अज्ञानरुपी अंधारात अडखळणा-या...

माझ्या चिमुकल्या पावलांना,

ज्ञानरुपी प्रकाशाची वाट दाखविणारे ...

महान दिपस्तंभ माझे परम श्रध्दास्थान

माझे आदर्श माझ्या जडणघडणीतील ...

सर्वश्रेष्ठ मुर्तीकार आणि ज्यांच्यासाठी माझे जीवन, बस्स !

ज्यांच्यासाठीच माझा प्रत्येक श्वास अशा...

ति. श्री. दादा व सौ. आई

यांच्या चरणी...

ही विनम्र शोध शब्दांजली

.... अमोल

MANAGEMENT OF POST HARVEST DISEASES OF SWEET ORANGE (*Citrus sinensis* L. Osbeck)

by

Kale Amol Dnyanoba
(Reg.No. 05/104)

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
POST GRADUATE INSTITUTE
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2007

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part there of has not been submitted by me or other person to any other University or Institute for a Degree or Diploma

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

(A.D. Kale)

Dated : 15/06/2007

Dr. S.V. Bulbule
Jr. Plant Pathologist,
All India Coordinated Research Project on Citrus,
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Rahuri - 413 722, Dist. Ahmednagar,
Maharashtra State, INDIA

CERTIFICATE

This is to certify that the thesis entitled, **"MANAGEMENT OF POST HARVEST DISEASES OF SWEET ORANGE *(Citrus sinensis* L. Osbeck)"** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (Maharashtra State) in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE** (AGRICULTURE) in **PLANT PATHOLOGY**, embodies the results of a piece of *bona fide* research work carried out by **MR. AMOL DNYANOBA KALE,** under my guidance and supervision and that no part of this thesis has been submitted for any other degree, diploma or publication in other form.

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

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

Dated : 15/06/2007

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Research Guide

Dr. A.S. Jadhav
Associate Dean,
Post Graduate Institute,
Mahatma Phule Krishi Vidyapeeth,
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Maharashtra State, INDIA

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Place : MPKV, Rahuri
Dated : / /2007

(A.S. Jadhav)

ACKNOWLEDGEMENTS

"Coming together is beginning, carrying together is progress and keeping together is success". In everyone's life, the day arise when he has to shape his feelings in words. Even though carrying the feelings is very difficult, their expression becomes necessary at every time. Each and every sentiments can be captured in words but a little of them sets the one at ease for me. The spell has come to gather the words for expressing my gratitude towards all the people who shaped my career.

It is unforgettable experience in my life to fortunate enough to work under the inspiring guidance of Dr. S.V. Bulbule, Junior Plant Pathologist, All India Coordinated Research Project on Citrus, Department of Horticulture, MPKV, 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, painstaking efforts taken for scrutinizing the manuscript and unfailing enthusiasm throughout the study since its conception to completion.

I am immensely grateful to the members of my Advisory Committee, Dr. S.G. Borkar, Head, Department of Plant Pathology and Agricultural Microbiology, Dr. S.N. Gohil, Horticulturist, All India Coordinated Research Project on Citrus, MPKV, Rahuri and Dr. A.P. Gaikwad, Associate Professor of Plant Pathology and Agricultural Microbiology for their valuable guidance, keen interest for carrying out the present research work, which is something that I shall never forget.

I am thankful to Dr. A.S. Jadhav, Associate Dean, Post Graduate Institute, M.P.K.V., Rahuri and all the academic staff of PGI for their continuous encouragement to students, valuable guidance and whole hearted cooperation.

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

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

I am also thankful to Dr. V.K. Mandhare, Senior Scientist, Seed Pathology and Shri. B.C. Game, Senior Research Assistant, Seed Pathology for taking microphotographs and their help during the course of these investigations.

I offer my thanks to my collegues Atul, Baba, Dipak, Kashinath, Nandkumar, Pramod, Shivaji, Rajendra, Rahul and Pallavi for their co-operation during my post graduation study and research work.

I offer my special thanks to my seniors Shri. Kiran Sawant-Patil and Shri. Sharif Shaikh for their cooperation during my research work.

My heart is filled with sweet memories of my Jolly friends Yogesh, Raju, Mahesh, Pradeep, Suraj, Datta, Pravin, Yuvraj, Rahul, Mangesh, Sunil, Dhanaji, Santosh, Ravidada for their excellent company, warmer affections and co-operations during my study. Generally words give wings to thoughts and feelings. But sometimes words fail inadequately to express the depth of feelings. Mere words can't express my whole hearted sense of gratitude to my friends Ravi, Tanaji, Ajit, Pavan, Mahesh[2], Sunil[2], Sanjay, Abhiman, Vinayak, Anil, Sidheshwar, Samadhan, Rahul, Somanath and Vishwavijay who have been a source of inspiration and encouragement throughout my study period. I must express my indebtedness, appreciation and thanks to Manisha[2], Dhanashri, Kalawati, Shubhangi, Sarswati, Amaraja, Madhuri, Dipali, Rupali[2], Rani and Aruna for their love, moral support and encouragement.

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LIST OF ABBREVIATIONS

%	:	Per cent
/	:	Per
^{0}C	:	Degree Celsius
et al.	:	Et alli (and others)
Fig.	:	figure
kg	:	Kilogram(s)
ha	:	Hectare(s)
viz.	:	Vide licet (namely)
@	:	At the rate of
e.g.	:	Exampli Gratia (for example)
etc.	:	Et cetra
h, hrs.	:	Hours
mm	:	Millimeter
µ	:	Microns
No.	:	Number
i.e.	:	Idest (that is)
ppm	:	Parts per million
RH	:	Relative humidity
min.	:	Minutes
wt.	:	Weight
NA	:	Nutrient agar
g	:	Gram
lit.	:	Litre
m	:	Metre
ml	:	Millilitre
cm	:	Centimetre
s, sec.	:	Seconds
a.i.	:	Active ingredient
cv.	:	Cultivar
Sr.	:	Serial
mg	:	Milligram
m/s.	:	Messers
var.	:	Variety
Ltd.	:	Limited
No.	:	Number
dia.	:	Diameter

ABSTRACT

MANAGEMENT OF POST HARVEST DISEASES OF SWEET ORANGE (*Citrus sinensis* L. Osbeck)

BY

Amol Dnyanoba Kale

A candidate for the degree of

MASTER OF SCIENCE (AGRICULTURE)

in

Plant Pathology

Mahatma Phule Krishi Vidyapeeth,

Rahuri - 413 722

2007

Research Guide	:	Dr. S.V. Bulbule
Department	:	Plant Pathology and Agril. Microbiology

Sweet orange (*Citrus sinensis* L. Osbeck) is one the most important commercial citrus cultivars in India. It occupies as the third largest fruits industry after mango and banana. The crop is found to be severely infected by several diseases. However, post harvest diseases caused by *Colletotrichum gloeosporioides* and *Penicillium digitatum* are a severe threat to the sweet orange crop causing severe losses in storage and transit. Therefore, attempts were made to study the symptomatology, morphological, cultural and physiological characters of the pathogens, *in vitro* efficacy of different fungicides, botanicals and effect of different physical and chemical post harvest treatments.

The causal organisms were isolated from affected fruits of sweet orange cv. Mosambi showing symptoms of post harvest diseases and were identified to be *Colletotrichum gloeosporioides* Penz. and *Penicillium digitatum* causing anthracnose and green mould, respectively. The pathogenicity of both the pathogens was confirmed on the healthy fruits using detached fruit technique.

The pathogen *Colletotrichum gloeosporioides* showed brown discolouration and sunken lesion on the fruit surface and involving the albedo portion. In advanced stages, the brown discolouration covered the entire fruit to cause rotting. In case of green mould disease caused by *Penicillium digitatum,* symptoms appeared initially as soft water soaked area and in advance stages white mycelial growth developed and the mass of green spore covered the entire fruit surface.

In case of *Colletotrichum gloeosporioides,* the mycelium was closely septate and irregularly branched. Acervuli looked dark brown to black in colour. They were globose to saucer or irregular in shape. Setae were light 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 light brown in muscilaginous masses or in acervuli.

Penicillium digitatum showed branched, septate and hyaline mycelium. The conidiophores were very short, smooth walled, single or less often in synnemata ending in phialides which

produced conidia in chains.. Conidia were smooth walled, dull, dark green, varying in form and dimensions, but usually elliptical.

The media Czapek's dox agar, potato dextrose agar, Asthana Hawkers, nutrient agar, Richard's agar, Sabourauds agar and M_2 agar were excellent for growth and sporulation of *Colletotrichum gloeosporioides*. The fungus *Penicillium digitatum* showed excellent growth and sporulation on potato dextrose agar.

The cardinal temperature range for growth of *C. gloeosporioides* and *P. digitatum* was 10 to 30 ^{0}C and 20 to 30 ^{0}C, respectively. However, the optimum temperatures for growth and sporulation of *C. gloeosporioides* and *P. digitatum* was 25 to 30 ^{0}C and 20 to 25 ^{0}C, respectively.

The fungicides *viz.*, hexaconazole (0.1 %), propiconazole (0.1 %) and difenconazole (0.1 %) completely inhibited growth of *C. gloeosporioides*, while, the fungicides chlorothalonil (0.25 %), captan (0.25 %), mancozeb (0.25 %), carbendazim (0.1 %), hexaconazole (0.1 %), propiconazole (0.1 %), difenconazole (0.1 %) and iprodione + carbendazim (0.25 %) completely inhibited the growth of *P. digitatum*.

The *Allium sativum* (garlic) extract and *Azadirachta indica* (neem) extracts (3 %) were effective in inhibiting *C. gloeosporioides* and *P. digitatum*, respectively.

Among the various physical and chemical post harvest treatments, inoculation of fruits before heating at 46 ^{0}C for 300 min. was most effective in the control of post harvest diseases of both the pathogens.

INTRODUCTION

1. INTRODUCTION

The citrus fruits are extensively grown in the tropical and subtropical regions of the world. It occupies as the third largest fruit industry after mango and banana in India. It is grown in 0.482 million ha area producing approximately 4.258 million ton's of fruits per annum. Maharashtra accounts for a total 180.3 thousand ha area with the production of 941.9 thousand tons of the citrus fruit. India is the sixth largest citrus producer contributing 4.8 per cent of the world's total citrus production (Singh, 2001).

Citrus fruits possess greater adaptability to different climatic conditions and so are grown with equal success even in some favourable parts of temperate regions of the world. It is a crop adaptable to wide range of soils, terrain, planting and cultural arrangements and over 100 nations reported citrus production in 1980 (Reitz, 1984).

Most of the taxonomists have a general agreement that Himalayan region and South China are the places of origin for most citrus fruits (Rajput and Haribabu, 1985). The oranges are belived to have been originated in China, the lime in India, the citron in Persia and India, the lemon in Arabia and the pummelo in Malayan Archipelago (De Condolle, 1886). The most important commercial citrus cultivars in India are the mandarin (*Citrus reticulata* Blanco) followed by sweet orange (*Citrus sinensis* L. Osbeck) and acid lime (*Citrus aurantifolia* Swingle). They are grown mainly in the states of Maharashtra, Andhra

Pradesh, Punjab, Karnataka, Uttar Pradesh and Bihar. In Maharashtra the sweet oranges are grown mainly in Aurangabad, Jalna, Ahmednagar, Pune and Solapur districts. In ndia, the oranges and limes perhaps deserve to rank as the oremost among citrus fruits. Being second only to plantains, hey occupy a prominent place in the fruit production of the ountry (Singh, 2000).

The world citrus production was 93.75 million tonnes wherein the contribution of sweet orange was 71 per cent, angerine group 13 per cent, lemon and limes 10 per cent and grape fruit 6 per cent.

Citrus occupies about 9 per cent of the total area nder various fruits in country (Bose *et al.,* 2001). The nandarin, sweet orange and acid lime shares 41, 23 and 23 per ent respectively, of all citrus fruits produced in the country. In erms of area, Maharashtra tops (93,193 ha) followed by Andhra Pradesh (56,116 ha), Punjab (37,464 ha), Karnataka, Uttar Pradesh and Bihar. However, highest productivity was obtained n Tamil Nadu (17.6 tonnes/ha) followed by Madhya Pradesh and Gujarat (16 tonnes/ha) (Singh, 2000).

Citrus fruits are delicious, refreshing to eat, provide itamins, minerals and are available throughout the year. They re mostly consumed as fresh, particularly mandarins, sweet ranges, pummelo and grapefruit. Mandarins and sweet oranges re also used in the preparation of squashes and cordials (Rajput nd Haribabu, 1985). In sweet orange, the principal constituent f the edible portion are sugars (glucose and sucrose) and acids

(primarily citric acid and little of malic acid). It is also a good source of vitamin-C, vitamin-A, thiamine, riboflavin and niacin. The rind of citrus fruits is rich in pectin, certain essential oils and glucosides (hesperidin in oranges). Total soluble solid (TSS) in fruit juice of sweet orange varies from 8-12 per cent, while titrable acidity usually ranges from 0.5-1.5 per cent usually for oranges and mandarins and has a TSS : acid ratio of 8:1. Citrus oil and citric acid are byproducts obtained from culled fruits. The oils are largely used as flavouring extracts and to some extent in perfumes and soap industry.

Sweet orange is known to be affected by diseases caused by several fungi, bacteria and viral pathogens. Among the fungal pathogens *Phytophthora* spp. induces root rot, foot rot, collar rot, gummosis of trunk and branches, leaf fall and fruit rot diseases. Twig blight or wither tip or anthracnose is caused by *Colletotrichum gloeosporioides*, sooty mould is induced by *Capnodium citri* and *Cladosporium* species. Pre-harvest stem end rot is caused by several pathogens including *Diplodia natalensis*, *Phomopsis citri* and *C. gloeosporioides* inducing fruit drop, thereby seriously affecting the marketable fruit yields (Sawant and Bulbule, 2000). Other fungal diseases of sweet orange include pink disease (*Pellicularia salmonicolor*), powdery mildew *Acrosporium tingitaninum*), scab (*Elsinoe fawcetti*) and dry root rot (*Fusarium solani*).

There are three broad categories of primary causes of losses in perishables : parasitic (biological), non-parasitic physiological) and physical (mechanical). The parasitic,

biological or pathological decay is incited by various agents ranging from fungi, bacteria, virus and nematodes during harvesting, handling, storage, transport and marketing. The various post harvest fruit rots of citrus caused during storage and transit are by *Penicillium digitatum, Penicillium italicum, Geotrichum candidum, Aspergillus niger, Aspergillus fumigatus, Alternaria citri, Alternaria alternata, Botryodiplodia theobromae, Rhizopus stolonifer, Colletotrichum gloeosporioides* and *Curvularia unata* (Naqvi, 2001). In India almost all the citrus produce is marketed fresh in domestic market and less than 1 per cent is being exported/processed. Around 18-23 per cent produce is being lost to post harvest diseases and rough handling of Nagpur mandarin trade in India. The post harvest infections may take place through wounds and/or injuries arising during harvest and handling of fruits.

Post harvest diseases can be controlled by physical and chemical treatments. The physical treatments include heat therapy, low temperature storage and radiation, while chemical treatments include the use of antibiotics, growth regulators, fungicides, oils, chemicals and vapour emitting compounds (Mehrotra *et al.,* 1998). Chemical fungicides currently provide the primary means for controlling post harvest decay in citrus fruit. However, consumer demands for pesticide free food and the development of pathogenic strains that are resistant to currently used fungicides necessitates the development of alternative methods or fungicide free safe post harvest treatments for decay control (Porat *et al.,* 2002). Therefore, realizing the importance of

the problem, it was thought worthwhile to carry out research work both on applied and fundamental aspects of the problem as:

1. To study the microorganisms associated with post harvest diseases of sweet orange from different markets, their isolation and identification studies.
2. To prove the pathogenicity of isolated organism.
3. To study the disease symptomatology.
4. To study the morphological, cultural and physiological characters of associated pathogens.
5. To evaluate the bioefficacy of different control measures against the pathogen.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Historical

Genus *Colletotrichum* (Corda) belongs to the division Eumycota, Sub-division Deuteromycotina, form class Coelomycetes, form order Melanconiales and form family Melanconiaceae.

Genus *Penicillium* belongs to the division Eumycota, sub-division Ascomycotina, form class Euascomycetes, form order Eurotiales and form family Trichocomaceae.

Bulliard (1809) used the name *Mucor penicillatus* for "broom" or brush like forms and provided the first reasonably adequate illustration of mold unquestionally representing a *Penicillium.*

The name *Penicillium* applied to a genus of fungi first appeared in Link's "Observationae" (1809) in which he described very briefly the genus and three species *P. glaucum, P. candidum* and *P. expansum.*

Link (1824) abandoned earlier named species *P. expansum* and called all the green penicillia, *P. glaucum.*

Corda (1837) differentiated *Colletotrichum* from that of *Gloeosporium* on the basis of presence or 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 as *Colletotrichum*

gloeosporioides based on the basis of presence of setae in the acervulus.

Saccardo (1884) followed by Wehmer (1894) reported *Penicillia* active in destruction of citrus fruit.

Wehmer (1895) published his studies of the *Penicillia* occurring upon rotting fruit. He figured and described the destructive effects of *Penicillium expansum* on apples, pears and grapes, but under the name *P. glaucum.* The olive coloured rot of oranges, already described by Saccardo as *Penicillium digitatum* in 1880 and distributed in Mycotheca Italic was *P. olivaceum* and the soft rot organism with blue green colours was correctly regarded as new and named *P. italicum.*

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.

Zaleski (1927) described new thirty-five species and one variety of *Penicillium* from the forest soils of Poland.

Grove (1937) considered *Colletotrichum* essentially as *Gloeosporium* producing setae in the acervulus, so he considered the binomial *Colletotrichum gloeosporioides* a conidial stage of *Gloeosporioides cingulata.*

The genus *Colletotrichum* has a very wide range of behavorial pattern in nature ranging from saprophytes to specialized parasitic strain with a narrow host range. Over 1000 *formae species* of *Colletotrichum* have been described on the basis of diseases caused on various hosts. Sutton (1980) has

given key and description to the 22 species recognized in this genus based on cultural characters. *Colletotrichum* species cause "anthracnose" (literally means 'coral like' leaf spot disease) of several crops and is therefore commonly referred to as anthracnose fungus. The important pathogenic species of *Colletotrichum* are *gloeosporioides, falcatum, capsici, lini, coffeanum, lindemuthianum, orbiculare, lagenarium, graminicola, dematium, circinus, coccodes, higginstanim, musae, truncatum* etc.

2.2 Occurrence

Nolla (1926) revealed that the anthracnose of mango, avacado and citrus fruits appeared to be connected mainly with the attack by *Colletotrichum gloeosporioides.*

Chaudhari and Singh (1933) reported that all commercial species of citrus are attacked by *Colletotrichum gloeosporioides* in Punjab.

Muller (1933) in Brazil reported *Colletotrichum gloeosporioides* causing the disease in grapefruit, Rangpur lime, sweet orange and especially on sweet and sour lemon.

Baker (1935) observed that fruit rot of grapefruit caused by *Penicillium digitatum* may occur in severe form soon after fruits have been taken into storage and the chief factors responsible are bruising or wounding and the profuse distribution of the spores of the fungus. The *Colletotrichum gloeosporioides* is characteristic of fruit which have been kept in storage for a considerable time and is by far the most serious

cause of wastage when fruits may have lost as much as 10 per cent of their moisture content.

Smith (1936) reported that citrus withertip or anthracnose in Jamaica were most commonly associated with the fungus *Colletotrichum gloeosporioides*.

Fawcett (1936) reported that stem end rot of citrus caused by *Colletotrichum gloeosporioides* is common in all citrus growing areas of the world and is more destructive in warm and humid regions.

Baker and Wardlaw (1937) observed the infection of *Colletotrichum gloeosporioides, Penicillium digitatum* and *P. italicum* in storage of grapefruit and mango.

Cheema *et al.* (1937) reported the occurrence of *Penicillium* fruit rot of citrus in India.

Singh and Hamid (1942) found that moulds are associated with the spoilage of sweet orange and mandarin fruits in cold storage and the estimated spoilage was 50 per cent.

Hafiz (1953) stated that Malta oranges as compared to santra, grapefruit and lemon suffered the most by *C. gloeosporioides*.

Subramanian *et al.* (1973) reported the losses from decay in mandarins to the tune of 20-30 per cent; considered associated mainly with fungal infection which developed even with slight bruises on rind which were common in harvesting processes. The comparative efficacy of some fungicides were tested against *C. gloeosporioides, P. digitatum, P. italicum* and *Diplodia natalensis*.

Pelser (1975) reported the pathogen responsible for fruit rot of sweet orange in storage as *C. gloeosporioides* Penz.

Kaul and Lall (1975) reported that besides *Penicillium italicum* and *P. digitatum, Aspergillus niger, Geotrichum candidum* and *Fusarium spp.* were responsible for decay of citrus in storage in Himachal Pradesh.

Ram and Naidu (1976) reported that fungi responsible for storage decay of Coorg orange are *Penicillium digitatum, Phomopsis citri, Diplodia natalensis* and *Sclerotinia sclerotiorum.*

Sumbali and Mehrotra (1981) reported that many species of *Penicillium* e.g., *P. expansum, P. digitatum* and *P. italicum* contribute higher percentage of the airspora and were found to be the cause of green and blue mold of oranges.

Tuset (1984) recorded 15 species and their relative incidence in storage and marketing, causing rots in citrus, are predominated by *Penicillium* and *Alternaria* species. These fungal infection occur even after storing fruits at 2-4^{0}C for two months.

Salerno and Cutuli (1985) observed that the infection by *C. gloeosporioides* in citrus may remain latent until after harvest and the disease manifest during transport and storage.

Gardner *et al.* (1986) reported that green (*Penicillium digitatum)* and blue (*P. italicum*) rots occur in all citrus growing areas and often constitutes the predominant type of decay.

Naqvi and Dass (1994) reported that the fruit rots due to moulds incidence may vary from 0-100 per cent depending upon the storage conditions. In Maharashtra, losses of 43 and

47 per cent of mandarins in trucks and train transport, respectively have been reported.

Babu and Reddy (1998) reported post harvest diseases of sweet orange caused by C*ladosporium cladosporioides, Curvularia* (Cochliobolus) *pallescens, Cochliobolus speicifer, Fusarium oxysporum, Macrophoma* sp., *Nigrospora* (Khuskia) *oryzae, Phoma sorghina* and *Syncephalastrum racemosum.*

The common anthracnose fungus has been recorded in most citrus growing countries including USA, Belize, Australia, Japan, India, South Africa and Israel (Sharma and Rana, 1999).

Sharma and Gupta (2000) reported that green and blue mold of citrus is caused by *Penicillium digitatum* and *Penicillium italicum* respectively.

The various post harvest rots are caused during storage and transit by *Penicillium digitatum, P. italicum, Geotrichum candidum, Aspergillus niger, A. fumigatus, Alternaria citri, A. alternata, Botryodiplodia theobromae, Rhizopus stolonifer* and *Curvularia lunata* (Naqvi, 2001). In India, almost all the citrus produce is being exported or processed. Around 18-23 per cent produce is being lost to post harvest diseases and rough handling of Nagpur mandarin trade in India. The post harvest infections may take place through wound and or injuries arising during harvest and handling of fruits.

Ismail and Zhang (2004) revealed that green mold of citrus is caused by *Penicillium digitatum* and *Colletotrichum gloeosporioides* causes anthracnose in citrus fruits.

2.3 Symptomatology

Fawcett and Berger (1927) observed initially a water soaked area on the rind of fruit which breaks easily. Such area soon becomes covered with white mould and coloured spores form at the centre of lesion. In green mould rot, there is usually a broad band of white beyond the sporing area and this rot predominates at moderate temperatures, emitting a foul smell on complete rotting.

Baker (1935) observed green mould of grapefruit (*P. digitatum*) lesion as water soaked, soft tissue and at first not discoloured. It enlarges rapidly, sometimes becoming sunken, and with a growth of white mycelium and finally a powdery mass of green spores appear on the surface. The entire fruit is reduced to a soft mass covered with the green spores of the fungus. In case of *C. gloeosporioides*, the lesion is firm, dark brown and leathery; sunken one or sometimes soft water soaked lesions of a peculiar ashy-grey colour. The fruit generally shows a peculiar light pinkish colouration in the affected rind, extending also in the pulp. Conidia are abundantly produced in pinkish acervuli on the outside.

Fawcett (1936) distinguished green mold of citrus fruits (*Penicillium digitatum*) from other common diseases of citrus fruits and described that lesions on the rind start as small, water soaked areas which extend rapidly; a wide white

margin of mycelium ahead of central green areas is seen as the lesion expands; the white area of mycelium is wrinkled and with a pasty appearance; green spore masses are confined to the fruit surface.

Tanaka (1968) reported that the citrus fruits disfigured by tear stain pattern on the peel in case of post harvest stem end rot is caused by *Colletotrichum gloeosporioides.*

Salerno and Cutuli (1985) reported that fruits of citrus undergo severe rotting, with formation of dark sunken lesion in case of stem end rot caused by *Colletotrichum gloeosporioides.*

Ullasa (1993) observed symptoms on stored kinnow fruits as infection of *C. gloeosporioides* usually starting from stem end and brown necrotic spots gradually spreading towards blossom end and covered upper half of the entire fruits in severe cases.

Aulakh *et al.,* (1998) described that *Penicillium digitatum* cause watery rot during transit and storage of citrus in which the rind breaks easily on pressing and the affected area get covered with green mouldy growth and the fruit emits a foul smell.

Sharma and Gupta (2000) described that green mold causes brownish and watery spot which may crack easily even with little pressure and such fruits are later on covered with green mouldy growth. Gradually entire fruit rots and may be reduced to decomposed mass, producing numerous conidia which act as secondary source of inoculum.

Ismail and Zhang (2004) reported that green mold of citrus due to *Penicillium digitatum* in early infection shows area as a soft watery spot and sometime it is referred as a clear rot. As the lesion progresses, white mycelia develops and these produce green spores. Infection takes place through wounds and fruit decay begins at these infected injury sites. The white mycelium develops into a broad zone surrounding the sporulating area. They have also reported that the anthracnose lesions caused by *Colletotrichum gloeosporioides* are initially silvery grey and leathery, being similar in firmness and elevation to adjacent healthy rind tissue. The infected rind becomes brown to greyish black and softens as the rot progress. Lesions vary in size and are irregular in shape. Pink spores may form on the lesion surface in humid environments.

2.4 Isolation

Baker (1935) isolated *Colletotrichum gloeosporioides* and *Penicillium digitatum* among the primary organisms of leather rot and soft rot disease of 'Marsh' grapefruit in storage.

Baker and Wardlaw (1937) isolated *C. gloeosporioides, P. digitatum* and *P. italicum* from grapefruit and mango encountered in the storage.

Davis and Smoot (1965) isolated *Penicillium digitatum* from infected orange fruits.

Kavanagh and Wood (1967) isolated *Penicillium digitatum* from lesions on Valencia orange.

Phelps (1968) isolated species of *Colletotrichum, Fusarium, Curvularia* and *Pestalotia* from citrus fruit.

Sinha *et al.*, (1972) isolated *Colletotrichum gloeosporioides* from stalk end rot of citrus.

Pelser (1975) isolated and identified the pathogen responsible for fruit rot of sweet orange in storage as *C. gloeosporioides* Penz.

Khilare and Gangawane (1997) isolated *Penicillium digitatum* from infected fruits of Mosambi collected from different local markets in Maharashtra.

Sholberg (1998) isolated *Penicillium digitatum* from infected fruits of citrus.

Shellie (1998) isolated *Penicillium digitatum* from Rio Red grapefruit and maintained on Potato dextrose agar.

Whiteside *et al.*, (1998) isolated the pathogen responsible for post harvest anthracnose and identified it as *C. gloeosporioides* from susceptible tangerine cultivars.

Dantas *et al.*, (2003) isolated *Colletotrichum gloeosporioides* from the orange fruits causing post harvest anthracnose.

Muniz *et al.*, (2003) isolated *Colletotrichum gloeosporioides* from the post harvest disease affected orange fruits.

2.5 Pathogenicity

Baker and Wardlaw (1937) reported the pathogenicity of *C. gloeosporioides, P. digitatum* and *P. italicum* from grapefruit and mango in storage studies.

Eckert *et al.*, (1968) inoculated the lemon fruits with *Penicillium digitatum* and proved its pathogenicity.

Sinha *et al.,* (1972) isolated *Alternaria citri* and *Colletotrichum gloeosporioides* from styler end rot and stalk end rot of citrus respectively and confirmed their pathogenicity.

Cheema *et al.,* (1976) observed six isolates of *Colletotrichum gloeosporioides* (*Glomerella cingulata*) obtained from six sweet orange varieties differing in pathogenicity on fruits of the varieties blood red and pineapple.

