ETIOLOGY AND MANAGEMENT OF DIE BACK DISEASE OF MANGO GRAFTS IN NURSERY

By

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(2006 - 11 - 123)

THESIS

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DECLARATION

I, **Sangeetha C** (2006-11-123) hereby declare that this thesis entitled '**Etiology and management of die back disease of mango grafts in nursery**' is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **'Etiology and management of die back disease of mango grafts in nursery'** is a bonafide record of research work done independently by **Ms. Sangeetha C (2006-11-123)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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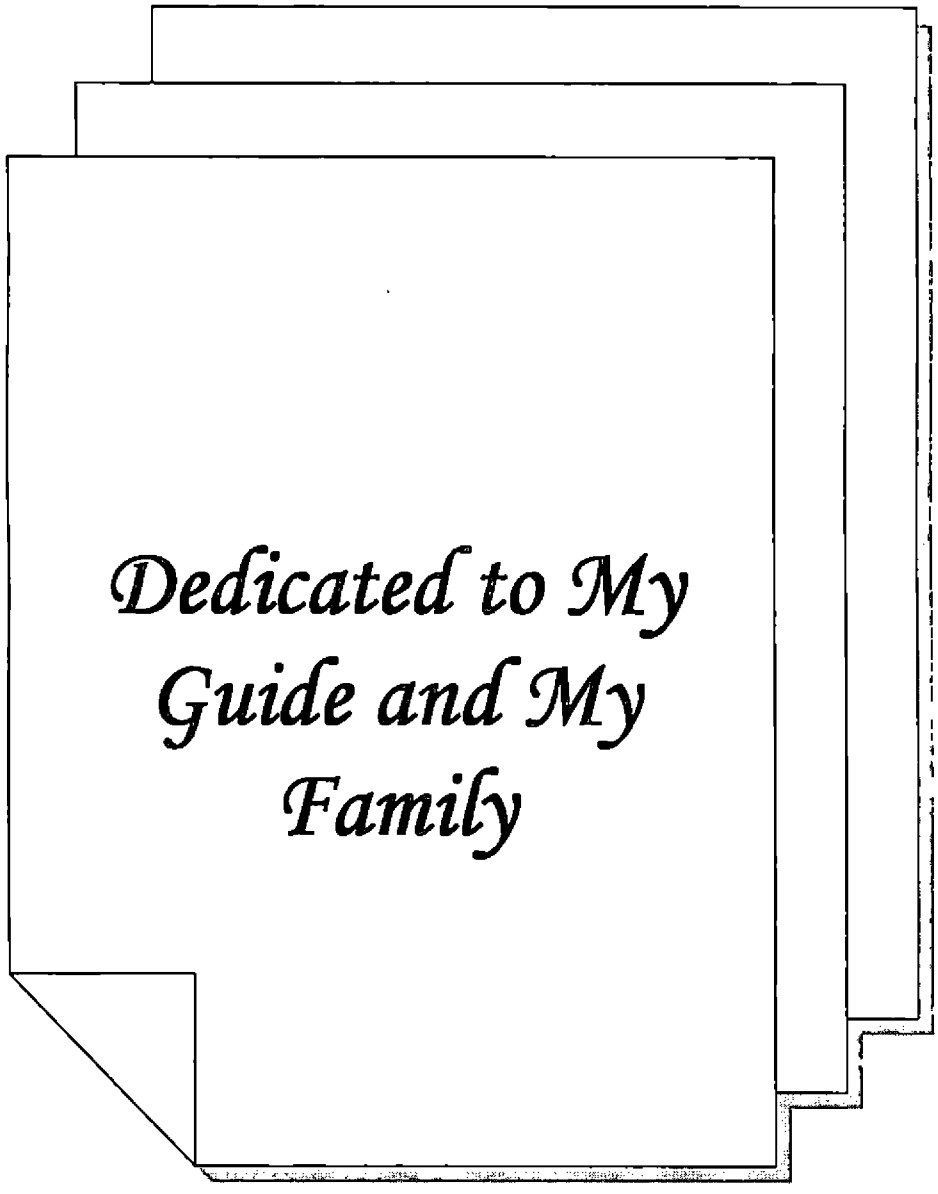
Dedicated to My
Guide and My
Family

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Introduction

1. INTRODUCTION

Mango (*Mangifera indica* L.) belonging to Family Anacardiaceae is one of the important commercially grown fruit crops of the country. It is considered as the King of fruits. A native of southeastern Asia, and cultivated in India for the last 4000years, its cultivation has gradually been extended to other tropical and subtropical countries of the world (Mukherjee, 1953; Popenoe, 1927). It is also grown in about 87 countries but nowhere it is so greatly valued as in India, where it occupies about 70 per cent of the area devoted to fruit crops with a total production of 8.21million tonnes. India has the richest collection of mango cultivars and is the world's largest producer of mango (FAO, 2002). The fruit is very popular with the masses due to its wide range of adaptability, high nutritive value, delicious taste and excellent flavour. It is a rich source of vitamin A and C. Good mango varieties contain 20 per cent total soluble sugars. The acid content of ripe desert fruit varies from 0.2 to 0.5 per cent and protein content is about one per cent.

Mango is not considered as a commercial crop of Kerala, but mango trees are inevitable components of homesteads of the state. The total estimated area under mango cultivation in this state is 75,911 ha with an annual production of 323,517 tonnes. Commercial orchards of mango are being established in Palakkad district, where the climatic conditions are more suitable for this tree. The mango population consists of both seedling and grafted trees but the commercial orchards are of grafted trees only. The cultivated varieties include Alphonso, Bennet Alphonso, Bangalora, Banganapally, Neelum, Kalapady, Guddadat and Priyor.

Mango is affected by a number of diseases at all stages of its development i.e. from nursery to harvest. Die back is one of the important diseases of mango caused by *Colletotrichum gloeosporioides* (Penz.) Sacc and *Botryodiplodia theobromae* Pat

at all stages of its growth (Plate 1). The first report on die back of mango caused by *B. theobromae* in India was given by Gupta and Zachariah (1945). In orchards, both young and old trees are affected by die back disease particularly under conditions of neglect. So it is posing a serious threat to successful cultivation of mango. This disease has been recognized as a major bottleneck in raising the mango nursery due to mass mortality of seedlings. Young grafted plants also suffer from rapid necrosis and drying up of leaves. It is considered as one of the major constraints in mango nurseries. The disease incidence may occur at any time of the year, but it is most conspicuous during October to November. Only limited literature is available on the various aspects of this disease. In this context, the present study is taken up, which is expected to give more informations about die back disease of mango. The research programme entitled "Etiology and management of die back disease of mango grafts in nursery" envisaged the following aspects.

- Isolation and Identification of pathogens from infected mango grafts.
- Cultural and morphological characters of die back pathogens.
- *In vitro* evaluation of fungicides and antagonists against die back pathogens.
- *In planta* evaluation on effect of selected fungicides and antagonists against die back disease.
- Screening of mango varieties for resistance against die back disease.

Plate 1: Die back of mango grafts






Review of Literature

2. REVIEW OF LITERATURE

Mango is an important fruit and is subjected to a number of diseases at all stages of its development i.e. from nursery to the consumption of fruits. Many types of agents cause diseases in mango and of these, fungi cause the largest number of diseases. Many diseases of mango were reported from different parts of India. Mango disease has been investigated and also measures to combat them have been worked out in different countries. Die back is one of the serious diseases of mango. It has spread widely in different parts of India. This disease is commonly known as "Mango Quick Decline" and "Mango Killer".

2.1 Symptomatology

2.1.1 Symptomatology of die back disease

Die back is a severe disease in mango growing orchards. The symptoms produced by different organisms were reported by different scientists. Rath *et al.* (1978) from Orissa reported that die back of mango exhibited withering of tip, twig blight and bark cankers. They reported that dieback was caused by *Botryodiplodia*, *Rhizoctonia*, *Colletotrichum* and *Sclerotium* species. Burhan (1987) reported that die back disease caused by *B. theobromae* was originated from green shoot and leaf petiole of mango seedlings. He observed the primary symptoms as development of necrotic dots on tip of shoot and at the base of leaf petiole. The upper leaves were healthy, green in colour and gradually turned brown accompanied by upward rolling of leaf margin. Savant and Raut (2000) reported die back of mango stone grafts incited by *C. gloeosporioides* and *B. theobromae* and described the symptoms produced by both the pathogens. The symptoms incited by *C. gloeosporioides*

included the development of dark brown circular or irregular spots on leaves resulting in to elongated black necrotic patches. The development of shot holes, crinkling of leaves, drying and shredding of leaves and finally severe shedding of leaves were also observed. The development of acervuli was observed on affected shoots. They observed internal discolouration and external darkening of the tender shoot and bark on plants infected by *B. theobromae*. The development of die back was mostly faster from the leaf tip towards petiole through midrib. The affected leaves turned yellowish brown and rolled upwards. The affected shoots became dark, shriveled and shedding of leaves was observed. The pycnidial bodies were developed on the affected parts. Kurien *et al* (2001) explained the symptom produced by *C. gloeosporioides* as light brown coloured spots with red coloured margin on leaves of cashew which later enlarged and coalesced to cause leaf blight symptom. In cashew seedlings, *C.gloeosporioides* caused anthracnose disease exhibited oval, reddish brown, shiny water soaked lesions scattered over the leaf surface (Venkateswarlu and Krishnamurthy, 2003). Garg *et al.* (2004) reported anthracnose disease caused by *C.gloeosporioides* in guava. This disease appeared as lesions on the fruits. In initial stage fruit showed spots and later changed to light brown discolouration of the skin. The lesion coalesced and white mycelial growth appeared on the margin of the spot and produced salmon pink spore masses under humid atmosphere.

2.1.2 Symptomatology of leaf blight disease

Macrophoma blight caused by *Macrophoma mangiferae* sp.nov was reported by Hingorani and Sharma (1956) on mango leaves which produced irregular brown coloured spots having raised dark purplish margin with black pycnidial bodies on the necrotic area. Another important disease reported in mango in 1960 by Sarkar was the grey blight caused by *Pestalotiopsis mangiferae*. The disease was characterized by the presence of brown spots developed from margin or tip of the lamina of matured

leaves during winter season. The spots were coalesced to form large lesion with tan coloured margin in which the central region turned greyish white or light olive grey with dark dots of acervuli. In case of severe infection he observed shedding of leaves. Philip (1973) explained the symptoms of seedling blight of cashew caused by *Cylindrocladium scoparium* and she reported wilting and withering of seedlings, rotting of underground portion of the stem. She observed necrotic lesions on young leaves of seedling, which later coalesced and resulted in flaccidity and yellowing followed by rotting of petiole and premature defoliation. Stem infection was observed under high humid condition resulted in stem rot and die back of apical shoot. Root rotting was also observed resulted in discolouration and decay of root.

Mordue (1980) also described the grey leaf spot of mango caused by *P.mangiferae*. The spots vary in size from a few mm to several cm in length, dark, with raised border and silvery grey coloured upper surface of leaves and grey to brown coloured lower surface of leaves. Another leaf spot caused by *Phoma sorghina*, was reported on mango (Prakash and Raoof, 1985). They observed the symptom as small, irregular, oval to roughly circular brown spots with dark brown margin and yellow halo on young leaves. In advanced stage, the spots coalesced to form large spots, measuring up to 14 mm in size. The symptoms they observed were similar to anthracnose but in anthracnose, the spots were smaller and no cracking were noticed on spotted area. In 1987, Prakash and Srivastava studied leaf spot of mango caused by *A. alternata*. They described the symptoms as small, brownish circular spots on the surface of leaves and fruits and as black patches on the twigs. The spots gradually enlarged and became irregular black and form larger patches. Search of literature on *Cylindrocladium mangiferae* in mango revealed no information on disease incidence. Disease caused by other species of *Cylindrocladium* was reported by earlier workers. *Cylindrocladium quinqueseptatum* causing seedling blight, extensive defoliation and die back of young plants of

Eucalyptus sp was reported by Ivory *et al.* (1993). Beena *et al.* (1994) also reported collar rot and wilt of clove seedlings incited by *Cylindrocladium camelliae.* On young seedlings, the disease appeared as small dark brown to black lesions on collar region and eventually spread to larger area. They also observed brown discolouration of vascular bundles in affected stem and infected seedlings showed premature yellowing of leaves.

Later, many workers reported leaf blight symptoms on a variety of hosts caused by *Pestalotiopsis* sp. (Rivera and Wright, 2000; Karakaya, 2001; Tagne and Mathur, 2001; Trapero *et al.*, 2003). Kurien *et al.* (2001) reported that *Pestalotia* produced dark brown spots on leaves of cashew which enlarged and caused blighting of leaves. Khalequzamman *et al.* (2003) reported that the leaf spot of sapota was caused by *Pestalotia sapotae* was first appeared as numerous small, reddish- brown specks on the leaf lamina. These specks gradually enlarged to form more circular spots measured 1-3 mm in diameter and fully developed spots had grayish centre. Keith *et al.* (2006) reported that scab disease of guava was caused by *Pestalotiopsis* sp. They observed symptoms on leaves as gray or light brown lesions surrounded by dark brown borders and on fruits as brown raised corky, necrotic lesions. *Cylindrocladium scoparium* was reported from the affected mastic tree seedling, where it caused several symptoms as leaf spot, stem lesions, blight and crown rot (Polizzi *et al.,* 2006). Later, Vitale and Polizzi (2008) first reported leaf spots, stem lesions and defoliation of *Pistacia lentiscus* caused by *C. pauciramosum* and *C. scoparium.*

2.2 Etiology

2.2.1 Die back disease

Many organisms were associated with die back disease. This destructive disease is known to be prevalent in India and other mango growing countries of the world. Mango anthracnose was first reported by Collins in 1903. In India, the disease was first noticed by McRae (1924). Stevens (1926) isolated *B. theobromae* which caused die back disease in mango from Florida. Later, Gupta and Zachariah (1945) reported *B. theobromae* as causal organism of die back of mango for the first time in India. Vaheeduddin (1954) from Hyderabad reported that die back of mango was caused by *Fusarium* sp. Agnolini and Guitiani (1977) reported that anthracnose of cashew was caused by *C. gloeosporioides* and was noticed in every cashew growing area of the world. Rath *et al.* (1978) reported that *B. theobromae, P.mangiferae, Phoma* sp, *C. gloeosporioides, Sclerotium rolfsii and Rhizoctonia solani* were associated with mango die back as prevalent in Orissa. Fitzell (1979) isolated *Colletotrichum acutatum* from leaves, panicles and fruit of mango showing symptoms of anthracnose. Pathak (1981) reported that mango anthracnose (*C.gloeosporioides*) caused severe losses in India and other mango growing countries. Anon. (1983) reported that *Colletotrichum, Fusarium* and *B. theobromae* were isolated from dry twigs of cashew.

Jadeja and Vaishnav (1984) reported that anthracnose caused by *C.gloeosporioides* and leaf blight caused by *Phyllosticta* sp and *Pestalotia* sp were the major diseases in mango orchard of Saurashtra region of Gujarat. Later, *C.gloeosporioides* was reported to be pathogenic on several fruit crops like mango, papaya and guava (Dianere *et al.*, 1985). Patnaik *et al.* (1987) observed the inflorescence blight caused by *B. theobromae* in cashew varieties at Bhubaneswar,

Orissa. From Himachal Pradesh Patial (1988) reported the association of several pathogens with die back of mango *viz., B. theobromae, P. mangiferae, C.gloeosporioides, Fusarium* sp., *Aspergillus* sp. and *Xanthomonas* sp. He also observed that *B. theobromae* Pat was the major pathogen associated with mango die back. Later Varma and Balasundaram (1990) also investigated the shoot die back of cashew and isolated *B. theobromae* from the dead tissues of cashew. Shukla and Chowdhury (1991) showed the association of *B. theobromae, C. gloeosporioides* and *Fusarium solani* with die back of rose. Twig blight of sapota caused by *B.theobromae* was reported by Patel and Chauhan (1993). Later, Uchida *et al.* (1996) noticed papaya seedling blight and damping off caused by *C. gloeosporioides*. Ferrari *et al.*(1996) reported the die back and gummosis caused by *L. theobromae* on citrus trees. Ploetz *et al.* (1996) reported that the decline syndromes in mango were caused by *Alterneria alternata, Cladosporium* sp, *Colletotrichum* sp, *Pestalotiopsis* sp. and *Phomopsis* sp. Savant and Raut (2000) reported that dieback of mango stone grafts caused by *C. gloeosporioides* and *B. theobromae* either alone or in combination was a major disease syndrome widely prevalent in the Konkan region of Maharashtra. Kurien *et al* (2001) also reported die back of cashew seedlings incited by *C.gloeosporioides* as an important disease in nurseries of cashew. Dama *et al.* (2004) identified the causal agent of anthracnose disease isolated from affected cashew seedlings as *C. gloeosporioides*. Gaikwad and Sawant (2005) reported that fruit rot (anthracnose) of custard apple incited by *C. gloeosporioides* was an important disease in Maharashtra.

2.2.2 Leaf blight diseases

Hingorani and Sharma (1956) reported *Macrophoma* blight of mango caused by *Macrophoma mangiferae*. Singh and Tandon (1967) isolated *Alternaria tenuis* from banana, guava and mango and found that it was pathogenic under artificial

condition. Later, Yadava and Udainarain (1970) reported *A. alternata* from the fallen stem of mango. Rajendran (1971) observed that the leaf spot of sapota as the most common and serious disease causing considerable yield loss in every year which was caused by *P. sapotae*. Philip (1973) reported *Cylindrocladium scoparium* as a new seedling blight pathogen on cashew seedlings from Kerala. In India, *P. mangiferae* was reported in mango by Pandey and Mohammad (1975); Vala *et al.* (1975). Madhukar and Reddy (1976) reported *A. alternata* caused leaf spot disease on pomegranate in India.

Cylindrocladium quinquiseptatum caused leaf blight disease on clove was reported by Wilson *et al.* (1972) and Sulochana *et al.* (1982). Abraham and Kumari (1980) reported the leaf spot disease of cashew caused by *C. gloeosporioides* in Kerala. Prakash and Raoof (1985) isolated *A. alternata* from mango leaves causing leaf spot disease. Naik *et al.* (1986) also reported the leaf spot disease of cashew caused by *C. gloeosporioides* in Orissa. Sharma *et al* (1987) reported that leaf disease of chicku caused by *P. sapotae*. Intini (1987) reported that among the diseases which contributed to decline of cashew cultivation in Tanzania, powdery mildew caused by *Oidium anacardi*, the anthracnose caused by *C. gloeosporioides*, the die back of shoot and inflorescence caused by *Phomopsis anacardi* and two leaf diseases caused by *Pestalotia heterocornis and Psudocercospora anacardi* were more important.

Kurien *et al* (2001) reported various leaf blight diseases in cashew seedlings caused by *C. gloeosporioides*, *Phytopthora palmivora* and *Pestalotia* sp. Khalequzamman *et al.* (2003) reported that the leaf spot of sapota was caused by *Pestalotia sapotae* in Bangladesh. Kumar *et al.* (2006) found that *Alternaria* rot or black spot of mango was caused by *A. alternata*. Farr *et al.* (2007) from USA reported fruit rot of pomegranate caused by *Alternaria* sp. Tziros *et al.* (2008) first observed fruit decay in pomegranate caused by *A. alternata* in Greece.

2.3 Cultural and morphological characters

2.3.1 Die back pathogens

2.3.1.1 *Colletotrichum* sp

Kumar (1999) identified C. lindemuthianum based on the cultural and morphological characters. He observed grey coloured slow growing colonies turned to dark with compact aerial mycelium and reverse side of the colony almost black. He also observed that formation of acervuli in culture with setae. Conidia hyaline, cylindrical with both ends obtuse, aseptate, uninucleate and measured 14.2-17.75 µm in size

Chowdhry and Varshney (2000) studied the cultural and morphological characters of *C. gloeosporioides* under *in vitro* condition and they observed abundant whitish aerial mycelium in the colony and noticed slimy pinkish spore mass and acervuli in the growth. They studied morphological characters, the conidia were straight, obtuse at the apex, measured 9-24×3-4.5 µm in size and observed acervuli without setae.

Davis (2003) described the cultural and morphological characters of *C.gloeosporioides*. She observed the colony initially as pinkish white later turned brown with greyish white colour and pink pigmentation. Hyphae branched, hyaline with 3.8 ×11.6-19.4 µm in size and setae dark brown with 1-2 septa.

Bag (2004) also studied morphological characters of *C. gloeosporioides* from flower and fruit drop of papaya. He observed brown to black coloured mycelium, septate, acervulus dark, cushion shaped with long slender, septate and black coloured

setae. The spores were short, hyaline, cylindrical, single celled and 13-18×4-5 µm in size.

Gaikwad and Sawant (2005) reported thirteen isolates of *C. gloeosporioides* obtained from custard apple. They observed significant difference in the various isolates of *C. gloeosporioides* in size, shape and colour of mycelium, acervuli, setae, conidia, appressoria, perithecia, asci and ascospores.

2.3.1.2 *Botryodiplodia* sp.

Based on the cultural and morphological characters Punithalingam (1976) identified *B. theobromae* as the causal agent of die back disease in many crops. He described the colonies as grey to black, fluffy with abundant aerial mycelium and reverse side of the colony black in colour. Pycnidia simple, or compound, often aggregated stromatic, ostiolate and 5mm wide. Conidiophores were hyaline, cylindrical, septate and rarely branched. Conidia initially unicellular, hyaline, oblong, thick walled, base truncate and matured conidia were uniseptae reddish brown coloured and measured 20-30×10-15 µm in size.

Ferrari *et al.* (1996) identified the causal agent of die back and gummosis disease of citrus as *Lasiodiplodia theobromae* on the basis of colony characters. Colonies grew quickly and were initially white, turned to black after 10 days. The fungus produced white cottony compact mycelial growth that later turned into mouse grey due to heavy conidial production. Conidia were fusiform, single celled and varying in size. The conidia measured 10.36 -15.5 × 2.58 -5.16µm (12.8×2.89) in size on the host and 10.32 - 20.62 × 2.58 - 5.16µ (13.5 × 3.61) on the PDA after 7 - 10 days of growth.

The cultural and morphological characters of *Lasiodiplodia theobromae* obtained from corm rot of elephant foot yam were described by Mali *et al.* (2005). He observed that ready growth of the fungus on PDA medium and produced light gray fluffy and aerial mycelium. The culture became dark colour in advanced stage. The mycelium was immersed, branched, septate and brown. The young hyphae were thin and sub hyaline turned thick, closely septate and brown in colour when matured and measured 4 - 11.7 µm in width. Pycnidia were immersed, globose, dark brown or black bodies in groups of 2 - 6 and measured 125 - 430 µm in diameter. The pynidiospores were acrogenous, hyaline, thin walled and one celled when young and measured 16.77 × 9.65µm when matured slowly became septate, dark brown, thick walled and ovoid to elongate with truncate base. They had longitudinal striations and measured 22.60 × 12.05 µm.

2.3.2 Leaf blight pathogens

2.3.2.1 *Pestalotiopsis* sp.

Mordue (1980) described the cultural and morphological characters of *P.mangiferae*, the causal organism of grey leaf spot of mango. The colonies on PDA showed dense white aerial mycelium and acervuli developed from small clumps of hyphae and form conspicuous greenish black slimy spore masses. Conidia fusiform, slightly curved, septate and measured 19.8 × 5.6 µm size, and 3 median cells equidistantly euseptate, olivaceous brown, thick walled, 10 - 16 µm long; apical and basal cells thin walled, hyaline, its wall extended at the apex to form 3 appendages with 3-25 µm long.

2.3.2.2 *Cylindrocladium* sp.

Perusal of literature revealed no information on leaf blight disease caused by *Cylindrocladium mangiferae* on mango. There were reports on morphological characters of other species of *Cylindrocladium* causing disease in other crops.

Nirwan and Singh (1967) described the morphological characters of *C.scoparium* isolated from leaf spot of *Madhuca indica*. Spores were cylindrical, hyaline, one septate measured 41.6 - 48.0 × 4.0 - 4.9 µm and were held together by sticky slime. The conidia were borne on the tips of dichotomously or trichotomously branched sterigmata.

Beena *et al.* (1994) studied the cultural and morphological characters of *C.camelliae* causing collar rot and wilt in clove seedlings. They observed the white mycelium turned to reddish brown, septate with penicillate branching of conidiophore and with sterile filament. The primary and secondary branches of the conidiophores were non septate. The phialide sterigmata on the secondary conidiophores attached the cylindrical, hyaline and biseptate conidia. The sterile filament was single, elongated and thick walled and the vesicle was lanceolate or ellipsoidal.

Leaf rot of coconut caused by *C. scoparium* was described by Srinivasan and Gunasekaran (1995). The culture appeared as white with a brown shade, cottony and produced abundant sclerotial bodies in the central portion. Conidiophores measured up to 0.5mm in length and 7.5 µm width near the base, and dichotomously branched near the apex. The conidia were cylindrical with rounded ends, measured 50-58×5-6 µm, hyaline, smooth, straight with distinctly a single transverse septum in the middle.

2.3.2.3 *Drechslera* sp.

Chowdhry and Shrivastava (2000) identified *D. australiensis* based on the cultural and morphological characters. They observed conidia ellipsoidal or oblong, rounded at the ends, pale brown to reddish brown, 3-5 pseudo septate and measured 12-14×6-10µm in size. The conidiophores were flexuous, geniculate, septate and reddish brown.

2.3.2.4 *Alternaria* sp.

Sharma *et al.* (2000) described the morphological characters of *A. alternata*. The conidia long, often branched chain, obclavate, obpyriform, short cylindrical beak, pale to mid golden brown, smooth and measured 20-50 × 9-15 µm in size, beak 2-5 µm thick. The conidiophores were single, straight and pale to mild olivaceous.

Davis (2003) studied the cultural and morphological characters of *A. alternata* from ivy gourd and reported that the fungus took only seven days to complete full growth in 9 cm Petri dishe. The growth initially showed whitish colour and turned to dark brownish black colour. The mycelial growth had a velvety appearance with dark purplish tinge on the upper side and reverse side of the colony was almost black. She described the morphological characters, conidia measured 19.5-31.2 × 7.8-15.6 µm in size with 3-5 transverse septa and 1-3 longitudinal septa.

Resmi (2005) described the cultural and morphological character of *A.alternata* causing leaf blight disease in bottle gourd. She observed the colony brownish grey, thick, velvety and reverse side of the colony was black. She also noticed the morphological characters of hyphae which was branched, brownish grey with 4.03 × 16.12 -28.21 µm in size. The conidiophores branched single or groups

and having 80.6-100.75 µm length with 3-6 septa, conidia formed in chain, straight, obclavate, smooth, brown and measured 12.09-52.39×4.03-16.12 µm in size.

Mangala *et al.*, (2006) described the conidial characters of *A. alternata*. The conidia were obclavate, beaked spiny, pigmented with relatively thin 1-9 longitudinal and 0-4 transverse and oblique septa, measured 45.77-47.85 µm in length and 14.38-16.42 µm in breadth. Tziros *et al* (2008) reported the fruit rot of pomegranate caused by *A. alternata*. They observed the cultures grew rapidly on PDA, initially white and turned to grey. Conidiophores were short, septate, branched and green to brown. The conidia were obpyriform with cylindrical beak, ovoid or ellipsoidal and produced in long chains, single but most often branched. Conidia measured 10-21 µm in length and 4-10 µm in width.

2.4 Disease management

Uses of plant protection chemicals and antagonists in the management of a disease have practical importance. So many workers have conducted studies on these aspects.

2.4.1 Fungicides for management of dieback and leaf blight diseases

A perusal of the literature revealed few reports on the *in vitro* and *in vivo* studies with fungicides on die back and leaf blight diseases of mango. However, there are reports on the effect of fungicides on the die back causing pathogens infecting different crops other than mango graft. West (1934) reported that *C. gloeosporioides*, which attacked tender portions of mango shoot, could be easily controlled by the application of Bordeaux mixture at one per cent concentration. Tandon *et al.* (1955)

tested by dusting the leaves with zinc sulphate that controlled the grey blight disease of mango but when they applied similar dusting on fruits which failed to control the rot.

According to Narain and Panigrahi (1971) spraying of blitox 50 at 0.5 per cent gave good control of die back and fruit rot of chilli. Misra and Singh (1971) tested the efficacy of Bordeaux mixture, Blitox 50 and Fytolan and organic fungicides Dithane z-78 and Captan against the growth of *Alternaria tenuis* and *Helminthosporium oryzae* using poisoned food technique. They reported that captan was superior to other fungicides for both the organisms followed by three copper fungicides. Later, Sohi *et al.* (1973) found that the mango variety, Alphonso was highly susceptible to stem end rot pathogen, *B. theobromae* and they concluded that it could be effectively controlled by three sprayings of carbendazim (0.1%) at fortnight intervals commencing from 45 days prior to the expected harvest. Singh and Srivastava (1978) observed that fungal diseases of mango grafts could be controlled by the application of blitox, or benlate at the concentration of 0.2 per cent in scion at the time of veneer grafting of mango. Later, Maity and Biswas (1980) while doing epicotyl grafting of mango reported that fungal infection could be controlled by dipping scions and seedlings in 0.2 per cent captan solution for few minutes before grafting.

Kanwar and Jawanda (1983) while topworking in mango trees through side grafting used Bordeaux paste for protection against fungal infection. Jadeja and Vaishnav (1984) conducted a field trial against anthracnose and leaf blight diseases in mango. They reported that spraying with copper oxychloride @0.2per cent plus zineb @0.2 per cent followed by wettable sulphur @0.2 per cent before flowering, carbendazim @0.03per cent at pea size fruit formation stage and zineb @0.2 per cent before maturation of stone reduced the incidence of both the diseases. Raju and Rao (1984) tested the efficacy of Dithane M-45 and Blitox-50 against anthracnose and

fruit rot diseases of chilli. Das and Mahanta (1985) reported that Bavistin completely inhibited *P. palmarum* Cke under *in vitro* condition. Kumar and Lal (1985) tested the efficacy of twelve fungicides at 100ppm concentration against *Drechslera maydis* causing leaf blight of maize. They reported that among the twelve fungicides, captafol was found to be the best. Later, Singh and Agarwala (1987) found that Benlate (200ppm) gave maximum control against anthracnose of citrus var. kagzi lime and kinnow and also proved Dithane M-45 and captan were the least effective.

Eswaramurthy *et al.* (1988) tested the efficacy of ten fungicides against die back and fruit rot of chilli caused by *Colletrotrichuim capsici* and *Alterneria solani.* They reported that Foltaf (0.2per cent) was the most effective chemical against both the diseases. It was followed by Fytolan (0.25per cent), Bavistin (0.1 per cent) against fruit rot of chilli and fytolan (0.25per cent) and hinosan (0.1per cent) against die back of chilli. Fitzell and Peak (1988) reported that application of prochloraz plus copper at fortnight.intervals during flowering gave higher yields than copper plus mancozeb and mancozeb alone which failed to control mango anthracnose. Patial (1988) identified six organisms *viz., B. theobromae, P. mangiferae,* C. *gloeosporioides, Fusarium* sp, *Aspergillus* sp and *Xanthomonas* sp as pathogens of die back of mango. He found that three sprayings of captan (0.3per cent) along with pruning of diseased twigs were more effective in controlling the die back disease. Ramaswamy *et al.* (1988) evaluated fungicides against *Pestalotiopsis psidii* and they found that Dithane M-45 showed good result. Khalequzamman *et al* (1988) also sprayed five fungicides against grey blight of coconut caused by *P. palmarum* and observed that carbendazim (0.1 per cent) and mancozeb (0.2 per cent) showed better control of the disease. Singh *et al.* (1989) reported the efficacy of six fungicides against canker and die back disease of pear caused by *B. theobromae.* They found that carbendazim was the most effective in inhibiting the growth of the pathogen at 200ppm followed by copper oxychloride at 2000ppm under *in vitro* condition. They also conducted field trial and

reported that the maximum disease control was achieved with copper oxychloride (0.3per cent) followed by carbendazim (0.1 per cent) and Bordeaux mixture at the ratio (8:8:250). Datar *et al.* (1990) reported that the lowest incidence of chilli anthracnose was observed in plots treated with mancozeb @ 0.25 per cent followed by carbendazim (0.2 per cent), captafol (0.2 per cent) and copper oxychloride (0.25 per cent).

