STUDIES ON ANTHRACNOSE OF GUAVA (*Psidium guajava* L.) CAUSED BY *Colletotrichum gloeosporioides* Penz.

by

SHRI. KIRAN ARUN SAWANT-PATIL
(Reg. No. 04/116)

A thesis submitted to the

**MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI – 413 722, DIST. - AHMEDNAGAR,
MAHARASHTRA, INDIA.**

in partial fulfilment of the requirements for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

**DEPARTMENT OF PLANT PATHOLOGY
AND AGRICULTURAL MICROBIOLOGY**

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2006

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part thereof has not been submitted by me or any other person to any other University or Institute for a degree or diploma

Place : M.P.K.V., Rahuri

Date : 31 / 05 /2006

(K. A. Sawant-Patil)

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CERTIFICATE

This is to certify that the thesis entitled, **"Studies on anthracnose of guava (*Psidium guajava* L.) caused by *Colletotrichum gloeosporioides* Penz."** submitted to Mahatma Phule Krishi Vidyapeeth, Rahuri, for the award of degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY,** embodies the results of a *bona fide* research carried out by **SHRI. KIRAN ARUN SAWANT-PATIL** under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been acknowledged.

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ACKNOWLEDGEMENTS

It is unforgettable experience in my life to fourtunate enough to work under the inspiring guidance of Dr. A. P. Gaikwad, Associate Professor of Plant Pathology, Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri and Chairman of my Advisory Committee. I find the words too inadequate to express my feelings to gratitude for his scholarly suggestions, ever- willing help, keen interest, pains taking efforts taken for scrutinizing the manuscript and unfailing enthusiasm throughout the study since its conceptions to completion.

I am immensely grateful to the members of my Advisory Committee Dr. G.N.Dake, Professor of Plant Pathology, Dr. B. V. Garad, Associate Professor of Horticulture, and Dr. K. S. Raghuwanshi, Junoir Plant pathologist, AICRP on Aridzone fruits MPKV, Rahuri for their valuable suggestions during the course of studies.

I also wish to express my profound sense of gratitude to Dr. S.G.Borkar, Head, Department of Plant Pathology and Agricultural Microbiology, Dr. D. M. Sawant, Associate Dean, College of Agreiculture, Pune-5 for their valuable guidance, keen interest for carrying out present research work is something that shall never forget.

I express my sincere thanks to Dr. P.V. Wani, Dr. C. D. Deokar, Dr. B. G. Barhate, Dr. S. V. Bulbule, Dr. (Mrs.) P.D.Patil, for their excellent teaching and persistant help in academic career.

I am thankful to Shri. R.B. Sonawane, Shri. S.J. Bade, Shri. A.S. Kharade, Shri. P.S. Pandhare, Shri. P. D. Lahare, and other staff members of Department of Plant pathology and Agricultural Microbiology for their help and kind co-operation.

I am also thankful to Prof. S.B. Gawade, Assistant Professor and Shri. B.C. Game, Senior Research Assistant, Seed Pathology for taking microphotographs and their help during the course of this investigations.

I offer my thanks to my collegues Gopal, Pranay, Sandip, Sharif, Swapnil and all senior, junior and collegues of Plant pathology and Agricultural Microbiology for co-operation during my post graduation study and research work.

I shall fail my duties if I forget the names of my friends Amit, Amol, Amar, Bapusaheb, Balasaheb, Goraksha, Ganesh, Mukund, Nitin, Pradeep, Praamod, Sachin, Sacchitanand, Sandip, Shantaram, Sharad, Vinayak and all my seniors and juniors who are in my heart for their excellent company, warmer affections and co-operation during my study at MPKV, Rahuri.

I offer my special thanks to Shri. Amar Mohite (MRO, SBI), Shri. Nitin Satpute (AO, BOM) and Shri. Sachin Kasabe (MRO, SBI) for their moral and economic support during research and thesis work.

The heartiest blessings of my Mama (Chadankar family), who always encouraged and extended unending support with which I am stepping my carrer. Words are insufficient to express my immense and indebtedness to my parents Sau. Aai, Shri. Tatya and brother Vikram(Aba), Sister Asha (Madam) are the source of my inspiration. Their contribution is beyond acknowledgement.

I am indebted to the authers whose literature have been cited.

I offer my thanks to any other source of contribution that might have been inadvertently left out.

Last but not least, I also entend my thanks to the pains taking efforts taken by Raj Computers, MPKV, Rahuri for metriculous work in computer typing and printing my thesis with great precision.

Place : M. P. K. V., Rahuri
Date :31 / 05 /2006

(K. A. Sawant-Patil)

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LIST OF ABBREVIATIONS

@	At the rate of
BOD	Biological oxygen demand
C.D.	Critical difference
cm	Centimeter (s)
CRD	Completely randomized design
C.V.	Coefficient of variation
^{0}C	Degree celsius
DFT	Detached fruit technique
EC	Emusifiable concentrate
et al.	Et alli (and others)
etc.	Et cetera (and so forth)
Fig.	Figure (s)
GR	Growth rate
hrs	hour (s)
i.e.	Id est (That is)
m	Meter (s)
µ	micrometer
MBIM	Mycelium bit inoculation method
mm	Millimeter
µm	Millimicron
/	per
pp	Page (s)
PDA	Potato dextrose agar
PDI	Percent disease intensity
S.E. ±	Standard error
spp.	Species
viz.	Videlicet (namely)
WP	Wettable powder

ABSTRACT

STUDIES ON ANTHRACNOSE OF GUAVA (*Psidium guajava* L.) CAUSED BY *Colletotrichum gloeosporioides* Penz.

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KIRAN ARUN SAWANT-PATIL

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Research Guide	:	**Dr. A. P. Gaikwad**
Department	:	**Plant Pathology and Agril. Microbiology**

Guava (*Psidium guajava* L.), the apple of the tropics, is one of the most common fruits in India. The area under guava is increased only due to high economic returns obtained from this crop. The crop is found to be badly infected by several diseases. However, anthracnose caused by *Colletotrichum gloeosporioides* is becoming severe threat to guava crop. The disease was noticed in high intensity during *Kharif,* 2004 in Horticultural garden at MPKV., Rahuri. Therefore, the attempts were made to study the symptomatology of disease; morphological, cultural and physiological characters of the pathogen; reaction of different varieties to the disease and *in vitro* efficacy of different fungicides and bioagents against pathogen, *C. gloeosporioides.*

The causal organism was isolated from affected fruits of guava showing typical anthracnose symptoms and identified as *Colletotrichum gloeosporioides* Penz. The pathogenicity of the isolated fungus was confirmed on the healthy fruits.

Disease symptoms started as production of many small, water soaked lesions on fruit surface. Slowly spots turned light brown in colour, about 0.5 to 1.0 mm in diameter and were shallow. In advanced stage the spots enlarged in size up to 3-5 mm in diameter, became more depressed, circular and redish-brown to light ashy at center and dark brown to black at margins. The diseased portion was comparatively harder, dry and in some cases small cracks developed in them. Dark brown to black, circular, very small spots were noticed on leaf lamina.

The mycelium of the pathogen was closely septate, irregularly branched and vacuolated. Acervuli looked orange in colour, which later changed to dark brown to black in colour. They were globose to saucer or irregular in shape. Setae were ashy-brown to dark brown, septate, stiff, straight or bending. The conidiophores were short, simple, thickly arranged and hyaline. The conidia were single celled, cylindrical in shape, hyaline when single, but orange to light brown in mucilaginous masses or in acervuli.

The synthetic media *viz.,* peptone glucose, Coon's and Nutrient agars, while non-synthetic media *viz.,* oat meal and potato dextrose agars were the excellent media for growth and sporulation of the fungus.

The cardinal temperature range for growth of pathogen was 10 - 35^{0}C. While, optimum for growth and abundant sporulation was in between 25 - 30^{0}C.

Out of eleven guava varieties, Behat seedling was resistant to anthracnose, while remaining were highly susceptible.

The fungicides *viz.,* Bordeaux mixture (1.0%), tricyclazole (0.1%), difenoconazole (0.1%), propiconazole (0.1%) and hexaconazole (0.1%) were best as they completely inhibited the pathogen growth under laboratory conditions.

Among four *Trichoderma* sp., *T. hamatum* was the most effective biological agent against *C. gloeosporioides* under *in vitro.*

INTRODUCTION

I. INTRODUCTION

One of the most gregarious of fruit trees, the guava , *Psidium guajava* L., of the myrtle family (Myrtaceae), is almost universally known by its common english names or its equivalent in other languages. In Spanish, the tree is *guayabo* or *guayavo*, the fruit *goyave* or *goyavier ;* the Dutch *guyaba*, *goeajaaba*; the Surinamese, *guave* or *goejaba* and the Portuguese *goiaba* or *goaibeira.* Hawaiians call it guava or *Kuawa.* In Guam it is *abas.* In Malaya, it is generally known either as guava or *jamba batu,* but has also numerous dialectal names as it does in India. In tropical Africa and the Phillipines where the name *'bayabas'* is often applied. Various tribal names like *Pichi, Posh, Enandi,* etc. are employed among the Indians (Morton, 1987).

In addition, in different states of our country the guava is called by various names like *Amrood* (North-Hindi States, Punjab), *Peru* (Maharashtra), *Jama* (Andhra Pradesh), *Pijuli* (Orissa), *Pera* (Kerala), *Sebe* (Karnataka), *Madhuri* (Assam), *Peyara* (Bengal), *Koyya* (Tamil Nadu) and *Jamphal* (Gujarat).

Guava (*Psidium guajava*), the apple of the tropics, is one of the most common fruits in India. It claims to be the fourth most important fruit in area and production after mango, banana and citrus (Bose and Mitra,1990). It is now widely grown all over the tropics and subtropics. Records suggest that it has been in cultivation in India since early 17th century and gradually became a crop of commercial significance. Guava is quite hardy, prolific bearer and highly remunerative even without much care.

Guava is believed to have originated in tropical America and has spread to most of the tropical and subtropical countries of the world. It is grown commercially in Cuba, Malaysia, Myanmar, Hawaiian, Islands,

Venezuela, Australia, South Africa, Bangladesh, Brazil, Colombia, Cameroon, Mexico, Peru, Thailand, Sudan, Kenya and India.

In India, it is successfully grown in Uttar Pradesh, Bihar, Madhya Pradesh, Maharashtra, West Bengal, Orissa and Tripura. Uttar Pradesh is considered as the most important guava producing state of India and Allahabad-Varanasi region has the reputation of growing the best quality guava in the country as well as in the world. In India, during the year 2002-2003, the area under cultivation of guava was 2.2 lakh hectare and production was 17.8 lakh tonnes (Anonymous, 2004).

Guava fruit is a berry and has very thin skin. The fruits have a characteristic gritty texture due to presence of stone cells and have sweet aroma. The fruit is an excellent source of vitamin 'C' (299 mg/100 gm) [Singh, 2002] and pectin, but has low energy (66 cal/100 g) and protein (1%) and has about 17 per cent dry matter and 83 per cent moisture. The fruit is also rich in minerals like phosphorus (23-37 mg/100 gm), calcium (14-30 mg/ 100 gm), iron (0.6 – 1.4 mg / 100 gm) [Sadhu and Chattopadhyay,2001] as well as vitamins like niacin, panthotenic acid, thiamine, riboflavin and vitamin A.

It is normally consumed fresh as a dessert fruit. Excellent salad, pudding, jam, jelly, nectar concentrate and syrup can be made from guava fruit. In some countries, the leaves are used for curing diarrhoea, and also for dyeing and tanning.

Guava is a hardy crop and like many other fruit crops it does not require huge initial capital investment for establishing orchards. Similarly, it is grown profitably without much exacting technological involvement. Due to these advantages, more growers are taking up the guava cultivation. Maharashtra is the leading guava producing state with total area of 8,486 hectare and production of 1,20,083 tonnes in the year

2003-04 (Anonymous, 2005). As such, the area under this crop is showing an increasing trend in Western Maharashtra (traditional guava growing area), mainly in Ahmednagar, Nashik, Pune and Jalana districts. The expansion in the area is due to the Economic Guarantee Scheme (EGS) of State Government.

Though guava is a hardy crop, it is observed to be infected by diseases like fruit canker (*Pestalotiopsis psidii*), wilt (*Fusarium* sp., *Cephalosporium* sp. and *Macrophomina phaseolina*), stem canker (*Physalospora psidii*), dieback (*Hendersonwia toruloidea*), anthracnose (*Colletotrichum gloeosporioides*), red rust (*Aldacephaleuros virescens*) and parasitic flowering plant Giant mistletoe (*Dentrophtoe falcate, D. longiflorus*).

Among these diseases, anthracnose (*Colletotrichum gloeosporioides* Penz.) appears sporadically wherever the crop is grown in the country. The disease severity varies according to climatic conditions. It is severe mostly during the rainy season when there is high humidity and moderate temperature. The fruits of all age groups are badly infected by the disease, which show circular, brown to black coloured lesions that enlarge in size. The saffron coloured sporulation can often be observed on the fruit under high relative humidity. Infection also occurs on young shoots and leaves. The disease was noticed in serious form on some varieties of guava in the orchard at Mahatma Phule Krishi Vidyapeeth, Rahuri during monsoon as well as post monsoon period of the year 2004. The review of literature reveals that no detailed work has been done in the past on anthracnose of guava caused by *Colletotrichum gloeosporioides.* Therefore, it was felt essential to study different aspects of the disease in details. Thus, the present studies were carried out with the following objectives:

1. To prove the pathogenicity of causal agent.
2. To study the symptomatology of disease.
3. To study the morphological, cultural and physiological characters of pathogen.
4. To study the reaction of available varieties to anthracnose and
5. To evaluate the bioefficacy of available fungicides and bioagents against the pathogen under *in vitro*.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Historical

Genus *Colletotrichum* (Corda) belongs to the division / phylum Deuteromycota, sub-division Deuteromycotina, form class Coelomycetes, form order Melanconiales and form family Melanconiaceae.

Corda (1837) differentiated *Colletotrichum* from that of *Gloesporium* on the basis of presence and absence of setae in the acervulus, respectively.

The fungus, *Colletotrichum gloeosporioides* Penz. was first described by Penzing (1882) as *Vermicularia gloeosporioides.* Later in 1887, he transferred it into genus *Colletotrichum.* Before this, Saccardo (1884) also named the fungus *Colletotrichum gloeosporioides* based on the basis of presence of setae in the acervulus.

Clausen (1912) considered the distinguishing features between the two genera *Colletotrichum* and *Gloeosporium* by the presence of setae in the former and the presence of coarsely granular plasma in the spores in the later. Grove (1937) considered *Colletotrichum* essentially as *Gloeosporium* producing setae in the acervulus, so he considered the bionomial *Colletotrichum gloeosporioides*, a conidial stage of *Glomerella cingulata.*

The genus *Colletotrichum* has a very wide range of behavioral patterns in nature ranging from saprophytes to specialized parasitic strains with a narrow host range. Over 1000 forma species of *Colletotrichum* have been described on the basis of diseases caused on various hosts. Sutton (1980) has given key and descriptions to the 22 species recognized in this genus based on the cultural characters. *Colletotrichum* species cause "anthracnose "(literally means 'coral like'

leaf spot disease) of several crops and is, therefore, commonly referred to as the anthracnose fungus. The important pathogenic species of *Colletotrichum* are *gloeosporioides*, *falcatum, capsici, coffeanum, lindemuthianum, orbiculare, lagenarium, graminicola, lini, dematium, circinans, coccodes, higginsianum, musae, truncatum*, etc.

2.2 Occurrence of *Colletotrichum* spp. on guava plants

The pathogen *Glomerella cingulata* (a sexual stage of *C. gloeosporioides*) was reported for the first time to cause anthracnose of guava from Uttar Pradesh, India by Mehata (1951).

Venkatakrishnaih (1954) described the occurrence of *Pestalotiopsis psidii* in Mysore on fruits and leaves of *Psidium guajava* along with another fungus *Glomerella psidii (Colletotrichum psidii)*. Sharma *et al.* (1981) observed that a major proportion of guava fruits was damaged between harvest and consumption owing to anthracnose caused by *Glomerella cingulata* (*C. gloeosporioides*). Similar kind of anthracnose was also observed by Singh and Sharma (1981 and 1982) in Haryana. whereas Chand *et al.*(1985-86) reported that the *Colletotrichum psidii* is a causal organism of guava anthracnose from Hissar (Haryana).

A fortnightly survey of Jaipur Markets for two years during 1983 and 1984 yielded a number of fungi causing rotting of guava fruits in both years. Maximum number of fruits were spoiled by *Colletotrichum* rot followed by *Botrydiplodia* rot (Mujumdar and Pathak, 1989).

Guava fruits were infected by various fungal pathogens during storage in Assam resulting in complete rotting (90%) of the fruits within 7-10 days. The fungal pathogen mainly responsible for the rotting of guava fruits in storage was reported as *Colletotrichum gloeosporioides* by Das and Bora (1993).

Rahman (2003), observed that *Pestalotiopsis psidii, Colletotrichum gloeosporioides* and *Botryodiplodia theobromae* were the causal organism of guava anthracnose in Pakistan.

2.3 Isolation, inoculation and pathogenicity of *C. gloeosporioides*

2.3.1 Isolation, inoculation and pathogenicity of *C. gloeosporioides* infecting guava

Tondon and Singh, (1969) proved the pahogenicity of *C. psidii* causing anthracnose of guava, in situ. The leaves and stem of seedlings as well as new leaves, buds, flowers and fruits were infected. The older leaves were infected only if injured with limited spots, however young as well as mature fruits infected in any condition readily. While, spread of infection was very rapid on ripe fruits.

Tricita *et al.* (1975) studied the pathogenicity of *Colletotrichum gloeosporioides* by cross inoculations of the isolates from guava and grape, which showed that both isolates could infect both fruits producing typical anthracnose symptoms.

C. gloeosporioides was isolated from different tropical fruit crops *viz.,* banana, mango, pawpaw, corambola (*Averrhoa carambola*), guava and wax apple (*Syzygium* spp.) by Yang and Chuang (1994) who found that most of these isolates were pathogenic to their original hosts.

Wounded and unwounded fruits of guava Cv. Balady were inoculated by Abdel (2000) with 10^6 spores/ml of *Colletotrichum gloeosporioides* (*Glomerella cingulata*) isolates (CM-1, CM-2, CM-3) for pathogenicity test. *C. gloeosporioides* was reisolated from inoculated fruits picked at different stages to determine infection time. Results

showed that CM-1 inoculated wounded fruits developed 100 per cent disease incidence.

The pahogenicity of *Colletotrichum gloeosporioides* (*Glomerella cingulata*) and *Pestalotia psidii* (*Pestalotiopsis psidii*) was tested on fruits of eight guava cultivars by Kader and Rahman (2001). Both pathogens caused anthracnose on fruits of all cultivars.

2.3.2 Isolation, inoculation and pathogenicity of *C. gloeosporioides* infecting other fruit crops

Rathod (1994) also isolated and proved the pathogenicity of the fungus *C. gloeosporioides* inciting fruit rot of mango. On inoculation, the symptoms were initiated as typical black sunken spots within 72 hrs in case of unwounded and 48 hrs in wounded fruits of mango. Then, Gaikwad (2002) confirmed pathogenicity of four isolates of *C. gloeosporioides* by inoculating the cultures on healthy fruits of custard apple under field as well as *in vitro.*

2.4 Symptomatology

As regards symptomatology, very negligible work has been done in guava.

Venkatakrishniah (1952) reported scab or canker type symptoms from Mysore state on young, green as well as mature fruits of guava. Incipient infection manifested storage rot of fruits. Tondon and Agarwal (1954) reported a 'die back' of main branches of guava resulting in the death of plant. The symptoms started from the tips of affected branches and progressed downward. The causal agent was *Colletotrichum gloeosporioides.*

Tondon and Singh (1968) observed small spots of pin-head first on unripe fully grown fruits during rainy season, which gradually attained

size of 5-6 mm in diameter. They were dark brown to black, sunken, circular and had minute black stomata in the center of the lesions with spore masses. The diseased portion was comparatively harder than the healthy and often developed cracks when the infection was severe. Tender twigs showed wither tip symptoms.

Midha and Chohan (1968) noticed that spread of infection of *C. gloeosporioides* was very rapid on fully mature green fruits, whereas young fruits did not normally take infection. They have also observed the leaf spot symptoms during the months of May-July and cankerous symptoms on fruits during September-October in Banglore. In addition the disease was serious under storage.

Tricita *et al.* (1975) observed three types of anthracnose symptoms on guava fruits. The infection in early fruit development caused circular, dry, raised and cankered pustules or spots on the unripe fruits. While, as the fruits grown and enlarged the surface of the spots broke open and when infection was severe, the infected fruits became mummified and black.

Pathak (1980) reported that anthracnose of guava was caused by *Gloeosporium psidii* (*Glomerella psidii*). He observed that the affected plants died back from the top of the branch. Similarly, shoots, leaves and fruits were readily affected. The growing tips gradually turned dark brown and the black necrotic areas extened backward causing die back.

