VARIABILITY OF *Colletotrichum* ISOLATES INCITING ANTHRACNOSE IN MANGO (*Mangifera indica* L.)

By

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(2014-11-219)

THESIS

Submitted in partial fulfilment of the requirement

for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR – 680 656

KERALA, INDIA

2016

DECLARATION

I, hereby declare that this thesis entitled is a '**Variability of *Colletotrichum* isolates inciting anthracnose in mango (*Mangifera indica* L.)**' bonafide record of the research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of my degree, diploma, associateship, fellowship or other similar title, of any other University or Society

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CERTIFICATE

Certified that this thesis entitled '**Variability of *Colletotrichum* isolates inciting anthracnose in mango (*Mangifera indica* L.)**' is a bonafide record of the research work done independently by **Ms. Priyanka B.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her

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We, the undersigned members of advisory committee of **Ms. Priyanka B.**, a candidate for the degree of **Master of Science in Agriculture** with major field in **Plant Pathology**, agree that the thesis entitled **'Variability of *Colletotrichum* isolates inciting anthracnose in mango (*Mangifera indica* L.)'** may be submitted by **Ms. Priyanka B (2014-11-219)**, in partial fulfilment of the requirement for the degree

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ACKNOWLEDGEMENT

And so comes the time to look back on the path traversed during the endeavor and to remember the faces and spirits behind the action with a sense of gratitude Nothing of significance can be accomplished without the acts of assistance, words of encouragement and gestures of helpfulness from the other members of the society

First and foremost I bow to the Almighty whose grace had endowed me the inner strength, patience, will power and good health which made me to complete this venture successfully

It is with great pleasure I express my deep sense of gratitude and sincere thanks to Dr S Beena, Professor, Department of Plant Pathology, College of Horticulture, Vellanikkara, chairperson of my advisory committee for her valuable guidance, inspiring advices, critical comments, constant supervision, keen interest, support and encouragement from the very early stage of my research work till the end This work would not have been possible without her unfailing support in the preparation of the manuscript

My sincere thanks to Dr Anita Cherian K,, Professor and Head, Department of Plant Pathology College of Horticulture, Vellanikkara, member of Advisory Committee for the valuable guidance, timely suggestions and help rendered in the conduct of the experiment.

I am deeply obliged to Dr.Reshmy vijayaraghavan, Assistant Professor, Department of Plant Pathology College of Horticulture, Vellanikkara, for invaluable help, guidance and critical assessment throughout the period of work

I wish to extend my wholehearted gratitude to Dr Abida P S , Professor and Head, Department of Plant Breeding and Genetics, RARS, Pattambi, member of my advisory committee for his timely help, constant support and valuable guidance for the successful completion of the research work

I take this opportunity to express my heartiest gratitude to Dr Koshy Abraham, and Professor and Head (ret), Department of Plant Pathology, Dr T. J. Rehumath Niza, Professor (Rtd), Dr T Sheela Paul, Professor (Rtd), Dr Sally K, Mathew , Professor (Rtd) and Dr. Deepa James, Teaching Assistant, Department of Plant Pathology, College of Horticulture, Vellanikkara for their boundless help accorded during the research and thesis work

I wish to express my deep sense of gratitude to Dr. S Krishnan, Associate Professor and Head, Department of Agricultural Statistics, College of Horticulture, Vellanikkara for his valuable suggestions, immense help and guidance during the statistical analysis of the data

My thanks to Mrs Sindhu, Agriculture officer, Muthalamada for her valuble time in extending help for sample collection in Chulliyarmedu.

I express my sincere thanks to my classmates Reshma Raj T, Praveen N M and Shreeja for being with me all time with their affection and kind help during my research thesis work. I duly acknowledge the encouragement, moral support, precious suggestions and timely persuasions by my dear seniors, Mrs. Hima, Mrs Remya J. S, Mr Ahmed Mujataba, Ms Mridha George, Mr. Ajit Kumar, Mrs Aswini, Mrs Arathikrishnan, Mrs Sumbula, , not only in my research work but also throughout my P G programme I have infinite pleasure to express whole hearted thanks to my loving juniors for their love, innumerable help and support especially Deepa Pawar, Amrutha, Femi, Nusarath, Ramya, Debbasish, Nagendra, Raghu, Umesh and Sunil.

I am thankful to Research Assistants and Prasanna chechi for immense help rendered during my research and thesis work.

I thank my dear friends Yan sing, Sabira, Learou, Vsavi, Supritha, Ashwini, Indraji, Shobha, Manjushree, Rekha, Reddy, Mangalam, Manjesh, Shridhar, Dharshan, Sameer, Rajanand, Suma, Ajankya, Abhishek Singh, Sree Vithya, Libi Antony,Vinu Shree and all my Karnataka friends in KAU for the unconditional support, help, timely valuable suggestions and encouragement which gave me enough mental strength and perseverance to get through all odds and tedious circumstances and immense thanks to all M Sc classmates for their moral support and encouragement

I am forever beholden to my beloved Parents, Prashanth, Rouf Ahmad, Mohan, Pratik Chevalikar, Jeevan, Vijayaraghavendra, Devendra, Sushma, Madhuri and Zahir Basha sir for their unfathomable love, boundless affection, personal sacrifice, incessant inspiration and constant prayers, which supported me to stay at tough tracks and helped me for the successful completion of this programme

I am thankful to Dr P N Choudry, Former Principle Scientist, IARI, New Delhi for his valuable help in identification of cultures and for his encouragement and support

I wish to express my sincere thanks to Dr Nirmala Devi, Dr Jesy Thomas, Shakila and all others of administrative office for their whole hearted cooperation and timely assistance

I gratefully thank Aravind chettan, Computer club, COH, Thrissur for their technical assistance in the preparation of the manuscript

I owe special thanks to the Library and Dr A. T Frances, Librarian, College of Horticulture, Vellanikkara who guided me in several ways, which immensely helped for collection of literature for writing my thesis

I express my deep sense of gratitude to Kerala Agricultural University for the financial and technical support for persuasion of my study and research work

A word of apology to those I have not mentioned in person and a note of thanks to one and all who worked for the successful completion of this endeavour

Priyanka B.

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INTRODUCTION

Mango (*Mangifera indica* L), one of the most important fruit crops in tropics and subtropics is known as 'King of Asiatic fruits' Mango leads up to 50 per cent of tropical fruits in the world (Jedele *et al* , 2003) Globally, India ranks first in mango production with an area of 2516 0 thousand ha and production of 184 31 lakh tonnes which contributes 42 2 per cent of world's total mango production Mangoes account about 22 9 per cent of the total fruit production in India (NHB, 2014) Due to diverse production and vast area grown, mango is infected with a number of diseases In Kerala, Palakkad district ranks first in mango production and the most commonly cultivated mango varieties include Muvandan, Alphonso, Neelum, Chandrakaran, Banganapalli, Prior, Sindhooram, Kalapady and Guddadat

Colletotrichum gloeosporioides (Penz) Penz & Sacc is a facultative parasite of anthracnose The telomorphic stage of this pathogen is *Glomerella cingulata,* belongs to the Class *Sodariomycetes* of Phylum *Ascomycota* This pathogen produces acervuli within the host tissue at mitotic phase of its life cycle The genus *Colletotrichum* is considered as one of the important plant pathogenic fungi, recently designated as the world's eighth most important pathogen (Dean *et al* , 2012) This pathogen attacks all plant parts from seedling to fruiting stage in the humid and sub humid tropical zones causing great economic loss in cereals, vegetables, fruits, grasses, plantation and medicinal plants

One of the constraints in the production of mango is the diseases, of which, anthracnose caused by *C gloeosporioides* is the most severe one In India, McRae (1924) noticed many diseases of mango in Bengal in which he mentioned about mango anthracnose This disease was first reported by Stevens and Pierce (1933) from Bombay Many scientists reported *C gleosporoides* as a causal agent along with many other spp *C asianum, C cordylinicola, C fructicola, C horii, C kahawae* subsp *kahawae, C musae,*

C nupharicola, C psidii, C siamense, C theobromicola, C tropicale C acutatum C simmondsii, C fioriniae, C lupine from different countries All the varieties and cultivars of mango are known to be affected by the pathogen

Literature survey showed the existence of several distinct populations among the isolates of *Colletotrichum gloeosporioides* It was reported even in 1957, by Arx that *C gloeosporioides* had more than 600 synonyms and showed many morphological and physiological variations. Even though *C gloeosporioides* was reported as the major causal agent of mango anthracnose, there were reports on the association of *C acutatum* (Fitzell, 1979) and *C gloeosporioides* var *minor* (Fitzell and Peak, 1984) with this disease In India, association of two genetically distinct populations of C *gloeosporioides* with mango anthracnose was reported by Chowdappa and Kumar (2012)

Perusal of literature revealed many reports on the variability in phenotypic and pathogenic characteristics of *Colletotrichum* sp from other parts of India But information on the variability in the cultural, morphological and pathogenic characters of anthracnose pathogen of mango is limiting from Kerala Hence, the present study was taken up to elucidate information on the characters of the pathogen attacking different varieties of mango commonly cultivating in Kerala which will throw light on the existence of variability, if any, among the different isolates of the pathogen The research programme entitled 'Variability of *Colletotrichum* isolates inciting anthracnose in mango (*Mangifera indica* L) ' envisaged the following aspects

1 Isolation of *Colletotrichum* sp from diseased specimens of mango

2 Symptomatology of anthracnose disease of mango

3 Variability in phenotypic characters of *Colletotrichum* isolates

4 Pathogenic variability of *Colletotrichum* isolates

5 Vegetative compatibility of *Colletotrichum* isolates

6 Variability of *Colletotrichum* isolates in sensitivity to plant protection chemicals

Review of literature

2. REVIEW OF LITERATURE

2.1. History

The fungal plant pathogen, *Colletotrichum* was first reported by Tode in 1790 under the genus *Vermicularia* and the name *Colletotrichum* was first proposed by Penzig in 1882, based on the characters of *Vermicularia gloeosporioides* where the pathogen was collected from *Citrus* orchard in Italy Corda (1831) erected the genus *Colletotrichum* in which he reported *Colletotrichum lineola* from *Apiaceae* family, in the Czech Republic This genus was then considered under "*Coelomycetes*" In 1904, Rolfs gave description of the fungus, occurred on various citrus trees and fruits in Florida In India, the fungus was first reported by Butler (1918) on coffee leaves

Taxonomic classifications of *Colletotrichum* were given by Arx and Muller (1954) which was known as the "von Arxian" taxonomic and was based on conidial shape and size *Colletotrichum* species estimation varied from periods where it ranged from 11 by Arx (1957), 22 by Sutton (1980) based on host specificity, shape and size of conidium, appressorium and presence of sclerotia In 1992 Sutton found about 40 species of *Colletotrichum* Sixty species were given in the Dictionary of the Fungi (Kirk *et al*, 2008), whereas there were 688 names (Anon , 2009) and 820 names in *Index Fungorum* (Anon , 2016) The genus *Colletotrichum* is a facultative parasite recently designated as the world's eighth most important plant pathogen (Dean *et al*, 2012)

Glomerella as sexual stage of *Colletotrichum* species was first reported by Shear and Wood (1907) Small (1926) also concluded *Glomerella cingulata* as sexual stage of *Colletotrichum gloeosporioides* This pathogen associated with 470 different hosts *Colletotrichum gloeosporioides* (Penz) Penz & Sacc is the most universal and extensively dispersed plant pathogen in the world (Jeffries *et al*,1990, Sutton, 1992, Cannon *et al*, 2000) This pathogen widely recorded in tropical and subtropical regions but also prevalent in temperate countries This

species was recognised as latent pathogen causing postharvest disease (Prusky and Plumbley, 1992, Pernezny and Marlatt 1993) The species which showed host specificity were intended to use as bio herbicides (Watson *et al*, 2000, Goodwin, 2001, Kaewchai *et al*, 2009)

Mango anthracnose was first noticed and reported from Puerto Rico by Collins (1903) and later on reported the incidence by Higgins (1906) from Hawaii Many scientists from different countries noticed mango anthracnose *viz* Fawcett (1907) from Florida, Cardin(1910) from Cuba, Taro (1929) from Columbia, Bitancounrt (1938) from Brazil, Traub and Robinson (1938) from United States and Sattar and Mallik (1939) from Pakistan

In India, McRae (1924) noticed many diseases of mango and also mentioned about mango anthracnose from Bengal This disease was first reported by Stevens and Pierce (1933) from Bombay Sattar and Malik (1939) reported severity of anthracnose from Punjab and noticed severe losses in India due to this disease They also reported the causative agents of the disease as *Glomerella cingulata* (Ston) Spauld and Sch *Colletotrichum gloeosporioides* Penz and *Gloeosporium mangiferae* P Henn The pathogen showed aggression on all plant parts *viz* leaves and petiole as well as on stem Mathur (1953) stated that *Colletotrichum gloeosporoides* as the main factor for the decay of cold stored mangoes in India Pathak (1981) reported that mango faced market crises due to post harvest damages caused by anthracnose disease This disease attacked many plant parts and manifested with symptoms *viz*, leaf spot, die back, necrosis, fruit infection, rots etc Ploetz and Prakash (1997) reported that post harvest infection was the most damaging which resulted in 15-20 per cent economical loss In the same year (1997), Dodd *et al* stated that anthracnose caused by *Colletorichum gloeosporioides* (Penz) Penz and Sacc , was the major field and post harvest disease in all mango growing areas of the world

Alfonso was one of the most popular varieties of mangoes grown in India (Subramanyam *et al*, 1972) In Kerala, maximum damage was observed in Neelum whereas, the variety Edward was reported to be resistant (Pandey *et al*,

2012) Many workers considered *C gloeosporioides* as a cumulative species composed of diverse sub population Fitzell (1979) reported *C acutatum* and Fitzell and Peak (1984) reported *C gloeosporioides* Penz var *minor* as the inciting agents of anthracnose disease in mango

Similarly there were many reports on variability in phenotypic, pathogenic and genetic characters of this pathogen Sutton (1992) described 7 formae speciales of *C gloeosporioides* and recognized the species as a heterogeneous group with a great variation in morphological characters Different levels of genetic variations according to host have been widely reported for *C gloeosporioides* (Hayden *et al*, 1994, Freeman *et al*, 1998) Serra *et al* (2006) suggested effective methods for differentiating the isolates of *C gloeosporioides* from cashew and mango based on pathogenicity, isozyme analysis and RAPD They also reported variations in comdial morphology, appressoria formation, sporulation and vegetative compatibility among different isolates of this pathogen Difference in host specificity of *C gloeosporioides* was due to distinct genetic clades (Abang *et al*, 2002, Munaut *et al*, 2002, MacKenzie *et al*, 2007) In India, the existence of two genetically distinct populations of *C gloeosporioides* in mango was reported by Chowdappa and Kumar (2012) Figueiredo *et al* (2012) observed genetic and pathogenic diversity in 18 isolates of *C gloeosporioides* from cashew and the isolates were clustered into two groups using UPGMA analysis of the RAPD and RFLP data

2.2. Symptomatology of anthracnose

Sattar and Mallik (1939) noticed anthracnose of mango from Punjab causing severe damage and studied the symptoms of this disease They observed drooping down of leaves, black discolouration on petiole and necrotic spots on twigs spreading from tip downwards Dickman and Alvarez (1983) observed *Colletotrichum gloeosporioides* causing anthracnose on papaya and noticed lesions on leaves which were circular to irregular with greyish centre, which

coalesced and later led to shot holes They also observed dark circular lesions on leaf petiole and deep lesions on fruits

Arauz (2000) described symptoms produced on mango caused by *Colletotrichum* sp He observed infection on leaves, twigs, inflorescence and fruits of mango Infection on leaves was noticed as irregular shaped black necrotic spots and also large necrotic spots along the leaf margins Fungus invaded into twigs and caused die back symptom Panicle anthracnose or blossom blight was noticed as elongated dark grey to black lesions In field condition, fruits of smaller size were aborted and larger fruits were also noticed Lesions of 2 cm size with indefinite border were observed on the fruit surface which was coalesced to cover large area of fruits In the advanced stage of the infection acervulus and salmon pink spore masses were noticed on the lesions Freire *et al* (2002) reported anthracnose of cashew from the north-eastern region of the Brazil They observed symptoms on young seedlings and adult plant and reported that the initial symptoms appeared as water soaked lesion and later the colour of the spots turned orange brown to light reddish brown and in severe condition leaves and fruits became totally blighted and dropped off which resulted in reduction of yield up to 50 per cent

Gosh *et al* (2003) observed the symptoms of mango anthracnose as spots with varied size (2-6 mm) They observed more than 90 per cent humidity and 30°C temperature were the conducive conditions for the infection to enlarge and cause rotting symptoms They stated that under high temperature and dry climate, fungus could not spread and hence drop off to form shot hole symptom On inflorescence or panicle axis, black spots were observed which became dry and withered before the fruit set Black spots were noticed on fruits and hardening of pulp under the infected area was also noticed In the same year (2003), Lim and Manicom described anthracnose symptom on guava They noticed water soaked light brown lesions on leaves, necrotic lesions on flowers and young branches and circular dry lesions, canker like pustules on mature fruits Under severe infection, the ripen fruit were mummified

Pitkethley and Conde (2007) explained the symptoms of mango anthracnose on leaves, twigs and fruits They observed infections on leaf margin and development of semicircular spots on young bronze coloured leaves Twigs appeared dark coloured from the tip backwards They observed large sunken spots followed by cracking of fruits On ripened fruits latent infection was seen which appeared as large greenish black areas on fruits sometimes leaves may defoliate Newly formed fruits showed large sunken spots Nelson (2008) noticed symptoms of mango anthracnose noticed on all plant parts On panicles, small blackish brown spots were observed which coalesced and enlarged, and killed the panicle before fruit set On leaves, the lesions begun as small angular, brown to black spots, later enlarged and during dry weather, lesions dropped off and formed shot hole symptom On petioles, twigs and stem typical black lesions were noticed Prominent sunken black spots were observed on fruits Dropping of premature fruits and rotting of severely infected fruits were observed second type of symptom noticed on fruits was " tear stain" symptom leading to " alligator skin" development

Sergeeva *et al* (2008) reported that *Colletotrichum acutatum* and *C gloeosporioides* were responsible for leaf spot of olives in Australia Infection was noticed from the edges of the leaves and also in midrib Light to dark brown spots and necrotic area with irregular shape of 1-3 cm diameter were observed Salmon coloured spore mass was also seen on affected leaves Anthracnose of dragon fruit caused by *C gloeosporioides* showed the characteristic symptoms of reddish brown lesions with cholrotic halos on stem and fruits In moisturized room, symptoms were found increased to form rotting of stem and fruits (Masyahit *et al*, 2009) Guettia *et al*, (2014) surveyed for the occurrence of mango fruit anthracnose in Abidjan of West Africa They observed symptoms after five to seven days of harvest, on which dark brown necrotic, sunken and irregular lesions with different size were observed The lesions spread rapidly on the surface and in advanced stage showed rotting symptoms Tear-stain lesions flow off from the stem-end to basal part of the fruits was noticed In Northern Tunisia, Rhaiem and Taylor (2016) observed typical dieback and wither-tip of

twigs in different varieties of citrus in major citrus producing area On leaves, circular light brown flat areas with purple margins were noticed whereas black grey spot were noticed on fruits Black coloured fructifications were observed on the surface of twigs

2.3. Cultural and morphological characters

Sattar and Mallik (1939) observed the development of acervulus of *Colletotrichum gloeosporioides* on diseased area of mango They isolated the pathogen and studied the phenotyphic character Acervulus of 80-20 µm size was observed in the culture Setae produced rarely which was dark cylindrical, continuous, fuliginous and measured about 40-90 x4-6 µm size The conidia were borne on well developed distinct conidiophores and they were hyaline one celled, straight oval or cylindrical and size ranges from 8-20 x 5-7 µm, with presence of oil globules Bose *et al* (1973) reported that comdial size of *Colletotrichum gloeosporioides* varied from 11-16 x -6 µm and acervuli measures about 115-467 x 15-22 µm size

Yee and Sariah (1993) isolated *Colletotrichum gloeosporioides* from cocoa and studied cultural characters Colony colour varied from white to olivaceous grey with aerial mycelium At the centre of the colony, orange conidial pustules were formed Smoky grey colour was observed on reverse side of the colony They also observed the morphological characters like conidial shape as cylindrical with obtuse ends, uninucliate with 5-22 flm x 2-6 flm formed in setose acervuli or as solitary phialides on mycelium Acervulus dark and measured about 60- 240 flm in diameter Setae thin and prolific, dark brown to black, 50- 170 flm long, straight or slightly curved with 1-4 septate and swollen at the base little curved towards the apex

Chowdhry and Varshney (2000) studied phenotyphic characters of *Colletotrichum gloeosporioides* under *in vitro* conditions and observed culture whitish mycelial colony Conidia were straight and obtuse at the apex and measured about 9-24 x 3-4 5 µm They also observed acervuli without seta and

also observed slimy spore mass in the culture *Colletotrichum gloeosporioides* colonies from *Aloe vera* appeared as white to grey, thick fluffy mycelium with dark orange coloured spore masses and on reverse side was white colour Conidia were, one celled, hyaline, ovoid to ablong and dumbbell shaped Comdial size varied from 12 5-18 x 3-5 µm Conidiophores were short, simple and erect Acervuli were formed in 15- 20 days of inoculation Setae were 1-4 septate, brown and ranged from 42-150 x 4-5 µm (Avasthi *et al*, 2001)

Davis (2003) reported *Colletotrichum gloeosporioides* from the leaf of ivy gourd (*Coccina grandis* (L) *voigt*) and described the cultural characters as pinkish white coloured colony initially and later turned to brown with greyish white colour Hyaline and branched hypha with 3 8 x 11 6- 19 4 µm size and she also observed one to two septate, dark brown setae Bag (2004) isolated *Colletotrichum gloeosporioides* from flower and fruits of papaya and observed cultural characters Mycelium was black and septate Spores were short, cylindrical, single celled and measured 13-18 x 4-5 µm Acervuli were dark and cushion shaped Setae were black, sepatate and slender

Based on the cultural characters of 13 isolates of *C gloeosporioides* from custard apple, Gaikwad and sawant (2005) concluded that there was significant difference in the various isolates of *Colletotrichum gloeosporioides* in size shape and colour of hyphae, conidia, setae, acervuli, perithecia, approsoria, asci and ascospores The isolates of *Colletotrichum* from citrus crop were found to produce grey and salmon cottony colonies, isolates from tree tomato formed orange colonies with hyaline or grey mycelium Isolates from mango showed wide variation from greyish white to orange thick cottony mycelium with numerous orange comdial masses The isolates from mango produced large sized comdia of 21 5 µm, whereas isolates from tomato and lime showed smaller sized comdia (Martinez *et al*, 2009) Masyahit *et al* (2009) identified the pathogen causing anthracnose of dragon fruit as *Colletotrichum gloeosporioides* and described colony as whitish orange with hyaline and septate hypha, capsule like

one celled conidia measured about 6-10 x 2-2 5 *µm* Circular, conidia bearing acervuli on conidigenous cell

Sangeetha (2009) obtained 7 isolates of *Colletotrichum gloeosporioides* from mango die back, observed slow growth rate of the isolates which took 6 days for full growth Colony was smooth and greyish white and later became thick and greyish black Pink coloured spore masses were observed on 10 days after incubation Acervulus formation was absent Conidia was hyaline, cylindrical, both ends were obtuse and single celled with globules, size varied from 11 9- 13 25 x 5 µm Hypha was branched and hyaline with 2 88- 3 26 µm width and septation at an interval of 17 66- 18 80 µm Sikirou *et al* (2011) defined cultural characters of *Colletotrichum gloeosporioides* inciting leaf twister disease in onion Initially mycelia were found white to grey and later turned to brown Conidia were aseptate, unicellular, hyaline and oval to cylindrical with round ends which measured about 10-20 µm x 3-5 µm size They observed numerous acervuli in the culture which were globouse to irregular shaped, dark brown to black coloured

Choi *et al* (2012) reported *Colletotrichum gloeosporioides* from tulip and explained, the cultural characters on PDA medium They observed white mycelia initially and later turned to grey Formation of salmon coloured spore mass was noticed in the culture Conidia were cylindrical and ovoid, measured about 10- 18 x 3-5 µm size Nilamudeen *et al* (2013) isolated *Colletotrichum gloeosporioides* from holy basil (*Ocimum tenuiflorum* L) in Kerala and explained the cultural characters of four days old fungal colonies They noticed produced white mycelium pinkish spore mass in the culture turned to pink Conidia were straight, and bullet shaped with oil globule at the centre and measured 3x 1 5 mm

Darshana *et al* (2014) studied *Colletotrichum gloeosporioides* causing leaf spot of ginger (*Zingiber officinale* Rosc) in some parts of Kerala and

Karnataka They observed change in conidial shape and were cylindrical with round ends, elliptical or dumbbell shaped and measured 12 2- 18 3 x 6 1- 6 9 µm size Gautam (2014) also reported that the conidia produced by *C gloeosporioides* were one celled, hyaline, straight cylindrical or dumbbell and obtuse at apices with size varied from 10-15 x 5-7 µm The colonies were woolly or cottony, greyish white to pale brown and mycelium hyaline, septate and branched Setae were dark brown, long and sepatate

The characterisation of the causal agent of mango anthracnose disease in Ghana was done by Honger (2014) He explained the cultural characters of *Colletotrichum gloeosporioides* as greyish white to dark grey aerial mycelia hyaline, unicellur, short, cylindrical with obtuse ends conidia He observed the development of acervuli in the culture which measured about 500 µm Setae with 4-8 X 200 µm were also observed in acervuli

Ismail *et al* (2015) characterised *Colletotrichum* sp obtained from anthracnose of mango in Italy Colonies were initially white turning purplish-grey on the upper surface with dense, raised, cottony mycelium Conida are hyaline cylindrical to ellipsoid with obtuse end on both ends of the spore and it measured about 11 2-13 8 x 1- 5 7 µm size

Suvarna *et al* (2015) collected 20 different isolates of *Colletotrichum gloeosporioides* isolates from mango The isolates produced white coloured colonies initially grey and later changed to whitish grey Mycelium septate and conidia was cylindrical, hyaline, aseptate with round ends and size ranged from 13 04- 16 62 x 4 08 – 5 46 µm On reverse side of the culture, concentric rings with light grey to dark grey discolouration were observed

2.4. Vegetative compatibility of *Colletotrichum* spp.

Vegetative compatibility test described by Puhalla (1985) was based on the ability of different isolates of same species forming heterokaryons by *nit* mutants (unable to utilize nitrate as a sole nitrogen source) and was used to study

genetic variation and characterization of population of asexual plant pathogenic fungi Brooker *et al* (1991) reported vegetative compatibility grouping between the isolates of *Colletotrichum* spp and in between *C gloeosporioides* reported by Freeman *et al* (1996) and Yang and Sweetingham (1998) Isolates that showed the ability of anastomose were considered as same vegetative compatibility group (VCG) The isolates within VCG showed more similar genetically than from the other VCGs Beynon *et al* (1995) selected six isolates of *Colletotrichum kahawae* from 47 isolates showing self-incompatiblity from coffee berry, which enabled the formation among *nit1* and *nit3* mutants of the 45 VGC were to be identified Freeman and Katan (1997) selected 113 isolates were belonged to single vegetative compatibility group out of 115 isolates obtained from foliar and root infecting *Colletotrichum* sp from strawberry in Israel Heilmann *et al* (2006) performed vegetative compatibility analysis exploiting *nit* mutants on 112 *Colletotrichum coccodes* isolates from potato where they selected 88 isolates and grouped into six VCGs and twenty-four isolates found incompatibility with any tester isolate Serra *et al* (2006) grouped isolates of *Colletotrichum gloeosporioides* from mango and cashew into two vegetative compatible groups VCG 1 with 4 isolates and VCG 2 with 2 isolates when the isolates exhibited the formation of stable heterokaryon

Shcolmck *et al* (2007) categorised 176 isolates of *Colletotrichum coccodes* from symptomatic tissues of potato tubers and stems into vegetative compatibility groups They grouped 153 isolates into eight VCGs whereas 23 isolates were not categorized into any of the VCGs and showed incompatibility with other isolates Anna *et al* (2010) found eight of the nine isolates proved to be self-compatible based on pairings of *nit* mutants from the different *Colletotrichum truncatum* isolates due to the formation of dense heterokaryons Among the 47 isolates of *C lindemuthianum* common bean (*Phaseolus vulgaris* L), Barcelos *et al* , 2011 observed the self-incompatibility of six isolates, where hercterokaryons were not observed at the line of intersection between colonies derived from same isolate

2.5. Pathogenic variability

Freeman and Shabi (1996) isolated forty two isolates of *Colletotrichum gloeosporioides* from almond, apple, avocado, mango and pecan Among the isolates inoculated on different host, the isolates from avocado and mango recorded significant difference in the lesion size but the isolates from mango showed less aggressiveness than isolates from apple

Cross inoculation studies conducted by Sanders and Korsten (2003), showed that isolates of *Colletotrichum gloeosporioides* from mango and avacado produced symptoms on other hosts such as guava, chili and papaya Guava found more susceptible to the isolates from mango and avacado and they were also concluded that *Colletotrichum* strains can infect more than one host and single host can also be infected by many *Colletotrichum* species Mango and banana fruits were found more susceptible to the isolates of *Colletotrichum gloeosporioides* from papaya and the isolates from mango and banana were also found infected on papaya But theses isolates showed least infection on grapes They observed highest lesion size in papaya (29 0 mm) and in mango (26 8mm) (Kumara and Rawal2004)

Colletotrichum gloeosporioides isolates from mango were cross inoculated on the detached mango, citrus and guava fruits, twigs and leaves The isolates produced 100 per cent typical symptom on mango and guava leaves but did not produce any symptoms on the citrus leaves (Sharma and verma, 2007) Cross inoculation of *C gloeoesporioides* from papaya, rambutan and mango expressed symptoms on all hosts Lesion diameter was highest when cross inoculated on respective hosts than on other hosts (Wijeratnam *et al*, 2008)

Martinez *et al* (2009) tested 30 isolates of *Colletotrichum gloeoesporioides* obtained from mango, on the mango fruits Among them only 19 isolates produced first symptoms on fourth day after inoculation They also observed negative results when inoculated on Tahiti lime and tree tomato crops

as there was no symptoms production and based on this they concluded existence of host specificity in the isolates Giblin *et al* (2010) showed the less susceptibility of isolates of isolatesof *Colletotrichum gloeoesporioides* from mango and avocado fruits whereas these isolates showed more aggressiveness to mango fruits