Sharma and Kaul (1990) reported the efficiency of Bordeaux mixture (one per cent) to control the die back disease of olive caused by *C. gloeosporioides*. Mohanan *et al.* (1991) observed that *C. gloeosporioides* caused foliar diseases in cocoa. They tested carbendazim and mancozeb against the three isolates of *C. gloeosporioides*. The growth of all the three isolates was completely inhibited in the presence of carbendazim at 5 and 10µg/ml medium. Joshi and Raut (1992) observed that the severe disease of young clove trees caused by *Pestalotiopsis versicolor* was best controlled by 0.1 per cent carbendazim sprays applied 3 times at 15 days interval. Selvan *et al.* (1993) reported the effectiveness of systemic fungicides in disease management. They observed the result of the superiority of these fungicides in the field coincided with the *in vitro* evaluation of fungicides like thiophanate methyl, carbendazim and tridemorph, which revealed that these fungicides inhibited the mycelial growth of *P. palmarum* even at lower concentration of 0.05 - 0.2 per cent. Sharma *et al.* (1993) observed that fruit dipping in carbendazim (0.1 per cent) and thiabendazole (0.1 per cent) was highly effective to check mango anthracnose in storage. Yadav and Narain (1993) studied the chemical control of *Alternaria* blight of chickpea caused by *A. alternata*. Here, among the 10 different fungicides tested, the best control was given by Difolatan @ 0.2 per cent, applied 3 times at 10 days interval. Mancozeb and ziram were found less effective.

Sharma and Badiyala (1994) observed the efficiency of carbendazim as the most effective fungicides against stem end rot of mango caused by *B. theobromae* which was followed by Bordeaux mixture and aureofungin. Sharma and Gupta (1994) tested nine fungicides against canker and die back disease of mango caused by *B. theobromae*. They observed the maximum disease control with Bordeaux mixture (one per cent) followed by Bordeaux mixture (0.8 per cent) and carbendazim (0.1per cent). Ebenezar and Subramanian (1995) proved also the effectiveness of Bordeaux mixture (one per cent), carbendazim (0.1per cent) and Bordeaux mixture (0.8 per cent) to manage the die back disease of acid lime caused by *C. gloeosporioides*. Bindu (1996) reported Bordeaux mixture one per cent was found to be effective in preventing the spread of the fungus caused seedling die back in cashew. Verma and Singh (1996) noticed young mango plants infected by *Macrophoma mangiferae*, causing leaf blight disease and reported that it could be successfully controlled by four sprays of Captaf (0.3 per cent) which was followed by Bordeaux mixture (one per cent), Captafol (0.2per cent), Bavistin (0.1 per cent) and Topsin -M (0.1 per cent). Majumdar and Pathak (1997) found that Bavistin and Dithane M-45 were effective against fruit rot of guava caused by *L. theobromae*. Banik *et al.* (1998) reported that the fungicides Bavistin showed complete inhibition of mango anthracnose (*C. gloeosporioides*) at 400ppm. It was followed by Captaf (450ppm), Topsin M (450ppm) and Kavach (550ppm) under *in vitro* condition.

Karthikeyan and Bhaskaran (1998) evaluated fungicides for controlling the leaf blight of coconut caused by *P. palmarum* and found that carbendazim and mancozeb completely inhibited the mycelial growth of the pathogen at 500µg/g under *in vitro*. Studies conducted by Srinivasan and Gunasekaran (1998) indicated that the fungicides copper oxychloride and carbendazim were very effective in controlling the infection of *C. gloeosporioides* causing leaf rot in coconut.

Kumar (1999) reported that carbendazim(0.1 per cent) and mancozeb (0.2 per cent) were very effective in reducing the infestation of *C. lindemuthianum* in cowpea. Kurien *et al* (2001) reported that 0.1 per cent carbendazim, 0.2 per cent copper oxychloride, mancozeb and zineb and one per cent Bordeaux mixture were effective for the management of leaf blight disease of cashew seedlings caused by *C.gloeosporioides* and *Pestalotia* sp. Vrinda (2002) observed that the fungicides copper oxychloride and carbendazim were very effective in controlling the infection of *C. gloeosporioides* causing leaf rot in coconut. Deepthy (2003) found that carbendazim (0.1 per cent) and copper oxychloride (0.2 per cent) and quinalphos 0.05 per cent were effective against *C. gloeosporioides* caused die back disease of cashew. She also reported that zineb (0.2 per cent) was not effective against the pathogen.

Davis (2003) tested the effectiveness of fungicides against *C. gloeosporioides* and *A. alternata.* She found that copper hydroxide was more effective against two pathogens which followed by copper oxychloride and captan. Mancozeb was least effective against both pathogens. Gupta *et al.* (2005) tested the effectiveness of fungicides *viz.* carbendazim, mancozeb, hexaconazole and copper oxychloride at 50ppm against the growth of *C. lindemuthianum.* Maximum inhibition was observed by carbendazim and mancozeb showed the least effect. Ekbote (2005) reported the lowest per cent disease index of chilli fruit rot with carbendazim (0.1per cent) and hexaconazole(0.1per cent) and the highest per cent disease index with mancozeb (0.3per cent). Priya (2005) tested the *in vitro* sensitivity of *C. gloeosporioides* to three different copper fungicides. She found that Bordeaux mixture at all three concentrations (0.5, 1.0 and 1.5 per cent) recorded cent per cent inhibition. Copper hydroxide and copper oxychloride recorded maximum inhibition of 82.9 per cent and 75.5 per cent respectively at 0.3 per cent concentration and least per cent inhibition of 50.7 per cent and 67.7 per cent respectively at 0.1 per cent. Kumar *et al.* (2006) reported that systemic and non systemic fungicides, tricyclazole and iprodione

respectively were more effective against black spot of mango caused by *A. alternata*. Sharma and Verma (2007) observed that complete inhibition of *C. gloeosporioides* causing mango anthracnose was obtained by systemic fungicides *viz.* Bavistin and Topsin-M at concentration of 100µg/ml. Among non- systemic fungicides chlorothalonil and Dithane M-45 provided complete growth inhibition at 1000 µg/ml followed by Captaf and Bordeaux mixture. Patil *et al.* (2007) tested the efficacy of twelve fungicides against *C. gloeosporioides* causing anthracnose in guava and was completely inhibited by Bordeaux mixture, tricyclazole, difenoconazole, propiconazole and hexaconazole. They also observed that carbendazim and captan were moderately effective and ready made Bordeaux mixture, copper oxychloride, propineb and mancozeb were less effective. Sanjay *et al* (2008) reported that the systemic fungicides were better than contact fungicides against grey blight disease of tea. The highest yield was recorded in the plots treated with systemic fungicides which were not only due to the disease control but also to their phytotonic effect.

2.4.2 Insecticide in disease management

Nambiar *et al.* (1973) reported the involvement of both the insect and fungus causing inflorescence blight in cashew. They recommended cumin @ 0.1 per cent in combination with dimecron @ 0.03 per cent to control the disease. Bhaskaran *et al.* (1976) reported synergistic action of ziram when mixed with fenthion under *in vitro* condition. In their field studies, combination of quinalphos plus edifenphos, parathion plus edifenphos, parathion plus mancozeb, phosalane plus mancozeb were found more effective against *D. oryzae*. Panda *et al.* (1986) found the combined spraying of Bordeaux mixture 1 per cent and Hildan 0.05 per cent on the panicle has reduced the die back incidence. The fungus identified as *B. theobromae* and reported flower thrips were observed to cause feeding injuries for the entry of the fungus. Later, Khan *et al.* (1989) tested the fungicidal properties of chlorpyrifos, fenvalerate, fenthion, and

monocrotophos singly and in combination with various fungicides under *in vitro* condition and reported that chlorpyrifos had a very good fungicidal activity. They found that the combination of chlorpyrifos plus captan, chlorpyrifos plus zineb, monocrotophos plus captafol, chlorpyrifos plus captafol, chlorpyrifos plus carbendazim, chlorpyrifos plus tridimefon also having fungicidal activity against *Drechslera oryzae*. Kalpana (1992) found the fungicidal property of quinalphos and also observed synergistic effect of carbendazim in combination with quinolphos against *Pyricularia oryzae*.

Bindu (1996) also reported that combined application of insecticide and fungicide was more effective in the control of inflorescence blight and die back symptom of cashew. In an experiment conducted at Cashew Research Station, Madakkathara, Kerala during 1999-2001 it was found that quinalphos @ 0.05 per cent plus copper oxychloride @ 0.2 per cent during flushing and endosulfan @ 0.05 per cent plus mancozeb @ 0.2per cent during flowering stages effectively controlled TMB- anthracnose complex (Kurian *et al.*, 2001). In anthracnose and tea mosquito endemic areas, application of monocrotophos @ 0.05 per cent plus 0.2 per cent copper oxychloride at the time of flushing, quinolphos @ 0.05 per cent plus mancozeb 0.2 per cent during flowering and carbaryl @ 0.1 per cent at the time of nut initiation is the current recommendation of the Kerala Agricultural University (KAU, 2002). Deepthy (2003) observed that the fungicides carbendazim, copper oxychloride and their combination with imidacloprid, quinalphos, azadirachtin and carbaryl gave cent per cent inhibition against *C. gloeosporioides*.

2.4.3 Antagonists in disease management

Biological control can be defined as reduction of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms

other than man (Cook and Baker, 1983). Today, antagonistic interactions have been exploited in the area of biological control of plant pathogens. Potential agents for biocontrol activity are rhizosphere competent fungi and bacteria which in addition to their antagonistic activity are capable of inducing growth responses by either controlling minor pathogens or producing growth stimulating factors (Cook and Weller,1986). Moreover, biological control being ecofriendly, it is more attractive proposition of crop protection especially, where products are export oriented. In contrast to agrochemicals which get leached off during incessant rains, biocontrol agents gets stabilized once efficient strains that fit into the concerned ecological niche are introduced into a given environment. Also, biocontrol agents fit well with organic farming, a proposition which is gaining popularity in recent times (Harman *et al.*, 1989).

Patil (1992) reported the antagonistic nature of *Trichoderma* sp against mango fruit rot incited by *Lasiodiplodia theobromae* and *Rhizopus arrhizus*. They observed that *Trichoderma* sp was effectively controlled both the pathogens. Korsten *et al.* (1993) found that mango fruits were dipped in *Bacillus licheniformis* (isolates B250 and B251) and gave effective control against anthracnose and stem end rot diseases. In the same year, Meah *et al* (1993) also reported the effectiveness of *Bacillus subtilis* against *B. theobromae* causing mango stem end rot. Koomen and Jeffries (1993) observed the effectiveness of *Pseudomonas fluorescens*588 against the anthracnose disease of mango. They found that the application of *Pseudomonas fluorescens*588 has significantly reduced the development of anthracnose.

Gupta *et al.* (1995) tested the effectiveness of *Trichoderma* sp and *Gliocladium virens* against *B. theobromae* causing stem canker and death of mulberry

saplings and also reported that isolates of *T. viride* exhibited stronger antagonistic activity compared to *G. virens*. Radhakrishan *et al.* (1995) also reported that *Trichoderma* sp, *Gliocladium* sp and *Bacillus subtilis* were antagonistic to *B.theobromae* by reducing the mycelial growth and conidial production under *in vitro* due to the production of fungal toxic metabolites. Majumdar and Pathak (1995) also found that *T. viride and T. harzianum* were effective against guava fruit rot incited by *B. theobromae*.

Bankole and Adebanjo (1996) reported that the isolates of *T. viride* were effective in inhibiting the *in vitro* and *in vivo* growth of *C. truncatum*, the causal agent of brown blotch disease of cowpea. Kumar (1999) observed the effectiveness of *T. viride* against anthracnose disease of vegetable cowpea caused by *C.lindemuthianum*. Gawad (2000) observed the inhibitory effect of *Bacillus subtilis* against *C. gloeosporioides* and reduced disease incidence in guava fruits. In India, talc based bioformulations have been reported to be effective in the management of several crop diseases (Nandakumar et al., 2001). Davis (2003) reported that *T. viride* and *T. harzianum*, followed by *A. niger* were effective against *Cercospora coccinae*, *C. gloeosporioides* and *A. alternata*, the leaf spot pathogens of ivygourd. *Pseudomonas fluorescens* also was effective and recorded more than 50 per cent reduction on the growth of pathogens. Bhuvaneswari and Rao (2003) under *in vitro* evaluation it was observed that the nonpathogenic *Fusarium* sp isolated from healthy mango fruits gave 73 per cent inhibition against C. *gloeosporioides* causing mango anthracnose under *in vitro*. Vivekananthan *et al.* (2004) observed that *P. fluorescens* (FP_7) + Chitin treatment reduced mango anthracnose incidence to 60 per cent over control and its efficacy was superior to standard fungicides, carbendazim in field conditions. Adebanjo and Bankole (2004) tested the two isolates of *T. viride* and three strains of *B. subtilis* against cowpea anthranose pathogen. They observed significant reduction anthracnose disease incidence and severity by two isolates of

T.viride. Pathania *et al.* (2004) screened *T. harzianum, T.hamatum, T. virens, T. viride,* and *B. subtilis* against *C. capsici* causing anthracnose in bell pepper. Among these antagonists, *T. hamatum* gave the highest inhibition zone when applied as seed treatment. Later, Sharma *et al.* (2005) tested the effect of different formulations of *T. harzianum* against *C. capsici* causing die back and fruit rot of chilli. They reported cent per cent inhibition on the symptom expression, conidial germination and mycelial growth of *C. capsici* by *T. harzianum*. Priya (2005) reported that *T. viride* was effective against C. *gloeosporioides* and *C. capsici* and followed by *A. niger*. *Pseudomonas fluorescens* was also effective and recorded more than 30 per cent reduction on the growth of both pathogens under *in vitro* evaluation.

Vivekananthan *et al.* (2006) tested different biocontrol agents *viz.*, Rhizobacterial strains FP7 and pf1 of *P. fluorescens*, *Bacillus subtilis* (Bs-1) and *Saccharomyces cerevisiae* (SC-1) against C. *gloeosporioides* causing anthracnose disease in mango. They found that the rhizobacterial strains FP7 in combination with chitin significantly reduced the growth of *C. gloeosporioides* in *in vitro* as well as in field condition.

2.5 Host resistance

Vander plank (1968) suggested that the difference in disease resistance exhibited by the genotypes may be due to different types of interaction between pathogen and the genotypes. Many factors are responsible for the resistant type of reaction shown by the genotype. This may include insufficient inoculation load, absence of pathogenic races to that genotype, unfavourable environmental condition and nutrient status of the soil in which the crops were cultivated (Yarwood 1978, Khan 1989).

Deepthy (2003) studied the varietal reaction of cashew under confined conditions against tea mosquito bug (TMB). She reported that none of the varieties were immune to TMB and all the varieties showed variability in their degrees of susceptibility. She found that the variety H-1600, Madakkathara-2 and Kanaka recorded minimum tea mosquito bug damage. The varieties Anakkayam-1, Madakkathara-1 and Sulabha recorded higher TMB damage.

Davis (2003) tested the nineteen genotypes of ivy gourd against different leaf spot diseases and observed highly resistant reaction in eight genotype showing CI value ranged from 0.4 to 3.6.

2.6 Estimation of total Phenol content

Presence of phenols and their oxidized products in the plant tissue is toxic to the growth and development of pathogens. Phenolic compounds were implicated in a wide variety of resistant reaction of plants to fungal attacks (Walker and Stahman, 1955; Kosuge, 1969). Nachiappan and Baskaran (1982) found that resistant varieties of mango contained more total and OD phenols. Later, Atri *et al.* (1985) reported the effectiveness of phenolic compounds against *B. theobromae.* Four phenolic compounds *viz,* B-naphthol, L- naphthol, vanillin and pyrocatechol proved considerable effectiveness against mycelial growth of *B. theobromae,* the incitant of fruit rot of sand pear. B-naphthol was found to be strongly anticellulolytic and anti respiratory. According to Nicholson and Hammerschmidt (1992), the phenolic compounds were the main toxic chemicals produced to inhibit pathogen or its activities.

Marie (2001) estimated the phenol content of different mango varieties and recorded the highest value of 37.10mg/g in Vellaikolumban followed by Olour (28.29 mg/g) and minimum value was recorded by Kalapady (18.73 mg/g) followed by Moovandan (21.51mg/g).

Materials and Methods

3. MATERIALS AND METHODS

The present study on "Etiology and management of die back disease of mango grafts in nursery" was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara, during the year 2007 - 2008. The details of materials used and the techniques adopted for the investigation are described below.

3.1 Survey and collection of diseased samples

A survey was conducted in different nurseries where mango grafts were raised such as Central Nursery (CN) of KAU, Vellanikkara, College orchard (CO) attached to College of Horticulture, Vellanikkara, Agricultural Research Station (ARS), Mannuthy and seven private nurseries *viz.*, South Indian nursery (SIN), Raja nursery (RN), Shalimar nursery (SN) and National rose garden (NRG) at Mannuthy, Murali nursery (MN), Kairali nursery (KN) and EASF nursery at Pannancheri, to study the occurrence of die back disease of mango grafts and to collect disease specimens for the experimental studies. During the survey various types of leaf blight infections on mango grafts were also observed and collected specimens of die back and leaf blight symptoms for the isolation of pathogens for further studies.

3.2 Isolation of pathogens

3.2.1 From die back symptom

The young twigs showing die back symptom were collected separately from the above mentioned areas and brought to the laboratory. They were observed under the microscope by preparing slides from infected areas. The disease specimens were washed under tap water and cut it in to small bits. They were surface sterilized with one per cent sodium hypochlorite solution and then

washed three times with sterile water and transferred to sterile Petri dishes containing Potato Dextrose Agar (PDA) medium. The pathogens grown on the medium were purified, sub cultured periodically and maintained for further investigation.

3.2.2 From leaf blight symptoms

The pathogens associated with leaf blight diseases were isolated by adopting the same procedure described in 3.2.1. The cultures of pathogens obtained in PDA medium were purified and sub cultured periodically and maintained for further investigation.

3.3 Pathogenicity test

3.3.1 Pathogens associated with die back symptoms

The pathogenicity of organisms associated with die back disease of mango graft was proved by artificial inoculation under *in planta* condition. For that healthy mango grafts were procured from Central Nursery of KAU, Vellanikkara and were inoculated with the pathogens on the young tissue of the twigs with and with out giving pin prick. The pathogens isolated from leaf blight diseases were also inoculated on the young tissues of the mango graft to find out their effect on die back incidence. Plants inoculated with sterile water served as control. The inoculated plants were kept under humid chamber and observed for the symptom expression. The pathogens were reisolated from the twigs showing symptoms and compared with the original culture.

3.3.2 Pathogens associated with leaf blight symptoms

3.3.2.1 *In vitro* condition

The pathogenicity of the different isolates of pathogens causing leaf blight disease was proved by artificial inoculation under *in vitro* condition. For that healthy mango leaves were collected from the mango orchard, washed under tap water and then disinfected with 70 per cent ethyl alcohol. The leaves were inoculated on both surfaces separately with the mycelial disc of different isolates of pathogens and proved the pathogenicity as explained in 3.3.1.

3.3.2.2 *In planta* condition

To prove the pathogenicity under *in planta* condition, healthy mango grafts were procured from Central nursery, Vellanikkara and the leaves were inoculated with the pathogens by giving with and without pinprick and proved the pathogenicity as described in 3.3.1.

3.4 Symptomatology

Symptoms produced by die back and the leaf blight pathogens of mango grafts under natural and artificial conditions were studied in detail.

3.5 Identification of pathogens

The pathogens associated with die back and leaf blight diseases were identified based on the cultural and morphological characters.

3.5.1 Cultural characters

Cultural characters of the different isolates of pathogens such as colour, shape, texture of fungal colony, formation of fruiting bodies, growth rate and sporulation on the PDA medium were studied in detail.

3.5.2 Morphological characters

Morphological characters of different isolates of pathogens in pure culture were studied. Permanent slides were prepared from the pure culture of all the isolates by slide culture technique (Riddle, 1950). Using micrometry, measurements on the size of hyphae, length and breadth of conidia of all the pathogens were recorded. Camera lucida drawings and photomicrographs of hyphae and conidia of all the pathogens were also made. These characters were compared with the characters given in CMI descriptions of Pathogenic Fungi and Bacteria and in textbook "Hyphomycetes" (Subramanian, 1971), to identify the different pathogens. For further confirmation, the cultures were sent to "National Center of Fungal Taxonomy", at New Delhi.

The morphological characters of different isolates of the same pathogen were analyzed with Euclidean co-efficient and was clustered by the Unweighed Pair Group Average Method (UPGAM). (UPGAM: Sneath and Sokal, 1973) using NTSYS pc 2.02 software to produce grouping. The genetic dissimilarity matrix was also computed.

3.6 Management of die back diseases of mango grafts

The effectiveness of fungicides and antagonistic organisms against die back pathogens was tested under *in vitro* condition. The *in vitro* evaluation of fungicides and antagonists was also carried out against the leaf blight pathogens along with die back causing organisms.

3.6.1. *In vitro* evaluation of fungicides against die back pathogens

In *in vitro* evaluation, eight different fungicides were tested against die back and leaf blight pathogens by poison food technique (Zentmeyer, 1995). For that 100 ml of PDA medium was taken in 250 ml conical flask and sterilized. Three different concentrations of each fungicide were mixed separately with the medium taken in conical flask and poured into sterilized Petri dishes @20 ml per plate. Mycelial disc of 10mm diameter were cut from actively growing seven day old cultures of the pathogens and placed at the centre of each Petri dish containing poisoned medium. Three replications were maintained for each concentration of all fungicides. Medium without fungicide served as control. The radial growth of the fungal colony was recorded up to and when the control plates were fully covered with fungal growth.

The per cent inhibition of growth over control was calculated by the formula suggested by Vincent (1927)

$$\text{Per cent inhibition of growth} = \frac{C - T}{C} \times 100$$

Where

C = Growth of pathogen in control (mm)

T = Growth of pathogen in treatment (mm)

Table1: Fungicides used for *in vitro* evaluation against dieback and leaf blight pathogens

Chemical name	**Trade name**	**Concentrations (Per cent)**
$Cuso_4$ + lime	Bordeaux mixture	0.5,1,1.5

Copper oxychloride	Fytran 50% WP	0.2,0.3,0.4
Copper hydroxide	Kocide 101 77%WP	0.05,0.15,0.25
Mancozeb	Indofil M-45 75%WP	0.2,0.3,0.4
Zineb	Indofil Z-78 75%WP	0.2,0.3,0.4
Captan	Captaf 50%WP	0.2,0.3,0.4
Hexaconazole	Contaf 5%SC	0.05,0.1,0.15
Carbendazim	Bavistin 50%WP	0.05,0.1,0.2

3.6.2 *In vitro* evaluation of fungal antagonists against die back and leaf blight pathogens

Fungal antagonists against die back and leaf blight pathogens were tested by dual culture method (Skidmore and Dickinson, 1976). For that 20 ml of PDA medium was transferred in to sterilized Petri dishes. After solidification of medium mycelial disc of 10mm diameter was cut from actively growing culture of the fungal pathogens and placed in the centre of one half of the Petri dish. The fungal antagonist was transferred and placed at the centre of the other half of the same Petri dish. Three replications were maintained for each treatment and the pathogen and antagonist grown as monoculture served as control. The plates were examined for the antagonistic activity and the measurements on the radial growth of pathogen and antagonist were taken daily till the control plates attained full growth. Per cent inhibition was calculated as per the formula given in 3.6.1. The nature of reaction of the antagonist on the pathogen was studied by following the method given by Purkayastha and Bhattacharya (1982).

Types of reaction

Homogenous : Free intermingling of hyphae

Over growth : Pathogen over grown by antagonists

Cessation of growth : Cessation of growth at line of contact

Aversion : Development of clear zone of inhibition

3.6.3 *In vitro* evaluation of bacterial antagonist against die back and leaf blight pathogens

The standard culture of the bacterial antagonist *viz. Pseudomonas fluorescens* was used to test the antagonistic effect against die back and leaf blight pathogens by dual culture method (Utkhede and Rahe, 1983). Mycelial disc of actively growing culture of pathogen of 10mm diameter size was transferred to the centre of the Petri dish containing PDA medium. The bacterium was inoculated as a line of streak on one side of the pathogen. The inoculated Petri dishes were incubated at room temperature and observations on growth of pathogen were taken at regular interval. Petri dish inoculated with pathogen alone were also maintained which served as control. Three replications were maintained for each isolates. The per cent inhibition of mycelial growth of pathogen over control was calculated by the same method as given in 3.6.1.

3.7 *In planta* evaluation of different fungicides and antagonists against dieback and leaf blight diseases

Two experiments were laid out under *in planta* condition to study the efficiency of fungicides, bioagents and one commonly used insecticide against die back and leaf blight pathogens of mango grafts. The recommended dose of fungicides bioagents and insecticide were used for the experiments.

3.7.1 First experiment

The first experiment was conducted during July to September, 2007 with all the eight fungicides, and insecticide quinolphos were included in this experiment. The commercial formulations of bioagents were used. The foliar application of treatments was given on 15 days after the establishment of grafts and four sprayings were given at 15 days interval.

The details of the first experiment are as follows.

Variety : Priyor
Design : CRD
Replications : 3
Number of treatments : 12
Number of plants/ treatment : 39

Source – Mango grafts were procured from Department of Pomology and Flouriculture.

Table 2: Details of treatments used in first experiment

Tr.No	Fungicides	Concentrations (Per cent)
T1	Bordeaux mixture	1.0
T2	Hexaconazole	0.1
T3	Copper oxychloride	0.3
T4	Mancozeb	0.3
T5	Zineb	0.3
T6	Captan	0.3
T7	Carbendazim	0.1

T8	Copper hydroxide	0.15
T9	*Pseudomonas fluorescens*	2.0
T10	*Trichoderma viride*	2.0
T11	Quinalphos	0.05
T12	Control	-

3.7.2 Second experiment

Based on the result of *in vitro* evaluation and the first *in planta* experiment, a second experiment was conducted during October- December, 2007. The most effective fungicides and bioagents were selected and used in this experiment.

The details of the second experiment are as follows.

Variety : Neelum
Design : CRD
Replication : 3
Number of treatments : 8
Number of plants/ Treatment : 21

Source – Mango grafts were procured from Department of Pomology and Flouriculture

Table 3: Details of treatments used in second experiment

Tr.No	Fungicides	Concentrations (per cent)
T1	Bordeaux mixture	1.0
T2	Copper hydroxide	0.15
T3	Captan	0.3
T4	Hexaconazole	0.1
T5	Carbendazim	0.1
T6	*P. fluorescens*	2.0
T7	*T. viride*	2.0
T8	Control	-

The treatments were given in the same way as that of the first experiment.

3.7.3 Observations

Observations on disease incidence and severity were recorded on 10 days after each spraying. The biometric observations *viz.*, number of leaves and height of the plant were taken10 days after last spray.

3.7.3.1 Assessment of disease incidence

For assessing the disease incidence, total number of plants and plants infected by die back and leaf blight pathogens in each treatment were recorded separately. The per cent disease incidence (PDI) of die back and leaf blight diseases were calculated separately using the formula.

$$\text{Percent disease incidence (PDI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

3.7.3.2 Assessment of disease severity

Disease severity of die back disease from each treatment was calculated using 0-4 scale as detailed below (Ambika and Abraham, 1979).

Score chart for assessment of disease severity of dieback disease

Grade	Description
0	No necrotic lesions/ streaks
1	Up to 3 necrotic lesions/streaks-general vigour of flushes un affected
2	4-6 coalescing or non- coalescing lesions/ streaks- general vigour of flushes affected
3	Above 6 coalescing or non- coalescing lesions/ streaks-general vigour of flushes affected
4	Lesions/ streaks confluent and drying of affected flushes

From each treatment the disease severity of leaf blight disease was calculated by observing all leaves of each plant for the per cent infection. The leaves were scored using 0-9 scales as detailed below (KAU, 1996).

Score chart for assessment of disease severity of leaf blight disease

Grade	Description
0	No symptoms
1	1-10 per cent leaf area infected
3	>10-25 per cent leaf area infected
5	>25-50 per cent leaf area infected
7	>50-75 per cent leaf area infected
9	>75 per cent leaf area infected

Per cent disease severity was calculated using the formula suggested by Wheeler (1969).

$$\text{Per cent disease severity (PDS)} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves observed} \times \text{Maximum diseased grade}} \times 100$$

3.7.4 Biometric observations

3.7.4.1 Plant height

Height of the plants from the end of scion to growing tip, in each treatment was recorded at 10days after the last spray.

3.7.4.2 Number of leaves

The total number of leaves in plants of each treatment was recorded at 10 days after last spray.

3.8 Screening of mango varieties for die back disease

Mango grafts of different varieties maintained at ARS, Mannuthy and in one private nursery National Rose Garden at Mannuthy were screened separately under natural condition for resistance to die back disease by recording PDI and PDS. They were also screened for leaf blight diseases by taking the same observations.

From the PDI and PDS, Coefficient of infection (CI value) was calculated using the formula suggested by Datar and Mayee (1981).

$$\text{Coefficient of infection (CI value)} = \frac{\text{Per cent disease incidence} \times \text{Per cent disease severity}}{100}$$

Based on the CI value, the varieties were categorized into five groups as shown below.

Sl. No	CI value	Category
1	0-4	Highly resistant (HR)
2	4.1-9	Resistant (R)
3	9.1-19	Moderately resistant (MR)
4	19.1-39	Moderately susceptible (MS)
5	39.1-69	Susceptible (S)
6	69.1-100	Highly Susceptible(HS)

3.9 Estimation of phenol

Phenol content present in the leaves of healthy plants of different varieties of mango was estimated to know the pre infectional biochemical defense mechanism of these varieties. Phenol was estimated in those varieties that showed high resistance and high susceptibility to foliar infection.

3.9.1 Estimation of phenol by spectrophotometer

Total phenol was estimated as per the protocol of Malick and Singh (1980). One gram of leaf was weighed and ground using a pestle and mortar in 10 times by volume of 80 per cent methanol. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was saved and the residue was re-extracted with five times the volume of 80 per cent methanol. Centrifuged and the supernatants were pooled together. The supernatant was evaporated to dryness and the residue was dissolved in 5 ml of distilled water. From the above residue dissolved, different aliquots (0.2-2ml) were pipetted out in to a test tube and the volume was made up with 3ml-distilled water. Folin –ciacalteau's reagent (0.5ml) was added to the test tube. After 3 min, 2 ml of 20 per cent sodium bi carbonate (Na_2CO_3) solution was added to each tube and mixed thoroughly. The tubes were placed in boiling water bath for exactly one minute. Cooled and the absorbance was measured at 650nm (Spectronic-20D+) against a reagent blank. A standard curve was prepared with different concentration of catechol.

3.10 Statistical analysis

Analysis of variance was performed on the data collected in various experiments using the statistical package MSTAT (Freed, 1986). Multiple comparisons among treatment means were done using DMRT.

Results

4. RESULTS

The present investigation was carried out to study the etiology, symptomatology, varietal screening and management of die back disease of mango grafts in nursery. Samples of infected mango grafts were collected from different nurseries and pathogens were isolated and identified. *In vitro* and *in planta* evaluations of the fungicides and antagonists were carried out to know their efficacy in reducing disease incidence, severity and improving plant biometric characters. The results of experiments are presented below.

