CATALOGUING AND DOCUMENTATION OF DISEASES OF SYMPODIAL ORCHIDS IN KERALA

BY

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(2020-11-005)

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THESIS

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DEPARTMENT OF PLANT PATHOLOGY
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2023

DECLARATION

I, hereby declare that the thesis entitled **"CATALOGUING AND DOCUMENTATION OF DISEASES OF SYMPODIAL ORCHIDS IN KERALA"** is a bonafide record of research work done by me during the course of research and that this thesis 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 **"CATALOGUING AND DOCUMENTATION OF DISEASES OF SYMPODIAL ORCHIDS IN KERALA"** is a bonafide record of research work done independently by Ms. Anjali P. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to him.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction

1. INTRODUCTION

Orchids are well-known ornamental plants that belong to the second largest family of flowering plants, Orchidaceae, and constitute about 7 per cent of angiosperms and nearly 40 per cent of monocots (Kumaria, 2013). They exhibit an incredible range of diversity in size, shape, colour and long vase life, which makes orchid growing, a highly profitable industry all over the world. About 25,000 to 35,000 species of orchids in nearly 750 genera have been reported so far, of which 1,331 species and 186 genera are confined to India (De *et al.,* 2015).

Based on the growth habit, orchids are grouped into sympodial and monopodial types. Monopodial orchids produce a single growing point on a non-terminal shoot, whereas in sympodial orchids, a terminal shoot produces new growth from the old shoot base (Hew and Yong, 2004; Staples and Herbst, 2005).

Orchids can be categorized into five groups depending on their habitat: epiphytic, terrestrial, saprophytes, lithophytes and climbers. More than 50 per cent of the orchid species found in India are epiphytic, and only around 30 per cent are terrestrial (Singh *et al*., 2013).

The floriculture industry has been shifting in favour of more expensive, exotic orchids that are being grown as cash crops in both developed and developing nations. Many of them, particularly those belonging to the genera *Dendrobium* sp., *Cattleya* sp., *Cymbidium* sp., *Paphiopedilum* sp., *Arachnis* sp., *Phalaenopsis* sp., and *Vanda* sp., are highly valued and in high demand for their cut flowers and potted plants, and they have made significant contributions to global trade in floriculture (Singh *et al*., 2013). Though both monopodial and sympodial orchids are equally used in commercial cultivation, sympodial types including *Cymbidium* sp., *Dendrobium* sp. etc., rank higher in the export market. In addition to being used as ornamental plants, orchids have a wide range of other uses, such as in the nutraceutical and pharmaceutical sectors, food, jewellery, dry flowers, perfumery, aromatherapy, cosmetics and flavouring industries (Prajapati, 2013).

The majority of orchid species prefer thriving in tropical regions with abundant rainfall and no dry spells (Moudi *et al.,* 2013). The shade requirement for orchid cultivation ranges from 30 to 50 per cent with a desired light intensity of 15,000 to 60,000 Lux (Rajeevan *et al.,* 2017). For different species of orchids, temperature between 10°C and 28°C at night and between 16°C and 35°C during the day with relative humidity of 75 to 80 per cent are essential (Talukdar, 2013). Kerala provides an ideal climate for commercial orchid cultivation, with high humidity and moderate temperatures accompanied by abundant rainfall. Among the floriculture crops grown in Kerala, orchids have drawn attention since they are high value crops with high productivity and suitability, both in urban and rural areas. Currently, production in coastal regions of Kerala, particularly in Cochin and Thiruvananthapuram, primarily satisfies domestic demand for cut flowers and potted plants (Rajeevan *et al.,* 2017).

However, production of quality flowers in orchids is hindered by many biotic factors, especially fungal, bacterial and viral diseases (Wu *et al*., 2011). These diseases lower the vigor of the plant, flower quality, thereby reducing its marketability and incurring significant economic losses to the orchid industry. As the diseases of orchids have turned problematic in plant quarantine too, the significant contribution towards the study of orchid diseases is of high value (Sowanpreecha and Rerngsamran, 2018). A perusal of the literature reveals that not much work has been done on documentation of various diseases associated with sympodial orchids. Hence, the present study was undertaken with the following objectives:

- Survey on the occurrence of diseases and collection of infected samples
- Isolation of pathogens and its pathogenicity studies
- Symptomatology of diseases
- Characterisation and identification of pathogens
- *In vitro* evaluation of fungicides and biocontrol agents against major pathogens
- Bioassay experiment

Review of literature

2. REVIEW OF LITERATURE

Orchids are the most fascinating and highly valued ornamental plants all around the world for their diversity in size, shape, colour and longevity. They belong to the family Orchidaceae, the largest family of perennial herbs, contain over 763 genera and about 28,000 species worldwide (Christenhusz and Byng, 2016).

Orchids are perennial herbaceous plants with two different growth habits: monopodial growth and sympodial growth. monopodial orchids show a single growing point on a non-terminal shoot, whereas in sympodial orchids, a terminal shoot produces new growth from the old shoot base (Hew and Yong, 2004; Staples and Herbst, 2005). Orchids are also divided into two categories based on the support requirements needed for growth: epiphytic and terrestrial. Epiphyte orchids grow on other plants and absorb moisture and nutrients from the air, moss, and bark. On the other hand, terrestrial orchids are rooted in the ground. Depending on the species, mature plants may flower continuously or just once or twice a year and it take two to ten years to attain reproductive maturity. Flowers can endure from one day to more than ten weeks and can be produced as single blooms, floral clusters, or lengthy inflorescences (Hew and Yong, 2004).

The industrial production of sympodial orchids significantly benefits the economies of many ASEAN (Association of South East Asian Nations) countries (Hew, 1994; Laws, 1995). Currently, orchids are dominating the cut flower and potted plant trade and hold sixth position among the top ten cut flowers in the world's floriculture industry (Chugh *et al.,* 2009, De and Debnath, 2011). Although both monopodial and sympodial orchids are cultivated commercially, sympodial orchids (*Dendrobium* sp., *Cymbidium* sp., *Oncidium* sp., *Cattleya* sp. etc.) are more popular in the export market. According to Cheamuangphan *et al.* (2013), 85 per cent of the world's cut flower orchid species were *Dendrobium* sp., followed by 15 per cent each of Phalaenopsis and *Cymbidium* sp. In addition to their ornamental value, some orchid species have been reported to have medicinal properties. Studies revealed that some species of *Dendrobium* sp. possess anticancer, antifungal, antioxidant, anti-inflammatory, and

immuno-enhancing activities (Venkateswarlu *et al.,* 2002; Sattayasai *et al.,* 2009; Song *et al.,* 2012; Yang *et al.,* 2014; Kim *et al.,* 2015).

However, orchid cultivation is often challenged by several biotic factors especially diseases. The majority of orchid diseases are caused by bacterial, viral, or fungal infection (Wu *et al.*, 2011, Sudha and Rani, 2016, Moon *et al.,* 2017). Recent climatic variations and increased exchange of planting material between different countries have also contributed to the incidence and spread of different diseases (Sowanpreecha and Rerngsamran, 2018, Srivastava *et al.,* 2018). The significant contributions to the investigation of orchid diseases are of high value as orchid diseases currently pose a problem for plant quarantine as well (Sowanpreecha and Rerngsamran, 2018). Hence, controlling diseases is vital for maintaining the quality of orchids. The literatures pertaining to the diseases affecting sympodial orchids are reviewed in this chapter.

2.1 OCCURRENCE OF DISEASES

2.1.1. Fungal diseases

Kevorkian (1940) first described many fungal pathogens affecting several orchids in Puerto Rico. He mentioned different diseases such as anthracnose caused by *Colletotrichum* spp. and *Gloeosporium* spp., rust disease caused by *Uredo* sp. and *Puccinia* spp., leaf spot diseases caused by *Phoma* sp., *Macrophoma* sp*., Diplodina* sp*., Hendersonia* sp., and *Cercospora* spp., rhizome infections caused by *Phytophthora* sp. and *Fusarium* sp. in Cattleya*,* and root rot caused by *Sclerotium rolfsii.* According to Uchida and Aragaki (1991b), *Phytophthora palmivora* is an important pathogen in many orchids *viz. Dendrobium* sp., *Cattleya* sp., *Epidendrum* sp., *Vanda* sp., *Paphiopedilum* sp. and *Laeliocattleya* sp. causing severe symptoms such as black rot, root rot, damping off, and blossom spots were found in Hawaii. They also reported the occurrence of *Phytophthora nicotianae* in many orchids. *Phytophthora cinnamomi* and *Phytophthora cactorum* were also found to cause diseases in *Cymbidium* sp. in Hawaii (Uchida and Aragaki, 1991b). A study conducted by Orlikowski and Szkuta (2006) in Polonica showed that *P. palmivora* was the causal organism of rot diseases in five

orchid species. Black rot of orchids caused by *P. cactorum* and *P. palmivora* was first reported by Cating *et al.* (2010) in Florida.

Studies showed that several *Fusarium* spp. cause diseases in orchids worldwide. It was reported that *Fusarium proliferatum* was the causal organism of black spots in *Cymbidium* hybrids in Korea (Chang *et al.,* 1998) and yellow and black spot disease in *Cymbidium* sp. caused by the same pathogen in Japan (Ichikawa and Aoki, 2000). They suggested that the different symptoms were caused by two different races of *F. proliferatum* (Ichikawa and Aoki, 2000). Aoki *et al.,* (2001) isolated and characterised *Fusarium fractiflexum* from *Cymbidium* spp. in Japan, which caused yellow leaf spot. *Fusarium subglutinans* causing leaf spot on *Cymbidium* spp. was reported by Han *et al.* (2015) for the first time in Korea.

Latiffah *et. al.* (2009) identified and characterised 29 *Fusarium* isolates from the root and stem portions of *Dendrobium* sp. They characterised 14 *Fusarium* isolates from the root portion of *Dendrobium* sp., among which six were *Fusarium oxysporum*, and the remaining four were *Fusarium solani* and *F. proliferatum* isolates. They identified seven *F. oxysporum*, three *F. solani* and five *F. proliferatum* isolates from the stem rot of *Dendrobium* sp. Wilt disease in *Dendrobium officinale* caused by *F. oxysporum*, leaf spot disease in *Cymbidium* sp. caused by *F. proliferatum* and black spot in *D. officinale* caused by *Cladosporium oxysporum* were reported in China for the first time (Zhang *et al.*, 2017; Wang *et al.,* 2018; Xiao *et al.,* 2021). The first report of the pathogen, *Fusarium equiseti,* the causal organism of dieback disease in *D. officinale* in China was given by Guo *et al.* (2020). Leaf blotch caused by *Fusarium sacchari* in *Dendrobium antennatum* in Malaysia and stem rot caused by *Fusarium kyushuense* on *D. officinale* in China were first reported by Mohd (2021) and Cao *et al.* (2022a), respectively. Mirghasempour *et al.* (2022) identified the causal organism of dieback disease in *D. officinale* as *Fusarium* spp. in China.

McMillan *et al.* (2008) reported Cercospora leaf spot caused by *Cercospora dendrobii* on *D. antennatoum*. Tao *et al.* (2011) isolated *Pythium vexans* causing stem rot of *Dendrobium* sp. in Yunnan Province of China. Tao *et al.* (2011) identified *P. nicotianae* as the inciting agent of blight disease in *Dendrobium* spp. in China. Ma *et al.* (2020) reported *Colletotrichum fructicola* causing anthracnose in *D. officinale* from

China. A study conducted by Fernandez-Herrera *et al.* (2020) showed that *Colletotrichum karstii* was the causal organism of anthracnose disease in *Dendrobium nobile* in Mexico. Southern blight of *Dendrobium huoshanense* caused by *S. rolfsii* was identified by Cheng *et al.* (2023).

The other fungal diseases reported in orchids include *C. oxysporum* causing black spot on *D. officinale* (Xiao *et al.,* 2018), *Trichoderma longibrachiatum* causing black leaf spot on *D. nobile* (Sarsaiya *et al.,* 2019), *Alternaria alternata* causing leaf spot disease on *Dendrobium candidum* (Wang *et al.,* 2020), and *Neopestalotiopsis clavispora* causing leaf spot on *D. officinale* (Cao *et al.*, 2022b).

The leaf spot pathogen *Neoscytalidium orchidacearum* on *Cattleya lueddemanniana* var. *lueddemanniana* was reported from Thailand by Suwannarach *et al.* (2018). Different species of the same genus, *Neoscytalidium dimidiatum,* causing leaf blight in Cattleya × hybrid were identified by Chang *et al.* (2020) for the first time in Taiwan. The causal agent of anthracnose disease in *Cattleya walkeriana* was identified as *C. karstii* (Silva *et al.,* 2021). The first report of *Rhizoctonia solani* causing leaf blight on Cattleya × hybrid was given by Hsieh *et al.* (2023).

Sreedharan *et al.* (1994), first identified *Glomerella cingulata* causing anthracnose in *Oncidium* sp. in Kerala. Later, Youlian, *et al.,* (2011) reported *Colletotrichum boninense* from *Oncidium flexuosum.* Liu *et al*. (2014) reported *Colletotrichum* spp. in *Spathoglottis plicata.*

Park *et al.* (1996) investigated the anthracnose disease in *Cymbidium* sp. caused by *Colletotrichum gloeosporioides* in Korea, and they also found *C. gloeosporioides* was pathogenic to *Dendrobium* sp. and *Phalaenopsis* sp. Silva *et al.* (2008) characterised *Phyllosticta capitalensis,* the causal organism of leaf and pseudobulb diseases in *Bifrenaria harrisoniae*. Leaf spot disease was noticed on *Epidendrum secundum* caused by *Pseudocercospora epidendri* in Araponga, Brazil (Silva and Pereira, 2011). Chowdappa *et al.* (2012) characterised *C. gloeosporioides*, the causal organism of anthracnose diseases of orchids in India. Park *et al.* (2013) gave the first report of anthracnose in *Cymbidium* sp. caused by *C. gloeosporioides* in Korea. Collar rot caused by *S. rolfsii* in in Phalaenopsis was reported by Meera *et al.* (2016) from

Kerala. The leaf blight symptom caused by *Curvicladiella* sp. on *Paphiopedilum* sp. were reported by Song *et al.* (2020).

Floral diseases are frequently noticed in many orchids worldwide, which lower the quality of blossoms and limit their market potential. Blossom blight caused by *Botrytis cinerea* on *Dendrobium* spp. was reported by Ito and Aragaki (1977) in Hawaii. Blossom disease caused by *P. palmivora* and *P. nicotianae* and flower spots and blights caused by *Colletotrichum coccodes* were also noticed in Hawaii (Uchida and Aragaki, 1991a). Several fungal diseases affecting blooms of orchids, such as blossom blight or grey mould caused by *B. cinerea,* blossom flecks and small spots caused by *Exserohilum rostratum*, *A. alternata*, *Bipolaris sorokiniana*, *Bipolaris setariae* and *Bipolaris* spp., yellow spot caused by *P. capitalensis,* and flower spots caused by *F. proliferatum* were observed in Hawaii (Leonhardt and Sewake, 1999). Dissanayake (2015) identified the inciting agent of bud wilt disease in *Dendrobium* spp. as *F. proliferatum*. A flower spot disease caused by *Phoma exigua* was reported in *Phalaenopsis* spp. by Meera *et al.* (2016).

2.1.2. Bacterial diseases

Bacterial diseases are now prevalent in orchid growing tracts and pose a major threat to the industry. Complete death of plants was observed, especially during the rainy season (Uchida, 1995). Various bacterial diseases reported in different orchids are described below.

Chuenchitt *et al.* (1983) first reported a bacterial disease in *Dendrobium* sp. caused by *Pseudomonas gladioli* in Nongkham district in Japan during the rainy season. A bacterial brown spot disease on *Cymbidium* spp. was reported by Tsuchiya *et al.* (1986) in Fukuoka, Japan during the summer season. The bacterial pathogen *Erwinia chrysanthemi* associated with leaf spot and soft rot symptom in *Phalaenopsis* sp. was isolated and characterised by Jin *et al.* (1994) from Korea. The same pathogen, *E. chrysanthemi* was identified in *Dendrobium* sp. and *Phalaenopsis* sp. by Lee *et al.* (1999) from Korea. Bacterial black spot caused by *Burkholderia andropogonis* on *Odontoglossum* sp. and intergeneric hybrid orchids were studied by Takahashi *et al.* (2004). The bacterium *Burkholderia gladioli* was identified in various genera of

orchids, including *Oncidium* sp., *Dendrobium* sp., *Cattleya* sp. and *Miltonia* sp. causing leaf spot diseases in Hawaii (Keith *et al.,* 2005). The first report of *Dickeya* sp. causing bacterial soft rot on *Tolumnia* spp. was given by Cating *et al.* (2009). Meera (2012) reported *Erwinia* sp. causing soft rot on *Phalaenopsis* sp. in Kerala. You *et al.* (2016) reported bacterial brown spot in *Phalaenopsis* spp. caused by *B. gladioli* in China for the first time. Moon *et al*. (2017) isolated and characterised *B. gladioli,* causing disease in *Cymbidium* sp. in Korea. Balamurugan *et al.* (2020) characterised *Dickeya fangzhongdai* which caused bacterial soft rot disease on *D. nobile* in India.

2.1.3. Viral diseases

Virus diseases pose a serious threat to orchid cultivation. They reduce vigour, bloom quality and marketability, incurring significant economic losses. Jensen (1950) studied *Cymbidium mosaic virus* (CyMV) on *Cymbidium* spp. from California. After then *Odontoglossum ringspot virus* (ORSV) was identified in *Odontoglossum grande* from USA by Jensen and Gold (1951). The virus was also found in *Cattleya* sp., causing flower variegation and in *Cymbidium* spp., as Cymbidium diamond mottle disease (Jenson and Gold, 1952). Inouye (1966) reported OGRV in Cymbidium from Japan, and he also found CyMV in *Cattleya* sp. and *Cymbidium* sp. (Inouye, 1966). So far, more than 50 viruses have been identified as infecting most of the cultivated orchids (Lawson and Hsu, 1995; Chang *et al.,* 2005; Navalienskiene *et al.,* 2005).

Goff and Corbett (1977) reported Tomato ringspot virus infection in *Cymbidium* sp. producing chlorotic leaf streak symptoms. Okemura (1983) studied the incidence and expression of CyMV in *Dendrobium* hybrids. Hu *et al.* (1993) detected CyMV, ORSV, TSWV and potyviruses in various orchids in Hawaii, and they found that most of the *Dendrobium* spp. were infected with CyMV. Three viruses, CyMV, ORSV and unidentified Potyviruses were reported from eight orchid genera (Inouye and Gara, 1996). Tanaka *et al.* (1997) detected ORSV and CyMV viruses by rapid immunofilter paper assay. These viruses were also identified in Thai orchids by immuno-capillary zone electrophoresis (Eun and Wong, 1999).

Blanchfield *et al.* (2001) identified orchid fleck virus by reverse transcriptase-polymerase chain reaction (RT-PCR). Based on the same technique, Sherpa *et al.*

(2003) identified CymMV in many *Cymbidium* hybrids. OGRV was also reported in orchids from Sikkim (Sherpa *et al*., 2006). Bhat *et al.* (2006) identified the virus in *Vanilla planifolia* from Karnataka and Kerala. A potyvirus infection in *Cymbidium* spp. was also found in the northern region of India (Singh *et al*., 2007). Incidence of CyMV and ORSV affecting *Oncidium* spp. and *Orchid fleck dichorhavirus* affecting *Cymbidium* sp. were reported in China (Liu *et al.,* 2013). The first report of orchid fleck virus on orchids in Mexico was given by Otero-Colina *et al.* (2021). At least ten viruses in cultivated orchids have been reported in India (Pant *et al.,* 2020).

2.2. SYMPTOMATOLOGY

Several researchers have carried out many studies on symptomatology of various fungal diseases affecting sympodial orchids.

2.2.1. Symptomatology of fungal diseases

2.2.1.1. Foliar diseases

Selenophoma leaf spot caused by *Selenophoma* sp. was recorded by Abiko *et al.* (1976) in Japan. Symptoms appeared as minute brown spots on the leaves that developed into round or irregular spots. Later, the affected leaves became discoloured and defoliated. Numerous fruiting bodies of the fungi were observed in the diseased area. Sarsaiya *et al.* (2019) described the symptom caused by *T. longibrachiatum* as black circular spots on leaves of *D. nobile*. Wang *et al.* (2020) described Alternaria leaf spot on *D. candidum* as small brown to black spot which turned into circular or elliptical greyish white necrotic lesions. The dried-out region fell off and produced shot holes.

The leaf spot symptom caused by *N. clavispora* on *D. officinale* in China was first described by Cao *et al.* (2022). The symptom initially appeared as small circular necrotic spots, gradually turned sunken spots with black margin.

The anthracnose caused by *C. fructicola* on *D. officiale* produced small circular brown spots. Later the spots turn to sunken lesions with dark brown margin. Fernandez-Herrera *et al.* (2020) described the anthracnose symptom caused by *C. karstii* on *D. nobile* in Mexico. The symptoms were characterised as sunken circular spots with dark centre surrounded by brown halo. In advanced stages, the spots coalesced to form large necrotic lesions.

Cheng *et al.* (2023) detailed the symptomatology of southern leaf blight of *D. huoshanense* caused by *S. rolfsii* as withering of leaves, formation of thick mycelial mat at the base of the stem, with the presence of numerous globular sclerotia. Gradually the whole stem showed yellowing and withering. The infected stem collapsed and numerous sclerotia were formed over the withered surface.

Ichikawa and Akoi (2000) reported two types of leaf spot symptoms, yellow spot and black spot in *Cymbidium* sp., caused by *F. subglutinans* and *F. proliferatum*. The yellow spot symptom was characterised by minute, water-soaked patches on leaves. Gradually the lesion became sunken, enlarged and turned brownish red with yellow margin. Later, the lesion coalesced to form necrotic patches. In black spot, the symptom initiated as minute speckles on the leaves, which later enlarged to form irregular black spots. Young leaves were found to be more susceptible to the disease. Another leaf spot symptom caused by *F. subglutinans* in *Cymbidium* sp. described as small yellow spots on leaves that gradually turned into sunken, brown to black spot with yellow halo. In advanced stages, the diseased portion fell down leaving shot holes. Leaf deformation was observed in severe cases (Han *et al.,* 2015). According to Chang *et al.* (1998), different types of leaf spot symptoms were produced by *F. proliferatum* on *Cymbidium* sp. These symptoms were observed as black, minute speckles with yellow halo or black irregular or circular sunken spots. Dekham and Kanchanawate (2020) reported yellow leaf spot in *Rhynchostylis gigantea* as 1-2 mm yellow patches without distinct margin. Later, the patches coalesced to form dark brown to black necrotic area. In advanced stages the entire plant turned brown and wilted. Silva *et al.* (2011), explained the symptom produced by *P. epidendri* in *E. secundum* as irregular, pale brown distinct leaf spots with black outer margin that were scattered over the leaves.

The symptoms of anthracnose in *Cymbidium* spp. were described as dark brown or black lesions on the margin or the tip of leaf with concentric rings formed by acervuli. In advanced stage, the leaves became completely brown with many concentric rings on dead tissues (Park *et al.,* 2020).

Uchida and Aragaki (1991b) found that seedlings of *Dendrobium* sp. were highly susceptible to *Phytophthora* spp. infection, which caused damping off. The

symptoms on seedlings described by water- soaked lesions on different plant parts such as stem, root and pseudobulbs caused rotting and death of plants. Root rot symptom could also cause chronic weakening of plant and growth retardation.

The infection caused by *Phytophthora multivesiculata* on *Cymbidium* spp. was first reported by Hill (2004). Here symptoms were found on both leaves and pseudostems. Dark green lesions appeared on mature leaves, which rapidly became water-soaked irregular patches under humid conditions. Under dry condition, the development of lesions was inhibited and turned brown. The stem infection caused by *P. multivesiculata* showed greenish grey discoloration followed by flaccidity of stem tissues. The pathogen also caused infection on pseudobulb with blue- black or purplish brown internal discoloration and a sour odour (Hill*,* 2004).

The first report of leaf blight disease caused by *Lasiodiplodia theobromae* on *Catasetum fimbriatum in* Brazil was given by Lopes *et al.* (2009). The symptoms initiated as chlorotic spots and gradually became necrotic with yellow halo. Later, the spots coalesced to cause leaf blight symptom.

The infection of *Phytophthora* spp. on *Dendrobium* sp. caused leaf spot symptom, which initiated as small water-soaked spots. Later, these spots enlarged and turned into irregular blistered or scalded spots with greenish centre and dark green water-soaked margins. Under moist conditions, these lesions turned grey or brownish black in colour, followed by rapid yellowing and the death of the plant. Under dry condition, the spots became brown and sunken. The infection of *P. palmivora* on mature orchids produced dry, sunken lesions with pale brown centre and dark brown margin. The leaves gradually turned yellow and fell off. The pathogen also caused leaf rot symptoms by producing black lesions on leaves, followed by leaf abscission (Uchida and Aragaki, 1991).

The leaf spot symptom caused by *N. orchidacearum* in *Cattleya* spp. was described by Suwannarach *et al.* (2018). The symptoms initiated as dark, circular spots that enlarged from 5 to 20 mm in size and became elliptical in shape. The leaf spots gradually turned dark brown to black centre with yellow to brown margin. Silva *et al.* (2021) described the symptoms of anthracnose in *C. walkeriana* caused by *C. karstii* in

detail. The symptom was characterised by irregular, circular to elliptical lesions with 15-50 x 10-30 mm diameter. Later, the lesions became sunken with salmon coloured spore mass.

The infection of *L. theobromae* in *Cattleya* sp. was reported by Cabrera and Cundom (2013) in Argentina. The symptoms were initiated as necrotic water-soaked spots with chlorotic halo on stem and pseudobulbs, whereas in leaves, chlorotic areas appeared along the edges, gradually turned brown. The symptoms produced on the roots of Cattleya by *L. theobromae* were discoloration and degradation of parenchymal tissues, along with shedding of bark. The leaf blight caused by *R. solani* in *Cattleya* hybrid produced dark brown necrotic lesions surrounded by chlorotic halo on the lower leaves (Hsieh *et al.,* 2023).

2.2.1.2. Rot diseases

Cane rot caused by *P. palmivora* initiated from the sheath or as lesion on the canes of *Dendrobium* sp. The pathogen entered the cane through apical buds or affected leaves, and the lesion progressed downward, causing browning of canes externally with internal black discoloration. In advanced stage, the diseased canes became weak and fell over the pot (Uchida and Aragaki, 1991b).

Huang *et al.* (2014) reported stem rot in jewel orchid caused by *F. oxysporum* f. sp. *anoectochili* in Taiwan. The symptom initiated as water-soaked lesions on leaves and stems, followed by shrinking of the stem, later leading death of plants. Mycelial growth was observed on the lesion surface, and vascular discoloration was also found with some isolates.

Cating *et al.* (2010) described the black rot symptoms caused by *P. palmivora* and *P. cactorum.* The symptom initiated as tiny black lesions on the base of pseudostems and roots. The lesion rapidly covered the entire pseudobulbs and leaves and eventually caused the death of the plant.

Cao *et al.* (2022a) described the symptoms of stem rot in *D. officinale* caused by *F. kyushuense* as greyish black lesions, gradually spread to vascular region, causing discoloration and wilting of the plant.

The infection of *S. rolfsii* in *Cymbidium* spp. in Korea was first described by Han *et al.* (2012). The pathogen caused wilting and death of the plant, and numerous sclerotia and white mycelial growth were observed on pseudostems, leaf sheaths and leaf bases. In advanced stage, the pseudobulb became rotten and wilted.

Root rot caused by *Fusarium* spp. in *Cymbidium* spp. was observed by Benyon *et al.* (1996). The disease was characterised by root decay, caused yellowing and withering of leaves due to impaired water and nutrient absorption. The brownish black rot from roots that extended into pseudobulbs and leaves, later leading to death of the plants.

Kim *et al.* (2002) described the dry rot symptom observed on *Cymbidium* spp. by *Fusarium* spp. as irregular dark brown lesions on the pseudobulbs extending to the base of the leaves. Gradually, the leaves became yellow and turned into blight. The affected pseudobulb became black and rotten.

2.2.1.3. Wilt diseases

The symptomatology of wilt disease caused by *S. rolfsii* on Indian orchids was studied by Bag (2003). The disease initiated on pseudobulbs of *Coelogyne corymbose* as basal rot, followed by yellowing and defoliation of leaves, which gradually caused drying and death of the plant. The diseased regions were covered with numerous brown sclerotia with white mycelial growth.

Zhang *et al.* (2017) documented the symptoms of wilt disease in *D. officinale* as yellowing of leaves starting from the bottom, followed by defoliation. Gradually, the collar region and roots turned brown, causing death of the whole plant.

2.2.1.4. Dieback

Dieback symptom caused by *Fusarium* spp. in *D. officinale* initially appeared as chlorotic leaves, that later resulted in blight were and wilting of plants. The shoot tips showed dark brown necrosis, gradually turned into dieback symptom, eventually led to death of plants (Mirghasempour *et al.,* 2022).

2.2.1.5. Floral diseases

Botryris blossom blight is a serious floral disease in *Dendrobium* spp., and the symptom appeared as watery soft rot followed by shedding of flowers. The disease

initiated as brown, water-soaked flecks on the petals. These specks turned into elliptical shape and reached 12 mm length (Ito and Aragaki, 1977).

Phytophthora spp. were also found to cause infection on flowers of *Dendrobium* sp. The symptom initiated as tiny water-soaked spots on petals, gradually enlarged, and caused translucent rots. Infected flowers turned brownish black due to the invasion of secondary pathogens. The floral infection by *Phytophthora* spp. on blossoms of *Vanda* sp. caused spots and dark brown rots. *C. coccodes* produced circular to oval, restricted lesions on petals with dark margin. The spots were surrounded by salmon coloured spore mass. Blossoms infected with *Phyllosticta* sp. produced pale pinkish to blue blemishes of 1 to 3 mm size. In advanced stage, the petals turned dark and covered with mycelia (Uchida and Aragaki, 1991a).

2.2.2. Symptomatology of bacterial diseases

Chuenchitt *et al.* (1983) documented the symptoms of bacterial disease caused by *P. gladioli* in *Dendrobium* spp. The symptom was characterised as water-soaked yellow to green lesions on the upper leaves that rapidly caused rotting. Gradually, the infected leaves became brown and fell off. Upon severe infection, stem and shoot became discoloured and collapsed. In young nursery bed, the pathogen produced dark green rot.

Balamurugan *et al.* (2020) described the soft rot disease caused by *D. fangzhongdai* in *D. nobile* as water-soaked lesion on the leaf margin that later turned into irregular green lesions. The lesion enlarged rapidly, causing soft rot symptom. Later, the infected leaves shed leaving bare stem and the rotten tissues emit a foul odour. The affected stem appeared spongy with yellowing externally, and brown coloured disintegrated tissues were observed internally. The presence of bacterial ooze from the cut end of the leaves confirmed bacterial etiology.

2.3 ISOLATION OF PATHOGENS

Latiffah *et al*. (2009) isolated *Fusarium* isolates from roots and stems of *Dendrobium* sp. by excising tissues of more than 3 mm in size followed by surface sterilization with 1 per cent sodium hypochlorite (NaOCl) for two min. The bits were

rinsed in sterile water and kept on blotting paper for drying. The bits were placed in peptone-pentachloro-nitrobenze agar (PPA) and incubated in 27±1^{0}C.

Wang *et al.* (2020) isolated *A. alternata* from *D. candidum* by cutting small bits from infected tissues taken from the margin of the lesions. The bits were surface sterilized in 70 per cent ethanol for 30 s and 1 per cent mercuric chloride ($HgCl_2$) solution for 1 min followed by rinsing in sterile water. The bits were placed in potato dextrose agar (PDA) amended with 150 µg/ml of cefotaxime sodium salt and incubated at 28°C for three days under dark condition.

Cao *et al.* (2022a) described the method of isolation of *F. kyushuense* from stem rot of *D. officinale*. The necrotic tissues (3 x 3 mm) were excised from the infected stem and surface sterilized with 10 per cent NaOCl for 1 min, followed by rinsing in sterile water three times. The surface sterilized tissues were blot dried and placed on potato dextrose agar (PDA), followed by incubation under dark condition at 25°C. They also isolated pathogen, *N. clavispora,* causing leaf spot in *D. officinale,* was isolated by cutting tissues (5 × 5 mm) between healthy and necrotic areas followed by disinfection of tissues in 10 per cent NaOCl for 1 min. The bits were washed three times in distilled water and then dried on blotting paper. The tissues were placed on PDA and then incubated at 25ºC in biochemical incubator under dark condition.

According to Schaad *et al.* (2001), isolation of bacterial pathogens was done by collecting ooze from surface sterilized infected plants parts and streaking the ooze into nutrient agar (NA) medium, followed by incubation at 28 ± 1 °C for 72 h. Meera (2016) isolated *E. chrysanthemi* from *Phalaenopsis* sp. by surface sterilizing the affected tissue with one per cent NaOCl, and then washed in three times in sterile water and crushed the tissues on a sterilized glass slide to get bacterial ooze. The bacterial suspension was streaked on nutrient agar (NA) medium to get single colonies of the bacterium and incubated for 48 h at room temperature (26 ± 2ºC).

2.4 PATHOGENICITY STUDIES

To test the pathogenicity of *P. nicotianae* two to three-year-old plants of *Dendrobium* spp. were selected. 6 mm agar plugs of the fungus were placed on the wounded and intact stems. The inoculated plants were then misted with water and

maintained in polythene bags for 48 h. The control plants were inoculated with sterile agar plugs. Plants were maintained inside the green house at 26ºC day and 18ºC night temperatures, and inoculated plants were observed daily for symptom development (Tao *et al.,* 2011).

Zhang *et al.* (2017) tested the pathogenicity of *F. oxysporum* isolated from wilt disease in *D. officinale*. The conidial suspension (10^5 conidia /ml) of isolates was prepared, and the roots of *Dendrobium* sp. at six leaf stage were dipped in spore suspension for 2 h. The inoculated plants were then transplanted into pots containing pine bark and poured with 20 ml spore suspension. Similar treatment was done for control plants using sterile water. Plants were kept in green house and observed for symptom development. The collar region and roots turned brown and later, all the inoculated plants died.

Pathogenicity studies were conducted by Wang *et al.* (2020) for *A. alternata* isolated from leaf spot on *D. candidum* from China. The conidial suspension (1×10^6 spores /ml) was sprayed on one year old *D. candidum* seedlings. Control plants were also kept by treating them with sterile water. The treated plants were kept in growth chambers at 25 ± 0.5°C at 90 ± 5 per cent relative humidity. 85 per cent of the seedlings inoculated with pathogen took infection, and the symptom appeared as black spots and necrotic lesions, while there was no infection observed for control plants.

To test the pathogenicity of *S. rolfsii* isolated from *D. huoshanense*, mycelial discs were inoculated on wounds made on the stem base. The control plants were maintained without inoculation of the fungus. The inoculated plants were incubated at 26 ± 1 °C for 72 h in polyethylene bags with 90 per cent relative humidity and 16 h light and 8 h dark photoperiod. The mycelial growth was observed on the wounds, and leaves surrounding the point of inoculation turned yellow after two days. Sclerotia developed after six days, and stems collapsed after 12 days with yellowing and defoliation of leaves. The pathogen was re-isolated from the infected tissues, thereby proved Koch's postulates (Cheng *et al.,* 2023).

Keith *et al.* (2005) carried out the pathogenicity test for *B. gladioli* from orchids in Hawaii. The healthy plants of three years old *Cattleya* sp. and 2.5 years old

Dendrobium sp., *Oncidium* sp. and *Miltonia* sp. were selected for pathogenicity studies. The leaves were inoculated with sterilized toothpick smeared with bacterial culture. The treated plants were kept in polyethene bags and placed in a dew chamber in the laboratory at 24ºC with relative humidity of 70 per cent. Symptoms developed after two to three days.

2.5. CULTURAL AND MORPHOLOGICAL CHARACTERISATION OF PATHOGENS REPORTED FROM SYMPODIAL ORCHIDS

2.5.1. *Fusarium* spp.

Srivastava (2014) described the cultural and morphological characters of five *Fusarium* spp. such as *F. proliferatum*, *F. oxysporum*, *F. solani*, *Fusarium poae* and *Fusarium semitectum* isolated from orchids in detail. *F. proliferatum* produced colony with violet pigmentation on the reverse side when cultured in PDA. Macroconidia were straight, slender, 3-4 septate and produced in orange sporodochia. Microconidia were non septate with club shaped and flat base. Chlamydospores were absent. The macroconidia of *F. oxysporum* were falcate to straight with three septa found in orange sporodochia. Microconidia were oval, elliptical and nonseptate. Chlamydospores were single celled, terminal or intercalary, and abundantly produced in the culture. The reverse of the colony showed pale violet pigmentation. *F. solani* were characterised by the production of white to cream colonies in PDA with light greenish pigmentation on the reverse side. Macroconidia were 3-7 septate, straight to slightly curved with round ends, and less frequently produced in green to blue sporodochia, whereas microconidia were 0-3 septate and reniform or ellipsoidal in shape. Chlamydospores were one or two celled and terminal. *F. poae* produced single celled napiform macroconidia on urn shaped monophialides and appeared as grape bunch whereas macroconidia were rarely produced on culture and were characterised as 1-3 septate, short and falcate. *F. semitectum* produced slightly curved, 3-5 septate macroconidia formed in orange sporodochia whereas mesoconidia were 3-5 septate and spindle shaped. *F. denticulatum* produced microconidia and mesoconidia from finger like polyphialides. Macroconidia were slightly falcate and slender with 3-4 septa and formed in orange sporodochia. The pathogen produced orangish mycelia with orange reverse side, whereas microconidia were single or bicelled with oval to obovoid in shape.

The pathogen *F. subglutinans* causing leaf spot symptom isolated from *Cymbidium* spp. produced white to pinkish aerial mycelia that were submerged in the centre and fluffy in the outer region. The colony attained full growth within four days with an average diameter of 55 mm. Conidiophores were branched or unbranched, with monophialides or polyphialides. Macroconidia were 3-5 septate, straight to slightly curved with a size of 32.5-75 x 2.5-4 µm whereas microconidia were allantoid to ellipsoid in shape, aseptate with a size of 8-18 x 2.5- 3 µm (Han *et al.,* 2015).

Dekham and Kanchanawatee (2020) characterised the morphological and cultural features of *F. sacchari,* causing yellow leaf spot on *R. gigantea.* The colonies of the pathogen were initially yellowish orange and later turned purplish white on PDA. The fungus produced cream coloured, globular sporodochia on carnation leaf agar (CLA) media. Macroconidia were septate, slightly falcate, slender with, a size of 47.38 ± 4.8 µm and 2.58 ± 0.3 µm and produced abundantly on CLA media, whereas production of macroconidia in PDA media were found to be low. Microconidia were slender and oval in shape with 0-1 septa and produced abundantly, both in CLS and PDA. The size of the microconidia was 8.85 ± 2.3 x 1.74 ± 0.3 µm. Chlamydospores were single, verrucose and rarely produced on PDA.

Latiffah *et al.* (2009) identified seven isolates of *F. oxysporum,* four isolates of *F. solani* and four isolates of *F. proliferatum* from root rot symptom and seven *F. oxysporum* and three *F. solani* from stem symptom. *F. oxysporum* produced oval to kidney shaped microconidia and straight to slightly curved macroconidia with abundant production of chlamydospores. For *F. solani,* microconidia were produced on long monophialides. Microconidia were oval and wider, whereas macroconidia were stout and straight. Chlamydospores were abundantly produced in the culture. *F. proliferatum* produced microconidia on short chain with V-shaped polyphialides. Macroconidia were straight and sickle shaped. The production of chlamydospores could not be observed.

Zhang *et al.* (2017) described the cultural and morphological features of *F. oxysporum* associated with wilt disease of *D. officinale*. The pathogen produced colourless to pale orange colonies on potato sucrose agar (PSA). The fungus produced 3-5 septate, slightly curved macroconidia with size of 21-49.5 x 3.5-4.8 µm. The microconidia were 1-2 septate and bacilliform in shape with a size of 49.5 x 3.5 -4.8

μm. Chlamydospores were round or oval, singly or in pairs and terminal or intercalary, with a size of 5.5-7.1 × 3.6-6.2 μm.

F. sacchari isolated from *D. antennatum* produced peach to whitish colony on PDA with violet pigment on the reverse side. Macroconidia were 3-5 septate, slender with curved apical cell and produced in orange sporodochia. Microconidia were aseptate, oval in shape, and abundantly produced in the culture. The size of the microconidia and macroconidia were 6.80 ± 1.3 × 2.0 ± 0.5 μm and 38.2 ± 3.6 × 3.6 ± 0.7 μm respectively. Chlamydospores were absent (Mohd, 2021).

Cao *et al.* (2022a) isolated and characterised *F. kyushuense* from *D. officinale*. The culture produced yellow to reddish white aerial mycelium on PDA with red pigmentation on the reverse side. Microconidia were 0-1 septate, clavate to ellipsoidal with a size of 5.2-17.6 x 2.3-4.7 μm. Macroconidia were 3-5 septate with 32.4-51.7 x 3.9-5.3 μm in size.

Mirghasempour *et al.* (2022) described five genera of Fusarium such as *Fusarium fujikuroi*, *Fusarium concentricum*, *Fusarium nirenbergiae*, *Fusarium stilboides*. and *Fusarium curvatum* associated with the dieback disease of *D. officinale* in China. *F. fujikuroi* produced white, aerial floccose mycelia that gradually turned grey violet. Microconidia were 0-1 septate, oval shaped with flattened base. Macroconidia were 3-6 septate, long, slender and produced in pale orange sporodochia on the CLA media. The colonies of *F. concentricum* were yellowish white, velutinous to lanose aerial hyphae with concentric rings on the PDA. Macroconidia were 3-5 septate, slender, fusiform to naviculate with foot shaped basal and beaked apical cells. Microconidia were aseptate, fusoid to obovoid and produced on mono or poly-phialides. Chlamydospores were absent. The colonies of *F. nirenbergiae* burly-wood to vinaceous colour with flocculent aerial hyphae on the PDA. Microconidia were 0-1 septate, oval to falcate and formed on the tip of the phialides. Macroconidia were 3 septate, crescent shaped, hyaline with foot shaped basal cells and semi papillate apical cells. Chlamydospores were aseptate, spherical and produced intercalary or terminally. Orange sporodochia were formed in CLS medium. *F. nirenbergiae* colonies were pale vinaceous to burly-wood colour, with abundant flocculent aerial hyphae on the PDA. Sporodochial conidia also formed small false heads on the tips of the phialides, lucid,

oval or falcate with 0-1-septa. Macroconidia were hyaline, generally 3-septate, and in the shape of crescents or sickles with an attenuated to semi-papillate, curved apical and foot-shaped basal cells. The globose to spherical, aseptate chlamydospores were further produced terminally or intercalary. The aerial mycelium also formed abundantly bright orange sporodochia on the CLA media. *F. stilboides* formed velvety and cottony mycelia with maroon pigmentation on PDA. Aerial conidia were 3-5 septate, cylindrical, smooth walled with pointed apices and straight to flexuous centre with foot cells. Macroconidia were 0-1 septate, elliptical or obovoid. Chlamydospores were also observed. *F. curvatum* produced abundant pinkish white floccose arial mycelia. Microconidia were 0-1 septate, falcate to ellipsoid, hyaline and formed on false heads at the tip of the phialides. Macroconidia were 2-4 septate, banana shaped with blunt basal cells and papillate to blunt apical cells. Chlamydospores were absent.

F. equiseti, causing dieback disease in *D. officinale* was characterised by Guo *et al.* (2020). The fungus produced white, cottony textured colonies with pale luteous reverse colony colour when kept under dark condition for four days. The average diameter of the colony was 4.8 cm. Under alternating light and dark condition, pinkish orange colonies were produced with circular growth patterns and undulating edges. The reverse of the colony was light orange, with an average diameter of 6.0 cm. The fungus produced smooth macroconidia with fusiform to clavate shape. The average length and width of the conidia were 19.67 µm and 3.58 µm respectively. The primary conidiophore was 1-2 branched, whereas secondary conidiophore was 2-4 branched.

2.5.2. *Sclerotium* spp.

Bag *et al.* (2003) characterised *S. rolfsii* causing wilt disease in orchids in India. The pathogen produced white, dense, aerial mycelia on PDA. Hyphae were septate with the presence of clamp connection. The fungi produced numerous sclerotia of 0.5-1.99 mm in size. The sclerotia were spherical or ellipsoidal, initially white and later turned brown in colour.

S. rolfsii was isolated from Sclerotium rot in *Cymbidium* spp. from Korea by Han *et al.* (2012). The fungus produced aerial mycelia with narrow strands of hyphae with 3-9 µm width. Hyphae were septate with the presence of clamp connection. The

fungus produced numerous spherical sclerotia with a diameter of 0.5 to 1.99 mm. Cheng *et al.* (2023) characterised *S. rolfsii* causing southern blight of *D. huoshanense.* The fungus produced white mycelia which produced sclerotia after seven days of incubation.

2.5.3. *Phytophthora* spp.

P. multivesiculata was recorded in *Cymbidium* spp. from New Zealand by Hill (2004). The fungus attained 43 mm diameter after four days of incubation. The hyphae showed characteristic catenulate swellings. The fungus produced aplerotic, spherical and smooth walled oospores (24-42 µm diameter) with amphigynous antheridia. Cating *et al.* (2010) characterised *P. cactorum* and *P. palmivora* associated with black rot of orchids. The zoosporangia of *P. cactorum* were oval to ellipsoidal or spherical whereas *P. palmivora* produced ovoid to elliptical zoosporangia. Both species produced papillate zoosporangia. *P. cactorum* was homothallic while *P. palmivora* was heterothallic. The antheridia produced by *P. cactorum* attached to the sides of the oogonium whereas antheridia of *P. palmivora* surrounded by the oogonium stalk.

The cultural and morphological characterisation of *P. nicotianae* causing leaf blight in *Dendrobium* spp. was done by Tao *et al.* (2011). The fungal colony appeared in rosette pattern on PDA. The thickness of the hyphae was 2.62-6.94 µm with hyphal swellings. The sporangia were pyriform, ellipsoid or ovoid to spherical with an average dimension of 46.20 x 34.89 µm. The length and breadth ratio were recorded as 0.95-1.95. Chlamydospores were globular, thick walled, intercalary or terminal with a diameter of 30.15 µm. The sex organs were produced after 15 to 30 days of incubation at 24ºC. Oogonia were globular and smooth with an average diameter of 24.04 µm whereas antheridia were spherical to short cylindrical and amphigynous type.

2.5.4. *Colletotrichum* spp.

C. gloeosporioides causing anthracnose disease in orchids was morphologically characterised by Chowdappa *et al.* (2012). They characterised 25 isolates of *C. gloeosporioides* from different orchids. Different isolates produced varying colony colours such as white to grey or pink to grey or orange with dark grey, orange or white on the reverse side with regular margin and uniform growth pattern. The growth rate of

isolates ranged from 6.5 to 9.3 mm except for strain OORC31 which showed a growth rate of 4.7 mm per day. The isolates produced hyaline or brown mycelia with sparse or abundant, loose, floccose or compact growth. The conidia were cylindrical with one apex pointed and another apex rounded or both apices rounded. The size of conidia varied from 7.57-15.50 × 3.38-7.52 µm. The longer conidia were produced by the isolate OORC19 with a length of 15.50 µm while shorter conidia were produced by the isolate OORC19 with a length of 7.57 µm.

The morphology of *Colletotrichum cymbidiicola* causing anthracnose disease in *Cymbidium* sp. was studied by Park *et al.* (2020). The fungus produced greyish, cottony aerial mycelium on PDA and attained a diameter of 80 mm after seven days. Conidiophores were hyaline, short, simple and septate with cushion-shaped acervuli of 62.5-150 µm long. The setae of acervuli were brown, verruculous in the upper part with 2 to 4 septa. Appressoria were lobate, brown with a size of 5-15 x 5-8.5 µm. Conidia were hyaline, single celled, cylindrical, with a prominent scar having a size of 14-16 x 5-6.1 µm.

Anthracnose on *D. officinale* caused by *C. fructicola* were morphologically characterised by Ma *et al.* (2020). The fungus produced whitish to grey mycelia with cottony texture after seven days of incubation on PDA. The conidia were hyaline, aseptate, cylindrical and rounded ends with two oil globules. The size of the conidia was 11.3 to 19.1 × 4.2 to 5.8 µm. The appressoria were obovoid to ellipsoid and medium to dark brown. Fernandez-Herrera *et al.* (2020) studied the cultural and morphological characters of *C. karstii* causing anthracnose disease in *D. nobile*. The fungus produced white to pink mycelia. Conidia were single celled, cylindrical with round ends. The size of the conidia ranged from10.8-15.5 x 5.3-6.8 µm.

2.6. MOLECULAR CHARACTERISATION OF PATHOGENS

Wang *et al.* (2020) characterised the fungal pathogen *A. alternata* by amplification and sequencing of internal transcribed spacer (ITS) region, RNA polymerase II β subunit (RPB2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Alternaria major allergen (Alt a1) gene and partial sequence of the β-Tubulin gene (TUB2) by using primers ITS1/ITS4, RPB2DF/RPB2DR, GDF/GDR,

Alt-for/Alt-rev, T1/T2 respectively. They found 99 per cent nucleotide similarity with corresponding sequences KU324792, MG736307, MK210169, MG199093 and MK050502 which were the reference isolates of *A. alternata* in GenBank and these sequences were deposited in gene bank with accession numbers, MN173832, MN307738, MN307737, MN313882 and MN175551 respectively.

The molecular characterisation was conducted for causal organism of Fusarium wilt disease in *D. officinale*. They adopted direct colony PCR method to amplify the ITS of rDNA, elongation factor (EF) β-tubulin (Tu) using the primers ITS4/ITS5, EF1/EF2, T1/T22 respectively. The four isolates were classified in *F. oxysporum* clade by the phylogenetic trees constructed using the ITS, TEF1 and β-tubulin sequences and these isolates were deposited in China general microbiological culture collection centre. The accession numbers of the isolates were CGMCC3.18018, CGMCC3.18020, CGMCC3.18025 and CGMCC3.18019 (Zhang *et al.,* 2017).

Ma *et al.* (2020) identified the causal organism of anthracnose disease on *D. officinale* by amplifying the ITS region and partial sequences of TUB2 and ACT and using ITS1 / ITS4, T1 / T2 and ACT512F / ACT783R respectively. The resultant sequences of ITS, ACT and TUB2 and showed 99.62, 99.14, 99.42 per cent identity with corresponding sequences of *C. fructicola*. The sequences of ITS, ACT and TUB2 were deposited in gene bank with accession numbers of MN173821, MN175550 and MN175549 respectively.

The causal organism of dieback diseases caused by *Fusarium* sp. on *D. officinale* were identified at species level by amplifying rDNA ITS, IGS and EF using primers of ITS1/ITS4, IGS11/IGS12 and EF1/EF2. As a result of cluster analysis, it was found that *F. equiseti* and the fungus under study were clustered in the same branch, showing 96 per cent identity and the pathogen was finally confirmed as *F. equiseti* (Guo *et al*., 2020).

Cao *et al.* (2022a) characterised causal organisms of stem rot disease caused by *Fusarium* sp. at molecular level by amplifying translation elongation factor-1a (TEF-la) and second largest subunit (RPB2) of RNA polymerase through PCR. BLASTn analysis revealed 99.2 per cent similarity with the TEF-1a sequence and 99.9 per cent

similarity with RPB2 sequence of *F. kyushuense*. The sequences were deposited in the NCBI GenBank nucleotide database with accession numbers OL548913 and OL548912.

Species level identification of the causal organism of leaf spot disease in *D. officinale* in China was done by Cao *et al.* (2022b). ITS, translation elongation factor1a (TEF-1a) and b-tubulin gene (TUB2) were amplified and the resultant sequences were deposited in NCBI GenBank with the following accession numbers of OK631881, OK655896 and OK655895 respectively. BLASTn analysis was carried out for the sequences and found nucleotide similarity with *N. clavispora* with accession numbers MG729690, MH423940 and MG740736 and similarity per cent of 100, 99.8 and 100 per cent respectively.

Balamurugan *et al.* (2020) characterised the species causing soft rot disease in *D. nobile* in India by amplifying 16S rRNA gene sequences. The DNA was extracted, and PCR analysis was done with universal primers 27F and 1492R. The PCR product was curated using Finch TV and the contigs were assembled using DNA Baser version v5 followed by BLAST analysis of the contig sequences. The result from BLAST analysis revealed that all the three isolates showed nucleotide identity with type strain JS5 [DSM 101947] of *D. fangzhongdai* having similarity per cent ranging between 99.35-99.93 per cent. These sequences of strains were deposited in GenBank with accession numbers MN400213, MN400217 and MN400214.

To confirm the identity of the pathogen causing southern leaf blight *D. huoshanense,* complete internal transcribed spacer (ITS) rDNA region and the large subunit (LSU) region, the elongation factor-1a gene (EF1a), MB-1 was amplified and sequenced using ITS1/ITS4, NL1/NL4, EF1-983F/EF1-2218R respectively. They showed 100 per cent sequence identity with *S. rolfsii* having accession numbers of MN610008.1 (ITS) and MW322687.1 (EF1a) while 99.84 per cent sequence similarity for MT225781.1 for LSU sequence (Cheng *et al.*, 2023).

2.7. DISEASE MANAGEMENT

2.7.1. Biological control

Sen (2006) evaluated the antagonistic activity of fluorescent Pseudomonas BRL1 against three pathogens, such as *Erwinia carotovora*, *F. oxysporum* and *Mucor hiemalis* f. *hiemalis* under *in vitro* and *in vivo* condition. The *in vitro* studies showed that it inhibited growth of fungal pathogens *F. oxysporum* and *M. hiemalis* f. *hiemalis* by 68 and 60 per cent respectively with a clear inhibition zone indicating the biocontrol efficacy of fluorescent Pseudomonas BRL1 against *E. carotovora.*

Meera *et al.* (2016) tested the efficacy of *Pseudomonas fluorescens* (0.5 %) against *E. chrysanthemi* and observed 65.56 per cent inhibition against the bacterial pathogen. They also evaluated *P. fluorescens* in combination with fresh cowdung extract (2 % + 0.5 %) and found 57.77 per cent inhibition of *E. chrysanthemi* which was less than *P. fluorescens* alone.

The studies conducted by Sowanpreecha and Rerngsamran (2018) showed that different anti-*P. palmivora* protein produced by *P. aeruginosa* RS1 were effective against *P. palmivora* causing black rot disease in orchids. *P. aeruginosa* RS1 showed 50 per cent inhibition of growth of *P. palmivora* on V8 agar medium. Proteins were eluted from culture filtrate of *P. palmivora* and each protein band was analysed by LC/MS. It was found that these proteins were included in three groups such as protease, chitin binding protein, catalase, indicating the antifungal activity of isolated proteins. The scanning electron microscopy studies also revealed that these proteins altered the normal growth and caused hyphal elongation of *P. palmivora*.

Basnet *et al*. (2022) investigated the biocontrol efficacy of *Bacillus megaterium* TRS-3 against *S. rolfsii,* causing crown rot disease in *Oncidium* spp. The antagonist inhibited the growth of pathogen by 55.5 per cent as compared to control under *in vitro* studies. They found that some volatile compounds released by *B. megaterium* TRS-3 reduced the growth of fungal mycelia under *in vitro* condition. The extracted compounds from bacterial lawn were mixed with media at 1:5 ratio and 100 per cent inhibition was found against *S. rolfsii.* The sclerotia also did not germinate when treated with the compounds extracted from *B. megaterium* TRS-3. Studies were carried out

under field condition as well and found a disease incidence of 42 per cent for treatment plants, while the control plants exhibited disease incidence of 92 per cent. Apart from biocontrol activity, the antagonist also induced biochemical defence mechanism in plants. They elicited the activity of β-1,3- glucanase, chitinase and peroxidase, which resulted in host resistance against pathogens.

The studies performed by Prapagdee *et al*. (2012) showed that crude extracts of antifungal compounds produced by *B. subtilis* SSE4 could inhibit the growth of *C. gloeosporioides* causing anthracnose disease in *Dendrobium* sp. They used different chemicals for the extraction of culture filtrate, and maximum inhibition percentage was observed for ethyl acetate and hexane extracts with 76.2 ± 5.2 and 75.3 ± 6.5 per cent respectively under *in vitro* condition. Bioassay studies were also conducted to evaluate the efficacy of antifungal compounds extracted by ethyl acetate and hexane against the pathogen and found promising result under *in planta* condition as well.

Kuenpech *et al*. (2014) studied the antifungal activity *of B. subtilis* isolate B6 against *C. gloeosporioides,* causing anthracnose disease in lady's slipper orchid. They evaluated the effect of both B6 filtrated culture media (FM) and B6 non-filtrated culture media (NFM) against the fungal pathogen, and both FM and NFM inhibited hyphal growth and spore germination. However, the efficacy of B6 non-filtrated culture medium treatment was found to be higher than filtrated culture medium and the treatment with four-day culture medium highly effective than treatment with two-day and six-day culture medium. In addition to culture filtrate, liquid and powder formulation of B6 were also evaluated against the pathogen and found to be effective in controlling disease. Chi *et al.* (2022) tested the efficacy of *B. subtilis* against soft rot disease of epiphytic orchids caused by *D. fangzhongdai* under *in vitro* condition and found strong inhibition against the pathogen, while only mild inhibition was observed under *in vivo* condition.

The antagonistic potential of *B. velezensis* SK71 was tested against the bacterial brown spot pathogen *Acidovorax avenae* subsp. *cattleyae* and found to be a promising natural biocontrol agent (Akarapisan *et al*., 2020). The antagonist, *B. velezensis* SK71 was isolated from the rhizosphere of a terrestrial orchid and tested *in vitro* against *A. avenae* subsp. *cattleyae* by paper disc method. An inhibition zone of 2.2 cm was

observed on the treatment plates, indicating the antagonistic ability of *Bacillus velezensis* SK71 in controlling *A. avenae* subsp. *cattleyae.* They found that the antagonistic ability of *B. velezensis* SK71was due to the production of antibiotics and hydrolytic enzymes. The formulation of *B. velezensis* SK71 with diatomite powder, glucose, carboxymethyl cellulose and K_2HPO_4 was also tested against the pathogen and found to be effective in suppressing the growth of bacterial pathogen. *In vivo* studies were also conducted to test the effectiveness of antagonists by bulb treatment and spraying method, and the studies showed that SK71 had a 53.34 per cent efficacy in controlling bacterial brown spot disease in terrestrial orchids.

Safeer *et al.* (2022) found that the root endophyte *Piriformospora indica* was effective in controlling CymMV along with antiviral formulation. The roots were inoculated with *P. indica* and the plants were treated with Virus Ex −1 ml L^{-1} as foliar spray which was found to be effective in controlling CymMV in *Dendrobium* spp.

2.7.2. Botanicals

The causal organism bud wilt disease caused by *F. proliferatum* in *Dendrobium* spp. was inhibited by different plant extracts, among which methanol extracts from sweet flag (25 %) showed maximum inhibition (77 %) followed by wild basil extract with 55 per cent inhibition (Dissanayake, 2015).

Kaewduangta *et al.* (2017) studied the efficacy of essential oils and crude extract from five plants; galangal, lemongrass, ginger, turmeric and garlic in controlling anthracnose disease in *Dendrobium* sp. caused by *Colletotrichum* sp. *In vitro* studies showed that essential oils from ginger at a concentration of 8,000 ppm and lemon grass at 500 ppm caused complete inhibition of fungal pathogen while other treatments were not effective against the pathogen. The crude extracts from garlic and galangal caused 100 per cent inhibition at 80,000 and 10,000 ppm respectively. The efficacy of essential oils and crude extracts were also studied by modified detachment leaf technique, and it was found that essential oil from ginger at 8,000 ppm caused 100 per cent inhibition of fungus.

The studies conducted by Meera *et al.* (2016) showed that *P. fluorescens* in combination with fresh cowdung extract (2 % + 0.5 %) when treated with *E.*

chrysanthemi showed 55.92 per cent inhibition of *E. chrysanthemi* causing soft rot in *Phalaenopsis* sp.

2.7.3. Chemical control

McMillan (2011) evaluated seven fungicides such as Dithane (mancozeb) 32.0 fl oz, Cleary's 3336 (thiophanate methyl) 6.0 fl oz, Pageant (pyraclostrobin + boscalid) 12 oz, Insignia (pyraclostrobin) 4 oz, Heritage (azoxystrobin) 4 oz, Insignia (pyraclostrobin) 8 oz, and Pageant (pyraclostrobin + boscalid) 6 oz against *C. gloeosporioides* on *Dendrobium* spp. The highest efficiency was shown by Insignia at 8.0 oz (pyraclostrobin) and Pageant (pyraclostrobin + boscalid) at 12.0 oz followed by Heritage (azoxystrobin) and Cleary's 3336 (thiophanate methyl).

Meera *et al.* (2016) investigated the efficacy of six fungicides against different pathogens isolated from *Phalaenopsis* spp. The fungicides selected for *in vitro* studies were fenamidone 10 % + mancozeb 50 % (0.1 %), carbendazim 12 % + mancozeb 63 % (0.15 per cent), cymoxanil 8 % + mancozeb 64 % (0.2 %) and carbendazim 25 % + iprodione 25 % (0.2 %). These fungicides were tested against the pathogen *viz. F. oxysporum*, *C. gloeosporioides*, *S. rolfsi* and *P. exigua* and it was found that all the fungicides showed complete inhibition of *C. gloeosporioides*, *S. rolfsi* and *F. oxysporum* except *P. exigua*. All the fungicides except carbendazim 12 % + mancozeb 63 % and cymoxanil 8 % + mancozeb 64 % showed 100 per cent inhibition of *P. exigua*.

McMillan *et al.* (2008) tested the efficacy of fungicides against *C. dendrobii* on *D. antennatoum* under *in vivo* condition and found that the plant treated with mancozeb, thiophanate methyl, BAS 516 04 F (pycadostrobin + boscalid), azoxystrobin and pyraclostrobin were effective against the fungus as compared to control, while higher efficacy shown by pyraclostrobin 20 %WG at 226.8 g per 379 L of water and **C**BASF 516 04 (pycadostrobin + boscalid) F 38 %WG at 340.2 g per 379 L of water as compared to other treatments and control.

Almanza-Alvarez *et al.* (2017) investigated the effectiveness of fungicides such as benomyl, thiabendazole and thiophanate methyl against pathogenic fungi isolated from *Laelia* spp. under *in vitro* condition. The result showed that thiabendazole

significantly inhibited both *F. solani* and *F. proliferatum* after five days. There were no significant differences in inhibition of *F. proliferatum* after ten days while an inhibitory effect was observed for *F. solani* up to 25 days. Benomyl showed significant inhibition of *Aspergillus fumigatus* after five days whereas thiabendazole showed moderate inhibition. All of the fungicides that were evaluated, however, had no effect on the growth of *C. karstii*, *C. boninense* and *F. oxysporum.*

Su *et al*. (2018) enlisted several fungicides that were effective against different fungal diseases in orchids. The fungicides recommended for *Fusarium* spp. were prochloraz 25 % EC (emulsifiable concentrate) diluted 2000 times, prochlorate manganese 50 % WP (wettable powder) diluted 6000 times, and pyraclostrobin 23.6 % EC diluted 3000 times. For *Phytophthora* spp., fungicides such as oxine-copper 33.5 % SC diluted 1500 times, propamocarb hydrochloride 66.5 % SL diluted 1000 times and etridiazole 35 % WP diluted 1500 times. Anthracnose disease caused by *Colletotrichum* spp. was controlled by propineb 70 % WP diluted 500 times, mancozeb + myclobutanil 62.25 % WP diluted 600 times and prochloraz 25 % EC diluted 2500 times. The fungicides suggested for southern blight were mepronil 75 % WP diluted 1000 times, tolclofos-methyl 50 % WP diluted 1000 times and flutolanil 50 % WP diluted 3000 times. Gray mold was controlled by iprodione 50 % WP diluted 1500 times, thiophanate-methyl 78 % WP diluted 2500 times, and procymidone 50 % WP diluted 2000 times.

Singh *et al.* (2012) documented various chemical management strategies against different diseases in orchids. Spraying of Bavistin (0.1 %) and Dithane M-45 (0.2 per cent) were effective against leaf spot diseases caused by *Phyllostictina* sp., *Colletotrichum* sp., *Gloeosporium* sp. and *Cercospora* sp. and brown speck and blight of flowers caused by *B. cinerea* while Pythium black rot and Phytophthora heart rot were controlled by fosetyl- Al and metalaxyl. Wettable sulphur was recommended for managing rust disease caused by *Hemileia americana*. The bacterial soft rot disease caused by *E. carotovora* was controlled by Streptocycline (0.1 %) and bacterial brown spot caused by *Xanthomonas cattleyae* was controlled by Agrimycin / tetracycline.

Meera *et al.* (2016) evaluated the efficacy of Streptocycline at 200 ppm against *E. chrysanthemi,* causing soft rot pathogen of *Phalaenopsis* sp., and found 30.74 per cent inhibition. Chi *et al.* (2022) tested the efficacy of different chemicals against *D. fangzhongdai,* causing soft rot in epiphytic orchids, under *in vitro* condition. Among the chemicals evaluated under *in vivo* condition, antibiotics such as streptomycin sulphate, erythromycin + streptomycin sulphate and found highly effective in controlling pathogens, followed by Cu-bismerthiazol and cuprous oxide.

Srivasthava (2014) investigated the efficacy of fungicides such as triticonazole a.i. at 20 %, azoxystrobin a.i. at 50 %, and pyraclostrobin a.i. at 25 % against *F. oxysporum*, *F. proliferatum*, *F. subglutinans* and *F. solani*. The results showed that triticonazole was the most effective fungicide against all the *Fusarium* isolates both under *in vitro* and *in vivo* condition followed by pyraclostrobin and azoxystrobin. Triticonazole showed 92 per cent reduction in mycelial growth of the fungi under *in vitro* condition whereas pyraclostrobin and azoxystrobin showed inhibition percentage of 68 per cent and 50 per cent respectively.

Pant *et al.* (2020) suggested that meristem tip culture was very effective method for eliminating viruses in orchids and Morel (1960) first employed this technique for developing virus-free *Cymbidium* sp. from diseased plants. Production of CymMV-free plants reportedly had excellent success, however, ORSV cases had only limited success. Production of CymMV-free plants had high success rate as compared to ORSV, which showed only partial success (Ishii, 1974). Yap *et al.* (1999) reported that ribavirin (virazole) was used to raise *Oncidium goldiana* by tissue culture method, and 43 per cent virus free plants were obtained by the application of virazole at 10 mgL^{-1} for 30 days. Kuehnle (1996) found that ribavirin or dithiouracil were effective for getting CymMV-free plants. According to Freitas-Astua and Rezende (1998), meristem culture in combination with virazole was used for getting 83 to 100 per cent ORSV-free orchids. Chang *et al.* (2005) developed coat-protein mediated resistance against CymMV in *Dendrobium* sp., and these transgenic plants showed resistance against severe strain of CymMV. Liao *et al.* (2004) developed transgenic *Phalaenopsis* sp. for the management of CymMV.

Materials & Methods

3. MATERIALS AND METHODS

The project 'Cataloguing and documentation of diseases of sympodial orchids in Kerala' was conducted in the Department of Plant Pathology, College of Agriculture, Kerala Agricultural University, Vellanikkara during the period 2021-2022. The details of the materials used and the techniques adopted during the course of experiments are described below.

3. 1. SURVEY ON THE OCCURRENCE OF DISEASES OF SYMPODIAL ORCHIDS

Purposive sampling surveys were conducted in different orchid growing areas of Kerala, and documentation of several diseases in sympodial orchids was done based on the disease incidence and severity data. Three districts *viz*., Thrissur, Ernakulam, and Thiruvananthapuram were chosen, and the locations of the survey in each district are described below. Samples were collected from each location for further study and symptoms on each plant part were closely examined and documented. Each symptom was catalogued by designating a specific code related to that location. The cultural practices adopted by the orchids growers of the survey location was also recorded.

Table 3.1. Details of survey locations

District	Location	Month of survey
Thrissur	Vellanikkara (VK)	June 2022, November 2022
	Vadanapally (VP)	July 2022
	Madakkathara (MT)	June 2022, September 2022
	Irinjalakuda (IK)	August 2022
Ernakulam	Ambunadu (AM)	July 2022
	Vyttila (VL)	April 2022
Thiruvananthapuram	Kottukal (KO)	May 2022
	Vellayani (VY)	May 2022

The disease incidence and severity on infected plants exhibiting different types of symptoms were noted during the course of survey.

The per cent disease incidence was calculated using the formula given by Wheeler (1969).

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

A standard score chart with 0-5 scale given in the following table was used to estimate the per cent disease severity (PDS) for all foliar diseases (Meera, 2012).

Table 3.2. Disease score chart for assessing foliar diseases

Grade	Percentage of infection
0	No infection
1	1 - 10
2	>10 - 25
3	>25 - 50
4	>50 - 75
5	> 75

Similar types of symptoms were graded in the field utilising the standard score chart on representative samples. Later, using formula given by Wheeler (1969), the per cent disease severity was determined for each type of symptom separately.

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

3.1.2. Correlation of disease incidence and severity with light intensity

The light intensities of different survey locations in Thrissur, Ernakulam and Thiruvananthapuram were measured using Lux meter and correlated with per cent disease incidence and per cent disease severity of that particular location using Statistical Package for Social Sciences (SPSS) software.

3.2.ISOLATION OF PATHOGENS FROM DISEASED SAMPLES AND PATHOGENICITY STUDIES

3.2.1. Isolation of fungal pathogens

Diseased specimens collected from the fields were brought to the lab, and thoroughly washed under running water to eliminate the saprophytes and dust adhered to them. Tissue segmentation method was employed to isolate the fungal pathogens from the infected samples (Rangaswamy and Mahadevan, 1999). The infected region was made into bits of size 1.0 to 1.5 cm^2 containing both healthy and diseased portions using sterile blade, followed by surface sterilization with 1 per cent sodium hypochlorite solution for 1 min. To remove the traces of sodium hypochlorite, the bits were subjected to three consecutive washings in sterile water for 1 min each and kept in sterile blotting paper under aseptic conditions. Meanwhile, the potato dextrose agar (PDA) that had been melted and cooled was aseptically dispensed into sterile Petri dishes. The bits were placed in the Petri dishes with solidified medium and kept for incubation at room temperature (26 ± 2°C) for five days in inverted position. The growth of the fungal pathogen from the bits was observed from the initial days of incubation and the culture was then purified using either single hyphal tip method or single spore isolation method. The fungal cultures thus obtained were maintained on PDA slants and stored at 4°C in the refrigerator for further studies.

3.2.2. Isolation of bacterial pathogens

Naturally infected orchid plants exhibiting symptoms were collected and bits were surface sterilized by dipping in 2 per cent sodium hypochlorite solution for 1 min followed by washing thrice in sterile water. The diseased tissue was then macerated in sterile water, and a loopful of the resultant suspension was streaked over the solidified nutrient agar (NA) medium in Petri dishes by quadrant streak method and there after the plates were incubated in inverted position at room temperature (26 ± 2°C). Typical single colonies were selected after 48 h of incubation, purified and maintained on NA slants (Schaad *et al.*, 2001).

3.2.3. Pathogenicity studies

3.2.3.1. Pathogenicity of fungal pathogens

The pathogenicity of isolated cultures was performed by mycelial bit inoculation method (MBIM) (Rocha *et al*., 1998), and Koch's postulates were proved by re-isolation of the pathogens from the artificially inoculated leaves. Fresh leaves collected from the field were surface sterilized with 70 per cent ethyl alcohol and pricks were made on the leaves using sterilized needle. Fungal discs taken from seven to eight-day-old cultures were inoculated on the pricked portion. Leaves inoculated with sterile water served as control. The inoculated area was covered with moist cotton, and the inoculated leaves were maintained in a humid chamber, and the symptoms developed were recorded. Re-isolation was done from the corresponding leaves and compared with the original isolate.

