ETIOLOGY AND CHARACTERIZATION OF DISEASES OF ANTHURIUM (*Anthurium andraeanum* L.) IN KERALA

By

NITHA RAFI

(2019-11-106)

THESIS

Submitted in partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
VELLANIKKARA, THRISSUR- 680656
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2021

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ABSTRACT OF THE THESIS

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DECLARATION

I, Nitha Rafi (2019-11-106) hereby declare that the thesis entitled **"Etiology and characterization of diseases of anthurium (*Anthurium andraeanum* L.) in Kerala"** is a bonafide record of research done by me during the course of research and that the thesis has not been previously formed for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara

Date: 24/11/2021

Nitha Rafi

(2019-11-106)

CERTIFICATE

Certified that this thesis entitled **"Etiology and characterization of diseases of anthurium (*Anthurium andraeanum* L.) in Kerala"** is a record of research work done independently by Ms. Nitha Rafi (2019-11-106) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellanikkara

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Introduction

1. INTRODUCTION

Anthurium (*Anthurium andraeanum* L.) originated in the rainforests of Brazil located between Colombia and Ecuador, is one among the most popular cut flowers in the international flower market. It is a coarse textured, herbaceous perennial, evergreen and slow growing flowering plant mostly prized for its unique, colourful and long lasting spathes. These spathes adjoin the creamy white with yellow tinged spadix that bears numerous minute actual flowers on them resembling a pigtail and hence the name tailflower. The leathery, green, heart shaped leaves of different size are arranged spirally in long slender petioles in a clustered fashion. The spathes and leaves possess ornamental value. Anthurium cultivation and trade already established internationally has opened new avenues for young Indian entrepreneurs due to its high export potential in cut flower market.

Anthurium being the genus of more than 1000 flowering plant species, is the biggest of the family *Araceae* which includes about 114 genera and almost 3750 species. *A. antioquiense*, a wild type bacterial blight resistant cultivar having tulip type upright spathe was cross hybridized with *A. andraeanum* possessing wider spathe and popular in cut flower industry to produce thousands of improved hybrids with desirable traits. Thus, the progenitor and source of variability of the modern anthurium cultivars is *A. andraeanum* (Alvarez, 2018). The two most cultivated anthurium species for red coloured spathe are *A. andraeanum* and *A. scherzerianum* where the former is characterised by a straight and rigid spadix and the later is having a wavy and coiled spadix. The popular commercially cultivated varieties of *A. andraeanum* are Anneke 141, Cuba, Claudia 108, Lydia 420 belonging to Dutch type and Ozaki, Kaumana, Nitta belonging to Hawaiian type. The often cultivated varieties in Indian climate are Tropical Red, Mauritius Red and Flame having red spathe, Acropolis and Lima White having white spathe, Abe Pink and Passion having pink spathe, Sunshine Orange and Nitta having orange spathe and Midori possessing green coloured spathe (KAU, 2016).

Anthurium is a tropical flowering plant that grows best in areas with a higher humidity of up to 90 per cent, a maximum day temperature of 28°C and night temperature falling between 15 °C and 22 °C. It prefers 80 per cent shade with bright indirect light since the frequency of flowering and the flower quality is reduced when

directly exposed to a light intensity greater than 2000 foot candles (TNAU, 2016). Currently, the leading producer and exporter of anthurium in the international flower trade is Netherlands followed by Mauritius. The major anthurium producers are Hawaii, Indonesia, Japan, Thailand, Singapore and Taiwan (IASRI, 2012). The highly demanded and traded anthurium types in world market is *A. andreanum* followed by *A. scherzerianum*, *A. clarinervium* (Velvet cardboard anthurium), *A. watermaliense* (Black anthurium) and *A. hookeri* (Bird's nest anthurium).

In India, anthurium cultivation which is still in the budding stage, is greatly confined to the Western and Eastern Ghats along with the North- East humid regions. The major producer states are Karnataka, Kerala, Andhra Pradesh and North- Eastern states making an annual production of 3230 tonnes with the highest production reported from Assam and Meghalaya. Karnataka which ranked 4th in production is a potent anthurium-export zone in India as Coorg anthuriums are gaining huge popularity in flower markets of Delhi and Mumbai. Recently, the cultivation is also focussed around Nasik, Pune, Kochi and Thiruvananthapuram (Seemanthini and Chandrashekar, 2018).

Anthurium is first among the preferred floriculture crops suitable for Kerala since it is suitable in the prevailing agro ecological environment of the state especially in the districts of Wayanad, Idukki, Malappuram and Ernakulum. An economic analysis conducted by Steephen *et al.* (2013) to study the possibilities and potentials of anthurium cultivation in Kerala concluded that anthurium oriented agribusiness was highly profitable among the farmers of the state that showed a calculated benefit:cost ratio of 1.3 to 2.2.

However, the cultivation is often challenged by many biotic factors especially diseases. Fungal, bacterial, viral and nematode infections are reported in anthurium worldwide. Among these diseases, bacterial blight, spadix rot, stem and root rots are the most devastating ones. Spadix rot caused by *Colletotrichum* sp. and *Xanthomonas* anthurium blight greatly reduced the flower production, deteriorated flower quality, and created huge economic loss that resulted in shut down of many agribusiness entrepreneurs. Stem rots, root rots and bacterial wilt diseases destroyed whole plants that caused a complete decline in the production downsizing the export. The best example is the collapse of Hawaiian anthurium hub due to the widespread occurrence

of bacterial blight, which was once the largest producer and exporter of anthurium in the world.

Over the last few years in Kerala, a boom in the cultivation of anthurium was observed where these were raised at small scale and large scale which boosted the state's agribusiness status. Since this flowering herb is amenable for planting under the warm humid climate of Kerala, it is gaining wide spread attention among the farmers dwelling in both cities and villages. This is also because the crop assures a sustainable income for the farmers, which could truly encourage women entrepreneurs to get into its cultivation. Due to its characteristic flower type and comparably long lasting flowers produced throughout the year, the growers are guaranteed of an assured revenue for atleast five to six years. Recent climatic variations also contribute to the incidence of new disease symptoms. A perusal of literature reveals that not much work has been done on documentation of various diseases associated with anthurium. Hence the current study was proposed to identify and document various diseases of anthuriums grown in Kerala followed by the characterization of pathogens associated with each disease. Hence this study was carried out based on the following objectives:

- Identification and documentation of various diseases affecting anthurium
- Characterization and identification of the associated pathogens
- *In vitro* evaluation of fungicides and biocontrol agents against selected pathogens

Review of literature

2. REVIEW OF LITERATURE

Anthurium (*Anthuium andraeanum* L.) also known as Painters Palette and Hawaiian Heart, is a herbaceous perennial, partially epiphytic plant commercially grown for its attractively coloured and waxy spathe along with green shiny foliage. The leathery spathe is often doubted as a part of the flower which is a modified leaf (bract) protecting the spadix bearing flowers on them. This ornamental flowering plant which is a native of Tropical America ranked eleventh in the global flower trade and second among the tropical flowers showing great export potential. For optimal growth and flower yield, an 80 percent shade level is desirable. The plant grows well in an environment with a relative humidity of at least 60 per cent, a temperature of 18-28°C and a light intensity level of up to 30 per cent. This coarse textured, slow growing plant flowers throughout the year under tropical conditions. Belonging to the family Araceae, *Anthurium andraeanum* got its genus name *Anthurium* from the Greek words Oura and Anthos which both indicate tailflower (Wimalsiri, 2019). One of the most cultivated species of anthurium was named *Anthurium andraeanum* as an honour to Edouard Francois Andre, the French horticulturist who first discovered and detailed the plant (Puccio, 2019).

Popularly known as Flamingo flowers, anthurium come in the frontline of cut-flower production and its cultivation is more confined to Western Ghats and North Eastern region with an annual production of 3230 tons (NHB, 2015). Light intensity and shade level are the two determining factors in anthurium flower production, where they flourish best under bright but indirect light. The characteristics of the potting media like excellent water holding capacity, optimum aeration and lightness of the materials along with the specific environmental conditions favor the incidence of many diseases in anthurium which is a major constrain in its production. A detailed review of records describing various diseases affecting anthurium is dealt with in this chapter.

2.1 OCCURRENCE OF DISEASES

Since the first description many decades ago several reports on various diseases caused by fungus, bacteria, virus, nematode *etc.* were found to affect the crop. Anthracnose, spadix rot, root rot, stem rot and bacterial blight are the major diseases of anthurium reported worldwide. Among these diseases, anthracnose caused by

Colletotrichum sp. and bacterial blight by *Xanthomonas axonopodis* were listed as the most devastating infections that occurred in most of the anthurium growing tracts globally. When such infections are not effectively managed, they cause farmers to lose money by lowering yield and compromising crop life span.

2.1.1 Fungal diseases

Various fungal pathogens affecting the above ground parts of anthurium like leaves, spathes, spadix and stems have been described by many authors worldwide. The review of reports of various fungal pathogens affecting anthurium, the region from where the disease was reported and corresponding year of study are listed in Table 2.1

Table 2.1: Occurrence of fungal diseases of anthurium

Sl. No.	Disease	Year of report	Country/Region	Reference
1.	*Pythium* rot	1960	Germany	Sauthoff and Krober, 1960
2.	*Phytophthora* rot	1991	Brazil	Pitta *et al.*, 1991
3.	*Rhizoctonia* root rot	1994	Hawaii	Guo and Ko, 1994
4.	Anthracnose	1997	Kerala	Naseema *et al.*,1997
5.	*Pestalotiopsis* leaf spot	1998	Brazil	Amorim, 1998
6.	*Sclerotium* leaf spot	2007	Peru	Bastos, 2007
7.	*Myrothecium* Leaf spot	2010	Brazil	Quezado, 2010
8.	*Alternaria* leaf blight	2018	India	Selvaraj and Ambalavanan, 2018
9.	*Lasiodiplodia* spadix rot	2019	Thailand	Daengsuwan *et al.*, 2019
10.	*Phytopythium* Stem and leaf wilt	2019	Korea	Park *et al*., 2019

Pythium was found to infect the roots and caused decay, appearance of dark patches and sloughing off of root tissues which leads to complete root destruction and wilting of the plant (Sauthoff and Krober, 1960). A widespread occurrence of anthurium root rot in Hawaii was claimed to be caused by *P.splendens, P.spinosum,* and *P.vexens* isolated from 11 cultivars of anthurium (Guo and Ko, 1994). Later the disease was reported from Jamaica (Huttan and Edman, 2002) and Florida (Norman and Ali, 2018).

Bateman (1961) remarked that *Pythium* rot was favoured by lower temperature and wet soil conditions while high temperature and dry soil conditions promoted *Rhizoctonia* stem and root rot. A highly destructive incidence of *Rhizoctonia* sp. was reported from Hawaii which caused more than 80 per cent loss of seedlings in nursery due to severe root rot and vascular wilt (Guo and Ko, 1994). Decayed roots showed characteristic mycelial growth which distinguished the disease from *Pythium* and *Phytophthora* rot. The pathogen was later reported from Trinidad in association with the plant parasitic nematode *Radopholus similis* infection (Hosein and Farzan, 2001).

The decline of anthurium cultivation was mainly due to the deterioration of flower quality resulted from the appearance of blue spots on spadix, spathe wilting, spathe or spadix darkening (Croat, 1988). Anthracnose also known as spadix rot or black nose disease (Kamemoto, 1988) caused by *Colletotrichum gloeosporioides* occurs in tropical and subtropical conditions (Nishijima, 1994). In Kerala, the first record of anthracnose was given by Naseema *et al.* (1997) from Trivandrum. A survey conducted in Aleppey District by Santhakumari *et al.* (2001) reported the incidence of a rotting complex involving *Xanthomonas* sp. and *Colletotrichum gloeosporioides* for the first time in Kerala which resulted in 100 percent death of plants. This disease which led to massive flower loss in quantity and quality had a maximum percent disease incidence of 56.66 in Dindugal district of Tamil Nadu (Rex *et al*., 2019). For the first time in Brazil, *Colletotrichum theobromicola* was reported, developing anthurium anthracnose, which was characterized by necrotic spots on the petals (Chaves *et al.*, 2020).

Black rot, another fungal disease found in anthurium characterised by rotting of spathe, spadix, leaves, petiole and stem which was caused by *Phytophthora*

citrophthora was first verified in Brazil (Pitta *et al.*, 1991). The pathogen caused severe leaf blight, root die back and eventual death of the whole plant in nurseries and field which affected the commercial quality of flowers. Later the disease was identified to be caused by different species of *Phytophthora* like *P.tropicalis, P.palmivora* from Brazil and *P.nicotianae* var *parasitica* from Venezuela and Korea (Jee *et al.*, 1998; Aponte *et al*., 2003 ; Paim *et al.*, 2006).

A fungal disease characterized by dark-brown spots on anthurium leaves and flowers was discovered by Amorim in August 1999 in Penedo-AL, Brazil which was caused by *Pestalotiopsis guepinii.*

A serious leaf spot disease caused by *Myrothecium roridum* was reported in Brazil which resulted in severe leaf necrosis (Quezado *et al*., 2010). The report from Korea revealed that the pathogen present in the plant culture media resulted in secondary infection when reused as it colonised on the host cells after the plant died (Kwon *et al*., 2014). The first report of *M.roridum* in China documented more than 20 per cent infected plant seedlings (Ben *et al*., 2015).

The first report of alternaria leaf blight caused by *Alternaria alternata* in India was verified from anthurium growing tracts of Kerala and Tamil Nadu. The disease characterised by black to brown irregular spots with concentric rings and marginal blight was limited to the leaf (Selvaraj and Ambalavanan, 2018).

Daengsuwan *et al.* (2019) described that *Lasiodiplodia theobromae* was responsible to cause spadix rot with pale brown to dark brown discoloration and black rot with sporulation at the tip of the spadix.

Stem and leaf wilt followed by complete death of the plant was caused by *Phytopythium vexans* due to rotting of stem or affected root resulted in severe damages to anthurium seedlings in Korea (Park *et al*., 2019).

2.1.2 Bacterial diseases

A wide variety of pathogenic bacteria causing most devastating diseases in anthurium were reported so far from different countries. A perusal of literature showed that the major declines of anthurium cultivation happened worldwide due to the wide

occurrence and spread of bacterial diseases. A list of diseases caused by bacterial pathogens in anthurium are given in Table 2.2

Table 2.2: Occurrence of bacterial diseases of anthurium

Sl. No.	Disease	Pathogen	Region/ Country	Year	Reference
1.	Leaf blight	*Xanthomonas axonopodis pv. dieffenbachiae*	Hawaii	1972	Hayward, 1972
			California	1985	Cooksey, 1985
			Florida	1990	Hoogasian, 1990
			Netherlands	1998	Sathyanarayana *et al*., 1998
			Turkey	2003	Aysana *et al*., 2003
			Poland	2008	Pulawska *et al*., 2008
2.	Leaf spot	*Pseudomonas* sp.	French West Indies	1985	Prior *et al*., 1985
			Trinidad	1992	Dilbar, 1992
3.	Wilt	*Ralstonia solanacearum*	Florida	1999	Norman and Yuen, 1999
			Martinique	2002	Wicker *et al*., 2002

Duffy (2000) reported that the single most serious threat to the cut flower industry was systemic bacterial blight caused by *Xanthomonas axonopodis pv. dieffenbachiae* which retained pathogenicity over a period of four months persisting in residues. The disease was first observed in the early 1970s in Hawaii (Hayward, 1972) that lessened the production from approximately 30 million stems in 1980 to 15.6 million stems in 1990 (Shehata, 1992). Since then, numerous outbreaks of this disease were reported in California (Cooksey, 1985), Florida (Hoogasian, 1990), Netherlands (Sathyanarayana *et al*., 1998), Turkey (Aysana *et al*., 2003) and Poland (Pulawska *et al*., 2008).

The first record of bacterial leaf spot caused by *Pseudomonas* sp. resulting in black necrotic lesions and eventual plant death was reported by Prior *et al*. (1985) in French West Indies and later in Trinidad (Dilbar, 1992).

Norman and Yuen (1999) made the first report of bacterial wilt in pot anthurium production and confirmed the pathogen associated as *Ralstonia solanacearum* from Florida. *Anthurium* sp. cultivated in the wetter and elevated areas of Martinique developed characteristic greasy water soaked lesions followed by severe wilting due to *Ralstonia solanacearum* infestation (Wicker *et al*., 2002).

2.1.3 Viral diseases

Several reports of virus infection in anthurium plants have been given by researchers worldwide. Viruses infecting anthurium globally or elsewhere are given in Table 2.3.

Table 2.3: Occurrence and distribution of viral diseases of anthurium

Sl. No.	Virus	Year of report	Place/ Region	Reference
1.	Dasheen mosaic virus	2003	Brazil	Lima *et al.*, 2003
		2019	Turkey	Koc, 2019
2.	Cucumber mosaic virus	2013	Brazil	Miura *et al*., 2013
		2013	Iran	Zavareh *et al.*, 2013
3.	Tomato spotted wilt virus	1999	Hawaii	Uchida *et al*., 1999
		2012	Florida	Norman and Ali, 2018
		2016	Turkey	Fidan *et al.*, 2016
4.	Impatiens necrotic spotvirus	1989	Dutch	Werkman *et al.*, 1989
		2002	Czech Republic	Mertelik *et al.*, 2002
5.	Groundnut bud necrosis virus	2020	Bengaluru	Amruta *et al*., 2020

Among the viruses identified, Dasheen mosaic virus (DsMV) which is transmitted by aphids in a non-persistent manner, by vegetative plant propagation and by mechanical inoculation (Zettler *et al*., 1978) is distributed globally in commercially grown anthuriums (Elliott *et al.*,1997). A high incidence of more than 60 per cent was observed in Brazil which exhibited symptoms such as mosaic, chlorotic strips along their foliar veins, reduced plant development, bract distortions and colour modifications

in the flowers (Lima *et al.*, 2003). A reverse transcription polymerase chain reaction involving immune precipitation (IP-RT-PCR) confirmed that it belongs to the genus *Potyvirus* (Lima and Nascimento, 2015). Later this virus was reported from Turkey and the sequencing of fractional coat protein gene along with the 3'UTR area of the viral genome was performed (Koc, 2019).

Anthurium was identified as the host of Cucumber mosaic virus (CMV) for the first time in Brazil which tested positive for the varieties Jureia, Garoa, Eidibel and Astral (Miura *et al*., 2013). In the same year, the virus was detected from Simba and Calore varieties of anthurium from Iran which showed disease symptoms like mosaic, blistering, yellowing, malformation and deformations on leaves (Zavareh *et al.*, 2013).

Tomato spotted wilt virus (TSWV) was firstly described in Hawaii causing severe damage to young shoots of anthurium varieties Aristocrat, Red Hot and Tropic Fire (Uchida *et al*., 1999). Later the virus was reported from Florida in commercially grown anthurium plants showing water soaked flecks, yellow-green patches, yellowish spots with brown pitting and blights (Norman and Ali, 2018). Fidan *et al.* (2016) claimed the presence of this virus from Turkey and confirmed that it belongs to the genus *Orthotospovirus* showing 96-99 per cent sequence similarity with different pepper isolates of TSWV.

The expansion of *Frankliniella occidentalis*, the major vector of *Orthotospovirus* had a great impact in the discovery of Impatiens necrotic spot virus (INSP) infecting anthurium plants, another virus belonging to the genus *Tospovirus*. Werkman *et al.* (1989) reported the incidence of INSP in *Anthurium scherzerianum* from Dutch. Later it was confirmed in *Anthurium andraeanum* from Czech Republic showing symptoms which include chlorotic to necrotic ringspots on leaves, systemic chlorosis, stunting and eventual plant death and observed spherical enveloped virus particles through transmission electron microscope (Mertelik *et al.*, 2002).

Groundnut bud necrosis virus (GBNV) has been reported as an emerging virus in anthurium plants. Amruta *et al.* (2020) confirmed GBNV in greenhouses of Regional Horticultural Research and Extension Centre Campus, Bengaluru showing a heavy disease incidence of 80 per cent in anthurium varieties Acropolish, Cheers, Fire,

Tropical and Xavia. The sequence analysis revealed that it had 98 per cent nucleotide similarity with N gene of a GBNV tomato isolate from Coimbatore (Amruta *et al*., 2020).

2.2 SYMPTOMATOLOGY

The following is a review of studies on the symptomatology of various anthurium diseases caused by fungi, bacteria and virus.

2.2.1 Leaf and spathe diseases

According to research, the major fungal genera that cause leaf disease in anthurium are *Colletotrichum, Myrothecium, Alternaria, Pestalotiopsis* and *Sclerotium.* Bacteria mostly responsible for blight and leaf spot are *Xanthomonas axonopodis* pv. *dieffenbachiae* and *Pseudomonas* sp. respectively.

Anthracnose caused by *Colletotrichum* sp. was seen on old leaves in the majority of instances, and the lesions were mostly found on the tips or margins of the leaves. The initial minute, sunken and uneven light brown spots of 0.5 cm diameter later turned necrotic surrounded by yellow halo. On the lesion, black acervuli proliferated in large numbers (Rex *et al*., 2019). The acervuli produced spore masses that were salmon pinkish in colour when grown in humid weather (Kagivata, 1990).

Myrothecium, which usually infected seedlings, caused dark brown sub-circular specks on leaves that grew to be more than 10 mm in diameter. In later stage, under elevated humidity black sporodochia containing white mycelial tufts, as well as black spore masses were produced (Ben *et al*., 2015).

In case of leaf blight caused by *Alternaria alternata,* the symptoms began as little circular to irregular brown lesions on the leaves with concentric rings that eventually grew larger along with marginal blight. Symptoms were only found on leaves, not on any other plant parts (Selvaraj and Ambalavanan, 2018).

Paim *et al*. (2006) identified leaf blight caused by *Phytophthora citrophthora* in anthurium plants grown in green house surrounded by cocoa plants, that developed typical water soaked lesions later forming dark necrotic patches which spread to the whole leaf surfaces giving a blighted appearance.

According to Nishijima (1994), *Xanthomonas campestris* pv. *dieffenbachiae* (syn. *X. dieffenbachiae*), the causal agent of bacterial leaf blight caused two distinct forms of symptoms. The first were foliar or leaf symptoms, which appeared when the infection started in the leaf or spathe. The second form of symptom was systemic or vascular, which developed when the bacteria established itself in the stem and distributed to other regions of the plant. The initial infection occurred through stomata and hydathodes which resulted in the appearance of water soaked spots mainly concentrating at leaf margins. The most prevalent symptom was marginal necrosis that advanced to the centre of the leaves, however interveinal necrotic patches surrounded by chlorotic halos were also found. On petioles, there were basipetal or acropetal vascular discolorations, as well as longitudinal splits with significant yellow oozing. The petioles as well as the flowers of systemically infected plants, easily detach from the stem. When removed from the stem, the tips of these petioles exhibit noticeable dark patches, and when cut across, the petiole has thin brown lines or streaks which correspond to infected vascular tissues. The leaf sheaths, newly budding leaf and flower buds, or the entire plant are eventually perished.

Prior *et al*. (1985) reported that anthurium leaves infected with *Pseudomonas* sp. produced discrete, angular, greasy patches on the under surface near veins and margins, as well as on spathes, are early symptoms. These lesions quickly become into big, black necrotic patches that become greyish black as the leaves age. Necrotic regions have greasy borders and narrow, intense chlorotic halos around them. On spathes, characteristic black necrotic lesions with violet halos are most common. Infections can spread to veins, resulting in soft rot of diseased tissue, general yellowing and eventual death of the whole plant.

2.2.2 Spadix rots

Daengsuwan *et al.* (2019) described that spadix rot caused by *Lasiodiplodia theobromae* appeared as light brown to black discoloration at the tip of the anthurium spadix and subsequently expanded throughout the spadix. At a later phase of infection, spores were released leading to the complete rotting of the spadix lowering anthurium production both in terms of quality and quantity. In case of black nose disease caused by *Colletotrichum* sp., minute brown to black discoloration developed on individual

flowers on the spadix forming diamond shaped necrotic patches. At later stages, when a greater number of tepal tissues were infected, the whole spadix was covered with the fungal mycelium causing dry rot and eventually fall off (Norman and Ali, 2018).

2.2.3 Stem and root diseases

According to Campoverde *et al.* (2017) *Pythium* and *Phytophthora* rot of anthurium typically affect the root tissues showing an initial symptom of water soaking on leaves, general yellowing followed by necrosis of the above ground plant parts particularly leaves and flowers. *Phytophthora* infected spadix showed dark brown to black angular lesions which slowly spread to corresponding spathe tissues making them distorted. The most affected plant part being the root developed dark lesions of decay along with sloughing off of more than 80 percent of infected tissues. The whole plant after infected with these oomycetes fungi got wilted and deteriorated causing a complete economical loss to the commercial growers. Often with *Pythium* and *Phytophthora* rots, superficial fungal growth on infected plants parts was not seen.

Park *et al.* (2019) described that *Phytopythium vexans* caused wilting symptoms in 5 per cent of seedlings in an anthurium farm located in Korea. The disease initiated with the development of water-soaked lesions followed by dark discoloration on the lower regions of the infected stems. Later on, the infection spread into leaves leading to yellowing, loss of turgor and wilting of the plant as a whole. White mycelial strands of the fungus were seen on the rotted stem tissues under wet and humid conditions leading to eventual death of the plants.

In case of *Rhizoctonia* root rot, young, soft and immature stems developed water soaked lesions that led to complete collapse of the infected tissues resulting in wilting of the plant. One of the characteristic symptoms of the disease was the appearance of dark brown discoloured patches throughout the lower stems and infected roots. Under water logged and humid conditions, infection spread to upper parts of the plant including leaves, petioles, spathes and spadix. Deteriorated roots have fungal mycelial strands over the surface which distinguished damping off caused by *Rhizoctonia* from *Pythium* and *Phytophthora* root rots (Norman and Ali, 2018).

The symptoms of bacterial wilt caused by *Ralstonia solanacearum* were observed as mild chlorosis with the appearance of small greasy, water soaked lesions on the under surface of the leaf tissues. During disease progression, infected leaves and spathes developed necrotic patches surrounded by greasy borders. Leaf veins, petioles, young stem tissues along with the tender portions at the point of attachment of petiole and the leaf showed bronze discoloration followed by sudden wilting of the whole plant that resembled a water stressed plant. Rotting of roots and lower stem regions with immense bacterial ooze was also noticed at a later stage of infection (Wicker *et al.,* 2002).

2.2.4 Viral diseases

Miura *et al.* (2013) reported that anthurium plants infected with cucumber mosaic virus showed characteristic symptoms like chlorotic spots, systemic mosaic, leaf distortion and blistering along with spathe deformation.

A significant incidence of dasheen mosaic virus was observed in anthurium plants during a survey conducted in commercial farms of Guaramiranga country which exhibited symptoms like mosaic patterns, chlorotic streaks on leaves and spathes, discoloration and deformation of flower spadix, bractless flowers and overall reduction in growth and vigour of the affected plants (Lima *et al.*, 2003)

A survey conducted in the commercial anthurium glasshouses in Czech Republic revealed the incidence of Impatiens necrotic spot virus of which the symptoms were mostly restricted to the leaves making it more difficult to detect. Plants showing the symptoms of the virus infection such as chlorotic spots were highly infested with thrips, *Frankliniella occidentalis* (Mertelik *et al.*, 2002).

The symptoms of Tomato spotted wilt virus infection were initially observed as minute chlorotic patches on leaves which gradually spread to the leaf surfaces forming large circular, light greenish spots with yellowish margin having brownish central shallow pits. As the disease progressed, the leaves showed blighted appearance, young shoots and tender tissues of the plants were killed resulting in a complete loss in yield (Uchida *et al.*, 1999).

According to Amruta *et al.* (2020), plants infected with Groundnut bud necrosis virus showed symptoms like dark necrotic spots on flower spathes, stalks, petioles and leaves as well as spathe distortions which showed a per cent disease incidence of 80 per cent.

2.3 INFLUENCE OF WEATHER PARAMETERS ON DISEASE

Favourable environmental factors such as rainfall, relative humidity, temperature, and other weather variables have a positive impact on disease development and dissemination in a susceptible host- virulent pathogen interaction. Chaves *et al.* (2020) reported that the spadix rot pathogen, *Colletotrichum theobromicola* caused severe infection during hot moist weather with copious spore production and germination in wet conditions. The presence of water along the margins of anthurium leaves enhanced the germ tube formation of the pathogen spores since the spores were hygroscopic in nature (Guimaraes and da Paz-Lima, 2017). In case of spadix rot caused by *Lasiodiplodia theobromae,* infection occurred especially in high-temperature, high-relative-humidity environments and the ideal temperature for pathogen proliferation was around 30 degrees Celsius (Daengsuwan *et al.*, 2019). Prolonged monsoon periods were the most ideal condition for stem rot due to *Phytopythium vexans* and under wet conditions fungal mycelia were found profusely on infected stems (Park *et al.,* 2019). The per cent disease incidence and severity of *Pythium* and *Phytophthora* root rot were maximum during rainy periods and the optimum temperature for hyphal penetration, spore germination and dissemination ranges from 15 – 20 degree Celsius. In addition to heavy rainfall, overhead irrigation and flooded soils enhanced disease severity by increasing leaf wetness, sporangial spread and activity of soil fungi respectively (Campoverde *et al*., 2017). Guo and Ko (1994) reported that the root rot caused by *Rhizoctonia* sp. was more severe under warm soil temperature of about 17 to 32 °Cand a medium soil moisture level of 60 to 65 per cent and also less aerated plant roots were more susceptible to the pathogen. According to Hubballi *et al.* (2010), *Alternaria alternata* infection was highly recorded at a temperature range of 15 to 30 °Cand the ideal temperature for the formation of appressorium was 25 °Cwhere 100 per cent relative humidity resulted in maximum spore germination. The per cent incidence of bacterial blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* on anthurium

plants was found to be more during warm and dry seasons at a greenhouse temperature of 30 to 35 °Cwhere colonisation and establishment of the pathogen on the susceptible cultivars advanced rapidly (Fukui *et al.,* 1996). Disease outbreaks are minimized by reducing shade house temperatures by aeration or by growing anthurium at a high altitude with a cooler temperature range of 18 to 24 °C(Norman and Ali, 2018). According to Norman and Yuen (1999), bacterial wilt caused by *Ralstonia solanacearum* rapidly progresses at warmer temperatures of more than 28 °Cwhere cooler weather enhanced bacterial colonization on anthurium plants as latent infections at a slow pace.

2.4 ISOLATION AND PATHOGENICITY STUDIES

To study various pathogen characters and their roles in disease development, one must isolate the associated pathogen from the diseased plant parts in pure cultures. In order to prove the pathogenicity of the isolated pathogens in the corresponding plant host, Robert Koch formulated a set of criteria known as Koch's postulates. According to Rex *et al*. (2019) *Colletotrichum gloeosporioides*, the causal agent of anthracnose in anthurium was isolated from infected leaf tissue by cutting out small bits from the margins of lesions followed by surface sterilization with 10 per cent of NaOCl for 5 mins followed by three washings with sterile distilled water. These bits were then transferred to sterile petri plates containing solidified potato dextrose agar (PDA) media and incubated at 28 °Cfor 7 days. Pure culture of the fungus was made by picking out mycelial tips from the established cultures and growing them in fresh PDA media followed by preparation of a spore suspension of 5 x 10^6 conidia/ml concentration. To prove the pathogenicity of the fungus, four inoculation methods were employed such as leaf clipping method, pin pricking method, spray inoculation method and injury by carborundum powder method. In the first technique, a pair of sterile scissors was dipped in the already prepared spore suspension of pathogen and was used to cut the marginal ends of three selected leaves of a healthy anthurium plant. In the second method, sterile needle was dipped in the spore suspension and pin pricks were given on the leaves to transfer the inoculum. In both the methods, the treated and control plants were maintained in green house at a temperature of 27 °Cby preserving adequate moisture. sprayed on to healthy plant leaves maintained in shade nets and the control plants were

sprayed with sterile distilled water alone. After 14 days of inoculation, typical symptoms developed on the treated leaves kept at a temperature of 30 degree Celsius.

Chaves *et al.* (2020) prepared the conidial suspension of 10^6 spores/ml from one week old culture of *Colletotrichum theobromicola* grown in potato dextrose agar media and inoculated healthy anthurium flowers with 10 µl of the suspension after giving pin pricks with a sterile needle. After inoculation, the treated flowers along with the control were maintained in a humid chamber for 24 hours and at a temperature of 25 °Cfor symptom development.

Selvaraj and Ambalavanan (2018) employed tissue segment method for isolation of *Alternaria alternata* from blighted leaves of anthurium and the purified cultures were used to prepare a 6 x 10^4 spore suspension after filtering out the hyphal debris by sieving though a cheese cloth, to prove the pathogenicity by single leaf inoculation technique.

Lasiodiplodia theobromae, the causal agent of anthurium spadix rot, was isolated by tissue transplantation method and for the establishment of pathogenicity Daengsuwan *et al.* (2019) conducted agar plug method for which four cultivars Angel, Casino, Cheers, and Pistache were selected. For this 48 hour old fungal culture was taken to obtain agar plugs of diameter 5 mm and these plugs were then placed onto the spadix which were surface sterilized. The inoculated spadices were kept in plastic bags to retain humidity and the corresponding symptom development occurred after 2 days.

For the isolation of *Myrothecium roridum*, Kwon *et al.* (2014) collected diseased anthurium plants showing characteristic symptoms, soaked for 5 minutes in sterile water while being shaken followed by centrifugation at 3500 rpm at 10 mins and removed the supernatant. After dilution with sterile water, the inoculum was plated onto sterile PDA media and kept for incubation at 25 °Cfor 72 hours. Pure culture of the pathogen raised by single spore isolation technique was used to make a spore suspension of 10^9 conidia/ml and inoculated anthurium leaves to establish pathogenicity following wound/drop inoculation technique. After one week of inoculation subcircular lesions developed on the tissues along with black sporodochia and thick dark spore mass.

Park *et al*. (2019) prepared hyphal suspension of *Phytopythium vexans* from mycelial mats grown on potato dextrose agar media for 2 weeks and the sterile soil in pots were inoculated with the inoculum suspension before planting the seedlings that resulted in the development of wilting symptoms on the inoculum treated plants with respect to the control plants.

Paim *et al.* (2006) conducted pathogenicity evaluation of *Phytophthora citrophthora, P. citrophthora, P. tropicalis* and *P. palmivora* in detached anthurium spathes and leaves. For this, mycelial plugs of the pathogen having a diameter of 5 mm were cut out from 7 days old culture grown in carrot agar and subsequently inoculated on the surface of the healthy plant parts taken and covered the bits with wet cotton bits to maintain the humidity. After 3 days of incubation, dark brown lesions formed on the inoculated sites, while pin pricked, healthy, uninoculated leaves and spathes remained as such without any symptoms.

The pathogenicity of *Xanthomonas axonopodis pv. dieffenbachiae* was tested on healthy leaves of anthurium plants by infiltrating them with the bacterial suspension containing 1 x 10^7 cfu/ml at 1 cm wide inoculation sites. The plants were maintained at 28°C temperature with a relative humidity of not more than 80 per cent. In 10 days of incubation time period, small water soaked lesions appeared at and around the inoculation sites that gradually enlarged showing the characteristic bacterial infection symptoms from which *Xanthomonas axonopodis* pv. *dieffenbachiae* was re-isolated proving Koch's postulates (Pulawska *et al.,* 2008).

Prior *et al.* (1986) isolated *Pseudomonas* sp. from infected anthurium leaves showing characteristic symptoms by crushing the infected leaf tissues in sterile distilled water followed by streaking the inoculum on sterile solidified King's medium B. Later pathogenicity was proved by spray inoculating *Pseudomonas* onto healthy leaves of anthurium which developed dark water soaked lesions from the point of inoculation after 5 weeks of inoculation.

Norman and Yuen (1999) conducted pathogenicity evaluation of *Ralstonia solanacearum* in anthurium, tomato, triploid banana and pothos. They used two methods to inoculate the selected healthy plants with the pathogenic bacteria. In the first

method, healthy plant stems were stab inoculated with a needle immersed in a bacterial suspension of 1 x 10^8 cfu/ml while in the other method 10 ml of the suspension was drenched onto the root system after making slight wounds almost 1.5 cm away from the collar region using a sterile sharp knife in order to promote better penetration of the inoculum for aiding infection. After two weeks of inoculation, plants showed characteristic yellowing, development of necrotic patches, vascular discoloration in petioles followed by wilting and death.

2.5 CHARACTERISATION OF PATHOGENS

After isolation of the pathogens from the infected tissues and conductance of pathogenicity assessments, characterization was done based on cultural, morphological and molecular aspects to identify, confirm and study the associated pathogens.

2.5.1 Fungi

On the basis of cultural and morphological characters fungal pathogens were characterized to the genus level. Fungi are identified based on cultural traits such as colony morphology, colour, texture, growth rate and pattern, kind of sporulation, pigmentation of the culture on the upper side and reverse side of petri plates, presence and nature of fruiting bodies. Light microscopy was helpful in determining the presence of septations and branching patterns in fungal hyphae, conidial and spore characters like its type, shape, color, dimensions, various fruiting bodies and reproductive structures which were the defining morphological characters.

2.5.1.1 **Cultural and morphological characterization**

Pythium splendens, *P. spinosum*, *P. vexans* causing root rot in anthurium produced thick, white, fluffy mycelia when grown on PDA media (Guo and Ko, 1994). Pythium possessed coenocytic, hyaline hyphae up to 6.2 μm length, globose oogonia having a diameter of 20 μm and present at the terminal ends of hyphae, antheridium with a dimension of 14 x 14 μm (Ashwathy *et. al.*, 2017).

Leaf blight and root rot caused by *Phytophthora citrophthora* on *Anthurium andraeanum* produced white cottony mycelia which were petaloid in shape. The morphological characters of the pathogen were the presence of both terminal and

intercalary chlamydospores throughout the hyaline hyphae with intermittent production of papillate sporangia having length x breadth dimension of 53.5 µm x 27.4 µm and the depth of papillae was measured as 5.4 µm (Paim *et al*., 2006).

According to Sharma *et al.* (2005), *Rhizoctonia solani*, the causal agent of root rot of anthurium showed a mycelial colour variation from white to brown when grown on PDA media and produced light brown coloured sclerotial bodies towards the marginal ends of the mycelial tufts which had a size of 2 mm.

Colletotrichum gloeosporioides, the causal agent of anthracnose in anthurium appeared whitish orange initially which later developed light grey coloured aerial mycelia while the back side of the petri plate showed a greyish green shade to the culture (Rex *et al*., 2019). According to Phoulivong *et al.* (2010), *C. gloeosporioides* produced characteristic one celled, oblong, hyaline and slightly curved conidia measuring a length of 15 µm and a breadth of 7 µm and when aggregated they appeared salmon in colour. Papade *et al.* (2019) reported that the mycelia of the pathogen grow to a radius of 8.5 cm within 8 days of inoculation and the conidia of *C. gloeosporioides* possessed a centrally located oil globule and the acervuli of the pathogen appeared black in colour with the characteristic hyaline conidia forming a mass at the centre.

According to Amorim (1999), *Pestalotiopsis guepinni*, the causal agent of anthurium leaf spot produced 5- celled conidia out of which 3 central cells were light brown in colour and terminal 2 cells were hyaline bearing branched appendages at the tip. Jeewon *et al.* (2003) described the measurements of the apical and basal appendages of the conidia as less than 25 µm.

Bastos (2007) described that *Sclerotium rolfsi* causing leaf spot in *Anthurium plowmanii* showed typical white, fluffy to fibrous, dense mycelial tuft when grown on PDA media. After 3 weeks of incubation, small to medium sized, light brown coloured, globose sclerotial bodies were produced. Initially these structures appeared white that gradually turned to brown on maturation having a size of up to 3 mm.

Myrothecium roridum causing anthurium leaf spot formed whitish initial mycelial growth with the development of intermittent concentric rings that are dark green in colour. The floccose mycelia later developed sporodochia after 5 days of

incubation at a temperature of 25 degree Celsius. Hyaline and oblong conidia having a dimension of 7.6 x 2.8 μm were formed on the tips of hyaline, branched conidiophores (Quezado, 2010).

According to Selvaraj and Ambalavanan (2018), *Alternaria alternata* causing leaf blight on anthurium produce light brown to olivaceous culture with dark brown to green reverse side, light brown straight and branched conidiophores, short beaked pale brown with prominent septations and smooth texture. Sporulation was favoured under adequate moisture and a temperature of up to 32 °C.

The fast growing fungus, *Lasiodiplodia theobromae* causing spadix rot of anthurium initially produced light greyish fluffy aerial mycelia which turned dark grey to black within 3 days of incubation. Black coloured conidiomata with shiny blackish tar like fluid formed in the culture after one month when incubated at a temperature of 28 °Cthat gradually produced black dusty conidia. Young conidia were hyaline and unicellular without septations but when they got matured conidia appeared light brown, 2 celled with a prominent septa and a thick outer wall and measuring 14.96 × 24.00 μm (Daengsuwan *et al.,* 2019).

Phytopythium vexans causing stem rot of anthurium produced white, slightly fluffy and dense mycelial growth on PDA media with terminal, globose, papillate sporangia having a length of up to 38 μm and a breadth of 28 μm and small zoospores measuring 9 μm diameter (Polat *et al*., 2017)

2.5.2.2 ***Molecular characterisation***

Chaves *et al*. (2020) characterized six fungal isolates associated with spadix rot of anthurium in Brazil and inferred that gene sequence analysis ITS, GAPDH, CAL, CHS, ACT (GenBank Accession No. URM8227) confirmed the pathogen to be *Colletotrichum theobromicola*.

To confirm the fungal pathogen associated with anthracnose in anthurium, Rex *et al.* (2019) extracted the corresponding fungal DNA and amplified the DNA using ITS1/ITS4 forward and reverse primers followed by sequencing the PCR product from Eurofins Genomics India Pvt Ltd, Bangalore. The ITS sequence (GenBank accession number MH479426) obtained was 92 per cent identical with *C. gloeosporioides*

deposited in the same database when analysed using BLAST program (Altschul *et al*., 1990).

The fungal isolate causing leaf blight in anthurium was confirmed to be *Alternaria alternata* by amplifying the internal transcribed spacer (ITS) region of extracted DNA using pathogen specific primers ITS-F/ITSR of its 18S rDNA. The pathogen (ID.NO. 8580.12) was further validated by Indian Type Culture Collection Centre, IARI, New Delhi (Selvaraj and Ambalavanan, 2018).

By using mini preparation method (Saitoh *et al*., 2006), DNA of the fungal isolate causing spadix rot in anthurium was extracted and the ITS of the nuclear rRNA and the elongation factor 1- α were amplified with universal primers ITS1/ITS4 and EF1-728F/ EF2 primers respectively (Carbone & Kohn, 1991). Macrogen Sequencing Service was used for sequencing the selected gene regions. The GenBank Accession No.LC496371 corresponding to ITS region and the GenBank Accession No.LC496372 corresponding to EF1-α sequence showed 100 per cent sequence similarity with *Lasiodiplodia theobromae* sequences in NCBI when analysed using BLAST after aligning the sequences in MEGA version 10 application (Daengsuwan *et al*., 2019).

Molecular characterization of the internal transcribed spacer (ITS) along with 5.8S nuclear rDNA of the fungal isolate causing characteristic leaf spot in anthurium was conducted by Ben *et al.* (2015) using universal primers. The amplified sequences (Accession no. KF761292) when blasted in the Gen Bank database showed 100 percent similarity with *Myrothecium roridum* isolates (Accession no. KC469695 and AJ301995).

Park *et al*. (2019) extracted DNA of the fungal pathogen isolated from rotted roots of anthurium plants following the protocols enlisted in DNeasy Plant Mini Kit and PCR amplified the ITS and cytochrome oxidase subunit II mitochondrial DNA sequences using ITS1/ITS4 and Cox2-F/Cox2-RC4 primer pairs respectively. Further sequencing in BIOFACT database followed by blasting in NCBI BLASTn revealed that the obtained PCR products (Gen Bank Accession No.MH478301 for ITS, Gen Bank Accession No MH492367 for cox2) showed 100 percent and 99 percent similarity with *Phytopythium vexans.* Taking *P. vexans* as the reference fungal isolate, a phylogenetic

tree was constructed with neighbour- joining tree as the model in the application MEGA version 7 (Tamura *et al*., 2016 and Panth *et al*., 2021).

2.5.2 Bacteria

Xanthomonas phaseoli pv. *dieffenbachiae,* the causal agent of bacterial blight of anthurium was reported to be a gram negative, rod shaped, aerobic, motile bacteria possessing a single polar flagellum, produced bright yellow pigmented, slimy colonies when streaked onto King's media B (Chase *et al*., 1992). The results of biochemical tests revealed that the bacterium showed positive reaction in catalase test, gelatin hydrolysis, starch hydrolysis, tributyrin hydrolysis, casein hydrolysis, H_2S production, acid production from mannose, lactose, sucrose and negative reaction for indole production, nitrate and oxidase reduction and urea test (Lelliott and Stead, 1987 and Chase *et al.*, 1992).

Pulawska *et al.* (2008) confirmed the identity of *X. axonopodis pv. dieffenbachiae* in three different ways i) by one of the serological methods immunofluorescence test using polyclonal antibodies, ii) PCR amplification with pathogen specific primers PXad and NXad and iii) on the basis of amplified fragment length polymorphism.

Janse and Emmeloord (2009) described three growth media specific to *X. axonopodis pv. dieffenbachiae* where the bacteria showed different cultural characteristics. On NCTM4 media, bacterial colonies formed 3 days after inoculation which appeared round, slimy, convex and yellowish. On Cellobiose starch medium (CS), bacterial strains were distinguished based on their ability to hydrolyse starch present in the media such that the strains which could hydrolyse starch developed a clear zone around the colony. On modified esculin trehalose medium (ET), the compound esculin was hydrolysed by the bacteria resulting in the development of a dark pigment around the colonies.

According to Prior *et al.* (1985), *Pseudomonas* sp. – the causal agent of anthurium leaf spot was a rod shaped, gram negative, motile bacteria with a unipolar flagellum forming dull white to creamy, round, non- fluorescent colonies with definite margins when grown on King's B media.

Ralstonia solanacearum causing bacterial wilt of anthurium when inoculated on TZC media produce characteristic mucoid, dark pink to red pigmented whirling egg like colonies which were the cultural traits that distinguished *Ralstonia* from other bacteria while on the nutrient media, they form flat, creamy and slimy colonies. Morphologically they were gram negative in reaction, rod shaped, aerobic, motile with a unipolar flagellum and had an average length of up to 1.5 µm (Norman and Yuen, 1999). To confirm the bacteria, Wicker *et al.* (2002) did molecular characterisation of the pathogen isolated from wilted anthurium in French West Indies and thus they amplified the bacterial DNA with *Ralstonia solanacearum* specific primer pairs 759/760 and obtained 280 base pair long amplicon.

2.5.3 Virus

Miura *et al*. (2013) described the mechanical transmission of Cucumber mosaic virus by extracting initial inoculum from infected spathes rather than the infected leaves due to the excessive presence of secondary metabolites in leaves that hindered the transmission of viral particles and prepared in a 2 per cent solution of polyvinylpyrrolidone with 0.5 per cent of sodium sulphate followed by applying the extract on to the leaves of healthy anthurium plants at 4 leaved stage after rubbing them with carborundum powder. The characteristic symptoms of the virus appeared in the treated leaves after 90 days of inoculation. When the extract was applied on to the carborundum rubbed leaves of healthy plants belonging to the families Amaranthaceae, Araceae, Chenopodiaceae and Solanaceae, symptoms developed within 5 days of inoculation. From the different infected plant tissues, total RNA was extracted using RNeasy Plant Mini Kit which was then amplified with CMV1 forward and CMV2 reverse primers.

To confirm the presence of Dasheen mosaic virus (DMV) in leaves and flowers of symptomatic anthurium plants, Lima *et al.* (2003) conducted indirect ELISA with DMV- specific antiserum where the suspected samples showed absorption values three times greater than the value of the healthy sample at an absorption spectrum of 405 nm. Light microscopic images of the virus infected tissues showed the presence of inclusion bodies which is a distinguishing character of *Potyvirus* genus. Koc (2019) conducted the molecular characterisation of DMV that infected anthurium plants in Turkey by

extracting total RNA from various infected parts like leaves, spathe, peduncle, stems and used MJ1/MJ2 primer pair for the amplification in RT-PCR. The coat protein genes of the virus were amplified by MJ1/MT4 primers and obtained a 719 base pairs long amplicon. Further sequencing and BLAST analysis of resultant amplicons confirmed the virus associated to be Dasheen mosaic virus.

The morphological characterisation of Impatiens necrotic spot tospovirus was conducted by Mertelik *et al.* (2002) by analysing the systemically infected leaf sample stained with 1 per cent uranyl acetate through a transmission electron microscope and they observed distinct enveloped particles which appeared spherical in shape.

Direct Antigen Coating (DAC)- ELISA was conducted using polyclonal antibodies to identify the presence of Groundnut bud necrosis virus (GBNV) in which the infected samples showed an absorbance value up to 1.4 which was three times higher than the value shown by healthy sample at 405 nm. To further confirm the identity of the virus, total RNA from the infected plants were extracted using Plant Total RNA Kit and using GBNV- specific primer pairs obtained amplicons which was then sequenced and blasted (Amrutha *et al.*, 2020).

2.6 DISEASE MANAGEMENT

A study was conducted by Hara *et al.* (1990) to evaluate the ecological and economic benefits of following integrated pest management (IPM) practises in anthurium cultivation to ward off thrips, false spider mite and anthracnose. The application of mancozeb 80 WP twice at bi-weekly interval reduced the anthracnose injury to a level less than 2 per cent and therefore a 96 per cent reduction in the application of fungicide was obtained.

Another study conducted by Kalidas (2013) regarding the various management aspects of *Colletotrichum* blight in anthurium, garlic extract (*Allium sativum*) was found a very efficient phytoextract in inhibiting the pathogen while among chemical fungicides propiconazole, carbendazim and copper oxychloride could inhibit the growth and sporulation the pathogen when screened by poison food technique. Sonawane and Patel (2017) conducted *invitro* evaluation of efficiency of various bioagents in inhibiting the growth of *Colletotrichum gloeosporioides* causing anthracnose in

anthurium by three different methods. *Trichoderma viridae* was found to be the superior bioagent that caused maximum inhibition in growth of the blight pathogen followed by *T. herzianum* and *T. longibrachiatum.* All the bioagents tested could inhibit the pathogen growth more than 50 per cent, with *T. viride* causing 68.84, 79.59 and 65.51 per cent inhibition under dual culture, antagonist at centre and pathogen at centre methods respectively.

A study conducted by Sabitha (2002) to evaluate the biological management of anthurium bacterial blight with botanicals, it was found that spray application of crude extract of neem cake along with *Tagetes erecta* could reduce the disease incidence by 85 per cent and the bactericidal effect of *T. erecta* was same as that of streptocycline at 100 ppm. Dhanya and Mary (2006) in a study to evaluate the efficacy of various eco-friendly compounds in controlling bacterial blight found that the anthurium plants grown under greenhouse conditions showed 100 per cent disease resistance when treated with 5 sprays of turmeric powder and streptocycline together followed by *Pseudomonas* and captan giving up to 97 per cent inhibition. A foliar spray containing 0.1 percent streptomycin sulphate, 0.1 percent copper oxychloride along with 0.1 percent fosetyl aluminium significantly reduced the intensity of *Xanthomonas* blight of anthurium by 85.33 percent when compared to the control (Suganyadevi *et al*., 2015). According to Suganyadevi *et al*. (2016), the growth and proliferation of *Xanthomonas axonopodis* pv. *dieffenbachiae* was highly inhibited by streptomycin sulphate used at 2000 ppm followed by several antibacterial compounds produced by *Bacillus mojavensis* and *B. subtilis*. The efficacy of gentamycin, a superior antibacterial antibiotic was much less than streptomycin sulphate in inhibiting the pathogenic bacterial growth. Dhanya *et al.* (2020) conducted a greenhouse trial to study the effect of systemic resistance in anthurium plants induced against *Xanthomonas axonopodis* pv. *dieffenbachiae* where the application of *Pseudomonas taiwanensis*, a rhizobacteria resulted in more than 80 per cent of reduction in disease development. Increased production of defence related compounds like poly phenol oxidase enzyme, other proteins and phenolic compounds resulted by the application of mancozeb and salicylic acid along with the rhizobacteria.

Materials and methods

3. MATERIALS AND METHODS

The present study focussed on the etiology and characterization of diseases of anthurium (*Anthurium andraeanum* L.) in Kerala was conducted at the Department of Plant Pathology, College of Agriculture under Kerala Agricultural University, Vellanikkara during the academic years 2019-2021. A detailed description of the materials utilized and methodologies adopted for the conduct of this research project is presented in this section.

3.1 SURVEY ON THE OCCURRENCE OF DISEASES OF ANTHURIUM

The documentation of several diseases in anthurium was carried out based on the disease incidence and severity data collected from surveys conducted in various anthurium growing regions of Kerala. For the purposive sampling survey six districts were selected *viz.* Thrissur, Ernakulam, Kozhikode, Malappuram, Palakkad and Wayanad. The details of the selected locations within each district are showed in Table 3.1 and Plate 1. The whole plant and plant parts were examined keenly for the incidence of any disease symptoms and the diseased samples collected thus were separately packed and brought to the laboratory for further analysis. Cataloguing of each disease type was conducted by designating a particular code to the sample related to the location from where it was collected.

3.1.1 Cataloguing of diseased samples

The plant samples showing characteristic disease symptoms were catalogued by the abbreviations corresponding to the kind of symptom observed in the field and location of purposive sampling survey. Accordingly different natural symptoms examined were abbreviated as LS (leaf spot), LB (leaf blight), LW (wilt), RR (root rot), SR (inflorescence rot), leaf malformations (ML) and locations as KMA for Kumaranellur, PBA for Perambra, VCN for Vellanikkara Central Nursery, VFN for Vellanikkara Floriculture shade house, MNT for Mannuthy, CKD for Chirakkekode, IJK for Irinjalakuda, KKY for Koorkenchery, OLK for Ollukkara, OKM for Oorakam, OLR for Ollur, TLR for Thalore, TBM for Therambam, NLB for Nilambur, PNM for Panamaram and ALV for Aluva.

3.1.2 Collection of infected samples

The whole plants irrespective of healthy or diseased were examined thoughout the survey to identify and compare the pathogen infection, development and expression of symptoms under natural conditions followed by tagging of infected plant parts. Various plant parts with symptoms were collected and packed separately in polythene bags, labelled and brought to the laboratory for further studies.

Plant samples suspected with fungal diseases were incubated in moist chamber as high humidity can induce sporulation and accelerate disease symptom development, if any. The fruiting bodies, spores and fungal tissues thus produced were observed under microscope for pathogen identification followed by isolation by tissue segmentation method.

In the case of plant samples showing bacterial diseases, the first level confirmation of pathogen was done by ooze test directly from the field and the corresponding samples were taken to the laboratory for bacterial isolation.

In the case of virus suspected plant samples showing characteristic symptoms like mosaic patterns, malformations and vein banding, the infected plant parts were taken to the laboratory under chilled conditions in ice boxes and kept in deep freezer at -20°C for further identification and characterization studies. The diseased plants collected as a whole were maintained under protected conditions in the virology net house of Plant Pathology department, College of Agriculture, Vellanikkara.

3.1.3 Assessment of disease incidence

The occurrence of various symptoms was recorded from the corresponding fields or shade houses during the sampling survey. Percent disease incidence was estimated for each category of symptom by the formula given by Wheeler (1969) as given below:

$$PDI = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

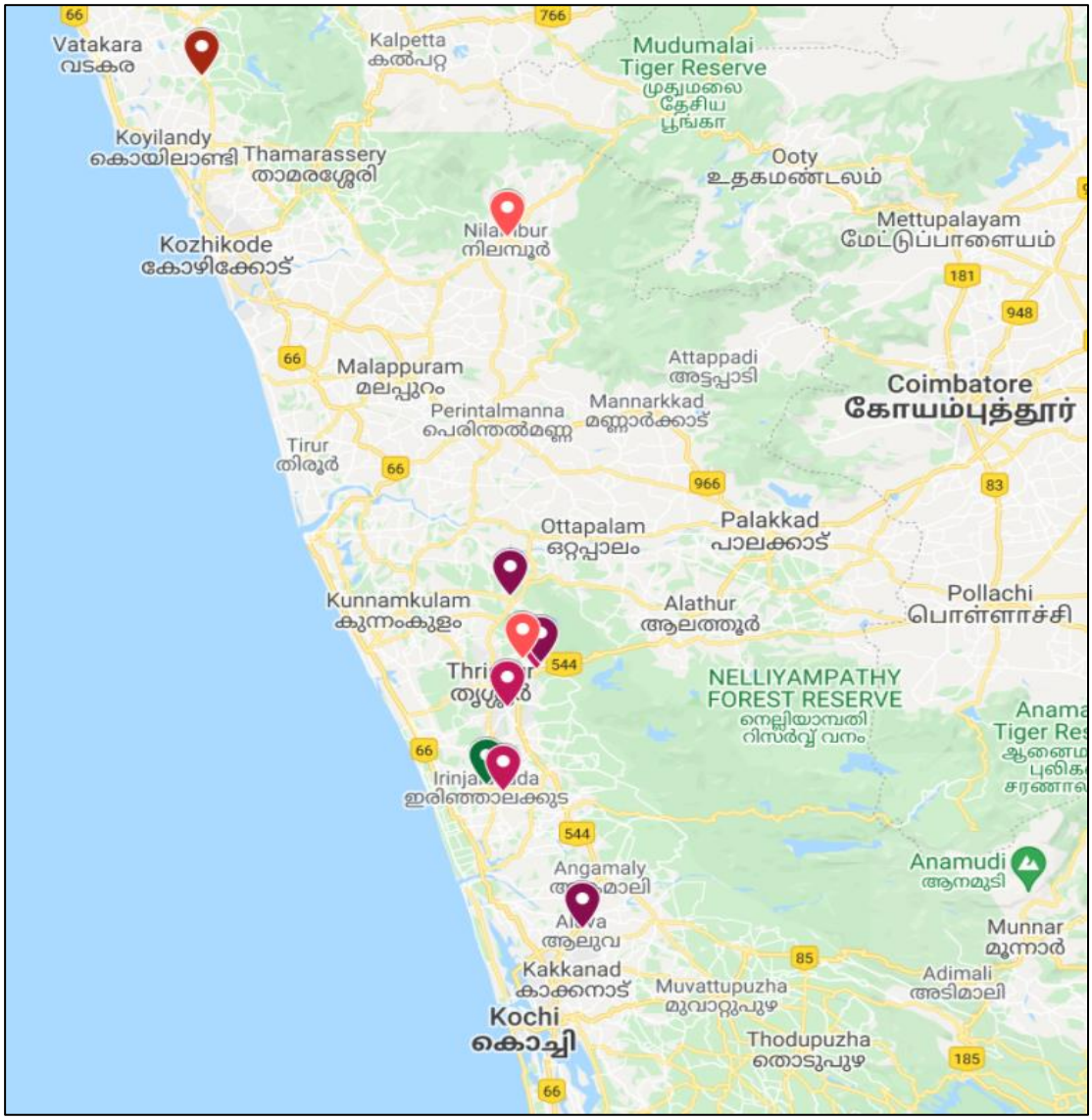


Plate 1: Locations of purposive sampling survey

- Thrissur- Vellanikkara, Mannuthy, Chirakkekode, Irinjalakuda, Koorkenchery, Oorakam, Ollukkara, Ollur, Thalore, Therambam,
- Kozhikode- Perambra
- Malappuram- Nilambur
- Palakkad- Kumaranellur
- Wayanad- Panamaram
- Ernakulam- Aluva

Table 3.1: Details of locations surveyed

Sl. No.	District	Place
1.	Palakkad	Kumaranellur
2.	Kozhikode	Perambra
3.	Thissur	Vellanikkara: central nursery
		Vellanikkara: Floriculture shade house
		Mannuthy
		Chirakkekode
		Irinjalakuda
		Koorkenchery
		Ollukkara
		Oorakam
		Ollur
		Thalore
		Therambam
4.	Malappuram	Nilambur
5.	Wayanad	Panamaram
6.	Ernakulam	Aluva

3.1.4 Assessment of disease severity

For foliar, floral and root diseases, the percent disease severity was calculated using standard score charts. A disease score chart of 0-5 scale developed by Dhanya and Mary (2006) was followed for leaf blights, a score chart of 0-9 scale was followed for leaf spots, floral diseases and root rots (TNAU, 2016) and a standard scire chart given by Sseruwagi *et al.* (2004) as given in Table 3.2, Table 3.3 and Table 3.4.

Table 3.2: Disease score chart for assessing leaf blight severity

Grade	Infection (%)	Extend of disease development
0	0	No infection
1	1-5	Small lesions on leaves
2	6-25	Lesions along with yellowing of 1-2 leaves
3	26-50	Lesion size (1.2x5 cm) along with yellowing of 1-2 leaves
4	51-75	Yellowing of all leaves with blackening of petiole
5	76-100	Complete death of plant

Table 3.3: Disease score chart for assessing leaf spot, foliar disease and root rot severity

Disease scale	Infection (%)
0	0
1	Less than 1
3	1-10
5	11-25
7	26-50
9	More than 50

Table 3.4: Disease score chart for assessing viral diseases

Disease scale	Extend of disease development
0	No symptom
1	Mild chlorosis and distortions
2	Pronounced mosaic pattern and distortions
3	Severe distortions of two thirds of leaves and general reduction of leaf size
4	Severe distortions of two thirds of leaves and general reduction of leaf size

Disease severity was calculated by assigning appropriate scores to the samples showing similar symptoms but various gradation of symptom development using the above-mentioned standard disease score charts. The formula given by Wheeler (1969) was then used to estimate the per cent disease severity of each type of symptoms.

$$PDS = \frac{\text{Sum of all disease ratings x 100}}{\text{Total number of samples scored x maximum disease grade}}$$

3.2 ISOLATION OF PATHOGENS FROM INFECTED SAMPLES

Various infected plant parts like leaves, spathes, spadices, flowers, stems and roots showing characteristic disease symptoms were thoroughly washed in running tap water to eliminate the superficially adhering saprophytic organisms and other solid particles.

3.2.1 Isolation of fungal pathogens

The technique used for the isolation of fungal pathogens from the infected samples was tissue segmentation method (Rangaswamy, 1958). Thoroughly washed specimens were taken and using sterile sharp blade plant tissue showing typical symptom along a section of adjacent healthy tissue was cut out into square bits of 0.5 to 1.0 cm size. The tissue bits thus having visibly diseased and healthy portions together were surface sterilized in one per cent sodium hypochlorite solution for 30 sec to 1 min followed by three consecutive washings in sterile water for 1 min each to eliminate the traces of sodium hypochlorite. The excess water around the tissue bit was removed by keeping the sample bits in a sterile blotting paper under aseptic conditions. Meanwhile, molten and cooled potato dextrose agar (PDA) [Appendix I] was poured into sterile Petri plates aseptically. After solidification of the media, sample tissue bits were kept in the plates and incubated at room temperature (26 + 2°C) for a period of five days. Fungal growth from the bits were observed during the initial days of incubation and the culture was purified by single hyphal tip method or single spore isolation method. Fungal cultures thus established were maintained in PDA slants and preserved for further studies at 4°C under refrigerated conditions.

3.2.2 Isolation of bacterial pathogens

Surface sterilised sample tissues were macerated in small amount of sterile distilled water on a clean glass slide and the collected ooze was then streaked aseptically on to solidified nutrient agar plated Petri dishes by quadrant streaking method. Later the plates were kept in an inverted position for incubation at a temperature of 26 °C for 24 to 72 h. After incubation, single colonies of bacterial pathogens formed were selected and streaked on to freshly prepared sterile solidified nutrient agar media poured plates. The purified cultures were stored for further studies under refrigerated conditions in nutrient agar slants and sterile water in glass vials.

3.3. PATHOGENICITY STUDIES

The fungal and bacterial cultures isolated and purified from the diseased samples collected from various survey locations were then subjected to pathogenicity studies in accordance with Koch’s postulates. Each pathogen isolates were artificially inoculated

on to healthy plant parts where symptom expression was noticed under field conditions. The inoculated spots were observed for the symptom development and incubation period was also noticed.

3.3.1 Pathogenicity of fungal pathogens

To study the characteristic symptoms produced by fungal pathogens on artificial inoculation, Mycelial Bit Inoculation Method (MBIM) explained by Rocha *et al.* (1998) was followed. Mycelial discs of fully grown fresh fungal cultures of 8.0 mm diameter were placed on pin prick injuries made on freshly detached leaves, spathes, flowers or on whole plants. Fungal discs were preferably kept on adaxial surface of the leaves and around the spadices where the symptoms were naturally observed (Rex *et al.*, 2019).

3.3.2 Pathogenicity of bacterial pathogens

To establish the pathogenicity of bacterial isolate, a loopful of 72 h old culture of bacteria was added to freshly made nutrient broth and the optical density was adjusted to 0.3 nm (OD_{600}) with a concentration of 10^8 cfu/ml. Thee month old healthy anthurium plants were selected as test plants. After watering, the test plants were retained in moist chamber for 24-48 h pre-inoculation for better disease development and maximum disease progression. The leaves of the test plants were injected with the bacterial suspension (1x 10^8 cfu/ml) specifically into the veins. The experiment was conducted in detached leaves also. The inoculated test plants were kept for symptom development at room temperature. Observations regarding pathogen infection and related host reactions in terms of symptom appearance were recorded each day post-inoculation. When the test plants showed characteristic symptoms, re- isolation of the inoculated bacterial pathogen was done as described in section 3.2.2.

3.4 SYMPTOMATOLOGY

The characteristic symptoms produced by various pathogens on anthurium under natural and artificial conditions were clearly studied and documented.

3.4.1 Symptomatology under natural conditions

During the sampling survey, naturally infected plants were examined and the typical symptoms expressed in various plant parts were recorded to identify the kind of pathogen associated with the symptoms *viz*. fungus, bacteria or virus.

3.4.2 **Symptomatology under artificial conditions**

The pathogens isolated from different samples were re-inoculated on to the respective plant parts from which they were isolated to study the phases of symptom expression in the host under favourable conditions provided artificially. The artificial inoculation of fungus and bacteria were done on anthurium plant parts following standard procedures described in section 3.3.1 and 3.3.2. After inoculation the respective plants or the plant parts were monitored regularly and various disease aspects like colour, size, nature of lesions and number of days required for initiation of symptoms were noted.

3.5 CHARACTERIZATION AND IDENTIFICATION OF PATHOGENS

3.5.1 Characterization of fungal pathogens

The identification of fungal pathogens was done based on the cultural and morphological characters and the molecular characterization of selected fungal pathogens were also done.

3.5.1.1 Cultural characterization

The fungal pathogens isolated from various samples were subcultured on sterilized and solidified potato dextrose agar (PDA) and kept for incubation at room temperature (26 ± 2°C). Visual observations like growth rate of the fungus, colour of mycelia, pattern of growth, texture of mycelia, pigmentation, sporulation, presence of fruiting bodies, production of different fluids/ooze in the culture, colour on the reverse side of the Petri plate were noted.

3.5.1.2 Morphological characterisation

The morphological characters of the fungus which include hyphal colour hyphal branching pattern, presence of septations, presence of conidia, septations on conidia,

colour, shape and size of spores, dimensions of spores, type and size of fruiting bodies were recorded with the help of microscope.

3.5.1.3 Identification of fungal pathogens

The respective fungal pathogens isolated from each sample were identified by comparing the cultural and morphological characters with CMI descriptions of plant pathogenic fungi and bacteria (CMI, 1964).

3.5.2 Characterization and identification of bacterial pathogens

Cultural, morphological, biochemical and molecular characterization were done for the identification of bacterial pathogens isolated from diseased anthurium plants.

3.5.2.1 Cultural characterization

The bacterial isolates obtained from diseased samples were streaked on to solidified nutrient agar (NA) [Appendix I] media in sterile Petri plates and then incubated at room temperature for 48 h in inverted position. Observations on several visual characters like colony colour, texture and mucoidal nature were recorded.

3.5.2.2 Morphological characterisation

Gram's staining technique and Electron microscopy were used for the morphological characterization of the bacterium that revealed the exact size and shape of the pathogenic bacteria.

3.5.2.2.a Gram's staining

A single drop of sterile distilled water was poured in the middle of a clean glass slide under aseptic conditions. A loopful of bacterial inoculum was taken from a 48 h old culture and mixed thoroughly with two to three drops of sterile water to form a thin bacterial smear over the glass slide. The smear was air dried and heat fixed followed by flooding with Crystal violet (primary stain) for 1 min. The stain was then washed gently in running tap water. Later the bacterial smear was flooded with iodine solution for 1 min and thereafter washed with 95 per cent alcohol (decolourizer). The slide was then washed with tap water to remove the decolourizer and counter stained with safranin and kept for 1 min. After staining, the slide was washed again in running tap water and air

dried. The stained and air dried sample in the glass slide was then observed under the microscope at 40X and 100X magnification.

3.5.2.2.b Scanning electron microscopy

Scanning electron microscopy (SEM) to capture the thee-dimensional image of the bacterium was conducted at Central Instrumentation Laboratory, College of Veterinary and Animal Sciences, Mannuthy, Thissur. The procedure followed for scanning electron microscopy was a small modification of the procedure described by Lopes *et al.* (2012).