4.1. Survey

Survey was conducted in ten nurseries *viz,* Central nursery of Kerala Agricultural University, Vellanikkara, College orchard of mango attached to College of Horticulture, Vellanikkara, Agricultural Research Station (ARS) Mannuthy and seven private nurseries *viz*., South Indian nursery, Raja nursery, Shalimar nursery and National rose garden at Mannuthy, Murali nurseries, Kairali and EASF at Panancheri. From these nurseries specimens of die back and leaf blight infections were collected and the pathogens were isolated.

4.2. Isolation of pathogens

4.2.1. From die back symptom

The pathogens associated with die back disease were isolated from the naturally infected mango grafts collected from ten nurseries. The isolation yielded two different fungal pathogens on PDA medium and was found belonging to *Colletotrichum* sp. and *Botryodiplodia* sp. From seven nurseries *Colletotrichum* sp. was isolated whereas *Botryodiplodia* sp. was isolated from two nurseries. All the isolates of these pathogens were purified and sub

cultured at frequent intervals and was identified up to species level based on cultural and morphological characters described later in this chapter.

4.2.2. From leaf blight symptom

The pathogens associated with leaf blight disease were isolated on PDA medium from naturally infected leaves of mango grafts collected from different locations. Five different fungal pathogens were isolated on PDA medium and were found belonging to, *Colletotrichum* sp., *Pestalotiopsis* sp., *Cylindrocladium* sp., *Drechslera* sp. and *Alternaria* sp. Among these, *Colletotrichum* sp. was isolated from seven different nurseries, *Pestalotiopsis* sp. from three nurseries and *Drechslera* sp., *Cylindrocladium* sp. and *Alternaria* sp. form two nurseries each. All the isolates of these pathogens were purified and sub cultured at frequent intervals. All these fungi were identified up to species level based on cultural and morphological characters described later in this chapter.

4.3. Pathogenicity test

4.3.1. Pathogens associated with die back symptoms

The pathogenicity of the two pathogens isolated from die back symptom was proved by artificial inoculation under *in planta* condition. *Colletotrichum* sp. and *Botryodiplodia* sp. inoculated separately on the shoot tip of mango grafts produced typical symptom of die back on the third day after inoculation (DAI). The symptoms were observed only on plants inoculated with pin pricks. Among the grafts inoculated with the pathogens isolated from leaf blight symptoms, only those plants inoculated with *Cylindrocladium* sp. produced the symptom of die back. *Pestalotiopsis* sp., *Drechslera* sp. and *Alternaria* sp. were failed to produce symptom on shoot tip.

Re-isolation of the pathogens from the artificially inoculated shoot yielded the fungi same as that in the respective original cultures.

4.3.2. Pathogens associated with leaf blight symptoms

The pathogenicity of fungi associated with leaf blight symptoms was carried out in *in vitro* and *in planta* conditions.

4.3.2.1. *In vitro* condition

The pathogenicity of the five pathogens isolated from different locations was proved by artificial inoculation under *in vitro* condition. Inoculation on severed mango leaves produced leaf blight symptoms within three to four DAI. The leaves inoculated with the cultures of *Colletotrichum* sp. and *Cylindrocladium* sp. with and without giving pin prick on upper and lower surface produced the symptoms on three DAI. The initial infection by *Pestalotiopsis* sp., *Drechslera* sp. on upper surface of leaves with pin prick was observed on four DAI whereas *Alternaria* sp. inoculated with pin prick on lower surface produced the symptoms on three DAI. The leaves maintained as control did not show any symptoms. Reisolation of the pathogens from the artificially inoculated leaves yielded the fungi same as that in the respective original cultures.

4.3.2.2. *In planta* condition

The pathogenicity of the five pathogens was also proved by artificial inoculation under *in planta* condition. The leaf blight pathogens were inoculated with and without giving pin prick on upper and lower surface of the leaves in mango grafts. The leaves inoculated with the cultures of *Colletotrichum* sp., *Pestalotiopsis* sp., *Cylindrocladium* sp. and *Alternaria* sp. with pin prick on upper surface produced leaf blight symptoms on four DAI.

Typical symptom of leaf blight was noticed on both surfaces of leaves inoculated after giving pin prick with *Drechslera* sp. on five DAI. No symptoms were observed on grafts maintained as control. Reisolation of the pathogens from the artificially inoculated leaves yielded the fungi same as that in the respective original cultures.

4.4. Symptomatology

The study on symptomatology of die back disease under natural and artificial conditions revealed that the nature of symptoms varied with different pathogens viz., *Colletotrichum* sp. and *Botryodiplodia* sp. that were isolated from the die back infected plants. The leaf blight symptoms were also studied under natural and artificial conditions and slight variation in symptoms produced by *Colletotrichum* sp., *Pestalotiopsis* sp., *Cylindrocladium* sp., *Drechslera* sp. and *Alternaria* sp. was observed. But in different locations, the variation in symptoms produced by the same pathogens was not observed.

4.4.1. Symptomatology of die back disease

4.4.1.1. *Colletotrichum* sp.

The infection initiated as small necrotic spot on the tip of the shoot and at the base of leaf petiole. These spots later coalesced and developed large brownish black necrotic area. Gradually the infection spread downwards and reached up to a length of 2-3cm from the shoot tip. The internal tissues were also infected and showed brownish black discolouration. Initially the leaves were healthy and green in colour but later lost its vigour and drooped from the twig. Gradually the leaves dried up, shrivelled, rolled and became brownish black in colour and fell down from the infected twigs (Plate 2a).

Under *in planta* condition, black coloured streaks were developed on the twig, which were inoculated with pure culture as well as the spore suspension of the pathogen after giving pin prick. Later, these streaks coalesced and formed black necrotic lesions on the inoculated area.

4.4.1.2. *Botryodiplodia* sp.

Initial infection was observed as black discolouration on the shoot tip. Discolouration and darkening of bark was observed on young green twigs. These dark lesions were increased in size and later spread downwards and resulted in typical die back symptom. The infection was also spread to internal tissues and caused death of tissues. The upper leaves became unhealthy and gradually turned black, brittle and rolled upwards. In advanced stage, leaf shedding was observed. Occasionally black discolouration was observed on leaf margin, which later spread to petiole and young twigs and resulted in die back symptom. The pathogen produced its fruiting body, pycnidium on the infected area which appeared as dark dots (Plate 2b).

On artificial inoculation, it produced black necrotic lesions on young green twigs, which spread downwards and produced die back symptom. Black necrotic lesions were produced on leaves inoculated under *in vitro* and *in planta* conditions.

4.4.2. Symptomatology of leaf blight disease

4.4.2.1. *Colletotrichum* sp.

Young leaves were found highly susceptible to infection. The initial symptom incited by *Colletotrichum* sp. was the development of dark brown circular spot with yellow halo which coalesced to form elongated black necrotic patches. In advanced stages of infection, shot hole symptom was

Plate 2: Symptomatology of die back disease

a. *Colletotrichum* sp.

b. *Botryodiplodia* sp.

noticed. Other symptoms like drying and shedding of leaves were also observed. The development of acervuli was noticed on affected leaves as black dots (Plate 3a).

Under *in planta* condition, brown coloured necrotic spots were noticed on the leaves on four DAI. Small necrotic area was produced on leaves inoculated under *in vitro* condition.

4.4.2.2. *Pestalotiopsis* sp.

Mature leaves were found infected by *Pestalotiopsis* sp. The symptom was initiated on leaf margin and / leaf tip as light brown necrotic area with irregular dark brown margin. Later, it spread towards midrib as large blighted area with silvery grey coloured centre on upper surface and grey to brown coloured area on lower surface of leaves. Development of acervuli, the fruiting body of the pathogen was observed which appeared as dark dots on the blighted area (Plate 3b).

Artificial inoculation of *Pestalotiopsis* sp. on healthy leaves produced minute brown spots on the inoculated area on third DAI. Gradually, it increased in size and resulted in large blighted grey coloured area with dark brown margin under *in vitro* and *in planta* conditions.

4.4.2.3. *Cylindrocladium* sp.

Young leaves of mango grafts were found susceptible to *Cylindrocladium* sp. The symptoms appeared first as small circular discolouration which later developed in to small brown spots. Two or three spots were coalesced to form irregular blighted area with yellow halo on the leaves. Premature leaf shedding was noticed in severely infected plants (Plate3c).

The artificial inoculation of *Cylindrocladium* sp., on leaves produced dark brown necrotic area under *in planta* condition. Later, it enlarged and coalesced to form large blighted area on leaves. The same symptom was observed on leaves inoculated under *in vitro* condition. Young twig of mango grafts inoculated with this pathogen produced dark brown streaks on shoot tip, which spread downwards and resulted in death of young twig. The upper leaves drooped and dried up.

4.4.2.4. *Drechslera* sp.

Middle aged leaves were found infected by *Drechslera* sp. The symptom appeared as dark brown coloured spots on the tip of leaves, which later coalesced to form large brownish black coloured blighted area (Plate 3d).

The plants on artificial inoculation with *Drechslera* sp. initially produced small black coloured spots on the tip of leaves, which later enlarged and coalesced to form black coloured blighted area. The pathogen produced same type of symptom on artificially inoculated severed leaves.

4.4.2.5. *Alternaria* sp.

Symptoms produced by *Alternaria* sp. on mango leaves were appeared as small dark brownish irregular spots scattered all over the leaf lamina. The spots were enlarged and became irregular black coloured and produced large blighted area. Yellow halo was observed around the blighted area. The matured leaves were found infected by this organism (Plate 3e).

Under artificial inoculation of *Alternaria* sp., small black lesions were developed on leaves under *in planta* condition. Black coloured blighted area was also observed under *in vitro* condition.

Plate 3: Symptomatology of leaf blight disease

a. *Colletotrichum* sp.

b. *Pestalotiopsis* sp.

c. *Cylindrocladium* sp.

d. *Drechslera* sp.

e. *Alternaria* sp.

4.5. Identification of the pathogen

The pathogen isolated from die back and leaf blight diseases were grown on PDA medium and identified based on cultural and morphological characters.

4.5.1. Cultural characters of die back pathogens

Colony characters *viz.*, colour, shape, texture and growth of all isolates of die back pathogens were studied.

4.5.1.1. *Colletotrichum* sp.

Not much variation in the cultural characters of seven isolates of *Colletotrichum* sp. obtained from different locations was observed. The colony showed a medium rate of growth and attained 9cm growth in Petri dish in six DAI. Initially the colony was smooth in texture and greyish white in colour. Later, when it matured became thick and greyish black in colour. The reverse side of the colony was greyish black in colour. Sporulation of the culture was observed on third DAI. Pink coloured spore mass was observed on 10 DAI (Plate 4a). Development of acervuli was not noticed in all isolates of the pathogen.

4.5.1.2. *Botryodiplodia* sp.

Compared to other pathogens, colonies of *Botryodiplodia* sp. showed a fast growth and attained full growth in five DAI in 9cm diameter Petri dish. Initially the growth was feeble and grey in colour. Later, the colour changed to greyish black and the colony showed a fluffy aerial growth. Aggregation of grey coloured mycelium and production of pycnidia in the centre of the colony were observed on six DAI. The pycnidia were regular, round in shape, smooth

and greyish black in colour (Plate 4b). The reverse side of the colony was black.

4.5.2. Cultural characters of leaf blight pathogens

4.5.2.1. *Colletotrichum* sp.

The cultural characters of *Colletotrichum* sp. isolated from leaf blight symptom were same as that isolated from die back symptom.

4.5.2.2. *Pestalotiopsis* sp.

A medium rate of growth was noticed on PDA medium and completed full growth in Petri dish in six DAI. The young colony was pure white in colour and showed a thick growth on medium. On seven DAI, development of black spore masses was observed on the surface of pure white coloured colony (Plate 4c). The reverse side of the colony was white in colour.

4.5.2.3. *Cylindrocladium* sp.

The colony of *Cylindrocladium* sp. was slow growing on PDA medium and attained 9cm diameter growth in nine DAI at room temperature. Initially the culture showed a feeble white coloured growth with reddish brown tinge on aerial mycelium. Later, on ten DAI the growth became thick and white colour changed to reddish brown and small water soaked like patches developed on the cultural growth (Plate 4d). Reddish brown discolouration was observed on reverse side of the colony.

4.5.2.4. *Drechslera* sp.

Colony showed a medium rate of growth and attained 9cm diameter growth in six DAI at room temperature. Initially the colonies were greyish white in colour. Later turned to greyish black in colour and had a thick mycelial growth (Plate 4e). The reverse side of the colony was black in colour.

4.5.2.5. *Alternaria* sp.

The colony was slow growing compared to *Colletotrichum* sp. and attained full growth on Petri dish on seven DAI at room temperature. Initially the aerial mycelium was grey and later changed to greyish black in colour on eight DAI (Plate 4f). The colony became thick, smooth and the reverse side of the colony was black in colour.

4.5.3. Morphological characters of die back pathogens

The morphological characters of die back pathogens *viz. Colletotrichum* sp. and *Botryodiplodia* sp. were studied and the measurements of hyphae and conidia of different isolates of the pathogens are given in Table 4.

4.5.3.1. *Colletotrichum* sp.

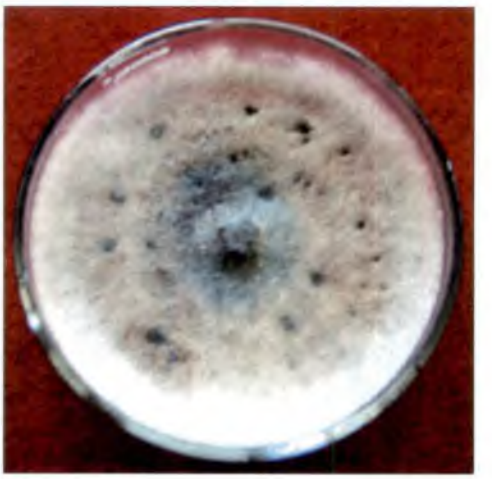
Slight variations in the morphological characters of seven different isolates of *Colletotrichum* sp. obtained from different locations were observed. The hyphae branched, hyaline with 2.88 - 3.26µm wide and septate at an interval of 17.66 -18.80µm. Conidia hyaline, cylindrical with both ends round, single celled, oil globules present and 11.9 -13.25 × 5 µm in size (Plate 5a, Fig.1). Based on the cultural and morphological characters, the organism was identified as *Colletotrichum gloeosporioides* (Penz) Sacc and was further confirmed by

Plate 4: Cultural characters of die back and leaf blight pathogens

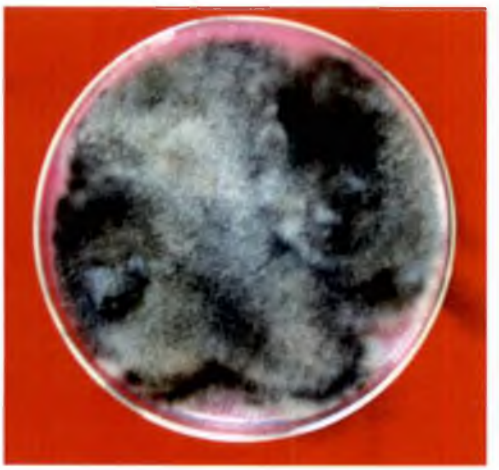
a. *Colletotrichum* sp.

b. *Botryodiplodia* sp.

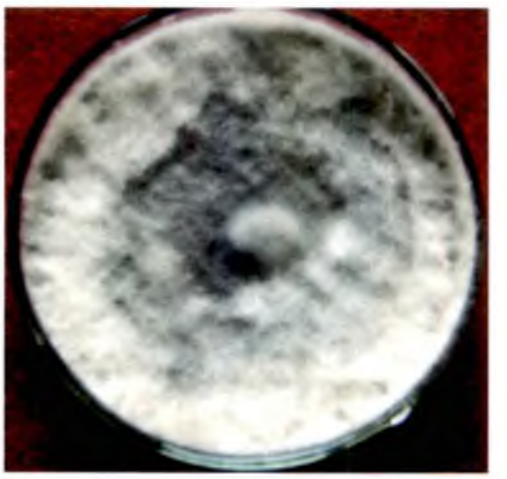
c. *Pestalotiopsis* sp.



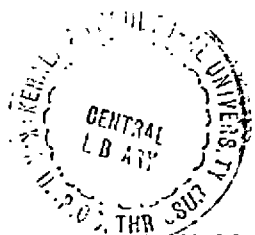
d. *Cylindrocladium* sp.



e. *Drechslera* sp.



f. *Alternaria* sp.



"National Center of Fungal Taxonomy", New Delhi (NCFT, No. 2169.08 - 2172.08).

4.5.3.2 *Botryodiplodia* sp.

Hyphae branched, young hyphae hyaline, when matured turned to brown, 5µm wide and septate at an interval of 17.5 - 35µm. Conidia initially unicellular, hyaline, oval shaped with round ends, matured conidia were brownish black in colour, uniseptate, thick walled and 15 - 25 × 12.5 - 17.5µm in size, pycnidia thick, black, ostiolate, produced spores inside on conidiophore (Plate 5b, Fig. 2). Based on these characters and on comparing the characters described by Subramanian (1971), the pathogen was identified as *Botryodiplodia theobromae* Pat and further confirmed by "National Center of Fungal Taxonomy", New Delhi (NCFT, No. 1770.07).

4.5.4 Morphological characters of leaf blight pathogens

The morphological characters of different pathogens isolated from leaf blight disease are given in Table 5.

4.5.4.1 *Colletotrichum* sp.

The morphological characters of the isolates of *Colletotrichum* sp. from die back and leaf blight diseases were found same. Hence only one isolate was used for further studies.

4.5.4.2 *Pestalotiopsis* sp.

Hyphae branched, hyaline, with 1.5 - 4µm wide and septate at an interval of 5 -18.5 µm. Conidia fusiform, thick walled, central three cells olivaceous brown, apical and basal cells thin walled and hyaline, 15-22.5µm long and

Table 4: Morphological characters of different isolates of die back pathogens

Sl. No	Isolates	Locations	Hyphal cell			Conidia		
			Length* (µm)	Width* (µm)	Colour	Length* (µm)	Width* (µm)	Colour
1.	*C.gloeosporioides*	CO	18.24	3.17	Hyaline	11.9	5.00	Hyaline
		NRG	17.76	3.07	Hyaline	13.25	5.00	Hyaline
		EASF	17.66	2.88	Hyaline	12.85	5.00	Hyaline
		KN	17.95	3.07	Hyaline	13.25	5.00	Hyaline
		SN	18.24	3.07	Hyaline	13.25	5.00	Hyaline
		SIN	18.24	3.17	Hyaline	12.35	5.00	Hyaline
		CN	18.80	3.26	Hyaline	13.0	5.00	Hyaline
2.	*B.theobromae*	ARS	17.5-35	5.00	Brown	15-25	12.5-17.5	Dark brown
		CO	17.5-35	5.00	Brown	15-25	12.5-17.5	Dark brown

* Mean of 50 observations

CO - College orchard
NRG - National Rose Garden
KN - Kairali Nursery
SN - Shalimar Nursery
EASF - Evangelical Social Action Forum
SIN - South Indian Nursery
ARS - Agricultural Research Station

7.5µm wide. The apical cell extended to form 2 and 3 appendages with 10-14 µm long, basal cell truncate with a single centrally inserted endogenous appendage (Plate 5c, Fig. 3). Based on the characters, the organism was identified as *Pestalotiopsis mangiferae* (Henn.) Steyaert and was later confirmed by "National Center of Fungal Taxonomy", New Delhi (NCFT, No. 1774.07).

4.5.4.3 *Cylindrocladium* sp.

Hyphae branched, reddish brown in colour, 2.4 - 4.8 µm wide and septate at an interval of 16.8 -120µm. Showed penicilliate branching of conidiophores with sterile filament; primary, secondary and tertiary branches hyaline and non septate, phialides hyaline, conidia cylindrical, septate and hyaline 36 × 4.8µm in size. Sterile filament ends in globular vesicle (Plate 5d, Fig. 4). Based on the characters the organism was identified as *Cylindrocladium mangiferae* sp. nov. and further confirmed by "National Center of Fungal Taxonomy", New Delhi (NCFT, No. 1772.07).

4.5.4.4 *Drechslera* sp.

Hyphae branched, hyaline, when matured turned to brownish black, 5µm wide and septate at an interval of 15 - 32.5µm in size. Conidiophores brown, septate and sympodula type (Plate 5e, Fig. 5). Conidia brown, thick walled, cylindrical and straight with round ends. 3 - 6 septate, 7.5 - 25 × 5 - 7.5µm in size. Based on the characters the organism was identified as *Drechslera australiensis* (Bugnicourt) Subram & Jain ex M.B.Ellis and further confirmed by "National Center of Fungal Taxonomy", New Delhi (NCFT, No. 1771.07).

4.5.4.5 *Alternaria* sp.

Hyphae branched, initially hyaline turned to brown, with 5µm wide, septate at an interval of 15-30µm. Condiophores produced singly in culture, brown and septate. Conidia formed in single, brown, obclavate, straight, 25 - 60 × 7.5 - 15 µm in size with 2 - 6 transverse and 1 - 2 longitudinal septa, beak prominent, 1-2 septate and 5 - 32.5µm long (Plate 5f, Fig. 6). Based on these characters the organism was identified as *Alternaria alternata* (Fries) Keissler.

4.5.5 Cluster analysis of different isolates of *C. gloeosporioides* based on morphological characters

Genetic dissimilarity index (DI) of seven different isolates of *C.gloeosporioides* was computed from morphological characters as Euclidean co-efficient using NTSYS pc 2.02 soft ware. The results are presented in Table 6. The dendrogram was constructed by using Unweighed Pair Group Average Method (UPGMA) as shown in Fig. 7. The lowest dissimilarity of 0.19 was observed between two isolates obtained from Kairali and National rose garden. It was followed by 0.29 between the isolates of Kairali and Shalimar nursery. The highest dissimilarity of 1.48 was observed between isolates obtained from College orchard and National rose garden followed by 1.43 between isolates from College orchard and Kairali. The isolates were grouped in to two clusters A and B. In cluster A the highest dissimilarity co efficient of 1.22 was recorded in isolates obtained from Central nursery and EASF. Cluster A was further divided in to two sub clusters A_1 and A_2. In sub cluster A_2 the highest dissimilarity co efficient of 0.53 was recorded in isolates obtained from Kairali and EASF.

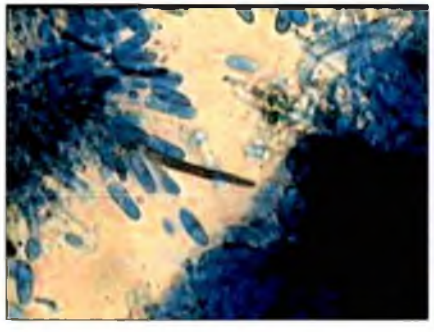
Table 5: Morphological characters of different isolates of leaf blight pathogens

Sl. No	Organisms	Hyphal cell (µm)		Conidia (µm)		Appendage (µm)
		Length*	Width *	Length*	Width *	Length*
1	*C.gloeosporioides*	3.07	17.5	5.00	12.85	-
2	*P.mangiferae*	5-18.5	1.5-4	15-22.5	7.5	10-14
3	*C.mangiferae*	16.8-120	2.4-4.8	36.00	4.8	-
4	*D.australiensis*	15-32.5	5.00	7.5-25	5-7.5	-
5	*A.alternata*	15-30	5.00	25-60	7.5-15	-

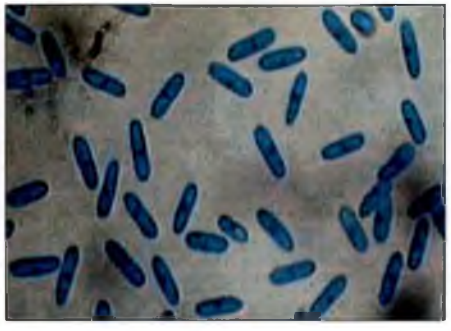
* Mean of. 50 observations

Plate 5: Morphological characters of die back and leaf blight pathogens

a. *C. gloeosporioides*

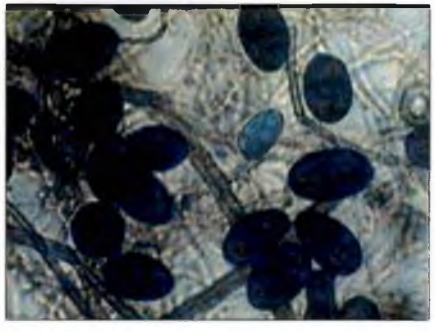


Acervulus



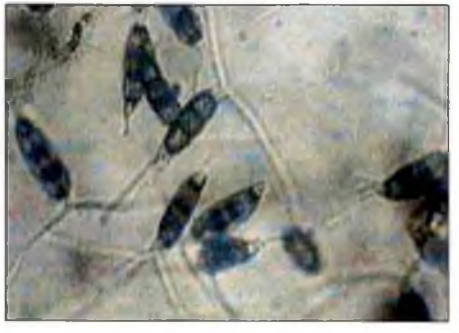
Conidia

b. *B. theobromae*



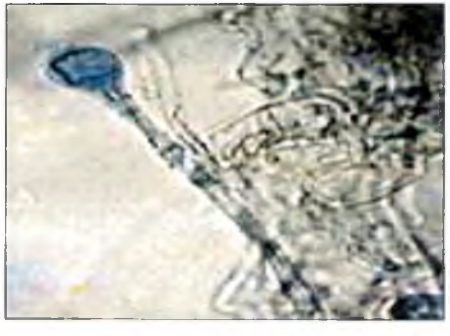
Conidia

c. *P.mangiferae*



Conidia

d. *C. mangiferae*



Sterile filament

Conidial apparatus

e. *D. australiensis*

Conidiophore and Conidia

f. *A. alternata*

Conidia

Fig. 1: Hyphae and conidia of *C. gloeosporioides*

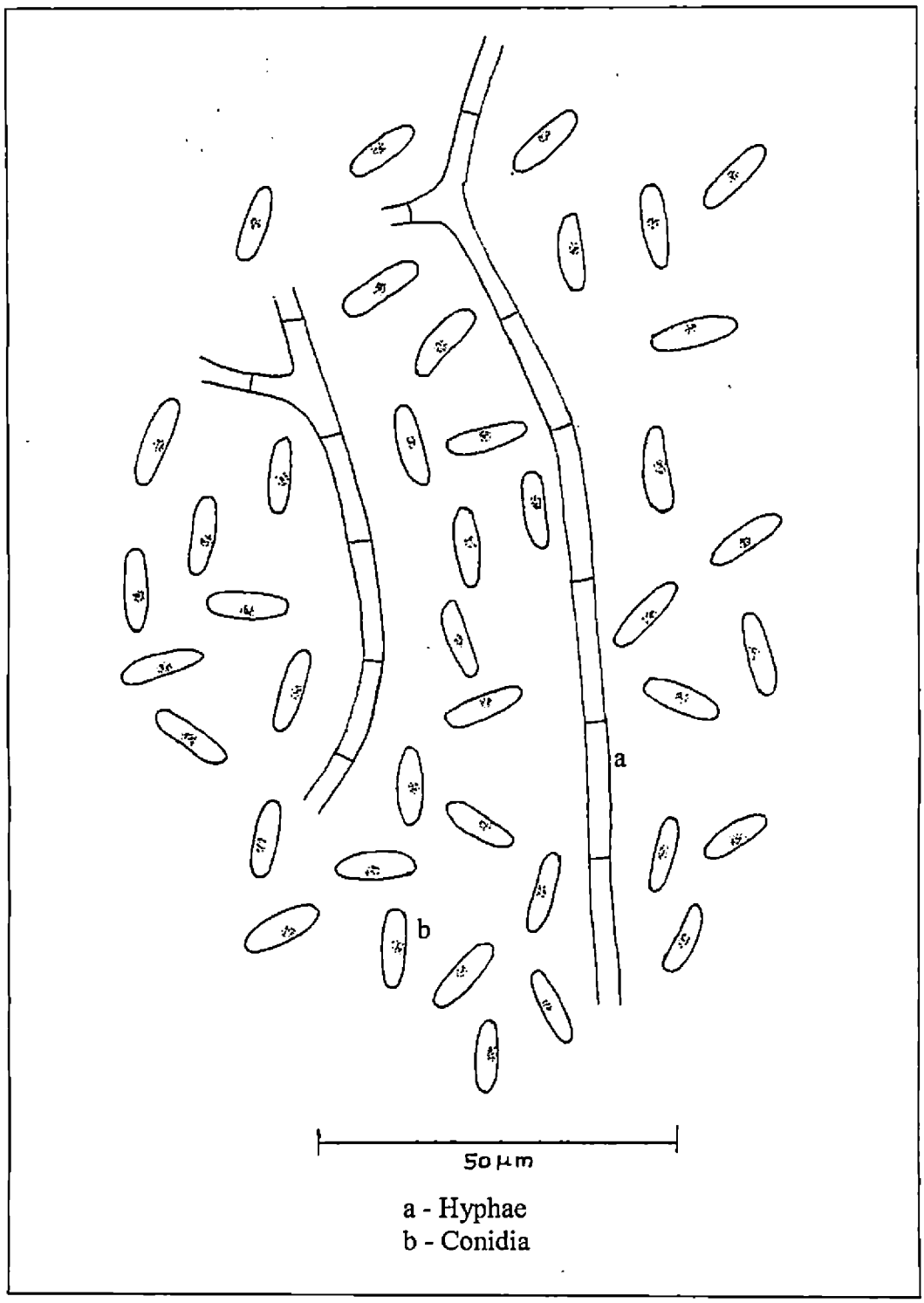
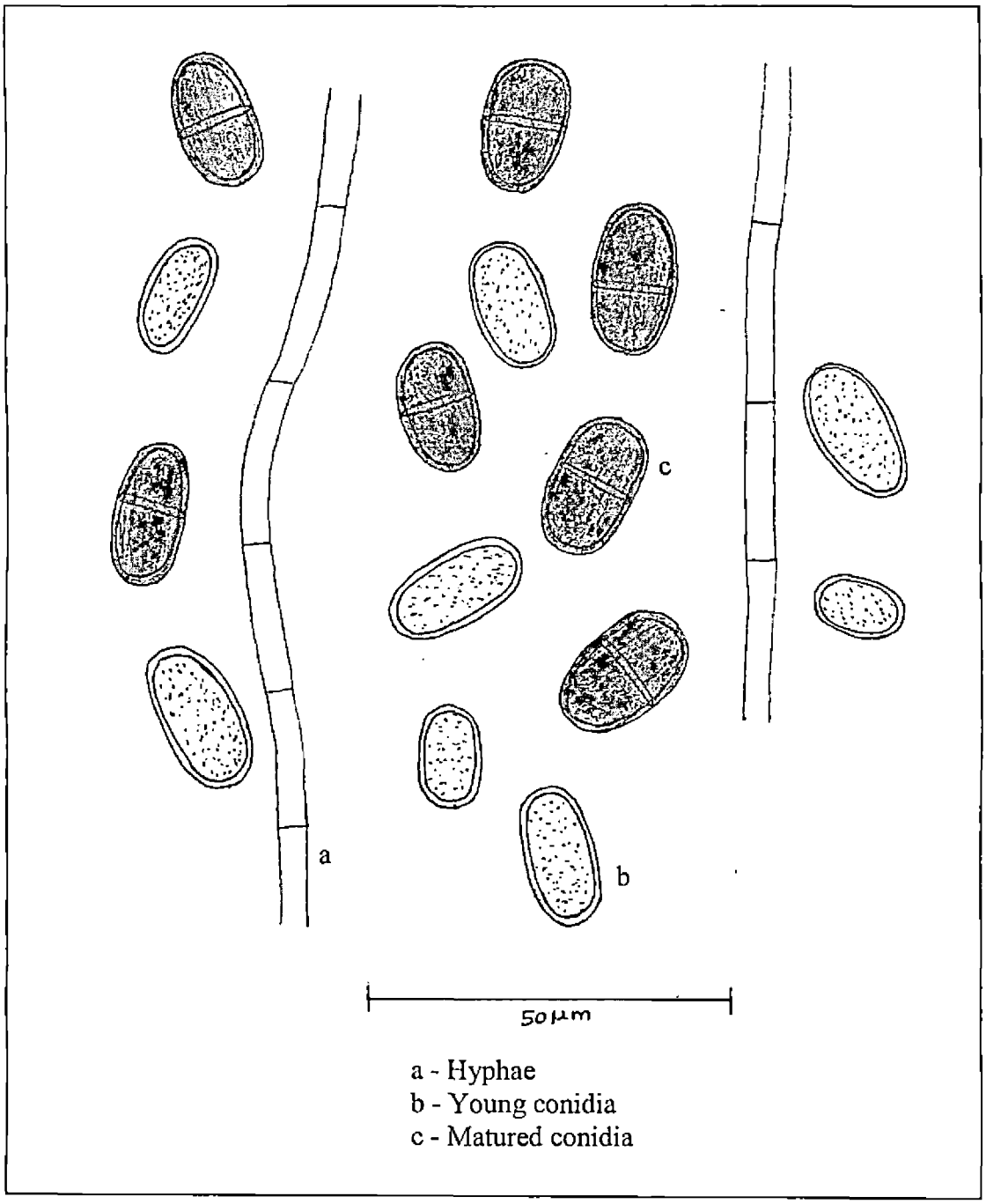
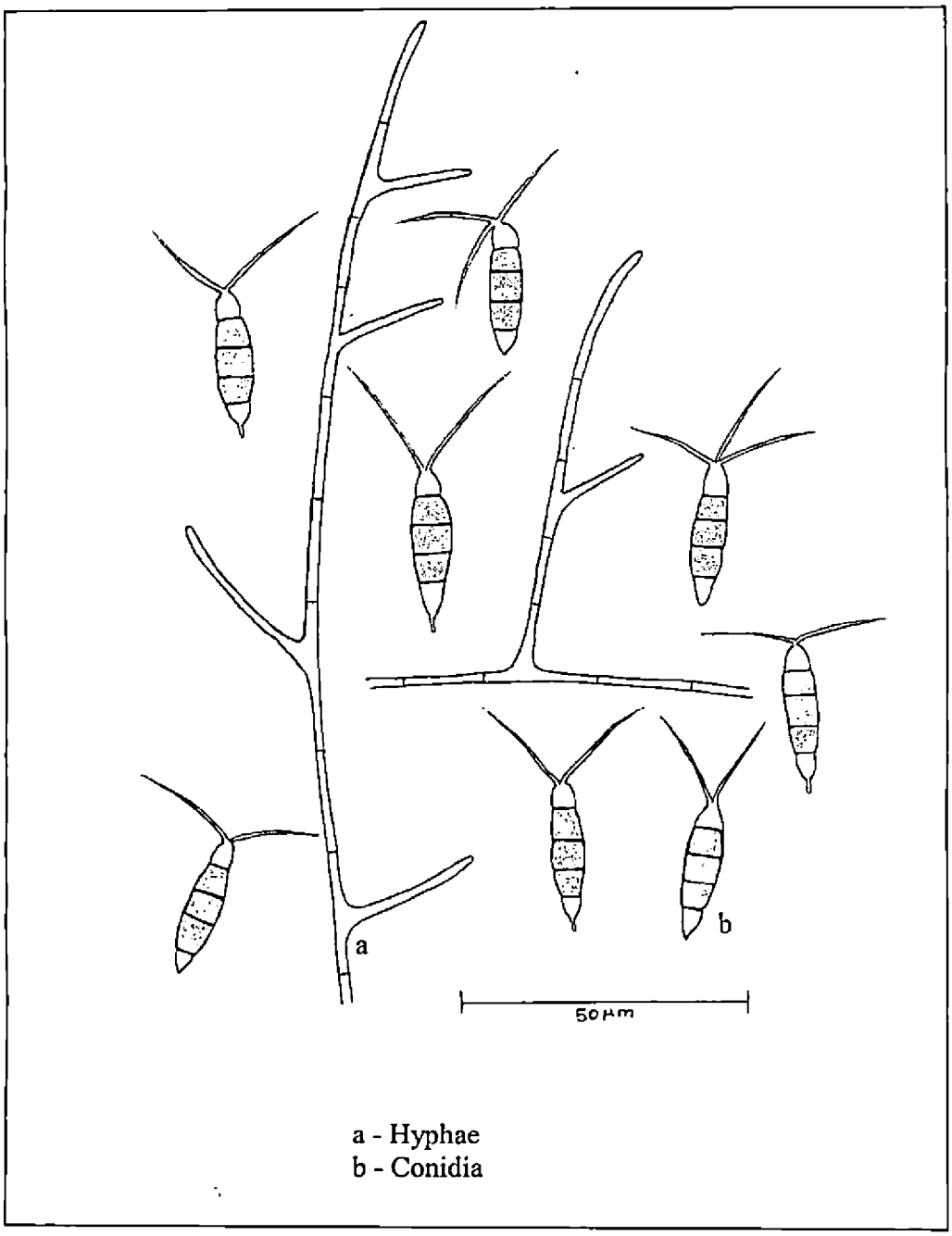