The role of Cx-cellulases has been suggested in both the *Penicillium* sp. in early stages of pathogenesis in orange cv. Valencia (Barkai-Golan and Karadamid, 1991).

Agostini *et al.,*(1992) distinguished three strains of *C. gloeosporioides* from citrus in Florida, USA and characterized them as FGG, SGO and KLA. The SGO strain reproduced all symptoms of the disease on sweet orange and Persian lime. The KLA strain produced typical lime anthracnose symptoms on key lime, but SGO isolates produced only a mild mottle of key lime leaves.

Timmer *et al.,* (1998) isolated *Colletotrichum gloeosporioides* from post harvest anthracnose of citrus fruits and proved the pathogenicity.

Sholberg (1998) inoculated citrus fruit with *Penicillium digitatum* and proved its pathogenicity.

Holmes and Eckert (1999) collected the isolates of *Penicillium digitatum* and *P. italicum* and confirmed its Pathogenicity on lemon fruits.

Muniz (2003) collected samples of orange and lemon, isolated the fungi on PDA medium and tested their pathogenicity

by wound inoculation on healthy fruits. *Colletotrichum gloeosporioides* (*Glomerella cingulata*) was the most frequently fungi isolated apart from *Trichoderma viride* on orange, *Fusarium lateritium* (*Gibberella baccata*) and *F. subglutinans* on lemon.

Benyahia *et al.* (2003) isolated *Colletotrichum gloeosporioides* from infected fruits of citrus showing tear stain pattern and confirmed its pathogenicity.

2.6 Morphology of fungus

Singh and Sinha (1954) observed the conidia of *Colletotrichum gloeosporioides* isolated from *citrus paradisi* as oval to oblong with both the ends equally rounded. They contain, sometimes, one or two globules and may or may not be with granular protoplasm. Conidia on potato dextrose agar after maturity measured 7.24-16.29 µm in length and 2.72-6.34 µm in breadth or 12.59 x 3.66 µm in size. Acervuli are pink and later turn black. The acervuli measure 100-305 µ in diameter. Setae appear when cultures are about a month or more in age. They are broad, pointed and black, measuring 54.2 to 145.9 µ in length.

Patil (1969) observed that the conidia of *Colletotrichum gloeosporioides* on maturity measured 8.5-13.0 µm x 3.5-6.25 µm.

Barnett and Barry (1972) described the morphological characters of *Penicillium* Link, wherein conidiophores arise from the mycelium singly or less often in synnemata, branched near the apex, penicillate, ending in phialides. Conidia were hyaline or

brightly coloured in mass, 1-celled mostly globose or ovoid, in dry basipetal chains.

Gupta and Madaan (1977) observed that the fruit rot of ber caused by *Colletotrichum gloeosporioides* Penz. consists of narrow, sparsely septate hyphae, which are first hyaline and then turn into dark brown colour. Acervuli are formed abundantly on the affected host surface. Conidiophores are simple, hyaline. The conidia develop on the apex of each conidiophore. They are broadly oval to oblong with rounded ends, non-septate, sometimes contain 1-2 globules, measuring 13-6 x 4-6 μm.

Raper and Thom (1984) described the morphological characters of *Penicillium digitatum.* The conidiophores are typically very short, commonly ranging from 30 to 100 μ by 4.0 to 5.0 μ, smooth walled (although granular appearing hyphae are occasionally observed under oil), arising from submerged hyphae or from the basal mycelial felt in colonies upon malt or other nutrient rich media; penicilli typically biverticillate and asymmetrical but varying greatly in dimensions and complexity, rarely appearing monoverticillate or unbranched, with branches, metulae and sterigmata often poorly defined, consisting of branches varying greatly in length and bearing either metulae and sterigmata, or sterigmata only; metulae variable in form and dimensions, commonly ranging from 15 to 30 μ by 4.0 to 6.0 μ and bearing sterigmata in variable but always in limited numbers, sterigmata equally variable and ranging from 15 to 28 μ by 3.5 to 5.0 μ, usually producing chains of elliptical conidia,

but occasionally terminating in swollen vesicular cells; conidia smooth-walled, dull dark green in mass, varying greatly in form and dimensions, ranging from subglobose to long cylindrical in shape but usually elliptical, commonly 3.5 to 5.0 μ by 3.0 to 3.5 μ at first, then 6.0 to 8.0 μ by 4.0 to 6.0 μ and occasionally upto 10 to 12 μ by 6.0 to 8.0 μ with substantial differences in form and dimensions of conidia commonly occurring within the same chain.

Agostini *et al.*,(1992) studied morphological characters of three strains of *Colletotrichum gloeosporioides* (*Glomerella cingulata*) and observed that the fast growing grey isolates from necrotic and senescent tissue had large conidia, mostly with both apices rounded, produced abundant setae and had large lobulate appressoria. The slow growing orange isolates associated with citrus post bloom fruit drop produced clavate, deeply pigmented appresoria. The slow growing isolates with deep orange pigmentation from key limes affected by lime anthracnose produced round, smaller, less pigmented appressoria. The slow growing orange and key lime isolates had smaller conidia, most with one fusiform apex and rarely produced setae.

Sumbali (2000) reported that the mycelium of *C. gloeosporioides* causing anthracnose of guava is intercellular, branched and light brown in colour. Each acervulus consists of closely packed, hyaline, unbranched conidiophores bearing conidia at the tips. Conidia are sub-hyaline, pinkish in mass,

oblong to cylindrical, obtuse at the apex, 1-celled, 9-24 x 3-4.5 µm.

Sharma (2000) reported that the mycelium of *C. gloeosporioides* causing anthracnose of mango consist of narrow, sparsely septate hyphae, hyaline at first, but slightly darker at later stages. Acervuli are produced abundantly as tangled sub epidermal masses of hyphae. Numerous closely packed conidiophores arise from this place. From the apex of each conidiophore one or more conidia develop. Setae are common in acervuli. Conidia are pinkish in mass but hyaline individually. They are oval to oblong, with rounded ends, non-septate and sometimes contain 1-2 globules and measure about 12-16 x 4-6 µm. On germination, conidial germ tubes produce dark appressoria.

Sharma and Gupta (2000) reported that conidiophores of *Penicillium digitatum* arise from the mycelium singly or less often in synnemata branched near the apex to form a brush like conidia bearing apparatus, ending in phialides which pinch off conidia in chains. These conidia are hyaline, one celled, globose or ovoid and produced basipetally.

Hande (2001) studied the morphological characters of the fungus, *Colletotrichum gloeosporioides* from curry leaf and noticed that the mycelium of the fungus was septate, irregularly branched, vacuolated, hyaline and measured 3.81 µm in width, when old. Initially acervuli looked light orange and later changed to dark brown to black in colour, rectangular to irregular in shape and measured 60.01 x 48.22 µm. Setae measured 73.40 x

3.52 µm. Conidiophores were short, simple, thickly arranged on acervuli and 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. He also noticed the sexual stage of fungus i.e. *Glomerella cingulata*. The perithecia were light to dark brown, circular to subglobose, which measured 110.96 x 113.2 µm. Asci were either straight or slightly bent at the middle and spored and measured 60.40 x 8.23 µm in size.

Gaikwad (2002) during study of fruit rot of custard apple recorded that the mycelium of *Colletotrichum gloeosporioides* was closely septate, irregularly branched, vacuolated of width 3.02 µm. Acervuli measured 188.02 x 147.80 µm, while setae length was 111.70 µm. The conidia were oblong to cylindrical, oval with 11.52 µm length. The conidiophores were short, septate, simple, thickly arranged and were hyaline and later turned very light brown to ashy brown. The chlamydospores were globuse or ovate, thick walled, intercalary in chain or terminal and yellowish-brown to ashy brown and 8.64 µm in diameter.

Sharma (2005) reported that the *Penicillium spp* causing blue mould produce dense mycelial growth with blue green or dull green conidia, which are formed in chains. The reproductive structure resembles a small brush. The conidia are green or blue green in mass, elliptical to globose, measuring 4.5-5.0 µm in diameter.

2.7 Growth and cultural characters

Singh and Sinha (1954) observed that *Colletotrichum gloeosporioides* grows well on 2 per cent potato dextrose agar, oat meal agar and Leoninan's agar media.

Dawkhar (1970) observed that Richard's agar medium was good for growth and sporulation of *Colletotrichum gloeosporioides.*

Mallikarjunaiah (1972) observed a good growth and abundant sporulation of *Colletotrichum gloeosporioides* in potato dextrose agar.

Raper and Thom (1984) described that cultivated in the laboratory, members of the *Penicillium digitatum* series are characterised by their sparse growth and limited spore production upon Czapek's solution agar and other comparable synthetic media based upon comparatively pure sugars and inorganic nitrogen sources. They are equally characterized by their luxuriant growth and abundant sporulation upon malt extract and potato dextrose agar and on Czapek's solution agar to which amendments such as steep liquor or yeast extract have been added.

Raper and Thom (1984) reported that colonies of *Penicillium citrinum* on steep agar growing more rapidly, attaining a diameter of 2.5 to 3.0 cm in 10 to 12 days at room temperature, heavly sporing and more closely furrowed, but in general exhibiting the same basic features as on Czapeks dox; exudate limited to abundant, pale yellow; reverse in yellow to light brown shades. They also reported that some strains of

Penicillium italicum from citrus fruits grow rapidly and fill the entire agar plate or tube, others are more restricted and seldom exceed 3.0 to 4.0 cm in diameter on Czapek's agar ever after three or four weeks, but all strains grow more luxuriantly on malt extract and steep agars, and on Czapek's there is some evidence that many strains suffered from a limited nutrient deficiency. They observed that colonies of *Penicillium expansum* on steep agar grow more rapidly than on Czapek's agar and attain a diameter of 5.5 to 6.0 cm in 8 to 10 days.

Patil (1989) observed that onion host leaf extract, potato dextrose agar and potato carrot agar gave good growth and sporulation of *Colletotrichum gloeosporioides.*

Ekbote *et al.*,(1997) studied the effect of synthetic and non-synthetic liquid and solid media on growth and sporulation of *Colletotrichum gloeosporioides.* They reported maximum growth of the fungus on Richard's agar and Potato dextrose agar media followed by Czapek's agar. In liquid media, maximum dry mycelial weight was noticed on Richard's broth.

Wahid (1999) tested five isolates of *Colletotrichum gloeosporioides* (*Glomerella cingulata*) for their ability to grow on potato dextrose agar, Czapek's yeast agar and guava decoction agar. Growth, sporulation and spore sizes exhibited some variations amongst these isolates.

Growth and cultural characters of *Colletotrichum gloeosporioides* causing fruit rot of custard apple was studied by Gaikwad (2002) on twelve synthetic and fifteen non-synthetic media. The synthetic media *viz.*, M_2 agar and Neopeptone

glucose agar were best for growth and sporulation followed by Sabourauds agar and Asthana Hawker's agar, while oat meal agar was the excellent non-synthetic medium for growth and sporulation.

2.8 Physiological studies

2.8.1 Effect of temperature

Ryall and Pentzer (1904) described that *Penicillium* spp. causing blue mould rot of cane fruits grows slowly at 0°C (32°F), but quite rapid at 10-27°C (50° to 80°F).

Ames (1915) reported that *Penicillium digitatum* from orange is able to germinate and grow at 30°C but no conidia are formed at this temperature. The thermal death point for *P. digitatum* was 58.0° to 58.5°C and the optimum temperature for growth of *P. digitatum* was around 25°C.

Fawcett and Berger (1927) observed that oranges kept at 90.5°F, which is above the maximal limit for *Penicillium italicum* and *P. digitatum*, are not decayed during 28 days exposure. They reported that the green moulds of citrus grows more rapidly at moderate temperatures.

Doron (1922) reported the optimum temperature for spore germination of *Penicillium digitatum* as 25°C.

Sattar and Malik (1939) reported the optimum temperature for the sporulation of *C. gloeosporioides* to be 30 °C.

Singh and Sinha (1954) observed that *C. gloeosporioides* grow well in different agar media at temperatures ranging from 20 to 28 °C.

Tandon (1967) reported that *C. gloeosporioides* can grow within the temperature range of 10 to 35°C and the optimum being 25°C.

Patil (1969) studied the temperature relationship of *Colletotrichum gloeosporioides* causing anthracnose of guava and observed that the fungus could thrive well between 10°C to 35°C. Optimum temperature for the growth was observed to be between 25 to 30°C.

Gordillo (1981), Ahmed (1985), Kim *et al.* (1986) and Hegde *et al.* (1993) reported that the growth and sporulation of *Colletotrichum gloeosporioides* was maximum between 25-35°C and 5-6.5 pH.

According to Prakash and Srivastava (1987), the pathogen's growth (*C. gloeosporioides*) is optimum at 25°C and ceases beyond 35°C, whereas optimum temperature for infection is 25°C.

Agostini *et al.*,(1992) observed that the three strains of *Colletotrichum gloeosporioides* (*Glomerella cingulata*) affecting citrus had optimum temperature for growth 23-27°C. But the fast growing isolates grew better at 31°C than the slow growing orange and key lime isolates.

Plaza *et al.*, (2003) observed that germination and growth of *Penicillium digitatum, Penicillium italicum* and *Geotrichum candidum* were markedly influenced by temperature and water activity, lag times were longer and germination and growth rates were slower when conditions of temperature and water activity were far from optimum. All studied species were

able to germinate over a range of 4-30°C at 0.995 aw although in non-optimal conditions, *P. digitatum* only reached 40-60 % of germinated conidia.

Cerruto *et al.,*(2004) observed optimum temperatures for mycelial growth of *P. digitatum* and *Lasiodiplodia theobromae* to be 25° and 30°C respectively.

Lahlali *et al.,*(2006) showed that *Penicillium digitatum* can grow best at ambient temperatures.

2.9 Evaluation of different control measures against *Colletotrichum gloeosporioides* and *P. digitatum.*

2.9.1 *In vitro* evaluation of fungicides

Tsai (1969) has reported from Taiwan that the post harvest treatment with orthocide, piomycin or dithane M-45 gives good control of papaya rot incited by *Colletotrichum gloeosporioides.*

Sohi *et al.,* (1973) reported satisfactory control of anthracnose of mango by dipping fruits in benomyl (500 ppm) and thiabendazole (500 ppm). Similarly, spray or dip treatment of carbendazim or thiophanate methyl (0.1 %) protects the fruits against post harvest decay.

Tandon *et al.,* (1975) reported that *Aspergillus niger* and *Penicillium spp.* were effectively checked by bavistin, cupramar and ziride. They reported that dithane Z-78, dithane M-45 and captan can effectively control the blue mold rot of grape caused by *Penicillium* spp.

Bolkan *et al.,*(1976) found that Brazilian papaya fruits dipped for 3 min. in either benomyl or thiabendazole solution

with a sticker at 500 mg/l gave substantial reduction in post harvest decay caused by *C. gloeosporioides*, *Gloeosporium* sp. and *Ascochyta carica-papayae.*

Ram and Naidu (1976) studied the fungicidal control of post harvest decay of summer as well as monsoon in Coorg orange and found that the post harvest decay of summer crop fruit was negligible, whereas, it was quite severe in monsoon oranges. The fungi mainly involved in post harvest decay of citrus fruit were *Phomopsis citri, Diplodia natalensis, Penicillium digitatum* and *Sclerotinia sclerotiorum.* The storage loss due to these fungi was effectively control by dipping the fruits for two min. in 0.1 per cent TBZ, bavistin, calixin and benlate.

Kumbhare and Chaudhari (1979) reported that carbendazim, benlate were the most effective fungicides against *Colletotrichum gloeosporioides* and *Botrydiplodia theobromae* in sweet orange.

Basant Ram *et al.,* (1983) reported that treatment before storage with benomyl, bavistin and thiabendazole (1000 ppm) effectively checks the rot in citrus caused by *Colletotrichum gloeosporioides.*

Rao (1984) observed Imazalil WP and EC formulations were highly effective for citrus fruits and showed a decay reduction index of 76.2 to 96.7 during a storage period of 36 days against *Penicillium* fruit rots.

Datar and Ghule (1988) reported that dipping of banana fruits in carbendazim (1000 ppm) for 10 minutes

protected the banana fruits from rotting caused by *Penicillium* Sp and *Colletotrichum* species.

Ullasa and Rawal (1988) reported benlate, baycor (1000 ppm) were highly effective, followed by prochloraz and bavistin for control of stem end rot of kinnow mandarin.

Cohen (1989) observed treatment of lemons with fenpropimorph and flutriafol at a packing house reduce post harvest decay due to molds in lemon by upto 94 per cent by arresting lesions and sporulation at inoculation sites.

Barkai and Apelbaum (1991) observed that treatment of oranges with sodium-o-phenyl-phenate for 3 min., heated to 45 ^{0}C resulted in 87.94 % reduction in the incidence of decay.

Berton *et al.,* (1992) reported that post harvest dip treatment of peaches with iprodione reduced *Monilinia* spp., *Penicillium* spp. and *Alternaria* spp. particularly when calcium chloride was applied before harvest.

Hegde *et al.,*(1992) conducted *in vitro* trials with seven fungicides against anthracnose fungus *Colletotrichum gloeosporioides* of arecanut and revealed that the fungicides viz. copper oxychloride and Mancozeb were good.

Ali *et al.,* (1993) ascertained that among seven fungicides, bavistin (100 ppm) and propiconazole (200 ppm) were found to be the most effective for complete inhibition of *Colletotrichum gloeosporioides* in *in vitro* while ready Bordeaux mixture was not effective.

Thakare and Patil (1995) recorded satisfactory inhibition of fungal growth of *Colletotrichum gloeosporioides* by

copper oxychloride at 0.2, 0.3 and 0.4 per cent concentration in *in vitro* fungicide tests.

Majumdar and Pathak (1997) reported benlate (0.1%) as most effective against *Colletotrichum* rot in both pre and post inoculation treatments in guava.

Kader and Rahman (2001) tested the sensitivity of *Colletotrichum gloeosporioides* and *Pestalotia psidii* to fungicides causing anthracnose of guava *in vitro* and observed that score 250 EC (difenconazole), knowin 50 WP (carbendazim) and bavistin (carbendazim) were equally effective in inhibiting the mycelial growth of *Colletotrichum gloeosporioides*.

Gaikwad (2002) noticed that benomyl (0.1 %), Prochloraz (0.1 %), propiconazoble (0.1 %), Bordeaux mixture (1.0 %) and thiophanate methyl (0.1 %) were effective in the order mentioned for the control of fruit rot (*Colletotrichum gloeosporioides*) of custard apple.

2.9.2 *In vitro* evaluation of plant extracts

Ahmed and Agnihotri (1972) worked on the antifungal properties of seventy one plant extracts using the cold and boiled water extracts in the "spore glass slide germination test". They observed that the extracts of *Canna indica* L., *Cenchrus catharticus* Pelile, *Allium cepa* L., *Allium sativum* Linn., *Lowsonia inermis* Linn., *Argemone mexicana* Linn. and *Datura stramonium* Linn. inhibited the spore germination of *Alternaria brassicae* (Sacc.), *Colletotrichum papayee* (*P. Henn*) and *Helminthosporium* species.

Narain and Satapathy (1977) studied the antifungal characteristics of leaf, flower, stem and root extracts of two varieties of *Vinca rosea* against *Helminthosporium nodulosum, Sclerotium rolfsii, Pestalotia* species, *Fusarium oxysporum, Colletotrichum* species and *Aspergillus niger.* These extracts inhibited spore germination, sporulation and mycelial growth of test fungi.

Yadav (1986) screened fifteen plant species for their fungitoxicity using the acetone extraction method. Out of them only clove extract of *Allium sativum* and leaf extracts of *Ocimum sanctum, Nerium indicum* Mill., *Cleome isocandra* Linn. and *Vinca rosea* caused complete inhibition of spore germination and mycelial growth of *Alternaria solani* (Cell and Mart.) L.R. Jones & Grout, *Aspergillus niger* V. Tiegh, *Colletotrichum capsici* (Syd.) *Butl.* and Bisby and sclerotial germination of *Macrophomina phaseolina.*

Godara and Pathak (1995) reported that among the five plant extracts screened under *in vitro* studies, tulsi leaf extract proved highly effective against germination of spores of *Penicillium italicum.*

Shivpuri *et al.,* (1997) reported the fungitoxic properties of extracts of ten plant species against five pathogenic fungi *viz., Alternaria brassicola, Colletotrichum capsici, Fusarium oxysporum, Rhizoctonia solani* and *Solerotinia sclerotiorum.* The results revealed that leaf extracts of *Azadirachta indica, Datura stramonium, Ocimum sanctum, Polyantha longifolia* and *Vinca rosea* were the most fungitoxic against all the test fungi.

Khilare and Gangawane (1997) reported that *Azadirachta indica* was highly effective in controlling the *Penicillium digitatum* both individually and mixture with thiophanate methyl (700 µg/ml). They also reported *Calotropis procera, Terminalia chebula, Curcuma longa, Ocimum sanctum* and *Zingiber officinale* extracts were also effective.

Obagwu and Korsten (2003) applied water and ethanol extracts of garlic cloves to artificially inoculated citrus fruits to test their efficacy against *Penicillium digitatum* and *P. italicum* and reported that the treatment 1 % garlic extract + oil was as effective as the fungicide treatment in controlling both green and blue mold of Valencia orange.

Sinha *et al.,*(2004) tested the fungicidal properties of different extracts against *Colletotrichum capsici* and observed that the radial growth of the fungus was lowest (30.93 mm) with *Ocimum sanctum* and was highest (45.5 mm) with neem as compared to control (71.4 mm). The sporulation was highest with bakayan extract and lowest with neem and *Cuscuta reflexa* as compared to control.

2.9.3 Effect of physical and chemical treatments on post harvest diseases of sweet orange

Wardlaw and Leonard (1937) reported control of decay of citrus fruit due to *Botryodiplodia theobromae, Colletotrichum gloeosporioides* and *Penicillium digitatum* by hot water treatment at 43.3^{0}C for 20 seconds.

Akamine and Arisumi (1953) showed that in papaya, post harvest phase of anthracnose can be controlled by submerging fruits in water at 46 to 49°C for 20 mins.

Smoot and Melvin (1965) demonstrated that immersion in hot water at 128°F for 5 minutes reduced the incidence of decay of oranges and tangerines as much or more than sodium o-phenylphenate, a standard fungicide.

Tandon (1961) found that 2 per cent bleaching powder (calcium hypochlorite) and 0.2 per cent flit 40 (N-trichloromethylmercapto-4-cyclohexane 1,2-dicarboximide) as 5 and 10 min fruit dip respectively, were most effective in controlling moulds of sweet orange and madarins.

Smoot and Segall (1963) have immersed 5 cultivars of mango in hot water ranging from 130 to 134.5 °F (54.5-56.9 °C) for 5-7 min. and obtained a significant reduction in the anthracnose infection. A slightly lower temperature of 125 °F (51.7 °C) at 15 min in also effective.

Eckert and Kolbezen (1964) observed that aqueous solutions of 0.5 % 2-aminobutane of pH less than 9 and at a temperature of 45 °C controlled *Penicillium digitatum* and *P. italicum* by submerging orange and lemon fruits for at least 1 min. in the solution. It's efficiency significantly improved by not rnsing the fruits after treatment.

Smoot and Melvin (1965) showed that heat treatment controls green mould rot (*Penicillium digitatum*) and stem end rot (*Diplodia natalensis*) of Florida orange.

Tandon and Singh (1968) suggested hot water treatment of fruits for 15 mins at 50-55 ^{0}C as also dipping mango fruits at 55^{0}C for 5 mins in water having benomyl or thiabendazole for anthracnose.

Brodrick *et al.,* (1972) reported that the post harvest rots caused by *C. gloeosporioides, Ascochyta spp.* and *Rhizopus spp.* in papaya can be controlled when a hot water dip (50^{0}C, 5 min.) is followed by a treatment with benomyl.

Spalding and Redder (1972) reported that hot water treatment (55^{0}C for 5 min) alone controlled anthracnose of mango for three weeks and benomyl or TBZ added to hot water gave control for four weeks.

Patel *et al.,* (1973) observed that fumigation with benzylisothiocynate controlled anthracnose of papaya and the post harvest phase could be controlled by submerging fruits in hot water at 46-49 ^{0}C for 20 minutes.

Subramanian *et al.,* (1973) observed thiabendazole (500 ppm) as dip treatment for 2-3 minutes or thiabendazole 0.1 % incorporated in waxol-0-12 was effective under refrigeration against blue and green moulds of oranges.

Quimo and Quimo (1974) have observed that a post harvest dip of mangoes in water containing 600-1000 ppm benomyl along with 0.05 % tween 40 for 10 min., effectively inhibits the decay caused by *C. gloeosporioides.*

Khare and Dhingra (1974) reported sodium carbonate, sodium hydroxide and sodium borate at 2 and 1 per cent

concentrations completely checked the growth of *Colletotrichum gloeosporioides* in culture.

Akamine (1977) reported 20 minutes hot water dip at 47^{0}C to be effective for controlling storage decay of mangoes in Hawaii.

El-Goorani and Sommer (1979) reported that adding 9 % carbon monoxide to air or controlled atmospheres slowed rot development by *P. digitatum* and *P. italicum* in oranges at 12.5 ^{0}C. The rates of rot development caused by *P. digitatum* in oranges incubated in air + carbon monoxide, in controlled atmosphere and in controlled atmosphere + carbon monoxide were reduced by 67, 45 and 94 %, respectively.

Barmore *et al.*, (1983) showed that polyethylene film packing in grapefruits (*Citrus paradisi*) limited the rate of oxygen utilization during decay process and reduces losses due to decay.

The papaya fruits inoculated with *C. gloeosporioides* and stored at low pressure (15 mm of Hg, 10 ^{0}C, 21d) developed less anthracnose rot during the subsequent 5 days of ripening at room temperature (Chau and Alvarez, 1983).

Chitzanidis *et al.*, (1987) reported that fumigation of packing houses with formaldehyde and driol for 4 hours has been found to reduce number of viable spores of *P. digitatum* and *P. italicum* on culture and plastic surface.

Ben-Yehoshua *et al.*, (1987) and Strange and Eckert (1994) showed that curing fruit at 32-36 ^{0}C for 36-72 hours is an effective method for control of green and blue moulds.

The food preservatives e.g. potassium sorbate, sodium benzoate and sodium propionate were effective against green mould when applied to Valencia oranges equivalent to Dox-Hex treatment used for citrus (Hall *et al.*1989).

Huang *et al.,* (1991) observed susceptibility of orange fruits due to wounds to *Penicillium digitatum* decreased during heating at 25 ^{0}C and more so at 30 ^{0}C due to lignification and phenylalanine ammonia lyase (PAL) activity which increased many folds.

McGuire (1991) reported immersion of fruit in water at 46 ^{0}C for 90-115 min. or hot forced air at 48 ^{0}C for 150 minute in 3 cultivars of mango reduced the anthracnose caused by *Colletotrichum gloeosporioides* by 60-89 %.

Jagdish Chandra and Pathak (1992) observed that severity of the rots in mango inoculated with *Diplodia natalensis, Colletotrichum gloeosporioides, Aspergillus niger* and *Rhizopus arrhizus* was significantly reduced by wrapping the fruits in plastic film (0.002 cm) than control on 5th and 8th day of inoculation. However, the disease was less on 5th day as compared to 8th day.

Saaiman and Lonsdale (1994) found hot water (55^{0}C for 2 mins.) having prochloraz (81 g a.i./100 l) to be effective in reducing anthracnose of mango.

Sharma *et al.,* (1994) observed that dipping mangoes for 1 to 3 mins. in hot water at 52 ^{0}C containing 0.1 % benomyl was effective in controlling *C. gloeosporioides* and *Botryodiplodia theobromae.*

Smilanick *et al.*,(1995) showed that inoculated lemon fruits immersed in solutions of 2 per cent sulfur dioxide at 45°C for 150 sec. followed by two fresh water rinses and ethanol 10 per cent at 45 °C for 150 sec. without rinsing controlled green mould of lemon caused by *Penicillium digitatum* without causing injury to the fruit.

Shellie and Skaria (1998) reported that lesions of grapefruits developing from wounds inoculated with *Penicillium digitatum* just prior to heating for 300 min. in 46 °C moist forced air developed less rapidly during 4 days of storage at 21 °C.

Aulakh *et al.*,(1998) reported that careful handling during picking and packing followed by fumigation with sulphur dioxide (0.5 % SO_2 in air) for 20 minutes gives effective control.