3.2.3.2. Pathogenicity of bacterial pathogens

For determining pathogenicity of bacterial isolates, a loopful of 48 h old culture of bacteria was added to freshly prepared nutrient broth and incubated at 26 ± 2°C for two days. Healthy leaves of orchids were collected and the bacterial suspension was injected into the veins. The inoculated leaves were kept in moist humid chamber and observed for symptom development. If the inoculated leaves showed characteristic symptom, re-isolation was done from the corresponding leaves.

3.3. SYMPTOMATOLOGY OF DISEASES

A detailed study on symptoms produced by various pathogens under natural and artificial condition was conducted. Under natural conditions, characteristic symptoms caused by fungi and bacteria on orchid plants were studied and documented, while the symptomatology under artificial conditions was carried out by inoculating different isolates on healthy plant parts as mentioned in section 3.2.3. Observations regarding the progression of disease such as size of the lesion and duration for disease development were taken.

3.4. CHARACTERISATION AND IDENTIFICATION OF PATHOGENS

Cultural and morphological features of selected fungal and bacterial pathogens were studied and based on the colony characters and morphological features, the isolates were identified up to generic level. Species level identification was done through molecular techniques.

3.4.1. Characterisation of fungal pathogens

3.4.1.1. Cultural characterisation of fungi

An agar disc of 8 mm diameter of fungal pathogen was placed in the centre of a sterile Petri plate containing solidified PDA medium followed by incubation at room temperature (26 ± 2ºC). The cultural traits of different fungal pathogens such as colour, texture, growth rate, growth pattern, sporulation, pigmentation, colour on the reverse side of Petri plates, and the presence of fruiting bodies were studied in detail.

3.4.1.2. Morphological characterisation of fungi

The morphological characters of the pathogens like colour of hyphae, branching pattern, septation of hyphae, presence of conidia, conidial septation, type, shape and size of spores, length and breadth of spores and presence of sexual structures were studied. For morphological observations, a mycelial bit of the fungus was mounted on a sterile slide with a drop of lactophenol stain and examined under a light microscope equipped with an image capture system. Measurement of the fungal structures were taken and average values of the measurements were recorded. The fungal pathogens isolated from each diseased sample were tentatively identified at genus level by comparing the cultural and morphological characteristics with CMI (Commonwealth mycological institute) descriptions of plant pathogenic fungi and bacteria.

3.4.1.3. Molecular characterisation of fungal pathogens

The species level identification of fungal isolates was done based on molecular characterisation by isolating the DNA of fungus, and sequencing its ITS (Internal transcribed spacer) rDNA region. After studying cultural and

morphological characteristics, ITS-rDNA gene sequencing of fungal cultures was done at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram.

3.4.1.3.1. DNA isolation of fungi

Using NucleoSpin® Plant II Kit, the fungal DNA was extracted (Macherey-Nagel).

Procedure

- 100 mg of the fungal tissue was homogenized in a mortar and pestle using liquid nitrogen, and the powdered mycelium was dispensed to a microcentrifuge tube.
- 400 µl of buffer (PL1) was added to the tube and vortexed for 1 min.
- 10 µl of RNase A reagent was added and mixed by inverting the tubes followed by incubation at 65°C for 10 min.
- The lysate was transferred to a Nucleospin filter, followed by centrifugation at 11000 rpm for 2 min.
- The supernatant obtained was mixed with 450 µl of buffer PC thereafter transferred to a Nucleospin Plant II column followed by centrifugation for 1 min.
- The flow through liquid was removed and 400 µl of buffer PW1 was added.
- The contents were centrifuged at 11000 rpm for 1 min followed by discarding the flow through liquid in the column.
- After that, 700 µl buffer PW2 was added, centrifuged at 11000 rpm and flow through liquid was discarded.
- 200 µl of PW2 mixed with the contents and centrifuged at 11000 rpm for 2 min to dry silica membrane in the column.
- The column was then transferred to a 1.7 ml tube and the contents were mixed with 50 µl of buffer PE followed by incubation at 65°C for 5 min. Then the column was again centrifuged at 11000 rpm for 1 min.
- The eluted DNA was stored at 4°C.

3.4.1.3.2. Agarose gel electrophoresis

The quality of eluted DNA of the fungus was confirmed by agarose gel electrophoresis.

- 1 µl of 6X gel-loading dye (0.25 per cent bromophenol blue, 30 per cent sucrose in TE buffer pH-8.0) was thoroughly mixed with DNA solution.
- After mixing with dye, DNA solution was loaded to the wells in 0.8 per cent agarose gel made in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide.
- Electrophoresis was performed in 0.5X TBE buffer at a voltage of 75 V until bromophenol blue dye migrated to the bottom of the gel.
- The gel was examined by UV transilluminator (Genei) and captured the image on a CCD camera equipped with a UV emission filter/Gel documentation system (Bio-Rad).

3.4.1.3.3. PCR Analysis

The amplification of ITS region fungal DNA was performed in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). The components of reaction mixture were 2X Phire Master Mix (5µl), forward primer (0.25 µl), reverse primer (0.25 µl), purified fungal template DNA (1 µl) and distilled water (4 µl). The primers used for PCR amplification are given in the Table 3.3.

The amplification process was accomplished by three steps: denaturation, annealing and extension. The first step was carried out with an initial denaturation of DNA at 98°C for 30 s, which was followed by 40 cycles of denaturation at 98°C for 5 s, annealing at 58°C for 10 s and then extension at 72°C for 15 s. Final extension step as carried out at 72°C for 60 s after completing of 40 cycles of amplification.

Table 3.3. Details of primers used for fungal DNA characterisation

Target	Primer Name	Direction	Sequence (5' 3')
ITS	ITS-1F	Forward	TCCGTAGGTGAACCTGCGG
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC

3.4.1.3.4. Gel documentation and ITS- rDNA gene sequencing

The PCR products were run in 1.2 per cent agarose gel prepared in 0.5X TBE buffer with 0.5 µg per ml of ethidium bromide. 1 µl of 6X gel loading dye was added to 4 µl of PCR product and mixed thoroughly. The resultant mixture was loaded to the wells and electrophoresis were conducted through gel with 0.5X TBE buffer. 2-log DNA ladder (NEB) was taken as the molecular standards. When the dye reached one third of the gel, electrophoresis was completed and the gels were visualized in a UV transilluminator (Genei) and the images of the gel were taken using Bio- Rad Gel documentation system. Sequencing reaction was done in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol.

The components in the PCR reaction mixture are shown in the Table 3.4 and the steps in the PCR amplification for sequencing is given in the Table 3.5.

Table 3.4. Components of PCR mix for ITS sequencing reaction

Sl. No.	Component	Quantity (µl)
1	5X Sequencing buffer	1.9
2	Forward primer	0.3
3	Reverse primer	0.3
4	Sequencing mix	0.2
5	Sequencing mix	0.2
6	ExoSAP treated PCR product	1.0
7	Distilled water	6.6

Table 3.5. PCR amplification programme of ITS sequencing

Steps	Temperature (°C)	Duration
Initial denaturation	96	2 min
denaturation	96	30 s
Annealing	50	40 s
Extension	60	4 min
Final extension	4	α

3.4.1.3.5. In silico analysis of ITS sequences

The sequences were analysed using BLASTN search against NCBI nr database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

3.4.2. Characterisation of bacterial pathogens

3.4.2.1. Cultural characterisation of bacteria

A loopful of bacteria was taken and streaked onto a sterile Petri plate containing solidified NA medium and the Petri dish was kept for incubation at room temperature (26 ± 2ºC). Colony characters such as colour, shape, texture and pigmentation were observed.

3.4.2.2. Morphological characterisation of bacteria

Gram staining was performed to study the morphological characters of bacteria. On a clean glass slide, a smear of 24 h old bacterial culture was prepared and allowed the smear to air dry and the smear was heat fixed by passing the slide through the flame three to four times. The heat fixed smear was stained with crystal violet for 1 min, excess stain was washed off and then flooded with Gram's iodine for 1 min. The smear was decolourized with 95 per cent ethanol for 10-20 s, and immediately washed off the alcohol with water. The smear was then stained with counter stain safranin for 30 s, and excess stain was washed off. The slide was blot dried and observed under oil immersion objective. The morphological characters of the bacteria like colour, shape and size of the cell were studied (Schaad, 2001).

The cultural, morphological and biochemical characters of the isolated bacteria were studied and compared with descriptions in Bergey's Manual of Systematic Bacteriology (Aneja, 2003).

3.4.2.3. Biochemical characterisation

Various biochemical tests were conducted for the identification of the bacterial pathogens. Biochemical tests such as starch hydrolysis, hydrolysis of gelatin, casein hydrolysis, urease production, Indole production, citrate fermentation, potassium

hydroxide (KOH) test, catalase test, oxidation test methyl red test (MR test) and Voges Proskauer's (VP test) test were performed.

3.4.2.3.1. KOH Test

A drop of 3 per cent KOH was placed on a microscopic slide. Fresh bacterial colony was taken by inoculation loop, mixed with KOH and observed for the viscous string formation between the mixture and inoculation loop (Schaad, 2001).

3.4.2.3.2. Catalase test

A drop of 30 per cent H_2O_2 was added on a microscopic slide and a loopful of bacterial culture was mixed with H_2O_2. After a few seconds, the slides were observed for the bubble formation (Facklam and Elliott, 1995).

3.4.2.3.3. Oxidase test

The test is conducted by rubbing fresh culture of bacteria on oxidase disc impregnated with 1 per cent tetramethyl-P diamine dihydrochloride and observed for the colour change (Schaad, 2001).

3.4.2.3.4. Gelatin hydrolysis test

Gelatin deep tubes were prepared by dispensing gelatin containing nutrient agar media into test tubes, followed by autoclaving. The tubes were cooled in an upright position. Bacterial culture was inoculated by stabbing half inch into the medium and the observation was made after two days of incubation (Holding and Collee, 1971).

3.4.2.3.5. Indole production test

Tryptone broth was prepared by dissolving 10 g of peptone in 1 L of distilled water in test tubes followed by sterilization. The tubes containing broth were inoculated with the bacterial culture and an uninoculated control was also kept. The inoculated tubes were incubated for 48 h at 35^{o}C. After incubation, Kovac's reagent (1 ml) was added to each tube including control and the tubes were shaken gently for 10-15 min. (Holding and Collee, 1971).

3.***4.2.3.6. Methyl red test***

Methyl red Voges Proskauer (MRVP) broth tubes were prepared and autoclaved. The tubes were inoculated with bacteria, and one tube was kept as control which was uninoculated. The inoculated tubes were incubated for 48 h at 35^{o}C. After that five drops of methyl red indicator were added to each test tube, and observed for the colour change (Holding and Collee, 1971).

3.4.2.3.7. Voges Proskauer's test

MRVP broth tubes were inoculated with bacteria and incubated at 35^{o}C for 48 h. Twelve drops of VP reagent I and two to three drops of VP reagent II were added to the tubes. The tubes were gently shaken for 30 s by removing the cotton plug to come in contact with oxygen. The tubes were kept 15-30 min for completing the reaction (Holding and Collee, 1971).

3.4.2.3.8. Citrate fermentation test

Citrate utilization test was carried out by preparing Simmond's citrate agar slants, and inoculating the tubes with bacterial culture. The tubes were incubated at 37^{o}C for 24-48 h. The tubes were observed for colour change and compared with control which was uninoculated (Dowson, 1957).

3.4.2.3.9. Hydrogen sulphide production test

Sulphide Indole Motility (SIM) agar slants were stab inoculated with bacteria, and incubated for 48 h at 37^{o}C. The inoculated tubes were observed for the presence or absence of black colouration along the line of stab inoculation (Holding and Collee, 1971).

3.4.2.4. Potato soft rot test

For further identification of bacterial pathogen, potato soft rot test was conducted according to the procedure of Lelliot *et al.* (1966), to determine their ability to cause soft rot infection on potato tubers. The potato slices were inoculated with needle pricking method.

3.4.2.5. Molecular characterisation of bacterial pathogens

The species level identification of bacterial cultures were done based on molecular characterisation. It was done by isolating the DNA of bacteria and sequencing of 16S rDNA region. After studying cultural, morphological and biochemical characteristics, 16S rDNA gene sequencing of bacterial cultures was done at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram.

3.4.2.5.1. Bacterial DNA isolation

- The isolation of genomic DNA of bacteria was performed using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions. The protocol followed is given below:

- Bacterial culture was transferred to a microcentrifuge tube and 180 µl of TI buffer and 25 µl of proteinase K were added. The mixture was incubated at 56°C in a water bath.

- After complete lysis, 5 µl of RNase A (100 mg/ml) was added to the tubes and kept for incubation at room temperature for 5 min.

- After incubation, 200 µl of B3 buffer was added and incubated at 70°C for 10 min

- Thereafter, 210 µl of 100 per cent ethanol was added to the mixture and the contents were subjected to vortexing.

- The suspension was then pipetted into NucleoSpin® Tissue column kept in a 2 ml collection tube and centrifuged at 11000 rpm for 1 min.

- After centrifugation, the NucleoSpin® Tissue column was transferred to a 2 ml tube and washed with 500 µl of BW buffer. Wash step was repeated using 600 µl of B5 buffer.

- After washing, the NucleoSpino Tissue column was placed in a clean 1.5 ml tube and DNA was extracted using 50 µl of BE buffer.

3.4.2.5.2. Agarose gel electrophoresis and PCR amplification

The quality of isolated DNA was confirmed using agarose gel electrophoresis. The samples containing 5 µl of eluted DNA and 1 µl of 6X gel loading buffer (0.25 per cent bromophenol blue, 30 per cent sucrose in TE buffer pH-8.0) were loaded to each well in 0.8 per cent agarose gel prepared with 0.5X TBE (Tris-Borate-EDTA) buffer having 0.5 µg per ml ethidium bromide. 0.5X TBE was used as the running buffer. Electrophoresis was carried out at 75 V until the dye in the buffer reached at the bottom of the gel. After finishing the process, DNA bands were visualized by UV transilluminator (Genei) and the documentation of the gel image was performed using Gel documentation system (Bio-Rad).

The PCR amplification of 16S rRNA gene was done in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems). Information on the primers used for the amplification reaction, composition and volume of PCR mix is given in Table 3.6 and 3.7 respectively.

Table 3.6. Details of primer used for bacterial genomic DNA characterisation

Target	Primer Name	Direction	Sequence (5' 3')
16S rRNA	16S-RS-F	Forward	CAGGCCTAACACATGCAAGTC
	16S-RS-R	Reverse	GGGCGGWGTGTACAAGGC

Table 3.7. Components of PCR mix for ITS amplification reaction

Sl. No.	Component	Quantity (µl)
1	5X Sequencing Buffer	1.9
2	Forward Primer	0.3
3	Reverse Primer	0.3
4	Sequencing Mix	0.2
5	Exosap treated PCR product	1.0
6	Distilled water	6.6

PCR amplification profile:

96ºC	-	2 min	30 cycles
96ºC	-	30 s	
50ºC	-	40 s	
60ºC	-	4 min	
4ºC	-	∞	

3.4.2.5.3. Agarose Gel electrophoresis and sequencing of 16S rRNA gene

The visualization of PCR product using AGE and further sequencing of bacterial 16S rRNA were performed in accordance with the protocol described in the section 3.6.1.4 using 16S-RS forward and reverse primers.

3.4.2.5.4. In silico analysis of 16S rRNA sequences

The sequence analysis was carried out using BLASTn search against NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to obtain the best aligned sequences.

3.5 *In vitro* EVALUATION OF FUNGICIDES AND BIOAGENTS AGAINST MAJOR PATHOGENS

3.5.1. *In vitro* evaluation of fungicides against major fungal pathogens

The efficacy of different fungicides against major pathogens affecting sympodial orchids was evaluated under *in vitro* condition by poison food technique (Zentmeyer, 1955). Seven different fungicides at three different dosage levels which include lower, recommended and higher doses (Table 3.8) were used for the experiment, whereas for Bordeaux mixture, only one dose i.e., 1 per cent was selected. Each fungicide at different doses was thoroughly mixed with 100 ml PDA to get homogenous concentration and thereafter, 20 ml each of the poisoned medium was then poured into sterilized Petri dishes and allow to solidify. 8 mm mycelial discs of the fungus were cut out with a flame-sterilized cork borer, and each one was placed in the centre of the plates. Plates containing media without fungicides served as control. Completely randomised design (CRD) was used for the experiment, with 23 treatments and three replications for each treatment. Observations were taken until the pathogen

had fully grown on the control plates. The percentage of inhibition of growth of the pathogen by the fungicidal action was obtained using the formula given by Vincent (1927).

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

C - Radial growth of pathogen in control (cm)

T - Radial growth of pathogen in treatment (cm)

Table 3.8. Fungicides used for *in vitro* evaluation along with their doses

Treatment No.	Treatments	Concentration (%)
T_1	Copper hydroxide	0.1, 0.2, 0.3
T_2	Hexaconazole	0.05, 0.1, 0.15
T_3	Propineb	0.1, 0.2, 0.3
T_4	Difenoconazole	0.05, 0.1, 0.15
T_5	Carbendazim 12 % + Mancozeb 64 %	0.1, 0.2, 0.3
T_6	Cymoxanil 8 % + Mancozeb 64 %	0.1, 0.25, 0.3
T_7	Azoxystrobin	0.05, 0.1, 0.15
T_8	Bordeaux mixture	1.0
T_9	Control	

3.5.2. *In vitro* evaluation of bio control agents against major fungal pathogens

The growth inhibition of fungal pathogens was tested with biocontrol agents *viz., Trichoderma asperellum* (KAU reference culture) and *Psedomonas fluorescens* (KAU reference culture), PGPR-II and PGPM. *T. asperellum* (KAU reference culture) and *P. fluorescens* (KAU reference culture) were evaluated against the pathogens using dual culture technique (Morton and Stroube, 1955), whereas PGPR-II and PGPM obtained from Kerala Agricultural University were evaluated using poison food technique. PGPR-II is a microbial consortium of *P. fluorescens*, *Bacillus megaterium* and *Lactobacillus* spp. whereas PGPM contains *T. viride, T. harzianum, B. megaterium* and *P. fluorescens*.

3.5.2.1. In vitro evaluation of T. asperellum against major fungal pathogens

For the evaluation of biocontrol efficacy of *Trichoderma asperellum*, 8 mm mycelial disc from five-day-old culture of the pathogen was placed at a distance of 2 cm from the periphery of a sterilized Petri plate with PDA medium. The inoculated plate was incubated for 48 h. A mycelial disc of 8 mm diameter was later cut from a five-day-old *T. asperellum* culture using a flame-sterilized cork borer and placed on the opposite side 2 cm away from the periphery. Three replications of each treatment were kept, and daily observations were taken until the pathogen attained full growth in the control plates. Using the key created by Webber and Hedger (1986), the forms of interactions between the pathogen and the fungal antagonist (*T. asperellum*) were analysed. Different types of interactions thus described were:

1. Intermingling of hyphae
2. Over growth of the antagonist on the pathogen
3. Mutual inhibition with pigmented band at the point of contact
4. Mutual inhibition with a clear zone between the fungal colonies
5. Extreme inhibition of the pathogen

3.5.2.2. In vitro evaluation of P. fluorescens against major fungal pathogens

For evaluating the antagonistic activity of *P. fluorescens* against major fungal pathogens, mycelial disc (8 mm) of five days old culture of the pathogen was inoculated at the centre of Petri plate and bacterial antagonist, *P. fluorescens* was streaked on either side of the pathogen at 2 cm from the periphery of the Petri dish. Three replications were kept for each treatment and the pathogen grown as monoculture served as the control. Observation was made until the pathogen attained full growth in control. The formula provided by Vincent (1927) was used to assess the percentage of inhibition pathogen by biocontrol agents.

3.5.2.3. In vitro evaluation of PGPR- II and PGPM formulation against major fungal pathogens

In vitro evaluation of antagonistic activity of PGPR-II and PGPM formulations against major fungal pathogens was done in accordance with poison food technique (Zentmeyer, 1955). 2 g of formulation (PGPR II and PGPM) was added to 100 ml of

sterilized PDA media followed by thorough shaking to get a uniform concentration of the formulation. The mixture was then poured into sterilized plates at a rate of 20 ml per plate. 8 mm mycelial disc of the fungal pathogen was placed at the centre of the plate and the plates containing media without formulation were served as the control. Three replications were kept for the treatment and the observations were taken until the pathogen attained full growth in control plate. The per cent inhibition of the growth of pathogen by bio control agents was computed by the formula given by Vincent (1927).

3.5.3. *In vitro* evaluation of antibiotics, fungicides and biocontrol agents against bacterial pathogen

3.5.3.1. In vitro evaluation of fungicides and antibiotics against bacterial pathogen

The efficacy of fungicides *viz.,* copper hydroxide, Bordeaux mixture and antibiotic Streptocycline against bacterial pathogens under *in vitro* condition was evaluated by agar well diffusion method (Perez *et al*., 1990). Three different concentrations (lower, recommended, and higher dose) of Streptocycline and copper hydroxide were tested along with the recommended dose of Bordeaux mixture (Table 3.9). Fresh formulations of the chosen concentrations of each fungicide and antibiotic were made aseptically in sterile distilled water. A loopful of the bacteria was taken from a 48 h old culture, and mixed with 50 ml of nutrient broth to yield sufficient bacterial population for making pathogen lawn, followed by incubation at 27 ± 2°C for three days. The dense bacterial inoculum was spread onto the Petri dish containing solidified NA medium using sterile L rod. After that 5 mm wells were made on the NA plates using sterilized cork borer and inoculated plates were placed in inverted position for 20 min under sterile condition. Then two to three drops of prepared concentrations of fungicidal and antibiotic solution were added to the wells. Drops of sterile water

Table 3.9. Chemicals used for *in vitro* evaluation along with their doses

Treatment No.	Treatment	Concentration (%)
T_1	Copper hydroxide	0.1, 0.2, 0.3
T_2	Streptocycline	0.01, 0.02, 0.025
T_3	Bordeaux mixture	1.0
T_4	Control	

were added to the wells made on inoculated plates and kept as control. Each treatment was maintained in three replications, along with the control. The zone of inhibition for each treatment was observed after incubation.

3.5.4 *In vitro* evaluation of biocontrol agents against bacterial pathogen

The antagonistic activity of biocontrol agents such as *T. asperellum* (KAU reference culture), *P. fluorescens* (KAU reference culture),) PGPR-II and PGPM against pathogenic bacteria was recorded by inhibition zone method (Bharti *et al*., 2020).

3.5.4.1 In vitro evaluation of T. asperellum and P. fluorescens against bacterial pathogen

In vitro study of antagonistic activity of *T. asperellum* (KAU reference culture) against bacterial pathogen was carried out based on of inhibition zone technique (Bharti *et al*., 2020). A loopful of three-day-old bacterial culture was mixed with 20 ml of nutrient broth followed by incubation in a shaker at 37°C and 129 rpm for 72 h. Then 0.1 ml of dense bacterial inoculum was pipetted out and spread uniformly onto the Petri dish containing solidified nutrient agar medium using sterile L rod. A mycelial disc of 8 mm size was cut out from an actively growing culture of *T. asperellum*, placed on the centre of the plates, and kept for incubation at 37ºC. The antagonistic activity of *P. fluorescens* was tested by taking a loopful of culture of antagonist and inoculating at the centre of the plate containing pathogen seeded media. Four replications of each treatment were maintained and the plates were kept for incubation at 37°C for 72 h. The plates were observed for the presence of inhibition zone between pathogenic bacteria and the antagonist and the measurements were recorded.

3.5.4.2 In vitro evaluation of PGPR-II and PGPM against bacterial pathogen

The antagonistic activity of PGPR-II and PGPM formulations against bacteria was tested using KAU filter paper disc method (Sain, 2010) and agar well diffusion method (Perez *et al*., 1990). 2 g of formulation was added to 100 ml of sterile distilled water taken in 250 ml conical flask followed by thorough mixing. After that, Whatman No.1 filter paper discs with a size of 5 mm diameter were immersed in PGPR-II solution for 5 min. A loopful of the bacteria was taken from a 48 h old culture and mixed with

50 ml of nutrient broth to yield a dense bacterial inoculum followed by incubation at 37°C for 72 h with constant shaking. Then 0.1 ml of dense bacterial inoculum was pipetted out and spread uniformly onto the Petri dish containing solidified NA medium using sterile L rod. Pre-soaked Whatman No.1 filter paper discs were placed at the middle of the plates containing media inoculated with pathogenic bacteria and the filter paper discs immersed in sterile were used in control plates.

The efficacy of PGPM formulation was evaluated by the same procedure used for the study of antagonistic activity against the bacterial isolate. 2 g of the PGPM formulation was mixed with 100 ml of sterile water under aseptic condition. Instead of NA, PDA was used to grow and seed the pathogen followed by incubation. The plates were observed for inhibition zone. Another procedure for determining the efficacy of both PGPR-II and PGPM against bacteria was agar well diffusion method (Perez *et al*., 1990). 0.1 ml of dense bacterial inoculum was pipetted out and spread uniformly onto the Petri dish containing NA agar medium using sterile L rod. Later, 5mm wells were made on the inoculated NA plates using sterilized cork borer and a few drops of prepared concentration of PGPR-II and PGPM were added to the wells. In control plates, drops of sterile water were added to the wells made in pathogen inoculated NA plates inoculated with pathogen. After 72 hours of incubation, observations on the development of bacteria in the treated plates in comparison to the control plates were noted.

3. 6. BIOASSAY STUDIES ON DETACHED LEAVES OF SYMPODIAL ORCHIDS AGAINST MAJOR PATHOGENS

Bioassay was conducted to evaluate the efficacy of fungicides against major pathogens in sympodial orchids using detached leaf technique. The fungicide for *in planta* evaluation was selected based on the results of inhibition of growth of major pathogens under *in vitro* condition. The pathogen was inoculated on the detached leaf by Mycelial Bit Inoculation Method (MBIM) (Rocha *et al*.,1998), followed by spraying of prepared concentration of fungicide. Fresh leaves collected from the field were surface sterilized with 70 per cent ethyl alcohol and pricks were made on the leaves using sterilized needle. Fungal discs taken from seven to eight-day-old cultures were inoculated on the pricked portion. The inoculated area was covered with moist cotton,

followed by spraying of fungicide solution. The leaves inoculated with pathogen but without spraying fungicide served as control. The treated leaves were maintained in both moist chamber and in open condition. Observations were taken daily and disease severity index (DSI) was calculated for each treatment.

DSI (%) = [sum (class frequency × score of rating class)] / [(total number of plants) × (maximal disease index)] × 100 (Chiang *et al.*, 2017).

The reduction in DSI was calculated using the following formula.

$$\text{Reduction in DSI} = \frac{\text{DSI of control - DSI of treatment}}{\text{DSI of control}} \times 100$$

3.7 STATISTICAL ANALYSIS

The data obtained from *in vitro* studies were analysed using web application, General R-shiny based Analysis Platform Empowered by Statistics (GRAPES) developed by Department of Agricultural Statistics, College of Agriculture, Vellayani (Gopinath *et al*., 2020). Comparison of various treatment means was done by critical difference method.

Results

4. RESULTS

The current investigation on 'Cataloguing and documentation of diseases of sympodial orchids in Kerala' was conducted to study the symptomatology of various diseases affecting sympodial orchids in Kerala, characterisation of pathogens associated with diseases, their *in vitro* evaluation and bioassay studies. The studies were conducted in Department of Plant Pathology, College of Agriculture, Vellanikkara during 2021-2022. The results of experimental studies are presented below.

4.1. SURVEY, COLLECTION AND CATALOGUING OF DISEASED SAMPLES

Intensive surveys were carried out during May-November 2022 at different orchid growing tracts of Thrissur, Ernakulam and Trivandrum districts (Plate 1) Surveys were conducted in a total of eight locations of three districts (Table 4.1) with the objective to collect various diseased samples of infected leaves and flowers of sympodial orchids, to find out the incidence and severity of different diseases associated with each type of symptom and extent of light intensity observed in each location under polyhouse condition.

Table 4.1. Details of survey locations

Sl. No.	District	Location	Latitude/Longitude	Month of survey
1	Thrissur	Vellanikkara	10.5483° N/76.2826°E	June 2022 November 2022
2		Vadanappally	10.5028° N/76.0595° E	July 2022
3		Madakkathara	10.5612° N/76.2624° E	June 2022 September 2022
4		Irinjalakuda	10.3469° N/76.2074° E	August 2022
5	Ernakulam	Ambunadu	9.9368° N/76.3180° E	July 2022
6		Vyttila	10.0014° N/76.2828°E	April 2022
7	Thiruvananthapuram	Kottukal	8.3908° N/77.0446° E	May 2022
8		Vellayani	8.4316° N/76.9867° E	May 2022

Table 4.2. Designated codes for natural symptoms observed in *Dendrobium* spp. and *Oncidium* spp.

Sl. No.	District	Location	Symptom
Dendrobium spp.			
1	Thrissur	Madakkathara	MT DLB1
2			MT DLB2
3			MT DLS
4		Vellanikkara	VK DLB
5			VK DLS
6			VK DFS1
7			VK DFS2
8			VK DTB
9		Irinjalakkuda	IK DLB
10			IK DLS
11		Vadanappally	VP DLS
12			VP DBR
13			VP DPB1
14			VP DPB2
15			VP DFS
16			VP DTB
17	Ernakulam	Ambunadu	AM DLB
18			AM DW
19		Vyttila	VL DLB
20			VL DLS
21	Thiruvananthapuram	Vellayani	VY DLB
Oncidium spp.			
1	Thrissur	Vellanikkara	VK OLB1
2			VK OLB2
3			VK OLS1
4			VK OLS2
5			VK OLS3
6		Irinjalakkuda	IK OLS1
7			IK OLS2
8		Vadanappally	VP OLS
9	Ernakulam	Ambunadu	AM OLB1
10			AM OLB2
11			AM OLB3
12		Vytilla	VL OLB1
13			VL OLB2
14	Thiruvananthapuram	Kottukal	KO OLB
15			KO OW
16		Vellayani	VY OLS

Location: MT- Madakkathara, VK-Vellanikkara, IK-Irinjalakkuda, VP-Vadanappally AM-Ambunadu VL-Vyttila, VY-Vellayani KO-Kottukal

Crop: D- *Dendrobium* spp., O-*Oncidium* spp.

Symptom: LB-Leaf blight, LS-Leaf spot, BR-Black rot, SR-Soft rot PB-Petal blight, FS-Flower spot, TB-Twig blight, W-Wilt

Plate 1 :Locations of survey

Vellanikkara

Vadanappally

Madakkathara

Irinjalakkuda

Vyttila

Ambunadu

Vellayani

Kottukal

Disease symptoms observed in each location of survey in each district under polyhouse condition were tabulated in Table 4.2 and Table 4.3. The diseased samples were designated based on the type of symptom along with the name of location and the name of the crop. The major symptoms included leaf blight, leaf spot, wilt, black rot, soft rot, twig blight, petal blight and flower spot which are abbreviated as LB, LS, W, BR, SR, TB, PB and FS respectively. The diseased samples were collected from different sympodial orchids viz., *Dendrobium* spp., *Cattleya* spp., *Oncidium* spp. and *Spathoglottis* spp. as these were the major sympodial orchids being grown in Kerala and these were abbreviated as D, C, O and S respectively while designating the codes for symptoms and isolates.

The different cultural practices adopted in orchid growing tracts were recorded during the survey at different locations in Thrissur, Ernakulam, and Thiruvananthapuram. The potting mixtures used for epiphytic orchids such as *Dendrobium* spp., *Cattleya* spp., and *Oncidium* spp. were coconut husk pieces, brick pieces, sawdust, charcoal, moss etc. whereas ground orchids (*Spathoglottis* spp.) were grown in soil. The containers used for growing orchids were earthen pots, plastic baskets, coconut husk blocks etc. Back bulbs and keikis were used as propagation material for sympodial orchids. Repotting was done when the roots outgrew the container. In most of the orchid nurseries, watering and adequate drainage were ensured, and fertilizers, mostly NPK formulations such as 20:20:20 or 10:10:10, were given at two-week interval. Plant protection chemicals such as mancozeb, carbendazim 12 % + mancozeb 63 % etc. were commonly used for managing leaf spot diseases, and foliar application of Streptocycline was given for managing bacterial diseases. Different insecticides such as imidacloprid, thiomethoxam etc. were used for controlling various sucking pests, and miticides for reducing mite attack. Apart from chemicals, different botanicals, soap solution and *Pseudomonas fluorescens* were also used for managing pests and diseases. The harvesting of flowers was done prior to their maturity and carried out in the evening.

Table 4.3. Designated codes for natural symptoms observed in *Cattleya* spp. and *Spathoglottis* spp.

Sl. No.	District	Location	Symptom
Cattleya spp.			
1	Thrissur	Vadanappally	VP CBR
2			VP CSR
3	Ernakulam	Ambunadu	AM CLB1
4			AM CLB2
5	Thiruvananthapuram	Vellayani	VY CLB
6			VY CLS
Spathoglottis spp.			
1	Thrissur	Madakkathara	MT SLB1
2			MT SLB2
3			MT SLB3
4			MT SLB4
5			MT SLS
6	Thiruvananthapuram	Kottukal	KO SLB

Location: MT-Madakkathara, VP-Vadanappally, AM- Ambunadu, VY-Vellayani, KO-Kottukal

Crop: C- *Cattleya* spp. S- *Spathoglottis* spp.

Symptom: LB-Leaf blight, LS-Leaf spot, BR-Black rot, SR-Soft rot

4.1.1. Assessment of disease incidence and disease severity

During the survey conducted in different orchid growing locations, diseased plant parts showing different kinds of symptoms were categorized separately and per cent disease incidence (PDI) and per cent disease severity (PDS) were calculated for each symptom. The data regarding PDI and PDS for each symptom noticed in different sympodial orchids from different locations of three districts are detailed in Table 4.4, 4.5, 4.6 & 4.7.

4.1.1.1. PDI and PDS of different symptoms obtained from Dendrobium spp.

During the survey conducted in four locations of Thrissur district, six different kinds of symptoms were recorded from *Dendrobium* spp. *viz*. leaf blight (4 no.), leaf spot (4 no.), black rot (1 no.), flower spot (3 no.), petal blight (2 no.), and twig blight (2 no.). Among the leaf blight diseases, highest disease incidence was observed for MT DLB2 obtained from Madakkathara followed by MT DLB1 noticed in same location with PDI of 60 per cent and 50 per cent respectively whereas highest per cent disease severity (36.67 %) was noticed for MT DLB1 followed by MT DLB2 (27.9 %). The lowest disease incidence (32.0 %) and disease severity (10.5 %) were observed for leaf blight collected from Irinjalakkuda (IK DLB). Different types of leaf spot diseases were noticed in Thrissur district, among which highest PDS (12 %) were recorded for IK DLS followed by VP DLS with PDS of 10.58 per cent. MT DLS and VK DLS showed lowest disease severity with PDS of 2.67 per cent and 5.1 per cent respectively. Another type of foliar symptom, VP DBR observed only at Vadanappally showed PDI of 18.18 per cent and PDS of 5.8 per cent. Among the floral symptoms collected from Thrissur, VK DFS1 showed highest disease severity of 19.3 per cent followed by VP DPB2 with 12.14 per cent. A disease (VK DTB) was recorded infecting twigs of *Dendrobium* sp. with 41.8 per cent disease incidence with 14.5 per cent severity. Another twig blight symptom noticed in Vadanappally (VP DTB) showed disease incidence of 32.0 per cent with severity of 10.2 per cent.

Among the symptoms collected from Ernakulam district, leaf blight, leaf spot and wilt symptoms were predominant. Of the symptoms observed, highest disease incidence and severity were recorded for leaf spot disease (VL DLS) obtained from Vyttila region

Table 4.4. PDI and PDS of diseases in *Dendrobium* spp. and their correlation with light intensity

Sl. No.	District	Location	Symptom	PDI (%)	PDS (%)	Light Intensity (Lux)
1	Thrissur	Madakkathara	MT DLB1	50.00	36.67	7683
2			MT DLB2	60.00	27.90	8769
3			MT DLS	10.00	2.67	9213
4		Vellanikkara	VK DLB	41.00	15.40	8892
5			VK DLS	18.10	5.10	11164
6			VK DFS1	34.70	19.30	7980
7			VK DFS2	29.50	8.70	8891
8			VK DTB	41.80	14.50	8895
9		Irinjalakkuda	IK DLB	32.00	10.50	9342
10			IK DLS	23.50	12.00	9342
11		Vadanappally	VP DLS	27.27	10.58	9100
12			VP DBR	18.18	5.80	9102
13			VP DPB1	33.33	10.33	9102
14			VP DPB2	30.43	12.14	9102
15			VP DFS	17.50	5.30	9102
16			VP DTB	32.00	10.20	9102
17	Ernakulum	Ambunadu	AM DLB	42.30	18.90	7548
18			AM DW	28.90	22.10	7548
19		Vyttila	VL DLB	37.50	19.80	9354
20			VL DLS	54.50	23.80	9354
21	TVM*	Vellayani	VY DLB	76.47	34.10	6319
		Correlation coefficient		**-0.603***	**-0.694***	

* - Significant at 5 % level

*TVM- Thiruvananthapuram

with a disease severity of 23.8 per cent. The leaf blight symptom obtained from Vyttila (VL DLB) showed 37.5 per cent disease incidence with 19.8 per cent disease severity. The wilt symptom (AM DW) showed disease incidence of 28.9 per cent and the diseased plant was completely destroyed. A leaf blight symptom (VY DLB) observed from Thiruvananthapuram district found to be more severe in that area with a disease incidence of 76.47 per cent with 34.1 per cent disease severity.

The data of PDI and PDS of diseases in *Dendrobium* spp. from various locations in Kerala revealed that leaf blight (VY DLB) noticed in Thiruvananthapuram was the most serious disease among all the symptoms observed, with highest disease incidence (76.47 %) followed by leaf blight observed from Madakkathara (MT DLB2) and leaf spot disease obtained from Vyttila (VL DLS), with PDI of 60 and 54.5 per cent respectively. Whereas, disease severity was highest for the leaf blight disease obtained from Madakkathara (MT DLB1) with 36.67 per cent followed by VY DLB and MT DLB2 with PDI of 34.1 per cent and 27.9 per cent respectively (Table 4.4).

4.1.1.2. PDI and PDS of different symptoms obtained from Oncidium spp.

During surveys conducted in *Oncidium* spp. growing tracts of Thrissur, symptoms like leaf spot and leaf blight were found to be more common and severe. Two different leaf blight symptoms, VK OLB1 and VK OLB2, were observed from Vellanikkara showing PDI of 10 and 18.1 per cent respectively. Six different types of leaf spot diseases were obtained from different orchid nurseries of Oncidium in Thrissur district, among which most severe symptom was shown by VK OLS1 with PDI 76.92 per cent and PDS 46.9 per cent. The leaf spot symptom (IK OLS1) obtained from Irinjalakkuda showed per cent disease severity of 13.6 per cent with PDI of 28.5 per cent. Another type of leaf spot symptom (VK OLS2) obtained from Vellanikkara was found to be moderately severe with 27.5 per cent disease incidence with PDS of 12.4 per cent. The other leaf spot symptoms, VK OLS3 and IK OLS2, were found to be less severe with PDS of 3.2 and 4.1 per cent respectively.

Table 4.5. PDI and PDS of diseases in *Oncidium* spp. and their correlation with light intensity

Sl. No.	District	Location	Symptom	PDI (%)	PDS (%)	Light Intensity (Lux)
1	Thrissur	Vellanikkara	VK OLB1	10.00	5.10	11,164
2			VK OLB2	18.10	4.40	11028
3			VK OLS1	76.92	46.90	7564
4			VK OLS2	27.50	12.40	7564
5			VK OLS3	7.60	3.20	8967
6		Irinjalakkuda	IK OLS1	28.50	13.60	9342
7			IK OLS2	18.60	4.10	9342
8		Vadanappally	VP OLS	26.60	10.50	9102
9	Ernakulum	Ambunadu	AM OLB1	24.60	12.30	7548
10			AM OLB2	20.10	8.70	7548
11			AM OLB3	52.00	28.50	7548
12		Vyttila	VL OLB1	12.50	7.87	9354
13			VL OLB2	23.00	14.30	9354
14	TVM*	Kottukal	KO OLB	25.00	13.50	7986
15			KO OW	12.50	11.25	7986
16		Vellayani	VY OLS	27.00	12.50	6319
		Correlation coefficient		**-0.439**	**-0.457**	

*TVM- Thiruvananthapuram

All the symptoms obtained from Ernakulam district were leaf blight among which AM OLB3 was most severe with PDS of 28.5 per cent followed by VL OLB2 with PDS of 14.3 per cent. The lowest PDS of 7.87 per cent was shown by the symptom VL OLB1 found in Vyttila region of Ernakulam. Three different kinds of symptoms viz., leaf blight, leaf spot, and wilt were obtained from Thiruvananthapuram district, among which VY OLS showed the highest disease incidence (27 %) whereas the severity of VY OLS, KO OW and KO OLB were comparatively low with 12.5, 11.25 and 13.5 per cent respectively.

Among the symptoms observed from different locations in three districts, leaf spot symptoms were found to be more frequent and severe, and the most severe leaf spot symptom, VK OLS1 was observed in Vellanikkara with the highest PDI (76.92 %) and PDS (46.9 %). Among the leaf blight symptoms, leaf blight obtained from Ambunadu region (AM OLB3) showed high severity with calculated PDI and PDS of 52 per cent and 28.5 per cent respectively (Table 4.5).

4.1.1.3. PDI and PDS of different symptoms obtained from Cattleya spp.

During the survey, two different leaf blight symptoms were obtained from Ambunadu region, which were designated as AM CLB1 and AM CLB2, with a disease severity of 13.4 and 14.52 per cent respectively. The leaf blight symptom (VY CLB) obtained from Vellayani region also showed similar per cent disease severity (14.7 %). Among all the leaf blight symptoms obtained from different districts, the per cent disease incidence was highest for the sample AM CLB2 (31.57 %). The leaf spot symptom (VY CLS) obtained from Vellayani region showed maximum per cent disease incidence (70.8 %) among all the foliar diseases obtained from *Cattleya* spp., whereas the disease severity was highest for the soft rot symptom obtained from Vadanappally region (VP CSR) with a PDS of 66.67 per cent and caused complete death of plants (Table 4.6).

4.1.1.4. PDI and PDS of different symptoms obtained from Spathoglottis spp.

Different types of fungal infections were observed in *Spathoglottis* spp. from different locations in Thrissur and Thiruvananthapuram districts. Four different leaf

Table 4.6. PDI and PDS of diseases in *Cattleya* spp. and their correlation with light intensity

Sl. No.	District	Location	Symptom	PDI (%)	PDS (%)	Light Intensity (Lux)
1	Thrissur	Vadanappally	VP CBR	20.00	18.50	6540
2			VP CSR	66.67	48.50	6540
3	Ernakulum	Ambunadu	AM CLB1	26.30	13.40	7548
4			AM CLB2	31.57	14.52	8255
5	TVM*	Vellayani	VY CLB	27.70	14.70	6319
6			VY CLS	70.80	26.90	6319
		Correlation coefficient		**-0.419**	**-0.378**	

Table 4.7. PDI and PDS of diseases in *Spathoglottis* spp. and their correlation with light intensity

Sl. No.	District	Location	Symptom	PDI (%)	PDS (%)	Light Intensity (Lux)
1	Thrissur	Madakkathara	MT SLB1	48.00	29.79	7683
2			MT SLB2	29.10	21.10	10,501
3			MT SLB3	36.00	23.40	9143
4			MT SLB4	25.00	7.37	10,953
5			MT SLS	29.41	22.40	9987
6	TVM*	Kottukal	KO SLB	38.00	18.62	7986
		Correlation coefficient		**-0.658**	**-0.943****	

* - Significant at 5 % level ** - Correlation is significant at 10 % level

*TVM- Thiruvananthapuram

blight symptoms and a leaf spot symptom were noticed in Madakkathara location of Thrissur district, among which MT SLB1 showed the highest disease incidence and severity with 48 per cent and 29.79 per cent respectively, whereas MT SLB4 was found to be less severe with a PDS of 7.37 per cent. A leaf spot symptom was noticed from the same location with a disease incidence of 29.41 per cent and a severity of 22.4 per cent. The leaf blight symptom (KO SLB) obtained from Kottukal region of Thiruvananthapuram showed 38 per cent disease incidence with 18.62 per cent disease severity (Table 4.7).

4.1.2. Correlation of light intensity with fungal diseases

The per cent disease incidence and severity of various diseases in sympodial orchids were correlated with light intensity at different locations to study the relationship between light intensity and disease severity. PDI and PDS of various symptoms obtained from different locations and the light intensities were recorded from corresponding locations during the survey.

A light intensity range of 6319-11,164 Lux was recorded from different survey locations of *Dendrobium* spp. observed under polyhouse condition (Table 4.4). A negative correlation was observed for all the diseases in different orchids, which revealed that the severity of diseases increased gradually with a decrease in the light intensity of the prevailing location. A correlation coefficient of 0.694 was obtained for the per cent disease severity in *Dendrobium* spp. with light intensity, whereas the correlation coefficient obtained for the per cent disease incidence was -0.603. By analysing the correlation coefficient value, it was found that both PDI and PDS were negatively correlated with light intensity. The highest light intensity recorded was 11,164 Lux from Vellanikkara during November 2022 (VK DLS) where the PDI and PDS values were found to be less, whereas maximum disease incidence and severity were observed in Vellayani during May 2022 (VY DLB), where the light intensity recorded was minimum (6319 Lux).

The correlation coefficients for PDI and PDS of diseases in *Oncidium* spp. were found to be -0.439 and -0.457 respectively, showing a negative correlationship with light intensity. A lower PDI and PDS were observed for leaf blight (VK OLB1) from

Vellanikkara during November 2022, where the recorded light intensity was 11,164 Lux (Table 4.5). Correlation studies were also conducted for diseases of *Cattleya* spp., with a correlation coefficient of -0.419 for PDI and -0.378 for PDS showing negative correlation without much significance (Table 4.6). The data obtained from correlation studies of diseases of *Spathoglottis* spp. with light intensity showed a significant negative correlation with a correlation coefficient of -0.943 and -0.658 for PDI and PDS respectively (Table 4.7). Higher light intensity (10,953 Lux) was recorded from Madakkathara during September 2022, where the PDS and PDI were found to be minimum. Thus, the correlation studies between light intensity and severity of diseases in orchids revealed that the light intensity of a particular location had a significant negative influence on the occurrence and severity of diseases in that location.

4.2. ISOLATION OF PATHOGENS AND PATHOGENICITY STUDIES

4.2.1. Isolation of pathogens associated with diseases

Diseased samples collected from different sympodial orchids from various locations were brought to the laboratory, and the pathogens were isolated as per the procedure mentioned under materials and methods 3.2.1., and the list of isolated pathogens from each symptom are given in Table 4.8, 4.9, 4.10 and 4.11. The purified fungal and bacterial cultures were maintained by periodical subculturing in potato dextrose agar (PDA) and nutrient agar (NA) slants respectively.

4.2.2. Pathogenicity test of different isolates

The pathogens isolated from different sympodial orchids were tested for pathogenicity under *in vitro* conditions by artificial inoculation on detached healthy leaves following Koch's postulates as described in materials and methods 3.2.3. The descriptions of symptomatology under artificial conditions of each pathogen are given in the Table 4.12, 4.13, 4.14 and 4.15. The incubation period, progression of lesion size, and nature of the symptoms were recorded. Most of the fungal pathogens showed an incubation period between two to three days, whereas bacterial pathogen initiated symptom expression within one to two days.

Table 4.8. Isolates corresponding to different symptoms collected from *Dendrobium* spp.

Sl. No.	Location	Symptom	Isolate
1	Madakkathara	MT DLB1	MT DF1
2		MT DLB2	MT DF2
3		MT DLS	MT DF3
4	Vellanikkara	VK DLB	VK DF1
5		VK DLS	VK DF2
6		VK DFS1	VK DF3
7		VK DFS2	VK DF4
8		VK DTB	VK DF5
9	Irinjalakkuda	IK DLB	IK DF1
10		IK DLS	IK DF2
11	Vadanappally	VP DLS	VP DF1
12		VP DBR	VP DF2
13		VP DPB1	VP DF3
14		VP DPB2	VP DF4
15		VP DFS	VP DF5
16		VP DTB	VP DF6
17	Ambunadu	AM DLB	AM DF1
18		AM DW	AM DF2
19	Vyttila	VL DLB	VL DF1
20		VL DLS	VL DF2
21	Vellayani	VY DLB	VY DF

F- Fungus

4.3. SYMPTOMATOLOGY

Symptomatology studies on diseases of sympodial orchids were carried out both under natural and artificial conditions. Symptomatology under natural conditions was studied under field conditions during the survey whereas symptom development under artificial conditions was observed by inoculating pathogens on detached healthy plant parts.

4.3.1. Symptomatology under natural and artificial conditions

During the survey conducted in different orchid growing tracts, distinct symptoms caused by different pathogens on various sympodial orchids were studied in detail and explained in the following section. This includes foliage symptoms, floral diseases, twig blight, and wilt symptoms. The symptomatology was also studied under artificial conditions.

4.3.A. Symptomatology of diseases in *Dendrobium* spp. under natural and artificial conditions

4.3.A.1. Foliar symptoms

The foliage diseases observed in *Dendrobium* spp. during the survey include leaf blight, leaf spot and black rot. Seven leaf blight symptoms and five types of leaf spot symptoms were obtained (Table 4.12).

A. Leaf blight

A.1. MT DLB1

The symptoms of MT DLB1 initially appeared as pale orangish, concentric circular, water soaked lesions on leaves with a prominent yellow halo. Later, the affected leaves completely turned yellow followed by drying and withering of entire plant. Another type of symptom observed was leaf blight, in which blight started from the tip and extended downward with a wavy margin. Later, white cottony mycelial growth appeared over the surface of the leaves with numerous pale brown sclerotia. Infected leaves dried and detached from the plant and the whole plant got eventually died (Plate 2).

Plate 2 : Syptomatology of diseases in *Dendrobium* spp. under natural and artificial conditions

Natural

Artificial

Natural

Artificial

Natural

Artificial

Table 4.9. Isolates corresponding to different symptoms collected from *Oncidium* spp.

Sl. No.	Location	Symptom	Isolate
1	Vellanikkara	VK OLB1	VK OF1
2		VK OLB2	VK OF2
3		VK OLS1	VK OF3
4		VK OLS2	VK OF4
5			VK OF5
6			VK OF6
7		VK OLS3	VK OF7
8	Irinjalakkuda	IK OLS1	IK OF1
9		IK OLS2	IK OF2
10	Vadanappally	VP OLS	VP OF
11	Ambunadu	AM OLB1	AM OF1
12		AM OLB2	AM OF2
13		AM OLB3	AM OF3
14	Vyttila	VL OLB1	VL OF1
15		VL OLB2	VL OF2
16	Kottukal	KO OLB	KO OF1
17		KO OW	KO OF2
18	Vellayani	VY OLS	VY OF

F- Fungus

Under artificial conditions, water soaked, translucent, circular to irregular lesions of 1.2 cm^2 in size appeared on leaves one day after inoculation (DAI). The lesions enlarged in size, later, the entire leaves got rotten, and numerous sclerotia appeared on the rotten surface.

A.2. MT DLB2

The leaf blight symptom obtained from Madakkathara initiated as yellowing, starting from the tip of the leaves, followed by drying of the leaves causing blighted appearance for the leaves. Later, greyish patches were observed on the affected portion.

Upon artificial inoculation, the leaves initially showed water soaked lesions on the inoculated areas 1 DAI followed by complete yellowing. Later, the leaves were rotten, and mycelial growth was observed on the leaves.

A.3. VK DLB

The symptom initially appeared as white, elongated, papery textured lesion on leaves with light brown margin. The lesion expanded longitudinally, causing blight of leaves and the affected areas fell off at later stages.

Under artificial conditions, water soaked lesions were developed 1 DAI, followed by complete yellowing of the leaves within three days. In advanced stages, rotting of leaves with the presence of white mycelial growth was observed (Plate 2).

A.4. IK DLB

The leaf blight symptom (IK DLB) collected from Irinjalakkuda was similar to the symptom VK DLB noticed in Vellanikkara. The initial symptom developed as white, papery spots surrounded by dark brown margin. Later, the spots coalesced over the leaves causing leaf blight symptom. Infected area fell off, giving shot hole appearance (Plate 2a).

When the actively growing fungus was inoculated on the healthy leaves, dull white, papery textured lesions were produced 3 DAI. The lesions became restricted, sunken, and surrounded by a brown margin.

Table 4.10. Isolates corresponding to different symptoms collected from *Cattleya* spp.

Sl. No.	Location	Symptom	Isolate
1	Vadanappally	VP CBR	VP CF
2		VP CSR	VP CB
3	Ambunadu	AM CLB1	AM CF1
4		AM CLB2	AM CF2
5	Vellayani	VY CLB	VY CF1
6		VY CLS	VY CF2

Table 4.11. Isolates corresponding to different symptoms collected from *Spathoglottis* spp.

Sl. No.	Location	Symptom	Isolate
1	Madakkathara	MT SLB1	MT SF1
2			MT SF2
3		MT SLB2	MT SF3
4		MT SLB3	MT SF4
5		MT SLB4	MT SF5
6		MT SLS	MT SF6
7	Kottukal	KO SLB	KO SF1
8			KO SF2
9			KO SF3

F- Fungus

B- Bacteria

A.5. AM DLB

The symptom obtained from Ambunadu region of Ernakulam showed a white extended papery textured, irregular lesion that began at the margin of the leaves and later to the entire leaves. The infected areas became brittle and fell off at later stages (Plate 2a).

Upon artificial inoculation, water soaked lesions developed 1 DAI, and led to complete yellowing of leaves within three days. White mycelial growth was observed on the leaves.

A.6. VL DLB

The symptom (VL DLB) obtained from Vyttila region of Ernakulam initially appeared as a dull black water soaked lesion on the upper surface of the leaves, later turning brownish to black irregular sunken lesion with a diffused yellow halo.

Under artificial conditions, dark water soaked lesions were produced 2 DAI followed by complete yellowing and rotting of leaves. The lesion quickly enlarged in size with the production of abundant mycelial growth over the leaf surface. Salmon coloured spore mass appeared on the lesion 7 DAI (Plate 2a).

A.7. VY DLB

The symptom obtained from Vellayani region of Thiruvananthapuram was characterised by a dirty white papery textured lesion starting from the tip of the leaves, which later enlarged and developed as blight. The blight portion became brittle and fell off at the later stages (Plate 2a).

Under artificial conditions, water soaked lesions were formed 1 DAI, followed by complete yellowing of leaves within three days, and the leaves got rotten with the presence of white mycelial growth over the surface of the leaves.

B. Leaf spot diseases

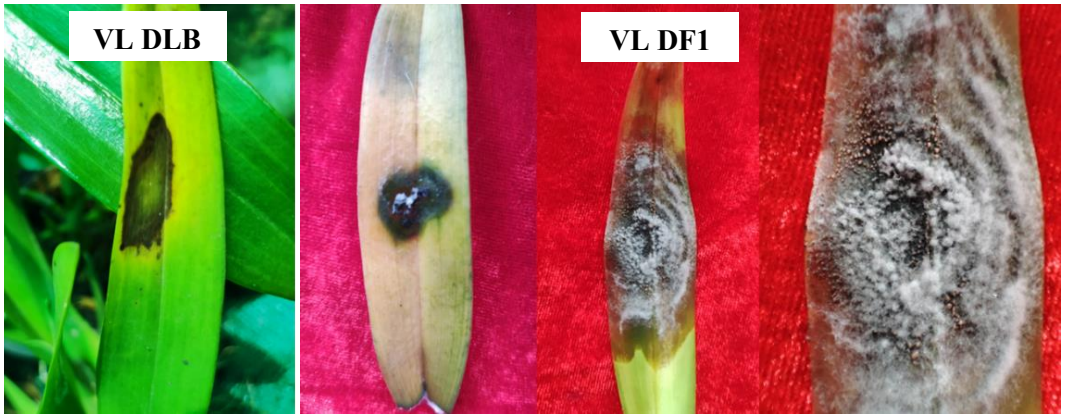
B.1. MT DLS

A leaf spot symptom obtained from Madakkathara initiated as dull brownish spots. Later, the spots enlarged in size and were surrounded by inconspicuous yellow halo.

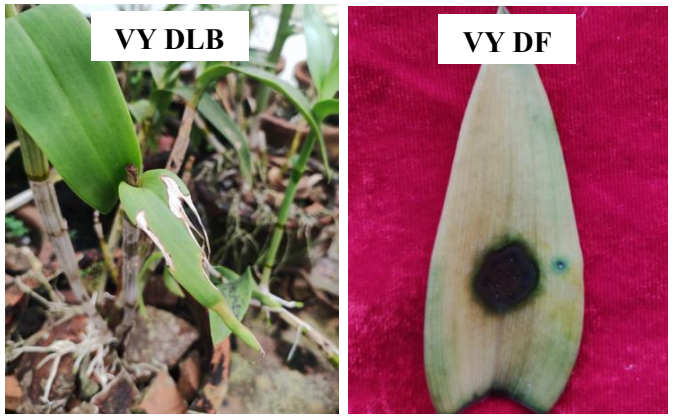
Plate 2a : Symptomatology of diseases in *Dendrobium* spp. under natural and artificial conditions

Natural **Artificial**

Natural **Artificial**



Natural **Artificial**



Natural **Artificial**

Table 4.12. Pathogens isolated from *Dendrobium* spp., their incubation period and symptoms upon artificial inoculation

Sl. No.	Isolate	Pathogen	Incubation period (days)	Symptoms on artificial inoculation	Reisolation (+/-)
1	MT DF1	Fungus	1	Water-soaked translucent circular to irregular lesions, later rotting and appearance of sclerotia	+
2	MT DF2	Fungus	1	Yellowing and rotting	+
3	MT DF3	Fungus	3	Greyish black, circular and sunken spot with prominent yellow halo	+
4	VK DF1	Fungus	1	Yellowing and rotting	+
5	VK DF2	Fungus	1	Dull black coloured circular to oval shaped water-soaked lesion with yellow halo later rotting of leaves	+
6	VK DF3	Fungus	2	Small brown spots with white halo	+
7	VK DF4	Fungus	2	Off white to brownish lesion gradually become papery textured with light brown margin	+
8	VK DF5	Fungus	3	Necrotic irregular spots coalesce and turned into irregular lesions, followed by yellowing and drying of twigs with fructifications of fungi	+
9	IK DF1	Fungus	3	Dull white, papery textured lesion with brown margin	+
10	IK DF2	Fungus	1	Yellowing and rotting	+
11	VP DF1	Fungus	2	Greyish black, circular and sunken lesion with yellow halo	+
12	VP DF2	Fungus	1	Initially black coloured water-soaked lesion later rotting of leaves	+

Upon artificial inoculation, greyish-black, circular and sunken spot were produced 3 DAI, and the average size of the lesions was 1.3 cm^2. The spots were surrounded by prominent yellow halo (Plate 2b).

B.2. VK DLS

The symptom initially appeared as black necrotic circular to oval spots along the midvein of the leaves. Later, the spots developed into a sunken lesion.

Upon artificial inoculation, a black circular to oval spot appeared 1 DAI. Gradually, the spot enlarged in size and covered the whole leaves caused rotting of the leaves 6 DAI (Plate 2b).

B.3. IK DLS

The symptom of leaf spot obtained from Irinjalakkuda initially appeared as dull greyish, circular, water soaked spots on leaves, which later enlarged in size and resulted in complete rotting of the leaves. Another symptom observed for the same pathogen was rotting with the presence of white mycelial growth in the affected region (Plate 2b).

Under artificial inoculation, the symptom initiated as a water soaked translucent lesion developed 2 DAI. The lesion quickly covered the whole leaves resulting in extensive yellowing and rotting within five days. The lesion was covered with a white mycelial mass.

B.4. VP DLS

The initial symptom of VP DLS was appeared as small dull black coloured circular to oval shaped water soaked lesions. Later, the lesion expanded in size and was surrounded by yellow halo. Infected area was covered with dull white mycelial growth on the underside of the lesion, and eventually affected leaves got decayed (Plate 2b).

Upon artificial inoculation greyish black circular lesion was observed 2 DAI. Later, the lesion turned slightly sunken surrounded by a yellow halo.

Plate 2b : Symptomatology of diseases in *Dendrobium* spp. under natural and artificial conditions

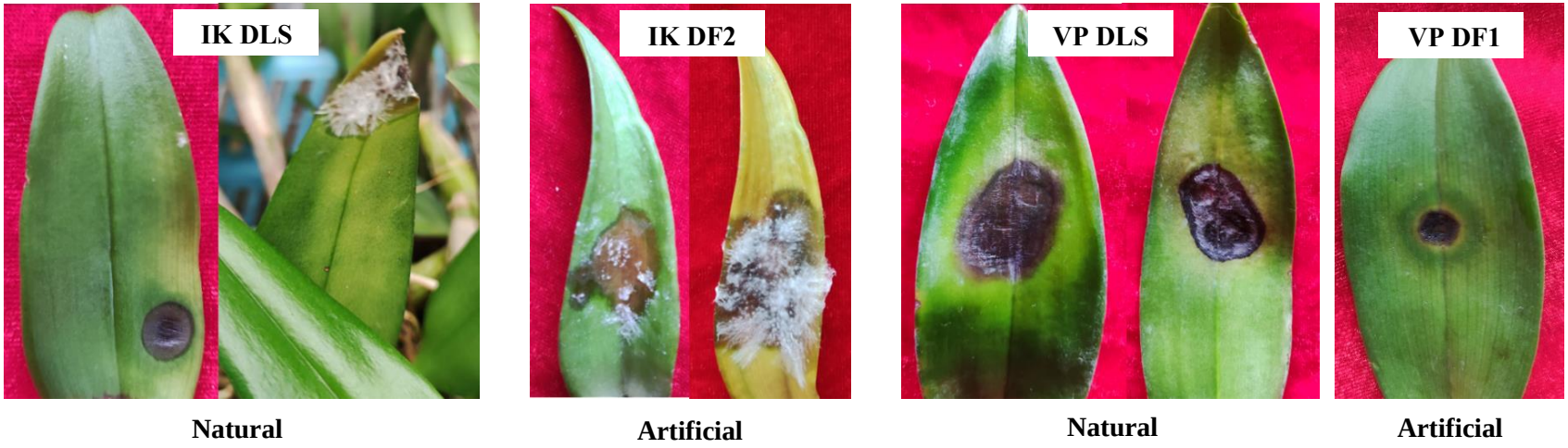


Table 4.12. Pathogens isolated from *Dendrobium* spp., their incubation period and symptoms upon artificial inoculation (Contd…)

Sl. No.	Isolate	Pathogen	Incubation period (days)	Symptoms on artificial inoculation	Reisolation (+/-)
13	VP DF3	Fungus	2	Brown, irregular, water-soaked lesions turn to papery in texture with straw coloured centre and brown margin	+
14	VP DF4	Fungus	3	Dull black lesion followed by drying of petals	+
15	VP DF5	Fungus	1	Brownish to black coloured, circular and sunken lesion	+
16	VP DF6	Fungus	3	Necrotic patches coalesce to form blight with yellow halo, later drying of twigs	+
17	AM DF1	Fungus	1	Yellowing and rotting	+
18	AM DF2	Fungus	1	Yellowing and rotting	+
19	VL DF1	Fungus	2	Yellowing and rotting of leaves with the presence of salmon coloured spore mass	+
20	VL DF2	Fungus	2	Dark brown, water-soaked lesion with yellow halo, later rotting of leaves with salmon coloured spore mass	+
21	VY DF	Fungus	1	Yellowing and rotting	+

B.5. VL DLS

The leaf spot symptom obtained from Vyttila initiated as minute, dull black coloured necrotic spots over the leaves with a faint yellow halo. Later, the spots became enlarged and sunken.

Under artificial conditions, dark brown, water soaked lesion with chlorotic halo appeared over the leaf surface 2 DAI. The lesion enlarged quickly and caused the rotting of leaves 6 DAI. Mycelial growth and salmon coloured spore mass appeared on the lesion at later stages of infection (Plate 2c).

C. Black rot

C.1. VP DBR

The symptom initially appeared as a black, water soaked lesion at the margin of the leaves. Later, the lesion extended from the margin to the centre, covering the whole leaf area. The lesion was surrounded by a prominent yellow halo. The affected portion became decayed and covered with thin mycelial strands of the fungus, causing the death of the plant (Plate 2c).

Upon artificial inoculation, the pathogen produced a black coloured lesion 1 DAI, which enlarged rapidly and caused complete rotting of leaves within four days.

4.3.A.2. Wilt

A.1. AM DW

Infected plants showed yellowing and drying of leaves from the base of the plants, which gradually spread into successive leaves. Necrotic patches were observed on the dried leaves, and the leaves got detached from the plant at a later stage of infection. Eventually, the entire plant completely wilted and dried (Plate 2c).

Upon artificial inoculation, water soaked lesions were produced 1 DAI, followed by complete yellowing within fourth days. Later, the leaves got rotten, and mycelial growth appeared over the leaves.

Plate 2c : Symptomatology of diseases in *Dendrobium* spp. under natural and artificial conditions

Natural

2 DAI

Artificial

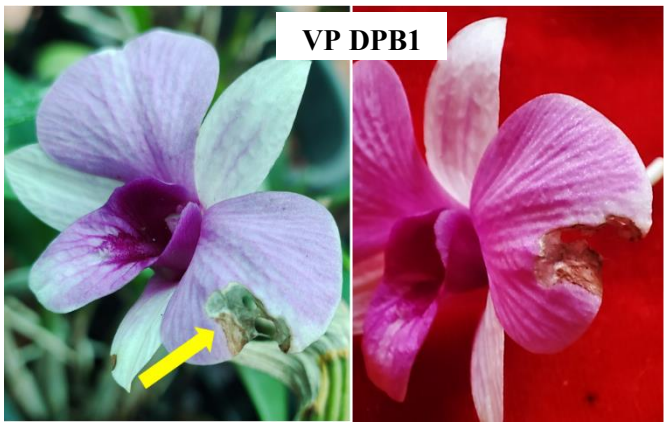
6 DAI

Natural

Artificial

Natural

Artificial



Natural

Artificial

4.3.A.3. Floral diseases

A. Petal blight

A.1. VP DPB1

Small, semi-circular spots appeared on the margins of the petals with a greyish to light brown centre and a dark brown margin. These lesions coalesced to form petal blight. The affected portions fell off, leaving shot holes on the petals (Plate 2c).

When the pathogen inoculated on the petals, brownish irregular water soaked lesions of size 0.8 cm^2 were produced 2 DAI, which later turned papery in texture with a straw-coloured centre and brown margin.

A.2. VP DPB2

The symptom initially appeared as black necrotic lesions at the tip of the petals and gradually progressed inward, causing blight and deformation of the petals.

Under artificial inoculation, dull black lesions appeared 3 DAI, followed by drying of the petals covered with dull white mycelia (Plate 2d).

B. Flower spot

B.1. VK DFS1

The symptom initially appeared as small brownish semi-circular spots on the margin of the petal, which later enlarged in size with a dull white centre and prominent dark brown margin. The affected portion fell off, leaving shot holes (Plate 2d).

Upon artificial conditions, small brown spots were produced on the petals 2 DAI. The spots were surrounded by white halo.

B.2.VK DFS2

The symptom noticed in flowers of *Dendrobium* spp. was characterised by minute brown spots at the lower surface of petals.

Under artificial conditions, off white to brownish coloured lesion appeared on the inoculated area 2 DAI, which gradually became papery textured with light brown margin (Plate 2d).

B.3. VP DFS

The flower spot (VP DFS) initially appeared as small, dark brown, oval to irregular spots on the petal with a thick brown margin. The spots enlarged in size and were surrounded by a prominent light green halo at later stages (Plate 2d).

Under artificial inoculation, a brownish black coloured circular lesion was produced 1 DAI. Later, the lesion enlarged in size and became sunken.

4.3.A.4. Twig blight

A.1. VK DTB

The symptoms of twig blight initially appeared as oval necrotic spots on the twigs. Later, they turned into slightly sunken lesions. In severe cases, these lesions coalesced together and resulted in blight of twig (Plate 2d).

Under artificial conditions, the pathogen produced necrotic, irregular spots on the twig surface 3 DAI. Later, the lesion became elongated, caused yellowing and drying of twigs with the appearance of salmon coloured spore mass over the twig surface 7 DAI.

A.2. VP DTB

The twig blight (VP DTB) noticed in the Vadanappally region showed necrotic lesions at the tip of the younger twigs. The lesion coalesced and extended downward, causing dieback and death of the twigs.

Under artificial condition, black coloured necrotic patches were produced 3 DAI, which coalesced to produce twig blight. Later, the lesion became sunken and the infected twig turned yellow and dried (Plate 2d).

Plate 2d : Symptomatolgy of diseases in *Dendrobium* spp. under natural and artificial conditions

Natural

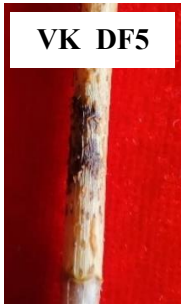
Artificial

Natural Artificial

Natural

Artificial

Natural



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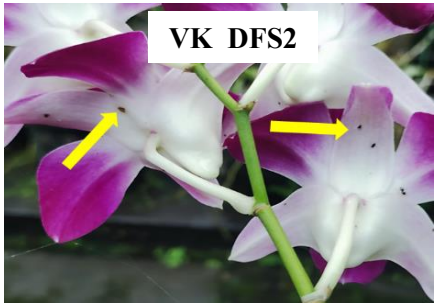


Natural

Artificial

Natural

Artificial

4.3.B. Symptomatology of diseases in *Oncidium* spp. under natural and artificial conditions

4.3.B.1. Foliar symptoms

Leaf blight and leaf spot were the major foliage diseases observed in *Oncidium* spp. Seven types of leaf blight symptoms and five types of leaf spot symptoms were obtained during the survey (Table 4.13).

A. Leaf blight diseases

A.1. VK OLB1

The symptom initiated as blight from the tip of leaves and gradually extended downward. The blight was greyish to light brown in colour and it was surrounded by a yellow halo. Black coloured pycnidia were arranged in a wavy fashion over the infected area (Plate 3).

Upon artificial inoculation, the pathogen produced a dark brown, irregular lesion 1 DAI. The lesion gradually enlarged in size and was surrounded by a faint yellow halo.

A.2.VK OLB2

The leaf blight symptom (VK OLB2) obtained from Vellanikkara initiated as greyish blight that started from the tip and progressed downward, causing leaf blight. The lesion was surrounded by a brown margin with a slight yellow halo (Plate 3).

Under artificial conditions, the symptom initially appeared as greyish spots at the point of inoculation, which expanded to become large lesions surrounded by brownish margin.

A.3. AM OLB1

Leaf blight symptom obtained from Ambunadu region of Ernakulam produced greyish to straw-coloured lesions starting from the leaf tip and extending downward was surrounded by a dark brown margin (Plate 3).

Table 4.13. Pathogens isolated from *Oncidium* spp., their incubation period and symptoms upon artificial inoculation

Sl. No.	Isolate	Pathogen	Incubation period (days)	Symptoms on artificial inoculation	Reisolation (+/-)
1	VK OF1	Fungus	1	Dark brown, irregular lesion with faint yellow halo	+
2	VK OF2	Fungus	2	Greyish lesion surrounded by brown margin	+
3	VK OF3	Fungus	1	Black irregular and sunken necrotic lesion	+
4	VK OF4	Fungus	1	Light brown lesion, later results in blight	+
5	VK OF5	Fungus	3	Dark brown, irregular lesion expands lengthwise and results in blight	+
6	VK OF6	Fungus	2	Greyish white lesion with black margin	+
7	VK OF7	Fungus	2	Greyish white, oval to irregular spot with distinct dark brown margin	+
8	IK DF1	Fungus	2	Dark brown, circular to irregular water-soaked lesion, later results in leaf blight	+
9	IK OF2	Fungus	2	Black necrotic lesions coalesce to cause blight	+
10	VP OF	Fungus	2	Dark brown to black circular spots with salmon coloured spore mass	+
11	AM OF1	Fungus	2	Greyish white spots surrounded by conspicuous, irregular, dark brown to black margin	+

Plate 3 : Symptomatology of diseases in *Oncidium* spp. under natural and artificial conditions

Natural

Artificial

Natural

Artificial

Natural

Artificial

Natural

Artificial

Under artificial condition, the pathogen produced greyish spots 3 DAI. The average size of the spots was 1.1 cm^2. Gradually, the spot became greyish-white surrounded by a conspicuous, irregular, dark brown to black margin.