Carranza *et al.* (2002) observed that anthracnose symptoms on fruits of common guava in Buenos Aires, Argentina. Symptoms of grayish, circular, sunken spots approximately 5 mm long were observed only on the surface of green unripe fruits. In humid conditions, acervuli containing salmon-pink masses of spores and dark setae were found

within lesions. The pathogen was identified as *Glomerella cingulata* (anamorph *C. gloeosporioides*), based on morphological characters.

2.5 Morphology of the fungus

The morphological studies of *C. gloeosporioides* isolated from mango carried out by Patil (1968) revealed that the average width of mycelium on PDA was 4.10 µm ranging from 3.25 to 5.0 µm. The fungus produced acervuli both on culture as well as on host. In culture, they developed within 6 to 7 days after inoculating, initially pink in colour, later changed to reddish. The conidia measured 8.5 to 13.0 x 3.5 to 6.25 µm.

According to Bose *et al.* (1973) the acervuli of *C. gloeosporioides* infecting mango were sub-epidermal and measured 115 to 216 x 95 to 112 µm. The size of the conidia varied from 11 to 16 x 4 to 6 µm. Further, Singh (1978) described the species of *C. gloeosporioides* from mango and recorded that mycelium consisted of narrow, sparsely septate hyphae, which were at first hyaline but later turned slightly black in colour. The conidia were broadly oval to oblong with rounded ends, non-septate and some times contained 1 to 2 globules and measured 12 to 16 x 4 to 6 µm in size.

Ghosh and Ikram (1980) studied the *C. gloeosporioides* causing leaf blight of *Arucaria bidwilli* and observed that the acervuli were formed on the upper surface of the leaves. They also noticed that conidia were cylindrical to oblong, one celled, hyaline to light pink in colour, continuous with rounded ends measuring 10 to 21 x 3 to 5 µm in size, produced on simple cylindrical conidiophores, which were 10 to 20 µm long and 3 to 4 µm broad.

Thereafter, Hasabnis (1984) observed that mycelium of *C. gloeosporioides* from mango when grown on PDA was conspicuous, well developed, septate, hyaline at first, later on becoming slightly dark.

Conidia measured 13.8 µm (12 to 16 µm) x 4.7 µm (4 to 6 µm) in size. Thakare (1991) found that the mycelium of the fungus *C. gloeosporioides* causing leaf blight of chrysanthemum was septate, hyaline, branched, vacuolated and average width of the hyphae was 3.84 µm. Conidiophores were short, simple, hyaline and closely packed together. The conidia were cylindrical, single celled, hyaline and their size ranged from 8.19 to 16.59 x 2.94 to 7.35 µm. The acervuli were dark brown with septate setae.

Rathod (1994) studied the morphology of the fungus *C. gloeosporioides* inciting fruit rot of mango. He observed the average measurements of different structures as mycelial width 4.20 µm, conidia 12.70 x 4.67 µm and setae 73.37 x 4.11 µm.

Recently, Hande (2001) studied the morphological characters of the fungus, *C. gloeosporioides* from curry leaf. He noticed that mycelium of the fungus was septate, irregularly branched, vacuolated, hyaline and measured 3.81 µm in width when became old. Initially acervuli looked light orange, which later changed to dark brown to black in colour, rectangular to irregular in shape and measured 60.01 x 48.22 µm. The setae were septate, brown,straight or bending stiff and measured 73.40 X 3.52 µm. Conidiophores were short, simple, thickly arranged on acervuli and were hyaline. Further, he observed that conidia were oblong to cylindrical in shape with rounded ends, single celled, hyaline with two or more vacuoles and measured 12.69 x 3.86 µm.

Morphological observations of *C. gloeosporioides* from custard apple fruit recorded by Gaikwad (2002) revealed that the mycelium was closely septate, irregularly branched, vacuolated and measured 4.64 µm (2.16 to 7.25 µm).The acervuli were globose to saucer or irregular in shape, dark brown to black and measured 188.02 x 147.80 µm. While, setae were ashy brown to dark, septate, stiff, straight or bending

and measured 111.70 x 2.70 µm. The number of setae were 7.47 (1-20). Further, he noticed that the conidia were oblong to cylindrical, obtuse, single celled, hyaline and vacuolated with 11.52 x 4.03 µm in size. The conidiophores were short, septate, simple, thickly arranged and were hyaline, later turned light brown to ashy-brown.

Gutierrez *et al.* (2002) suggested genetic variability among isolated strains of *C. gloeosporioides* obtained from guava fruits on the basis of morphology, cultural and pathological characters.

2.6 Growth and cultural characters of *C. gloeosporioides*

The cultural characters of *C. gloeosporioides* causing anthracnose of mango reported by Mishra and Mahmood (1960) revealed that Richard's agar medium provided good growth while, Czapek's Dox agar medium supported good sporulation among synthetic media. Among non-synthetic media potato dextrose agar medium was the best. Singh *et al.* (1966) reported excellent growth of *C. gloeosporioides* causing anthracnose of *Dioscorea* plant on potato dextrose agar, oat meal agar and corn meal agar. Further, good growth and sporulation was observed on host extract medium and Czapek's Dox agar while fair to poor growth was on Richard's agar and Asthana Hawker's agar.

Bose *et al.* (1973) found that *C. gloeosporioides* causing die-back of mango grew maximum on Czapek's Dox agar medium followed by PDA and peptone dextrose medium. However, Marathe *et al.* (1973) observed that the oat meal agar was the best medium for growth and sporulation of *C. gloeosporioides* causing twig blight of castor.

Shinde (1988) studied cultural characters of *C. gloeosporioides* causing dieback of hybrid tea roses. He noticed good growth of the fungus on Richard's agar medium and Sabourand's agar medium, while poor growth on leaf extract and Czapek's Dox medium.

Then, Hegde *et al.* (1989) noticed that the growth of *C. gloeosporioides* from *Areca catechu* was best on potato dextrose agar and Sabourand's agar media, while oat meal, Coon's and bean juice agars favoured maximum sporulation.

The cultural characters of *C. gloeosporioides* causing fruit rot of mango observed by Rathod (1994) revealed that mango pulp agar, Richard's agar and Coon's agar favoured abundant growth and sporulation followed by oat meal agar. Good growth and sporulation was recorded on Czapek's Dox agar and Sabourand's agar. Further, Ekbote *et al.* (1997) also observed maximum radial growth of *C. gloeosporioides* (*G.cingulata*), causing anthracnose of mango, on Richard's agar and potato dextrose agar, followed by Czapek's Dox agar.

Growth and cultural characters of *C. gloeosporioides* causing fruit rot of custard apple were studied by Gaikwad (2002) on 12 synthetic and 15 non-synthetic media. The synthetic media *viz.*, M_2 agar and Neopeptone glucose agar were the best for growth and sporulation, followed by Sabourand's agar and Asthana Hawker's agar, while oat meal agar was the excellent non-synthetic medium for growth and sporulation and next in order were custard apple fruit pulp decoction agar, PDA, Potato agar and bean meal agar.

2.7 Effect of temperatures on growth and sporulation of *C. gloeosporioides*

Patil (1968) studied the temperature relationship of *C. gloeosporioides* inciting anthracnose of mango. He observed that fungus could thrive between 10 to 30^0C. Optimum temperature for the growth was observed between 25 to 30^0C.

Dawkhar (1970) reported the maximum mycelial growth of *C. gloeosporioides* causing fruit rot of papaya at optimum temperature of

25^{0}C. While it failed to grow below 20^{0}C and above 40^{0}C. Thereafter, Gunasekaran (1979) reported that *C. gloeosporioides* from citrus grew best at optimum temperature of 25 -27^{0}C and no growth was observed at 34-35^{0}C. Whereas, Doornik (1982) observed maximum mycelial growth of *C. gloeosporioides* causing anemone leaf curl at 25^{0}C, while it did not take place above 35^{0}C and below 20^{0}C.

Naik *et al.* (1984) reported 20-30^{0}C as optimum temperature for *C. gloeosporioides* causing anthracnose of betelvine. In addition, Ahmed (1985) also reported that temperature range for growth and sporulation of *C. gloeosporioides* causing anthracnose of many tropical and subtropical fruits was 15-35^{0}C with an optimum of 20-30^{0}C. Further, Kim *et.al.* (1986) observed the optimum temperature for two strains of *C. gloeosporioides* causing anthracnose of chilli was between 25 to 28^{0}C.

The studies on effect of different temperatures on growth and sporulation of *C. gloeosporioides* causing fruit rot of mango by Rathod (1994) revealed that the fungus could grow at a wide range of temperatures (15-35^{0}C). There was no growth below 10^{0}C and beyond 40^{0}C.The maximum growth and sporulation was observed at 27±1^{0}C. Good growth and sporulation were also observed at 25^{0}C and 30^{0}C.

Abdel (2000) while studying the influence of temperature on growth, fruit infection and sporulation of *C gloesporioides* inciting anthracnose of guava noticed that all isolates grew at a wide range (5 - 49^{0}C), with an optimum temperature of 30^0 and 25^{0}C for infection and sporulation, respectively.

2.8 Varietal Resistance

Eight different varieties of guava, *viz.,* Apple shaped, seedling, Behat coconut, L-49 (Sardar), Allahabad safeda, Apple guava, Guava

red and Red fleshed and four species of *Psidium* (*P. chinense, P. cattleianum* var. *lucidium, P. guineese* and *P. molle*) were found susceptible but the spots remained very small on Apple guava. *P. chinense* resisted leaf infection whereas, *P. molle* and Beumount were highly susceptible. The variety Allahabad safeda developed heavy infection on fruits at Hessarghatta (Anonymous, 1974).

The fungi *C. gloeosporioides* and *Pestalotia psidii* (*Pestalotiopsis psidii*) were tested on fruits of eight cultivars of guava by Kadar and Rahaman (2001). Both pathogens caused anthracnose on fruits of all the eight cultivars. Allahabad, Kanchannagar, Mulundapuri, Seedless, Selim and Swarupkathi were highly to moderately susceptible to *C. gloeospdrioides.* Disease severity was significantly lower on Kazipeyara and Thaiking as compared to the other six cultivars. No line was found resistant when inoculated with both pathogens.

Rahman *et al.* (2003) observed that no any tested variety was resistant against anthracnose pathogen (*C. gloeosporioides).* The pear shaped fruits had less susceptibility than elliptical round fruits.

2.9 Bioefficacy of fungicides against *C. gloeosporioides* under *in vitro*

2.9.1 Bioefficacy of fungicides against *C. gloeosporioides* infecting guava

Tondan and Singh (1969) reported that Bordeaux mixture was more effective than copper oxychloride and cuprous oxide for control of *C. gloeosporioides* from guava crop.

Butt *et al.* (1995) also reported that the growth and acervulus formation of *Gloeosporium psidii* (*C. gloeosporioides*) the cause of anthracnose of guava, were strongly inhibited by thiophanate methyl and also by benomyl.

Kader and Rahman (2001) tested the sensitivity of *C. gloeosporioides* to fungicides causing anthracnose of guava in *in vitro.* Score 25 EC (difenoconazole) , Tilt 25 EC (propiconazole) and Bavistin (carbendazim) were equally effective in inhibiting the mycelial growth of *C. gloeosporioides.*

2.9.2 Bioefficacy of fungicides against *C. gloeosporioides* infecting other fruit crops

Ali *et al.* (1993) ascertained that among seven fungicides, Bavistin (100 ppm) and propiconazole (200 ppm) were most effective for complete inhibition of *C. gloeosporioides* inciting anthracnose of tea under *in vitro,* while ready Bordeaux mixture was not effective. During the same year, Smith and Black (1993) studied the *in vitro* activity of 28 fungicides against *Colletotrichum* sp. (*G. cingulata*), a causal agent of strawberry anthracnose and crown rot. The mycelial growth was reduced by all fungicides tested at concentration of 50 ppm a.i..However, benomyl and propiconazole severely limited growth even at very low (0.05%, a.i.) dose and mancozeb and chlorothalonil reduced the colony diameter by 20 per cent at the same concentration.

Rathod (1994) tested eight fungicides, out of that those carbendazim and benomyl (0.05, 0.1 and 0.2% concentration) were found effective in inhibiting *C. gloeosporioides* inciting fruit rot of mango. Later, thiophanate methyl and mancozeb were found effective by Lai *et al.* (1995) against leaf blight pathogen, *C. gloeosporioides* of *amomum villosum.*

Thakare and Patil (1995) recorded good control of *C. gloeosporioides* by copper oxychloride at 0.2, 0.3 and 0.4 per cent concentration in *in vitro* fungicides tests. Later, Padalkar *et al.* (1996) reported that mancozeb (0.2 and 0.25%), carbendazim (0.1 and 0.2%), thiophanate methyl (0.05 and 0.1%) and Bordeaux mixture (1.0 and 2.0 %),

gave good control of leaf spot pathogen (*C. gloeosporioides*) of arecanut in *in vitro* tests. In addtion, Akthar *et al.* (1998) in their *in vitro* chemical control trial against anthracnose pathogen (*C. gloeosporioides*) of mango reported that thiophanate methyl, mancozeb and benomyl were most effective at all concentrations.

In vitro evaluation of eight different fungicides at 10, 50, 100, 250 and 500 ppm concentrations by Das *et al.* (1998) against *C. gloeosporioides* inciting blossom blight of mango revealed that propiconazole completely inhibited the linear mycelial growth at 50 $\mu g\ ml^{-1}$. However, the higher concentration (500 $\mu g\ ml^{-1}$) of mancozeb and copper oxychloride were moderately effective.

The *in vitro* studies carried out by Gaikwad (2002) regarding efficacy of different fungicides against *C. gloeosporioides* revealed that the effectiveness of fungicides *viz.,* benomyl, prochloraz, propiconazole, Bordeaux mixture, thiophanate methyl, hexaconazole, carbendazim and difenoconazole in the order of their efficacy merit.

2.10 Efficacy of bioagents against *C. gloeosporioides* under *in vitro*

Banu *et al.* (1990) noticed that biocontrol agent, *T. harzianum* was equally as effective as the hot water treatment (55^0C) against seed-borne fungi *viz., C. dematium* and *C. capsici* by seed treatment method of chilli. Narendra Singh (1992) reported that *T. harzianum* was a strong inhibitor of *Colletotrichum falcatum* under *in vitro.*

Michereff *et al.* (1993) observed a significant reduction in the growth of *C. graminicola,* a causal agent of sorghum anthracnose with the culture filtrate of *T. viride, T. koningii* and *T. harzianum* under laboratory condition. Suzzi *et al.* (1995) reported the antagonistic activity of *S. cerevisiae* against fungal pathogen, *viz., C. acutatum.* The

Trichoderma spp. ranked next in order mentioned in their antagonistic effect against the pathogen.

Jeyalakshmi (1998) screened seven *Trichoderma* spp., seven isolates of *Pseudomonas fluorescens, two isolates of Fluorescent pseudomonad, Bacillus subtilis* and one yeast (*Saccharomyces cerevisiae)* againt *Colletotrichum capsici,* both *in vitro* and on the plant. Among the fungal antagonists, *S. cerevisiae* exhibited the maximum reduction of the mycelial growth followed by *T. viride, T. hamatum, T. pileatus* and *T. harzianum.*

Ravi *et al.* (1999) tested six species of *Trichoderma (T. hamatum, T. harzianum, T. koningii, T. pseudokoningii, T. viride* and *T. longibrachiatum)* for their antagonistic activity against seed borne *C. lindemuthianum* in french bean. Among these, *T. viride* recorded the maximum inhibition of mycelial growth and spore germination followed by *T. harzianum* in dual culture technique.

Rajathilagam and Kannabiran (2001) recorded considerable reduction in the biomass and synthesis of DNA, RNA and protein of *C. capsici* cultures due to non-volatile antibiotic (NVAC) extracted in chloroform from *T. viride.*

D'souza *et al.* (2001) observed promising results of eight isolates of *T. harzianum* against fungal pathogen of betelvine, *C. capsici* under *in vitro* conditions by dual culture plate technique.

MATERIAL AND METHODS

3. MATERIAL AND METHODS

The material used and methods followed during present investigation were as follows :

3.1. Material

3.1.1 Source of isolate

The infected fruit samples of grava were collected for isolation of pathogen responsible for anthracnose from the guava orchards, Department of Horticulture, M.P.K.V., Rahuri.

3.1.2 Laboratory instruments and equipments

Various laboratory instruments used during the study were autoclave, inoculation chamber (Laminar air flow unit), biological oxygen demand incubator (BOD), binocular research microscope, refrigerator, electronic and physical balance, occular and filar micrometer, haemocytometer, ordinary as well as microphotography cameras, etc.

3.1.3 Glasswares

Different types of Corning and Borosil brand glasswares used were conical flasks, petriplates, test tubes, glass rods, slides, cover slips, funnels, beakers, measuring cylinders, pipettes, spirit lamps, decicater, etc.

3.1.4 Culture media

Eight synthetic (Table 2) and eight non-synthetic (Table 3) solid culture media were used to study the growth and cultural characters of the fungus.

3.1.5 Chemicals

The chemicals used for different studies were of analytical grade and of standard firms *viz.,* M/S Merck (India) Pvt. Ltd., Mumbai, Glaxo Laboratories (India) Pvt. Ltd., Mumbai, Bolts/ Qualigens Laboratories, Mumbai, Hi Media Laboratories (India) Pvt. Ltd., Mumbai, etc.

3.1.6 Different varieties of guava for screening against pathogen

Eleven varieties (Table 5a and 5b) for their reaction to pathogen, were collected from guava germplasm plot, Department of Horticulture, M.P.K.V., Rahuri.

3.1.7 Biological agents

The isolates of *Trichoderma* sp. *viz., T. viride, T. hamatum, T. harzianum* and *T. koningii* were obtained from Culture Bank, Department of Plant Pathology and Agricultural Microbiology, M.P.K.V.,Rahuri.

3.1.8 Miscellaneous material

Other petty items included atomizer, non-absorbent cotton, butter paper, blotter paper, filter papers, inoculating needle, forceps, scissors, spirit lamp, cork borer, foot rule, muslin cloth, stock solution of $HgCl_2$ (0.1 %), sodium hypochloride (0.5%), spirit, rubber bands, polypropylene bags, wrapping tape (15 µm thickness), glass marking pens, sticky labels, cork borers, etc.

Colour of the fungal colony was judged by using Methuen Handbook of Colour (Kornerup and Wanscher, 1967).

3.1.9 Fungicides tested against pathogen, *C. gloeosporioides*

Sr. No.	Trade name	Common name	Chemical name	Source
1	Bordeaux mixture	Bordeaux mixture	$CuSO_4$ + $Ca(OH)_2$ in equal proportions	--
2	Bordo	Ready made Bordeaux mixture 25 % WP	25 % metalic copper	Rallis India Ltd. , Mumbai.
3	Bavistin	Carbendazim 50 % WP	2-(Methoxy-carbamoyl) benzimidazole	BASF India Ltd. , Mumbai.
4	Blitox–50	Copper oxychloride 50 % WP	Copper oxychloride containing 50 % metalic copper	Rallis India Ltd. , Mumbai.
5	Captan	Captan 50% WP	N-Trichloromethyl-thiotetrahydro-phtalimide	Rallis India Ltd., Mumbai.
6	Contaf	Hexaconazole 5 % EC	(RS)-(2,4-dichlorophenyl)-1-(1H-1,2,4-trizole-1 yi) hexan-2-OI (IUPAC)	Rallis India Ltd. , Mumbai.
7	Indofil M-45	Mancozeb 70 % WP	Zinc ion and manganous ethylene bis dithiocarbamate	Indofil Chem. Co., Mumbai.
8	Kavach	Chlorothalonil 75 % WP	Tetrachloroisophthalonitrite	Syngenta Agro Chemicals, Mumbai.
9	Score	Difenoconazole 25 % EC	1-{2-[4-(4-chlorophenoxy)-2-chlorophenyl-(4-methyl-1,3-dioxolan-2-yl)-methyl]}-1H-2,2,4-trizole	Syngenta Agro Chemicals, Mumbai.
10	Sivic	Tricyclazole 75 % WP	5-methyl-1-2-4 – triazole (3,4-b) benzothiazole	Nagarjuna Agri chem. Ltd., Hyderabad
11	Tilt	Propiconazole 25 % EC	1-[2-(2,4-dichlorophenyl) – 4-propyl-1,3-dioxolan-2-yl-methyl]-1H-1,2,4-triazole	Syngenta Agro Chemicals, Mumbai.
12	Antracol	Propineb 70% WP	Zinc propylene bisdithiocarbamate	Bayer Crop Science, Himattnagar, Gujarat.