Sholberg (1998) observed that citrus fruits (grapefruit, lemon and oranges) inoculated with *Penicillium digitatum* and placed in air tight chambers containing acetic acid (1.9 or 2.5 µl/l), formic acid (1.2 µl/l) and propionic acid (2.5 µl/l) for 30 min. aerated, injured and incubated under 20 °C for 3 to 6 days showed reduction in decay from 86 to 11 % by all three acids.

Lanza *et al.*,(1998) reported that holding citrus fruit for 3 days at 32°C in a water saturated atmosphere conducive to wound healing and detrimental to *P. digitatum* development (curing) was found to be very effective in reducing decay in 24 h old *P. digitatum* infections of lemons and Valencia late oranges. The integrated treatments, which involved immersing lemon, cv. Femminello Siracusano fruits with incipient (24 h) *P. digitatum* infections in 10% ethanol at 45°C for 180 s, followed by curing at

32°C for 1 day were as effective as dipping fruits in 1 g a.i./litre imazalil. Immersion of lemon in a 3% solution of sodium carbonate plus hot water treatment at 52°C were as effective as imazalil treatment.

Roy (2000) observed application of hot water in various citrus fruit species at 2 min. dip in water at 53°C or 56°C markedly reduced the decay caused by *Penicillium digitatum* and *Penicillium italicum.* In Marsh grapefruit, a combined hot water (52°C) + growth regulator (50 ppm Progibb + 200 ppm 2-4,D) treatment reduced the decay.

Porat *et al.,*(2000) showed that a minimum exposure period of 20 s at 56 °C is required to inhibit *Penicillium digitatum in vitro* spore germination in citrus. *In vivo* studies carried out by rinsing and brushing citrus fruits 24 h after artificial inoculation with *P. digitatum* spore suspension indicated that hot water brushing at 56, 59 and 62 °C for 20 s reduced decay development in infected wounds to only 20, 5 and less than 1%, respectively of that in untreated control fruits.

Erkan and Pekmezci (2000) reported that wrapping of fruits of 'washington naval' oranges in diphenyl impregnated paper resulted in the lowest percentages of chilling injury and wrapped fruits could be stored for more than 4 months at 5 °C with minimal quality loss.

Om Prakash and Pandey (2000) showed that hot water (52°C) alone for 30 min was found very effective in controlling the anthracnose of mango caused by *Colletotrichum gloeosporioides.*

Brown and Chambers (2000) found that application of polyhexamethylene biguanide (1000-4000 ppm) to citrus fruits provided significant control of stem end rot caused by *Diplodia natalensis,* sour rot (*Geotrichum citriaurantii*) and green mould (*Penicillium digitatum*) in mandarins, Orlando tangelo and sweet oranges.

Palou *et al.,* (2001) showed that hot water controlled blue mould at 50 to 55^{0}C temperature near those that injured fruit and its effectiveness declined after 14 days of storage sodium carbonate and sodium bicarbonate were superior to hot water. Temperature of sodium carbonate solution influenced effectiveness more than concentration or immersion period. Sodium carbonate applied for 150 s at 45^{0}C at 3 to 4 % reduced decay more than 90 per cent. Sodium bicarbonate applied at room temperature at 2 to 4 per cent reduced blue mould by more than 50 per cent. In another set of experiments, treatment of sodium bicarbonate at room temperature, sodium carbonate at 45^{0}C and hot water at 45^0 reduced blue mould incidence on artificially inoculated oranges by 6, 14 and 27 per cent respectively, after 3 weeks of storage at 3^{0}C. These experiments reduced green mould incidence by 6, 1 and 12 per cent respectively, while incidence among controls of both moulds was about 100 per cent.

Smilanick and Sorenson (2001) showed that incidence of green mould of citrus caused by *Penicillium digitatum* was reduced by 80 % or more by the immersion of lemons or oranges

for 1-4 min in warm (40.6-43.3^{0}C) liquid lime sulfur solution that contained 0.75 % (wt./vol.) calcium polysulfide.

Teixido *et al.*,(2001) showed that the efficacy of *Pantea agglomerans* for the control of green mold was improved when combined with sodium bicarbonate resulting in complete and 97.6 % reduction of decay incidence at 3 and 20^{0}C compared to untreated controls.

Porat *et al.*, (2002) reported that application of hot water rinsing and brushing (62 ^{0}C for 20 s), 2 % sodium bicarbonate (baking soda) or *Candida oleophila* (108 cells/ml) 24 h after inoculation grapefruit with *P. digitatum* reduced development in the infected wounds by 68, 61 or 23 %, respectively. The combination of any two of these treatments or all three of them together reduced decay by 87-89 % from the level in untreated control fruit.

Tijsken *et al.*,(2003) reported dry air at 48.5 ^{0}C for 4 hr alone or in combination with TBZ (thiabendazole) decreased chilling injury intensity and fungal development of *Colletotrichum gloeosporioides* causing decay in papaya. The best effect was achieved by combining dry heat treatment and TBZ without causing heat injury.

Cerruto *et al.*,(2004) observed that curing citrus fruit at 35 ^{0}C for 48 h achieved better green mould control than a shorter time of 24 h. The combination of thiabendazole drench (500 ppm) and curing of wounded 'Valencia' oranges and inoculated 'Flame' grapefruit reduced both green mold and stem end rot by more than 93 %.

Venditti *et al.*, (2005) observed that oranges cv. Biondo Comune wounded and treated with 5 % Na_2CO_3 and inoculated with *P. digitatum* or *P. italicum* conidia 3 days post treatment, the decay percentage as compared to untreated wounds was reduced by 97.2 and 93.9 %, respectively.

MATERIAL AND METHODS

3. MATERIAL AND METHODS

3.1 Material

3.1.1 Collection of samples

The affected diseased sweet orange cv. Mosambi (*Citrus sinensis*) fruits were collected from the Agricultural Produce Market Committee (APMC), Rahuri; APMC, Ahmednagar; APMC, Nashik; APMC, Pune; APMC, Pandharpur and APMC, Akluj.

3.1.2 Culture media

Potato dextrose agar (PDA) was used for isolating the fungus. Different synthetic and non-synthetic solid culture media were used to study the morphological, growth and cultural characteristics of fungus (Booth, 1971).

3.1.3 Glasswares

Different types of Corning and Borosil glasswares were used in the experimental work. The common glasswares were petriplates, test tubes, conical flasks, measuring cylinder, glass rods, microslides, cover slips, beakers, pipette, graduated glass cylinders, etc.

3.1.4 Equipment and instruments

The common laboratory equipments and instruments used were autoclave, hot air oven, incubator, laminar flow air chamber, refrigerator, electronic weighing balance, research microscope, water bath, physical balance, thermometer, haemocytometer, filar and stage micrometer, mortar and pestle etc.

3.1.5 Miscellaneous materials

Miscellaneous materials *viz.*, marking pencils, rubber bands, sticky labels, muslin cloth, blotting paper, inoculating needle, forceps, spirit lamp, scale, filter paper, cork borer, moisture chamber, mixer, footrule, funnel, glass jar, wire sieve, dessicator, thin wrapping tape, polyethylene bags, mercuric chloride and sodium hypochlorite stock solution, bags etc. were used.

3.1.6 Chemicals

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

The details of different chemicals used for control of post harvest diseases are as below.

Sr. No.	Name of chemical	Chemical formulae	Source
1.	Glacial Acetic acid	CH_3COOH	S.d. Fine Chem. Ltd., Boisar
2.	Ethanol	C_2H_5OH	S.d. Fine Chem. Ltd., Mumbai
3.	Sodium bisulfite	$NaHSO_3$	Loba Chemie, Bombay
4.	Sodium carbonate	Na_2CO_3	Glaxo India Ltd., Bombay
5.	Sodium bicarbonate	$NaHCO_3$	Glaxo India Ltd., Bombay

3.1.7 Fungicides

The details of the fungicides used are given as below.

Sr. No.	Trade name	Common name	Chemical name	Source
1.	Bavistin	Carbendazim 50 % WP	2-Methoxy-carbamoyl benzimidazole	BASF India Ltd., Mumbai.
2.	Blitox-50	Copper oxychloride 50 % WP	Copper oxychloride containing 50 % metallic copper	Rallis India Ltd., Mumbai.
3.	Captaf	Captan 50 % WP	N-trichloromethylthiotetra hydropthalimide	Rallis India Ltd., Mumbai.
4.	Contaf	Hexaconazole 5 % EC	(Rs)-(2,4-dichloropheynyl)-1-(1H-1, 2,4-trizole-1-yi) hexan-2-01 (IUPAC)	Rallis India Ltd., Mumbai.
5.	Indofil M-45	Mancozeb 70 % WP	Zinc ion and Manganese ethylene bisdithiocarbamate	Indofil Chemical Co., Mumbai.
6.	Kavach	Chlorothalonil 75 % WP	Tetrachloroisophthalo-nitrate	Syngenta Agrochemicals, Mumbai.
7.	Quintal	Iprodione 25 % + carbendazim 25 % WP	3(3,5-dichlorophyenyl)-N-(1-methylethyl)-2, 4-dioxo-1-imidazolidine carboxamide + 2-(methoxycarbonylamino) – benzimidazole	Bayer (India) Limited, Chennai.
8.	Score	Difenconazole 25 % WP	1-{2-[4-chlorophenoxy) - 2-chlorophenyl-(4-methyl-1, 3-dioxolan-2-yi)-methyl]}-1H-2, 2, 4-triazole	Syngenta Agrochemicals, Mumbai.
9.	Tilt	Propiconazole 25 % EC	1-[2-(2,4-dichorophenyl) -4-Propyl-1, dioxolan-2-yi-methyl]1H-1, 2, 4-triazole	Syngenta Agrochemicals, Mumbai.

3.2 Methods

3.2.1 Isolation

Several isolations were carried out from the affected sweet orange fruits by employing tissue isolation method (Tuite, 1969; Booth, 1971). Isolations were carried out from the rind portion and diseased tissues cut into small pieces were disinfected by dipping in 1:1000 mercuric chloride solution for 1-2 minutes followed by thorough washing in three changes of sterile water to remove the traces of mercuric chloride. These pieces were then kept on sterile blotting paper to remove the excess moisture and plated on potato dextrose agar (PDA) medium in petri plates, which were already sterilized, poured and cooled under aseptic conditions. The inoculated plates were incubated at 27 $\pm$ 1 ^{0}C.

The petridishes were daily observed for the growth of fungi. When the growths of more than one fungi were observed, individual mycelium was carefully transferred with inoculating needle to sterilized PDA plates as well as slants and incubated at 27 $\pm$ 1 ^{0}C. The individual cultures were purified by repeated sub-culturing on PDA in plates and were maintained on PDA slants for further detailed study.

3.2.2 Maintenance of culture

Isolated fungi were sub-cultured on PDA slants and kept at 27 $\pm$ 1 ^{0}C for seven days. When maximum growth was noticed, such slants were preserved in refrigerators at 5 to 10 ^{0}C. The cultures were sub-cultured once in month to maintain the viability of the fungus.

3.2.3 Inoculation and pathogenicity studies

Inoculation experiment was carried out by first growing the fungi on potato dextrose agar medium at room temperature for seven days. These cultures were used to inoculate healthy fruits of sweet orange cv. Mosambi. Under laboratory conditions, the pathogenicity was proved by detached fruit technique (Gaikwad, 2002). For this purpose, freshly harvested medium sized fruits of Mosambi were used. Fruits were stored at room temperature for two days to determine any incipient infection and those which showed any kind of symptoms were discarded. Before inoculation, the remaining healthy fruits were washed with tap water, air dried, surface sterilized with 0.5 per cent sodium hypochlorite solution for 45 seconds (Agostini *et al.*, 1992) followed by thorough but gentle rinsing with three changes of sterile water. The fruits were injured by pinprick method over the surface of fruit. The inoculation was made by using 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.

Inoculated fruits were incubated in sterilized moisture chamber. The inner part of moisture chamber was covered with moistened cotton to maintain proper humidity for disease development. The moistening of cotton was carried out every 12 hours. The relative humidity and temperature in the moisture

chamber during the period of infection and disease development ranged from 85 to 95 per cent and 20-28 ^{0}C, respectively. The uninoculated set of fruits was also kept as a control. For this, few drops of sterile water were placed on fruits of same size and age and covered with sterilized cotton. The observations for disease appearance were recorded starting 24 hours from inoculation till appearance of visible symptoms at 12 hours interval. The final observations for kind of symptoms were recorded until 10 days after inoculation.

3.2.4 Reisolation

The reisolation was carried out from artificially inoculated fruits showing typical symptoms by adopting tissue isolation method. The fungi thus obtained were transferred and maintained separately on PDA slants for comparison with the original culture and for subsequent studies.

3.2.5 Identification of cultures

Identification of cultures was done at the laboratory of Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri and was confirmed from "Disease Diagnosis Centre", Department of Plant Pathology and Agril. Microbiology, PGI, MPKV, Rahuri.

3.2.6 Preparation of inocula for further studies

Pure culture grown on potato dextrose agar medium were used for morphological, cultural, physiological and fungicidal evaluation studies. These studies were carried out by

inoculating the medium with the fungal disc of five mm diameter from the periphery of seven day old culture.

3.2.7 Symptomatology

The symptoms produced by the microorganisms on sweet orange cv. Mosambi were studied from the naturally infected fruits as well as artificially inoculated fruits.

3.2.7.1 Symptoms under natural conditions

The disease symptoms produced on the fruits and its further progress of development were studied in detail. The observations regarding initiation of symptoms, development of symptoms, colour of lesion, its growth habit on fruit were recorded by following standard methodology.

3.2.7.2 Symptoms under *in vitro* condition

The healthy fruits of sweet orange cv. Mosambi were inoculated by detached fruit technique as described previously and the symptoms were noted.

3.2.8 Morphology of the fungus

Morphological characters of the microorganisms infecting sweet orange cv. Mosambi were studied from culture growth on potato dextrose agar (PDA) for 5 to 10 days at 27 $\pm$ 1 ^{0}C (Chowdhary and Varshney, 2000).

In case of the morphological characters of different structures *viz.*, mycelium, acervuli, chlamydospore, setae, conidiophore, phialides and conidia observations were taken by adopting slide culture technique. The length and width of acervulus, setae and conidia were measured and the width of mycelium, chlamydospore and phialides was also measured. The

length : width ratio of acervulus and conidia was calculated from the length and width measurements. The number of segments per setae was also calculated. The length of conidiophore and phialid was measured.

The morphological structures of fungus was measured by preparing slides using cotton blue stain. Twenty observations for each structure was taken and each observation was multiplied by calibration factor (calculated from divisions of filar needed for completing 10 divisions of stage) to give measurements in μm (micrometer).

3.2.9 Growth and cultural characters of *Colletotrichum gloeosporioides* and *Penicillium digitatum*

The cultural characters of both the fungus were studied on different culture media *viz.*,

Synthetic media

i. Nutrient agar
ii. M_2 agar
iii. Ashbys agar
iv. Sabourauds glucose agar
v. Richards agar
vi. Czapek's dox agar
vii. Asthana Hawker

Non-synthetic media

i. Potato dextrose agar
ii. Tap water agar
iii. Corn meal agar
iv. Wheat meal agar
v. Prunne agar

These media were prepared according to methods and procedures described by Tuite (1969) and Booth (1971).

Twenty ml of each of the above melted and cooled media at 45 ^{0}C was poured into 100 mm diameter petriplates with each medium replicated thrice. These plates were allowed to solidify and then, five mm diameter fungal mycelial disc of test microorganism was inoculated at the centre with the help of sterile cork borer. These plates were incubated at 27 $\pm$ 1 ^{0}C for 8-10 days. Colony diameter were recorded by averaging the linear growth of colony in two directions. Cultural characteristics namely topography, type of margin, colour of colony and sporulation on different media for each plate were recorded. 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)

x = Initial value

x + 1 = Value after 48 hrs.

3.2.10 Physiological studies

3.2.10.1 Effect of temperature on growth and sporulation of *C. gloeosporioides* and *P. digitatum*

Twenty ml of potato dextrose agar medium was poured into previously sterilized 100 mm diameter petriplates for

each temperature replicated thrice. After cooling, these plates were inoculated at the centre with the five mm diameter fungal mycelial disc. The plates were incubated at 0, 5, 10, 15, 20, 25 and 30 ^{0}C for seven days. Colony diameter were recorded by averaging the linear growth of colony in two directions for each plate of each culture (Gottileb, 1950). Observations were recorded in tabular form.

3.2.11 Evaluation of different control measures against *C. gloeosporioides* and *P. digitatum*

3.2.11.1 *In vitro* evaluation of fungicides

Poisoned food technique (Horsefall, 1957) was employed to evaluate the efficacies of different fungicides against pathogens associated with post harvest diseases of sweet orange cv. Mosambi. Potato dextrose agar medium was prepared and distributed in 100 ml quantities in 250 ml Erlenmeyer flasks. After sterilization, flasks were cooled to 45 ^{0}C and fungicides were added to the medium under aseptic conditions so as to obtain the concentrations of chlorothalonil 0.25 %, captan 0.25 %, copper oxychloride 0.25 %, mancozeb 0.25 %, carbendazim 0.1 %, hexaconazole 0.1 %, propiconazole 0.1 %, difenconazole 0.1 % and iprodione + carbendazim 0.25 %. Flasks were shaken thoroughly and then poured in triplicate plates for each chemical. These plates were inoculated with five mm fungal mycelial disc of seven day old culture grown on potato dextrose agar and were incubated at 27 $\pm$ 1 ^{0}C for seven days. Plates with PDA medium without fungicides served as control.

Observations on colony diameter and sporulation were recorded on eighth day after inoculation. Radial growth was measured and the results were expressed as per cent inhibition of mycelial growth (Vincent, 1947) over control using the formula,

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

Where,

I = Per cent inhibition of fungal growth

C = Growth in mm on the 8th day after inoculation in control.

T = Growth in mm on 8th day after inoculation in treatment

3.2.11.2 *In vitro* evaluation of plant extracts against *C. gloeosporioides* and *P. digitatum*

1. Plant and their parts used in experiment

Sr. No.	Plant species	Common name	Parts used
1.	*Nerium indicum* Mill	Kanher	Leaves
2.	*Ocimum sanctum* Wild.	Tulas	Leaves
3.	*Lantana camera*	Ghaneri	Leaves
4.	*Azadirachta indica*	Neem	Leaves
5.	*Allium sativum* Linn.	Garlic	Cloves
6.	*Vinca rosea* Linn.	Sadaphuli	Leaves
7.	*Acacia arabica* Willd.	Babul	Leaves
8.	*Tagetes erecta*	Zendu (Marigold)	Leaves
9.	*Polyantha longifolia*	Ashoka	Leaves

2. Method of plant extraction

Ten grams of the plant materials was weighed and thoroughly washed. The plant material was then crushed in mortar and pestle by adding 10 ml of sterilized distilled water. The crude material was then expressed through double layered muslin cloth and filter paper (Whatman No. 1). The filtrate thus obtained was used in the experiments (Bambode and Shulka, 1973).

3. Effect of plant extracts on mycelial growth of test fungi on solid media

The effect of plant extracts on mycelial growth was studied using poisoned food technique. All the nine plant extracts were tried against the test fungi. The whole crude plant extracts were supplemented to sterilized PDA in 1:2 proportion (Tripathi and Dixit, 1977) and the 'poisoned' medium was poured in petriplates of 10 cm diameter. A 5 mm mycelial disc of test fungus was cut from periphery of seven day old culture. It was aseptically inoculated in the centre of the medium. Each set was replicated thrice. The control set were run side by side by using sterilized distilled water instead of plant extracts. The petriplates were incubated at 27 $\pm$ 1 ^{0}C for 7 days.

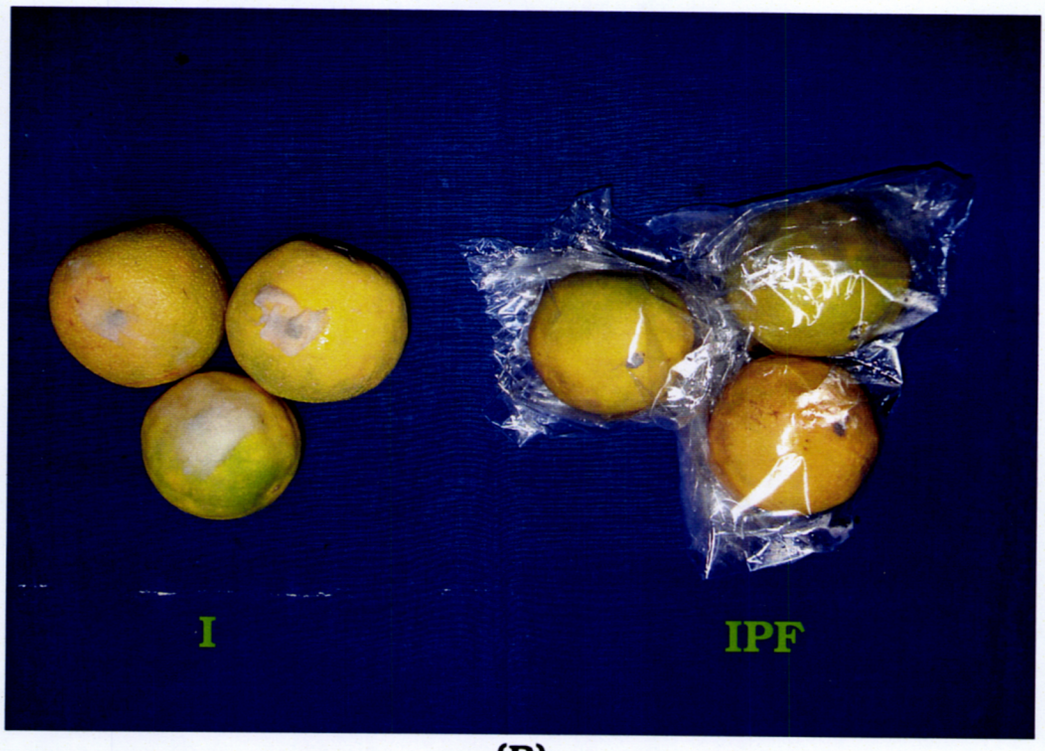
Observations were recorded on the colony diameter, topography colour of colony and sporulation.

3.2.11.3 Effect of different post harvest treatments for the management of post harvest diseases of sweet orange cv. Mosambi

Sweet orange cv. Mosambi fruits of uniform size and maturity were washed, surface sterilized with 0.5 % sodium hypochlorite, air dried and inoculated with the pathogens *viz.*, *Colletotrichum gloeosporioides* and *Penicillium digitatum* at a spore density of 10^6 spores/ml or a 2 mm agar disc of a seven day old culture. The fruits were then subjected to different post harvest treatments as per the treatment schedule. Individual treatments were replicated thrice and appropriate control was maintained. Observations were recorded on increase or decrease of lesion diameter at each wound inoculation site and the average for each fruit calculated. The per cent disease control was worked out for each treatment. Final observations were recorded, when all the fruits from control were totally decayed. Treated fruits were stored under ambient conditions (25-27 ^{0}C) and evaluated at 4 days interval. The treatment details for the different post harvest treatments were as follows.

1. Plastic film wrapping (20 μ) of fruit (Plate 1 A and B).
2. Fumigation with acetic acid (2.5 %) for 30 min.
3. Inoculation, before heating at 46 ^{0}C for 300 min.
4. Inoculation, after heating at 46 ^{0}C for 300 min.
5. Immersion in hot water at 45 ^{0}C for 150 sec. (Plate 2)
6. Immersion in 10 % ethanol at 45 ^{0}C for 150 sec.
7. Immersion in 2 % sulphur dioxide at 45 ^{0}C for 150 sec. followed by 2 sterile water rinses.

(A)



(B)

Plate 1. **Effect of plastic film wrapping on post harvest anthracnose (*C. gloeosporioides*) and green mould (*P. digitatum*) of sweet orange cv. Mosambi; A = *C. gloeosporioides*; B = *P. digitatum*; where I = fruits inoculated with *C. gloeosporioides* and *P. digitatum*, respectively and IPF = inoculated fruits wrapped in plastic film (20 μ) separately for both pathogens**

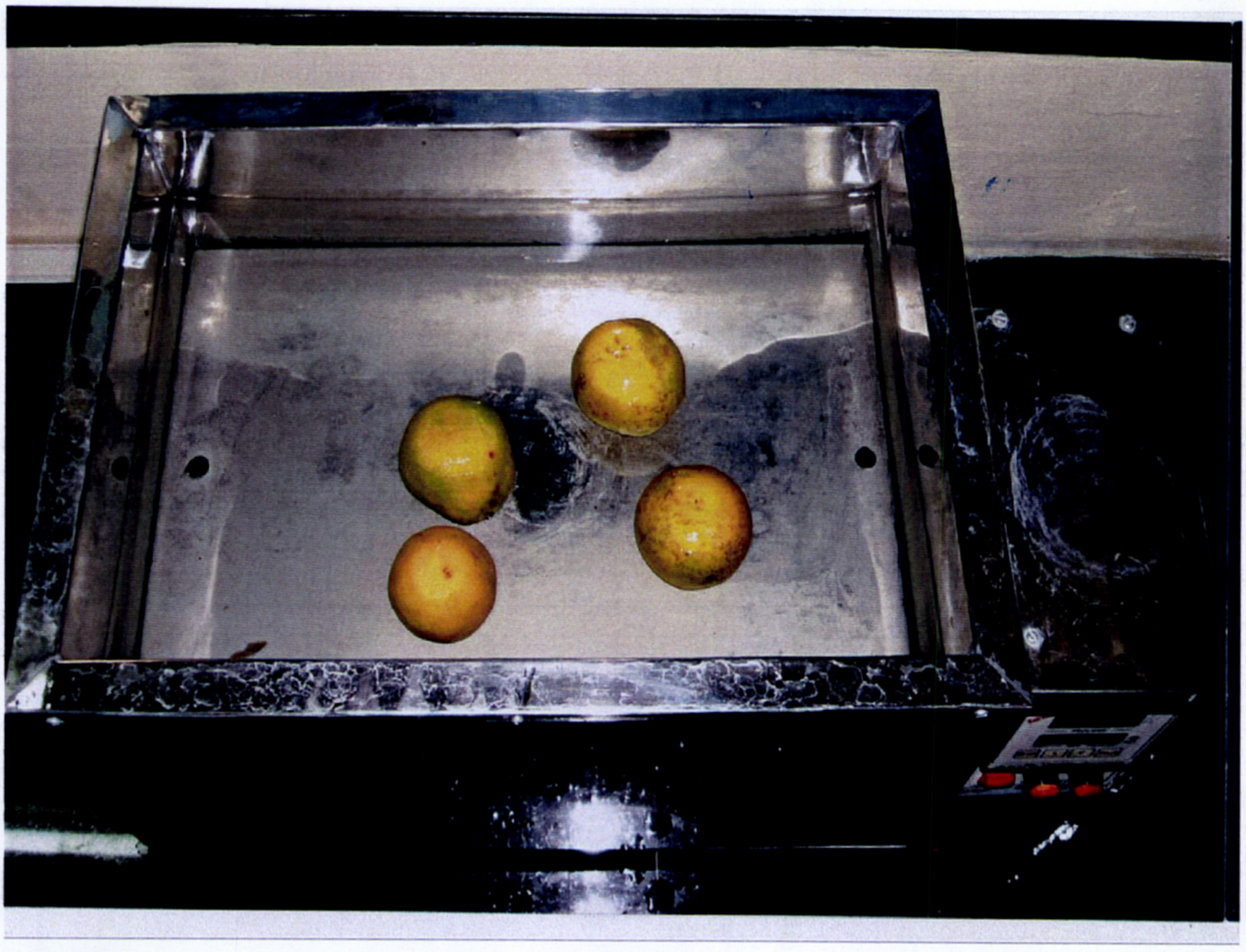


Plate 2. **Hot water treatment : Fruits placed in hot water bath at 46 ^{0}C for 150 sec.**

8. Immersion in 3 % sodium carbonate at 45°c for 150 sec. followed by 2 sterile water rinses.
9. Immersion in 3 % sodium bicarbonate for 150 sec. followed by 2 sterile water rinses.
10. Control.

3.2.12 Statistical analysis

The data obtained on cultural studies, physiological studies, nutritional studies, fungal inhibition studies and post harvest treatment studies were subjected to statistical analysis using standard statistical methods (Panse and Sukhatme, 1985). For this, completely randomized design was used for studying the analysis of variance.

EXPERIMENTAL RESULTS

4. EXPERIMENTAL RESULTS

The present studies were carried out on post harvest diseases of sweet orange cv. Mosambi in respect of isolation, identification, pathogenicity, reisolation of pathogen, symptomatology, morphology, cultural and physiological characters, efficacy of different fungicides, botanicals (plant extracts) and various post harvest treatments. The results are presented here as under :

4.1 Isolation, identification, pathogenicity and reisolation of pathogens

4.1.1 Isolation

Isolations were made by tissue isolation method from the affected fruits of sweet orange cv. Mosambi collected from different markets which showed disease symptoms. The pure culture of the fungus was obtained and was later maintained for further studies by frequent subculturing.

