.3.1.1 Observations on variation in conidial morphology

After conducting the *in vitro* evaluation (3.3.1) of fungicides, nutrient and bio-control agents on mycelial growth, variations in morphology of conidia produced in the above experiment were observed. A strand of aged mycelium transferred from the Petri plate cultures containing each amended treatment was placed on a glass slide and observed under the microscope (40 x). The shape and size of conidia obtained from each treatment were compared with those transferred from control.

3.3.2 Effect of chemical and non-chemical treatments on germination of conidia of anthracnose pathogen

A separate experiment was conducted for estimating inhibition of conidial germination of the most virulent isolate (C1) of the anthracnose pathogen by the different chemical and non-chemical treatments tested in 3.3.1

Conidial suspension of the virulent isolate of the pathogen was prepared separately by incorporating the tested concentrations of the nutrient, fungicide and bio-agents *viz.,* azoxystrobin (0.1 per cent), KAU talc based formulation of *T. viride,* (2 per cent), KAU talc based formulation of *P. fluorescens* (two per cent), azoxystrobin (0.1 per cent) + KAU talc based formulation of *P. fluorescens* (2 per cent), potassium silicate (0.5 per cent) and mancozeb (0.4 per cent). Conidial suspensions of most virulent isolate (C1) of the pathogen were prepared and incorporated separately with the different treatments. One µl from each of the treatment amended suspensions was transferred at five minutes interval to separate cavity slides and incubated for 24 h in moisture chamber at (25 ± 2°C). A drop of lactophenol cotton blue was then placed over conidial suspension on each of the cavity slides. The slides were observed under the microscope (40 x) for recording the percentage inhibition of conidial germination of the pathogen (Imtiaj *et al.*, 2005).

3.3.1 b) Assay of bio control agents on inhibition of growth of the pathogen by dual culture method (Huang and Hoes, 1976)

Design : CRD

Replications : 3

Treatments : 2

The fungal and bacterial antagonists *viz., T. viride and P. fluorescens* respectively were tested for inhibitory action against anthracnose leaf spot pathogen by the dual culture technique (Mishra, 2010) using the cultures of antagonists obtained from Department of Microbiology, College of Agriculture ,Vellayani.

For evaluating the efficacy of the fungal antagonist a mycelial disc of *T. viride* (5 mm dia) was placed at a distance of 2.5 cm away from periphery of the Petri plate containing PDA. An agar disc of the pathogen of the similar size (5 mm dia.) was placed opposite to this and 2.5 cm away from the periphery of the plate. The plates were incubated at (25 ± 2°C) and mycelial growth of the pathogen in each inoculated plate was recorded by measuring the colony diameter.

Antagonistic activity of *P. fluorescens* was evaluated by streaking a 24 hold culture of the bacterial bio control agent as a 4 cm line (2.5 cm away from edge of the plate) on Petri plate containing PDA medium. A 5 mm mycelial disc of the pathogen was placed opposite to the streak at the distal point (2.5 cm away from the edge) of dish, perpendicular to the bacterial streak (Georgakopoulos *et al.* 2002). Plates were incubated at room temperature (25 ± 2°C) and mycelial growth of the pathogen in each inoculated plate was recorded by measuring the colony diameter which was expressed in mm.

In the above two experiments, a 5 mm agar disc of the pathogen placed at one end at a distance of the 2.5 cm from periphery of Petri plate without the inoculation of bio-control agents, served as control.

Observation on the extent of mycelial growth of the most virulent isolate of the pathogen used in the experiment, was recorded 24 h after inoculation and continued up to the period when the fungal growth completely covered the Petri dish in the control (Petri plate containing PDA without any amendment). The diameter of the mycelial growth of the virulent pathogen was measured in each Petri plate containing the respective treatments.

Per cent inhibition of the pathogen over control was calculated according to the following formula (Vincent, 1927)

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

where,

I- Per cent growth of inhibition

C - Mycelial growth of the pathogen in control (colony diameter measured in mm)

T - Mycelial growth of the pathogen in treatment (colony diameter measured in mm).

3.4 Management of anthracnose leaf spot of snake gourd in green house study

A green house study was conducted at Instructional Farm, College of Agriculture, Vellayani to evaluate the efficacy of three most effective treatments that were screened in the *in vitro* study (3.3) for reducing the anthracnose disease. The virulent isolate of the anthracnose pathogen was artificially inoculated on adaxial side of third, fourth, fifth, sixth and seventh leaf from bottom of the plant for inducing the leaf spot symptom of the disease. Disease incidence, disease severity / percentage disease index (PDI) and biometric observation such as root length, shoot length and number of leaves of snake gourd plants in different treatments were recorded. The experiment was conducted in green house as follows;

Cultivar : Kaumudi

Location : Instructional Farm, College of Agriculture Vellayani

Design : CRD

Replications : 5

Treatments : 5

T_1, T2 and T_3 : Three most effective treatments screened in the *in vitro* study.

T_4 : Foliar spray of mancozeb, 0.4 per cent (treated check)

T_5 : Absolute control

Seeds of snake gourd cultivar Kaumudi were sown in pro-trays filled with potting mixture consisting of vermi-compost and coir pith in 1:1 ratio. The snake gourd seedlings were transplanted to UV stabilized 600 gauge 150 micron grow bags of size 40 X 24 X 24 cm^3 which were filled with potting mixture (sand, soil and cowdung in 1:1:1 ratio), at the time of emergence of first two leaves. Each growbas contained two plants and three replications were maintained for each treatment. Virulent isolate of anthracnose pathogen was artificially inoculated after wounding the leaf with pin pricks on adaxial side of third, fourth, fifth, sixth and seventh leaf.

The effective treatments screened in the *in vitro* study along with treated check were applied after the initial appearance of symptoms on the foliage after artificial inoculation of the pathogen. Two sprays were given at an interval of fifteen days. The following observations were recorded during the course of experiment

3.8.4 Disease Incidence

Observations on incidence of anthracnose disease were recorded from twenty four hours after inoculation up to a period of seven days. Disease incidence was calculated according to formula of Agrios (2005) and Hossain *et al.*, (2010).

$$\text{Disease Incidence} = \frac{\text{Number of infected leaves}}{\text{Total number of leaves observed}} \times 100$$

3.8.5 Percent Disease Index Disease severity

Symptoms of the disease were scored based on 0-9 scale developed by Goncalves *et al.*, (1997) and disease severity was calculated using the score ratings, based on formula of Mc kinney, (1923) as follows:

$$\text{Disease Severity} = \frac{\text{Sum of all ratings}}{\text{Total number of leaves}} \times \frac{100}{\text{maximum rating}}$$

Biometric observations of root length, shoots length and number of leaves was also recorded after thirty days after the treatment applications. Datas were statistically analysed in completely randomised design (CRD).

Results

4. RESULTS

4.1 ISOLATION OF PATHOGEN FROM DISEASED LEAVES OF SNAKE GOURD PLANTS SHOWING SYMPTOMS OF ANTHRACNOSE CAUSED BY *Colletotrichum* sp.

Survey were conducted in snake gourd fields of Thiruvananthapuram district (8.36° N latitude and 77.01° E longitude) *viz.,* in Instructional Farm, Department of Olericulture, College of Agriculture, Vellayani, and at Kalliyoor, Kakkamoola and Palapoor during October 2014 where snake gourd was cultivated on a large scale.

4.1.1 Symptom development, incidence and severity of anthracnose of snake gourd plants in snake gourd fields of different locations.

Nature of symptom development, incidence and severity of anthracnose disease were recorded from the plants in the snake gourd fields consisting of more than 200 plants selected in five different locations of Thiruvananthapuram district. Four surveys were conducted at ten days interval in each location for studying the nature of symptom development and assessment of incidence and severity of the anthracnose disease that was prevalent in the snake gourd fields.

4.1.1.1 Symptom development of anthracnose leaf spot

In the selected snake gourd fields 10 plants were pre-tagged at random in order to collect 5 leaf samples of the anthracnose disease from each plant at ten days interval. Symptoms of leaf samples from these tagged plants, during each survey, were classified into different stages starting from the initial symptom up to the maturity of the disease. Initial symptoms of the disease were recorded on immature green leaves approximately fourth to fifth leaf from the top of each plant.

Symptoms on the foliage initially appeared as small water soaked brown lesions which were transformed into necrotic areas surrounded by yellow halo.

Gradually, the lesion enlarged in size and covered the leaf surface. As necrosis progressed the lesion exhibited concentric zonations, gradually enlarged and covered the entire foliage within a period of seven days. Finally the infected leaves shriveled, wrinkled and dried up after nine to ten days. During the final stage of survey severe defoliation of many plants were observed (Plate 2-4).

Disease incidence (DI) and severity of anthracnose disease affecting snake gourd plants were assessed in each location during the survey from a random sample of fifty plants. The cultivar Kaumudi was cultivated in all the locations surveyed. Results of the study indicated that among the different locations surveyed, maximum DI (90.00 per cent) was observed in snake gourd field of Instructional Farm, College of Agriculture, Vellayani, which was followed by the disease incidence of snake gourd field of Department of Olericulture, College of Agriculture, Vellayani (84.00 per cent). Snake gourd fields at Kalliyoor and Kakkamoola recorded a disease incidence of 80.00 per cent and 82.00 per cent respectively and were comparable. Lowest DI (70.00 per cent) was recorded in snake gourd field surveyed in Palapoor.

Disease severity of snake gourd plants in the field was calculated by observing the symptoms on leaves collected randomly from fifty snake gourd plants of each location during every survey.

Maximum percentage disease index (PDI) / disease severity (44.22) was observed in snake gourd field of Instructional Farm, College of Agriculture, Vellayani, which was followed PDI of snake gourd field of Department of Olericulture, College of Agriculture, Vellayani (40.66). Plants surveyed in snake gourd fields of Kalliyoor and Kakkamoola recorded PDI of 37.33 and 30.22 respectively which were comparable. Lowest PDI (21.89) was recorded in snake gourd field of Palapoor. (Table 3; Fig-1)

Initial symptoms of brown lesion with yellow

Yellowing and necrosis as symptom advanced

Entire leaf started to dried up at severe stages

Plate 2. Symptoms of anthracnose of snake gourd in field

Location I, II, III and IV	
Stages of symptom development	**Symptoms**
Symptoms started from the lower leaf of the plants. Initial symptom appeared as small greenish yellow to pale brown surrounded by a yellow halo.	
In the next stage of symptom development, the lesion turned dark brown and began to expand and enlarge length wise	
As the disease progressed, there was coalesion of several lesions formed on the leaf. Brown lesions covered on entire leaf and which became chlorotic and the leaf started to dry.	
In the final stage of disease development, the entire leaf turned brown, shriveled and dried up.	

Plate 3. Symptom development of anthracnose in snake gourd fields of location I, II, III and V.

Location IV	
Stages of symptom development	**Symptoms**
Symptoms started from the lower leaf of the plants. Initial symptom appeared as small pale brown lesion surrounded by an yellow halo.	
In the next stage of symptom development, colour of lesion turned to dark brown and it expanded and enlarged length wise.	
The brown lesion enlarged accompanied by colour change in the form of concentric zonations	
As the disease progressed, there was coalesion of several lesions covered the entire leaf which became chlorotic and the leaf started to dry	
In the final stage of disease development, the entire leaf turned brown, shriveled and dried up	

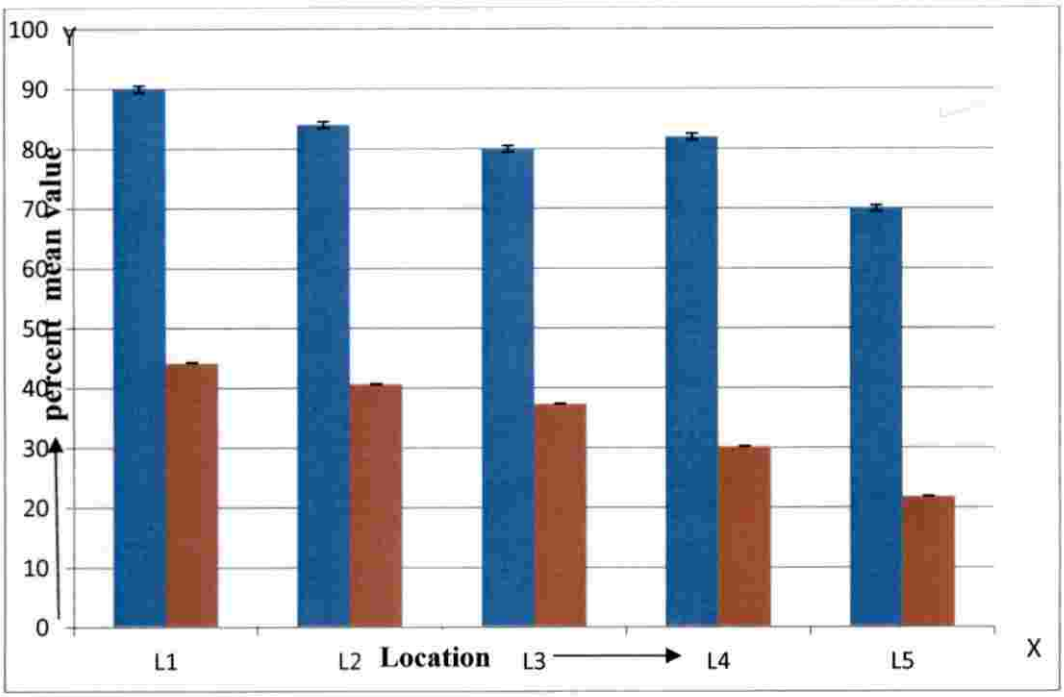
Plate 4. Symptom development of anthracnose of snake gourd in location I

Table 3. Mean Disease Incidence and disease severity of infected leaves in snake gourd fields of different locations in Thiruvananthapuram district.

Sl.no	Locations	Mean Disease incidence (%)*	Mean Percentage Disease Index /Severity (%)*
1	Snake gourd field of Instructional Farm - Vellayani	90.00 ± 0.57 [a]	44.22 ± 0.08[a]
2	Snake gourd field of Department of Olericulture	84.00 ± 0.52 [b]	40.66± 0.08 [b]
3	Snake gourd field of Kalliyoor	80.00 ± 0.54 [d]	37.33± 0.05 [c]
4	Snake gourd field of Kakkamoola	82.00 ± 0.54[c]	30.22 ± 0.05[d]
5	Snake gourd field of Palapoor	70.00 ± 0.52 [e]	21.89± 0.02[e]
	CD (0.05)	0.762	0.011

*Mean of five replications

Figures followed by same letter do not differ significantly according to one way ANOVA at P= 0.05



Blue- DI

Red- PDI

L1- IF-Vellayani

L2- Department of Olericulture

L3- Kalliyoor

L4- Kakkamoola

L5- Palapoor

Fig. 1. Incidence and severity of anthracnose of snake gourd in different locations

4.1.2 Isolation of pathogen

The pathogen associated with anthracnose leaf spot of snake gourd was isolated from infected leaves of the crop for conducting subsequent studies on integrated management of the disease.

Five fungal isolates with similar cultural characters were consistently obtained from leaf samples collected from the various surveyed locations, as indicated in Table 4. The isolates produced grey to olivaceous green mycelial growth on PDA and on examining the older regions of mycelia growth, straight, cylindrical and hyaline conidia were observed. The isolates were tentatively identified as *Colletotrichum* sp on the basis of the morphological characters studied and were designated as C1, C2, C3, C4 and C5.

4.2 STUDIES ON PATHOGENICITY

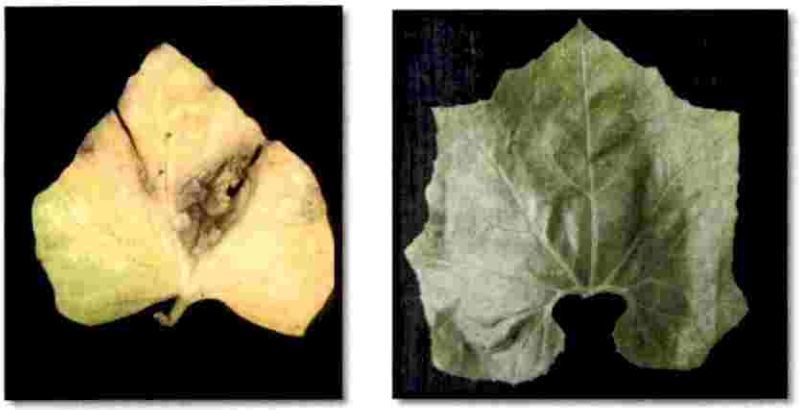
The pathogenicity of the five isolates of *Colletotrichum* sp. obtained in 4.1.2 were confirmed by artificial inoculation on both detached leaf as well as on intact leaves of 30 days-old-potted plants of *T. cucumerina* L. (cultivar- Kaumudi).

4.2.1 On detached leaf

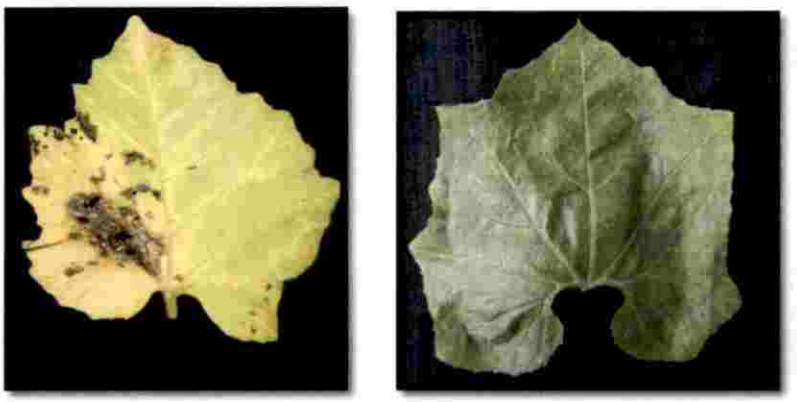
Among the five isolate tested by the detatched leaf technique, the isolate C1 (Instructional Farm-Vellayani) recorded the largest lesion size (2.53 cm^2) which was significantly larger compared to lesion size produced by all other isolates. Symptoms appeared as small brown lesion which gradually enlarged and covered the entire foliage. Nature of symptoms that developed after inoculation with different isolates was almost similar (Plate 5-6). Lesion size of the isolate C2 (2.03 cm^2) was significantly higher than those produced by the isolates C3 (1.83 cm^2) and C5 (1.56 cm^2). The isolate C4 recorded the minimum lesion size of (1.33 cm^2). Re-isolation of the pathogen from symptoms produced by artificial inoculation with each isolate, resulted in fungal cultures which similar in morphological characters of the respective original isolates (Table 5).

Table 4. Details of surveyed locations and fungal isolates obtained.

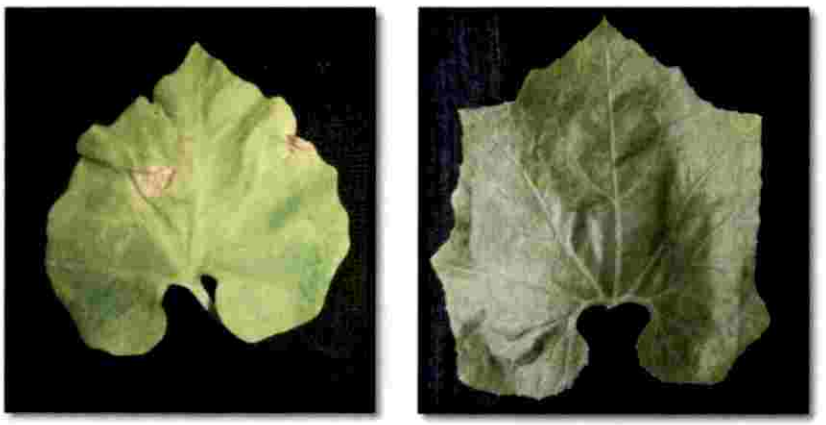
Designated isolate	Locations	Acerage	Cultivar used	Crops surrounding the locations
C1	Snake gourd field of Instructional Farm - Vellayani	25 cents	Kaumudi	Chilli, cowpea, bitter gourd
C2	Snake gourd field of Department of Olericulture- Vellayani	25 cents	Kaumudi	Chilli, cowpea
C3	Snake gourd field of Kalliyoor	25 cents	Kaumudi	Chili, bitter gourd
C4	Snake gourd field of Kakkamoola	25 cents	Kaumudi	Chilli, banana
C5	Snake gourd field of Palapoor	10 cents	Kaumudi	Nil



a) Inoculated Control



b) Inoculated Control



c) Inoculated Control

a) Location- IF- Vellayani
b) Location- Department of Olericulture
c) Location- Kalliyoor

Plate 5. Symptom development of anthracnose of anthracnose in pathogenicity tests with isolates C1, C2 and C3 of *Colletotrichum* sp. on detatched leaf.

d) Inoculated

Control

e) Inoculated

Control

d) Location- Kakkamoola
e) Location- Palapoor

Plate 6. Symptom development of anthracnose in pathogenicity tests with isolates C4 and C5 of *Colletotrichum* sp. on detatched leaf.

Table 5. Details of pathogenicity test of different isolates of *Colletotrichum* sp. on detached leaf.

Isolate	Locations	Pathogenicity	Time taken for onset of symptom	Mean Lesion size recorded at advanced stages of infection* (cm^2)
C1	Snake gourd field of Instructional Farm - Vellayani	Pathogenic	5	$2.53^a \pm 0.173$
C2	Snake gourd field of Department of Olericulture- Vellayani	Pathogenic	6	$2.03^b \pm 0.111$
C3	Snake gourd field of Kalliyoor	Pathogenic	6	$1.83^c \pm 0.173$
C4	Snake gourd field of Kakkamoola	Pathogenic	8	$1.33^e \pm 0.152$
C5	Snake gourd field of Palapoor	Pathogenic	6	$1.56^d \pm 0.057$
CD(0.05)				0.100

*Mean of three replications

Figures followed by same letter do not differ significantly according to one way ANOVA at P= 0.05

b) On potted plants of T. cucumerina (cultivar- Kaumudi)

In the pathogenicity tests conducted on leaves of 30-days old potted plants of *T. cucumerina* (cultivar- Kaumudi), maximum lesion size (10.06 cm^2) was recorded in leaves inoculated with the isolate C1 (IF-Vellayni) which was significantly greater than lesion size produced by remaining isolates. This was followed by isolate C2 (7.63 cm^2) which produced lesions which were significantly larger than those produced by C5 (2.83 cm^2) and C3 (2.06 cm^2) and C4 (1.53 cm^2) respectively. Minimum lesion size (1.53 cm^2) was recorded in leaves inoculated with isolate C4 (Table 6). The virulence rating of each isolates was also proportional to the lesion size produced.

4.2.2 Symptom development in pathogenicity test

Description of different stages of development which were observed in the inoculated plants, are presented in (Plate 7-11). Symptoms were classified into four stages. No symptoms were observed in plants inoculated with plain agar that did not contain any fungal inoculum. Characteristic stages of symptom development are represented in Table 7.

4.2.3 Incubation period (IP) and Disease development period (DDP) of anthracnose pathogen

Incubation period (IP) is defined as time measured in days between inoculation and the appearance of initial symptom (Cannon *et al.*, 2008). In pathogenicity tests conducted on potted plants of cultivar - Kaumudi, using mycelial fragments of the different isolates obtained, the incubation period recorded ranged from 5 to 8 days. Among the five isolates, an incubation period of 5 days was observed for the isolate C1, with the initial appearance of brown spot after inoculating the fungal isolate. An incubation period of 6 days was recorded for the isolates C2, C3, C5 and C4 had an incubation period of 8 days.

Table 6. Details of pathogenicity tests of different isolates of *Colletotrichum* sp. on potted plants of *Trichosanthes cucumerina* L. (cultivar- Kaumudi).

Isolate	Locations	Pathogenicity	Virulence rating*	Mean Lesion size (cm)*
C1	Snake gourd field of Instructional Farm - Vellayani	Pathogenic	+++++	10.06^{a} ±0.152
C2	Snake gourd field of Department of Olericulture- Vellayani	Pathogenic	++++	7.63^{b} ±0.057
C3	Snake gourd field of Kalliyoor	Pathogenic	++	2.06^{d} ± 0.057
C4	Snake gourd field of Kakkamoola	Pathogenic	+	1.53^{e} ± 0.115
C5	Snake gourd field of Palapoor	Pathogenic	++	2.83^{c} ± 0.115
CD(0.05)				0.157

* Mean of three replications

+: 1cm^2-2 cm^2

++: 2.1cm^2-4 cm^2

+++: 4.1cm^2-6cm^2

++++:6.1cm^2-8 cm^2

+++++: 8.1cm^2-10 cm^2

Figures followed by same letter do not differ significantly according to one way ANOVA at P= 0.05

Inoculated

Control

Stage	Description	Symptom
1	Appearance of small brown spot with yellow halo 5 days after inoculation	
2	Lesion expanded on leaf surface, surrounded by an yellow halo 9 days after inoculation	
3	Lesion became necrotic 13 days after inoculation	
4	Lesion expanded over the entire leaf which became wrinkled and deformed 16 days after inoculation	

Plate 7. Symptom development in pathogenicity test of isolate C1 (Instructional Farm –Vellayani) on potted plants.

Inoculated

Control

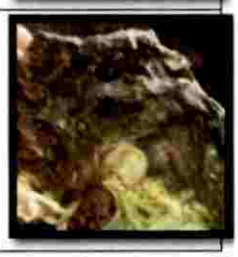
Stage	Description	Symptom
1	Appearance of brown spot with yellow halo 7 days after inoculation	
2	Lesion enlarged and expanded surrounded by yellow halo ii days after inoculation	
3	Lesion became necrotic 14 days after inoculation	
4	As necrosis expanded leaf became wrinkled and deformed 17 days after inoculation	

Plate 8. Symptom development in pathogenicity test of isolate C2 (Department of Olericulture -Vellayani) on potted plants.

Inoculated

Control

Stage	Description	Symptom
1	Appearance of brown spot with yellow halo 7 days after inoculation	
2	Lesion enlarged and expanded with yellow halo 11 days after inoculation	
3	Lesion become necrotic 14 days after inoculation	
4	Necrosis expanded and leaf became wrinkled and deformed 17 days after inoculation	

Plate 9. Symptom development in pathogenicity test of isolate C3 (Kalliyoor) on potted plants.

Inoculated

Control

Stage	Description	Symptom
1	Appearance of small brown spots 9 days after inoculation	
2	Brown spots turned to small lesion surrounded by yellow halo and which later showed concentric zonations 15 days after inoculation	
3	Lesion expanded with yellow halo and became necrotic 20 days after inoculation	
4	Necrosis extended towards the margin 23 days after inoculation	

Plate10. Symptom development in pathogenicity test of isolate C4 (Kakkamoola) on potted plants.

