CATALOGUING AND DOCUMENTATION OF FUNGAL DISEASES OF GERBERA

by

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(2014-11-237)

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THESIS

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DEPARTMENT OF PLANT PATHOLOGY
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2016

DECLARATION

I, hereby declare that the thesis entitled **"CATALOGUING AND DOCUMENTATION OF FUNGAL DISEASES OF GERBERA"** is a bonafide record of research work done by me during the course of research and that this thesis has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"CATALOGUING AND DOCUMENTATION OF FUNGAL DISEASES OF GERBERA"** is a bonafide record of research work done independently by Mr. Praveen N. M. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to him.

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EXTERNAL EXAMINER

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Introduction

1. INTRODUCTION

Gerbera, a perennial herb, native to tropical regions of South America, Africa and Asia belongs to Asteraceae family. It is the most popular cut flower with increasing commercial significance. Gerbera, also known as Transvaal daisy, African daisy, Barberton daisy or Verdt daisy is prized for its large daisy like blooms. It is named after a German botanist, Traugott Gerber. It is grown throughout the world for its wide spectrum of colours, shapes, excellent vase life and handling. According to the global trend in floriculture, gerbera occupies the fourth place among cut flowers (Sujatha *et al.,* 2002). Broek *et al.* (2004) that production of gerbera was approximately US$ 220 million in 2001 representing 70 million stems sold in US alone whereas Netherlands produces 420 million stems of gerbera per year which procure 145 million Netherlands guilders.

Gerbera is one of the most demanding cut flowers in Europe but a major portion of it is imported from other countries including India (Anonymous, 2006). It fetches an excellent price in the international market and contributes greatly to the export earnings of the country because of its graceful appearance, hardiness and long shelf life (Aswath and Rao, 2006). In India, gerbera is grown commercially for export as well as the domestic market, and its commercial production is largely centered in Pune and Bangalore (Anonymous, 2006). Gerbera grows well in open tropical and subtropical conditions, however, under temperate climatic conditions, it needs to be protected from frost and hence cultivated in greenhouses. Production of quality flowers of gerbera is carried out under protected conditions as these require partial shade (Singh, 2006).

One of the important constraints that limit the production of quality flowers in gerbera is the severe incidence of diseases. The crop is affected by various fungal, bacterial and viral diseases which reduce the plant vigour, flower quality and market value, thus causing significant losses to the commercial cut

flower industry (Moorman, 1995). Even though many diseases of gerbera are reported internationally and from India, no systematic study has been conducted in Kerala.

Hence, considering the emerging trend towards the industry favouring gerbera as a major ornamental crop and keeping in view of its economic value and visualizing the seriousness of the disease, the present investigation was undertaken to identify the fungal diseases of gerbera occurring in the state of Kerala during different seasons and also to catalogue and document the same. The study encompasses with the following objectives.

- Survey on the occurrence of fungal diseases and collection of diseased samples
- Isolation of pathogend and its pathogenicity
- Symptomatology of diseases
- Characterization and identification of pathogens
- *In vitro* evaluation of fungicides and biocontrol agents against major fungal pathogens
- *In vivo* management of major diseases

Review of Literature

2. REVIEW OF LITERATURE

Gerbera, a popular ornamental crop among florists, is cultivated throughout the world for cut flower as well as for ornamental potted plants. The first authorized description about the cut flower crop was made in Curtis' botanical magazine by J. D. Hooker in 1989, and thereafter it was named as *Gerbera jamesonii* Bolus ex. Hook f. (Das and Singh, 1989). Among the various species of gerbera, only single species, Gerbera *jamesonii* is cultivated throughout the world under wide range of climatic conditions. Though Richard Irwin Lynch initiated breeding programmes in gerbera in 1890, it has been commercialized globally by only the beginning of 20^{th} century when Netherlands became the major production centre. Gerbera having a unique position in cut flower industry has very good export potential because of its graceful appearance, hardiness, ability to withstand transportation and long shelf life. In India, it is being cultivated in polyhouses and Hi-Tech floriculture projects for better yield and quality. However, over the past few years several important pests and diseases have gained considerable importance and pose serious threat to the cultivation and production of gerbera in India. Since gerbera flourishes well under the warm humid tropical climate of Kerala, the crop is prone to infection by different fungal pathogens which thrive well under such conditions.

2.1 FUNGAL DISEASES AND ASSOCIATED PATHOGENS

A perusal of the literature revealed that studies on foliar, root and floral fungal diseases have been reported globally from gerbera growing tracts of Brazil, Mexico, Poland, Italy, Holland, Bulgaria, North of Iran, China, Korea and India. Beaumont and Gregory (1937) was the first to describe a leaf spot disease on gerbera in England and they found that *Ascochyta* gerbera*e* Maffei is the pathogenic agent causing the disease.

Kulibaba (1972) reported fourteen fungal pathogens from Soviet Russia from gerbera grown during July- August under polyhouses. According to them,

foliage pathogens noticed were *Phyllosticta* gerbera*e, Alternaria dauci, Botrytis cinerea, Cladosporium herbarum, Alternaria tenuis* (*A. alternata*), *Albugo tragopogonis* where as the root infective fungal pathogens identified were *Rhizoctonia violacea* (*Helicobasidium purpureum*) as well as *Phytophthora cryptogea, Fusarium oxysporum, Verticillium dahliae, V. albo-atrum* and *Sclerotium* (*Corticium*) *rolfsii.*

Ghosh (1998) opined that *Alternaria* blight caused by *Alternaria alternata* (Fr.) Keissler was the important disease affecting gerbera in Indian climatic conditions. Zaccaria *et al.* (2000) observed the most devastating pathogens in Italy under open field conditions were *Botrytis cinerea, Sphaerotheca fusca*, *Sclerotinia sclerotiorum,* besides, *Phytophthora cryptogea* under hydroponics. Likewise, Jurkovic and Cosic (2006) enlisted major disease causing pathogens on gerbera as *Rhizoctonia solani, Pythium*, *Fusarium* spp., *Thielaviopsis basicola, Phytophthora cryptogea, P. parasitica* (*P. nicotianae* var. *parasitica*), *Erysiphe cichoracearum* and *Botrytis cinerea.* Recent studies by Ferronato *et al.* (2008) showed eight plant pathogenic fungi infecting gerbera *viz., Erysiphe cichoracearum, Pythium* sp., *Phytophthora* sp., *Fusarium oxysporum, Cercospora gerberae, Botrytis cinerea, Albugo tragopogonis* (*Pustula tragopogonis*) and *Capnodium* sp.

Nagrale *et al.* (2012) reported several diseases from India like foot rot caused by *Pythium irregualre, Phytophthora cryptogea* and *Rhizoctonia solani*, wilt by *Fusarium oxysporum*, Sclerotium rot by *Sclerotium rolfsii*, grey mould by *Botrytis cinerea* and powdery mildew by *Erysiphe cichoracearum.*

2.1.1 Foliage diseases

Many workers have conducted several studies on diseases affecting foliage of gerbera plants. Sen and Gupta (1996) recorded two new pathogenic fungi, *Alternaria tenuis* and *Colletotrichum gloeosporioides* infecting leaves of gerbera

from Himachal Pradesh, India. Severe incidence of leaf blight caused by *Alternaria* spp. was observed in gerbera grown in polyhouses (Gosh, 1998; Mirkova and Konstatinova, 2003; Nagrale, 2007; Farhood and Hadian, 2012). Bhat *et al.* (2013) recorded the highest mean disease incidence of 60.72 per cent with a mean disease intensity of 24.96 per cent *Alternaria* blight in various districts of Kashmir Valley. According to Panda *et al.* (2014) seedling blight of gerbera caused by *Macrophomina phaseolina* was reported under protected condition.

Vazquez *et al.* (2000) reported white rust of gerbera caused by *Albugo tragopogonis* for the first time from Mexico. Yeasmin and Shamsi (2013) isolated phylloplane fungal species like *Alternaria* spp., *Aspergillus* spp., *Bipolaris hawaiiensis, Chaetomella raphigera, Cladosporium cladosporoides, Curvularia* spp., *Colletotrichum* spp., *Fusarium* spp., *Phomopsis* sp., *Penicillium* sp., *Pestalotia* sp., *Rhizopus* stolonifera and *Trichoderma viride.* Among the isolates, *Alternaria citrii*, *Alternaria tennuissima*, *Colletotrichum capcisi*, *Colletotrichum dematium*, *Colletotrichum coffeanum* and *Curvularia clavata* were found to be pathogenic causing blight and anthracnose diseases of gerbera plants.

Folk and Tusnadi (1984) proposed two new serious diseases of gerbera *viz.,* powdery mildew caused by *Oidium erysiphoides* f. sp. *gerberae* and downy mildew by *Bremia lactucae* Regel. Schickedanz (1993) also noticed for the first time *Bremia lactucae* causing downy mildew symptoms during summer in green houses in Germany. Likewise, this disease was reported for the first time from Argentina, Brazil, Germany and Poland in gerbera during autumn season cultivated in greenhouses (Wolcan, 2010). Baiswar *et al.* (2010) documented anamorphic *Podosphaera* sp. from *Gerbera jamesonii* for the first time from India. First report of powdery mildew caused by *Golovinomyces cichoracearum* from Italy on potted 'Nini Yellow' gerbera was observed by Troisi *et al.* (2010). Duarte *et al.* (2013) published first incidence of downy mildew disease caused by *Plasmopara halstedii* during winter from Brazil.

2.1.2 Floral diseases

A perusal of the literature revealed that reports on floral diseases of gerbera are meagre and scanty. However, attempts have been made to include some of the available literature on diseases affecting flowers of gerbera apart from foot rots, blights, damping off, wilts and fruit rots from field as well as in storage. Rao and Ullasa (1970) reported an outbreak of blossom blight in gerbera flowers from India which was usually sporadic in nature and was confined to prolonged wet and cool weather when surveyed during two successive monsoon periods. Granke and Hausbeck (2013) noticed the signs of powdery mildew disease on petals of gerbera flowers apart from its infection on foliage.

2.1.3 Root diseases

Attempts have been made by researchers to study the phytopathological problems caused by soil borne pathogens in floriculture industry. Arx (1952) observed for the first time heavy mortality of gerbera grown under glasshouse in Netherlands due to foot rot disease. Scholten (1970) made an extensive review of research conducted on the etiology of wilt diseases in Netherlands and reported that *Phytophthora cryptogea, Verticillium alboatrum* and *Verticillium dahlia* were the pathogens associated with wilt disease. From India, *Sclerotium* rot disease of gerbera was reported by Singh (1973) where the disease causing agent was identified as *Sclerotium rolfsii* affecting the above ground parts of the crop. Orlikowski (1976) isolated 32 fungi from 120 wilted plants among which *Phytophthora cryptogea* was the most frequently observed root pathogen, followed by *Fusarium oxysporum, Botrytis cinerea, Pythium sylvaticum, Rhizoctonia solani* and *Thielaviopsis bassicola*.

Scholten (1970) and Hyeong *et al.* (1996) reported about a destructive disease of gerbera *viz*., foot rot caused by *Phytophthora cryptogea* besides *Verticillium* wilt. Minuto *et al.* (2004) reported the presence of *Fusarium* wilt on

gerbera grown on soilless media as well as on soil in Northern Italy. Wolcan and Edizioni (2004) observed sudden wilting and basal rot symptoms in Argentina during autumn and spring seasons which further progressed through stem causing necrosis and the pathogen was identified as *Sclerotinia sclerotiorum.* Occurrence of leaf blight in gerbera grown inside greenhouses in Italy due to *Phytophthora tentaculata* was reported by Cristinzio *et al.* (2006).

2.2 SEASONAL INFLUENCE OF OCCURRENCE OF DISEASES

Plant diseases are always influenced by the interaction between host, pathogen and environment. It is well known that a disease progresses due to an infectious pathogen as well as due to favorable of host factors, weather parameters like temperature, relative humidity, rainfall, number of rainy days and epidemiological conditions.

Gaumann (1950) reviewed the effect of various environmental factors that influenced the establishment of parasitic relationship with plant diseases. Vigodsky (1969) while studying the factors causing *Sclerotinia* wilt on gerbera opined that besides inoculum rate, colonization period, age and vigour of the plant, climatic conditions also play a crucial role in disease development. Later, Singh (1973) observed an upsurge in disease incidence of wilt caused by *Sclerotinia sclerotiorum* on gerbera from 35-68 per cent to 90 per cent relative humidity and at an above average temperature (34^{o}C). Salinas and Verhoeff (1995) observed spotting on gerbera ray florets caused by *Botrytis cinerea,* a post-harvest disease which was highly influenced by environmental factors. According to them, the temperature range of 18-25^{o}C with high relative humidity was found congenial for the pathogen. Kerssies (1994) also opined that incidence of petal spotting enhanced as the storage temperature increased under post-harvest conditions.

Change of weather from autumn to spring increased the incidence of *Sclerotinia* wilt of gerbera in Argentina (Wolcan and Edizioni, 2004). Garibaldi *et al.* (2008) observed first symptom development of *Fusarium* wilt on gerbera during hottest period during summer with an average temperature of 27^{o}C. Ghosh *et al.* (2009) reported that *Alternaria alternata* (Fr.) Keissler and *Cercospora gerberae* causing leaf blight and leaf spot disease respectively were found to be very severe during summer to pre-rainy months and the per cent disease (PDI) and per cent disease severity (PDS) recorded for *Alternaria* leaf blight was 42.5 and 16.40 per cent and in the case of *Cercospora* leaf spot, 37.85 and 12.95 per cent. An outbreak of powdery mildew was reported by Troisi *et al.* (2010) in African daisy during summer which was previously unfamiliar in Italy. A validation to above study, Kumar *et al.* (2012) specified weather parameters most congenial for powdery mildew were relative humidity (80-95%), moderate temperature (20-28^{o}C) and low light intensity or shade. Leah *et al.* (2012) assessed factors affecting severity of powdery mildew in gerbera by correlation studies and reported that the severity of the disease was correlated negatively with temperature and leaf wetness whereas positively correlated with relative humidity. Severe symptoms of downy mildew in gerbera were noticed during winter season in Brazil (Duarte *et al.* 2013).

2.3 PATHOGENICITY OF ISOLATES

Pathogenicity test was introduced by Robert Koch in the 21st century, as preliminary confirmation method for proving the significance of an isolate from a diseased specimen in causing disease. Many success stories have been reported on different methods of pathogenicity test of foliar and root pathogens in various crop plants.

Warkentin *et al.* (1995) carried out pathogenicity test for the powdery mildew pathogen in pea by detached leaf assay method where susceptible leaves placed above cotton saturated with sucrose solution (5%) maintained in a Petri

dish which was then inoculated with the conidial mass of optimum density. According to them, they reported that the symptoms were observed ten days of inoculation and thus confirmed pathogenicity. Similarly, Baiswar *et al.* (2010) confirmed pathogenicity of *Podosphaera* sp. on *Gerbera jamesonii* by dusting fungal conidia on healthy plants.

Several authors have documented the pathogenicity of *Curvularia lunata, C. pallescens* and *C. gladioli* by inoculating conidial suspension on leaves of other ornamental plants like lotus, gladiolus and canna (Rath and Bhal, 1972; Kolse *et al.*, 2000; Cui and Sun, 2012; Pawar *et al.,* 2012; Torres *et al.* 2013).

Garibaldi and Minuto (2007) noticed wilt symptoms and vascular discolorations in gerbera while testing pathogenicity of *Fusarium oxysporum* by dipping in a conidial suspension (5×10^7 conidia/ml). For testing pathogenicity of *Phytophthora cryptogea* on African daisy, Hong *et al.* (2008) inoculated the isolate multiplied in vermiculite in the root zone and symptoms were noticed 7-14 days of inoculation. Pathogenicity of *P. cryptogea* in *Rhododendron* spp. and *Camelia* spp. was also done by placing agar plugs (7mm) of the culture on the adaxial surface of the host after giving injury. Plants were incubated for ten days for symptom development inside humid growth chamber (90%) at 24^{o}C (Yakabe *et al.*, 2009). Similar inoculation method with detached leaf was reported by Moralejo *et al.* (2009) for testing pathogenicity of *Phytophthora* spp. which were isolated from different ornamentals. Ramyabharathi *et al.* (2014) detailed pathogenicity test of *F. oxysporum.* f. sp. *gerberae* in one month old gerbera plants by inoculating the pathogen after mass multiplied in sand-maize medium.

Many authors pointed out the pathogenicity studies by Mycelia Droplet Inoculation Technique (MDIT) and Mycelia Bit Inoculation Method (MBIM) suggested by Munaut *et al.* (1997) and Rocha *et al.* (1998). Pathogenicity of *Alternaria alternata* on gerbera was proved by spray inoculation @ 100ml/pot of spore suspension (2 x 10^5cfu/ml) of the pathogen which caused typical symptoms

of *Alternaria* leaf blight (Mirkowa and Konstantinova, 2003 and Farhood and Hadian, 2012). Likewise, Nagrale *et al.* (2012) confirmed the pathogenicity of *Alternaria alternata* in gerbera by MBIT and MDIT method. However, Spoelder *et al.* (2013) carried out pathogenicity test of *A. alternata* and *A.solani* by detached leaf assay on potato and compared lesion size and noticed that unlike *A. solani, A. alternata* failed to form lesions by detached leaf assay.

2.4 SYMPTOMATOLOGY

Several researchers have carried out many studies on symptomatology of various fungal diseases affecting gerbera plants.

2.4.1 Leaf spot

Ascochyta leaf spot caused by *Ascochyta* gerbera*e* Maffei was recorded by Beaumont and Gregory (1937) in Italy. Symptoms appeared as minute granular spots of 2-10mm size surrounded by a purple margin most conspicuous in the early stages. These spots subsequently coalesced, resulting in shriveling of older leaves.

Vazquez *et al.* (2000) observed white erumpent sori on undersurface of chlorotic spots which matched the chlorotic spots seen on the upper surface showing typical symptoms of white rust disease. Symptoms of *Alternaria* leaf spot were studied by Mirkova and Konstantinova (2003) in Bulgaria and according to them, leaves show typical development of small, scattered brown dots, which gradually enlarged and coalesced to form large, oval, circular or irregular, brown to black lesions with concentric rings.

Ferronato *et al.* (2008) described *Alternaria* leaf spot as pale brown to grey colour surrounded by a purple ring which when coalesced, turned into larger areas of necrotic tissue. Farhood and Hadian (2012) reported initial stage of

Alternaria leaf spot infection in green house as brown, small, scattered spots on the leaves that gradually become round or irregular. These spots coalesce to affect large areas of leaves and cause defoliation. Shi *et al.* (2012) studied the symptoms of leaf spot caused by *Corynespora cassicola* in gerbera from China which were round or irregular, with greyish centre surrounded by dark brown borders of 5-15 mm diameter.

2.4.2 Powdery mildew

Ferronato *et al.* (2008) while reviewing fungal diseases of gerbera in the State of Parana, Brazil opined that powdery mildew caused by *Erysiphe cichoracearum* showed white or light greyish powdery growth on upper surface of leaves denoting conidiophore or conidial growth. Troisi *et al.* (2010) observed white mycelial growth of *Golovinomyces cichoracearum* on adaxial surface of leaf which later turned yellow. Similarly, Baiswar *et al.* (2010) reported that symptoms of *Podosphaera* sp. on gerbera were more prominent on upper leaves which later turned necrotic.

2.4.3 Downy mildew

Wolcan (2010) studied two different genera, *Bremia* and *Plasmopara* causing downy mildew diseases on gerbera grown in many European and South American countries. Duarte *et al.* (2013) documented the symptoms of *Plasmopara* as dense whitish sporulation on abaxial surface whereas adaxial leaf lamina turned yellow which later became brown with intense blighting of leaves of the whole plant. They opined that *P. halstedii* was reported for the first time in *G. jamesoni in* Brazil, though it has been commonly recorded on numerous hosts belonging to the Asteraceae family. According to them, symptoms appeared as irregularly shaped, yellow discoloration on upper surface of leaf, later turning to dark brown, reaching 5cm long, which was vein delimited during winter season. The lesions were localised to the leaf margins or in the centre of the lamina with

typical white greyish sign of downy mildew on the lower surface of leaves and as the infection progressed, lesions coalesced turning necrotic.

2.4.4 Petal blight

Kerssies (1994) noticed grey mold disease caused by *Botrytis cinerea* with visible symptoms on leaves and petals and observed the same during vegetative, flowering and post-harvest stage of the cut flower crop. Ferronato *et al.* (2008) supported the fact that small brown spots appeared on petals under high humid condition which later turned pale brown, resulting in rotting of entire florets.

2.4.5 Wilt and root rot

Skadow (1981) and Hyeong *et al.* (1996) detailed the symptomatology of *Phytophthora cryptogea* infecting gerbera as wilting of leaves, discoloration of stem, foot rot symptoms and decay of primary roots. Thery reported that apart from *Phytophthora, Fusarium* sp. also caused wilting symptoms where the crop finally dried with leaves still attached. Minuto *et al.* (2004) differentiated symptoms of *Phytophthora cryptogea* and *Fusarium oxysporum* causing wilt disease in gerbera as the latter infected plants and did not show symptoms of collapse while plants infected with *P. cryptogea* wilt unilaterally showing yellowing of leaves. Garibaldi *et al.* (2008) also described similar unilateral yellowing with vascular streaks as typical symptoms of *Fusarium* wilt.

Wolcan and Edizioni (2004) described crown rot symptoms caused by *Sclerotinia sclerotiorum* on potted gerbera plants in Argentina with yellowing and grey or tan discoloration of leaves along with stem necrosis above the collar region which later progressed and produced black and irregular sclerotia. Several workers elucidated the symptomatology of *Fusarium oxysporum, Phytophthora* sp. and *Pythium* sp. which showed similar symptoms like wilt and root rot (Coutinho, 2001; Caldari, *et al.,* 2005; Ferronato *et al.,* 2008). Troisi *et al.* (2009)

reported yellowing of leaves and symptoms of wilting due to *F. oxysporum* f. sp. *tracheiphilum* and *F. oxysporum* f. sp. *chrysanthemi* in southwest Spain and Italy.

2.5. CHARACTERISATION OF PATHOGENS

Among the various diseases studied in gerbera, pathogens which are endemic and economically important are characterized.

2.5.1 *Alternaria* spp.

Mirkova and Konstantinova (2003) isolated *Alternaria* spp. and studied colony morphology and determined sporulation of the pathogen. Fang *et al.* (2010) reported leaf spot disease on gerbera and the pathogen was identified as *Alternaria tenuissima* which was confirmed by morphological and by molecular analysis. Similar studies on *Alternaria* sp. the leaf spot pathogen was carried out by Farhood and Hadian (2012) in North Iran where they identified the causal agent as *A.alternata* by studying the morphological characteristics of the pathogen. The fungus produced effuse, olivaceous, black colonies with dark olive green margins and abundant branched septa with golden brown mycelium. The conidiophores were branched, straight, pale brown to olive brown. The pale brown conidia of the isolates were catenated in long, sometimes produced branched chains of 5-12 spores. The size of conidia varied from 20-63 µm in length and 9-18 µm in width and usually ovoid to ellipsoid or obclavate with short conical beak at the tip. Conidia were with two to three transverse septa and usually several longitudinal septa. Nagrale *et al.* (2012) similarly studied morphological characters of *A. alternata* (Fr.) Keisslar infecting gerbera where the conidiophore measured 42.26 µm in length, 4.29 µm in width with length: width ratio 9.85. Conidia were olivaceous to dark brown in colour, varied in shape from obclavate to mostly ellipsoidal, muriform having tapered apex with 1 to 3 longitudinal and 2-10 transverse septa.

2.5.2 *Colletotrichum* spp.

Often, pathogens like *Colletotrichum gloeosporioides* and *C. capsici* infect flower crops like orchids, gerbera and carnation causing anthracnose. Cabrera *et al.* (2003) characterized orchids infecting *C. gloeosporioides.* The conidia were cylindrical with both apices rounded; or with one apex rounded and the other end pointed. The conidia was hyaline, oblong with rounded ends formed abundantly on potato dextrose agar (PDA) culture where the size varied from 16.0-24.0 x 4.0-6.0 µm and the setae were straight and dark.

2.5.3 *Podosphaera* spp. and *Golovinomyces* spp.

Powdery mildew is considered as the most common and destructive disease of gerbera plants. Farr and Rossman (2009) first reported powdery mildew disease caused by *Podosphaera* sp. in gerbera in India. Similar findings were noticed by Baiswar *et al.* (2010) where they confirmed the disease incidence of powdery mildew through characteristic features of the pathogen, *Podosphaera* sp. According to them, presence of catenate conidia with fibrosin bodies as the characteristic feature of *Podosphaera* sp. which differentiated it morphologically from *Golovinomyces cichoracearum*.

2.5.4 *Plasmopara* spp. and *Bremia* spp.

Farr and Rossman (2009) revealed the presence of ectophytic mycelium of downy mildew pathogen with indistinct appresorium in gerbera where the pathogen was identified as *Plasmopara* sp. with catenate conidia and fibrosin bodies under scanning electron microscope. Foot cells of conidiophores measured 39–58 x 10–12 µm dimension with two or three shorter cells, terminated with elliptical conidia produced in chains having dimension ranging from 20–24 x 18–22 µm. The basal septum of conidiophore was adjacent to the mycelium. Wolcan (2010) carried out a detailed description of downy mildew pathogen, *Bremia*

lactucae. According to him the pathogen was dichotomously branched, hyaline producing branched conidiophores with tips ending in swollen vesicles (7–9 µm) bearing 3–5 sterigmata (3.7–7.4 µm) measured a length of 475–625 x 10–13 µm. Conidia were pale olivaceous in colour and ovoid to globose in shape (22.2–25.9 x 18.5–25.9 µm). Duarte *et al.* (2013) studied features of hyaline, aseptate, straight, cylindrical, hypophyllous sporangiphore with 650 × 5-10 × 6.5- 13 µm dimensions of *Plasmopara halstedii* causing downy mildew disease bearing globose to ovoidal, 20 to 28 µm long and 13 to 18 µm wide, hyaline, smooth sporangia.

2.5.5 *Fusarium* spp.

Characteristics of *Fusarium dianthi* was studied by Kumar *et al.* (2014) which caused *Fusarium* wilt in carnation. The fungal growth on PDA was initially white and later turned light pink to orange. Macroconidia sparse and fusoid with 2-3 septa and measured 17-24 x 3.5-4 µm. Microconidia were abundant, hyaline, continuous, ovoid and measured 4.5-8 x 2-3.5 µm and chlamydospores were hyaline and spherical.

2.5.6 *Phytophthora* spp.

Erwin and Ribeiro (1996) observed distinctive morphological characteristics of *Phytophthora* sp. which included abundant hyphal swellings in sterile soil extract and hemp seed water cultures that was non papillated, persistent, internally proliferating producing obpyriform sporangia having mean dimensions of 36 × 26 µm and mean length/breadth ratios ranging from 1.30 to 1.53. Farr and Rossman (2009) opined that globally, *P.cryptogea* has over 140 reported hosts in approximately 50 plant families and among the reported hosts, approximately 40 were ornamental crops. They noticed that *P.cryptogea* cultures had a petaloid pattern on PDA and no growth was observed at 35°C.

2.5.7 Minor pathogens

Shi *et al.* (2012) isolated *Corynespora cassiicola* from gerbera which was rarely seen and therefore considered as a minor pathogen. According to them, the pathogen produced grey, floccose colonies with brown or olivaceous cylindrical, straight and unbranched conidiophores with 2-7 septa and 25-83 × 4-7 µm in size. Conidiogenous cells were olivaceous or brown, cylindrical and 11-21 × 4-6 µm in size. Conidia were borne singly or in chains of 2-5, brown, cylindrical, straight to slightly curved, 2 to 8 pseudosepta, and 70.4 × 7.3 µm in size, with a conspicuous hilum.