Protocol

A loopful of bacterial cells were collected from a 48 h old culture with a flame sterilized inoculation loop and suspended in 1.5 ml of phosphate buffer saline (PBS) having a pH value of seven. The samples were centrifuged at 6000 rpm for 5 min. The supernatant was discarded after centrifugation and 1.5 ml of PBS was added followed by a centrifugation at 6000 rpm for 5 min. Then, the supernatant was removed and 1.5 ml of 2.5 per cent glutaraldehyde was added and the mixture was vortexed. After thorough mixing, the samples were kept for incubation at 37°C for 1.5 h giving intermittent vortexing. The contents were centrifuged after incubation at 6000 rpm for 5 min and washed three times thoroughly with PBS. The bacterial pellet thus formed was serially dehydrated with ethanol (30%, 50%, 70%, 80% and 90%) and incubated at 37°C for 10 min. After centrifugation at 6000 rpm for 5 min, the supernatant was removed and to the cell contents 1.5 ml of 100% ethanol was added and incubated for 1h. After that, the samples were centrifuged at 6000 rpm for 5 min. The supernatant was carefully removed without disturbing the pellets and was air dried. Then 4µl sample was smeared on each stub and sputter coated with gold particles at vacuum ($8x10^{-2}$ mBar). Nitrogen was used for creating vacuum to sputter coat the bacterial cells. After 30 min, the samples were taken out of sputter coater and kept inside scanning electron microscope for visualising the cells. Trial and error method was followed for standardizing optimum working distance and magnification to obtain images of high resolution.

3.5.2.3 Biochemical characterization

A series of biochemical tests were carried out for the identification of bacteria. The various tests based on which the bacterium was characterized were amylase production test, indole production test, gelatin hydrolysis test, citrate test, casein hydrolysis test, MR test, VP test, catalase test, oxidase test, hydrogen sulphide production test, potassium hydroxide test. The media composition used for various tests are given in Appendix II.

3.5.2.3.a Starch hydrolysis test / Amylase production test

Starch agar was prepared and autoclaved at 121°C and 15 lbs pressure for 30 min. The molten and cooled medium was then poured in to sterilized Petri plates aseptically. A central streak of the isolated bacteria was given on the solidified media and incubated at room temperature for 72 h. After attaining proper bacterial growth in the plates, the surface was flooded with Lugol's iodine solution using a dropper. The plates flooded with iodine was kept for 30 sec. After removing excess iodine, observations were recorded.

3.5.2.3.b Indole production test

In test tubes, 5 ml of freshly prepared 1 per cent tryptone broth was added and autoclaved. After sterilization, purified bacterial isolates were inoculated in to the medium and an uninoculated control tube was maintained. Both the treated and untreated test tubes were incubated at 37°C for 48 h. After incubation, each of the test tubes were added with 1 ml of Kovac's reagent and proper shaking was given for 10-15 min.

3.5.2.3.c Gelatin hydrolysis test

Gelatin agar was prepared and poured in to test tubes for autoclaving. To the sterilized gelatin tubes, bacterial culture was stab inoculated and one control tube was maintained without inoculation. The tubes were then incubated at room temperature for 3-6 days. After incubation period, tubes were transferred to a refrigerator and kept at 4°C for 10-15 min.

3.5.2.3.d Citrate utilization test

Simmond's citrate agar was prepared and 5 ml of the media was transferred to test tubes followed by autoclaving. The sterilized tubes were taken out and citrate agar slants were prepared. To the sterilized tubes, bacterial colonies were streaked and kept for incubation at room temperature for 48 h. After incubation period, test tubes were examined for colour change.

3.5.2.3.e Casein hydrolysis test

To perform the test, 100 ml of skim milk agar was prepared and autoclaved. The molten and cooled media was then poured in to sterile plates under aseptic conditions and bacteria was streaked on to it. To observe the hydrolysing of casein by the test bacteria, care was taken to give a single streak at the centre of the plate and a control plate was maintained.

3.5.2.3.f Methyl red test

MRVP broth was prepared in test tubes and autoclaved. To the sterilised test tubes containing 5 ml of the broth, test bacterium was inoculated and incubated for 48 h at 35°C along with an uninoculated control. After incubation, five drops of methyl red pH indicator were added to each test tube and observed for the colour change. The development of red colour was recorded as positive reaction whereas no colour change in the broth was taken as negative reaction.

3.5.2.3.g Voges Prausker's test

To the sterilized MRVP broth in the test tubes, 12 drops of VP reagent I and two to three drops of VP reagent II was added followed by gentle shaking of the tubes for 30 sec without cotton plugs for the bacterial cells to come in contact with oxygen. After proper shaking, keep the test tubes as such for 15 min in order to complete the reaction and observed the colour change. The development of pink/purple colour was taken as a positive reaction.

3.5.2.3.h Catalase test

A clean glass slide was taken and poured a few drops of 30 per cent H_2O_2. A loopful of bacterial culture was taken and inoculated to the glass slide over hydrogen peroxide. After inoculation, observed the glass slide for the presence of gas bubbles.

3.5.2.3.i Oxidase test

A clean glass slide was taken and placed the oxidase disc over it. With a flame sterilised inoculation loop, a single bacterial colony was picked up and smeared on the disc. Observed for a colour change on the oxidase disc within 10 sec.

3.5.2.3.j Hydrogen sulphide production test

SIM agar media was prepared in test tubes and autoclaved. The bacterial isolates were stab inoculated into the sterilized media and kept for incubation at 37°C for 48 h. After incubation, the tubes were examined for the presence of black colouration along the line of stab inoculation.

3.5.2.3.k Potassium hydroxide test

Thee per cent potassium hydroxide solution was freshly prepared and three to four drops were poured on to a clean glass slide using a dropper. A loopful of bacterial culture was taken with an inoculation loop and mixed with the solution. After proper mixing of the bacteria, the loop was gently pulled up and observed for fine thread of bacteria along the loop.

3.5.2.3 Identification of bacterial pathogen

The cultural, morphological and biochemical characters of the bacteria isolated were examined and compared with CMI descriptions of plant pathogenic fungi and bacteria (CMI, 1964), Bergey's Manual of Systematic Bacteriology (Aneja, 2003), several literatures based on which the bacteria were identified (Mahuku *et al.* (2006); Sathyanarayana *et al.* (1998); Soustrade *et al.* (2000) and Toves (2008)).

3.5.3 Characterisation of virus

Virus suspected samples collected during sampling survey were subjected to primary analysis for confirmation of virus particles based on morphological characters.

3.5.3.2 Morphological characterisation

To detect the presence of virus particles and study the morphology, symptomatic leaves and spathe tissues collected from suspected plants were subjected to transmission electron microscopic studies. The samples were sent to Advance Centre for Plant Virology, IARI, New Delhi.

The protocol used for transmission electron microscopy was leaf dip method. Symptomatic leaf or the spathe was cut in to bits of $1cm^2$ area and crushed in 0.1 M phosphate buffer of pH 7 which contain nicotine sulphate (1%) over a clean glass slide. After thorough mixing, a drop of the sample was transferred on to carbon coated grid used in electron microscope and kept for 2 min. Later the grid was washed with ten drops of distilled water and stained with two per cent of uranyl acetate. Using a filter paper the excess quantity of stain was removed from the edges of the grid followed by examining the sample though transmission electron microscope.

3.6 MOLECULAR CHARACTERIZATION

3.6.1 Molecular characterization of fungal isolates

The species level identification of all the fungal isolates were done based on molecular characterization by isolating fungal DNA and sequencing corresponding ITS-rDNA region. The purified fungal cultures after studying cultural and morphological characters were sent to Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram for ITS-rDNA gene sequencing.

3.6.1.1 DNA isolation procedure

The fungal DNA was isolated by using NucleoSpin® Plant II Kit (Macherey-Nagel)

- 100 mg of the fungal mycelium was homogenized using liquid nitrogen in a prechilled mortar and pestle and the powdered mixture was transferred to a microcentrifuge tube.
- 400 µl of buffer PL1 was added and vortexed for 1 min followed by adding 10 µl of RNase A solution.
- The tubes were incubated at 65ºC for 10 min and the contents were transferred to a Nucleospin filter thereafter centrifuged at 11000 rpm for 2 min.

- The supernatant was transferred to a Nucleospin Plant II column and 450 µl of buffer PC was added and centrifuged for 1min.
- The flow though liquid was discarded and to the column 400 µl of buffer PW1 was added and eventually centrifuged at 11000 rpm for 1 min followed by removing the supernatant from the contents in the column.
- Then 700 µl PW2 was added, centrifuged at 11000 rpm and flow though liquid was discarded.
- Finally, another 200 µl of PW2 was added and centrifuged at 11000 rpm for 2 min for drying the silica membrane in the column containing the mixture.
- After centrifugation, the column was transferred to a 1.7 ml centrifuge tube and 50 µl of buffer PE was added followed by incubating tubes at 65^{o}C for 5 min.
- The column was finally centrifuged at 11000 rpm for 1 min to elute the DNA.
- The eluted DNA obtained thus was stored at 4^{o}C.

3.6.1.2 Agarose gel electrophoresis

The quality of isolated DNA was confirmed using agarose gel electrophoresis.

- 5 µl of isolated fungal DNA was mixed with 1 µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0).
- Thoroughly mixed DNA samples were then loaded to each of the wells in 0.8% agarose gel prepared with 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide.
- Electrophoresis was carried out in 0.5X TBE buffer at 75 V until bromophenol blue dye reached to the bottom of the gel.
- DNA bands were visualised in UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.6.1.3 PCR Analysis

The amplification of fungal ITS region of isolated DNA was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). Reaction mixture contained 5 µl of 2X Phire Master Mix, 0.25 µl of forward primer, 0.25 µl of reverse primer, 1 µl of purified fungal template DNA and 4 µl of distilled water. The details of primers used for PCR amplification are given in the Table 3.5. The

amplification process was carried out with an initial denaturation at 98^{o}C for 30 sec, followed by 40 cycles of denaturation at 98^{o}C for 5 sec, annealing at 58^{o}C for 10 sec and extension at 72^{o}C for 15 sec. After the completion of 40 cycles of amplification, a final extension step was performed at 72^{o}C for 60 sec.

Table 3.5: Details of primer used for fungal DNA characterization

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

3.6.1.4 Gel documentation and ITS- rDNA gene sequencing

The PCR products obtained after the completion of reactions were run in 1.2 per cent agarose gel prepared with 0.5X TBE buffer containing 0.5 µg per ml of ethidium bromide to visualise the DNA banding pattern. 4 µl of PCR product mixed with 1 µl of 6X gel loading dye was loaded to the wells and the process was performed using 0.5X TBE buffer as running buffer. 2-log DNA ladder (NEB) was taken as the ladder to compare and identify the corresponding size of the DNA bands. When the dye moved one third of the gel, electrophoresis was done and the gels were visualized in a UV transilluminator (Genei) and the images of visualised DNA bands were captured using Bio- Rad Gel documentation system.

Sequencing reaction was done in a PCR thermal cycler (GeneAmp 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 3.6 and the steps in the PCR amplification for sequencing is given in the Table 3.7

Table 3.6: Components of PCR mix for ITS sequencing reaction

Sl. No.	Component	Quantity
1.	5X Sequencing Buffer	1.9µL
2.	Forward Primer	0.3µL
3.	Reverse Primer	0.3µL
4.	Sequencing Mix	0.2µL
4.	Sequencing Mix	0.2µL
5.	ExoSAP treated PCR product	1µL
6.	Distilled water	6.6µL

Table 3.7: PCR amplification programme of ITS sequencing

Steps	Temperature	Duration	
Initial denaturation	96°C	2 min	
Denaturation	96°C	30 sec	30 cycles
Annealing	50°C	40 sec	
Extension	60°C	4 min	
Final extension	4°C	∞	

3.6.1.5 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).

3.6.2 Molecular characterization of bacterial pathogens

The bacterial pathogens associated with the diseases were identified and characterized to the species level by 16S rRNA gene sequencing.

3.6.2.1 **Bacterial genomic DNA isolation**

The isolation of genomic DNA from bacterial pathogens was performed using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions. The protocol followed is as follows:

- A portion of bacterial culture was transferred to a microcentrifuge tube and 180 µl of T1 buffer and 25 µl of proteinase K were added followed by an incubation at 56°C in a water bath.
- After complete lysis, 5 µl of RNase A (100 mg/ml) was added to the tubes and incubated at room temperature for 5 min.
- Thereafter, 200 µl of B3 buffer was added and incubated at 70°C for 10 min.
- After incubation, 210 µl of 100 per cent ethanol was added to the mixture and the contents were thoroughly mixed by vortexing.
- The mixture was then pipetted into NucleoSpin® Tissue column placed in a 2 ml collection tube and centrifuged at 11000 rpm for 1 min.
- After centrifugation, the NucleoSpin® Tissue column was transferred to a new 2 ml tube and washed with 500 µl of BW buffer. Wash step was repeated using 600 µl of B5 buffer.

- After washing the NucleoSpin® Tissue column was placed in a clean 1.5 ml tube and DNA was eluted out using 50 µl of BE buffer.

3.6.2.2 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% bromophenol blue, 30% sucrose in TE buffer pH= 8.0) were loaded to each wells in 0.8% 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 for the process. Electrophoresis was performed at 75 V until the dye in the buffer reached at the bottom of the gel. After the completion of the process, DNA bands were visualized in a UV transilluminator (Genei) and the documentation of the gel image was carried out using Gel documentation system (Bio-Rad).

The PCR amplification of 16S rRNA gene was performed in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). Information on the primers used for the amplification reaction, composition and volume of PCR mix and amplification profile is given in Table 3.8 and 3.9 respectively.

Table 3.8: Details of primer used for bacterial genomic DNA characterization

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

Table 3.9: Components of PCR mix for amplification reaction

Sl. No.	**Component**	**Quantity**
1.	2X Phire Master Mix	5.0µL
2.	Forward Primer	0.25µL
3.	Reverse Primer	0.25µL
4.	DNA	1.0µL
5.	Distilled water	4µL

PCR amplification profile:

95 °C	-	5.00 min	
95 °C	-	30 sec	35 cycles
60 °C	-	40 sec	
72 °C	-	60 sec	
72 °C	-	7.00 min	
4 °C	-	∞	

3.6.2.3 Agarose Gel electrophoresis and sequencing of 16S rRNA gene

The visualization of PCR product using AGE and further sequencing of bacterial 16S rRNA were carried out following the protocols described in the section 3.6.1.4 using 16S-RS forward and reverse primers.

3.6.2.4 *In silico* analysis of 16S- rRNA sequences

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

3.7 CORRELATION OF WEATHER PARAMETER

Data regarding various weather parameters like maximum and minimum temperature, rainfall and relative humidity of survey locations in Thrissur, Malappuram, Palakkad, Wayanad, Kozhikode and Ernakulam that affect disease progression were collected from the official site of Marksim DSSAT weather file generator (http://gismap.ciat.cgiar.org/MarkSimGCM/). The details obtained from each location were compared and corelated with the fungal disease severity observed in that particular location.

3.8 *IN VITRO* EVALUATION OF FUNGICIDES AND BIOCONTROL AGENTS AGAINST MAJOR FUNGAL PATHOGENS

3.8.1 *In vitro* evaluation of fungicides against major fungal pathogens

In vitro evaluation of eight different fungicides was conducted against selected fungal pathogens at thee doses *viz*. lower, recommended and higher doses (Table 3.10) by following the protocol of poisoned food technique (Zentmyer, 1955). For the experiment, only one dose of Bordeaux mixture was used *i.e.* one per cent was taken.

According to the protocol, the chemicals were measured and added to 100 ml sterilized PDA separately and mixed thoroughly to get a uniform concentration. The poisoned media was then poured in to sterilized Petri plates at the rate of 20 ml per plate. After solidification of the media, eight mm mycelial discs of fungal pathogens were cut out using a flame sterilised cork borer and placed each at the centre of the plates. Plates poured with media without fungicide served as the control.

The experiment was performed using Completely Randomised Block Design with 22 treatments and each treatment replicated four times.

Observations from the treatment plates and control plates were recorded and compared until full growth of the test fungus was attained in the control plates. The fungicidal effect on the pathogen was measured as per cent inhibition using the formula given by Vincent (1927).

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

C – Growth of the pathogen in the control

T – Growth of the pathogen in treatment

3.8.2 *In vitro* evaluation of biocontrol agents against major fungal pathogens

The inhibition in growth of fungal pathogens isolated during the sampling survey by several biocontrol agents *viz. Trichoderma sp*. (KAU reference culture), *Pseudomonas fluorescens* (KAU reference culture), PGPR mix- II (Plant Growth Promoting Rhizobacteria) and PGPM (Plant Growth Promoting Microorganisms) was evaluated using dual culture technique (Johnson and Curl, 1972), ring inoculation method and poisoned food technique respectively. PGPR-II contained *P. fluorescens, Bacillus megaterium* and *Lactobacillus* whereas PGPM was a microbial consortia of *T. viride*, *T. harzianum*, *B. megaterium* and *P. fluorescens.*

Table 3.10: Fungicides and their doses

Sl. No.	Fungicide	Concentration (Per cent)
1.	Copper hydroxide	0.1, 0.2, 0.3
2.	Hexaconazole	0.05, 0.1, 0.15
3.	Propineb	0.1, 0.2, 0.3
4.	Difenoconazole	0.05, 0.1, 0.15
5.	Carbendazim 12% + Mancozeb 64%	0.1, 0.2, 0.3
6.	Cymoxanil 8% + Mancozeb 64%	0.1, 0.25, 0.3
7.	Azoxystrobin	0.05, 0.1, 0.15
8.	Bordeaux mixture	1.0

3.8.2.1 *In vitro* evaluation of *Trichoderma* sp. against major fungal pathogens

To evaluate the interaction effect of *Trichoderma* sp., sterilized Petri plates were poured with 20 ml of molten and cooled PDA media followed by placing an eight mm mycelial disc of fungal pathogen taken from five days old culture at a distance of 2 cm from the periphery. The plates were incubated at room temperature for 48 h. From five days old culture of *Trichoderma* sp., another mycelial disc of eight mm diameter was cut out using a flame sterilized cork borer and kept at a distance of 2 cm from the periphery at the opposite side. PDA media containing pathogen disc alone served as the control plates. Four replications of each treatment were kept and observations were recorded daily till the growth of the pathogen was completed in control plates.

The kind of interaction observed in the treatment plates between the fungal pathogen and the antagonist was evaluated. Various interactions thus detailed 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 colonies
5. Extreme inhibition of the pathogen

3.8.2.2 *In vitro* evaluation of *Pseudomonas fluorescens* against major fungal pathogens

The antagonistic activity of *Pseudomonas fluorescens* was tested against major fungal pathogens by bacterial ring inoculation technique (Adetuyi and Cartwright, 1985). The caps of screw cap vials were autoclaved and the lower margin of the autoclaved caps were dipped in bacterial lawn of *Pseudomonas fluorescens*. The inoculum coated caps were then pressed gently on the centre of Petri plate containing solidified PDA such that a continuous and uniform bacterial ring was formed. From five days old culture of test fungus, a mycelial disc of 8 mm diameter was cut out using a cork borer and placed at the centre of the 48 h old *Pseudomonas* ring. Plates inoculated with pathogen without the bacterial ring served as control. For each isolate, four replications were maintained and all the plates were incubated until a complete growth of the test fungus was observed in the control plates. The radial length of the fungal growth was measured and recorded. Thereafter, the per cent inhibition of the fungal pathogen was calculated using the formula given by Vincent *et al.* (1927).

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

C – Growth of the pathogen in the control

T – Growth of the pathogen in treatment

3.8.2.3 *In vitro* evaluation of PGPR- II formulation against major fungal pathogens

In vitro antagonistic activity of PGPR-II formulation was tested against major fungal pathogens by following the protocol of poisoned food technique (Zentmyer, 1955). Two grams of the formulation was weighed and added to 100 ml pre- sterilized, molten and cooled PDA media under aseptic conditions. The contents were shaken thoroughly to obtain a homogenous concentration of the formulation. PDA containing PGPR-II formulation was then poured to the sterile Petri plates at the rate of 20 ml per plate. After solidification of the media, an 8 mm mycelial disc of the fungal pathogen was inoculated at the centre of the plate. PDA without the formulation was poured to Petri plates and inoculated with the fungal disc of 8 mm diameter served as the control

plates. Each selected fungal isolate was replicated in four plates and were incubated until full growth of the pathogen was obtained in the control plates. The radial growth of the pathogen was measured in the treatment plates and compared with that of the control plates. Using the measurements, per cent inhibition of the growth of the test pathogen over the control was calculated using the formula given by Vincent *et al.* (1927).

$$\text{Per cent inhibition} = \frac{C - T}{C}$$

C – Growth of the fungal pathogen in the control

T – Growth of the fungal pathogen in x 100

3.8.2.4 *In vitro* evaluation of PGPM formulation against major fungal pathogens

The antagonistic effect of PGPM formulation was evaluated against major fungal isolates by poisoned food technique (Zentmyer, 1955). To 100 ml sterilized PDA, two grams of the formulation was added and shaken thoroughly to get a uniform concentration of the product. The molten and cooled PDA containing the formulation was transferred to sterile Petri plates such that each plate contained 20 ml of the formulation mixed growth media. Thereafter, an 8 mm of the pathogen disc was placed at the centre of the plate and the PDA plate inoculated with the pathogen without the formulation were maintained as control. The radial mycelial growth in each of the four treatment plates inoculated with a test pathogen was measured and per cent inhibition in the growth of the fungal pathogen in treatment plates over control plates was calculated by the formula given by Vincent *et al*. (1927).

$$\text{Per cent inhibition} = \frac{C - T}{C} \text{ X } 100$$

C – Growth of the fungal pathogen in the control

T – Growth of the fungal pathogen in treatment

3.9 *IN VITRO* EVALUATION OF ANITIBIOTICS, FUNGICIDES AND BIOCONTROL AGENTS AGAINST BACTERIAL PATHOGEN

3.9.1 *In vitro* evaluation of antibiotics and fungicides against bacterial pathogen

The sensitivity of bacterial isolate towards fungicides *viz*. copper hydroxide, Bordeaux mixture and antibiotic streptocycline was tested by filter paper disc assay (Madavi *et al*., 2020). Three different concentrations (lower, recommended and higher dose) of copper hydroxide and streptocycline were taken while only recommended dose was selected for Bordeaux mixture (Table 3.10). The selected concentrations of all the fungicides and the antibiotic were formulated freshly in sterile distilled water aseptically. To obtain sufficient bacterial cells for preparing pathogen lawn, a loopful of the bacterial inoculum was taken from a 48 h old culture and mixed with 50 ml of nutrient broth. The inoculated broth media was incubated at 27 ± 2°C for three days. Using sterile cotton wool sticks, the thick bacterial inoculum was swab inoculated on to the Petri plates containing solidified nutrient agar medium. The inoculated plates were kept inverted in a sterile environment for 20 min. Thereafter, pre- sterilised Whatman No. 42 filter paper discs measuring 5 mm in diameter were soaked in prepared concentrations of the fungicides and antibiotic for 5 min. The well soaked discs were placed at the centre of each plate and incubated at 27± 2°C for 48 h. NA plates containing bacterial inoculum and filter paper dipped in sterile water served as the control plates. Three replications of each treatment were maintained along with the control. After incubation, the zone of inhibition was recorded for each treatment.

Table 3.11: Chemicals used and their doses

Sl. No.	Chemicals used	Concentration (Per cent)
1.	Copper hydroxide	0.1, 0.2, 0.3
2.	Streptocycline	0.01, 0.02, 0.025
3.	Bordeaux mixture	1.0

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

The *in vitro* effect of antagonistic biocontrol agents such as *Trichoderma sp*. (KAU reference culture), *Pseudomonas fluorescens* (KAU reference culture), PGPR mix- II (Plant Growth Promoting Rhizobacteria) and PGPM (Plant Growth Promoting Microorganism) was evaluated by inhibition zone method (Piddock, 1990).

3.9.2.1 *In vitro* evaluation of *Trichoderma sp*. and *Pseudomonas fluorescens* against bacterial pathogen

The *in vitro* study to evaluate the efficacy of antagonistic organisms *viz*. *Trichoderma sp*. (KAU reference culture) and *Pseudomonas fluorescens* (KAU reference culture) was carried out following the procedure of inhibition zone technique (Chapke *et al*., 2020). A loopful of three days old bacterial inoculum was added to 20 ml of nutrient broth in a 100 ml conical flask and incubated in a shaker at 37°C and 120 rpm for 72 h. After incubation, 10 ml of the heavy bacterial suspension was transferred aseptically to 100 ml of molten and cooled nutrient agar media with constant stirring. The thoroughly mixed nutrient media was poured to sterile Petri plates and allowed to solidify. Thereafter, an 8 mm mycelial disc from an actively culture of *Trichoderma* sp. was placed at the middle of the plate. In case of *Pseudomonas fluorescens*, a loopful culture was taken and inoculated at the centre of the Petri plate containing the pathogen seeded media. Each treatment was replicated four times and all the plates were then incubated at 37°C for 72 h. Measurements of the inhibition zone produced around the bacterial pathogen by the antagonists were recorded. The efficacy of *Trichoderma* sp. against the bacterial pathogen was also tested by streak method where two parallel streaks of the bacteria were given at a distance of 2 cm from two ends of the Petri plate containing 8 mm fungal disc at the centre. Observations regarding the alterations in the growth of bacteria were recorded.

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

The antagonistic efficacy of PGPR-II mix and PGPM formulations (KAU based) were tested against the bacterial pathogen by filter paper disc method (Sain, 2010). To 100 ml presterilized distilled water in a 250 ml conical flask, two grams of PGPR-II formulation was added aseptically (recommended dosage of formulation = 20 grams per litre) and thoroughly mixed in a shaker at 120 rpm for 30 min. Thereafter, Whatman No.1 filter paper discs of 5 mm diameter were soaked in the homogeneous PGPR-II solution for 5 min. A heavy bacterial suspension was prepared by taking a loopful of pathogen inoculum from a 48h old culture plate and transferred to 50 ml of nutrient broth in a 250 ml conical flask. The inoculum mixed nutrient broth was incubated at 37°C for 72 h with intermittent shaking. Then, 20 ml of bacterial inoculum

was taken from the broth and added to 100 ml of cooled nutrient agar media aseptically. After proper mixing, the media was poured into sterile Petri plates and kept for solidification. Pre-soaked filter paper discs were kept at the centre of the solidified media seeded with the pathogen whereas paper disc dipped in sterile water were used in the control plates.

In case of PGPM formulation, the same procedure was followed for the evaluation of antagonistic activity against the bacterial isolate. The formulation was added to sterile distilled water at the rate of two grams per 100 ml and potato dextrose agar was taken for multiplying and seeding the pathogen instead of NA. The treatment plates along with the control were incubated at room temperature for 48 h and observations on the development of inhibition zone were recorded and studied.

As a second procedure, the efficacy of both formulations was tested by streak method. In this method, the growth media was seeded with corresponding formulation at the rate of two grams per 100 ml and poured to sterile Petri plates under aseptic conditions. On the solidified media, a central streak of the bacterial pathogen was given. Bacteria streaked over growth media without the formulations served as control. Observations on the growth of bacteria in the treatment plates when compared to the control plates were recorded after 72 h of incubation.

3.10 Statistical analysis

The analysis of data recorded from the in vitro studies was performed using web agri-stat package (WASP 2.0). Transformations of the resultant data were done if required. Critical difference method was followed for comparing various treatment means.

Results

4. RESULTS

The research on "Etiology and characterization of diseases of anthurium (*Anthurium andraeanum* L.) in Kerala" was conducted to characterize and document various diseases affecting anthurium cultivated in the state. The experimental studies regarding the research work were carried out in the Department of Plant Pathology, College of Agriculture, Vellanikkara during the period 2019-2021. The results of various studies conducted during the research are described below.

4.1 SURVEY AND CATALOUGING OF DISEASED SAMPLES FROM ANTHURIUM GROWING TRACTS OF KERALA

To identify, assess and evaluate diseases occurring in anthurium, purposive sampling surveys were conducted in selected anthurium growing locations of six districts of Kerala *viz*. Thrissur, Palakkad, Malappuram, Kozhikode, Wayanad and Ernakulam (Plate 2 & 3). Surveys were carried out in fifteen locations of six districts in order to assess diseased samples and the extent of shade observed in each location is mentioned in Table 4.1. During the survey, various kinds of diseased samples including infected roots, stems, leaves, spathes and flowers were collected separately. Details about the type of symptoms expressed under natural conditions to assess disease incidence and severity and extent of shade provided in the shade houses were recorded.

Different types of symptoms studied from the surveyed locations are given in table 4.2. The collected symptoms included leaf spots, leaf blights, spadix rots, root rots, mosaic patterns on leaves and wilting. Among the symptoms observed, leaf spot was the most commonly and repeatedly found disease in almost all the surveyed locations except in Aluva, Panamaram, Chirakkekode and Mannuthy. Leaf blight was a very serious symptom observed in Vellanikkara (Anthurium shade house under Department of Floriculture and Landscape Architecture) and Ollukkara. Root rot was another kind of symptom recorded from Vellanikkara.

Table 4.1 Details of survey locations and extent of shade

Sl. No.	District	Location/ Abbreviation	Agro Ecological Unit (AEU)	Geographical co-ordinates	Month of survey	Extent of shade
1	Palakkad	Kumaranellur /KMA	Northern central laterite (AEU 10)	10.7872/76.0476	October, 2020	Not under shade net
2	Kozhikode	Perambra /PBA	Northern laterites (AEU 11)	11.5640/75.7564	October, 2020	Not under shade net
3	Thrissur	Vellanikkara: central nursery /VCN	Northern central laterite (AEU 10)	10.5452/76.274	October, 2020	Plants in shade house
		Vellanikkara: Floriculture shade house /VFN	Northern central laterite (AEU 10)	10.5452/76.274	October, 2020	Plants in shade house
		Mannuthy /MNT	Northern central laterite (AEU 10)	10.5363/76.2651	October, 2020	Plants in polyhouse
		Chirakkekode /CKD	Northern central laterite (AEU 10)	10.5578/76.2905	November, 2020	Plants under shade net
		Irinjalakuda /IJK	Kole land (AEU 6)	10.3469/76.2074	November, 2020	Plants under shade net

Table 4.1 Details of survey locations and extent of shade (Cntd…)

		Koorkenchery /KKY	Northern central laterite (AEU 10)	10.5053/76.2105	January, 2020	Not under shade net
		Ollukkara/ OLK	Northern central laterite (AEU 10)	10.5319/76.2523	January, 2020	Not under shade net
		Oorakam/ OKM	Kole land (AEU 6)	11.0647/76.0102	March, 2021	Partially in shade
		Ollur/ OLR	Northern central laterite (AEU 10)	10.4825/76.2387	April, 2021	40% of plants under shade net
		Thalore/ TLR	Northern central laterite (AEU 10)	10.4547/76.2535	July, 2021	Plants under shade net
		Therambam/ TBM	Northern central laterite (AEU 10)	10.5636/76.2666	July, 2021	Plants under shade net
4	Malappuram	Nilambur /NLB	Northern high hills (AEU 15)	11.2855/76.2386	July, 2021	Plants under shade net
5	Wayanad	Panamaram/ PNM	Wayanad central plateau (AEU 20)	11.7381/76.0740	December, 2020	Not under shade net
6	Ernakulam	Aluva/ ALV	Southern high hills (AEU 14)	10.1004/76.3570	March, 2021	Plants under shade net

(Central Nursery, College of Agriculture) where the root tissues sloughed off and rotted thereby the infection spread to above ground plant parts. Wilting of the petioles, leaves and tender shoot portions of anthurium plants were found to be a serious problem in National Rose Garden of Mannuthy. Inflorescence rot was seen in four locations *viz.* Mannuthy, Chirakkekkode, Aluva and Panamaram that reduced the quality as well as market potential of the cut flower. In inflorescence rot, the central long spadix bearing numerous very small flowers were found decayed initially which spread to the spathe. Appearance of mosaic patterns on the leaves were examined in Vellanikkara (Thrissur).

After collection of various diseased specimens, cataloguing of symptoms was done by assigning codes containing abbreviations of survey location and kind of symptom collected. Each symptom was studied further in accordance with the assigned codes until the pathogen causing the symptom was identified and confirmed.

Purposive sampling survey in Thrissur district were conducted in ten locations *viz.* Vellanikkara, Mannuthy, Chirakkekkode, Irinjalakuda, Koorkkenchery, Ollukkara, Oorakam, Ollur, Thalore and Therambam. Sixteen kinds of symptoms were collected which included leaf spots, leaf blight, root rot, wilting, inflorescence rot and mosaic on leaves. Two different types of leaf spots (VCNLS1, VCNLS2), one severe form of root rot (VCNRR) and plants showing mosaic on leaves were collected from Vellanikkara (Central Nursery, COA). Another kind of leaf spot (VFNLS) and leaf blight (VFNLB) were observed in anthurium plants raised in the shade house of Department of Floriculture and Landscape Architecture, COA, Vellanikkara. The symptoms MNTSR and MNTLW were collected from Mannuthy. A different kind of infection on the spadix (CKDSR) was observed in the fields of Chirakkekode. Several leaf spots *viz*. IJKLS, KKYLS, OKMLS, OLRLS, TLRLS and TBMLS were collected from Irinjalakuda, Koorkkenchery, Oorakam, Ollur, Thalore and Therambam respectively. A leaf blight (OLKLB) symptom was also recorded from Ollukkara.

VELLANIKKARA
Floriculture shade house

VELLANIKKARA
Central nursery

THERAMBAM

IRINJALAKUDA

KOORKKENCHERY

THALORE

MANNUTHY

CHIRAKKEKODE

Plate 2: Surveyed locations

OLLUR

NILAMBUR

PERAMBRA

OORAKAM

ALUVA

Plate 3: Surveyed locations

Table 4.2 Details of symptoms and designated codes

Sl. No	District	Location	Symptoms/ Abbreviation	Designated Code
1	Palakkad	Kumaranellur (KMA)	Leaf spot (LS)	KMALS
2	Kozhikode	Perambra (PBA)	Leaf spot (LS)	PBALS
3	Thrissur	Vellanikkara (VCN)	Root rot (RR)	VCNRR
			Leaf spot 1 (LS1)	VCNLS1
			Leaf spot 2 (LS2)	VCNLS2
			Leaf mosaic (ML)	VCNML
		Vellanikkara (VFN)	Leaf blight (LB)	VFNLB
			Leaf spot (LS)	VFNLS
		Mannuthy (MNT)	Inflorescence rot (SR)	MNTSR
			Leaf wilt (LW)	MNTLW
		Chirakkekode (CKD)	Inflorescence rot (SR)	CKDSR
		Irinjalakuda (IJK)	Leaf spot (LS)	IJKLS
		Koorkenchery (KKY)	Leaf spot (LS)	KKYLS
		Ollukkara (OLK)	Leaf blight (LB)	OLKLB
		Oorakam (OKM)	Leaf spot (LS)	OKMLS
		Olloor (OLR)	Leaf spot (LS)	OLRLS
		Thalore (TLR)	Leaf spot (LS)	TLRLS
		Therambam (TBM)	Leaf spot (LS)	TBMLS
4	Malappuram	Nilambur (NLB)	Leaf spot 1 (LS)	NLBLS
5	Wayanad	Panamaram (PNM)	Inflorescence rot (SR)	PNMSR
6	Ernakulam	Aluva (ALV)	Inflorescence rot (SR)	ALVSR

Three kinds of leaf spots (KMALS, PBALS and NLBLS) were obtained from the survey in the districts of Palakkad, Kozhikode and Malappuram. KMALS was collected from Kumaranellur of Palakkad district while PBALS from Perambra of Kozhikode district and NLBLS Nilambur of Malappuram district.

In Wayanad district, Panamaram was the surveyed location from where one kind of inflorescence rot (PNMSR) was collected. During the survey in the district of Ernakulam, only one nflorescence rot (ALVSR) was obtained from Aluva.

4.1.1 Assessment of Per cent disease incidence and Per cent disease severity

The various symptoms observed within the plant parts were categorized and described separately for the accurate assessment of per cent disease incidence (PDI)/ per cent disease severity (PDS). The calculated values of PDI and PDS corresponding to each disease symptom is detailed in table 4.3.

Nine leaf spots (VCNLSI, VCNLS2, VFNLS, IJKLS, KKYLS, OKMLS, OLRLS, TLRLS and TMBLS), one root rot (VCNRR), two leaf blights (VFNLB and OLKLB), wilting of leaves and petioles (MNTLW), two inflorescence rots (MNTSR and CKDSR) and one mosaic symptom on leaves (VCNML) were collected from Thrissur district. Among the observed disease symptoms, root rot (VCNRR) collected from Vellanikkara showed a maximum disease incidence of 47.33 per cent and disease severity of 64 per cent. Out of the nine leaf spots, IJKLS showed the greatest incidence of 36 per cent followed by VFNLS showing an incidence of 33%. The utmost disease severity among leaf spots was seen in TLRLS (42%) followed by VCNLS1(37.77%). Leaf blight (VFNLB) was recorded with a disease severity of 40 per cent even though the incidence was only 27.63%. The wilting symptom collected from Mannuthy (MNTLW) appeared with an incidence of 21 per cent. A disease severity of 24.44 per cent and an incidence of 31 per cent was observed for inflorescence rot (MNTSR).

The leaf spot KMALS collected from Palakkad district had an incidence of 40 per cent but the severity recorded 33.33%. The leaf spot samples collected from Perambra of Kozhikode district (PBALS) showed an elevated disease incidence and severity of 73.66 per cent and 58.23 per cent respectively. NLBLS surveyed and collected from Malappuram district was recorded with an incidence of 80 per cent and

Table 4.3: Disease incidence and severity

SL. NO	LOCATION	DESIGNATED CODE	PDS (%)	PD1 (%)
1	Kumaranellur (KMA)	KMALS	33.33	40.00
2	Perambra (PBA)	PBALS	58.23	73.66
3	Vellanikkara (VCN)	VCNRR	64.00	47.33
		VCNLS1	37.77	20.00
		VCNLS2	28.88	13.33
		VCNML	44.00	16.34
4	Vellanikkara (VFN)	VFNLB	40.00	27.63
		VFNLS	36.00	33.00
5	Mannuthy (MNT)	MNTSR	24.44	31.00
		MNTLW	-	21
6	Chirakkekode (CKD)	CKDSR	9.13	15.72
7	Irinjalakuda (IJK)	IJKLS	37.03	36.00
8	Koorkenchery (KKY)	KKYLS	21.00	27.00
9	Ollokkara (OLK)	OLKLB	33.00	25.00
10	Oorakam (OKM)	OKMLS	25.00	33.33
11	Ollur (OLR)	OLRLS	25.00	15.5
12	Therambam (TBM)	TBMLS	33.33	30.00
13	Thalore (TLR)	TLRLS	42.00	28.00
14	Nilambur (NLB)	NLBLS	46.66	80.00
15	Panamaram (PNM)	PNMSR	16.71	23.30
16	Aluva (ALV)	ALVSR	32.12	59.00

PDI: Per cent disease incidence

PDS: Per cent disease severity

the leaf spot infection was highly serious that it showed a severity value of 46.66%.

From Wayanad and Ernakulam districts, only inflorescence rots were collected. These two rot symptoms *i.e*. PNMSR (from Panamaram of Wayanad) and ALVSR (from Aluva of Ernakulam) were recorded with an incidence of 23.3 per cent and 59 per cent respectively. The two isolates showed a disease severity of 16.71 per cent (PNMSR) and 32.12% (ALVSR).

4.2 ISOLATION OF PATHOGENS

The diseased plant samples like infected leaves, stems, roots, spathes and spadix collected from various survey locations were properly labelled and brought to the laboratory for further analysis. Based on the protocols detailed in the sections 3.2.1 and 3.2.2, the isolation of pathogens from the collected diseased samples were done and the purified cultures were maintained in PDA/NA slants by recurrent sub culturing.

4.3 PATHOGENICITY OF VARIOUS ISOLATES

The *in vitro* pathogenicity studies of all the isolated pathogens (included 18 fungal isolates and 2 bacterial isolates) associated with the infected plant specimens obtained from the surveyed locations were conducted by artificial inoculation on healthy plant parts followed by reisolation as per the procedure given in sections 3.3.1 and 3.3.2. The post inoculation changes in the host tissue showing the symptom development are described in section 4.4.2. The details of the pathogenicity studies are shown in Table 4.4.

The time period between the inoculation of the pathogen and the development of initial symptom (incubation period) was recorded. The incubation period of various fungal pathogen ranges between two to four days whereas it was eight to nine days for the bacterial pathogen to initiate the symptom development in the host tissue.

4.4 SYMPTOMATOLOGY

Symptoms appeared in naturally infected anthurium plants were studied during the survey and those developed by artificial inoculation were documented during *in vitro* pathogenicity studies.

Table 4.4 Isolated pathogens and time taken to develop symptoms on artificial inoculation

Sl. No.	Isolate	Pathogen	Incubation period (days)	Symptoms on artificial inoculation	Reisolation (+/-)
1	KMALS	Fungus	2	Small, black spots with white cottony mycelia	+
2	PBALS	Fungus	2	Minute black circular spots with slight yellow halo later expanded irregularly	+
3	VCNRR	Fungus	2	Dark brown, water soaked lesions followed by rotting	+
4	VCNLS1	Fungus	4	Brown necrotic lesions with chlorotic halo	+
5	VCNLS2	Fungus	3	Minute irregular brown spots coalesced to necrotic lesions surrounded by yellow halo	+
6	VFNLB	Bacteria	8	Water soaked, brown lesions with yellowish margins, vascular discolouration and rotting	+
7	VFNLS	Fungus	2	Brown oval to triangular greasy spots with black fructifications	+
8	MNTSR	Fungus	2	Dark brown water soaked lesions in spathe, rotting in spadix with mycelia	+
9	MNTLW	Fungus	4	Brown lesions followed by wilting and yellowing	+
10	CKDSR	Fungus	3	Light brown lesions with white mycelia on the spadix	+

Table 4.4 Isolated pathogens and time taken to develop symptoms on artificial inoculation (Cntd…)

Sl. No.	Isolate	Pathogen	Incubation period (days)	Symptoms on artificial inoculation	Reisolation (+/-)
11	IJKLS	Fungus	3	Dark brown lesions surrounded by chlorotic halo and white mycelial growth over the lesions	+
12	KKYLS	Fungus	2	Dark brown water soaked lesions expanded to cover the whole leaf lamina	+
13	OLKLB	Bacteria	9	Brown and dried lesions followed by yellowing of surrounding tissues	+
14	OKMLS	Fungus	4	Dark brown patch surrounded by light brown border. Mycelial growth observed on the lesions	+
15	OLRLS	Fungus	4	Light brown necrotic region with a prominent yellow halo	+
16	NLBLS	Fungus	2	Dark brown to black necrotic patch	+
17	TLRLS	Fungus	3	Water soaked lesions in brown colour surrounded by yellow halo	+
18	TBMLS	Fungus	2	Brown spots with chlorotic halo	+
19	PNMSR	Fungus	2	Dark grey to brown coloured spots with dark brown margin	+
20	ALVSR	Fungus	2	Minute black water soaked lesions coalesced to necrotic patch, rotting occurred in spadix, appearance of white mycelia on the rotted area	+

4.4.1 Symptomatology under natural conditions

A detailed description of different symptoms observed on various parts of diseased anthurium plants during the sampling survey conducted in six districts of Kerala is given in the upcoming paragraphs. These comprise of foliar symptoms, root rots, inflorescence rots, wilting and mosaic symptoms.

4.4.1.1 Foliar symptoms

The symptoms produced in leaves of the infected plants include leaf spots and leaf blight. Twelve leaf spot samples (KMALS, PBALS, VCNLS1, VCNLS2, VFNLS, IJKLS, KKYLS, OKMLS, OLRLS, TLRLS, TBMLS and NLBLS) and two leaf blight samples (VFNLB and OLKLB) were obtained from various locations of the state during the survey.

Leaf spot (KMALS)

The leaf spot (KMALS) was obtained from Kumaranellur of Palakkad district. Tropical Red was the raised variety. Initially symptoms emerged as very small, almost round shaped, light shaded brownish spots which later became dark brown in colour. Rarely the spots deviated from round shapes. Gradually, prominent yellow halos became visible around the margins of the spots. Mostly, the spots measured an average diameter of 0.8 to 1.0 cm. Slightly raised, minute black fruiting bodies of the fungal pathogen associated with the symptom were clearly visible towards the center of the matured spots. Spots were more concentrated towards the midrib portions (Plate 4).

Leaf spot (PBALS)

Leaf spot (PBALS) was collected from Perambra of Kozhikode and the spot was characterized by dark greyish brown lesion arising mainly from the leaf borders and apparently progressing towards the centre, spreading in all directions resulting in drying of whole leaf during the later stages of lesion maturation. The symptom started as minute, irregular grey spots which turned to brownish lesions measuring two to three centimetre longitudinally. There were less prominent yellow halos around the dark brown borders of the lesions. Thin line of yellow halos was restricted to the lesion

margins and was not spreading to the nearby green tissues. Slightly greyish fruiting bodies of the pathogen were seen on the dried portion of the lesions (Plate 4).

Leaf spot (VCNLSI)

The leaf spot, VCNLS1 was collected from Vellanikkara of Thrissur district. The diseased leaves showed small black coloured, irregular spots mainly concentrating between the veins. The average size of the spots was 0.3 to 0.6 cm. The young developing spots had a less distinct rusty appearance on maturation became black in colour. During later stages of infection, these spots were surrounded by faint chlorotic halos (Plate 4).

Leaf spot (VCNLS2)

Another leaf spot obtained during the survey in Vellanikkara of Thrissur district was VCNLS2. Initially, the spots were circular in shape and were lightly purple tinged with spreading yellow halo around them. During later stages of development, faint concentric rings formed intermittently throughout the dark greyish lesion area and the round shape of the young spot was transforming to an irregular pattern with most of the spots appearing in a polygonal pattern. The spots were intensively seen on and around the marginal portions of the leaf (Plate 4).

Leaf spot (VFNLS)

The leaf spot, VFNLS was collected from Vellanikkara (Thrissur district) and the plants were maintained under shade house. Symptoms produced on matured leaves appeared as black coloured irregular patches surrounded by thick yellow halo. The young and tender leaves were not infected. Along with the developing symptom, the yellow halo spread to the adjacent healthy tissues at a quicker rate. Rough and slightly sunken spots measured 0.8 to 1.0 cm in size. Fructifications of the fungal pathogen producing the symptom were seen on spots in later stages (Plate 4).

Leaf spot (IJKLS)

The symptoms in the leaf spot sample IJKLS collected from Irinjalakuda of Thrissur district were visualized initially as papery white coloured oval shaped spots with definite margins. Borders of the spots were distinctly coloured in bright paddy

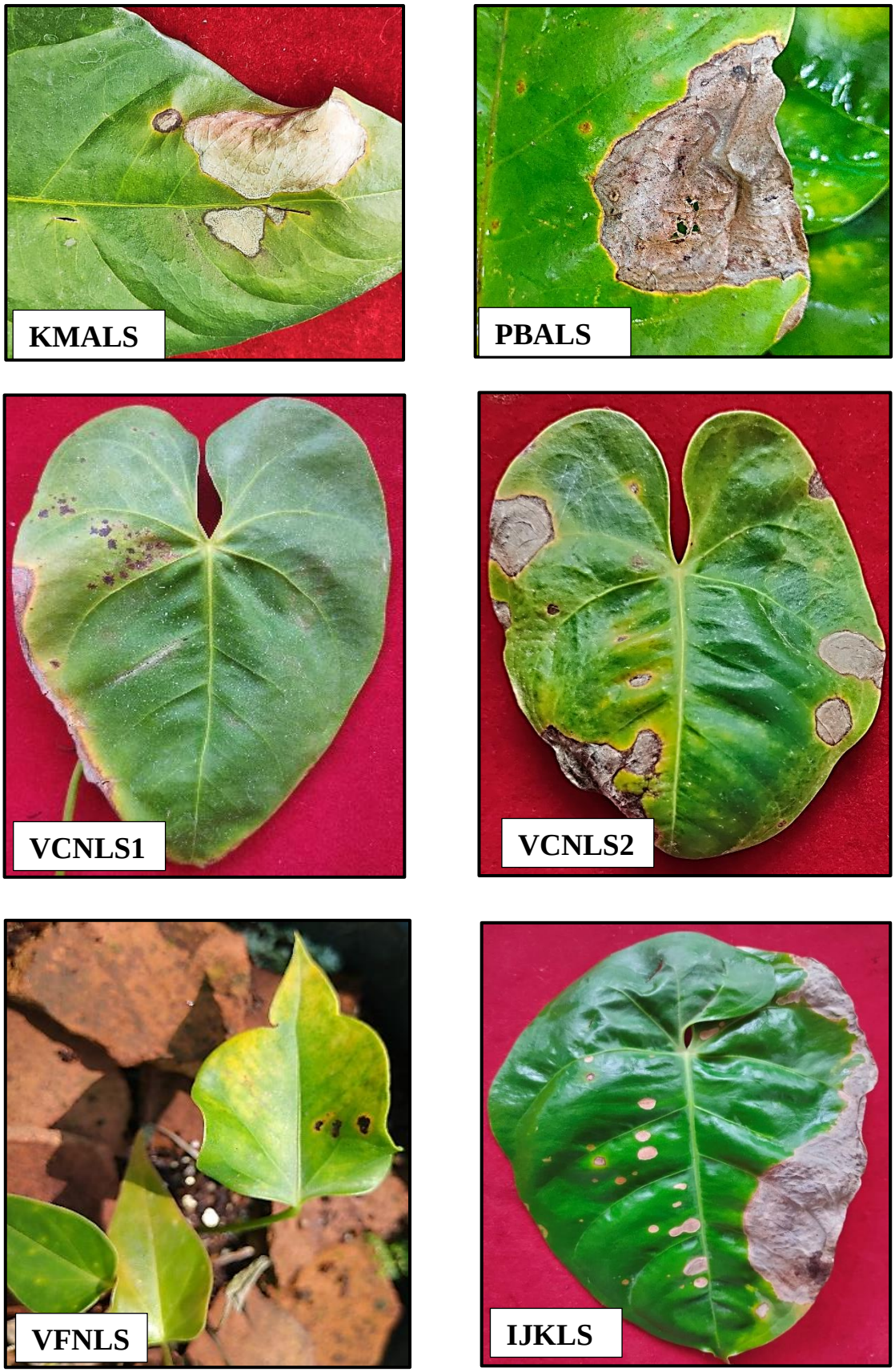


Plate 4: Symptoms under natural conditions – leaf spots

straw colour. The texture of the spots was dry and papery such that many neighboring spots coalesced to form a blighted appearance. Yellow halos were absent around the spots but the tissues were slightly chlorotic (Plate 4).

Leaf spot (KKYLS)

During the survey in Koorkenchery of Thrissur district, the leaf spot KKYLS was collected. The formation of these characteristic spots was focused mainly on matured anthurium leaves. A well defined colour zonation was visible in the spots where the inner portions were greyish brown coloured and were surrounded by thick dark coloured margins followed by another faint brick red coloured patch. The lesions started developing from the edges of the leaf towards the midrib and measured an average of 3.0 cm in length and 1.5 to 2.0 cm in width. The plant tissues surrounding the spots gradually turned yellow (Plate 5).

Leaf spot (OKMLS)

One of the leaf spot samples (OKMLS) collected from Oorakam of Thrissur district was distinguished by the appearance a few cylindrical shaped spots with round ends, coloured in dull grey and measuring 0.5 to 0.8 mm longitudinally. The spots when young had a very dark brownish borders surrounded by highly prominent bright yellow halos. When many spots developed nearby, they coalesced and formed irregular dark patches mostly concentrated towards the edges of the infected leaves (Plate 5).

Leaf spot (OLRLS)

The symptoms appeared in the leaf spot OLRLS obtained from Ollur of Thrissur district were characterized by marginal necrosis of infected leaves when turned severe. The spots were surrounded by less prominent concentric zonation during the initial stages. Thereafter, the spots covered the whole leaf lamina making the infection more severe. The infected leaves later became blighted and the diseased portions were surrounded by thick and smudged yellow halos. As the infection was fast spreading to the healthy leaves, the whole plant seemed in a dried appearance (Plate 5).

Leaf spot (TLRLS)

A survey of anthurium plants grown in a shade house in Thalore (Thrissur district) revealed a kind of leaf spot (TLRLS) infecting most of the plants cultivated in the particular location. The spots appeared grey in colour and was textured like a transparent thin plastic film. This upper layer of infection was followed by a thick papery textured dark grey coloured tissue layer. Initially, the spots were oval in shape and measuring an average size of 0.7 to 1.0 cm which later turned irregular. Spots were limited by thin line of dark brown margins and were surrounded by definite yellow halos. During later stages of disease progression, several spots expanded and coalesced, dried and the central portion of infected tissues fell off making shot holes in the leaves. The underside of the spots was charcoal black coloured with slight yellow halo (Plate 5).

Leaf spot (TBMLS)

The symptoms observed on the leaf spot sample TBMLS collected from Therambam of Thrissur district was distinguished by the appearance of creamy white patches over the infected leaves. The symptom initially visualized as minute irregular spots which then expanded and became oval to irregular shape. The spots mainly formed near the edges of the infected leaves. Small, raised, black fructifications of the pathogen were visible over the surface of the spots. Such lesions were mainly observed on the older and matured leaves than the young leaves. During later stages of infection, shot holes were formed in the infected regions due to the shedding of severe lesions (Plate 5).

Leaf spot (NLBLS)

This leaf spot was obtained during the survey in Nilambur of Malappuram district. NLBLS began the infection in mature and tender leaves as small grey tinged light brownish spots, circular to oval in shape. The spots were spreading to the whole leaf lamina. These spots were limited by thick and dark brown margins overlapped with yellow halos. The infection was seen progressing to the nearby spathe and spadix tissues causing them to rot completely at a later stage. When the infection developed at the marginal ends of the leaves, the symptom exhibiting portions dried and shot holes were

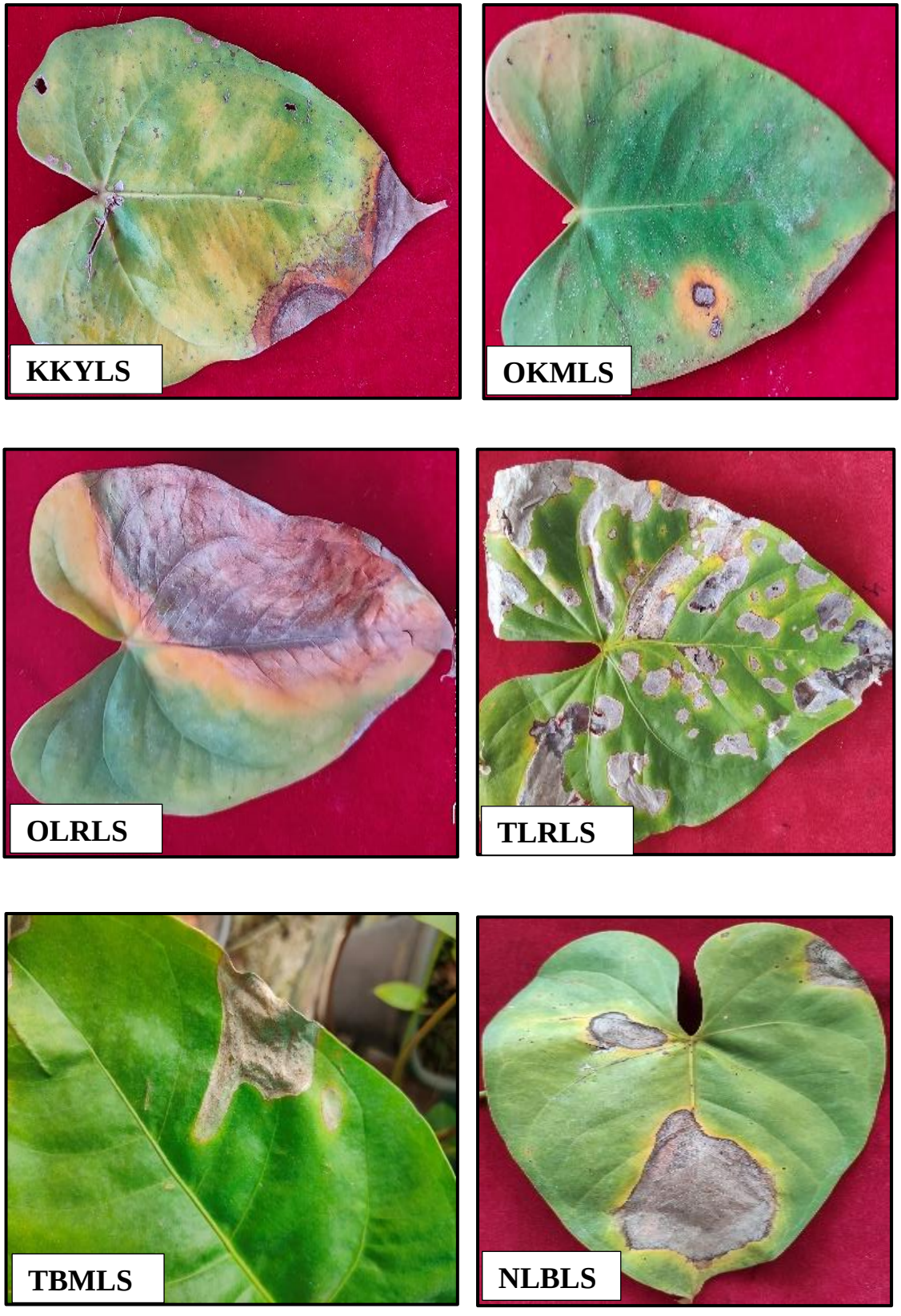


Plate 5: Symptoms under natural conditions – leaf spots

formed. The spadix was easily pulled out of the spathe-petiole junction at a heavily infested stage (Plate 5).

Leaf blight (VFNLB)

The leaf blight sample VFNLB collected from Vellanikkara of Thrissur district showed marginal necrosis as the prominent symptom. The symptomatic leaves were initially characterized by the presence of 'V' shaped necrotic patches developing from the margins of the leaves and gradually progressing towards the midrib regions. The water soaked diseased patches were surrounded by chlorotic halos. When the infection progressed to the petioles, the corresponding tissues showed vascular discolouration. The spread of the disease was from one plant to adjoining plant kept in a row (Plate 6).

Leaf blight (OLKLB)

The leaf blight symptom OLKLB was collected during the survey in Ollukkara of Thrissur district. Initially the infected regions appeared slightly yellow in colour and had a water-soaked appearance. As the infection progressed, a greater number of leaf tissues were killed resulting in the appearance of necrotic areas surrounded by chlorotic halos. These water-soaked spots occurred along the edges of the leaves on upper as well as lower portions and were gradually diffusing towards the center in a 'V' shaped pattern. In most of the infected leaves, the spread of the symptoms was through the veins thus transforming the veins look yellowish and chlorotic. In later stages of infection, the diseased tissues got dried, necrotized and even fell off (Plate 6).

4.4.1.2 Root rot (VCNRR)

Rotting of anthurium roots was observed in young plantlets grown in the shade house of Vellanikkara (Thrissur district). The infected seedlings of anthurium showed yellowing and wilting symptoms on the upper portions. Young leaves were intensely affected than the mature leaves in an infected plant. Initially the green leaves completely turned to yellow followed by wilting and drying. The affected petioles got completely rotten and dried leading to shedding of the wilted leaves. Diseased stem and roots showed water soaking and developed brownish lesions over the surface. Infected stems and petioles became flaccid and got deteriorated. On the roots, dark patches of rotting

and decay were observed and most of the root tissues sloughed off due to excessive rotting (Plate 7).

4.4.1.3 Inflorescence rots

During the sampling survey, four types of inflorescence rots were identified and recorded from three different districts viz. Thrissur, Wayanad and Ernakulum and were catalogued as MNTSR, CKDSR, PNMSR and ALVSR.

Inflorescence rot (MNTSR)

The spadix rot (MNTSR) was collected from Mannuthy of Thrissur. The spadix that bears minute flowers over them was seen severely affected when compared to the spathe surrounding it. The disease appeared at the tips of the spadix, initiating as very small dark brown diamond shaped spots further spreading to the basal tissues as brown lesions covering the whole spadix causing yellowing and dry rot. The infection gradually spread to the spathe and developed dark grey to brown dry lesions surrounded by prominent yellow halo. Fructification of the fungal pathogen associated with the symptom was visible over the surface of lesions on the spathe. At a later stage of infection, diseased portions at the tip of the spadix were detached (Plate 8).

Inflorescence rot (CKDSR)

This type of rot was observed and collected from Chirakkekode of Thrissur district. Due to this kind of infection, the quality and demand of the flower in the cut flower market was badly affected. The infection emerged at the junction of spadix and spathe from where it got gradually spread to the whole spathe, spadix and petioles. Initially, the affected tissues showed a light brownish to dull brick red colour variation. Later, these infected portions became water soaked and rotted such that the spadix got easily pulled off. Unlike MNTSR, in this type of inflorescence rot the infection was progressing from the base of spadix towards the tip. The petioles when affected showed a light brownish discolouration followed by rotting (Plate 8).

Inflorescence rot (PNMSR)

PNMSR was a kind of inflorescence rot obtained from Panamaram of Wayanad district. The symptoms were initially noticed in the spathe of infected cut flowers. Dark

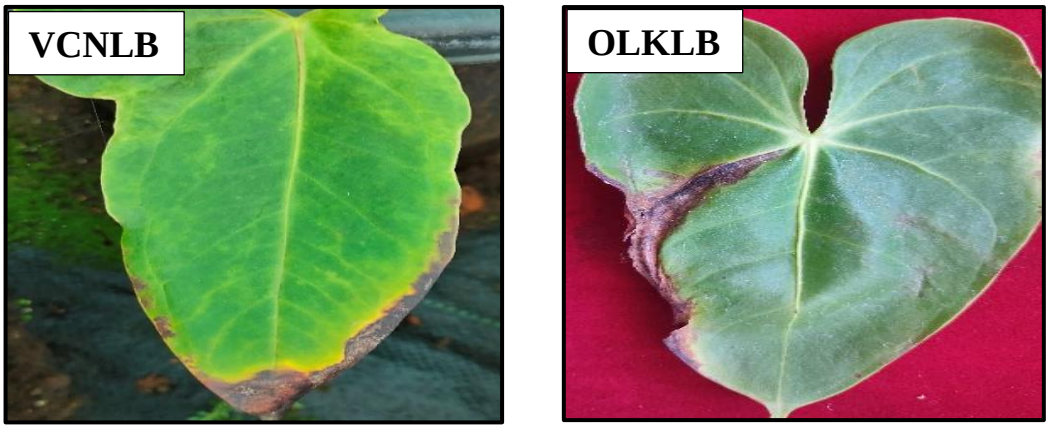


Plate 6: Symptoms under natural conditions – leaf blight

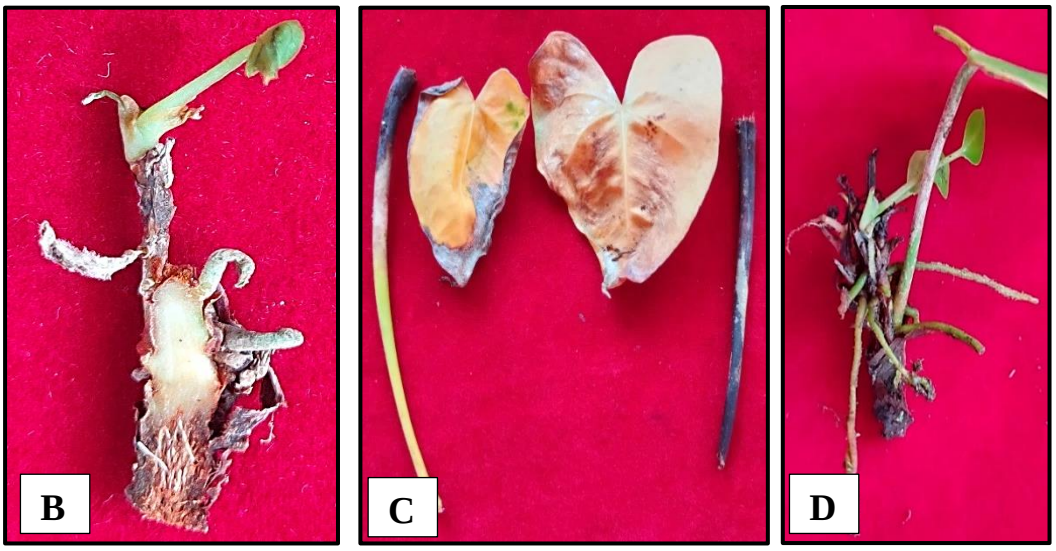


Plate 7: Symptoms under natural conditions – Root rot

A: Yellowing of leaves
B: Vascular discoloration in collar region
C: Wilted leaves and rotted petioles
D: Sloughing off and rotting of root

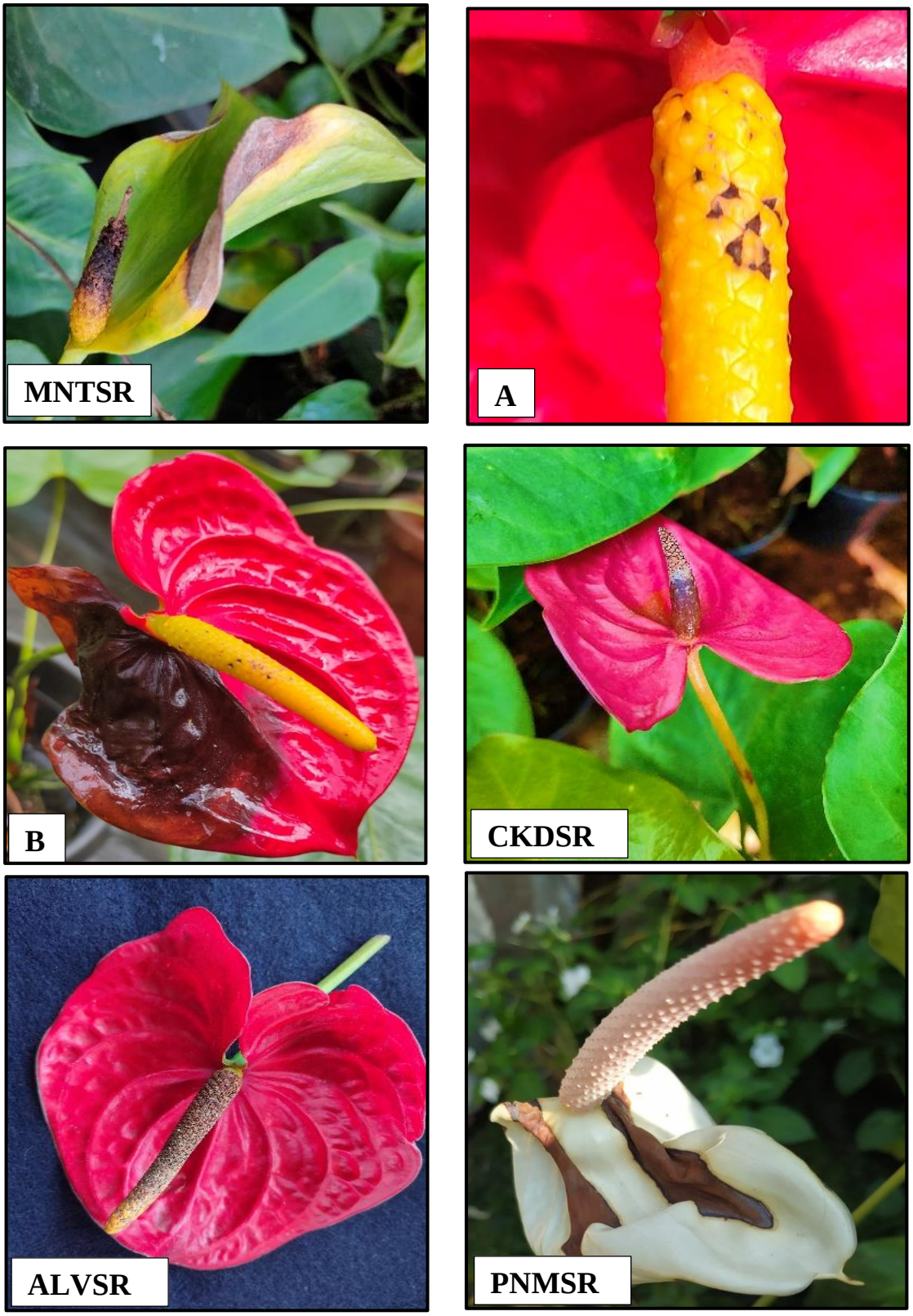


Plate 8: Symptoms under natural conditions-inflorescence rots

Symptoms associated with MNTSR

A: Triangular shaped black necrotic spots on spadix

B: Rotting of spathe tissues

brown, irregular lesions limited by brownish black margins developed on the spathe. Thereafter the lesions extended to wider areas finally covering the whole spathe tissues. In severe cases, the infection spread to the corresponding spadix and caused complete rotting of the flowers affecting its quality. Fruiting bodies of the fungal pathogen were visible on the surface of the flowers (Plate 8).

Inflorescence rot (ALVSR)

The inflorescence rot ALVSR was collected from Aluva of Ernakulum district. This kind of infection mainly affected the spadix and deteriorated the quality of the cut flower. Infection initiated from the very tip of spadix as diamond shaped angular spots that were dark brown to black in colour. A few to small number of spots were formed during initial stage of infection which increased in number and size as the disease progressed. After spreading completely over the spadix, the infection gradually extended to the spathe tissues. This was a kind of dry rot and black coloured fruiting bodies were seen on the surface of infected spathe / spadix (Plate 8).