Fig. 2 : Hyphae and conidia of *B. theobromae*



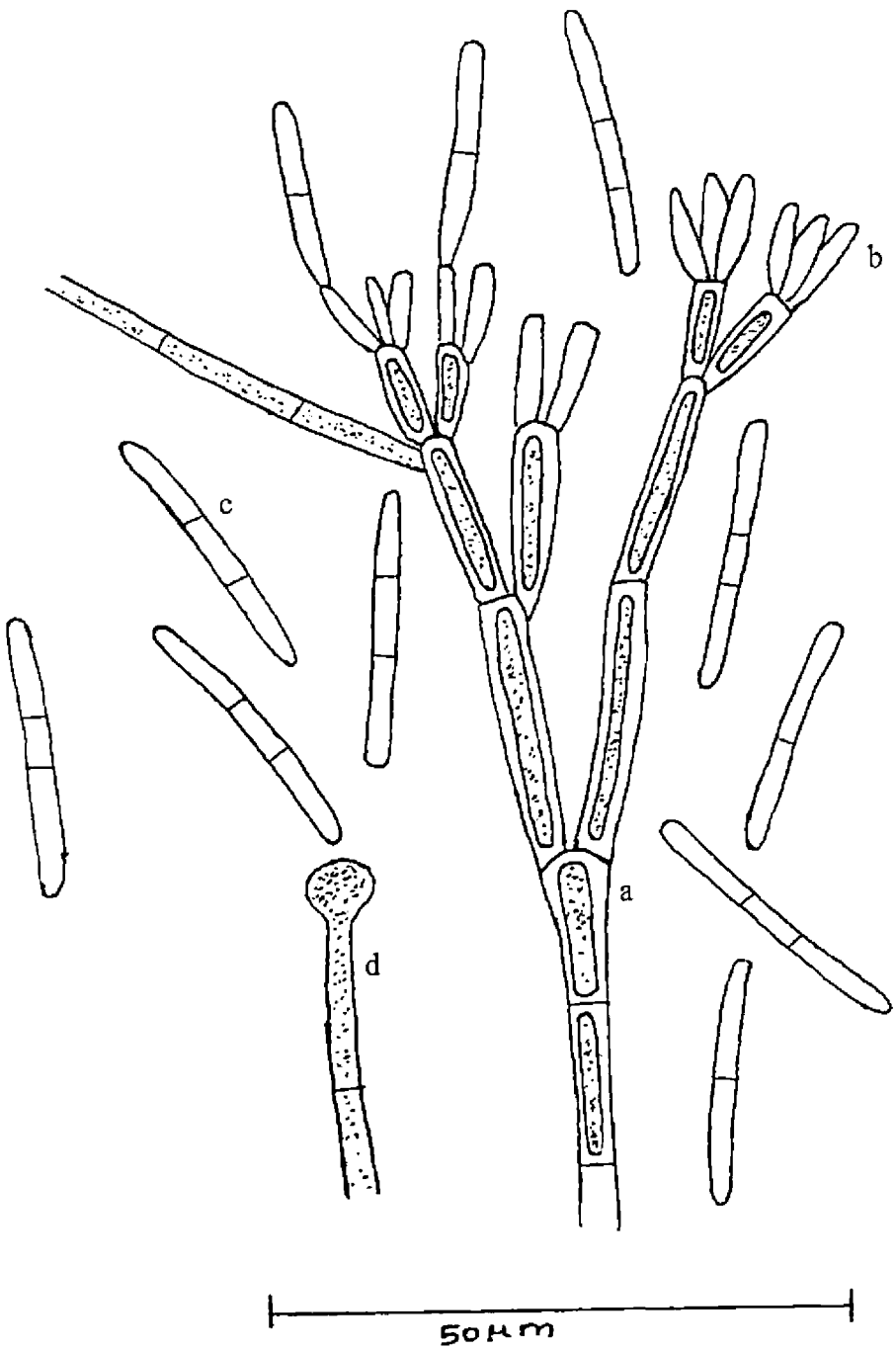
a - Hyphae
b - Young conidia
c - Matured conidia

Fig. 3: Hyphae and conidia of *P. mangiferae*



a - Hyphae
b - Conidia

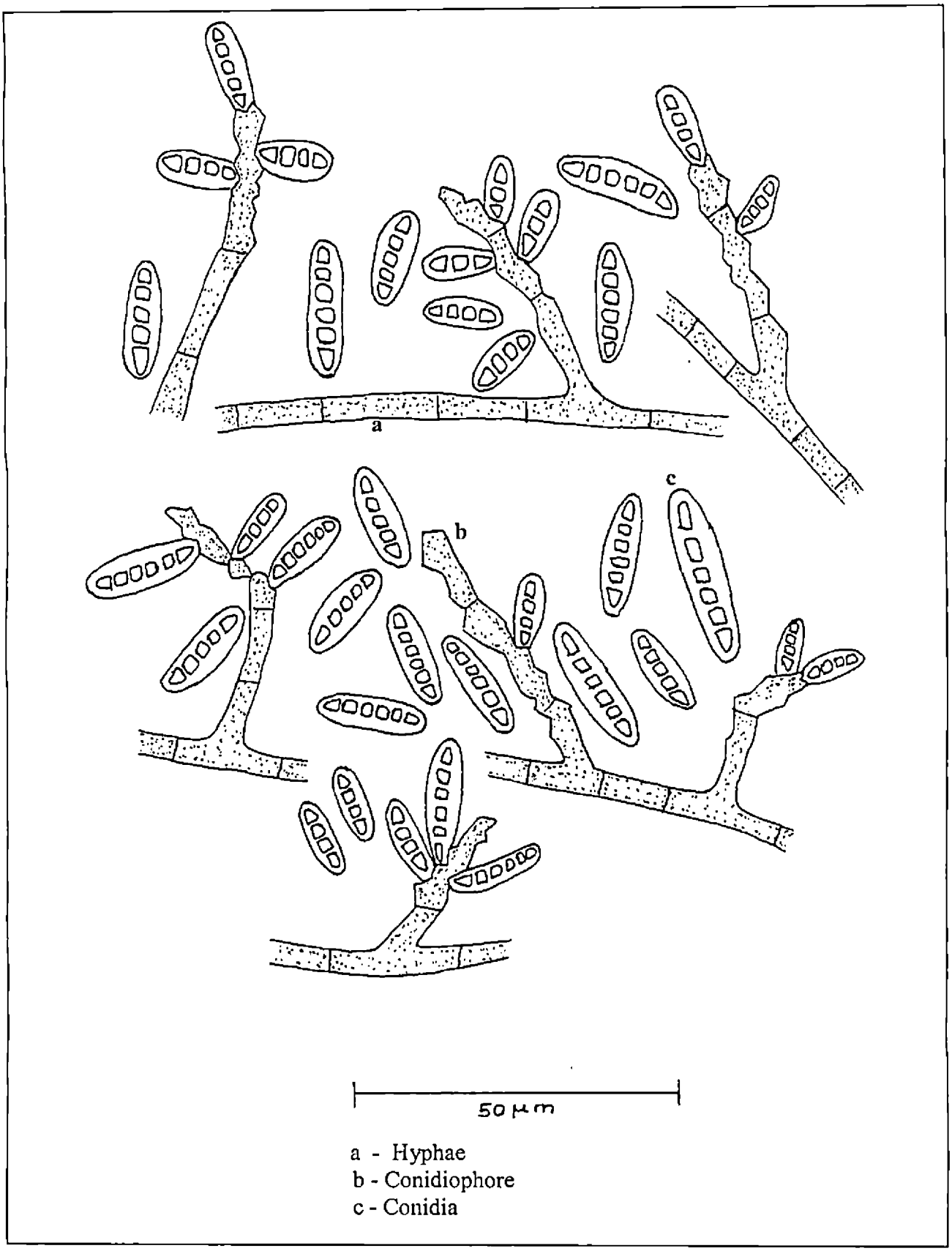
Fig. 4: Conidial apparatus of *C. mangiferae*



a - Conidiophore b - Phialides

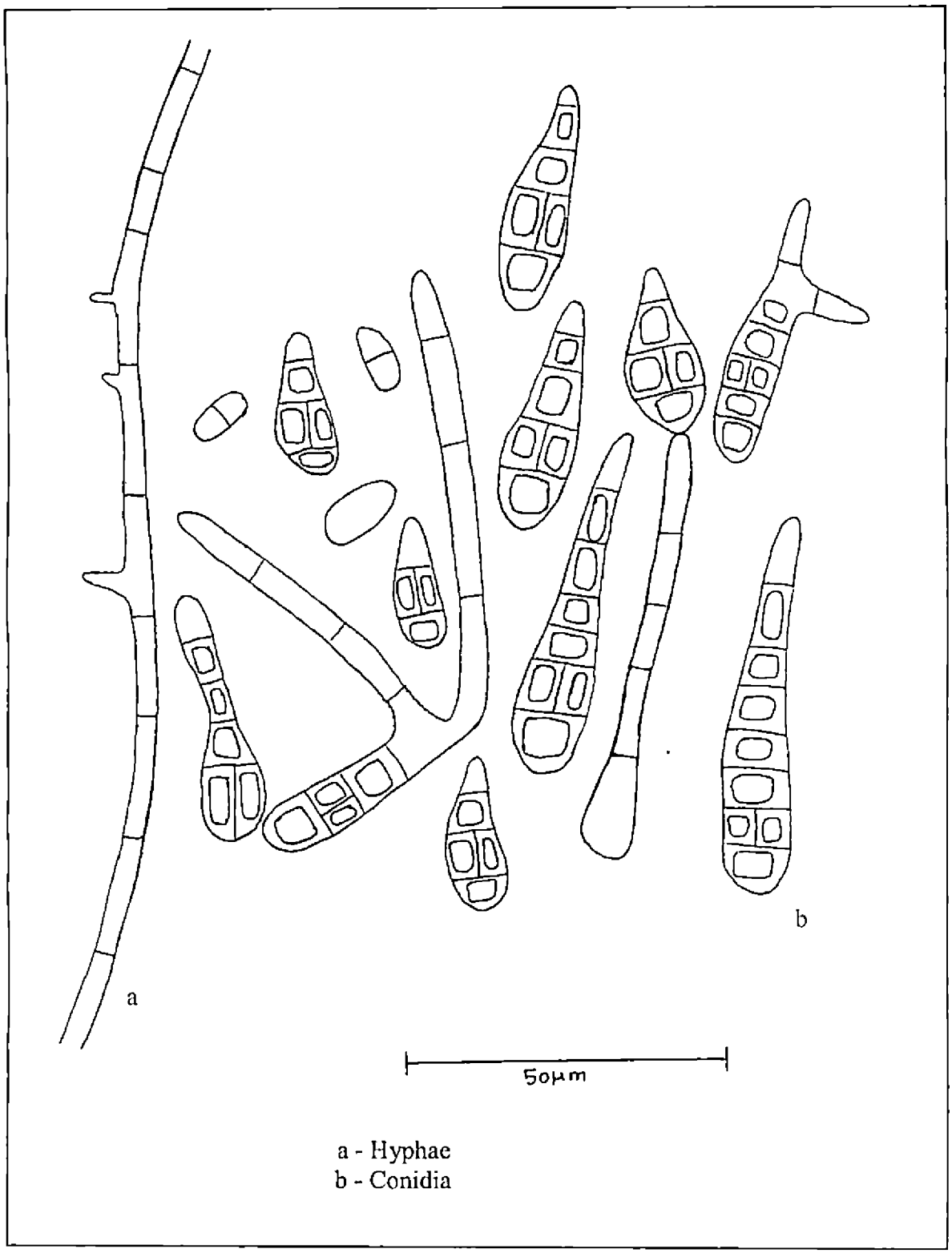
c - Conidia d - Sterile filament

Fig. 5: Hyphae, conidiophore and conidia of *D. australiensis*



a - Hyphae
b - Conidiophore
c - Conidia

Fig. 6: Hyphae and conidia of *A. alternata*

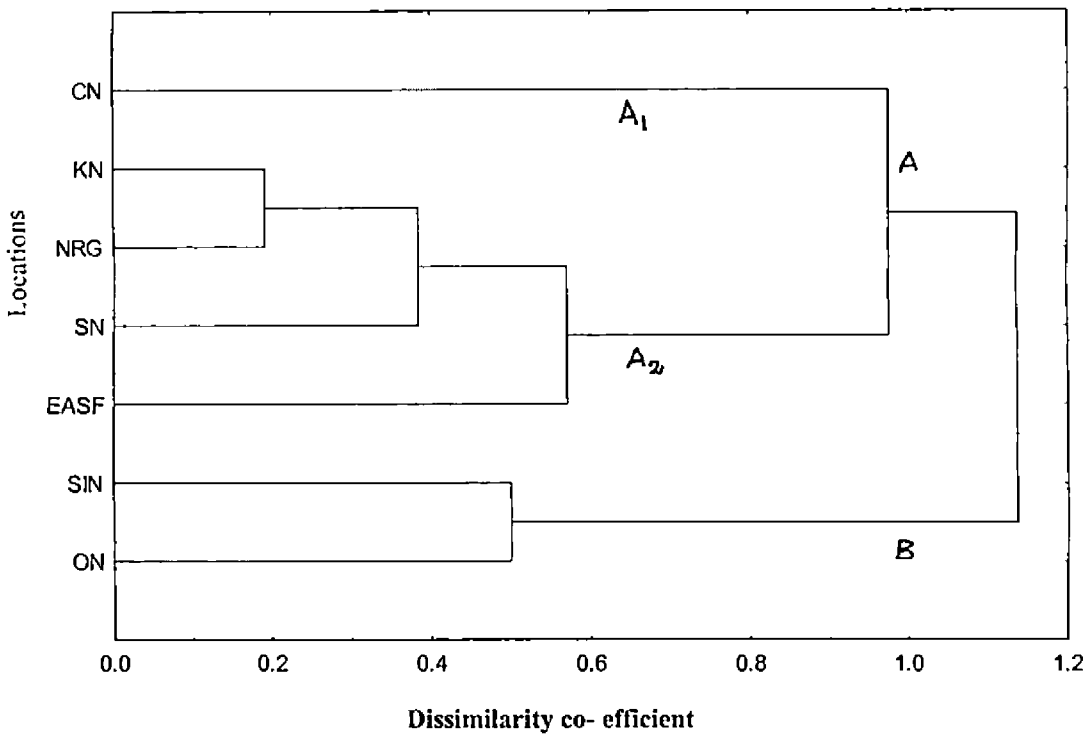


a - Hyphae
b - Conidia

Table 6: Dissimilarity matrix of *C. gloeosporioides* isolates based on morphological characters

Sl.No	Locations	CN	SIN	CO	KN	SN	EASF	NRG
1	CN	0.00						
2	SIN	0.87	0.00					
3	CO	1.29	0.50	0.00				
4	KN	0.92	0.95	1.43	0.00			
5	SN	0.66	0.91	1.40	0.29	0.00		
6	EASF	1.22	0.82	1.19	0.53	0.73	0.00	
7	NRG	1.10	1.02	1.48	0.19	0.48	0.45	0.00

Fig. 7: Cluster analysis of different isolates of *C. gloeosporioides*



4.6 Management of die back and leaf blight diseases

The efficacy of chemicals and antagonistic organisms against the die back pathogens were evaluated under *in vitro* and *in planta* conditions. Along with that the evaluation of leaf blight pathogens were also carried out. The results of the *in vitro* evaluations are given in Table 7 to 15.

4.6.1 *In vitro* evaluation of fungicides against die back pathogens

4.6.1.1 *C. gloeosporioides*

The inhibitory efficacy of different fungicides at three different concentrations on the growth of *C. gloeosporioides* is given in Table 7. From the data, it is evident that the treatments differed significantly with each other in their efficiency in inhibiting the growth of *C. gloeosporioides*. Among the eight fungicides tested, Bordeaux mixture and carbendazim at all three concentrations recorded cent per cent inhibition on the growth of *C.gloeosporioides*. So the observations taken in these two treatments (Bordeaux mixture and carbendazim) were not included in the statistical analysis since these treatments were proved as superior than all other treatments. In the remaining treatments, the maximum inhibition on the growth of pathogen was recorded by the highest concentration of all fungicides. Hexaconazole at 0.15 per cent concentration was found to be the best among the remaining fungicides, which recorded 81.4 per cent inhibition over control. But it was on par with hexaconazole at 0.1 and 0.05 per cent concentrations recording 80.2 and 77.0 per cent inhibition respectively. All the three concentrations of copper oxychloride, mancozeb and zineb recorded less than 50 per cent inhibition of pathogen over control. Among them, zineb at all the three concentrations were found less effective to the pathogen. Zineb at 0.3 per

Table 7 : *In vitro* evaluation of fungicides against *C. gloeosporioides*

Tr. No	Treatments	Concentrations (Per cent)	Colony diameter (mm)* DAI				Per cent inhibition over control
			2	4	6	7	
1	Bordeaux † mixture	0.5	0	0	0	0	100
		1.0	0	0	0	0	100
		1.5	0	0	0	0	100
2	Copper oxychloride	0.2	17.3^{bc}	40.0^{b}	52.3^{de}	52.0^{d}	42.2
		0.3	13.3^{efg}	36.2^{c}	50.5^{e}	52.0^{d}	42.2
		0.4	11.0^{hi}	31.3^{de}	48.3^{e}	49.0^{d}	45.6
3	Copper hydroxide	0.05	13.7^{ef}	22.7^{f}	31.7^{g}	33.3^{f}	63.0
		0.15	11.7^{ghi}	21.5^{f}	27.8^{gh}	29.3^{fg}	67.4
		0.25	11.2^{hi}	15.0^{ghi}	24.2^{h}	24.7^{h}	72.6
4	Mancozeb	0.2	14.0^{e}	32.8^{d}	55.3^{cd}	58.7^{c}	34.8
		0.3	12.2^{fgh}	29.7^{e}	50.8^{e}	53.2^{d}	40.9
		0.4	11.7^{ghi}	30.5^{de}	49.5^{e}	50.7^{d}	43.7
5	Captan	0.2	13.5^{efg}	20.8^{f}	31.7^{g}	38.3^{e}	68.5
		0.3	11.8^{ghi}	17.0^{g}	33.3^{g}	33.0^{f}	63.3
		0.4	11.7^{ghi}	16.0^{gh}	23.7^{h}	25.7^{gh}	71.4
6	Hexaconazole	0.05	10.3^{hi}	13.0^{hij}	17.8^{i}	20.7^{i}	77.0
		0.1	10.0^{i}	12.5^{ij}	15.7^{i}	17.8^{i}	80.2
		0.15	10.0^{i}	10.5^{j}	15.2^{i}	16.7^{i}	81.4
7	Carbendazim †	0.05	0	0	0	0	100
		0.1	0	0	0	0	100
		0.2	0	0	0	0	100
8	Zineb	0.2	18.2^{b}	42.7^{b}	64.3^{b}	66.7^{b}	25.9
		0.3	16.0^{cd}	42.3^{b}	64.7^{b}	66.8^{b}	25.8
		0.4	15.0^{de}	35.8^{c}	58.5^{c}	60.3^{c}	33.0
9	Control	-	29.7^{a}	60.8^{a}	83.0^{a}	90.0^{a}	-

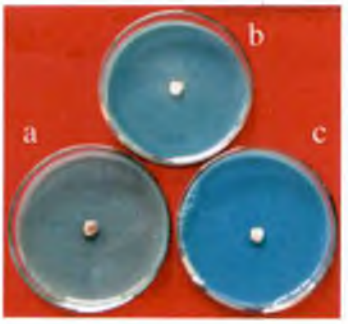
DAI - Days After Inoculation

* Mean of three replications

† - Not included in statistical analysis

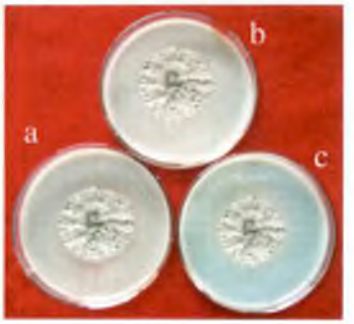
In each column figures followed by same letter do not differ significantly according to DMRT

Plate 6: *In vitro* evaluation of fungicides against *C. gloeosporioides*



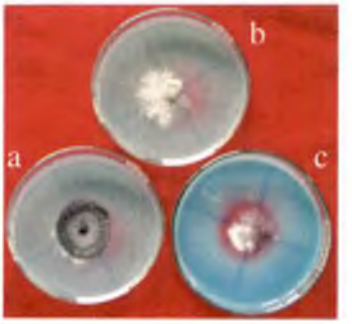
Bordeaux mixture

a - 0.5% conc
b -1.0 % conc
c - 1.5 % conc



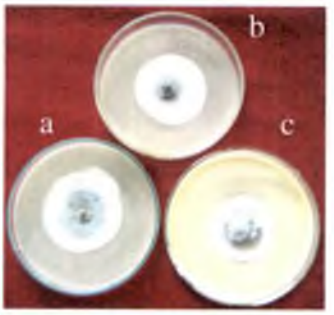
Copper oxychloride

a - 0.2 % conc
b - 0.3 % conc
c - 0.4 % conc



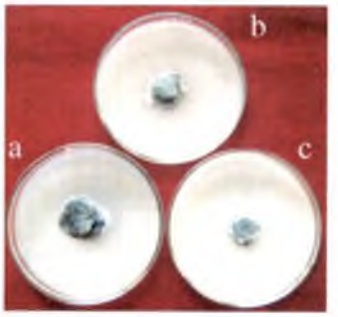
Copper hydroxide

a - 0.05 % conc
b - 0.15 % conc
c - 0.25 % conc



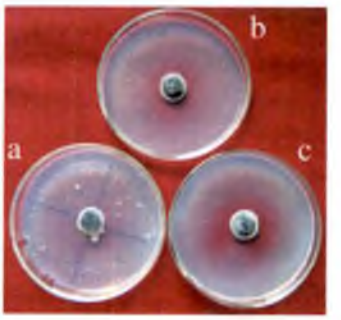
Mancozeb

a - 0.2 % conc
b - 0.3 % conc
c - 0.4 % conc



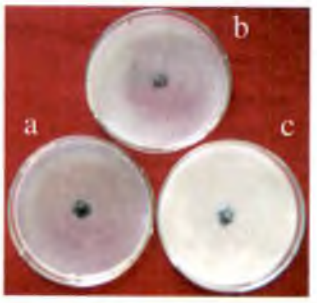
Captan

a - 0.2 % conc
b - 0.3 % conc
c - 0.4 % conc



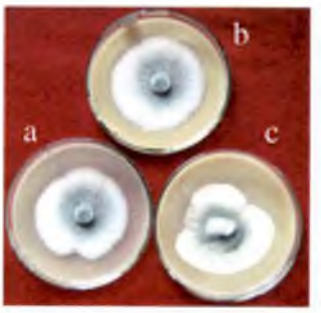
Hexaconazole

a - 0.05 % conc
b - 0.1 % conc
c - 0.15 % conc



Carbendazim

a - 0.05 % conc
b - 0.1 % conc
c - 0.2 % conc

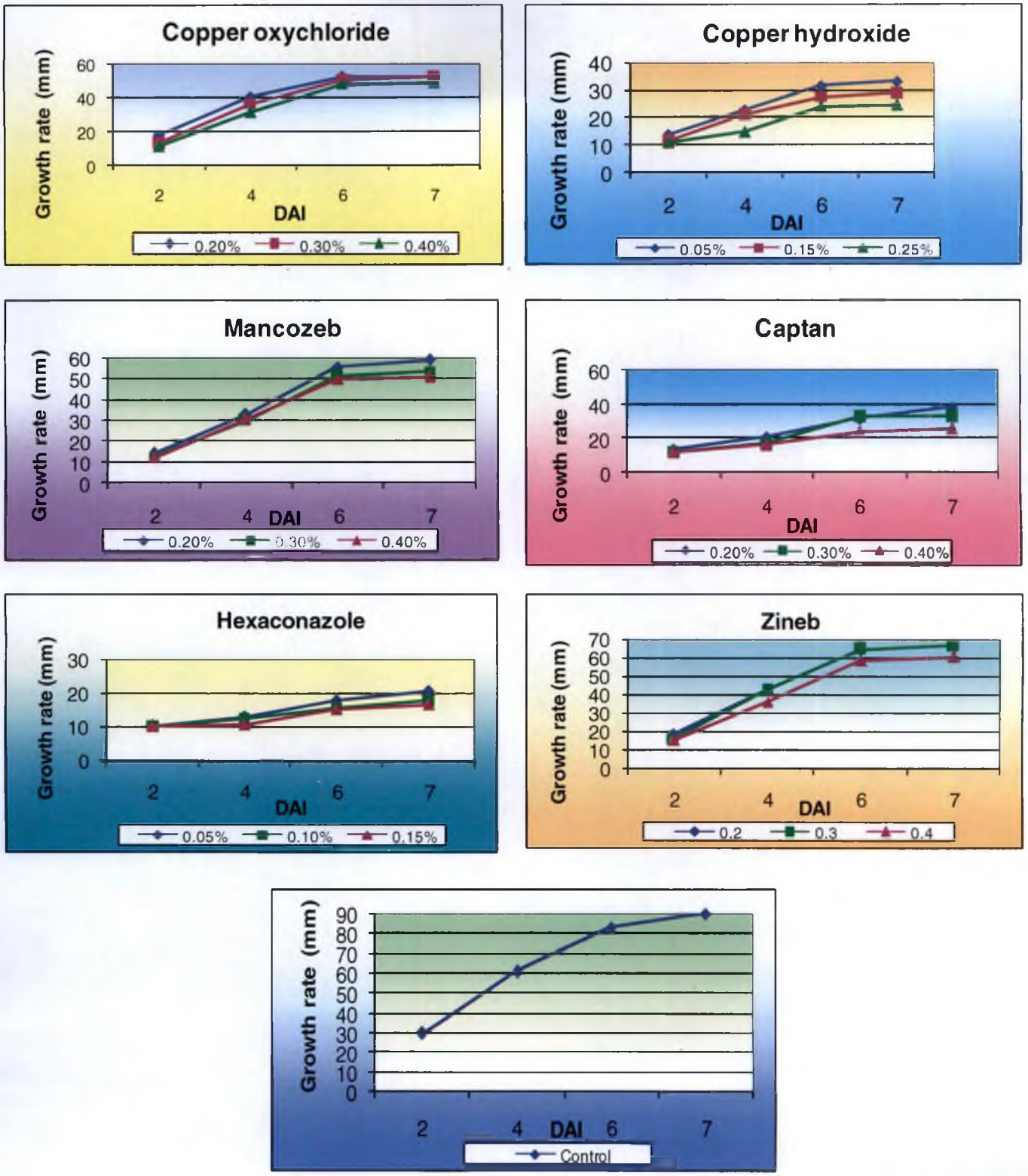


Zineb

a - 0.2 % conc
b - 0.3 %conc
c - 0.4 %conc

Control

Fig 8: *In vitro* evaluation of fungicides against *C. gloeosporioides*



cent concentration recorded the least inhibition of 25.8 per cent over control (Plate 6, Fig. 8).