Inoculated

Control

Stage	Description	Symptom
1	Appearance of small brown spot with yellow halo 6 days after inoculation	
2	Spots expanded as lesion on leaf surface with yellow halo 10 days after inoculation	
3	Lesion became mild necrotic 14 days after inoculation	
4	Necrosis expanded and foliage finally dried off 17 days after inoculation	

Plate 11. Symptom development in pathogenicity test of isolate C5 (Palpoor) on potted plants.

Table 7. Characteristic stages of symptom development of anthracnose on snake gourd leaves

Stages	Descriptions
1	Infection starts with small brown spots surrounded by yellow halo
2	As the brown spot enlarged, surrounding yellow halo the spot became distinct
3	Later spots become necrotic
4	Finally the leaf starts to dry up.

Disease Development Period (DDP) is defined as the time measured in days between inoculation and appearance of mature lesions. Disease development period ranged from 3 to 5 days in different isolates. DDP of three days was recorded in isolates C1, C2 and C5. DDP in isolate C3 and C4 were 4 days and 5 days respectively. (Table 8).

4.2.3 Cultural and morphological characters of the different isolates

Results of the cultural and morphological characters of the five pathogenic isolates are presented below

4.2.3.1 Cultural characters

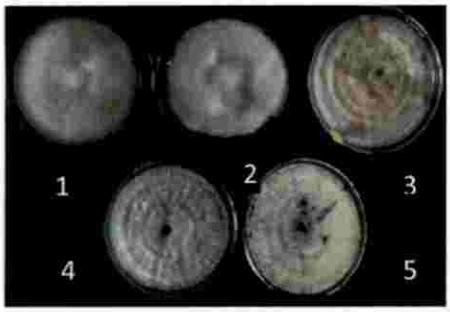
Cultural characters of the different isolates observed on PDA, as mentioned in 3.2.4.1, are presented below

The isolates C1 and C2 had fluffy, cottony mycelia growth with regular margin and took 5-7 days for completing the mycelial growth (9 cm dia. Petri plate). The surface mycelium of C3 and C4 showed concentric ring pattern with regular margins, while the isolate C5 showed sparse mycelia growth and the concentric ring pattern was not so prominent on the upper surface (Plate 12). The colony color of C1 and C2 isolates varied from white to light grey on the upper surface and the isolate C2 had a pink tinge on the reverse of Petri plate. On the reverse side of the Petri plate, the colony colour of C1 and C4 was light grey and that of C3 and C5 were grey to black. In the isolate C5 concentric ring patterns were clearly visible on rear view. After 9 to 10 days, fungal colonies of isolate C1, C2, C3 and C5, turned to olivaceous green and C4 became dark grey, on reverse side of Petri plate.

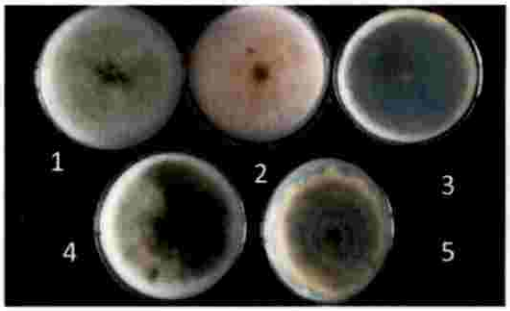
Mean diameter of mycelium in isolate C1 was 9 cm, 6 days after inoculation. Isolate C2 attained nine cm diameter of mycelial growth seven days after inoculation. Isolate C4 completed mycelial growth on PDA 7 days after inoculation while C3 and C5 took 9 days for completing growth. Time taken for the spore mass production also varied with the isolates. Spore mass production was observed in the isolates C1, C2,

Table 8.Incubation period and disease development Period (DDP) of different isolates estimated in pathogenicity test.

Sl no	Designated isolates	No. of days taken for initial symptom development	Disease Development Period(DDP) days
1	C1	5	3
2	C2	6	3
3	C3	6	4
4	C4	8	5
5	C5	6	3



a) Surface view



b) Rear view

Isolates

1- C1- Instructional Farm -Vellayani

2 - C2- Department of Olericulture – Vellayani

3- C3-Kalliyoor

4-C4-Kakkamoola

5-C5-Palapoor

Plate 12. Isolates of anthracnose pathogen obtained from surveyed locations.

C3, C4 and C5 after 10, 12, 13, 15 and 13 days respectively. The colour of spore mass in all the isolates varied from light orange to bright orange (Table 9).

4.2.3.2 Growth of pathogenic isolates on natural, semi-synthetic and synthetic solid media.

Five isolates of *Colletotrichum* spp. were screened on different natural, semi-synthetic and synthetic media following the procedure described in 3.2.4.

On solid media

The isolate C1 completed its growth in 5, 8, 7, 6, 5 and 6 days on oat meal agar, carrot agar, Sabouraud's agar, Czapek's (Dox) agar, Richards' agar and PDA media respectively. Isolate C2 completed their growth in 6, 8, 8, 8, 6 and7, C3 in 7, 8, 8, 9, 7 and 7; and C5 in 8, 9, 8, 8, 7 and 7 days in oat meal agar, carrot agar Sabouraud's agar, Czapek's (Dox) agar, Richards' agar and PDA media respectively. Least growth was recorded by the isolate C4 in all the media (Table 10; Plate 13).

All the five isolates produced maximum growth in oat meal agar followed by PDA. In synthetic medium maximum growth was observed in Richards' agar for all the five isolates. Miiumum growth for all isolates was observed in carrot agar. Among all the isolates C1 produced maximum growth of 9 cm on oat meal agar and Richards' agar. Isolate C3 also produced maximum growth in oat meal agar. The isolates produced profuse white growth on oat meal agar, PDA and Richards' agar, transparent growth was observed on carrot agar and sparse growth in Czapek's (Dox) agar and Sabouraud's agar. C1 completed its growth (9 cm dia.) *viz.,* oat meal agar, PDA and Richards' agar in 5 days. The isolate C2, C3, C4 and C5 completed the growth on oat meal agar and Richards' agar in 6, 7, 9 and 8 days respectively. The four isolates took 7 days for completing their growth on semi synthetic medium, PDA.

Table 9. Colony characters of isolates of *Colletotrichum* sp. on potato dextrose agar medium.

Isolates	DTCP	Growth pattern	Colony colour (Aerial)	Colony colour (Rear)	Spore mass colour	DTSP
C1	6	Dense	White	Light grey	Creamy white	10
C2	7	Dense	White	Light pink to grey	Creamy white	12
C3	9	Sparse	Creamy white to grey	Grey to black	Creamy white	13
C4	7	Sparse	White to grey	Dark Grey to black	Light orange.	15
C5	9	Sparse	Creamy white to grey	Grey to black	Creamy white	13

DTCP- Days taken to cover 9 cm Petri plate

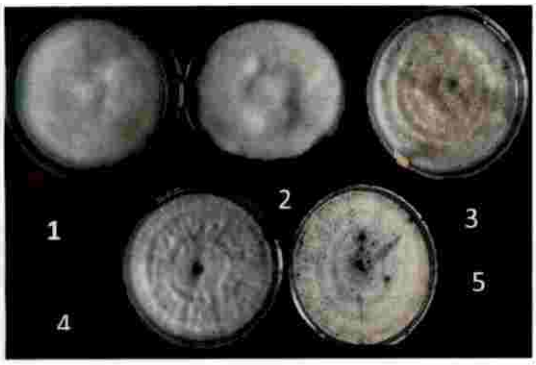
DTSP- Days taken for spore mass production

Table 10. Growth of isolates of *Colletotrichum* sp. in natural, semi-synthetic and synthetic solid media

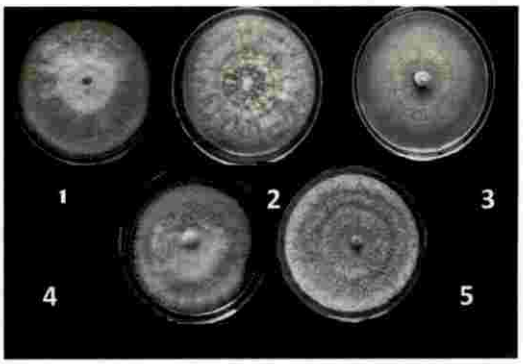
Media	Diameter (cm)- [7 days after inoculation]*					Days taken for competing mycelial growth in 9cm Petri plate				
	C1	C2	C3	C4	C5	C1	C2	C3	C4	C5
Oat meal Agar	$9.00^{a}\pm0.05$	$9.00^{a}\pm0.05$	$9.00^{a}\pm0.05$	$8.90^{a}\pm0.05$	$8.90^{a}\pm0.00$	5	6	7	9	8
Carrot Agar	$6.40^{c}\pm0.10$	$5.86^{d}\pm0.05$	$4.26^{d}\pm0.05$	$4.60^{d}\pm0.05$	$4.00^{d}\pm0.10$	8	8	8	9	9
Sabouraud's agar	$7.93^{b}\pm0.05$	$6.40^{c}\pm0.05$	$7.40^{b}\pm0.05$	$7.90^{b}\pm0.05$	$7.40^{c}\pm0.05$	7	8	8	9	8
Czapek's (Dox) Agar	$8.00^{b}\pm0.05$	$8.36^{b}\pm0.05$	$7.20^{c}\pm0.05$	$7.70^{c}\pm1.00$	$7.83^{b}\pm0.05$	6	8	9	9	8
Richards' Agar	$9.00^{a}\pm0.00$	$9.00^{a}\pm0.05$	$8.90^{a}\pm0.05$	$8.90^{a}\pm0.00$	$8.83^{a}\pm0.05$	5	6	7	8	7
PDA	$8.93^{a}\pm0.05$	$8.83^{b}\pm0.05$	$8.90^{a}\pm0.05$	$8.90^{a}\pm0.05$	$8.80^{a}\pm0.05$	6	7	7	7	7
CD(0.05)	0.100	0.122	0.136	0.145	0.125	-	-	-	-	-

*Mean of three replications

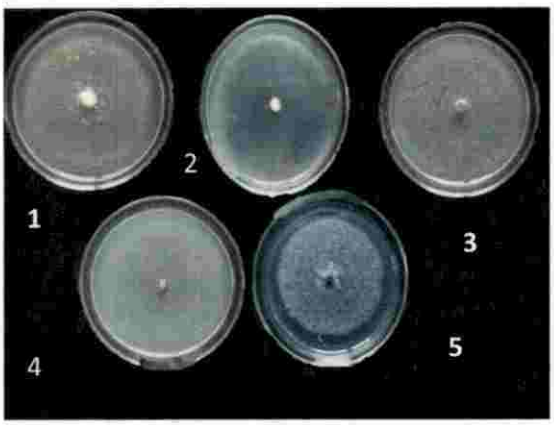
Figures followed by same letter do not differ significantly according to one way ANOVA at P= 0.05



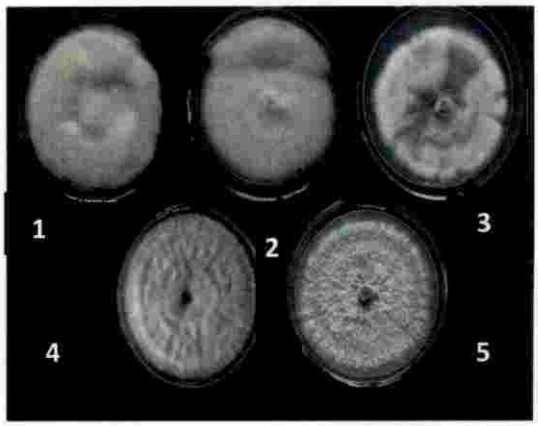
a) PDA medium



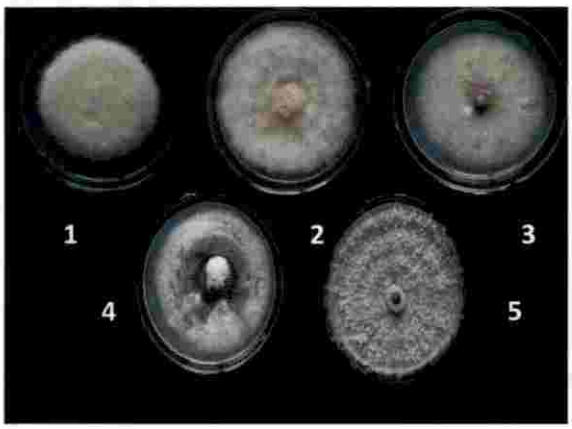
b) oat meal agar



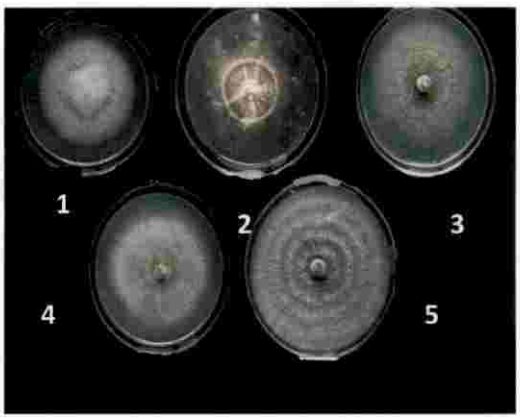
c) carrot Agar



d) Richard's medium



e) Czapek's (Dox)Agar



f) Sabouraud's Agar

1) IF – Vellayani
2) Department of Olericulture
3) Kalliyoor
4) Kakamoola
5) Palapoor

Plate 13. Growth of different isolates on different natural, semi-synthetic and synthetic solid media.

On liquid media (Broth)

Screening of five isolates of *Colletotrichum* sp. in liquid media was done following the procedures mentioned in 4.2.3.

Ten days after inoculation of the five isolates in liquid media, maximum mycelial dry weight was recorded in oat meal broth (natural medium) as well as Richards' broth (synthetic medium) and minimum mycelial development was observed in carrot broth and Sabouraud's broth. The mean mycelia weights of the isolates C1, C2, C3, C4 and C5 in oat meal broth were 166 mg, 160 mg, 151.3 mg, 128 mg and 152 mg respectively while they produced 413 mg, 393 mg, 336.3 mg, 262 mg and 386.2 mg of mycelial dry weight in Richards' medium. C4 recorded least mycelial dry weight (Table 11; Fig 2).

***4.2.3.3 Effect of different sources of light on growth of isolates of Colletotrichum* sp.**

Effect of different light sources *viz.*, exposure to alternate cycles of 12 h of fluorescent light and darkness (185 nm - 253.7nm), continous exposure to fluorescent light (185 nm - 253.7 nm), light emitting diode light (L.E.D - 365 nm) and ultra violet light (U.V. - 10-380 nm) for 45 min, of the pathogenic isolates were studied on best medium suitable for growth as screened in 4.2.4.3 and 4.2.4.4 (oat meal agar and Richards' agar respectively). Maximum growth of isolates C1, C2, C3, C4 and C5 were attained when the culture was exposed alternatively to 12 h of fluorescent light and darkness for a period of 7 days. It was followed by continous exposure of isolates to fluorescent light and LED light. Least mycelial growth was observed when the isolates were exposed to UV light for 45 min. Control recorded maximum mycelial growth of 9 cm Petri plate (Table-12-15; Plate-14-15).

Time taken for spore mass production in Richards' medium ranged from 9 to 12 days for C1, 10 to 13 days for C2, 12 to 14 days for C3, 14 to 15 days for C4 and 12 to 13 days for C5 when the cultures were exposed to alternate cycles of 12 h light and darkness, fluorescent light, L.E.D light and UV light respectively. Similarly time

Table 11. Mycelial dry weight of different isolates of *Colletotrichum* sp. in natural, semi-synthetic and synthetic liquid media ten days after inoculation.

Media	Mean Mycelial dry weight* (mg) - [10 days after inoculation]				
	C1	C2	C3	C4	C5
Oat meal broth	166.00^{c}± 3.21	160.00^{c} ±2.51	151.30^{c}±1.52	128.00^{b} ± 1.52	152.00^{c}±0.15
Carrot broth	13.30^{e}±0.17	11.00^{f}±0.30	10.60^{e} ±0.05	10.10^{e}±0.11	11.20^{e}±0.10
Sabouraud's broth	60.00^{d} ±1.00	52.50^{e}±0.11	51.00^{d} ±1.00	20.00^{d} ±0.57	53.00^{d} ±1.73
Czapek's (Dox) broth	340.00^{b} ±1.52	337.30^{b}± 1.00	314.60^{b}±0.57	102.00^{c} ±1.00	326.00^{b}±2.51
Richards' Broth	413.00^{a}±3.21	393.00^{a}±2.51	336.30^{a}±1.52	262.00^{a}±1.52	386.30^{a}±0.15
CD(0.05)	2.180	1.324	1.962	1.397	1.922

*Mean of three replications

Figures followed by same letter do not differ significantly according to one way ANOVA at P= 0.05

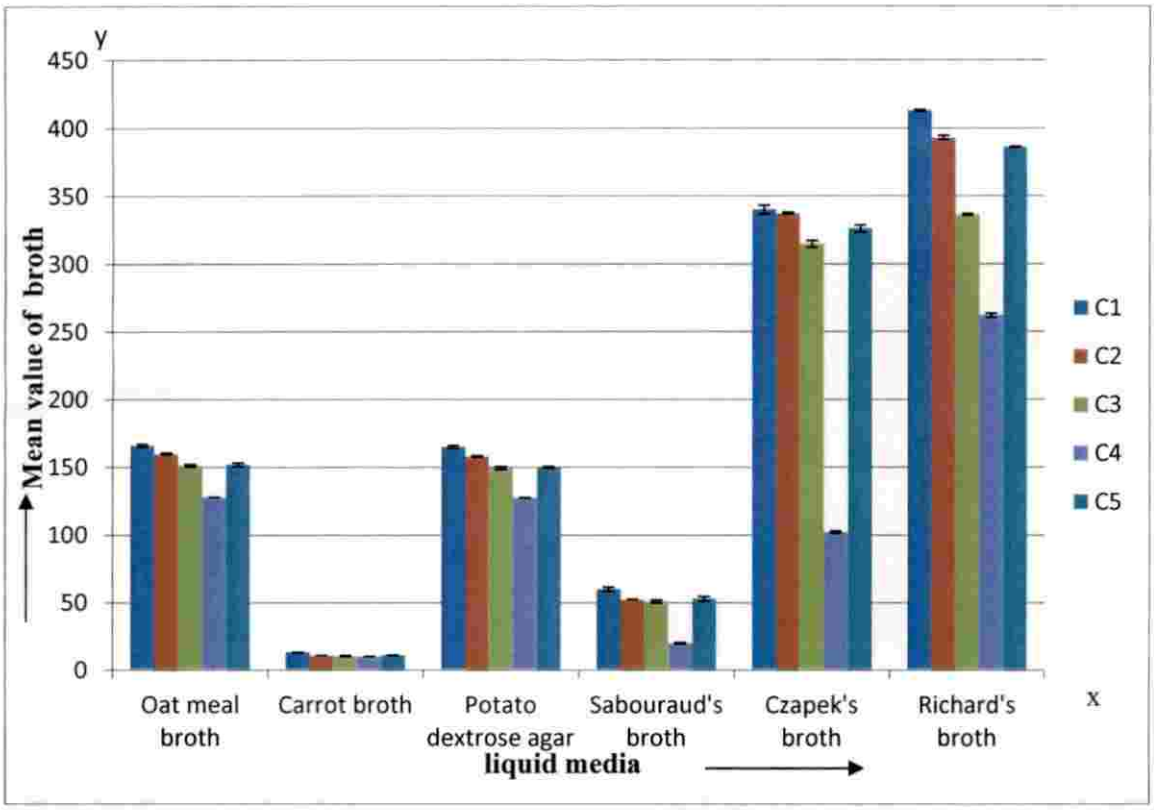


Fig. 2. Growth of different isolates of *Colletotrichum* sp. in natural, semi-synthetic and synthetic liquid media after ten days.

Table 12. Effect of different sources of light on mycelial growth of isolates of *Colletotrichum* sp. on oat meal agar.

Treatments	Mean Diameter of mycelial growth of *Colletotrichum* sp. on oat meal agar (cm)*				
	C1	C2	C3	C4	C5
Fluorescent light given alternatively at 12 h of light and 12 h of darkness	$9.00^{a}\pm0.00$	$9.00^{a}\pm0.05$	$8.92^{a}\pm0.05$	$8.90^{a}\pm0.05$	$9.00^{a}\pm0.05$
Fluorescent light	$8.93^{b}\pm0.05$	$8.73^{b}\pm0.05$	$8.80^{a}\pm0.05$	$8.90^{a}\pm0.05$	$8.76^{b}\pm0.00$
LED	$7.66^{c}\pm0.05$	$7.26^{c}\pm0.05$	$7.50^{b}\pm0.05$	$7.26^{b}\pm0.051$	$7.43^{c}\pm0.05$
UV	$1.26^{d}\pm0.05$	$1.10^{d}\pm0.05$	$0.61^{c}\pm0.05$	$0.23^{c}\pm0.05$	$0.31^{d}\pm0.05$
Control(day light)	9.00^{a}	8.90^{a}	8.90^{a}	8.80^{a}	8.90^{a}
CD(0.05)	0.122	0.100	0.099	0.099	0.083

*Mean of three replications **Figures followed by same letter do not differ significantly according to one way ANOVA at P= 0.05

Table 13. Effect of different sources of light on mycelial growth of *Colletotrichum* sp. on Richards' agar.

Treatments	Mean Diameter of mycelial growth of *Colletotrichum* sp. on Richards' agar*				
	C1	C2	C3	C4	C5
Fluorescent light given alternatively at 12 h of light and 12 h of darkness	$8.90^{a}\pm0.00$	$8.92^{ab}\pm0.05$	$8.82^{a}\pm0.00$	$8.80^{a}\pm0.05$	$8.90^{ab}\pm0.05$
Fluorescent light	$8.80^{b}\pm0.00$	$8.66^{b}\pm0.05$	$8.70^{b}\pm0.05$	$8.82^{a}\pm0.05$	$8.81^{b}\pm0.00$
LED	$7.36^{c}\pm0.05$	7.50 ± 0.05	$7.36^{c}\pm0.05$	$7.56^{c}\pm0.05$	$7.46^{c}\pm0.05$
UV	$0.21^{d}\pm0.05$	$0.32^{d}\pm0.05$	$0.56^{d}\pm0.05$	$0.22^{d}\pm0.05$	$0.32^{d}\pm0.05$
Control (day light)	9.00^{a}	9.00^{a}	8.90^{a}	8.50^{b}	8.90^{a}
CD(0.05)	0.100	0.100	0.122	0.100	0.099

L.E.D- Light Emitting Diode

U.V. - Ultra Violet

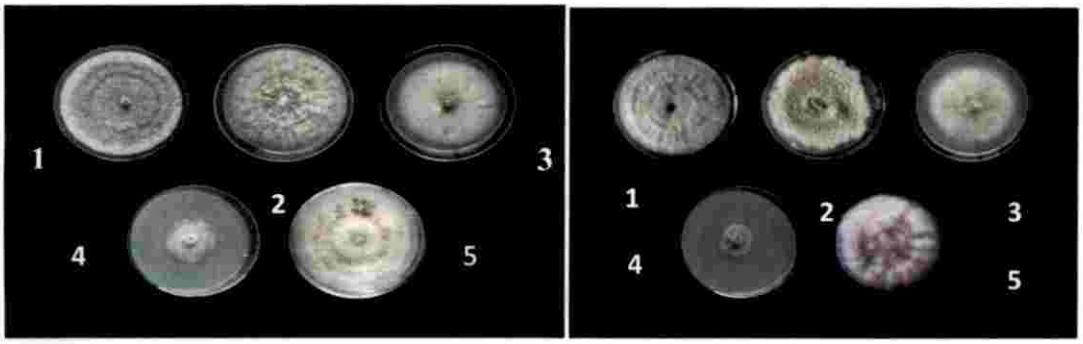
*Mean of three replications **Figures followed by same letter do not differ significantly according to one way ANOVA at P= 0.05

Table 14. Effect of different light sources on sporulation of different isolates of *Colletotrichum* sp. on oat meal agar.

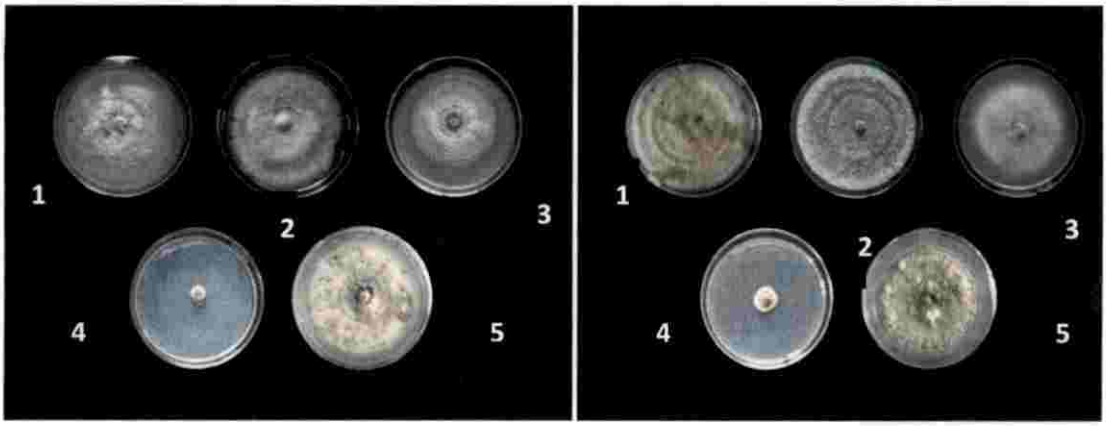
Treatments	Time taken for spore mass production on oat meal agar				
	C1	C2	C3	C4	C5
Fluorescent light given alternatively at 12 h of light and 12 h of darkness	10	11	11	14	12
Fluorescent light	11	12	13	15	13
L.E.D light	12	13	13	15	13
UV light	-	-	-	-	-
Control (day light)	11	13	13	15	12
Colour of spore mass	Bright orange	Bright orange	Bright orange	Light orange	Bright orange

Table 15. Effect of different light sources on sporulation of different isolates of *Colletotrichum* sp. on Richards' agar.