4.4.1.4 Wilting (MNTLW)

One kind of plant sample showing wilting symptom (MNTLW) was collected from Mannuthy of Thrissur district. The diseased plants were wilted, dried and collapsed. Both the mature as well as young seedlings were showing the symptoms. The leaves and petioles were initially affected showing severe chlorosis. Infected leaves and flowers became hard and turned brittle. Marginal necrosis, yellowing and blighting were prominent in these leaves. Petioles and stems showed vascular discolouration. The affected seedling dried within days and the mature plants wilted completely. The sample showed negative result for ooze test (Plate 9).

4.4.1.5 Virus like symptoms

Symptoms resembling virus infection were noticed in anthurium plants raised in the shade house of Central Nursery in Vellanikkara of Thrissur district. The symptoms collected are described below.

Two to three leaves of a plant exhibited mosaic patterns on the upper surface. Pale yellow to yellow green patches were seen irregularly arranged on the leaves in a

wavy pattern resembling mosaic. This type of symptom appeared on whole leaf lamina. Along with the symptomatic leaves, normal leaves were also present in the plants (Plate 10).

The flowers showed colour alterations when compared to healthy flowers. There were chlorotic patches and spots on the lower as well as upper surfaces of the spathes. The length of the spadix was reduced and the overall size of the flowers were small unlike normal flowers.

The natural flexibility of the leaves was markedly reduced making the virus suspected leaves more brittle and stiffer. Numerous yellow spots were seen on the infected leaves due to the unequal distribution of chlorophyll.

4.4.2 Symptomatology under *in-vitro* conditions

In vitro pathogenicity studies were conducted by Mycelial Bit Inoculation Method (MBIM). The corresponding healthy plant parts from where the pathogens were initially isolated were selected for artificial inoculation. After the transfer of inoculum, the respective samples were incubated for symptom development. The changes that occurred at and around the inoculated sites post-inoculation are detailed below.

4.4.2.1 Foliar diseases

KMALS, PBALS, VCNLS1, VCNLS2, VFNLS, IJKLS, KKYLS, OKMLS, OLRLS, TLRLS, TBMLS, and NLBLS isolates showed characteristic symptoms of leaf spot whereas VFNLB and OLKLB showed leaf blighting on artificial inoculation.

Leaf spot KMALS

The fungal pathogen isolated from the leaf spot (KMALS) which was collected from Kumaranellur (Thrissur district) produced similar symptoms when inoculated on to healthy leaves of anthurium. The initial symptoms appeared within 48 h of inoculation at the site of infection. White mycelial growth of the fungus was seen on the lower surface of the leaf. Water soaked dark brown, circular spots were developed which later extended to form large spots measuring three to four centimetre in diameter. The spots were surrounded by chlorotic halos within one week of inoculation (Plate 11)

Plate 9: Symptoms under natural conditions – Wilt

Plate 10: Symptoms under natural conditions – Virus like symptoms

VCNML-A: Chlorotic patches on leaves

VCNML-B: Mosaic on leaves

Leaf spot PBALS

The pathogen associated with PBALS collected from Perambra (Kozhikode district) when reinoculated on disease free anthurium leaves produced typical spots mostly irregular in shape. Two days after inoculation, dusty white mycelial strands along with yellow spore mass were visible on the upper and lower leaf surface. Progressively, the light brownish lesions extended over the leaf surface mainly through the veins and covered the whole leaf lamina within 13 days post inoculation. Initially a pale chlorotic halo was present around the spot that later disappeared (Plate 11).

Leaf spot VCNLS1

VCNLS1 was the leaf spot collected from Vellanikkara region of Thrissur district. The fungal pathogen causing the disease was isolated and reinoculated on healthy young leaves of anthurium. The infection started within five days of inoculation which initially exhibited a symptom of tissue discolouration at and around the site of pathogen entry. On the following days, brown lesions started developing which were surrounded by prominent chlorotic halo (Plate 11).

Leaf spot VCNLS2

The fungal pathogen associated with the leaf spot VCNLS2 recorded form Vellanikkara (Thrissur district) was inoculated on matured healthy leaves. The symptoms appeared within 3 days of incubation. Minute and irregular brown coloured round shaped spots were formed initially which then coalesced to form big circular spots coloured in purplish brown. The margins of the spots were surrounded by highly prominent yellow halos (Plate 11).

Leaf spot VFNLS

The leaf spot VFNLS, collected from Vellanikkara region (College of Agriculture) of Thrissur district on inoculation to the healthy leaves produced characteristic disease symptoms. The spots were oval to triangular in shape. The centre of the spots was brown in colour followed by a light brownish layer of tissues whereas the margins were showing dark brownish pigmentation. Over the surface of the spots, black fructifications of the pathogen were visible within one week of inoculation. In

this type of infection, yellow halo was absent but the tissues neighbouring the inoculation site turned chlorotic (Plate 11).

Leaf spot IJKLS

The kind of leaf spot sample collected from Irinjalakuda of Thrissur district which was catalogued as IJKLS showed distinguishing symptoms on inoculated leaves. Symptoms were visible within three days after inoculation. Water soaked and irregular shaped lesions developed on the leaves which had the pathogen mycelial growth over the centre of the infected region. The lesions expanded gradually within one week and the infected leaves turned chlorotic along with brown discolouration (Plate 11).

Leaf spot KKYLS

KKYLS was the leaf spot collected from Koorkkenchery of Thrissur district. The pathogen causing the disease was able to infect the healthy tissues by 2nd day post inoculation. The symptoms appeared as small brown coloured spots which later expanded very rapidly to cover most of the leaf lamina. White mycelial tufts of the pathogen were visible at the lesion site (Plate 11).

Leaf spot OKMLS

The fungal pathogen associated with the leaf spot (OKMLS) obtained from Oorakam region of Thrissur district produced symptom on healthy leaves following inoculation within a period of four days. The symptoms appeared as water soaked spots extending irregularly towards all sides. The spots were initially light brown coloured which on maturation turned to thick brown. Mycelial grown was seen over the surface of the spots (Plate 11).

Leaf spot OLRLS

OLRLS, the leaf spot isolate obtained from Ollur of Thrissur district infected the inoculated leaves within four days of inoculum transfer. The symptoms appeared as blackish brown circular spots surrounded on all sides by chlorotic halos. Later the spots expanded in a cylindrical fashion longitudinally and the spots were then margined with thick and bright yellow halo. Water soaking of the spots gradually reduced and the

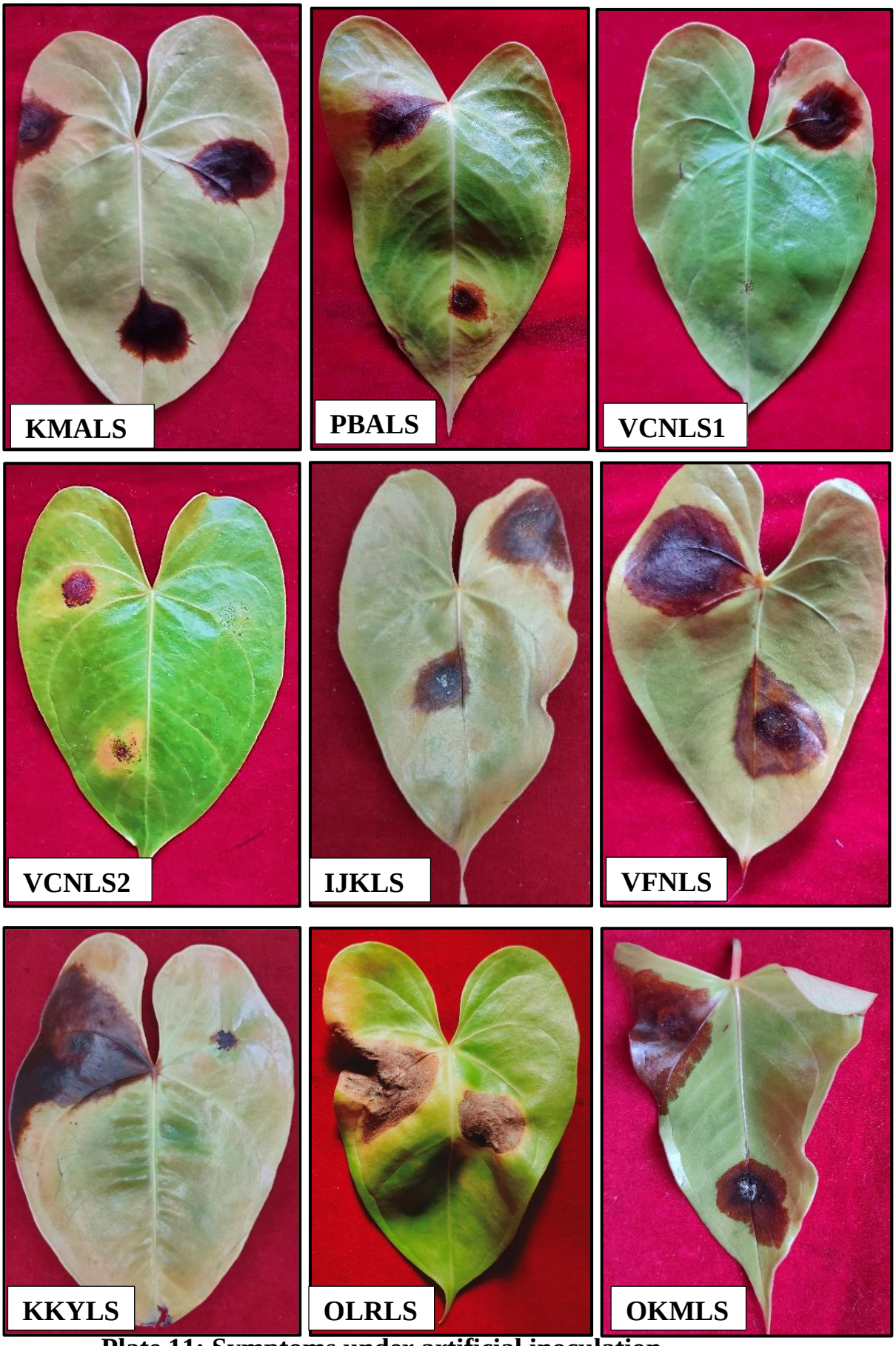


Plate 11: Symptoms under artificial inoculation

lesions got dried by 12th day of inoculation. The yellow halo was still prominent around the dried lesions which extended towards the margins of the infected leaves (Plate 11).

Leaf spot TLRLS

TLRLS was the leaf spot isolate collected during the survey in Thalore region of Thrissur district. Actively growing pathogen isolate was inoculated on healthy leaves of anthurium. The pathogen produced symptoms within three days of inoculation. The infection started as slight brownish discolouration which later expanded irregularly and pigmented in dark brown. During initial stages, a faint chlorotic halo was present which gradually turned to bright and thick yellow halo (Plate 12).

Leaf spot TBMLS

The pathogen causing leaf spot disease (TBMLS) was inoculated on young and disease-free leaves of anthurium. The infection appeared on second day after inoculation. Water soaking was prominent around the spots. Faint brown coloured spots were initially round in shape which then expanded to form bigger sized spots surrounded by light chlorotic halo. Black coloured fruiting bodies of the pathogen were visible on the spots. Mostly, the spots expanded through midribs and veins to cover the whole leaf surface (Plate 12).

Leaf spot NLBLS

NLBLS was the leaf spot sample collected from a homestead in Nilambur region of Malappuram district. The fungal pathogen associated with the collected symptom infected on healthy leaves within two days of inoculation. Dark brown to black spots, oval to irregular in shape developed and expanded throughout the leaf lamina in a very rapid rate. Yellow halo was absent around the spots. The infected tissues were water soaked and caused complete rotting of the infected leaf tissues. Brownish spots were also seen scattered around the major spots in an irregular pattern (Plate 12).

4.4.2.2 Inflorescence rots

Inflorescence rot MNTSR

MNTSR was the inflorescence rot isolate collected from Mannuthy of Thrissur district. The fungal pathogen isolated from the sample was artificially inoculated on to the tip and base of the spadix and on the spathe. The symptoms started appearing within 48 h of inoculation. The infected tissues showed brownish discolouration and dry rot was observed at the tip of the spadix which resulted in the detachment of the tip portion (Plate 12).

Inflorescence rot CKDSR

The fungal pathogen associated with CKDSR obtained from Chirakkekode of Thrissur district was inoculated on healthy spadix tissues. The incubation period of the pathogen was three days. Initially the upper tip of the spadix appeared brownish in colour. Gradually, the discolouration spread to the base of the spadix. White mycelial growth of the fungal pathogen was visible over the spadix (Plate 12).

Inflorescence rot PNMSR

The pathogen associated with the inflorescence rot PNMSR gathered from Panamaram of Wayanad district was inoculated artificially on healthy spathe. The pathogen initiated the infection within two days. The water soaked lesions formed initially later turned to dark brown necrotic patches and progressively covered the whole spathe surface. Fungal mycelia were seen on the surface of infected lesions (Plate 12).

Inflorescence rot ALVSR

ALVSR, the spadix rot isolate collected from Aluva region of Ernakulum district showed similar symptoms when artificially inoculated on healthy spathe and spadix. The pathogen started infection within two days of inoculation. The spots showed characteristic brown water soaked lesions that gradually expanded towards the middle and covered the entire spathe tissues. The infected tips of the spadix appeared necrotic and lesions spread to the petioles that later became rotted. White mycelial growth was visible on the surface of infected spathe and spadix (plate 12).

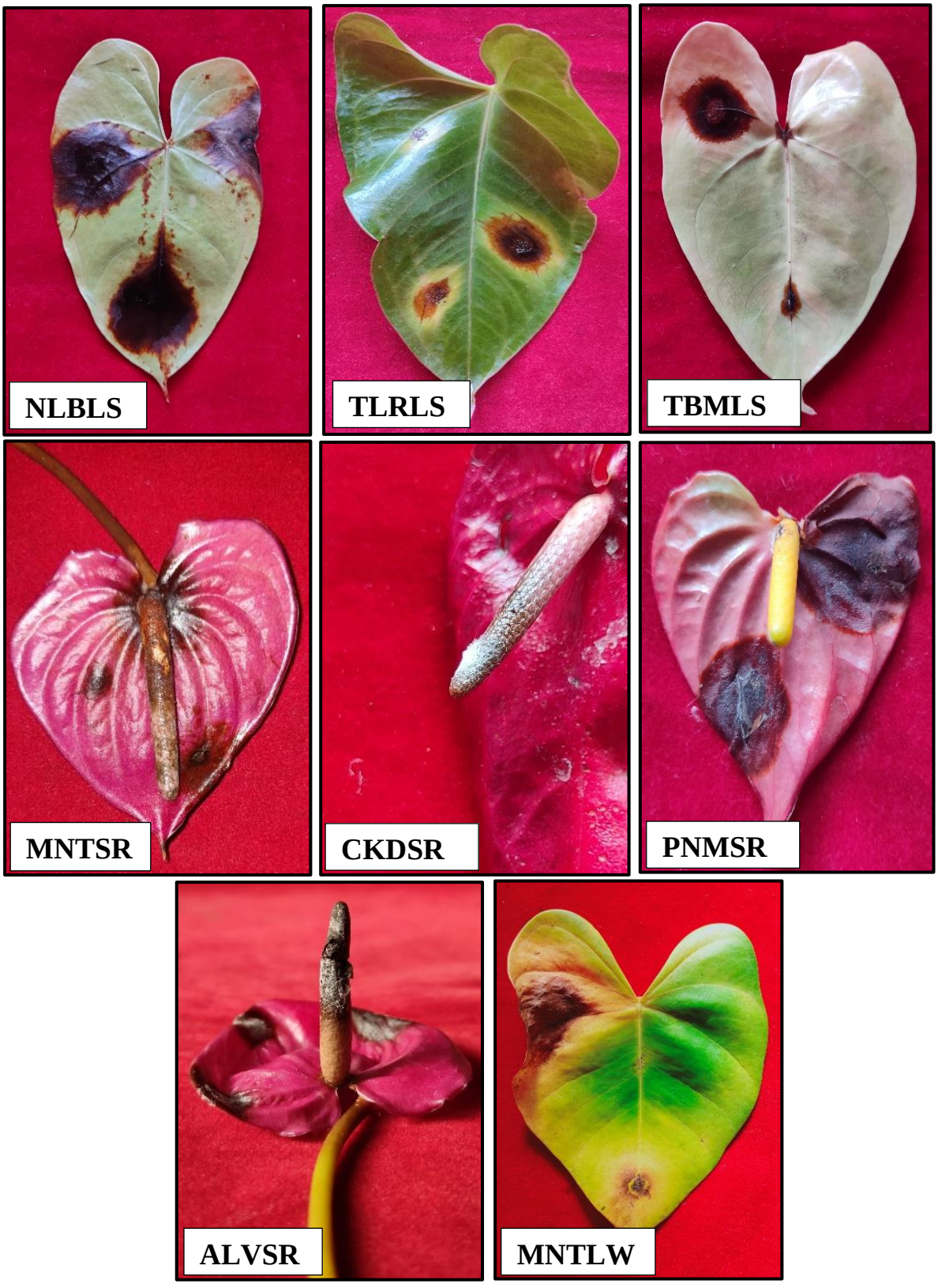


Plate 12: Symptoms under artificial inoculation

4.4.2.3 Wilting and drying of leaves

The pathogen associated with the wilting and drying of leaves (MNTLW) collected from Mannuthy (Thrissur district) was artificially inoculated on to the healthy leaf of anthurium. The initial symptoms appeared within four days of inoculation. The pathogen caused severe yellowing of the inoculated leaf followed by the development of dry and necrotic patches. The dry lesions enlarged and covered the entire leaf lamina. By the time, the whole leaf got dried and became chlorotic with prominent yellowing over the surface. The leaf veins also exhibited yellowing symptoms. At a later stage, the leaf appeared wilted and blighted severely (Plate 12).

Leaf blight VFNLB

The leaf blight pathogen isolated from the diseased sample VFNLB collected from Vellanikkara (Thrissur district) was artificially injected to the veins of the healthy leaves of anthurium following the protocol explained in section 3.3.2. When inoculated to the veins of detached leaf, it took eight days for the pathogen to start the infection. The incubation period recorded was much higher than those of the fungal pathogens. Symptoms appeared as prominent water-soaked lesions progressing through the major veins of the inoculated leaf. Light to dark brown lesions spread completely over the leaf lamina and finally the infected leaf rotted off. The leaf tissues surrounding the lesions were chlorotic. Marginal yellowing of the infected leaf tissue was another symptom observed. As the infection spread to the petiolar region, cracking of petioles and rotting was observed along with vascular discolouration (Plate 13).

The pathogen suspension when inoculated to the leaves of a live plant, symptoms started appearing on 13th day of inoculation. Initially, yellowing was observed around the inoculated vein. Later, brown-coloured patches appeared along with severe yellowing. By the 23rd day of inoculation, complete blighting of the infected leaf was observed. During later stages of infection, the diseased leaf got dried and detached from the plant as the infection spread to the petiole (Plate 13).

Leaf blight OLKLB

The bacterial pathogen associated with leaf blight isolate OLKLB was artificially injected to the veins of a detached healthy leaf. After an incubation period

of nine days, the symptoms were expressed around the margins of the leaves as small water soaked brown lesions surrounded by faint yellow halo. As the lesion progressed towards the midrib, it became brightly coloured and the yellow halo seemed more prominent. Those necrotic patches that are developed from the leaf margins extended in a 'V' shaped pattern whereas those that initiated infection from the petiolar end spread through the veins (Plate 13).

Severe leaf blighting was observed in the inoculated live plants. Infection progressed after an incubation period of 15 days. The water-soaked light brownish lesions expanded towards the midrib and was surrounded by deep yellow halo. Thereafter, the infected leaf showed yellowing followed by blighting in a severe form. The diseased petioles showed vascular discolouration. Finally, the blighted leaf got detached from the plant after a time period of 45 days (Plate 13).

4.4.2.4 Root rot

The root rot pathogen isolated from the sample VCNRR collected during the survey in Vellanikkara (Central Nursery, KAU) of Thrissur district, initiated infection within two days of inoculation on healthy detached leaves. The symptoms appeared as small, oval shaped water-soaked spots which were light brown in colour. Later the spots expanded throughout the leaf lamina very rapidly such that the whole leaf got chlorotic followed by rotting within 10 days of inoculation. Yellow halo was absent around the lesions. The spots were surrounded by faint chlorotic halos (Plate 14).

The pathogenicity of the fungal isolate was studied in live plant by inoculating healthy anthurium plants with hyphal suspensions. Two week old fungal mycelial mats were collected from actively growing cultures of the pathogen in PDA and correspondingly hyphal suspensions were prepared. To the collar region of healthy plants, pathogen was inoculated by spraying followed by drenching the soil with fungal suspension. Within three weeks of inoculation, rotting of the collar region, roots, petioles and leaves were noticed which resulted in complete yellowing and blighting of certain leaves severely infected by the pathogen. The roots of corresponding plants showed sloughing off symptoms as seen in the naturally infected plants. The petioles of infected leaves and stems showed vascular discolouration (Plate 14)

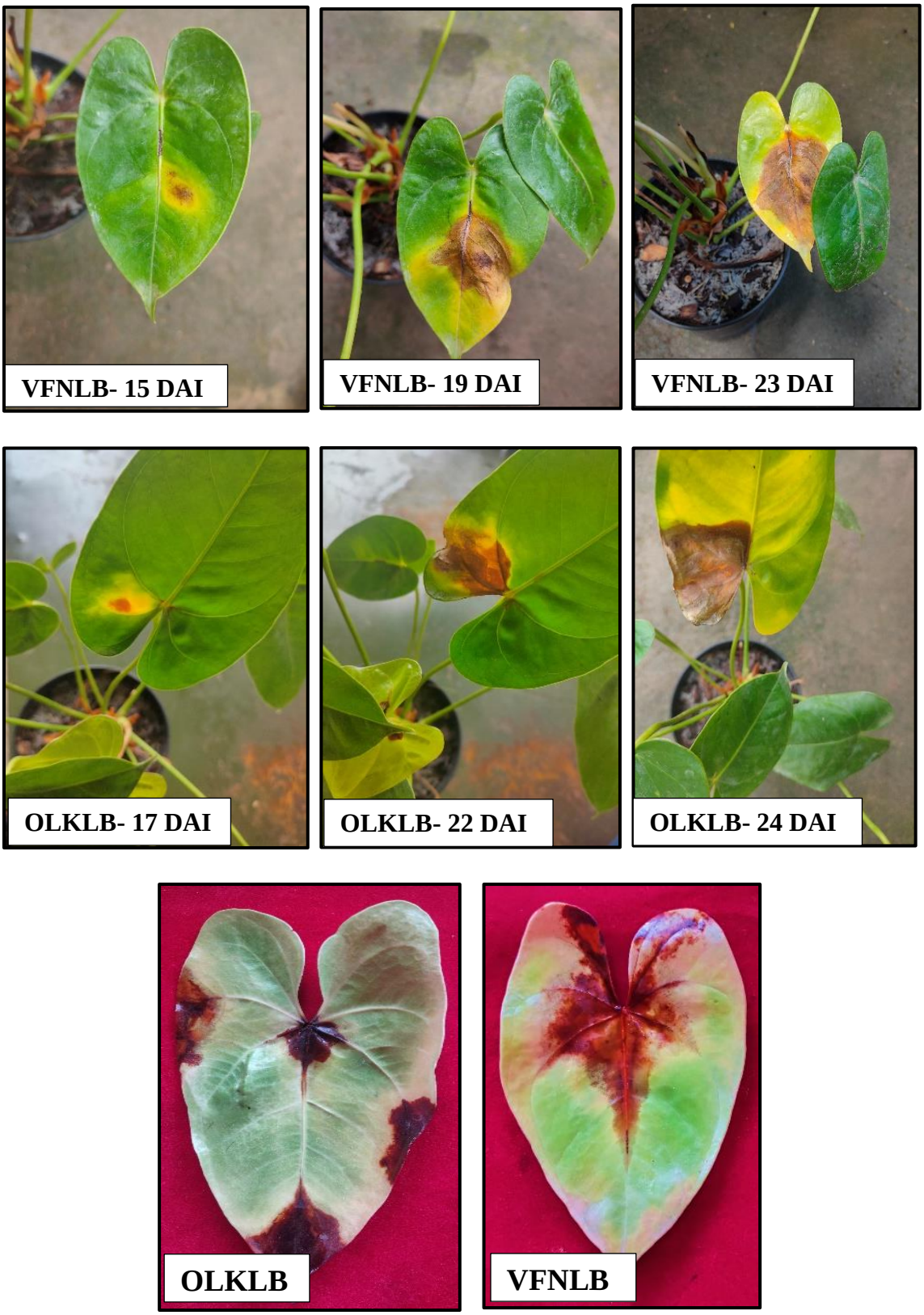


Plate 13: *In vitro* pathogenicity of leaf blight pathogens on live plants and detached leaves

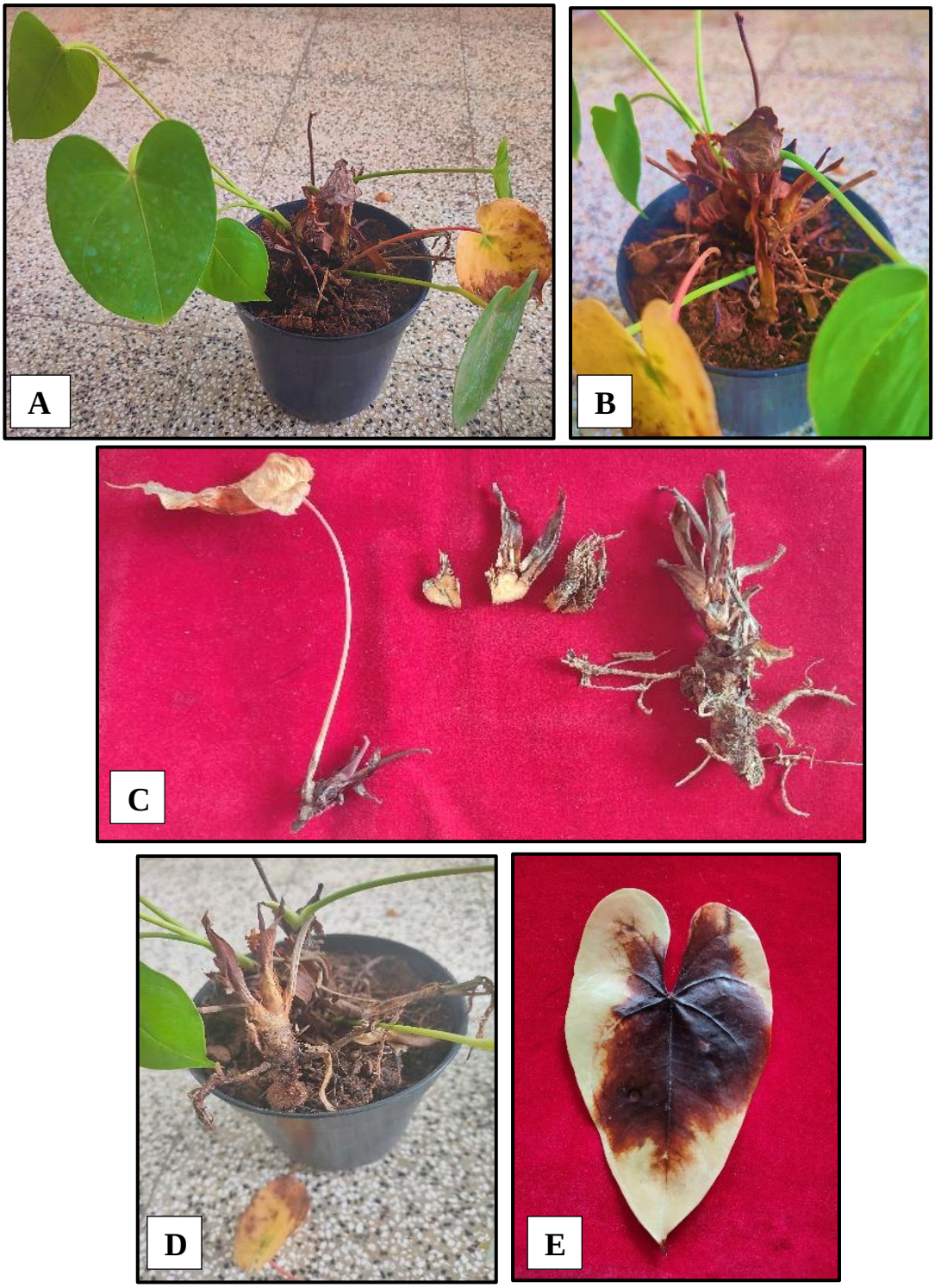


Plate 14: Symptoms under artificial inoculation (VCNRR)

A: Wilting of inoculated plant
B: Drying of collar region
C: Vascular discolouration of petiole, stem and root
D: Rotting of stem and root
E: Symptoms of artificial inoculation on detached leaf

4.5 CHARACTERIZATION OF ISOLATED PATHOGENS

The kind of pathogen causing disease symptoms like leaf spots, wilting, root rot, and spadix rots was found to be fungus and those which caused leaf blighting was identified as bacterial pathogen based on the isolations and *in vitro* pathogenicity studies conducted.

4.5.1 Characterization of fungal pathogens

Fungal pathogens isolated and purified from the associated symptoms were studied further based on the cultural and morphological characters. Among the diseased samples collected, the isolates KMALS, PBALS, VCNRR, VCNLS1, VCNLS2, MNTSR, MNTLW, VFNLS, IJKLS, KKYLS, OKMLS, OLRLS, TLRLS, TBMLS, NLBLS, PNMSR, ALVSR and CKDSR were fungal pathogens. The cultural and morphological characterization of the isolates were done for further identification.

4.5.1.1 Cultural and morphological characters of fungal isolates

The cultural characters of the fungus studied were the colour, texture, growth rate, growth pattern, fructifications, sporulation and pigmentation on the reverse side of the Petri plates. Thereafter, the morphological characters like colour, branching pattern of hyphae, and hyphal and conidial septations, colour, shape and dimensions of spores and presence of fruiting bodies (Table 4.5 and 4.6).

PATHOGENS ASSOCIATED WITH LEAF SPOT SYMPTOMS

Twelve pathogens causing leaf spot were isolated from the diseased samples collected during purposive sampling survey conducted in six districts. The isolates are catalogued as KMALS, PBALS, VCNLS1, VCNLS2, VFNLS, IJKLS, KKYLS, OKMLS, OLRLS, TLRLS, TBMLS and NLBLS.

Pathogen associated with leaf spot KMALS

KMALS was collected from a homestead at Kumaranellur (Palakkad district). After two days of incubation, white and cottony textured fungal mycelia were seen arising from the diseased bits kept for isolation on PDA plates. The fungus produced white coloured aerial mycelia which was later tinged with very light grey (Plate 15).

Dark greyish green pigmentation with dull white mycelia were seen scattered in the culture which was visible at the reverse side of the plate. Dark orange coloured spore mass was found on the tuft of aerial mycelia. A complete growth of the pathogen was observed in the Petri plate within seven days of incubation at room temperature. The recorded growth rate was 1.5 cm per day (Table 4.5).

Slide culture technique was adopted for studying the morphological characters of the isolate followed by making microscopic observations. The fungal hyphae were hyaline and septate. Bullet shaped, hyaline and one celled conidia with an oil globule at the centre were observed. The average dimensions of the conidia were 14.11 μm (length) x 4.26 μm (breadth) (Plate 21).

On the basis of the above mentioned characters, KMALS associated pathogen was identified as *Colletotrichum gloeosporioides.*

Pathogen associated with leaf spot PBALS

The fungal pathogen isolated from the leaf spot sample PBALS collected from Perambra of Kozhikode district was subcultured on PDA in sterilised Petri plates. The results of the study revealed that the pathogen produced white, fluffy aerial mycelia with dark grey pigmentations found diffusing through the growth media on the reverse side of the plate (Plate 15). The fungus produced salmon orange coloured conidial mass at the centre and margins of the plate. The fungal mycelia covered the whole surface of the Petri plate within seven days and the growth rate was 1.5 cm per day (Table 4.5).

The pathogen produced single celled and hyaline conidia across the colony. The shape of the conidia was cylindrical with obtuse ends, and was straight with no septations. At the centre of the conidia, oil globules were prominently seen. The average size of the conidia was 19.24 μm (length) x 4.11 μm (breadth) (Plate 21).

Based on the fungal colony and morphological attributes, the pathogen causing the leaf spot disease PBALS was identified as *Colletotrichum gloeosporioides.*

Pathogen associated with leaf spot VCNLS1

On isolation the pathogen associated with the leaf spot VCNLS1 produced white to dark brown coloured floccose aerial mycelia on PDA within three days of incubation. The thick and brown tinged fungal growth later turned to olivaceous green blended with dark brown which showed a dusty appearance in the growth media (Plate 15). The pathogen completed full growth in the Petri plate of nine centimetre diameter within five days with a growth rate of 1.8 cm per day (Table 4.5). The lower side of the PDA plate showed deep brown pigmentation.

The microscopic observation on the morphology of fungus showed that the hyphae was brown coloured, septate and branched. The conidia were unicellular, hyaline and ellipsoid in shape. Brown coloured, bulged and round shaped chlamydospores were seen arranged in chains. The average dimensions of the conidia were recorded as 6.10 μm (length) x 2.55 μm (breadth) (Plate 21).

Based on the detailed cultural and morphological characters, the pathogen responsible for the infection causing leaf spot VCNLS1 was identified at the genus level as *Phoma* sp.

Pathogen associated with leaf spot VCNLS2

The pathogen isolated from the leaf spot sample VCNLS2 observed from Vellanikkara of Thrissur district was grown and studied on PDA plates which showed uniform mycelial growth in all directions. Initially, the fungal colonies appeared in faint concentric growth rings which were covered abundantly with thick aerial mycelia. The upper surface of the culture showed white and dark green blended effused mycelial growth which later exhibited tinges of brown within. The thick hairy growth of the fungus was gradually progressing in round shape. The lower surface of the Petri plates showed bright green pigmentations. The pathogen completed the growth in the PDA plate within eight days and the observed growth rate was 1.125 cm/day (Plate 15).

The morphological study on the pathogen isolate revealed that the hyphae were pale brown coloured and the conidia produced were showing high degree of variation in morphology as well as dimensions. The conidial shapes varied from obclavate to cylindrical with some of them appearing straight and others were slightly curved. The

brown coloured conidia possessed 3 to 14 pseudoseptations. Variations were evident in the conidial dimensions also and the average length recorded was 83.82 μm and average width was 9.30 μm (Plate 21).

The genus level identification of pathogen was done based on characters detailed and it was identified as *Corynespora* sp.

Pathogen associated with leaf spot VFNLS

The pathogen causing leaf spot symptom VFNLS collected from Vellanikkara of Thrissur district produced greyish white floccose aerial mycelia which later turned to dark brownish green in colour (Plate 15). The fungus completed growth inside the Petri plate within a time period of seven days and the growth rate was observed as 1.28 cm per day (Table 4.5).

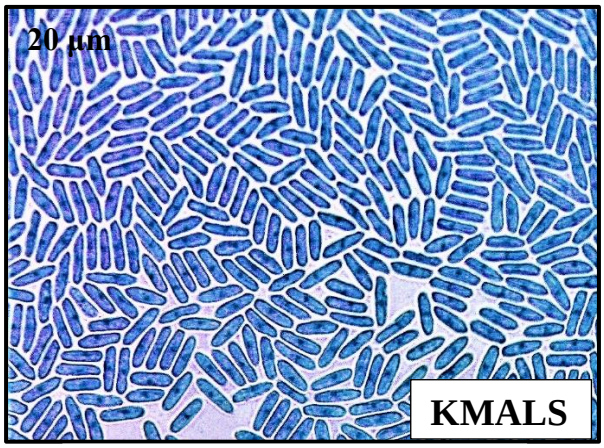
The hyphae were hyaline, which later turned to brown and was septate. The conidia were one celled, hyaline and cylindrical with round ends. The average length of conidia was 17.34 μm and breadth was 3.81 μm. Dark brown to black coloured setae were seen on the culture which developed from brown coloured acervuli. The average length of setae was 59.23 μm (Plate 21).

Based on the cultural and morphological characters observed, the isolate was identified as *Colletotrichum gloeosporioides*.

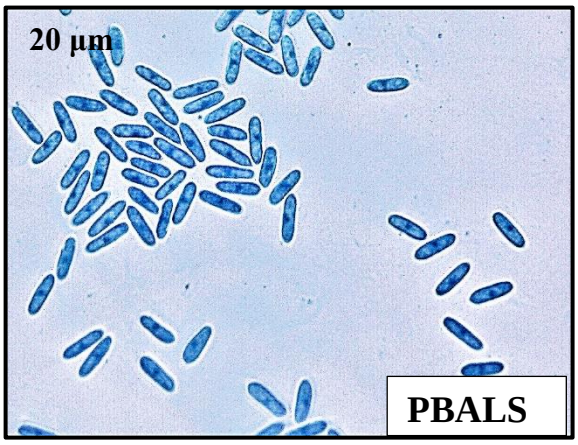
Pathogen associated with leaf spot IJKLS

The leaf spot pathogen found associated with IJKLS collected from Irinjalakuda of Thrissur district was isolated and cultured on PDA. The fungus produced creamy white, slightly compacted, thick and shrunken mycelia. The reverse side of the PDA plate appeared in dull white tinged in pale tan colour. The growth of the fungal pathogen was completed in the Petri plate (9 cm diameter) within six days and the recorded growth rate was 1.5 cm per day (Table 4.5). After 12 days of incubation at room temperature, yellowish creamy liquid (conidial exudation from the ostioles) was found oozing from the mycelial tufts (Plate 15).

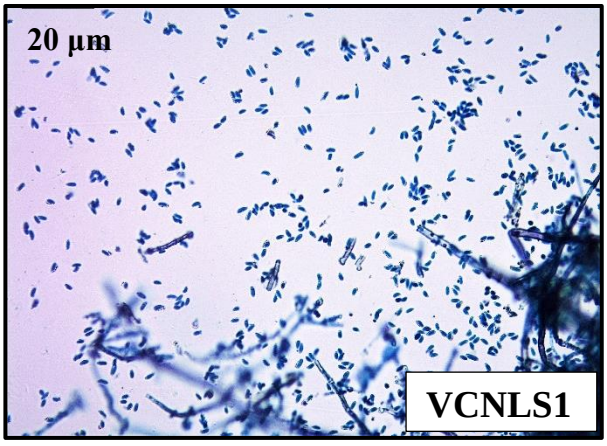
The microscopic observations of the isolate revealed that the fungal colonies produced alpha conidia that are hyaline, aseptate, elliptical and guttulate. In some of the



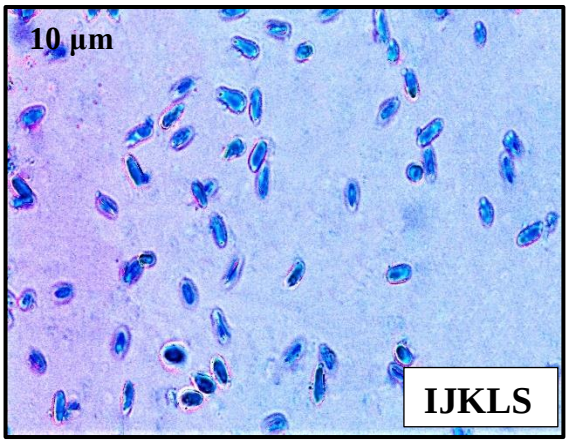
Colletotrichum gloeosporioides



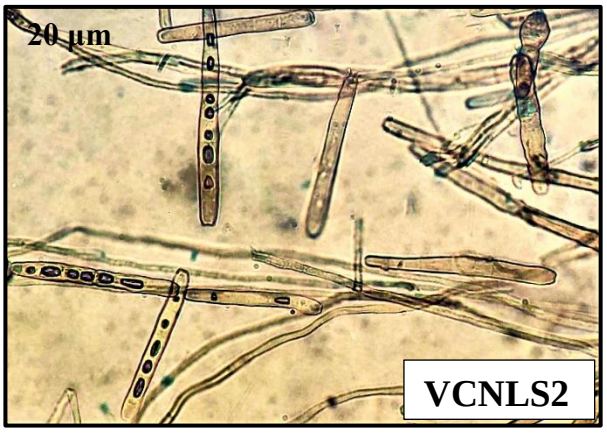
Colletotrichum gloeosporioides



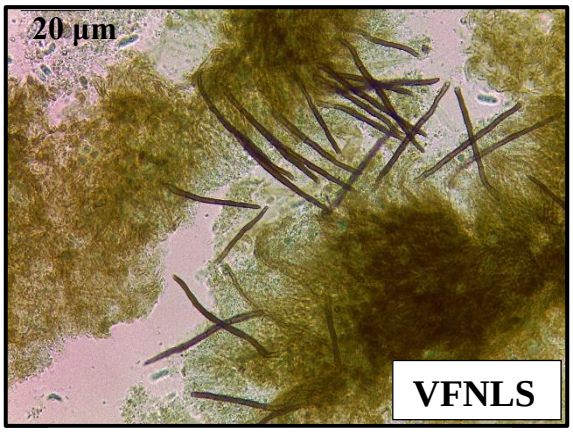
***Phoma* sp.**



***Phomopsis* sp.**



***Corynespora* sp.**

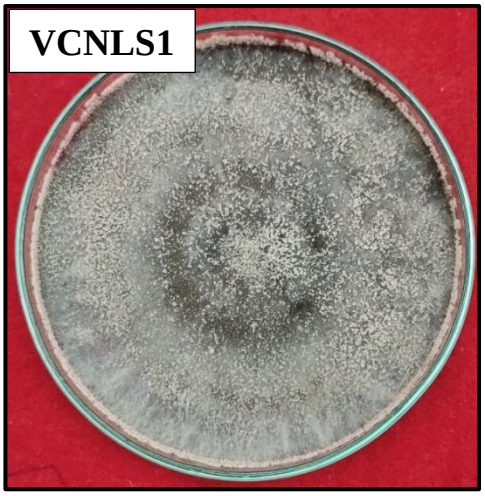


Colletotrichum gloeosporioides

Plate 21: Morphological characters of leaf spot isolates

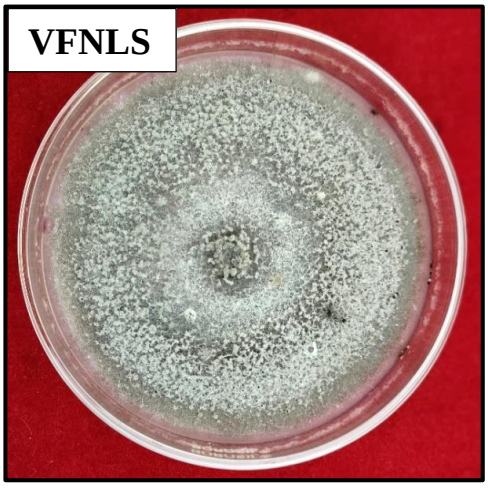
Colletotrichum gloeosporioides

Colletotrichum gloeosporioides



***Phoma* sp.**

***Corynespora* sp.**



Colletotrichum gloeosporioides

***Phomopsis* sp.**

Plate 15: Cultural characters of leaf spot isolates

spores, one end was slightly obtuse and other end acute and measured an average length of 5.9 μm and breadth of 2.3 μm (Plate 21).

The cultural and morphological characters observed supported the identification of pathogen causing the leaf spot IJKLS as *Phomopsis* sp. at the genus level.

Pathogen associated with the leaf spot KKYLS

The cultural characters of the fungal pathogen associated with the leaf spot KKYLS collected from a homestead in Koorkkenchery of Thrissur district was studied by culturing the fungus on PDA plates. The initial growth was observed within three days as white cottony fungal mycelia and it expanded to cover the Petri plates completely in five days. The growth rate of the fungal isolate was 1.8cm per day (Table 4.5). The white aerial mycelia later turned to creamy white tinged with dark brown. The reverse side of the plate was seen brownish and dark brown coloured pycnidia was found scattered. These brightly coloured pycnidial bodies were partially embedded in the media with the black coloured necks protruding out from the surface. Yellowish creamy conidial drops were found oozing from the ostiole (Plate 16).

The morphological observations taken with a microscope revealed that the pathogen produced both alpha conidia and beta conidia. The hyphae were hyaline, branched and septate. The beta conidia (walking stick shaped) were hyaline, filiform and curved with no septations. The average length of the beta conidia was 18.43 μm and average width was 1.06 μm. Occasionally alpha conidia was produced and were less in number compared to beta conidia. The hyaline, aseptate and biguttulate alpha conidia were elliptical to sub cylindrical in shape. The average size of alpha conidia was recorded as 6.3 μm (lenth) x 3.5 μm (breadth) (Plate 22).

The pathogen was identified as *Phomopsis* sp. based on the observed cultural and morphological characters.

Pathogen associated with the leaf spot OKMLS

The pathogen causing leaf spot OKMLS was isolated on PDA medium and produced white coloured woolly mycelia abundantly which later turned to brown. After six days, a complete growth of the fungus was seen over the surface of the Petri plate

(9 cm in diameter) with a growth rate of 1.6 cm per day (Table 4.5). Two to three days after full growth, the mycelia turned brownish concentrating more on the reverse side of the culture and black coloured pycnidial bodies were seen scattered in the media (Plate 16).

The morphological characters of the fungus showed that the isolate produced hyaline hyphae with septations and the alpha conidia developed were hyaline and elliptical with round ends. The average dimensions of the conidia were 6.3 µm (length) x 3.5 µm (breadth) while the beta conidia measured an average length of 18.43 µm and average width of 1.06 µm (Plate 22).

Based on the observed cultural and morphological characters, the pathogen was identified as *Phomopsis* sp. at the genus level.

Pathogen associated with leaf spot OLRLS

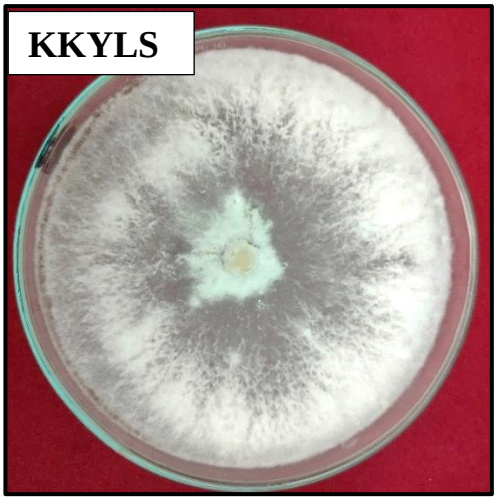
This leaf spot causing pathogen collected from Ollur of Thrissur district was isolated on PDA. On isolation, the fungus produced thick, raised and dense dark grey to green mycelia which later showed brown pigmentation. The fungal culture produced dark greenish brown colour when viewed through the lower surface of the plate. The growth rate of the fungus was 1.0 cm per day which grew over the PDA plate of diameter 9 cm within nine days of incubation (Table 4.5). The young olivaceous green coloured effused mycelia appeared velvety and thick hairy when matured (Plate 16).

Under microscope, fungal conidia of varying size were found solitary and in chains of three to five. Pale brown to sub hyaline pigmented conidia were variously shaped *viz.* cylindrical to obclavate. The straight to slightly curved conidia showed pseudoseptations and measured an average length of 74.53 µm x 8.32 µm. The hyphae were brown coloured, septate and branched (Plate 22).

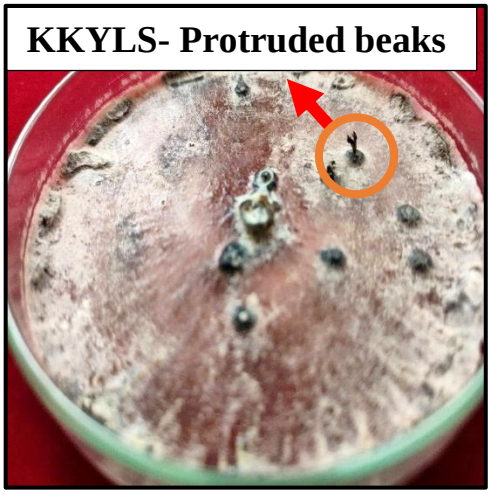
The pathogen was identified as *Corynespora* sp. on the basis of the cultural and morphological characters observed.

Pathogen associated with leaf spot TBMLS

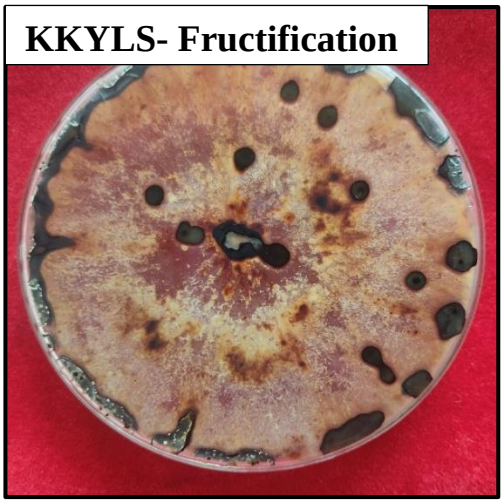
The fungal pathogen associated with the leaf spot TBMLS obtained from Therambam of Thrissur district produced olivaceous green tinged whitish aerial mycelia



***Phomopsis* sp.**

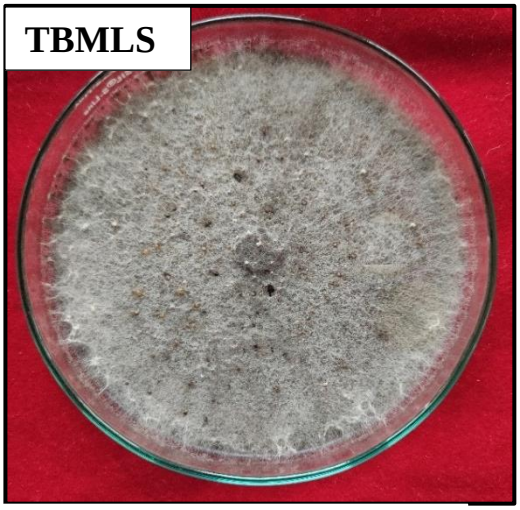


***Phomopsis* sp.**

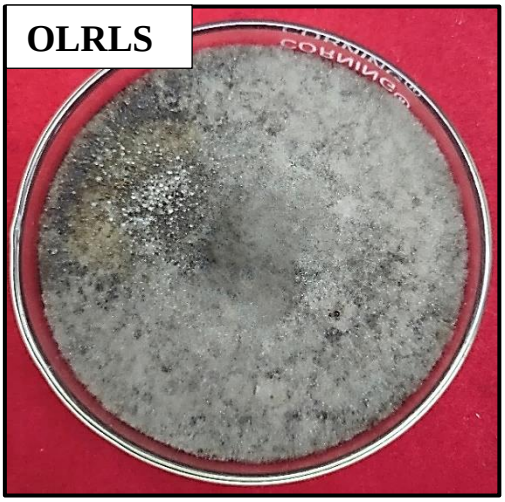


***Phomopsis* sp.**

***Phomopsis* sp.**



Colletotrichum gloeosporioides



***Corynespora* sp.**

Plate 16: Cultural characters of leaf spot isolates

initially which later turned to dark grey. The reverse side of the media was pigmented with dark greyish green. The pathogen grew completely over the PDA surface within six days of incubation with a growth rate of 1.5 cm per day (Table 4.5). Yellow coloured spore mass and black coloured acervuli were evidently seen on the mycelial surface after 10 days of complete growth (Plate 16).

The pathogen was morphologically characterised under light microscope which showed the presence of hyaline, one celled conidia which were bullet shaped with round ends and contained oil globules at the centre. Dark brown coloured acervuli were present with an average length of 146.8µm typically with black coloured unbranched setae having an average length of 52.27 µm. The dimensions of conidia range from 12 to 18 µm in length (average 15.28 µm) and 4 to 5 µm in breadth (average 4.01 µm) (Plate 22).

Based on the detailed cultural and morphological characteristics, the pathogen was identified as *Colletotrichum gloeosporioides*.

Pathogen associated with the leaf spot TLRLS

The isolation of the pathogen infecting anthurium and causing leaf spot TLRLS was done in PDA and that yielded fungus with white cottony growth and undulating margins. Concentric zonations developed in the culture that later turned to dark brown in colour. The reverse side of the Petri plate was tinged in light cream or slightly buff coloured. After ten days of incubation at room temperature, black coloured conidiomata developed and found scattered in the immersed mycelia (Plate 17). The fungus covered the entire PDA plate surface within nine days and showed a growth rate of 1 cm per day (Table 4.5).

Th fungus developed characteristic five celled, fusiform conidia with four to five septations. The middle three cells were brown coloured whereas the outer cells were hyaline. The appendages on the apical cells were two to four in number and the average length was recorded as 15.23 µm. Only one appendage was noticed in the basal cell measuring an average of 4.5µm length. The average dimensions of the conidia were recorded as 28.30 µm (length) x 6.86 µm (breadth) (Plate 22). The fungus produced hyaline hyphae.

Based on the characteristics observed, the pathogen was identified as *Pestalotiopsis* sp.

Pathogen associated with leaf spot NLBLS

The pathogen isolated from the leaf spot sample NLBLS collected from Nilambur of Malappuram district grew vigorously over the surface of PDA. Dull grey coloured straight and fluffy aerial mycelia covered the entire surface of PDA plate within three days of incubation. Later thick mycelial growth was observed which changed to black colour within 14 days of incubation. After four weeks of incubation, black coloured pycnidia developed in the culture which initially appeared soft and later turned hard. From the tip of these raised structures, black and shiny liquid oozed out giving a tarry appearance. Later, spores were released from these structures resembling black dust (Plate 17). The recorded growth rate of the fungal pathogen was five centimetre per day (Table 4.5).

The pathogen produced brown coloured and septate hyphae with numerous mature and immature conidia. When young, the conidia shaped oval and was hyaline, aseptate and possessed thick cell wall with no striations. Matured conidia were brown coloured, oval shaped, and septate that gave two equal portions of conidia. Prominent longitudinal striations were visible along the matured conidia. The average size of conidia was recorded as 26.40 μm (length) x 14.70 μm (breadth) (Plate 22).

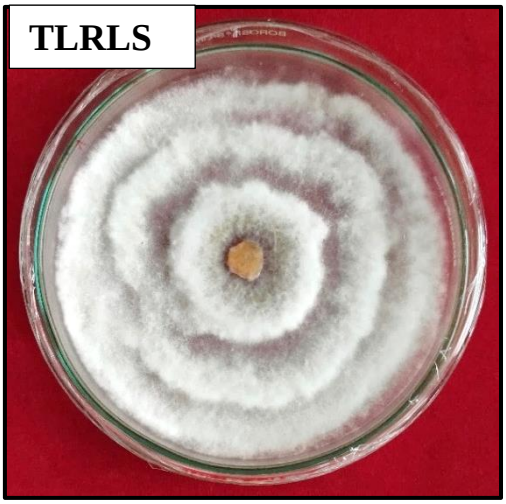
Based on the cultural and morphological characters, the pathogen was identified as *Lasiodiplodia theobromae.*

PATHOGEN ASSOCIATED WITH ROOT ROT SYMPTOM

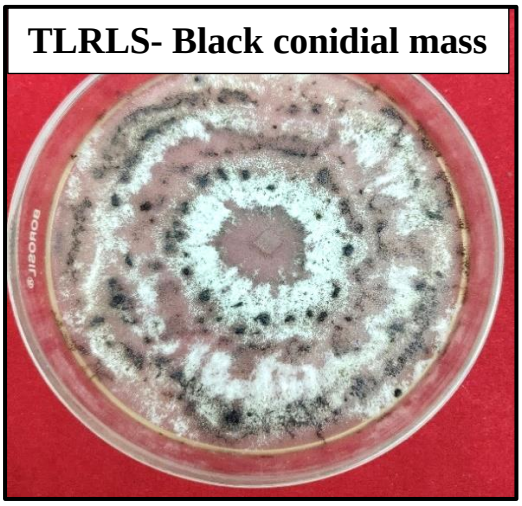
One fungal pathogen was isolated from the sample VCNRR showing symptoms such as root rot, petiole discolouration, leaf yellowing and drying. The diseased samples were collected from Vellanikkara region of Thrissur district.

Pathogen associated with VCNRR

The fungal pathogen causing root rot symptom in anthurium plants was isolated on PDA. From the infected tissue bits, white coloured blooming aerial mycelia developed resembling floral patterns and covered the entire PDA plate (9 cm diameter)



Pestalotiopsis **sp.**



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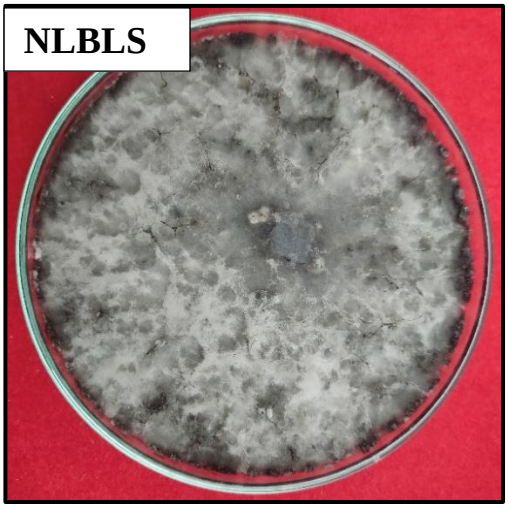
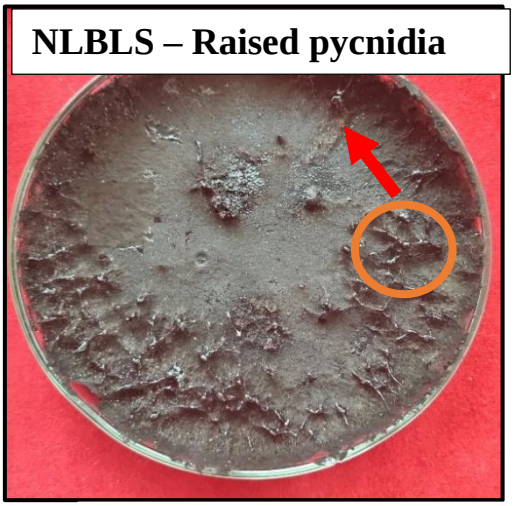


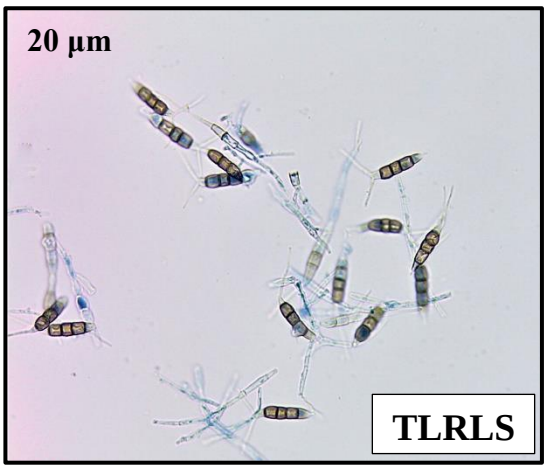
Lasiodiplodia theobromae



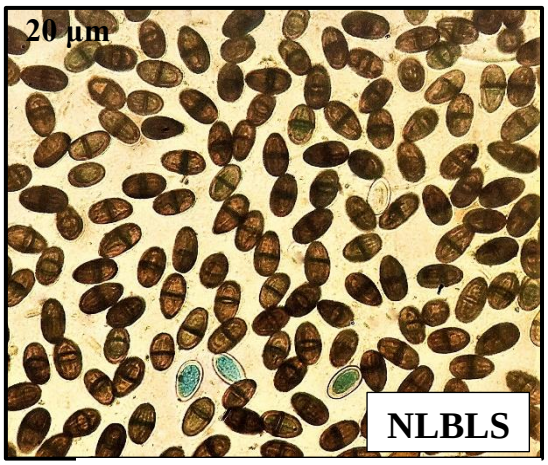
Lasiodiplodia theobromae

Lasiodiplodia theobromae

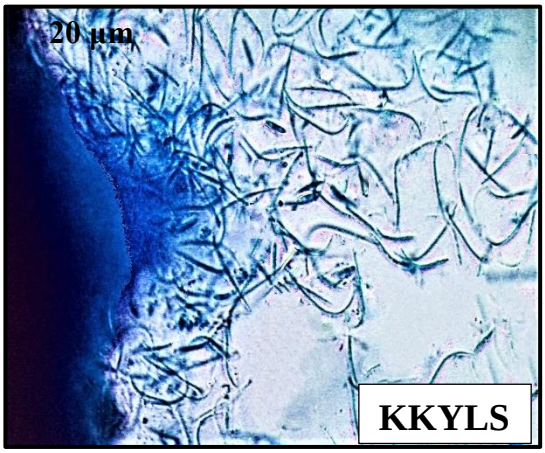
Plate 17: Cultural characters of leaf spot isolates



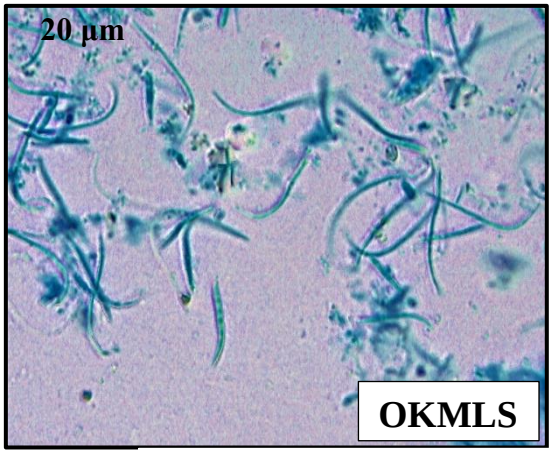
***Pestalotiopsis* sp.**



Lasiodiplodia theobromae



***Phomopsis* sp.**



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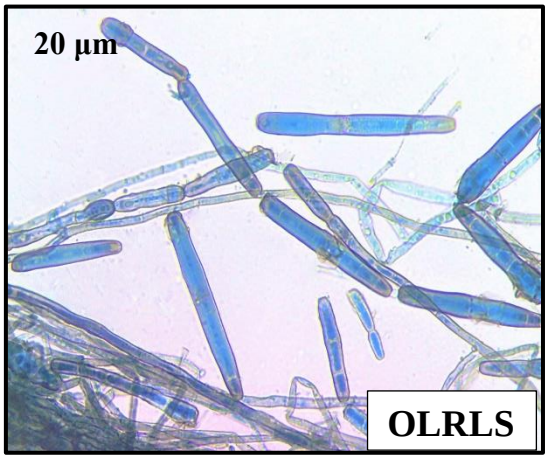
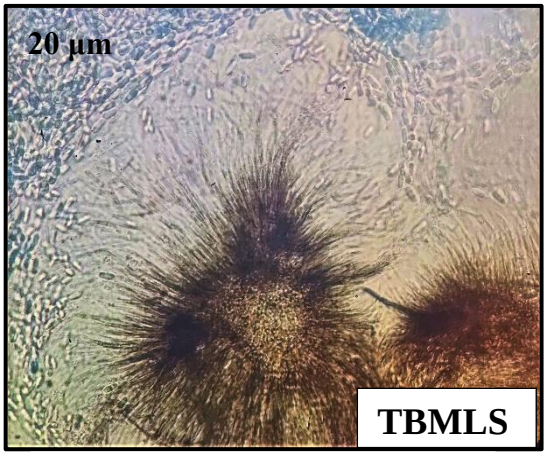


***Corynespora* sp.**



Colletotrichum gloeosporioides

Plate 22: Morphological characters of leaf spot isolates

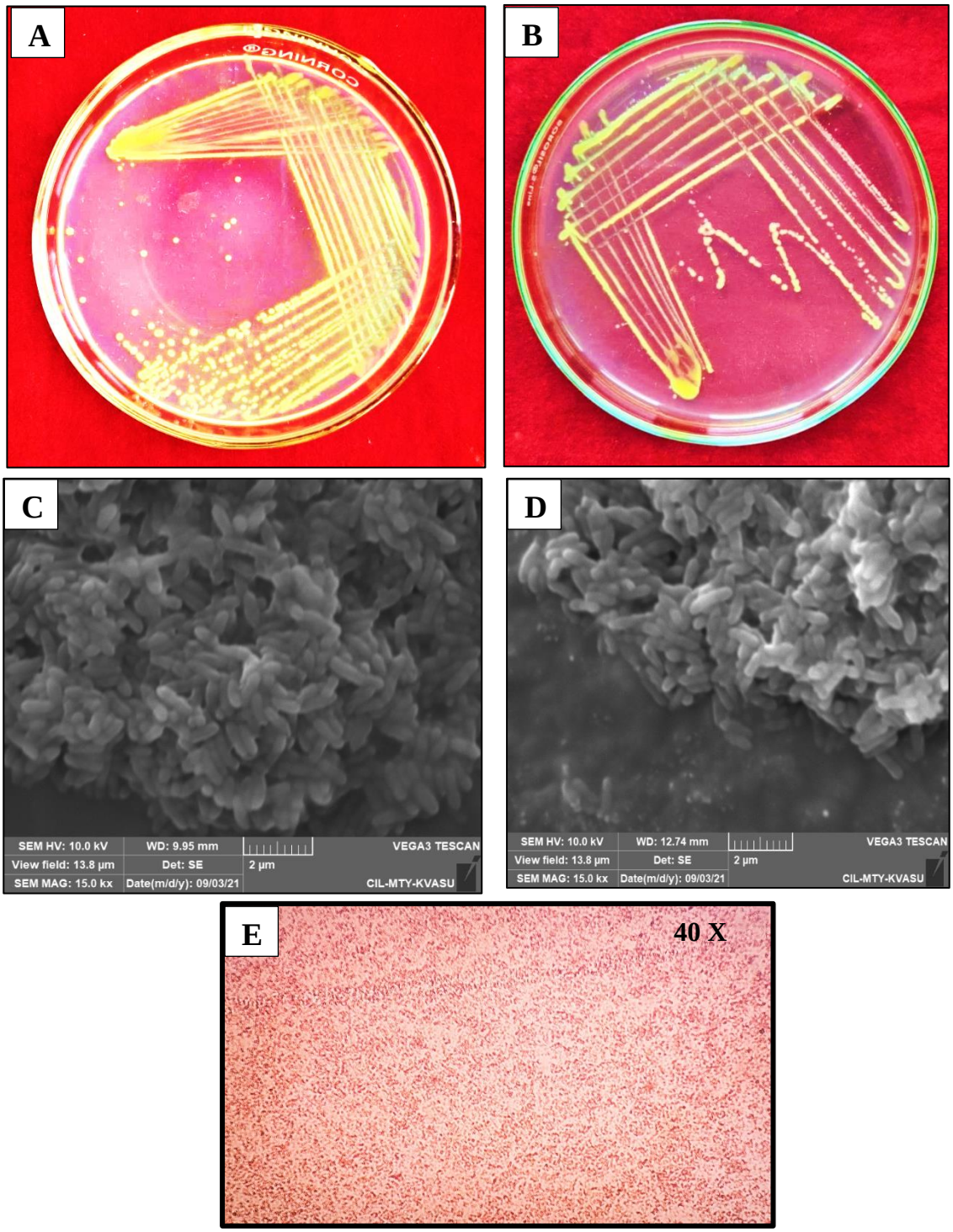


Plate 24: Cultural and morphological characters of bacterial isolates associated with leaf blight samples

A: Cultural characters of VFNLB on nutrient agar
B: Cultural characters of OLKLB on nutrient agar
C: Scanning Electron Micrograph of VFNLB
D: Scanning Electron Micrograph of OLKLB
E: Gram staining of leaf blight isolate

within four days of incubation. The growth rate of the fungus was recorded as 2.25 cm per day (Table 4.5). White and slightly fluffy mycelia were developed later with no visual signs of sporulation on the surface of the culture or on the media (Plate 18).

The morphological characters of the fungus were studied using light microscope and the observations revealed that the fungal isolate collected from VCNRR was an oomycete. The hyphae were hyaline, coenocytic and freely branching. The characteristic sporangia of the fungus were globose to subglobose, mostly terminal and papillate or non papillate. The size of sporangia ranges from 17.64 μm to 21.87 μm in diameter (Plate 23).

The pathogen was identified as *Phytopythium* sp. at the genus level based on the cultural and morphological characters studied.

PATHOGEN ASSOCIATED WITH WILTING SYMPTOM

The anthurium plants surveyed from Mannuthy of Thrissur district which showed wilting symptoms were isolated in PDA and the associated pathogen was confirmed to be a fungus. The fungal isolate MNTLW was further studied based on cultural and morphological characters.

Pathogen associated with MNTLW

The fungal pathogen causing wilting of leaves and discolouration of petioles in anthurium plants produced white coloured aerial mycelia which subsequently turned to pinkish in colour and later developed dark purple pigmentation on PDA plates. The thick mycelia radiated to all directions uniformly and covered the surface of the PDA media within eight days of incubation (Plate 19). The recorded growth rate of the fungus was 1.125 cm/day (Table 4.5).

The pathogen produced hyaline and septate hyphae and two kinds of conidia *viz*. microconidia and macroconidia. The hyaline, septate macroconidia measured 24 μm x 2.55 μm whereas the hyaline, aseptate microconidia measured 8.0 μm x 3.0 μm. Microconidia were elliptical in shape while macroconidia were spindle shaped and slightly curved (Plate 23). Based on the cultural and morphological characters, the pathogen was identified at the genetic level as *Fusarium* sp.