Lakshmi *et al* (2011) studied variation in cross infectivity among the *C gloeosporioides* isolates and reported that the isolates showed maximum per cent disease index, virulence index and minimum incubation period on their original host Among the selected fruit crops cashew, custard apple, mango, acid lime, papaya and pomegranate were less susceptible to this pathogen Variation in cross infectivity of *C acutatum* was studied on two different varieties of strawberry, variety Pegasus found more susceptible to *C acutatum* than the variety Elkas (Stankova *et al* , 2011) Devamma *et al.* (2012) studied variability among *Colletotrichum gloeosporioides* based on the per cent disease incidence, the isolates were classified into less virulent, moderately virulent and highly virulent They recorded maximum PDI of 76 30 per cent and less PDI of 12 55%

Pathogenicity testing of ten strains representing five species of *Colletotrichum* was carried out on chili, papaya, orange, rose apple, mango and guava using a wound drop technique Chili and rose apple were infected by the strain of *Colletotrichum asianum* isolated from coffee, whereas the strain isolated from mango infected only chili and mango (Phouhvong *et al* , 2012)

Honger (2014) studied the pathogenic variability of *Colletotrichum gloeosporioides* from the mango through cross infection and mango was found more susceptible to the pathogen compared to avocado and papaya Thirty isolates of *Colletotrichum gloeosporioides* Penz were inoculated on mango, pomegranate, mandarin, sweet orange, guava and custard apple Guava and custard apple were found to be less susceptible as compared to mango, orange,

pomegranate and sweet orange Mango was found susceptible to eighteen isolates (Joshi and Sawant, 2014) Sharma and Kulshrestha (2015) studied pathogenic variability of *Colletotrichum gloeosporioides* on detached leaves of various fruits *viz* , apple, guava, kiwi, mango and peach and observed symptom expression within 5-7 days of inoculation They noticed maximum pathogenicity on kiwi leaves and minimum pathogenicity on mango leaves

2.6. Evaluation of plant protection chemicals against *Colletotrichum* spp.

Deshmukh *et al* (1999) conducted *in vitro* studies of ten fungicides against *Colletotrichum gloeosporioides* causing anthracnose of anthurium They observed hundred per cent inhibition on the growth of the pathogen in Petri dishes by Bordeaux mixture (1 and 2%) copper oxychloride (0 2 and 0 3%), fosetyl-AL (0 1 and 0 2%), carbendazim (0 1 and 0 2%) and thiophenate methyl (0 1 and 0 2%) was very effective in complete controlling of fungal growth in Petri dish (cent per cent inhibition) Washathi and Bhargava (2000) reported that *Colletotrichum dematium* from chickpea was inhibited completely by tetra methyl thiuram disulphide (0 25%), mancozeb (0 2-0 25%), carbendazim (0 05-0 1%) and benomyl (0 15%) and concluded that this fungicides were very effective for the control of the pathogen

Patel and Joshi (2000) also observed cent per cent inhibition of *Colletotrichum gloeosporioides* isolates from turmeric leaf spot by systemic fungicides viz carbendazim, thiophanate methyl, propiconazole, hexaconazole and triclazole *In vitro* studies using different fungicides against *Colletotrichum* sp responsible for premature yellowing and bean shedding in vanilla showed that thiophanate methyl even at very low concentration i e 100 ppm was highly inhibitory to the fungus followed by carbendazim (250 ppm) or carbendazim + mancozeb mixture (200 ppm) (Bhai *et al*, 2003) Ashok (2005) reported that among contact fungicides evaluated against *Colletotrihum gloeosporioides* Penz obtained from vanilla Mancozeb showed best result (77 65 %) in inhibiting this

fungi It was observed that the fungicidal efficiency of mancozeb was found on par with copper sulphate (74 81%) and captan (70 97%)

Sharma and Veram (2007) observed complete inhibition of *Colletotrichum gloeosporioides* by carbendazim and metyl thiophanate fungicides whereas among the contact fungicide kavach and dithane M-45 showed cent per cent inhibition of growth at higher concentration of 1000 µg/ml followed by captan and Bordeaux mixture (1%) Sundravadana *et al* (2007) observed the efficacy of azoxystrobin against *Colletotrichum gloeosporioides* under lab conditions and recorded the complete inhibition of mycelial growth at 1 and above ppm on solid and liquid PDA medium

Filoda (2008) reported that the fungicide safurn was most effective chemical to inhibit the growth of *Colletotrichum gloeosporioides* isolated from anthracnose on *Hypericum perfo ratum* L causing anthracnose whereas Amistar showed less inhibitory property at 0 05 per cent, but significant inhibition was observed at 0 1 per cent Prashanth *et al* (2008) evaluated four systemic and contact fungicides each, under *in vitro* condition against *C gloeosporioides* Carbendazim, difenoconazole and carbendazim+ mancozeb were found to be most effective in reducing radial growth of fungus at higher concentrations (0 1%) Carbendazim+ mancozeb recorded maximum inhibition of 89 2 per cent, less inhibition of 53 7 per cent was recorded in chlorothalanil Propmeb and mancozeb showed results of 64 0% and 64 8 per cent inhibition respectively In the same year (2008) Jadhav *et al* isolated *Colletotrichum gloeosporioides* Penz from leaf spot of Kokum (*Garcinia indica* Choisy) seedlings and isolates were screened against nine fungicides under *in vitro* condition mancozeb + carbendazim (0 25%), propiconazole (0 1%), carbendazim (0 1%), tricylazole (0 15%) showed cent per cent inhibition of mycelial growth of the pathogen

Among seven systemic fungicides selected carbendazim, thiophanate-methyl and prochoraz at 50 ppm showed cent per cent inhibition on the growth

of *Colletotrichum gloeosporioides* from mango Copper oxycholride at 1000 ppm showed hundred per cent inhibition among selected two non systemic fungicides and mancozeb showed less effective against pathogen inhibition (Mathew *et al*, 2009) Tasiwal *et al* (2009) evaluated ten fungicides at 0 05%, 0 1% and 0 15% concentrations against *Colletotrichum gloeosporioides* from papaya Carbendazim was found to be very effective among all selected fungicides and showed cent per cent inhibition Propiconazole was effective only at 0 15% and gave 100 per cent inhibition followed by hexaconazole (94 33%) and triadiamefon (94 14%) at 0 15 per cent Among the five contact fungicides only Captan at 0 15 per cent was found highly effective and showed hundred per cent inhibition followed by macozeb (88 61%) at 0 15 per cent Patil *et al* (2010) tested fungicides on *Colletotrichumm gloeosporioides* from leaf blight of sapota under laboratory conditions Hexaconazole (0 1%), copper oxychloride (0 25%), difenconazole (0 1%), and propiconazole (0 1%) showed complete inhibition on the growth of the pathogen Benomyl (0 1%) and carbendazim (0 1%) were also found elective and showed 91 67 per cent and 90 00 per cent inhibition over control Mancozeb was found to be less effective

Nargund *et al* (2012) evaluated six systemic and six non systemic fungicides under *in vitro* conditions against *Colletotrichum gloeosporioides* isolated from anthracnose of pomegranate Iprobenfos showed 87 99 per cent inhibition of the pathogen followed by propiconazole which showed 87 10 per cent Among contact fungicides carbendazim + mancozeb recorded 75 10 per cent inhibition followed by captan (60 77%) They observed copper oxychloride as least effective against the pathogen Barhate (2012) evaluated eight fungicides under *in vitro* conditions against *C melongenae* causing fruit rot of brinjal Among them propiconazole (0 1%), carbendazim (0 1%) and hexaconazol (0 1%) showed cent percent inhibition of the pathogen and were recorded as most effective Singh *et al* (2012) studied *in vitro* effect of six fungicides for the management of *Colletotrichum gloeosporioides* causing coffee anthracnose and

found that hexaconazole 5% EC @ 250 ppm Carbendazım 50 % WP @ 500 ppm were most effective for the ınhıbıtıng the growth of pathogen

Ahmed *et al* (2014) studıed five fungıcıdes agaınst *C gloeosporıoıdes* of beetle vıne under *ın vıtro* condıtıon where propıconazole, tebuconazole and trıcyclazole showed complete ınhıbıtıon (100%) on the growth of the pathogen In the same year (2014), Azad *et al* tested the efficacy of fungıcıdes usıng poıson food technique agaınst *Colletotrıchum gloeosporıoıdes* Bavıstın (150 ppm), Kavach (500 ppm) was found the most effective ın ınhıbıtıng cent per cent growth of the pathogen Indofil M-45 and Blıtox-50 showed sımılar magnıtude of ınhıbıtıon Among four fungıcıdes selected, Bavıstın was found as the best even at 250 µg/ml concentratıon ınhıbıtıng complete growth of the fungus

Among the seven fungıcıdes evaluated agaınst *Colletotrıchum truncatum* causıng pod blıght of soybean by poısoned food technique, Deepthı *et al* (2015) reported that hexaconazole and tebuconazole were found to be best effectıve fungıcıdes for ınhıbıtıng pathogen and recorded 65% per cent and 83 37 per cent ınhıbıtıon respectıvely Jagpat (2015) reported that carbendazım + mancozeb at 0 3 % showed 82 10 percent ınhıbıtıon of mycelıal growth of *Colletotrıchum capsıcı* from turmerıc leaf blıght followed by chlorothalonıl wıth 75 80 per cent and captan 63 48 per cent The systemıc fungıcıdes hexaconazole recorded 32 82 per cent ınhıbıtıon and was recorded as least effectıve agaınst the pathogen Kale and Ukesh (2015) obtaıned 14 ısolates of *Colletotrıchum gloeosporıoıdes* and 6 ısolates of *Colletotrıchum dematıum* from soybean Among the three fungıcıdes evaluated agaınst the pathogen, propıconazole at 0 1% concentratıon showed ınhıbıtıon ın the range of 41 83 to 100 per cent Azoxystrobın recorded 51 73 to 100 per cent of fungal ınhıbıtıon, whereas hexaconazole showed 31 63 to 100 per cent ınhıbıtıon on the growth of the both pathogens

Materials and Methods

3. MATERIALS AND METHODS

The study on "Variability of *Colletotrichum* isolates inciting anthracnose in mango (*Mangifera indica* L)" was conducted in the Department of Plant Pathology, College of Horticulture, Vellamkkara, Thrissur during the period 2014-2016 The details of the materials used and the different techniques adopted for the investigation are described below

3.1. Collection of diseased specimens of mango

Diseased specimens of mango showing typical anthracnose symptoms on leaves, petiole, twigs and fruits were collected from Government farms, farmer's fields and private nurseries in Thrissur and Palakkad districts Diseased fruit samples were also collected from vegetable markets of Thrissur and Palakkad districts The varieties of mango commonly cultivated in Kerala *viz*, Muvandan, Neelum, Alphonso, Banganapalli, Prior, Sindhooram and Chandrakaran were selected for the study The locations from where the diseased specimens of mango were collected are given in Table 1

Table 1. Locations of collection of diseased specimens of mango

Districts	Sl. No.	Locations
Thrissur	1	Central nursery, KAU, Vellanikkara
	2	Sales counter, KAU, Mannuthy
	3	Krishi Vigyan Kendra, Thrissur
	4	Antony's nursery farm, Mannuthy
	5	Farmer's field, Angamali
	6	Farmer's field, Kodakara
	7	Farmer's field, Pattikkad
	8	Farmer's field, Puthukkad
	9	Vegetable markets, Mannuthy and Thrissur
Palakkad	1	Regional Agricultural Technological Training Centre, Malampuzha
	2	Farmers' fields, Chulliyarmedu

3.2. Isolation of pathogen

The diseased specimens collected from different locations were brought to the laboratory and washed thoroughly under tap water to remove dust and saprophytes present on the surface To check the signs of the pathogen associated with it, scrapings were taken from the infected area and mounted on a clean glass slide using lactophenol stain and covered with a clean cover slip The slides were observed under microscope to see the morphology of *Colletotrichum* sp Isolation of the pathogen was carried out on sterilized Potato Dextrose Agar (PDA) medium Infected area along with some healthy portions were cut into small bits and were surface sterilized with one per cent sodium hypochlorite solution for one minute followed by washing in three changes of sterile water The surface sterilized bits were transferred to Petri dishes mediated with solidified PDA medium All Petri dishes were incubated at room temperature (26 ± 2°C) and observed next day onwards for the growth of the pathogen Slides were prepared from the cultures of each isolates separately and observed under microscope to confirm the purity of pathogen Contaminated cultures were discarded The pure cultures of the isolates were maintained under refrigerated condition (4°C) by periodical subculturing for further research work

3.3. Pathogenicity test

Pathogenicity of all isolates obtained from seven different varieties of mango from different locations was tested by inoculating the pure culture of each isolate on the corresponding variety Inoculation was carried out on healthy leaves, twigs and fruits of respective variety Healthy twigs with healthy leaves and fruits of different varieties were collected and cleaned by washing with tap water From the actively growing pure culture of each isolate, a small bit of 10 mm culture was inoculated aseptically on the corresponding plant parts with an inoculation needle On leaves, inoculation was given on both the sides after giving pinpricks Then, the inoculated area was covered with a thin layer of moistened cotton The inoculated twigs with leaves were dipped in a conical flask containing water and covered with moistened polythene cover to maintain

sufficient humidity and incubated at room temperature Similarly the isolates of *Colletotrichum* sp obtained from twigs and fruits of different varieties were inoculated to the respective varieties of mango following this procedure given above Healthy leaves, twigs and fruits inoculated with blank PDA disc with or without pinprick served as control These were observed from next day onwards for the development of symptoms Observations on number of days taken for symptom initiation, colour, shape and size of the infected area were recorded The pathogen was reisolated from the artificially inoculated area on PDA medium and characters of the reisolated cultures were compared with that of the respective original cultures of the isolates

3.4. Symptomatology of anthracnose

Symptomatology of anthracnose disease in selected varieties of mango was studied under both natural and artificial conditions Symptoms developed on leaves, young twigs and fruits were recorded in detail under field condition These observations were recorded from the locations mentioned in 3 1 and variations in symptom expression if any, on different plant parts were noticed Symptoms developed on artificial inoculation during pathogenicity test were recorded and compared with symptoms noticed under field conditions

3.5. Variability in phenotypic characters of different isolates of *Colletotrichum* sp.

The phenotypic characters *viz*, cultural and morphological characters of different isolates of *Colletotrichum* sp from different varieties of mango were studied to find out variations if any, among them

3.5.1. Cultural characters

The cultural characters of different isolates of *Colletotrichum* sp were studied on PDA and Green Bean Agar (GBA) medium (Correll, 1993) (Appendix 1) Different characters of cultures such as colour, texture, shape, presence of zonation, colour on the reverse side of the medium, formation of

spores pink spore mass and acervuli in the culture were recorded on both the media

3.5.1.1. Preparation of GBA medium

Washed 400 g of beans thoroughly with water and cut into small pieces Beans were cooked in 500 ml of water, grounded it and took the extract To that, added molten agar (20 g), mixed thoroughly and made up the volume to one litre Transferred the medium to 250 ml conical flask @125 ml/ flask and sterilized in autoclave at 121^0C and 15 lb pressure for 20 mm

To study the cultural characters, 10 mm diameter disc of different isolates of *Colletotrichum* sp were cut from the actively growing cultures and transferred to the centre of Petri dishes mediated separately with PDA and GPA media All the Petri dishes were incubated at room temperature (26 ± 2 °C) Three replications were maintained for each isolates and for each medium Growth rate of each isolate was recorded by taking measurements on radial growth everyday till it attained full growth in Petri dishes The other cultural characters mentioned in 3 5 1 were also recorded

3. 5. 2. Morphological characters

The morphological characters of hyphae, spores, setae and fruiting body produced by different isolates of *Colletotrichum* sp were studied Colour and size of hypha, colour, shape and size of spores, and colour and size of setae of different isolates were recorded For that, permanent slides were prepared separately from different isolates and observed under microscope The size of the hyphae, spores and setae were recorded using image analyser Microphotographs of various structures of different isolates of *Colletotrichum* sp were also taken

2 Mutual inhibition with clear zone (MC)

3 Mutual inhibition of thick mycelial strand (MT)

4 Intermingling of hyphae (I)

5 Overgrowth (O)

3.8. *In vitro* evaluation on pathogenic variability of different isolates of *Colletotrichum* sp.

Variability in the pathogenicity of different isolates of *Colletotrichum* sp from seven varieties of mango was evaluated under *in vitro* condition The isolates obtained from each variety were cross inoculated on other varieties of mango The study was conducted on healthy detached leaves and fruits of mango and the isolates were inoculated as per the procedure given in 3 3 Three replications were maintained for each isolate inoculated on each variety of mango Leaves/ fruits of mango inoculated with medium alone served as control Observations as mentioned in 3 3 were recorded

3.9. Identification of pathogen

The cultural and morphological characters of different isolates of *Colletotrichum* sp were studied in detail and the characters were compared with the characters given in CMI descriptions of Pathogenic Fungi and Bacteria to identify the pathogen Based on these characters, the isolates were tentatively identified as *Colletotrichum* sp and for the confirmation aggressive seven isolates, each from a variety of mango were sent to National Centre for Fungal Taxonomy (NCFT), New Delhi

3.10. Evaluation on sensitivity of isolates of *Colletotrichum* sp. against plant protection chemicals

Any variation in the sensitivity of different isolates of *Colletotrichum* sp to commonly used plant protection chemicals in mango was evaluated under *in vitro* condition using poisoned food technique (Zentmeyer, 1955) The experiment was conducted on PDA medium and plant protection chemicals used for the study are furnished below

Table 2. Plant protection chemicals evaluated against different isolates of *Colletotrichum* sp.

A. Contact fungicides			
Sl. No.	**Chemical name**	**Trade name**	**Concentration (%)**
1	Bordeaux mixture	-	1
2	Copper hydroxide 77WP	Kocide	0 15
3	Mancozeb 75 WP	Indofil-M45 WP	0 3
B. Systemic fungicides			
1	Carbendazim 50 WP	Bavistin	0 1
2	Tebuconazole 25 9 EC	Folicur	0 1
3	Hexaconazole 5 EC	Contaf	0 1
4	Azoxystrobin 18 2 W/W	Amistar	0 15
C. Insecticides			
1	Malathion 50 EC	Malathion	0 1
2	Dimethoate 30 EC	Roger	0 05

Double strength PDA medium (potato 400 g, dextrose 40 g and agar 40 g in 1000 ml of water) was used for the study To the 250 ml conical flasks, 5 ml of

this medium was transferred and sterilized Weight of required quantity of plant protection chemicals was taken and exposed to UV light in laminar air flow chamber for 30 min for sterilization Fungicide and insecticide solutions were prepared by mixing the weighed chemicals separately in 50 ml of sterile water After melting and cooling of 50 ml of PDA medium, the fungicide and insecticide solutions were added to the medium in separate conical flasks to get the required concentrations of the chemicals Then transferred the chemicals mixed medium of 15-20 ml to separate Petri dishes and allowed to solidify From the seven days old pure culture of different isolates of *Colletotrichum* sp , 10 mm discs were taken and transferred to the centre of the Petri dishes mediated with the poisoned medium Petri dishes containing PDA medium alone served as control Three replications for each chemical and each isolate of pathogen were maintained Incubated all Petri dishes under room temperature and observations were taken on next day onwards for the growth of the pathogen Measurements on radial growth of the pathogen were recorded daily till attained full growth in the control plates

The percent inhibition on the growth of different isolates of the pathogen was calculated by using the formula given by Vincent (1947)

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

PI = Per cent inhibition

C = Growth of the pathogen in control (mm)

T = Growth of the pathogen in dual culture (mm)

3.11. STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was performed on the data using the statistical package Multiple comparisons among treatment means were done using Duncan's Multiple Range Test (DMRT)

Results

4. RESULTS

The objective of the present study on "Variability of *Colletotrichum* isolates inciting anthracnose in mango (*Mangifera indica* L)" was to investigate the variation among different isolates of *Colletotrichum* sp causing anthracnose disease on different varieties of mango grown in Thrissur and Palakkad districts of Kerala Variability among isolates with respect to symptomatology, phenotypic characters, pathogenicity, vegetative compatibility and sensitivity to plant protection chemicals were studied in detail and results are presented below

4.1. ISOLATION OF PATHOGEN

The pathogen associated with the diseased samples of seven varieties of mango *viz* , Muvandan, Neelum, Prior, Banganapalh, Alphonso, Sindhooram and Chandrakaran was isolated on Potato Dextrose Agar (PDA) medium A total of 30 isolates of *Colletotrichum* sp were collected from different locations of Thrissur and Palakkad districts (Table 3) Among these, six isolates were obtained from the variety Muvandan, eight from Neelum, four each from Prior, Banganapalh and Alphonso, three from Sindhooram and one from Chandrakaran Among the 30 isolates 18 isolates were obtained from leaves, 8 from twigs and four from fruits of these seven varieties of mango The isolates were named after variety and the diseased plant parts from where it was isolated All the 30 isolates were purified and maintained as pure cultures by subculturing at frequent intervals

Table 3. Details of different isolates obtained from Thrissur and Palakkad districts

Sl. No.	Isolates	Variety	Locations/places	Plant parts
1	ML1	Muvandan	KVK, Thrissur*	Leaves
2	ML2	Muvandan	Farmer's field, Chulıyarmedu**	Leaves
3	MI	Muvandan	Farmer's field, Pattıkkad *	Inflorescence
4	MT1	Muvandan	ATIC, Mannuthy*	Twigs
5	MT2	Muvandan	Mango orchards, Vellanıkkara**	Twigs
6	MF	Muvandan	Farmer's field , Pattıkkad*	Fruits
7	NL1	Neelum	Mango orchards, Vellanıkkara*	Leaves
8	NL2	Neelum	Farmer's field, Chulıyarmedu**	Leaves
9	NL3	Neelum	Farmer's field, Chulıyarmedu**	Leaves
10	NT1	Neelum	Mango orchards, Vellanıkkara*	Twigs
11	NT2	Neelum	Regional Agricultural Technological Training Centre, Malampuzha**	Twigs
12	NF1	Neelum	Vegetable market, Mannuthy*	Fruits
13	NF2	Neelum	Vegetable market, Thrissur*	Fruits
14	NF3	Neelum	Vegetable market, Thrissur*	Fruits
15	PL1	Prior	Regional Agricultural Technological Training Centre, Malampuzha**	Leaves
16	PL2	Prior	Antony's nursery, Mannuthy*	Leaves
17	PI	Prior	Mango orchards, CoH, Vellanıkkara*	Inflorescence
18	PT	Prior	Farmer's field, Chulıyarmedu**	Twigs
19	BL1	Banganapallı	Mango orchards, Vellanıkkara*	Leaves
20	BL2	Banganapallı	Farmer's field, Chulıyarmedu**	Leaves
21	BL3	Banganapallı	Farmer's field, Chulıyarmedu**	Leaves
22	BT	Banganapallı	Farmer's field, Chulıyarmedu**	Twigs
23	AL1	Alphonso	Mango orchards, Vellanıkkara*	Leaves
24	AL2	Alphonso	Farmer's field, Chulıyarmedu**	Leaves
25	AL3	Alphonso	Regional Agricultural Technological Training Centre, Mallampuzha**	Leaves
26	AL4	Alphonso	Farmer's field, Chulıyarmedu**	Leaves
27	CT	Chandrakaran	Mango orchards, Vellanıkkara*	Twigs
28	ST	Sındhooram	Mango orchards, Vellanıkkara*	Twigs
29	SL1	Sındhooram	Mango orchards, Vellanıkkara*	Leaves
30	SL2	Sındhooram	Farmer's field, Puthukkad*	Leaves

ML Isolates from Muvandan leaves
MI Isolate from Muvandan Inflorescence
MT- Isolates from Muvandan twigs
MF Isolate from Muvandan fruits
PL- Isolates from Prior leaves
PI Isolate from Prior inflorescence
PT- Isolate from Prior twigs
SL Isolates from Sindhooram leaves
* Thrissur district

NL Isolates from Neelum leaves
NT Isolates from Neelum Twigs
NF Isolates from Neelum fruits
BL- Isolates from Banganapalli leaves
BT Isolate from Banganapalli twigs
AL Isolates from Alphonso leaves
CT Isolate from Chandrakaran twigs
ST Isolate from Sindhooram twigs
** Palakkad district

4.2. PATHOGENICITY

Pathogenicity of all the 30 isolates of *Colletotrichum* sp was proved on their respective hosts The observations on the symptom development were recorded from second day after inoculation (DAI) (Plate 1a & 1b)

4.2.1. Isolates of *Colletotrichum* sp. from Muvandan

The six isolates of pathogen obtained from the variety Muvandan took two to five days for symptom expression on leaves and fruits The isolate MT2 initiated symptom on second days after inoculation (DAI) On fifth day the infection enlarged and became dark brown, irregular shaped and 13 mm diameter sized lesions with dark grey mycelial growth on the surface of the inoculated area The isolates *viz*, ML1, ML2, MI and MF recorded initial symptom expression on third DAI with diameter of 4, 6, 8, 9 mm respectively without any mycelial growth The isolates MT1 initiated symptom as minute black irregular spot of one millimetre size and was observed on fourth DAI

4.2.2. Isolates of *Colletotrichum* sp. from Neelum

On the leaves and fruits, the isolates NL1, NT1, NF1, NF2 and NF3 produced symptoms on second DAI These isolates showed brown to dark black lesions with size of 11, 7, 8, 12, 10 mm diameter respectively The isolates NL2 and NT2 initiated symptoms on third DAI with white mycelial growth on the inoculated area The isolates NL3 recorded symptom initiation on fourth DAI and produced small angular brown spots measuring 2 mm diameter

4.2.3. Isolates of *Colletotrichum* sp. from Prior

The initial symptom development on leaves was recorded on second DAI by all the four isolates of pathogen The isolate from Prior variety (PL1) produced the largest lesion of 12 mm diameter and was irregular, light brown with black thin margin the other three isolates *viz*, PL2, PI and PT showed brown to dark

Plate 1. Pathogenicity of *Colletotrichum* isolates under *in vitro* condition

Plate 1a On leaves

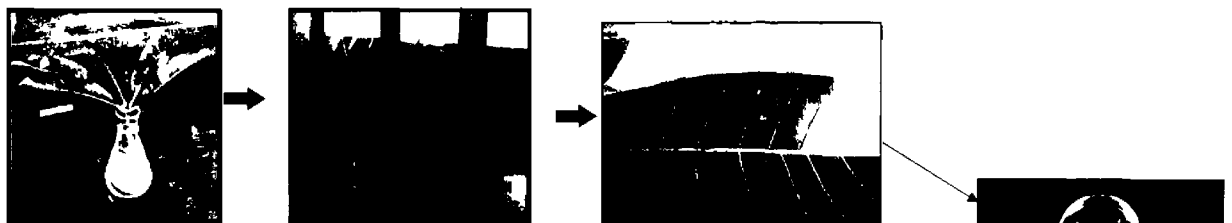
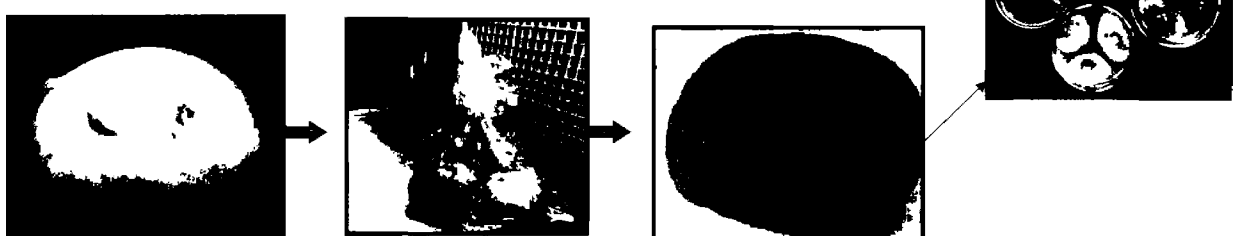


Plate 1b On fruits



brown lesions without any margin and were measured about 10, 9, 4 mm respectively

4.2.4. Isolates of *Colletotrichum* sp. from Banganapalli

On third DAI initial symptom expression was recorded by all the four isolates All these isolates showed similar symptoms development, such as angular, dark brown lesions sized 4, 10, 3, 12 mm by BL1, BL2, BL3 and BT isolates respectively Grey coloured mycelial growth was noticed on the pinpricked area inoculated with the isolate BT

4.2.5. Isolates of *Colletotrichum* sp. from Alphonso

All the four isolates from the variety Alphonso expressed initial symptom development on second DAI Brown to black irregular lesions with 6, 9, 10 and 11 mm diameter size was recorded by the isolates AL1, AL2, AL3 and AL4 respectively

4.2.7 Isolates of *Colletotrichum* sp. from Chandrakaran

The isolate CT obtained from the variety Chandrakaran showed the initial infection on third DAI On the infected area it produced dark brown lesions of 11 mm and on that thin mycelial growth was also noticed

4.2.6. Isolates of *Colletotrichum* sp. from Sindhooram

The isolate SL1 showed irregular, light brown lesions with pale red centre bounded with dark margin measured 12 mm diameter, which initiated on second DAI SL2 and ST recorded first symptom expression on third day The isolate SL2 showed irregular light brown lesions with dark margins sized about 9 mm diameter, whereas ST showed 4 mm dark black lesions without any mycelial growth on the inoculated area

4.3. SYMPTOMATOLOGY

Symptomatology of anthracnose disease was recorded under natural condition during the survey conducted for the collection of diseased specimens from seven selected varieties of mango Slight variation in the symptoms developed on the leaves of different varieties was recorded

4.3.1. Symptoms produced by *Colletotrichum* sp. on selected varieties of mango under natural conditions.

Symptomatology of the anthracnose disease on the selected seven varieties *viz*, Muvandan, Neelum, Prior, Banganapalli, Alphonso, Chandrakaran and Sindhooram were observed under natural condition

4.3.1.1. Symptoms on variety Muvandan

Symptoms of disease were observed on the seedlings and mature trees on leaves, twigs, inflorescence and fruits On seedlings, leaf spot and die back were noticed as the main symptoms On the young leaves irregular shaped brownish black spots were developed which later enlarged and centre portion fell down and showed shot hole symptom Leaf margins were also infected and produced similar type of infection The infected area later tear off and showed irregular shaped leaf margins Die back of the young twig was another symptom observed in nursery plants Small black lesions were developed on the tip of the growing twig which later coalesced and spread downward and resulted in dieback symptom (Plate 2a)