2.6 MANAGEMENT OF FUNGAL DISEASES

Soil and crop plants being the inhabitant of many microorganisms includes pathogens whose activities are influenced by many physical, chemical and biological interactions on it. Since any single method would be of little effect to contain the disease, an integrated approach would be an ideal strategy to tackle the complex. Integrated approaches for plant disease management are practiced nowadays with combination of cultural as well as biological control measures and strategy of chemical control were reserved as the last resort when all other methods have been utilized.

2.6.1 Chemical control

According to Hirooka and Ishii (2013) chemical control of disease plays a significant role in preventing losses due to plant diseases and the most important method of protecting plants against fungal attack is the use of fungicides. A perusal of literature revealed that reports on fungicidal toxicity on pathogen infecting gerbera meagre and scanty. However, attempt has been made to include some available literature on the fungicidal action of similar pathogens infecting other crop plants.

2.6.1.1 In vitro evaluation of fungicides

Mohanty *et al.* (2014) observed the effectiveness of hexaconazole and carbendazim (12%) + mancozeb (64%) against *Colletotrichum capsici* under *in vitro* causing leaf spot on gerbera. Apet *et al.* (2014) noticed the sensitiveness of *Alternaria alternata* isolated from gerbera to systemic fungicides *viz.,* propiconazole, hexaconazole, thiophanate methyl, carbendazim and difenoconazole and the non systemic fungicides like copper oxychloride, captan, mancozeb, propineb and cymoxanil + mancozeb at 500 and 1000 ppm concentration.

Mathivanan and Prabavathy (2007) tested per cent reduction of mycelial growth of *Alternaria helianthi* using contact and systemic fungicides and they observed 100, 75.5 and 58.8 per cent inhibition with carbendazim + mancozeb, carbendazim and mancozeb + metalaxyl respectively. Contradictory to the above findings, Thaware *et al.* (2010) noticed least per cent inhibition with carbendazim on *Alternaria* leaf blight pathogen of cowpea and complete inhibition with propiconazole, mancozeb and difenoconazole. Similarly, Parveen *et al.* (2013) concluded that carbendazim and mancozeb as better systemic and contact fungicides against *Alternaria alternata.* Roopa *et al.* (2014) noticed inhibition of above 80 per cent with hexaconazole and the least with propineb against *Alternaria solani.*

Shashidhara *et al.* (2008) carried out *in vitro* evaluation and attained complete inhibition of *Phytophthora capsici* with Akomin, Aliette 80WP, Bordeaux mixture, Melody duo 66.75 WP, Profiler 71.04 WDG, Ridomil MZ 72 WP and Secure 60WDG at highest concentration of 0.3 per cent. Kaur *et al.* (2009) opined that Ridomil Gold 68 WP, Ridomil MZ 72 WP and Curzate M 8 were the most promising fungicides under *in vitro* evaluation against *Phytophthora nicotianae*. Cent per cent inhibition of *Phytophthora* spp. by Curzate M-8 was recorded by Boughalelleb *et al.* (2006) and Kaur and Verma (2009). However, Mtasa *et al.* (2014) noticed complete inhibition of *Phytophthora*

sp. with a combination fungicide carbendazim (12%) + mancozeb (64%) (Saaf). Sumbula (2015) noticed complete inhibition of *Phytophthora* sp. causing nutmeg leaf fall with Bordeaux mixture (1%), copper hydroxide (2g/l), copper oxychloride (2.5g/l), potassium phosphonate (3ml/l) and the combination fungicides iprovalicarb + propineb (1.5 and 2.0g/l) and cymoxanil + mancozeb (2g/l).

Kumhar *et al.* (2015) reported the effectiveness of copper oxychloride 50WP at 0.2, 0.25 and 0.3 per cent against *Fusarium solani* causing die back disease in tea whereas at the same concentration, copper hydroxide recorded inhibition ranging from 60 to 80 per cent. Fareed *et al.* (2015) observed less than 55 per cent inhibition with Ridomil, Score, copper oxychloride, Cabriotop and Antracol against *Fusarium oxysporum.*

2.6.1.2 In vivo evaluation of fungicides

a) Foliage diseases

Kumar *et al.* (2011) assessed management of chrysanthemum leaf blight pathogen, *Alternaria alternata* under *in vivo* condition with fungicides and they noticed that per cent disease severity was reduced to maximum extent while treating with hexaconazole (0.1%) followed by chlorothalonil (0.2%) and mancozeb (0.2%). Similar to the above study, Balai and Singh (2013) proved the efficacy of foliar sprays of mancozeb, copper oxychloride, proximain, iprodione, chlorothanlonil and carbendazim against *Alternaria* leaf blight of pigeon pea. Nagrale *et al.* (2012) studied the effect of of Bordeaux mixture (0.6%), tricyclazole (0.1%) and iprodione + carbendazim (0.1%) for management of *Alternaria* blight in polyhouse and observed more than 90 per cent inhibition with all the three fungicides.

Folk and Tusnadi (1984) observed good control of powdery mildew disease caused by *Oidium erysiphoides* f. sp. *gerberae* by spraying with elemental sulphur or dinocap (Karathane FN-57 0.05% + Karathane 0.05%) as well as with systemic fungicides benomyl (Chinoin Fundazol 50 WP 0.1%), bupirimate (Nimrod 25 EC 0.1%), fenarimol (Rubigan 12 EC 0.03%), pyrazophos (Afugan 0.1%) or thiophanate-methyl (Topsin-M WP 0.08%). Sansiviero *et al.* (1995) reported the effectiveness of diethofencarb (25%) + carbendazim (25%) (Sumiko), tebuconazole (10%) + tolyfluanid (40%) (Folicur), fenbuconazole (5%) (Indar 5F), vinclozolin (10%) + thiram (64%) (Silbos DF), fludioxonil (50%), procymidone (50%) (Sumisclex), dichlofluanid (50%) (Euparen) and procymidone (12.3%) + thiram (49%) (Sialex T) against grey mould on gerbera. McMillan and Vendrame (2006) studied the efficacy of fungicides *viz.,* Compass 50 WDG, Systhane 40 WSP, Heritage 50 WSP and found that alternative sprays of the three fungicides at 14 days interval were found to be effective against powdery mildew of gerbera. Kumar *et al.* (2013) observed the efficacy of wettable sulphur and carbendazim at different concentration for the management of powdery mildew under polyhouse condition. According to them, wettable sulphur at 2.5 gl^{-1} and carbendazim 2 gl^{-1} reduced disease severity of powdery mildew.

Pawar *et al.* (2012) observed complete inhibition of *Curvularia lunata, Curvularia pallescens* with mancozeb (0.2%) followed by tricyclazole (0.1%), Companion (mancozeb +carbendazim) (0.25%) and zineb (0.2%) which resulted in a per cent inhibition above 75 over control. Similarly, Gadage and Patil (1977) found that mancozeb (0.2%) and zineb (0.2%) were effective in controlling *C. lunata* under *in vitro* condition.

b) Wilt and root diseases

Soil drenching of Aaterra (etridiazole) (0.2%) or Dexon (fenaminosulf) (0.15%) twice at fortnightly intervals gave good control against root rot and collar rot caused by *Phytophthora* sp. (Raicu *et al.,* 1981). According to Leski (1984),

soil drenching of Ridomil 25WP (Metalaxyl) at fortnightly intervals showed exceptional management of downy mildew caused by *Bremia lactucae* Regal. Hilal *et al.* (2000) reported the efficacy of vitavax or thiram followed by Monceren-Combi (dichlofluanid + pencycuron) against *Fusarium oxysporum.* Lebrun (2002) observed significant control with a mixture of oxadixyl and cymoxanil against *Phytophthora cryptogea* infecting gerbera. Kaur *et al.* (2009) revealed that Ridomil Gold 68 WP, Ridomil MZ 72 WP and Curzate M- 8 as the most promising fungicides which could effectively control *Phytophthora nicotianae* causing citrus root rot. Fareed *et al.* (2015) observed maximum reduction of *Fusarium* wilt of cucumber when treated with Ridomil (metalaxyl) and Score (difenoconazole) compared to Antracol (propineb) and Cabriotop (pyraclostrobin 50% + metiram 55%).

2.6.2 Biological control

Biocontrol agents are ideal microbial antagonists to several plant pathogens which suppressed the disease or pathogen population not only by producing various metabolites but also by inducing defence enzymes which had been found to be a novel way whereby plants defend themselves from pathogenic attack (Bharati *et al.,* 2004). Nowadays, biocontrol of plant diseases have received increased attention in view of the hazardous impact of pesticides and other agro-chemicals on the ecosystem. Hence, the focus of plant disease management has been shifted from chemical pesticides to ecofriendly management using biopesticides thus, minimizing the hazard of development of pesticide resistance strains of plant pathogens and also restricts the hazardous effect of increased use of agro-chemicals on the environment and human health. Numerous studies have showed that biological control offers an environmental friendly alternative to protect plants from both soil borne and foliar pathogens (Weller *et al.,* 2002).

Liu and Baker (1980) demonstrated genus *Trichoderma* as the potential biocontrol agent against plant pathogenic fungi. Natarajan and Manibhushanarao

(1996) suggested the use of fungal antagonists against fungal pathogens which gained considerable attention and appeared to be promising as a viable supplement to chemical control. Similarly, the soil borne fluorescent pseudomonads have received particular attention because of their catabolic versatility, excellent root colonizing ability and their capacity to produce a wide range of enzymes and metabolites that favour the plants to withstand varied biotic and abiotic stress conditions (Ramamoorthy *et al.,* 2001; Saravanakumar *et al.,* 2007).

2.6.2.2 In vitro evaluation of bioagents

Padgan and Gade (2006) reported the efficacy of *Trichoderma viride* and *Pseudomonas fluorescens* with soil borne pathogens of gerbera*, Fusarium oxysporum* f. sp. gerbera*e* and *Pythium* sp. Chavan and Hegde (2009) made similar observations on the wilt pathogen, *F. solani.* Likewise, Perveen and Bokhari (2012) observed that different strains of *Trichoderma viride* could inhibit *F. oxysporum* under *in vitro* conditions.

Grasso *et al.* (2003) experimented antagonistic *Fusarium* spp. and *Trichoderma* spp. isolated from gerbera rhizosphere against root rot caused by *Phytophthora cryptogea* in a soilless system. Similar study on management of *Fusarium* wilt in gerbera grown in soilless system was carried out by Minuto *et al.* (2008). The results showed that, combination of antagonistic *Fusarium* spp. or *Trichoderma* spp. or *Streptomyces griseoviridis* with slow sand filtration significantly reduced the disease. Pandey (2010) proved the efficiency of *Trichoderma viride* and *Trichoderma harzianum* against *Alternaria alternata* isolated from *Capsicum frutescens*. Jamwal and Jamwal (2011) noticed *T. viride, T.virens* and *T.harzianum* could manage the root rot complex pathogens of gerbera *viz., Fusarium oxysporum* f. sp. *gerberae and Pythium irregulare.* Taj and Kumar (2012) assessed efficacy of *T. harzianum* against different flower and fruit crops under *in vitro* condition. According to them, application of *T. harzianum*

restricted the growth of *Colletotrichum gloeosporioides* causing leaf spot on gerbera by 76.25 per cent. Choudhari *et al.* (2012) noticed antagonistic property of *T. viride, T. harzianum* and *Pseudomonas fluorescens* against *Fusarium* solani. Manjunath *et al.* (2012) stated that the bacterial antagonist, *Pseudomonas fluorescens* restricted the growth of *Fusarium oxysporum* f. sp. *udum* and *C. gloeosporioides* below 50 per cent.

Shashidhara *et al.* (2008) recorded 55 to 70 per cent inhibition of *Phytophthora capsici* with *Trichoderma* spp. and *Pseudomonas* spp. Mir *et al.* (2011) recorded 75 and 90 per cent inhibition of *Rhizoctonia* sp., *Phytophthora* sp. and *Fusarium* sp. with different strains of *T. viride.* An attempt was made by Apet *et al.* (2014) to study *in vitro* evaluation of bioagents *viz., T.viride, T. koningii, T. hamatum, T. harzianum, T. lignorum* and *P. fluorescens* against *Alternaria alternata* causing leaf blight on gerbera and they noticed that the highest per cent inhibition of test pathogen was recorded with *T. viride* followed by *T. hamatum.* Maurya *et al.* (2014) obtained maximum inhibition of *Alternaria alternata* with the bacterial antagonist, *P. fluorescens*. Tapwal *et al.* (2015) elucidated the inhibitory action of *Trichoderma* spp. on different seed borne pathogens *viz., Curvularia lunata, F. oxysporum, A.alternata, C.gloeosporioides* and *Rhizoctonia solani.*

2.6.3 Integrated disease management of fungal pathogens

Paradikovic *et al.* (2000) documented the effect of *T. harzianum*, isolate T-22 against the pathogen, *F. oxysporum* f. sp. *gerbera* causing wilt disease in gerbera. Likewise, Lingan *et al.* (2014) studied the potential of combinations of biocontrol agents against *Fusarium* wilt of gerbera. The talc-based formulation of two bioagents, *P. fluorescens* and *T.viride* were tested against the disease through rooted cuttings as dipping, soil application and foliar spray which significantly reduced wilt incidence to 3.3 per cent in first year and 7.0 per cent in the following year which was on par with carbendazim treatment. Orlikowski *et al.*

(2004) studied the combination effect of bioagents with chemical fungicides and succeeded in management of *Phytophthora cryptogea* infection on gerbera using *T. viride* in combination with furalaxyl. An attempt was made by Kshirgar *et al.* (2008) to manage root rot disease by *Rhizoctonia solani* Kuhn and reported that *T. viride* was found to be effective against the disease. Moreover, integration of *T. harzianum* with propineb (0.25%) showed high efficacy in controlling the disease and inferred that application of bioagents along with chemical fungicides showed superior results over the individual treatments. Moyer and Peres (2008) tested efficacy of biofungicide product, Cease (*Bacillus subtilis* QST 713) against powdery mildew of gerbera caused by *Erysiphe cichoracearum*.

Materials & Methods

3. MATERIALS AND METHODS

The present study on ″Cataloguing and documentation of fungal diseases of gerbera" was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara during the period 2014-16. The detailed description of materials used and methods followed during the course of the experiment are given below.

3.1 SURVEY ON THE OCCURRENCE OF FUNGAL DISEASES OF GERBERA

A purposive sampling survey on the occurrence of fungal diseases of gerbera was carried out in three districts *viz.,* Wayanad, Malappuram and Thrissur for the collection of diseased samples. The samples showing typical symptoms of fungal diseases were collected from gerbera grown both under polyhouses and in open field conditions. The survey was conducted during the months of July-August, November-December and March-April to get a complete profile on the occurrence of diseases prevailing during rainy, winter and summer season. The details regarding the places surveyed are given in Table 3.1.

Table 3.1. Locations of sampling survey in various districts

Sl. No.	District	Location
1.	Thrissur	Vellanikkara
		Madakathara
		Chalakudy
2.	Malappuram	Anakkayam
3.	Wayanad	Chulliyode
		Ambalavayal

3.1.1 Assessment of disease incidence and disease severity

The disease incidence and disease severity was recorded from the surveyed areas based on different types of symptoms produced by different pathogens. Per cent disease incidence (PDI) was calculated using the formula given by Wheeler (1969).

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

The severity of disease for all foliage diseases except powdery mildew was assessed by adopting a standard score chart of 0-5 scale as cited in Table 3.2. The score chart of 0-6 scale developed by Kumar *et al.* (2012) was followed for powdery mildew disease (Table 3.3).

Table 3.2. Score chart for severity of diseases on leaves

Grade	Description
0	No symptom
1	>0-10 per cent leaf area infected
2	>10-25 per cent leaf area infected
3	>25-50 per cent leaf area infected
4	>50-75 per cent leaf area infected
5	>75 per cent leaf area infected

Table 3.3. Score chart for severity of powdery mildew disease on leaves

Grade	Description
0	No powdery mildew
1	1-20 per cent of the leaf area with powdery growth
2	21-40 per cent of the leaf area with powdery growth
3	41-60 per cent of the leaf area with powdery growth
4	61-80 per cent of the leaf area with powdery growth
5	81-99 per cent of the leaf area with powdery growth
6	100 per cent of the leaf area with powdery growth

$$\text{Per cent disease severity (PDS)} = \frac{\text{Sum of all numerical ratings}}{\text{Total no. of leaves observed X Maximum disease grade}} \times 100$$

Per cent disease severity for powdery mildew disease was recorded by observing the upper leaf surface during the initial stage of growth and on the lower surface of leaves at later stages of growth.

3.1.2 Meteorological data

The meteorological factors such as maximum and minimum temperature, relative humidity and rainfall influencing the crop and pathogen prevailing in the polyhouse and open condition during the survey period were recorded from all the locations of Wayanad, Malappuram and Thrissur district and the mean disease severity was correlated with different weather parameters.

3.2 ISOLATION OF PATHOGENS

The pathogens causing various fungal diseases of gerbera except the obligate parasites were isolated from the samples showing distinct type of

symptom on naturally infected plant parts *viz.,* leaf, root and flower. The infected samples collected, washed under running tap water and were cut into small bits consisting of both healthy and infected portions using a sterile blade and were disinfected with sodium hypochlorite (1%) for one minute and subsequently three washings were given using sterilized distilled water and the excess moisture in the sample bit was dried with sterilized blotting paper. Such surface sterilized bits were then placed aseptically on solidified Potato Dextrose Agar (PDA) medium in sterile Petri dishes. The plates were then incubated at room temperature (26± 2^{o}C). The growth of fungi noticed after four to six days of incubation was subsequently subcultured to solidified PDA in sterile Petri dishes. The isolates were thereafter purified by single hyphal tip method and then maintained in PDA slants by periodic sub culturing and stored under refrigerated condition at 4^{o}C for further studies.

3.3 PATHOGENICITY OF ISOLATES

The pathogenicity of isolates obtained was proved by following Koch's postulates. It was proved by artificial inoculation of cultures on healthy plants or plant parts to observe whether the isolates are capable of reiterating typical symptoms under artificial conditions.

3.3.1 Pathogenicity test for foliage and flower diseases

For pathogens infecting leaves and flowers, except for obligate parasites like powdery mildew disease artificial inoculation was done by two methods *viz,* Mycelial Bit Inoculation Method (MBIM) (Rocha *et al.,* 1998) and Micro Droplet Inoculation Technique (MDIT) (Munaut *et al.,* 1997) except for powdery mildew disease.

3.3.1.1 Mycelial Bit Inoculation Method (MBIM)

Mycelial discs of size 8 mm diameter, taken from seven day old culture, were inoculated in inverted position on detached leaves or flowers as well as on live plants after giving injury by pinprick method on adaxial surface of leaf lamina or flowers. Humidity was provided by placing a small moist cotton swab over it. The inoculated leaves, flowers and live plants were kept under humid chamber for incubation and observed daily for appearance of symptoms.

3.3.1.2 Micro Droplet Inoculation Technique (MDIT)

Micro droplet inoculation technique was done on three month old gerbera plants grown in growbags. The plants were kept in moist chamber for 48h prior to inoculation to provide maximum humidity for infection and disease development. Then seven day old pure cultures of different isolates grown on PDA were taken and the spores produced were harvested by gently scrapping the culture and added to 100ml sterile water. This was then filtered through muslin cloth to get a final spore suspension containing 10^5 spores ml^{-1}. The inoculation was done on plants by spraying the spore suspension using a hand atomizer after giving injury by pinpricks on healthy leaves and flowers. The plants were incubated in moist humid chamber. After 48h of inoculation, the bags were removed from moist chamber and transferred to the platform under shade net and sprayed with sterilized water for 3-4 times daily upto five days from inoculation.

3.3.1.3 Inoculation of obligate parasite

For obligate parasites causing powdery mildew diseases, pathogenicity was proved by detaching an infected leaf containing a single colony and inoculating onto a fresh healthy leaf. The whole leaf sample was covered with plastic bag and observations were taken for symptom development. Uninoculated

plants were maintained under same condition and were treated as control (Warkentin *et al.,* 1995).

3.3.2 Pathogenicity test for root diseases

3.3.2.1 Spore suspension method

Spore suspension of the isolate from 10 day old culture with inoculum concentration $3x10^6$ cfu ml^{-1} was prepared. This spore suspension of the pathogen was poured in the rhizosphere region after giving injury on the root (Shashi and Vishwa, 2005).

3.3.2.2 Soil inoculation method

In soil inoculation method, the isolates were mass multiplied in carrot discs. For this carrot discs (100g) were autoclaved and used as substrate for mass multiplying the pathogen. The mass multiplied inoculum was added @5g in the root zone after giving an injury in the root and thereafter the plants were kept for incubation in the net house under prevailing conditions (Sumbula, 2015). Observations on root rot incidence in the plants were recorded from 15 days after inoculation.

The pathogens were re-isolated from artificially inoculated plant parts. The cultural and morphological characters of the isolates were studied and compared with that of the original one. The isolates thus obtained were maintained on PDA slants and used for further studies.

3.4 SYMPTOMATOLOGY OF THE DISEASES

Symptoms of fungal diseases on gerbera were studied under natural conditions during the survey. Symptoms produced by each pathogen on artificial inoculation of gerbera plants were also recorded.

The symptomatology of diseases under artificial conditions were studied by inoculating, the pathogens causing diseases in gerbera as per standard procedure as mentioned in 3.3.1 and 3.3.2 respectively. The inoculum was prepared with the zoospore/conidial suspension or culture discs of actively growing mycelia with sporangia/conidia of the respective pathogens. Inoculation was done on leaves, flowers and roots depending upon the symptoms of the disease from which the pathogens were isolated. Observations on variations in symptom development like lesion size and time for symptom development were also recorded.

3.5 CHARACTERISATION AND IDENTIFICATION OF PATHOGENS

The associated fungal pathogens isolated were identified based on their cultural and morphological characters.

3.5.1 Cultural characters

The cultural characters exhibited by different pathogens were recorded by visual observations. The pathogens were grown aseptically in sterilized Petri dishes containing solidified PDA by placing agar discs of 8mm in the centre of the Petri plate. The plates were kept for incubation at 26±2^{o}C and the distinguishing characters were recorded upto 7 days at an interval of 24h. Variations in colony characteristics, pigmentation, growth pattern and growth rate of each isolate were studied.

3.5.2 Morphological characters

Morphological characters were studied by slide culture technique (Riddle, 1950). These slides were observed for various fungal structures *viz.,* type of mycelium, branching pattern, type of spores, their shape, size, L/B ratio, septal

distance and presence of sexual structures if any. Microphotographs and measurements of fungal structures were taken assisted by the software Ultrascope.

For obligate parasites like powdery mildew diseases, a temporary mount of fungal pathogen was prepared by using a strip of transparent cellophane tape (10 cm long) which was held in between the thumb and the forefinger. The sticky side of the tape was firmly pressed onto the leaf surface of a sporulating colony. After gently removing the cellophane tape, the sticky surface carrying fungal spores and hyphae was carefully placed over drops of lactophenol cotton blue kept at the centre of a clean glass slide. The tape was gently pressed and the extended ends of the tape is held over the ends of the slide and observed under the light microscope where the characteristics of spores and sporulating strucutures were studied (Narayanasamy, 2011).

For further confirmation, the isolates were sent to National Center for Fungal Taxonomy (NCFT), New Delhi where the cultures were also deposited under different accession numbers.

3.6 *In vitro* EVALUATION OF FUNGICIDES AND BIOAGENTS AGAINST PATHOGENS

The efficacy of fungicides and bioagents were tested against isolated pathogens under aseptic conditions. The fungicides were evaluated under *in vitro* against the pathogens by employing the poison food technique (Zentmeyer, 1955). In the case of biocontrol agents, dual culture method suggested by Skidmore and Dickinson (1976) was used for testing efficacy of the reference cultures of KAU *viz, Trichoderma viride* and *Pseudomonas fluorescens* against isolated pathogens.

3.6.1 *In vitro* evaluation of fungicides by Poison Food technique

The fungicides depicted in Table 3.4 were used for *in vitro* evaluation. An appropriate quantity of fungicide was added to 100ml sterilized molten PDA

medium so as to get the final required concentrations. The medium was mixed thoroughly before plating. After solidification of medium 8 mm mycelial disc from actively growing culture of the test pathogen was cut with a sterile cork borer and placed in the centre of each Petri dish and incubated at 26±1^{o}C. The experiment was planned with CRD and each media poisoned with fungicide was poured in three Petri plates. Non toxicated media was poured into Petri plates and kept as control. Observations on radial growth and sporulation of the isolated pathogens were recorded daily until the control plates showed full growth. The per cent inhibition of mycelial growth was calculated by using the formula suggested by Vincent (1927).

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

C= Growth of fungus in control (mm), T= Growth of fungus in treatment (mm)

Table 3.4. Fungicides used for *in vitro* evaluation against pathogens

Sl. No.	Chemical name	Trade name	Concentration (Per cent)
1.	Bordeaux mixture	-	0.5, 1, 1.5
2.	Copper hydroxide 77WP	Kocide	0.15, 0.2, 0.25
3.	Hexaconazole 5EC	Mega master	0.05, 0.1, 0.15
4.	Propineb 70WP	Antracol	0.25, 0.3, 0.35
5.	Difenoconazole 25EC	Score	0.02, 0.05, 0.1
6.	Carbendazim 12% + Mancozeb 64% WP	Saaf	0.15, 0.2, 0.25
7.	Cymoxanil 8% + Mancozeb 64% WP	Curzate	0.15, 0.2, 0.25
8.	Tebuconazole 250EC	Folicur	0.1, 0.15, 0.2
9.	Pyraclostrobin 20WG	Headline	0.05, 0.1, 0.15

3.6.2 *In vitro* evaluation of bio agents by dual culture technique

Biocontrol agents *viz., Pseudomonas fluorescens* and *Trichoderma viride* the reference cultures of KAU were tested for the efficacy on mycelial growth of fungal pathogen by following the dual culture technique (Dennis and Webster, 1971).

3.6.2.1 Fungal antagonist

The fungal pathogen and the fungal antagonist, *Trichoderma viride* were inoculated as dual cultures after giving due consideration for the growth rate of both the pathogen and the antagonist. Mycelial disc (8mm) of fungal pathogen from seven day old culture grown on PDA was inoculated aseptically 2cm away from the periphery of the sterilized Petri plate containing PDA medium and 8 mm mycelial disc of *T. viride* was inoculated in the same PDA plate 3.5 cm away from the pathogen disc. The plates were incubated at room temperature (26±1^{o}C). Three replications were maintained for each isolate. The fungal antagonist and the pathogen grown in monoculture served as control. The growth measurements were taken at regular intervals after 24h of inoculation of the antagonist upto five days till the control plates reached full growth. The per cent inhibition of mycelial growth of the pathogen was calculated using the formula as mentioned in 3.6.1. The nature of antagonistic reaction of *T. viride* tested against the pathogen was assessed by following the method of Purakayastha and Bhattacharya (1982) and assigned into four categories.

- Homogenous - Free intermingling hyphae
- Overgrowth - Pathogen overgrown by test organism
- Cessation of growth - Cessation of growth at the line of contact
- Aversion - Development of clear zone of inhibition

3.6.2.2 Bacterial antagonist

Pseudomonas fluorescens, the bacterial antagonist, was evaluated against different fungal pathogenic isolates by simultaneous antagonism (Utkhede and Rahe, 1983). A loopful of the bacterial culture was streaked at both the ends of Petri plate (2cm from edge of the plate) plated with PDA medium and mycelial disc (8mm diameter) of seven day old culture was placed in the centre of the Petri plate. Monoculture of the pathogen served as control and the plates were incubated at room temperature (26±1^{o}C). Three replications were maintained for each isolate. Observations on growth of the fungus were recorded at regular intervals till full growth of the pathogen was attained in control plates. Per cent inhibition of mycelial growth of the pathogen was calculated as mentioned in 3.6.1.

