CATALOGUING AND MANAGEMENT OF MAJOR DISEASES OF MONOPODIAL ORCHIDS

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THESIS

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DECLARATION

I, hereby declare that the thesis entitled **"Cataloguing and management of major diseases of monopodial orchids."** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Cataloguing and management of major diseases of monopodial orchids."** is a bonafide record of research work done independently by **Ms. Meera T.M. (2010-11-122)** under my guidance and supervision and that it has not formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Introduction

1. INTRODUCTION

Orchids are the largest group of flowering plants belong to the most diverse family Orchidaceae, consists of about 800 genera and 25,000 species and lakh man-made hybrids. Orchids have varying habitats but epiphytic orchids dominate the trade. They are also classified as monopodials (stems having a vertical growth, non-branching, with aerial roots) and sympodials (stems having a horizontal growth, producing pseudobulbs in clusters with no aerial roots).They are unique group of plants and exhibit incredible range of diversity in size, shape, number, colour and fragrance of flower. Orchids are well known throughout the world for their beautiful, myriad shaped, multi coloured, and long lasting quality flowers and constitute an order of royalty in the world of ornamental plants. Orchids are abundant in tropical countries in South East Asia, South and Central America and South Africa. India is blessed with an abundance of orchid flora with Himalayas as their main habitat and others scattered in Eastern and Western Ghats.

In recent years due to change in the aesthetic values of the society and the awareness of the export potentiality of the flowers, a radical change has taken place in the cultivation of orchids as an enterprise by most of the farmers and industrialists. The orchids are marketed both as potted plants and as cut-flowers. In the past few years, the orchid trade has increased both in volume and value throughout the world.

Monopodials have recently gained popularity due to the availability of large number of varieties and hybrids including intergeneric ones that show a wide range of variability in floral characters. The cost of production is comparatively less as most of them growing under open condition. According to Rajeevan (2011), monopodial orchids are classified into three types based on mode of growth. They

are short stemmed epiphytes (Phalaenopsis), intermediates (Basket Vanda and Mokara) and tall growing orchids (Arachnis, Aranthera, Aranda, Terete and semiterete Vanda).

Many diseases are reported from orchids and some of them are major production constraints. Diseases of orchids are caused by fungi, bacteria, and viruses. They are classified as leaf spots, flower blights, and root, stem and pseudobulb rots, which are the most serious. Several plant diseases caused by phytopathogens lead to impact in both quality and quantity of orchid production and resulted in profit loss. Even though literature on diseases of sympodial orchids is available, it is meager in the case of monopodial orchids.

In Kerala, most of the orchid growers are non-conventional agriculturists and are mostly unaware of disease problems and their management. As, no systematic work has been carried out on orchid diseases in Kerala, the orchid growers have to depend on reports from other countries for the information on diseases and its management. So an investigation has been undertaken to study in detail the symptomatology, etiology and management of different diseases associated with monopodial orchids *viz*., Phalaenopsis, Basket Vanda, Mokara and Arachnis.

The investigation was carried out on the following aspects of disease

- Symptomatology of diseases
- Characterisation of pathogens
- Seasonal influence on occurrence of disease
- Disease management

Review of Literature

2. REVIEW OF LITERATURE

Orchid is an important commercial flower crop grown in India. The plant is getting popularity due to its profitable proposition. Orchids are noted for their bewitchingly beautiful, long-lasting flowers, widely differing in shape, size and colour.

They have varying habitats but epiphytic orchids dominate the trade. Based on the growth habit, orchids are classified as monopodials and sympodials. Monopodial orchids have a main stem which continue to grow year after year and sympodials have a main stem which terminate growth at the end of each season. Based on growth habit, monopodials are grouped into, short stemmed epiphytes, intermediates and tall growing ones. Phalaenopsis is a short stemmed epiphyte, Mokara and basket Vanda are intermediates whereas Arachnis, Aranthera, terete and semiterete vanda are tall growing types of Monopodial orchids. The important monopodial orchids grown in Kerala are Phalaneopsis, Arachnis, Vanda, Ascocentrum, Rhyncostylis (Rajeevan, 2011).

In the present investigation, diseases of monopodial orchids *viz*., Phalaenopsis (*Phalaenopsis* sp.), Basket Vanda (*Vanda* sp.), Mokara, a trigeneric hybrid orchid (*Arachnis* x *Ascocentrum x Vanda),* and Arachnis *(Arachnis sp.)* were studied in detail.

2.1. DISEASE OCCURRENCE

According to Uchida (1994*) Phytophthora palmivora, P. nicotianae and P. cactorum* are the common black rot pathogens of Vanda, Dendrobium, Cattleya and Cymbidium. Jin *et al*. (1994) isolated *Erwinia chrysanthemi* from Phalaenopsis showing soft rot symptom in Korea Republic. Sreedharan *et al.* (1994) first reported the moderate to severe outbreak of anthracnose in orchids of Kerala incited by *Glomerella cingulata*. Uchida (1994) reported fungal pathogens

frequently associated with common orchid genera in Hawaii include *Alternaria* sp., *Bipolaris* sp., *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Lasiodiplodia theobromae*, *Phoma* sp., *Phytophthora cactorum*, *Phytophthora palmivora*, *Sclerotium rolfsii*, *Cercospora* sp., *Pythium ultimum* etc. Duff (1997) reported major pathogens of orchids in the northern territory as *Erwinia carotovora*, *Fusarium* sp., *Alternaria alternata*, *Sclerotium rolfsii*, *Colletotrichum gloeosporioides* etc.

SoonYeong *et al.* (1998) first reported the infection of *Phytophthora palmivora* in Cheju Island as the causal organism of crown rot of Cymbidium. Wey (1988) from a survey concluded that soft rot (*Erwinia chrysanthemi*), black rot (*Phytophthora palmivora*) are the important diseases of orchids in Taiwan. Bag (2006) reported orchid wilt caused by *Sclerotium rolfsii* on Vanda group of orchids in Sikkim. Bag (2007) concluded that out of 29 native orchid genera belonging to 85 species, only four orchid species were found free from infection by *Colletotrichum gloeosporioides*.

Cating *et al.* (2008) first reported the bacterial soft rot on Vanda orchids caused by *Erwinia chrysanthemi* in United States. Yadav *et al.* (2010) first reported leaf spot of orchids caused by *Alternaria alternata i*n Uttarkhand. Bag (2011) pointed out major diseases of orchids in Sikkim as Black rot (*Phytophthora* sp.), Sclerotium wilt (*Sclerotium rolfsii*), anthracnose (*Colletotrichum gloeosporioides*), leaf spot (*Fusarium* sp.) etc. Pant *et al* (2011) described the most important diseases in the north eastern states of India. The main diseases are black rot (*Phytophthora* sp.), anthracnose (*Colletotrichum gloeosporioides*), orchid wilt (*Sclerotium rolfsii*), leaf blight (*Fusarium oxysporum*), leaf spot (*Alternaria alternata, Fusarium* sp.), soft rot (*Erwinia* sp.) etc.

Important disease reported from Kerala are leaf spot caused by *Colletotrichum* and *Gloeosporium*, collar rot and orchid wilt caused by *Sclerotium*

rolfsii (KAU, 2011). Rajeevan (2011) listed out the main diseases of orchids in Kerala and they include leaf spot (*Colletotrichum* and *Gleosporium*), leaf blight (*Pythium*), collar rot (*Sclerotium*, *Fusarium*) and black rot (*Phytophthora*).

2.2. SYMPTOMATOLOGY

2.2.1.Fusarium wilt

Kim *et al.* (2002) reported that root rot of moth orchid, Phalaenopsis was caused by *Fusarium* spp. including *F. solani, F. oxysporum and F. proliferatum* and resulted in 30 per cent of disease incidence in some greenhouses. The symptoms were characterized by dark-brown to black discoloration on roots which rotted in dry condition. Lower leaves of severely diseased plants turned yellow and blight later.

Lee *et al.* (2002) explained the symptoms of dry rot on cymbidium caused by *Fusarium* sp. Symptoms on the basal parts of the leaves appeared as brown to dark-brown, irregularly advancing lesions from the pseudobulbs. Infected leaves generally showed yellowing as the disease developed, then later showed blighting. Infected pseudobulbs and roots turned dark-brown to black and became rotten in dry condition. The strongly pathogenic isolates of *Fusarium* sp. induced severe dry rot of pseudobulbs and roots of the host plants. The symptoms progressed up to the basal part of the leaves, which later caused blight of the entire plant.

Hsieh (2005) reported wilting, yellow leaves and root rot of Phalaenopsis infected by *F. solani* and *F. proliferatum*. Kim and Chun (2007) also reported root and basal stem rot of moth orchid. Wilting occurred on orchid plants at initial stage and the infected leaves turned yellow to red. The diseased leaves abscised after a short period of time, and eventually the infected orchid plants died.

Su *et al*. (2010) reported symptoms of Phalaenopsis Fusarium wilt in detail. Plants first showed symptoms on the lower leaves which turned yellow with black sheath rot. The diseased leaves abscised after a short period of time and eventually the infected orchid plants died. Several small red granules (perithecia) with white mycelia were usually observed on the discolored sheath surface. In some cases, surfaces of roots and stalks displayed brown to black rot with perithecia.

Palmer (2011) explained the symptoms of Fusarium wilt in orchids as severe yellowing of leaves, collapse of the leaf stalk and wilting of entire plant. The rhizome of infected orchid developed a pink discolouration which eventually spread through the whole rhizome and to the pseudobulb.

2.2.2. Collar rot

Bag (2003) explained the symptoms produced by *Sclerotium rolfsii* on some Indian orchids. Symptoms include basal rot of pseudo bulb, yellowing and detachment of leaf from the psedobulb. Numerous small brown coloured sclerotia and white mycelial growth appeared in the leaf bases which resulted in the death of entire plant.

Bag (2004) observed two new orchid hosts *Phaius flavus* and *Paphiopedilum venustum* of *S.rolfsii* in Sikkim. On *Phaius flavus*, the disease caused basal rotting of pseudobulbs during the initial stages of infection. The leaves became yellow and detached from the pseudobulbs. Gradually, the disease spread upwards until the entire plant turned brown to black and died. On *Paphiopedilum venustum*, the disease appeared at the collar region as a soft rot, which gradually moved upwards, resulting in leaf collapse. Numerous small brown spherical sclerotia and white mycelial growth were observed on the pseudobulbs, leaf base and along the leaf sheaths.

Bag (2006) reported orchid wilt on Vanda group of orchids caused by *S. rolfsii*. He reported that the disease caused basal rot on pseudostem at initial stage and the disease gradually spread upwards, the leaves turned yellow and got detached from the stems and ultimately caused the mortality of infected plants.

Cating *et al.* (2009a) reported symptoms caused by *S. rolfsii* on Ascocentrum and Ascocenda orchids in Florida. Affected Ascocentrum and Ascocenda orchids were severely wilted at the apex, while around the base of the plants, tan, soft, water-soaked lesions were present. As the lesions progressed, leaves around the base of the plants began to fall off, leaving the stem bare. After 2 days, white, flabellate mycelium was seen progressing up the stem and numerous, tan-to-brown sclerotia were appeared.

2.2.3.Anthracnose

Uchida (1994) explained the symptoms caused by *Colletotrichum gloeosporioides* in orchids as small restricted, black, sunken lesion on the leaves especially at the leaf base. She also explained the symptoms of black rot of Vanda caused by *Phytophthora palmivora*, *P. nicotianae*, *P. cactorum* and *P. cinnamomi* as water-soaked brown to brownish black becoming yellow as the disease progressed and pseudobulb and canes are readily rotted through leaf or root infection.

Suk Young *et al.* (1996) described the symptoms produced by anthracnose fungi *C. gloeosporioides* as dark brown to black spots appeared on naturally infected leaves of orchids and severe infection resulted in leaf blight or plant death.

According to Akrapikulchart (2009), at first the spots of anthracnose of Dendrobium may occur on the leaves or tubers and vary from yellowish to light brown and more or less circular, soft and sunken. Lesion may coalesce quickly to form large necrotic areas that may contain small black fungal structures. The

surfaces of the lesion become covered with the wet, gelatinous spores from acervulus with numerous setae.

2.2.4. Black rot

Batchelor (1980) explained about black rot of Cattleya caused by *Phytophthora cactorum* and *Pythium ultimum*. They attack any part of an orchid, generally working from the bottom up or the top down. From there, the infection can spread the entire length of the rhizome. If the infection begins in leaf or pseudobulb, it may begin as a purplish-black softened spot, but can likewise spread downward with alarming speed.

Ann (1995) observed that *Phytophthora* infection on Phalaenopsis, Dendrobium and Oncidium developed rapidly and turned dark green or light brown, the affected leaves became yellow and dropped and eventually the whole plant died. *P. palmivora* induced typical black rot symptoms on leaves and pseudostems of affected Cattleya and *Cymbidium* spp. *Phytophthora* spp. mainly attacked pseudostems and young buds to cause yellowing, wilting and death of affected plants.

SoonYeong *et al.* (1998) reported the infection of *P. palmivora* as the causal pathogen of Phytophthora crown rot of Cymbidium. The disease initiated at the basal portion of the infected plant and progressed upwards to the younger leaves. Soon after, distinct water-soaking lesions appeared on the lower leaves and the plant became wilted, blighted and died.

Bamba and Wall (2007) explained the symptoms of black rot of Vanda as black necrotic areas radiating from the base of the leaf and with distinct margins and white fungal growth on the necrotic areas. Rot progressed on the plant at three weeks' time.

Cating *et al.* (2009b) explained the symptoms produced by *Phytophthra palmivora* and *P.cactorum* in orchid as small black lesions on the roots or basal

portion of the pseudobulb. Later these lesions enlarged and spread to entire pseudo bulb and leaf.

2.2.5. Alternaria leaf spot

Symptoms of leaf spot caused by *Alternaria alternata* on *Cymbidium* sp. in Uttarakhand was explained by Yadav *et al.* (2010). Pathogen produced symptoms that started from the tip or margin of the leaves and progressed up to the basal part of the leaves, which later caused blight of the entire plant.

2.2.6. Fusarium leaf spot

Ichikawa and Aoki (2000) studied the symptoms of leaf spot of Cymbidium caused by *Fusarium subglutinans* and *F. proliferatum*. They observed two types of spots *viz.*, yellow spot and black spot. Yellow spot was characterized by small water-soaked patches appeared on the leaves which subsequently enlarged, center became sunken, turned reddish brown and surrounded by yellowish swellings without definite borders. In the black-spot type of the disease, minute black speckles appeared on the leaves at an early stage and then enlarged to irregular, angular black spots.

2.2.7. Soft rot

Abdullah and Kadzimin (1993) studied symptoms of bacterial soft rot of orchids caused by *Erwinia chrysanthemi*. Initial symptom was a water soaked rot which enlarged rapidly with no apparent yellowing of the margin after 1-2 days during wet periods. On seedlings, soft rot commonly occurs at the base of the leaves, thus resulting in the death of the plants soon after infection. Jin *et al.* (1994) isolated *E. chrysanthemi* from Phalaenopsis and explained the symptoms of soft rot and leaf spot.

2.2.8. Bacterial black spot

A bacterial black spot caused by *Burkholderia* sp. was observed by Takahashi *et al.* (2004) in orchids in Japan. The typical symptoms on the leaves were dark or black spots with yellow halo. A bacterial disease characterized by small to large leaf spots with or without water-soaking or soft rots was observed on various orchid genera, including *Dendrobium*, *Oncidium*, *Miltonia* spp. and hybrids. The pathogenic bacterium was identified as *B. gladioli* (Keith *et al.*, 2005).

2.3. CHARACTERISATION OF PATHOGENS

2.2.1. *Fusarium* sp

Ichikawa and Aoki (2000) described the cultural and morphological characters of *Fusarium subglutinans* causing leaf spot disease of Cymbidium orchid. Fungus produced a white to beige colony with a purple center on the surface and on the reverse side, beige to yellowish pink colony with a purple center. The colony surface was cottony to powdery with abundant aerial mycelia. Macroconidia were falcate, 1-5-septate, hyaline, and smooth, 41 - 77 µm x 4.3 - 5.5 µm in size. Microconidia were produced in false heads and were ovoid to ellipsoid, 0-1-septate, hyaline, smooth, 8 - 26 µm x 3.6 - 4.8 µm. Conidiophores were cylindrical, branched or unbranched, with mono- and polyphialides on their apices and chlamydospores were not observed.

Lee *et al.* (2002) explained the morphological characteristics of *Fusarium oxysporum* of Cymbidum orchid. Microconidium was oval to cylindrical in shape with 3-25 x 2-5µm in size and that of macroconidium was 16 - 60 x 2 - 6µm with 3 - 5 septate.

Zakaria *et al.* (2009) reported the morphological characters of *Fusarium oxysporum* associated with root and stem rot of Dendrobium orchid. It was identified based on the production of microconidia in false heads borne on short monophialides. The microconidia were oval to kidney shaped and macroconidia straight to slightly curved and abundant chlamydospore.

2.2.2. *Sclerotium* sp

Kuekulvong (2008) explained about cultural and morphological characters of *Sclerotium rolfsii* in Dendrobium orchid. Silky white hyphae tend to aggregate into rhizomorphic cords. In culture, the whole area of Petri plate was rapidly covered with mycelium including aerial hyphae which may cover the lid of plate. Sclerotia began to develop after 4-7 days of mycelial growth. Two types of hyphae were produced, coarse, straight large cells (2 - 9µm x 150 - 250µm) have two clamp connections at each septation and other type was slender hyphae (1.5 - 2.5µm diameter) which tend to grow irregularly and lack clamp connections.

According to Hajara (2011) the growth of *S. rolfsii* infecting fruit crops and ornamentals was white and fluffy, hyphae branched and septate, breadth of hyphae ranged from 4.25 to 8.93 µm and the length ranged from 52.6 to 196.32 µm.

2.2.3. *Colletotrichum* sp

Conidia of anthracnose fungi *Colletotrichum gloeosporioides* infecting Dendrobium averaged 15.9 µm in length and 5.4 µm in width (Uchida, 1994). Cabrera *et al.* (2003) explained the characters of *C. gloeosporioides* infecting orchids in north east of Argentina. Culture on potato-glucose-agar yielded abundant, gray aerial mycelium and unicellular, hyaline, oblong conidia, with rounded ends. Conidial size ranged from 16.0 to 24.0 µm x 4.0 to 6.0 µm and setae were straight and dark.

Davis (2003) studied about *C. gloeosporioides* of ivy gourd and observed the colony as fast growing, dark brown in colour and with pink pigmentation after few days. Hyphae branched, hyaline with 3.8 µm width and septate at an interval of 11.6 - 19.4µm. Conidia hyaline, cylindrical with both ends round, aseptate with 11.7 x 3.9 µm in size.

Wijesekara and Agarwal (2006) studied taxonomic characters of *C. gloeosporioides* of various crops and they explained the colonies with sparse to wool mycelium, at first creamy white turning to greyish black with age. Conidia single celled, hyaline, straight, cylindrical, ellipsoid or slightly curved and measured 8.54 - 21.95µm in size.

2.2.4. *Phytophthora* sp

Culture of *Phytophthora cactorum* usually slightly radiate with uniform slight aerial mycelium. Hyphae normally less than 6µm wide and irregularly swollen without characteristic hyphal swellings. Sporangia abundant on the media, broadly and regularly ellipsoid or ovoid to obpyriform, 36 - 50 x 28 - 35 µm, apex with a conspicuous hemispherical papilla with apical thickening upto 5 µm deep, deciduous with a pedicel up to 4µm long occluded by the septal plug. Oogonia 19 - 38 µm diameter, spherical or tapering to the base, wall thin and colourless or slightly yellow. Oospore aplerotic, 20 - 26 µm diameter, wall colourless and 2 µm in diameter. Anthredia nearly spherical to irregularly club shaped, always seen close to oogonial stalk (Waterhouse and Waterston, 1966).

Yeh *et al.* (1998) isolated black rot pathogen *P. palmivora* from diseased Cattleya plants and all tested isolates formed white colonies without special patterns on medium. Sporangia were elongated ellipsoid or elongated ovoid, papillate, and deciduous with pedicel length less than 5 µm. Average range of

length and width of sporangia were 44.3 - 51.0 x 26.1 - 29.7 µm with L/W ratios of 1.61 - 1.75.

2.2.5. *Alternaria* sp

As literature on cultural and morphological characters of the pathogen from orchids is not available, that of from other crops is mentioned here. According to Davis (2003), *Alternaria alternata* formed dark brownish black coloured colony and had a velvety appearance with dark purplish tinge on the upper surface. Hyphae brownish black with 3.8 - 5.8 µm width, septate at 15.4 - 30.9 µm intervals. Size of conidia observed was 19.5 - 31.2µm x 7.8 - 15.6 µm with 3 - 5 transverse and 1 - 3 longitudinal septa.

Resmi (2005) also studied the cultural and morphological characters of *A. alternata* of cucurbits. She observed brownish grey, thick and velvety colony. Hyphae brownish grey coloured with 4.03 x 16.12 - 28.21 µm in size and conidia formed in chain, straight, obclavate, smooth, brown and measured 12.09 - 52.39 x 4.03 - 16.12µm in size.

According to Sangeetha (2009), *A. alternata* a leaf blight pathogen of mango seedlings initially formed a grey coloured mycelium which later changed to greyish black coloured and the colony was thick and smooth. She also noted the morphology of the pathogen. Hyphae brown coloured with 5 µm width and septate at an interval of 15 - 30 µm. Conidia brown, obclavate, straight, 25 - 60 x 7.5 - 15 µm in size with 2 - 6 transverse and 1 - 2 longitudinal septa

2.2.6. *Botryodiplodia* sp

As literature on cultural and morphological characters of the pathogen from orchids is not available, that of from other crops is mentioned here. Punithalingam (1976) identified *Botryodiplodia theobromae,* the die back pathogen of many crops

based on cultural and morphological characters. He observed the colony as grey to black, fluffy and with abundant aerial mycelium. Pycnidia simple or compound, often aggregated and ostiolate and 5 mm wide. Conidia measured 20 - 30 x 10 - 15 µm in size and was reddish brown coloured.

According to Sangeetha (2009), *Botryodiplodia* of mango seedlings colony showed a fast growth and was greyish black with fluffy aerial mycelium. Pycnidia were regular, round in shape, smooth and greyish black in colour. Hyphae branched, 5 µm wide and septate at an interval of 17.5 - 35 µm. size of matured conidia were 15 - 25 x 12.5 - 17.5 µm, uniseptate and brownish black in colour.

2.2.7. *Erwinia* sp.

Erwinia chrysanthemi forms a greyish white to creamy white, smooth, round, shining, flat to slightly raised, rapidly growing colony on the medium after 24 h of incubation. Gram-negative, non- spore forming rods, occupying singly or in pairs. Sizes of cells ranged from 0.5 - 0.8 x 1 - 2.5 µm and are motile with peritrichous flagella (Bradbury, 1977).

Abdullah and Kadzimin (1993) reported characters of soft rot bacteria *Erwinia* sp in Phalaenopsis and Dendrobium. *E.chrysanthemi* produced blue pigment on Glucose yeast extract calcium carbonate (GYCA) on first day of incubation and on modified Yeast extract dextrose calcium carbonate medium (YDC), pigment production was variable and was observed only on the third or fourth day. *E. carotovora* pv. *carotovora* did not produced any pigment on both YDC and Glucose yeast extract calcium carbonate (GYCA). On Nutrient Agar, bacteria produced small translucent colonies that could not be differentiated. Rod shaped bacteria with peritrichous flagella was the distinct feature of the bacteria.

YungAn and ChengPin (2006) developed a medium for the isolation and differentiation of *E. chrysanthemi* from other *Erwinia* spp. based on the production of blue-pigmented indigoidine. The medium, named NGM consists of nutrient agar supplemented with one per cent glycerol, that induces pigment production, and 2mM MnCl that further enhances colour development. All tested strains of *E. chrysanthemi* developed dark brownish to blue colonies easily distinguishable from other *Erwinia* spp. The results indicate that pigment production on the NGM medium is a very stable property and can be used as a phenotypic property to differentiate *E. chrysanthemi* from other *Erwinia* spp

2.2.8. *Burkholderia* sp

According to Keith *et al.* (2005), *Burkholderia gladioli* produced pale yellow, opaque, round colonies with entire margins on Nutrient Broth Yeast Extract agar medium and brownish-yellow, non mucoid colonies on Yeast Dextrose Calcium Carbonate Medium. The bacteria are gram-negative, aerobic rods.

2.3. SEASONAL INFLUENCE OF DISEASE

Kim *et al.* (2002) reported that root rot of moth orchid was caused by *Fusarium* spp. resulted in 30 per cent of disease incidence in some greenhouses.

