STUDIES ON THE MICROBIAL DISEASES OF *DENDROBIUM* HYBRID (ORCHIDS)

THESIS SUBMITTED
UNDER THE FACULTY OF SCIENCE TO THE
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

MICROBIOLOGY

By

B. CHRISTUDHAS WILLIAMS

Department of Biotechnology
Cochin University of Science and Technology
Kochi - 682 022

August 1999

DEPARTMENT OF BIOTECHNOLOGY
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
COCHIN 682 022

Ph: 0484-559267 **Email:** biotech@md3.vsnl.net.in

CERTIFICATE

This is to certify that the work presented in thesis entitled **"STUDIES ON THE MICROBIAL DISEASES OF *DENDROBIUM* HYBRID (ORCHIDS)"** is based on the original work done by **Mr. B. Christudhas Williams** under my guidance and supervision, at the Department of Biotechnology and no part of the work has been included in any other thesis for the award of any degree.

Dr. M. Chandasekaran,
Professor & Head

Cochin-22
9-8-1999.

DECLARATION

I here by declare that the work presented in thesis entitled **"STUDIES ON THE MICROBIAL DISEASES OF *DENDROBIUM* HYBRID (ORCHIDS)"** is based on the original research carried out by me at the Department of Biotechnology, Cochin University of Science and Technology, under the guidance of Dr. M. Chandrasekaran, Professor and Head, Department of Biotechnology, and no part thereof has been presented for the award of any other degree.

B. Christudhas Williams

Cochin-22
9-8-1999

ACKNOWLEDGEMENT

I have great pleasure in expressing my sincere gratitude and obligation to Dr. M. Chandrasekaran, Professor & Head, Department of Biotechnology, Cochin University of Science and Technology, for the excellent guidance, encouragement and help throughout the course of my work.

I take this opportunity to thank Dr. Rosamma Philip, Lecturer, School of Marine sciences for her cooperation as Doctoral Committee member for my PhD Programme. and Dr. G.S. Selvam, Dr. C.S. Paulose and Dr. Padma Nambisan, Faculty members of the Department of Biotechnology for their kind co-operation.

I feel extremely happy to thank, Prof. Dr. N.Unnikrishnan Nair, Pro-Vice Chancellor, CUSAT, Prof. Dr. S. Muraleedharan Nair, Head of the Department, Dr.P.G. Sankaran and Mr Nambodhiri of Dept.of Statistics, and Dr. Jeevananth, Department of Mathematics, Union Christian College, Alwaye for their valuable help in statistical analyses, and Dr.Abi .N. Eldo, and Shri Ehtirajan, Scientist, TCM Ltd, Kalamassery, for all their timely help, encouragement and support.

The support, encouragement and timely help which I received from my lab-mates, Dr.P V.Mohanan, Dr. P V.Suresh, Dr.G. Nagendra Prabhu, Dr, K.S.Shylaraj, Dr.K.Jayachandran, Shri A.Sabu, Shri.S.Rajeevkumar, Mrs Keerthi, Shri,M.K.Sureshkumar, and Dr.Dasan, MBBS, cannot be expressed in words. I wish to express my sincere thanks to all other colleagues also.

I also thank gratefully all those orchid farm owners for extending their kind cooperation in giving permission to use their farm as my experimental farm for data collection.

I am thankful to the Head, Department of Atmospheric Sciences, School of marine Sciences for providing meteorological data.

I acknowledge and thank the non-teaching staff of the Department of Biotechnology for their support and cooperation. I also thank the authorities of Cochin University of Science and Technology for providing necessary facilities for carrying out the work. Financial support from the University in the form of a research fellowship is gratefully acknowledged.

I am also indebted to my wife and children for their patience and moral support extended to me for spending my time on this research work.

B.Christudhas Williams

CONTENTS

INTRODUCTION

1.Introduction

1.1 Preface

ORCHIDS, the most beautiful in gods creation, comprise a unique group of plants. Orchid flowers are reckoned as 'queen of flowers' because of their enchanting beauty of structure and color variations and enduring quality. Orchid flowers are great mimics in nature resembling bees, wasps, spiders, scorpions, monkey man frog etc (Joseph,1990).

Orchids are used for decoration at wedding ceremonies, Easter, Christmas, as bouquets and flower arrangement during conferences, Mothers day receptions, and as corporate and personal gifts. Increased demand for orchids has promoted their commercial cultivation and trade in almost all the developed and developing countries. Consequently there is a great demand for hybrid orchids, especially *Dendrobium* sp in the floriculture industry.

Orchids are highly specialized and largest group of flowering plants of unique biology, flowers of fantastic architectural beauty, color combination and the most complicated flower structure among the monocotyledons. It is a perennial herb; terrestrial, epiphytic, or saprophytic, sometimes vine-like, the terrestrial with fibrous or with thickened tuberous or cord-like roots; seeds very abundant, minute, often fusiform, without endosperm, the embryo undifferentiated (Lawrence1951). Taxonomically, they represent the most highly evolved family among

monocotyledons with 600-800 genera and 25000-35000 species, under the family Orchidaceae (Arditti 1979). Besides, man made specific, inter generic hybridization orchids are more than the species population.

The name 'orchid', has come from a Greek word *Orchis* meaning testicle and had been given by Theophrastus (370-285 B.C), the Greek philosopher and the pupil of Plato and Aristotle fancied resemblance of the paired underground tubers of the European terrestrial orchid, to the testicles of the dog.

Geographical Distribution of Orchids

Orchids have a wide range of distribution found even in frozen areas of Alaska, the snow covered areas of the Himalayas and the deserts of Africa and Australia (Polunin 1959, Stearn 1960). They are broadly classified into temperate and tropical areas of the Northern hemisphere such as, Europe, Northern Asia and North America. Tropical orchids are both terrestrial and epiphytes.

The island of Sri Lanka, which lies nearest to South India and has almost the same climatic conditions, is reported to harbour180 species of orchids from and maintains a considerable amount of individuality, as is evidenced by the 104 species, which are endemic to the island. Among them, there are only 37 species which South India and Sri Lanka share to gether(Hooker.1895). In Singapore, out of the 179 species recorded, 45 are terrestrials. They accompany only 25% of the total number of native orchid species, which is typical for a tropical rain forest (Yam Tim Wing 1993). The climate range in Hawaii is conducive to the growth of many orchid genera, allowing for large collections among hobbyists and stimulating commercial

production. Several commercial orchid nurseries in Hawaii breed and propagate a wide variety of species and hybrids (Uchida, 1995).

Ecology

The tropical climate is characterized by a high amount of rainfall, contributed by the south west and north east monsoons, with thick diversified flora and fauna, there is no prolonged dry season, and even these dry months have a mean rainfall of 3-4 inches. The temperature is high, ranging between 70-90°F at sea level. The heavy rainfall and high temperature provide the atmosphere with a high relative humidity. This warm, humid tropics is the home of epiphytic orchids.

In the equatorial region, i.e., 7-12 North and South of the Equator, the climate is equable throughout the year, with no great variation in temperature. The rainfall is distributed uniformly throughout the year, although there are two yearly peaks consequent upon the sun's journey north and south of the Equator. The monopodial forms of orchids, characterized by their terminal growing point, reach their zenith of development under these climatic conditions. Temperature in the equatorial belt is usually maintained at 70-90°F, and plants continue to grow almost throughout the year. The various *Vandas*, *Angraecums*, *Stauropsis*, etc. come under this category.

As the distance from the equator increases, most of the rain fall gets confined to one particular season, the rest of the year getting only sporadic showers. The epiphytic orchids which are more dependent on rainfall than those species which are rooted in soil, develop storage organs which serve as protection against possible scarcity of atmospheric moisture. Most of the deciduous sympodial forms include

Dendrobium, Bulbophyllum, Cattleya, etc. With the increase of distance from the equator, the amount of rainfall and warmth and resultant humidity also decrease and the epiphytic species are completely replaced by terrestrial forms with underground tubers, which are adapted to survive by hibernating during the unfavorable conditions.

South India lies between 8^0 - 20^0 north of the equator and between 70^0 - 85^0 in longitude. The mean temperature is 27^0C, at sea level, which it enjoys with Northern Australia, New Guinea, Philippines, Indonesia, Malaysia, Sri Lanka, and major part of South Africa, parts of Brazil, Guyana, Columbia, Venezuela, Panama, the Honduras and Guatemala.

The southernmost regions of peninsula, which lie on the northern fringes of the equatorial belt and extends north-south touching Indian Ocean at its southern most tip, is flanked on the west by the Arabian Sea and on the East by the Bay of Bengal. The combination of the geographical factors discussed above along with the yearly excursions of the sun towards the north and south of the equator, render these southern coastal regions exposed to both South West and North East monsoons. During the hot summer months of June and July, when the sky would be constantly overcast, it would go on raining for days at a stretch. The temperature at this time of the year may vary between 75-80^0F at day time and between 60-75^0F during night. The relative humidity is almost always at or near saturation point. Since north-east monsoon winds travel mostly over land area before they enter South India, the showers these winds bring are far less heavy than those of south-west monsoon.

During this season, south-western regions of Western Ghats experience a very predictable weather of bright sunny morning and cloudy afternoons with thundershowers. From December to February, when the sun is at its southern zenith, weather is comparatively dry with little or no rain, and the temperature drop down to as low as 60^0F. The months of March, April and May are the hottest of the year, and the temperature soar to 90-100^0F with occasional showers to relieve the monotony of this summer heat (Abraham & Vatsala 1981).

Distribution of orchids in peninsular India

Orchids from 9% of our flora and are the largest botanical family of higher plants in India. It is estimated that about 1,300 species (140 genera) of orchids are found in our country with Himalayas as their main home and others scattered in Eastern and western Ghats. The efforts of Wight, Hooker, Fischer, Blatter & McCann Santapau and Kapadia, nearly 240 species in about 70 genera of orchids have been reported from South India and nearly 60% of the species are epiphytic, the remaining 40% constituting the terrestrial flora (Abraham & Vatsala 1981).

The following is the distribution of orchids species in different regions of India.

North-Western Himalayas ca 200 species

North-Eastern India ca 800 species

Western Ghats ca 300 species

North-Eastern India owing to its peculiar gradient and varied climatic conditions contains largest group of temperate, sub-tropical orchids.

India has a very large variety of orchids and hilly regions have one or the other orchid flowering almost throughout the year. The diversity is so large that there are large-flowered, terrestrial, epiphytic and also saprophytic orchids. In general terrestrial orchids are more common in North-Western India, epiphytic orchids in North-Eastern India and small flowered orchids in Western Ghats. The largest terrestrial genus is *Habenaria* (ca 100 spp.) and the largest epiphytic genus is *Dendrobium*(ca 70 spp.). Most of the *Paphiopedilum* (lady's slipper) species are restricted to N.E Himalayas except for *P. druryi* which has been reported from Kerala but now is almost extinct from its original habitat.

Some orchids are endemic to Indian Species are so ornamental and in demand that their natural populations have been over exploited. Some species in the genera like *Arundina* , *Cymbidium, Coelogyne, Dendrobium, Paphiopedilum, Renanthera,* and *Vanda* are almost extinct. The provisional list of 150 endangered plants of India includes many orchids like *Acanthephippium sylhetense, Anoectochilus sikkimensis, Aphyllorchis montana, Arachnanthe clarkei, Arundina graminifolio, Cymbidium macrorhizon, Dendrobium densiforum, Didiciea cunninghamii, Eria crassicaulis, Galeola lindleyana, Gastrodia Exilis, Paphiopedilum fairanum, P. druryi, Pleione humilis, Renanthera imschootiana, Vanda coerulea, V. pumila* and *V. roxburghi.*

The list of plants banned or restricted for export from India formerly included a few orchids but now include all orchids growing wild. The convention of International Trade in Endangered Species of Fauna and Flora (CITES), ratified by India, places all species of Orchidaceae under Appendix II, meaning thereby that their

trade will be only through export permits. Steps have also been taken to conserve Indian native species by establishing orchidaria, sanctuaries and germplasm conservation centers. Botanical survey of India has established two orchidaria one at Shillong and other at Yercaud to conserve rare and endangered species. The ICAR research complex at Shillong, the Indian Institute of Horticultural Research at Hessaraghatta and the Indian Botanic Gardens at Calcutta maintain collections of orchids in their orchidaria. Some states have also established orchid sanctuaries in Sikkim at Singtom and Deorali and in Arunachal Pradesh at Tapi.

Majority of the cultivated orchids are native of tropical climates and are found in abundance in India in the state of Assam, Meghalaya, West Bengal, Karnataka and Kerala. Kalimpong, Shillong, Trivandrum, Bangalore , Yercaud and almost all the coastal areas of India are the places most suitable for the for the cultivation of orchids.

Commercial cultivation of orchid in India,

Commercial cultivation of orchid in India which started in1992 in four acres of land has grown up to 120 acres. Growing of orchids in India commercially is not organised and is still in the hands of hobbyist and few dealers who mainly depends on wild collections from forest to meet a large part of their foreign and local demands, due to which some of the orchids-growing areas are now without any orchid and very rare species are now facing the danger of depletion. The spikes which are produced in the orchid farms are absorbed by the domestic market. In spite of ideal climatic

condition available for orchid cultivation, their production at large scale is not achieved.

World market for Orchid

The orchids represent the first floricultural crop successfully mass propagated, through tissue culture techniques and commercial application of micro propagation is being increasingly realized for this group of great ornamental orchids. The major cut-flower producers are Thailand, Singapore, Australia, New Zealand, Malaysia, Netherlands, and Taiwan and the major consumers are the United States, Japan, Germany, France, Italy etc.

Presently, Netherlands is the largest producer of orchids for cut-flower under glasshouse conditions, while in Thailand, the orchid cut-flower industry has become the major foreign exchange earner (Guptha1994).

In 1992, Singapore exported S$23,247,000 of orchid cut-flowers and S$3,141,000 of orchid plants. Japan, the leading importer of orchid cut-flowers consumes some 40% of the worlds supply (Koay Sim Huat 1993).

The commercial value of various orchid crops in Hawaii approximated $11 million in 1990 (Anonymous 1990-1991). In 1989, Hawaii exported nearly 3.2 million sprays, mostly to the U.S. mainland, which imported over 5.6 million *Dendrobium* sprays from foreign countries (Wanitprapha *et. al* 1991).