4.1.2 Inoculation

The inoculation studies using detached fruit inoculation technique could infect the sweet orange cv. Mosambi fruit and cause disease symptoms. The symptoms on fruits appeared within 1-2 days of inoculation, whereas no symptoms appeared in the uninoculated fruits.

4.1.3 Reisolation

The reisolations were made from artificially inoculated sweet orange cv. Mosambi fruits that showed typical symptoms

of disease, yielded the fungal culture which was identical to the original isolates.

4.1.4 Identification

The fungus isolated from the post harvest disease affected fruits and inoculated fruits were identified as *Colletotrichum gloeosporioides* Penz. causing anthracnose and *Penicillium digitatum* causing green mould of sweet orange at the Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri and was confirmed from the "Disease Diagnosis Centre", Department of Plant Pathology and Agricultural Microbiology, PGI, MPKV, Rahuri.

4.2 Symptomatology

The symptoms of post harvest disease caused by *Colletotrichum gloeosporioides* and *Penicillium digitatum* in sweet orange cv. Mosambi were studied from the disease samples collected from the market (naturally infected fruits) as well as artificially inoculated fruits in the laboratory by detached fruit inoculation technique.

4.2.1 Symptoms from naturally infected fruits

4.2.1.1 Symptoms from naturally infected fruits by *Colletotrichum gloeosporioides*

Generally *Colletotrichum gloeosporioides* infects the sweet orange cv. Mosambi fruit showing brown discolored lesions on the surface of fruit and progressing as dark brown to blackish sunken lesions or patches (Plate 3). The affected fruits later

(I) (H)

Plate 3. **Symptoms on sweet orange cv. Mosambi fruit samples naturally infected with *Colletotrichum gloeosporioides*; I = Infected, H = Healthy**

(I) (H)

(I)

Plate 4. **Symptoms on sweet orange cv. Mosambi fruit samples naturally infected with *Penicillium digitatum*; I = Infected, H = Healthy**

become brownish entirely and rot. Brown discolouration was also seen in the outer portion of the albedo.

4.2.1.2 Symptoms from naturally infected fruits by *Penicillium digitatum*

In case of fruits infected naturally with *Penicillium digitatum* showed white mould visible on the surface. When the mould growth sporulates the rot appears as olive-green in the centre and surrounded by a broad white zone of mycelial growth at the advancing margins, beyond which there is an area of soft rind (Plate 4).

4.2.2 Symptoms under *in vitro* conditions

4.2.2.1 Symptoms on artificially infected fruits by *C. gloeosporioides*

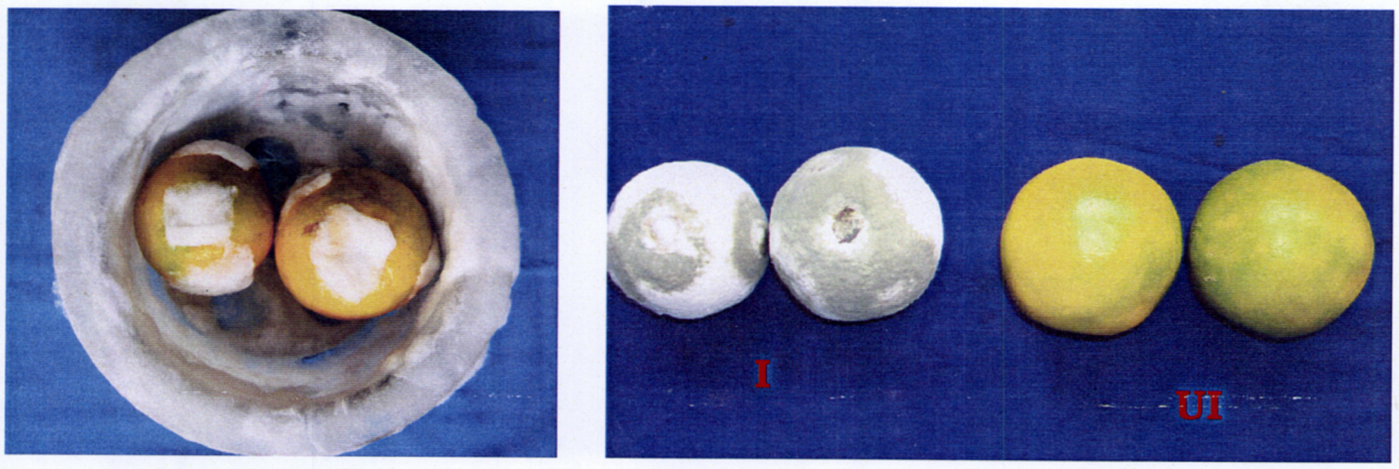
The sweet orange cv. Mosambi fruits inoculated with *Colletotrichum gloeosporioides* by the detached fruit technique with mycelial bit inoculation method showed brown discoloured lesion on fruit rind at the inoculation site (Plate 5). The brown discolouration starts within 48 hours from inoculation starting from inoculation site and covers the entire surface of fruit within 12-15 days. The typical disease symptoms include formation of brown sunken lesions and brown discoloured outer portion of the albedo. The white mycelial growth was observed over the lesions under high humid conditions.

4.2.2.2 Symptoms on artificially infected fruits by *P. digitatum*

In case of *Penicillium digitatum,* the sweet orange cv. Mosambi fruits inoculated by the detached fruit technique with

(A) (B)

Plate 5. **Pathogenicity of *Colletotrichum gloeosporioides* in sweet orange under *in vitro* by detached fruit technique; A = fruits inoculated with *C. gloeosporioides* and kept in humidity chamber; B = symptoms developed *C. gloeosporioides* under *in vitro* condition (I = Inoculated, UI = uninoculated fruits)**



(A) (B)

Plate 6. **Pathogenicity of *Penicillium digitatum* in sweet orange under *in vitro* by detached fruit technique; A = fruits inoculated with *P. digitatum* and kept in humidity chamber;B = symptoms developed *P. digitatum* under *in vitro* condition (I = Inoculated, UI = uninoculated fruits)**

mycelial bit inoculation method showed the early infection area as a soft water soaked spot within 2-3 days of inoculation at the site of inoculation. As the lesion progresses, white mycelial growth develops, later a powdery mass of green spores appearing over the surface within 10-12 days. Surrounding this sporulating area (Plate 6), a broad zone of while mycelial growth can be observed.

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 *Colletotrichum gloeosporioides* and *Penicillium digitatum* shown in Tables 1 and 2, respectively are as below.

4.3.1 Morphology of C. *gloeosporioides*

Mycelium

The fungus *Colletotrichum gloeosporioides* produced profuse white mycelial growth on potato dextrose agar, which later on ageing turned dull white, light to dark grey. The mycelium was septate, hyaline, irregularly branched and vacuolated in early growing stage. Hyphae were thin in early stage, which become moderately thick on aeging (Plate 7a). It measured 4.04 μm (1.50 to 6.01 μm) in width.

Acervulus

The fungus produced acervuli in culture, in about 10-12 days after subculturing and were firm on the medium. Most of the acervuli were produced within mycelial mat, they looked dark brown to black in colour (Plate 7b). They were globose to

saucer or irregular in shape. The acervuli (including setae) measured 190.39 µm (120.24 to 344.05 µm) x 114.76 µm (57.87 to 180.04 µm). The base of acervulus was dark brown to black, while remaining portion was light brown in colour.

Table 1. Measurement of different morphological structures of *C. gloeosporioides* from sweet orange

Sr. No.	Morphological structure	Measurement parameters (µm)			
		Length	Width	L:W ratio	No./unit
1.	Mycelium	-	4.04 (1.50-6.01)	-	-
2	Acervulus	190.39 (120.24-344.05)	114.76 (57.87-180.04)	1.67 (1.12-2.18)	-
3.	Setae	78.31 (43.08-136.32)	2.62 (1.5-3.84)	-	4.2* (2-8)
4.	Conidia	11.46 (9.185-13.193)	4.72 (4.34-6.84)	2.47 (1.44-3.03)	-
5.	Conidiophore	53.52 (33.73-75.81)	-	-	-
6.	Chlamydospores	-	6.54 (5.01-9.018)	-	-

(Figures in parenthesis indicate range of measurement).

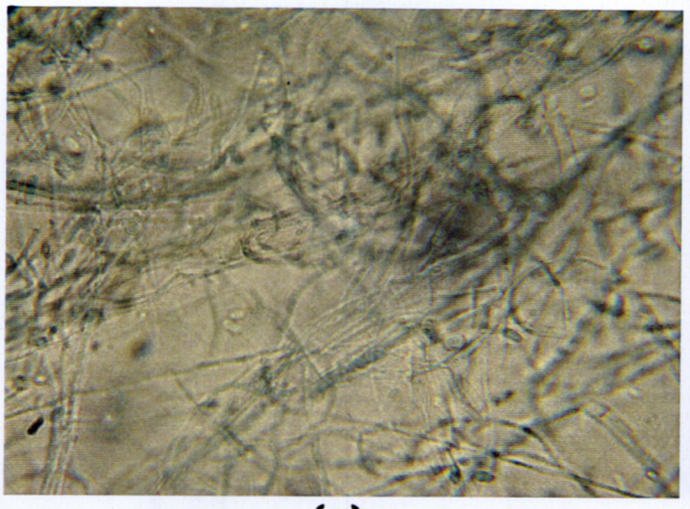
Where :

: Not applicable/not observed

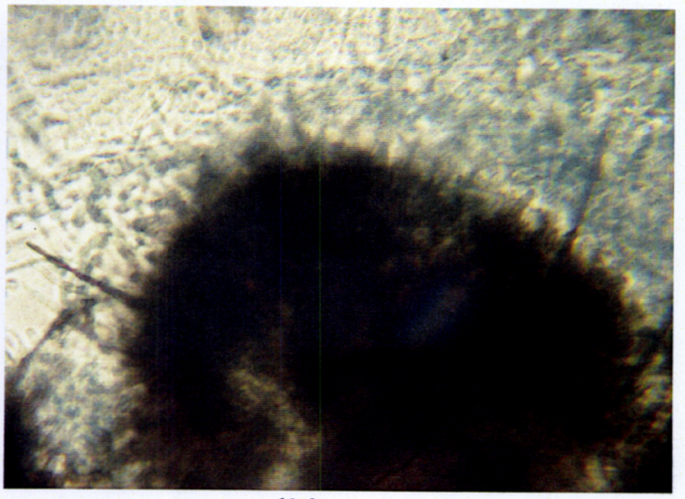
: No. of setae per acervulus

Setae

Setae (Plate 7b) were irregularly arranged throughout the acervulus in the culture. They were light brown to dark brown, septate (2-8), stiff, straight or bending. The setae were



(a)



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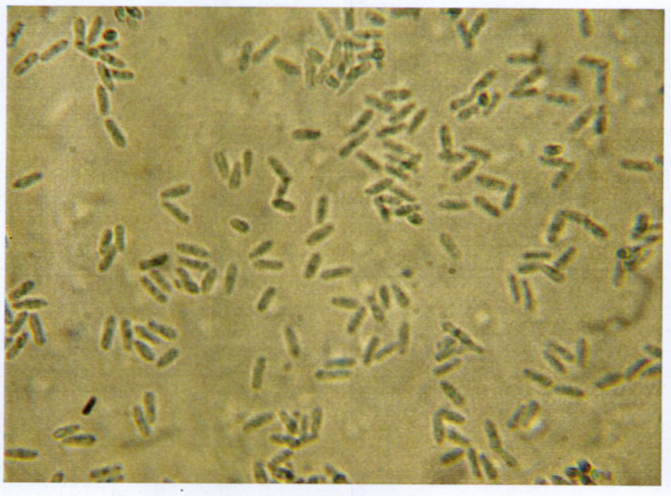

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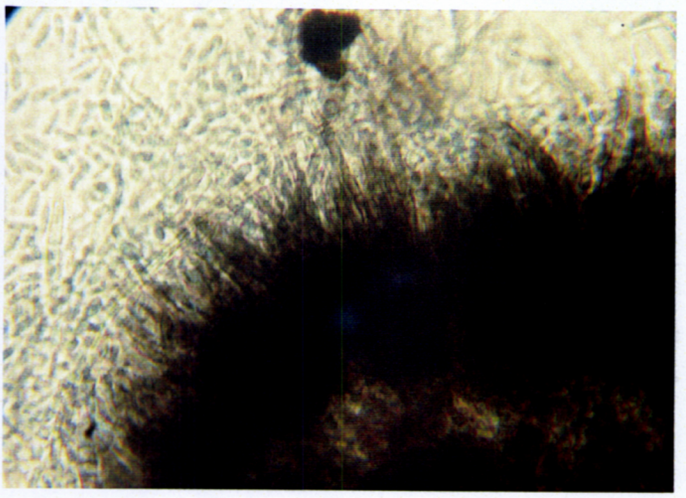

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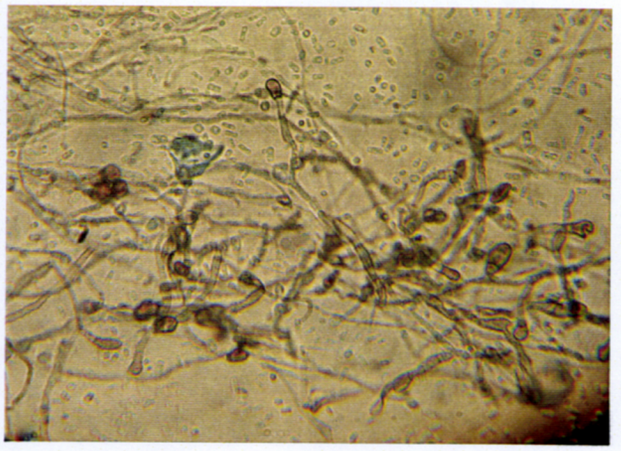


(e)

Plate 7. **Morphostructures of *C. gloeosporioides* causing post harvest anthracnose of sweet orange cv. Mosambi; a = Mycelium; b = Acervulus with setae; c = Conidia, d = Acervulus with conidiophores and conidia; e = Chlamydospores**

wider at the base and tapering towards tip. They were varying in length which measured 78.31 µm (43.08-136.32 µm) and width 2.62 µm (1.5-3.84 µm).

Conidia

The conidia (Plate 7c) were oblong to cylindrical in shape with rounded ends. They were single celled, hyaline when single, but light brown in muscilagenous masses or in acervuli. On an average, they measured 11.46 µm (9.185-13.193 µm) in length and 4.72 µm (4.34-6.84 µm) in width. The length width ratio was 2.47 µm.

Conidiophores

The conidiophores (Plate 7d) were short, septate, simple, thickly arranged and were hyaline when young, but turned very light brown to ashy brown when older. They were broader at base, while became quite narrow at tips and measured about 53.52 µm (33.73-75.81 µm) in width.

Chlamydospores

The chlamydospores (Plate 7e) noticed in the culture after 20 days were globose or ovate, thick walled, intercalary in chain or terminal and yellowish-brown to ashy-brown in colour. They measured 6.54 µm (5.01-9.018 µm) in diameter.

4.3.2 Morphology of *P. digitatum*

Mycelium

The fungus *Penicillium digitatum* produced olive green coloured mycelial growth (Plate 8a) on potato dextrose agar. The mycelium was branched, septate and hyaline. It measured 4.44 µm (2.84 to 5.18 µm) in width.

Conidiophore

The conidiophore (Plate 8b) were typically very short, smooth walled, arising from submerged hyphae or from basal mycelial felt singly or less often in synnemata branched near the apex to form brush like conidia bearing apparatus, ending in phialides which bear conidia in chains. The conidiophores measured about 38.58 µm (33.07-48.26 µm) in width.

Table 2. Measurement of different morphological structures of *P. digitatum* from sweet orange

Sr. No.	Morphological structure	Measurement parameters (µm)	
		Length	Width
1.	Mycelium	-	4.44 (2.84-5.18)
2.	Conidia	4.33 (3.674-5.01)	3.31 (3.006-3.674)
3.	Phialides (Sterigmata)	19.96 (17.20-22.88)	3.50 (2.50-5.01)
4.	Conidiophore	38.58 (33.07-48.26)	-

(Figures in parenthesis indicate range of measurement).

Where : - : Not applicable/not observed

Phialides

Phialides or sterigmata (Plate 8b) were equally variable usually producing chains of elliptical conidia, but occasionally terminating in swollen vesicular cells and measured about 19.96 µm (17.20-22.88 µm) x 3.50 µm (2.50- 5.01 µm).

Conidia

Conidia (Plate 8c) were smooth walled, dull dark green in mass, varying greatly in form and dimensions, ranging from subglobose to long cylindrical in shape, but usually elliptical and

measured about 4.33 µm (3.674-5.01 µm) x 3.31 µm (3.006-3.674 µm).

4.4 Growth and cultural characters

4.4.1 Growth and cultural characters of *C. gloeosporioides*

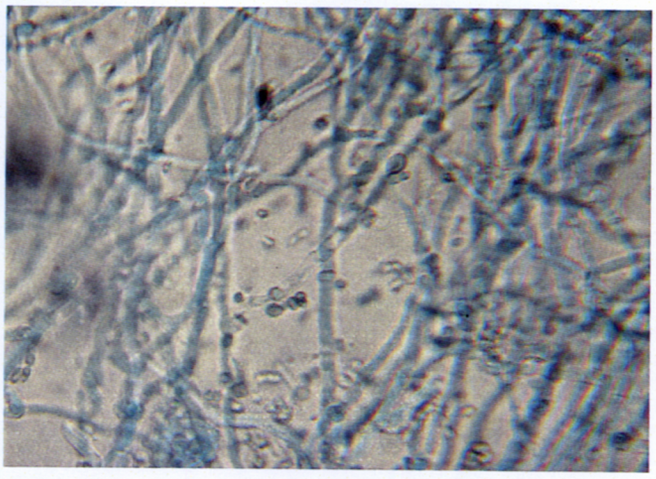
The growth and colony character of the fungus on different synthetic and non-synthetic media are presented in Table 3. The treatment differences in respect of colony diameter at 48 hours interval, growth rate and spore count were statistically significant. Different synthetic and non-synthetic media evaluated for growth characters exhibited varying degree of growth rates, mean colony diameter and spore count.

Growth

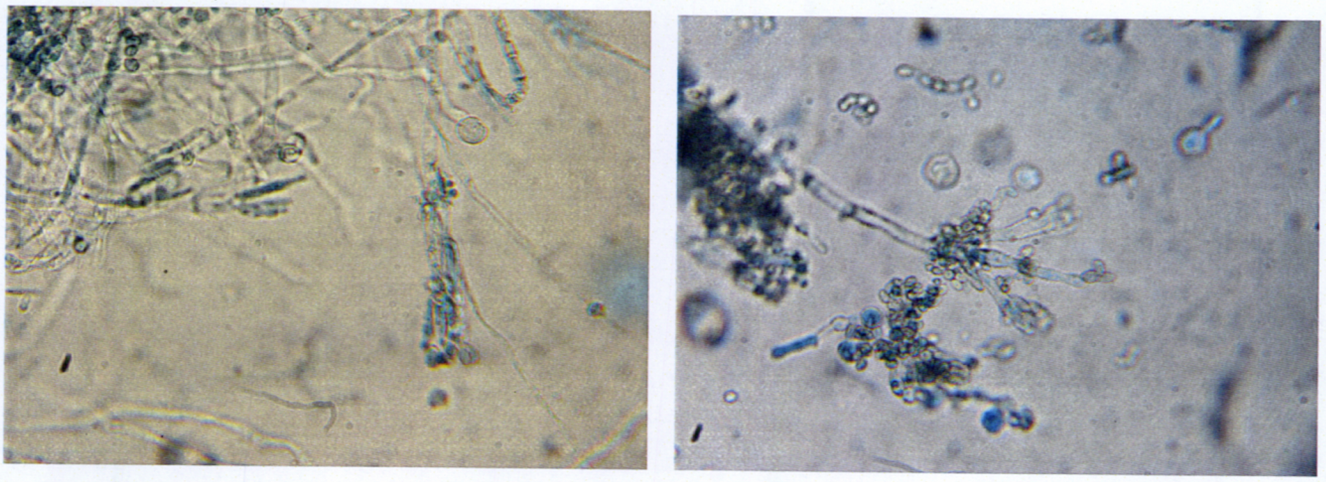
Growth attained on different synthetic and non-synthetic media is illustrated in Plate 9. The maximum mean growth rate of 0.47 mm hr^{-1} was recorded on Czapek's dox agar and potato dextrose agar followed by Asthana Hawkers, nutrient agar, Richards agar, Sabourauds agar and M_2 agar. Maximum colony diameter was observed on Czapek's dox agar as 9.10 cm diameter (Fig. 1) followed by Asthana Hawkers, potato dextrose agar, Sabourauds agar, nutrient agar and Richard's agar media.

Sporulation

Significantly highest spore count (0.78 x 10^4 conidia cm^{-2}) was recorded in potato dextrose agar followed by M_2 agar, Nutrient agar and Asthana Hawkers. The fungus did not sporulate on Czapek's dox agar media.



(a)



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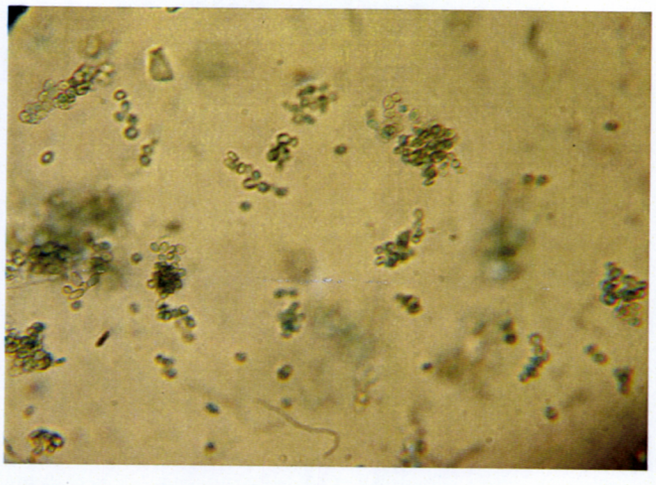

(c)

Plate 8. **Morphostructures of *P. digitatum* causing post harvest green mould of sweet orange cv. Mosambi; a = Mycelium; b = Conidiophores with phialides bearing conidia; c = Conidia**

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

Sr. No.	Name of agar media	Colony diameter (cm) and growth rate (mm h^{-1})					Growth	Sporulation		Colony characters			
		48	96	144	192	Mean growth rate		Count ($x10^4$ cm^{-2})	Degree	Colour	Margin	Shape	Nature
1.	Asthana Hawker's	3.4 (0.70)	6.7 (0.69)	8.6 (0.39)	9.0 (0.08)	0.46	++++	0.60	+++	White	Entire	Circular	Submerged transparent mycelium with broad concentric ring and orange coloured fruiting bodies on surface.
2.	Czapek's Dox	3.08 (0.64)	6.41 (0.69)	8.96 (0.53)	9.10 (0.02)	0.47	++++	-	-	Cottony white	Entire	Circular	Aerial, raised mycelium, thick fungal mat with slight broad concentric ring
3.	Richard's	1.76 (0.36)	5.5 (0.77)	7.88 (0.49)	8.68 (0.16)	0.44	++++	0.51	++	Cottony white with orange shade	Entire	Circular	Aerial, raised mycelium with abundant orange coloured fruiting bodies on surface
4.	Sabouraud's	2.78 (0.57)	5.98 (0.66)	8.58 (0.54)	8.81 (0.04)	0.44	++++	0.44	++	White to light green	Entire	Circular	Sub aerial to sub merged mycelium without zone
5.	Nutrient	3.0 (0.62)	5.75 (0.57)	8.50 (0.57)	8.73 (0.04)	0.45	++++	0.62	+++	White with orange shade	Entire	Circular	Aerial, raised mycelium with slight concentric ring and orange coloured fruiting bodies.
6.	M_2	3.41 (0.71)	6.18 (0.57)	8.2 (0.42)	8.4 (0.04)	0.43	+++	0.68	+++	Orange to light brown	Entire	Roughly circular	Sub aerial to submerged mycelium with slight concentric ring
7.	Ashby's	2.18 (0.45)	4.51 (0.48)	5.68 (0.24)	6.98 (0.27)	0.36	++	0.22	+	Dull white	Entire	Roughly circular	Sub aerial to submerged sparse mycelial growth

Table 3. contd...

Sr. No.	Name of agar media	Colony diameter (cm) and growth rate (mm h^{-1})					Growth	Sporulation		Colony characters			
		48	96	144	192	Mean growth rate		Count ($x10^4$ cm^{-2})	Degree	Colour	Margin	Shape	Nature
8.	P.D.A	3.12 (0.65)	5.8 (0.56)	8.15 (0.49)	8.95 (0.16)	0.47	++++	0.78	++++	White with orange shade	Entire	Circular	Aerial, raised mycelial growth with orange coloured fruiting bodies irregularly scattered over surface
9.	Corn meal	1.58 (0.32)	3.61 (0.42)	5.25 (0.34)	6.05 (0.16)	0.31	++	0.10	+	Ashy white	Entire	Circular	Submerged, scanty mycelium without concentric ring
10.	Wheat meal	2.47 (0.51)	4.78 (0.35)	6.81 (0.42)	7.51 (0.14)	0.35	+++	0.11	+	Dull white	Entire	Circular	Sub aerial to submerged mycelium with orange to brown bodies and slight concentric ring
11.	Prunne	2.13 (0.44)	4.71 (0.54)	7.06 (0.49)	8.00 (0.19)	0.41	+++	0.17	+	Dull white to grey	Entire	Circular	Aerial, raised mycelium without concentric ring
12.	Tap water	1.83 (0.38)	3.63 (0.37)	5.33 (0.35)	6.01 (0.14)	0.31	+	0.006	+	Dull white	Entire	Circular	Totally submerged very sparse mycelial growth
	S.E. ±	0.19	0.21	0.20	0.15	0.11		0.01					
	CD at 5 %	0.56	0.62	0.58	0.45	0.32		0.03					

(Figures in parenthesis are growth rates)

Where + = Scanty, ++ = Moderate, +++ = Good, ++++ = Abundant/profuse, - = Nil

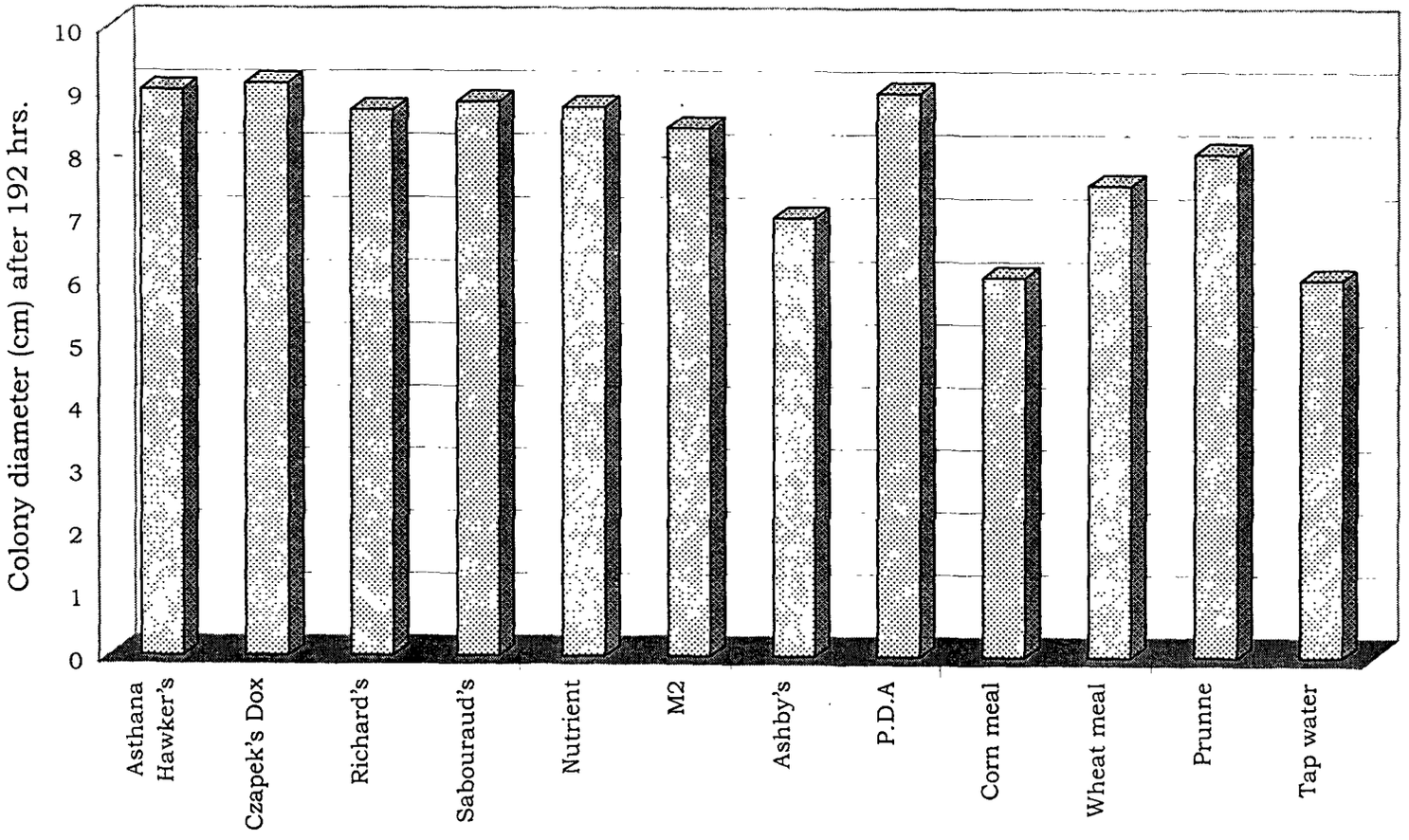

Fig. 1. Colony diameter of *C. gloeosporioides* on different synthetic and non-synthetic media

4.4.2 Growth and cultural characters of *P. digitatum*

The growth and colony diameter of the fungus on different synthetic and non-synthetic media are presented in Table 4. The treatment difference in respect of colony diameter at 48 hours interval as well as spore count and growth rate were statistically significant. Different synthetic and non-synthetic media evaluated for growth characters exhibited varying degree of growth rates, mean colony diameter and spore count.