Table 4.5: Growth rate of different fungal isolates on PDA

Sl. No.	Code	Mean colony diameter (cm) Days of inoculation										Days for full growth	Growth rate
		1	2	3	4	5	6	7	8	9	10		
1	KMALS	1.75	3.0	5.3	6.2	8.2	9.0					6	1.5
2	PBALS	1.8	3.2	5.4	7.2	8.3	9.0					6	1.5
3	VCNRR	2.8	5.7	7.9	9.0							4	2.25
4	VCNLS1	1.9	3.6	5.2	7.4	9.0						5	1.8
5	VCNLS2	2	3.1	4.0	5.5	6.0	7.2	8.0	9.0			8	1.125
6	VFNLS	1.6	3.2	5.2	6.2	7.2	8.8	9.0				7	1.28
7	MNTSR	1.9	3.5	5.3	6.8	8.3	9.0					6	1.5
8	MNTLW	1.7	2.5	3.5	4.7	5.3	7.3	8.4	9.0			8	1.125

Table 4.5: Growth rate of different fungal isolates on PDA (Cntd…)

Sl. No.	Code	Mean colony diameter (cm) Days of inoculation										Days for full growth	Growth rate
		1	2	3	4	5	6	7	8	9	10		
9	CKDSR	1.1	3.0	5.1	6.4	8.5	9.0					6	1.5
10	IJKLS	1	3.6	5.7	7.1	8.8	9.0					6	1.5
11	KKYLS	1.5	3.1	5.6	8.0	9.0						5	1.8
12	OKMLS	2.9	4.9	6.5	8.2	9.0						5	1.8
13	OLRLS	1.8	2.5	3.6	4.7	5.8	6.8	7.6	8.4	9.0		9	1.0
14	TBMLS	1.7	3.4	5.5	6.9	7.9	9.0					6	1.5
15	PNMSR	2.0	3.3	4.4	5.6	6.7	7.5	8.5	9.0			8	1.125
16	ALVSR	1.9	3.3	4.6	6.3	7.4	8.3	9.0				7	1.28
17	NLBLS	3.6	6.5	9.0								3	3.0
18	TLRLS	1.8	2.4	2.9	3.6	4.4	5.7	6.4	7.1	9.0		9	1.0

PATHOGENS ASSOCIATED WITH INFLORESCENCE ROTS

Four samples of inflorescence rot were collected during the survey from different locations in six districts. The collected samples were MNTSR, CKDSR, PNMSR and ALVSR.

Pathogen associated with inflorescence rot MNTSR

The pathogen associated with inflorescence rot (MNTSR) obtained from Mannuthy of Thrissur district was isolated and studied by growing on PDA. The fungus produced white fluffy aerial mycelia that later turned to dull greyish white (Plate 20). The reverse side of the plate showed greenish pigmentation and yellowish spore mass was seen on the surface of the fungal culture. Full growth of the fungus was obtained in the Petri plate (9 cm diameter) within six days with a growth rate of 1.5 cm per day (Table 4.5).

The conidia were hyaline, one celled, bullet shaped with round ends. Oil globules were clearly visible at the centre of the conidia. The average dimensions of the conidia were measured to be 13.93 µm length x 4.82 µm breadth (Plate 23).

Based on the cultural and morphological characters observed, the pathogen associated with MNTSR was identified to be *Colletotrichum gloeosporioides*.

Pathogen associated with inflorescence rot CKDSR

The pathogen causing the inflorescence rot CKDSR produced white coloured thick and profuse mycelia which later was tinged in yellow and became compacted. After 10 days of full growth, minute black coloured concentric pycnidia developed on the culture with prominent yellow oozing from the tips of these black structures. Within four weeks of incubation, numerous black coloured pycnidia developed within the culture and was seen submerged inside the medium. The reverse side of the plate was yellowish orange in colour (Plate 20). A complete growth of the pathogen was observed in six days of incubation with a growth rate of 1.5 cm per day (Table 4.5).

Morphological characterisation studies of the pathogen conducted showed the presence of two kinds of conidia *viz.* alpha conidia and beta conidia. Alpha conidia were hyaline, one celled, elliptical to sub cylindrical and guttulate and the average size of

conidia was recorded as 6.8 μm length x 2.9 μm breadth. The beta conidia resembled the form of a walking stick and was filiform, curved and hyaline with an average size of 19.08 μm length x 1.73 μm breadth (Plate 23).

The pathogen causing the spadix rot CKDSR was identified as *Phomopsis* sp. based on the cultural and morphological observations.

Pathogen associated with inflorescence rot PNMSR

The pathogen causing inflorescence rot PNMSR collected from Panamaram of Wayanad district produced dull white fluffy aerial mycelia when isolated on PDA and later turned to grey with green pigmentations. The lower side of the fungal culture was dark grey in colour when viewed through the reverse side of the Petri plate (Plate 20). The fungus attained full growth on the surface of the media within eight days of incubation and the recorded growth rate was 1.125 cm per day (Table 4.5).

The pathogen produced hyaline hyphae. Numerous conidiophores were developed from well developed stroma. Acervuli were produced without setae. Conidiophores were hyaline, densely packed and short. Conidia were hyaline, non septate, single celled and cylindrical with obtuse ends. The average dimension of the conidia was 18 .24 μm length x 4.23 μm breadth. Oil globules were prominently observed at the centre of the conidia. (Plate 23).

On the basis of cultural and morphological characters observed, the pathogen was identified as *Colletotrichum gloeosporioides*.

Pathogen associated with the inflorescence rot ALVSR

The pathogen which caused inflorescence rot of anthurium ALVSR collected from Aluva of Ernakulum district produced grey coloured fluffy mycelia on isolation plate. The grey colour of the fungal culture was later turned to dark grey tinged with green and the reverse side of the plate was dark grey coloured. The pathogen showed irregular growth pattern. A complete growth of the fungal pathogen was recorded within a week of incubation in Petri plates of diameter 9 cm. After 10 days of incubation,

Table 4.6: Cultural and morphological characters of fungal pathogens

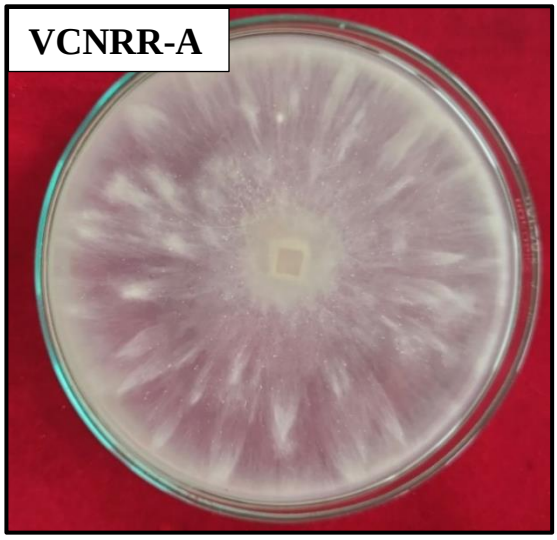
Isolate	Cultural characters	Reverse side of the Petri plate	Morphological characters				Associated pathogen
			Hyphae	Shape of conidia	Colour of conidia	Dimensions of conidia	
KMALS	Bright white coloured fluffy aerial mycelia	White mycelia with greyish green pigmentation	Hyaline, septate, branched	Bullet shaped	Hyaline	14.11 µm x 4.26 µm	*Colletotrichum gloeosporioides*
PBALS	Fluffy white aerial mycelia	Dark greyish green in white mycelia	Hyaline, septate, branched	Bullet shaped	Hyaline	19.24 µm x 4.11 µm	*Colletotrichum gloeosporioides*
VCNLS1	Dark brown tinged with olivaceous green mycelia, Dusty texture	Deep brown	Brown coloured, septate, branched	Ellipsoid	Hyaline	6.10 µm x 2.55 µm	*Phoma* sp.
VCNLS2	Dark greenish brown thick hairy mycelia	Bright green	Pale brown	Obclavate to cylindrical	Brown	83.82 µm x 9.30 µm	*Corynespora* sp.
VFNLS	White floccose aerial mycelia later turned to dark brownish green	Greenish brown	Hyaline	Cylindrical with round ends	Hyaline	17.34 µm x 3.81 µm	*Colletotrichum gloeosporioides*
IJKLS	Creamy white, slightly compacted, thick and shrunken mycelia	Dull white tinged in pale tan colour	Hyaline, septate	Ellipsoid	Hyaline	5.9 µm x 2.3 µm	*Phomopsis* sp.

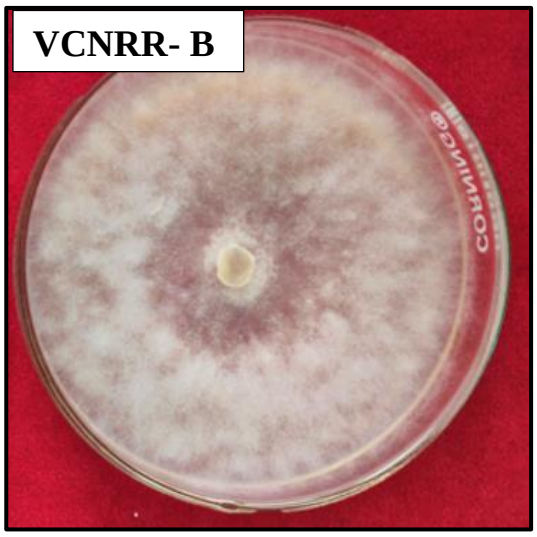
Table 4.6: Cultural and morphological characters of fungal pathogens (Cntd…)

Isolate	Cultural characters	Reverse side of the Petri plate	Morphological characters				Associated pathogen
			Hyphae	Shape of conidia	Colour of conidia	Dimensions of conidia	
KKYLS	White mycelial growth later turned to creamy white tinged with dark brown	Brown	Hyaline, branched, septate	Alpha conidia – elliptical to sub-cylindrical Beta conidia – Filiform	Both are hyaline	Beta conidia – 19.81 µm x 1.23 µm Alpha conidia – 6.7 µm x 2.7 µm	*Phomopsis* sp.
OKMLS	White coloured woolly mycelia later turned to light brown colour	Light brown	Hyaline, septate, branched	Alpha conidia: Ellipsoid Beta conidia: Filiform	Hyaline	Alpha conidia- 6.3 µm x 3.5 µm Beta conidia 18.43 µm x 1.06 µm	*Phomopsis* sp.
OLRLS	Dark greenish brown thick and hairy mycelia	Dark green	Pale brown	Obclavate to cylindrical	Brown	74.53 µm x 8.32 µm	*Corynespora* sp.
TLRLS	White cottony growth with undulating margins later turned dark brown	Buff	Hyaline, septate	Fusiform	Median cells - dark brown outer cells – hyaline	28.30 µm x 6.86 µm	*Pestalotiopsis sp.*
TBMLS	Olivaceous green tinged whitish aerial mycelia initially which later turned to dark greenish grey	Dark greyish green	Hyaline, septate, branched	Bullet shaped	Hyaline	15.28 µm x 4.01 µm	*Colletotrichum gloeosporioides*

Table 4.6: Cultural and morphological characters of fungal pathogens (Cntd…)

Isolate	Cultural characters	Reverse side of the Petri plate	Morphological characters				Associated pathogen
			Hyphae	Shape of conidia	Colour of conidia	Dimensions of conidia	
NLBLS	Dull grey coloured fluffy aerial mycelia later turned black	Black	Brown, septate	Oval	Brown	26.40 µm x 14.70µm	*Lasiodiplodia theobromae*
VCNRR	White blooming aerial mycelia	Dull white to cream	Hyaline, aseptate, branching	Sporangia were globose to subglobose, mostly terminal and papillate/ non papillate. The size ranged from 17.64 µm to 21.87 µm in diameter			*Phytopythium sp.*
MNTLW	Pinkish white mycelia subsequently turned dark purple	Purple	Hyaline, septate	Microconidia-ellipsoid Macroconidia –Spindle shaped	Hyaline	Microconidia – 8.0 µm x 3.0 µm Macroconidia – 24 µm x 2.55 µm	*Fusarium sp.*
MNTSR	White and fluffy mycelia	Dark green in white mycelia	Hyaline, septate, branched	Bullet shaped	Hyaline	13.93 µm x 4.82 µm	*Colletotrichum gloeosporioides*
CKDSR	White profuse mycelia later became compacted	Dull white	Hyaline, branched, septate	Alpha conidia-elliptical Beta conidia – Filiform	Both are hyaline	Beta conidia – 19.08 µm x 1.73 µm Alpha conidia – 6.8 µm x 2.9 µm	*Phomopsis* sp.
PNMSR	Grey fluffy mycelia	Dark grey	Hyaline, septate, branched	Bullet shaped	Hyaline	18.24 µm x 4.23 µm	*Colletotrichum gloeosporioides*
ALVSR	Dark grey fluffy growth	Dark greyish green	Hyaline, septate, branched	Bullet shaped	Hyaline	20.63 µm x 4.57 µm	*Colletotrichum gloeosporioides*



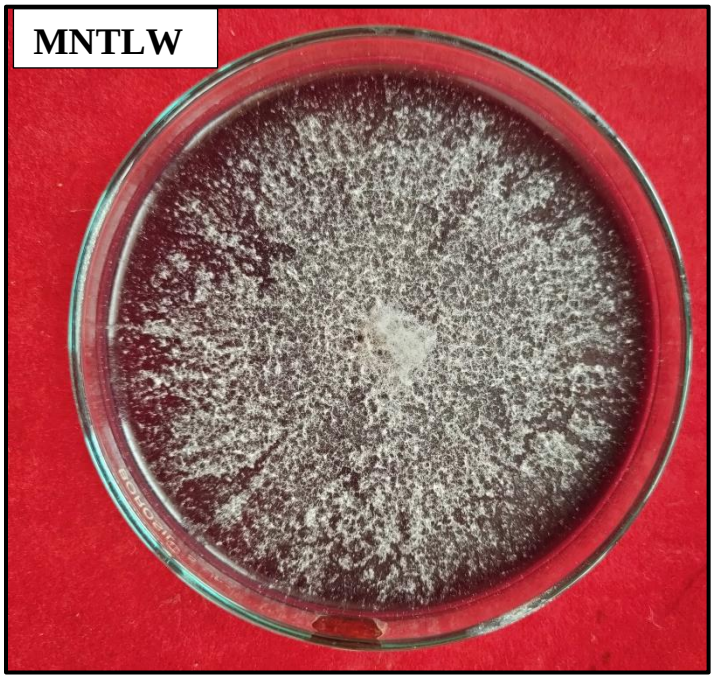


***Phytopythium* sp.** ***Phytopythium* sp.**

Plate 18: Cultural characters of root rot isolate

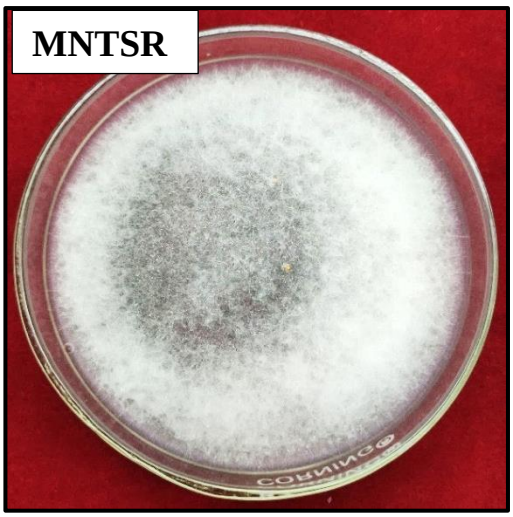
VCNRR-A: Initial mycelia in floral bloom appearance

VCNRR-B: Fluffy growth of the fungus developed gradually

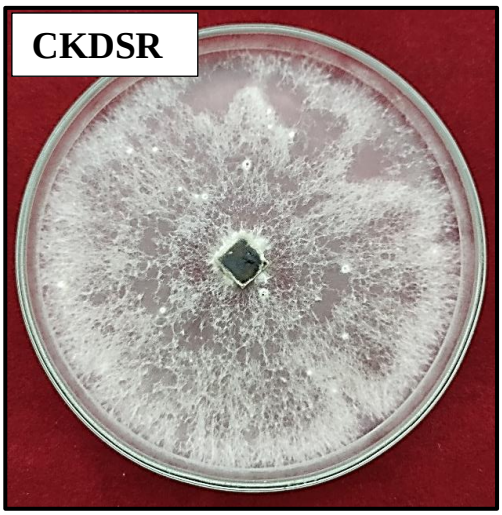


***Fusarium* sp.**

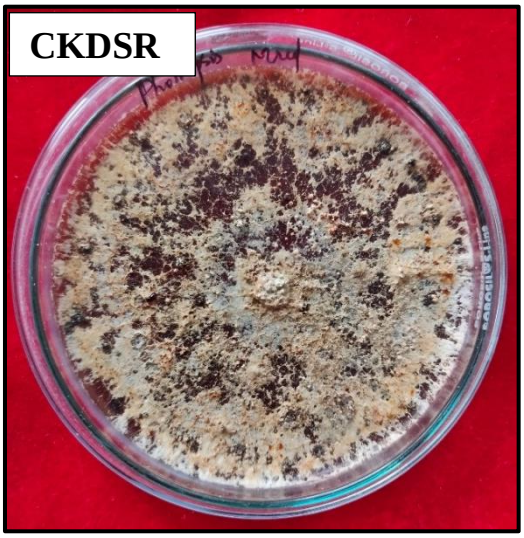
Plate 19: Cultural characters of wilt isolate



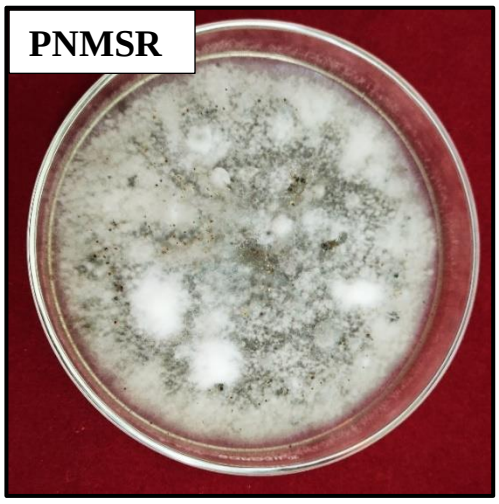
Colletotrichum gloeosporioides



***Phomopsis* sp.**



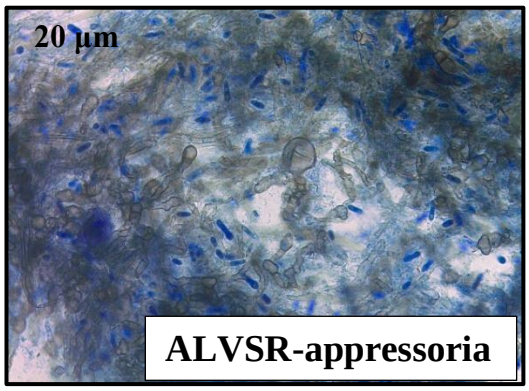
***Phomopsis* sp. - Black pycnidia & yellow ooze**



Colletotrichum gloeosporioides

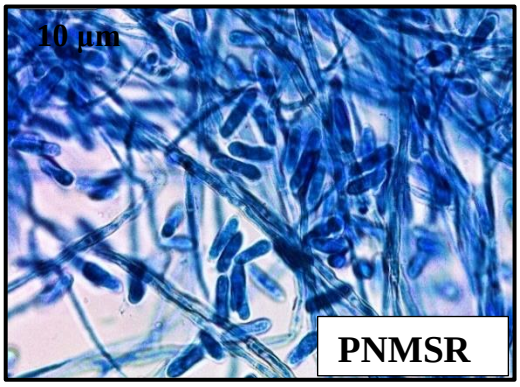
Colletotrichum gloeosporioides

Plate 20: Cultural characters of inflorescence rot isolates

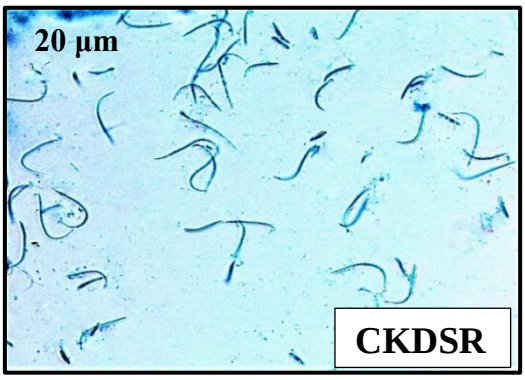


Colletotrichum gloeosporioides

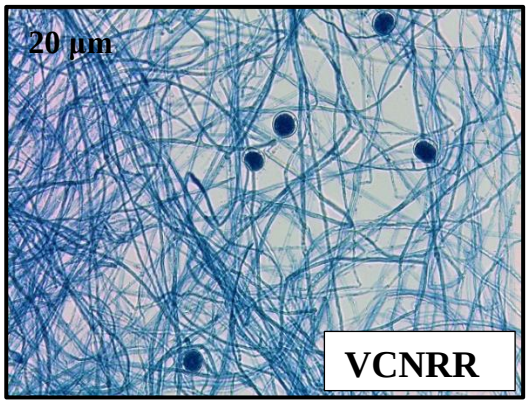
Colletotrichum gloeosporioides



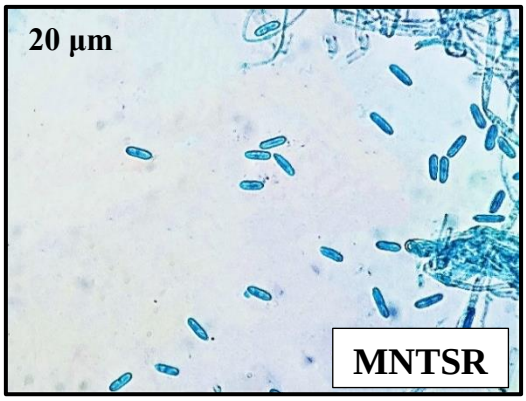
Colletotrichum gloeosporioides



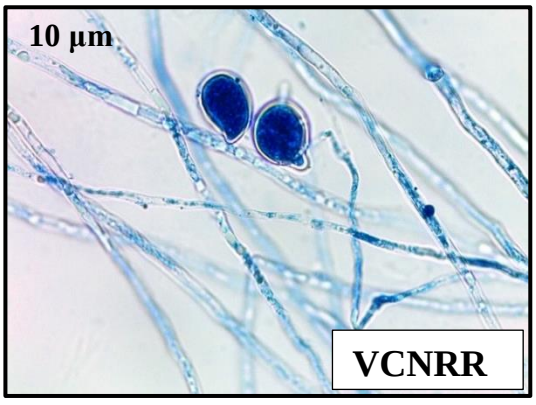
***Phomopsis* sp.**



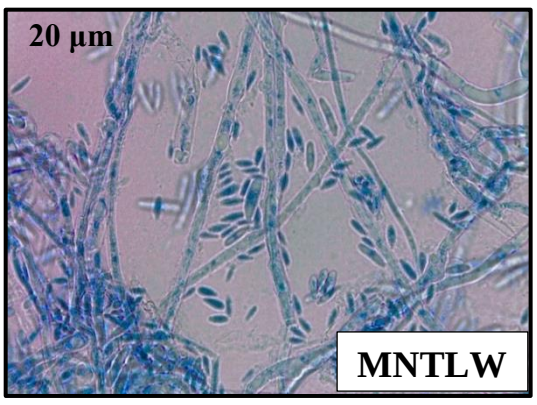
***Phytopythium* sp.**



Colletotrichum gloeosporioides



***Phytopythium* sp.**



***Fusarium* sp.**

Plate 23: Morphological characters of inflorescence rot, root rot and wilt isolates

yellow spore mass was clearly seen on the fungal mycelial tufts (Plate 18). The growth rate of the fungus was 1.28cm per day (Table 4.6)

The fungus developed hyaline, septate and branched hyphae which later turned to light brown in colour. The various structures like appressoria and chlamydospores were visible throughout the mycelia. Conidia were hyaline, single celled, and one celled with oil globules at the centre and measured an average size of 20.63 µm length x 4.57 µm breadth (Plate 23).

Based on the cultural and morphological characters, the pathogen was identified as *Colletotrichum gloeosporioides*.

4.5.2 Characterisation of isolated bacterial pathogen

The cultural, morphological and biochemical characters of the bacterial pathogens isolated from infected samples collected during the sampling survey in various anthurium growing tracts of six district of the state were used for the characterisation and identification of the corresponding pathogens. The cultural characters of the bacterial colonies were studied without any magnifying devices and the characters observed were the colour, pigmentation, texture and mucoidal nature of the developing colonies. The morphology of bacterial cells such as shape and size were observed under light microscope (40X) and with the aid of Electron microscope (15000X)

4.5.2.1 Characterization based on cultural characters

During the purposive sampling survey, two pathogenic bacterial isolates causing leaf blight symptoms *viz*. VFNLB and OLKLB were obtained from Vellanikkara and Ollukara regions of Thrissur district respectively. Colony growth was started within 72 hours of incubation in nutrient agar. Both the isolates produced small to medium sized, circular, smooth and yellowish colonies that were highly mucoid in nature. The bacterial colonies were thick, convex and slightly raised (Plate 24).

4.5.2.2 Characterization based on morphological characters

The morphology of bacterial cells was initially studied under light microscope with a magnification of 40 X and100 X and revealed that both the isolates *viz*. VFNLB

and OLKLB were rod in shape. Short rods stained in pale reddish were visible in Gram's staining reaction which confirmed that the isolates were gram negative.

Further detailing of morphological characters were done using scanning electron microscope (Tescan Vega-3 LMU) from Central Instrumentation laboratory, College of veterinary and Animal science, Mannuthy. The electron microscopy analysis confirmed that the bacterial isolates associated with leaf blight of anthurium (VFNLB and OLKLB) were rod shaped with a size of 1.25 to 1.38 µm in length and 0.35 to 0.42 µm breadth (Plate 24). The microscopic image was captured at a working distance of 9.95 mm and a SEM voltage of 10.0 kV which was fixed by trial and error method.

From the observations gathered and studied, both the isolates showed similar morphological and cultural characters as that of *Xanthomonas* sp.

4.5.2.3 Characterization based on biochemical tests

Several biochemical tests *viz*. hydrolysis of starch, hydrolysis of gelatin, casein hydrolysis, indole production test, citrate test, MR test, VP test, catalase test, potassium hydroxide test, oxidase test and hydrogen sulphide production test were carried out to further confirm the identity of bacterial isolates after performing cultural and morphological characterization (Plate 25) (Table 4.7).

Starch hydrolysis/ Amylase production test

The bacterial isolates associated with leaf blight symptom were tested positive for starch hydrolysis as they were capable of producing amylase enzyme. Around the central single streak of bacteria, a clear zone was developed when the Petri plate was flooded with iodine solution. This indicated that the starch molecules in the media was hydrolysed by amylase enzyme that appeared yellowish orange in colour when compared to rest of the area which contained starch and appeared in blue since iodine provide blue colour in the presence of starch.

Gelatin hydrolysis test

The two bacterial isolates collected from VFNLW and OLKLB showed positive reactions in gelatin hydrolysis test. The semisolid gel like consistency of gelatin was converted to liquid like after the incubation period. Thus, it was confirmed that both the

isolates were capable of producing gelatinase enzyme so that the gelatin media on which bacteria were cultured didn't get solidified after keeping in refrigerated condition at 4°C.

Casein hydrolysis test

Both the isolates tested positive for casein hydrolysis test. After 72 h of incubation, a clear zone developed prominently around the bacterial streak as the isolates were capable of producing the enzyme caseinase which degraded the casein in the media.

Indole production test

After 48 h of incubation, the two bacterial isolates cultured in one per cent tryptone broth showed negative results for the production of indole. On addition of Kovac's reagent, the reaction failed to give cherry red coloured layer over the surface of the media.

Citrate test

The bacteria isolated from the infected samples VFNLB and OLKLB showed positive result for citrate test. Within 24 h of incubation, the bacterial colonies were capable of utilizing the available citrate in the growth media and the byproduct formed thus (CO_2) raised the pH and as a result of which a blue colour developed in the corresponding slants.

Methyl red test

The bacterial isolates showed negative reaction in methyl red test as there developed no red colouration on addition of methyl red indicator. The isolates grown in the test broth were unable to utilise glucose contained in the media and thereby no acids were produced which resulted in a higher pH of the media. Methyl red indicator which usually turns reddish under acidic conditions remained yellow as the pH was higher.

Voges – Proskauer test

The two isolates of leaf blight were not capable of producing acetylmethylcarbinol formed usually during glucose fermentation. After the addition of

VP reagent I and VP reagent II to the broth containing bacteria, no colour change was observed. Thus, the isolates tested negative for VP test as these failed to show a pinkish red colouration at the surface of the media.

Catalase test

Both the isolates obtained from Vellanikkara (VFNLB) and Ollukkara (OLKLB) tested catalase positive. When a loopful of bacterial cells were added and mixed with 30 per cent hydrogen peroxide solution, oxygen bubbles evolved rapidly and that indicated the ability of the isolates to produce catalase enzyme.

Potassium hydroxide test

The bacterial isolates associated with leaf blight VFNLB and OLKLB were KOH positive. On mixing a loopful of bacterial inoculum in three per cent of KOH solution, fine and slimy viscous threads were formed when the inoculation loop was raised and that confirmed the gram negative nature of the isolates.

Oxidase test

The two bacteria isolates causing leaf blight symptom tested negative for oxidase reaction as there was no purple colour development in the oxidase disc. These were not capable of producing the enzyme, cytochrome C oxidase and thus the reagent present in the disc (tetramethyl-P-phenylene diamine) remained without any change in the colour.

Hydrogen sulphide production test

The bacterial pathogens showed negative results for hydrogen sulphide production test. Thus, both the isolates were incapable of reducing sulphur to hydrogen sulphide.

Based on the results of various biochemical reactions conducted as described before, the isolates associated with the leaf blight samples collected from Vellanikkara (VFNLB) and Ollukkara (OLKLB) of Thrissur district were identified and confirmed as *Xanthomonas* sp.

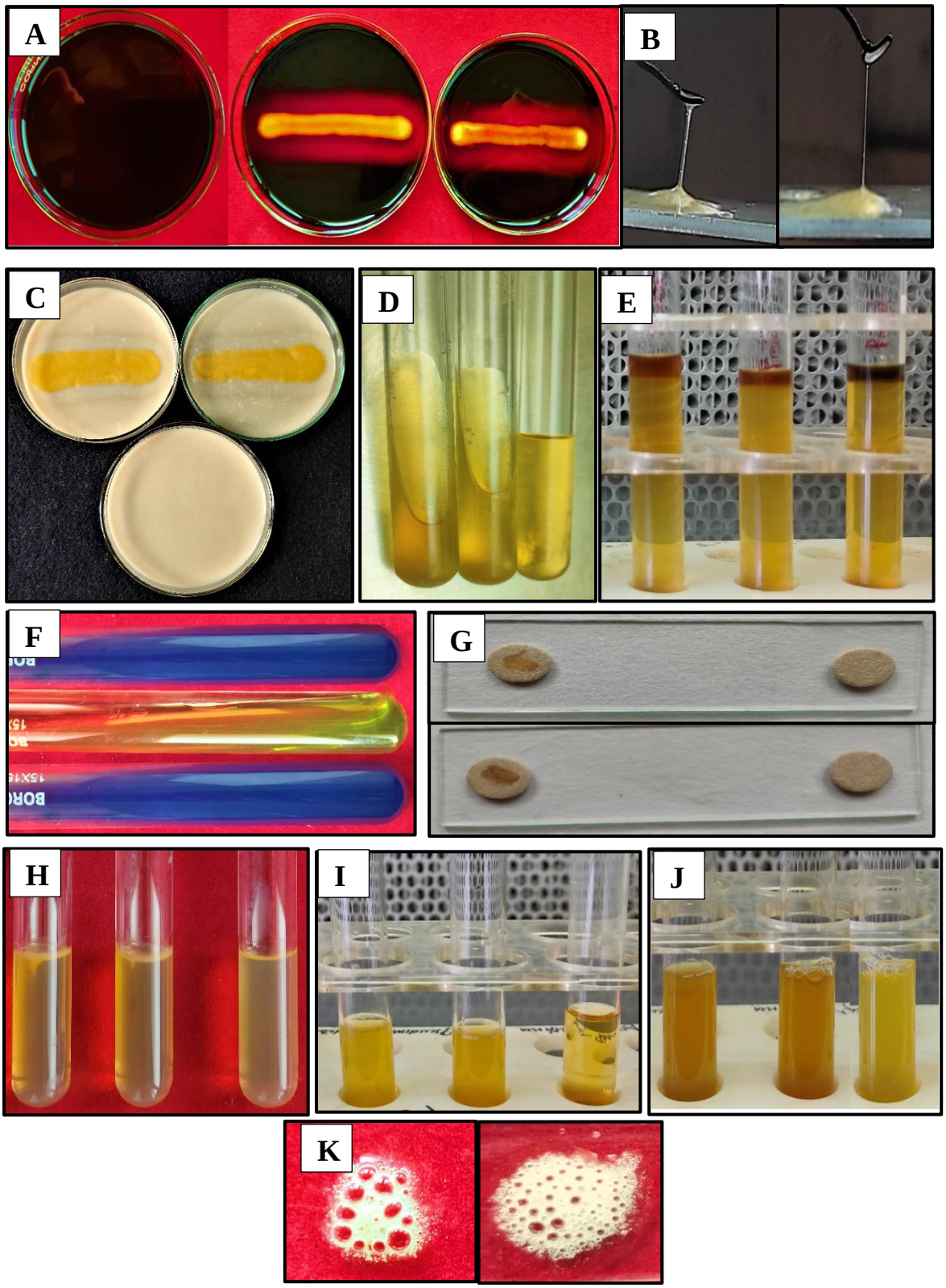


Plate 25: Biochemical characterization of bacteria associated with leaf blight

A: Amylase production test
B: KOH test
C: Casein hydrolysis test
D: Gelatin hydrolysis test
E: Indole production test
F: Citrate test
G: Oxidase test
H: Hydrogen sulfide production test
I: MR test
J: VP test
K: Catalase test

Table 4.7: Biochemical reactions of bacterial isolates

Sl. No.	Biochemical test	Reaction		Reaction of *Xanthomonas* sp.	Reference
		VFNLB	OLKLB		
1.	Amylose production test	+	+	+	Jovit *et al.* (2016)
2.	Hydrolysis of Gelatin	+	+	+	Pernezny *et al*. (2003)
3.	Casein hydrolysis	+	+	+	Goszczynska and Serfontein (1998)
4.	Indole production	-	-	-	Arshad *et al*. (2015)
5.	Citrate test	+	+	+	Sarker *et al*. (2017)
6.	MR test	-	-	-	Gitatis *et al*. (1987)
7.	VP test	-	-	-	Monllor (1991)
8.	Catalase test	+	+	+	Lia *et al*. (2018)
9.	Potassium hydroxide test	+	+	+	Jadhav *et al*. (2018)
10.	Oxidase test	-	-	-	Haider *et al.* (2020)
11.	Hydrogen sulfide production test	-	-	-	Meena *et al.* (2018)

Table 4.8: Identification of pathogens associated with different symptoms

Sl. No.	Designated code	Disease	Associated pathogen
1.	Leaf spot	KMALS	*Colletotrichum gloeosporioides*
2.	Leaf spot	PBALS	*Colletotrichum gloeosporioides*
3.	Root rot	VCNRR	*Phytopythium* sp.
4.	Leaf spot	VCNLS1	*Phoma* sp.
5.	Leaf spot	VCNLS2	*Corynespora* sp.
6.	Leaf spot	VFNLS	*Colletotrichum gloeosporioides*
7.	Inflorescence rot	MNTSR	*Colletotrichum gloeosporioides*
8.	Leaf wilt	MNTLW	*Fusarium* sp.
9.	Inflorescence rot	CKDSR	*Phomopsis* sp.
10.	Leaf spot	IJKLS	*Phomopsis* sp.
11.	Leaf spot	KKYLS	*Phomopsis* sp.
12.	Leaf spot	OKMLS	*Phomopsis* sp.
13.	Leaf spot	OLRLS	*Corynespora* sp.
14.	Leaf spot	TLRLS	*Pestalotiopsis* sp.
15.	Leaf spot	TBMLS	*Colletotrichum gloeosporioides*
16.	Leaf spot	NLBLS	*Lasiodiplodia* sp.
17.	Inflorescence rot	PNMSR	*Colletotrichum gloeosporioides*
18.	Inflorescence rot	ALVSR	*Colletotrichum gloeosporioides*
19.	Leaf blight	VFNLB	*Xanthomonas sp.*
20.	Leaf blight	OLKLB	*Xanthomonas sp.*

4.5.3 Characterization of virus

The virus suspected plant samples collected from Vellanikkara (Central Nursery, Kerala Agricultural University) in Thrissur district were sent for morphological characterisation of virus particles (if any).

4.5.3.1 Morphological characterization of virus particles

The suspected samples of virus infection were subjected to transmission electron microscopic studies at Advanced Centre for Plant Virology, I. A. R. I., New Delhi. The protocol of leaf dip method described by Bhat *et al.* (2004) was used for the characterization under JEM1011 electron microscope. The results revealed that no virus particles were present in the suspected samples. Based on these results, it was confirmed that the virus-like symptom seen on the spathe and leaves of collected anthurium plants were not caused by virus infection.

4.6 MOLECULAR CHARACTERIZATION

After the cultural and morphological characterization of isolates (both fungus and bacteria) to identify the corresponding genus which the pathogen belonged to, molecular characterization was done for species level identification and confirmation. The ITS regions of fungal DNA and 16S-rRNA regions of bacterial genomic DNA were PCR amplified using specific primers at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram. The sequences obtained were analysed using BLASTN search against NCBI nr database.

4.6.1 Fungal pathogens

The fungal isolates collected from different locations in six districts during the sampling survey were characterised up to genus level based on cultural and morphological characters as *Colletotrichum gloeoesporioiodes, Corynespora* sp., *Phytopythium* sp., *Phomopsis* sp., *Pestalotiopsis* sp., *Phoma* sp., *Lasiodiplodia theobromae* and *Fusarium* sp. Molecular characterization of these isolates were done by isolation of fungal DNA and amplification of ITS region at Rajiv Gandhi centre for Biotechnology (RGCB), Thiruvananthapuram.

4.6.1.1 Isolate VCNRR

The cultural and morphological characterization of the fungal pathogen associated with root rot symptom VCNRR identified the genus to be *Phytopythium* sp. The Gel image revealed that amplicon of 500bp length of the ITS region was obtained (Plate 28). In the BLASTN analysis, the sequence showed 99.77 per cent identity with *Phytopythium vexans* isolate TS-7 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence (MW426381.1) with 100 per cent query cover. Along with this, the sequence showed 99.07 per cent similarity with the accessions of the same pathogen (KP183960.1, KP183933.1, MH478301.1) (Fig 2a). The evolutionary relationship between *Phytopythium vexans* (VCNRR) and other top hits obtained from BLASTN analysis in NCBI nr database revealed that the pathogen associated with the root rot symptom VCNRR was more related to the accession of *Phytopythium vexans* (MT647272.1) reported in *Prunus serotina* from USA and distantly related to the accessions of the same pathogen MF196965.1 and MF196965.1 (Fig 3a).

4.6.1.2 Isolate VCNLS2

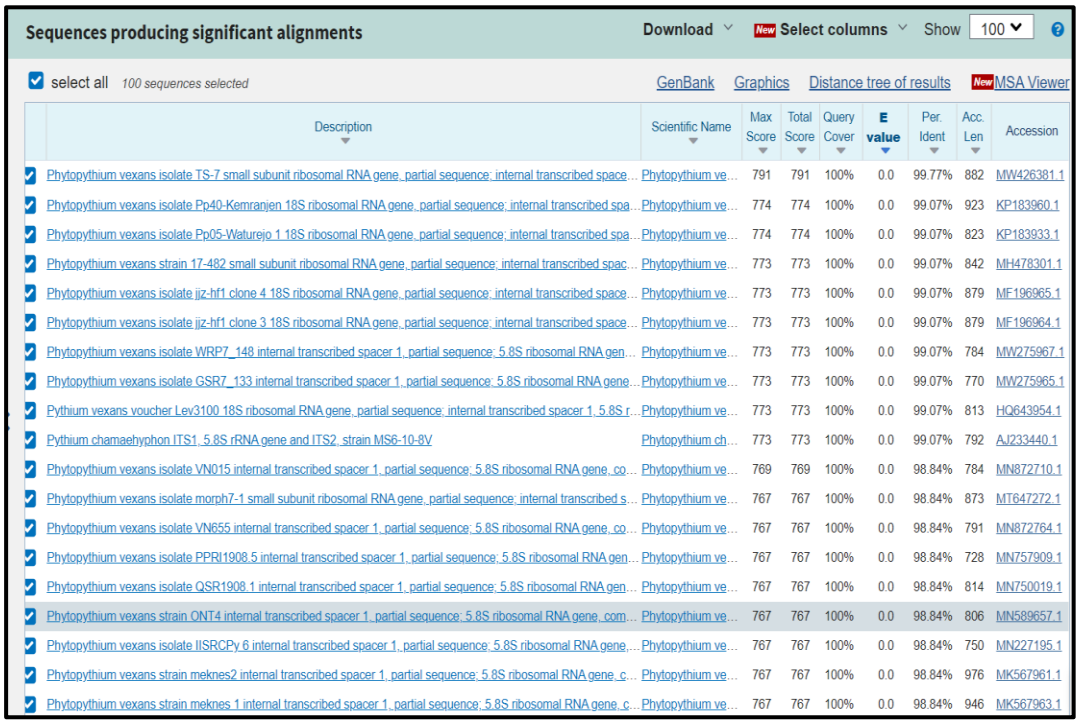
Based on the cultural and morphological characters, the causal agent of leaf spot VCNLS2 collected from Vellanikkara of Thrissur district was confirmed as *Corynespora* sp. The Gel image revealed that amplicon of 500bp length of the ITS region was obtained (Plate 26). The BLASTN analysis revealed that the pathogen showed 99.11 per cent identity with *Corynespora cassiicola* isolate FXSB-1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence with 98 per cent query cover (MK139711.1) (Fig 2b). Mega X software was used to analyse the phylogenetic relationship between isolate and the accessions of *Corynespora cassiicola* obtained in the BLASTN analysis and found maximum relationship with *Corynespora cassiicola* (MN809263.1) associated with cowpea in Ghana (Fig 3b).

5’CACCCTTCCACGTGAACCGTTTTGTTTTGCTTTCGAGTGCTTTTGTTGCGCTC
GGAGCATGTTTTGGGCTTCGCTGCTGGCGCTTGATTGTGCTGGCGGCTCGAGG
CCATCAAGCGGCGTTTTGAGTGTGTGTTCGCATACAATTAAAACGTCCAAACC
TTTTAAACCCATTTGATTGAAAACTGAACTATACTGTGGGGACGAAAGTCCTC
GCTTTGAAACTAGATAACAACTTTCAGCAGTGGATGTCTAGGCTCGCACATCG
ATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTGCAGGATTCAGTGA
GTCATCGAACTTTTGAACGCATATTGCACTTTCGGGTTACGCCTGGAAGTATGT
CTGTATCAGTGTCCGTACACTAAACTTGCGTCTCTTCCGTCGTGTAGTCGTCGG
TTGTTTGATTGCAGATGTGAAGTTGGTCTCGCGATCTGTTGCTTGTCGACAGGG
TTGCGAGTCCCTTTAAAGTCGGACGCGTGTTTTTCCGTTTTGTGCTTGATGGGG
TGCGGCTGCGGCCGTGTCTGCTGGCGGGTCCGGTGACCTTTGGCGATGGCATG
AGAGTGGATTGCTCGATTTGCGGTATGTTAGGCTTCGGCTTTGACAATGCAGCT
TATTGGGTGTGTTCGCTTGGCTGTTGCTGTATGGGTGAGCTGGATGGTCGGTGG
ATGCGTTGTTGCGTGTCGTTTTTCATTGAGTGCGTTTCGGTTGTCGTCGCCATTT
GGGAATTTGTGGTCTCTAAGGAGAGCATCTCATTTGGACCTGATATCAGACAA
GAATAA3’

Fig 1 a: Nucleotide sequence of ITS gene of *Phytopythium vexans* (VCNRR)

5’TTCTTTCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGAATCCCTACTCTGA
TCCAAGGTCAAAATTCTGGTGTGGGGGGGCTCATGGTGCGCCGACCCGCAGC
CACTTCAGCGCTTGCTCTGCTGCGCTCGTGGCCTGCTGGGAACCGGCCGCCAA
TGCTTTTGAGGCGAGTCCAGGCGCGCGGAGGCGGGACAGACGCCCAACACCA
AGCTAGGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCTAAGGAATA
CCAAAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAAT
TCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAG
ATCCGTTGTTGAAAGTTGTAAATAGTTTGTTTTGTTTTGTTCAGACGTGTACTT
CTTGTACTGCAATGGGTTTGTGGTGGGTCCCCGTTGGCAGGCGAGCTCTGCCG
AGGAAACGAGAGGTGCTCATAAACAAAGGGTGCTATCTCGAAGGGGGCGAG
GCCCCTACGATAATGATCCTTCCGCAGGTTCACCTACGAAAGAAAG3’

Fig 1 b: Nucleotide sequence of ITS gene of *Corynespora cassiicola* (VNCLS2)

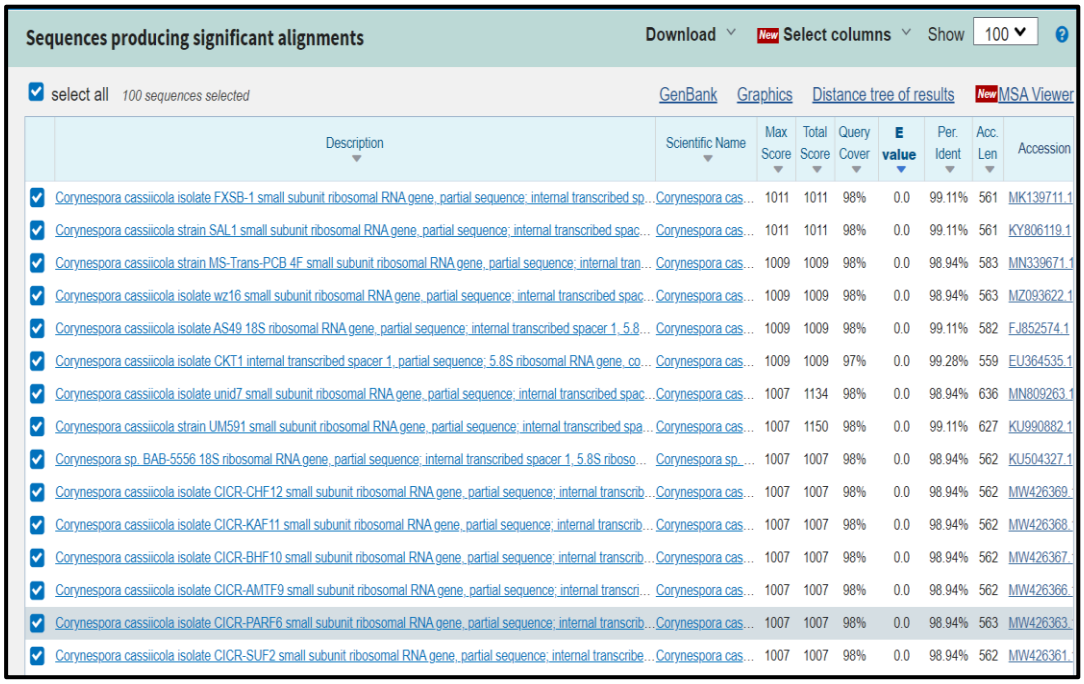


Sequences producing significant alignments — Download — New Select columns — Show 100

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

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Phytopythium vexans isolate TS-7 small subunit ribosomal RNA gene, partial sequence; internal transcribed space…	Phytopythium ve…	791	791	100%	0.0	99.77%	882	MW426381.1
Phytopythium vexans isolate Pp40-Kemranjen 18S ribosomal RNA gene, partial sequence; internal transcribed spa…	Phytopythium ve…	774	774	100%	0.0	99.07%	923	KP183960.1
Phytopythium vexans isolate Pp05-Waturejo 1 18S ribosomal RNA gene, partial sequence; internal transcribed spa…	Phytopythium ve…	774	774	100%	0.0	99.07%	823	KP183933.1
Phytopythium vexans strain 17-482 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac…	Phytopythium ve…	773	773	100%	0.0	99.07%	842	MH478301.1
Phytopythium vexans isolate jiz-hf1 clone 4 18S ribosomal RNA gene, partial sequence; internal transcribed space…	Phytopythium ve…	773	773	100%	0.0	99.07%	879	MF196965.1
Phytopythium vexans isolate jiz-hf1 clone 3 18S ribosomal RNA gene, partial sequence; internal transcribed space…	Phytopythium ve…	773	773	100%	0.0	99.07%	879	MF196964.1
Phytopythium vexans isolate WRP7_148 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen…	Phytopythium ve…	773	773	100%	0.0	99.07%	784	MW275967.1
Phytopythium vexans isolate GSR7_133 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene…	Phytopythium ve…	773	773	100%	0.0	99.07%	770	MW275965.1
Pythium vexans voucher Lev3100 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S r…	Phytopythium ve…	773	773	100%	0.0	99.07%	813	HQ643954.1
Pythium chamaehyphon ITS1, 5.8S rRNA gene and ITS2, strain MS6-10-8V	Phytopythium ch…	773	773	100%	0.0	99.07%	792	AJ233440.1
Phytopythium vexans isolate VN015 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, co…	Phytopythium ve…	769	769	100%	0.0	98.84%	784	MN872710.1
Phytopythium vexans isolate morph7-1 small subunit ribosomal RNA gene, partial sequence; internal transcribed s…	Phytopythium ve…	767	767	100%	0.0	98.84%	873	MT647272.1
Phytopythium vexans isolate VN655 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, co…	Phytopythium ve…	767	767	100%	0.0	98.84%	791	MN872764.1
Phytopythium vexans isolate PPRI1908.5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen…	Phytopythium ve…	767	767	100%	0.0	98.84%	728	MN757909.1
Phytopythium vexans isolate QSR1908.1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen…	Phytopythium ve…	767	767	100%	0.0	98.84%	814	MN750019.1
Phytopythium vexans strain ONT4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, com…	Phytopythium ve…	767	767	100%	0.0	98.84%	806	MN589657.1
Phytopythium vexans isolate IISRCPy 6 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene,…	Phytopythium ve…	767	767	100%	0.0	98.84%	750	MN227195.1
Phytopythium vexans strain meknes2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, c…	Phytopythium ve…	767	767	100%	0.0	98.84%	976	MK567961.1
Phytopythium vexans strain meknes 1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, c…	Phytopythium ve…	767	767	100%	0.0	98.84%	946	MK567963.1

Fig 2 a: BLASTN text output of nucleotide sequence of ITS gene of *Phytopythium vexans* (VCNRR)



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

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

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Corynespora cassiicola isolate FXSB-1 small subunit ribosomal RNA gene, partial sequence; internal transcribed sp…	Corynespora cas…	1011	1011	98%	0.0	99.11%	561	MK139711.1
Corynespora cassiicola strain SAL1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac…	Corynespora cas…	1011	1011	98%	0.0	99.11%	561	KY806119.1
Corynespora cassiicola strain MS-Trans-PCB 4F small subunit ribosomal RNA gene, partial sequence; internal tran…	Corynespora cas…	1009	1009	98%	0.0	98.94%	583	MN339671.1
Corynespora cassiicola isolate wz16 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac…	Corynespora cas…	1009	1009	98%	0.0	98.94%	563	MZ093622.1
Corynespora cassiicola isolate AS49 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8…	Corynespora cas…	1009	1009	98%	0.0	99.11%	582	FJ852574.1
Corynespora cassiicola isolate CKT1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, co…	Corynespora cas…	1009	1009	97%	0.0	99.28%	559	EU364535.1
Corynespora cassiicola isolate unid7 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac…	Corynespora cas…	1007	1134	98%	0.0	98.94%	636	MN809263.1
Corynespora cassiicola strain UM591 small subunit ribosomal RNA gene, partial sequence; internal transcribed spa…	Corynespora cas…	1007	1150	98%	0.0	99.11%	627	KU990882.1
Corynespora sp. BAB-5556 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S riboso…	Corynespora sp…	1007	1007	98%	0.0	98.94%	562	KU504327.1
Corynespora cassiicola isolate CICR-CHF12 small subunit ribosomal RNA gene, partial sequence; internal transcrib…	Corynespora cas…	1007	1007	98%	0.0	98.94%	562	MW426369.1
Corynespora cassiicola isolate CICR-KAF11 small subunit ribosomal RNA gene, partial sequence; internal transcrib…	Corynespora cas…	1007	1007	98%	0.0	98.94%	562	MW426368.1
Corynespora cassiicola isolate CICR-BHF10 small subunit ribosomal RNA gene, partial sequence; internal transcrib…	Corynespora cas…	1007	1007	98%	0.0	98.94%	562	MW426367.1
Corynespora cassiicola isolate CICR-AMTF9 small subunit ribosomal RNA gene, partial sequence; internal transcri…	Corynespora cas…	1007	1007	98%	0.0	98.94%	562	MW426366.1
Corynespora cassiicola isolate CICR-PARF6 small subunit ribosomal RNA gene, partial sequence; internal transcrib…	Corynespora cas…	1007	1007	98%	0.0	98.94%	563	MW426363.1
Corynespora cassiicola isolate CICR-SUF2 small subunit ribosomal RNA gene, partial sequence; internal transcribe…	Corynespora cas…	1007	1007	98%	0.0	98.94%	562	MW426361.1

Fig 2 b: BLASTN text output of nucleotide sequence of ITS gene of *Corynespora cassiicola* (VCNLS2)

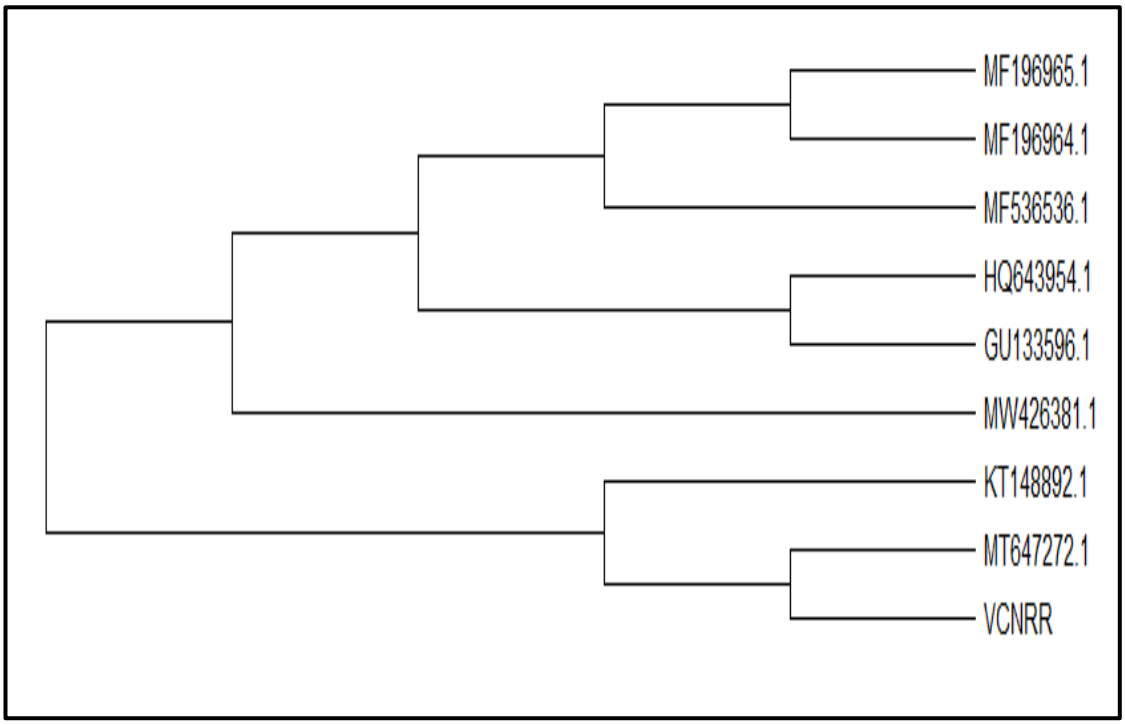


Fig 3 a: Phylogenetic tree of *Phytopythium vexans*

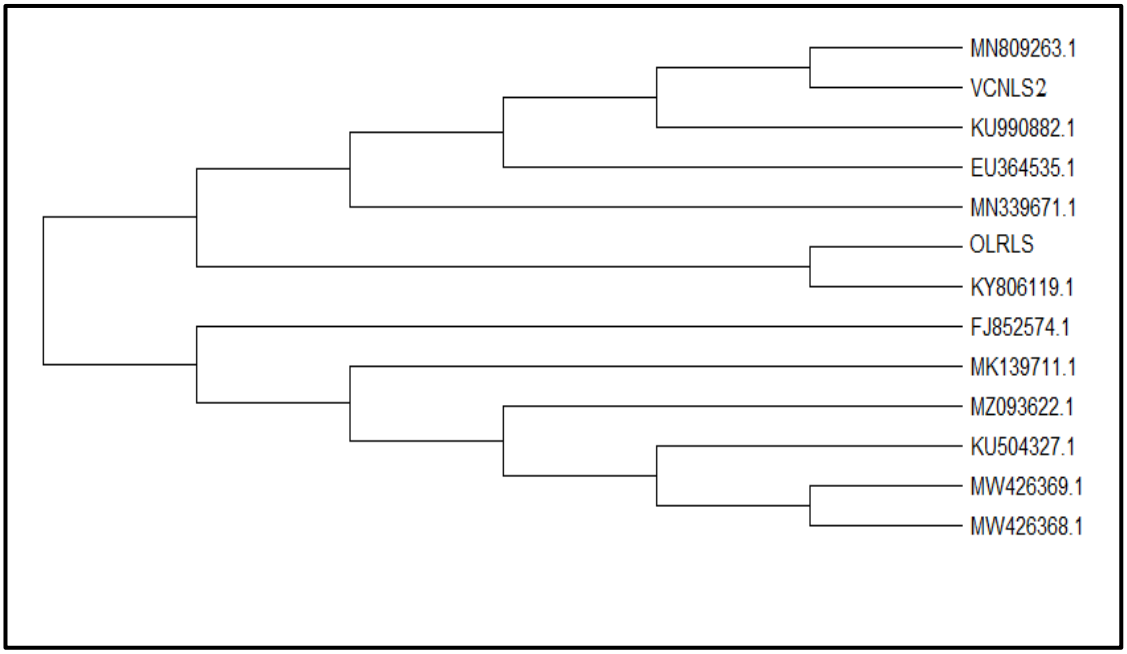


Fig 3 b: Phylogenetic tree of *Corynespora cassiicola*

4.6.1.3 Isolate IJKLS

The pathogen associated with the leaf spot IJKLS collected from Irinjalakuda region of Thrissur district was confirmed to be of the genus *Phomopsis* sp. based on the characters observed in cultural and morphological studies. The Gel image revealed that amplicon of 500bp length of the ITS region was obtained (Plate 26).The BLASTN analysis of ITS sequences of the isolate in NCBI nr database showed 96.01 per cent identity with *Diaporthe phaseolorum* isolate B3155 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence with a query cover of 66 per cent (MT043777.1) (Fig 2c). A maximum phylogenetic relationship of the isolate was obtained with the accession of *D. phaseolarum* (MT043777.1) infecting tamarind and distant relationship with the accessions of the same pathogen (KX519728.1 and KX510128.1) when related with different accessions of the same pathogen in Mega X software (Fig 3c).

4.6.1.4 Isolate KKYLS

The Gel image revealed that amplicon of 500bp length of the ITS region was obtained (Plate 26). *In silico* analysis of the ITS sequences of the leafspot pathogen (KKYLS) which was confirmed to be of the genus *Phomopsis* sp., showed a sequence similarity of 95.54 per cent with *Phomopsis heveicola* strain XJJH-3 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence with a query cover of 71 per cent (KY379053.1). The accession of *Diaporthe tulliensis* (telomorph of *Phomopsis heveicola*) showed 95.20 per cent (MG832547.1) sequence similarity with the nucleotide sequence of the isolate KKYLS confirming the pathogen as *Phomopsis heveicola* with the sexual stage as *Diaporthe* tulliensis (Fig 2d). The phylogenetic relationship of the isolate KKYLS with other accessions of the same pathogen obtained from the BLASTN analysis was performed in Mega X software and found a maximum relationship with the accession of *P. heveicola* (MW775533.1) and distantly related to the accessions of same pathogen (MH930430.1 and EF423529.1) (Fig 3d).

4.6.1.5 Isolate OKMLS

The genus of the fungal isolate associated with the leaf spot sample OKMLS obtained from Oorakam of Thrissur district was confirmed to be *Phomopsis* sp. based on the cultural and morphological characters studied. The Gel image revealed that amplicon of 500bp length of the ITS region was obtained (Plate 27).The BLASTN analysis in NCBI nr database showed 99.82 per cent identity with *Phomopsis heveicola* strain XJJH-3 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence with a query cover of 71 per cent (KY379053.1) with a query cover of 98 per cent and a maximum score of 1048 (Fig 2e). Hence the pathogen was confirmed to be *Phomopsis heveicola* (Fig 3e). The isolate was more phylogenetically related to the accession of *Phomopsis heveicola* (MW775533.1) when analysed with Mega X software (Fig 3d).

4.6.1.6 Isolate OLRLS

The pathogen causing leaf spot OLRLS collected from Ollur of Thrissur district was confirmed to be *Corynespora* sp. on the basis of the cultural and morphological characters studied. The Gel image revealed that amplicon of 500bp length of the ITS region was obtained (Plate 29). *In silico* analysis of the ITS sequences of OLRLS in NCBI database showed a 99.65 per cent sequence similarity with *Corynespora cassiicola* isolate NTOU 4260 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence with 45 per cent query cover (MZ422956.1) (Fig 2f). Thus, the pathogen responsible for the leaf spot OLRLS was confirmed to be *Corynespora cassiicola.* In Mega X software, the phylogenetic relationship of the isolate with other accessions of *C. cassiicola* was analysed and found a maximum relationship with the accession KY806119.1 infecting *Clinacanthus nutans* from Malaysia (Fig 3b).