4.6.1.2 *B. theobromae*

From the data given in Table 8, it was found that the treatments differed significantly with each other in their efficiency against the growth of *B. theobromae*. Bordeaux mixture at all the concentrations recorded cent per cent inhibition of pathogen over control. Hence this treatment was not included in statistical analysis. Among the remaining treatments carbendazim 0.1 and 0.2 per cent concentration showed cent per cent inhibition of the pathogen and was followed by carbendazim 0.05 per cent concentration. It recorded 76.9 per cent inhibition over control, which was significantly superior to all the concentrations of all other fungicides. All the three concentrations of hexaconazole, copper oxychloride 0.3 and 0.4 per cent, copper hydroxide 0.25 per cent and captan 0.3 and 0.4 per cent concentrations recorded more than 50 per cent inhibition of pathogen over control. Mancozeb and zineb 0.2 per cent concentration recorded the least inhibition of growth (35.6 per cent) over control (Plate 7, Fig. 9).

4.6.2 *In vitro* evaluation of fungicides against leaf blight pathogens

4.6.2.1 *C. gloeosporioides*

The isolates of *C. gloeosporioides* obtained from leaf blight disease was found to be the same as that of die back pathogen, and hence separate evaluation of this isolate was not carried out.

Table 8: *In vitro* evaluation of fungicides against *B. theobromae*

Tr. No	Treatments	Concentrations (Per cent)	Colony diameter (mm)*			Per cent inhibition over control
			DAI			
			2	4	6	
1	Bordeaux † mixture	0.5	0	0	0	100
		1.0	0	0	0	100
		1.5	0	0	0	100
2	Copper oxychloride	0.2	23.5^{hi}	37.7^{efg}	45.3^{ef}	49.7
		0.3	21.8^{hi}	34.0^{gh}	42.8^{fg}	52.4
		0.4	20.7^{ijk}	31.5^{hi}	38.3^{hi}	57.4
3	Copper hydroxide	0.05	23.7^{h}	37.5^{efg}	46.7^{def}	48.1
		0.15	21.3^{hij}	36.8^{efg}	45.7^{ef}	49.2
		0.25	18.3^{kl}	29.0^{ij}	40.7^{gh}	54.8
4	Mancozeb	0.2	34.7^{cd}	45.7^{bc}	58.0^{b}	35.6
		0.3	31.0^{ef}	42.8^{cd}	50.2^{cd}	44.2
		0.4	28.7^{fg}	37.7^{efg}	46.3^{ef}	48.6
5	Captan	0.2	30.2^{efg}	38.5^{ef}	46.8^{de}	48.0
		0.3	29.2^{fg}	36.5^{fg}	44.8^{ef}	50.2
		0.4	27.3^{b}	35.5^{fg}	43.3^{efg}	51.9
6	Hexaconazole	0.05	20.8^{hijk}	28.8^{ij}	40.5^{gh}	55.0
		0.1	18.5^{jkl}	27.3^{j}	35.7^{i}	60.3
		0.15	16.3^{l}	23.3^{k}	31.3^{j}	65.2
7	Carbendazim	0.05	10.7^{m}	14.8^{l}	20.8^{k}	76.9
		0.1	10.0^{m}	10.0^{m}	10.0^{l}	100
		0.2	10.0^{m}	10.0^{m}	10.0^{l}	100
8	Zineb	0.2	38.8^{b}	49.0^{b}	58.0^{b}	35.6
		0.3	36.0^{c}	40.7^{de}	50.7^{c}	43.7
		0.4	32.5^{de}	40.7^{de}	47.0^{de}	47.8
9	Control	-	46.0^{a}	82.5^{a}	90.0^{a}	-

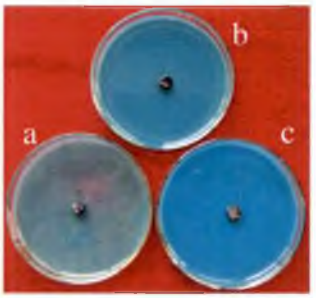
DAI - Days After Inoculation

* Mean of three replications

† - Not included in statistical analysis

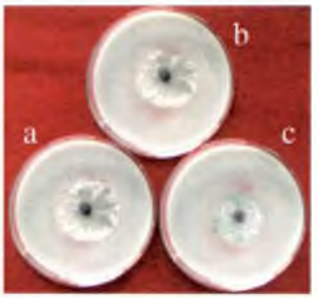
In each column figures followed by same letter do not differ significantly according to DMRT

Plate 7: *In vitro* evaluation of fungicides against *B. theobromae*



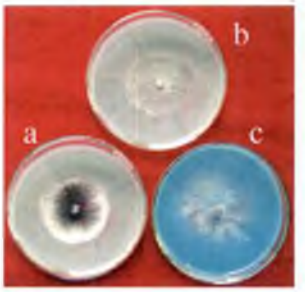
Bordeaux mixture

a - 0.5% conc
b -1.0 % conc
c - 1.5% conc



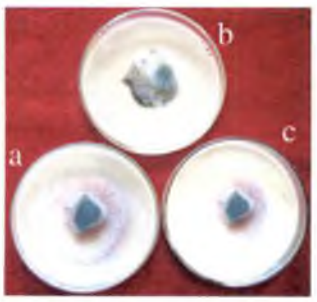
Copper oxychloride

a - 0.2 % conc
b - 0.3 % conc
c - 0.4% conc



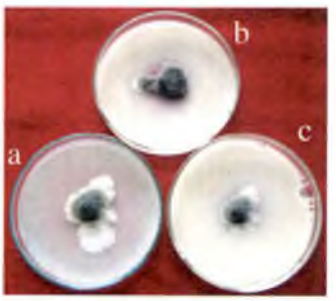
Copper hydroxide

a - 0.05% conc
b - 0.15 % conc
c -0.25 % conc



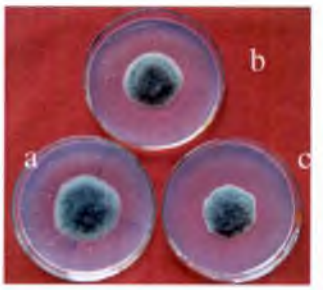
Mancozeb

a - 0.2 % conc
b - 0.3 % conc
c - 0.4 % conc



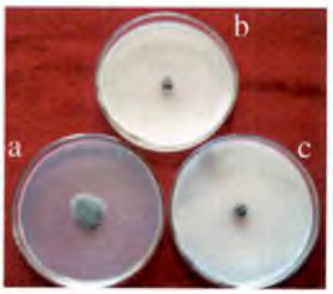
Captan

a - 0.2 %conc
b - 0.3 % conc
c - 0.4 %conc



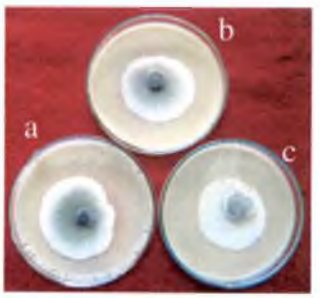
Hexaconazole

a - 0.05 % conc
b - 0.1 % conc
c - 0.15 % conc



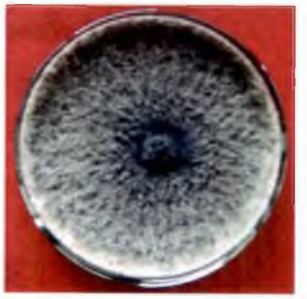
Carbendazim

a - 0.05 % conc
b - 0.1 % conc
c - 0.2 %conc

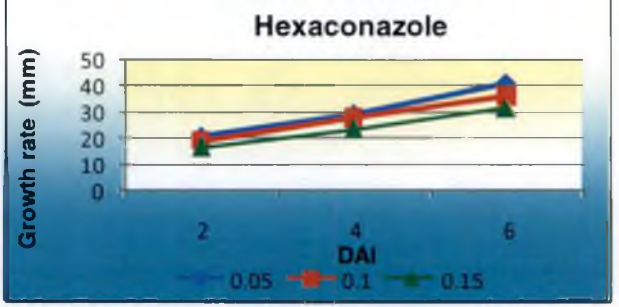


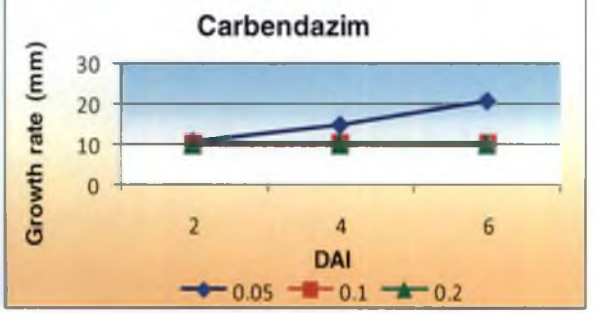
Zineb

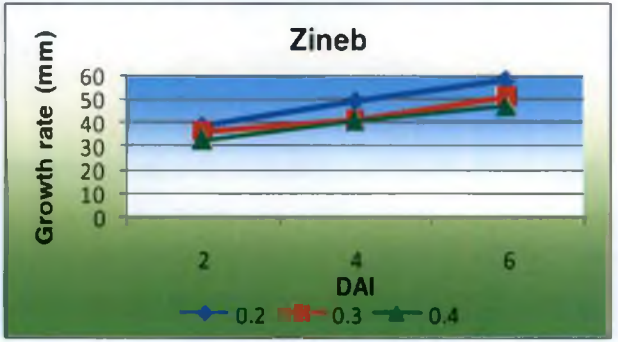
a - 0.2 % conc
b - 0.3 % conc
c - 0.4 % conc



Control


Hexaconazole
Growth rate (mm)
50
40
30
20
10
0
2
4
6
DAI
0.05
0.1
0.15


Carbendazim
Growth rate (mm)
30
20
10
0
2
4
6
DAI
0.05
0.1
0.2


Zineb
Growth rate (mm)
60
50
40
30
20
10
0
2
4
6
DAI
0.2
0.3
0.4

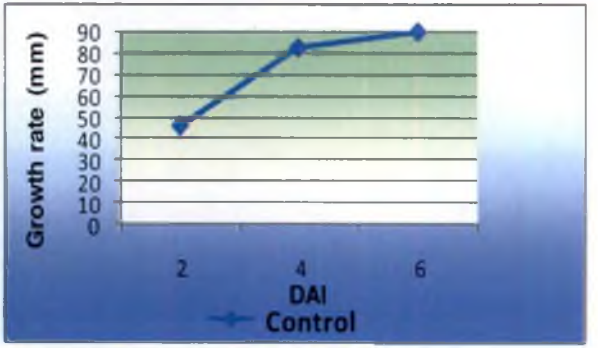
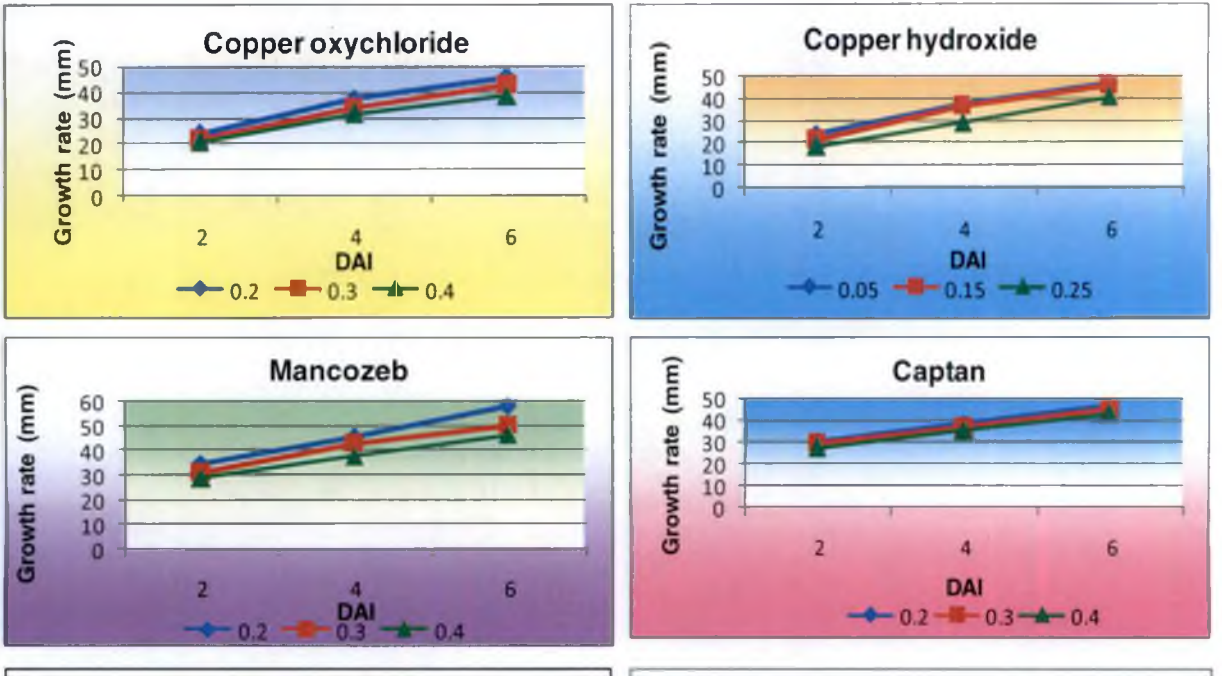

Growth rate (mm)
90
80
70
60
50
40
30
20
10
0
2
4
6
DAI
Control

Fig 9: *In vitro* evaluation of fungicides against *B. theobromae*



4.6.2.2 *P. mangiferae*

From the data furnished in Table 9, statistical difference among the treatments in their efficiency against the growth of *P. mangiferae* was observed. Bordeaux mixture and carbendazim at all three concentrations recorded cent per cent inhibition on the growth of the pathogen and hence they were not included in statistical analysis. Among the other fungicides, the maximum inhibition of 85.3 per cent over control was recorded by captan at 0.4 per cent concentration. But it was on par with the other two concentrations of captan (0.2 and 0.3 per cent), copper hydroxide 0.25per cent, zineb 0.4 per cent, hexaconazole and mancozeb at all the three concentrations. Copper hydroxide at 0.05 per cent, recorded the lowest inhibition over control (11.6 per cent).

4.6.2.3 *C. mangiferae*

The data on the inhibitory effect of different fungicides on *C. mangiferae* are given in Table 10. The statistical analysis of data showed significant difference among the treatments on their inhibitory effect on the growth of the pathogen. Bordeaux mixture and carbendazim at all concentrations completely inhibited the growth of *C. mangiferae*. So these two treatments were not included in the statistical analysis. Among the other fungicides, copper hydroxide 0.25 per cent concentration recorded the maximum inhibition of the pathogen (84.6 per cent) over control. It was also on par with 0.15per cent of copper hydroxide where the growth inhibition over control was recorded as 78.5 per cent. All the three concentrations of copper oxychloride, captan, zineb, copper hydroxide 0.05 per cent and hexaconazole 0.05 and 0.1 per cent and mancozeb 0.2 and 0.3 per cent recorded less than 50 per cent inhibition of pathogen over control. The lowest inhibition on growth of the pathogen was noticed at 0.3 per cent concentration of captan, which

Table 9: *In vitro* evaluation of fungicides against *P. mangiferae*

Tr. No	Treatments	Concentrations (Per cent)	Colony diameter (mm)*			Per cent inhibition over control
			DAI			
			2	4	6	
1	Bordeaux † mixture	0.5	0	0	0	100
		1.0	0	0	0	100
		1.5	0	0	0	100
2	Copper oxychloride	0.2	11.2^{c}	44.8^{bc}	67.5^{c}	25.0
		0.3	10.0^{c}	44.7^{bc}	71.0^{bc}	21.1
		0.4	10.0^{c}	42.0^{c}	62.8^{c}	30.2
3	Copper hydroxide	0.05	19.0^{b}	52.0^{b}	79.5^{ab}	11.6
		0.15	13.3^{c}	20.5^{de}	37.2^{d}	58.8
		0.25	10.2^{c}	11.5^{e}	24.2^{efg}	73.2
4	Mancozeb	0.2	10.0^{c}	15.7^{de}	16.0^{fg}	83.6
		0.3	10.0^{c}	14.8^{de}	18.0^{fg}	83.0
		0.4	10.0^{c}	12.2^{e}	16.0^{fg}	84.1
5	Captan	0.2	14.3^{c}	17.3^{de}	19.0^{fg}	78.9
		0.3	13.3^{c}	15.0^{de}	16.8^{fg}	81.3
		0.4	10.7^{c}	12.3^{e}	13.2^{g}	85.3
6	Hexaconazole	0.05	10.0^{c}	16.2^{de}	18.7^{fg}	79.3
		0.1	10.0^{c}	14.2^{de}	17.0^{fg}	81.1
		0.15	10.0^{c}	13.3^{e}	15.8^{fg}	82.4
7	Carbendazim †	0.05	0	0	0	100
		0.1	0	0	0	100
		0.2	0	0	0	100
8	Zineb	0.2	10.0^{c}	20.8^{de}	27.0^{def}	70.0
		0.3	10.0^{c}	22.8^{d}	31.2^{de}	65.4
		0.4	10.0^{c}	12.3^{e}	15.0^{fg}	83.3
9	Control	-	39.5^{a}	75.7^{a}	90.0^{a}	-

DAI - Days after inoculation

* Mean of three replications

† - Not included in statistical analysis

In each column figures followed by same letter do not differ significantly according to DMRT

Table 10: *In vitro* evaluation of fungicides against *C. mangiferae*

Tr. No	Treatments	Concentrations (Per cent)	Colony diameter (mm)* DAI					Per cent inhibition over control
			2	4	6	8	9	
1	Bordeaux † mixture	0.5	0	0	0	0	0	100
		1.0	0	0	0	0	0	100
		1.5	0	0	0	0	0	100
2	Copper oxychloride	0.2	11.8^{ghi}	13.3^{gh}	14.7^{g}	48.3^{ef}	51.5^{e}	42.8
		0.3	13.8^{defghi}	14.2^{gh}	13.7^{g}	55.8^{de}	65.7^{cd}	27.1
		0.4	14.2^{defgh}	14.7^{gh}	15.0^{g}	46.7^{ef}	50.0^{ef}	44.4
3	Copper hydroxide	0.05	13.0^{efghi}	36.7^{cd}	38.0^{cd}	64.3^{cd}	69.2^{c}	23.2
		0.15	12.7^{fghi}	14.8^{gh}	14.8^{g}	16.3^{h}	19.3^{g}	78.5
		0.25	10.3^{hi}	11.7^{h}	12.3^{g}	13.2^{h}	13.8^{g}	84.6
4	Mancozeb	0.2	12.2^{fghi}	19.0^{fgh}	42.3^{bc}	56.3^{de}	65.5^{cd}	27.2
		0.3	11.0^{ghi}	18.3^{fgh}	30.0^{de}	46.7^{ef}	54.3^{de}	39.7
		0.4	10.0^{i}	11.5^{h}	16.5^{fg}	30.0^{g}	37.2^{f}	58.7
5	Captan	0.2	23.3^{b}	41.7^{bc}	61.7^{a}	79.0^{ab}	84.7^{ab}	6.00
		0.3	23.3^{b}	45.3^{ab}	66.2^{a}	85.0^{a}	89.5^{a}	0.50
		0.4	18.3^{c}	34.3^{d}	48.7^{b}	61.3^{cd}	68.2^{c}	24.3
6	Hexaconazole	0.05	16.0^{cdef}	25.5^{ef}	34.3^{cd}	43.3^{ef}	47.7^{ef}	47.1
		0.1	16.8^{cde}	24.3^{ef}	33.3^{cde}	41.8^{fg}	46.2^{ef}	48.8
		0.15	16.8^{cde}	20.7^{fg}	24.7^{ef}	38.2^{fg}	41.3^{ef}	54.1
7	Carbendazim †	0.05	0	0	0	0	0	100
		0.1	0	0	0	0	0	100
		0.2	0	0	0	0	0	100
8	Zineb	0.2	14.7^{cdefg}	30.3^{de}	48.7^{b}	67.8^{bcd}	76.2^{bc}	15.4
		0.3	14.7^{cdefg}	30.5^{de}	48.5^{b}	67.8^{bcd}	76.7^{bc}	14.9
		0.4	17.7^{cd}	33.3^{d}	51.5^{b}	70.5^{bc}	78.5^{abc}	12.8
9	Control	-	30.0^{a}	51.0^{a}	68.8^{a}	85.7^{a}	90.0^{a}	-

DAI - Days After Inoculation

* Mean of three replications

† - Not included in statistical analysis

In each column figures followed by same letter do not differ significantly according to DMRT

recorded only 0.50 per cent inhibition over control. It was also on par with captan (0.2 per cent) and zineb (0.4 per cent).

4.6.2.4 *D. australiensis*

The data showed significant difference among treatments on their inhibitory effect against this fungus (Table11). Bordeaux mixture at all concentrations recorded cent per cent inhibition and it was not included in statistical analysis. Among the other treatments 0.1 per cent hexaconazole, recorded the maximum inhibition of 88.2 per cent over control, but it was also on par with other two concentrations of the fungicide. Copper hydroxide at 0.15 and 0.25 per cent concentrations were recorded 75.2 and 77.7 per cent inhibition of the pathogen over control respectively. Copper hydroxide at 0.05 per cent, zineb 0.4 per cent and the three concentrations of copper oxychloride were on par with each other in their efficacy to inhibit the growth of pathogen and recorded a range of 46.1 to 63.2 per cent inhibition over control. Carbendazim at 0.05 per cent concentration showed the lowest inhibition of the pathogen (35.7 per cent) over control.

4.6.2.5 *A. alternata*

The data (Table 12) revealed that Bordeaux mixture at all the three concentrations showed cent per cent inhibition on the growth of *A.alternata* and was not included in the statistical analysis. Among the remaining treatments the maximum inhibition on the growth of pathogen was recorded by 0.1 per cent hexaconazole (71.3 per cent) and it was on par with hexaconazole 0.15 per cent which showed 70.4 per cent inhibition on the growth of pathogen over control. In all the three concentrations of carbendazim, 0.05 per cent hexaconazole, 0.4 per cent copper oxychloride, 0.15 and 0.25 per cent copper hydroxide, more than 50 per cent inhibition of growth was observed. All the

Table 11: *In vitro* evaluation of fungicides against *D. australiensis*

Tr. No	Treatments	Concentrations (Per cent)	Colony diameter (mm)* DAI			Per cent inhibition over control
			2	4	6	
1	Bordeaux † mixture	0.5	0	0	0	100
		1.0	0	0	0	100
		1.5	0	0	0	100
2	Copper oxychloride	0.2	15.5^{bcde}	24.7^{bcdef}	34.2^{def}	62.1
		0.3	13.7^{def}	22.0^{def}	32.3^{def}	46.1
		0.4	11.7^{ef}	22.5^{cdef}	33.2^{def}	63.2
3	Copper hydroxide	0.05	18.0^{bcd}	28.0^{bcdef}	35.2^{def}	61.0
		0.15	14.5^{def}	17.8^{efg}	22.3^{efg}	75.2
		0.25	12.0^{ef}	15.7^{fg}	20.0^{fg}	77.7
4	Mancozeb	0.2	20.3^{b}	37.2^{b}	51.7^{bc}	42.7
		0.3	14.7^{cdef}	25.7^{bcdef}	38.3^{cd}	57.4
		0.4	14.0^{def}	25.8^{bcdef}	40.3^{cd}	55.2
5	Captan	0.2	20.2^{b}	28.8^{bcde}	38.7^{cd}	57.1
		0.3	18.2^{bcd}	26.7^{bcdef}	38.8^{cd}	56.8
		0.4	20.0^{b}	28.3^{bcde}	38.7^{cd}	57.1
6	Hexaconazole	0.05	10.0^{f}	10.0^{g}	11.3^{g}	87.4
		0.1	10.0^{f}	10.0^{g}	10.7^{g}	88.2
		0.15	10.0^{f}	10.0^{g}	11.7^{g}	87.1
7	Carbendazim	0.05	19.8^{bc}	34.8^{bc}	57.8^{b}	35.7
		0.1	15.5^{bcde}	27.2^{bcdef}	45.5^{bcd}	49.4
		0.2	16.2^{bcde}	29.8^{bcde}	45.7^{bcd}	49.3
8	Zineb	0.2	19.8^{bc}	34.0^{bcd}	44.3^{bcd}	50.7
		0.3	15.3^{bcde}	28.0^{bcdef}	36.2^{cde}	59.8
		0.4	15.7^{bcde}	26.2^{bcdef}	34.8^{def}	61.3
9	Control	-	39.2^{a}	70.5^{a}	90.0^{a}	-

DAI - Days after inoculation

* Mean of three replications

† - Not included in statistical analysis

In each column figures followed by same letter do not differ significantly according to DMRT

Table 12: *In vitro* evaluation of fungicides against *A. alternata*

Tr. No	Treatments	Concentrations (Per cent)	Colony diameter (mm)* DAI				Per cent inhibition over control
			2	4	6	7	
1	Bordeaux † mixture	0.5	0	0	0	0	100
		1.0	0	0	0	0	100
		1.5	0	0	0	0	100
2	Copper oxychloride	0.2	14.2^{b}	26.8^{b}	38.7^{bcd}	46.8^{cde}	48.0
		0.3	13.3^{bc}	18.5^{cdef}	29.0^{e}	45.5^{ef}	49.4
		0.4	12.5^{cdef}	16.8^{def}	24.2^{f}	42.7^{f}	52.6
3	Copper hydroxide	0.05	11.5^{fghij}	27.2^{b}	37.7^{cd}	48.3^{bcde}	46.3
		0.15	10.5^{jk}	17.8^{edef}	29.2^{e}	37.2^{f}	58.7
		0.25	10.5^{jk}	15.2^{ef}	27.0^{e}	35.5^{gh}	60.6
4	Mancozeb	0.2	12.7^{cde}	30.0^{ab}	40.0^{bc}	50.8^{b}	43.6
		0.3	12.0^{defgh}	27.7^{b}	37.8^{cd}	49.0^{bcd}	45.6
		0.4	11.5^{fghij}	27.7^{b}	36.3^{d}	47.0^{cde}	47.8
5	Captan	0.2	13.0^{cd}	28.7^{b}	39.0^{bcd}	49.2^{bcd}	45.4
		0.3	12.2^{defg}	26.5^{b}	37.0^{d}	45.7^{def}	49.3
		0.4	11.7^{efghi}	25.2^{bc}	36.8^{d}	47.5^{bcde}	47.2
6	Hexaconazole	0.05	11.3^{ghij}	15.3^{ef}	20.5^{g}	31.3^{i}	65.2
		0.1	10.8^{ijk}	13.5^{f}	17.5^{h}	25.8^{j}	71.3
		0.15	11.0^{hijk}	13.3^{f}	17.8^{h}	26.7^{j}	70.4
7	Carbendazim	0.05	10.7^{ijk}	22.5^{bcde}	28.5^{e}	35.7^{gh}	60.0
		0.1	10.2^{k}	17.7^{cdef}	26.7^{e}	32.8^{hi}	63.5
		0.2	10.0^{k}	15.5^{ef}	27.0^{e}	32.8^{hi}	63.5
8	Zineb	0.2	12.5^{cdef}	25.0^{bc}	41.0^{b}	50.3^{bc}	44.1
		0.3	10.7^{ijk}	24.2^{bcd}	38.5^{bcd}	49.2^{bcd}	45.4
		0.4	10.5^{jk}	24.2^{bcd}	36.5^{d}	47.8^{bcd}	46.9
9	Control	-	19.0^{a}	36.5^{a}	76.5^{a}	90.0^{a}	-

DAI - Days After Inoculation

* Mean of three replications

† - Not included in statistical analysis

In each column figures followed by same letter do not differ significantly according to DMRT

three concentrations of mancozeb, captan and zineb recorded less than 50 per cent inhibition of pathogen over control. Mancozeb at 0.2 per cent recorded the lowest inhibition of the pathogen (43.6 per cent).

4.6.3 *In vitro* evaluation on antagonistic effect of bioagents against die back and leaf blight pathogens

The inhibitory effect of standard cultures of fungal and bacterial antagonists against the die back and leaf blight pathogens of mango was studied under *in vitro* condition by dual culture method. Standard cultures of *Trichoderma viride* (KAU), *Trichoderma harzianum* (IISR) and *Pseudomonas fluorescens* (KAU) were used for the study. The results are presented in Table 13 to 15.

4.6.3.1 Evaluation of fungal antagonists against die back pathogens

4.6.3.1.1 *C. gloeosporioides*

Initially the growth of pathogen was greater than both the antagonists (Table 13). But, on the third day onwards the growth rate of both the antagonists was found to be faster than the pathogen. On the third day, *T. viride* measured 48.7mm growth as against 37.8mm diameter growth of pathogen and *T. harzianum* measured 49.1mm as against 42.6mm growth of pathogen. Both the antagonists showed over growth on fourth day onwards and completed over growth of the pathogen by both the antagonists was recorded in seven DAI.

4.6.3.1.2 *B. theobromae*

From data (Table 13), the growth of both the antagonists became faster in dual culture and mono culture compared to the pathogen on the third day

Table 13: *In vitro* evaluation of fungal antagonists against die back pathogens

Treatments		DAI (Colony diameter in mm)*														Antagonism
		1		2		3		4		5		6		7		
		T	A	T	A	T	A	T	A	T	A	T	A	T	A	
C.gloeosporioides/ T.viride	D	29.0	28.2	35.5	39.2	37.8	48.7	30.5	59.5	20.5	69.1	9.40	80.6	0	90	Over growth
	M	29.5	26.3	36.8	39.9	40.9	52.2	53.1	60.9	62.7	73.4	78.7	90.0	90	90	
C.gloeosporioides/ T.harzianum	D	29.3	28.0	39.5	38.7	42.6	49.1	32.1	57.9	24.2	65.3	12.3	77.7	0	90	Over growth
	M	29.5	26.5	36.8	39.4	40.9	49.8	53.1	56.7	62.7	69.5	78.7	83.3	90	90	
B.theobromae/ T.viride	D	28.7	27.1	43.0	46.2	36.4	53.6	20.1	69.8	6.20	83.8	0.00	90.0			Over growth
	M	29.9	27.4	46.5	43.4	53.5	58.9	68.9	79.9	79.2	89.4	90.0	90.0			
B.theobromae/ T.harzianum	D	28.2	26.6	41.2	40.8	33.1	54.9	19.5	70.3	5.80	84.2	0.00	90.0			Over growth
	M	29.9	29.7	46.5	44.1	53.5	58.7	68.9	78.2	79.2	88.1	90.0	90.0			

DAI – Days After Incubation
* Mean of five replications

T - Test organism
A - Antagonist
D - Dual culture
M - Mono culture

onwards and over growth of antagonists on pathogen was also observed on the third day. *T.viride* measured 53.6mm diameter growth against 36.4mm of pathogen and *T. harzianum* showed 54.9mm diameter against 33.1mm growth of pathogen. On third day after incubation the growth rate of *T. viride* and *T. harzianum* became faster and completed the over growth and cent per cent inhibition of pathogen was recorded on six DAI.