Cerkauskas (2001) described the influence of atmospheric temperature and soil temperature on occurrence of southern blight of tomato caused by *S.rolfsii*. High temperatures (above 30° C) and high soil moisture favor disease development while low soil moisture favors survival of the sclerotia. The germination of sclerotia is most abundant at the soil surface.

Wey (1988) observed that infection of *Erwinia chrysanthemi* occured in the summer months and that of *Phytophthora palmivora* and *P. nicotianae* var. *parasitica* throughout the year in orchid houses.

GeowChing and WenHuei (1998) studied the influence of temperature and relative humidity on the incidence of soft rot bacteria *E. chrysanthemi*. There was a higher incidence of soft rot of orchid caused by *Erwinia* sp. when the temperature was above 26º C. The occurrence and symptoms of soft rot were affected by temperature, relative humidity and inoculum concentration. Percentage incidence of rotting was very high, at usually more than 80 per cent relative humidity, at high temperatures (above 28ºC), and at high inoculum concentration (1.0 x 1010 c.f.u/ml). The rot incidence was effectively reduced if the relative humidity and inoculum concentration were lowered. Greenhouse temperatures of 25 - 30 degrees and a higher humidity in the night during the summer are suitable for the growth of Phalaenopsis in Taiwan. However, such conditions are also favourable for leaf rot. YungChun *et al.* (1999) found that bacterial soft rot incidence and rainfall were positively correlated. Laboratory studies showed that the optimum temperature for soft rot development was 20 - 32º C, and that high relative humidity (97.5 - 100%) and an inoculum density of over 105 c.f.u./ml are necessary to cause soft rot.

Yeh *et al.* (1998) observed that the optimum temperature for direct germination of sporangia of *Phytophthora palmivora* causing black rot of Cattleya was at 24º C. No zoospores were formed at 35ºC and RH below 80 per cent. Gupta (1999) reported that a temperature range of 18 - 22ºC is favorable for disease development in apple by *Phytophthora cactorum* and 18ºC is found to be the most conducive for disease development under Indian conditions.

Uchida (1994) reported that winter months in Hawaii are conducive to diseases caused by *Colletotrichum gloeosporioides* in orchids and foliar diseases are readily reproduced at green house temperatures of 20 - 25ºC. She also observed that at 31ºC, *C. gloeosporioides* grows and sporulates whereas at this temperature, *C.coccodes* are greatly inhibited and produced small, black stromata.

Davis (2003) observed that *Alternaria alternata* infection in Ivygourd was severe during summer months and so high temperature, and moderate relative humidity and rainfall were the favourable climatic factors for the pathogen.

2.4. DISEASE MANAGEMENT

2.4.1. Fusarium wilt

Wedge and Elmer (2008) reported that fludioxonil (Medallion), triflumizole (Terraguard) and azoxystrobin (Heritage) provided adequate control of Fusarium wilt if applied during the early infection period. Chlorothalonil provided excellent protective activity while the systemic fungicide azoxystrobin provided suppression of Fusarium wilt. Kumar and Dubey (2001) observed that *Fusarium oxysporum* infecting pea causing collar rot of pea was completely inhibited by Carbendazim and Benlate at 0.025 and 0.05 per cent concentrations.

2.4.2. Collar rot

Laha *et al*. (1996) studied the effectiveness of *Pseudomonas fluorescens* against *Sclerotium rolfsii* of cotton and they found that the inhibition zone ranged from 3mm to 14mm. They also observed that, the antagonistic effect of fluorescent Pseudomonas was reduced in ferric chloride amended medium indicating the production of some antifungal compound. Kolte and Raut (2007) tested the inhibitory effects of different fungicides against *S. rolfsii*. Among the fungicides, mancozeb, difenoconazole and hexaconazole resulted in the total inhibition of radial growth.

2.4.3. Anthracnose

Uchida (1994) reported that benomyl is highly sensitive to *Colletotrichum gloeosporioides* causing anthracnose of Dendrobium. Davis (2003) observed that

Mancozeb 0.3 per cent concentration showed least inhibition of 21.84 per cent against *C. gloeosporioides*, a leaf spot pathogen of Ivy gourd. Deepthy (2003) found that carbendazim (0.1 per cent) and copper oxychloride (0.2 per cent) were effective against *C. gloeosporioides*. Prapagdee *et al.* (2008) suggested soil-borne *Streptomyces hygroscopicus* for biocontrol of anthracnose disease caused by *C. gloeosporioides* in orchid. Disease inhibition was due to antifungal metabolites in the culture filtrates. Sangeetha (2009) noticed the *in vitro* inhibitory effect of *Pseudomonas fluorescens* against die back and leaf blight pathogens of mango seedlings. The per cent inhibition observed against *C. gloeosporioides*, *Botryodiplodia theobromae* and *Alternaria alternata* were 50.33, 60.99 and 38.49 per cent respectively.

2.4.4. Black rot

Uchida (1991) recommended Captan, Dithane M-45 and Physan 20 for the management of black rot of orchids. Leu (1994) used metalaxyl for the control of orchid black rot induced by *Phytophthora palmivora*. He found that application of metalaxyl once in every eight weeks resulted in almost 100 per cent disease control. Uchida (1994) suggested fungicides like metalaxyl and etridiazole for the management of *Phytophthora* in orchid. Ann (2001) reported that application of high concentration (>1000 ppm) of neutralized phosphorous acid could directly protect plants by inhibition and interference of the mycelial growth and sporangial production of *Phytophthora* sp. and other members of Oomycetes. Disease reduction was associated with increase in production and accumulation of phytoalexins, phenolic compounds or other antifungal substances. Cating *et al.* (2009b) suggested prophylactic applications of foestyl-AL (Aliette, Flanker, Prokoz Avalon), potassium phosphite (Alude, Fungi-Phite, Topaz), propamocarb hydrochloride (Banol), trifloxystrobin (Compass), dimethomorph (Stature),

mefenoxam (Subdue), etridiazole (Terrazole 35%) to reduce the infection by *P. palmivora* and *P. cactorum* causing black rot in orchids.

2.4.5. Leaf spot diseases

Sharma and Badiyala (1994) reported that carbendazim is the most effective fungicide against stem end rot of mango caused by *Botryodiplodia theobromae* which was followed by Bordeaux mixture and aureofungin. According to Sangeetha (2009), carbendazim showed hundred per cent inhibition of *B.theobromae* at 0.1 and 0.2 per cent concentrations. She also found that carbendazim showed more than 60 to 63.5 per cent inhibition on the growth of *Alternaria alternata*.

2.4.6. Bacterial diseases

Agusni and Rumawas (1978) reported that Oxytetracycline and Chloramphenicol were effective for the control of *Erwinia carotovora* var. *carotovora* on moth orchids and also recommended aqueous solution of mercuric chloride as the cheapest and equally effective treatment. Uchida (1995) recommended Agribrom for the control of bacterial disease of orchids. YungChun *et al*. (1999) observed that the growth of *E. chrysanthemi* causing soft rot in Oncidium was inhibited by lincomycin (1000 ppm) and streptomycin (125 ppm) in the *in vitro* studies. Antibiotic bactericide (oxytetracycline/streptomycin mixture WP) was most effective for controlling the bacterial black spot caused by *Burkholderia andropogonis* of orchid (Takahashi *et al.*, 2004). Keith *et al*. (2005) reported that the minimum inhibitory concentration (MIC) of cupric sulfate among Copper resistant strains of *B. gladioli* infecting orchid ranged from 50 to 1,000 µg/ml and the MIC of streptomycin for streptomycin resistant strain was 50 to 100 µg/ml.

Materials and Methods

3. MATERIALS AND METHODS

The present study on Cataloguing and management of major diseases of monopodial orchids was conducted in the Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur during the period from November 2010 to June 2012. The details of the materials used and the techniques adopted for the investigation are described below.

3.1. SURVEY AND COLLECTION OF DISEASED SAMPLES OF MONOPODIAL ORCHIDS

Purposive sampling survey was conducted in the orchidarium of AICRP on Floriculture improvement in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara as well as in the farms of selected orchid growers in Thrissur district to study the various diseases prevalent in these areas.

3.2. ISOLATION OF PATHOGENS

Pathogens associated with diseases of monopodial orchids were isolated from the diseased samples.

3.2.1. Isolation of fungal pathogens

The infected portions showing symptoms of diseases were collected separately and brought to the laboratory and were observed under microscope by preparing the slides from the infected areas. Samples were washed under tap water and dried using blotting paper. Small bits of infected leaf portions along with some healthy areas were surface sterilized with one per cent sodium hypochlorite and then washed in three changes of sterile water. Medium used for isolation of pathogens

was Potato Dextrose Agar (PDA). The fungi grown on the medium were purified, sub cultured and maintained for further investigation.

3.2.1. Isolation of bacterial pathogens

The bacterial pathogens were isolated from naturally infected plants showing typical symptoms. Infected samples were washed thoroughly, cut into small bits, surface sterilized with one per cent sodium hypochlorite and then washed in three changes of sterile water and crushed on a sterilized glass slide to get bacterial suspension. This suspension was streaked on Nutrient Agar (NA) medium to get single isolated colonies of the bacterium. The plates were incubated for 48h at room temperature (26 ± 2ºC). Characteristic single colonies were selected and purified by repeated streaking on NA medium. The pure culture was maintained in slants as well as in sterile water under refrigerated condition.

The composition of media used for isolation is given in Appendix I.

3.3. PATHOGENICITY

The pathogenicity of the organisms associated with different diseases was tested by artificial inoculation of the pathogen into the respective healthy plants or plant parts.

3.3.1. *In vitro* condition

Healthy plant parts collected from the field were washed under tap water and wiped with 70 per cent ethyl alcohol. For fungal pathogens, the leaves/flowers were inoculated with actively growing seven to eight days old mycelial growth of the pathogen after giving pinprick and for bacterial pathogens a thick 24h old culture suspension of the isolated bacteria was inoculated by giving pinprick. Leaves inoculated with sterile water served as control. Moist cotton was placed over inoculated area and the inoculated leaves were kept under humid chamber and

observed daily for the appearance of symptom. The respective pathogens were re-isolated from the infected leaves or plants and compared with the original culture.

3.3.2. *In vivo* condition

Under *in vivo* condition, the respective pathogens were inoculated on the plant either on leaf, collar portion or on root. The inoculation was carried out as explained on 3.3.1. Plants inoculated with sterile water kept as control. The inoculated plants were placed under humid chamber and observed for the symptom expression. The pathogens were re-isolated from the inoculated plants showing symptoms and compared with the original culture.

3.4. SYMPTOMATOLOGY OF DISEASES

Symptoms produced by different pathogens on monopodial orchids under natural and artificial conditions were studied in detail.

3.5. CHARACTERISATION OF FUNGAL AND BACTERIAL PATHOGENS

The cultural and morphological characters of the fungal and bacterial pathogens were studied on PDA and NA media respectively for the characterisation of the organisms.

3.5.1. Cultural and morphological characters of different fungal pathogens

An eight mm diameter disc of fungal pathogen was inoculated into a sterile Petri dish containing PDA medium and was incubated at room temperature. Cultural characters of pathogen such as rate of growth, colour and formation of fruiting body in the medium were studied in detail.

For studying morphological characters slide was prepared. For that, to a sterile slide, a drop of lactophenol stain was poured and to that a mycelial bit was

placed and viewed under a phase contrast microscope. Morphological characters of pathogens like size of hyphae and spore were studied.

3.5.2. Cultural and morphological characters of different bacterial pathogens

For studying cultural characters of the bacterial pathogens, a loop full of bacteria was taken and streaked on a sterile Petri dish containing solidified NA medium and was kept for incubation at room temperature (26 ± 2ºC). Characters such as colony nature, colour and texture were observed.

For studying the morphology of bacteria, gram staining was done. A thin smear of bacteria was made on a sterile glass slide and was air dried and heat fixed for some time. Crystal violet was poured on the smear for 30 seconds and washed with sterile water. Poured Grams iodine solution for 60 seconds, washed it with 95 per cent ethyl alcohol and was again washed with distilled water and air dried. Safranine was applied on the smear for 30 seconds, washed with sterile water and blot dried with absorbent paper, air dried and observed colour, shape, and size of cell.

3.5.3. Identification of fungal pathogens

The fungi associated with the diseases were identified based on the cultural and morphological characters and was further confirmed by National Centre of Fungal Taxonomy, New Delhi.

3.5.4. Identification of bacterial pathogens

The bacteria were identified at species level based on molecular characters in two private laboratories namely Scigenom, Kakanad and Vision Centre, Angamaly as per the following procedure. DNA was isolated from the liquid culture and electrophoresed in 1% Agarose gel and visualized under UV. 16S r DNA gene was PCR amplified with forward and reverse primers. Amplicon was electrophoresed in a

1% Agarose gel and visualized under UV. Concentration of the amplicon was checked in a Nanodrop ND 2000. The amplicon was purified using Nucleospin purification column (Macherey-Nagel). Sequencing of amplicon with forward and reverse primers in ABI 3730xl cycle sequencer was done. Forward and reverse sequences were assembled and contig was generated after trimming the low quality bases. The sequence analysis was carried out using bioinformatic tool BLAST of NCBI. Based on maximum identity score first few sequences were selected and aligned using multiple sequence alignment software MultAlin. Dendrogram was constructed.

3.6. SEASONAL INFLUENCE ON THE OCCURRENCE OF DISEASES

The orchid plants maintained in the orchidarium of AICRP on Floriculture Improvement in the Department of Pomolgy and Floriculture, College of Horticulture, Vellanikkara were observed for one year to study the seasonal influence on the occurrence of various diseases. Short stemmed epiphyte, Phalaenopsis was grown under rain shelter, Mokara and Basket Vanda were grown under polyhouse and tall growing orchid, Arachnis was grown under open condition. Per cent disease incidence and per cent disease severity and weather parameters like temperature and relative humidity were recorded for that purpose.

3.6.1. Assessment of disease incidence

For assessing the disease incidence, number of infected plants and total number of plants were recorded and per cent disease incidence was calculated using the formula.

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

3.6.2. Assessment of disease severity

Disease severity was recorded for leaf spot diseases on ten randomly selected marked plants for leaf spot diseases. Bottom five leaves of the plant were selected and disease was scored using 0-5 scale as detailed below.

Table1. Score chart for disease severity

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

Per cent Disease Severity was calculated using the following formula suggested by Wheeler (1969).

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

3.7. MANAGEMENT OF PATHOGENS ASSOCIATED WITH DISEASES OF ORCHIDS

An *in vitro* evaluation on the effectiveness of fungicides, antibiotics, *Pseudomonas fluorescens* and cowdung extract was carried out for the management of pathogens causing diseases in orchids by standard procedures.

3.7.1. *In vitro* evaluation against fungal pathogens

Fungicides and the bacterial antagonist *P. fluorescens* (liquid formulation) were evaluated by Poisoned Food Technique (Zentmyer, 1955) against all isolated fungal pathogens. For the evaluation, 100 ml of PDA was taken in 250 ml conical flask and sterilized at 1.05 $kgcm^{-2}$ pressure for 20 minutes. Required quantity of fungicides/*P.fluorescens* were mixed separately with the medium to get desired concentration and poured into sterile Petri dishes @ 20 ml per plate. Mycelial disc of 8mm diameter were cut from actively growing seven day old culture of the fungal pathogen and placed at the centre of each Petri dish containing poisoned medium. Three replications were maintained for each treatment. Media without fungicides served as control. The details of treatments used for *in vitro* evaluation are given in Table 2. The inoculated Petri dishes were incubated at 28±2ºC. The diameter of the fungal colony was recorded upto and when growth of fungus in the control plates were fully covered the medium.

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

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

PI = Per cent inhibition on growth

C = Growth of pathogen in control (mm)

T = Growth of pathogen in treatment (mm)

Table 2. Treatments used for *in vitro* evaluation against fungal pathogens

Treatment No.	Treatments	Trade Name	Concentration (%)
T1	Potassium phosphonate 40%+ Mancozeb 75%	Akomin + Indofil M-45	0.2 + 0.15
T2	Cymoxanil 8% + Mancozeb 64%	Curzate M8	0.2
T3	Carbendazim 12%+ Mancozeb 63%	Saaf	0.15
T4	Carbendazim 25% + Ipridione 25%	Quintal	0.2
T5	Fenamidone 10% + Mancozeb 50%	Sectin	0.1
T6	*P. fluorescens* (Liquid)		0.5
T7	Control		

3.7.2. *In vitro* evaluation against bacterial pathogens

In vitro evaluation against bacterial pathogens was carried out using agar well diffusion method (Perez *et al*., 1990) against all isolated bacterial pathogens. The antibiotic streptocycline, fresh cowdung extract, the bacterial antagonist *Pseudomonas fluorescens* and a combination of cowdung extract and *P.fluorescens* were used for the study. The efficacy was calculated and expressed as per cent inhibition using the formula given in 3.7.1.

For *in vitro* evaluation, 20 ml of Nutrient Agar medium was poured into sterile Petri dishes. After solidification of the medium, 0.1 ml of 48h old bacterial suspension (prepared by adding one loop full of bacteria in 10ml water and incubated for 48 hour) was poured on the solidified medium and spread with spreader. An 8

mm diameter well was made at the centre of the medium with a sterilized cork borer. Required concentrations of treatments were prepared in sterile water and from that 0.1 ml was transferred in to the well made at the center of the plate. Three replications were maintained for each treatment. Sterile water was added to the well served as control. The inoculated Petri dishes were incubated at room temperature (26 ± 2ºC). The details of treatments used for the evaluation are given in Table 3. The diameter of the inhibition zone was recorded until it reached maximum.

Table 3. Treatments used for *in vitro* evaluation against bacterial pathogens

Treatment No.	Treatment	Concentration
T1	Streptocycline	200ppm
T2	Fresh cowdung extract	2%
T3	*Pseudomonas fluorescens*	0.5%
T4	Fresh cowdung extract + *P. fluorescens* (liquid formulation)	2% +0.5%
T5	Control	

3.8. STATISTICAL ANALYSIS

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

Results

4. RESULTS

The present investigation on the "Cataloguing and management of major diseases of monopodial orchids" was carried out to study symptomatology of diseases of orchids, characterization of pathogens associated with diseases, seasonal occurrence of various diseases of monopodial orchids and *in vitro* evaluation on management of pathogens. The results of the study are presented below.

4.1. SURVEY AND COLLECTION OF DISEASED SPECIMENS OF MONOPODIAL ORCHIDS

A survey was conducted in the orchidarium of AICRP on Floriculture Improvement in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara and many orchid growers of Thrissur district *viz*., Bhavana Orchids, Cheroor; Jones Gardenia, Thrissur; Pearl Orchids, Perinjanam; National Rose garden, Madakkathara; Shobha Orchids, Kanimangalam and Teeose Nursery, Mannuthy. Diseased specimens were collected from these places and the pathogens were isolated. The diseases noted during the survey are given below (Table 4).

Table 4. Diseases of monopodial orchids observed during survey

Sl No.	Disease	Type of orchid	Location
1	Fusarium wilt	Phalaenopsis	Vellanikkara
2	Collar rot	Phalaenopsis	Vellanikkara
3	Flower spot	Phalaenopsis	Vellanikkara
4	Anthracnose	Phalaenopsis	Cheroor, Perinjanam
5	Soft rot	Phalaenopsis	Vellanikkara, Perinjanam
6	Heart rot	Basket Vanda	Madakkathara, Kanimangalam

7	Sclerotium wilt	Basket Vanda	Madakkathara
8	Leaf spot	Basket Vanda	Vellanikkara,Perinjanam, Madakkathara
9	Bacterial wilt	Basket Vanda	Perinjanam
10	Anthracnose	Mokara	Thrissur
11	Leaf spot	Mokara	Vellanikkara
12	Anthracnose	Arachnis	Vellanikkara, Perinjanam
13	Leaf spot	Arachnis	Vellanikkara

Viral diseases of monopodial orchids were not observed from any of the surveyed places.

4.2. Isolation of pathogens associated with diseases

Different pathogens causing diseases in monopodial orchids collected from various places were isolated by the procedure as described earlier and the details of isolated pathogens are given in the Table 5.

4.2.1. Isolation of fungal pathogens

The fungal pathogens were isolated from the naturally infected specimens collected from surveyed places. The isolation yielded different fungal pathogens *viz*., *Fusarium* sp. (Phalaenopsis and Arachnis), *Sclerotium* sp. (Phalaenopsis and Basket Vanda), *Phoma* sp., *Colletotrichum* sp. (Phalaenopsis, Mokara and Arachnis), *Phytophthora* sp., *Alternaria* sp. and *Botryodiplodia* sp. All these pathogens were purified and sub cultured at frequent intervals on PDA medium.

4.2.1. Isolation of bacterial pathogens

The bacterial pathogens were isolated from the naturally infected samples collected from surveyed places. The isolation yielded two bacterial pathogens *viz*., *Erwinia* sp. and *Burkholderia* sp.

Table 5. Fungal and bacterial pathogens isolated from diseased specimens

Type of orchid	**Disease**	**Pathogen**
Phalaenopsis	Fusarium wilt	*Fusarium* sp.
Phalaenopsis	Collar rot	*Sclerotium* sp.
Phalaenopsis	Flower spot	*Phoma* sp.
Phalaenopsis	Anthracnose	*Colletotrichum* sp.
Phalaenopsis	Soft rot	*Erwinia* sp.
Basket Vanda	Heart rot	*Phytophthora* sp.
Basket Vanda	Sclerotium wilt	*Sclerotium* sp.
Basket Vanda	Leaf spot	*Botryodiplodia* sp.
Basket Vanda	Bacterial wilt	*Burkholderia* sp.
Mokara	Anthracnose	*Colletotrichum* sp.
Mokara	Leaf spot	*Alternaria* sp.
Arachnis	Anthracnose	*Colletotrichum* sp.
Arachnis	Leaf spot	*Fusarium* sp.

4.3. PATHOGENICITY

The pathogenicity of fungi/ bacteria associated with different diseases was tested by artificial inoculation on healthy plants (*in vivo*) or detached plant parts (*in vitro*).

4.3.1. *In vitro* condition

Phoma sp., *Colletotrichum* sp., *Sclerotium* sp., *Botryodiplodia* sp., *Alternaria* sp., *Fusarium* sp. were inoculated on detached plant parts. *Phoma* sp. inoculated on Phalaenopsis flower produced symptoms after four days of inoculation. Anthracnose symptoms were produced in Phalaenopsis after nine days of inoculation of

Colletotrichum sp. Wilt pathogen *Sclerotium* sp. infecting Basket Vanda started to produce symptom on third day of inoculation. In Mokara, anthracnose symptoms were started after five days of inoculation of *Colletotrichum* sp. and blighting developed after 15 days of inoculation. Leaf spot pathogen *Alternaria* sp. infecting Mokara developed typical spot at 13 days after inoculation. Pathogens of Arachnis *viz.*, *Colletotrichum* sp. and *Fusarium* sp. produced symptoms of anthracnose and leaf spot at ten and eight days after inoculation of pathogen. The pathogens were re-isolated from the infected portion and showed same cultural characters as that of the original culture.

4.3.2. *In vivo* condition

The pathogens *viz.*, *Fusarium* sp., *Sclerotium* sp., *Phytophthora* sp., *Erwinia* sp., and *Burkholderia* sp. were inoculated on whole plant. *Fusarium* sp. inoculated on leaf lamina produced leaf spot symptoms instead of yellowing and discolouration of leaf base at five days of inoculation. *Sclerotium* sp. causing collar rot of Phalaenopsis developed typical symptom of rotting within five days afer inoculation and sclerotia developed after 10 days. Bacterial pathogen, *Erwinia* sp. produced complete rotting of Phalaenopsis leaves within two days of inoculation. *Phytophthora* sp. infecting Basket Vanda produced heart rot symptom after four days of inoculation. Bacterial pathogen *Burkholderia* sp. of Basket Vanda developed symptoms of yellowing, discolouration of leaf base and leaf detachment after 12 days of inoculation. The pathogens were re-isolated from the infected portions and showed same cultural characters as that of the original culture.

4.4. SYMPTOMATOLOGY OF DISEASES

Symptomatology of various diseases was studied under natural and artificial conditions and the details are given below.

4.4.1. Fusarium wilt of Phalaenopsis

Under natural condition, the important symptoms noticed were chlorosis, stunting and wilting of plants. The symptoms initiated as yellowing of leaves. Later the leaf base showed a black discolouration and the leaves got detached from the plant. The leaves appeared shrivelled and ultimately the whole plant wilted and died (Plate 1A).

Under artificial conditions, the symptom appeared on the leaf as black spot after five days of inoculation. Even though leaf base and roots were inoculated symptoms were not observed in these parts (Plate 1A).

