

INVESTIGATIONS ON BLACK BANDED DISEASE  
OF MANGO

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JUNE, 2015

**INVESTIGATIONS ON BLACK BANDED DISEASE OF  
MANGO**

*Thesis submitted to the  
University of Agricultural Sciences, Dharwad  
in partial fulfillment of the requirements for the  
Degree of*

**MASTER OF SCIENCE (AGRICULTURE)**  
*in*  
**PLANT PATHOLOGY**

*By*  
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**CERTIFICATE**

This is to certify that the thesis entitled "INVESTIGATIONS ON BLACK BANDED DISEASE OF MANGO" submitted by Mr. CHIRAG GAUTAM for the degree of MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY to the University of Agricultural Sciences, Dharwad is a record of research work carried out by him during the period of his study in this university, under my guidance and supervision, and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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## ACKNOWLEDGEMENT

At this very outset, my reverences towards “Almighty GOD” would definitely deserve the most special mention, for his eternal love, unseizing help, guidance, kindness and blessing which guarded me in completing the present task. I take this opportunity to look back on the path traversed during the course of this endeavor and to remember the guiding faces behind the task with a sense of gratitude.

I express my deep sense of gratitude and profound indebtedness, reverence and thanks to Dr. VIRUPAKSHA PRABHU H., Associate Professor, Department of Plant Pathology, College of Agriculture, Dharwad and esteemed Chairman of my Advisory Committee for his ablest guidance, close counseling and sustained interest, constant encouragement throughout the study period. I also thank him for his constructive criticism of the study and valuable suggestions without which this study have not taken this shape and progressed in disciplined manner. In fact, his kind and outstanding nature has been overwhelming and it is my privilege fortune to have an opportunity to work under him and I sincerely and proudly confess that it has been a great privilege for me to have been one of his students.

It is rather difficult to express in words my sincere and heartfelt gratitude to the members of my Advisory Committee, Dr. V. B. Nargund, Professor, Department of Plant Pathology, Dr. J. C. Mathad, Professor, Department of Horticulture and Dr. Kiran K. Mirajkar, Assistant Professor, Department of Biochemistry, College of Agriculture, Dharwad, University of Agricultural Sciences, Dharwad for their constructive criticism, valuable suggestions and overwhelming support to pursue my research programme is deeply acknowledged, with due respect.

I place on record with sincerity the indebtedness to Dr. A. S. Byadgi, Professor and Head, Department of Plant Pathology, University of Agricultural Sciences, Dharwad, Dr. Yashoda Hegde, Dr. M. S. Patil, Dr. V. I. Benagi, Department of Plant Pathology, University of Agricultural Sciences, Dharwad, other Professors and Staff Members, of the Department for their constant cooperation and encouragement throughout my degree programme.

I feel the inadequacy of diction in expressing my sincere heartfelt gratitude and affectionate regards to my beloved parents, Smt. Maya Gautam, Shri Deveki Nandan Gautam, my brother Deepak Gautam and all the members of the family and relatives always backed me throughout my life.

Friendship is the most important ingredient in the recipe of life and it adds more flavour when that is from different states with different language and culture. I am fortunate to have a myriad of friends here. I am thankful to my beloved friends, seniors and juniors

I am very much thankful to M/s. Anup Computers, Dharwad for their neat and timely editing manuscript.

..... Omission of any names in this brief acknowledgement does not mean lack of gratitude

**DHARWAD  
JUNE, 2015**

**(Chirag Gautam)**

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# 1. INTRODUCTION

Mango (*Mangifera indica* L.) described as the “king of fruits” or “super fruit”, known for its strong aroma, delicious taste and high nutritive value, is a prominent horticultural crop of India. It belongs to the family *Anacardiaceae*, having chromosome number of  $2n = 40$ . Indo-Burma region is considered as centre of origin for mango. It is the most important crop among the tropical and sub tropical fruits, grown in more than 110 countries of the world. India ranks first among world's mango producing countries accounting for about 65% of the world's mango production. Other major mango producing countries include China, Thailand, Mexico, Pakistan, Philippines, Indonesia, Brazil, Nigeria and Egypt.

In India, it is grown over an area of 2.46 mha with production of 17.3 mt (Anon., 2013). Among all states, Andhra Pradesh stands first in area and production followed by Uttar Pradesh and Karnataka. Other major mango growing states are Bihar, Gujarat, Maharashtra, Orissa, Tamil Nadu, West Bengal, Madhya Pradesh, Kerala, Haryana and Punjab. In Karnataka, the area under mango cultivation is 1.78 lakh ha with a production of 1.78 mt and productivity is 10 t/ha (Anon., 2014).

Mango is a perennial, branching, evergreen tree approximately 10-15 m tall. Vegetative flush can occur one to three times in a year, but flush occurring during June to July period is more conducive for flowering. Normal flowering season is December to March. Flowering duration is three weeks. It's fruit is a large, fleshy drupe containing a laterally compressed stone housing the seed. Upon harvesting at maturity, hydrolytic processes are triggered and the depletion of starch, the breakdown of insoluble pectin occurs. The onset of ripening is accompanied by a five-fold increase in heat production (Krishnamurthy and Subramanyam, 1973), followed by an increase in ethylene production, catalase and peroxidase activities. Degrading enzymes such as amylase, chlorophyllase, cellulase, pectin-esterase, polygalacturonase and polyphenoloxidase were reported in ripening mangoes (Fuchs *et al.*, 1980).

Mango cultivars vary considerably in fruit size, colour, shape, flavour, texture and taste. Mango fruits are rich in vitamin A (3894 IU/100g), vitamin C (27.7mg/100g), flavonoids, carotenes, sterol, aromatic acids, essential oils, fatty acids and phenolics. It is a nutritive fruit containing most of the essential substances needed by the human body (Pandey and Dinesh, 2010).

Mango and its parts have been used for various medicinal purposes. In India fruits are used as a laxative and diuretic. Fruit sap has been used to treat pain of bee and scorpion stings. Fruits are eaten as a kidney tonic and to cure headaches. Half ripe fruit is eaten with salt and honey for treating gastrointestinal disorders, bilious disorders, blood disorders and scurvy. Extracts of unripe fruits, bark, stems and leaves possess antibiotic activity against gram positive and gram negative bacteria (Brindha, 2012). It has been reported that the fruits can be processed into dry mango, mango pickle, mango jelly or can be eaten cooked (Crane *et al.*, 2006).

The natural spread of the genus *Mangifera* is limited to the Indo- Malaysia, extending from India to the Philippines and New Guinea in the east. The number of valid species in the genus *Mangifera* is 57 as listed by Kostermans and Bompard (1993). India is considered to be home of four species *M. andumanica*, *M. sylvatica*, *M. khasiana* and *M. camptosperma* apart from *M. indica* (Mukharjee, 1985). *M. indica* is the most important economical species, which has close relationship with *M. longipes*. It is believed that the common English term Mango and the botanical name *Mangifera indica* L. originated from Indian name “mangas”. Literal meaning of *Mangifera indica* is an “Indian plant bearing “mangas” (Radha and Varghese, 2012).

Although India is the largest producer of mango, the productivity is low. This may be due to the wide range of climatic conditions, environmental situation in which the mango grows and the diversity of the associated disease problems. Over 140 diseases, insect pests and physiological disorders are known to cause damage to the crop at all stages of its development, right from the plant in the nursery to the fruit in

storages or transit, yet there are few diseases which are of great economic importance (Omprakash, 1998).

Some important diseases are anthracnose, powdery mildew, malformation, die back, stem end rot, leaf blight, red rust, pink disease, sooty mould, bacterial canker, scab, gummosis, blossom blight and phoma blight.

Earlier black banded disease of mango was considered as a minor disease but for the last three to four years it is becoming severe in all the mango growing regions. The causal fungus *Peziotrichum corticolum* (Masse) Subramanian was described on the bark of trees in Poona, India by Masse (Hughes, 1980). Later on, it has been reported from Goa, West Bengal, Karnataka, Maharashtra, Bihar, Orissa, Andhra Pradesh, Kerala, Andaman & Nicobar Islands and Tamil Nadu in mild to severe form (Naqvi, 2004; Ploetz and Prakash, 1997 and Om Prakash and Srivastava, 1987).

The pathogen grows superficially on the bark of trees forming large, dark black, irregular, girdle-like infection patches and hence the name. According to Pandey and Dinesh (2010) black velvety growth of fungus is sometimes found on the midribs and veins of leaves also.

It is necessary to conduct survey of the disease to get comprehensive information on disease distribution, level of severity, extent of spread and to locate hot spots. As the information available on the causal fungus *Peziotrichum corticolum* is very less, it is necessary to conduct morphological, cultural and biochemical studies of the pathogen.

There is no information available on the management of this disease by fungicides and biorationals. Hence studies need to be under taken to assess the efficacy of various systemic and contact fungicides under *in vitro* conditions. The information of disease management using new effective fungicides is unknown. Therefore, it is necessary to test the field efficacy of some recently available new fungicides for the effective management of the disease.

In view of the above facts, the present investigation was therefore initiated to elucidate some of the aspects of the pathogen, host and relative damage caused by pathogen with the following objectives

1. Survey of the disease in North Karnataka.
2. Morphological, cultural and biochemical studies of the pathogen.
3. *In vitro* and *in vivo* evaluation of chemicals and biorationals.
4. Screening of genotypes under natural conditions for disease resistance.

## 2. REVIEW OF LITERATURE

Black banded disease of mango is caused by *Peziotrichum corticolum* Masee. Presently, very little work on this disease and organism has been done and literature available is very scarce. Hence, in the present review, related diseases of mango like anthracnose and die back are included.

### 2.1 History and symptomatology

#### 2.1.1 History

The disease was first time recorded by Masee (1901) from Poona, India. He described *Rhinocladium corticola* Masee as a causal agent of black banded disease of mango (Saccardo, 1906). Subramanian (1956) renamed the pathogen as *Peziotrichum corticola* (Masee) Subram. (described as '*Corticolum*').

Presently both names are used as synonyms. In present study *Peziotrichum corticolum* is used as causal agent of black banded disease of mango.

Taxonomic position of *Peziotrichum corticolum* (Anon., 2015)

Kingdom	: Fungi
Phylum	: Ascomycota
Sub-division	: Pezizomycotina
Class	: Sordariomycetes
Sub-class	: Hypocreomycetidae
Order	: Hypocreales
Family	: Nectriaceae
Genus	: Peziotrichum
Species	: Corticolum

### 2.1.2 Symptomatology

The disease was noticed on the midribs and veins of leaves, twigs and branches of mangoes as black velvety fungal growth. The incidence of the disease was very low on the main branches. It presented a characteristic and conspicuous black banded appearance. The infected portions of the bark usually showed the mycelia growth and clusters of conidiophores. The mycelial growth drops off in the summer months leaving light black bands in the affected portions. The fungus was confined to the surface of bark. (Reddy *et al.*, 1961; Prakash and Srivastava, 1987).

According to Mukherjee and Litz (2009) the causal fungus, *Rhinocladium corticola* Masee (described as '*Corticolum*') affected mango leaves and branches formed a black, velvety mass of hyphae on affected surfaces in conspicuous blotches or bands. The fungus was restricted to the outer portions of bark.

Patil and Dangat (2012) conducted microscopic studies on the pathogen and revealed that, *Peziotrichum corticolum* grew superficially on the bark of trees forming extensive, spreading, dark black, irregular and girdle-like infection patches, hence the name 'Black Banded disease'. The young spreading mycelium near the periphery of the infection bands was white or nearly hyaline.

## 2.2 Survey of the disease in North Karnataka

Patil and Dangat (2011) carried out a survey of black banded disease of mango in Kolhapur district in Maharashtra, India during the years 2008-2010 and reported that disease incidence ranged from 28.5 per cent to 100 per cent and the disease was in a medium to severe state of infection. Black banded disease has been recorded on wild and all hybrid varieties of mango.

Venkataravanappa (2002) reported that in field survey of Northern and Southern districts of Karnataka showed diverse incidence form of anthracnose on

mango, such variation in disease incidence was usually attributed to environmental variations and variability in pathogenic fungus.

Sangeetha (2003) conducted the survey on mango anthracnose in some of the regions of South India during 2001-2002 and recorded maximum (54.50) per cent disease incidence in Devanahalli region of Karnataka and lowest (27.71 PDI) in Tiruvur region of Andhra Pradesh.

Lakshmi *et al.* (2011) recorded disease severity based on the percentage of leaf or fruit area affected by anthracnose disease.

### 2.2.1 Pathogenicity

Venkataravanappa (2002) reported that pathogenicity was proved by spraying spore suspension (106 spores/ml) of fungus *Colletotrichum gloeosporioides* on five month old mango seedlings and infection occurred twelve days after inoculation.

## 2.3 Morphological, cultural and biochemical studies of the pathogen

### 2.3.1 Morphology

According to Mukherjee and Litz (2009) fungus *Rhinocladium corticolum* causing black banded disease of mango produced intricately, branched, septate, olivaceous hyphae measuring 5-7  $\mu\text{m}$  in diameter. Erect hyphae had globose, olivaceous, densely and minutely tuberculate conidia measuring 15-18  $\mu\text{m}$ .

According to Patil and Dangat (2012), fungus *Peziotrichum corticolum* produces dark brown, profusely branched, septate hyphae (3-6 $\mu$ ) which were interspersed with vertical, erect, straight or bent aggregations of hyphae which converge above. 'Aleurispores' or conidia are produced singly and terminally at the tips of branches. The conidia were one-celled, pale brown, globose, smooth-walled; 12-18.5 $\mu$  in diameter (Subramanian, 1956).

Pandya and Vala (2001) studied histopathology of *Rhinocladium corticolum* to know about the penetration of the pathogen in different microparts of the infected

stem. The penetration of the pathogen on the bark of infected mango twigs was observed through lenticels from where it moved to epidermal cell, cork layer, cork cambium and finally settled in the cortex region without affecting the vascular tissues. The hyphae were observed intracellular and presence of haustoria or any other specialized organ for absorption of nutrients was not observed.

Venkataravanappa (2002) studied morphological characters of six isolates of *Colletotrichum gloeosporioides*, indicated that, hyphae septate, conidiomata acervuli and conidiophores were hyaline, conidia one celled, hyaline, straight, cylindrical, rounded at both ends, with one to three oil globules in the conidium. The conidia measured 10.9-20.6  $\mu\text{m}$  length and 4.39 to 6.65  $\mu\text{m}$  width.

Adhikary *et al.* (2013) studied the morphology of the fungus *Colletotrichum gloeosporioides* causing anthracnose of mango on infected host tissue. Microscopic examination of infected tissue revealed that acervuli were saucer shaped, measuring 141-381  $\times$  33-90  $\mu$  with an average of 170  $\times$  42  $\mu$ . The acervulus was covered with a mucilaginous mass and containing numerous conidia measuring 8-20  $\times$  4-7  $\mu$ . Dark brown to black setae were arising through this mass; they were erect in habit, measuring 32-76  $\times$  1-4  $\mu$ . Conidia were hyaline, single celled and smooth walled.

### 2.3.2 Cultural characters

Prashanth (2007) reported that, among non/semi synthetic media maximum radial growth and good sporulation of *Colletotrichum gloeosporioides* was recorded on PDA followed by oatmeal agar, while least growth and poor sporulation was observed on host leaf extract media. Among the synthetic media, maximum growth and fair sporulation were recorded on Richards' agar and least growth with poor sporulation was recorded on Czapek's (Dox) agar. In case of broth, maximum dry mycelial weight was recorded in Richards' broth and least dry mycelial weight was recorded in host leaf extract broth after thirteen days of incubation.

Vinod and Benagi (2009) worked on papaya anthracnose caused by *Colletotrichum gloeosporioides* and they reported that among different solid media Richards' agar showed good growth and sporulation. The fungus in liquid media recorded good growth upto 10 days of incubation and decreased after eleventh day.

Adhikary *et al.* (2013) studied cultural characters of the fungus *Colletotrichum gloeosporioides* causing anthracnose of mango on PDA culture media. The fungal colony from 10 days old culture on PDA media was white with smooth margins. The mycelium was hyaline, superficial, septate and branched. The aerial mycelium was white. Sporulation was abundant with maximum fruiting bodies at the centre of the plate and profuse mycelium growth was found towards the periphery. The acervuli and conidia from the culture measured  $130\text{-}252 \times 28\text{-}76\mu$  and  $7\text{-}11 \times 2\text{-}5 \mu$  respectively.

### 2.3.3 Biochemical studies

#### 2.3.3.1 Sugars

Sugars are precursors for synthesis of phenols, phytoalexins, lignin and callose. Hence, they play an important role in defense mechanism of plants. In general, the infection by some pathogens bring changes in respiratory pathway and photosynthesis which are the vital processes taking place inside the plant leading to wide fluctuations in sugars (Kiralý and Farkas, 1962; Kuc, 1966 and Klement and Goodman, 1967).

Horsfall and Dimond (1957) assigned a major role for sugars in disease resistance. They classified the diseases as high sugar diseases and low sugar diseases. Low sugar diseases occur severely when host sugar content is low and high sugar diseases occur when host sugar content is high.

The disease reaction has been correlated with the sugar level in different crop plants. Generally high levels of total sugars, reducing sugars and non-reducing sugars

in the host plant were stated to be responsible for disease resistance (Bateman and Millar, 1966 and Jayapal and Mahadevan, 1968).

Patil and Dangat (2011a) conducted biochemical studies while working on black banded disease. There was considerable decrease in the contents of reducing and total sugars as well as RNA and DNA contents of infected bark.

#### 2.3.3.2 Phenols

Plant tissues contain a large number of phenolic compounds. The most important of which are simple phenols, coumarine, most flavonoids, certain amino acids, prosthetic groups, some enzymes, plant pigments and complex derivatives such as lignins. Phenolic substances are known to participate in a number of physiological processes which are essential for growth and development, such as oxidation reduction reactions, lignification and stimulation as well as inhibition of auxin activity. Phenolic compounds occur in a variety of simple and complex forms. Simple phenols such as, cinnamic, coumarine, caffeic, protocatechuic, chlorogenic and quinic acid exhibit antimicrobial activities.

Infection in certain diseases is characterized by increased synthesis of certain precursors of phenolic compounds and oxidation products of phenolics, such as quinones which exhibit more toxicity to microorganisms than their reduced forms. Positive correlation between the amount of phenolic content and degree of resistance to plant disease has been evidenced by several workers.

It has been frequently observed that phenol accumulation takes place in all the infected plant tissues but more rapid accumulation of phenolics takes place in incompatible host pathogen complex than in the compatible ones (Kiraly and Farkas, 1962).

Tomiyama (1963) reported that the accumulation of phenolics in diseased plants was a common phenomenon observed in many host pathogen interactions. The

increase in phenolics concentration might arise from the release of phenol from their glucosides by the enzyme glucosidase of their host or pathogen (Pridham, 1965).

Patil and Dangat (2011a) reported that phenol content of the infected bark increased as compared to that in healthy bark.

## 2.4 *In vitro* and *in vivo* evaluation of chemicals and biorationals

Narasimhudu *et al.* (1987) conducted studies at Regional Fruit Research Station, Anantharajupet to find out effective chemical control of black banded disease of mango. They found that Bavistin at 0.1% concentration gave good control. The addition of Rogor did not give better results than Bavistin alone.

Patil and Dangat (2012) studied on efficacy of some fungicides; plant extracts and combination of both for the control of black banded disease *in vivo*. Fungicides used for treatments were Contaf, Sulphur, Care, Bordeaux mixture, Himil Gold, Kavach and Multineem oil. Out of these Sulphur, Care, Contaf, Multineem oil + Kavach and Multineem oil + Himil gold had shown promising results in arresting the growth of the pathogen. Extracts of plants used were *Nicotiana tabacum* L., *Lantana camara* L., *Polyalthia longifolia* (Sonner.) Thw. and *Justicia adhatoda* L. Among these *L. camara* and *P. longifolia* showed most effective control of disease. Combinations of all the plant extracts with the above mentioned fungicides were also tested, *viz.* *Nicotiana* + Contaf, *Nicotiana* + Sulphur, *Lantana* + Care, *Lantana* + Himil Gold, *Lantana* + Bordeaux, *Lantana* + Sulphur, *Polyalthia* + Care, *Polyalthia* + Bordeaux, *Polyalthia* + Sulphur, *Justicia* + Sulphur, *Justicia* + Himil Gold showed effective results.

Bhuvanewari and Rao (2001) reported that mango fruits inoculated with *T. viride* remained free from *Pestalotia* sp., *Colletotrichum gloeosporioides*, *A. niger*, *A. flavus*, *L. theobromae*, *R. stolonifer* and *M. phaseolina* infection indicating their suppression by the antagonistic nature of *T. viride*. In addition, *T. Viride* suppressed

the growth of *Penicillium purpurogenum*, *Penicillium sp.* and *Phoma sp.* even as the inoculated fruits were rotten.