In matured tree, infections were observed on leaves, twigs, inflorescence and a fruits On the tender leaves, small black coloured dot like spots were formed which later coalesced to form large sized, irregular, reddish brown spots In advanced stage, the centre portion fell off and showed typical shot hole symptom On matured leaves two types of symptoms were observed Small sized (1 2 mm diameter), round shaped, reddish brown to black spots with black margin were observed along the midrib and veins Later these spots enlarged to a

size of 4-5 mm diameter and became light brown to black with distinct black margin

Another symptom observed on the leaves was the development of small sized irregular shaped greyish white spots with dark margin (Plate 2a) Later they coalesced and formed large sized irregular spots with dark margin In the centre of spots dark dots were observed which indicated the presence of fruiting body of the pathogen In advanced stage, this infected area dried up, fell down and produced shot hole symptom In cases where the infection observed on leaf margin, the advanced stage of the infection resulted in irregular shape of leaves Infection was noticed on young twigs of the matured tree where elongated black lesions were developed on the twigs which later enlarged and resulted in die back symptom

Symptoms of anthracnose disease in the fruits were not observed in the field Symptoms were observed on half ripened to completely ripened fruits after the harvest Large number of small pin point like dots were observed on the fruit surface Some dots were enlarged and became black coloured, round, sunken spots without any definite margin Later these spots coalesced and became large lesions covering about half of the fruit surface In early stage, it was observed that the infection was restricted only on the rind, but in advanced conditions rotting of the entire fruit was noticed The pathogen produced fruiting body on the fruit which appeared as dark dots on the lesions In some fruits tear stain symptom was observed, in which the sunken spots were developed in line from the stalk end to the tip of the fruit (Plate 2a)

4.3.1.2. Symptoms on variety Neelum

Small reddish brown to black coloured minute spots were observed on the leaf lamina near the veins and midrib Some spots were developed on the veins and the centre of the spots fell down and showed shot hole symptom Large greyish white coloured lesions with dark brown margin were also developed on the leaves Fruiting body formation was clearly visible on the infected area as small black dots In advanced stage, the infected area teared off and produced large

sized holes on the leaves Leaf margin and leaf tips were also infected by the pathogen and that resulted in drying up of the leaves Symptoms observed on twigs were similar to that noticed on the variety Muvandan (Plate 2b) On fruits, small dark to large lesions covering about half of the fruit surface from the stalk base was observed

4.3.1.3. Symptoms on variety Prior

Small round to irregular shaped reddish brown spots were observed on young leaves Such spots have dark brown margin and light yellow halo Most of the spots remained as distinct On some leaves, the leaf tip showed the formation of large brownish black lesions with dark margin and light yellow halo Shot hole symptom was also observed on leaves Infection was observed on twigs as black lesions which later spread and caused dieback symptoms (Plate 2c)

4.3.1 4. Symptoms on variety Banganapalli

Leaves and twigs were found to be infected by the pathogen On tender leaves, reddish brown, irregular spots bound by the veins were observed These spots were enlarged and coalesced which resulted in drying of tender leaves On leaf petiole, black discolouration was noticed that resulted in defoliation In matured leaves, black irregular spots were observed with or without yellow halo near the midrib, veins and veinlets Spots coalesced and enlarged to cover large area In some leaves it was found that, these spots remained as distinct black spot with reddish tinge, in the centre Shot hole symptom was also observed On young twigs, infection was noticed where the pathogen produced black lesions which enlarged and caused dieback symptom (Plate 2d) Clear tear stain symptom was observed on the fruits from the stalk to tip

Plate 2. Symptomatology of anthracnose disease of mango

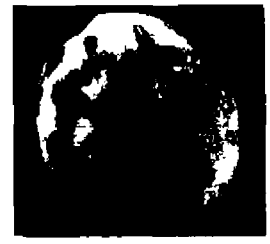
Plate 2a. Symptoms on variety Muvndan

Black spots with shot hole

Lesion with margin

Twig infection

Blossom blight



Sunken spots

Tear stain

Plate 2b. Symptoms on variety Neelum

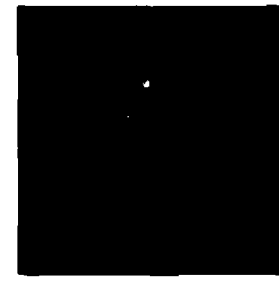
Lesion near veins

Infection on leaf margin

Greyish white spots

Lesion on twigs

Die back



Lesion on fruits

Plate 2c. Symptoms on variety Prior

Spots near veins

Lesions on petiole

Lesions on twig

4.3.1.5. Symptoms on variety Alphonso

Small reddish brown to black distinct spots with brown margin were observed on the leaves of the variety Alphonso Most of these spots were found bounded by the veins and veinlets and were surrounded by yellow halo Later the centre portion of the spots fell down and showed shot hole symptom

Symptoms similar to that developed in the variety Muvandan were also observed in the variety Alphonso Irregular shaped greyish to light brown coloured spots with dark brown margin and light yellow halo were observed on the leaves They were also bounded by the veins and few such spots coalesced and formed dark brown lesions Dropping of the infected parts resulted in the formation of large gap in that area Infection on twigs was noticed and this pathogen produced black coloured and finally that resulted in dieback (Plate 2e)

4.3.1 6. Symptoms on variety Chandrakaran

Symptoms of the anthracnose diseases were recorded from leaves, twigs, and fruits of matured tree On leaves the infection started as small round to irregular shaped spots with light brown margin and yellow halo Infection was also noticed along the leaf margin where the lesions were developed with dark brown margin and yellow halo In advanced stage, the infected area fell down from the leaf margin and resulted in irregular shape of the leaves

Infection on young twigs first appeared as small black dots which later enlarged and developed into black lesions Ripened fruits were infected by the pathogen and produced large sized black sunken spots with fimbriated margin on fruit surface These spots were coalesced and caused infection on large area of mango fruit (Plate 2f) Small clear sunken spots were covered all over the fruit surface causing mummification at advanced stage

4.3.1.7. Symptoms on variety Sindhooram

Symptoms of the anthracnose disease were observed on leaves and twigs Symptoms noticed were almost similar to that of other varieties like Muvandan On leaves and twigs small reddish brown to black spots with dark margin and light yellow were observed The spots developed were near to the veins of the leaves and appeared to be distinct On some leaves large reddish brown lesions were developed on leaf margin which later dried and fell down giving an irregular shape to the leaves Black lesions formed on the twigs coalesced and resulted in dieback symptom (Plate 2g)

4.3.2. Symptomatology in artificial condition

Symptomatology of the anthracnose disease on artificial condition was recorded during the pathogenicity test The symptoms developed on different plant parts of these varieties were appeared to be almost same Not much variation in the symptom expression was noticed compared to natural condition In general, all the 30 isolates produced light brown to black coloured irregular shaped lesions on their respective variety of mango In the variety Sindhooram, the isolate SL1 produced light brown lesions with pale red centre bounded with dark margin on the leaves The size of the lesions developed by the isolates on the different varieties was measured on five DAI and found variation in the size of the infected area developed by different isolates It was varied from 2 mm to 13 mm in diameter on 5 DAI The isolate MT2 produced the largest lesion having 13 mm diameter with dark grey mycelial growth on the infected area The isolate MT1 and AL3 showed small sized spots of two millimetres in diameter In the field condition, yellow halo was observed around the spots in all varieties except Muvandan and Neelum But in artificial condition, yellow halo formation was not observed

Plate 2d. Symptoms on variety Banganapalli

Lesions on young leaves

Lesion on margin

Fear stun

Plate 2e. Symptoms on variety Alphonso

Spots with yellow halo

Lesions on margin

Die back

Plate 2f. Symptoms on variety Chandrakaran

Infection on margin

Twig infection

Sunken spots

Plate 2g. Symptoms on variety Sindhooram

Lesion on young leaves

Infection on margin

Die back

4.4. VARIABILITY IN PHENOTYPIC CHARACTERS OF SELECTED ISOLATES OF *COLLETOTRICHUM* SP.

Phenotypic character of the 30 isolates of *Colletotrichum* sp obtained from the selected varieties of mango was subjected in detail to know the variations in the cultural and morphological characters The results are presented below

4.4.1. Cultural characters

Cultural characters such as colour, texture and shape of the colony, development of zonations in the culture, colour on reverse side of the culture, production of spores, acervulus and spore mass formation and growth rate of the selected isolates were studied on two different media *viz*, PDA and GBA Observations were taken till 30 days after incubation (DAI) The results are presented in Tables 4 to 6

4.4.1.1. Cultural characters of different isolates of *Colletotrichum* sp. on PDA medium

4.4.1.1.1. Isolates of *Colletotrichum* sp. from Muvandan

The colony characters of six isolates of *Colletotrichum* sp from Muvandan variety were studied on PDA medium and the results are presented in Table 4 Slight variation in the colony characters among these isolates was observed All the isolates except ML1 produced light grey to dark grey coloured colony growth The isolate MF showed thin cottony texture of the mycelium whereas ML1 and ML2 produced thick woolly and MI, MT1 and MT2 showed thick cottony texture All the isolates except MT1 showed regular shape of the colony Zonation in the cultures was observed only in ML2 and MT1 isolates All isolates showed light greenish grey colour development on reverse side of the culture (Plate 3)

Conidia formation was observed in all the isolates and it took 5-7 days for development of conidia The acervulus formation and pink spore mass formation

were observed in isolates *viz*, ML1, MT1, and MF It took 2-5 days for the development of pink spore mass after the acervulus formation

4.4.1.1.2. Isolates of *Colletotrichum* sp. from Neelum

The data presented in the Table 4 showed variations in cultural characters among the eight isolates of Neelum The colony colour of these isolates varied from white to dark grey White colony was observed in NF3 whereas dark grey colour was noticed in NL2 and NL3 The isolates showed thin or thick cottony growth except NL2, where it showed thick woolly texture In all isolates regular growth was noticed except in NF2 where the colony growth was irregular Zonation pattern of cultural growth was observed only m NL2 The isolates NF2 and NF3 showed pale white to light grey colour on the reverse side of the Petri dishes whereas, all other isolates showed light greenish grey to black colour on the reverse side of the culture (Plate 3)

Spore formation was noticed in 5-8 DAI The isolate NF1 took longest time of 8 days for spore formation whereas, the isolates NT2, NF2 and NF3 took the shortest period of 5 days for spore development Among the eight isolates, only three isolates produced acervulus *viz*, NT1, NF1 and Nf3 which took 12-20 days for the acervulus formation Pink spore mass formation was noticed only in NT1 (21 DAI) and NF3 (22 DAI)

4.4.1.1.3. Isolates of *Colletotrichum* sp. from Prior

The isolates PL1, PI and PT showed smoky white with thick cottony growth, whereas the isolate PL2 showed slight variation from other isolates and produced white coloured thin cottony culture (Table 5) Only PI isolate showed irregular colony growth and zonation was not observed in the culture of any isolates In PL1 and PL2, pale white to white colour was noticed on the reverse side of the colony whereas, PI and PT showed black to greyish black colour development The isolates from Prior took 6-7 days for the spore formation Acervulus and pink spore mass formation were not observed in these isolates (Plate 4)

Table 4. Cultural characters of different isolates of *Colletotrichum* sp. from Muvandan and Neelum on PDA medium

Sl No	Isolates	Colony characters							
		Colour	Texture	Shape	Zonation	Colour on reverse side of culture	Spore formation (DAI)	Acervulus formation (DAI)	Pink spore mass formation (DAI)
1	ML1	White	Thick woolly, fluffy at centre	Regular	No	Light grey	5	12	17
2	ML2	Light grey	Thick woolly	Regular	Yes	Dark grey	6	-	-
3	MI	Light grey with white	Thick cottony	Regular	No	Dark grey	7		
4	MT1	Light grey	Thick cottony	Irregular	Yes	Light grey	5	14	16
5	MT2	Light grey with white	Thick cottony, fluffy at centre	Regular	No	Light grey	5		
6	MF	Dark grey	Thin cottony	Regular	No	Dark grey	6	12	16
7	NL1	Light grey	Thin cottony	Regular	No	Grey	6		
8	NL2	Dark grey	Thick woolly	Regular	Yes	Grey	7	-	
9	NL3	Dark grey	Thin cottony, fluffy at centre	Regular	No	Black	6	-	-
10	NT1	Whitish grey	Thick cottony	Regular	No	Dark grey	7	17	21
11	NT2	Light grey	Thin cottony	Regular	No	Black	5		-
12	NF1	Smoky white	Thick cottony cushion	Regular	No	Dark grey	8	12	-
13	NF2	Light grey with white	Thick cottony, fluffy at centre	Irregular	No	Light grey	5	-	-
14	NF3	White	Thick cottony fluffy at centre	Regular	No	Pale white	5	20	22

ML Isolates from Muvandan leaves

MI- Isolate from Muvanda Inflorescence

MT- Isolates from Muvandan twigs

MF Isolate from Muvandan fruits

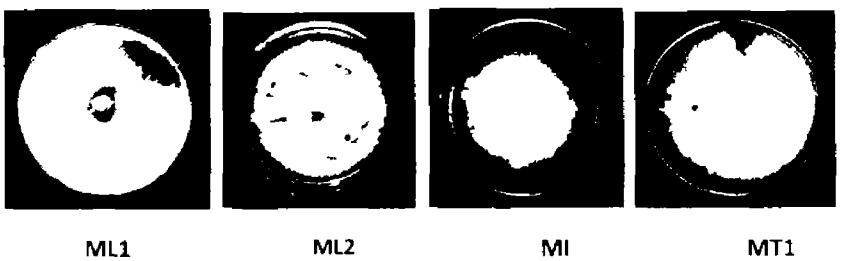
NL- Isolates from Neelum leaves

NT Isolates from Neelum Twigs

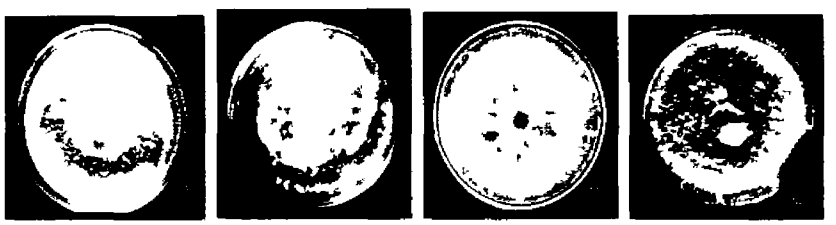
NF- Isolates from Neelum fruits

DAI- days after incubation

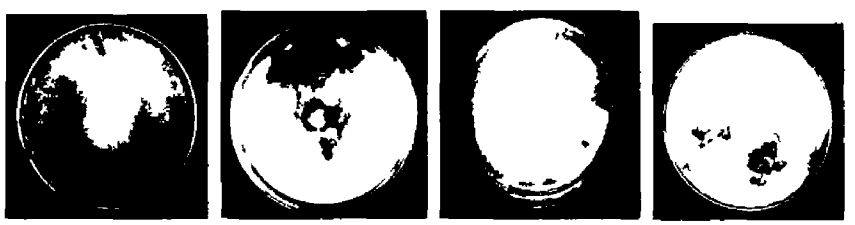
Plate 3 Cultural characters of *Colletotrichum* isolates from Muvandan and Neelum on PDA medium



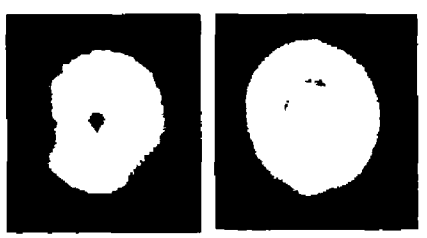
ML1 ML2 MI MT1



MT2 MF NL1 NL2



NL3 NT1 NT2 NF1



NF2 NF3

4.4.1.1.4. Isolates of *Colletotrichum* sp. from Banganapalli

Among the isolates from Banganapalli, BL3 and BT isolates showed similar cultural characters such as grey coloured thick cottony mycelia (Table 5) The isolates BL1 showed light grey and thick woolly growth whereas in BL2 smoky white with thick cottony mycelial cushion was observed All isolates showed regular to dark grey colour development The isolate BL3 showed spore formation on fourth day and all other isolates produced spore on sixth day Acervulus formation was observed only in two isolates *viz* , BL1 and BT which was noticed on 13 and 16 DAI respectively Pink spore mass formation was observed only in the isolate BL1 which was noticed on 19 DAI (Plate 4)

4.4.1.1.5. Isolates of *Colletotrichum* sp. from Alphonso

Not much variation in the cultural characters was observed among the isolates from Alphonso (Table 6) The colour of the colony was found to vary from smoky white to light grey All isolates produced thick cottony growth except in AL4 where it showed thin cottony mycelia Irregular growth was noticed only in AL1 Zonation was not observed in any isolates All isolates showed dark grey to black colour development on reverse side of the culture Spore formation was observed in 4 to 6 DAI and the isolates AL1 and AL3 took four and six days respectively for the spore formation Acervulus and pink spore mass formation were absent only in one isolate, AL1 which was recorded as 15 and 19 DAI respectively (Plate 4)

4.4.1.1.6. Isolates of *Colletotrichum* sp. from Chandrakaran

The isolate CT from Chandrakaran showed white, thick cushion like mycelial growth with woolly texture (Table 6) The culture showed regular shaped growth with zonation On the reverse side of the dish, grey coloured concentric rings were noticed The spore development was noticed on 7 DAI and the acervulus formation and pink spore mass formation were noticed on 12 and 18 DAI respectively (Plate 4)

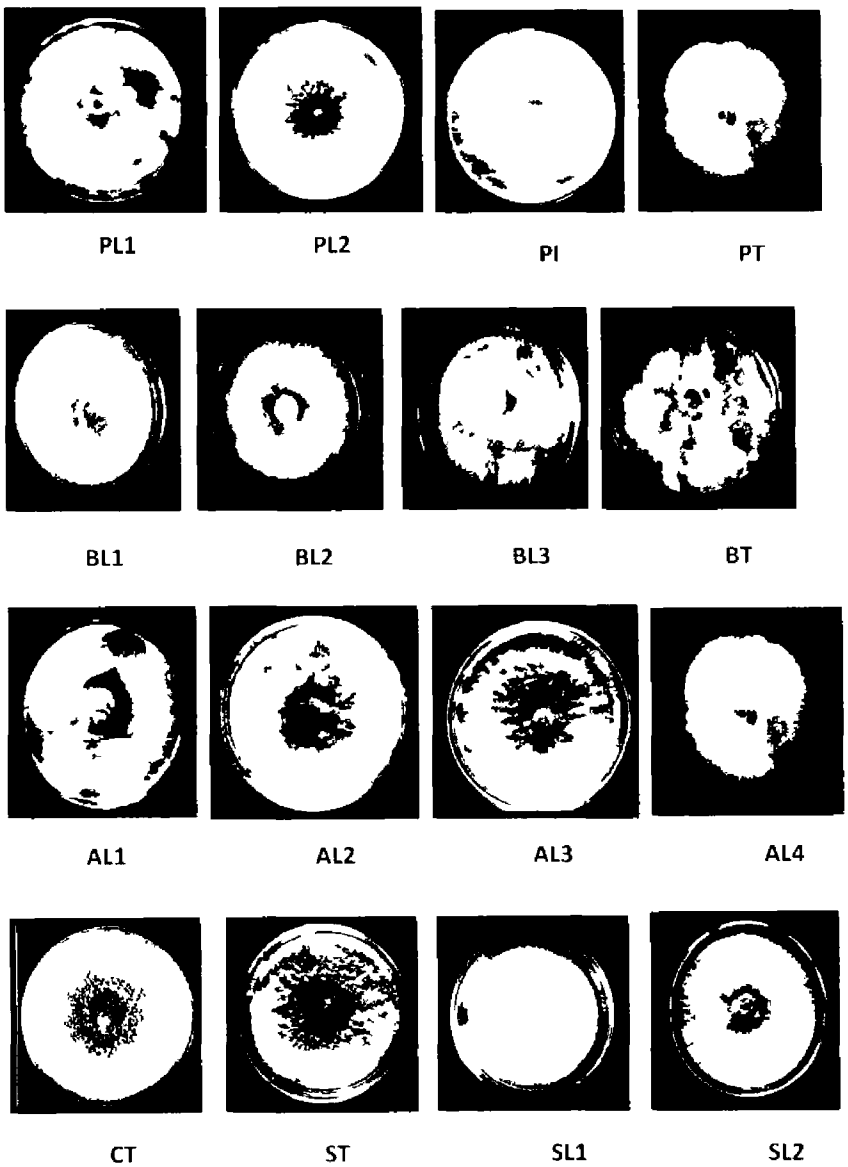
4.5. VEGETATIVE COMPATIBILITY OF DIFFERENT ISOLATES OF *COLLETOTRICHUM* SP.

The vegetative compatibility of 30 isolates of *Colletotrichum* sp was studied under lab condition by dual culture method to find out vegetative compatible group among them The results are given in Table 19, from the data it was recorded that all the isolates showed compatible reactions with other isolates which was found in the range of 2 to 9 isolates All the isolates were found compatible with the respective same isolates and showed mutual intermingling of hyphae in the Petri dishes The highest compatible combinations were observed in the BL2 which showed free intermingling of hyphae of eight other isolates of *Colletotrichum* sp *viz*, MI, MT2, NL3, NT2, NF3, PL1, BL1 and BL3 It was followed by ML1 which recorded the maximum compatibility with seven other isolates The lowest vegetative compatible reaction was recorded in isolates *viz*, PI, PT, BT, AL1, SL1 and SL2 which showed only one compatible pair (PIxST, PTxNT1, BTxMF, AL1xAL2, SL1xPL1 and SL2xML1)

The vegetative compatibility of six isolates from Muvandan was evaluated with the isolates from other varieties The results showed that the isolate ML1 recorded maximum compatible group of seven with other isolates and was followed by MI and MT2 which recorded three compatible pairs each Among the 8 isolates from Neelum variety, four isolates each were compatible with five other isolates of pathogen which included the isolates from Muvandan, Neelum, Prior, Alphonso and Sindhooram

The four isolates from the variety Prior *viz*, PL1, PL2, PI and PT, recorded the compatible reactions of the vegetative growth with one isolate from Muvandan, four from Neelum, three from Sindhooram, two from Alphonso and one from Banganapalli The highest compatible pairs were recorded by PL2 (6 nos) which included two isolates each from Neelum, Alphonso and one each from Banganapalli and Sindhooram The isolates from the variety Banganapalli did not produce any compatible reactions with the isolates from the Alphonso and Sindhooram Only one isolate, BL1 showed intermingling of hypha with the

Plate 4 Cultural characters of *Colletotrichum* isolates from Priot Banganapalli, Alphonso, Chandrakaran, Sindhooram on PDA medium



4.4.1.1.7. Isolates of *Colletotrichum* sp. from Sindhooram

The colony colour among the isolates was found to vary from white to light grey (Table 6) Thick cottony growth was observed in ST and SL2 The isolate SL1, showed thick mycelial cushion with woolly surface All isolates showed regular colony growth without any zonation On the reverse side of the Petri dishes small dark grey to small black spots (SL2) were observed Spore formation was observed in 3- 7 days The isolate ST, took the shortest period of three days and the isolate SL1 took the longest period of seven days for spore formation Acervulus (21 DAI) and spore mass (25 DAI) was noticed only in the isolate SL1 (Plate 4)

4.4.1.2. Cultural characters of selected isolates of *Colletotrichum* sp. On GBA medium

The cultural characters of 30 isolates of *Colletotrichum* sp were studied on GBA medium (Tables 7 to 9) The isolates showed slight variation in the in the colony characters The colour of the colony varied from white to smoky white to light grey All the isolates except five *viz* , MI, NT2, PL2, PT and AL4 showed white coloured colony on GBA medium Colony texture was not much varied where most of the isolates showed thick cottony cushion growth Thin cottony growth was observed only in MI, NL3, NT2, NF3 and PT isolates All isolates showed regular growth without any zonation Colony colour on the reverse side of the culture varied from white to black colour with small dark spots in the isolates like MT2, ST and SL2 The isolates which showed white (PT, CT, and SL1) to pale white (NF3, PL1, BL1, AL1 and AL2) colour on the reverse side of the culture remained as such without any colour development even after prolonged incubation (Plate 5 & 6)

Spore formation on GBA medium was observed on three to five DAI Among the 30 isolates, 14 isolates showed the spore development on three DAI, 9 isolates on four DAI and 7 isolates on five DAI Development of acervulus and pink spore mass were not observed in any of the isolates on GBA medium

Table 6. Cultural characters of different isolates of *Colletotrichum* sp. from Alphonso, Chandrakaran and Sindhooram on PDA medium

Sl. No.	Isolates	Colony characters							
		Colour	Texture	Shape	Zonation	Colour on reverse side of culture	Spore formation (DAI)	Acervulus formation (DAI)	Pink spore mass formation (DAI)
1	AL1	Smoky white	Thick cottony cushion	Irregular	No	Black	4	15	21
2	AL2	Light grey	Thick cottony	Regular	No	Black	5	-	-
3	AL3	Light grey	Thick cottony	Regular	No	Dark grey	6	-	-
4	AL4	Light grey	Thin cottony	Regular	No	Dark grey	5	-	-
5	CT	White	Thick cushion, woolly	Regular	Yes	Grey	7	12	18
6	ST	Light grey	Thin cottony	Regular	No	Dark grey	3	-	-
7	SL1	White	Thick cushion, woolly	Regular	No	Black	7	21	25
8	SL2	Pale white	Thick cottony	Regular	No	Black	4	-	-

AL- Isolates from Alphonso leaves
CT- Isolate from Chandrakaran twigs
DAI Days after incubation
SL Isolates from Sindhooram leaves
ST- Isolate from Sindhooram twigs

Table 7. Cultural characters of different isolates of *Colletotrichum* sp. from Muvandan and Neelum on GBA medium

Sl. No.	Isolates	Colony characters					
		Colour	Texture	Shape	Zonation	Colour on reverse side of culture	Spore formation (DAI)
1	ML1	White	Thick cottony cushion	Regular	No	Light grey	3
2	ML2	White	Thick cottony cushion	Regular	No	Dark grey	3
3	MI	Light grey	Thin cottony	Regular	No	Light grey	3
4	MT1	White	Thick cottony cushion	Regular	No	Light grey	4
5	MT2	White	Thick cottony, fluffy at centre	Regular	No	Light grey	3
6	MF	White	Thick cottony cushion	Regular	No	Light grey	3
7	NL1	White	Thick cottony cushion	Regular	No	Dark grey	4
8	NL2	White	Thick cottony cushion	Regular	No	Black	5
9	NL3	White	Thin cottony	Regular	No	Black	3
10	NT1	White	Thick cottony cushion	Regular	No	Dark grey	3
11	NT2	Smoky white	Thin cottony	Regular	No	Dark grey	4
12	NF1	White	Thick cottony cushion	Regular	No	Dark grey	3
13	NF2	White	Thick cottony cushion	Regular	No	Grey spots	4
14	NF3	White	Thin cottony	Regular	No	Pale white	3

ML Isolates from Muvandan leaves
MI Isolate from Muvanda Inflorescence
MT Isolates from Muvandan twigs
MF-Isolate from Muvandan fruit

NL- Isolates from Neelum leaves
NT- Isolates from Neelum Twigs
NF- Isolates from Neelum fruits
DAI-days after incubation

Table 8. Cultural characters of different isolates of *Colletotrichum* sp. from Prior and Banganapalli on GBA medium

Sl. No.	Isolates	Colony characters					
		Colour	Texture	Shape	Zonation	Colour on reverse side of culture	Spore formation (DAI)
1	PL1	White	Thick cottony cushion	Regular	No	Pale white	3
2	PL2	Smoky white	Thick cottony,	Regular	No	Light grey	4
3	PI	White	Thick cottony cushion	Regular	No	Greyish	4
4	PT	Light grey	Thin cottony	Regular	No	White	3
5	BL1	White	Thick cottony	Regular	No	Pale white	5
6	BL2	White	Thick cottony cushion	Regular	No	Dark grey	5
7	BL3	White	Thick cottony cushion	Regular	No	Light grey	4
8	BT	White	Thick cottony , fluffy at centre	Regular	No	Black	5

PL-Isolates from Prior leaves
PI- Isolate from Prior inflorescence
PT- Isolates from Prior twigs
BL Isolates from Banganapalli leaves
BT- Isolate from Banganapalli twigs
DAI days after incubation

Table 9. Cultural characters of different isolates of *Colletotrichum* sp. from Alphonso, Chandrakaran and Sindhooram on GBA medium

Sl. No.	Isolates	Colony characters					
		Colour	Texture	Shape	Zonation	Colour on reverse side of culture	Spore formation (DAI)
1	AL1	White	Thick cottony cushion	Regular	No	Pale white	4
2	AL2	White	Thick cottony cushion	Regular	No	Pale white	3
3	AL3	White	Thick cottony	Regular	No	Light grey	3
4	AL4	Light grey	Thick cottony	Regular	No	Light grey	5
5	CT	white	Thick cottony cushion	Regular	No	White	5
6	ST	white	Thick cottony cushion	Regular	No	Light grey	3
7	SL1	white	Thick cottony cushion	Regular	No	White	5
8	SL2	white	Thick cottony	Regular	No	Light grey	4

AL Isolates from Alphonso leaves
CT- Isolate from Chandrakaran twigs
DAI- Days after incubation
SL- Isolates from Sindhooram leaves
ST- Isolate from Sindhooram twigs

Plate 5. Cultural characters of *Colletotrichum* isolates from Muvanda and Neelum on GBA medium