3.7 MOLECULAR CHARACTERISATION OF MAJOR PATHOGENS OF GERBERA

Based on the previous studies on per cent disease incidence and per cent disease severity, three pathogens *viz., Alternaria tenuissima, Phytophthora cryptogea* and *Fusarium solani* were identified as the major pathogen. Hence, these were subjected to molecular characterisation prior to the *in vivo* management study for the final confirmation regarding its identity. The molecular characterization was carried out at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram by ITS sequencing to identify at species level. Sequence analysis and nucleotide homology of each pathogen were analysed through the BLASTn programme of NCBI (http:// ncbi.nlm.nhm.gov/blast).

3.8 *In vivo* EVALUATION OF FUNGICIDES AND BIOCONTROL AGENTS ON MAJOR FUNGAL DISEASES OF GERBERA

A pot culture experiment was laid out for the management of the most severe and predominant fungal pathogens using the promising treatments obtained under *in vitro* studies. The experiment was carried out during May 2016 at College of Horticulture, Vellanikkara. The details of the experiment are as follows:

Design	: CRD
Number of treatments	: 7
Replications	: 5
Variety	: Natasha

Three major pathogens *viz., Phytophthora cryptogea, Fusarium solani* and *Alternaria tenuissima* were selected for the study. The pathogens were selected based on the severity of disease observed during the survey period at various locations. The treatments were decided based on the *in vitro* efficacy of fungicides and biocontrol agents as carried out in 3.6.

3.8.1 Preparation of potting mixture and planting

Tissue culture plants of gerbera variety, Natasha were raised in growbags of size 35×20×20 cm. The bags were filled with potting mixture which consisted of soil, sand and cowdung at 1:1:1. The growbags were kept in net house and irrigated regularly. All the cultural operations were carried out as per the Package of Practice of Horticultural Crops (Anonymous, 2007).

3.8.2 Treatment application

Gerbera plants were challenge inoculated with the pathogen by Micro Droplet Inoculation Technique (MDIT) or by root inoculation as described in 3.3.2. Treatments were given as foliar spray or soil drench depending upon the

symptom development by the pathogen. First spray and / or drench were given on symptom appearance after challenge inoculation of the pathogen and subsequent two sprays were given at ten days interval. However, in the case of biocontrol agent, prophylactic application of *Trichoderma viride* in the respective treatments was given ten days prior to challenge inoculation of the pathogen. Treatment details are shown in Table 3.5, Table 3.6 and Table 3.7.

3.8.3 Observations recorded

Observations on type of symptoms, disease incidence and disease severity were recorded at 10 days interval after each treatment.

Table 3.5 Details of experiment with the pathogen, *Alternaria tenuissima*

Sl. No.	Treatments (Foliar spray)		Conc. (%)
	Common name	Trade name	
1.	T_1- Carbendazim 12% + Mancozeb 64% WP	Saaf	0.2
2.	T_2- Cymoxanil 8% + Mancozeb 64% WP	Curzate M-8	0.2
3.	T_3- Hexaconazole 5EC	Mega master	0.1
4.	T_4- Difenoconazole 25EC	Score	0.05
5.	T_5- Propineb 70WP	Antracol	0.3
6.	T_6- *Trichoderma viride*	-	2.0
7.	T_2- Control	-	-

Table 3.6 Details of experiment with the pathogen, *Phytophthora cryptogea*

Sl. No.	Treatments (Soil drench and foliar spray)		Conc. (%)
	Common name	**Trade name**	
1.	T_1- Propineb 70WP	Antracol	0.3
2.	T_2- Cymoxanil 8% + Mancozeb 64% WP	Curzate M-8	0.2
3.	T_3- Carbendazim 12% + Mancozeb 63% WP	Saaf	0.2
4.	T_4- Copper hydroxide 77 WP	Kocide	0.2
5.	T_5- Hexaconazole 5 EC	Mega master	0.1
6.	T_6- *Trichoderma viride*	-	2.0
7.	T_7- Control	-	-

Table 3.7 Details of experiment with the pathogen, *Fusarium solani*

Sl. No.	Treatments (Soil drench)		Conc. (%)
	Common name	**Trade name**	
1.	T_1- Carbendazim 12% + Mancozeb 64% WP	Saaf	0.2
2.	T_2- Tebuconazole 250EC	Folicur	0.15
3.	T_3- Copper hydroxide 77WP	Kocide	0.2
4.	T_4- Pyraclostrobin 20WG	Headline	0.05
5.	T_5- Difenoconazole 25EC	Score	0.1
6.	T_6- *Trichoderma viride*	-	2.0
7.	T_7- Control	-	-

3.9 Statistical analysis

Data was subjected to analysis of variance (ANOVA). Data sets were analyzed using statistical package for social scientists (SPSS) version 21. Levels of significance, means and standard error were obtained for various data sets. Multiple comparisons between the treatment means, where the F test was significant was done with Duncan's Multiple Range Test (DMRT). The data wherever needed was subjected to appropriate transformation as suggested by Gomez and Gomez (1984).

Results

4. RESULTS

The present study on "Cataloguing and documentation of fungal diseases of gerbera*"* was carried out to identify various fungal pathogens infecting gerbera and to study the symptomatology, etiology and management of diseases. The results of the investigation carried out during 2014-16 are presented below:

4.1 SURVEY AND COLLECTION OF DISEASED SAMPLES

A purposive sampling survey was conducted in three districts *viz.,* Thrissur, Malappuram and Wayanad for the collection of diseased samples of gerbera and recorded the disease incidence and severity during the months of July-August, November-December and March-April during the year 2014-16. The diseased specimens were collected from these locations and the pathogens were isolated. The diseases observed in each location of each district and also under poly house and open filed conditions are presented in Table 4.1 and Table 4.2.

Based on the different types of symptoms produced by different pathogens the foliage diseases were abbreviated as LB for leaf blight, LS for leaf spot, PM for powdery mildew. Diseases like root rot, wilt and petal blight observed during the survey were mentioned as such.

4.1.1 Assessment of disease incidence and disease severity

Per cent disease incidence (PDI) and per cent disease severity (PDS) were recorded for distinct types of symptom of fungal diseases developed at every location during the survey. The details of PDI and PDS of three districts are furnished in Table 4.3, 4.4 and 4.5 respectively.

During the survey conducted in three seasons in three locations of Thrissur district the fungal diseases recorded in gerbera grown under polyhouses were LB-1, LB-3, LS-1, wilt and root rot. It was observed that LB-1 was noticed in all the seasons and in all locations *viz.,* Vellanikkara, Madakkathara and Chalakudy

Plate 4.1. Survey under open field condition in Malappuram district

Plate 4.2. Survey under polyhouse condition in Thrissur district

whereas LB-3 was observed in a hydroponic unit of gerbera maintained at CoH, Vellanikkara. LB-1 recorded a PDI ranging from 41.4 per cent to 74.7 per cent with the maximum from Vellanikkara during the months of March-April and minimum from Chalakudy during November-December. Similarly, the maximum PDS of 16.0 per cent was noticed only in Vellanikkara during the winter season (November-December) with the PDI of 53.1 per cent and PDS of 9.1 per cent respectively. The fungal disease, LS-1 was noticed from gerbera grown in Madakkathara, root rot from Vellanikkara and wilt from Chalakudy area. Among all the diseases observed, LS-1 recorded the highest PDI and PDS of 78.2 and 19.4 per cent respectively in Madakkathara during March-April. Disease intensity of root rots and wilt was more pronounced in July-August of which one was observed in the hydroponic unit in Vellanikkara with PDI of 69.44 per cent and that of wilt was observed in Chalakudy with PDI of 15.5 per cent.

In Malappuram district, survey was conducted in Anakkayam where diseases like LB-1, LS-2 and petal blight were observed. LB-1 was observed in all three seasons with a PDI of 76.1, 78.8 and 82.8 per cent respectively during November-December, March-April and July-August. However, the PDS ranged from 6.45 to 10.2 per cent only. Petal blight was noticed in gerbera plants showing a very low disease intensity of 3.98 per cent in July-August followed by 4.3 per cent in November-December.

Survey was conducted in mainly two locations of Wayanad district *viz.*, Ambalavayal and Chulliyode. Diseases like LB-1, LB-2, PM and petal blight were found in Ambalavayal whereas only LB-1 and PM were observed from Chulliyode. LB-1 was observed in all seasons with PDI ranging from 7.81 to 48.1 per cent where the lowest PDI was observed in November-December and the highest was noticed in March-April from Ambalavayal. However, LB-2 though noticed in all the three seasons, the disease was observed only in Ambalavayal with a maximum PDI of 72 per cent and a minimum of 62.1 per cent in July-August and November-December. Likewise, PDS of LB-2 ranged from 15.8 to 17.3 per cent. Petal blight was recorded only from Ambalavayal with a very low PDI of 4.3 per cent during the winter season. Powdery mildew was the most

Table 4.1 Diseases of gerbera observed during the survey period

Sl. No.	District	Location	Disease
1.	Thrissur	Madakkathara	Leaf blight 1 (LB-1)
			Leaf spot 1 (LS-1)
		Vellanikkara	Leaf blight 1 (LB-1)
			Root rot
			Leaf blight 3 (LB-3)
		Chalakudy	Wilt
			Leaf blight 1 (LB-1)
2.	Malappuram	Anakkayam	Leaf blight 1 (LB-1)
			Leaf spot 2 (LS-2)
			Petal blight
3.	Wayanad	Ambalavayal	Leaf blight 1 (LB-1)
			Leaf blight 2 (LB-2)
			Powdery mildew (PM)
			Petal blight
		Chulliyode	Leaf blight 1 (LB-1)
			Powdery mildew (PM)

Table 4.2 Diseases of gerbera observed under open field conditions and polyhouse

Sl.No.	District	Diseases	
		Open field	Polyhouse
1.	Thrissur	-	LB-1, LS-1, Root rot, Wilt
2.	Malappuram	LB-1, LS-2, Petal blight	-
3.	Wayanad	LB-1, LB-2, Powdery mildew, Petal blight	-

Table 4.3 Per cent disease incidence and severity of fungal diseases of gerbera in Thrissur district

Sl. No.	Location	Disease	Period					
			July-Aug		Nov- Dec		March-April	
			PDI	PDS	PDI	PDS	PDI	PDS
1.	Vellanikkara	LB-1	62.7	15.3	65.2	13.2	74.7	16.0
		LB-3	-	-	53.1	9.1	-	-
		Root rot	69.4	-	-	-	-	-
2.	Madakkathara	LB-1	63.3	6.88	66.1	7.53	69.4	7.67
		LS-1	45.0	9.2	41.5	6.0	78.2	19.4
3.	Chalakudy	LB-1	43.4	11.3	41.4	12.4	47.2	13.4
		Wilt	15.5	-	-	-	-	-

PDI- Per cent disease incidence; PDS- Per cent disease severity
LB-1- Leaf blight 1; LB-3- Leaf blight 3; LS-1- Leaf spot 1

Table 4.4 Per cent disease incidence and severity of fungal diseases of gerbera in Malappuram district

Sl. No.	Location	Diseases	Period					
			July- Aug		Nov- Dec		March-April	
			PDI	PDS	PDI	PDS	PDI	PDS
1.	Anakkayam	LB-1	82.8	10.2	76.1	6.45	78.8	9.6
		LS-2	-	-	43.1	14.3	40.2	13.4
		Petal blight	3.98	-	4.3	-	-	-

PDI- Per cent disease incidence; PDS- Per cent disease severity
LB-1- Leaf blight 1; LS-2- Leaf spot 2

Table 4.5 Per cent disease incidence and severity of fungal diseases of gerbera in Wayanad district

Sl. No.	Locations	Disease	Period					
			July- Aug		Nov- Dec		March-April	
			PDI	PDS	PDI	PDS	PDI	PDS
1.	Ambalavayal	LB-1	9.4	7.3	7.81	11.3	12.7	10.6
		LB-2	72.0	17.1	62.1	15.8	67.4	17.3
		PM	-	-	93.6	51.3	67.1	32.7
		Petal blight	-	-	4.3	-	-	-
2.	Chulliyode	LB-1	41.4	12.3	35.3	13.7	48.1	13.2
		PM	-	-	95.2	57.4	73.1	49.8

PDI- Per cent disease incidence; PDS- Per cent disease severity
LB-1- Leaf blight 1; LB-2- Leaf blight 2; PM- Powdery mildew

devastating disease observed within the district with a PDI of 93.6 and 95.2 per cent in Ambalavayal and Chulliyode during November-December. Per cent disease severity of the disease was also found to be very high compared to other diseases observed during the survey with the maximum of 57.4 per cent at Chulliyode and a minimum of 32.7 per cent at Ambalavayal.

4.1.2 Correlation of weather parameters with fungal diseases

During sampling survey, the extent of intensity and severity were recorded for each fungal disease and was correlated with the weather parameters *viz.,* temperature, relative humidity (RH) and rainfall prevailing in each location. The meteorological data for the period October 2014 to April 2016 of various districts are presented in Table 4.6, Table 4.7 and Table 4.8 and details regrading correlation studies are presented in Table 4.9, 4.10 and 4.11.

4.1.2.1 Leaf blight-1 (LB-1)

The results of the study showed that the disease LB-1 was noticed in all the locations of the three districts surveyed and in all the three seasons and was found significant in all locations. The correlation study of LB-1 with three weather parameters at Ambalavayal indicated that the disease was positively correlated with temperature whereas relative humidity and rainfall had a negative correlation (Table 4.8). As all the three factors have significant role in disease development, severity of LB-1 fluctuated with the variation in weather parameters. A total of 96 mm rainfall received during November-December at Ambalavayal coupled with RH of 80.27 per cent and mean temperature of 22.39^{o}C resulted in a maximum PDS of 12.32 per cent when compared to the PDS observed in summer with 8.80 per cent eventhough the disease was positively correlated with temperature. Hence, rainfall and relative humidity also plays an equal role in disease development as well. In Vellanikkara, incidence of LB-1 was found positively correlated with temperature but had no significant influence with

Table 4.6 Weather parameters of Thrissur district

Month	Maximum temperature (^{o}C)	Minimum temperature (^{o}C)	Relative humidity (%)	Rainfall (mm)
2014				
November	31.6	23.2	72	85.3
December	31.9	23.2	65	151.2
2015				
January	32.5	22.1	58	0
February	34.3	23.0	55	0
March	35.8	24.9	63	72
April	34.0	24.6	77	162.2
May	32.9	24.7	80	259.0
June	31.0	23.7	85	629.8
July	30.3	23.5	85	510.1
August	29.5	23.7	83	320.8
September	31.3	23.7	81	248.2
October	31.9	24.1	79	203.8
November	31.6	23.8	75	151.2
December	31.9	23.3	65	88.3
2016				
January	33.2	23.0	56	23.8
February	35.3	23.5	57	11.4
March	36.3	25.2	67	9.8
April	35.8	26.2	69	25.8

Table 4.7 Weather parameters of Malappuram district

Month	Maximum temperature (^{o}C)	Minimum temperature (^{o}C)	Relative humidity (%)	Rainfall (mm)
2014				
October	28.8	18.9	99.5	276.00
November	28.13	17.85	82.5	99.60
December	27.9	17.37	79.9	27.00
2015				
January	26.7	16.00	74.7	0.00
February	32.41	16.00	71.8	0.00
March	36.75	19.33	76.5	6.80
April	34.93	19.43	78.1	128.60
May	29.64	19.64	82.5	236.90
June	28.26	18.51	86.4	434.00
July	27.50	18.38	87.5	265.00
August	28.29	18.58	86.1	245.60
September	28.65	18.78	88.00	190.00
October	28.64	19.16	86.2	181.20
November	28.50	18.38	80.00	106.80
December	27.64	16.60	75.7	21.40

Table 4.8 Weather parameters of Wayanad district

Month	Maximum temperature (^{o}C)	Minimum temperature (^{o}C)	Relative humidity (%)	Rainfall (mm)
2014				
October	27.5	18.57	81.59	92
November	27.15	16.79	76.65	50
December	27.47	16.6	81.32	15
2015				
January	27.34	16.1	83.31	0.4
February	27.65	16.23	78.23	0.00
March	27.5	17.1	79.21	2.2
April	31.00	19.38	77.67	187.2
May	29.81	19.24	78.54	175.0
June	24.08	18.17	83.61	490.0
July	23.81	18.57	80.14	180.0
August	25.50	18.44	81.28	128.4
September	26.03	17.61	79.32	176.0
October	26.60	18.12	79.26	122.2
November	26.86	18.14	81.12	106.0
December	26.40	18.20	80.43	59.8
2016				
January	26.56	18.31	80.13	0.00
February	27.43	18.23	79.13	0.00
March	28.21	18.30	77.71	0.00
April	28.81	18.13	78.12	0.00

Table 4.9 Correlation of weather parameters with development of fungal diseases of gerbera

Sl. No.	District	Location	Disease	Correlation coefficient		
				Temperature	Relative humidity	Rainfall
1.	Wayanad	Ambalavayal	LB-1	0.317**	-0.323**	-0.340**
			LB-2	NS	NS	0.297**
			PM	NS	NS	NS
		Chulliyode	LB-1	NS	NS	NS
			PM	-0.292*	0.292*	-0.292*
2.	Malappuram	Anakkayam	LB-1	NS	0.28*	0.289*
			LS-2	NS	NS	NS
3.	Thrissur	Vellanikkara	LB-1	0.208†	NS	NS
		Madakathara	LB-1	0.236*	-0.246*	-0.253*
			LS-1	NS	NS	NS

† - Significant at 10% level * - Significant at 5% level ** - Significant at 1% level

Table 4.10. Range statistics of the weather factors for the three locations and three seasons

Sl. No.	District	Location	Disease	Correlation coefficient		
				Mean temperature (^{o}C)	Relative humidity (%)	Rainfall (mm)
1.	Wayanad-Ambalavayal, Chulliyode	S1	LB-1, LB-2, PM	21.43	81.68	266.13
		S2		22.39	80.27	96.0
		S3		23.14	78.37	63.13
2.	Malappuram-Anakkayam	S1	LB-1, LS-2	23.26	86.67	314.87
		S2		23.15	80.63	103.13
		S3		26.48	75.47	45.1
3.	Thrissur-Vellanikkara, Madakkathara	S1	LB-1, LS-1	26.95	84.33	486.9
		S2		27.76	73.0	147.77
		S3		29.43	65.0	78.06

S1- Monsoon (July-August), S2- Winter (Nov-Dec), S3- Summer (March-April)

Table 4.11. Per cent disease severity of fungal diseases of gerbera during different seasons

Sl.No.	Disease	Wayanad			Malappuram			Thrissur			CD value
		S1	S2	S3	S1	S2	S3	S1	S2	S3	
1.	LB-1	5.48	12.32	8.80	12.16	16.64	12.48	9.28	7.52	8.0	2.29
2.	LB-2	12.32	8.64	14.88	-	-	-	-	-	-	1.85
3.	LS-1	-	-	-	-	-	-	6.56	7.36	8.16	NS
4.	LS-2				11.04	11.36	9.76	-	-	-	NS
5.	PM		50.72	49.12	-	-	-	-	-	-	5.41
			47.2	33.6							

S1: Monsoon (July-August), S2- Winter (Nov-Dec), S3- Summer (March-April)

rainfall and relative humidity. Likewise, in Madakkathara, though a positive correlation was noticed with temperature, the reverse was shown with RH and rainfall. Highest mean PDS of 9.28 per cent was recorded in Thrissur district during the monsoon with a mean temperature of 26.95^{o}C, RH of 84.33 per cent and rainfall of 486.9 mm. In Malappuram district, though temperature was negatively correlated the weather conditions like RH and rainfall were observed to be most congenial for crop infection as it was positively correlated and showing the highest per cent disease severity (16.64%) during November-December.

4.1.2.2 Leaf blight-2 (LB-2)

In the present study, LB-2 was observed typically only in the high range tract, Ambalavayal of Wayanad district where the disease was correlated positively with temperature with no significant influence with relative humidity and rainfall. Maximum per cent severity of 14.88 per cent was observed during summer followed by 12.32 per cent during monsoon and the least 8.64 per cent in cool weather condition. This shows that a low temperature of 22.39^{o}C coupled with average RH of 80.27 per cent and rainfall of 96.0 mm can reduce the disease intensity compared to the PDS observed during winter and the rainy season.

4.1.2.3 Leaf spot-1 (LS-1)

The disease LS-1 was observed only in Madakkathara of Thrissur district and mean per cent disease severity was found unaffected though the weather parameters deviated considerably. Though no considerable variation was observed in temperature during three seasons, relative humidity varied from 65-84.33 per cent and rainfall from 78.06-486.9 mm.

4.1.2.4 Leaf spot-2 (LS-2)

The disease LS-2 was noticeable only in Malappuram district where a non-significant relation existed between weather parameters in disease development which infers that the disease was least navigated by the climatological factors. The mean per cent disease severity was observed as 11.04, 11.36 and 9.76 per cent during rainy, winter and summer seasons respectively.

4.1.2.5 Powdery mildew

Powdery mildew existed in two locations *viz.,* Ambalavayal and Chulliyode of Wayanad district with more severity at Chulliyode. Mean per cent disease severity was maximum during November-December with 50.72 per cent in Chulliyode and 47.2 per cent in Ambalavayal. In Ambalavayal, the disease was non-significant and no correlation existed between weather parameters on disease progress. But, in Chulliyode, correlation studies revealed that it was significant with positive correlation to relative humidity and a reverse relation existed with temperature and rainfall. The weather data clearly depicts that at a low rainfall of 96 mm and above average relative humidity of 80.27 per cent during November-December was the congenial factor influencing the disease development. But during summer, decline in relative humidity (78.37%) and rainfall (63.13 mm) caused a slight reduction in mean per cent disease severity of 49.12 per cent and 33.6 per cent at Chulliyode and Ambalavayal respectively.

4.2 ISOLATION OF PATHOGENS

Isolation of different fungal pathogens, except obligate pathogens causing diseases in gerbera were carried out from naturally infected samples collected from various locations. The pathogens were isolated from leaves, roots and flowers,

purified by single hyphal tip method and maintained on PDA by periodic sub culturing.

4.3 PATHOGENICITY OF ISOLATES

The pathogenicity of different isolates from gerbera were studied by artificial inoculation under *in vivo* condition. Inoculation of pathogens for pathogenicity test varied with different types of diseases *viz.,* foliage, flower and root diseases. Methods followed for inoculation of pathogens on foliage, flower and root diseases are described in 3.3.1 and 3.3.2. Details of the symptoms observed after inoculation of each pathogen are described below.

4.3.1 Foliage and flower diseases

4.3.1.1 Leaf blight 1 (LB-1)

The pathogenicity test of LB-1 was done by Mycelial Bit Inoculation Method (MBIM) and Mycelial Droplet Inoculation Technique (MDIT). Among the two methods, MDIT method could establish the pathogenicity of the isolate. The result showed that the fungus could infect gerbera leaves and caused typical symptoms of disease after three days of inoculation. Initially the symptoms on leaves appeared as dark black coloured water soaked lesions which later enlarged thereby covering the entire leaf lamina.

4.3.1.2 Leaf blight 2 (LB-2)

Establishment of pathogenicity of LB-2 was carried out by MDIT though MBIM was also attempted. Initially symptoms were produced within three days of

inoculation. Small spots appeared near the margin of the leaves unlike the entire leaf lamina. Size of the lesion covered an area of 3.2 cm^2 after four days of inoculation where the lesion gradually enlarged in size causing complete blightening of leaves.

4.3.1.3 Leaf blight 3 (LB-3)

Confirmation of pathogenicity of the isolate causing LB-3 was effectively done by both MBIM and MDIT. Inoculation of the pathogen causing LB-3 was produced typical symptoms of the disease on second day of inoculation. Typical circular to oval shaped black coloured lesions were developed on the marginal area of leaves which enlarged in size and spread gradually through veinlets. Inoculation of spore suspension of 10^5 cfu/ml on live plant produced more vivid symptoms than by mycelial disc method.

4.3.1.4 Leaf spot 1 (LS-1)

MDIT and MBIM were employed for testing pathogenicity of isolate causing LS-1. Mycelial disc of the pathogen causing LS-1 was inoculated on detached leaf of gerbera. Symptoms appeared on the fourth day of inoculation as typical circular spots on leaf lamina. Similar symptoms were produced on three month old gerbera plants when plants were inoculated by MDIT method.

4.3.1.5 Leaf spot 2 (LS-2)

MBIM used for pathogenicity test showed typical symptoms of the disease. Symptoms like light to grey coloured and, circular to oval shaped lesions were produced second day after inoculation when mycelial disc of the pathogen was placed

on detached leaf. Variation in lesion size was noticed which spread all along the leaf lamina.

4.3.1.6 Powdery mildew (PM)

For proving pathogenicity of powdery mildew disease, the infected samples were inoculated onto fresh healthy leaves whereby the symptoms appeared three weeks after inoculation. The development of symptom after inoculation was very slow due to obligate nature of the pathogen. Dispersed white powdery growth was observed above the leaf lamina, thus confirmed the pathogenicity of the isolate.

4.3.1.7 Petal blight

Pathogenicity of the isolate causing petal blight was confirmed by MDIT. After inoculation by spray suspension of the isolate, typical blightening symptoms on petals were observed four days after inoculation. Symptom appeared as light brown coloured blightened streaks which were formed longitudinally along the petals. Later, the blightened portions were developed into shot holes.

4.3.2 Root diseases

4.3.2.1 Root rot

The fungal pathogen causing root rot disease were inoculated on detached leaf where symptoms like black water soaked lesions appeared on leaf lamina on third day of inoculation. Spraying of 10^5 cfu/ml spore suspension on three month old live plants also initiated typical symptoms. In live plants, symptoms initiated from the

petiole as pale water soaked lesion which later turned black and progressed further causing wilting of the entire plant.

4.3.2.2 Wilt

Pathogenicity of wilt was examined on three month old live plants following spore suspension method as mentioned in 3.3.2.1. A spore suspension of 10 ml containing 10^5 cfu/ml was poured in the rhizosphere region after providing an injury to root. Initial symptom was detected nine days after inoculation as typical yellowing of leaf lamina which gradually spread causing rotting of roots.

For every pathogens inoculated, except for obligate parasite, after typical symptom development, pathogens were reisolated from the diseased samples following the same isolation protocol mentioned earlier. The pure culture of the isolates thus obtained was transferred in PDA slants for comparison with original culture. Hence, proved the pathogenicity of each isolate separately. For obligate parasite like powdery mildew, pathogenicity was confirmed by microscopic diagnosis of the pathogens in the diseased samples.

4.4 SYMPTOMATOLOGY OF DISEASES

Studies on symptomatology of fungal diseases of gerbera were carried out both under natural and artificial conditions.

4.4.1 Symptomatology under natural condition

During the purposive sampling survey, distinct symptoms caused by various pathogens were observed in gerbera plants. Generally four types of symptoms were noticed *viz.,* foliage symptoms, root rot, wilt and flower blight.