Kumar *et al.* (2007) evaluated variability in fungicidal resistance or sensitivity among the *Colletotrichum gloeosporioides* by poisoned food technique using potato dextrose agar with fungicides. They recorded five isolates which were highly sensitive (>90% inhibition) and two isolates were sensitive (>75% inhibition) to Carbendazim at 50 ppm concentration whereas five isolates were highly resistant (>40 inhibition) and two isolates were resistant to Mancozeb at 1000 ppm concentration and all the isolates were resistant (40-60% inhibition) to Copper oxychloride at 1000 ppm concentration.

Narasimhadu (2007) evaluated bioefficacy of Score 25 EC (difenconazole) against powdery mildew and anthracnose on mango in field condition. The results indicated that the fungicide Score 25 EC @ 1 ml/l water was significantly superior to other treatments like Carbendazim 1 g/l, Mancozeb 3 g/l and Blitox 3g/l in controlling both powdery mildew and anthracnose.

Sharma *et al.* (2010) conducted field experiment for evaluation of seven fungicides against mango anthracnose on mango variety Alphonso. Fungicides used were Carbendazim 0.1 %, Thiophanate methyl 0.1%, Mancozeb 0.2%, Chlorothalonil 0.2 %, Propineb 0.2%, Saaf 0.2% and Tricyclazole 0.1%. They totally gave three sprays, one at the onset of disease symptoms on new flesh, second and third sprays at 10 days intervals. The per cent disease control was recorded 10 days after third spray. Result showed that Saaf was effective (82.34 % disease control) in management of disease followed by Carbendazim (77.25 % disease control).

Thahir *et al.* (2010) isolated ninety six native potential antagonists from phylloplane and leaf endophytes during the roving survey conducted in different regions of Andhra Pradesh. Of the 96 antagonists screened by dual culture technique, four fungal and six bacterial antagonists identified as fungicidal tolerant potential

bioagents. Further these antagonists were evaluated for their compatibility with four systemic fungicides viz., Carbendazim (50ppm), Thiophanate-methyl (50 ppm), Propiconazole (25 ppm), Hexaconazole (25 ppm) and two non-systemic fungicides viz., Mancozeb (1000 ppm) and Copper oxychloride (1000 ppm) to manage the disease anthracnose of mango through an integrated approach by combining bioagents and economic use of chemicals.

## 2.5 Screening of genotypes under natural conditions for disease resistance

Reddy *et al.* (1961) reported Neelum, Alampur Baneshan, Nawab Pasand, Kovaji Patel and Sambandham were highly susceptible to black banded disease of mango. About 40 varieties were found free from disease including *Mangifera odorata*.

Venkataravanappa (2002) screened ten mango cultivars for their reaction against mango anthracnose under natural and artificial conditions. Two cultivars viz., Mallika and Alampur Beneshan showed moderately susceptible reaction where as eight cultivars showed susceptible reaction.

Sangeetha (2003) screened 97 germplasms against anthracnose. The results revealed that none were resistant, 21 were moderately resistant, 61 susceptible and 15 highly susceptible. Among the moderately resistant genotypes, H-165 recorded the least per cent disease index. Neeleshan was recorded to be highly susceptible to this disease.

## 3. MATERIAL AND METHODS

The present investigations on black banded disease of mango were conducted during 2014-2015 at the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad. The field experiments related to management of the disease were carried out during 2014-15 at the Department of Horticulture, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad.

Dharwad is situated in northern transitional zone (Zone 8) of Karnataka state at 15°15'N latitude, 75°7'N longitude and at an altitude of 774.0 m above mean sea level. The material used and methodology adopted during the course of investigations are presented in this chapter.

### 3.1 General laboratory procedures

#### 3.1.1 Glassware cleaning

For all laboratory experimental studies, Corning and Borosil glassware were used. Wherever required, they were kept in the cleaning solution containing 60 g potassium dichromate ( $K_2Cr_2O_7$ ) and 60 ml. of concentrated sulphuric acid ( $H_2SO_4$ ) in one litre of water for a day. Then, they were cleaned by washing with detergent followed by rinsing several times in tap water and finally in distilled water.

#### 3.1.2 Sterilization

All the glassware were sterilized in an autoclave at 1.1 kg/cm<sup>2</sup> pressure for 20 minutes and further sterilized in hot air oven at 160° C for two hr. Sterilization of both solid and liquid media was achieved by autoclaving at 1.1 kg/cm<sup>2</sup> (121.6° C) pressure for 15 minutes for all the laboratory studies.

The plant tissues were surface sterilized in (1.0%) sodium hypochlorite solution for 60 seconds followed by three changes in sterile distilled water. All

cultural studies were conducted in aseptic condition under laminar flow. The tips of inoculation needle, forceps and cork borers were sterilized under flame.

### 3.2 Survey of the Disease in North Karnataka

An intensive roving survey was conducted in mango orchards of Dharwad, Haveri, Belgaum and Gadag districts of North Karnataka during the year 2014-15. In each village, three to four orchards were selected and in each orchard, five trees were examined randomly. The farmers were interviewed, fields were surveyed and observations were recorded for both disease incidence and severity then severity was scored by using 0-7 scale.

#### 3.2.1 Per cent disease incidence (I)

Disease Incidence (I) is the number of diseased plants (X) divided by the total number of plants evaluated (N).

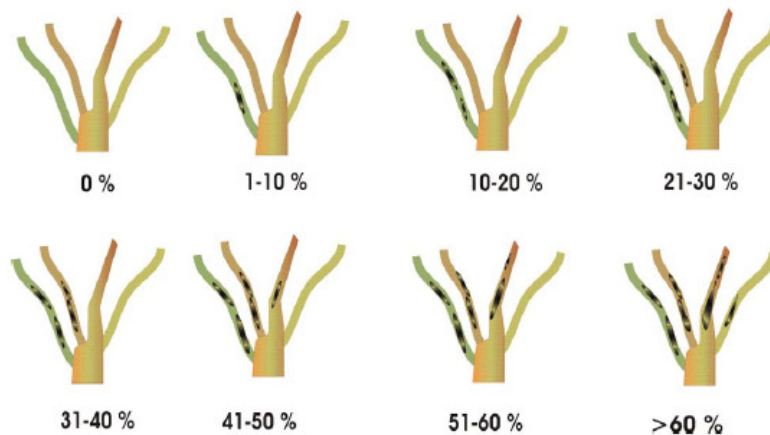
$$I = \frac{X}{N} \times 100$$

#### 3.2.2 Disease severity(S)

Severity (S) was estimated by the number of black bands on the trees and number of affected branches. Four branches, each measuring 3.0 m in length, were selected uniformly for each selected tree and the severity was estimated based on the area covered by the velvety growth of the fungal pathogen. Lengths of the black bands were measured and per cent area of infection over total area was calculated and scored by using 0-7 scale.

A disease severity scale for black banded disease was developed in line with the sudden death syndrome of mango developed by Masood *et al.* (2010), as there is no existing scale available for this disease.

Per cent disease severity on branches of mango tree was recorded according to scale (0-7)



Disease severity scale for mango black banded disease on stem

Rating	Severity (%)
0	No disease symptoms
1	1-10 % area of infection over total area observed
2	11-20 % area of infection over total area observed
3	21-30 % area of infection over total area observed
4	31-40 % area of infection over total area observed
5	41-50 % area of infection over total area observed
6	51-60 % area of infection over total area observed
7	>60 % area regarded as maximum severity

Per cent disease index (PDI)

Per cent disease index was calculated by using formula given below

$$\text{PDI} = \frac{\text{Sum of individual disease ratings on stem}}{\text{Total number of branches observed} \times \text{Maximum disease score}} \times 100$$

### 3.2.3 Isolation, Identification and proving the Pathogenicity of the fungus

#### 3.2.3.1 Collection and isolation of the pathogen

The twigs of mango (*Mangifera indica* L.) showing the typical symptoms of the disease were collected from silver jubilee orchard, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. The standard tissue isolation procedure was followed to isolate the pathogen. The infected bark bits were surface sterilized with sodium hypochlorite (1.0%) solution for 60 seconds. These bits were thoroughly washed in sterile distilled water to remove the traces of sodium hypochlorite if any, then aseptically transferred to sterilized Petriplates (1-2 bits of infected bark portion per Petri dish) containing Potato Dextrose Agar (PDA). The Petriplates were incubated at room temperature ( $27 \pm 1^{\circ}\text{C}$ ) and observed periodically for the growth. Bit of fungal growth developed from the infected tissue was transferred to PDA slants and incubated at  $27^{\circ} \pm 1^{\circ}\text{C}$  for 12 days. Then such slants with pure culture were used for further studies.

#### 3.2.3.2 Identification of the pathogen

Identification of *Peziotrichum corticolum* was achieved by studying morphological characters of the same pathogen as described by Mukherjee and Litz (2009) and Patil and Dangat (2012). Koch postulates were proved to identify and to confirm the pathogenicity of isolated pathogen.

### 3.2.2.3 Maintenance of the cultures

The fungus was sub-cultured on Potato dextrose agar (PDA) slants and allowed to grow at  $27 \pm 1^\circ\text{C}$  for 16 days, such slants were preserved in refrigerator at  $5^\circ\text{C}$  and maintained.

Sub-culturing was done once in a month, such cultures were used throughout the study; virulence of the fungus was maintained by passing through the host after every three months.

### 3.2.2.4 Proving the pathogenicity

Mango seedlings of aged three to four years were grown in the earthen pots filled with sterilized soil. Pathogenic culture of *P. corticolum* was grown on PDA for 15 days. Injury on seedlings was made by removing upper layers of the bark with the help of a sharp, sterilized knife. With sterilized spatula, mycelial disc was inoculated on the injury. Then the inoculated area was added with small quantity of sterile distilled water and covered with the help of transparent adhesive tape. Two controls were maintained as explained here under.

Control I: Injury + media + sterile distilled water

Control II: Sterile distilled water

All plants were covered with polythene bags for 48 hr to maintain humidity. After 48 hr of incubation, polythene bags were removed and the plants were kept in greenhouse. Observations were made for symptom development periodically. Re-isolation was made from the spots which were showing typical symptoms on inoculated plants. The isolated culture was compared with the original culture to confirm the pathogen.

### 3.3 Morphological, Cultural and Biochemical studies of the pathogen

#### 3.3.1 Cultural characteristics of *P. corticolum* on different solid media

Dharwad isolate was grown on different media viz., Potato dextrose agar, Host extract dextrose agar, Czapek's malt agar, Richards agar, Oat meal agar, V-8 juice agar and Potato carrot agar and observations were recorded for radial growth in (mm) and sporulation of the pathogen.

The composition and preparation of the above mentioned synthetic and semi-synthetic media were obtained from Ainsworth and Bisby's 'Dictionary of the Fungi' by Ainsworth (1971) and plant pathological methods, fungi and bacteria by Tuite (1969). The composition and preparation of different media are given below-

##### 1. Potato Dextrose Agar (PDA)

Potato	200.00 g
Dextrose	20.00 g
Agar	20.00 g
Distilled water	1000.00 ml

Potato slices were boiled in 500 ml distilled water for 20 minutes. Extract was filtered through muslin cloth. Dextrose (20 g) was added in this extract and mixed well. Agar was melted in another 500 ml distilled water, both solutions were mixed and the volume made up to 1000 ml and autoclaved at 121.6°C at 1.1 kg/cm<sup>2</sup> for 15 min.

##### 2. Host extract dextrose agar (HEDA)

Healthy mango twig bark	200.00 g
Agar-agar	20.00 g
Dextrose	20.00 g
Distilled water	1000.00 ml

Mango bark of healthy plant was boiled in 500 ml water for 30 min. Extract was collected by filtering through muslin cloth. Dextrose (20 g) was added in this extract and mixed well. The agar agar was melted in 500 ml water, both the solutions were mixed and the volume was made up to 1000 ml and then autoclaved.

### 3. Czapek's malt agar

Malt extract	40.00 g
Sucrose	30.00 g
Sodium nitrate	2.00 g
Potassium chloride	0.50 g
Magnesium sulphate	0.50 g
Ferrous sulphate	0.01 g
Dipotassium phosphate	1.00 g
Agar	20.00 g
Distilled water	1000.00 ml

Agar was melted in 500 ml distilled water and rest of the ingredients were thoroughly dissolved in another 500 ml distilled water. Both the preparations were mixed and the final volume was made up to 1000 ml and then autoclaved.

### 4. Richards Agar

Potassium nitrate ( $\text{KNO}_3$ )	10.00 g
Potassium monobasic phosphate ( $\text{KH}_2\text{PO}_4$ )	5.00 g
Magnesium sulphate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ )	2.50 g
Ferric chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ )	0.02 g
Sucrose ( $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ )	50.00 g
Agar agar	15.00 g
Distilled water	1000.00 ml

Agar was melted in 500 ml distilled water and rest of the ingredients were thoroughly dissolved in another 500 ml distilled water. Both the preparations were mixed and the final volume was made up to 1000 ml and then autoclaved.

#### 5. Oat meal agar

Oat flakes	30.00 g
Agar-agar	20.00 g
Distilled water	1000.00 ml
pH	7.00

Oat flakes (30.00 g) were boiled in 500 ml of distilled water for 20 min and the extract was filtered through a muslin cloth. Agar agar was melted separately in 500 ml of water. Both the solutions were mixed thoroughly. The volume was made up to 1000 ml distilled and then autoclaved.

#### 6. V-8 juice agar (V8JA)

V-8 Juice (100 ml)	8.30 g
L-Asparagine	10.00 g
Yeast extract	2.00 g
Calcium carbonate (CaCO <sub>3</sub> )	2.00 g
Glucose	2.00 g
Agar-agar	20.00 g
Distilled water	1000.00 ml

All the ingredients except agar agar were dissolved one by one in 400 ml distilled water and agar was dissolved separately in 500 ml distilled water and mixed with the above solution and the volume was made up to 1000 ml before sterilization.

### 7. Potato carrot agar

Grated potato	20.00 g
Grated carrot	20.00 g
Agar agar	20.00 g
Distilled water	1000.00 ml

Grated vegetables (potato and carrot) were boiled for 1 hr. in the tap water, strained through fine sieve, agar was added. Later boiled over water bath till agar dissolved, then volume was made upto 1000 ml sterilized at  $1.1 \text{ kg/cm}^2$  for 15 min.

Twenty ml. of media (PDA) was poured aseptically into sterilized Petriplates and kept for solidification. After solidification, 5 mm discs of the *Peziotrichum corticolum* were cut using a cork borer and a single disc was placed at the center of Petridish. Each set of experiment replicated thrice and they were incubated at  $27 \pm 1 \text{ }^\circ\text{C}$  for 15 days. Cultural characters such as the colony diameter, colony colour, type of colony margin and sporulation were recorded.

### 3.3.2 Cultural characteristics of *P. corticolum* on liquid media

Two liquid media namely, potato dextrose broth and Richards' broth were used to study growth phase of *P. corticolum*. The composition and preparation of both liquid media used were the same as that of solid media except that the agar was not added.

Thirty ml. of broth was added into each of 100 ml conical flask and sterilized at  $1.1 \text{ kg/cm}^2$  pressure for 15 minutes at  $121^\circ\text{C}$ . After sterilization, these flasks were allowed to cool and then inoculated with 5 mm disc from 15 days old culture and incubated at room temperature. Each treatment was replicated three times. The culture was filtered through Whatman No. 42 filter paper of 9.0 cm diameter, which was dried to a constant temperature at  $60^\circ\text{C}$  in an electric oven prior to filtration. The

mycelial mat on the filter paper was thoroughly washed with sterile distilled water to remove traces of salts likely to be associated with it. Three flasks were harvested at 48 hrs after incubation and subsequent harvesting was done at an interval of two days up to 20 days. The filter paper along with the mycelial mat were dried to a constant weight at 60°C and weighed immediately on a digital balance. The difference between final and initial weight of filter disc was taken as the weight of the mycelial mat. The data were analyzed statistically.

Dry mycelial weight (mg) = Total weight of filter paper along with mycelia – Initial weight of filter paper

### 3.3.3 Spore Morphology

The spores produced in the black velvety growth of fungus on the host bark were observed. Length and breadth of spores were measured using motic images in computer (400 X). The average size of the spore was calculated.

### 3.3.4 Biochemical studies

Extraction of bark material in alcohol

Five grams of bark material was extracted in ethanol as per the procedure followed by Jaypal and Mahadevan (1968). The precipitate was removed by filtering the alcohol extract through Whatman No. 1 filter paper and the filtrate was made up to 25 ml with 80 per cent alcohol. Reducing sugars, non-reducing sugars, total sugars and phenols were estimated in alcohol extract of fresh healthy and diseased bark material.

#### 3.3.4.1 Sugars estimation

Reducing sugars

The reducing sugar was estimated following Nelson's modification of Somogyi's method (Nelson, 1944).

## Reagents

### A. Alkaline copper reagent

Solution A: Twenty five gram of anhydrous sodium carbonate, 25 g of sodium potassium tartarate, 20 g of sodium bicarbonate and 200 g of sodium sulphate were dissolved in about 800 ml of distilled water and final volume was made up to one litre.

Solution B: Fifteen grams of copper sulphate was dissolved in distilled water and one or two drops of concentrated sulphuric acid was added and made up to 100 ml with distilled water.

Solution A and B were mixed in 24:1 (v/v) proportion just before use.

### B. Arsenomolybdate reagent

Twenty five gram of ammonium molybdate was dissolved in 450 ml of distilled water. 21 ml of concentrated sulphuric acid was added and mixed with the above solution. 2. 3 g of sodium orthoarsenate was dissolved in 25 ml of distilled water. The above two solutions were mixed by stirring and placed in an incubator at 37°C for 24-48 hr. The reagent was stored in brown bottle.

## Procedure

One ml of each sample (alcoholic extract) was pipetted to a test tube. To each 1 ml of extract, 1 ml of mixture of solution A and B was added. The test tubes were heated on a hot water bath for 20 min. The tubes were then cooled under running tap water. After cooling 1 ml of arsenomolybdate reagent was added. The above solution was diluted to 15 ml after 15 min. The absorbance of the solution was measured in spectrophotometer at 510 nm. The amount of reducing sugars was determined by using standard curve prepared.

### Acid hydrolysis of non-reducing sugar and its estimation as reducing sugar

Non-reducing sugar was first hydrolyzed with the help of diluted Hydrochloric acid (HCl). The hydrolysate was neutralized and the reducing sugar was estimated by Nelson Somogyi's method (Nelson, 1944).

#### Reagents

1. 0.1 and 1 N Hydrochloric acid and 1 N sodium hydroxide.
2. Phenolphthalein indicator solution in alcohol.

#### Procedure

One ml each of alcohol extract was taken in a test tube and to it 1 N HCl was added. The test tubes were kept on hot water bath at 50<sup>0</sup>C for 20 minutes. After cooking, one drop of indicator was added and mixed well. To the solution, 1 N sodium hydroxide was added drop wise till the colour turned pink due to excess alkali. The excess alkali was reneutralized with 0.1 N hydrochloric acid till the solution became colourless. Then the volume was made up to 5 ml. From this, 1 ml was taken and reducing sugar present in hydrolysate was estimated by Nelson - Somogyi's method. The reducing sugar in the hydrolysate was a measure of total sugar. To get the quantity of non-reducing sugar, the quantity of reducing sugar was subtracted from total sugar.

#### 3.3.4.2 Phenol estimation

The total phenols present in plant samples was estimated by following folin ciocalteau reagent method.

#### Reagents

1. Folin - ciocalteau reagent (FCR, 1%)
2. Sodium carbonate (2%)

## Procedure

One ml each of alcoholic extract was taken in a test tube to which one ml of Folin ciocalteau reagent was added followed by two ml of sodium carbonate solution. The tubes were shaken well and heated in a hot water bath for exactly one minute and then cooled under running tap water. The content developed was diluted to 20 ml with distilled water and its absorbance was read at 650 nm in the spectrophotometer. The amount of phenols present in the sample was calculated from a standard curve prepared from catechol.

## 3.4 *In vitro* and *in vivo* evaluation of Chemicals and Biorationals

### 3.4.1 *In vitro* evaluation of fungicides against *P. corticolum*

The efficacy of five non-systemic and three combi- products and three systemic fungicides were tested against *P. corticolum* for growth inhibition on the potato dextrose agar media using poisoned food technique under *in vitro* conditions.

### 3.4.2 Poisoned food technique

Poisoned food technique (Shravelle, 1961) was followed to test the efficacy of the fungicides. The pathogen *P. corticolum* was grown on suitable solid medium in Petriplates for ten days prior to experiment. Fungicide suspension was prepared in that medium by adding requisite quantities of fungicides to obtain the desired concentration. Poisoned medium was poured in each of the sterilized petriplates. Mycelial disc of 5 mm was taken from the periphery of the ten days old culture and placed in the centre of petriplate and incubated at  $28 \pm 2^\circ\text{C}$  till growth of the fungus reaches the periphery in the control plate. Three replications were maintained for each treatment. The colony diameter was measured in two directions and average was worked out. The per cent inhibition of growth was calculated by using the formula given by Vincent (1947).