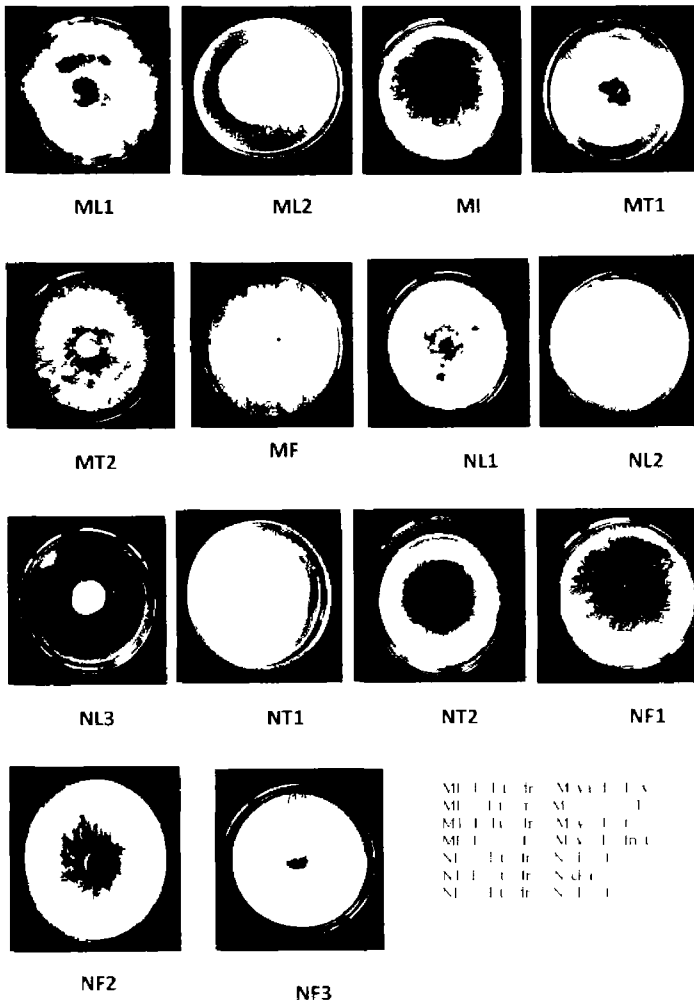
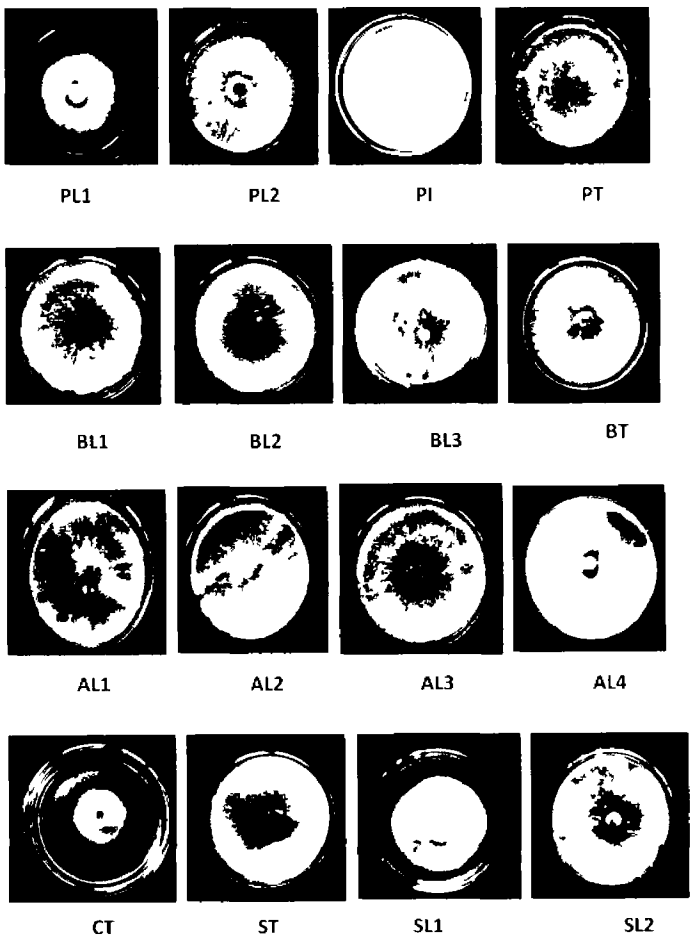


Plate 6. Cultural characters of *Colletotrichum* isolates from Prior, Banganapalli, Alphonso, Chandrakaran and Sindhooram on GBA medium



4.4.1.3. Growth rate of selected isolates of *Colletotrichum* sp. on PDA medium.

On PDA medium, variation in the growth rate of selected isolates of *Colletotrichum* sp was observed It took two to seven days to complete full growth (90 mm) on Petri dish The isolates PT, BL2, BL3, and AL4 took the shortest time of two days, while the isolates CT and SL1 took the longest time of seven days to complete full growth on Petri dish Among the 30 isolates, 11 and 12 isolates from different varieties recorded full growth on three and four DAI respectively (Table 10)

4.4.1.4. Growth rate of different isolates of *Colletotrichum* sp. on GBA medium

The growth rate of 30 isolates of *Colletotrichum* sp from seven varieties of mango was recorded on GBA medium (Table 11 & 12) Variation in the growth rate of different isolates was observed Among them, 22 isolates showed full growth on Petri dish, which took 5-12 days for 90 mm growth The remaining eight isolates could not complete the full growth even at 12 DAI The isolate BL2 recorded the shortest period of five days and the isolate AL1 recorded the largest period of 12 days to complete 90mm growth in Petri dishes The isolates ML1 and MI from Muvandan variety stop the growth on seven and eight DAI and recorded 48 5 mm and 73 1 mm respectively The isolate NL3 and CT recorded 19 0 mm and 24 6 mm growth on 9 DAI respectively and did not showed any further growth on subsequent days

4.4.1.5. Grouping of Colletotrichum isolates based on growth rate on PDA and GBA medium

The average rate of growth of all 30 isolates of pathogen on PDA and GBA was calculated from the measurements on radial growth of isolates recorded daily from the date of inoculation The results are given in the Table 13 Based on the difference in the growth rate (mm/day, the 30 isolates were categorized

Table 10. Growth rate of different isolates of *Colletotrichum* sp. on PDA medium

Sl. No.	Isolates	Colony diameter after days of incubation (cm)*							Growth rate (mm/day)
		1	2	3	4	5	6	7	
1	ML1	32 3	61 1	83 3	90 0				19 2
2	ML2	27 3	59 8	90 0					31 2
3	MI	30 5	62 2	86 8	90 0				19 0
4	MT1	27 0	53 0	79 0	90 0				21 0
5	MT2	3 6	74 0	81 0	90 0				18 0
6	MF	1 11	87 0	90 0					39 0
7	NL1	19 0	36 0	72 0	90 0				23 2
8	NL2	29 4	52 0	79 8	90 0				20 2
9	NL3	20 3	47 6	88 9	90 0				23 2
10	NT1	37 1	68 3	90 0					26 4
11	NT2	22 3	52 7	69 4	84 3	90 0			16 9
12	NF1	29 3	51 5	71 1	90 0				20 2
13	NF2	34 8	59 3	90 0					27 6
14	NF3	32 6	59 0	86 1	90 0				19 1
15	PL1	39 7	79 2	90 0					25 1
16	PL2	12 1	58 5	84 1	90 0				25 9
17	PI	17 3	88 8	90 0					36 3
18	PT	65 2	90 0						24 8
19	BL1	40 7	78 7	90 0					24 5
20	BL2	42 0	90 0						48 0
21	BL3	54 0	90 0						36 0
22	BT	29 7	74 1	90 0					30 1
23	AL1	47 3	86 2	90 0					21 3
24	AL2	28 7	54 4	75 8	90 0				20 4
25	AL3	42 0	88 0	90 0					24 0
26	AL4	48 0	90 0						42 0
27	CT	11 0	27 0	39 0	61 0	74 0	88 0	90 0	13 0
28	ST	11 1	63 1	90 0					39 4
29	SL1	10 0	15 0	32 0	53 0	68 0	79 0	90 0	14 2
30	SL2	28 5	64 2	81 2	90 0				20 5

ML-Isolates from Muvandan leaves
MI Isolate from Muvandan Inflorescence
MT Isolates from Muvandan twigs
MF- Isolate from Muvandan fruits
PL- Isolates from Prior leaves
PI Isolate from Prior inflorescence
PT Isolate from Prior twigs
SL Isolates from Sindhooram leaves
ST Isolate from Sindhooram twigs
NL1 Isolates from Neelum leaves
NT- Isolates from Neelum twigs
NF- Isolates from Neelum fruits
BL- Isolates from Banganapalli leaves
BT Isolate from Banganapalli twigs
AL- Isolates from Alphonso leaves
CT- Isolate from Chandrakaran twigs
* Mean of three replications

Table 11. Growth rate of different isolates of *Colletotrichum* sp. From Muvandan and Neelum on GBA medium

I. No.	Isolates	Colony diameter after days of incubation (mm)*												Growth rate (mm/day)*
		1	2	3	4	5	6	7	8	9	10	11	12	
1	ML1	13 2	19 5	25 6	31 0	42 5	48 3	48 5	48 5	48 5	48 5	48 5	48 5	5 0
2	ML2	11 5	23 3	34 7	48 6	62 3	78 3	83 9	89 7	90 0				9 8
3	MI	12 4	17 6	23 2	35 7	45 9	51 5	65 4	73 1	73 1	73 1	73 1	73 1	8 6
4	MT1	11 9	19 1	26 3	36 8	43 5	52 7	59 1	63 2	75 5	82 3	90 0		7 9
5	MT2	12 1	21 5	28 6	33 7	44 4	52 9	65 5	71 8	76 8	88 2	90 0		7 3
6	MF	10 5	19 2	28 2	40 8	57 3	64 3	78 5	86 1	90 0				9 9
7	NL1	11 8	14 3	26 5	39 1	50 6	63 7	76 8	82 6	88 8	90 0			9 6
8	NL2	10 0	15 2	19 2	23 5	27 8	31 3	39 9	45 4	52 1	52 6	52 6	52 6	5 2
9	NL3	10 0	10 6	12 0	12 5	13 3	15 4	16 6	17 2	19 0	19 0	19 0	19 0	11 0
10	NT1	14 8	27 6	38 5	44 4	49 6	58 8	64 6	72 9	79 0	85 2	88 0	88 0	7 4
11	NT2	17 9	31 0	48 5	62 2	74 4	90 0							14 0
12	NF1	19 8	28 2	33 3	47 1	58 5	65 2	78 7	90 0					10 0
13	NF2	13 9	22 1	36 6	48 9	59 6	67 8	74 9	81 6	88 6	90 0			9 3
14	NF3	14 6	28 2	34 5	47 9	53 2	67 2	74 6	89 6	90 0				9 4

ML- Isolates from Muvandan leaves

MI- Isolate from Muvanda Inflorescence

MT Isolates from Muvandan twigs

MF Isolate from Muvandan fruits

NL Isolates from Neelum leaves

NT- Isolates from Neelum twigs

NF- Isolates from Neelum fruits

* Mean of three replications

Table 12. Growth rate of different isolates of *Colletotrichum* sp. from Prior, Banganapalli, Alphonso, Chandrakaran and Sindhooram on GBA medium

Sl. No.	Isolates	Colony diameter after days of incubation (mm)*												Growth rate (mm/ day)*
		1	2	3	4	5	6	7	8	9	10	11	12	
1	PL1	10 0	15 5	21 2	29 9	34 5	44 6	57 0	63 7	65 5	71 0	71 0	71 0	6 0
2	PL2	11 6	17 5	25 5	37 1	49 7	64 5	72 6	79 1	89 1	90 0			9 6
3	PI	16 7	28 9	35 6	48 8	63 2	79 6	90 0						12 2
4	PT	11 5	14 5	26 6	34 7	45 5	53 9	67 3	78 9	84 9	90 0			9 1
5	BL1	13 1	25 5	38 2	52 6	66 5	75 7	87 2	90 0					10 9
6	BL2	21 6	44 4	67 9	84 4	90 0								17 1
7	BL3	19 2	38 9	51 5	67 6	88 9	90 0							14 0
8	BT	13 6	19 4	29 3	37 5	44 2	62 4	71 2	79 1	82 6	90 0			8 6
9	AL1	12 4	19 4	28 5	36 6	43 2	49 6	57 9	66 6	71 3	78 5	87 9	90 0	7 0
10	AL2	17 2	24 2	37 3	48 4	61 2	78 2	84 9	90 0					10 4
11	AL3	13 6	15 6	23 3	34 6	43 9	55 5	65 3	77 1	81 5	87 9	90 0		7 7
12	AL4	12 7	22 5	36 6	47 2	61 1	73 2	86 2	90 0					11 0
13	CT	10 6	10 4	13 5	15 8	15 9	17 6	19 4	20 5	24 6	24 6	24 6	24 6	1 7
14	ST	13 9	1 69	24 4	31 5	43 2	56 6	64 4	71 2	85 6	90 0			8 0
15	SL1	11 5	15 9	19 5	25 2	28 1	30 0	33 2	35 0	39 6	42 5	53 0	53 0	4 2
16	SL2	14 5	24 2	37 4	49 6	53 3	65 2	74 8	90 0					10 7

PL- Isolates from Prior leaves
PI Isolate from Prior inflorescence
PT Isolate from Prior twigs
ST- Isolate from Sindhooram twig
SL Isolates from Sindhooram leaves
BL Isolates from Banganapalli leaves
BT- Isolate from Banganapalli twigs
AL- Isolates from Alphonso leaves
CT- Isolate from Chandrakaran twig
* Mean of three replications

into two morphological groups (Chowdappa and Kumar, 2012) The isolates which showed less than 30 mm growth per day grouped s fast growing on PDA medium The slow growing isolate were grouped under Type I cultures and fast growing cultures under Type II cultures On PDA medium eight isolates *viz*, ML2, MF, PL3, BL2, BL3, BT, AL4 and ST where grouped under Type II cultures which recorded >30 mm/day growth All other isolates except those eight recorded <30 mm/day growth and they were grouped under Type I cultures Among the eight Type II cultures, the isolates BL2 recorded the highest growth rate of 48 mm/day It was followed by AL4 (42 mm/day), MF (39 4 mm/day PL3 (36 3 mm/day) and BL3 (36mm/day) Among the Type I cultures, the isolate CT recorded the lowest growth rate of 13 mm and this isolate NF2 recorded the highest growth rate of 27 6 mm/day

Similarly the growth/day of all the 30 isolates was calculated on GBA medium where the isolates which recorded <10 mm/day grouped under Type I cultures and these recoded >10mm/day grouped under Type II cultures Nine isolates *viz*, NT2, NF1, PL3, BL1, BL2, BL3, AL2, AL4 and SL2 were grouped under Type II cultures which showed >10 mm growth per day All other isolates were grouped under Type I cultures which recorded <10 mm growth/day The isolate BL2 recorded the highest growth rate of 17 1 mm/day among the Type II cultures It was followed by BL3 and NT2 (14mm/day), PL3 (12 2 mm/day) and AL4 (11 mm/day) Among the type I cultures, the lowest rate of growth was exhibited by NL3 (1 1 mm/day) and the highest by ML2 (9 8 mm/day)

Table 13. Grouping of *Colletotrichum* as based on growth rate on PDA and GBA media

Medium	Growth rate	Group	Isolates
PDA	Group I (> 30 mm/ day)	I (Fast growing)	ML2, MF, PI, BL2, BL3, BT, AL4, ST
	Group II (< 30 mm/ day)	II (Slow growing)	ML1, ML3, MT1, MT2, NL1, NL2, NL3, NT1, NT2, NF1, NF2, NF3,PL1, PL2, PT, BL1, AL1, AL2, AL3, CT, SL1, SL2
GBA	Group I (> 10 mm/ day)	I (Fast growing)	NL3, NT2, NF1, PI, BL1, BL2, BL3, AL2, AL4, SL2
	Group II (<10 mm/ day)	II (Slow growing)	ML1, Ml2, ML3, MT1, MT2, MF, NL1, NL2, NT1, NF2, NF3, PL1, PL2, PT, BT, AL1, AL3, CT, ST, SL1

ML, MT, MF-Isolates from Muvandan
NL, NT, NF Isolates from Neelum
PL, PI and PT Isolates from Prior
BL and BT Isolate from Banganapalli
AL- Isolates from Alphonso
CT Isolate from Chandrakaran
SL, ST Isolates from Sindhooram
PDA Potato Dextrose Agar
GBA- Green Bean Agar

4.4.2. Morphological characters of different isolates of *Colletotrichum* sp.

4.4.2.1. Isolates of *Colletotrichum* sp. from Muvandan

The data on morphological characters of six isolates of *Colletotrichum* sp from Muvandan are depicted in Table 14 Variation in the size of spore was observed the colour of hyphae vary from light grey to dark grey and greyish brown hyphal colour was noticed in the isolate MI The size of hypha was observed in the range of 26 20-36 40 x 3 33-5 16 µm The conidia are cylindrical with obtuse ends with an average size of 13 50-16 99 x 3 40-5 16 µm The conidia of the isolates MF produced the large sized conidia in the range of 12 64-22 62 x 3 86-6 73 µm Development of acervulus was obtained in MT1 which were dark brown to black, septate and 104 90-142 30 x 2 08-3 45 µm size (Plate 7) Whereas in ML1 and MF setae formation was not observed (Plate 9b)

4.4.2.2. Isolates of *Colletotrichum* sp. from Neelum

The observations on characters of hyphae spore and setae of eight isolates of *Colletotrichum* sp from Neelum are given in Table 15 The colour of hyphae was observed in the range of hyaline to brown The isolates NL1 and NF3 showed hyaline hypha Except these two all other isolates showed coloured hypha The hyphal size measured as 28 64-39 67 x 3 44-5 00 µm The shape of conidia developed by all the eight was recorded as cylindrical with obtuse ends with an average size of 14 43-15 64 x 4 16-5 03 µm The large sized conidia were recorded in the isolate NT2 (13 91-17 26 x 3 03-6 48 µm) Production of setae was observed in NL3 and was recorded as dark brown to black, septate and 96 47-111 76 x 3 04-4 00 µm size (Plate 7) Setae formation was not observed in isolates NF1 and NF3 (Plate 9b)

4.4.2.3. Isolates of *Colletotrichum* sp. from Prior

Morphological characters of four isolates of *Colletotrichum* sp from Prior are given in Table 16 The colour of hyphae was observed in the range of hyaline to brown with size in the range of 28 47-36 43 x 3 43-5 31 µm Conidia of all four isolates were cylindrical with obtuse ends with an average size of 14 39-

Table 14. Morphological characters of different isolates of *Colletotrichum* sp. from Muvandan

Sl. No	Isolates	Hyphal characters		Spore characters			Setae characters		
				Shape	Size* (µm)				
		Colour	Size *(µm)		Average	Range	Colour	Septation	Size** (µm)
1	ML1	Light grey	30 65x 5 16	Cylindrical with obtuse ends	14 49 x 3 87	10 91-16 49 x 3 22-4 51	-	-	
2	ML2	Dark grey	28 98 x 3 67	Cylindrical with obtuse ends	16 99 x 5 16	11 22 20 25 x 2 28 4 60	-	-	-
3	MI	Greyish brown	36 4 x 3 33	Cylindrical with obtuse ends	15 64 x 3 40	11 09-15 07 x 2 82-4 90		-	
4	MT1	Light grey	31 65 x 4 1	Cylindrical with obtuse ends	13 52 x 3 99	11 26 15 91 x 2 38 5 02	Dark brown to black	Septate	104 90-142 30 x 2 08- 3 45
5	MT2	Light grey	26 2 x 5 00	Cylindrical with obtuse ends	14 25 x 3 86	10 47-18 76 x 1 97-6 99	-	-	-
6	MF	Dark grey	32 45 x4 78	Cylindrical with obtuse ends	15 25 x 3 94	12 64-22 62 x 3 86-6 73		-	-

ML Isolates from Muvandan leaves
MI Isolates from Muvandan Inflorescence
MT Isolates from Muvandan twigs
MF Isolate from Muvandan Fruits

* Mean of 20 replications
** Mean of 5 replications

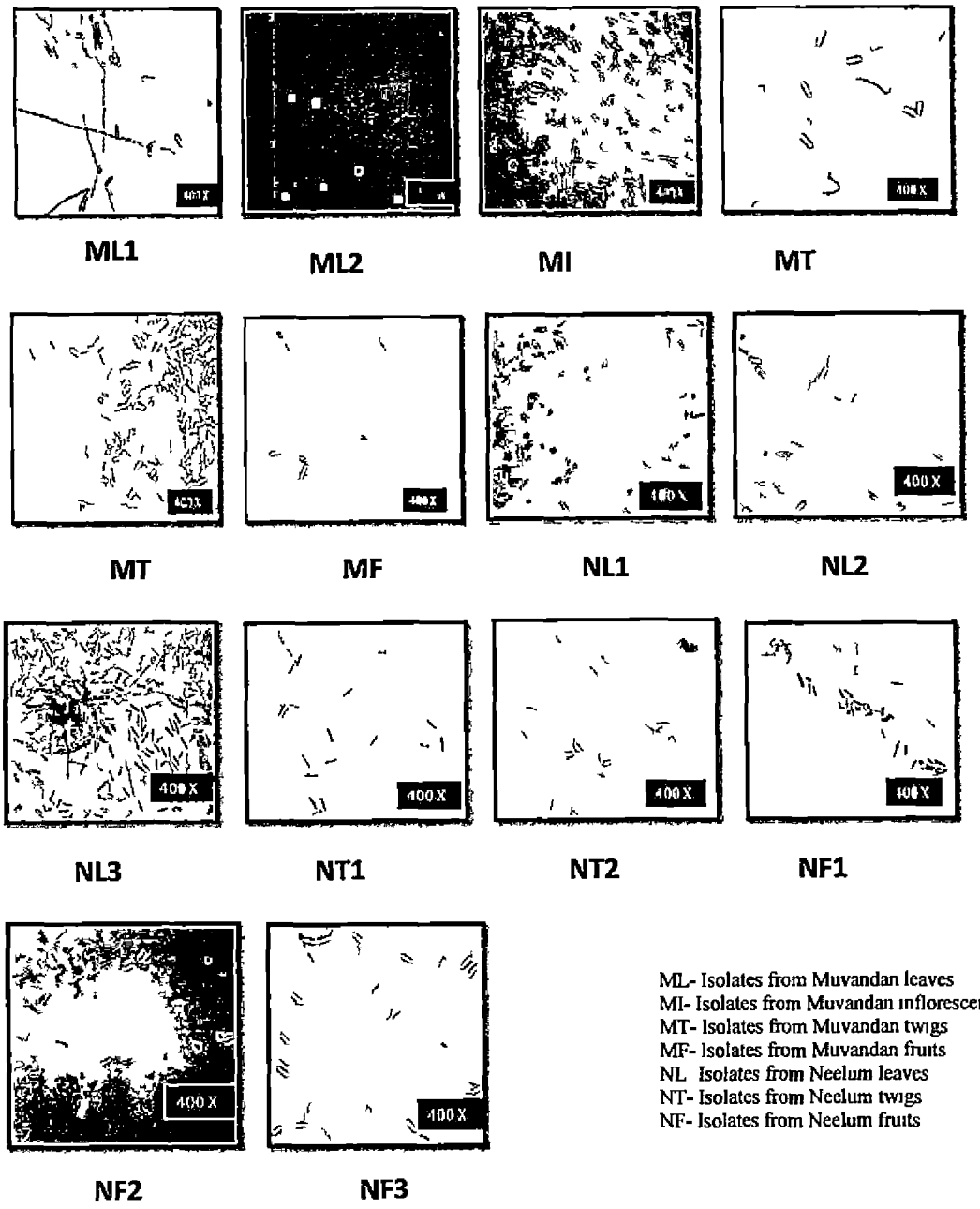
Table 15. Morphological characters of different isolates of *Colletotrichum* sp from Neelum

Sl No.	Isolates	Hyphal characters		Spore characters			Setae characters		
				Shape	Size* (µm)				
		Colour	Size* (µm)		Average	Range	Colour	Septation	Size** (µm)
1	NL1	Hyaline	34 89 x 3 90	Cylindrical with obtuse ends	14 83 x 4 69	10 73-17 46 x 1 74-5 64	-	-	
2	NL2	Greyish brown	32 66 x 4 24	Cylindrical with obtuse ends	14 69 x 4 46	11 41-19 73 x 1 94-7 37	-		-
3	NL3	Brown	31 87 x 4 74	Cylindrical with obtuse ends	15 45 x 4 46	10 74-20 22 x 3 34 7 22	-	-	
4	NT1	Greyish brown	28 64 x 3 93	Cylindrical with obtuse ends	14 46 x 4 88	13 55-18 82 x 1 63-6 76	Dark brown to black	Septate	96 47 111 76 x 3 04-4 0
5	NT2	Brown	29 33 5 00	Cylindrical with obtuse ends	15 21 x 4 16	13 91 17 26 x 3 03-6 48	-	-	-
6	NF1	Brown	32 87 x 3 44	Cylindrical with obtuse ends	14 92 x 4 43	12 86-20 64 x 2 54 7 84		-	
7	NF2	Dark grey	39 67 x 4 49	Cylindrical with obtuse ends	14 43 x 5 03	13 01 22 70 x 2 54-6 81	-		-
8	NF3	Hyaline	30 98 x 4 48	Cylindrical with obtuse ends	15 64 x 4 22	12 12-20 55 x 1 89 6 77	-	-	

NL Isolates from Neelum leaves
NT- Isolates from Neelum Twigs
NF- Isolates from Neelum fruits

* Mean of 20 replications
** Mean of 5 replications

Plate 7. Spores of *Colletotrichum* isolates from Muvandan and Neelum



ML- Isolates from Muvandan leaves
MI- Isolates from Muvandan inflorescence
MT- Isolates from Muvandan twigs
MF- Isolates from Muvandan fruits
NL Isolates from Neelum leaves
NT- Isolates from Neelum twigs
NF- Isolates from Neelum fruits

15 74 x 3 76-4 30 µm Acervulus setae formation were not observed in these isolates (Plate 8)

4.4.2.4. Isolates of *Colletotrichum* spp. from Banganapalli

The data on morphological characters of four isolates from Banganapalli are given in Table 16 The hyphal characters were observed in the range of 26 58-31 87 x 3 26-5 15 µm All isolates were cylindrical with obtuse ends with an average size of 15 23-16 58 x 4 02-4 61 µm Large sized conidia was noticed in the isolate BL1 (13 76-21 09 x 2 31-7 42 µm) Dark brown to black coloured setae formation was observed in BL1 which were septate with 111 20-142 00 x 2 45-4 02 µm size (Plate 8 & 9a)

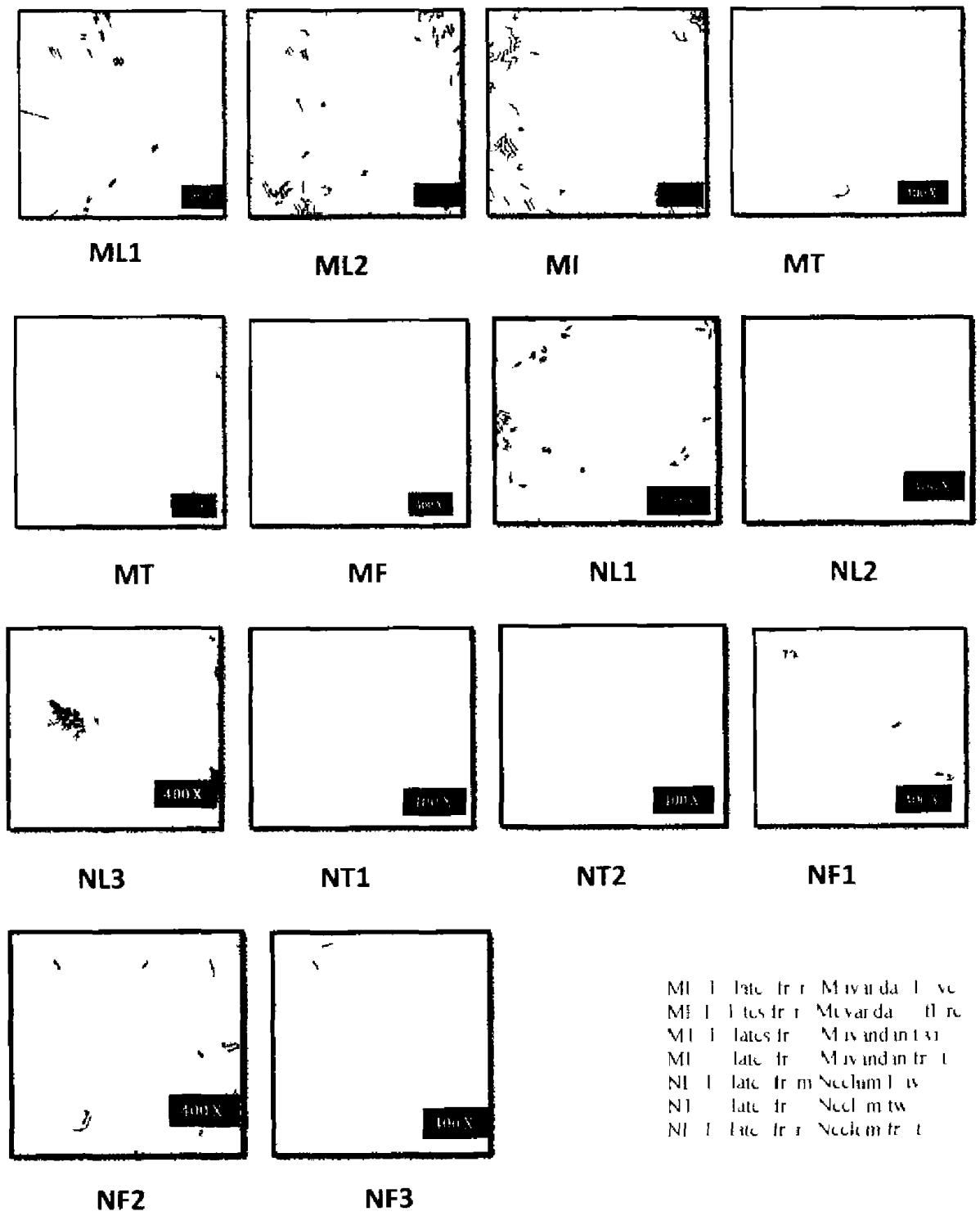
4.4.2.5. Isolates of *Colletotrichum* sp. from Alphonso

Morphological characters of four isolates from the variety Alphonso are given in Table 17 The isolates AL3 and AL4 showed hyaline hyphae whereas dark grey and dark brown coloured hypha was observed in AL1 and AL2 respectively The size of hyphal cell was observed in the range of 28 10-35 12 x 3 75-4 92 µm The conidia were cylindrical with obtuse ends with an average size of 15 27-16 59 x 3 64-4 69 µm Large sized comdial development was observed in the isolate AL1 (14 78-17 74 x 2 78-6 66 µm) The setae formation in acervulus was noticed in AL1 which produced dark brown to black, septate setae with 98 78-121 00 x 2 45-3 56 µm size (Plate 8)

4.4.2.6. Isolates of *Colletotrichum* sp. from Chandrakaran

The isolate CT from the variety Chandrakaran produced light grey coloured hypha with 34 65 x 3 43 µm size (Table 17) Conidia were cylindrical with obtuse ends and an average size of 15 47 x 4 05 µm Setae formation was observed in the culture, which was dark brown to black, septate and 95 83-137 07 x 2 70-4 75 µm size (Plate 8)