In both the die back pathogens the mechanism of antagonism was observed to be over growth of pathogens.

4.6.3.2 Evaluation of fungal antagonists against leaf blight pathogens

The result of the evaluation of fungal antagonists against leaf blight pathogens are presented in Table 14.

4.6.3.2.1 *P. mangiferae*

From the first DAI onwards, the growth of *T.viride* was found to be greater than the test organism. *T. harzianum* showed fast growth rate compared to that of pathogen as evidenced after three days of incubation. On the third day of incubation, growth of *T. viride* was 48.7mm against 41.3mm of pathogen. *T. harzianum* showed 46.2mm against 43.7mm growth of pathogen. On fourth day after incubation, *T. viride* and *T. harzianum* started over growing pathogen. The cent per cent inhibition of growth of pathogen by both the antagonists was recorded on six DAI. The mechanism of antagonism noticed in *T. viride* and *T. harzianum* was over growth on the pathogen.

4.6.3.2.2 *C. mangiferae*

The initial growth of pathogen was less compared to that of antagonists in both mono and dual cultures which was evidenced from first

Table 14: *In vitro* evaluation of fungal antagonists against leaf blight pathogens

Treatments		DAI (Colony diameter in mm)*																		Antagonism
		1		2		3		4		5		6		7		8		9		
		T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	
P.mangiferae/	D	24.7	27.6	38.6	41.1	41.3	48.7	21.4	66.8	14.3	74.7	0	90							over growth
T. viride	M	26.4	26.8	39.5	40.3	46.2	58.6	56.8	70.2	73.8	81.9	90	90							
P.mangiferae/	D	24.1	25.1	39.1	38.6	43.7	46.2	24.6	65.1	15.2	74.0	0	90							over growth
T. harzianum	M	26.4	24.8	39.5	38.1	46.2	54.7	56.8	69.8	73.8	81.9	90	90							
C. mangiferae/	D	14.2	23.8	19.8	29.7	28.5	46.8	32.3	56.8	20.8	69.2	10.2	79.8	0	90	0	90	0	90	over growth
T. viride	M	14.8	24.2	20.6	30.3	30.9	47.7	34.4	59.1	42.9	70.9	52.8	80.7	69.1	90	80.2	90	90	90	
C. mangiferae/	D	14.6	22.7	20.2	29.4	30.4	45.9	33.2	55.4	22.2	67.8	12.6	77.4	0	90	0	90	0	90	over growth
T. harzianum	M	14.8	24.1	20.6	30.1	30.9	47.2	34.4	58.9	42.9	69.9	52.8	80.2	69.1	90	80.2	90	90	90	
D.australiensis/	D	24.9	27.8	34.5	39.4	40.2	49.0	28.1	61.7	9.8	80.2	0	90							over growth
T. viride	M	26.1	28.4	37.4	40.2	48.9	52.1	59.5	70.6	72.2	86.9	90	90							
D.australiensis/	D	25.8	27.6	35.6	37.8	39.8	50.9	25.8	64.1	7.4	82.6	0	90							over growth
T. harzianum	M	26.1	28.1	37.4	39.1	48.9	53.1	59.5	69.9	72.2	87.4	90	90							
A. alternata/	D	24.6	27.1	36.6	40.1	40.8	52.4	20.8	69	9.8	80.2	0	90	0	90					over growth
T. viride	M	25.2	27.5	39.8	42.4	49.2	57.6	57.8	76.2	69.9	90.0	80.2	90	90	90					
A. alternata/	D	24.9	24.3	36.5	38.4	40.2	49.6	23.6	65.1	13.4	76.2	0	90	0	90					over growth
T. harzianum	M	25.2	24.6	39.8	39.6	49.2	54.4	57.8	68.2	69.9	82.6	80.2	90	90	90					

T - Test organism A - Antagonist D - Dual culture M - Mono culture

DAI - Days after incubation

* Means of five replications

DAI on wards. On fifth day both the antagonists started over growing the pathogen. *T. viride* measured 69.2mm diameter against 20.8mm of pathogen and *T. harzianum* showed 67.8mm diameter against 22.2mm of pathogen on fifth DAI. *T. viride* and *T. harzianum* recorded complete inhibition of pathogen on seven DAI.

.4.6.3.2.3 *D. australiensis*

The growth recorded by both the antagonists was higher in mono and dual culture than the pathogen from the initial stage onwards. *T. viride* and *T. harzianum* showed over growth on the pathogen from fourth DAI and the measurement of *T.viride* showed 61.7mm against 28.1mm of pathogen, *T. harzianum* showed 64.1mm against 25.8mm of pathogen. Both the antagonists recorded cent per cent inhibition of pathogen which was observed on six DAI.

4.6.3.2.4 *A. alternata*

The antagonists showed a faster growth in initial stage on wards in both mono and dual culture than the pathogen. *T.viride* and *T. harzianum* showed over growth on the pathogen from fourth DAI and the measurement of *T. viride* recorded 69.0mm against 20.8mm of pathogen, *T. harzianum* showed 65.1mm against 23.6mm of pathogen. Complete inhibition of pathogen by the antagonist was recorded in six DAI.

4.6.3.3 *In vitro* evaluation of bacterial antagonist against die back and leaf blight pathogens

The inhibitory efficacy of standard culture of *P. fluorescens* (KAU) was evaluated against die back and leaf blight causing organisms and data are presented in the Table 15. Among the two die back pathogens the highest per cent inhibition of growth (60.99 per cent) was recorded by *B. theobromae* and

C. gloeosporioides showed only 50.33 per cent inhibition of growth. Among the leaf blight causing pathogens, *P. mangiferae* (57.78 per cent), *D. australiensis* (56.31per cent) recorded more than 50 per cent inhibition of growth over control, whereas *C. mangiferae, A. alternata* showed 42.16 and 38.49 per cent inhibition of growth over control respectively.

Table 15: *In vitro* evaluation of *P. fluorescens* against die back and leaf blight pathogens

Tr.No	Pathogens	Per cent inhibition *
1	*C. gloeosporioides*	50.33
2	*B. theobromae*	60.99
3	*P. mangiferae*	57.78
4	*C. mangiferae*	42.16
5	*D. australiensis*	56.31
6	*A .alternata*	38.49

*** Mean of five replications**

4.6.4 *In planta* evaluation of fungicides and antagonists against die back and leaf blight diseases in mango grafts

The effect of different fungicides and antagonistic organisms on the management of die back and leaf blight diseases of mango grafts was studied by giving four sprayings on naturally infected mango grafts at an interval of 15 days after the establishment of the grafts. The disease incidence and severity of die back and leaf blight were recorded separately before the first spray and ten days after each spray (Plate 8).

Plate 8: Die back and leaf blight symptoms observed during *in planta* experiment

Table 16: Effect of various treatments on per cent disease incidence of die back of mango grafts

Tr. No	Treatments	Per cent disease incidence at 10days after last spray				Per cent reduction over control
		R_1	R_2	R_3	Mean	
T_1	Bordeaux mixture (1%)	5.13	2.56	-	2.56	91.67^{a}
T_2	Hexaconazole(0.1%)	2.56	7.69	5.13	5.13	80.56^{ab}
T_3	Copper oxychloride (0.3%)	2.56	2.56	2.56	2.56	91.67^{a}
T_4	Mancozeb (0.3%)	15.38	20.51	17.95	17.95	30.56^{c}
T_5	Zineb (0.3%)	12.82	17.95	15.38	15.38	40.02^{c}
T_6	Captan (0.3%)	15.38	10.26	12.82	12.82	50.01^{bc}
T_7	Carbendazim (0.1%)	7.69	5.13	2.56	5.13	80.56^{ab}
T_8	Copper hydroxide (0.15%)	5.13	5.13	5.13	5.13	80.56^{ab}
T_9	*P. fluorescens* (2%)	12.82	7.69	2.56	7.69	70.01^{abc}
T_{10}	*T. viride* (20 g/l)	10.26	7.69	12.82	10.26	59.98^{bc}
T_{11}	Quinalphos (0.05%)	5.13	10.26	7.69	7.69	70.01^{abc}
T_{12}	Control	30.77	25.64	20.51	25.64	-

In each column figures followed by same letter do not differ significantly according to DMRT

4.6.4.1 Per cent disease incidence of die back disease of mango grafts

The data on per cent disease incidence of die back and per cent reduction of disease incidence over control on 10 days after the last spray was given in Table 16 and Fig.10.

From the data it was observed that the lowest disease incidence was recorded in plants sprayed with one per cent Bordeaux mixture (T_1) and 0.3 per cent copper oxychloride (T_3) which showed 91.67 per cent reduction of die back incidence over control. It was followed by the treatments T_2 (hexaconazole 0.1per cent), T_7 (carbendazim 0.1per cent) and T_8 (copper hydroxide 0.15per cent) which recorded 80.56 per cent reduction over control. The treatments T_9 and T_{11} recorded 70.01 per cent reduction over control. Statistically all these treatments were on par with each other. Among the treatments except control the maximum disease incidence was observed in T_4 (mancozeb 0.3%) which showed only 30.56 per cent reduction of disease incidence over control. The control plants recorded the highest disease incidence of 25.64 per cent.

4.6.4.2 Per cent disease severity of die back disease of mango grafts

From the data presented in the Table 17 and Fig.11, it was observed that the lowest disease severity (1.28 per cent) was recorded in plants treated with one per cent Bordeaux mixture (T_1), 0.3 per cent copper oxychloride (T_3), 0.1 per cent carbendazim (T_7) and 0.15 per cent copper hydroxide (T_8) which showed 90.47 per cent reduction in disease severity over control. These treatments were on par with T_2, T_9, T_{10} and T_{11} in which the reduction of disease severity over control was ranged from 75.39 to 84.94 per cent. The lowest reduction in disease severity was observed in mancozeb 0.3 per cent (30.14 per cent) which was on par with zineb (0.3 per cent) which recorded 35.69 per cent reduction over control.

Table 17: Effect of various treatments on per cent disease severity of die back of mango grafts

Tr.No	Treatments	Per cent disease severity at 10days after last spray				Per cent reduction over control
		R_1	R_2	R_3	Mean	
T_1	Bordeaux mixture (1%)	1.92	-	1.92	1.28	90.47[a]
T_2	Hexaconazole (0.1%)	-	3.85	1.92	1.92	84.94[a]
T_3	Copper oxychloride (0.3%)	1.92	1.92	-	1.28	90.47[a]
T_4	Mancozeb (0.3%)	7.69	9.61	9.61	8.97	30.14[c]
T_5	Zineb (0.3%)	9.61	5.77	9.61	8.33	35.69[c]
T_6	Captan (0.3%)	1.92	5.77	5.77	4.49	65.08[b]
T_7	Carbendazim (0.1%)	-	3.85	-	1.28	90.47[a]
T_8	Copper hydroxide (0.15%)	1.92	-	1.92	1.28	90.47[a]
T_9	*P. fluorescens* (2%)	3.85	1.92	1.92	2.56	79.35[ab]
T_{10}	*T. viride* (20g/l)	1.92	3.85	3.85	3.21	75.39[ab]
T_{11}	Quinalphos (0.05%)	1.92	3.85	1.92	2.56	79.35[ab]
T_{12}	Control	13.46	11.54	13.46	12.82	-

In each column figures followed by same letter do not differ significantly according to DMRT

Fig. 10 Effect of different treatments on per cent reduction of incidence and severity of die back over control

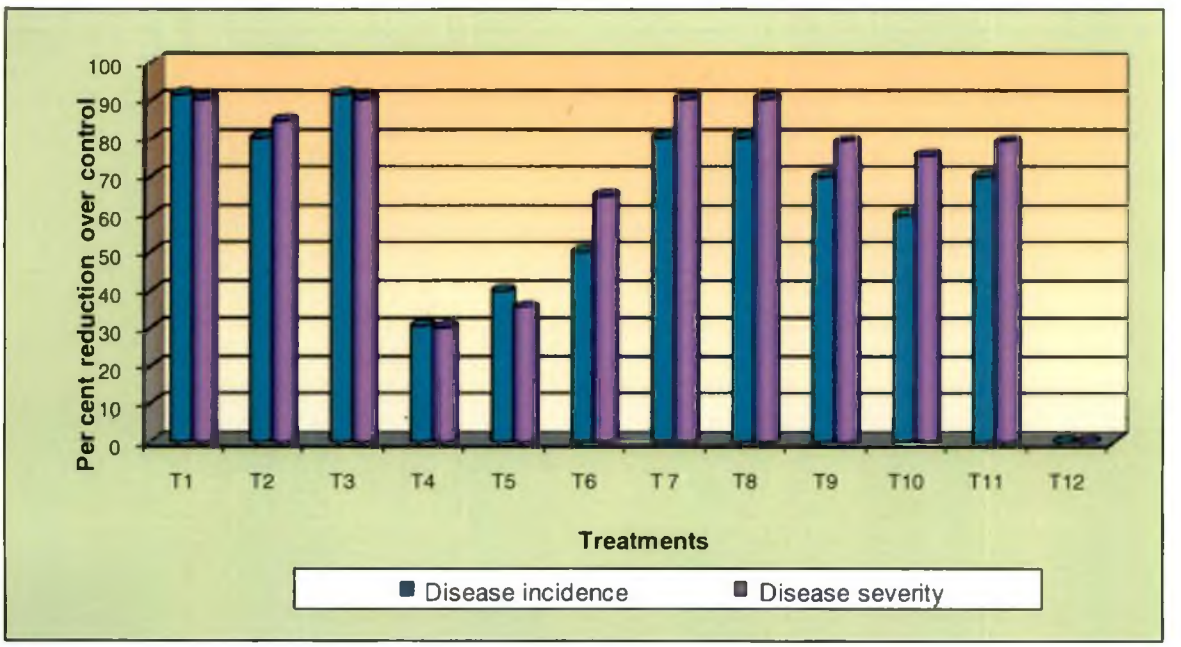
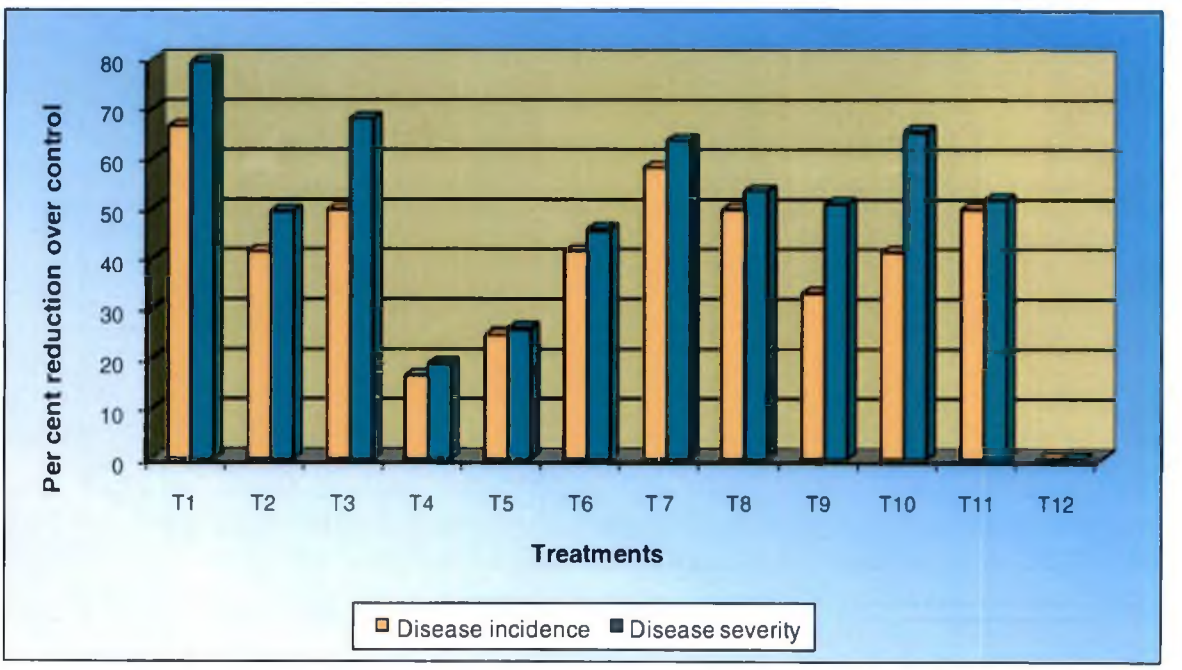


Fig. 11 Effect of different treatments on per cent reduction of incidence and severity of leaf blight over control



4.6.4.3 Per cent disease incidence of leaf blight disease of mango grafts

From the data presented in the Table 18 and Fig.11, it was observed that there was significant difference among the treatments on per cent reduction of disease incidence over control which was recorded on 10 days after fourth spraying. Before first spraying the treatments copper oxychloride and *P. fluorescens* were observed free from leaf blight disease incidence. Carbendazim recorded the minimum disease incidence of 2.50 and was followed by Bordeaux mixture, zineb and control plants which were recorded 2.56 per cent disease incidence. On 10 days after first spraying, the plants in treatment T_7 recorded the minimum disease incidence of 2.50 per cent. It was followed by T_1 and T_3 which showed PDI of 2.56 and 2.55 respectively. The maximum disease incidence was recorded by the plants under the control (15.40 per cent). The minimum disease incidence was recorded in T_1 and T_7 on ten days after second spraying which showed 5.13 and 5.10 per cent. It was followed by T_2, T_3, T_8 and T_9 which were recorded 7.69, 7.65, 7.67 and 7.64 per cent disease incidence. The maximum PDI was recorded in control (T_{12}) (15.50 per cent). Ten days after third spraying the treatments T_1 (Bordeaux mixture) and T_7 (carbendazim) recorded the minimum disease incidence of 7.69 per cent. It was followed by T_3, T_8 and T_{11} which recorded 10.20, 10.25 and 10.24 per cent disease incidence respectively. The treatment T_{12} (control) recorded the maximum PDI (20.51 per cent).

Observations on disease incidence on 10 days after fourth spraying, the treatment T_1 (Bordeaux mixture) recorded the minimum disease incidence of 10.28 and 66.59 per cent reduction of disease incidence over control. It was followed by the treatments T_2, T_3, T_6, T_7, T_8, T_{10} and T_{11} which were recorded more than 40 per cent reduction in disease incidence over control and the treatments T_4, T_5 and T_9 were recorded less than 40 per cent reduction over control.

Table 18: Effect of various treatments on per cent disease incidence of leaf blight of mango grafts

Tr. No	Treatments	Before first spraying	Per cent disease incidence at 10 DAS *				Per cent reduction over control
			First spray	Second spray	Third spray	Fourth spray	
T_1	Bordeaux mixture (1%)	2.56	2.56	5.13	7.69	10.28	66.59^{a}
T_2	Hexaconazole (0.1%)	5.13	5.13	7.69	12.81	17.95	41.66^{ab}
T_3	Copper oxychloride (0.3%)	-	2.55	7.65	10.20	15.38	50.02^{ab}
T_4	Mancozeb (0.3%)	5.13	10.19	12.80	15.37	25.64	16.68^{c}
T_5	Zineb (0.3%)	2.56	7.69	15.40	17.90	23.07	25.02^{bc}
T_6	Captan (0.3%)	-	5.13	10.23	12.82	17.92	41.76^{ab}
T_7	Carbendazim (0.1%)	2.50	2.50	5.10	7.69	12.85	58.35^{ab}
T_8	Copper hydroxide (0.15%)	5.13	5.13	7.67	10.25	15.38	50.02^{ab}
T_9	*P. fluorescens* (2%)	-	5.13	7.64	15.38	20.51	33.35^{abc}
T_{10}	*T. viride* (20 g/l)	5.13	10.26	12.20	12.80	17.99	41.53^{ab}
T_{11}	Quinalphos (0.05%)	5.13	5.13	7.69	10.24	15.35	50.11^{ab}
T_{12}	Control	2.56	15.40	15.50	20.51	30.77	-

DAS – Days After Spraying

* Mean of three replications

In each column figures followed by same letter do not differ significantly according to DMRT

Table 19: Effect of various treatments on per cent disease severity of leaf blight of mango grafts

Tr.No	Treatments	Before first spraying	Per cent disease severity at10 DAS *				Per cent reduction over control
			First spray	Second spray	Third spray	Fourth spray	
T_1	Bordeaux mixture (1%)	1.48	1.19	1.52	1.83	2.27	79.63[a]
T_2	Hexaconazole (0.1%)	2.22	2.83	4.71	5.50	5.62	49.64[ab]
T_3	Copper oxychloride(0.3%)	1.93	2.19	2.17	3.42	3.55	68.22[a]
T_4	Mancozeb(0.3%)	1.78	2.51	5.72	8.23	9.02	19.14[c]
T_5	Zineb (0.3%)	1.33	4.56	5.22	7.62	8.25	26.08[bc]
T_6	Captan (0.3%)	2.52	3.12	3.52	5.16	6.03	45.97[abc]
T_7	Carbendazim (0.1%)	1.19	1.79	2.87	3.57	4.02	64.00[a]
T_8	Copper hydroxide(0.15%)	2.07	3.74	5.14	5.08	5.14	53.91[ab]
T_9	*P. fluorescens* (2%)	2.37	3.17	4.71	5.15	5.44	51.23[ab]
T_{10}	*T. viride* (20 g/l)	1.04	2.80	3.13	3.58	3.84	65.62[a]
T_{11}	Quinalphos (0.05%)	2.67	3.52	4.43	5.09	5.35	52.03[ab]
T_{12}	Control	1.63	4.79	6.57	9.28	11.16	-

DAS - Days After Spraying

* - Mean of three replications

In each column figures followed by same letter do not differ significantly according to DMRT

4.6.4.4 Per cent disease severity of leaf blight disease of mango grafts

The data on the per cent disease severity of leaf blight disease are presented in Table 19 and Fig.11, before first spraying the slight variations were observed on PDS among the treatments. The minimum and maximum disease severity was observed in treatments T_{10} and T_{11} which recorded 1.04 and 2.67 per cent respectively.

On 10 days after first spraying, the minimum disease severity of 1.19 per cent was recorded in T_1 (Bordeaux mixture). It was followed by T_7 which showed 1.79 per cent of disease severity. The maximum disease severity was recorded in T_{12} which showed 4.79 per cent. On ten days after second spraying the disease severity was recorded the range of 1.52(T_1) - 6.57(T_{12}) per cent. The minimum per cent disease severity was recorded by T_1 and maximum per cent disease severity was recorded by T_{12} (control).

On 10 days after third spraying, the treatment T_1 recorded the minimum disease severity of 1.83 per cent. It was followed by the treatments T_3 and T_7 which showed the PDS of 3.42 and 3.57 respectively. The treatments T_2, T_6, T_8, T_9 and T_{11} were recorded the disease severity in the range of 5.08-5.50per cent. The treatments T_4 (mancozeb) and T_5 (zineb) showed the per cent disease severity of 8.23 and 7.62. The plants under T_{12} (control) recorded the maximum per cent disease severity of 9.28.

On 10 days after fourth spraying, Bordeaux mixture (T_1) recorded the minimum per cent disease severity of 2.27 and showed 79.63 per cent reduction of disease severity over control. It was followed by copper oxychloride (T_3) which recorded 68.22 per cent reduction of disease severity over control. The treatments T_7, T_8, T_9, T_{10} and T_{11} which were recorded more than 50 per cent reduction over control. The treatment T_4 (mancozeb) recorded the minimum disease severity 19.14 per cent reduction over control.

Table 20: Effect of various treatments on height and number of leaves of mango grafts during *in planta* experiment

Tr.No	Treatments	Plant * height(cm)	Number * of leaves
T_1	Bordeaux mixture	5.95	7.20
T_2	Hexaconazole	6.18	5.43
T_3	Copper oxy chloride	4.39	6.44
T_4	Mancozeb	4.29	5.94
T_5	Zineb	4.66	5.52
T_6	Captan	5.16	5.11
T_7	Carbendazim	5.39	7.25
T_8	Copper hydroxide	4.73	7.27
T_9	*P. fluorescence*	7.34	7.80
T_{10}	*T. viride*	5.99	6.60
T_{11}	Quinalphos	4.93	5.16
T_{12}	Control	3.64	4.93

* Mean of three replications

4.6.4.5 Effect of various treatments on height and number of leaves of mango grafts

The data on the observations on height and number of leaves of mango grafts after the fourth spray are presented in Table 20.

The plants sprayed with *P. fluorescens* recorded the maximum height of plant (7.34cm) and maximum number of leaves (7.80). It was followed by hexaconazole (6.18cm) in height of plant and copper hydroxide (7.27) in number of leaves. Among the other treatments, T1, T2, T6, T7 and T10 recorded more than 5cm height and all treatments except control produced more than 5 leaves. The lowest height of plant (3.64cm) and no of leaves (4.93) were observed in control plants.

4.6.5 *In planta* evaluation of selected fungicides and antagonists against die back and leaf blight diseases in mango grafts

The most effective fungicides against die back and leaf blight diseases were selected from *in vitro* evaluation and first experiment under *in planta* condition and these selected fungicides and antagonists were again evaluated for their fungicidal efficiency.

4.6.5.1 Per cent disease incidence and severity of die back disease of mango grafts

The observations on per cent disease incidence and per cent disease severity of die back taken on 10 days after the last spraying are given in Table 21 and 22, Fig.12. Statistical analysis showed significant difference among the treatments on per cent reduction of disease incidence whereas no significant difference was observed in per cent reduction of disease severity over control. Among the treatments the lowest disease incidence (9.52 per cent) and disease severity (2.38 per cent) was recorded by the treatments T_1 (Bordeaux mixture,

Table 21: Effect of selected treatments on per cent disease incidence of die back of mango grafts.

Tr.No	Treatments	Per cent disease incidence at 10days after spray				Per cent reduction over control
		R1	R2	R3	Mean	
T1	Bordeaux mixture (1%)	-	14.29	14.29	9.52	71.44[a]
T2	Copper hydroxide (0.15%)	14.29	9.52	19.05	14.29	57.13[ab]
T3	Captan (0.3%)	19.05	23.81	28.57	23.81	28.56[b]
T4	Hexaconazole (0.1%)	19.05	14.29	9.52	14.29	57.13[ab]
T5	Carbendazim (0.1%)	9.52	9.52	23.81	14.29	57.13[ab]
T6	*P. fluorescens* (2%)	19.05	23.81	14.29	19.05	42.84[ab]
T7	*T. viride* (20 g/l)	23.81	9.52	23.81	19.05	42.84[ab]
T8	Control	28.57	33.33	38.10	33.33	-

In each column figures followed by same letter do not differ significantly according to DMRT

Table 22: Effect of selected treatments on per cent disease severity of die back of mango grafts

Tr.No	Treatments	Per cent disease severity at 10 DAS				Per cent reduction over control
		R_1	R_2	R_3	Mean	
T1	Bordeaux mixture (1%)	3.57	3.57	-	2.38	80.02[a]
T2	Copper hydroxide (0.15%)	7.14	3.57	3.57	4.76	60.03[a]
T3	Captan (0.3%)	3.57	7.14	10.71	7.14	40.05[a]
T4	Hexaconazole (0.1%)	3.57	3.57	3.57	3.57	70.03[a]
T5	Carbendazim (0.1%)	7.14	-	3.57	3.57	70.03[a]
T6	*P. fluorescens* (2%)	7.14	7.14	-	4.76	60.03[a]
T7	*T. viride* (20 g/l)	3.57	7.14	7.14	5.95	50.04[a]
T8	Control	10.72	17.87	7.14	11.91	-

DAS – Days after spraying

In each column figures followed by same letter do not differ significantly according to DMRT

1%) which showed 71.44 per cent and 80.02 per cent reduction of die back incidence and severity over control respectively. It was followed by T_2, T_4 and T_5 in per cent reduction of disease incidence (57.13) and T_4 and T_5 in per cent reduction of disease severity (70.03) over control.

The bioagents *P. fluorescens* (T_6) and *T. viride* (T_7) were recorded only 42.84 per cent reduction on disease incidence over control. The treatment T_3 (captan 0.3per cent) was found to be the least effective in reducing disease incidence which showed only 28.56 per cent reduction over control. The statistical analysis of data given in Table 22 showed no significant difference among the different treatments. All the treatments were found on par with each other in per cent reduction of die back disease severity over control and it ranged from 40.05 to 80.02 per cent. All treatments except T_3 (captan 0.3 per cent) recorded more than 50 per cent reduction of disease severity over control. Captan recorded the lowest value of 40.05 per cent disease reduction over control.

4.6.5.2 Per cent disease incidence and severity of leaf blight disease of mango grafts

From the data given in the Table 23 and 24, Fig.13, it was observed that before first spraying the leaf blight disease incidence was recorded by all treatments except T_1 (Bordeaux mixture), T_4 (Hexaconazole) andT_7 (*T. viride*). On 10 days after first spraying it was observed that, the disease incidence and severity was noticed in all treatments except T_1. Among the other treatments the lowest disease incidence of 4.73 per cent was recorded by the treatment T_2, T_4 and T_6 (copper hydroxide, hexaconazole and *P. fluorescens* respectively) and the lowest disease severity of 0.81 per cent was recorded by the treatment T_4 (hexaconazole).