A.4. AM OLB2

The symptom was characterised by dark brownish blight alternating with light brown and dark brown concentric zonations. The lesion enlarged in size. The lesion was surrounded by a yellow halo and white mycelial growth appeared on the lesion.

Under artificial inoculation, the symptom appeared 3 DAI. It was characterised by the development of a dark brownish to black lesion that expanded in size and measured 4.1 cm^2 (Plate 3).

A.5. AM OLB3

Another leaf blight symptom observed in Ambunadu region initially produced greyish to straw-coloured blight from the tip which gradually enlarged and extended inward causing leaf blight symptom. The lesion was surrounded by a dark brown margin with a pale yellow halo (Plate 3a).

Under artificial conditions, the pathogen produced a brownish to grey coloured water soaked lesion 2 DAI with an average size of 3.2 cm^2, which later extended over the leaves, causing blight and complete drying of leaves.

A.6. VL OLB1

The symptom noticed in *Oncidium* spp. from Vyttila region initiated as dull, black necrotic lesion on the leaf lamina. Gradually, the lesion enlarged in size and was surrounded by a prominent yellow halo caused leaf blight (Plate 3a).

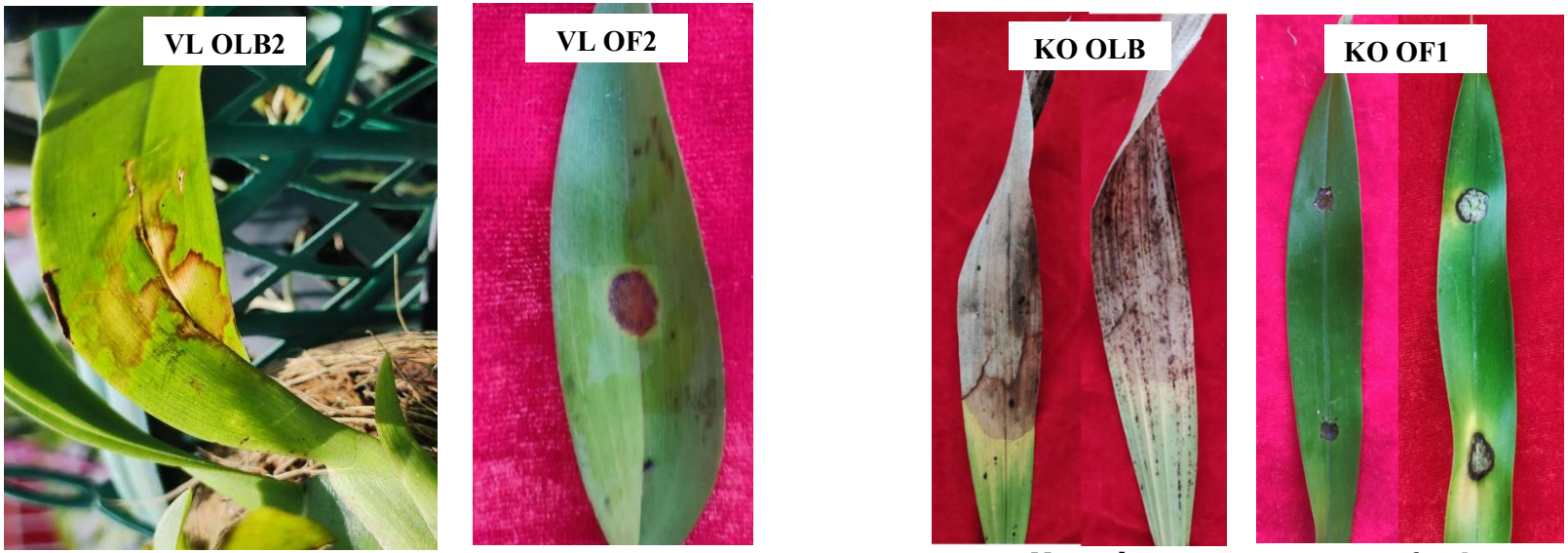
Upon artificial inoculation, the symptom appeared as a circular to irregular, dark brown, water soaked lesion with dark brown margin. The lesion was surrounded by pale yellow halo.

Table 4.13. Pathogens isolated from *Oncidium* spp., their incubation period and symptoms upon artificial inoculation (Contd…)

Sl. No.	Isolate	Pathogen	Incubation period (days)	Symptoms on artificial inoculation	Reisolation (+/-)
12	AM OF2	Fungus	3	Dark brownish to black lesion	+
13	AM OF3	Fungus	2	Brownish to grey coloured water-soaked lesion, followed by complete drying of leaves	+
14	VL OF1	Fungus	1	Circular to irregular dark brownish water-soaked lesion with dark brown margin surrounded by pale yellow halo	+
15	VL OF2	Fungus	1	Circular to irregular water-soaked transparent lesion with yellow halo	+
16	KO OF1	Fungus	2	Circular to irregular black sunken spots with distinct greyish to white centre surrounded by dark brown to black margin with yellow halo	+
17	KO OF2	Fungus	1	Yellowing and rotting	+
18	VY OF	Fungus	3	Light brownish irregular water-soaked lesion with pale chlorotic halo	+

Plate 3a : Symptomatology of diseases in *Oncidium* spp. under natural and artificial conditions

Natural Artificial Natural Artificial



Natural Artificial Natural Artificial

A.7. VL OLB2

A peculiar type of leaf blight symptom collected from Vyttila (VL OLB2) appeared as an irregular, dirty-white, water soaked lesion. The lesion was surrounded by a brown margin which extended over the leaf lamina causing leaf blight (Plate 3a).

Under artificial conditions, circular to irregular water soaked transparent lesions were developed on the inoculated area 1 DAI, with a size of 1.4 cm^2. The lesions were surrounded by yellow halo.

A.8. KO OLB

The symptom which started as greyish to light brown lesion, started at the tip of the leaves and progressed downward, causing leaf blight and drying of foliage. Black patches were observed on the diseased portions. The affected region was surrounded by a yellow halo. Numerous black coloured acervuli were produced over lesion (Plate 3a).

When pathogen was inoculated on healthy leaves, circular to irregular black sunken spots were produced 2 DAI. Gradually, the lesion enlarged in size and developed a distinct greyish to white centre surrounded by dark brown to black margin with yellow halo.

B. Leaf spot diseases

B.1. VK OLS1

The leaf spot symptom noticed in *Oncidium* spp. produced small, black necrotic spots that were scattered throughout the leaf lamina (Plate 3b).

Under artificial conditions, black irregular necrotic lesion was observed on the inoculated area 1 DAI, and the lesion became slightly sunken.

B.2. VK OLS2

The leaf spot symptom collected from Vellanikkara showed brownish to black necrotic specks appeared as patches on the leaves and scattered over the leaf surface.

Three fungal isolates were obtained from the symptom mentioned above, and these pathogens produced three different kinds of symptoms upon artificial inoculation.

In the first type (VK OF4), light brownish lesions appeared 1 DAI, which gradually expanded and covered almost two-thirds of the leaf causing leaf blight. In the second type, (VK OF5), dark brownish irregular lesions were produced 3 DAI, which expanded lengthwise caused blight symptom. The third pathogen (VK OF6), upon artificial inoculation produced irregular greyish lesion 3 DAI, which later became greyish white with black margin (Plate 3b).

B.3. VK OLS3

The symptom obtained from Vellanikkara showed leaf spot disease, which initially appeared as small brownish spots and later, coalesced to form dark brownish sunken lesions surrounded by yellow halo (Plate 3b).

Under artificial inoculation, the symptom appeared as greyish-white oval to irregular spots with distinct dark brown margins.

B.4. IK OLS1

The leaf spot symptom obtained from Irinjalakkuda initially appeared as black, irregular necrotic spots on the lower surface of the leaves. Gradually, the spots coalesced and turned into irregular lesions that were slightly sunken at the lower surface of the leaves (Plate 3b).

Upon artificial inoculation, dark brown, circular to irregular water soaked lesions were produced 2 DAI. Later, the lesions turned into blight and extended over the leaves causing leaf blight.

B.5. IK OLS2

Initially, the symptoms of IK OLS2 developed as minute, irregular, rusty sunken spots on the upper surface of the leaves. Later, the spots turned purplish black with pale yellow halo. The spots coalesced to form larger lesions (Plate 3c).

Under artificial conditions, the pathogen produced black necrotic lesions 2 DAI, and later the lesions coalesced to develop leaf blight.

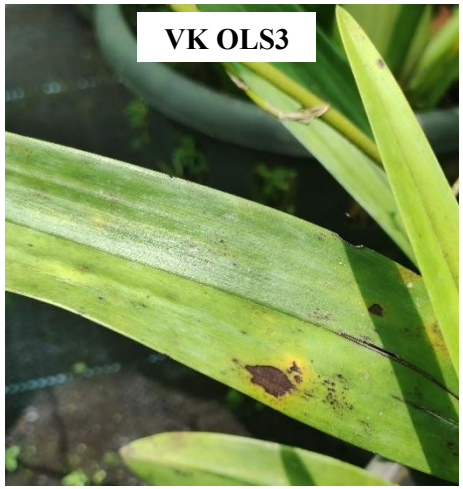
Plate 3b : Symptomatology of diseases in *Oncidium* spp. under natural and artificial conditions

Natural

Artificial

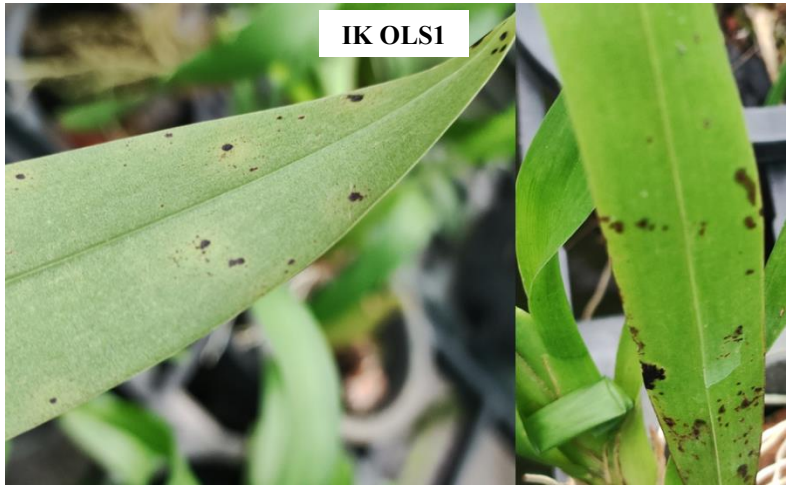
Natural

Artificial



Natural

Artificial



Natural

Artificial

B.6. VP OLS

The symptom initially appeared as a brownish spot, which later turned into circular or irregular spots with straw coloured centre and dark brown margin.

Under artificial conditions, the pathogen produced dark brown to black circular spots 3 DAI. Later, the spots enlarged in size with the presence of salmon-coloured spore mass at the centre of the lesion (Plate 3c).

B.7. VY OLS

The leaf spot symptom (VY OLS) obtained from Vellayani produced small, minute, dark brownish, irregular spots on leaves with prominent chlorotic halo (Plate 3c). When the pathogen was inoculated artificially on healthy leaves, produced light brownish irregular water soaked lesions 3 DAI surrounded by a pale chlorotic halo.

4.3.B.2. Wilt

A.1. KO OW

The leaf rot symptom noticed in Kottukal region initiated as a brownish black water soaked lesion on roots and pseudo stems. As a result of the root infection, the leaves of the affected plant turned yellow. Gradually, the lesion extended into upper plant parts and caused rotting and toppling of the entire plant (Plate 3c).

Under artificial conditions, the pathogen initially produced a transparent, water soaked brown lesion 1 DAI, with lesion size of 2.8 cm^2. Gradually, the lesion became enlarged and surrounded by prominent chlorotic halo. The lesion extended and covered the leaves, causing complete rotting.

4.3.C. Symptomatology of diseases in *Cattleya* spp. under natural and artificial conditions

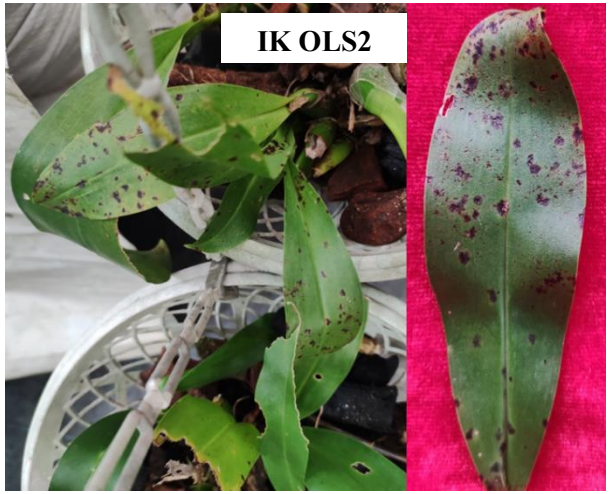
4.3. C.1. Foliar symptoms

Leaf blight, leaf spot, black rot and soft rot were the major foliage diseases observed in *Cattleya* spp. (Table 4.14).

Table 4.14. Pathogens isolated from *Cattleya* spp., their incubation period and symptoms upon artificial inoculation

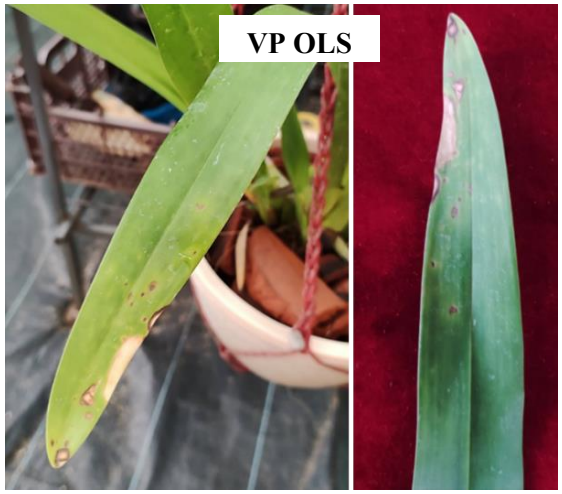
Sl. No.	Isolate	Pathogen	Incubation period (days)	Symptoms on artificial inoculation	Reisolation (+/-)
1	VP CF	Fungus	1	Initially black lesion, later results in rotting of whole leaf	+
2	VP CB	Bacteria	1	Water-soaked lesion covers the whole leaf surface resulting in soft rot	+
3	AM CF1	Fungus	1	Initially black lesion later the leaves completely turned into yellow	+
4	AM CF2	Fungus	2	Dark brown, irregular lesion with greyish white centre and dark brown to black margin	+
5	VY CF1	Fungus	1	Dark brown to black sunken lesion, with greyish centre and dark brown margin	+
6	VY CF2	Fungus	2	Greyish white, irregular sunken leaf spot with thick black margin	+

Plate 3c : Symptomatology of diseases in *Oncidium* spp. under natural and artificial conditions

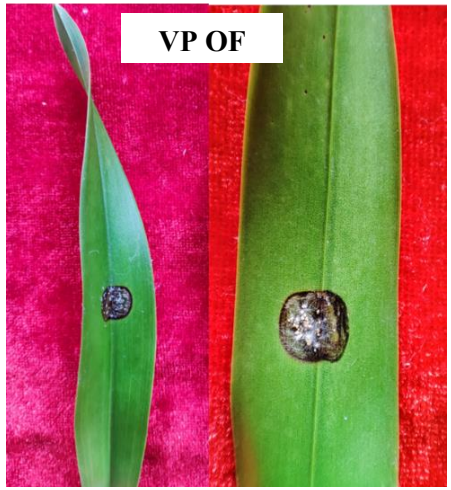


Natural

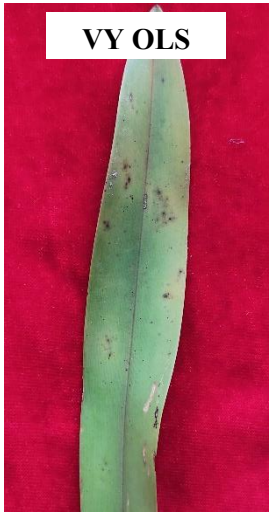
Artificial



Natural



Artificial



Natural

Artificial

Natural

Artificial

A. Leaf blight diseases

A.1. AM CLB1

The leaf blight symptom obtained from *Cattleya* spp. initially produced a light brownish leaf blight at the margins of the leaves, gradually extended through the margins to the leaf lamina. The infected portion became papery and brittle and fell off at later stages (Plate 4).

When the pathogen was inoculated on healthy leaves, dark black lesions were produced at the inoculated area 1 DAI, followed by complete yellowing of the entire leaves 4 DAI.

A.2. AM CLB2

The initial symptom of leaf blight (AM CLB2) was characterised as greyish brown lesions starting from the leaf tip that later extended into the leaf lamina, causing leaf blight followed by drying. Dull black patches appeared on the blight parts at later stages (Plate 4).

Under artificial conditions, dark brown, irregular lesion was observed 3 DAI, which later developed into a greyish white centre with a dark brownish to black margin.

A.3.VY CLB

The symptom initiated as a brownish to black water soaked lesion on the younger leaves, and the affected leaves remained unopened. Black sunken patches appeared on the infected leaves, and the decayed leaves could be easily pulled out (Plate 4).

Under artificial conditions, initially a dark brownish to black lesion was observed 2 DAI. Later, the spot became sunken with a greyish centre and dark brown margin. The spots enlarged into lesions and attained a size of 5.2 cm^2 10 DAI.

B. Leaf spot diseases

B.1. VY CLS

The symptom was characterised by greyish white irregular sunken leaf spot with a thick brown margin. The spots enlarged and coalesced to form blight symptom.

Similar symptom was produced upon artificial inoculation, which was described by the appearance of black necrotic lesion 3 DAI. Later, the lesion turned into a greyish white irregular sunken leaf spot with a thick black margin (Plate 4).

C. Black rot

C.1. VP CBR

The symptom of black rot disease initiated as black lesions from the tip of the leaves or on the margin of the leaves and extended inward to the leaf lamina, causing complete blackening of entire leaves and eventually leading to death of the plant

Under artificial condition, black lesions were produced 1 DAI, similar to the natural symptom. The lesion rapidly covered the entire leaves and caused complete blackening of leaves within four days (Plate 4).

D. Soft rot

D.1. VP CSR

The soft rot symptom observed in *Cattleya* spp. appeared as small, dull black, water soaked lesion at the tip of the leaves, which enlarged rapidly and resulted in soft rot of the leaves. The macerated leaf tissues emitted a foul smell. The affected leaves were seen hanging down from the plants (Plate 4).

Under artificial conditions, the pathogen produced a water soaked lesion 1 DAI, followed by rapid decay and rotting of leaves within four days. The affected leaves produced ooze with a foul smell, which confirmed bacterial etiology. Upon potato soft rot test, the bacterial culture produced characteristic symptom of the disease on the potato slices, hence confirmed it as soft rot pathogen.

4.3.D. Symptomatology of diseases in *Spathoglottis* spp. under natural and artificial conditions

4.3.D.1. Foliar symptoms

Leaf blight was the major symptom observed in *Spathoglottis* spp. Along with five types of leaf blight symptoms, one leaf spot symptom was also collected from various locations (Table 4.15).

Plate 4 : Symptomatology of diseases in *Cattleya* spp. under natural and artificial conditions

A. Leaf blight diseases

A.1. MT SLB1

The symptom of leaf blight (MT SLB1) obtained from Madakkathara was characterised by brown coloured lesions surrounded with yellow halo that extended lengthwise and turned into large lesions with straw coloured centre with dark brown margin (Plate 5).

Two different fungal isolates were obtained from the mentioned symptom, which on artificial inoculation, produced two different types of symptoms. The first isolate, (MT SF1), produced dark brown oval spots with chlorotic halo 3 DAI. Later, the entire leaf lamina turned purple with yellow halo. The second isolate, (MT SF2) also produced the similar symptom of first isolate, (MT SF1). The symptom appeared on the leaves 2 DAI.

A.2. MT SLB2

The symptom initiated as greyish to brown blight starting from the tip of the leaves and extended inward, covering the whole leaf lamina. Concentric zonations were observed on the lesions with pale yellow halo (Plate 5).

Under artificial conditions, dark brownish to black, oval to irregular lesions were appeared 2 DAI. Later, the entire leaves became purple, surrounded by chlorotic halo.

A.3. MT SLB3

Another type of leaf blight symptom obtained from Madakkathara was characterised as dark brownish, water soaked lesion starting from the base of the leaf and extending along the midvein to the tip of the leaf. The lesions were surrounded by prominent yellow halo (Plate 5).

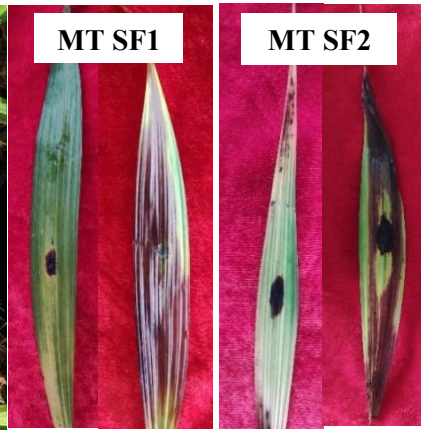
Under artificial conditions, brown lesions were observed on the inoculated leaves surrounded by yellow halo. Later, brown blight developed at the tip of the leaves, with chlorotic halo which gradually extended downward and resulted in leaf blight.

Table 4.15. Pathogens isolated from *Spathoglottis* spp., their incubation period and symptoms upon artificial inoculation

Sl. No.	Isolate	Pathogen	Incubation period (days)	Symptoms on artificial inoculation	Reisolation (+/-)
1	MT SF1	Fungus	3	Dark brown, oval spot with chlorotic halo, later leaves turn into purple	+
2	MT SF2	Fungus	2	Brown, oval spot with chlorotic halo, later the leaves turned purple	+
3	MT SF3	Fungus	2	Dark brown to black, oval to irregular lesion. later leaves turn purple	+
4	MT SF4	Fungus	2	Brown lesion surrounded with yellow halo, later leads to leaf blight symptom	+
5	MT SF5	Fungus	2	Grey coloured irregular spot surrounded by dark brown to black margin	+
6	MT SF6	Fungus	3	Dark brown irregular lesion surrounded by yellow halo	+
7	KO SF1	Fungus	2	Brown water-soaked lesion, later turned into purplish in colour	+
8	KO SF2	Fungus	3	Brownish to black, oval shaped lesion, followed by yellowing of whole leaf	+
9	KO SF3	Fungus	2	Brownish to black irregular shaped lesion surrounded by chlorotic halo, later cause blight and drying of leaves	+

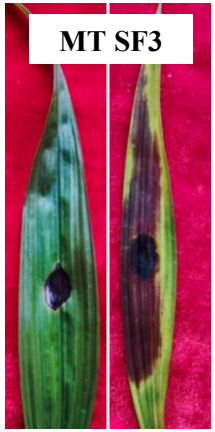
Plate 5 : Symptomatolgy of diseases in *Spathoglottis* spp. under natural and artificial conditions

Natural

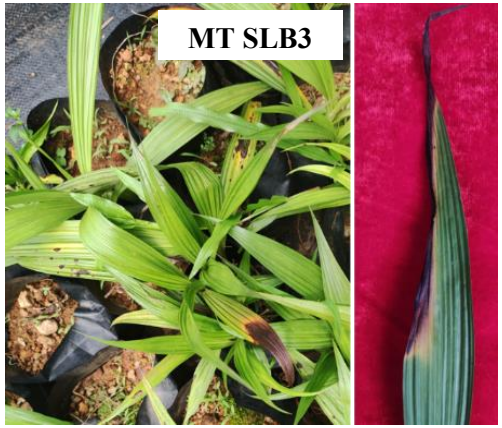


Artificial

Natural



Artificial



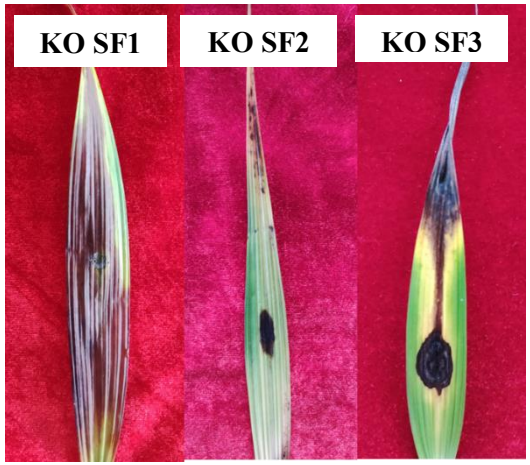
Natural

Artificial

Natural

Artificial

Natural



Artificial

Natural

Artificial

A.4. MT SLB4

Fourth type of leaf blight (MD GLB4) from Madakkathara, initially appeared as brown to straw coloured irregular lesions on leaves with dark brown margin surrounded by yellow halo. The lesions later expanded over the leaves and the blight parts fell off leaving shot holes (Plate 5).

Under artificial conditions, the pathogen produced grey coloured irregular spots surrounded by dark brown to black margins.

A.5. KO SLB

The leaf blight symptom obtained from Vellanikkara showed light brown to dark brown water soaked blight starting from the base and extending to the tip. The blight was surrounded by a prominent yellow halo.

Three fungal isolates were obtained from the symptom upon isolation, and different types symptoms were produced upon artificial inoculation. The first isolate, (KO SF1) produced brown water soaked lesions 2 DAI. Later, entire leaves turned purple in colour. Whereas the second isolate, (KO SF2), produced oval shaped brownish to black lesion 3 DAI, followed by complete yellowing of leaves. The third isolate, (KO SF3), showed brownish to black irregular shaped lesions with chlorotic halo. Later, blight began from the tip, followed by drying of the leaves (Plate 5).

B. Leaf spot diseases

B.1. MT SLS

The leaf spot was initiated as brown circular to oval spots, which later enlarged in size, surrounded by dark brown margin with dark brown concentric zonations.

Under artificial inoculation, the pathogen produced dark brown, irregular lesion 4 DAI, surrounded by a yellow halo (Plate 5).

4.4. CHARACTERISATION OF PATHOGENS ISOLATED FROM SYMPODIAL ORCHIDS

Isolation of the pathogens from different symptoms and their pathogenicity revealed that the isolates causing leaf blight, leaf spots, wilt, floral diseases, twig blight and black rot symptoms were fungal pathogens while the soft rot symptom was due to bacteria. These pathogens were further characterised by cultural, morphological and molecular techniques.

4.4.1. Cultural and morphological characterisation of fungal pathogens

The cultural characters observed were colour, texture, growth rate, growth pattern, sporulation, colour on the reverse side of Petri plates and presence of fruiting bodies. The various microscopic observations like colour of hyphae, branching pattern, septation, presence of conidia, conidial septation, types, shape, colony dimensions and presence of sexual structures were studied.

4.4.1.A. Cultural and morphological characterisation of fungi obtained from *Dendrobium* spp.

Cultural and morphological characters of fungal pathogens isolated from *Dendrobium* spp. are detailed in Table 4.16.

A.1. MT DF1

The colony was cottony white with tufts of longer hyphae, and an even sheet of aerial mycelium made up of clear mycelial strands which attained full growth on a 90 mm Petri dish at five days of incubation (DOI) with an average growth rate of 1.8 cm per day. The under surface of the culture plate was white Sclerotia were developed 15 DOI as pale brown structures, which later changed to dark brown colour (Plate 6). Numerous sclerotia, smooth and spherical, were produced and seen scattered over the culture. Primary hyphae were hyaline and septate and showed clamp connections at the septa. Based on cultural and morphological features, the pathogen was tentatively identified as *Sclerotium* sp.

Table 4.16. Cultural and morphological characters of fungal pathogens in *Dendrobium* spp.

Sl. No.	Isolate	Cultural characters	Reverse side of Petri plate	Days for full growth	Growth rate (cm/day)	Morphological characters				Pathogen identified
						Hyphae	Shape of conidia	Colour of conidia	Dimension of the conidia (400X)	
1	MT DF1	Cottony white, aerial mycelium	White	5	1.80	Hyaline	Sclerotia developed			*Sclerotium* sp.
2	MT DF2	White coloured mycelia later become pinkish white	Pinkish white	8	1.12	Hyaline, septate, and branched	Macroconidia- septate, fusiform shaped, slightly curved with tapering ends	Hyaline	5.2 x 1.43 µm	*Fusarium* sp.
3	MT DF3	Dark grey to black, effuse, velvety, immersed mycelium	Black	7	1.28	Septate, brown and branched	Dark brown, curved at the 3rd cell from the base and asymmetrical with pale brown end cells	Dark brown	19.6 x 8.64 µm	*Curvularia* sp.
4	VK DF1	Dull white coloured aerial, mycelia with pinkish tinge, woolly in texture	Purple	8	1.12	Hyaline, branched and septate	Microconidia- ellipsoid, unicellular Macroconidia- septate, fusiform shaped, slightly curved with tapering ends	Hyaline	Microconidia- 3.2 x 1.1 µm Macroconidia- 8.6 x 2.0 µm	*Fusarium* sp.
5	VK DF2	Dull white, fluffy mycelia with woolly texture	Dull greenish to purple	8	1.12	Hyaline and septate	Aseptate, fusiform shaped microconidia	Hyaline	2.7 x 0.89 µm	*Fusarium* sp.
6	VK DF3	Dull greyish white woolly mycelia, later change into dark greyish to black	Black	8	1.12	Septate, initially hyaline turned into brown	Elongated, cylindrical with tapering end	Hyaline	19.78 x 5.26 µm	*Colletotrichum* sp.

A.2. MT DF2

The pathogen produced white mycelia, which later became pinkish white with filamentous margins. The mycelial growth was very sparse at the centre and cottony at the periphery with flat growth. (Plate 6). The colony attained 90 mm of growth at 8 DOI with a growth rate of 1.12 cm per day. Hyphae were hyaline, septate and branched. The fungus produced numerous macroconidia, which were hyaline, fusiform shaped, slightly curved at the middle and septate (three to five septa) with a size of 5.2 x 1.43 µm. The pathogen was tentatively identified as *Fusarium* sp. based on cultural and morphological features.

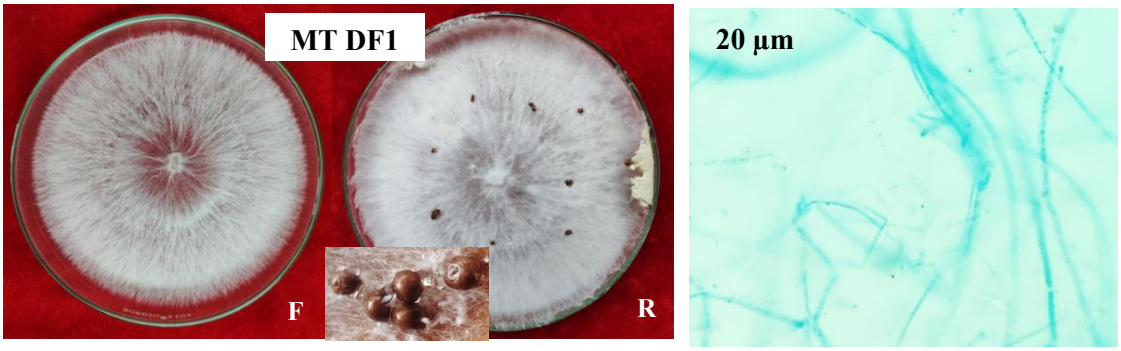
A.3. MT DF3

Colonies were dark grey to black, effuse, velvety, immersed mycelium with concentric zonations, later turned flat, black on maturity attained 90 mm growth at 7 DOI with an average growth rate of 1.28 cm per day. Vegetative hyphae were septate, brown and branched. Conidia were dark brown, curved at the third cell from the base, and asymmetrical with pale brown end cells with a size of 19.6 x 8.64 µm. Based on the above characters, the isolate was tentatively identified as *Curvularia* sp.

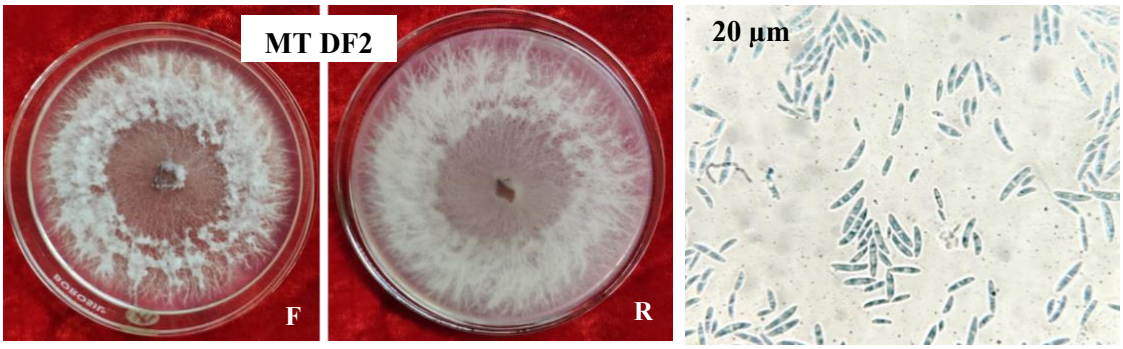
A.4. VK DF1

The isolate (VK DF1) obtained from Vellanikkara produced dull white mycelia with a pinkish tinge and serrated margin (Plate 6). The mycelia were aerial, woolly in texture, moderately raised, dome shaped, with pinkish to purplish coloration on the reverse side, and attained full growth at 8 DOI with an average growth rate of 1.12 cm per day. The pathogen produced hyaline, branched and septate hyphae with the presence of microconidia and macroconidia. Microconidia were hyaline, ellipsoid, unicellular, and scattered freely in the mycelial mat with a size of 3.2 x 1.1 µm. macroconidia were hyaline, septate, fusiform shaped and slightly curved and tapered at the ends. The average size of macroconidia was 8.6 x 2.0 µm, which were scarcely produced in the culture as compared to microconidia. Based on cultural and morphological features, the pathogen was tentatively identified as *Fusarium* sp.

Plate 6 : Cultural and morphological characters of fungal pathogens in *Dendrobium* spp.



***Sclerotium* sp.**



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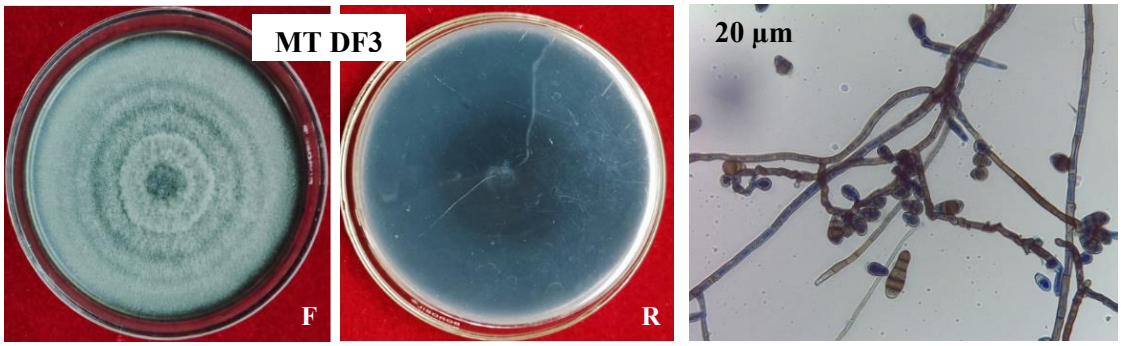
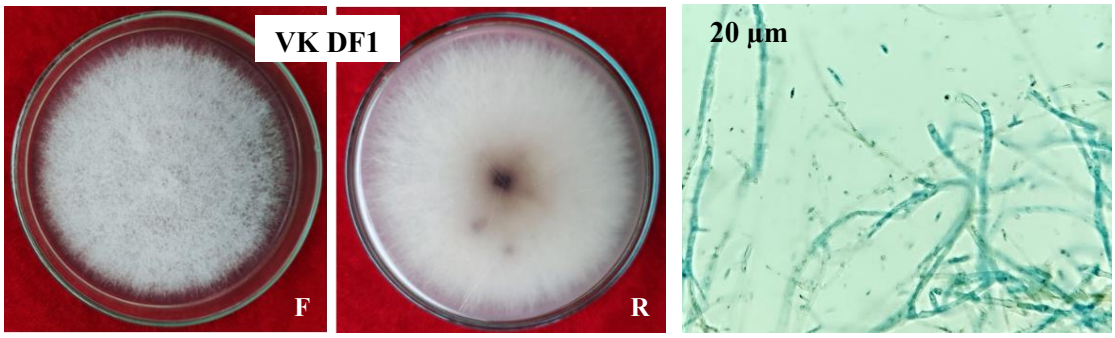


***Curvularia* sp.**



***Fusarium* sp.**

F- Front side R- Rear side

Table 4.16. Cultural and morphological characters of fungal pathogens in *Dendrobium* spp. (Contd…)

Sl. No.	Isolate	Cultural characters	Reverse side of Petri plate	Days for full growth	Growth rate (cm/day)	Morphological characters				Pathogen identified
						Hyphae	Shape of conidia	Colour of conidia	Dimension of the conidia (400X)	
7	VK DF4	Yellowish white cottony mycelia with fluffy growth	Yellowish white with dull yellow tinge at the centre	8	1.50	Hyaline, septate and branched	Hyaline	Microconidia fusiform shaped tapered at both the ends	4.90 x 2. 63 µm	*Fusarium* sp.
8	VK DF5	Woolly dull white mycelia, later become olivaceous grey	Greyish black with dirty white margin	7	1.12	Septate, initially hyaline and turned into brown	Hyaline	Conidia elongated, cylindrical with acute apex	14.12 x 3.58 µm	*Colletotrichum* sp.
9	IK DF1	Dull white, delicate, woolly, aerial mycelia	Light purplish tinge	7	1.28	Hyaline and septate	Microconidia- aseptate, carenate Macroconidia- 1-3 septate, hyaline	Hyaline	Microconidia-. 4.0 x 1.0 µm Macroconidia- 9.1 x 2.0 µm	*Fusarium* sp.
10	IK DF2	Dull white, cottony mycelia	Dull white	5	1.80	Hyaline and septate	Sclerotia developed			*Sclerotium* sp.
11	VP DF1	Dull white aerial cottony mycelia initially, later become greyish white	Deep yellowish pigmentation	11	0.81	Hyphae branched and septate	Obclavate shape with beak	Pale brown to olive brown	50.0 x 18.2 µm	*Alternaria* sp.
12	VP DF2	White cottony aerial mycelia with petaloid to stellate growth pattern	White	6	1.50	Hyaline, aseptate and branched	Sporangia hyaline, ovoid and non-papillate with 44.8 µm x 36.0 µm size			*Phytophthora* sp.

A.5. VK DF2

The pathogen (VK DF2) isolated from the leaf spot symptom produced dull white fluffy mycelia with woolly texture which later turned slightly pinkish with a dull greenish to purple reverse (Plate 6a). The fungus attained full growth at 9 DOI with an average growth rate of 1.12 cm per day. Vegetative hyphae were hyaline, septate, and branched. The colony produced numerous aseptate, fusiform shaped microconidia with a size of 2.7 x 0.89 µm. The pathogen was tentatively identified as *Fusarium* sp.

A.6. VK DF3

The fungus that caused flower spots in *Dendrobium* sp. initially produced dull greyish-white woolly mycelia which later changed into dark greyish to black in colour with a black reverse side (Plate 6a). The colony was characterised by flat, submerged growth with regular margin completed 90 mm growth in Petri dish at 8 DOI with an average growth rate of 1.12 cm per day. Acervuli were produced at 10 DOI. associated with abundant conidial mass. Hyphae were initially hyaline, which later turned brown. Conidia were elongated and cylindrical with tapering ends, and an oil globule was present at the centre of the cell. The size of the conidia was 19.78 x 5.26 µm. Based on the above features, pathogen was tentatively identified as *Colletotrichum* sp.

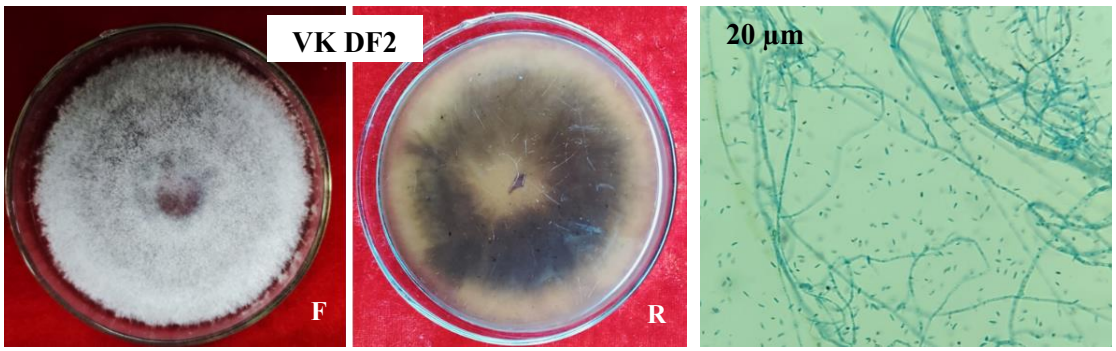
A.7. VK DF4

The fungus isolated from the flower spot produced yellowish white, cottony mycelia with fluffy a growth pattern. The fungus attained full growth at 8 DOI with an average growth rate of 1.5 cm per day. The reverse side of the culture appeared yellowish white with a dull yellow tinge at the centre. The mycelia were hyaline, septate and branched. Numerous fusiform microconidia were observed that were intermingled with mycelia. The size of the microconidia measured was 4.90 x 2. 63 µm. Based on cultural and morphological features, the pathogen was tentatively identified as *Fusarium* sp.

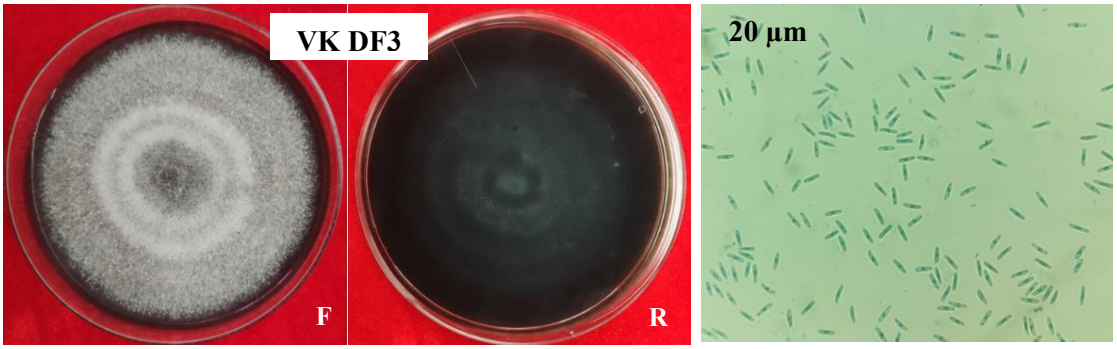
A.8. VK DF5

The pathogen associated with twig blight produced woolly, dull white mycelia initially and later became olivaceous grey with flat and even growth. The reverse side of the colony appeared greyish black with dirty white margin (Plate 6a). The fungus

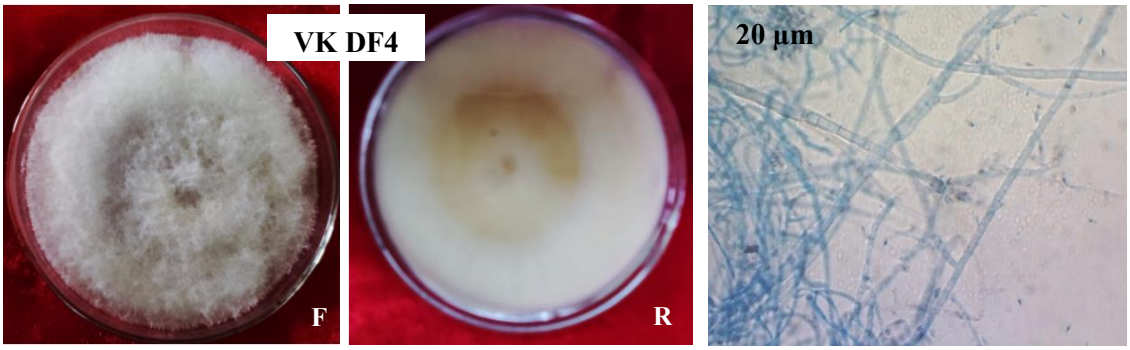
Plate 6a : Cultural and morphological characters of fungal pathogens in *Dendrobium* spp.



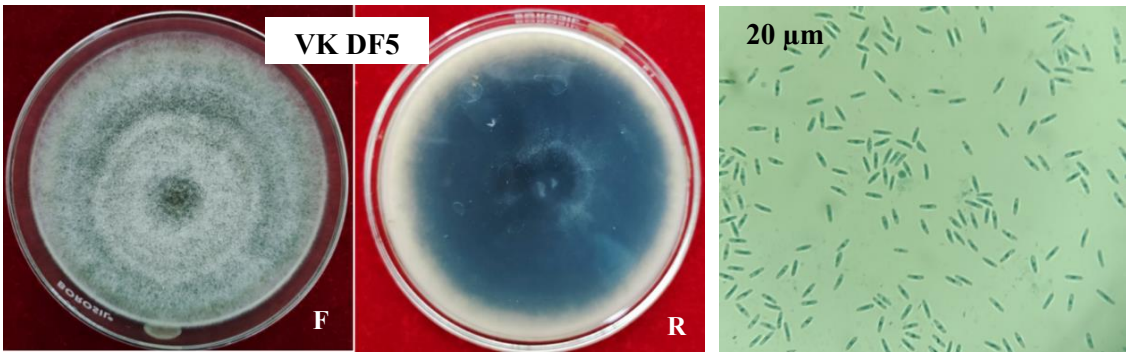
***Fusarium* sp.**



***Colletotrichum* sp.**



***Fusarium* sp.**



***Colletotrichum* sp.**

F- Front side R- Rear side

attained full growth at 7 DAI. Acervuli were minute and black and produced at 11 DOI with an average growth rate of 1.12 cm per day. Hyphae were septate, branched, and initially hyaline, which later turned brown. Conidia were elongated, cylindrical with acute apices and an oil globule was present at the centre of the cell. The average size of the conidia was measured as 14.12 x 3.58 µm. Based on the above features, pathogen was tentatively identified as *Colletotrichum* sp.

A.9. IK DF1

The pathogen causing the leaf blight symptom (IK DF1) produced dull white, delicate and woolly aerial mycelia that were slightly dome shaped at the centre with filamentous and irregular margin. The pathogen completed full growth (90 mm) after 7 DOI with an average growth rate of 1.28 cm per day. A light purplish tinge was observed on the reverse side over time (Plate 6b). The hyphae were hyaline and septate. The culture produced numerous aseptate, carenate microconidia, and one to three septate, hyaline macroconidia with sizes of 4.0 x 1.0 µm and 9.1 x 2.0 µm respectively. The pathogen was tentatively identified as *Fusarium* sp.

A.10. IK DF2

The pathogen causing leaf spot (IK DLS) produced dull white cottony mycelial strands with sparse growth at the centre and pluffy at its edges (Plate 6b). Numerous sclerotia were formed by mycelial aggregation within 10-15 days. The sclerotia were round, smooth, initially dull white, later turning light brown, and seen scattered over the culture. The fungus attained full growth at 5 DOI with an average growth rate of 1.8 cm per day. Based on cultural and morphological features, the pathogen was tentatively identified as *Sclerotium* sp.

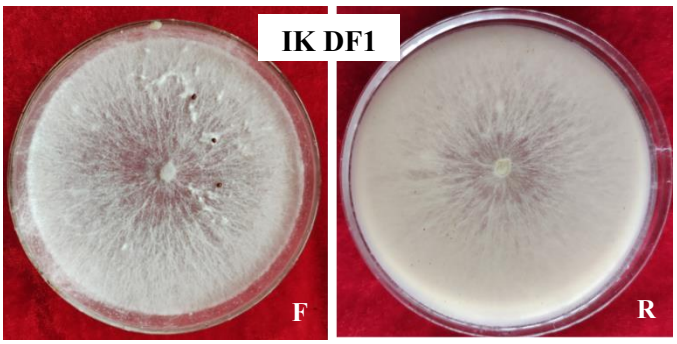
A.11. VP DF1

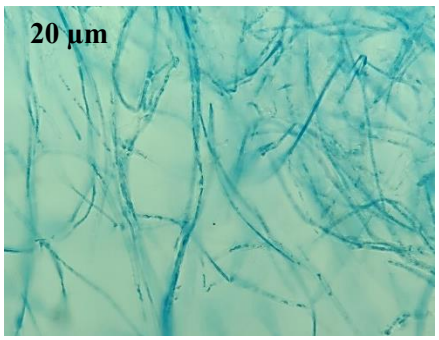
The leaf spot causing pathogen (VP DF1) initially produced dull white aerial cottony mycelia, which later became greyish white, slightly dome shaped with a circular and serrate margin. The reverse of the plate showed deep yellowish pigmentation towards

Table 4.16. Cultural and morphological characters of fungal pathogens in *Dendrobium* spp. (Contd…)

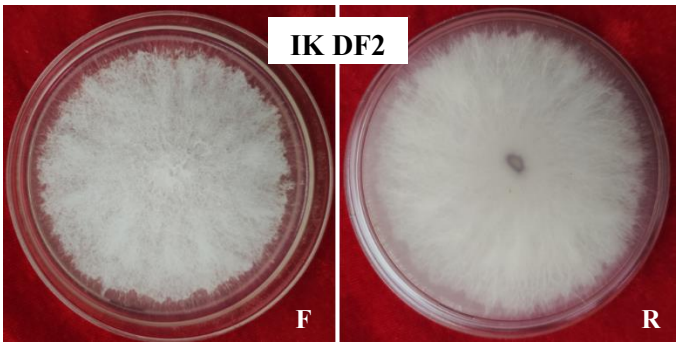
Sl. No.	Isolate	Cultural characters	Reverse side of Petri plate	Days for full growth	Growth rate (cm/day)	Morphological characters				Pathogen identified
						Hyphae	Shape of conidia	Colour of conidia	Dimension of the conidia (400X)	
13	VP DF3	Greyish white fluffy and woolly mycelia	Greenish grey in colour with dark greyish patches	7	1.28	Septate, initially hyaline and turn into brown	Cylindrical with blunt end	Hyaline	15.28 x 3.80 µm	*Colletotrichum* sp.
14	VP DF4	Dull white aerial cottony mycelia initially, later become greyish white	Deep yellowish pigmentation	11	0.81	Hyphae branched and septate	Obclavate shape with beak	Pale brown to olive brown	51.1 x 19.2 µm	*Alternaria* sp.
15	VP DF5	Pure white moderately fluffy, woolly aerial mycelia	White	7	1.28	Hyaline, septate and branched	Hyaline	Microconidia- fusiform shape tapered at both the ends Macroconidia- septate, fusiform shaped, slightly curved with tapering ends	Microconidia 3.8 x 1.1µm Macroconidia 17.19 x 3.18 µm	*Fusarium* sp.
16	VP DF6	Greyish woolly mycelia, moderately fluffy gradually become felted and turn into dark grey	Greenish grey to greyish black	7	1.28	Hyphae were septate, initially hyaline, turn into brown	Hyaline	Cylindrical	15.28 x 4.22 µm	*Colletotrichum* sp.
17	AM DF1	Fluffy, dull white, woolly mycelia	Reddish purple	9	1.00	Hyaline and septate	Microconidia	Hyaline	2.9 x 1.0 µm	*Fusarium* sp.

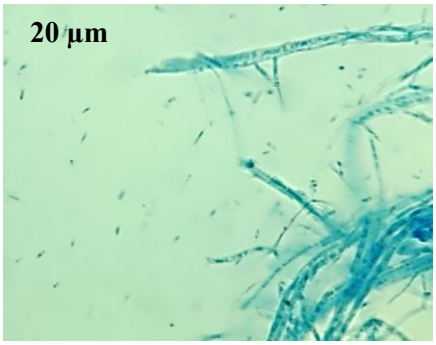
Plate 6b : Cultural and morphological characters of fungal pathogens in *Dendrobium* spp.



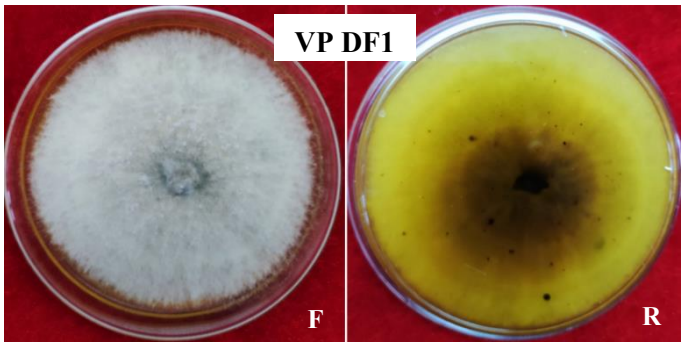


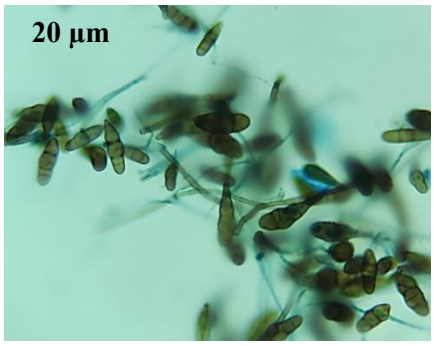
***Sclerotium* sp.**



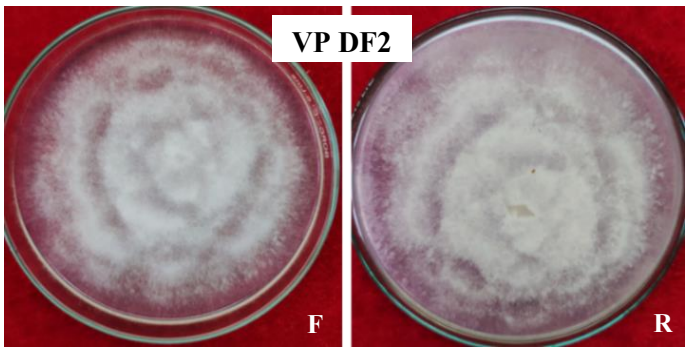


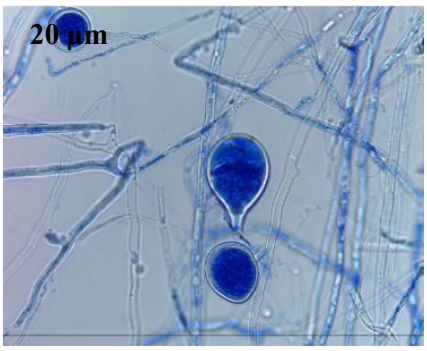
***Fusarium* sp.**





***Alternaria* sp.**





***Phytophthora* sp.**

F- Front side R- Rear side

the periphery and yellowish brown towards the centre (Plate 6b). The fungusattained full growth at 11 DOI with an average growth rate of 0.81 cm per day. Hyphae were branched, septate, and initially hyaline later turned brownish in colour. Conidia were pale brown to olive brown, obclavate or muriform shaped with a beak at the tip. The average size of the conidia was 50.0 x 18.2 µm with two to three transverse and one to two longitudinal septa. Based on cultural and morphological characters, the pathogen was tentatively identified as *Alternaria* sp.

A.12. VP DF2

The mycelial growth of the fungal pathogen (VP DF2) obtained from black rot symptom appeared as white cottony aerial mycelia with a petaloid to stellate growth pattern (Plate 6b). The reverse side of the culture was white, and the fungus attained 90 mm growth on the Petri plate at 6 DOI with an average growth rate of 1.5 cm per day. Hyphae were hyaline, aseptate and branched. The sporangia were hyaline, ovoid, and non-papillate, with a size of 44.8 µm x 36.0 µm. Based on cultural and morphological features, the pathogen was tentatively identified as *Phytophthora* sp.

A.13. VP DF3

The fungus (VP DF3) causing petal blight in flowers produced greyish, white, fluffy and woolly mycelia with serrated margin. The culture later turned greyish-green with dark greyish patches on the reverse side (Plate 6c). The growth of the fungus was radial and attained full growth at 7 DOI with an average growth rate of 1.28 cm per day. The fungus produced pin head shaped black coloured acervuli after 12 days. Hyphae were septate, branched, hyaline, and gradually turned brown in colour. Conidia were one celled, hyaline, bullet-shaped and had a central oil globule. The average size of the conidia was 15.28 x 3.80 µm. The pathogen was tentatively identified as *Colletotrichum* sp.

A.14. VP DF4

The petal blight causing pathogen (VP DPB2) produced dull white aerial cottony mycelia, which later became greyish white, slightly dome shaped with a circular and serrated margin (Plate 6c). The reverse of the plate showed deep yellowish

pigmentation towards the periphery and yellowish brown towards the centre. The fungus attained full growth at 11 DOI with an average growth rate of 1.81 cm per day. Hyphae were branched, septate and initially hyaline, and later turned brownish in colour. Conidia were pale brown to olive brown, obclavate or muriform shaped, with beak at the tip. Average size of the conidia was 51.1 x 19.2 µm with two to three transverse and one to two longitudinal septa. Based on cultural and morphological features, the pathogen was tentatively identified as *Alternaria* sp.

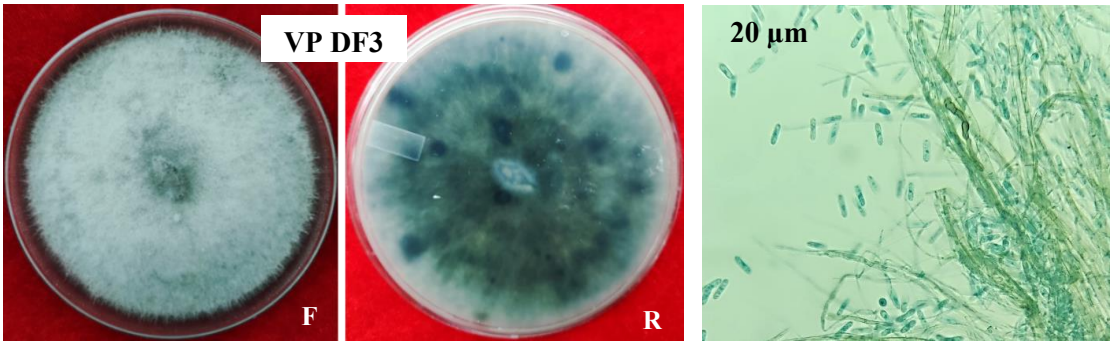
A.15. VP DF5

The fungus obtained from a flower spot of *Dendrobium* spp. showed pure white, moderately fluffy and woolly aerial mycelia with clumps of mycelia at the periphery of the colony (Plate 6c). The reverse side also appeared as white with no pigment production. The fungus attained full growth at 7 DOI with an average growth rate of 1.28 cm per day. The hyphae were hyaline, septate and branched. Microconidia and macroconidia were also observed with sizes of 13.8 x 1.1µm and 17.19 x 3.18 µm respectively. The pathogen was tentatively identified as *Fusarium* sp.

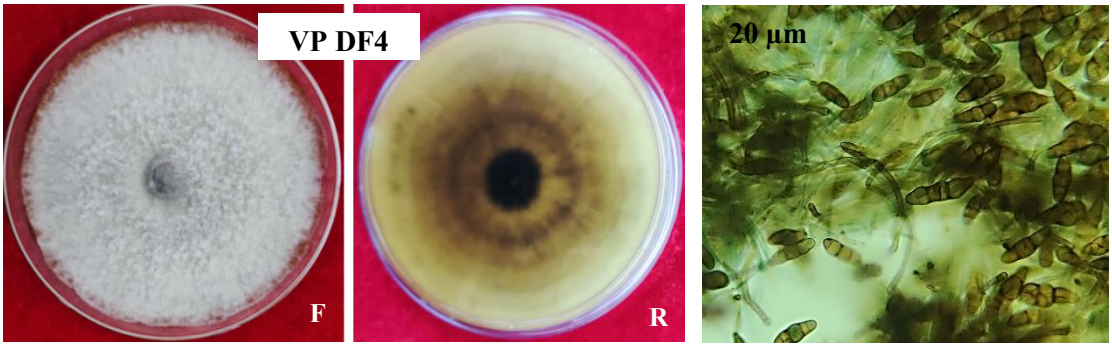
A.16. VP DF6

The fungus isolated from twig blight (VP DTB) initially produced greyish woolly mycelia, moderately fluffy, gradually became felted and turned dark grey with whitish grey mycelial clumps (Plate 6c). The fungus attained full growth at 7 DOI with an average growth rate of 1.28 cm per day. The culture produced acervuli at 12 DOI with salmon coloured spore mass. The reverse side of the colony appeared greenish grey to greyish black in colour. The hyphae were branched, septate, initially hyaline and gradually turned brown in colour. Conidia were one celled, hyaline, bullet shaped and had a central oil globule. The average size of the conidia was 15.28 x 4.22 µm. The pathogen was tentatively identified as *Colletotrichum* sp.

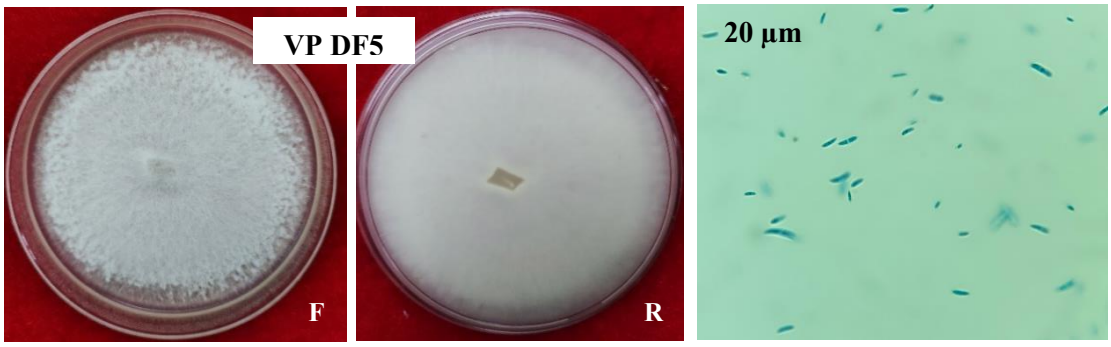
Plate 6c : Cultural and morphological characters of fungal pathogens in *Dendrobium* spp.



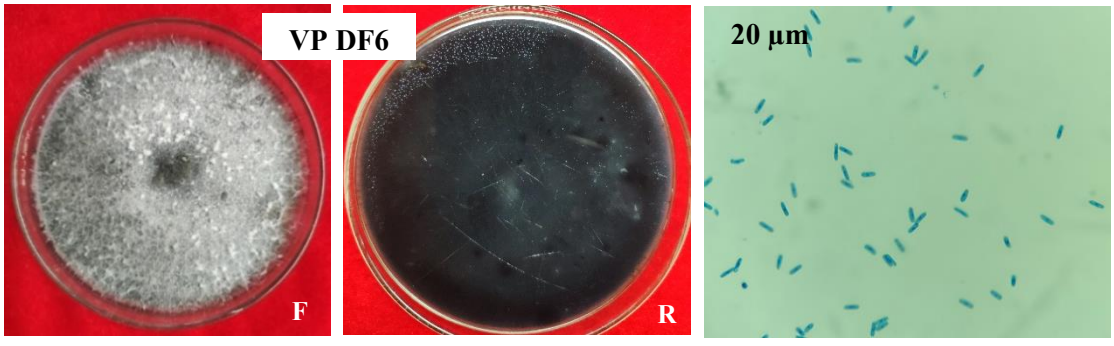
***Colletotrichum* sp.**



***Alternaria* sp.**



***Fusarium* sp.**



***Colletotrichum* sp.**

F- Front side R- Rear side

Table 4.16. Cultural and morphological characters of fungal pathogens in *Dendrobium* spp. (Contd…)

Sl. No.	Isolate	Cultural characters	Reverse side of Petri plate	Days for full growth	Growth rate (cm/day)	Morphological characters				Pathogen identified
						Hyphae	Shape of conidia	Colour of conidia	Dimension of the conidia (400X)	
18	AM DF2	Pinkish to white woolly aerial mycelia, later turn deep reddish to purple	Reddish purple	8	1.28	Hyaline and septate	Microconidia-Fusiform shaped tapered at both the ends	Hyaline	3.9 x 1.2 µm	*Fusarium* sp.
19	VL DF1	Light pinkish, woolly mycelia with greyish patches	Light pink	8	1.12	Hyaline and septate	Bullet shaped	Hyaline	10.84 x 4.34 µm	*Colletotrichum* sp.
20	VL DF2	Mycelia woolly, moderately fluffy	Light greyish with whitish creamy colour having light greyish centre	8	1.12	Brown, branched and septate	Oblong to bullet shaped	Hyaline	11.2 x 4.0 µm	*Colletotrichum* sp.
21	VY DF	Dull white, fluffy, cottony mycelia with pinkish tinge	Purple	9	1.00	dark pinkish to brown	Microconidia-fusiform shaped tapered at both the ends	Hyaline	3.0 x 0.98 µm	*Fusarium* sp.

A.17. AM DF1

The leaf blight pathogen (AM DF1) obtained from Ambunadu region produced fluffy, dull white woolly mycelia with filamentous and undulating margin (Plate 6d). The fungus completed full growth after nine days of incubation with an average growth rate of 1.0 cm per day. The reverse side of the petri dish appeared as reddish purple, with the presence of mycelial clumps over the colony. The culture produced hyaline and septate hypha with numerous microconidia of size 2.9 x 1.0 µm. Based on cultural and morphological characters, the pathogen was tentatively identified as *Fusarium* sp.

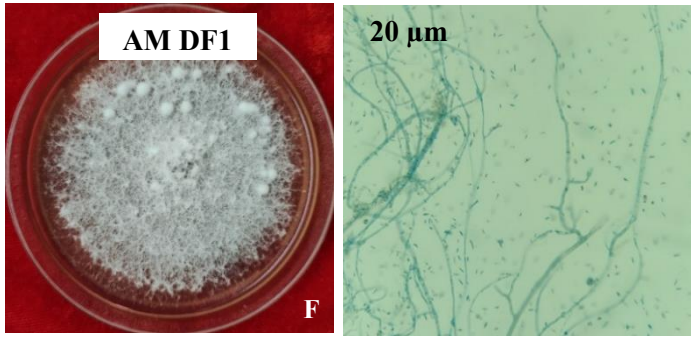
18. AM DF2

The pathogen (AM DF2) obtained from the wilt symptom showed light pinkish to white, woolly aerial mycelia with compact growth. The mycelia were slightly raised at the centre with an undulating margin. The base of the culture became deep reddish to purple with time. The reverse side of the Petri plate showed purple pigmentation. The fungus attained 90 mm growth at 9 DOI and the average growth rate was 1.28 cm per day. Hyphae were hyaline and septate with the production of numerous microconidia. Microconidia were single celled, fusiform shaped and tapered at both ends with a size of 3.9 x 1.2 µm. The pathogen was tentatively identified as *Fusarium* sp.

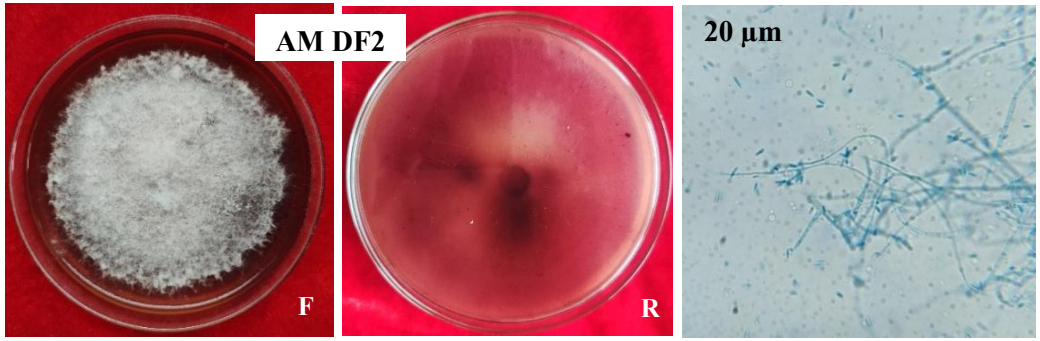
A.19.VL DF1

The leaf blight (VL DF1) symptom obtained from Vyttila produced light pinkish, woolly mycelia that were slightly raised at the middle with a light pinkish reverse side. The aerial portion of the mycelia gradually turned grey in colour and appeared as a light pinkish colony with greyish patches on the upper surface (Plate 6d). The pathogen completed 90 mm growth at 8 DOI and the average growth rate was 1.12 cm per day. After 15 days, numerous sclerotia were produced all over the culture which were scattered over the colony. Later, salmon coloured spore mass was produced with numerous conidia. Conidia were one celled, hyaline, bullet shaped and had a central oil globule. The average size of the conidia was 10.84 x 4.34 µm. Based on the above observations, the pathogen was tentatively identified as *Colletotrichum* sp.

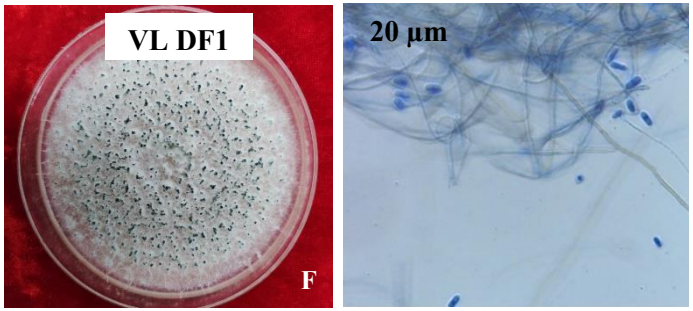
Plate 6d : Cultural and morphological characters of fungal pathogens in *Dendrobium* spp.



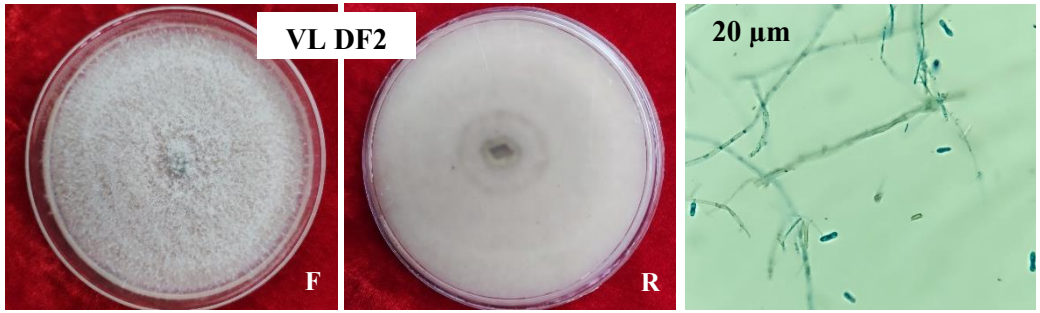
***Fusarium* sp.**



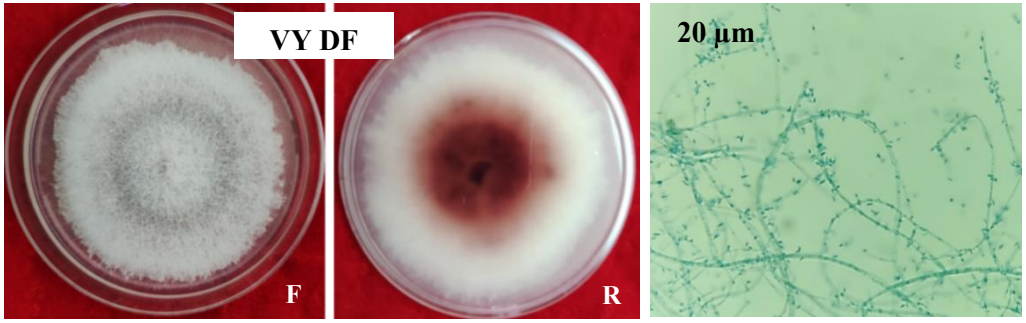
***Fusarium* sp.**



***Colletotrichum* sp.**



***Colletotrichum* sp.**



***Fusarium* sp.**

F- Front side R- Rear side

A.20. VL DF2

The pathogen (VL DF2) isolated from the leaf spot symptom (VL DLS) produced cream-coloured colonies on PDA. The mycelia were woolly, moderately fluffy, with regular and serrated margin (Plate 6d). Aerial mycelia later turned light greyish, with whitish creamy reverse side having light greyish centre. Sclerotial bodies were developed after ten days of incubation and were scattered over the medium. Acervuli were produced after 15 days of inoculation and were associated with salmon coloured spore mass. The fungus completed full growth on 90 mm petri dish after eight days of incubation with an average growth rate of 1.12 cm per day. Later salmon coloured spore mass was produced with numerous conidia. Conidia were one celled, hyaline, bullet shaped and had a central oil globule. The average size of the conidia was 11.2 x 4.0 µm. Based on the above observations, the pathogen was tentatively identified as *Colletotrichum* sp.