In Thailand, the worlds largest producer of *Dendrobium*, the cultivar *Sonia* accounts for over 70% of the *Dendrobium* grown, whereas University of Hawaii cultivars dominate production in Hawaii. The estimated 3-year cost for a 1.5-`acre

farm in Hawaii (excluding land cost of $30,000-$150,000/acre)exceeds $200,000, whereas a similar farm cost less than $4,000 in Thailand (Wanitprapha *et.al* 1991). Hawaiis growers receive higher wholesale prices for products of better quality than those from competing nations (Wanitprapha *et.al* 1991). A tremendous number of genera are grown in the state, including a number of species that thrive in cooler areas, for example, cultivars of *Cymbidium, Dendrobium,* and *Phalaenopsis.* Over 100 nurseries in Hawaii produce orchids, with many specializing in selected genera of potted plants or in flower production (Anonymous 1989).

Diseases in Orchids

The common diseases on orchids under cultivation in the different climatic and edaphic conditions in Kerala is yet to be made since their wide spread cultivation. Nevertheless the common diseases of the orchids known in general are caused by viruses, fungi, bacteria, insects, etc (Joseph, 1990).

Orchids like other plants are attacked by fungi, bacteria, nematodes, and viruses. More than 100 diseases have been reported on orchids. Some diseases such as the black rot caused by fungi *Phytophthora cactorum* (Led. & Cohn) Schroet. and *Pythium ultimum* Trow., and bacterial brown spot caused by *Pseudomonas cattleyae* (Pavareno) Savulescu can cause death of plant. Similarly several diseases like leaf spots, stem rot, soft rot, root rot, leaf blight etc cause severe loss in the orchid production in any commercial cultivation farm. More than 32 diseases are known to occur on orchids. In some cases the same virus has been known to produce more than one diseases in different species, the most common are Cymbidium mosaic virus As

control measures all infected plants should be isolated to prevent spreading of the disease.

The most commonly reported insects pests on orchids are thrips, aphids, spidermite, soft scale, mealy bugs, orchid weevil, snail and slugs. These insects pests harm the plants in many ways. They feed on tender young shoot, suck the sap and damage the young bud and shoots and also act as the carrier of different diseases. Fortunately all these can be controlled by effective insecticides like Parathion, Malathion, BHC, Aldrin, Dieldrin, etc. Metaldehyde has proved to be very effective in killing.

Consequently, in the light of increased interest in orchid cultivation and commercial production for export and domestic use, there is a need for field data and detailed information on occurrence of diseases and influence of environmental parameters on disease outbreak in order to have an efficient disease management and reduce loss in orchid production.

Indian scenario on orchid cultivation, epidemiology and farm management is not well understood, and scientifically documented when compared to other orchid producing countries. In this context the present study assumes due importance and significance with reference to generation of a data bank on occurrence of diseases and statistical modeling, which could have long term impact in the design and development of appropriate strategies for effective farm management.

1.2. Review of literature

A critical survey of the available literature on orchid diseases revealed that the quantum of literature available is limited to very few which are given below. Most of the reports are on specific orchids and specific diseases associated with them.

The black rot fungi, *Phytophthora cactorum* Schroet. and *Pythium ultimum* Trow., and bacterial brown spot caused by *Pseudomonas cattleyae* (Pavareno) Savulescu are organisms that can cause death (Burnett1986).

Bacterial soft rot is a common disease of *Dendrobium*, especially when the conditions are very wet. The casual organism was found to be *Pseudomonas gladiolii* (Ganapathi & Chinathambi 1988).

Disease organisms that attack orchid plants in the Northern Territory of Australia, are bacterial soft rot caused by possibly *Erwinia carotovora* on *Dendrobium, Oncidium*, a rapid collapse of bulb and stem tissues resulting in leaf loss. *Pseudomonas cattleyae* has been reported on *Dendrobium, Oncidium, Rhynchostylis* as small brown soft water soaked areas (John,1997).

In the United States, fungal pathogens frequently associated with common orchid genera include: *Aecidium graebnerianum* Henn., *Alternaria* spp., *Bipolaris* spp., *Botrytis cinerea* Pers.: Fr., *Cercospora* spp., *Coletotrichum gloeosporium* (Penz.) Penz. & Sacc. IN Penz., *Fusarium oxysporum* Schlechtend.: Fr., *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., Phoma spp., *Phyllosticta capitalensis* Henn., *Phytopthora cactorum* (Lebert& Cohn)J.Schrot., *Phytopthora*

palmivora (E.J.Butler) E.J. Butler, *Pseudocercospora* spp., *Puccinia cypripedii* Arth. &Holw., *Pythium splendens* H. Braun, *Pythium ultimum* Trow, *Rhizoctonia solani* Kuhn, *Sclerotium rolfsii* Sacc., Septoria spp., *Sphenospora kevorkianii* Linder, and *Uredo* spp.(Farr, *et al* 1989). These organisms are associated with flower blights, leaf rots or spots, stem or pseudo bulb rot, root rots, plant decline, and seedling damping-off.

Some of these pathogens have been noted to occur in other parts of the world (Sutabutr 1980 & Kamjaipai 1984). Geographic isolation and state agricultural quarantine regulations have prevented the establishment of rusts in Hawaii, but all other fungal pathogens listed above are now known to occur in Hawaii.

The rapid growth of the orchid industry in Hawaii during the 1980s was accompanied by a proliferation of disease with unknown etiologies. Little research-based information was available for the many pathogens associated with diseases of orchids. Research efforts in Hawaii, were focused on diseases of hybrid *Dendrobium*, with support for studies coming from commercial growers and state agencies, and international growers. These studies also serve as a foundation for investigations of problems in other genera, such as *Cattleya, Cymbidium, Epidendrum*, and *Vanda* (Uchida 1994).

Bacterial diseases are common on orchids and have been especially troublesome in wet areas of Hawaii. The most serious are rots of *Dendrobium, Oncidium*, and *Phalenopsis* plants. *Erwinia chrysanthemi* Burkholder, McFadden, and Dimock and *Pseudomonas gladioli* Severini pv. *gladioli* have been identified as

pathogens in *Dendrobium* fields. Wounding is not needed for disease initiation, and young tissue is the most susceptible (Uchida 1994).

The aerial surfaces of plants are colonized by a characteristic micro flora consisting of bacteria, yeasts and filamentous fungi. Although representatives of each of these major groups may be found on leaves at any time of the year, there is an underlying seasonal succession (Blakeman 1985). As leaves open at the start of the growing season, the initial colonists are predominantly bacteria. Yeasts dominate during the middle of the growing season, followed by filamentous fungi, spores of which commence to germinate as leaves pass their peak of activity. Hyphae of filamentous fungi may later enter the tissues as minor pathogens causing yield losses (Dickinson 1981). The micro flora associated with aerial plant parts is predominantly composed of saprophytes. However, plant microbiologists are becoming increasingly aware of the significance of plant pathogens (particularly bacteria) which adopt a saprophytic resident phase in this habitat (Hirano & Upper 1983).

Bacteria may not be entirely confined to the surface of the plant; internal populations may be present in intracellular spaces of sub stomatal chambers. Surface population of bacteria may become internal as a result of heavy rain inducing water-soaking of leaf tissue. This may be the cause of observed increases in out break of brown spot *(Pseudomonas syringae)* following heavy natural rain or simulated rain applied from a substantial height above the crop (Hirano & Upper 1988).

Bacteria associated with aerial plant surfaces are mainly Gram-negative rods with a higher proportion of chromogenic forms than from other habitats. The most

common genera are *Pseudomonas*, *Eerwinia*, and *Xanthomonas*, together with the less well defined group flavobacteria. Many saprophytic organisms are not readily identifiable to species level but a few are well known in this habitat. Strains of *Ps. syringae* are the most widely distributed colonists on foliar surfaces; many of these are saprophytic but some are pathovars able to attack a specific host under appropriate conditions. Fluorescent pseudomonads also occur widely on aerial plant surfaces. These are distinguishable by their green fluorescence on Kings medium B and positive oxidase reaction. *Erwinia herbicola*, a yellow pigmented saprophyte, is present widely as an epiphyte, especially on fruit trees where it may often be associated with the fire blight pathogen, *E. amylovora*, with which it competes (Blakeman. 1985).

Hirano & Upper (1983) list 27 different bacterial pathogens which exist as epiphytes on over 30 hosts belonging to both temperate and tropical genera. Despite numerous pathovars being represented, these belong to only three bacterial genera, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. Gram-positive bacterial pathogens, e.g. *Corynebacterium*, are not represented as epiphytes.

The majority of plant pathogenic bacteria have only a very limited capacity to survive in soil. It is likely that most bacterial pathogens obtained from soil have been present as colonists of shoot or root debris. *Pseudomonas syringae* pv. *glycinea* and pv. *syringae* on soybeans and beans respectively and X.*campestris* pv. *glycinea* and pv. *vesicatoria* on soybean and tomato respectively can all be recovered from soil where debris harboring these pathogens has been present (Hirano & Upper 1983).

The airborne population of bacteria appears to be largely derived from the foliage of crop and wild plants. Sampling of bacterial cells from the air above the crops has shown that approximately four times greater numbers of cells occurred over a crop of lucerne than over bare soil at the same time (Lindemann *et al.* 1982). This and other evidence suggests that plant foliage represents a major source of air borne bacteria, including pathogens.

Plants in different stages of maturity may contribute made to the bacterial cell count above the crop. There were more bacteria above the mature winter wheat field than a young maize field (Lindemann *et al.* 1982).

Haulm destruction of potato foliage has been shown to result in release into the air of cells of the bacterium which caused tuber soft rot and black leg of stems of potato, i.e. *Erwinia carotovora* subsp. *atroseptica*. This bacterium can also develop as epiphytic population on healthy potato leaves (Burgess 1989) where it may be dispersed short distances to neighbouring plants by rainsplash.

1.3 Objectives of the present study

There is no baseline data available at present on the nature of various diseases that occur in a orchid population, under cultivation, in any commercial orchid farm maintained by small scale entrepreneurs who invest considerable amount of money, effort and time. The available data on type of disease symptoms, causative agent, , nature of pathogens, as to bacteria or fungi or any other biological agents, and their source, appropriate and effective control measures could not be devised, for large scale implementation and effective management, although arbitrary methods are being practiced by very few farms. Further influence of seasonal variations and environmental factors on disease outbreak is also not scientifically documented and statistically verified as to their authenticity.

In this context, the primary objective of the present study was to create a data bank on the following aspects

- Occurrence of different disease symptoms in *Dendrobium* hybrid over a period of one year covering all seasons
- Variations in the environmental parameters at the orchid farms
- Variations in the characteristics of water used for irrigation in the selected orchid farm
- Microbial population associated with the various disease symptoms
- Isolation and identification of bacteria isolated from diseased plants
- Statistical treatment of the quantitative data and evolving statistical model

MATERIALS AND METHODS

2. Materials and methods

2.1 Study area

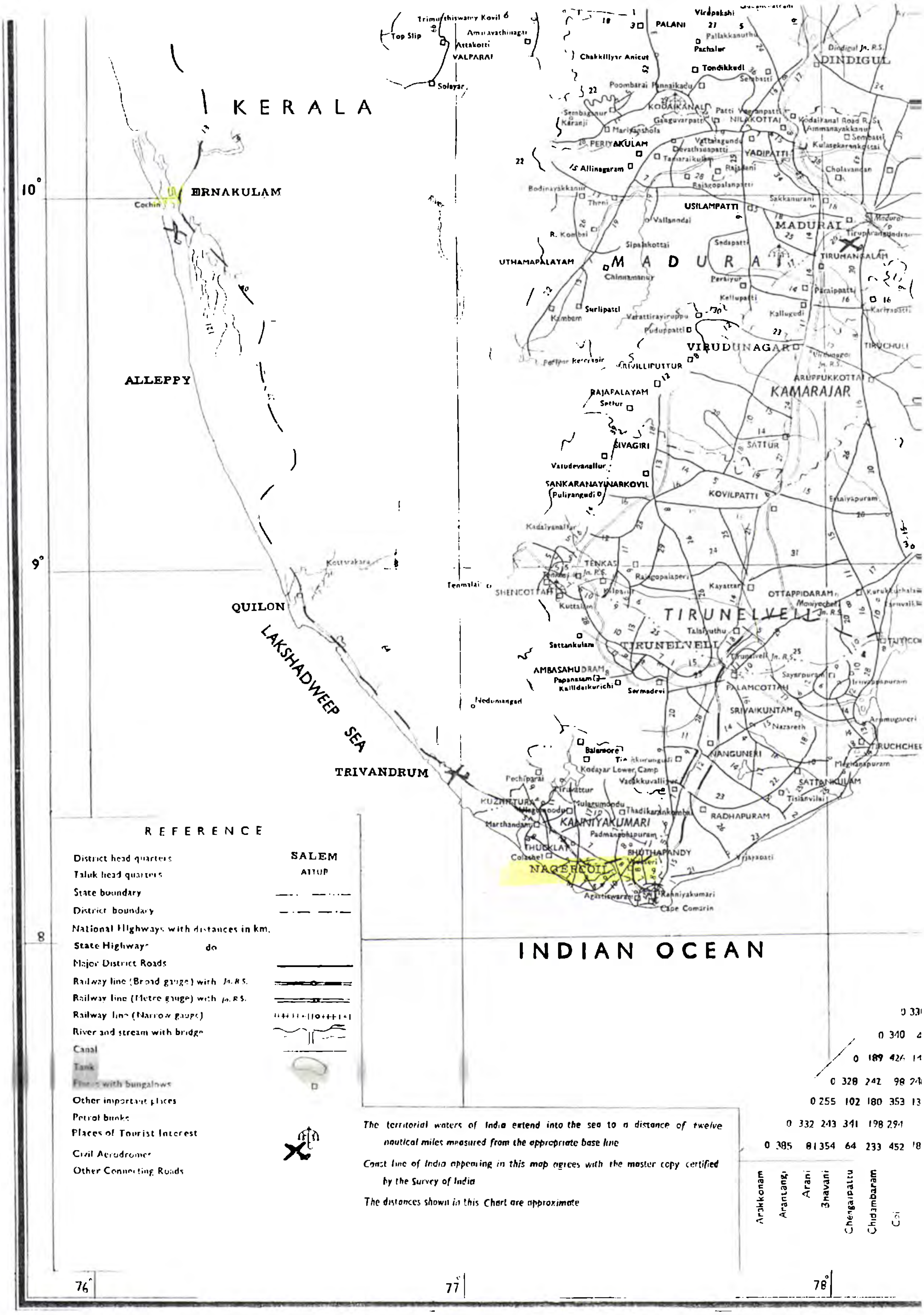
The present study was conducted on *Dendrobium* hybrid orchids under cultivation in small scale commercial orchid farms managed by entrepreneurs, in selected places, located in Kanyakumari (Tamilnadu) and Ernakulam (Kerala) Districts in the peninsular India, which is known to be conducive for orchid cultivation (Joseph 1990).