On 10 days after the second spray, the treatment T_1 recorded the lowest incidence of 4.73 per cent and severity of 1.39 per cent. It was followed by T_2 and T_4 which showed the disease incidence of 9.47 per cent and in disease severity, it

Fig. 12 Effect of selected treatments on per cent reduction of incidence and severity of die back over control

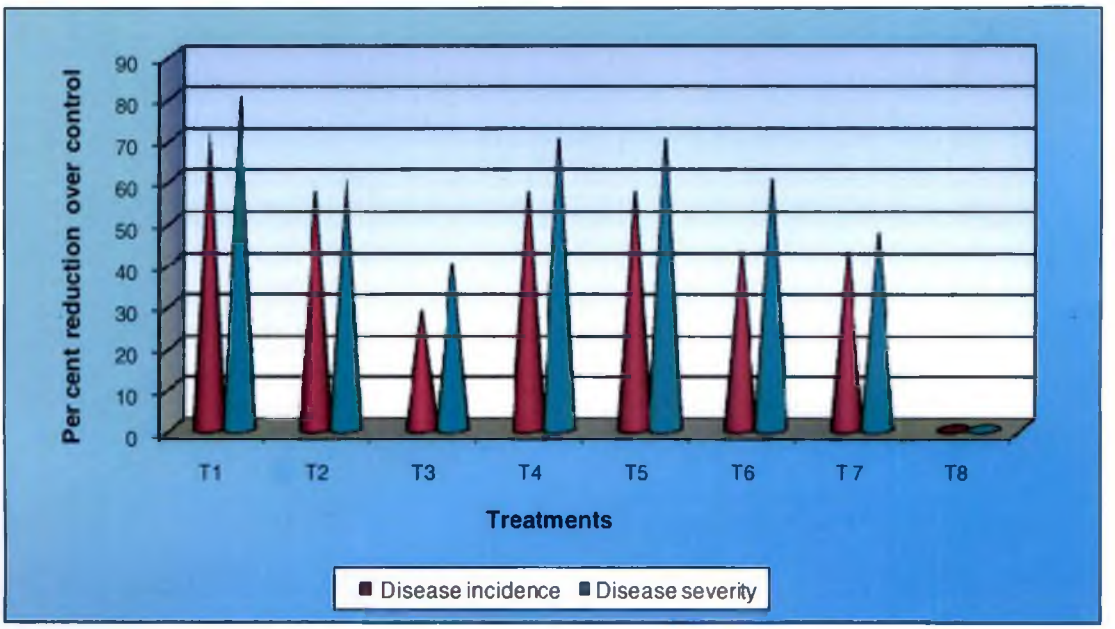


Fig. 13 Effect of selected treatments on per cent reduction of incidence and severity of leaf blight over control

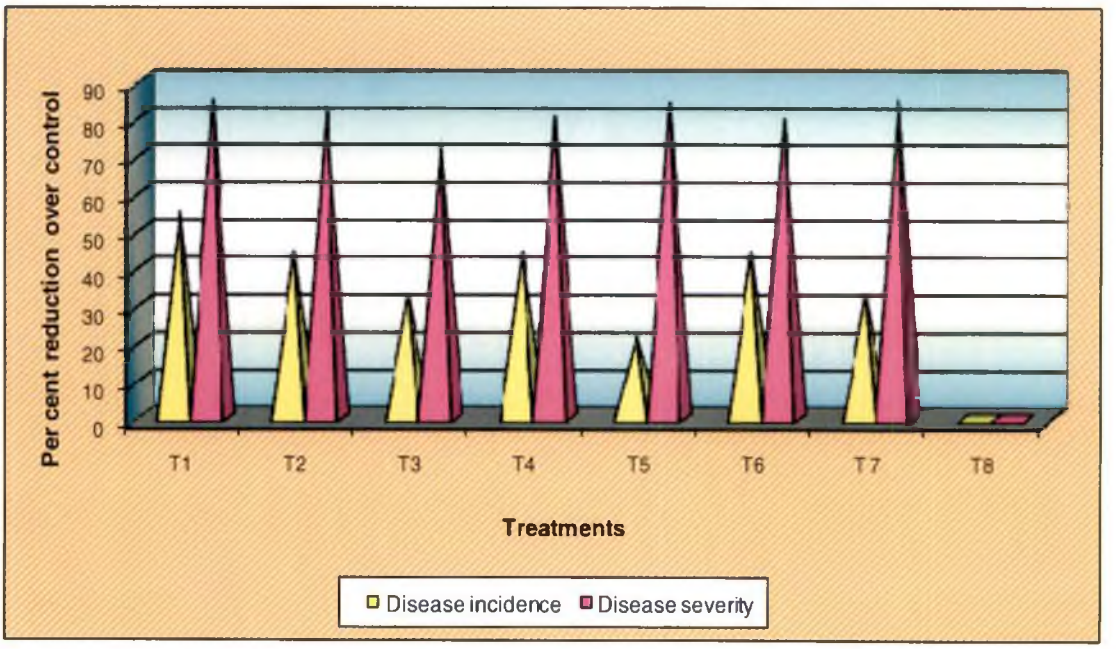


Table 23: Effect of selected treatments on per cent disease incidence of leaf blight of mango grafts

Tr.No	Treatments	Before first spraying	Per cent disease incidence at 10 DAS *				Per cent reduction over control
			First spray	Second spray	Third spray	Fourth spray	
T_1	Bordeaux mixture (1%)	-	-	4.73	9.47	19.00	55.61[a]
T_2	Copper hydroxide (0.15%)	4.73	4.73	9.47	14.29	23.73	44.56[a]
T_3	Captan (0.3%)	4.73	9.52	14.29	19.00	28.50	33.41[a]
T_4	Hexaconazole (0.1%)	-	4.73	9.47	14.23	23.73	44.56[a]
T_5	Carbendazim (0.1%)	9.56	14.29	18.97	23.73	33.27	22.27[a]
T_6	*P. fluorescens* (2%)	4.73	4.73	9.52	14.23	23.73	44.56[a]
T_7	*T. viride* (20g/l)	-	9.52	14.29	18.97	28.50	33.41[a]
T_8	Control	4.73	23.73	28.50	33.27	42.80	-

DAS - Days after spraying

* Mean of three replications

In each column figures followed by same letter do not differ significantly according to DMRT

Table 24: Effect of selected treatments on per cent disease severity of leaf blight of mango grafts

Tr. No	Treatments	Before first spraying	Per cent disease severity at10 DAS *				Per cent reduction over control
			First spray	Second spray	Third spray	Fourth spray	
T_1	Bordeaux mixture (1%)	-	-	1.39	3.55	4.07	85.64 [a]
T_2	Copper hydroxide (0.15%)	1.20	1.57	3.28	3.68	4.99	82.39[a]
T_3	Captan (0.3%)	1.43	2.03	2.20	3.15	7.43	73.79[a]
T_4	Hexaconazole (0.1%)	0.81	0.81	3.14	3.74	5.42	80.88[a]
T_5	Carbendazim (0.1%)	1.96	2.03	3.31	3.36	4.28	84.90[a]
T_6	*P. fluorescens* (2%)	2.17	2.78	3.01	3.58	5.62	80.18[a]
T_7	*T. viride* (20 g/l)	-	1.63	1.87	2.71	4.09	85.57[a]
T_8	Control	2.54	18.47	19.48	21.71	28.35	-

DAS - Days after spraying

* Mean of three replications

In each column figures followed by same letter do not differ significantly according to DMRT

was followed by T_7 (*T. viride*) which recorded 1.87 per cent. The maximum incidence (28.50 per cent) and severity (19.48 per cent) of disease were observed in control.

On 10 days after third spraying the minimum disease incidence (9.47per cent) and severity (2.71per cent) were recorded by the treatments T_1 (Bordeaux mixture) and T_7 (*T. viride*) respectively. The plants under control recorded the maximum incidence and severity of 33.27 and 21.71 per cent respectively.

The observation noticed on 10 days after fourth spraying also showed the lowest incidence and severity of disease in plants under T_1. It recorded 55.61 per cent reduction in disease incidence and 85.64 per cent reduction in disease severity over control. The maximum disease incidence (42.80 per cent) and disease severity (28.35 per cent) were recorded by the control. Among the other treatments T_2, T_4 and T_6 recorded more than 40 per cent reduction in disease incidence and the treatments T_3, T_5 and T_7 were recorded less than 40 per cent reduction of disease incidence over control. All treatments except captan (T_3) recorded more than 80 per cent reduction in disease severity over control.

4.6.5.3 Effect of selected treatments on height and number of leaves of mango grafts

Observations on height of plant and number of leaves are presented in Table 25 after the fourth spray. The maximum height of plant (6.68cm) and number of leaves (10.50) were recorded in treatment T_6. It was followed by the treatment T_5 in both height (6.22) and number of leaves (10.44) of mango grafts. Among the remaining treatments except control all the treatments recorded more than 5 cm in the height of plant. In number of leaves, the treatment T_2 and T_4 recorded 10.02 and 10.15 leaves respectively. The lowest height of plant (3.69cm) and number of leaves (6.38) was observed in control.

Table 25: Effect of selected treatments on height and number of leaves of mango grafts during *in planta* experiment

Tr. No	Treatments	Plant height(cm)*	Number of leaves*
T_1	Bordeaux mixture	5.54	9.42
T_2	Copper hydroxide	6.18	10.02
T_3	Captan	5.57	8.59
T_4	Hexaconazole	5.34	10.15
T_5	Carbendazim	6.22	10.44
T_6	*P. fluorescence*	6.68	10.50
T_7	*T. viride*	5.94	9.67
T_8	Control	3.69	6.38

* Mean of three replications

4.7 Screening of varieties of mango grafts for resistance against die back and leaf blight diseases

Different varieties of mango grafts maintained at Agricultural Research Station (ARS), KAU, Mannuthy and in a private nursery at Mannuthy were screened for resistance against die back and leaf blight disease of mango. The results are presented in Table 26 to 29.

4.7.1 Screening of varieties of mango grafts for resistance against die back

Thirteen varieties of mango grafts maintained at ARS, Mannuthy were screened against die back disease under natural condition. From the data (Table 26), it was found that the varieties showed variation in resistance to die back. Among the thirteen varieties none of them were found completely free of disease. However, three varieties *viz.*, Columbu, Alphonso and Mulgoa showed low CI value ranged from 2.09 to 2.81 and were found to be highly resistant to die back. Among these, Mulgoa showed the lowest CI value of 2.09. Six varieties Kadiri, Sindhu, Mallikā, Neelum, Mundappa and Chandrakaran showed resistance reaction against die back and CI value ranged from 4.83 to 8.00. Nadasala, Banganapalli and Priyor showed moderately resistant reaction against die back. Only one variety (Amrutham) was found moderately susceptible to die back and recording maximum CI value of 31.88.

Naturally infected mango grafts were observed from National Rose Garden (NRG), Mannuthy for resistance against die back disease (Table 27). Among the fifteen varieties observed, three varieties *viz.*,, Alphonso, Mulgoa, Chandanam were highly resistant to die back with the CI value ranged from 0.63-3.61. Banganapalli, Priyor, Columbu, Muvandan, Jahangir, Chandrakaran, Black &rose and Neelum were showed resistant reaction against die back and the CI value ranged from 4.1-8.3. Vatta mango and Kottaya paramban were

Table 26: Evaluation of different varieties of mango grafts for resistance against die back disease from Agricultural Research Station

Sl. No	Varieties	Per cent disease incidence	Per cent disease severity	Coefficient of infection	Disease reaction
1	Kadiri	30.0	17.50	5.25	R
2	Sindhu	28.6	17.86	5.11	R
3	Mallika	35.0	20.00	7.00	R
4	Amrutham	75.0	42.50	31.88	MS
5	Neelum	35.3	13.67	4.83	R
6	Columbu	20.0	11.25	2.25	HR
7	Nadasala	25.0	20.00	15.00	MR
8	Mundappa	30.0	17.50	5.25	R
9	Banganapally	40.0	25.00	10.00	MR
10	Alphonso	25.0	11.25	2.81	HR
11	Chandrakaran	40.0	20.00	8.00	R
12	Priyor	45.0	26.25	11.81	MR
13	Mulgoa	16.7	12.50	2.09	HR

Table 27: Evaluation of different varieties of mango grafts for resistance against die back disease from National Rose Garden

Sl.No	Varieties	PDI	PDS	Coefficient of infection	Disease reaction
1	Banganapally	25	19.28	4.82	R
2	Himayuddin	45	35.00	15.80	MR
3	Alphonso	30	12.02	3.61	HR
4	Priyor	25	16.25	4.06	R
5	Columbu	25	18.00	4.50	R
6	Mulgoa	10	6.25	0.63	HR
7	Vatta mango	50	42.50	21.30	MS
8	Muvandan	25	21.30	5.30	R
9	Kottaya paramban	50	42.50	21.25	MS
10	Jahangir	35	23.80	8.30	R
11	Chandanam	15	13.80	2.10	HR
12	Chandrakaran	25	16.30	4.10	R
13	Black and rose	20	21.30	4.30	R
14	Neelum	30	20.00	6.00	R
15	Thotta puri	45	20.98	9.44	M R

showed moderately susceptible to die back and recorded high CI value of 21.30 and 21.25 respectively.

4.7.2 Screening of mango grafts for resistance against leaf blight disease

From the data on screening of mango grafts maintained at ARS, Mannuthy against leaf blight disease under natural condition (Table 28), it was found that the varieties showed variation in resistance to leaf blight disease. Columbu, Alphonsa and Mulgoa showed that CI value ranged from 2.19 - 3.51 and were found to be highly resistant to leaf blight disease. Among these varieties Mulgoa showed the lowest CI value of 2.19. Kadiri, Sindhu, Banganapalli and Chandrakaran showed resistance reaction to leaf blight and the CI value ranged from 4.36 - 5.40. Mallika, Amrutham, Neelum, Nadasala, Mundappa and Priyor showed moderate resistance to leaf blight disease.

Naturally infected mango grafts were observed from NRG, Mannuthy for resistance against leaf blight disease (Table 29). Among the fifteen varieties observed, three varieties (Alphonso, Mulgoa and Chandrakaran) were highly resistant to leaf blight and CI value ranged from 0.80 - 3.61. Himayuddin and Priyor were showed resistance to leaf blight. Columbu, Chandanam, Jahangir and Neelum were found to be moderately resistant to leaf blight. Vatta mango, Muvandan, Banganapalli, Kottaya paramban, Black &rose and Thotta puri showed moderately susceptible reaction to leaf blight.

4.8 Estimation of total phenol content in different varieties of mango

Total phenol in the methanol extracts of six varieties of mango grafts was estimated and the results are presented in Table 30. The highest phenol content (393.69µg/g) was recorded by Alphonso variety. It was followed by Mulgoa which recorded 283.59µg of total phenol per gram of plant sample. The phenol content of other varieties *viz.*, Chandrakaran, Banganapalli Priyor, and Neelum was in the range of 114.49 to 171.75µg/g.

Table 28: Evaluation of different varieties of mango grafts for resistance against leaf blight disease from Agriculture Research Station

Sl. No	Varieties	PDI	PDS	Coefficient of infection	Disease reaction
1	Kadiri	40.00	13.50	5.40	R
2	Sindhu	35.71	14.14	5.05	R
3	Mallika	50.00	24.53	12.27	MR
4	Amrutham	50.00	25.64	12.82	MR
5	Neelum	53.30	18.85	10.05	MR
6	Columbu	35.00	10.02	3.51	HR
7	Nadasala	40.00	24.17	9.67	MR
8	Mundappa	45.00	21.27	9.57	MR
9	Banganapally	50.00	9.07	4.54	R
10	Alphonso	35.00	7.65	2.68	HR
11	Chandrakaran	45.00	9.69	4.36	R
12	Priyor	45.00	20.88	9.40	MR
13	Mulgoa	35.00	6.25	2.19	HR

Table 29: Evaluation of different varieties of mango grafts for resistance against leaf blight disease from National Rose Garden

Sl.No	Varieties	PDI	PDS	Coefficient of infection	Disease reaction
1	Banganapally	90.00	25.42	22.88	MS
2	Himayuddin	25.00	19.38	4.85	R
3	Alphonso	30.00	12.02	3.61	HR
4	Priyor	28.20	16.25	4.58	R
5	Columbu	45.00	36.92	16.60	MR
6	Mulgoa	10.60	7.58	0.80	HR
7	Vatta mango	85.00	42.59	20.73	MS
8	Muvandan	90.00	40.32	36.29	MS
9	Kottaya paramban	90.00	35.56	32.00	MS
10	Jahangir	55.00	32.77	18.02	MR
11	Chandanam	43.30	21.07	9.12	MR
12	Chandrakaran	19.30	15.61	3.01	HR
13	Black and rose	75.00	26.60	19.95	MS
14	Neelum	57.11	16.27	9.27	MR
15	Thotta puri	85.00	35.11	29.84	MS

Table 30: Total phenol content in different varieties of mango grafts

Sl.No	Mango varieties	Phenol content (µg/g)
1	Alphonso	393.69
2	Chandrakaran	171.73
3	Mulgoa	283.59
4	Banganapally	114.49
5	Priyor	171.75
6	Neelum	132.70

Discussion

DISCUSSION

Mango is an important commercial fruit crop of India. India is the world's largest producer of mango, which is grown through out the tropics and subtropics the world over. It contains rich source of essential nutrients and vitamins. Even though, mango is affected by a number of diseases at all stages of its development i.e. from nursery to harvest, die back is one of the important diseases of mango caused by *C.gloeosporioides* (Penz.) Sacc and *B. theobromae* Pat at all stages of its growth. Mango anthracnose was first reported by Collins in 1903. In India anthracnose disease was first reported by Mc Rae (1924).The first report on die back of mango caused by *B. theobromae* in India was given by Gupta and Zachariah (1945). In nurseries, the mango grafts were found severely infected by the disease. Complete drying up of the plants was occured if it was not controlled properly. In some nurseries, severe mortality of mango grafts was also observed due to die back disease. In view of the above facts, an investigation was carried out to study various aspects of die back disease of mango grafts in nursery particularly the symptomatology, etiology, disease management and varietal resistance.

To study the occurrence of die back diseases and for the collection of diseased sample, surveys were conducted in ten nurseries including the College orchard, College of Horticulture, Vellanikkara; Central Nursery, Vellanikkara; Agricultural research station (ARS), KAU, Mannuthy; National rose garden (NRG); Shalimar nursery; Raja nursery and South Indian nursery at Mannuthy; Murali nursery; Kairali nursery and EASF at Panancheri.

During the survey mango grafts were found infected by various types of leaf blight diseases along with die back. To study the influence of leaf blight pathogens on die back incidence, disease samples of both the diseases were collected separately

and pathogens were isolated on PDA medium. The isolation of pathogens from these symptoms yielded different types of fungal growth and showed the association of two fungi *viz. Colletotrichum* sp. and *Botryodiplodia* sp. from die back disease and *Colletotrichum* sp., *Pestalotiopsis* sp., *Cylindrocladium* sp., *Drechslera* sp. and *Alternaria* sp. from leaf blight disease.

Pathogenicity of these organisms was proved by artificial inoculation on mango grafts and also on severed mango leaves under *in vitro* condition. The symptoms produced under artificial conditions were same as that observed under natural condition. On artificial inoculation, the pathogen causing die back disease produced symptoms only on plants inoculated after giving pin pricks. The same pathogens causing die back disease were reported on mango by many workers from different parts of India. Rath *et al.* (1978); Patial (1988); Savant and Raut (2000) reported that *B. theobromae* and *C. gloeosporioides* were associated with die back of mango. The artificial inoculation of leaf blight pathogens under *in vitro* and *in planta* condition produced symptoms on inoculated leaves. All leaf blight causing pathogens except *Cylindrocladium* sp. were failed to produce die back symptom on artificial inoculation. *Cylindrocladium* sp. infected the young twigs and showed the death of tissues from tip downwards.

Symptomatological study of a disease is an important part in the diagnosis of a disease. In the infection chain, the symptom expression is the last stage and based on the symptom, the disease can be easily diagnosed and thereby help to adopt effective management practice. So the symptomatology of the die back disease occurred at the different locations were studied in detail. In this study, it was found that the symptoms produced by the same pathogen at all the ten locations were almost same. In die back disease caused by *Colletotrichum* sp., small necrotic spot on the tip of shoot and at the base of leaf petiole were observed initially. Later, they coalesced

and developed large brownish black necrotic area. This infection spread gradually down wards and reached up to a length of 2-3cm from the shoot tip. The leaves attached to the infected shoot also dried up, shrivelled and became brownish black in colour and fell down from the infected twigs. Whereas, in case of die back caused by *Botryodiplodia* sp., black discolouration and darkening of bark of young green twigs were observed at first. These dark lesions increased in size and spread down wards and resulted in typical die back symptom. The leaves present on infected twigs turned black, brittle and rolled up wards. Leaf shedding was also observed in severe infection. Occasionally infection by this pathogen was seen on leaf margin which later spread to petiole and young twigs and resulted in die back symptom. The pathogen produced dark coloured pycnidium on the infected area which appeared as small dark dots. Savant and Raut (2000) also described same type of symptom on mango stone grafts caused by *C. gloeosporioides* and *B. theobromae*.

Symptoms of leaf blight disease caused by five different pathogens were also studied in detail. In addition to die back disease (twig infection) *Colletotrichum* sp. was also found infecting the young leaves of mango grafts and caused leaf blight disease. It produced small circular dark brown spots with yellow halo. These spots coalesced and resulted in large blighted area. The pathogen produced the fruiting body acervulus, which appeared as dark dots on infected area. *Colletotrichum* sp. also showed its characteristic symptom, i.e. the shot hole formation on young leaves. In case of *Pestalotiopsis* sp., the symptom initiated either from the margin or from tip of the matured leaves as light brown necrotic area with irregular dark brown margin. Gradual spreading of the infection was observed towards the midrib with silvery grey coloured centre on upper surface and grey to brown coloured area on lower surface of the leaves. Like *Colletotrichum* sp., this pathogen also produced its fruiting body acervulus on the blighted area as dark dots. Mordue (1980) described the same type of symptom caused by *P. mangiferae* on mango. Other workers also described the

grey blight symptoms on other crops caused by *Pestalotiopsis* sp. (Khalequzamman *et al.*, 2003; Keith *et al.*, 2006). Hence, the observation made in this study was also in line with the earlier reports.

The other three pathogens *viz.*, *Cylindrocladium* sp., *Drechslera* sp. and *Alternaria* sp. initially produced dark brown spots on leaves. In all these infections, the spots were coalesced and produced large brown coloured blighted area on leaves. Yellow halo around the infected area was observed in symptoms produced by *Cylindrocladium* sp. and *Alternaria* sp. Young leaves were infected by *Cylindrocladium* sp. and hence premature leaf shedding was noticed in severely infected plants. Middle aged and matured leaves of mango grafts were found infected by *Drechslera* sp. and *Alternaria* sp. respectively. Prakash and Srivastava in 1987 reported same type of symptom on leaf, twig and fruits of mango caused by *A.alternata.* Search of literature did not reveal any reports on leaf blight disease caused by *Drechslera* sp. and *Cylindrocladium* sp. on mango.

From the die back symptom two pathogens *viz. Colletotrichum* sp. and *Botryodiplodia* sp. were isolated. *Colletotrichum* sp. was isolated from seven different locations whereas *Botryodiplodia* sp. was obtained only from two locations. All the isolates of same pathogens showed almost same type of cultural and morphological characters. *Colletotrichum* sp. showed a medium growth rate compared to *Botryodiplodia* sp. which showed a slight fast growth rate and completed the 9cm growth in six and five days respectively. The matured colony of *Colletotrichum* sp. was thick and greyish black in colour and it showed pink coloured spore masses in the culture but the development of acervuli was not observed in the culture. A slight variation in conidial measurements of different isolates of *Colletotrichum* sp. obtained from seven locations was observed. The conidia showed 11.9 - 13.25 x 5 µm in size, hyaline and single celled with oil globules. The cultural

and morphological characters of *Colletotrichum* sp. observed were found same as those described by earlier workers (Davis, 2003; Bag, 2004 and Gaikwad and Sawant, 2005). Thus one of the pathogens causing die back of mango grafts by producing necrotic spots on young twig and also at the base of leaf petiole was identified as *Colletotrichum gloeosporioides* (Penz) Sacc and was confirmed by NCFT, New Delhi (NCFT No.2172.08).

Based on the morphological characters of various isolates of *C.gloeosporioides* obtained from seven different locations, genetic dissimilarity index was computed using NTSYS pc 2.02 soft ware. Clustering was done as per UPGMA method of Sneath and Sokal (1973) and the clustering pattern showed slight variability among the isolates. The seven isolates were grouped in to two clusters A and B. The highest dissimilarty co-efficient was noticed in isolates obtained from central nursery and college orchard. Cluster A was further divided in to two sub clusters A_1 and A_2. The lowest dissimilarity co-efficient was recorded between Kairali (KN) and National rose garden (NRG). Resmi (2005) reported variability in different species of *Alternaria* isolated from cucurbitaceous vegetable by calculating the genetic dissimilarity index using NTSYS pc. 2.02 soft ware.

The culture of *Botryodiplodia* sp. showed greyish black coloured fluffy aerial growth and formation of pycnidia in the colony on six DAI. No variation was observed in the conidial characters of the isolates obtained from two different locations. The colony characters and conidial measurements recorded by this pathogen were found comparable to those reported by earlier workers (Punithalingam, 1976 and Ferrari *et al*, 1996). Thus the other pathogen causing die back of mango graft by producing black lesions on young green twigs was identified as *Botryodiplodia theobromae* Pat and was further confirmed by NCFT, New Delhi (NCFT No.1770.70).

In addition to *C. gloeosporioides*, four more pathogens *viz. Pestalotiopsis* sp., *Drechslera* sp., *Cylindrocladium* sp. and *Alternaria* sp. were also isolated from leaf blight symptom. All these organisms were isolated on PDA medium and they showed good growth on this medium. The cultural and morphological characters of *Colletotrichum* sp. isolated from leaf blight symptom were found similar to the characters of *Colletotrichum gloeosporioides* (Penz) Sacc and thereby confirmed it as one of the pathogens of leaf blight disease.

Even though a good colony growth of all the other four pathogens were observed on PDA medium, *Cylindrocladium* sp. took more days (9 days) to complete the 9cm growth in Petri dish and was followed by *Alternaria* sp. which took 7 days. But a medium growth rate of six days was observed in case of *Pestalotiopsis* sp. and *Drechslera* sp. The matured colonies of all these pathogens were observed as thick but in different colour. *Pestalotiopsis* sp. showed a pure white growth with black spore masses, *Cylindrocladium* sp. appeared as in reddish brown colour whereas *Drechslera* sp. and *Alternaria* sp. showed greyish black coloured mycelial growth. All these pathogens produced conidia in the culture. The hyphal and conidial characters and measurements of hyphae and conidia were taken. Based on these characters and on comparing the characters given in CMI Descriptions of Pathogenic Fungi and Bacteria (No. 676), the pathogen causing grey coloured leaf blight symptom on mango leaves was identified as *Pestalotiopsis mangiferae* (Henn.) Steyaert. The identification of the fungus was further confirmed by NCFT, New Delhi (NCFT No.1774.07). The pathogen producing irregular blighted area on young leaves resulted in leaf shedding was identified as *Cylindrocladium* sp. National Center of Fungal Taxonomy, New Delhi identified the culture as a new pathogen on mango as *Cylindrocladium mangiferae* sp.nov (NCFT No.1772.07). The other two pathogens causing large brownish black blighted area were identified as *Drechslera*

australiensis (Bugnicourt) Subram & Jain ex M.B.Ellis and *Alternaria alternata* (Fries) Keissler. The identity of *D. australiensis* (Bugnicourt) Subram & Jain ex M.B.Ellis was confirmed by NCFT, New Delhi (NCFT No.1771.07). Earlier, many workers studied on the cultural and morphological characters of *Pestalotiopsis mangiferae* (Mordue, 1980), *Drechslera australiensis* and *Alternaria alternata* (Davis, 2003; Resmi, 2005 and Tziros *et al.* 2008). *C. mangiferae* sp. nov is a new pathogen on mango causing leaf blight symptom and is the first report on mango. But characters of other species of *Cylindrocladium* were reported by many workers (Beena *et al*, 1994; Srinivasan and Gunasekaran, 1995).

The main objective of the present study was the management of die back disease and also the leaf blight disease associated with the die back disease. For this, at first an *in vitro* evaluation of different fungicides and antagonists were carried out. Fungicides such as Bordeaux mixture, copper oxychloride, copper hydroxide, mancozeb, captan, hexaconazole, carbendazim and zineb each at three different concentrations were evaluated against die back and leaf blight causing pathogens. Among these fungicides, Bordeaux mixture at all the three concentrations recorded hundred per cent inhibition on the growth of all the pathogens. The effectiveness of Bordeaux mixture against die back pathogens of many crops was reported by many workers (Sharma and Kaul (1990); Sharma and Badiyala (1994); Sharma and Gupta (1994) and Ebenezar and Subramanian (1995) Patil *et al.* (2007)) and hence the present result is in conformity with the earlier reports. It was followed by carbendazim which showed hundred per cent inhibition on the growth of *C. gloeosporioides*, *P.mangiferae* and *C. mangiferae* at all the three concentrations. But it was not effective against *D. australiensis* which recorded less than 50 per cent inhibition over control by all the three concentrations of the fungicide. Srinivasan and Gunasekaran (1998) and Vrinda (2002) observed the fungicidal effect of carbendazim in controlling leaf rot of coconut caused by *C. gloeosporioides*. Khalequzamman *et*

al. (1988); Joshi and Raut (1992) also obtained the same result, and reported 0.1 per cent carbendazim as most effective against *Pestalotia* sp. Carbendazim showed hundred per cent inhibition of *B. theobromae* at 0.1 and 0.2 per cent concentrations whereas at 0.05 per cent concentration, it recorded only 76.9 per cent inhibition over control. Sohi *et al.* (1973) also reported the effectiveness of 0.1 per cent carbendazim for the management of *B. theobromae* causing stem end rot of mango. All the three concentrations of carbendazim showed more than 60 to 63.5 per cent inhibition on the growth of *A. alternata*. Carbendazim failed to show hundred per cent inhibition on the growth of *D. australiensis* and *A. alternata*. This observation confirmed the ineffectiveness of carbendazim against coloured dematiaceous fungi whereas the other systemic fungicide, hexaconazole was found very effective to control the growth of *D. australiensis* and recorded more than 85 per cent inhibition on the growth of this pathogen by all the three concentrations tested. Similarly, this fungicide at 0.15 per cent concentration recorded the maximum inhibition of 81.4 and 82.4 per cent on the growth of *C. gloeosporioides* and *P. mangiferae* respectively, but statistically it was on par with the data recorded by 0.1 per cent concentration. The fungicide hexaconazole was found to be very effectively inhibiting the growth of *C.gloeosporioides* causing anthracnose disease in guava (Patil *et al.*, 2007). Studies conducted by Gupta *et al.* (2005) also reported that hexaconazole was effective against the growth of *C. lindemuthianum*. Hexaconazole at 0.15 per cent concentration recorded the maximum inhibition (65.2 per cent) on growth of *B.theobromae* whereas at 0.1 per cent concentration, it showed the maximum inhibition on the growth of *A. alternata* (71.3 per cent) over control. This fungicide was found least effective in inhibiting the growth of *C. mangiferae* which showed only 54.1 per cent inhibition on the growth of this pathogen at the highest concentration of 0.15 per cent. A search of literature on the effectiveness of hexaconazole on die back and leaf blight pathogens of mango revealed no information.

In addition to Bordeaux mixture, two more copper fungicides *viz.* copper oxychloride and copper hydroxide were also used for the *in vitro* evaluation. On comparing the data recorded by these two fungicides, it was revealed that copper hydroxide was more effective than copper oxychloride to control the growth of all the five pathogens. Copper hydroxide at the highest concentration (0.25 per cent) recorded the maximum inhibition of all the pathogens and among them, more than 70 per cent inhibition on the growth of *C. gloeosporioides* (72.6 per cent), *P. mangiferae* (73.2 per cent), *C. mangiferae* (84.6 per cent) and *D. australiensis* (77.7 per cent) was observed. In the case of copper oxychloride, the maximum value recorded was 63.2 per cent inhibition on the growth of *D. australiensis* by the highest concentration (0.4 per cent) of the fungicide and it was followed by 0.2 per cent concentration which recorded 62.1 per cent. Copper oxychloride, at all the three concentrations recorded less than 60 per cent inhibition on the growth of all other pathogens over control and that was observed in the range of 21.1 to 57.4 per cent. It was found to be least effective against *P. mangiferae* and 0.3 per cent concentration of the fungicide recorded only 21.1 per cent inhibition of *P. mangiferae* over control.

Captan, a heterocyclic nitrogen compound known as Kittleson's killer was found to be the most effective to control *P. mangiferae* which recorded more than 75 per cent inhibition on the growth of this pathogen by all the three concentrations tested. But, from the data it was observed that this fungicide was least effective to inhibit the growth of *C. mangiferae* and 0.3 per cent concentration of this fungicide recorded only 0.5 per cent inhibition on the growth of this pathogen. Captan recorded 63.3 to71.4 per cent inhibition of *C. gloeosporioides*, 48.0 to 51.9 per cent on growth of *B. theobromae*, 56.8 to 57.1 per cent on growth of *D. australiensis* and 45.4 to 49.3 per cent on growth of *A. alternata* inhibition over control. Singh and Agarwala (1987) also reported the least effectiveness of captan against anthracnose pathogen.

Contradictory to that Patial (1988) reported the most effectiveness of captan at 0.3 per cent concentration against die back disease.

Similar to captan, mancozeb and zineb recorded the maximum inhibition of growth in *P. mangiferae*. Mancozeb recorded more than 80 per cent inhibition on growth of *P. mangiferae* at all the three concentrations whereas zineb, only 0.4 per cent concentration recorded more than 80 per cent inhibition on the growth of this pathogen. In 1988, Ramaswamy *et al.* and Khalequzamman *et al.* reported that Dithane M-45 showed good result against *Pestalotiopsis* sp. But the mancozeb and zineb showed less than 50 per cent inhibition on the growth of *C. gloeosporioides, B.theobromae, C. mangiferae* and *A. alternata* except mancozeb at 0.4 per cent concentration which recorded 58.7 per cent inhibition on growth of *C. mangiferae.* But both the fungicides showed more than 50 per cent inhibition on the growth of *D.australiensis* except mancozeb at 0.2 per cent concentration which recorded 42.7 per cent inhibition on growth of *D. australiensis*. Earlier many workers reported the least effectiveness of zineb (Deepthy, 2003) and mancozeb (Singh and Agarwala.1987 and Fitzell and Peak.1988) against *C. gloeosporioides* in different crops. But contradictory to that, many workers reported the inhibitory effect of mancozeb against plant pathogens in many crops (Majumdar and Pathak. 1997; Kumar 1999; Gupta *et al.*, 2005).