3.2 Methods

In present investigations different methods used are given below :

3.2.1 Isolation, inoculation and pathogenicity of *C. gloeosporioides*

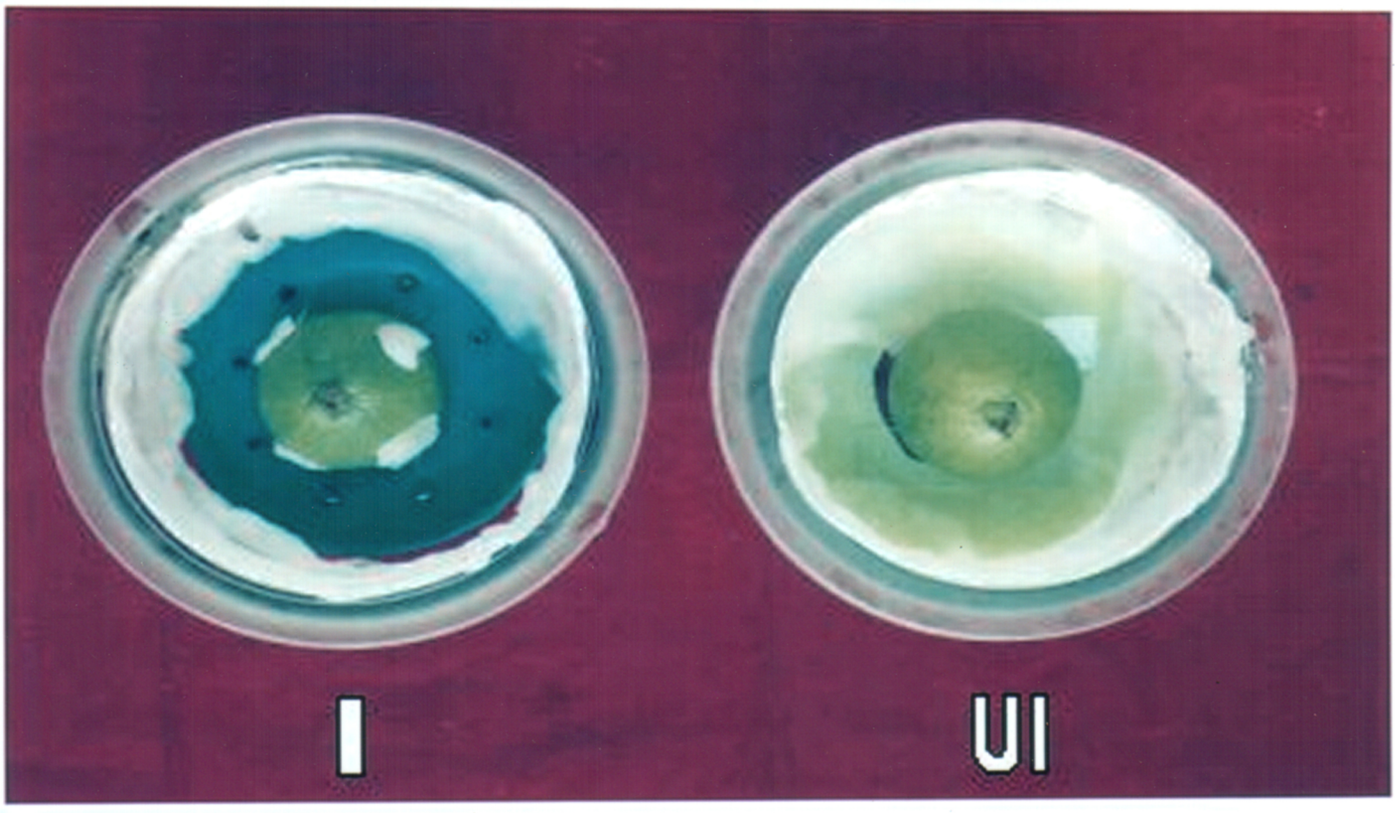
3.2.1.1 Isolation and maintenance of culture

The anthracnose infected fruits of guava were collected for isolation from Horticulture Garden, M.P.K.V., Rahuri. The affected parts of the fruits were cut into smaller pieces with a sterile scalpel and these were disinfected with mercuric chloride solution (0.1%) for one minute and subsequently three washings were given in sterilized water. The samples were dried by sterillized blotting paper. Isolation was made by standard agar plate technique (APT) on PDA. The plates were then incubated at 27±1^{0}C temperature. The fungal growth noticed after four days of inoculation was subsequently subcultured on PDA slants for obtaining pure culture. The pure culture of the fungus thus obtained was maintained on PDA slants in refrigerator at 5 ^{0}C temperature for further studies.

3.2.1.2 Pathogenicity under laboratory condition (Detached fruit technique)

Under laboratory condition, the pathogenicity was proved by detached fruit technique (Gaikwad, 2002). The fully grown immature fruits (95 to 105 days old) were collected along with long fruit stalk (3-4 inches). Before inoculation the fruits were washed with tap water, air dried, surface disinfected with 0.5 per cent sodium hypochloride solution for 45 seconds (Agostini *et al.,* 1992) followed by thorough but gentle rinsing with sterilized water.

Thereafter, the fruits were kept in decicater by inserting the fruit stalk in sterilized water.The inner walls of decicater and lid were covered



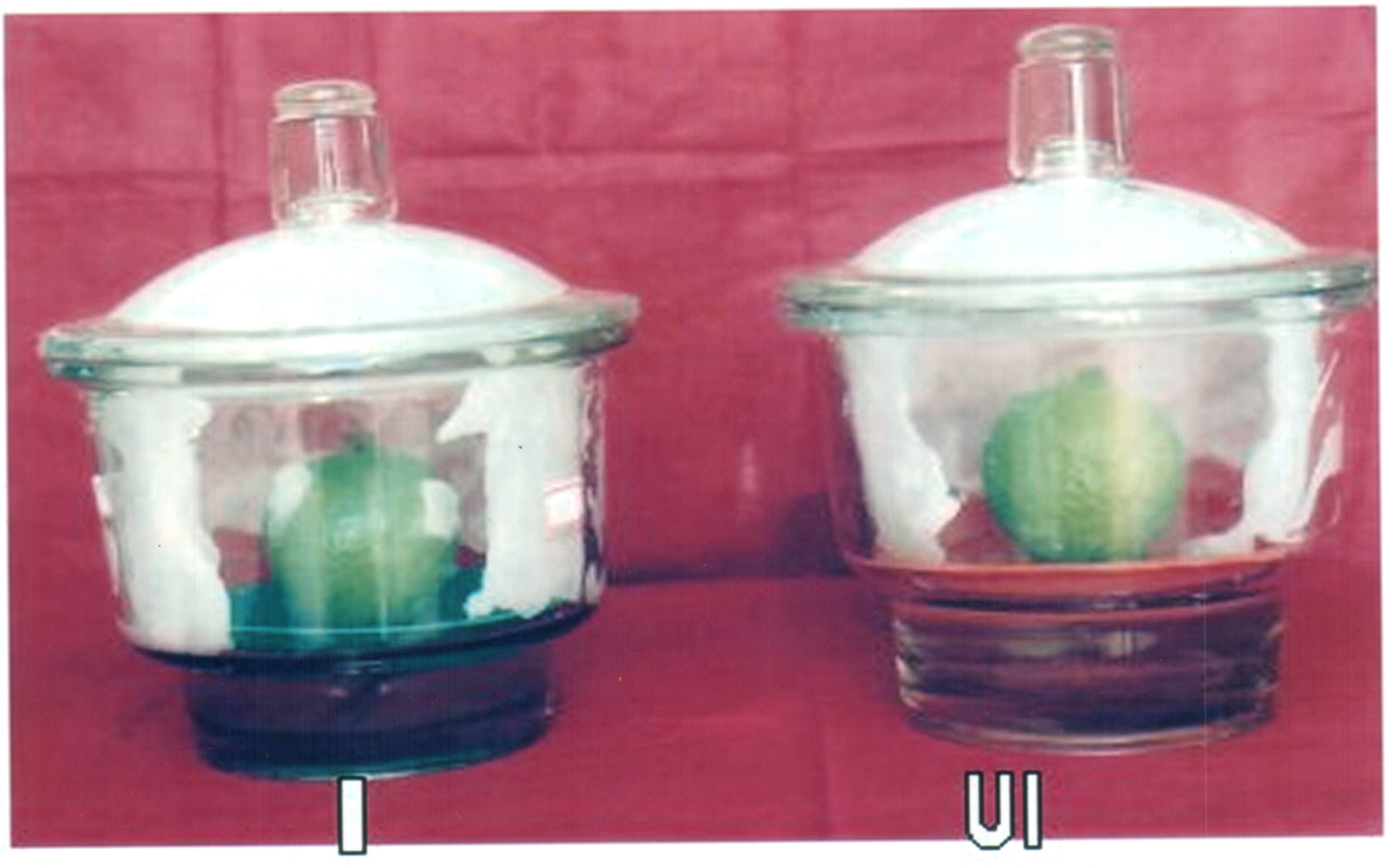


Fig. 1. Pathogencity – Methodology
Artificial epiphytotics by detached fruit technique under *in vitro* conditions.

I - Inoculated, UI - Uninoculated

with a layer of wet cotton to provide maximum humidity for infection and disease development. It was done to provide 24 hours pre-inoculation incubation of fruits as suggested by Manandhar *et al.* (1995). Next day the fruits were removed from the decicater and inoculation was made at the site of fruit (surface, except stem end and blossom end portion) as described by Korsten *et al.* (1994). The injury was made by pin pricking at four equdistance point on the outer surface of fruit. The inoculation was made by mycelial bit inoculation method (MBIM) [Rocha *et al.* 1998]. The mycelial discs of uniform size (5 mm diameter) were placed in inverted position (i.e. the side of fungal growth on wounded portion) and covered with small wet cotton swab to provide moisture for conidial germination and infection. The inoculated fruits were again kept in the decicater. The mycelial bit and cotton swab were removed two days after inoculation. Adequate control set of uninoculated fruits was provided (Fig.1). Five fruits were inoculated alongwith a uninoculated control set. The observations for disease appearence were recorded starting 24 hour from inoculation till appearance of visible symptoms at 12 hours interval. The final observations for kind of symptoms were recorded 10 days after inoculation

3.2.1.3 Reisolation

The reisolation was carried out from artificially infected fruits in the same way as described earlier. The isolate of the fungus thus obtained was transferred on PDA slants for comparison with original culture. The pure culture slants thus obtained were used for subsequent studies.

3.2.2 Symptomatology

The symptoms produced by *C. gloeosporioides* in guava were studied from the naturally infected plants in the field as well as artificially inoculated fruits.

3.2.2.1 Symptoms under natural field conditions

The disease symptoms produced on different plant parts were studied in detail. The observations regarding initiation of symptoms, developmental stages of different kind of symptoms, time required for attainment of typical symptoms of disease, number of spots, size of spots and kind of symptoms produced on different plant parts were recorded by following standard methodology. The magnifying lens (10 X) was used where needed. The size of the spots was measured with the help of scale, which had 0.5 mm gradation. The close up photographs of different kind of symptoms on various plant parts were taken by using micro/magnifying lenses.

3.2.2.2 Symptoms under *in vitro* conditions

The fruits of highly susceptible variety of guava (Allahabad safeda) were inoculated by detached fruit technique as described under 3.2.1.2. The symptoms were noted as per 3.2.2.1.

3.2.3 Morphology of the fungus

Morphological characters of the pathogen, *C. gloeosporioides* infecting guava, were studied from the culture growth on potato dextrose agar (PDA) for 5 to 10 days at 25 $\pm$ 1^{0}C. As suggested by Chowdhry and Varshney (2000), observations regarding morphological characters of different structures *viz.*, mycelium (young and matured), acervuli, setae, conidiophore and conidia were noted by adopting slide culture technique. The microscopic measurements were recorded with the help of filar micrometer. Averages based on 50 observations for each structure, recorded from 5 different slides of 10 randomly selected individuals from

each slide. The measurements for young and old mycelium were recorded from five and ten days old cultures, respectively.

3.2.4 Growth and cultural characters of *C. gloeosporioides*

The fungus, *C. gloeosporioides* was grown on different media by using agar plate technique in order to study its growth and cultural characters on different media. Sixteen different synthetic (Table 2) and non-synthetic media (Table 3) included in the studies. A set of quadruplicate plates was maintained for each medium.

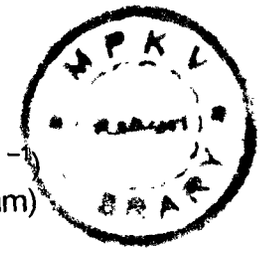
The inoculated plates were then incubated at 27 $\pm$ 1^{0}C temperature in BOD incubator in inverted position. The observations on mean colony diameter and sporulation were recorded at 48 hrs interval, while spore count and other growth characters were noted ten days after inoculation.

The spore count was measured with the help of haemocytometer as per the standard methodology. The growth rate of the fungus on each medium was calculated as follows:

$$GR = \frac{S_{x+1} - S_x}{T_{x+1} - T_x}$$

Where,

GR = Growth rate (mm hr^{-1})
S = Colony diameter (mm)
T = time (hrs)



3.2.5 Effect of different temperatures on growth and sporulation of *C. gloeosporioides*

The effect of different levels of temperature on the growth and sporulation was studied. PDA medium was prepared, autoclaved at 1.54 kg/cm^2 for 15 minutes, poured in plates in equal quantities, allowed to solidify and inoculated with fungal discs of 0.5 cm diameter. The plates were incubated in BOD incubators at different temperatures (0, 5, 10, 15, 20, 25, 30, 35 and 40^{0}C) for ten days. Each treatment was replicated

four times. Observations on colony diameter, growth characters and sporulation were recorded as mentioned under 3.2.4.

3.2.6 Varietal reaction

The study of varietal reaction or varietal screening was carried out to find out resistant varieties against the pathogen *C. gloeosporioides* causing anthracnose of guava. Eleven varieties (Table 5a and 5b) were tested against anthracnose pathogen under natural field as well as *in vitro* conditions.

3.2.6.1 Varietal raction under natural field conditions

The reaction of fruits of different varieties of guava against *C. gloeosporioides* was studied from guava, germplasm collection plot of Department of Horticulture at M.P.K.V., Rahuri. The fruits were allowed for natural infection of disease. The observations for anthracnose were recorded on 5 plants of each variety and three branches of each tree by adopting 0-7 grade score card.

3.2.6.2 Varietal reaction by detached fruit technique

Fresh, fully developed immature healthy fruits of different varieties of guava that had more or less similar age were selected. The fruits were collected, brought to the laboratory and inoculated by the MBIM as described under 3.2.1.2. Uninoculated fruits served as control. A set of quadruplicate fruits was maintained for each variety. Observations on development of symptoms and spore counts were recorded 10 days after inoculation. The disease severity was recorded by 0-7 grade score card as cited below.

Disease reaction classification for anthracnose of guava

Disease Index	Fruit area infection (%)	Symptoms under		Reactions
		Field	*In vitro*	
0	-	Nil	Nil	Immune (I)
1	< 1	Healthy looking fruits, only minute black, pin point, few spots, sometimes yellowish specks may be observed	Healthy looking fruits only minute black, pin point, few spots, sometimes yellowish specks may be observed	Highly resistant (HR)
2	1.01 to 10.0	Fruits with small, light brownish - black spots of about 0.5 to 1.0 mm in diameter and 5 to 20 spots	Fruits with small, brown to dark brown discoloured area of about 0.5 to 2.0 mm in diameter with or without yellow halo	Resistant (R)
3	10.01 to 20.0	Fruits with small, slightly sunken reddish brown spots of 1.0 to 2.0 mm in size and 10 to 25 spots	Light brown discolouration of about 5 to 10 mm in diameter, without any fungal growth	Moderately resistant (MR)
4	20.01 to 30.0	Fruits with medium sized, moderately sunken light brown to black spots of about 2 to 3 mm in diameter and 20 to 25 spots	Fruits with brown discolouration of about 10 to 20 mm in diameter, but < 30 % fruit surface infection, with or without fungal growth	Moderately susceptible (MS)
5	30.01 to 40.0	Fruits with medium to large sized, dark brown to black spots of about 3 to 5 mm in diameter and 50-100 spots scattered all over the fruit, with or without sub-epidermal acervuli	Fruits with brown to chocolate discolouration of about 20-30 mm in diameter, but < 40 per cent area of fruit with scanty to moderate ashy - white fungal growth with or without acervuli.	Susceptible (S)
6	40.01 to 50.0	Fruits with large deeply sunken, dark brown to black spots of about 5 to 6 mm in size and few to many isolated but adjoining spots with sub epidermal acervuli	Fruits with dark brown to chocolate discolouration of about 30-40 mm in diameter, but < 50 per cent area of fruit surface with good ashy- white fungal growth containing saffron acervuli of the pathogen.	Highly susceptible (HS)
7	> 50.01	Fruits with several typical anthracnose type spots which coalesce with each other resulting into blackening of fruits with or without orange to black, pin point sub-epidermal fungal bodies.	Fruits with dark brown to chocolate discolouration covering > 50 per cent area of fruit surface, with luxuriant ashy - white fungal growth containing numerous orange – black acervuli of the pathogen, finally resulting into semi – soft rot of fruits.	Highly susceptible (HS)

Percent disease incidence and intensity were calculated by the formula:

$$\text{Incidence} = \frac{\text{No. of fruits infected}}{\text{Total No. of fruits examined}} \times 100$$

$$\text{PDI} = \frac{\text{Total numerical rating}}{\text{Total No. of fruits examined} \times \text{Maximum rating (i.e. 7 rating)}} \times 100$$

The highest intensity shown by each fruit was taken into consideration for judging the reaction of individual variety. On the basis of kind of symptoms and PDI, different guava varieties were ranked as immune to highly susceptible.

3.2.7 Bioefficacy of fungicides against *C. gloeosporioides* under *in vitro*

Twelve fungicides (Table 6) were evaluated in the laboratory by adopting poisoned food technique as described by Horsefall (1957).

An appropriate quantity of required concentration of each fungicide was added in previously sterilized and moderately cooled (41-45^{0}C) 100 ml PDA separately in 250 ml conical flasks. The flasks were then shaken well to ensure uniform distribution of fungicides in the basal medium. The medium was then poured in quadruplicate in sterilized petriplates and were inoculated at the center with an uniform disc of 0.5 cm diameter of seven days old culture. Plates without fungicides served as control. Then all plates were incubated 27±1^{0}C temperature for 10 days. Observations on colony diameter, growth characters and sporulation were recorded at 24 hrs interval starting 48 hrs after incubation.

An inhibition of the fungal growth in each treatment was calculated by using following formula given by Vincent (1947)

$$I = \frac{100(C - T)}{C}$$

Where, I = Per cent growth inhibition

C = Growth (mm) in control after ten days

T = Growth (mm) in treatment after ten days.

3.2.8 Efficacy of bioagents against *C. gloeosporioides* under *in vitro.*

Four species of *Trichoderma* bioagents *viz., T.viride, T. harzianum, T. hamatum* and *T. koningii* were tested for their bioefficacy against *C. gloeosporioides*. *In vitro* trial was laid out in petriplates by dual inoculation technique (Brodbent *et al.* 1971).

The pure cultures of the bioagents were grown on PDA for 7 days until the petriplate were fully covered. The disc of 0.5 cm diameter of pathogen and bioagents each were cut with sterile cork borer from the peripheral growth zone and transferred them aseptically on PDA in petriplate. The fungal discs were placed in a plate in such a manner so that the pathogen and bio-control agent get equal oppourtunity for growth.

EXPERIMENTAL RESULTS

4. EXPERIMENTAL RESULTS

The present studies were carried out on anthracnose of guava caused by *C. gloeosporioides* in respect of isolation, identification, pathogenicity, reisolation of pathogen, symptomatology, morphology, cultural and physiological characters, reaction of varieties and efficiency of fungicides and bioagents under *in vitro*. The results are presented hereunder :

4.1 Isolation, inoculation, pathogenicity, reisolation and identification of the pathogen

4.1.1 Isolation

Isolations were made from affected fruits of the guava and the pure culture of the fungus thus obtained was later maintained for further studies by subculturing.

4.1.2 Inoculation and pathogenicity

The inoculation studies showed that the fungus could infect the guava fruits and caused disease. The small brown to light chocolate, circular to irregular discolourated areas appeared on the fruits just below the mycelial bit 48 hours after inoculation. Slowly the spots turned ashy at center and surrounded by dark brown margins. During initial stage of disease development the spots resembled typical anthracnose symptoms (Fig.2A, 3A and 3B). However, observations recorded ten days after inoculation revealed that the entire fruit turned dark brown to chocolate and covered with ashy white mycelial growth of the fungus (Fig. 2B). Similarly, in some fruits the ashy-white fungal growth on the spots was surrounded by numerous orange-black fungal bodies (acervuli) in the concentric rings (Fig. 3C and 3 D).

4.1.3 Reisolation

Reisolations from inoculated and diseased guava fruits yielded the fungal culture which was identical to the original isolate.