2.1.1 Ernakulam District

The topography of the district is, high and low land with back water, situated between 9.5^0 - 10.5^0 latitude and 76^0 - 77^0 longitude. The district is marked by the Arabian Sea on the west, Western Ghats with thick vegetation on the east, and by the thick Horticultural and Agricultural lands on to the south, and the north. The average rainfall is between 250 -300cm. The district is thickly populated and highly polluted. Three stations, were selected from this District, for regular observation and collection of samples.

2.1.1.1. Station I

The selected orchid farm is located in the low land area surrounded by hills and with paddy fields on two sides, and with coconut and banana plantations on the other sides. Approximately 40,000 plants are cultivated in 2.5 acres with a flat 50% shading net roofing structure, at the height of 8 feet from soil, which is flat and



Reg. No. 5/1986 (Thiruvalluvar Andu 201) (C. S. O.) 10,00,000 Revenue Department Publis

placed as a single stretch (Plate-1). The plants are planted in earthen pots with a diameter of 4-6 inches on an elevated (3 foot), concrete and metal platforms. The air circulation with in the farm is very poor. The major source of water for the farm is the bore-well, and during some months the plants are directly exposed to rain. Watering of plants is done with the help of sprinklers.

2.1.1.2 Station II

The topography of the Station II is a plain land with no ups and downs in the near by area. The farm is located at a central place which is surrounded by lands with Horticultural crops and few houses on the three sides and by a broad road. Approximately 5,000 plants are under care, under the terrace roofing structure with 50% shading net. The plants are planted in earthen pots on metallic stands (Plate-2). The major source of water for the farm is the well, and during some months the plants are directly exposed to rain. Watering of plants is done with the help of sprinklers.

2.1.1. 3 Station III

This station is located in a place bracketed by broad and narrow roads on two sides of the farm, and with residences on the other two sides. Approximately 4,500 plants are maintained on concrete and metallic platforms. The plants are planted in earthen pots with charcoal as the media under 50% shading net. The height of the roof is approximately 10 feet height, and the air circulation is poor (Plate-3). The main source of water for the farm is the municipal water and during some months the plants are directly exposed to rain. Watering of plants is done with the help of hose pipe, and not sprinkled.

Plate -1 Showing Station-1

Plate -2 Showing Station-2

2.1.2 Kanyakumari District

Two stations were selected from Nagercoil, the Head Quarters of Kanyakumari District. This town is situated in the southern most peninsular India, between 77° - 78° longitude, and 8° - 9° latitude. The District is bordered by Arabian Sea and Indian Ocean respectively on the West and South. Northern side is marked with terrain hills and valleys. The Western Ghats starts from here, with thick flora and fauna, and most part of the eastern region is dry land, excluding a small area surrounded by the Bay of Bengal. The topography of the district is uneven land with many ups and downs. The annual rainfall is 150 to 200cm, distributed throughout the year. Two stations, were selected from this District, for regular observation and collection of samples.

2.1.2.1 Station IV

The orchid farm is located in a residential area where the topography of the land is slightly elevated. Approximately 2,000 plants are maintained on the roof top of the house with 50% shading net at 6 feet high. The farm is surrounded by residential complexes on three sides with mixed horticultural crops, and by a broad road on the Southern side (Plate-4). The main source of water for the farm is the municipal water, which is stored in a large cement tanks. Occasionally rain water is collected in large plastic containers and used. Watering done by hose, and not sprinkled.

Plate -3 Showing Station-3

Plate -4 Showing Station-4

2.1.1.2.2 Station V

The topography of the station-V is highly elevated. About 2,500 plants are accommodated on the roof of the house with 50% black shading net at the height of 7 feet. The farm is surrounded with thick residential area with horticultural crops and a narrow road on the Western side (Plate-5). The source of water for the farm is from either the bore-well or the municipal water. Water is usually collected, and stored in a large cement tanks and used for irrigation of plants. Watering done by hose, and not sprinkled.

2.2 Samples

2.2.1 Orchid

Dendrobium was considered as the subject for the study

Dendrobium belongs to the family Orchidaceae. The characteristics of *Dendrobium* a perrineal herb, epiphytic, pseudobulb with 2 or more leaves; flowers with lateral sepals. Adnate to the foot of the column to form a mentum. Lip lobed; mid lobe much larger than the side lobes(and often crenulatte to pectinate; pollinia four, ovoid or oblong.

Dendrobium hybrid orchid was monitored for the occurrence of various diseases over a period of one year covering all seasons in a year. Leaves, flower, stem, root and bud portions of the plant were considered as samples for various analyses during the course of study.

Plate -5 Showing Station-5

2.2.2 Environmental samples

Air, and water from the orchid farm were considered as environmental samples for various analyses during the course of study.

2.3. Collection and transportation of samples

Samples were collected at regular intervals of one month, over a period of one year, during 1996-1997 from all the 5 stations selected for study. Orchid samples, both fresh and diseased, were collected aseptically in a sterile polyethylene bags. Water samples were collected in sterilized bottles. All the samples collected were transported to the laboratory and subjected to various analyses as described elsewhere.

2.4. Environmental Characteristics

Meteorological data for the three stations selected in Ernakulam district was obtained from the Department of Atmospheric Sciences, School of Marine Sciences, Cochin University of Science and Technology, Cochin-682016. Data on temperature and humidity, for the two stations selected in Kanyakumari district was obtained from the Rice Research Station, Nagercoil. Data on rainfall in the selected stations of Kanyakumari district was obtained from the Public relation office at Nagercoil

2.4.1. Atmospheric Temperature

Maximum and minimum temperatures of the atmosphere at the selected station of study were recorded for the period of 5 days including sampling day and the average data is presented under results.

2.4.2. Humidity

Humidity prevailing in the sampling area was recorded for the period of 5 days including sampling day and the average data is presented under results.

2.4.3. Rainfall

Data on total rainfall for the period of 5 days including sampling day, at the selected study area, were recorded and the average data is presented under results.

2.5 Characteristics of water used in Orchid farm

2.5.1 Color and Odor

Color and odor of the water samples were assessed by sensory evaluation from the field.

2.5.2 Temperature

Temperature of water samples were recorded, using a sensitivity (1/10) thermometer (0-100^{0}C), immediately after collection

2.5.3 pH

Water samples were subjected to measurement of pH, which was carried out using a Systronics digital pH meter-335, after standardization using buffer solution of pH 4.0 and 7.0.

2.5.4 Hardness

Hardness is the property of water which prevents lather formation with soap and increase the boiling point of water APHA (1989). Calcium and magnesium are the principal cations imparting hardness. The anions responsible for hardness are

mainly bicarbonate, carbonate, sulfate, chloride, nitrate, and silicate. Total hardness of water was estimated according to the methods of APHA(1989)

1. A known volume (50 ml) of sample was added with 1 ml of buffer solution. (*Preparation of solution* 16.9 gm of NH_4CL in 143 ml of conc. NH_4OH + 1.179 g of disodium salt of EDTA and 0.78 g of M_gSO_4 in 50 ml of distilled water; final volume made upto 250 ml.)
2. 100 to 200 mg of Erichrome Black-T indicator was added to the contents and titrated against EDTA solution (0.01 M was prepared by dissolving 3.723 g of disodium salt of EDTA in one liter of distilled water and stored in a pyrex bottle).

3.The end point was the color change from wine red to blue.

4.Calculation .

$$\text{Total hardness } CaCO_3 \text{ mg/l} = \frac{\text{Vol.of EDTA x M.of EDTA x 100 x 1000}}{\text{Volume of sample}}$$

2.5.5 Total Alkalinity

Total alkalinity of water was estimated according to the methods of APHA(1989)

1. A known volume (50 ml) of sample was taken in 250 ml conical flask, and added with 2-3 drops of phenolphthalein indicator to the sample. Titrated with 0.02N H_2SO_4 , till the pink color disappears.
2. Recorded the buret reading, added with 2-3 drops of Methyl Orange indicator and resumed the titration to the appearance of a very faint orange color

3. Calculation :

$$\text{Total alkalinity as } CaCO_3 \text{ mg/l} = \frac{\text{Titration value x N x 100 x 1000}}{\text{Volume of sample}}$$

2.6 Disease symptoms

The affected plant parts (leaves, stem, root, bud, and flower) develop visible changes to the naked eye. The following disease symptoms were observed in hybrid *Dendrobium* during the period of study. Each disease symptom is described below (Source of information :- farm workers) and is supported with photographs for reference.

2.6.1 Yellowing of leaves(Plate- 6).

The yellow color will start in any part of the leaf and spread through out the leaf with in two days during the unfavorable seasons, the surface both abaxil and adaxial is smooth not interrupted with any other symptoms.

2.6.2 Brown dots on leaves(Plate - 7).

The green leaves very often affected with brown or black dots on the abaxial side of the leaves. Usually it is very small and slightly elevated.

2.6.3 Leaf rot (Plate -8).

The infected portion is yellow, very soft, watery and produce rotten smell. The symptoms start either side of the leaf. The infection is very fast and usually it was observed as more than 30% infection in a leaf

Plate-6 - Yellowing of Leaves

2.6.4 Brown spots on flower (Plate-9).

The brown spot on flowers occurs on the adaxial side of the petals and sepals. The symptoms show round to elliptical, center dark to light in the outer. Usually observed in white color flowers rarely on dark colors

2.6.5 Bud wilting (Plate10).

Sudden change in color of young and mature buds leads to abscision and wilting the other floral buds have no change.

2.6.6 Stem rot (Plate-11).

Water soaking appears in any portion of the mature stem and rapidly spread to leaves, flower spikes and roots resulting in death of plant

2.6.7 Dried stem (Plate-12).

Normally observed in the back pseudobulbs (not very old), with out any leaves. The lose of water and changing color from the normal (green) to yellowish with ridges and groves, soft to touch like cotton. The new pseudobulbs are healthy with green leaves.

2.6.8. Root rot (Plate-13).

Observed in the new and old shoots. The fleshy root white and solid, and change in the color from white to brown and soft to touch, with out any parenchymatous tissue.

2.6.9. Flower drooping (Plate 14)

Sudden change in the texture of the freshness of the petals and sepals ie change in color bright to fade and exposing the veins.

Plate-8 - Leaf rot

Plate-10 - Bud wilting.

Plate-11 - Stem rot

Plate-12 - Dried Stem

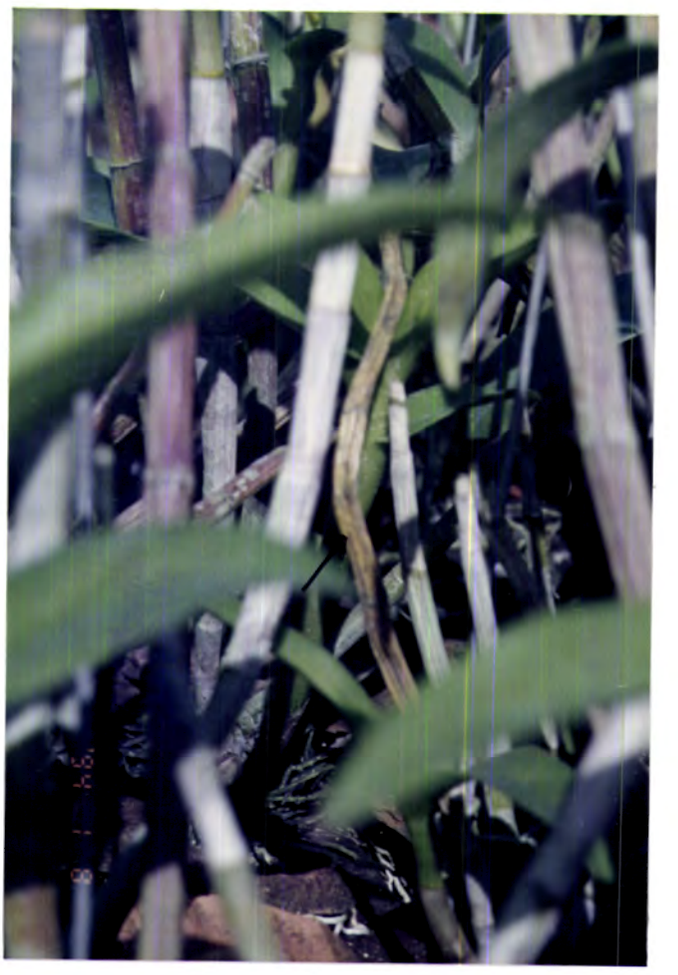


Plate-13 -Root rot.

Plate-14 -Flower drooping.

2.7 Sensory evaluation of Diseased condition

The intensity of disease, and the frequency of distribution were observed by sensory evaluation using a 5 point score(Table 1). The intensity of the diseased plant parts were calculated in terms of percentage of total surface area of the sample portion; and the frequency of distribution is calculated in terms of percentage of infected plants among the total number of a given population

2.8 Enumeration of Microbial population

2.8.1.Enumeration of bacterial Population

Bacterial population associated with air, water and hybrid *Dendrobium* samples was enumerated employing standard microbiological procedures

2.8.1.1Medium:

Nutrient agar medium(HI), available commercially, as ready made medium, was used for enumeration, isolation and maintenance of bacteria, during the period of study.

2.8.1.2. Preparation of samples of Plant materials

Fresh or diseased Leaves (10 leaves in number), were taken (5 pieces of , $1cm^2$ size, from each leaf randomly selected,) and ground in a mortar and pestle, using 10 ml of the sample blank(prepared using physiological saline(0.85% NaCl) as 90ml aliquot in 250 ml conical flasks and autoclaved). The prepared sample was used for preparation of serial dilution, and subsequent inoculation.

In the case of samples of stem, bud, flower and root, about 10g (wet weight) of the sample, randomly pooled from many plant samples, was ground in a mortar

Table 1. Evaluation index used for assessing Percentage of surface area with disease symptoms/ Percentage of plants with disease symptoms in a given Population

Percentage of surface area with disease symptoms/ Percentage of plants with disease symptoms in a given Population	Point score
1-20	1
21-40	2
41-60	3
61-80	4
81-100	5

Score -1 represents the initial stage of any particular disease

Score –2-3 is the progressive stage of the disease an

Score –4 represents that the plant is in the pre-death condition due to any particular disease

Score –5 represents that the plant is dead condition due to any particular disease.

and pestle, using 10 ml of the sample blank (prepared using physiological saline(0.85% NaCl) as 90ml aliquot in 250 ml conical flasks and autoclaved). The prepared sample was used for preparation of serial dilution, and subsequent inoculation.