4.4.2. Collar rot of Phalaenopsis

Under natural conditions, first symptom observed was yellowing of leaves. Later white mycelial growth appeared and rotting of infected area took place. In the advanced stage, numerous light brown sclerotia were formed on the infected area and infected leaf got detached from the plant (Plate 1B).

Upon artificial inoculation, the symptom started as water soaked spot and rotting was more prominent than yellowing compared to natural infection and resulted in complete rotting within five days. Light brown sclerotia were appeared on the infected plant within 10 days of inoculation (Plate 1B).

4.4.3. Flower spot of Phalaenopsis

Under natural conditions, flower spot caused by *Phoma* sp. appeared as minute, brown coloured sunken spots on flower petals. Symptoms were prominent in white coloured flowers than flowers of other colour. In advanced stage, these spots coalesced to form moderate sized brown lesion. At the centre of the lesion, pycnidia were appeared as black dots (Plate 1C).

Plate 1. Symptomatology of diseases of Phalaenopsis

Natural

Artificial

A. Fusarium wilt

Natural

Artificial

B. Collar rot

Natural

Artificial

C. Flower spot

Under artificial condition, symptoms were produced as minute brown to black coloured spots on inoculated flower petal after four days of inoculation of pathogen (Plate 1C).

4.4.4. Anthracnose of Phalaenopsis

The symptoms of anthracnose were initiated as water soaked areas on upper surface and chlorotic spot on the corresponding lower surface of the leaf. Later it turned to brown to black irregular spot with brown margin surrounded by prominent yellow halo. In severe cases, these spots coalesced together resulted in the blighting of leaf. Spot was sunken on the upper surface and numerous acervuli appeared on the infected area (Plate 1D).

Under artificial conditions, the symptom produced was same as that in the natural condition and was visible after nine days of pathogen inoculation. The spot appeared prominently on the upper side of the leaf with a yellow halo (Plate 1D).

4.4.5. Soft rot of Phalaenopsis

Under natural conditions, the bacterial disease initiated as greenish water soaked spot on the leaves. Later the spot spread to entire leaf within two days and resulted in liquefaction of leaf tissues and rotting of entire leaf. A characteristic foul smell was also associated with rotting. If control measures were not taken, the spread of the disease was very fast (Plate 1E).

Upon artificial inoculation of bacteria, the symptom started as greenish water soaked spot 24 h after inoculation and complete rotting occurred within 48 h (Plate 1E).

Plate 1. Symptomatology of diseases of Phalaenopsis

Natural

Artificial

Anthracnose

Natural

Artificial

E. Soft rot

4.4.6. Heart rot of Basket Vanda

Symptoms of heart rot of Basket Vanda caused by *Phytophthora* sp. was initiated in the topmost two young leaves. At first, brown water soaked areas appeared on the leaf base, later it turned to black necrotic lesion and resulted in rotting of entire leaf. Yellowing symptom was also noticed in the infected plant. In advanced stage, mycelial growth was observed on the infected leaf (Plate 2A).

Under artificial condition, symptoms were started after two days of pathogen inoculation and were similar to that of natural conditions. Complete rotting of the leaf occurred on fourth day of pathogen inoculation (Plate 2A).

4.4.7. Sclerotium wilt of Basket Vanda

Under natural conditions, yellowing of leaves was observed as initial symptom of Sclerotium wilt. Dry rotting was another symptom which spread from the pseudostem to leaf base and to the entire leaf. In advanced stage, white mycelial growth and numerous light brown coloured sclerotia were appeared in the infected area after seven days (Plate 2B).

Under artificial condition, the inoculated leaf showed yellowing and rotting on third day after inoculation. Later light brown sclerotia were also appeared on the infected areas (Plate 2B).

4.4.8. Leaf spot of Basket Vanda

The symptom of leaf spot caused by *Botryodiplodia* sp. was characterized by greyish white coloured spot with thick black margin. The lesion enlarged in size resulted in blighting of large area as the time progressed. The fungus produced its fruiting body, pycnidia at the centre of the lesion as black dots. Symptoms were also observed on the psudostem of the plant (Plate 2C).

Plate 2. Symptomatology of diseases of Basket Vanda

A. Heart rot

Natural

Artificial

B. Sclerotium wilt

Plate 2. Symptomatology of diseases of Basket Vanda

Natural

Artificial

C. Bacterial wilt

Natural

Artificial

D. Leaf spot

Under artificial condition, the symptom started after four days of inoculation on leaf. Oval to spindle shaped greyish white spot was developed within 12 days of inoculation (Plate 2C).

4.4.9. Bacterial wilt of Basket Vanda

Under natural condition, the symptoms of bacterial wilt started as brown discolouration of leaf base. Yellowing, wilting of plant and drying of roots were the other symptoms of infection. Leaves got detached from the plant and the progression of the diseases lead to mortality of plant (Plate 2D).

Artificial inoculation of bacteria at the leaf base resulted in brown discolouration, yellowing of leaf and leaf detachment after 12 days of inoculation (Plate 2D).

4.4.10. Anthracnose of Mokara

Under natural condition, symptom started from tip of leaf as brown lesion which later spread to form large necrotic area measuring about one-fourth area of the leaf. Ultimately the infected leaf turned chlorotic. The acervuli of the fungus was seen as black dots on the infected area of the leaf (Plate 3A).

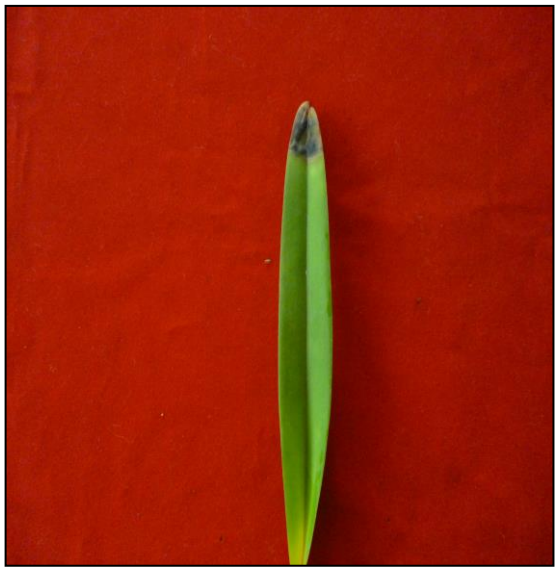
Under artificial condition, the symptom started on the inoculated leaf after five days and blighting was appeared after 15 days of inoculation. Chlorosis of leaves was not observed (Plate 3A).

4.4.11. Leaf spot of Mokara

Incidence of leaf spot caused by *Alternaria* sp. was high and almost all leaves were infected. Round to spindle shaped spot with brown margin and off-white centre with a vertical splitting was the characteristic symptom under natural condition (Plate 3B).

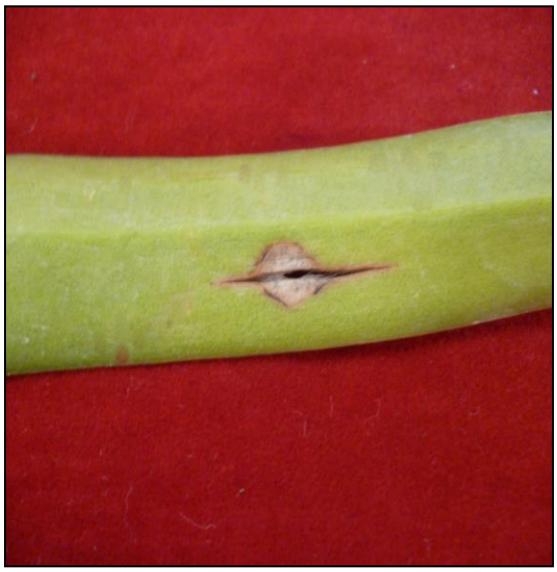
Plate 3. Symptomatlology of diseases of Mokara

Natural



Artificial

A. Leaf spot



Natural

Artificial

B. Anthracnose

Under artificial condition, oval to spindle shaped light brown coloured spot with characteristic vertical splitting was produced 13 days after inoculation of leaf (Plate 3B).

4.4.12. Anthracnose of Arachnis

Dark brown sunken leaf spot with yellow halo was the characteristic symptom under natural condition. Spot was more sunken on the upper surface of the leaf. Fruiting body of the fungus was appeared at the center of the spot as black dots. Later infection developed as blight and had a burned appearance (Plate 4A).

Upon artificial inoculation of leaf, an irregular brown spot with a black margin was developed on the upper surface after 10 days of inoculation. On the lower surface, the spot was sunken and pink coloured spore mass of the fungus was observed. Slight yellow halo was appeared around the spot on both surface of leaf (Plate 4A).

4.4.13. Leaf spot of Arachnis

Under natural condition, oval to spindle shaped grey coloured spot with a black margin was the characteristic symptom of the Fusarium leaf spot. On the corresponding lower surface, a brown colour was observed. Occasionally shredding of infected tissues was also noticed (Plate 4B).

Under artificial condition, spindle shaped grey coloured spot was developed after eight days of inoculation of the leaf. But tissue shredding symptom was not observed (Plate 4B).

4.5. CHARACTERISATION OF FUNGAL AND BACTERIAL PATHOGENS

The pathogens causing different diseases were characterized and identified by studying cultural and morphological characters.

Plate 4. Symptomatology of diseases of Arachnis

Natural

Artificial

A. Anthracnose

Natural

Artificial

B. Leaf spot

4.5.1. Cultural and morphological characters of different fungal pathogens

Fungal pathogens *viz*., *Fusarium* sp., *Sclerotium* sp., *Phoma* sp., *Colletotricum* sp., *Phytophthora* sp., *Botryodiplodia* sp., *Alternaria* sp. were characterized by studying cultural and morphological characters.

4.5.1.1. *Fusarium* sp in Phalaenopsis

Cultural and morphological characters of *Fusarium* sp. which caused Fusarium wilt of Phalaenopsis were studied in detail. Initially a sparse white mycelial growth was seen, and slight pink pigmentation was observed on the third day of incubation. Mycelium was delicate and compact. Pink colour was more prominently observed on the reverse side of the Petri dish. The fungus completed full growth in Petri dish (nine cm diameter) by six days at room temperature (26 ± 2ºC) (Plate 5A).

Hyphae hyaline, branched, septate with 3.85 to 5.09 x 24.01 to 39.33 µm size. Macroconidia hyaline, straight to sickle shaped 3 - 5 septate, pointed ends with size 22.65 - 30.06 x 3.32 - 4.41µm. Microconidia hyaline, oval, non septate with 5.71 - 9.22 x 2.70 - 3.26 µm size.

4.5.1.2. *Sclerotium* sp in Phalaenopsis

The causal organism of collar rot of Phalaenopsis was studied in detail. Colony was white with an even sheet of aerial mycelium with clear mycelial strands and with tufts of longer hyphae. Colony had a fast growth and attained nine cm growth in five days at room temperature. Sclerotial development was started at seven DAI as a white structure which later changed to light brown and then to dark brown colour. Sclerotium was round, smooth and shiny in appearance. Number of sclerotia produced in the culture after 20 days of incubation was in the range of 60 - 100 (Plate 5B).

Hyphae hyaline, septate, branched with 3.26 - 4.32 x 74.97 -92.56 µm size

4.5.1.3. *Phoma* sp in Phalaenopsis

Cultural and morphological characters of pathogen causing flower spot of Phalaenopsis were studied in detail. On PDA medium, initially a white mycelial growth was developed, and which turned to greenish black on the fourth day of incubation and later to greyish black. Reverse side of Petri dish was black in colour. Fungus attained nine cm diameter growth in eight days at room temperature (Plate 5C).

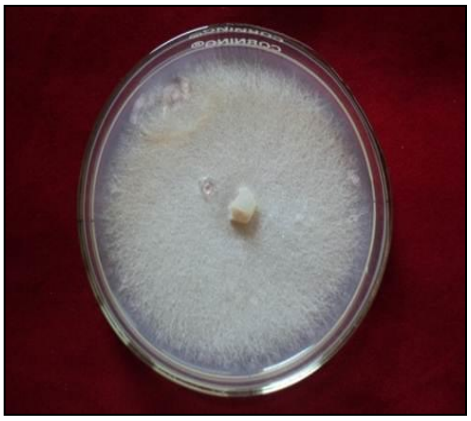
Hyphae initially hyaline, turned brown, septate, branched with 2.58 - 4.83 x 13.61-30.47µm size. Conidia hyaline, cylindrical with one end round and other end tapering, aspetate with 4.27-5.80 x 1.81-3.09 µm size.

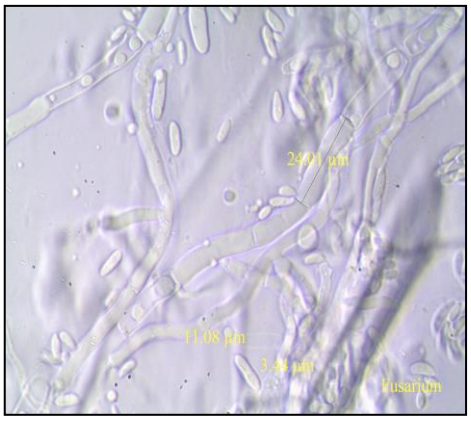
4.5.1.4. *Colletotrichum* sp in Phalaenopsis

The anthracnose pathogen was characterised by observing the cultural and morphological characters. Initially a cottony white mycelial growth was observed which turned to greyish white and then to greyish black colour with pink sporulation. Reverse side of the Petri dish appeared black in colour. Fungus attained nine cm diameter growth in eight days at room temperature. Pinkish spore mass and acervuli appeared after 14 days of incubation (Plate 5D).

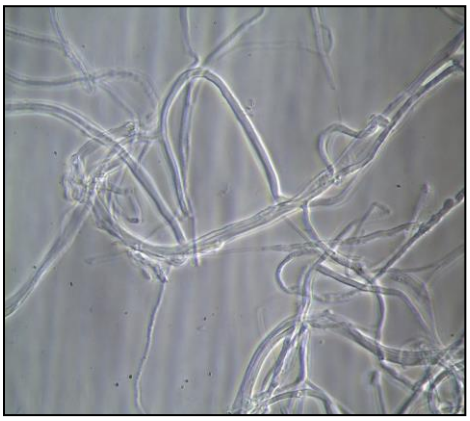
Hyphae initially hyaline, later changed to greyish white coloured, branched with 2.53-5.01µm width and septate at an interval of 12.53 - 42.66µm. Conidia hyaline, cylindrical with both ends round, aseptate, with an oil globule and 11.15 -13.42 x 2.5 - 3.65 µm in size. Conidiophores club shaped, hyaline and non septate. Setae dark brown, septate with swollen base and tapering apex.

Plate 5. Cultural and morphological characters of pathogens of Phalaenopsis

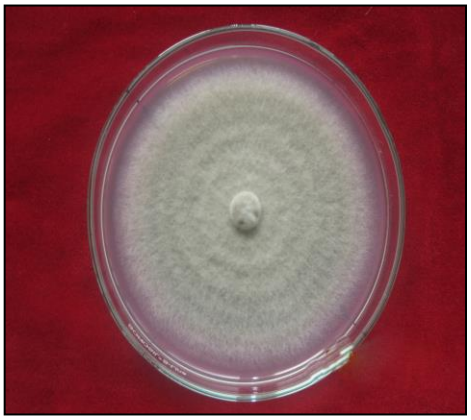


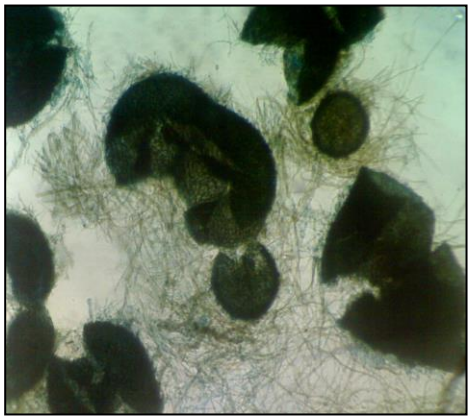


A. *Fusarium oxysporum*



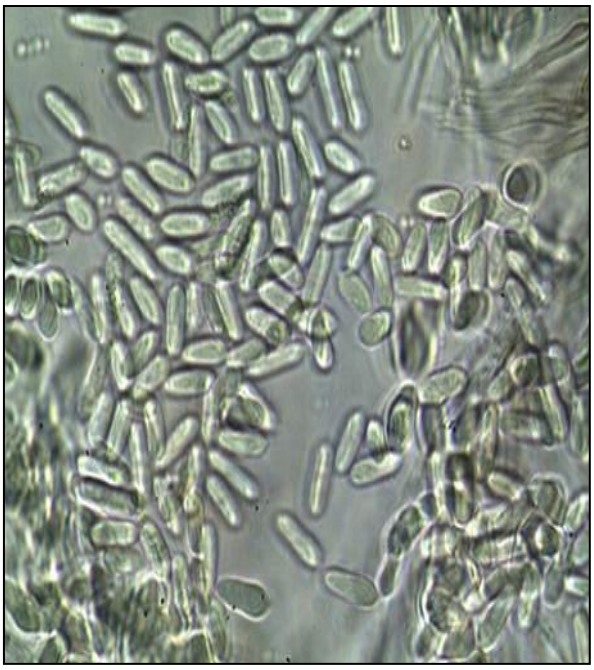
B. *Sclerotium rolfsii*



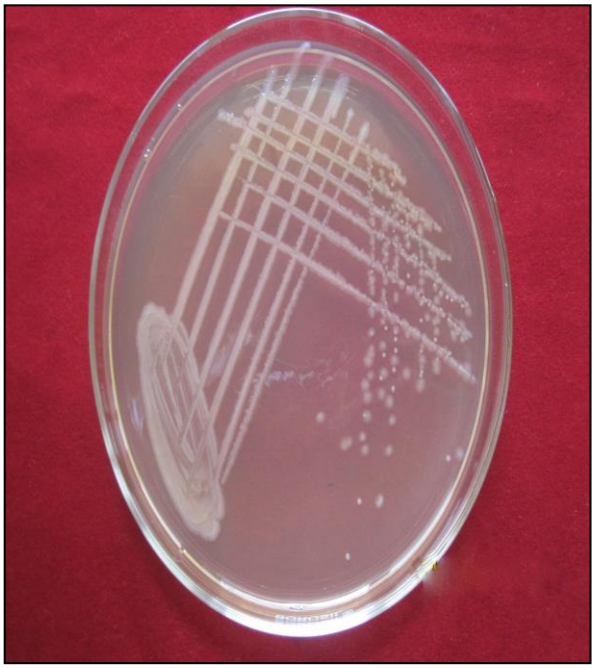


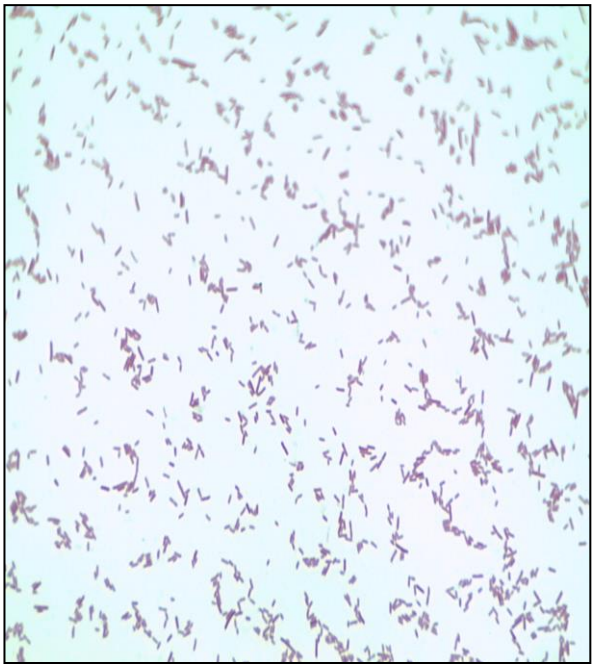
C. *Phoma exigua*

Plate 5. Cultual and morphological characters of pathogens of Phalaenopsis



D. *Colletotrichum gloeosporioides*





E. *Erwinia chrysanthemi*

4.5.1.5. *Phytophthora* sp in Basket Vanda

Causal organism of heart rot of Basket Vanda was studied in detail by observing cultural and morphological characters. Mycelial growth appeared as cottony wool, uniformly dense and white in colour. Culture was slightly radiating and overlapping mycelial growth gave a petalloid appearance to colony. Fungus was fast growing and completed nine cm diameter growth in six days at room temperature (Plate 6A).

Hyphae hyaline , aseptate and 5.01-8.71 µm width . Sporangia ovoid to obpyriform with a conspicuous hemispherical apically thickened papilla with size 36 - 50 x 29.5 - 35 µm.

4.5.1.6. *Sclerotium* sp in Basket Vanda

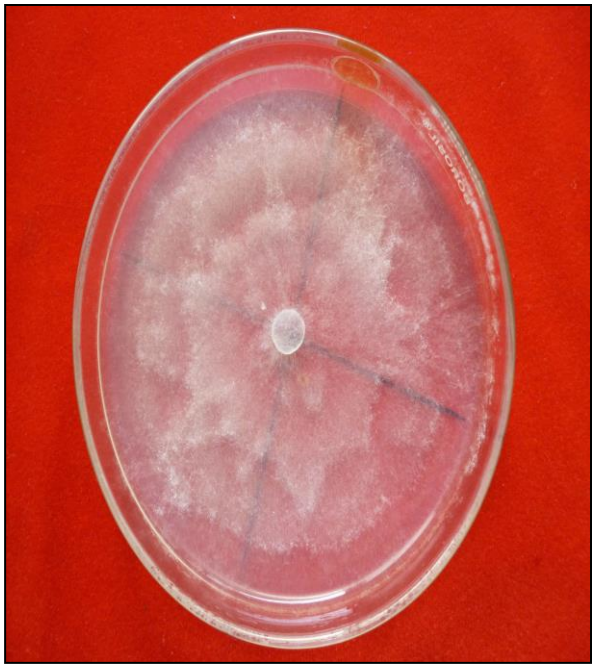
Colony was white with an even sheet of aerial mycelium with clear mycelial strands and with tufts of longer hyphae. Colony had a fast growth and attained nine cm diameters in five days at room temperature. Sclerotial development was started after seven days as a white structure, later which changed to light brown and then to dark brown colour. Sclerotium was round, smooth and shiny in appearance. Number of sclerotia produced in the culture after 20 days was in the range of 60-100 (Plate 6B).

Hyphae hyaline, septate, branched with 3.25 - 4.32 x 74.97 - 94.35 µm size.

4.5.1.7. *Botryodiplodia* sp in Basket Vanda

Initially greyish white mycelium was observed, which changed its colour to greyish black and the colony showed a fluffy aerial growth. Deep black colour was appeared in the reverse side of the colony. Fungus showed a fast growth and attained nine cm diameter growth in three days at room temperature. Pycnidia development

Plate 6. Cultural and morphological characters of pathogens of Basket Vanda



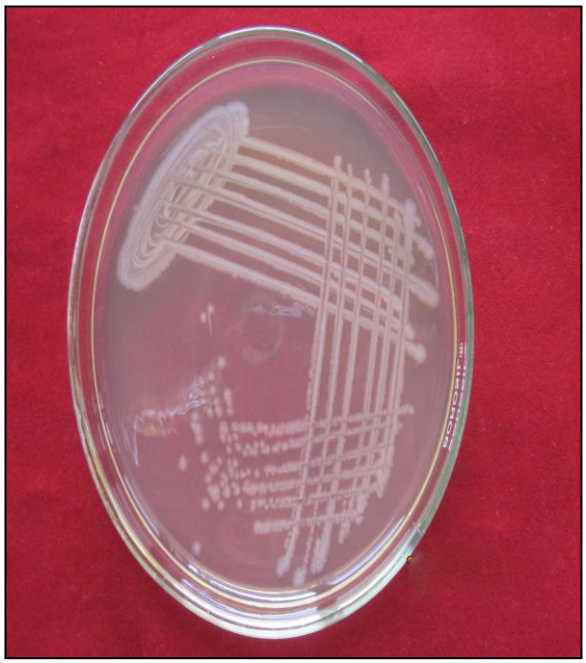
A. *Phytophthora cactorum*

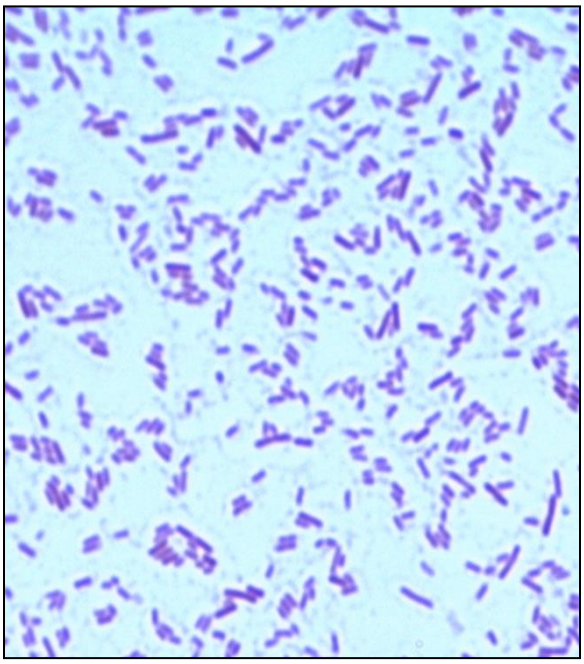
Plate 6. Cultural aı ıs of Basket Vanda

B. *Sclerotium rolfsii*

Plate 6. Cultural and morphological characters of pathogens of Basket Vanda

C. *Botryodiplodia theobromae*





D. *Burkholderia gladioli*

started at eight days after incubation and was often aggregated, tough and was black in colour (Plate 6C).