Plate 7. Spores of *Colletotrichum* isolates from Muvandan and Neelum



15 74 x 3 76-4 30 µm Acervulus setae formation were not observed in these isolates (Plate 8)

4.4.2.4. Isolates of *Colletotrichum* spp. from Banganapalli

The data on morphological characters of four isolates from Banganapalli are given in Table 16 The hyphal characters were observed in the range of 26 58-31 87 x 3 26-5 15 µm All isolates were cylindrical with obtuse ends with an average size of 15 23-16 58 x 4 02-4 61 µm Large sized conidia was noticed in the isolate BL1 (13 76-21 09 x 2 31-7 42 µm) Dark brown to black coloured setae formation was observed in BL1 which were septate with 111 20-142 00 x 2 45-4 02 µm size (Plate 8 & 9a)

4.4.2.5. Isolates of *Colletotrichum* sp. from Alphonso

Morphological characters of four isolates from the variety Alphonso are given in Table 17 The isolates AL3 and AL4 showed hyaline hyphae whereas dark grey and dark brown coloured hypha was observed in AL1 and AL2 respectively The size of hyphal cell was observed in the range of 28 10-35 12 x 3 75-4 92 µm The conidia were cylindrical with obtuse ends with an average size of 15 27-16 59 x 3 64-4 69 µm Large sized conidial development was observed in the isolate AL1 (14 78-17 74 x 2 78-6 66 µm) The setae formation in acervulus was noticed in AL1 which produced dark brown to black, septate setae with 98 78-121 00 x 2 45-3 56 µm size (Plate 8)

4.4 2.6 Isolates of *Colletotrichum* sp. from Chandrakaran

The isolate CT from the variety Chandrakaran produced light grey coloured hypha with 34 65 x 3 43 µm size (Table 17) Conidia were cylindrical with obtuse ends and an average size of 15 47 x 4 05 µm Setae formation was observed in the culture, which was dark brown to black, septate and 95 83-137 07 x 2 70-4 75 µm size (Plate 8)

Table 16. Morphological characters of different isolates of *Colletotrichum* sp. from Prior and Banganapalli

Sl. No.	Isolates	Hyphal characters		Spore characters			Setae characters		
				Shape	Size* (µm)				
		Colour	Size* (µm)		Average	Range	Colour	Septation	Size** (µm)
1	PL1	Light grey	28 47 x 4 96	Cylindrical with obtuse ends	15 37 x 4 30	11 78-19 32 x 1 35 6 66	-	-	-
2	PL2	Hyaline	31 56 x 4 24	Cylindrical with obtuse ends	14 39 x 4 25	10 73-17 76 x 3 21-5 95	-	-	-
3	PI	Brown	30 54 x 5 31	Cylindrical with obtuse ends	15 74 x 3 76	13 27- 19 84 x 1 44- 4 94	-	-	
4	PT	Brown	36 43 x 3 43	Cylindrical with obtuse ends	14 85 x 4 30	10 58-18 39 x 2 14-6 53		-	
5	BL1	Light grey	31 87 x 5 15	Cylindrical with obtuse ends	16 12 x 4 28	13 76-21 09 x 2 31- 7 42	Dark brown to black	Septate	111 20 142 00 x2 45 4 02
6	BL2	Grey	27 93 x 3 26	Cylindrical with obtuse ends	15 23 x 4 61	11 24-18 22 x 2 75-6 92	-		-
7	BL3	Hyaline	26 58 x 3 75	Cylindrical with obtuse ends	16 58 x 4 26	12 66 22 21 x 1 93 5 32	-		-
8	BT	Dark grey	29 29 x 3 66	Cylindrical with obtuse ends	15 63 x 4 02	10 64-17 88 x 3 33 7 38	-	-	

PL Isolates from Prior leaves
PI Isolate from Prior inflorescence
PT isolate from Prior twigs
BL Isolates from Banganapalli leaves
BT- Isolate from Banganapalli twigs
* Mean of 20 replications
** Mean of 5 replications

4.4.2.7. Isolates of *Colletotrichum* sp. from Sindhooram

Morphological characters of three isolates from the variety Sindhooram are given in Table 17 All the isolates produced light grey coloured hyphal cells, measured in the range of 29 42-34 13 x 3 98-4 68 µm size Conidia were cylindrical with obtuse ends with an average size of 14 98-15 43 x 4 06-4 16 µm Large sized conidia was observed in SL1 (12 78-19 32 x 3 44-6 66 µm) Development of setae was observed in the isolate SL1 which were dark brown to black, septate and measured 86 32- 124 09 x 2 86-4 30 µm size (Plate 8 & 9a)

4.4.3. Cluster analysis of *Colletotrichum* sp. isolates based on cultural and morphological characters.

Cluster analysis of 30 isolates of *Colletotrichum* from different varieties of mango was computed from cultural and morphological characters as similarity coefficient using NTSYS pc 2 02 software (Fig 1) The dendrogram was constructed using the unweighted pair group method with arithmetic mean (UPGMA) The maximum variation among the isolates was recorded at 10 per cent similarity where two clusters were formed *viz*, A1 and A2 Only three (ML1, MT1 and NF3) isolates were included in Cluster A1 showing dissimilarity of 90 per cent from all other isolates which were included in A2

At 15 per cent similarity four subclusters were formed *viz*, B1, B2, B3 and B4 These sub clusters were included in A1 and A2 The Cluster A2 branched into three clusters (B2, B3, B4) including maximum number of isolates Among them, the isolate only BL1 included in the cluster B3 and showed highest variation from other isolates In sub cluster B4, five isolates were categorized, one each from Muvandan (MF), Alphonso (AL1), Neelum (NT1), Chandrakaran (CT), and Sindhooram (SL1), whereas Cluster B2 included maximum number of isolates and showed more than 70 per cent dissimilarity among the 30 isolates Only seven isolates showed maximum similarity of 56 per cent between them and which were included in with three groups *viz*, Group I (PL1, PL3, AL3), Group II (ML3, PL2), Group III (MT2, AL2) These three groups were categorised under the sub clusters B2 (Table 18)

Table 17. Morphological characters of different isolates of *Colletotrichum* sp. from Alphonso, Chandrakaran and Sindhooram

Sl. No.	Isolates	Hyphal characters		Spore characters			Setae characters		
				Shape	Size* (µm)				
		Colour	Size* (µm)		Average	Range	Colour	Septation	Size** (µm)
1	AL1	Dark grey	31 67 x 4 86	Cylindrical with obtuse ends	16 08 x 4 69	14 78-17 74 x 2 78 6 66	Dark brown to black	Septate	98 78- 121 00 x2 45-3 56
2	AL2	Brown	35 12 x 4 92	Cylindrical with obtuse ends	15 27 x 4 16	14 06-17 82 x 2 72-4 93	-		-
3	AL3	Hyaline	30 16x 4 13	Cylindrical with obtuse ends	15 57 x 3 64	12 72 18 86 x 1 61- 5 56		-	-
4	AL4	Hyaline	28 10 x 3 75	Cylindrical with obtuse ends	16 59 x 3 97	13 39-21 63 x 1 95-5 79	-		-
5	CT	Light grey	34 65 x 3 43	Cylindrical with obtuse ends	15 47 x 4 05	15 64-25 64 x 4 04-7 02	Dark brown to black	Septate	95 83 137 07 x2 72- 4 75
6	ST	Light grey	32 47 x 3 98	Cylindrical with obtuse ends	15 43 x 4 16	12 12 19 22 x 1 93-6 77	-		-
7	SL1	Light grey	34 13 x 4 59	Cylindrical with obtuse ends	14 98 x 4 06	12 78-19 32 x 3 44- 6 66	Dark brown to black	Septate	86 32 124 09 x2 86 4 30
8	SL2	Light grey	29 42 x4 68	Cylindrical with obtuse ends	15 00 x 4 14	10 73 17 56 x 3 21 5 94		-	-

AL- Isolates from Alphonso leaves
CT- Isolate from Chandrakaran twigs
ST Isolate from Sindhooram twigs
SL Isolates from Sindhooram leaves
* Mean of 20 replications
** Mean of 5 replications

Plate 8. Spores of *Colletotrichum* isolates from Prior, Bnganapalli, Alphonso, Chandrakaran and Sindhooram

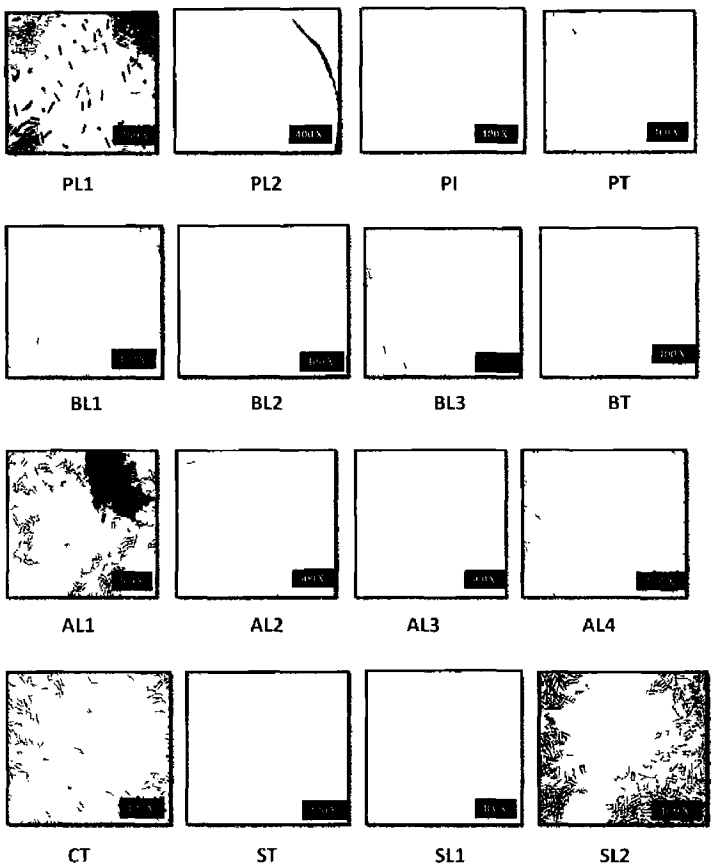
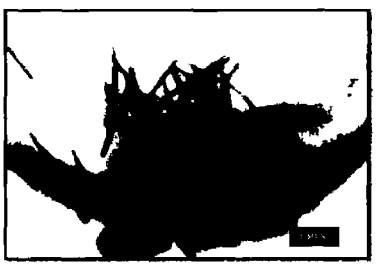


Plate 9. Development of acervuli in the culture of *Colletotrichum* sp.

Plate 9a. Acervulus with setae

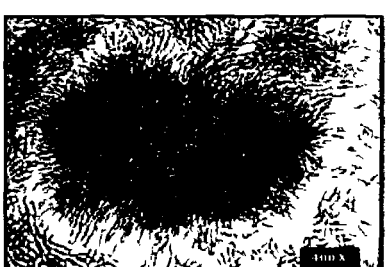
BL1



SL1

Plate 9b. Acervulus without setae

NL3



ML

Fig 1 Dendrogram of 30 different isolates of *Colletotrichum* from mango

Table 18 Grouping of isolates based on cluster analysis

Clusters	Isolates
B1	ML1 MT1 NL3
B2	ML2 PL1 PL3 AL3 BL1 NL2 BL3 SL1 BL2 ML3 PL2 NL1 MT2 AL2 NL2 AL4 PL1 NL2 NL3 NL1 SL2
B3	BL1
B4	MF AL1 NL1 CL1 SL1

4.5. VEGETATIVE COMPATIBILITY OF DIFFERENT ISOLATES OF *COLLETOTRICHUM* SP.

The vegetative compatibility of 30 isolates of *Colletotrichum* sp was studied under lab condition by dual culture method to find out vegetative compatible group among them The results are given in Table 19, from the data it was recorded that all the isolates showed compatible reactions with other isolates which was found in the range of 2 to 9 isolates All the isolates were found compatible with the respective same isolates and showed mutual intermingling of hyphae in the Petri dishes The highest compatible combinations were observed in the BL2 which showed free intermingling of hyphae of eight other isolates of *Colletotrichum* sp *viz*, MI, MT2, NL3, NT2, NF3, PL1, BL1 and BL3 It was followed by ML1 which recorded the maximum compatibility with seven other isolates The lowest vegetative compatible reaction was recorded in isolates *viz*, PI, PT, BT, AL1, SL1 and SL2 which showed only one compatible pair (PIxST, PTxNT1, BTxMF, AL1xAL2, SL1xPL1 and SL2xML1)

The vegetative compatibility of six isolates from Muvandan was evaluated with the isolates from other varieties The results showed that the isolate ML1 recorded maximum compatible group of seven with other isolates and was followed by MI and MT2 which recorded three compatible pairs each Among the 8 isolates from Neelum variety, four isolates each were compatible with five other isolates of pathogen which included the isolates from Muvandan, Neelum, Prior, Alphonso and Sindhooram

The four isolates from the variety Prior *viz*, PL1, PL2, PI and PT, recorded the compatible reactions of the vegetative growth with one isolate from Muvandan, four from Neelum, three from Sindhooram, two from Alphonso and one from Banganapalh The highest compatible pairs were recorded by PL2 (6 nos) which included two isolates each from Neelum, Alphonso and one each from Banganapalh and Sindhooram The isolates from the variety Banganapalli did not produce any compatible reactions with the isolates from the Alphonso and Sindhooram Only one isolate, BL1 showed intermingling of hypha with the

Table 19 Evaluation of vegetative compatibility- Compatible combination of *Colletotrichum* isolates showing intermingling of hyphae

Sl No.	Isolates	Isolates showing intermingling of hyphae (I)	No of isolates
1	ML1	ML1, ML2,MI NL1, NF1, AL2, CT, SL2MF1, AL2,	8
2	ML2	ML2, ML1, MT1, NL2	5
3	MI	MI ML1, NT1, PL1, BL2	5
4	MT1	MT1, ML2, MT2	5
5	MT2	MT2, MT1, NL1,BL2, NT2	5
6	MF	MF, BT, NL2, ML	4
7	NL1	NL1, ML1, MT2 NL2, NL3,NF1	6
8	NL2	NL2,ML2,MF, PL2 AL4	5
9	NL3	NL3 NL1 NF1, PL2 BL3	5
10	NT1	NT1,MI, NF2, PT, AL3 ST	6
11	NT2	NT2, MT2, NF2,BL1	6
12	NF1	NF1 ML1, NL1,,NL3	4
13	NF2	NF2,NT1, AL2, AL3, AL4	5
14	NF3	NF3, PL1, BL2	3
15	PL1	PL1, MI, NF3 PL2, BL2	5
16	PL2	PL2, NL2, NL3,SL1	4
17	PI	PI ST	2
18	PT	PT, NT1, PL2	3
19	BL1	BL1 NT2, PL1, BL2, CT	5
20	BL2	BL2, MI, MT2, NL3, NF3, BL1, BL3, PL1, NT2	9
21	BL3	BL3, NL3, BL2	3
22	BT	BT MF	2
23	AL1	AL1 AL2	2
24	AL2	AL2, ML1, NF2, PL2, AL1	5
25	AL3	AL3, NT1, NF2, PL2	4
26	AL4	AL4, NL2, NF2	3
27	CT	CT, ST ML1 PT	4
28	ST	ST NT1 PI, CT	4
29	SL1	SL1 PL1	2
30	SL2	SL2, ML1, PL2	3

ML MI MT MF Isolates from Muvandan
PL PI PT Isolates from Prior
AL Isolates from Alphonso
SL ST Isolates from Sindhooram
NL NT NF Isolates from Neelum
BL BT Isolate from Banganapalli
CT Isolate from Chandrakaran

isolate from Chandrakaran Among the four isolates from this variety, BL2 recorded maximum number (8 nos) of compatible pairs with other isolates of pathogen

Among the four isolates from the variety Alphanso, AL2 recorded the highest compatible pairs (4 nos) with one isolate each from Muvandan (ML1), Neelum (NF2), Alphonso (AL1), Prior (PL2) The isolates from Chandrakaran (CT) showed compatible pairs with three other isolates *viz* , ML1, BL1 and ST This isolates did not showed compatible intermingling hyphae with the isolates from Neelum, Alphanso and Prior

The isolates obtained from the twig of the variety Sindhooram (ST) recorded compatible reactions with three other isolates whereas the isolates from leaves *viz* , SL1 and SL2 showed vegetative compatibility with one isolate each from Prior (PL1) and Muvandan (ML1) respectively

All possible combinations of 30 isolates of the pathogen except those given in Table 20-22 recorded either overgrowth of isolates, development of clear zone in between the isolates or mycelial thickness at the meeting point Hence those pairs of isolates were recorded as incompatible pairs (Plate 10)

4.6. EVALUATION OF PATHOGENIC VARIABILITY AMONG THE SELECTED ISOLATES OF *COLLETOTRICHUM* SP.

Evaluation on the pathogenic efficiency of 30 isolates of pathogen obtained from seven varieties of mango was conducted to know variability in the pathogenic character of the organism For that the isolates from one variety of mango was cross inoculated to the different varieties and observed for the symptoms developed Then the isolates were subjected to virulence rating to access the pathogenic variability Observation on the days taken for initial infection and characters of symptoms developed were recorded The results of the study are furnished in the Table 23-30

Table 20. Evaluation of vegetative compatibility- Incompatible combinations of *Colletotrichum* isolates showing mutual inhibition with clear zone

Sl. No.	Isolates	Isolates showing	No. of isolates
1	ML1	NL3, NF3 PL1, BL1, AL1, AL4, ST	7
2	ML2	NL1 NF3 PL2 PI, PT, AL1, AL4	7
3	MI	MT1, MF NL3, PL2	4
4	MT1	MI, BL1 BL2, ST, SL2	5
5	MT2	MF, NT1 PL2, AL2	4
6	MF	MI, MT2 NT1, PL2 PI, SL1	6
7	NL1	ML2, NT2 NF3, PI, AL1, AL4, CT	7
8	NL2	NF3, BT AL2, SL2	4
9	NL3	ML1, MI NT2, CT	4
10	NT1	MT2, MF, NT2, NF1, NF3 PI, BL2 AL4 SL1	9
11	NT2	NL1, NL3 NT1, NF3 PT, BL3, AL3, ST	8
12	NF1	NT1 NF3, BL1 AL4 SL2	5
13	NF2	BL2 AL1, CT, SL1	4
14	NF3	ML1, ML2 NL1 NL2 NT1 NT2 NF1, PT, BL3 ST	10
15	PL1	ML1, BT AL1, AL2	4
16	PL2	ML2, MI MT2, PI, AL1, CT	6
17	PI	ML2, MF NL1 NT1 PL2, BL2, AL1, SL1	8
18	PT	ML2 NT2, NF3 AL3, ST	5
19	BL1	ML1, MT1 NF1, BL3, ST	5
20	BL2	MT1 NT1, NF2 PI, BT, AL1	6
21	BL3	NT2, NF3 BL1, AL1	4
22	BT	NL2 PL1 BL2, AL4	4
23	AL1	ML1, ML2, NL1, NF2, PL1, PL2 PI, BL2, BL3, AL3, SL1	11
24	AL2	MT2, NL2, PL1, SL1, SL2	5
25	AL3	NT2, PT AL1 CT SL1	5
26	AL4	ML1, ML2, NL1, NT1, NF1 BT ST, SL1	8
27	CT	NL1, NL3 NF2, PL2, AL3	5
28	ST	ML1, MT1 NT2, NF3, PT, BL1, AL4	7
29	SL1	MF, NT1 NF2 PI AL1, AL2 AL3, AL4	8
30	SL2	MT1, NL2, NF1, AL2	4

ML MI MT MF Isolates from Muvandan

PL PI PT Isolates from Prior

AL Isolates from Alphonso

SL ST- Isolates from Sindhooram

NL NT NF Isolates from Neelum

BL BT Isolate from Banganapalli

CT Isolate from Chandrakaran

Table 21. Evaluation of vegetative compatibility- Incompatible combinations of *Colletotrichum* isolates showing over growth

Sl. No.	Isolates	Isolates showing over growth
1	ML1	→ MT1, MT2, MF, NL2 NT1, NT2, PL2, PT, BL2, BL3, BT, AL3, SL1
		←NF2, PI
2	ML2	→MT2, MF, NT1, NT2, PL1, BT, AL2, CT, SL1
		←MI, NL3 NF1, NF2 , BL1, BL2, BL3, AL3, ST, SL2
3	MI	→NF3, PT, BL3, CT, ST, SL1, SL2
		←MT2, NL1, NL2, NT2, NF1, NF2, PI, BL1, BT, AL1, AL2, AL4
4	MT1	→MF, NL1, NL2, NL3, NT1, NT2, NF1, NF2, NF3, PL1, PL2, PI, BL3, AL2, AL3, AL4, CT
		← PT, BT, AL1, SL1
5	MT2	→PL1, PI, BT, CT, SL1
		←NL2, NL3, NF1, NF3, PT, BL1, AL1, AL3, AL4, ST, SL2
6	MF	→NF2, NF3, PL1, PT, BL2, BL3, AL3, ST
		←NL1, NL3, NT2, NF1, PL2, AL1, AL2, AL4, SL2
7	NL1	→NT1, NF2 PL1, PL2, PT, BT, AL2, AL3, ST, SL1, SL2
		←BL1, BL2, BL3
8	NL2	→NL3, NT1, NF2, PI, PT, CT, SL1
		←NT2, NF1, PL1, BL1, BL2, BL3, AL1, ST
9	NL3	→NT1, NF3, PI, PT, BL1 SL1
		←NF2, BT, AL1, AL2, AL3, ST, SL2
10	NT1	→PL1, PL2, BL1, BL3, BT, AL1, AL2, CT
		←SL2
11	NT2	→NF1, CT
		←PL1, PL2, PI BL2, BT, AL1, AL2, AL4, SL1, SL2
12	NF1	→NF2, BL3, ST
		←PL1, PL2, PI, PT, BL2, BT AL1, CT, SL1
13	NF2	→NF3, PL1, PL2, PI, PT, BL1, BL3, BT, ST
		←SL2
14	NF3	→PI, BL1, AL4, SL1 SL2
		←PL2, BT, AL1, AL2, AL3 CT

Sl. No.	Isolates	Isolates showing over growth
15	PL1	→PT, BL1, BL3, AL3, AL4, CT, ST
		←PI, BL2, SL2
16	PL2	→PT, BL2, BL3, BT, CT, SL1
		←AL4
17	PI	→BL1, BT, AL2, AL3, AL4, CT, SL2
		←PT, BL3
18	PT	→BL1, BL2, BL3, BT, AL2, CT, SL1, SL2
		←AL1, AL4
19	BL1	→AL2, AL3, AL4, SL1, SL2
		←AL1
20	BL2	→AL2, AL3, AL4, CT, ST, SL1, SL2
21	BL3	→BT, AL2, AL3, AL4, CT, ST, SL1, SL2
22	BT	→AL2, AL3, CT, ST, SL1, SL2
		←AL1
23	AL1	→CT, ST
		←AL4, SL2
24	AL2	→AL3, CT, ST
		←AL4
25	AL3	→AL4, ST, SL2
26	AL4	→CT, SL2
27	CT	→SL1
		←SL2
28	ST	→SL1
		←SL2
29	SL1	←SL2

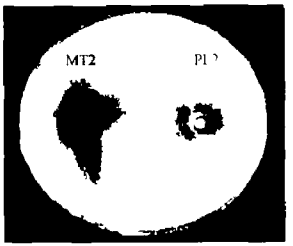
ML, MI, MT, MF Isolates from Muvandan
PL, PI, PT Isolates from Prior
AL Isolates from Alphonso
SL, ST Isolates from Sindhooram
NL, NT, NF Isolates from Neelum
BL, BT Isolate from Banganapalli
CT Isolate from Chandrakaran
a→**b** a over growth on b
a←b b over growth on a

Table 22. Evaluation of vegetative compatibility- Incompatible combinations of *Colletotrichum* isolates showing thick mycelial strand

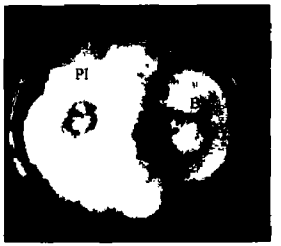
Sl. No.	Isolates	Isolates showing thick mycelial strand
1	MI	AL3
2	MT2	NF2, BL3
3	MF	BL1, CT
4	NL2	AL3
5	NL3	PL1
6	PL1	NL3, PL2
7	PL2	PL1
8	BL1	MF, BT
9	BL3	MT2
10	BT	BL1
11	AL3	MI, NL2
12	CT	MF

MI- Isolate from Muvanda Inflorescence
MT Isolate from Muvandan twigs
MF Isolate from Muvandan fruits
NL- Isolates from Neelum leaves
NF Isolates from Neelum fruits
PL Isolates from Prior leaves
BL Isolates from Banganapalli leaves
CT Isolate from Chandrakaran twigs
AL- Isolates from Alphonso leaves

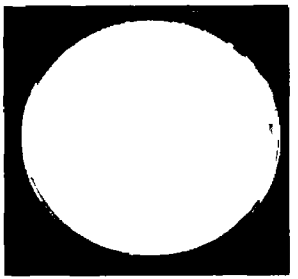
Plate 10. Evaluation of vegetative compatibility of different isolates of *Colletotrichum* sp. from seven varieties of mango



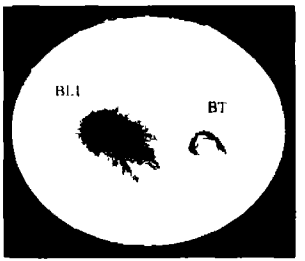
Intermingling of hyphae



Mutual inhibition with clear zone



Over growth



Thick mycelial strand

MT Isolate from Muvandan twig
FL Isolate from Fern leaves
FI Isolate from Fern inflorescence
BL Isolate from Banganapalli leaves

CT Isolate from Chandrakaran twig
AL Isolate from Alphonso leaves
BT Isolate from Banganapalli twigs
BI Isolate from Banganapalli fruit

4.6.1.1. Pathogenic variability of different isolates of *Colletotrichum* sp. on the variety Muvandan

Among the 24 isolates of *Colletotrichum* sp inoculated on the leaves of the variety Muvandan, infection was observed on the leaves inoculated with 16 isolates (Table 23) Symptoms development was not recorded in eight isolates which included NL2, NT1, NT2, PT, BL3, AL3, AL4 and SL2 The symptoms developed by all isolates were almost same and it showed the development of brown to black coloured, irregular to angular shaped spots without any specific margin The isolates PL2and AL2 produced angular shaped spots On the inoculated area mycelial growth was not observed in three isolates from Prior, two from Neelum and one each from Banganapalli and Alphonso

The symptom initiation by the different isolates was recorded on two to four DAI All the isolates from Neelum except NL3, isolates from Banganapalli except BL1, isolates from Prior except PL2, isolates from Alphonso except AL2 and isolates from Sindhooram except SL1 recorded the short period of less than three days for the symptom initiation The isolates BL1, AL2 and SL1 recorded four days for the symptom initiation The size of the spot was measured on 5 DAI and it ranged from 3-13 67 mm, where smallest measurement was recorded in SL1 (3 mm) which initiated on 4 DAI and noticed highest sized spot in BT and NF3 (13 67 mm)

4.6.1.2. Pathogenic variability of different isolates of *Colletotrichum* sp. on the variety Neelum

Totally 15 isolates showed infection on Neelum variety, out of which four isolates were from Muvandan, three each from Prior and Alphonso, two each from Banganapalli and Sindhooram and one from Chandrakaran (Table 24) These isolates took 2-3 66 days for symptom initiation in which five isolates produced first symptom on 2-3 DAI The isolates ML1, MF, PL1 and AL1 showed symptom initiation on 3-4 DAI The spots developed were appeared pale red with back margin (MF) to black spots without any margin (MI, PL1, AL1) Angular shaped spots were noticedin MI and BT and all other isolates produced

Table 23. Evaluation of pathogenic variability of different isolates of *Colletotrichum* sp. on Muvandan variety

Sl. No.	Characters of symptoms						
	Isolates	No of days taken to symptom initiation*	Colour	Shape	Margin	Presence or absence of mycelia	Size of the spots on 5 DAI (mm)**
1	NL1	2 00[a]	Dark brown	Irregular	No	+	7 66[ef]
2	NL3	3 00[cd]	Brown	Irregular	No	+	3 66[ij]
3	NF1	2 00[a]	Brownish black	Irregular	No	-	6 66[fg]
4	NF2	2 33[b]	Brown	Irregular	No	+	9 00[de]
5	NF3	2 00[a]	Brown	Irregular	No		13 67[a]
6	PL1	2 00[a]	Dark brown	Irregular	No	-	10 67[bc]
7	PL2	3 00[cd]	Dark brown	Angular	No		5 33[gh]
8	PI	2 00[a]	Brown	Irregular	Dark black, thin	-	11 67[b]
9	BL1	4 00[d]	Brown	Irregular	No		4 67[hi]
10	BL2	2 33[b]	Brown	Irregular	No	+	10 33[bcd]
11	BT	2 00[a]	Dark brown	Irregular	No	+	13 67[a]
12	AL1	2 66[bc]	Brownish black	Irregular	No	+	9 67[cd]
13	AL2	4 00[d]	Dark black	Angular	No	-	2 33[j]
14	CT	2 66[bc]	Brown	Irregular	No	+	7 33[f]
15	ST	2 00[a]	Black	Irregular	No	+	11 33[b]
16	SL1	4 00[d]	Black	Angular	No	+	3 00[ij]
	Control		-	-	-	-	-

NL NF Isolates from Neelum
PL PI – Isolates from Prior
BL BT Isolates from Banganapalli
AL – Isolates from Alphonso
CT isolate from Chandrakaran
ST SL Isolates from Sindhooram

\+ Presence of mycelia on symptom
Absence of mycelia on symptom
* Mean of three replications
DAI- Days after inoculation

Table 24. Evaluation of pathogenic variability of different isolates of *Colletotrichum* sp. on Neelum variety