2.8.1.3. Preparation of Serial Dilutions

Serial dilution blanks were prepared using physiological saline(0.85%NaCl). Prepared blanks were dispensed as 9ml aliquots in test tubes, autoclaved, and used.

Samples of water and plant materials, (leaves, flowers, stem, bud and root) were subjected to serial dilutions, as per the conventional procedures, as and when necessary.

2.8.1.4. Plating procedures.

In the case of aerial flora, previously prepared nutrient agar plates, poured earlier in to pre sterilized petri plates and solidified, were used. In the Orchid farm the nutrient agar medium in the petri plates were exposed to air for one hour at, or slightly above the plantation canopy

Whereas, in the case of water and other plant materials, pour plate technique was employed for inoculation of the prepared samples, at desired dilutions, in triplicate, in to the nutrient agar medium. After plating, the plates were incubated at 30°C for 3-5 days.

All the colonies developed on the nutrient agar plates were counted and the bacterial population was expressed as colony forming unit (CFU) per ml in the case of water, and as CFU per cm^2 or g in the case of plant materials.

2.8.2. Enumeration of Fungal population

Fungal population associated with air, water and hybrid *Dendrobium* samples, both fresh and diseased, were enumerated employing standard microbiological procedures.

2.8.2.1.Medium:

Potato Dextrose agar medium(PDA)(HI) commercially available as ready made medium was routinely used during the period of study. The dehydrated media was dissolved in distilled water, autoclaved and used as per the specifications HI.

2.8.2.2. Preparation of samples of Plant materials

Samples of plant materials were prepared as described under section 2.8.1.2

2.8.2.3. Preparation of Serial Dilutions

Serial dilution blanks and serial dilution of samples were done as mentioned under section 2.8.1.3

2.8.2.4.Plating procedures.

In the case of aerial flora, the Potato Dextrose agar media was poured earlier in to pre sterilized petri plates, cooled and solidified were used. The petri plates were exposed to the air for 10-15mt exposed at, or slightly above the plantation canopy

Whereas in the case of water and other plant materials, pour plate technique was employed for inoculation of samples, in triplicate, in to the potato dextrose agar medium. After plating, the plates were incubated at 30°C for 5-7 days, and colony counts were made. The population was expressed as colony forming unit (CFU) per ml in the case of water and as CFU per cm^2/g in the case of plant materials.

2.9 Isolation and Identification of bacteria

1 All single celled colonies of bacteria developed on nutrient agar medium(NA) plates, when plated for enumeration of bacterial population, were picked and subculture on nutrient agar slants.

2 Cultures were purified by repeatedly streaking on nutrient agar plates.

3 They were identified to various genera based on their morphological and biochemical characteristics, as outlined in the Bergey's Manual of Systamatic Bacteriology V0l. 1-4 (1989)

4 All the purified cultures were maintained on agar slants and subcultured periodically (once in 15 days). One set of stock cultures were maintained under refrigeration (4°C), and another set stored under mineral oil at –80°C

2. 10 Statistical analyses

Statistical analyses of the data obtained for the different variables were carried out using SPSS soft ware package. Correlation coefficient analysis, Multiple regression, and Multiple Linear regression Model were done for the variables including bacterial and fungal population associated with the disease symptoms, air, water, environmental parameters and water characteristics, for all the Stations.

RESULTS

3. Results

3.1 Occurrence of various diseases in hybrid *Dendrobium* in different Stations

Station I

During the course of study, in general the percentage of infected plants in Station varied between 5-26%, with the maximum in the month of July '97.(Fig. 2) Percentage of infected plants were very limited to less than 5% during the months of November'96, April '97 and October '97. During the rainy season (south west monsoon period), *ie* during the months of June to September '97 the percentage of infected plants were relatively more compared to other seasons

In general the hybrid orchids in Station I showed 8 different disease symptoms which included yellowing of leaves, brown dots, bud wilting, root rot, dried stem, brown spot on flowers, stem rot, and leaf rot, during the course of the study. (Fig. 3)

Among the different diseases, yellowing of leaves, brown dots and dead root were observed among the diseased plants throughout the period of study. Among these three diseases, the yellowing and brown dots in leaves were predominant. Whereas, the dead root disease was observed with a lesser population. Other diseases were observed only during certain period of the course of study.

FIGURE-2

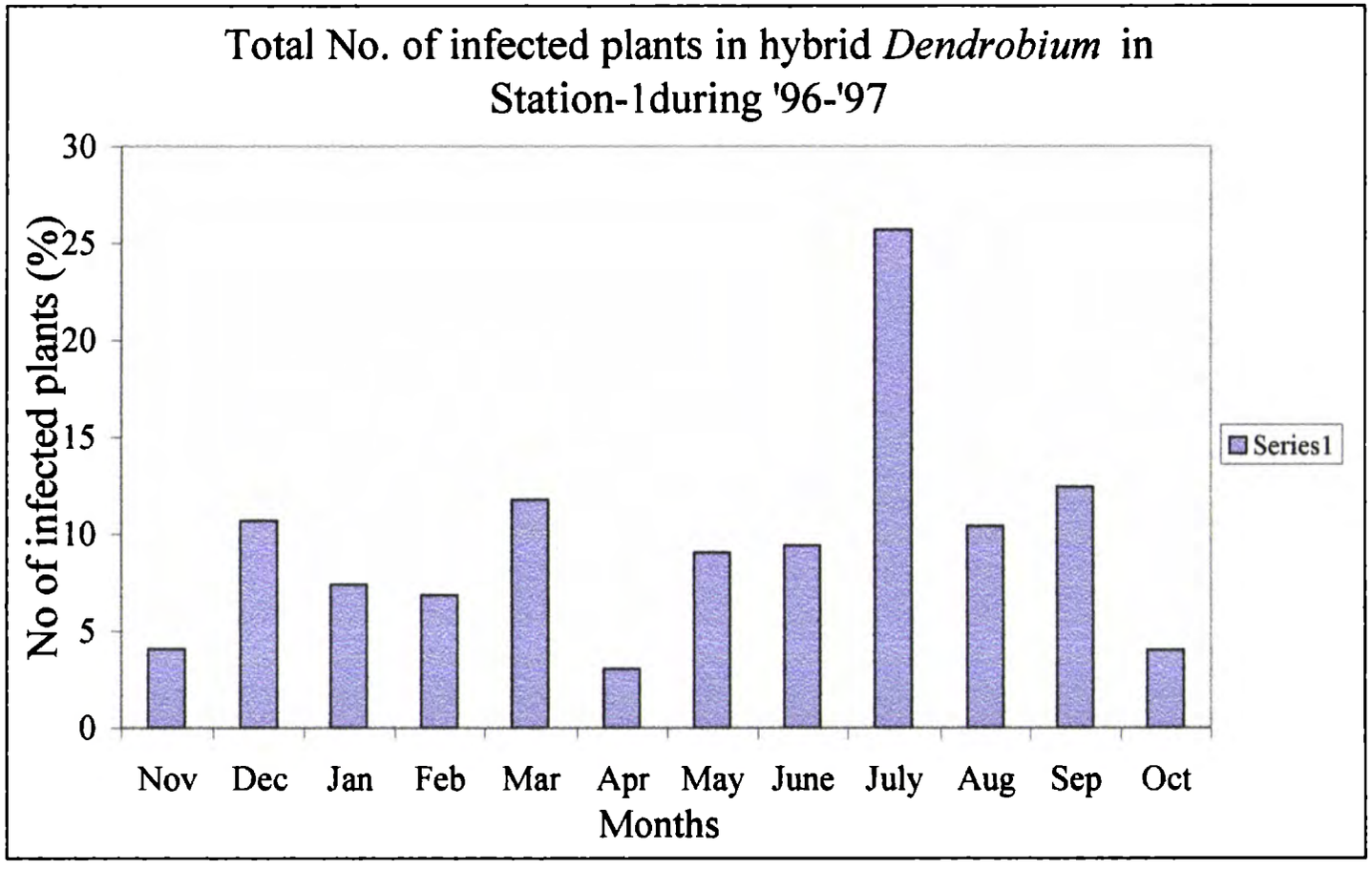
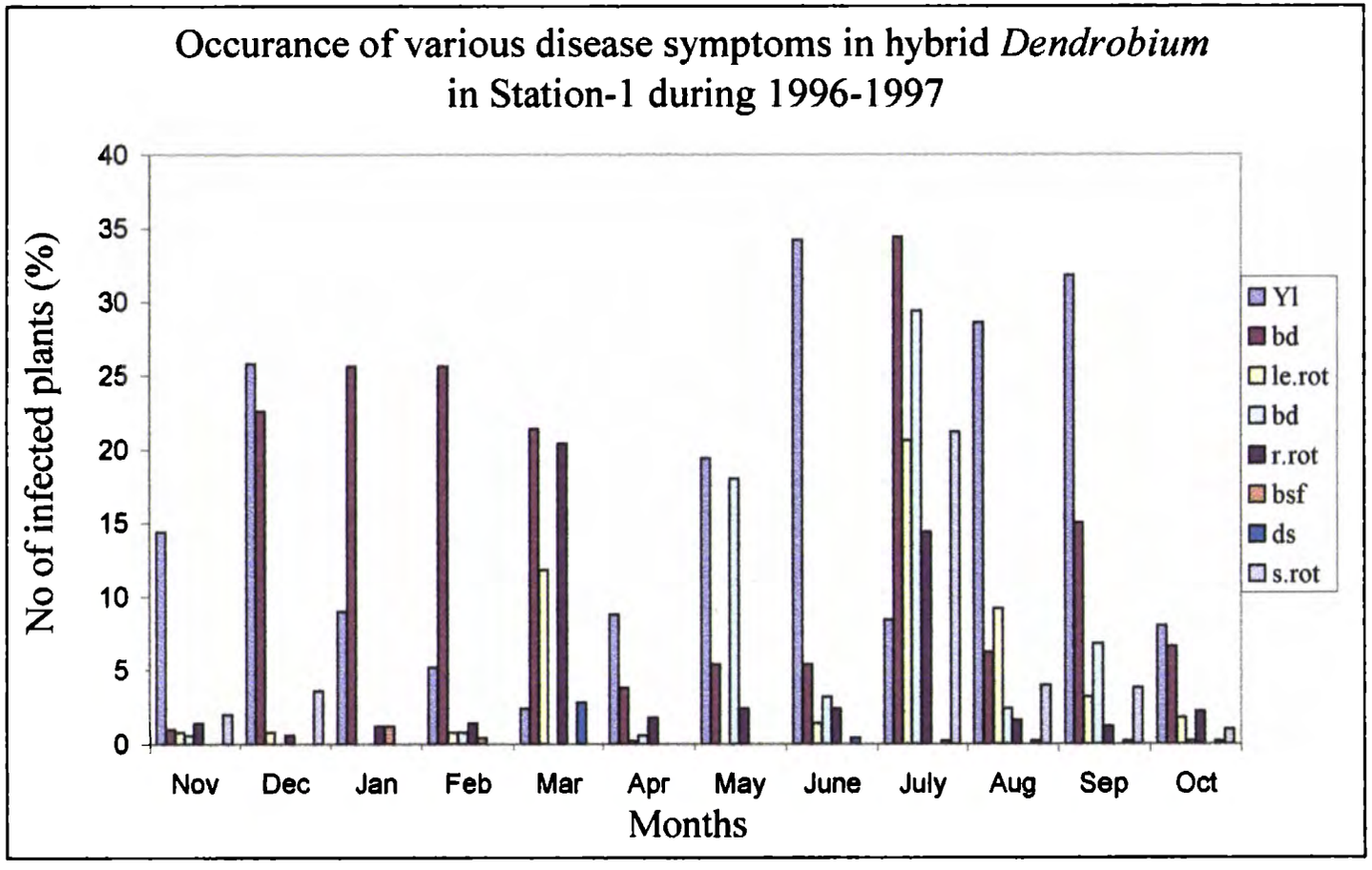

Total No. of infected plants in hybrid *Dendrobium* in Station-1during '96-'97
No of infected plants (%)
30
25
20
15
10
5
0
Nov
Dec
Jan
Feb
Mar
Apr
May
June
July
Aug
Sep
Oct
Months
Series1

FIGURE-3


Occurance of various disease symptoms in hybrid *Dendrobium* in Station-1 during 1996-1997
No of infected plants (%)
40
35
30
25
20
15
10
5
0
Nov
Dec
Jan
Feb
Mar
Apr
May
June
July
Aug
Sep
Oct
Months
Yl
bd
le.rot
bd
r.rot
bsf
ds
s.rot

Yellowing of leaves in *Dendrobium* hybrids was observed throughout the year with a minimum of 2.4 % in March'97 to a maximum of 34.2% in June'97 in the plant population. The yellowing of leaves was predominant during June to September '97 (31.8%), the rainy season in the west coast of India. Month of December '96 also witnessed a high level of yellowing of leaves in the plant population(25.8%).

Brown dots on leaves in *Dendrobium* hybrids was observed throughout the year with a minimum of 1.0 % in November '96 to a maximum of 34.4% in July '97, in the plant population. This disease was predominant during the period from December '96(22.6%) to March'97 (21.4%).

Leaf rot in hybrid *Dendrobium* varied from a minimum of 0.2% in April'97 to a maximum of 20.6% in July'97, in the plant population, followed by March'97 (11.8%) and August'97(9.2%). There was no incidence of this disease during the months of January and May '97.

Bud wilting disease in hybrid *Dendrobium* varied from a maximum of 29.4% of the plant population in July'97 to a minimum of 0.2 % in October'97, followed by May'97 (18%), compared to other months. There was no incidence of this disease condition during the months of December'96, January'97 and March '97.

Root rot diseases in *Dendrobium* hybrids was observed throughout the year with a minimum of 0.6 % in December '96 and a maximum of 20.4% during March, '97 in the plant population. This disease was also relatively high in July,'97 and very less in the population (less than 3.0%) during the rest of the period of study.

Dried stem in hybrid *Dendrobium* was recorded at a very low percentage of 0.2% in the plant population during the months of July'97 to October'97, except in March'97(when a maximum of 2.8 % were recorded) and June'97(04%). There was no incidence of this disease during the months of November'96 to February, and during April - May '97.