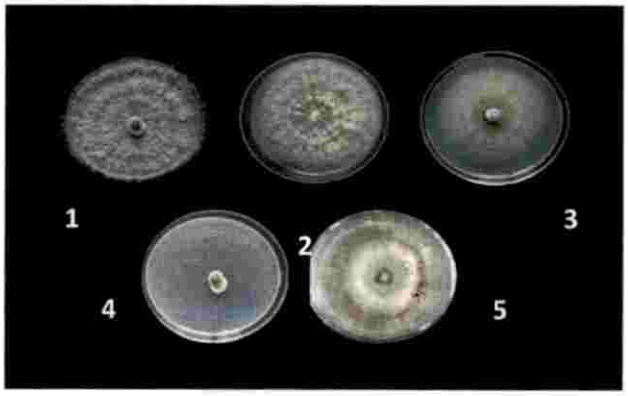
Treatments	Time taken for spore mass production on Richards' agar				
	C1	C2	C3	C4	C5
luorescent light iven alternatively t 12 h of light and 2 h of darkness	9	10	12	14	12
luorescent light	10	12	12	14	13
.E.D light	12	13	14	15	13
JV light	-	-	-	-	-
ontrol (day light)	10	12	13	15	13
olour of spore ass	Bright orange	Bright orange	Bright orange	Light orange	Bright orange



C1 (IF-Vellayani) C2 (Department of Olericulture)



C3 (Kalliyoor) C4 (Kakkamoola)

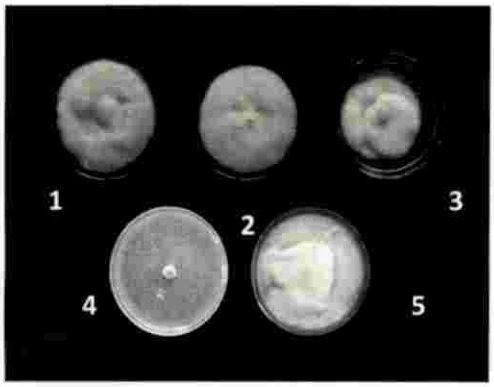


C5 – Palapoor

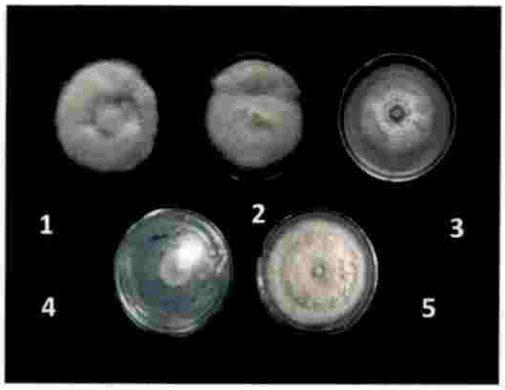
Different light sources

1. Alternate light and dark
2. Fluorescent light
3. L.E.D light
4. U.V light
5. Control

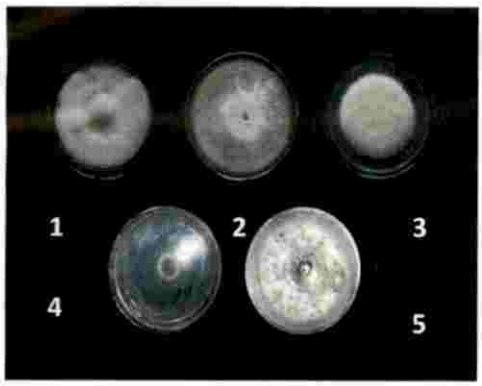
Plate 14. Influence of light sources on growth of *Colletotrichum* sp. oat meal agar.



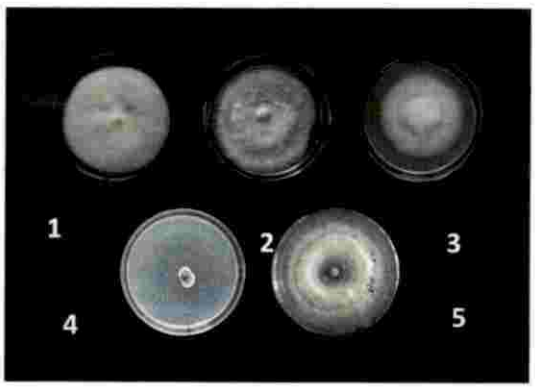
a) C1 (IF-Vellayani)



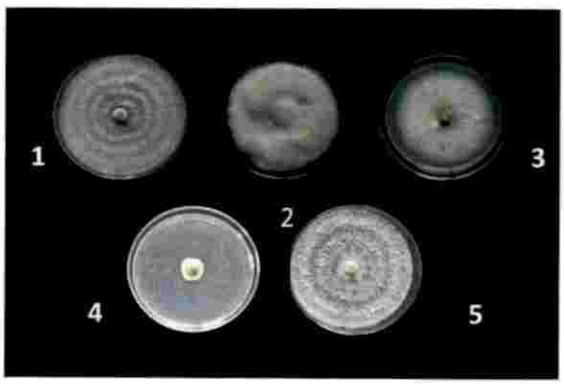
b) C2 (Dept. of Olericulture)



c) C3- Kalliyoor



d) C4- Kakkamoola



e) C5- Palapoor

Different sources of light

1. Alternate light and darkness
2. Fluorescent light
3. L.E.D light
4. U.V light
5. Control

Plate 15. Influence of light sources on growth of *Colletotrichum* sp. on Richards' agar

taken for spore mass production in oat meal agar ranged from 10-12 days in C1, 11 to 13 days in C2, 11 to 13 days in C3, 14 to 15 days in C4 and 12 to 13 days in C5 when the cultures are exposed to alternately to light (12 h), and darkness (12 h), fluorescent light, L.E.D light and UV light respectively. Colour of spore masses in isolates C1, C2, C3 and C5 were bright orange and in isolate C4 it was light orange.

4.2.3.4 Morphological characters

Hyphae of all four isolates (C1, C2, C3 and C5) were hyaline and septate with a width ranging from 2.22 µm - 3.86µm. The isolate C1 had maximum hyphal width of 3.86 µm and minimum hyphal width was recorded in the isolate C4 (1.92µm).

Conidia produced in all the isolates were hyaline and cylindrical with obtuse to slightly round ends. The average conidial size of four isolates (C1, C2, C3 and C5) was 11 - 15 µm in length and 4 µm - 5 µm in breadth. Maximum average size (13.14 µm x 4.82 µm) was recorded for conidia produced by isolate C1 which was followed by C2 (12.28 µm x 4.73 µm), C3 (11.40 µm x 4.52 µm) and C5 (11.3 µm x 4.48 µm. The least conidial size was observed in isolate C4 (10.52 µm x 4.40 µm). (Table 16; Plate 16).

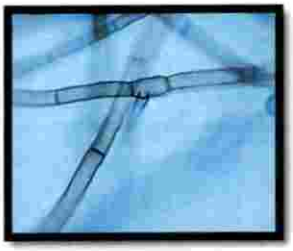
Acervuli bearing setae were produced in aged culture of each of the five isolates. The acervuli were varied from ovoid, irregular in shape, brown in colour and their diameters ranged from 85.52 - 123.25 µm Setae were few in number, brown, straight to slightly curved, swollen at the base and narrowing towards the apex.

Based on the above characters recorded in morphological and cultural studies the isolates C1, C2, C3, C4 and C5 were tentatively identified as *Colletotrichum* sp. Among these, four isolates (C1, C2, C3 and C5) were identified as *C. coffeanum* F. Noack and the isolate C4 was identified as *C. musae* (Berk. & M.A. Curtis) Arx by National Fungal Culture Collection of India (NFCCI), Pune (Acession no. NFFCI/2015-8/AKC/2293-03/SKS/DKM and NFFCI/2015-8/AKC/2207/SKS/DKM) respectively.

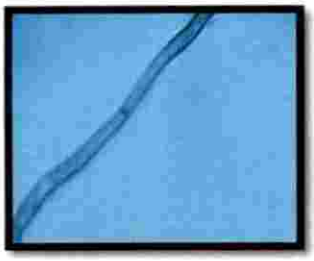
Table 16. Microscopic observations of different isolates of *Colletotrichum* sp. obtained from surveyed locations

Isolate	Shape of conidia	Mean Conidial dimension (Length x Breadth) (µm)**	Colour of conidia	Mean Hyphal dimension Breadth) (µm)*	Diameter of acervulus (µm) *	No of setae
C1	Cylindrical with obtuse to slightly round ends.	13.14x 4.82	Hyaline	3.86	123.25	15.00
C2	Cylindrical with obtuse to slightly round ends.	12.28 x 4.73	Hyaline	3.50	112.56	12.00
C3	Cylindrical with obtuse to slightly round ends.	11.40x 4.52	Hyaline	2.65	98.13	10.00
C4	Ellipsoidal	10.52 x 4.40	Hyaline	1.92	82.52	6.00
C5	Cylindrical with obtuse to slightly round ends.	11.30 x 4.48	Hyaline	2.22	105.67	11.00

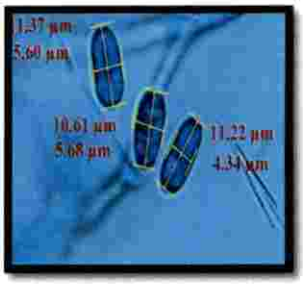
*Mean of ten replications, ** Mean of fifteen replication



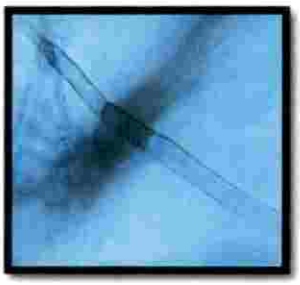
a) Hyphae of C1 b) Acervuli of C1 c) Spore of C1

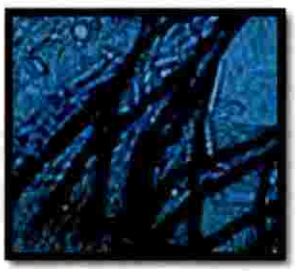


a)Hyphae of C2 b) Acervuli of C2 c) Spore of C2



a)Hyphae of C3 b) Acervuli of C3 c) Spore of C3





a)Hyphae of C4 b) Acervuli of C4 c) Spore of C4

a) Hyphae of C4 b) Acervuli of C4 c) Spore of C4

Plate 16. Hyphae, acervuli and conidia of different isolates of *Colletotrichum* sp.

4.3 *In vitro* evaluation of chemicals and bio-control agents against anthracnose pathogen

The most virulent isolate (C1 from IF- Vellayani), screened on the basis of pathogenicity study, incubation period and disease development period and identified as *C. coffeanum* at NFFCI, Pune was used for testing against different chemicals and bio-control agents.

4.3.1 a) Assay of chemicals and bio-control agents on growth inhibition of the pathogen by poisoned food

The *in vitro* inhibition of *C. coffeanum* by fungicides, nutrient and bio control agents was evaluated by conducting the poison food technique on PDA (Nene and Thapliyal, 1993). Six treatments were used for the *in vitro* study (Table 17; Plate 17; Fig-3).

Results of the experiment revealed that there was 100 per cent inhibition of mycelial growth of *C. coffeanum* on PDA amended with nutrient, potassium silicate at the field dose (0.5 per cent) which was on par with inhibition recorded on the medium amended with mancozeb at field dose (0.4 per cent) which also resulted in 100 per cent inhibition of mycelial growth of the pathogen. The above two treatments were significantly superior. Amendment of the medium with KAU talc based formulation of *P. fluorescens* (2 per cent) that recorded an inhibition of 88.83 per cent which was on par with amendment of the medium with KAU talc based formulation of *T. viride* (2 per cent) resulted in 87.33 per cent mycelial inhibition. This was followed by amendment with combination of azoxystrobin (0.1 per cent) + KAU talc based formulation of *P. fluorescens* (2 per cent) which recorded 82.56 per cent mycelial inhibition and was superior to amendment with azoxystrobin (0.1 per cent) which resulted in 61.10 per cent mycelial inhibition. There was no inhibition of mycelial growth of the pathogen in the control.

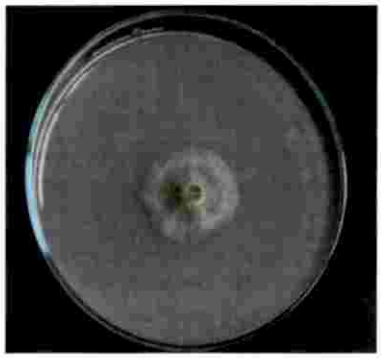
Table 17. *In vitro* management of chemical fungicides and talc based formulations of bio agents against. C1 isolate of *Colletotrichum* sp.

Treatments	Fungicides and bio-control agents	Mean Percentage mycelial inhibition of C1 isolate (cm*)
		Field dose
T1	Azoxystrobin (0.1 per cent)	61.10± 0.67 (51.62)[d]
T2	Azoxystrobin (0.1 per cent) + KAU talc based formulation of *P. fluorescens* (2.0 per cent)	82.56± 1.21 (65.33)[c]
T3	KAU talc based formulation of *T. viride* (2.0 per cent)	87.33 ± 1.68 (69.17)[b]
T4	KAU talc based formulation of *P. fluorescens* (2.0 per cent)	88.83 ± 2.25 (70.53)[b]
T5	Potassium silicate (0.1 per cent)	100.00 ± 0.00 (89.04)[a]
T6	Mancozeb (0.4 per cent)	100.00 ± 0.00 (89.04)[a]
Control		0.00
CD (0.05)		1.965

*Mean of three replication

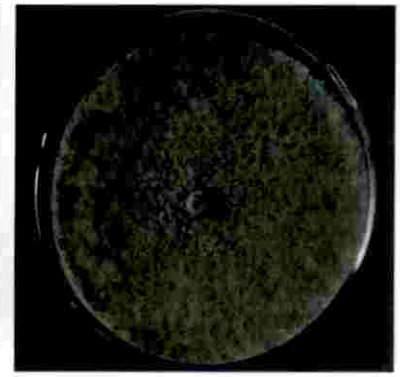
- Figures is parenthesized denote arc sin transformation

Figures followed by same letter do not differ significantly according to one way ANOVA at P= 0.05



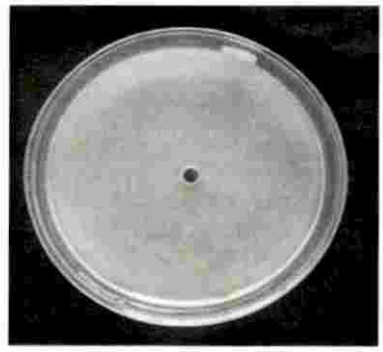
Azoxystrobin (0.1 %)

Azoxystrobin (0.1%) + *P. fluorescens* (2.0%)



T. viride (2.0 %)

P. fluorescens (2.0 %)

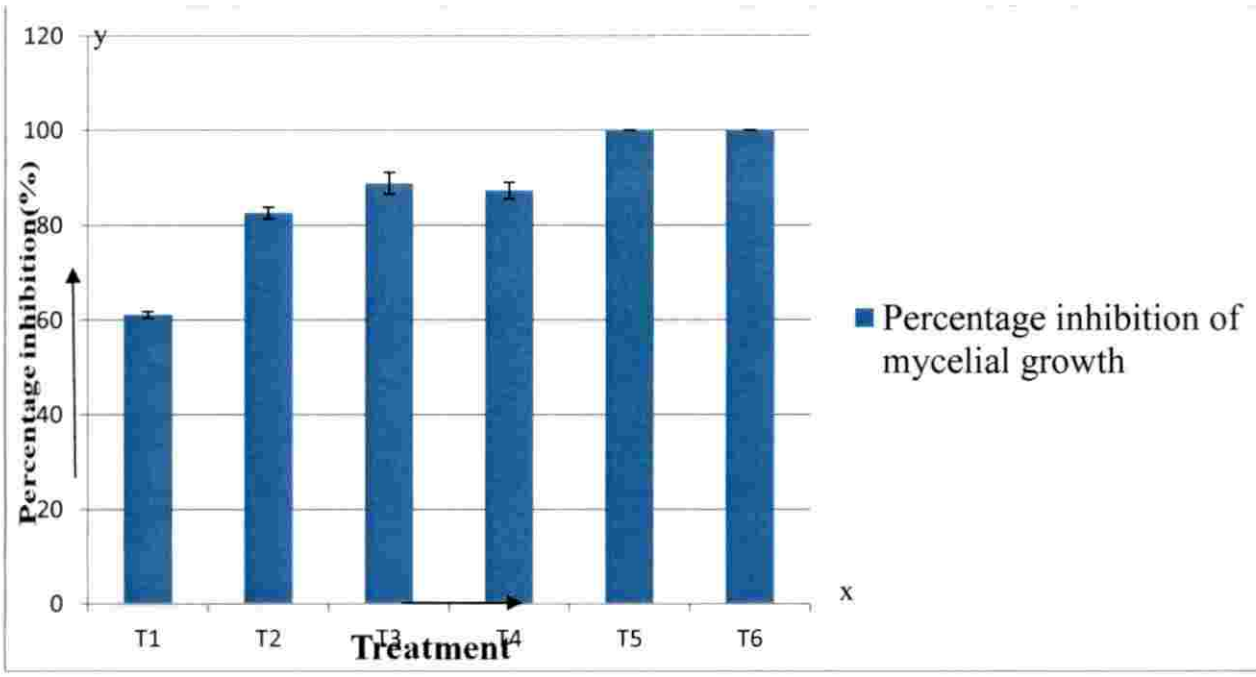


Potassium silicate (0.5 %)

Mancozeb (0.4 %)

Control

Plate 17. Effect of fugicides, foliar nutrient and talc based formulations of bio-control agents on mycelial growth of C1 isolate on PDA.



T1- azoxystrobin, T2- azoxystrobin + *P. fluorescens*, T3- *P. fluorescens*

T4- *T.viride,* T5- potassium silicate, T6- mancozeb

Fig . 3. Percentage inhibition of C1 isolate of *Colletotrichum* sp. due to fungicides, nutrient, bio-control agents and combination of fungicide and bio-control agent.

4.3.1.1 Observations on variation in conidial morphology

Variations in the conidial morphology were observed in medium amended with fungicides as well as bio-control agents in poison food technique (4.3.1). Distinct variations were observed in the size and shape of the conidia in different treatments. In medium amended with the fungicide azoxystrobin, average conidial dimensions of 10.28 µm x 4.26 µm were recorded at 0.1 per cent. Amendment of medium with the bio-control agent, *T. viride,* resulted in conidial size of 10.12 µm x 4.18 µm at 2 per cent which was followed by amendment with *P. fluorescens* (2 per cent) concentration in which average conidial size of 10.08 µm x 4.02 µm. Amendment of medium with combination of azoxystrobin (0.1 per cent) + *P. fluorescens* (2 per cent) recorded a conidial size of 10.21µm x 4.23 µm. (Table-18; Plate 18). Results of variations in conidial morphology were not recorded in media amended with potassium silicate and mancozeb due to absence of mycelial growth in respective treatments.

4.3.2 Effect of chemical and non-chemical treatments on germination of conidia of anthracnose pathogen

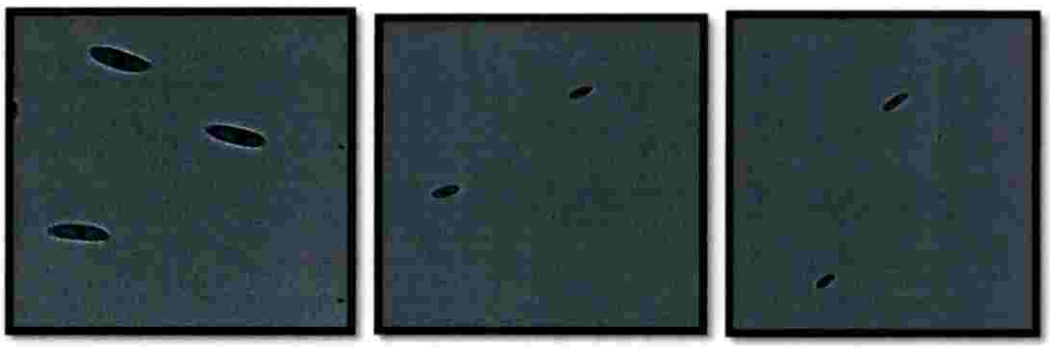
A separate experiment was conducted to evaluate the effects of chemical and non-chemical treatments on germination of conidia of *C. coffeanum.*

The *in vitro* studies on conidial germination revealed that recommended dosage of KAU talc based formulation of *T. viride* and KAU talc based formulation of *P. fluorescens* recorded 1.33 per cent and 1.66 per cent mean spore germination with 97.34 per cent and 96.68 per cent reduction over the control respectively. This was followed by mancozeb (0.4 per cent) and azoxystrobin (0.1 per cent) which were recorded 2.33 per cent and 3.00 per cent mean spore germination with 95.34 per cent and 94.00 per cent reduction over the control respectively. In the combination of azoxystrobin (0.1 per cent) + KAU talc based formulation of *P. fluorescens* (2 per cent) there was 2.33 per cent mean spore germination and 95.34 per cent reduction

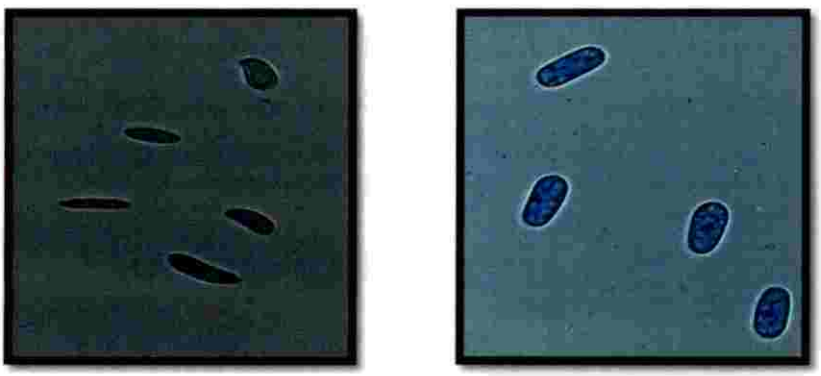
Table 18. Variations in conidial morphology of C1 isolate tested against fungicides and talc based formulations of bio-control agents

Treatment amended in medium	Concentration of amended treatment	Spore dimension* (µm)	Shape of spore
Azoxystrobin	0.1 per cent	10.28x 4.26	Small, cylindrical and elongated with pointed ends.
KAU talc based formulation of *T. viride*	2.0 per cent	10.12x 4.18	Small, cylindrical and elongated with pointed ends.
KAU talc based formulation of *P. fluorescens*	2.0 per cent	10.08x 4.02	Small, cylindrical and elongated with pointed ends.
Azoxystrobin + KAU talc based formulation of *P. fluorescens*	0.1 per cent + 2.0 per cent	10.21x4.23	Small, cylindrical and elongated with pointed ends.
Potassium silicate	0.5 per cent	Spores absent	Spores absent
Mancozeb	0.4 per cent	Spores absent	Spores absent
Control		13.14 x 4.82	

* Mean of three replications



Azoxystrobin (0.1%) *P.fluorescens* (2.0 %) *T. viride (*2.0 %)



Azoxystrobin (0.1%) + *P.fluorescens* (2.0%)

Control

Plate 18. Effect of fungicides, foliar nutrient and talc based formulations of bio-control agents on conidial morphology of C1 isolate of *Colletotrichum* sp.

over the control. Hundred per cent spore germination was recorded in control. Maximum mean spore germination was recorded in potassium silicate (0.5 per cent) compared to other treatments (Table 19).

b) Antifungal assay by dual culture method

The fungal antagonist *T. viride* inhibited growth of *C. coffeanum* by 90.58 per cent and the bacterial antagonist *P. fluorescens* resulted in 70.58 per cent inhibition of mycelial growth of the pathogen (Table-20; Plate-19).

4.4 Management of anthracnose leaf spot of snake gourd in green house study

A green house study was conducted at Instructional Farm, College of Agriculture, Vellayani to evaluate the efficacies of three best treatments that were screened in the *in vitro* study (3.3). The virulent isolate of the anthracnose pathogen C1, (*C. coffeanum)* was artificially inoculated on adaxial side of leaf, twenty days after transplanting, for inducing the leaf spot symptom of the disease. Disease incidence, disease severity and biometric observations such as root length, shoot length and number of leaves of snake gourd plants in different treatments were recorded. Treatments for the greenhouse study are given in Table 21.

Two applications of treatments were made at fifteen days interval starting frominitial appearance of symptoms. The per cent disease incidence and disease severity were recorded after first and second days of application of treatments and the biometric observations were recorded at the end of the experiment.

4.4.1 Disease Incidence (DI)

Observations of the symptoms of anthracnose disease on the leaves were recorded 24 h after artificial inoculation of *C. coffeanum* and continued upto 45 days. (Table 22; Plate 20-23). Disease incidence was calculated according to Agrios, (2005) and Hossain *et al.,* (2010) as mentioned in 3.3.1.

Table 19 . Effect of fungicides and talc based formulations of bio-control agents on conidial germination of C1 isolate.

Treatments	Mean spore germination*	Reduction over the control
	Medium	Medium
Azoxystrobin (0.1 per cent)	2.00[b]±1.00	96.00± 2.00 (78.71)[a]
KAU talc based formulation of *T. viride*(2.0 per cent)	1.33 [b]± 0.57	97.34±0.00 (80.73)[a]
KAU talc based formulation of *P. fluorescens* (2.0 per cent)	1.66 [b]± 0.57	96.68±1.14 (79.59)[a]
Azoxystrobin (0.1%) +KAU talc based formulation of *P. fluorescens* (2.0 per cent)	2.33 [b]± 1.15	95.34± 2.30 (77.83)[a]
Potassium silicate (0.5 per cent)	8.66[a]± 1.52	82.68±3.05 (65.45)[b]
Mancozeb (0.4 per cent)	2.00 [b]± 1.00	96.00± 2.00 (78.71)[a]
Control	100	-
CD (0.05)	1.828	4.805

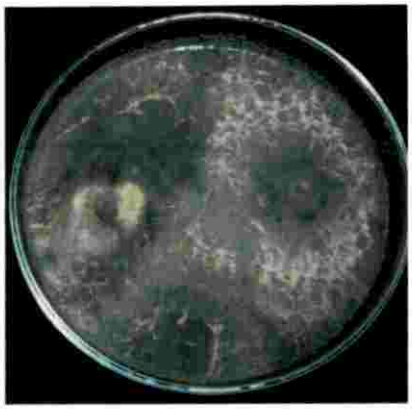
*Mean of three replication. 50 spores were observed.

- Figures is parenthesized denote arc sin transformation

Figures followed by same letter do not different significantly according to one way ANOVA at P= 0.05

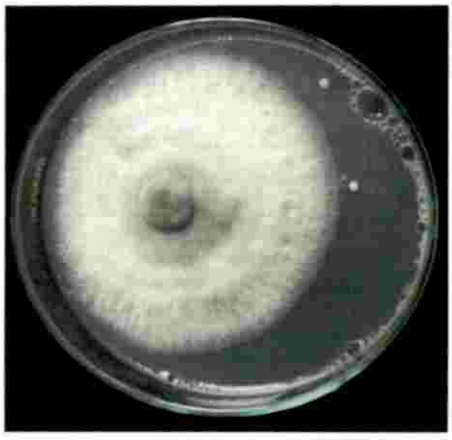
Table 20. *In vitro* suppression of mycelial growth of C1 isolate tested by dual culture technique with cultures of bio-control agents.