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

Where,

I = Per cent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

The list of fungicides used along with their concentration is given below.

List of fungicides

Sl. No.	Common name	Trade name	Concentrations (%)			
Systemic fungicides						
1.	Hexaconazole	Contaf 5% EC	0.05	0.10	0.15	
2.	Propiconazole	Tilt 25% EC	0.05	0.10	0.15	
3.	Difenconazole	Score 25% EC	0.05	0.10	0.15	
Contact fungicide/s						
1.	Bordeaux mixture		0.5	0.75	1.0	1.25
2.	Chlorothalonil	Kavach 75% WP	0.15	0.20	0.25	0.3
3.	Copper oxychloride	Blitox 50% WP	0.15	0.20	0.25	0.3
4.	Cuprous hydroxide	Kocide 77% WP	0.15	0.20	0.25	0.3
5.	Mancozeb	Indofil M-45 75% WP	0.15	0.20	0.25	0.3
Combiproducs						
1.	Carbendazim 12% + Mancozeb 63% WP	Saaf 75%WP	0.15	0.20	0.25	0.3
2.	Hexaconazole 4% + Zineb 68% WP	Avatar 72% WP	0.15	0.20	0.25	0.3
3.	Captan 70% + Hexaconazole 5% WP	Taqat 75% WP	0.15	0.20	0.25	0.3

### 3.4.3 *In vitro* evaluation of botanicals against *P. corticolum*

The present investigation was carried out to evaluate the extracts of five plant species to know the presence of fungitoxicant properties against *P. corticolum*.

#### 3.4.3.1 Preparation of plant based products

Fresh healthy plant parts of 100 g (leaves/kernel) as indicated below were collected and washed with distilled water and air dried and crushed in 100 ml of sterile water. The crushed product was tied in muslin cloth and filtrate was collected. The prepared solution gave 100 per cent, which was further diluted to required concentrations of 5 and 10 per cent. The extracts were tested against *P. corticolum* on

the PDA using poisoned food technique under *in vitro* condition as described earlier. The per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947).

Sl. No.	Plants product/extract	Plant part used
1.	<i>Lantana camara</i>	Leaf
4.	Neem kernel extract	Kernel
5.	Neem leaf extract	Leaf
3.	<i>Nerium oleander</i>	Leaf
2.	<i>Parthenium hysterophorus</i>	Leaf

#### 3.4.4 *In vitro* evaluation of bioagents against *P. corticolum*

The efficacy of four bioagents was tested against *P. corticolum* for radial growth inhibition on the potato dextrose agar media using dual culture technique under *in vitro* condition. List of bioagents used against *P. corticolum* are mentioned below.

- (i) *Trichoderma harzianum*
- (ii) *Bacillus subtilis*
- (iii) *Pseudomonas fluorescens*
- (iv) *Verticillium lecanii*

##### 3.4.4.1 Dual culture test

Bioagents were evaluated for their efficacy following dual culture technique. The bioagents and the test fungus were inoculated side by side on a single Petridish containing solidified PDA medium. Five replications were maintained for each treatment with one control by maintaining only pathogen separately. They were incubated for 12 days. The diameter of the colony of both bioagents and the pathogen was measured in two directions and average was recorded. Per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947).

### 3.4.5 *In vivo* evaluation of Chemicals and Biorationals against *P. corticolum*

An experiment was conducted at Main Agricultural Research Station, UAS Dharwad, during 2014-15 to manage the black banded disease of mango. The variety, Alphonso was selected and sprayed with different fungicides and biorationals. The experiment included nine treatments and one check with three replications. Three sprays were given after rubbing of black bands with the help of gunny bag, in 10 days interval. Disease severity was calculated before and after 10 days of final spray and then per cent disease reduction over control (PDRC) was calculated.

PDBS-PDAS

$$\text{PDRC} = \frac{\text{PDBS} - \text{PDAS}}{\text{PDC}} \times 100$$

PDC

Where-

PDRC= Per cent Disease Reduction over Control

PDBS= Per cent Disease Severity before Spray

PDAS= Per cent Disease Severity after Spray

PDC = Per cent Disease Severity in Control

Details of experiment are given hereunder

Location	: Main Agricultural Research Station
Variety	: Alphonso
Age of the plants	: 16-20 years
Treatments	: 10
Number of plants /treatment	: 5
Replication	: 3
Design	: RBD
Year	: 2014-15

## Details of treatments

Treatments			Concentration (%)	Quantity/liter
	Common name	Trade name		
T1	Bordeaux mixture		1	1:1:100
T2	Bordeaux paste		10	10:10:100
T3	Carbendazim 12% + Mancozeb 63% WP	Saaf 75% WP	0.3	3g
T4	Copper oxychloride	Blitox 50% WP	0.3	3g
T5	Difenconazole	Score 25% EC	0.1	1ml
T6	Hexaconazole 4% + Zineb 68% WP	Avatar 72% WP	0.3	3g
T7	Neem Kernel Extract		10	100 ml
T8	Propiconazole	Tilt 25% EC	0.1	1ml
T9	<i>Trichoderma harzianum</i>		1	10g
T10	Untreated control		-	-

### 3.5 Screening of genotypes under natural conditions for disease resistance

This experiment was conducted at Main Agricultural Research Station, UAS Dharwad, during 2015. Twelve genotypes namely Alphonso, Dilpasand, Fernandin, Jahangir, Mallika, Mulgoa, Neeleshan, Neelgoa, Neelum, Pairi, Ratna, Swarna and Totapuri were subjected to screening. Disease severity was calculated for the selected genotypes and comparison for disease resistance among different genotypes of mango was accomplished under natural conditions.

### 3.6 Statistical analysis

The experimental data collected were analyzed statistically for its significance of difference by the normal statistical procedure adopted for completely randomized design and interpretation of data was carried out in accordance with Walter (1997). The level of significance used in 'F' and 'T' test was  $P=0.05$  and  $P=0.01$ . Critical differences were calculated wherever 'F' test was significant.

## 4. EXPERIMENTAL RESULTS

The results of the experiments conducted on various aspects on black banded disease of mango (*Mangifera indica*) during the period 2014 to 2015 with reference to survey of disease; morphological, cultural characters of pathogen and biochemical studies of the disease; *in vitro* and *in vivo* evaluation of chemicals, biorationals and screening of genotypes under natural conditions for disease resistance are presented here under.

### 4.1 Survey of the Disease in North Karnataka

Roving survey was undertaken during 2014 – 2015 to assess the prevalence and severity of black banded disease in major mango growing areas of Dharwad, Haveri, Belagavi and Gadag districts by taking two to three orchards in each village, three villages in each taluk and one to three taluk in every districts as explained in the “Material and Methods” and results are presented in Table 1, Plate 1 and Fig. 1.

Mean per cent disease severity of different villages ranged from 3.72 to 27.39 and the highest per cent disease severity (27.39) was recorded in Narendra village of Dharwad district followed by Karambal village (22.46) of Belagavi district and Gabbur village (20.58) of Dharwad district. The least per cent disease severity (3.72) was recorded in Hulikatti (Gadag).

Mean per cent disease index (PDI) of different villages ranged from 9.53 to 47.63 and the highest PDI (47.63) was recorded in Narendra village of Dharwad district followed by PDI (42.87) for both Karambal village of Belagavi and Gabbur village of Dharwad district. The least PDI (9.53) was recorded in Hulikatti (Gadag).

Among the different talukas, per cent disease severity ranged from 11.69 to 20.64. Dharwad (Dharwad) taluk recorded highest per cent disease severity of 20.64, followed by 17.33 in Hubballi (Dharwad) taluk and 16.13 in Bilahongal (Belagavi) taluk. The least per cent disease severity (11.69) was recorded in Gadag (Gadag) taluk.

Table 1. Severity of black banded disease in major mango growing areas of North Karnataka during 2014-15

SI No	District	Taluk	Village	Area of orchard (Ha)	Type of soil	Genotype	Age of the tree (Year)	Per cent disease incidence (I) (%)	Disease severity (S) (%)	Grade	Mean disease severity (%)	PDI		
1.	Belagavi	Belagavi	Bagewadi	0.36	Red	Totapuri	15	61.11	11.08	2	12.42	28.58		
				0.18	Red	Alphonso	10	83.33	13.75	2				
			Bastawad	0.35	Loamy	Totapuri	25	74.29	12.42	2	15.63	28.58		
					Black	Alphonso	25	88.57	18.83	2				
				0.80	Black	Alphonso	18	62.5	16.92	2			15.25	28.58
						Alphonso	12	77.78	13.58	2				
		Mean for Taluk											14.43	28.58
		Bilahongal	M.K. Hubballi	0.30	Loamy	Alphonso	25	100	14.08	2	17.04	28.58		
				0.42	Red	Alphonso	20	100	20	2				
			Neginhal	0.30	Loamy	Alphonso	20	93.33	15.83	2	16.33	28.58		
					Loamy	Alphonso	18	62.50	16.83	2				
				0.24	Loamy	Alphonso	18	62.50	16.83	2				
			Udikeri	0.40	Black	Alphonso	12	60	13.58	2	15.00	28.58		
		0.30		Black	Alphonso	15	83.33	16.42	2					
		Mean for Taluk											16.13	28.58
		Khanapura	Devalatti	0.30	Red	Neelum	18	53.33	12.50	2	6.25	14.29		
				0.25	Red	Alphonso	5	0	0	0				
			Halakarni	0.60	Loamy	Alphonso	15	75	18.83	2	19.42	33.34		
					Black	Alphonso	12	80	15.33	2				
				0.24	Loamy	Alphonso	20	79.17	24.08	3				
			Karambal	0.35	Loamy	Alphonso	18	77.14	23	3	22.46	42.87		
		0.15			Alphonso	30	80	21.92	3					
		Mean for Taluk											16.04	30.62
		Mean for District											15.53	29.26
		2.	Dharwad	Dharwad	Chikkamalligwada	0.35	Black	Totapuri	15	66.67	8.5	1	18.31	28.58
						0.70	Black	Alphonso	12	80	18.83	2		
						0.40	Black	Alphonso	20	100	27.58	3		
					Itigatti	1.00	Black	Alphonso	25	94.74	22.17	3	19.19	33.34
0.25	Black					Mallika	15	33.33	7.25	1				
0.75	Black					Alphonso	18	91.67	28.17	3				

Table 1. Contd.....

Sl No	District	Taluk	Village	Area of orchard (Ha)	Type of soil	Genotype	Age of the tree (Year)	Per cent disease incidence (I) (%)	Disease severity (S) (%)	Grade	Mean disease severity (%)	PDI		
			MARS, UAS	0.60	Red	Alphonso	18	100	26.01	3	17.68	28.58		
				0.45	Red	Mallika	15	100	9.35	1				
		Narendra	0.35	Black	Alphonso	10	86.67	23.58	3	27.39	47.63			
			0.45	Black	Alphonso	15	86.11	24.83	3					
			0.40	Black	Alphonso	13	87.50	33.75	4					
		Mean for Taluk											20.64	34.53
		Hubballi	Gabbur	0.40	Black	Alphonso	15	66.67	20.42	3	20.58	42.87		
				1.50	Black	Alphonso	17	75	20.33	3				
				1.2	Black	Alphonso	18	68	21.00	3				
			Murarhalli	0.65	Loamy	Alphonso	18	70	17.17	2	16.36	33.34		
				0.80	Black	Alphonso	12	66.67	11.33	2				
				0.35	Black	Alphonso	20	88.57	20.58	3				
			Nuluvi	0.60	Black	Alphonso	10	16.67	13.97	2	15.06	28.58		
				0.55	Black	Mallika	15	17.86	7.58	1				
				0.35	Black	Alphonso	30	100	23.67	3				
		Mean for Taluk											17.33	34.93
		Kalghatagi	Dummavada	0.25	Loamy	Alphonso	18	100	16.67	2	18.33	28.58		
				0.48	Black	Alphonso	12	100	19.50	2				
				0.60	Black	Alphonso	15	91.67	18.83	2				
			Hirehonnehalli	0.20	Red	Alphonso	25	90	17.17	2	13.75	23.82		
				0.80	Loamy	Alphonso	12	80	16.00	2				
				0.16	Black	Mallika	17	53.33	8.08	1				
			Joddahalli	0.35	Black	Neelum	30	83.33	8.00	1	9.67	19.05		
				0.65	Black	Alphonso	10	64.29	12.17	2				
				0.44	Black	Alphonso	10	67.69	8.83	1				
		Mean for Taluk											13.92	23.82
		Mean for District											17.29	31.09
		3.	Gadag	Gadag	Hoolageri	0.25	Red	Alphonso	18	100	21.58	3	19.25	35.73
0.45	Red					Mallika	23	66.67	16.92	2				

Table 1. Contd.....

Sl No	District	Taluk	Village	Area of orchard (Ha)	Type of soil	Genotype	Age of the tree (Year)	Per cent disease incidence (I) (%)	Disease severity (S) (%)	Grade	Mean disease severity (%)	PDI		
			Hosahalli	0.32	Black	Alphonso	4	0	0	0	12.11	19.05		
				0.80	Red	Alphonso	15	100	16.42	2				
				0.35	Black	Alphonso	15	93.75	19.97	2				
			Hulikatti	0.25	Loamy	Alphonso	3	0	0	0	3.72	9.53		
				0.40	Red	Alphonso	8	0	0	0				
				0.36	Red	Alphonso	20	77.27	11.17	2				
Mean for Taluk											11.69	21.44		
Mean for District											11.69	21.44		
	Haveri	Byadagi	Chikkabasur	0.30	Loamy	Alphonso	15	60	16.92	2	15.64	28.58		
						0.28	Loamy	Neelum	17	46.42			13.58	2
						0.18	Loamy	Alphonso	12	88.89			16.42	2
					Dummihala	0.25	Black	Alphonso	35	100	12.75	2	14.72	28.58
						0.28	Black	Alphonso	18	82.14	19.50	2		
						0.23	Black	Neelum	18	47.82	11.92	2		
					Kummur	0.48	Loamy	Alphonso	10	60	14.33	2	11.06	19.05
						0.28	Red	Alphonso	4	0	0	0		
						0.85	Red	Alphonso	20	87.50	18.83	2		
Mean for Taluk											13.81	25.40		
			Hanagal	Akkialur	0.30	Loamy	Alphonso	15	83.33	19.75	2	17.89	28.58	
						0.32	Red	Alphonso	18	78.13	19.58			2
						0.45	Loamy	Neelum	20	66.67	14.33			2
					Honkana	0.35	Black	Alphonso	10	40	7.25	1	16.96	28.58
						0.48	Black	Alphonso	28	93.33	22.42	3		
				Kallapura	1.50	Red	Alphonso	40	100	23.58	3	7.58	28.58	
					0.80	Loamy	Neelum	13	85	9.67	1			
				Tilavalli	0.50	Black	Mallika	15	75	13.92	2	19.38	35.73	
					0.60	Black	Alphonso	18	81.82	24.83	3			
Mean for Taluk											15.45	30.37		
Mean for District											14.63	27.89		



a) Belagavi district



b) Dharwad district



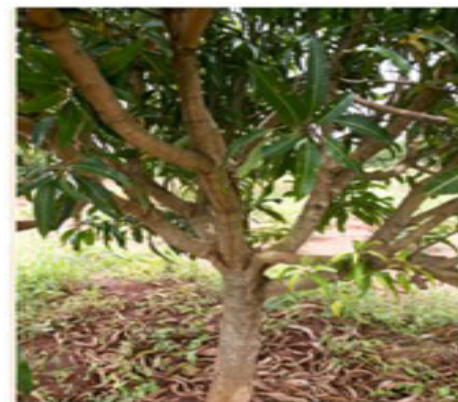
c) Gadag district



d) Haveri district

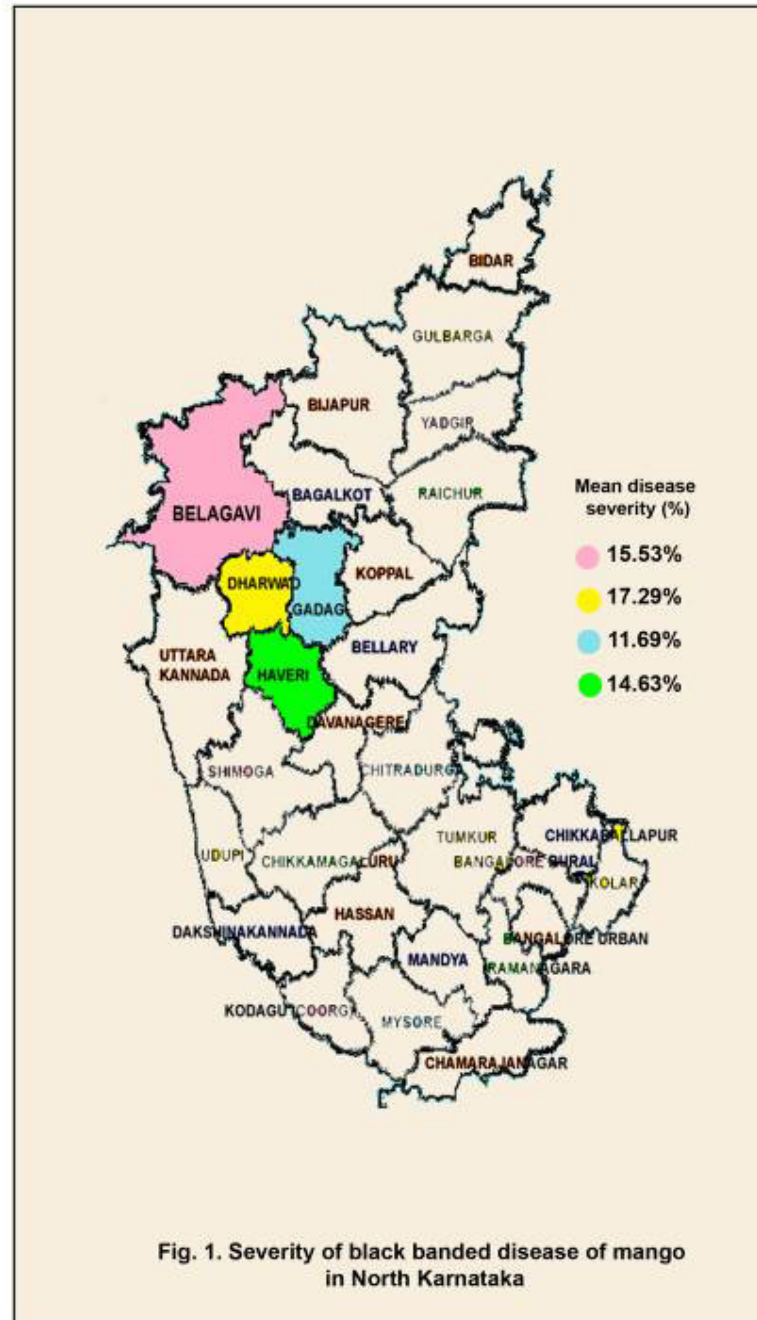


e) Taking observation during survey



f) Young plant free from disease

**Plate 1: Severity of black banded disease on mango observed during survey**



Among the different talukas, PDI ranged from 21.44 to 34.93. Hubballi (Dharwad) taluk recorded highest PDI of 34.93, followed by 34.53 in Dharwad (Dharwad) taluk and 30.62 in Khanapura (Belagavi) taluk. The least PDI (21.44) was recorded in Gadag (Gadag) taluk.

District-wise severity of black banded disease of mango surveyed during 2014-15, depicted that (Table 1a and Fig. 2), Dharwad district recorded maximum per cent disease severity (17.29 %) as well as maximum PDI (31.09) followed by Belagavi (15.53 % and 29.26 respectively) and Haveri (14.63 % and 27.89 respectively). Lowest per cent disease severity (11.69 %) and lowest PDI of 21.44 was recorded in Gadag district.

Among the varieties, Alphonso recorded highest, 17.43 per cent disease severity and PDI (32.11) whereas, Totapuri recorded least, 10.67 per cent disease severity and Mallika recorded least PDI (19.05) (Table 1b and Fig 3).

Looking into the age of plants, irrespective of genotypes (Table 1c and Fig. 4), highest per cent disease severity (18.80) and highest PDI (33.72) was observed in old age (16-20 years) trees compared to young age trees (5-10 years). Trees having age upto 5 years were found to be disease free.

Looking into the age of plants of different genotypes (Table 1d and Fig. 5), Alphonso and Neelum recorded highest per cent disease severity (20.48 % and 13.08 % respectively) for 16 to 20 years aged plants. Whereas, Mallika and Totapuri recorded highest per cent disease severity (16.92 % and 12.42 % respectively) for equal or more than 21 years aged plants.