Hyphae brownish black coloured, septate with 3.46 - 6.22 x 14.19 - 28.06 µm size. Pycnidium was black coloured and osteolate. Conidia initially hyaline later brown coloured, cylindrical with both ends round, septate with a single septa and two cells and 20.80 - 25.83 x 10.42 - 15.59µm size.

4.5.1.8. *Colletotrichum* sp in Mokara

The anthracnose pathogen of Mokara was studied in detail by observing the cultural and morphological characters. Initially colony showed a greyish white sparse mycelial growth and later changed to greyish black coloured mycelium. Full growth on Petri dish (nine cm diameter) was obtained in seven days at room temperature. Pinkish spore mass and acervulai developed by 12 days after incubation and these were seen more towards periphery (Plate 7A).

Hyphae initially hyaline, later changed to greyish white coloured, branched with 5.51 - 6.80 x 21.22 - 25.1 µm size. Conidia hyaline, cylindrical with both ends round, aseptate, with one oil globules and 10.75 - 11.83 x 3.61 - 5.66 µm in size. Conidiophores club shaped, hyaline, non septate. Setae were not observed in the acervulus.

4.5.1.9. *Alternaria* sp in Mokara

Cultural and morphological characters of the pathogen which caused leaf spot of Mokara were studied in detail. Initially mycelial growth was white in colour, later it turned to greenish grey and then to greyish brown. The mycelial growth had a velvety appearance and conspicuous concentric zonations were observed in the culture after five days. The lower side of the colony was black in colour. Fungus was

Plate 7. Cultural and morphological characters of pathogens of Mokara

A. *Colletotrichum gloeosporioides*

B. *Alternaria alternata*

slow growing and completed nine cm diameter growth in nine days at room temperature (Plate 7B).

Hyphae branched, initially hyaline, turned brownish black, septate with 2.45 - 4.74 x 12.3 - 23.03 µm width. Conidia formed in chain or sometimes free, straight, obclavate, smooth, rostrate, the basal cells rounded, pale to dark brown, 31.54 - 60.26 x 8.61 - 11.84 µm and with 3 - 7 transverse and 1 - 3 longitudinal septae.

4.5.1.10. *Colletotrichum* sp in Arachnis

Characterization of anthracnose fungi was done by studying its cultural and morphological characters. Initially pale white, cotton wool like mycelial growth was observed and later it slightly turned to greyish black colour. Lower surface of the colony was more black compared to upper surface. Colony was fast growing and attained nine cm diameter growth in six days at room temperature (Plate 8A).

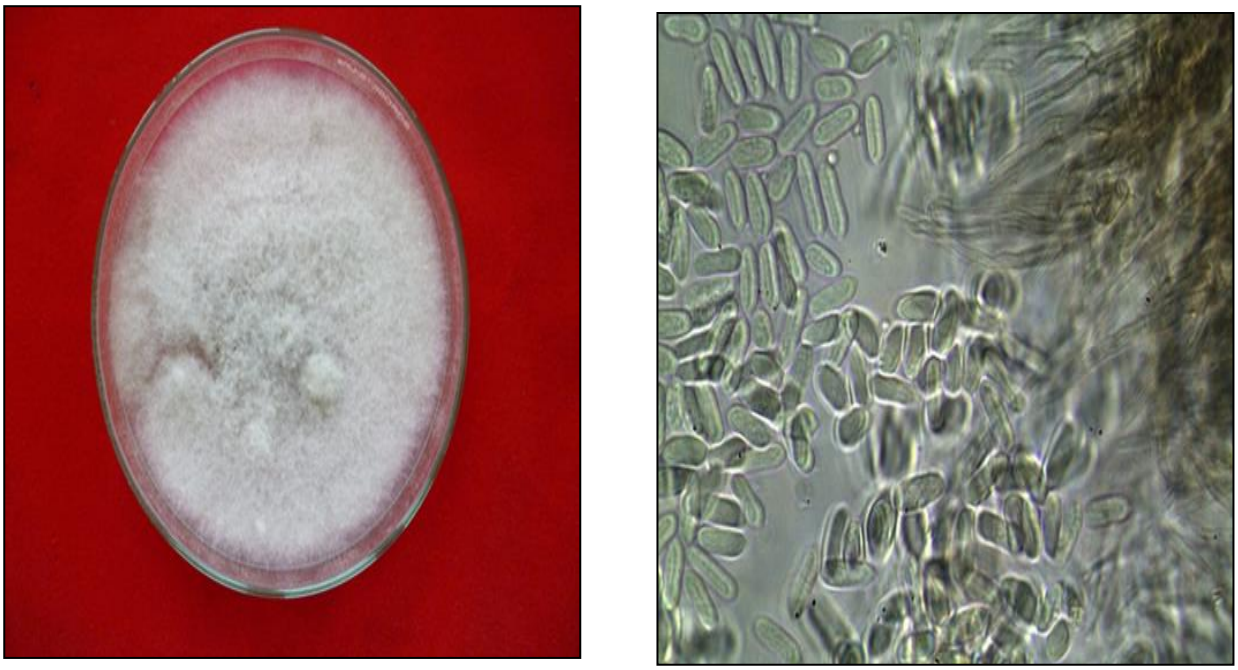
Hyphae initially hyaline, later changed to greyish white coloured, branched with 2.58 – 4.49 x 14.06 - 27.43 µm size. Conidia hyaline, cylindrical with both ends round, aseptate, with one oil globules and 12.05 - 15.31 x 3.84 - 5.60 µm in size. Conidiophores club shaped, hyaline and non septate. Setae dark brown, septate with swollen base and tapering apex. .

4.5.1.11. *Fusarium* sp in Arachnis

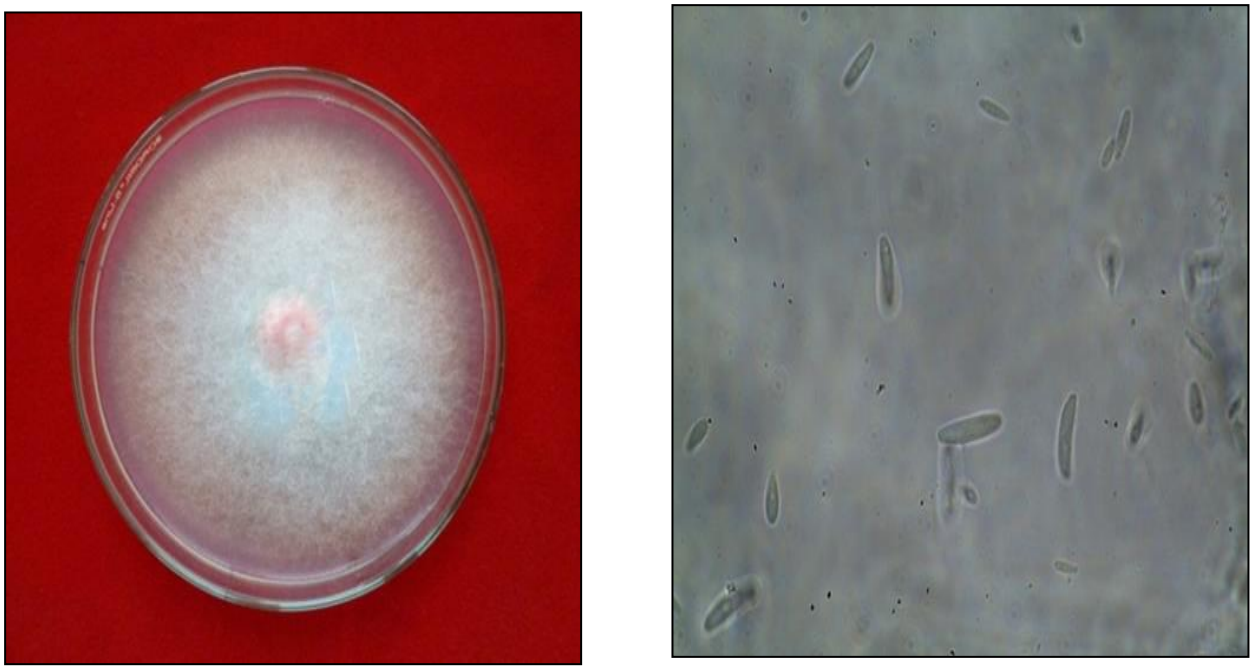
Characteristics of leaf spot pathogen of Arachnis were studied in detail. Initially the fungus had a white delicate mycelial growth. Later a slight pinkish coloured pigmentation was appeared and reverse side of the colony was dark purple in colour. The colony was slow growing and attained nine cm diameter growth in nine days at room temperature (Plate 8B).

Hyphae hyaline, branched, septate with 3.85 - 5.04 x 22.50 - 25.15 µm size. Macroconidia hyaline, straight to sickle shaped 3 - 5 septate, pointed ends with size

Plate 8. Cultural and morphological characters of pathogens of Arachnis



A. *Colletotrichum gloeosporioides*



B. *Fusarium oxysporum*

22.65 - 30.06 x 3.32 - 4.41µm. Microconidia hyaline, oval or ellipsoid, non septate with 5.71 - 9.22 x 2.70 - 3.26 µm size.

4.5.2. Cultural and morphological characters of bacterial pathogens

Cultural and morphological characters of Bacteria *viz.*, *Erwinia* sp. and *Burkholderia* sp.were studied. The details are given below.

4.5.2.1. *Erwinia* sp in Phalaenopsis

The cultural characteristics of bacteria which caused soft rot of Phalaenopsis were studied in detail. On Nutrient Agar medium, bacteria produced small greyish white to creamy white, smooth, round, glistening, slightly raised translucent colonies (Plate 5E).

Bacterium was gram-negative, non-spore forming straight rods with round ends and occurred in groups.

4.5.2.2. *Burkholderia* sp in Basket Vanda

Cultural and morphological characters of the pathogen which caused wilt of Basket Vanda were studied in detail. On nutrient agar medium, bacteria produced pale yellow, opaque, round colonies with entire margins (Plate 6D).

Bacteria were gram negative, rod shaped and non-spore forming.

4.5.3. Identification of fungal pathogens

Based on the study of cultural and morphological characters, eleven fungal pathogens were identified. The identification of six fungal pathogens was further confirmed by National Centre of Fungal Taxonomy, New Delhi.

Table 6. Identification of fungal pathogens

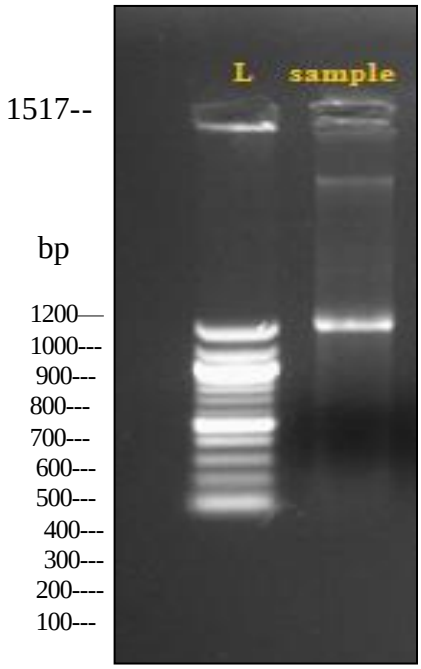
Sl No	Type of orchid	Disease	Identification of pathogen	Identification No.
1	Phalaenopsis	Fusarium wilt	*Fusarium oxysporum*	NCFT, No.4590.11
2	Phalaenopsis	Collar rot	*Sclerotium rolfsii*	Nil*
3	Phalaenopsis	Flower spot	*Phoma exigua*	NCFT, No.4591.11
4	Phalaenopsis	Anthracnose	*Colletotrichum gloeosporioides*	Nil*
5	Basket Vanda	Heart rot	*Phytophthora cactorum*	NCFT, No.4592.11
6	Basket Vanda	Sclerotium wilt	*Sclerotium rolfsii*	Nil*
7	Basket Vanda	Leaf spot	*Botryodiplodia theobromae*	NCFT, No.4593.11
8	Basket Vanda	Anthracnose	*Colletotrichum gloeosporioides*	Nil*
9	Mokara	Leaf spot	*Alternaria alternata*	NCFT, No.4594.11
10	Arachnis	Anthracnose	*Colletotrichum gloeosporioides*	Nil*
11	Arachnis	Leaf spot	*Fusarium oxysporum*	NCFT, No.4595.11

*Identified based on cultural and morphological studies and comparison with reference culture.

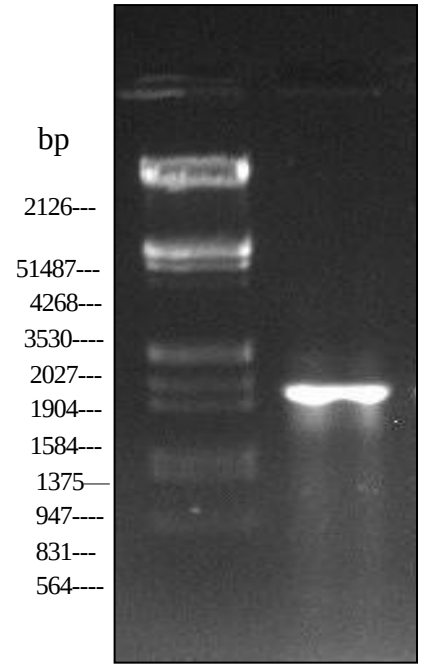
4.5.3. Identification of bacterial pathogens

The bacterial pathogens were identified based on molecular characterisation by SciGenom, Kakanad and Vision Centre, Angamaly. By amplification of 16srRNA gene, one amplicon of about 1500 bp was obtained for *Burkholderia* sp.(Plate 9B) and 1200 bp was obtained in the case of *Erwinia* sp.(Plate 9A) and it was purified. Sequence analysis was carried out and nucleotide homology was found out for bacterial pathogen of Basket Vanda and the same was identified as *Burkholderia*

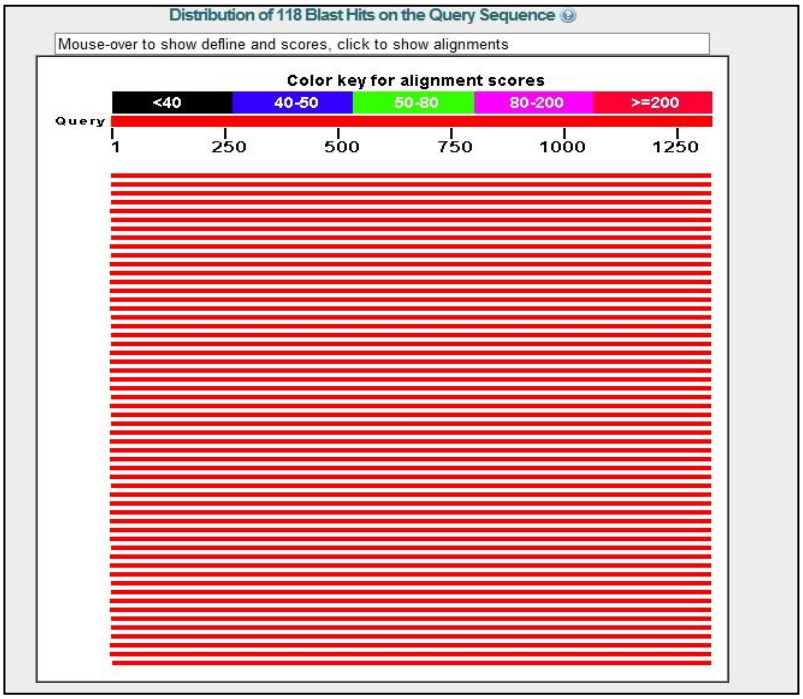
Plate 9. Amplification of 16S rRNA gene of bacteria



A.*Erwinia chrysanthemi*



B. *Burkholderia gladioli*



Descriptions

Legend for links to other resources: UniGene GEO Gene Structure Map Viewer PubChem BioAssay

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AB713571.1	Dickeya sp. SUPP2739 gene for 16S rRNA, partial sequence, str	2444	2444	99%	0.0	99%
AB713570.1	Dickeya sp. SUPP2738 gene for 16S rRNA, partial sequence, str	2444	2444	99%	0.0	99%
AB713554.1	Dickeya sp. SUPP40 gene for 16S rRNA, partial sequence, strain	2444	2444	99%	0.0	99%
AB713537.1	Dickeya sp. SUPP1399 gene for 16S rRNA, partial sequence, str	2444	2444	99%	0.0	99%
FM946179.1	Pectobacterium chrysanthemi partial 16S rRNA, isolate SCH-01	2444	2444	100%	0.0	99%
AB713566.1	Dickeya sp. SUPP1164 gene for 16S rRNA, partial sequence, str	2440	2440	99%	0.0	99%
AB713562.1	Dickeya sp. SUPP420 gene for 16S rRNA, partial sequence, strai	2440	2440	99%	0.0	99%
AB713550.1	Dickeya sp. SUPP2451 gene for 16S rRNA, partial sequence, str	2438	2438	99%	0.0	99%
HM590191.1	Erwinia chrysanthemi strain miandian2 16S ribosomal RNA gene,	2438	2438	100%	0.0	99%
AB713535.1	Dickeya sp. SUPP2586 gene for 16S rRNA, partial sequence, str	2435	2435	99%	0.0	99%
JN940859.1	Dickeya sp. PA1 16S ribosomal RNA gene, partial sequence	2433	2433	100%	0.0	99%
HQ287573.1	Dickeya sp. 0827-2 16S ribosomal RNA gene, partial sequence	2433	2433	100%	0.0	99%
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HM590189.1	Erwinia chrysanthemi strain yunnan 16S ribosomal RNA gene, pa	2433	2433	100%	0.0	99%
GQ293897.1	Erwinia chrysanthemi strain 1015-1 16S ribosomal RNA gene, pa	2433	2433	100%	0.0	99%
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AB713564.1	Dickeya sp. SUPP1152 gene for 16S rRNA, partial sequence, str	2429	2429	99%	0.0	99%
AB713568.1	Dickeya sp. SUPP2735 gene for 16S rRNA, partial sequence, str	2427	2427	99%	0.0	99%
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HM590194.1	Erwinia chrysanthemi strain sangzhi2 16S ribosomal RNA gene, p	2427	2427	100%	0.0	99%
HM590193.1	Erwinia chrysanthemi strain sangzhi1 16S ribosomal RNA gene, p	2427	2427	100%	0.0	99%
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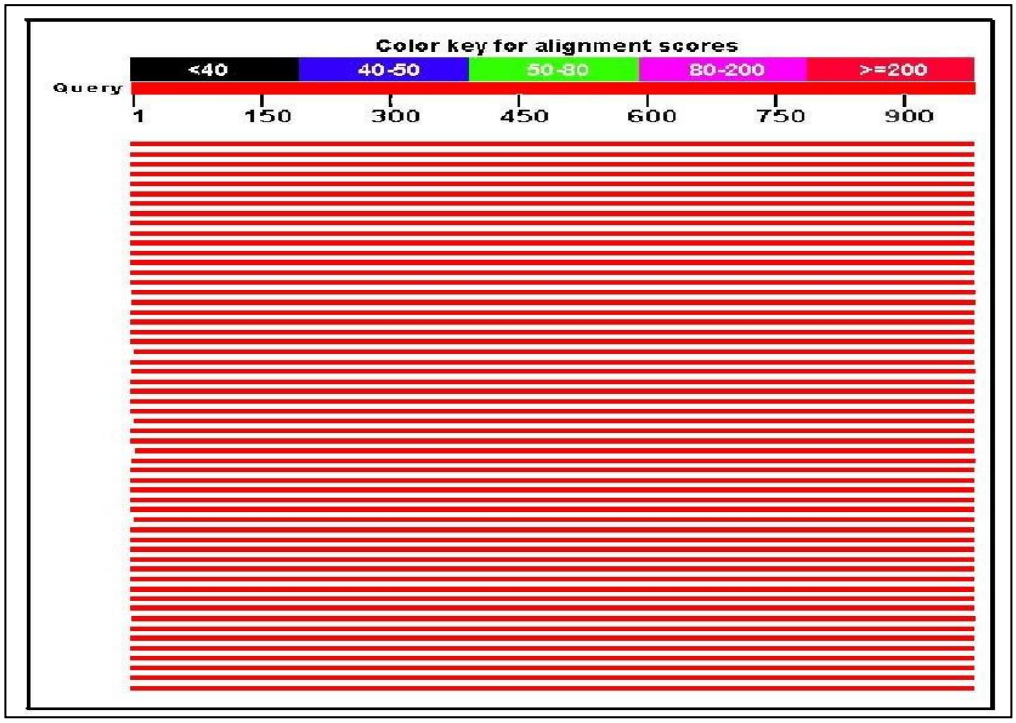
JQ867399.1	Erwinia chrysanthemi strain Y4 16S ribosomal RNA gene, partial	2422	2422	100%	0.0	99%

Fig.1. Sequence analysis of *Erwinia chrysanthemi*



Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
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HQ624993.1	Bacterium SZ6-2 16S ribosomal RNA gene, partial sequence	1799	1799	100%	0.0	99%	
HM231304.1	Burkholderia gladioli strain 343-3318 16S ribosomal RNA gene, partial sequence	1799	1799	100%	0.0	99%	
GU936679.1	Burkholderia gladioli pv. alliicola strain CFBP 2422 16S ribosomal RNA gene, partial sequence	1799	1799	100%	0.0	99%	
GU479033.1	Burkholderia gladioli pv. gladioli 16S ribosomal RNA gene, partial sequence	1799	1799	100%	0.0	99%	
EJ593642.1	Uncultured bacterium clone JAB A7 16S ribosomal RNA gene, partial sequence	1799	1799	100%	0.0	99%	
EF193645.1	Burkholderia gladioli strain PA17.2 16S ribosomal RNA gene, partial sequence	1799	1799	100%	0.0	99%	
EF193644.1	Burkholderia gladioli strain PA14.4 16S ribosomal RNA gene, partial sequence	1799	1799	100%	0.0	99%	
EF193643.1	Burkholderia gladioli strain PA13.5 16S ribosomal RNA gene, partial sequence	1799	1799	100%	0.0	99%	
EF193642.1	Burkholderia gladioli strain PA11.1 16S ribosomal RNA gene, partial sequence	1799	1799	100%	0.0	99%	
EF088208.1	Burkholderia gladioli strain S10 16S ribosomal RNA gene, partial sequence	1799	1799	100%	0.0	99%	
AY268167.1	Burkholderia gladioli strain 1993027208 16S ribosomal RNA gene, partial sequence	1799	1799	100%	0.0	99%	
AY353696.1	Burkholderia sp. 45250588-5 16S ribosomal RNA gene, partial sequence	1799	1799	100%	0.0	99%	
DQ355168.1	Burkholderia gladioli strain 223gr-1 16S ribosomal RNA gene, partial sequence	1799	1799	100%	0.0	99%	
AB354157.2	Bacterium 2a-Bj4 gene for 16S rRNA, partial sequence	1797	1797	99%	0.0	99%	
AB354152.2	Bacterium 1b-Mt10 gene for 16S rRNA, partial sequence	1797	1797	99%	0.0	99%	
AY297695.1	Burkholderia gladioli 16S ribosomal RNA gene, partial sequence	1796	1796	100%	0.0	99%	
HQ624992.1	Bacterium SZ6-1 16S ribosomal RNA gene, partial sequence	1794	1794	100%	0.0	99%	
	Burkholderia gladioli strain OM1 16S						

Fig.2. Sequence analysis of *Burkholderia gladioli*

gladioli from Vision centre, Angamaly (Identification No. VSS/107). Along with nucleotide homology, phylogenetic analysis was also carried out and soft rot bacteria of Phalaenopsis was identified as *Erwinia chrysanthemi* from SciGenom, Kakanad (Identification No.338-0).