Sl no	Bio-control agent	Mycelial growth (cm)	Control (cm)	Percentage inhibition(%)
1	*T. viride*	0.8	8.5	90.58
2	*P. fluorescens*	2.5	8.5	70.58



T. viride

P. fluorescens



Control

Plate 19. *In vitro* suppression of mycelial growth of C1 isolate of *Colletotrichum* sp. by bio control agents.

Table 21. Effective treatments tested against C1 isolate in green house study.

	Treatments	Dosage
T1	Foliar spraying of Potassium silicate	0.5 per cent
T2	Basal application of *T. viride*	2.0 per cent
T3	Foliar spraying of *P. fluorescens*	2.0 per cent
T4	Foliar spraying of mancozeb (treated check)	0.4 per cent
T5	Inoculated control	

Table 22. Mean disease Incidence (DI) after application of treatments in green house study.

Treatment	Mean Disease Incidence (DI)*	
	DI after first treatment application	DI after second treatment application
Basal application of KAU talc based formulation of *T. viride* (2 per cent)-basal	4.84± 2.14 (12.47) [bc]	3.38± 1.67 (10.33) [bc]
Foliar spray of potassium silicate (0.5 per cent)	1.64± 1.73 (5.80)[a]	0.71± 0.65 (3.88)[a]
Foliar spray of KAU talc based formulation of *P. fluorescens* (2.0 per cent)	4.08± 1.88 (11.31) [bc]	2.09±1.78 (7.25) [ab]
Foliar spray of Mancozeb (0.4 per cent)	2.38± 1.55 (7.92) [ab]	0.98± 1.02 (4.49)[a]
Control	6.16± 2.09 (14.21) [c]	6.38± 1.08 (14.58) [c]
CD (0.05)	4.987	4.428

* Mean of five replications

- Figures is parenthesized denote arc sin transformation
- Figures followed by same letter do not different significantly according to one way ANOVA at P= 0.05

Plate 20. General view of pot culture experiment

Appearance of small brown spot with yellow halo expands on leaf surface

Brown spot with yellow halo expands on leaf surface and leaf started to dry

Plate 21. Symptoms after artificial inoculation

Plant sprayed with 0.5 per cent potassium silicate

Inoculated control

Plant sprayed with 2 per cent *P. fluorescens*

Inoculated control

Plate 22. Effect of potassium silicate and *P. fluorescens* on snake gourd anthracnose.

Plant treated with 2 per cent *T. viride*

Inoculated control

Plant sprayed with 0.4 per cent mancozeb

Inoculated control

Plate 23. *In vivo* effect of bio-control agent *T.viride* and fungicide mancozeb on snake gourd anthracnose.

After the first application, disease incidence in the treatments reduced to 1.64 - 6.16 per cent. Among the treatments, lowest mean disease incidence (1.64 per cent) was recorded in plants sprayed with potassium silicate (0.1 per cent) which significantly reduced the incidence when compared to that of control. This was on par with foliar application of mancozeb (0.4 per cent) which recorded 2.38 per cent disease incidence which was also on par with that of plants sprayed with KAU talc based formulation of *P. fluorescens* (2 per cent) with a disease incidence of 4.08 per cent and basal application of KAU talc based formulation of *T. viride* which recorded disease incidence of 4.84 per cent. Disease incidence was highest (6.16 per cent) in plants maintained as control.

Disease incidence after second application of treatments was significantly low (0.71 per cent) in plants that were sprayed with 0.1 per cent potassium silicate which was on par with plants sprayed with mancozeb (0.4 per cent), (DI - 0.98 per cent) and plants sprayed with KAU talc based formulation of *P. fluorescens* (2 per cent) that recorded disease incidence of 2.09 per cent and was on par with disease incidence observed in plants that received basal application of talc based formulation of *T. viride* (3.38 per cent). Highest disease incidence (6.38 per cent) was recorded in plants maintained as control.

4.4.2 Percentage Disease Index (PDI) / Severity.

Disease scoring was done using 0-9 scale of score chart developed by Goncalves *et al.,* (1997), 24 h after artificial inoculation of the anthracnose pathogen *C. coffeanum,* and continued at three days interval up to 45 days. The PDI / severity was calculated using score rating according to the formula of Mc Kinney, (1923) as follows:

Percent Disease Index (PDI) / Severity

$$= \frac{\text{Sum of all ratings}}{\text{Total number of leaves}} \times \frac{100}{\text{maximum rating.}}$$

Percentage disease suppression was also recorded in each treatment in comparison with control.

After first application of treatments, lowest disease severity (1.64 per cent) was recorded in plants sprayed with potassium silicate (0.5 per cent) resulting in 69.74 per cent disease reduction compared to control. This was on par with severity recorded in plants that received foliar application of 0.4 per cent, mancozeb (2.38 per cent) with disease reduction of 56.08 per cent. Plants sprayed with talc based formulation of *P. fluorescens* (2 per cent) recorded 3.53 per cent disease severity and 34.87 per cent disease reduction and was on par with that of plants received basal application of KAU talc based formulation of *T. viride* in which disease incidence of 4.58 per cent and percentage disease reduction of 15.49 were recorded. Inoculated control recorded highest disease severity of 5.42 per cent.

Disease severity recorded after second application, in different treatments ranged from 0.71 to 6.53 per cent. Among the treatments lowest disease severity (0.71 per cent) was recorded in plants sprayed with potassium silicate (0.1 per cent) which significantly reduced disease by 89.12 per cent compared to control. This was on par with that of plants which received foliar application of mancozeb (0.4 per cent) that recorded 0.98 per cent disease severity with 84.99 per cent disease reduction and foliar application of KAU talc based formulation of *P. fluorescens* (2 per cent) which recorded in 3.24 per cent disease severity with 50.38 per cent disease reduction. These treatments were significantly superior to plants that received basal application of KAU talc based formulation of *T. viride* which recorded a disease

severity of 3.31 per cent and disease reduction of 49.31 per cent. Inoculated control recorded highest disease severity (6.53 per cent) (Table 23).

Effect of different treatments on growth parameters of snake gourd plants revealed that foliar application of potassium silicate (0.5 per cent) recorded an average shoot length of 327 cm, root length of 15.30 cm and with an average of 86 number of leaves per plant and was significantly superior to other treatments. This was followed by foliar spray of mancozeb 0.4 per cent (325 cm shoot length, 14.70 cm root length and 82 numbers of leaves per plant) which was significantly better than foliar spray of KAU talc based formulation of *P. fluorescens* (2 per cent) (314 cm shoot length, 13.20 cm root length and 80 number of leaves per plant) and basal application of KAU talc based formulation of *T. viride* (2 per cent) which recorded (296 cm shoot length, 11.40 cm root length and 78 number of leaves per plant compared to control (Table 24).

Table 23. Mean percent disease index (PDI)/ disease severity after application of treatments in green house study.

Treatment	Mean Disease severity (%)*		Percentage disease suppression	
	15th day after first treatment application	15th day after second treatment application	15th day after first treatment application	15th day after second treatment application
Basal application of KAU talc based formulation of *T. viride* (2.0 per cent)	4.58± 1.58 (11.99) [bc]	3.31± 2.01 (10.17)[b]	15.49	49.31
Foliar spray of potassium silicate (0.5per cent)	1.64± 1.73 (5.80)[a]	0.71± 0.65 (3.88)[a]	69.74	89.12
Foliar spray of KAU talc based formulation of *P. fluorescens* (2.0 per cent)	3.53± 1.48 (10.62) [bc]	3.24± 1.47 (6.88) [ab]	34.87	50.38
Foliar spray of Mancozeb (0.4 per cent)	2.38± 1.66 (7.92) [ab]	0.98± 1.03 (4.49)[a]	56.08	84.99
Control	5.42± 1.65 (13.35)[c]	6.53± 1.18 (14.75)[c]		
CD (0.05)	4.670	4.367		

* Mean of five replications

- Figures is parenthesized denote arc sin transformation

Table 24. Effect of different treatments on growth parameters of snake gourd

Treatments	Mean number of leaves*	% increase over the control	Mean shoot length (cm)*	% increase over the control	Mean root length (cm)*	% increase over the control	Mean number of infected leaves*	% Decrease of infected leaves
Basal application of KAU talc based formulation of *T. viride* (2.0 per cent)	$78^{c} \pm 1.14$	14.10	$296^{c} \pm 2.40$	11.16	$23.22^{b} \pm 1.22$	19.45	$12^{c} \pm 0.54$	40.00
Foliar application of potassium silicate (0.5 per cent)	$86^{a} \pm 1.81$	22.09	$327^{a} \pm 4.77$	23.39	$28.32^{a} \pm 0.48$	44.66	$3^{a} \pm 1.34$	85.00
Foliar application of KAU talc based formulation of *P. fluorescens* (2 per cent)	$80^{b} \pm 1.51$	16.25	$314^{b} \pm 2.96$	18.49	$24.49^{b} \pm 0.65$	18.59	$8^{ab} \pm 1.14$	60.00
Foliar application of Mancozeb (0.4 per cent)	$82^{b} \pm 1.41$	22.38	$325^{a} \pm 3.83$	22.64	$27.70^{a} \pm 0.72$	41.65	$3^{a} \pm 0.83$	85.00
Control	$67^{d} \pm 4.39$		$265^{d} \pm 4.33$		$19.58^{c} \pm 0.98$		$20^{d} \pm 0.70$	
CD (0.05)	3.64		4.91		1.45		1.264	

- * Mean of five replications *Figures is parenthesized denote arc sin transformation
- Figures followed by same letter do not different significantly according to one way ANOVA at P= 0.05

Discusssion

5. DISCUSSION

The present investigation entitled "Integrated management of anthracnose of snake gourd" was conducted at the Department of Plant Pathology, College of Agricuture, Vellayani, in order to study the symptomatology, etiology followed by the management of the disease using chemicals and bio-control agents. Surveys on prevalence and assessment of the disease in different locations, studies of the pathogenic isolates obtained from the surveyed locations followed by *in vitro* and *in vivo* experiments conducted for screening effective chemicals and bio-control agents for controlling the disease, were the major aspects dealt with, in this programme.

Surveys were conducted in five different snake gourd fields located in Instructional Farm of College of Agricuture, Vellayani, Department of Olericulture of College of Agricuture, Vellayani, Kakkamoola, Palapoor and Kaliyoor, of Thiruvanathapuram district, in order to determine the prevalence of anthracnose of snake gourd in the fields and also to study the nature and symptom development of the disease, which were conducted at 10 days interval with a total of four surveys conducted from October 2015 to November 2015. Further a comparative assessment of anthracnose disease in the surveyed locations was also made by finding out the disease incidence (DI) and percentage disease index (PDI) of the disease.

In all the surveyed locations symptoms of anthracnose appeared both on immature and mature leaves but this was more severe, and spread rapidly on the older and mature leaves. Symptoms started as small, oblong or elliptical (1-2 mm dia.) straw to greenish yellow coloured sunken spots on the leaf surface and were surrounded by pale yellow halo. As the spots enlarged into more or less elliptical to oblong brown lesions (2-3 cm in dia.), the surrounding halo also expanded bearing a distinct yellow colour. Later these lesions coalesced and turned into dark necrotic patches while the leaf margins shriveled, finally causing the leaves to dry up. Slight variations from these general symptoms were observed in the anthracnose affected plants of the snake gourd fields of Palapoor, wherein the symptomatic leaves

exhibited concentric zonations consisting of numerous black acervuli on the enlarged lesions, which were distinct giving a target board appearance

Anthracnose is one of the most common diseases affecting cucurbitaceous crops. In addition to snake gourd, anthracnose can affect cucumber, cantaloupe, chayote, citron, gherkin, gourd, honeydew melon, muskmelon, watermelon, and many non-cucurbit species. The disease causes serious economic losses to several economically important vegetable crops, worldwide. However pumpkin and squash are rarely affected by the disease (Wasilwa *et al.,* 1993). The name 'anthracnose', derived from Greek word meaning 'coal', is common term used for designating plant diseases characterized by very dark, sunken lesions containing spores (Issac, 1992). Sitterly and Keinath (1996) described the symptoms of anthracnose in cucumber in which minute oblong to ellipsoidal lesions which were pale yellow to brown in colour with yellow halo, were produced which spread over the entire leaf. In some cases centers disintegrated and fell off leading to a shot hole appearance. Snake gourd anthracnose develops in all above ground parts of the plant causing leaf spot, blight and fruit rot during growing season. Severe epidemics of the disease occurred during warm and wet climate which resulted in early defoliation, yield loss and lower quality of fruits (Damm *et al.,* 2013).

Disease incidence (DI) and severity/percentage disease index (PDI) observed in the fields during the period of survey ranged from 70 per cent to 90 per cent and 21.89 per cent to 44.22 per cent respectively. The highest disease parameters were recorded in the fields of Instructional Farm, College of Agriculture, Vellayani followed closely by those of Deptartment of Olericulture, College of Agriculture, Vellayani. Lowest (DI and PDI) were observed in the snake gourd fields of Palapoor. There was large scale and continuous cultivation of the crop in the fields of Instructional Farm, College Of Agriculture, Vellayani and Department of Olericulture, College of Agriculture, Vellayani whereas the acreage under the crop was comparatively less (10 cents) in Palapoor. Besides, there were other

cucurbitaceous crops like cucumber, pumpkin and bittergourd surrounding the fields of Instructional Farm, College of Agriculture, Vellayani and Department of Olericulture, College of Agriculture, Vellayani while the snakegourd field in Palapoor was located in an isolated area with no other crops cultivated nearby.

There are very few reports of anthracnose affecting snake gourd in the fields of Kerala, in spite of the wide prevalence of the disease as observed in the present survey conducted at Thiruvananthapuram district. Very high intensity (72 per cent) of the disease was recorded from Banglore many years back in 1979. It is only very recently that the extensive cultivation of snake gourd was taken up by farmers which may be due to the growing awareness of nutrients and medicinal values of this vegetable. Moreover cultivation of the crop is also more economical when compared to the other major trailing cucurbitaceous crop *viz.*, bittergourd which is seriously affected by diseases and pests and often cause much loss to farmers. Therefore the recent large scale and continuous cultivation of the crop might have resulted in cumulative increase of the pathogen inoculum which accounts for the greater spread and severity of anthracnose disease of snakegourd, in the fields. As there are no reports, so far, on the incidence of anthracnose of snake gourd in Kerala, this is the first study in which the symptom of anthracnose affecting foliage of snake gourd plant is described. However, worldwide, anthracnose disease has recently been considered to be particularly important wherever cucurbits are cultivated. High infections may cause formation of numerous leaf lesions and vine defoliation resulting in poor quality fruit and yield loss in grapes (Egel, 2014). Cucumber and pumpkin were included among the 470 different host genera of anthracnose pathogen of *C. gloeosporoioides* (Sharma and Kulshrestha, 2015). Disease incidence of anthracnose in bottle gourd was 14 per cent in Chittagong regions of Bangladesh as reported by Hossain *et al.*, (2010).

Attempts were also made for isolating the pathogen from leaf samples exhibiting typical symptoms of the disease that were collected from different

surveyed locations. Five isolates of the pathogen obtained on PDA were designated as C1, C2, C3, C4 and C5. Microscopic examination of these isolates revealed that they were almost similar in morphological characters producing septate, hyaline hyphae and cylindrical to ellipsoidal conidia. Based on morphological characters, the five isolates were identified as *Colletotrichum* sp. The fungus *Colletotrichum* is one of the major plant pathogenic genera responsible for causing anthracnose disease on a variety of hosts, from trees to grasses. Different species of the pathogen were reported from a variety of plant hosts including cereals and grasses, legumes, vegetables, perennial crops and tree fruits in India (Gautham, 2014). Field losses due to *Colletotrichum* sp. have been reported to be more than 60 per cent in the United States (Thompson and Jenkins, 1985). Earlier findings revealed *C. lagenarium* (synonym *C. orbiculare*) as the causal agent of leaf, stem and fruit anthracnose of cucurbits (Prakash *et al.,* 1974). *C. orbiculare* was recognized worldwide as the pathogen causing anthracnose in cucurbits (Palenchar *et al.*, 2009). This fungus caused infections on leaves, stems, and fruits of cucumber plants and severely hindered cucumber crop production (Thompson and Jenkins, 1985 and Wasilwa *et al.*, 1993). Further, Rampersad (2010) isolated the pathogen *C. gloeosporioides* from surface-sterilized tissues of symptomatic plants and reported for the first time that anthracnose is caused by *C. gloeosporioides* in pumpkin in Trinidad. According to Li and Zhang (2014), there was a heavy loss due to fruit rot caused by *C. gloeosporioides* in *Trichosanthes kirilowii* Maxim, a species within the gourd family which is cultivated in China for its edible seeds and medicinal roots.

When each of the five isolates (C1 to C5) was artificially inoculated on detached leaves of snake gourd plants (cultivar-Kaumudi) it was observed that all the isolates produced small black lesions which spread and covered almost the entire leaves within 3 to 5 days. Although the typical symptoms were not produced on the inoculated leaves, this technique helped in the preliminary detection of infectivity of the isolates on the host plant. Following this, pathogenicity tests of all the five

isolates were conducted by adopting the routine methods of proving Koch's postulates. When five isolates of the pathogen were artificially inoculated on 30-days–old potted plants of snake gourd (cv-.Kaumudi), almost similar symptoms were produced by all the isolates of the pathogen. Initial symptom appeared as small circular brown lesion which enlarged gradually and was surrounded by a yellow halo. As the lesions enlarged, they became irregular and necrotic and the yellow halo faded. Finally the necrotic area covered almost the entire leaf which shriveled along the margins and finally dried up. However slight variations were noticed in symptoms produced by isolate C4 isolate in which dark concentric zonations were distinct as the circular spots enlarged.

Suhag and Duhan (1984) inoculated detatched ten days old leaves of bottle gourd with 100-200 conidia/ml of *C. lagenarium,* kept on moist blotter in glass Petri dish at 25-30°C. They maintained 100 per cent relative humidity for 24 h and got the best result of pathogenicity of the fungus. Artificial inoculation methods *in vitro* are commonly used to test the pathogenicity of a fungal species, as it is easy to control environmental conditions (Photita *et al.,* 2004). Common inoculation methods for pathogenicity testing included drop inoculation and wound /drop inoculation (Kanchana-udomkan *et al.,* 2004 and Cai *et al.*, 2009), micro-injection and spraying with high pressure guns (Freeman (1996); Lin *et al.* (2002); AVRDC, (2003); Sharma *et al.,*(2005) and Than *et al.* (2008). In pathogenicity tests, six pumpkin plants (cv. Jamaican squash) for each of five isolates of *C. gloeosporioides* were spray inoculated with a conidial suspension (1.0×10^6 conidia/ml). Negative controls were sprayed with sterile distilled water. In repeated tests, plants were symptomatic of infection seven days post inoculation. There were no symptoms on control plants and Koch's postulates were fulfilled with the re-isolation of the pathogen from symptomatic leaf tissues.

Pathogenicity studies conducted with the five isolates also revealed that the isolate C1 recorded maximum lesion size of 2.53 cm^2 on detatched leaf and 10.06

cm^2 on 30-days old potted plants. Smallest lesion size was recorded for isolate C4 both on detatched (1.33 cm^2) and on thirty days old potted plants (1.53 cm^2). Lesion area was evaluated by measuring length and width of typical anthracnose lesion developed on coffee berries, from 1 to 15 days after inoculation (Prihastuti *et al.*, 2009).

The time taken for the onset of disease denoted as incubation period (IP) and DDP is the days taken for the symptom to become mature lesions. C1 isolate (Instructional Farm, Vellayani) had shortest IP and DDP, and was therefore screened as the most virulent isolate for conducting subsequent studies on integrated management of the disease. Palenchar (2009) reported that initial symptoms of anthracnose appeared on cucumber leaves six days after artificial inoculation with *C.orbiculare*. In general, the first symptom as anthracnose lesion appeared on the inoculated leaf three days after inoculation cucumber leaves. The brown lesions enlarged with time, up to 5 days after the appearance of initial symptom and coalesced each other to form large diseased area on the leaves (Negishi, 2011). Symptom expression of anthracnose in noni was 8 days as reported by Manjunath *et al.*, (2011) and in chilli it was recorded as 5 days according to Linu and Jisha, (2013).

Detailed studies of morphological and cultural characters of the five isolates obtained from the surveyed locations were conducted at Department of Plant Pathology, College of Agriculture, Vellayani in order to identify the pathogen.

Differentiation among the *Colletotrichum* morpho-groups based on traditional methods such as conidial shape and size appeared to be useful as the differences of conidial size, both in length and width of conidia, were statistically significant (Simmonds 1965; Sutton 1962; 1965; 1966; 1968; 1980 and von Arx (1981). Identification of species of *Colletotrichum* had relied primarily on morphological differences such as colony color, size, and shape of conidia, optimal temperature, growth rate, presence or absence of setae, and existence of the teleomorph, *Glomerella* (Freeman *et al.*, 1998). Morpho-taxonomic criteria such as shape and size

of conidia, morphology and size of acervuli, number of setae, response to temperature on potato dextrose agar medium (PDA) and host specificity, as well as molecular identification techniques were used for identification of *Colletotrichum* sp. (Sutton, 1992; Freeman and Kartan, 1998; Lui *et al.,* 2007). Morphological characterization conducted in present study indicated that the isolates produced septate hyaline hyphae of width ranging from 1.92 µm to 3.86 µm and cylindrical to ellipsoidal conidia of average size ranging from 10.52 µm to 13.14 µm X 4.02 µm to 4.40 µm. Average width of the acervuli of five isolates ranged from 82.52 µm to 123.25 µm, bearing 6 to 15 numbers of setae. Bose *et al.*, (1973), observed that the size of conidia of *C. gloeosporides* varied from 11 µm to 16 µm x 4 µm to 6 µm and 13.8 µm x 4.8 µm with ellipsoidal shape. The conidial size varied from 10.5 µm to 15 µm x 6 µm to 7 µm with cylindrical to ellipsoidal in shape (Chowdappa *et al.,* 2012). According to Shilpa (2015) *C. gloeosporoides* produced acervuli bearing 15 to 30 number of setose.

Culture studies of all the five isolates indicated the growth of creamy white to greyish coloured mycelium, with light to dark orange coloured spore masses, on PDA. The characters of all the five isolates observed in this study conformed to the sporulation, pigmentation and colony characters of *C. gloeosporoides* as reported by Denobys and Baurdy (1995) in strawberry, Kuramae *et al., (*1997) in citrus, Schwarts and Gent, (2007) in cucurbits and Manjunath (2010) in noni. Chowdappa *et al.,* (2015) reported that *Colletotrichum* sp. produced white to grayish white colonies on Richards' agar and PDA.

Thus based on the results of the studies on morphological and cultural characters all the five isolates of the snakegourd anthracnose pathogen obtained from different locations of Thiruvanathapuram district, were tentatively identified as *C. gloeosporoides.*

Comparative assessments of the different isolates were also made in the cultural and morphological studies. Cultural studies revealed that oat meal medium, potato

dextrose medium and Richards' medium were most suitable solid and liquid media for the growth of all the isolates of the pathogen. Exposure of fungal cultures to fluorescent light and darkness, alternatively for a period of 12 h, up to duration of 7 days, resulted in maximum growth and sporulation of all the isolates.

With respect to studies on morphological characters *viz.*, hyphal width, size of conidia and acervuli, it was observed that the isolates C1, C2, C3 and C5 were larger (IF- Vellayani, Department of Olericulture, Kalliyoor and Palapoor) compared to the isolate C4. Similarly, the number of setae on the acervuli was greater in each of the four isolate C1, C2, C3 and C5 when compared to isolate C4.

Studies on cultural characters also indicated that the isolate C4 differed from the remaining four isolates. Isolate C4 recorded sparse growth and produced white to grey aerial mycelium on PDA. It took 15 days for spore mass production and the colour of spore mass was light orange. Meanwhile the isolates C1, C2, C3 and C5 produced creamy white to white aerial mycelium, took 10 to 13 days to produce spore masses which were dark orange in colour. Thus the isolates of *Colletotrichum* sp. infecting snake gourd, obtained in the present study, varied with respect to sporulation, pigmentation and colony characters.

The isolate C4 obtained from the snake gourd fields of Kakkamoola therefore differed slightly from the remaining isolates with respect to nature of symptom development as well as its morphological and cultural characters.

Morphological characterization of the five isolates, that were tentatively identified as *C. gloeosporioides* were further performed at National Fungal Collection Culture India (NFCCI), Pune in order to ascertain their identities. Based on these characterization studies, the identities of isolate C1, C2, C3 and C5 were revealed as *C. coffeanum* F. Noack and were assigned the accession number NFFCI/2015-8/AKC/2293-03/SKS/DKM while the isolate C4 was identified as *C. musae* (Berk. & M.A. Curtis) Arx and was assigned the accession number NFFCI/2015-8/AKC/2293-

07/SKS/DKM. However phylogenetic analysis of these isolates needs to be conducted, in order to confirm their actual identities. In many of earlier reports, the conidial size of *C. coffeanum* ranged from 10 µm - 18 µm x 4 µm - 5 µm (Firman and Waller, (1977); Sutton (1980); von Arx (1981) and Holiday (1989). In morphological descriptions of *C. coffeanum* isolated from coffee plants, Prihastui *et al.,* (2009) indicated that the conidial size of the pathogen ranged from 12 µm - 18 µm x 4 µm - 5µm and they produced dark brown acervuli bearing less number of setae. With regard to cultural studies conducted earlier by Mc Donald, (1926) and Rayner,(1952) in Kenya, they reported that *C. coffeanum* inciting coffee berry disease (CBD) produced slow growing, cottony, dark-greenish grey colonies. Prihausthi *et al.,* (2009) observed that the pathogen *C. coffeanum* produced creamy white to olivaceous green mycelium with bright orange coloured spore mass. Thus it was evident from this study that the morphological and cultural characters of the four isolates were similar to the observations recorded in the earlier reports which therefore authenticates the identification of the four isolates, *viz.,* C1, C2, C3 and C5 as *C. coffeanum.*

The isolate C4 obtained from Kakkamoola identified as *C. musae*, produced morphological and cultural characters that varied from the other four isolates by the production of sparse white to grey aerial mycelium on PDA which took 15 days for spore mass production. Unlike the other four isolates, C4 produced light orange coloured spore masses. Von Arx (1981) included *C.musae* under *C. gloeosporioides* as specific to Musa, while Sutton (1980, 1982) accepted this as distinct species which was supported by recent molecular work (L. Cai, pers. comm). This taxon has been reported as the major causal organism of anthracnose and also responsible for causing crown rot, blossom end rot and tip rot of banana (Nazriya *et al.,* 2007). Striking differences with regard to morphological characters of isolate C4 were their hyphal width that was narrower (1.92 µm) than those of other isolates, ellipsoidal shape and smaller size of their conidia and less number of setae borne by the acervulus. Lim *et*

al., (2002) reported that conidia of *C. musae* were aseptate, hyaline, mostly ellipsoidal, ranging from 10 µm - 18 µm and 4 µm - 9 µm. Thus based on the cultural and morphological characters of the five isolates identified as *C. musae* and *C. coffeanum* in the present study and were in agreement with those observed by Berk *et al.,* (1957) and Noack, (1993) who reported these pathogens for the first time. Thangamani *et al.,* (2011) observed that the pathogen *C. musae* produced coloured acervuli in case of anthracnose in banana.