4.4.1.1 Foliage symptoms

The major foliage symptoms observed during the survey were leaf blights, leaf spots and powdery mildew. Among the leaf blights, three types of symptoms were recorded and among leaf spots, only two types of leaf spots were noticed.

a) Leaf blight-1 (LB-1)

The initial symptoms on leaves appeared as yellow circular dots scattered on the leaf lamina which advanced rapidly to light brown to dark brown patches with concentric zonations inside the spots. Later these spots coalesced to form large lesions covering the entire leaf lamina resulting in complete blightening of the leaves [Plate 4.3 (a)].

b) Leaf blight-2 (LB-2)

Symptoms produced in LB-2 disease were similar to that produced in LB-1. Marginal leaf blightening was the common symptom observed for both LB-1 and LB-2. However, in LB-2, small, circular brown necrotic, concentric spots occurred which never scattered in leaf lamina as observed in LB-1. These spots formed near the margin of the leaf lamina later coalesced resulting in withering, extensive drying and shedding of leaves [Plate 4.3 (b)].

c) Leaf blight-3 (LB-3)

Symptom initiated as black water soaked lesions on upper surface of leaf lamina. Water soaked lesions later enlarged to form blightened areas which appeared as circular or sub circular often with grey or black coloured concentric zonations with

Plate 4.3. Symptomatology of diseases

a. Leaf blight 1

b. Leaf blight 2

c. Leaf blight 3

d. Leaf blight 4

e. Powdery mildew

f. Petal blight

a black border on the margin of the leaves [Plate 4.3 (c)]. On older lesions, dark sporodochia surrounded by white hyphal tuft were observed.

d) Leaf spot-1 (LS-1)

Leaf spot-1 was observed on matured leaves where the disease symptom was initiated by development of small yellow spots analogous to symptom development of *Alternaria* leaf spot. Symptoms appeared as circular, pale to dark brown necrotic spots with definite borders scattered over the leaf lamina. Each individual spot appeared as deep sunken necrotic spots, delimited by major veins and further coalesced to form large blightened areas [Plate 4.3 (d)].

e) Leaf spot-2 (LS-2)

Initially the symptoms of LS-2 developed as small, yellow-brown flecks, often with a light green halo on the upper surface of the leaves. Later, the spots turned grey coloured, circular to oval with chlorotic irregular patches on the entire leaf lamina.

f) Powdery mildew

Symptoms appeared as distinct white powdery mould on the upper surface of leaf lamina. These spots later enlarged to form white powdery mat which gradually turned pale yellow to brown [Plate 4.3 (e)]. It was observed that immature leaves were severely affected compared to mature ones leading to complete death of the plant.

4.4.1.2 Flower blight

Flower or petal blight was the only floral disease observed during the survey. Coloured, soft petals turned pale brown due to the infection by the pathogen. Shot holes were also noticed in the necrotic centre of the blightened portion of the petal [Plate 4.3 (f)].

4.4.1.3 Root disease

a) Root rot

Stem or collar rot resulted in fatal infection with complete death of the plant. Initially a dark brown lesion appeared on the stem through collar portion which later extended upto the root hairs [Plate 4.3 (g)]. Foliar yellowing and defoliation were the general aerial symptoms noticed in the affected plants.

b) Wilt

Dark brown discoloration appeared on fine lateral roots which gradually spread to the main tap root. The aerial symptoms due to wilt were that of foliar yellowing and sometimes with black water soaked lesion and later defoliation. During advanced stages of infection, complete decaying and disintegration of internal tissues were observed [Plate 4.3 (h].

4.4.2 Symptomatology under artificial condition

Artificial inoculation of all the fungal pathogens isolated from various locations was done for studying symptomatology under artificial condition as per the protocol mentioned in 3.3.1 and 3.3.2. Development of symptoms under artificial inoculation was similar to that observed in field under natural conditions. Lesion size

and time for symptom development after inoculation of pathogens are shown in Table 4.12.

On leaves, the pathogen causing LB-1 produced initial symptoms within 48h with a lesion size of 1.2 cm^2 and full development of symptoms occurred within 6 to 8 days with lesion size ranging from 6.1-7.8 cm^2. The infected leaves defoliated within one to two weeks on live plants. In LB-2, the pathogen initiated the disease symptoms only within three days of inoculation with a lesion size of 2.4 cm^2 on the fourth day. Likewise in LB-3, the pathogen developed symptoms two days after inoculation with a minute lesion size of 0.8 cm^2 which gradually enlarged and attained a lesion size 8.4 cm^2 on the 10^{th} day of inoculation. Leaf spot 1 (LS-1) showed first symptom of disease development after 3^{rd} day of inoculation with 0.4 cm^2 sized lesion on leaf surface. Similarly, leaf spot 2 (LS-2) also showed early symptoms on leaf surfaces on third day of inoculation where size of lesion varied from 1.4 cm^2 to 2.8 cm^2 as the disease progressed upto 10 days after inoculation. Powdery mildew disease took 18 days to initiate symptom after inoculation, and later the leaves turned necrotic.

Pathogen causing root rot produced typical black coloured lesions on both leaves and roots after four days of inoculation where the size of the lesion formed on leaf lamina later enlarged from 3.6 to 8.2 cm^2 at 10 days after inoculation. Wilt disease causing pathogens showed late response of symptom development on the crop. Initial symptom appeared only after eight days of incubation and lesion size on leaf lamina measured 3.8 cm^2 on 10^{th} days of inoculation.

4.5 CHARACTERISATION AND IDENTIFICATION OF PATHOGEN

The fungal pathogens isolated from different diseased samples from different locations were subjected to cultural and morphological studies for characterisation and thereby identification of the isolates. A detailed description regarding each isolate is presented below.

4.5.1 Cultural and morphological characters

4.5.1.1 Leaf blight-1 (Alternaria sp.*)*

The pathogen causing LB-1 was observed in Madakkathara and Vellanikkara of Thrissur district as well as Chulliyode of Wayanad district. The fungus produced black fluffy growth of mycelia in PDA medium with white bordered periphery which attained full growth in Petri plate by 12 days after inoculation [Plate 4.4 (a)]. Reverse of the culture in Petri dish was found dark black in colour. Conidiophores cylindrical, scattered or gregarious, pale grey yellow, straight or curved, geniculate, simple or branched. Spores olivaceous to dark brown coloured with varied shape from obclavate to mostly ellipsoidal, muriform having tapered apex with 1-3 longitudinal and 2-10 transverse septa having 22.76-60.90 µm x 6.45-13.01 µm dimension [Plate 4.5 (a)]. Based on these cultural and morphological characters, the fungal pathogen was confirmed as *Alternaria* sp.

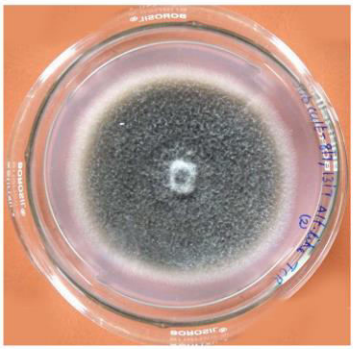
4.5.1.2 Leaf blight-2 (Alternaria sp.*)*

The disease LB-2 was prevalent in all districts, which were surveyed. Mycelia developed aerial hyphae on greyish white colonies which later turned olive-green to black in PDA, exhibiting dense growth in the media [Plate 4.4 (b)]. The size of

g. Root rot

h. Wilt

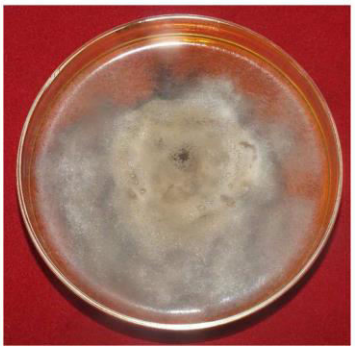
Plate 4.4. Cultural characters of pathogens



a. ***Alternaria alternata***

b. ***Alternaria tenuissima***

c. ***Myrothecium roridum***



d. ***Ulocladium chartarum***

conidia varied from 23.86 to 48·69 µm long and 7.34 to 16·32 µm wide with olivaceous to dark brown colour. Horizontal and vertical septations of conidia varied from 1-8 and 0-2 respectively [Plate 4.5 (b)]. Conidia were significantly large. Based on these characters this isolate was also identified as *Alternaria* sp.

4.5.1.3 Leaf blight-3 (Myrothecium sp.*)*

The pathogen causing LB-3 disease was observed in hydroponics unit maintained in CoH, Vellanikkara. The colony colour of the isolates varied from white, floccose, concentric-ringed with irregular shapes of dark green to black sporodochia [Plate 4.4 (c)]. Conidiophores 2-4 branches at each node while phialides were hyaline, cylindrical, in whorls of 3-5 and measured 13 to 16 × 2.0 µm. Conidia hyaline, one-celled, rod-shaped with rounded ends and measured 5 to 10.74 × 2.0 µm [Plate 4.5 (c)]. Based on these characters, the pathogen was identified as *Myrothecium* sp.

4.5.1.4 Leaf spot-1 (Ulocladium sp.*)*

The disease LS-1 was confined to gerbera grown in polyhouses observed only in Thrissur district of Chalakudy and Madakkathara area. The pathogen initiate the growth from diseased sample in PDA as pure white mycelial growth which later transformed into typical yellow to dark orange brown pigmentation in PDA media with greyish white mycelial growth [Plate 4.4 (d)]. Spores obovoid, non-beaked and produced olivaceous to dark brown coloured conidia having dimensions ranging from 26.54-51.54 µm x 15.16-40.24 µm [Plate 4.5 (d)].. These characteristics confirm the identity as *Ulocladium* sp.

Table 4.12. Differential response of artificial inoculation of fungal pathogens on gerbera plants

Sl. No.	Symptom	Lesion size (cm^2)					Days of first symptom development
		Days after inoculation					
		2	**4**	**6**	**8**	**10**	
1.	Leaf blight 1	1.2	3.4	6.1	7.2	7.8	2
2.	Leaf blight 2	0	2.4	3.6	5.7	6.8	3
3.	Leaf blight 3	0.8	2.2	3.9	6.1	8.4	2
4.	Leaf spot 1	0	0.4	1	1.5	2.3	3
5.	Leaf spot 2	0	1.4	2.0	2.4	2.8	4
6.	Wilt	0	0	0	0	3.8	9
7.	Root rot	0	3.6	5.8	7.1	8.2	8
8.	Powdery mildew	-	-	-	-	-	18

4.5.1.5 Leaf spot-2 (Curvularia sp.*)*

The disease LS-2 was observed in Anakkayam of Malappuram district. Colonies of the isolate was effuse, producing grey mycelium in PDA mediated Petri plates which later turned black [Plate 4.4 (e)]. Mycelia on maturity turned dark brown coloured, septate and produced anastomosis structures which form sympodially proliferating conidiogenous cells. Conidia three septated, slightly curved measuring 12-28 x 6-12 µm of conidia. Based on these cultural and morphological characters, the isolate was identified as *Curvularia* sp.

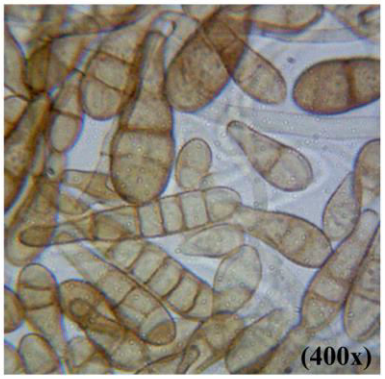
4.5.1.6 Powdery mildew (Erysiphe sp. *and Podosphaera* sp.*)*

Powdery mildew was the most severe disease observed during the survey in the high range zone, Ambalavayal of Wayanad district. Two varied type of spores of powdery mildew PM-1 and PM-2 were identified from the diseased samples collected from Wayanad district. PM-1 produced hyaline, septate mycelia with globose conidia with irregular peripheral end formed in a chain and those causing PM-2 produced superficial, hyaline, coenocytic mycelium with oval or ellipsoidal, catenate conidia with dimension ranging from 22.1-30.18 x 13.36-18.08µm formed in unbranched erect conidiophores. Based on these characters, PM-1 was identified as *Golovinomyces cichoracearum* (*Erysiphe cichoracearum*) and PM-2 as *Podosphaera* sp. [Plate 4.5 (e)], [Plate 4.5 (f)].

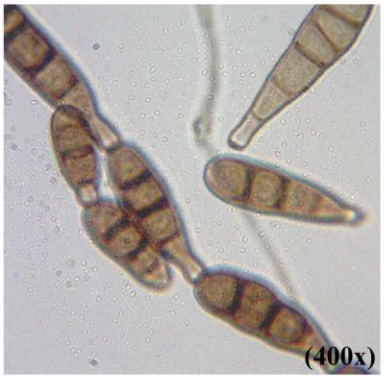
4.5.1.7 Petal blight (Curvularia sp.*)*

The extent of intensity of floral blight in gerbera being very sparse was observed in Anakkayam and Ambalavayal regions of Malappuram and Wayanad districts. The colony colour of the isolate was dark, velvety, rapid growing with thin and suppressed growth on PDA which completed full growth in Petri plate within 12 days

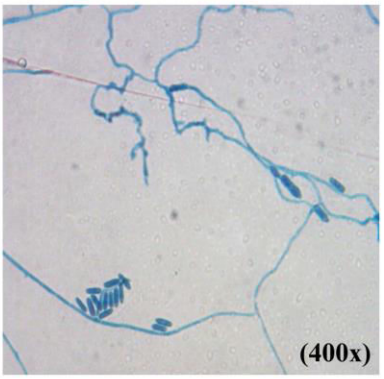
Plate 4.5. Morphological characters of pathogens



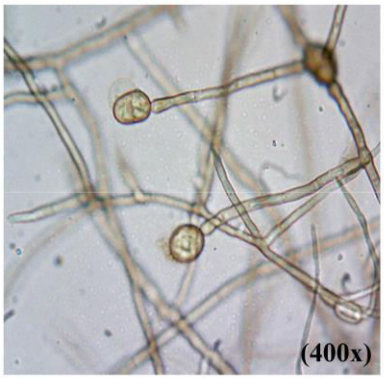
a. ***Alternaria alternata***



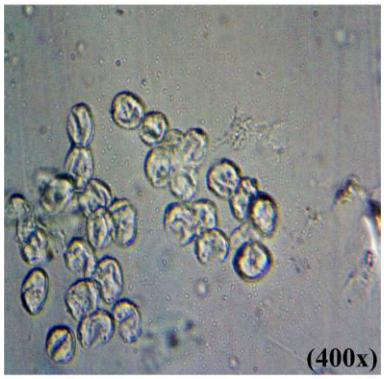
b. ***Alternaria tenuissima***



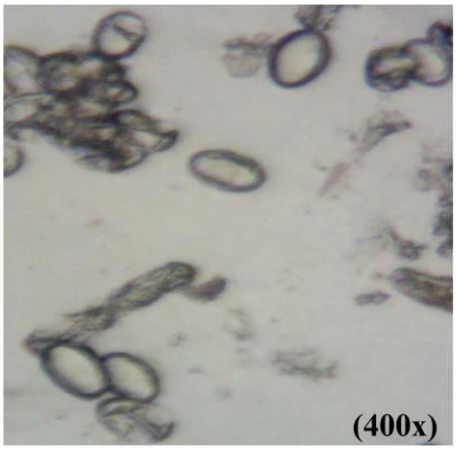
c. ***Myrothecium roridum***



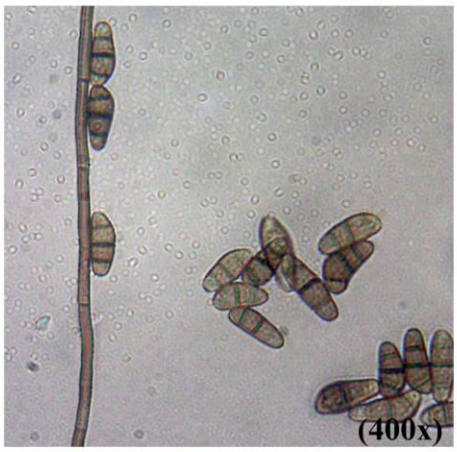
d. ***Ulocladium chartarum***



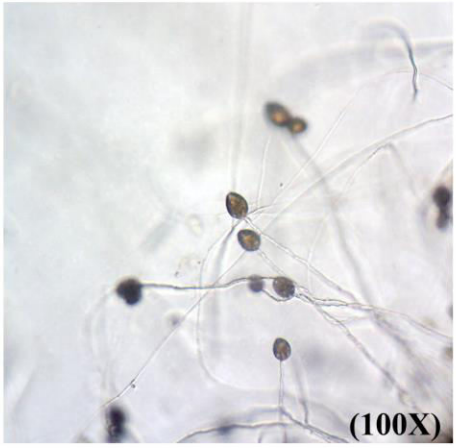
e. ***Golovinomyces cichoracearum***



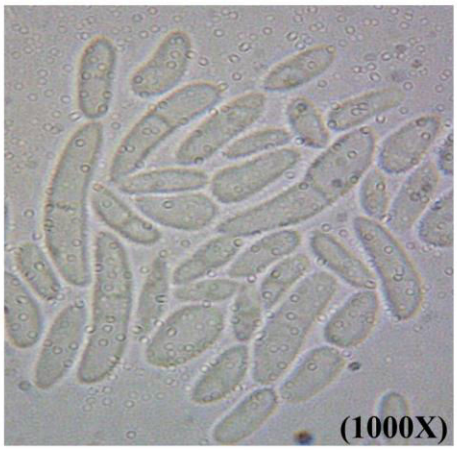
f. *Podosphaera sp.*



g. *Curvularia lunata*



h. *Phytophthora cryptogea*



i. *Fusarium solani*

[Plate 4.4 (f)]. The length and breadth of the conidia of the isolate ranged from 20–32 × 9–15 µm with 3 septa formed in geniculate growth manner in pale brown septate conidiophore. Basal and apical cells of the conidia was pale brown leaving the other cells brown or dark brown coloured with smooth, curved at third cell from base [Plate 4.5 (g)]. Based on these characters the isolate was identified as *Curvularia* sp.

4.5.1.8 Root rot (Phytophthora sp.*)*

The disease was observed in gerbera grown under hydroponic system maintained at CoH Vellanikkara, where the whole plants parts were severely infected. The pathogen isolated on PDA media which formed white cottony growth [Plate 4.4 (g)]. Hyphae were branched, hyaline, coenocytic with sporangia oval to obpyriform shaped, nonpapillate, borne either terminally or laterally on the sporangiophores in a simple sympodial fashion. Dimension of sporangia ranged from 32.5-57.5 x 25-35 µm [Plate 4.5 (h)]. Full growth of the culture was attained in Petri plate by eight days. Based on these characters the isolate was confirmed as *Phytophthora* sp.

4.5.1.9 Wilt (Fusarium sp.*)*

The disease was observed in the polyhouses of Chalakudy area of Thrissur district. The isolate produced light pinkish aerial mycelium which attained full growth in PDA mediated Petri dish within nine days [Plate 4.4 (h)]. Pathogen typically produced both macro- and microconidia from slender phialides. Macroconidia hyaline, two to several-celled, fusiform to sickle-shaped, mostly with an elongated apical cell and pedicellate basal cell of size 28-42 x 4-6 µm.

Table 4.13. Identification of fungal pathogens

Sl No.	Symptom	Pathogen	ID. No.
1.	Leaf blight -1 (LB-1)	*Alternaria alternata*	7915.15
2.	Leaf blight -2 (LB-2)	*Alternaria tenuissima*	7946.15
3.	Leaf blight -3 (LB-3)	*Myrothecium roridum*	7948.15
4.	Leaf spot -1 (LS-1)	*Ulocladium chartarum*	7945.15
5.	Leaf spot -2 (LS-2)	*Curvularia pallescens*	7947.15
6.	Powdery mildew -1 (PM-1)	*Golovinomyces cichoracearum*	8046.16
7.	Powdery mildew -2 (PM-2)	*Podosphaera* sp.	8047.16
8.	Petal blight	*Curvularia lunata*	7949.15
9.	Root rot	*Phtyophthora cryptogea*	7950.15
10.	Wilt	*Fusarium solani*	8045.16

Microconidia one or two-celled, hyaline, smaller than macroconidia, pyriform, fusiform to ovoid, straight or curved with 8-16 x 2-4.5 µm dimension [Plate 4.5 (i)]. Based on the above mentioned characteristics the pathogen was identified as *Fusarium* sp.

Based on the above cultural and morphological characters of the pathogens studied, coupled with symptomatology and pathogenicity, the pathogens could be identified upto the genus level. Further confirmation of the isolates were carried out at National Center for Fungal Taxonomy (NCFT), New Delhi where the cultures were maintained in the depository with proper identification numbers (ID. No.) (Table 4.13).

4.6 *In vitro* EVALUATION OF FUNGICIDES AND BIOAGENTS AGAINST PATHOGENS

Under *in vitro* condition, the efficacy of different chemicals and biocontrol agents were evaluated against different pathogens isolated from gerbera. The protocol used for *in vitro* evaluation of pathogens against fungicides was poison food technique and those with bioagents was by dual culture technique respectively as described in 3.6.1 and 3.6.2. Details of the experiment are presented in Table 4.7, 4.8, 4.9 and 4.10.

4.6.1 *In vitro* evaluation of fungicides

The pathogens *viz., Alternaria tenuissima, Alternaria alternata, Myrothecium roridum, Ulocladium chartarum, Curvularia pallescens, Curvularia lunata, Fusarium solani* and *Phytophthora cryptogea* were tested against three concentrations of nine fungicides. Inhibition of mycelial growth of different pathogens varied significantly with different fungicides at different concentrations.

4.6.1.1 Alternaria alternata

The data revealed that fungicides *viz.,* difenoconazole 25EC (Score), propineb 70WP (Antracol), hexaconazole 5EC (Mega master) and tebuconazole 250EC (Folicur) at all three concentrations and the highest concentration of Saaf at 0.15 per cent were found promising against *Alternaria alternata* as they showed cent per cent inhibition against the pathogen (Table 4.14). Other chemicals like cymoxanil 8% + mancozeb 64% (Curzate M-8) showed 66.10, 71.11 and 74.99 per cent inhibiton of the pathogen at concentrations of 0.02, 0.05 and 0.1 per cent respectively. Pyraclostrobin 20WG (Headline) at all concentration showed an inhibition of above 80 per cent. Similar trend of inhibition was observed for the three concentrations of carbendazim 12% + mancozeb 63% (Saaf) with 71.66 to 100 per cent inhibition. Likewise, all the concentrations of copper hydroxide 77WP (Kocide) inhibited *Alternaria alternata* from 79.43 to 81.66 per cent (Plate 4.6).

4.6.1.2 Alternaria tenuissima

Cent per cent inhibition of *Alternaria tenuissima* was achieved by chemicals like carbendazim 12% + mancozeb 63% (Saaf), cymoxanil 8% + mancozeb 64% (Curzate M-8), hexaconazole 5EC (Mega master), tebuconazole 250EC (Folicur), difenoconazole 25EC (Score) at all three concentrations (Table 4.14). Complete inhibition of the pathogen by propineb 70WP (Antracol) was noticed at the highest concentration of 0.35 per cent whereas as an inhibition of above 80 per cent was observed at 0.3 per cent and 0.25 per cent of the fungicide. Pyraclostrobin 20WG (Headline) inhibited the pathogen ranging from 73.33 to 81.11 per cent at three different concentrations of 0.5, 0.1 and 0.15 per cent. Copper hydroxide 77WP (Kocide) at all concentrations could inhibit the pathogen above 70 per cent (Plate 4.7).

Plate 4.6.*In vitro* evaluation of fungicides against *Alternaria alternata*

a. Propineb 70WP

b. Difenoconazole 25EC

Plate 4.7.*In vitro* evaluation of fungicides against *Alternaria tenuissima*

a. Copper hydroxide 77WP

b. Cymoxanil 8% + Mancozeb 64%

Plate 4.8.*In vitro* evaluation of fungicides against *Myrothecium roridum*

a. Bordeaux mixture

b. Pyraclostrobin 20WG

4.6.1.3 Myrothecium roridum

From the data in Table 4.14, it was evident that the fungicides *viz.,* propineb 70 WP (Antracol), cymoxanil 8% + mancozeb 64% (Curzate M-8), pyraclostrobin 20WG (Headline), carbendazim 12% + mancozeb 63% (Saaf), copper hydroxide 77WP (Kocide), difenoconazole 25EC (Score), tebuconazole 250EC (Folicur), Bordeaux mixture except hexaconazole 5EC (Mega master) at all the three concentrations showed cent per cent inhibition of the pathogen. Hexaconazole 5EC showed a per cent inhibition more than 70 per cent at all three concentrations (Plate 4.8).

4.6.1.4 Ulocladium chartarum

Among the nine fungicides tested (Fig.4.1 to 4.9), six chemicals showed completly inhibited the mycelial growth of the pathogen, *Ulocladium chartarum.* Chemicals *viz.,* carbendazim 12% + mancozeb 64% (Saaf) , cymoxanil 8% + mancozeb 64% (Curzate M-8), pyraclostrobin 20WG (Headline), tebuconazole 250EC (Folicur), copper hydroxide 77WP (Kocide) and propineb 70WP (Antracol) at all the three concentrations tested recorded 100 per cent inhibition (Table 4.15). Hexaconazole 5EC (Mega master) at 0.05, 0.1 and 0.15 per cent showed an inhibition ranging from 55.92 to 68.62 per cent inhibition whereas difenoconazole 25EC (Score) at 0.02, 0.05 and 0.1 per cent recorded an inhibition of 52.21, 69.62 and 74.07 per cent respectively. Moreover, Bordeaux mixture at all the three concentrations showed an inhibition of above 75 per cent only (Plate 4.9).

Table 4.14. *In vitro* evaluation of fungicides against *Alternaria tenuissima, Alternaria alternata* and *Myrothecium roridum*

Sl.No.	Fungicide	Conc. (%)	*Per cent Inhibition		
			Alternaria alternata	*Alternaria tenuissima*	*Myrothecium roridum*
1.	Carbendazim 12% +Mancozeb 63% (Saaf)	0.15	71.66 (8.47)^{jk}	100 (10)^{a}	100 (10)^{a}
		0.2	81.11 (9.0)^{e}	100 (10)^{a}	100 (10)^{a}
		0.25	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
2.	Cymoxanil 8% +Mancozeb 64% (Curzate M-8)	0.15	66.10 (8.13)^{l}	100 (10)^{a}	100 (10)^{a}
		0.2	71.11 (8.43)^{j}	100 (10)^{a}	100 (10)^{a}
		0.25	74.99 (8.66)^{i}	100 (10)^{a}	100 (10)^{a}
3.	Propineb (Antracol 70WP)	0.25	100 (10)^{a}	79.99 (8.94)^{d}	100 (10)^{a}
		0.3	100 (10)^{a}	82.77 (9.09)^{b}	100 (10)^{a}
		0.35	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
4.	Pyraclostrobin (Headline 20WG)	0.05	80.55 (8.98)^{fg}	73.33 (8.56)^{i}	100 (10)^{a}
		0.1	83.85 (9.16)^{b}	78.32 (8.85)^{e}	100 (10)^{a}
		0.15	83.85(9.16)^{c}	81.11 (9.01)^{c}	100 (10)^{a}
5.	Copper hydroxide (Kocide 77WP)	0.15	79.43 (8.91)^{h}	73.88(8.60)^{h}	100 (10)^{a}
		0.2	80.55 (8.98)^{f}	76.44 (8.74)^{g}	100 (10)^{a}
		0.25	81.66 (9.04)^{d}	78.32 (8.85)^{f}	100 (10)^{a}
6.	Hexaconazole (Mega master 5EC)	0.05	100 (10)^{a}	100 (10)^{a}	73.33 (8.56)^{d}
		0.1	100 (10)^{a}	100 (10)^{a}	78.32 (8.85)^{b}
		0.15	100 (10)^{a}	100 (10)^{a}	76.11 (8.72)^{c}
7.	(Folicur 250EC) Tebuconazole	0.1	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
		0.15	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
		0.2	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
8.	Difenoconazole (Score 25EC)	0.02	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
		0.05	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
		0.1	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
9.	Bordeaux mixture	0.5	39.44 (6.28)^{o}	57.77 (7.60)^{l}	100 (10)^{a}
		1	44.99 (6.71)^{n}	61.66 (7.85)^{k}	100 (10)^{a}
		1.5	46.66 (6.83)^{m}	66.10 (8.13)^{j}	100 (10)^{a}
	CD (0.05)		0.906	0.79	1.28

***** Mean of the three replications

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

$\sqrt{x} + 0.5$ transformed values are given in parantheses

4.6.1.5 Curvularia pallescens

The pathogen was tested against all the nine fungicides (Fig.4.1 to 4.9), and the result revealed that all the fungicides except Bordeaux mixture and copper hydroxide 77WP (Kocide) completely inhibited the mycelial growth at all three concentrations evaluated. Both the copper fungicides at all three concentrations could inhibit the pathogen ranging from 72.59 to 78.94 per cent (Table 4.15) (Plate 4.10).