Sl. No.	Characters of symptoms						
	Isolates	No. of days taken to symptom initiation *	Colour	Shape	Margin	Presence or absence of mycelia	Size of the spots on 5 DAI (mm)*
1	ML1	3 66^{d}	Darkish brown	Irregular	No	+	4 33^{f}
2	MI	2 33^{ab}	Black	Angular	No	-	9 00^{e}
3	MT1	2 00^{d}	Brown	Irregular	No	-	10 33^{dc}
4	MF	3 33^{cd}	Pale red	Irregular	Black	+	5 00^{f}
5	PL1	3 00^{bcd}	Black	Irregular	No	+	11 67^{cd}
6	PL2	2 33^{ab}	Brown	Irregular	No	-	4 66^{f}
7	PT	2 00^{a}	Dark brown	Irregular	No	+	13 33^{bc}
8	BL2	2 66^{abc}	Dark brown	Irregular	No	+	15 00^{ab}
9	BT	2 00^{d}	Brown	Angular	No	-	8 66^{e}
10	AL1	3 66^{d}	Black	Irregular	No	-	4 66^{f}
11	AL2	2 66^{abc}	Brownish black	Irregular	No	-	8 66^{e}
12	AL3	2 66^{abc}	Dark brown	Irregular	No		12 00^{cd}
13	CT	2 00^{a}	Brown	Irregular	No		15 33^{a}
14	SL1	2 00^{a}	Dark black	Irregular	No		11 66^{cd}
15	SL2	2 66^{abc}	Brown	Irregular	No		9 00^{e}
	Control	-	-	-	-	-	-

ML MF – Isolates from Muvandan
PL PT – Isolates from Prior
BL BT – Isolates from Banganapalli
AL– Isolates from Alphonso
* Mean of three replications

CT – Isolate from Chandrakaran
SL Isolates from Sindhooram
+ Presence of mycelia on symptom
Absence of mycelia on symptom
DAI Days after inoculation

irregular shaped spots Mycelial growth on the inoculated area was noticed in five isolates *viz* , ML1, MF, PL1, PT, BL2 On fifth day after inoculation the size of the spots were observed to be in the range of 4 33mm to 15 33 mm The isolate from Chandrakaran recorded the maximun size of 15 33 mm on 5 DAI

4.6.1.3. Pathogenic variability of different isolates of *Colletotrichum* sp. on the variety Prior

Out of 26 isolates of *Colletotrichum* spp inoculated on the Prior variety, only eight isolates produced symptom on the leaves of Prior Among them two isolates each from Muvandan and Neelum and one isolate each from Banganapalli, Alphonso, Chandrakaran and Sindhooram The isolates took two to four days for symptom expression and the isolates AL2 took the maximum period of four days to cause symptom These isolates produced pale brown to black, angular to irregular spots without any margin Mycelial growth on symptom was observed on leaves inoculated with the isolates NL1 and CT Largest spot was noticed in MT2 (12 33 mm) followed by NL1(10 mm) and smallest spot was observed in AL2 (4 67 mm) on 5 DAI (Table 25)

4.6.1.4. Pathogenic variability of different isolates of *Colletotrichum* sp. on the variety Banganapalli

Among the 26 isolates, 12 isolates produced symptom on this variety The isolates took two to four days for symptom expression (Table 26) Most of the isolates produced brown to black coloured spots except PL1, which showed the formation of pale red spot Angular spot was noticed only in MT1 Margin was not observed in any spot except in the spot produced by PL1, in which thin dark red margin was observed Mycelial growth on the inoculated area was observed only in three isolates *viz* , MI, PL1, SL2 Four isolates (MI, MT1, AL4, SL2) showed small spots measured less than 5 mm The isolate AL4 showed smallest spot of 4 33 mm and NF1 and NF2 showed large sized spots of 16 66 mm

Table 25. Evaluation of pathogenic variability of different isolates of *Colletotrichum* sp. Prior variety

Sl No.	Characters of symptoms						
	Isolates	No. of days taken to symptom initiation*	Colour	Shape	Margin	Presence or absence of mycelia	Size of the spots on 5 DAI (mm)*
1	MT1	3 33	Black	Irregular	No	-	6 00 ^{cd}
2	MT2	2 00	Dark brown	Irregular	No	-	12 33 ^{a}
3	NL1	2 33	Brown	Irregular	No	+	10 00 ^{ab}
4	NT2	3 33	Black	Angular	No	-	5 33 ^{cd}
5	BL3	2 66	Pale brown	Irregular	No	-	7 00 ^{bcd}
6	AL2	4 00	Black	Angular	No	-	4 67 ^{d}
7	CT	2 66	Brown	Irregular	No	+	8 33 ^{bc}
8	SL1	3 00	Light brown	Irregular	No	-	6 33 ^{cd}
	Control		-		-	-	-

MT – Isolates from Muvandan
NL Isolates from Neelum
BL Isolate from Banganapalli
AL Isolate from Alphonso
CT Isolate from Chandrakaran

+ Presence of mycelia on symptom
- Absence of mycelia on symptom
* Mean of three replications
DAI Days after inoculation
SL Isolate from Sindhooram

Table 26. Evaluation of pathogenic variability of different isolates of *Colletotrichum* sp. on Banganapalli variety

Sl. No.	Characters of symptoms						
	Isolates	No. of days taken to symptom initiation*	Colour	Shape	Margin	Presence or absence of mycelia	Size of the spots on 5 DAI (mm)*
1	ML1	2 00 [a]	Dark brown	Irregular	No	No	10 00 [e]
2	MI	3 33 [cde]	Dark brown	Irregular	No	Yes	5 66 [de]
3	MT1	3 00 [bcd]	Brown	Angular	No	No	6 00 [de]
4	MT2	2 66 [abc]	Dark black	Irregular	No	No	9 33 [c]
5	NF1	2 00 [a]	Brown	Irregular	No	No	16 66 [a]
6	NF2	2 00 [a]	Brown	Irregular	No	No	16 66 [a]
7	PL1	2 00 [a]	Pale red	Irregular	Dark red, thin	Yes	13 66 [b]
8	AL1	2 33 [ab]	Black	Irregular	No	No	10 00 [e]
9	AL3	2 33 [ab]	Black	Irregular	No	No	7 66 [cd]
10	AL4	4 00 [e]	Brownish black	Irregular	No	No	4 33 [e]
11	CT	2 66 [abc]	Black	Irregular	No	No	13 33 [b]
12	SL2	3 66 [de]	Black	Irregular	No	Yes	5 00 [e]
	Control		-	-	-	-	

ML MI MT Isolates from Muvandan
NF – Isolates from Neelum
PL isolate from Prior
AL Isolates from Alphonso
CT isolate from Chandrakaran

\+ Presence of mycelia on symptom
Absence of mycelia on symptom
* Mean of three replications
DAI Days after inoculation
SL Isolate from Sindhooram

4.6.1.5. Pathogenic variability of different isolates of *Colletotrichum* sp. on the variety Alphonso

Sixteen isolates showed symptoms on Alphonso, out of which five isolates were from Muvandan, four from Neelum, two each from Prior, Banganapalli and Sindhooram and one isolate from Chandrakaran (Table 27) The isolates took 2-3 33 days for symptom initiation and developed dark grey to black coloured spots without any margin Only two isolates (ML2, NF1) showed angular shaped spots developed near veins and all other isolates produced irregular shaped spots Mycelial growth was observed in six isolates *viz*, ML1, MF, NL1, NF1, PL1, and ST The largest spot was observed in SL2 (15 33 mm) and smallest in PI (4 66 mm).

4.6.1.6. Pathogenic variability of different isolates of *Colletotrichum* sp. on the variety Chandrakaran

Among the 29 isolates inoculated only 12 isolates developed symptom in Chandrakaran variety (Table 28) These isolates produced within four DAI Two isolates (NT2, NF2) showed pale red coloured spots, four isolates (MT2, NT1, PL1, PI) produced brown to dark brown coloured spots and six isolates (ML1, NF3, AL3, NL1, NL2, BL3) showed brownish black to black coloured spots Prominent margin was not observed in any symptom produced by the isolates All isolates showed irregular shaped spots except NT1, which produced angular spot In seven isolates, mycelial growth noticed on the infected area Smallest spot was observed on the PT 2 (3mm) and the largest spot was seen in NF3 (15 33 mm) on 5 DAI

4.6.1.7. Pathogenic variability of different isolates of *Colletotrichum* sp. on the variety Sindhooram

Twenty seven isolates of *Colletotrichum* spp were inoculated, among them only 13 isolates produced symptoms on this variety (Table 29) Out of which, one isolate was from Chandrakaran, two isolates each from Muvandan, Prior, Banganapalli, Alponso and four isolates from Neelum Five isolates showed first

Table 27. Evaluation of pathogenic variability of different isolates of *Colletotrichum* sp. on Alphonso variety

Sl No	Characters of symptoms						
	Isolates	No. of days taken to symptom initiation*	Colour	Shape	Margin	Presence or absence of mycelia	Size of the spots on 5 DAI (mm)*
1	ML1	3 33 ^{c}	Brown	Irregular	No		5 00^{g}
2	ML2	2 00 ^{a}	Dark brown	Angular	No		11 00 ^{cd}
3	MI	2 66 ^{abc}	Black	Irregular	No	-	7 00 ^{efg}
4	MT1	3 00 ^{bc}	Brown	Irregular	No	+	5 00 ^{g}
5	MF	2 00 ^{a}	Black	Irregular	No	+	12 00 ^{abc}
6	NL1	3 00^{bc}	Brown	Irregular	No	+	6 00 ^{fg}
7	NT1	2 66 ^{abc}	Brown	Irregular	No	-	8 66 ^{de}
8	NF1	2 00 ^{a}	Black	Angular	No	+	11 00 ^{cd}
9	NF3	2 00 ^{a}	Black	Irregular	No	-	14 33 ^{ab}
10	PL1	2 33 ^{bc}	Brown	Irregular	No	+	10 33 ^{cd}
11	PI	3 33 ^{c}	Brown black	Angular	No	-	4 66 ^{g}
12	BL1	3 33 ^{c}	Black	Irregular	No	-	5 33 ^{fg}
13	BT	3 33 ^{c}	Black	Irregular	No	-	6 00 ^{fg}
14	CT	3 00 ^{bc}	Brown	Irregular	No	-	7 66 ^{ef}
15	SL2	2 00 ^{a}	Dark grey	Irregular	No	-	15 33 ^{a}
16	ST	3 00 ^{bc}	Dark brown	Irregular	No	+	6 66 ^{efg}
	Control			-			-

ML MI MT MF- Isolates from Muvandan
NL NT NF– Isolates from Neelum
PL PI Isolates from Prior
BL BT Isolates from Banganapalli
CT Isolate from Chandrakaran
SL ST Isolates from Sindhooram

\+ Presence of mycelia on symptom
Absence of mycelia on symptom
* Mean of three replications
DAI Days after inoculation

Table 28. Evaluation of pathogenic variability of different isolates of *Colletotrichum* sp on Chandrakaran variety

Sl. No.	Characters of symptoms						
	Isolates	No. of days taken to symptom initiation*	Colour	Shape	Margin	Presence or absence of mycelia	Size of the spots on 5 DAI (mm)*
1	ML1	3 00 ^{bc}	Brownish black	Irregular	No	+	7 00 ^{cd}
2	MT2	3 66 ^{c}	Brown	Irregular	No	+	3 00 ^{f}
3	NL1	3 00 ^{bc}	Black	Irregular	No	-	6 66 ^{cde}
4	NL2	3 00 ^{bc}	Black	Irregular	No	+	5 33 ^{def}
5	NT1	2 00 ^{a}	Brown	Angular	No	-	13 00 ^{a}
6	NT2	2 00 ^{a}	Pale red	Irregular	No	-	13 33 ^{a}
7	NF2	2 00 ^{a}	Pale red	Irregular	No	-	13 67 ^{a}
8	NF3	2 00 ^{a}	Brownish black	Irregular	No	+	15 33 ^{a}
9	PL1	3 66 ^{c}	Dark brown	Irregular	No	+	4 33 ^{ef}
10	PI	2 66 ^{ab}	Dark brown	Irregular	No	-	8 00 ^{bc}
11	BL1	2 00 ^{a}	Black	Irregular	No	+	10 33 ^{b}
12	AL3	2 66 ^{ab}	Brownish black	Irregular	No	+	8 67 ^{bc}
	Control				-		-

ML MT – Isolates from Muvandan
NL NT NF – Isolates from Neelum
PL PI – Isolates from Prior
BL – Isolate from Banganapalli
AL Isolate from Alphonso

\+ Presence of mycelia on symptom
Absence of mycelia on symptom
* Mean of three replications
DAI Days after inoculation

Table 29. Evaluation of pathogenic variability of different isolates of *Colletotrichum* sp. on Sindhooram variety

Sl. No.	Characters of symptoms						
	Isolates	No. of days taken to symptom initiation*	Colour	Shape	Margin	Presence or absence of mycelia	Size of the spots on 5 DAI (mm)*
1	MI	2 00[a]	Dark brown	Angular	No	-	9 66[bc]
2	MT2	3 00[bc]	Brown	Irregular	No	+	7 33[de]
3	NL3	3 33[a]	Pale red	Irregular	No		5 00[fg]
4	NT1	2 66[abc]	Brownish black	Irregular	No	+	7 33[de]
5	NF1	2 66[abc]	Black	Angular	No	-	5 66[efg]
6	NF3	2 00[a]	Brown	Irregular	No	-	11 00[b]
7	PL1	3 00[ab]	Dark brown	Irregular	No	-	6 33[def]
8	PI	2 33[ab]	Black	Irregular	No	+	10 00[bc]
9	BL1	2 00[a]	Dark black	Irregular	No	-	10 33[bc]
10	BT	2 00[a]	Black	Irregular	No	+	14 67[a]
11	AL1	2 33[ab]	Black	Irregular	No	+	8 33[cd]
12	AL4	2 00[a]	Brownish black	Irregular	No		11 33[b]
13	CT	3 00[bc]	Brown	Irregular	No	+	4 00[g]
	Control					-	-

MI MT Isolates from Muvandan
NL NT NF Isolates from Neelum
PL PI Isolates from Prior
BL BT Isolates from Banganapalli

+ Presence of mycelia on symptom
- Absence of mycelia on symptom
* Mean of three replications
DAI Days after inoculation

symptom on second DAI whereas the rest of isolates (eight) took more than two days to produce infection They produced brown to dark brown spots without any margin Angular spots were observed in the isolates MI and NF1 and except these two isolates showed irregular shaped spots In the infection caused by the isolates *viz* , MT2, NT1, PI, BT1, AL1 and CT, mycelial growth was observed Small sized spot was produced by CT (4 mm) and large sized spots in BT (14 67 mm) followed by AL4, NF3, BL and PI in which spots were measured 10- 11 33 mm size

4.6.2. Virulence rating of isolates based on pathogenic variability

Based on the efficiency of each isolate to infect number of varieties (Table 30) and time taken to symptom expression, the 30 isolates were grouped into three as highly virulent (HV), moderately virulent (MV) and less virulent (LV) isolates The isolate CT from the variety Chandrakaran was found to infect all the seven varieties and was followed by PL1 and NF3 which showed infection on six varieties Accordingly NF3, PL1 and CT were grouped as highly virulent, 16 isolates including four each from Muvandan and Neelum, three from Alphonso, two each from Banganapalli and Sindhooram and one from Prior as moderately virulent, and 11 isolates including two each from Muvandan, Prior and Banganapalli, three from Neelum and one each from Alphonso and Sindhooram were grouped under less virulent isolates (Table 31)

4.6.3. Identification of most virulent isolates of *Colletotrichum* sp.

The isolates which showed high virulence in pathogenic characters were selected from each variety of mango and were identified based on the phenotypic characters The cultural and morphological characters of the seven isolates were compared with the characters described in the CMI descriptions of Pathogenic Fungi and Bacteria and were tentatively identified as *Colletotrichum gloeosporioides* (Penz) Penz & Sacc For further confirmation cultures were sent to NCFT, New Delhi and identified as *Colletotrichum gloeosporioides* (Penz) Penz & Sacc (Table 32)

Table 30. Pathogenic variability- response of mango varieties against *Colletotrichum* sp

Isolates	Muvandan	Neelum	Prior	Banganapalli	Alphonso	Chandrakaran	Sindhooram	No of varieties susceptible for isolates
ML1	+	+		+	+	+		4
ML2	+	-	-	-	+		-	2
Ml	+	+		+	+	-	+	5
MT1	+	+	+	+	+	-		5
MT2	+	-	+	+		+	+	5
MF	+	+	-		+	-	-	3
NL1	+	+	+	-	+	+	-	5
NL2		+	-	-	-	+		2
NL3	+	+	-		-	-	+	2
NT1		+		-	+	+	+	4
NT2	-	+	+	-		+	-	3
NF1	+	+		+	+	-	+	5
NF2	+	+	-	+		+	-	4
NF3	+	+		+	+	+	+	6

3 6 Cluster analysis

Based on the cultural and morphological characters such as days taken for full growth in Petri dishes, growth rate in PDA and GBA media, spore formation, pink spore mass formation, length and breadth of spore, acervulus formation and days taken to initial symptom expression during pathogenicity test were considered to check the similarity between the thirty isolates Similarity among the 30 isolates was also checked through NTSYS pc 2 02 software, by using Unweighted Pair Group Method with Arithmetic mean (UPGMA) and plotted dendrogram

3.7. Evaluation of vegetative compatibility of different isolates of *Colletotrichum* sp.

The vegetative compatibility of different isolates of *Colletotrichum* sp obtained from seven different varieties of mango was evaluated under *in vitro* on PDA medium by dual culture technique (Dennis and Webster, 1971) From the actively growing culture of different isolates, mycelial disc of 10 mm diameter was taken and transferred to a Petri dish mediated with PDA The discs were placed at 4 5 cm apart at the centre of each half of a Petri dish The dual cultures were prepared with all possible combinations of thirty different isolates obtained from seven different varieties of mango Single isolates placed at the centre of one half of the Petri dish served as control Three replications were maintained for each combination of isolates All dishes were incubated at room temperature and observed for the growth of the isolates The type of reaction between the isolates was recorded by the method given by Webber and Hedger (1986) Observations on the type of reactions were taken till full growth attained in control plates

Types of reaction

1 Extreme inhibition of pathogen (E)

Isolates	Muvandan	Neelum	Prior	Banganapalli	Alphonso	Chandrakaran	Sindhooram	No of varieties susceptible for isolates
PL1	+	+	+		+	+	+	6
PL2	+	+	+					3
PI	+	-	+		+	+	+	5
PT		+	+	-	-			2
BL1	+			+	+	+	+	5
BL2	+	+	-	+		-		3
BL3			+	+				2
BT	+	+		+	+	-	+	5
AL1	+	+		+		-	+	5
AL2	+	+	+	-		-		3
AL3		+		+		+		4
AL4		-		+			+	3
CT	+	+	+	+	+	+	+	7
ST	+			-	+		-	3
SL1	+	+	+		-	-		4
SL2		+		+	+	-		4

ML MI MT MF Isolates from Muvandan
PL PI PT Isolates from Prior
AL Isolates from Alphonso
SL ST Isolates from Sindhooram

NL NT NF Isolates from Neelum
BL BT Isolate from Banganapalli
CT Isolate from Chandrakaran

Table 31. Virulence rating of isolates based on pathogenic variability

Rating	Isolates
Highly virulence	CT, NF3, PL1
Moderately virulence	ML1,MI, MT1, MT2, NL1, NT1, NF1, NF2, PI, BL1, BT AL1, AL2, AL3, SL1, SL2
Least virulence	ML2, MF, NL2, NL3, NT2, PL2, PT, BL2, BL3, AL4, ST

ML MT, MF Isolates from Muvandan
NL, NT NF Isolates from Neelum
PL1, PI and PL2 Isolates from Prior
BL BT- Isolate from Banganapalli
AL Isolates from Alphonso
CT Isolate from Chandrakaran
SL, ST Isolates from Sindhooram

Table 32. Identification of *Colletotrichum* isolates by NCFT

Sl. No.	Isolates	Identification	I.D. No. of NCFT
1	MT1	*Colletotrichum gloeosporioides* (Penz) Penz & Sacc	8214 16
2	NL2	*Colletotrichum gloeosporioides* (Penz) Penz & Sacc	8215 16
3	PL1	*Colletotrichum gloeosporioides* (Penz) Penz & Sacc	8216 16
4	AL2	*Colletotrichum gloeosporioides* (Penz) Penz & Sacc	8217 16
5	BL1	*Colletotrichum gloeosporioides* (Penz) Penz & Sacc	8218 16
6	CT	*Colletotrichum gloeosporioides* (Penz) Penz & Sacc	8219 16
7	SL2	*Colletotrichum gloeosporioides* (Penz) Penz & Sacc	8220 16

NCFT- National Centre for Fungal Taxonomy
MT Isolates from Muvandan
NL Isolates from Neelum
PL Isolates from Prior
BL Isolate from Banganapalli
AL Isolates from Alphonso
CT Isolate from Chandrakaran
SL Isolates from Sindhooram

4.7. *IN VITRO* EVALUATION OF PLANT PROTECTION CHEMICAL AGAINST ISOLATES OF *COLLETOTRICHUM* SP.

The sensitivity of different isolates of *Colletotrichum* sp against plant protection chemical was evaluated by poisoned food technique under *in vitro* condition The study on effect of seven fungicides and two insecticides were done and the results are given in the Table 33- 36

4.7.1. Isolates of *Colletotricum gloeosporioides* from Muvandan

Statistical analysis of data given in the Table 33 showed significant difference in the effect of treatments on the growth of the pathogen Among the seven fungicides evaluated, carbendazim (0 1%), tebuconazole (0 1%), hexaconazole (0 1%), and azoxystrobin (0 1%) recorded cent per cent inhibition on the growth of all the six isolates of pathogen from Muvandan variety Bordeaux mixture (1%) recorded complete inhibition on the growth of ML2, MI, MT2 and MF isolates, whereas copper hydroxide (0 15%) recorded the same in MT1, MT2, and MF isolates Mancozeb (0 3%) showed the highest per cent inhibition of 81 85 in the isolate ML2 and lowest inhibition (69 63%) in the isolate MF The lowest inhibition on the growth of all isolates of pathogen except ML2 over control was recorded by the insecticide dimethoate (0 05%) and it ranged from 52 59 to 74 00 per cent The insecticide malathion (0 1%) recorded 60 74 to 78 14 per cent inhibition on the growth of the isolates of *Colletotrichum gloeosporioides* (Fig 2, Plate 11)

4.7.2. Isolates of *Colletotricum* sp. from Neelum

The results of the *in vitro* evaluation given in the Table 34 showed significant difference in the effect of treatments against the eight isolates of the pathogen from the Neelum variety Cent per cent inhibition on the growth of all isolates was recorded by carbendazim (0 1%), tebuconazole (0 1%), hexaconazole (0 1%), and azoxystrobin (0 1%) and Bordeaux mixture (1%) Copper hydroxide (0 15%) showed hundred per cent inhibition in all isolates except NT2 (82 22%) and NF3 (50 74) In mancozeb (0 3%), complete inhibition

Table 33. *In vitro* evaluation of plant protection chemical against different *Colletotricum* sp. isolates from Muvandan

Chemicals	Concentration (%)	Isolates of *Colletotrichum* sp.					
		PIOC*					
		ML1	ML2	ML3	MT1	MT2	MF
Bordeaux mixture	1	90 37[b]	(100)[a]	(100)[a]	69 63[c]	(100)[a]	(100)[a]
Copper hydroxide 77WP	0 15	85 18[b]	70 74[e]	78 88[b]	(100)[a]	(100)[a]	(100)[a]
Mancozeb 75 WP	0 3	79 25[c]	81 85[b]	72 22[c]	79 63[b]	73 70[b]	69 63[b]
Carbendazim 50 WP	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Tebuconazole 25.9 EC	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Hexaconazole 5 EC	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Azoxystrobin 18 2 W/W	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Malathion 50 EC	0 1	76 30[c]	78 14[c]	68 14[d]	68 88[c]	62 22[c]	60 74[c]
Dimethoate 30 EC	0 05	74 00[c]	75 15[c]	60 37[e]	54 44[d]	52 59[d]	58 90[c]
CD (0.05)		5 91	2 79	3 21	4 61	4 78	4 15

PIOC Per cent inhibition over control
* Mean of three replications
In each column figures followed by same letter do not differ significantly according to DMRT
ML Isolates from Muvandan leaves MT Isolates from Muvandan twigs
MI Isolate from Muvandan Inflorescence MF Isolate from Muvandan fruits

Table 34. ***In vitro*** **evaluation of plant protection chemical against different** ***Colletotricum*** **sp. isolates from Neelum**

Chemicals	Concentration (%)	Isolates of *Colletotrichum* sp.							
		PIOC*							
		NL1	NL2	NL3	NT1	NT2	NF1	NF2	NF3
Bordeaux mixture	1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Copper hydroxide 77WP	0 15	(100)[a]	(100)[a]	(100)[a]	(100)[a]	82 22[b]	(100)[a]	(100)[a]	50 74[d]
Mancozeb 75 WP	0 3	80 74[b]	90 00[b]	84 44[b]	(100)[a]	81 48[b]	(100)[a]	(100)[a]	68 88[c]
Carbendazim 50 WP	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Tebuconazole 25 9 EC	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Hexaconazole 5 EC	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Azoxystrobin 18 2 W/W	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Malathion 50 EC	0 1	65 55[c]	71 11[c]	75 92[c]	66 29[c]	82 59[b]	76 66[b]	62 96[c]	(100)[a]
Dimethoate 30 EC	0 05	72 59[d]	59 26[d]	77 03[c]	74 81[b]	81 48[b]	72 22[c]	72 96[b]	77 77[b]
CD (0 05)		3 28	7 25	2 34	5 45	2 841	2 201	1 640	2 405

PIOC Per cent inhibition over control
* Mean of three replications
In each column figures followed by same letter do not differ significantly according to DMRT
NL Isolates from Neelum leaves NF Isolates from Neelum fruits
NT Isolates from Neelum Twigs

Fig 2 Effect of plant protection chemicals against *Colletotrichum* isolates from Muvandan

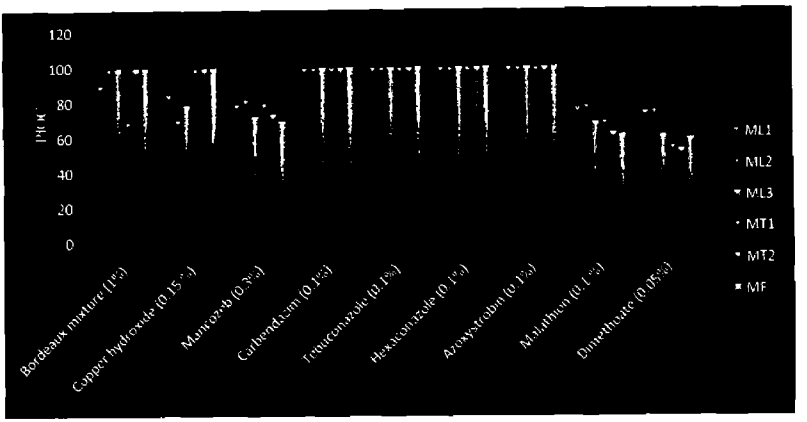
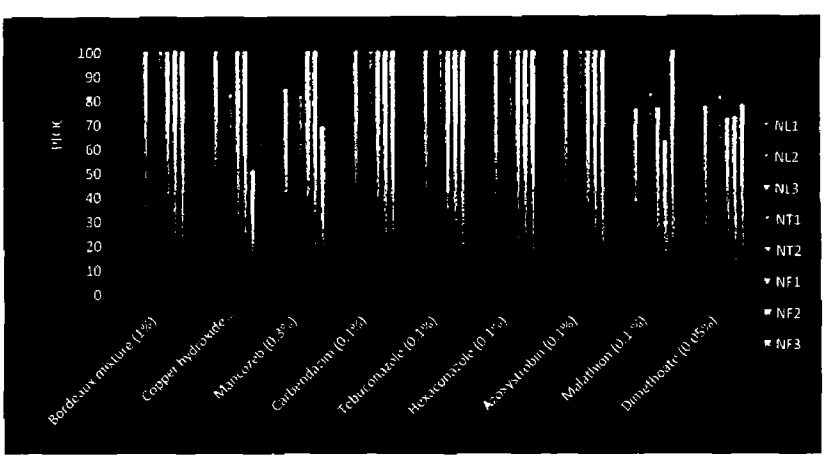


Fig 3 Effect of plant protection chemicals against *Colletotrichum* isolates from Neelum



on the growth was noticed in NT1, NF1 and NF2 isolates Among the insecticides, the inhibition was observed in the range of 59 26 to 100 per cent where malathion (0 1%) recorded cent per cent inhibition of the isolate NF3 (Fig 3, Plate 11)

4.7.3. Isolates of *Colletotricum* sp. from Prior and Banganapalli

The data given in the Table 35 revealed significant difference in the effect of treatments on the growth of isolates of the pathogen from Prior and Banganapalli All fungicides except mancozeb recorded hundred per cent inhibition on the growth of four isolates each from the Prior and Banganapalli Mancozeb (0 3%) showed the inhibition on the growth in the range of 72 96-100 per cent, where the growth of the isolates BL2 and BL3 were completely inhibited by the fungicide treatment The insecticides recorded 55 55 (BL2) to 83 70 (PT) per cent inhibition on the growth of different isolates of pathogen (Fig 4 & 5, Plate 11)

4.7.4. Isolates of *Colletotricum* sp. from Alphonso, Chandrakaran, Sindhooram

Four isolates from Alphonso, one isolate from Chandrakaran and three isolates from Sindhooram varieties were evaluated for their sensitivity to palnt protection chemicals and the data are presented in the Table 36 Statistical analysis of the data showed significant difference among the treatments on inhibiting the growth of isolates All fungicides except copper hydroxide (0 15%) and mancozeb (0 3%) recorded cent per cent inhibition on the growth of all isolates from these three varieties of mango Copper hydroxide (0 15%) recorded hundred per cent inhibition of all isolates except ST isolate (84 44%), whereas mancozeb (0 3%) showed cent per cent inhibition of only two isolates *viz* , CT and SL1 The insecticides, malathion (0 1%) and dimethoate (0 05%) recorded the inhibition on the growth of isolates in the range of 50 74 to 81 85 per cent (Fig 6-9, Plate 11)

Table 35. ***In vitro*** **evaluation of plant protection chemical against different** ***Colletotrichum*** **sp. isolates from Prior and Banganapalli**