Brown spots on flower stalks of hybrid *Dendrobium* was observed in the plant population only during the months of January'97(1.2%) and in February'97(0.4%), and was absent in the population during the remaining period of study.

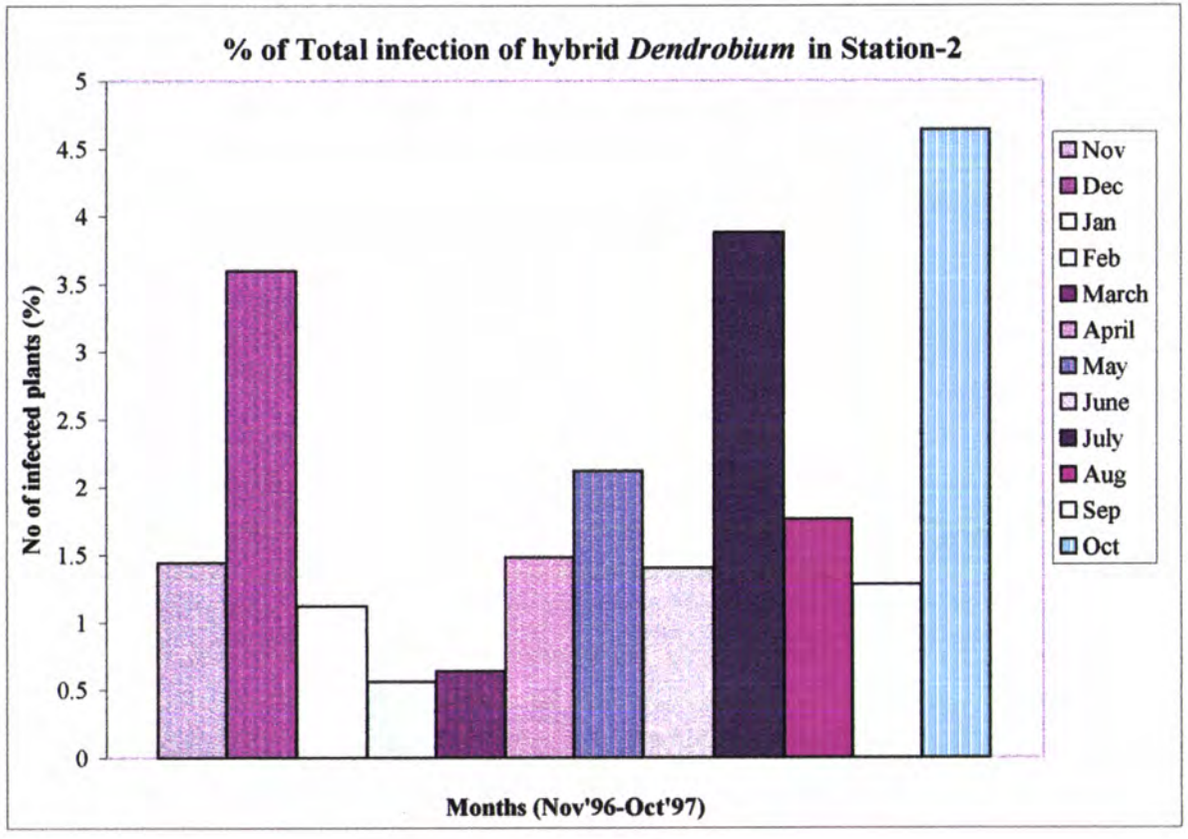
Stem rot disease, in hybrid *Dendrobium*, varied from a maximum of 21.2% in July'97 to a minimum of 1.0% in October'97, compared to other months, when they were less than 4%. Interestingly there was no incidence of this disease during the period from January to June '97.

Station II

Data presented in (Fig-4) indicated that, in general hybrid *Dendrobium* plants in Station II were healthy, since only less than 5% of the total plants were infected by diseases during the entire period of study. Nevertheless, months of December'96, July'97 and October '97 witnessed relatively more percentages of diseases in the plant population compared to other months.

Altogether five types of diseases, including yellowing of leaves, brown dots on leaves, bud wilting , leaf rot and flower drooping, were observed during the period

Figure-4


% of Total infection of hybrid *Dendrobium* in Station-2
No of infected plants (%)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Months (Nov'96-Oct'97)
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sep
Oct

of study. Among these diseases only yellowing of leaves was observed throughout the year.(Fig-5)

Yellowing of leaves in hybrid *Dendrobium*, which was observed throughout the year in the plant population, varied from a minimum of 1.8 % in November'96 and February'97 to a maximum of 15.2% in July'1997. In general, this disease was less than 10% in the population during the rest of the period of study, excluding July'97. Comparatively the incidence of this disease condition was predominant during the period from May'97 to September'97.

Brown dots on leaves in hybrid *Dendrobium* was observed throughout the period from November '96 to July'97 continuously, with a maximum of 5.4% in November 96 and a minimum of 0.6% in July 1997 in the population. This disease was absent in the population during the months of August'97 to October'97. From the results it is inferred that this disease condition is not associated to rainy season, as it was recorded at low levels (0.6-0.8%) when present during June and July'97.

Whereas, leaf rot, Bud wilting and Flower drooping in hybrid *Dendrobium* were incurred only during the months of December'96(9.4%), July'97(3.6%) and October'97(20.4%) respectively, and not incurred in the plant population during the rest of the period of study.

Station III

Results presented in (Fig-6) indicated that, in general, hybrid *Dendrobium* plants were healthy in Station III, since only less than 4% of the total plants were

Figure-5

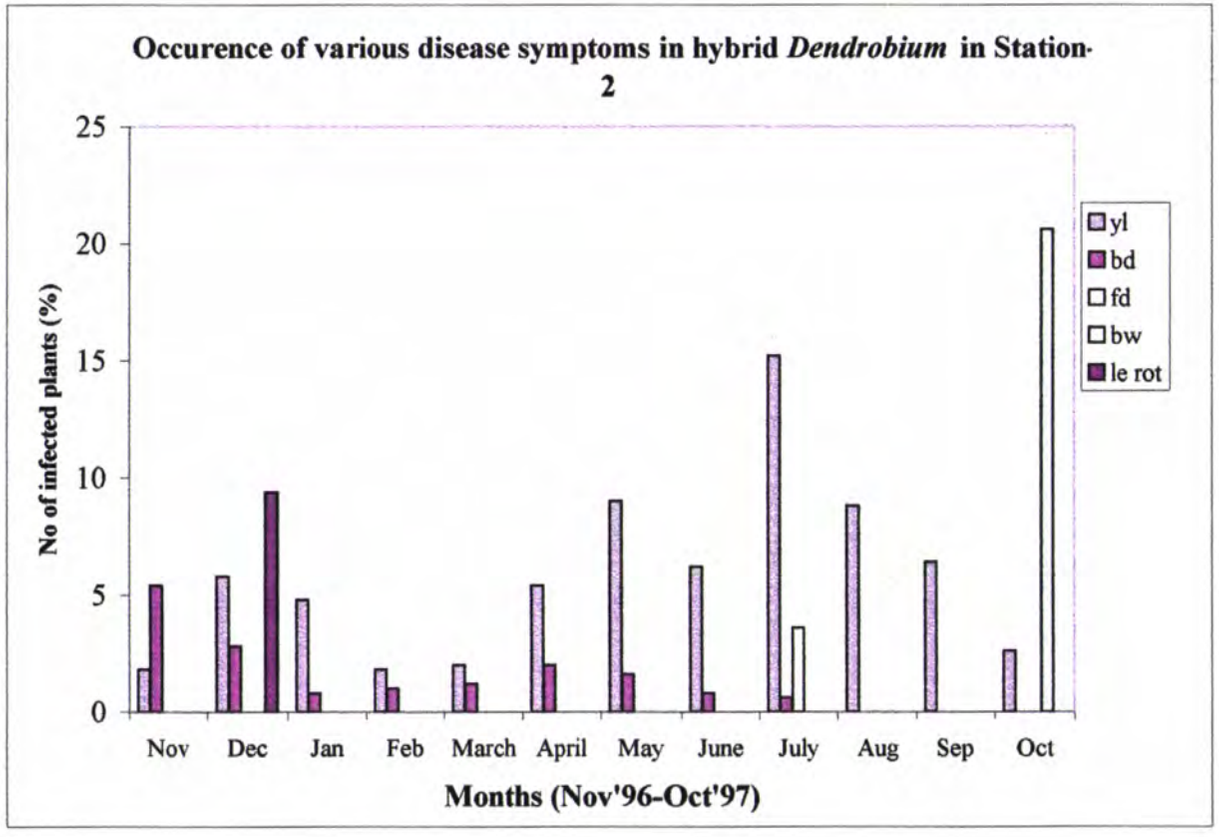
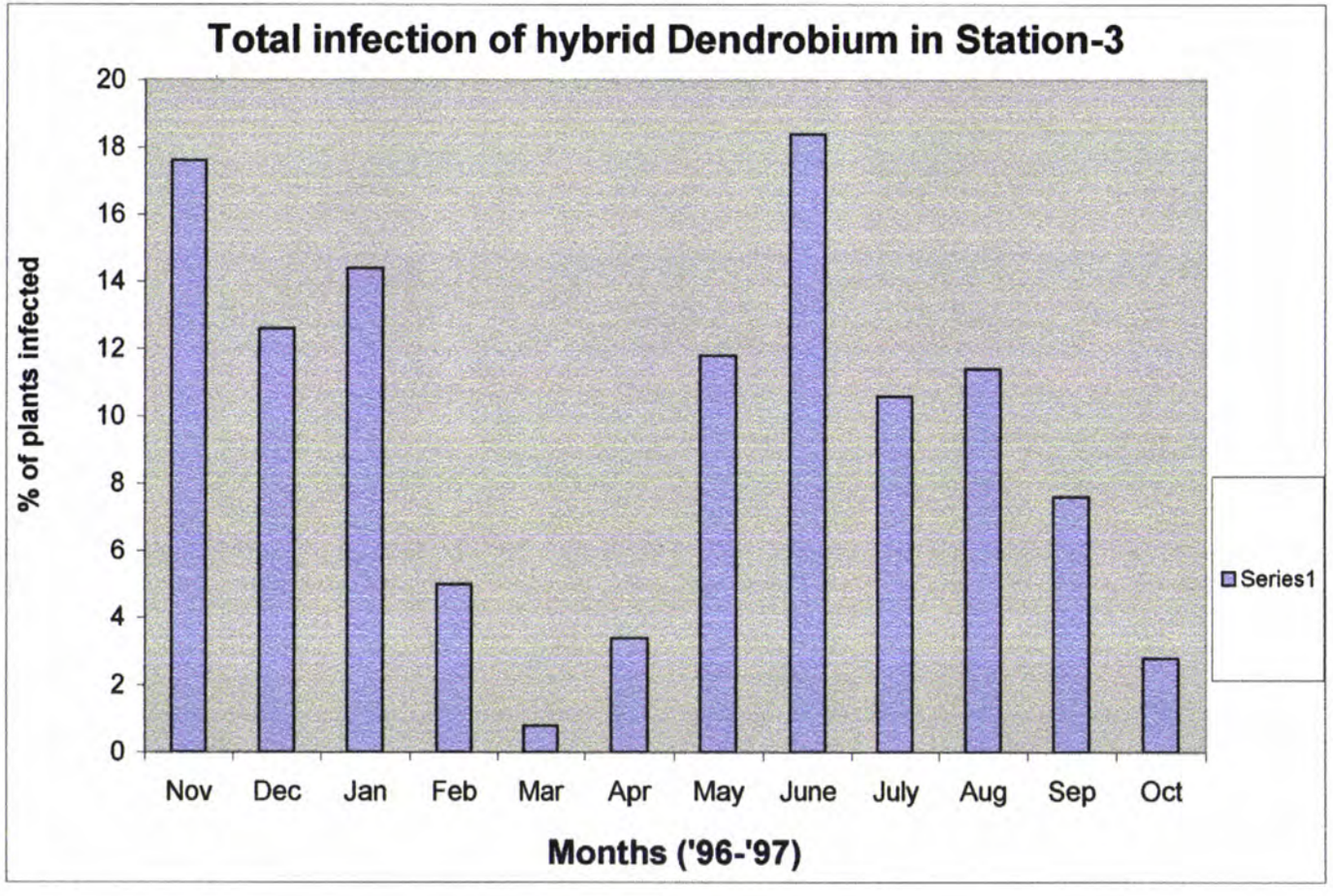

Occurence of various disease symptoms in hybrid *Dendrobium* in Station-2
No of infected plants (%)
25
20
15
10
5
0
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sep
Oct
Months (Nov'96-Oct'97)
yl
bd
fd
bw
le rot

Figure - 6


Total infection of hybrid Dendrobium in Station-3
% of plants infected
20
18
16
14
12
10
8
6
4
2
0
Nov
Dec
Jan
Feb
Mar
Apr
May
June
July
Aug
Sep
Oct
Months ('96-'97)
Series1

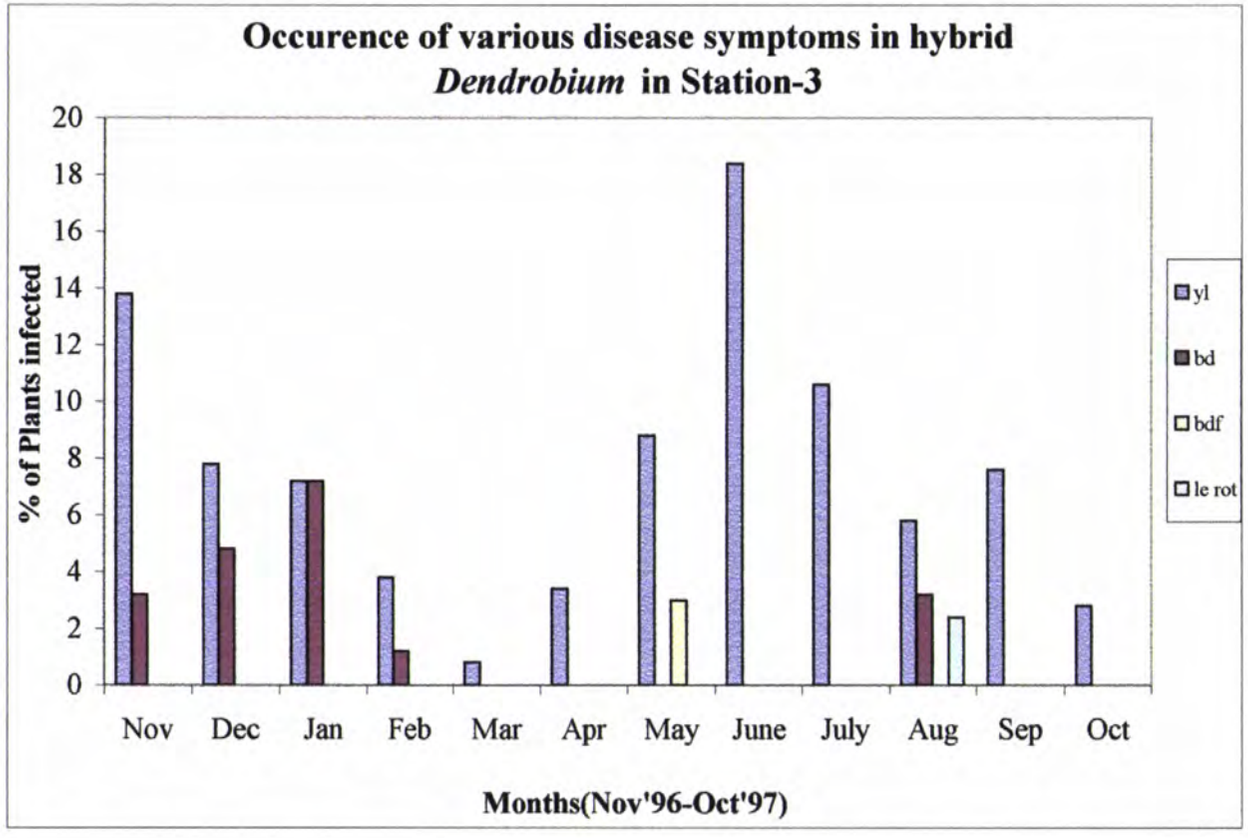
infected by diseases during the entire period of study. More plants were diseased during November'96 and June'97 compared to February'97, March'97 and October '97 which witnessed relatively less percentage of diseased plants in the population.