4.6.1.6 Curvularia lunata

Most of the chemicals *viz.,* cymoxanil 8% + mancozeb 64% (Curzate M-8), propineb 70WP (Antracol), hexaconazole 5EC (Mega master), tebuconazole 250EC (Folicur), copper hydroxide 77WP (Kocide) at all three concentrations exhibited 100 per cent inhibition of the pathogen (Table 4.16) (Fig.4.1 to 4.9). Also, carbendazim 8% + mancozeb 63% (Saaf) and pyraclostrobin 20WG (Headline) at the higher two concentrations showed cent per cent inhibition. Difenoconazole 25EC (Score) at 0.02, 0.05 and 0.1 per cent concentration recorded 81.66, 84.44 and 85.66 per cent inhibition respectively (Plate 4.11).

4.6.1.7 Phytophthora cryptogea

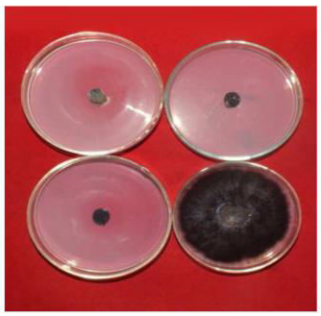
In vitro evaluation of six chemicals *viz.,* propineb 70WP (Antracol), cymoxanil 8% + mancozeb 64% (Curzate M-8), carbendazim 12% + mancozeb 63% (Saaf), copper hydroxide 77WP (Kocide), tebuconazole 250EC (Folicur) and Bordeaux mixture at all three concentrations showed cent per cent inhibition of the pathogen (Table 4.16) (Fig.4.1 to 4.9). Apart from that, the higher concentrations of hexaconazole 5EC (Mega master) at 0.1 and 0.15 per cent and the maximum concentration of pyraclostrobin 20WG (Headline) showed 100 per cent inhibition.

Plate 4.9. *In vitro* evaluation of fungicides against *Ulocladium chartarum*

a. Propineb 70WP

b. Cymoxanil 8% + Mancozeb 64%

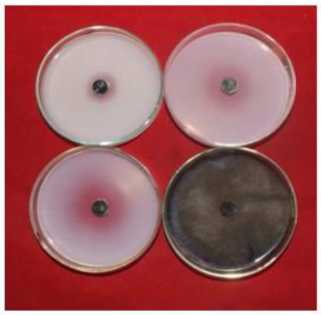
Plate 4.10.*In vitro* evaluation of fungicides against *Curvularia pallescens*



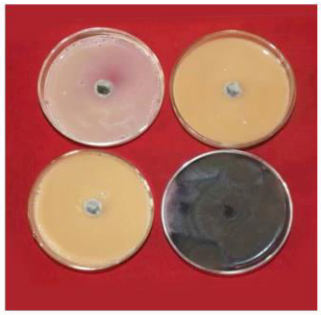
a. Hexaconazole 5EC

b. Carbendazim 12% + Mancozeb 63%

Plate 4.11.*In vitro* evaluation of fungicides against *Curvularia lunata*



a. Tebuconazole 250EC



b. Pyraclostrobin 20WG

Table 4.15 *In vitro* evaluation of fungicides against *Ulocladium chartarum* and *Curvularia pallescens*

Sl.No.	Fungicide	Conc. (%)	*Per cent Inhibition	
			Ulocladium chartarum	*Curvularia pallescens*
1.	Carbendazim 12% +Mancozeb 63% (Saaf)	0.15	100 (10)[a]	100 (10)[a]
		0.2	100 (10)[a]	100 (10)[a]
		0.25	100 (10)[a]	100 (10)[a]
2.	Cymoxanil 8% +Mancozeb 64% (Curzate M-8)	0.15	100 (10)[a]	100 (10)[a]
		0.2	100 (10)[a]	100 (10)[a]
		0.25	100 (10)[a]	100 (10)[a]
3.	Propineb (Antracol 70WP)	0.25	100 (10)[a]	100 (10)[a]
		0.3	100 (10)[a]	100 (10)[a]
		0.35	100 (10)[a]	100 (10)[a]
4.	Pyraclostrobin (Headline 20WG)	0.05	100 (10)[a]	100 (10)[a]
		0.1	100 (10)[a]	100 (10)[a]
		0.15	100 (10)[a]	100 (10)[a]
5.	Copper hydroxide (Kocide 77WP)	0.15	100 (10)[a]	73.88 (8.59)[f]
		0.2	100 (10)[a]	76.38 (8.74)[d]
		0.25	100 (10)[a]	78.94 (8.88)[b]
6.	Hexaconazole (Mega master 5EC)	0.05	55.92[i]	100 (10)[a]
		0.1	58.14[h]	100 (10)[a]
		0.15	68.62[g]	100 (10)[a]
7.	(Folicur 250EC) Tebuconazole	0.1	100 (10)[a]	100 (10)[a]
		0.15	100 (10)[a]	100 (10)[a]
		0.2	100 (10)[a]	100 (10)[a]
8.	Difenoconazole (Score 25EC)	0.02	52.21[j]	100 (10)[a]
		0.05	69.62[f]	100 (10)[a]
		0.1	74.07[e]	100 (10)[a]
9.	Bordeaux mixture	0.5	75.55[d]	72.59 (8.52)[g]
		1	76.66[c]	74.42 (8.62)[e]
		1.5	79.99[b]	77.25 (8.78)[c]
	CD		0.567	0.636

*Mean of the three replications
In each column figure followed by same letter do not differ significantly according to DMRT.
$\sqrt{x} + 0.5$ transformed values are given in parantheses

Difenoconazole 25EC (Score) at all three concentration *viz.,* 0.02, 0.05 and 0.1 per cent showed comparatively lesser inhibition to the pathogen with 39.16, 46.44 and 60.55 per cent inhibition respectively (Plate 4.12).

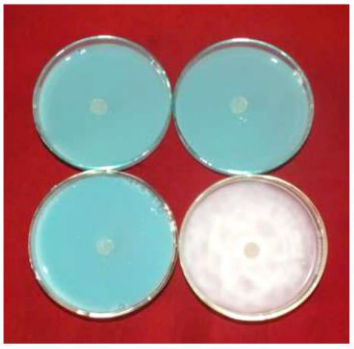
4.6.1.8 Fusarium solani

Cent per cent inhibition of *Fusarium solani* was observed at all three concentrations of carbendazim 12% + mancozeb 63% (Saaf) (0.05, 0.1, 0.15%), pyraclostrobin 20WG (Headline) (0.05, 0.1, 0.15%), and tebuconazole 250EC (Folicur) (0.05, 0.1, 0.15%) (Table 4.17) (Fig.4.1 to 4.9). Other chemicals like cymoxanil 8% + mancozeb 64% (Curzate M-8), hexaconazole 5EC (Mega master) and propineb 70WP (Antracol) showed very poor inhibition of the pathogen. An inhibition of 58.33 per cent was noticed with 0.15 per cent of Curzate and 71.11 per cent inhibition with the highest concentration of 0.25 per cent of the fungicide. Hexaconazole (Mega master) showed 61.66 to 71.11 per cent inhibition for the lowest concentration of 0.05 and the highest concentration of 0.15 per cent respectively (Plate 4.13).

4.6.2 *In vitro* evaluation of bio agents

Fungal antagonist, *Trichoderma viride* and bacterial antagonist, *Pseudomonas fluorescens* were tested against eight fungal pathogens isolated from gerbera and is presented in Table 4.17 and Table 4.18 respectively. The protocol followed for evaluation of biocontrol agents has been detailed in 3.6.2.1 and 3.6.2.2.

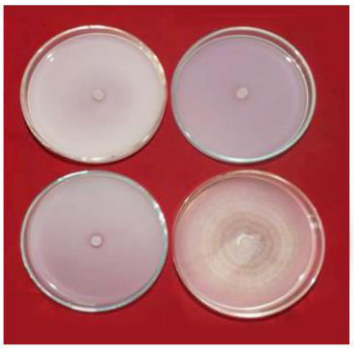
Plate 4.12.*In vitro* evaluation of fungicides against *Phytophthora cryptogea*



a. Carbendazim 12% + Mancozeb 63%

b. Cymoxanil 8% + Mancozeb 64%

Plate 4.13.*In vitro* evaluation of fungicides against *Fusarium solani*



a. Tebuconazole 250EC

b. Pyraclostrobin 20WG

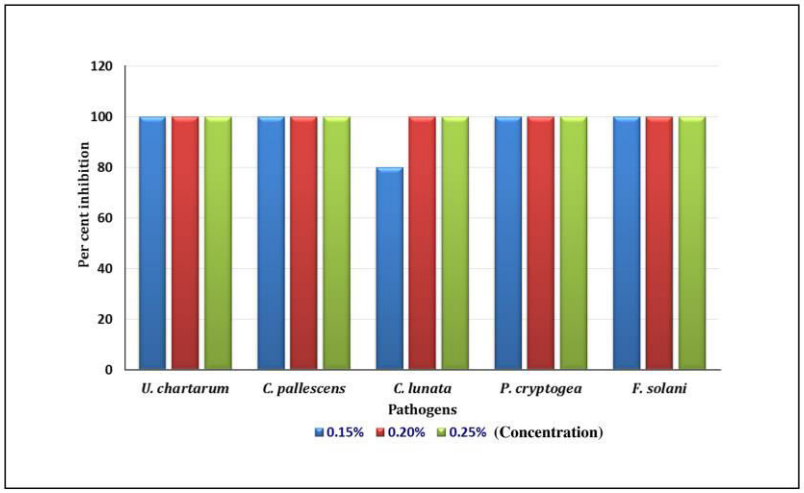


Fig 4.1 Efficacy of carbendazim 12% + mancozeb 63% against leaf spot, petal blight & root pathogens

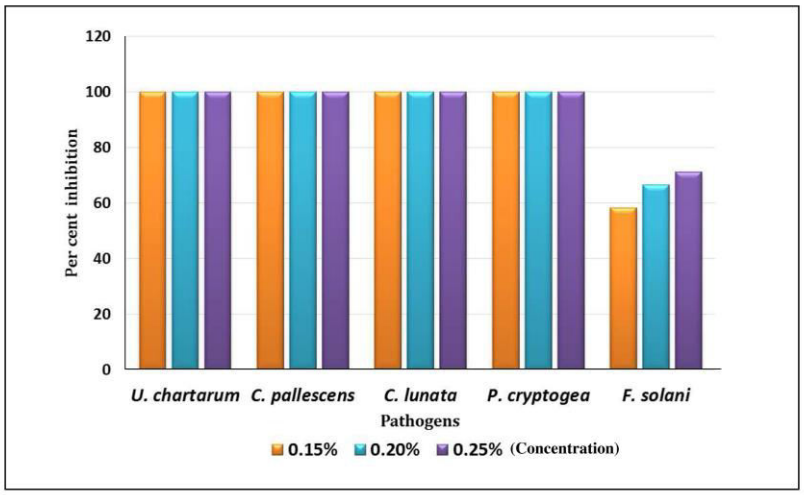


Fig 4.2 Efficacy of cymoxanil 8% + mancozeb 64% against leaf spot, petal blight & root pathogens

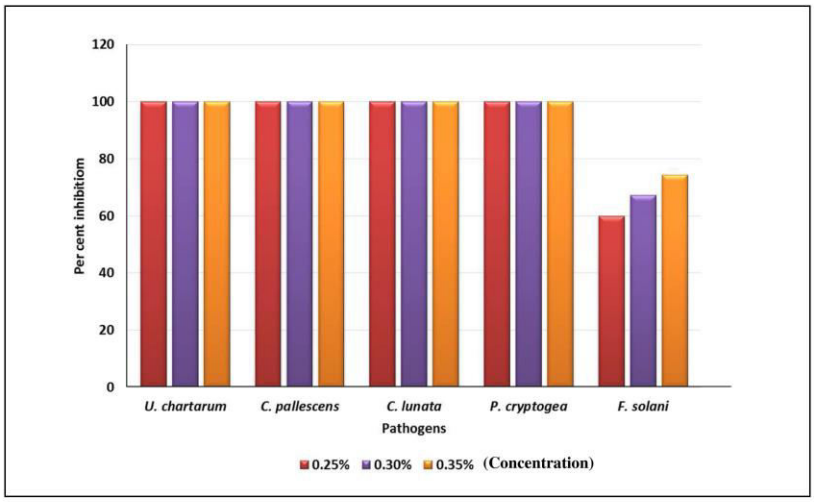


Fig 4.3 Efficacy of propineb 70WP against leaf spot, petal blight & root pathogens

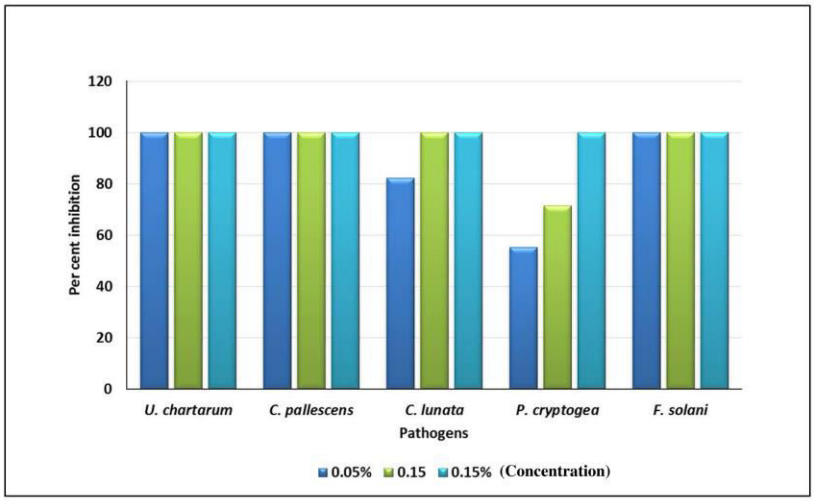


Fig 4.4 Efficacy of pyraclostrobin 20WG against leaf spot, petal blight & root pathogens

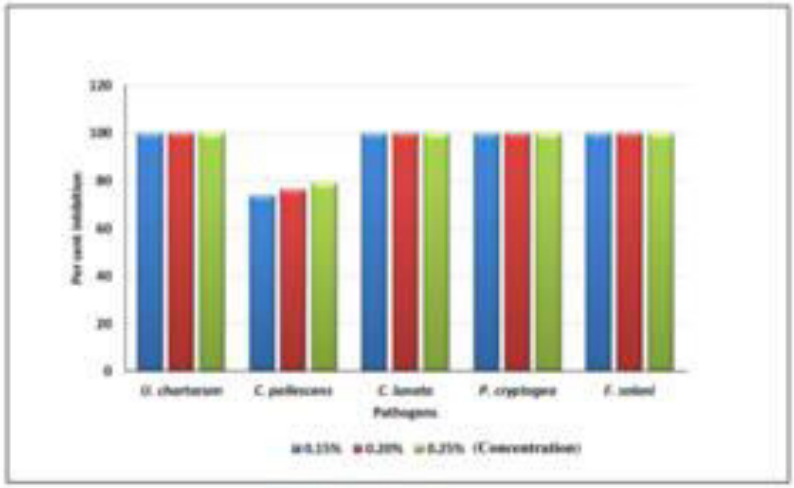


Fig 4.5 Efficacy of copper hydroxide 77WP against leaf spot, petal blight & root pathogens

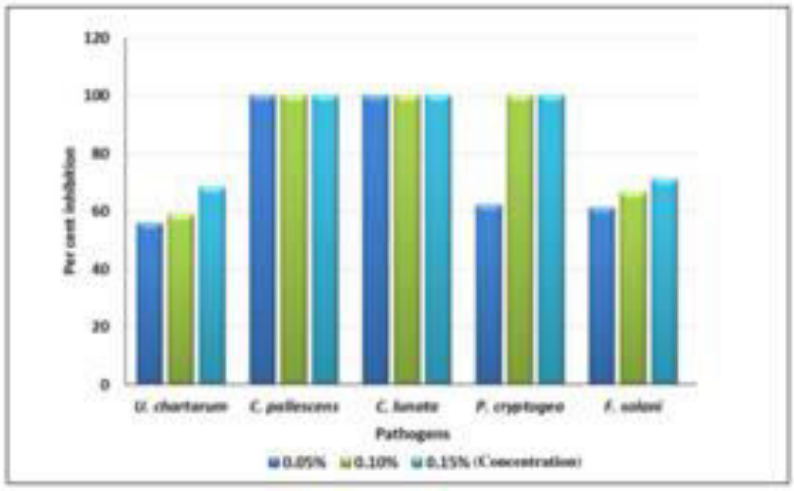


Fig 4.6 Efficacy of hexaconazole 5EC against leaf spot, petal blight & root pathogens

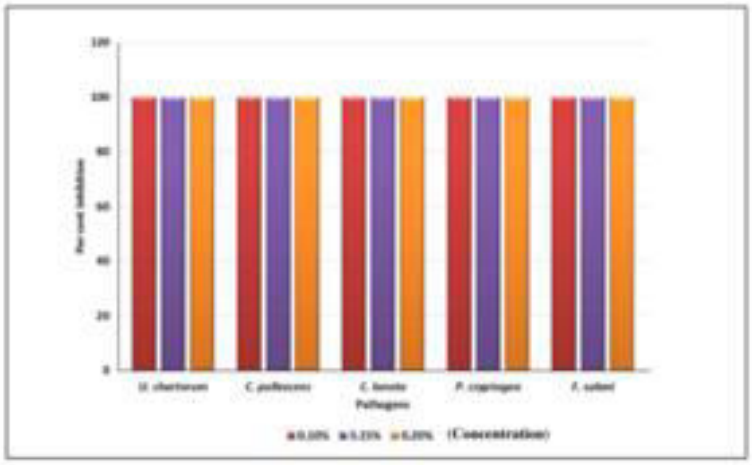


Fig 4.7 Efficacy of tebuconazole 250EC against leaf spot, petal blight & root rot pathogens

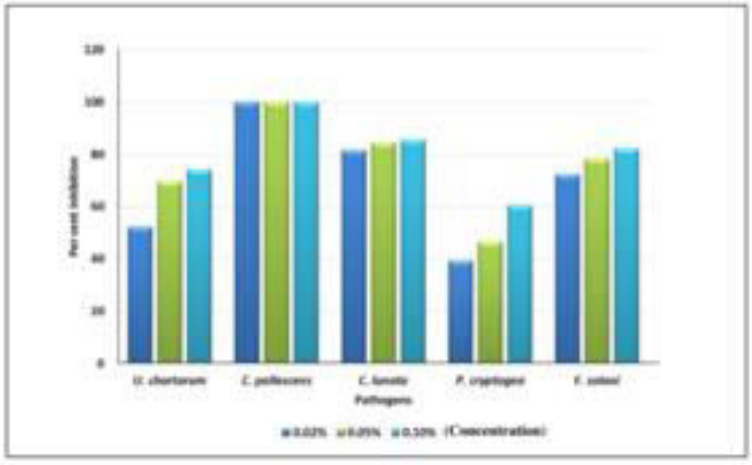


Fig 4.8 Efficacy of difenoconazole 25EC against leaf spot, petal blight & root rot pathogens

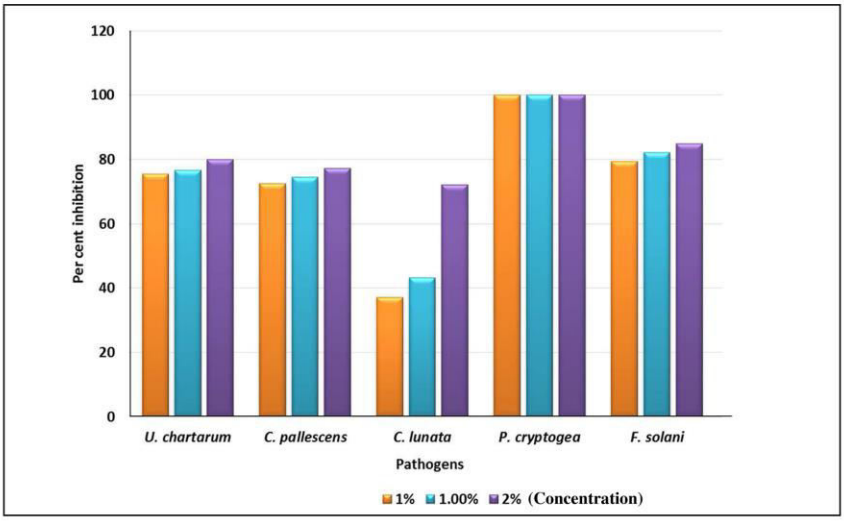


Fig 4.9 Efficacy of Bordeaux mixture against leaf spot, petal blight & root rot pathogens

Table 4.16. *In vitro* evaluation of fungicides against *Phytophthora cryptogea, Fusarium solani* and *Curvularia lunata*

Sl.No.	Fungicide	Conc. (%)	*Per cent Inhibition		
			Curvularia lunata	*Phytophthora cryptogea*	*Fusarium solani*
1.	Carbendazim 12% +Mancozeb 63% (Saaf)	0.15	80.88 (8.99)^{f}	100 (10)^{a}	100 (10)^{a}
		0.2	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
		0.25	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
2.	Cymoxanil 8% +Mancozeb 64% (Curzate M-8)	0.15	100 (10)^{a}	100 (10)^{a}	58.33 (7.64)^{m}
		0.2	100 (10)^{a}	100 (10)^{a}	66.66 (8.16)^{j}
		0.25	100 (10)^{a}	100 (10)^{a}	71.11 (8.43)^{hi}
3.	Propineb (Antracol 70WP)	0.25	100 (10)^{a}	100 (10)^{a}	59.99 (7.75)^{l}
		0.3	100 (10)^{a}	100 (10)^{a}	67.22 (8.20)^{i}
		0.35	100 (10)^{a}	100 (10)^{a}	74.44 (8.63)^{f}
4.	Pyraclostrobin (Headline 20WG)	0.05	82.22 (9.06)^{d}	55.33 (7.44)^{e}	100 (10)^{a}
		0.1	100 (10)^{a}	71.56 (8.46)^{b}	100 (10)^{a}
		0.15	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
5.	Copper hydroxide (Kocide 77WP)	0.15	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
		0.2	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
		0.25	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
6.	Hexaconazole (Mega master 5EC)	0.05	100 (10)^{a}	63.23 (7.95)^{c}	61.66 (7.85)^{k}
		0.1	100 (10)^{a}	100 (10)^{a}	66.66(8.16)^{ij}
		0.15	100 (10)^{a}	100 (10)^{a}	71.11 (8.43)^{h}
7.	(Folicur 250EC) Tebuconazole	0.1	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
		0.15	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
		0.2	100 (10)^{a}	100 (10)^{a}	100 (10)^{a}
8.	Difenoconazole (Score 25EC)	0.02	81.66 (9.04)^{e}	39.16 (6.26)^{f}	72.22 (8.50)^{g}
		0.05	84.44 (9.19)^{c}	46.44 (6.82)^{e}	78.33 (8.85)^{e}
		0.1	85.55 (9.25)^{b}	60.55 (7.78)^{d}	82.22 (9.07)^{cd}
9.	Bordeaux mixture	0.5	37.21(6.10)^{i}	100 (10)^{a}	79.41 (8.91)^{d}
		1	43.33 (6.58)^{h}	100 (10)^{a}	82.22 (9.07)^{c}
		1.5	72.22 (8.50)^{g}	100 (10)^{a}	84.99 (9.22)^{b}
	CD		0.51	0.525	1.93

***** Mean of the three replications
In each column figure followed by same letter do not differ significantly according to DMRT.
$\sqrt{x}$ + 0.5 transformed values are given in parantheses

4.6.2.1 Alternaria alternata

Mycelial disc of *Alternaria alternata* and *T. viride* were allowed to grow in PDA mediated sterile Petri plate and it was observed that *T. viride* overgrew and restricted the growth of pathogen by 61.1 per cent after nine days of inoculation [Plate 4.14(a)]. The bacterial antagonist, *Pseudomonas fluorescens* was streaked at the two ends of PDA mediated sterile Petri plate and it was noticed that the antagonist could inhibit the pathogen by 46.6 per cent [Plate 4.15(a)].

4.6.2.2 Alternaria tenuissima

The reponse of *T. viride* to the fungal pathogen, *A. tenuissima* showed better control of the pathogen by overgrowth mechanism of inhibition. The fungal antagonist, *T. viride* inhibited the pathogen by 63.3 per cent [Plate 4.14(b)]. *P. fluorescens* inhibited the pathogen by 57.7 per cent showing a low per cent of inhibition than the fungal antagonist [Plate 4.15(b)].

4.6.2.3 Myrothecium roridum

In the case of *M. roridum,* growth of the pathogen was restricted upto 70 per cent by the fungal antagonist, *T. viride* whereas the bacterial antagonist inhibited the pathogen by only 42.2 per cent. *T. viride* showed antagonistic mechanism of overgrowth for restricting the growth of the fungal pathogen [Plate 4.14(c)], [Plate 4.15(c)].

Plate 4.14.*In vitro* evaluation of *Trichoderma viride* against fungal pathogens

a. *Alternaria alternata*

b. *Alternaria tenuissima*

c. *Myrothecium roridum*

d. *Ulocladium chartarum*

e. *Curvularia pallescens*

f. *Curvularia lunata*

g. *Phytophthora cryptogea*

h. *Fusarium solani*

Plate 4.15.*In vitro* evaluation of *Pseudomonas fluorescens* against fungal pathogens

a. *Alternaria alternata*

b. *Alternaria tenuissima*

c. *Myrothecium roridum*

d. *Ulocladium chartarum*

e. *Curvularia pallescens*

f. *Curvularia lunata*

g. *Phytophthora cryptogea*

h. *Fusarium solani*

4.6.2.4 Ulocladium chartarum

While evaluating *P. fluorescens* against *Ulocladium chartarum* by dual culture technique, a clear zone of inhibition demarcating the growth of pathogen and bacteria was observed which displayed an aversion type of growth as the pathogen approached the antagonist when streaked on both sides of the Petri plate. The bacterial antagonist showed a maximum of 55.5 per cent inhibition of mycelial growth of pathogen [Plate 4.14(d)]. The antagonist, *T. viride* restricted the growth of the fungal pathogen, *Ulocladium chartarum,* by 66.6 per cent through overgrowth mechanism of inhibition [Plate 4.15(d)].