Chemicals	Concentration (%)	Isolates of *Colletotrichum* sp.							
		PIOC*							
		PL1	PL2	PL3	PT	BL1	BL2	BL3	BT
Bordeaux mixture	1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Copper hydroxide 77WP	0 15	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Mancozeb 75 WP	0 3	80 74[b]	90 00[b]	84 44[b]	90 00[b]	72 96[b]	(100)[a]	(100)[a]	81 85[b]
Carbendizim 50 WP	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Tebuconazole 25 9 EC	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Hexaconazole 5 EC	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Azoxystrobin 18 2 W/W	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Malathion 50 EC	0 1	65 55[d]	71 11[c]	75 92[c]	82 22[c]	69 63[b]	55 55[c]	76 29[b]	63 33[c]
Dimethoate 30 EC	0 05	72 59[c]	59 25[d]	77 03[c]	83 70[c]	73 70[b]	64 44[b]	71 48[c]	62 22[c]
CD (0 05)		3 28	7 25	2 35	5 17	5 77	2 29	2 07	2 40

PIOC Percent inhibition over control
* Mean of three replications
In each column figures followed by same letter do not differ significantly according to DMRT

PL Isolates from Prior leaves
PI Isolate from Prior inflorescence
PT Isolate from Prior twigs

BL Isolates from Banganapalli leaves
BT Isolate from Banganapalli twigs

Table 36. *In vitro* evaluation plant protection chemical against isolates of *Colletotrichum* sp. from Alphonso, Chandrakaran and Sindhooram isolates

Chemicals	Concentration (%)	Isolates of *Colletotrichum* sp							
		PIOC*							
		AL1	AL2	AL3	AL4	CT	ST	SL1	SL2
Bordeaux mixture	1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Copper hydroxide 77WP	0 15	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	84 44[b]	(100)[a]	(100)[a]
Mancozeb 75 WP	0 3	89 25[b]	75 92[c]	90 00[b]	94 81[a]	(100)[a]	71 85[c]	(100)[a]	84 07[b]
Carbendazim 50 WP	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Tebuconazole 25.9 EC	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Hexaconazole 5 EC	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Azoxystrobin 18 2 W/W	0 1	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]	(100)[a]
Malathion 50 EC	0 1	80 00[c]	81 48[b]	65 18[d]	72 59[b]	55 55[c]	68 51[d]	54 07[b]	75 56[c]
Dimethoate 30 EC	0 05	70 37[d]	70 74[c]	81 85[c]	76 29[b]	62 59[b]	68 51[d]	50 74[c]	65 18[d]
CD (0 05)		5 67	1 68	5 15	6 03	2 32	2 37	3 02	1 75

PIOC Per cent inhibition over control
* Mean of three replications
In each column figures followed by same letter do not differ significantly according to DMRT

AL Isolates from Alphonso leaves
CT Isolate from Chandrakaran twigs
ST Isolate from Sindhooram twigs
SL Isolates from Sindhooram leaves

Fig 4 Effect of plant protection chemicals against *Colletotrichum* isolates from Prior

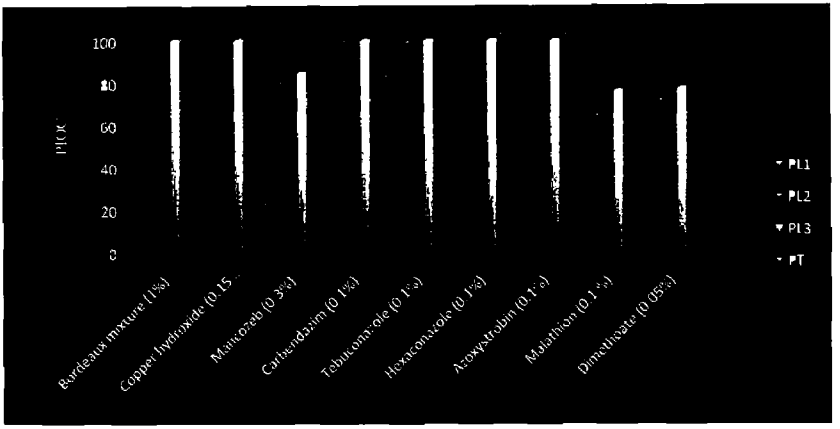


Fig 5 Effect of plant protection chemicals against *Colletotrichum* isolates from Banganapalli

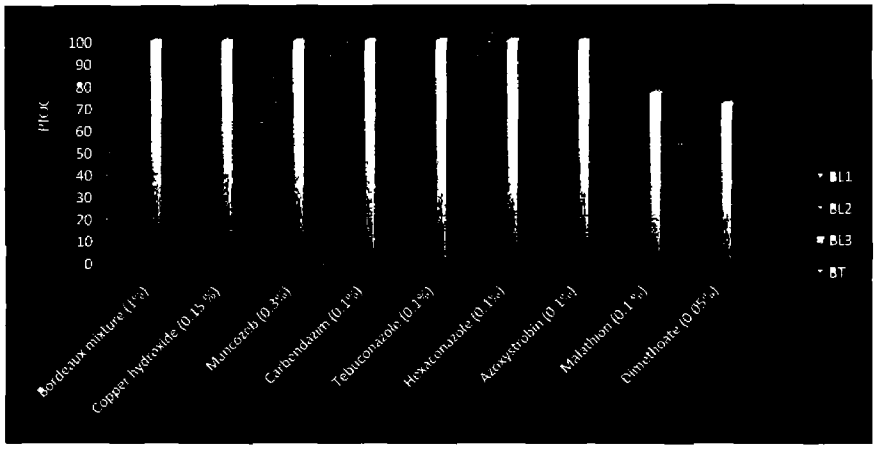


Fig 6 Effect of plant protection chemicals against *Colletotrichum* isolates from Alphonso

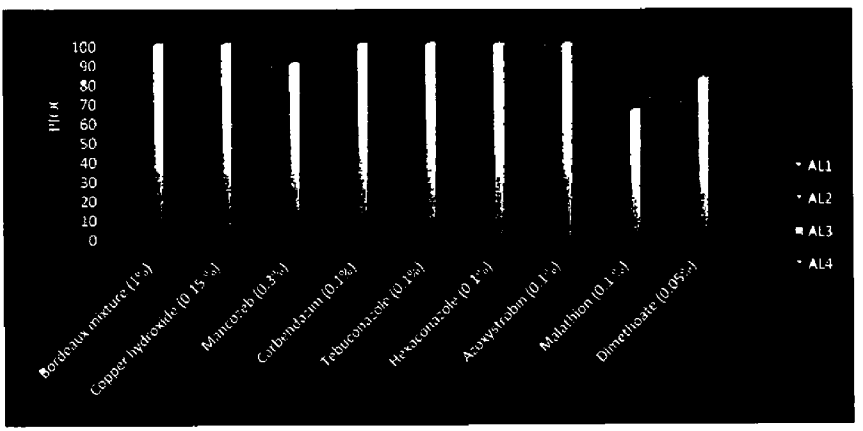


Fig 7 Effect of plant protection chemicals against *Colletotrichum* isolates from Chandrakaran

Fig 8 Effect of plant protection chemicals against *Colletotrichum* isolates from Sindhooram

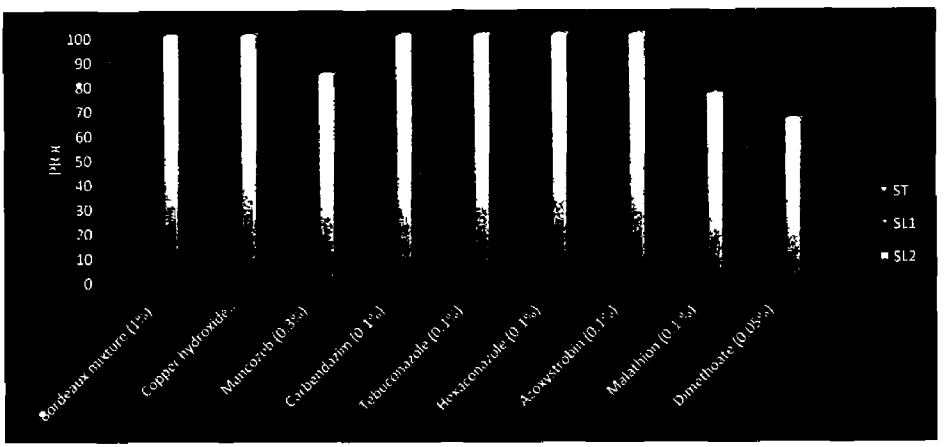
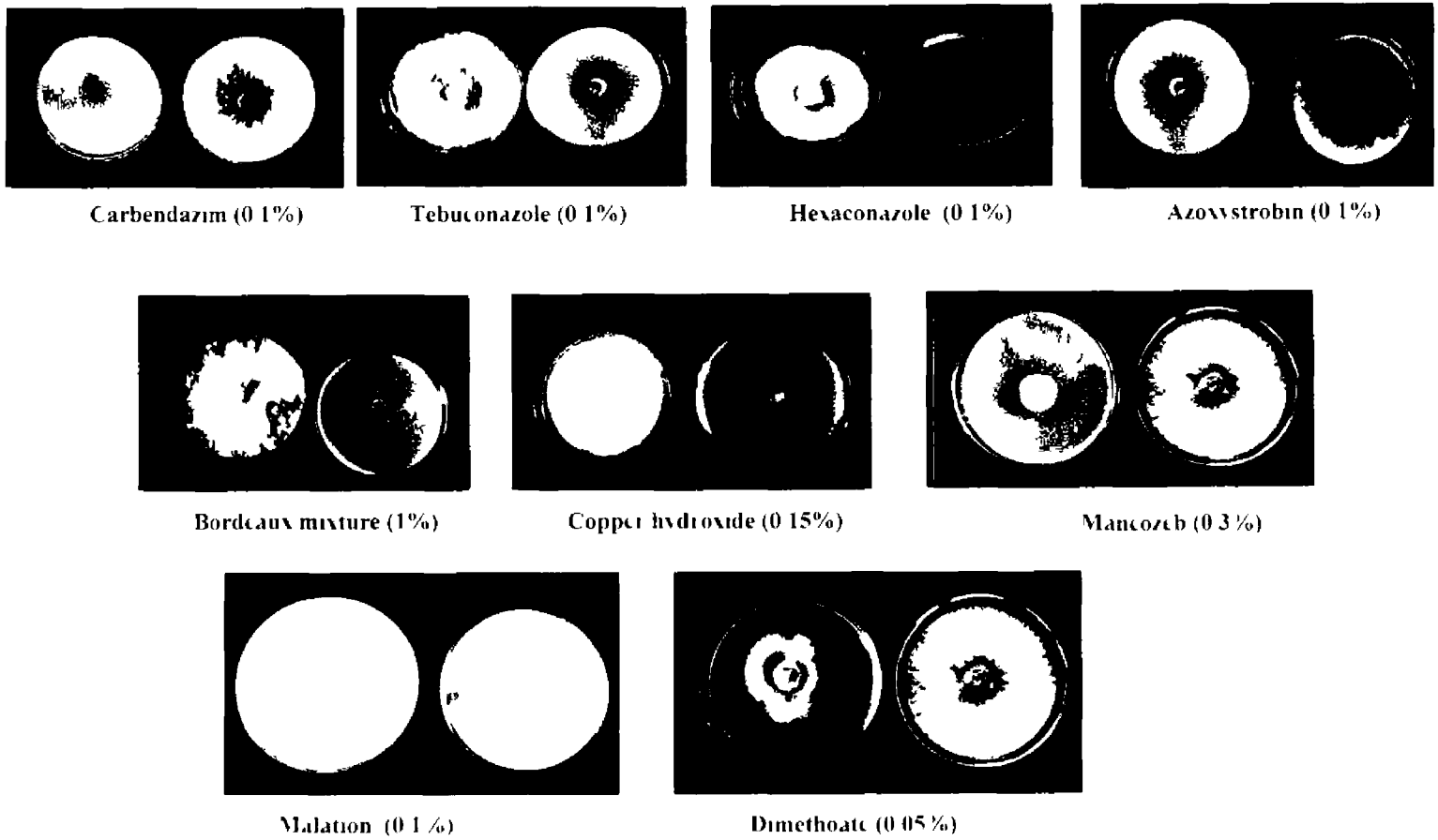


Plate 11. *In vitro* evaluation of plant protection chemicals



Carbendazim (0.1%) Tebuconazole (0.1%) Hexaconazole (0.1%) Azoxystrobin (0.1%)

Bordeaux mixture (1%) Copper hydroxide (0.15%) Mancozeb (0.3%)

Malation (0.1%) Dimethoate (0.05%)

From the results of the *in vitro* evaluation of the plant protection chemicals against the isolates of pathogen, it was recorded that four systemic fungicides *viz*, carbendazim (0 1%), tebuconazole (0 1%), hexaconazole (0 1%), and azoxystrobin (0 1%) were highly effective for the management of anthracnose pathogen *Colletotrichum gloeosporioides* and these fungicides recorded hundred per cent inhibition on the growth of all the 30 isolates of pathogen obtained from seven varieties of mango

Discussion

5. DISCUSSION

Mango (*Mangifera indica* L) is an important fruit crop of tropical and subtropical countries It is considered as 'King of fruits' and regarded as one of the world's most important fruit crops India stands first in global mango production but compared to other states, contribution from Kerala to the national production is low

One of the constraints in production of mango is the disease of which, anthracnose is the major pre and postharvest disease of the crop (Ploetz, 2003, Prakash, 2004) Anthracnose caused by *Colletotrichum gloeosporioides* is the most severe and wide spread disease and the pathogen is known to attack all stages of plant growth The fruits infected at field condition carry the pathogen into storage and may cause latent infection Hence, *Colletotrichum* sp is considered as the most important post harvest pathogen Most of the varieties and cultivars of mango are highly susceptible to this pathogen and almost every parts of the tree are attacked and produced symptoms like leaf spots, dieback, anthracnose, fruit rot etc Moreover, the pathogen has a wide host range and is responsible for the anthracnose disease in many other fruit crops

Literature review revealed many reports on the variability in the phenotypic characters of the anthracnose pathogen, *Colletotrichum* sp Ploetz (1999) reported that isolates from mango comprises genetically and pathogenically distinct population of this species There are reports on the association of *Colletotrichum acutatum* as one of the causal agent of mango anthracnose (Fitzell, 1979, Jayasinghe and Fernando, 2009) Chowdappa and Kumar (2012) also reported the presence of two genetically distinct populations of *Colletotrichum gloeosporioides* in mango from India But reports on variability study in *Colletotrichum* sp causing anthracnose disease of mango in Kerala were not revealed in literature review In this background this study was taken up to elucidate the existence of variability if any, among the isolates of *Colletotrichum* causing anthracnose of common varieties of mango growing in Kerala

This study was started with the collection of diseased specimens of mango for the isolation of the anthracnose pathogen For that, purposive sampling surveys were conducted at different locations of Thrissur and Palakkad districts This included different farmer's fields, private nurseries, mango orchards and sales counters of KAU in Thrissur district and RATTC, Mallampuzha and farmers' fields at Chulliyarmedu in Palakkad districts The mango varieties commonly cultivated in Kerala *viz*, Muvandan, Neelum, Prior, Banganapalli, Alphonso, Chandrakaran and Sindhooram were selected and diseased specimens from leaves, twigs, inflorescence and fruits were collected from these varieties for the isolation of pathogen

The isolation was carried out on Potato Dextrose Agar (PDA) medium and finally 30 isolates of *Colletotrichum* sp were obtained which included six isolates from Muvandan, eight from Neelum, four each from Prior, Banganapalli and Alphonso, three from Sindhooram and one from Chandrakaran Among them, 16 isolates were obtained from leaves, eight from twigs, four from fruits and two from inflorescence of these seven varieties Earlier workers were also isolated the anthracnose pathogen from different fruit crops on Potato Dextrose Agar (PDA) medium (Lakshmi *et al*, 2011, Phoulivong *et al*, 2012 Singh *et al*, 2012, Rhaiem *et al*, 2016)

To know the potential capacity of the pathogen on their respective host, pathogenicity test was carried out The pathogenicity of all the 30 isolates was proved on their respective host All the isolates recorded symptom expression on two to five days after inoculation (DAI) All the four isolates from Prior and Alphonso, one isolate from Muvandan (MT2) and five isolates from Neelum (NL1, NT1, NF1, NF2, NF3) showed the initial symptom on two DAI and these were considered as the most virulent isolates The incubation period i e, the time between inoculation and symptom expression, is considered as one of the important factors for deciding the virulence of the pathogen These 14 isolates proved to be as the most virulent in attacking and producing symptoms on their respective host during the pathogenicity test Phoulivong *et al* (2012) reported that *Colletotrichum* isolates from mango were highly pathogenic when

reinoculated on mango Similar line of work was carried out by Pandey *et al* (2012) and Guettia *et al* (2014) and reported the high pathogenic efficiency of *Colletotrichum* isolates on mango

During the survey, the symptoms produced by this pathogen on different plant parts of these seven selected varieties were studied in detail Similarly, the symptomatology on artificial condition was studied during the pathogenicity test Under the field conditions, variations in the symptoms produced by the pathogen on the seven different varieties were observed In the variety Muvandan, infection was observed on all plant parts of the seedlings and matured trees Leaf spot, shot hole and die back symptoms were mainly seen on seedlings On trees, leaves, twigs, inflorescence and fruits were found infected by the pathogen On leaves two types of symptoms were observed On young leaves, small sized back spots were initiated which later coalesced to irregular reddish brown spots without any yellow halo Later shot hole symptom was observed in these leaves

On matured leaves reddish brown to black round shaped distinct spots with black margin were noticed Large irregular shaped greyish white spots with dark margin were also observed on leaves On these spots, acervulus formation was noticed as black dots In all these symptoms, shot hole formation or dropping off of the infected area was also observed Infections on leaf margin and leaf tip were also noticed and that resulted in irregular shape of the leaves These types of irregular shaped greyish white spots were also observed in the variety Alphonso But in Alphonso, yellow halo was noticed around these spots In all other varieties like Neelum, Prior, Banganapalli, Alphonso, Chandrakaran and Sindhooram, reddish brown spots with yellow halo were observed on the leaves and these spots were bound by the vein or veinlets The intensity of yellow halo may vary as it was observed in Banganapalli with or without yellow halo and in Sindhooram with light yellow halo In all varieties development of shot hole was found to be a common symptom irrespective of the size of the spots In all seven varieties, twig infection was observed as small black lesions which later spread and resulted in dieback symptom Earlier workers also reported the same type of anthracnose symptom incited by the pathogen on leaves and twigs of mango

(Arauz, 2000, Sangeetha, 2009, Nelson, 2008, Rhaiem and Taylor, 2016) Anthracnose symptoms were observed on the fruits of Muvandan, Neelum, Banganapalli and Chandrakaran On fruits of Muvandan, small black coloured pin point like spots were observed in large number Later some spots enlarged and developed into sunken spots Development of fruiting body of the pathogen, acervulus, was observed on the surface of sunken spots These types of small sized spots were not observed in Neelum and Chandrakaran Tear stain symptom was noticed in some fruits of Muvandan and Banganapalli in which the sunken spots were developed in line from stalk end to the tip of the fruit Tear stain symptom was developed due to the spread of the pathogen, along with the fruit sap during the harvesting of fruits Usually the anthracnose symptoms on fruits are not observed in the field condition on unrippened fruits Infection by the pathogen may occur in the field but the symptom development is usually observed during storage or transportation So there are chances for the spreading of the pathogen along with the fruit sap which later developed as black sunken spots, which is the latent infection by the pathogen Hence this pathogen, *Colletotrichum* sp is also considered as post harvest pathogen Arauz, 2000, Nelson, 2008 and Guettia *et al*, 2014 reported the development of tear stain symptom on mango fruits infected by *Colletotrichum* sp Nelson (2008) observed tear stain symptoms on mango fruits with or without superficial cracking of the epidermis which lead to 'alligator skin' effect Hence the observations made in this study on the symptomatology of anthracnose on various plant parts of different varieties of mango in Kerala are in conformity with earlier reports

Symptomatology of the anthracnose disease on artificial condition was recorded during the pathogenicity test In general, all the 30 isolates produced light brown to black coloured irregular shaped lesions on their respective variety of mango In the variety Sindhooram, the isolate SL1 produced light brown lesions with pale red centre bounded with dark margin on the leaves The size of the lesions developed by the isolates on the different varieties was measured on five DAI and found variation in the size of the infected area developed by different isolates It was varied from 2 mm to 13 mm in diameter on 5 DAI The

isolate MT2 produced the largest lesion having 13 mm diameter with dark grey mycelial growth on the infected area The isolate MT1 and AL3 showed small sized spots of two millimetres in diameter In the field condition, yellow halo was observed around the spots in all varieties except Muvandan and Neelum But in artificial condition, yellow halo formation was not observed Pandey *et al* (2012) reported that pathogen invasion and symptom development process are similar in both natural infection and artificial inoculation which presented no significant difference between the expressions

The main objective of this study is to find out variability in the phenotypic characters of different isolates of *Colletotrichum* sp For that the cultural and morphological characters of the isolates were studied in detail on two different media *viz*, Potato Dextrose Agar (PDA) and Green Bean Agar (GBA) Perusal of literature revealed many reports on the variability in the cultural and morphological characters of this pathogen and many workers grouped the isolates of *Colletotrichum gloeosporioides* as fast growing and slow growing isolates based on the rate of growth *Colletotrichum* sp is an anamorphic fungus classified under the *Phylum Ascomycota* which produces conidia from the fruiting body, acervulus So the characters of acervulus like presence or absence of setae, pink spore mass formation etc, are important in a variability study Hence, these cultural characters along with the morphological characters of hyphae, conidia and setae of acervulus, of all the 30 isolates were also studied in details

Variation in the cultural characters among the 30 isolates was observed on PDA medium The isolates like ML1, NF3, PL2, CT and SL1 produced white coloured mycelial growth Whereas, some isolates like MF, NL2 and NL3 showed dark grey mycelial growth Except these eight isolates, the other isolates showed smoky white to light grey coloured mycelial growth and among them some produced fluffy growth at the centre of the culture in Petri dishes (MT2, NF2, NF3) Out of the 30 isolates, only four isolates showed irregular shaped cultural growth, whereas all other isolates recorded regular growth in the culture Similarly only four isolates showed zonations in the cultural growth and among

them the isolates CT showed clear zonations in the culture Difference in the colour development on the reverse side of the culture was also observed Usually the pathogen *Colletotrichum gloeosporioides* showed dark grey to black colour on the reverse side But in these 30 isolates, a variation in the colour development was noticed and observed white/ light grey/ brownish grey/ dark grey/ black colour on the back side of the culture The isolate SL2 showed small dark coloured dots on the reverse side of the culture

Among the various colony characters of a fungal pathogen, the formation of spores, fruiting body etc are very important In the culture, this pathogen can produce conidia directly from the mycelium On prolonged incubation, acervulus and pink spore mass formation can occur in the culture The time taken for spore development in the culture was studied by preparing slides from different parts of culture from second day after the incubation It was revealed that, the different isolates took three to eight days for spore formation in the culture One isolate from the fruit of Neelum variety (NF1) took the longest period of eight days whereas the isolate from the twig of Sindhooram (ST) took the minimum time of three days for the spore formation In general, majority of the isolates took five to seven days for the spore formation So a clear variation was observed in the spore development by these 30 isolates Similarly all the isolates did not produce acervulus and pink spore mass in the culture Only 11 isolates produced acervulus and among them, only in nine isolates the pink spore mass formation was noticed When this pathogen produce acervulus in the culture, large number of conidia will be produced from the conidiophores present in the acervulus When these spores clustered, appeared as light pink mass From the observation recorded, it was found that even though acervulus was formed, the isolates NF1 and BT could not produce enough number of comdia and hence they couldn't show the pink coloured spore mass formation in the culture Similarly variations in the cultural characters of different isolates of the anthracnose pathogen was reported by many earlier workers (Zakaria and Bailey, 2000, Benyahia, 2003, Honger, 2014, Ismail *et al*, 2015, Suvarna *et al*, 2015) Suvarna *et al* (2015) studied the cultural characters of 20 isolates of *C gloeosporioides* causing

mango anthracnose in Thirupati and reported variations in the colony characters such as growth, shape, colour, texture and zonation among these isolates

The cultural characters of these 30 isolates were also studied on GBA medium But unlike PDA medium, only slight variation was observed in the colony characters. Most of the isolates produced white coloured, thick cottony cushion textured regular shaped cultural growth without any zonation Colour on the reverse side of the culture varied from white to black with small dots in three isolates

Regarding the spore formation, 14 isolates showed the spore formation on three DAI, 9 isolates on four DAI and 7 isolates on five DAI So on GBA medium, it was found that the isolates showed conidia faster than on PDA medium There are reports by earlier workers on the use of GBA medium for the fast spore formation by Correll *et al* (1993), Jeun *et al* (2005) and Impullitti (2010) They used GBA medium for enhancing sporulation in *Colletotrichum lagenarium*, *C orbiculare* and *Phialophora gregata* Hence, the present results are in line with the earlier reports But acervulus and pink spore mass formation were not observed in any of the isolates on GBA medium

Another important cultural character studied was the growth rate of the different isolates of the pathogen on PDA and GBA media The results revealed wide variation in the radial growth exhibited among the isolates and also between the media On PDA medium, two to seven days were taken whereas on GBA medium it took five to twelve days to complete the growth Some isolates *viz*, ML1, MI, NL2, NL3, NT1, PL1, CT, SL1 could not complete 90 mm growth even after 12 days after incubation The isolate NL3 recorded only 19 mm growth at 12 DAI and it stopped the growth there From this, it is concluded that PDA medium was found to be more suitable for the fast growth of isolates of the pathogen, but early sporulation was recorded in GBA medium From the radial growth of isolates recorded on PDA and GBA media, the growth rate (mm/day) on these two media was calculated It was found that the growth rate on PDA medium was more than that on GBA medium Then the 30 isolates were

categorised into Group I and II based on their growth rate On PDA medium, those isolates which showed > 30 mm growth per day were included under Group I whereas those showed < 30 mm per day were included under Group II Accordingly in Group I, only eight isolates were categorised and the remaining 22 isolates were included under Group II The Group I isolates were the fast growing and Group II were the slow growing on PDA medium Similarly, those isolates which showed >10 mm per day growth on GBA medium were considered as fast growing and included under Group I and those showed < 10 mm per day growth were the slow growing and included under Group II Accordingly the Group I of 10 isolates showed fast growth and Group II of 20 isolates showed slow growth on GBA medium After the grouping of isolates it was observed that, four isolates *viz* , PI, BL, BL3 and AL4 were included under Group I of PDA and GBA media and they were the fastest growing ones among the 30 isolates obtained from seven varieties of mango Grouping of isolates based on the growth rate was carried out by many workers (Zakaria and Bailey, 2000, Abang, 2002, Chowdappa and Kumar 2012) Chowdappa and Kumar (2012) categorised 79 isolates of *Colletotrichum gloeosporioides* from mango anthracnose into two morphological groups as Type I and Type II cultures based on the growth rate on Potato Dextrose Agar medium Hence, the reports of earlier workers support the observations made in this study

Study on the morphological characters of the different isolates of the pathogen is also important for the investigation of variability among the isolates For this the characters of hyphae, spores and setae developed in cultures were observed under the microscope and their size, shape and colour were recorded The colour of hypha ranged from hyaline to greyish brown with 26 20- 39 67 x 3 33- 5 31 µm size So variations in the colour and size of hyphae were observed Regarding the conidial characters, all isolates produced hyaline cylindrical shaped conidia with obtuse ends, and without oil globules But variation was observed in the size of conidia and it varies with isolates The average size of conidia was recorded in the range of 13 52-16 99 x 3 40- 5 16 µm Pandey *et al* (2012) concluded that the variation in size of the spore of *Colletotrichum* was

due to secondary conidial formation which showed variation in shape and size Acervulus development was observed in 11 isolates grown on PDA medium, but acervulus with setae was observed only in six isolates *viz*, MT1, NL3, BL1, AL1, CT and SL1

All the six isolates produced acervulus in the culture with dark brown, septate setae The size of the setae was found to vary with isolate It was recorded in the range of 86 32-142 30 x 2 08-4 75 µm The other five isolates produced acervulus in the culture without setae These observations made on the variations in characters of hyphae, conidia and fruiting body are in line with the reports (Chowdappa and Kumar, 2012, Pandey *et al*, 2012, Suvarna *et al*, 2015, Kubi, 2016) Development of acervulus without setae in the cultures of *Colletotrichum gloeosporioides* was reported by Zakaria and Bailey (2000) and Benyahia (2003) In this study also acervulus development without setae was observed in six isolates and hence, the earlier reports support the present observation

After the detailed study of cultural and morphological characters of all the 30 isolates of the pathogen, cluster analysis was carried out and completed using the similarity coefficient way using NTSYS pc 2 02 software Based on this dendrogram was generated using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) Among the 30 isolates of *Colletotrichum* sp seven isolates showed maximum similarity of 56 per cent, and were categorised into Group I (PL1, PL3, AL3) Group II (ML3, PL2) and Group III (MT2, AL2) Maximum variation was recorded at 10 per cent similarity level, where the 30 isolates were grouped into two major clusters *viz*, A1 and A2, accommodating three and 27 isolates respectively

Another objective of the study was to find out the compatible group of isolates among these 30 isolates of the pathogen It was studied under *in vitro* condition by dual culture method and observations were recorded based on different types of reactions suggested by Webber and Hedger (1986) All the possible combinations of 30 isolates were taken and that resulted in 465

combinations While taking observations, mainly four types of reactions were noticed ie intermingling of hypha (I), mutual inhibition with clear zone (MC), mutual inhibition with thick mycelial strand (MT) and over growth (O) Out of the 465 combinations 76 combinations of isolates showed intermingling of hyphae with each other and that indicated the compatible reactions between them In between them, the highest compatible combinations (8 nos) of isolates were recorded by BL2 and was followed by ML1 (7 nos) with isolates from other varieties In some compatible groups, certain specific compatible reactions were observed Among the six isolates from Muvandan, MT1 was found compatible with MT1, ML2 and MT2 and it is not at all compatible with any other isolates, whereas ML1 showed compatible reactions with any one isolate from all other varieties except Prior The isolates ML2, MI, MT2 and MF showed incompatible reaction with all isolates from Alphonso, Chandrakaran and Sindhooram Similarly isolates from Neelum variety, NL1 and NF1 recorded compatible reactions only with the isolates from Muvandan and Neelum varieties It was also noticed that all the isolates from the variety Neelum except NT1 was incompatible with all isolates from Sindhooram and Chandrakaran The isolate NT1 was incompatible with CT but was compatible with one isolate from Sindhooram (ST) It is specifically noticed that certain isolates *viz*, PI, BT, AL1, and SL1 showed compatible reaction with only one another isolates from other variety *viz*, ST, MF, AL2, PL1 respectively