A.21.VY DF

The pathogen (VY DF) causing leaf blight symptom obtained from Vellayani initially produced dull white, fluffy cottony mycelia with pinkish tinge. The margin of the colony was irregular and submerged (Plate 6d). The reverse of the colony showed dark pinkish to brown pigmentation at its central portion. The colony attained 90 mm growth at 9 DOI and the average growth rate was 1.0 cm per day. Hyphae were hyaline and septate with the production of numerous microconidia. Microconidia were single celled and fusiform shaped, tapered at both the ends, had a size of 3.0 x 0.98 µm. The pathogen was tentatively identified as *Fusarium* sp.

4.4.1.B. Cultural and morphological characterisation of fungi obtained from *Oncidium* spp.

Cultural and morphological characters of fungal pathogens isolated from *Oncidium* spp. are detailed in Tables 4.17

B.1. VK OF1

The leaf blight pathogen obtained from *Oncidium* spp. initially produced greyish green woolly mycelia, turned dark grey colour over time, with dark grey to black reverse side. The fungus attained full growth at 4 DOI and the average growth rate was 2.25 cm per day. Pycnidia formation was observed after 15 days of incubation. The mycelia were septate, initially hyaline, which later turned dark brown. The conidia were oval in shape, initially colourless and aseptate which gradually turned into dark brown and bicelled. The conidial size was recorded as 24.42 x 17.61 µm. Based on cultural and morphological characteristics, the pathogen was tentatively identified as *Diplodia* sp.

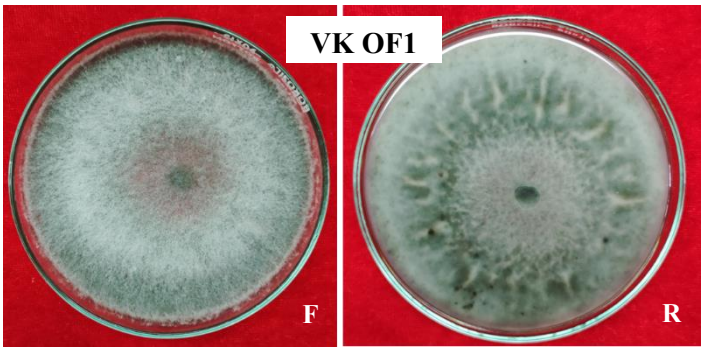
B.2. VK OF2

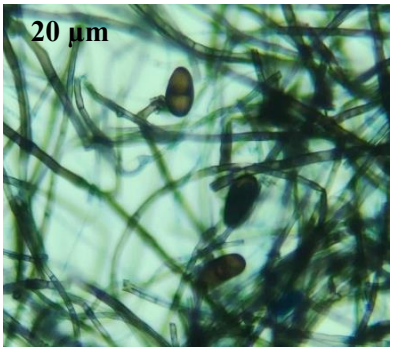
The pathogen associated with the leaf blight symptom obtained from Vellanikkara produced greyish white, woolly mycelia with a fluffy growth pattern (Plate 7). The growth of the fungus was regular and even, with serrated margin. The reverse side of the plates produced dark greyish patches, and the fungal colony attained 90 mm growth at 7 DAI with an average growth rate of 1.28 cm per day. Acervuli were produced at 9 DOI. Hyphae were septate, branched, hyaline, and gradually turned brown in colour with the presence of hyphal swellings. Moderate production of conidia was observed, which were hyaline, single celled and cylindrical to dumbbell shaped with an uneven margin. The average size of the conidia was 17.46 x 6.06 µm. The pathogen was tentatively identified as *Colletotrichum* sp.

B.3. VK OF3

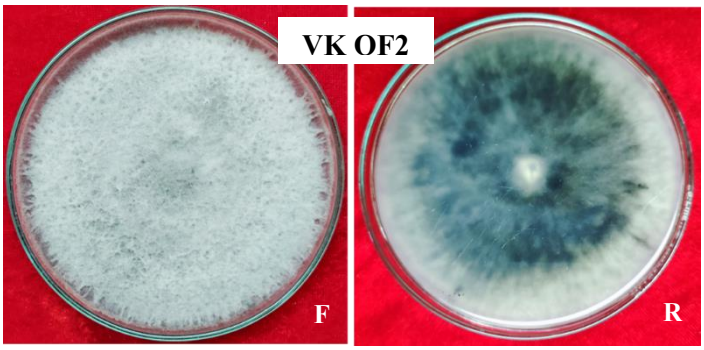
The pathogen associated with the leaf spot symptom produced dull white and woolly mycelia, which later turned into olivaceous grey with flat and even growth (Plate 7). The reverse side of the colony appeared greyish black in colour. The fungus attained 90 mm growth at 7 DOI and the average growth rate was 1.28 cm per day. Acervuli were minute and black and produced at 11 DOI. Hyphae were septate, branched and initially hyaline, which later turned brown. Conidia were elongated and cylindrical, with acute ends. An oil globule was present in the middle of the hyaline cell. The size

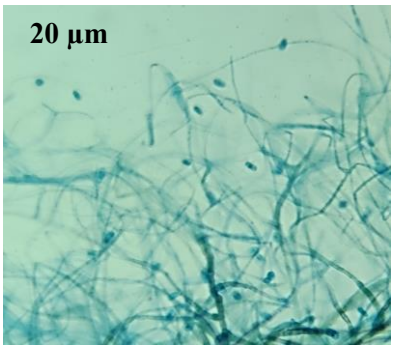
Plate 7 : Cultural and morphological characters of fungal pathogens in *Oncidium* spp.



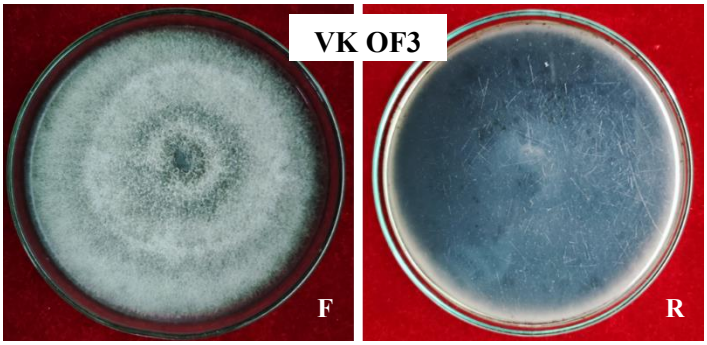


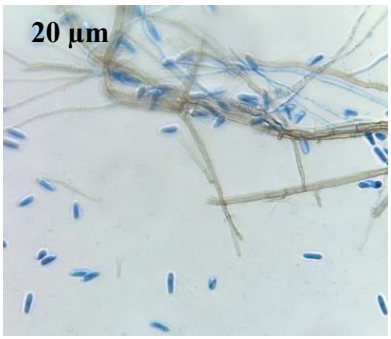
***Diplodia* sp.**



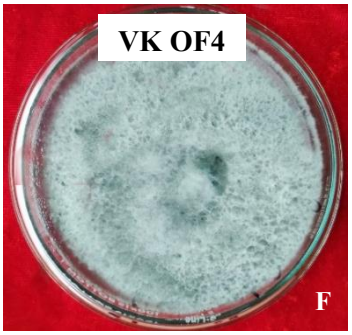


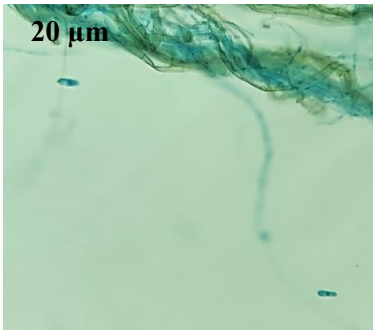
***Colletotrichum* sp.**

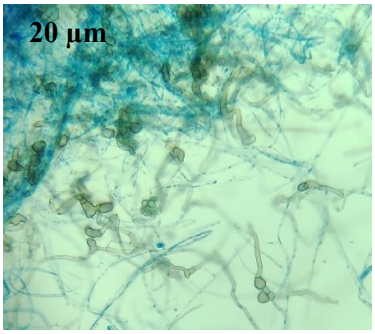




***Colletotrichum* sp.**







Hyphal swellings

***Colletotrichum* sp.**

F- Front side R- Rear side

Table 4. 17. Cultural and morphological characters of fungal pathogens in *Oncidium* spp.

Sl. No.	Isolate	Cultural characters	Reverse side of Petri plate	Days for full growth	Growth rate (cm/day)	Morphological characters				Pathogen identified
						Hyphae	Shape of conidia	Colour of conidia	Dimension of the conidia (400X)	
1	VK OF1	Greyish green, woolly mycelia	Dark grey to black	4	2.25	Septate, hyaline, turn into dark brown	Oval	Dark brown	24.42 x 17.61 µm	*Diplodia* sp.
2	VK OF2	Greyish white, woolly mycelia with fluffy growth pattern	Dark greyish patches	7	1.28	Septate, branched and hyaline	Cylindrical to dumbbell	Hyaline	17.46 x 6.06 µm	*Colletotrichum* sp.
3	VK OF3	Woolly, dull white mycelia initially, later become olivaceous grey	Greyish black with dirty white margin	7	1.28	Septate, branched, initially hyaline, turn into dark brown	Hyaline	Elongated, cylindrical with acute end	19.0 x 3.9 µm	*Colletotrichum* sp.
4	VK OF4	Woolly, fluffy, greyish mycelia	Dark greyish	6	1.5	Septate, branched initially hyaline, turn into dark brown	Hyaline	Bullet shaped with broad and narrow opposite ends	14.48 x 4.22 µm	*Colletotrichum* sp.
5	VK OF5	Light pinkish, woolly mycelia	Light pink	7	1.28	Septate, branched initially hyaline, turn into dark brown	Cylindrical	Hyaline	13.37 x 3.80 µm	*Colletotrichum* sp.
6	VK OF6	Greyish white, fluffy and woolly mycelia	Grey	7	1.28	Septate, branched initially hyaline, turn into dark brown	Cylindrical and elongated	Hyaline	14.36 x 3.69 µm	*Colletotrichum* sp.

of the conidia was measured as 19.0 x 3.9 µm. The pathogen was tentatively identified as *Colletotrichum* sp. based on the above features.

B.4. VK OF4

The culture associated with leaf spot disease initially produced woolly, fluffy and greyish mycelia attained full growth at 6 DOI with an average growth rate of 1.5 cm per day. The growth was regular with an even margin and on the reverse side, the colony showed a dark grey (Plate 7). The hyphae were branched and septate, which were initially hyaline and later became dark brown in colour. The conidial production was scanty. Conidia were bullet shaped with broad and narrow opposite ends. The average size of conidia was 14.48 x 4.22 µm. Based on cultural and morphological characteristics, the pathogen was tentatively identified as *Colletotrichum* sp.

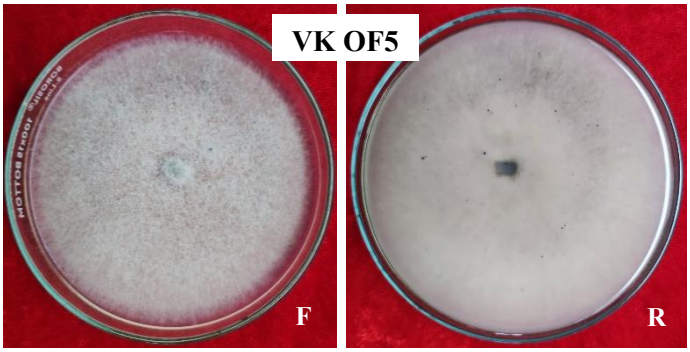
B.5.VK OF5

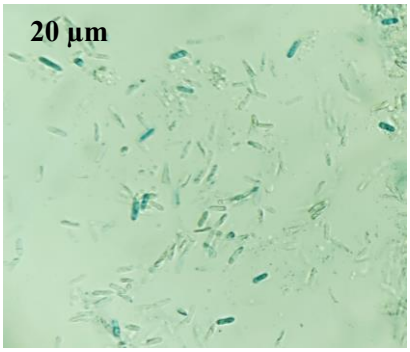
The leaf spot symptom obtained from Vellanikkara produced light pinkish, woolly and flat subaerial mycelia with a light pink reverse side (Plate 7a). The aerial portion of the mycelia gradually turned greyish pink in colour and attained full growth (90 mm) at 8 DOI and the average growth rate was 1.28 cm per day. The hyphae were branched, septate, initially hyaline, and later turned dark brown. Conidia were one celled, hyaline, cylindrical, slightly elongated, and contained two oil globules per cell. The size of the conidia was 13.37 x 3.80 µm. Based on the above features, the pathogen was tentatively identified as *Colletotrichum* sp.

B.6. VK OF6

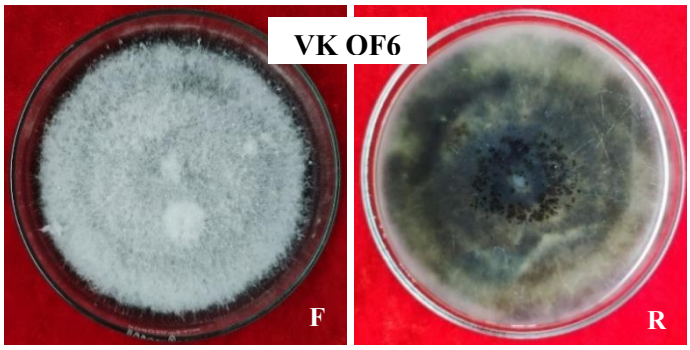
The fungus associated with leaf spot disease initially produced greyish-white, fluffy and woolly mycelia, which completed full growth at 7 DOI and the average growth rate was 1.28 cm per day. The mycelia gradually became felted and produced acervuli at 10 DOI. The hyphae were branched and septate, which were initially hyaline and later dark brown in colour. Conidia were produced abundantly in the culture, and conidia were characterised as one celled, hyaline, cylindrical and elongated with an oil globule per cell (Plate 7a). The size of the conidia was 14.36 x 3.69 µm.

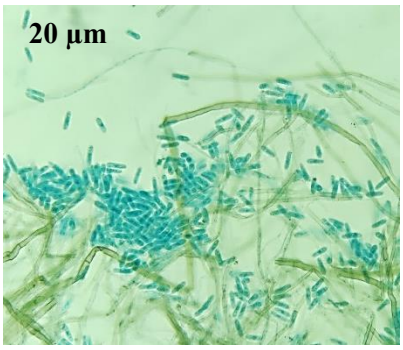
Plate 7a : Cultural and morphological characters of fungal pathogens in *Oncidium* spp.



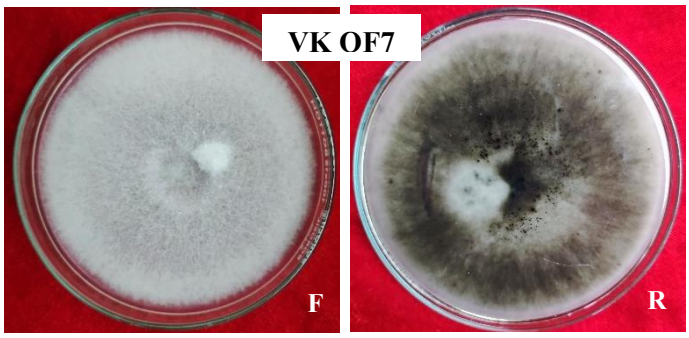


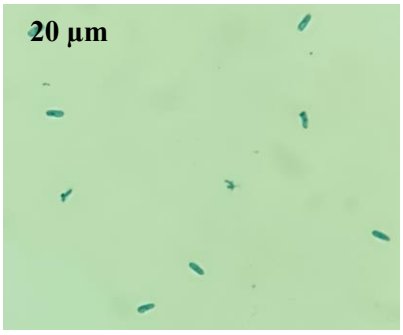
***Colletotrichum* sp.**



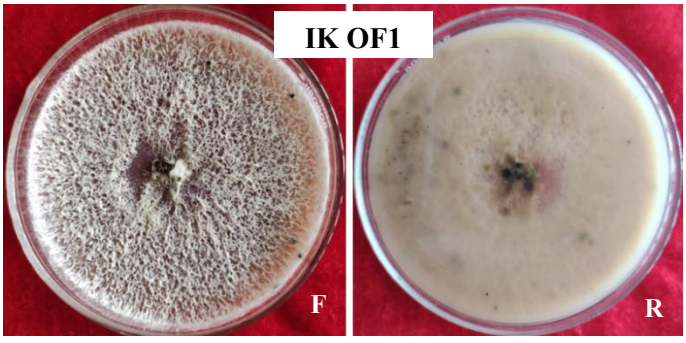


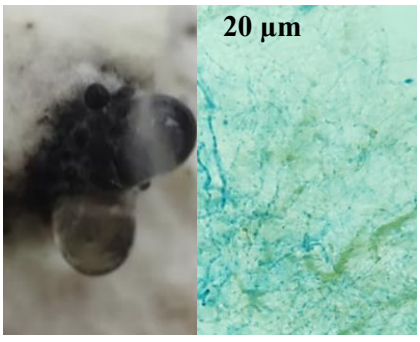
***Colletotrichum* sp.**





***Colletotrichum* sp.**





***Diaporthe* sp.**

F- Front side R- Rear side

Table 4. 17. Cultural and morphological characters of fungal pathogens in *Oncidium* spp. (Contd…)

Sl. No.	Isolate	Cultural characters	Reverse side of Petri plate	Days for full growth	Growth rate (cm/day)	Morphological characters				Pathogen identified
						Hyphae	Shape of conidia	Colour of conidia	Dimension of the conidia (400X)	
7	VK OF7	Dull white, woolly, fluffy mycelia gradually turn greyish white	Greenish grey	7	1.28	Septate, branched initially hyaline, turn into dark brown	Bullet shaped with broad and narrow opposite ends	Hyaline	14.48 x 4.22 µm	*Colletotrichum* sp.
8	IK OF1	Dirty white flat, sparse and spreading cottony aerial mycelium	Yellowish to dirty white	6	1.50	Hyaline and septate	No conidia observed			*Diaporthe* sp.
9	IK OF2	White cottony, aerial mycelium with diurnal zonation	Light orangish yellow and black patches	9	1.00	Hyaline	Straight, fusiform to slightly clavate	Middle three brown cells and outer two hyaline cells	33.63 x 6.95 µm	*Pestalotia* sp.
10	VP OF	Greyish, woolly and fluffy mycelium which gradually become dark grey	Greyish black	7	1.28	Septate, branched initially hyaline, turn into dark brown	Cylindrical and elongated	Hyaline	16.0 x 3.7 µm	*Colletotrichum* sp.
11	AM OF1	Greyish white, woolly textured mycelia turn into dark grey	Concentric greyish zonation surrounded by greyish white region with greenish grey periphery	8	1.12	Septate, branched, initially hyaline, turn into dark brown	Bullet shaped	Hyaline	16.24 x 4.90 µm	*Colletotrichum* sp.
12	AM OF2	Greyish, cottony, aerial mycelia with compact growth turn into brownish grey	Dark brownish with concentric zonations	10	0.90	Septate, branched. Initially hyaline, turn into dark brown	Muriform	Light olivaceous to dark brown	49.0 x 15.87 µm	*Alternaria* sp.

Based on the above characters, the pathogen was tentatively identified as *Colletotrichum* sp.

B.7. VK OF7

The fungus associated with the leaf spot symptom produced dull white, woolly and fluffy mycelia, which gradually turned greyish white (Plate 7a). The reverse side of the culture appeared as greenish grey. The fungus attained 90 mm growth at 7 DOI and the average growth rate was 1.28 cm per day. Black pinhead acervuli were produced after 11 DOI. The hyphae were branched and septate, which were initially hyaline and later dark brown in colour. The conidia were characterised as one-celled, hyaline and cylindrical or bullet shaped, with a broad and narrow opposite end. A single oil globule was present at the centre of conidial cell. The size of the conidia was 14.48 x 4.22 µm. Based on cultural and morphological characteristics, the pathogen was tentatively identified as *Colletotrichum* sp.

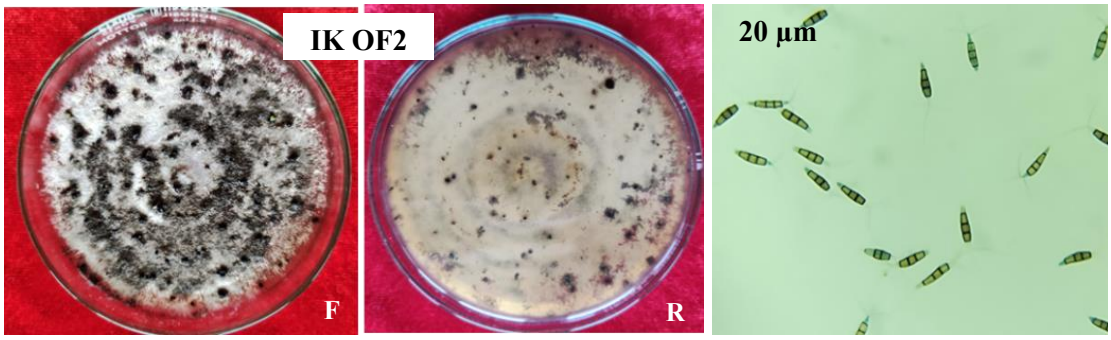
B.8. IK OF1

The pathogen causing leaf spot disease produced dirty white, flat, sparse aerial mycelium with cottony texture. The colony produced yellowish green pigmentation at later stages (Plate 7a). The fungus attained 90 mm growth at 6 DOI with an average growth rate of 1.5 cm per day. Black, irregular pycnidial conidiomata embedded in the culture at 20 DOI. The reverse side appeared yellowish to dirty white with dull greenish pigmentation. A dull white ooze appeared on the conidiomata after 30 days of incubation. Hyphae were hyaline and septate. No sporulation could be observed. Based on the above characters, the pathogen was tentatively identified as *Diaporthe* sp.

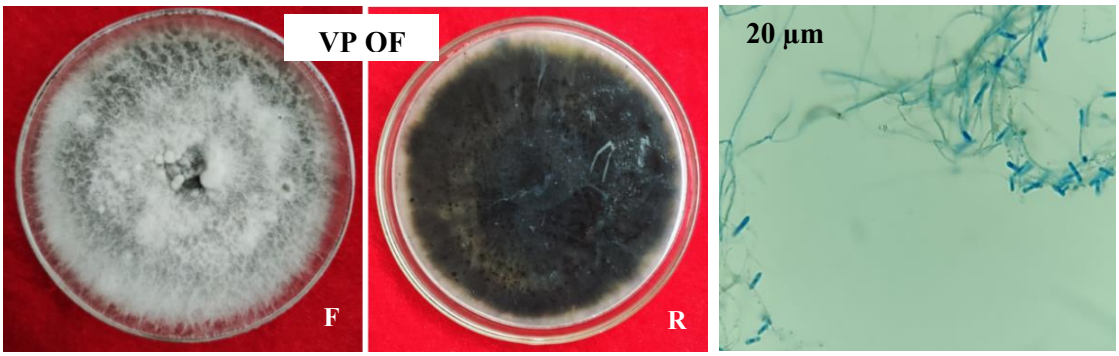
B.9. IK OF2

The fungus isolated from the leaf spot symptom produced white cottony aerial mycelium, which diffused towards the advancing edge and was denser on the older part of the colony with diurnal zonation (Plate 7b). The culture attained full growth at 9 DOI with an average growth rate of 1.0 cm per day. Abundant scattered acervuli with black slimy spore mass were developed gradually. The reverse side appeared light orangish yellow with black patches. Fungus produced hyaline hyphae with

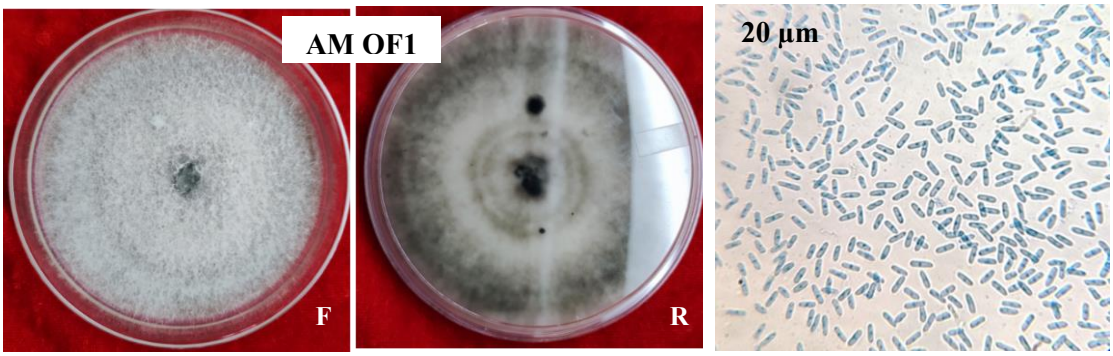
Plate 7b : Cultural and morphological characters of fungal pathogens in *Oncidium* spp.



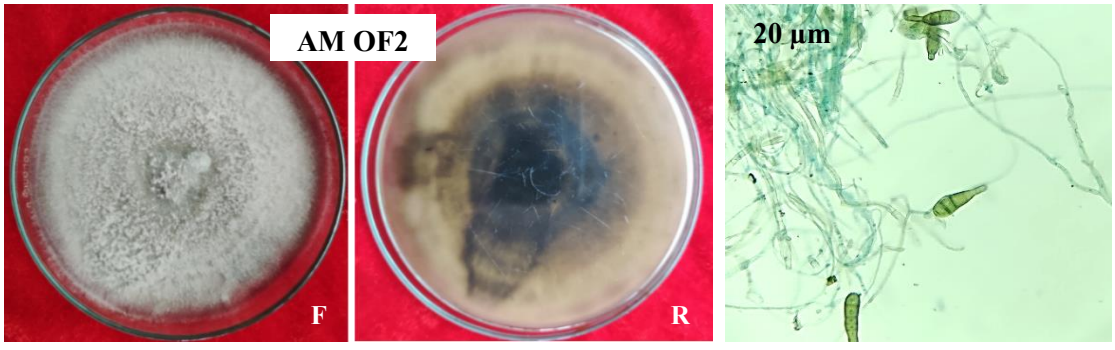
***Pestalotia* sp.**



***Colletotrichum* sp.**



***Colletotrichum* sp.**



***Alternaria* sp.**

F- Front side R- Rear side

characteristic five celled conidia. Conidia were straight, fusiform to slightly clavate, with three to four septa. The three cells in the middle were brown, whereas the outer two cells were hyaline. The basal appendages were hyaline, straight, with an average size of 4.4 µm. Apical appendages were two in number, with an average length of 15.2 µm. The average dimension of conidia was 33.63 x 6.95 µm. Based on cultural and morphological characteristics, the pathogen was tentatively identified as *Pestalotia* sp.

B.10. VP OF

The pathogen associated with leaf spot disease produced greyish, woolly and fluffy mycelia, which gradually turned dark grey in colour. The fungus showed radial growth with serrated margin. The reverse side of the Petri dish appeared greyish-black. The pathogen attained full growth after 7 DOI with an average growth rate of 1.28 cm per day. The hyphae were branched and septate, which were initially hyaline and later dark brown in colour. The conidia were characterised as one celled, hyaline, cylindrical and elongated with the presence of an oil globule. The average dimension of conidia was 16.0 x 3.7 µm. The pathogen was tentatively identified as *Colletotrichum* sp.

B.11. AM OF1

The leaf blight associated pathogen obtained from Ambunadu region initially produced greyish white, woolly textured mycelia which gradually turned into dark grey with the production of acervuli. The colony was flat with even growth and regular margin. The reverse of the Petri dish showed concentric greyish zonation surrounded by greyish white region with greenish grey periphery (Plate 7b). The fungus attained 90 mm growth at 8 DOI with an average growth rate of 1.12 cm per day. The hyphae were branched and septate which were initially hyaline and later became dark brown in colour. Conidia were abundant, hyaline and bullet shaped with the presence of an oil globule at the centre with a size of 16.24 x 4.90 µm. Based on cultural and morphological characteristics, the pathogen was tentatively identified as *Colletotrichum* sp.

B.12. AM OF2

The leaf blight pathogen collected from Ambunadu region produced greyish, cottony textured aerial mycelia with compact growth. The margin of the culture was slightly irregular and mycelia turned into brownish grey over time (Plate 7b). The reverse side of the petri dish appeared as dark brown with concentric zonations. The fungus attained 90 mm growth at 10 DOI with an average growth rate of 0.9 cm per day. The hyphae were branched and septate which were initially hyaline and later turned into dark brown in colour. Conidia were light olivaceous to dark brown in colour, obclavate or muriform shaped with one to two longitudinal and three to five transverse septa had a narrow beak at one end. The average length and width of conidia were 49.0 x 15.87 µm respectively. The beak size was measured 7.9 µm. The pathogen was tentatively identified as *Alternaria* sp.

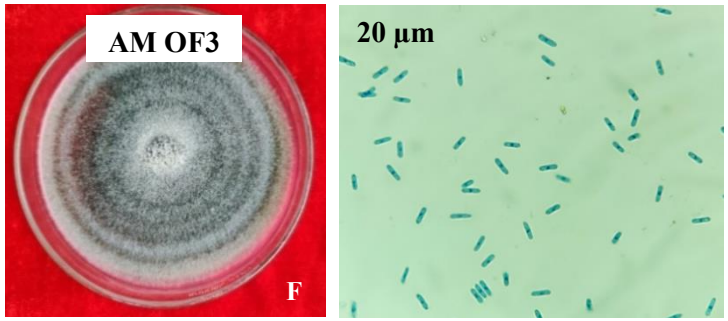
B.13. AM OF3

The pathogen isolated from leaf blight disease in *Oncidium* sp. showed greyish, woolly sub aerial mycelia with alternate dark greenish to grey zonations (Plate 7c). The colony was flat with entire margin and the reverse side of the culture appeared grey in colour. The fungus attained full growth at 7 DOI with an average growth rate of 1.28 cm per day. The hyphae were branched and septate which were initially hyaline and later became dark brown in colour. The conidia were aseptate and cylindrical with blunt end. An oil globule was seen at the middle of the hyaline cell. The size of conidia measured as 17.0 x 4.29 µm. Based on cultural and morphological characteristics, the pathogen was tentatively identified as *Colletotrichum* sp.

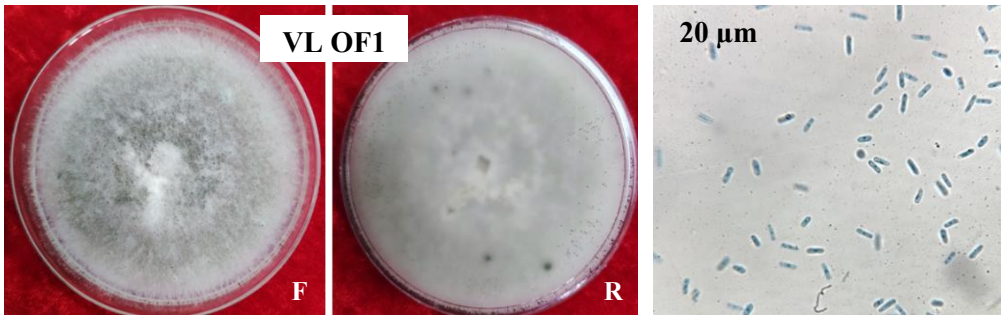
B.14. VL OF1

The leaf blight pathogen obtained from Vyttila initially produced light pinkish, woolly mycelia with slightly raised centre and light pink reverse side. The aerial portion of the mycelia gradually turned grey in colour and appeared as light pinkish colony with greyish patches on the upper surface of the colony. The fungus attained 90 mm growth at 8 DOI with an average growth rate of 1.12 cm per day. Numerous sclerotia were produced at 15 DOI. Later, salmon-coloured spore mass was produced with numerous conidia. Conidia were one celled, hyaline, bullet shaped had a central oil globule. The

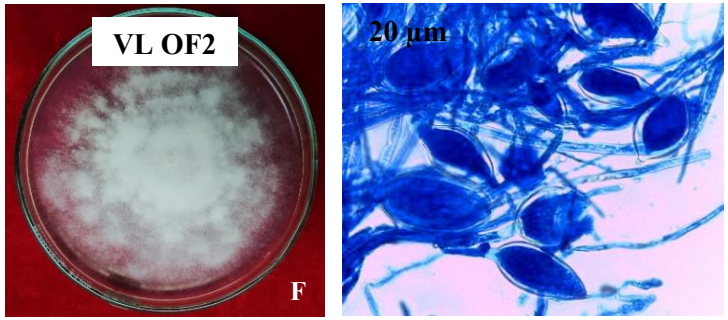
Plate 7c : Cultural and morphological characters of fungal pathogens in *Oncidium* spp.



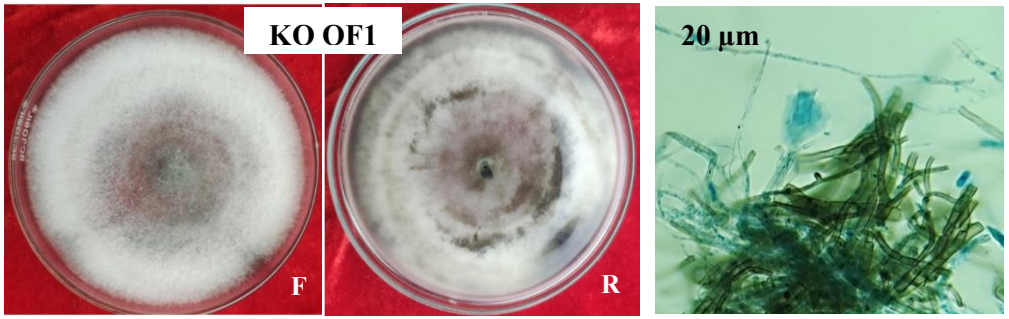
***Colletotrichum* sp.**



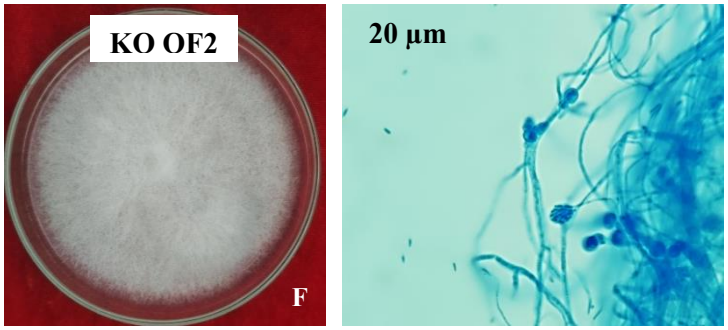
***Colletotrichum* sp.**



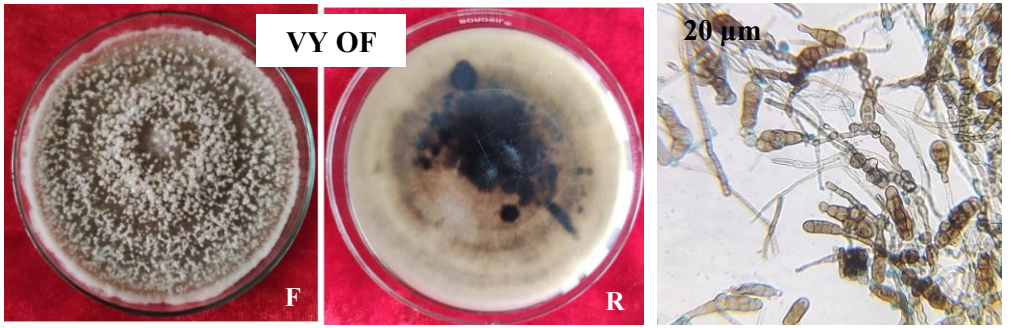
***Phytophthora* sp.**



***Colletotrichum* sp.**



***Fusarium* sp.**



***Alternaria* sp.**

F- Front side R- Rear side

Table 4. 17. Cultural and morphological characters of fungal pathogens in *Oncidium* spp. (Contd…)

Sl. No.	Isolate	Cultural characters	Reverse side of Petri plate	Days for full growth	Growth rate (cm/day)	Morphological characters				Pathogen identified
						Hyphae	Shape of conidia	Colour of conidia	Dimension of the conidia (400X)	
13	AM OF3	Greyish woolly sub aerial mycelia with alternating dark greenish to grey zonations	Grey	7	1.28	Septate, branched hyaline, turn into dark brown	Conidia aseptate, cylindrical with blunt end	Hyaline	17.0 x 4.29 µm	*Colletotrichum* sp.
14	VL OF1	Light pinkish, woolly mycelia	Light pink	8	1.12	Septate, branched, initially hyaline, turn into dark brown	Bullet shaped	Hyaline	18.0 x 4.2 µm	*Colletotrichum* sp.
15	VL OF2	Cottony mycelia with restricted, tufted and irregular growth pattern	White	7	1.28	Hyaline and aseptate	Ovoid semi papillate sporangium	Hyaline	32.48 x 9.8 µm	*Phytophthora* sp.
16	KO OF1	White, woolly, aerial mycelia gradually turn into greyish white	Greenish grey	7	1.28	Septate, branched initially hyaline, turn into dark brown	Aseptate and bullet shaped	Hyaline	17.0 x 4.29 µm	*Colletotrichum* sp.
17	KO OF2	White delicate, woolly mycelia with sparse growth	White	9	1.00	Hyaline and septate	Fusiform microconidia	Hyaline	2.8 x 0.8 µm	*Fusarium* sp.
18	VY OF	Greyish to light brown fluffy, aerial mycelia with cottony texture turn into dark brownish with concentric zonations	Brownish to black	11	0.81	Brown	Obclavate to muriform shape	Light olivaceous to dark brown	51.44 x 16.88 µm	*Alternaria* sp.

size of the conidia was 18.0 x 4.2 µm. Based on cultural and morphological characteristics, the pathogen was tentatively identified as *Colletotrichum* sp.

B.15. VL OF2

The leaf blight associated pathogen obtained from Vyttila region produced cottony mycelia with restricted, tufted and irregular growth pattern where clumps of mycelia alternated with sparse mycelial growth (Plate 7c). The colony was flat, uneven with irregular margin. The fungus attained 90 mm growth at 7 DAI with an average growth rate of 1.28 cm per day. The hyphae were hyaline and aseptate, with abundant production of elongated and ovoid semi-papillate sporangia with an average size of 32.48 x 9.8 µm. Pathogen was tentatively identified as *Phytophthora* sp.

B.16. KO OF1

The fungus associated with the leaf blight symptom obtained from Kottukal region initially produced white, woolly aerial mycelia, gradually turned greyish white and became felted (Plate 7c). The reverse side of the colony produced greenish grey patches. The fungus attained 90 mm growth after 7 DOI with an average growth rate of 1.28 cm per day. The hyphae were branched and septate which were initially hyaline, later became dark brown in colour. The conidia were aseptate and bullet shaped. An oil globule was seen at the middle of the hyaline conidial cell. The conidial production was scanty, with an average size of 17.0 x 4.29 µm. Based on cultural and morphological characteristics, the pathogen was tentatively identified as *Colletotrichum* sp.

B.17. KO OF2

The pathogen associated with wilt disease in *Oncidium* spp. produced white, delicate and woolly mycelia with sparse growth (Plate 7c). The fungal colony was even, flat with entire margin, and attained full growth at 9 DOI with an average growth rate of 1.0 cm per day. Hyphae were hyaline and septate. Fusiform shaped microconidia were scattered among the mycelia with size of 2.8 x 0.8 µm. Chlamydospores were terminal or intercalary. The pathogen was tentatively identified as *Fusarium* sp.

B.18. VY OF

The fungus isolated from leaf spot disease produced greyish to light brown, fluffy aerial mycelia with cottony texture, gradually turned into dark brown in colour. The reverse side appeared as brownish to black with concentric zonations (Plate 7c). The fungus attained full growth at 11 DOI with an average growth rate of 0.81 cm per day. Initially the hyphae were hyaline and irregularly branched which gradually turned greyish to brown, and septate. Conidia were light olivaceous to dark brown in colour with obclavate to muriform shape. One end of the conidia was tapered with a beak measuring an average size of 8.7 µm. Conidia were septate with one to two longitudinal and three to six transverse septa, with an average length and width of 51.44 x 16.88 µm respectively. The pathogen was tentatively identified as *Alternaria* sp.

4.4.1.C. Cultural and morphological characterisation of fungi obtained from *Cattleya* spp.

Cultural and morphological characters of fungal pathogens isolated from *Cattleya* spp. are detailed in table 4.18.

C.1.VP CF

The mycelial growth of the fungal pathogen (VP CF) obtained from black rot symptom appeared as white, cottony and aerial mycelia with petaloid growth pattern (Plate 8). The reverse side of the culture was white and fungus attained 90 mm growth at 6 DOI with an average growth rate of 1.5 cm per day. Hyphae were hyaline, aseptate and branched. The sporangia were hyaline, ovoid and papillate with a size of 46.2 x 36.6 µm. Based on cultural and morphological characteristics, the pathogen was tentatively identified as *Phytophthora* sp.

C.2. AM CF1

The pathogen (AM CF1) associated with the leaf blight symptom showed light pinkish to white, woolly aerial mycelia with compact growth. The mycelia were slightly raised at the centre with an undulating margin (Plate 8). The base of the culture became deep reddish to purple with time. The reverse side of the Petri plate showed purplish pigmentation. The fungus attained full growth at 7 DOI with an average growth rate of

1.28 cm per day. Hyphae were hyaline and septate with the production of numerous microconidia. Microconidia were single and fusiform shaped with tapering ends. The average size of the microconidia was 2.50 x 0.71 µm. The pathogen was tentatively identified as *Fusarium* sp.

C.3. AM CF2

The fungus isolated from leaf blight disease produced greyish mycelia with woolly texture, and sparse growth, which appeared flat colony on PDA (Plate 8). Later mycelia, gradually turned dark greyish to black in colour, attained full growth at 7 DOI with an average growth rate of 1.28 cm per day. The hyphae were branched and septate, which were initially hyaline and later turned dark brown in colour. Acervuli were not observed. Perithecia were hyaline, with clustered asci observed at 14 DOI. Sickle shaped, aseptate hyaline ascospores with a size of 18.0 x 8.1µm were observed. Based on cultural and morphological characteristics, the pathogen was tentatively identified as *Colletotrichum* sp.

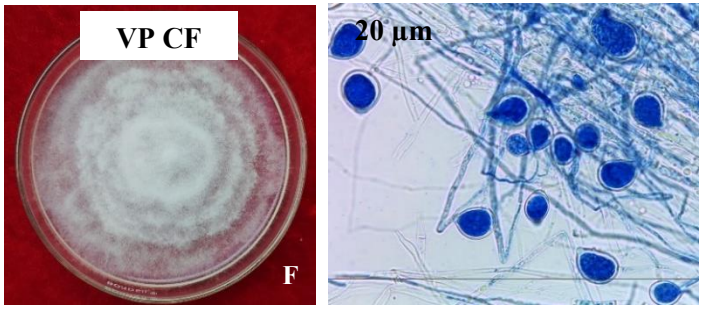
C.4.VY CF1

The pathogen causing the leaf blight symptom initially produced greyish aerial and woolly mycelia, later became dark greyish to black in colour (Plate 8). The fungus attained full growth at 8 DOI with an average growth rate of 1.12 cm per day. The reverse side appeared as dark grey in colour. Acervuli were produced at 14 DOI with abundant production of conidia. Conidia were bullet shaped, hyaline and one celled with a single oil globule. The size of the conidia observed was 14.0 x 5.1 µm. Perithecia with hyaline and clustered asci were observed at 16 DOI. The ascospores were aseptate, hyaline and sickle shaped, with an average size of 14.0 x 5.1 µm. The pathogen was tentatively identified as *Colletotrichum* sp.

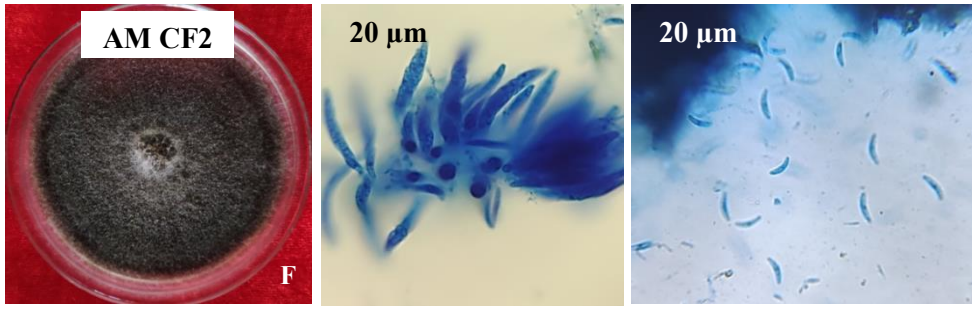
C.5.VY CF2

The fungal pathogen cultured on PDA attained full growth at 4 DOI by forming dull white, abundant, fluffy, aerial mycelia, turned olive-grey or grey over time (Plate 8). The average growth rate of the fungus was 1.25 cm per day. The reverse side of the Petri plate became greyish to black. Pycnidia were observed after two weeks of

Plate 8 : Cultural and morphological characters of fungal pathogens in *Cattleya* spp.



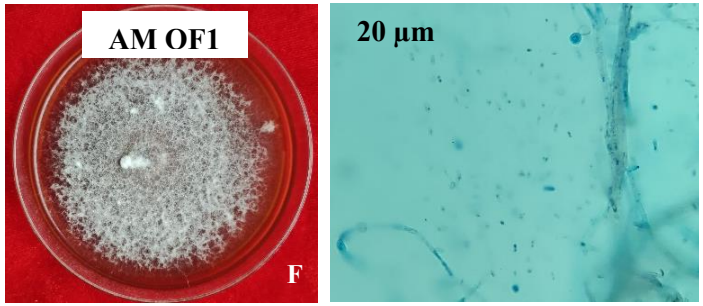
***Phytophthora* sp.**



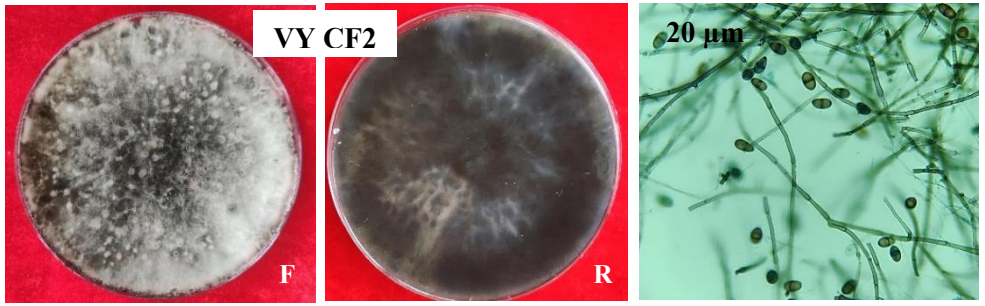
Asci

Ascospores

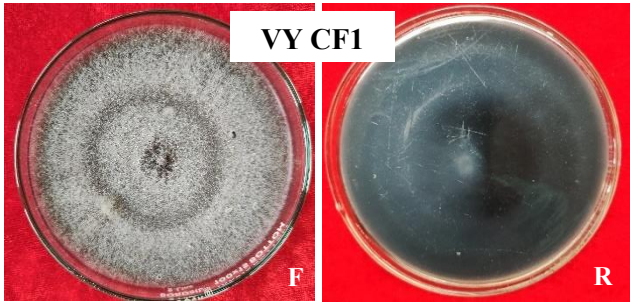
***Colletotrichum* sp.**



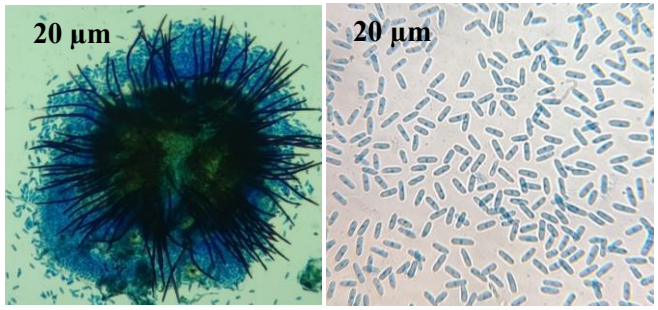
***Fusarium* sp.**



***Lasiodiplodia* sp.**

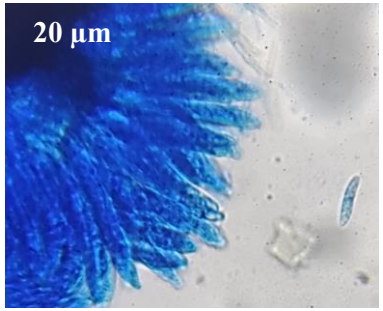


***Colletotrichum* sp.**



Acervuli

Conidia



Asci and ascospores

F- Front side R- Rear side

Table 4.18. Cultural and morphological characters of pathogens in *Cattleya* spp.

Sl. No.	Isolate	Cultural characters	Reverse side of Petri plate	Days for full growth	Growth rate (cm/day)	Morphological characters				Pathogen identified
						Hyphae	Shape of conidia	Colour of conidia	Dimension of the conidia (400X)	
1	VP CF	White cottony aerial mycelia with petaloid growth pattern	White	6	1.50	Hyaline, aseptate and branched	Sporangia- hyaline, ovoid and papillate with a size of 46.2 x 36.6 µm			*Phytophthora* sp.
2	AM CF1	Light pinkish to white woolly aerial mycelia deep reddish to purple	Purplish pigmentation	7	1.28		Microconidia are single celled fusiform shaped	Hyaline	2.50 x 0.71 µm	*Fusarium* sp.
3	AM CF2	Woolly flat greyish mycelia with sparse growth	Grey	7	1.28	Septate, branched initially hyaline, turn into dark brown	Sexual spores- sickle shaped aseptate hyaline ascospores of 18.0 x 8.1µm size were produced inside the ascus			*Colletotrichum* sp.
4	VY CF1	Greyish aerial, woolly mycelia later become dark greyish to black in colour	Dark grey patches on the reverse side	8	1.12	Septate, branched initially hyaline, turned into dark brown	Bullet shaped	Hyaline	14.0 x 5.1 µm	*Colletotrichum* sp.
5	VY CF2	Dull white, abundant, fluffy, aerial mycelia, turned olive-grey or grey	Greyish to black	4	2.25	Brown	Ellipsoid to ovoid	Brown	20.0 x 8.6 µm	*Lasiodiplodia* sp.
6	VP CB	Creamy yellow, translucent, flat colonies, initially circular later become wavy outer margin	Creamy yellow	2		-	-	-	-	*Pectobacterium* sp.

incubation. The immature conidia of the fungal isolate were unicellular, hyaline, ellipsoid-ovoid, broadly round at the apex with truncate or round base with thick walls. Conidia on maturation turned brown in colour with transverse septa and longitudinal striations. The length and breadth of the conidia were 20.0 x 8.6 µm. Based on the above features, The pathogen was tentatively identified as *Lasiodiplodia* sp.

4.4.1.D. Cultural and morphological characterisation of fungi obtained from *Spathoglottis* spp.

Cultural and morphological characters of fungal pathogens isolated from *Spathoglottis* spp. are detailed in Table 4.19.

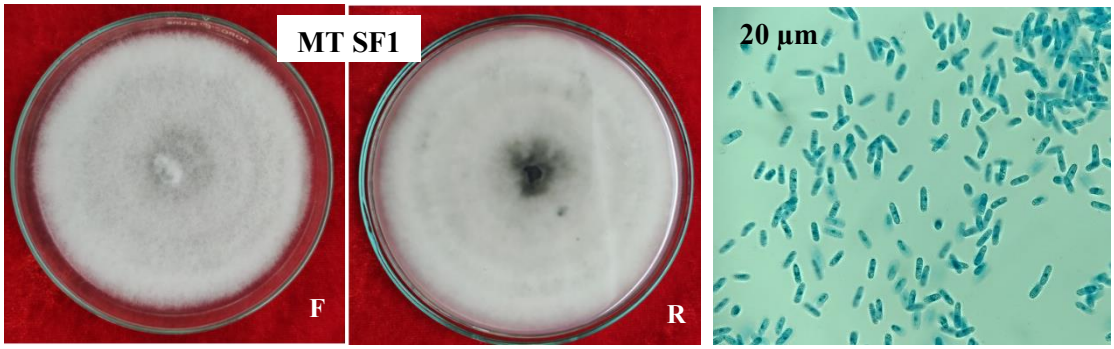
D.1. MT SF1

The leaf blight causing pathogen obtained from *Spathoglottis* spp. initially produced white, fluffy and woolly textured mycelia that later turned dull white with greenish grey patches. The reverse of the petri dish showed white colour with dark greenish patches at the centre. The colony attained 90 mm growth at 7 DOI with an average growth rate of 0.9 cm per day. The mycelia were hyaline, septate and branched. The conidia produced abundantly and were intermingled with hyphae. The conidia were bullet-shaped, hyaline and single celled with a central oil globule (Plate 9). The size of conidia was measured as 14.0 x 5.9 µm. Based on the above characters, The pathogen was tentatively identified as *Colletotrichum* sp.

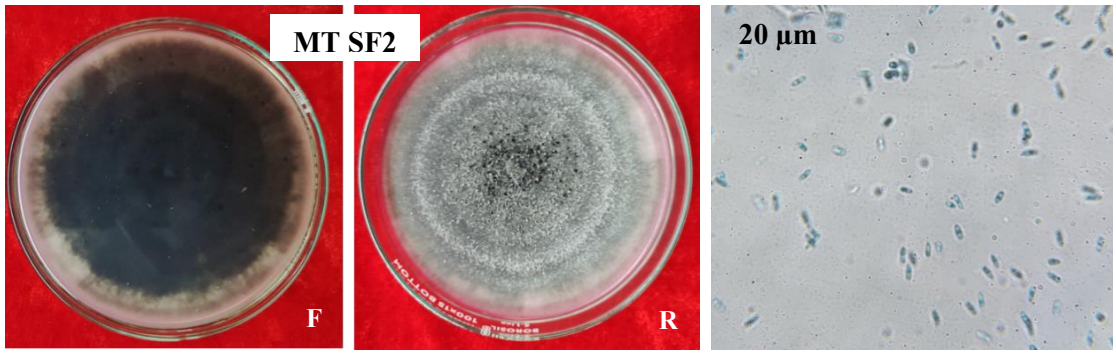
D.2. MT SF2

The fungus obtained from leaf blight symptom produced flat, subaerial greyish to dull black, woolly mycelia with irregular margin (Plate 9). The colony showed concentric zonation with black reverse side. The colony attained full growth after ten days of incubation, and the pycnidia were produced after 17 days of incubation with an average growth rate of 1.28 cm per day. The hyphae were, branched, septate and hyaline turned brown. The conidia were hyaline, unicellular, and oval shaped. Each conidium had two oil droplets inside with a size of 1.55 x 0.75 µm. The pathogen could not be identified based on cultural and morphological features.

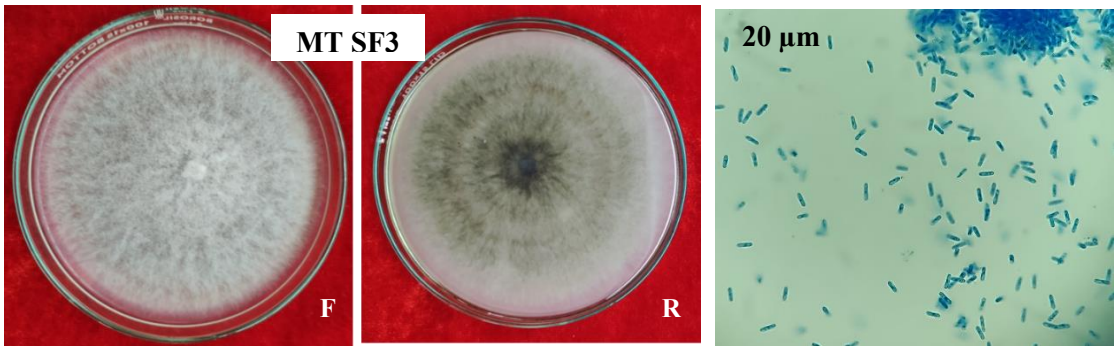
Plate 9 : Cultural and morphological characters of fungal pathogens in *Spathoglottis* spp.



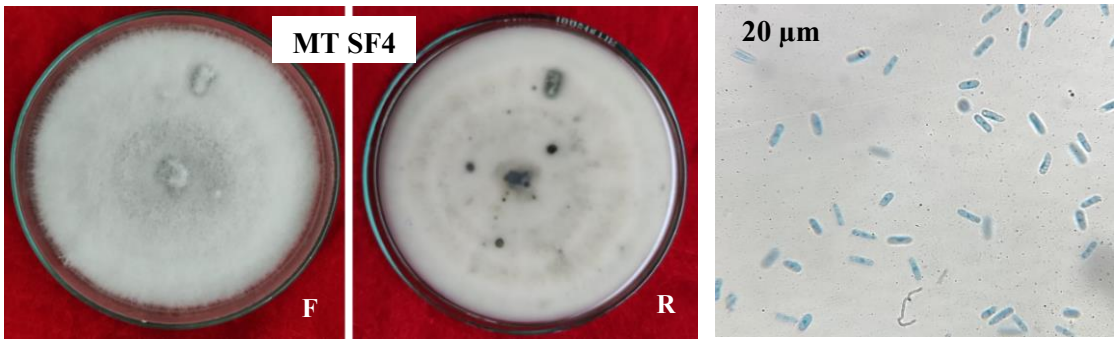
***Colletotrichum* sp.**



Unidentified species



***Colletotrichum* sp.**



***Colletotrichum* sp.**

F- Front side R- Rear side

Table 4.19. Cultural and morphological characters of fungal pathogens in *Spathoglottis* spp.

Sl. No.	Isolate	Cultural characters	Reverse side of Petri plate	Days for full growth	Growth rate (cm/day)	Morphological characters				Pathogen identified
						Hyphae	Shape of conidia	Colour of conidia	Dimension of the conidia (400X)	
1	MT SF1	White fluffy, woolly mycelia later turn dull white with greenish grey patches	Dark greenish patches at the centre	7	1.28	Hyaline, septate and branched	Bullet shaped hyaline	Hyaline	14.0 x 5.9 µm	*Colletotrichum* sp.
2	MT SF2	Subaerial greyish to dull black woolly mycelia with irregular margin	Black	10	0.9	Brown	Oval	Hyaline	1.55 x 0.75 µm	*Unknown*
3	MT SF3	Greyish to dull white woolly mycelia	Grey concentric patches	9	1.00	Branched, septate, initially hyaline, later become dark brown	Cylindrical	Hyaline	15.0 x 8.2 µm	*Colletotrichum* sp.
4	MT SF4	White fluffy, woolly textured mycelia later turn dull white with greenish grey patches	Dark greenish patches	7	1.28	Hyaline, septate and branched	Bullet-shaped	Hyaline	14.9 x 6 .2 µm	*Colletotrichum* sp.
5	MT SF5	White fluffy, woolly textured mycelia turn dull white with greenish grey	Dark greenish patches	7	1.28	Hyaline, septate and branched	Bullet-shaped	Hyaline	15.2 x 7.0 µm	*Colletotrichum* sp.

D.3. MT SF3

The fungus isolated from leaf blight symptom produced greyish to dull white woolly mycelia with greenish to grey concentric patches on the reverse side (Plate 9). The fungal colony was flat, subaerial with sparse growth and attained 90 mm growth at 9 DOI with an average growth rate of 1.0 cm per day. The hyphae were branched, septate, initially hyaline and later dark brown. The conidia were hyaline, one celled cylindrical to bullet shaped with an oil globule at the centre. The conidial size recorded was 15.0 x 8.2 µm. Based on cultural and morphological characteristics, the pathogen was tentatively identified as *Colletotrichum* sp.

D.4. MT SF4

The leaf blight causing pathogen initially produced white, fluffy and woolly textured mycelia that later turned dull white with greenish grey patches (Plate 9). The reverse of the petri dish was white with dark greenish patches. The colony attained 90 mm growth after seven days with an average growth rate of 1.28 cm per day. The hyphae were hyaline, septate, and branched. The conidia were bullet-shaped hyaline, single celled with an oil globule at its centre. The size of the conidia measured was 14.9 x 6 .2 µm. Based on the above features, The pathogen was tentatively identified as *Colletotrichum* sp.

D.5. MT SF5

The leaf blight pathogen MT GF5, initially produced white, fluffy and woolly textured mycelia that later turned dull white with greenish grey patches (Plate 9a). The reverse of the petri dish showed white with dull green patches. The colony attained 90 mm growth at 7 DOI with an average growth rate of 1.28 cm per day. The mycelia were hyaline, septate and branched. The conidia were characterised as bullet-shaped hyaline and single celled with abundant production. The size of the conidia was 15.2 x 7.0 µm. Based on cultural and morphological features, the pathogen was tentatively identified as *Colletotrichum* sp.

Table 4.19. Cultural and morphological characters of fungal pathogens in *Spathoglottis* spp. (Contd…)

Sl. No.	Isolate	Cultural characters	Reverse side of Petri plate	Days for full growth	Growth rate (cm/day)	Morphological characters				Pathogen identified
						Hyphae	Shape of conidia	Colour of conidia	Dimension of the conidia (400X)	
6	MT SF6	White fluffy, woolly textured mycelia	Dark greenish patches	7	1.28	Hyaline, septate and branched	Bullet-shaped	Hyaline	15.7 x 7.4 µm	*Colletotrichum* sp.
7	KO SF1	Greyish, compact aerial mycelia, gradually turn greenish grey	Grey	8	1.12	Mycelia were hyaline, septate and branched	Bullet-shaped	Hyaline	16.0 x 7.5 µm	. *Colletotrichum* sp.
8	KO SF2	Dull white, delicate woolly mycelia	White	8	1.12	Hyaline and septate	Macroconida - single celled fusiform shaped	Hyaline	2.5 x 0.73 µm	*Fusarium* sp.
9	KO SF3	Grey mycelia with cottony texture	Dark grey	7	1.28	Cylindrical shaped	Bullet-shaped	Hyaline	17.3 x 9.1 µm	*Colletotrichum* sp.

D.6. MT SF6

The leaf spot pathogen initially produced white, fluffy and woolly textured mycelia that later turned dull white with greenish grey patches (Plate 9a). The reverse of the petri dish showed white with dark greenish patches. The colony attained 90 mm growth after 7 DOI with an average growth rate of 1.28 cm per day. The mycelia were hyaline, septate and branched. The conidia were bullet-shaped hyaline and single celled, with an oil globule at the centre of the cell. The size of conidia was measured as 15.7 x 7.4 µm. The pathogen was tentatively identified as *Colletotrichum* sp.

D.7. KO SF1

The fungus causing leaf blight symptom produced greyish, compact aerial mycelia which gradually turned greenish grey in colour. The mycelia attained 90 mm growth at 8 DOI with an average growth rate of 1.12 cm per day. The fungal colony was slightly raised at the centre with even margin. The mycelia were hyaline, septate and branched with abundant production of conidia. The conidia were bullet-shaped hyaline and single celled with a central oil globule. with an average size of 16.0 x 7.5 µm. The pathogen was tentatively identified as *Colletotrichum* sp. based on the above features.

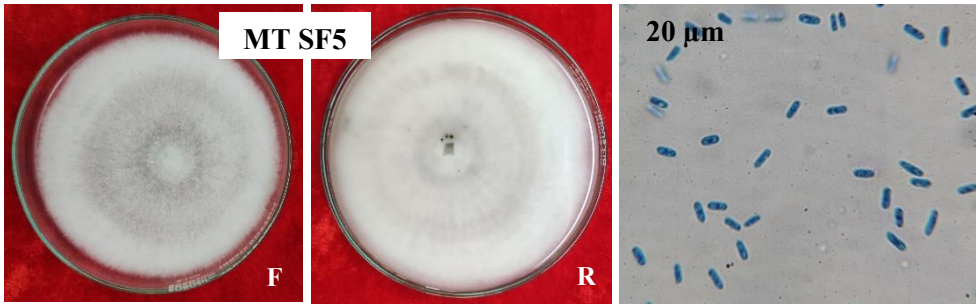
D.8. KO SF2

The pathogen associated with leaf blight symptom produced dull white, delicate woolly mycelia with very sparse growth. (Plate 9a). The culture attained 90 mm growth at 8 DOI with an average growth rate of 1.12 cm per day. The mycelia were hyaline and septate. Macroconidia were scattered over the mycelia with a size of 2.5 x 0.73 µm. Based on cultural and morphological features, the pathogen was tentatively identified as *Fusarium* sp.

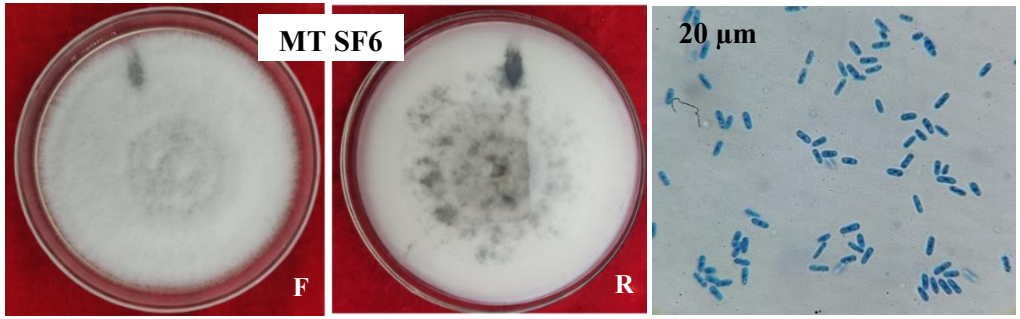
D.9. KO SF3

The fungus isolated from leaf blight disease produced grey mycelia with cottony texture (Plate 9a). The colony was flat with mycelial clumps on the surface of the culture with dark grey reverse side. The mycelia attained 90 mm growth at 7 DOI with

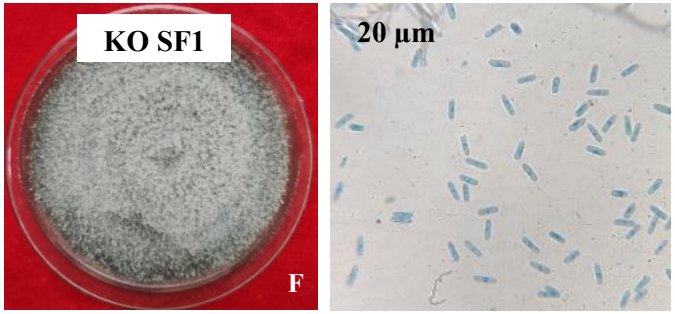
Plate 9a : Cultural and morphological characters of fungal pathogens in *Spathoglottis* spp.



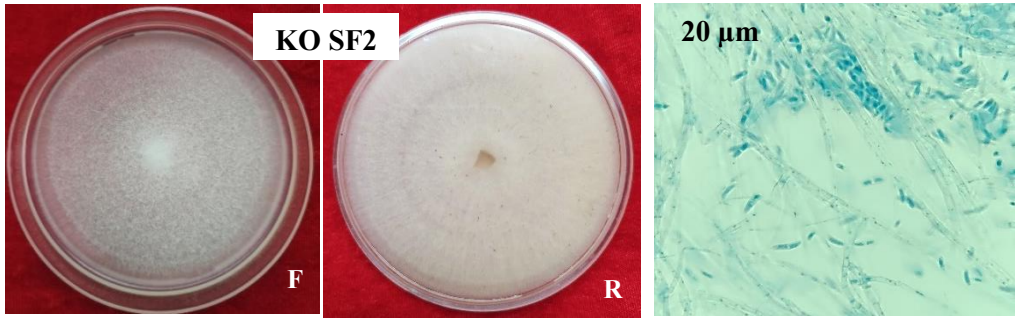
***Colletotrichum* sp.**



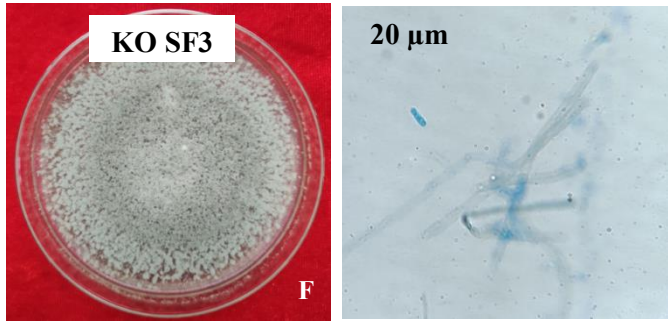
***Colletotrichum* sp.**



***Colletotrichum* sp.**



***Fusarium* sp.**



***Colletotrichum* sp.**

F- Front side R- Rear side

an average growth rate of 1.28 cm per day. with scanty production of conidia. The conidia were cylindrical in shape and without oil globules. The size of conidia was measured as 2.5 x 0.73 µm. The pathogen was tentatively identified as *Colletotrichum* sp.

4.4.2. Characterisation and identification of bacterial pathogen

Cultural, morphological and biochemical characters of bacterial pathogen (VP CB) obtained from *Cattleya* spp. were studied in detail The findings are given below.

4.4.2.1. Cultural characterisation

The bacterial pathogen produced yellowish translucent white, small and flat colonies on nutrient agar medium after 72 hours of incubation (Plate 10). The colony margin was initially circular later became wavy (Table 4.18).

4.4.2.2. Morphological characterisation

Microscopic studies revealed that bacterium was rod shaped. Bacteria were observed as pink colonies, and gram staining reaction confirmed it as gram negative bacteria (Plate 10).

4.4.2.3. Biochemical characterisation

Several biochemical tests *viz.* potassium hydroxide test, catalase test, oxidase test, hydrolysis of gelatin, indole production test, methyl red test, Voges-Proskauer test, citrate test, and hydrogen sulphide production test, were carried out to confirm the identity of bacterial isolates after characterising cultural and morphological features (Table 4.20).

A. Potassium hydroxide (KOH) test

The bacterial pathogen was KOH positive. When bacterial inoculum was mixed with three per cent of KOH solution, slimy, viscous threads were observed on the inoculation loop, indicated gram-negative nature of the bacteria (Plate 10).

B. Catalase test

When a loopful of bacteria was mixed with 30 per cent H_2O_2 solution, oxygen bubbles were formed which confirmed the ability of the isolates to produce catalase enzyme (Plate 10).

C. Oxidase test

The bacteria tested negative for oxidase reaction as there was no purple colour formation in the oxidase disc, confirmed the inability of the bacteria to produce cytochrome C oxidase enzyme. (Plate 10).

D. Gelatin hydrolysis test

The bacterium tested positive for gelatin hydrolysis test. Solidified gelatin in the tubes got liquified after 3 DOI at room temperature, which confirmed the presence of gelatinase enzyme produced by the bacteria. (Plate 10).

E. Indole production test

Bacteria showed negative result for the production of indole. The reaction failed to give cherry red coloured layer over the surface of the medium upon the addition of Kovac's reagent (Plate 10).

F. Methyl red test

The bacteria tested positive for methyl red test as there was development of red colour upon addition of methyl red indicator, thus indicated the acid producing ability of the bacteria (Plate 10).

G. Voges-Proskauer test

The bacteria tested negative for Voges-Proskauer test as it failed to show a pinkish red coloration at the surface of the medium, which indicated its inability to ferment glucose (Plate 10).