4.6. SEASONAL INFLUENCE ON THE OCCURRENCE OF DISEASES

The influence of season on the occurrence of different diseases of monopodial orchids were observed by recording per cent disease incidence and severity from orchidarium of AICRP on Floriculture Improvement in the Department of Pomology and Floriculture , College of Horticulture, Vellanikkara. During the study, the main diseases noticed in the orchidarium were Fusarium wilt, Collar rot and Soft rot of Phalaenopsis, Botryodiplodia leaf spot of Basket Vanda, Alternaria leaf spot of Mokara and Fusarium leaf spot of Arachnis. Phalaenopsis was grown in rainshelter, Basket Vanda and Mokara in poly house and Arachnis in open condition.

4.6.1. Assessment of disease incidence

Per cent disease incidence of Fusarium wilt, collar rot and soft rot of Phalaenopsis, Botryodiplodia leaf spot of Basket Vanda, Alternaria leaf spot of Mokara and Fusarium leaf spot of Arachnis were calculated and the data are given in Table 7 and 8. The per cent disease incidence was correlated with the temperature and relative humidity recorded in the growing structure and rainfall received in open condition. The details of temperature, relative humidity and rainfall recorded are given in Table 9, 10 and 11.

4.6.1.1. Fusarium wilt of Phalaenopsis

From the data presented in Table 7, it was observed that severe infection of Fusarium wilt was noticed during November with maximum disease incidence of

10.41 per cent. It was found to decrease in the subsequent months and further increased from June onwards. Disease was not observed in the month of March and May. During the month of November, minimum temperature was 25.50ºC, maximum temperature was 34.57ºC, mean temperature was 30.04ºC, relative humidity was 58.36 per cent and the rainfall was 49.60 mm (Table 9).

4.6.1.2. Collar rot of Phalaenopsis

Incidence of collar rot by *Sclerotium rolfsii* was more during the month of December with a maximum disease incidence of 6.52 per cent (Table 7). During this month, minimum temperature of 24.65ºC and the maximum temperature of 34.67ºC with a mean temperature of 29.66ºC, relative humidity of 54.53 per cent and rainfall of 2.40 mm (Table 9) were recorded.

4.6.1.3. Soft rot of Phalaenopsis

From the data given in Table 7, it was observed that soft rot caused by *Erwinia chrysanthemi* was more during rainy months (June - August). The maximum incidence of 10 per cent was recorded in June followed by 8.93 per cent in July and 7.50 per cent in August. From Table 9, it was observed that during these months, minimum temperature of 24.83 - 25.60ºC and the maximum temperature of 33 - 33.59ºC were recorded with a mean temperature of 28.93º-29.59ºC. The relative humidity ranged from 78.70- 79.42 per cent with high rain fall of 647.30 - 731.80 mm . Disease was not observed in the months of February - April and in December.

4.6.1.4. Botryodiplodia leaf spot of Basket Vanda

From the data presented in the Table 8, it was observed that incidence of Botryodiplodia leaf spot was high in almost all months and was varied between 64 - 84 per cent with maximum in the month of June. Minimum temperature of 26.90ºC,

Table 7. Per cent incidence of diseases of Phalaenopsis

Period	Per cent disease incidence		
	Fusarium wilt (*Fusarium* sp.)	Collar rot (*Sclerotium* sp.)	Soft rot (*Erwinia* sp.)
May'11	0	0	2.00
June	7.05	0	10.00
July	4.30	2.50	8.93
August	6.25	2.94	7.50
September	4.30	1.20	2.40
October	5.20	2.85	6.70
November	10.41	0	7.25
December	4.30	6.52	0
January '12	4.30	2.00	2.17
February	2.54	2.80	0
March	0	0	0
April	0	0	0

PDI – Per cent Disease Incidence

PDS – Per cent Disease Severity

maximum temperature of 34.52ºC with a mean temperature of 30.71 ºC was noticed in the month of June (Table 10). Relative humidity and rainfall observed during this month were 83 per cent and 551.50mm respectively.

4.6.1.5. Alternaria leaf spot of Mokara

In Mokara, almost all plants were infected by the pathogen *Alternaria alternata* and the disease incidence was varied between 79.48 - 92.3 per cent (Table 8). The maximum incidence was noticed in the month of June. During this month, minimum temperature of 26.9 ºC and the maximum temperature of 34.52ºC were recorded with a mean temperature of 30.71ºC (Table 10). Relative humidity and rainfall observed during this month were 83 per cent and 551.50mm respectively.

4.6.1.6. Fusarium leaf spot of Arachnis

In Arachnis, cent per cent incidence of Fusarium leaf spot was recorded throughout the year (Table 8).

4.6.2. Assessment of disease severity

The per cent disease severity was recorded for Botryodiplodia leaf spot of Basket Vanda, Alternaria leaf spot of Mokara and Fusarium leaf spot of Arachnis and the data are given in Table 8.

4.6.2.1. Botryodiplodia leaf spot of Basket Vanda

From the data given in Table 8, it was observed that severity of Botryodiplodia leaf spot of Basket Vanda was in the range of 15.20 - 28 per cent and the maximum infection was recorded in the month of June. During this month, minimum temperature of 26.90 ºC and maximum temperature of 34.52ºC were recorded with a mean temperature of 30.71ºC (Table 10). Relative humidity and rain fall during the month of June was 83 per cent and 551.50 mm respectively.

4.6.2.2. Alternaria leaf spot of Mokara

The per cent disease severity of Alternaria leaf spot of Mokara is given in the Table 8 and was in the range of 12.80 - 21.60 per cent. High disease severity was recorded in the months of April - June (21.20 -21.60 per cent). During these periods, minimum temperature ranged from 26.90 - 29.92 ºC and the maximum temperature ranged from 34.52 - 39.17 ºC with a mean temperature of 30.71 - 34.54ºC (Table 10). Rainfall and relative humidity during these months were in the range of 101.90 - 551.50 mm and 69.93 - 83 per cent respectively.

4.6.2.3. Fusarium leaf spot of Arachnis

From the data presented in the Table 8, it was observed that severity of Fusarium leaf spot of Arachnis was ranged between 13.20 - 24.10 per cent and maximum was in June. During this month, minimum temperature was 23.90 ºC and maximum temperature was 30.10ºC with a mean temperature of 27ºC. Relative humidity and rainfall were 85 per cent and 551.50 mm respectively. The weather data are presented in the Table 11.

Table 8.Per cent incidence and severity of leaf spot diseases of Basket Vanda, Mokara and Arachnis

Period	Leaf spot of Basket Vanda (*Botryodiplodia* sp.)		Leaf sot of Mokara (*Alternaria* sp.)		Leaf spot of Arachnis (*Fusarium* sp.)	
	PDI	PDS	PDI	PDS	PDI	PDS
July'11	76.92	15.20	79.48	12.80	100	18.00
August	76.00	20.00	82.05	14.00	100	16.70
September	72.00	21.20	82.05	15.60	100	19.60
October	76.00	23.20	80.00	15.60	100	13.20
November	64.00	24.40	82.50	18.80	100	22.40
December	68.00	24.40	82.05	20.00	100	15.60
January'12	80.00	25.20	80.95	20.00	100	18.80
February	76.00	25.20	84.61	20.40	100	21.20
March	72.00	25.60	83.33	20.80	100	23.00
April	76.00	26.40	87.17	21.20	100	15.40
May	84.00	26.40	87.17	21.20	100	19.60
June	84.00	28.00	92.30	21.60	100	24.10

PDI – Per cent Disease Incidence

PDS – Per cent Disease Severity

Table 9.Temperature and relative humidity in rain shelter from May'11 to April'12

Month	Temperature (ºC)			Average RH (%)
	Max Temp	Min Temp	Mean Temp	
May'11	39.17	28.32	33.74	65.96
June	33.59	25.60	29.59	78.73
July	33.04	24.83	28.93	78.7
August	33.00	25.23	29.11	79.42
September	34.84	25.39	30.11	72.76
October	38.28	26.49	32.38	64.79
November	34.57	25.50	30.04	58.36
December	34.67	24.65	29.66	54.53
January'12	35.83	23.67	29.75	52.62
February	38.02	25.03	31.52	46.85
March	37.47	25.89	31.68	54.77
April	38.34	26.52	32.43	65.63

Table 10. Temperature and relative humidity in poly house from July'11 to June'12

Month	Temperature (ºC)			Average RH (%)
	Max Temp	Min Temp	Mean Temp	
July'11	33.50	25.21	29.35	82.50
August	33.67	25.92	29.79	81.24
September	35.38	26.41	30.89	77.13
October	38.60	26.54	32.57	71.95
November	35.57	26.42	30.99	68.36
December	34.98	25.12	30.05	64.68
January'12	36.11	24.85	30.48	52.62
February	36.56	25.98	31.27	48.95
March	37.96	26.56	32.26	56.48
April	38.50	28.10	33.30	69.93
May	39.17	29.92	34.54	78.00
June	34.52	26.90	30.71	83.00

Table 11.Weather data at monthly intervals from July 2011 to June 2012

Period	Temperature(°C)			Average Relative Humidity (%)	Total Rainfall (mm)
	Max. Temp.	Min.Temp.	Mean Temp.		
July'11	29.00	23.55	26.27	88.40	731.80
August	29.40	22.87	26.13	87.25	688.90
September	30.07	23.10	26.58	83.50	435.20
October	31.84	23.40	27.62	78.30	383.40
November	31.52	22.92	27.22	66.75	49.60
December	32.05	22.57	27.31	61.00	2.40
January'12	32.50	21.50	27.00	56.40	0
February	35.32	22.17	28.75	56.75	0
March	35.20	31.87	33.54	67.25	3.50
April	34.38	24.82	29.60	73.40	101.90
May	32.60	25.30	28.95	76.00	117.30
June	30.10	23.90	27.00	85.00	551.50

4.7. MANAGEMENT OF PATHOGENS ASSOCIATED WITH DISEASES OF ORCHIDS

The efficacy of fungicides, antibiotics, *Pseudomonas fluorescens* and fresh cowdung extract were evaluated against different pathogens of monopodial orchids under in vitro condition. Fungicides such as potassium phosphonate 40% + mancozeb 75%, cymoxanil 8% + mancozeb 64% , carbendazim 12% + mancozeb 63%, carbendazim 25% + ipridione 25% , fenamidone 10% + mancozeb 50% and biocontrol agent *Pseudomonas fluorescens* (liquid 0.5%) were evaluated against fungal pathogens. For management of the bacteria, different treatments evaluated were Streptocycline 200ppm, fresh cowdung extract 2%, *P. fluorescens* (0.5% liquid) and fresh cowdung extract 2% + *P. fluorescens* (0.5% liquid) were used.

4.7.1. *In vitro* evaluation against fungal pathogens

In vitro evaluation was carried out against fungal pathogens *viz., Fusarium oxysporum* (Phalaenopsis and Arachnis), *Sclerotium rolfsii* (Phalaenopsis and Basket Vanda), *Phoma exigua* (Phalaenopsis), *Colletotrichum gloeosporioides* (Phalaenopsis, Mokara and Arachnis), *Phytophthora cactorum* (Basket Vanda), *Botryodiplodia theobromae* (Basket Vanda) and *Alternaria alternata* (Mokara). The fungicides used for the study were potassium phosphonate 40% + mancozeb 75%, cymoxanil 8% + mancozeb 64%, carbendazim 12% + mancozeb 63%, carbendazim 25% + ipridione 25%, fenamidone 10% + mancozeb 50%. The efficacy of bio control agent *P. fluorescens* (0.5% of liquid formulation) was also tested against the fungal pathogens.

4.7.1.1. *Fusarium oxysporum* from Phalaenopsis

The data on the efficacy of different fungicides and *P. fluorescens* on the growth of *Fusarium oxysporum* is given in Table 12. All fungicides *viz.*, potassium phosphonate 40% + mancozeb 75% , cymoxanil 8% + mancozeb 64%, carbendazim 12% + mancozeb 63%, carbendazim 25% + ipridione 25%, fenamidone 10% + mancozeb 50% showed cent per cent inhibition of the pathogen, while *P. fluorescens* showed 61.85 per cent inhibition over control (Plate 10 A).

4.7.1.2. *Sclerotium rolfsii* from Phalaenopsis

The results given in Table 13 revealed that all the combination fungicides *viz.*, potassium phosphonate 40% + mancozeb 75%, cymoxanil 8% + mancozeb 64%, carbendazim 12% + mancozeb 63%, carbendazim 25% + ipridione 25%, fenamidone 10% + mancozeb 50% used in the study showed complete inhibition on the growth, and the *P. fluorescens* showed 64.8 per cent inhibition over control. The observation was taken when the control plates recorded 90 mm growth by five days of incubation (Plate 10 B).

4.7.1.3. *Phoma exigua* from Phalaenopsis

Fungicides *viz.*, potassium phosphonate 40% + mancozeb 75%, carbendazim 25% + ipridione 25% and fenamidone 10% + mancozeb 50% showed cent per cent inhibition of fungus while carbendazim 12%+ mancozeb 63% and cymoxanil 8% + mancozeb 64% showed 87.41 and 79.63 per cent inhibition of the fungus respectively. *P.fluorescens* showed 52.97 per cent inhibition of the fungus (Table14). The complete growth of control plate was recorded after five days of incubation at room temperature (Plate 10 C).

Table 12.*In vitro* evaluation of fungicides and *P. fluorescens* against *Fusarium oxysporum* from Phalaenopsis

Treatment No.	Treatment Details	Concentration (%)	Mean Diameter (mm)	Per cent inhibition over control
1	Potassium Phosphonate 40%+ Mancozeb 75%	0.2 + 0.15	0	100
2	Cymoxanil 8% + Mancozeb 64%	0.2	0	100
3	Carbendazim 12% + Mancozeb 63%	0.15	0	100
4	Carbendazim 25% + Ipridione 25%	0.2	0	100
5	Fenamidone 10% + Mancozeb 50%	0.1	0	100
6	*P. fluorescens*(Liquid)	0.5	34.33	61.85
7	Control		90	

*Mean of three replications

Table 13. *In vitro* evaluation of fungicides and *P. fluorescens* against *Sclerotium rolfsii* from Phalaenopsis

Treatment No.	Treatment Details	Concentration (%)	Mean diameter (mm)	Per cent inhibition over control
1	Potassium Phosphonate 40% + Mancozeb 75%	0.2 + 0.15	0	100
2	Cymoxanil 8% + Mancozeb 64%	0.2	0	100
3	Carbendazim 12% + Mancozeb 63%	0.15	0	100
4	Carbendazim 25% + Ipridione 25%	0.2	0	100
5	Fenamidone 10% + Mancozeb 50%	0.1	0	100
6	*P. fluorescens* (Liquid)	0.5	31.66	64.80
7	Control		90	

*Mean of three replications

Table 14. *In vitro* evaluation of fungicides and *P. fluorescens* against *Phoma exigua* from Phalaenopsis

Treatment No.	Treatment Details	Concentration (%)	Mean diameter (mm)	Per cent inhibition over control
1	Potassium Phosphonate 40% + Mancozeb 75%	0.2 + 0.15	0	100
2	Cymoxanil 8% + Mancozeb 64%	0.2	18.33	79.63
3	Carbendazim 12% + Mancozeb 63%	0.15	11.33	87.41
4	Carbendazim 25% + Ipridione 25%	0.2	0	100
5	Fenamidone 10% + Mancozeb 50%	0.1	0	100
6	*P. fluorescens* (Liquid)	0.5	42.33	52.97
7	Control		90	

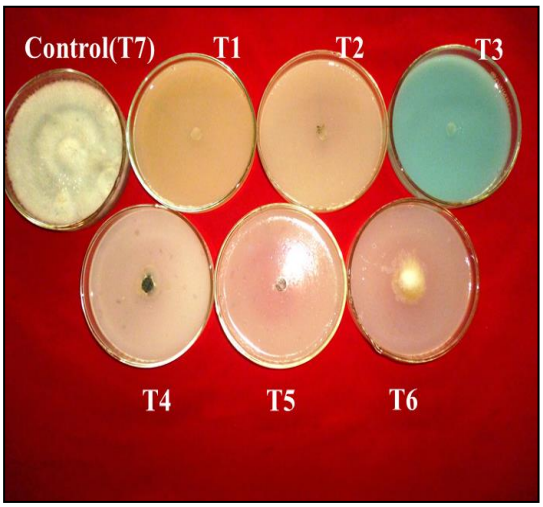
*Mean of three replications

Plate 10. *In vitro* evaluation against fungal pathogens of Phalaenopsis

A.*Fusarium oxysporum*

B.*Sclerotium rolfsii*

C. *Phoma exigua*



D. *Colletotrichum gloeosporioides*

4.7.1.4. *Colletotrichum gloeosporioides* from Phalaenopsis

The data on the efficacy of different fungicides and *P. fluorescens* are given in the Table 15. All the five fungicides tested gave complete inhibition of the pathogen, and the treatment with *P. fluorescens* showed 86.30 per cent inhibition on the growth over control (Plate 10 D). The complete growth of control plate was recorded after eight days of incubation at room temperature.

4.7.1.5. *Phytophthora cactorum* from Basket Vanda

From the data given in the Table 16, fungicides *viz.*, potassium phosphonate 40% + mancozeb 75% , cymoxanil 8% + mancozeb 64%, carbendazim 12% + mancozeb 63%, fenamidone 10% + mancozeb 50% recorded cent per cent inhibition of the pathogen and it was found that carbendazim 25% + ipridione 25% showed 64.44 per cent inhibition. *P. fluorescens* gave 83.33 per cent inhibition of the fungus. The efficiency of *P. fluorescens* was found to be higher than the fungicide carbendazim 25% + ipridione 25% in the *in vitro* evaluation against *P. cactorum* (Table 15). The maximum growth of 90mm was recorded in the control plate after six days of incubation (Plate .11 A)

4.7.1.6. *Sclerotium rolfsii* from Basket Vanda

The data given in Table 17 showed that all the fungicides used in the study gave complete inhibition of the fungus, and *P. fluorescens* gave 68.89 per cent inhibition (Plate 11 B).The maximum growth of 90 mm was recorded in the control plate after five days of incubation.

Table 15. *In vitro* evaluation of fungicides and *P. fluorescens* against *Colletotrichum gloeosporoiodes* from Phalaenopsis

Treatment No.	Treatment Details	Concentration (%)	Mean diameter (mm)	Per cent inhibition over control
1	Potassium Phosphonate 40% + Mancozeb 75%	0.2 + 0.15	0	100
2	Cymoxanil 8% + Mancozeb 64%	0.2	0	100
3	Carbendazim 12% + Mancozeb 63%	0.15	0	100
4	Carbendazim 25% + Ipridione 25%	0.2	0	100
5	Fenamidone 10% + Mancozeb 50%	0.1	0	100
6	*P. fluorescens* (Liquid)	0.5	22.33	86.30
7	Control		90	

*Mean of three replications

Table 16. *In vitro* evaluation of fungicides and *P. fluorescens* against *Phytophthora cactorum* from Basket Vanda

Treatment No.	Treatment Details	Concentration (%)	Mean diameter (mm)	Per cent inhibition over control
1	Potassium Phosphonate 40%+ Mancozeb 75%	0.2 + 0.15	0	100
2	Cymoxanil 8% + Mancozeb 64%	0.2	0	100
3	Carbendazim 12% + Mancozeb 63%	0.15	0	100
4	Carbendazim 25% + Ipridione 25%	0.2	32	64.44
5	Fenamidone 10% + Mancozeb 50%	0.1	0	100
6	*P. fluorescens* (Liquid)	0.5	15	83.33
7	Control		90	

Table 17. *In vitro* evaluation of fungicides and *P. fluorescens* against *Sclerotium rolfsii* from Basket Vanda

Treatment No.	Treatment Details	Concentration (%)	Mean diameter (mm)	Per cent inhibition over control
1	Potassium Phosphonate 40%+ Mancozeb 75%	0.2 + 0.15	0	100
2	Cymoxanil 8% + Mancozeb 64%	0.2	0	100
3	Carbendazim 12% + Mancozeb 63%	0.15	0	100
4	Carbendazim 25% + Ipridione 25%	0.2	0	100
5	Fenamidone 10% + Mancozeb 50%	0.1	0	100
6	*P. fluorescens* (Liquid)	0.5	28	68.89
7	Control		90	

*Mean of three replications

4.7.1.7. *Botryodiplodia theobromae* from Basket Vanda

The data on the inhibitory effect of different fungicides and *P. fluorescens* are given in Table 18. All the fungicides *viz*., potassium phosphonate 40% + mancozeb 75%, cymoxanil 8% + mancozeb 64%, carbendazim 12% + mancozeb 63%, carbendazim 25% + ipridione 25%, fenamidone 10% + mancozeb 50% inhibited the fungus completely, while *P. fluorescens* gave 77.03 per cent inhibition and 20.66 mm growth of fungus by three days when control plate showed full growth (Plate 11C).

4.7.1.8. *Colletotrichum gloeosporioides* from Mokara

The efficacy of different fungicides and *P. fluorescens* are given in the Table 19. All the fungicides used in the treatment against *C. gloeosporioides* gave complete inhibition of the fungus, and the *P. fluorescens* gave 75.92 per cent inhibition and 21.67 mm fungal growth when growth of fungus in the control plate attained full growth of 90mm at seven days of incubation at room temperature (Plate 12 A).

4.7.1.9. *Alternaria alternata* from Mokara

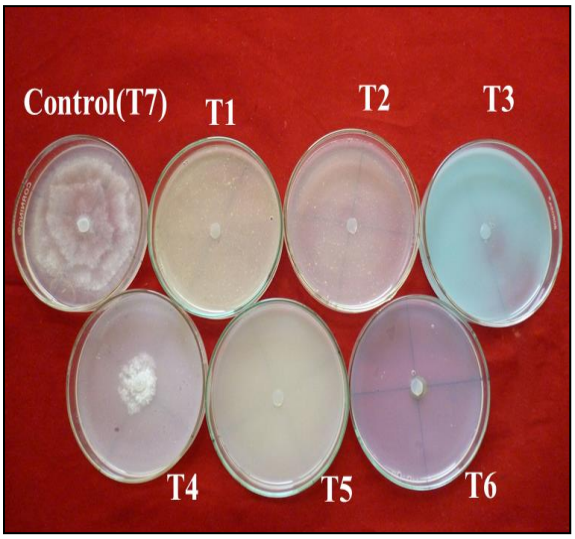
From the data given in the Table 20, fungicides viz., potassium phosphonate 40%+ mancozeb 75%, cymoxanil 8% + mancozeb 64%, carbendazim 25% + ipridione 25%, recorded cent per cent inhibition of the fungus whereas carbendazim 12% + mancozeb63% and fenamidone10% + mancozeb50% gave 87.03 per cent inhibition of the fungus. *P. fluorescens* gave 83.33 per cent inhibition of the fungus (Plate 12B). The maximum growth of 90 mm was recorded in the control plate after nine days of incubation at room temperature.