Summing up the results of *in vitro* evaluation of fungicides against die back and leaf blight pathogens, it was found that Bordeaux mixture at 0.5, 1.0 and 1.5 per cent concentrations was most effective to control all the six pathogens since it recorded hundred per cent inhibition on growth of these pathogens. Similarly carbendazim also recorded hundred per cent inhibition of *C. gloeosporioides, P.mangiferae, C. mangiferae* and *B. theobromae* except at the lowest concentration of 0.05 per cent which was observed in case of *B. theobromae*. This fungicide did not

show hundred per cent inhibition of coloured fungi *viz. D. australiensis* and *A. alternata*. Hexaconazole at 0.1 and 0.15 per cent showed more than 80 per cent inhibition on the growth of *C. gloeosporioides*, *P. mangiferae,* whereas all the three concentrations recorded more than 80 per cent inhibition on the growth of *D. australiensis*. Compared to copper oxychloride, copper hydroxide recorded better results especially at the highest concentration of 0.25 per cent. All the three concentrations of captan, mancozeb and zineb were found most effective to control the growth of *P. mangiferae*.

Even though chemical fungicides are very effective to manage the diseases, the continuous use of fungicides is not recommended for the management of a disease. It may lead to the development of resistant strains of pathogen, and may cause residual toxicity. An alternative to these problems is the use of bioagents for the management of plant diseases. So an *in vitro* evaluation of fungal and bacterial antagonists against the die back and leaf blight pathogens of mango was carried out. The standard culture of *T. viride* (KAU), *T. harzianum* (IISR) and *P. fluorescens* (KAU) were used for the study. From the data, it was revealed that all the three antagonists tested were very effective for the control of all the pathogens. The two fungal antagonists *viz.*, *T. viride* and *T. harzianum* showed complete over growth on the pathogens of die back and leaf blight diseases. *C. gloeosporioides* and *C.mangiferae* were found completely over grown by both the antagonists on seven DAI whereas in case of *B. theobromae*, *P. mangiferae*, *D. australiensis* and *A.alternata*, it took only six days for the overgrowth. So both the fungal antagonists were found equally effective for inhibiting the growth of all the pathogens. There were many reports on the antagonistic efficiency of *T. viride* and *T. harzianum* against many fungal plant pathogens (Gupta *et al.*, 1995; Majumdar and Pathak, 1995; Adebanjo and Bankole, 2004 and Sharma *et al.*, 2005.) and hence the earlier reports are supportive to the present results.

Per cent inhibition on the growth of all the pathogens due to bacterial antagonist, *P. fluorescens* was evaluated. The data showed more than 50 per cent inhibition on the growth of *C. gloeosporioides*, *B. theobromae*, *P. mangiferae* and *D. australiensis* and among them, the maximum inhibition of growth was observed in *B. theobromae* (60.99 per cent) and was followed by *P. mangiferae* (57.78 per cent), *D. australiensis* (56.31 per cent) and *C. gloeosporioides* (50.33 per cent). The lowest inhibition was observed in *A. alternata* (38.49 per cent). The efficacy of *P.fluorescens* as a good antagonist is a proved fact and there were many reports on the effective use of this antagonist against many diseases (Koomen and Jeffries, 1993; Vivekananthan *et al.*, 2004 and Vivekananthan *et al.*, 2006) reported the effectiveness of *P. fluorescens* against anthracnose of mango.

After the *in vitro* evaluation of fungicides and antagonists, an *in planta* experiment was conducted two times to know the effect of various treatments on the management of die back and leaf blight diseases. From the field observations and search of literatures, it was observed that the die back disease of mango grafts was always associated with an insect attack. The injuries made by the insect predispose the plants for infection and also the die back pathogen could easily enter the plant through the wounds made by the insect. Hence in the treatment, an insecticide viz. quinalphos was also included. So in the first *in planta* experiment the recommended dose of all fungicides and the commercially available bioagents *viz. T. viride* and *P.fluorescens* which were used for the *in vitro* evaluation and the insecticide, quinalphos @ 0.05 per cent were included. The observations made on per cent disease incidence and per cent disease severity of die back disease of mango revealed that all the treatments were superior to control. The incidence of dieback disease was observed only on control plants in the early stage of the experiment. Ten days after the last application of treatments, the highest reduction in disease incidence was

observed in plants sprayed with Bordeaux mixture and copper oxychloride (91.67 per cent). It was followed by hexaconazole, carbendazim and copper hydroxide (80.56 per cent). The bacterial antagonist and insecticide, quinalphos recorded 70.01 per cent reduction over control. The observation on per cent disease severity revealed that all treatments were superior to control. The highest reduction in disease severity (90.47 per cent) was observed in plants treated with one per cent Bordeaux mixture, 0.3 per cent copper oxychloride, 0.1 per cent carbendazim, 0.15 per cent copper hydroxide and all these treatments were found statistically superior than all other treatments. The efficiency of copper fungicides *viz.* Bordeaux mixture, copper oxychloride, copper hydroxide and the systemic fungicides hexaconazole and carbendazim for the management of plant diseases was reported earlier by many workers. Sharma and Kaul (1990), Ebenezar and Subramanian (1995) reported that Bordeaux mixture at one per cent concentration was the best to control *C. gloeosporioides*. Similarly, Sharma and Gupta (1994) showed the effectiveness of one per cent Bordeaux mixture against *B. theobromae*. The effectiveness of carbendazim against *C. gloeosporioides* and *B. theobromae* was reported by Sohi *et al.* (1973); Singh *et al.* (1989); Sharma and Badiyala (1994); Deepthy (2003) and Sharma and Verma (2007). Gupta *et al.* (2005) and Patil *et al.* (2007) reported the complete inhibition on the growth of *Colletotrichum* sp. by hexaconazole. The treatments T9 (*P. fluorescens*), T10 (*T.viride*) and T11 (quinalphos) were also on par with the above treatments. From this, it was revealed that both the bioagents and even the insecticide quinalphos were equally efficient to the copper fungicides and systemic fungicides in the management of die back disease of mango. The fungicidal property of quinalphos was earlier reported by Kalpana (1992); Bindu (1996) and Deepthy (2003) and hence our observations confirm the earlier reports. Similarly the antifungal antagonistic property of *T. viride* and *P. fluorescens* was also reported by many workers (Patil, 1992; Koomen and Jeffries, 1993; Majumdar and Pathak, 1995; Bankole and Adebanjo, 1996; Vivekananthan *et al.*, 2004 and Vivekananthan *et al.*, 2006).

In this experiment, along with die back disease, leaf blight symptoms were also developed on mango leaves and PDI and PDS of leaf blight disease were also calculated. From the data, significant difference among the treatments was observed. After the fourth spraying with chemicals and antagonists the highest reduction in disease incidence (66.59 per cent) and disease severity (79.63 per cent) were recorded in plants sprayed with one per cent Bordeaux mixture. But in both disease incidence and severity, Bordeaux mixture was on par with all treatments except mancozeb and zineb. The bioagents and the insecticide, quinalphos were found efficient in reducing disease incidence and disease severity. Among the treatments, Bordeaux mixture, copper oxychloride, carbendazim, copper hydroxide and quinalphos recorded more than 50 per cent reduction over control. The effectiveness of copper fungicides and systemic fungicides against leaf blight pathogens was reported by many workers (Sharma and Gupta, 1994; Gupta *et al.*, 2005; Ekbote, 2005; Patil *et al.*, 2007 and Sanjay *et al.*, 2008). Mancozeb at 0.3 per cent showed the lowest reduction of disease incidence over control (16.68 per cent). Earlier several reports are there on the low efficiency of mancozeb compared to other fungicides. Yadav and Narain (1993) reported the least effectiveness of mancozeb against *A. alternata* in chickpea. Singh and Agarwala (1987) also reported the least effectiveness of Dithane M 45 against anthracnose of citrus. But contradictory to that, Datar *et al.*1990 showed the lowest incidence of chilli anthracnose in plants treated with mancozeb @ 0.25 per cent.

A second experiment under *in planta* condition was carried out in which the best treatments from the *in vitro* evaluation and also from the first *in planta* experiment were used. Thus seven treatments were selected *viz.* one per cent Bordeaux mixture, 0.15 per cent copper hydroxide, 0.3 per cent captan, 0.1 per cent hexaconazole, 0.1 per cent carbendazim and bioagents, *T. viride* and *P. fluorescens* to confirm the result of first experiment. Observations on PDI and PDS of die back

and leaf blight diseases were taken separately and data did not show any significant difference among the treatments except in the die back incidence. The highest per cent reduction of disease severity over control was exhibited by Bordeaux mixture (T_1) (80.02 per cent) and was on par with all other treatments. Similarly the highest reduction in PDI was also noticed in plants sprayed with Bordeaux mixture (T_1, 71.44 per cent). But it was on par with all other treatments except T_3 (captan 0.3 per cent) which recorded only 28.56 per cent reduction over control. It was concluded that all the five fungicides *viz.*, Bordeaux mixture, copper hydroxide, hexaconazole and carbendazim and captan were equally effective for the management of disease severity of die back of mango grafts. Moreover it was found that both the antagonists, *T. viride* (20g/ l) and *P. fluorescens* (2%) were also equally efficient to fungicides in the management of die back disease. Vivekananthan *et al.*2004 reported the efficacy of *P. fluorescens* as superior to carbendazim for the management of mango anthracnose in field condition. As discussed earlier, these observations are in conformity with the earlier reports. Sohi *et al.* (1973) and Sharma and Badiyala (1994) observed that 0.1 per cent carbendazim was very effective to control die back of mango caused by *B. theobromae.* Similarly Ebenezar and Subramanian (1995); Bindu (1996) and Patil *et al.* (2007) reported the effectiveness of one per cent Bordeaux mixture to control *C. gloeosporioides* causing diseases in citrus, cashew and guava respectively.

Observation on PDI and PDS of leaf blight symptoms were also taken during the second experiment conducted under *in planta* condition and observed no significant difference among the treatments. Here also the highest reduction of disease incidence and severity over control was observed in the treatment T_1 (Bordeaux mixture). But it was on par with all other treatments. Hence, it was concluded that all the five fungicides tested and the fungal and bacterial antagonists were equally effective in the management of leaf blight disease of mango.

The next part of the investigation was screening of different varieties of mango for resistance against die back and leaf blight diseases because host resistance is an important factor in pathogenesis and which in some extent determines the infection by a pathogen. Varietal resistance is a type of biological control in which the host itself plays the role of an antagonist. Use of resistant varieties is the most simple, practical, effective and economical method of plant disease management. Screening of large number of varieties will be helpful for locating highly resistant one to die back disease, which can be further utilized for the development of resistant varieties in breeding programme. With this idea, mango grafts of different varieties maintained at Agricultural Research Station, Mannuthy and in a private nursery, National Rose Garden, Mannuthy were screened for resistance against die back and leaf blight disease under field condition. The varieties Alphonso, Mulgoa and Columbu were found highly resistant to die back disease at ARS, Mannuthy, whereas the varieties Alphonso, Mulgoa and Chandanam maintained at NRG showed high resistance to this disease. The CI value of these highly resistant varieties ranged from 0.63 to 3.61. The varieties Neelum and Chandrakaran at both the locations showed resistant reaction to die back disease; showing CI value in the range of 4.1 to 8. The variety Amruthum at ARS, Vatta mango and Kottayaparamban at NRG were recorded moderately susceptible reaction and showed CI value ranged from 21.25 to 31.88. Out of thirteen varieties at ARS and sixteen varities at NRG screened, none of the varieties showed highly susceptible reaction to die back disease.

Varietal screening against leaf blight disease revealed highly resistant reaction in Alphonso and Mulgoa at both the locations against this disease and recorded CI value ranged from 0.80 to 3.61. The variety Columbu also showed highly resistant reaction against leaf blight at ARS with a CI value of 3.51. Neelum variety recorded moderate resistance in both the locations whereas Chandrakaran showed resistant

reaction at ARS, and highly resistant at NRG. None of the varieties maintained at ARS recorded susceptability to this disease. But seven varieties at NRG showed moderately susceptible reaction ranged from 19.95 to 36.29 which included the local varieties *viz.* Vatta mango, Muvandan and Kottayaparamban.

From the data it was concluded that the varieties Alphonso and Mulgoa were recorded as best varieties since they showed highly resistant reaction to both die back and leaf blight diseases. Vander plank (1968) suggested that the difference in disease resistance exhibited by the genotypes may be due to different types of interaction between pathogen and the genotypes that affected. Many factors are responsible for the resistant type of reaction shown by the genotype. This may include insufficient inoculum load, absence of pathogenic races to that genotype, unfavourable environmental condition and nutrient status of the soil in which the crops were cultivated (Yarwood 1978, Khan 1989).

Phenolic compounds have an important role in defence mechanism of plants which give resistance to pathogens. Certain phenols are already present in the plant while others are produced only after the infection. Total phenol content of plant will give an indication of host resistance to pathogen. According to Nicholson and Hammerschmidt (1992), the phenolic compounds were the main toxic chemicals produced to inhibit pathogen or its activities. They observed antibiotic phenols in all plants investigated. With this view the total phenol content of different mango varieties which showed either high resistance or susceptibility to infection were estimated.

From the data it was observed that the highest total phenol content of 393.69µg/g was recorded by Alphonso variety and was followed by Mulgoa which recorded 283.59µg/g. During the screening for resistance to die back and leaf blight

disease, both these varities recorded high resistance reaction against both the diseases. The lowest total phenol content was observed in Banganapally (114.49µg/g) and all other varieties recorded the total phenol content in the range from 132.70 to 171.75µg/g. Marie (2001) reported the total phenol content of different mango varieties, and she recorded the minimum value in Kalapady (18.73 mg/g) followed by Moovandan (21.51mg/g). Even though a resistant to moderately resistant reaction was observed in Priyor and Neelum varieties against die back and leaf blight, they recorded total phenol content of 171.75 and 132.70µg/g respectively.

Summing up the results of this investigation, it was revealed that, two fungal pathogens *viz.*, *C. gloeosporioides* and *B. theobromae* were associated with the die back disease and five pathogens *viz.*, *C. gloeosporioides*, *P. mangiferae*, *C.mangiferae*, *D. australiensis* and *A. alternata* were the causal agents of leaf blight disease of mango grafts. The *in vitro* evaluation of different fungicides proved the efficiency of Bordeaux mixture even at 0.5 per cent concentration as the most effective fungicide and which recorded hundred per cent inhibition of the growth of all the six pathogens. The two fungal antagonist *viz.*, *T. viride* (KAU) and *T.harzianum* (IISR) showed complete inhibition on the growth of all the six pathogens whereas the bacterial antagonist *P. fluorescens* showed more than 50 per cent inhibition on the growth of *C. gloeosporioides*, *B. theobromae*, *P. mangiferae* and *D. australiensis*.

From the results of the *in planta* evaluation of fungicides and antagonists, it was found that all the five fungicides *viz.*, Bordeaux mixture (one per cent), copper hydroxide (0.15 per cent), hexaconazole (0.1 per cent), carbendazim (0.1 per cent) and captan (0.3 per cent) and two bioagents *viz.*, *T. viride*(KAU) (20g/l) and *P.fluorescens* (KAU) (2.0%) were equally efficient in the management of die back and leaf blight disease of mango grafts.

Screening of different varieties of mango grafts showed highly resistant reaction in Alphonso and Mulgoa to die back and leaf blight diseases. The highest total phenol content of 393.69 µg/g was recorded in Alphonso variety and was followed by Mulgoa which recorded 283.59 µg/g of total phenol per gram of plant sample.

Summary

SUMMARY

The present investigation on "Etiology and management of die back disease of mango grafts in nursery" was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2007-2008 to study various aspects, particularly the etiology, symptomatology, varietal reaction and management of die back disease.

Surveys were conducted at ten different nurseries *viz*., Central nursery, KAU, Vellanikkara; College orchard, COH, Vellanikkara; Agricultural Research station, Mannuthy; Raja nursery; Shalimar nursery; National rose garden and South Indian nursery at Mannuthy; EASF, Kairali and Murali nursery at Panancheri on the occurrence of die back disease of mango grafts. In the nurseries leaf blight disease was also noticed along with die back disease. Pathogens associated with these diseases were isolated and proved the pathogenicity by artificial inoculation under *in vitro* and *in planta* conditions. Isolation of pathogen on PDA medium yielded *Colletotrichum* sp. and *Botryodiplodia* sp. from die back symptom and *Colletotrichum* sp., *Pestalotiopsis* sp., *Cylindrocladium* sp., *Drechslera* sp. and *Alternaria* sp. from leaf blight symptom.

Studies on symptomatology showed that die back and leaf blight symptoms produced by the respective pathogens at different locations were same. The die back caused by *Colletotrichum* sp. was observed initially on young twigs as small necrotic spots on the tip of shoot and at the base of leaf petiole. Later, they coalesced and developed large brownish black necrotic area and spread downwards and reached up to a length of 2-3 cm from the shoot tip. The leaves on the infected shoot dried up and fell down whereas in case of *Botryodiplodia* sp., black discolouration and darkening of the bark of young green twigs were observed at first. This infection spread downwards and resulted in typical die back symptom. The leaves turned black, brittle and rolled upwards. In severe infection leaf shedding was also observed. The leaf blight caused by *Colletotrichum* sp. was

noticed on young leaves and appeared as dark brown circular spot with yellow halo which coalesced to form large blighted area. The pathogen produced the fruiting body, acervulus, which appeared as dark dots on infected area and also showed shot hole symptom. *Pestalotiopsis* sp. infected matured leaves and produced light brown necrotic area with irregular dark brown margin from the leaf tip or margin. The infection spread towards the midrib with silvery grey coloured area on upper surface and grey to brown colour on lower surface. Like *Colletotrichum* sp. this pathogen also produced its fruiting body acervulus on the blighted area as dark dots.

The other three pathogens *viz.*, *Cylindrocladium* sp., *Drechslera* sp. and *Alternaria* sp. initially produced dark brown spots on leaves. In all these infections, the spots were coalesced and produced large brown coloured blighted area on leaves. Yellow halo around the infected area was observed in symptoms produced by *Cylindrocladium* sp. and hence pre mature leaf shedding was noticed in severely infected plants. Middle aged and matured leaves of mango grafts were found infected by *Drechslera* sp. and *Alternaria* sp. respectively.

Cultural and morphological characters of the six pathogens were studied on PDA medium. *Colletotrichum* sp. showed medium rate of growth and the colony was greyish white initially which later turned to greyish black in colour. Pink coloured spore masses were observed in the culture. The hyphae septate and showed 17.66 -18.80 × 2.88 - 3.26 µm in size. Conidia hyaline, aseptate and measured 11.9 - 13.25 × 5µm with oil globules. The organism was identified as *Colletotrichum gloeosporioides* (Penz.) Sacc and confirmed by National Center of Fungal Taxonomy, New Delhi (NCFT No 2172.08). The result of the genetic dissimilarity index computed by the seven different isolates of *C. gloeosporioides* showed a slight variability among the isolates. The seven isolates were grouped in to two clusters A and B. The highest dissimilarity co efficient was noticed in isolates obtained from Central nursery and College orchard. Cluster A was further

divided in to two sub clusters A_1 and A_2. The lowest dissimilarity co-efficient was noticed in isolates from Kairali and National rose garden.

The culture of *Botryodiplodia* sp. showed black coloured fluffy aerial growth and formation of pycnidia on artificial medium. The hyphae septate and measured 17.5-35 × 5µm in size. Conidia initially unicellular, hyaline, when matured became bicelled, brownish black and measured 15 - 25 × 12.5 - 17.5µm. Based on the characters, the organism was identified as *Botryodiplodia theobromae* Pat and confirmed by National Center of Fungal Taxonomy, New Delhi (NCFT No. 1770.70).

Pestalotiopsis sp. showed medium rate of growth and the culture was pure white in colour. Black spore masses were observed on the surface of colony. Hyphae measured 1.5 - 4 × 5 - 18.5µm in size and hyaline. Condia fusiform, five celled, central three cells coloured, end cells hyaline and measured 15 - 22.5 × 7.5 µm with 2 or 3 appendages. The organism was identified as *Pestalotiopsis mangiferae* (Henn.) Steyaert and further confirmed by National Center of Fungal Taxonomy, New Delhi (NCFT No. 1774. 07).

Cylindrocladium sp. showed white growth with reddish brown tinge on aerial mycelium. Later the slow growing colonies turned to reddish brown in colour. Hyphae septate, reddish brown and measured 16.8 - 120 × 2.4 - 4.8 µm in size. Showed penicillate conidial apparatus with non septate primary, secondary and tertiary branching of conidiophore, phialides hyaline. Conidia hyaline, cylindrical and measured 36 × 4.8 µm in size. Based on the characters, the organism was identified as *Cylindrocladium* sp. National Center of Fungal Taxonomy, New Delhi identified this fungus as *Cylindrocladium mangiferae* sp. nov (NCFT No. 1772.07) as a new pathogen on mango. Hence the leaf blight disease caused by *Cylindrocladium mangiferae* sp. nov is the first report on mango.

Colonies of *Drechslera* sp. were greyish white which later turned to greyish black in colour and showed medium rate of growth. Hyphae septate and hyaline at first, when matured turned to brownish black and measured 15 - 32.5 × 5µm in size. Conidiophores brown, septate and sympodula type. Conidia thick walled, light brown and measured 7.5 - 25 × 5 - 7.5µm in size with 3 - 6 septate. Based on the characters the organism was identified as *Drechslera australiensis* (Bugnicourt) Subram & Jain ex M.B.Ellis and confirmed by National Center of Fungal Taxonomy, New Delhi (NCFT No. 1771. 07).

Alternaria sp. showed grey coloured slow growing colonies which later turned to black. The hyphae septate, hyaline to brown and 15 - 30 × 5µm in size. Conidia brownish black in colour and measured 25 - 60 × 7.5 - 15µm with 2 - 6 transverse and 1 - 2 longitudinal septa, beak prominent 5 - 32.5µm length. Based on these characters the organism was identified as *Alternaria alternata* (Fries) Keissler.

An *in vitro* experiment was conducted to evaluate the effectiveness of fungicides and antagonists against die back pathogens. The pathogens isolated from leaf blight symptoms were also included in the *in vitro* evaluation. Among the fungicides, Bordeaux mixture at all the three concentrations recorded cent per cent inhibition on the growth of all the six pathogens. Carbendazim showed cent per cent inhibition on the growth of *C. gloeosporioides*, *P. mangiferae* and *C.mangiferae* at all the three concentrations but it was not very effective against *D.australiensis* which recorded less than 50 per cent inhibition over control by all the three concentrations. Hexaconazole was found effective to control the growth of *D. australiensis* and recorded more than 85 per cent inhibition over control on the growth of this pathogen. Copper hydroxide was found more effective than copper oxychloride to control the growth of all the pathogens except *B.theobromae*. Captan was found to be the most effective to control *P. mangiferae* at all the three concentrations which recorded more than 75 per cent inhibition on the growth of this pathogen. Mancozeb at all the three concentrations and zineb at

0.4 per cent concentration recorded more than 80 per cent inhibition over control on the growth of *P. mangiferae*.

The two fungal antagonists *viz., T. viride* (KAU) and *T. harzianum*(IISR) showed complete inhibition on the growth of all the six pathogens whereas the bacterial antagonist, *P. fluorescens* showed more than 50 per cent inhibition on the growth of *C. gloeosporioides, B. theobromae, P. mangiferae* and *D.australiensis*.

After the *in vitro* evaluation of fungicides and antagonists, an *in planta* experiment was conducted two times to know the effect of various treatments on the management of die back and leaf blight diseases. Observations on per cent disease incidence and per cent disease severity of die back and leaf blight disease revealed that all the treatments were superior to control. The data recorded during the first experiment revealed that ten days after the last application of treatments, the highest reduction in disease severity (90.47 per cent) was observed in plants treated with one per cent Bordeaux mixture, 0.3 per cent copper oxychloride, 0.1 per cent carbendazim, 0.15 per cent copper hydroxide. The treatments T9 (*P.fluorescens*), T10 (*T. viride*) and T11 (quinalphos) were also on par with the above treatments. From this, it was noticed that both the bioagents and even the insecticide quinalphos were equally efficient like copper fungicides and systemic fungicides in the management of die back disease of mango grafts. The highest reduction in leaf blight incidence (66.59 per cent) and disease severity (79.63 per cent) over control were recorded in plants sprayed with one per cent Bordeaux mixture and was on par with all other treatments except mancozeb and zineb.

The results of the second experiment revealed that the highest per cent reduction of die back disease incidence (71.44 per cent) and severity (80.02 per cent) over control was exhibited by one per cent Bordeaux mixture and was on par with all other treatments. Similarly Bordeaux mixture recorded the highest reduction of leaf blight disease incidence (55.61 per cent) and severity (85.64 per

cent) over control, and was on par with all other treatments. Hence it was concluded that all the five fungicides *viz.*, one per cent Bordeaux mixture, 0.15 per cent copper hydroxide, 0.3 per cent captan, 0.1 per cent hexaconazole and 0.1 per cent carbendazim and the bioagents *viz.*, *T. viride* (20 g/ lit) and *P. fluorescens* (2 per cent) were equally effective in the management of die back and leaf blight disease of mango grafts.

The data on the screening of mango grafts of different varieties revealed that the two varieties, *viz.*, Alphonso and Mulgoa were highly resistant to die back and leaf blight diseases. The highest total phenol content of 393.69µg/g was recorded in Alphonso and was followed by Mulgoa which recorded 283.59 µg/g of total phenol per gram of plant sample.

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ETIOLOGY AND MANAGEMENT OF DIE BACK DISEASE OF MANGO GRAFTS IN NURSERY

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ABSTRACT OF THE THESIS

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ABSTRACT

A study on "Etiology and management of die back disease of mango grafts in nursery" was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2007 - 20008 to study various aspects, particularly the etiology, symptomatology, varietal reaction and management of die back disease.

Surveys were conducted at ten different nurseries on the occurrence of die back disease of mango grafts and during the survey different leaf blight diseases were also noticed along with die back incidence. Pathogens associated with these diseases were isolated and proved pathogenicity by artificial inoculation under *in vitro* and *in planta* conditions. Isolation of pathogen on PDA medium yielded *Colletotrichum* sp. and *Botryodiplodia* sp. from die back symptom and *Colletotrichum* sp., *Pestalotiopsis* sp., *Cylindrocladium* sp., *Drechslera* sp. and *Alternaria* sp. from leaf blight symptom.

Studies on symptomatology showed that die back and leaf blight symptoms produced by the respective pathogens at different locations were same, but different pathogens produced different type of symptom on mango grafts. The die back caused by *Colletotrichum* sp. was observed as small necrotic spots on the tip of shoot and at the base of leaf petiole which later coalesced and developed large brownish black necrotic area. The leaves on the infected shoot dried up and fell down. Black discolouration and darkening of the bark of young green twigs were observed in mango grafts infected by *Botryodiplodia* sp. This infection spread downwards and resulted in typical die back symptom. In severe infection leaf shedding was also observed. The leaf blight caused by *Colletotrichum* sp. was also noticed on young leaves and appeared as dark brown circular spot with yellow halo which coalesced to form large blighted area. The pathogen produced the fruiting body, acervulus, on infected area and also showed shot hole symptom. *Pestalotiopsis* sp. infected matured leaves and produced light brown necrotic area

from the leaf tip or margin which later spread towards the midrib with silvery grey coloured area on upper surface of leaf. The other three pathogens *viz.*, *Cylindrocladium* sp., *Drechslera* sp. and *Alternaria* sp. initially produced dark brown spots on leaves. In all these infections, the spots were coalesced and produced large brown coloured blighted area on leaves.

Cultural and morphological characters of the six pathogens were studied on PDA medium. Based on the etiological studies and cultural and morphological characters of organisms the pathogens causing die back disease were identified as *Colletotrichum gloeosporioides* (Penz.) Sacc and *Botryodiplodia theobromae* Pat and the leaf blight inciting pathogens were identified as *Colletotrichum gloeosporioides* (Penz.) Sacc, *Pestalotiopsis mangiferae* (Henn.) Steyaert, *Cylindrocladium mangiferae* sp. nov, *Drechslera australiensis* (Bugnicourt) Subram & Jain ex M.B.Ellis and *Alternaria alternata* (Fries) Keissler. There was no earlier report of *C. mangiferae* on mango and hence it is the first report of this fungus as a leaf blight pathogen on mango. The result of the genetic dissimilarity index computed by the seven different isolates of *C. gloeosporioides* showed a slight variability among the isolates.

An *in vitro* experiment was conducted to evaluate the effectiveness of fungicides and antagonists against die back and leaf blight pathogens. Among the fungicides, Bordeaux mixture at all the three concentrations recorded cent per cent inhibition on the growth of all the six pathogens. Carbendazim showed cent per cent inhibition on the growth of *C. gloeosporioides*, *P. mangiferae* and *C. mangiferae* at all the three concentrations but it was not very effective against *D. australiensis*. Hexaconazole was found effective to control the growth of *D. australiensis* and recorded more than 85 per cent inhibition over control. The two fungal antagonists *viz.*, *T. viride* (KAU) and *T. harzianum* (IISR) showed complete inhibition on the growth of all the six pathogens whereas the bacterial antagonist, *P. fluorescens* showed more than 50 per cent inhibition on the growth of *C. gloeosporioides*, *B.theobromae*, *P. mangiferae* and *D. australiensis*.

After the *in vitro* evaluation of fungicides and antagonists, an *in planta* experiment was conducted two times to know the effect of various treatments on the management of die back and leaf blight diseases. Observations on per cent disease severity of die back and leaf blight disease revealed that all the treatments were superior to control. The data recorded on ten days after the last application of treatments during the first experiment revealed that the highest reduction in disease severity (90.47 per cent) was observed in plants treated with one per cent Bordeaux mixture, 0.3 per cent copper oxychloride, 0.1 per cent carbendazim, 0.15 per cent copper hydroxide. The treatments T9 (*P. fluorescens*), T10 (*T. viride*) and T11 (quinalphos) were also on par with the above treatments. The highest reduction in disease severity of leaf blight (79.63 per cent) over control was recorded in plants sprayed with one per cent Bordeaux mixture and was on par with all other treatments except mancozeb and zineb.

The results of the second experiment revealed that the highest per cent reduction in disease severity of die back (80.02 per cent) over control was exhibited by one per cent Bordeaux mixture and was on par with all other treatments. Similarly Bordeaux mixture recorded the highest reduction in disease severity of leaf blight (85.64 per cent) over control, and was on par with all other treatments. Hence it is concluded that all the five fungicides *viz.*, one per cent Bordeaux mixture, 0.15 per cent copper hydroxide, 0.3 per cent captan, 0.1 per cent hexaconazole and 0.1 per cent carbendazim and the bioagents *viz.*, *T. viride* (20 g/ lit) and *P. fluorescens* (2.0 per cent) were equally effective in the management of die back and leaf blight disease of mango grafts.

The data on the screening of mango grafts of different varieties revealed that the two varieties, *viz.*, Alphonso and Mulgoa were highly resistant to die back and leaf blight diseases. The highest total phenol content of 393.69µg/g was recorded in Alphonso and was followed by Mulgoa which recorded 283.59 µg/g of total phenol per gram of plant sample.