Fig. 2. Pathogenicity - Symptoms

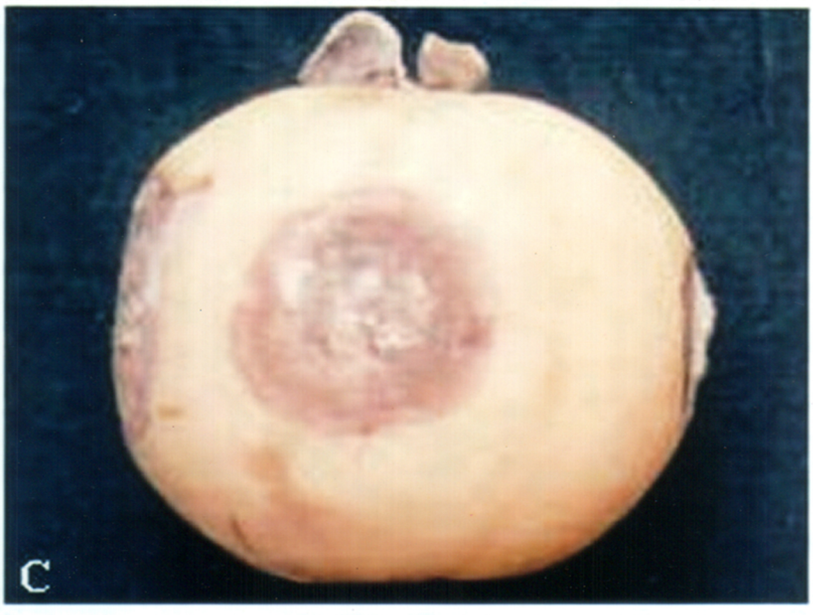
A. Anthracnose symptoms under *in vitro* by detached fruit technique

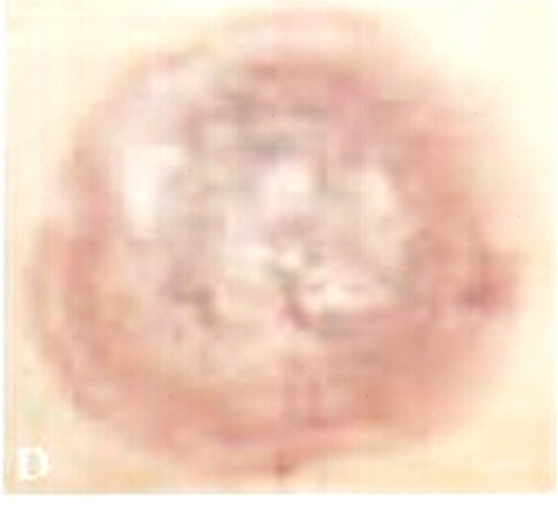
B. Inoculated guava fruit totally covered with ashy fungal growth at advanced stage of the disease

I - Inoculated, UI - Uninoculated

A. Typical anthracnose symptoms on guava fruits showing ashy center with dark margins

B. Enlarged view of typical anthracnose symptoms on guava fruits showing pinkish-ashy center with dark margins





Enlarged view of the spot (C)

Ashy-white fungal growth at the center of the spot (C) surounded by pinkish orange fruiting bodies in concentric rings (D)

Fig. 3. Pathogenicity – symptoms
Different stages of symptom development under *in vitro* by detached fruit technique due to *C. gloeosporioides* on guava fruits.

4.1.4 Identification

The fungus was identified as *Colletotrichum gloeosporioides* Penz. at Department of Plant pathology and Agricultural Microbiology, PGI, MPKV., Rahuri and also confirmed from the Mycologist, Agarkar Research Institute (MACS), Pune-4.

4. 2 Symptomatology

The symptoms of anthracnose caused by *C. gloeosporioides* were studied from the naturally infected plants in the field as well as artificially inoculated fruits in the laboratory by detached fruit technique.

4.2.1 Symptoms under natural field conditions

Generally the disease infected all above ground plant parts *viz.*, fruits, leaves and twigs. In the present investigations the most conspicuous symptoms of disease were appeared on the fruit surface. The young green fruits showed trace infection. The spots on these fruits were very minute and brown to dark brown in colour. On the contrary, the fully grown immature fruits were found to be badly affected by the disease. Formation of grey-brown to black spots during rainy to post rainy season was quite common. Initially, disease symptoms started as production of many, small, water soaked lesions on the surface of fruits. Slowly spots turned light brown in colour about 0.5 to 1 mm in diameter and were shallow (Fig. 4A and 4B -1,2). In the advanced stage the spots enlarged in size upto 3 to 5 mm in diameter became more depressed, circular and redish- brown to light ashy at center and dark brown to black at margins (Fig. 4A and 4B-3,4). In severe infections more number (224 spots/fruit) of spot were produced on fruits (Fig. 4A). Subsequently, they coalesce to form larger, irregular depressed areas on the fruit surface. The diseased portion was comparatively harder, dry and in some cases small cracks developed in them. The fruits did not develop soft rot symptoms. Symptoms on leaves were not so prominent as that of anthracnose appearing

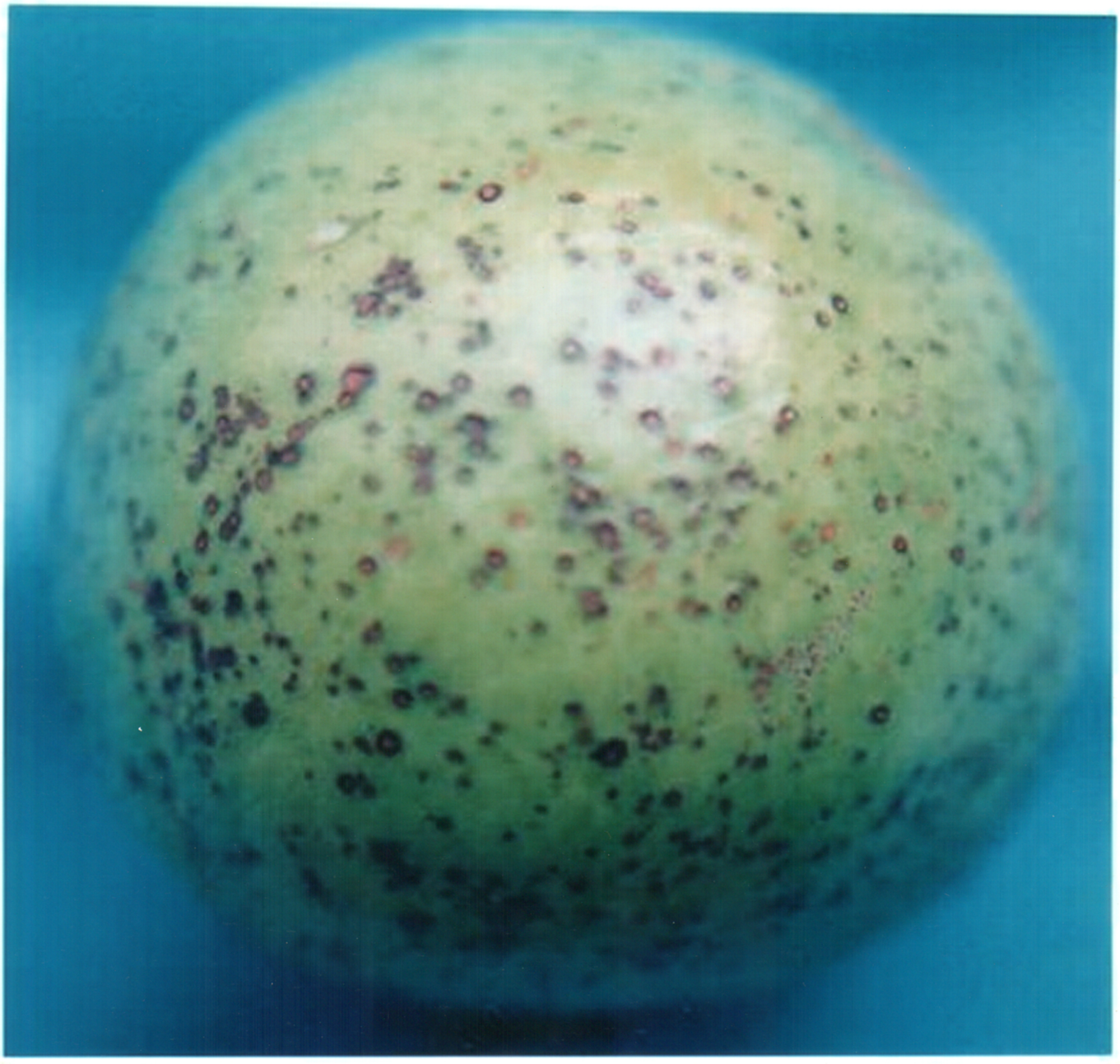


Fig. 4A. Symptomatology
Dark brown to black, depressed sunken, circular spots developed on guava fruits due to *C. gloeosporioides*

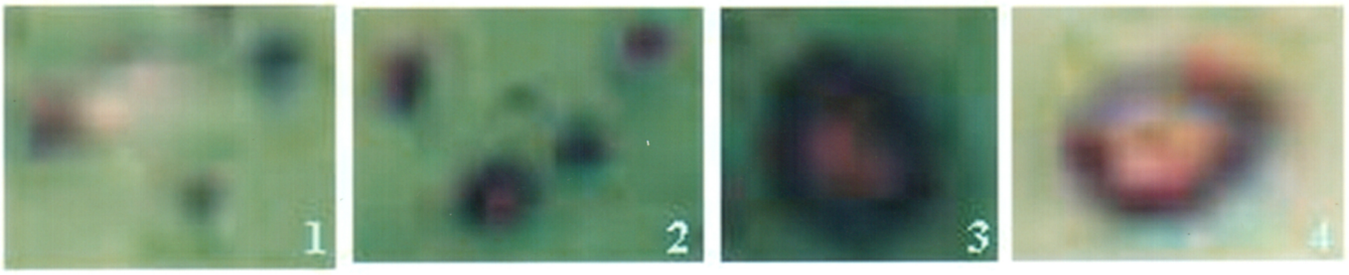


Fig. 4B. Symptomatology
Anthracnose spot development stages on the guava fruits
1. Initiation of disease with pin point spots,
2. Dark brown developing spots,
3. Well developed spots with light centre and
4. Well developed spot with pinkish-brownish centre and dark brown margins

Fig. 5. Symptoms of anthracnose on guava leaves produced by *C. gloeosporioides* under natural field conditions

D. Dark brown to black pinhead spots on leaf

D- Diseased leaf, H - Healthy leaf

on other crops. Dark brown to black, circular, very small spots were noticed on leaf lamina, which did not develop into typical anthracnose symptoms (Fig.5).

Infection of young succulent shoots resulted into twig blight. Initial brown to straw coloured discolouration changed to ashy-white in colour with sub epidermal black bodies. However, the twig infection was very trace.

4.2.2 Symptoms under *in vitro* conditions

The pathogen inoculated fruits of Allahabad Safeda, a highly susceptible variety of guava, by detached fruit technique with mycelial bit inoculation method showed brown to chocolate discolouration of fruit rind. The discolouration started within 48 hours from inoculation and covered complete surface of fruit within further 7 - 8 days. Initially, typical anthracnose type spots i.e. ashy centers surrounded by dark brown margins were visible on the fruit at inoculation site (Fig.3A and B). Further, the disease symptom included formation of concentric rings on the fruit surface. Three concentric rings in the form of white cottony growth at center followed by dull white mycelial zone and finally saffron to chocolate coloured marginal zone. The outer zone had abundant orange to dark brown spore bodies i.e. acervulli (Fig.3C and D). Subsequently concentric spots developed, coalesced and coverd entire fruit with ashy white mycelium (Fig.2B). Finally, soft pliable rot developed emiting bad smell.

4.3 Morphology of the fungus

Morphological observations of the fungus were recorded by adopting slide culture technique. The measurements of different morphological structures of the *C. gloeosporioides* are presented in Table 1.

Table 1. Measurement of different morphological structures of *C. gloeosporioides* inciting anthraconose of guava

Sr. No.	Morphological structures	Measurement parameters (µm)			
		Length	Width	L:W ratio	No/unit
1	Mycelium (young)	-	3.4 (1.67-5.51)	-	-
2	Mycelium (old)	-	4.84 (2.5-180.04)	-	-
3	Acervulus	206.33 (99.66 - 344.0)	116.17 (49.51-180.04)	1.77 (2.01 - 1.91)	-
4	Setae	88.64 (41.8 - 141.6)	3.00 (1.67-5.01)	-	7.00* (3-16)
5	Conidia	11.57 (8.35 - 18.37)	5.33 (4.34-6.68)	2.17 (1.92-2.75)	-
6	Conidiophores	53.52 (33.4 - 76.15)	-	-	-

Note :

- = Not applicable / not observed

* = Number of setae per acervulus

Mycelium

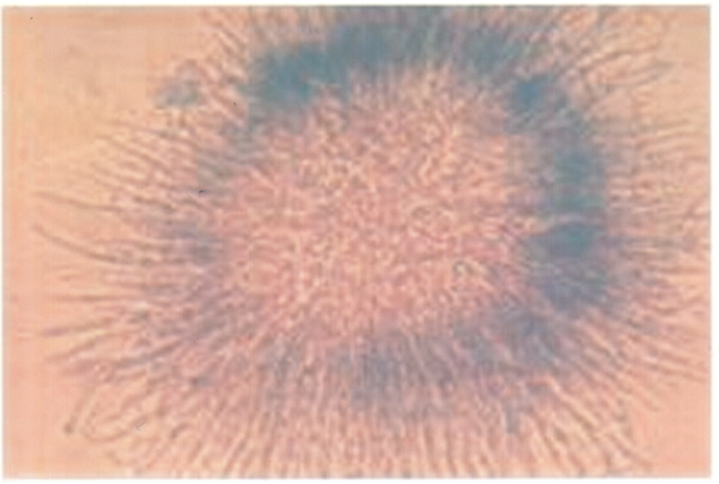
The fungus produced profuse white mycelial growth on PDA, which later on turned dull white to slightly grey in colour. The mycelium was closely septate, irregularly branched and vacuolated. In the early growing stage, hyphae were thin (3.40 µm in diameter), narrow, hyaline but become slightly thick (4.84 µm diameter) as they grew old.

Acervuli

The fungus produced acervuli both on host as well as in culture. On fruits in the field they were sub-epidermal while in artificially inoculated fruits they were prominent on the surface embeded in the mycelial growth. In culture, acervuli were produced in about 4 to 5 days after sub culturing and were firm on the medium. Most of the acervuli were produced within the mycelial mat. Initially, acervuli looked orange in colour, which later changed to dark brown to black in colour (Fig.6C). They were globose (Fig.6A) to saucer or irregular (Fig. 6B) in shape. The acervuli (including setae) measured 206.33 µm (99.66 to 344.0 µm) x 116.17 µm (49.51 to 180.04 µm) in size. The base of the acervulus was dark brown to black, while remaining portion was light brown in colour.

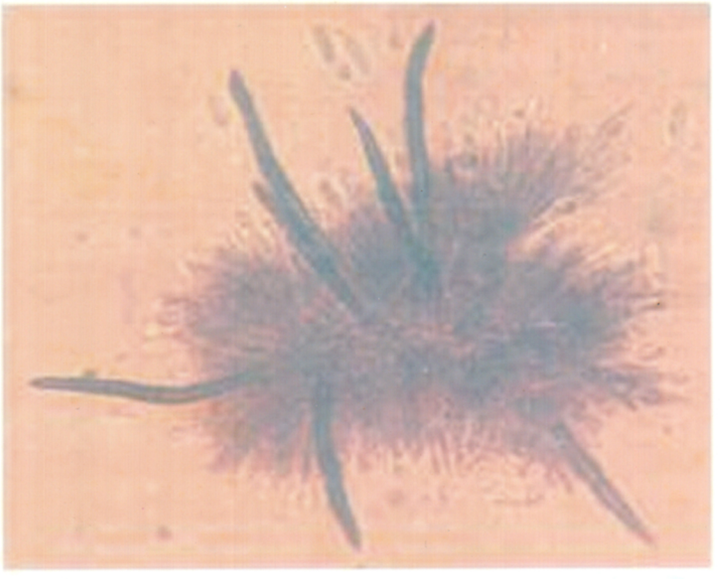
Setae

Setae (Fig.6C) were irregularly arranged throughout the acervulus in the culture. They were ashy - brown to dark brown, septate (4-5), stiff, straight or bending. At base, septation was at shorter distance, while at longer distance from mid to tip. The setae were wider at the base (4.01 µm) and tapering towards tip (2.0 µm), which was obtuse. They were varying in length, which measured 88.64 µm (41.8 to 141.46 µm) and the average width was 3.0 µm (1.67 to 5.01 µm). The number of setae per acervulus varied greatly with an average of 7.0 (3.0 to 16).

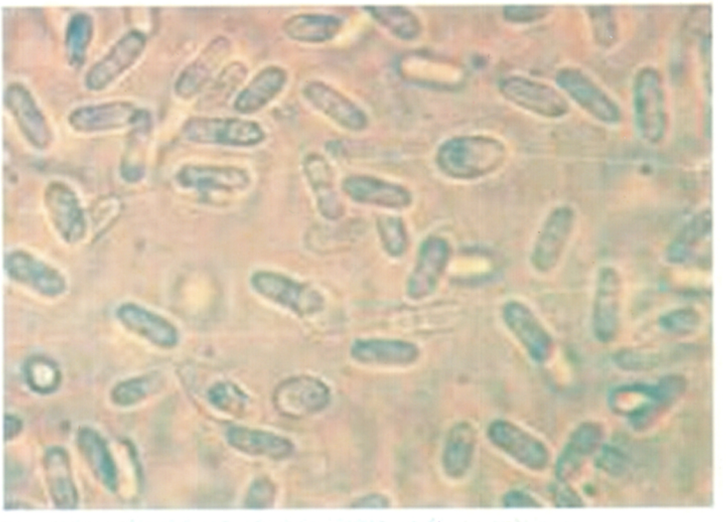


A. Globose acervulus with conidiophores

B. Acervulus with conidiophores and conidia



C. Acervulus with conidiophores, conidia and setae



D. Conidia of *C. gloeosporioides*

Fig. 6. Morphostructures of *C. gloeosporioides* causing anthracnose of guava (40 x 10X)

Conidiophores

The conidiophores (Fig.6A) were short, simple, thickly arranged and were hyaline. They were broader at base, while became quite narrow at tips, which were bearing conidia (Fig.6B). The height of condiophores was 53.52 µm (33.4 to 76.15 µm)

Conidia

The conidia (Fig.6D) were oblong to cylindrical in shape. They were single celled, hyaline when single, but orange to light brown in mucilaginous masses or in acervuli. On an average they measured 11.57 µm (8.35 to 18.37 µm) in length and 5.33 µm (4.34 – 6.68 µm) in width with length : breadth ratio of 2.17 µm (1.92 to 2.75 µm).

4.4 Growth and cultural characters of *C. gloeosporioides*

4.4.1 Growth and cultural characters on synthetic media

The growth and colony characters of fungus on different synthetic media are presented in Table 2. The treatment differences in respect of colony diameter at every 48 hours interval as well as sporulation time and spore count were statistically significant. The synthetic culture media evaluated for growth characters exhibited varying degree of growth rates, mean colony diameter, sporulation time and spore count.

Growth

Growth attained on different synthetic media is illustrated in Fig.7A. The maximum mean growth rate of 0.37 mm hr^{-1} was recorded on different media *viz.,* Richard's agar, Sabourand's agar, Czapek's agar, peptone glucose agar and nutrient agar. Though the growth rate was similar on these media, the colony diameter varied slightly, i.e. 9.0, 8.93, 8.90, 8.86 and 8.80 cm on Richard's agar, Sabourand's agar, Czapek's agar, peptone glucose agar and nutrient agar, respectively, which were

Table 2. Colony diameter, growth rate, sporulation and growth characters of *C. gloeosporioides* from guava on different synthetic media.

Sr. No.	Name of agar media	Colony diameter (cm) and growth rate (mm hr $^{-1}$)						Growth	Sporulation			Colony characters			
		48	96	144	192	240	Mean		Time (hr)	Count (X10^{4} cm^{-2})	Degree	Colour	Margin	Shape	Nature
1	Asthana Hawker's	1.7 (0.35)	4.0 (0.48)	6.2 (0.46)	7.3 (0.23)	8.15 (0.18)	0.34	+++	130	0.64	+++	Cottony white	Entire	Circular	Transparent, scanty, flat mycelium with light concentric rings
2	Czapek's Dox	1.4 (0.29)	4.15 (0.57)	6.00 (0.39)	7.9 (0.40)	8.9 (0.21)	0.37	+++	-	-	-	Pinkish - white	Entire	Circular	Aerial, raised, thick fungal growth without concentric rings
3	Richard's	2.05 (0.43)	4.5 0.51)	7.1 (0.54)	8.1 (0.20)	9.0 (0.19)	0.37	+++	144	0.51	++	Light pinkish	Entire	Circular	Highly aerial, raised mycelium with light concentric zones
4	Sabourand's	2.2 (0.46)	4.5 (0.48)	7.0 (0.52)	8.2 (0.25)	8.93 (0.16)	0.37	+++	96	0.41	++	Pinkish - white	Entire	Circular	Flat mycelial growth with circular rings containing fungal spore bodies
5	Peptone glucose	2.0 (0.42)	3.6 (0.33)	6.23 (0.55)	7 8 (0.32)	8.86 (0.22)	0.37	+++	96	0.72	++++	Yellowish- white	Entire	Circular	Aereal, raised mycelium with abundant brown to black bodies irregularly placed at centre
6	Elliot's	1.7 (0.35)	3.7 (0.42)	5.83 (0.45)	7.40 (0.32)	8.1 (0.15)	0.34	+++	-	-	-	White	Slightly serrated	Roughly circular	Sub-aerial to sub merged mycelium without concentric rings
7	Coon's	2.2 (0.46)	4.63 (0.51)	6.5 (0.39)	7.40 (0.19)	8.5 (0.23)	0.36	+++	144	0.77	++++	Ashy - white to pinkish white	Entire	Circular	Aerial, raised mycelium thick fungal mat without concentric rings and ashy white fungal bodies
8	Nutrient	1.7 (0.46)	3.6 (0.40)	6.0 (0.50)	7.73 (0.36)	8.80 (0.22)	0.37	+++	96	0.65	+++	White	Entire	Circular	Aerial raised mycelial growth with distinct concentric rings
	S.E. ±	0.06	0.07	0.1	0.11	0.06	**0.06**		2.66	0.01					
	C.D. at 5%	0.18	0.21	0.31	0.33	0.2	**NS**		7.97	0.04					
	C.V (%)	3.33	1.77	1.65	1.46	0.81	**16.9**		3.02	2.84					

Note : + = Scanty, ++ = Moderate, +++ = Good, ++++ = Abundant/profuse, - = Nil, Figures in parenthesis are growth rates, NS = Non significant,

The bold figures regarding SE, CD and CV are of growth rate.

at par with each other. The media *viz.,* Conn's, Asthana Hawker's (AHA) and Elliot's agars also recorded good colony diameter of 8.50, 8.15 and 8.10 cm and growth rates of 0.36, 0.34 and 0.34 mm hr^{-1}, respectively.