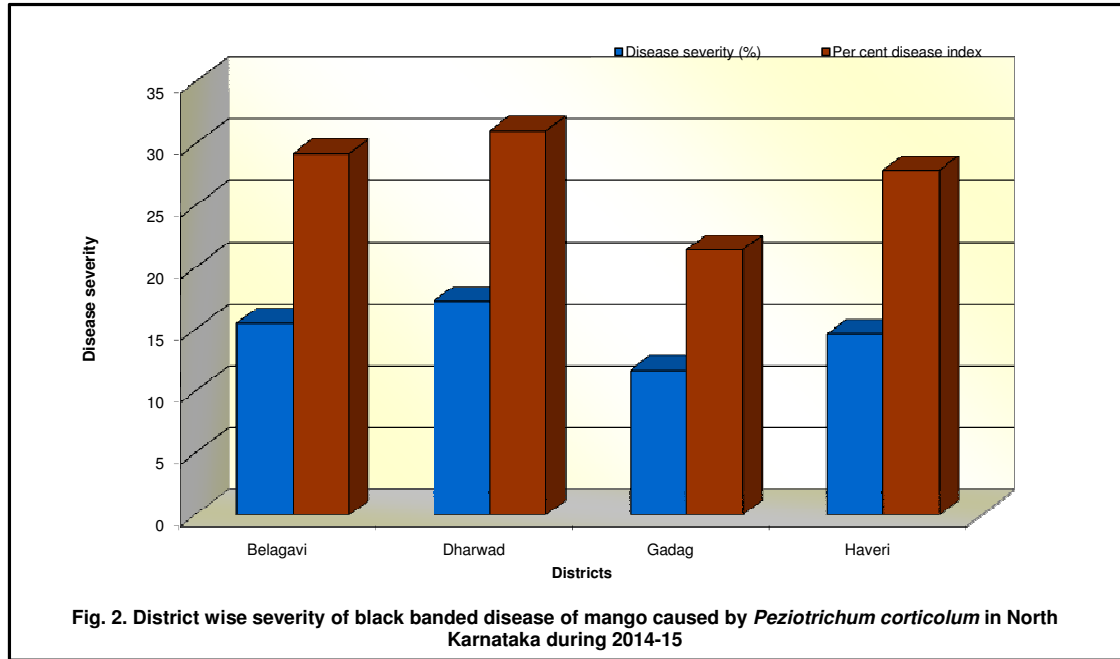
Looking into the age of plants of different genotypes (Table 1e and Fig. 6), Alphonso, Mallika and Totapuri recorded highest PDI (36.52, 28.58 and 28.58 respectively) for equal or more than 21 years aged plants. Whereas, Neelum recorded highest PDI (28.58) for 16 to 20 years aged plants.

Table 1a. District wise severity of black banded disease of mango caused by *Peziotrichum corticolum* in North Karnataka during 2014-15

Sl No.	District	Disease severity (%)	Per cent disease index
1.	Belagavi	15.53	29.26
2.	Dharwad	17.29	31.09
3.	Gadag	11.69	21.44
4.	Haveri	14.63	27.89

Table 1b. Variety-wise severity of black banded disease of mango caused by *Peziotrichum corticolum* in North Karnataka during 2014-15

Sl. No.	Genotypes	Mean disease severity (%)	Mean per cent disease index
1.	Alphonso	17.43	32.11
2.	Mallika	10.75	19.05
3.	Neelum	11.67	23.82
4.	Totapuri	10.67	23.82



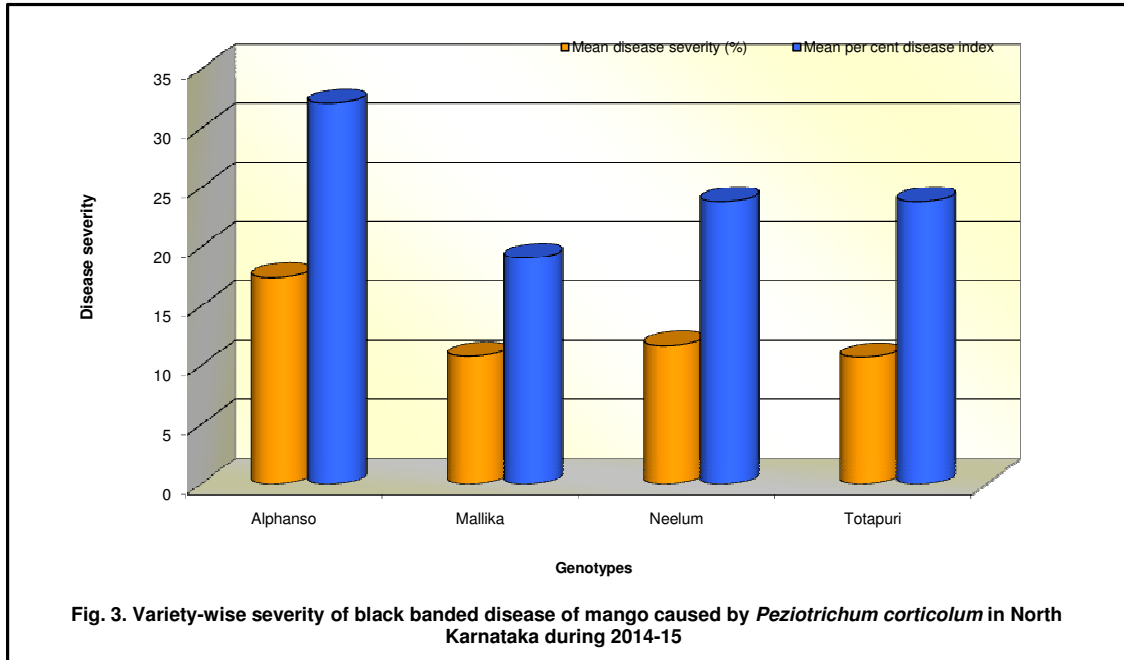


Table 1c. Age-wise severity of black banded disease of mango caused by *Peziotrichum corticolum* (Irrespective of genotypes) in North Karnataka during 2014-15

Sl No.	Age of plants (years)	Disease severity (%)	Per cent disease index
1.	Up to 5	0	0
2.	5 - 10	11.74	23.22
3.	11 - 15	15.92	28.01
4.	16 - 20	18.80	33.72
5.	≥21	17.83	33.34

- No plants observed

Table 1c. Age-wise severity of black banded disease of mango caused by *Peziotrichum corticolum* (Irrespective of genotypes) in North Karnataka during 2014-15

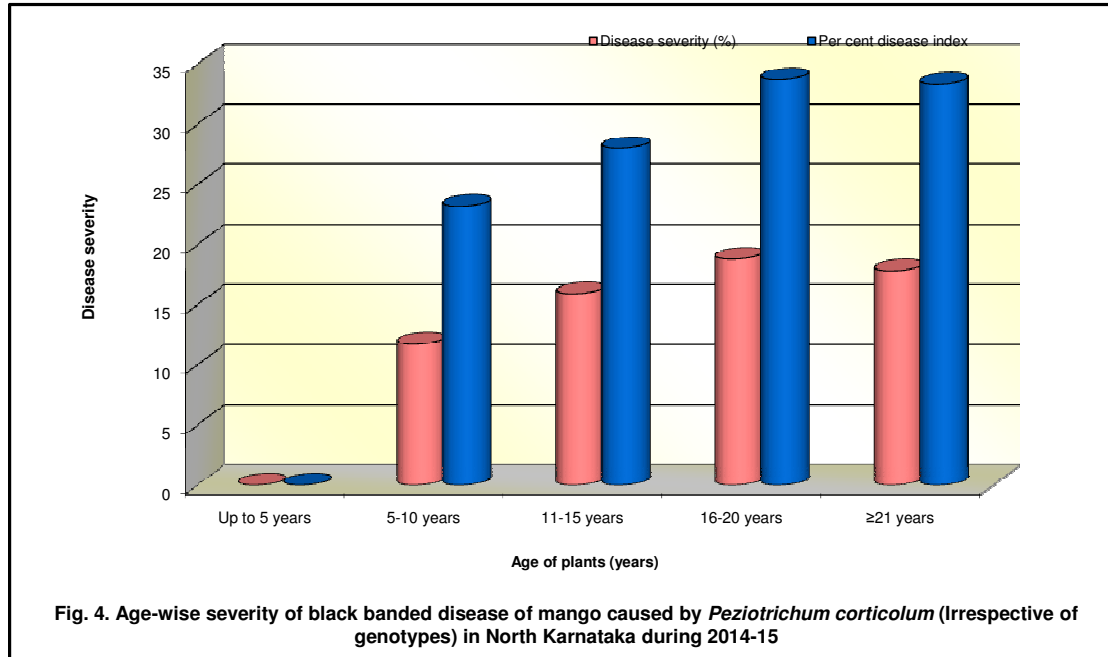
Sl No.	Age of plants (years)	Disease severity (%)	Per cent disease index
1.	Up to 5	0	0
2.	5 - 10	11.74	23.22
3.	11 - 15	15.92	28.01
4.	16 - 20	18.80	33.72
5.	≥21	17.83	33.34

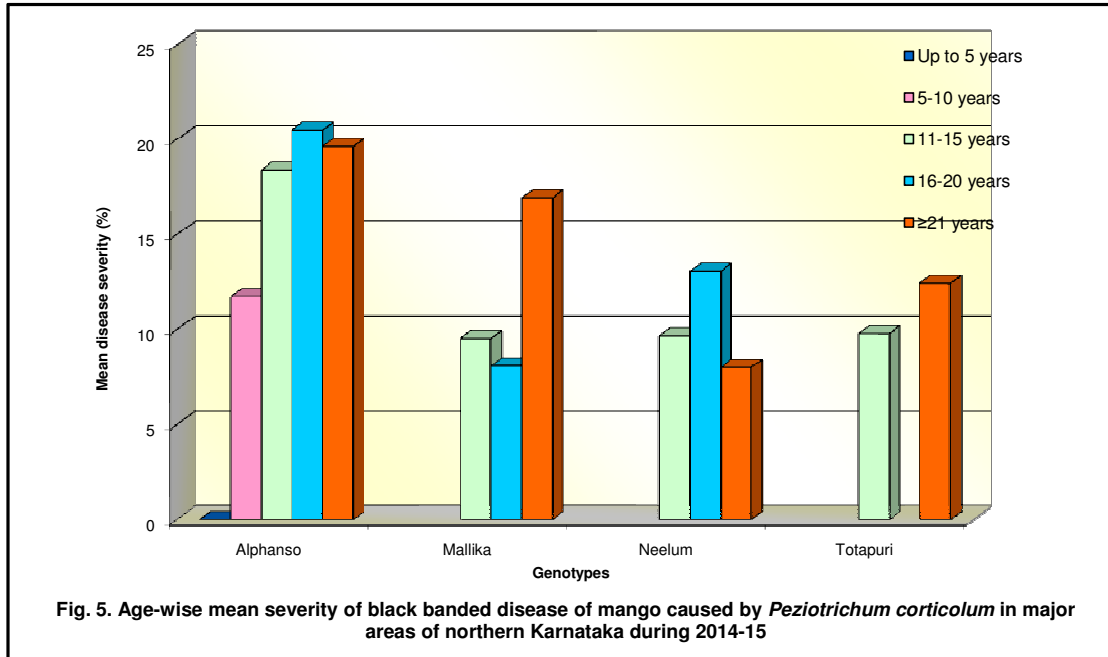
- No plants observed

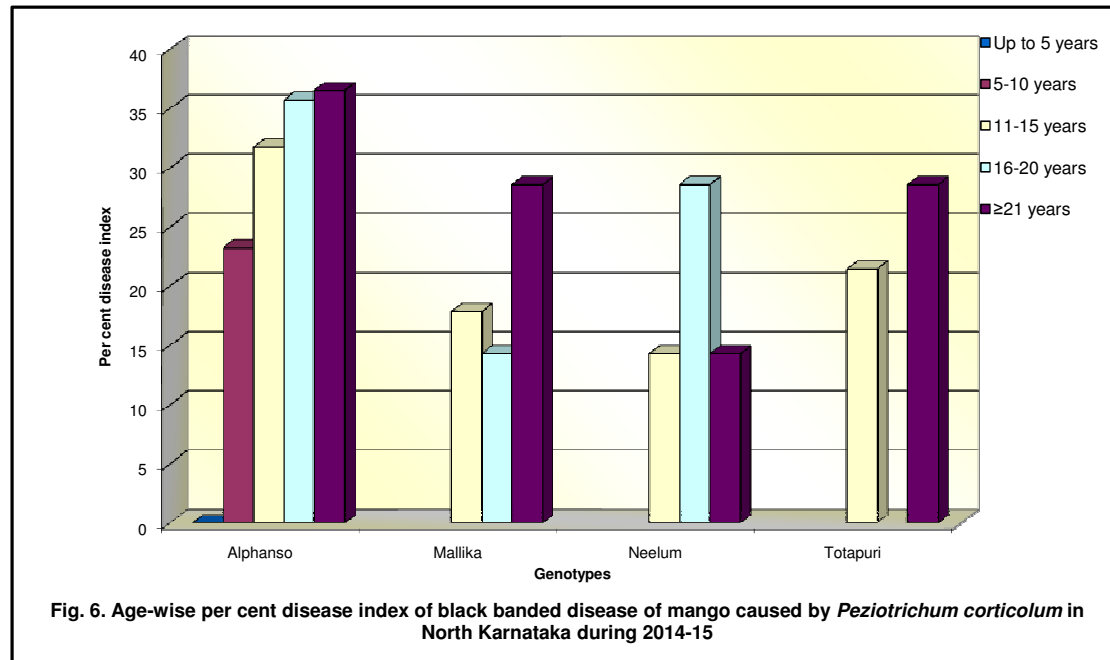
Table 1d. Age-wise per cent disease severity of black banded disease of mango caused by *Peziotrichum corticolum* in North Karnataka during 2014-15

Sl. No.	Age of plants (years)	Disease severity (%)			
		Genotypes			
		Alphonso	Mallika	Neelum	Totapuri
1.	Up to 5	0	-	-	-
2.	5 - 10	11.74	-	-	-
3.	11 - 15	18.37	9.53	9.67	9.79
4.	16 - 20	20.48	8.08	13.08	-
5.	≥21	19.62	16.92	8	12.42

- No plants observed







### 4.1.1 Symptomatology

The typical black banded symptoms were observed on twigs, branches, leaf petiole, midrib and veins of leaves. Symptoms were rarely produced on main trunk of the tree. Inflorescences were found to be free from symptoms.

On branches and twigs, symptoms are characterized by typical black, irregular, superficial velvety fungal growth. The size of fungal growth increased with the advancement of the disease which resulted in large, black coloured, girdle like velvety bands. Velvety appearance of bands is due to fungal mycelium which aggregate together and erect, perpendicular to bark. The young spreading mycelium near the periphery of the infection bands is white or nearly hyaline. In infected trees, symptoms are more conspicuous on young branches and twigs than on old branches. The velvety mycelial growth drops off in the summer months leaving light black coloured, bands in the affected portions.

On leaves, black velvety fungal growth can be seen on midrib and veins. Girdling of petioles by black velvety fungal growth is also common (Plate 2).

### 4.1.2 Isolation of the pathogen

Standard tissue isolation technique was followed to obtain causal agent from the infected bark portion showing typical black banded symptoms.

### 4.1.3 Proving pathogenicity

Fungus was isolated from infected bark of mango tree and pure culture was obtained by subsequent sub-culturing and such culture was used for pathogenicity test as described in "Material and Methods" (Plate 3).

On tenth day of inoculation, typical black velvety fungal growth was seen on twigs. On 13<sup>th</sup> day, area between velvety fungal growths became black. The boundary of black fungal growth was light white in colour. The fungus was reisolated and



a) On new branches



b) On old branches



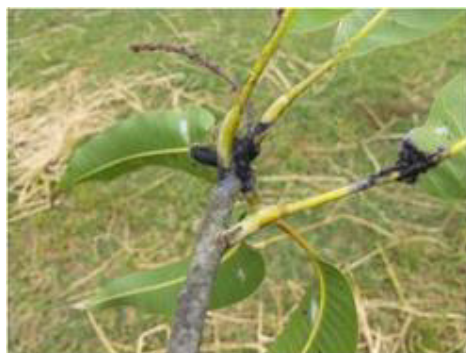
c) On midrib and veins of leaves



c) On midrib and veins of leaves



d) Close view of black velvety symptoms



e) On leaf petiole



f) Microscopic view of velvety growth

**Plate 2: Symptoms of black banded disease of mango**

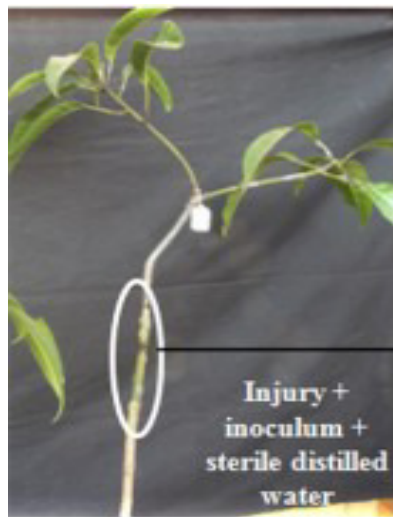


**Control 1: Injury + media + sterile distilled water**



**Control 2: Sterile distilled water**

b



**Inoculated plant (Injury + inoculum + sterile distilled water)**



**Initial stage of symptom expression**

**Plate 3: Proving pathogenicity**

pathogenic culture thus obtained was compared with the original culture of *P. corticolum*.

#### 4.1.4 Identification of the pathogen

Identification of the fungus was carried out based on the morphological characters of the isolated fungus. The fungus in the present study produced septate mycelium. Isolated fungal culture was white. After twenty days, white colour changed to brown and subsequently black colour. Fungal hypha was white in early days later it changed to brown colour (Plate 4).

### 4.2 Morphological, Cultural and Biochemical studies of the pathogen

#### 4.2.1 Morphological characters

##### 4.2.1.1 Spore morphology

In the present study, conidia of *P. corticolum* obtained from infected bark were measured and compared with respect to their spore morphology. The conidia were single-celled, pale brown, globose, smooth-walled and measured 11.25 - 17.36  $\mu$  in diameter. Further conidia were transferred to water agar and germination of conidia was observed (Plate 4).

##### 4.2.1.2 Mycelium morphology

Mycelium of *P. corticolum* obtained from infected bark and culture was septate, brown in colour and measured 3.2 - 6.82  $\mu$  in width (Plate 4).

#### 4.2.2 Cultural characters

##### 4.2.2.1 Cultural characteristics of *P. corticolum* on different solid media

Diversity in cultural and morphological characters of *P. corticolum* were studied in five non synthetic / semi synthetic and two synthetic media at room temperature  $27 \pm 1^\circ\text{C}$  as described in "Material and Methods" and the results obtained are presented in Table 2; Fig. 7 and Plate 5.



Culture up-to 20 days



Culture after 20 days



Spores



Mycelium



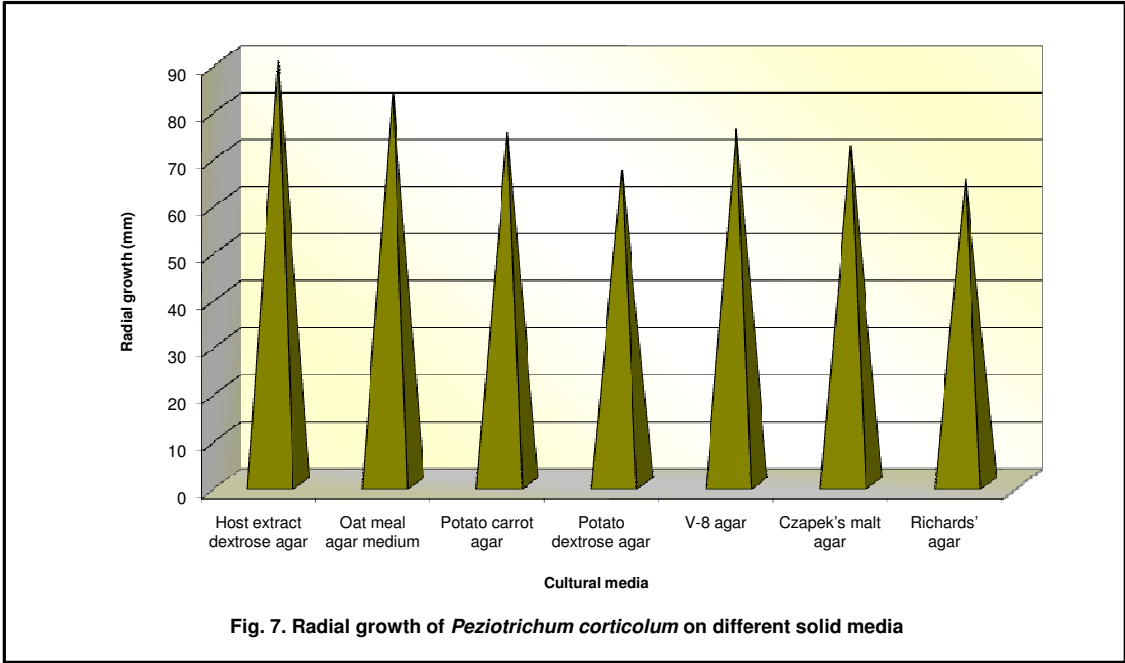
Germinated spores in agar plate

Plate 4: Culture and morphology of *Peziotrichum corticolum*

**Table 2. Cultural and morphological characters of *Peziotrichum corticolum* on different solid media**

Sl. No.	Different Media	Colony characters				
		Radial growth (mm) <sup>#</sup> (12 DAI)	Colour	Type of growth	Pigmentation	Margin
1.	Host extract dextrose agar	90.0	White	Flat growth circular	White	Sparse Smooth
2.	Oat meal agar medium	83.3	Grayish off white	Flat growth irregular	Gray	Smooth
3.	Potato carrot agar	74.7	White	Flat growth circular	Gray	Medium Smooth
4.	Potato dextrose agar	66.7	White	Flat growth circular	White	Smooth
5.	V-8 agar	75.3	Yellowish White	Flat growth circular	Yellow	Sparse Smooth
6.	Czapek's malt agar	72.0	White	Flat growth irregular	White	Irregular
7.	Richards' agar	64.7	White	Sparse irregular	Yellow	Sparse Irregular
S.Em.±		0.56				
CD at 1%		2.37				

# Mean of three replications  
DAI= Days after incubation





- |                               |                         |
|-------------------------------|-------------------------|
| 1. Host extract dextrose agar | 2. Potato dextrose agar |
| 3. Czapeck's malt agar        | 4. Richards' agar       |
| 5. Oat meal agar              | 6. V-8 agar             |
| 7. Potato carrot agar         |                         |

**Plate 5: Growth of *Pezizotrichum corticolum* on different solid media after 12 days**

The radial growth, colony characters and sporulation of the fungi were recorded, when the maximum growth was attained on any one of the tested media. The effect of different culture media on the growth of fungi differed significantly. Maximum radial growth of *P. corticolum* was recorded on host extract dextrose agar (90.00 mm), which was found to be significantly superior to all other media followed by oat meal agar (83.3 mm), V-8 agar (75.3 mm), potato carrot agar (74.70 mm), Czapek's malt agar (72.00 mm) and potato dextrose agar (66.7 mm). The least radial growth was recorded in Richards' agar (64.7 mm). Potato dextrose agar (66.7 mm) and Richards' agar (64.7 mm) were on par with each other. Further same on par results were found for V-8 agar (75.3 mm) and potato carrot agar (74.70 mm). The non synthetic / semi synthetic media recorded maximum growth compared to synthetic media.