4.6.2.5 Curvularia pallescens

Bacterial antagonist, *Pseudomonas fluorescens* inhibited the pathogen by 53.3 per cent whereas the fungal antagonist restricted the growth of the pathogen, *C. pallescens* by 64.4 per cent. *T. viride* inhibited the pathogen by overgrowth mechanism of inhibition though *P. fluorescens* did not show any variation in the growth pattern of the pathogen [Plate 4.14(e)], [Plate 4.15(e)].

4.6.2.6 Curvularia lunata

Curvularia lunata when inoculated separately with the fungal and bacterial antagonist by dual culture technique, the pathogen exhibited a less inhibitory per cent of 52.2 by overgrowth mechanism of action whereas *P. fluorescens* showed 57.7 per cent inhibition with a better antagonistic action compared to that of *T. viride* [Plate 4.14(f)], [Plate 4.15(f)].

Table 4.17. Per cent inhibition of fungal pathogens by *Trichoderma viride*

Sl. No.	Pathogen	Per cent inhibition of pathogen	Antagonistic reaction
1.	*Alternaria alternata*	61.1	O
2.	*Alternaria tenuissima*	63.3	O
3.	*Myrothecium roridum*	70.0	O
4.	*Ulocladium chartarum*	66.6	A
5.	*Curvularia pallescens*	64.4	O
6.	*Phytophthora cryptogea*	68.8	O
7.	*Fusarium solani*	48.8	H
8.	*Curvularia lunata*	52.2	O

H - Homogenous
O - Overgrowth
C - Cessation of growth
A - Aversion

Table 4.18. Per cent inhibition of fungal pathogen by *Pseudomonas fluorescens*

Sl. No.	Pathogen	Per cent inhibition of pathogen
1.	*Alternaria alternata*	46.6
2.	*Alternaria tenuissima*	57.7
3.	*Myrothecium roridum*	42.2
4.	*Ulocladium chartarum*	55.55
5.	*Curvularia pallescens*	53.33
6.	*Phytophthora cryptogea*	44.4
7.	*Fusarium solani*	22.2
8.	*Curvualria lunata*	38.8

4.6.2.7 Phytophthora cryptogea

Trichoderma viride and *Pseudomonas fluorescens* were evaluated against *Phytophthora cryptogea* and found that *T. viride* was antagonistic against the pathogen showing 68.8 per cent inhibition. It was noticed that *T. viride* overgrew the pathogen. [Plate 4.14(g)]. *P. cryptogea* was also inhibited by *P. fluorescens* showing an inhibition of 44.4 per cent which was less than that exhibited by *T. viride* [Plate 4.15(g)].

4.6.2.8 Fusarium solani

The pathogen *Fusarium solani* and the fungal antagonist, *T. viride* showed homogenous growth while evaluating the efficacy of the antagonist by dual culture method [Plate 4.14(h)]. The pathogen exhibited an inhibition of 48.8 per cent by the fungal antagonist whereas the bacterial antagonist, *P. fluorescens* inhibited only 22.2 per cent showing very poor antagonistic action against soil borne pathogen [Plate 4.15(h)].

4.7 MOLECULAR CHARACTERISATION OF MAJOR PATHOGENS OF GERBERA

The existence survey carried out on per cent disease incidence (PDI) and per cent disease severity (PDS) of fungal diseases revealed that predominance of three pathogens *viz., Alternaria tenuissima, Phytophthora cryptogea* and *Fusarium solani* on gerbera grown in Kerala. Hence, these isolates were subjected to molecular characterisation for final confirmation regarding its identity upto the species level. Hence these were subjected to molecular characterisation for final confirmation regarding its identity upto species level. The molecular characterisation of the

pathogens was carried out at Rajiv Gandhi Centre for Biotechnology (RGCB), Thriuvananthapuram. Sequence analysis and nucleotide homology of each pathogen were analysed through the online BLASTn programme of NCBI. Details of the result of sequence comparison of three isolates are presented in Table 4.19, 4.20, 4.21 and Table 4.22 respectively.

4.7.1 Sequence comparison of *Alternaria tenuissima* isolate

Sequence homology search for *Alternaria* culture revealed that among the 100 hits in blast, cent per cent identity was noticed against *Alternaria tenuissima* strain NBt5L2 (Accession KU204747.1) with 99 per cent query coverage. Moreover, 99 per cent identity was observed with strains of *Alternaria alternata* isolate Ps-06 (Accession KU671333.1), *Alternaria gaisen* strain NBt7H2 (Accession KU204746.1), *Alternaria solani* (Accession KX090416.1) and *Alternaria arborescens* (Accession KU293592.1) and *Alternaria arborescens* strain EECC636 (Accession KP942949.1) with cent per cent query coverage. Hence sequence analysis of the *Alternaria* culture showed homology with *A. tenuissima* having 99 per cent identity and cent per cent query coverage as the same was identified earlier through cultural and morphological characterisation.

4.7.2 Sequence comparison of *Phytophthora cryptogea* isolate

Comparison of nucleotide sequence of *Phytophthora* culture revealed that two species of *Phytophhtora* were homologous to each other *viz., P. cryptogea* and *P. drechsleri* to the maximum extent. Cent per cent Query coverage was recorded for three strains of *P. cryptogea* which showed 98 per cent identity for *Phytophthora cryptogea* isolate 92-225 (Accession EU000138.1), *Phytophthora cryptogea* isolate

Table 4.19. Sequence homology observed for *Alternaria tenuissima* in BLASTn analysis as per BLAST results

Sl. No.	Description	Max. score	Query coverage (%)	E value	Identity (%)	Accession
2.	*Alternaria tenuissima* strain NBt5L2	977	100	0.0	99	KU204747.1
3.	*Alternaria gaisen* strain NBt7H2	977	100	0.0	99	KU204746.1
4.	*Alternaria alternata* isolate Ps-06	977	100	0.0	99	KU671333.1
5.	*Alternaria solani*	977	100	0.0	99	KX090416.1
6.	*Alternaria arborescens*	977	100	0.0	99	KU293592.1
7.	*Alternaria arborescens* strain EECC636	977	100	0.0	99	KP942949.1

Table 4.20. Sequence homology observed for *Phytophthora cryptogea* in BLASTn analysis as per BLAST results

Sl.No.	Description	Max. score	Query coverage (%)	E value	Identity (%)	Accession
1.	*Phytophthora cryptogea* isolate 92-225	1406	100	0.0	98	EU000138.1
2.	*Phytophthora cryptogea* isolate 279	1404	100	0.0	98	KP070716.1
3.	*Phytophthora cryptogea* isolate PH050	1404	100	0.0	98	KF042255.1
4.	*Phytophthora drechsleri* isolate TBF0052A29	1387	100	0.0	97	KU726771.1
5.	*Phytophthora drechsleri* isolateTBF0049A14	1387	100	0.0	97	KU726768.1
6.	*Phytophthora drechsleri* isolate TBF0037A05	1387	100	0.0	97	KU726763.1

Table 4.21. Sequence homology observed for *Fusarium solani* in BLASTn analysis as per BLAST results

Sl. No.	Description	Max. score	Query coverage (%)	E value	Identity (%)	Accession
1.	*Fusarium solani* strain D113	977	100	0.0	100	KU377510.1
2.	*Fusarium solani* strain CBS138803	977	100	0.0	100	KU296243.1
3.	*Fusarium solani* strain CBS138805	977	100	0.0	100	KU296242.1
4.	*Fusarium solani* strain cv554	977	100	0.0	100	KU296241.1
5.	*Fusarium solani* strain CBS 138812	977	100	0.0	100	KU296238.1
6.	*Fusarium solani* strain cv714	977	100	0.0	100	KU296237.1

Table 4.22 Genomic sequence of *Alternaria tenuissima, Phytophthora cryptogea* and *Fusarium solani*

Pathogen	Sequence (5'-3')
Alternaria tenuissima	GGAGGGATCATTACACAAATATGAAGGCGGGCTGGAATCTC TCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGC GTACTTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAA ACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATT AATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCAT CGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTG CAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGC CCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGT ACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTT TGCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTG GTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGG TCTAGCATCCATTAAGCCTTTTTTCAACTTTTGACCTCGGATC AGGTAGGGATACCCGCTGAACTTAA
Phytophthora cryptogea	TCATTACCACACCTAAAAAACTTTCCACGTGAACCGTATCAA CCTTTTTAAATTGGGGGCTTCCGTCTGGCCGGCCGGTTCTCGG CTGGCTGGGTGGCGGCTCTATCATGGCGACCGCCTGGGCCTC GGCCTGGGCTAGTAGCGTATTTTTTAAACCATTCCTAATTACT GAAAAAACTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATA GCAACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAA GAACGCTGCGAACTGCGATACGTAATGCGAATTGCAGGATTC AGTGAGTCATCGAAATTTTGAACGCATATTGCACTTCCGGGT TAGTCCTGGGAGTATGCCTGTATCAGTGTCCGTACACTAAAC TTGGCTCCCTTCCTTCCGTGTAGTCGGTGGATGGGGACGCGC AGATGTGAAGTGTCTTGCGGCTGGTCTTCGGTCCGGCTGCGA GTCCTTTTAAATGTACTACACTGTACTTCTCTTTGCTCGA
Fusarium solani	AGGGATCATTACCGAGTTATACAACTCATCAACCCTGTGAAC ATACCTATAACGTTGCCTCGGCGGGAACAGACGGCCCCGTAA CACGGGCCGCCCCCGCCAGAGGACCCCCTAACTCTGTTTCTA TAATGTTTCTTCTGAGTAAACAAGCAAATAAATTAAAACTTT CAACAACGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAG CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATC ATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGC GGGCATGCCTGTTCGAGCGTCATTACAACCCTCAGGCCCCCG GGCCTGGCGTTGGGGATCGGCGGAAGCCCCCTGCGGGCACA ACGCCGTCCCCCAAATACAGTGGCGGTCCCGCCGCAGCTTCC ATTGCGTAGTAGCTAACACCTCGCAACTGGAGAGCGGCGC

279 (Accession KP070716.1) and *Phytophthora cryptogea* isolate PH050 (Accession KF042255.1). The three strains of *P. drechsleri* recorded an identity per cent of 99 per cent query coverage of 96 per cent among which the maximum score of 1441 was noticed with the isolate NC103. Hence, the observations on the cultural and morphological studies were in line with the result achieved through molecular characterisation.

4.7.3 Sequence comparison of *Fusarium solani* isolate

Sequence comparison of nucleotide of *Fusarium* isolate showed cent per cent identity with different strains of *Fusarium solani.* This sequence also exhibited 100 per cent query coverage with *Fusarium solani* strain D113, *Fusarium solani* strain CBS138803, *Fusarium solani* strain CBS138805, *Fusarium solani* strain cv554, *Fusarium solani* strain CBS 138812 and different strains of *Fusarium solani.* Hence, the isolate which was earlier identified as *Fusarium solani* by cultural and morphological characters was further confirmed with the result of molecular characterisation.

4.8 *In vivo* EVALUATION OF FUNGICIDES AND BIOCONTROL AGENTS ON MAJOR FUNGAL DISEASES OF GERBERA

Based on the previous studies on per cent disease incidence (PDI) and per cent disease severity (PDS) three major pathogens *viz., Alternaria tenuissima, Phytophthora cryptogea* and *Fusarium solani* were selected as the major disease causing pathogens. Hence an experiment was laid out to study the efficacy of selected fungicides and biocontrol agents on the management of the three major fungal diseases of gerbera. Treatments were selected based on the efficacy shown in the *in vitro* evaluation studies and was applied after first symptom appearance. The

experiment was conducted as described in material and methods and observations on per cent disease incidence and per cent disease severity were recorded. Details of the results are furnished below:

4.8.1. Management of *Alternaria tenuissima*

The pathogen, *Alternaria tenuissima* was challenge inoculated on three month old plants by Mycelia Droplet Inoculation Technique (MDIT) and leaf blight symptoms were observed ten days after inoculation (Plate 4.16). Observations on disease incidence and disease severity for each treatment are tabulated in Table 4.23. It was noticed that per cent disease incidence (PDI) ten days after challenge inoculation was ranged from 20 to 100 per cent with a maximum in treatment T_7 (control) and minimum in T_3 (Mega master). Data on PDS ten days after inoculation revealed that there was no significant difference among the treatments however the maximum PDS was recorded in T_1 (Saaf) closely followed by T_4 (Score), T_5 (Antracol), T_7 (Control) and minimum in T_6 (*T. viride*) followed by T_2 (Curzate M-8).

From the data, it is evident that, after application of each treatment there was a significant reduction in disease severity. PDS was recorded consecutively after ten days of first and second spray. Per cent reduction over control was calculated and it was observed that maximum reduction in disease severity was recorded with T_5 (propineb) (0.3%) (70.7%), T_7 (*Trichoderma viride*) (2%) (68.2%) and the minimum with T_1 (Saaf) (46.3%) respectively. Though the per cent disease severity between the treatments were found insignificant to each other, treatments like T_6 (*Trichoderma viride*) (2%), T_5 (propineb 70WP) (0.3%) and T_5 (difenoconazole) (0.05%) showed promising results after the first treatment spray whereas T_1 (carbendazim (12%) + mancozeb (63%)) (0.2%) exhibited the least per cent reduction of 46.3 per cent.

Plate 4.16.*In vivo* evaluation of fungicides and biocontrol agents against *Alternaria tenuissima*

a. Experiment plot

b. Challenge Inoculation

c. Symptom appearance

Observations on PDS recorded 10 days after application of second treatment spray showed the significant difference among treatments where the disease severity reduced drastically with an overall per cent reduction of 63.6 to 90.9 per cent. The data revealed that a minimum PDS of 1.6 was noticed against T_1 (Saaf) which were on par with all other treatments. Moreover, maximum per cent reduction of 90.9 per cent was attained against T_1 (carbendazim (12%) + mancozeb (63%)) followed by T_2 (cymoxanil (8%) + mancozeb (64%)) and T_5 (propineb 70WP) with 86.3 per cent reduction in disease severity. Among the various treatments the bioagent T_6 (*Trichoderma viride*) (2%) was the least effective which recorded a PDS of 6.4 with 63.3 per cent efficiency over control.

4.8.3 Management of *Phytopthora cryptogea*

In the present study, *Phytophthora cryptogea* was challenge inoculated by root inoculation and also by giving a foliar spray of spore suspension on three month old gerbera plant. The first symptom appearance on leaves and roots was observed eight days after inoculation and the treatments were applied twice at an interval of ten days (Plate 4.17). Observations on disease incidence are presented in Fig 4.10. Among the various treatments, cent per cent disease incidence was observed in treatments T_1 (Antracol 70WP), T_4 (copper hydroxide 77WP) and T_7 (control) while the other treatments recorded a PDI of 80 per cent.

It was evident from the data that per cent disease incidence was reduced comparatively after the first treatment where the PDI was reduced to 80 per cent in treatments T_1 (Propineb 70WP) (0.3%), T_2 (cymoxanil (8%) + mancozeb (64%)) (0.2%), T_3 (carbendazim (12%) + mancozeb (64%)) (0.2%), T_5 (hexaconazole 5EC) (0.1%), and T_6 (*Trichoderma viride*) (2%). Similarly, observations on per cent disease incidence after second spray revealed that three treatments *viz.,* T_1 (propineb 70WP) (0.3%), T_2 (cymoxanil (12%) + mancozeb (64%)) (0.2%) and T_4 (0.2%)

Plate 4.17.*In vivo* evaluation of fungicides and biocontrol agents against *Phytophthora cryptogea*

a. Experiment plot

b. *Phytophthora* multiplied for challenge inoculation

c. Symptom appearance

(copper hydroxide 77WP) showed a low PDI of 60 per cent with the maximum in T_7 (control) (100%). The remaining treatments T_3 (Saaf), T_5 (hexaconazole 5EC) (0.1%) and T_6 (*Trichoderma viride*) (2%) recorded a PDI of 80 per cent respectively.

4.8.3 Management of *Fusarium solani*

In vivo management study of *Fusarium* wilt in gerbera was carried out by inoculating spore suspensions of *Fusarium solani* into the rhizosphere region of three month old plants (Plate 4.18). The disease symptoms of *Fusarium* wilt was noticed only 20 days after inoculation and observations on disease incidence recorded are depicted in Fig 4.11. The per cent reduction of disease was analysed and documented at weekly intervals after each treatments.

The results of the analysis revealed that, per cent disease incidence noticed 20 days after inoculation ranged from 40 to 100 per cent where highest per cent disease incidence of 100 per cent was observed with T_1 (carbendazim (12%) + mancozeb (64%)) (0.2%) and the lowest for T3 (copper hydroxide) (0.2%) (40%). All other treatments recorded comparatively higher disease incidence ranging from 60 in treatment T5 (difenconazole 25EC) (0.1%) and T_7 (Control) to 80 in T_4 (pyraclostrobin 20WG) (0.05%) and T_6 (*Trichoderma viride*) (2%) respectively. Per cent disease incidence (PDI) recorded seven days after first treatment showed a drastic reduction in incidence ranging from 20 to 60 per cent with the lowest incidence in T_3 (copper hydroxide 77WP) (0.2%) and the highest in T_7 (control) (60%). Likewise, seven days after the second treatments zero per cent disease incidence was recorded with T_1 (Saaf) (0.2%), T_5 (Score) (0.1%) and T_6 (*Trichoderma viride*) (2%) which was closely followed by T_2 (Folicur) (0.15%), T_3 (Kocide) (0.2%) and T_4 (Headline) (0.05%) with 20 per cent incidence where the highest PDI was registered with T_7 (control).

Plate 4.18.*In vivo* evaluation of fungicides and biocontrol agents against *Fusarium solani*

a. Experiment plot

b. Challenge inoculation

c. Symptom appearance

Table 4.23. Effect of treatments on per cent disease incidence and per cent disease severity of *Alternaria tenuissima*

Treatment no.	Treatments (Foliar spray)	Concentration (%)	10 days after inoculation		10 days after first spray		10 days after second spray	
			*PDI	*PDS	*PDS	Per cent disease reduction over control	*PDS	Per cent disease reduction over control
T_1	carbendazim 12% + mancozeb 64% WP (Saaf)	0.2	80	15.2	8.8	46.3	1.6 (1.6)[b]	90.9
T_2	cymoxanil 8% + mancozeb 64% (Curzate)	0.2	60	9.6	7.2	56.0	2.4 (2.4)[b]	86.3
T_3	hexaconazole 5EC (Mega master)	0.2	20	12.0	7.2	56.0	4.0 (4.0)[b]	77.2
T_4	difenconazole 25EC (Score)	0.05	80	11.2	5.6	65.8	2.4 (2.4)[b]	86.3
T_5	propineb 70WP (Antracol)	0.3	80	11.2	4.8	70.7	3.2 (3.2)[b]	81.8
T_6	*Trichoderma viride*	2	40	8.0	5.2	68.2	6.4 (6.4)[b]	63.6
T_7	Control	-	100	11.2	16.4	-	17.6 (17.6)[a]	-
	CD (0.05)		-	NS	NS	-	9.12	-

***** Mean of the five replications

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

$\sqrt{x} + 0.5$ transformed values are given in parantheses

PDI - Per cent disease incidence

PDS - Per cent disease severity

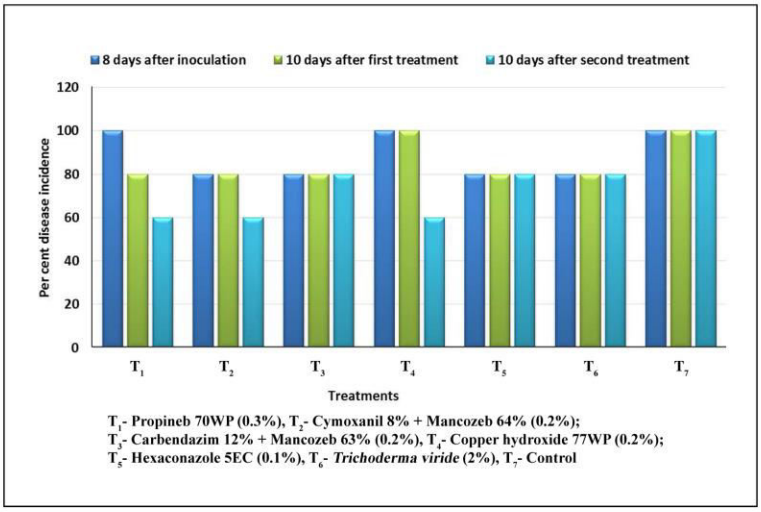


Fig 4.10 Effect of treatments on per cent disease incidence of *Phytophthora* root rot

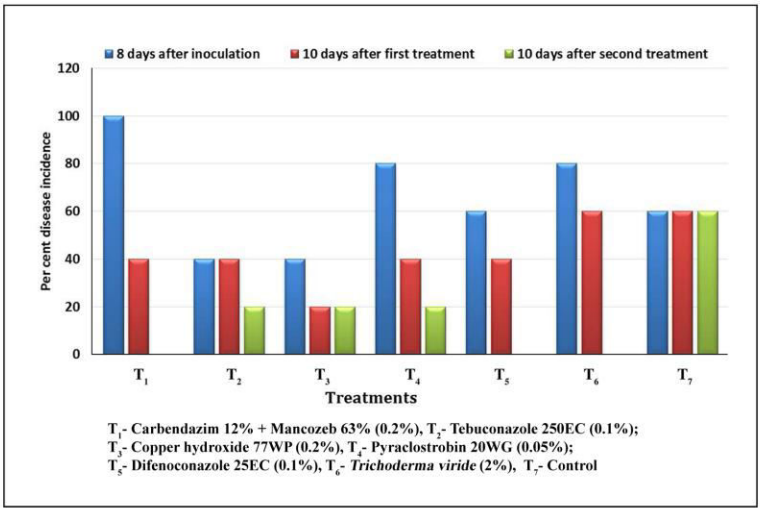


Fig 4.11 Effect of treatments on per cent disease incidence of Fusarium wilt

Discussion

5. DISCUSSION

Cut flowers, one of the most profitable crops has emerged as an important industry mainly to cater to the needs of the demand in the global market. *Gerbera jamesonii* Bolus, a very attractive, commercial cut flower crop ranks fourth in the international cut flower trade (Sujatha *et al.,* 2002). These plants are grown throughout the world in a wide range of climatic conditions and are in great demand in the floral industry as cut flower as well as potted plant due to its beauty, colour, long vase life and ability to rehydrate after long transportation. The flowers being hardy, can withstand rigorous transportation and a long shelf life. However, the plant is subjected to infection by a number of diseases which limit its production potential and market value. This has posed a serious threat to gerbera cultivation especially in the warmer humid tropics of Kerala. Interestingly, several researchers have observed many fungal diseases infecting the crop *viz.,* leaf blight (*Alternaria* spp.), seedling blight (*Macrophomina phaseolina*), white rust (*Albugo tragopogonis*), powdery mildew (*Oidium erysiphoides* f. sp. *gerberae, Golovinomyces cichoracearum*), downy mildew (*Bremia lactucae* Regel., *Plasmopara halstedii*), root rot (*Phytophthora cryptogea*) and wilt (*Fusarium* sp., *Verticillium* sp.) resulting in heavy crop losses (Folk *et al.*, 1985; Hyeong *et al.*, 1996; Vazquez *et al.,* 2000; Minuto *et al.*, 2004; Nagrale, 2007; Troisi, *et al.,* 2010; Duarte *et al.,* 2013).

A perusal of the literature revealed that no attempts have been made so far for the identification and documentation of fungal diseases of gerbera in Kerala. Hence, it is pertinent to have a detailed investigation to identify various fungal pathogens infecting gerbera and to study its etiology, symptomatology and the management of diseases. Thus, being the first attempt, the present research work is focused to catalogue and document fungal diseases of gerbera in Kerala.

5.1 SURVEY, COLLECTION AND ISOLATION OF FUNGAL DISEASES

In order to study the diversity and distribution of fungal diseases of gerbera, a purposive sampling survey was initiated in 2014-16 in three districts *viz.,* Thrissur, Malappuram and Wayanad during rainy, winter and summer seasons to get a complete profile of diseases in all seasons. The survey was conducted to assess the disease incidence, disease severity, to record the symptoms of various fungal diseases and to collect diseased specimens for isolation of various pathogens of gerbera. During the survey, occurrence of three leaf blights, two leaf spots, two powdery mildew, one each of root rot, wilt and petal blight were the ten diseases observed. Leaf blights and leaf spots were found distributed throughout the districts surveyed compared to root rot and flower blight. This is in agreement with the findings of Kulibaba (1972) who surveyed different locations of Soviet Russia and observed various fungal diseases of gerbera except petal blight. Similar line of work was recorded by Nagrale (2007) and Ferronato *et al.* (2008).

Diseased samples collected during the survey were used for isolation of pathogens. The isolates were tentatively identified upto genus level based on the cultural and morphological characters and also by comparing with the description given in CMI Descriptions of Pathogenic Fungi and Bacteria. The pathogen causing leaf blights (LB-1 and LB-2) were tentatively identified as *Alternaria* spp., LB-3 as *Myrothecium* sp., leaf spots (LS-1 and LS-2) as *Ulocladium* sp. and *Curvularia* sp., root rot as *Phytophthora* sp., wilt as *Fusarium* sp. and petal blight as *Curvularia* sp.

5.1.1 Assessment of per cent disease incidence, per cent disease severity and correlation with weather parameters

Weather conditions play a predominant role in determining the cause and severity of epidemics. Accurate and timely reports of host-pathogen-weather

records are essential for identification, management and prevention of plant diseases. Hence, an attempt was made to study the role of different weather parameters *viz.,* rainfall, relative humidity and temperature on infection and development of fungal diseases of gerbera.

The disease dynamics in terms of seasonal variations of incidence and severity since November 2014 to April 2016 showed that among the various diseases, LB-1 (*Alternaria* sp.) was noticed in all the seasons through out the tracts of observation but with differential intensity. Correlation studies of LB-1 with weather parameters revealed that the disease observed in various locations showed disparity in relation with the climatic factors. In Ambalavayal, the severity of the disease was found positively correlated with temperature whereas the reverse relation existed with relative humidity (RH) and rainfall. As all the three factors have significant role in disease development, severity of LB-1 fluctuated with the variation in weather parameters and the highest mean per cent disease severity (PDS) of 12.32 per cent was recorded in cool weather conditions. Mean PDS of LB-1 in Malappuram district was recorded maximum during winter season (16.64%) which absolutely followed a positive correlation with RH and rainfall. In Thrissur district, LB-1 was observed at two locations *viz.,* Vellanikkara and Madakkathara which exhibited a differential correlation result. In Madakkathara, the disease was influenced by all the three weather parameters with a positive correlation with temperature and a reverse relation with RH and rainfall whereas the disease was biased positively only with variation in temperature. Highest mean PDS of 9.28 per cent was recorded in Thrissur district during the monsoon season with a mean temperature of 26.95^{o}C, RH of 84.33 per cent and rainfall of 486.9 mm.

In general, weather parameters which recorded congenial for the occurrence of LB-1 during all the three seasons ranged from a mean temperature of 21.43-29.43^{o}C, RH of 65-86.67 per cent and rainfall of 96 to 486 mm. It is also interesting to note that the disease LB-1, caused by *Alternaria* sp. is observed in

gerbera plants grown both under polyhouses and open field conditions. Several studies have shown the occurrence of blight disease on gerbera incited by *Alternaria alternata* in most of the green house and polyhouses (Ghosh, 1998; Mirkova and Konstantinova, 2003; Farhood and Hadian, 2012 and Nagrale, *et al.,* 2012). The above findings were closely supported by Gud *et al.* (2007) where they studied different weather parameters for the spread of *Alternaria* leaf blight disease in safflower. According to them, rainfall of 334.8 mm, 88 to 93 per cent RH with a mean temperature 21 to 32^{o}C was found congenial for rapid buildup of *Alternaria* blight in safflower. Ghosh *et al.* (2009) opined that the results were found comparable with the present study as the incidence of *Alternaria* blight reached its peak during summer to pre-rainy months with a PDI of 42.5 per cent and PDS of 16.40 per cent, thus showing a positive correlation with temperature.