Sporulation

Significantly highest spore count (0.77 X 10^4 conidia cm^{-2}) was recorded in Coon's agar. It was followed by peptone glucose, nutrient, Asthana Hawker's, Richard's and Sabourand's agars, which sporulated 0.72, 0.65, 0.64, 0.51 and 0.41 x 10^4 conidia cm^{-2}, respectively. While, the fungus did not sporulate on Czapek's Dox agar and Elliot's agar.

Sporulation time

The significantly minimum time of 96 hours was required for sporulation of *C. gloeosporioides* on Sabourand's agars, peptone glucose agar and nutrient agar, while on Richard's agar and Coon's agar fungus sporulated significantly very late (i.e. 144 hours).

Thus, the synthetic media *viz.,* peptone glucose agar, nutrient agar and Coon's agar were best for growth and sporulation of *C. gloeosporioides* .

4.4.2 Growth and cultural characters on non-synthetic media

The data in Table 3 revealed that the differences in respect of colony diameter, sporulation time and spore count were statistically significant.

Growth

The non-synthetic media exhibited varying degree of growth characters as indicated in Table 3. The growth attained by the pathogen on different non-synthetic media is shown in Fig.7B.

Table 3. Colony diameter, growth rate, sporulation and growth characters of *C. gloeosporioides* from guava on different non-synthetic media.

Sr. No.	Name of agar media	Colony diameter (cm) and growth rate (mm hr $^{-1}$)						Growth	Sporulation			Colony characters			
		48	96	144	192	240	Mean		Time (hr)	Count (X10 ^{4} cm $^{-2}$)	Degree	Colour	Margin	Shape	Nature
1	PDA	1.8 (0.38)	4.0 (0.46)	6.85 (0.58)	7.9 (0.23)	9.0 (0.23)	0.38	+++	96	0 71	++++	Cottony - white	Entire	Circular	Aerial, raised mycelial growth with broad concentric rings
2	Carrot	2.2 (0.46)	4.4 (0.46)	7.45 (0.64)	8.1 (0.14)	9.1 (0.23)	0.39	+++	240	0 05	+	Dull white	Entire	Circular	Sub-merged, moderately sparse mycelial growth without concentric rings
3	Guava leaf decoction	1.5 (0.31)	3.1 (0.33)	5.0 (0.40)	6.5 (0.31)	7.9 (0.29)	0.33	+++	-	-	-	Ashy - white	Entire	Circular	Flat, scanty mycelial growth without concentric rings
4	Guava pulp decoction	2.1 (0.43)	4.65 (0.53)	7.25 (0.54)	8.0 (0.16)	9.1 (0.23)	0.39	+++	-	-	-	Blakish with dull white	Serrated	Irregular to circular	Aerial mycelial growth with several marked concentric rings
5	Guava rind decoction	1.8 (0.38)	3.65 (0.39)	6.45 (0.58)	8.0 (0.32)	8.8 (0.17)	0.37	+++	-	-	-	Cottony - white	Entire	Circular	Aerial mycelial growth without concentric rings
6	Guava rind + pulp decoction	1.95 (0 40)	4 05 (0.44)	6.90 (0 60)	7 95 (0 22)	8 90 (0 2)	0 37	+++	-	-	-	Pale grey to ashy	Entire	Circular	Aerial and profuse mycelial growth without concentric rings
7	Corn meal	1.3 (0.27)	2.05 (0.16)	3.5 (0.30)	5.6 (0.44)	6.5 (0.19)	0.27	+	192	0.10	+	White	Entire	Circular	Transparent scanty mycelial with few brown fungal bodies at centre
8	Oat meal	1.95 (0.40)	3.9 (0.40)	6.0 (0.44)	7.8 (0.38)	9.0 (0.25)	0.37	++++	60	0.83	+++	Ashy-brown to dull white	Entire	Circular	Aerial, raised mycelial growth without distinct fungal bodies of saffron to dark colour
	S.E. ±	0.07	0.05	0.08	0.12	0.08	**0.06**		2.35	0.03					
	C.D. at 5%	0.2	0.15	0.22	0.36	0.23	**NS**		7.06	0.08					
	C.V. (%)	3.77	1.34	1.22	1.59	0.92	**17.18**		3.2	12.63					

Note : + = Scanty, ++ = Moderate, +++ = Good, ++++ = Abundant/profuse, - = Nil, Figures in parenthesis are growth rates,

NS = Non significant, The bold figures regarding SE, CD and CV are of growth rates.

The non-synthetic media containing carrot agar and guava fruit pulp decoction showed maximum colony diameter (9.10 cm) and mean growth rate (0.39 mm hr^{-1}) of the pathogen. However, they were at par with Potato dextrose, guava pulp + rind decoction and oat meal agars, which recorded 9.0, 8.9 and 9.0 cm colony diameter and growth rate of 0.38, 0.37 and 0.37 mm hr^{-1}, respectively. Whereas, least colony diameter and growth rate were recorded in guava leaf decoction and corn meal agars as 7.90 and 6.5 cm and 0.29 and 0.27 mm hr^{-1}, respectively.

Sporulation

The significantly maximum sporulation (0.83 x 10^4 spores cm^{-2}) was noticed in oat meal agar (OMA) followed by potato dextrose agar (0.71 x 10^4 spores cm^{-2}). Whereas, corn mean agar (0.1 x 10^4 conidia cm^{-2}) and carrot agar (0.047 x 10^4 conidia cm^{-2}) yielded significantly least spores of the pathogen. There was no sporulation on any host decoction media.

Sporulation time

The pathogen sporulated within significantly minimum (60 hrs) time on oat meal agar and was followed by PDA (96 hrs). On the other hand, carrot agar took significantly maximum time (240 hrs) to initiate sporulation, followed by corn meal agar (192 hrs). On host decoction media pathogen did not sporulate.

Therefore, the results revealed that oat meal agar and PDA were the excellent non-synthetic media for growth and sporulation of *C. gloeosporioides* and next in order was corn meal agar.

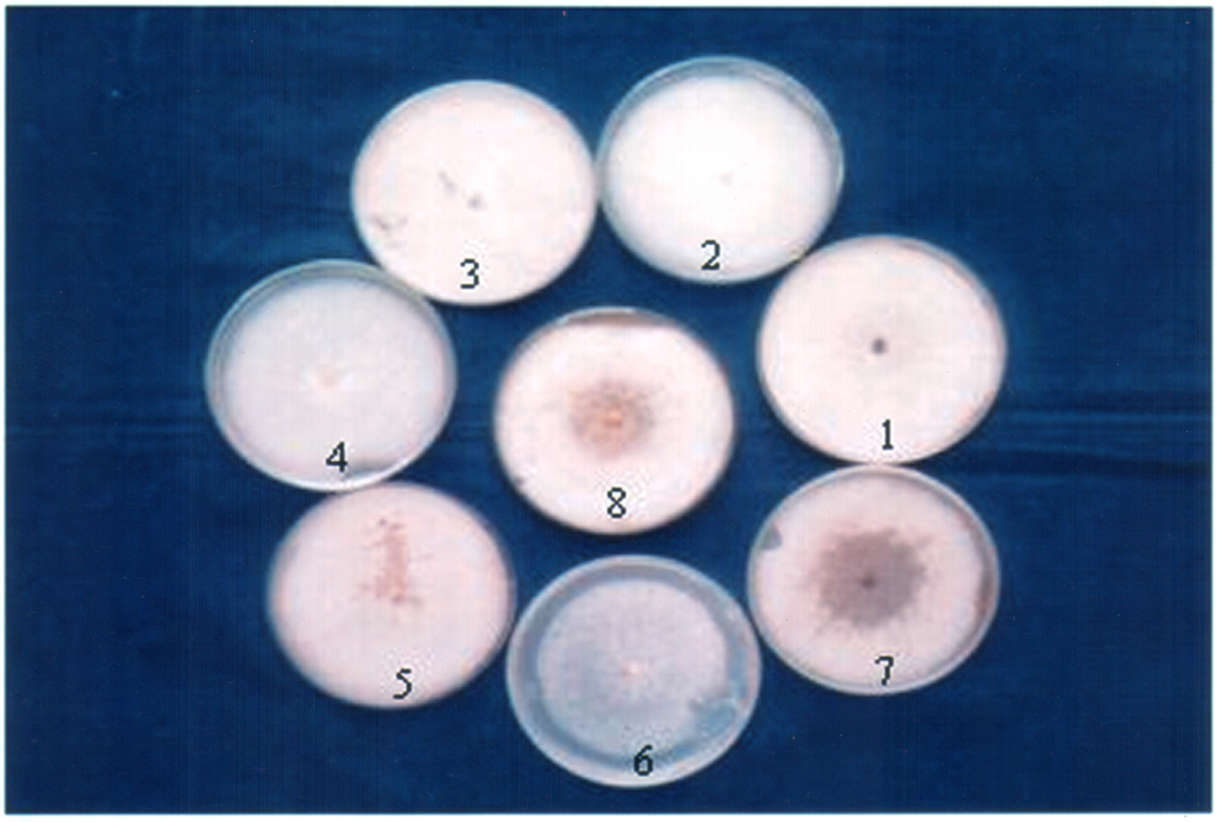


Fig 7A. Effect of synthetic agar media on growth and sporulation of *C. gloeosporioides*

1. Asthana Hawker's, 2. Czapek's Dox, 3. Richard's, 4. Sabourands 5. Peptone glucose, 6. Elliot's, 7. Conn's, 8. Nutrient agars

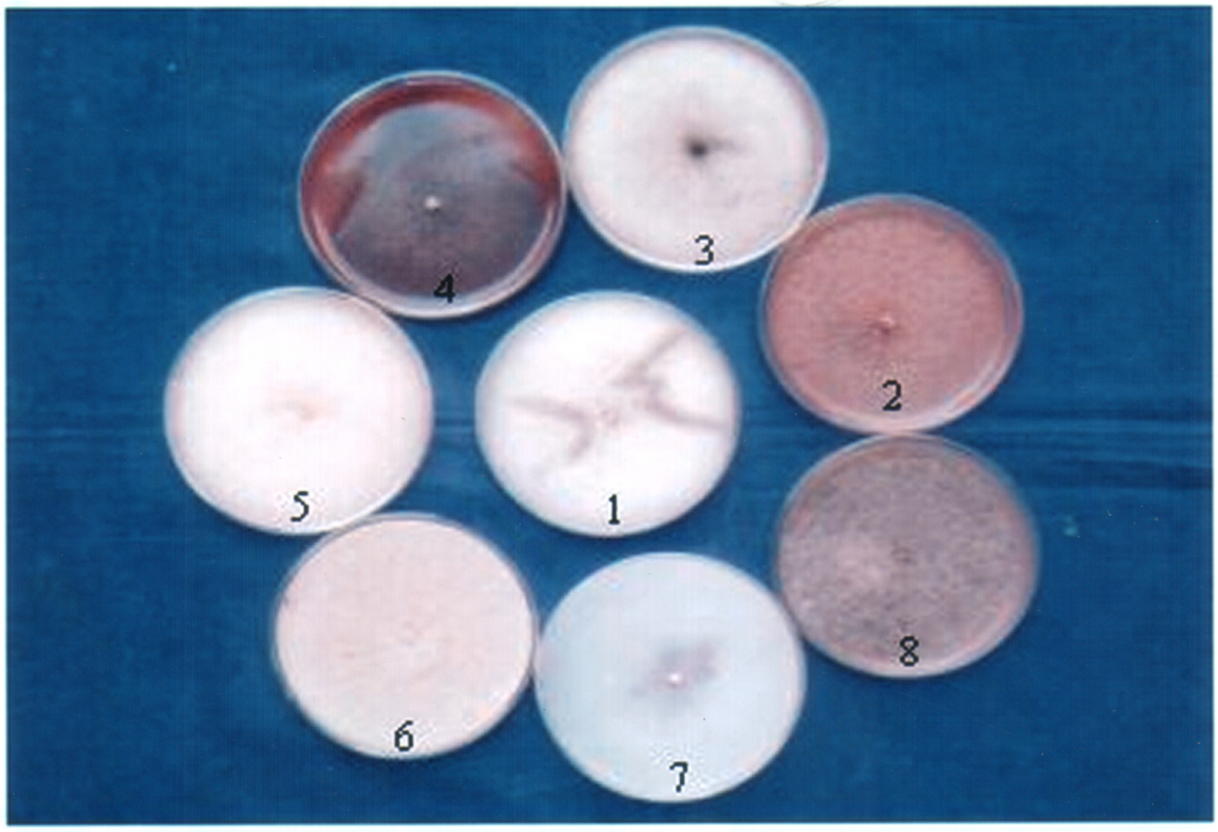


Fig 7B. Effect of non-synthetic agar media on growth and sporulation of *C. gloeosporioides*

1. Potato dextrose, 2. Carrot, 3. Guava leaf decoction, 4. Guava pulp decoction, 5. Guava rind decoction, 6. Guava pulp + rind decoction, 7. Corn meal and 8. Oat meal agars

4.5 Effect of different temperatures on growth and sporulation of *C. gloeosporioides*

The studies were carried out to assess the growth and sporulation of *C. gloeosporioides* at different temperatures. The growth and colony characters of fungus at different temperatures are presented in Table 4. The temperatures evaluated for growth characters exhibited varying degree of growth rates, mean colony diameters, sporulation time and spore count. The treatment differences in respect of all these characters were statistically significant. Growth attained at different temperatures is illustrated in Fig.8.

Growth

The maximum mean growth rate of 0.38 mm hr^{-1} was noticed at 25^0C and 30^0C temperatures. Similarly, significantly maximum colony diameter of 9.1 cm and 9.0 cm respectively were recorded at 25^0C and 30^0C, which were at par with each other. Good growth of fungus (8.6 cm and 7.3 cm colony diameter) was observed at 20^0C and 15^0C temperature, while it was poor (5.5 cm and 5.2 cm) at 35^0C and 10^0C.

Sporulation

The significantly maximum spores (0.72×10^4 cm^{-2}) were counted at 25^0C temperature followed by 30^0C temperature level (0.70×10^4 cm^{-2}), which were at par with each other. It was followed by 35^0C at which moderate sporulation of 0.40×10^4 cm^{-2} was recorded. The fungus sporulated very trace at 20^0C (0.14×10^4 cm^{-2}), while there was no sporulation at 10^0C and 15^0C. Further, it was observed that the lower (0 and 5^0C) and higher temperature of 40^0C did not support the pathogen growth.

Significantly minimum time of only 96 hours was required for sporulation of *C. gloeosporioides* at 25 and 30^0C temperatutres. On

Table 4. Effect of different temperature levels on growth and sporulation of *C. gloeosporioides* under *in vitro*.

Sr. No.	Temperature (^{0}C)	Colony diameter (cm) and growth rate (mm hr^{-1})						Growth	Sporulation			Colony characters			
		48	96	144	192	240	Mean		Time (hr)	Count (X10^4 cm^{-2})	Degree	Colour	Margin	Shape	Nature
1	0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-
2	5	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-
3	10	0.9 (0.19)	1.5 (0.13)	3.35 (0.39)	4.2 (0.18)	5.2 (0.20)	0.22	+	-	-	-	White	Entire	Circular	Sub-merged to sub-aerial mycelium having poor growth without acervuli
4	15	1.5 (0.31)	2.9 (0.29)	4.5 (0.33)	6.12 (0.35)	7.3 (0.24)	0.3	+	-	-	-	White	Entire	Circular	Raised mycelial growth without sporulation
5	20	2.5 (0.52)	4.3 (0.38)	5.7 (0.29)	7.8 (0.44)	8.6 (0.17)	0.36	++	144	0.14	+	White	Entire	Circular	Aerial, raised cottony white mycelial growth with scanty sporulation
6	25	3.1 (0.65)	5.6 (0.52)	6.93 (0.27)	8 4 (0.31)	9 1 (0.16)	0.38	+++	96	0.72	++++	White	Entire	Circular	Aerial mycelium growth with several orange bodies
7	30	2.33 (0.48)	4.1 (0.38)	7.14 (0.65)	8.1 (0.19)	9.0 (0.19)	0.38	+++	96	0.70	++++	White	Entire	Circular	Aerial, raised mycelium growth with orange bodies within mycelial mat
8	35	2.3 (0.25)	2.3 (0.25)	3.4 (0.23)	4.6 (0.25)	5.5 (0.19)	0.23	+	144	0.40	++	White	Entire	Circular	Sub merged mycelial growth with few orange to brown fungal bodies above mat
9	40	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-
	S.E. ±	0.07	0.12	0.1	0.1	0.06	**0.05**		1.60	0.02					
	C.D. at 5%	0.2	0.36	0.3	0.32	0.19	**0.14**		4.71	0.07					
	C.V %	5.53	5.32	2.97	2.5	1.3	**23.66**		2.97	10.60					

Note : + = Scanty, ++ = Moderate, +++ = Good, ++++ = Abundant/profuse, - = Nil, Figure in parenthesis are growth rates,
The bold figures regarding SE, CD and CV are of growth rate.

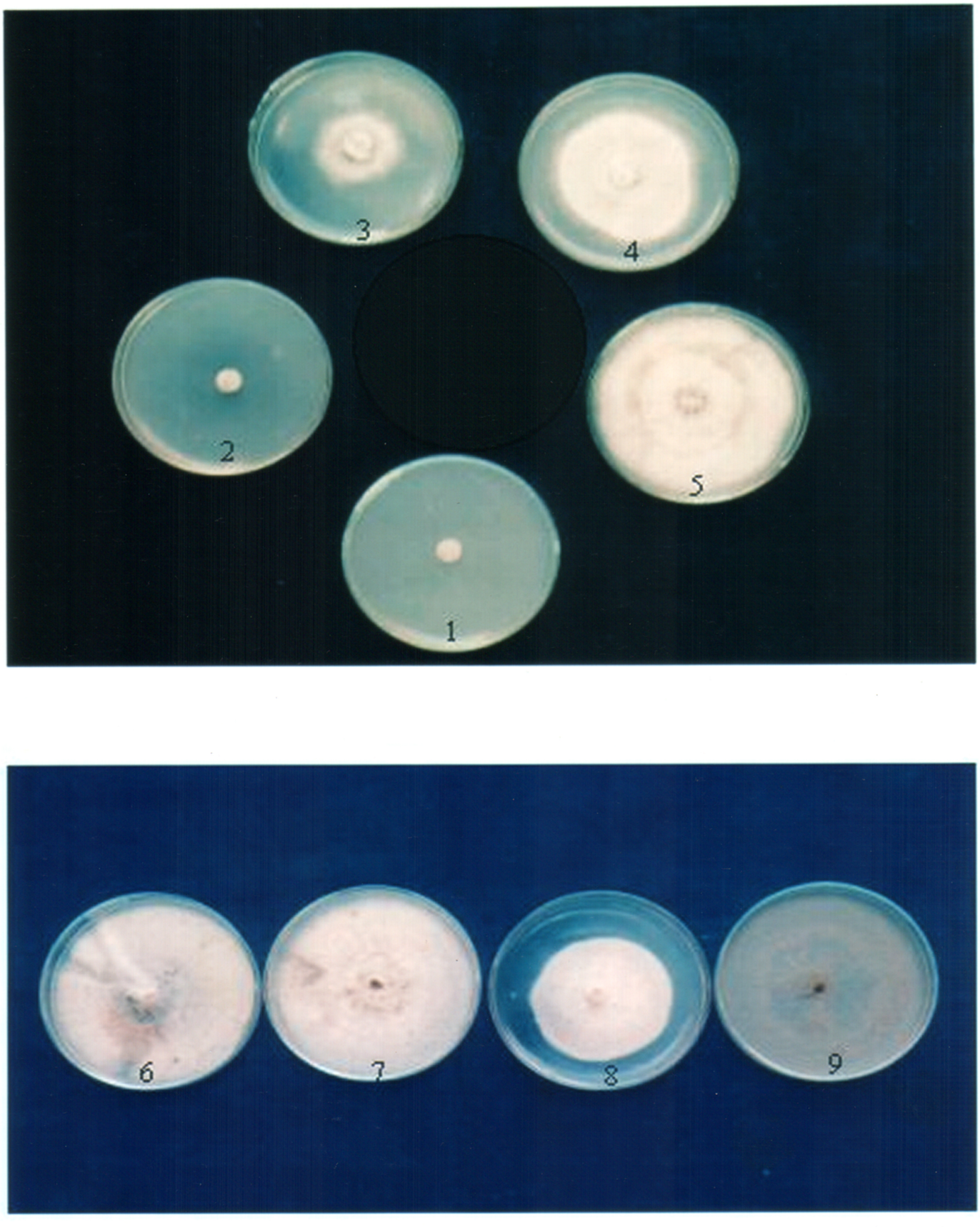


Fig. 8. Growth and sporulation of *C. gloeosporioides* at different temperatures

1. 0 °C	2. 5 °C	3. 10 °C
4. 15 °C	5. 20 °C	6. 25 °C
7. 30 °C	8. 35 °C	9. 40 °C

the contrary, the temperature levels of 20 and 35^{0}C took significantly maximum time (144 hrs.) to initiate sporulation.