Mycelium colour varied from white to light gray. The growth varied from flat to sparse. Pigmentation in the media also varied from brownish yellow to gray. Spores were not produced in any media used for study.

#### 4.2.2.2 Cultural characteristics of *P. corticolum* on different liquid media

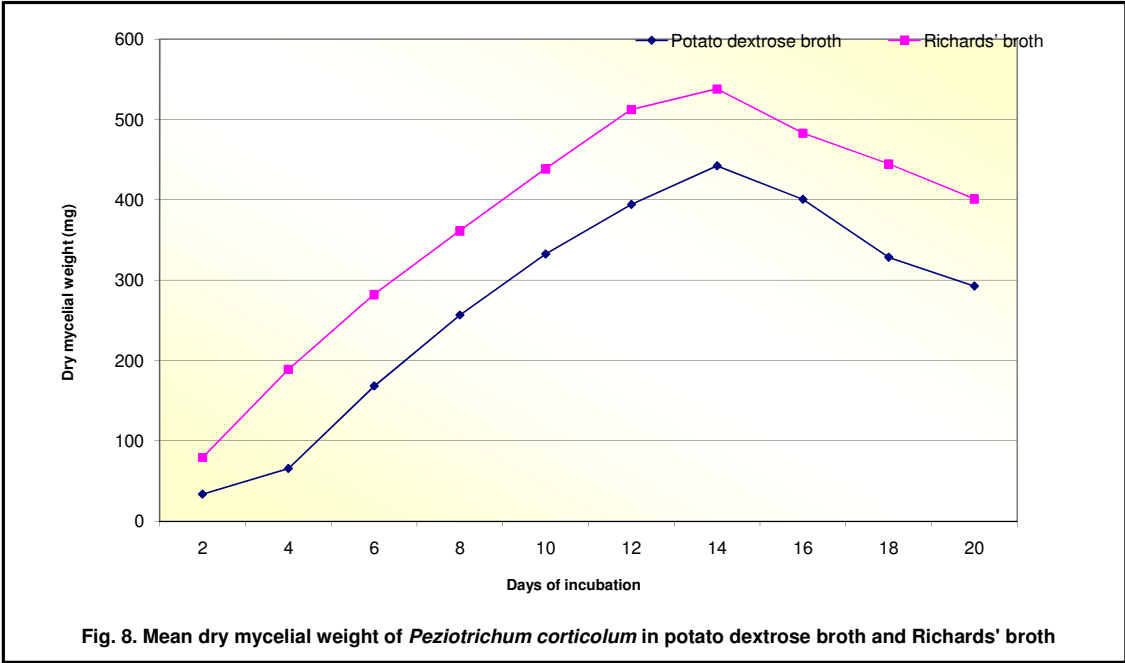
The experiment was conducted as mentioned in "Material and Methods" to ascertain the period for the maximum growth of the fungus by dry mycelial weight method using two liquid media viz., potato dextrose broth and Richards' broth, starting from the 2<sup>nd</sup> day to 20<sup>th</sup> day. The results obtained are presented in the Table 3 and Fig. 8.

It is evident from the data that there were significant differences in the different incubation periods. Dry mycelial weight of *P. corticolum* recorded gradual increase in potato dextrose broth and Richards' broth, starting from second day (33.67 mg and 79.33 mg respectively) and reached peak growth on 14<sup>th</sup> day (442.33 mg and 538.00 mg respectively) and remained significantly superior to remaining treatments. Later the dry mycelial weight declined to reach 292.67 mg and 401.00 mg

Table 3. Growth phase of *Peziotrichum corticolum* in liquid media

Days after incubation (DAI)	Dry mycelial weight (mg) <sup>#</sup>		Mean
	Potato dextrose broth	Richards' broth	
2	33.67	79.33	56.50
4	65.67	189.00	127.33
6	168.33	282.00	225.17
8	256.67	361.33	309.00
10	332.67	438.67	385.67
12	394.33	512.33	453.33
14	442.33	538.00	490.17
16	400.67	483.00	441.83
18	328.67	444.67	386.67
20	292.67	401.00	346.83
Mean	271.57	372.93	
Source	DAI (A)	Broth (B)	A × B
S.Em ±	1.63	0.73	2.30
CD at 1%	6.23	2.79	8.82

<sup>#</sup> Mean of three replications



respectively on 20<sup>th</sup> day of incubation. Mean dry mycelial weight of *P. corticolum* recorded maximum (490.17 mg) on 14<sup>th</sup> day of incubation. The dry mycelial weight on 12<sup>th</sup> and 16<sup>th</sup> day in potato dextrose broth remained on par with dry mycelial weight on 20<sup>th</sup> day in Richards' broth. Similarly on par results were recorded on 14<sup>th</sup> day in potato dextrose broth and 10<sup>th</sup> and 18<sup>th</sup> day of incubation in Richards' broth. **4.2.3 Biochemical studies**

#### 4.2.3.1 Total sugar

The total sugar recorded in different genotypes of mango under the influence of *P. corticolum* is presented in Table 4 and Fig. 9. The differences due to genotypes were found significant.

The results revealed that there was a decrease in total sugar content from healthy to diseased bark and differed significantly among genotypes, type of bark (healthy and diseased) and their interaction.

The maximum mean total sugar was recorded (1.55 mg/g fresh weight) in Alphonso and minimum (1.36 mg/g fresh weight) in Mallika. Alphonso recorded maximum total sugar content in healthy (1.74 mg/g fresh weight) and Alphonso together with Ratna recorded maximum total sugar content in diseased (1.36 mg/g fresh weight) condition and there was significant difference over other genotypes.

Mallika recorded least total sugar content in both cases, healthy (1.62 mg/g fresh weight) and diseased (1.1 mg/g fresh weight) condition.

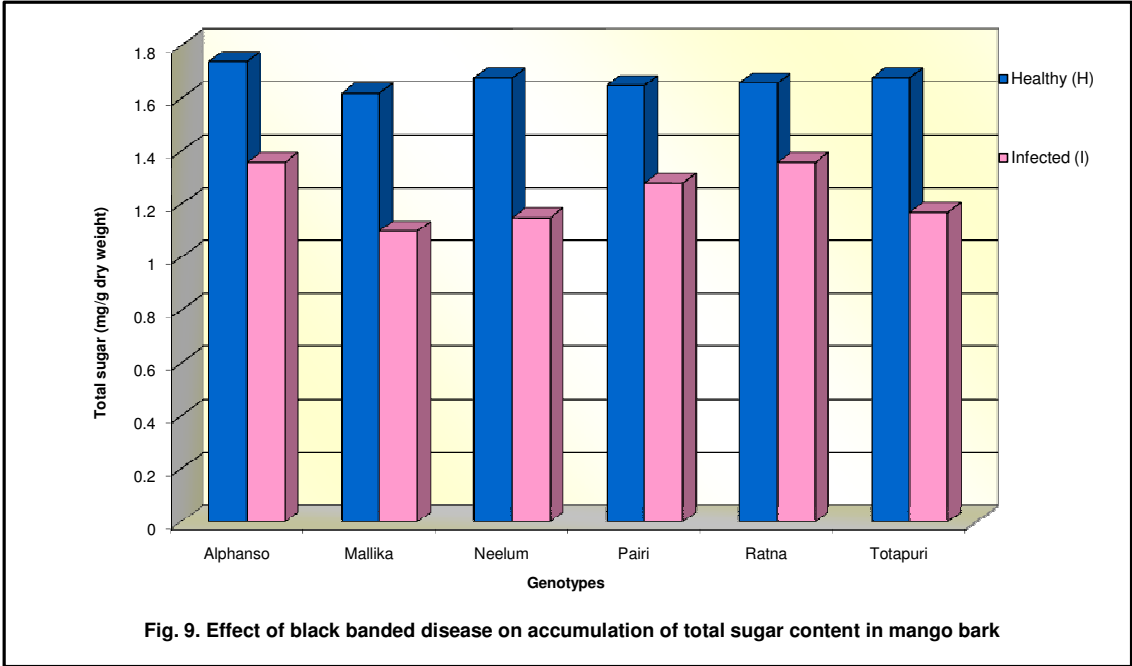
Mallika recorded maximum per cent decrease of total sugar content (32.10%) whereas Ratna recorded least per cent decrease of total sugar content (18.07%).

#### 4.2.3.2 Reducing sugar

The reducing sugar recorded in different genotypes of mango under the influence of *P. corticolum* is presented in Table 5 and Fig. 10.

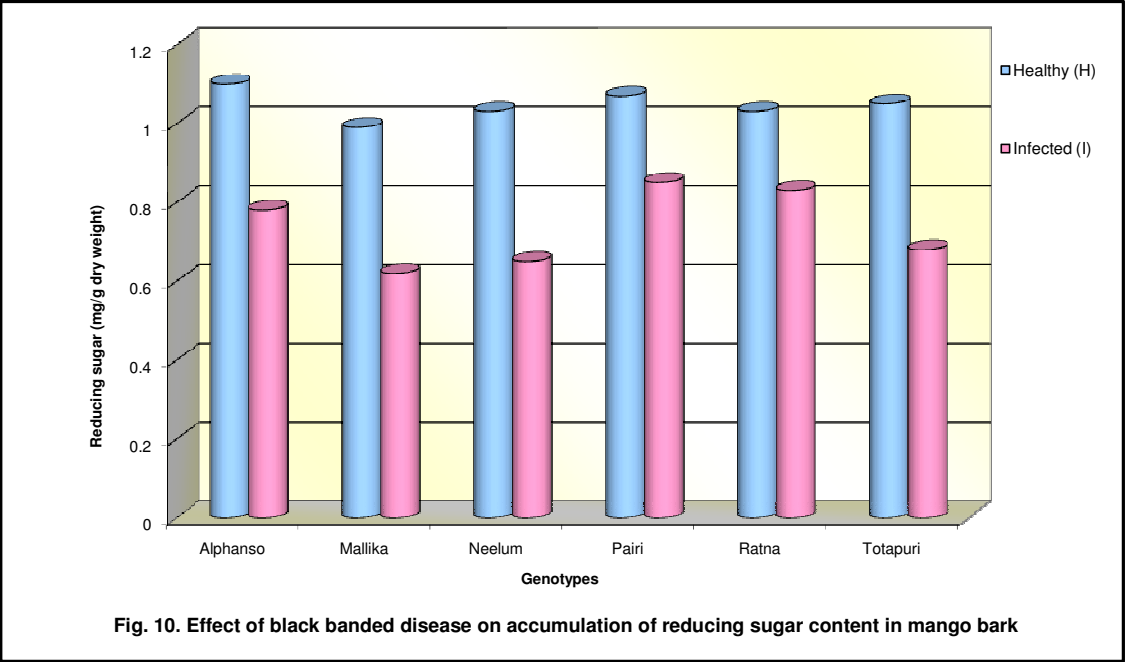
**Table 4. Effect of black banded disease on accumulation of total sugar content in mango bark**

Sl. No.	Genotypes	Total sugar (mg/g dry weight)			
		Healthy (H)	Infected (I)	Mean	Per cent decrease over healthy
1.	Alphonso	1.74	1.36	1.55	21.84
2.	Mallika	1.62	1.10	1.36	32.10
3.	Neelum	1.68	1.15	1.42	31.55
4.	Pairi	1.65	1.28	1.47	22.42
5.	Ratna	1.66	1.36	1.51	18.07
6.	Totapuri	1.68	1.17	1.43	30.36
Mean		1.67	1.24	1.45	26.06
Source		Genotypes (G)	Treatment (T)		G × T
S.Em±		0.02	0.01		0.02
CD at 1%		0.07	0.04		0.10



**Table 5. Effect of black banded disease on accumulation of reducing sugars in mango bark**

Sl. No.	Genotypes	Reducing sugar (mg/g dry weight)			
		Healthy (H)	Infected (I)	Mean	Per cent decrease over healthy
1.	Alphonso	1.10	0.78	0.94	29.09
2.	Mallika	0.99	0.62	0.81	37.37
3.	Neelum	1.03	0.65	0.84	36.89
4.	Pairi	1.07	0.85	0.96	20.56
5.	Ratna	1.03	0.83	0.93	19.42
6.	Totapuri	1.05	0.68	0.87	35.24
	Mean	1.05	0.74	0.89	29.76
	Source	Genotypes (G)	Treatment(T)	G × T	
	S.Em±	0.03	0.02	0.04	
	CD at 1%	0.12	0.07	0.17	



The differences due to genotypes were found significant. The results revealed that there was a decrease in reducing sugar content from healthy to diseased bark and differed significantly among genotypes, type of bark (healthy and diseased) and their interaction.

The maximum mean reducing sugar was recorded (0.96 mg/g fresh weight) in Pairi and minimum (0.81 mg/g fresh weight) in Mallika. Alphonso recorded maximum reducing sugar content in healthy (1.1 mg/g fresh weight) and Pairi recorded maximum reducing sugar content in diseased (0.85 mg/g fresh weight) condition and there was significant difference over other genotypes.

Mallika recorded least reducing sugar content in both healthy (0.99 mg/g fresh weight) and diseased (0.62 mg/g fresh weight) condition.

Mallika recorded maximum per cent decrease of reducing sugar content (37.37%) whereas Ratna recorded least per cent increase of reducing sugar content (19.42%).

#### 4.2.3.3 Non-reducing sugar

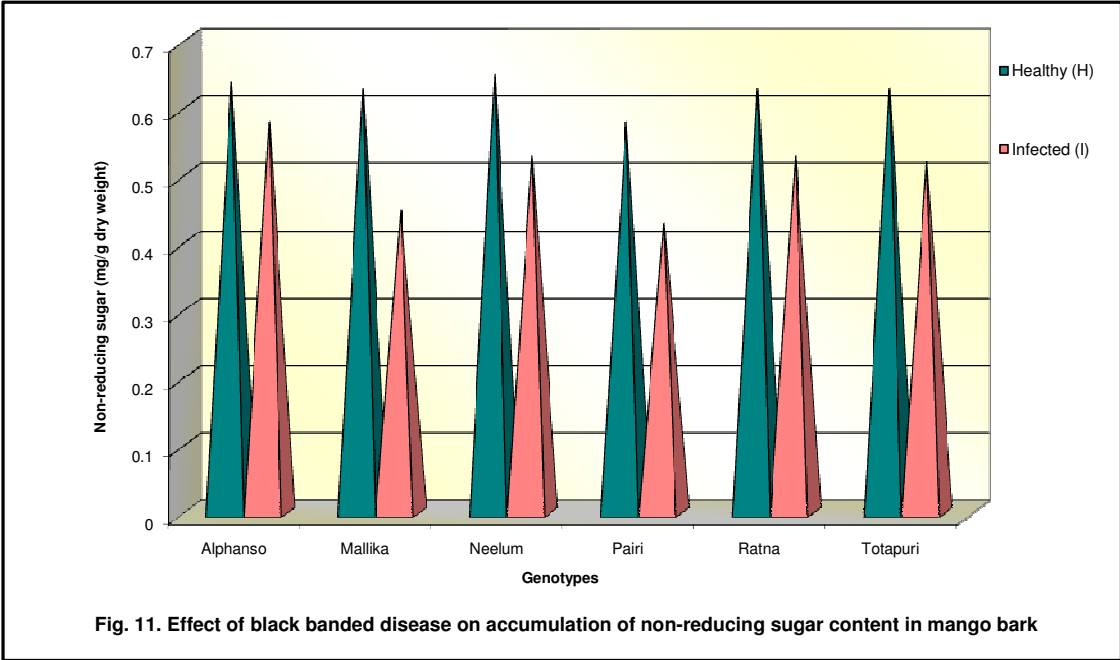
The non-reducing sugar content recorded in different genotypes of mango under the influence of *P. corticolum* is presented in Table 6 and Fig. 11. The differences due to genotypes were found significant.

The results revealed that there was a decrease in non-reducing sugar content from healthy to diseased bark and differed significantly among genotypes, type of bark (healthy and diseased) and their interaction.

The maximum mean non reducing sugar was recorded (0.61 mg/g fresh weight) in Alphonso and minimum (0.51 mg/g fresh weight) in Pairi. Neelum recorded maximum non-reducing sugar content in healthy (0.65 mg/g fresh weight) and Alphonso recorded maximum non-reducing sugar content in diseased (0.58 mg/g fresh weight) condition and there was significant difference over other genotypes.

**Table 6. Effect of black banded disease on accumulation of non-reducing sugar content in mango bark**

Sl. No.	Genotypes	Non-reducing sugar (mg/g dry weight)			
		Healthy (H)	Infected (I)	Mean	Per cent decrease over healthy
1.	Alphonso	0.64	0.58	0.61	9.38
2.	Mallika	0.63	0.45	0.54	28.57
3.	Neelum	0.65	0.53	0.59	18.46
4.	Pairi	0.58	0.43	0.51	25.86
5.	Ratna	0.63	0.53	0.58	15.87
6.	Totapuri	0.63	0.52	0.58	17.46
	Mean	0.63	0.51	0.57	19.27
	Source	Genotypes (G)	Treatment (T)	G × T	
	S.Em±	0.01	0.01	0.02	
	CD at 1%	0.05	0.03	0.07	



Pairi recorded least non-reducing sugar content in both healthy (0.57 mg/g fresh weight) and diseased (0.43 mg/g fresh weight) condition.

Mallika recorded maximum per cent decrease of non-reducing sugar content (28.57%) whereas Alphonso recorded least per cent increase of non-reducing sugar content (9.38%).

#### 4.2.3.4 Total Phenol

The total phenol recorded in different genotypes of mango under the influence of *P. corticolum* is presented in Table 7 and Fig. 12. The differences due to genotypes were found significant.

The results revealed that there was an increase in total phenol content from healthy to diseased bark and differed significantly among genotypes, type of bark (healthy and diseased) and their interaction.

The maximum mean total phenol was recorded (1.95 mg/g fresh weight) in Mallika and minimum (1.51 mg/g fresh weight) in Ratna. Mallika recorded maximum total phenol content in both healthy (1.62 mg/g fresh weight) and diseased (2.28 mg/g fresh weight) condition and there was significant difference over other genotypes.

Ratna recorded least total phenol content in both healthy (1.36 mg/g fresh weight) and diseased (1.65 mg/g fresh weight) condition.

Mallika recorded maximum per cent increase of total phenol content (40.74%) whereas Alphonso recorded least per cent increase of total phenol content (20.29%).