Altogether four types of diseases, including yellowing of leaves, brown dot on leaves, brown spots on flower and leaf rot, were observed in hybrid *Dendrobium* in Station III during the period of study. Among these diseases only yellowing of leaves was observed throughout the year (Fig-7)

Yellowing of leaves, which was recorded throughout the year, varied from a minimum of 0.8 % in March'97 to a maximum of 18.4% in June'1997 in the plant population. In general, this disease showed a declining trend in the population from November '96(13.8%) to a minimum(0.8 %) in March'97, and later increased gradually to a maximum(18.4%) in June'87. However, again there was a decline in the number of plants during July'97 to October'97(2.8%). This kind of observation was not incurred in other Stations during the period of study.

Brown dots on leaves in hybrid *Dendrobium* was observed only during 5 months, from November '96 to February'97 continuously, with a maximum of 7.2% in January'97 and a minimum of 1.2% in February'97 in the plant population. Nevertheless months of November'96, December'96(3.2% and 4.8% respectively) recorded this disease in a progressive manner leading to a maximum in January'97. This disease was absent in the population during the months of March'97 to July'97 and during September and October'97.

FIGURE - 7


Occurence of various disease symptoms in hybrid
Dendrobium in Station-3
% of Plants infected
20
18
16
14
12
10
8
6
4
2
0
Nov
Dec
Jan
Feb
Mar
Apr
May
June
July
Aug
Sep
Oct
Months(Nov'96-Oct'97)
yl
bd
bdf
le rot

Whereas, Brown spots on flower and leaf rot, in hybrid *Dendrobium* plant population, were incurred only during the months of March (0.8%), May'97(3.0%) and August'97(2.4%) respectively, and were not incurred in the population during the rest of the period of study.

Station IV

Station IV, in general, showed very less number of infected (less than 1.5%) hybrid *Dendrobium* plants during the entire period of study (Fig-8). Comparatively more plants were infected during February'97 compared to other months.

Altogether three types of diseases, including yellowing of leaves, brown dot on leaves, and leaf rot, in hybrid *Dendrobium* plants, were observed during the period of study. Among these diseases only yellowing of leaves was observed throughout the year.(Fig-9)

Yellowing of leaves was observed in 0.8% - 3.4% of plants, with a minimum of 0.8 % during December'96 and April'97, and a maximum during January'1997 in the plant population. In general, this disease showed a declining trend in the population from January'97(3.4%) to a minimum during April'97(0.8%) and later increased gradually to a maximum in August'97(2.8%).

Brown dot on leaves in hybrid *Dendrobium* was observed only during 3 months, from December '96 to February'97 continuously, with a maximum of 2.2% in December '96 and a minimum of 0.8% in January and February'97, in the plant population.

FIGURE-8

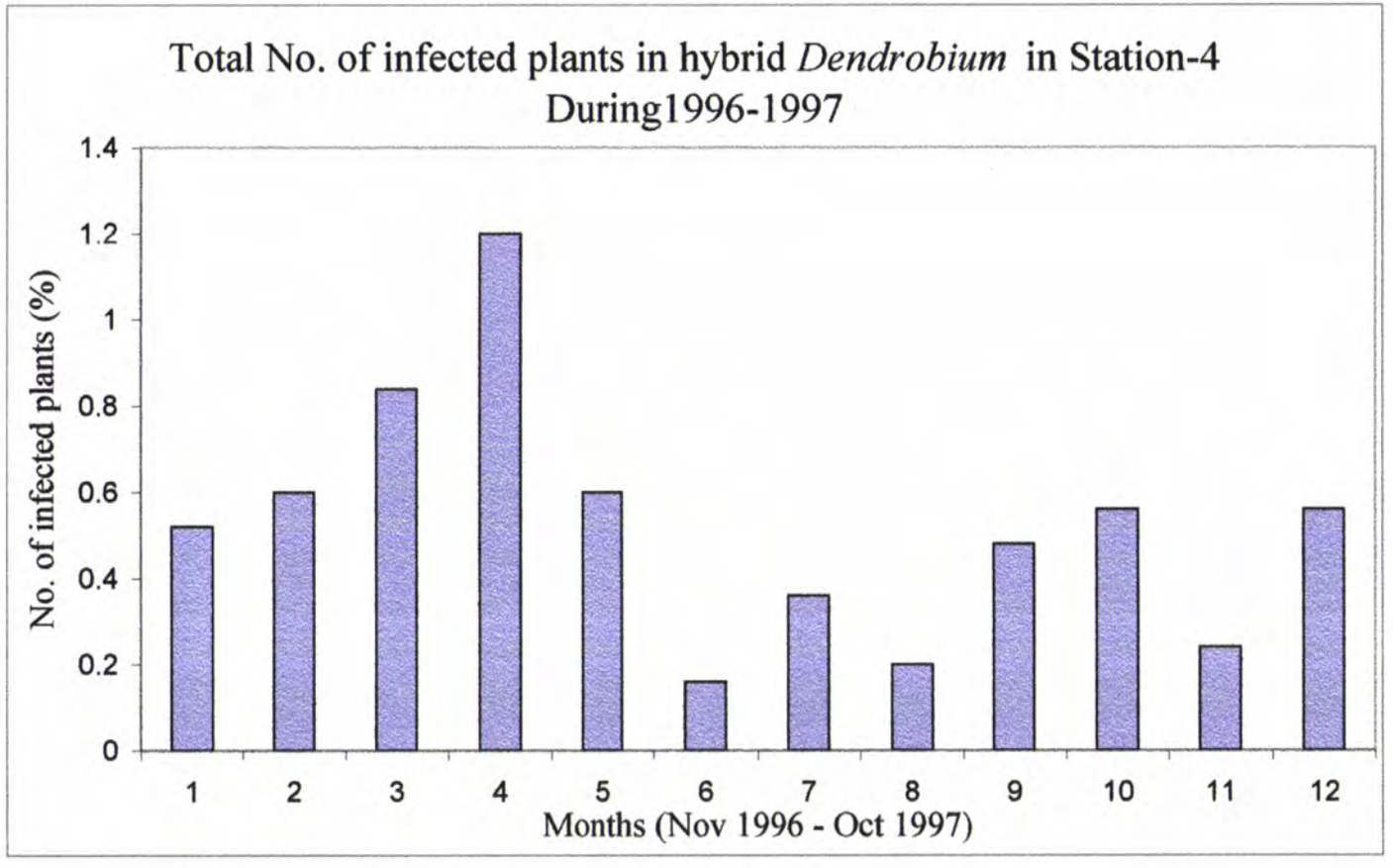
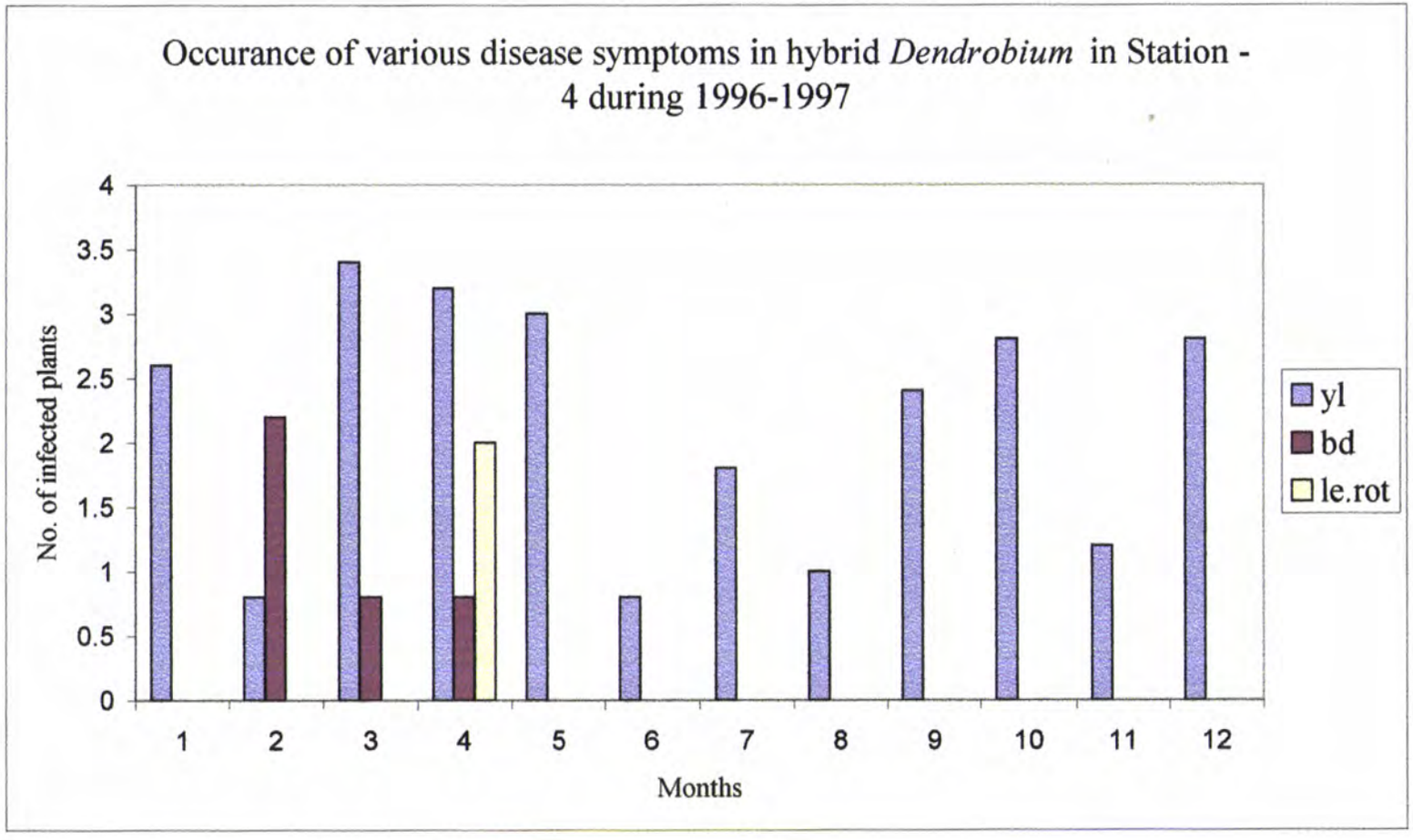

Total No. of infected plants in hybrid *Dendrobium* in Station-4 During1996-1997
No. of infected plants (%)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
7
8
9
10
11
12
Months (Nov 1996 - Oct 1997)

FIGURE-9


Occurance of various disease symptoms in hybrid *Dendrobium* in Station - 4 during 1996-1997
No. of infected plants
4
3.5
3
2.5
2
1.5
1
0.5
0
1
2
3
4
5
6
7
8
9
10
11
12
Months
yl
bd
le.rot

Whereas, diseased conditions of leaf rot in hybrid *Dendrobium* was incurred only during the months of February'97 (2.0%), and were not incurred in the population during the rest of the period of study.

Station V

Station V, in general, showed very less number of infected (less than 1.5%) hybrid *Dendrobium* plants during the entire period of study (Fig-10). Comparatively more plants were infected during January'97, when compared to other months.

Altogether only two types of disease, including yellowing of leaves, and brown dot on leaves, in hybrid *Dendrobium* plants, were observed during the period of study. Among these diseases only yellowing of leaves was observed throughout the year.(Fig-11)

Yellowing of leaves was observed with a maximum in January'1997(3.2 %) and a minimum in February'97(1.0 %) in the plant population. Further same level (3.0%) of diseased plants were recorded during November and December'96, and July and August'97. Comparatively yellowing of leaves was incurred in lesser population during the months of February, March and June '97.