Thus, the optimum temperature for maximum growth and sporulation of the fungus *C. gloeosporioides* was observed to be ranging between 25^{0}C and 30^{0}C.

4.6 Varietal reaction

The reaction of the fruits of different guava varieties to pathogen *C. gloeosporioides* was studied under natural field conditions and also in laboratory by detached fruit technique (DFT) and mycelial bit inoculation method (MBIM). The results in Table 5a and 5b indicate that the difference in respect of diseases intensity among eleven guava varieties under field as well as *in vitro* were statistically significant.

4.6.1 Disease reaction under natural field condition

The attack of *C. gloeosporioides* causing anthracnose was observed on all guava varieties under field conditions (Table 5a). Significantly highest disease intensity (48.0%) was noticed on the fruits of Allahabad safeda followed by Sardar (41.6%) and Makhmalabad Safeda (40.03%) which were at par with each other and showed highly susceptible reaction. Inaddition the varieties *viz.*, Behat coconut (37.6%), Apple colour (37.6%) and chettedar (34.4%) recorded susceptible reaction. The moderately susceptible varieties were seedless (29.6%), Basti (28.0%), Nagpur seedless (26.0%) and Pear shaped (25.4%) which were at par with each other. Behat seedling (5.4%) was the only variety having resistant disease reaction.

Table 5a : Reaction of different guava varieties to anthracnose caused by *C. gloeosporioides* under natural field condition.

Sr. No.	Variety	Per cent Disease Incidence	Per cent Disease Intensity	Reaction
1.	Allahabad safeda	35.41 (36.483)	48.0 (44.040)	HS
2.	Seedless	30.50 (33.473)	29.6 (32.704)	MS
3.	Sardar	21.0 (27.243)	41.6 (40.122)	HS
4.	Behat seedling	15.13 (22.747)	5.4 (13.388)	R
5.	Makhamalabad safeda	26.66 (30.970)	40.03 (38.906)	HS
6.	Pear shaped	21.83 (27.730)	25.4 (29.232)	MS
7.	Nagpur seedless	15.34 (23.543)	26.0 (30.206)	MS
8.	Behat coconut	16.12 (23.277)	37.6 (37.578)	S
9.	Chettedar	17.59 (24.020)	34.4 (37.578)	S
10.	Basti	18.91 (25.703)	28.0 (31.628)	MS
11.	Apple colour	29.47 (32.873)	37.6 (37.588)	S
	S.E.± C.D. at 5% CV (%)	2.56 7.56 15.85	3.47 9.90 22.97	

Note :

HR = Highly resistant
R = Resistant
MR = Moderately resistant
MS = Moderately susceptible
S = Susceptible
HS = Highly susceptible

Figures in parenthesis are arc sin transformed values.

Table 5 b : Reaction of different guava varieties to anthracnose caused by *C. gloeosporioides* under *in vitro*.

Sr.No.	Variety	PDI	Sporulation		Symptoms	Reaction
			Count ($x 10^4 cm^{-2}$)	Degree		
1.	**Allahabad safeda**	90.0 (70.767)	2.41	++++	All fruits covered with ashy white moderately raised mycelium with coloured fungal bodies. A soft pliable rot developed and fruit decayed slowly.	HS
2.	**Seedless**	67.50 (55.328)	0.51	+	Spots with ashy white center and with grey brown margin. Later on fruit covered with raised cottony white mycelium without visible sporulating bodies.	HS
3.	**Sardar**	72.5 (58.692)	2.51	++++	Concentric zones with ashy white center and brown coloured margin with saffron coloured acervuli.	HS
4.	**Behat seedling**	7.0 (15.212)	0.41	+	Mycelium was white, submerged scanty had small brown spots of discolouration with concentric zones while with trace pinkish bodies.	R
5.	**Makhamalabad safeda**	71.25 (57.750)	2.6	++++	The distinct layer of mycelium on fruit surface white cottony growth at center and numerous pinkish acervuli around margin.	HS
6.	**Pear shaped**	62.5 (52.400)	0.37	+	Two distinct zones i.e. white center surrounded by brownish – ashy fungal growth.	HS
7.	**Nagpur seedless**	55.0 (47.885)	0.84	+	Concentric zones of blackish- white mycelium chocolate discolouration of fruit.	HS
8.	**Behat coconut**	55.0 (47.885)	0.97	+	Irregular ashy white fungal growth on surface of fruit	HS
9.	**Chettedar**	60.0 (50.895)	1.17	++	Chocolate discolouration of fruit with ashy white mycelial growth.	HS
10.	**Basti**	55.0 (52.700)	-		Fungus grows irregularly with first discolouration of rind and than cottony cover of fungus	HS
11.	**Apple colour**	70.0 (56.923)	2.37	++++	Submerged irregular profuse ashy white growth of fungus with abundant pinkish acervuli	HS
	S.E.±	2.93	0.17			
	C.D. at 5%	8.42	0.51			
	CV (%)	5.70	13.54			

Note : + = Scanty, ++ = Moderate, +++ = good , ++++ = Abundant / Profuse, HR = Highly resistant, R = Resistant, MR = Moderately resistant HS = Highly susceptible, MS = Moderately susceptible, S = Susceptible, Figures in parenthesis are arc sin transformed values.

Fig. 9. Severity of *C. gloeosporioides* anthracnose on different guava varieties

1. Allahabad safeda, 2. Seedless, 3. Sardar, 4. Behat seedling, 5. Makhamalabad safeda, 6. Pear shaped, 7. Nagpur seedless, 8. Behat coconut, 9. Chettedar, 10. Basti, 11. Apple colour

4.6.2 Disease reaction under *in vitro* condition

All the tested varieties were highly susceptible to the anthracnose caused by *C. gloeosporioides,* except Behat seedling, which was resistant and recorded just 7.0 per cent diseases intensity (Table 5b).

The varieties *viz.,* Allahabad safeda, Sardar, Seedless, Behat coconut, Chettedar, Nagpur seedless, Pear shaped and Basti all were highly susceptible, but differed significantly from each other in respect of diseases intensity (Fig.9). Further, it was noticed that the anthracnose intensity was quite more by DFT as compared to field conditions.

Sporulation

The highest sporulation (2.6 x 10^4 cm^{-2}) of pathogen was noticed on the fruits of Makhamalabad seedling. This was followed by Sardar (2.51 x 10^4 cm^{-2}) , Allahabad safeda (2.41 x 10^4 cm^{-2}) and Apple colour (2.37 x 10^4 cm^{-2}). The variety, Behat seedling that had resistant reaction produced very trace sporulation (0.41 x 10^4 cm^{-2}). It is very interesting to note that the highly susceptible variety, Basti did not support any sporulation of the fungus on infected fruits. Similarly, other highly susceptible varieties *viz.,* Behat coconut, Seedless, Nagpur seedless and Pear shaped were supported trace sporulation of 0.37, 0.51, 0.84 and 0.97 x 10^4 cm^{-2}, respectively.

Therefore, among eleven varieties only Behat seedling was resistance to *C. gloeosporioides* causing anthracnose.

4.7 Bioefficacy of fungicides against *C. gloeosporioides* under *in vitro*

The results of *in vitro* trial regarding bioefficacy of fungicides against *C. gloeosporioides* are presented in Table 6 and Fig.10.

Table 6. Effect of different fungicides on growth and sporulation of *C. gloeosporioides* under *in vitro*.

Sr. No.	Fungicides	Conc. (%)	Colony diameter(cm) and growth rate i.e. GR (*mm hr^{-1}*) hours after inoculation						Growth		Sporulation		
			48	96	144	192	240	Mean	Degree	Inhi-bition (%)	Time	Degree	Count
1	Bordeaux mixture	1.00	0.00	0.00	0.00	0.00	0.00	0.00	-	100	_	_	0.00
2	*Ready Bordeaux* mixture	1.00	1.20 **0.25**	2.70 **0.31**	4.10 **0.29**	5.20 **0.23**	6.10 **0.19**	**0.25**	+++	26.5	96	+	0.08
3	Chlorothalonil	0.25	0.00	0.90 **0.19**	1.85 **0.20**	3.00 **0.24**	4.10 **0.23**	**0.17**	+	50.6	144	+	0.07
4	Tricyclazole	0.10	0.00	0.00	0.00	0.00	0.00	0.00	-	100	_	_	0.00
5	Copper oxychloride	0.25	1.20 **0.25**	2.60 **0.30**	4.30 **0.35**	5.70 **0.30**	6.20 **0.10**	**0.26**	+++	25.3	-	-	0.00
6	Propineb	0.25	0.00 **0.00**	1.50 **0.31**	3.20 **0.35**	4.50 **0.27**	5.47 **0.20**	**0.23**	++	34.1	144	+	0.08
7	Difenoconazole	0.10	0.00	0.00	0.00	0.00	0.00	0.00	-	100	-	-	0.0
8	Carbendazim	0.10	0.00 **0.00**	0.00 **0.00**	0.65 **0.14**	0.80 **0.03**	1.00 **0.04**	**0.04**	+	87.95	-	-	0.00
9	Captan	0.25	0.00 **0.00**	0.70 **0.15**	1.25 **0.12**	2.00 **0.16**	2 50 **0.11**	**0.11**	+	69.87	-	-	0.00
10	Mancozeb	0.25	0.00 **0.00**	2.00 **0.42**	3.60 **0.33**	5.70 **0.44**	6.80 **0.23**	**0.28**	+++	18.1	192	++	0.10
11	Propiconazole	0.10	0.00	0.00	0.00	0.00	0.00	0.00	-	100	-	-	0.00
12	Hexaconazole	0.10	0.00	0.00	0.00	0.00	0.00	0.00	-	100	-	-	0.00
13	Control	-	2.05 **0.42**	5.45 **0.71**	6.95 **0.31**	7.81 **0.18**	8.30 **0.10**	**0.35**	+++	-	96	+++	0.53
	S.E. ±		0.02	0.018	0.08	0.08	0.09	0.05		0.52	0.85		0.01
	C.D. at 5 %		0.07	0.05	0.24	0.23	0.26	0.13		1.50	2.45		0.03
	C.V. (%)		7.10	1.50	4.20	3.00	2.86	36.15		0.83	1.91		14.42

Note: + = Scanty, ++ = Moderate, +++ = Good, ++++ = Abundant/profuse, - = Nil

Figures in bold are growth rates.

Table 7. Effect of different fungicides on colony characters of *C. gloeosporioides* under *in vitro.*

Sr. No.	Fungicides	Conc. (%)	Colony characters			
			Colour	Margin	Shape	Nature
1	**Bordeaux mixture**	1.00	-	-	-	-
2	**Ready Bordeaux mixture**	1.00	Ashy to dull white	Entire	Circular	Mycelium slightly raised at centre and submerged at margin
3	**Chlorothalonil**	0.25	Pale white	Serrated	Irregular	Raised mycelium
4	**Tricyclazole**	0.10	-	-	-	-
5	**Copper oxychloride**	0.25	Light brown to ashy white	Entire	Circular	Flat, scanty growth of mycelium towards peripheri
6	**Propineb**	0.25	Pinkish - white	Entire	Circular	Slightly raised mycelium growth
7	**Difenoconazole**	0.10	-	-	-	-
8	**Carbendazim**	0.10	Ashy - white	Entire	Circular	Aerial, very scanty mycelium growth
9	**Captan**	0.25	White	Entire	Circular	Raised mycelium growth
10	**Mancozeb**	0.25	Pinkish - white	Entire	Circular	Highly aerial and profuse growth of mycelium
11	**Propiconazole**	0.10	-	-	-	-
12	**Hexaconazole**	0.10	-	-	-	-
13	**Control**	-	Cottony white	Entire	Circular	Highly aerial and profuse mycelium growth

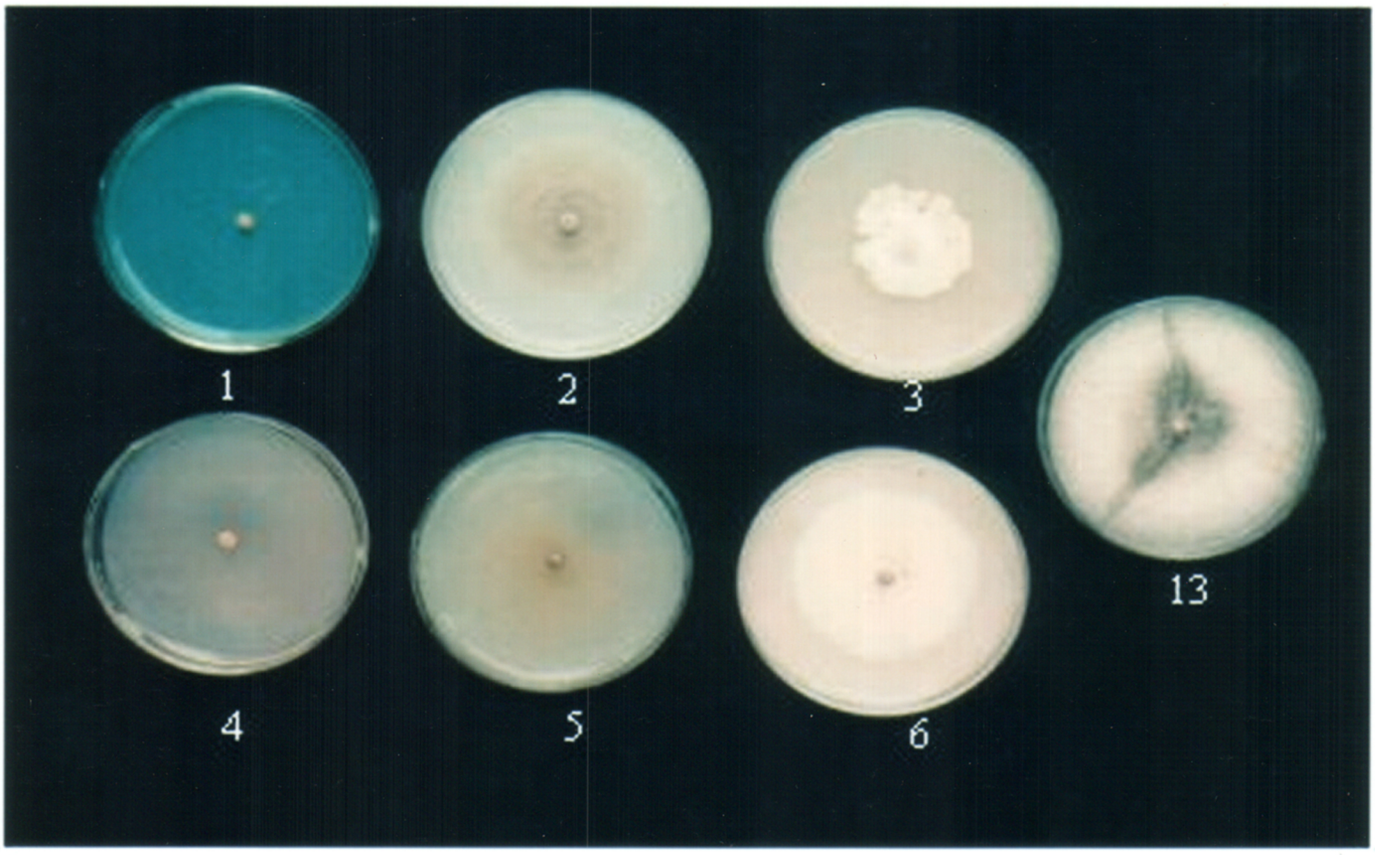
Growth

The treatment differences in respect of colony diameter and growth rate inhibition percentage and sporulation time were statistically significant. All fungicidal treatments showed significantly least colony diameter and mean growth rate as compared to control. The growth of the fungus was completely inhibited by the fungicides *viz.,* Bordeaux mixture, tricyclazole, difenoconazole, propiconazole and hexaconazole, thereby showed 100 per cent inhibition of the pathogen (Fig.10).

The next fungicides in order of superiority were carbendazim and captan wherein the colony diameter was just 1.0 and 2.5 cm, which showed minimum mean growth rate of 0.04, and 0.11 mm hr^{-1}, respectively. Thus, these fungicides showed significantly maximum pathogen inhibition of 87.95 and 69.87 per cent, respectively as compared to rest of the fungicides. The next fungicides in order were chlorothalonil and propineb those showed colony diameter of 4.1 and 5.5 cm, the growth rate of 0.17, 0.23 mm hr^{-1} and inhibition of 50.6 and 34.1 per cent, respectively. On the contrary, the fungicides *viz.,* ready Bordeaux mixture (Bordo), copper oxychloride and mancozeb were least effective, which had higher growth rate of 0.25, 0.26 and 0.28 mm hr^{-1}, and thereby significantly least inhibition of the fungus i.e. 26.5, 25.3 and 18.1 per cent, respectively.

Sporulation

The fungicides *viz.,* ready Bordeaux mixture, chlorothalonil, propineb and mancozeb were not only ineffective in inhibiting the fungal growth but also failed to suppress the sporulation of pathogen. Though the fungicides, carbendazim and captan showed little fungal growth they did not support the sporulation of pathogen.



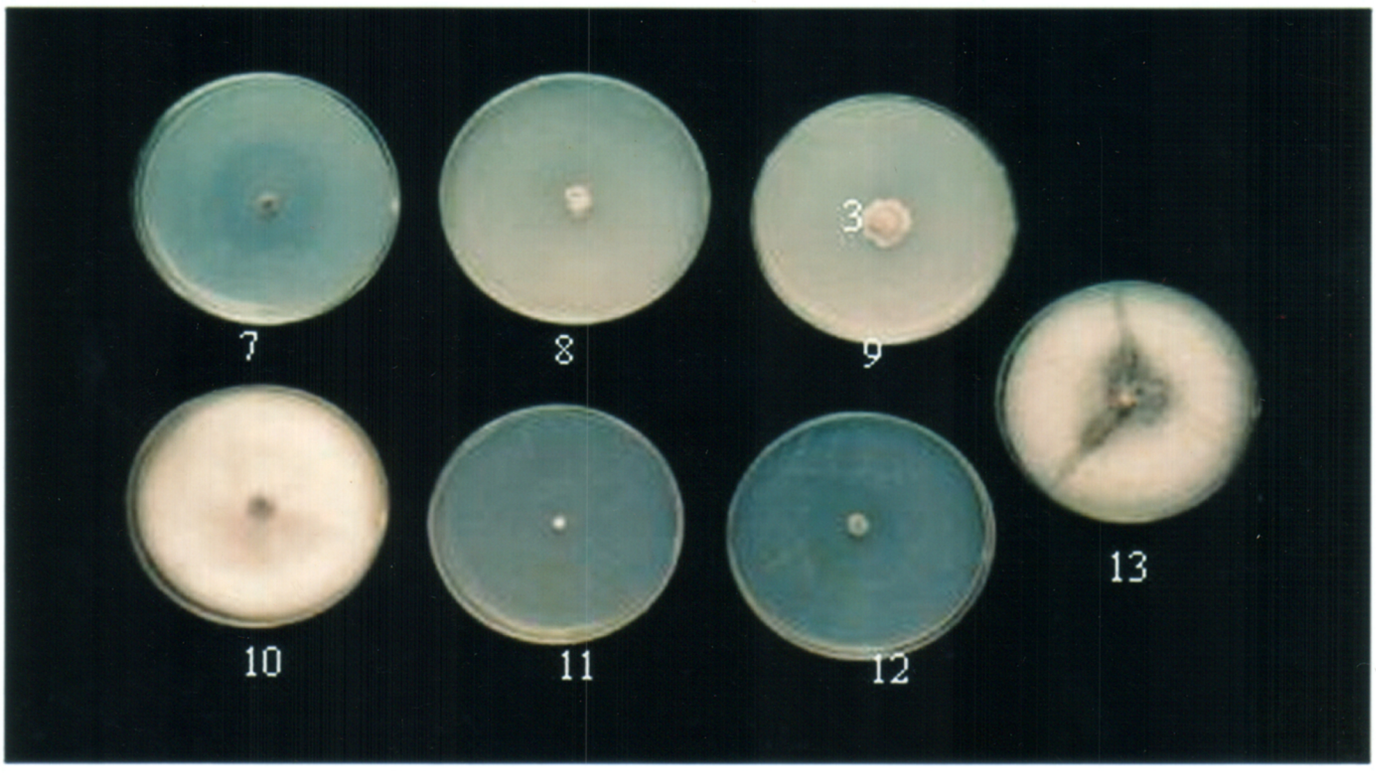


Fig. 10. Effect of fungicides on growth and sporulation of *C. gloeosporioides* under *in vitro*

1. Bordeaux mixture, 2. Ready B.M., 3. Chlorothalonil,
4. Tricyclazole, 5. Copper oxychloride, 6. Propineb,
7. Difenoconazole, 8. Carbendazim, 9. Captan
10. Mancozeb, 11. Propiconazole, 12. Hexaconazole,
13. Control

These results indicated that some fungicides completely inhibited growth as well as sporulation, others failed to suppress growth and also sporulation while few failed in either of these. This showed their varied mode of action against the pathogen under *in vitro* studies. Thus, the results indicated that the fungicides *viz.,* Bordeaux mixture, tricyclazole, difenoconazole, propiconazole and hexaconazole were the best followed by carbendazim and captan.