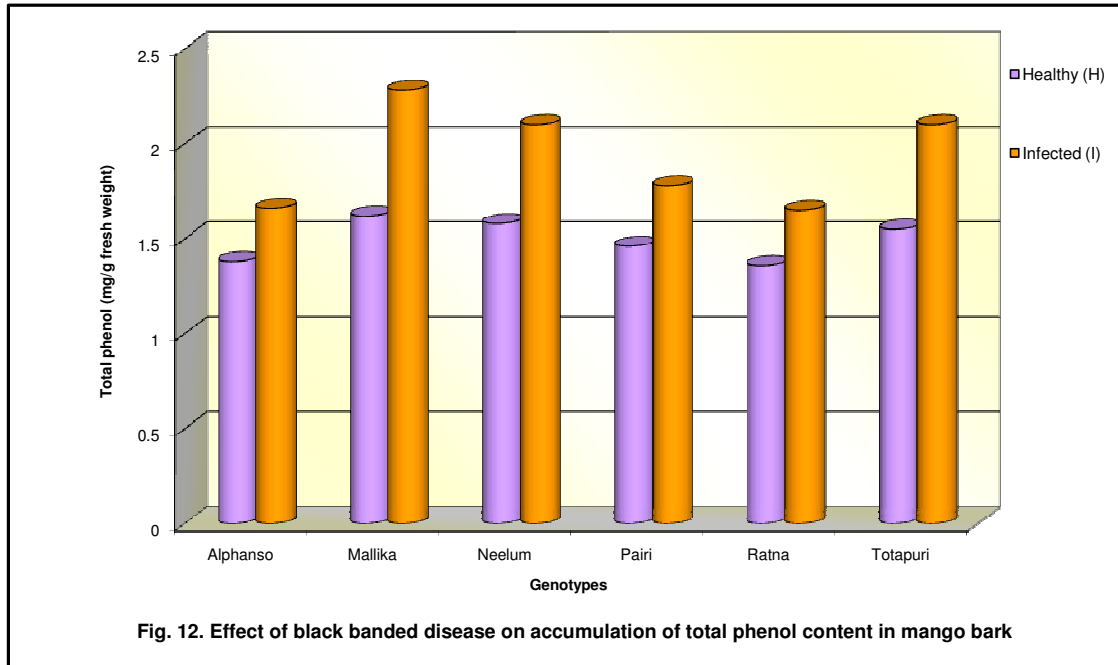
### 4.3 *In vitro* and *In vivo* evaluation of Chemicals and Biorationals

#### 4.3.1 *In vitro* evaluation of fungicides against *P. corticolum*

Screening of fungicides was done against *P. corticolum* under laboratory condition by following poisoned food technique as described in "Material and Methods". Three systemic, five non-systemic fungicides and three combi products

**Table 7. Effect of black banded disease on accumulation of total phenol content in mango bark**

Sl. No.	Genotypes	Total phenol (mg/g fresh weight)			
		Healthy (H)	Infected (I)	Mean	Per cent increase over healthy
1.	Alphonso	1.38	1.66	1.52	20.29
2.	Mallika	1.62	2.28	1.95	40.74
3.	Neelum	1.58	2.10	1.84	32.91
4.	Pairi	1.46	1.78	1.62	21.92
5.	Ratna	1.36	1.65	1.51	21.32
6.	Totapuri	1.55	2.10	1.83	35.48
	Mean	1.49	1.93	1.71	28.78
	Source	Genotypes (G)	Treatment(T)	G × T	
	S.Em±	0.04	0.02	0.06	
	CD at 1%	0.17	0.10	0.23	



were evaluated against *P. corticolum* in laboratory at three, four and four concentrations respectively by poisoned food technique.

#### 4.3.1.1 *In vitro* evaluation of non systemic and combi fungicides against *P. corticolum*

The results with respect to inhibition of mycelial growth of *P. corticolum* at four concentrations of five non-systemic fungicides and three combi products were recorded and presented in Table 8; Fig. 13, Plate 6 and Plate 7.

Data from the table revealed that, the efficacy of different non-systemic fungicides, concentrations and their interaction on per cent inhibition of mycelial growth of *P. corticolum* differed significantly.

Among all five non-systemic fungicides, maximum mean per cent inhibition (100%) of *P. corticolum* was recorded for Bordeaux mixture, Copper oxychloride and Mancozeb which were significantly superior to all other fungicides followed by Cuprous hydroxide (96.39%). Least mean per cent inhibition was noticed in Chlorothalonil (73.92%). Bordeaux mixture, Copper oxychloride and Mancozeb recorded 100% inhibition of mycelial growth irrespective of concentration. For Cuprous hydroxide maximum per cent inhibition of mycelial growth (100%) was recorded at 0.25 and 0.3 per cent concentration. Chlorothalonil showed maximum per cent inhibition of mycelial growth (84.44%) at 0.3 per cent concentration.

At 0.3 per cent concentration, Copper oxychloride, Cuprous hydroxide and Mancozeb recorded highest per cent inhibition of mycelial growth (100%) of fungus which was significantly superior to other fungicides followed by Chlorothalonil (84.44%) which showed least inhibition of mycelial growth of fungus.

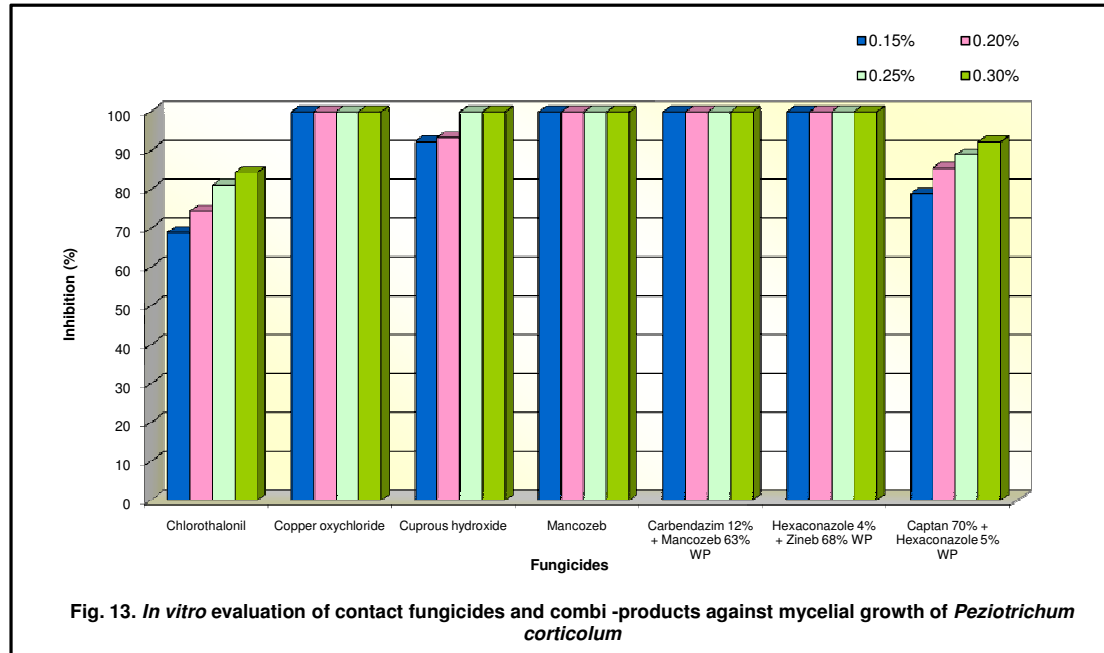
At 0.15 per cent concentration, maximum per cent inhibition of mycelial growth (100%) of the fungus was recorded in Copper oxychloride and Mancozeb. The least per cent inhibition (68.89%) of fungus was in Chlorothalonil.

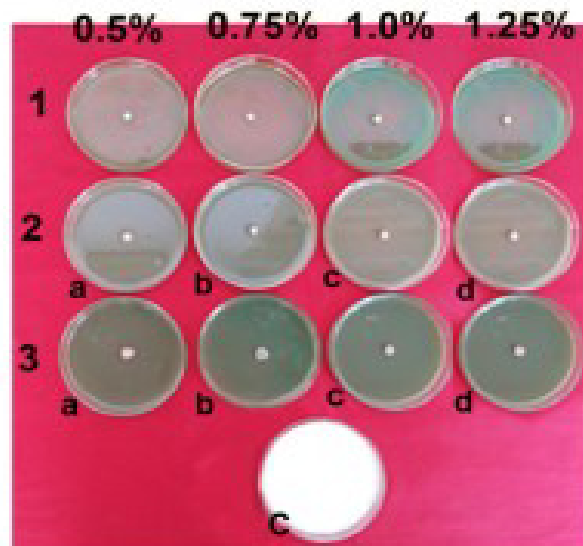
**Table 8. *In vitro* evaluation of contact fungicides and combi -products against mycelial growth of *Peziotrichum corticolum***

Common name	Trade name	Inhibition (%)				
		Concentrations (%)				Mean
		0.15	0.20	0.25	0.30	
<b>Contact fungicides</b>						
Chlorothalonil	Kavach 75% WP	68.89 <sup>#</sup> (56.08) <sup>*</sup>	74.44 (59.61)	81.11 (64.21)	84.44 (66.74)	73.92 (61.66)
Copper oxychloride	Blitox 50% WP	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
Cuprous hydroxide	Kocide 77% WP	92.22 (73.78)	93.33 (75.01)	100.00 (89.96)	100.00 (89.96)	96.39 (82.18)
Mancozeb	Indofil M-45 75% WP	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
<b>Combiproducs</b>						
Carbendazim 12% + Mancozeb 63% WP	Saaf 75% WP	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
Hexaconazole 4% + Zineb 68% WP	Avatar 72% WP	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
Captan 70% + Hexaconazole 5% WP	Taqat 75% WP	78.89 (62.23)	85.56 (67.67)	88.89 (70.50)	92.22 (73.80)	86.39 (68.65)
Source		Fungicides (F)		Concentration (C)	F × C	
S.Em. ±		0.13		0.10	0.26	
CD at 1%		0.49		0.37	0.98	
Bordeaux mixture	-	Concentrations (%)				Mean
		0.50	0.75	1.00	1.25	
		100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)

\* = Arcsine values

# = Mean of three replications

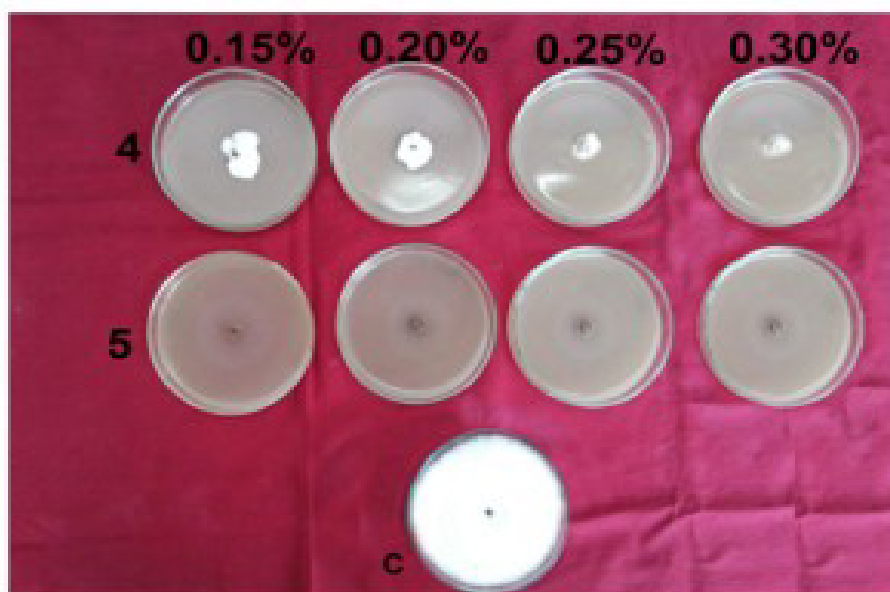




1 - Bordeaux mixture  
2 - Copper oxychloride  
3 - Cuprous hydroxide

a - 0.15%  
b - 0.20%  
c - 0.25%  
d - 0.30%

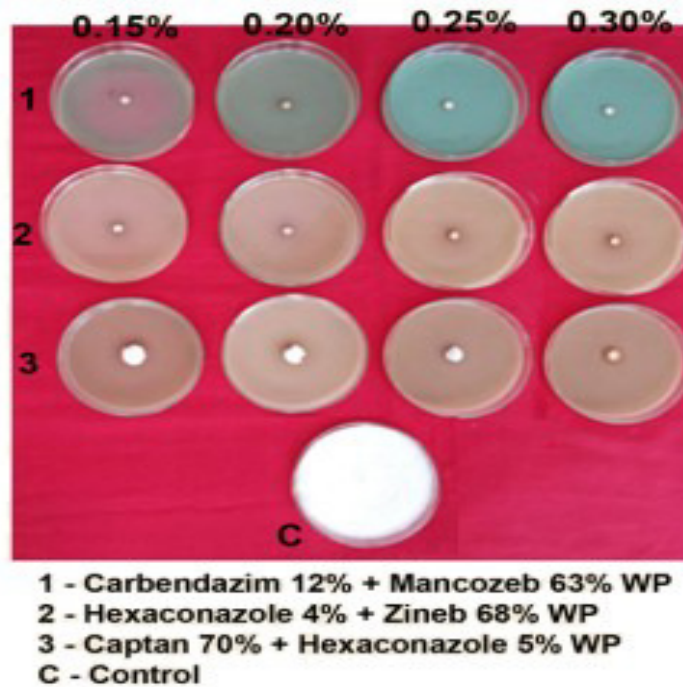
C - Control



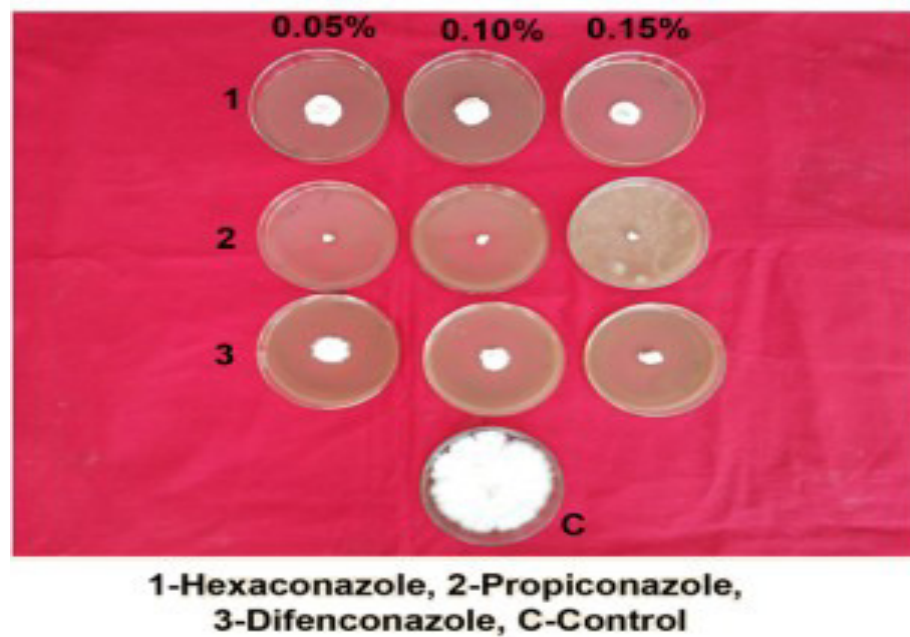
4 - Chlorothalonil  
5 - Mancozeb

C-Control

Plate 6: *In vitro* evaluation of Non systemic fungicides against *Pezizotrichum corticolum*



**Plate 7: *In vitro* evaluation of Combi products against *Peziotrichum corticolum***



**Plate 8: *In vitro* evaluation of systemic fungicides against *Peziotrichum corticolum***

Among the three combi products, Carbendazim + Mancozeb, Hexaconazole + Zineb recorded cent per cent inhibition (100%) which were significantly superior to all other fungicides followed by Captan + Hexaconazole (86.39%) which was least among combi products.

Carbendazim + Mancozeb and Hexaconazole + Zineb recorded cent per cent inhibition of mycelial growth irrespective of concentration. Captan + Hexaconazole recorded (78.89%) inhibition of mycelia growth at 0.15 per cent concentration and (92.22%) inhibition at 0.3 per cent concentration.

#### 4.3.1.2 *In vitro* evaluation of systemic fungicides against *P. corticolum*

Data with respect to inhibition of mycelial growth of *P. corticolum* at three concentrations of three systemic fungicides were recorded and presented in Table 9; Fig. 14 and Plate 8.

It was observed that, fungicides, concentrations and their interaction differed significantly with respect to inhibition of the mycelial growth of *P. corticolum*.

Among three systemic fungicides, maximum mean per cent inhibition of growth of *P. corticolum* was observed in Propiconazole (95.56%) which was significantly superior to all other fungicides followed by Difenconazole (80.74%). The least mean per cent inhibition of fungus was recorded in Hexaconazole (74.07%).

Among the tested three concentrations, 0.15 per cent concentration of all fungicides was significantly found superior to 0.1 and 0.05 per cent.

At 0.05 per cent concentration, maximum per cent inhibition of mycelial growth (93.33%) of the fungus was recorded in Propiconazole, followed by Difenconazole (72.22%). The least per cent inhibition of fungus was recorded in Hexaconazole (68.89%).

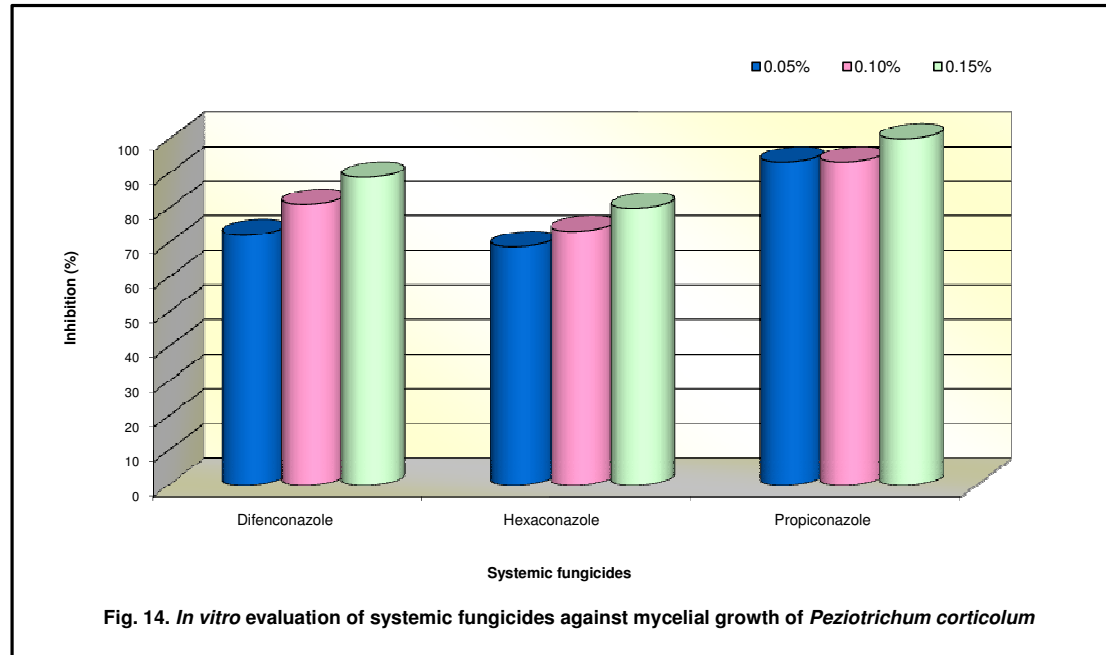
At 0.1 per cent concentration, maximum per cent inhibition of mycelial growth (93.33%) of the fungus was recorded in Propiconazole, followed by Difenconazole

**Table 9. *In vitro* evaluation of systemic fungicides against mycelial growth of *Peziotrichum corticolum***

Common name	Trade name	Inhibition (%)			
		Concentration (%)			Mean
		0.05	0.10	0.15	
Difenconazole	Score 25% EC	72.22 <sup>#</sup> (58.17) <sup>*</sup>	81.11 (64.21)	88.89 (70.50)	80.74 (64.29)
Hexaconazole	Contaf 5% EC	68.89 (56.08)	73.33 (58.89)	80.00 (63.41)	74.07 (59.46)
Propiconazole	Tilt 25% EC	93.33 (75.01)	93.33 (75.01)	100.00 (89.96)	95.56 (79.99)
Source		S.Em $\pm$		CD at 1%	
Fungicides (F)		0.39		1.59	
Concentration (C)		0.39		1.59	
F $\times$ C		0.68		2.76	

<sup>\*</sup>=Arcsine values

<sup>#</sup>=Mean of five replications



(81.11%). The least per cent inhibition of fungus was recorded in Hexaconazole (73.33%).

At 0.15 per cent concentration, maximum per cent inhibition of mycelial growth (100%) of the fungus was recorded in Propiconazole, followed by Difenconazole (88.89%). The least per cent inhibition of fungus was recorded in Hexaconazole (80%).

At all the tested concentrations among systemic fungicides, Propiconazole recorded highest inhibition of mycelia growth.