Growth

Growth attained on different synthetic and non-synthetic media is illustrated in Plate 10. The maximum mean growth rate of 0.29 mm hr^{-1} was recorded on potato dextrose agar followed by Prunne agar. Maximum colony diameter was observed on potato dextrose agar as 7.31 cm (Fig. 2). The fungus did not grow on Czapek's dox agar, M_2 agar, Ashbys agar, corn meal agar, wheat meal agar and tap water agar media.

Sporulation

Highest sporulation (0.56 x 10^4 conidia cm^{-2}) was recorded in potato dextrose agar media, while the media nutrient agar also showed good sporulation. The fungus did not sporulate on Asthana Hawker, Czapek's dox, Richards, M_2, Ashbys, corn meal, wheat meal, Prunne and Tap water agar media.

Table 4. Colony diameter, growth rate, sporulation and characters of *P. digitatum* on different synthetic and non-synthetic media

Sr. No.	Name of agar media	Colony diameter (cm) and growth rate (mm h^{-1})						Growth	Sporulation		Colony characters			
		48	96	144	192	240	Mean growth rate		Count ($x10^4$ cm^{-2})	Degree	Colour	Margin	Shape	Nature
1.	Asthana Hawker's	0.6 (0.12)	0.8 (0.04)	0.96 (0.03)	1.26 (0.06)	1.4 (0.02)	0.05	+	-	-	White	Entire	Roughly circular	Totally submerged, very sparse mycelial growth
2.	Czapek's dox	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	Richard's	-	0.68 (0.14)	0.86 (0.04)	1.08 (0.04)	1.26 (0.04)	0.05	+	-	-	White	Entire	Circular	Totally submerged, very sparse white coloured mycelial growth
4.	Sabouraud's	2.3 (0.5)	2.6 (0.06)	2.83 (0.05)	3.03 (0.04)	3.26 (0.05)	0.14	++	0.14	+	Green	Entire	Roughly circular	Green coloured, slightly raised mycelial growth surrounded by white mycelial zone at advancing margin
5.	Nutrient	1.4 (0.3)	1.95 (0.10)	2.46 (0.1)	2.98 (0.1)	3.5 (0.1)	0.14	++	0.40	+++	Dark green	Entire	Circular	Dark green coloured, slightly raised mycelial growth surrounded by mycelial zone at advancing margin
6.	M_2	-	-	-	-	-	-	-	-	-	-	-	-	-
7.	Ashby's	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4. contd...

Sr. No.	Name of agar media	Colony diameter (cm) and growth rate (mm h^{-1})						Growth	Sporulation		Colony characters			
		48	96	144	192	240	Mean growth rate		Count ($x10^4$ cm^{-2})	Degree	Colour	Margin	Shape	Nature
8.	P.D.A	5.3 (1.1)	5.9 (0.12)	6.41 (0.1)	6.93 (0.1)	7.31 (0.07)	0.29	++++	0.56	++++	Green	Entire	Roughly circular	Green coloured slightly raised mycelial growth surrounded by white mycelial zone at advancing margin
9.	Corn meal	-	-	-	-	-	-	-	-	-	-	-	-	-
10.	Wheat meal	-	-	-	-	-	-	-	-	-	-	-	-	-
11.	Prunne	4.05 (0.8)	4.68 (0.13)	5.23 (0.11)	5.61 (0.08)	6.03 (0.09)	0.24	+++	-	-	Dirty white	Entire	Circular	Totally submerged, very sparse dirty white coloured mycelial growth
12.	Tap water	-	-	-	-	-	-	-	-	-	-	-	-	-
	S.E. ±	0.03	0.03	0.02	0.02	0.03	0.06		0.004					
	CD at 5 %	0.11	0.11	0.08	0.07	0.10	0.18		0.01					

(Figures in parenthesis are growth rates)

Where + = Scanty, ++ = Moderate, +++ = Good, ++++ = Abundant/profuse, - = Nil

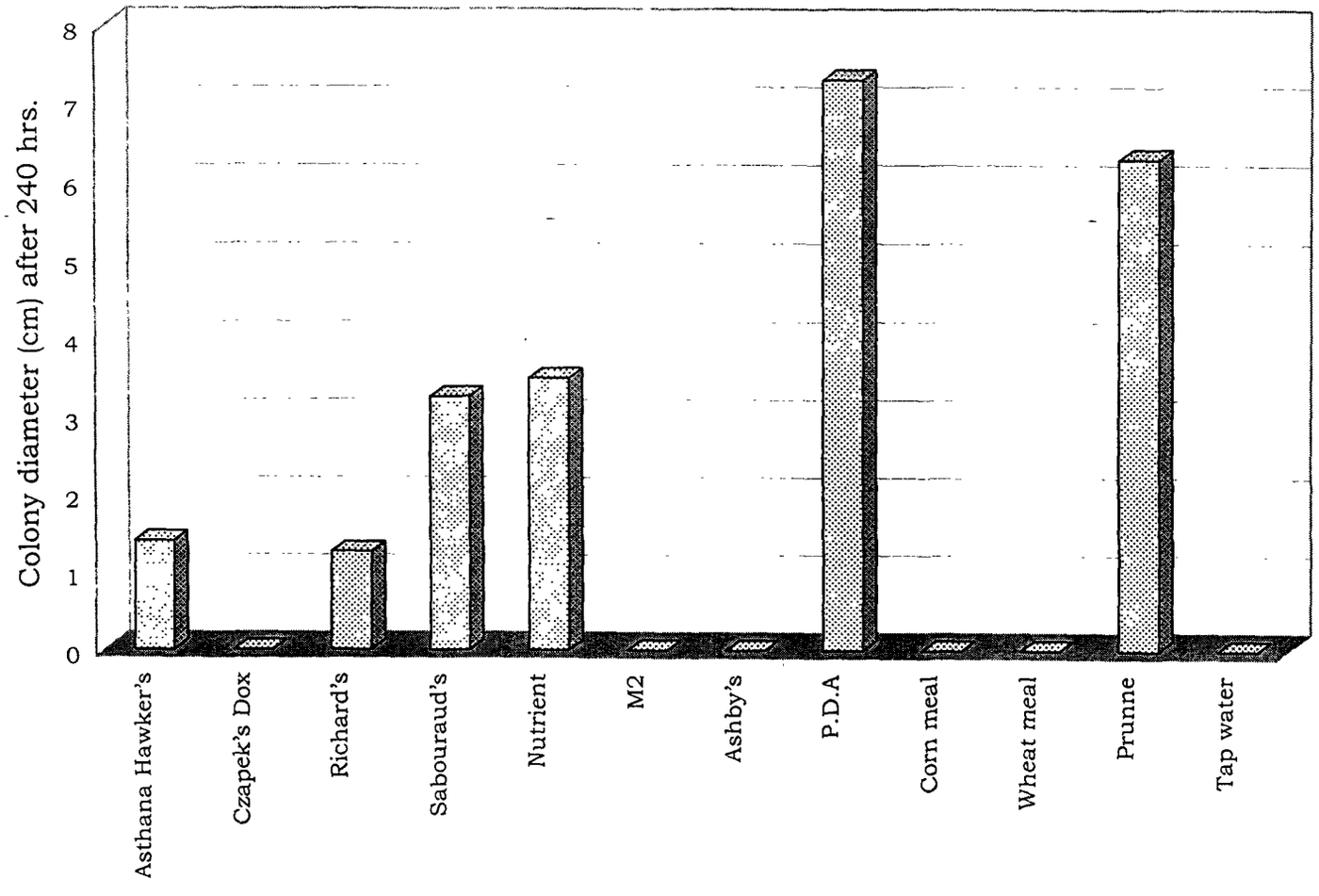

Fig. 2. Colony diameter of *P. digitatum* on different synthetic and non-synthetic media

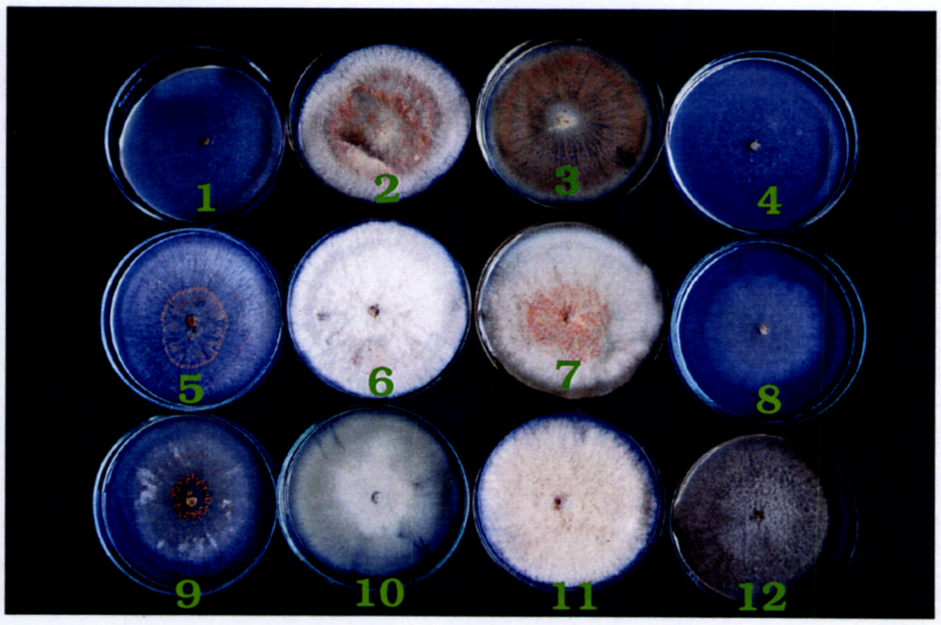

Plate 9. **Effect of different synthetic and non-synthetic media's on growth and sporulation of *C. gloeosporioides*; 1. Tap water agar; 2. Nutrient agar; 3. M_2 agar; 4. Ashby's agar; 5. Asthana Hawker; 6. Czapek's Dox; 7. Potato dextrose agar; 8. Corn meal agar; 9. Wheat meal agar; 10. Sabouraud's agar; 11. Richards agar; 12. Prunne agar**

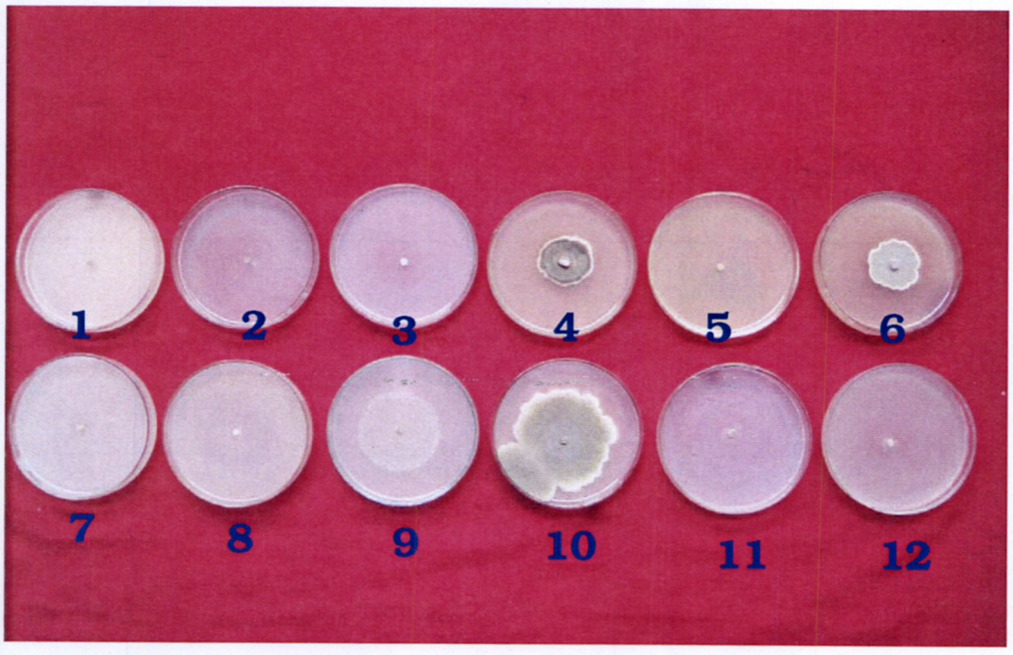

Plate 10. **Effect of different synthetic and non-synthetic media's on growth and sporulation of *P. digitatum*; 1. Ashby's agar; 2. Tap water agar; 3. Czapek's Dox; 4. Nutrient agar; 5. M_2 agar; 6. Sabouzraud's agar; 7. Corn meal agar; 8. Wheat meal agar; 9. Prunne agar; 10. Potato dextrose agar; 11. Asthana Hawker; 12. Richards agar**

4.5 Physiological studies

4.5.1 Effect of temperature on growth and sporulation of *C. gloeosporioides*

The studies were carried out to know the effect of different temperatures and assess the temperature requirements of *Colletotrichum gloeosporioides* for growth and sporulation. The growth and colony character of fungus at different temperatures revealed significant differences and the data presented in Table 5. The temperatures evaluated for growth characters, colony diameter and the sporulation at different temperatures is illustrated in Plate 11.

The fungus could grow between the temperatures of 10 to 30 ^{0}C, whereas no growth and sporulation was observed at 0 and 5 ^{0}C (Fig. 3). The maximum growth was observed at 25 ^{0}C with maximum colony diameter of 8.95 cm, while maximum sporulation was observed at 25 and 30 ^{0}C. The optimum temperature range for growth and sporulation was observed between 25 and 30 ^{0}C. Scant growth and sporulation was observed at temperatures 10 and 15 ^{0}C.

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

Table 5. Effect of different temperatures on growth and sporulation of *Colletotrichum gloeosporioides* on solid media

Sr. No.	Temperature (°C)	Mean colony diameter after 7 days (cm)	Sporulation	Colony character			
				Topography	Type of margin	Colony colour	Growth character
1.	0	0.00	-	-	-	-	-
2.	5	0.00	-	-	-	-	-
3.	10	2.85	+	Raised	Circular	White	White, raised, mycelial growth towards periphery
4.	15	3.30	+	Raised	Circular	White	White, raised, mycelial growth towards periphery
5.	20	8.25	++	Slightly raised	Circular	White	Aerial, slightly raised mycelial growth
6.	25	8.95	+++	Raised	Circular	White	White, raised, mycelial growth towards periphery
7.	30	8.33	+++	Raised	Circular	White	White, raised, mycelial growth towards periphery
	S.E. ±	0.08					
	C.D. at 5 %	0.25					

Where, +++ = Good sporulation, ++ = Moderate sporulation, + = Scanty sporulation, - = No sporulation

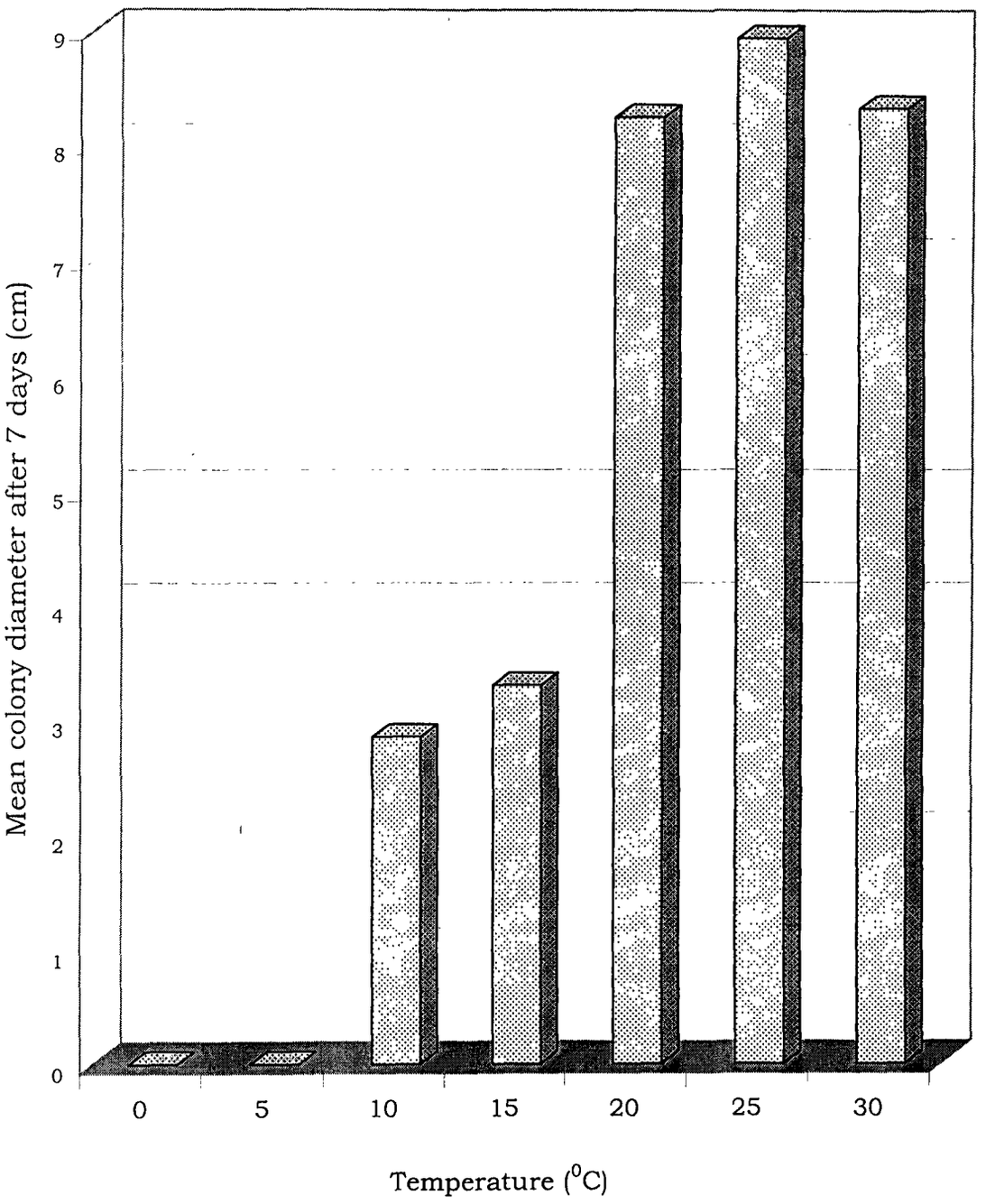

Fig. 3. Effect of different temperatures on growth of *Colletotrichum gloeosporioides* on solid media

4.5.2 Effect of temperatures on growth and sporulation of *P. digitatum*

The studies were carried out to know the effect of different temperatures and assess the temperature requirements of *Penicillium digitatum* for growth and sporulation. The growth and colony characters of fungus at different temperatures revealed significant differences and the data presented in Table 6. The temperatures evaluated for growth characters, colony diameter and the sporulation attained at different temperatures is illustrated in Plate 12.

The fungus could grow between the temperatures 20 to 30 ^{0}C, whereas no growth and sporulation was observed at 0, 5, 10 and 15 ^{0}C (Fig. 4). The maximum growth was observed at 25 ^{0}C with maximum colony diameter of 7.70 cm, while maximum sporulation was observed at 20 and 25 ^{0}C. The optimum temperature range for growth and sporulation was observed 20 to 25 ^{0}C. Scant growth and sporulation was observed at temperature of 30 ^{0}C.

Thus, the temperature range for maximum growth and sporulation of the fungus *Penicillium digitatum* was observed to be ranging between 20 to 25 ^{0}C.

Table 6. Effect of different temperatures on growth and sporulation of *Penicillium digitatum* on solid media

Sr. No.	Temperature (°C)	Mean colony diameter after 7 -days (cm)	Sporulation	Colony character			
				Topography	Type of margin	Colony colour	Growth character
1.	0	0.00	-	-	-	-	-
2.	5	0.00	-	-	-	-	-
3.	10	0.00	-	-	-	-	-
4.	15	0.00	-	-	-	-	-
5.	20	7.45	+++	Slightly raised	Roughly circular	Green	Green coloured, slightly raised mycelial growth with white mycelial zone at advancing margin
6.	25	7.70	+++	Slightly raised	Roughly circular	Green	Green coloured, slightly raised mycelial growth with white mycelial zone at advancing margin
7.	30	4.08	+	Flat	Roughly circular	Green	Green coloured, flat mycelial growth with white mycelial zone at advancing margin
	S.E. ±	0.06					
	C.D. at 5 %	0.16					

Where, +++ = Good sporulation, ++ = Moderate sporulation, + = Scanty sporulation, - = No sporulation

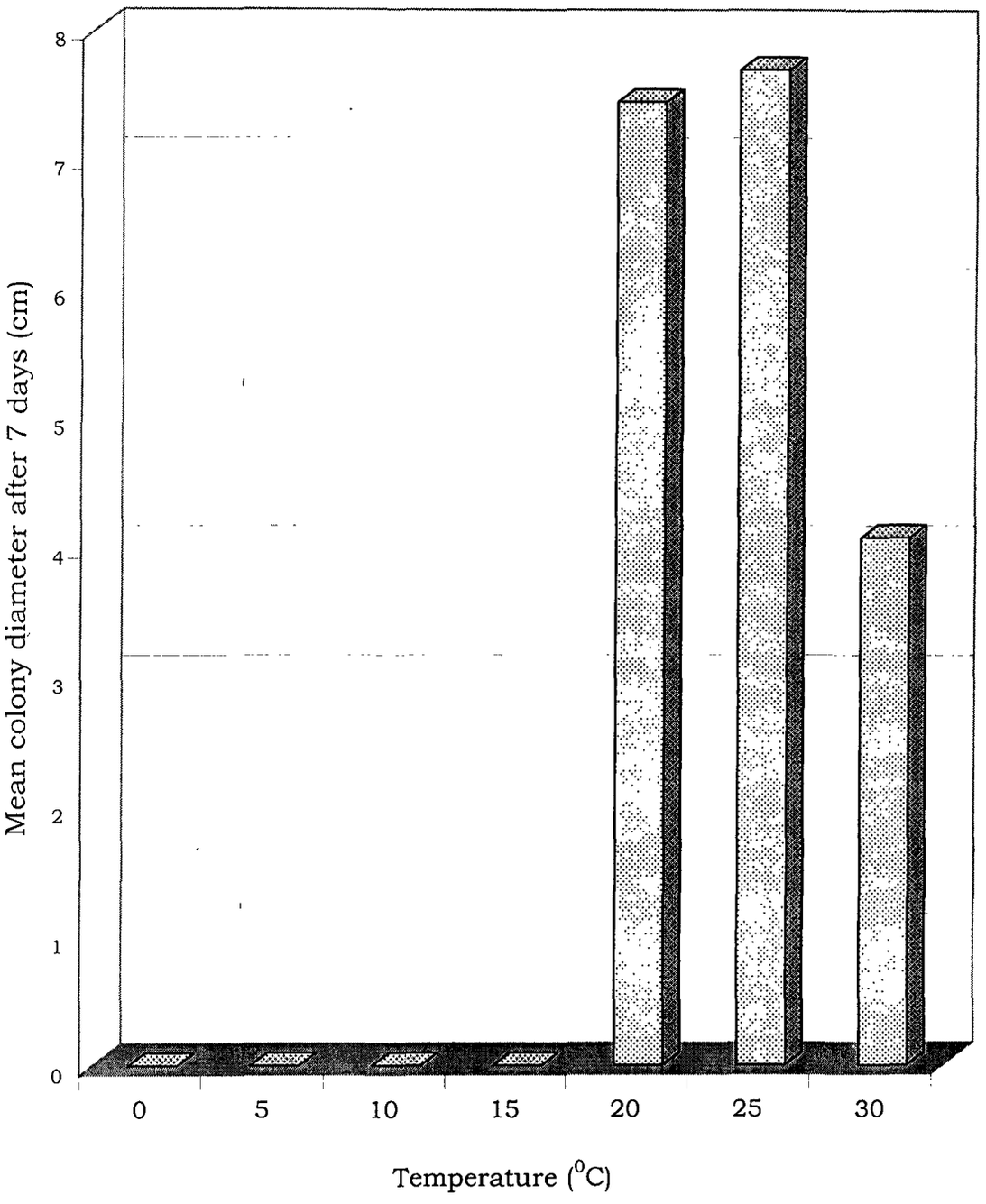

Fig. 4. Effect of different temperatures on growth of *Penicillium digitatum* on solid media

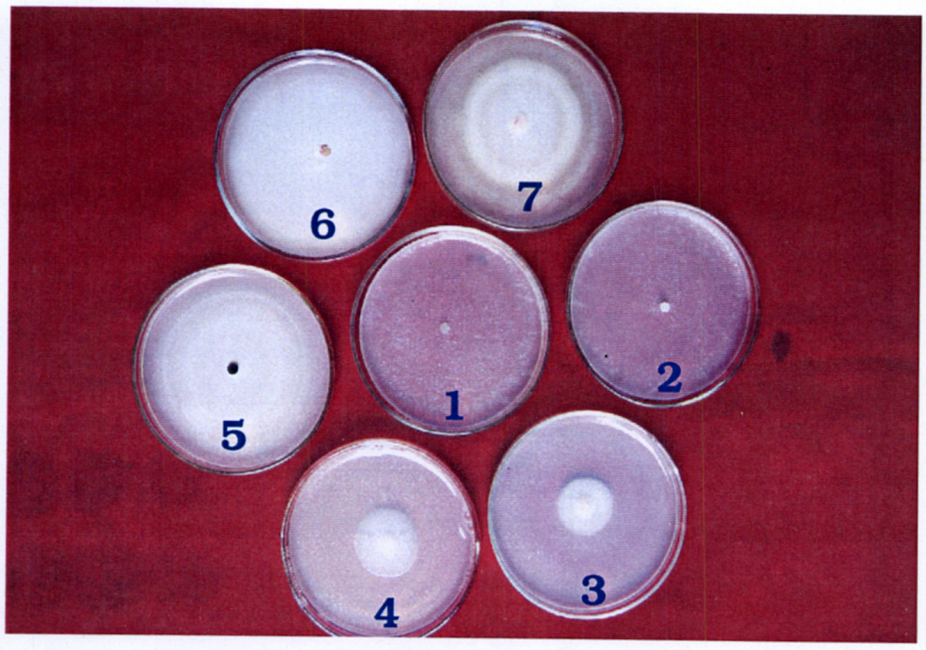

Plate 11. **Growth and sporulation of *C. gloeosporioides* at different temperatures; 1. 0°C; 2. 5°C; 3. 10°C; 4. 15°C; 5. 20°C; 6. 25°C; 7. 30°C**

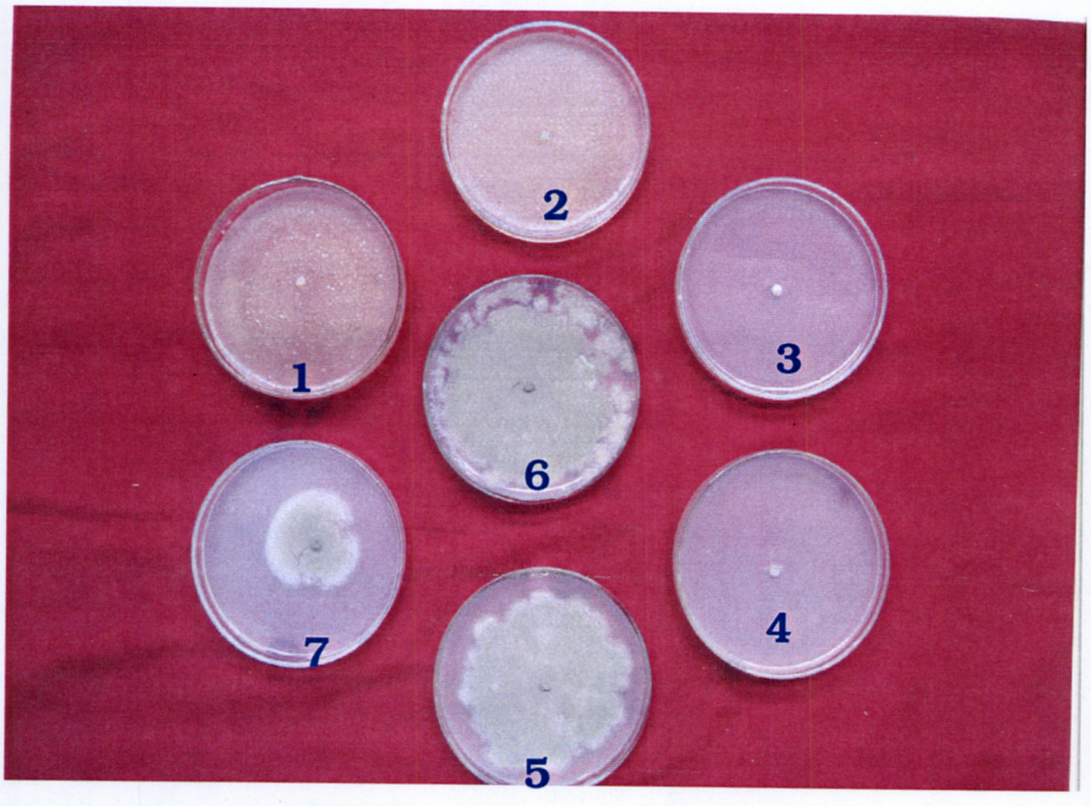

Plate 12. **Growth and sporulation of *P. digitatum* at different temperatures; 1. 0°C; 2. 5°C; 3. 10°C; 4. 15°C; 5. 20°C; 6. 25°C; 7. 30°C**

4.6 Evaluation of control measures

4.6.1 *In vitro* evaluation of fungicides

4.6.1.1 *In vitro* evaluation of fungicides against *C. gloeosporioides*

The results of evaluation of fungicides against *Colletotrichum gloeosporioides in vitro* are presented in Table 7. Significant differences were observed amongst different fungicides evaluated. All the fungicidal treatments showed significantly low colony diameter as compared to control treatments in the observations recorded after 7 days.