The isolates C1, C2, C3 and C5 were specifically identified as *C. gloeosporioides* during the study conducted at College of Agriculture, Vellayani, due to the characteristic shape of their cylindrical conidia, hyphal width....which conformed to the characters described in the earlier reports (Sutton, 1980,give other names also). The name *C. kahawae* was assigned by Waller *et al.,* 1993 to the pathogen *C. coffeanum* which caused infection specifically on coffee plants. At the same time, Sreenivasaprasad *et al.,* (1993) showed that *C. kahawae* is very close to *C. gloeosporioides* on the basis of rDNA sequences. Affinity of these two species (*C. kahawae* and *C. gloeosporioides*) was based on a single base difference in rDNA sequence data which is used to discriminate related taxa due to limited number of informative sites identified and their underlying difference is very small. However the studies conducted later by Cannon *et al.,* (2008) indicated that that *C. kahwae* is closely related to *C. gloeosporioides* based on rDNA-ITS sequence analysis. Therefore revelations of these previous studies conformed to the results of the present investigation in which four isolates (C1, C2, C3 and C5) were identified initially as *C. gloeosporioides* and were later confirmed as *C. coffeanum.*

In many of the earlier reports coffee plant was exclusively indicated as the host plant of *C. coffeanum.* However, recently the pathogen has been reported to infect other host plants like gerbera (Yaling *et al.,* 2015). Similarly the pathogen *C. musae* has several host plants other than banana (*Musa* spp.) and has been reported to be pathogenic to apple (*Malus pumila*), mango (*Mangifera indica*), avocado (*Persea*

americana), guava (*Psidium guajava*) and *Vigna* sp. (Sutton and Waterston 1970). Recently, Mahadtanapuk *et al.,* (2007) found that *C.musae* caused anthracnose disease on curcuma flowers (*Curcuma alismatifolia* Gagnep). Similar reports were made by Daundasekera *et al.,* (2008); Da Silva *et al.,* (2008) and Niroshini and Karunaratnte, (2009) in mango, avocado and guava respectively. Therefore, this study also forms the first report of a cucurbitaceous vegetable being infected by *C. coffeanum* and *C. musae*. However confirmation on the identity of the two species by molecular characterization is essential as a definite identification of *Colletotrichum* sp. based on morphology is difficult on account of the overlapping ranges of conidial and colony characters of isolates and also because the variations in morphology were accepted for isolates within a species as reported by Sutton, (1992). In this context it is necessary to highlight the need for further molecular characterization in order to confirm the identities of the pathogenic isolates of anthracnose disease of snakegourd obtained during the present study.

Apart from the culture studies for identification of the pathogen, the five isolates of anthracnose pathogen obtained in the study were also examined for their growth on natural semi-synthetic and synthetic media, both in solid and liquid states. Among the natural media, semi-synthetic and synthetic media tested both in solid and liquid state, the media *viz.,* oat-meal agar (OMA), potato dextrose agar (PDA) and Richards' media were found to be the best media for growth of all the five pathogenic isolates. Isolate C1 of *C. coffeanum* completed the growth (9 cm dia.) on the three media *viz.,* OMA, PDA and Richards' agar in 5 days while the isolates C2, C3 and C5 of *C. coffeanum* completed growth on the same media within a period of 6-8 days. The isolate C4 of *C. musae* required 9 days for the completion of growth in the respective media. These results are in confirmation with those obtained in the study of Durairaj, (1956) in case of *C. capsici* and those of Ekbote *et al.,* (1997); Akthar and Singh, (2000); Sudhakar, (2000) and Rani and Murthy, (2004) in case of *C. gloeosporioides* where maximum growth was recorded in PDA medium, Richards'

and oat meal agar. Kenchaiah, (1975) reported highest mycelial growth of *C. gloeosporiodes* in Richards' broth. Hiremath *et al.* (1993) and Ekbote *et al.* (1997) also observed maximum dry mycelia growth of *C. gloeosporioides* in Richards' broth. In the present study fungus reached maximum dry mycelial weight when it was incubated for 10 days,in the above mentioned broth and thereafter it showed decreased dry mycelial weight which indicated the occurance of autolysis. Lilly and Barnett, (1951) reportedphenomenon of antolysis of fungi after the attainment of maximum growth when there cellular enzymes begin to digest the various cell constituents. Ekbote, (1994) and Hiremath *et al.* (1993) also recorded maximum mycelial dry weight in oat meal, PDB and Richards' medium in case of *C. gloeosporioides*. Mesta (1996) and Chidanandaswamy (2001) reported good growth of *C. gloeosporiodes* on Richards' broth. Mello *et al.*, (2004) found that oat meal agar medium was best media for growth of *C. gloeosporioides.*

The influence of exposure to different light sources (alternate cycles of 12 h fluorescent light and 12 h darkness; continuous exposure to fluorescent light for 12 h; continuous exposure for 12 h to Light Emitting Diode (L.E.D) and exposure for 45 min to ultraviolet light followed by normal diurnal conditions on the growth and sporulation of the cultures of the pathogenic isolates were recorded, revealed that all the isolates when exposed to 12 h of alternate light and dark conditions, recorded maximum growth and sporulation compared to exposure to other treatments. Least growth was recorded when isolates were exposed to UV light for a period of 45 min. Chowdhuary (1936) observed that continuous light or darkness inhibited sporulation of *C. graminicola*, while the cultures exposed to alternate light and darkness sporulated earlier. Exposure of *C. gloeosporioides* to alternate light and darkness for a consecutive period of 12 h resulted in maximum growth and sporulation (Kamanna, (1996); Sudhakar, (2000); Alexander *et al.*, (2004); Ashoka, (2005); Narendra Kumar, (2006) and Soltani *et al.*,(2014). Bokhari *et al.*, (2013) reported that exposure

of *C. musae* to UV light for a period of 45 min inhibited mycelial growth of the pathogen.

The most virulent isolate, C1 isolate which was identified *C. coffeanum*, was tested in subsequent studies against different chemical and non-chemical agents and their combinations in order to detect their efficacies in inhibiting the pathogen. The chemicals used in the *in vitro* assay were the fungicides azoxystrobin and mancozeb and the nutrient potassium silicate. Amendment of the medium with recommended dosage (0.1 per cent) of the fungicide azoxystrobin, recorded 61.10 percent reduction of mycelia growth over the control which was increased to 82.56 per cent when the same fungicide was combined with KAU talc based formulation of *P. fluorescens*.

Sundaravadana *et al.*, (2006) observed that the mycelial growth inhibition over the control was more than 60 per cent when three concentrations (0.05, 0.10 and 1 per cent) of azoxystrobin were tested against *C. gloeosporoiodes*. Sundaravadana *et al.*, (2007) studied the *in vitro* efficacy of azoxystrobin on mango leaf and panicle anthracnose pathogen (*C. gloeosporioides*) and reported that azoxystrobin at 1.0 ml, 2.0 ml and 4.0 ml/l showed 65.39 per cent, 68.29 per cent and 69.62 per cent mycelial inhibition respectively. Anand *et al.*, (2010) reported that azoxystrobin (0.1 per cent) resulted in minimum fruit incidence in chilli. Adhikari *et al.*, (2013) reported that azoxystrobin at (0.1 per cent, 0.50 per cent and 1 per cent) slightly inhibited the mycelial growth of *C. gloeosporiodes* in mango.

The contact fungicide mancozeb was used as treated check as it is used widely used for the management of anthracnose disease. There was 100 per cent inhibition of mycelia growth when the fungicide, mancozeb at recommended concentration (0.4 per cent) was amended in the growth medium. Srivastava and Soni (1993) reported that mancozeb (0.25 per cent) was effective for control of anthracnose pathogen both under laboratory and field conditions. The low dose of 100 mg mancozeb / l PDA reduced the growth of the pathogen by 49.21 per cent after 7 days, which remained

the same even after 14 days (Hussain *et al.,* 2008). He also reported that the fungicide mancozeb which is a derivative of dithiocarbamic acid is toxic to fungi because they are metabolized to isothiocyanate radicals inside the pathogen cells, which inactivate the –SH group of aminoacids and enzymes. Shinde *et al.,* (2012) reported that mancozeb (300 ppm concentration) showed highest inhibitory effect on *C. capsici* and recorded maximum inhibition of 100 per cent and least mycelia dry weight. Sileshi *et al.,* (2014) reported that when three synthetic fungicides at different concentrations were evaluated by the poisoned food technique against the bean anthracnose pathogen *C. lindemuthianum,* there was least mycelia growthof the pathogen in medium amended with mancozeb at 250 ppm and there was no growth at all, of the mycelium in media amended with mancozeb at 500 ppm compared to the other two fungicides *viz.,* folpan.

There was hundred per cent inhibition of mycelial growth of *C. coffeanum* when the medium was amended with potassium silicate (0.5 per cent). Potassium silicate had direct inhibitory effect on fungal growth of *Colletotrichum* sp. (Kaiser *et al.,* 2005 and Bekker *et al.,* 2009). Soluble potassium silicate completely suppressed the mycelial growth of *C. gloeosporiodes* at a concentration of 40 ml / l (Polanco *et al.,* 2014). Bekker *et al.,* (2013) reported that the complete mycelial inhibition of the anthracnose pathogen when it was amended with different concentrations of potassium silicate due to the effect of higher pH resulting from the addition of the nutrient.

The present study indicated the remarkable antagonistic activity of KAU talc based formulations of bio-control agents *P. fluorescens* and *T. viride* under *in vitro* conditions. Amendment of medium with *P. fluorescens* (2 per cent) inhibited the mycelial growth of *C. coffeanum* by 88.83 per cent and with *T. viride* (2 per cent) by 87.33 per cent respectively. Capacity of the *C. gloeosporioides* by the bio-control agents *viz., P. fluorescens* and *T. viride* had been suggested as early as in 1976 by Lenne and Parbery. Several plant growth promoting fungal antagonists such as

Trichoderma spp. (Papavizas, 1980) and rhizhobacteria such as *P. fluorescens* (Kloepper *et al.*, 1992) had been reported to be promising in controlling various plant pathogens.

Antagonist fungi especially *Trichoderma* spp. and the bacteria *P. fluorescens* have been widely used for the control of a number of plant pathogens (Rini and Sulochana, 2007). Ngullie *et al.*, (2010) tested seven antagonists against *C. gloeosporioides*, and observed that *P. fluorescens* (2 per cent) exerted the maximum inhibition (67.42 per cent) of mycelial growth of pathogen followed by *T. viride* (2 per cent) and *Bacillus subtilis* (2 per cent) that inhibited mycelial growth of *C. gloeosporioides* by (63.34 per cent) and (56.86 per cent) respectively, compared to the control. Chidanandaswamy, (2001) reported that *P. fluorescens* inhibited the growth rate of *C. capsici* causing leaf spot of turmeric followed by the fungal antagonists *viz.*, *T. harzianum* (Rifai) and *T. viride* (Pers) under *in vitro* conditions. *P. fluorescens* (Pf1) was a potential bio-control agent used for to control of majority of diseases by seed, soil and foliar treatments, mainly due to its efficient antagonistic activity against various phyto-pathogens (Bharathi *et al.*, 2004). According to several studies, the different compounds like HCN, siderophore, chitinase and protease produced by *P. fluorescens* are known to inhibit mycelial growth in several *Colletotrichum* sp. (Vidhyasekaran *et al.*, 1997 and Viswanathan and Samiyappan, 1999). Mixing of culture filtrates of bio-agents with fungicides to control plant pathogens, have been reported (Fan and Tian, 2001 and Yoshida *et al.*, 2001). Barathi *et al.*, (2004); Srinivas *et al.*, (2006); Muthukumar *et al.*, (2010) and Anand *et al.*, (2010) reported that *P. fluorescens* (2 per cent) strongly inhibited the growth of *C. capsici* under *in vitro* conditions. *P. fluorescens* produced compounds like pseudobactin, HCN, Salicylic acid, pyrolnitrine, pyocyanin which induced systemic resistance in host plant or showed specific interference with fungal pathogens (Ongena *et al.*, 1999; Velazhahan *et al.*, 1999; Dave and Dube, 2000; Gupta *et al.*,

2001; Pandey *et al.,* 2006; Hofte and Bekker, 2007; Reddy *et al.*, 2008 and Muthukumar *et al.,* 2010).

Antagonistic activity of *Trichoderma* for controlling wide range of microbes was well studied, documented and demonstrated for more than seven decades ago (Weindling, 1934). Sivakumar *et al.,*(2000) studied the effect of *T. koningii*, *T. harzianum* and *T. viride* on *C. coccodes* and reported that *T. koingii* exhibited highest antagonism against the pathogen. Mandeep *et al.,* (2006) recorded that *T. viride* reduced the mycelial growth of *C. capsici* by 52.5 per cent followed by *T. virens* (38.12 per cent). Antagonistic ability of *Trichoderma* isolates against *C. capsici* causing anthracnose in chilliwere recorded in many earlier studies (Jeyalakshmi and Seetaraman, 1996 and Srinivas *et al.,* 2006). Intana *et al.,* (2007) tested two species of *Trichoderma* (*T. viride and T. harzianum*) against *C. capsici* and recorded mycelial inhibition of 83.00 and 75.50 per cent respectively. Padder *et al.,* (2010) reported that *T. viride* and *T. harzianum,* recorded 69.21 per cent and 64.20 per cent mycelia growth inhibition against *C. lindemuthianum* respectively.

Inhibition of mycelial growth was attributed to mycoparasitism and antagonism (Sturz *et al.,* 1998). *Trichoderma* strains have the ability to produce several lytic enzymes such as chitinases and 1, 3-β-glucanases (Horvath *et al.*, 1998). These enzymes played a key role in the lysis of cell walls of plant pathogens during the antagonistic action of *Trichoderma* spp. (Jeyalakshmi and Seetharaman, 1999 reported that *Trichoderma viride* reduced the mycelial growth of *Colletotrichum* sp. either by growing over the pathogen, or causing coiling, lysis and abnormalities on the hyphae. Strains of *Trichoderma* produced antifungal metabolites which had the ability to suppress the mycelial growth of the phyto-pathogens (Viterbo *et al.*, 2002). Volatile component of *Trichoderma* sp. suppressed the mycelial growth of *C. capsici* as reported by Ajith *et al.,* (2010). The antimicrobial metabolites produced by *T. viride* were effective against several plant pathogens like *C. lagenarium, C. acutatum* and *C. gloeosporiodes.*

The treatments tested for inhibition of mycelia growth of *C. coffeanum*, were also evaluated for their effects on germination of spores of the fungus. Azoxystrobin (0.1 per cent) recorded 2.00 per cent mean spore germination with 96.00 per cent reduction over the control. Mode of action of new generation fungicide, azoxystrobin is by blocking the transfer of electrons from cytochrome b to cytochrome c thereby creating an energy deficiency which leads to fungal death. Evidence of this effect on fungi was observed in spore mortality and spore inhibition Harrison and Tedford, (2002). Hsiang *et al.*, (2004) reported that azoxystrobin at 1.0-100 µg a.i / ml eliminated germination of uredospores of *P. hemerocallidis*. Conidial germination and appressorial formation in black rot pathogen (*G. bidwelli*) of grape was inhibited by azoxystrobin as reported by Hoffman and Wilcox, (2003). Archana, (2009) reported that as the concentration of the fungicide increased, the extent of inhibition of sporangial germination also increased. Complete inhibition of the sporangial germination of *P. viticola* was recorded by the addition of azoxystrobin from 300 ppm onwards. Ahiladevi *et al.*, (2014) reported that azoxystrobin at 0.12 per cent was very effective in reducing mycelia growth, dry weight and spore germination by 83.27 per cent, 69.78 per cent, 81.39 per cent respectively. Spore germination and zoospore motility are developmental stages of fungi that are particularly sensitive to azoxystrobin (Leinhos *et al.*, 1997 and Bartlett *et al.*, 2002). Therefore the study also revealed the azoxystrobin inhibited spore germination more than the mycelial growth of the pathogen.

As far as potassium silicate (0.5 per cent) was concerned, it recorded 8.66 per cent mean spore germination resulting in 82.68 per cent reduction over the control when compared to other treatments. Wainwright, (1993) reported that the potassium silicate acted as a nutrient source and stimulated spore formation and germination of *C. gloeosporiodes*. Singh *et al.*, (1990) also observed that potassium silicate acted as a nutrient source and thereby increased the conidial germination (7.25 per cent) of *C. gloeosporiodes* while the fungicide mancozeb resulted in a mean spore germination

of 2.20 per cent with 96 per cent reduction compared to control. Imtiaj *et al.*, (2005) reported that mancozeb completely inhibited conidial germination of *C. gloeosporiodes* when spore suspension was treated at high concentration of 500 ppm.

Meanwhile KAU talc based formulations of *T. viride* and *P. fluorescens* also inhibited spore germination of the pathogen by 97.34 per cent and 96.68 per cent respectively. Govindasamy and Balasubramanian (1989) reported the reduction in germination rate of uredospore of *P. arachidis* when treated with *T. viride*. Iqbal *et al.*, (1994) observed that the antagonistic fungi *T. harzianum* was effective in suppressing the spore germination of *C. falcatum*. Secretions of enzymes such as endochitinase, chitobiosidase glucan 1,3 β galactosidase by *Trichoderma* spp. strongly inhibited the spore germination of plant pathogens during infection (Tronsmo and Hjeljord, 1997). Similar reports was also made by Jeyalakshmi *et al.*, (1998) who observed the effect of *Trichoderma* in inhibiting the spore germination of *C. capsici* which was only 1.34 per cent. Intana *et al.*, (2007) reported that two wild strains of *T. harzianum* inhibited conidial germination of *C. gloeosporiodes* and observed that antifungal metabolites were responsible for spore inhibition. *C. capsicii* recorded 1.56 per cent mean spore germination when it was amended with two per cent formulation of *P. fluorescens* in chilli (Bharathi *et al.*, 2004). Sanders *et al.*, (2011) suggested that besides the inhibition of mitochondrial respiration, spore germination and mycelia growth, azoxystrobin also has antisporulant activity and therefore, it could be used as a protectant, curative and eradicative material due to its translaminar and systemic properties.

Distinct variations were observed in the size and shape of the conidia due to the different treatments tested *in vitro*. There was reduction in size of conidia from the normal size of 13.14 µm x 4.82 µm to a smaller size that ranged from 10.08 µm - 10.28 µm in length and 4.02 µm - 4.28 µm in width. Morphological studies of *C. gloeosporioides* were also performed earlier by many researchers. Recently, Akthar and Singh (2007); Sangdee *et al.*, (2011); Christopher *et al.*, (2013) and Masoodi *et*

al., (2013) had also observed variability in morphologyas well as pathogenicity among isolates of *Colletotrichum* sp. causing anthracnose in chilli. Zivkovic *et al.,* (2010) reported variation in conidial morphology sch as shape and size of the conidia of *C. gloeosporioides.*

Dual culture method in antagonistic study showing the relationship (interactions) between the two organisms in which one was a bio-control agent, while the second organism was the studied fungal species (Huang and Hoes, 1976).

In vitro assay of bio-control agents on inhibition of growth of the pathogen *C. coffeanum* by dual culture method revealed that the fungal antagonist *T. viride* recorded 90.58 per cent and bacterial antagonist *P. fluorescens* recorded 70.58 per cent mycelial inhibition respectively. *P. fluorescens* was found to be effective against *C. capsici* and recorded 75.60 per cent mycelial inhibition (Suthin *et al.*, 2009). Recently significant reduction in mycelial growth of *Colletotrichum* sp. due to *P. fluorescens* in dual culture were reported by Pallavi *et al.,* (2012).

Michereff *et al.,* (1993) studied the *in vitro* inhibition of *Trichoderma* sp. against *C. gramnicola* in sorghum and reported that there was no interaction in paired culture, but inhibition zones were observed. Benitez *et al.* (2004) isolated chemicals like harzianic acid, tricholin and viridine, which played a vital role in antagonistic behavior of *Trichoderma. Trichoderma* produced certain antimicrobial metabolites which were highly effective against a wide range of fungal plant pathogens such as, *C. lagenarium, C. acutatum* and *C. gloeosporioides* (Yan *et al* 2001 and Svetlana *et al.,* 2010). *In vitro* results obtained using dual culture technique recorded that *T. harzianum* strain IMI 392433 significantly inhibited the mycelial growth, conidial germination, elongation of germ tube and disease severity against *C. capsici* (Rahman *et al.,* 2013).

Effective control of *Colletotrichum* diseases usually involves the use of a combination of cultural control, biological control, chemical control and intrinsic

resistance (Wharton and Dieguez-Uribeondo, 2004). The effective treatments screened in the laboratory assay were tested for their efficacies on snake gourd plants under greenhouse conditions. Results of the experiment revealed that plants sprayed with potassium silicate had low incidence (10.71 per cent) and severity (0.71 per cent) of the disease compared to other treatments and recorded 89. 12 per cent disease suppression when compared to control. Potassium may cause reduction in disease severity by various modes of action, including regulation of photosynthesis, respiration and osmotic pressure regulation (Kettlewell *et al.,* 2000). Sun *et al.,* (2002) reported reduction in incidence of anthracnose disease in cucumber caused by *C. orbiculare* by the application of silicon. Studies conducted in cucumber leaves investigating the process of infection of plants showed that resistance to infection can be acquired by the expression of protein rich in proline together with presence of silica at the site of pathogen penetration (Kauss *et al.*, 2003). An average of 63 per cent reduction of angular leaf spot of bean by the application of potassium silicate was reported by Moraes *et al.,* (2005). Silicon amendments proved to be effective in controlling both soil borne and foliar fungal diseases in cucumber, rice, sugarcane, turf and several other plant species (Datnoff *et al.*, 2007). Polanco *et al.,* (2014) reported that foliar application of potassium silicate resulted in satisfactory control of anthracnose in common bean, probably due to the formation of physical barrier as a result of deposition of silicon on leaf surface or due to the osmotic effect of silicate sprayed on to the leaf. Anthracnose severity in common bean was reduced by 41 per cent by foliar application of potassium silicate.

Plants sprayed with mancozeb also exhibited higher percentage of disease suppression (84.99 per cent) compared to control. Foliar application of mancozeb (0.4 per cent) recorded satisfactory control against *C. coccodes* causing anthracnose in tomato as reported by Johtson (1986). Fungicides zineb (0.2 per cent) and mancozeb (0.4 per cent) were effective against chilli anthracnose as reported by Mallaraju and Swami, (1988). Efficacies of mancozeb (0.2 per cent) spray in reducing the disease

incidence in chilli were reported by Das and Mohanthy (1988) and Paramasivan *et al.,* (2008). Foliar application of 0.4 per cent mancozeb recorded 65.50 per cent disease suppression in common bean (Amin *et al.,* 2014).

Plants sprayed with KAU talc based formulations of *T. viride* and *P. fluorescens* recorded 49.31 per cent and 50.38 per cent disease reductions respectively. Management of diseases in different crops by the bacterial bio-control agent *P. fluorescens* either as bacterial suspension or through different formulations have been highlighted in several earlier reports of Vidhyasekharan *et al.,* (1997) and Viswanathan and Samiyappan, (1999). Ngullie *et al.*, (2009) evaluated the efficacy of plant disease antagonists and reported that *T. viride* (2 per cent) showed a disease reduction of 61.41 per cent in chilli. *T. viride* isolates were also reported to control anthracnose disease of chili (Intana *et al.*, 2007). Ramkumar *et al.,* (2012) reported 12 percent disease index and 48.12 per cent disease incidence in case of turmeric anthracnose when plants were sprayed with *P. fluorescens* (2 per cent). This suppression of disease was attributed to several mechanisms pertaining to *P. fluorescens* such as the impact of antifungal compounds produced due to systemic resistance induced in the host plant. *Trichoderma* strains significantly reduced anthracnose disease in chilli by 69.52 - 81.39 per cent (Rahman *et al.*, 2013).

Effect of different treatments on growth parameters of snake gourd plants revealed that plants treated with foliar application of potassium silicate (0.5 per cent) recorded an average shoot length of 327 cm, root length of 15.30 cm and produced an average of 86 numbers of leaves per plant and was significantly better than other treatments. This was followed by foliar spray of mancozeb 0.4 per cent (325 cm shoot length, 14.70 cm root length and 82 leaves per plant) which gave better result than plants sprayed with KAU talc based formulation of *P. fluorescens* (2 per cent) (314 cm shoot length, 13.20 cm root length, 80 leaves per plant) and basal application of KAU talc based formulation of *T. viride* (2 per cent) which recorded 296 cm shoot length, 11.40 cm root length and produced an average 78 number of leaves per plant

compared to control plants. Effects of all the treatments on growth parameters were significantly different from each other and were superior to control. There was significant increase in plant growth, leaf area index (LAI) and leaf area duration (LAD) in common bean due to foliar application of potassium silicate (Polanco *et al.*, 2013). Separate foliar application of *P. fluorescens* (2 per cent) and mancozeb (0.4 per cent) at 30 days after transplanting increased mean plant height, number of leaves and flowers per plant in a study conducted to control anthracnose of chilli (Suthin *et al.,* 2013). Growth promoting effects by *Trichoderma* has been reported in crops like cucurbits by Chang *et al.*, (1986) and Windham *et al.,* (1986). Fungal antagonist *Trichoderma* increased average shoot length, root length, shoot weight and root weight, vigour index of the crops *viz.,* sweet gourd, snake gourd, cowpea, cucumber and okra (Yeasmin, 2004). Ninteen isolates of *Trichoderma* promoted growth of cucumber up to 100 per cent and extended protection to cucumber from anthracnose disease up to 88.3 per cent when given as basal application (Veronica *et al.,* 2011).