Mycelial inhibition of *P. corticolum* by Propiconazole at 0.05 per cent (93.33%) and 0.1 per cent (93.33%) remained on par with each other. Further mycelial inhibition by Hexaconazole at 0.1 per cent concentration (73.33%) remained on par with Difenconazole at 0.05 per cent (72.22%). Further mycelial inhibition by Hexaconazole at 0.15 per cent concentration (80%) remained on par with Difenconazole at 0.1 per cent (81.11%).

#### 4.3.2 *In vitro* evaluation of botanicals against *P. corticolum*

An experiment was conducted to assess the antifungal activity of five plant extracts as described under “Material and Methods” and the results are presented in Table 10; Fig. 15 and Plate 9.

The effect of plant extracts on the per cent inhibition of mycelial growth of *P. corticolum* at three concentrations differed significantly.

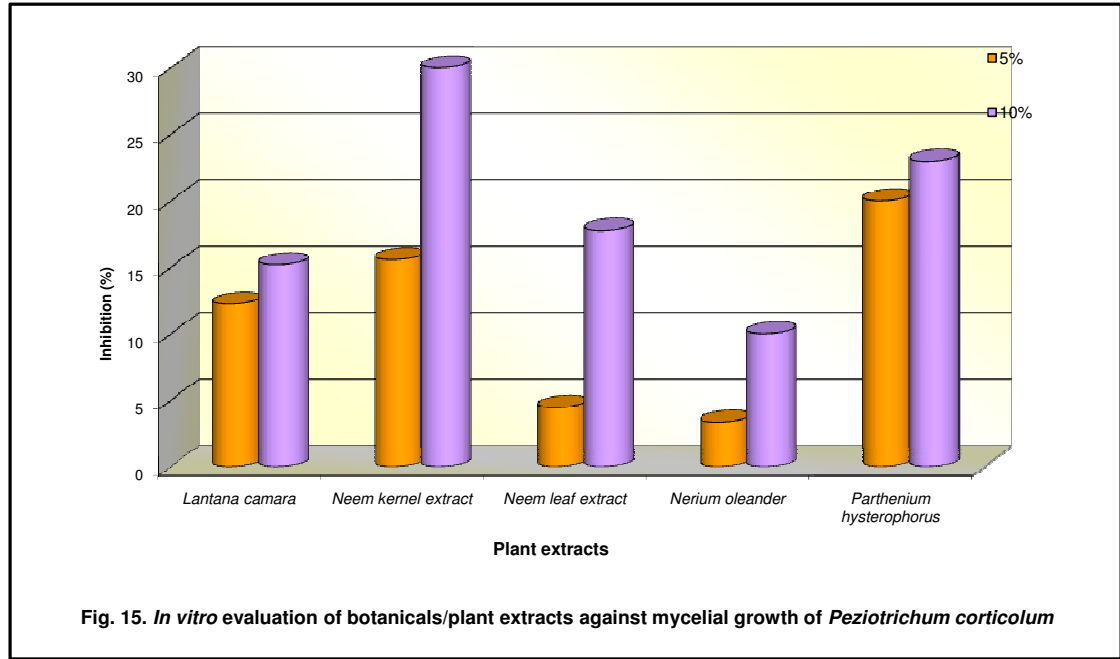
Among the five plant extracts, maximum mean per cent inhibition of mycelial growth (22.78%) was recorded in neem kernel extract which was significantly superior to other all treatments tested, followed by *Parthenium* leaf extract (21.48%), *Lantana camara* leaf extract (13.70%), neem leaf extract (11.11%). Least inhibition was recorded in *Nerium* leaf extract (0.56%).

**Table 10. *In vitro* evaluation of botanicals/plant extracts against mycelial growth of *Peziotrichum corticolum***

Sl. No.	Name of plant	Plant part used	Per cent Inhibition		
			Concentration (%)		Mean
			5	10	
1.	<i>Lantana camara</i>	Leaf	12.22 <sup>#</sup> (20.44) <sup>*</sup>	15.19 (22.90)	13.70 (21.67)
2.	Neem kernel extract	Kernel	15.56 (23.21)	30.00 (33.20)	22.78 (28.20)
3.	Neem leaf extract	Leaf	4.44 (12.10)	17.78 (24.92)	11.11 (18.51)
4.	<i>Nerium oleander</i>	Leaf	3.33 (10.06)	10.00 (18.36)	6.67 (14.21)
5.	<i>Parthenium hysterophorus</i>	Leaf	20.00 (26.55)	22.96 (28.61)	21.48 (27.58)
Mean			11.11 (18.47)	19.19 (25.60)	15.15 (22.04)
Source			Botanicals (B)	Concentration (C)	B × C
SEm±			0.69	0.43	0.97
CD at 1%			2.76	1.75	3.90

<sup>#</sup> = Mean of five replications

<sup>\*</sup> = Arcsine transformed values





1. *Bacillus subtilis*      2. *Pseudomonas fluorescens*  
 3. *Verticillium lecanii*    4. *Trichoderma harzianum*  
 C - Control

Plate 9: *In vitro* evaluation of plant extracts against *Pezizotrichum corticolum*



1. *Lantana camara*      2. *Parthenium hysterophorus*  
 3. *Nerium oleander*    4. Neem kernel extract  
 5. Neem leaf extract    C- Control

Plate 10: *In vitro* evaluation of bioagents against *Pezizotrichum corticolum*

At 10 per cent concentration of plant extracts, maximum of 30 per cent inhibition of mycelial growth was recorded in neem kernel extract followed by *Parthenium* leaf extract (22.96%), neem leaf extract (17.78%), *Lantana camara* leaf extract (15.19%). Least inhibition was recorded in *Nerium* leaf extract (10%).

At 5 per cent concentration of plant extracts, maximum of 20 per cent inhibition of mycelial growth was recorded in *Parthenium* leaf extract followed by neem kernel extract (15.56%), *Lantana camara* leaf extract (12.22%) and neem leaf extract (4.44%). Least inhibition was recorded in *Nerium* leaf extract (3.33%).

Mycelial inhibition of *P. corticolum* by *Nerium* leaf extract at 5 per cent (3.33%) remained on par with neem leaf extract at 5 per cent (4.44%). Further at 10 per cent, mycelial inhibition by *Lantana camara* leaf extract (15.19%) remained on par with neem leaf extract (17.78%). Further mycelial inhibition by neem kernel extract at 5 per cent (15.56%) remained on par with *Lantana camara* leaf extract at 10 per cent (15.19%).

#### 4.3.3 *In vitro* evaluation of bioagents against *P. corticolum*

Efficacy of bacterial and fungal bioagents was studied under *in vitro* condition by following dual culture method as described in “Material and Methods” and the results are presented in Table 11, Fig. 16 and Plate 10.

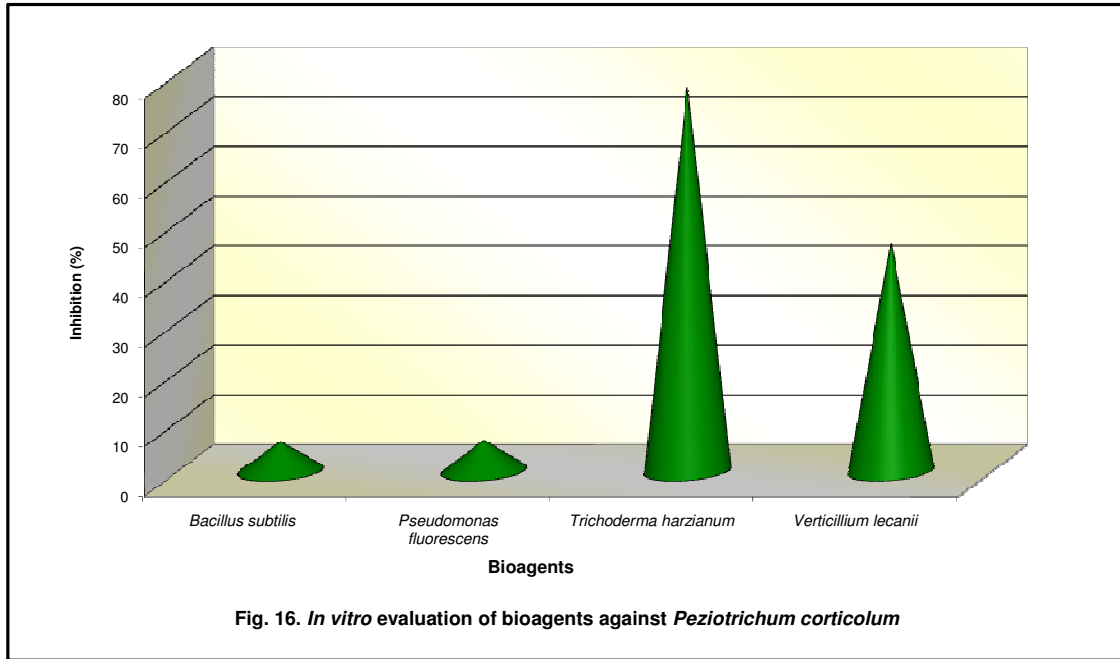
There were significant differences among all the tested bioagents. *Trichoderma harzianum* (77.22%) was found to be significantly superior in inhibiting the mycelial growth of *P. corticolum* followed by *Verticillium lecanii* (45.83%) and *Pseudomonas fluorescens* (6.11%). The least inhibition of mycelial growth of *P. corticolum* was recorded in *Bacillus subtilis* (5.83%). Further *Pseudomonas fluorescens* and *Bacillus subtilis* remained on par with each other.

**Table 11. *In vitro* evaluation of bioagents against *Pezizotrichum corticolum***

Sl. No.	Bioagent	Per cent inhibition
1.	<i>Bacillus subtilis</i>	5.83 <sup>#</sup> (13.82) <sup>*</sup>
2.	<i>Pseudomonas fluorescens</i>	6.11 (14.19)
3.	<i>Trichoderma harzianum</i>	77.22 (61.48)
4.	<i>Verticillium lecanii</i>	45.83 (42.58)
S.Em±		1.03
C.D at 1%		4.29

<sup>#</sup> Mean of five replications

<sup>\*</sup> Arcsine transformed values



#### 4.3.4 *In vivo* evaluation of fungicides and bioagent against black banded disease of mango during 2014-15

This study was undertaken to evaluate the relative efficacy of different fungicides and bioagent for management of black banded disease of mango during 2014-15 (Plate 11).

The experiment was conducted at Main Agricultural Research Station, UAS Dharwad, with nine treatments and one untreated control as described in "Material and Methods". Totally three sprays were given at an interval of 10 days. The observation on black banded disease was recorded at 10 days interval. Further these observations were converted into per cent disease severity. The per cent disease reduction over control was worked out. Statistically analyzed data are presented in the Table 12 and Fig. 17 and Plate 12.

Results of the experiment revealed that per cent disease severity reduction over control was maximum for Bordeaux paste (80.60%), followed by Bordeaux mixture (20.70%), Copper oxychloride (17.93%), Carbendazim 12% + Mancozeb 63% WP (10.93%), Hexaconazole 4% + Zineb 68% WP (8.12%), Propiconazole (5.58%) and Difenconazole (1.87%). Least per cent disease severity reduction over control (0.00%) was recorded for neem kernel extract and *Trichoderma harzianum*.

#### 4.4 Screening of genotypes under natural conditions for disease resistance

Twelve genotypes were subjected to screening under natural condition as described in "Material and Methods" and results are presented in Table 13 and Fig. 18.

No genotype was found to be immune, resistant and moderately resistant. Among all genotypes under study, Mallika showed comparatively the least susceptibility against *P. corticolum* followed by Neelum.



**Plate 11: Orchard view**

**Table 12. *In vivo* evaluation of fungicides and bioagent (after seven days of third spray) against black banded disease of mango in orchard during 2014-15**

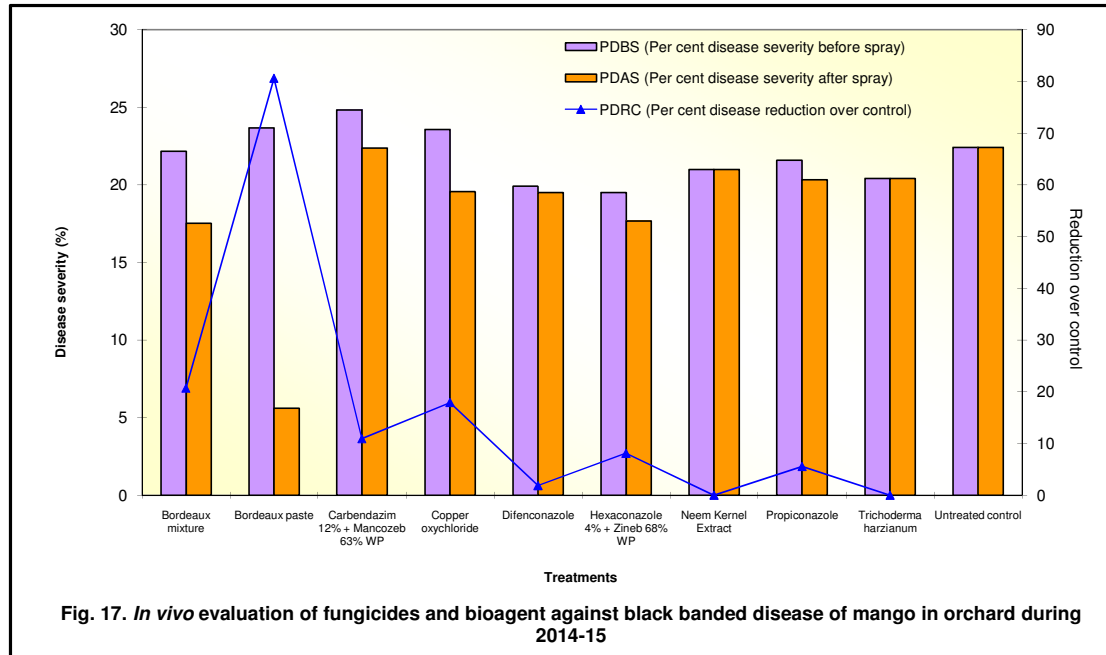
Treatments		Concentration (%)	Disease severity (%)		
			Before spray (PDBS)	After spray (PDAS)	Reduction over control (PDRC)
T1	Bordeaux mixture	1.0	22.17 (28.08)*	17.53 (24.74)	20.70 (27.05)
T2	Bordeaux paste	10.0	23.67 (29.10)	5.60 (13.68)	80.60 (63.84)
T3	Carbendazim 12% + Mancozeb 63% WP	0.3	24.83 (29.88)	22.38 (28.22)	10.93 (19.30)
T4	Copper oxychloride	0.3	23.58 (29.04)	19.56 (26.24)	17.93 (25.04)
T5	Difenconazole	0.1	19.92 (26.50)	19.50 (26.19)	1.87 (7.86)
T6	Hexaconazole 4% + Zineb 68% WP	0.3	19.50 (26.19)	17.68 (24.85)	8.12 (16.55)
T7	Neem Kernel Extract	10.0	21.00 (27.26)	21.00 (27.26)	0.00 (0.00)
T8	Propiconazole	0.1	21.58 (27.67)	20.33 (26.79)	5.58 (13.66)
T9	<i>Trichoderma harzianum</i>	1.0	20.42 (26.85)	20.42 (26.85)	0.00 (0.00)
T10	Untreated control	-	22.42 (28.25)	22.42 (28.25)	-
SEm±			0.35	0.57	-
CD at 5%			1.06	1.72	-

\* Arcsine transformed values

PDRC= Per cent Disease Reduction over Control

PDBS= Per cent Disease Severity before Spray

PDAS= Per cent Disease Severity after Spray





**Infected branches before rubbing with gunny cloth**



**Infected branches after rubbing with gunny cloth**



**Treated branches (after rubbing with gunny cloth) with Bordeaux paste**



**After seven days of treatment : No sign of disease symptoms from the infected branches**

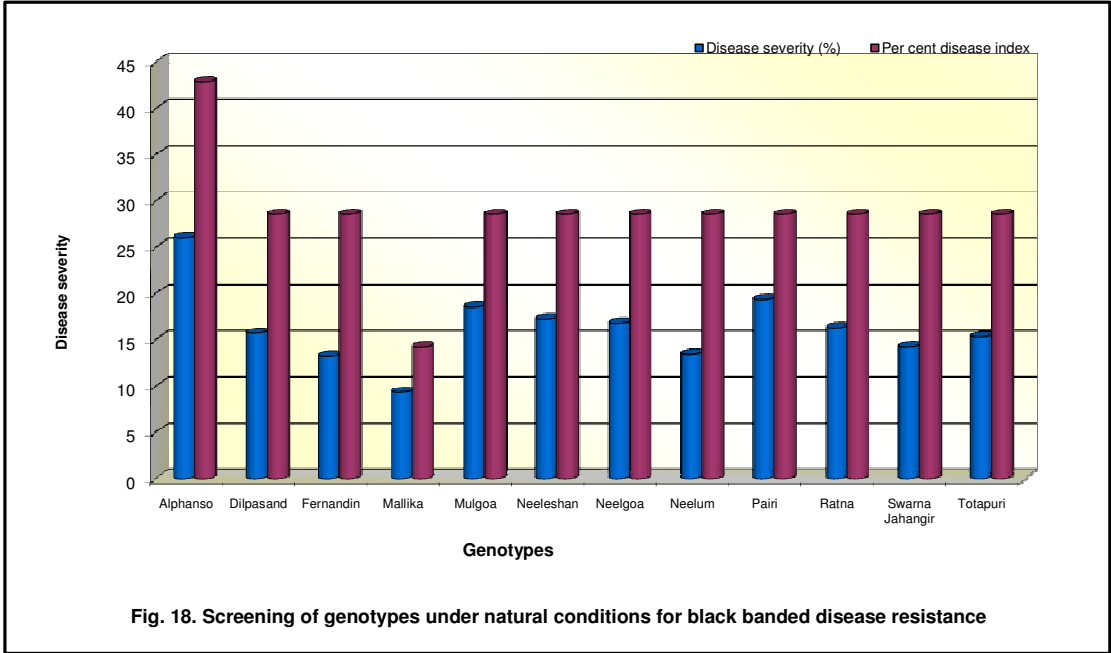


**Control (Rubbing with gunny cloth + water spray)**

**Plate 12: Management of black banded disease**

**Table 13. Screening of genotypes under natural conditions for black banded disease resistance**

Sl. No.	Genotypes	Disease severity (%)	Per cent disease index
1.	Alphonso	26.01	42.87
2.	Dilpasand	15.75	28.58
3.	Fernandin	13.22	28.58
4.	Mallika	09.35	14.29
5.	Mulgoa	18.6	28.58
6.	Neeleshan	17.25	28.58
7.	Neelgoa	16.82	28.58
8.	Neelum	13.42	28.58
9.	Pairi	19.36	28.58
10.	Ratna	16.35	28.58
11.	Swarna Jahangir	14.3	28.58
12.	Totapuri	15.32	28.58



## 5. DISCUSSION

Mango is regarded as “king of fruits” because of its captivating flavor, excellent taste, attractive fragrance, irresistible sweetness and beautiful shades of colour both inside and outside of the fruit. It is the national fruit of India, Pakistan and Philippines.

Although mango is considered to be a hardy tree it is susceptible to various diseases, insect pests and physiological disorders. Among the various fungal diseases, black banded disease caused by *Peziotrichum corticolum* (Massee) Subramanian has assumed to be one of the serious problems in northern Karnataka. The disease was first time reported by Massee (1901) from Poona, India. The pathogen grows superficially on the bark of trees forming large, dark black, irregular, girdle-like infection patches and hence the name. The disease is also noticed on the midrib and veins of leaves.

In recent years the disease has become more severe in northern Karnataka. Therefore, investigations were carried out on various aspects of the pathogen with respect to symptomatology, severity of disease in different locations, morphological, cultural aspects of pathogen, biochemical changes in infected plants, evaluation of fungicides, bioagents and botanicals against the disease in both laboratory and orchard and screening of genotypes under natural condition for disease resistance for developing an effective disease management approach. The results so obtained are discussed here under.

### 5.1 Survey of the disease in North Karnataka

Survey on the incidence and severity of black banded disease of mango revealed the magnitude of the problem on hand and served as a precursor for evolving the management strategies.

The work was initiated on survey to know the severity and distribution of the disease in major mango growing areas of Dharwad, Haveri, Belagavi and Gadag districts of northern Karnataka.

Survey and surveillance form the basis for any successful plant protection that depends on early detection of the disease followed by timely adoption of control measures. Hence, in the present investigation, roving survey was undertaken for one year in major mango growing areas of northern Karnataka to assess the incidence and severity of black banded disease. During the survey, it was generally observed that, disease incidence on new branches was more than old branches of trees in most of the areas surveyed. Likewise, disease incidence was less in the orchard with wide spacing as it helps in reducing relative humidity.

The survey also supplements the information about intensity and existence of biotypes in particular geographical locations. The severity of black banded disease of mango expressed as per cent disease severity. The survey also revealed that the severity of black banded disease was depended on location, age of the plant, variety and various environmental factors like temperature, relative humidity, pattern of rainfall and also existence of variability in the pathogen.