4.6.1.7 Isolate TLRLS

Based on cultural and morphological characterization, the fungal pathogen associated with the leaf spot sample TLRLS was identified to be *Pestalotiopsis* sp. The

5’ACACTCCGTAACCGGAGGAGCATATCGATAAGCGGAGGAGCATATCTGT
AACCGGAGGAGCATATCTCTTCACGGAGGACCCTAGCAATAAGGGGAGGA
CCCGCCGGCGGCCGGAAGACTCTTGTTTCTATAGTAAAACTCTGAGTAAAA
AACATAGATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCG
ATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGT
GAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGC
ATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCTGGCTTGGTGATGGGGC
ACTGCCTTCTAACGAGGGCAGGCCCTGAAATCTAGTGGCGAGCTCGCTAGG
ACCCCGAGCGTAGTAGTTATATCTCGTTCTGGAAGGCCCTGGCGGTGCCCT
GCCGTTAAACCCCCAACTTCTGAAAATTTGACCTCGGATCAGGTAGGAATA
CCCGCTGAGGTTACGCACACAGGTTCACCTAAGGGACATATCAATAAGCG
GAGGAGCATATCAATAAGCGGAGGAGCATATCAATAAGCGGAGGAGCATA
TCAATAAGCGGAGGAGCATATCAATAAGCGGAGGAGCATATCAATAAGCG
GAGGAGCATATCAATAA3’

Fig 1 c: Nucleotide sequence of ITS gene of *Diaporthe phaseolarum* (IJKLS)

5’TCCTCCGCTTATTGATATGCTCCTCCGCTTATTGATATGCTCCTCCGCTTAT
TGATATGCTCCTCCGCTTATTGATATGCTCCTCCGCTTATTGATATGCTCCTC
CGCTTATTGATATGCCCCGTAGGTGAACCTGAGGAGGAATGGCTCACCGCG
TATTCATACCTGATCCGAGGTCAAAATTTCAGAAGTTGGGATGCTTAACGGC
AGGGCACCGACAGGGCCCTCCAGCAAAGAGATATAACTAATACGCTCGGG
GTCCTGGCGAGCTCGCCACTAGATTTCAGGGCCCGCCCTTTTGCAAAGGCG
GTGCCCCAACACCAAGCCAGGCTTGAGGGTTGAAATGACGCTCGAACAGGC
ATGCCCTCCGGAATACCAGAGGGCGCAATGTGCGTTCAAAGATTCGATGAT
TCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATC
GATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTCATTATGTTTTT
TACTCAGAGATTCACTAAGAAACAAGAGTTCGTTTGGCCGCCGGCGGGCTC
CTCCCTGTCTCCAGGGGGCCTCGCTGAGGAGGCCGGCCACCGCCGAGGCAA
CAGTATAGTCGTATAAGTTCATCAAAGGGTCCTCCGGCGATGCGCCGATAT
GCGCGTTCCAGCAATGATCCCTCCGCAGGTTCACCTACGGAAGCATATCAA
TAAGCGGAGGAGCATATCAATAAGCGGAGGAGCATATCAATAAGCGGAGG
AGCATATCATTAAGCG3’

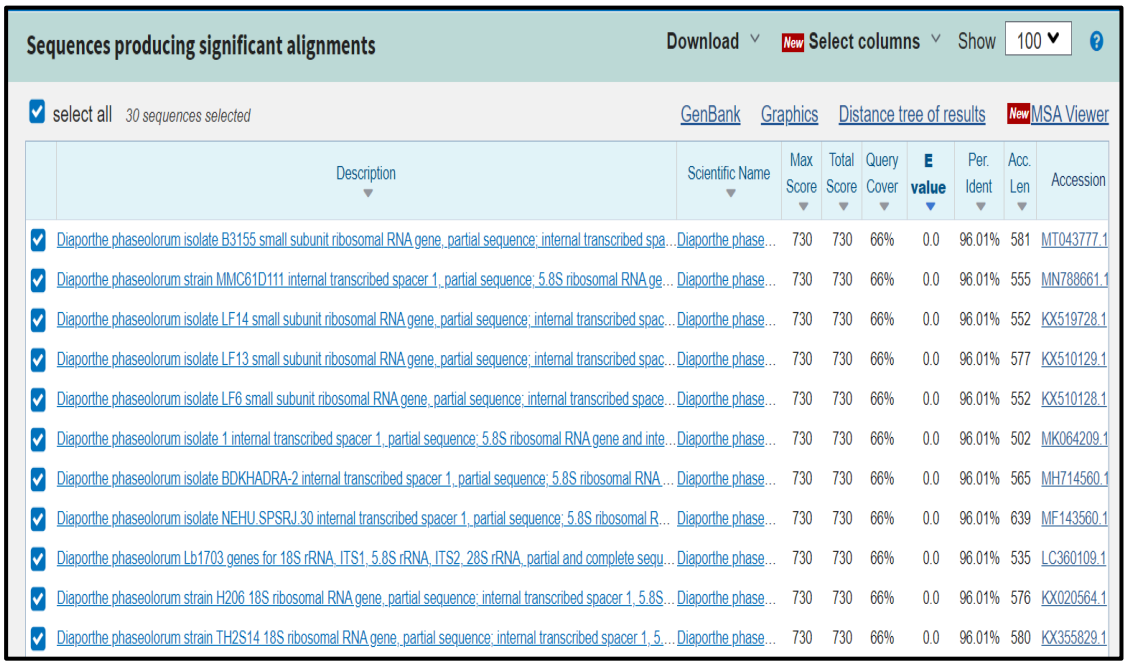
Fig 1 d: Nucleotide sequence of ITS gene of *Phomopsis heveicola* (KKYLS)

5’TGATTCTTCCCGCTTATTGATATGCTTAAGTTCAGCGGGTATTCCTAC
CTGATCCGTAGGTCAAAATTTCACGAAGTTGGGGGTTTAACGGCAGGG
CACCGCCAGGGCCTTCCAGAACGAGATATAACTACTACGCTCGGGGTC
CTGGCGAGCTCGCCACTAGATTTCAGGGCCCGCCCTTTTCCAAAGGCG
GTGCCCCAACACCAAGCCAGGCTTGAGGGTTGAAATGACGCTCGAACA
GGCATGCCCTCCGGAATACCAGAGGGCGCAATGTGCGTTCAAAGATTC
GATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGC
GTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGAT
TCATTATGTTTTTTACTCAGAGATTCACTAAGAAACAAGAGTTTGTTTG
GCCGCCGGCGGGCTGCTCCCTGTCTCCAGGGGGCCTCGGTGAGGAGGC
CGGCCAGCGCCGAGGCAACAGTATAGGTATAAGTTCACAAAGGGTTTC
TGGGTGCGCCGAAGCGCGTTCCAGCAATGATCCCTCCGCAGGTTCACC
TAG3’

Fig 1 e: Nucleotide sequence of ITS gene of *Phomopsis heveicola* (OKMLS)

5’AACAAATCAATACGCTCGAGGTAGCACCCTTTGTTCGGAGGACCATA
TCATTAACCGGAGGAGCTCATCTGTAAACGGAGAACCATATCAATACC
CGGAGGAACTATAAGTACACGTCAGGAGCATACAAAACAAACTATGA
ACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCAATAAAAAACGC
ATCGAAATGCGATAAGTACTGTGAATTGAAGAATTCAGTGAATCATCG
AATCTTTGAACGCACATTGCGCCCTTTGGTATTCCTTAGGGCATGCCTG
TTCGAGCGTCATTTCAACCCTCAAGCCTAGCTTGGTGTTGGGCGTCTGT
CCCGCCTCCGCGCGCCTGGACTCGCCTCAAAAGCATTGGCGGCCGGTTC
CCAGCAGGCCACGAGCGCATCAGAGCAAGCGCTGAAGTGGCTGCGGGT
CGGCGCACCATTGATAAGCCCCCACACAACAGTTATATACTTTTTGGAA
CTCTGGAATAATCCTGCGAAAAGCGATACGTTCCTTGCATTACAGAAAT
CATCCTACCATATCAAATATATGATCATACAGAGGACGACAAGATCAA
TAAGCGGAGGAGCATATCAATAAGCGAGAGGAGCATATCAATAAGCG
GAAGAGCATATCAATAAGCGGAGGAGCATATCAATAAGCGGAGGAGC
ATATCAATAAGCGGAGGAGCATATCAATAAGCGGAGGAGCATATCAAT
AAGCGGAGGAGCATATCAATAAGCGGAGGAGCATATCAATAAGCGGA
GGAGCATATCAATAAGCGAGGAGCAT3’

Fig 1 f: Nucleotide sequence of ITS gene of *Corynespora cassiicola* (OLRLS)



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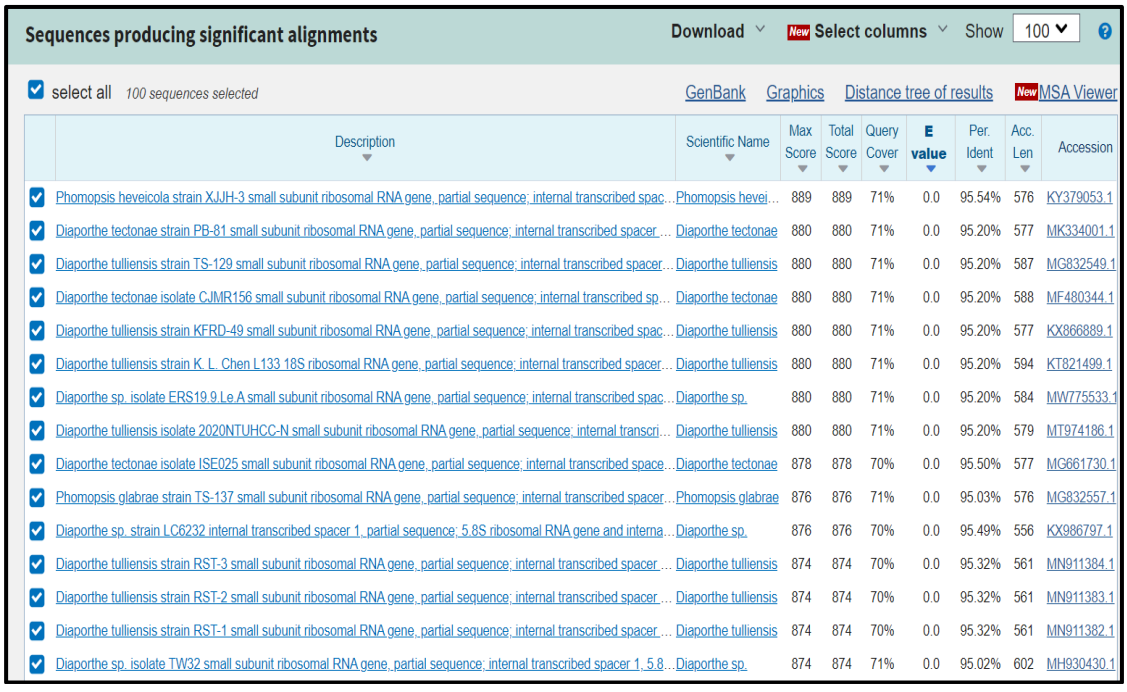
select all 30 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Diaporthe phaseolorum isolate B3155 small subunit ribosomal RNA gene, partial sequence; internal transcribed spa...	Diaporthe phase...	730	730	66%	0.0	96.01%	581	MT043777.1
Diaporthe phaseolorum strain MMC61D111 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ge...	Diaporthe phase...	730	730	66%	0.0	96.01%	555	MN788661.1
Diaporthe phaseolorum isolate LF14 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Diaporthe phase...	730	730	66%	0.0	96.01%	552	KX519728.1
Diaporthe phaseolorum isolate LF13 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Diaporthe phase...	730	730	66%	0.0	96.01%	577	KX510129.1
Diaporthe phaseolorum isolate LF6 small subunit ribosomal RNA gene, partial sequence; internal transcribed space...	Diaporthe phase...	730	730	66%	0.0	96.01%	552	KX510128.1
Diaporthe phaseolorum isolate 1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inte...	Diaporthe phase...	730	730	66%	0.0	96.01%	502	MK064209.1
Diaporthe phaseolorum isolate BDKHADRA-2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ...	Diaporthe phase...	730	730	66%	0.0	96.01%	565	MH714560.1
Diaporthe phaseolorum isolate NEHU.SPSRJ.30 internal transcribed spacer 1, partial sequence; 5.8S ribosomal R...	Diaporthe phase...	730	730	66%	0.0	96.01%	639	MF143560.1
Diaporthe phaseolorum Lb1703 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequ...	Diaporthe phase...	730	730	66%	0.0	96.01%	535	LC360109.1
Diaporthe phaseolorum strain H206 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S...	Diaporthe phase...	730	730	66%	0.0	96.01%	576	KX020564.1
Diaporthe phaseolorum strain TH2S14 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5,...	Diaporthe phase...	730	730	66%	0.0	96.01%	580	KX355829.1

Fig 2 c: BLASTN text output of nucleotide sequence of ITS gene of *Diaporthe phaseolarum* (IJKLS)

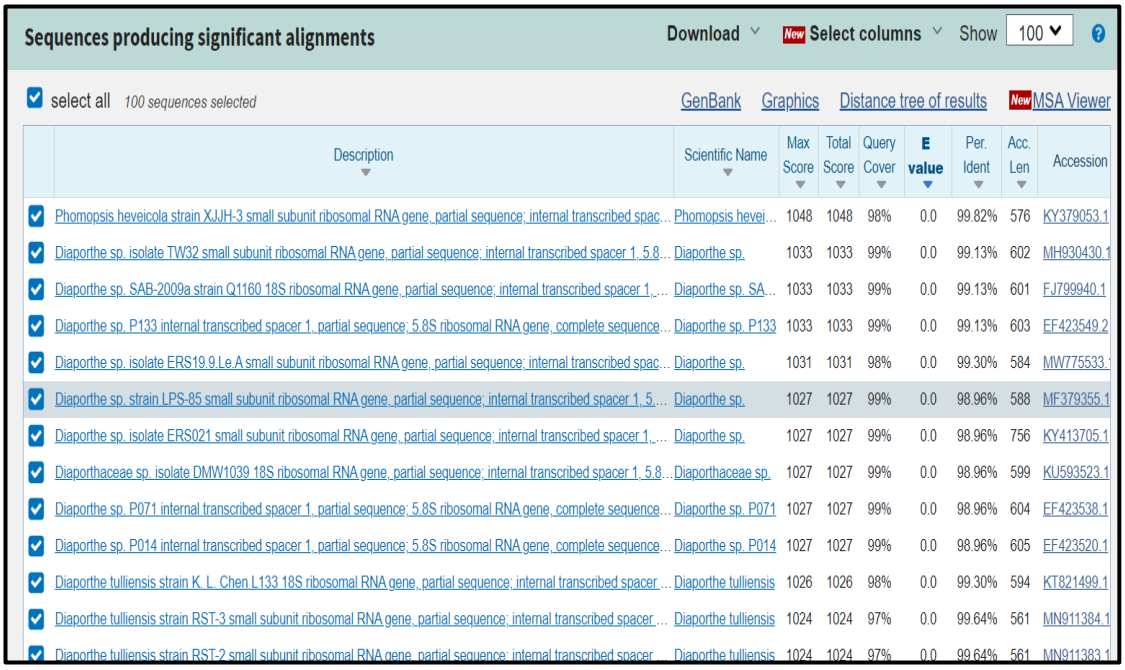


Sequences producing significant alignments — Download — Select columns — Show 100

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
Phomopsis heveicola strain XJJH-3 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Phomopsis hevei...	889	889	71%	0.0	95.54%	576	KY379053.1
Diaporthe tectonae strain PB-81 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer ...	Diaporthe tectonae	880	880	71%	0.0	95.20%	577	MK334001.1
Diaporthe tulliensis strain TS-129 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer...	Diaporthe tulliensis	880	880	71%	0.0	95.20%	587	MG832549.1
Diaporthe tectonae isolate CJMR156 small subunit ribosomal RNA gene, partial sequence; internal transcribed sp...	Diaporthe tectonae	880	880	71%	0.0	95.20%	588	MF480344.1
Diaporthe tulliensis strain KFRD-49 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Diaporthe tulliensis	880	880	71%	0.0	95.20%	577	KX866889.1
Diaporthe tulliensis strain K. L. Chen L133 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ...	Diaporthe tulliensis	880	880	71%	0.0	95.20%	594	KT821499.1
Diaporthe sp. isolate ERS19.9.Le.A small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Diaporthe sp.	880	880	71%	0.0	95.20%	584	MW775533.1
Diaporthe tulliensis isolate 2020NTUHCC-N small subunit ribosomal RNA gene, partial sequence; internal transcri...	Diaporthe tulliensis	880	880	71%	0.0	95.20%	579	MT974186.1
Diaporthe tectonae isolate ISE025 small subunit ribosomal RNA gene, partial sequence; internal transcribed space...	Diaporthe tectonae	878	878	70%	0.0	95.50%	577	MG661730.1
Phomopsis glabrae strain TS-137 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer ...	Phomopsis glabrae	876	876	71%	0.0	95.03%	576	MG832557.1
Diaporthe sp. strain LC6232 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and interna...	Diaporthe sp.	876	876	70%	0.0	95.49%	556	KX986797.1
Diaporthe tulliensis strain RST-3 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer ...	Diaporthe tulliensis	874	874	70%	0.0	95.32%	561	MN911384.1
Diaporthe tulliensis strain RST-2 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer ...	Diaporthe tulliensis	874	874	70%	0.0	95.32%	561	MN911383.1
Diaporthe tulliensis strain RST-1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer ...	Diaporthe tulliensis	874	874	70%	0.0	95.32%	561	MN911382.1
Diaporthe sp. isolate TW32 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8...	Diaporthe sp.	874	874	71%	0.0	95.02%	602	MH930430.1

Fig 2 d: BLASTN text output of nucleotide sequence of ITS gene of *Phomopsis heveicola* (KKYLS)

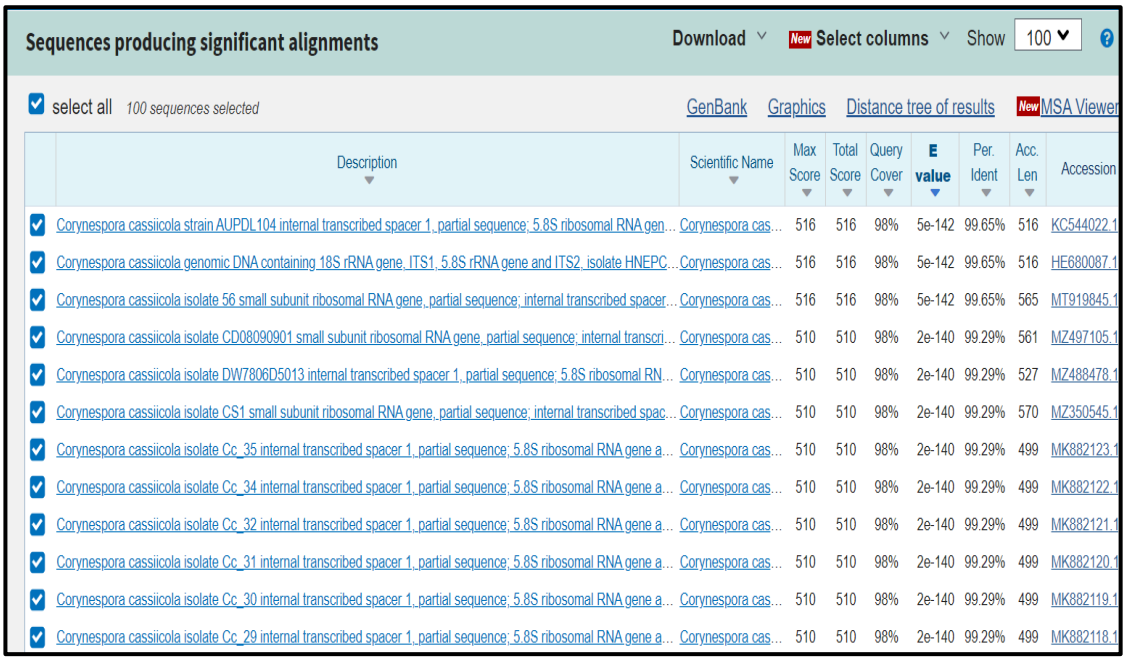


Sequences producing significant alignments — Download — New Select columns — Show 100

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

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Phomopsis heveicola strain XJJH-3 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Phomopsis hevei...	1048	1048	98%	0.0	99.82%	576	KY379053.1
Diaporthe sp. isolate TW32 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8...	Diaporthe sp.	1033	1033	99%	0.0	99.13%	602	MH930430.1
Diaporthe sp. SAB-2009a strain Q1160 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, ...	Diaporthe sp. SA...	1033	1033	99%	0.0	99.13%	601	FJ799940.1
Diaporthe sp. P133 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence...	Diaporthe sp. P133	1033	1033	99%	0.0	99.13%	603	EF423549.2
Diaporthe sp. isolate ERS19.9.Le.A small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Diaporthe sp.	1031	1031	98%	0.0	99.30%	584	MW775533.1
Diaporthe sp. strain LPS-85 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5....	Diaporthe sp.	1027	1027	99%	0.0	98.96%	588	MF379355.1
Diaporthe sp. isolate ERS021 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1,...	Diaporthe sp.	1027	1027	99%	0.0	98.96%	756	KY413705.1
Diaporthaceae sp. isolate DMW1039 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8...	Diaporthaceae sp.	1027	1027	99%	0.0	98.96%	599	KU593523.1
Diaporthe sp. P071 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence...	Diaporthe sp. P071	1027	1027	99%	0.0	98.96%	604	EF423538.1
Diaporthe sp. P014 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence...	Diaporthe sp. P014	1027	1027	99%	0.0	98.96%	605	EF423520.1
Diaporthe tulliensis strain K. L. Chen L133 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ...	Diaporthe tulliensis	1026	1026	98%	0.0	99.30%	594	KT821499.1
Diaporthe tulliensis strain RST-3 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer ...	Diaporthe tulliensis	1024	1024	97%	0.0	99.64%	561	MN911384.1
Diaporthe tulliensis strain RST-2 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer ...	Diaporthe tulliensis	1024	1024	97%	0.0	99.64%	561	MN911383.1

Fig 2 e: BLASTN text output of nucleotide sequence of ITS gene of *Phomopsis heveicola* (OKMLS)



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

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

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Corynespora cassiicola strain AUPDL104 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen...	Corynespora cas...	516	516	98%	5e-142	99.65%	516	KC544022.1
Corynespora cassiicola genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene and ITS2, isolate HNEPC...	Corynespora cas...	516	516	98%	5e-142	99.65%	516	HE680087.1
Corynespora cassiicola isolate 56 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer...	Corynespora cas...	516	516	98%	5e-142	99.65%	565	MT919845.1
Corynespora cassiicola isolate CD08090901 small subunit ribosomal RNA gene, partial sequence; internal transcri...	Corynespora cas...	510	510	98%	2e-140	99.29%	561	MZ497105.1
Corynespora cassiicola isolate DW7806D5013 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RN...	Corynespora cas...	510	510	98%	2e-140	99.29%	527	MZ488478.1
Corynespora cassiicola isolate CS1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Corynespora cas...	510	510	98%	2e-140	99.29%	570	MZ350545.1
Corynespora cassiicola isolate Cc_35 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Corynespora cas...	510	510	98%	2e-140	99.29%	499	MK882123.1
Corynespora cassiicola isolate Cc_34 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Corynespora cas...	510	510	98%	2e-140	99.29%	499	MK882122.1
Corynespora cassiicola isolate Cc_32 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Corynespora cas...	510	510	98%	2e-140	99.29%	499	MK882121.1
Corynespora cassiicola isolate Cc_31 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Corynespora cas...	510	510	98%	2e-140	99.29%	499	MK882120.1
Corynespora cassiicola isolate Cc_30 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Corynespora cas...	510	510	98%	2e-140	99.29%	499	MK882119.1
Corynespora cassiicola isolate Cc_29 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Corynespora cas...	510	510	98%	2e-140	99.29%	499	MK882118.1

Fig 2 f: BLASTN text output of nucleotide sequence of ITS gene of *Corynespora cassiicola* (OLRLS)

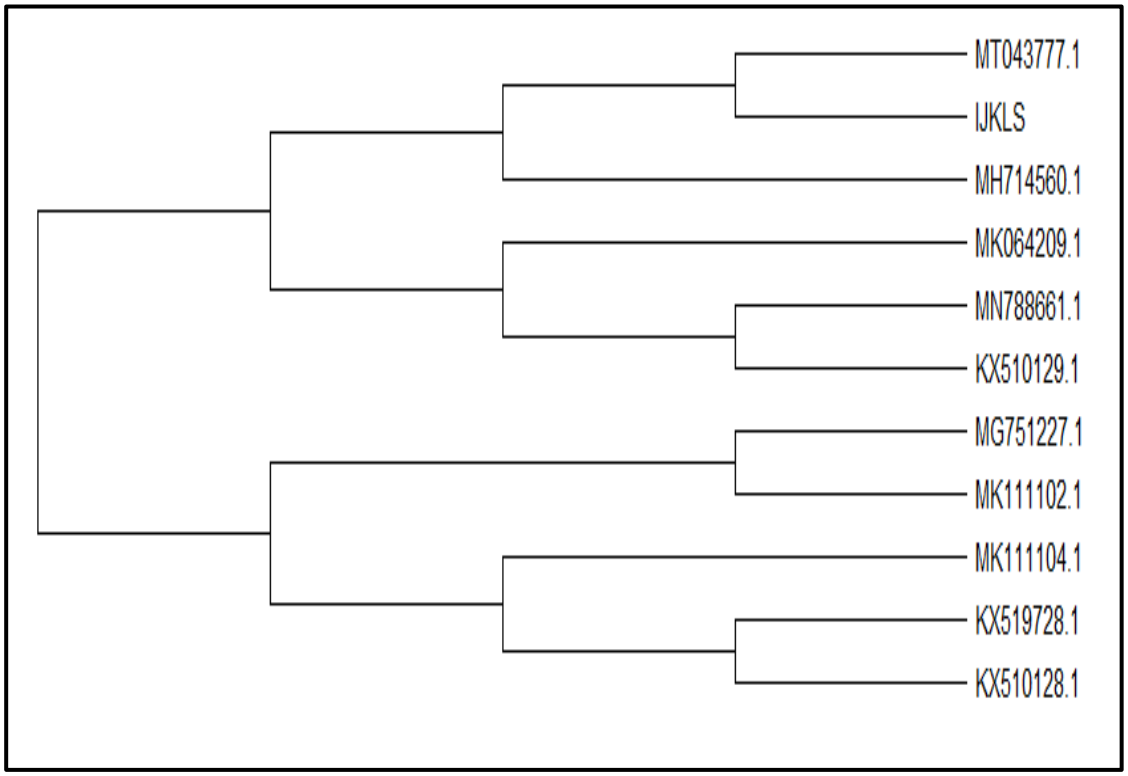


Fig 3 c: Phylogenetic tree of *Diaporthe phaseolarum*

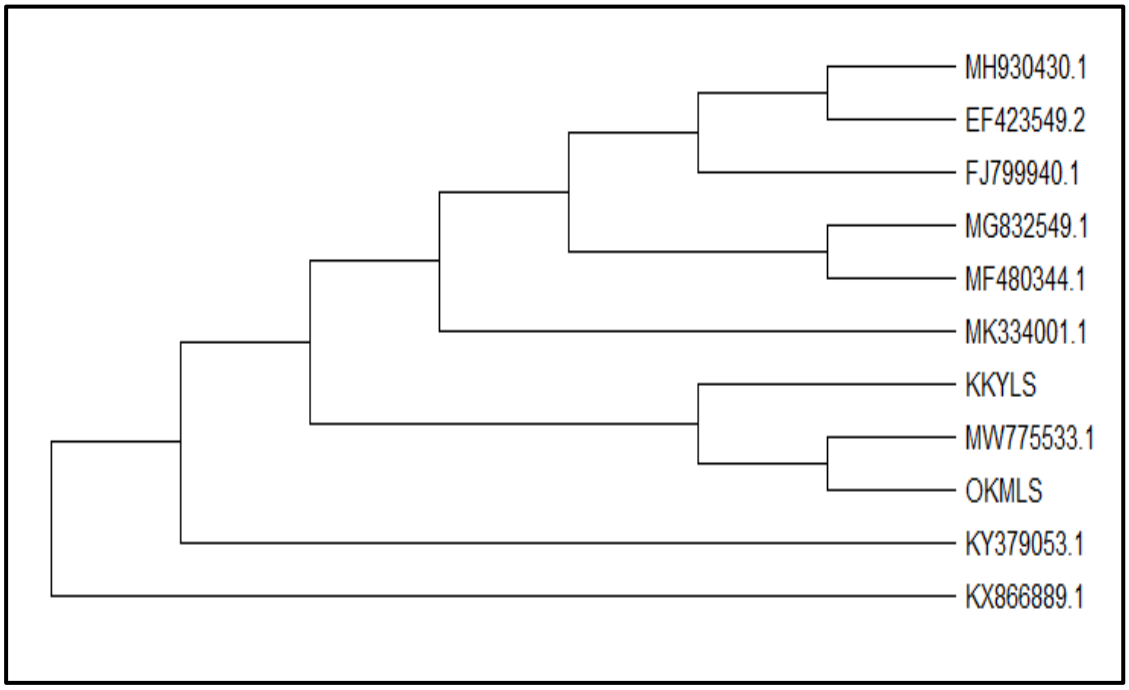


Fig 3 d: Phylogenetic tree of *Phomopsis heveicola*

ITS sequences of the isolate obtained was BLASTN analysed in NCBI nr database and found that these sequences showed 99.81 per cent identity with *Pseudopestalotiopsis theae* strain LCM 985.01 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence with 100 per cent query cover (MF495464.1) (Fig 2g). The Gel image revealed that amplicon of 500bp length of the ITS region was obtained (Plate 29). Thus, the pathogen which was identified as *Pestalotiopsis* sp. based on cultural and morphological characters were confirmed as *Pseudopestalotiopsis theae* on the basis of molecular analysis. The evolutionary relationship of the isolate TLRLS with other accessions of the same pathogen, *Pestalotiopsis thea* and *Pestalotiopsis* sp. when analysed in Mega X software revealed that the isolate showed maximum similarity with the accession of *Pseudopestalotiopsis thea* (MH472583.1) infecting *Ixora chinensis* from China and the isolate was distantly related to *Pestalotiopsis thea* (EF423551.1) (Fig 3e).

4.6.1.8 Isolate NLBLS

Based on the cultural and morphological characteristics, the pathogen isolated from the leaf spot sample NLBLS collected from Nilambur region of Malappuram district was confirmed to be *Lasiodiplodia theobromae*. The Gel image revealed that amplicon of 500bp length of the ITS region was obtained (Plate 26). The BLASTN analysis of nucleotide sequence of ITS gene of the fungal isolate in NCBI database showed a sequence identity of 93.28 per cent with *Lasiodiplodia theobromae* BU-DLa 02 genes for ITS1, 5.8S rRNA, ITS2, partial and complete sequence with a query cover of were more than 93 per cent similar to the accessions of *Lasiodiplodia theobromae* with a query cover of 79 % (LC468781.) (Fig 2h). The evolutionary relationship of the pathogen associated with the leaf spot NLBLS collected from Nilambur with other accessions of the same pathogen showed a maximum relationship with the accession LC468781.1 reported in dragon fruit from Bangladesh and distantly related to the same pathogen (KT325577.1) associated with *Anacardium* sp. (fig 3f).

5’AACTCCCAACCCATGTGAACTTTACCTTTTGTTGCCTCGGCAGAGGTT
ACCTGGTACCTGGAGACAGGTTACCCTGTAGCAACTGCCGGTGGACTA
CTAAACTCTTGTTATTTTATGTAATCTGAGCGTCTTATTTTAATAAGTCA
AAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCA
GCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGA
ATCTTTGAACGCACATTGCGCCCATTAGTATTCTAGTGGGCATGCCTGT
TCGAGCGTCATTTCAACCCTTAAGCCTAGCTTAGTGTTGGGAATTTACA
GTTATGTAATTCCTGAAATACAACGGCGGATCTGTGGTATCCTCTGAGC
GTAGTAAATTATTTCTCGCTTTTGTTAGGTGCTGCAGCTCCCAGCCGCT
AAACCCCCAATTTTTTGTGGTTGACCTCGGATCAGGTAGGAATACCCGC
TGAACTTAAGCATATCAATAAGCGGAG3’

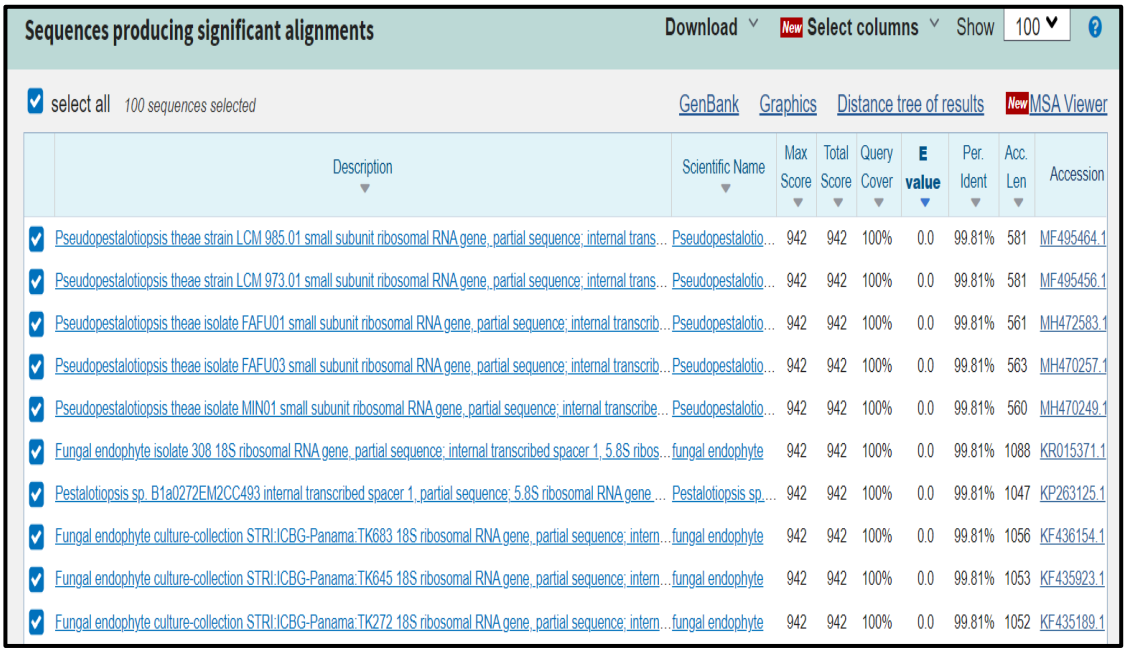
Fig 1 g: Nucleotide sequence of ITS gene of *Pseudopestalotiopsis thea* (TLRLS)

5’GTACTAGCTCCGGCTCGAGTCTCCCCACCCTTTGTGAAGTACCTCTGT
TGCTTTGGAGGATCCGGCCGCCAAAGGACCTTCAAACTCCAGTCAGTA
AACGCAGACGTCTGATAAACAAGTTAATAAACTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAA
GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC
ATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTAC
AACCCTCAAGCTCTGCTTGGAATTGGGCACCGTCCTCACTGCGGACGC
GCCTCAAAGACCTCGGCGGTGGCTGTTCAGCCCTCAAGCGTAGTAAAA
TACACCTCGCTAAGGAGCGGTTGGCGTCGTCACTCGCCGGACGAACAT
TCAATAAGCGGAGAAGCATATCAATCAAGTCGGATGACCATTCGGATC
AGGTAGGGATACCCGCTGAACTTAAGCATATCGTTAACCTGAGGTGCA
TATCAATAAGCGGAGGAGCATATCAATAAGCGGAGGAGCATATCAATA
AGCGGAGGAGCATATCAATAAGCGGAGGAGCATATCAATAAGCGGAG
GAGCATATCAATAATCGGAGGAACAGAA3’

Fig 1 h: Nucleotide sequence of ITS gene of *Lasiodiplodia theobromae* (NLBLS)

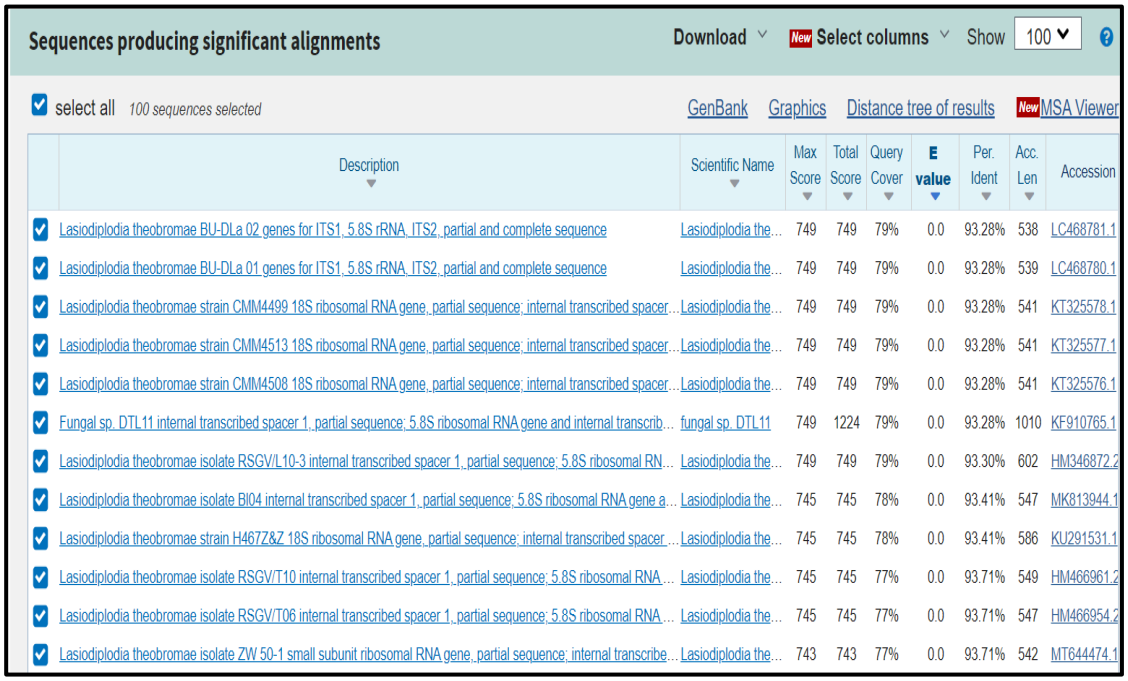
5’TACAATCACTAAGCGGGGAGCATACCAATATGCGGAGGAGCATATAT
CATACCCGGACGAGCAAAACGGGACGGCCAGGAACAGGACCCCTAAA
CTATGATTCTATATGTAACTTCAGAATAAAACCATAAATAAATCAAAA
CTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCA
AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC
TTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCG
AGCGTCATTTCAACCCTCAAGCCCCCGGGTTTGGTGTTGGGGATCGGCG
AGCCCTTGCGGCAAGCCGGCCCCGAAATCTAGTGGCGGTCTCGCTGCA
GCTTCCATTGCGTAATAGTAAAACCCTCGCAACTGGTACGCGGCGCGG
CCAAGCCGTTAAACCCCCAACTTCTGAATGTTGACCTCAAAA3’

Fig 1 i: Nucleotide sequence of ITS gene of *Fusarium fujikuroi* (MNTLW)



Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Pseudopestalotiopsis theae strain LCM 985.01 small subunit ribosomal RNA gene, partial sequence; internal trans...	Pseudopestalotio...	942	942	100%	0.0	99.81%	581	MF495464.1
Pseudopestalotiopsis theae strain LCM 973.01 small subunit ribosomal RNA gene, partial sequence; internal trans...	Pseudopestalotio...	942	942	100%	0.0	99.81%	581	MF495456.1
Pseudopestalotiopsis theae isolate FAFU01 small subunit ribosomal RNA gene, partial sequence; internal transcrib...	Pseudopestalotio...	942	942	100%	0.0	99.81%	561	MH472583.1
Pseudopestalotiopsis theae isolate FAFU03 small subunit ribosomal RNA gene, partial sequence; internal transcrib...	Pseudopestalotio...	942	942	100%	0.0	99.81%	563	MH470257.1
Pseudopestalotiopsis theae isolate MIN01 small subunit ribosomal RNA gene, partial sequence; internal transcribe...	Pseudopestalotio...	942	942	100%	0.0	99.81%	560	MH470249.1
Fungal endophyte isolate 308 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribos...	fungal endophyte	942	942	100%	0.0	99.81%	1088	KR015371.1
Pestalotiopsis sp. B1a0272EM2CC493 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...	Pestalotiopsis sp....	942	942	100%	0.0	99.81%	1047	KP263125.1
Fungal endophyte culture-collection STRI:ICBG-Panama:TK683 18S ribosomal RNA gene, partial sequence; intern...	fungal endophyte	942	942	100%	0.0	99.81%	1056	KF436154.1
Fungal endophyte culture-collection STRI:ICBG-Panama:TK645 18S ribosomal RNA gene, partial sequence; intern...	fungal endophyte	942	942	100%	0.0	99.81%	1053	KF435923.1
Fungal endophyte culture-collection STRI:ICBG-Panama:TK272 18S ribosomal RNA gene, partial sequence; intern...	fungal endophyte	942	942	100%	0.0	99.81%	1052	KF435189.1

Fig 2 g: BLASTN text output of nucleotide sequence of ITS gene of *Pseudopestalotiopsis thea* (TLRLS)

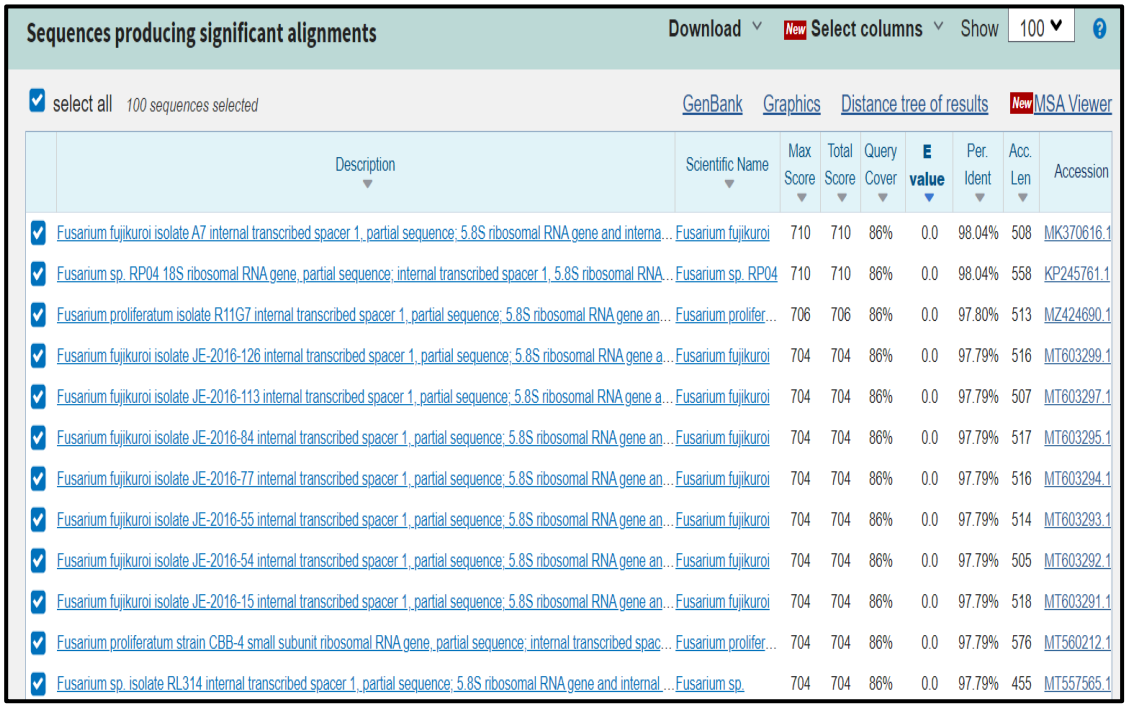


Sequences producing significant alignments — Download — New Select columns — Show 100

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

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Lasiodiplodia theobromae BU-DLa 02 genes for ITS1, 5.8S rRNA, ITS2, partial and complete sequence	Lasiodiplodia the...	749	749	79%	0.0	93.28%	538	LC468781.1
Lasiodiplodia theobromae BU-DLa 01 genes for ITS1, 5.8S rRNA, ITS2, partial and complete sequence	Lasiodiplodia the...	749	749	79%	0.0	93.28%	539	LC468780.1
Lasiodiplodia theobromae strain CMM4499 18S ribosomal RNA gene, partial sequence; internal transcribed spacer...	Lasiodiplodia the...	749	749	79%	0.0	93.28%	541	KT325578.1
Lasiodiplodia theobromae strain CMM4513 18S ribosomal RNA gene, partial sequence; internal transcribed spacer...	Lasiodiplodia the...	749	749	79%	0.0	93.28%	541	KT325577.1
Lasiodiplodia theobromae strain CMM4508 18S ribosomal RNA gene, partial sequence; internal transcribed spacer...	Lasiodiplodia the...	749	749	79%	0.0	93.28%	541	KT325576.1
Fungal sp. DTL11 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcrib...	fungal sp. DTL11	749	1224	79%	0.0	93.28%	1010	KF910765.1
Lasiodiplodia theobromae isolate RSGV/L10-3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RN...	Lasiodiplodia the...	749	749	79%	0.0	93.30%	602	HM346872.2
Lasiodiplodia theobromae isolate BI04 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Lasiodiplodia the...	745	745	78%	0.0	93.41%	547	MK813944.1
Lasiodiplodia theobromae strain H467Z&Z 18S ribosomal RNA gene, partial sequence; internal transcribed spacer...	Lasiodiplodia the...	745	745	78%	0.0	93.41%	586	KU291531.1
Lasiodiplodia theobromae isolate RSGV/T10 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ...	Lasiodiplodia the...	745	745	77%	0.0	93.71%	549	HM466961.2
Lasiodiplodia theobromae isolate RSGV/T06 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ...	Lasiodiplodia the...	745	745	77%	0.0	93.71%	547	HM466954.2
Lasiodiplodia theobromae isolate ZW 50-1 small subunit ribosomal RNA gene, partial sequence; internal transcribe...	Lasiodiplodia the...	743	743	77%	0.0	93.71%	542	MT644474.1

Fig 2 h: BLASTN text output of nucleotide sequence of ITS gene of *Lasiodiplodia theobromae* (NLBLS)



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

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

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Fusarium fujikuroi isolate A7 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and interna...	Fusarium fujikuroi	710	710	86%	0.0	98.04%	508	MK370616.1
Fusarium sp. RP04 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA...	Fusarium sp. RP04	710	710	86%	0.0	98.04%	558	KP245761.1
Fusarium proliferatum isolate R11G7 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an...	Fusarium prolifer...	706	706	86%	0.0	97.80%	513	MZ424690.1
Fusarium fujikuroi isolate JE-2016-126 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Fusarium fujikuroi	704	704	86%	0.0	97.79%	516	MT603299.1
Fusarium fujikuroi isolate JE-2016-113 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Fusarium fujikuroi	704	704	86%	0.0	97.79%	507	MT603297.1
Fusarium fujikuroi isolate JE-2016-84 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an...	Fusarium fujikuroi	704	704	86%	0.0	97.79%	517	MT603295.1
Fusarium fujikuroi isolate JE-2016-77 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an...	Fusarium fujikuroi	704	704	86%	0.0	97.79%	516	MT603294.1
Fusarium fujikuroi isolate JE-2016-55 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an...	Fusarium fujikuroi	704	704	86%	0.0	97.79%	514	MT603293.1
Fusarium fujikuroi isolate JE-2016-54 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an...	Fusarium fujikuroi	704	704	86%	0.0	97.79%	505	MT603292.1
Fusarium fujikuroi isolate JE-2016-15 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an...	Fusarium fujikuroi	704	704	86%	0.0	97.79%	518	MT603291.1
Fusarium proliferatum strain CBB-4 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Fusarium prolifer...	704	704	86%	0.0	97.79%	576	MT560212.1
Fusarium sp. isolate RL314 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal ...	Fusarium sp.	704	704	86%	0.0	97.79%	455	MT557565.1

Fig 2 i: BLASTN text output of nucleotide sequence of ITS gene of *Fusarium fujikuroi* (MNTLW)

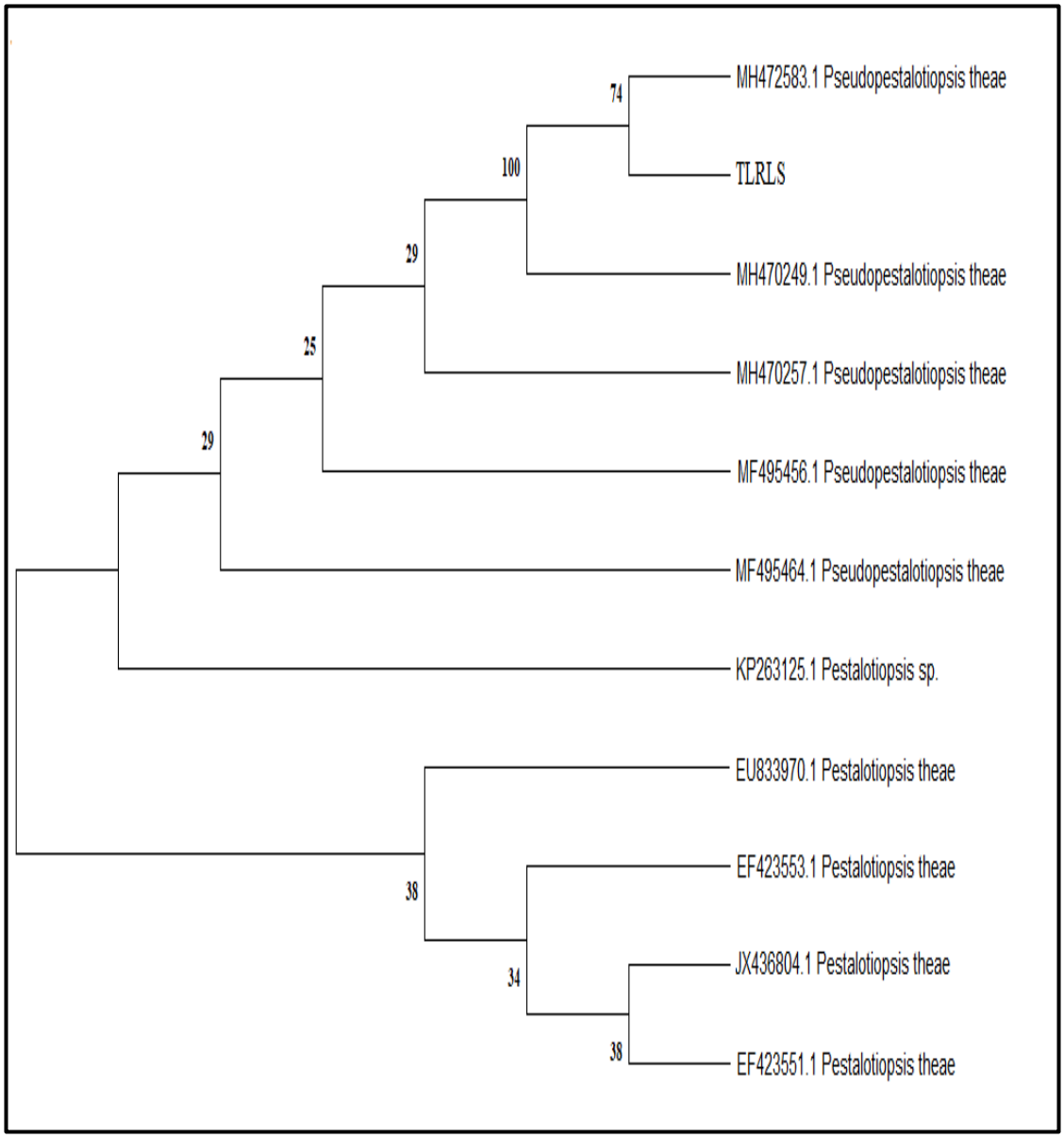


Fig 3 e: Neighbor- joining phylogenetic tree of *Pseudopestalotiopsis thea* constructed in Mega X software with bootstrap values

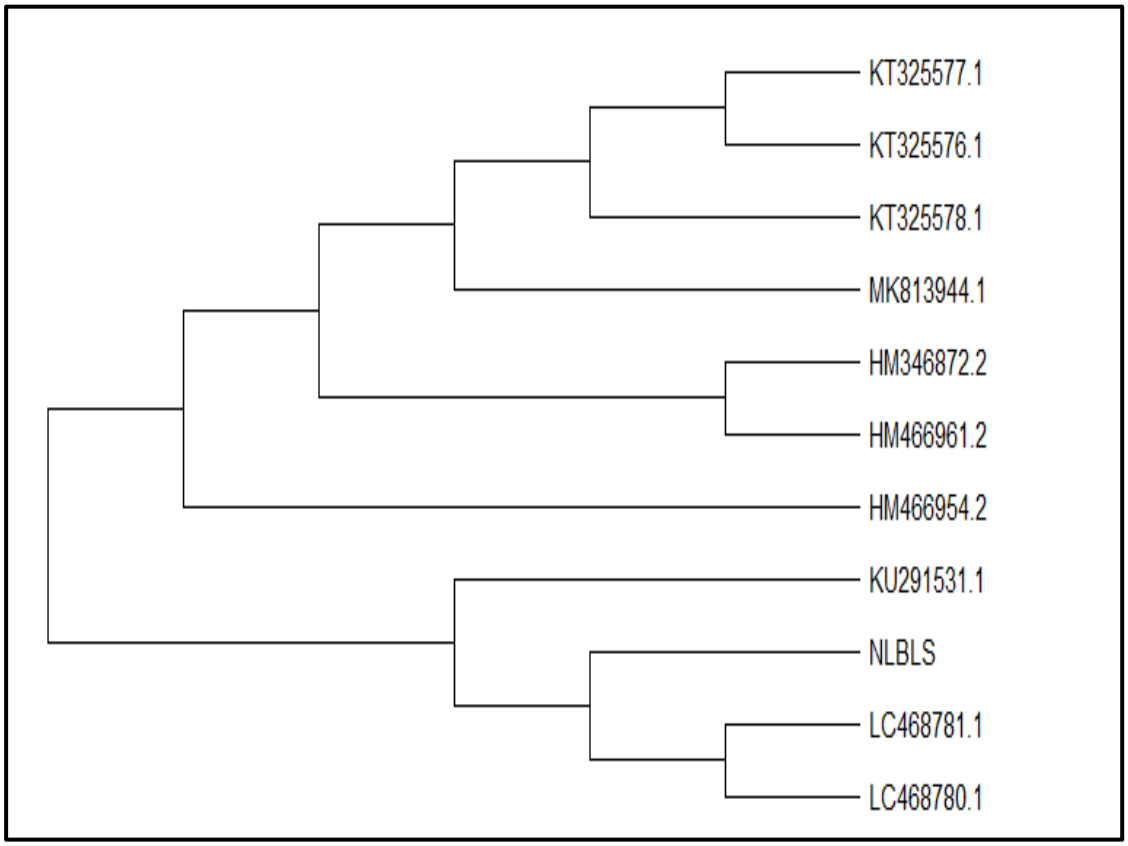


Fig 3 f: Phylogenetic tree of *Lasiodiplodia theobromae*

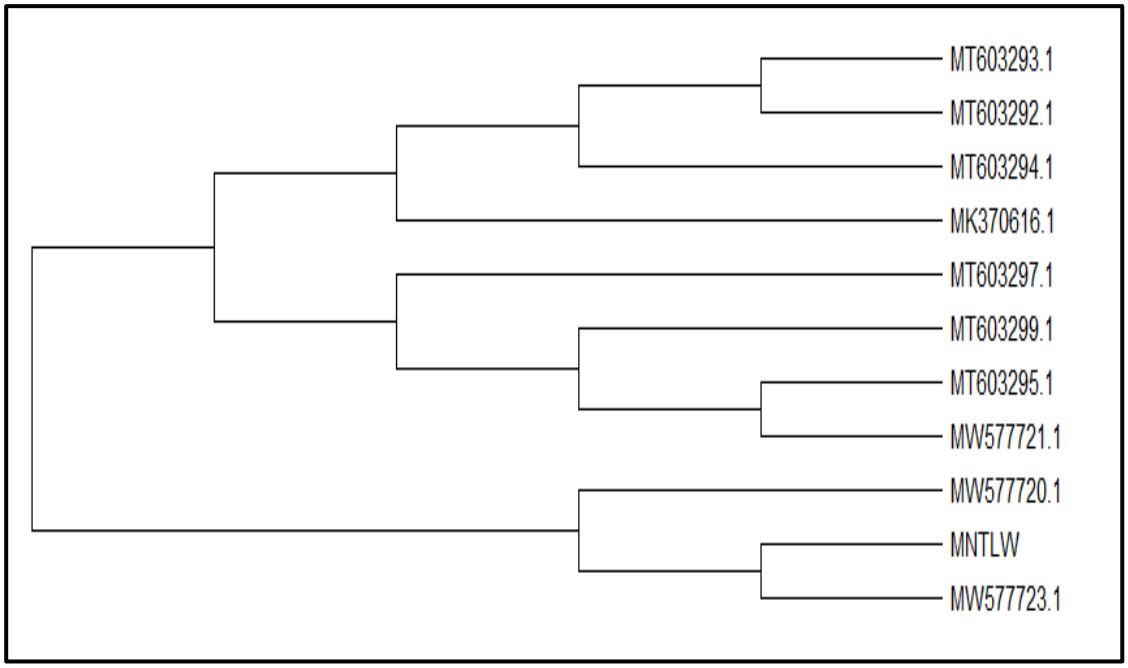


Fig 3 g: Phylogenetic tree of *Fusarium fujikuroi*

4.6.1.9 Isolate MNTLW

The BLASTN analysis of ITS region of the isolate MNTLW in NCBI nr database showed a sequence similarity of 98.04 per cent with *Fusarium fujikuroi* isolate A7 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence with a query cover of 86 per cent (MK370616.1) (Fig 2i). The Gel image revealed that amplicon of 500bp length of the ITS region was obtained (Plate 30). Thus, the pathogen associated with wilting was confirmed to be *Fusarium fujikuroi*. Mega X software was used to find the evolutionary relationship of the pathogen with other accessions of the same pathogen obtained from the top hits in NCBI database. The analysis revealed that the wilt isolate MNTLW was evolutionary more related to the accession of *F. fujikuroi* (MW577721.1) reported from Iraq (Fig 3g).

4.6.1.10 Isolate KMALS

Based on the cultural and morphological characters, the pathogen associated with the leaf spot KMALS collected from Kumaranellur (Palakkad district) was identified to be *Colletotrichum gloeosporioides*. The Gel image revealed that amplicon of 500bp length of the ITS region was obtained (Plate 29). The nucleotide sequences of ITS region of the fungal genome were BLASTN analysed in NCBI database and observed 100 per cent sequence similarity with *Colletotrichum citri-maximae* isolate SR-D1FGL-S1 internal transcribed spacer 1, partial sequence; and large subunit ribosomal RNA gene, partial sequence with a query cover of 99 per cent (MN535219.1), *Colletotrichum gloeosporioides* isolate A1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence (MN067750.1) with 99 per cent query cover, *Colletotrichum siamense* isolate ST1022 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence with a query cover of 99 per cent (MW699607.1)and *Colletotrichum alienum* strain LA-8 internal transcribed spacer 1, partial sequence; with 99 per cent query cover (MH371126.1) (Fig 2j).

4.6.1.11 Isolate VFNLS

The BLASTN analysis of ITS sequences of the isolate VFNLS in NCBI database showed 91.86 per cent identity with *Colletotrichum queenslandicum* isolate NENLS internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence with a query cover of 95% (MW055667.1). Various accessions of different species of *Colletotrichum* such as *C. aenigma* (MZ930213.1, KU642475.1), *C. gloeosporioides* (JF710564.1, JF710559.1, MF143557.1) and *C. siamense* (MK041891.1, MZ725042.1, MT729945.1, MG642980.1) also showed an identical sequence similarity of 97.13 per cent and a query cover of 70 per cent with the ITS sequences of VFNLS (Fig 2k). The Gel image revealed that amplicon of 500bp length of the ITS region was obtained (Plate 27).

4.6.1.12 Isolate TBMLS

The cultural and morphological characterisation of the isolate TBMLS revealed that the pathogen belonged to the genus of *Colletotrichum gloeosporioiodes*. The Gel image revealed that amplicon of 500bp length of the ITS region was obtained (Plate 27). The nucleotide sequence of ITS gene of fungal DNA was subjected to nucleotide blast analysis in NCBI database and found that these sequences showed 96.72 per cent similarity with *Colletotrichum queenslandicum* isolate NENLS internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence with a query cover of 98 % (MW055667.1). It showed 98.80 per cent similarity and 76 per cent query cover with other accessions of the same pathogen (MN547511.1 and MK119210.1) (Fig 2l). Thus, the pathogen associated with the leaf spot TBMLS was confirmed as *C. queenslandicum*.

4.6.1.13 Isolate MNTSR

The *in silico* analysis of the inflorescence rot pathogen MNTSR revealed the identity of the pathogen as *Colletotrichum gloeosporioides*. The BLASTN analysis of the ITS sequences in NCBI database showed an identity percentage of 98.20 and query cover of 90 per cent with *Colletotrichum gloeosporioides* strain Y18 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

(MT729940.1) and *Colletotrichum* sp. 2 JM-2013 isolate CJBL2-37 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence (KC895547.1). The Gel image revealed that amplicon of 500bp length of the ITS region was obtained (Plate 27). Among the top hits obtained in the database, the accessions of *C. gloeosporioides* and *C. siamense* were showing 90 per cent query cover and 97.99 per cent identity with the ITS sequences of MNTSR (Fig 2m).

4.6.1.14 Isolate PBALS

The pathogen associated with the leaf spot PBALS was identified as *Colletotrichum gloeosporioides* based on cultural and morphological characters. The Gel image revealed that amplicon of 500bp length of the ITS region was obtained (Plate 27). On BLASTN analysing the ITS sequences of the pathogen in NCBI nr database, the isolate showed a sequence similarity of 95.07 per cent and a query cover of 87 per cent with *Colletotrichum gloeosporioides* isolate OBC1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, and 28S ribosomal RNA gene, partial sequence (JF710559.1). But the isolate also showed similarity with the accessions of *C. siamense* (MZ725042.1 and MT729945.1) where a sequence similarity of 94.87 per cent and a query cover of 88 per cent were found (Fig 2n).

4.6.1.15 Isolate PNMSR

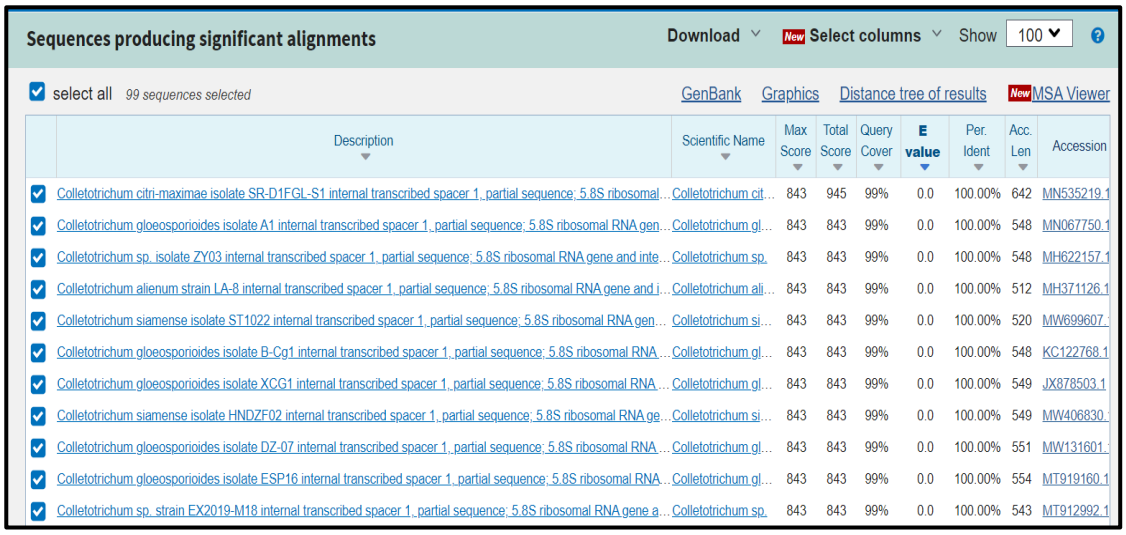
The BLASTN analysis of nucleotide sequences of ITS gene in the genome of fungal isolate PNMSR in NCBI database showed a sequence similarity of 95.10 per cent with *Colletotrichum queenslandicum* isolate NENLS internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence with a query cover of 100 % (MW055667.1). But the sequences showed 99.38 per cent identity with *Colletotrichum aotearoa* strain BL27 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence with a query cover of 78 % (MN273085.1) (Fig 2o). The Gel image revealed that amplicon of 500bp length of the ITS region was obtained (Plate 27).

5’TAACCCTTTGTGACATACCTATAACTGTTGCTTCGGCGGGTAGGGTCT
CCGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGG
AGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGC
AAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGA
AGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA
ATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGC
ATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGG
GCCCTACAGCTGATGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGG
AGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGAGGGA
CTCTTGCCGTAAAACCCCCTAA3’

Fig 1 j: Nucleotide sequence of ITS gene of *Colletotrichum gloeosporioides* (KMALS)

5’CCCCCATCCCCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCG
CCGGAGGATAACCAAACTCTGATTTAACGACGCTTCTTCTGAGTGGTAC
AAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCG
ATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCA
GTGAATCATGGAATCTTCGAACGCACATTGCGCCCGCCAGCATTCTGG
CGGGCATGCCCGTTCGAGCGTCATTTCAACCCTCAAGCTATGCTTGCTG
TAGGGGCCCTACAGTTGCAGTAGGCCCTCAAAGCATATCAATAAGCCT
AGGAGCATATCAATAAGCGGAGGAGCATATCAATAAGCGGAGGAGCA
TATCAATAAGCGGAGGAGCATATCAATAAGCGGAGGAGCATATCAATA
AGCGGAGAGCATCA3’

Fig 1 k: Nucleotide sequence of ITS region of *Colletotrichum gloeosporioides* (VFNLS)

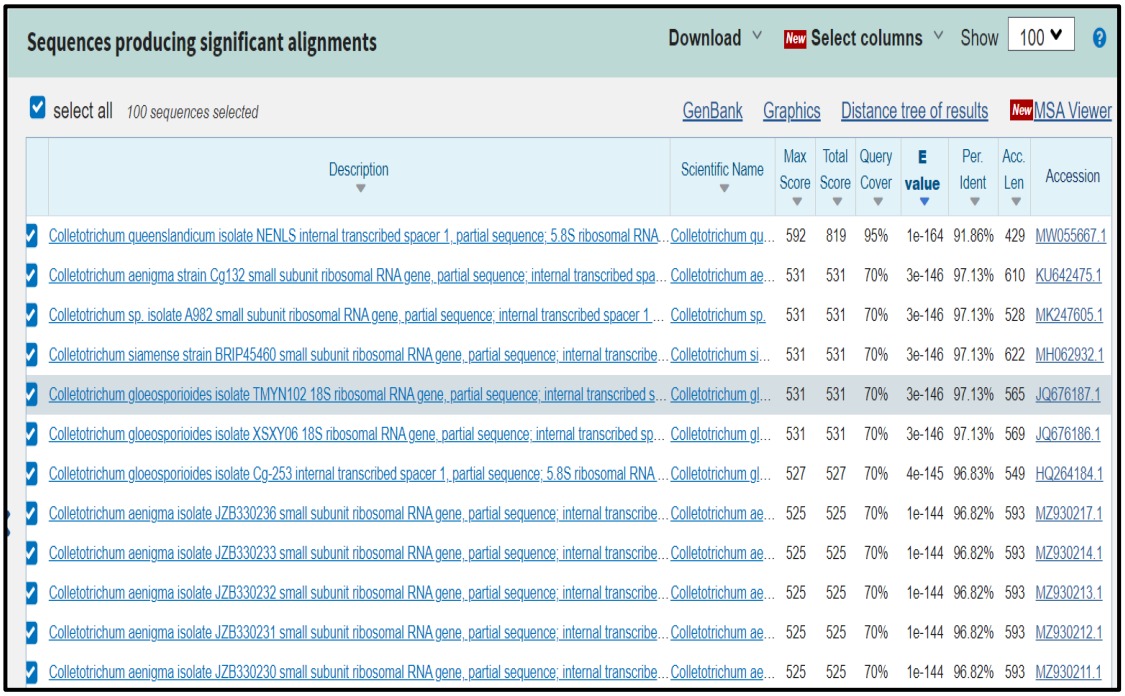


Sequences producing significant alignments — Download — Select columns — Show 100

select all 99 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 citri-maximae isolate SR-D1FGL-S1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal...	Colletotrichum cit...	843	945	99%	0.0	100.00%	642	MN535219.1
Colletotrichum gloeosporioides isolate A1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen...	Colletotrichum gl...	843	843	99%	0.0	100.00%	548	MN067750.1
Colletotrichum sp. isolate ZY03 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inte...	Colletotrichum sp.	843	843	99%	0.0	100.00%	548	MH622157.1
Colletotrichum alienum strain LA-8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i...	Colletotrichum ali...	843	843	99%	0.0	100.00%	512	MH371126.1
Colletotrichum siamense isolate ST1022 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen...	Colletotrichum si...	843	843	99%	0.0	100.00%	520	MW699607.1
Colletotrichum gloeosporioides isolate B-Cg1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA...	Colletotrichum gl...	843	843	99%	0.0	100.00%	548	KC122768.1
Colletotrichum gloeosporioides isolate XCG1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA...	Colletotrichum gl...	843	843	99%	0.0	100.00%	549	JX878503.1
Colletotrichum siamense isolate HNDZF02 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ge...	Colletotrichum si...	843	843	99%	0.0	100.00%	549	MW406830.1
Colletotrichum gloeosporioides isolate DZ-07 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA...	Colletotrichum gl...	843	843	99%	0.0	100.00%	551	MW131601.1
Colletotrichum gloeosporioides isolate ESP16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA...	Colletotrichum gl...	843	843	99%	0.0	100.00%	554	MT919160.1
Colletotrichum sp. strain EX2019-M18 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Colletotrichum sp.	843	843	99%	0.0	100.00%	543	MT912992.1

Fig 2 j: BLASTN text output of nucleotide sequence of ITS gene of *Colletotrichum gloeosporioides* (KMALS)



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
Colletotrichum queenslandicum isolate NENLS internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA...	Colletotrichum qu...	592	819	95%	1e-164	91.86%	429	MW055667.1
Colletotrichum aenigma strain Cg132 small subunit ribosomal RNA gene, partial sequence; internal transcribed spa...	Colletotrichum ae...	531	531	70%	3e-146	97.13%	610	KU642475.1
Colletotrichum sp. isolate A982 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1...	Colletotrichum sp.	531	531	70%	3e-146	97.13%	528	MK247605.1
Colletotrichum siamense strain BRIP45460 small subunit ribosomal RNA gene, partial sequence; internal transcribe...	Colletotrichum si...	531	531	70%	3e-146	97.13%	622	MH062932.1
Colletotrichum gloeosporioides isolate TMYN102 18S ribosomal RNA gene, partial sequence; internal transcribed s...	Colletotrichum gl...	531	531	70%	3e-146	97.13%	565	JQ676187.1
Colletotrichum gloeosporioides isolate XSXY06 18S ribosomal RNA gene, partial sequence; internal transcribed sp...	Colletotrichum gl...	531	531	70%	3e-146	97.13%	569	JQ676186.1
Colletotrichum gloeosporioides isolate Cg-253 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA...	Colletotrichum gl...	527	527	70%	4e-145	96.83%	549	HQ264184.1
Colletotrichum aenigma isolate JZB330236 small subunit ribosomal RNA gene, partial sequence; internal transcribe...	Colletotrichum ae...	525	525	70%	1e-144	96.82%	593	MZ930217.1
Colletotrichum aenigma isolate JZB330233 small subunit ribosomal RNA gene, partial sequence; internal transcribe...	Colletotrichum ae...	525	525	70%	1e-144	96.82%	593	MZ930214.1
Colletotrichum aenigma isolate JZB330232 small subunit ribosomal RNA gene, partial sequence; internal transcribe...	Colletotrichum ae...	525	525	70%	1e-144	96.82%	593	MZ930213.1
Colletotrichum aenigma isolate JZB330231 small subunit ribosomal RNA gene, partial sequence; internal transcribe...	Colletotrichum ae...	525	525	70%	1e-144	96.82%	593	MZ930212.1
Colletotrichum aenigma isolate JZB330230 small subunit ribosomal RNA gene, partial sequence; internal transcribe...	Colletotrichum ae...	525	525	70%	1e-144	96.82%	593	MZ930211.1

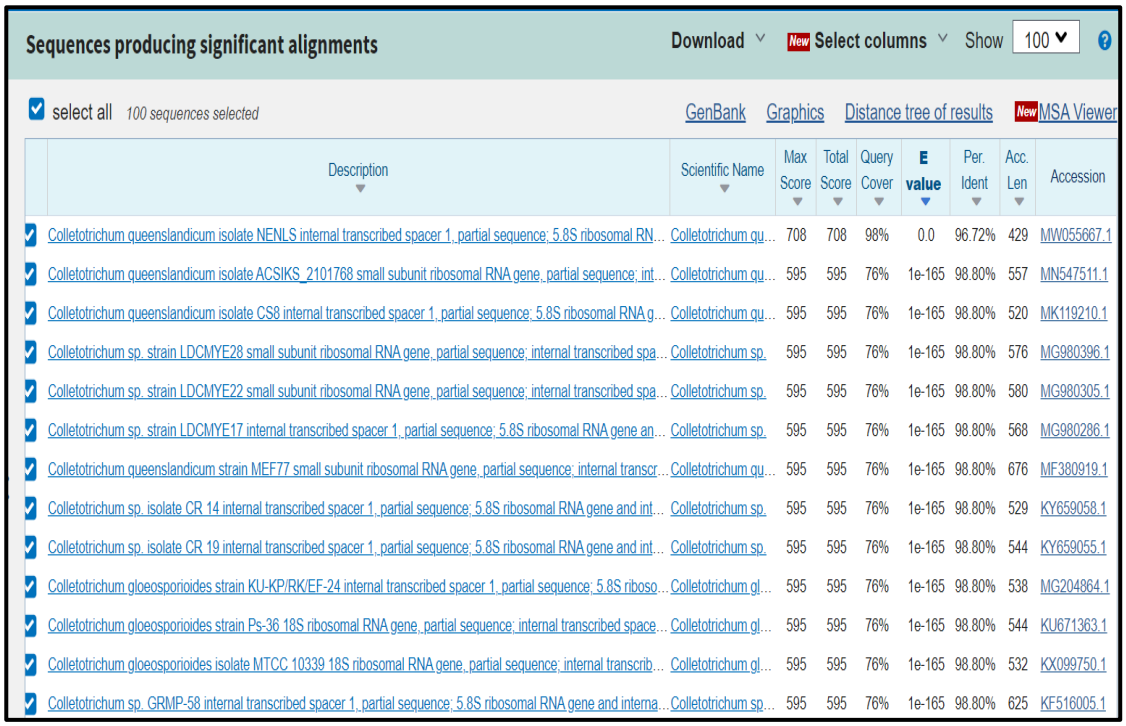
Fig 2 k: BLASTN text output of nucleotide sequence of ITS gene of *Colletotrichum gloeosporioides* (VFNLS)

5’CTGTTTCCCTCCCCCCCGGGCGGGTCGGCGCTCGCCGGAGGATAACC
AAACTCTGATTTAACGACGTTTCTTTTGAGTGGTACAAGCAAATAATCA
AAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCA
GCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGA
ATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGT
TCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACA
GCCGATGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCTTCCT
TAAGCGGTGAAGCTTATCATTACGCGGAGGAGCATATCAATAAGCGGA
GGAGCATATCACTAAGCGGAGGAGCATATCAATAAGCGGAGAGCATTC
3’

Fig 1 l: Nucleotide sequence of ITS region of *Colletotrichum gloeosporioides* (TBMLS)

5’TCGCTCTCACCCTTTGTGACATACCTATAACTGTTGCTTCGGCGGGTA
GGGTCTCAGCGACCCTCCCGGCCTCCCACCTCCGGGCGGGTCGGCGCC
CGCCGGAGGATAACCAAACACTGATTTAACGACGTTTCTTCTGAGTGG
TACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCA
TCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
TCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCT
GGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGG
TGTTGGGGCCCTACAGCTGATGTACGCCCTCAAAGGAAGTGGCAGACC
TCTCTCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATC
CGGACGGACTCCGGCGGTGCACCCCCTAAGCGGCGAAGCAGTCAATAA
ATCGAGAGCAT3’

Fig 1 m: Nucleotide sequence of ITS region of *Colletotrichum gloeosporioides* (MNTSR)