It was observed that among the nine fungicides tried, hexaconazole (0.1 %) and propiconazole (0.1 %) completely inhibited the growth of fungus followed by 0.1 % difenconazole (91.96 %). As regards the sporulation, scanty sporulation was observed in chlorothalonil, captan, mancozeb and carbendazim. The fungicides copper oxychloride, hexaconazole, propiconazole, difenconazole and iprodione (25 %) + carbendazim (25 %) inhibited sporulation completely. In case of control, good growth of fungus and sporulation was observed (Plate 13).

The fungicides *viz.*, captan, iprodione (25 %) + carbendazim (25 %), copper oxychloride, chlorothalonil and appeared mancozeb to be less effective in inhibiting the fungus, while carbendazim failed to inhibit the fungus growth.

The results indicated that the fungicides hexaconazole 0.1 %, propiconazole 0.1 % and difenconazole 0.1 % inhibited growth and sporulation (Fig. 5) of *Colletotrichum gloeosporioides* under *in vitro* conditions.

Table 7. Effect of different fungicides on growth and sporulation of *Colletotrichum gloeosporioides* on solid media

Sr. No.	Fungicides	Conc. (%)	Mean colony diameter after 7 days (cm)	Per cent inhibition	Sporu-lation	Colony character			
						Topography	Type of margin	Colony colour	Growth character
1.	Chlorothalonil	0.25	4.61	49.23	+	Flat	Circular	White	Slow, whitish, flat mycelial growth spreading slowly
2.	Captan	0.25	4.23	53.41	+	Slightly raised	Circular	White	Slow, whitish, slightly raised, mycelial growth towards periphery
3.	Copper oxychloride	0.25	4.41	51.43	-	Flat	Circular	White	Slow, whitish flat mycelial growth spreading slowly
4.	Mancozeb	0.25	5.03	44.60	+	Raised	Circular	White	Slow, whitish, raised mycelial growth, spreading slowly
5.	Carbendazim	0.1	7.10	21.80	+	Slightly raised	Roughly circular	White	Fast, thick, luxuriant mycelial growth spreading uniformly
6.	Hexaconazole	0.1	-	100	-	-	-	-	-
7.	Propiconazole	0.1	-	100	-	-	-	-	-

Table 7. contd...

Sr. No.	Fungicides	Conc. (%)	Mean colony diameter after 7 days (cm)	Per cent inhibition	Sporu-lation	Colony character			
						Topography	Type of margin	Colony colour	Growth character
8.	Difenconazole	0.1	0.73	91.96	-	Raised	Circular	White	White growth, restricted around inoculated mycelial block
9.	Iprodione 25 % + carbendazim 25 %	0.25	4.25	53.19	-	Slightly raised	Roughly circular	White	Slow, whitish, slightly raised mycelial growth spreading slowly
10.	Control	-	9.08	-	+++	Raised	Circular	White	Full, thick, luxuriant, white mycelial growth spreading rapidly towards periphery covering completely in 7 days
	S.E. ±		0.13	-					
	C.D. at 5 %	-	0.38	-					

Where, +++ = Good sporulation, + = Scanty sporulation, - = No sporulation

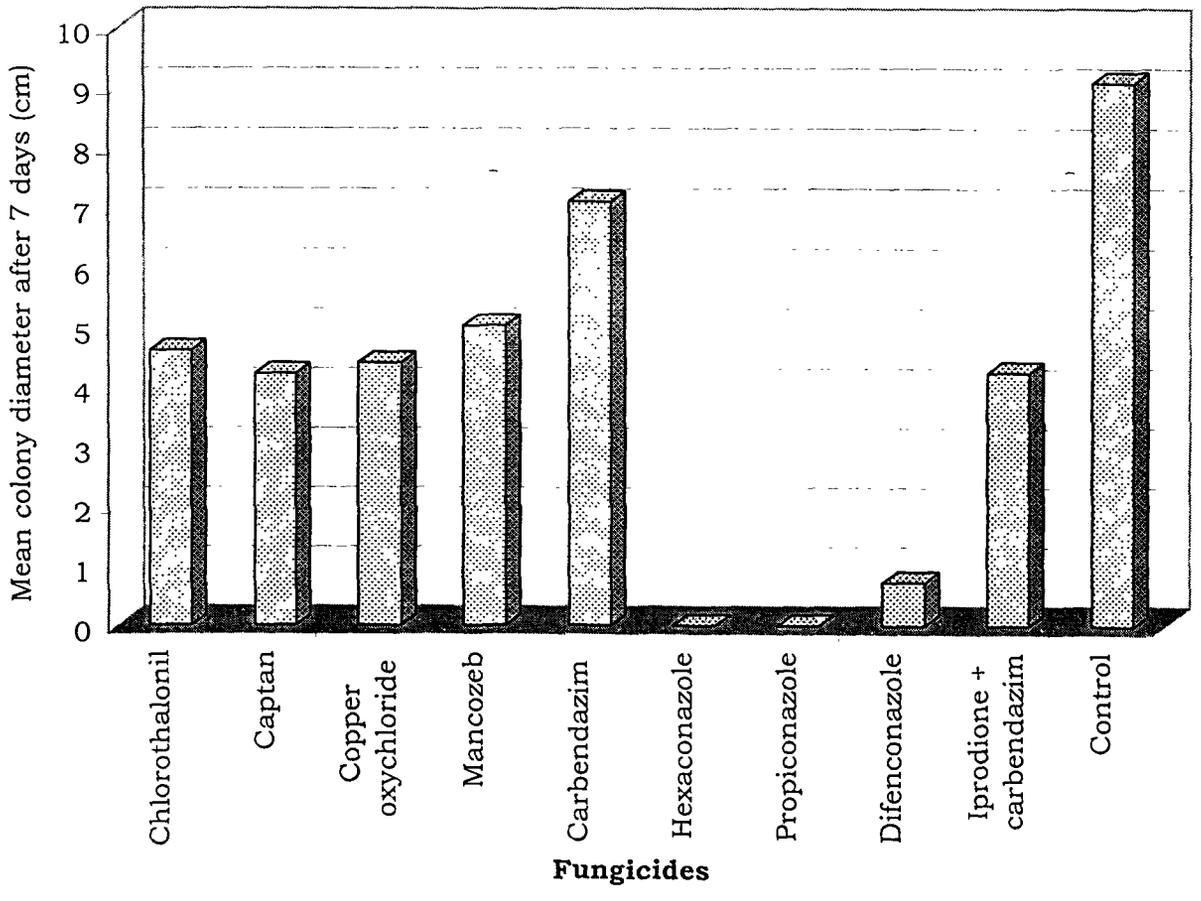

Fig. 5. Effect of different fungicides on growth of *Colletotrichum gloeosporioides* on solid media

4.6.1.2 *In vitro* evaluation of fungicides against *P. digitatum*

The results of evaluation of fungicides against *Penicillium digitatum in vitro* are presented in Table 8. Significant differences were observed amongst different fungicides evaluated. All the fungicides showed significantly low colony diameter as compared to control treatments in the observations recorded after 7 days.

It was observed that among the nine fungicides tried, chlorothalonil (0.25 %), captan (0.25 %), mancozeb (0.25 %), carbendazim (0.1 %), hexaconazole (0.1 %), propiconazole (0.1 %), difenconazole (0.1 %) and iprodione (25 %) + carbendazim (25 %) completely inhibited the growth of fungus (Plate 14).

As regards the sporulation, no sporulation was observed in any fungicide. In case of control, good growth of fungus and sporulation was observed.

The fungicide copper oxychloride was less effective in inhibiting the fungus and inhibited 63.38 % growth of the fungus.

The results indicated that chlorothalonil 0.25 %, captan 0.25 %, mancozeb 0.25 %, carbendazim 0.1, hexaconazole 0.1 %, propiconazole 0.1 %, difenconazole 0.1 % and iprodione (25 %) + carbendazim (25 %) 0.25 % inhibited growth and sporulation of *Penicillium digitatum* (Fig. 6) under *in vitro* conditions.

Table 8. Effect of different fungicides on growth and sporulation of *Penicillium digitatum* on solid media

Sr. No.	Fungicides	Conc. (%)	Mean colony diameter after 7 days (cm)	Per cent inhibition	Sporu-lation	Colony character			
						Topography	Type of margin	Colony colour	Growth character
1.	Chlorothalonil	0.25	-	100	-	-	-	-	-
2.	Captan	0.25	-	100	-	-	-	-	-
3.	Copper oxychloride	0.25	2.38	63.38	-	Flat	Circular	Grey	Slowly, grey coloured mycelial growth spreading slowly
4.	Mancozeb	0.25	-	100	-	-	-	-	-
5.	Carbendazim	0.1	-	100	-	-	-	-	-
6.	Hexaconazole	0.1	-	100	-	-	-	-	-
7.	Propiconazole	0.1	-	100	-	-	-	-	-
8.	Difenconazole	0.1	-	100	-	-	-	-	-
9.	Iprodione 25 % + carbendazim 25 %	0.25	-	100	-	-	-	-	-
10.	Control	-	6.50	-	+++	Flat	Roughly circular	Green	Fast, greenish coloured mycelial growth with white mycelial zone at advancing margin
	S.E. ±	-	0.02	-					
	C.D. at 5 %	-	0.07	-					

Where, +++ = Good sporulation, + = Scanty sporulation, - = No sporulation

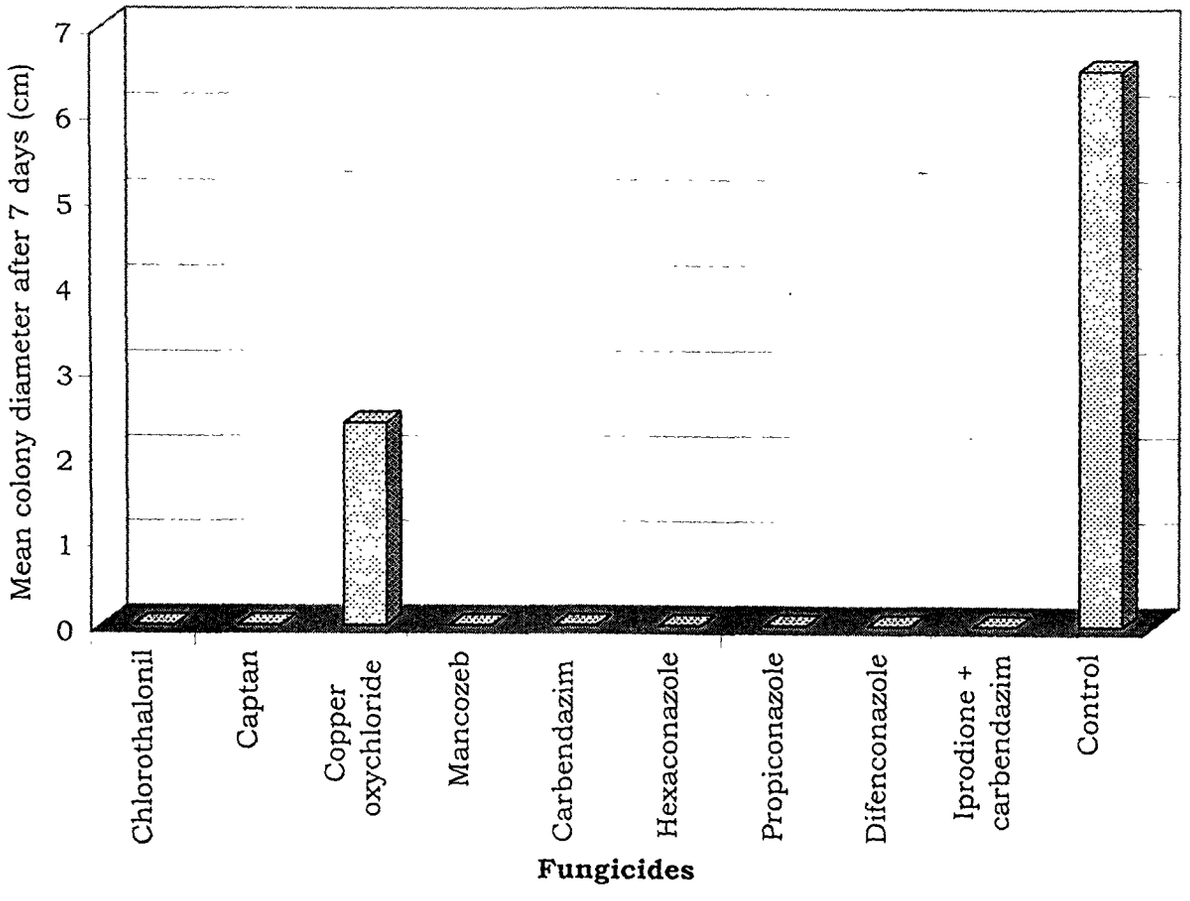

Fig. 6. Effect of different fungicides on growth of *Penicillium digitatum* on solid media

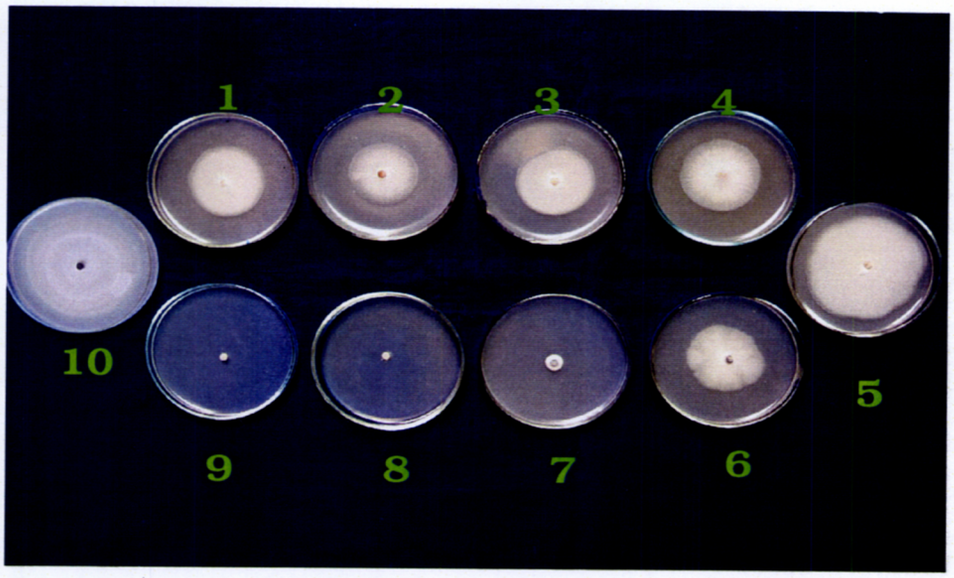

Plate 13. **Effect of fungicides on growth and sporulation of *C. gloeosporioides*; 1. Chlorothalonil 0.25 %; 2. Captan 0.25 %; 3. Copper oxychloride 0.25 %; 4. Mancozeb 0.25 %; 5. Carbendazim 0.1 %; 6. Quintol 0.25 %; 7. Difenconazole 0.1 %; 8. Propiconazole 0.1 %; 9. Hexaconazole 0.1 %; 10. Control**

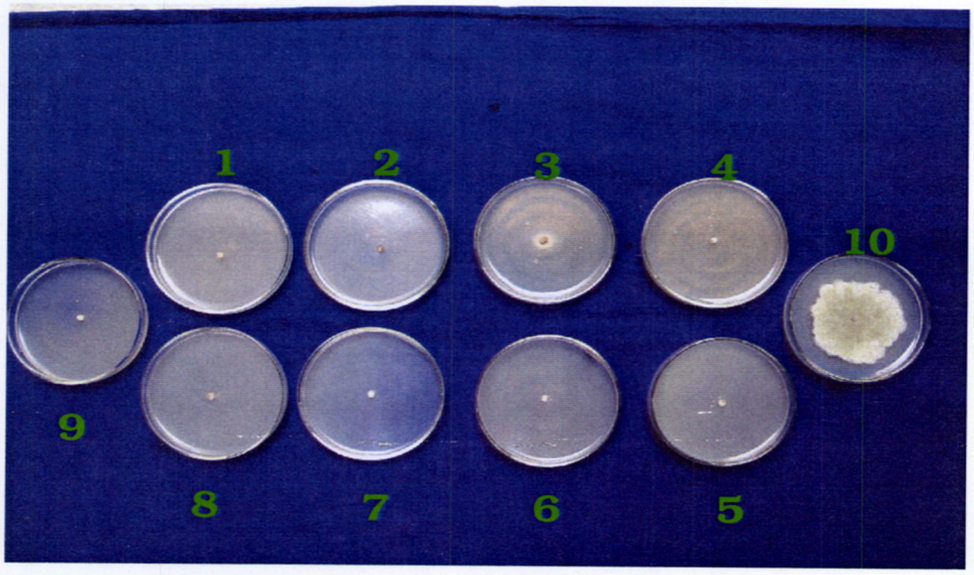

Plate 14. **Effect of fungicides on growth and sporulation of *P. digitatum*; 1. Chlorothalonil 0.25 %; 2. Captan 0.25 %; 3. Copper oxychloride 0.25 %; 4. Mancozeb 0.25 %; 5. Difenconazole 0.1 %; 6. Propiconazole 0.1 %; 7. Hexaconazole 0.1 %; 8. Carbendazim 0.1 %; 9. Quintol 0.25 %; 10. Control**

4.6.2 *In vitro* evaluation of plant extracts against

4.6.2.1 *In vitro* evaluation of plant extracts against C. *gloeosporioides*

The results of *in vitro* evaluation of botanicals (plant extracts) against *Colletotrichum gloeosporioides* are presented in Table 9. All the treatments showed significantly low colony diameter and sporulation as compared to control after seven days (Plate 15).

Amongst different botanicals tried against *Colletotrichum gloeosporioides Allium sativum* extract was observed most effective showing highest inhibition of growth (57.73 %) followed by *Polyantha longifolia, Acacia arabica* and *Lantana camera* having 55.00, 52.24 and 49.22 % inhibition, respectively (Fig. 7). *Vinca rosea, Nerium indicum, Ocimum sanctum* and *Azadirachta indica* showed less percentage of inhibition *viz.,* 36.47, 35.48, 34.59 and 33.16 %, respectively.

Moderate sporulation was observed in *Tagetes erecta,* while scanty sporulation was observed in *Azadirachta indica,* kahner and *Vinca rosea,* other botanicals did not support sporulation. Incase of control good growth and sporulation was observed.

The botanical *Tagetes erecta* failed to inhibit the growth of the fungus.

The result indicated *Allium sativum, Polyantha longifolia* and *Acacia arabica* extracts to be effective in inhibiting the growth of *Colletotrichum gloeosporioides* under *in vitro* conditions.

Table 9. Effect of different botanicals on *Colletotrichum gloeosporioides* in solid media

Sr. No.	Plant extract	Mean colony diameter after 7 days (cm)	Per cent inhibition	Sporulation	Colony character			
					Topography	Type of margin	Colony colour	Growth character
1.	*Azadirachta indica*	6.06	33.16	±	Raised	Circular	White	White, raised mycelial growth
2.	*Nerium indicum*	5.85	35.48	+	Raised	Circular	White	White, raised mycelial growth
3.	*Vinca rosea*	5.76	36.47	+	Raised	Circular	White	White, raised mycelial growth
4.	*Acacia arabica*	4.33	52.24	-	Slightly raised	Circular	White	White, raised mycelial growth
5.	*Allium sativum*	4.01	57.73	-	Raised	Circular	White	White, raised mycelial growth
6.	*Ocimum sanctum*	5.93	34.59	-	Raised	Roughly circular	White	White, raised mycelial growth
7.	Polyantha longifolia	4.08	55.00	-	Raised	Irregular	White	White, raised mycelial growth
8.	*Tagetes erecta*	7.00	22.73	++	Raised	Circular	White	White, raised mycelial growth
9.	*Lantana camera*	4.60	49.22	-	Slightly raised	Circular	White	White, slightly raised mycelial growth
10.	Control	9.06	-	+++	Raised	Circular	White	White, raised luxuriant mycelial growth
	S.E. ±	0.11						
	CD at 5 %	0.32						

Where, +++ = Good sporulation, ++ = Moderate sporulation, + = Scanty sporulation, - = No sporulation

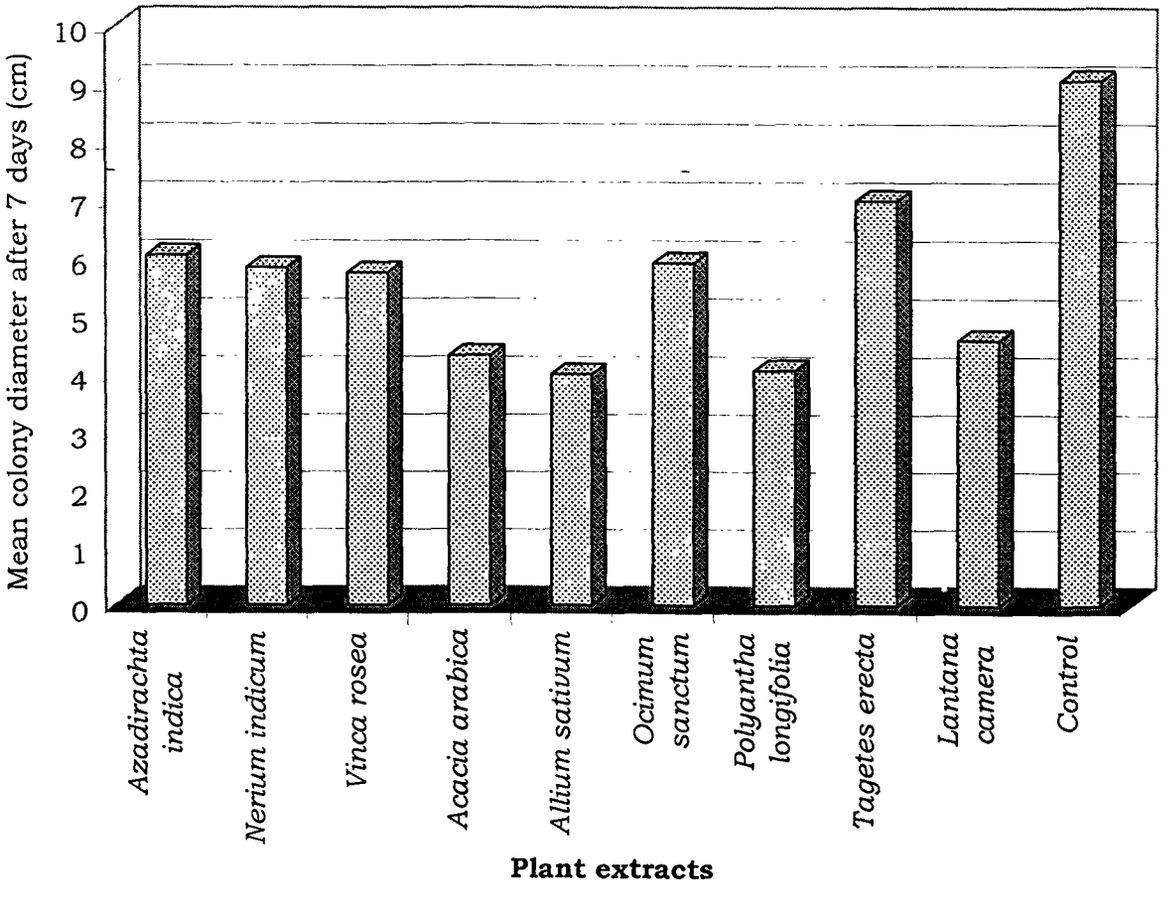

Fig. 7. Effect of different plant extracts on growth of *Colletotrichum gloeosporioides* on solid media

4.6.2.2 *In vitro* evaluation of plant extracts against *P. digitatum*

The results of *in vitro* evaluation of botanicals (plant extracts) against *Penicillium digitatum* are presented in Table 10. all the treatments showed significantly low colony diameter and sporulation as compared to control after seven days (Plate 16).

Amongst different botanicals tried against *Penicillium digitatum*, *Azadirachta indica* extract was observed most effective showing highest inhibition of growth (76.12 %), followed by *Ocimum sanctum*, *Acacia arabica*, *Nerium indicum* and *Lantana camera* having percentage of inhibition *viz.*, 51.80, 49.63, 47.03 and 45.44 %, respectively (Fig. 8). *Vinca rosea*, *Allium sativum* and *Polyantha longifolia* showed less percentage of inhibition *viz.*, 36.61, 35.89 and 34.00 %, respectively.

Moderate sporulation was observed in *Tagetes erecta*, while scanty sporulation was observed in *Vinca rosea*, *Allium sativum* and *Polyantha longifolia*. Other botanicals did not support sporulation. In case of control, good growth and sporulation was observed.

The botanical *Tagetes erecta* failed to inhibit the growth of the fungus.

The results indicated *Azadirachta indica*, *Ocimum sanctum* and *Acacia arabica* extracts to be effective in inhibiting the growth of *Penicillium digitatum* under *in vitro* conditions.

Table 10. Effect of different botanicals on *Penicillium digitatum* in solid media

Sr. No.	Plant extract	Mean colony diameter after 7 days (cm)	Per cent inhibition	Sporulation	Colony character			
					Topography	Type of margin	Colony colour	Growth character
1.	*Azadirachta indica*	1.65	76.12	-	Slightly raised	Circular	Green	Green, slightly raised mycelial growth with white thick mycelial zone at advancing margin
2.	*Nerium indicum*	3.66	47.03	-	Slightly raised	Circular	Green	Green, slightly raised mycelial growth with white mycelial zone at advancing margin
3.	*Vinca rosea*	4.38	36.61	+	Flat	Circular	Green	Green, flat mycelial growth with white mycelial zone at advancing margin
4.	*Acacia arabica*	3.48	49.63	-	Flat	Circular	Dull white	Dull, white coloured scanty mycelial growth with zone at advancing margin
5.	*Allium sativum*	4.43	35.89	+	Slightly raised	Circular	Green	Green, slightly raised mycelial growth with white thick mycelial zone at the advancing margin
6.	*Ocimum sanctum*	3.33	51.80	-	Flat	Circular	Green	Green, flat mycelial growth with white thin mycelial zone at advancing margin

Table 10 contd...