In the case of LB-2 (*Alternaria* sp.), the disease was observed typically in the high range tract of Ambalavayal area of Wayanad district where the highest PDS of 14.88 per cent was noticed during summer as the correlation studies with weather parameters hold the same conclusion. Thus, gerbera grown with a lower temperature coupled with an above average relative humidity and a low rainfall may bring down the disease to some extent but at such a juncture the temperature plays the havoc as there will be sudden upsurge in temperature immediately after the wet spells.

The disease, LB-3 (*Myrothecium* sp.) was observed only during November-December at Vellanikkara area of Thrissur district where gerbera was grown in a hydroponic unit. Hence, it may be inferred that the pathogen favours low temperature and high moisture condition for perpetuation and spread of the disease. The above conclusions are in agreement with the inference stated by Chase (1984) who pointed out that a temperature ranging from 21-27^{o}C with a high relative humidity favours the disease incidence. The PDI and PDS recorded for LB-3 were 53.1 and 9.1 per cent respectively.

The foliage diseases, LS-1 (*Ulocladium* sp.) and LS-2 (*Curvularia* sp.) were found non-significant during all varying climatic factors. It is noticed that the disease LS-1, was recorded only where gerbera was grown under polyhouses of Thrissur district whereas LS-2 was observed under open conditions in Malappuram district. Eventhough, the diseases were not much influenced by climatic factors, the former recorded a highest mean PDS during March-April with 8.16 per cent and the latter during November-December with 11.36 per cent. However, the present result is contradictory to the report by Pawar *et al.* (2012) who observed *Curvularia* blight caused by *Curvularia lunata* and *Curvularia gladioli* in gladiolus during rainy season. In the case of *Ulocladium* sp., there are reports saying that species of *Ulocladium* could grow at a temperature ranging from 5-34^{o}C and requires constant relative humidity of above 75 per cent.

Powdery mildew was the most devastating disease of gerbera observed during the survey causing significant economic losses. The disease was observed only in Wayanad district during winter and summer season. Highest PDI and PDS of 95.2 and 57.4 per cent respectively were recorded during November-December in Chulliyode of Wayanad district. Correlation studies revealed that in Chulliyode, the disease was positively correlated to relative humidity and exhibited a reverse relation with temperature and rainfall. The correlation analysis that clearly depicted the major weather factors that influenced the spread of powdery mildew were low temperature, high relative humidity and sparse but less intense rainfall. This might be the one of the reason why powdery mildew was not noticed during the monsoon season. As a testimonial to the above conclusion, Kumar *et al.* (2012) detailed weather parameters most congenial for powdery mildew which included high relative humidity (80-95%), moderate temperature (20-28^{o}C) and low light intensity or shade. Similarly, the results are in accordance with Leah *et al.* (2012), who showed that the disease exhibited positive correlation with RH and negative with temperature.

Among the root diseases infecting gerbera, root rot and wilt were monitored by recording per cent disease incidence. Root rot disease caused by *Phytophthora* sp. recorded high incidence of 69.44 per cent which was observed in a hydroponic unit whereas comparatively low PDI of 15.5 per cent was observed for wilt disease caused by *Fusarium* sp. Here both the diseases were observed in gerbera grown under polyhouse conditions. Several studies have shown that greenhouse conditions favour luxurious growth of crops which favour the development of plant pathogens especially root rots causing economic losses to the growers (Rajendran *et al.,* 2014). Recent evidences suggest that *Phytophthora* rot and *Fusarium* wilt are the most widespread and destructive diseases of many ornamental crops (Padghan and Gade, 2006; Ampuero *et al.,* 2008 and Rajendran *et al.,* 2014). In this study it was found that both the diseases occurred during monsoon season in Thrissur district and the results thus obtained are in line with the report by Ampuero *et al.* (2008) where they stated that weather conditions like temperature ranging from 15-30^{o}C and long, frequent soil saturation periods as in the case of hydroponics favour the development of *Phytophthora* rot. Similarly, Gardinar (1987) experimented the influence of variation in temperature on the occurrence of *Fusarium* wilt of chrysanthemum under protected condition. He opined that symptoms like chlorosis of one or two leaves were observed at a temperature of 24^{o}C whereas the symptoms got extended into necrosis and wilting of plants as the temperature increased upto 29^{o}C and complete wilting of plant observed at 32^{o}C. Mina and Dubey (2010) also elucidated the most important environmental variables inciting *Fusarium* wilt as, soil temperature, soil moisture along with atmospheric temperature.

Another disease which recorded the lowest intensity among all the diseases noticed during the survey period was petal blight (*Curvularia* sp.) in gerbera. The disease was observed in Ambalavayal of Wayanad district as well as in Anakkayam of Malappuram district. Though the intensity of the disease was very sparse in both the districts (3.98-4.3%), the disease was found very detrimental to the commercial cut flower production. The reason for petal blight

was attributed to the monsoon season and the cool weather conditions that make the flowers vulnerable to disease attack. Similar observations on the progression of the disease in relation to climatic factors have been made by Rath and Dhal (1972) and Pawar *et al.* (2012) where they observed the disease caused by *Curvularia pallescens* and *Curvularia lunata* on *Canna indica* and *Curvularia gladioli* on gladiolus during the rainy season.

5.2 PATHOGENICITY OF ISOLATES

Pathogenicity of each isolate showing distinct symptoms was tested by artificial inoculation on whole plant or on detached leaves. The methods employed for testing pathogenicity varied with the nature of isolate, either obligate or facultative and types of diseases like foliage, root and flower diseases. MBIM method (Rocha *et al.,* 1998) and MBIT method (Munaut *et al.,* 1997) of inoculation of pathogen were followed for proving pathogenicity of foliage and flower isolates, whereas for root isolates, soil inoculation method was followed. Garibaldi and Minuto (2007) and Farhood and Hadian (2012) experimentally proved pathogenicity of *Fusarium oxysporum* and *Alternaria* leaf blight isolates by inoculating spore suspension into healthy gerbera plants. Likewise, the Koch postulates as pathogenicity test for fungal pathogen, *Phytophthora,* is in congruence with the results of Ampeuro *et al.* (2008) where they observed isolates of *Phytophthora cryptogea* pathogenic on petunia after crown and root inoculation with mycelial fragments and after soil inoculation with zoospores. Further, the results of the present study are in line with the findings of Cui and Sun (2012) and Pawar *et al.* (2012) where they could prove pathogenicity of *Curvularia lunata* and *Curvularia gladioli* in lotus and gladiolus respectively. Moreover, Baiswar *et al.* (2010) confirmed the pathogenicity of the powdery mildew pathogen, *Podosphaera* sp. in gerbera by dusting conidia on healthy plants. Kwon *et al.* (2014) also contributed to the study of pathogenicity of *Myrothecium* sp. in anthurium. Pathogenicity test of *Myrothecium* sp. and *Ulocladium* sp. isolated from gerbera by artificial inoculation proved the pathogenic nature of the isolates.

Reports of *Ulocladium* sp. as a disease causing agent was unusual among agricultural crops, though, Zitter and Hsu (1990) could isolate and prove the pathogenic nature of *Ulocladium sp.* from cucumber causing leaf spot disease. Hence the pathogenicity test carried out in the present investigation proved that all the isolates were disease causing agents in gerbera reproducing distinct symptoms observed as in naturally infected plants.

5.3 SYMPTOMATOLOGY OF THE PATHOGENS

An attempt was made further to study the symptomatology of fungal diseases both under natural and artificial conditions. The pathogen, *Alternaria* caused leaf blight disease (LB-1) on gerbera which produced, yellow circular dots on leaf lamina and later turned into dark brown concentric rings that further coalesced and caused blightening symptom. Another leaf blight disease (LB-2) also showed typical symptoms of *Alternaria* leaf blight. However, in LB-2 the symptoms appeared as small, circular necrotic spots near the margin of leaves which resulted in withering, drying and shedding of leaves. Many authors have reported the occurrence of *Alternaria alternata* showing typical leaf blight symptoms of LB-1 mentioned earlier (Ghosh, 1998; Nagrale, 2007 and Farhood and Hadian, 2012). The above depiction on symptomatology of *Alternaria* causing leaf blight 2 (LB-2) was in agreement with the findings of Honda *et al.* (2001) who noticed *Alternaria tenuissima* leaf spot in broad bean.

During the period of study, *Myrothecium* sp. caused LB-3 disease which was isolated from a hydroponic unit of gerbera which produced symptoms like black water soaked lesions which later enlarged causing blightening of leaves. Mmbaga *et al.* (2010) reported pathogenic nature of *Myrothecium roridum* causing leaf spot in garden hydrangea where disease symptoms noticed in garden hydrangea due to *Myrothecium* sp. was in corroboration with that observed in gerbera.

The organisms associated with leaf spot 1 (LS-1) was *Ulocladium* sp. and that of LS-2 and petal blight were *Curvularia* spp. The former pathogen caused extensive damage on leaves like circular, pale to dark brown necrotic spots with definite borders scattered on leaf lamina and the latter showed typical symptoms as small, yellow-brown flecks, often with a light green halo on the upper surface of leaves which later turned into circular to oval chlorotic irregular patches. The occurrence of *Ulocladium* sp. on gerbera has not been reported so far and thus, it needs further detailed investigation to ascertain the extent of damage caused by the pathogen. However, symptoms of *Curvularia* leaf spot and petal blight are in line with that of Rath and Dhal (1972), Kolse *et al.* (2000), Pawar *et al.* (2012), Cui and Sun (2012) and Torres *et al.* (2013) where they noticed similar leaf spots on ornamental plants like canna, gladiolus and lotus respectively.

Powdery mildew disease in gerbera is the most common and destructive disease that reduces the commercial value of the crop. Symptomatological studies of this disease were found to be as similar as that observed in other crops. Symptoms were more prominent on upper leaves where white powdery mould appeared on the leaf lamina which later turned necrotic. The description of powdery mildew symptom was in conformity with the findings putforth by other workers (Ferronato *et al.,* 2008; Farr and Rossman, 2009; Baiswar *et al.* 2010 and Troisi *et al.,* 2010).

Among the root rot and wilt disease, symptoms of *Phytophthora* rot initiated as dark, black coloured lesion on leaves and stem through collar region which later extended to root hairs, apart from foliar yellowing and defoliation. Several workers like Skadow (1981) and Hyeong *et al.* (1996) detailed the symptomatology of root rot in gerbera as wilting of leaves, discoloration of stem and foot rot which was found similar to that described earlier. Symptoms of *Fusarium* wilt in gerbera appeared as dark brown discolouration on lateral roots which gradually spread to main tap roots, along with foliar yellowing and defoliation. The above description of symptoms of *Fusarium* wilt was comparable

with the report of Garibaldi *et al.* (2008) where they described unilateral yellowing and vascular streaks as typical symptoms of *Fusarium* wilt in gerbera.

5.4 CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF PATHOGENS

Studies were further carried out for the identification of fungal pathogens. The fungal pathogens were identified based on cultural and morphological characters. Further confirmation of the pathogen was assured by National Centre of Fungal Taxonomy (NCFT), New Delhi where species level identification was carried out.

Isolate of *Alternaria sp.* causing leaf blight 1 (LB-1) produced olivaceous to dark brown spores with varied shape from obclavate to mostly ellipsoidal, muriform having tapered apex with 1-3 longitudinal and 2-10 transverse septa having 22.76-60.90µm x 6.45-13.01µm dimension formed in cylindrical, scattered or gregarious, pale grey yellow, straight or curved, geniculate, simple or branched conidiophores. Likewise, LB-2 produced olivaceous to dark brown coloured conidia of dimensions 23.86-48·69µm x 7.34-16·32µm wide. All these characters were in accordance with those reported by Nagrale *et al.* (2012) who studied similar morphological characters of the pathogen. Based on the above characters, coupled with symptomatology and pathogenicity, the isolates were identified as *Alternaria alternata* and *Alternaria tenuissima.*

Another leaf blight (LB-3) causing pathogen, *Myrothecium* sp.*,* was subjected to characteristion study and revealed that the pathogen produced white, floccose, concentric-ringed colonies on PDA with irregular shapes of dark green to black sporodochia. Moreover, hyphae were hyaline, conidiophores formed as 2-4 branches at each node while phialides hyaline, cylindrical and measured 13-16 x 2.0µm dimension. Conidia hyaline, single celled, rod-shaped with rounded ends and measured 5-10.74 x 2.0µm. The cultural and morphological characters of the

present study were consistent with the description of *Myrothecium roridum* causing leaf spot in begonia (Fujinawa *et al.,* 2016) and the isolate obtained in the present study was thus confirmed as *Myrothecium roridum.*

The disease leaf spot 1 (LS-1) caused by *Ulocladium* sp. was observed in a polyhouse of Thrissur district. The isolate produced obovoid, non-beaked, olivaceous to dark brown coloured conidia having dimensions ranging from 26.54-51.54µm x 15.16-40.24µm. The pathogen on PDA appeared as greyish white with yellowish pigmentation. Vannini *et al.* (2000) and Zarandi and Sharzei (2015) reported the causal agent of leaf spot disease of *Quercus pubescens* and lemon verbena leaf spot by *Ulocladium chartarum.* These characteristics confirmed the identity as *Ulocladium chartarum.*

Leaf spot disease (LS-2) was observed in Anakkayam area of Malappuram district where gerbera was grown in open fields. The disease was caused due to *Curvularia pallescens* which appeared on PDA as effuse colony producing grey mycelium which later turned black. Conidia three septated, slightly curved measuring 12-28 x 6-12µm. Olufolaji (1983) studied growth and sporulation of *C. pallescens* had detailed the cultural and morphological characters in different media which was found comparable with the present study. Thus, the pathogen was confirmed as *Curvularia pallescens*.

Powdery mildew disease caused by obligate pathogen was observed only in the high ranges of Wayanad district. Morphological characterisation of the isoates revealed the existence of two distinct pathogens *viz., Erysiphe* sp. and *Podosphaera* sp. Light microscopy revealed the presence of hyaline, septate mycelia, globose conidia with irregular peripheral end formed in chains where the characters were similar to that of *Erysiphe* sp. Troisi *et al.* (2010) from Italy while studying etiology of powdery mildew in gerbera reported *Erysiphe cichoracearum* as the causative agent. *Podosphaera* sp. produced superficial, hyaline, coenocytic mycelium with oval or ellipsoidal, catenate conidia with dimension ranging from

22.21-30.18µm x 13.36-18.08µm formed in unbranched erect conidiophores where these characters are in conformity with those reported by Baiswar *et al.* (2010) where they detailed the morphological characters *Podosphaera* sp. in *Gerbera jamesonii* from India. Based on the host and morphological characteristics, the powdery mildew pathogens were identified as *Golovinomyces cichoracearum* (previously known as *Erysiphe cichoracearum*) and *Podosphaera* sp.

Root rot pathogen, *Phytophthora* sp. was isolated from gerbera grown in a hydroponic unit from Thrissur district. The isolate produced uniformly dense white cottony growth on PDA. The hypha was branched, hyaline, coenocytic with oval to obpyriform sporangia, nonpapillate borne either terminally or laterally on the sporangiophores in a simple sympodial fashion. Dimension of sporangia ranged from 32.5-57.5 x 25-35 µm. These characters are in agreement with that reported by Erwin and Ribeiro (1996) in gerbera and the pathogen was finally identified as *Phytophthora cryptogea.*

Petal blight of gerbera was observed in Wayanad and Malappuram districts which were caused due to the same pathogen, *Curvularia lunata.* Cultural and morphological characters revealed that, the pathogen isolated in PDA was dark, velvety, rapid growing colony showing thin and suppressed growth. Basal and apical cells of the conidia was pale brown leaving the other cells brown or dark brown coloured with smooth, curved at third cell from base. Dimensions of the conidia ranged from 20–32 × 9–15µm with three septa formed in geniculate growth manner in pale brown septate conidiophore. Description of Pawar *et al.* (2012) on the characteristics of *Curvularia lunata* causing leaf spot disease in gladiolus and depiction of growth and sporulation characters of *Curvualaria lunata* by Lal *et al.* (2014) were found comparable with the characteristics of the pathogen in the present study. Thus the identity of petal blight causing pathogen in gerbera was confirmed as *Curvularia lunata.*

Similarly, cultural and morphological characters of *Fusarium* sp. causing wilt disease in gerbera were studied. The pathogen produced typical hyaline, micro and macro conidia from slender phialides. Macroconidia two to several-celled, fusiform to sickle-shaped, mostly with an elongated apical cell and pedicellate basal cell of size 28-42 x 4-6 µm. Microconidia one or two-celled, hyaline, smaller than macroconidia, pyriform, fusiform to ovoid, straight or curved with 8-16 x 2-4.5 µm dimension. The above descriptions were comparable with the characteristics of isolate obtained from *Fusarium* wilt of carnation (Kumar *et al.,* 2014). Moreover, the cultural characters of the pathogen isolated from gerbera was in conformity with that isolated from carnation as both showed white fungal growth initially and later turned into light pink to orange in PDA media. Based on the above characteristics, the isolate causing wilt was identified as *Fusarium solani.*

5.5 DISEASE MANAGEMENT

Plant diseases contributed for a loss of 6 to 7 per cent of ornamental production in United States which accounted for 23.2 million dollar outlay during 2011 itself (Williams-Woodward, 2011). Thus, disease management plays significant role at every aspects of crop production from seedling to post harvest stages. Use of disease resistant planting materials, cultural, biological and chemical control methods were the essential strategies employed under plant disease management.

5.5.1 *In vitro* evaluation of chemical fungicides against the pathogens

One of the most important strategy of protecting plants against fungal pathogens is the use of fungicides. However, these fungicides have to be used judiciously according to the need and kind of organism involved. Hence, an attempt was made to evaluate fungicides under *in vitro* conditions which provide useful preliminary information regarding its efficacy against a pathogen within the shortest period of time and thereby serve as guide for further field level testing.

In the present investigation, *in vitro* evaluation of nine fungicides including contact fungicides like copper fungicides (Bordeaux mixture, copper hydroxide 77WP), propineb (77WP), systemic fungicides *viz.,* triazoles (hexaconazole 5EC, difenoconazole 25EC, tebuconazole 250EC), strobilurins (pyraclostrobin 20WG) along with combination chemicals (carbendazim 12% + mancozeb 63% WP and cymoxanil 8% + mancozeb 64% WP) were tested against eight fungal pathogens which have been isolated from gerbera. Triazole group of systemic fungicides *viz.,* hexaconazole 5EC (Mega master), difenoconazole 25EC (Score) and tebuconazole 250EC (Folicur) along with propineb 70WP (Antracol) at all three concentrations obtained cent per cent inhibition of *Alternaria alternata*. Copper hydroxide 77WP and Bordeaux mixture being the two copper fungicides tested against *A. alternata*, better inhibition was recorded with copper hydroxide 77WP whereas the least was noticed with Bordeaux mixture. Pyraclostrobin 20 WG at all concentrations, combination fungicide, carbendazim 12% + mancozeb 63% WP (Saaf) at 0.05 and 0.1 per cent recorded a per cent inhibition of above 75 percent. However, comparatively less inhibition was noticed with cymoxanil (8%) + mancozeb (64%), showing an inhibition of 65 to 75 per cent at all three concentrations.

Several researchers evaluated the efficiency of triazoles against *A. alternata* where comparable results were obtained by Thaware *et al.* (2010). Contradictory to the above findings, Apet *et al.* (2014) observed 77.4 per cent inhibition of *A. alternata* of gerbera with difenoconazole (0.1%). However, according to them, the pathogen showed 94.5 per cent inhibition with hexaconazole 5EC (0.1%). Similarly, Ginoya and Gohel (2015) observed inhibitory effect of tebuconazole 250EC and hexaconazole 5EC against *Alternaria alternata* of gerbera which are agreeable with the present study. The inhibitory action of propineb 70WP in the study was in accordance with that of Hegde (1988) who also observed cent per cent inhibition of *A. alternata.* Mathivanan and Prabavathy (2007) noticed the sensitiveness of cymoxanil (8%) + mancozeb (64%) against *Alternaria helianthi* which obtained 47.2 per cent inhibition. Also,

the findings of Waghe *et al.* (2015) on evaluation of *Alternaria helianthi* with 0.1 and 0.2 per cent carbendazim (12%) + mancozeb (63%) attained an inhibition of above 85 per cent which is in line with the present finding.

The pathogen, *Alternaria tenuissima* causing leaf blight disease in gerbera was evaluated against nine chemical fungicides under *in vitro* conditions. It was observed that the two combination fungicides *viz.,* carbendazim (12%) + mancozeb (63%) (Saaf), cymoxanil (8%) + mancozeb (64%) (Curzate M-8) along with three triazoles *viz.,* hexaconazole 5EC, difenoconazole 25EC (Score) and tebuconazole 250EC (Folicur) recorded cent per cent inhibition whereas strobilurin, pyraclostrobin 20WG showed maximum per cent inhibition of 81.11 per cent at 0.15 per cent respectively. Per cent inhibition of 57.77 per cent was recorded with Bordeaux mixture compared to copper hydroxide 77 WP (Kocide) which showed a superior performance of 70 to 80 per cent. The efficacy of triazoles in the present study is in agreement with previous studies who evaluated the chemicals against *Alternaria* spp. (Mallikarjun, 1996; Arunkumar, 2006; Patel and Choudhary, 2010 and Parveen *et al.,* 2013). Roopa *et al.* (2014) compared the efficacy of propineb 70WP, hexaconazole 5EC and difenoconazole 25EC against *Alternaria* sp. and observed that least inhibition was shown by propineb and the maximum with hexaconazole 5EC (0.1%) which is found in corroboration with the present study.

In the case of *Myrothecium roridum,* all the chemical fungicides except hexaconazole 5EC restricted the growth of the fungus completely at all three concentrations tested. An *in vitro* assay of fungicides with *M. roridum* by Duval *et al.* (2009) revealed that copper hydroxide 77WP and tebuconazole 25EC was found effective in inhibiting *M. roridum* with a per cent inhibition of 85 and 90 per cent which is almost in congruence with the present finding. The study also supports the results of Daivasikamani *et al.* (2013) where they noticed inhibition of *Myrothecium roridum* with tebuconazole. The observations obtained in the present study are in tune with the reports of Dighule *et al.* (2011) and Mourya *et*

al. (2009) where they noticed 69 to 82 per cent inhibition of *M. roridum* with propineb 70WP.

Ulocladium chartarum, reported to be a new pathogen causing leaf spot in gerbera was subjected to *in vitro* evaluation against fungicides. The results revealed that the pathogen was inhibited cent per cent by carbendazim 12% + mancozeb 63% (Saaf), cymoxanil 8% + mancozeb 64% (Curzate M-8), pyraclostrobin 20WG (Headline), tebuconazole 250EC (Folicur), copper hydroxide 77WP (Kocide) and propineb 70WP (Antracol) at all the three concentrations. Other fungicides like Bordeaux mixture, hexaconazole 5EC (Mega master) and difenoconazole 25EC (Score) showed an inhibition per cent of less than 75 per cent. Since there is very scanty literature available on *Ulocladium chartarum*, there are no reports on the effect of fungicides on the said pathogen.

Nine fungicides while evaluated against *Curvularia pallescens* under *in vitro* condition, achieved remarkably good control of the pathogen by most of the chemicals. Among the fungicides evaluated, all the chemicals showed a cent per cent inhibition at all the three concentrations except for the two copper fungicides, Bordeaux mixture and copper hydroxide 77WP (Kocide). Bordeaux mixture and copper hydroxide 77WP (Kocide) restricted the growth of the pathogen with almost similar per cent inhibition ranging from 73.0 to 78.0 percent respectively. Pawar *et al.* (2012) recorded 66.5 and 85.4 per cent inhibition of *Curvularia pallescens* with Bordeaux mixture and Curzate M-8 which is in accordance with the present study. Several workers also pointed out the *in vitro* inhibitory effect of *Curvularia pallescens* with different fungicides (Grewal and Payak, 1976, Singh *et al.,* 1997 and Lakpale, 2011).

In vitro evaluation of nine fungicides against *Curvularia lunata* found that cymoxanil (8%) + mancozeb (64%) (Curzate M-8), propineb 70WP (Antracol), copper hydroxide 77WP (Kocide), hexaconazole 5EC (Mega master) and tebuconazole 250EC (Folicur) completely restricted the radial growth of

mycelium at all three concentrations along with the highest concentration of carbendazim (12%) + mancozeb (63%) (Saaf) and pyraclostrobin 20WG (Headline). The highest concentration of difenoconazole 25EC (Score) (0.1%) and Bordeaux mixture (1.5%) could restrict the growth ranging from 70 to 85 per cent. Similar results were observed by different workers. Sumangala *et al.* (2008) noticed per cent inhibition of above 90 with *Curvularia lunata* when evaluated with difenoconazole 25EC (Score) which was comparable with that of the present study. The result of the present study also supports the findings of previous workers who tested the efficacy of different fungicides against *Curvularia* pathogen in crops like cotton (Gadage and Patil, 1977), coriander (Prasad, 1982), gladiolus (Kolse *et al.,* 2000), mung bean (Arun and Tomar, 2005) and sweet potato (Thenge *et al.,* 2008).

In vitro evaluation of nine chemicals against *Phytophthora cryptogea* recorded cent per cent inhibition with six chemicals *viz.,* propineb 70WP (Antracol), cymoxanil (8%) + mancozeb (64%) (Curzate M-8), carbendazim (12%) + mancozeb (63%) (Saaf), copper hydroxide 77WP (Kocide), tebuconazole 250EC (Folicur) and Bordeaux mixture at all the three concentrations. The above findings are in validation with Shashidhara *et al.* (2008) and Kaur *et al.* (2009) where they observed the efficiency of Bordeaux mixture and cymoxanil + mancozeb on *Phytophthora* sp. Cent per cent inhibition of carbendazim (12%) + mancozeb (63%) (Saaf) was also confirmed by the study of Mtasa *et al.* (2014). Moreover, cent per cent inhibition was also recorded by 0.1 and 0.15 per cent of hexaconazole 5EC (Mega master) and at the highest concentration of pyraclostrobin 20WG (Headline) whereas difenoconazole 25EC (Score) showed least inhibition of the pathogen. According to Lebrun (2002) chemicals preferred to *Phytophthora* root rot in gerbera were grouped under phenyl amides like furalaxyl, fosetyl Al, combination of oxadixyl and cymoxanil.

A total inhibition of radial growth of *Fusarium solani* was recorded with fungicides *viz.,* carbendazim 12% + mancozeb 63% (Saaf) (0.05, 0.1, 0.15%),

pyraclostrobin 20WG (Headline) (0.05, 0.1, 0.15%) and tebuconazole 25EC (Folicur) (0.05, 0.1, 0.15%) when evaluated by poison food technique. Hexaconazole 5EC at all concentrations, inhibited *F. solani* which was in accordance with the results of Taskeen-Un-Nisa *et al.* (2011) and Kumhar *et al.* (2015) where they reviewed 67.8 per cent inhibition of *F. oxysporum* with hexaconazole 5EC. However, the results on copper hydroxide 77WP were in contrary to the present study where they observed a per cent inhibition of 60 to 80 with the fungicide. Fareed *et al.* (2015) observed 50 per cent reduction of mycelial growth of *F. oxysporum* with difenoconazole 25EC (Score) and propineb 77WP (Antracol) which was comparable with the present study.