Brown dot on leaves in hybrid *Dendrobium* was observed only during 4 months, from November '96 to February'97 continuously, with a maximum in January'97(3.2%) and a minimum in November'96(0.4 %). Interestingly this disease showed a progressive increase in the population during November'96 to January'97

FIGURE-10

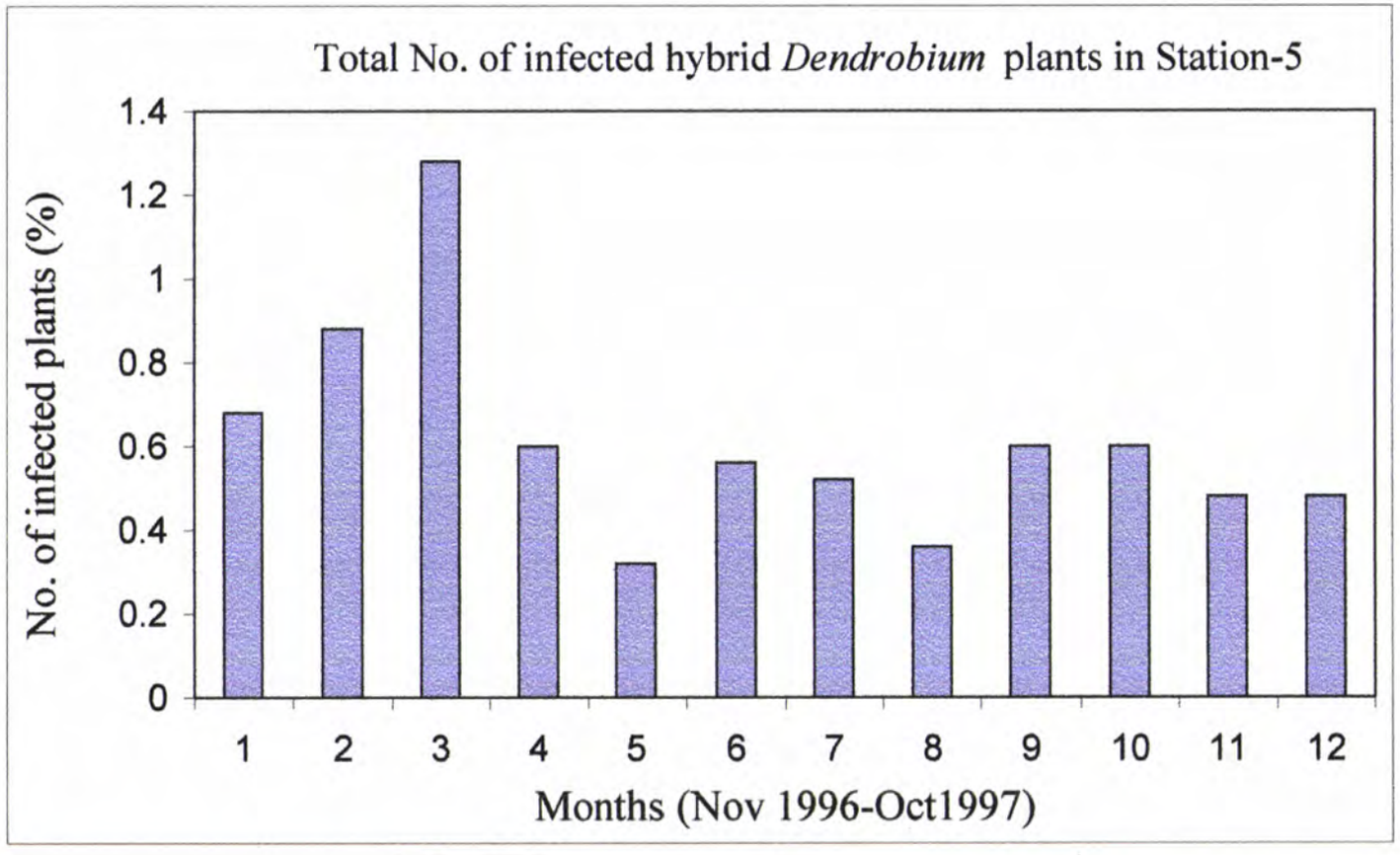
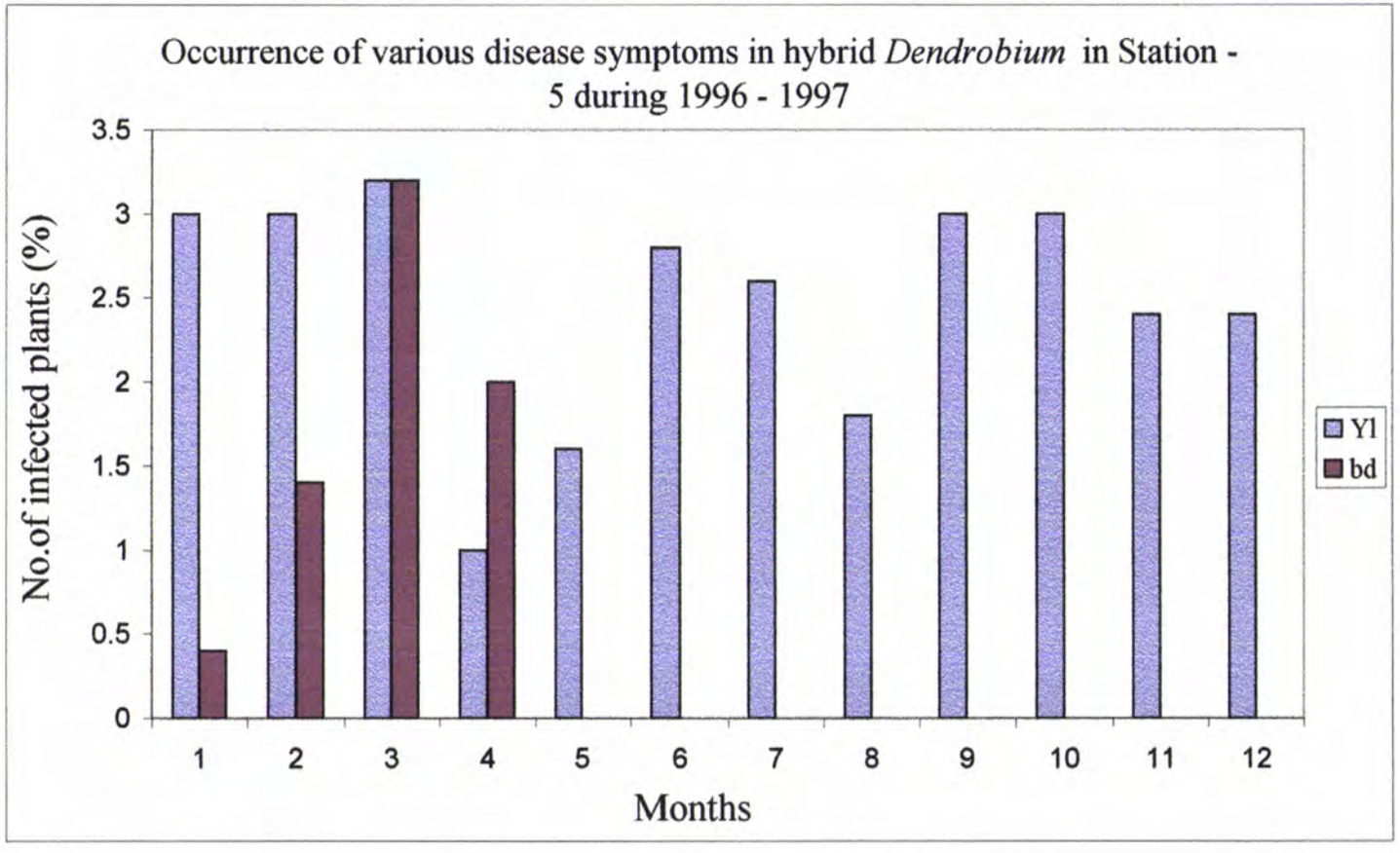

Total No. of infected hybrid *Dendrobium* plants in Station-5
No. of infected plants (%)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
7
8
9
10
11
12
Months (Nov 1996-Oct1997)

Figure- 11


Occurrence of various disease symptoms in hybrid *Dendrobium* in Station - 5 during 1996 - 1997
No.of infected plants (%)
3.5
3
2.5
2
1.5
1
0.5
0
1
2
3
4
5
6
7
8
9
10
11
12
Months
Yl
bd

Comparison of percent infection in hybrid *Dendrobium* plant population in the five different Stations

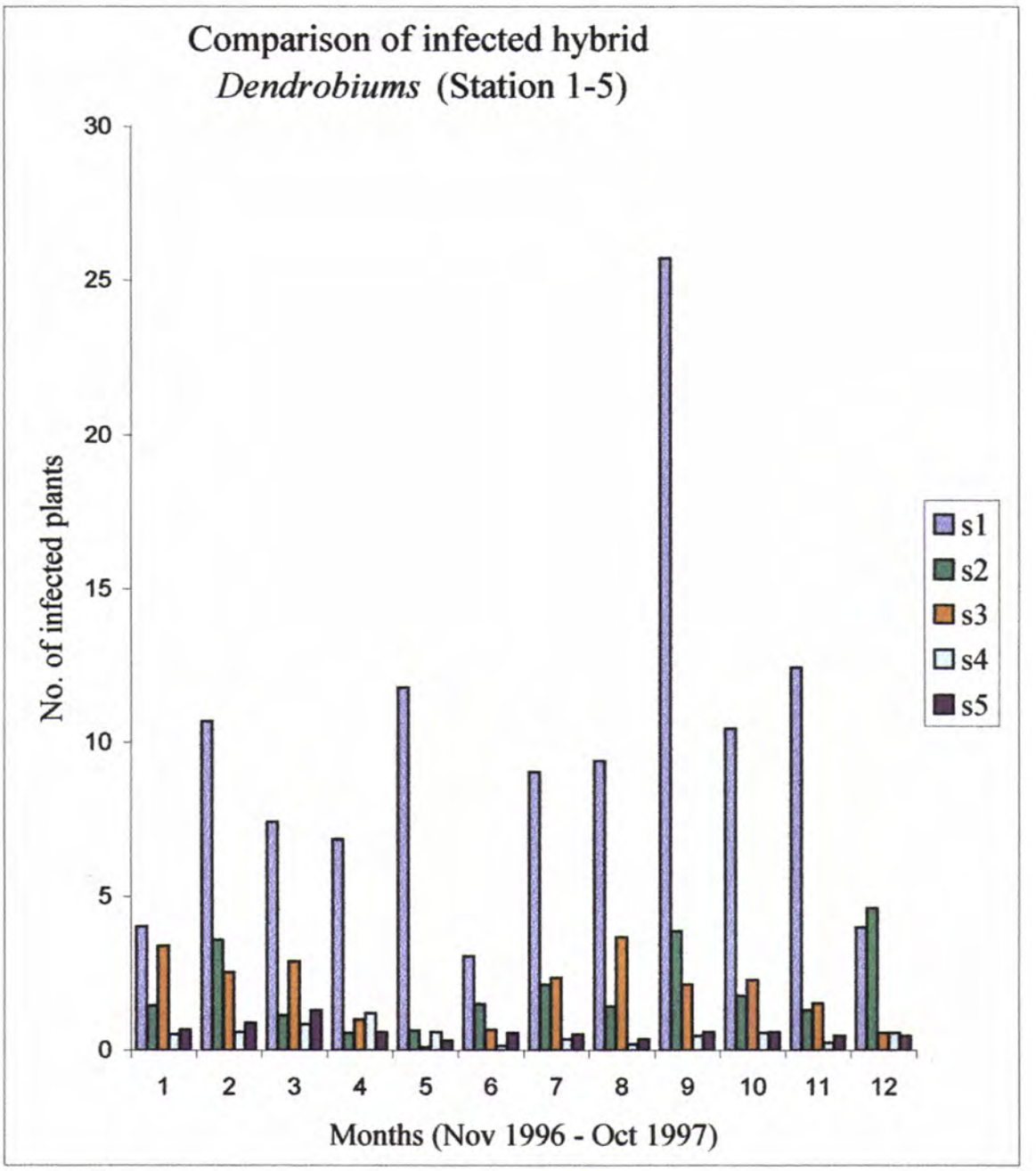
Results presented in (Fig-12) very clearly testify that, Kanyakumari District recorded very less percentage of diseased plants in the population during the period of study, compared to Ernakulam District which recorded relatively a higher percentage of diseased plants.

Among the five different stations, selected for monitoring the occurrence of various diseases in hybrid *Dendrobium* plants, Station I , located in Ernakulam District, showed comparatively larger population of infected plants which varied from 3.04%(April'97) to 25.72%(July'97). Station IV, located in Kanyakumari District of Tamil nadu, showed comparatively lesser population of infected plants which varied from 0.16%(April'97) to 1.2% (February'97) during the period of study.

Among the three stations selected in Ernakulam District, Station III recorded very less diseased population (0.1%(March'97) to 3.68% (June'97)). Nevertheless, Station II located in Ernakulam District, also recorded very less diseased population (0.56% (February'97) to 4.64 % (October'97)).

Both stations selected in Nagercoil of Kanyakumari District of Tamil Nadu showed only very less percentage of diseased plants. Even Station V, located in Kanyakumari District of TamilNadu, showed comparatively lesser population of infected plants which varied from 0.36% (June'97 and October'97) to 1.28% (January'97) during the period of study.

Figure -12


Comparison of infected hybrid
Dendrobiums (Station 1-5)
No. of infected plants
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
Months (Nov 1996 - Oct 1997)
s1
s2
s3
s4
s5

3.2 Environmental characteristics of the Study area

3.2.1 Temperature of Atmosphere

Maximum Temperature

Maximum Temperature of Atmosphere in the 5 stations did not show much variation during the period of study (Table-2) In general temperature varied between 28.0°C and 33.8° for the stations and particularly from 29.2° C to 33.4° C for Station I; from 29.0 ° C to 33.6° C in Station II; from 29.0 ° C to 33.8° C in Station III; from 28.0 ° C to 32.0 ° C in Station IV; and from 28.0 ° C to 31.7° C in Station V.

Higher temperatures around 33.0° C was observed with the stations located at Ernakulam District during summer compared to Kanyakumari Disrtrict

Minimum Temperature

Minimum Temperature of Atmosphere recorded in the 5 different stations showed variation during the period of study (Table-3) In general temperature varied between 21.0°C and 27.6° C for the stations and particularly from 24.2° C to 26.0° C for station I; from 23.2° C to 26.0° C in Station II; from 22.2° C to 24.6° C in Station III; from 21.5° C to 27.6° C in Station IV; and from 21.0 ° C to 27.1° C in Station V

Higher temperatures around 27.6° C was observed with the stations located at Kanyakumari Disrtrict during January and February '97 compared with Ernakulam District, which recorded very low temperatures around 24.0° C. Variations in the minimum temperatures were high in Kanyakumari District compared to Ernakulam District during the course of study.