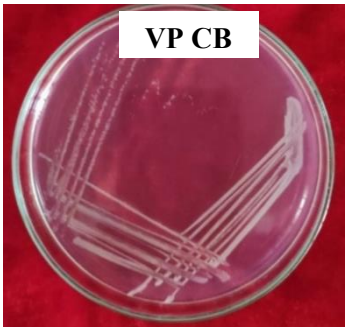
H. Citrate test

The bacteria showed a positive result for the citrate test after 24 h of incubation, indicating its ability to utilize citrate compound (Plate 10).

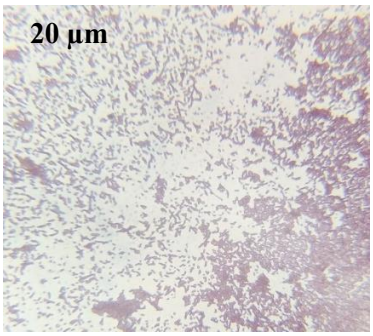
I. Citrate test

The bacteria showed a positive result for the citrate test after 24 h of incubation, indicating its ability to utilize citrate compound (Plate 10).

Plate 10 : Cultural, morphological and biochemical characters of bacterial pathogen (*Pectobacterium aroidearum)* in *Cattleya* spp.

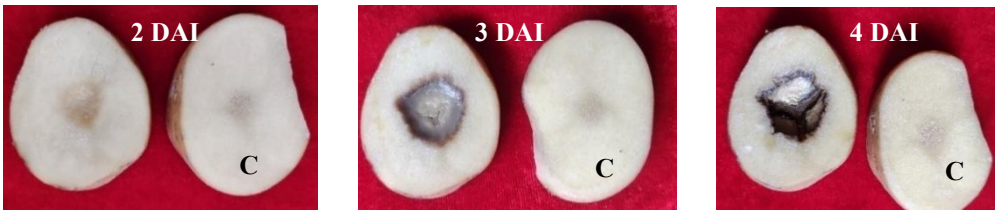


Cultural characters



Gram staining

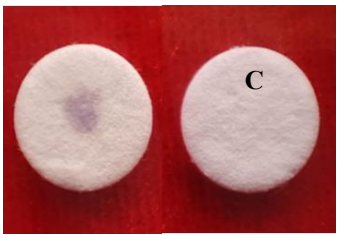
Ooze test



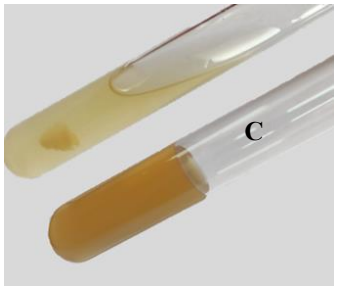
Potato soft rot test (+)

KOH test (+)

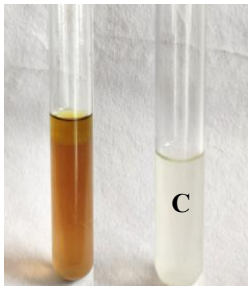
Catalase test (+)



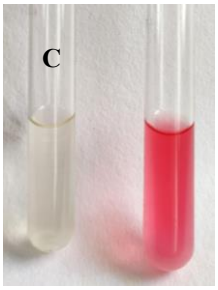
Oxidase test (-)



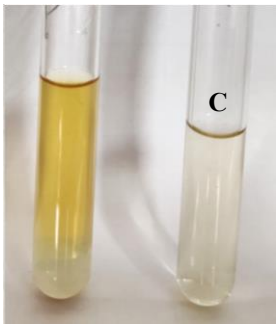
Gelatin hydrolysis test (+)



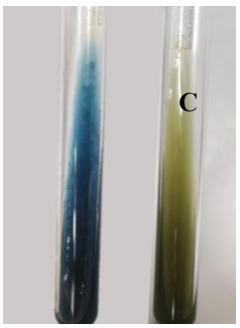
Indole production test (-)



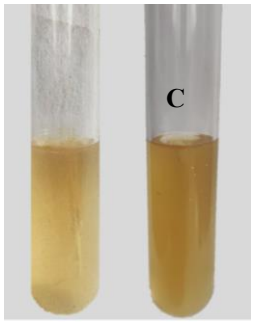
MR test (+)



VP test (-)



Citrate fermentation test (+)



H_2S production test (-)

C- Control

J. Hydrogen sulphide production test

The bacterial pathogen showed negative results for hydrogen sulphide production test, indicating its inability to reduce sulphur to hydrogen sulphide (Plate 10).

Based on the cultural, morphological and biochemical characters, the pathogen was tentatively identified as *Pectobacterium* sp.

4.4.3. Molecular characterisation of major pathogens

After cultural and morphological characterisation, molecular characterisation of the major pathogens was done for species level identification. Nine fungal cultures and one bacterial culture were sent for molecular identification based on the incidence and severity of the diseases observed during the survey. These pathogens were identified and listed in Table 21.

The isolate VL DF1 causing leaf spot symptom in *Dendrobium* spp. was characterised by amplification and sequencing of large subunit ribosomal ribonucleic acid (LSU rRNA) gene using primers LROR and LR7, and found 100 per cent identity with *Colletotrichum boninense* strain CBS241.78 having accession number DQ286167.1 with query coverage of 96 per cent (Fig. 4.1 & 4.2).

Another isolate VY DF, causing leaf blight symptom in *Dendrobium* spp., was characterised by amplification and sequencing of the internal transcribed spacer (ITS) region using primers ITS-1F/ITS-4R. BLASTn analysis revealed 100 per cent identity with *Fusarium proliferatum* strain DWBM-2-2-1 having accession number ON527497.1with 99 per cent query coverage (Fig. 4.3 & 4.4).

The isolate MT DF1 associate with leaf blight in *Dendrobium* spp. was identified by amplification and sequencing of the ITS region, using primers ITS-1F/ITS-4R. BLASTn analysis showed 93.29 per cent identity with *Athelia rolfsii* strain XFTH. The accession number of the identical sequence was JQ340325.1 with a query coverage of 99 per cent (Fig. 4.5 & 4.6).

The pathogen VP DF1, causing leaf spot symptom in *Dendrobium* spp., was characterised by amplification and sequencing of the ITS region using primers ITS-

1F/ITS-4R. BLASTn analysis revealed 100 per cent identity with *Alternaria alternata* strain NFMLCH44 376 having accession number KM458821.1 with 41 per cent query coverage (Fig. 4.7 & 4.8).

Another isolate VY DF, causing leaf blight symptom in *Dendrobium* spp., was characterised by amplification and sequencing of the LSU rRNA region using primers LROR and LR7. BLASTn analysis revealed 99.75 per cent identity with *Fusarium oxysporum* having accession number CP052041.1 with 100 per cent query coverage (Fig. 4.9 & 4.10).

The pathogen (VK OF3) isolated from leaf spot disease in *Oncidium* spp. was characterised by amplification and sequencing of LSU rRNA gene using primers LROR and LR7 and found 100 per cent identity with *Colletotrichum* sp. AR3750. The accession number of the identical sequence was DQ286216.1, with a query coverage of 97 per cent (Fig. 4.11 & 4.12).

The leaf spot causing pathogen, IK OF1 isolated from *Oncidium* spp., was characterised by amplification and sequencing of LSU rRNA gene using primers LROR and LR7. The BLASTn analysis revealed that the fungus exhibited 99.86 per cent identity with *Diaporthe tulliensis* isolate SF24 with 99.86 per cent similarity and 100 per cent query coverage (Fig. 4.13 & 4.14).

The isolate VY CF2 causing leaf spot disease in *Cattleya* spp. was identified by amplification and sequencing of LSU rRNA gene using primers LROR and LR7. The BLASTn analysis showed 97.13 per cent identity with *Lasiodiplodia theobromae* isolate VTCA having accession number KC442316.1 with 94 per cent query coverage. The culture was deposited in NCBI GenBank with an accession number of OQ348267 (Fig. 4.15 & 4.16).

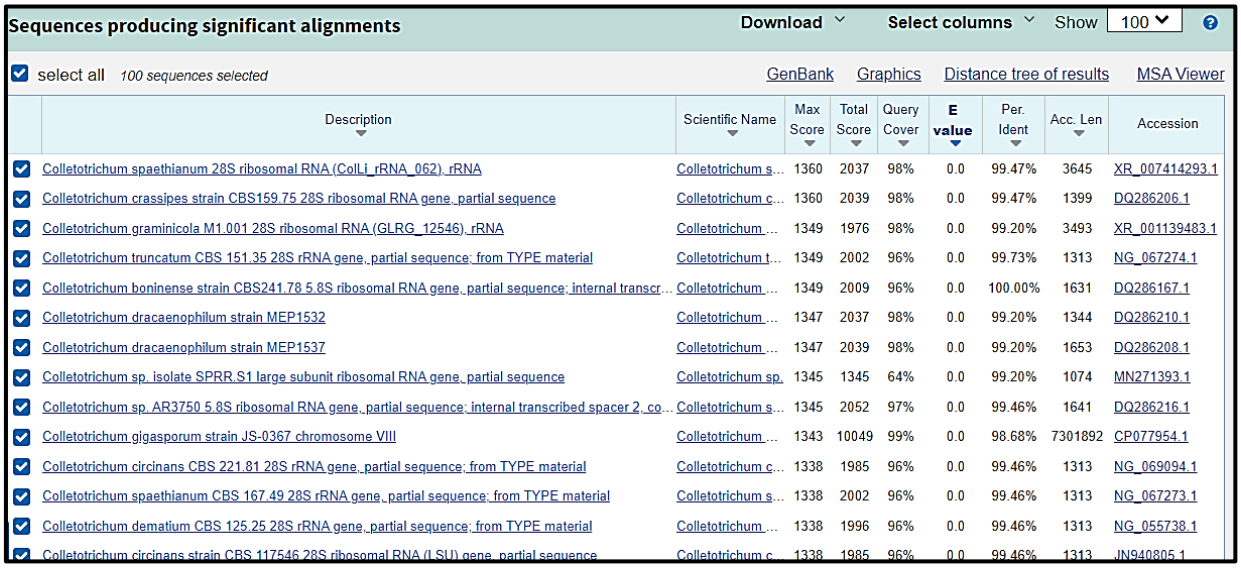
The fungus MT SF1, causing leaf blight symptom in *Spathoglottis* spp., was characterised by amplification and sequencing of LSU rRNA gene using primers LROR and LR7. The BLASTn analysis revealed that the fungus exhibited 99.87 per cent identity with *Colletotrichum gloeosporioides* strain RP205 of accession number JF441221.1 with 95 per cent query coverage (Fig. 4.17 & 4.18).

Table 4.20. Biochemical reaction of bacterial isolate

Sl. No.	Biochemical test	Reaction of bacterial isolate (VP CB)
1	Potassium hydroxide test	+
2	Catalase test	+
3	Oxidase test	–
4	Gelatin hydrolysis test	+
5	Indole production test	–
6	Methyl red test	+
7	Voges-Proskauer test	–
8	Citrate test	+
9	Hydrogen sulphide production test	–

Table 4.21. Molecular identification of pathogens with accession numbers

Sl. No.	Isolate	Pathogen	Sequence ID
1	VL DF1	*Colletotrichum boninense*	DQ286167.1
2	VY DF	*Fusarium proliferatum*	ON527497.1
3	MT DF1	*Athelia rolfsii* (*S. rolfsii*)	JQ340325.1
4	IK DF1	*Fusarium Oxysporum*	CP052041.1
5	VP DF1	*Alternaria alternata*	KM458821.1
6	VK OF3	*Colletotrichum* sp.	DQ286216.1
7	IK OF1	*Diaporthe tulliensis*	OM228732.1
8	VY CF2	*Lasiodiplodia theobromae*	KC442316.1
9	MT SF1	*Colletotrichum gloeosporioides*	JF441221.1
10	VP CB	*Pectobacterium aroidearum*	MH549224.1



Sequences producing significant alignments — Download — Select columns — Show 100

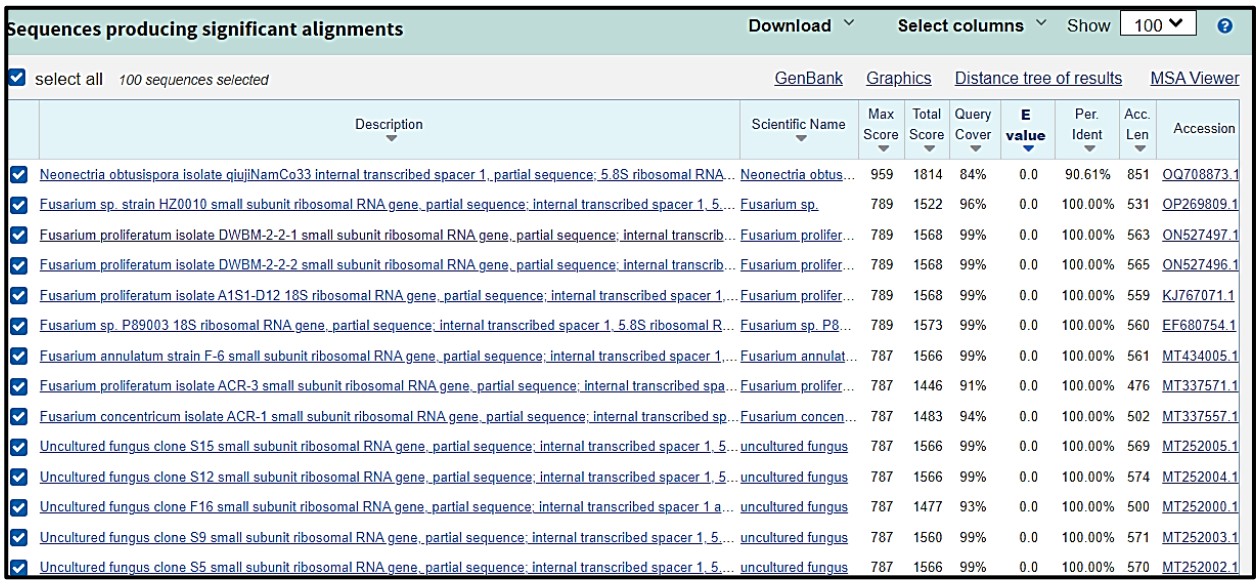
select all 100 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Colletotrichum spaethianum 28S ribosomal RNA (ColLi_rRNA_062), rRNA	Colletotrichum s...	1360	2037	98%	0.0	99.47%	3645	XR_007414293.1
Colletotrichum crassipes strain CBS159.75 28S ribosomal RNA gene, partial sequence	Colletotrichum c...	1360	2039	98%	0.0	99.47%	1399	DQ286206.1
Colletotrichum graminicola M1.001 28S ribosomal RNA (GLRG_12546), rRNA	Colletotrichum ...	1349	1976	98%	0.0	99.20%	3493	XR_001139483.1
Colletotrichum truncatum CBS 151.35 28S rRNA gene, partial sequence; from TYPE material	Colletotrichum t...	1349	2002	96%	0.0	99.73%	1313	NG_067274.1
Colletotrichum boninense strain CBS241.78 5.8S ribosomal RNA gene, partial sequence; internal transcr...	Colletotrichum ...	1349	2009	96%	0.0	100.00%	1631	DQ286167.1
Colletotrichum dracaenophilum strain MEP1532	Colletotrichum ...	1347	2037	98%	0.0	99.20%	1344	DQ286210.1
Colletotrichum dracaenophilum strain MEP1537	Colletotrichum ...	1347	2039	98%	0.0	99.20%	1653	DQ286208.1
Colletotrichum sp. isolate SPRR.S1 large subunit ribosomal RNA gene, partial sequence	Colletotrichum sp.	1345	1345	64%	0.0	99.20%	1074	MN271393.1
Colletotrichum sp. AR3750 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, co...	Colletotrichum s...	1345	2052	97%	0.0	99.46%	1641	DQ286216.1
Colletotrichum gigasporum strain JS-0367 chromosome VIII	Colletotrichum ...	1343	10049	99%	0.0	98.68%	7301892	CP077954.1
Colletotrichum circinans CBS 221.81 28S rRNA gene, partial sequence; from TYPE material	Colletotrichum c...	1338	1985	96%	0.0	99.46%	1313	NG_069094.1
Colletotrichum spaethianum CBS 167.49 28S rRNA gene, partial sequence; from TYPE material	Colletotrichum s...	1338	2002	96%	0.0	99.46%	1313	NG_067273.1
Colletotrichum dematium CBS 125.25 28S rRNA gene, partial sequence; from TYPE material	Colletotrichum ...	1338	1996	96%	0.0	99.46%	1313	NG_055738.1
Colletotrichum circinans strain CBS 117546 28S ribosomal RNA (LSU) gene, partial sequence	Colletotrichum c...	1338	1985	96%	0.0	99.46%	1313	JN940805.1

Fig. 4.1. BLASTn text output of nucleotide sequence of LSU gene of *Colletotrichum boninense* (VL DF1)

AGGGGGAGGAAAGGAAAACCACAGGGATTGCCTCAGTAACGGCGAGTGAAGCGGCAAC
AGCTCAAATTTGAAATCTGGCCCGTCCGGGTCCGAGTTGTAATTTGCAGAGGATGCTTTT
GGCGCGGTGCCTTCCGAGTTCCCTGGAACGGGACGCCATAGAGGGTGAGAGCCCCGTAC
GGTTGGACACCAAGCCTTTGTAAAGCTCCTTCGACGAGTCGAGTAGTTTGGGAATGCTGC
TCAAAATGGGAGGTATATTTCTTCTAAAGCTAAATACCGGCCAGAGACCGATAGCGCAC
AAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGGGTTAAACAGCACGTGAA
ATTGTTAAAAGGGAAGCGCTTGTGACCAGACTTGCGCCCGGTGAATCACCCGACGGTTCT
CCGGGGGCTCACGCCCTAGGCTTCGTCACTGACCTCCACGTCCGCCTACTCCTCAGCGCA
TCGTTTCTACGCTGAGGGCGAGGTATGGGTGAGACGCTTGAGCGCCATCCATTTTCAGGG
CTAGTACATTCGGCAGGTGAGTTGTTACACAGTCCTTAGCGGATTCCGACTTCCATGGCC
ACCGTCCTGCTGTCAAGATGTACTAACACCTTTTGTGGTGTCTGATGAGCGTCTACTCTG
GCACCTTAACCTCGCGTTCGGTTCATCCCGCATCGCCAGTTCTGCTTACCAAAAATGGCC
CACTAGTGTTGATACATTCGAATGCCCACGTTCAACTAAGTAACAAGGGCTTCTTACATA
TTTAAAGTTTGAGAATGGATGAAGGCAATATAGCGCCCCCGAGTCCCTAATCATTCGCTT
TACCTCATAAAACTGAGTTCAACACTGCTATCCTGAGGGAAACTTCGGCGGAAACCAGC
TACTAGAAGGTTCGATTAGTCTTTCGCCCCCATGCGCATATTTGACGATCGATTTGCACG
TCAGAACCGCTGCGAGCCTCCACCAGAGTTTCCTCTGGCTTCACCCTATACACGCATAGT
TCACCTTCTTTCGGGTCCAACCCTATATGCTCTTACTCAAATCCATCCGAGAACATCAGG
ATCGGTCGATGATGCGCCGAAGCTCTCACCTGCGTTCACTTTCATTACGCGTAGGGGTTT
AACACCCAAACACTCGCACATAAGGTTGACTCCC

Fig. 4.2. Nucleotide sequence of LSU region of *Colletotrichum boninense* (VL DF1)



Sequences producing significant alignments — Download — Select columns — Show 100

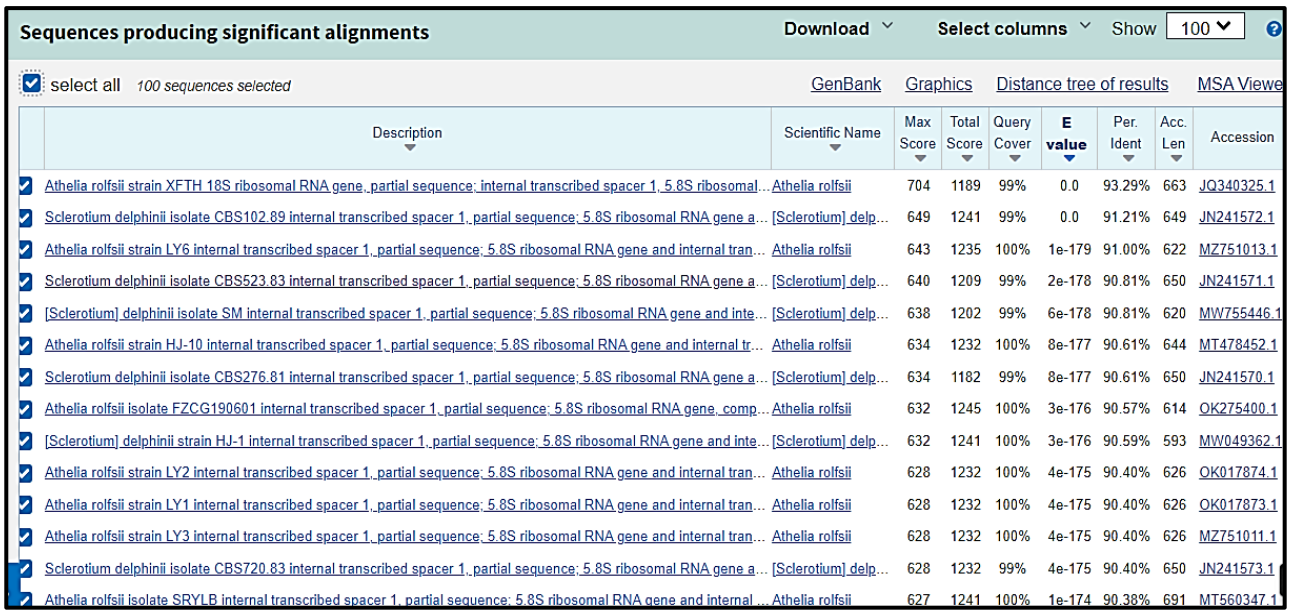
select all 100 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Neonectria obtusispora isolate qiujiNamCo33 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA...	Neonectria obtus...	959	1814	84%	0.0	90.61%	851	OQ708873.1
Fusarium sp. strain HZ0010 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5....	Fusarium sp.	789	1522	96%	0.0	100.00%	531	OP269809.1
Fusarium proliferatum isolate DWBM-2-2-1 small subunit ribosomal RNA gene, partial sequence; internal transcrib...	Fusarium prolifer...	789	1568	99%	0.0	100.00%	563	ON527497.1
Fusarium proliferatum isolate DWBM-2-2-2 small subunit ribosomal RNA gene, partial sequence; internal transcrib...	Fusarium prolifer...	789	1568	99%	0.0	100.00%	565	ON527496.1
Fusarium proliferatum isolate A1S1-D12 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,...	Fusarium prolifer...	789	1568	99%	0.0	100.00%	559	KJ767071.1
Fusarium sp. P89003 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal R...	Fusarium sp. P8...	789	1573	99%	0.0	100.00%	560	EF680754.1
Fusarium annulatum strain F-6 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1,...	Fusarium annulat...	787	1566	99%	0.0	100.00%	561	MT434005.1
Fusarium proliferatum isolate ACR-3 small subunit ribosomal RNA gene, partial sequence; internal transcribed spa...	Fusarium prolifer...	787	1446	91%	0.0	100.00%	476	MT337571.1
Fusarium concentricum isolate ACR-1 small subunit ribosomal RNA gene, partial sequence; internal transcribed sp...	Fusarium concen...	787	1483	94%	0.0	100.00%	502	MT337557.1
Uncultured fungus clone S15 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...	uncultured fungus	787	1566	99%	0.0	100.00%	569	MT252005.1
Uncultured fungus clone S12 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...	uncultured fungus	787	1566	99%	0.0	100.00%	574	MT252004.1
Uncultured fungus clone F16 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 a...	uncultured fungus	787	1477	93%	0.0	100.00%	500	MT252000.1
Uncultured fungus clone S9 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5....	uncultured fungus	787	1560	99%	0.0	100.00%	571	MT252003.1
Uncultured fungus clone S5 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5....	uncultured fungus	787	1566	99%	0.0	100.00%	570	MT252002.1

Fig. 4.3. BLASTn text output of nucleotide sequence of ITS gene of *Fusarium proliferatum* (VY DF)

GACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAAATAAATCAAAACT
TTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGT
AATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCA
GTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCCCGGGTTTGGT
GTTGGGGATCGGCGAGCCCTTGCGGCAAGCCGGCCCCGAAATCTAGTGGCGGTCTCGCT
GCAGCTTCCATTGCGTAGTAGTAAAACCCTCGCAACTGGTACGCGGCGCGGCCAAGCCG
TTAAACCCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTA
ACCATATCATAACGCAATGGAAGCTGCAGCGAGACCGCCACTAGATTTCGGGGCCGGCT
TGCCGCAAGGGCTCGCCGATCCCCAACACCAAACCCGGGGGCTTGAGGGTTGAAATGAC
GCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAAAGATTCGA
TGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTGCTGCGTTCTTCATCGATG
CCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTTATTTATGGTTTTACTCAGAAGTT
ACATATAGAAACAGAGTTTAGGGGTCCTCTGGCGGGCCGTCCCGTTTTACCGGGAGCGG
GCTGATCCGCCGAGGCAACAATTGGTATGTTCACAGGGGTTTGGGAGTTGTAAACTCGG
TAATGATCCCTCCGCAGGTTCCTAC

Fig. 4.4. Nucleotide sequence of ITS region of *Fusarium proliferatum* (VY DF)



Sequences producing significant alignments — Download — Select columns — Show 100

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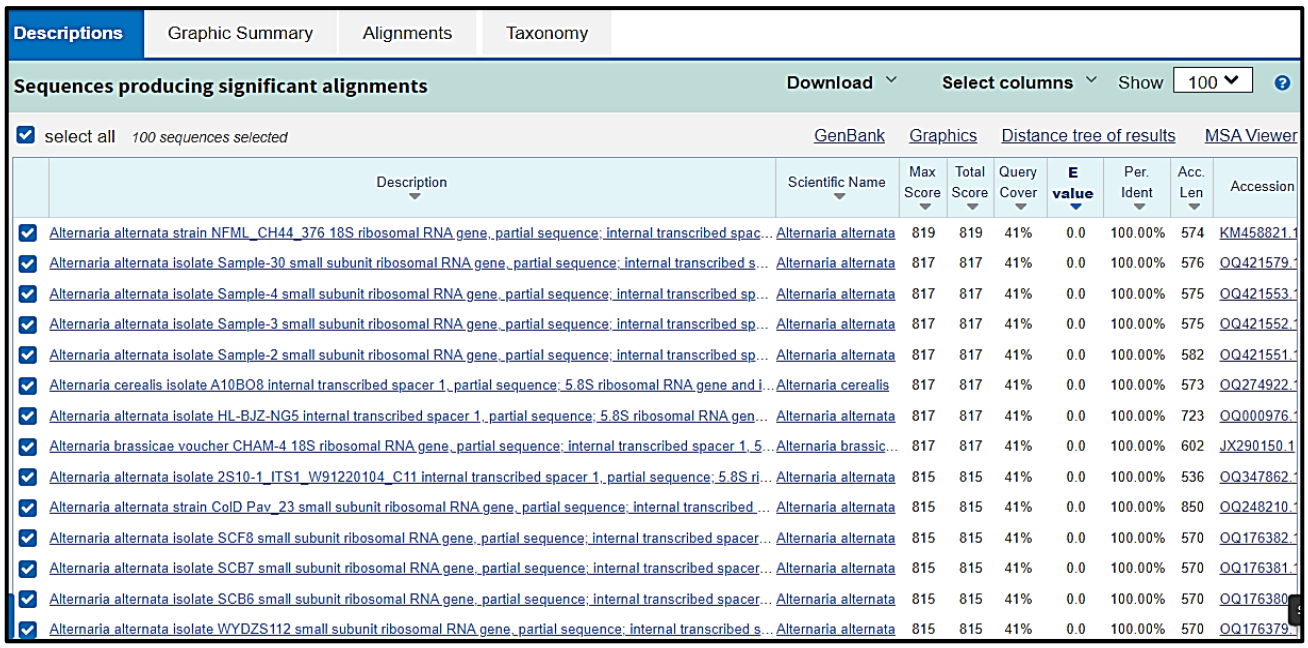
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Athelia rolfsii strain XFTH 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal...	Athelia rolfsii	704	1189	99%	0.0	93.29%	663	JQ340325.1
Sclerotium delphinii isolate CBS102.89 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	[Sclerotium] delp...	649	1241	99%	0.0	91.21%	649	JN241572.1
Athelia rolfsii strain LY6 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal tran...	Athelia rolfsii	643	1235	100%	1e-179	91.00%	622	MZ751013.1
Sclerotium delphinii isolate CBS523.83 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	[Sclerotium] delp...	640	1209	99%	2e-178	90.81%	650	JN241571.1
[Sclerotium] delphinii isolate SM internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inte...	[Sclerotium] delp...	638	1202	99%	6e-178	90.81%	620	MW755446.1
Athelia rolfsii strain HJ-10 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal tr...	Athelia rolfsii	634	1232	100%	8e-177	90.61%	644	MT478452.1
Sclerotium delphinii isolate CBS276.81 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	[Sclerotium] delp...	634	1182	99%	8e-177	90.61%	650	JN241570.1
Athelia rolfsii isolate FZCG190601 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, comp...	Athelia rolfsii	632	1245	100%	3e-176	90.57%	614	OK275400.1
[Sclerotium] delphinii strain HJ-1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inte...	[Sclerotium] delp...	632	1241	100%	3e-176	90.59%	593	MW049362.1
Athelia rolfsii strain LY2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal tran...	Athelia rolfsii	628	1232	100%	4e-175	90.40%	626	OK017874.1
Athelia rolfsii strain LY1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal tran...	Athelia rolfsii	628	1232	100%	4e-175	90.40%	626	OK017873.1
Athelia rolfsii strain LY3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal tran...	Athelia rolfsii	628	1232	100%	4e-175	90.40%	626	MZ751011.1
Sclerotium delphinii isolate CBS720.83 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	[Sclerotium] delp...	628	1232	99%	4e-175	90.40%	650	JN241573.1
Athelia rolfsii isolate SRYLB internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal ...	Athelia rolfsii	627	1241	100%	1e-174	90.38%	691	MT560347.1

Fig. 4.5. BLASTn text output of nucleotide sequence of ITS gene of *Athelia rolfsii* (*Sclerotium rolfsii*) (MT DF1)

TCTGGAGAAATCCTGACTATGATTACTCTATATAACTCTTATTGTATGTTACATAGAACGATC
TCATATTGAAGCTTTGTTTTTTTTTCCAGTTTCTCTTAATTGAAAAATACACAACTTTCCACAA
CGGAACTCTTGGCTCTTGCATCCATTAAAAACGCCACCAAATGCCATAAATAATGGGAATTG
CAAAATCCCGTGAATCCTCCAATCCTTTAACCCCCCTTGCCCCCTTTGGTATTCCCAAGGGCA
TGCCTGTTTGAAAATCATTAAATTCCCCACCTTACCAATTTTTGTATTTGTCCAGGCTTGGAT
GTGAAAATTGCTAATTAAAAATATCTGAATGGCTCTCTTTAAAACTATTAATAAGACATATA
AAAATGCCCGCCGTTGGTGTGATAATATGTCCACCCCTATACCAAAGGGGAATCCAGCTTGG
ATGCACTACTTATAAAATCATGCGCATATATCTTGCATGGCGTAGACATATTATCACACCAA
CCGCAGGCATTTCTATATGTCCTACTAATAATTTTAAAGAGAGCCAGTCAGATATTCTTAACT
AGCAACTCTCACATCCAGCCTTGACAAATACAAAAATTTGTAAGGTTGAGAATTTAATGACT
CTCAAACAGGCATGCCCCTCGGAATACCAAAGGGCGCAAGGTGCGTTCAAAGATTCGATGA
TTCACTGGATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCAAGA
GCCAAGAGATCCGTTGTTGAAAGTTGTGTATTTTTCAATTAAGAGAAACTTGTAAAAAAAAC
AAAGC

Fig. 4.6. Nucleotide sequence of ITS region of *Athelia rolfsii* (*Sclerotium rolfsii*) (MT DF1)



Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 100

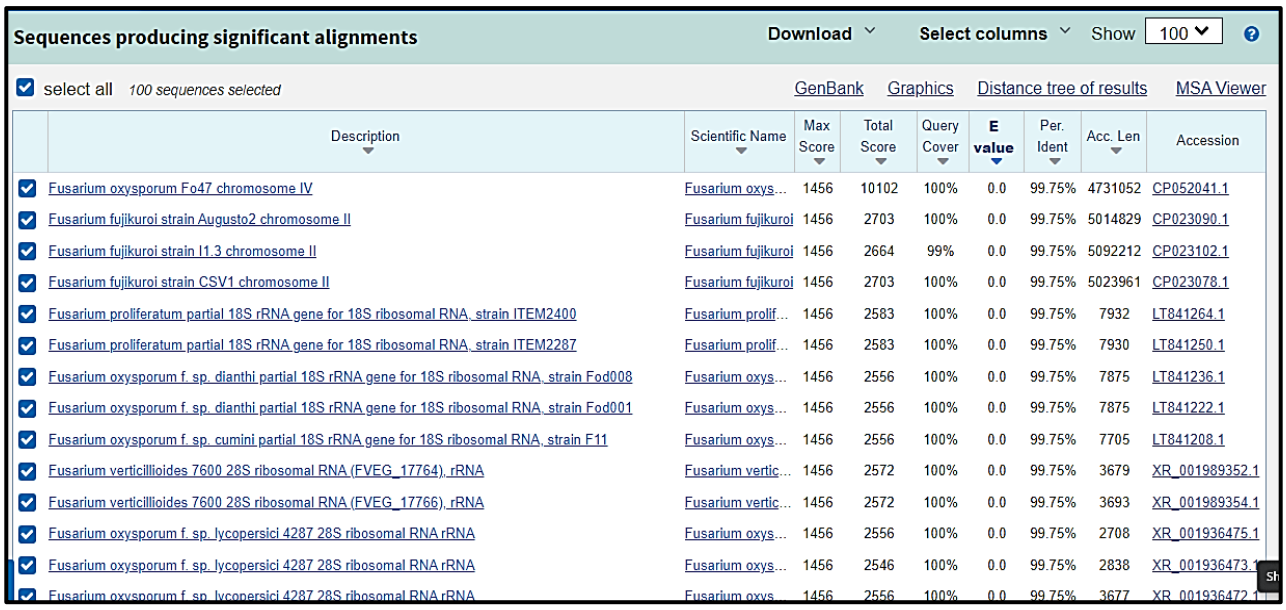
select all 100 sequences selected — GenBank | Graphics | Distance tree of results | MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Alternaria alternata strain NFML_CH44_376 18S ribosomal RNA gene, partial sequence; internal transcribed spac...	Alternaria alternata	819	819	41%	0.0	100.00%	574	KM458821.1
Alternaria alternata isolate Sample-30 small subunit ribosomal RNA gene, partial sequence; internal transcribed s...	Alternaria alternata	817	817	41%	0.0	100.00%	576	OQ421579.1
Alternaria alternata isolate Sample-4 small subunit ribosomal RNA gene, partial sequence; internal transcribed sp...	Alternaria alternata	817	817	41%	0.0	100.00%	575	OQ421553.1
Alternaria alternata isolate Sample-3 small subunit ribosomal RNA gene, partial sequence; internal transcribed sp...	Alternaria alternata	817	817	41%	0.0	100.00%	575	OQ421552.1
Alternaria alternata isolate Sample-2 small subunit ribosomal RNA gene, partial sequence; internal transcribed sp...	Alternaria alternata	817	817	41%	0.0	100.00%	582	OQ421551.1
Alternaria cerealis isolate A10BO8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i...	Alternaria cerealis	817	817	41%	0.0	100.00%	573	OQ274922.1
Alternaria alternata isolate HL-BJZ-NG5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen...	Alternaria alternata	817	817	41%	0.0	100.00%	723	OQ000976.1
Alternaria brassicae voucher CHAM-4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...	Alternaria brassic...	817	817	41%	0.0	100.00%	602	JX290150.1
Alternaria alternata isolate 2S10-1_ITS1_W91220104_C11 internal transcribed spacer 1, partial sequence; 5.8S ri...	Alternaria alternata	815	815	41%	0.0	100.00%	536	OQ347862.1
Alternaria alternata strain ColD Pav_23 small subunit ribosomal RNA gene, partial sequence; internal transcribed ...	Alternaria alternata	815	815	41%	0.0	100.00%	850	OQ248210.1
Alternaria alternata isolate SCF8 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer...	Alternaria alternata	815	815	41%	0.0	100.00%	570	OQ176382.1
Alternaria alternata isolate SCB7 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer...	Alternaria alternata	815	815	41%	0.0	100.00%	570	OQ176381.1
Alternaria alternata isolate SCB6 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer...	Alternaria alternata	815	815	41%	0.0	100.00%	570	OQ176380.1
Alternaria alternata isolate WYDZS112 small subunit ribosomal RNA gene, partial sequence; internal transcribed s...	Alternaria alternata	815	815	41%	0.0	100.00%	570	OQ176379.1

Fig. 4.7. BLASTn text output of nucleotide sequence of ITS gene of *Alternaria alternata* (VP DF1)

CCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAATAA
TTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGA
TAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTT
GGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGG
GCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTT
TCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTT
TCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGA
GGACGCTTATTGATATGCCGCAGGTTCACCTACGGATGTTCCTCCGCTTATTGATATGCCGCA
GGTTCACCTACGGATGTTCCTCCGCTTATTGATATGCCGCAGGTTCACCTACGGATGTTCCTC
CGCTTATTGATATGCCGCAGGTTCACCTACGGATGTTCCTCCGCTTATTGATATGCCGCAGGT
TCACCTACGGATGTTCCTCCGCTTATTGATATGCCGCAGGTTCACCGACGGATGTTCCTCCGC
TTATTGATGTGCCGCAAGTTCAGCTAGGGATGTTCCTCCGCTTATTGATATGCCGCAGGTTCA
CCTACGGATGTTCCTCCGCTTATTGATATGCCGCAGGTTCACCTACGGATGTTCCTCCGCTTAT
TGATATGCCGCAGGTTCACCTACGGATGTTCCTCCGCTTATTGATATGCCGCAAGTTCACCTA
CAGATGTTCCTCCGCTTATTGATATGCCGCAGGTTCACCTACAGATGTTCCTCCGCTTATTGAT
ATGCCGCAAGTTCAAAAACAGAGGTTCCTCCAGGTGAACCTGCGCCATATGAACACGCGGAG
GAGCATCCCTAGGTGAACCTGCGGCATATCAATAACCGAAGGAACATCCCTAGGTGAACCTG
CGGCA

Fig. 4.8. Nucleotide sequence of ITS region of *Alternaria alternata* (VP DF1)



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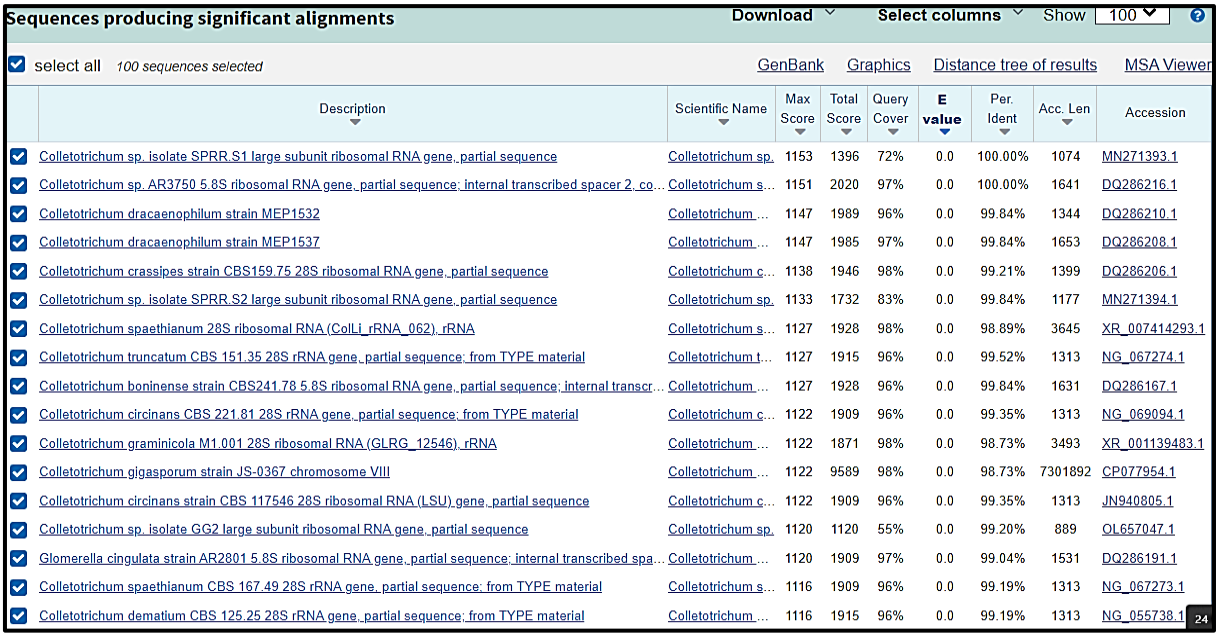
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Fusarium oxysporum Fo47 chromosome IV	Fusarium oxys…	1456	10102	100%	0.0	99.75%	4731052	CP052041.1
Fusarium fujikuroi strain Augusto2 chromosome II	Fusarium fujikuroi	1456	2703	100%	0.0	99.75%	5014829	CP023090.1
Fusarium fujikuroi strain I1.3 chromosome II	Fusarium fujikuroi	1456	2664	99%	0.0	99.75%	5092212	CP023102.1
Fusarium fujikuroi strain CSV1 chromosome II	Fusarium fujikuroi	1456	2703	100%	0.0	99.75%	5023961	CP023078.1
Fusarium proliferatum partial 18S rRNA gene for 18S ribosomal RNA, strain ITEM2400	Fusarium prolif…	1456	2583	100%	0.0	99.75%	7932	LT841264.1
Fusarium proliferatum partial 18S rRNA gene for 18S ribosomal RNA, strain ITEM2287	Fusarium prolif…	1456	2583	100%	0.0	99.75%	7930	LT841250.1
Fusarium oxysporum f. sp. dianthi partial 18S rRNA gene for 18S ribosomal RNA, strain Fod008	Fusarium oxys…	1456	2556	100%	0.0	99.75%	7875	LT841236.1
Fusarium oxysporum f. sp. dianthi partial 18S rRNA gene for 18S ribosomal RNA, strain Fod001	Fusarium oxys…	1456	2556	100%	0.0	99.75%	7875	LT841222.1
Fusarium oxysporum f. sp. cumini partial 18S rRNA gene for 18S ribosomal RNA, strain F11	Fusarium oxys…	1456	2556	100%	0.0	99.75%	7705	LT841208.1
Fusarium verticillioides 7600 28S ribosomal RNA (FVEG_17764), rRNA	Fusarium vertic…	1456	2572	100%	0.0	99.75%	3679	XR_001989352.1
Fusarium verticillioides 7600 28S ribosomal RNA (FVEG_17766), rRNA	Fusarium vertic…	1456	2572	100%	0.0	99.75%	3693	XR_001989354.1
Fusarium oxysporum f. sp. lycopersici 4287 28S ribosomal RNA rRNA	Fusarium oxys…	1456	2556	100%	0.0	99.75%	2708	XR_001936475.1
Fusarium oxysporum f. sp. lycopersici 4287 28S ribosomal RNA rRNA	Fusarium oxys…	1456	2546	100%	0.0	99.75%	2838	XR_001936473.1
Fusarium oxysporum f. sp. lycopersici 4287 28S ribosomal RNA rRNA	Fusarium oxys…	1456	2556	100%	0.0	99.75%	3677	XR_001936472.1

Fig. 4.9. BLASTn text output of nucleotide sequence of LSU gene of *Fusarium oxysporum* (IK DF1)

GATTGCCCTAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCTGGCTCTCGGGCC
CGAGTTGTAATTTGTAGAGGATACTTTTGATGCGGTGCCTTCCGAGTTCCCTGGAACGGGACG
CCATAGAGGGTGAGAGCCCCGTCTGGTTGGATGCCAAATCTCTGTAAAGTTCCTTCGACGAGT
CGAGTAGTTTGGGAATGCTGCTCTAAATGGGAGGTATATGTCTTCTAAAGCTAAATACCGGCC
AGAGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTA
AAAAGTACGTGAAATTGTTGAAAGGGAAGCGTTTATGACCAGACTTGGGCTTGGTTAATCATC
TGGGGTTCTCCCCAGTGCACTTTTCCAGTCCAGGCCAGCATCAGTTTTCCCCGGGGGATAAAG
GCTTCGGGAATGTGGCTCTCTTCGGGGAGTGTTATAGCCCGTTGTGTAATACCCTGGGGGGGGA
CTGAGGTTCGCGCATCTGCAAGGATGCTGGCGTAATGGTCATCAACGACCCGTCTTGAAACAC
GGACCAAGGGAGTCGTCTTCGTATGCGAGTGTTCGGGTGTCAAACCCCTACCCTAGGCTTCGT
CACTGACCTCCACGTCCGCCTACTCCTCAGGGCATCGTTTCTACCCTGAGGGCGAGGTATGGG
TGAGACGCTTGAGCGCCATCCATTTTCAGGGCTAGTACATTCGGCAGGTGAGTTGTTACACAG
TCCTTAGCGGATTCCGACTTCCATGGCCACCGTCCTGCTGTCAAGATGTACTAACACCTTTTGT
GGTGTCTGATGAGCGTCTACTCTGGCACCTTAACCTCGCGTTCGGTTCATCCCGCATCGCCAGT
TCTGCTTACCAAAAATGGCCCACTAGTGTTGATACATTCGAATGCTCACGTTCAATTAAGCAA
CAAGAGCTTCTTACATATTTAAAGTTTGAGAATGGATGAAGGCTAAATAGCGCCCCCGAGTCC
CTAATCATTCGCTTTACCTCATAAAACTGAGTTCAACACTGCTATCCTGAGGGAAACTTCGGCG
GAAACCAGCTACTAGAAGGTTCGATTAGTCTTTCGCCCCCATGCCCATATTTGACGATCGATTT
GCACGTCAGAACCGCTGCGAGCCTCCACCAGAGTTTCCTCTGGCTTCACCCTATACAGGCATA
GTTCACCTTCTTTCGGGTCCGGCCCCGTATGCTCTTACTCAAATCCATCCGAGAACATCAGGAT
CGGTCGATGATGCGCCGAAGCTCTCACCTGCGTTCACTTTCATTACGCGTAGGGGTTTGACACC
CGAACACTCGCATACGAAGACGACTCCTTGGTCCGTGTTTCAAGACGGGTCGTTGATGACCAT

Fig. 4.10. Nucleotide sequence of LSU region of *Fusarium oxysporum* (IK DF1)



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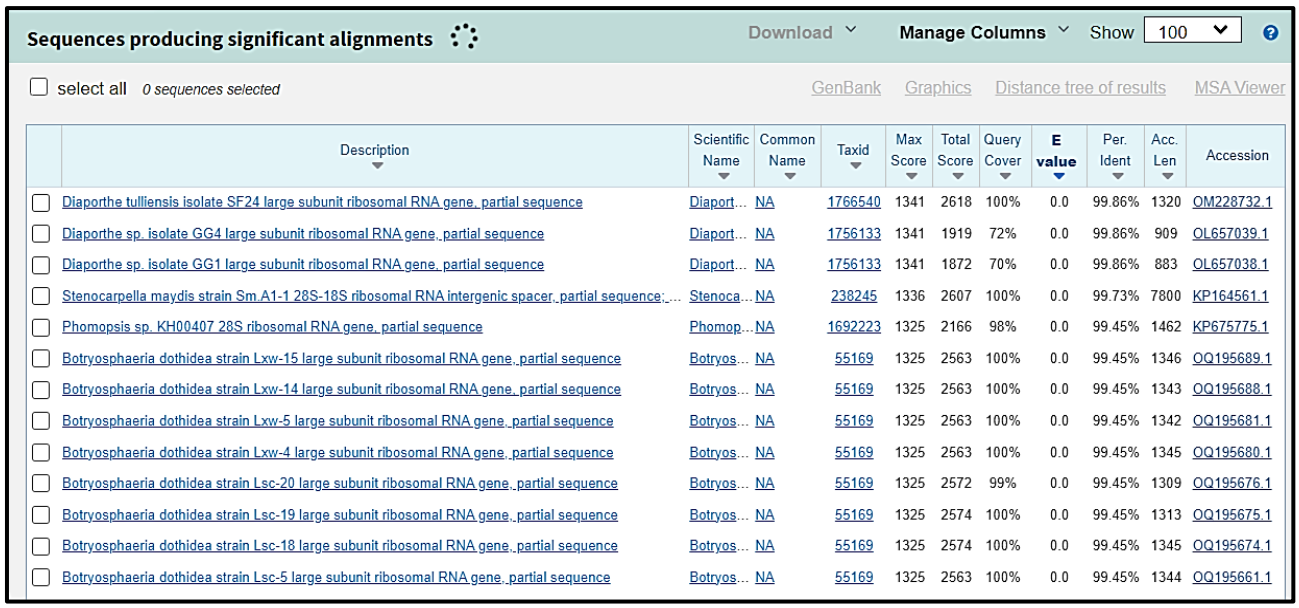
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Colletotrichum sp. isolate SPRR.S1 large subunit ribosomal RNA gene, partial sequence	Colletotrichum sp.	1153	1396	72%	0.0	100.00%	1074	MN271393.1
Colletotrichum sp. AR3750 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, co...	Colletotrichum s...	1151	2020	97%	0.0	100.00%	1641	DQ286216.1
Colletotrichum dracaenophilum strain MEP1532	Colletotrichum ...	1147	1989	96%	0.0	99.84%	1344	DQ286210.1
Colletotrichum dracaenophilum strain MEP1537	Colletotrichum ...	1147	1985	97%	0.0	99.84%	1653	DQ286208.1
Colletotrichum crassipes strain CBS159.75 28S ribosomal RNA gene, partial sequence	Colletotrichum c...	1138	1946	98%	0.0	99.21%	1399	DQ286206.1
Colletotrichum sp. isolate SPRR.S2 large subunit ribosomal RNA gene, partial sequence	Colletotrichum sp.	1133	1732	83%	0.0	99.84%	1177	MN271394.1
Colletotrichum spaethianum 28S ribosomal RNA (ColLi_rRNA_062), rRNA	Colletotrichum s...	1127	1928	98%	0.0	98.89%	3645	XR_007414293.1
Colletotrichum truncatum CBS 151.35 28S rRNA gene, partial sequence; from TYPE material	Colletotrichum t...	1127	1915	96%	0.0	99.52%	1313	NG_067274.1
Colletotrichum boninense strain CBS241.78 5.8S ribosomal RNA gene, partial sequence; internal transcr...	Colletotrichum ...	1127	1928	96%	0.0	99.84%	1631	DQ286167.1
Colletotrichum circinans CBS 221.81 28S rRNA gene, partial sequence; from TYPE material	Colletotrichum c...	1122	1909	96%	0.0	99.35%	1313	NG_069094.1
Colletotrichum graminicola M1.001 28S ribosomal RNA (GLRG_12546), rRNA	Colletotrichum ...	1122	1871	98%	0.0	98.73%	3493	XR_001139483.1
Colletotrichum gigasporum strain JS-0367 chromosome VIII	Colletotrichum ...	1122	9589	98%	0.0	98.73%	7301892	CP077954.1
Colletotrichum circinans strain CBS 117546 28S ribosomal RNA (LSU) gene, partial sequence	Colletotrichum c...	1122	1909	96%	0.0	99.35%	1313	JN940805.1
Colletotrichum sp. isolate GG2 large subunit ribosomal RNA gene, partial sequence	Colletotrichum sp.	1120	1120	55%	0.0	99.20%	889	OL657047.1
Glomerella cingulata strain AR2801 5.8S ribosomal RNA gene, partial sequence; internal transcribed spa...	Colletotrichum ...	1120	1909	97%	0.0	99.04%	1531	DQ286191.1
Colletotrichum spaethianum CBS 167.49 28S rRNA gene, partial sequence; from TYPE material	Colletotrichum s...	1116	1909	96%	0.0	99.19%	1313	NG_067273.1
Colletotrichum dematium CBS 125.25 28S rRNA gene, partial sequence; from TYPE material	Colletotrichum ...	1116	1915	96%	0.0	99.19%	1313	NG_055738.1

24

Fig. 4.11. BLASTn text output of nucleotide sequence of LSU gene of *Colletotrichum* sp. (VK OF3)

CTGGAAAGAGGGACCAACAGGGATTGCCTCAGTAACGGCGAGTGAAGCGGCAACAGCT
CAAATTTGAAATCTGGCCCCCCGGGTCCGAGTTGTAATTTGCAGAGGATGCTTTTGGCG
CGGTGCCTTCCGAGTTCCCTGGAACGGGACGCCATAGAGGGTGAGAGCCCCGTACGGTT
GGACACCAAGCCTGTGTAAAGCTCCTTCGACGAGTCGAGTAGTTTGGGAATGCTGCTCA
AAATGGGAGGTATATTTCTTCTAAAGCTAAATACCGGCCAGAGACCGATAGCGCACAA
GTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGGGTTAAACAGCACGTGAAAT
TGTTAAAAGGGAAGCGCTTGTGACCAGACTTGCGCCCGGTGAATCACCCAGCTCTCGCG
GCTGGGGCACTTTGCCGGCTCAGGCCAGCATCAGCTCGCCGTCGGGGACAAAAGCTTCG
GGAACGTAGCTCTAACCGTCCCAAACCGGGGGCCTAACGCCCTAGGCTTCGTCACTGAC
CTCCACGTCCGCCTACTCCTCAGCGCATCGTTTCTACGCTGAGGGCGAGGTATGGGTGA
GACGCTTGAGCGCCATCCATTTTCAGGGCTAGTACATTCGGCAGGTGAGTTGTTACACA
GTCCTTAGCGGATTCCGACTTCCATGGCCACCGTCCTGCTGTCAAGATGTACTAACACCT
TTTGTGGTGTCTGATGAGCGTCTACTCTGGCACCTTAACCTCGCGTTCGGTTCATCCCGC
ATCGCCAGTTCTGCTTACCAAAAATGGCCCACTAGTGTTGATACATTCGAATGCCCACG
TTCAGCTAAGTAACAAGGGCTTCTTACATATTTAAAGTTTGAGAATGGATGAAGGCAAT
ATAGCGCCCCCGAGTCCCTAATCATTCGCTTTACCTCATAAAACTGAGTTCAACACTGCT
ATCCTGAGGGAAACTTCGGCGGAAACCAGCTACTAGAAGGTTCGATTAGTCTTTCGCCC
CCATGCGCATATTTGACGATCGATTTGCACGTCAGAACCGCTGCGAGCCTCCACCAGAG
TTTCCTCTGGCTTCACCCTATACACGCATAGTTCACCTTCTTTCGGGTCCAACCCTATATG
CTCTTAC

Fig. 4.12. Nucleotide sequence of LSU region of *Colletotrichum* sp. (VK OF3)



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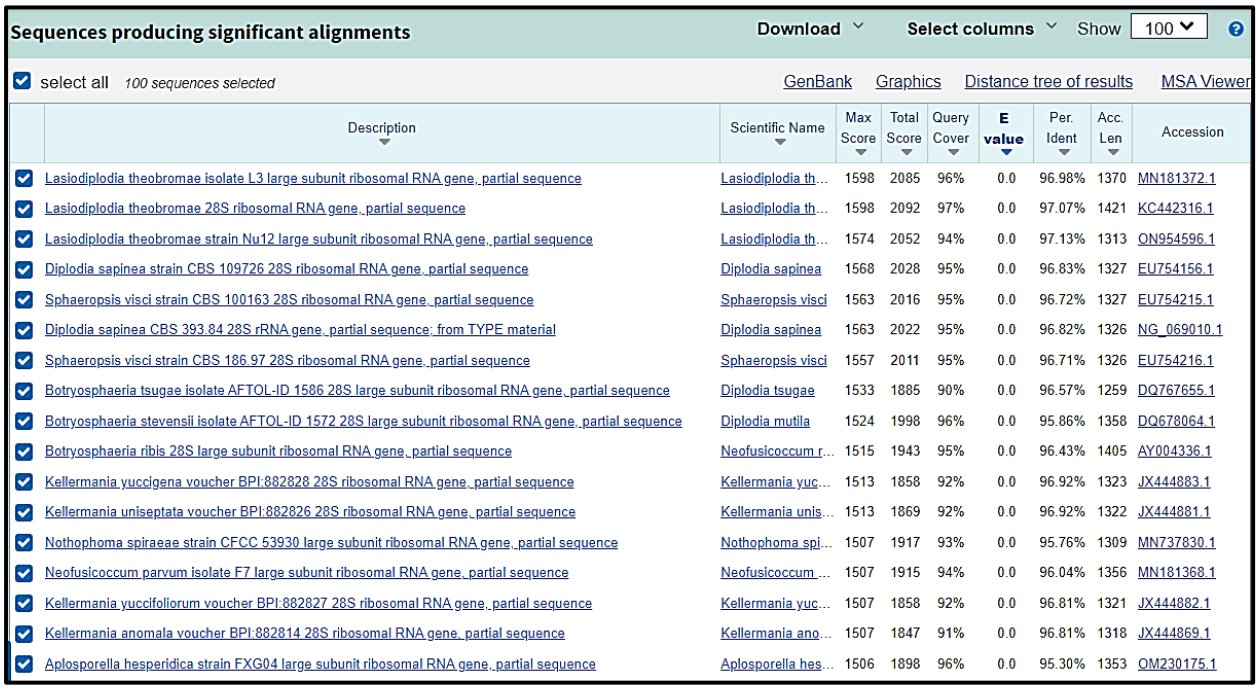
Description	Scientific Name	Common Name	Taxid	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Diaporthe tulliensis isolate SF24 large subunit ribosomal RNA gene, partial sequence	Diaport...	NA	1766540	1341	2618	100%	0.0	99.86%	1320	OM228732.1
Diaporthe sp. isolate GG4 large subunit ribosomal RNA gene, partial sequence	Diaport...	NA	1756133	1341	1919	72%	0.0	99.86%	909	OL657039.1
Diaporthe sp. isolate GG1 large subunit ribosomal RNA gene, partial sequence	Diaport...	NA	1756133	1341	1872	70%	0.0	99.86%	883	OL657038.1
Stenocarpella maydis strain Sm.A1-1 28S-18S ribosomal RNA intergenic spacer, partial sequence;...	Stenoca...	NA	238245	1336	2607	100%	0.0	99.73%	7800	KP164561.1
Phomopsis sp. KH00407 28S ribosomal RNA gene, partial sequence	Phomop...	NA	1692223	1325	2166	98%	0.0	99.45%	1462	KP675775.1
Botryosphaeria dothidea strain Lxw-15 large subunit ribosomal RNA gene, partial sequence	Botryos...	NA	55169	1325	2563	100%	0.0	99.45%	1346	OQ195689.1
Botryosphaeria dothidea strain Lxw-14 large subunit ribosomal RNA gene, partial sequence	Botryos...	NA	55169	1325	2563	100%	0.0	99.45%	1343	OQ195688.1
Botryosphaeria dothidea strain Lxw-5 large subunit ribosomal RNA gene, partial sequence	Botryos...	NA	55169	1325	2563	100%	0.0	99.45%	1342	OQ195681.1
Botryosphaeria dothidea strain Lxw-4 large subunit ribosomal RNA gene, partial sequence	Botryos...	NA	55169	1325	2563	100%	0.0	99.45%	1345	OQ195680.1
Botryosphaeria dothidea strain Lsc-20 large subunit ribosomal RNA gene, partial sequence	Botryos...	NA	55169	1325	2572	99%	0.0	99.45%	1309	OQ195676.1
Botryosphaeria dothidea strain Lsc-19 large subunit ribosomal RNA gene, partial sequence	Botryos...	NA	55169	1325	2574	100%	0.0	99.45%	1313	OQ195675.1
Botryosphaeria dothidea strain Lsc-18 large subunit ribosomal RNA gene, partial sequence	Botryos...	NA	55169	1325	2574	100%	0.0	99.45%	1345	OQ195674.1
Botryosphaeria dothidea strain Lsc-5 large subunit ribosomal RNA gene, partial sequence	Botryos...	NA	55169	1325	2563	100%	0.0	99.45%	1344	OQ195661.1

Fig. 4.13. BLASTn text output of nucleotide sequence of LSU gene of *Diaporthe tulliensis* (IK OF1)

ATTGCCCTAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCTGGCTTCGGCCCGA
GTTGTAATTTGCAGAGGATGTTTCTGGCGCGGTGCCTTCCGAGTTCCCTGGAACGGGACGCCA
CAGAGGGTGAGAGCCCCGTATGGTCGGACACCAAGCCTGTGTGAAACTCCTTCAACGAGTCG
AGTAGTTTGGGAATGCTGCTCTAAATGGGAGGTAAATCTCTTCTAAAGCTAAATACCGGCCAG
AGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACCTTGAAAAGGGGGTTAAA
TAGTACGTGAAATTGTTGAAAGGGAAGCACTTATGACCAGACTTGGGCCGGGCGGCTCATCAG
GGGTTCTCCCCTGTGCACTCCGCCCGGCACAGGCCAGCATCGGTTTTCGCGGGGGGGATAAGAC
CGACGGGAACGTAGCACCCTCCGGGGTGTGTTATAGCCCGGCGGACGATACCCTCGCGGGGA
CCGAGGTCCGCGCTCCGCAAGGATGCTGGCGTAATGGTCATCAGTGACCCGTCTTGAAACACG
GACCAAGGAGTCGTCCATTAGAGCGAGCGTTTGGGTGTAAAACCCCGCACGCGTAATGAAAG
TGAACGCAGGTGAGAGCTTCGGCGCATCATCGACCGATCCTGATGTTCTCGGAAGGATTTGAG
TAAAGAGTTTTAACGGACGGACCCGAAAGACAGGCTTCGTCACTGACCTCCACGGCCGCCTAC
TCCCCAGGGCATCGTTTCTACCCTGGGGGCGGGGTATGGGCGGTACGCTTGAGCGCCATCCAT
TTTCAGGGCTAGTACATTCGGCAGGTGAGTTGTTACACAGTCCTTAGCGGATTCCGACTTCCAT
GGCCACCGTCCTGCTGTCAAGATGTACTAACGCCTTTTGTGGTGTCTGATGAGCGTACACTCTG
GCGCCTTAACCCCGCGTTCGGTTCATCCCGCATCGCCAGTTCTGCTTACCAAAAATGGCCCACT
AGTGTTGGTACATTCGAATGCCCACGTTCAACTAAGTAACAAGGGCTTCTTACATATTTAAAG
TTTGAGAATGGATGAAGGCAATATAGCGCCCCCGAGTCCCTAATCATTCGCTTTACCTCATAA
AACTGAAGATCAACACTGCTATCCTGAGGGAAACTTCGGCGGTAACCAGCTACTAGACAGTTC
GATTAGTCTTTCGCCCCCATGCTCATATTTGACGATCGATTTGCACGTCAGAACCGCTGCGAGC
CTCCACCAGAGTTTCCTCTGGCTTCACCCTATACAAGCATAGTTCACTGTCTTTCGGGTCCGTC
CGTTAAAACTCTTACTCAAATCCTTCCGAGAACATCAGGATCGGTCGATGATGCGCCGAAGCT
CTCACCTGCGTTCACTTTCATTACGCGTGCGGGTTTTACACCCAAACGCTCGCTCTAAATGGA

Fig. 4.14. Nucleotide sequence of LSU region of *Diaporthe tulliensis* (IK OF1)



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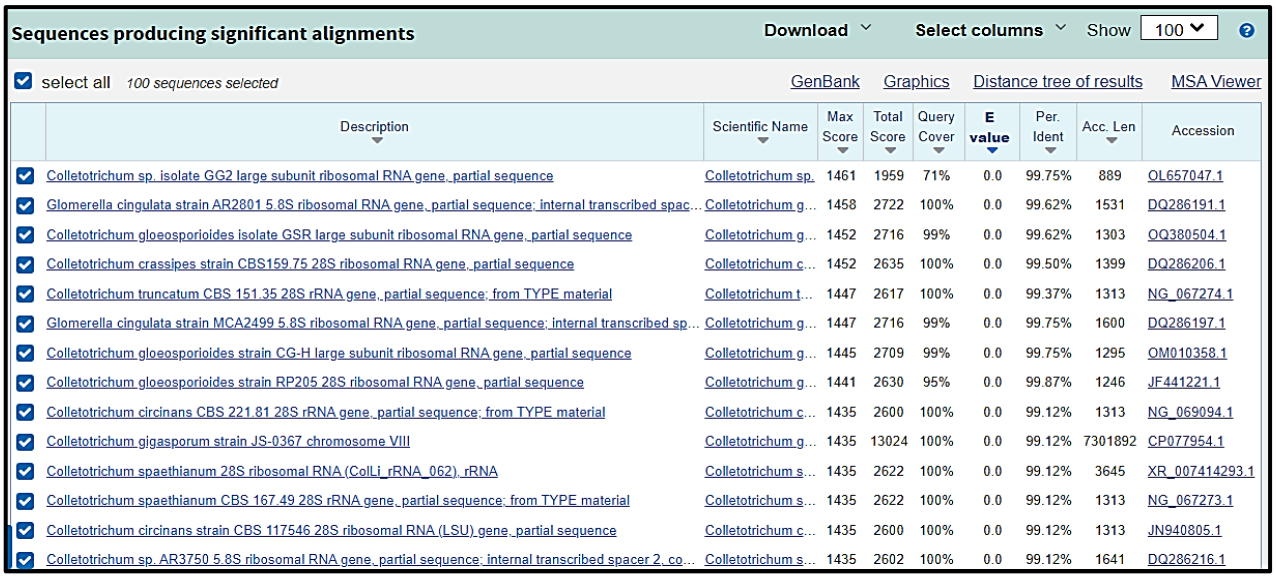
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Lasiodiplodia theobromae isolate L3 large subunit ribosomal RNA gene, partial sequence	Lasiodiplodia th...	1598	2085	96%	0.0	96.98%	1370	MN181372.1
Lasiodiplodia theobromae 28S ribosomal RNA gene, partial sequence	Lasiodiplodia th...	1598	2092	97%	0.0	97.07%	1421	KC442316.1
Lasiodiplodia theobromae strain Nu12 large subunit ribosomal RNA gene, partial sequence	Lasiodiplodia th...	1574	2052	94%	0.0	97.13%	1313	ON954596.1
Diplodia sapinea strain CBS 109726 28S ribosomal RNA gene, partial sequence	Diplodia sapinea	1568	2028	95%	0.0	96.83%	1327	EU754156.1
Sphaeropsis visci strain CBS 100163 28S ribosomal RNA gene, partial sequence	Sphaeropsis visci	1563	2016	95%	0.0	96.72%	1327	EU754215.1
Diplodia sapinea CBS 393.84 28S rRNA gene, partial sequence; from TYPE material	Diplodia sapinea	1563	2022	95%	0.0	96.82%	1326	NG_069010.1
Sphaeropsis visci strain CBS 186.97 28S ribosomal RNA gene, partial sequence	Sphaeropsis visci	1557	2011	95%	0.0	96.71%	1326	EU754216.1
Botryosphaeria tsugae isolate AFTOL-ID 1586 28S large subunit ribosomal RNA gene, partial sequence	Diplodia tsugae	1533	1885	90%	0.0	96.57%	1259	DQ767655.1
Botryosphaeria stevensii isolate AFTOL-ID 1572 28S large subunit ribosomal RNA gene, partial sequence	Diplodia mutila	1524	1998	96%	0.0	95.86%	1358	DQ678064.1
Botryosphaeria ribis 28S large subunit ribosomal RNA gene, partial sequence	Neofusicoccum r...	1515	1943	95%	0.0	96.43%	1405	AY004336.1
Kellermania yuccigena voucher BPI:882828 28S ribosomal RNA gene, partial sequence	Kellermania yuc...	1513	1858	92%	0.0	96.92%	1323	JX444883.1
Kellermania uniseptata voucher BPI:882826 28S ribosomal RNA gene, partial sequence	Kellermania unis...	1513	1869	92%	0.0	96.92%	1322	JX444881.1
Nothophoma spiraeae strain CFCC 53930 large subunit ribosomal RNA gene, partial sequence	Nothophoma spi...	1507	1917	93%	0.0	95.76%	1309	MN737830.1
Neofusicoccum parvum isolate F7 large subunit ribosomal RNA gene, partial sequence	Neofusicoccum ...	1507	1915	94%	0.0	96.04%	1356	MN181368.1
Kellermania yuccifoliorum voucher BPI:882827 28S ribosomal RNA gene, partial sequence	Kellermania yuc...	1507	1858	92%	0.0	96.81%	1321	JX444882.1
Kellermania anomala voucher BPI:882814 28S ribosomal RNA gene, partial sequence	Kellermania ano...	1507	1847	91%	0.0	96.81%	1318	JX444869.1
Aplosporella hesperidica strain FXG04 large subunit ribosomal RNA gene, partial sequence	Aplosporella hes...	1506	1898	96%	0.0	95.30%	1353	OM230175.1

Fig. 4.15. BLASTn text output of nucleotide sequence of LSU gene of *Lasiodiplodia theobromae* (VY CF2)

AGGGAGGGAAAGGAACCAAAAGGGGATTGCCTTAGTAACGGCGAGTGAAGCGGCAACAGC
TCAAATTTGAAAGCTGGCCCTTTTAGGGTCCGCATTGTAATTTGTAGAGGATGATTCGGCGA
GGGCTCCTGCCTAAGTCCCCTGGAACGGGGCGTCATAGAGGGTGAGAATCCCGTATGCGGT
AGGTTGCCTTAGCCATGTGAATCTCCTTCGACGAGTCGAGTTGTTTGGGAATGCAGCTCTAA
ATGGGAGGTAAATTTCTTCTAAAGCTAAATACCGGCCAGAAATCCTTGCTGACGGGGGATAC
TCCCAAGGCTTCGTCACGGGCCTCCAACGGCCTGCCTACTCGCCAGGGCATCGTATCTACCC
TGGCGGCGATGTATGGGTAGCACGCTTGAGCGCCATCCATTTTCAGGGCTAGTTCATTCGGC
AGGTGAGTTGTTACACACTCCTTAGCGGATTCCGACTTCCATGGCCACCGTCCTGCTGTCTAG
ATGAACTAACACCTTTTGTGGTGTCTGATGAGCGTGCATTCCGGCACCTTAACATCGCGTTCG
GTTCATCCCGCATCGCCAGTTCTGCTTACCAAAAATGGCCCACTAGTAACGGTACATTCAAG
TGTCCACGTTCAACTAAGTAACAAGGACTTCTTACATATTTAAAGTTTGAGAATAGGTTAAG
GCTGTTTCAGCCCCAAGGCCTCTAATCATTCGCTTTACCTCATAAAACTGAATACGTTACTGC
TATCCTGAGGGAAACTTCGGCAGGAACCAGCTACTAGATGGTTCGATTAGTCTTTCGCCCCT
ATACCCAAATTTGACGATCGATTTGCACGTCAGAACCGCTGCGAGCCTCCACCAGAGTTTCC
TCTGGCTTCACCCTATTCAGGCATAGTTCACCATCTTTCGGGTCCCAACAGCTATGCTCTTGC
TCAAATCCATCCGAAGACATCAGGATCGGCCGATGGTGCACCCTTGCGGGTTCCCACCTCCG
TTCACTTTCATTACGCGTACGGGTTTGACACCCGAACACTCGCATAGATGTTAGACTCCTTGG
TCCGTGTTTCAGACGGGCCGCTTACACCATTACGCCCGCATCCTTGCCGAAGCGAGTTCCTC
AGTCCAGGCTGGCCGCATTGCAACCCCGGCCTATTACACTCTCGGAGAGAGCACATTCCCGA
GGCTTTATCCGACTGCCCGAACCTGATGCTGACTGCCGCTAAAGAGAATGGCGAGTCAAGG

Fig. 4.16. Nucleotide sequence of LSU region of *Lasiodiplodia theobromae* (VY CF2)



Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Colletotrichum sp. isolate GG2 large subunit ribosomal RNA gene, partial sequence	Colletotrichum sp.	1461	1959	71%	0.0	99.75%	889	OL657047.1
Glomerella cingulata strain AR2801 5.8S ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum g...	1458	2722	100%	0.0	99.62%	1531	DQ286191.1
Colletotrichum gloeosporioides isolate GSR large subunit ribosomal RNA gene, partial sequence	Colletotrichum g...	1452	2716	99%	0.0	99.62%	1303	OQ380504.1
Colletotrichum crassipes strain CBS159.75 28S ribosomal RNA gene, partial sequence	Colletotrichum c...	1452	2635	100%	0.0	99.50%	1399	DQ286206.1
Colletotrichum truncatum CBS 151.35 28S rRNA gene, partial sequence; from TYPE material	Colletotrichum t...	1447	2617	100%	0.0	99.37%	1313	NG_067274.1
Glomerella cingulata strain MCA2499 5.8S ribosomal RNA gene, partial sequence; internal transcribed sp...	Colletotrichum g...	1447	2716	99%	0.0	99.75%	1600	DQ286197.1
Colletotrichum gloeosporioides strain CG-H large subunit ribosomal RNA gene, partial sequence	Colletotrichum g...	1445	2709	99%	0.0	99.75%	1295	OM010358.1
Colletotrichum gloeosporioides strain RP205 28S ribosomal RNA gene, partial sequence	Colletotrichum g...	1441	2630	95%	0.0	99.87%	1246	JF441221.1
Colletotrichum circinans CBS 221.81 28S rRNA gene, partial sequence; from TYPE material	Colletotrichum c...	1435	2600	100%	0.0	99.12%	1313	NG_069094.1
Colletotrichum gigasporum strain JS-0367 chromosome VIII	Colletotrichum g...	1435	13024	100%	0.0	99.12%	7301892	CP077954.1
Colletotrichum spaethianum 28S ribosomal RNA (ColLi_rRNA_062), rRNA	Colletotrichum s...	1435	2622	100%	0.0	99.12%	3645	XR_007414293.1
Colletotrichum spaethianum CBS 167.49 28S rRNA gene, partial sequence; from TYPE material	Colletotrichum s...	1435	2622	100%	0.0	99.12%	1313	NG_067273.1
Colletotrichum circinans strain CBS 117546 28S ribosomal RNA (LSU) gene, partial sequence	Colletotrichum c...	1435	2600	100%	0.0	99.12%	1313	JN940805.1
Colletotrichum sp. AR3750 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, co...	Colletotrichum s...	1435	2602	100%	0.0	99.12%	1641	DQ286216.1

Fig. 4.17. BLASTn text output of nucleotide sequence of LSU gene of *Colletotrichum gloeosporioides* (MT SF1)

GATTGCCTCAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCTGGCCCTAGGCCC
GAGTTGTAATTTGCAGAGGATGCTTTTGGTGCGGTGCCTTCCAAGTTCCCTAGAACGGGACGC
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CCCGAGTCCCTAATCATTCGCTTTACCTCATAAAACTGAGTTCAACACTGCTATCCTGAGGGAA
ACTTCGGCGGAAACCAGCTACTAGAAGGTTCGATTAGTCTTTCGCCCCCATGCGCATATTTGA
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CATCAGGATCGGTCGATGATGCGCCGAAGCTCTCACCTGCGTTCACTTTCATTACGCGTAGGG
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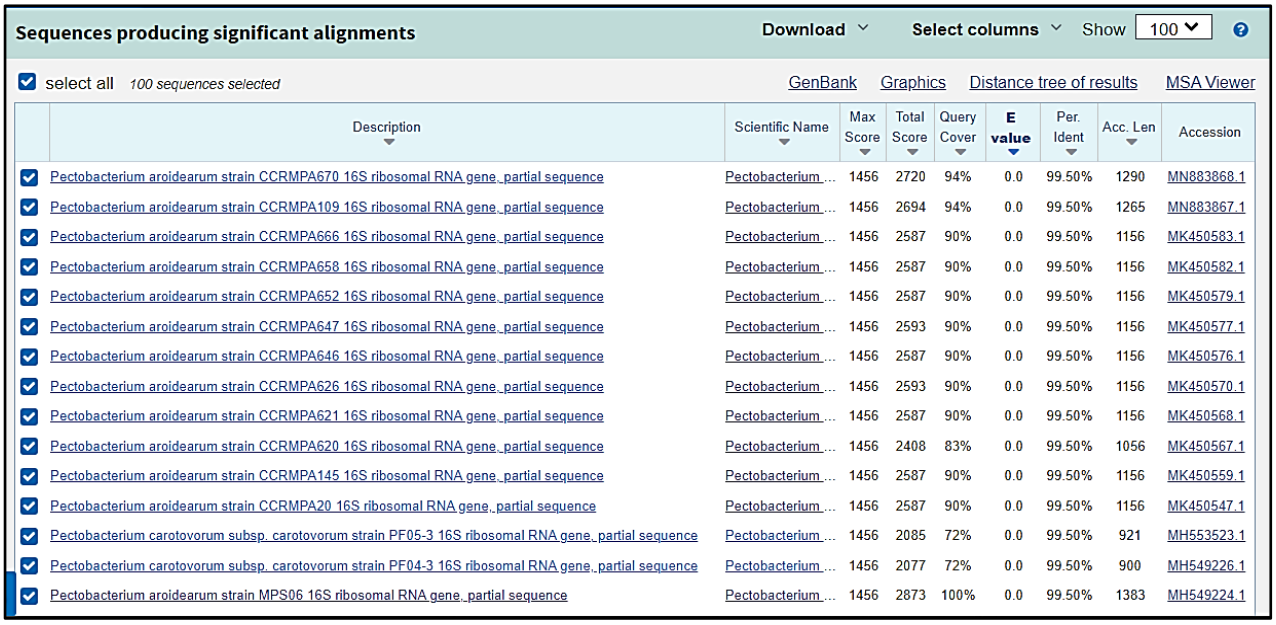
TGATGACCATTTATGCCAGCATCCTTGCGGAGCGCGT

Fig. 4.18. Nucleotide sequence of LSU region of *Colletotrichum gloeosporioides* (MT SF1)



Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Pectobacterium aroidearum strain CCRMPA670 16S ribosomal RNA gene, partial sequence	Pectobacterium ...	1456	2720	94%	0.0	99.50%	1290	MN883868.1
Pectobacterium aroidearum strain CCRMPA109 16S ribosomal RNA gene, partial sequence	Pectobacterium ...	1456	2694	94%	0.0	99.50%	1265	MN883867.1
Pectobacterium aroidearum strain CCRMPA666 16S ribosomal RNA gene, partial sequence	Pectobacterium ...	1456	2587	90%	0.0	99.50%	1156	MK450583.1
Pectobacterium aroidearum strain CCRMPA658 16S ribosomal RNA gene, partial sequence	Pectobacterium ...	1456	2587	90%	0.0	99.50%	1156	MK450582.1
Pectobacterium aroidearum strain CCRMPA652 16S ribosomal RNA gene, partial sequence	Pectobacterium ...	1456	2587	90%	0.0	99.50%	1156	MK450579.1
Pectobacterium aroidearum strain CCRMPA647 16S ribosomal RNA gene, partial sequence	Pectobacterium ...	1456	2593	90%	0.0	99.50%	1156	MK450577.1
Pectobacterium aroidearum strain CCRMPA646 16S ribosomal RNA gene, partial sequence	Pectobacterium ...	1456	2587	90%	0.0	99.50%	1156	MK450576.1
Pectobacterium aroidearum strain CCRMPA626 16S ribosomal RNA gene, partial sequence	Pectobacterium ...	1456	2593	90%	0.0	99.50%	1156	MK450570.1
Pectobacterium aroidearum strain CCRMPA621 16S ribosomal RNA gene, partial sequence	Pectobacterium ...	1456	2587	90%	0.0	99.50%	1156	MK450568.1
Pectobacterium aroidearum strain CCRMPA620 16S ribosomal RNA gene, partial sequence	Pectobacterium ...	1456	2408	83%	0.0	99.50%	1056	MK450567.1
Pectobacterium aroidearum strain CCRMPA145 16S ribosomal RNA gene, partial sequence	Pectobacterium ...	1456	2587	90%	0.0	99.50%	1156	MK450559.1
Pectobacterium aroidearum strain CCRMPA20 16S ribosomal RNA gene, partial sequence	Pectobacterium ...	1456	2587	90%	0.0	99.50%	1156	MK450547.1
Pectobacterium carotovorum subsp. carotovorum strain PF05-3 16S ribosomal RNA gene, partial sequence	Pectobacterium ...	1456	2085	72%	0.0	99.50%	921	MH553523.1
Pectobacterium carotovorum subsp. carotovorum strain PF04-3 16S ribosomal RNA gene, partial sequence	Pectobacterium ...	1456	2077	72%	0.0	99.50%	900	MH549226.1
Pectobacterium aroidearum strain MPS06 16S ribosomal RNA gene, partial sequence	Pectobacterium ...	1456	2873	100%	0.0	99.50%	1383	MH549224.1

Fig. `4.19. BLASTn text output of nucleotide sequence of 16SrRNA gene of *Pectobacterium aroidearum* (VP CB)

GCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAA
CGGTAGCTAATACCGCATAACGTCTTCGGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCAT
CAGATGTGCCCAGATGGGATTAGCTGGTAGGTGAGGTAATGGCTCACCTAGGCGACGATCCC
TAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGG
AGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTG
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CATCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAAT
ACGGAGGGTGCAAGCGTTAATCGGAATGACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTA
AGTCAGATGTGAAATCCCCGAGCTTAACTTGGGAACTGCATTTGAAACTGGCAAGCTAGAGT
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CCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGGAG
CAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGTGCC
CTTGAGCGTGGCTTCCGGGAGCTAACGCGTTAAATCGACCGCCCTGGGGACTACGATTACTA
GCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGTACTTTATGA
GGTCCGCTTGCCCTCGCGAGGTCGCTTCTCTTTGTATACGCCATTGTAGCACGTGTGTAGCCC
TACTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGT
CTCCTTTGAGTTCCCGGCCGAACCGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGCGGGA
CTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCAGAGTTCC
CGAAGGCACCAAAGCATCTCTGCTAAGTTCTCTGGATGTCAAGAGTAGGTAAGGTTCTTCGC
GTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTT
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GTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCCCCCCTCTACAAG
ACTCTAGCTTGCCAGTTTCAAATGCAGTTCCCAAGTTAAGCTCGGGGGATTTCACATCTGACT

Fig. 4.20. Nucleotide sequence of 16SrRNA region of *Pectobacterium aroidearum* (VP CB)

The bacteria (VP CB) causing soft rot symptom observed in *Cattleya* spp. was identified by *in silico* analysis of the 16S ribosomal RNA sequence of the bacteria in NCBI database showed 99.5 per cent similarity with *Pectobacterium aroidearum* strain MPS06 of accession number MH549224.1, with a query coverage of 100 per cent (Fig. 4.19 & 4.20).