Table 18. *In vitro* evaluation of fungicides and *P. fluorescens* against *Botryodiplodia theobromae* from Basket Vanda

Treatment No.	Treatment Details	Concentration (%)	Mean diameter (mm)	Per cent inhibition over control
1	Potassium Phosphonate 40% + Mancozeb 75%	0.2 + 0.15	0	100
2	Cymoxanil 8% + Mancozeb 64%	0.2	0	100
3	Carbendazim 12% + Mancozeb 63%	0.15	0	100
4	Carbendazim 25% + Ipridione 25%	0.2	0	100
5	Fenamidone 10% + Mancozeb 50%	0.1	0	100
6	*P. fluorescens* (Liquid)	0.5	20.67	77.03
7	Control		90	

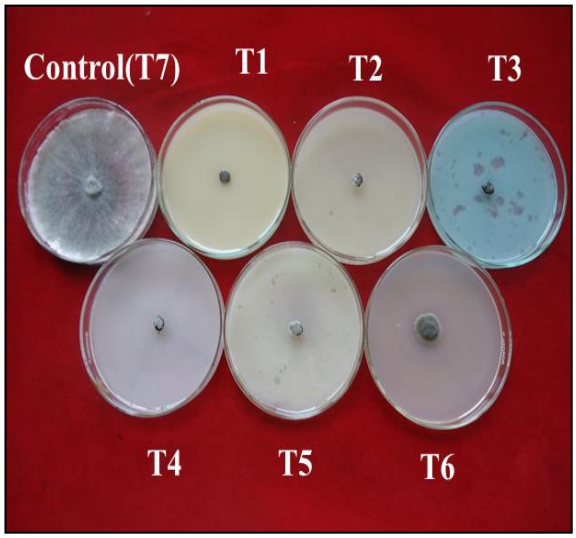
*Mean of three replications

Plate 11. *In vitro* evaluation against fungal pathogens of Basket Vanda



A.*Phytophthora cactorum*

B.*Sclerotium rolfsii*



C.*Botryodiplodia theobromae*

Table 19. *In vitro* evaluation of fungicides and *P. fluorescens* against *Colletotrichum gloeosporioides* from Mokara

Treatment No.	Treatment Details	Concentration (%)	Mean diameter (mm)	Per cent inhibition over control
1	Potassium Phosphonate 40% + Mancozeb 75%	0.2 + 0.15	0	100
2	Cymoxanil 8% + Mancozeb 64%	0.2	0	100
3	Carbendazim 12% + Mancozeb 63%	0.15	0	100
4	Carbendazim 25% + Ipridione 25%	0.2	0	100
5	Fenamidone 10% + Mancozeb 50%	0.1	0	100
6	*P. fluorescens* (Liquid)	0.5	21.67	75.92
7	Control		90	

*Mean of three replications

Table 20. *In vitro* evaluation of fungicides and *P. fluorescens* against *Alternaria alternata* from Mokara

Treatment No.	Treatment Details	Concentration (%)	Mean diameter (mm)	Per cent inhibition over control
1	Potassium Phosphonate 40%+ Mancozeb 75%	0.2 + 0.15	0	100
2	Cymoxanil 8% + Mancozeb 64%	0.2	0	100
3	Carbendazim 12% + Mancozeb 63%	0.15	11.66	87.03
4	Carbendazim 25% + Ipridione 25%	0.2	0	100
5	Fenamidone 10% + Mancozeb 50%	0.1	11.66	87.03
6	*P. fluorescens* (Liquid)	0.5	15	83.33
7	Control		90	

*Mean of three replications

4.7.10. *Colletotrichum gloeosporioides* from Arachnis

The efficacy of different fungicides and *P. fluorescens* on the growth of *Colletotrichum gloeosporioides* is given in Table 21. All fungicides gave cent per cent inhibition of fungus, while *P. fluorescens* showed 24 mm growth against full growth in control at six days after incubation with an inhibition of 73.33 per cent of fungus (Plate 12 C).

4.7.1.11. *Fusarium oxysporum* from Arachnis

From the data given in the Table 22, it was found that the fungicides *viz*., potassium phosphonate 40% + mancozeb 75% , cymoxanil 8% + mancozeb 64%, carbendazim 12% + mancozeb 63%, carbendazim 25% + ipridione 25% showed cent per cent inhibition of the fungus while fenamidone 10% + mancozeb 50% showed 70 per cent inhibition. Bio control agent *P. fluorescens* gave 68.52 per cent inhibition of the fungus (Plate 12 D). Full growth of 90mm was recorded in the control plate after nine days of incubation.

4.7.2. *In vitro* evaluation against bacterial pathogens

In vitro evaluation was done against the bacterial pathogens *viz*., *Erwinia chrysanthemi*, and *Burkholderia gladioli* with streptocycline (200 ppm), fresh cowdung extract 2%, *Pseudomonas fluorescens* 0.5% and fresh cowdung extract 2% + *P. fluorescens* 0.5%.

4.7.2.1. *Erwinia chrysanthemi* from Phalaenopsis

The efficacy of antibiotic, cowdung and *P. fluorescens* against *E. chrysanthemi* causing soft rot in Phalaenopsis is given in the Table 23. Among the treatments used against *E. chrysanthemi*, *P. fluorescens* showed maximum inhibition (65.56 %) of the bacteria. It was on par with fresh cowdung extract 2% + *P. fluorescens* 0.5% and fresh cowdung extract 2% which recorded 57.77 and 55.92 per

Table 21. *In vitro* evaluation of fungicides and *P. fluorescens* against *Colletotrichum gloeosporioides* from Arachnis

Treatment No.	Treatment Details	Concentration (%)	Mean diameter (mm)	Per cent inhibition over control
1	Potassium Phosphonate 40% + Mancozeb 75%	0.2 + 0.15	0	100
2	Cymoxanil 8% + Mancozeb 64%	0.2	0	100
3	Carbendazim 12% + Mancozeb 63%	0.15	0	100
4	Carbendazim 25% + Ipridione 25%	0.2	0	100
5	Fenamidone 10% + Mancozeb 50%	0.1	0	100
6	*P. fluorescens* (Liquid)	0.5	24	73.33
7	Control		90	

*Mean of three replications

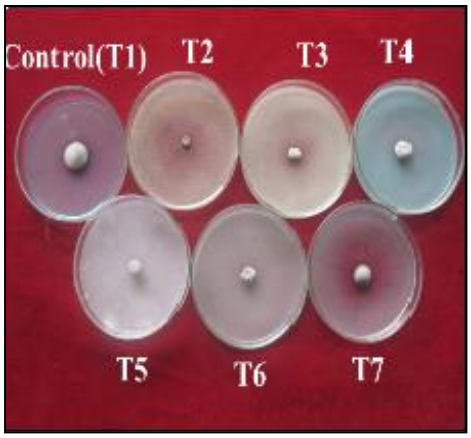
Table 22. *In vitro* evaluation of fungicides and *P. fluorescens* against *Fusarium oxysporum* from Arachnis

Treatment No.	Treatments	Concentration (%)	Mean diameter (mm)	Per cent inhibition over control
1	Potassium Phosphonate 40% + Mancozeb 75%	0.2 + 0.15	0	100
2	Cymoxanil 8% + Mancozeb 64%	0.2	0	100
3	Carbendazim 12% + Mancozeb 63%	0.15	0	100
4	Carbendazim 25% + Ipridione 25%	0.2	0	100
5	Fenamidone 10% + Mancozeb 50%	0.1	27	70
6	*P. fluorescens* (Liquid)	0.5	28.33	68.52
7	Control		90	

*Mean of three replications

Plate 12. *In vitro* evaluation against fungal pathogens of Mokara and Arachnis

A. *Colletotrichum.gloeosporioides*



B. *Alternaria alternata*

Mokara

C. *Colletotrichum.gloeosporioides*

D. *Fusarium oxysporum*

Arachnis

Table 23. *In vitro* evaluation of antibiotic, cowdung and *P. fluorescens* against *Erwinia chrysanthemi* from Phalaenopsis

Treatment No.	Treatment Details	Concentration	Mean diameter (mm)	Per cent inhibition over control
1	Streptocycline	200 ppm	62.33[b]	30.74
2	Fresh cowdung extract	2%	39.67[a]	55.92
3	*P. fluorescens* (Liquid)	0.5%	31.00[a]	65.56
4	Fresh cowdung extract + *P. fluorescens* (Liquid)	2% + 0.5%	38.00[a]	57.77
5	Control		90	

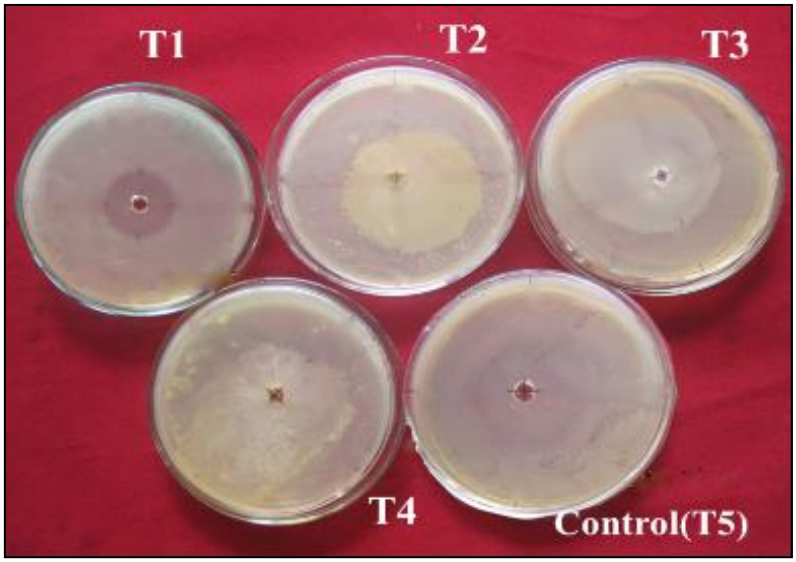
Table 24. *In vitro* evaluation of antibiotic, cowdung and *P. fluorescens* against *Burkholderia gladioli* from Basket Vanda

Treatment No.	Treatment Details	Concentration	Mean diameter (mm)	Per cent inhibition over control
1	Streptocycline	200 ppm	64.67[b]	28.14
2	Fresh cowdung extract	2%	38.33[a]	57.41
3	*P. fluorescens* (Liquid)	0.5%	46.00[a]	48.89
4	Fresh cowdung extract + *P. fluorescens* (Liquid)	2% + 0.5%	51.67[ab]	42.59
5	Control		90	

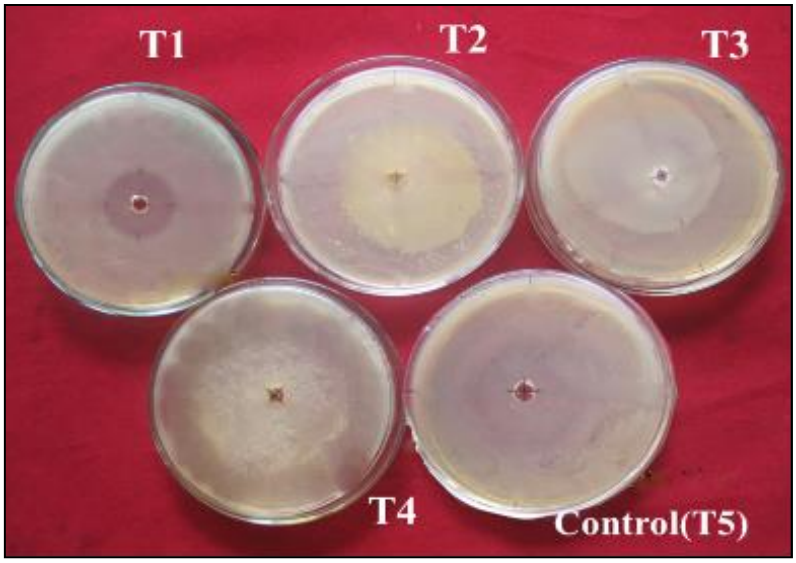
*Mean of three replications

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

Plate 13. *In vitro* evaluation against bacterial pathogens of Phalaenopsis and Basket Vanda



A . *Erwinia chrysanthemi* of Phalaenopsis

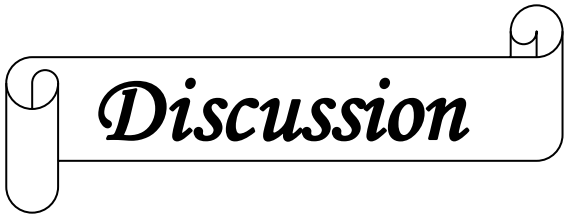


B. *Burkholderia gladioli* of Basket Vanda

cent inhibition respectively. Streptocycline 200 ppm differ significantly from the other treatments and showed least inhibition (30.74 per cent) to the pathogen (Plate 13 A).

4.7.2.2. *Burkholderia gladioli* from Basket Vanda

The efficacy of antibiotic, cowdung and *P. fluorescens* against *B. gladioli* causing wilt of Basket Vanda was given in the Table 24. Among the treatments used against bacteria, fresh cowdung extract 2% showed maximum inhibition of 57.41 per cent and was on par with *P.fluorescens* (0.5%) and fresh cowdung extract 2%+ *P. fluorescens* 0.5% (Liquid) which showed an inhibition of 48.89 per cent and 42.59 per cent respectively. Streptocycline showed least inhibition of 28.14 per cent and it differed significantly from other treatments (Plate 13 B).


Discussion

5. DISCUSSION

Orchids represent the most highly evolved family among monocotyledons and comprise a unique group of plants, exhibiting an incredible range of diversity in size, shape and colour of their flowers. In cut flower trade they occupy top position among all the flowering plants. Orchids reported to comprise over 800 genera, 25,000 species and a lakh man-made hybrids. They have varying habitats but epiphytic orchids dominate the trade. They are classified as monopodials and sympodials (Rajeevan, 2011).

Monopodials have a main stem which continue to grow year after year. Their stem have a vertical growth with aerial roots whereas sympodials terminate the growth at end of each season and the stem have a horizontal growth, producing pseudobulbs in clusters and aerial roots are absent. *Phalaenopsis, Renanthera, Arachnis, Ascocentrum, Rhyncostylis, Vanda* etc are the important monopodial orchids grown in Kerala.

The crop is affected by various diseases affecting its production potential and market value. Black rot (*Phytophthora* sp.), anthracnose (*Colletotrichum* sp.), orchid wilt (*Sclerotium rolfsii*), soft rot (*Erwinia* sp.) and leaf spot (*Alternaria* sp., *Fusarium* sp.) were the important orchid diseases reported by earlier workers (Wey, 1988; Duff, 1997; Bag, 2011; Pant *et al*., 2011). These diseases were reported from various parts of India and abroad. But literature on diseases of orchids is very meager from Kerala and that of monopodial orchids is practically nil. In view of the above facts, an investigation was carried out to study about various diseases of monopodial orchids prevalent in Kerala, its symptomatology, pathogen characterization, seasonal influence on occurrence and management of diseases.

At first, a survey was conducted in various locations of Thrissur district to understand the occurrence of various diseases and collected diseased samples.

Different types of diseases were noticed and isolation of pathogens yielded 11 fungi and two bacteria. Different fungal pathogens isolated were *Fusarium* sp. (2 isolates), *Sclerotium* sp. (2 isolates), *Phoma* sp., *Colletotrichum* sp. (3 isolates), *Phytophthora* sp., and *Alternaria* sp. The bacterial pathogens isolated were *Erwinia* sp. and *Burkholderia* sp.

The pathogenicity of these organisms was proved by artificial inoculation on whole plant or on the detached plant part. Symptomatology was studied under natural and artificial conditions. The symptoms produced under artificial conditions were almost similar to those observed under natural conditions.

In Phalaenopsis, symptomatology of fusarium wilt, collar rot, flower spot, anthracnose and soft rot were studied. The symptoms produced by *Fusarium* sp causing fusarium wilt of Phalaenopsis comprised of yellowing, detachment of leaf, discolouration of leaf base and wilting of the whole plant. According to Hsieh (2005) Phalaenopsis *F. solani* and *F. proliferatum* infection of Phalaenopsis resulted in wilting, yellow leaves and root rot. Kim and Chun (2007) also reported *F. oxysporum* causing similar symptoms of root rot and basal rot symptoms in Phalaenopsis. Wedge and Elmer (2008) studied Fusarium wilt of Phalaenopsis and symptoms reported were chlorosis, wilting and stunting of plant and shriveling and water soaking of leaves. Water soaking of leaves was not observed in the present study whereas the other symptoms observed were similar to the earlier reports.

In the case of collar rot of Phalaenopsis caused by *Sclerotium* sp., yellowing of leaves was observed initially, later mycelial growth, rotting of plant and sclerotial formation were observed. Bag (2003) also explained the symptoms of Dendrobium wilt caused by *Sclerotium rolfsii* in Dendrobium. The symptoms included basal rot of pseudobulb, yellowing and detachment of leaves, development of mycelial growth and formation of numerous small brown coloured sclerotia. In the present study, the symptoms observed were same as reported in the case of Dendrobium.

Flower spot of Phalaenopsis was a fungal disease caused by *Phoma* sp. and it appeared as minute brown coloured sunken spot on flower petals and later these spots coalesced to form brown lesion. Even though *Phoma* sp. was reported as a pathogen of orchids (Uchida, 1994), the details on the symptomatology and host range are not available. *Botrytis cinerea* is the common pathogen reported to cause flower spot in orchids (Jones, 2003).

Anthracnose caused by *Colletotrichum* sp. was observed in Phalaenopsis, Mokara and Arachnis. In Phalaenopsis, brown to black irregular sunken spot with brown margin and yellow halo was observed. In Mokara, the symptom started from tip of leaf as brown lesion which later spread to form large necrotic area. In Arachnis, large brown sunken leaf spot with yellow halo was the symptom. The symptoms produced by the pathogen were of different types in different monopodial orchids. Uchida (1994) explained the symptoms of *C. gloeosporioides* infection in Dendrobium as small restricted, black, sunken lesion on leaf base. Suk Young *et al.* (1996) reported the symptoms of *C. gloeosporioides* infection in Cymbidium as dark brown to black spots on the leaves, leaf blight and plant death. Even though sunken spots and blighting symptoms described in the above reports were similar to the symptoms of anthracnose in the present study, yellow halo of spots observed in Phalaenopsis and Arachnis and yellowing of leaves of Mokara were not reported by the earlier workers.

A bacterial disease commonly found in Phalaenopsis was soft rot caused by *Erwinia* sp. and it initiated as greenish water soaked spots on leaves and finally resulted in rotting of entire leaf. According to Abdullah and Kadzimin (1993) initial symptom of soft rot in Phalaenopsis and Dendrobium was water soaked rot which enlarged rapidly without yellow margin. Geow Ching and Wen Huei (1998) also explained the symptoms of bacterial soft rot of Phalaenopsis. According to them, the symptoms include water soaking, liquefaction of leaf tissue and rotting of leaf. In the

present study also, similar symptoms were observed along with a characteristic foul smell.

In Basket Vanda, the diseases *viz*., heart rot, sclerotium wilt, leaf spot and bacterial wilt were studied. Heart rot incited by *Phytophthora* sp. was observed during survey in certain localities. Water soaking of leaf base, development of black necrotic lesions and rotting of entire leaf were the symptoms of the disease. According to Uchida (1994), *Phytophthora* infection of Vanda caused water soaked lesions later changed to brown or brownish black, finally leaves become yellow as on age. Bamba and Wall (2007) reported *Phytophthora* infection of Vanda. The symptoms reported were necrotic areas most often radiating from the base of the leaf with distinct margins and white fungal growth on the necrotic areas. In the present study also symptoms were similar to that reported earlier.

Symptom of sclerotium wilt of Basket Vanda was initiated as yellowing of leaves. Later dry rotting spread from pseudostem to leaf base and to entire leaf. Orchid wilt of Vanda incited by *Sclerotium rolfsii* was reported by Bag (2006). The disease caused basal rot of pseudostem at the initial stage. The disease gradually spread upward and the leaves turned yellow and got detached from the stem and ultimately caused the mortality of infected plants. In the present study, *S.rolfsii* caused dry rot in Basket Vanda, and wet rot in Phalaenopsis. All other symptoms observed here were similar to that of earlier reports.

Botryodiplodia leaf spot of Basket Vanda was characterised by greyish white coloured spot with black margin which later led to blighting of leaves. Varma and Bilgrami (1977) reported the pathogen from *Vanda roxberghi*, a medicinal orchid. *Lasiodiplodia* sp. had been reported as orchid pathogen by Uchida (1994). But the details on host plant and symptomatology are not available. **This is the first record of Basket Vanda as the host of pathogen, *Botryodiplodia* sp.**

Bacterial wilt of Basket Vanda caused by *Burkholderia* sp. was a minor disease which was observed in one of the places of the survey. It was characterized by brown discolouration at the leaf base, yellowing, wilting of plant and drying of roots. The bacterium *Burkholderia gladioli* was reported earlier by Keith *et al*. (2005) as a leaf spot pathogen of orchid from Hawaii. But in the present study, pathogen caused yellowing and wilting. Hence it is the first report of the wilt symptom caused by the pathogen.

Alternaria leaf spot of Mokara, an important leaf spot disease was characterized by round to spindle shaped spot with brown margin and off-white centre and with a vertical splitting along the spot. According to Yadav *et al*. (2010), symptom of leaf spot caused by *Alternaria alternata* in *Cymbidium* sp. started from the tip or margin of the leaf and progressed up to the basal part resulted in leaf blighting. But in the present study, the symptoms were not started from tip or margin but were appeared throughout the leaf. Vertical splitting of the spot noticed in the present study was not reported earlier. Even though size of leaf spot was increased, they did not coalesce and not resulted in leaf blighting. All these shows that, even though the pathogen is same, symptoms were different in Cymbidium and Mokara**. It is the first report of Mokara (a trigeneric hybrid of Arachnis x Ascocentrum x Vanda) as a host of *Alternaraia alternata*.**

Leaf spot of Arachnis by *Fusarium* sp. was a major disease of the crop observed in the study. Oval to spindle shaped grey coloured spot with black margin was the characteristic symptom. Tissue shredding symptom of leaf was also observed in the infected area. Ichikawa and Aoki (2000) explained the symptoms of leaf spot caused by *Fusarium* sp. in Cymbidium orchid. They observed two types of spots *viz*., yellow spot and black spot. Yellow spot was characterized by small water-soaked patches appeared on the leaves which subsequently enlarged, center became sunken, turned reddish brown and surrounded by yellowish swellings without

definite borders. In the black-spot type of the disease, minute black speckles were appeared on the leaves at an early stage and then enlarged as angular to irregular black spots. Pant *et al.* (2011) reported leaf spot of orchid caused by *Fusarium* sp. But this is the first report of Arachnis as a host of leaf spot disease caused by *F.oxysporum*. The symptoms observed in the Cymbidium were different from that observed in the present study. The same pathogen also caused wilt disease of Phalaenopsis. That might be due to strain variation of the pathogen.

The cultural and morphological characters of pathogens were studied for proper identification of the organisms. *Fusarium oxysporum* of Phalaenopsis attained nine cm diameter growth in six days and the colony was compact and the reverse side of the dish was slightly pink coloured whereas *F. oxysporum* of Arachnis was slow growing and completed nine cm diameter growth in nine days and the reverse side of the dish was dark purple in colour. The difference in cultural characters of *F. oxysporum* affecting Phalaenopsis and Arachnis shows that they may be of different strains of the pathogen. The morphological characters observed in the study were comparable to that of *F. oxysporum* explained by Booth (1970). The identity of the pathogen was further confirmed by National Centre of Fungal Taxonomy, New Delhi and both the pathogens were identified as *F. oxysporum* (NCFT, No. No.4590.11 and No. 4595.11)

The pathogen *Sclerotium rolfsii* causing collar rot of Phalaenopsis and wilt of Basket Vanda was a fast growing fungus. It produced white colony with an even sheet of aerial mycelium and tuft of longer hyphae. Fungus produced numerous sclerotia at 15 DAI as surviving structures in the culture. Hyphae hyaline, 3.26 - 4.32 µm width and septate at an interval of 74.97 - 92.56µm. Kuekulvong (2008) explained the cultural and morphological characters of *S. rolfsii* affecting Dendrobium which was similar to that observed in the present study. He explained

the size of hyphae as 2 - 9 µm x 150 - 250 µm with two clamp connections at each septation.

The colony of *Phoma exigua* of Phalaenopsis appeared as greenish black to greyish black colour and attained full growth in eight days at room temperature. The fungus formed fruiting body pycnidia in the culture. Conidia hyaline, aseptate, 4.27-5.80 x 1.81-3.09 µm in size and were cylindrical with one end round, other end tapering. Selvanathan *et al*. (2011) explained the conidial characters of *Phoma exigua* affecting *Calotropis gigantea* as 5.5 - 10 X 2.5 - 3.5 µm size and straight, ellipsoid, often biguttulate and septate. The size of conidia of *P.exigua* isolated from orchid is almost similar to the previous report, but was without septa. The identity of the pathogen was confirmed by National Centre of Fungal Taxonomy, New Delhi (NCFT, No. 4591.11).

The colony of *Colletotrichum gloeosporioides* of Phalaenopsis was greyish black in colour with pink sporulation. The same pathogen infecting Mokara showed a greyish white coloured mycelium with pink spore mass. In Arachnis, the same pathogen produced cotton wool like greyish white mycelium with pink sporulation. This showed that cultural variation exists in *C. gloeosporioides* affecting different orchids. Setae were absent in the acervulus of *C. gloeosporioides* of Mokara but were present in that of Phalaenopsis and Arachnis. According to Uchida (1994), conidial size of *C. gloeosporioides* affecting Dendrobium was 15.9 µm x 5.4µm. Wijesekara and Agarwal (2006) studied taxonomic characters of *C. gloeosporioides* of various vegetable crops. According to them, colony showed sparse to wooly aerial mycelium and was greyish black in colour. Akrapikulchart (2009), explained that conidia of *C. gloeosporioides* affecting Dendrobium is straight cylindrical, apex obtuse with 12 - 17 x 3.5 – 6 µm size. The size of conidia observed in the current study was within the range as that reported earlier.

Soft rot pathogen, *Erwinia chrysanthemi* of Phalaenopsis formed small greyish white to creamy white smooth round colonies in nutrient agar medium. The characters of *Erwinia* sp. explained by Abdullah and Kadzimin (1993) in Phalaenopsis and Dendrobium were similar to that observed in the present study. Apart from cultural studies, the pathogen was identified by molecular methods to confirm the identity from SciGenom, Kakanad, Ernakulam.