5.5.2 *In vitro* evaluation of biocontrol agents against the pathogens

Although various fungicides have promising results in management of fungal diseases, there is always a problem of phytotoxicity and fungicidal residue leading to environmental pollution. Hence, ecofriendly management of fungal diseases involving biocontrol agents have been used extensively to control both foliar and soil borne pathogens where they are found to inhibit disease causing pathogens or provide protection by enhancing defense mechanism. Considering the importance and use of biocontrol agents, the reference cultures of KAU, *Trichoderma viride* and *Pseudomonas fluorescens* were evaluated for the efficiency against the fungal pathogens under *in vitro* conditions.

In vitro evaluation of two microbial antagonists *viz., Trichoderma viride* and *Pseudomonas fluorescens* were tested against the eight fungal pathogens isolated from gerbera. Both the fungal and bacterial antagonists restricted the radial growth of all the pathogens. The fungal antagonist, *Trichoderma viride* showed 61.1 per cent inhibition of *Alternaria alternata* which restricted the pathogen by overgrowth mechanism of inhibition. The results obtained are in line with Pandey (2010) and Rajkonda *et al.* (2011) where they revealed that fungal antagonist, *T. viride* and *T. harzianum* could delay the growth of *A. alternata* through overgrowth mechanism of inhibition. Similarly, another study by Taj and

Kumar (2012) on *in vitro* evaluation of biocontrol agents showed that, various strains of *Trichoderma harzianum* inhibited radial growth of the *Alternaria* sp. isolated from gerbera by 52.6 to 75.0 per cent. Dual culture technique of *Alternaria alternata* with bacterial antagonist, *Pseudomonas fluorescens* recorded a per cent inhibition of 46.6 per cent which was entirely analogous with the result of Maurya *et al.* (2014) where they observed a maximum inhibition of 48.1 per cent and a minimum of 44.4 per cent inhibition of *A. alternata.* Several other workers also contributed to the above findings in the *in vitro* evaluation of the fungal antagonist against *Alternaria alternata* (Harman, 2006; Brisa *et al.*, 2007 and Akbari and Parakhia, 2007).

In vitro studies with *Alternaria tenuissima* revealed that the two biocontrol agents, *Trichoderma viride* and *Pseudomonas fluorescens* achieved 63.3 and 57.7 per cent inhibition of the pathogen. However, contradictory to the above result, Ambuse *et al.* (2012) reported 80 per cent inhibition of *Alternaria tenuissima* with *Trichoderma viride.* Similar results were noticed by Taj and Kumar (2012) and Roopa *et al.* (2014). Moreover, Manjunatha *et al.* (2012) noticed very less inhibition of 20 per cent of *Alternaria sesami* with *Pseudomonas fluorescens*.

Dual culture technique of *Myrothecium roridum* along with *Trichoderma viride* noticed 70.0 per cent inhibition of the test pathogen. This result was consistent with Dighule *et al.* (2011) who reported 75 per cent inhibition of *M. roridum.* However, contrary to the above study Kale and Ukesh (2015) attained an inhibition ranging from 3.4 to 43.9 per cent of *M. roridum* isolated from soybean. In the case of *Pseudomonas fluorescens,* comparatively less inhibition of the pathogen (42.2%) was noticed. Amongst the biocontrol agents evaluated against *Ulocladium chartarum*, *Trichoderma viride* attained 66.7 per cent inhibition of the pathogen whereas 55.6 per cent inhibition was recorded with *Pseudomonas fluorescens*. *Ulocladium chartarum and Myrothecium roridum* being new pathogens reported from gerbera a search through the literature did not give any relevant information on *in vitro* studies with the biocontrol agents.

In reviewing the effect of antagonists against *Curvularia pallescens,* it was observed that *Trichoderma viride* and *Pseudomonas fluorescens* could inhibit the pathogen by 64.4 and 53.3 per cent respectively. Pawar *et al.* (2012) pointed out 83.3 per cent inhibition of the pathogen with *T. viride* and *P. fluorescens.* However, consistent results were obtained by Kithan and Daiho (2014) where they also reported 53.3 per cent inhibition of *Curvularia pallescens* with *P. fluorescens.*

Phytophthora cryptogea when evaluated against the biocontrol agents recorded a per cent inhibition of 68.8 and 44.4 with *Trichoderma viride* and *Pseudomonas fluorescens.* Perusal of the literature suggests that the present findings are in agreement with the reports by the other researchers. Shashidhara *et al.* (2008), Mir *et al.* (2011) and elucidated the efficacy of different strains of *Trichoderma* spp against *Phytophthora* by overgrowth mechanism of antagonism. Anith *et al.* (2002) and Paul and Sarma (2006) confirmed the efficacy of different strains of *P. fluorescens* against *Phytophthora capsici.* Similar results of inhibition by different strains of *P. fluorescens* and *Trichoderma* sp. were previously reported by several authors (Anandaraj *et al.,* 1995; Jahagirdar, 1998; Jubina and Girija, 1998; Anith and Manomohandas, 2001; Rajan *et al.,* 2002; Sid *et al.,* 2003; Zhang *et al.,* 2010; Hernandez *et al.,* 2011; Jagtap *et al.,* 2012 and Sumbula, 2015).

Data on the *in vitro* evaluation of the pathogen, *Fusarium solani* with the biocontrol agent showed that *T. viride* could inhibit the pathogen only by 48.8 per cent and *P. fluorescens* by 22.2 per cent. However, Jamwal and Jamwal (2011) and Taj and Kumar (2012) reported maximum inhibition of *Fusarium oxysporum* f. sp. *gerberae* (71.6%) and minimum inhibition of *Fusarium oxysporum* f. sp. *dianthi* (48.2%) by the biocontrol agent. Many workers have also reported the efficacy of *T. viride* and *P. fluorescens* against *F. oxysporum* and *F. solani* (Rini and Sulochana, 2007; Chavan and Hegde, 2009; Perveen and Bokhari, 2012; Choudhary *et al.,* 2012; Toua *et al.*, 2013; Lakshmidevi *et al.,* 2015 and Haggag *et al,* 2015).

The study on *in vitro* antagonism of *T. viride* against *Curvularia lunata* revealed that the fungal antagonist restricted the pathogen to 52.2 per cent by overgrowth mechanism whereas *Pseudomonas fluorescens* could restrict the pathogen upto 38.8 per cent only. Similar findings on inhibitory action of *T. viride* was proposed by previous workers (Chaudhary and Prajapati, 2004; Gangwar *et al.*, 2004 and Lal *et al.,* 2006). According to Bisht *et al.* (2013) and Tapwal *et al.* (2015) per cent inhibition was maximum when *Trichoderma harzianum* was used as the antagonist against *C. lunata*. Likewise, Kithan and Daiho (2014) observed better inhibition of *Curvularia lunata* with *P. fluorescens*.

5.5.3 *In vivo* evaluation of fungicides and bioagents on major fungal diseases of gerbera

In vivo management of plant diseases are usually based upon the results of the studies carried out under *in vitro* conditions where chemical or antagonists or plant extracts or any other antimicrobial agents are tested against the pathogen under aseptic conditions. Though the efficacy of fungicides and biocontrol agents can be proved under *in vitro* conditions effectively, it is evident that efficiency of the same cannot be ascertained under natural conditions. Moreover, the effect of antagonist always show a drop in inhibition on pathogen when the study is gradually shifted from *in vitro* set up to field conditions. Thus, it is significant to test the effect of fungicides and biocontrol agents under natural conditions which have been proved efficient under *in vitro* conditions.

Reading back the results of the survey conducted in three districts, three pathogens *viz., Alternaria tenuissima, Phytophthora cryptogea* and *Fusarium solani* were found to cause complete crop failure as *Alternaria tenuissima* (LB-2) recorded a per cent disease severity of 15 to 17 per cent in all the three seasons compared to *Alternaria alternata* (LB-1) which showed a PDS of only 6 to 16 per cent. Similarly, *Phytophthora cryptogea* and *Fusarium solani* being the root pathogens caused severe crop loss especially under polyhouse condition where the

crop is densely populated, genetically uniform and relative humidity is generally more than 60 per cent. Hence, the three pathogens though identified by morphological and cultural characters, were subjected to molecular characterization prior to *in vivo* evaluation so as to confirm the identity upto the species level. The molecular characterisation of the cultures were carried out at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram where they revealed that *Alternaria* and *Phytophthora* cultures showed sequence homology of 98 per cent with *A. tenuissima* and *Phytophthora cryptogea* respectively whereas the sequence of *Fusarium* culture showed cent per cent homology with *Fusarium solani.* Thus, the results of cultural and morphological characters were found in conformity with the results of molecular characterization.

Keeping all these in mind and for better understanding of the efficacy of fungicides, antagonists under natural conditions, an *in vivo* experiment was laid out to study the effect of fungicides and biocontrol agents selected from previous *in vitro* evaluation in the management of fungal diseases caused by *Alternaria tenuissima, Phytophthora cryptogea* and *Fusarium solani*

5.5.3.1 Management of Alternaria leaf blight

In vivo experiment carried out to find the effect of fungicides and the biocontrol agents *Trichoderma viride* against the pathogens revealed a per cent disease severity ranging from 8 to 15 per cent, ten days after challenge inoculation of the pathogen. Though, there was no significant difference among the treatments after ten days of first treatment spray, it was observed that T_5 (Propineb 70WP) and T_6 (*Trichoderma viride*) exhibited maximum inhibition while, the least per cent reduction of disease over control was noticed with T_1 (carbendazim (12%) + mancozeb (64%)) (0.2%) whereas the remaining treatments *viz.,* T_2 (cymoxanil (8%) + mancozeb (64%)), T_3 (carbendazim (12%) + mancozeb (63%)) and T_4 (copper hydroxide 77WP) recorded an efficiency of more than 55 per cent over control. After the second treatment spray, a significant difference was recorded on

the PDS among the treatments with T_1 (carbendazim (12%) + mancozeb (63%)) showing the highest per cent reduction of 90.9 per cent over control. While, the biocontrol agent T_6 (*Trichoderma viride*) showed the 63.9 per cent reduction after ten days of second spray. The effectiveness of *Trichoderma viride* on the pathogen during the first treatment spray may be attributed to the activation of defence responses in the plant and not due to its direct action on the pathogen. These findings are in tune with the reports of many workers who studied the effect of fungicides and biocontro agents on *Alternaria* leaf blight of gerbera and other ornamental crops. Kumar *et al.* (2011) noticed the effectiveness of hexaconazole (0.1%), carbendazim (12%) + mancozeb (63%) (0.2%) and propineb 70WP (0.2%) in reducing the incidence of chrysanthemum leaf blight caused by *Alternaria alternata* under field conditions which is found agreeable with the present study. Similarly, Nagrale *et al.* (2012) noticed efficacy of propineb (0.25%), difenoconazole (0.1%), hexaconazole (0.1%) against *Alternaria* leaf blight in gerbera under *in vivo* conditions. Balai and Singh (2013) also proved the efficacy of fungicides and bioagents against *Alternaria tenuissima* in pigeon pea.

5.5.3.2 Management of Phytophthora root rot

The results of the *in vivo* experiment against *Phytophthora cryptogea* after challenge inoculation revealed that, among all the treatments cent per cent disease incidence was noticed in treatments in treatments T_1 (propineb 70WP), T_4 (copper hydroxide 77WP) and T_7 (control) while the other treatments recorded a PDI of 80 per cent. However, after ten days of first and second spray, a comparative reduction in the disease incidence was noticed after two treatment sprays. The data revealed that treatments T_1 (propineb 70WP) (0.3%), T_2 (cymoxanil (8%) + mancozeb (64%)) (0.2%), T_3 (carbendazim (12%) + mancozeb (63%)) (0.2%) and T_4 (copper hydroxide 77WP) (0.2%) could reduce the incidence to some extent whereas T_5 (hexaconazole 5EC) (0.1%) and T_6 (*Trichoderma viride*) (2%) were incompetent in the management of the disease. With regards to the ineffectiveness of *T. viride* under field conditions, the main reasons attributed is that, under *in*

vivo condition, the establishment of the antagonists might have been affected by competition with the other soil pathogens. It is well known that biological control depends on the establishment and maintenance of a threshold population of antagonists in soil. Hence, more care should be taken to develop better delivery techniques and to understand more about the soil ecology so that the antagonist activity can be enhanced. Contradictory to the above study Fravel (2005) reviewed several biocontrol products against different ornamental diseases under green houses and nurseries. Similarly, Grasso *et al.* (2003) and Gade (2012) evaluated the efficiency of *Trichoderma viride* against *Phytophthora* root rot in gerbera and citrus respectively. The present finding is in agreement with the studies of previous workers who reported the efficacy of chemical fungicides in the management of *Phytophthora* foot rot (Kaur *et al.,* 2009; Meyer and Hausbeck, 2013; Sumbula and Mathew, 2015).

5.5.3.3 Management of Fusarium wilt

Attempts were made to study the effect of fungicides and biocontrol agents against *Fusarium* wilt in gerbera where first treatment spray was made after challenge inoculation of the pathogen. It is evident from the data that after first and second treatment spray, the treatment T_1 (carbendazim (12%) + mancozeb 63%) (0.2%) was found very effective as it could considerably reduce the disease incidence. Similarly, treatment T_5 (difenoconazole 25EC) (0.1%), T_6 (*Trichoderma viride*) (2%), T_2 (tebuconazole 250EC) (0.15%), T_3 (copper hydroxide 77WP) (0.2%) and T_4 (pyraclostrobin 20WG) (0.05%) performed well in reducing the disease incidence. Sharma (2000) opined that application of *Trichoderma* sp. along with potting media effectively suppressed *Fusarium* wilt in carnation. Similarly, Padghan and Gade (2006) also noticed efficacy of different strains of *Trichoderma* spp. in the management of root rot complex of gerbera caused by *Fusarium oxysporum* f. sp. *gerberae* and *Pythium irregulare*. Thus, both the studies were found comparable with the results of the present investigation. According to Raj *et al.* (2005) and Chandel and Tomer (2007), the

combination fungicide Saaf at 0.2 per cent could effectively reduce the disease incidence of *Fusarium* wilt in gladiolus which was in accordance with the present finding. It well said that the results of *in vitro* studies do not always correlate with the results of *in vivo* experiments. Hence, care should be taken while selecting the fungicides and biocontrol agents for large scale field experiments after giving a provision for conducting several *in vitro* and *in vivo* trials.

Recalling back the results obtained with respect to survey conducted in three districts *viz.,* Wayanad, Malappuram and Thrissur on fungal diseases of gerbera*,* three leaf blights caused by *Alternaria alternata, Alternaria tenuissima, Myrothecium roridum,* two leaf spots by *Ulocladium chartarum*, *Curvularia pallescens,* two powdery mildew pathogens, *Golovinomyces cichoracearum* and *Podosphaera* sp., petal blight by *Curvularia lunata* and root rot and wilt by *Phytophthora cryptogea* and *Fusarium solani* are documented in gerbera from Kerala. Among the various diseases reported, the most destructive diseases observed in gerbera during the survey were *Alternaria* leaf blight, powdery mildew, root rot and wilt. Search on literature revealed very few studies regarding the pathogen, *Ulocladium* sp. and *Myrothecium* sp. infecting other crop plants. It is worthwhile to mention that this may be the first report of leaf blight and leaf spot caused by *Myrothecium roridum* and *Ulocladium chartarum* on gerbera. Moreover, the observations made through the studies have strongly warranted that *in vitro* and *in vivo* results with fungicides and biocontrol agents against pathogens do not always reflect what happens in the field. Hence, the study should be complemented by varietal screening and multilocational field trials to prove the effectiveness of the aforesaid fungicides and biocontrol agents in the management of fungal diseases of gerbera. It may be concluded that the present study has enlightened our knowledge on the various fungal diseases of gerbera prevailing in Kerala and thrown light on the management of the major dreadful disease infecting the crop.

Summary

6. SUMMARY

Gerbera, a commercial cut flower crop stands in the fourth place in the international market. The crop is inflicted with a number of fungal diseases which reduces the economic value in the floriculture trade. Hence, the present study "Cataloguing and documentation of fungal diseases of gerbera" was proposed with an objective to examine the causal agents of diseases, to study their symptomatology, cultural, morphological as well as management of fungal pathogens.

1. A purposive sampling survey in three districts *viz.,* Thrissur, Malappuram and Wayanad during three seasons under polyhouse and open field conditions revealed the incidence of leaf blights, leaf spots, powdery mildew, flower disease and root rot diseases.

   - Based on the distinct symptoms, diseases were categorized into leaf blight 1 (LB-1), leaf blight 2 (LB-2) and leaf blight 3 (LB-3), leaf spot 1 (LS-1) and leaf spot 2 (LS-2) and powdery mildew as foliage diseases, petal blight as flower disease and root rot and wilt as root diseases.
   - In Thrissur, diseases like LB-1, LB-3, LS-1, root rot and wilt were observed where LS-1 disease recorded a PDI and PDS of 78.2 and 19.4 per cent followed by LB-1 with PDI and PDS of 74.7 and 16.0 per cent respectively. For root diseases, PDI of 69.4 and 15.5 per cent were recorded for root rot and wilt diseases.
   - Survey in Malappuram district revealed that among LB-1, LS-2 and petal blight, LB-1 recorded highest PDI and PDS of 82.8 and 10.2 per cent whereas petal blight was the only flower disease observed during the survey.
   - In Wayanad, LB-1, LB-2, petal blight and powdery mildew were observed where powdery mildew recorded a PDI and PDS of 95.2 and 57.4 per cent.

- Leaf blight, LB-1 was observed in gerbera grown both under protected and open field conditions whereas LB-2 was confined to open and LB-3 under protected field conditions.

2. Correlation studies between disease development and weather parameters showed the seasonal influence of disease occurrence.

- The disease LB-1 was found positively correlated with temperature and reverse relation existed between relative humidity and rainfall at Wayanad and Malappuram, whereas, it was found positively correlated with temperature in Thrissur district
- Disease LB-2 was observed only in hilly tracts of Wayanad where it was correlated positively with temperature with no significant influence with relative humidity and rainfall.
- Diseases like LS-1 and LS-2 did not show any significant correlation with weather parameters.
- Powdery mildew disease showed negative correlation with temperature and rainfall whereas positive correlation with relative humidity which attributed to the high per cent disease severity during winter season.

3. Isolation of pathogens from diseased samples collected during the survey yielded eight isolates and pathogenicity was established by Mycelial Bit Inoculation Method (MBIM) for foliage diseases and Micro Droplet Inoculation Technique (MDIT) for foliage, root and flower diseases.

4. Symptomatology studies were carried out both under natural and artificial conditions.

- Leaf blight 1 (LB-1) under natural conditions, exhibited scattered yellow chlorotic spots on the leaf lamina which converted into dark brown concentric rings which coalesced to form marginal blightening symptoms.

- Leaf blight 2 (LB-2) showed marginal blightening symptom without the formation of concentric rings.
- Leaf blight 3 (LB-3) was found infecting younger leaves where the symptoms initiated with the formation of water soaked lesions which enlarged into blightened areas.
- Leaf spot 1 (LS-1) produced scattered dark brown necrotic spots whereas in LS-2, minute numerous spots showing shot hole symptoms were noticed.
- Symptoms of powdery mildew showed white powdery growth on adaxial surface of leaf lamina which later turned into necrotic lesions.
- Petal blight was noticed as blightening of petals with shot holes.
- In the case of root rot disease, apart from rotting of roots, leaves showed dark water soaked lesion whereas in wilt disease, yellowing of leaves was also observed.

5. Cultural and morphological characterisation of pathogens were carried out which was confirmed to the species level with the reports of National Centre for Fungal Taxonomy (NCFT), New Delhi.

- Leaf blight 1 (LB-1) pathogen was identified as *Alternaria alternata,* LB-2 as *Alternaria tenuissima,* LB-3 as *Myrothecium roridum,* powdery mildew pathogens as *Golovinomyces cichoracearum* and *Podosphaera* sp., petal blight pathogen as *Curvularia lunata,* root rot pathogen as *Phytophthora cryptogea* and wilt pathogen as *Fusarium solani.*

6. *In vitro* evaluation of fungicides and bioagents against fungal pathogens revealed that:

- Bordeaux mixture inhibited the radial growth of *M. roridum* and *P. cryptogea* which showed comparatively less inhibition with other pathogens.

- Propineb 70WP at all three concentrations attained total inhibition of *A. alternata, M. roridum U. chartarum, C. pallescens, C. lunata* and *P. cryptogea* while, copper hydroxide 77WP showed cent per cent inhibition against *M. roridum, U. chartarum, C. lunata, P. cryptogea* and *F. solani.*

- *Alternaria alternata* showed 80 to 100 per cent inhibition of the pathogen where cent per cent inhibition was observed with hexaconazole 5EC, tebuconazole 250EC and difenoconazole 25EC.

- Systemic fungicides *viz.,* carbendazim 12% + mancozeb 63%, cymoxanil 8% + mancozeb 64%, tebuconazole 250EC, hexaconazole 5EC and difenoconazole 25EC restricted the growth of *A. tenuissima* showing cent per cent inhibition.

- In the case of *M. roridum,* except hexaconazole 5EC, all other fungicides showed cent per cent inhibition. All systemic fungicides except hexaconazole 5EC and difenoconazole 25EC showed complete inhibition of *U. chartarum.*

- Cent per cent inhibition of *C. pallescens* was noticed with all systemic fungicides.

- Systemic fungicides like carbendazim 12% + mancozeb 63%, cymoxanil 8% + mancozeb 64%, tebuconazole 250EC, hexaconazole 5EC against *Curvularia lunata* and *Phytophthora cryptogea* showed cent per cent inhibition whereas *Fusarium solani* exhibited a varying inhibition of 58 to 100 per cent.

- *In vitro* evaluation of bioagents revealed that fungal antagonist, *Trichoderma viride* showed per cent inhibition of 48.8 to 70 per cent of all the fungal pathogens tested when compared to the bacterial antagonist, *Pseudomonas fluorescens* which showed 22.2 to 57.7 per cent only.

7. Three pathogens *viz., A. tenuissima, P. cryptogea* and *F. solani* were subjected to molecular characterisation which was carried out at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram where the results showed cent per cent identity with *Fusarium solani,* 99 and 98 per cent with *A. tenuissima* and *P. cryptogea.*

8. *In vivo* evaluation of *Alternaria* leaf blight, *Phytophthora* root rot and *Fusarium* wilt were carried out using fungicides and bioagents which showed promising result under *in vitro* evaluation.

- Carbendazim 12% + mancozeb 63% showed 90 per cent disease reduction against *Alternaria* leaf blight.
- Propineb 70WP, cymoxanil 8% + mancozeb 64% (0.2%) and copper hydroxide 77WP (0.2%) showed 40 per cent disease reduction against *Phytophthora* root rot.
- Cent per cent disease reduction was noticed with carbendazim 12% + mancozeb 63% (0.2%), difenoconazole 25EC (0.1%) copper hydroxide (0.2%) and *Trichoderma viride* (2%) against *Fusarium* wilt.

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Abstract

CATALOGUING AND DOCUMENTATION OF FUNGAL DISEASES OF GERBERA

by

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ABSTRACT OF THE THESIS
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ABSTRACT

Gerbera, a commercial cut flower crop, has widespread acceptance in the international market due to its vibrant colours, shapes, excellent vase life and handling quality. One of the major limiting factors which reduce the commercial value of the crop is the incidence of diseases. Hence, the present investigation was undertaken to identify the fungal diseases of gerbera occurring in Kerala.

A purposive sampling survey was conducted in three districts *viz.,* Thrissur, Malappuram and Wayanad during rainy (July-August), winter (November-December) and summer (March-April) seasons for monitoring disease occurrence and sample collection both under protected and open field conditions. Three leaf blights (LB-1, LB-2 and LB-3), two leaf spots (LS-1 and LS-2) and powdery mildew (PM) were the foliage diseases observed during the survey. Petal blight was the only flower disease noticed apart from root rot and wilt disease. Per cent disease incidence (PDI) and per cent disease severity (PDS) assessed during the survey revealed that powdery mildew showed the highest per cent disease incidence and per cent disease severity. Besides, per cent disease severity (PDS) of leaf blight-2 and root rot was also comparatively higher. Correlation studies of weather parameters with disease severity could elucidate the seasonal influence in disease development.

Eight pathogens were isolated from diseased samples and pathogenicity of each isolate was proved by Mycelial Bit Inoculation Method (MBIM) for foliage diseases and Micro Droplet Inoculation Technique (MDIT) for foliage, flower and root diseases. Pathogenicity test for each disease also aided in relating symptoms of the disease under artificial conditions. Under natural conditions, leaf blights, LB-1 and LB-2 showed marginal blightening with distinct symptom development and black water soaked lesions noticed for LB-3 disease. Leaf spot-1 (LS-1) appeared as dark brown necrotic spots scattered on the leaf lamina between the major veins whereas leaf spot-2 (LS-2) produced numerous spots with shot holes.

Petal blight showed blightening resulting in shot holes whereas, white powdery growth appeared on the leaf lamina in the case of powdery mildew disease. Rotting of roots were noticed for root rot as well as for wilt disease.

Cultural and morphological characterisation of each pathogen was carried out for the identification of pathogens and the identity was confirmed by National Center for Fungal Taxonomy (NCFT), New Delhi. The pathogens causing LB-1, LB-2 and LB-3 were identified as *Alternaria alternata*, *Alternaria tenuissima* and *Myrothecium roridum* respectively. The pathogens responsible for leaf spots (LS-1 and LS-2) were identified as *Ulocladium chartarum* and *Curvularia pallescens* and for powdery mildew as *Golovinomyces cichoracearum* and *Podosphaera* sp. *Curvularia lunata* was identified as the causal organism of petal blight. Moreover, root rot and wilt disease observed during the survey were caused due to *Phytophthora cryptogea* and *Fusarium solani* respectively.

In vitro management studies of each pathogen revealed that tebuconazole 250EC and propineb 70WP showed promising results against foliage diseases. For root diseases, systemic fungicides like hexaconazole 25EC, tebuconazole 250EC, pyraclostrobin 20WG, combination fungicide, carbendazim 12% + mancozeb 63% and contact fungicides like copper hydroxide 77WP and Bordeaux mixture performed exceptionally well by inhibiting the radial growth of the pathogen. Biocontrol agents like *Trichoderma viride* and *Pseudomonas fluorescens* tested against each pathogen showed that the fungal antagonist was found more efficient than the bacterial antagonist. Prior to the management studies, the identity of pathogens were reassured by analyzing the sequence of the pathogens in Blastn programme where the sequencing have been carried out at RGCB, Thiruvananthapuram. Management of three major pathogens *viz., Alternaria tenuissima, Phytophthora cryptogea* and *Fusarium solani* with chemical fungicides and bioagents were carried out under *in vivo* conditions. Compared to other systemic and contact fungicides, the combination fungicide, carbendazim 12% + mancozeb 63% (0.2%) recorded 90 per cent reduction of *Alternaria* leaf

blight disease. The per cent disease incidence of *Phytophthora* root rot was reduced considerably by the application of contact fungicides, propineb 70WP (0.3%) cymoxanil 8% + mancozeb 64% (0.2%) and copper hydroxide 77WP (0.2%). Fungicides like carbendazim 12% + mancozeb 63% (0.2%), difenoconazole 25EC (0.1%), copper hydroxide 77WP (0.2%) and *Trichoderma viride* (2%) showed noticeable reduction in per cent disease incidence of *Fusarium* wilt.

It is worthwhile to mention that this is the first report of *Ulocladium chartarum* and *Myrothecium roridum* on gerbera. It may be concluded that the present study has enlightened our knowledge on the various fungal diseases of gerbera prevailing in Kerala and thrown light on their management aspects.