4.5. *IN VITRO* EVALUATION OF CHEMICALS AND BIOCONTROL AGENTS AGAINST MAJOR PATHOGENS

The efficacy of different chemicals and biocontrol agents was tested against major pathogens by *in vitro* evaluation. The pathogens were selected based on PDI and PDS (Table 4.22).

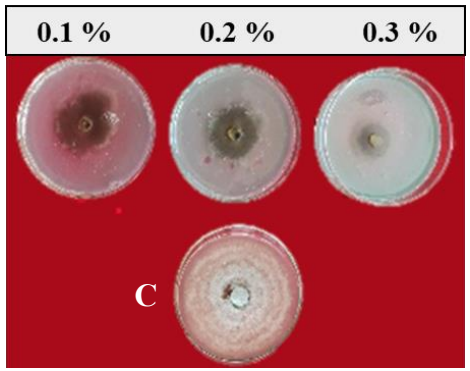
4.5.1. *In vitro* evaluation of chemicals against major pathogens

The experiment was carried out in CRD with 23 treatments and 3 replications each per treatment. Selected pathogens viz., *C. boninense* (VL DF1), *F. proliferatum* (VY DF), *S. rolfsii* (MT DF1), *A. alternata* (VP DF1), *Colletotrichum* sp. (VK OF3), *L. theobromae* (VY CF2) and *C. gloeosporioides* (MT SF1) were evaluated against seven fungicides at three doses (lower dose, recommended dose, and higher dose) and Bordeaux mixture at recommended dose (1 %) by poisoned food technique. The fungicides used for the study were copper hydroxide, hexaconazole, propineb, difenoconazole, carbendazim 12 % + mancozeb 63 %, cymoxanil 8 % + mancozeb 64 %, azoxystrobin and Bordeaux mixture. The bacterial pathogen, *P. aroidearum* (VP CB) was tested against different chemicals such as Streptocycline, copper hydroxide

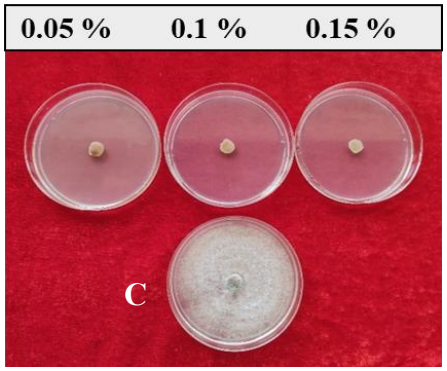
Table 4.22. Major pathogens of sympodial orchids selected for *in vitro* evaluation

Sl. No.	Pathogen	Designated code	Associated symptom
1	*Colletotrichum boninense*	VL DF1	Leaf blight (VL DLB)
2	*Fusarium proliferatum*	VY DF	Leaf blight (VY DLB)
3	*Sclerotium rolfsii*	MT DF1	Leaf blight (MT DLB1)
4	*Alternaria alternata*	VP DF1	Leaf spot (VP DLS)
5	*Colletotrichum* sp.	VK OF3	Leaf spot (VK OLS1)
6	*Lasiodiplodia theobromae*	VY CF2	Leaf spot (VY CLS)
7	*Colletotrichum gloeosporioides*	MT SF1	Leaf blight (MT SLB1)
8	*Pectobacterium aroidearum*	VP CB	Soft rot (VP CSR)

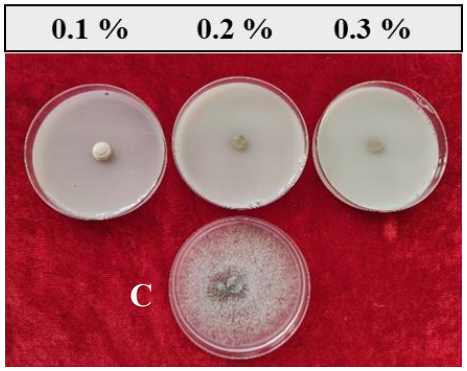
Plate 11 : *In vitro* efficacy of fungicides against *Colletotrichum boninense* (VL DF1)



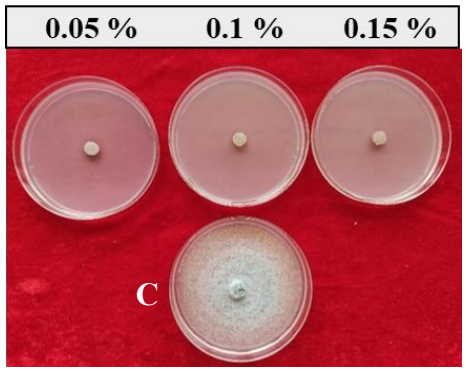
Copper hydroxide



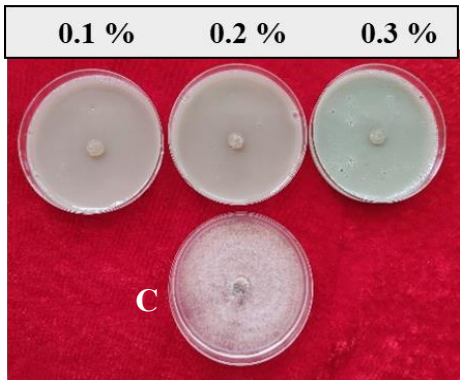
Hexaconazole



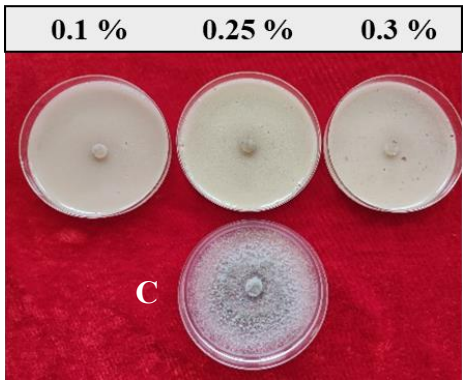
Propineb



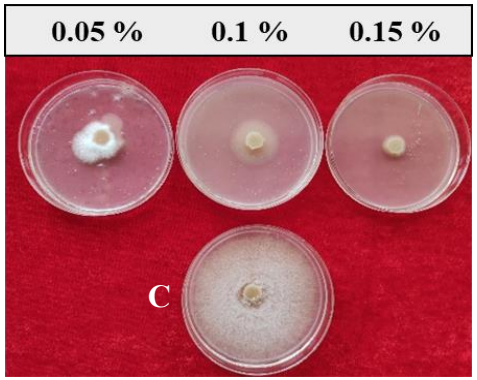
Difenoconazole



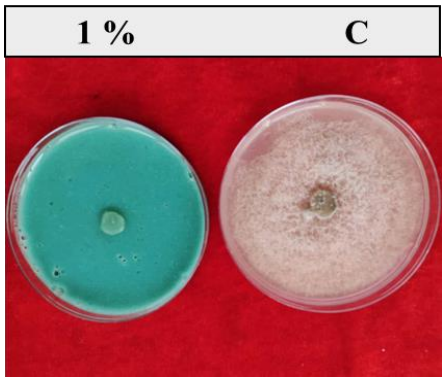
Carbendazim 12%+ Mancozeb 63%



Cymoxanil 8% + Mancozeb 64%



Azoxystrobin



Bordeaux mixture

C- Control

and Bordeaux mixture. The growth of the pathogens was found inhibited by various chemicals at different concentrations, and the inhibition varied significantly.

4.5.1.1. In vitro evaluation of fungicides against Colletotrichum boninense (VL DF1)

Among the fungicides tested, the fungicides *viz.* hexaconazole, propineb, difenoconazole, carbendazim 12 % + mancozeb 63 % and cymoxanil 8 % + mancozeb 64 % showed 100 per cent inhibition of mycelial growth at all the concentrations and Bordeaux mixture (1 %) showed complete inhibition. Copper hydroxide at 0.1, 0.2 and 0.3 per cent showed an inhibition ranging from 48.75 to 62.92 per cent, whereas azoxystrobin at 0.05, 0.1 and 0.15 per cent recorded an inhibition of 58.75, 59.58 and 79.58 per cent respectively (Table 4.23 & Plate 11).

4.5.1.2. In vitro evaluation of fungicides against Fusarium proliferatum (VY DF)

Hundred per cent inhibition of *F. proliferatum* was observed in all three concentrations of copper hydroxide, hexaconazole, carbendazim 12 % + mancozeb 63 % and 1 per cent Bordeaux mixture. Propineb at its higher concentration (0.3 %), difenoconazole at its recommended and higher concentration completely inhibited the fungal growth. Azoxystrobin at 0.05, 0.1 and 0.15 per cent showed an inhibition percentage of 60.83, 67.5 and 72.91 per cent respectively. Cymoxanil 8 % + mancozeb 64 % found to be the least effective fungicide with an inhibition percentage of 29.58, 38.75 and 49.16 at 0.1, 0.25 and 0.3 per cent respectively (Table 4.23 & Plate 12).

4.5.1.3. In vitro evaluation of fungicides against Sclerotium rolfsii (MT DF1)

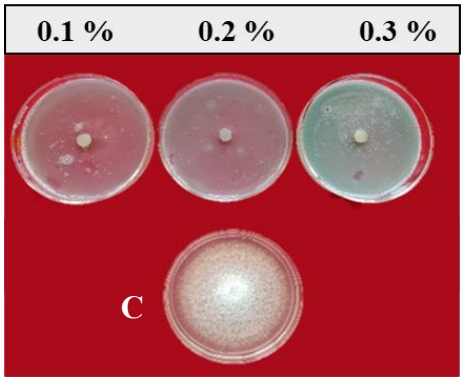
The data revealed that fungicides *viz*. hexaconazole, propineb, difenoconazole, carbendazim 12 % + mancozeb 63 %, cymoxanil 8 % + mancozeb 64 % at all dosages and 1 per cent Bordeaux mixture were found promising against *S. rolfsii* and showed 100 per cent inhibition against the pathogen. Azoxystrobin, when applied at a higher dose (0.15 %), resulted in 100 per cent inhibition of fungal pathogen, whereas at a lower dose (0.05 %) and at the recommended dose (0.1 %), the inhibition recorded was 71.67 and 82.5 per cent respectively. Copper oxychloride was found to be the least effective fungicide, showing 32.08, 52.91 and 87.5 per cent inhibition of the pathogen at concentrations of 0.1, 0.2 and 0.3 per cent respectively (Table 4.24 & Plate 13).

Table 4.23. *In vitro* evaluation of fungicides against *Colletotrichum boninense* (VL DF1) and *Fusarium proliferatum* (VY DF)

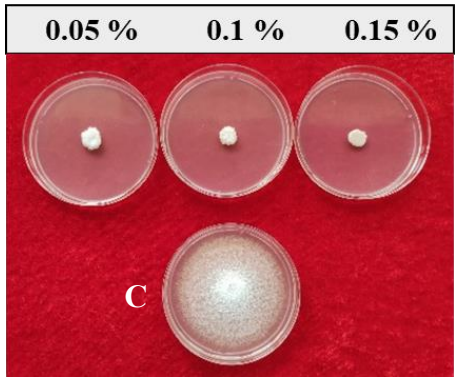
Sl. No.	Fungicide	Concentration (%)	*Per cent inhibition	
			Colletotrichum boninense	*Fusarium proliferatum*
1.	Copper hydroxide	0.1	48.75 (6.99)^{e}	100.0 (10)^{a}
		0.2	58.33 (7.67)^{d}	100.0 (10)^{a}
		0.3	62.92 (7.96)^{c}	100.0 (10)^{a}
2.	Hexaconazole	0.05	100.0 (10)^{a}	100.0 (10)^{a}
		0.1	100.0 (10)^{a}	100.0 (10)^{a}
		0.15	100.0 (10)^{a}	100.0 (10)^{a}
3.	Propineb	0.1	100.0 (10)^{a}	52.08 (7.22)^{f}
		0.2	100.0 (10)^{a}	86.25 (9.29)^{b}
		0.3	100.0 (10)^{a}	100.0 (10)^{a}
4.	Difenoconazole	0.05	100.0 (10)^{a}	90.41(9.50)^{b}
		0.1	100.0(10)^{a}	100.0 (10)^{a}
		0.15	100.0 (10)^{a}	100.0 (10)^{a}
5.	Carbendazim 12 % + Mancozeb 63 %	0.1	100.0 (10)^{a}	100.0 (10)^{a}
		0.2	100.0 (10)^{a}	100.0 (10)^{a}
		0.3	100.0 (10)^{a}	100.0 (10)^{a}
6.	Cymoxanil 8 % + Mancozeb 64 %	0.1	100.0 (10)^{a}	29.58 (5.43)^{h}
		0.25	100.0(10)^{a}	38.75 (6.22)^{g}
		0.3	100.0 (10)^{a}	49.16 (7.01)^{f}
7.	Azoxystrobin	0.05	58.75 (7.69)^{d}	60.83 (7.80)^{e}
		0.1	59.58 (7.70)^{cd}	67.50 (8.21)^{d}
		0.15	79.58 (8.94)^{b}	72.91 (8.54)^{c}
8.	Bordeaux mixture	1.0	100.0 (10)^{a}	100.0 (10)^{a}
CV			1.584	1.461
CD (0.05 %)			0.246	0.2

***** Mean of three replications
In each column means followed by same letter do not differ significantly according to DMRT √ (x + 0.5) transformed values are given in parentheses

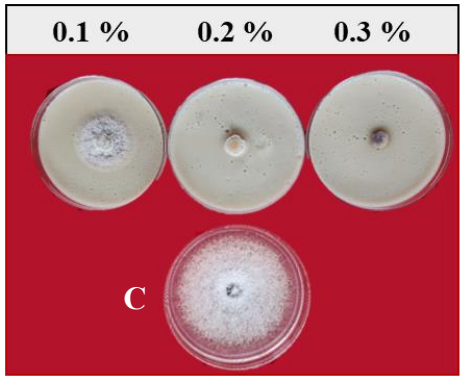
Plate 12 : *In vitro* efficacy of fungicides against *Fusarium proliferatum* (VY DF)



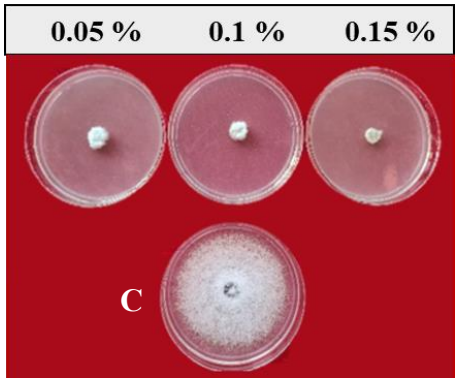
Copper hydroxide



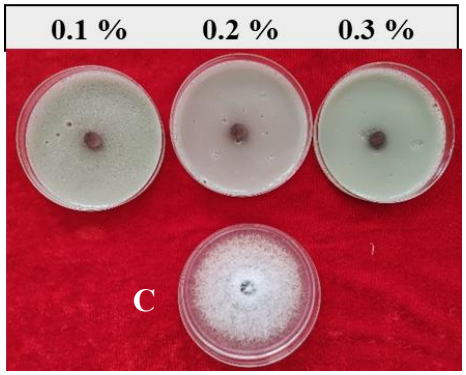
Hexaconazole



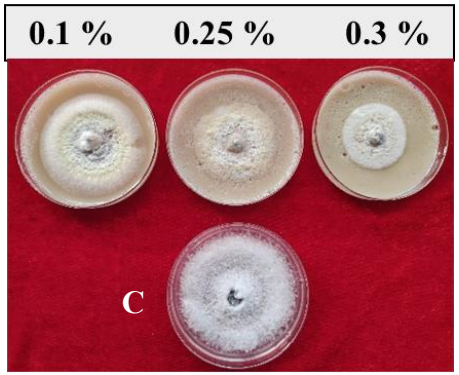
Propineb



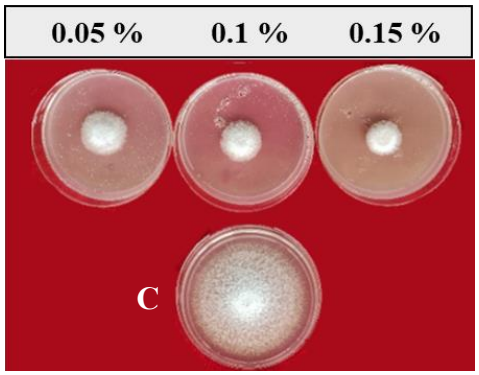
Difenoconazole



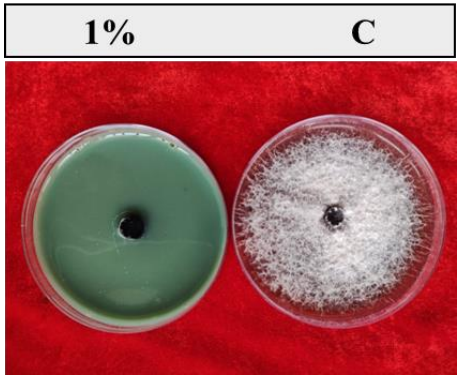
Carbendazim 12%+ Mancozeb 63%



Cymoxanil 8% + Mancozeb 64%



Azoxystrobin



Bordeaux mixture

C- Control

4.5.1.4 In vitro evaluation of fungicides against Alternaria alternata (VP DF1)

The data given in Table 4.24 revealed that hexaconazole was the only fungicide showing complete inhibition of the pathogen at all concentrations. Difenoconazole and copper hydroxide showed 83.33 and 79.16 per cent inhibition of fungal growth at their higher concentration and Bordeaux mixture at recommended dose (1 %) showed 76.25 per cent inhibition of fungal growth. Carbendazim 12 % + mancozeb 63 % and cymoxanil 8 % + mancozeb 64 % at their higher dosages showed an inhibition per cent of 65.0 and 57.08 per cent respectively, whereas the per cent inhibition of fungal growth by propineb at different doses ranged from 42.08 to 51.67 per cent. Azoxystrobin was found to be the least effective fungicide, showing inhibition percentage of 30.41 per cent at its highest concentration (Plate 14).

4.5.1.5. In vitro evaluation of fungicides against Colletotrichum sp. (VK OF3)

Complete inhibition of *Colletotrichum* sp. was observed in all three doses of carbendazim 12 % + mancozeb 63 % (0.1, 0.2, and 0.3 %) and the recommended dose of 1 per cent Bordeaux mixture (1 %). Copper hydroxide, difenoconazole and propineb at their higher concentrations showed complete inhibition of mycelial growth of the pathogen. Hexaconazole at 0.05, 0.1 and 0.15 per cent recorded inhibition per cent of 38.33, 52.91 and 79.58 per cent respectively. Cymoxanil 8 % + mancozeb 64 % inhibited the growth by 66.25 per cent at their higher concentration of 0.3 per cent. Azoxystrobin at 0.05, 0.1 and 0.15 per cent showed lesser inhibition against the pathogen ranging from 52.08 to 62.91 per cent (Table 4.25 & Plate 15).

4.5.1.6. In vitro evaluation of fungicides against Lasiodiplodia theobromae (VY CF2)

Hundred per cent inhibition of *L. theobromae* was observed in all three concentrations of carbendazim 12 % + mancozeb 63 %, cymoxanil 8 % + mancozeb 64 % and propineb and 1 per cent Bordeaux mixture. Hexaconazole at its higher concentration showed maximum inhibition percentage (100 %), whereas higher concentration of difenoconazole showed an inhibition of 74.58 per cent. Copper hydroxide at 0.1, 0.2 and 0.3 per cent showed an inhibition percentage of 58.33, 73.75 and 82.5 per cent respectively. Azoxystrobin at 0.05, 0.1 and 0.15 per cent showed an inhibition ranging from 37.08- 50.0 per cent and was found to be least effective against the pathogen (Table 4.25 & Plate 16).

Table 4.24. *In vitro* evaluation of fungicides against *S. rolfsii* (MT DF1) and *Alteranria alternata* (VP DF1)

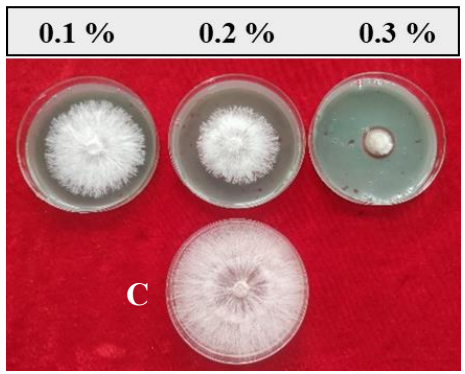
Sl. No.	Fungicide	Concentration (%)	*Per cent inhibition	
			S. rolfsii	*Alternaria alternata*
1	Copper hydroxide	0.1	32.08 (5.64)^{e}	73.33 (8.56)^{cd}
		0.2	52.91 (7.30)^{d}	76.67 (8.75)^{bc}
		0.3	87.50 (9.37)^{b}	79.16 (8.90)^{bc}
2	Hexaconazole	0.05	100.0 (10)^{a}	100.0 (10)^{a}
		0.1	100.0 (10)^{a}	100.0 (10)^{a}
		0.15	100.0 (10)^{a}	100.0 (10)^{a}
3	Propineb	0.1	100.0 (10)^{a}	42.08 (6.49)^{h}
		0.2	100.0(10)^{a}	47.08 (6.86)^{gh}
		0.3	100.0 (10)^{a}	51.67 (7.19)^{fg}
4	Difenoconazole	0.05	100.0 (10)^{a}	65.88 (8.11)^{e}
		0.1	100.0(10)^{a}	69.16 (8.32)^{de}
		0.15	100.0 (10)^{a}	83.33 (9.11)^{b}
5	Carbendazim 12 % + Mancozeb 63 %	0.1	100.0 (10)^{a}	52.91(7.27)^{fg}
		0.2	100.0 (10)^{a}	57.91(7.61)^{f}
		0.3	100.0 (10)^{a}	65.0 (8.04)^{e}
6	Cymoxanil 8 % + Mancozeb 64 %	0.1	100.0 (10)^{a}	49.16 (7.01)^{g}
		0.25	100.0 (10)^{a}	51.66 (7.19)^{fg}
		0.3	100.0(10)^{a}	57.08 (7.55)^{f}
7	Azoxystrobin	0.05	71.67 (8.48)^{c}	21.25 (4.61)^{i}
		0.1	82.50 (9.11)^{b}	25.83 (5.08)^{j}
		0.15	100.0 (10) a	30.41(5.52)^{k}
8	Bordeaux mixture	1.0	100.0 (10)^{a}	76.25 (8.73)^{bcd}
CV			3.074	3.310
CD (0.05 %)			0.484	0.424

***** Mean of three replications

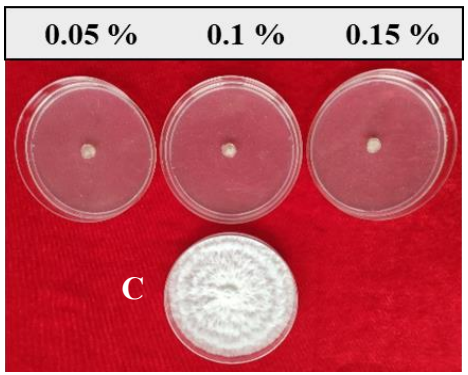
In each column means followed by same letter do not differ significantly according to DMRT

√ (x + 0.5) transformed values are given in parentheses

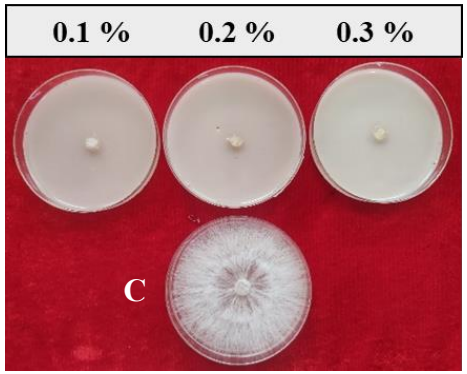
Plate 13 : *In vitro* efficacy of fungicides against *Sclerotium rolfsii* (MT DF1)



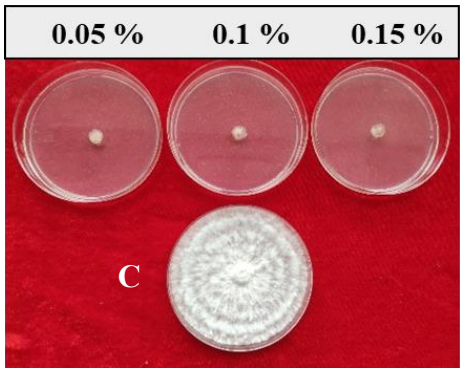
Copper hydroxide



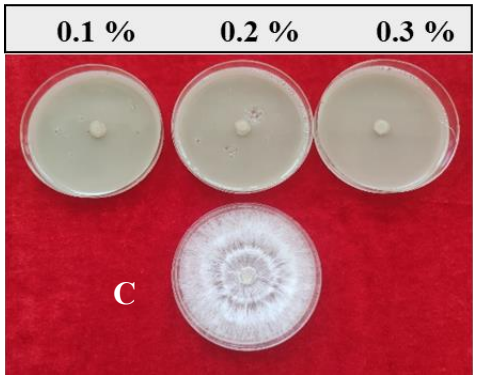
Hexaconazole



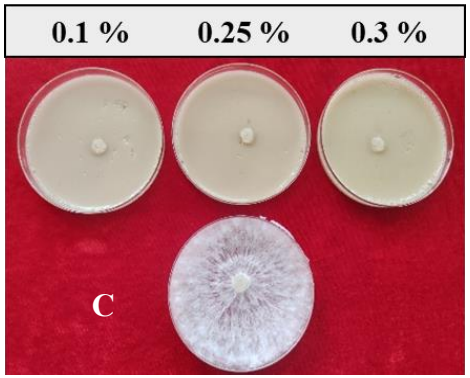
Propineb



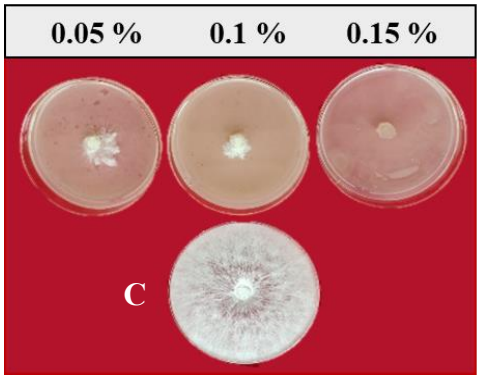
Difenoconazole



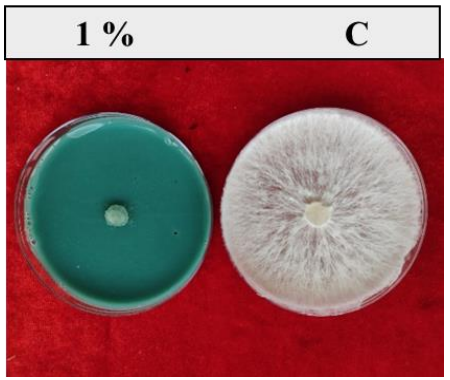
Carbendazim 12%+ Mancozeb 63%



Cymoxanil 8% + Mancozeb 64%



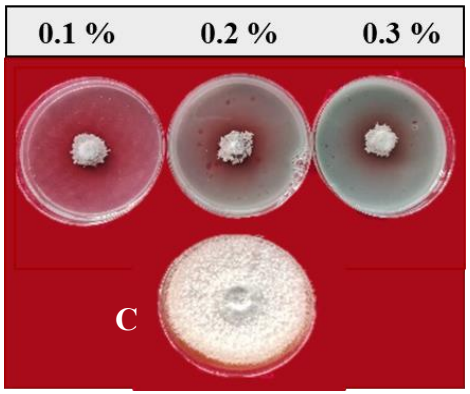
Azoxystrobin



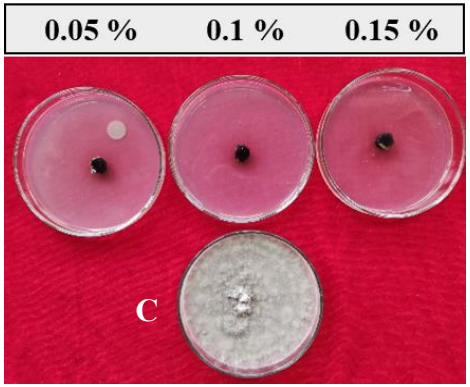
Bordeaux mixture

C- Control

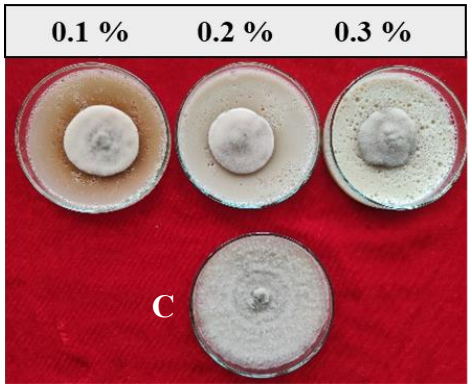
Plate 14 : *In vitro* efficacy of fungicides against *Alternaria alternata* (VP DF1)



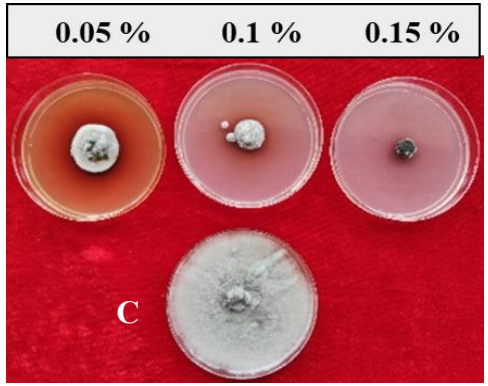
Copper hydroxide



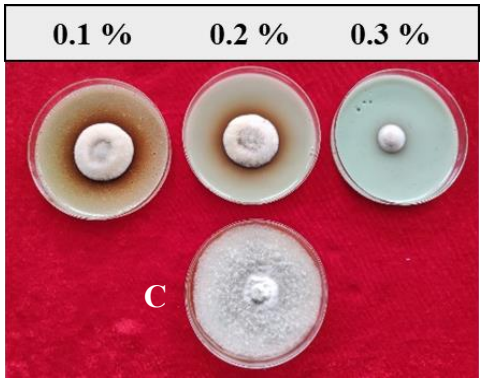
Hexaconazole



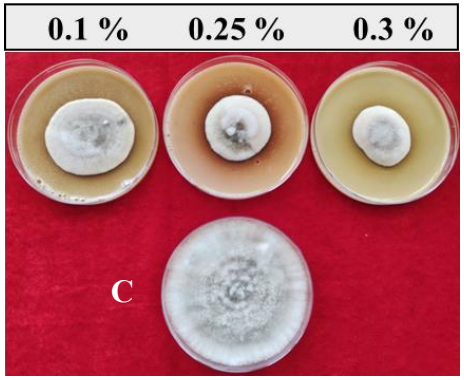
Propineb



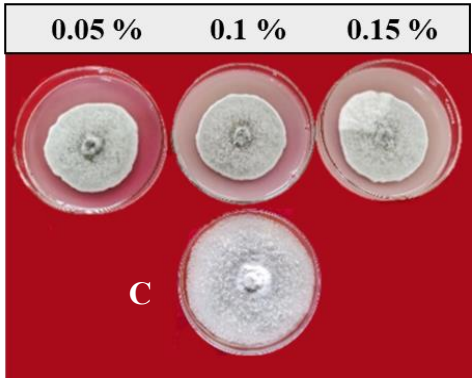
Difenoconazole



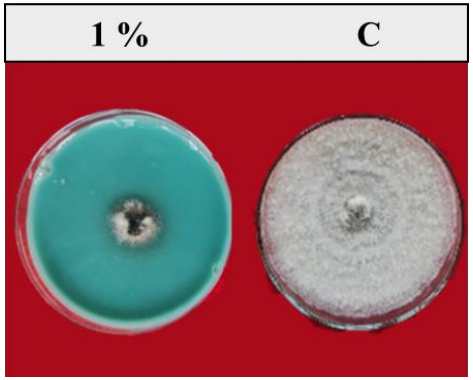
Carbendazim 12%+ Mancozeb 63 %



Cymoxanil 8 % + Mancozeb 64 %



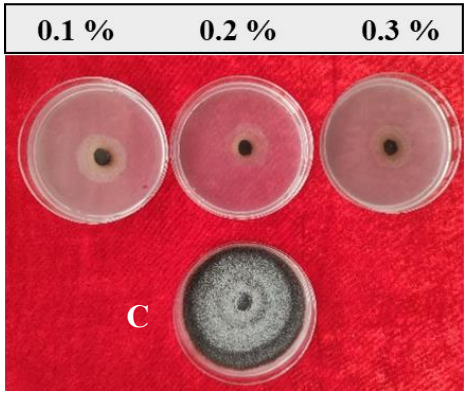
Azoxystrobin



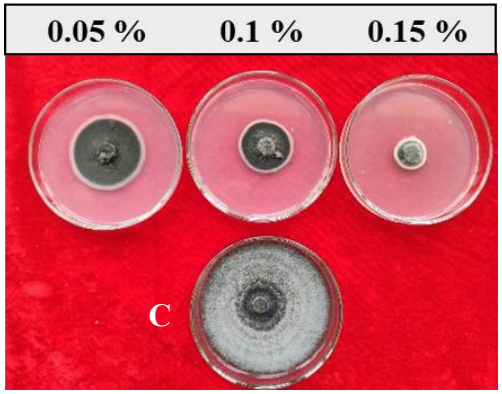
Bordeaux mixture

C- Control

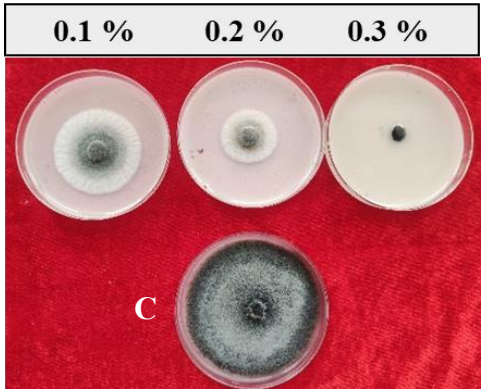
Plate 15 : *In vitro* efficacy of fungicides against *Colletotrichum* sp. (VK OF3)



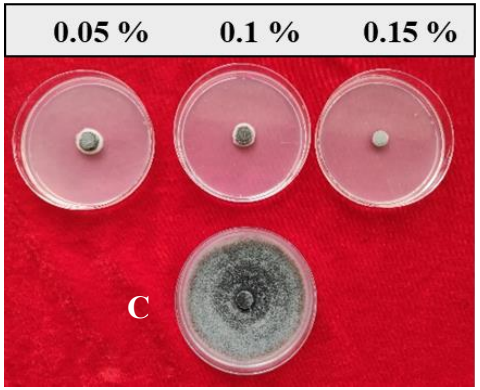
Copper hydroxide



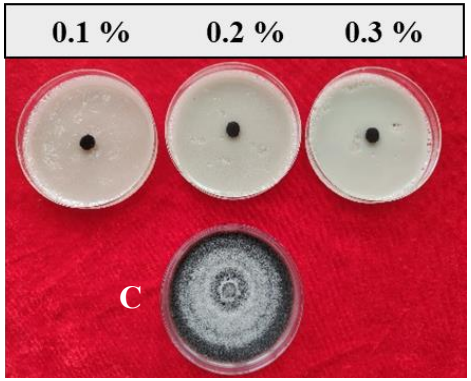
Hexaconazole



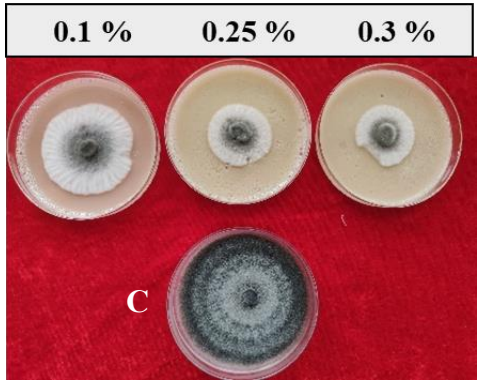
Propineb



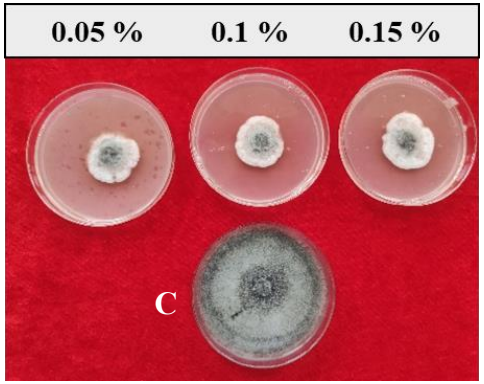
Difenoconazole



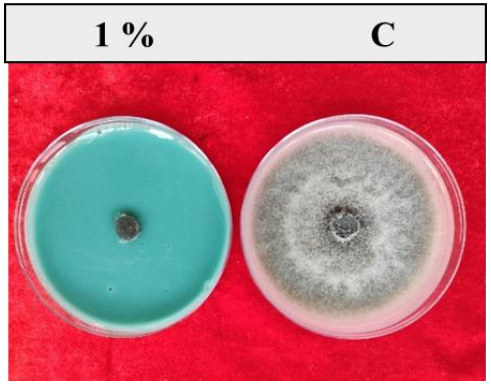
Carbendazim 12%+ Mancozeb 63%



Cymoxanil 8% + Mancozeb 64%



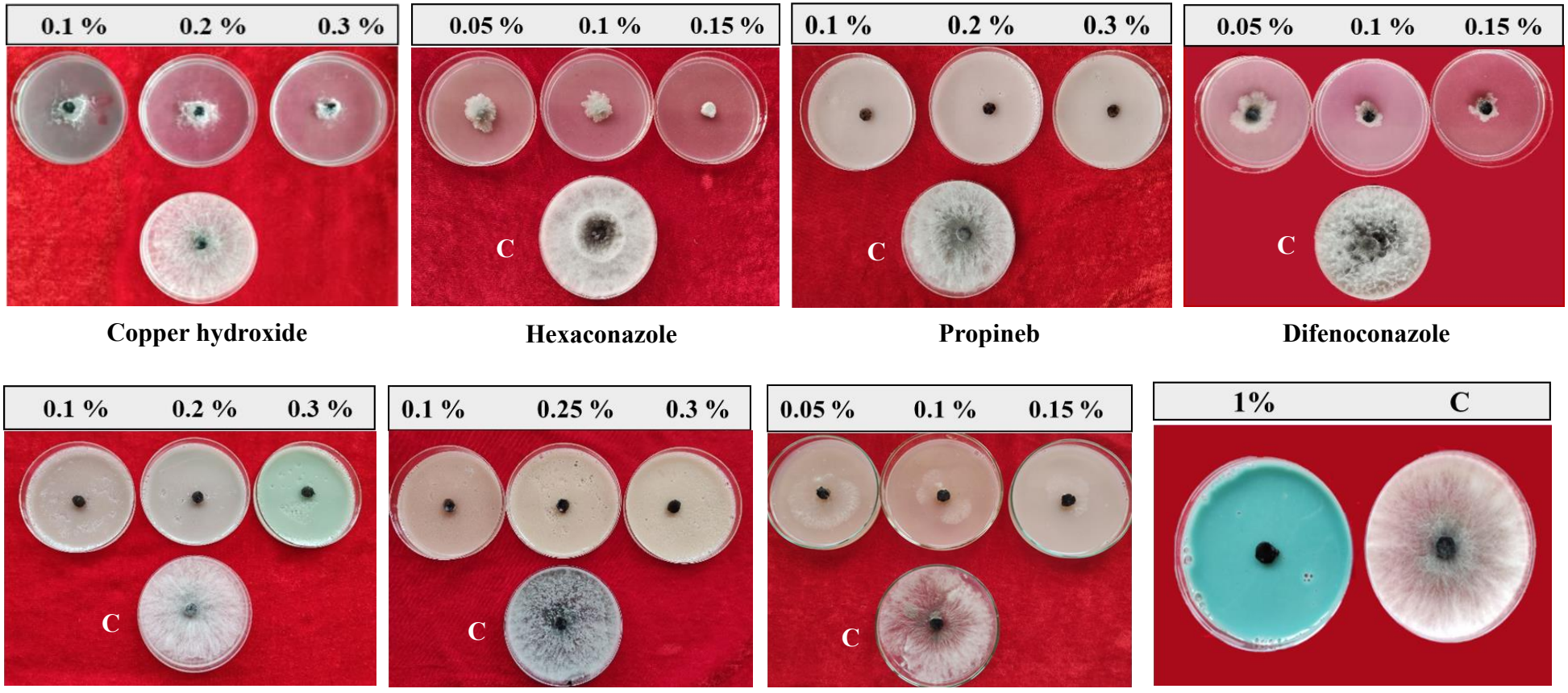
Azoxystrobin



Bordeaux mixture

C- Control

Plate 16 : *In vitro* efficacy of fungicides against *Lasiodiplodia theobromae* (VY CF2)



Copper hydroxide | **Hexaconazole** | **Propineb** | **Difenoconazole**

Carbendazim 12%+ Mancozeb 63% | **Cymoxanil 8% + Mancozeb 64%** | **Azoxystrobin** | **Bordeaux mixture**

C- Control

4.5.1.7. In vitro evaluation of fungicides against Colletotrichum gloeosporioides (MT SF1)

Complete inhibition of *C. gloeosporioides* was observed in all three doses of carbendazim 12 % + mancozeb 63 % (0.1, 0.2 and 0.3 per cent) and the recommended dose of 1 per cent Bordeaux mixture (Table 4.25). Hexaconazole and difenoconazole, at their higher dose showed complete inhibition of the pathogen.

Copper hydroxide and propineb at their higher concentrations showed inhibition percentage of 72.5 and 88.33 per cent respectively. Azoxystrobin at 0.05, 0.1 and 0.15 per cent, inhibited the pathogen by 28.33, 35.42 and 47.5 per cent respectively. Cymoxanil 8 % + mancozeb 64 % at 0.1, 0.25 and 0.3 per cent showed an inhibition ranging from 11.25 to 38.33 per cent and were found to be least effective against the pathogen (Plate 17).

4.5.1.8. In vitro evaluation of chemicals against Pectobacterium aroidearum (VP CB)

Among the chemicals treated against *P. aroidearum* under *in vitro* conditions, copper hydroxide showed a maximum inhibition percentage of 25.92 per cent at its higher concentration (0.3 %), whereas Streptocycline at 200 and 250 ppm showed per cent inhibition of 13.33 and 16.67 respectively and no inhibition was noticed at a lower concentration (100 ppm). The fungicide Bordeaux mixture at 1 per cent showed an inhibition percentage of 15.5 per cent (Table 4.26 & Plate 18).

Table 4.26. In vitro evaluation of chemicals against Pectobacterium aroidearum (VP CB)

Sl. No.	Chemical	Concentration (%)	*Per cent inhibition
1	Streptocycline	0.01	0.0 (0.22)^{f}
		0.02	13.33 (3.65)^{e}
		0.025	16.67 (4.08)^{c}
2	Copper hydroxide	0.1	13.33 (3.65)^{e}
		0.2	22.22 (4.72)^{b}
		0.3	25.92 (5.09)^{a}
3	Bordeaux mixture	1.0	15.50 (3.95)^{d}
CV			0.653
CD (0.05 %)			0.042

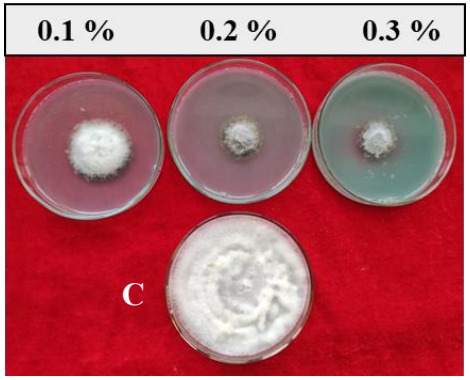
***** Mean of three replications
In each column means followed by same letter do not differ significantly according to DMRT
√ (x + 0.5) transformed values are given in parentheses

Table 4.25. *In vitro* evaluation of fungicides against *Colletotrichum* sp. (VK OF3), *Lasiodiplodia theobromae* (VY CF2) and *Colletotrichum gloeosporioides* (MT SF1)

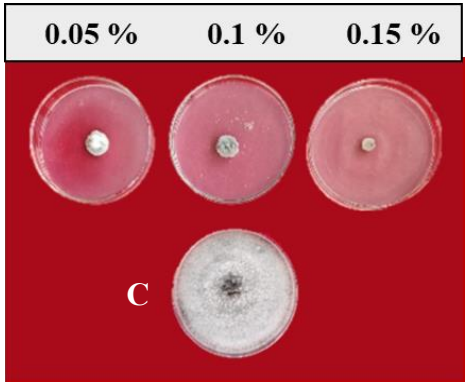
Sl. No.	Fungicide	Conc. (%)	*Per cent inhibition		
			Colletotrichum sp.	*Lasiodiplodia theobromae*	*Colletotrichum gloeosporioides*
1	Copper hydroxide	0.1	62.92 (7. 96)^{cd}	58.33 (7.62)^{f}	47.91 (6.92)^{d}
		0.2	91.25 (9.55)^{ab}	73.75 (8.58)^{cd}	66.67 (8.16)^{c}
		0.3	100.0 (10)^{a}	82.50 (9.08)^{b}	72.50 (8.51)^{bc}
2	Hexaconazole	0.05	38.33 (6.23)^{f}	69.58 (8.34)^{d}	80.42 (8.96)^{b}
		0.1	52.91 (7.3)^{b}	70 (8.36)^{d}	94.58 (9.7)^{a}
		0.15	79.58 (8.91)^{b}	100.0 (10)^{a}	100.0 (10)^{a}
3	Propineb	0.1	35.41 (5.95)^{f}	100.0 (10)^{a}	27.50 (5.22)^{f}
		0.2	86.66 (9.27)^{b}	100.0 (10)^{a}	54.58 (7.35)^{d}
		0.3	100.0 (10)^{a}	100.0 (10)^{a}	88.33 (9.78)^{a}
4	Difenoconazole	0.05	78.75 (8.90)^{b}	63.75 (7.98)^{e}	76.67 (8.76)^{b}
		0.1	81.25 (9.04)^{b}	70.41 (8.39)^{cd}	95.0 (9.74)^{a}
		0.15	100.0 (10)^{a}	74.58 (8.63)^{c}	100.0 (10)^{a}
5	Carbendazim 12 % + Mancozeb 63 %	0.1	100.0 (10)^{a}	100.0 (10)^{a}	100.0 (10)^{a}
		0.2	100.0 (10)^{a}	100.0 (10)^{a}	100.0 (10)^{a}
		0.3	100.0 (10)^{a}	100.0 (10)^{a}	100.0 (10)^{a}
6	Cymoxanil 8 % + Mancozeb 64 %	0.1	35.833 (6.02)^{f}	100.0 (10)^{a}	11.25 (3.35)^{h}
		0.25	53.75 (7.35)^{de}	100.0 (10)^{a}	19.58 (4.42)^{g}
		0.3	66.25 (8.16)^{c}	100.0 (10)^{a}	38.33 (6.19)^{e}
7	Azoxystrobin	0.05	52.08 (7.24)^{e}	37.08 (6.09)^{i}	28.33 (5.32)^{f}
		0.1	60.83 (7.83)^{cde}	45.0 (6.71)^{h}	35.42 (5.95)^{e}
		0.15	62.91 (7.96)^{cd}	50.0 (7.07)^{g}	47.50 (6.89)^{d}
8	Bordeaux mixture	1.0	100.0 (10)^{a}	100.0 (10)^{a}	100.0 (10)^{a}
	CV		4.990	1.814	3.907
	CD (0.05 %)		0.702	0.267	0.512

***** Mean of three replications
In each column means followed by same letter do not differ significantly according to DMRT
√ (x + 0.5) transformed values are given in parentheses

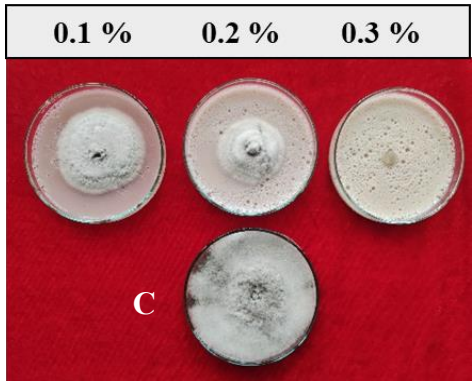
Plate 17 : *In vitro* efficacy of fungicides against *Colletotrichum gloeosporioides* (MT SF1)



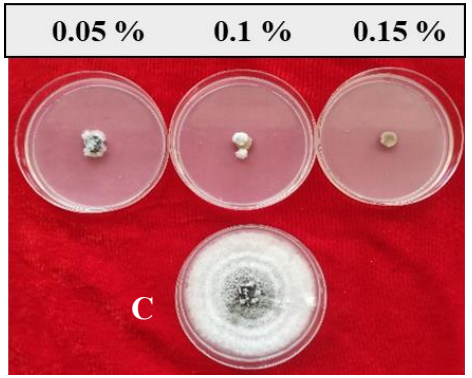
Copper hydroxide



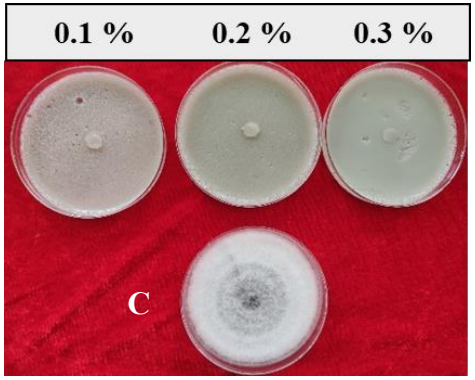
Hexaconazole



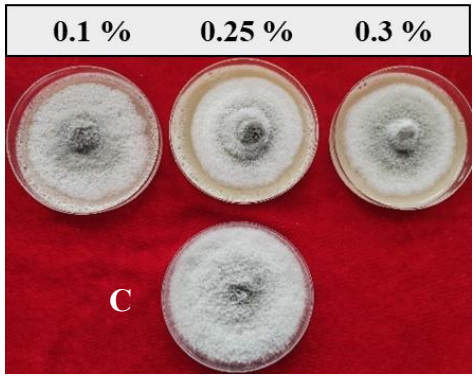
Propineb



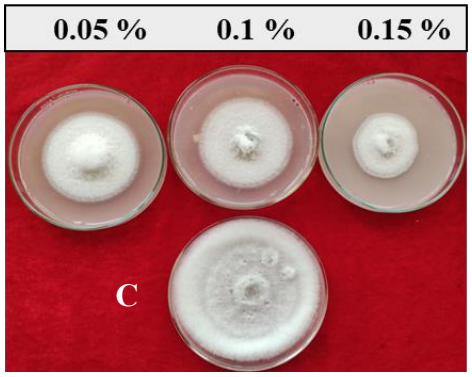
Difenoconazole



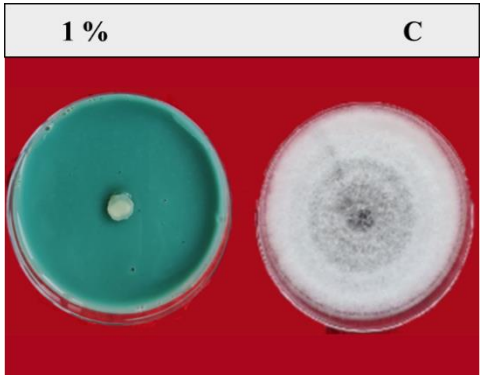
Carbendazim 12%+ Mancozeb 63%



Cymoxanil 8% + Mancozeb 64%



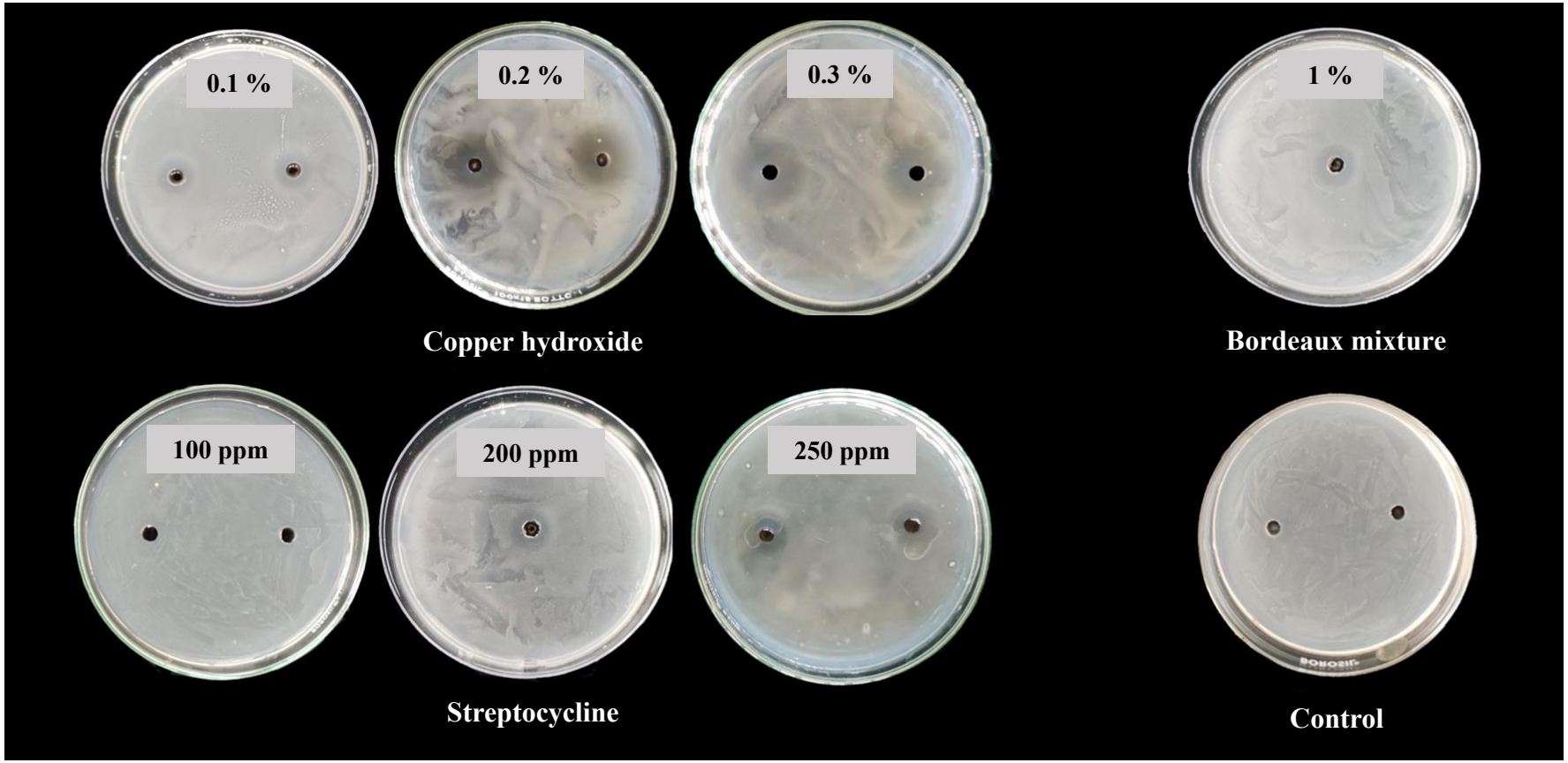
Azoxystrobin



Bordeaux mixture

C- Control

Plate 18 : *In vitro* efficacy of chemicals against *Pectobacterium aroidearum* (VP CB)



4.5.2. In vitro evaluation of biocontrol agents against major pathogens

Biocontrol agents viz. *Trichoderma asperellum* (KAU reference culture), *Pseudomonas fluorescens* (KAU reference culture), PGPR-II (KAU formulation) and PGPM (KAU formulation) were evaluated against the major pathogens obtained from sympodial orchids during the survey. The efficiency of biocontrol agents viz. *T. asperellum* and *P. fluorescens* against fungal pathogens was tested *in vitro* by dual culture technique (Morton and Stroube, 1955), whereas PGPR- II and PGPM were evaluated by poisoned food technique (Vincent, 1927). The efficacy of biocontrol agents against bacterial pathogen was performed by agar diffusion test or disc diffusion test.

4.5.2.1. In vitro evaluation of biocontrol agents against major fungal pathogens

4.5.2.1.1. In vitro evaluation of Trichoderma asperellum against major fungal pathogens

The biocontrol agent *T. asperellum* was evaluated against the major fungal pathogens by dual culture technique. It was observed that, the most promising result was obtained against the pathogen *A. alternata,* showing a maximum inhibition percentage of 91.16 per cent (Table 4.27). The pathogens such as *F. proliferatum*, *C. boninense, S. rolfsii, Colletotrichum* sp. and *C. gloeosporioides* exhibited moderate per cent inhibition ranging from 53.06 to 61.9 per cent. However, the lowest per cent inhibition of 20.41 per cent was shown against the pathogen *L.. theobromae*. *T. asperellum* inhibited the pathogens in different ways, which were recorded in detail. Interaction of the antagonist with *C. boninense* produced a slight demarcation at the meeting point with reduced growth whereas a clear inhibition zone was observed for *F. proliferatum, Colletotrichum sp., C. gloeosporioides* and *L. theobromae. A. alternata* was inhibited by overgrowth of *T. asperellum* (Plate 19).

4.5.2.1.2. In vitro evaluation of P. fluorescens against major fungal pathogens

The bacterial antagonist, *P. fluorescens,* was evaluated against the major pathogens and found that only *Colletotrichum* sp. (VK OF3) exhibited comparatively higher inhibition and the other pathogens showed less inhibition. However, reduction in mycelial growth was observed for pathogens, and different types of interaction of *P.*

fluorescens with fungal pathogens were studied. The inhibition pattern of the pathogens has been designated in Table 4.27. Mycelial thickening was observed for *F. proliferatum* and *C. gloeosporioides,* whereas *A. alternata* was inhibited by lysis and overgrowth. Mycelial thickening, lysis and overgrowth were observed for *C. boninense,* while no inhibition was observed for *S. rolfsii* and *L. theobromae* (Plate 20).

4.5.2.1.3. In vitro evaluation of PGPR-II against major fungal pathogens

In vitro evaluation studies of PGPR-II against major fungal pathogens showed that, complete inhibition of 100 per cent exhibited by *C. boninense* and *C. gloeosporioides*. This was followed by *A. alternata, Colletotrichum* sp., and *S. rolfsii,* with inhibition percentage of 79.58, 74.58 and 72.91 per cent respectively. The least inhibition of 50.41 per cent was noticed for *L. theobromae* (Table 4.27 & Plate 21).

4.5.2.1.4. In vitro evaluation of PGPM against major fungal pathogens

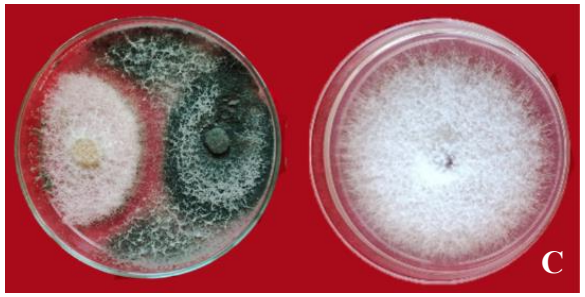
During *in vitro* evaluation, PGPM showed varied per cent inhibition of the major fungal pathogens. The maximum percentage of 100 per cent inhibition was exhibited by C. *boninense* and *L. theobromae*. It was followed by *C. gloeosporioides, Colletotrichum.* sp., *A. alternata* and *S. rolfsii*, with inhibition per cent of 84.58, 77.91, 77.08 and 75 per cent respectively. The least inhibition was noticed for *F. proliferatum,* with inhibition percentage of 60.41 per cent (Table 4.27 & Plate 22).

4.5.2.2. In vitro evaluation of biocontrol agents against bacterial pathogen (Pectobacterium aroidearum)

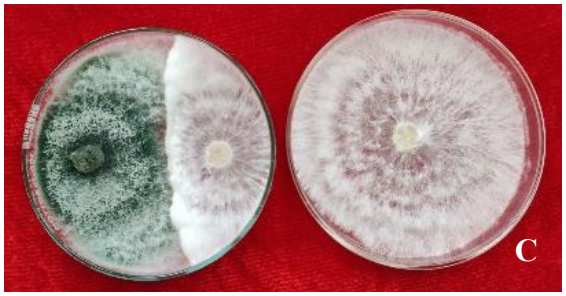
The different biocontrol agents viz. *P. fluorescens*, *T. asperellum*, PGPR-II and PGPM were evaluated *in vitro* against *P. aroidearum,* and no inhibition was found in the case of *P. fluorescens*, PGPR-II and PGPM. However, *T. asperellum* exhibited a lower inhibition percentage of 26.67 per cent against the bacterial pathogen (Table 4.27 & Plate 23).