The pathogens *viz., Phytophthora cactorum, Botryodiplodia theobromae* and *Burkholderia gladioli* infecting Basket Vanda were characterised and identified by studying cultural and morphological characters. Growth of *Phytophthora cactorum* of Basket Vanda was cotton wool like with white coloured mycelium and petalloid appearance due to radiating growth. The hyphal and sporangial characters were similar to that of *P. cactorum* described by Waterhouse and Waterson (1966) .The identity of the pathogen was confirmed by National Centre of Fungal Taxonomy, New Delhi (NCFT, No.4592.11).

The colony of *Botryodiplodia theobromae* of Basket Vanda was very fast growing compared to other pathogens and was deep black in colour. Pycnidia development started at eight DAI in the culture and matured conidia were two celled and brown whereas immature conidia were hyaline and single celled. Similar cultural and morphological characters were described by Punithalingam (1976) for *B. theobromae* which confirmed its identity. The fungus was also got identified from National Centre for Fungal Taxonomy, New Delhi (NCFT, No.4593.11).

Burkholderia gladioli of Basket Vanda produced pale yellow, opaque, round colonies with entire margin. Bacterium was gram negative and rod shaped. Kieth *et al*. (2005) explained the similar characters of *Burkholderia gladioli* from Dendrobium, Oncidium and Miltonia causing black spot in these crops. The bacterium was identified by molecular methods by Vision Scientific, Angamaly, Ernakulam.

The pathogens *viz*., *Alternaraia alternata* and *Colletotrichum gloeosporioides* of Mokara were characterised and identified by studying cultural and morphological characters. *Alternaria alternata*, a leaf spot pathogen of Mokara produced a greenish grey velvety colony with distinct zonations. Conidia were obclavate, dark brown coloured with both longitudinal and transverse septa. Resmi (2005) reported *Alternaria alternata* of cucurbits with similar cultural and morphological characters. The identity of the pathogen was confirmed from National Centre for Fungal Taxonomy, New Delhi (NCFT No.4594.11).

Seasonal influence on the occurrence of important diseases was studied for one year by observing the plants maintained in orchidarium of AICRP on Floriculture Improvement in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara. Important diseases observed were Fusarium wilt, collar rot and soft rot of Phalaenopsis, Botryodiplodia leaf spot of Basket Vanda, Alternaria leaf spot of Mokara and Fusarium leaf spot of Arachnis. Per cent disease incidence of the above mentioned diseases were recorded whereas per cent disease severity was recorded for leaf spot diseases such as Botryodiplodia leaf spot, Alternaria leaf spot and Fusarium leaf spot. Phalaenopsis were maintained in the rain shelter, Basket Vanda and Mokara were in poly house and Arachnis plants were in open condition.

From the data, it was observed that Fusarium wilt of Phalaenopsis was more during the months of November and June (Fig. 3). It may be due to the influence of temperature prevailed during that period. Mean temperature of these months varied between 29.59 - 30.04ºC which was optimum for the growth and survival of *Fusarium* sp. But during the months with high mean temperature of 31.68 - 33.74ºC, the wilt incidence was zero. Kim *et al*. (2002) reported that root rot of moth orchid caused by *F. solani, F. oxysporum* and *F. proliferatum* resulted in 30 per cent of disease incidence in some greenhouses. Wedge and Elmer (2008) has reported that

temperature stress and high relative humidity (RH) favours the incidence of Fusarium wilt in Phalaenopsis, Cattleyas and Oncidium. But in the present study, RH in the month of November was 58.36 per cent where as that of June was 78.73 per cent. Months with zero disease incidence recorded RH in the range of 54.77- 65.96 per cent. This indicated that, even though RH was optimum for the growth of pathogen, disease may not occur if temperature is above the optimum range. Hence temperature had a major role in the occurrence of fusarium wilt than RH.

Collar rot was more prevalent during December with an incidence of 6.52 per cent (Fig. 4). During that month, the mean temperature was 29.66ºC and RH was 54.53 per cent. Disease was also occurred during July- August when temperature was in the range of 28.93 - 29.11ºC and RH was 78.7 - 79.42 per cent. Incidence of the disease was also noticed when temperature was 32.38ºC and RH was 64.79 per cent (October) and when temperature was 31.52ºC and RH was 46.85 per cent (February). Singh *et al*. (2007) reported that high incidence of collar rot of lentil caused by *Sclerotium rolfsii* was recorded in the month of November, when the temperature ranged from 20 to 28ºC and the RH ranged from 65 to 77 per cent and the lowest mortality was recorded during February when the atmospheric temperature ranged from 9 to 23ºC and the average RH varied from 68 to 78 per cent. In the present study incidence was noticed at a temperature of 29 - 32ºC and RH of 47 - 79 per cent. As in the case of Fusarium wilt, temperature played a major role than RH in the occurrence of Collar rot.

Soft rot of Phalaenopsis was more during rainy months (June - August) and during November (Fig. 5). In June - August, temperature ranged from 28.93 - 29.59ºC, relative humidity was nearly 80 per cent and rainfall was in the range of 551.50 - 731.80 mm. In summer months of February - April, the temperature was high and relative humidity was low and therefore no incidence of disease. Geowching and WenHuei (1998) reported that higher incidence of soft rot of

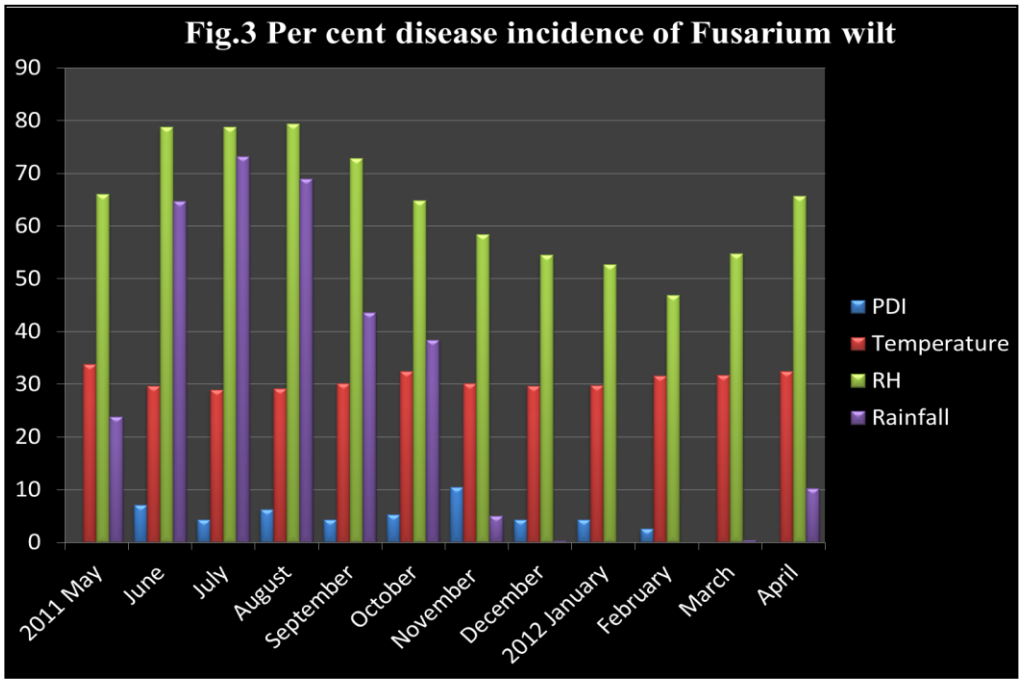


Fig.3 Per cent disease incidence of Fusarium wilt
90
80
70
60
50
40
30
20
10
0
2011 May
June
July
August
September
October
November
December
2012 January
February
March
April
PDI
Temperature
RH
Rainfall

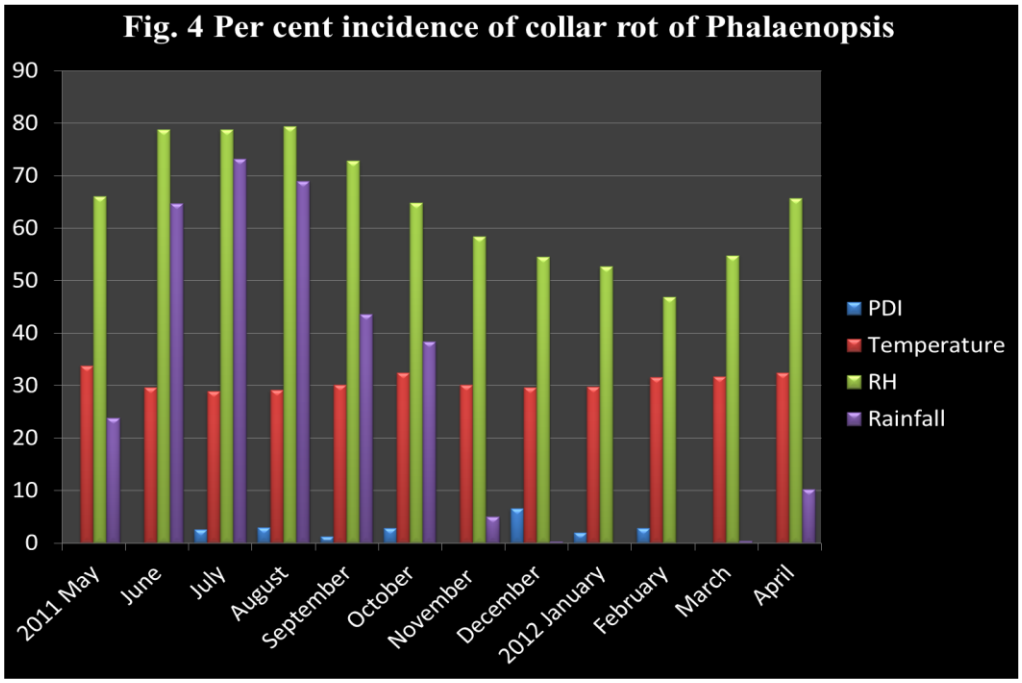


Fig. 4 Per cent incidence of collar rot of Phalaenopsis
90
80
70
60
50
40
30
20
10
0
2011 May
June
July
August
September
October
November
December
2012 January
February
March
April
PDI
Temperature
RH
Rainfall

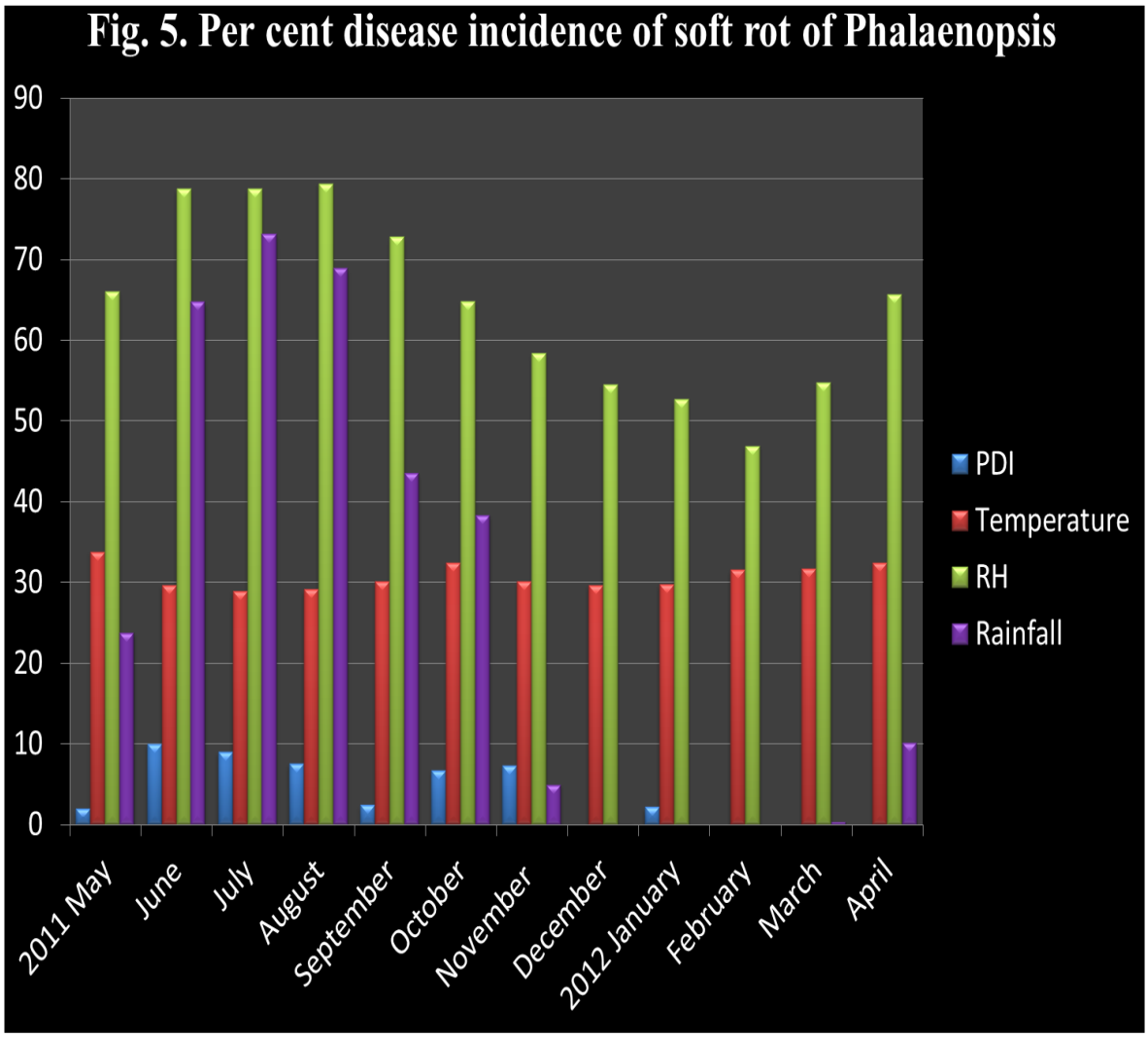


Fig. 5. Per cent disease incidence of soft rot of Phalaenopsis
90
80
70
60
50
40
30
20
10
0
2011 May
June
July
August
September
October
November
December
2012 January
February
March
April
PDI
Temperature
RH
Rainfall

Phalaenopsis was observed when the temperature was about 26ºC and a relative humidity greater than 80 per cent. Huang and Tze chung (2008) also reported that the spread of soft rot bacteria of Phalaenopsis is favoured by a temperature of 25 - 30ºC and high humidity. In the present study also, the influence of temperature, relative humidity and rainfall played a major role in the occurrence of this disease.

Per cent disease incidence of Botryodiplodia leaf spot was maximum (84%) during the months of May and June whereas maximum disease severity (28%) was occurred in June (Fig.6). A gradual increase in disease incidence and severity was observed from first month of observation to the last month of observation even under varied temperature and relative humidity. The secondary spread of the pathogen may be the reason for higher disease severity and incidence in the last months of observation.

In the case of Alternaria leaf spot of Mokara, the per cent disease incidence and severity was maximum (92.3% and 21.6% respectively) during the month of June (Fig. 7). A progressive increase was noticed in the incidence and the severity of the disease throughout the period of observation. According to Davis (2003), *Alternaria alternata* infection in Ivy gourd was severe during summer months because of the presence of comparatively high temperature, moderate relative humidity and favourable rainfall. In the present study even though incidence and severity were higher in summer months (87.17% and 21.2% respectively) maximum incidence and severity were noticed in the month of June which was the last month of observation. Hence, as in the case of Botryodiplodia leaf spot of Basket Vanda, the secondary spread of the pathogen might be the reason for the higher incidence and severity of the disease in the last month of observation.

In Arachnis, cent per cent disease incidence was recorded throughout the period of observation (Fig. 8). This may be due to the presence of leaf spot in any of the leaf, as the plant is tall growing type and has many leaves. The severity showed

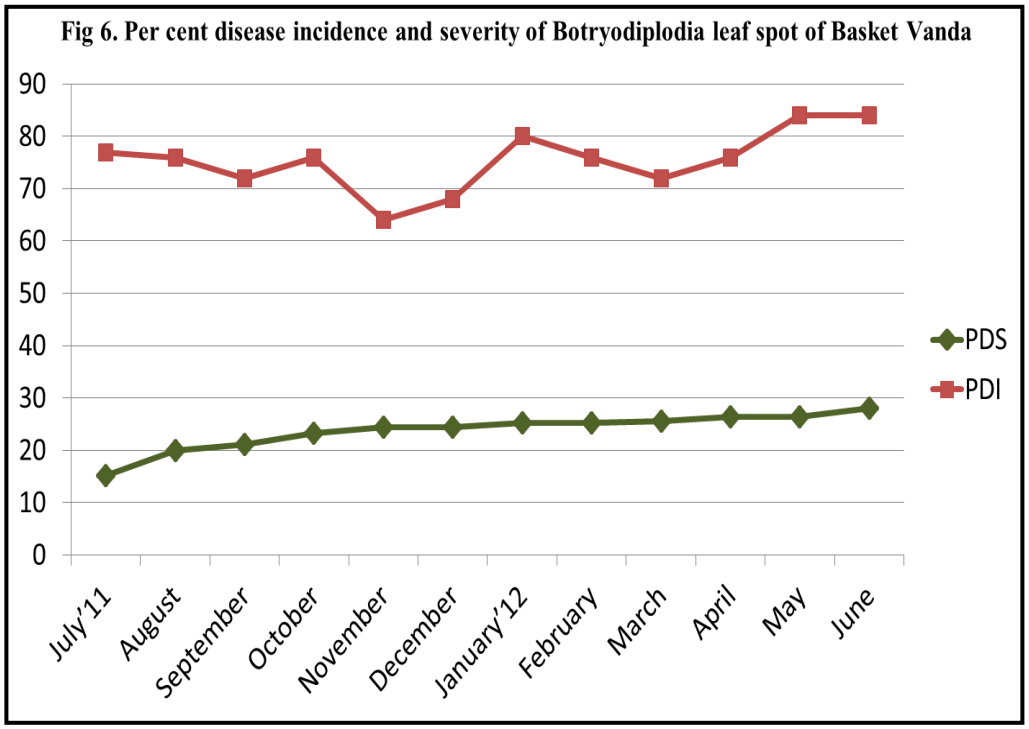


Fig 6. Per cent disease incidence and severity of Botryodiplodia leaf spot of Basket Vanda
90
80
70
60
50
40
30
20
10
0
July'11
August
September
October
November
December
January'12
February
March
April
May
June
PDS
PDI

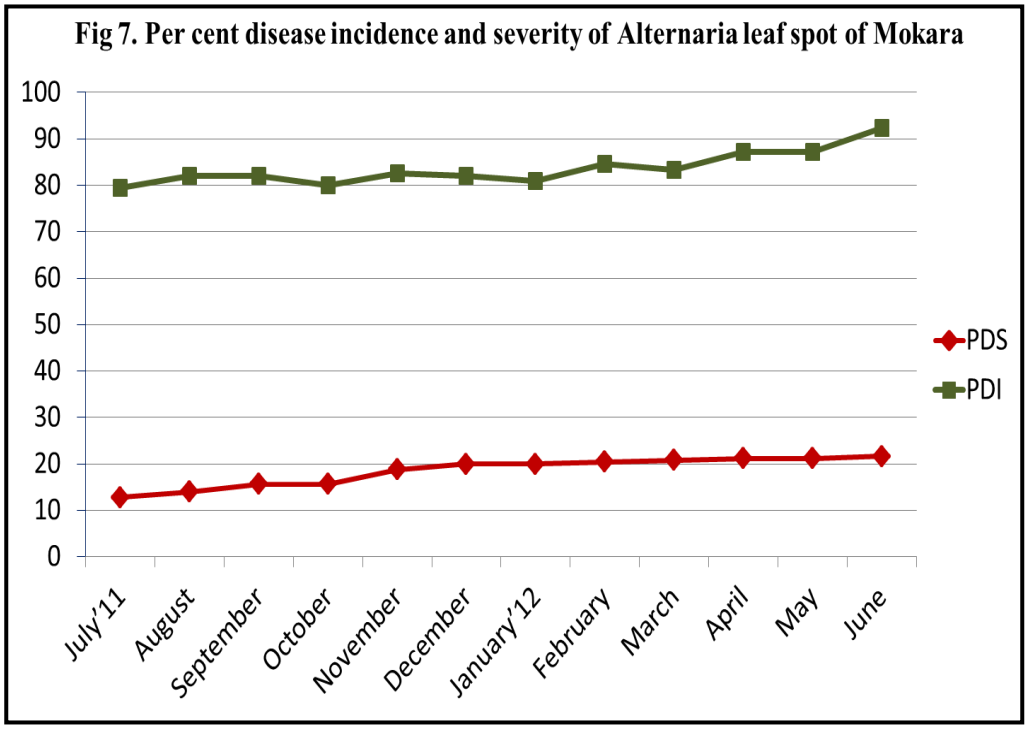
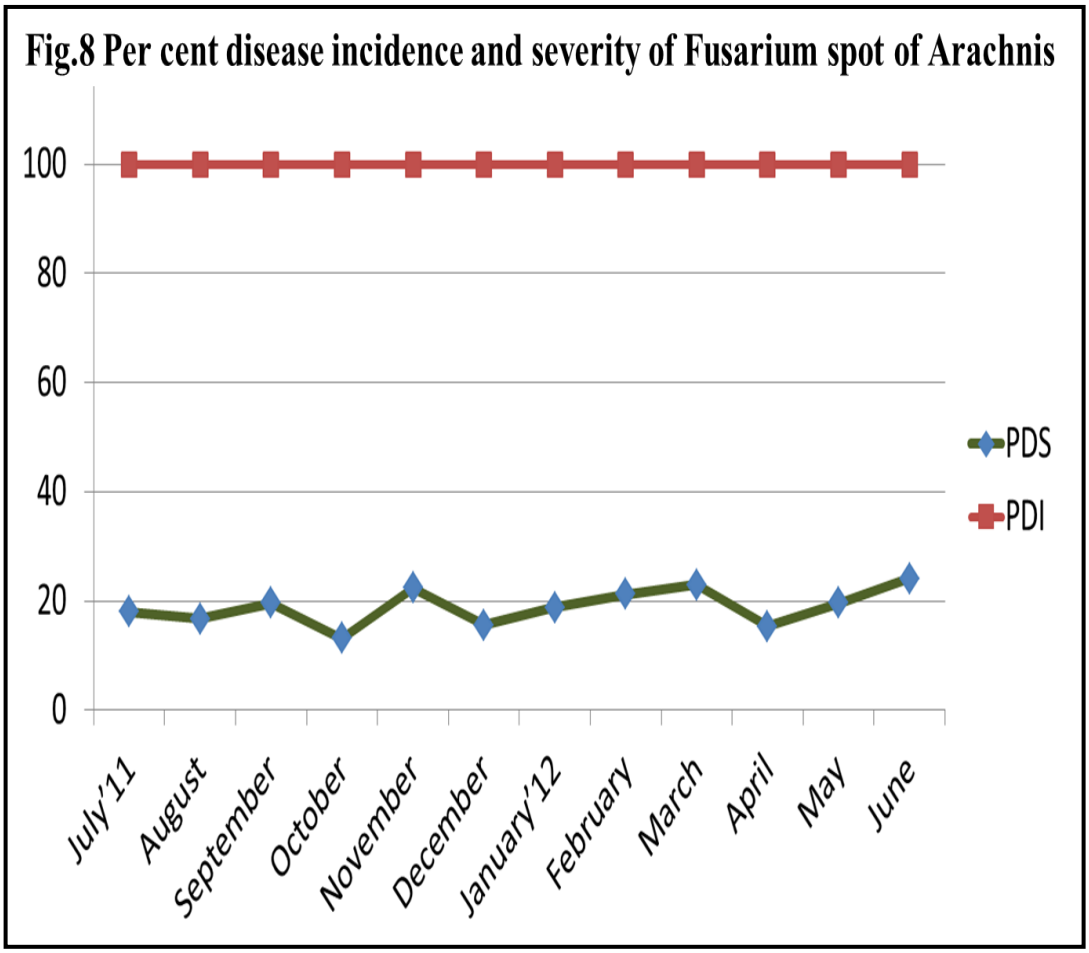


Fig 7. Per cent disease incidence and severity of Alternaria leaf spot of Mokara
100
90
80
70
60
50
40
30
20
10
0
July'11
August
September
October
November
December
January'12
February
March
April
May
June
PDS
PDI

Fig.8 Per cent disease incidence and severity of Fusarium spot of Arachnis



varied trend and reached maximum in June. Since the leaves of Arachnis became senile faster than that of Basket Vanda and Mokara, new leaves were included in the observed set of leaves and caused fluctuations in per cent disease severity during the observed period. Since the plants were grown in open condition, rainfall may also be contributed to high disease severity during June.