Sr. No.	Plant extract	Mean colony diameter after 7 days (cm)	Per cent inhibition	Sporulation	Colony character			
7.	*Polyantha longifolia*	4.56	34.00	+	Slightly raised	Circular	Green	Greenish, slightly raised mycelial growth, white mycelial zone at advancing margin
8.	*Tagetes erecta*	4.75	31.26	++	Flat	Circular	Green	Greenish, flat mycelial growth with white thick but loose mycelial zone at advancing margin
9.	*Lantana camera*	3.77	45.44	-	Flat	Circular	Green	Greenish flat mycelial growth with white mycelial zone at advancing margin
10.	Control	6.91	-	+++	Flat	Roughly circular	Green	Greenish, flat mycelial growth with white mycelial zone at advancing margin
	S.E. ±	0.10						
	CD at 5 %	0.31						

Where, +++ = Good sporulation, ++ = Moderate sporulation, + = Scanty sporulation, - = No sporulation

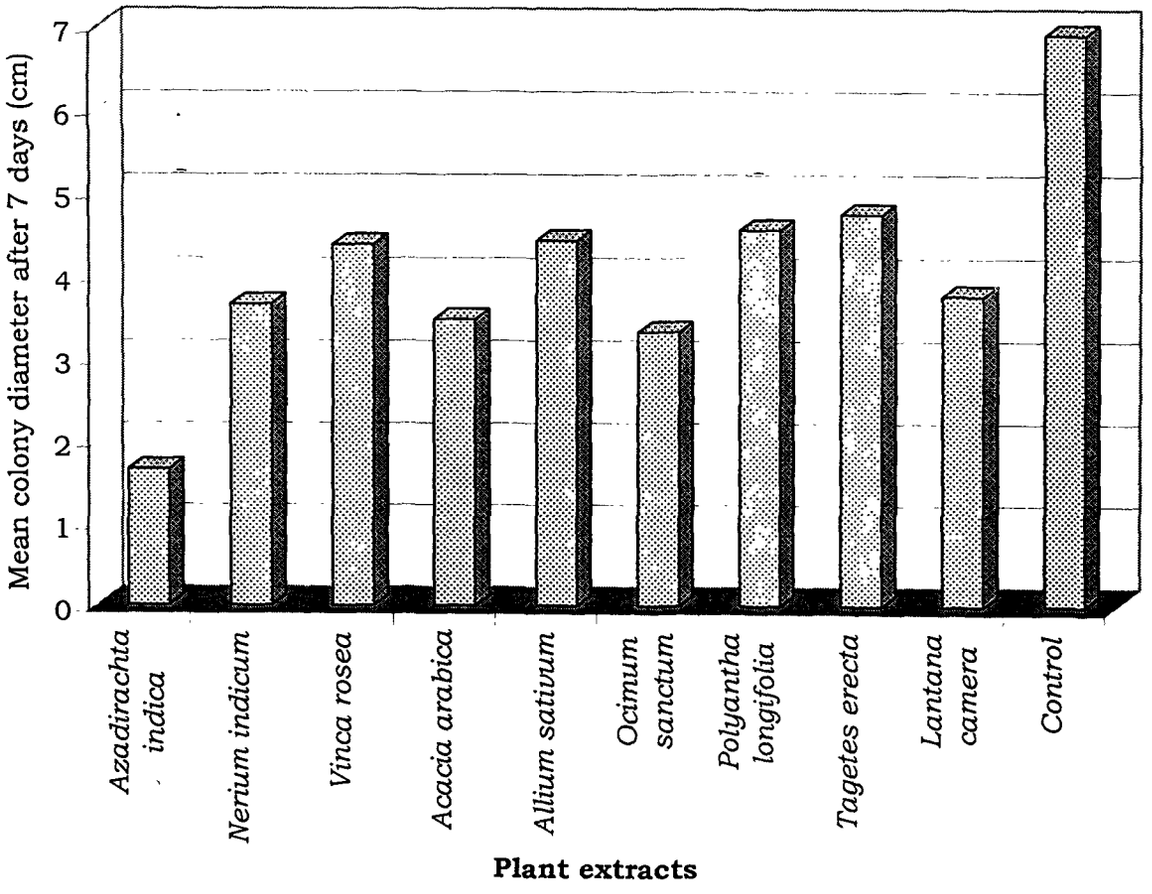

Fig. 8. Effect of different plant extracts on growth of *Penicillium digitatum* on solid media

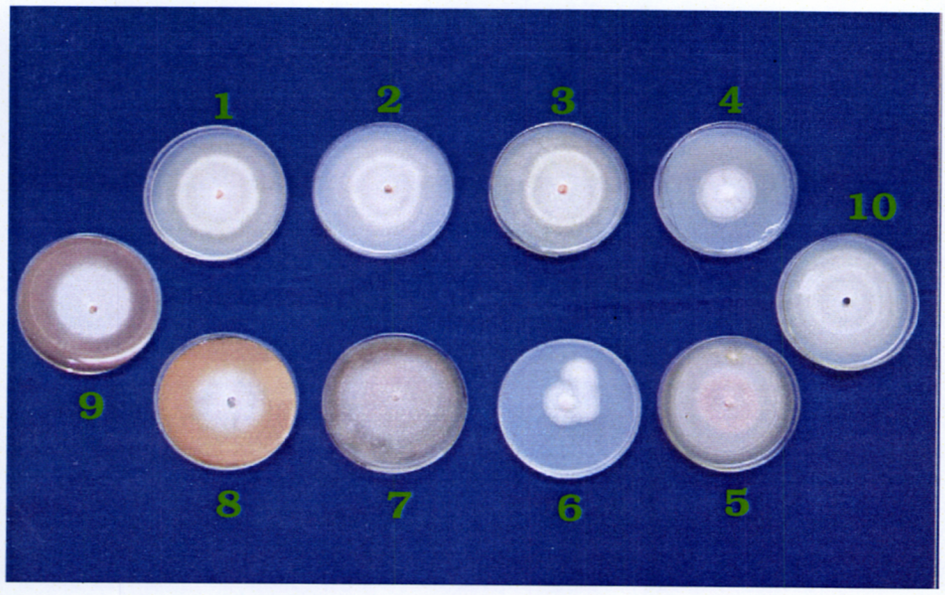

Plate 15. ***In vitro*** **evaluation of different plant extracts against** ***C. gloeosporioides*****; 1.** ***Azadirachta indica*** **(Neem); 2.** ***Nerium indicum*** **(Kahner); 3.** ***Vinca rosea*** **Linn. (Sadaphuli); 4.** ***Allium sativum*** **Linn.(Garlic); 5.** ***Tagetes erecta*** **(Marigold); 6.*Polyantha longifolia*** **(Ashoka); 7.** ***Ocimum sanctum*** **Wild. (Tulas); 8.** ***Acacia arabica*** **Willd. (Babul); 9.** ***Lantana camera*** **(Ghaneri); 10. Control**

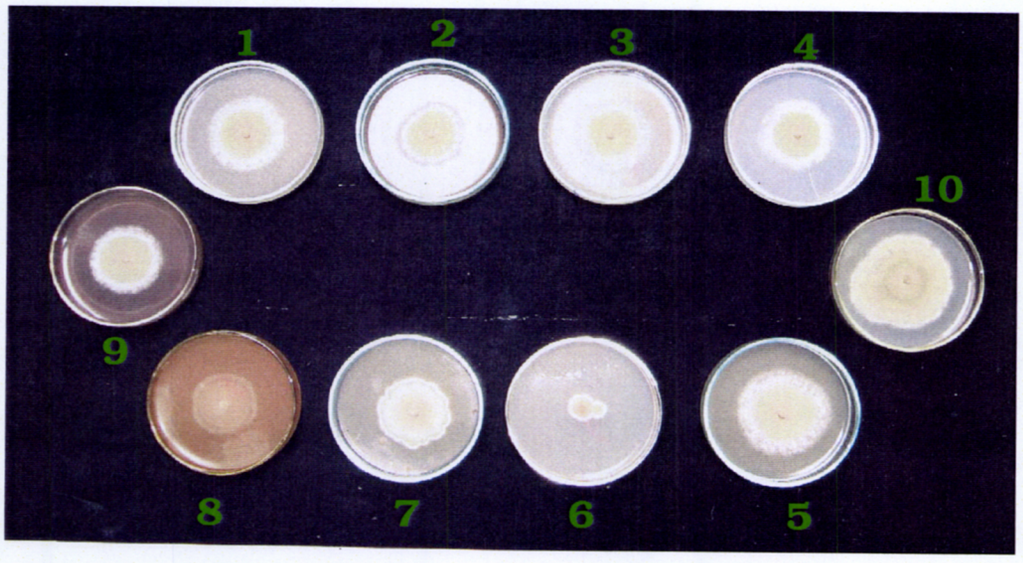

Plate 16. ***In vitro*** **evaluation of different plant extracts against** ***P. digitatum*****; 1.** ***Polyantha longifolia*** **; 2.** ***Nerium indicum*** **(Kahner); 3.** ***Vinca rosea*** **Linn. (Sadaphuli); 4.** ***Allium sativum*** **Linn.(Garlic); 5.** ***Tagetes erecta*** **(Marigold); 6.** ***Azadirachta indica*** **(Neem); 7.** ***Ocimum sanctum*** **Wild. (Tulas); 8.** ***Acacia arabica*** **Willd. (Babul); 9.** ***Lantana camera*** **(Ghaneri); 10. Control**

4.6.3 Effect of different physical and chemical treatments on post harvest diseases of sweet orange cv. Mosambi

4.6.3.1 Effect of different physical and chemical treatments on posts harvest anthracnose disease caused by *Colletotrichum gloeosporioides*

The results of evaluation of different physical and chemical post harvest treatments on *Colletotrichum gloeosporioides* are presented in Table 11. Significant differences were observed amongst the different treatments evaluated. All the treatments showed significantly low lesion diameter as compared to control treatment in the observations recorded after 4, 8 and 12 days (Plate 17).

It was observed that among the nine treatments tried, the treatment inoculation of fruits before heating at 46 ^{0}C for 300 min. showed highest disease control (70.71 %) with minimum lesion diameter of 2.70 cm as compared to control. It was followed by inoculation of fruits after heating at 46 ^{0}C for 300 min. showing disease control of 59.21 % and lesion size of 3.76 cm (Fig. 9).

The treatment immersion in 10 % ethanol at 45 ^{0}C for 150 sec. also showed good control of disease as 55.20 % with a lesion size of 4.13 cm. Treatments immersion in 3 % sodium carbonate at 45 ^{0}C for 150 sec., immersion in 2 % sulfur dioxide at 45 ^{0}C for 150 sec., immersion in 3 % sodium bicarbonate for 150 sec. and fumigation with 2.5 % acetic acid showed less

Table 11. Effect of different post harvest treatments on *Colletotrichum gloeosporioides* in sweet orange cv. Mosambi

Sr. No.	Treatment name	Lesion diameter at days (cm)			Per cent disease control at days		
		4	8	12	4	8	12
1.	Plastic film wrapping (20 μ)	1.65	3.57	5.75	55.16	40.00	37.63
2.	Fumigation with 2.5 % acetic acid	1.60	3.45	5.60	56.52	42.01	39.26
3.	Inoculation, before heating at 46 ^{0}C for 300 min.	0.50	1.45	2.70	86.41	75.63	70.71
4.	Inoculation, after heating at 46 ^{0}C for 300 min.	0.78	1.78	3.76	78.80	70.08	59.21
5.	Immersion in hot water at 45 ^{0}C for 150 sec.	1.70	3.65	5.95	53.81	38.65	35.36
6.	Immersion in 10 % ethanol at 45 ^{0}C for 150 sec.	0.88	2.10	4.13	76.08	64.70	55.20
7.	Immersion in 2 % sulfurdioxide at 45 ^{0}C for 150 sec. f.b. 2 sterile water rinses	1.35	3.16	5.20	63.31	46.89	43.60
8.	Immersion in 3 % sodium carbonate at 45 ^{0}C for 150 sec. f.b. 2 sterile water rinses	1.10	2.81	4.90	70.11	52.77	46.85
9.	Immersion in 3 % sodium bicarbonate for 150 sec. f.b. 2 sterile water rinses	1.45	3.40	5.50	60.59	42.86	40.35
10.	Control	3.68	5.95	9.22	-	-	-
	S.E. ±	0.24	0.27	0.22			
	C.D. at 5 %	0.73	0.82	0.66			

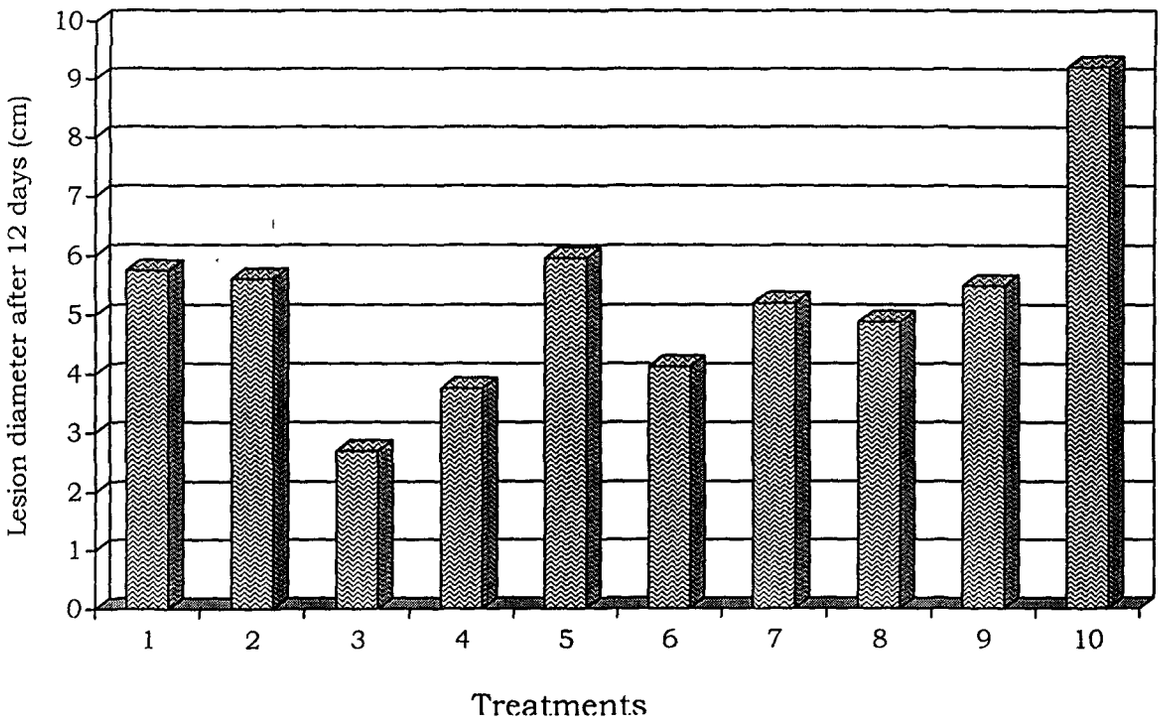

Fig. 9. Effect of different post harvest treatments on *Colletotrichum gloeosporioides* in sweet orange cv. Mosambi

1. Plastic film wrapping (20 μ)
2. Fumigation with 2.5 % acetic acid
3. Inoculation, before heating at 46 ^{0}C for 300 min.
4. Inoculation, after heating at 46 ^{0}C for 300 min.
5. Immersion in hot water at 45 ^{0}C for 150 sec.
6. Immersion in 10 % ethanol at 45 ^{0}C for 150 sec.
7. Immersion in 2 % sulfurdioxide at 45 ^{0}C for 150 sec. f.b. 2 sterile water rinses
8. Immersion in 3 % sodium carbonate at 45 ^{0}C for 150 sec. f.b. 2 sterile water rinses
9. Immersion in 3 % sodium bicarbonate for 150 sec. f.b. 2 sterile water rinses
10. Control

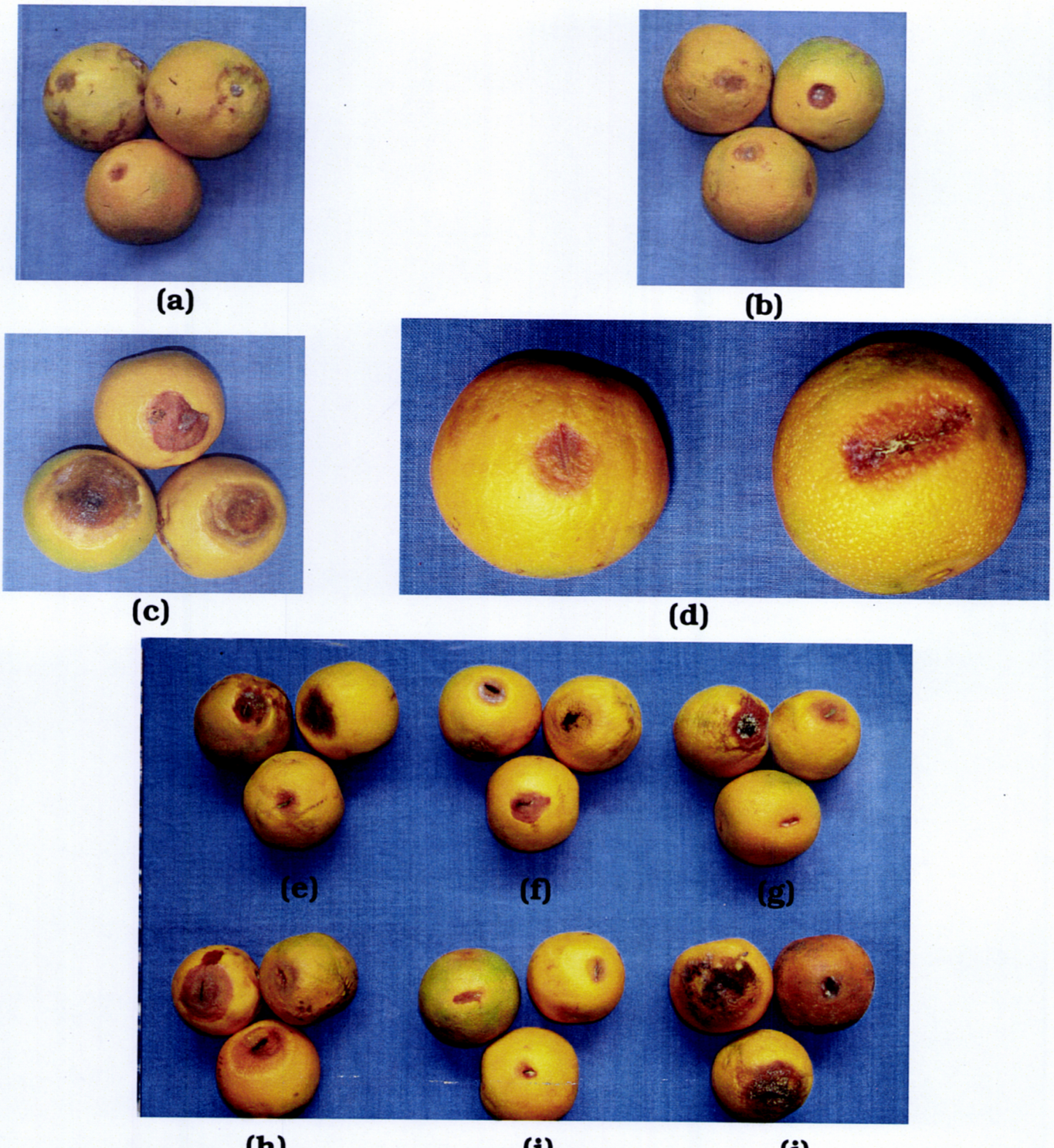

Plate 17. **Effect of different physical and chemical treatments on post harvest anthracnose disease of sweet orange cv. Mosambi caused by *C. gloeosporioides;* a) Inoculation before heating at 46 ^{0}C for 300 min.; b) Inoculation after heating at 46 ^{0}C for 300 min.; c) Plastic film wrapping (20 μ); d) Fumigation with 2.5 % acetic acid; e) Immersion in 3 % $NaHCO_3$ for 150 sec. f.b. 2 sterile water rinses; f) Immersion in 3 % Na_2CO_3 at 45 ^{0}C for 150 sec. f.b. 2 sterile water rinses; g) Immersion in 2 % SO_2 at 45 ^{0}C for 150 sec. f.b. 2 sterile water rinses; h) Hot water at 45 ^{0}C for 150 sec.; i) Immersion in 10 % ethanol at 45 ^{0}C for 150 sec.; j) Control**

control of disease as 46.85, 43.60, 40.35 and 39.26 %, respectively.

The treatments plastic film wrapping (20 μ) and hot water treatment at 45 ^{0}C for 150 sec. had low effectiveness in controlling the disease.

The results indicated that the treatments *viz.*, inoculation of fruits before heating at 46 ^{0}C for 300 min. was most effective in disease control followed by inoculation after heating at 46 ^{0}C for 300 min. and immersion in 10 % ethanol at 45 ^{0}C for 150 sec. were effective for the control of post harvest decay of sweet orange cv. Mosambi caused by *Colletotrichum gloeosporioides.*

4.6.3.2 Effect of different physical and chemical treatments on post harvest green mould disease caused by *Penicillium digitatum*

The results of evaluation of different physical and chemical treatments on post harvest disease (green mold) caused by *Penicillium digitatum* are presented in Table 12. Significant differences were observed amongst the different treatments evaluated. All the treatments showed significantly low lesion diameter as compared to control treatment in the observations recorded after 4, 8 and 12 days (Plate 18).

It was observed that among the nine treatments tried, inoculation of fruits before heating at 46 ^{0}C for 300 min. showed highest disease control of 86.52 % and minimum lesion size of 1.21 cm as compared to control. It was followed by inoculation of

Table 12. Effect of different post harvest treatments on *Penicillium digitatum* in sweet orange cv. Mosambi

Sr. No.	Treatment name	Lesion diameter at days (cm)			Per cent disease control at days		
		4	8	12	4	8	12
1.	Plastic film wrapping (20 μ)	0.70	1.55	4.10	75.82	74.46	54.34
2.	Fumigation with 2.5 % acetic acid	0.61	1.30	3.72	78.67	78.58	58.57
3.	Inoculation, before heating at 46 ^{0}C for 300 min.	0.50	0.66	1.21	82.51	89.12	86.52
4.	Inoculation, after heating at 46 ^{0}C for 300 min.	0.58	1.10	2.70	79.72	81.88	69.93
5.	Immersion in hot water at 45 ^{0}C for 150 sec.	0.95	1.75	4.95	66.78	71.17	44.87
6.	Immersion in 10 % ethanol at 45 ^{0}C for 150 sec.	0.85	1.70	4.63	70.28	71.99	48.44
7.	Immersion in 2 % sulfurdioxide at 45 ^{0}C for 150 sec. f.b. 2 sterile water rinses	1.11	2.30	5.50	61.18	62.11	38.75
8.	Immersion in 3 % sodium carbonate at 45 ^{0}C for 150 sec. f.b. 2 sterile water rinses	1.67	4.07	6.30	41.60	32.95	29.84
9.	Immersion in 3 % sodium bicarbonate for 150 sec. f.b. 2 sterile water rinses	1.40	3.93	5.98	51.05	35.25	33.40
10.	Control	2.86	6.07	8.98	-	-	-
	S.E. ±	0.13	0.17	0.20	-	-	-
	C.D. at 5 %	0.39	0.51	0.59	-	-	-

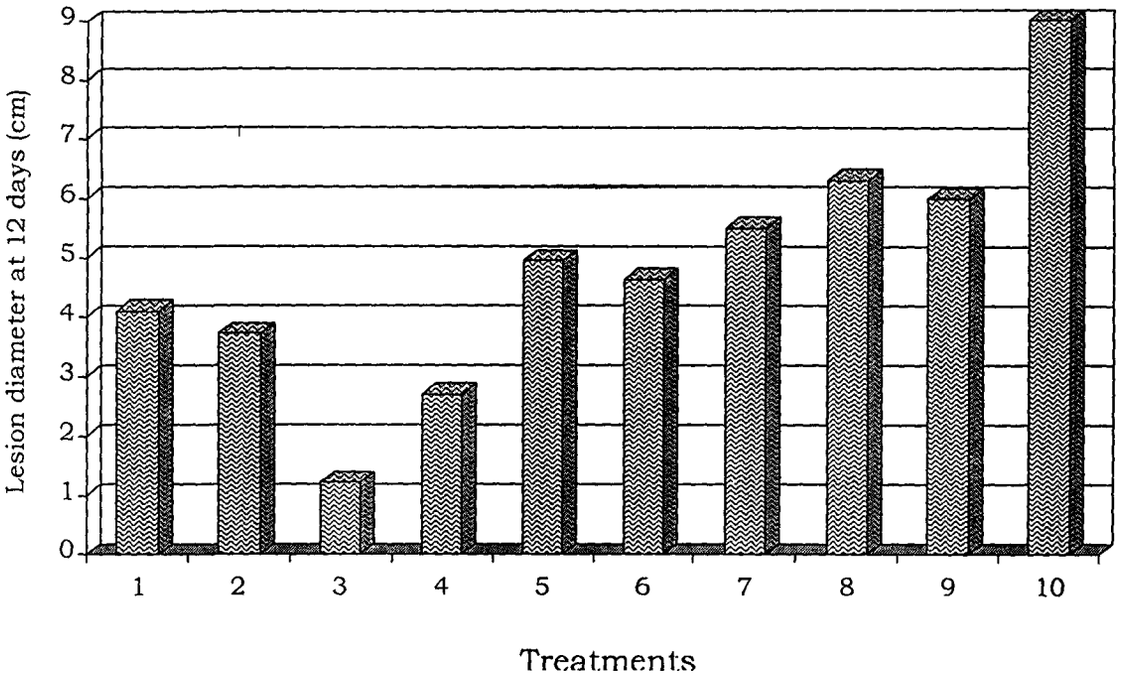

Fig. 10. Effect of different post harvest treatment on *Penicillium digitatum* in sweet orange cv. Mosambi

1. Plastic film wrapping (20 μ)
2. Fumigation with 2.5 % acetic acid
3. Inoculation, before heating at 46 ^{0}C for 300 min.
4. Inoculation, after heating at 46 ^{0}C for 300 min.
5. Immersion in hot water at 45 ^{0}C for 150 sec.
6. Immersion in 10 % ethanol at 45 ^{0}C for 150 sec.
7. Immersion in 2 % sulfurdioxide at 45 ^{0}C for 150 sec. f.b. 2 sterile water rinses
8. Immersion in 3 % sodium carbonate at 45 ^{0}C for 150 sec. f.b. 2 sterile water rinses
9. Immersion in 3 % sodium bicarbonate for 150 sec. f.b. 2 sterile water rinses
10. Control

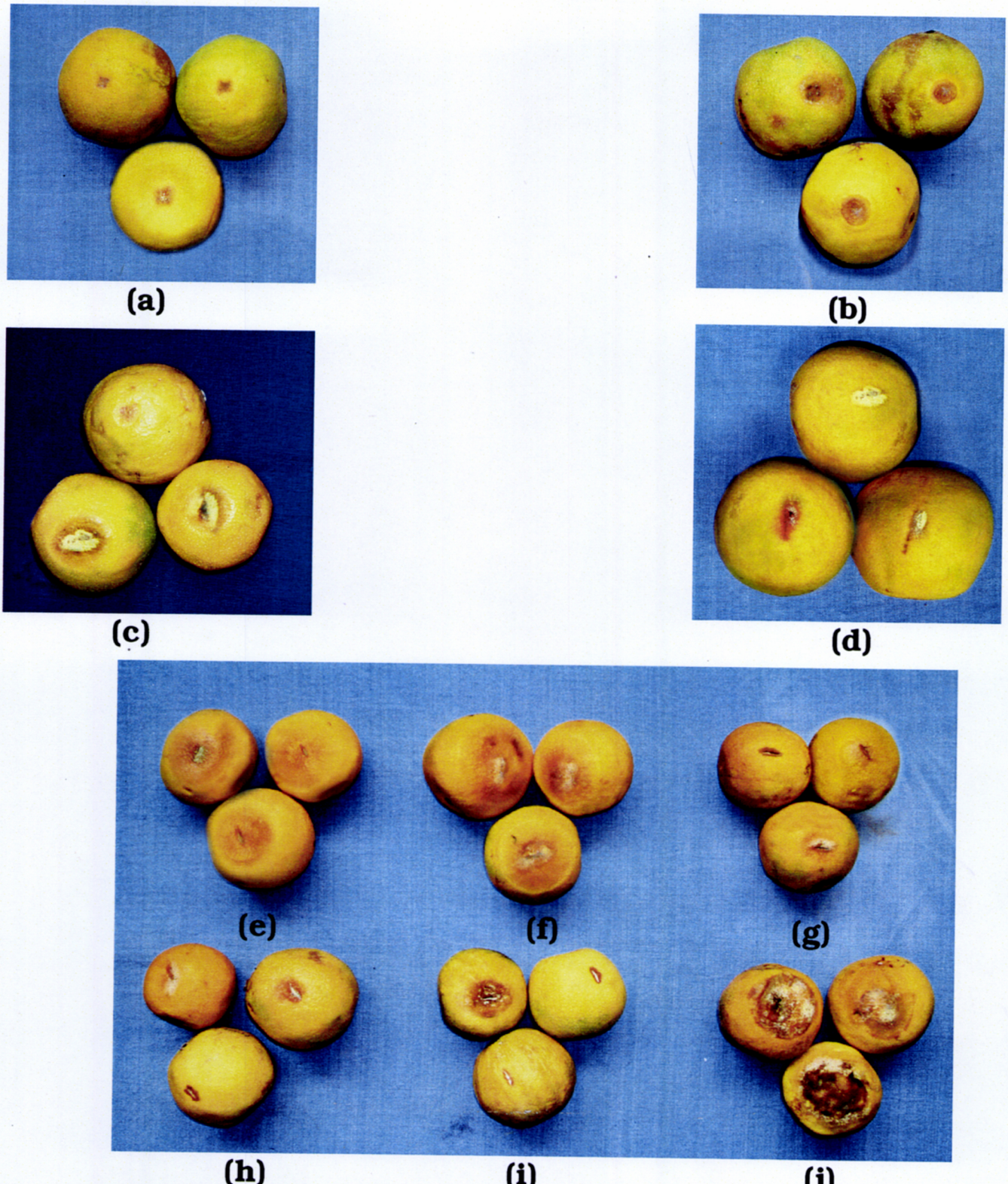

Plate 18. **Effect of different physical and chemical treatments on post harvest green mold disease of sweet orange cv. Mosambi caused by *P. digitatum;* a) Inoculation before heating at 46 ^{0}C for 300 min.; b) Inoculation after heating at 46 ^{0}C for 300 min.; c) Plastic film wrapping (20 μ); d) Fumigation with 2.5 % acetic acid; e) Immersion in 3 % $NaHCO_3$ for 150 sec. f.b. 2 sterile water rinses; f) Immersion in 3 % Na_2CO_3 at 45 ^{0}C for 150 sec. f.b. 2 sterile water rinses; g) Immersion in 2 % SO_2 at 45 ^{0}C for 150 sec. f.b. 2 sterile water rinses; h) Hot water at 45 ^{0}C for 150 sec.; i) Immersion in 10 % ethanol at 45 ^{0}C for 150 sec.; j) Control**

fruits after heating at 46 ^{0}C for 300 min. with 69.93 % disease control and lesion size of 2.70 cm (Fig. 10).