As a result of the extensive use of fungicides for a very long time, pathogens have gradually acquired resistance to those chemicals. Moreover, the fungicide residues have had bad effects on both human health and the environment (Zhang *et al.*, 2015). On account of fungicide residues detected in harvested cucumber fruits, it is necessary to find new alternatives of control like natural products, mineral salts, bio-agents and others which are considered to be more effective and safe for environment and human health (Farias *et al.*, 2011). Many investigators used potassium silicate as foliar or soil applications for reducing the severity of powdery mildew in cucumber, grape, strawberry, tomato and wheat (Lee *et al.,* 2000; Yildirim *et al.,* 2002; Be'lange *et al.,* 2003; Kanto *et al.,* 2006 and Yan *et al.,* 2006). Besides, foliar and root applications of silicon reduced the severity of the powdery mildew on cucumber leaves (Lee *et al.,* 2000).

The present study has also revealed the prospects of utilizing the mineral nutrient, potassium silicate for control of anthracnose disease as well as increase in

bio-metric characters of snake gourd which is an important vegetable crop of Kerala. Besides, this study also has highlighted the scope for integrating the fungicide mancozeb with bio-control agents like *P. fluorescens* and *T. viride* which were was also highly beneficial for the disease management as well as in improving the growth parameters of the crop under green house conditions. Such integration of fungicide mancozeb with bio-control agents will also be useful in minimizing the detrimental effects of continous and intensive use of this fungicide which is otherwise very effective in disease management. In the background of the promising results obtained from this thesis project especially from the use of potassium silicate, trials for confirming the beneficial effects of the treatments in field grown snake gourd plants would be useful for the vegetable growers in Kerala.

SUMMARY

6. SUMMARY

The study entitled "Integrated management of anthracnose in snake gourd (*Tricosanthes cucumerina* L.)" was conducted at the College of Agriculture Vellayani from 2014 to 2016, encompassing the main objective of making a comparative evaluation of the efficacy of foliar application of bio-control agents and newer chemical molecules for the management of anthracnose diseases of snake gourd.

Surveys were conducted in five locations of snake gourd fields in and around College of Agriculture, Vellayani, Thiruvananthapuram district. Symptoms of anthracnose were predominant in snake gourd fields of different locations *viz.*, Instructional Farm, College of Agriculture, Vellayani (Location I), Department of Olericulture, College of Agriculture, Vellayani (Location II), Kalliyoor (Location III), Kakamoola (Location IV) and Palapoor (Location V) where an average of more than 50 per cent disease incidence was observed when 50 plants were examined in each location.

Nature of symptom development was recorded on the pre-tagged plants in each of the surveyed fields. The disease appeared on leaves as small circular spots that coalesced to form large elliptical spots and under severe conditions, defoliation of affected plant occurred. Variations in symptom expression were noticed in location-IV (snake gourd fields of Kakkamoola) where brown irregular lesions on the leaves changed to concentric ring like appearance which is generally referred to as target board symptom. Initial appearance of anthracnose symptoms were observed in lower leaves of the plants and symptoms were severe on third to fifth leaves from bottom of each plant. Maximum mean disease incidence (90.00 per cent) and mean percentage disease index (44.22) were recorded in the snake gourd fields of Instructional Farm - College of Agriculture, Vellayani while in snake gourd fields of Palapoor, mean disease incidence (70.00 per cent) and mean percentage disease index (21.89) were minimum.

Laboratary studies pertaining to etiology of the disease were conducted by both microscopic examinations of leaf samples collected from surveyed locations as well as by isolation of the pathogen from these specimens. Five isolates of the pathogen designated as C1, C2, C3, C4 and C5, produced the typical diagnostic symptoms of anthracnose in pathogenicity tests conducted under *in vitro* conditions on detatched leaf and on leaves of 30-days old potted plants of cultivar Kaumudi. Re-isolation from these specimens yielded fungal cultures that were morphologically similar to original isolates that were inoculated.

Among the different isolates tested for pathogenicity studies, the isolate C1 obtained from Instructional Farm, College of Agriculture, Vellayani was most virulent resulting in maximum lesion size of 2.53 cm^2 on detatched leaf and 10.06 cm^2 on leaves of potted plants whereas the minimum lesion size of 2.03 cm^2 on detatched leaf and 7.63 cm^2 on leaves of potted plant were recorded when leaves were inoculated with the isolate of isolate C4 obtained from Kakkamoola. C1 isolate also recorded the shortest incubation period and disease development period, and was therefore screened as the most virulent isolate for conducting subsequent studies on integrated management of the disease.

Microscopic examinations of the five isolates conducted in the morphological study revealed that the isolates produced septate hyaline hyphae of width ranging from 1.92 µm to 3.86 µm and cylindrical to ellipsoidal conidia ranging in size from 10.52 µm to 13.14 µm X 4.02 µm to 4.40 µm. Average width of the acervuli of five isolates ranged from 82.52 µm to 123. 25 µm, bearing 6 to 15 numbers of setae. Culture studies of all the five isolates indicated the growth of creamy white to greyish coloured mycelium, with light to dark orange coloured spore masses, on PDA. Results of the studies on morphological and cultural characters confirmed that all the five isolates belonged to the *Colletotrichum gloeosporoides* based on similar characters reported earlier (Bose *et al.*, 1973; Chowdappa *et al.*, 2012 and Shilpa, 2015).

Comparative assessments of the different isolates were also made in the cultural and morphological studies. Cultural studies revealed that oat meal medium, potato dextrose medium and Richards' medium were most suitable solid and liquid media for the growth of all the isolates of the pathogen. Exposure of fungal cultures to fluorescent light and darkness, alternatively for a period of 12 h, up to duration of 7 days, resulted in maximum growth and sporulation of all the isolates.

With respect to studies on morphological characters *viz.,* hyphal width, size of conidia and acervuli, it was observed that the isolates C1, C2, C3 and C5 were larger (IF- Vellayani, Department of Olericulture, Kalliyoor and Palapoor) compared to the isolate C4. Similarly, the number of setae on the acervuli was greater in each of the four isolate C1, C2, C3 and C5 when compared to isolate C4. Thus the isolate C4 (snake gourd fields of Kakkamoola) differed slightly from the remaining isolates, in nature of symptom development as well as morphological characters.

Further morphological characterization of the five isolates, that were tentatively identified as *C. gloeosporiodes,* were performed at National Fungal Collection Culture India (NFCCI), Pune. Based on these characterization studies, the identities of isolate C1, C2, C3 and C5 were reveaied as *C. coffeanum* F. Noack and were assigned the accession number NFFCI/2015-8/AKC/2293-03/SKS/DKM while the isolate C4 was identified as *C. musae* (Berk. & M.A. Curtis) Arx and was assigned the accession number NFFCI/2015-8/AKC/2293-07/SKS/DKM. However phylogenetic analysis of these isolates needs to be conducted, in order to confirm their actual identities.

Investigation on the efficacy of certain chemical and biological agent were tested against the most virulent isolate of the pathogen C1 (IF- Vellayani), both in laboratory and under green house conditions *In vitro* test of newer molecule potassium silicate (0.5 per cent) and the fungicide mancozeb (0.4 per cent) recorded 100 per cent inhibition of mycelial growth of isolate C1. Amendment of potato dextrose agar medium with combination of azoxystrobin (0.1 per cent) + KAU talc

based formulation of *P. fluorescens* (2 per cent) resulted in 82.56 per cent mycelial inhibition and was superior to amendment of the medium, merely with azoxystrobin (0.1 per cent) which gave only 61.1 per cent mycelial inhibition. Among the bio-control agents tested, amendment of PDA with KAU talc based formulation of *T. viride* (2 per cent) resulted in 87.33 per cent mycelial inhibition while KAU talc based formulation of *P. fluorescens* (2 per cent) amended in the medium inhibited mycelial growth of the pathogen by 88.83 per cent when compared to control. Least inhibition of mycelial growth was observed in control plates.

Pure cultures of bio control agents with out the addition of carrier material (talc) were also tested against C1 isolate of the pathogen by dual culture technique. Fungal antagonist *T. viride* inhibited growth of the pathogenic isolate by 90.58 per cent and the bacterial antagonist *P. fluorescens* resulted in 70.58 per cent inhibition of mycelial growth of the pathogen.

Impact of the chemical and bio-control agents on the germination and spore characters of the pathogen, were also observed in a separate experiment. Results of the experiment revealed that all the 50 spores in control plates germinated whereas the treatments *viz.,* recommended dosages of KAU talc based formulations of *T. viride* and *P. fluorescens*, the fungicides mancozeb and azoxystrobin and combination of azoxystrobin and KAU, talc based formulation of *P. fluorescens* recorded mean spore germination of 1.33 per cent, 1.66 per cent, 2.33 per cent, 3.00 per cent and 2.33 per cent respectively. Maximum spore germination (8.66 per cent) was recorded, when conidial suspension was treated with potassium silicate at a concentration of 0.5 per cent.

Distinct variations were also observed in the size of the conidia due to different treatments. Conidial size of the pathogen in control was 13.14 µm X 4.82 µm while the size was reduced when the media was amended with the fungicide azoxystrobin (10.28 µm x 4.26 µm, KAU talc based formulations of bio-control agents *viz., T. viride* (10.12 µm x 4.18 µm), *P. fluorescens* (10.08 µm x 4.02µm) and combination

of azoxystrobin + *P. fluorescens* (10.21 µm x 4.23 µm) at their recommended concentrations. Thus the conidia became smaller in size in the media amended with both chemical and biological treatments when compared to those in the control.

From the above *in vitro* experiments conducted, chemical potassium silicate (0.5 per cent), bio control agents *T. viride* and *P. fluorescens* were screened as the most effective treatments in inhibiting the growth of the anthracnose pathogen and were further tested in the pot culture study.

Based on the results of the above laboratory studies, three most effective treatments were screened and tested for their efficacies in controlling anthracnose disease in green house conditions. Percentage disease index / disease severity recorded in different treatments after second treatment application, ranged from 0.71 per cent to 6.53 per cent. Lowest disease severity was recorded in plants sprayed with 0.1 per cent potassium silicate (0.71 per cent) which significantly reduced disease by 89.12 per cent compared to control. Foliar application of 0.4 per cent mancozeb also recorded almost similar percentage reduction of anthracnose disease (84.99 per cent) in green house study.

With regard to bio-control agents, foliar application of KAU talc based formulation of *P. fluorescens* (2 per cent) and basal application of KAU talc based formulation of *T. viride,* (2 per cent) resulted in moderate reduction of disease severity (50.38 per cent and 49.31 per cent) respectively.

Effect of different treatments on growth parameters of snake gourd plants revealed that that foliar application of 0.5 per cent potassium silicate resulted in highest shoot length of 327 cm, root length of 15.30 cm and produced an average number of 86 leaves per plant and significantly improved the growth of the plantwhen compared to other treatments. This was followed by foliar spray of 0.4 per cent mancozeb (325 cm shoot length, 14.70 cm root length, 82 leaves per plant) which was significantly better than foliar spray of KAU talc based formulation of *P.*

fluorescens, 2 per cent (314 cm shoot length, 13.20 cm root length and 80 leaves per plant) and basal application of KAU talc based formulation of *T. viride*, 2 per cent (296 cm shoot length, 11.40 cm root length and 78 number of leaves per plant when compared to control.

Detailed studies on the symptomatology, nature of different isolates of the pathogen and management of the disease have been investigated for the first time in Kerala, through the present study. Slight variations in symptom development of anthracnose were observed in the surveys conducted in different snake gourd fields near Vellayani which corresponded to the variations in the isolates of the pathogen that was characterized as *Colletotrichum gloeosporiodes*. The preliminary trials, conducted both under *in vitro* and *in vivo* conditions, for evaluating chemical compounds and biological agents against the anthracnose pathogen of cucurbitaceous crops, revealed the remarkable inhibiting property of the nutrient potassium silicate, hitherto not reported in Kerala, as well as fungicide mancozeb, against the pathogen C1 isolate of *Colletotrichum* causing anthracnose of snake gourd. The bacteria antagonist, *P. fluorescens* and fungual antagonist *T. viride* though not as effective as potassium silicate, were also prospective components especially for integrated management of the disease. Thus this study opens a new avenue for the development of novel compounds, biological agents and their combinations for an integrated disease management strategy that does not have any negative impact on the environment.

References

7. REFERENCE

Abdel-Kader, M. M., El-Mougy, N. S., El-Gammal, N. G., Abd-El-Kareem, F., and Abd-Alla, M. A. 2012. Laboratory evaluation of some chemicals affecting pathogenic fungal growth. *J. Appl. Sci. Res.* 8: 523-530.

Adhikary, N. K., Dey, S., and, Tarafdar, J. 2013. Studies on morphology of mango anthracnose disease causing fungus *Colletotrichum gloeosporioides* (Penz.) Penz .and Sacc. and efficacy of azoxystrobin against the fungus under *in vitro* and *in vivo* conditions. *The Bioscan.* 8(2): 493–497.

Agrios, G.N. 2005. *Plant Pathology (5th edition).* Elsevier-Academic Press, San Diego, CA, 922 pp.

Ahiladevi, P., Vinothini, K., and Prakasam, V. 2014. Induced systemic resistence against powdery mildew (*Uncinula necator*) by azoxystrobin 8.3 % + mancozeb 64.7 % along with bio-control agent. *Wudpecker J. of Agric. Res.* 3(4): 074 – 080.

Ainsworth, G.C. 1961. *Dictionary of fungi.* Commonwealth Mycological Institute, Kew Surrey, England. 547p.

Ajith, P. S. and Lekshmi, D. N. 2010. Effect of volatile and non-volatile compounds from *Trichoderma* spp. against *Colletotrichum capsici* incitant of anthracnose on bell peppers. *Cult.* 8(9): 265–269.

Akthar, J. and Singh, M. K. 2007. Studies on variability of *Colletotrichum capsici* causing chilli anthracnose. *Indian Phytopathol.* 60 (1): 63-67.

Alexander, F. S., Mello, C., Zamboni, M., and Ivan, P. B. 2004. Development of *Colletotrichum gloeosporioides* isolated from green pepper in different culture media, temperatures and light regimes. *Sci. Agric.* 61(5): 542-544.

Amarjit, K. S. and Chander, M. 2006. Effect of different culture media on the growth and sporulation of *Colletotrichum gloeosporioides* causing guava anthracnose. *Plant Dis.Res.* 21: 224.

Amin, M., Teshele, J., and Tesfay, A. 2014. Evaluation of bio-agents Seed treatment against *Colletotrichum lindemuthianum*, in Haricot Bean anthracnose under field condition. *Res. In Pl. Sci.* 2(1) : 22-26.

Anamika, A., Rhoda, S., and Nath, P., 2014. Survey of anthracnose disease in chilli crop in rewa region. *Int. J. Sci. and Res.* 3 (8): 1851-1854.

Anand, T. and Bhaskaran, R. 2009. Exploitation of plant products and bio-agents for eco-friendly management of chilli fruit rot disease. *J. of Plant Prot. Res.* 49 (2): 186–197.

Anand, T., Chandrasekaran, A., Kuttalam, S., Senthilraja, S., and Samiyappan, R. 2010. Integrated control of fruit rot and powdery mildew of chilli using the biological agent *Pseudomonas fluorescens* and a chemical fungicide. *Biocontrol.* 52: 1-7.

Archana, S. 2009. Studies on the evaluation of azoxystrobin 23 SC against downy mildew and powdery mildew of grapevine. M.Sc. (Ag.) thesis, TamilNadu Agricultural University, Coimbatore, 55p.

Ashoka, S. 2005. Studies on fungal pathogens of vanilla with special reference to *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. M.Sc.(Ag.) thesis, University of Agricultural Science, Dharwad, 62p.

Ashraf, S. and Zuhaib, M. 2013. Fungal biodiversity: A potential tool in the plant disease management. *Indian Phytopathol.* 55 (2): 43-48

Ashutosh, P., Brajesh, K. P., Sailemdar, R., and Lallan, P. Y. 2011. *In vitro* screening for resistance to anthracnose (*Colletotrichum gloeosporioides*) in different mango (*Mangifera indica*) hybrids. *Tunisian J. of plant prot.* 6 (2): 99-108.

Avinash, T. S. and Ravishankar, V. 2013. Identification of diverse fungi related with selected cucurbitaceous vegetables. *J. of Agric. Techol.* 9 (7): 1837–1848.

AVRDC. 2002 – Pepper diseases- Anthracnose. *AVRDC International Co-operators 2011* [on-line]. Available: http://www.avrdc.org/LC/ pepper/anthracnose.html. [08 June 2003].

Azad, I. and Azad, A. 2015. Effect of biocontrol agents against *Colletotrichum capsici* causing anthracnose of chilli (*Capsicum annuum* L.). *Int. J. of Biol, Pharmacy and Allied Sci.* 4 (5): 3194–3201.

Bartlett, D. W., Clough J. M., Godwin J. R., Hall, A. A., Hamer, M., and Parr-Dobrzanski, B. 2002. The strobilurin fungicides. *Pest Manag. Sci.* 58: 649–662.

Be´lange, R. R., Benhamou, N., and Menzies, J. G. 2003. Cytological evidence of an active role of silicon in wheat resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici). Phytophatol.* 93: 402-412.

Bekker, T. F., Kaiser, C., and Labuschagne, N. 2009. The antifungal activity of potassium silicate and the role of pH against selected plant pathogenic fungi *in vitro. S. Afr. J. Plant Soil* 26(1): 25-26.

Benitez, M .S., Tustas, F. B., Rotenberg, D., Kleinhenz, M. D., Cardina, J., Stinner, D., Miller, S. A and Gardener, B. B. M. 2007. Multiple statistical approaches of

com munity fingerprint data reveal bacterial populations associated with general disease suppression arising from the application of different organic field management strategies. *Soil Biol. and Biochem.* 39: 2289-2301.

Bharathi, R., Vivekananthan, R., Harish, S., Ramanathan, A., and Samiyappan, R. 2004. Rhizobacteria based bio-formulations for the management of fruit rot infection of chillies. *Crop Prot.* 23: 835-843.

Bokhari, N. A., Siddiqui, I., Siddique, K. P. I., Rizwana, H., and Soliman, D. A. W. 2013. Management of anthracnose of banana by UV irradiation. *J. of Anim. and Plant Sci.* 23 (4): 1211–1214.

Bose, S.K., Sindhan, G. S., and Pandey, B. N. 1973. Studies on the die back disease of mango in the Tarai region of Kumaon. *Progressive Hortic.* 5:41-53.

Cai, L., Hyde, K. D., Taylor, P. W. J., Weir, B. S., Waller, J., Abang, M. M., Zhang, J. Z., Yang, Y. L., Phoulivong, S., Liu, Z. Y., Prihastuti, H., Shivas, R. G., Mc Kenzie, E. H. C., and Johnston, P. R. 2009 . A polyphasic approach for studying *Colletotrichum*. *Fungal Diversity.* 39: 183–204.

Cannon, P. F., Buddie, A. G. and Bridge, P. D. 2008. The typification of *Colletotrichum gloeosporioides*. *Mycotaxon* 104: 189-204.

Chang, Y. C., Baker, R., Kleifeld, O. and Chet, I. 1986. Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. *Plant Dis.* 70: 145–148.

Chidanandaswamy, B. S. 2001. Studies on *Colletotrichum capsici* (Syd.) Butler and Bisby causing leaf spot of turmeric (*Curcuma longa L.*), M.Sc. (Ag) thesis, University of Agricultural Sciences, Dharwad, 63p.

Chowdhuary, S. 1936. A disease of *Zea mays* caused by *Colletotrichum graminicola* (Ces) Wilson. *Indian J. Agric. Sci.* 6: 833-843.

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

Christopher, D. J., Sujith R. T., and Suha, K. R. 2013. Morphological, pathological and molecular variability of *Colletotrichum capsici* causing anthracnose of chilli. *Ann. Pl. Prot. Sci.* 2 (2): 309-514.

Corda, A. C. I. 1831. *Die Pilze Deutschlands* (ed. J.Sturm). Deutschlands Flora, *Abtheilung.* 3: 1-144.

Daykin, M. E and Millholand, R. D. 1984. Ripe rot of muscadine grapes caused by *Colletotrichum gloeosporides* and its control. *Phytopathol.* 74 : 710-714.

Da Silva, M. B., Da Costa, A. S. V., Rufini, J. C. M., Galvao, E. R. and Zambolim, L. 2008. Thermotherapy and prochloraz to control anthracnose on 'Prata Ana' bananas in post harvest conditions. *Summa Phytopathologica.* 34: 364-365.

Damm, U., Cannon, P. F., Liu, F., Barreto, R. W., Guatimosim, E., and Crous, P. W. 2013. The *Colletotrichum orbiculare* species complex: Important pathogens of field crops and weeds. *Fungal Diversity.* 61 (1): 29-59.

Das, S. R. and Mohanty, K. L. 1988. Management of die back/ twig blight of chilli with fungicides. *Indian J. Pl. Prot.* 16: 109-111.

Datnoff, L. E., Snyder, G. H. and Korndörfer, G. H. 2001. Silicon in Agriculture. *Am. J. of Phtopathol.* 17: 132-140.

Datnofft, L. W., Elmer. N., and Huber, D. 2007. Mineral nutrition and plant disease. *Am. J. of Phtopathol.* 23 : 32-34.

Daundasekera, W. A. M., Joyce, D. C., Adikaram, N. K. B. and Terry, L. A. 2008. Pathogen-produced ethylene and the *Colletotrichum musae*-banana fruit pathosystem. *Aust. Plant Pathol.* 37: 448-453.

Dave, D. P. and Dube, H.C. 2000. Detection and chemical characterization of siderophores of rhizobacterial fluorescent *pseudomonads*. *Indian Phytopathol.* 53:97-98.

Delacroix, G. 1897. De la tavelure goyaves produite par le *Gloeosporium psidii* nov. sp. G. Del. *Bulletin de la Société Mycologique de France.* 19: 143–145

Dennis, C. and Webster, J. 1971. Antagonistic properties of species group of *Trichoderma* production of non-volatile antibiotics. *Trans. Br. Mycol. Soc.* 57: 41-48.

Denobys, B. and Baudry, A. 1995. Species identification and pathogenicity strictly of *Colletotrichum* strains isolated from strawberry using morphological and cultural characteristics. *Phytopathol.* 85: 53-57.

Ding, Y., Chu, J., Gu, Z., and Zhuang, Q. 2007. Effect of different factors on growth and spore production of a *Colletotrichum* sp., a potential biocontrol agent for waterhyacinth. *J. Aquat. Plant Manag.* 80(2) : 233-234.

Durairaj, V. 1956. Growth of *Colletotrichum capsici* in pure culture. *J. Indian Bot. Sci.* 35: 409-413.

Egel, D. S. 2014. Vegetable diseases: Anthracnose of cucumber, muskmelon, and watermelon. *Phytopathol.* 103 (2): 180-183.

Ekbote, S. D., Padaganur, G. M., Patil, M. S. and Chattannavar, S. N. 1997. Studies on the cultural and nutritional aspects of *Colletotrichum gloeosporioides*, the causal organism of mango anthracnose. *J. Mycol. Pl. Path.* 27: 229-230.

Epstein, E. 1999. Silicon. *Annu.Rev. Plant Physiol. Plant Mol.Biol.* 50: 641- 664.

Fan, O. and Tian, S. 2001. Postharvest biological control of grey mold and blue mold on apple by *Cryptococcus albidus* (Saito) Skinner. *Postharvest Biol. and Technol.* 21: 257-358.

FAOSTAT (online) (2010). *Food and Agriculture Organization of the United Nations* - Rome, Italy. http://faostat.fao.org/default.aspx?lang=en.

Farias, L. J, and Orozco, S. M. 2011. Effect of polyethylene mulch color on aphid populations, soil temperature, fruit quality, and yield of watermelon under tropical conditions. *New Zealand. J. of Crop and Hortic. Sci.* 25 :369-374

Farr, D. F. and Rossman, A. Y. 2013. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved April 5, 2013, from http://nt.ars-grin.gov/fungaldatabases/

Firman, I. D. and Waller, J. M. 1977. Coffee berry disease and other *Colletotrichum* diseases of coffee. *Phytopathol.* 20 (2): 12-14.

Freeman, S. and Katan, T. 1998. Identification of *Colletotrichum* species responsible for anthracnose and root necrosis of strawberry in Israel. *Phytopathol.* 87: 516-521.

Freeman, S., Shabi, E. and Katan, T. 2000. Characterization of *Colletotrichum acutatum* causing anthracnose of anemone (*Anemone coronaria* L.). *Appl. and Environ. Microbiol.* 66: 5267-5272.

Gautham, A. K. 2014. The genera *Colletotrichum*: an incitant of numerous new plant diseases in India. *J. of new biol. repots*. 3 (1): 09-21.

Georgakopoulos, D. G., Fiddaman, P., Leifert, C. and Malathrakis, N. E. 2002.Biological control of cucumber and sugar beet damping–off caused by *Pythium ultimum* with bacterial bacterial and fungal antagonists. *J.of Appl. Microbiol.* 92:1078-1084.

Goncalves, M., Paiva, T., Ramos, T. and Guilleminault, C. 1997.Anthracnose of curcubits. *Am. J. of phytopathol.* 125: 2091-2096

Govindasamy, V., and Balasubrarnanian, R. 1989. Antifungal metabolites from *Trichoderma harzianum*. *J. of Nat. Products*. 50: 1799-1809.

Gubler, W. D., and Eastburn, D. M. 1988. Research progress report: Anthracnose in California. *Adv. Strawberry Prod.* 7: 47-50.

Gupta, S. K., Gupta, P. P., and Kaushik, C. D. 2001. Changes in leaf peroxidase, polyphenol oxidase, catalase and total phenols due to Alternaria leaf blight in *Brassica* species. *Indian J. Mycol. Plant Pathol.* 25: 175-180.

Handique, P. K., Das, S., Dutta, P., and Chakravorty, R. 2008. Leaf spot disease in clones of *Perseabombycina* caused by *Colletotrichum gloeosporioides*. *J. Mycol Pl Pathol.* 38: 322 -324.

Harrison, S. and Tedford, E. 2002. Quadris, a novel fungicide for disease control in rice. *In:Proceeding of the temperate rice conference*, 29-31 January 2002, IRRI . pp. 289-294.

Hiremath, S.V., Hiremath, P.C. and Hedge, R. K. 1993.Studies oncultural characters of *Colletotrichum gloeosporioides* a causal agent of Shisham blight in Karnataka *J. Agric. Sci.* 6: 30-32.