Plate 19 : *In vitro* efficacy of *Trichoderma asperellum* against major pathogens

***Colletotrichum boninense* (VL DF1)**

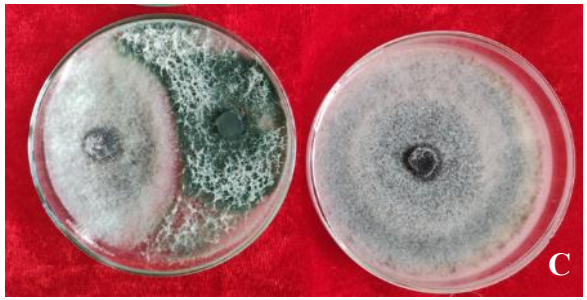


***Fusarium proliferatum* (VY DF)**

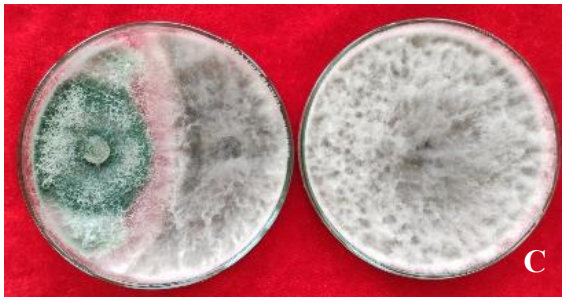


***Sclerotium rolfsii* (MT DF1)**

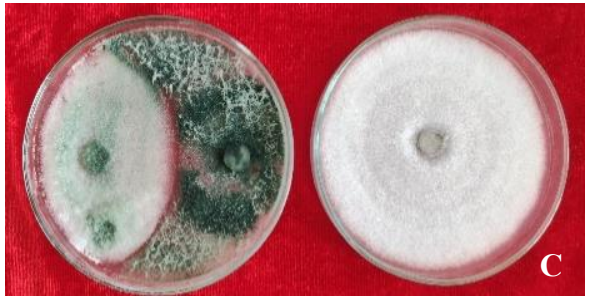
***Alternaria alternata* (VP DF1)**



***Colletotrichum* sp. (VK OF3)**



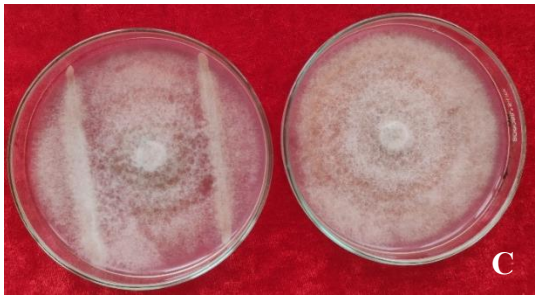
***Lasiodiplodia theobromae* (VY CF2)**



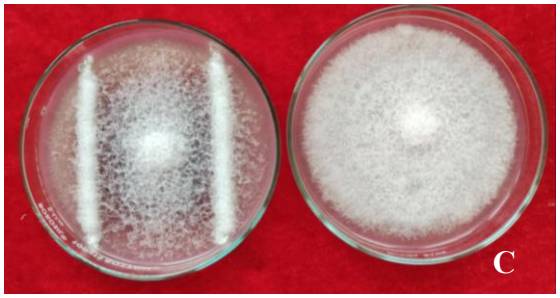
***Colletotrichum gloeosporioides* (MT SF1)**

C- Control

Plate 20 : *In vitro* efficacy of *Pseudomonas fluorescens* against major pathogens

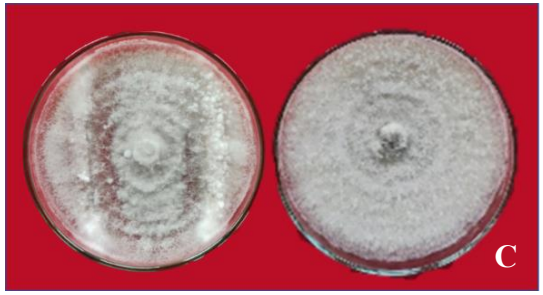


***Colletotrichum boninense* (VL DF1)**

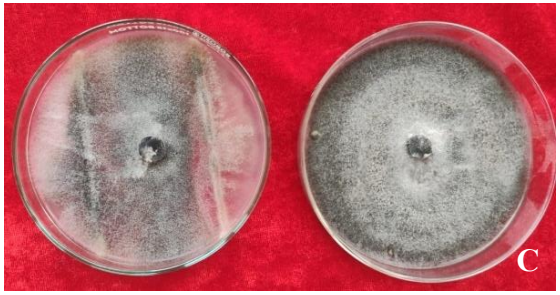


***Fusarium proliferatum* (VY DF)**

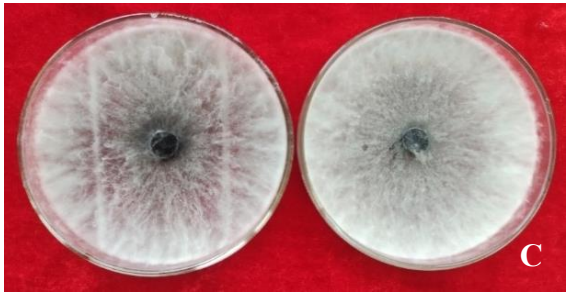
***Sclerotium rolfsii* (MT DF1)**



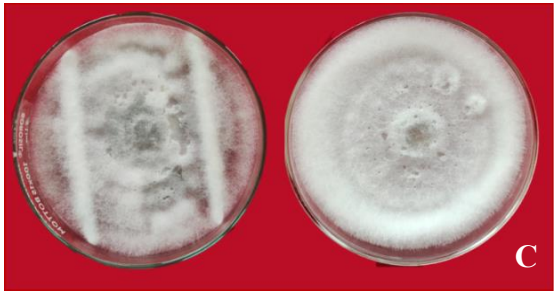
***Alternaria alternata* (VP DF1)**



***Colletotrichum* sp. (VK OF3)**



***Lasiodiplodia theobromae* (VY CF2)**



***Colletotrichum gloeosporioides* (MT SF1)**

C- Control

Plate 21 : *In vitro* efficacy of PGPR II against major pathogens

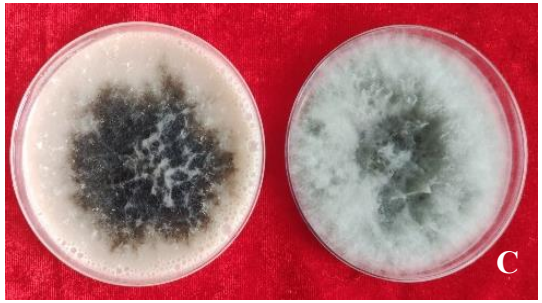
***Colletotrichum boninense* (VL DF1)**

***Fusarium proliferatum* (VY DF)**

***Sclerotium rolfsii* (MT DF1)**

***Alternaria alternata* (VP DF1)**

***Colletotrichum* sp. (VK OF3)**



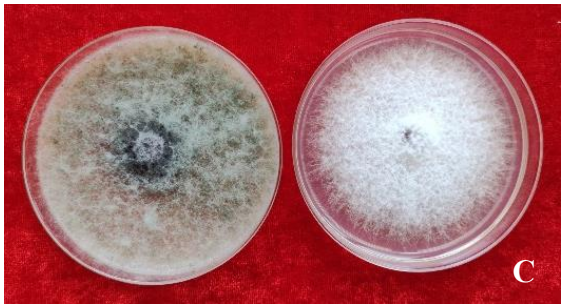
***Lasiodiplodia theobromae* (VY CF2)**

***Colletotrichum gloeosporioides* (MT SF1)**

C- Control

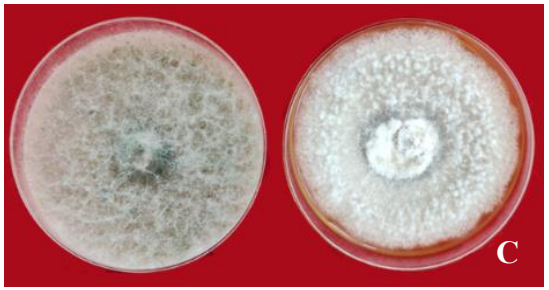
Plate 22 : *In vitro* efficacy of PGPM against major pathogens

***Colletotrichum boninense* (VL DF1)**



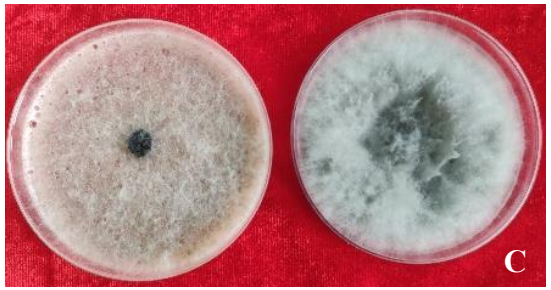
***Fusarium proliferatum* (VY DF)**

***Sclerotium rolfsii* (MT DF1)**



***Alternaria alternata* (VP DF1)**

***Colletotrichum* sp. (VK OF3)**

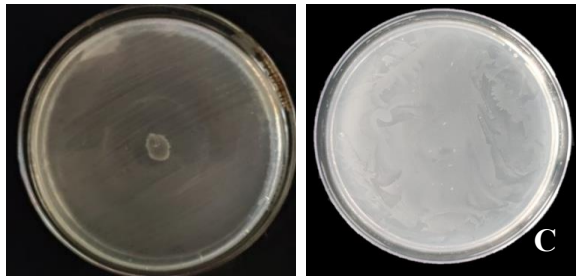


***Lasiodiplodia theobromae* (VY CF2)**

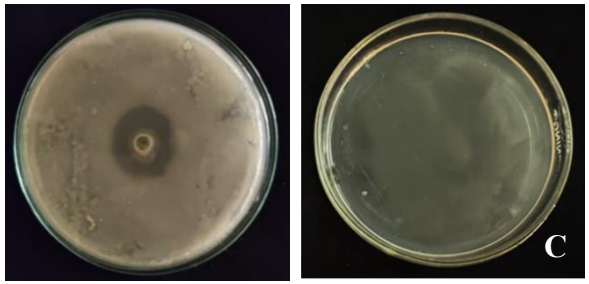
***Colletotrichum gloeosporioides* (MT SF1)**

C- Control

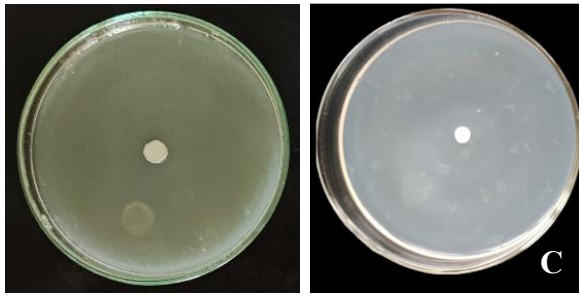
Plate 23 : *In vitro* efficacy of biocontrol agents against *Pectobacterium aroidearum* (VP CB)



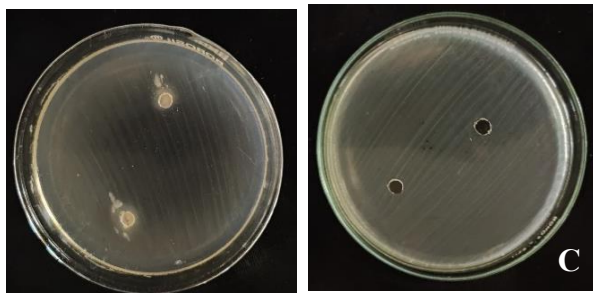
Pseudomonas fluorescens



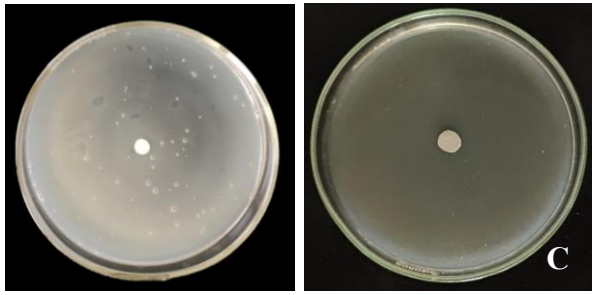
Trichoderma asperellum



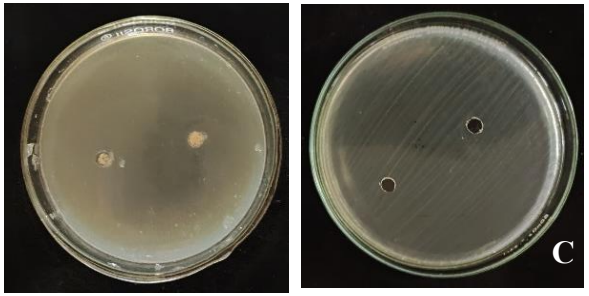
PGPR II- Paper disc method



PGPR II- Well diffusion method



PGPM- Paper disc method



PGPM- Well diffusion method

C- Control

Table 4.27. *In vitro* evaluation of biocontrol agents against major pathogens

Biocontrol agent	Per cent inhibition of pathogen (%)							
	Colletotrichum boninense (VL DF1)	*Fusarium proliferatum* (VY DF)	*Sclerotium rolfsii* (MT DF1)	*Alternaria alternata* (VP DF1)	*Colletotrichum* sp. (VK OF3)	*Lasiodiplodia theobromae* (VY CF2)	*Colletotrichum gloeosporioides* (MT SF1)	*Pectobacterium aroidearum* (VP CB)
Trichoderma asperellum	61.22	61.90	58.50	91.16	57.14	20.41	53.06	26.67
**Pseudomonas fluorescens*	+	+	-	++	+++	-	+	-
PGPR II	100.0	70.0	72.91	79.58	74.58	50.41	100.0	-
PGPM	100.0	60.41	75.0	77.08	77.91	100.0	84.58	-

*No per cent inhibition can be calculated. Only inhibition pattern can be designated as follows:

- → No inhibition
+ → Weak
++ → Moderate/ high
+++ → High

4.6. Bioassay studies on detached leaves of sympodial orchids against major pathogens

Bioassay experiment was conducted to evaluate the efficacy of fungicide, against the major pathogens such as *F. proliferatum, C. boninense, L. theobromae, S. rolfsii* and *Colletotrichum* sp. The pathogens were selected based on their highest PDI and PDS (Table 4.28). The combination fungicide, carbendazim 12 % + mancozeb 63 % was found to be the most effective fungicide from *in vitro* evaluation, and hence it was selected for bioassay studies.

Bioassay experiment study was conducted for *F. proliferatum* (VY DF) causing leaf blight disease in *Dendrobium* spp. and a disease severity index (DSI) of 3.6 per cent was noticed in fungicide treated leaves whereas 100 per cent DSI was found in control leaves 3 DAI. A reduction in disease severity of 96.4 was observed when treated with above said fungicide indicating high efficacy of fungicide against the pathogen (Plate 24).

The efficiency of the fungicide was evaluated against *C. boninense* (VT DF1) affecting *Dendrobium* spp. and revealed that no significant infection could be observed on treated leaves as compared to control leaves. DSI for fungicide treated leaves was calculated as 2.8 per cent which was found negligible, when compared to control leaves with 83 per cent disease severity. The percentage reduction in disease severity was calculated as 96.67 per cent, which indicated that the fungicide was highly effective against *C. boninense* (Plate 24).

The fungicide was also evaluated against the pathogen *S. rolfsii* (MT DF1) affecting *Dendrobium* sp., and 95.7 per cent reduction in disease severity was observed 5 DAI. The calculated DSI for the treatment was 4.3 per cent compared to control, and hence the fungicide was found effective against *S. rolfsii* under *in planta* condition. *Colletotrichum* sp. (VK OF3) causing leaf spot in *Oncidium* spp. was carried out using the above-mentioned fungicide, which resulted in 90.4 per cent reduction in disease severity whereas, *L. theobromae* (VY CF2) in *Cattleya* sp. showed a reduction in severity of only 21.17 per cent (Plate 24a).

Plate 24 : Bioassay studies on detached leaves of sympodial orchids against major pathogens

Moist chamber

Open condition

2 DAI

6 DAI

Control

***Colletotrichum boninense* (VL DF1)**

Moist chamber

Open condition

3 DAI

4 DAI

Control

***Fusarium proliferatum* (VY DF)**

Thus, the study revealed that carbendazim 12 % + mancozeb 63 % was highly effective against *C. boninense* (VT DF1), followed by *F. proliferatum* (VY DF), *S. rolfsii* (MT DF1) and *Colletotrichum* sp. (VK OF3), whereas the fungicide was found least effective in controlling *L. theobromae* (VY CF2) under *in planta* condition.

Table 4. 28. Bioassay studies on detached leaves of sympodial orchids against major pathogens

Sl. No.	**Pathogen**	**Fungicide** Carbendazim 12 % + Mancozeb 63 % (0.2 %)		**Percentage reduction in disease severity (%)**
		DSI (Disease severity index)		
		Treatment (%)	Control (%)	
1	*Colletotrichum boninense* (VT DF1)	2.8	83.0	96.67
2	*Fusarium proliferatum* (VY DF)	3.6	100.0	96.4
3	*Sclerotium rolfsii* (MT DF1)	4.3	100.0	95.7
4	*Colletotrichum* sp. (VK OF3)	1.2	12.6	90.4
5	*Lasiodiplodia theobromae* (VY CF2)	10.8	13.7	21.17

Plate 24a : Bioassay studies on detached leaves of sympodial orchids against major pathogens

Moist chamber Open condition 3 DAI 5 DAI Control

***Sclerotium rolfsi*i (MT DF1)**

Moist chamber Open condition 3 DAI 7 DAI Control

***Colletotrichum* sp. (VK OF3)**

Moist chamber Open condition 5 DAI Control

***Lasiodiplodia theobromae* (VY CF2)**

Discussion

5. DISCUSSION

Orchids are bewitchingly beautiful flowers known for their long vase life, incredible range of diversity in size, shape and colour, and being grown as both cut flower and potted plants (Singh *et al.*, 2013). They belong to Orchidaceae which is one of the largest families of flowering plants. Nearly 30,000 species in 600 genera with 1.5 lakh man-made hybrids of orchids have been reported worldwide (Rajeevan *et al*., 2017). India contributes about 7 per cent of the world's orchid genetic diversity, with 1300 species in 184 genera (Meena and Medhi, 2013). Among the top ten cut flowers, orchids rank sixth in global trade. Orchids are categorised into monopodial and sympodial types based on their growth habit. The stems of monopodial orchids emerge from a single bud, and leaves are added from the apex annually, whereas sympodial orchids produce a series of adjacent shoots with limited growth along with pseudobulbs (Sailo, 2014). Though both monopodial and sympodial orchids are equally used in commercial cultivation, sympodial types (*Cymbidium* sp., *Dendrobium* sp., *Cattleya* sp. etc.) rank higher in the export market. The vast majority of orchids are grown in Kerala due to favourable climatic conditions (Rajeevan *et al*., 2017). However, orchid cultivation is challenged by many biotic factors, especially fungal, bacterial and viral diseases, which limit its production potential and market value. Several researchers have documented many diseases affecting different sympodial orchids such as anthracnose (*Colletotrichum* spp.), southern leaf blight (*Sclerotium rolfsii*), stem rot (*Fusarium kyushuense*), wilt (*Fusarium oxysporum* and *S. rolfsii*), blossom blight (*Botrytis cineraria*) and soft rot (*Dickeya fangzhongdai)* in *Dendrobium* sp., Anthracnose (*Colletotrichum* spp.), leaf spot (*Lasiodiplodia theobromae*), leaf blight (*Rhizoctonia solani)* and black rot (*Phytophthora palmivora*) in Cattleya, *Cymbidium mosaic virus* (CyMV) and *Odontoglossum ringspot virus* (ORSV) in *Cymbidium* sp., resulting in heavy crop losses (Jensen, 1950; Jensen and Gold, 1951; Ito and Aragaki, 1977; Uchida and Aragaki, 1991b; Bag, 2003; Cabrera and Cundom, 2013. ; Zhang *et al.*, 2017; Balamurugan *et al.,* 2020; Fernandez-Herrera *et al.*, 2020; Ma *et al*., 2020; Silva *et al.,* 2021; Cao *et al.*, 2022a; Cheng *et al.*, 2023; Hsieh *et al.,* 2023).

However, not much work has been conducted on documentation of various diseases associated with sympodial orchids. Hence, the present investigation is

proposed with an objective to identify the diseases affecting sympodial orchids grown in Kerala, characterisation of associated pathogens and *in vitro* studies.

5. 1. SURVEY

An intensive sampling survey was carried out during May-November 2022 at different orchid growing tracts in Thrissur, Ernakulam and Trivandrum districts to investigate the diversity and distribution of diseases in sympodial orchids. Surveys were conducted in a total of eight locations in three districts, and disease samples were collected from *Dendrobium* spp., *Cattleya* spp., *Oncidium* spp. and *Spathoglottis* spp. as these were the major sympodial orchids being grown in Kerala. A total of 49 symptoms were from various locations, among which 31 samples were from Thrissur, 12 samples were from Ernakulam, and 6 samples were obtained from Thiruvananthapuram from various sympodial orchids. Different symptoms such as leaf blight (7 nos.), leaf spot (5 nos.), wilt (1 no.), black rot (1 no.), twig blight (2 nos.), petal blight (2 nos.) and flower spot (3 nos.) were collected from *Dendrobium* spp.

The occurrence of leaf blight symptoms in *Dendrobium* spp. was reported by Tao *et al.* (2011) and Cheng *et al.* (2023). Different types of leaf spot symptoms were observed by McMillan *et al.* (2008), Sarsaiya *et al.* (2019), Wang *et al.* (2020) and Cao *et al.* (2022a), black rot symptom was reported by Cating *et al.* (2010) wilt disease in *Dendrobium* spp. was reported by Zhang *et al.* (2017), and dieback was observed by Mirghasempour *et al.* (2022). Similarly, study regarding floral diseases like flower spot was reported by Leonhardt and Sewake (1999), while Uchida and Aragaki (1991a) and Meera *et al.* (2016) reported flower spot symptoms in various orchids.

In *Oncidium* spp., leaf blight (8 nos.), leaf spot (7 nos.) and wilt (1 nos.) symptoms were collected from different locations. The symptomatology studies on diseases in oncidium haven't been conducted much. Different symptoms like black rot, soft rot and leaf blight diseases were noticed in Cattleya during the survey. The results are in agreement with the findings of Ark (1951), Keith *et al.* (2005) and Silva-Cabral (2019) who reported black rot, soft rot and anthracnose symptoms in *Cattleya* spp. respectively. Leaf blight (4 nos.) and leaf spot (1 no.) were collected from *Spathoglottis* spp. from Thrissur and Thiruvananthapuram districts. No much studies have been conducted on

diseases in *Spathoglottis* spp. However, leaf blight symptom caused by *Colletotrichum* spp. in *Spathoglottis plicata* was mentioned by Brooks (2002).

PDS and PDI were calculated for each symptom from *Dendrobium* sp., and the highest PDI and were recorded for VY DLB (76.47 %) and highest PDS were recorded for MT DLB1 (36.67 %). In *Oncidium* sp., the highest PDI and PDS were recorded for VK OLS1 with 76.92 per cent and 46.90 per cent respectively. In *Spathoglottis* spp., highest PDI and PDS were recorded for MT SLB1 with 48.0 and 29.79 per cent respectively. From the PDS and PDI data, it was found that in *Dendrobium* sp., leaf blight symptoms were more common and severe, whereas in *Oncidium* sp., leaf spot symptoms were more severe. In *Cattleya* sp. leaf spot symptoms were most frequently noticed. However, soft rot symptom showed highest severity in *Cattleya* sp. In *Spathoglottis* sp., leaf blight symptoms were most commonly observed with high severity too. Similar studies were conducted by Joko *et al.* (2011), who recorded per cent disease incidence and severity of bacterial soft rot symptom in *Phalaenopsis* sp. from seven surveyed locations of Indonesia and found maximum PDI and PDS of 100 per cent and 46.2 per cent respectively.

The incidence and severity of epidemics in crop plants are primarily determined by weather conditions. Hence, an attempt was made to study the role of light intensity on infection and the development of diseases in sympodial orchids. PDS and PDI values were correlated with light intensity at different survey locations, and a negative correlation was obtained for all the values, which revealed that the severity of diseases increased gradually with a decrease in the light intensity of the prevailing location. A significant negative correlation was observed between light intensity and disease incidence and severity in *Dendrobium* sp. and *Spathoglottis* sp. while no much significance was found for *Oncidium* sp. and *Cattleya* sp. The results were found to be comparable with findings of Amsalem *et al.* (2006) who found that, radiation of 7000 lux was detrimental to powdery mildew caused by *Sphaerotheca macularis* f. sp. *fragariae* on strawberry, and concluded that greater light intensities resulted in a reduction in disease severity.

5.2. ISOLATION OF PATHOGENS AND PATHOGENICITY STUDIES

The isolation of the pathogens from diseased specimens collected from different sympodial orchids was isolated by tissue segmentation method (Rangaswamy, 1958), and pathogenicity test was conducted for each isolate obtained from distinct symptoms by artificial inoculation of the pathogen on detached plant parts. The mycelial bit inoculation method (Rocha *et al*., 1998) was followed for proving the pathogenicity of different isolates obtained from foliage, flowers and twigs.

A total of 21 fungal isolates were obtained from *Dendrobium* spp. from various survey locations (Thrissur -6, Ernakulam -4 and Thiruvananthapuram -1). All the isolates exhibited distinct symptoms upon artificial inoculation, and these isolates could be reisolated proving Koch's postulates. Tao *et al.* (2011) experimentally proved the pathogenicity of *Phytophthora nicotianae* causing infection in *Dendrobium* spp. Likewise, Dissanayake (2015) proved the pathogenicity of *Fusarium proliferatum* in flower buds of *Dendrobium* sp. by spraying conidial suspension (1 x 10^6 conidia /ml) of the isolates. Zhang *et al.* (2017) could prove the pathogenicity of *F. oxysporum* causing wilt disease in *Dendrobium officinale*. The pathogenicity test for *Colletotrichum* spp. in *Dendrobium* sp. is in congruence with the results of Ma *et al.* (2019) and Fernandez-Herrera *et al.* (2020). Moreover, method followed by Wang *et al.* (2020) was found parallel to the present study as they could prove the pathogenicity of *Alternaria alternata* in *Dendrobium candidum.* Likewise, the pathogenicity of *S. rolfsii* in *Dendrobium huoshanense* was proved by of Cheng *et al*. (2023). Hence, the pathogenicity test carried out in the present study confirmed that all the isolates were pathogens of *Dendrobium* spp. reproducing distinct symptoms observed as in naturally infected plants. Eighteen fungal isolates were obtained from *Oncidium* spp. from different tracts (Thrissur -10, Ernakulam -5 and Thiruvananthapuram -3) and all the isolates proved pathogenicity test. Five fungal isolates (Thrissur-1, Ernakulam-2 and Thiruvananthapuram-1) and one bacterial isolate (Thrissur) were obtained from *Cattleya* spp. and nine fungal isolates were obtained from *Spathoglottis* sp. (Thrissur -6, Thiruvananthapuram -3).

5.3. SYMPTOMATOLOGY

5.3.1. Symptomatology of diseases in *Dendrobium* spp.

The leaf blight symptoms collected from *Dendrobium* spp. from various locations (MT DLB2, VK DLB, IK DLB, AM DLB and VY DLB) were found to be similar and these symptoms initially appeared as white, papery textured lesions produced from the tip or margin of the leaves. These lesions coalesced to cause leaf blight, and the affected areas fell off at later stages. The pathogens isolated from these symptoms were identified as *Fusarium* spp. There are no reports of symptoms similar to those mentioned above yet. All *Fusarium* isolates causing leaf blight, except IK DLB, produced water-soaked lesions on the inoculated areas followed by complete yellowing and rotting of leaves. The similar kind of artificial symptom was observed for *Fusarium sacchari* causing leaf blotch in *Dendrobium antennatum* in Malaysia (Mohd, 2021), while IK DLB produced dull white, papery textured sunken lesions with brown margin on artificial inoculation.

Apart from leaf blight, *Fusarium* spp. also caused leaf spot disease (VK DLS) which was described as black circular sunken spot along the mid vein. The symptoms match with that of black spot caused by *F. proliferatum* in *Cymbidium* hybrids (Chang *et al.,* 1998). Upon artificial inoculation, black circular to oval spots were produced, followed by rotting of the leaves. A wilt symptom collected from Ambunadu (AM DW) caused wilting and drying of plants. Upon artificial inoculation, the leaves became water-soaked, followed by complete yellowing and rotting. Similar symptoms produced under natural and artificial condition were reported by Zhang *et al.* (2017) in *D. officinale*. The disease is characterised by the yellowing of leaves starting from the bottom, followed by defoliation. Gradually, the collar region and roots turned brown, causing the death of the whole plant. Fusarium produced two different kinds of flower spots on *Dendrobium* sp. flowers. The flower spot symptom VK DFS2 appeared as minute brown spots at the lower surface of the petals, while VP DFS was described as dark brown spots with green halo. *Sclerotium* sp. produced different types of symptoms at different stages of disease development. The leaf blight symptom obtained from Madakkathara (MT DLB1) initially appeared as orangish, concentric circular, water-soaked lesions on leaves with a prominent yellow halo. Later, the

affected leaves completely turned yellow, followed by drying and withering of entire plant. Another type of symptom recorded was leaf blight, in which blight started from the tip and extended downward with a wavy margin, whereas the leaf spot, IK DLS, appeared as dull greyish, circular, water-soaked spots on leaves. Similar symptoms were previously reported by Cheng *et al.* (2023), who detailed the leaf blight caused by *S. rolfsii* in *D. huoshanense* as the withering of leaves, formation of a thick mycelial mat at the base of the stem, and the presence of numerous globular sclerotia.

Colletotrichum isolates produced different kinds of symptoms on various plant parts, such as leaf blight, leaf spot, flower spot, petal blight and twig blight. The leaf blight symptoms VL DLB was described as brownish to black irregular sunken lesions with diffused yellow halo. These symptoms are in agreement with anthracnose caused by *Colletotrichum karstii* on *D. nobile* (Fernandez-Herrera *et al.,* 2020). VL DLS appeared as minute, dull black coloured necrotic sunken spots over the leaves with a faint yellow halo. Under artificial conditions, both isolates produced similar symptoms which appeared as dark brown, water-soaked lesion with prominent yellow halo. The lesion enlarged quickly and caused the rotting of leaves with the development of mycelia and salmon coloured spore mass.

Curvularia spp. produced dull brownish spots on leaves with chlorotic halo. Upon artificial inoculation, the pathogen produced greyish-black, circular and sunken spot with prominent yellow halo. These findings are in agreement with Rinchen *et al.* (2023), who observed black-brown circular spot on the leaves caused by *C. lunata.* Streda (2013) noticed rusty brown spots on *Dendrobium* sp. flowers caused by *Curvularia* spp.

Petal blight (VP DPB1) caused by *Colletotrichum* sp. produced brownish blight from the margin and later fell off, whereas flower spot symptom (VK DFS1) appeared as brownish semi-circular spots at margins. The pathogen isolated from VP DPB1 produced brownish, irregular water-soaked lesions upon artificial inoculation, while the flower spot pathogen produced small brown spots with a white halo. The findings are in line with Uchida and Aragaki, (1991a), who observed flower spots in *Dendrobium* sp. caused by *C. coccodes* and Jadrane (2012) reported petal blight symptom caused by *C. karstii* in Phalaenopsis orchids.

The pathogen also produced blight symptoms on the twigs (VK DTB and VP DTB). No report of twig infection caused by *Colletotrichum* sp. in *Dendrobium* spp. have been published yet. However, the symptoms match with the findings of Chowdappa *et al.* (2012), who observed necrotic lesions on *Cymbidium* sp., and Sharma *et al.* (2013), recorded similar symptoms of twig blight on *Catharanthus roseus*.

Other symptoms, such as black rot (VP DBR) caused by *Phytophthora* sp. caused complete decay and death of the plants. Upon artificial inoculation, the pathogen produced a black coloured, water-soaked lesion, which enlarged rapidly and caused complete rotting. The natural and artificial symptoms are in conformity with the observations of Cating *et al.* (2010), who observed black lesions on various plant parts of *Dendrobium* sp. *Alternaria* sp. produced black circular to oval shaped water-soaked lesion with chlorotic halo on leaves (VP DLS) and the pathogen produced dull black circular lesion with yellow halo under artificial inoculation. The pathogen also caused blight and deformation of petals (VP DPB2), that produced similar symptoms under artificial condition. No similar symptoms were reported in *Dendrobium* sp. caused by *A. alternata.*

5.3.2. Symptomatology of diseases in *Oncidium* spp.

Different types of blight symptoms were observed in *Oncidium* spp. caused by *Colletotrichum* isolates. Most of the leaf blight symptoms appeared as greyish to brown blight starting from the tip of the leaf and extending downward, causing drying of foliage. They produced similar kinds of symptoms under artificial inoculation. AM OLB1 appeared as greyish to straw-coloured leaf blight with concentric zonations. Under artificial condition, the pathogen produced greyish-white lesion with dark brown to black, irregular margin. Similar symptoms were observed for anthracnose disease in *Cymbidium* sp. caused by *C. cymbidiicola* (Park *et al.*, 2020). The leaf blight symptom caused by *Colletotrichum* sp. (VL OLB1) was characterised as dull, black necrotic lesion with prominent yellow halo. The isolate produced similar symptoms under artificial condition, while KO OLB appeared as greyish to light brown blight from tip with yellow halo. Similar symptoms were produced under artificial condition as well.

Different types of leaf spot diseases were observed in *Oncidium* spp., and the majority of the leaf spot symptoms were caused by various *Colletotrichum* isolates. Three types of leaf spot symptoms were obtained from Vellanikkara. VK OLS1 appeared as small, black necrotic spots, while VK OLS2 was characterised by black necrotic specks which appeared as patches. VK OLS1 produced similar symptoms under artificial condition, while the symptom VK OLS2 was caused by three different *Colletotrichum* isolates which upon artificial inoculation produced distinct symptoms. VK OLS3 appeared as brownish sunken lesions surrounded by yellow halo which on artificial inoculation produced greyish-white oval to irregular spots with distinct dark brown margins. The symptom produced by VK OLS3 matches with leaf spot diseases of *Liriope cymbidiomorpha* caused by *Colletotrichum* sp. (Yang *et al*., 2020) Leaf spot symptom obtained from Vadanappally (VP OLS) produced circular or irregular spots with a straw-coloured centre and dark brown margin. Under artificial conditions, the symptom appeared as dark brown to black circular spots with salmon-coloured spore mass at the centre of the lesion.

The leaf blight symptom AM OLB2 was characterised by dark brownish blight alternating with light brown and dark brown concentric zonations. The lesion was surrounded by a yellowish halo, with the appearance of mycelial growth on the lesion. Under artificial inoculation, the pathogen produced a dark brownish to black lesion. Akhtar *et al.* (2004) also reported similar lesions with concentric zonations on tomato caused by *A. alternata*. *Alternaria* spp. also caused leaf spot disease in *Oncidium* sp. (VY OLS) which was described by small, minute, dark brownish, irregular spots on leaves with chlorotic halo. Under artificial condition, light brownish irregular water-soaked lesions were observed.

Two types of leaf spot symptoms, IK OLS1 and IK OLS2 were observed on *Oncidium* sp., and the causal agents were identified as *Diaporthe tulliensis* and *Pestalotia* sp. respectively. IK OLS1 appeared as rusty, sunken spots on the upper surface of the leaves and later turned purplish black. Under artificial conditions, the pathogen produced black necrotic lesions, which later developed into leaf blight. The results are comparable with the findings of Gong *et al.* (2020), who described symptoms caused by *D. tulliensis* in *Coffea arabica*. IK OLS2 produced black, irregular necrotic,

irregular sunken spots on the lower surface of the leaves. Upon artificial inoculation, dark brown, circular to irregular water-soaked lesions were observed. The symptoms are comparable with the leaf spot symptom caused by *Neopestalotiopsis clavispora* on *D. officinale* (Cao *et al.*, 2022b).

The leaf blight symptom (VK OLB1) caused by *Diplodia* sp. initiated from tip and gradually extended downward. The blight was greyish to light brown in colour and surrounded by a yellow halo. Black coloured pycnidia were arranged on the lesion in a wavy fashion. Upon artificial inoculation, the pathogen produced a dark brown, irregular lesion with yellow halo. The findings are in line with the report of Lopes *et al.* (2009), who observed necrotic spots with yellow halo caused by *Lasiodiplodia theobromae* on *Catasetum fimbriatum.*

Wilt symptom, KO OW, caused by *Fusarium* sp. noticed in Kottukal, was characterised as brownish black water-soaked lesion on roots and pseudo stems. The leaves of the affected plant turned yellow. Gradually, the lesion extended into upper plant parts and caused the rotting and toppling of the entire plant. Under artificial conditions, the pathogen initially produced a transparent, water-soaked brownish lesion with prominent chlorotic halo. The lesion gradually extended and covered the leaves, causing complete rotting and wilting. Huang *et al.* (2014) reported identical symptoms in jewel orchid caused by *Fusarium oxysporum* f. sp. *anoectochili* under both natural and artificial condition.

5.3.3. Symptomatology of diseases in *Cattleya* spp.

Different types of symptoms, such as leaf blight, leaf spot, black rot and soft rot were noticed in *Cattleya* spp. Two different types of leaf blight symptoms were observed. The symptom obtained from Ambunadu (AM CLB1) showed greyish brown lesions, starting from the leaf tip that later extended into the leaf lamina, causing the blight and drying of whole leaves. Upon artificial inoculation, the pathogen produced black lesions followed by complete yellowing of the leaves. The leaf blight symptom obtained from Vellayani (AM CLB2) was characterised by brownish to black water-soaked lesion on the younger leaves. The affected leaves remained unopened. Black sunken patches appeared on the infected leaves, and the decayed leaves could be easily

pulled out. Under artificial conditions, dark brownish irregular lesion was observed which later developed into a greyish white centre with dark brownish to black margin. The observations are in agreement with the findings of Silva *et al.* (2021), who described the symptoms of anthracnose in *Cattleya walkeriana* caused by *Colletotrichum karstii.* The leaf spot symptom obtained from Vellayani (VY CLS) was characterised by greyish-white irregular sunken leaf spot with brown margin. The spots enlarged and coalesced to form leaf blight. Similar symptoms were produced under artificial condition as well. The symptoms match with the infection of *Lasiodiplodia theobromae* in *Cattleya* sp. (Cabrera and Cundom, 2013).

Black rot symptom (VP CBR) initiated as black lesions from the tip of the leaves or on the margin of the leaves progressing downward, causing complete blackening of leaves and eventually leading to the death of the plant. Cating *et al.* (2010) described black rot symptoms caused by *Phytophthora palmivora* and *P. cactorum.* The symptom initiated as tiny black lesions on the base of pseudo stems and roots. The lesion rapidly covered the entire pseudobulbs and leaves and eventually caused the death of the plant. The soft rot symptom caused by *Pectobacterium aroidearum* observed in *Cattleya* spp. appeared as small, dull black, water-soaked lesion at the tip of the leaves, which enlarged rapidly and resulted in soft rot of the leaves. The affected leaves hung down from the plants and macerated leaf tissues emitted a foul smell. Similar symptoms were also reported in *Cattleya* sp. caused by *Erwinia carotovora* causing soft rot (Ark, 1951).

5.3.4. Symptomatology of diseases in *Spathoglottis* spp.

Leaf blight symptoms caused by different *Colletotrichum* isolates were severe in *Spathoglottis s*pp. Four different leaf blight symptoms and one leaf spot symptom were obtained from Madakkathara, and all diseases were incited by *Colletotrichum* isolates. MT SLB1 was a combined infection by *Colletotrichum* sp. and an unidentified species. The symptom of leaf blight (MT SLB1) was characterised by brown coloured lesions surrounded by yellow halo that extended lengthwise and turned into straw-coloured blight with dark brown margin. The pathogens isolated from MT SLB1 produced similar symptoms on artificial inoculation, and the symptoms were characterised by dark brown oval spot with chlorotic halo. Later, the entire leaf lamina turned purple. Another leaf blight symptom, MT SLB2 produced greyish to brown

blight from the tip with concentric zonations surrounded by yellow halo. The fungus produced dark brownish to black, oval to irregular lesions upon artificial inoculation. Later, the whole leaves turned purple with chlorotic halo. MT SLB3 was characterised as dark brownish, water-soaked lesion with prominent yellow halo, while MT SLB4 initially appeared as brown to straw coloured irregular lesions with dark brown margin with yellow halo. The lesions coalesced to become blight and affected portion fell off leaving shot holes. Upon artificial inoculation, fungi isolated from MT SLB3 and MT SLB4 reproduced symptoms identical to their natural symptoms. The leaf spot symptom (MT SLS) appeared as greyish irregular spot surrounded by thick dark brown margin with concentric zonations. The symptoms produced by artificial inoculation were similar to the natural symptom. The leaf blight symptoms obtained from Kottukal showed light brown to dark brown water-soaked blight from tip with prominent yellow halo. The disease was a combined infection of two *Colletotrichum* spp. (KO SF1 and KO SF3) and one *Fusarium* sp. (KO SF2). Upon artificial inoculation, KO SF1 produced brown water-soaked lesions. Later, the leaves turned purplish in colour. KO SF2 produced oval shaped brownish to black lesions, and the leaves turned yellow. No symptomatology studies have been carried out in *Spathoglottis* sp. so far. This may be the first detailed study on symptomatology of different diseases in *Spathoglottis* sp.

5.4. CHARACTERISATION OF PATHOGENS ISOLATED FROM SYMPODIAL ORCHIDS

Based on the pathogenicity tests of the pathogens isolated from different symptoms on sympodial orchids, it was revealed that the isolates causing leaf blight, leaf spots, black rot, wilt, floral diseases and twig blight symptoms were fungal pathogens, while the soft rot symptom was due to bacteria. These pathogens obtained from different sympodial orchids were further characterised by cultural, morphological and molecular techniques.

5.4.1. Cultural and morphological characterisation of fungal pathogens

5.4.1.1. Cultural and morphological characterisation of fungal pathogens obtained from Dendrobium spp.

Different fungal isolates such as *Fusarium* spp. (9 nos.), *Colletotrichum* spp. (9 nos.), *Alternaria* spp. (2 nos.), *Sclerotium* spp. (2 nos.), *Phytophthora* sp. (1 no.) and *Curvularia* sp. (1 no.) were obtained from various symptoms collected from *Dendrobium* spp. Two isolates of *Sclerotium* spp. were obtained from Madakkathara (MT DF1) and Irinjalakkuda (IK DF2). The colony of MT DF1 was described as cottony white with tufts of hyphae and even sheet of arial mycelium with clear mycelial strands whereas IK DF2 produced dull white cottony mycelial strands with sparse growth at the centre and pluffy at its edges. Both cultures attained full growth at 5th DOI with a growth rate of 1.8 cm per day. Sclerotia formed at 10-15th DOI, and they were smooth, spherical and pale brown, which later changed to dark brown colour. Primary hyphae were hyaline and septate. The cultural and morphological features of the pathogens match with the descriptions of Cheng *et al.* (2023) who characterised *Sclerotium rolfsii* causing southern leaf blight in *Dendrobium huoshanense.*

The isolates MT DF2, VK DF1, VK DF2, VK DF4, IK DF1, VP DF5, AM DF1, AM DF2 and VY DF were identified as *Fusarium* spp. based on cultural and morphological features. The leaf blight pathogen, MT DF2, produced pinkish white mycelia that was sparse at the centre and cottony at the periphery, while VK DF1 produced pinkish white woolly mycelia that was moderately raised at the centre. The leaf spot pathogen, VK DF2, also showed similar colony characters. The growth rate of the three isolates was 1.12 cm per day. MT DF2 and VK DF2 produced numerous fusiform macroconidia with sizes of 5.2 x 1.43 µm and 2.7 x 0.89 µm respectively, while VK DF1 produced both micro and macroconidia. Microconidia were hyaline, ellipsoid, unicellular, and scattered freely in the mycelial mat with a size of 3.2 x 1.1 µm, while macroconidia were hyaline, septate, fusiform shaped, slightly curved and tapered at the ends with a size of 8.6 x 2.0 µm. The cultural and morphological characteristics of the isolates are in agreement with the findings of Srivastava (2014), who described different *Fusarium* isolates from orchids.

Two types of *Fusarium* isolates were obtained from floral symptoms that differed in cultural and morphological features. The *Fusarium* sp. obtained from flower spot (VK DF4) produced yellowish-white, cottony and fluffy mycelia with yellowish pigmentation on the reverse side with average growth rate of 1.5 cm per day. The mycelia were hyaline, septate and branched with numerous fusiform microconidia with a size of 4.90 x 2.63 µm. The cultural characters of VK DF4 are in line with descriptions given by Cao *et al.* (2022a). Another *Fusarium* isolate obtained from flower spot (VP DF5) produced pure white, moderately fluffy, and woolly aerial mycelia with clumps of mycelia at the periphery of the colony with a growth rate of 1.28 cm per day. The hyphae were hyaline, septate and branched. Microconidia and macroconidia were also observed with size of 3.8 x 1.1µm and 17.19 x 3.18 µm respectively. These features are consistent with *Fusarium subglutinans* causing leaf spot on *Cymbidium* spp. (Han *et al.*, 2015).

Fusarium isolates obtained from Ambunadu (AM DF1 and AM DF2) and Vellayani (VY DF) showed similar colony characters. The isolates produced fluffy, dull white woolly mycelia with filamentous and undulating margin. The reverse side of the Petri dish appeared deep reddish purple in colour. Both AM DF1 and VY DF had an average growth rate of 1.0 cm per day, whereas AM DF2 showed a growth rate of 1.28 cm per day. All the cultures produced hyaline and septate hypha with numerous microconidia of size ranging 2.9-3.9 x 0.98-1.2 µm. The characters of these cultures are in line with CMI description of pathogenic fungi and bacteria (Booth, 1969).

Fusarium sp. obtained from Irinjalakkuda (IK DF1) produced dull white, delicate and woolly aerial mycelia with filamentous and irregular margin. The culture was dome shaped with purplish tinge on the reverse side, and the average growth rate was 1.28 cm per day. The hyphae were hyaline and septate with numerous aseptate, carenate microconidia and one to three septate, hyaline macroconidia of sizes 4.0 x 1.0 µm and 9.1 x 2.0 µm respectively. These types characters of the fungi are not reported in orchids yet and are similar with findings of Hussain *et al*. (2012), who described the characters of *Fusarium oxysporum* isolated from guava wilt.

Another isolate obtained from leaf spot symptom (MT DF3) produced colonies that were dark grey to black, effuse, velvety, immersed mycelium with concentric

zonations, later turned flat, black on maturity, with an average growth rate of 1.28 cm per day. Hyphae were septate, brown and branched. Conidia were dark brown, curved at the third cell from the base, and asymmetrical with pale brown end cells, with a size of 19.6 x 8.64 µm. The characters of these culture are in line with the studies of Rinchen *et al.* (2023), who reported *Curvularia lunata* in *Bulbophylum reptans.*

Different *Colletotrichum* isolates were obtained from various symptoms, among which VK DF3 (isolate from flower spot) and VK DF5 (isolate from twig blight) produced similar colony characters. Theses cultures initially produced dull greyish-white woolly mycelia, which later changed into dark greyish to black in colour with a black reverse side. The colony had flat and submerged growth with an average growth rate of 1.12 cm per day. Acervuli were produced at 10-11 days of inoculation (DOI), associated with abundant conidial mass. Hyphae were initially hyaline, which later turned brown. Conidia were elongated and cylindrical with tapering ends, and an oil globule was present at the centre of the cell. The size of the conidia varies between 14.12- 19.78 x 3.58 -5.26 µm. The cultural and morphological features of the isolates were similar with *C. fructicola* causing anthracnose in *D. officinale* (Ma *et al.,* 2019).

The cultural and morphological characters of other *Colletotrichum* isolates, VL DF1 and VL DF2 were similar. The were produced light pinkish, woolly mycelia that were slightly raised at the middle with a light pinkish reverse side. The aerial portion of the mycelia gradually turned grey and appeared as a light pinkish colony with greyish patches on the upper surface. The growth rate of both pathogens was 1.12 cm per day and black, irregular, hard structures were formed all over the cultures after 15 days. Later, salmon coloured spore mass was produced, and the conidia were one celled, hyaline, bullet shaped, and had a central oil globule. The average size of the conidia was 10.84-11.2 x 4.0- 4.34 µm. The features of the cultures are in accordance with the descriptions of *C. boninense* by Moriwaki *et al.* (2003).

The twig blight isolate (VP DF6) initially produced greyish woolly, moderately fluffy mycelia that gradually became felted and turned dark grey with whitish grey mycelial clumps. The average growth rate of the fungus was 1.28 cm per day. The culture produced acervuli at 12 DOI with salmon coloured spore mass. The reverse side

of the colony appeared greyish black. The hyphae were branched, septate, initially hyaline, and gradually turned brown in colour. Conidia were one-celled, hyaline, bullet shaped, and had a central oil globule. The average size of the conidia was 15.28 x 4.22 µm. The cultures were identified as *Colletotrichum* spp. based on CMI descriptions of pathogenic fungi and bacteria (Mordue, 1971).

The cultural and morphological characters of *Alternaria* spp., VP DF1 (leaf spot pathogen) and VP DF4 (petal blight pathogen) were found to be similar. The colonies initially appeared as dull white cottony mycelia, which later became greyish white and slightly dome shaped with a circular and serrate margin. The reverse of the plate showed deep yellowish pigmentation. The fungus attained full growth at 11 DOI, with an average growth rate of 0.81 cm per day. Hyphae were branched, septate, and initially hyaline and they later turned brown. Conidia were pale brown to olive brown, obclavate or muriform shaped, with a beak at the tip. The average size of the conidia was 50.0-51.1 x 18.2-19.2 µm with two to three transverse and one to two longitudinal septa. The features of the isolates are in agreement with the findings of Wang *et al.* (2020), who characterised *A. alternata* from *D. candidum.*

The culture VP DF2 obtained from black rot symptom appeared as white, cottony aerial mycelia with a petaloid to stellate growth pattern. The reverse side of the culture was white, and the average growth rate of the fungus was 1.5 cm per day. Hyphae were hyaline, aseptate, and branched. The sporangia were hyaline, ovoid, and non-papillate, with a size of 44.8 x 36.0 µm. The findings are in accordance with the studies of Wongwan *et al.* (2021), who characterised *P. palmivora* in *Rhynchostylis gigantea.*

5.4.1.2. Cultural and morphological characterisation of fungal pathogens obtained from Oncidium spp.

Different types of *Colletotrichum* isolates viz., VK OF2, VK OF3, VK OF4, VK OF5, VK OF6, VK OF7, VP OF, AM OF1, AM OF3, VL OF1 and KO OF1 were obtained from *Oncidium* spp. from various leaf spot and leaf blight symptoms. VK OF2 (leaf blight pathogen) produced greyish white, woolly mycelia with a fluffy growth pattern with serrated margin. The reverse side of the plates produced dark greyish

patches, with an average growth rate of 1.28 cm per day. Acervuli were produced at 9 DOI. Hyphae were septate and branched, initially hyaline and later turning brown with characteristic swellings. Conidia were hyaline, single celled, and cylindrical to dumbbell shaped, with a size 17.46 x 6.06 µm. The cultural and morphological features of the isolates were similar with *Glomerella cingulata* causing anthracnose in *Oncidium* sp. in Kerala (Sreedharan *et al.,* 1994).

The leaf spot pathogen, VK OF3 produced woolly olivaceous grey mycelia with flat and even growth with greyish black reverse side. The average growth rate of the fungus was measured as 1.28 cm per day Acervuli were minute and black and produced at 11 DOI. Conidia were elongated and cylindrical, with acute ends. The size of the conidia was measured as 19.0 x 3.9 µm. The cultural and morphological features are in line with the characters of *Colletotrichum oncidii* reported by Liu *et al*. (2014).

There are three leaf spot pathogens isolated from symptom from Vellanikkara, VK OLS2, of which VK OF4 produced woolly, fluffy and greyish mycelia that attained full growth at 6 DOI with an average growth rate of 1.5 cm per day. Conidial production was scanty. Conidia were bullet shaped with broad and narrow opposite ends. The average size of conidia was 14.48 x 4.22 µm. No similar studies were found regarding the cultural features of the isolate. However morphological features were found similar to *Colletotrichum gloeosporioides* causing anthracnose disease in *Cymbidium* sp. Park *et al.* (1996). Another pathogen associated with the same symptom, VK OF5 produced light pinkish, woolly and flat subaerial mycelia with a light pink reverse side. The average growth rate was 1.28 cm per day. Conidia were one celled, hyaline, cylindrical, slightly elongated, and contained two oil globules per cell. The size of the conidia was 13.37 x 3.80 µm. The cultural characters are similar to *C. karstii,* causing anthracnose disease in *D. nobile* (Fernandez-Herrera *et al.*, 2020) while morphological characters are match with *Colletotrichum dacrycarpi* from Dacrycarpus (Liu *et al*., 2014). The third pathogen (VK OF6), isolated from VK OLS2, initially produced greyish-white, fluffy and woolly mycelia, with a growth rate of 1.28 cm per day. Acervuli produced at 10 DOI. Conidia were celled, hyaline, cylindrical and elongated with an oil globule per cell. The size of the conidia was 14.36 x 3.69 µm. No supporting literature were found regarding the cultural characters of the pathogen. However, the morphological

characters found similar with *C. gloeosporioides* causing anthracnose disease in *Cymbidium* sp. (Park *et al.,* 1996).

The leaf spot pathogen, VK OF7 produced dull white, woolly and fluffy mycelia, which gradually turned greyish white with greenish grey reverse side. The fungus produced acervuli at 11 DOI with a growth rate of 1.28 cm per day. The conidia were characterised as one-celled, hyaline, cylindrical or bullet shaped, with a broad and narrow opposite end. A single oil globule was present at the centre of conidial cell. The size of the conidia was 14.48 x 4.22 µm. The morphological characters are found similar to those of *C. cymbidiicola* causing anthracnose disease in *Cymbidium* sp. (Park *et al.*, 2020).

The pathogen associated with leaf spot (VP OF) produced greyish, woolly and fluffy mycelia with serrated margin. The reverse side of the Petri dish appeared greyish-black. The pathogen attained full growth after 7 DOI. The conidia were characterised as one celled, hyaline, cylindrical and elongated with the presence of an oil globule. The average dimension of conidia was 16.0 x 3.7 µm. The morphological characters of both the isolates found similar with *C. gloeosporioides*, causing anthracnose disease in *Phalaenopsis* sp. (Meera, 2016).

The leaf blight pathogen, AM OF1 produced dark grey mycelia with greyish concentric zonation on the reverse side of the Petri dish. The colony produced acervuli with a growth rate of 1.12 cm per day. Conidia were abundant, hyaline and bullet shaped, with the presence of an oil globule at the centre and a size of 16.24 x 4.90 µm. No supporting descriptions were found regarding the cultural characters of the pathogen. Another leaf blight pathogen obtained from Ambunadu region (AM OF3) showed greyish, woolly sub aerial mycelia with alternate dark greenish to grey zonations and grey reverse side. The fungus attained full growth at 7 DOI. The hyphae were branched and septate which were initially hyaline and later became dark brown in colour. The conidia were hyaline and cylindrical with blunt ends having size of 17.0 x 4.29 µm. No descriptions were found similar to the cultural features AM OF1and AM OF3. However, the morphological characters of both the isolates found similar with *C. gloeosporioides*, causing anthracnose disease in orchids (Chowdappa *et al.*, 2012).

The pathogen (VL OF1) associated with leaf spot disease, produced light pinkish grey, woolly mycelia with slightly raised centre and light pink reverse side. The fungus produced irregular hard structures which were immersed in the medium later producing salmon-coloured spore mass. The growth rate of the fungus was .12 cm per day and conidia were one celled, hyaline, bullet shaped with a size of 18.0 x 4.2 µm. The cultural and morphological features were found to be similar with characters of *C. boninense* obtained from *Oncidium flexuosum* (Youlian *et al.,* 2011). The leaf blight pathogen, KO OF1, produced white, woolly aerial mycelia that gradually turned greyish white with greenish grey reverse side. The average growth rate of the fungus was 1.28 cm per day. The conidia were aseptate and bullet shaped with central oil globule, and the size of the conidia was 17.0 x 4.29 µm. The cultural and morphological characters are similar to *C. gloeosporioides,* causing anthracnose disease in orchids (Chowdappa *et al.*, 2012).

Other pathogens isolated from different symptoms collected from *Oncidium* spp. were *Diplodia* sp., *Diaporthe* sp., *Pestalotia* sp. *Alternaria* sp., *Phytophthora sp.* and *Fusarium* sp. The leaf blight pathogen VK OF1 produced greyish green woolly mycelia, turned dark grey over time, with dark grey to black reverse side and an average growth rate of 2.25 cm per day. The mycelia were septate and initially hyaline, which later turned dark brown. The conidia were oval in shape, initially colourless and aseptate, which gradually turned into dark brown and bicelled. The conidial size was recorded as 24.42 x 17.61 µm. The cultural and morphological characters comparable with the features of *Lasiodiplodia theobromae* in *Cattleya* sp. (Cabrera and Cundom, 2013). The pathogen associated with leaf spot symptom, IK OF1, produced dirty white, flat, sparse aerial mycelium with cottony texture. The colony produced yellowish green pigmentation at later stages. The fungus attained 90 mm of growth at 6 DOI. Black, irregular conidiomata embedded in the culture at 20 DOI. A dull white ooze appeared on the conidiomata after 30 days of incubation. Hyphae were hyaline and septate. No sporulation could be observed. The results are comparable with the findings of Gong *et al*. (2020), who characterised *D. tulliensis* in *Coffea arabica* and the pathogen has not been reported in orchid so far.

Another leaf spot pathogen, IK OF2, produced white cottony aerial mycelium, which diffused towards the advancing edge and was denser on the older part of the colony with diurnal zonation. The culture attained full growth at 9 DOI. Abundant, scattered acervuli with black, slimy spore mass were developed gradually. The reverse side appeared light orangish yellow with black patches. Fungus produced hyaline hyphae with characteristic five-celled conidia. Conidia were straight, fusiform to slightly clavate, with three to four septa. The three cells in the middle were brown, whereas the outer two cells were hyaline. The basal appendages were hyaline and straight, with an average size of 4.4 µm. Apical appendages were two in number, with an average length of 15.2 µm. The average dimension of conidia was 33.63 x 6.95 µm. The cultural and morphological features are in accordance with the characters of *N. clavispora,* causing leaf spot in *Dendrobium* spp. (Cao *et al.,* 2022b).

The isolates AM OF2 and VY OF showed similar cultural and morphological characters. Both the isolates produced brownish grey, cottony textured, compact, aerial mycelia. The reverse side of the Petri dish appeared dark brown with concentric zonations. The growth rates of AM OF2 and VY OF were 0.9 cm and 0.81 cm per day respectively. The hyphae were branched and septate which were initially hyaline and later turning dark brown. Conidia were light olivaceous to dark brown, obclavate or muriform shaped, with one to two longitudinal and three to six transverse septa had a narrow beak at one end. The average length and width of conidia were 49.0-51.44 x 15.87-16.88 µm. The beak size was measured 7.9-8.7 µm. The cultures were identified as *Alternaria* spp. based on CMI descriptions of pathogenic fungi and bacteria (David,1988).

The leaf blight associated pathogen obtained from Vyttila (VL OF2) produced cottony mycelia with restricted, tufted and irregular growth pattern where clumps of mycelia alternated with sparse mycelial growth. The colony was flat, uneven, with irregular margin. The fungus attained 90 mm of growth at 7 DAI with an average growth rate of 1.28 cm per day. The hyphae were hyaline and aseptate, with abundant production of elongated and ovoid semi-papillate sporangia with an average size of 32.48 x 9.8 µm. The features of the isolates were found similar with *P. palmivora* causing black rot on *Rhynchostylis gigantea* (Wongwan, 2021). The pathogen

associated with wilt disease (KO OF2) in *Oncidium* spp. produced white, delicate and woolly mycelia with sparse growth. The fungal colony was even, flat with entire margin, and attained full growth at 9 DOI with an average growth rate of 1.0 cm per day. Hyphae were hyaline and septate. Fusiform shaped microconidia were scattered among the mycelia with a size of 2.8 x 0.8 µm. Chlamydospores were terminal or intercalary. The findings are in accordance with the cultural and morphological features of *F. oxysporum* causing vascular wilt disease in tomato (Nirmaladevi and Srinivas, 2012).

5.4.1.3. Cultural and morphological characterisation of fungal pathogens obtained from Cattleya spp.

Fungal isolates such as *Colletotrichum* spp. (2 nos.), *Fusarium* sp. (1 no.), *Lasiodiplodia* sp. (1 no.) and *Phytophthora* sp. (1 no.) and a bacterial isolate *Pectobacetrium* sp. were isolated from Cattleya. Both *Colletotrichum* isolates (AM CF2 and VY CF1) exhibited blight symptom and produced greyish black, woolly mycelia with sparse growth on PDA. The average growth rate of these isolates was 1.28 cm per day. The hyphae were branched and septate, initially hyaline and later turning dark brown in colour. Both cultures produced perithecia at 14-16 DOI, which were hyaline, with clustered asci. Sickle shaped, aseptate hyaline ascospores with a size of 14.0-18.0 x 5.1-8.1µm were observed. Conidia were not observed in the case of AM CF2 whereas VY CF1 produced conidia which were bullet shaped, hyaline and one celled with a single oil globule. The size of the conidia observed was 14.0 x 5.1 µm. The morphological features observed are in line with descriptions of *C. karstii* in *Cattleya walkeriana* (Silva *et al.*, 2021). Another leaf blight pathogen, AM CF1, produced pinkish to white, woolly and compact aerial mycelia. The colony was slightly raised at the centre with an undulating margin, and the reverse side of the culture were deep reddish to purple. The growth rate of the fungus was 1.28 cm per day. Hyphae were hyaline and septate, with the production of numerous microconidia. Microconidia were single and fusiform shaped with tapering ends. The average size of the microconidia was 2.50 x 0.71 µm. The cultural and morphological characters are in agreement with the findings of Srivastava (2014), who described different *Fusarium* isolates from orchids.

The *Phytophthora* sp. (VP CF) causing black rot symptom appeared as white, cottony and aerial mycelia with petaloid growth pattern which attained 90 mm growth at 6 DOI The sporangia were hyaline, ovoid and papillate with a size of 46.2 x 36.6 µm. The cultural and morphological characters are in line with studies of Wongwan *et al.* (2021), who characterised *P. palmivora* in *Rhynchostylis gigantea.*

The pathogen (VY CF2) isolated from leaf spot symptom produced dull white, abundant, fluffy, aerial mycelia, turned olive-grey or grey over time, with greyish to black on the reverse of the Petri plate. The average growth rate of the fungus was 1.25 cm per day. The immature conidia of the fungal isolate were unicellular, hyaline, ellipsoid-ovoid, broadly rounded at the apex, with truncate or round base with thick walls. On maturation, conidia turned brown in colour with transverse septa and longitudinal striations. The size of the conidia was 20.0 x 8.6 µm. The cultural and morphological characters were found to be similar with *L. theobromae* reported in in *Cattleya* sp. in Argentina (Cabrera and Cundom, 2013).

5.4.1.4. Cultural and morphological characterisation of fungi obtained from Spathoglottis spp.

Seven *Colletotrichum* spp., one *Fusarium* sp. and an unidentified culture were obtained from *Spathoglottis* spp., in which MT SF1, MT SF4, MT SF5 and MT SF6 were similar in cultural and morphological characters. These cultures initially produced white, fluffy and woolly textured mycelia that later turned dull white with greenish grey patches. The average growth rate of these cultures was 1.28 cm per day. The conidia were bullet-shaped, hyaline and single celled with a central oil globule. The size of the conidia was measured as 14.0-15.7 x 5.9- 47.4 µm. The cultural and morphological characters are similar to those of *C. gloeosporioides,* causing anthracnose disease in orchids (Chowdappa *et al.*, 2012).

MT SF3 isolated from leaf blight symptom produced greyish to dull white, woolly mycelia with greenish to grey concentric patches on the reverse side. The average growth rate of 1.0 cm per day. The hyphae were branched, septate, initially hyaline and later dark brown. The conidia were hyaline, one celled cylindrical to bullet shaped with an oil globule at the centre. The conidial size recorded was 15.0 x 8.2 µm.

No studies were found similar with the cultural characters of the fungi. However, the pathogen was identified as *Colletotrichum* sp., based on CMI description of pathogenic fungi and bacteria (Mordue, 1971).

The *Colletotrichum* isolate KO SF1, produced greenish grey compact aerial mycelia which was slightly raised at the centre with even margin. The average growth rate of the fungus was 1.12 cm per day. The conidia were abundant, bullet-shaped hyaline and single celled with a central oil globule, with size of 16.0 x 7.5 µm. Another *Colletotrichum* sp. (KO SF3) isolated from the same symptom, produced grey, cottony mycelia with dark grey reverse side. The mycelia attained 90 mm growth at 7 DOI with scanty production of conidia. The conidia were cylindrical in shape and without oil globules The size of the conidia were 2.5 x 0.73 µm.

The *Fusarium* isolate, (KO SF2) produced dull white, delicate woolly mycelia with very sparse growth. The culture attained 90 mm of growth at 8 DOI. The mycelia were hyaline and septate. Macroconidia were scattered over the mycelia, with a size of 2.5 x 0.73 µm. The cultural and morphological features were similar with *Fusarium subglutinans* causing leaf spot on *Cymbidium* spp. (Han *et al.*, 2015).

The fungal pathogen MT SF2 produced flat, subaerial greyish to dull black, woolly mycelia with irregular margin. The colony showed concentric zonation with black reverse side. The growth rate of the fungi was 0.9 cm per day and the pycnidia were produced at 17 DOI. The hyphae were, branched, septate and hyaline and turned brown. The conidia were hyaline, unicellular, and oval shaped. Each conidium had two oil droplets, with a size of 1.55 x 0.75 µm. The pathogen could not be identified by cultural and morphological features.

5.4.2. Cultural, morphological and biochemical characterisation of bacterial pathogen

Cultural, morphological and biochemical characters of bacterial pathogen (VP CB) obtained from *Cattleya* spp. were studied in detail. The bacterial pathogen

produced translucent, yellowish white, small and flat colonies on nutrient agar medium after 72 hours of incubation.

The colony margin was initially circular and later became wavy in shape. The cultural characters of the bacteria were found similar with the studies of Tang *et al.* (2021) who reported *Pectobacterium aroidearum* causing soft rot in carrot.

Microscopic studies revealed that the bacterium was rod shaped. Bacteria were observed as pink colonies, and gram staining reaction confirmed it as gram negative bacteria. The morphological characters are similar to the findings of Nabhan *et al.* (2013), who identified *Pectobacterium aroidearum* from monocotyledonous plants.

The bacteria also showed positive results for potassium hydroxide test, gelatin hydrolysis test, methyl red test and citrate test whereas the bacteria was negative for catalase test, indole production test, Voges-Proskauer test and hydrogen sulphide production test. Based on the results of different biochemical tests, the isolate was identified as *Pectobacterium* sp. The biochemical results are in conformity with the findings of Dickey *et al.* (1979), Nabhan *et al.* (2013), Dana *et al*. (2014), Gore *et al.* (2020) and Sangeetha *et al.* (2020).

5.4.3. Molecular characterisation of major pathogens

Molecular characterisation of major pathogens from *Dendrobium* spp., *Oncidium* spp., *Cattleya* spp. and *Spathoglottis* spp. was done for species level identification by amplification and sequencing of ITS or LSU region of fungal pathogens and 16S rRNA sequence of bacterial pathogen. Nine fungal cultures and one bacterial culture were selected based on incidence and severity of the diseases and sent for molecular identification.

The isolate VL DF1 causing leaf spot symptom in *Dendrobium* spp. was characterised by amplification and sequencing of large subunit ribosomal ribonucleic acid (LSU rRNA) using primers LROR and LR7, and found 100 per cent identity with *Colletotrichum boninense* strain CBS241.78 having accession number DQ286167.1 with query coverage of 96 per cent. The pathogen has also been reported in *Dendrobium kingianum* and *Cattleya* sp. by Moriwaki *et al.* (2003) in Japan. The pathogen, VY DF, causing leaf blight symptom in *Dendrobium* spp., was characterised by amplification

and sequencing of the internal transcribed spacer (ITS) region using primers ITS-1F/ITS-4R. BLASTn analysis revealed 100 per cent identity with *Fusarium proliferatum* strain DWBM-2-2-1 having accession number ON527497.1 with 99 per cent query coverage. Latiffah *et al.* (2009) identified *F. proliferatum,* causing root rot in D*endrobium* sp.

Another isolate, IK DF1, causing leaf blight symptom in *Dendrobium* spp., was characterised by amplification and sequencing of LSU rRNA sequence using primers LROR and LR7. BLASTn analysis revealed 100 per cent similarity with *Fusarium oxysporum having* accession number CP052041.1, with 99 per cent query coverage. *Similar report was given by* Zhang *et al.* (2017), who identified and characterised *F. oxysporum,* causing wilt in *D. officinale*. The isolate MT DF1 associated with leaf blight in *Dendrobium* spp. was identified by amplification and sequencing of ITS region using primers ITS-1F/ITS-4R. BLASTn analysis showed 93.29 per cent similarity with *Athelia rolfsii* strain XFTH. The accession number of the identical sequence was JQ340325.1, with a query coverage of 99 per cent. Similarly, Cheng *et al.* (2023) also reported *Athelia rolfsii* (*S. rolfsii*) causing blight in *D. huoshanense*.

The pathogen (VP DF1) associated with leaf spot disease in *Dendrobium* spp. was characterised by amplification and sequencing of ITS region using primers ITS-1F/ITS-4R. BLASTn analysis revealed 100 per cent similarity with *Alternaria alternata having* accession number KM458821.1, with 41 per cent query coverage. Similar report was given by Wang *et al.* (2010) who characterised *A. alternata* causing leaf spot disease in *D. candidum.* The pathogen (VK OF3) isolated from leaf spot disease in *Oncidium* spp. was characterised by amplification and sequencing of LSU rRNA gene using primers LROR and LR7 and found 100 per cent similarity with *Colletotrichum* sp. AR3750. The accession number of the identical sequence was DQ286216.1, with a query coverage of 97 per cent. Liu *et al.* (2014) previously reported *Colletotrichum arxii* from *Oncidium excavatum* in Netherlands. The leaf spot causing pathogen, IK OF1, isolated from *Oncidium* spp., was characterised by amplification and sequencing of LSU rRNA gene using primers LROR and LR7. The BLASTn analysis revealed that the fungus exhibited 99.86 per cent similarity with *Diaporthe tulliensis* isolate SF24,

having accession number OM228732.1, with 99.86 per cent similarity and 100 per cent query coverage. Yuan *et al.* (2009) reported *Phomopsis amygdali* as an endophyte in *D. nobile.* Gong *et al*. (2020) also characterised *D. tulliensis* in *Coffea arabica*. However, this may be the first report of *D. tulliensis* infection on *Oncidium* spp. in India and elsewhere.

The isolate VY CLS causing leaf spot disease in *Cattleya* spp. was identified by amplification and sequencing of LSU rRNA gene using primers LROR and LR7. The BLASTn analysis showed 97.13 per cent similarity with *Lasiodiplodia theobromae* isolate VTCA having accession number KC442316.1 with 94 per cent query coverage. The infection of *L. theobromae* in *Cattleya* sp. was reported by Cabrera and Cundom (2013) in Argentina. The pathogen was also reported to cause leaf blight in *Catasetum fimbriatum* (Lopes *et al.,* 2009). The fungus MT SF1, causing leaf blight symptom in *Spathoglottis* spp., was characterised by amplification and sequencing of LSU rRNA gene using primers LROR and LR7. The BLASTn analysis revealed that the fungus exhibited 99.87 per cent similarity with *Colletotrichum gloeosporioides* strain RP205 of accession number JF441221.1 with 95 per cent query coverage. Dentika *et al.* (2023) reported *C. gloeosporioides* in *S. splicata* from Caribbean Island of Guadeloupe. The pathogen was also reported in other sympodial orchids like *Dendrobium* sp. and *Cymbidium* sp. (Chowdappa *et al.,* 2012; Park *et al.*, 2013).

The bacteria (VP CB) causing soft rot symptom observed in *Cattleya* sp. was identified by *in silico* analysis of the 16S ribosomal RNA sequence of the bacteria in NCBI database, showed 99.5 per cent similarity with *Pectobacterium aroidearum* strain MPS06 of accession number MH549224.1 with a query coverage of 100 per cent. Nabhan *et al.* (2103) reported *P. aroidearum* as causal organism of soft rot in monocot plants. Limber and Friedman (1943) also reported *E. carotovora* as soft rot causing pathogen in *Cattleya* sp. However, this may be the first report of *P. aroidearum* causing soft rot in *Cattleya* sp.