The treatment fumigation with 2.5 % acetic acid was third best with 58.57 % disease control and lesion size of 3.72 cm.

The treatments plastic film wrapping (20 μ), immersion in 10 % ethanol at 45 ^{0}C for 150 sec. and hot water at 45 ^{0}C for 150 sec. showed 54.34, 48.44 and 44.84 per cent disease control, respectively.

The treatments immersion in 2 % sulfur dioxide at 45 ^{0}C for 150 sec., immersion in 3 % sodium carbonate at 45 ^{0}C for 150 sec. and immersion in 3 % sodium bicarbonate for 150 sec. were low in their effectiveness to control the disease.

The results indicated that, the treatments *viz.*, inoculation of fruits before heating at 46 ^{0}C for 300 min. followed by inoculation of fruits after heating at 46 ^{0}C for 300 min. and fumigation with 2.5 % acetic acid were effective for the control of post harvest green mold decay of sweet orange cv. Mosambi caused by *Penicillium digitatum.*

DISCUSSION

5. DISCUSSION

5.1 Collection of disease sample, isolation, inoculation and pathogenicity studies

In the present investigations, diseased sweet orange (*Citrus sinensis* L. Osbeck) cv. Mosambi fruit samples were collected from the Agricultural Produce Market Committee of Rahuri, Ahmednagar, Nashik, Pune, Akluj and Pandharpur, Based on the differences in the appearance of symptoms, the fruit samples were categorized into different groups.

The affected specimens were used for isolation, pure culture obtained and maintained for further studies. Isolations made from the diseased fruit samples yielded the fungus *Colletotrichum gloeosporioides* Penz. and *Penicillium digitatum* causing anthracnose and green mould, respectively. Based on the morphological and cultural characters, authentic literature published in journals and help from the scientists at the "Disease Diagnosis Centre" at the Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, MPKV, Rahuri, the identification of pathogen was made.

Several workers *viz.,* Nolla (1926), Muller (1933), Baker (1935), Fawcett (1936), Baker and Wardlaw (1937), Hafiz (1953), Phelps (1968), Pelser (1975) and Whiteside *et al.* (1998) have reported *C. gloeosporioides* to cause the disease. The pathogenicity tests were carried out using detached fruit inoculation technique under *in vitro* conditions for confirmation of the pathogen. The various workers *viz.,* Baker and Wardlaw

(1932), Sinha *et al.* (1972), Cheema *et al.* (1976), Timmer *et al.* (1998), Holmes and Eckert (1999), Muniz (2003) and Benyahia *et al.* (2003) have reported its pathogenicity on citrus fruits.

Similarly, *Penicillium digitatum was* reported by the workers *viz.,* Baker (1935), Fawcett (1936), Baker and Wardlaw (1937), Cheema *et al.* (1937), Davis and Smoot (1965), Kavanagh and Wood (1967), Kaul and Lall (1975), Ram and Naidu (1976), Sumbali and Mehrotra (1981), Tuset (1984), Gardner *et al.* (1986), Khillare and Gangawane (1997), Sholberg (1998), Shellie (1998), Sharma and Gupta (2000) and Ismail and Zhang (2004) to cause green mould disease in various citrus fruits. The pathogenicity test was conducted for the confirmation of the pathogen and as also reported by other scientists, the association of this pathogen *viz.,* Baker and Wardlaw (1937), Eckert *et al.* (1968) and Holmes and Eckert (1999).

Reisolations made from inoculated fruits yielded the fungus cultures similar to the original one, which was isolated and inoculated, thereby proving Koch's postulates.

5.2 Symptomatology

5.2.1 Symptoms produced by *Colletotrichum gloeosporioides*

The symptoms of post harvest fruit rot due to *Colletotrichum gloeosporioides* in sweet orange cv. Mosambi causing anthracnose are visible as brown sunken lesions, which later on coalesce and turn brownish to dark brown in colour. In later stage of symptom development, this brown discolouration progresses fast to cover and rot the fruit in 12-15 days. The

present observations were confirmatory to those observed by Baker (1935), Salerno and Cutuli (1985), Ullasa (1993) and Ismail and Zhang (2004).

The foregoing results revealed that the pathogen *Colletotrichum gloeosporioides* infects sweet orange fruits and cause rotting of fruits.

5.2.2. Symptoms produced by *Penicillium digitatum*

The symptoms of post harvest fruit rot *viz.*, green mould due to *Penicillium digitatum* in sweet orange cv. Mosambi is visible initially as soft water soaked spot, such areas of lesion in later stages enlarges and the entire fruit is covered with green spores/mass. A broad white mycelial zone or band develops beyond the green sporing area and in 10-12 days, the fruit rots. The present observations were confirmatory to those observed by Fawcett and Berger (1927), Baker (1935), Fawcett (1936), Aulakh *et al.* (1998), Sharma and Gupta (2000) and Ismail and Zhang (2004).

The foregoing results revealed that the pathogen *Penicillium digitatum* infects sweet orange to cause green mould disease of the fruits.

5.3 Morphology of the fungus

5.3.1 Morphology of *Colletotrichum gloeosporioides*

The morphological characters of the fungus were studied from the culture grown on potato dextrose agar (PDA) medium. Initially colonies of the fungus were whitish which later turned slight grey to darker than early stages. Mycelium of the fungus was septate and irregularly branched. Hyphae were thin

initially when young and become thick with age. They measured 4.04 μm (1.50-6.01 μm) in width on culture. The findings are in agreement with the results of Gupta and Madaan (1977) in respect of mycelium morphology. Further, the results recorded by Hande (2001), Gaikwad (2002) are similar to those of present findings as regards the general character of mycelium. The mycelial measurements are similar to those of Hande (2001).

The fungus produced acervuli on culture. Acervuli appeared dark brown to black at the base, while the remaining part was light brown. This finding is similar to Hande (2001). The acervuli were globose to saucer or irregular in shape. Further, the acervuli (including setae) measured 190.39 μm.

Setae were light to dark brown, septate (2-8), stiff, straight or bending, wider at base, while tapering towards the tip. Hande (2001) and Gaikwad (2002) also reported similar observations in respect of setae of *Colletotrichum gloeosporioides* acervulus from curry leaf and custard apple, respectively. However, the measurements of setae in the present studies were 78.31 x 2.62 μm. The conidiophores were hyline, thickely arranged broader at base and quite narrow at tips. These findings are in consonance with the results of Gaikwad (2002).

Most of the conidia were oblong to cylindrical in shape with rounded ends and non-septate, hyaline when single but light brown in muscilagenous masses or in acervuli. These findings are similar with those of Hande (2001) and Gaikwad (2002).Conidia may sometimes contain one or two globules. These findings are similar with Singh and Sinha (1954), Gupta

and Madaan (1977). The conidia measured 11.46 x 4.72 μm with length : breadth ratio 2.47. The measurements are nearly similar to those observed by Hande (2001) who found it as 12.69 x 3.86 μm and Gaikwad (2002) who found it as 11.52 x 4.86 μm.

5.3.2 Morphology of *Penicillium digitatum*

The morphological characters of the fungus were studied from the culture grown on PDA medium. Initially colonies of the fungus were whitish on PDA which later turned greenish. Mycelium of the fungus was branched, septate and hyaline. They measured 4.44 μm (2.84-5.18 μm) in width. The results recorded by Sharma (2005) are similar to those of present findings as regards the general characters of mycelium.

The conidiophores were typically very short, smooth walled arising from hyphae or from basal mycelial felt singly or in synnemata branched near the apex and ending in phialides bearing conidia in chains. They measured 38.58 μm (33.07-48.26 μm) in width. These findings are in agreement or similar with those observed by Raper and Thom (1984). Further the results recorded by Barnett and Barry (1972) and Sharma and Gupta (2000) are similar to those of present finding as regards general characters of the mycelium.

Phialides or sterigmata were equally variable bearing chains of conidia and measured about 19.96 μm (17.20-22.88 μm) in length and 3.50 μm (2.50 x 5.01 μm) in width. These findings obtained in present work coincide with the findings of Raper and Thom (1984).

Most of the conidia were smooth walled, dull, dark green in mass, varying greatly in form and dimensions, ranging from subglobose to long, cylindrical in shape, but usually elliptical and measured about 4.33 µm (3.674-5.01 µm) x 3.31 µm (3.006 x 3.674 µm). These findings are in agreement with the results Raper and Thom (1984) and Sharma (2005). Further, the results recorded by Barnett and Barry (1972) and Sharma and Gupta (2000) are similar to those of the present findings as regards the general characters of the conidia.

5.4 Growth and cultural characters

5.4.1 Growth and sporulation of *C. gloeosporioides* on different synthetic and non-synthetic media

The cultural characters of the fungus on different synthetic and non-synthetic media under study revealed that, maximum colony diameter (9.10 cm) was recorded on Czapek's dox agar media. This finding is an agreement with Ekbote *et al.*, (1997) who also observed good growth of *Colletotrichum gloeosporioides* on Czapek's dox agar media. Significantly highest sporulation count (0.78 x 10^4 conidia cm^{-2}) was recorded in potato dextrose agar media. This finding is in agreement with Mallikarjunaiah (1972) and Patil (1989).

The next media in order of superiority for growth were potato dextrose agar, Asthana Hawker's, nutrient agar, Richards agar, Sabourauds agar and M_2 agar.

The findings in respect of Sabourauds agar, Asthana Hawker and M_2 agar are in agreement with the results of

Gaikwad (2002) who reported good growth of *Colletotrichum gloeosporioides* on these media.

5.4.2 Growth and sporulation of *P. digitatum* on different synthetic and non-synthetic media

The cultural characters of the fungus on different synthetic and non-synthetic media under study revealed that, maximum colony diameter (7.31 cm), maximum growth rate (0.29 mm h^{-1}) and highest sporulation count (0.56 x 10^4 conidia cm^{-2}) was recorded on potato dextrose agar media. These findings are in agreement with Raper and Thom (1984) who also observed good growth of *Penicillium digitatum* on potato dextrose agar media.

Significantly less growth of the fungus *P. digitatum* was recorded on media *viz.,* Czapek's dox, M_2 agar, Ashbys agar, corn meal, wheat meal and tap water agar media.

The findings in respect of Czapek's dox are in agreement with the results of Raper and Thom (1984) who also observed sparse growth and limited spore production of *P. digitatum* on Czapek's dox agar media.

5.5 Physiological studies

5.5.1 Effect of temperature on growth and sporulation of *C. gloeosporioides*

The fungus *Colletotrichum gloeosporioides* could grow between the range 10 to 30 ^{0}C. The optimum temperature for growth and sporulation was observed to be between 25 to 30 ^{0}C. These findings are similar to those reported by Tandon (1967), Patil (1969), Gordillo (1981), Ahmed (1985), Kim *et al.* (1986),

Prakash and Srivastava (1987) and Hegde *et al.* (1993). Similarly, Agostini *et al.* (1992) observed that the fast growing isolates of *Colletotrichum gloeosporioides* grew better at 31 ^{0}C than slow growing orange and key lime isolates.

The growth and sporulation was not observed at 0 and 5 ^{0}C.

5.5.2 Effect of temperature on growth and sporulation of *P. digitatum*

The fungus *Penicillium digitatum* could grow between the range 20 to 30 ^{0}C. The optimum temperature for growth and sporulation was observed to be between 20 to 25 ^{0}C. These findings are similar to those reported by Doron (1922), Fawcett and Berger (1927) and Lahalali *et al.* (2006).

The growth and sporulation was not observed at 0, 5, 10 and 15 ^{0}C.

The findings in respect of 0 ^{0}C is in agreement with the results of Ryall and Pentzer (1904) who reported slow growth at 0 ^{0}C (32 ^{0}F).

5.6 Evaluation of different control measures

5.6.1 *In vitro* evaluation of fungicides

5.6.1.1 *In vitro* evaluation of fungicides against *C. gloeosporioides*

The results of different concentrations of fungicides *viz.*, hexaconazole 0.1 % and propiconazole 0.1 % indicated that they completely inhibited the growth of *Colletotrichum gloeosporioides* under *in vitro*, there by showing their effectiveness against the pathogen. Ali *et al.* (1993) observed

bavistin 100 ppm and propiconazole 200 ppm to inhibit *Colletotrichum gloeosporioides* in *in vitro*. Gaikwad (2002) observed propiconazole (0.1 %) and Bordeaux mixture 1 % to be effective against fruit rot (*C. gloeosporioides*) of custard apple.

Next in order were the fungicides difenconazole 0.1 %, captan 0.25 %, iprodione (25 %) + carbendazim (25 %) 0.25 % and copper oxychloride 0.25 % which showed 91.96, 53.41, 53.19 and 51.43 % inhibition of the fungus, respectively. These results are analogous to those reported by Kader and Rahman (2001) who observed difenconazole 250 EC, propiconazole 250 EC and carbendazim 50 WP to be equally effective in inhibiting the mycelial growth of *Colletotrichum gloeosporioides* under *in vitro* conditions.

Thus, the studies revealed the effectiveness of fungicides *viz.,* hexaconazole 0.1 %, propiconazole 0.1 % and difenconazole 0.1 % in the order of their efficacy.

5.6.1.2 *In vitro* evaluation of fungicides against *P. digitatum*

The *in vitro* studies by poisoned food technique indicated that the fungicides *viz.,* chlorothalonil (0.25 %), captan (0.25 %), mancozeb (0.25 %), carbendazim (0.1 %), hexaconazole (0.1 %), propiconazole (0.1 %) difenconazole (0.1 %) and iprodione + carbendazim (0.25 %) completely inhibited the growth of *P. digitatum* and thus showed their effectiveness against the pathogen. Copper oxychloride showed less effectiveness against the pathogen.

The results about highest effectiveness of carbendazim obtained in present work coincide with finding of Ram and Naidu (1976) and Datar and Ghule (1988). The results are also in consonance with the work of Tandon *et al.* (1975) who reported that mancozeb and captan was highly effective against *Penicillium* sp. for control of blue mould rot of grape.

Thus, *in vitro* studies revealed the effectiveness of fungicides *viz.,* chlorothalonil, captan, mancozeb, carbendazim, hexaconazole, propiconazole, difenconazole and iprodione + carbendazim.

5.6.2 *In vitro* evaluation of plant extracts

5.6.2.1 *In vitro* evaluation of plant extract against *C. gloeosporioides*

The results of evaluation of different botanicals (plant extracts) indicated that *Allium sativum* is most effective against *Colletotrichum gloeosporioides* exhibing highest percentage of inhibition (57.73 %). These findings are similar to those of Ahmed and Agnihotri (1972) who found *Allium sativum* as one of the botanical to inhibit the growth and sporulation of *Colletotrichum papayee.* Yadav (1986) also observed the effectiveness of *Allium sativum, Ocimum sanctum* against *Colletotrichum capsici.*

The next best botanical to inhibit (55.00 %) *Colletotrichum gloeosporioides* was *Polyantha longifolia* and the results are in accordance with Shivpuri *et al.* (1997) who found *Polyantha longifolia* most effective against *Colletotrichum capsici,*

Ocimum sanctum and *Tagets erecta* showed less effectiveness as inhibitor of the fungus.

Thus, the present investigations revealed the effectiveness of botanicals (plant extracts) *viz., Allium sativum, Polyantha longifolia, Ocimum sanctum* and *Lantana camera* extracts in inhibiting the growth of *Colletotrichum gloeosporioides* fungus.

5.6.2.2 *In vitro* evaluation of plant extracts against *P. digitatum*

The results of evaluation of different botanicals (plant extracts) indicated that *Azadirachta indica* is most effective against *Penicillium digitatum* exhibiting highest per cent of inhibition (76.12 %). The next best botanical to inhibit (51.80 %) *Penicillium digitatum* was *Ocimum sanctum.* These findings are similar to those of Khilare and Gangawane (1997) who found *Azadirachta indica* and *Ocimum sanctum* to inhibit the growth and sporulation of *Penicillium digitatum.* Godara and Pathak (1995) also observed the effectiveness of *Ocimum sanctum* leaf extract against *Penicillium italicum.*

Thus, the present investigations revealed the effectiveness of botanicals (plant extracts) *viz., Azadirachta indica, Ocimum sanctum, Acacia Arabica, Nerium indicum* and *Lantana camera* extracts in inhibiting the growth of *Penicillium digitatum* fungus.

5.6.3 Effect of different post harvest treatments

5.6.3.1 Effect of different physical and chemical treatments on anthracnose disease

The result of evaluation of different physical and chemical treatments indicated that the treatment inoculation of fruits before heating them at 46 ^{0}C for 300 min. is most effective against *Colletotrichum gloeosporioides,* exhibiting highest percentage of disease control i.e. 70.71 % with minimum lesion size of 2.70 cm as compared to control. McGuire (1991) found that hot forced air at 48 ^{0}C for 150 minute reduced the growth of *Colletotrichum gloeosporioides* in 3 cultivars of mango. Tijsken *et al.* (2003) observed dry air 48.5 ^{0}C for 4 hrs decreased the fungal development of *C. gloeosporioides* decay in papaya. The present findings are similar to the observations made by the above workers.

The next best treatment found to inhibit growth of *Colletotrichum gloeosporioides* is inoculation of fruits after heating at 46 ^{0}C for 300 min.

Thus, the present investigation revealed the effectiveness of post harvest treatments *viz.,* inoculation before heating at 46 ^{0}C for 300 min., inoculation after heating at 46 ^{0}C for 300 min. and immersion in 10 % ethanol at 45 ^{0}C for 150 sec. in inhibiting growth of *Colletotrichum gloeosporioides* fungus.

5.6.3.2 Effect of different physical and chemical treatments on green mould disease

The results of evaluation of different physical and chemical treatments indicated that the treatment inoculation of

fruits before heating at 46 ^{0}C for 300 min. is most effective against green mold of sweet orange cv. Mosambi caused by *Pencillium digitatum* exhibiting highest percentage of disease control of 86.52 % with minimum lesion size of 1.21 cm as compared to control. These findings are similar to those of Shellie and Skaria (1998) who found that wounds inoculated with *Penicillium digitatum* just prior to heating for 300 min. in 46 ^{0}C moist forced air developed less rapidly.

The next best treatment found to inhibit growth of *Penicillium digitatum* was inoculation of fruits after heating at 46 ^{0}C for 300 min. Several workers *viz.,* Smooth and Melvin (1965), Ben-Yehoshua *et al.,*(1987) and Huang *et al.,*(1991) reported that heat treatment reduced green mold (*Penicillium digitatum*) of sweet orange. The results of the present findings are similar for the observations made by different workers on the use of heat treatment for the control of green mold of citrus fruits.

The third best treatment was observed to be fumigation with 2.5 % acetic acid. This finding is similar to Sholberg (1998) who found that fumigation with acetic acid, formic acid and propionic acid for 30 min. showed reduction in decay due to *Penicillium digitatum.*

Thus, the present investigations revealed the effectiveness of post harvest treatments *viz.,* inoculation before heating at 46 ^{0}C for 300 min., inoculation after heating at 46 ^{0}C for 300 min. and fumigation with 2.5 % glacial acetic acid, in inhibiting the growth of *Penicillium digitatum* in sweet orange cv. Mosambi fruits.

SUMMARY AND CONCLUSIONS

6. SUMMARY AND CONCLUSIONS

6.1 Summary

The citrus occupies about 9 per cent of the total area under various fruits in the country. Amongst the citrus fruits, sweet orange constitute the major produce in India after mandarin. The crop is known to be severely affected by post harvest diseases *viz.*, anthracnose and green mould caused by *Colletotrichum gloeosporioides* and *Penicillium digitatum* respectively. The post harvest infections generally take place through wounds and/or injuries arising during harvest and handling of fruits. The fruits affected with *C. gloeosporioides* shows brown to dark brown sunken lesions on fruit, which progress fast to cause complete rotting of the fruit. *Penicillium digitatum* initially shows water soaked areas which later on is covered with green spore masses. The present studies were undertaken with the object to study the symptoms of disease, morphological, cultural physiological characters and efficacy of different control measures against the fungus.

The causal organisms were isolated from sweet orange cv. Mosambi fruits collected from different markets showing symptoms of the diseases. The causal fungus were identified as *Colletotrichum gloeosporioides* Penz. and *Penicillium digitatum.* On the basis of morphological and cultural characters, the Koch's postulates was proved on sweet orange fruits under laboratory conditions, using detached fruit inoculation technique.

The fungus *C. gloeosporioides* produced acervuli in culture. They were globose to saucer or irregular in shape, dark brown to black in colour and measured 190.39 x 114.76 µm. Setae were light brown to dark brown, septate, stiff, straight or bending wider at base and were 78.31 x 2.62 µm in size. Mycelium were white, which turn light to dark grey coloured in later stage. It was septate, hyaline, irregularly branched and 4.04 µm thick. The conidiophores were haline, thickly arranged broader at base and quite narrow at tips and measured 53.52 µm. Conidia were oblong to cylindrical, single celled, hyaline and measured 11.46 x 4.72 µm, with a length and breadth ratio of 2.47 µm.

The mycelium of the fungus, *Penicillium digitatum* was branched, septate and hyaline and measured about 4.44 µm in width. Conidiophores were very short, smooth walled, arising from submerged hyphae or from basal mycelial felt, singly or less often in synnemata, branched near the apex and were 33.58 µm in width. Phialides producing chains of conidia measured about 19.96 x 3.50 µm in size. Conidia were subglobose to long, cylindrical in shape, but usually elliptical and measured about 4.33 x 3.31 µm.

Cultural and physiological characters of both the fungus were also studied. *Colletotrichum gloeosporioides* showed good growth and sporulation on Czapek's dox agar and potato dextrose agar media respectively, while *Penicillium digitatum* showed good growth and sporulation on potato dextrose agar media. Temperature requirement studies indicated the minimum

and maximum temperature for *Colletotrichum gloeosporioides* as 10 and 30 ^{0}C, respectively. *Penicillium digitatum* grew between the temperatures 20 to 30 ^{0}C.

The evaluation of control measures for the fungus and the efficacy of different fungicides against *Colletotrichum gloeosporioides* revealed the high effectiveness of fungicides *viz.,* hexaconazole 0.1 %, propiconazole 0.1 %, difenconazole 0.1 %, captan (0.25 %) and iprodione + carbendazim (0.25 %). The fungicides chlorothalonil (0.25 %), captan (0.25 %), mancozeb (0.25 %), carbendazim (0.1 %), hexaconazole (0.1 %), propiconazole (0.1 %), difenconazole (0.1 %) and Iprodione + carbendazim (0.25 %) were effective in inhibiting *Penicillium digitatum.* The studies regarding efficacy of different plant extracts indicated the effectiveness of garlic, *Polyantha longifolia, Ocimum sanctum* and *Lantana camera* against *Colletotrichum gloeosporioides* under *in vitro* conditions; while the plant extracts *Azadirachta indica,* tulas, *Ocimum sanctum, Nerium indicum* and *Lantana camera* were showed their effectiveness against *Penicillium digitatum.*

In case of different physical and chemical post harvest treatments tried against *Colletotrichum gloeosporioides.* Inoculation before heating at 46 ^{0}C for 300 min. Inoculation after heating at 46 ^{0}C for 300 min. and immersion in 10 % ethanol at 45 ^{0}C for 150 sec. in this order were the best treatments. The treatments inoculation before heating at 46 ^{0}C for 300 min., inoculation after heating at 46 ^{0}C for 300 min. and fumigation

with 2.5 % glacial acetic acid in these order showed their effectiveness against *Penicillium digitatum.*

6.2 Conclusions

1. Isolation and identification studies revealed that the sweet orange cv. Mosambi fruit samples collected from different markets were infected by *Colletotrichum gloeosporioides* and *Penicillium digitatum.*
2. Koch's postulates of both the pathogens were proved on sweet orange fruits under *in vitro* conditions. The pathogen *Colletotrichum gloeosporioides* produces brown to black discoloured lesions on fruit and further causes fruit rot. *Penicillium digitatum* appears initially as soft watery spot which on progress shows olive green coloured rot.
3. Morphological measurements of the anthracnose fungus with respect to mycelium, acervuli, setae, chlamydospore and conidia revealed the organism to be *Colletotrichum gloeosporioides.* In case of green mould, the morphological measurements with respect to mycelium, phialides, conidiophores and conidia revealed the organism to be *Penicillium digitatum.*
4. Czapek's dox agar and potato dextrose agar supported good growth and sporulation of *Colletotrichum gloeosporioides,* respectively. Potato dextrose agar supported good growth and sporulation of *Penicillium digitatum.*
5. Good growth and sporulation of *Colletotrichum gloeosporioides* occurred in the temperature range 25 to 30

^{0}C and in case of *Penicillium digitatum* this range was about 20 to 25 ^{0}C.

6. Hexaconazole 0.1 %, propiconazole 0.1 % and difenconazole 0.1 %, captan 0.25 % and iprodione (25 %) + carbendazim (25 %) 0.25 % were found best for *Colletotrichum gloeosporioides* while the fungicides chlorothalonil 0.25 %, captan 0.25 %, mancozeb 0.25 %, carbendazim 0.1 %, hexaconazole 0.1 %, propiconazole 0.1 %, difenconazole 0.1 % and iprodione + carbendazim 0.25 % were effective for *Penicillium digitatum* under *in vitro* conditions.
7. The plant extracts *Allium sativum* and *Azadirachta indica* were most effective in inhibiting the fungus *Colletotrichum gloeosporioides* and *Penicillium digitatum*, respectively.
8. The post harvest treatment i.e. inoculation of fruits before heating at 46 ^{0}C for 300 min. was most effective for inhibition of both the organisms.

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7. LITERATURE CITED

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* Original not seen.

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8. VITA

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