Hindorf, H., 1970. *Colletotrichum* ssp. isolated from *Coffea arabica* L. in Kenya. Z. PflKrankh. *PflPath. Pfl. Schutz.* 77: 328–331.

Hoffman, L. E. and Wilcox, W. F. 2003. Factors influencing the efficacy of myclobutanil and azoxystrobin for control of grape black rot. *Plant Dis.* 87: 273-281.

Hofte, M. and Bakker, P. A. 2007 .Competition for iron and induced systemic resistance by siderophore of plant growth promoting rhizobacteria. *Soil Biol.* 12: 121-133.

Holliday, P. 1980. *Fungal diseases of tropical crops.*Cambridge university press, Cambridge. pp 22-30.

Horvath D. M., Huang D. J., and Chua N. H. 1998. Four classes of salicylate-induced tobacco genes. *Mol. Plant-Microbe Interaction.* 11: 895–905.

Hossain, M. F., Ara, N., and Akhter, B. 2013. Characterization and evaluation of snake gourd (*Trichosanthes anguina* L) genotypes. *Wudpecker J. of Agric. Res.* 2 (12): 330 – 334.

Hossain, M. T., Hossain, M. M., Baker, M. A., Rahman, A. K. M., and Uddin, S. N. 2010. Survey on major diseases of vegetable and fruit crops in Chittagong region. *Bangladesh J. Agric. Res.* 35 (3) : 423-429.

Hsiang, T., Cooks, S., and Zhao, Y. 2004. Studies on biology and control of lily rust in Canada. *The Daylily J.* 59: 47-57.

Huang, H. C. and Hoes, J. A. 1976. Penetration and infection of *Sclerotinia sclerotiorum* by *Coniothyrium minitans*. *Can J of Pl Pathol.* 54: 406–410.

Hubballi, M., Nakkeeran, S., Raguchander, T., Anand, T., and Renukadevi, P. 2011. Physiological characterisation of *Colletotrichum gloeosporioides*, the incitant of anthracnose disease of noni in India. *Arch. of Phytopathol and Plant Prot.* 44 (11): 1105–1114.

Hussain, A., Razik, F., and Khan, H. 2011. Integrated control of *Colletotrichum gloeosporioides* with biological and chemical agents in tomato. *Sarhad J. Agric.* 28 (1): 45-48.

Hussain, A., Raziq, F., and Khan, H. 2008. *In vitro* integrated control of *Colletotrichum gloeosporioides* with biological and chemical agents. *Sarhad J. of Agric.* 24 (1): 79-84.

Hyde, K. D., Cai, L., McKenzie, E. H. C., Yang, Y. L., Zhang, J. Z., and Prihastuti, H. 2009. *Colletotrichum*: a catalogue of confusion. *Fungal Diversity.* 39: 1-17.

Imtiaj, A., Rahman, S. A., Alam, S., Parvin, R., Farhana, K. M., Kim, S.B., and Lee, T. S. 2005. Effect of fungicides and plant extracts on the conidial germination of *Colletotrichum gloeosporioides* causing mango anthracnose. *Mycobiol.* 33 (4): 200-205.

Intana W, Suwanno T, Chamswarng C, Chantrapromma K, Ngamriabsakul. 2007. Increased efficacy for controlling anthracnose of chili using antifungal metabolites from mutant strains of *Trichoderma harzianum*. *Thai J. of Agric. Sci.* 40 (1-2): 65-72.

Issac, S. 1992. *Fungal Plant Interaction (2nd Edition.)*. Chapman and Hall press, London, 115p.

Iqbal, S. M., Rauf, C. A., Rahat, S., Akhtar, C. M. 1994. Antagonist to *Colletotrichum falcatum*, the cause of sugarcane red rot. *Sarhad J. of Agric.* 10: 575–579.

Jalal, S., Mahsa, Y. P. H., and Sonbol, N. 2014. Light, temperature, and aging dependent vegetative growth and sporulation of *Colletotrichum gloeosporioides* on different culture media. *J. of Med. Plants Res.* 8 (4): 208–216.

James, W. C., Shih, C. S., Hodgson, W. A., and Callbeck, L. C. 1973. A method for estimating the decrease in marketable tubers caused by potato late blight. *Am. Potato J.* 50: 19-23.

Jeffrey, C. 1990. An outline classification of the cucurbitaceae, In: Bates, D.M., Robionson, R.W. and Jeffrey C. (Eds.). Biology and utilization of the Cucurbitaceae, Ithaca and London: Cornell University. pp. 449-463.

Jeun, Y. C., Lee, Y. J., Kim, K. W., Kim, S. J., and Lee, S. W. 2008. Ultrastructures of *Colletotrichum orbiculare* in the leaves of cucumber plants expressing induced systemic resistance mediated by *Glomus intraradices* BEG110. *Am. Phytopathol.* 36 (4): 236–241.

Jeyalakshmi, C. and Seetharaman, K. 1999. Biological control of fruit rot and die-back of chilli with plant products and antagonistic microorganisms. *Plant Dis Res.* 13: 46-48.

Jeyalakshmi, C. and Seetharaman, K. 1999. Variation in *Colletotrichum capsici* isolates causing fruit-rot and die-back of chilli. *J. Soils Crops.* 4: 88.

Jeyalakshmi, C., Durairaj, P., Seetharam, K. and Sivaprakasam, K. 1996. Biocontrol of fruit rot and die back of chilli using antagonistic microorganisms. *Indian Phytopathol.* 51 (2): 180-183.

Jinyoung, L., Lim, T. H. and Cha, B. 2002.Isolation and identification of *Colletotrichum musae* from imported bananas. *Am. Phytpathol.* 30 (3): 361-363.

Johtson, S. A. 1986. Fungicide and Nematicide update: Current status of fungicide testing on vegetable crops. *The Am. Phytopathol. Soc.* 45 (2): 72-74.

Kaiser, C., Bekker, T. F. and Labuschagne, N. (2005).*In vitro* inhibition of mycelial growth of several phytopathogenic fungi, including *Phytophthora cinnamomi* by soluble silicon. *S. Afr. J. Plant Soil.* 23: 169-172.

Kamanna, B. C. 1996. Epidemiology and control of anthracnose diseasesof coffee incited by *Colletotrichum gloeosporioides* (Penz.)Penz. & Sacc.Ph.D..(Ag.) Thesis, University of Agriculture Science, Bangalore, 72p.

Kanchana-udomkarn C., Taylor, P. W. J., and Mongkolporn, O. 2004.Development of a bio-assay to study anthracnose infection of capsicum fruit caused by *Colletotrichum capsici. Agric. Sci.* 37: 293-297.

Kanto, T., Miyoshi, A., Ogawa, T., Maekawa, K., and Aino, M. 2006. Suppressive effect of liquid potassium silicate on powdery mildew of strawberry in soil. *J. Gen. Plant Pathol.* 72: 137-142.

Kauss, K. R., Franke, S., Gilbert, A., Dietrich., and Kroger., N. 2003. Silica deposition by a strongly cationic proline-rich protein from systemically resistant cucumber plants. *Plant J.* 33: 87-95.

Kenchaiah, B. 1975.Studies on chilli anthracnose caused by *Colletotrichum capsici* (Syd) Butler and Bisbyn Karnataka M.Sc. (Ag) Thesis, University of Agricultural Sciences, Bangalore, 75p.

Kettlewell, P. S., Cook, J. W., and Parry, D. W., 2000. Evidence for an osmotic mechanism in the control of powdery mildew disease of wheat by foliar-applied potassium chloride. *Europ. J. of Plant Pathol.* 106: 297–300.

Kim, H., Lim, T. H., Kim, J., Kim. Y. H., and Kim, H. T. 2009. Potential of cross-infection of *Colletotrichum* species causing anthracnose in persimmon and pepper. *Plant Pathol. J.* 25: 13–20.

Kloepper, J. W. 1992. Plant growth promoting rhizobacteria as biological control agents. In: Soil Microbial Technologies (Ed: Matting, B.) Marcel Dekker, New York, Pp. 142–152.

Koike, S. T., Gladder, P. and Paulus, A. O. 2007. *Vegetable diseases-a colour handbook. London, UK.* Manson Publishing Ltd. 220-228pp.

Kuramae, E. E., Lopes, C. R., Souza, N. L and Machado. 1997. Morphological and molecular characterization of *Colletotrichum* spp. from citrus orchads affected by post bloom fruit drop in Brazil. *Eur. J. of Plant Pathol.*103: 323-329.

Lee, J. S., Park, J. H., and Kim, S. K. 2000. Effect of soluble silicon on the severity of powdery mildew (*Sphaerotheca fuliginea*) in cucumber leaves. *J. Korean Soc. Hort. Sci.* 41: 356-360.

Leinhos, G.M.E., Gold, R. E., Düggelin, M. and Guggenheim, R. 1997.Development and morphology of *Uncinula necator* following treatment with the fungicides kresoxim- methyl and penconazol. *Mycol. Res.* 101: 1033-1046.

Lenné, J. M. *and* Parbery, D. G. 1976.Phyllosphere antagonists and appressoria formation in *Colletotrichum gloeosporioides. Trans. of the Br. Mycol. Soc:* 66: 334-336.

Li, Y. H and Zang, H. 2014. Anthracnose of cucumber. *Am. J of Phytopathol.* 60 (2): 32-34.

Lilly, V. G. and Barnett, H. L. 1951. *Physiology of the Fungi.* McGraw Hill Book Company, Inc., New York, pp. 464.

Lin, Q., Kanchana-udomkan, C., Jaunet, T. and Mongkolporn O. 2002. Genetic analysis of the resistance to pepper anthracnose caused by *Colletotrichum capsici. Thai J. of Agric. Sci.*35: 259-264.

Linu, M. S and Jisha, M. S. 2013. Effect of biocontrol agents against *Colletotrichum capsici* causing anthracnose of chilli (*Capsicum annum. L.*). *Int. J. Bio.Pharmacy and Allied Sci.* 2 (12): 2218-2223.

Mahadtanapuk, S., Sanguansermsri, M., Cutler, R. W., Sardsud, V and Anuntalabhochai, S. 2007. Control of anthracnose caused by *Colletotrichum musae* on *Curcuma alismatifolia* Gagnep. using antagonistic *Bacillus* spp. *Am. J. of Agric. and Biol. Sci.* 2 (2): 54-61.

Mallaraju, E. G. and Swamy, R. N. 1988. Effect of certain fungicides on the incidence of *Colletotrichum capsici* in chilli varieties K-1 and K-2. *South Indian Hort.* 36: 205-206.

Mandeep, K., Sharma, O. P. and Sharma, P. N. 2006. *In vitro* effect of *Trichoderma* species on *Colletotrichum capsici* causing fruit rot of chilli (*Capsicum annuum L.*). *Indian Phytopathol.*59 (2): 243-245.

Manjunath, 2011. Morphological and Molecular characterization of *Alternaria alternata* and *C.gloeosporioides,* incitants of leaf blight and anthracnose diseases of noni and their management, M.Sc.(Ag) Thesis, Tamil Nadu Agricultural University, Coimbatore, pp: 222.

Masoodi, L., Anwar, A., Ahmed, S. and Softi, T. A. 2013. Cultural, morphological and pathogenic variability in *Colletotrichum capsici* causing die-back and fruit rot of chilli. *Asian .J. Pl. Pathol.* 7(1): 29-41.

Mc Donald, J. 1926. A preliminary account of a disease of green coffee berries in Kenya. *Trans. Br. Mycol. Soc.* 11: 145-154.

Mc Govern, R. J. 1995. First report of fruit rot of *Capsicum chinense* caused by two *Colletotrichum* species. *Pl. Dis.* 79 (2): 212.

Mc Kinney, H. H. 1923. Influence of soil temperature and moisture on infection of wheat seedling by *Helmintosporium sativum. J. Agric. Res.* 26: 195-217.

Mello, A. F. S., Andressa, C. Z. M. and Ivan, P. B. 2004. Development of *Colletotrichum gloeosporioides* isolated from green pepper in different culture media, temperatures, and light regimes. *Agric. (Piracicaba, Braz.).*61: 542-544.

Menzies, J., Bowen, P., Ehret, D., and Glass, A. D. M. 1992. Foliar application of potassium silicate reduce severity in powdery mildew on cucumber, musk melon and zucchini squash. *J. Am. Soc. Hort. Sci.* 117: 902-905.

Mesta, R. K. 1996. Studies on fruit rot of chilli (*Capsicum annuum L.)* caused by *Colletotrichum capsici* (Sydow.) Butler and Bisby, M.Sc.(Ag) thesis, University of Agricultural Sciences, Dharwad, 66p.

Mesta, R. K., Kulkarni, V. R. and Rao, M. S. L. 2009. Identification of succeptible stages of fruit and role of bio chemical constituents in fruit rot of chilli *(Colletotrichum capsici). Int. J. Agric. Sci.*3 (1): 46-48.

Michereff, A., Sallam, D. and Harman, G. E. 1993. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathol.* 96, 190–194.

Mishra, P. K., Mukhopadhyay, A. N and Fox, R. T. V. 2010. *Annals of Applied Biology*. 137(3): 361- 364.

Moraes, S. R. G., Pozza, E. A., Alves, E., Pozza, A. S. A., Carvalho, J. G., Lima, P. H., and Botelho. 2005. Effect of silicon on incidence and severity of anthracnose in common bean. *Phytopathol.* 31 (2): 69-75.

Muthukumar, A., Eswaran, A., Nakeeran, S., and Sangeetha, G. 2010.Efficacy of plant extracts and biocontrol agents against *Pythium aphanidematum* inciting chilli damping off.*Crop Prot*. 29: 1483-1488.

Naik, M . K. 1985. Studies an anthracnose of betelvine (*Piper betel* Linn.) caused by *Colletotrichum gloeosporioides* (Penz.) Penz. And Sacc. M.Sc (Ag.) Thesis, University Agriculture Science, Bangalore, 82p.

Narendra Kumar, P. G. 2006. Studies on anthracnose of sorghum caused by *Colletotrichum graminicola* (Ces.) Wilson. M.Sc. (Ag.) Thesis, University Agriculture Science, Dharwad, 78p.

Nazriya, N. F., De Costa, D. M. and Azhaar, A. S. 2007. Genomic variation of *Colletotrichum musae* morphotype infecting banana varieties in Sri Lanka. *Proceedings of the Peradeniya University Research Session*, 2-4 February 2007, Srilanka. pp 1-2.

Negishi, H. Y., Yamaguchi, H., Shinohara, M., Kawabe., A and Arimoto, Y. 2011. Disease Resistance against anthracnose induced in Cucumber Leaves Treated Using Yeast Extract .*J. Mycol. Plant Pathol* 46: 181-188.

Nene, Y. L. and Thapliyal, P. N. 1993. *Fungicides in Plant Disease Control (3rd Edition.)*. Oxford and IBH Publishing Co. Pvt. Ltd. 691p.

Ngullie, M., Daiho, L. and Upadhyay, D. N. 2010. Biological management of fruit rot in world's hottest chilli (*Capsicum chinense jacq.*) . *J. Pl. Prot. Res.* 50 (3): 360-365.

Niroshini, W. K. R. and Karunararatne, A. M. 2009. Interactions of *Colletotrichum musae* and *Lasiodiplodia theobromaee* and their biocontrol by *Pantoea agglomerans* and *Flavobacterium sp.* in expression of crown rot of "Embul" banana. *BioControl* 54: 587-596.

Noack, D. 1993. Die Krankheiten des Kaffeebaumes in Brasilien. III. *Colletotrichum coffeanum* sp. Zeitschrift für Pflanzenkrankheiten. 2: 196-203.

Ongena, M. F. P, Daayf, P., Jaques, N., Thorart, T. C., Benhamou, P., Pauliz, N., Cornelis, R. K., and Belinger. 1999. Production of cucumber against Pythium root rot by fluorescent *pseudomonads*: predominant role of induced resistance over siderophores and antibiotics. *Plant Pathol.* 48: 66-76.

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. *Sci. Biol.* 2 (3): 72-76.

Palenchar, J., Danielle, D. T., Dantoff, L. E., Gevens, A. J., and Vallad, J. E. 2009. Cucumber anthracnose in Florida.*Am. Phytopathol.* 45 (2): 43-46.

Pandey, A., Pandey, B. K., and Muthukumar, M. 2012. *In vitro* Screening for resistance to anthracnose (*Colletotrichum gloeosporioides*) in different mango (*Mangifera indica*) hybrids. 6 (2): 24-25.

Pandey, A., Trivedi, P., Kumar, B., and Thorarat, L. S. 2006. Characterization of phosphate solubilizing and antagonistic strain of *Pseudomonas putida* isolated from a sub alpine location in the Indian Central Himalaya. *Curr.Microbiol.* 48:66-76.

Papavizas, G. V and Mumsden, R. D. 1980. Biological control of soil borne fungal propagules. *Ann. Rev.Phytopathol.* 18: 389-413.

Paramasivan, M. and Kalaimani, T. 2008. Management of chilli fruit rot caused by *Colletotrichum capsici. Indian. J .Pl. Prot.* 36: 102-107.

Paris, H. S. and Maynard, D. N. 2008. *Tricosanthes cucumerina* snakegourd In: Janick, J. and Paull, R.E. (ed.), Encyclopedia of fruits and nuts, *CAB Int.* Wallingford, Oxfordshire, UK. 312-313.

Peregrine, W. T. H., Ahmad, K., and Momin, M. 1984.Controlling anthracnose in water melon. *World Crops*. 36 (5): 184-185.

Photita, W., Lumyong, S., Lumyong, P., McKenzie, E. H. C., and Hyde, K. D. 2004. Are some endophytes of *Musa acuminata* latent pathogens?. *Fungal Diversity* 16: 131–140.

Photita, W., Taylor, P., Ford, R. W. J., Hyde, K. D., and Lumyong, S. 2005. Morphological and molecular characterization of *Colletotrichum* species from herbaceous plants in Thailand. *Fungal Diversity* 18: 117-133.

Polanco, L. R., Rodrigues, F., Moreria, E. N. 2014. Management of anthracnos in common bean by foliar sprays of potassium silicate, sodium molybdate and fungicide. *Pl. Dis.* 98: 84-89.

Prakash O., Sohi, H. S., and Sokhi, S. S. 1974. Studies on anthracnose disease of cucurbits caused by *Colletotrichum lagenarium* (Pass) E11.1 Halst.and their control. *Indian J. Hortic.* 31 (3): 278-282.

Pratibha, S., Kulshrestha, G., Gopal, M. and Kadu, L. N. 2004. Integrated management of chilli die back and anthracnose in Delhi region. *Indian Phytopathol.* 57 (4): 427-434.

Prihastuti, H., Cai, L., Chen, H., McKenzie, E. H. C. and Hyde, K. D. 2009. Characterization of *Colletotrichum* species associated with coffee berries in northern Thailand. *Fungal Diversity* 39: 89-109.

Pring, R .J., Latunde, A. O., Connell, R. J., Nash, C., Lucas, J. A., and Bailey, J. A. 1995. Infection process and identity of the hemibio-trophic anthracnose fungus (*Colletotrichum destructivum* O'Gara) from cowpea (*Vigna unguiculata* (L.)Walp.). *Mycol.Res.* 100: 1133-1141.

Promputtha, I., Lumyong, S., Lumyong, P., McKenzie, E. H. C. and Hyde, K. D. 2002. Fungal succession on senescent leaves of *Manglietia garrettiion* Doi Suthep-Pui National Park, northern Thailand. *Fungal Diversity.* 10: 89-100.

Rahman, M. A., Razvy, M. A., and Alam, M. F. 2013. Antagonistic activities of *Trichoderma* strains against chili anthracnose pathogen. *Int. J. for.Microbiol .and Mycol.* (1): 8-22.

Rai, M., Pandey, S. and Kumar, S. (2008). Cucurbit research in India: a retrospect Cucurbitaceae 2008, In: Pitrat, M. (Ed.), Cucurbitaceae 2008. Proceedings of

the IX EUCARPIA meeting on genetics and breeding of cucurbitaceae, INRA, Avignon, France.

Ramkumar., Soureche, R., Prabhakar, S. and Pandurangan, M. 2012. Role of antagonistic microbe *Pseudomonas fluorescens* on *Colletotrichum capsici* infecting *Curcuma longa*. *J. Pl. Pathol. Microb.*3: 146.

Rampersad, N. 2010. First report of anthracnose caused by *C. gloeosporoioides* in pumpkin in Trinidad, the university of west indies, department of life science, bio-technology laboratory, t. Augustine, Trinidad and Tobago, West Indies. 98 (8): 10-253.

Ramyasmruthi, S., Pallavi, O., Pallavi, S., Tilak, K., and Srividya, S. 2012. Chitinolytic and secondary metabolite producing *Pseudomonas fluorescens* isolated from Solanaceae rhizosphere effective against broad spectrum fungal phytopathogens. *Asian J. Plant Sci. and Res.* 2 (1): 16-24.

Rangaswami, G. 1958. An agar blocks technique for isolating soil micro organisms with special reference to Pythiaceous fungi. *Sci Cult.* 14: 24:85.

Ratanacherdchai, K., Wang, H., Lin, F., and Soytong, K. 2010. ISSR for comparison of cross- inoculation potential of *Colletotrichum capsici* causing chili anthracnose. *Microbiol Res.* 4 (1): 76–83.

Rayner, R. W. 1952. Coffee berry disease. A survey of investigations carried out up to 1950. *Eur. Agric. For. J.* 17: 130-150.

Reddy, B. P., Reddy, K. R. N., Subba Roa, B.P., and Roa, K. S. 2008. Efficacy of antimicrobial metabolites of *Pseudomonads fluorescens* against rice fungal pathogens. *Curr.Trends Biotechnol. Pharm.* 2: 178-182.

Rini, C. R., and Sulochana, K. K. 2007. Usefulness of *Trichoderma and Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. *J. of Tropic. Agric.* 45 (1-2): 21–28.

Sanders, G. M. and Korsten, L. 2003. A comparative morphology of South African avocado and mango isolates of *Colletotrichum gloeosporioides*. *Can. J.of Bot.* 81: 877-885.

Sanders, G. M., Korsten, L. and Wehner, F. C. 2011.Survey of Fungicide Sensitivity in *Colletotrichum gloeosporioides* from different avocado and mango production areas in South Africa. *Euro. J of Plant Pathol,* 106:745-752.

Sangdee, A., Sachan, S., and Khankhum, S. 2011. Morphological, pathological and molecular variability of *Colletotrichum capsici* causing anthracnose of chilli in North-east of Thailand. *Afr. J .Microbiol. Res.* 5 (25): 4368-4372.

Schwartz, H. F., and Gent, D. H. 2007. Cucurbits Anthracnose (Cucumber, Melon, Pumpkin, Squash and Zucchini). *Indian Phytopathol.* 77 (2): 32-35.

Sharma, M., and Kulshrestha, S. 2015. *Colletotrichum gloeosporioides*: An Anthracnose Causing Pathogen of Fruits and Vegetables. *Biosci.Biotechnol. Res. Asia.* 12 (2): 1233-1246.

Shinde, J. U., Bhadrashette, V. S. and Gawai, D.U. 2012. Efficacy of leaf extracts of some medicinal plants on growth *of Colletotrichum capsici* Butler and Bibsy. *Afr.J.Pl.Sci.* 8 (7): 353-355.

Shilpa, T. C. 2006.Integrated management of anthracnose in chilli (*Capsicum annum* L.) M.Sc. (Ag.) Thesis, Kerala Agriculture University, Trissur, 56p.

Sileshi, F., Mohammed, A., Thangavel, S., and Adugna, A. 2014. *In vitro* evaluation of some fungicides and bioagents against common bean anthracnose (*Colletotrichum lindemuthianum* Sacc.Magnus) Briosi Cavara. *Afr. J. of Microbiol. Res.* 8 (20): 2000-2005.

Simmonds, J. H. 1965. A study of the species of *Colletotrichum* causing ripe fruit rots in Queensland. *Qld. J. of Agric. and Anim.Sci.* 22: 437-459.

Singh, B. P., Singh, S. P. and Mohammad, A. 1990. Economic efficacy of different fungicides for the control of leaf spot of cauliflower. *Indian Phytopathol.* 43: 207-209.

Sitterly, W. R., and Keinath, A. P. 1996. Anthracnose. In: Zitter TA, Hopkins DL, Thomas CE (eds) Compendium of cucurbit diseases. APS Press, pp 24–25.

Sivakumar, D., Wieratnam, W. R.S., Wijesundera, R. L. C., Marikar, F. M. T. and Abeyesekere, M. 2000. Antagonist effect of *Trichoderma harzianum* on post harvest pathogen of Rambuttan (*Naphelium lappaceum*). *Phytoparasitica.* 28: 240-247.

Soltani, J., Haghighi, M. Y. P., and Nazeri, S. 2014. Light, temperature, aging dependent vegetative growth and sporulation of *Colletotrichum gloeosporioides* on different culture media. *J. of med. plants res.* 8 (4): 208-216.

Sreenivasaprasad, S., Brown, A. E. and Mills, P. R. 1993. Coffee berry disease pathogen in Africa: genetic structure and relationship to the group species *Colletotrichum gloeosporioides. Mycol Res.* 97: 995-1000.

Srinivas, C., Niranjana, S. R., Praveen, K. L., Chandra, N. S., and Shetty, H. S . 2006. Effect of fungicides and bioagents against *Colletorichum capsici* on seed quality of chilli. *Indian Phytopathol.* 59: 62-67.

Srivastava, K. K. and Soni, K. K. 1993.Seedling blight of *Albizzia falcataria* and its control. *Annu.Forestry.* 1:82-84.

Sturz A. V., Christie, B. R., and Matheson, B. G. 1998. Association of bacterial endophyte populations from red clover and potato crops with potential for beneficial allelopathy. *Can. J. of Microbiol.* 44: 162-167.

Sudhakar, 2000, Biology and management of Stylosanthes anthracnose caused by *Colletotrichum gloeosporioides* (Penz.)Penz.And Sacc. M.Sc. (Ag.) Thesis, University Agriculture Science, Dharwad, 68 p.

Suhag, N. and Duhan, T. 1984. A new method for identifying *Colletotrichum lagenarium* of water melon inoculating with detatched leaf and judging resistance with a standard. *Acta Hortic.* 3: 402-405.

Sun, W., Liang, Y. and Yang, Y. 2002. Influences of silicon and inoculation with *Colletotrichum lagenarium* on peroxidase activity in leaves of cucumber and their relation to resistance to anthracnose. *Sci. Agric. Sin.* 35:1560-1564.

Sundravadana, S., Alice, D., Kuttalam, S. and Samiyappan, R. 2007. Efficacy of azoxystrobin on *Colletotrichum gloeosporiodes* (penz.) penz and ssac. growth and on controlling mango anthracnose. *J. Agric. Biol. Sci.* 2(3): 10-15.