In vitro evaluation was carried out to select the best treatment for the control of all isolated pathogens of orchid. Fungicides *viz*., potassium phosphonate 40% + mancozeb 75% (0.2% + 0.15%), cymoxanil 8% + mancozeb 64% (0.2%), carbendazim 12% + mancozeb 63% (0.15%), carbendazim 25% + ipridione 25% (0.2%), fenamidone 10% + mancozeb 50% (0.1%) and biocontrol agent *Pseudomonas fluorescens* (liquid 0.5%) were evaluated against fungal pathogens. For the management of bacteria, treatments were Streptocycline 200ppm, fresh cowdung extract 2%, *P. fluorescens* 0.5% liquid and fresh cowdung extract 2% + *P. fluorescens* 0.5% liquid. All the fungicides used were effective in controlling the growth of fungal pathogens in general and varied with individual pathogen.

The *in vitro* evaluation of fungicides and *P. fluorescens* liquid against *Fusarium oxysporum* of Phalaenopsis revealed that all the fungicides *viz*., potassium phosphonate 40% + mancozeb 75%, cymoxanil 8% + mancozeb 64%, carbendazim 12% + mancozeb 63%, carbendazim 25% + ipridione 25%, fenamidone 10% + mancozeb 50% showed cent per cent inhibition of the fungus while *P. fluorescens* 0.5% showed 61.85% inhibition. Reports are not available on the evaluation of mixed fungicide formulation against *F. oxysporum* of Phalaenopsis. Kumar and Dubey (2001) observed that *F. oxysporum* infecting pea was completely inhibited by carbendazim at 0.025 and 0.05 per cent concentrations. Palmer (2011) recommended the fungicides like Chlorothalonil and Azoxystrobin to suppress *F. oxysporum* of orchid. In the present study, complete inhibition of the fungus was observed by the

use of mixed fungicide formulation. *P. fluorescens* also showed inhibitory action against the pathogen.

In the case of *Sclerotium rolfsii* causing collar rot of Phalaenopsis and wilt of Basket Vanda, all the combination fungicides *viz.*, potassium phosphonate 40% + mancozeb 75%, cymoxanil 8% + mancozeb 64%, carbendazim 12% + mancozeb 63%, carbendazim 25% + ipridione 25%, fenamidone 10% + mancozeb 50% inhibited the fungus completely and the *P.fluorescens* inhibited the fungus by 64.80 per cent and 68.89 per cent respectively. Laha *et al.* (1996) studied the effectiveness of *P. fluorescens* against *S. rolfsii* of cotton and found that the inhibition zone ranged from 3mm to 14 mm. Rangeshwaran and Prasad (2000) reported that sclerotium rot of sunflower caused by *S. rolfsii* was suppressed by *P.fluorescens* by 60 - 63 per cent in plants. Kolte and Raut (2007) studied the efficacy of fungicides for the management of *S. rolfsii* of orchids and found that mancozeb, difenoconazole and hexaconazole resulted in the total inhibition of the radial growth of the pathogen. In the present study also mancozeb combinations showed complete inhibition of radial growth of *S. rolfsii.*

In vitro evaluation of fungicides and *P.fluorescens* against *Phoma exigua*, the causal agent of flower spot of Phalaenopsis showed that mixed fungicides *viz.*, potassium phosphonate 40% + mancozeb 75%, carbendazim 25% + ipridione 25%, fenamidone 10% + mancozeb 50% recorded complete inhibition of pathogen cymoxanil 8% + mancozeb 64% and carbendazim 12% + mancozeb 63% inhibited the growth of pathogen by 79.63 per cent and 63 per cent respectively. Bio control agent *P.fluorescens* inhibited the fungus by 52.97 per cent. Even though *Phoma* sp. was recorded as an orchid pathogen, literature was not available on its management.

In vitro evaluation of *Colletotrichum gloeosporioides* causing anthracnose in Phalaenopsis, Mokara and Arachnis was carried out using different fungicides and *P.fluorescens*. All fungicides evaluated against *C. gloeosporioides* of Phalaenopsis,

Mokara and Arachnis gave cent per cent inhibition of fungus, while *P. fluorescens* inhibited the fungus by 86.30 per cent, 75.92 per cent and 73.33 per cent respectively. Uchida (1994) proved that *C. gloeosporioides* of orchid is highly sensitive to benomyl. SukYoung *et al*. (1996) also found that the pathogen is sensitive to benomyl and bitertanol. In the present study also, benzimidazole fungicide carbendazim combinations showed cent per cent inhibition of the pathogen.

In the case of *Phytophthora cactorum*, the causal organism of heart rot of Basket Vanda, it was found that fungicides *viz*., potassium phosphonate 40%+ mancozeb 75%, cymoxanil 8% + mancozeb 64%, carbendazim 12% + mancozeb 63%, fenamidone 10% + mancozeb 50% fungicides inhibited the fungus completely whereas carbendazim 25% + ipridione 25% inhibited the fungus by 64.44 per cent. Bio control agent *P. fluorescens* inhibited the fungal growth by 83.3 per cent. Cating *et al*. (2009b) recommended the fungicides *viz*., Alliete, potassium phosphonate, dimethomorph and etridiazole for the effective management of *P.palmivora* and *P.cactorum* causing black rot of orchid. In the present study also, potassium phosphonate and mancozeb combination showed cent per cent inhibition of the fungus.

In vitro evaluation of fungicides and *P.fluorescens* against *Botryodiplodia theobromae*, the leaf spot pathogen of Basket Vanda showed that all the fungicides tested recorded complete inhibition of the pathogen and *P.fluorescens* gave 77.03 per cent inhibition. According to Sangeetha (2009), carbendazim (0.2%) showed complete inhibition of *B.theobromae* in mango whereas mancozeb (0.3%) showed 48.6 per cent inhibition. In the present study, mixed formulation of carbendazim and mancozeb completely inhibited the pathogen.

In the case of leaf spot pathogen *Alternaria alternata* of Mokara, fungicidal combinations *viz*., potassium phosphonate 40% + mancozeb 75%, cymoxanil 8% +

mancozeb 64%, carbendazim 25% + ipridione 25% showed cent per cent inhibition whereas fungicides carbendazim 12% + mancozeb 63% and fenamidone 10% + mancozeb 50% inhibited the fungus by 87.03 per cent while all other fungicides gave cent per cent inhibition of the fungus. A study conducted by Davis (2003) showed that least inhibition of 52.66 per cent was recorded by mancozeb 0.3% against *A.alternata*, a leaf spot pathogen of Ivy gourd. But in the present study, mancozeb combinations *viz*., potassium phosphonate 40% + mancozeb 75% and cymoxanil 8% + mancozeb 64% showed cent per cent inhibition of the fungus and other mancozeb combinations *viz*., carbendazim 12% + mancozeb 63% and fenamidone 10% + mancozeb 50% showed more than 85 per cent inhibition.

For *Fusarium oxysporum*, the leaf spot pathogen of Arachnis, the fungicidal combinations *viz*., potassium phosphonate 40% + mancozeb 75%, cymoxanil 8% + mancozeb 64% , carbendazim 12% + mancozeb 63%, carbendazim 25% + ipridione 25% , gave cent per cent inhibition whereas fenamidone 10% + mancozeb 50% gave 70 per cent inhibition of pathogen. *P.fluorescens* inhibited the fungus by 68.52 per cent. All fungicide treatments including fenamidone 10% + mancozeb 50% showed complete inhibition of *F.oxysporum* of Phalaenopsis.

In vitro evaluation was done for the management of soft rot bacteria *Erwinia chrysanthemi* and wilt pathogen *Burkholderia gladioli* using streptocycline 200 ppm, fresh cowdung extract 2%, *P.fluoresecens* 0.5%, fresh cowdung extract 2% + *P.fluoresecens* 0.5%. For *E. chrysanthemi, P.fluorescens* showed maximum inhibition of 65.56 per cent followed by fresh cowdung extract 2% + *P.fluorescens* 0.5% and fresh cowdung extract 2% showing 57.77 per cent and 55.92 per cent respectively. Streptocycline 200 ppm showed the least inhibition of 30.74 per cent. This showed the superiority of *P. fluorescens* (0.5%) over the antibiotic streptocycline (200 ppm) in controlling *E.chrysanthemi*. Streptocycline caused lysis of the bacteria and it was indicated by the formation of clear zone around the well. In

all other treatments, inhibition of the pathogenic bacteria was by the growth of antagonistic bacteria.

For, *Burkholderia gladioli* of Basket Vanda, fresh cowdung extract 2% showed maximum inhibition of 57.41 per cent followed by *P.fluorescens* 0.5% and fresh cowdung extract 2% + *P.fluorescens* 0.5% with 57.41 and 48.89 per cent inhibition respectively. Streptocycline showed least inhibition of 28.14 per cent. Keith *et al.* (2005) reported that sensitive strains of *B.gladioli* were completely inhibited by copper fungicides and streptomycin. The strain of bacteria used in the study was not very sensitive to streptocycline 200 ppm and the bacterium was inhibited more effectively by other treatments.

Combined application of *P.fluorescens* and fresh cowdung extract is a recommended practice against bacterial diseases to get synergestic effect. But in the present study, combined application of fresh cowdung extract 2% and *P.fluorescens* 0.5% recorded slight reduction in antibacterial effect against *E.chrysanthemi* and *B.gladioli* even though statistically on par.

Summing up the results discussed so far, it was concluded that eleven fungal and two bacterial diseases of different monopodial orchids were observed and the pathogens were identified based on cultural, morphological/ molecular characters. Viral diseases were not observed during the survey. The symptomatology of all the diseases were studied both under natural and artificial condition. Variations were observed in disease incidence and not in disease severity with respect to seasons. The tested fungicides were very effective for the inhibition of all fungal pathogens and the bio control agent *P.fluorescens* showed 53 - 86 per cent inhibition of fungal pathogens. *P.fluorescens* and cowdung showed inhibition whereas streptocycline showed lysis of bacterial pathogens.


Summary

6. SUMMARY

The present investigation on "Cataloguing and management of major diseases of monopodial orchids" was carried out to study symptomatology, etiology, and management of various diseases of monopodial orchids.

A survey was conducted in different locations *viz*., orchidarium of AICRP on Floriculture improvement in the Department of Pomolgy and Floriculture, Vellanikkara and nurseries at Cheroor, Thrissur, Perinjanam, Madakkathara Kanimangalam and Mannuthy in Thrissur district. Different types of fungal and bacterial diseases were noticed whereas viral diseases were not noticed. Isolation of pathogen yielded eleven fungal pathogens and two bacterial pathogens. The pathogenicity of these organisms was proved by artificial inoculation either into plant or plant part

Symptomatology of various diseases produced by respective pathogens was studied in detail and symptom differed significantly. *Fusarium* sp. caused wilt in Phalaenopsis whereas it caused leaf spot in Arachnis. *Sclerotium* sp. produced collar rot in Phalaenopsis and dry rotting in Basket Vanda. Anthracnose fungi *Colletotrichum* sp. produced different symptoms of anthranose in Phalaenopsis, Mokara and Arachnis. In Phalaenopsis, the symptom was irregular sunken spot, and in Arachnis, round sunken spot whereas in Mokara the symptom was blighting of leaf. Heart rot of Basket Vanda caused by *Phytophthora* sp. resulted in rotting of younger leaves and was a serious disease. Botryodiplodia leaf spot of Basket Vanda was characterised by greyish white coloured spot with black margin and pycnidia at the centre. Alternaria leaf spot of Mokara, a common disease of Mokara was seen as round to oval shaped brown coloured spot with a vertical splitting. The bacterial diseases noted during the survey were soft rot of Phalaenopsis caused by *Erwinia* sp.

and wilt of Basket Vanda caused by *Burkholderia* sp. Water soaking and rotting of leaf were the symptom of soft rot whereas yellowing, wilting and detachment of leaves were the symptoms of wilt disease.

Cultural and morphological characters of fungal pathogens were studied in detail and they were identified. Colony of *Fusarium* sp., a wilt pathogen of Phalaenopsis showed a pink pigmentation whereas the same organism causing leaf spot in Arachnis showed a dark purple pigmentation. Size of macroconidia ranged from 22.65 - 30.06 x 3.32 - 4.41µm and was straight to sickle shaped and for microconidia 5.71 - 9.22 x 2.70 - 3.26 µm size, hyaline and oval shaped. Based on the characters, organism was identified as *Fusarium oxysporum* Schlecht.

Sclerotium sp. infecting Phalaenopsis and Basket Vanda showed a white coloured colony with even sheet of aerial mycelium. Surviving structure, sclerotia formed in the colony were round, smooth, shiny, and mustard shaped and was dark brown in colour. The hyphae measured 3.26 - 4.32 µm width, septate at an interval of 74.97 - 92.56 µm and were hyaline. The organism was identified as *Sclerotium rolfsii* Sacc.

Flower spot pathogen of Phalaenopsis, *Phoma* sp. formed a greyish black coloured colony in the medium. Conidia were small in size and measured 4.27 - 5.80 x 1.81 - 3.09 µm. The organism was identified as *Phoma exigua*.

Colletotrichum sp. isolated from Phalaenopsis, Mokara and Arachnis showed greyish white and greyish black coloured colony with pink sporulation after 12 - 14 days. Among the three, *Colletotrichum* from Mokara have no setae in acervulus whereas other two have setae. Size of conidia ranged 10.75 - 15.31 x 2.5 - 5.66 µm and was cylindrical in shape with both ends round. Based on these characters, the pathogen was identified as *Colletotrichum gloeosporioides* (Penz.) Sacc.

Colony of *Phytophthora* sp. causing heart rot of Basket Vanda appeared like cottony wool, uniformly dense and white in colour. Culture was slightly radiating and petalloid like due to overlapping mycelial growth. Fungus produced ovoid to obpyriform sporangia with papilla and was 36 - 50 x 29.5 - 35 µm in size. Based on these characters, the organism was identified as *Phytophthora cactorum* (Leb. & Cohn) Schroet.

Leaf spot pathogen of Basket Vanda, *Botryodiplodia* sp. formed a deep black coloured colony with dark coloured aggregated pycnidia. Conidia were double celled and brown in colour and with a size of 20.80- 25.83 x 10.42-15.59µm. Based on these characters the organism was identified as *Botryodiplodia theobromae* Pat.

Alternaria sp., a leaf spot pathogen of Mokara formed greyish brown coloured colony with velvety appearance. Conidium was 31.54 - 60.26 x 8.61 - 11.84 µm in size, obclavate, and pale to dark brown in colour and was with 3-7 transverse and 1-3 longitudinal septae. Based on all these characters, the organism was identified as *Alternaria alternata* Fr. Keissler.

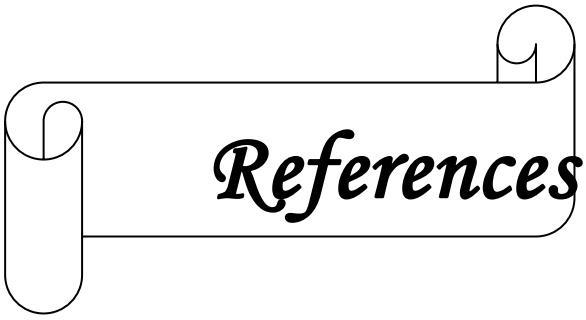
The soft rot bacteria, *Erwinia* sp. from Phalaenopsis, produced small greyish white to creamy white, smooth, round, glistening, slightly raised transluscent colonies. Bacterium was gram-negative, non-spore forming straight rods with round ends. The bacterium was identified as *Erwinia chrysanthemi* based on molecular techniques.

The bacterial wilt pathogen *Burkholderia* sp. produced pale yellow, opaque, round colonies with entire margins. Bacteria were motile, gram negative and rod shaped. Bacterium was identified as *Burkholderia gladioli* based on molecular techniques.

Seasonal influence on the incidence and severity of major diseases were studied for one year in the orchidarium of AICRP in Floriculture improvement in the Department of Pomology and Floriculture. The per cent disease incidence was

recorded for Fusarium wilt, collar rot and soft rot of Phalaenopsis, Botryodiplodia leaf spot of Basket Vanda, Alternaria leaf spot of Mokara and Fusaium leaf spot of Arachnis. The per cent disease severity was calculated for Botryodiplodia leaf spot of Basket Vanda, Alternaria leaf spot of Mokara and Fusaium leaf spot of Arachnis. The maximum incidence of Fusarium wilt was observed during the month of November and collar rot was during the month of December and soft rot during rainy months. Leaf spot diseases were not very much influenced by seasons. Disease severity of leaf spots was in the range of 12 - 28 per cent and it showed gradual increase during period of observation.

For the management of fungal pathogens, an *in vitro* evaluation was conducted on the effectiveness of fungicides and *P. fluorescens* (liquid - 0.5%) and for bacterial pathogens, effectiveness of streptocycline, *pseudomonas* and cowdung slurry. All the fungicides used *viz*., potassium phosphonate 40% + mancozeb 75% (0.2% + 0.15%), cymoxanil 8% + mancozeb 64% (0.2%), carbendazim 12% + mancozeb 63% (0.15%), carbendazim 25% + ipridione 25% (0.2%), fenamidone 10% + mancozeb 50% (0.1%) were effective in controlling fungi and the bio control agent *P.fluorescens* showed an inhibition of 53 - 86 per cent. For the management of bacterial pathogens, *P.fluorescens* (liquid-0.5%), fresh cowdung extract 2%, fresh cowdung extract 2% + *Pseudomonas* 0.5% (liquid) were more effective than 200 ppm streptocycline. Streptocycline caused lysis of pathogenic bacteria whereas *P.fluorescens* and cowdung caused inhibition of its growth.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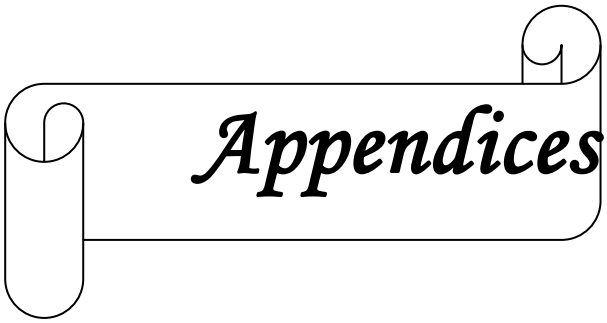
Appendices

APPENDIX I

MEDIA COMPOSITION

1. POTATO DEXTROSE AGAR

Potato	: 200 g
Dextrose	: 20.0 g
Agar	: 20.0 g
Distilled water	: 1000 ml

2. NUTRIENT AGAR

Peptone	: 10.0 g
Beef extract	: 5.0 g
Agar	: 20.0 g
Distilled water	: 1000 ml

APPENDIX II

Nucleotide sequence of bacterial pathogens

1. *Erwinia chryasanthemi*

AGCGGCGGACGGGTGAGTAATGTCTGGGGATCTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATA
CCGCATAACGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCACGCCATCGGATGAACCCAGATGGGATTAGCT
AGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACT
GAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTGATGCAGCCA
TGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGGTAAGGTTAATAACC
TTACCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCA
AGCGTTAATCGGAATGACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTTGGATGTGAAATCCCCGGGCTTA
ACCTGGGAACTGCATTCAAAACTGACAGGCTAGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAA
ATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAA
GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGTGCCCTT
GAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATG
AATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTT
GACATCCAGCGAAGACTGCAGAGATGCGGTCGTGCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCTGTCGTC
AGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTCTGTTGCCAGCACTACGGGTG
GGAACTCAGGGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGA
GTAGGGCTACACACGTGCTACAATGGCGTATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAG
TACGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGC
TACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTCAAA

2. *Burkholderia gladioli*

CCGTCCTCCTTGCGGTTAGACTAGCCACTTCTGGTAAAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAG
ACCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGCACTCGAGTTGCA
GAGTGCAATCCGGACTACGATCGGTTTTCTGGGATTAGCTCCCCCTCGCGGGTTGGCGACCCTCTGTTCCGACC
ATTGTATGACGTGTGAAGCCCTACCCATAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCCGGTTTGT
CACCGGCAGTCTCCCTAGAGTGCTCTTGCGTAGCAACTAAGGACAAGGGTTGCGCTCGTTGCGGGACTTAACCC
AACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTATCGGTTCTCTTTCGAGCACCCTCAGATC
TCTCCAAGGTTCCGACCATGTCAAGGGTAGGTAAGGTTTTTCGCGTTGCATCGAATTAATCCACATCATCCACC
GCTTGTGCGGGTCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGTCAACTTCACGC
GTTAGCTACGTTACTAAGGAAATGAATCCCCAACAACTAGTTGACATCGTTTAGGGCGTGGACTACCAGGGTAT
CTAATCCTGTTTGCTCCCCACGCTTTCGTGCATGAGCGTCAGTATTGGCCCAGGGGGCTGCCTTCGCCATCGGT
ATTCCTCCACATCTCTACGCATTTCACTGCTACACGTGGAAATTCTACCCCCCTCTGCCATACTCTAGCTTGCC
AGTCACCAATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCGGTCTTAACAAACCGCCTGCGCACGCTTTA
CGCCCAGTAATTCCGATTAACGCTCGCACCCTACGTATTACCGCGGCTGCTGCACGTAGTTAGCCGTGCTTATT
CTTCCGGTACCGTCATCCCCG

CATALOGUING AND MANAGEMENT OF MAJOR DISEASES OF MONOPODIAL ORCHIDS

By

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ABSTRACT OF THE THESIS

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COLLEGE OF HORTICULTURE

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ABSTRACT

Disease is one of the major production constraints in orchid cultivation. Hence an investigation has been undertaken to study the symptomatology, etiology and management of various diseases of important monopododial orchids *viz.*, Phalaenopsis, Vanda (Basket Vanda), Mokara and Arachnis.

A survey was conducted in different locations of Thrissur District *viz.*, orchidarium of AICRP on Floriculture Improvement, Department of Pomolgy and Floriculture, COH, Vellanikkara, nurseries at Cheroor, Thrissur, Perinjanam, Madakkathara and Kanimangalam. Different fungal and bacterial diseases were observed and isolation of pathogen yielded eleven fungal pathogens and two bacterial pathogens. The pathogenicity of these organisms was proved by artificial inoculation.

Symptomatology of various diseases was studied in detail under natural and artificial conditions. *Fusarium* sp. caused wilt symptom in Phalaenopsis and caused leaf spot in *Arachnis*. *Sclerotium* sp. caused collar rot in *Phalaenopsis* and dry rotting in Basket Vanda, Anthracnose pathogen, *Colletotrichum* sp. produced different symptoms in Phalaenopsis, Mokara and Arachnis. In Phalaenopsis, the symptom was irregular sunken spot, in Mokara leaf blighting and in Arachnis, round sunken spot. Heart rot was the symptom of *Phytophthora* infection in Basket Vanda. Botryodiplodia leaf spot of Basket Vanda was characterized by greyish white coloured spot with black margin and pycnidia at the centre. Alternaria leaf spot of Mokara, was round to oval shaped with a vertical splitting through the centre of the spot. Soft rot of Phalaenopsis by *Erwinia* sp. and bacterial wilt of Basket Vanda by *Burkholderia* sp. were the bacterial diseases observed during the survey. Water

soaking and rotting of leaves were the symptoms of soft rot while yellowing, wilting and leaf detachment were the symptoms of bacterial wilt.

Cultural characters and morphological characters of fungal pathogens were studied and they were identified at species level. Cultural, morphological and gram reaction of bacterial pathogens were studied and they were identified at species level by molecular techniques.

For the management of fungal pathogens, an *in vitro* evaluation was conducted with fungicides and liquid formulation of *Pseudomonas fluorescens*. Most of the fungicides revealed cent per cent inhibition on the growth where as *P. fluorescens* showed 53 - 86 per cent inhibition. For the management of bacterial pathogens, an *in vitro* evaluation was conducted with cowdung, liquid formulation of *P. fluorescens*, cowdung + *P. fluorescens* and Streptocycline and differences were observed in their inhibitory properties.

Seasonal influence on the incidence of diseases of monopodial orchids was studied for one year. Influence of temperature, humidity and rainfall was prominent in the incidence of Fusarium wilt, collar rot and soft rot of Phalaenopsis whereas not very prominent in the incidence and severity of Botryodiplodia, Alternaria and Fusarium leaf spot diseases of Basket Vanda, Mokara and Arachnis respectively . Incidence of Fusarium wilt was more in the month of November, collar rot in the month of December whereas bacterial soft rot was prominent in rainy months.