All isolates from Prior recorded incompatible reaction with all isolates from Alphonso and Chandrakaran Similarly all four isolates from Banganapalli showed incompatible reaction with all isolates from Alphonso and Sindhooram The four isolates obtained from the variety Alphonso recorded incompatibility with all isolates from Chandrakaran, Sindhooram and Banganapalli The isolates from Chandrakaran CT, showed compatible reaction with one isolate each from Sindhooram (ST) and Muvandan (ML1) and recorded incompatibility with all isolates from Neelum, Banganapalli, Prior and Sindhooram In this case, isolates from Sindhooram showed incompatible reaction against all isolates from Banganapalli and Alphonso

There are several earlier reports on the study of vegetative compatibility of different isolates of *Colletotrichum* spp because of its importance as asexual fungi The vegetative compatible groups can exchange genetic information through heterokaryosis and parasexual cycle The literature review revealed the use of nitrate nonutihty (*nit*) mutants to study the vegetative compatibility in *Colletotrichum* spp (Correll *et al* , 1993, Sanei and Razavi, 2011, Ben-Daniel *et al* , 2009) But in the present study compatibility was tested using dual culture technique and the observations were taken based on the type of reactions suggested by Webber and Hedger (1986) Hence to confirm the results of this study, further evaluation using *nit* mutants is required

From these observations it is clearly concluded that all the 30 isolates of *Colletotrichum* causing anthracnose disease in different varieties of mango are not one and the same This may be due to the genetic characters of different isolates Maybe due to genetically controlled characters and the genetic variability, typical compatibility/ incompatibility are more prominent in the isolates from Alphonso, Chandrakaran and Sindhooram Since, these isolates showed repeated incompatible reactions against isolates from other varieties

One of the factors which determine the virulence of a pathogen is its efficiency to attack more number of crops and cause severe damage to the crops Otherwise, if a pathogen has wide host range, that can be considered as a virulent one In this study, the virulence rate of the 30 isolates of *Colletotrichum* sp was studied by cross inoculating them on the selected seven varieties of mango The isolates were artificially inoculated and observations on the symptoms development were recorded From the data it was revealed that, all the isolates could not infect all the seven varieties of mango Variation in the pathogenicity was observed among the isolates Variation in time taken for initiating the first symptom, colour, shape and size of the spots were also observed

On the leaves of Muvandan, all the 24 isolates from other six varieties were inoculated, but only 16 isolates could infect Muvandan and produce the

symptoms Like that 15, 8, 16, 12, 13 and 12 isolates showed infection in Neelum, Prior, Alphonso, Banganapalh, Chanadrakaran and Sindhooram respectively These isolates recorded variations in their incubation period and took 2-4 days for initiating symptoms on the inoculated area Much variation was observed on the colour, shape and margin of the spots developed on the inoculated area Some isolates produced mycelial growth on the inoculated area, but it is not specific to a particular isolate or on a particular variety On five days after inoculation, the size of the spot developed was measured and it was found in the range of 3 mm to 16 66 mm From these observations it is revealed that among the isolates of *Colletotrichum* sp , slight host specificity is existing and that might be the reason for the inability of all the isolates to infect all the seven varieties During the cross inoculation experiments, Lakshmi *et al* (2011) observed variation in the level of host preferences by *Colletotrichum* isolates causing anthracnose in subtropical fruits

The isolate CT from the variety Chandrakaran was found to infect all the seven varieties and was followed by PL1 and NF3 which showed infection on six varieties So based on the efficiency of each isolate to infect number of varieties and time taken to symptom expression, these 30 isolates were grouped into three as highly virulent (HV), moderately virulent (MV) and less virulent (LV) isolates Accordingly NF3, PL1 and CT were grouped as highly virulent, 16 isolates including four each from Muvandan and Neelum, three from Alphonso, two each from Banganapalli and Sindhooram and one from Prior as moderately virulent, and 11 isolates including two each from Muvandan, Prior and Banganapalh, three from Neelum and one each from Alphonso and Sindhooram were grouped under less virulent isolates The cross infection potential of *Colletotrichum* isolates was tested by many earlier workers (Kumar and Rawal, 2004, Phouhvong *et al* , 2012, Sharma and Kulshrestha, 2015) and reported the variation in the pathogenic efficiency and narrow host range of different species of *Colletotrichum* Devamma *et al* (2012) reported significant difference with respect to pathogenicity among the seven isolates of *Colletotrichum gloeosporioides* causing mango anthracnose and based on the per cent disease

incidence they grouped the isolates as highly virulent, moderately virulent and less virulent Hence the result of this study is in line with the early reports

The vegetative compatibility and pathogenic efficiency of all the 30 isolates were compared to known whether any correlation existing between these characters The highly virulent isolates CT could infect all seven verities but showed incompatible reaction with all isolates from Neelum, Prior, Banganapalli and Sindhooram It showed vegetative compatibility with its own culture and one isolate each from Sindhooram (ST) and Muvandan (ML1) The isolates NF3 and PL1 recorded infection on six varieties except Prior and Banganapalli respectively In vegetative compatibility test it was found that the isolate NF3 was found compatible with the isolates NF3, PL1 and BL2 and was found incompatible with all isolates from Muvandan, Prior, Sindhooram and Chandrakaran Similarly in the case of PL1, it was compatible with only five isolates and incompatible with all isolates from Alphonso, Chandrakaran and Sindhooram From these observations, it was revealed that there is no correlation between the hyphal compatibility and pathogenic efficiency of an isolate But contradictory to this some isolates like PT and AL2 recorded inefficiency to attack some varieties and incompatibility reaction with isolates from those varieties These results showed a correlation between vegetative compatibility and pathogenic efficiency of an isolate By referring the earlier reports Brooker *et al* (1991) suggested the existence of positive correlation between vegetative compatibility and pathogenicity but they concluded that this correlation does not always hold

One isolate each from each variety of mango which showed high / moderate virulence in pathogenic characters were selected for the identification Based on the cultural and morphological characters, all the seven isolates were tentatively identified as *Colletotrichum gloeosporioides* To confirm this identity the cultures were sent to NCFT (National Centre for Fungal Taxonomy), New Delhi and identified as *Colletotrichum gloeosporioides* (Penz) Penz & Sacc Chowdappa and Kumar (2012) characterised 79 isolates of *Colletotrichum gloeosporioides* isolated from mango growing in India and they concluded that

the pathogen responsible for mango anthracnose in India belonged to two subpopulations of *C gloeosporioides* Suvarna *et al* (2015) identified the pathogen causing mango anthracnose in Kadapa and Chittur districts of Rayalaseema region as *Colletotrichum gloeosporioides* Penz based on the mycelial and comdial characters Hence the identification of pathogen causing mango anthracnose in Kerala is supportive to the reports of the earlier workers from other parts of India But contradictory to this, there are reports of *Colletotrichum acutatum* and *C asianum* (Abera *et al*, 2015) *C karstii* and *C kabawae* sub sp *ciggaro* (Ismail *et al*, 2015) as causal agent of anthracnose disease from Ethopia and Italy respectively

The last part of the investigation was the study on sensitivity of isolates of *Colletotrichum gloeosporioides* to the commonly using plant protection chemicals in mango Three contact fungicides *viz*, Bordeaux mixture (1%), copper hydroxide (0 15%) and mancozeb (0 3%), four systemic fungicides *viz*, carbendazim (0 1%), tebuconazole (0 1%), hexaconazole (0 1%) and azoxystrobin (0 1%) and two insecticides *viz*, malathion (0 1%) and dimethoate (0 05%) were used for the *in vitro* evaluation of the isolates of the pathogen by poisoned food technique. From the results, it was revealed that all the systemic fungicides were very effective and recorded cent per cent inhibition on the growth of all the 30 isolates of the pathogen and these four systemic fungicides were equally effective for the management of the anthracnose pathogen The observations on the efficiency of systemic fungicides evaluated in the present study are in favour to the reports of the earlier workers Sundravadana *et al* (2007) observed the complete inhibition of mycelial growth of *Colletotrichum gloeosporioides* under *in vitro* condition They also reported that azoxystrobin @2 ml/l showed significant reduction in the mango anthracnose disease incidence in the field experiments Adhikary *et al* (2013) also confirmed the efficiency of azoxystrobin for the management of mango anthracnose disease under *in vitro* and *in vivo* conditions They reported that azoxystrobin @100 ppm was found to be the optimum rate for the control of the disease at field level Similarly the efficiency of other systemic fungicides *viz*, carbendazim,

tebuconazole and hexaconazole was reported by many earlier workers (Mathew *et al*, 2009, Patil *et al*, 2010, Sreeja, 2014, Kadam *et al*, 2014, Kumar *et al*, 2015)

Among the contact fungicides evaluated, Bordeaux mixture (1%) recorded hundred per cent inhibition on the growth of all isolates except ML1 and MT1 from the variety Muvandan Copper hydroxide (0 15%) showed cent per cent inhibition of 24 isolates of pathogen and it recorded 50 74 per cent (NF3) to 85 18 per cent (ML1) inhibition on the growth of the remaining six isolates which included three isolates from Muvandan (ML1, ML2 and MI), two from Neelum (NT2 and NF3) and one from Sindhooram (ST) The efficiency of copper fungicides *viz*, Bordeaux mixture and copper hydroxide on inhibiting the growth of *Colletotrichum gloeosporioides* was reported by many earlier workers (Sharma and Verma, 2007, Parvathy and Girija, 2016, Pandey *et al*, 2012) Mancozeb at 0 3 per cent concentration exhibited inhibition on the growth of only seven isolates of pathogen which included NT1, NF1, NF2, BL2, BL3, CT and SL1 All the isolates showed the inhibition in the range of 68 88 to 94 8 per cent Among three contact fungicides tested mancozeb (0 3%) was found to be least effective for the control of anthracnose pathogen, even though it recorded complete inhibition of seven isolates of pathogen Similar results were reported by earlier workers and hence the observation made in this study is in favour of the reports of Tasiwal *et al* (2012), Chacko and Gokulapalan (2014) and Sreeja (2014)

On comparing the efficiency of insecticides, it was statistically found that malathion and dimethoate were least efficient in inhibiting the growth of the anthracnose pathogen Among them malathion 0 1 per cent was found more effective than dimethoate which recorded 60 74 to 78 14 per cent inhibition the growth of the pathogen Vijayaragavan (2003) reported the inhibiting effect of dimethoate against *Phytophthora capsici* causing *Phytophthora* disease in black pepper During the *in vitro* study she observed >80% inhibition on the growth of *P capsici* in all the three concentrations of the insecticides tested

From this study it was concluded that all systemic fungicides can be effectively used for the management of the pathogen Similarly, Bordeaux mixture (1%) and copper hydroxide (0 15%) recorded cent per cent inhibition on the growth of majority of isolates and hence they can be considered as good fungicides for the management of the anthracnose pathogen

Summing up the results of the study discussed so far, it may be pointed out that 30 isolates of *Colletotrichum* sp inciting anthracnose disease on various parts of seven varieties of mango were collected from various locations of Thrissur and Palakkad districts The pathogenicity of all the 30 isolates was proved on their respective host Variation in the symptom expression by this pathogen in different varieties was observed under natural condition, but on artificial condition, not much variation was observed Variation in the colony characters, sporulation, acervulus and pink spore mass formation and setae of acervulus was noticed The isolates PI, BL2, BL3 and AL4 were identified as fast growing isolates on PDA and GBA media and categorised under Group I based on the per day growth of the isolates Based on the phenotypic characters of the isolates, dendrogram was grouped and among the 30 isolates, maximum variation was recorded at 10 per cent similarity level where the clusters B3 included the isolate BL1 showed maximum dissimilarity with other isolates

The compatibility study recorded 76 compatible combinations among 456 total combinations of isolates The isolates ML1 and BL2 showed maximum compatible reactions with other isolates The isolates of pathogen also recorded variations in their pathogenic efficiency on the other varieties of mango This study concluded that NF3, PL1 and CT were the highly virulent isolates of *Colletotrichum* sp Evaluation of similarity of isolates against plant protection chemicals, showed the high sensitivity of all isolates to systemic fungicides All the four systemic fungicides recorded cent per cent inhibition on the growth of all the isolates Bordeaux mixture (1 %) and copper hydroxide (0 15 %) recorded cent per cent inhibition of 28 and 24 isolates respectively Among the two insecticides tested, the pathogen was found to be more sensitive to malathion (0 1%) compared to dimethoate at 0 05 per cent concentration

Summary

6. SUMMARY

The present investigation on 'Variability of *Colletotrichum* isolates inciting anthracnose in mango'(*Mangifera indica* L) was conducted in Department of Plant Pathology, College of Horticulture, Vellanikkara, to study the variation in different isolates of the pathogen, *Colletotrichum* sp causing anthracnose in different varieties of mango grown in Thrisssur and Pallakad districts of Kerala

For the isolation of pathogen, purposive sampling surveys were conducted in different locations of these districts The mango varieties *viz*, Muvandan, Neelum, Prior, Banganapalli, Alphonso, Chandrakaran, Sindhooram were selected for the study The pathogen associated with the diseased specimens was isolated on Potato Dextrose Agar (PDA) medium A total of 30 isolates of *Colletotrichum* sp were obtained which included six isolates from Muvandan, eight from Neelum, four each from Prior, Banganapalli and Alphonso, three from Sindhooram and one from Chandrakaran Among them, 16 isolates were obtained from leaves, eight from twigs, four from fruits and two from inflorescence of these seven varieties The pathogenicity of all the 30 isolates was proved on their respective host All the isolates recorded symptom expression on two to five days after inoculation (DAI) All the four isolates from Prior and Alphonso, one isolate from Muvandan (MT2) and five isolates from Neelum (NL1, NT1, NF1, NF2, NF3) showed the initial symptom on two DAI and these isolates were considered as most virulent isolates

Symptomatology of the anthracnose disease of mango was studied in detail under natural and artificial conditions Variations in the symptoms produced by the pathogen on the seven different varieties were observed under the field In the variety Muvandan, infection was observed on all plant parts of the seedlings and matured trees Leaf spot, shot hole and die back symptoms were mainly seen on seedlings On trees, leaves, twigs, inflorescence and fruits were found infected by the pathogen On leaves of old trees two types of symptoms were observed On young leaves, small sized back spots were initiated

which and later coalesced to irregular reddish brown spots without any yellow halo A later shot hole symptom was observed in these leaves Reddish brown to black round shaped distinct spots with black margin were noticed on matured leaves Large irregular shaped greyish white spots with dark margin were observed on leaves On these spots, acervulus formation was noticed as black dots In all these symptoms, shot hole formation or dropping off the infected area was also observed Infections on leaf margin and leaf tip were also noticed and that resulted in irregular shape to the leaves These types of irregular shaped greyish white spots were also observed in the variety Alphonso But in Alphonso, yellow halo was noticed around these spots In all other varieties, like Neelum, Prior, Banganapalli, Chandrakaran and Sindhooram, reddish brown spots with yellow halo were observed on the leaves and these spots were bound by the vein or veinlets The intensity of yellow halo may vary as it was observed in Banganapalli with or without yellow halo and in Sindhooram with light yellow halo In all varieties development of shot hole was found to be a common symptom irrespective of the size of the spots In all seven varieties, twig infection was observed as small black lesions which later spread and resulted in dieback symptom

On fruits of Muvandan, small black coloured pin point like spots were observed in large number Later some spots enlarged and developed into sunken spots Development of fruiting body of the pathogen, acervulus, was observed on the surface of sunken spots This type of small sized spots was not observed in Neelum and Chandrakaran Tear stain symptom was noticed in some fruits of Muvandan and Banganapalh in which the sunken spots were developed in line from stalk end to the tip of the fruit

Symptoms of the anthracnose disease on artificial condition were recorded during the pathogenicity test All the 30 isolates produced light brown to black coloured irregular shaped lesions on their respective variety of mango The isolate SL1 produced light brown lesions with pale red centre bounded with dark margin on the leaves of variety Sindhooram The size of the lesions developed by the isolates on the different varieties was measured on five DAI

and found variation in the size of the infected area (2 mm to 13 mm in diameter) The isolate MT2 produced the largest lesion having 13 mm diameter with dark grey mycelial growth on the infected area The isolate MT1 and AL3 showed small sized spots of two millimetres in diameter In the field condition, yellow halo was observed around the spots in all varieties except Muvandan and Neelum But in artificial condition, yellow halo formation was not observed

The phenotypic characters of the different isolates of *Colletotrichum* sp were studied in details on two different media *viz*, Potato Dextrose Agar (PDA) and Green Bean Agar (GBA) Variation in the cultural characters among the 30 isolates was observed on PDA medium The isolates like ML1, NF3, PL2, CT and SL1 produced white coloured mycelial growth, whereas some isolates like MF NL2 and NL3 showed dark grey mycelial growth Out of the 30 isolates, only four isolates showed irregular shaped cultural growth Similarly only four isolates showed zonations in the cultural growth and among them the isolates CT showed clear zonations in the culture Variation in the colour development on the reverse side of the culture was noticed and observed white/ light grey/ brownish grey/ dark grey/ black colour on the back side of the culture The isolate SL2 showed small dark coloured dots on the reverse side of the culture

Development of spores, acervulus and pink spore mass in the cultures of the disfferent isolates were recorded The isolates took three to eight days for spore formation in the culture One isolate from the fruit of Neelum variety (NF1) took the longest period of eight days whereas the isolate from twig of Sindhooram (ST) took the minimum time of three days for the spore formation In general, majority of the isolates took five to seven days for the spore formation So a clear variation was observed in the spore development by these 30 isolates Similarly all the isolates did not produce acervulus and pink spore mass in the culture Only 11 isolates produced acervulus and among them, only in nine isolates the pink spore mass formation was noticed

The cultural characters of all the isolates were also studied on GBA medium compared to PDA medium, slight variation was observed in the colony

characters on this medium Most of the isolates produced white coloured, thick cottony cushion textured regular shaped cultural growth without any zonation Colour on the reverse side of the culture varied from white to black with small dots in three isolates Fourteen isolates showed the spore formation on three DAI, 9 isolates on four DAI and 7 isolates on five DAI So on GBA medium, conidial formation was found to be faster than on PDA medium

The growth rate of the different isolates of the pathogen was recorded on PDA and GBA media The results revealed wide variation in the radial growth exhibited among the isolates and also between the media On PDA medium, two to seven days were taken whereas on GBA medium it took five to twelve days to complete the growth Some isolates *viz*, ML1, MI, NL2, NL3, NT1, PL1, CT, SL1 could not complete 90 mm growth on GBA medium even after 12 days of incubation From this, it is concluded that PDA medium was found to be more suitable for the fast growth of isolates of the pathogen, but early sporulation was recorded on GBA medium The growth rate (mm/day) on these two media was calculated Then the 30 isolates were categorised into Group I and II based on the growth rate After the grouping of isolates it was observed that, three isolates *viz*, PI, BL3 and AL4 were included under Group I of PDA and GBA media and they were the fastest growing isolates among the 30 isolates obtained from seven varieties of mango

Study on the morphological characters of the different isolates of pathogen is also important for the investigation of variability among the isolates For this the characters of hyphae, spores and setae developed in cultures were observed under the microscope and recorded the size, shape and colour of these structures The colour of hypha ranged from hyaline to greyish brown with 26 20- 39 67 x 3 33- 5 16 µm size All isolates produced hyaline cylindrical shaped conidia with obtuse ends, hyaline and without oil globules But variation was observed in the size of conidia and it varies with isolates The average size of conidia was recorded in the range of 13 52-16 99 x 3 40- 5 16 µm Acervulus development was observed in 11 isolates grown on PDA medium, but acervulus with setae was observed only in six isolates *viz*, MT1, NL3, BL1, AL1, CT and SL1 The

size of the setae was found vary with isolate It was recorded in the range of 86 32 – 142 30 x 2 08- 4 75 µm

After detailed study of cultural and morphological characters of all the 30 isolates of the pathogen, cluster analysis was carried out Dendrogram was generated using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) Maximum variation was recorded at 10 percent similarity level, where the 30 isolates were grouped into two major clusters *viz*, A1 and A2, accommodating three and 27 isolates respectively

Screening of vegetative compatible combinations of 30 isolates of the pathogen was another objective All the possible combinations of 30 isolates were taken and that results in 465 combinations Out of the 465 combinations 76 combinations of isolates showed intermingling of hyphae with each other and that indicated the compatible reactions between them Among the six isolates from Muvandan, MT1 was found compatible with MT1, ML2 and MT2 and it was not at all compatible with any other isolates whereas ML1 showed compatible reactions with any one isolate from all other varieties except Prior Similarly isolates from Neelum variety, NL1 and NF1 recorded compatible reactions only with the isolates from Muvandan and Neelum varieties It is specifically noticed that certain isolates *viz*, PI, BT, AL1, and SL1 showed compatible reaction with only one another isolates from other variety *viz*, ST, MF, AL2, PL1 respectively All isolates from Prior recorded incompatible reaction with all isolates from Alphonso and Chandrakaran Similarly all four isolates from Banganapalli showed incompatible reaction with all isolates from Alphonso and Sindhooram The four isolates obtained from this variety Alphonso recorded incompatibility with all isolates from Chandrakaran, Sindhooram and Banganapalli From these observations it is clearly concluded that all the 30 isolates of *Colletotrichum* causing anthracnose disease in different varieties of mango are not one and the same The genetic variability may be more prominent in the isolates from Alphonso, Chandrakaran and Sindhooram since these isolates showed repeated incompatible reaction against isolates from other varieties

Another important factor which determines the virulence of a pathogen is its efficiency to attack more number of crops and causing severe damage to the crops So the virulence rate of the 30 isolates of *Colletotrichum* sp was studied by cross inoculating them on the selected seven varieties of mango The observations on time taken for initiating the first symptom, colour, shape and size of the spots were recorded The data revealed variations in the pathogenicity among the isolates

On the leaves of Muvandan, all the 24 isolates from other six varieties were inoculated, but only 16 isolates could infect Muvandan and produce the symptoms Like that 15, 8, 16, 12, 13 and 12 isolates showed infection in Neelum, Prior, Alphonso, Banganapalli, Chanadrakaran and Sindhooram respectively Based on the efficiency of each isolate to infect number of varieties and time taken to symptom expression, they were grouped into three, as highly virulent (HV), moderately virulent (MV) and less virulent (LV) isolates Accordingly NF3, PL1 and CT were grouped as highly virulent, 16 isolates including four each from Muvandan and Neelum, three from Alphonso, two each from Banganapalli and Sindhooram and one from Prior as moderately virulent, and one of the 11 isolates including two each from Muvandan, Prior and Banganapalli, three from Neelum and one each from Alphonso and Sindhooram were grouped under less virulent isolates The vegetative compatibility and pathogenic efficiency of all the 30 isolates were compared to know whether any correlation existing between these characters The highly virulent isolate, CT recorded infection on all other six varieties during pathogenic variability study, but it recorded vegetative compatible reaction only with three other isolates From this observation, it was revealed that there is no correlation between the hyphal compatibility and pathogenic efficiency of an isolate

A total of seven isolates, one from each variety of mango which showed high/ moderate virulent in pathogenic characters were selected for the identification Based on the cultural and morphological characters, all the seven isolates were tentatively identified as *Colletotrichum gloeosporioides* To confirm this identity the isolates were sent to NCFT (National Centre for Fungal

Taxonomy), New Delhi and identified as *Colletotrichum gloeosporioides* (Penz) Penz & Sacc

The study on sensitivity of isolates of *Colletotrichum gloeosporioides* against the commonly using plant protection chemicals in mango was carried out by poisoned food technique Three contact fungicides *viz* Bordeaux mixture (1%), copper hydroxide (0 15%) and mancozeb (0 3%), four systemic fungicides *viz*, carbendazim (0 1%), tebuconazole (0 1%), hexaconazole (0 1%) and azoxystrobin (0 1%) and two insecticides *viz* malathion (0 1%) and dimethoate (0 05%) were used for the *in vitro* evaluation of the isolates of the pathogen by poisoned food technique From the results it was revealed that all the systemic fungicides were very effective and recorded cent per cent inhibition on the growth of all the 30 isolates of the pathogen and these four systemic fungicides were equally effective for the management of the anthracnose pathogen

The contact fungicide, Bordeaux mixture (1%) recorded hundred per cent inhibition on the growth of all isolates except ML1 and MT1 from the variety Muvandan Copper hydroxide (0 15%) showed cent per cent inhibition of 24 isolates of pathogen Mancozeb at 0 3 per cent concentration exhibited cent per cent inhibition on the growth of only seven isolates of pathogen All the isolates showed the inhibition in the range of 68 88 to 94 8 per cent over control On comparing the efficiency of insecticides, it was found that statistically malathion (1%) and dimethoate (0 05%) were least efficient in inhibiting the growth of the anthracnose pathogen Among them malathion 0 1 per cent was found more effective than dimethoate which recorded 60 74 to 78 14 per cent inhibition, the growth of the pathogen

It was concluded that all systemic fungicides can be effectively used for the management of the pathogen, since they exhibited cent per cent inhibition on the growth of all 30 isolates Similarly, Bordeaux mixture (1%) and copper hydroxide (0 15%) recorded cent per cent inhibition on the growth of majority of isolates and hence they can also be recommended as good fungicides for the management of the anthracnose pathogen

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Appendix

APPENDIX- I

COMPOSITION OF MEDIA USED

1. **Potato Dextrose Agar**

Potato	- 200g
Agar- Agar	-20g
Dextrose	-20g
Distilled water	-1000ml

2. **Green Bean Agar**

Green beans	- 400g
Agar- Agar	-20g
Distilled water	-1000ml

VARIABILITY OF *Colletotrichum* ISOLATES INCITING ANTHRACNOSE IN MANGO (*Mangifera indica* L.)

By

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(2014-11-219)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement

for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR – 680 656

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2016

ABSTRACT

The present investigation on "Variability of *Colletotrichum* isolates inciting anthracnose in mango (*Mangifera indica* L.)" was carried out in Department of Plant Pathology, College of Horticulture, Vellamkkara during 2014-2016 to elucidate the variability existing in *Colletotrichum* causing anthracnose of mango in terms of pathogenicity, phenotypic characters, compatibility of isolates and their sensitivity to plant protection chemicals

For the isolation of pathogen, purposive sampling surveys were conducted at different locations of Thrissur and Palakkad districts Disease samples were collected from seven selected varieties of mango *viz* Muvandan, Neelum, Prior, Banganapalli, Alphonso, Chandrakaran and Sindhooram Totally 30 isolates were obtained of which, six isolates were from Muvandan, eight from Neelum, four each from Prior, Banganapalli and Alphonso, one from Chandrakaran and three from Sindhooram Pathogenicity of all the thirty isolates was proved on their respective host and observed symptom development in two to three days

Symptomatology of anthracnose disease of mango was studied in detail under natural and artificial conditions Under natural condition, various types of symptoms like small black spots with shot hole, large spots with black margin, infection on leaf margin, lesions with or without halo and distortion of large area were observed on leaves of different varieties Twig infection with dieback symptom was observed on all varieties On the fruits of Muvandan and Neelum, black sunken spots and tear stain symptoms were noticed All these typical symptoms were not observed under artificial inoculation and in general, all the 30 isolates produced light brown to black irregular lesions on their respective varieties

Phenotypic characters of the 30 isolates were studied on Potato Dextrose Agar (PDA) and Green Bean Agar (GBA) media Variations in the cultural

characters like colony texture, shape, spore formation, development of acervulus and pink spore mass formation were recorded on both media Among the 30 isolates, only 11 isolates showed the development of acervulus of which, only nine isolates produced pink spore mass on PDA medium Variation in cultural characters was not observed on GBA medium and the isolates did not produce fruiting body on this medium But early spore formation was recorded by all the isolates on this medium Growth rate of each isolate on PDA and GBA media was calculated and based on which the isolates were grouped as fast growing (Group I) and slow growing (Group II) The isolates PI, BL2, BL3 and AL4 were recorded as fast growing isolates on both media

Morphological characters of hyphae, spores, and setae of the isolates showed variation The average size of comdia was recorded in the range of 13 52-16 99 x 3 40-5 16 µm Acervulus development was observed in 11 isolates on PDA medium, but acervulus with setae was noticed only in six isolates *viz*, MT1, NT1, BL1, AL1, CT and SL1 The size of setae was recorded in the range of 86 32-142 30 x2 08-4 75 µm Cluster analysis of all 30 isolates was done based on the cultural and morphological characters Maximum variation was recorded at 10 per cent similarity level, where 30 isolates were grouped into two major clusters *viz* A1 and A2 accommodating three and 27 isolates respectively

Screening for vegetative compatible combinations of the isolates was studied using dual culture technique Out of the 465 combinations, only 76 showed compatible reaction of intermingling of hyphae, while others showed incompatible reactions The isolates BL2 recorded the highest compatible conditions (8 nos) with other isolates To study the variations in the pathogenic efficiency, the isolates were cross inoculated on other varieties The results showed variations in the time taken for symptom development, spot size on five days after inoculation and number of varieties infected by the isolates The isolate, CT recorded infection on all seven varieties followed by NF3 and PL1 recorded infection on six varieties Based on the efficiency of each isolate to infect number of varieties and time taken to symptom expression, these 30

isolates were grouped into three as highly virulent (HV), moderately virulent (MV) and less virulent (LV) isolates Accordingly NF3, PL1 and CT were grouped as highly virulent isolates On the basis of phenotypic and pathogenic characters, seven virulent isolates of *C gloeosporioides*, one from each variety were sent to National Centre for Fungal Taxonomy (NCFT), New Delhi and the identity of these isolates was confirmed as *Colletotrichum gloeosporioides* (Penz) Penz & Sacc by NCFT with ID No 8214 16-8220 16

In vitro evaluation of plant protection chemicals (three contact fungicides, four systemic fungicides and two insecticides) was carried out to check the sensitivity of isolates All the four systemic fungicides showed cent per cent inhibition on the growth of 30 isolates and were found most effective for the control of *C gloeosporioides* Bordeaux mixture (1 %) and copper hydroxide (0 15%) recorded cent per cent inhibition on the growth of 28 and 24 isolates respectively The insecticides, malathion (0 1 %) and dimethoate (0 05%) also recorded inhibition of fungal growth in the range of 50 74 to 83 70 per cent

The results of the present study proved the existence of considerable phenotypic and pathogenic variability among the 30 isolates of *C gloeosporioides* obtained from seven mango varieties grown in Kerala This information could establish a base for the future investigation on the mechanism of genetic variability of the pathogen