5.5. DISEASE MANAGEMENT

Disease management is a crucial component of orchid cultivation, and appropriate management practices can avoid the spread of disease, maintain plant health, improve the yield and quality of flowers, and assure the long-term sustainability of orchid cultivation. Disease management by chemicals and biocontrol agents is considered to be the most effective tools for managing various diseases. In the current study, the efficacy of different chemicals and biocontrol agents was tested *in vitro* against major pathogens associated with sympodial orchids.

5.5.1. *In vitro* evaluation of chemicals against major pathogens

Among plant protection strategies, chemical control is an indispensable part of integrated disease management. Therefore, an effort was made to evaluate *in vitro* efficacy of fungicides against major pathogens in sympodial orchids.

In the current investigation*, in vitro* evaluation of eight fungicides including three systemic fungicides *viz*., hexaconazole 5 % EC (0.05, 0.1, 0.15 %), difenoconazole 25 % EC (0.05, 0.1, 0.15%) and azoxystrobin 2 % EC (0.05, 0.1, 0.15 %), three contact fungicides such as Bordeaux mixture (1%), copper hydroxide 77WP (0.1, 0.2, 0.3 %) and propineb 77WP (0.1, 0.2, 0.3 %), along with two combination fungicides viz., carbendazim 12 % + mancozeb 63 % WP (0.1, 0.2, 0.3 %) and cymoxanil 8 % + mancozeb 64% WP (0.1, 0.25, 0.3 %) were performed against seven fungal pathogens (*Colletotrichum boninense* (VL DF1), *Fusarium proliferatum* (VY DF), *Sclerotium rolfsii* (MT DF1)*, Alternaria alternata* (VP DF1), *Colletotrichum* sp. (VK OF3), *Lasiodiplodia theobromae* (VY CF2) and *Colletotrichum gloeosporioides* (MT SF1)) which have been isolated from different sympodial orchids. Apart from fungal pathogens, a bacterial pathogen, *Pectobacterium aroidearum* (VP CB) was also evaluated against different chemicals such as Streptocycline (100, 200, 250 mgl $^{-1}$), copper hydroxide (0.1, 0.2, 0.3 %) and Bordeaux mixture (1 %).

5.5.1.1. In vitro evaluation of fungicides against Colletotrichum boninense (VL DF1), Colletotrichum gloeosporioides (MT SF1) and Colletotrichum sp. (VK OF3)

Among the fungicides tested against *C. boninense* (VL DF1), the fungicides belong to triazole group *viz.* hexaconazole and difenoconazole, the contact fungicide *viz.* propineb and combination fungicides such as carbendazim 12 % + mancozeb 63 % and cymoxanil 8 % + mancozeb 64 %, showed 100 per cent inhibition of mycelial growth at all concentrations. Bordeaux mixture 1 % also exhibited complete inhibition of the pathogen, while the inhibition per cent of azoxystrobin ranged from 58.75 to 79.58 per cent (Fig.5.1). The per cent reduction in mycelial growth by copper hydroxide varied between 48.75 and 62.91 per cent at different concentrations. Hence, copper hydroxide was found to be the least effective fungicide against the pathogen followed by azoxystrobin. The efficacy of same fungicides was also tested against *Colletotrichum* sp. (VK OF3) isolated from *Oncidium* sp., and complete inhibition of the pathogen was observed in all three doses of carbendazim 12 % + mancozeb 63 % (0.1, 0.2, and 0.3 %) and 1 % Bordeaux mixture. Copper hydroxide, difenoconazole and propineb at their higher concentrations showed complete inhibition of mycelial growth of the pathogen.

In contrast to the results obtained for *C. boninense* (VL DF1), hexaconazole and cymoxanil 8 % + mancozeb 64 % did not show complete inhibition of *Colletotrichum* sp. (VK OF3). The inhibition per cent by hexaconazole and cymoxanil 8 % + mancozeb 64 % ranged from 38.33 to 79.58, and 35.83 to 66.25 per cent respectively. Azoxystrobin was least effective in controlling *Colletotrichum* sp. (VK OF3), which exhibited an inhibition per cent of 52.08 to 62.91 per cent, and the values at different concentrations were on par. Propineb at its lowest concentration (0.1 per cent) exhibited lowest inhibition per cent (35.41 %) among all the fungicides, whereas the pathogen was completely inhibited at higher concentration (0.3 %) of propineb (Fig.5.2).

The fungicides were also evaluated against *C. gloeosporioides* (MT SF1) isolated from *Spathoglottis* sp., and 100 per cent inhibition was observed in all three doses of carbendazim 12 % + mancozeb 63 % and the recommended concentration of Bordeaux mixture. Triazole fungicides (hexaconazole and difenoconazole) at their higher doses showed complete inhibition of the pathogen (Fig.5.3). The contact

fungicides, copper hydroxide and propineb at their higher concentrations also showed higher inhibition per cent of 72.5 and 88.33 per cent respectively. Azoxystrobin and cymoxanil 8 % + mancozeb 64 % exhibited less than 50 per cent inhibition only, and the range of inhibition per cent by azoxystrobin and cymoxanil 8 % + mancozeb 64 % were 28.33 to 47.5 per cent and 11.25 to 38.33 per cent respectively. Therefore, among the fungicides evaluated, the least effective fungicides against *C. gloeosporioides* (MT SF1) were cymoxanil 8 % + mancozeb 64 % followed by azoxystrobin.

The data obtained from evaluation of various fungicides against different *Colletotrichum* spp. from three different sympodial orchids revealed that, the efficacy of fungicides varied for different species of same genera. However, carbendazim 12 % + mancozeb 63 % and Bordeaux mixture (1 %) were found to be highly effective against all *Colletotrichum* isolates under study. The high sensitivity of carbendazim 12 % + mancozeb 63 % against the same pathogen was reported by Saju *et al.* (2012). Dalvi *et al.* (2022), who observed complete inhibition of mycelial growth of *C. gloeosporides* by carbendazim 12 % + mancozeb 63 % WP and Bordeaux mixture at 1 per cent also support our findings. The high efficacy of benzimidazole group of fungicides is due to their ability to inhibit the cell wall synthesis and energy production of fungi (Nene and Thapliyal, 1982).

Triazole group of fungicides (hexaconazole and difenoconazole) also showed promising results against different *Colletotrichum* isolates. The observations match the findings of Dev *et al.* (2016), who reported 100 per cent inhibition by hexaconazole and 84.84 per cent by difenoconazole. The effectiveness of triazoles fungicides may be attributed to their interference with the biosynthesis of fungal sterols and inhibition of ergosterol biosynthesis (Tatsumi *et al.,* 2023).

Azoxystrobin was found to be least effective since inhibition per cent at higher concentration varied between 47.5 and 79.58 for different *Colletotrichum* isolates. The

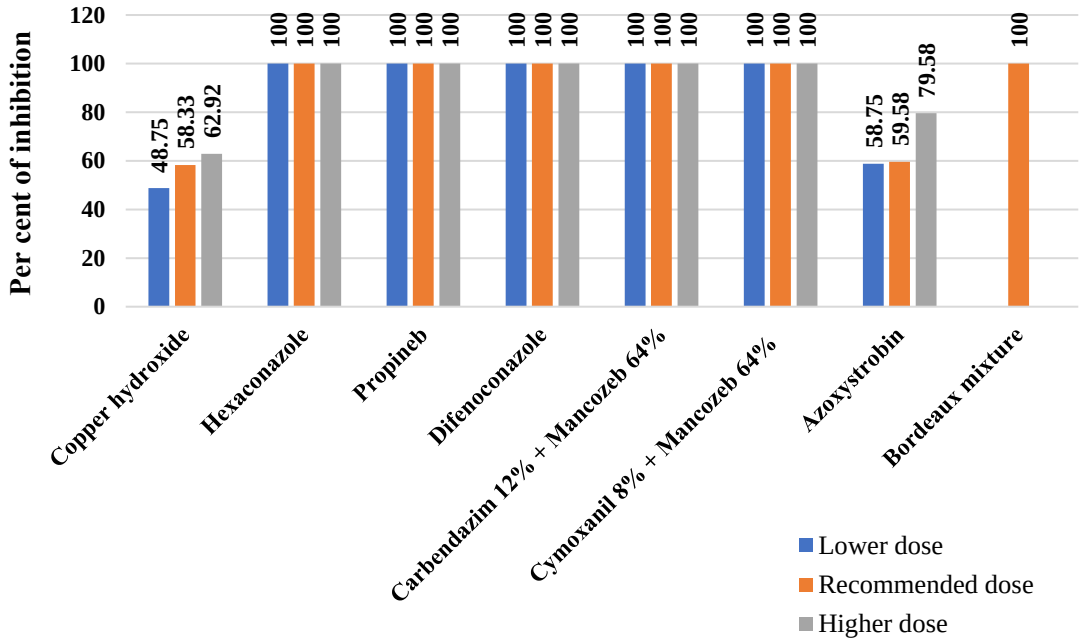


Fig. 5.1. *In vitro* efficacy of fungicides against *Colletotrichum boninense* (VL DF1)

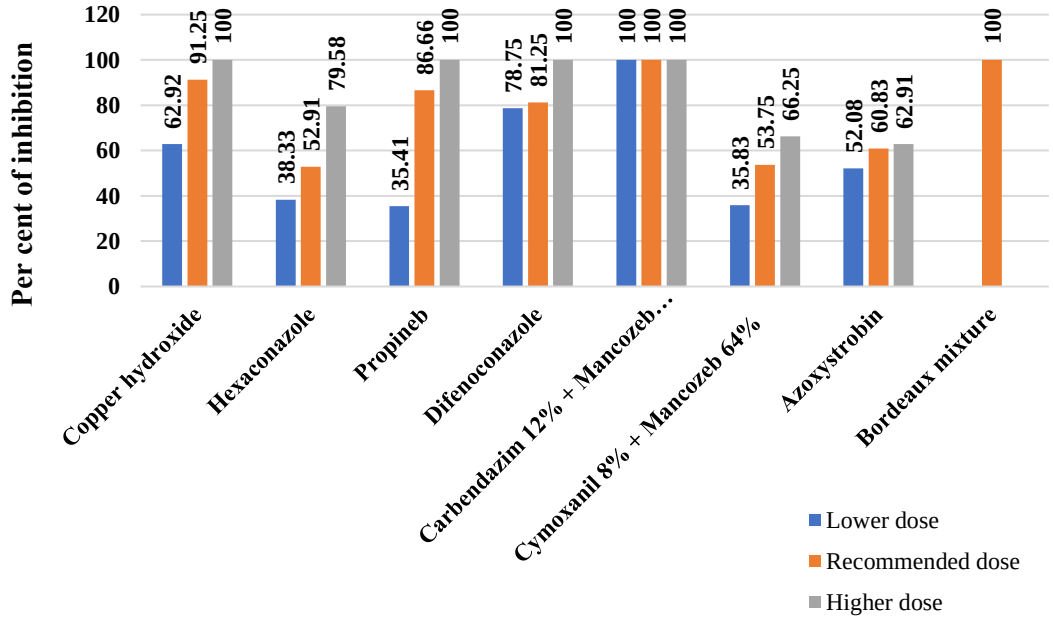


Fig. 5.2. *In vitro* efficacy of fungicides against *Colletotrichum* sp. (VK OF3)

findings are in agreement with the results of *in vitro* evaluation of azoxystrobin against *C. capsici* SCC1 isolate with 59.42, 63.94, 68.93 and 72.20 per cent inhibition at 50, 100, 150 and 200 ppm respectively (Begum *et al.,* 2015). Similar studies were conducted by Dev *et al.* (2016) who reported 51.97 per cent inhibition of *C. gloeosporioides* by the same fungicide. According to Dev and Narendrappa (2016), inhibition per cent of *C. gloeosporioides* by azoxystrobin was found on par at 0.5, 0.1 and 0.2 per cent dosages, and the inhibition per cent varied between 50.64 and 52.64 per cent, which is comparable with the current findings.

Cymoxanil 8 % + mancozeb 64 % was found effective only against *C. boninense,* while the fungicide was found least effective against *C. gloeosporioides* (MT SF1) and *Colletotrichum* sp. (VK OF3). The results are in consistence with the studies by Kumar *et al.* (2015) who found low efficacy of the fungicide, Mancozeb + cymoxanil (0.25 %) in controlling *C. capsici* under pot culture experiment.

Copper hydroxide at higher concentration (0.3 %) showed more than 60 per cent inhibition against all *Colletotrichum* sp. and the values varied between 62.92 and 100 per cent while the inhibition per cent of propineb (0.3 %) ranged from 88.33 to 100 per cent. The findings are in conformity with the observations of Dhavale *et al.* (2019), who found complete inhibition of growth of *C. gloeosporioides* by copper hydroxide 77 % WP and propineb 70 % WP under *in vitro* conditions. Dalvi *et al.* (2022) also recorded 100 per cent inhibition of mycelial growth of fungi by propineb 70 % WP at 0.2 per cent. However, propineb and copper hydroxide were less effective in controlling *C. gloeosporioides* (MT SF1) isolated from *Spathoglottis* spp.

5.5.1.2. In vitro evaluation of fungicides against Fusarium proliferatum (VY DF)

Another major pathogen, *F. proliferatum* (VY DF) causing leaf blight diseases in *Dendrobium* sp. was also evaluated against different fungicides, and a total inhibition of mycelial growth was recorded for copper hydroxide, hexaconazole, carbendazim 12 % + mancozeb 63 % at all concentrations, and Bordeaux mixture at 1 per cent concentration (Fig.5.4). The study conducted by Ullah *et al.* (2018) revealed the high efficacy of propineb in controlling *F. oxysporum* f. sp. *capsici.* Ghante *et al.* (2019) observed complete inhibition of *F. oxysporum* f. sp. *udum* by hexaconazole under *in*

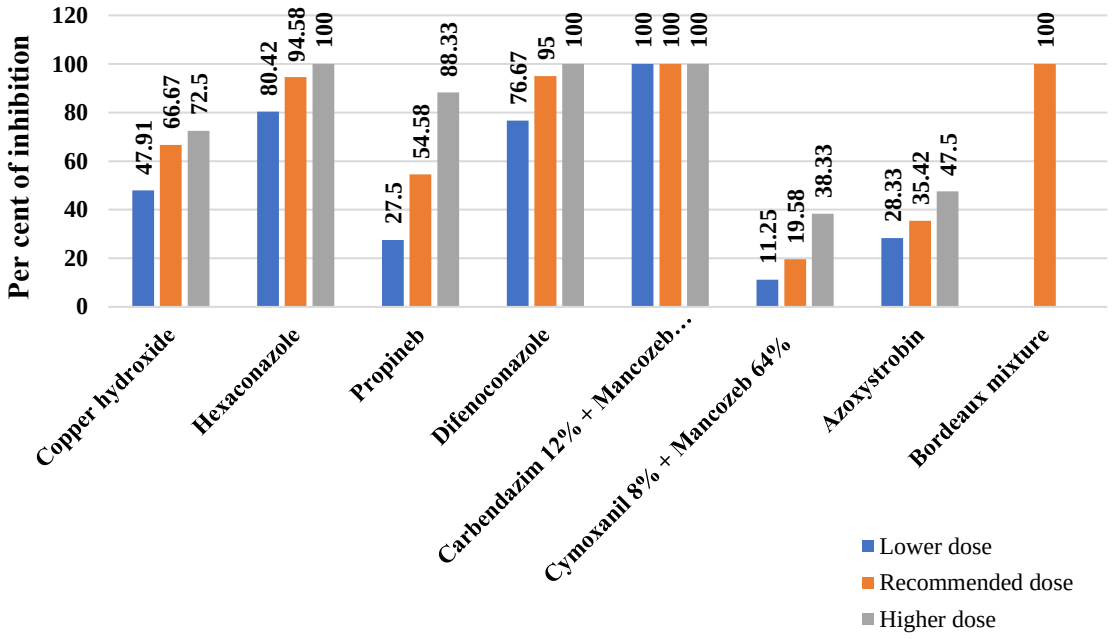


Fig. 5.3. *In vitro* efficacy of fungicides against *Colletotrichum gloeosporioides* (MT SF1)

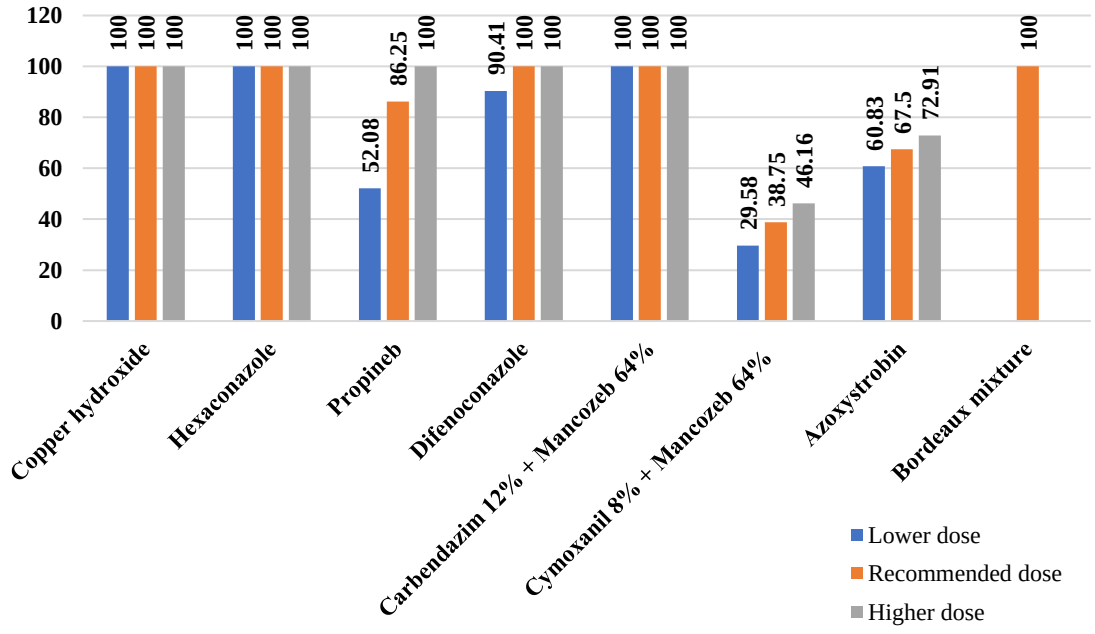


Fig. 5.4. *In vitro* efficacy of fungicides against *Fusarium proliferatum* (VY DF)

vitro condition. Propineb at its higher concentration (0.3 %) and difenoconazole at its recommended and higher concentration also completely inhibited the fungal growth. The findings are in conformity with studies of Vani *et al*. (2019) who found 79.7 per cent inhibition by *F. oxysporum* at 0.1 per cent concentration of difenoconazole 25 % EC and 86.6 per cent inhibition by propineb at 0.2 per cent dose. According to Gadhave *et al.* (2020), carbendazim 25 % + mancozeb 50 % WS and copper oxychloride 50 % WP were the most effective chemicals in controlling *F. oxysporum* f. sp. *lycopersici.* The data are in accordance with the observations of Rao *et al.* (2021), who recorded 92.96, 96.51 and 100 per cent of mycelial inhibition of *F. oxysporum* f. sp. *melongenae* by difenoconazole 25 % EC at 500, 1000 and 1500 ppm respectively.

Azoxystrobin at 0.05, 0.1 and 0.15 per cent showed an inhibition percentage of 60.83, 67.5 and 72.9 per cent respectively. The study is comparable with the findings of Vani *et al*. (2019), who found 55.8 per cent inhibition of mycelial growth of *F. oxysporum* by azoxystrobin at 0.1 per cent concentration. Cymoxanil 8 % + mancozeb 64 % found to be the least effective fungicides, with an inhibition per cent of 29.58, 38.75 and 49.16 at 0.1, 0.25 and 0.3 per cent respectively. A study conducted by Praveen (2016) showed poor inhibition of *Fusarium solani* by Cymoxanil 8 % + mancozeb 64 % under *in vitro* condition.

5.5.1.3. In vitro evaluation of fungicides against Sclerotium rolfsii (MT DF1)

Different fungicides were tested against *S. rolfsii* (MT DF1) causing leaf blight in *Dendrobium* sp. and 100 per cent efficacy was reported for hexaconazole, propineb, difenoconazole, carbendazim 12 % + mancozeb 63 %, cymoxanil 8 % + mancozeb 64 % at all doses, and Bordeaux mixture at 1 per cent dose (Fig.5.5). This is in conformity with the studies of Deepthi (2014), who found 100 per cent inhibition by hexaconazole against *S. Rolfsii* causing stem rot of groundnut and Manu *et al.* (2012) found maximum efficacy of hexaconazole and difenoconazole against *S. rolfsii.* The findings of Shirsole *et al*. (2019) revealed that propineb 70 % WP and carbendazim 12 % + mancozeb 63 % completely inhibited the mycelial growth of *S. rolfsii* even at 200 and 500 ppm concentrations. Sangeeta *et al.* (2022) also reported 100 per cent efficacy of carbendazim 12 % + mancozeb 63 % against *S. rolfsii*. Prasad *et al.* (2017) reported cent per cent inhibition of *S. rolfsii* by cymoxanil + mancozeb at recommended dose.

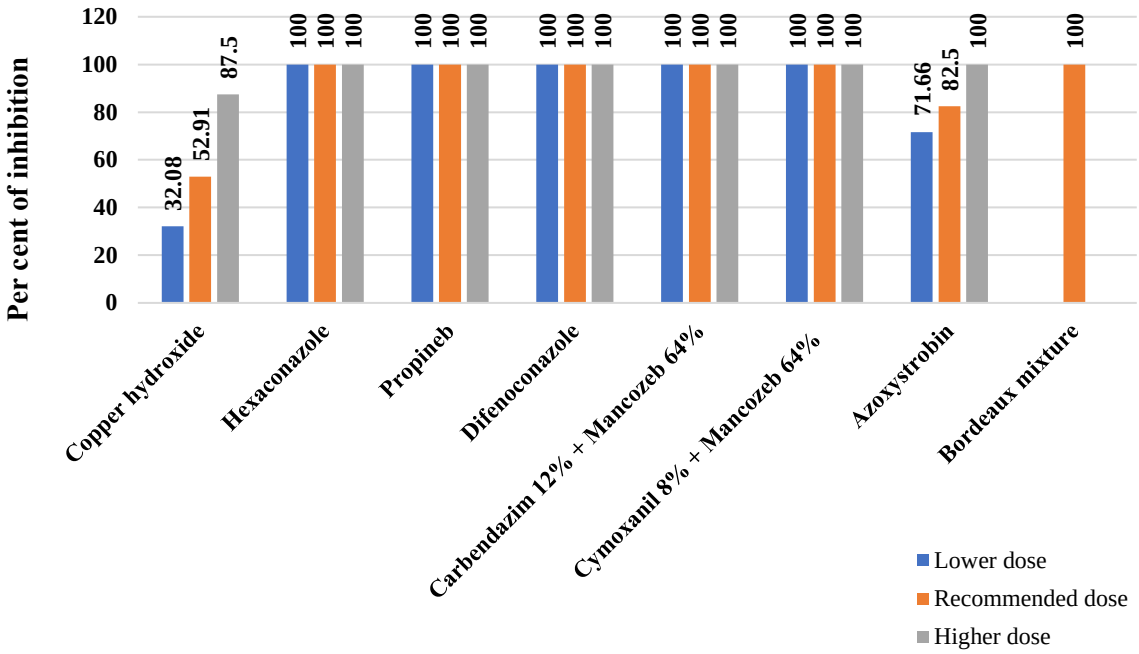


Fig. 5.5. *In vitro* efficacy of fungicides against *Sclerotium rolfsi*i (MT DF1)

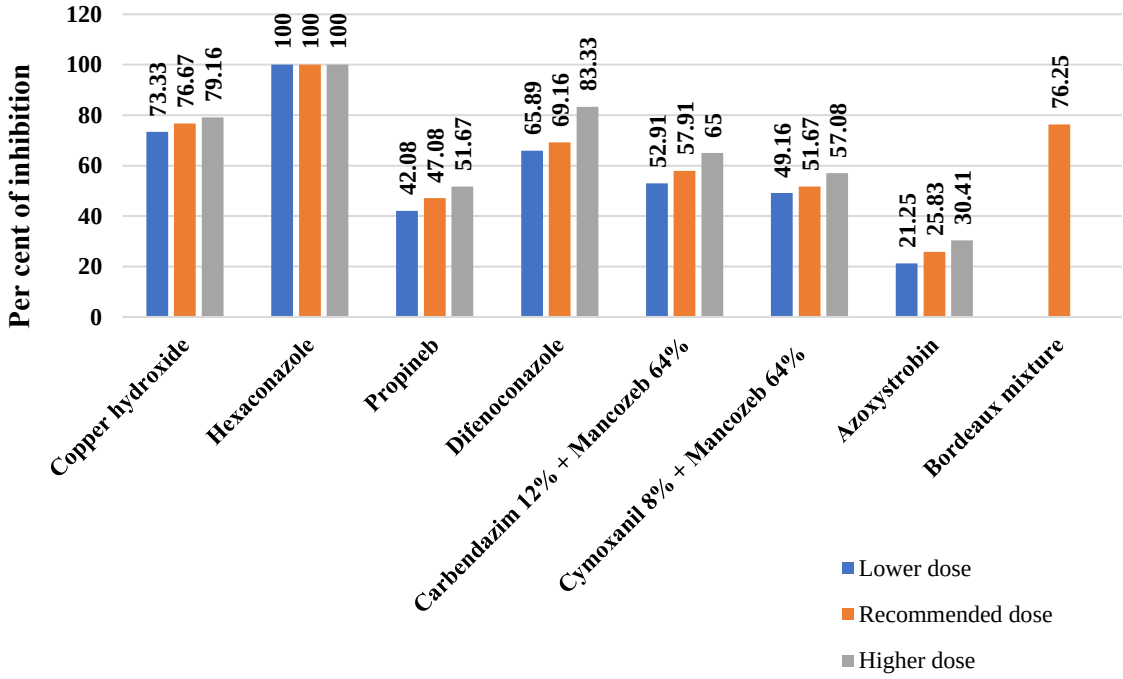


Fig. 5.6. *In vitro* efficacy of fungicides against *Alteranria alternata* (VP DF1)

Azoxystrobin, when applied at a higher dose (0.15 %), resulted in 100 per cent inhibition of fungal pathogen, whereas at a lower dose (0.05 %) and at the recommended dose (0.1 %), the inhibition recorded was 71.66 and 82.5 per cent respectively. The results are in conformity with studies by Patel *et al.* (2018), who recorded similar range of inhibition per cent by azoxystrobin. Copper hydroxide was found to be the least effective fungicide, showing 32.08, 52.91 and 87.5 per cent inhibition of the pathogen at concentrations of 0.1, 0.2 and 0.3 per cent respectively. This is in agreement with the findings of Patel *et al.* (2018) who recorded mean inhibition per cent of 70.42 per cent against *S. rolfsii.*

5.5.1.4 In vitro evaluation of fungicides against Alternaria alternata (VP DF1)

In vitro evaluation of fungicides against *A. alternata* (VP DF1) was performed, and complete inhibition of mycelial growth of the fungus was recorded for only hexaconazole. These observations are in conformity with Dasharathbhai *et al.* (2018) and Singh and Singh, (2006) recorded cent per cent growth inhibition by hexaconazole against *A. alternata.* Jewaliya *et al.* (2021) also reported 97.96 per cent mycelial inhibition against *A. alternata* by hexaconazole. Difenoconazole and copper hydroxide showed 83.33 and 79.16 per cent inhibition of fungal growth at their higher concentrations and Bordeaux mixture at 1 per cent showed 76.25 per cent inhibition of fungal growth (Fig.5.6). The findings are in conformity with Singh and Singh (2006) who recorded similar per cent of inhibition of the pathogen by copper hydroxide.

Carbendazim 12 % + mancozeb 63 % and cymoxanil 8 % + mancozeb 64 % at their higher dosages showed an inhibition per cent of 65.0 and 57.08 per cent respectively, whereas the per cent inhibition of fungal growth by propineb at different doses ranged from 42.08 to 51.66 per cent. The above findings are in accordance with the studies Zade *et al.* (2018) who found Cymoxonil + mancozeb was found highly effective in arresting growth of *A. alternata* and complete inhibition of the pathogen were observed. Jewaliya *et al.* (2021) and Singh and Singh (2006) observed similar per cent of inhibition by propineb against *A. alternata.* Shingne *et al.* (2020) found 93.15 per cent inhibition of the pathogen by carbendazim + mancozeb at 0.25 per cent dose. Azoxystrobin was found to be the least effective fungicide, showing inhibition

percentage of 30.41 per cent at its highest concentration (0.15 %). Meena *et al.* (2020) also recorded low inhibition per cent (25.17 %) by azoxystrobin against *A. alternata.*

5.5.1.5. In vitro evaluation of fungicides against Lasiodiplodia theobromae (VY CF2)

L. theobromae (VY CF2) causing leaf spot in *Cattleya* spp. was treated with different fungicides and hundred per cent inhibition of pathogen was observed in all three concentrations of carbendazim 12 % + mancozeb 63 %, cymoxanil 8 % + mancozeb 64 %, propineb and 1 per cent Bordeaux mixture (Fig.5.7).The findings are in conformity with Amrutha and Vijayaraghavan (2020) who reported cent per cent inhibition of carbendazim 12 % + mancozeb 63 % and cymoxanil 8 % + mancozeb 64 % at all three concentrations and 81.11 per cent inhibition by Bordeaux mixture. Hegde *et al.* (2013) also reported complete inhibition of the pathogen by carbendazim 12 % + mancozeb 63 %. Hexaconazole at its higher concentration showed maximum inhibition percentage (100 per cent), whereas higher concentration of difenoconazole showed an inhibition of 74.58 per cent. This is in agreement with the observations of Amrutha and Vijayaraghavan (2020) who recorded 76.67 per cent inhibition of mycelial growth at 0.15 per cent concentration.

Copper hydroxide at 0.1, 0.2 and 0.3 per cent showed an inhibition percentage of 58.33, 73.75 and 82.5 per cent respectively. Azoxystrobin at 0.05, 0.1 and 0.15 per cent showed an inhibition ranging from 37.08 to 50.0 per cent and was found to be least effective against the pathogen. The results are in line with the findings of Rafi (2021) who reported low efficacy of copper hydroxide and azoxystrobin against *L. theobromae* under *in vitro* condition.

5.5.1.6. In vitro evaluation of chemicals against Pectobacterium aroidearum (VP CB)

Among the chemicals treated against *P. aroidearum* under *in vitro* conditions, copper hydroxide showed a maximum inhibition percentage of 25.92 per cent at a higher concentration (0.3 %) (Fig.5.8). The results are in agreement with the reports of Kolomiiets *et al.* (2020) who found that *P. carotovorum* was sensitive to copper hydroxide. The antibiotic Streptocycline at 200 and 250 ppm showed per cent inhibition of 13.33 and 16.67 per cent respectively and no inhibition was noticed at 100 ppm. The

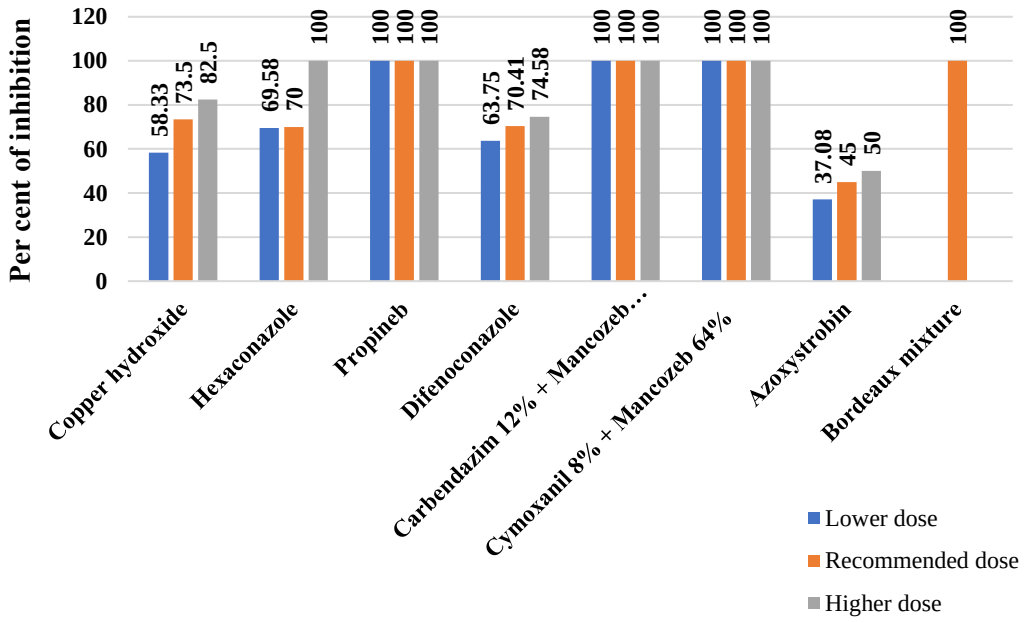


Fig. 5.7. *In vitro* efficacy of fungicides against *Lasiodiplodia theobromae* (VY CF2)

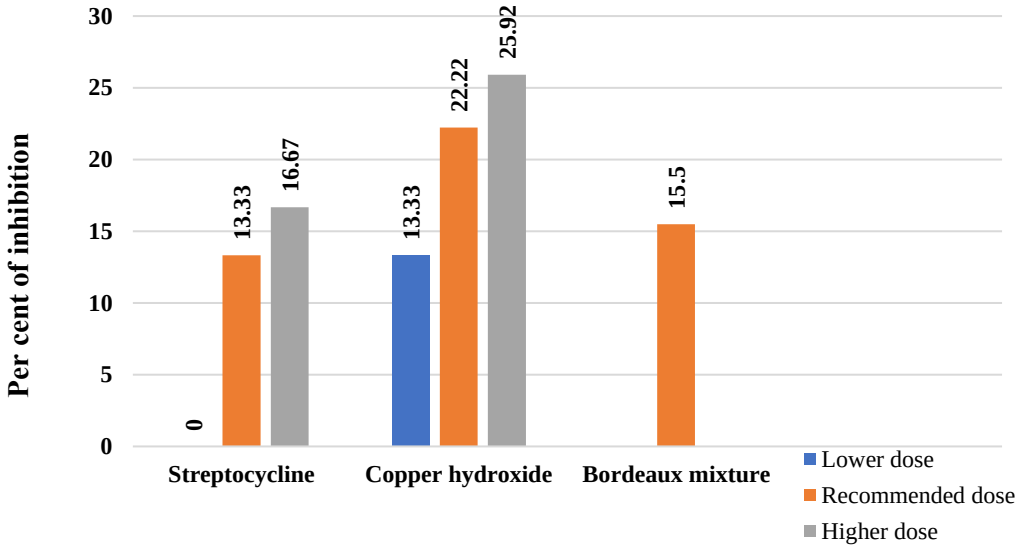


Fig. 5.8. *In vitro* efficacy of chemicals against *Pectobacterium aroidearum* (VP CB)

results contradict the previous report given by Vijayalaxmi *et al.* (2014) and who found significant inhibition of *P. carotovorum* by Streptocycline. The fungicide, Bordeaux mixture at 1 per cent showed a lesser inhibition percentage of 15.5 per cent which contradicts the findings of by Mailawati and Ginting (1999) who found significant reduction of *E. chrysanthemi* by 1 per cent Bordeaux mixture.

5.5.2. In vitro evaluation of biocontrol agents against major pathogens

5.5.2.1. In vitro evaluation of biocontrol agents against fungal pathogens

Biocontrol agents viz. *Trichoderma asperellum* (KAU reference culture), *Pseudomonas fluorescens* (KAU reference culture), PGPR-II and PGPM (KAU formulations) were evaluated against the major pathogens obtained from sympodial orchids during the survey. The efficiency of biocontrol agents viz. *T. asperellum* (KAU reference culture) and *P. fluorescens* (KAU reference culture) was tested *in vitro* by dual culture technique (Morton and Stroube, 1955), whereas PGPR- II and PGPM were evaluated by poisoned food technique (Zentmeyer,1955).

5.5.2.1.1. In vitro evaluation of Trichoderma asperellum against major fungal pathogens

The biocontrol agent *T. asperellum* was evaluated against the major fungal pathogens by dual culture technique. It was observed that the most promising result was obtained against the pathogen *A. alternata,* showing a maximum inhibition percentage of 91.16 per cent (Fig.5.9). Shingne *et al.* (2020) recorded 58.88 per cent inhibition of *A. alternata* by *T. asperellum*. Rai and Singh (2023) also found high effectiveness of *T. asperellum* against *Alternaria* sp. isolated from Ashwagandha (53.06 %) and Sarpagandha (53.51 %).

The pathogens such as *F. proliferatum*, *C. boninense, S. rolfsii, Colletotrichum* sp. and *C. gloeosporioides* exhibited moderate per cent inhibition ranging from 53.06 to 61.9 per cent (Fig.5.8). The findings are in agreement with the studies of Rai and Singh (2023), who recorded 56.36 per cent inhibition against *F. solani* isolated from Ashwagandha. Tapwal *et al.* (2015) also reported significant inhibition of *A. alternata*, *C. gloeosporioides* and *F. oxysporum* by *Trichoderma harzianum* and *Trichoderma*

viride. According to Ghanbarzadeh *et al.* (2014), the antagonist action of *Trichoderma* spp. was more on *F. proliferatum* than on *F. oxysporum*. A study conducted by Rana *et al.* (2016) showed that *T. harzianum* inhibited the growth of *Colletotrichum graminicola* by 66.81 to 74.88 per cent. The studies conducted by Vinod *et al.* (2009) and Deshmukh *et al.* (2010) found significant inhibition of *C. gloeosporioides* by *Trichoderma* spp. Sekhar *et al.* (2020) recorded an inhibition per cent of 33.87 per cent by *T. asperellum* against *S. rolfsii* which not in line with the current result. However, he recorded more than 50 per cent inhibition of *S. rolfsii* by *T. harzianum.* The lowest per cent inhibition (20.41 %) was recorded against the pathogen *L. theobromae*. The results were similar to the studies conducted by Bhadra *et al.* (2014), who found less inhibition of *L. theobromae* by volatile metabolites from *T. viride* (33.3 %), whereas the results are not agreeable with the finding of Amrutha and Vijayaraghavan (2020) who recorded 74.10 per cent mycelial growth inhibition of *L. theobromae* respectively by *T. asperellum. T. asperellum* inhibited the pathogens in different manner, which was studied in detail. Interaction of the antagonist with *C. boninense* produced a slight demarcation at the meeting point with reduced growth whereas a clear inhibition zone was observed for *F. proliferatum Colletotrichum sp., C. gloeosporioides* and *L. theobromae. A. alternata* was inhibited by overgrowth of *T. asperellum.* Amrutha and Vijayaraghavan (2020) observed that the inhibition of *L. theobromae* by *Trchoderma* sp. was due to overgrowth mechanism. The inhibition in growth of two interacting organisms in dual culture is due to competition, hyper parasitism and release of inhibitory substance by one or both organisms (Meena *et al.,* 2017).

5.5.2.1.2. In vitro evaluation of Pseudomonas fluorescens against major fungal pathogens

The bacterial antagonist, *P. fluorescens,* was evaluated against the major pathogens and found to be less effective against most of the pathogens except *Colletotrichum* sp. (VK OF3). These findings are in accordance with the studies by Rana *et al*. (2016), who found significant inhibition of *C. graminicola by P. fluorescens.* Dasharathbhai (2018) recorded 38.38 per cent inhibition of *A. alternata* by *P. fuorescens.* Gadhave *et al.* (2020) recorded 21.66 inhibition of *F. oxysporum* by *P. fluorescens,* which is comparable to the current study.

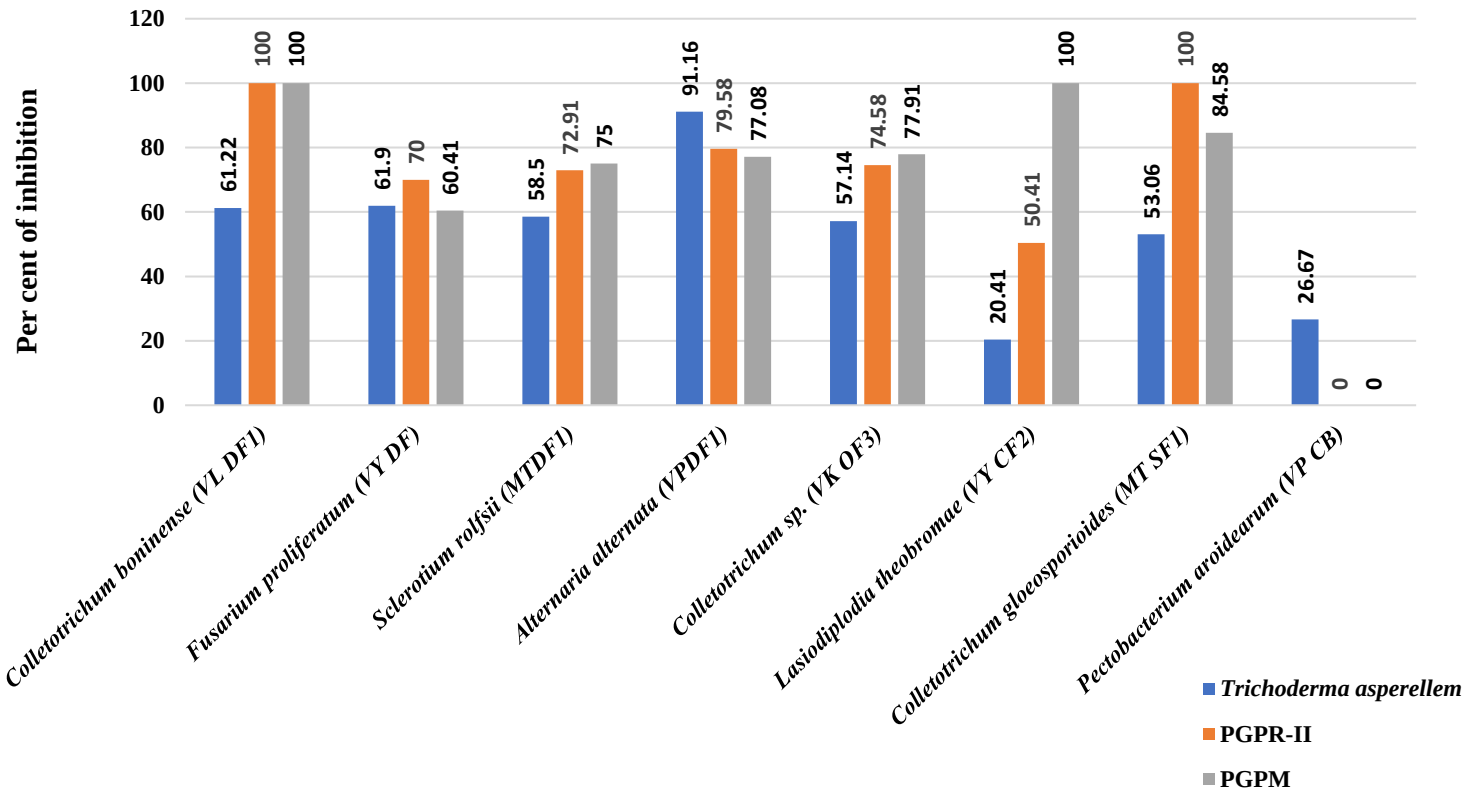


Fig. 5.9. *In vitro* efficacy of biocontrol agents against major pathogens of sympodial orchids

In contradiction to the above results, Akinbode and Ikotun (2008) found 53 per cent inhibition of *Colletotrichum destructivum* by *P. fluorescens.* The studies conducted by Ramanjaneyulu (2010) recorded 83.3 and 78,9 per cent of inhibition of *Fusarium solani* and *Alternaria tenuissima* by *P. fluorescens,* which is not in line with the present investigation.

However, reduction in mycelial growth was observed for pathogens, and different types of interaction of *P. fluorescens* with fungal pathogens were studied. Mycelial thickening was observed for *F. proliferatum* and *C. gloeosporioides,* whereas *A. alternata* was inhibited by lysis and overgrowth. Mycelial thickening, lysis and overgrowth were observed for *C. boninense,* while no inhibition was observed for *S. rolfsii* and *L. theobromae.* The results are in line with the observations of Manu *et al.* (2012) and Amrutha and Vijayaraghavan (2020), who found zero inhibition of *S. rolfsii and L. theobromae* by *P. fluorescens.*

5.5.2.1.3. In vitro evaluation of PGPR-II against major fungal pathogens

In vitro evaluation studies of PGPR-II against major fungal pathogens showed that *C. boninense* and *C. gloeosporioides* were completely inhibited by PGPR-II. This was followed by *A. alternata, Colletotrichum* sp., and *S. rolfsii,* with inhibition percentages of 79.58, 74.58 and 72.91 per cent respectively (Fig.5.8). The least inhibition of 50.41 per cent was noticed for *L. theobromae*. According to the previous report, plant growth promoting rhizobacteria function as effective antagonists of plant pathogens by different mechanisms such as competition, lysis, antibiosis, parasitism, and by inducing host plant resistance (Beneduzi *et al.,* 2012; Tariq *et al.,* 2017). Walia, *et al.* (2013) recorded 82.85 per cent inhibition of *F. oxysporum* by PGPR. Similarly, Ali *et al.* (2020) reported more than 60 per cent antifungal activity of the pathogen against *C. gloeosporioides, F. oxysporum, Fusarium moniliforme,* and *Colletotrichum falcatum,* which is comparable with the current result.

5.5.2.1.4. In vitro evaluation of PGPM against major fungal pathogens

During *in vitro* evaluation, PGPM showed different per cent inhibition of the major fungal pathogens. Hundred per cent inhibition was shown by C. *boninense* and *L. theobromae*. It was followed by *C. gloeosporioides, Colletotrichum* sp., *A. alternata*

and *S. rolfsii*, with inhibition per cent of 84.58, 77.9, 77.08 and 75 per cent respectively (Fig.5.8). The least inhibition was noticed for *F. proliferatum,* with inhibition percentage of 60.41 per cent. The antagonistic activity of PGPM against *L. theobromae* and *C. gloeosporioides* was comparable with the observations of Rafi (2021), who recorded complete inhibition of the pathogens when treated with PGPM. Earlier studies carried out by Sumbula (2015) also proved the potential inhibitory action of PGPM, which caused a complete reduction in the growth of the pathogen causing early blight in tomato.

5.5.2.1. In vitro evaluation of biocontrol agents against bacterial pathogen (Pectobacterium aroidearum)

Among the biocontrol agents evaluated, *T. asperellum* showed less inhibition percentage of 26.67 per cent against the bacterial pathogen (Fig.5.8). This is in conformity with the findings of Rashid *et al.* (2013) who found inhibition of *Erwinia carotovora* subsp. *Carotovora* by *T. harzianum* with inhibition zone of 16.33 mm. No inhibition of bacterial growth was found when treated with *P. fluorescens*, PGPR-II and PGPM. The results contradict to the report of Rashid *et al.* (2013), who found an inhibitory action of *P. fluorescens* against *E. carotovora var. carotovora.* Similarly, Meera (2016) also reported significant inhibition (65.56 %) of growth *of Erwinia chrysamthemi* causing soft rot in Phalaenopsis. The studies regarding evaluation of PGPR-II and PGPM against *P. aroidearum* have not been reported yet.

5.6. BIOASSAY STUDIES ON DETACHED LEAVES OF SYMPODIAL ORCHIDS AGAINST MAJOR PATHOGENS

Bioassay experiment was conducted to evaluate the efficacy of fungicide against the major pathogens such as *F. proliferatum, C. boninense, L. theobromae, S. rolfsii* and *Colletotrichum* sp. The pathogens were selected based on their highest PDI and PDS. The fungicide, carbendazim 12 % + mancozeb 63 % was found to be the most effective fungicide under *in vitro* evaluation, and hence it was selected for bioassay studies. The disease severity index (DSI) was calculated according to the formula given by Chiang *et al.* (2017).

Bioassay experiment study was conducted for *C. boninense* (VT DF1) affecting *Dendrobium* spp., and no significant infection could be observed on treated leaves as compared to control leaves. 96.6 per cent reduction in disease severity was noticed, indicating high effectiveness of fungicide against *C. boninense.* The efficiency of the fungicide was evaluated against *F. proliferatum* (VY DF) causing leaf blight disease in *Dendrobium* spp. and 96.4 per cent reduction in disease severity was observed when treated with above said fungicide indicating high efficacy of fungicide against the pathogen. The fungicide was also evaluated against the pathogen *S. rolfsii* (MT DF1) affecting *Dendrobium* sp., and 95.7 per cent reduction in disease severity was observed five days after inoculation. The calculated DSI for the treatment was 4.3 per cent compared to the control, and hence the fungicide was found effective against *S. rolfsii* was confirmed. *Colletotrichum* sp. (VK OF3) causing leaf spot in *Oncidium* spp. was carried out using the same fungicide, which resulted in 90.4 per cent reduction in disease severity, whereas, *L. theobromae* (VY CF2) in *Cattleya* sp. showed a reduction in severity of only 21.16 per cent.

Thus, the study revealed that carbendazim 12 % + mancozeb 63 % was highly effective against *C. boninense* (VT DF1), followed by *F. proliferatum* (VY DF), *S. rolfsii* (MT DF1) and *Colletotrichum* sp. (VK OF3), whereas the fungicide was found least effective in controlling *L. theobromae* (VY CF2) under *in planta* condition. Similar study was conducted by Prapagdee *et al.* (2012) who evaluated the efficacy of the antifungal compounds of *Bacillus subtilis* SSE4 extracted by ethyl acetate and hexane against *C. gloeosporioides* causing anthracnose disease in *Dendrobium* sp. and found promising result under *in planta* condition. No much studies have been conducted regarding *in planta* evaluation of fungicides in orchids. This may be the first attempt to study the evaluation of carbendazim 12 % + mancozeb 63 % under *in planta* condition. However, further evaluation fungicides under field condition are required for better understanding.

Summary

6. SUMMARY

Orchids are prized for their exotic beauty and long shelf life, making them one of the most popular cut flowers and ornamental plants worldwide. Currently, orchids hold sixth position among the top ten cut flowers in the world, among which, sympodial orchids rank higher in the export market. However, the crops are infected with fungal, bacterial and viral diseases, which reduce the economic value in the floriculture trade. Hence, the present study, 'Cataloguing and documentation of diseases of sympodial orchids in Kerala' was proposed with the objectives to document various diseases affecting sympodial orchids in Kerala, to identify of the pathogens, and to evaluate fungicides and biocontrol agents against selected pathogens under *in vitro* conditions.

A purposive sampling survey in eight locations of three districts *viz.,* Thrissur, Ernakulam and Thiruvananthapuram conducted revealed the incidence of leaf blight, leaf spot, wilt, black rot, soft rot, twig blight, petal blight and flower spot diseases in four different sympodial orchids *viz*., *Dendrobium* spp., *Cattleya* spp., *Oncidium* spp. and *Spathoglottis* spp. A total of 49 symptoms were obtained from various locations, among which 31 samples were collected from Thrissur, 12 samples from Ernakulam, and six samples from Thiruvananthapuram from the selected sympodial orchids. Sixteen symptoms from *Dendrobium* spp. (leaf blight (4 nos.), leaf spot (4 nos.), black rot (1 no.), petal blight (2 nos.), flower spot (3 nos.) and twig blight (2 nos.)), 8 samples from *Oncidium* spp. (leaf blight (2 nos.), leaf spot (6 nos.)), 2 samples from *Cattleya* spp. (black rot (1 no.), soft rot (1 no.)) and 5 samples from *Spathoglottis* spp. (leaf blight (4 nos.), leaf spot (1 no.)) were collected from Thrissur district. Four samples from *Dendrobium* spp. (leaf blight (2 nos.), leaf spot (1 no.), wilt (1 no.)), five leaf blight symptoms from *Oncidium* spp. and two leaf blight symptoms from *Cattleya* spp. were collected from Ernakulam district. From Thiruvananthapuram district, one leaf blight sample from *Dendrobium* spp., three samples from *Oncidium* spp. (leaf blight (1 no.), leaf spot (1 no.), and wilt (1 no.)) two samples from *Cattleya* spp. (leaf blight (1 no.) and leaf spot (1 no.)) and one leaf blight sample from *Spathoglottis* spp. were obtained.

PDS and PDI were calculated for each symptom from *Dendrobium* spp., and the highest PDI and PDS were recorded for VY DLB (76.47 %) and MT DLB1 (36.67 %) respectively. In *Oncidium* spp., the highest PDI and PDS were recorded for VK OLS1 with 76.92 per cent and 46.90 per respectively. In *Spathoglottis* spp., highest PDI (48.0 %) and PDS (29.79 %) were recorded for MT SLB1. From PDS and PDI data, it was found that, leaf blight symptoms were more common and severe in *Dendrobium* spp. whereas leaf spot symptoms more severe in *Oncidium* spp. In *Cattleya* spp., leaf spot symptoms were most frequently noticed, and soft rot symptom showed highest disease severity. In *Spathoglottis* sp., leaf blight symptoms were most frequent and severe. PDS and PDI values were correlated with light intensity at different survey locations. A negative correlation was obtained for all the values, which revealed that the severity of diseases increased gradually with a decrease in the light intensity of the prevailing location. The correlation values obtained for *Dendrobium* sp. and *Spathoglottis* sp. showed significant negative correlation of PDS and PDI with light intensity, whereas no much significant correlation was found for *Oncidium* sp. and *Cattleya* sp.

Isolation of pathogens from diseased samples collected during the survey yielded 52 fungal isolates and 1 bacterial isolate from various sympodial orchids. A total of 21 fungal isolates were obtained from *Dendrobium* spp. from various survey locations (Thrissur -16, Ernakulam -4, Thiruvananthapuram -1), 18 from *Oncidium* spp. (Thrissur -10, Ernakulam -5, Thiruvananthapuram -3), five fungal isolates (Thrissur-1, Ernakulam-2 and Thiruvananthapuram-2) and one bacterial isolate (Thrissur) from *Cattleya* spp. and nine from *Spathoglottis* spp. (Thrissur -6 and Thiruvananthapuram -3). All the isolates showed different symptoms upon artificial inoculation and these isolates could be reisolated and confirmed their pathogenicity.

The leaf blight symptoms collected from Dendrobium from various locations such as MT DLB2, VKDLB, IK DLB, AM DLB and VY DLB caused by *Fusarium* spp. were found to be similar, and these symptoms initially appeared as white papery textured lesions, and later coalesced to cause leaf blight. Apart from leaf blight, *Fusarium* spp. also caused leaf spot, wilt symptom, and flower spots (VK DFS2 and VP DFS). *Curvularia* spp. produced dull brownish spots on leaves with chlorotic halo.

Sclerotium spp. produced different types of symptoms at different stages. Initial symptoms appeared as water soaked spots on leaves, followed by quick rotting. *Colletotrichum* isolates produced different kinds of symptoms on various plant parts, such as leaf blight, leaf spot, flower spot, petal blight and twig blight. The leaf blight symptom, VL DLB was described as brownish to black irregular sunken lesions with diffused yellow halo, whereas leaf spot symptom (VL DLS) appeared as minute, dull black sunken necrotic spots. Other symptoms, such as black rot (VP DBR) caused by *Phytophthora* sp. led to complete decay and death of the plants. *Alternaria* sp. produced black, circular to oval, water-soaked lesion with chlorotic halo on leaves, and it also caused blight and deformation of petals (VP DPB2).

Colletotrichum isolates from *Oncidium* spp. produced different kinds of blight symptoms. Most of the leaf blight symptoms appeared as greyish to brown blight starting from the tip of the leaf and extending downward, caused drying of foliage. *Diplodia* sp. and *Phytophthora* sp. also caused leaf blight in *Oncidium* spp. Different types of leaf spot diseases were observed in *Oncidium* spp. and most of the leaf spot symptoms were caused by *Colletotrichum* spp. The symptomatology of different leaf spot symptoms caused by *Diaporthe* sp., *Pestalotia* sp., and *Alternaria* sp. and wilt symptom caused by *Fusarium* sp. were also studied.

Different types of symptoms, such as leaf blight, leaf spot, black rot and soft rot were noticed in *Cattleya* spp. The leaf blight symptom incited by *Fusarium* sp. caused blight and drying of whole leaves. *Colletotrichum* spp. isolated from two different kinds of blight symptoms (AM CLB2 and VY CLB) produced distinct symptoms under natural conditions. The leaf spot symptom obtained from Vellayani (VY CLS) caused by *Lasiodiplodia theobromae* was characterised by greyish white irregular, sunken leaf spot with brown margin. Black rot symptom (VP CBR) characterised as complete blackening of leaves and death of the plant and the causal organism proved to be *Phytophthora* spp. The bacterial soft rot, VY CSR was caused by *Pectobacterium* sp.

Leaf blight symptoms caused by different *Colletotrichum* isolates were severe in *Spathoglottis* spp. The symptoms of leaf blight (MT SLB1) were characterised by straw coloured blight with dark brown margin. Another leaf blight symptom, MT

SLB2, produced greyish to brown blight with concentric zonations. MT SLB3 was characterised as dark brownish, water-soaked lesion with yellow halo, while MT SLB4 appeared as straw coloured blight with dark brown margin. The leaf spot symptom, MT SLS produced irregular grey spots with brown margin. The leaf blight symptom obtained from Kottukal was due to combined infection of two *Colletotrichum* spp. (KO SF1 and KO SF3) and one *Fusarium* sp. (KO SF2).

The pathogens obtained from different sympodial orchids were characterised by cultural, morphological and molecular techniques. Based on these, different fungal isolates, such as *Fusarium* spp. (9 nos.), *Colletotrichum* spp. (9 nos.), *Alternaria* spp. (2 nos.), *Sclerotium* spp. (2 nos.), *Phytophthora* sp. (1 no.) and *Curvularia* sp. (1 no.) were obtained from various symptoms collected from *Dendrobium* spp. The pathogens isolated from different symptoms collected from *Oncidium* spp. were *Colletotrichum* spp., *Diplodia* sp., *Diaporthe* sp., *Pestalotia* sp*., Alternaria* sp., *Phytophthora sp.* and *Fusarium* sp*.* Fungal isolates such as *Colletotrichum* spp. (2 nos.), *Fusarium* sp. (1 no.), *Phytophthora* sp. (1 no.) and *Lasiodiplodia* sp. (1 no.), and a bacterial isolate, *Pectobacterium* sp. were isolated from *Cattleya* spp., and seven *Colletotrichum* spp., one *Fusarium* sp. and an unidentified fungal culture were obtained from *Spathoglottis* spp.

Molecular characterisation of major pathogens from *Dendrobium* spp., *Oncidium* spp., *Cattleya* spp. and *Spathoglottis* spp. was done for species level identification. Leaf blight pathogens, VL DF1, VY DF, IK DF1 and MT DF1 were identified as *Colletotrichum boninense, Fusarium proliferatum, Fusarium oxysporum and Sclerotium rolfsii* respectively, and the leaf spot pathogen, VP DF1 as *Alternaria alternata.* The leaf spot pathogens, VK OF3 and IK OF1 from *Oncidium* spp. were identified as *Colletotrichum* sp. and *Diaporthe tulliensis respectively.* The isolate, VY CLS causing leaf spot disease in *Cattleya* spp. was *Lasiodiplodia theobromae and* the fungus MT SF1, causing leaf blight symptom in *Spathoglottis* spp., was *Colletotrichum gloeosporioides.* The bacteria (VP CB) causing soft rot symptom in *Cattleya* spp. was identified as *Pectobacterium aroidearum.*

The efficacy of different chemicals was tested against major pathogens by *in vitro* evaluation. The selected pathogens *viz., C. boninense* (VL DF1), *F. proliferatum* (VY

DF), *S. rolfsii* (MT DF1)*, A. alternata* (VP DF1), *Colletotrichum* sp. (VK OF3), *L. theobromae* (VY CF2) and *C. gloeosporioides* (MT SF1) were evaluated against seven fungicides at three doses and 1 per cent Bordeaux mixture by poisoned food technique. The bacterial pathogen, *P. aroidearum* (VP CB) was also evaluated against different chemicals such as Streptocycline, copper hydroxide and Bordeaux mixture.

Among the fungicides tested, the fungicides *viz.,* hexaconazole, propineb, difenoconazole, carbendazim 12 % + mancozeb 63 % and cymoxanil 8 % + mancozeb 64 % showed 100 per cent inhibition of mycelial growth at all concentrations and 1% Bordeaux mixture showed complete inhibition of *C. boninense* (VL DF1).

Hundred per cent inhibition of *F. proliferatum* (VY DF) was observed in all three concentrations of copper hydroxide, hexaconazole, carbendazim 12 % + mancozeb 63 % and 1 per cent Bordeaux mixture. Propineb at its higher concentration (0.3 %) and difenoconazole at its recommended and higher concentration completely inhibited the mycelial growth of the pathogen.

Fungicides *viz*. hexaconazole, propineb, difenoconazole, carbendazim 12 % + mancozeb 63 %, cymoxanil 8 % + mancozeb 64 % at all dosages and 1 per cent Bordeaux mixture were found promising against *S. rolfsii* with 100 per cent inhibition against the isolate MT DF1.

Hexaconazole was the only fungicide showing complete inhibition of the pathogen *A. alternata* (VP DF1) at all concentrations. Complete inhibition of *Colletotrichum* sp. (VK OF3) was observed in all three doses of carbendazim 12 % + mancozeb 63 % (0.1, 0.2, and 0.3 per cent) and 1 per cent Bordeaux mixture.

Hundred per cent inhibition of *L. theobromae* was observed in all three concentrations of carbendazim 12 % + mancozeb 63 %, cymoxanil 8 % + mancozeb 64 % and propineb and 1 per cent Bordeaux mixture. Hexaconazole at its higher concentration showed maximum inhibition percentage (100 %), whereas higher concentration of difenoconazole showed an inhibition of 74.58 per cent.

Complete inhibition of *C. gloeosporioides* was observed in all three doses of carbendazim 12 % + mancozeb 63 % and 1 per cent Bordeaux mixture. Hexaconazole and difenoconazole, at their higher dose showed complete inhibition of the pathogen.

Among the chemicals treated against *P. aroidearum* under *in vitro* conditions, copper hydroxide showed maximum inhibition percentage (25.92 %) at its higher concentration (0.3 %), whereas Streptocycline at 200 and 250 ppm showed per cent inhibition of 13.33 and 16.67 respectively, and no inhibition was noticed at a lower concentration (100 ppm). Bordeaux mixture at 1 per cent showed inhibition of 15.5 per cent.

Biocontrol agents viz. *Trichoderma asperellum* (KAU reference culture), *Pseudomonas fluorescens* (KAU reference culture), PGPR-II and PGPM (KAU formulation) were evaluated against the major pathogens. *T. asperellum* was evaluated against major pathogens by dual culture technique. The most promising result was obtained against the pathogen *A. alternata,* showing a maximum inhibition percentage of (91.16 per cent). The pathogens such as *F. proliferatum*, *C. boninense, S. rolfsii, Colletotrichum* sp. and *C. gloeosporioides* exhibited moderate per cent inhibition, and lowest per cent inhibition was shown against the pathogen *L. theobromae*.

The bacterial antagonist, *P. fluorescens,* was evaluated against the major pathogens and found that only *Colletotrichum* sp. (VK OF3) exhibited maximum inhibition, while no inhibition was observed for *S. rolfsii* and *L. theobromae.* Other pathogens showed less inhibition.

In vitro evaluation studies of PGPR-II against major fungal pathogens showed that, complete inhibition of 100 per cent exhibited by *C. boninense* and *C. gloeosporioides*. This was followed by *A. alternata, Colletotrichum* sp., and *S. rolfsii.* PGPM showed 100 per cent inhibition of C. *boninense* and *L. theobromae*. It was followed by *C. gloeosporioides, Colletotrichum.* sp., *A. alternata* and *S. rolfsii*. The least inhibition was noticed for *F. proliferatum.*

The different biocontrol agents *viz., P. fluorescens*, *T. asperellum*, PGPR-II and PGPM were evaluated *in vitro* against *P. aroidearum,* and no inhibition was found in the case of *P. fluorescens*, PGPR-II and PGPM. However, *T. asperellum* exhibited a lower inhibition percentage (26.67 %) against the bacterial pathogen.

Bioassay experiment was conducted to evaluate the efficacy of fungicide, against the major pathogens such as *F. proliferatum, C. boninense, L. theobromae, S. rolfsii* and *Colletotrichum* sp. The pathogens were selected based on their highest PDI and PDS, and carbendazim 12 % + mancozeb 63 % was found to be the most effective

fungicide from *in vitro* evaluation, and hence it was selected for bioassay studies. The study revealed that carbendazim 12 % + mancozeb 63 % was highly effective against *C. boninense* (VT DF1), followed by *F. proliferatum* (VY DF), *S. rolfsii* (MT DF1) and *Colletotrichum* sp. (VK OF3), whereas the fungicide was found least effective in controlling *L. theobromae* (VY CF2) under *in planta* conditions.

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Abstract

CATALOGUING AND DOCUMENTATION OF DISEASES OF SYMPODIAL ORCHIDS IN KERALA

BY

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ABSTRACT OF THE THESIS

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Cataloguing and documentation of diseases of sympodial orchids in Kerala

ABSTRACT

Orchids are valued for their exotic beauty and long vase life, making them one of the most popular cut flowers and ornamental plants worldwide. Currently, orchids hold sixth position among the top ten cut flowers in the world, among which, sympodial orchids rank higher in the export market. However, orchid cultivation is challenged by many biotic factors especially fungal, bacterial and viral diseases, which reduce production potential and economic value. Hence, the present study, 'Cataloguing and documentation of diseases of sympodial orchids in Kerala' was proposed with the objective to document various diseases affecting sympodial orchids in Kerala.

A purposive sampling survey in orchid growing areas of three districts *viz.,* Thrissur, Ernakulam and Thiruvananthapuram conducted revealed the incidence of leaf blight, leaf spot, wilt, black rot, soft rot, twig blight, petal blight and flower spot diseases in four different sympodial orchids such as *Dendrobium* spp., *Cattleya* spp., *Oncidium* spp. and *Spathoglottis* spp. Among the 49 diseased samples collected from eight different locations, 21 diseased samples were obtained from *Dendrobium* spp., 16 from *Oncidium* spp., six from *Cattleya* spp. and six from *Spathoglottis* spp.

PDS and PDI were calculated for each symptom from *Dendrobium* spp., and the highest PDI and PDS were recorded for VY DLB (76.47 %) and MT DLB1 (36.67

%) respectively. In *Cattleya* spp. highest PDI and PDS were recorded for VY CLS (70.80 %) and VP CSR (48.5 %) respectively. In *Cattleya* spp. highest PDI and PDS were recorded for VY CLS (70.80 %) and VP CSR (48.5 %) respectively. In *Oncidium* spp., the highest PDI and PDS were recorded for VK OLS1 with 76.92 per cent and 46.90 per cent respectively. In *Spathoglottis* spp., highest PDI (48.0 %) and PDS (29.79 %) were recorded for MT SLB1. From the PDS and PDI data, it was found that, leaf blight symptoms were more common and severe in *Dendrobium* spp. whereas leaf spot symptoms were more severe in *Oncidium* spp. In *Cattleya* spp., leaf spot symptoms were most frequently noticed, and soft rot symptom showed highest disease severity. In *Spathoglottis* spp., leaf blight symptoms were most frequent and severe. PDS and PDI values were correlated with light intensity at different survey locations, and a negative correlation was observed.

Isolation of pathogens from diseased samples yielded 52 fungal isolates and one bacterial isolate from different sympodial orchids. A total of 21 fungal isolates were obtained from *Dendrobium* spp.*,*18 fungal isolates from *Oncidium* spp., five fungal isolates and one bacterial isolate from *Cattleya* spp. and nine fungal isolates from *Spathoglottis* spp. All the isolates showed different symptoms upon artificial inoculation and these isolates could be reisolated and confirmed as pathogens.

The pathogens were identified at generic level based on cultural and morphological characteristics. Different fungal isolates, such as *Fusarium* spp. (9 nos.), *Colletotrichum* spp. (6 nos.), *Alternaria* spp. (2 nos.), *Sclerotium* spp. (2 nos.), *Phytophthora* sp. (1 no.) and *Curvularia* sp. (1 no.) were obtained from various symptoms collected from *Dendrobium* spp. The pathogens isolated from *Oncidium* spp. were *Colletotrichum* spp. (11 nos.), *Diplodia* sp. (1 no.), *Diaporthe* sp. (1 no.), *Pestalotia* sp. (1 no.)*, Alternaria* sp. (2 nos.), *Phytophthora* sp. (1 no.) and *Fusarium* sp. (1 no.). Fungal isolates such as *Colletotrichum* spp. (2 nos.), *Fusarium* sp. (1 no.), *Lasiodiplodia* sp. (1 no.), and a bacterial isolate, *Pectobacterium* sp. were isolated from *Cattleya* spp, and *Colletotrichum* spp. (7 nos.), *Fusarium* sp. (1 no.) and an unidentified fungal culture were obtained from *Spathoglottis* spp.

Molecular characterisation of major pathogens from *Dendrobium* spp., *Oncidium* spp., *Cattleya* spp. and *Spathoglottis* spp. was done for species level

identification. Leaf blight pathogens, VL DF1, VY DF, IK DF1 and MT DF1 were identified as *Colletotrichum boninense, Fusarium proliferatum, Fusarium oxysporum and Sclerotium rolfsii* respectively, and the leaf spot pathogen, VP DF1 was identified as *Alternaria alternata.* The leaf spot pathogens, VK OF3 and IK OF1 from *Oncidium* were identified as *Colletotrichum* sp. and *Diaporthe tulliensis* respectively. The isolate, VY CF2 causing leaf spot disease in *Cattleya* was identified as *Lasiodiplodia theobromae and* the fungus MT SF1, causing leaf blight symptom in *Spathoglottis* was identified as *Colletotrichum gloeosporioides.* The bacteria (VP CB) causing soft rot symptom in *Cattleya* was identified as *Pectobacterium aroidearum.*

In vitro studies were carried out using chemicals and biocontrol agents against seven fungal pathogens and one bacterial pathogen. Based on the data, hexaconazole was the most effective among the systemic fungicides, which showed 100 per cent inhibition of *Colletotrichum boninense* (VT DF1), *Fusarium proliferatum* (VY DF), *Sclerotium rolfsii* (MT DF1), *Alternaria alternata* (VP DF1) and *Colletotrichum gloeosporioides* (MT SF1) at all three doses. The fungicide also showed 100 per cent inhibition of *Lasiodiplodia theobromae* (VY CF2) at higher dose, while it was least effective (38.33-79.58 %) against *Colletotrichum* sp. (VK OF3). Bordeaux mixture (1 %) was found to be the most effective contact fungicide, which showed complete inhibition of all pathogens under study except *Alternaria alternata* (76.25 %). Among two combination fungicides evaluated, carbendazim 12 % + mancozeb 63 % could inhibit all fungal pathogens except *Alternaria alternata* (52.91 to 65 %). Among the chemicals evaluated against *Pectobacterium aroidearum,* copper hydroxide showed a maximum per cent inhibition (25.92 %) followed by 1 % Bordeaux mixture (15.5 %) while streptocycline showed per cent inhibition ranging from 0-16.67 per cent.

Among the biocontrol agents evaluated, PGPM showed maximum inhibition per cent against the fungal pathogens (60.41-100 %) followed by PGPR-II (50.41-100 %) and *Trichoderma asperellum* (20.41-91.16 %), while *Pseudomonas fluorescens* was found to be least effective. In the case of *Pectobacterium aroidearum,* no inhibition was found when evaluated with *P. fluorescens*, PGPR-II and PGPM. However, *Trichoderma asperellum* exhibited a lower inhibition percentage (26.67 %) against the bacterial pathogen.

Bioassay studies revealed that carbendazim 12 % + mancozeb 63 % was highly effective against *Colletotrichum boninense* (VT DF1), followed by *Fusarium proliferatum* (VY DF), *S. rolfsii* (MT DF1) and *Colletotrichum* sp. (VK OF3), whereas the fungicide was found least effective in controlling *Lasiodiplodia theobromae* (VY CF2) under *in planta* condition.

Hence the study has thrown light upon the diseases affecting sympodial orchids in Kerala and disease management by chemicals and biocontrol agents under in *vitro* condition.