Suthin , R. T., and John, C. D, Ann , S. H. 2013. Evaluation of virulence and methods of inoculation of *Colletotrichum capsici* (SYD) Butler and Bisby. *Int. J. Agric. Sci.* 9 (2): 802-805.

Sutton, B. C. 1962. *Colletotrichum dematium* (Pers. ex Fr.) Grove and *C . trichellum* (Fr. ex Fr.) Duke. *Trans of the Br Mycol* Soc. 45: 222-232.

Sutton, B. C. 1965. Studies on the taxonomy of *Colletotrichum* Cda with special reference to *C. graminicola* (Ces.) Wilson. (Ph.D). Thesis, University of London. 45p.

Sutton, B. C. 1966. Development of fruitifications in *Colletotrichum graminicola* (Ces.) Wils. and related species. *Can J of Bot.* 44: 887-897.

Sutton, B. C. 1968. The appressoria of *C. graminicola* and *C. falcatum. Can.J. of Bot.* 46: 873-876.

Sutton, B. C. 1980. The Coelomycetes: Fungi imperfecti with pycnidia, acervuli and stromata. *Phytopathol* 38 (1) : 34-38.

Sutton, B .C. and Waterston, J. M. 1970. *Colletotrichum musae.* CMI Description of pathogenic fungi and bacteria. *Phytopathol* 32 (2): 222.

Sutton, B. C. 1992. The genus *Glomerella* and its anamorph *Colletotrichum.* In *Colletotrichum*: biology, pathology and control (eds. J.A. Bailey and M. J. Jeger). *J. of Zhejiang Univ Sci.* 11(1): 1-26.

Svetlana , Z. S., Stojanovic, Z., Ivanovic, V., Gavrilovic, T. P., and Jelica, B. 2010. Screening of antagonistic activity of microorganisms against *Colletotrichum acutatum. Indian Phytopathol.* 72 (2): 54-56.

TeBeest, D. O., Correll, J. C. and Weidemann, G. J. 1997. Specification and population biology in *Colletotrichum.* In: The Mycota V, part B (eds. K, Esser and P.A. Lemke). *Am. Phytopathol.* 24 (2): 157-168.

Than, P. P., Shivas, R. G., Jeewon, R., Pongsupasamit, S., Marney, T. S., Taylor, P. W. J. and Hyde, K. D. 2008. Epitypification and phylogeny of *Colletotrichum acutatum*. *Fungal Diversity*. 45 (1): 31-33.

Thangamani, P., Kuppusamy, P., Peeran, M. F., Gandhi, K., and Raguchander, T. 2011. Morphological and physiological characterization of *Colletotrichum musae* the causal organism of banana anthracnose. *World J. of Agric. Sci : 7* (6): 743-754.

Thompson, D. C. and Jenkins, S. F. 1985. Influence of cultivar resistance, initial disease, environment fungicide concentration and timing on anthracnose development and yield loss in pickling cucumbers. *Phytopathol.* 75: 1422-1427.

Timchenko, V. I .1977. Diseases of melons. *Indial Phytopathol.* 48 (2): 10:62.

Toofanee, S. B. and Dulymamode, R. 2002 Fungal endophytes associated with *Cordemoya integrifolia. Fungal Diversity.*11: 169- 175.

Tronsmo, A. and Hjeljord, L. 1997. Biological control with *Trichoderma* species. In: Boland GJ, Kuykendall LD (eds): Plant Microbe Interactions and Biological Control. Marcel Dekker, New York.

Velazhahan, R., Samiyappan, R., and Vidhyasekaran, P. 1999. Relationship between antagonistic activities of *Pseudomonas fluorescens* isolates against *Rhizoctonia solani* and their production of lytic enzymes. *Can. J. of Phytopathol.* 106: 244-250.

Veronica, N. D., Silva., Guzzo., Dias, S., Lucon., Mantovanello, C. M., Harakawa and Ricardo. 2011. Growth promotion and resistance indicator against

anthracnose in cucumber using *Trichoderma*-spp. *Pesq.agroper.bars.* 46.: 1609-1618.

Vidhyasekaran, P., Rabindran, R., Muthamilan, M., Nayar, K., Rajappan, K., Subramanian, N and Vasumathi, K. 1997.Development of powder formulation of *Pseudomonas fluorescens* for control of rice blast. *Plant Pathol.* 46:291-297.

Vincent, J. M. 1927. Distortion of fungal hyphae in presence of certain inhibitors. *Nature.* 159:850.

Viswanatan, R and Samiyappan, R. 1999. Induction of systemic resistance by plant growth promoting rizobacteria against red rot disease caused by *Colletotrichum falcatum* in sugarcane. *Proc.sugar.Techol.Assoc.India.* 61: 24-39.

Viswanathan, R., Padmanabhan, P., and Mohanraj, D. 1997. Growing virulence of red rot pathogen of sugarcane in Tamil Nadu. *Indian Sugar.* 47: 23 -30.

Viterbo, A., Ramot, O., Chernin, L and Chet, I. 2002. Significance of lytic enzymes from *Trichoderma* spp. in the bio control of fungal plant pathogens. *Am. J of Pl Pathol.* 81: 549-556.

Von Arx, J. A. 1957. Die Arten der Gattung Colletotrichum Cda. *Phytopathol.* 29: 413–4684.

Von Arx J. A. 1981. Die Arten der Gattung *Colletotrichum* Cda. *Phytopath Zeitschrift.* 29: 414-468.

Voorips, R. E., Finkers, R., Sanjaya, L., and Groenwold, R. 2004. QTL mapping of anthracnose (*Colletotrichum* spp.) resistance in a cross between *Capsicum annum* and *C. chinense. Theor. Appl. Genet.* 109: 1275-1282.

Waller, J. M, Lenne, J. M and Waller, S. J. 2002. Plant Pathologists's Pocket book. CABI, Wallingford, UK.

Waller, J.W., Bridge, P.D., Black, R. and Hakiza, G. 1993. Characterizationof the coffee berry disease pathogens, *Colletotrichum kahawae*. *Mycol. Res.* 97: 989-994.

Wasilwa, L. A., Correll, J. C., Morelock, T. E. and Mc New, R. E. 1993.Reexamination of races of the cucurbit anthracnose pathogen *Colletotrichum orbiculare*. *Am. J. of phytpathol.* 83: 1190- 1198.

Weinding, R. 1934. Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. *Phytopathol.* 24: 1153-1179.

Wharton, P. S. and Dieguez- Uribeondo, J. 2004. The biology of *Colletotrichum acutatum*. *Anales del jardin Botanico de Madrid.* 61: 3-22.

Whitaker, T. W. and Davis, G. N. 1962. Cucurbits: botany, cultivation and utilization. *Inter sci.* 32 (2): 250.

Windham, T. T., Elad, Y. and Baker, A. R. 1986. A mechanism for increased growth induced by *Trichoderma* spp. *Phytopathol.* 76: 518-521.

Yaling, L., Pongnak, W., and Kasem, S. 2015. Effect of different factors on sporulation of *Colletotrichum coffeanum*. *Aus . J . of phytopathol.* 11 (4): 997–1012.

Yan, S., Tao, S., Tao, X. H., Chen, L., Yun, S. C., and Zhang, Y. 2001. Broad-spectrum ntimicrobial activity and high stability of Trichokonins from

Trichoderma koningii SMF2 against plant pathogens. *FEMS Microbial LeM* . 260: 119-125.

Yan, Z., Dolstra, O., Prins, T. W., Stam, P., and Visser, P. B. 2006. Assessment of partial resistance to powdery mildew *Podosphaera pannosa* in a teraploid rose population using a spore suspension inoculums method. *Eur. J. Plant Pathol.* 114: 301-308.

Yeasmin, M. R 2004. Integrated Management of Seedling Diseases of Blackgram, Mungbean and Lentil, MSc (Ag) Thesis, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, 55p.

Yildirim. A., Onogur, E., and Irshad, M. 2002. Investigations on the efficacy of some natural chemicals against powdery Mildew [*Uncinula necator* (Schw.) Burr.] of Grape. *J. Phytopathol.* 150: 697-702.

Yoshid, S. S., Hiradate, T., Tsukamoto, K., and Shirata, A. 2001. Antimicrobial activity of culture filtrate of *Bacallus amylolasuefaciens* RC-2 isolated from mulberry leaves February. *Biol. Control.* 91: 2181–2187.

Zhang, Z., Jiang, W., Jian, Q., Song, W., Zheng ,Z., and Wang, D., 2015. Residues and dissipation kinetics of triazole fungicides difenoconazole and propiconazole in wheat and soil in Chinese fields. *Food Chem.* 168: 396–403.

Zitter, T. A., Hopkins D. L., and Thomas C. E. 1996. *Compendium of cucurbit diseases.* American Phytopathological Society Press, St Paul.

Zivkovic, S., Stojanovic, S., Ivanovic, Z., Gavrilovic, V., and Jelica, B. 2010. Screening of antagonistic activity of microorganisms against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides. Arch. of Biol. Sci.* 62 (3): 611-623.

INTEGRATED MANAGEMENT OF ANTHRACNOSE OF SNAKE GOURD

(*Trichosanthes cucumerina* L.)

by

ASWANI DEVI

(2014 - 11 - 140)

Abstract of the thesis

Submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM – 695522

KERALA, INDIA

2016

INTEGRATED MANAGEMENT OF ANTHRACNOSE OF SNAKE GOURD

(*Trichosanthes cucumerina* L.)

by

ASWANI DEVI

(2014 - 11 - 140)

Abstract of the thesis

Submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM – 695522

KERALA, INDIA

2016

ABSTRACT

The objective of present study entitled "Integrated management of anthracnose of snake gourd (*Trichosanthes cucumerina* L.)" was to make a comparative evaluation of foliar application of newer fungicides and biocontrol agents for the management of anthracnose disease of snake gourd. Surveys were conducted during October 2015 in snakegourd fields of five different locationsof Thiruvananthapuram district , *viz.*, Instructional Farm, College of Agriculture,Vellayani, Department of Olericulture, College of Agriculture, Vellayani, Kalliyoor, Kakkamoola and Palapoor. Maximum disease incidence (90.00 per cent) and disease severity/ percentage disease index (44.22) were recorded in the snake gourd fields of Instructional Farm, Vellayani whereas disease parameters were minimum (21.89 per cent and 70.00 per cent) in the fields of Palapoor

Pathogenicity tests revealed that the isolate C1 obtained from Instructional Farm, Vellayani produced maximum lesion size both on detatched leaf (2.53 cm^2) as well as and on intact leaves of 30-days old potted plant (10.06 cm^2). The smallest lesion size of 1.33 cm^2 on detatched leaf and 1.53 cm^2 on 30 days old potted plant was produced by the isolate C4 from Kakkamoola. Based on lesion size and virulence rating, C1 was screened as the most virulent isolate.

Results of the cultural studies among the five isolates, showed that potato dextrose medium, oat meal medium and Richard's medium were screened as the best media both in solid and liquid states for the growth of the tested isolates. Effect of different light sources (Fluorescent light: 253.7 - 185 nm, L.E.D light: 365 nm and UV light: 10 - 380 nm) on growth and sporulation of anthracnose pathogen indicated that the cultures exposed to fluorescent light and darkness for alternate cycles of 12 h of each day resulted in maximum mycelial growth and minimum days for sporulation for all the isolates. Growth was less when the cultures of the isolates were exposed to UV light for a period of 45 min.

The morphological characters studied indicated that average conidial size of isolate C1, C2, C3 and C5 ranged from 11.40µm- 13.14µm x 4.48µm-4.82 µm and that they were cylindrical in shape with obtuse ends. The isolate C4 was ellipsoidal in shape and had a conidial size of 10.52µm x 4.40 µm. The isolates C1, C2, C3 and C5 were further identified at National Fungal Collection Culture India (NFCCI) - Pune, as *C. coffeanum* F.Noack and the isolate C4 was identified as *C. musae* (Berk. & M.A. Curtis) Arx.

Isolate C1 of *C. coffeanum* which was earlier screened as the most virulent isolate was used for the subsequent studies conducted for the management of anthracnose disease in snake gourd. In the *invitro* assayconducted for screening of newer fungicides and bio control agents, effective for the inhibition of the isolate C1 of *C. coffeanum*, the nutrient potassium silicate (0.5 per cent) and fungicide mancozeb (0.4 per cent) resulted in hundred per cent mycelial inhibition while, KAU talc based formulations of the bio control agents *viz., P. fluorescens* (2 per cent) and *T. viride* (2 per cent) resulted in inhibition of 88.33 per cent and 87.33 per cent respectively and were selected for further evaluation in green house study. Minimum spore germination was recorded due to amendment of medium with *T. viride* (1.33 per cent). Conidia were also reduced in size when growth medium (PDA) were amended with tested chemicals and bio-control agents.

In the green house studies using the snake gourd variety Kaumudi, maximum disease suppression was observed when plants were sprayed with 0.5 per cent potassium silicate (89.12 per cent) and 0.4 per cent of fungicide mancozeb (84.99 per cent) at fifteen days interval. Biometric parameters observed were also maximum for plants sprayed with 0.5 per cent potassium silicate followed by mancozeb and KAU talc based formulation of bio-control agents *P. fluorescens* and *T. viride*.

Anthracnose affecting the foliage of snake gourd is a serious disease in snakegourd fields of Thiruvanathapuram district. The nutrient potassium silicate

which was tested for the first time in Kerala against a plant pathogen, *Colletotrichum* sp. is found to be a promising chemical for management of the disease.

The present study has also revealed the prospects of utilizing the mineral nutrient, potassium silicate for control of anthracnose disease as well as attainment growth promotion, flowering behavior, yield components and increases in yield of snake gourd which is an important vegetable crop of Kerala. Besides, this study also has highlighted the scope for integrating the fungicide mancozeb with bio-control agents like *P. fluorescens* and *T. viride* which were was also highly beneficial for the disease management as well as in improving the growth parameters of the crop under green house conditions. Such integration of fungicide mancozeb with bio-control agents will also be useful in minimizing the detrimental effects of continous and intensive use of this fungicide which is otherwise very effective in disease management. In the background of the promising results obtained from this thesis project especially from the use of potassium silicate, trials for confirming the beneficial effects of the treatments in field grown snake gourd plants would be useful for the vegetable growers in Kerala.

പടവലത്തിന്റെ കരിമ്പൂപ്പ് രോഗത്തിന്റെ സംയോജിത നിയന്ത്രണം

പടവലത്തിന്റെ ഇലകളിൽ കാണപ്പെടുന്ന കരിമ്പൂപ്പ് രോഗത്തിനെ രാസവസ്തുക്കളും ജൈവവസതുക്കളും സംയോജിതമായി ഉപയോഗിച്ചുള്ള നിയന്ത്രണത്തെ ക്കുറിച്ചാണ് ഈ പഠനം. കല്ലിയൂർ പഞ്ചായത്തിലെ അഞ്ച് പാടശേഖരങ്ങളിൽ (ഇൻസ്ട്രക്ഷണൽ ഫാം, കോളേജ് ഓഫ് അഗ്രികൾച്ചറൽ-വെള്ളായണി, ഡിപാർട്ട്മെന്റ് ഓഫ് ഒലറികൾച്ചർ-കോളേജ് ഓഫ് അഗ്രികൾച്ചർ -വെള്ളായണി, കാക്കാമൂല, കല്ലിയൂർ, പാലപ്പൂർ) നടത്തിയ സർവ്വേയിൽ രോഗവ്യാപ്തത, രോഗ തീവ്രത, രോഗലക്ഷണങ്ങൾ തുടങ്ങിയ പഠനങ്ങൾ നടത്തി.

സർവ്വേയുടെ അടിസ്ഥാനത്തിൽ ഇൻസ്ട്രക്ഷണൽഫാം വെള്ള ായണിയിൽ ആണ് പരമാവധി രോഗവ്യാപ്തത (90.00%) കൂടാതെ രോഗതീവ്രത (44.22%)രേഖപ്പെടുത്തിയത്.

ഇവിടെ നിന്ന് ശേഖരിച്ച ഇലകളുടെ രോഗലക്ഷണങ്ങളുള്ള സാംപിളുകളിൽ നിന്നും രോഗാണുവിന്റെ അഞ്ചു ഐസോലേറ്റുകൾ വേർതിരിച്ചെടുക്കുകയും അവയെ ഐസോലേറ്റ്-സി, ഐസോലേറ്റ്-സി-2, ഐസോലേറ്റ്-സി-3, ഐസോലേറ്റ്-സി-4, ഐസോ ലേറ്റ്-സി-5, എന്ന് നാമകരണം ചെയ്യുകയും ചെയ്തു. ഈ ഐസോലേറ്റുകൾ ഉപയോഗിച്ച് പടവലച്ചെടികളിൽ പഠനം നടത്തിയതിന്റെ അടിസ്ഥാനത്തിൽ ഐസോലേറ്റ്-സി1 (ഇൻസ്ട്ര ക്ഷണൽ ഫാം വെള്ളായണി) പരമാവധി തീവ്രത രേഖപ്പെടുത്തി.)

രോഗാണുവിന്റെ വളർച്ച വിവിധ ഖരരൂപത്തിലും ദ്രാവകരൂപത്തിലുമുള്ള മീഡിയ കളിൽ പരീക്ഷിച്ചതിന്റെ ഫലമായി ഉരുളക്കിഴങ്ങ്-ഡെക്സ്ട്രോസ് മീഡിയത്തിലും, ഓട്ട് മീൽ മീഡിയത്തിലും, റിച്ചാർഡ്സ് മീഡിയത്തിലും കുമിളിന്റെ പരമാവധി വളർച്ച രേഖപ്പെടുത്തിയ തിനാൽ ഈ മീഡിയങ്ങൾ രോഗാണുവിന്റെ വളർച്ചയക്ക് അനുയോജ്യമാണെന്ന് സ്ഥിതീകരിച്ചു.

ഫ്ലൂറസെന്റ് വെളിച്ചം 12 മണിക്കൂർ ഇടവിട്ട് ചാക്രികമായി നൽകിയതിന്റെ ഫലമായി പരമാവധി മൈസീലിയൽ വ്യാസവും, സ്പോറുലേഷനും രേഖപ്പെടുത്തി. എന്നാൽ 45 മിനിറ്റ് യു.വി വെളിച്ചം നൽകിയപ്പോൾ വളരെക്കുറവ് മൈസീലിയൽ വ്യാസമാണ് രേഖപ്പെടു ത്തിയത്.

രോഗാണുവിന്റെ ബാഹ്യമായ ഘടന പഠിച്ചപ്പോൾ, ഐസോലേറ്റ് സി-1, സി-2, സി-3 കൂടാതെ സി-4 ന്റെ കോനീഡിയയുടെ വലിപ്പം 11.40-13.14 X 4.48-4.82 മൈക്രോമീറ്റർ രേഖപ്പെടുത്തുകയും, ഐസോലേറ്റ് സി-4 നു 10.52 X 4.40 മൈക്രോമീറ്റർ രേഖപ്പെടുത്തുകയും ചെയ്തു.

എൻ.എഫ്.സി.സി.ഐ, പൂനയിൽ നടത്തിയ പഠനത്തിൽ സി-1, സി-2, സി-3, സി-5 ഐസോലേറ്റുകൾ കോളിറ്റോട്രിക്കം കോഫിയാനം എന്നും സി-4 ഐസോലേറ്റിനെ കോളിറ്റോട്രിക്കം മ്യൂസെയെന്നും സ്ഥിതീകരിച്ചു.

വിവിധ രാസവസ്തുക്കളുടേയും, ജീവാണുവളങ്ങളുടേയും രോഗാണുവിന് (കോളി റ്റോട്രിക്കം കോഫിയാനം-ഐസോലേറ്റ് സി-1) എതിരെയുള്ള കാര്യക്ഷമത ലബോറിട്ടറിയിൽ പരീക്ഷിച്ചപ്പോൾ പൊട്ടാസ്യം സിലിക്കേറ്റ് (0.5%), മാംങ്കോസെബ് (0.4%) ഏറ്റവും ഫലപ്രദമായി കണ്ടെത്തി. കാർഷിക സർവ്വകലാശാല വികസിപ്പിച്ചെടുത്ത ട്രൈക്കോഡർമ, സ്യൂടോമോ ണാസ് എന്നീ ജീവാണു കുമിൾനാശിനികളും രോഗാണുവിനെതിരെ ഫലപ്രദമായി കണ്ടു. ആയതിനാൽ മേൽപ്പറഞ്ഞ വസ്തുക്കളും ജീവാണു കുമിൾനാശിനികളും ഹരിത ഗൃഹപഠനത്തിനായി തിരഞ്ഞെടുത്തു. ഹരിതഗൃഹപഠനത്തിന്റെ ഫലമായി പൊട്ടാസ്യം സിലിക്കേറ്റ് (0.5%), മാംങ്കോസെബ് (0.4%) പരമാവധി രോഗനിയന്ത്രണം രേഖപ്പെടുത്തി. എന്നാൽ കാർഷിക സർവകലാശാല വികസിപ്പിച്ചെടുത്ത ജീവാണുവളങ്ങൾ അത്ര ഫലപ്രദമായിരുന്നില്ല എങ്കിലും ഒരു പരിധിവരെ രോഗത്തെ നിയന്ത്രിച്ചു.

ആയതിനാൽ പൊട്ടാസ്യം സിലിക്കേറ്റ് പൊട്ടാസ്യം സിലിക്കേറ്റ് (0.5%), മാംങ്കോസെബ് (0.4%) ജീവാണുവളങ്ങളായ ട്രൈക്കോഡർമ (2%), സ്യൂഡോമോണിസ് (2%) എന്നിവയും പടവലത്തിന്റെ കരിമ്പൂപ്പ് രോഗത്തിന്റെ സംയോജന നിയന്ത്രണത്തിനായി ഉപയോഗിക്കാം എന്നാണ് ഈ പഠനത്തിന്റെ കണ്ടെത്തൽ.

Appendices

APPENDIX-1

COMPOSITION OF MEDIA USED

1. Potato Dextrose Agar

Peeled and sliced potatoes	- 200.00g
Agar-agar	- 20.00g
Dextrose	- 20.00 g
Distilled water	- 20.00g

2. Richards' agar

Potassium Nitrate	- 10.00g
Di potassium phosphate	- 5.00 g
Magnesium sulphate	- 2.50 g
Ferric chloride	- 0.02 g
Sucrose	- 50.00g
Agar	- 20.00g
Distilled water	- 1000 ml

3. Czapek's (Dox) agar

Sucrose	- 30.00g
Sodium Nitrate	- 2.00g
K_2HPO_4	- 1.00 g
$MgSO_4.7H_2O$	- 0.50g
KCl	- 0.50g
$FeSO_4$	- 0.01g
Agar	- 20.00g
Distilled water	- 1000 ml

4. Sabouraud's agar

Glucose	- 40g
Peptone	- 10g

Agar - 20.00g

Distilled water - 1000 ml

5. Carrot agar

Carrot - 200.00g

Agar - 20.00g

Distilled water - 1000 ml

6. Oat meal agar

Oats - 30.00g

Agar - 20.00g

Distilled water - 1000

APPENDIX-II

COMPOSITION OF STAIN USED

1. Lactophenol- Cotton blue

Anhydrous lactophenol	- 67.00 ml
Distilled water	- 20.00 ml
Cotton blue	- 0.10 g

Anhydrous lactophenol was prepared by dissolving 20g phenol in 16 ml lactic acid in 3ml glycerol.

महाराष्ट्र विज्ञान वर्धिनी
आघारकर अनुसंधान संस्था
Maharashtra Association for the Cultivation of Science
AGHARKAR RESEARCH INSTITUTE
(An Autonomous Body under
the Department of Science and Technology, Govt. of India)

12/185

173959

National Fungal Culture Collection of India (NFCCI)-A National Facility

Sender: Ms. Aswani Devi, C/o Dr. Kamala Nayar, College of Agriculture, Kerala Agricultural Univeristy, Vellayani-695522, Kerala

Details of Fungus Identified

Sr.	Culture	Identification Remarks	Family
1.	Isolate-1	*Colletotrichum* sp. aff. *C. coffeanum* F. Noack	Glomerellaceae
2.	Isolate-2	*Colletotrichum* sp. aff. *C. coffeanum* F. Noack	Glomerellaceae
3.	Isolate-3	*Colletotrichum* sp. aff. *C. trichellum* (Fr.) Duke	Glomerellaceae
4.	Isolate-4	*Colletotrichum* sp. aff. *C. musae* (Berk. & M.A. Curtis) Arx	Glomerellaceae
5.	Isolate-5	*Colletotrichum* sp. aff. *C. coffeanum* F. Noack + *Aspergillus niger* gr.	Glomerellaceae

Note: The identity was confirmed solely based on morphological characters in *in-vitro* culture.

CONDITIONS AND REMARKS:

1. THE PARTY HAS DELIVERED THE SAMPLE AT ARI.
2. THE RESULTS HAVE BEEN OBTAINED ON CAREFUL ANALYSIS AND EXAMINATION OF THE SAMPLE ONLY AND IN THE CONDITION RECEIVED.
3. THIS REPORT SHOULD BE USED ONLY FOR ACADEMIC AND RESEARCH PURPOSES. IT SHOULD NOT BE USED AS AN EVIDENCE OF AUTHENTICITY IN ANY OFFICIAL/ GOVERNMENTAL/ LEGAL/STATUTORY CORRESPONADANCE OR CERTIFICATION. THE INSTITUTE SHALL NOT BE BOUND TO CONFIRM THE AUTHENTICITY BEFORE ANY LEGAL FORUM.
4. THE CONTENTS OF THIS REPORT ARE CONFIDENTIAL AND BEING DISCLOSED ONLY TO THE PARTY / SUPPLIER OF SAMPLE.
5. THE PARTY NEEDS TO ACKNOWLEDGE THE SERVICE (S) PROVIDED/RENDERED BY NFCCI-ARI (IN THESIS/REPORTS/PUBLICATION/BOOKS/MONOGRAPHS ETC.).
6. THE PARTY IS REQUESTED TO SUBMIT COPY/REPRINT OF PUBLICATION TO CURATOR, NFCCI BASED ON FUNGAL STRAINS DEPOSITED & ACCESSIONED IN NFCCI FOR OFFICIAL RECORD.

KERALA AGRICULTURAL UNIVERSITY CENTRAL LIBRARY KAU P.O. THRISSUR

Dr. S.K. Singh, Scientist
National Facility (NFCCI & FIS)
Biodiversity and Paleobiology
E-mail: nfcci.ari@gmail.com, singhsksingh@gmail.com
Phone: 020-25325103

NFCCI/2015-7/AKC 2254-06/PNS/SKS/DKM