From the results of survey, it was observed that, disease incidence ranged from zero per cent to 100 per cent. Young plants (upto 5 years) were found to be disease free and incidence was more on old aged trees. This may be due to lack of initial inoculum in case of young plants. Patil and Dangat (2011) reported disease incidence ranged from 28.5 to 100 per cent in Kolhapur district of Maharashtra.

Among all the districts, the maximum per cent disease severity was recorded in Dharwad (17.29%), which may be due to susceptibility of cultivars and favourable environmental condition *viz.*, optimum temperature and relative humidity, moisture conditions that must have favoured for the build up of inoculum and subsequently

showed increased disease severity. On the contrary, lowest per cent disease severity was recorded from Gadag (11.69%) district. This may be due to unfavourable environmental condition which reduced the buildup of inoculum thus reduced the severity. In general, the disease incidence and severity vary from season to season in different agro-climatic zones and varieties, which may be due to variation in pathogen, host varieties and or climatic conditions.

Among the varieties, Alphonso was found more susceptible with more mean per cent disease severity (17.43%). This disease was recorded from all the varieties irrespective of location. The present findings are in conformity with the work of Patil and Dangat (2011), who reported medium to high disease severity on all varieties and hybrids which were grown in Kolhapur district of Maharashtra.

### 5.1.1 Symptomatology

The typical black banded symptoms were observed on twigs, branches, leaf petiole, midrib and veins of leaves. Symptoms were rarely produced on main trunk of the tree.

On branches and twigs, symptoms are characterized by typical black, irregular, superficial velvety fungal growth. The size of fungal growth increases with the advancement of the disease which results in large, black coloured, girdle like velvety bands. Velvety appearance of bands is due to fungal mycelium which aggregate together and erect, perpendicular to bark. The young spreading mycelium near the periphery of the infection bands is white or nearly hyaline. In infected trees, symptoms are more conspicuous on young branches and twigs than on old branches. The velvety mycelial growth drops off in the summer months leaving light black coloured, bands in the affected portions

On leaves, black velvety fungal growth can be seen on midrib and veins. Girdling of petioles by black velvety fungal growth is also common.

Similar descriptions of the symptoms of black banded disease of mango were given by previous workers, Reddy *et al.* (1961); Prakash and Srivastava (1987); Mukherjee and Litz (2009) and Patil and Dangat (2012).

### 5.1.2 Collection and isolation

The field survey of disease conducted during 2014-15 in northern districts of Karnataka showed diverse incidence of black banded disease in different locations. Affected disease samples, showing typical black banded symptoms were collected from different locations during survey. Upon tissue isolation the pathogen was brought into pure culture and identified as *Peziotrichum corticolum* (Masse) Subramanian based on morphology and pathogenicity in accordance with the description given by Subramanian (1956); Mukherjee and Litz (2009) and Patil and Dangat (2012).

### 5.1.3 Pathogenicity

Pathogenicity test was proved following Koch's postulates. As this is the first attempt towards proving pathogenicity against *P. corticolum*, no supporting reference is available for conformity or for comparison.

### 5.1.4 Identification

The study on morphological characteristics of *Peziotrichum corticolum* indicated, septate hyphae measuring 3.2 - 6.82 $\mu$  in width, brown in colour. Conidia were single celled, pale brown, globose, smooth-walled; 11.25 - 17.36  $\mu$  in diameter. The findings are in agreement with the findings of earlier workers (Subramanian, 1956; Mukherjee and Litz, 2009; Patil and Dangat, 2012). Based on the mycelial and spore morphology, the fungus under study was identified as *Peziotrichum corticolum* (Masse) Subramanian.

## 5.2 Cultural studies

Fungi secure food and energy from the substrate upon which they live in nature. In order to culture the fungus in the laboratory it is necessary to furnish those essential elements and compounds in the medium for their growth and other life processes. Neither all media are equally good for all fungi, nor there any universal substrate or artificial medium upon which all fungi can grow. So, different media including, non synthetic, semi-synthetic and synthetic were tried for *P. corticolum*.

### 5.2.1 Cultural characteristics of *P. corticolum* on different solid media

Among the non or semi-synthetic and synthetic media used for growth and sporulation of *P. corticolum*, maximum radial growth was recorded on host extract dextrose agar (90.00 mm) and least radial growth was recorded in Richards's agar (64.7 mm). Sporulation was not observed in any media, used for study. Maximum radial growth of the fungus on host extract dextrose agar may be due to host's cell constituents in the media, supporting good fungal growth. Further Naqvi, (2004) reported maximum radial growth of *Pestalotiopsis mangiferae* causing grey blight of mango on host extract agar.

### 5.2.2 Cultural characteristics of *P. corticolum* on different liquid media

Mean dry mycelial weight of *P. corticolum* was recorded maximum (490.17 mg) on 14<sup>th</sup> day of incubation. Maximum dry mycelial weight of *P. corticolum* was recorded on 14<sup>th</sup> day in both liquid media namely potato dextrose broth and Richard's broth (442.33 mg and 538.00 mg respectively). Later the dry mycelial weight declined to reach 292.67 mg and 401.00 mg respectively on 20<sup>th</sup> day of incubation which may have been due to autolysis of the mycelium, accumulation of toxins and exhaustion of nutrients in the medium after incubation for optimum number of days.

Vinod and Benagi (2009) also reported autolysis of the fungus *Colletotrichum gloeosporioides* after optimum days of incubation. Further Ashoka (2005) reported

that dry mycelial weight of *Colletotrichum gloeosporioides* was obtained more in Richards' broth (394.00 mg) than potato dextrose broth (378.00 mg).

## 5.3 Biochemical studies

### 5.3.1 Sugar content

Sugars act as precursor for synthesis of phenolics, phytoalexins, lignin and cellulose which play an important role in defense mechanism of plants against invading pathogens.

In present study, lower levels of sugars were observed in diseased plants in all the six genotypes. Mean total sugar was recorded maximum (1.55 mg/g fresh weight) in Alphonso and minimum (1.36 mg/g fresh weight) in Mallika. Whereas, mean reducing sugar was recorded maximum (0.96 mg/g fresh weight) in Pairi and minimum (0.81 mg/g fresh weight) in Mallika.

By these results, it can be concluded that Mallika is more resistant when compared to Alphonso and other genotypes. The primary metabolites include carbohydrates and proteins, which are exploited by the pathogen for their growth and development. Therefore the level of total sugars and reducing sugar was decreased in case of diseased bark of mango. These primary metabolites also function as precursor for secondary metabolites, which play major role in plant defense. Therefore, in case of resistant varieties low total and reducing sugar was observed due to their role in the synthesis of secondary metabolites. Contrary to this, genotypes in which higher level of sugars was recorded can be considered as susceptible. Further, result of the experiment was supported by Patil and Dangat (2011).

### 5.3.2 Total phenol

Phenols have been found to play an important role in determining resistance or susceptibility of a host to parasitic infection. High concentration causes an instant lethal action by a general tanning effect while, low concentration causes gradual

effect on the cellular constituent of the parasite. If the concentration does not occur at toxic level, the inhibition will be obviously slow. Besides, the pathogen readily detoxifies low concentrations of the toxicants rather than high concentrations (Dasgupta, 1988).

There is significant positive correlation between total phenol content and disease resistance. In present study, lower levels of phenols were observed in healthy plants in all the six genotypes. Mean total phenol was recorded maximum (1.95 mg/g fresh weight) in Mallika and minimum (1.51 mg/g fresh weight) in Ratna. Mallika recorded maximum per cent increase of total phenol content (40.74%) whereas Alphonso recorded least per cent increase of total phenol content (20.29%).

Per cent disease severity was found to be less in Mallika in the present investigation. This indicates that total phenol content in the bark imparts resistance against the disease. Result of the experiment was further supported by Patil and Dangat (2011). The marked increase in phenolic content might be due to accumulation of phenolics from surrounding healthy tissues (Kiralý and Farkas, 1962 and Jayapal and Mahadevan, 1968).

## 5.4 *In vitro* and *in vivo* evaluation of chemicals and biorationals

### 5.4.1 *In vitro* evaluation of fungicides against *P. corticolum*

In the absence of resistant cultivars, use of fungicides to manage the disease is an old-age practice. When there is outbreak of epidemic for any reason perhaps use of fungicides is one of the best options available. These fungicides have to be used judiciously according to the need and kind of organism involved. Availability of new fungicides necessitates evaluation of fungicides under *in vitro* conditions to know their efficacy, and initiate spray schedule in field conditions.

*In vitro* evaluation of new synthetic molecules of fungicides is very much necessary before they are tried under field condition. Among all three groups of fungicides, non-systemic fungicides and combi products were found to be more effective than systemic fungicides. Three non systemic fungicides namely Bordeaux mixture, Copper oxychloride, Mancozeb and two combi products namely Carbendazim + Mancozeb and Hexaconazole + Zineb recorded cent per cent inhibition of mycelial growth of *P. corticolum*. Least mean per cent inhibition was noticed in Chlorothalonil (73.92%).

Among three systemic fungicides, maximum mean per cent inhibition of growth of *P. corticolum* was observed in Propiconazole (95.56%) and least mean per cent inhibition of fungus was recorded in Hexaconazole (74.07%).

At higher concentration most of the fungicides inhibited maximum mycelial growth but decreased with reduced concentration. Naik and Hiremath (1986) reported the complete inhibition of mycelial growth of *Colletotrichum gloeosporioides* with Copper oxychloride (0.1, 0.2 and 0.3%). Ashoka (2005) reported that Carbendazim + Mancozeb recorded cent per cent inhibition of mycelial growth of *Colletotrichum gloeosporioides*. Contrary to it, Venkataravanappa and Nargund (2002) reported tricyclazole as very effective under laboratory condition against *Colletotrichum gloeosporioides* among all the tested fungicides.

#### 5.4.2 *In vitro* evaluation of botanicals against *P. corticolum*

Extensive use of fungicides has led to various environmental problems, human health and their persistence in the fruits. To sort out these problems botanicals were tested in laboratory against *P. corticolum*.

Continuous use of chemical fungicides in the management of disease also brought new problems with them. Amongst them is pollution of air, water, soil, residual toxicity, development of resistance in the pathogen against chemicals there

by the need to apply them more with their escalating prices and harmful effects on non target organisms. Consequently, fungicides have been alarming the development of harmful environment for human beings. Contrary to the problems associated with use of synthetic chemicals, botanicals are environmentally non-pollutive, renewable, in-exhaustible, indigenously available, thus readily biodegradable relatively cost effective and hence constitute as a suitable plant protection in the strategy of integrated disease management. Hence, screening of plant products for its effective antifungal activity against the pathogen is essentially required to minimize the use of fungicides and considered as one of the components in the integrated disease management (Khadar, 1999 and Nagesh, 2000, Jayalakshmi, 2010).

At 10 per cent concentration of plant extracts, maximum of 30 per cent inhibition of mycelial growth was recorded in neem kernel extract and least inhibition was recorded in *Nerium* leaf extract (10%).

At 5 per cent concentration of plant extracts, maximum of 20 per cent inhibition of mycelial growth was recorded in *Parthenium* leaf extract followed by neem kernel extract (15.56%) and least inhibition was recorded in *Nerium* leaf extract (3.33%).

The effectiveness of Neem kernel extract as a pesticide may be due to phyto-constituents like alkaloids, glycosides, flavonoids and saponins which are antimicrobial in nature. Further, Shivapuri *et al.* (1997) noticed *Azadirachta indica*, as more fungitoxic among ten plant extracts.

#### 5.4.3 *In vitro* evaluation of bioagents against *P. corticolum*

Regular use of synthetic chemicals / fungicides in mango has been a norm in many orchards that brings with it many hazards such as pesticide residue, development of resistant strains among the pathogen, ecological consideration, etc.

Use of bioagents, now a days, is best and has been most emphasized and widely accepted practice as it is environmentally safe and can overcome the residual problems associated with heavy use of fungicides for management disease. Hence, the present investigation was taken up to screen the bioagents for effective management of black banded disease of mango.

Among the bioagents tried during present investigation *Trichoderma harzianum* was found to be best in inhibiting mycelial growth of *P. corticolum* (77.22%) followed by *Verticillium lecanii* (45.83%) and *Pseudomonas fluorescens* (6.11%) and least per cent inhibition of mycelial growth was observed in *Bacillus subtilis* (5.83%).

Present studies recorded significant mycoparasitism of *Trichoderma harzianum* on *P. corticolum* that caused lysis of the hyphae *in vitro*. Result of the experiment was further supported by Watve *et al.* (2009) according to them, maximum inhibition of *Colletotrichum gloeosporioides* causing leaf spot disease of jatropha was achieved by *Trichoderma harzianum*.

#### 5.4.4 *In vivo* evaluation of fungicides and bioagent against black banded disease of mango during 2014-15

By utilizing the *in vitro* information generated on bioassay of fungicides and bioagent, a field experiment was planned and executed during 2014-15 at Main Agricultural Research Station, UAS, Dharwad. Seven different fungicides (three non-systemic, two combi products and two systemic), one plant extract (Neem kernel extract) and one bioagent (*Trichoderma harzianum*) with untreated control were evaluated for their efficacy to control the black banded disease of mango.

Results of the experiment revealed that per cent disease severity reduction over control was maximum for Bordeaux paste (80.60%), followed by Bordeaux mixture (20.70%), Copper oxychloride (17.93%), Carbendazim 12% + Mancozeb 63% WP (10.93%),

Hexaconazole 4% + Zineb 68% WP (8.12%), Propiconazole (5.58%) and Difenconazole (1.87%). Least per cent disease severity reduction over control (0.00%) was recorded for neem kernel extract and *Trichoderma harzianum*.

Bordeaux mixture and other chemicals which were applied as spray were less effective than Bordeaux paste. It may be due to less persistence nature of sprayed chemical on the bark of the mango tree. Whereas Bordeaux paste persists more on the bark, thus more effective than sprayed chemicals.

Narasimhudu *et al.* (1987) reported that Bavistin at 0.1 % concentration gave good control. Bavistin was not used in present study. Patil and Dangat (2012) reported that Bordeaux mixture was showing promising results but Sulphur was more effective than Bordeaux mixture.

In present study Bordeaux paste gave best results. Even after six months of the treatment, there was no new fungal growth.

## 5.5 Screening of genotypes under natural conditions for disease resistance

The management of disease through host plant resistance has been an important choice in all crop improvement programme. Utilization of resistance is most simple, effective and economical method in the management of biotic stress. Besides, the resistant cultivars conserve natural resources and reduce the cost, time and energy when compared to other methods of disease management. Use of fungicides as paste or spray is not feasible for the farmers to control the black banded disease of mango and fungicides also have disadvantage of their persistence and development of new strains of pathogen. To avoid this situation, identifying the resistant cultivars against black banded disease is most significant one.

The screening of 12 genotypes of mango under natural conditions was done at UAS, Dharwad and results on per cent disease severity and PDI are presented in Table 13 and Fig. 18. The severity of disease (diseased area) is more reliable

compound to PDI, because PDI is same for different genotypes inspite of, genotypes differing in per cent disease severity (Table 13). The reason for this is, PDI is calculated based on grade/ scale which involve range of per cent disease severity into groups. This leads to incomplete conclusion. Hence, it is preferred to use the per cent disease severity which accounts for actual data from the tree.

In present study, no genotype was found to be immune, resistant and moderately resistant. Mallika showed comparatively the least susceptible reaction against *P. corticolum* followed by Neelum. Contrary to it, Reddy *et al.* (1961) reported that Neelum was highly susceptible to *P. corticolum*. This may be attributed to different agroclimatic conditions and variation in pathogen due to difference in locality.

### Future line of work

1. There is a need to find out conditions and nutrition requirements for sporulation *in vitro*
2. There is a need to study epidemiology of the disease
3. There is a need to study variability in pathogen
4. To find more feasible ways to manage the disease
5. To search for resistant genotypes with good orchard management practices.

## 6. SUMMARY AND CONCLUSION

An investigation on black banded disease of mango caused by *Peziotrichum corticolum* was carried out with reference to survey on the incidence and severity of black banded disease in orchards, morphological, cultural characters of the *P. corticolum*, biochemical changes in plants due to disease, evaluation of fungicides, botanicals and bioagents against the disease both under laboratory and field conditions and screening of genotypes under natural conditions. The results obtained are summarized here under.

An extensive roving survey was carried out in northern parts of Karnataka to assess the severity of black banded disease of mango. This study revealed that highest per cent disease severity was recorded in Dharwad district. Correspondingly, the lowest per cent disease severity was recorded in Gadag district. Among the varieties, Alphonso was found more susceptible and recorded highest per cent disease severity. The maximum disease severity was recorded in 16-20 years old plants.

The colony morphology in general indicated that fungus on potato dextrose agar produced white septate mycelium initially which later changed to brown and subsequently black. On the basis of morphological characters, the fungus was identified as *P. corticolum*. Pathogenicity of the fungus was proved.

The fungus *P. corticolum* exhibited diversified cultural characters on synthetic and semi / non-synthetic solid media. Maximum radial growth of the fungus (90.00 mm) was recorded on host extract dextrose agar whereas growth of the fungus was least on Richards' agar.

The maximum dry mycelial weight of *P. corticolum* was observed on 14<sup>th</sup> day of incubation and this period was considered as optimum period for fungal growth. Dry mycelial weight was more in Richards' broth than potato dextrose broth.

Mean total sugar was recorded maximum (1.55 mg/g fresh weight) in Alphonso and minimum (1.36 mg/g fresh weight) in Mallika.

Mean reducing sugar was recorded maximum (0.96 mg/g fresh weight) in Pairi and minimum (0.81 mg/g fresh weight) in Mallika.

Mean non reducing sugar was recorded maximum (0.61 mg/g fresh weight) in Alphonso and minimum (0.51 mg/g fresh weight) in Pairi.

Mean total phenol was recorded maximum (1.95 mg/g fresh weight) in Mallika and minimum (1.51 mg/g fresh weight) in Ratna.

Among five nonsystemic and three combi-products evaluated *in vitro*, Bordeaux mixture, Copper oxychloride, Mancozeb, Carbendazim + Mancozeb and Hexaconazole + Zineb recorded highest per cent inhibition of mycelial growth (100%). However least per cent inhibition of mycelium (84.44%) was recorded in Chlorothalonil at 0.3 per cent concentration.

In case of systemic fungicides, Propiconazole at 0.15 per cent recorded highest per cent inhibition of mycelium growth (100%) whereas, least per cent inhibition of mycelial growth (80%) was recorded in Hexaconazole at 0.15 per cent concentration.

Among plant extracts, neem kernel extract at 10 per cent concentration showed higher inhibition (30%) of mycelial growth of *P. corticolum* and least inhibition (0.56%) was recorded in Nerium leaf extract at 30 per cent concentration.

Four bioagents were tested against *P. corticolum* by following dual culture technique. Among four bioagents maximum inhibition (77.22%) of mycelial growth of the fungus was recorded in *T. harzianum*. The least inhibition of mycelial growth (5.83%) of the fungus was noticed in *Bacillus subtilis*.

The management of black banded disease of mango using different fungicides and biorationals *in vivo* indicated that Bordeaux paste was significantly effective. Neem kernel extract and *Trichoderma harzianum* were found to be least effective.

Of the twelve mangoes genotypes, screened for their reaction to black banded disease under natural conditions, Mallika showed comparatively low disease prevalence followed by Neelum. No genotype was found to be immune, resistant and moderately resistant.

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# INVESTIGATIONS ON BLACK BANDED DISEASE OF MANGO

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## ABSTRACT

Black banded disease of mango is becoming severe from last three to four years which affects twigs, branches, leaf petiole, midrib and veins of leaves. The severity of black banded disease was more in Dharwad district followed by Belagavi, Haveri and Gadag districts. The identity of the fungus was confirmed as *Peziotrichum corticolum*. The fungus showed maximum radial growth on host extract dextrose agar on 12<sup>th</sup> day after incubation at 27±1°C. Culture of *P. corticolum* exhibited diversity with respect to cultural characters like type of the growth, mycelial colour, pigmentation and colony margin. Maximum dry mycelial weight of the fungus was recorded on 14<sup>th</sup> day after incubation. Dry mycelial weight of the fungus was more in Richards' broth than potato dextrose broth. Total sugar content was maximum in Alphonso and minimum in Mallika. Reducing sugar content was maximum in Pairi and minimum in Mallika. Non reducing sugar content was maximum in Alphonso and minimum in Pairi. Total phenol content was maximum in Mallika and minimum in Ratna. Among the fungicides, bioagents and botanicals tested against the *P. corticolum*, Bordeaux mixture, Copper oxychloride, Mancozeb, Propiconazole, *Trichoderma harzianum* and neem kernel extract were superior, in inhibiting the mycelial growth of the fungus under *in vitro* condition. The bioefficacy of fungicides and bioagent which performed well *in vitro* condition were tested *in vivo* condition as well. Among them, Bordeaux paste at 10 per cent was effective in reducing the per cent disease severity of black banded disease. Mallika showed comparatively low susceptibility against *P. corticolum* under natural condition, followed by Neelum.