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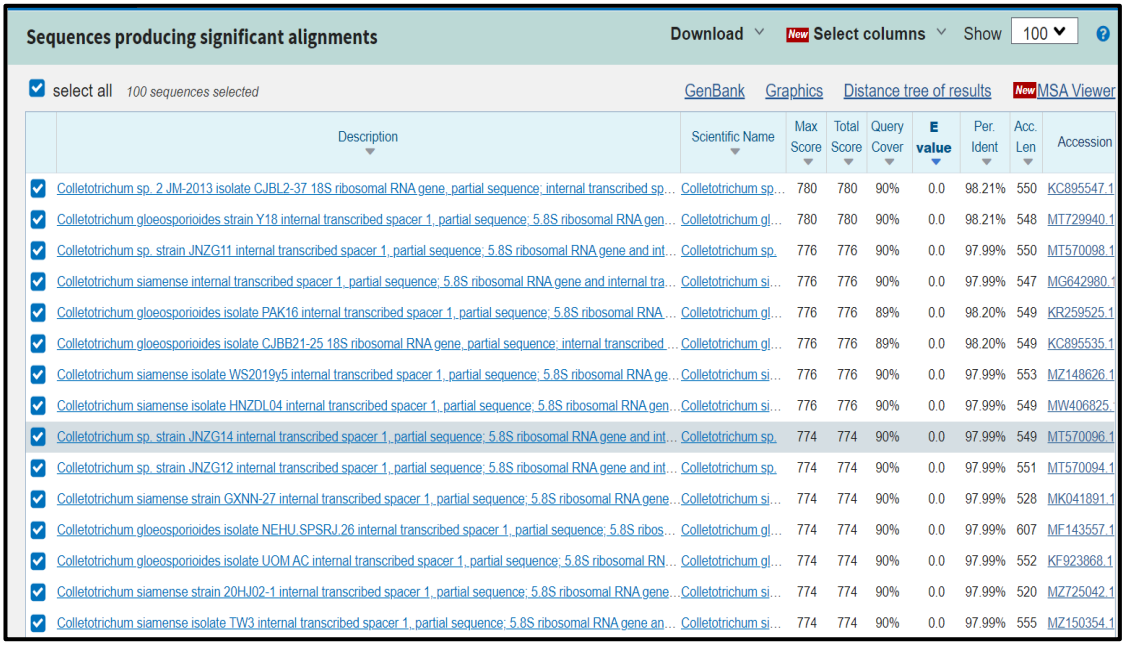
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Colletotrichum queenslandicum isolate NENLS internal transcribed spacer 1, partial sequence; 5.8S ribosomal RN...	Colletotrichum qu...	708	708	98%	0.0	96.72%	429	MW055667.1
Colletotrichum queenslandicum isolate ACSIKS_2101768 small subunit ribosomal RNA gene, partial sequence; int...	Colletotrichum qu...	595	595	76%	1e-165	98.80%	557	MN547511.1
Colletotrichum queenslandicum isolate CS8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA g...	Colletotrichum qu...	595	595	76%	1e-165	98.80%	520	MK119210.1
Colletotrichum sp. strain LDCMYE28 small subunit ribosomal RNA gene, partial sequence; internal transcribed spa...	Colletotrichum sp.	595	595	76%	1e-165	98.80%	576	MG980396.1
Colletotrichum sp. strain LDCMYE22 small subunit ribosomal RNA gene, partial sequence; internal transcribed spa...	Colletotrichum sp.	595	595	76%	1e-165	98.80%	580	MG980305.1
Colletotrichum sp. strain LDCMYE17 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an...	Colletotrichum sp.	595	595	76%	1e-165	98.80%	568	MG980286.1
Colletotrichum queenslandicum strain MEF77 small subunit ribosomal RNA gene, partial sequence; internal transcr...	Colletotrichum qu...	595	595	76%	1e-165	98.80%	676	MF380919.1
Colletotrichum sp. isolate CR 14 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int...	Colletotrichum sp.	595	595	76%	1e-165	98.80%	529	KY659058.1
Colletotrichum sp. isolate CR 19 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int...	Colletotrichum sp.	595	595	76%	1e-165	98.80%	544	KY659055.1
Colletotrichum gloeosporioides strain KU-KP/RK/EF-24 internal transcribed spacer 1, partial sequence; 5.8S riboso...	Colletotrichum gl...	595	595	76%	1e-165	98.80%	538	MG204864.1
Colletotrichum gloeosporioides strain Ps-36 18S ribosomal RNA gene, partial sequence; internal transcribed space...	Colletotrichum gl...	595	595	76%	1e-165	98.80%	544	KU671363.1
Colletotrichum gloeosporioides isolate MTCC 10339 18S ribosomal RNA gene, partial sequence; internal transcrib...	Colletotrichum gl...	595	595	76%	1e-165	98.80%	532	KX099750.1
Colletotrichum sp. GRMP-58 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and interna...	Colletotrichum sp...	595	595	76%	1e-165	98.80%	625	KF516005.1

Fig 2 l: BLASTN text output of nucleotide sequence of ITS gene of *Colletotrichum gloeosporioides* (TBMLS)



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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Colletotrichum sp. 2 JM-2013 isolate CJBL2-37 18S ribosomal RNA gene, partial sequence; internal transcribed sp...	Colletotrichum sp...	780	780	90%	0.0	98.21%	550	KC895547.1
Colletotrichum gloeosporioides strain Y18 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen...	Colletotrichum gl...	780	780	90%	0.0	98.21%	548	MT729940.1
Colletotrichum sp. strain JNZG11 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int...	Colletotrichum sp.	776	776	90%	0.0	97.99%	550	MT570098.1
Colletotrichum siamense internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal tra...	Colletotrichum si...	776	776	90%	0.0	97.99%	547	MG642980.1
Colletotrichum gloeosporioides isolate PAK16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA...	Colletotrichum gl...	776	776	89%	0.0	98.20%	549	KR259525.1
Colletotrichum gloeosporioides isolate CJBB21-25 18S ribosomal RNA gene, partial sequence; internal transcribed...	Colletotrichum gl...	776	776	89%	0.0	98.20%	549	KC895535.1
Colletotrichum siamense isolate WS2019y5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ge...	Colletotrichum si...	776	776	90%	0.0	97.99%	553	MZ148626.1
Colletotrichum siamense isolate HNZDL04 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen...	Colletotrichum si...	776	776	90%	0.0	97.99%	549	MW406825.1
Colletotrichum sp. strain JNZG14 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int...	Colletotrichum sp.	774	774	90%	0.0	97.99%	549	MT570096.1
Colletotrichum sp. strain JNZG12 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int...	Colletotrichum sp.	774	774	90%	0.0	97.99%	551	MT570094.1
Colletotrichum siamense strain GXNN-27 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Colletotrichum si...	774	774	90%	0.0	97.99%	528	MK041891.1
Colletotrichum gloeosporioides isolate NEHU.SPSRJ.26 internal transcribed spacer 1, partial sequence; 5.8S ribos...	Colletotrichum gl...	774	774	90%	0.0	97.99%	607	MF143557.1
Colletotrichum gloeosporioides isolate UOM AC internal transcribed spacer 1, partial sequence; 5.8S ribosomal RN...	Colletotrichum gl...	774	774	90%	0.0	97.99%	552	KF923868.1
Colletotrichum siamense strain 20HJ02-1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Colletotrichum si...	774	774	90%	0.0	97.99%	520	MZ725042.1
Colletotrichum siamense isolate TW3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an...	Colletotrichum si...	774	774	90%	0.0	97.99%	555	MZ150354.1

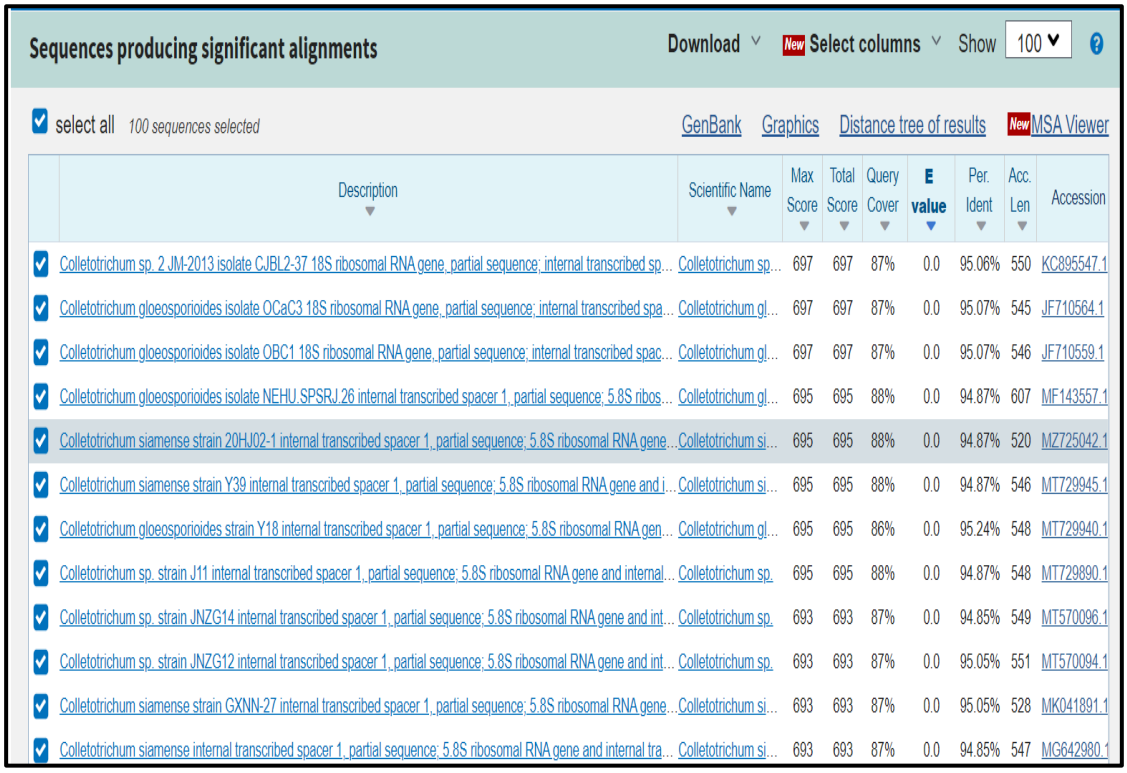
Fig 2 m: BLASTN text output of nucleotide sequence of ITS gene of *Colletotrichum gloeosporioides* (MNTSR)

5’GAGGTACGCTCTCACCCTTTGTGACATACCTATAACTGTTGATTCGGC
GGGTAGGGTATGAGCGACCCTCCCGGCCTCCCACCTCCGGGCGGGTCG
AGCGACCGCCGGAGGATAACCGAAGACTCTGATTTAACGACGTTTCTA
CTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTG
GTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAA
TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC
CAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGC
TCTGCTTGGTGTTGGGGCCCTACAAATGATGAAAGACCTCAAAGGTAG
ATGACGAACCCTCTCGGAGCCTCCTTTGCGTAGTAACCTTACCTCTCGC
ACGGGGATCCGGTCAGTATGCGGCGGAGCACCCACTAAGCGGCGGAGC
ATATCAATAAGCCGAGAGCATTCA3’

Fig 1 n: Nucleotide sequence of ITS gene of *Colletotrichum gloeosporioides* (PBALS)

5’GCCCGCCGGAGGATAACCAAACTCTGATTCAACGACGTTTCTTCTGA
GTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCT
GGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
GAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGC
ATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTG
CTTGGTGTTGGGGCCCTACAGCTGATGTAGGCCCTCAAAGGTAGTGGC
GGACCCTCCCGGAGCCTCCTAAGCGTAGTAAGCATACCTCTAGCCGGG
GGAGCATATCAATAAGCGGAGGAGCATATCACTAAGCGGAGGAGCAT
ATCAATAAGCGGAGAGCATCAT3’

Fig 1 o: Nucleotide sequence of ITS gene of *Colletotrichum gloeosporioides* (PNMSR)



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

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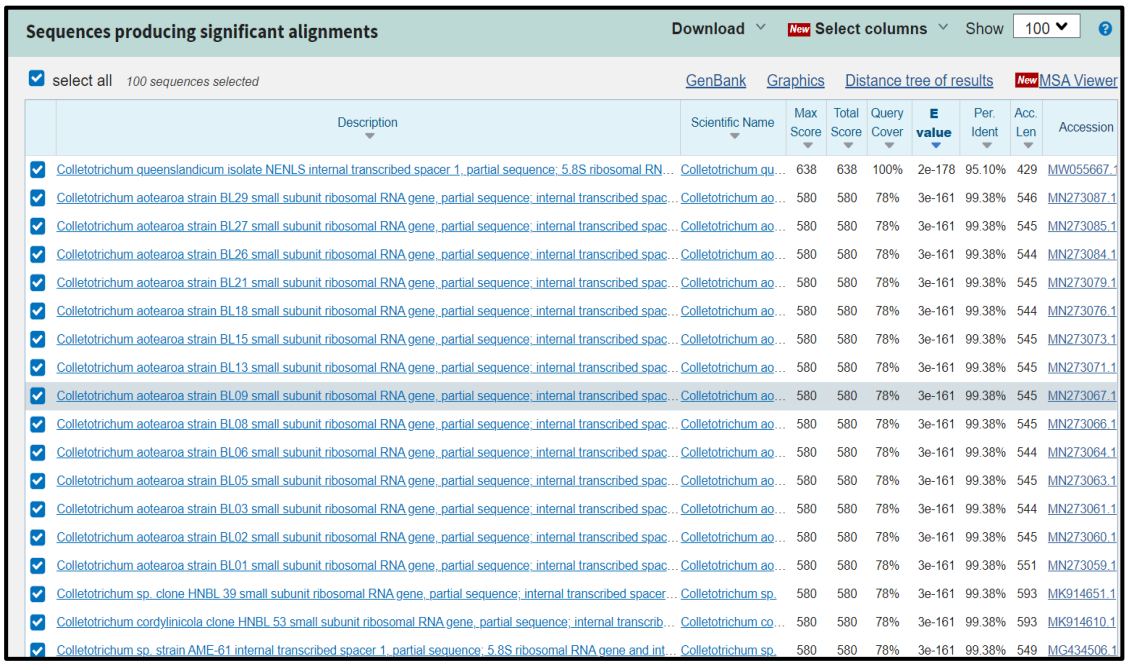
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Colletotrichum sp. 2 JM-2013 isolate CJBL2-37 18S ribosomal RNA gene, partial sequence; internal transcribed sp...	Colletotrichum sp...	697	697	87%	0.0	95.06%	550	KC895547.1
Colletotrichum gloeosporioides isolate OCaC3 18S ribosomal RNA gene, partial sequence; internal transcribed spa...	Colletotrichum gl...	697	697	87%	0.0	95.07%	545	JF710564.1
Colletotrichum gloeosporioides isolate OBC1 18S ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum gl...	697	697	87%	0.0	95.07%	546	JF710559.1
Colletotrichum gloeosporioides isolate NEHU SPSRJ 26 internal transcribed spacer 1, partial sequence; 5.8S ribos...	Colletotrichum gl...	695	695	88%	0.0	94.87%	607	MF143557.1
Colletotrichum siamense strain 20HJ02-1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Colletotrichum si...	695	695	88%	0.0	94.87%	520	MZ725042.1
Colletotrichum siamense strain Y39 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i...	Colletotrichum si...	695	695	88%	0.0	94.87%	546	MT729945.1
Colletotrichum gloeosporioides strain Y18 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen...	Colletotrichum gl...	695	695	86%	0.0	95.24%	548	MT729940.1
Colletotrichum sp. strain J11 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal...	Colletotrichum sp.	695	695	88%	0.0	94.87%	548	MT729890.1
Colletotrichum sp. strain JNZG14 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int...	Colletotrichum sp.	693	693	87%	0.0	94.85%	549	MT570096.1
Colletotrichum sp. strain JNZG12 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int...	Colletotrichum sp.	693	693	87%	0.0	95.05%	551	MT570094.1
Colletotrichum siamense strain GXNN-27 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Colletotrichum si...	693	693	87%	0.0	95.05%	528	MK041891.1
Colletotrichum siamense internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal tra...	Colletotrichum si...	693	693	87%	0.0	94.85%	547	MG642980.1

Fig 2 n: BLASTN text output of nucleotide sequence of ITS gene of *Colletotrichum gloeosporioides* (PBALS)



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

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

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Colletotrichum queenslandicum isolate NENLS internal transcribed spacer 1, partial sequence; 5.8S ribosomal RN...	Colletotrichum qu...	638	638	100%	2e-178	95.10%	429	MW055667.1
Colletotrichum aotearoa strain BL29 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum ao...	580	580	78%	3e-161	99.38%	546	MN273087.1
Colletotrichum aotearoa strain BL27 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum ao...	580	580	78%	3e-161	99.38%	545	MN273085.1
Colletotrichum aotearoa strain BL26 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum ao...	580	580	78%	3e-161	99.38%	544	MN273084.1
Colletotrichum aotearoa strain BL21 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum ao...	580	580	78%	3e-161	99.38%	545	MN273079.1
Colletotrichum aotearoa strain BL18 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum ao...	580	580	78%	3e-161	99.38%	544	MN273076.1
Colletotrichum aotearoa strain BL15 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum ao...	580	580	78%	3e-161	99.38%	545	MN273073.1
Colletotrichum aotearoa strain BL13 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum ao...	580	580	78%	3e-161	99.38%	545	MN273071.1
Colletotrichum aotearoa strain BL09 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum ao...	580	580	78%	3e-161	99.38%	545	MN273067.1
Colletotrichum aotearoa strain BL08 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum ao...	580	580	78%	3e-161	99.38%	545	MN273066.1
Colletotrichum aotearoa strain BL06 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum ao...	580	580	78%	3e-161	99.38%	544	MN273064.1
Colletotrichum aotearoa strain BL05 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum ao...	580	580	78%	3e-161	99.38%	545	MN273063.1
Colletotrichum aotearoa strain BL03 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum ao...	580	580	78%	3e-161	99.38%	544	MN273061.1
Colletotrichum aotearoa strain BL02 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum ao...	580	580	78%	3e-161	99.38%	545	MN273060.1
Colletotrichum aotearoa strain BL01 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Colletotrichum ao...	580	580	78%	3e-161	99.38%	551	MN273059.1
Colletotrichum sp. clone HNBL 39 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer...	Colletotrichum sp.	580	580	78%	3e-161	99.38%	593	MK914651.1
Colletotrichum cordylinicola clone HNBL 53 small subunit ribosomal RNA gene, partial sequence; internal transcrib...	Colletotrichum co...	580	580	78%	3e-161	99.38%	593	MK914610.1
Colletotrichum sp. strain AME-61 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int...	Colletotrichum sp.	580	580	78%	3e-161	99.38%	549	MG434506.1

Fig 2 o: BLASTN text output of nucleotide sequence of ITS gene of *Colletotrichum gloeosporioides* (PNMSR)

4.6.1.16 Phylogenetic analysis

Among the 18 fungal isolates collected during sampling survey, seven isolates (KMALS, PBALS, VFNLS, TBMLS, MNTSR, PNMSR, ALVSR) were confirmed to be of the genus, *Colletotrichum*. Mega X software was used to analyse the evolutionary relationship of different *Colletotrichum* isolates obtained during the survey with other species of the same pathogen obtained during BLASTN analysis in NCBI nr database. The evolutionary relationship of KMALS, PBALS, VFNLS, TBMLS, MNTSR and PNMSR with four accessions each of *Colletotrichum gloeosporioides*, *C. siamense*, *C. aenigma*, *C. aotearoa*, *C. queenslandicum* and *C. truncatum* was analysed by constructing a neighbor-joining tree using Mega X. The phylogenetic tree revealed that all the accessions were related to each other and the species diversification study based on ITS sequencing alone was not possible as the different species of *Colletotrichum* were clustering together (Fig 3h). Based on the analysis, it was confirmed that the five isolates KMALS, PBALS, VFNLS, MNTSR and PNMSR belonged to *C. gloeosporioides* species complex.

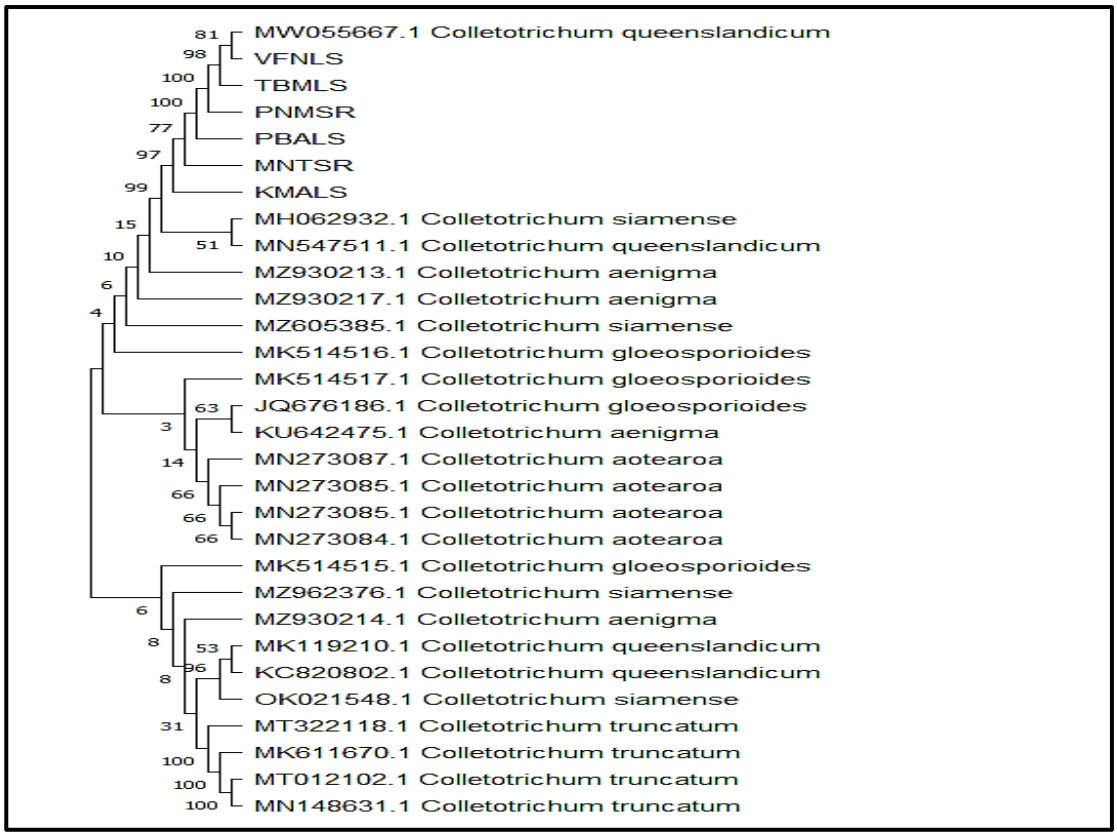


Fig 3h: Neighbor-joining phylogenetic tree constructed in Mega X showing relationship between various species of the genus *Colletotrichum*

4.6.2. Bacterial pathogen

The bacterial pathogens associated with the leaf blight samples VFNLB collected from Vellanikkara and OLKLB collected from Ollukkara of Thrissur district were identified as *Xanthomonas* sp. based on the cultural, morphological and biochemical characters. Further confirmation of the identity of these isolates were done by molecular characterization of the genomic DNA of corresponding bacteria. The bacterial DNA was isolated and amplification of 16S rRNA was carried out using standard protocols.

4.6.2.1 Gel electrophoresis of PCR product

Bacterial DNA sample amplified with 16S-RS-F and 16S-RS-R primers were observed by electrophoresis on 1.2 % agarose gel. The DNA bands were visualised under a UV transilluminator followed by capturing the gel image using Gel documentation system (Bio- Rad). Amplicons of size 1.5 kB were obtained (Plate 31).

4.6.2.3 *In silico* analysis of bacterial genome

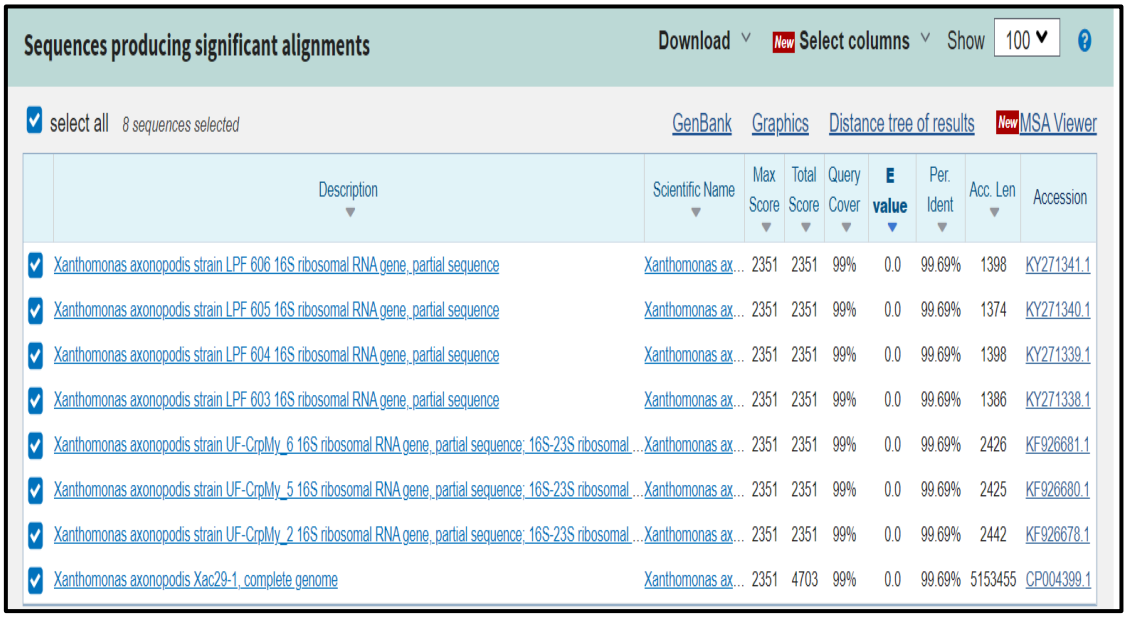
In silico analysis of nucleotide sequences of the two bacterial isolates associated with leaf blight symptom (VFNLB and OLKLB) was done using BLASTN search against NCBI nr database. The results revealed that the nucleotide sequence of VFNLB and OLKLB were similar to *Xanthomonas axonopodis* strain LPF 606 16S ribosomal RNA gene, partial sequence (KY271341.1) with a sequence similarity percentage of 99.69 and 100 respectively. The query cover of VFNLB was 99 per cent and OLKLB was 100%. Thus, the pathogen causing leaf blight in anthurium (VFNLB and OLKLB) were identified as *Xanthomonas axonopodis* (Fig 5a and Fig 5b). The evolutionary relationship these two isolates with the top hit accessions of the same pathogen in NCBI database was analysed in Mega X and found that both the isolates were closely related to the same pathogen (KY271338.1) reported in *Schinus terebinthifolius* and *Mabea fistulifera* from Brazil (Fig 6).

5’TCTGCGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACT
CCAATCCGGACTGAGATAGGGTTTCTGGGATTGGCTTGCCCTCGCGGG
TTTGCAGCCCTCTGTCCCTACCATTGTAGTACGTGTGTAGCCCTGGTCG
TAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCA
CCGGCGGTCTCCTTAGAGTTCCCACCATTACGTGCTGGCAACTAAGGA
CAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGA
GCTGACGACAGCCATGCAGCACCTGTCTCACGGTTCCCGAAGGCACCA
ATCCATCTCTGGAAAGTTCCGTGGATGTCAAGACCAGGTAAGGTTCTTC
GCGTTGCATCGAATTAAACCACATACTCCACCGCTTGTGCGGGCCCCC
GTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGCGA
ACTTAACGCGTTAGCTTCGATACTGCGTGCCAAATTGCACCCAACATCC
AGTTCGCATCGTTTAGGGCGTGGACTACCAGGGTATCTAATCCTGTTTG
CTCCCCACGCTTTCGTGCCTCAGTGTCAGTGTTGGTCCAGGTAGCCGCC
TTCGCCACGGATGTTCCTCCCGATCTCTACGCATTTCACTGCTACACCG
GGAATTCCGCTACCCTCTACCACACTCTAGTGACCCAGTATCCACTGCA
ATTCCCAGGTTGAGCCCAGGGCTTTCACAACAGACTTAAACCACCACC
TACGCACGCTTTACGCCCAGTAATTCCGAGTAACGCTTGCACCCTTCGT
ATTACCGCGGCTGCTGGCACGAAGTTAGCCGGTGCTTATTCTTTGGGTA
CCGTCAGAACAATCGGGTATTAACCGACTGCTTTTCTTTCCCAACAAAA
GGGCTTTACAACCCGAAGGCCTTCTTCACCCACGCGGCATGGCTGGAT
CAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGA
GTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGACCA
GCTACGGATCGTCGCCTTGGTGGGCCTTTACCCCGCCAACTAGCTAATC
CGACATCGGCTCATTCAACCGCGCGAAGCCCGAAGGTCCTCCGCTTTC
ACCCGTAGGTCGTATGCGGTATTAGCGTAAGTTTCCCTACGTTATCCCC
CACGAAAGAGTAGATTCCGATGTATTCCTCACCCGTCCGCCACTCGCC
ACCCATAAGAGCA3’

Fig 4 a: Nucleotide sequence of ITS gene of *Xanthomonas axonopodis* (VFNLB)

5’CTAGAGCTTGCTCTTATGGGTGGCGAGTGGCGGACGGGTGAGGAATA
CATCGGAATCTACTCTTTCGTGGGGGATAACGTAGGGAAACTTACGCT
AATACCGCATACGACCTACGGGTGAAAGCGGAGGACCTTCGGGCTTCG
CGCGGTTGAATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGG
CCCACCAAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCAC
ACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG
AATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGGGTG
AAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAAAGCAG
TCGGTTAATACCCGATTGTTCTGACGGTACCCAAAGAATAAGCACCGG
CTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAC
TCGGAATTACTGGGCGTAAAGCGTGCGTAAGTGGTGGTTTAAATCTGTT
GTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGTGGATACTTGGGTCA
CTAGAGTGTGGTAGAGGGTAGCGGAATTCCCGGTGTAGCAGTGAAATG
CGTAGAGATCGGGAGGAACATCCGTGGCGAAGGCGGCTACCTGGACCA
ACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGAT
ACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCA
ATTTGGCACGCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGG
AGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCAC
AAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTAC
CTGGTCTTGACATCCACGGAACTTTCCAGAGATGGATTGGTGCCTTCGG
GAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAG
ATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCA
GCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGA
GGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCT
ACACACGTACTACAATGGTAGGGACAGAGGGCTGCAAACCCGCGAGG
GCAAGCCAATCCCAGAAACCCTATCTCAGTCCGGATTGGAGTCTGCAA
CTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGGTCAAGGAT3’

Fig 4 b: Nucleotide region of ITS region of *Xanthomonas axonopodis* (OLKLB)



Sequences producing significant alignments

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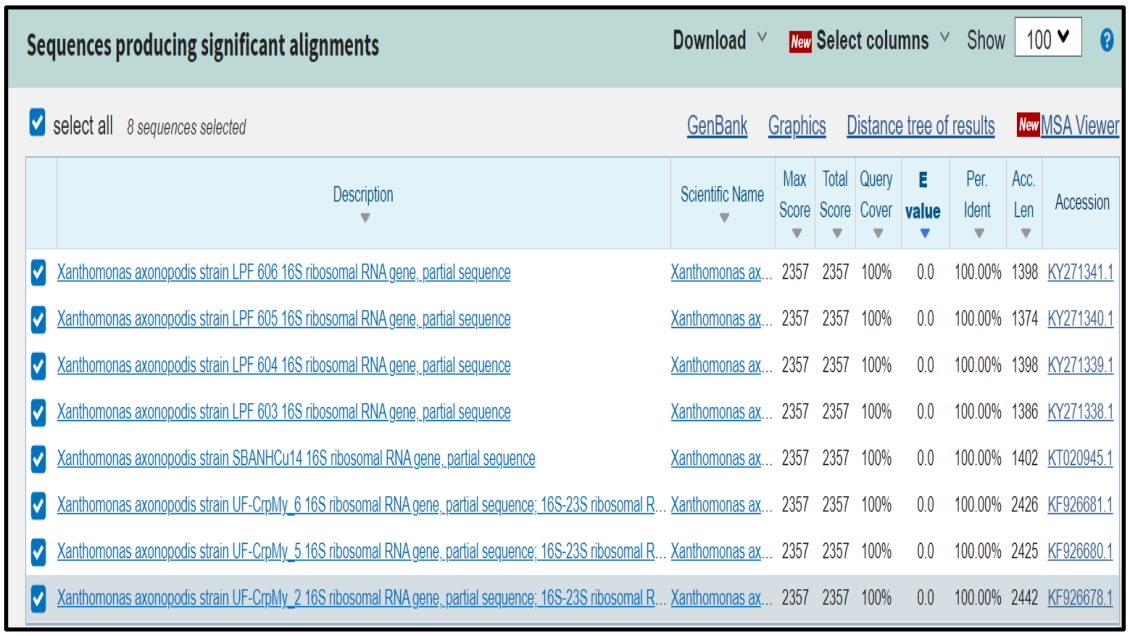
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Xanthomonas axonopodis strain LPF 606 16S ribosomal RNA gene, partial sequence	Xanthomonas ax...	2351	2351	99%	0.0	99.69%	1398	KY271341.1
Xanthomonas axonopodis strain LPF 605 16S ribosomal RNA gene, partial sequence	Xanthomonas ax...	2351	2351	99%	0.0	99.69%	1374	KY271340.1
Xanthomonas axonopodis strain LPF 604 16S ribosomal RNA gene, partial sequence	Xanthomonas ax...	2351	2351	99%	0.0	99.69%	1398	KY271339.1
Xanthomonas axonopodis strain LPF 603 16S ribosomal RNA gene, partial sequence	Xanthomonas ax...	2351	2351	99%	0.0	99.69%	1386	KY271338.1
Xanthomonas axonopodis strain UF-CrpMy_6 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal ...	Xanthomonas ax...	2351	2351	99%	0.0	99.69%	2426	KF926681.1
Xanthomonas axonopodis strain UF-CrpMy_5 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal ...	Xanthomonas ax...	2351	2351	99%	0.0	99.69%	2425	KF926680.1
Xanthomonas axonopodis strain UF-CrpMy_2 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal ...	Xanthomonas ax...	2351	2351	99%	0.0	99.69%	2442	KF926678.1
Xanthomonas axonopodis Xac29-1, complete genome	Xanthomonas ax...	2351	4703	99%	0.0	99.69%	5153455	CP004399.1

Fig 5 a: BLASTN text output of nucleotide sequence of ITS gene of *Xanthomonas axonopodis* (VFNLB)



Sequences producing significant alignments

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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Xanthomonas axonopodis strain LPF 606 16S ribosomal RNA gene, partial sequence	Xanthomonas ax...	2357	2357	100%	0.0	100.00%	1398	KY271341.1
Xanthomonas axonopodis strain LPF 605 16S ribosomal RNA gene, partial sequence	Xanthomonas ax...	2357	2357	100%	0.0	100.00%	1374	KY271340.1
Xanthomonas axonopodis strain LPF 604 16S ribosomal RNA gene, partial sequence	Xanthomonas ax...	2357	2357	100%	0.0	100.00%	1398	KY271339.1
Xanthomonas axonopodis strain LPF 603 16S ribosomal RNA gene, partial sequence	Xanthomonas ax...	2357	2357	100%	0.0	100.00%	1386	KY271338.1
Xanthomonas axonopodis strain SBANHCu14 16S ribosomal RNA gene, partial sequence	Xanthomonas ax...	2357	2357	100%	0.0	100.00%	1402	KT020945.1
Xanthomonas axonopodis strain UF-CrpMy_6 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal R...	Xanthomonas ax...	2357	2357	100%	0.0	100.00%	2426	KF926681.1
Xanthomonas axonopodis strain UF-CrpMy_5 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal R...	Xanthomonas ax...	2357	2357	100%	0.0	100.00%	2425	KF926680.1
Xanthomonas axonopodis strain UF-CrpMy_2 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal R...	Xanthomonas ax...	2357	2357	100%	0.0	100.00%	2442	KF926678.1

Fig 5 b: BLASTN text output of nucleotide sequence of ITS gene of *Xanthomonas axonopodis* (OLKLB)

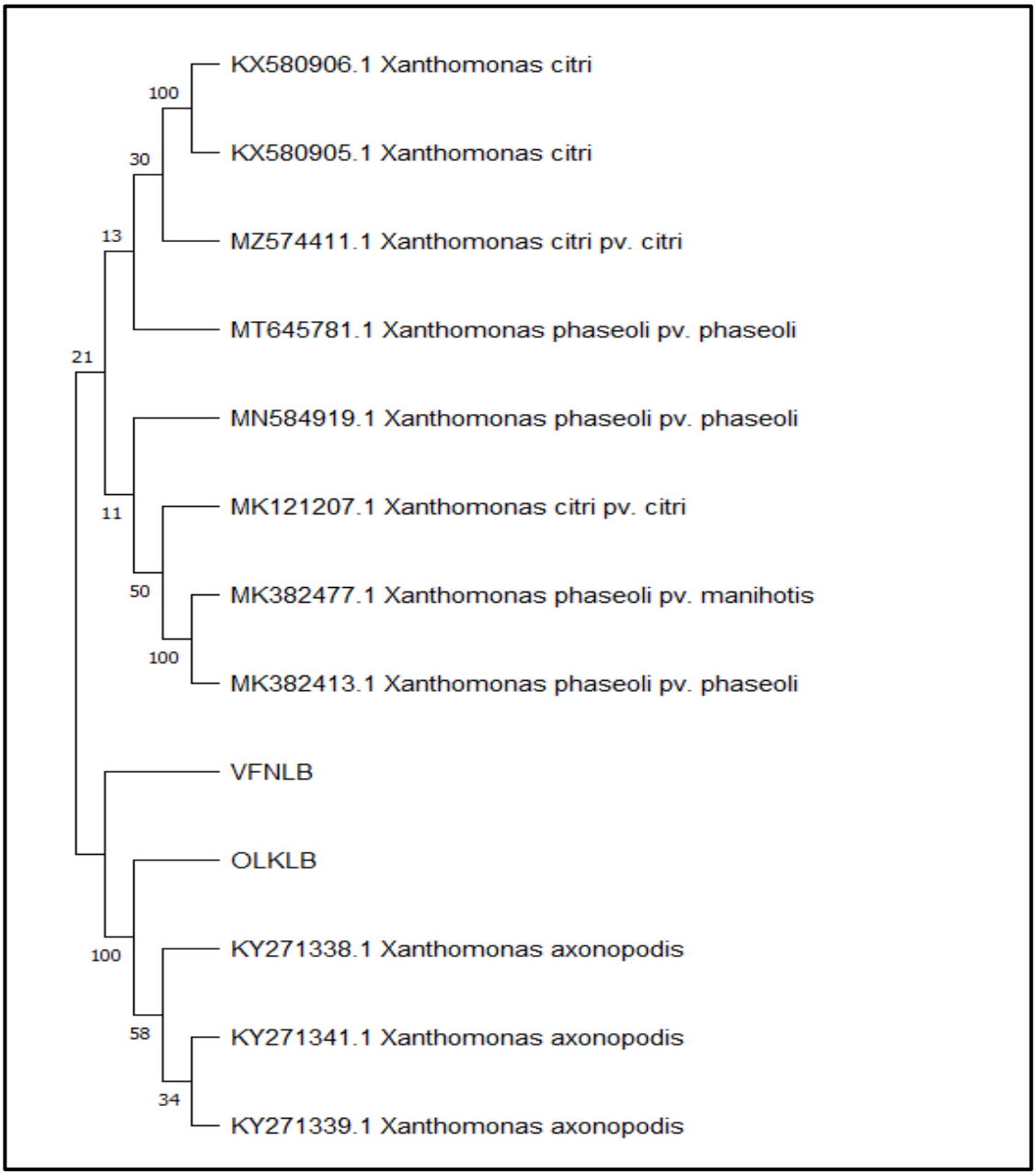

Fig 6: Neighbor-joining phylogenetic tree of *Xanthomonas axonopodis* constructed in Mega X

4.7 CORRELATION OF WEATHER PARAMETERS WITH DISEASE SEVERITY OF FUNGAL DISEASES

The studies based on cultural and morphological characters revealed that 18 out of 21 isolates collected during the sampling survey in six districts were fungal pathogens. Therefore, the percentage of fungal disease severity was correlated with weather parameters *viz*. maximum and minimum temperature, relative humidity and rainfall prevailing in the corresponding location in order to determine the impact of these variables on disease severity. The data regarding various weather parameters were obtained from the official site of MarkSim (Table 4.9) and the relation between each weather parameter and the per cent diseases severity was obtained by correlation analysis.

4.7.1 Impact of rainfall (precipitation) on severity of fungal diseases

The severity of fungal infections on various plant parts was correlated with the average value of rainfall for the three months before the month of survey. According to the findings, rainfall has a substantial positive correlation with the fungal disease severity and the correlation coefficient was 0.386** (Fig 7a).. The leaf spot sample (PBALS) collected from Perambra in the Kozhikode district, where rainfall was heavier, had the highest PDS of 58.23%.

4.7.2 Impact of relative humidity on severity of fungal diseases

The relationship between relative humidity and the extent of disease severity was investigated using a correlation analysis that compared average relative humidity values to percent disease severity**.** A positive correlation was observed which revealed that the severity of fungal diseases increased gradually with an increase in the relative humidity of the prevailing location. Th correlation coefficient obtained in the analysis was 0.510** (Fig 7b). A lower disease severity of 16.71 was seen in the spadix rot sample (PNMSR) collected from Panamaram of Wayanad district where the relative humidity recorded was the lowest among the survey locations.

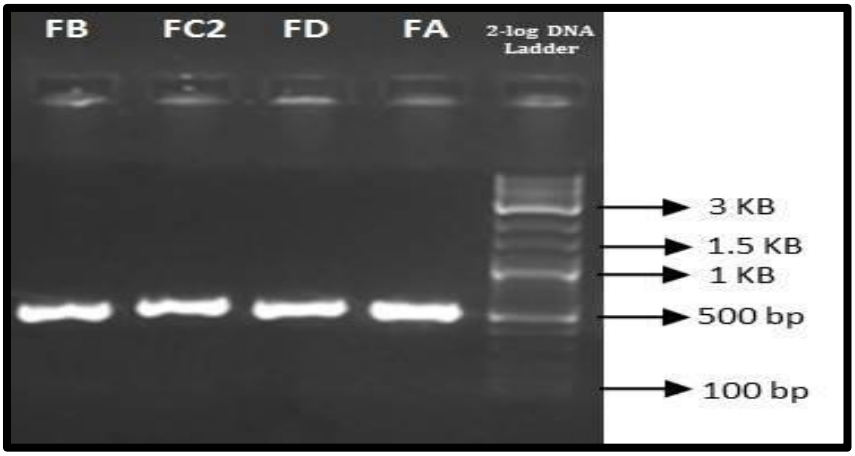

Plate 26: Gel profile of PCR-

FB: *Phomopsis* sp. (KKYLS)
FC2: *Corynespora* sp. (VCNLS2)
FD: *Lasiodiplodia* sp. (NLBLS)
FA: *Diaporthe* sp. (IJKLS)

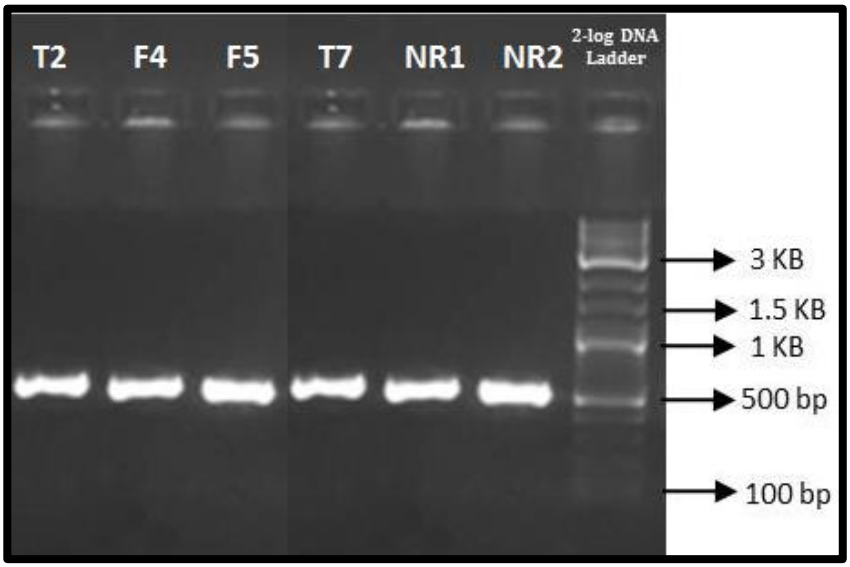

Plate 27: Gel profile of PCR-

T2: *Colletotrichum* sp. (TBMLS)
F4: *Phomopsis* sp. (OKMLS)
F5: *Colletotrichum* sp. (MNTSR)
T7: *Colletotrichum* sp. (PBALS)
NR1: *Colletotrichum* sp. (PNMSR)
NR2: *Colletotrichum* sp. (VFNLS)

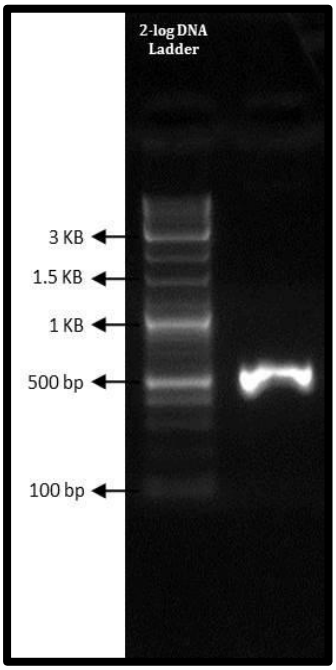

Plate 28: Gel profile of PCR- *Phytopythium* sp. (VCNRR)

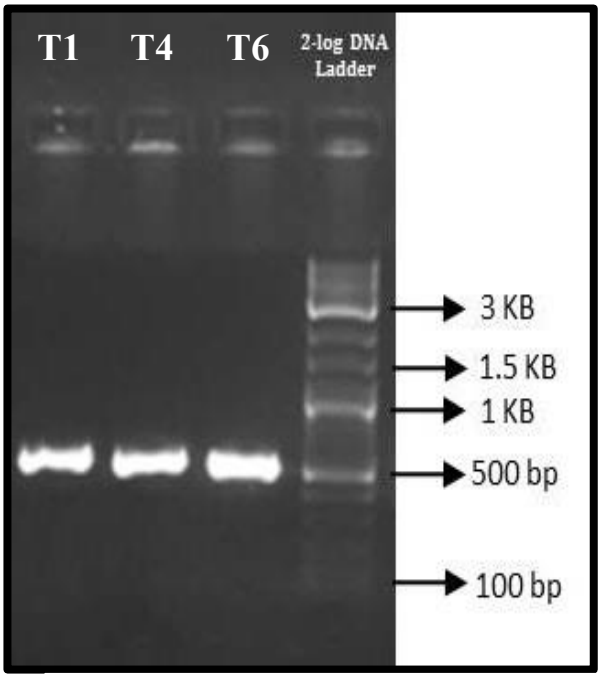

Plate 29: Gel profile of PCR-
T1: *Corynespora* sp. (OLRLS)
T4: *Colletotrichum* sp. (KMALS)
T6: *Pseudopestalotiopsis* sp. (TLRLS)

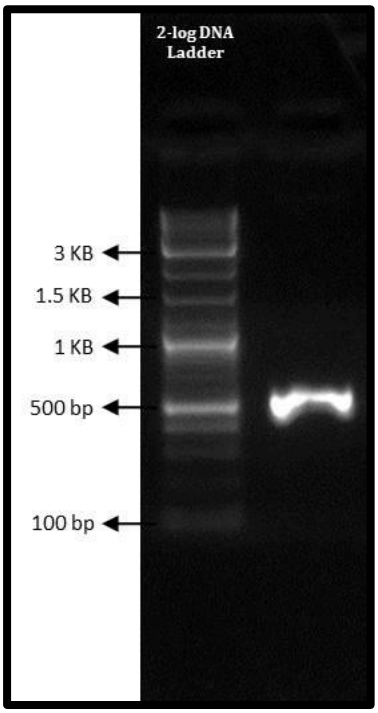

Plate 30: Gel profile of PCR- *Fusarium* sp. (MNTLW)

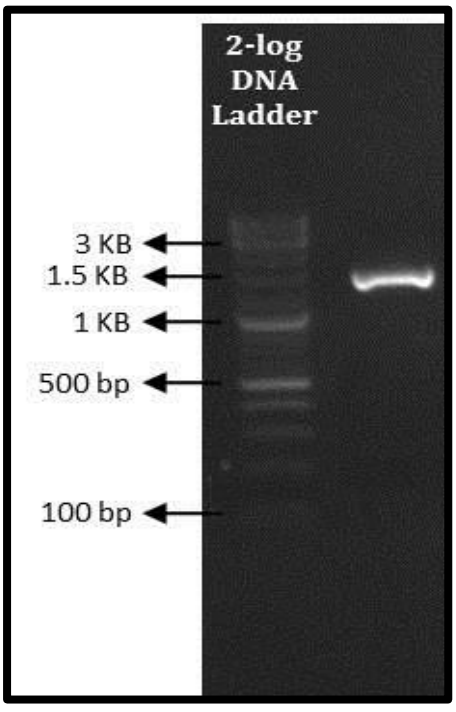

Plate 31: Gel profile of PCR- *Xanthomonas* sp.

4.7.3 Impact of temperature on severity of fungal diseases

Temperature of the surveyed locations, contrary to the parameters *viz.* rainfall and relative humidity, had a negative correlation with the disease severity and showed a correlation coefficient of (-) 0.537** (Fig 7c). Thus, the severity of fungal diseases decreased as the temperature rose.

Table 4.9: Meteorological data of the surveyed locations

SL. No.	Fungal isolates	Locations	PDI (%)	PDS (%)	Mean temperature (°C)	Relative humidity (%)	Rainfall (mm per day)
1.	KMALS	Kumaranellur	40	33.33	25.115	88.31	12.48
2.	PBALS	Perambra	73.66	58.23	26.475	85.62	16.29
3.	VCNRR	Vellanikkara	47.33	41.00	26.525	87.12	14.76
4.	VCNLS1	Vellanikkara	20	37.77	26.525	87.12	14.76
5.	VCNLS2	Vellanikkara	13.33	28.88	26.525	87.12	14.76
6.	VFNLS	Vellanikkara	33	36	26.525	87.12	14.76
7.	MNTLW	Mannuthy	21	42.22	25.635	81.15	11.62
8.	MNTSR	Mannuthy	31	24.44	25.635	81.15	11.62
9.	IJKLS	Irinjalakkuda	36	37.03	27.17	86.44	4.86
10.	KKYLS	Koorkkenchery	27	21	26.37	89.50	4.72
11.	OKMLS	Oorakam	33.33	25	30.605	58.56	2.04
12.	OLRLS	Ollur	15.5	25	26.915	88.25	8.68
13.	TLRLS	Thalore	28	42	27.175	87.19	8.64
14.	TBMLS	Therambam	30	33.33	27.26	87.21	8.68
15.	CKDSR	Chirakkekode	15.72	9.13	25.635	81.50	11.62
16.	PNMSR	Panamaram	23.3	16.71	33.53	58.31	1.89
17.	ALVSR	Aluva	59	32.12	28.995	64.00	2.32
18.	NLBLS	Nilambur	80	46.66	21.93	81.31	6.20

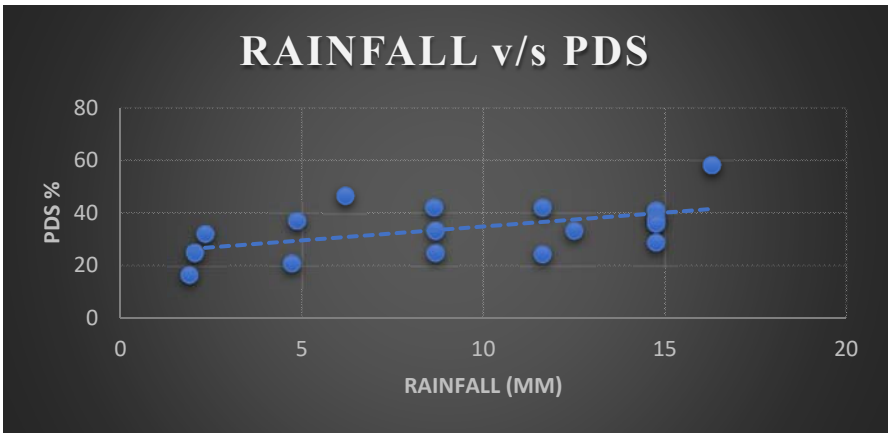

Fig 7 a: Correlation of rainfall with per cent disease severity

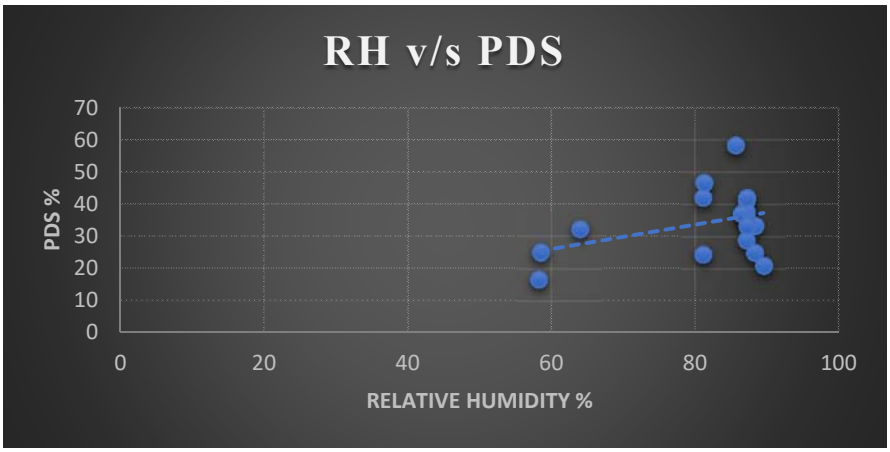

Fig 7 b: Correlation of relative humidity with per cent disease severity

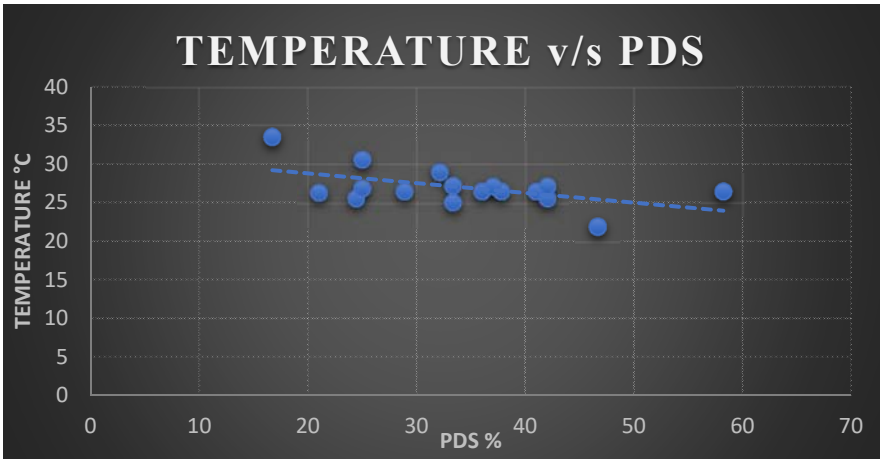

Fig 7 c: Correlation of temperature with per cent disease severity

4.8 *In vitro* evaluation of fungicides and biocontrol agents against major fungal pathogens

Among the various leaf spot diseases, NLBLS and PBALS collected form Nilambur (Malappuram district) and Perambra (Kozhikode district) showed a greater disease incidence and severity percentage. ALVSR collected from Aluva of Ernakulum district showed a higher disease incidence of 59 per cent among the four inflorescence rot symptoms. The root rot disease of anthurium (VCNRR) collected form Vellanikkara (Thrissur district) recorded a severity of 64 %. Thus, the pathogens associated with these symptoms were selected as major pathogens in the course of survey and tested the efficacy of various fungicides and antagonists *in vitro* (Table 4.10).

Table 4.10: Major fungal pathogens selected for *in vitro* analysis

Sl. No.	Pathogens	Designated code	Associated symptoms
1.	*Lasiodiplodia theobromae*	NLBLS	Leaf spot
2.	*Phytopythium vexans*	VCNRR	Root rot
3.	*Colletotrichum* sp.	ALVSR	Inflorescence rot
4.	*Colletotrichum* sp. (*C. gloeosporioides* species complex)	PBALS	Leaf spot

.4.8.1 *In vitro* evaluation of fungicides

The experiment was conducted in CRD with 22 treatments and 4 replications each. Seven fungicides at three doses (lower dose, recommended dose and higher dose) and Bordeaux mixture at recommended dose (1 %) were chosen for performing the evaluation study. The inhibitory action of these fungicides was tested against the selected pathogens by using poison food technique.

4.8.1.1 *In vitro* evaluation of fungicides against *Lasiodiplodia theobromae*

The fungicides *viz*. carbendazim 12 % + mancozeb 64 %, cymoxanil 8 % + mancozeb 64 %, propineb and difenoconazole were found highly effective against the

pathogen as there was complete inhibition of mycelial growth at lower, recommended and higher doses of these fungicides. Copper hydroxide resulted in 35.55, 58.88 and 62.22 per cent inhibition at lower (0.1 %), recommended (0.2 %) and higher doses (0.3 %) respectively. An inhibition percentage of 88.89 was observed in the PDA plates poisoned with 0.15 per cent of hexaconazole (higher dose) when compared with the lower and recommended doses (72.22 and 82.22 % inhibition respectively). Azoxystrobin when applied at recommended dose (0.1 %) resulted in 73.33 per cent inhibition of fungal growth with a mean colony diameter of 5.4 cm. At lower dose of 0.05 per cent, the inhibition recorded was 67.78 per cent with a mean colony diameter of 5.6 cm and at higher dose (0.15 %), the diameter of the colony observed was only 5.0 showing an inhibition of 81.11 per cent. Bordeaux mixture was also found very effective against *Lasiodiplodia theobromae* as no growth of the pathogen was observed at the recommended dosage of one per cent (Table: 4.11; Plate 32).

4.8.1.2 *In vitro* evaluation of fungicides against selected root rot pathogen *Phytopythium vexans* (VCNRR)

Four fungicides used in the experiment completely inhibited the pathogen growth in the PDA media poisoned with the three doses of these fungicides (lower dose, recommended dose and higher dose). Two combination fungicides *viz*. carbendazim + mancozeb (Saaf) and cymoxanil + mancozeb (Curzate) along with propineb and 1 per cent Bordeaux mixture showed a complete inhibition of the pathogen. At the higher and recommended doses (0.3 and 0.2 % respectively) of copper hydroxide, the pathogen failed to grow radially over the poisoned media whereas at the lower dose of 0.1 per cent, a mean fungal colony diameter of 1.5 cm was observed which counted to 83.33 percentage inhibition. In the case of hexaconazole, a higher inhibition percentage of 83.33 was observed at higher dose (0.15 %) and at recommended dose of 0.1 per cent, the inhibition recorded was 74.44 per cent with a mean colony diameter of 2.3 cm. When the same fungicide was applied at lower dose (0.05 %), the inhibition observed was 42.22 percent and the fungus showed a radial growth of 5.2 cm in diameter. When propineb was given at recommended dose of 0.2 per cent, the inhibition observed was 81.11 per cent and at lower dose (0.1 %), the radial growth of the fungus was 2.2 cm in

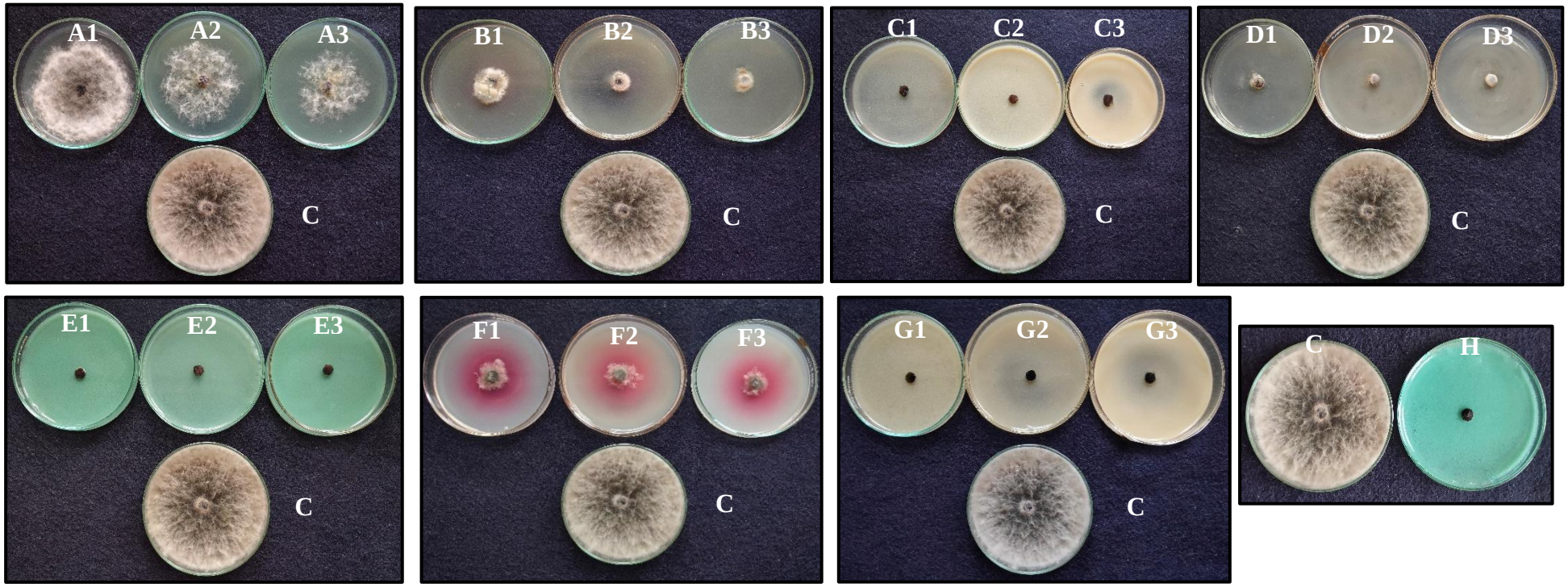

Plate 32: *In vitro* evaluation of fungicides against *Lasiodiplodia theobromae*

A1: Copper hydroxide (0.1%), A2: Copper hydroxide (0.2%), A3: Copper hydroxide (0.3%)
B1: Hexaconazole (0.05%), B2: Hexaconazole (0.1%), B3: Hexaconazole (0.15%)
C1: Propineb (0.1%), C2: Propineb (0.2%), C3: Propineb (0.3%)
D1: Difenoconazole (0.05%), D2: Difenoconazole (0.1%), D3: Difenoconazole (0.15%)
E1: Carbendazim 12%+ Mancozeb 64% (0.1%), E2: Carbendazim 12%+ Mancozeb 64% (0.2%), E3: Carbendazim 12%+ Mancozeb 64% (0.3%)
F1: Azoxystrobin (0.05%), F2: Azoxystrobin (0.1%), F3: Azoxystrobin (0.15%),
G1: Cymoxanil 8% + Mancozeb 64% (0.1%), G2: Cymoxanil 8% + Mancozeb 64% (0.25%), G3: Cymoxanil 8% + Mancozeb 64% (0.5%)
H: 1% Bordeaux mixture C: Control plate

diameter showing an inhibition percentage of 75.56 whereas at higher dose of 0.3 percent, an inhibition percentage of 83.33 was noticed (Table: 4.12; Plate 33)

4.8.1.3 *In vitro* evaluation of fungicides against selected inflorescence rot pathogen *Colletotrichum sp.* (ALVSR)

All the fungicides except copper hydroxide (lower and recommended dose) were highly effective against the selected inflorescence rot pathogen (ALVSR) at all the three doses *i. e.* lower, recommended and higher doses. These fungicides showed cent per cent inhibition of the pathogen growth. Copper hydroxide showed an inhibition percentage of 78.89 at recommended dose of 0.2 per cent with a mean cony diameter of 1.91 cm whereas an inhibition of 29.66 per cent was observed at lower dose and the mean colony diameter noticed was 6.34 cm. Bordeaux mixture at 1 per cent completely inhibited the pathogen with no visible fungal growth in the poisoned media (Table:4.13; Plate 34).

4.8.1.4 *In vitro* evaluation of fungicides against selected leaf spot pathogen *Colletotrichum sp.* (PBALS)

Propineb, difenoconazole and the two combination fungicides (Carbendazim + mancozeb and cymoxanil + mancozeb) were highly effective against this leaf spot pathogen as no fungal growth was observed on the poisoned PDA plates inoculated with the pathogen mycelial bits. Copper hydroxide at the recommended dose (0.2%) provided 48.89 per cent inhibition with a mean colony diameter of 4.6 cm and at higher dose of 0.3 per cent, an inhibition of 56.67 was noticed whereas the fungicide inhibited the fungal growth even at lower dose of 0.1 per cent restricting the radial growth of the pathogen at 5.81 cm diameter. All the three doses of hexaconazole *i.e*. lower dose (0.05 %), recommended dose (0.1 %) and higher dose (0.15 %) showed different percentages of inhibition over the fungal growth *viz.* 37.78 per cent, 52.22 per cent and 82.33 per cent respectively. The mean colony diameter of the fungus was only 4.40 cm at the recommended dose of hexaconazole. Azoxystrobin when applied at recommended dose (0.1 %) showed an inhibition percentage of 66.67 and the colony diameter was restricted to 3 cm whereas 47.78 per cent of inhibition in the radial growth of the fungal pathogen was observed when the fungicide was applied at a lower dose. The mean diameter of

the fungal colony was noticed to be 2.4 cm when azoxystrobin was applied at a higher dose of 0.15 per cent (Table:4.14; Plate 35).

4.8.2 *In vitro* evaluation of biocontrol agents against fungal pathogens

The antagonistic efficiency of antagonists viz. *Trichoderma* sp. (KAU reference culture) against the major fungal pathogens was carried out by dual culture method, *Pseudomonas fluorescens* (KAU reference culture) by ring inoculation method and PGPR – II (KAU formulation) and PGPM (KAU formulation) by poisoned food technique respectively. The pathogens selected for *in vitro* evaluation were *Lasiodiplodia theobromae* (NLBLS), *Phytopythium vexans* (VCNRR), *Colletotrichum* sp. (ALVSR) and *Colletotrichum* sp. (PBALS) which showed maximum disease incidence/ severity.

The observations revealed that *Trichoderma* sp. was highly competent in inhibiting the growth of the test pathogens. Cent per cent inhibition was noticed against the two isolates of *Colletotrichum* sp. (ALVSR and PBALS). In the dual culture assay, the radial growth of *Lasiodiplodia theobrome* and *Phytopythium vexans* were inhibited by *Trichoderma* sp. at a percentage of 70 and 60 respectively (Plate 36).

One of the potent bacterial antagonists, *Pseudomonas fluorescens* showed an inhibition percentage of 82.22 per cent and 77.78 per cent against the *Colletotrichum* sp. isolates (PBALS and ALVSR) respectively. In case of *Phytopythium vexans*, no inhibition was observed whereas an inhibition of 63.34 per cent was noticed in the growth of *Lasiodiplodia theobromae* (Plate 37).

The KAU formulations of PGPR II and PGPM were highly effective in the control of these pathogens. Complete inhibition of the fungal pathogens was observed against PGPM whereas PGPR-II showed cent per cent inhibition in the growth of all the three isolates except *Lasiodiplodia theobromae* which was inhibited by 90 per cent (Table:4.16; Plate 38 & 39).