4.8 Efficacy of bioagents against *C. gloeosporioides* under *in vitro*

The studies on the inhibitory effect of an antagonistic biological agents, *Trichoderma* spp. against the pathogen *C. gloeosporioides* by dual inoculation technique showed a significant variation in the reduction of the pathogen growth (Table 8 and Fig.11).

The biological agents *viz., T. harzianum, T. hamatum, T. viride* and *T. koningii* were tested against the *C. gloeosporioides* under *in vitro.* Among the bioagents, *T. hamatum* was found to be the most effective wherein the colony diameter of the pathogen was significantly least (2 cm) and thereby recorded maximum inhibition (76.20%) of mycelial growth. It was followed by *T. harzianum* (45.0%), *T.viride* (42.50%) and *T. koningii* (41.66%), which showed moderate inhibition of mycelial growth of pathogen.

Thus, *in vitro* studies revealed that the antagonist *T. hamatum* was most effective bioagent against *C. gloeosporioides* followed by *T. harzianum, T. viride* and *T. koningii* in the order of their effectiveness.

Table 8. : *In vitro* effect of bioagents on growth and inhibition of *C. gloeosporioides*

Sr. No.	Bioagents	Mycelial growth of pathogen* (cm)	Growth inhibition (%)
1	*T. harzianum*	4.63	45.00
2	*T. hamatum*	2.00	76.20
3	*T. viride*	4.83	42.50
4	*T. koningii*	4.90	41.66
5	Control	8.40	-
	SE ±		0.10
	CD at 5%		0.30
	CV (%)		1.95

Note : * = Mean of three replications

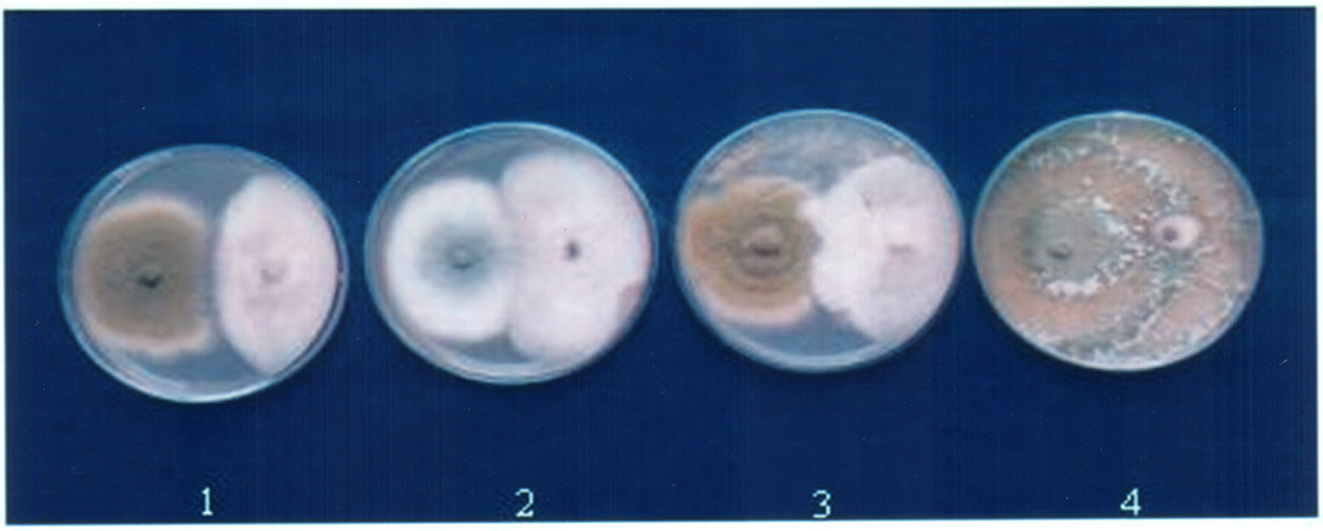


Fig. 11. Efficacy of different species of *Trichoderma* against *C. gloeosporioides* under *in vitro*

1. *Trichoderma koningii*,
2. *Trichoderma viride*
3. *Trichoderma harzianum*
4. *Trichoderma hamatum*

DISCUSSION

5. DISCUSSION

Guava (*Psidium guajava*), the apple of the tropics, is one of the most common fruits in India. Guava fruit is a berry and has very thin skin, so it is normally consumed fresh as a dessert fruit. The fruit has a characteristic gritty texture due to presence of stone cells and have sweet aroma. The fruit is excellent source of vitamin C and pectin. In addition, salad, pudding, jam, jelly, nectar concentrate and syrup can be made from it.

The area under guava is increased only due to high economic returns obtained from this crop. The crop is found to be badly infected by several diseases. However, anthracnose caused by *C. gloeosporioides* is becoming severe threat to guava crop. The disease was noticed in high intensity during *kharif* 2004 in Horticultural garden at MPKV; Rahuri. Hence, looking to the economic importance of crop, severity of disease and future threat to the farmers, the present investigations were planned to study the forecoming aspects.

5.1. Isolation, inoculation and pathogenicity of *C. gloeosporioides*

In the present investigation, diseased guava fruits showing typical symptoms of disease were collected from Horticultural garden of MPKV., Rahuri in the month of October, 2004. Isolations made from diseased fruits yielded the fungus *C. gloeosporioides* Penz., The pathogenicity test was carried out by detached fruit technique with mycelial bit inoculation method (MBIM) under *in vitro*. Reisolation made from the artificially inoculated guava fruits yielded fungus similar to original one, which was isolated and inoculated, thereby proving Koch's postulates. Thus, it was confirmed that the fungus, *C. gloeosporioides* was pathogenic to guava fruits in *in vitro* pathogenicity tests. The kind of symptoms produced

during initial growth stage of disease development in pathogenicity tests were typical anthracnose type.

The pathogenicity tests carried out in the present investigations are in agreement with the findings of Tricita *et al.* (1975), Yang and Chuang (1994) Abdel (2000) and Kader and Rahman (2001) who also proved the Koch's postulate of *C. gloeosporioides* on guava (*Psidium guajava*).

In addition to this, different scientists (Rathod, 1994 and Gaikwad, 2002) also proved the Koch's pastulates of *C. gloeosporioides* (on mango and custard apple, respectively), which is in conformity with the pathogenicity studies carried out in the present work.

5.2 Symptomatology

The symptoms of anthracnose caused by *C. gloeosporioides* were studied from naturally infected plants as well as artificially inoculated fruits by detached fruit technique.

5.2.1 Symptoms under field conditions

In the field anthracnose started right from immature young fruits. However, Midha and Chohah (1968) did not observe infection of young green fruits that is not agreeing with present results. The present finding is in conformity with the results of Carranza *et al.* (2002) who noticed that the anthracnose symptoms due to *C. gloeosporioides* appeared on the young green fruits. Whereas, the symptoms like sunken, large sized spots up to 5 cm long as observed by him on young and green fruits did not notice in the present investigation, whereas in present finding the spots were very minute and dark brown. The infection of disease on young fruits was very trace, however, the fully grown immature fruits were found to be badly infected by the disease. These finding are in agreement with the symptoms reported by Tondan and Singh (1968) and Midha and Chohan (1968) who also observed heavy infection of disease on fully matured green fruits. The typical symptoms of anthracnose

on the guava fruits included sunken, circular, ashy-black spots of 3 to 5 mm in size, which coalesced to form large, irregular depressed areas on the fruit surface. These symptoms are in consonance with the reports of the Tondan and Singh, (1968). Whereas, the cankerous, raised spots as recorded by Midha and Chohan (1968) and Tricita *et al.* (1975), similarly, Salmon – pink masses of spores on the infected portions of guava fruits due to *C. gloeosporioides* as reported by Tondan and Singh (1968) and Carranza *et al.* (2002) were not observed in the present investigation.

5.2.2 Symptoms under *in vitro*

The sympytoms under *in vitro* condition by detached fruit technique and mycelial bit inoculation method started within 48 hours of inoculation showing brown to chocolate discolouration of fruit rind and covered entire fruit surface within 7 - 8 days. The typical symptoms included formation of fungal concentric rings with numerous pinkinsh to chocolate brown coloured fruiting bodies consisting of spore masses. The symptoms in respect of spore masses are in agreement with the findings of Tondon and Singh (1968) and Carranza *et al.* (2002).

5.3 Morphology of the fungus

The various morphological structures *viz.*, mycelium, acervuli, setae, conidiophores and conidia of *C. gloeosporioides* were studied. The mycelium of the fungus was closely septate, irregulally branched and vacuolated and measured 4.84 µm in diameter turning to slightly grey at maturity. The present findings about mycelium colour are in conformity with the results of Singh (1978) and Hasabnis (1984) who also noticed dark coloured mycelium of *C. gloeosporioides* infecting mango. Similarly, the results recorded by Thakare (1991), Hande (2001) and Gaikwad (2002) with *C. gloeosporioides* from chrysanthemum, curry leaf and custard apple, respectively are tallying in respect of general characters of mycelium. The mycelial measurements from the present study do not

tally with the earlier work carried out by different scientists (Patil,1968; Thakare, 1991 and Hande,2001), but slightly nearer to the reports of Rathod (1994) and Gaikwad (2002). In culture the acervuli were produced within 4-5 days which looked dark pinkish initially and later turned dark brown to black. Earlier, Patil (1968) observed that the acervuli of *C. gloeosporioides* from mango were produced within 6-7 days after inoculation that is dissimilar from the present results. However, the findings regarding colour of aceervuli are in agreement with the work carried out by Hande (2001). On diseased host surface the acervuli were subepidermal, which is similar to the reports of Bose *et al.,* (1973) who also noticed subepidermal acervuli of *C. gloeosporioides* in mango.

The acervuli were globose to saucer or irregular in shape and measured 206.33 x 116.17 µm in size. The results are in conformity regarding the shape of acervuli as noticed by Gaikwad (2002), while not tallying with Hande (2001), who observed rectangular acervuli. The size of the acervuli reported in present studies is not in agreement with the work did by earlier scientists.

The setae were ashy - brown to dark brown, 4-5 septate, stiff, straight or bending and wider at base, while tapering towards tip. These findings are in consonance with the reports of Hande (2001) and Gaikwad (2002) who also reported similar observations of *C. gloeosporioides* from curry leaf and custard apple, respectively. The measurements of setae in the present studies (88.64 µm x 3.0 µm) are not in agreement with any worker. But average number of setae per acervulus (7) and width of seatae (3.0 µm) are nearly matching with record of Gaikwad (2002).

The conidiophores were hyaline, thickly arranged broader at base and quite narrow at tips. These findings are in consonance with the results of Hande (2001) and Gaikwad (2002). Whereas, studied of Ghosh

and Ikram (1980) revealed that the conidiophores of *C. gloeosporioides* were cylindrical and measured 10-20 µm in length, which is not in agreement with present findings, wherein the length of conidiophores was 54.78 µm (33.4 to 76.15 µm).

The conidia were hyaline, single celled, oblong to cylindrical with rounded end and measured 11.57 x 5.33 µm. The results in respect of conidial shape and other general characters are conformity with the findings of (Singh, 1978; Ghosh and Ikram, 1980 and Hande, 2001). The conidial measurement in respect of length nearly match with that recorded by Gaikwad (2002), but not tallying with that observed by Patil (1968), Bose *et al.* (1973), Hasabnis (1994) and Singh (1978) in respect of both length and breadth.

5.4 Growth and cultural characters of *C. gloeosporioides*

5.4.1 Cultural characters of *C. gloeosporioides* on synthetic media

The cultural characters of fungus on different synthetic media under study revealed that the media *viz.,* peptone glucose agar, nutrient agar and Coon's agar were the best for growth and sporulation of fungus. The results in respect of peptone glucose agar are in conformity with the findings of Bose *et al.* (1973) and Gaikwad (2002) who noticed the similar effect of this medium on *C. gloeosporioides* infecting mango and custard apple, respectively. Similarly, Hegde *et al. (*1989) and Rathod (1994) observed Coon's agar as a best medium for *C. gloeosporioides* causing anthracnose of arecanut and mango, respectively which is in agreement with present studies. While, the effectiveness of nutrient agar as good medium for *C. gloeosporioides* is not observed previously by any worker.

In addition to peptone glucose, Coon's and nutrient agars, the next media in order of superiority were Asthana Hawker's, Richard's and

Sobourand's agars. The findings in respect of Richard's agar are in conformity with the investigations of Mishra and Mahmood (1960), Rathod (1994), Ekbote *et al.* (1997), and Shinde (1998), who also noticed this medium as good for *C. gloeosporioides* from different crops. However, the present findings differed from the report of Singh *et al.* (1966) who observed Richard's and Asthana Hawker's agars as fair to poor media for *C. gloeosporioides* causing anthracnose of *Dioscorea* plants. Further, the investigations as regards to Sarbourand's agar are tallying with the studies carried out by Shinde (1988), Hegde *et al.* (1989), Rathod (1994) and Gaikwad (2002) wherein they have also observed this medium as good for *C. gloeosporioides*. Similarly, Gaikwad (2002) noticed Asthana Hawker's agar as good medium for *C. gloeosporioides* which is in conformity with present findings.

5.4.2 Cultural characters of *C. gloeosporioides* on non-synthetic media

The cultural characters of the pathogen on different non-synthetic media under study indicated that oat meal and potato dextrose agars were the excellent media for growth and sporulation of *C. gloeosporioides* from guava and the next in order was corn meal agar. These results are exactly matching with Singh *et al.* (1960) who also reported excellent growth and sporulation of *C. gloeosporioides* from *Dioscorea* on these media.

In addition, Hegde *et al.* (1989) also observed the goodness of PDA and oat meal agar for *C. gloeosporioides,* which is tallying with present studies. Further, oat meal agar was also noticed to be best medium for *C. gloeosporioides* from different hosts by Marathe *et al.* (1973), Rathod (1994) and Gaikwad (2002), which is in consonance with present investigations.

The potato dextrose agar was found to be best medium for growth and sporulation of *C. gloeosporioides* infecting mango by Mishra (1960), Ekbote *et al.* (1997) and Gaikwad (2002), which is in agreement with present findings.

Rathod (1994) and Gaikwad (2002) reported the effectiveness of host decoction agar for growth and sporulation of *C. gloeosporioides* that is in agreement with present findings in respect of growth only, but not with sporulation, wherein the sporulation on host decoctions was very trace. On the contrary, Shinde (1988) observed poor growth of *C. gloeosporioides* on host decoction agar that is not in consoncnce with present investigations.

Therefore, the synthetic media *viz.,* peptone glucose, Coon's and nutrient agars, while non-synthetic media like oat meal, potato dextrose and corn meal agars were excellent for growth as well as sporulation of *C. gloeosporioides*, infecting guava.

5.5 Effect of temperatures on growth and sporulation of *C. gloeosporioides*

The fungus *C. gloeosporioides* could thrive well between a wide range of temperature (10-35^{0}C), while optimum temperature for growth was observed to be between 25-30^{0}C. The results in respect of optimum temperature are exactly matching with the work of Patil (1968), Dawkhar (1970), Kim *et al.* (1986) and Rathod (1994) who also noticed the similar range of optimum temperature for growth and sporulation of *C. gloeosporioides* from mango, papaya, chilli and mango, respectively. Similarly, the wide range of temperature (i.e.10-35^{0}C) is also in consonance with the findings of Patil (1968) who also recorded the same wide range of temperature for *C. gloeosporioides.* However, the results are not in agreement with the work of Dawkhar (1970) and Doornik (1982) who did not observe any growth of *C. gloeosporioides* from

papaya and anemone below 20^{0}C temperature. Similarly, a wide range of temperature for the pathogen under study is not tallying at all with the record of Abdel (2000) who noticed a very wide range of 5 - 49^{0}C for growth and sporulation of *C. gloeosporioides* from guava. The present findings are different from those of Doornik (1982) who observed that the pathogen failed to grow below 20^{0}C. However, Gunasekaran (1979) observed no growth of *C. gloeosporioides* at 34-35^{0}C.

Further, results about ceased the growth of pathogen above 40^{0}C are in agreement with the finding of Rathod (1994).

5.6 Varietal resistance

Use of resistant varieties is one of the cheapest, long lasting and economical methods of controlling plant diseases, particularly in fungus having a wide host range. In the present study, eleven varieties of guava were screened for their reaction against the fungus *C. gloeosporioides* under natural field and controlled conditions. It was revealed that, none of varieties were found to be completely free from disease infection.

The anthracnose disease reaction on guava fruits under natural field condition indicated that all the eleven varieties were infected by disease, however, the disease intensity varied considerably. The variety Behat seedling was resistant to the disease. On the contrary, remaining varieties showed moderately susaceptible to highly susceptible reaction to the anthracnose. The varieties *viz.,* Allahabad safeda, Sardar and Makhmalabad safeda were highly susceptible. Behat coconut, Apple colour and Chettedar had susceptible reaction, while moderately susceptible varieties were Seedless. Basti, Nagpur seedless and Pear shaped.

As like field studies, in *In vitro* results by detached fruit technique also revealed that out of eleven varieties only Behat seedling was

resistant to *C. gloeosporioides* causing anthracnose disease, while remaining all varieties were highly susceptible. Similar findings were also reported by Anon (1974) who found that the varieties *viz.,* Allahabad safeda, Apple guava, Seedling, Behat coconut and Sardar (L-49) were susceptible, which is in confimity with present work. The variety Allahabad Safeda was highly susceptible and showed maximum (90.0%) disease intensity that is in agreement with Anon (1974) who also observed that Allahabad safeda variety of guava was highly susceptible to *C. gloeosporioides.* Further, Rahman *et al.* (2003) found that the pear shaped fruits had less susceptibility than elliptical round fruits. Similar findings were also recorded in present investigation, wherein the variety Pear shaped showed less disease as compared to other varieties.

Hence, out of eleven guava varieties only Behat seedling was resistant to *C. gloeosporioides* causing anthracnose disease.

5.7 Bioefficacy of fungicides against *C. gloeosporioides* under *in vitro*

The *in vitro* studies by poisoned food technique indicated that the fungicides *viz.,* Bordeaux mixture, tricyclazole, dipenoconazole, propiconazole and hexaconazole completely inhibited the growth of *C. gloeosporioides* and thus showed their best effectiveness against the pathogen. Similarly, carbendazim and captan were moderately effective and ready Bordeaux mixture, copper oxychloride, propineb and mancozeb were less effective.

The results about highest effectiveness of Bordeaux mixture obtained in present work coincide with the findings of Tandon and Singh (1969), Padalkar *et al. (1996)* and Gaikwad (2002) who also noticed best efficacy of Bordeaux mixture against *C. gloeosporioides* from different crops. The results are also in consonance with the work of Kader and Rahman (2001) who reported that difenoconazole was highly effective

against *C. gloeosporioides* from guava but do not agree regarding highly effectiveness of carbendazim, wherein this fungicide was moderately effective in present studies.

Further, Ali *et al.* (1993), Smith and Black (1993), Das *et al.* (1998) and Gaikwad (2002) noticed the high effectiveness of propiconazole against *C. gloeosporioides* from different hosts, which is in conformity with the present results.

In addition, mancozeb was found to be most effective against *C. gloeosporioides* by Lai *et al.* (1995), Padalkar *et al.* (1996) and Akhtar *et al.* (1998) but this finding is not in agreement with the present achievements in which mancozeb was less effective. Gaikwad (2002) observed the moderate effectiveness of difenoconazole and hexaconazole, but in present findings compelete inhibition of growth of pathogen was noticed, thus differs from the work of Gaikwad.

Then copper oxychloride and ready Bordeaux mixture were recorded to be least effective in present studies. Similar findings were also reported by Gaikwad (2002), which is tallying with present findings. The results vary from the findings reported by Thakare and patil (1995) wherein copper oxychloride was most effective, while it was least effective in present work. Similarly, Ready Bordeaux mixture was also least effective that is in conformity with finding of Ali *et al.* (1993).

Thus, *in vitro* studies revealed the most effectiveness of fungicides *viz.,* Bordeaux mixture, tricyclazole, propiconazole, dipenconazole and hexaconazole and followed by moderate effectiveness of carbendazim and captan.