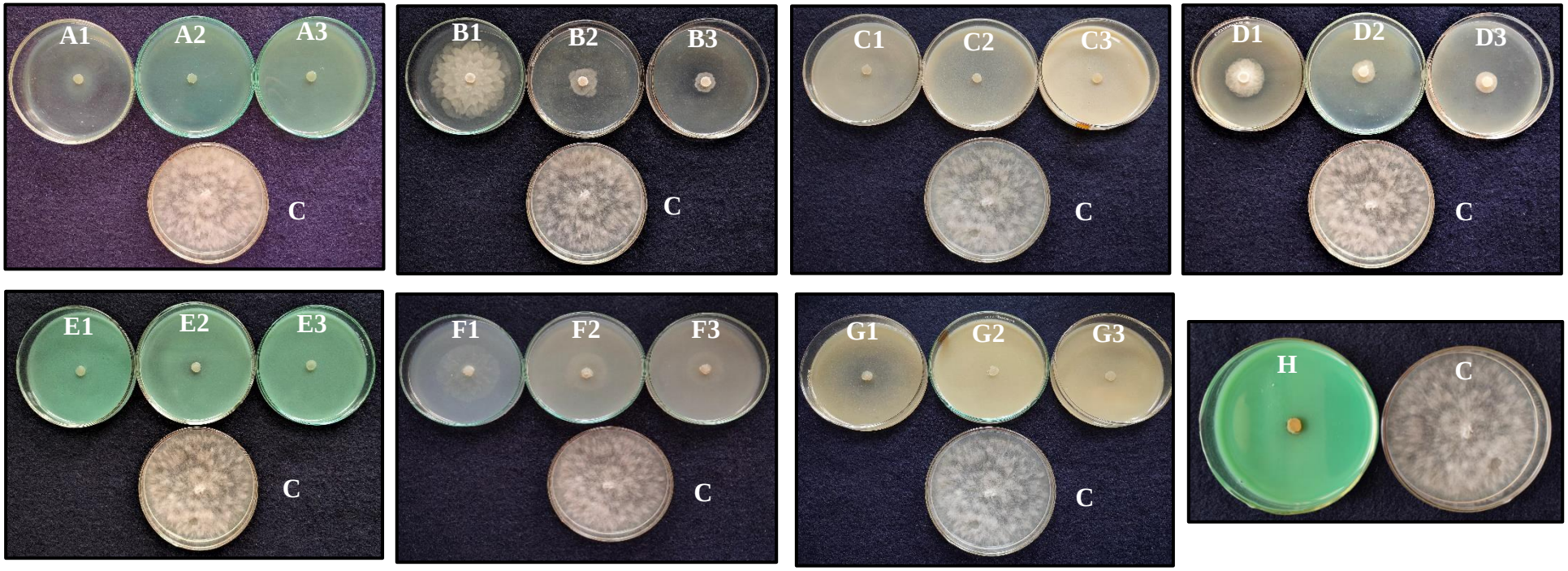

Plate 33*: In vitro* evaluation of fungicides against *Phytopythium vexans*

A1: Copper hydroxide (0.1%), A2: Copper hydroxide (0.2%), A3: Copper hydroxide (0.3%)
B1: Hexaconazole (0.05%), B2: Hexaconazole (0.1%), B3: Hexaconazole (0.15%)
C1: Propineb (0.1%), C2: Propineb (0.2%), C3: Propineb (0.3%)
D1: Difenoconazole (0.05%), D2: Difenoconazole (0.1%), D3: Difenoconazole (0.15%)
E1: Carbendazim 12%+ Mancozeb 64% (0.1%), E2: Carbendazim 12%+ Mancozeb 64% (0.2%), E3: Carbendazim 12%+ Mancozeb 64% (0.3%)
F1: Azoxystrobin (0.05%), F2: Azoxystrobin (0.1%), F3: Azoxystrobin (0.15%),
G1: Cymoxanil 8% + Mancozeb 64% (0.1%), G2: Cymoxanil 8% + Mancozeb 64% (0.25%), G3: Cymoxanil 8% + Mancozeb 64% (0.5%)
H: 1% Bordeaux mixture C: Control plate

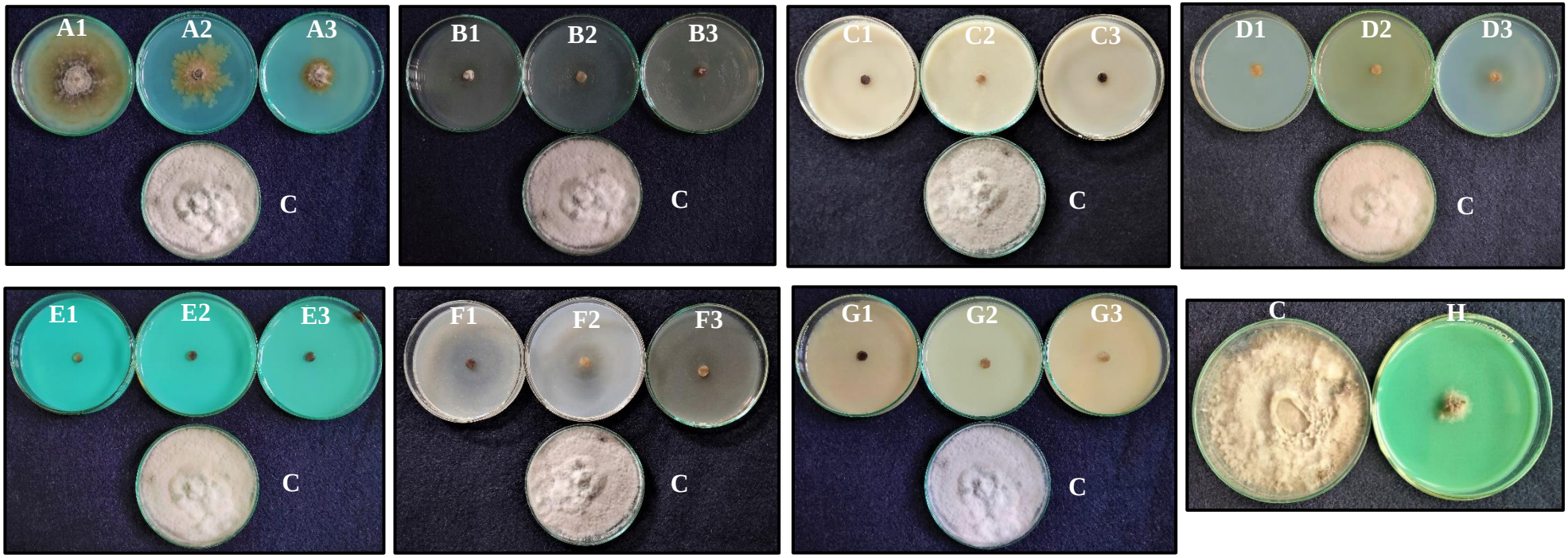

Plate 34*: In vitro* evaluation of fungicides against *Colletotrichum* sp. (ALVSR- inflorescence rot pathogen)

A1: Copper hydroxide (0.1%), A2: Copper hydroxide (0.2%), A3: Copper hydroxide (0.3%)
B1: Hexaconazole (0.05%), B2: Hexaconazole (0.1%), B3: Hexaconazole (0.15%)
C1: Propineb (0.1%), C2: Propineb (0.2%), C3: Propineb (0.3%)
D1: Difenoconazole (0.05%), D2: Difenoconazole (0.1%), D3: Difenoconazole (0.15%)
E1: Carbendazim 12%+ Mancozeb 64% (0.1%), E2: Carbendazim 12%+ Mancozeb 64% (0.2%), E3: Carbendazim 12%+ Mancozeb 64% (0.3%)
F1: Azoxystrobin (0.05%), F2: Azoxystrobin (0.1%), F3: Azoxystrobin (0.15%),
G1: Cymoxanil 8% + Mancozeb 64% (0.1%), F2: Cymoxanil 8% + Mancozeb 64% (0.25%), F3: Cymoxanil 8% + Mancozeb 64% (0.5%)
H: 1% Bordeaux mixture C: Control plate

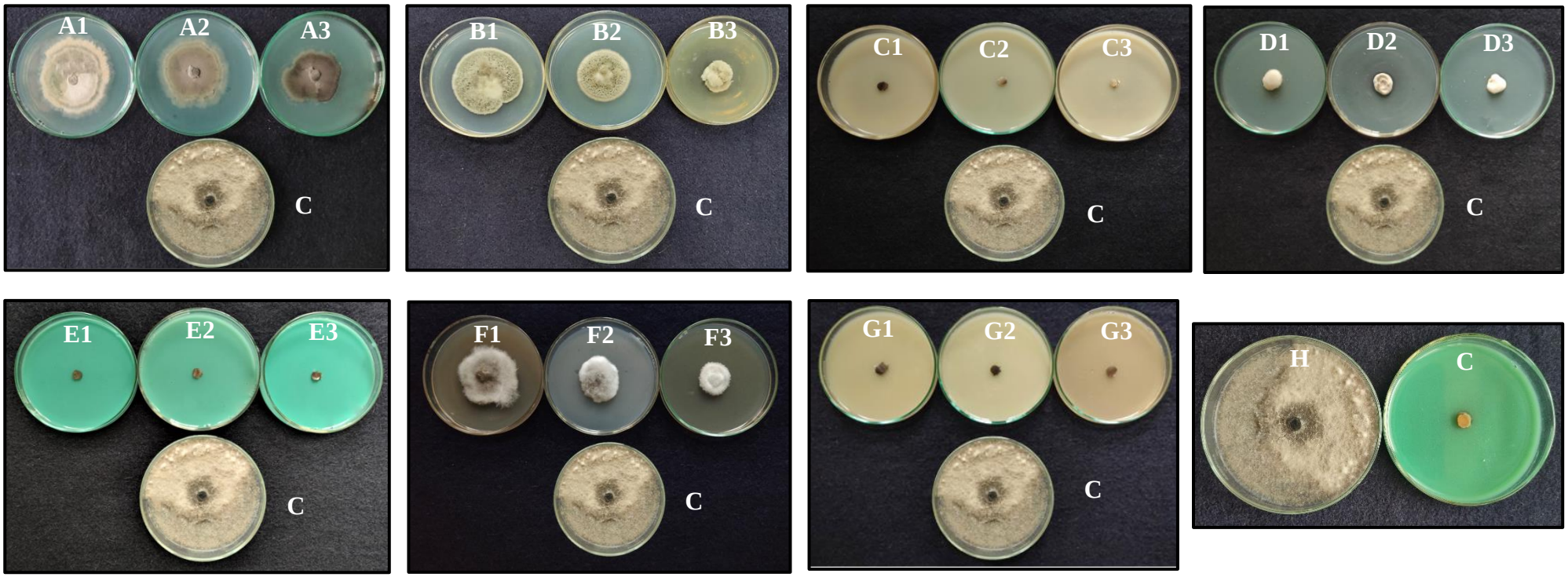

Plate 35: *In vitro* evaluation of fungicides against *Colletotrichum* sp. (PBALS- leaf spot pathogen)

A1: Copper hydroxide (0.1%), A2: Copper hydroxide (0.2%), A3: Copper hydroxide (0.3%)
B1: Hexaconazole (0.05%), B2: Hexaconazole (0.1%), B3: Hexaconazole (0.15%)
C1: Propineb (0.1%), C2: Propineb (0.2%), C3: Propineb (0.3%)
D1: Difenoconazole (0.05%), D2: Difenoconazole (0.1%), D3: Difenoconazole (0.15%)
E1: Carbendazim 12%+ Mancozeb 64% (0.1%), E2: Carbendazim 12%+ Mancozeb 64% (0.2%), E3: Carbendazim 12%+ Mancozeb 64% (0.3%)
F1: Azoxystrobin (0.05%), F2: Azoxystrobin (0.1%), F3: Azoxystrobin (0.15%),
G1: Cymoxanil 8% + Mancozeb 64% (0.1%), G2: Cymoxanil 8% + Mancozeb 64% (0.25%), G3: Cymoxanil 8% + Mancozeb 64% (0.5%)
H: 1% Bordeaux mixture C: Control plate

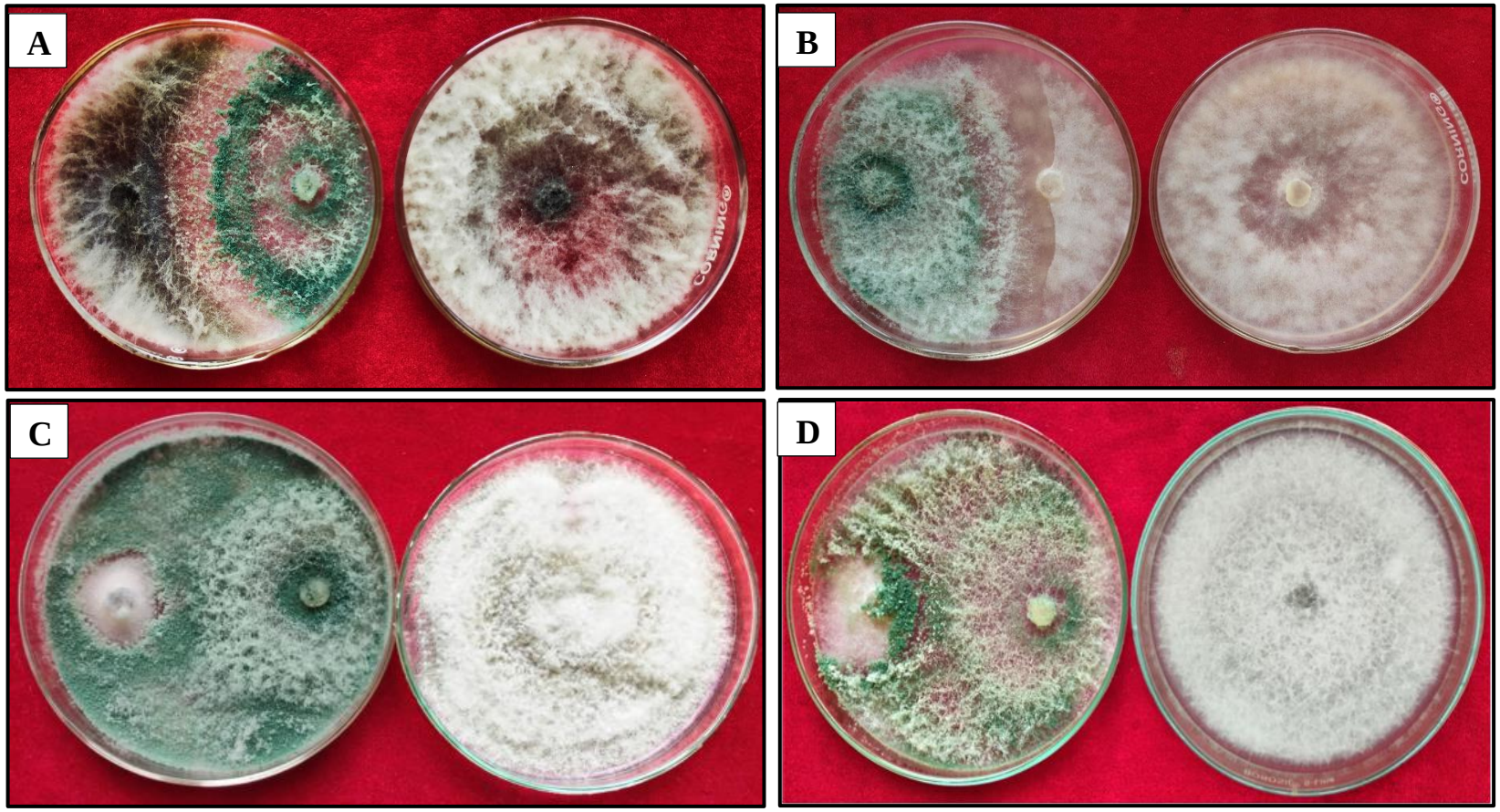

Plate 36: *In vitro* evaluation of *Trichoderma* sp. against fungal pathogens

A: *Trichoderma* sp. x *Lasiodiplodia theobromae*

B: *Trichoderma* sp. x *Phytopythium vexans*

C: *Trichoderma* sp. x *Colletotrichum* sp. (ALVSR- inflorescence rot pathogen)

D: *Trichoderma* sp. x *Colletotrichum* sp. (PBALS- leaf spot pathogen)

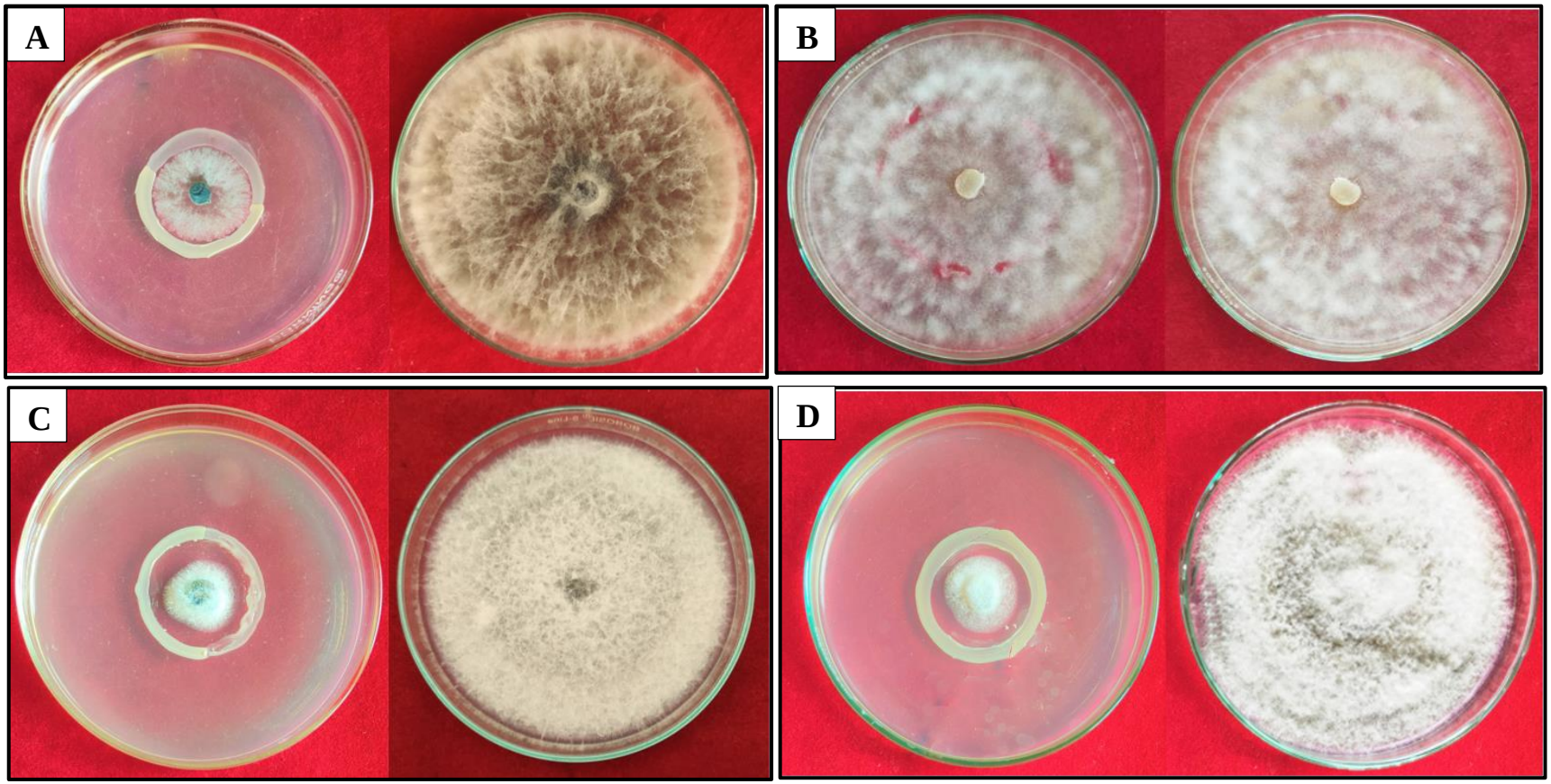

Plate 37: *In vitro* evaluation of *Pseudomonas fluorescens* against fungal pathogens

A: *Pseudomonas fluorescens* x *Lasiodiplodia theobromae*

B: *Pseudomonas fluorescens* x *Phytopythium vexans*

C: *Pseudomonas fluorescens* x *Colletotrichum* sp. (ALVSR- inflorescence rot pathogen)

D: *Pseudomonas fluorescens* x *Colletotrichum* sp. (PBALS- leaf spot pathogen)

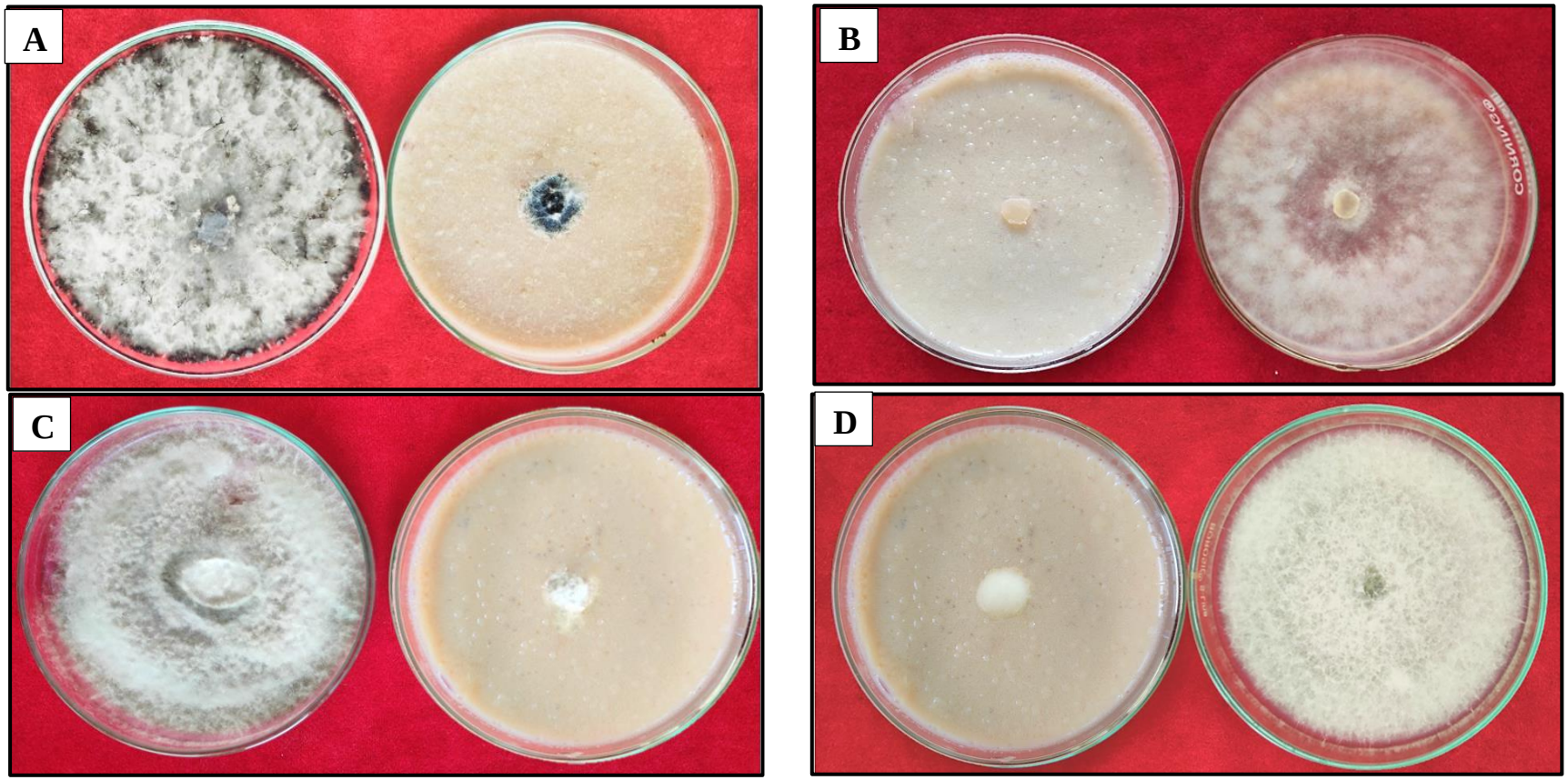

Plate 38: *In vitro* evaluation of PGPR-II against fungal pathogens

A: PGPR-II x *Lasiodiplodia theobromae*

B: PGPR-II x *Phytopythium vexans*

C: PGPR-II x *Colletotrichum* sp. (ALVSR- inflorescence rot pathogen)

D: PGPR-II x *Colletotrichum* sp. (PBALS- leaf spot pathogen)

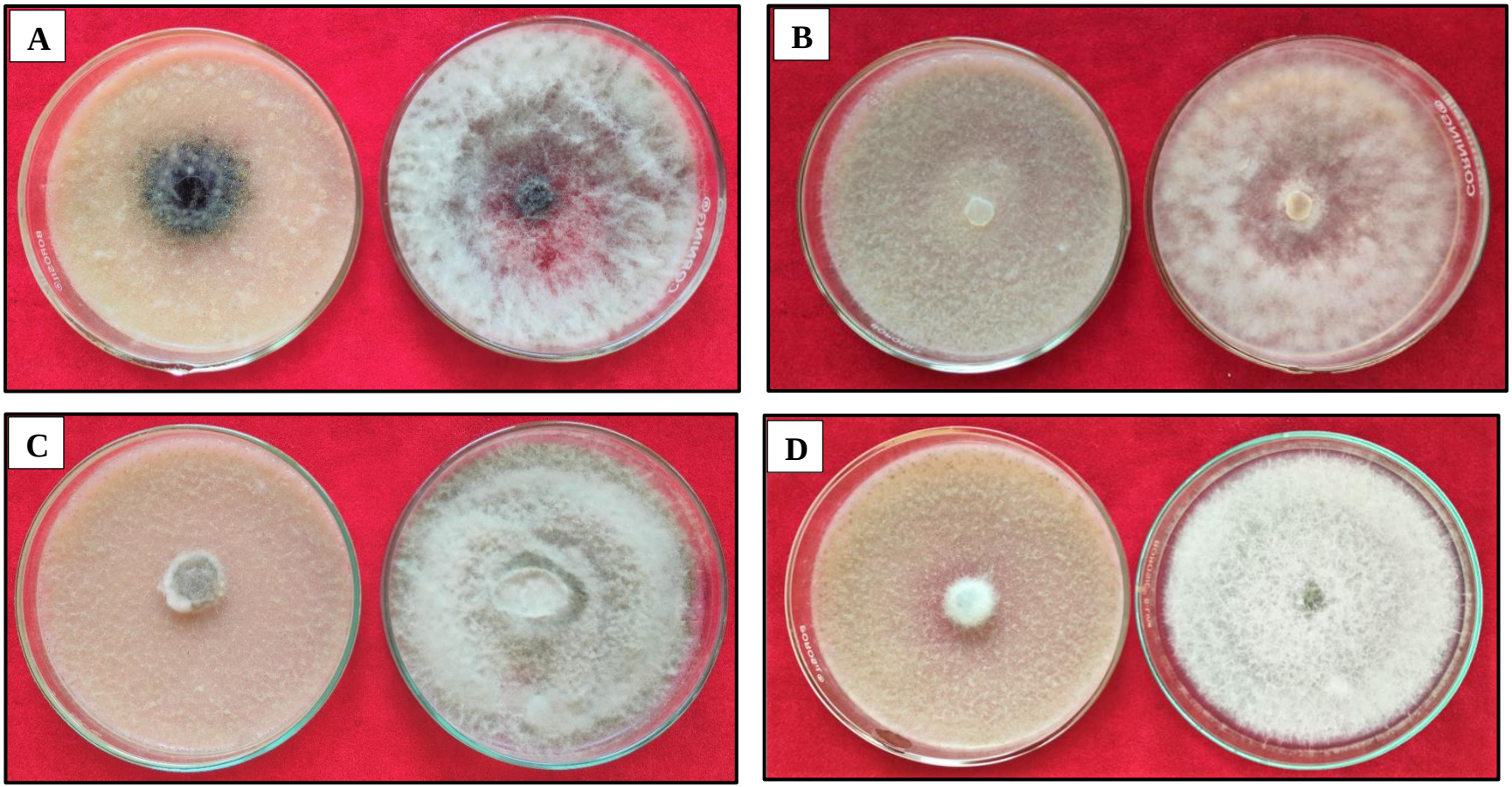

Plate 39: *In vitro* evaluation of PGPM against fungal pathogens

A: PGPM x *Lasiodiplodia theobromae*

B: PGPM x *Phytopythium vexans*

C: PGPM x *Colletotrichum* sp. (ALVSR-inflorescence rot pathogen)

D: PGPM x *Colletotrichum* sp. (PBALS-leaf spot pathogen)

4.9 *IN VITRO* EVALUATION OF ANITIBIOTICS, FUNGICIDES AND BIOCONTROL AGENTS AGAINST BACTERIAL PATHOGEN

4.9.1 *In vitro* evaluation of antibiotics and fungicides against bacterial pathogen

Two fungicides *viz*. copper hydroxide at three doses and Bordeaux mixture at 1 per cent and an antibiotic streptocycline were tested against *Xanthomonas axonopodis* by inhibition zone technique. Among the chemicals, streptocycline was found highly effective against the pathogen since the inhibition percentage obtained with this antibiotic was 27.78 per cent, 33.33 per cent and 38.89 per cent when applied at 100 ppm, 200 ppm and 250 ppm respectively. An inhibition zone of 2.2 cm was observed when copper hydroxide was used at the recommended dose of 0.2 per cent and an inhibition per centage of 12.22 and 28.89 were noticed at the lower dose (0.1%) and higher dose (0.3 %) respectively. The paper discs soaked in 1 per cent Bordeaux mixture produced an inhibition percentage of 30 against the pathogen (Table:4.15; Plate 40).

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

The antagonistic activity of *Trichoderma* sp. (KAU reference culture), *Pseudomonas fluorescens* (KAU reference culture), PGPR-II and PGPM (KAU formulations) was tested against *Xanthomonas axonopodis* by inhibition zone technique and streak method.

From the experiment it was evident that all the tested biocontrol agents showed different degree of inhibition. When *Trichoderma* sp. was used as an antagonist, an inhibition zone of 3.8 cm was observed within 48 h of incubation in the Petri plates when antagonist disc of 8 mm was placed at the centre. Thereafter bacterial population gradually decreased and finally no bacterial growth was visible as the fungal antagonist completely overgrew the culture (Plate 41; C & D). The interaction effect of *Trichoderma* sp. against the bacterial pathogen was also tested using streak method by swabbing bacterial suspension in parallel lines which were 2 cm away from the periphery of Petri plates containing mycelial disc of the antagonist. After 72 h of incubation, a slight reduction in the bacterial growth towards the inner region of swab was observed in the treatment plates where the fungal and bacterial growth overlapped. After five days of incubation, it was found that bacterial cells failed to grow where the

antagonist have grown and sporulated whereas reduction in growth of *Xanthomonas axonopodis* was noticed along the swab where the mycelial growth of *Trichoderma* sp. was only present without sporulation. Within a week of incubation, *Xanthomonas axonopodis* was completely overgrown and lysed by *Trichoderma* sp. as no bacterial cells were visible in the treatment plates compared control plates (Plate 41; A & B).

After an incubation period of 72 h, an inhibition zone of 2.3 cm was observed when a loopful culture of *Pseudomonas fluorescens* was inoculated at the centre of *Xanthomonas axonopodis* seeded media which showed an inhibition percentage of 34.45 % (Plate 42).

The KAU formulations of PGPR-II and PGPM showed different degree of inhibition on *Xanthomonas axonopodis*. Two methods were used to evaluate the efficacy of formulations on the bacterial pathogen. In the first method, a single central streak or three spot inoculations of the pathogen was given on the media seeded with each of the formulation whereas in the second method inhibition zone developed around the filter paper disc dipped in the respective formulation and placed over the media seeded with bacterial pathogen were evaluated. In the case of first method, no bacterial growth was observed along the streak and the inoculated spots in the treatment plates (both PGPR-II and PGPM) (Plate 43, 44, 46, 47) compared to the control plate. In the second method, a percentage inhibition in growth of 17.78 (Plate 45) and 20 (Plate 48) were recorded from PGPR-II and PGPM treatment plates respectively (Table: 4.17).

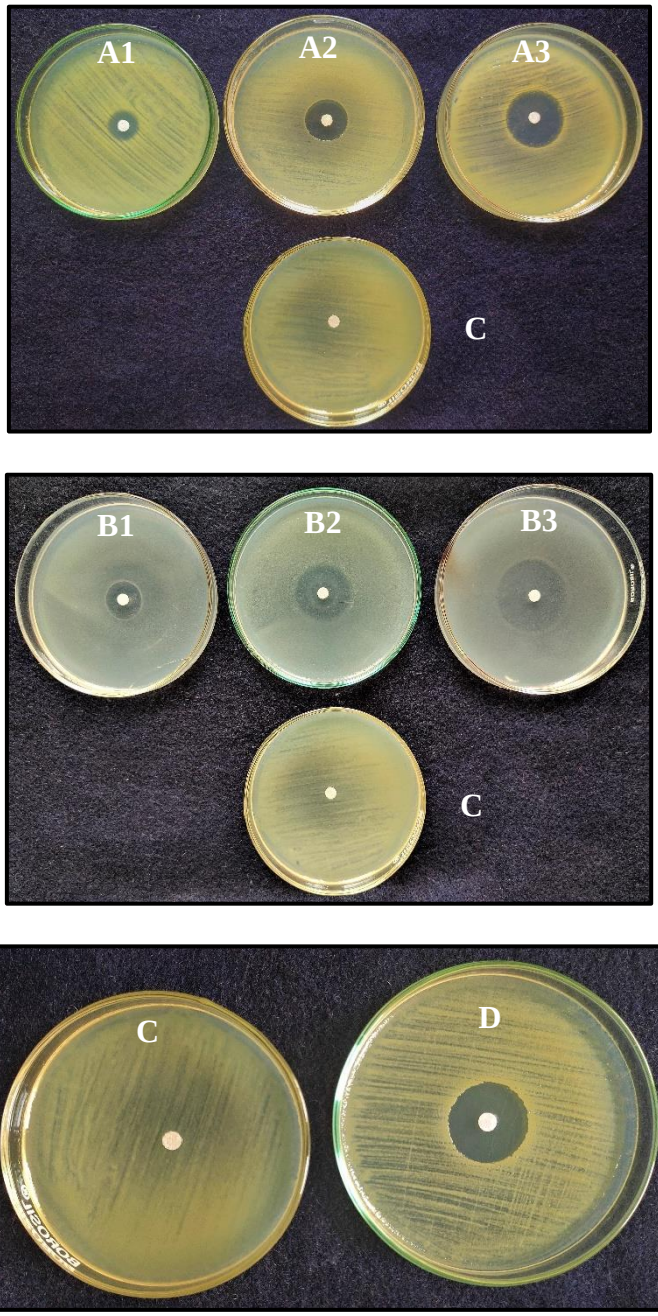

Plate 40: *In vitro* evaluation of chemicals against *Xanthomonas axonopodis*

A1: Copper hydroxide (0.1%)
A2: Copper hydroxide (0.2%)
A3: Copper hydroxide (0.3%)
B1: Streptocycline 100ppm
B2: Streptocycline 200ppm
B3: Streptocycline 250ppm
D: 1% Bordeaux mixture
C: Control plate

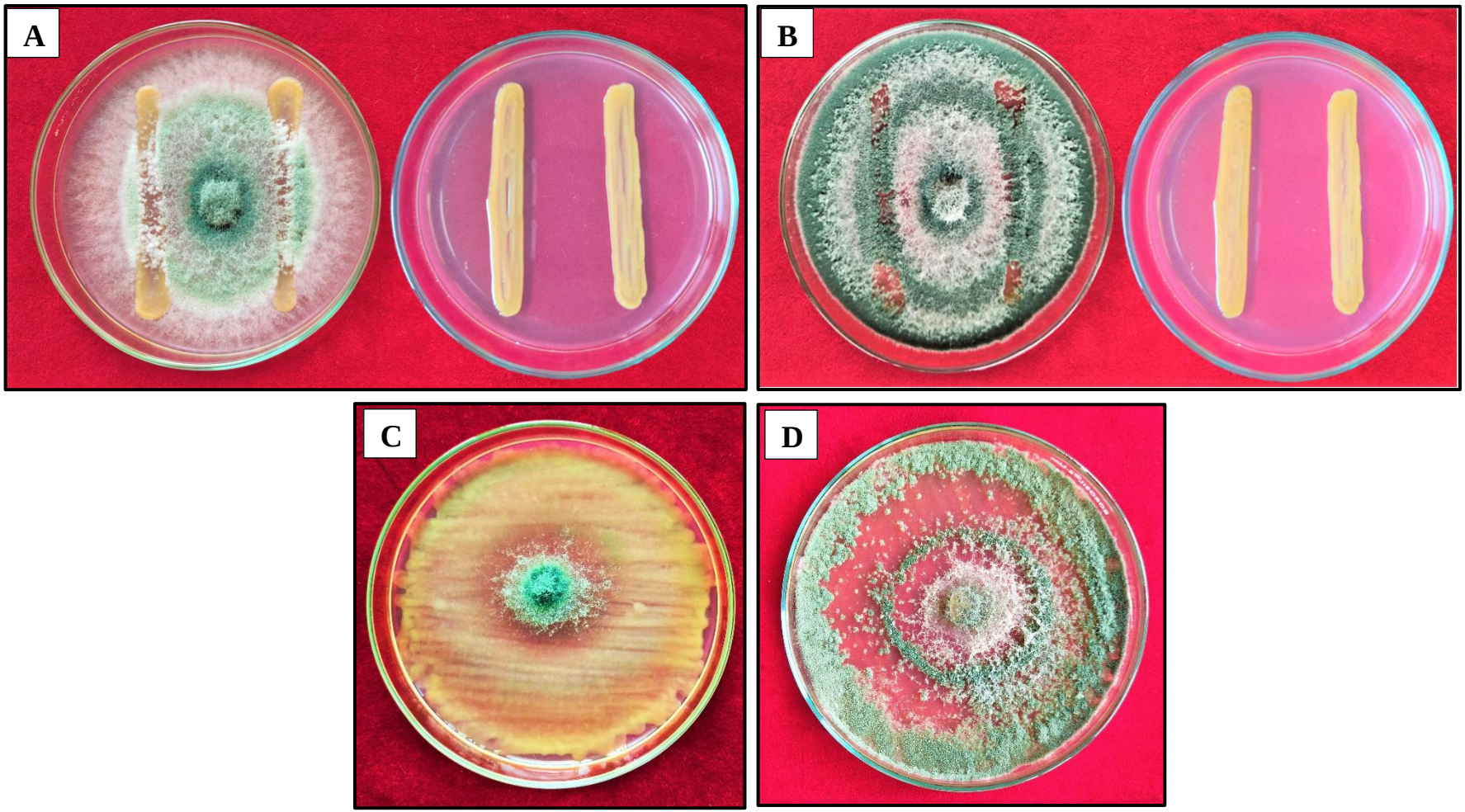

Plate 41: *In vitro* evaluation of *Trichoderma* sp. against *Xanthomonas axonopodis*

A: Observation after 5 days of incubation (Streak method)

B: Observation after 7 days of incubation (Streak method)

C: Formation of inhibition zone after 48 h (Inhibition zone technique)

D: Complete lysis of bacterial cells after 7 days of incubation ((Inhibition zone technique)

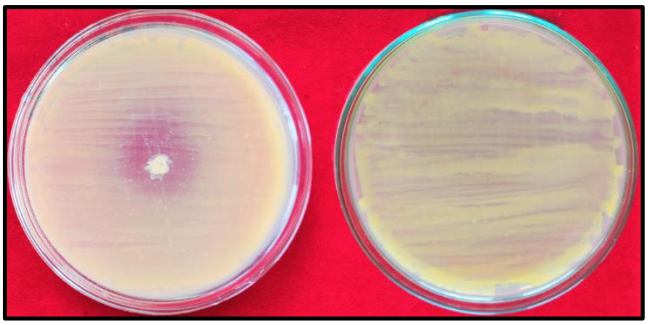

Plate 42: *P. fluorescens* x *X. axonopodis*

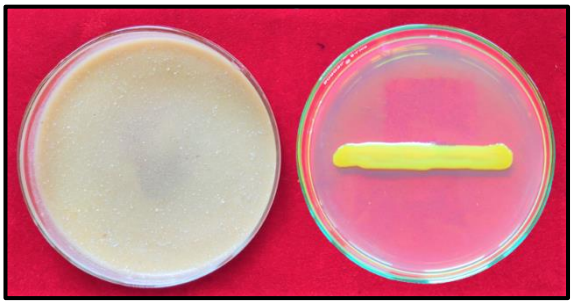

Plate 43: PGPR-II x *X. axonopodis*
(Streak method)

Plate 44: PGPR-II x *X. axonopodis*
(Spot inoculation method)

Plate 45: PGPR-II x *X. axonopodis*
(Inhibition zone technique)

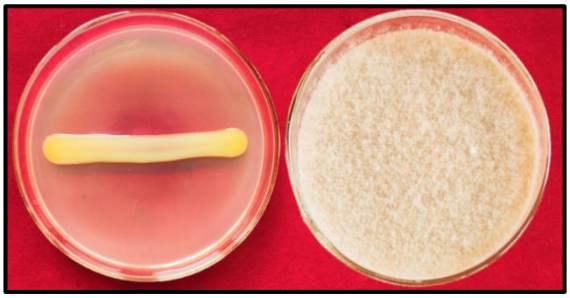

Plate 46: PGPM x *X. axonopodis*
(Streak method)

Plate 47: PGPM x *X. axonopodis*
(Spot inoculation method)

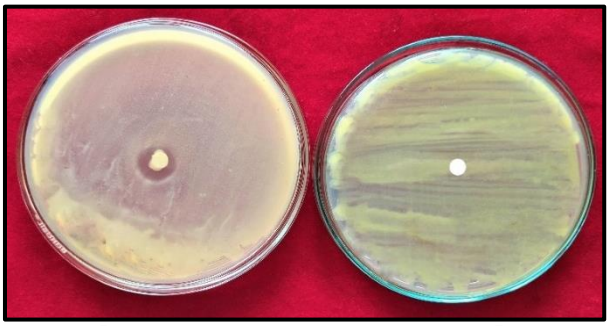

Plate 48: PGPM x *X. axonopodis*
(Inhibition zone technique)

Table 4.11: *In vitro* evaluation of fungicides against *Lasiodiplodia theobromae*

Sl No.	Fungicide	Concentration (%)	Mean colony diameter (cm)	Inhibition (%)	Transformed Value*
1.	Copper hydroxide	0.10	5.8	35.55	36.576[h]
		0.20	3.9	58.90	48.837[g]
		0.30	3.4	62.22	51.976[f]
2.	Hexaconazole	0.05	2.5	72.22	58.208[d]
		0.10	1.6	82.22	65.081[c]
		0.15	1.0	88.90	70.557[b]
3.	Propineb	0.10	0	100	90.000[a]
		0.20	0	100	90.000[a]
		0.30	0	100	90.000[a]
4.	Difenoconazole	0.05	0	100	90.000[a]
		0.10	0	100	90.000[a]
		0.15	0	100	90.000[a]
5.	Carbendazim 12% + Mancozeb 64%	0.10	0	100	90.000[a]
		0.20	0	100	90.000[a]
		0.30	0	100	90.000[a]
6.	Cymoxanil 8% + Mancozeb 64 %	0.10	0	100	90.000[a]
		0.25	0	100	90.000[a]
		0.30	0	100	90.000[a]
7.	Azoxystrobin	0.05	2.9	67.78	55.414[e]
		0.10	2.4	73.33	58.924[d]
		0.15	1.7	81.11	64.271[c]
8.	Bordeaux mixture	1.0	0	100	90.000[a]
	CV				1.520
	CD (0.05)				1.639
	CD (0.01)				2.176**

*Angular transformed values

**Significant at 1per cent

Table 4.12: *In vitro* evaluation of fungicides against *Phytopythium vexans*

Sl No.	Fungicide	Concentration (%)	Mean colony diameter (cm)	Inhibition (%)	Transformed value
1.	Copper hydroxide	0.10	1.5	83.33	65.908[h]
		0.20	0	100	90.000[a]
		0.30	0	100	90.000[a]
2.	Hexaconazole	0.05	5.2	42.22	40.511[f]
		0.10	2.3	74.44	59.666[d]
		0.15	1.5	83.33	65.926[c]
3.	Propineb	0.10	0	100	90.000[a]
		0.20	0	100	90.000[a]
		0.30	0	100	90.000[a]
4.	Difenoconazole	0.05	2.2	75.56	60.403[d]
		0.10	1.7	81.11	64.271[c]
		0.15	1.5	83.33	65.926[c]
5.	Carbendazim 12% + Mancozeb 64%	0.10	0	100	90.000[a]
		0.20	0	100	90.000[a]
		0.30	0	100	90.000[a]
6.	Cymoxanil 8% + Mancozeb 64 %	0.10	0	100	90.000[a]
		0.25	0	100	90.000[a]
		0.30	0	100	90.000[a]
7.	Azoxystrobin	0.05	4.0	55.56	48.191[e]
		0.10	1.7	81.11	64.300[c]
		0.15	1.0	88.89	70.667[b]
8.	Bordeaux mixture	1.0	0	100	90.000[a]
	CV				1.807
	CD (0.05)				1.955
	CD (0.01)				2.596

*Angular transformed values

**Significant at 1per cent

Table 4.13: *In vitro* evaluation of fungicides against *Colletotrichum sp.* (ALVSR) (Inflorescence rot pathogen)

Sl No.	Fungicide	Concentration (%)	Mean colony diameter (cm)	Inhibition (%)	Transformed Value*
1.	Copper hydroxide	0.10	6.34	29.66	32.904[c]
		0.20	1.91	78.89	62.587[b]
		0.30	0	100	90.000[a]
2.	Hexaconazole	0.05	0	100	90.000[a]
		0.10	0	100	90.000[a]
		0.15	0	100	90.000[a]
3.	Propineb	0.10	0	100	90.000[a]
		0.20	0	100	90.000[a]
		0.30	0	100	90.000[a]
4.	Difenoconazole	0.05	0	100	90.000[a]
		0.10	0	100	90.000[a]
		0.15	0	100	90.000[a]
5.	Carbendazim 12% + Mancozeb 64%	0.10	0	100	90.000[a]
		0.20	0	100	90.000[a]
		0.30	0	100	90.000[a]
6.	Cymoxanil 8% + Mancozeb 64 %	0.10	0	100	90.000[a]
		0.25	0	100	90.000[a]
		0.30	0	100	90.000[a]
7.	Azoxystrobin	0.05	0	100	90.000[a]
		0.10	0	100	90.000[a]
		0.15	0	100	90.000[a]
8.	Bordeaux mixture	1.0	0	100	90.000[a]
	CV				0.641
	CD (0.05)				0.780
	CD (0.01)				1.035**

*Angular transformed values

**Significant at 1per cent

Table 4.14: *In vitro* evaluation of fungicides against *Colletotrichum sp.* (PBALS) (Leaf spot pathogen)

Sl No.	Fungicide	Concentration (%)	Mean colony diameter (cm)	Inhibition (%)	Transformed Value*
1.	Copper hydroxide	0.10	5.81	35.55	36.544^{g}
		0.20	4.60	48.89	44.362^{f}
		0.30	3.90	56.67	48.832^{de}
2.	Hexaconazole	0.05	5.60	37.78	37.918^{g}
		0.10	4.40	52.22	45.954^{ef}
		0.15	1.60	82.33	65.119^{b}
3.	Propineb	0.10	0	100	90.000^{a}
		0.20	0	100	90.000^{a}
		0.30	0	100	90.000^{a}
4.	Difenoconazole	0.05	0	100	90.000^{a}
		0.10	0	100	90.000^{a}
		0.15	0	100	90.000^{a}
5.	Carbendazim 12% + Mancozeb 64%	0.10	0	100	90.000^{a}
		0.20	0	100	90.000^{a}
		0.30	0	100	90.000^{a}
6.	Cymoxanil 8% + Mancozeb 64 %	0.10	0	100	90.000^{a}
		0.25	0	100	90.000^{a}
		0.30	0	100	90.000^{a}
7.	Azoxystrobin	0.05	4.70	47.78	43.724^{f}
		0.10	3.0	66.67	50.235^{d}
		0.15	2.4	73.33	58.923^{c}
8.	Bordeaux mixture	1.0	0	100	90.000^{a}
	CV				2.981
	CD (0.05)				3.065
	CD (0.01)				4.070

*Angular transformed values

**Significant at 1per cent

Table 4.15: *In vitro* evaluation of chemicals against *Xanthomonas axonopodis*

Sl No.	Chemicals	Concentration (%)	Inhibition zone (cm)	Inhibition (%)	Transformed value*
1.	Copper hydroxide	0.10	1.1	12.22	19.379^{e}
		0.20	2.2	24.44	29.614^{d}
		0.30	2.6	28.89	32.501^{bcd}
2.	Streptocycline	0.01	2.5	27.78	31.795^{cd}
		0.020	3.0	33.33	35.256^{b}
		0.025	3.5	38.89	39.013^{a}
3.	Bordeaux mixture	1.0	2.7	30	33.200^{bc}
	CV				5.826
	CD (0.05)				3.218
	CD (0.01)				4.466**

*Angular transformed values

**Significant at 1per cent

Table 4.16: Per cent inhibition of major fungal pathogens by various biocontrol agents

Sl No.	Pathogen	*Per cent* inhibition over control			
		***Trichoderma* sp.**	***Pseudomonas fluorescens***	**PGPR**	**PGPM**
1.	*Lasiodiplodia theobromea*	70	63.34	100	90
2.	*Phytopythium vexens*	60	0	100	100
3.	*Colletotrichum sp.* (ALVSR)	100	77.78	100	100
4.	*Colletotrichum sp.* (PBALS)	100	82.22	100	100

Table 4.17: Interaction of *Xanthomonas axonopodis* and various biocontrol agents

Sl. No.	Biocontrol agents / Formulations	Growth of bacteria over control				
		Streak method			Inhibition zone method (Per cent inhibition)	
		3rd day	5th day	7th day	2nd day	7th day
1.	*Trichoderma* sp.	Normal growth with slight inhibition at the region of mycelial growth	Reduced growth, cell lysis at the region of fungal sporulation	No growth, complete lysis of cells, fungus overgrown and sporulated	42.22	100
2.	*Pseudomonas fluorescens*	-			34.45	
3.	PGPR-II	No growth			17.78	
4.	PGPM	No growth			20	

Discussion

5. DISCUSSION

Flowers have long been regarded as a symbol of charm and elegance, as well as a visual feast. As in the words of Luther Burbank, “Flowers always make people better, happier and more helpful; they are sunshine, food and medicine for the soul”, flowers are one of the best gifts of nature that upgrade value and aesthetics in human dwellings. These (both loose and cut flowers) serve as the finest media to express gratitude, love and care thus enhance the emotional well-being of mankind. Considering the multifarious elements of its utility such as bio-aesthetic planning, landscape architecture, outdoor and indoor gardening, social and religious importance, the demand for floricultural plants is increasing steadily. Besides the beauteousness and attractiveness, they own great economic value in the global flower trade and thus modern commercial floriculture play a major role in boosting the global economy. In many nations around the world, it has become one of the most valuable industries in agricultural sector (Taj *et al*, 2013). Since early 1990s, Indian floriculture sector has flourished much and is as promising or profitable as it is in Europe and the United States (Smitha, 2004). This has increased the possibility for floriculture to expand and diversify which lead to the budding of urban and rural entrepreneurs in the countryside. Anthurium (*Anthurium andraeanum* L.) a native of South America which comes in the front line of global cut flower trade has a huge demand in both international flower export and domestic markets due to its long lasting flowers, leathery foliage, long stalks and differently coloured spadix and spathe. This tropical plant which flushes and flowers well under shady conditions is mainly grown in the North Eastern states of the country such as Mizoram, Nagaland, Sikkim, Meghalaya and Arunachal Pradesh followed by Karnataka in the south (Rohmingliani and Thanga, 2014). Over thousand anthurium varieties cultivated globally, only around 13 varieties are grown in Kerala namley Midori, Lima White, Tropical, Agnihotri, Liver Red, Acropolis, Gino Orange and Cuba mainly in the districts of Wayanad, Ernakulam, Thrissur and Thiruvanathapuram. The agroclimatology of the State suits well for the cultivation of this shade loving crop and therefore anthurium is one of top listed options for the flower growers of Kerala. In recent times, the cultivation of this highly potent cut flower crop was challenged by many biotic factors and the climatic changes occurred in the past few years lead to the introduction of various new diseases and pests. Even though bacterial

blight and anthracnose were the two major diseases reported to be occurring on the crop globally, a systematic study on the diseases of anthurium was not conducted in the State so far.

Various studies revealed that the major constrain regarding anthurium cultivation was the incidence of diseases that deteriorated the quality and quantity of the commercially important plant parts such as spathes, spadix and leaves. Since the crop required 75 to 80 per cent of shade and indirect bright sun light during the whole day, these are grown under shade houses and therefore the crop should be monitored frequently to check the occurrence of disease symptoms. For the better management of diseases, the causal agents have to be identified and associated symptoms have to be documented so that farmers can save the crop from pathogen infections through early identification. Therefore, this research project was undertaken to assist farmers in identifying and managing various diseases affecting anthurium and thus assure quality output from their crop. As an initial step of this project, the pathogens associated with different symptoms on anthurium grown in Kerala have been identified and characterized.

5.1 SURVEY

During the period of 2019-2021, purposive sampling surveys were scheduled and conducted in anthurium growing tracts of six districts *viz.* Thrissur, Kozhikode, Wayanad, Malappuram, Palakkad and Ernakulam to monitor the incidence of various diseases in the crop and thereafter characterize the pathogens associated. During the sampling survey, each kind of disease symptom was keenly observed and compared with the healthy plants to calculate the disease incidence. Then the extent of severity of each type of infection was recorded by using standard score charts. The collected symptoms were catalogued by assigning unique codes for each type of diseased samples which were then labelled properly and taken to the laboratory for isolation, characterization and identification of associated pathogens. Six kinds of disease symptoms (leaf spot, leaf blight, wilting, root rot, spadix rot and appearance of mosaic patterns on leaves) were obtained from various locations. The cataloguing was done based on the symptoms observed under natural conditions and the assigned code contained the abbreviations of the location from where the particular sample was

collected followed by the kind of symptom recorded *viz*. leaf spot (LS), leaf blight (LB), wilting (LW), root rot (RR), inflorescence rot (SR) and appearance of mosaic patterns on leaf (ML). The survey was conducted in ten locations of Thrissur district and one location each of the other five districts. A total of twentyone different infected samples were collected from fifteen locations out of which sixteen samples were gathered from Thrissur district alone. During the survey, it was observed that many women entrepreneurs are engaged in the cultivation of anthurium in different regions of Thrissur district compared to other districts where commercial anthurium cultivation is still in its infancy.

Among the 21 isolates, twelve were leaf spots, two leaf blights, four inflorescence rots and one each root rot and wilt. Virus infection like symptoms such as mosaic patterns and chlorotic spots on leaves, chlorotic patches on flowers/spathes and leathery texture of leaves and flowers were noticed on the plant samples collected from Vellanikkara of Thrissur district. Leaf spot was the major symptom observed in majority of the locations except in the districts Wayanad and Ernakulum. Similar kind of infections in leaf spot samples obtained during the survey were also described in anthurium by Kagivata (1990), Naseema *et al.* (1997), Amorim (1999), Norman and Ali (2018), Daengsuwan *et al.* (2019), Rex *et al*. (2019) and Vithanage *et al.* (2021).

The infected plant parts showing the characteristic symptoms were separately labelled with the unique codes and brought to the laboratory for isolation and characterization of the related pathogens. Tissue segmentation method (Rangaswamy, 1958) was employed for the isolation of fungal pathogens in potato dextrose agar whereas bacterial ooze collected from the infected plant parts were streaked on to the nutrient agar for isolating bacterial pathogens. Based on the preliminary observations, it was found that leaf spots, wilt, root rot and inflorescence rots were caused by fungal pathogens and the leaf blight was due to bacterial infection. Based on the characteristic diagnostic symptoms, the appearance of mosaic patterns and chlorotic patches on leaves and spathes were thought to be caused by virus infection initially during the survey. Similar descriptions of symptoms expressed on virus infected leaves and flowers of anthurium plants were given by Miura *et al*. (2013) and Zavareh *et al.* (2013).

The disease severity of each symptom was assessed using standard score charts whereas disease incidence was estimated by counting number of infected plants out of total plants in the surveyed location. The observations obtained thus were equated in the formula given by Wheeler (1969) to calculate the per cent disease incidence and severity. Twelve leaf spot samples were collected during the survey which occurred in majority of the locations. KMALS, PBALS, VCNLS1, VCNLS2, VFNLS, IJKLS, KKYLS, OLKLS, OKMLS, TLRLS, TBMLS and NLBLS were the leaf spots collected from Kumaranellur, Perambra, Vellanikkara, Irinjalakkuda, Koorkkenchery, Ollur, Oorakam, Thalore, Therambam and Nilambur respectively. The ease with which pathogen spores or propagules spread through rain, irrigation water, insects, wind *etc*. might explain the preponderance of leaf spot symptoms. Among the leaf spot isolates, PBALS collected from Perambra of Kozhikode district showed highest disease severity of 58.23 per cent with an incidence of 73.66 per cent. High precipitation (16.29 mm per day) and relatively higher per cent of humidity (85.62%) prevailed in this location during the time of survey impacted strongly on the spread of disease. The extent of severity recorded for KMALS, VCNLS1, VCNLS2, VFNLS, IJKLS, KKYLS, OLKLS, OKMLS, TLRLS, TBMLS and NLBLS were 33.33, 37.77, 28.88, 36, 37.03, 21, 25, 25, 42, 33.33 and 46.66 per cent respectively.

Four inflorescence rot diseases MNTSR, CKDSR, PNMSR and ALVSR were obtained from the surveyed locations *viz*. Mannuthy, Chirakkekode, Panamaram and Aluva respectively. Out of four samples, the inflorescence rot recorded from Aluva (ALVSR) has the highest percentage of incidence (59%) and severity (32.12%). The disease incidence of inflorescence rot PNMSR was 23.3 per cent and the severity observed was less compared the sample ALVSR *i.e.* 16.71 %. This might be due to the variation in the climatic conditions such as relative humidity, average temperature of the day and precipitation prevailing in plains and high altitude areas that created an unfavourable condition for the pathogen to spread. Jones (2016) described that relative humidity and temperature can determine the duration of leaf wetness to a great extent which is directly related to the survival and spread of fungal pathogens. Inflorescence rot from Chirakkekode (CKDSR) showed the least incidence and severity per cent of

15.72 and 9.13 correspondingly. MNTSR was assessed with an incidence of 31 per cent but the severity was only 24.44 per cent.

During the survey in anthurium growing shade house of Vellanikkara region conducted in the month of October 2020, a root rot symptom VCNRR was collected and found more severe in anthurium seedlings. The disease showed a higher incidence per cent of 47.33 and the severity recorded was 64 per cent. The preliminary microscopic observations and the cultural characters of the fungus examined during the isolation revelated that the pathogen associated with this symptom was an oomycete. Park *et al*. (2019) recorded similar observations in a disease survey of anthurium conducted in Korea during the month of September, 2019 where five per cent of the seedlings surveyed were infected by the oomycete pathogen.

The wilting isolate MNTLW collected from Mannuthy showed an incidence per cent of 21. The samples tested negative in the ooze test and the isolation of the pathogen from infected leaf bits revealed that the pathogen associated with this wilting symptom was a fungus.

Pathogens responsible for infection in eighteen isolates collected during the purposive sampling survey were identified to be fungal pathogens from the preliminary isolation studies and microscopic observations. Among these 18 isolates, 7 samples (KMALS, PBALS, VFNLS, MNTSR, TBMLS, PNMSR and ALVSR) were found to be caused by *Colletorichum gloeosporioides* which was identified from the initial microscopic examination of the isolates. The survey revealed that the crop damage caused by this particular pathogen was very serious and it could be considered as an important widespread fungal pathogen that its incidence was recorded in all the surveyed districts except Malappuram. Santhakumari *et al*. (2001), Kalidas (2013), Rex *et al.* (2019) and Guarnaccia *et al*. (2019) also described inflorescence rot and leaf spot disease caused by *Colletotrichum* sp. which caused serious damage in anthurium plants globally.

Severe leaf blight symptoms were recorded from Vellanikkara and Ollukkara regions of Thrissur districts where the disease incidence was 27.63 % and 25 % respectively. The systemically infected plants were completely lost even though the

blighted leaves were removed from the plants. Several reports revealed that this disease caused a total loss to the anthurium growers if not managed properly and was responsible for major share of crop loss in the world (Hayward (1972); Cooksey (1985); Hoogasian (1990); Shehata (1992); Duffy (2000)).

During the survey, plants showing symptoms resembling virus infection were collected from the shade house in Vellanikkara. The symptoms like mosaic appearance on leaves, chlorotic spots on leaves and flowers and leathery texture of leaves were noticed in the infected plants. However, under further investigation using transmission electron microscopy it was confirmed that no virus particles were detected in the infected sample. Hence such symptoms might be due to nutritional imbalance.

5.2 PATHOGENICITY

The fungal and bacterial pathogens isolated from the diseased samples were subjected to pathogenicity studies to prove Koch's postulates. The pathogenic isolates were inoculated to the healthy plants/plant parts using various inoculation techniques based on the kind of pathogen (fungi or bacteria) and characteristics of symptoms. The pathogenicity of isolates collected from spathes, spadix and leaves were proved by following the protocols of Mycelial Bit Inoculation Method (MBIM) in which the fungal mycelial plugs collected from actively growing cultures were kept over the surface of healthy tissues as described by Rocha *et al*. (1998).

The fungal pathogens associated with the leaf spot samples KMALS, PBALS, VFNLS and TBMLS initiated infection within two days of inoculation and the symptoms developed on the inoculated healthy leaf tissues were identical to the observations recorded by Mathur (2000) and Kee *et al.* (2020) where the characteristic anthracnose symptoms were visible at the inoculated site within two days of incubation.

The leaf spot isolates VCNLS2, VCNLS1, OLRLS and TLRLS when inoculated on the healthy leaf tissues by the same MBIM produced small necrotic spots within four days of inoculation and later formed medium sized brown spots surrounded by yellow halo. Similar observations were obtained by Deon *et al*. (2012) and Kingsland (1986) where no necrotic patches were seen at one and two days after inoculation but developed pin point necrotic spots within five days of inoculation.

Three leaf spot isolates collected from the samples IJKLS, KKYLS and OKMLS produced dark brown lesions which were surrounded by slight to prominent yellow halo and the initial symptoms were noticed two to three days post inoculation. Kumar *et al*. (2019) found that healthy leaves of the brinjal showed earlier symptoms of *Phomopsis* infection within three days of inoculation under artificial conditions.

The fungal pathogen isolated from leaf spot sample NLBLS was inoculated on to the healthy leaves using MBIM and the black discolouration on the inoculated site developed two days post inoculation and that later developed in to dark necrotic spots. Similar observation of appearance of brown lesions on the inoculated spathes and spadix were reported by Daengsuwan *et al*. (2019) in anthurium plants. The leaf spot pathogen isolated from the infected sample TLRLS started developing the initial symptoms within three days of inoculation and the results obtained were similar to those described by Amorim (1999).

The pathogenicity of four inflorescence rot pathogens isolated from MNTSR, CKDSR, PNMSR and ALVSR were employed following the procedures of the same MBIM method and the same method was replicated in the experiments conducted by Pitta *et al*. (1991) to prove the pathogenicity of black rot pathogen infecting anthurium spadix. The isolates MNTSR and ALVSR produced water soaked lesions within two days of inoculation and the infection spread to the nearby tissues in a rapid rate. The spadix got rotten after the whole infected tissues turned dark brown in colour which led to the detachment of the rotten spadix from the respective spathes. After 14 days of inoculation, minute black coloured fruiting bodies were seen on the rotten tissues and the infection progressed to the nearby spathe tissue also. PNMSR initiated the infection in the spathe tissues within two days of inoculation and gradually progressed in a circular fashion to cover the whole spathe. Initially light brown coloured spots were formed which progressively turned to dark brown with immense water soaking that finally led to complete rotting of the flower. Black coloured fructifications of the associated pathogen were visible over the rotted surface of the leaf sample. Similar observations were recorded by Damm *et al*. (2019) in a study conducted to compare and analyse the pathogenicity of various *Colletotrichum* species. The fungal pathogen associated with CKDSR initiated the infection on spadix two days post inoculation.

Brown lesions were developed on the tips of the inoculated spadix that gradually progressed towards the base and white mycelial growth of the fungus was evident on the discoloured patches. Shishido *et al*. (2006) found similar observation of formation of water soaked brown lesions on the artificially inoculation cucurbits plant parts infected with *Phomopsis sclerotioides*.

The root rot pathogen isolate from VCNRR was subjected to pathogenicity tests following the procedures of MBIM in detached leaves to confirm the infection in leaves followed by drenching of mycelial suspension in the collar region of live plants to confirm the ability of pathogen to cause infection on root tissues. The detached leaves inoculated with mycelial bits showed initial signs of infection within 2 days of infection followed by extensive rotting of the young leaf. The healthy anthurium plant drenched with mycelial suspension showed symptoms such as wilting of leaf, rotting and discoloration of collar tissues, rotting of petiole of infected leaf, sloughing off and rotting of root tissues which is supported by the observations of Park *et al*. (2019) where the young seedlings of artificially infected anthurium showed vascular discoloration and rotting after two weeks of inoculation.

The isolate showing yellowing and wilting of leaves (MNTLW) collected from Mannuthy was artificially inoculated on to the veins and midrib of healthy anthurium leaves following MBIM and the associated pathogen produced initial symptoms of dry brown lesions on leaves within four days of inoculation. The lesions expanded and caused severe yellowing of leaves along with chlorosis of veins. The whole leaf dried within twelve days of inoculation. Ichikawa and Aokv (2000) obtained similar results in Cymbidium where the pathogen caused yellowing and drying of the inoculated leaves.

Two bacterial isolates associated with leaf blight disease of anthurium (VFNLB and OLKLB) were obtained during the sampling survey. The pathogenicity of both the isolates were proved in detached leaves initially followed by leaf injection of bacterial suspension in leaf veins of live anthurium plants. The symptom started developing within 13 to 15 days of inoculation on the live plants and extensive yellowing and blighting were seen at 23rd day of inoculation. The symptom expression by

Xanthomonas sp. (VFNLB and OLKLB) were in line with the observations obtained by Chase *et al.* (1992) for *Xanthomonas campestris* on different aroids.

5.3 SYMPTOMATOLOGY

Various symptoms produced by 21 isolates collected during the sampling survey conducted in anthurium growing regions of six districts of Kerala were observed and recorded both under natural and artificial conditions. The characteristic symptoms produced by the isolates under artificial inoculation is discussed in the section 5.2.

The leaf spot samples KMALS, PBALS, VFNLS and TBMLS, under natural conditions produced characteristic round to polygonal shaped, light coloured spots initially which later turned to brown coloured necrotic lesions mostly surrounded by mild to prominent yellow halos. Natural *et al.* (1994) documented similar symptoms caused by the leaf spot pathogen *Colletotrichum* sp. in anthurium plants. During later stages of infection *i. e*. after severe progression over the leaf surface, the pathogen produced numerous black coloured fruiting bodies mostly concentrated at the centre of the matured and older spots. However, PBALS, VFNLS and TBMLS produced typical brown spots that later formed dried lesions with fructifications towards the marginal areas whereas the isolate KMALS was seen concentrated at the midrib regions of the leaves. TBMLS and KMALS different from the other two isolates showed creamy white discolouration on the infected tissues.

Under natural conditions, VCNLS2 and OLRLS were characterized by round to irregular brown coloured dried spots with prominent yellow halos which appeared smudged when the disease progressed. During later stages of infection, faint to bright concentric zonations were clearly visible towards the edges of the spots that later covered the whole leaf lamina and appeared dried. Similar observations were recorded by Jinji *et al*. (2007) in rubber leaves infected by *Corynespora cassiicola*.

The leaf spot VCNLS1 was characterized by many small black colored, irregular spots mainly concentrating between the veins having and average size of 0.3 to 0.6 cm. Both the upper and lower surfaces of the lesions were dark black coloured. These observations were in line with the results given by Rashid *et al.* (2016) which showed

similar infections on the leaves and stems of tomato infected by *Phoma destructiva*. Shugufta *et al.* (2019) also reported similar symptom expression in beans.

The leaf spot samples IJKLS, KKYLS and OKMLS were characterized by the formation of grey to creamy white centered, oval to cylindrical spots initially which expanded over the leaf surface and developed yellow halos around during the later stages of infection. The isolate IJKLS differed from KKYLS and OKMLS in not having a yellow halo and the spots were still pigmented with creamy white. KKYLS and OKMLS with creamy white center developed brown coloured margins which was surrounded by yellow halo. Similar result was inferred by Xiaohui *et al.* (2004) in anthurium infected with *Phomopsis anthurii*.

The leaf spot isolate TLRLS appeared grey in colour over the upper layer of infection which was followed by a papery textured dark grey coloured tissue layer. These spots were delimited by thin brown margins and were surrounded by yellow halos. On disease progression, several spots coalesced, dried and shot holes were formed in the leaves. The underside of the spots appeared charcoal black surrounded by faint yellow halo. These observations were in agreement with the symptoms produced by *Pestalotiopsis* sp. in guava described by Keith *et al*. (2006).

NLBLS isolate was characterized by small grey blended light brownish spots with yellow halo, circular to oval in shape that were found spreading to the whole leaf lamina. On progression of the disease, nearby spathe and spadix got infected which rot completely at a later stage. Similar kind of rotting in spadix and spathe tissues were reported by Daengsuwan *et al*. (2019) in anthurium.

Rotting was the major disease found affecting anthurium flowers during the sampling survey. Four isolates of inflorescence rot pathogens were collected *viz*. MNTSR, CKDSR, PNMSR and ALVSR. Among the four isolates, MNTSR, PNMSR and ALVSR were characterized by dark brown to black irregular lesions surrounded by brown margins on the spathe and black coloured diamond shaped spots on the spadix initially. During later stages, the whole spathe and spadix got infected and rotted with the black fructifications of the pathogen on the rotten tissues. These observations were in line with the symptoms caused by *Colletotrichum* sp. in anthurium reported by

Aragaki and Ishii (1960). The symptoms shown by CKDSR were in congruence with those described above but the colour of the lesions on the spadix was light brown to dull brick red with a layer of thick brown tissues in between and dull white fungal growth were visible on the surface of spadix. Different from other three isolates, no fruiting bodies were noticed on the tissues. Similar type of symptoms was reported in strawberry by the infection of *Phomopsis* sp. (Ellis *et al*., 2000).

The root rot isolate VCNRR showed characteristic yellowing and wilting symptoms on the upper portions. The symptoms were severe in young leaves and initially the green leaves turned to yellow followed by wilting and drying. The affected petioles, stems and roots got completely rotten which showed water-soaked brownish lesions. On the roots, excessive rotting resulted in sloughing off of tissues. Identical symptoms were reported by Park *et al.* (2019) in anthurium seedlings infected with *Phytopythium vexans.*

MNTLW was the isolate associated with wilting symptoms obtained from the survey in Mannuthy of Thrissur dustrict. The isolate produced leaves that showed characteristic blighting and yellowing. The infected flowers and leaves appeared hard and brittle with severe chlorosis. The whole plant was wilted and the petioles were discoloured. These observations were in line with those given by Zhou *et al.* (2021) in *Polygonatum cyrtonema* caused by *Fusarium proliferatum*.