5.8 Efficacy of bioagents against *C. gloeosporioides* under *in vitro.*

In vitro studies regarding biological control of *C. gloeosporioides* causing anthracnose of guava by four *Trichoderma* spp. indicated that *T. hamatum* was the most effective, which recorded maximum inhibition (76.20%) of pathogen. This was followed by *T. harzianum* (45%), *T. viride* (42.50%) and *T. koningii* (40%). Jeyalakshmi *et al.* (1998) noticed that *T. viride* was more effective followed by *T. hamatum, T. harzianum* and *T. koningii* against *C. capsici,* which is more or less nearer to the present findings. Jeyalakshmi *et al.*(1998) reported more effectiveness of *T. viride,* while in present studies *T. hamatum* was most effective. The present results are also in agreement with Michereft *et al.* (1993) who observed the significant reduction in the growth of *C. graminicola,* a causal agent of sorghum anthracnose, with *T. viride, T. koningii* and *T. harzianum* under *in vitro.* Singh (1992) reported *T. harzianum* as strong inhibitor of *Colletotrichum falcatum* that is not in total conformity with present studeies, where *T. hamatum* was a strong inhibitor followed by *T. harzianum.* The earlier results in respect of good control of pathogen, *C. dematium* and *C. capsici* with *T. harzianum* and *T. viride* by seed treatment method in chilli by Banu (1990); *C. capsici* with *T. viride* by Rajathilagam and Kannabiran (2001) *and* C. capsici *from betelvine* with *T. harzianum* by D'souza *et al* (2001) are also in consonance with the present investigations.

Thus, *T. hamatum* was most effective bioagent against *C. gloeosporioides* causing anthracnose of guava, followed by *T. harzianum, T. viride* and *T. koningii.*

SUMMARY AND CONCLUSIONS

6. SUMMARY AND CONCLUSIONS

6.1 Summary

During last few years the guava crop was found to be badly infected by anthracnose disease in rainy and post rainy seasons. The disease was invariably noticed in *Kharif* seasons of 2004 and 2005 in the guava orchards of Horticulture Section at MPKV., Rahuri. Previously, it was a sporadic disease, hence not sufficient and detailed work has been carried out on this disease.

Therefore, the present studies were under taken with the objectives to study symptomatology, morphological, cultural and physiological characters; interaction of different guava varieties with the pathogen and bioefficacy of fungicides and bioagent against pathogen.

The causal organism was isolated from fruits of guava showing typical symptoms of disease and identified as *Colletotrichum gloeosporioides* Penz. on the basis of morphological characters. The Koch's postulate of the isolated pathogen was proved on guava fruits under laboratory conditions by detached fruit technique (DFT) and mycelial bit inoculation method (MBIM), from this, it was concluded that the fungus, *C. gloeosporioides* is a potential pathogen of anthracnose.

The pathogen affected mostly the fruits and caused symptoms like grey to brown, circular spots of 3-5 mm in diameter in initial stage and later on irregular, sunken spots, were observed only on the surface of fully matured unriped fruits during rainy to post rainy season. The diseased portion on the fruits was comparatively harder than the healthy. The infection spread rapidly on fully grown mature fruits.

The fungus produced mycelium with average width of 3.4 to 4.84 µm. Orange to brown-black acervuli measured 206.33 x 116.17 µm. Setae were ashy-brown to dark, septate, wider at base and measured 88.64 x 3.0 µm. The conidiophores were short, simple, thickly arranged, hyaline with average height of 53.32 µm. Conidia were oblong to cylindrical, hyaline, which measured 11.57 x 5.33 µm with length breadth ratio of 2.17.

The synthetic media *viz.,* peptone glucose agar, conn's agar and nutrient agar while, non-synthetic media, oat meal agar and PDA were the excellent for growth and sporulation of *C. gloeosporioides.*

Minimum, optimum and maximum temperatures for fungal growth were 10^0C, 25-30^0C, and 35^0C, respectively. The maximum growth and sporulation were between 25 to 30^0C. There was no growth of pathogen at 0^0C, 5^0C, and 40^0C.

Among 11 varieties, Behat seedling was resistant to fruit anthracnose while remaining all were highly susceptible.

The growth of the fungus was completely inhibited under *in vitro* by the fingicides *viz.,* Bordeaux mixture, tricyclazole, difenoconazole, propiconazole and hexaconazole. Among biological agants tested under *in vitro, T. hamatum* was most effective against *C. gloeosporioides* followed by *T. harzianum, T. viride* and *T. koningii.*

6.2 Conclusions

- The pathogen, *C. gloeosporioides* mostly infected fruits causing grey to brown, circular to irregular, sunken spots on fruit.
- The measurements of morphological structures of the pathogen slightly differ from the earlier measurements of *C. gloeosporioides.*
- The disease mostly occurred in rainy to post rainy season.
- The synthetic media *viz.,* peptone glucose agar, Conn's agar and nutrient agar, while non-synthetic media *viz.,* oat meal agar and potato dextrose agar were the excellent media for growth and sporulation of pathogen.
- The cardinal temperature range for growth of pathogen was 10-35^{0}C while optimum for growth and abundant sporulation was in between 25-30^{0}C.
- Behat seedling was resistant variety to *C. gloeosporioides.*
- The fungicides *viz.,* Bordeaux mixture, tricyclazole, difenoconazole, propiconazole and hexaconazole completely inhibited the pathogen growth under laboratory conditions and
- *T. hamatum* was the effective biological agent against *C. gloeosporioides* under *in vitro*, followed by *T. harzianum, T. viride* and *T. koningii.*

LITERATURE CITED

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Abdel Gawad. T. I. (2000). Anthracnose fruit rot disease of guava in El-minia, Egypt. Assiut-Jour. Agril. Sci. 31(4):89-107.

Agostini, J.P., Timmer, L.W. and Mitchell, D.J. (1992). Morphological and pathological characters of strains *C. gloesporioides* from citrus. Phytopathology 82(11):1377-1382.

Ahmed, K.M. (1985). Effect of temperature and light on the growth and sporulation of *C. gloesporioides* Penz. Bangladesh J. Botany 14(2):155-159.

Akhtar, K. P. ; Khan, A. I., Khan I. A. and Khan S. M. (1998). Studies on incidence and pathogenicity of *C. gloeosporioides* Penz. causing anthracnose of mango and its chemical control. Pakistan J. Phytopathology 10 (1) : 42-44.

Ali, M.A., Ali, M., Huq, M. and Ahmed, M. (1993). *In vitro* studies on fungicides against *Colletotrichum gloeosporioides* (Penz.) Sacc. The die-back of tea. Shri Lanka J. Tea Sci. 62 (1) : 25-31.

Anonymous (1974). Six monthly Report of the Indian Inst. of Hort. Res., Hessaragatta for the period of Jan. to June, 1974.

Anonymous (2004). Area and production of major horticultural crops. Economic survey, 2003-2004. Govt. of India pp.157.

Anonymous (2005). Districtwise production of major horticultural crops in Maharashtra State. Dept. of Agril., Govt. of Maharashtra.

Banu I.S., Shivanna, M.B. and Shetty, H.S. (1990). Efficacy of physical, chemical and Biological methods in reducing the seed borne fungal incidence in Chilli. Int. J. Tropical Plant Diseases 18:119-127.

Bose, T.K. and S.K. Mitra. (1990). Fruits: Tropical and subtropical 6:280-303.

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. Prog. Hort. 5(2) : 41-53.

Broadbent. P., Baker, K.F. and Waterworth, Y. (1971). Bacteria and actinomycetes antagonistic to fungi root pathogen in Australian Soil. Australian J. Boil. Sci. 24:925-944.

Butt, A.A., Nasir, M.A. and Bajwa, M.N. (1995). *In vitro* evaluation of different chemicals against *Gloeosporium psidii* the cause of anthracnose of guava. Pakistan J. Phytopathology 7(1) : 92-93.

Carranza, M., Larran, S., and Ronco, B. (2002). First report of *Glomerella cingulata* on common guava in Argentina. Plant Disease 86(4):440.

Chand, J.N., Gupta, P.C.and Madan, R.L. (1985-86). Diseases of guava, ber and Phalsa in India. Rev. Trop. Pl.Path. 2 : 235-261.

Chowdhry, P. N. and Varshney, A. (2000). Identification of different *Colletotrichum gloeosporioides* species. In manual on identification of plant pathogenic and biocontrol fungi of Agricultural importance. Edt. P.N. Chowdhry, Center of Advance studies in plant pathology, Division of Plant Pathology, IARI, New Delhi pp. : 73-78.

Clausen, R.E. (1912). A new fungus concerned in wither tip varieties of citrus media. Phytopathology 2 (6) : 217-235.

*Corda, A.C.I. (1837). Icons Fungorum cognitorum, 3 prague. (From Thesis "Studies on leaf spots of curry leaf [*Murrayya koenigii*]" by Hande J. M., 2001).

Das, M. and Bora, K.N. (1993). *Colletotrichum acutatum* a new fruit rotting pathogen of guava (*Psidium guajava* L.) in storage. Indian J. Mycol. Pl.Pathol. 23:331.

Das, S. K., Panda, S.N. and Pani, B.K. (1998). Evaluation of fungicides against *C. gloeosporioides* Penz. inciting blossom blight of mango. Environ. and Ecol. 16 (3) : 623-624.

Dawkhar, S.S. (1970). Studies on fruit rot disease of papaya (*Carica papaya* L.) incited by *C. gloeosporioides* Penz. in Maharashtra. M.Sc. (Agri.) Thesis, MPKV, Rahuri, Maharashtra, India.

D'Souza, Alvira; Jayanta K. Roy, Bibekananda Mahanty and Dasgupta, B. (2001). Screening of *Trichoderma harzianum* against major fungal pathogens of betelvine. Indian Phytopath. 54(3):340-345.

Doornik, A.W. (1982). Studies on anemone leaf curl disease caused by *C gloesporioides.* Annual Report. Laboratory for flower bud Resarch, Lsse Netherlands1983. pp.132.

Ekbote, S. D., Padaganur, G. M., Patil, M.S. and Chattanavar, S.N. (1997). Studies on the cultural and nutritional aspects of *Colletotrichum gloeosporioides,* the causal organism of mango anthracnose. Indian J. Mycol. Pl. Pathol. 27 (2) : 229-230.

Gaikwad, A. P. (2002). Studies on fruit rot of custard apple (*Annona squamosa* L.) caused by *Colletotrichum gloeosporioides* Penz. Ph.D. thesis submitted to M.P.K.V., Rahuri.

Ghosh, R. N. and Ikram, S. (1980). Leaf blight disease of *Araucana bidwilli.* Indian Phytopath. 33 (4) : 629-630.

Grove, W. B. (1937). British stem and leaf fungi. Vol. II. Cambridge Univ. Press, London and New York. (Coelomycetes, B.C. Sulton, 1973, pp : 521).

Gunasekaran, M. (1979). Physiological studies on pathogenically different isolates of *Colletotrichum gloeosporioides* Penz. the causal organism of citrus die back. M.Sc. (Agri.) Thesis, I.A.R.I. New Delhi.

*Gutierrez-Alonso, O., Nieto-Angel, D., Gultierrez-Alonso, J.G., Delgdillo-Sanchez, F., Dominguez-Alvarez, J.L. (2002). Morphological, cultural characteristics and pathogenicity of *Colletotrichum* spp. isolates obtained from guava fruits (*Psidium guajava* L.) Revista-Mexicana-de- Filopathologia 20(1):24-30.

Hande, J.M. (2001). Studies on leaf spots of curry leaf (*Murraya koenigii*). A M.Sc. (Agri.) Thesis, MPKV, Rahuri, Maharashtra, India.

Hasabnis, S.N. (1984). Studies on storage rot of mango (*Mangifera indica* L.) fruits caused by *C. gloeosporioides* Penz. and *Botrydipiodia theobromae* . A M.Sc. (Agri.) Thesis, KKV., Dapoli, Maharashtra, India.

Hegde, Y.R., Hegde, R.K. and Kulkarni, S. (1989). Perenation of *Colletotrichum gloeosporioides* (Penz.) and Sacc. a causal agent of anthracnose of arecanut. Current Res. 18 (7) : 98-100.

Horsefall, J.G. (1957). Principles of fungicidal action. Chronica Botanica Co, U.S.A.

Jeyalakshmi, C., Durairaj, P., Seetharaman, K. and Sivaprakasam, K. (1998). Biocontrol of fruit rot and die-back of chilli using antagonistic microorganism. Indian Phytopath. 51(2):180-183.

Jitendra Singh (2002). Basic Horticulture, Plant Growth Regulators pp. 120-131.

Kader, K.A. and Rahman, M.N. (2001). Pathogenicity of *Colletotrichum gloeosporioides* and *Pestalotia psidii* on guava friut and *in vitro* screening of some fungicides against them. Bangladesh J. Pl. Pathology 17 (1/2): 55-58.

Kim, W.G., Cho, E.K. and Lee. E.J. (1986). Two strains of *Colletotrichum gloeosporioides* causing anthracnose on pepper fruits. Korean J. Pl. Path. 2(2):107-113.

Kornerup, A. and Wanscher, J.H. (1967). Methuer Handbook of colour. Publ. By Methuen and Co. Ltd., 11 Newsletter lane, Londan, E.C.4.

Korsten, L., Sanders, G.M. and Grosse, W.E. (1994). Isolation and pathogenicity of avocado post harvest pathogens from Westalia and other avocado producing area. Yearbook, South Africa Avocado Growers association 17:46-48.

Lai, C.Y., Li, Z.K. and Lu, K.M. (1995). Trial on chemical control of leaf blight of *Amomum villosum*. Plant Protection 21 (4) : 16-18.

Manandhar, J.B., Hartman, G.L. and Wang, T.C. (1995). Anthracnose development of pepper fruits inoculated with *Colletotrichum gloeosporioides.* Plant Disease 79(4):380-383.

Marathe, C. V., Anahosur, K. H. and Narawade, U. G. (1973). A new twig blight disease of castor. Indian Phytopath. 26(3): 502-504.

Mehata, P.R. (1951). Observations on new and known crop diseases of plants in Uttar Pradesh. Plant. Pro. Bull., New Delhi 3:7-12.

Michereff, S.J., Menezer, M. and Mariane, R.L.R. (1993). Antagonism of *Trichoderma* sp. against *Colletotrichum graminicola,* an agent of sorghum anthracnose under laboratory conditions. Summa phytopathologica 19:14-17.

Midha, S.K. and Chohan, J.S. (1968). Factors affecting the production of pectinolytic enzyme by *Gloeosporium psidii,* the causal agent of fruit rot of guava (*Psidium guajava* L.) J. Res. Ludhiana. 5:395-40.

Mishra, A. P. and Mahmood, M. (1960). Effect of carbon and nitrogen nutrition on growth and sporulation of *Colletotrichum capsici.* J. Indian Bot. Soc. 39(2) : 314-321.

Morton, J. (1987). Guava. In : "Fruits of warm climates". Julia F. Mortin, Miomi, F.L. pp. 356-363.

Mujumdar, V. L. and Pathak, V.N. (1989). Incidence of major post-harvest diseases of guava fruits in Jaipur Markets. Indian Phytopath. 42:469.

Mujumdar, V. L. and Pathak, V.N. (1997) Control of fruit rot of guava by chemical fungicides. J. Mycol. Pl. Pathol., 27 (1) : 17-20.

Naik, M.K., Hiremath, P. G., Hegde, R. K. and Hiremath, S. V. (1984). Anthracnose of betelvine caused by *C gloesporioides* (Penz.) Sacc. in Karnataka, India. Pl. Path. News letter 2(2):13-14.

Narendra Singh (1992). Biological control of red rot disease of sugarcane. Indian Phytopath. 43 – 44 : Abst. 64

Padalkar, N.R., Mandokhot, A.M. and Fugro, P.A. (1996). Leaf spot disease of arecanut in Konkan region of Maharashtra state Indian Cocoa, Arecanut Spices J. 20 (4) : 111-112.

Pathak, V.N. (1980). Guava. In : "Diseases of fruit crops". Oxford and IBH Publishing Company. New Delhi pp : 151-164.

Patil, B.K. (1968). Anthracnose of mango (*Mangifera indica* L.) caused by *C. gloeosporioides* in M.S. M.Sc. (Agri.) Thesis, Univ. Poona, M.S., India.

Penzing, O. (1882). Fungi *Agrumicoli michelia*, vol. 2, December, 1882., pp : 385-498.

Rahman, M.A., Ansari, T.H., Meach, M. B. and Tetsashi Yoshhida. (2003). Prevalence and pathogenicity of guava anthracnose with special Emphasis on varietal reaction. Pakistan J. Bio. Sci. 6(3) :234-241.

Rajathilagam, R. and Kannabiran, B. (2001). Antagonistic effects of *Trichoderma viride* against anthracnose fungus *Colletotrichum capsici.* Indian Phytopath. 54(1):135-136.

Ramkrishnan, T.S. (1967). Diseases and Pests. The Mango: A Handbook I.C.A.R. New Delhi pp-150-168.

Rathod, H.L. (1994). Studies on fruit rot of mango (*Mangifera indica* L.) caused by *C. gloeosporioides* Penz. M. Sc.(Agri.) Thesis, M.P.K.V., Rahuri, Maharashtra, India.

Ravi, S., Dovajswamy, S., Valluvaparidasan, V.and Jeyalakshmi, C. (1999). Effect of biocontrol agents on seed borne *Colletotrichum* in french bean. Plant Dis.Res. 14(2):146-151.

*Rocha, J. de-R-de.s., Oliveira, N.T. de and Menezes, M. de. (1998). Comparision of inoculation methods efficiency for evaluation of *Colletotrichum gloeosporioides* isolates pathogenicity on passion fruits (*Passiflora edulis*). Brazilian – Archives of Biol. And Technol. 41(1) :145-153. (CAB Abst. : 981009638, 1989-1999/09).

Saccardo, P.A. (1884). Sylloge Fungorum. Vol. 3, pp. : 735.

Sadhu, M.K. and Chattopadhayay, T.K. (2001). Introductory Fruit Crops pp.282-289.

Sharma, S.K., Singh, J.P. and Daulta, B.S. (1981). Controlling anthracnose of guava caused by *Glomerella cingulata* by wax emulsion and oils. Haryana J. Hort. Sci. 10:186-189.

Shinde, S. D. (1988). Studies on die-back of Hybrid Tea roses. M.Sc. (Agri.) Thesis, MPKV, Rahuri, Maharashtra, India.

Singh, J.P. and Sharma S.K. (1981). Screening and chemical basis of resistance in guava varieties to anthracnose. (*Glomerella cingulata*). Haryana J. Hortic. Sci. 10:155-157.

Singh, J.P. and Sharma S.K. (1982). Controlling anthracnose of guava caused by *Glomerella cingulata* by fumigation. Indian Phytopathol. 35:273-276.

Singh, R. S. (1978). Plant Disease, IV. Ed. Oxford and IBH. Publishing Co., New Delhi pp : 365 and 386-392.

Singh, R. D., Prasad, N. and Mathur, R. L. (1966). Anthracnose of *Dioscorea alata* L. Indian Phytopath. 19 : 65 - 71.

*Smith, B.J. and Black, L. L. (1993). *In vitro* activity of fungicides against *Colletotrichum fragariae*. Acta Horticulture No. 348: 509-512.

Sutton, B.C. (1980). The coleomycetes. Common Wealth Mycological Institute, Kew, Surrey, England pp:696.

*Suzzi, G., Ramano, P., Ponti, I. and Montuschi, C. (1995). Natural Wine yeast as biocontrol agents. J. Appl. Bacteriol. 78:304-308.

Thakare, C.S. (1991). Studies on leaf blight of chrysanthemum caused by *C. gloeosporioides* (Penz.) and Sacc. M.Sc. (Agri.) Thesis, MPKV., Rahuri, Maharashtra, India.

Thakare, C.S. and Patil, P.Y. (1995). Studies on leaf blight of chrysanthemum caused by *C. gloeosporioides*. J. Maharashtra agric. Univ. 20 (1) : 49-52.

Tondon, I.N. and Singh, B. B. (1969). Studies on anthracnose of guava and its control. Indian Phytopath. 22:322-326.

Tondon, R.N. and R.K. Agarawal. (1954). Studies on die back of guava. Proc. Indian Acad Sci. 40B:102-109.

Tricita, H., Quimio, T.N. and Quimio, A.J. (1975). Notes on Philippine Grape and Guava Anthracnse. Plant Dis. Reptr. 59:221-224.

Venkatakrishniah, N.S. (1952). Proc. Indian Acad. Sci. Sect. B-36: 129-134.

Venkatakrishniah, N.S. (1954). *Pestalotiopsis psidii* on *Psidium guajava.* Curr. Sci. 23(1):164-165.

Vincent, J.M. (1947). Distortion of fungal hypae in the presence of certain inhibitors. Nature 159:350.

*Yang, H.R. and Chuang, J.U. (1994). Pathogenicity and zymogram of anthracnose fungi isolated from some fruits. Memorie of the College of Agriculture, National Taiwan University 34 (1) : 1- 8.

Zulfiqar, M., Brlansky, R.H. and Timmer, L.W. (1996). Infection of flower and vegetative tissues of citrus by *C. gloeosporioides* and *C. acutatum.* Mycologia 88(1):121-128.

* Originals not seen.

VITA

8. VITA

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of

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

Title of the thesis	:	Studies on anthracnose of guava (*Psidium guajava* L.) caused by *Colletotrichum gloeosporioides* Penz.
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