The leaf blight isolates VFNLB and OLKLB collected from Thrissur district showed characteristic marginal necrosis as the prominent symptom. Typical V shaped necrotic patches were found developing from the margins of the leaves and progressed towards the midrib. Prominent yellow / chlorotic halos were seen around the necrotic patches. The corresponding petioles showed vascular discoloration. The thick creamy white to yellowish ooze containing bacterial cells collected from the infected leaves confirmed the causal agent to be a bacterium. Similar symptoms were documented by Fukui (1996), Soustrade *et al*. (2000) and Kelaniyangoda and Wickramarathne (2009).

5.4 CHARACTERIZATION

The characterization and genus level identification of pathogens associated with different diseases of anthurium were done based on the cultural, morphological and biochemical (for bacterial isolates) characteristics.

The fungal pathogens were isolated on PDA and purified fungal cultures were subjected to cultural characterization to study the colour, texture, growth rate, growth pattern, sporulation, fructifications, pigmentations on the upper and lower side of the Petri plates. Slide culture technique was employed for the morphological characterisation of each isolate by documenting various microscopic observations like colour of hyphae, hyphal septations, branching patterns, conidial shape, size, dimensions and septations.

Twelve leaf spot isolates were obtained from six districts during the sampling survey. Among them, four leaf spot isolates *viz*. KMALS, PBALS, VFNLS and TBMLS were identified to be *Colletotrichum gloeosporoides*. KMALS and PBALS produced white coloured fluffy aerial mycelia which later turned to dull white and developed greyish green pigmentations on the reverse side of the plate. Yellowish orange spore mass was seen over the mycelia as the fungal culture matured and the microscopic observations revealed that the pathogen produced hyaline, septate and branched hyphae, the conidia were one- celled, hyaline, guttulate, bullet shaped with round ends and with a size ranging from 14.11 – 19.24 µm x 3.68 – 4.26 µm. These two isolates showed a regular growth and no setae was present. Conidia were produced in mass directly from the mycelia which appeared orange to yellow in colour. The observed characters were in line with the characters of *Colletotrichum siamense* coming under *Colletotrichum gloeosporioides* species complex as described by James *et al*. (2014), Ni *et al*. (2017) who detailed the characterization of *C. siamense* causing pepper spot disease and Ling *et al.* (2019) who identified *C. siamense* as causal agent of pepper spot disease in litchi. Thus, these two isolates were identified to be included in the *Colletotrichum* genus based on the reported cultural and morphological characters where the conidial size of *C. gloeosporioides* was ranging from 13.53 – 19.99 µm x 3.40 – 5.16 µm. The isolate VFNLS produced floccose white to dark grey coloured aerial mycelia that later turned to dark brownish green and the reverse side of the plate were pigmented with dark

brown tinged with green. Yellow to orange coloured spore mass was seen over the mycelia as the culture matured. The fungus produced acervuli with prominent setae. The cultural characters of the isolate TBMLS were slightly different from those of the other three isolates that it produced olivaceous green tinged aerial mycelia which later turned dark greyish greenish in colour. The texture of the fungal growth was more cottony, sparse and smooth rather than fluffy that was observed in the other three cultures. Slight zonations were visible along the radial growth of the pathogen and black fruiting bodies were seen on the culture. Thick orange coloured conidial mass were observed over the acervuli. These observations were in line with the cultural and morphological characters of *C. queenslandicum* described by James *et al.* (2014) which was characterized by the presence of greyish green scant aerial mycelia with scattered acervuli containing yellow ooze and with a conidial size ranging from 14 – 16 µm length and 3 – 6 µm width. A complete growth over Petri plates (9 cm diameter) was obtained within 6 to 7 days of incubation for these four leaf spot isolates. The cultural and morphological characters of the four leaf spot isolates were similar to the characters of *C. gloeosporioides* described by Weir *et al.* (2012).

Two leaf spot isolates VCNLS2 and OKMLS collected from Vellanikkara and Oorakam respectively were confirmed as *Corynespora* sp. which was characterised by the formation of thick and dense dark grey to green mycelia which later showed brown pigmentation. Both the isolates were hairy textured with dark green colour on the reverse side of the plate. The fungal colonies which developed faint concentric growth rings were later covered abundantly with effused aerial mycelia. A complete growth of the fungus was attained within eight days and the recorded growth rate was 1.12 cm per day. The cultural characteristics of *Corynespora* sp. were in line with the observations of Shimomoto *et al*. (2011) and Qi *et al*. (2011). The morphological characters observed in the slide culture revealed that the fungal isolates produced brown coloured, septate hyphae and the conidia were obclavate – cylindrical in shape, mostly straight, pale brown to sub-hyaline with 3-14 pseudoseptations, with an average dimension of 83.82 µm x 9.30 µm. Conidial observations of *Corynespora* sp. were found similar to the observations recorded by Nghia *et al*. (2008).

Three leaf spot isolates IJKLS, KKYLS and OKMLS caused by *Phomopsis* sp. developed white cottony mycelia which later turned compacted thick and shrunken after attaining full growth in the PDA plates of 9 cm diameter. The reverse side of the plate containing IJKLS showed white to tan coloured pigmentation whereas the isolates KKYLS and OKMLS appeared dark brown. All the isolates produced dark brown coloured pycnidial bodies which seemed partially immersed in the PDA and all the three isolates completed growth in five to six days with a growth rate of 1.6 to 1.8 cm per day. The cultural characters of *Phomopsis* sp. were in line with the findings of Gong *et al*. (2020). Hyaline, septate and branched mycelia were produced by the fungus with two types of hyaline, one celled conidia *i. e.* alpha conidia and beta conidia which were elliptical and filiform respectively. Beta conidia was absent in the isolate IJKLS and this might be due to the lack of any nutrients in the growth media since nutritional conditions can greatly influence the kind of spores produced in *Phomopsis* sp. as explained by Jensen (1983). The average measurement of beta conidia was 19.81 µm x 1.23 µm and alpha conidia was 6.9 µm x 2.4 µm. Similar results were recorded by Kanematsu *et al*. (1999) and Farr *et al.* (2002) confirming the pathogen to be *Phomopsis* sp.

TLRLS caused by *Pestalotiopsis* sp. developed white cottony mycelia with uneven margins and concentric zonations which later turned dark brown in colour. The lower side of the Petri plate were buff coloured and black coloured fruiting bodies were scattered in the media as the culture matured. The pathogen attained full growth in nine days with a growth rate of one centimetre per day. These observations were in agreement with the cultural characters of *Pestalotiopsis* sp. described by Maharachchikumbura *et al*. (2011). Pathogen produced five celled, fusiform conidia with four to five septations where the middle three cells were brown coloured and outer cells were hyaline. Two to four conidial appendages were present on the apical cells and only one appendage was seen in the basal cell. The average size of the conidia was 28.30 µm x 6.86 µm. Ren *et al*. (2013) observed similar characteristics and thus the pathogen was identified as *Pestalotiopsis* sp. at the genus level

NLBLS, another leaf spot isolate was confirmed to be *Lasiodiplodia* sp. and developed characteristic grey coloured fluffy mycelia that covered the entire surface of

Petri plate within two to three days with a vigorous growth rate of three centimetre per day. As the culture matured, black coloured mycelial growth was seen and the raised mycelia got suppressed. After one month of incubation, black coloured hard pycnidial bodies developed in the culture from the tip of which black liquid oozed out and black dusty spores were released. The pathogen produced brown septate hyphae and oval shaped conidia. The immature conidia were hyaline, aseptate and were having thick cell wall without striations. Mature conidia were brown coloured, septate and with longitudinal striations. The average size of conidia was 26.40 µm x 14.70 µm. The cultural and morphological characters were cent per cent matching with the characters of *Lasiodiplodia theobromae* described by Urbez-Torres *et al*. (2008).

The leaf spot VCNLS1was identified to be caused by *Phoma* sp. which produced dark brown floccose aerial mycelia that later turned to olivaceous green blended with dark brown with a dusty appearance. The reverse side of the plate was pigmented in deep brown. A complete growth of the pathogen was recorded within five days with a growth rate of 1.8 cm per day. Hyphae was brown septate and branched which produced unicellular, hyaline and ellipsoid conidia. Brown coloured round shaped chlamydospores were seen on the hypha. The average size of conidia was measured as 6.10 µm x 2.55 µm and the average size of pycnidia was 84.53 µm x 73.12 µm. Davidson *et al*. (2009) observed similar cultural and morphological characters for the pathogen *Phoma* sp.

Four isolates of inflorescence rot pathogens *viz*. MNTSR, CKDSR, PNMSR and ALVSR were collected from Thrissur, Wayanad and Ernakulum districts respectively. The cultural and morphological characters revealed that the pathogen associated with the isolates MNTSR, PNMSR and ALVSR were *Colletotrichum gloeosporioides*. The growth of the fungal isolate MNTSR was in a regular pattern and the mycelia were white in colour with fluffy appearance that resembled the leaf spot isolates KMALS and PBALS. The isolate PNMSR in contrary to MNTSR produced fluffy white to greyish green mycelia and the growth progressed in an irregular pattern. Raised and fluffy aerial growth was noticed unevenly over the surface with abundant orange coloured spore mass. This isolate produced acervuli without setae. Numerous conidiophores were present from where the conidia were released. In case of MNTSR, conidia were directly

produced from mycelia as in the isolates KMALS and PBALS where no acervuli was found in the culture. The isolate ALVSR appeared dark grey in colour with numerous masses of conidia in yellow colour. Acervuli was not produced in the culture as the fungus may produce conidia directly from mycelia. A regular pattern of radial growth was noticed with uneven aerial growth which appeared raised and fluffy. Numerous clavate to irregular shaped appressoria were distributed throughout the culture. The three isolates associated with the inflorescence rot disease showed variability in the cultural and morphological characters even though all these were *C. gloeosporioides*. Full growth of these isolates was observed in seven to eight days with a growth rate of 1.125 to 1.28 cm per day. Hyaline, non-septate, single celled, guttulate and bullet shaped conidia were produced by the pathogen with a size ranging from 13 to 21 µm length and 3 to 5 µm in breadth. The cultural and morphological characters were in consistent with the findings of Smith and Black (1990) confirming the pathogen to be *Colletotrichum gloeosprioides*.

Seven isolates of *Colletotrichum* sp. were collected from four leaf spots and three inflorescence rots. The morphological and cultural characters showed great variations among the isolates and thus they are not one and the same. The hyphal colour varied from hyaline to light brown and the conidial dimensions ranged from 13 to 20 µm in length and 3 to 5 µm in breadth. The variation in conidial size may be due to the formation of secondary conidia as described by Pandey *et al.* (2012).

The inflorescence rot pathogen CKDSR on isolation produced white profuse aerial mycelia which became compacted and was slightly pigmented in yellow. Black concentric pycnidia developed on the culture with yellow oozing. The reverse side of plate was tan in colour. The fungus attained full growth within six days with a growth rate of 1.6 cm per day. Two types of conidia *viz.* alpha conidia and beta conidia were seen under microscope. Alpha conidia were hyaline, one celled, elliptical and guttulate with a size of 6.8 µm x 2.9 µm whereas beta conidia were filiform, curved and hyaline and measured 19.08 µm length x 1.73 µm breadth. These observations were similar to the findings of Rosskopf *et al.* (2000) thus the pathogen was confirmed to be *Phomopsis* sp.

The isolate of root rot symptom VCNRR was characterized by white blooming radial mycelia resembling floral patterns and later developed white fluffy aerial mycelia over the surface with no visual signs of sporulation. The pathogen attained complete growth in the media within four days and the growth rate was 2.25 cm per day. The observation recorded were similar to cultural characters of *Phytopythium* sp. described by Prencipe *et al*. (2020). The hyphae of this oomycete were hyaline, aseptate and freely branching which produced characteristic globose to subglobose, mostly terminal and papillate/non papillate sporangia with an average diameter of 17.64 µm x 21.87 µm. Similar characters were studied and documented by Jabiri *et al*. (2020) while studying anthracnose of apple caused by *Phytopythium vexans.*

The fungal pathogen associated with wilting symptom MNTLW was identified as *Fusarium* sp. and it produced pinkish white mycelia that later turned dark purple. A full growth of fungus was obtained within eight days of incubation with a growth rate of 1.125 cm/day. Lebeau *et al*. (2019) observed similar kind of cultural characters with dark purple colouration of the culture while working with *Fusarium* sp. The fungus produced hyaline and septate hypha with both microconidia and macroconidia. Macroconidia was hyaline and septate with a size of 24 µm x 2.55 µm whereas microconidia were hyaline and aseptate that measured 8.0 µm (length) X 3.0 µm (breadth). These morphological characters were in congruence with the findings of Fravel *et al*. (2003).

Two pathogenic bacterial isolates associated with leaf blight symptoms *viz*. VFNLB and OLKLB were obtained from Vellanikkara and Ollukara regions of Thrissur district respectively. Both the isolates showed colony growth within 72 h of incubation and in nutrient agar they produced medium sized, circular, smooth and yellowish colonies with excessive mucus. These cultural characters were exactly matching with the observations of Mhedbi-Hajri *et al*. (2013) and Benagi and Nargund (2013) who described the colony characteristics of *Xanthomonas* sp. as yellowish, convex, raised, smooth and mucoid. The morphological characters such as size and shape of bacterial cells were studied under Scanning Electron Microscope (Tescan Vega-3 LMU) at Central instrumentation Laboratory, College of Veterinary and Animal Sciences, Mannuthy. The SEM images revealed the shape of these blight pathogens as short rods

with an average size of 1.39 µm x 0.36 µm. The morphological characters observed were in close proximity with the findings of Joseph (1997) and Lambani and Jahagirdar (2017). Both the isolates showed positive reaction towards amylose production test, hydrolysis of gelatin, casein hydrolysis, citrate test, catalase test and potassium hydroxide test and negative for indole production test, MR test, VP test, oxidase test and hydrogen sulfide production test. These observations were in line with the results shown by *Xanthomonas* sp. described by Gitatis *et al*. (1987), Monllor (1991), Goszczynska and Serfontein (1998), Pernezny *et al*. (2003), Arshad *et al*. (2015), Jovit *et al*. (2016), Sarker *et al*. (2017), Lia *et al*. (2018), Jadhav *et al*. (2018), Meena *et al*. (2018) and Haider *et al*. (2020).

5.5 MOLECULAR CHARACTERISATION OF ISOLATES

Cultural, morphological and biochemical (in case of bacterial pathogens) characteristics were used to identify and characterize fungal and bacterial pathogens from different locations during sampling survey. The molecular characterization of the selected isolates was carried out by the amplification of ITS region and 16S rRNA regions of isolated genomic DNA of fungus and bacteria respectively.

The root rot VCNRR pathogen was identified to be of the genus *Phytopythium* on the basis of the observed cultural and morphological characters. The comparison of nucleotide sequences corresponding to ITS region showed 99.77 per cent sequence similarity and 100 per cent query cover with a maximum score of 791 to the accession of *Phytopythium vexans* (MW426381.1). The sequence also showed 99.07 per cent similarity with the accessions of the same pathogen (KP183960.1, KP183933.1, MH478301.1). Therefore, the pathogen associated with the root rot symptom VCNRR collected from Vellanikkara was confirmed to be *Phytopythium vexans*. Park *et al*. (2019) also reported the incidence of *Phytopythium vexans* in anthurium seedlings. The perusal of literature shows that this might be the first report of *P. vexans* in anthurium in India.

The pathogen MNTLW associated with wilting symptom was confirmed to be *Fusarium fujikuroi* based on the cultural, morphological and molecular characters. The nucleotide sequences of the isolate showed more than 97 per cent identity with the

accessions of *F. fujikuroi*. The same pathogen was identified by Acuna *et al*. (1999) from infected leaves of *Dracaena reflexa* var. *angustifolia.* To our knowledge, this is the first report of *F. fujikuroi* infecting anthurium in India and elsewhere.

Two leaf spot pathogens VCNLS2 and OLRLS collected from Vellanikkara and Ollur respectively were characterized based on ITS sequencing. The isolates which were identified as *Corynespora* sp. in accordance with the cultural and morphological characters were confirmed as *Corynespora cassiicola* when molecular characterization was done. The similarity search showed more than 98 per cent sequence similarity with the accessions of *Corynespora cassiicola* MK139711.1 (99.11%), KY806119.1 (99.11%), MN339671.1 (98.94%) and EU364535.1 (99.28%) thus, confirming the pathogens to be *Corynespora cassiicola.* This might be the first report of *C. cassiicola* causing leaf spot in anthurium in India.

The comparison of ITS sequences of the leaf spot isolate IJKLS with other sequences in NCBI database showed 96.01 percent sequence similarity and 66 per cent query cover with the accessions of *Diaporthe phaseolarum* MT043777.1, MN788661.1, KX519728.1 and KX510129.1. Therefore, the pathogen was confirmed as *Diaporthe phaseolarum* based on the findings of cultural, morphological and molecular characters. Literatures show that this might be the first report of *D. phaseolarum* causing leaf spot in anthurium in India,

In silico analysis of ITS sequences of the leafspot pathogens KKYLS and OKMLS showed a sequence similarity of 95.54 per cent and 99.82 per cent with the accession of *Phomopsis heveicola* (KY379053.1) with a query cover of 78 per cent and 98 per cent respectively. Both the set of ITS sequences showed more than 95 per cent similarity with the accession of *Diaporthe tulliensis* MG832547.1 (teleomorph of *Phomopsis heveicola*). Therefore, the pathogen (KKYLS and OKMLS) causing leaf spot symptom were identified and confirmed as *Phomopsis heveicola* based on the cultural and morphological characters studied. This is the first report of *Phomopsis heveicola* causing leaf spot disease in anthurium in India and elsewhere.

The pathogen causing leaf spot symptom NLBLS was identified as *Lasiodiplodia* sp. based on the cultural and morphological characters. Several hits

obtained when the ITS sequences of the isolate were BLASTN analysed in NCBI nr database showed a sequence similarity of 93.28 per cent and a query cover of 79 per cent with the accessions of *Lasiodiplodia theobroma*e (LC468781.1, LC468780.1, KT325578.1, KT325577.1 and KF910765.1). Thus. The pathogen was confirmed to be *Lasiodiplodia theobromae* as the cultural and morphological characters observed were similar to *L. theobromae*. To our knowledge, this might be the first report of *L. theobromae* infecting anthurium in India.

The sequence comparison of the isolate TLRLS collected from Thalore of Thrissur district showed a sequence similarity of 99.81 per cent with a query cover of 100 per cent with the accessions of *Pseudopestalotiopsis theae* (MF495464.1, MF495456.1, MH472583.1, MH470257.1 and MH470249.1). Therefore, the pathogen identified as *Pestalotiopsis* sp. on the basis of cultural and morphological characters was confirmed as *Pseudopestalotiopsis theae* by ITS sequencing. Chen *et al.* (2021) analysed the genetic variation in the ITS region of nuclear ribosomal DNA of *Pestalotiopsis*, *Neopestalotiopsis* and *Pseudopestalotiopsis* and concluded that *Pestalotiopsis* is the ancestor of other two *Pestalotiopsis* like fungi. However, a quick examination of the cultural and conidial characteristics of these three fungi revealed that they are all members of the *Pestalotiopsis* genus (Lateef *et al*. 2018). This is the first report of *Pseudopestalotiopsis thea* causing disease in anthurium in India and elsewhere.

Four fungal leaf spot isolates (KMALS, PBALS, VFNLS and TBMLS) and three inflorescence rot isolates (MNTSR, PNMSR and ALVSR) were confirmed to be *Colletotrichum gloeosporioides* based on cultural and morphological characters. The nucleotide sequences of ITS region of the pathogen associated with leaf spot KMALS when BLASTN analysed in NCBI database showed a sequence similarity and query cover of 100 per cent each with the accessions of *C. citri-maximae* (MN535219.1), *C. gloeosporioides* (MN067750.1), *C. siamense* (MW699607.1) and *C. alienum* (MH371126.1). In the case of MNTSR, among the top hits obtained in the NCBI nr database, the accessions of *C. gloeosporioides* and *C. siamense* were showing 90 per cent query cover and 97.99 per cent identity with the ITS sequences of the pathogen. Likewise, the BLASTN analysis of ITS sequences of the isolate PBALS showed a

sequence similarity of 95.07 and 94.87 per cent and query cover of 87 and 88 per cent with the accessions of *C. gloeosporioides* (JF710559.1 and JF710564.1) and *C. siamense* (MZ725042.1 and MT729945.1) respectively. The nucleotide sequences of ITS region of the isolate VFNLS showed 95 per cent query cover and 91.86 per cent similarity with the accession of *Colletotrichum queenslandicum* (MW055667.1) but an identical sequence similarity of 97.13 per cent and query cover of 70 per cent were also obtained with the accessions of *C. aenigma, C. gloeosporioides and C. siamense.* The ITS sequences of the isolate TBMLS when blasted in NCBI database showed 96.72 per cent similarity and 98 per cent query cover with the accession of *C. queenslandicum* (MW055667.1). Several hits obtained from the BLASTN analysis of ITS sequences of the isolate PNMSR with others showed a sequence similarity of 95.10 per cent and query cover of 100 per cent with the accession of *Colletotrichum queenslandicum* (MW055667.1). But a greater sequence similarity of 99.38 per cent and comparatively lower query cover of 78 per cent were obtained with the accessions of *C. aotearoa* (MN273087.1, MN273085.1, MN273079.1, MN273073.1 and MN273076.1).

Among the six *Colletotrichum* isolates, the species level identification of only one isolate *i.e.* TBMLS was confirmed as *C. queenslandicum* based on ITS sequencing. Mega X software was used to further analyse the species diversification and evolutionary relationship of these six isolates viz. KMALS, PBALS, VFNLS, MNTSR, TBMLS, and PNMSR with various *Colletotrichum* species such as *C. gloeosporioides, C. siamense, C. aenigma, C. aotearoa, C. quenslandicum* and *C. truncatum.* All the accessions were related to each other and no significant difference was found between the analysed species on the basis of ITS sequencing data alone. Weir *et al*. (2012) described and detailed 22 species and one sub species coming under *Colletotrichum gloeosporioides* species complex which included *C. gloeosporioides, C. siamense, C. aenigma, C. aotearoa* and *C. queenslandicum* that shared more or less similar morphological characters and were unable to identify separately on the basis of ITS gene sequencing alone. Hence multiple genes and gene combinations such as glyceraldehyde-3-phosphate dehydrogenase and glutamine synthetase were isolated and sequenced to confirm the species within the complex separately. Molecular characterisation of different species within *C. gloeosporioides* species complex causing

anthracnose in persimmon were done by multiple gene sequencing such as internal transcribed spacer (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and actin (ACT) genes where ITS sequencing alone couldn't differentiate the species within the complex (Hassan *et al*., 2018). Therefore, the species level confirmation of pathogens associated with KMALS, PBALS, VFNLS, MNTSR and PNMSR require further molecular analysis with species specific gene isolation and characterisation. From the present study, we confirmed that the pathogens associated with KMALS, PBALS, MNTSR, PNMSR, TBMLS and VFNLS were included within the *Colletotrichum gloeoesporioides* species complex. Based on the ITS sequencing, BLASTN analysis in NCBI nr database and the neighbouring- joining phylogenetic tree constructed in Mega X software, the fungal pathogen which caused the leaf spot disease TBMLS was confirmed as *C. queenslandicum.*

The BLASTN tool was used to find the best matched sequences available in the NCBI database with the 16S rRNA sequences of the genomic DNA of two bacterial isolates associated with leaf blight symptom (VFNLB and OLKLB). With a sequence similarity percentage of 99.69 and 100, the nucleotide sequences of VFNLB and OLKLB were found to be comparable to *Xanthomonas axonopodis* accessions (KY271341.1, KY271340.1, KY271339.1 and KY271338.1). VFNLB had a 99 percent query cover, while OLKLB had a 100 percent query cover. Thus, the pathogens that caused anthurium leaf blight (VFNLB and OLKLB) has been identified as *Xanthomonas axonopodis*. Soustrade *et al*. (2000), Junhai *et al*. (2014) and Suganyadevi *et al*. (2015) described the infection of *X. axonopodis* on anthurium.

5.6 CORRELATION OF WEATHER PARAMETERS WITH PER CENT DISEASE SEVERITY

Meteorological data such as rainfall, relative humidity, maximum and minimum temperature of the surveyed locations were collected and studied to find the effect of these weather parameters on the severity of diseases prevailing in each location. It was inferred that the severity of fungal diseases was higher in the location where recorded rainfall was higher. Therefore, per cent disease severity was correlated with weather parameters and correlation analysis was performed.

A disease initiates and progresses when a susceptible host is infected by a virulent pathogen under favourable environmental conditions. A thorough understanding of epidemiology is required for predicting disease outbreak and to better manage the prevailing diseases. From the correlation analysis between per cent disease severity and various weather data collected from corresponding locations, it was inferred that amount of precipitation (mm/ day) was positively correlated with the disease severity and the correlation coefficient obtained was 0.386. Among the survey locations, maximum rainfall was recorded in Perambra of Kozhikode district from where the leaf spot disease PBALS with the highest PDS (58.23%) was collected. Likewise, a positive correlation coefficient of 0.510 was obtained when relative humidity and per cent disease severity was analysed *i. e.* the disease progression was found higher in areas of higher relative humidity. In agreement to this observation, a lower severity of inflorescence rot was noticed in the sample PNMSR collected from Panamaram where the relative humidity recorded was the lowest. In contrary to relative humidity and rainfall, mean temperature had a negative effect on the disease severity of fungal pathogens as the correlation coefficient obtained was (-) 0.537. These observations were compatible with those inferred by Alvarez (2018), Norman and Ali (2018) and Kalidas (2013).

5.7 DISEASE MANAGEMENT

The inhibitory effect of fungicides was tested against two major leaf spot pathogens (*Lasiodiplodia theobromae* and *Colletotrichum* sp. (PBALS)), one inflorescence rot pathogen (ALVSR (*Colletotrichum* sp.)) and the root rot pathogen (*Phytopythium vexans*). The experiment was performed using poisoned food technique with seven fungicides each at lower dose, recommended dose and higher dose *viz*. [copper hydroxide (0.1, 0.2, 0.3 %), hexaconazole (0.05, 0.10, 0.15 %), propineb (0.1, 0.2, 0.3 %), difenoconazole (0.05, 0.1, 0.15 %), carbendazim 12 % + mancozeb 64 % (0.1, 0.2, 0.3 %), cymoxanil 8 % + mancozeb 64 % (0.1, 0.25, 0.30 %), azoxystrobin (0.05, 0.1, 0.15 %)] and 1 per cent Bordeaux mixture.

Two combination fungicides (carbendazim + mancozeb and cymoxanil + mancozeb), propineb and difenoconazole were highly effective against *Lasiodiplodia theobromae* at all the tested doses. Complete inhibition of the pathogen was observed

in these cases and the treatment effect is on par. Amrutha and Vijayaraghavan (2020) reported cent percent inhibition of *L. theobromae* causing crown rot in strawberry when treated with carbendazim 12% + mancozeb 63% (Saaf) and cymoxanil 8% + mancozeb 64% (Curzate M8). Kanyakumari (2019) reported that two non systemic fungicides *viz*. propineb and mancozeb and a systemic combination fungicide (Carbendazim + mancozeb) showed cent per cent inhibition against *L. theobromae* associated with mulberry root rot. In a study conducted by Syed *et al*. (2014) difenoconazole (Score) was found to have high rate of inhibition against this pathogen that caused stem rot in mango. Th fungicidal effect of carbendazim was due to the binding of active ingredient with *β*-tubulin of the pathogen and difenoconazole inhibited sterol synthesis of fungus (Pereira *et al.*, 2012). A similar observation of inhibition effect of difenoconazole (triazole group of fungicide) on sterol biosynthesis pathway of the fungus affecting papaya was reported in Brazil by Li *et al*. (2020). The effectiveness of carbendazim against *L. theobromae* was described by Khanzada *et al*. (2005) and Bhatt and Jadeja (2010). An *in vitro* analysis conducted against the pathogen with four fungicides *viz*. Thiophanate-methyl, Carbendazim, Precure combi (Thiophanate-methyl + Diethofencarb) and Copper oxychloride provided a maximum inhibition of fungal growth by benzimidazole group of fungicides carbendazim, thiophanate methyl along with combination product Precure combi followed by copper oxy chloride (35.26 %) (Shahbaz *et al*, 2009). Among the fungicides tested, more than 50 per cent of inhibition was given by azoxystrobin and hexaconazole at lower, recommended and higher doses.

Three fungicides *i. e.* propineb, carbendazim + mancozeb and cymoxanil + mancozeb at three different doses resulted in cent per cent inhibition of *Phytopythium vexans* and copper hydroxide at recommended (0.2%) and higher dose (0.3%) completely inhibited the mycelial growth whereas lower dose of the same fungicide showed an inhibition percentage of 83.33. Similar result which showed complete pathogen inhibition was reported in the oomycete, *Phytophthora* sp. when sprayed with copper hydroxide (Meadows *et al*, 2011). Another study by Benfradj *et al*. (2016) also showed similar results in agreement with the effectiveness of copper hydroxide against *Phytophthora*, *Pythium* and *Phytopythium* where 100 per cent inhibition of mycelial growth was reported. Propineb was reported with an inhibition percentage of more than

80 in *Pythium* sp. (Duan *et al*., 2014). This dithiocarbamate fungicide prevents fungicide resistance in the pathogen as it has multisite action in various steps of respiratory chain. The two triazole fungicides *viz*. hexaconazole and difenoconazole showed more than 60 per cent inhibition at all the three doses (lower, recommended and higher dose). An inhibition of 58.7 per cent was observed in *Phytophthora capsici* when treated with a novel triazole fungicide as described by Wang *et al*. (2016). Azoxystrobin showed an inhibition of 55.56 per cent at a lower dose of 0.05 per cent, whereas at recommended and higher doses the pathogen was inhibited by more than 80 per cent which proved that azoxystrobin can be used for the better management of the pathogen. This was supported by Rende *et al.* (2012) who observed that the strobilurin fungicides *viz*. azoxysrobin and trifloxystrobin were highly effective in controlling *Phytophthora* blight in pepper plants. Therefore, strobilurins and triazoles were also effective against oomycetes along with the two combination fungicides and copper hydroxide tested against the pathogen. In a study conducted by Chaithra *et al*. (2019), to evaluate the *invitro* efficacy of seven systemic, eight non-systemic and six combination fungicides against *Pythium aphanidermatum*, the highest per cent inhibition of pathogen growth was recorded with carbendazim (systemic), mancozeb (contact) and the combination fungicide carbendazim + mancozeb. The carbendazim bears a striking similarity to one of the important plant secondary metabolites colchicine, which inactivates the spindle formation and inhibits mitosis and meiosis in both animal and plant cells. These chemicals impede spore germination and inhibit mycelial growth, as well as conidia generation in fungi by binding to the β tubulin and thus preventing spindle and microtubule formation.

Propineb, difenoconazole, carbendazim + mancozeb, cymoxanil + mancozeb and 1 per cent Bordeaux mixture were highly effective against *Colletotrichum* sp. (PBALS) at all the three doses *i. e.* lower dose, recommended dose and higher dose. When tested against *Colletotrichum* sp. (ALVSR), all the fungicides except copper hydroxide at lower dose (29.66% inhibition) and recommended dose (78.89% inhibition) showed cent per cent inhibition of fungal growth. Hexaconazole when applied at 0.05 per cent, resulted in 37.78 per cent inhibition whereas at recommended dose of 0.1 per cent and higher dose of 0.15 per cent, the inhibition observed were 52.22

and 83.33 per cent respectively in PBALS isolate. When treated with 0.05%, 0.1% and 0.15% azoxystrobin, PBALS showed inhibition in growth of 47.78%, 67.77% and 73.33% respectively. A study conducted by Piccirillo *et al*. (2018) on the sensitivity of various *Colletotrichum* sp. towards different QoI fungicides concluded that the sensitivity of isolates towards azoxystrobin, pyraclostrobin and trifloxystrobin varied greatly due to the changes in induction of alternative respiration pathways of the fungus. This might be the reason for differential sensitivity of both the *Colletotrichum* sp. isolates associated with PBALS and ALVSR to the same fungicides. Both the combination fungicides and triazole compounds were found to have high efficiency in controlling the radial growth of the two isolates (PBALS and ALVSR) and these observations were in line with the findings of Kaur *et al*. (2015) who reported a complete inhibition of *Colletotrichum* sp. when treated with difenoconazole and thiophanate methyl separately. Similar observations were documented by Kumar *et al*. (2015) a disease reduction of more than 73 per cent was obtained in chick pea with the application of triazole fungicides *viz*. tebuconazole and difenoconazole followed by hexaconazole. Mahesh *et al*. (2020) also revealed that difenoconazole resulted in 100 per cent inhibition of the pathogen whereas azoxystrobin at 0.05 per cent, 0.1 per cent and 0.15 per cent showed an inhibition of 69.63, 78.15 and 83.70 per cent respectively which is congruent to our findings. Bordeaux mixture at 1 per cent was also effective against the pathogen since no fungal growth was noticed in the poisoned media as copper ions induced membrane permeability of fungal cells and caused leakage of cell contents along with blockage of essential enzyme functions (McCallan, 1949).

Leaf blight caused by *Xanthomonas axonopodis* is a serious disease of anthurium which occur severely in all the anthurium growing tracts of the world that deteriorates the quality of this cut flower considerably. Three chemicals *viz*. copper hydroxide (0.1%, 0.2%, 0.3%), streptocycline (100 ppm, 200 ppm, 250 ppm) and 1% Bordeaux mixture were tested against the pathogen using filter paper disc method to find a better option of pathogen management *in vitro.* Among these chemicals, streptocycline showed maximum inhibition of the pathogen at 200 ppm and 250 ppm followed by 1% Bordeaux mixture which showed inhibition percentages of 38.89, 33.33 and 30 respectively at the three doses. The effect of 0.3 per cent of copper hydroxide

and 0.01 per cent of streptocycline was at par since an inhibition zone of 2.6 cm and 2.5 cm were observed around former and later treated plates correspondingly. Thakre *et al*. (2017) described similar results where streptocycline and Bordeaux mixture were found to have the greatest effect against the pathogen. The observations of Raju *et al*. (2012) and Raghuwanshi *et al*. (2013) were in congruence with our result.

The *in vitro* evaluation of antagonist ability of the bio control agents tested against fungal pathogens revealed that *Trichoderma* sp. was highly capable of inhibiting the overall growth of *Lasiodiplodia theobromae*, *Colletotrichum* sp. and *Phytopythium vexens* which showed an inhibition percentage of 70, 100 (ALVSR and PBALS) and 60 respectively. A study conducted by Bhadra *et al.* (2014) revealed that better control of *L. theobromae* was obtained with *Trichoderma viridae* when compared with the commercial fungicides tested. The antagonistic action of 20 isolates of *Trichoderma* sp. was evaluated by Shovan *et al*. (2008) where more than 90 percentage inhibition of radial growth of *Colletotrichum* sp. was reported. Another biocontrol agent, *Pseudomonas fluorescens* when tested against these pathogens provided inhibition of 63.34%, 77.78% and 82.22 % respectively against *L. theobromae*, *Colletorichum* sp. (ALVSR and PBALS) whereas no inhibition was noticed in the growth of *Phytopythium vexans*. These observations were in agreement with the findings of Benfradj *et al*. (2016), Seethapathy *et al*. (2016) and Sonawane and Patel (2017). PGPR-II formulation of KAU when applied at the rate of 20 grams per litre showed cent per cent inhibition of the pathogen growth for all the four pathogens tested. In case of PGPM, *L. theobromae* showed 90 per cent inhibition of radial growth whereas all the other three pathogens showed cent per cent inhibition.

The antagonistic effect of *Trichoderma* sp., *Pseudomonas fluorescens*, PGPR-II and PGPM (KAU reference cultures/ formulations) were tested against leaf blight pathogen, *Xanthomonas axonopodis* and it was found that all the tested antagonists showed different degrees of inhibition. An inhibition zone of 3.8 cm giving 42.22 per cent inhibition was observed when treated with *Trichoderma* sp. initially and thereafter bacterial population reduced gradually which may be due to the inhibitory compounds produced by the biocontrol agent during the interaction and finally the bacterial growth was completely inhibited as the fungal antagonist overgrow the culture. In streak

method, considerable suppression in growth and lysis of bacterial cells were observed after *Trichoderma* sp. sporulate and completely overgrew the culture which can be due to the production of antibacterial enzymes and secondary metabolites by the antagonist. When *Xanthomonas axonopodis* was streaked on both sides of Petri plate after 48 hours of keeping the mycelial disc of *Trichoderma* sp. at the centre, no growth of the bacteria was observed which may be due to the production of secondary metabolites, antimicrobial volatile compounds or antibiotics by the antagonistic fungi that already got diffused into the media before bacterial inoculation. Baazeem *et al*. (2021) reported that isolates of *Trichoderma hamatum* were capable of producing bactericidal and fungicidal compounds that restricted the pathogen growth to a great extent such as 6-pentyl-alpha-pyrone (volatile bioactive compound), proteases, amylases, chitinases, glucanases, and cellulases that resulted in complete lysis of the pathogens. This is in line with the observations of Mukherjee *et al.* (2006), Raju *et al.* (2012) and Bharti *et al.* (2020). When *Pseudomonas fluorescens* was tested against *Xanthomonas axonopodis*, an inhibition zone of 2.3 cm was observed with an inhibition percentage of 34.45 %. Balan *et al*. (2014) analysed the antagonistic ability of 8 bacterial isolates against *X. axonopodis* in anthurium and observed that *P. fluorescens* inhibited the bacterial growth showing an inhibition per cent of 21% and an inhibition zone of 1.83 cm. Thereafter, PGPR-II and PGPM (KAU formulations) showed an inhibition of 17.78 % and 20 % on the growth of pathogenic bacteria respectively. These observations are in line with the descriptions given by Bora *et al*. (2013) regarding the antagonistic effect of *Trichoderma* sp. and *Pseudomonas fluorescens* alone and in various combinations that inhibited the growth of different plant pathogens. Plant pathogens were inhibited by *Trichoderma* spp. through a variety of mechanisms including hyperparasitism, production of antibiotics and diffusible toxic substances, lysis of cells and pathogen overcrowding (Bora and Bora, 2020). Bacterial antagonists *viz*. *Pseudomonas fluorescens* and *Bacillus* sp. are known to limit the incidence of bacterial blight in anthurium by the production of various inhibitory chemicals such as ammonia, siderophores, different antibiotics and various other compounds (Balan *et al*. 2014). According to Stockwell *et al.* (2011), using biocontrol agents in a consortia formulation can increase effectiveness, stability, and reliability under a multitude of soil and environmental circumstances.

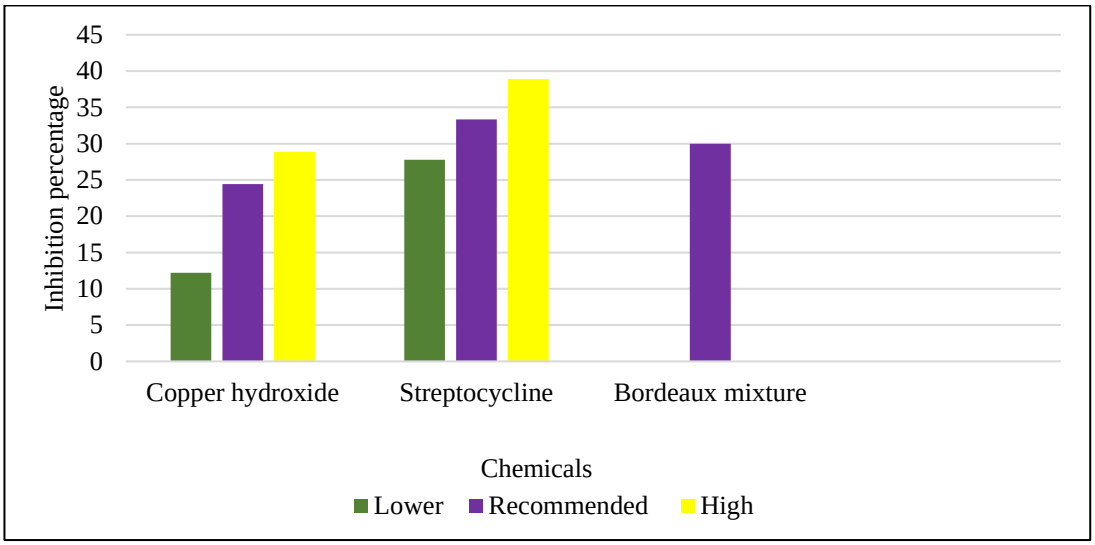

Fig 8a: *In vitro* evaluation of chemicals against *Xanthomonas axonopodis*

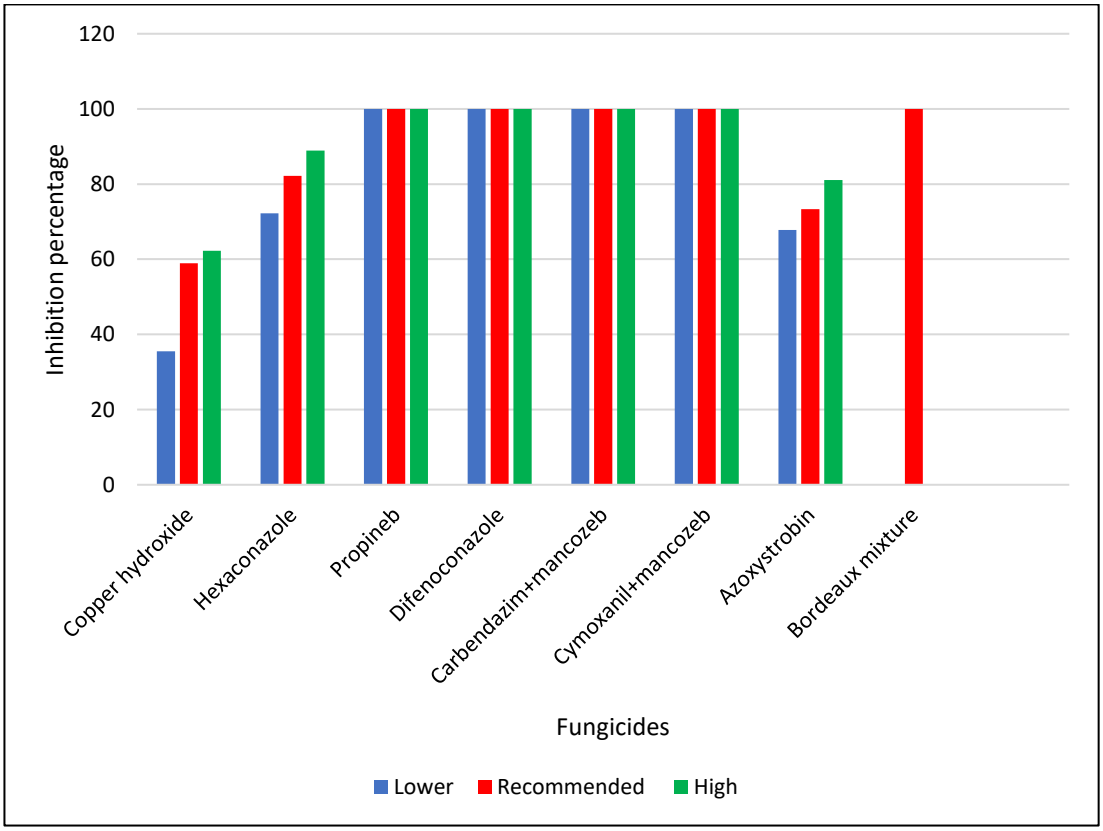

Fig 8b: *In vitro* evaluation of fungicides against *Lasiodiplodia theobromae*

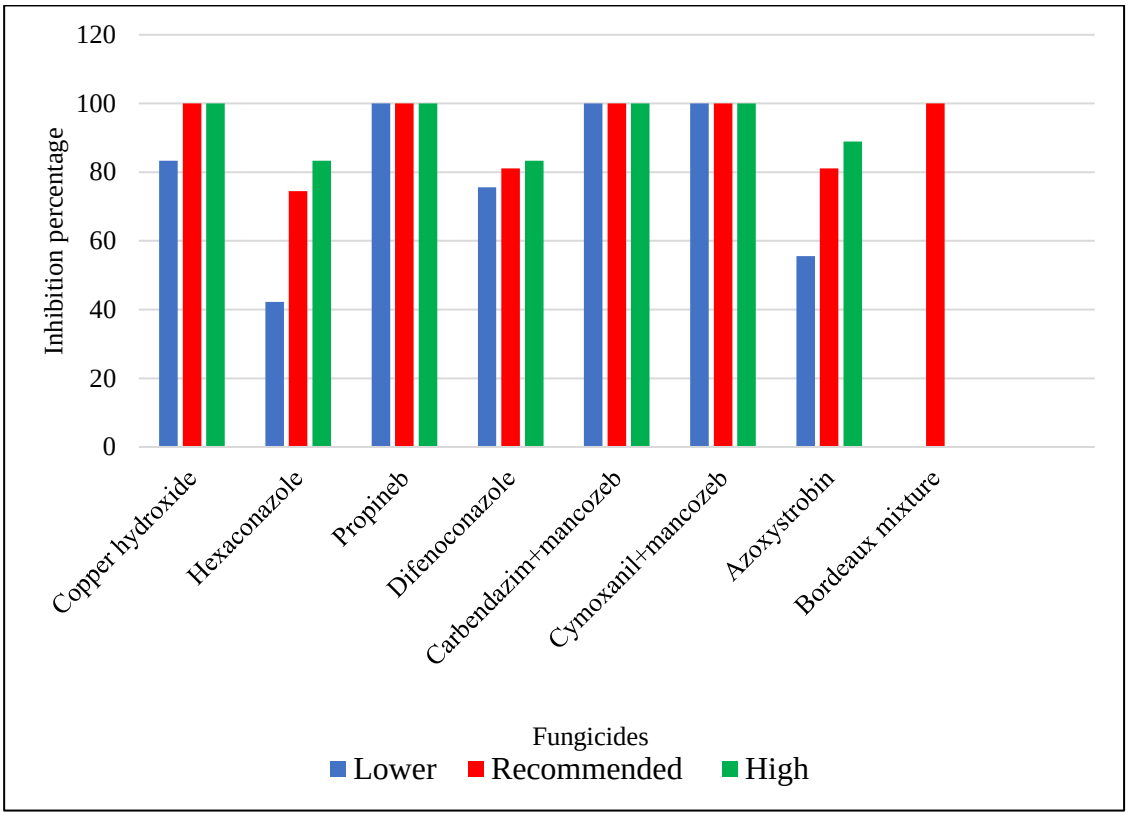

Fig 8c: *In vitro* evaluation of fungicides against *Phytopythium vexans*

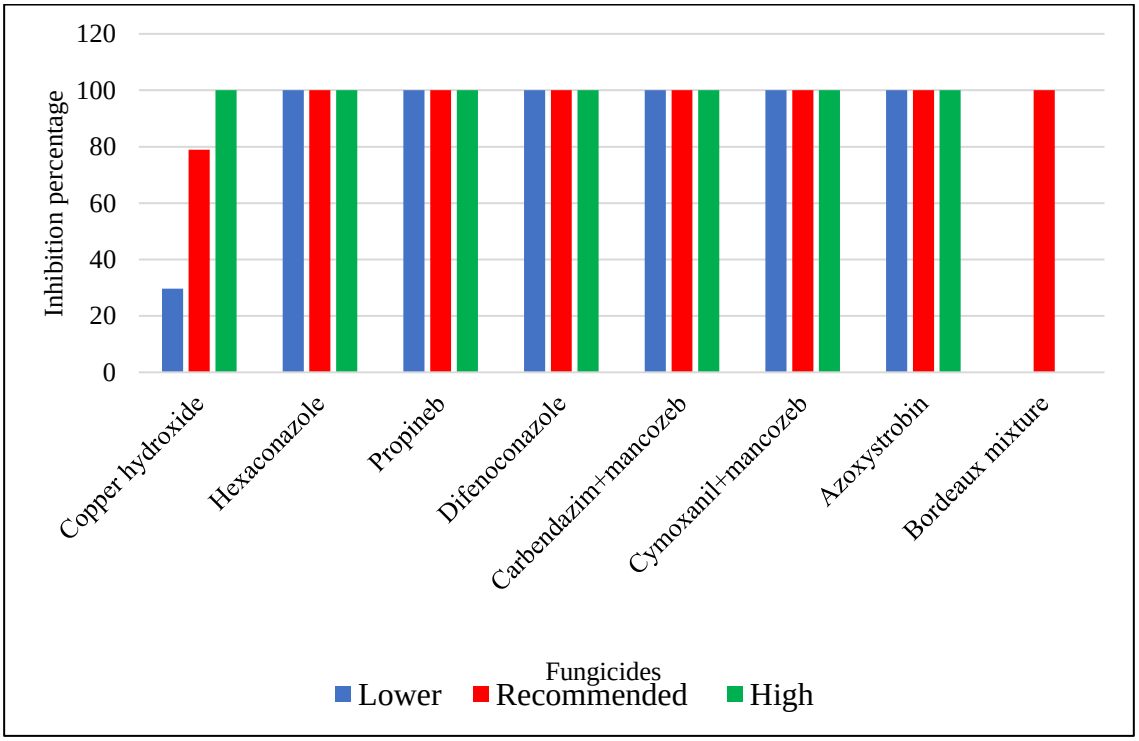

Fig 8d: *In vitro* evaluation of fungicides against *Colletotrichum* sp. (ALVSR) (Inflorescence rot)

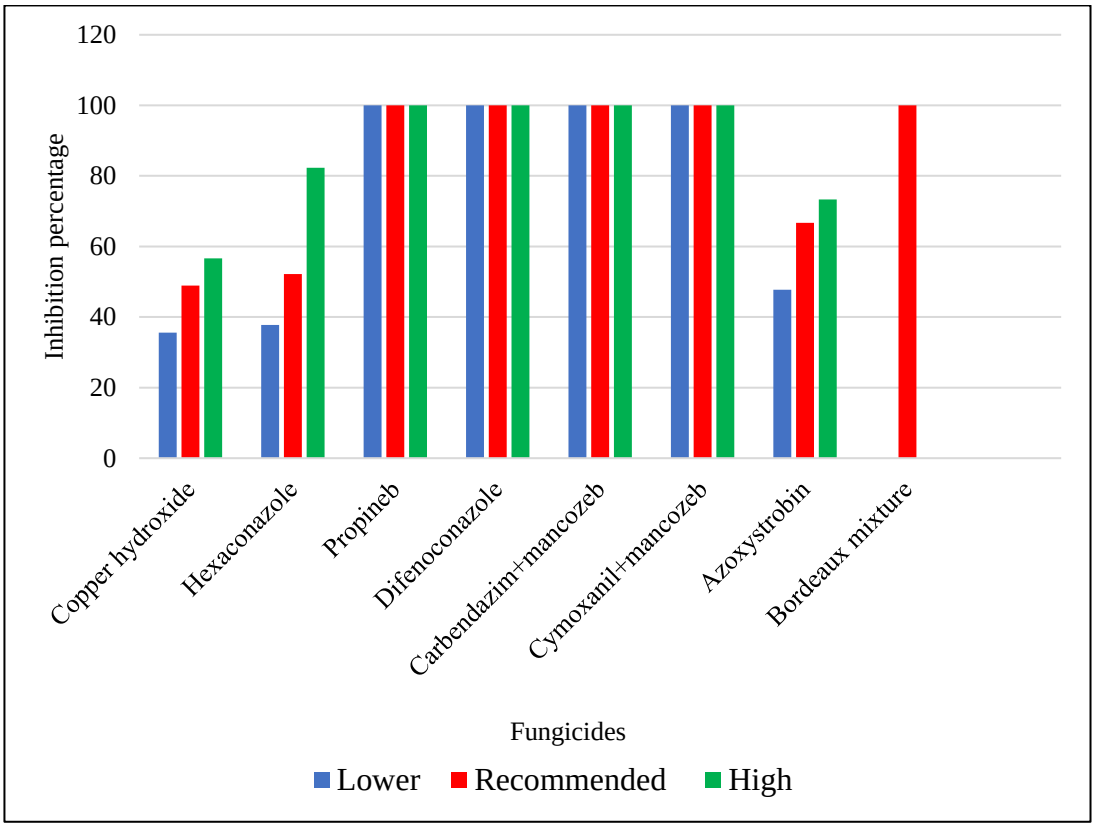

Fig 8e: *In vitro* evaluation of fungicides against *Colletotrichum* sp. (PBALS) (Leaf spot)

Summary

6. SUMMARY

Anthurium is a tropical flowering plant which is popular among cut flowers and is an emerging ornamental crop in Kerala. The commercial cultivation of anthurium is highly profitable under the current agroclimatic conditions of the state because of which many new entrepreneurs especially women are involved in large scale production of this cut flower. But the incidence of various diseases affected the quality and demand of both flowers and foliage which out listed the plant from domestic as well as global floriculture trade. Hence, the present research entitled "Etiology and characterization of diseases of anthurium (*Anthurium andraeanum* L.) in Kerala" aimed to study various diseases affecting anthurium, identify and characterize the pathogens associated with different diseases, analyse and document the set of symptoms produced by the infection under natural and artificial conditions, correlate weather parameters with the initiation and spread of diseases, and *in vitro* management of major fungal and bacterial pathogens.

Purposive sampling surveys in fifteen locations within six districts *viz*. Thrissur, Ernakulam, Kozhikode, Malappuram, Wayanad and Palakkad were conducted and thereby six kinds of diseases were collected such as leaf spots, leaf blights, root rots, inflorescence rots, wilt and mosaic symptoms. A total of twenty one symptoms were collected from various locations and catalogued as KMALS, PBALS, VCNLS1, VCNLS2, VFNLS, IJKLS, KKYLS, OKMLS, OLRLS, TLRLS, TBMLS, NLBLS (leaf spots), VFNLB and OLKLB (leaf blights), VCNRR (root rot), MNTLW (wilt), MNTSR, PNMSR, ALVSR, CKDSR (inflorescence rots) and VCNML (mosaic symptom).

Nine leaf spots (VCNLS1, VCNLS2, VFNLS, IJKLS, KKYLS, OKMLS, OLRLS, TLRLS, TBMLS), two inflorescence rots (MNTSR and CKDSR), one root rot (VCNRR), one wilt isolate (MNTLW) and virus infected symptom like mosaic patterns on leaves (VFNML) were collected from ten different locations within Thrissur district. Among these samples, maximum percentage of disease incidence and severity were showed by the root rot sample VCNRR (64 % and 47.33 %) collected from Vellanikkara. A maximum disease severity of 42 per cent was observed in the leaf spot TLRLS whereas the greatest percentage of disease incidence was recorded for the

isolate IJKLS (36 %) among the nine leaf spot isolates. The two inflorescence rot samples were recorded with a severity percentage of 24.44 and 9.13 respectively. Even though the plant samples which showed mosaic appearance on leaves measured a severity of 44 per cent, the incidence was only 16.34 per cent. It was later found that the appearance of these mosaic patterns on leaves was not due to any virus infection, on the basis of transmission electron microscopic study and the associated symptoms might be due to nutritional imbalance.

The leaf spot isolate NLBLS collected from Nilambur of Malappuram district marked the highest percentage of disease incidence (80 %) among the twelve leaf spot samples collected during the survey whereas the maximum diseases severity was recorded for the isolate PBALS (58.23 %) collected from Perambra of Kozhikode district.

Among the four inflorescence rot samples collected, ALVSR obtained from Aluva of Ernakulum district showed a maximum severity and incidence percentage of 32.12 and 59 respectively. A different kind of flower infection collected from Panamaram of Wayanad district showed a lower disease incidence percentage of 23.3.

The isolation and pathogenicity studies revealed that leaf spots, root rot, inflorescence rots and wilt were caused by fungal pathogens whereas leaf blight was due to bacterial pathogens. The data on agroclimatology of each surveyed location were gathered from the official website of Marksim based on which the correlation analysis between severity of fungal disease (PDS) and influence of weather was done. From the analysis, it was inferred that a positive relation existed between the two parameters (rainfall and relative humidity) and per cent disease severity while temperature showed a negative correlation. The correlation coefficients obtained were 0.386, 0.510 and -0.537 for rainfall, relative humidity and temperature respectively.

In vitro pathogenicity studies of various pathogens (both fungus and bacteria) associated with twenty diseased samples collected from six districts were performed and Koch's postulates were established. Different symptoms appeared in natural conditions and by artificial inoculation were documented. The leaf spot isolate KMALS was characterised by the presence of round to irregular shaped creamy white lesions

surrounded by dark brown margins and yellow halo. Black coloured fruiting body of the fungus was seen embedded in the infected tissues on maturation. The isolate PBALS showed large brownish lesions that expanded over the whole leaf lamina which got dried on disease progression. The leaf spot isolate VCNLS1 showed typical black coloured sunken and necrotic patches on the upper surface of leaves whereas the isolate VCNLS2 produced light purple tinged circular spots on leaves that later turned brown polygonal shaped spots with faint concentric rings. In contrast to VNCLS1, the isolate VFNLS was surrounded by prominent yellow halo around the irregular black lesions which was absent in the isolate VCNLS1. Well defined straw coloured round spots were distributed on anthurium leaves grown in Irinjalakkuda region of Thrissur district (IJKLS) that later coalesced to form creamy white coloured irregular patches. The leaf spot sample KKYLS was characterised by the presence of dark grey coloured inner infected layers surrounded by thick brown margins followed by brick red coloured outer layer whereas in the leaf spot OKMLS, dark grey centered spots bordered with dark brown were surrounded by highly prominent yellow halo. Another leaf spot sample OLRLS, showed dry brown lesions over the whole leaf lamina which were surrounded by yellow halo. The isolate TBMLS appeared straw coloured as in the leaf spot IJKLS but was irregular in shape and black fructifications were clearly visible on the centre of matured spots. In contrast to this, the isolate TLRLS, showed dark grey coloured thin upper layer of infection margined in dark brown which was followed by charcoal black coloured thick papery layer. The leaf spot isolate collected from Nilambur (Malappuram district) appeared irregular shaped pigmented in dark grey and limited by dark brown margins surrounded by yellow halo. The two leaf blight samples (VFNLB and OLKLB) showed severe marginal necrosis of the leaf tissues which showed a V shaped pattern of expansion towards the midrib region. The blighted tissues were surrounded by deep yellow halos and the infected petioles and stem portions showed vascular discoloration. Both these isolates produced yellowish ooze when the infected leaves were crushed in water confirming them to be caused by bacterial pathogens. In contrast to this, the wilt isolate collected from Mannuthy showed vascular discoloration and marginal necrosis along with mild wilting symptoms but was devoid of any mind of ooze from the infected portions which confirmed the causal agent to be a fungus. Severe yellowing of leaves and discoloration of infected petioles, peduncles, stems and roots were noticed in the

isolate VCNRR. When uprooted the infected plants showed reduced root growth, sloughing off of existing roots and rooting of the whole root tissues.

Four types of inflorescence rots *i. e.* MNTSR, CKDSR, PNMSR and ALVSR were collected from Thrissur, Wayanad and Ernakulam districts respectively. The samples MNTSR, PNMSR and ALVSR showed characteristic small angular necrotic spots initially on the segments of spadix which later expanded throughout the spadix, spathes and peduncles of infected plants. On maturation of the lesions, dark brown to black coloured fruiting bodies of pathogen were visible over the infected tissues. The isolate CKDSR showed initial brown discoloration arising from the basal regions of spadix and gradually progressed towards the tip and to the lower spathe tissues. Dark brown layers of infection were followed by brick red coloured outer layers which contained slight mycelial growth of the fungus.

The preliminary characterization of fungus up to the genus level was done by studying the cultural and morphological characters. The study revealed that four leaf spots (KMALS, PBALS, VFNLS, TBMLS) and three inflorescence rots (MNTSR, PNMSR, ALVSR) were caused by the infection of *Colletotrichum gloeosporioides*. The pathogen responsible for VCNLS1 was confirmed as *Phoma* sp. whereas *Corynespora* sp. was the causal agent of the leaf spots VCNLS2 and OLRLS. The pathogen causing IJKLS, KKYLS and OKMLS were identified as *Phomopsis* sp. whereas the fungal pathogens that caused leaf spot diseases TLRLS and NLBLS were identified to be *Pestalotiopsis* sp. and *Lasiodiplodia theobromae* respectively. The pathogen associated with the root rot sample VCNRR was identified as *Phytopythium* sp. whereas the wilting pathogen was confirmed as *Fusarium* sp. The bacterial pathogens responsible for leaf blight symptoms (VFNLB and OLKLB) were identified and confirmed as *Xanthomonas* sp. based on cultural, morphological and biochemical characters.

The molecular characterization of fungal pathogens was done by ITS gene sequencing and bacterial pathogens were characterized by the isolation of genomic DNA followed by amplification and sequencing of 16S rRNA region. BLASTN analysis of corresponding gene sequences was carried out in NCBI nr database and confirmed various pathogens. The leafspot disease TBMLS was caused by *Colletotrichum queenslandicum* whereas three other leaf spots (KMALS, VFNLS and

PBALS) along with two inflorescence rots (PNMSR and MNTSR) were caused by *Colletotrichum* sp. included in *C. gloeosporioides* species complex. The pathogen associated with VCNLS2 and OLRLS was identified as *Corynespora cassiicola*. Two leaf spots KKYLS and OKMLS were caused by the infection of *Phomopsis heveicola* whereas the leaf spot disease IJKLS was associated with the fungal pathogen *Diaporthe phaseolarum. Lasiodiplodia theobromae* was responsible for NLBLS and *Phytopythium vexans* was found associated with VCNRR. The wilt symptom MNTLW was caused by *Fusarium fujikuroi* and the leaf spot TLRLS was due to the infection of *Pseudopestalotiopsis thea*. The bacterial pathogens associated with leaf blight was confirmed as *Xanthomonas axonopodis*.

The inhibitory effect of eight fungicides and antagonistic ability of four bioformulations/biocontrol agents were tested against two leaf spot pathogens, one root rot pathogen and one inflorescence rot pathogen (two *Colletotrichum* sp. (PBALS and ALVSR), *L. theobromae*, *P. vexans*). Propineb, difenoconazole, carbendazim 12 %+ mancozeb 64 %, cymoxanil 8% + mancozeb 64 % and Bordeaux mixture were highly effective against *L. theobromae* and *Colletotrichum* sp. (PBALS) as it caused cent per cent inhibition of these two pathogens at all the three doses. Against *Phytopythium vexans*, the fungicides *viz*. propineb, azoxystrobin, carbendazim 12 %+ mancozeb 64 %, cymoxanil 8% + mancozeb 64 % and Bordeaux mixture caused complete inhibition of growth. All the tested fungicides were effective against the *Colletotrichum* isolate (ALVSR). *Trichoderma* sp., PGPR-II and PGPM greatly inhibited the fungal growth whereas *Pseudomonas fluorescence* showed maximum antagonistic effect over the pathogens, except *Phytopythium vexans*. The effect of three chemicals against *Xanthomonas axonopodis* was tested *in vitro* and found that streptocycline at 200 ppm and 250 ppm showed maximum inhibition of the pathogen followed by 1per cent Bordeaux mixture. *Trichoderma* sp., *Pseudomonas fluorescence*, PGPR-II and PGPM showed maximum inhibition over the pathogen.

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Appendices

Appendix I

Composition of media for isolation of pathogens (fungi and bacteria) from symptomatic samples

1. **Potato dextrose agar (PDA)**

   Potato-200 g

   Dextrose -20 g

   Agar-20 g

   Distilled water-1000 ml

2. **Nutrient agar (NA)**

   Peptone-20 g

   Beef extract-1g

   NaCl -5 g

   Agar - 20 g

   Distilled water - 1000 ml

   pH-6.5-7.5

Appendix II

Composition of media for biochemical characterisation of bacterial pathogens

1. **Starch agar medium**

   Soluble starch-20g

   Beef extract-3g

   Distilled water-IL

   Peptone-5g

   pH-7

2. **Nutrient gelatin agar medium**

   Peptone - 5g

   Beef extract-3g

   Gelatin - 120 g

   Distilled water - 1L

   pH-6.8

3. **Skim milk agar medium**

   Skim milk powder - 100g

   Agar - 20g

   Distilled water - 1L

   Peptone - 5g

   pH-7.2

4. **Tryptone broth (1%)**

   Peptone - 10g

   Distilled water – IL

5. **Simmond's citrate agar**

   Ammonium dihydrogen phosphate - 1g

   Dipotassium phosphate- Ig

   NaCl - 5g,

   Sodium citrate- 2g

   Magnesium sulphate- 0.2g

   Agar - 15g

   Bromothymol blue - 0.8g

   Distilled water - 1L

6. **SIM agar medium(H_2S) (pH 7.3)**

   Peptone - 30g

   Beef extract - 3 g

   Ferrous Ammonium Sulphate - 0.2 g

   Sodium thiosulphate - 0.025 g

   Agar - 3 g

   Distilled Water - 1000 ml

7. **MRVP broth (5 ml) (pH 6.9)**

   Peptone - 7 g

   Dextrose - 5 g

   Potassium phosphate - 5 g

   Distilled Water - 1000 ml

ETIOLOGY AND CHARACTERIZATION OF DISEASES OF ANTHURIUM (*Anthurium andraeanum* L.) IN KERALA

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Anthurium (*Anthurium andraeanum* L.), a native of Tropical America is a perennial herbaceous plant commercially grown for its attractive coloured spathe and green shiny foliage. These have great economic value in the global flower trade that led to the budding of many urban and rural anthurium entrepreneurs in the countryside, particularly in the state of Kerala. During the recent past, the cultivation of this high valued cut flower crop is challenged by many biotic factors and the changes in climate scenario occurred recently led to the emergence of various new pests and diseases. Hence the present study was undertaken to identify and document the diseases affecting anthurium grown in the state of Kerala and the characterization of associated pathogens.

Purposive sampling surveys were conducted in the anthurium cultivated locations of six districts *viz*. Thrissur, Ernakulam, Palakkad, Malappuram, Kozhikode and Wayanad during the period from October, 2020 to July, 2021. The incidence and severity of various symptoms were assessed and collected the samples for further studies. Twelve leaf spots (KMALS, PBALS, VFNLS, VCNLS1, VCNLS2, IJKLS, KKYLS, OKMLS, OLRLS, TLRLS, TBMLS and NLBLS) two leaf blights (VFNLB and OLKLB), one root rot (VCNRR), one wilt (MNTLW) four inflorescence rots (MNTSR, CKDSR, PNMSR and ALVSR) and one mosaic symptom (VCNML) were collected during the survey. Among the leaf spots, PBALS recorded the highest per cent disease severity (PDS) of 58.23 followed by NLBLS (46.66 %) and ALVSR recorded the highest severity of 32 per cent among inflorescence rots.

Isolation and pathogenicity studies of the associated pathogens yielded 18 fungal and two bacterial isolates. Symptomatology of these diseases were studied both under natural and artificial conditions. The fungal pathogens were characterized and identified by studying cultural and morphological characteristics. Based on these characters, leaf spot pathogens were identified as *Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Phoma* sp., *Phomopsis* sp., *Corynespora* sp., *Pestalotiopsis* sp. and those causing inflorescence rot as *Colletotrichum gloeosporioides* and *Phomopsis* sp. and root rot

pathogen as *Phytopythium* sp. and wilt pathogen as *Fusarium* sp. The bacterial isolates causing leaf blight were identified as *Xanthomonas* sp. based on cultural, morphological and biochemical characteristics. The viral like symptoms subjected to electron microscopy revealed the absence of any virus particles.

PCR amplification of Internal Transcribed Spacer (ITS) region of the fungal genome followed by sequencing and *in silico* analysis confirmed that the pathogen associated with TBMLS as *C. queenslandicum*, VCNRR as *Phytopythium vexans*, IJKLS as *Diaporthe phaseolarum*, KKYLS and OKMLS as *Phomopsis heveicola*, NLBLS as *Lasiodiplodia theobromae*, TLRLS as *Pseudopestalotiopsis thea*, VCNLS2 and OLRLS as *Corynespora cassiicola*, MNTLW as *Fusarium fujikuroi*. The pathogens associated with KMALS, PBALS, VFNLS, MNTSR, PNMSR and ALVSR belong to *Colletotrichum gloeosporioides* species complex. The bacterial isolates (VFNLB and OLKLB) were confirmed as *Xanthomonas axonopodis* through the amplification of 16S rRNA region of the genome followed by sequencing and *in silico* analysis.

An *in vitro* experiment was conducted to study the efficacy of fungicides and biocontrol agents/bioformulations against major fungal pathogens. Fungicides *viz*. propineb, difenoconazole, carbendazim 12 % + mancozeb 64 %, cymoxanil 8% + mancozeb 64 % and Bordeaux mixture were highly effective against *L. theobromae* and *Colletotrichum* sp. (PBALS) as it resulted in cent per cent inhibition of these two pathogens at all the three doses. In the case of *P. vexans*, fungicides *viz*. propineb, azoxystrobin, carbendazim 12 % + mancozeb 64 %, cymoxanil 8% + mancozeb 64 % and Bordeaux mixture were found to be effective even at a lower dose. Among the three chemicals tested against *Xanthomonas axonopodis*, maximum inhibition was recorded in the case of streptocycline. Dual culture and filter paper disc method revealed that the biocontrol agents/formulations *viz*. *Trichoderma* sp., PGPR-II and PGPM were effective against all the tested fungal and bacterial pathogens. *Pseudomonas fluorescens* was found effective against three fungal and the bacterial pathogen.