Table 2.. Atmospheric Temperature (maximum)recorded at the five stations of the study area during the period of study (expressed as T°C)

Month	Station I	Station II	Station III	Station IV	Station V
November'96	31.6	30.4	30.6	29.3	29.3
December'96	30.0	30.0	30.0	28.0	28.0
January'97	31.8	31.0	31.0	29.6	30.0
February'97	31.6	31.6	33.8	30.1	30.1
March'97	33.4	33.4	31.6	32.0	31.7
April'97	33.2	33.6	33.6	31.0	29.0
May'97	33.0	33.0	33.0	32.0	31.2
June'97	32.0	31.8	31.8	32.0	29.1
July'97	29.4	29.4	29.4	29.0	29.4
August'97	29.2	29.0	29.0	29.0	29.0
September'97	29.4	29.8	29.8	29.0	28.5
October'97	31.8	31.8	31.8	29.5	29.0

Table 3.. **Atmospheric Temperature (minimum)recorded at the five stations of the study area during the period of study (expressed as T°C)**

Month	Station I	Station II	Station III	Station IV	Station V
November'96	24.8	23.2	23.6	27.0	27.1
December'96	23.8	24.0	24.0	26.2	26.2
January'97	23.8	23.4	23.4	27.1	27.1
February'97	24.0	24.0	23.8	27.6	27.6
March'97	25.2	25.2	23.4	26.8	26.8
April'97	26.0	26.0	24.0	24.2	24.2
May'97	25.5	25.2	25.2	23.1	23.1
June'97	25.0	25.2	25.2	25.4	25.4
July'97	24.2	23.6	23.6	25.0	25.0
August'97	24.8	23.6	23.6	25.0	25.1
September'97	24.2	24.6	24.6	21.6	22.1
October'97	24.2	24.2	22.2	21.5	21.0

3.2.2 Humidity

Humidity of Atmosphere in the 5 different stations did not show much variation during the period of study (Table-4) In general humidity varied between 71% and 96% for the stations and particularly from 74-91% for station I; from 74-96% in Station II; from 74-96% in Station III; from 71-90% in Station IV; and from 71-90 % in Station V.

Higher levels of humidity around 90% was observed with all the stations during July'97. Whereas, all the three stations of Ernakulam District recorded humidity levels above 90% during December'96 and August'97 also. Stations II and III recorded highest level of 96% humidity during August'97, while stations of Kanayakumari district recorded only 90% humidity as maximum compared to stations of Ernakulam

Similarly lower levels of humidity (71% and 74% respectively for Kanyakumari and Ernakulam districts) were observed with all the stations during April'97.

3.2.3 Rainfall

Rainfall at the 5 different stations showed much variation during the period of study (Table-5). In general rainfall varied between 4 - 190cm in Ernaakulam District and between 4 - 105 cm in Kanyakumari District during the period of study, and particularly between 4 -190cm in Station I; between 9 –168 cm in Station II; between 9-168 cm in Station III; between 4-105.7 cm in Station IV; and between 4-105.7 cm in Station V.

Table 4. Humidity of Atmosphere recorded at the five stations of the study area during the period of study (expressed as %)

Month	Station I	Station II	Station III	Station IV	Station V
November'96	82.0	83.0	85.0	85.5	85.5
December'96	91.0	91.0	91.0	78.0	78.0
January'97	77.0	87.0	87.0	73.0	73.0
February'97	87.0	87.0	81.0	79.0	79.3
March'97	81.0	81.0	87.0	73.3	73.3
April'97	74.0	74.0	74.0	71.0	71.0
May'97	82.0	82.0	82.0	79.0	79.0
June'97	88.0	87.0	87.0	86.0	86.0
July'97	92.0	94.0	94.0	90.0	90.0
August'97	92.0	96.0	96.0	82.4	82.0
September'97	93.0	84.0	84.0	84.5	84.5
October'97	83.0	83.0	83.0	85.5	85.7

Table .5. Rainfall recorded at the five stations of the study area during the period of study (expressed in cm)

Month	Station I	Station II	Station III	Station IV	Station V
November'96	4.0	11.0	11.0	34.0	34.0
December'96	35.0	35.0	35.0	29.0	29.0
January'97	-	-	-	4.0	4.0
February'97	-	-	-	-	-
March'97	31.0	31.0	31.0	-	-
April'97	13.0	13.0	13.0	9.2	9.2
May'97	9.0	9.0	9.0	16.5	16.5
June'97	40.0	30.0	30.0	32.5	32.5
July'97	190.0	168.0	168.0	10.5	10.5
August'97	10.0	111.0	111.0	66.0	66.0
September'97	159.0	49.0	49.0	105.7	105.7
October'97	34.0	34.0	34.0	54.5	54.5

High levels of rainfall around 168cm was observed during July'97in the stations of Ernakulam District, and during September'97 in all the stations of Kanayakumari district. There was no rainfall during the months of January'97 and February'97 in Ernakulam District and during February and March'97 in Kanayakumari district. November'96, April and May'97 recorded poor rainfall in all the stations.

3.3 Charateristics of Water used in the Orchid farms

3.3.1 Water Temperature

Water samples used in the 5 stations did not show much variation in temperature during the period of study (Table-6) In general, temperature varied between 24.0°C and 31.0 °C for the stations and particularly from 26.5 °C to 28.0 °C for station I; from 26.0 °C to 31.0 °C in Station II; from 24.0 °C to 29.0 °C in Station III; from 26.0 °C to 29.0 °C in Station IV; and from 26..5 °C to 28.0 °C in Station V.

3.3.2 pH

Water samples used in the 5 stations were slightly alkaline in nature during certain months of study, However there was much variation in pH during the period of study (Table-7) and varied in general, between 7.30 and 8.71 for the stations and particularly from 7.30 (July'97) to 8.63(November'96) for Station I; from 7.50(June'97) - 8.71(August'97) in Station II; from 7.82(May'97)-8.71(August'97) in

Table .6. Water temperature recorded at the five stations of the study area during the period of study (expressed as T°C)

Month	Station I	Station II	Station III	Station IV	Station V
November'96	26.5	30.0	28.0	27.0	26.5
December'96	26.5	30.0	27.0	26.0	26.5
January'97	28.0	27.0	29.0	27.0	28.0
February'97	27.5	28.0	25.0	28.0	27.5
March'97	28.0	29.5	27.0	29.0	28.0
April'97	26.5	31.0	27.5	26.5	26.5
May'97	27.0	28.0	29.0	29.0	27.0
June'97	26.5	27.0	26.0	28.0	26.5
July'97	28.0	26.0	24.0	28.0	28.0
August'97	26.5	27.5	24.5	28.5	26.5
September'97	27.0	28.0	26.0	28.0	27.0
October'97	27.5	26.5	26.5	27.5	27.5

Table.7 .. pH of water of water recorded at the five stations of the study area during the period of study

Month	Station I	Station II	Station III	Station IV	Station V
November'96	8.63	8.61	7.92	8.03	8.14
December'96	8.60	8.57	8.13	7.74	7.90
January'97	8.62	8.61	7.93	7.75	7.50
February'97	8.53	8.33	8.20	8.70	7.82
March'97	8.60	8.40	8.10	8.27	8.11
April'97	8.60	8.59	7.98	8.21	7.50
May'97	8.56	8.60	7.82	7.92	7.50
June'97	8.50	7.50	7.91	8.35	7.65
July'97	7.30	7.71	7.78	7.79	8.02
August'97	8.59	8.71	8.71	8.11	7.93
September'97	8.60	8.61	7.83	7.92	7.70
October'97	8.20	8.60	8.58	8.06	7.90

Station III; from 7.74(December'96) - 8.7(February'97) in Station IV; and from 7.5 (April'97) - 8.14(November,96) in Station V.

3.3.3 Total Alkalinity

Water samples used in the 5 stations showed variation in terms of total alkalinity during the course of study in all the stations (Table-8). In general, total alkalinity varied between 32 ppm and 376ppm for the stations and particularly from 32 ppm(July'97) –376 ppm(May'97) for station I; from 56 ppm(July'97)-184 ppm(August'97)in Station II; from 40 ppm(July'97)-232 ppm(December'96) in Station III; from 72 ppm(December'96) - 344 ppm(February'97) in Station IV; and from 96 ppm (August'97) - 200 ppm (April97) in Station V.

Lowest and highest levels of alkalinity were recorded in Station I during July'97 and May'97 respectively, compared to other stations. Further Total alkalinity of water was high in Station I and Stations IV and V showed relatively lesser levels of total alkalinity, except for the months of February'97 (344ppm) and April'97(200ppm) at Station IV, and for the month of April'97(200 ppm) at Station V. The total alkalinity of water at Station III showed inconsistency and recorded fluctuations unlike in other Stations.

3.3.4 Hardness

Water samples used in the different 5 stations showed variation in terms of hardness during the course of study in all the stations(Table-9). In general, hardness varied between 10 ppm and 140 ppm for the stations and particularly from 10

Table 8.. **Total Alakalinity of water recorded at the five stations of the study area during the period of study (expressed as ppm)**

Month	Station I	Station H	Station III	Station IV	Station V
November'96	312	152	64	128	144
December'96	280	144	232	72	120
January'97	280	168	48	88	112
February'97	368	136	224	344	112
March'97	288	130	160	112	160
April'97	312	136	60	200	200
May'97	376	136	40	128	120
June'97	320	128	56	112	136
July'97	32	56	40	104	152
August'97	288	184	184	112	96
September'97	288	176	48	100	136
October'97	168	144	136	120	160

Table .9. Hardness of water recorded at the five stations of the study area during the period of study (expressed as ppm)

Month	Station I	Station II	Station III	Station IV	Station V
November'96	66	110	22.5	50	30
December'96	66	110	140	40	30
January'97	62	110	60	40	20
February'97	62	100	120	80	20
March'97	60	90	35	40	20
April'97	60	110	28	70	70
May'97	60	100	10	50	40
June'97	50	20	20	50	50
July'97	10	20	30	30	30
August'97	60	110	120	30	20
September'97	60	140	25	80	50
October'97	54	110	120	30	60

ppm(July'97)to 66 ppm(November and December'96) for station I; from 20 ppm(June and July'97) to 140 ppm(September'97) in Station II; from 10 ppm(May'97) to 140 ppm(December'96) in Station III; from 30 ppm(July, August and October'97) to 80 ppm (February and September'97) in Station IV; and from 20 ppm(January-March'97 and August'97) to 60 ppm(October'97) in Station V.

Water samples of Station II recorded higher hardness compared to other stations. Whereas, water samples of Station V recorded relatively a lesser level of hardness during the period of study. The hardness of water at Station III showed inconsistency and recorded wide fluctuations unlike in other Stations. During summer months, while Station II showed higher level of hardness Station III showed relatively very less level of hardness.

3.4 Microbial population associated with the diseased hybrid *Dendrobium* plants in different Stations

3.4.1 Bacterial population associated with air, water, fresh and diseased hybrid *Dendrobium* plants in different Stations

3.4.1.1 Bacterial population of atmospheric microbial flora

Bacterial population of atmospheric microbial flora at the 5 stations of the study area showed comparatively very less population (Table-10). In general, the population varied from a minimum of 3 CFU in Station I (April and May'97) to a maximum of 82 CFU in Station II (June'97). Particularly the bacterial population varied from 3 CFU (April and May'97) to 19 CFU(October'97) in Station I., from 6

Table .10 Bacterial population in the atmosphere(air) at the five stations of the study area during the period of study (expressed in cm)

Month	Station I	Station II	Station III	Station IV	Station V
November'96	16	20	16	52	18
December'96	6	12	19	40	12
January'97	9	10	6	0	32
February'97	8	14	13	26	12
March'97	5	24	21	36	21
April'97	3	16	13	22	17
May'97	3	6	11	18	15
June'97	9	82	4	32	6
July'97	16	61	21	43	22
August'97	18	12	14	53	12
September'97	8	10	27	42	6
October'97	19	46	34	43	16

CFU (May'97) to 82 CFU(June'97) in Station II; from 4CFU(June'97) to 34 CFU(October'97) in Station III; from 18 CFU (May'97) to 53 CFU(August'97) in Station IV; and from 6 CFU (June and September'97) to 32 CFU(January'97) in Station V. In general the bacterial population of atmospheric flora was comparatively high in Stations IV and II and lesser in Station I, III and V.

3.4.1.2 Bacterial population of water

Bacterial population of water used for irrigation at the 5 stations of the study area. In general the population varied from a minimum of 6 CFU/ml in Station V (December'96) to a maximum of $7.6x10^4$ CFU/ml in Station IV (May'97) (Table-11). Particularly the bacterial population varied from 30 CFU/ml (May'97) to 3.12 x 10^3CFU/ml (March'97) in Station I; from 10 CFU/ml (January'97) to 1.07 x 10^3CFU/ml (November'96) in Station II; from 110 CFU/ml (March'97) to $5.6x10^4$ CFU/ml (June'97) in Station III; from 40 CFU/ml (June'97) to $7.6x10^4$ CFU/ml (August'97) in Station IV; and from 6 CFU (December'96) to 3.0 x 10^3 CFU/ml (March'97) in Station V. Interestingly no colony appeared on the plates for the samples of Station I, analyzed for the months of January, February and June'97 and for month of May'97 for the Station II.

3.4.1.3 Bacterial population of fresh leaves

Bacterial population of fresh leaves of the hybrid *Dendrobium* at the 5 stations of the study area showed wide variation from a minimum of 4 CFU/cm2 in Station V

Table11 .. Bacterial population in the water used at the five stations of the study area during the period of study (expressed in cm)

Month	Station I	Station II	Station III	Station IV	Station V
November'96	80	1070	16000	760	76
December'96	200	80	680	1110	6
January'97	0	10	24000	150	8
February'97	0	120	160	5000	11
March'97	3120	60	110	5000	3000
April'97	1900	170	320	640	16
May'97	30	0	570	76000	11
June'97	0	160	56000	40	14
July'97	540	270	210	90	88
August'97	170	420	230	100	14
September'97	200	520	180	160	6
October'97	120	370	260	760	18

(January'97) to a maximum of 4.0 $x10^7$ CFU/cm2 in Station IV (October'97) (Table-12).

The bacterial population varied from 60 CFU/cm2 (November'96) to 3.9 x 10^5CFU/cm2 (August'97) in Station I; from 120 CFU (December'96) to 8.2 x 10^4CFU/cm2 (august'97) in Station II; from 820 CFU/cm2 (January'97) to 1.12 $x10^5$ CFU/cm2 (May'97) in Station III; from 600 CFU/cm2 (July'97) to $4.0x10^7$ CFU/cm2 (October'97) in Station IV and from 4 CFU/cm2 (January'97) to 7.5 x 10^4CFU/cm2 (April'97) in Station V.

Among the 5 Stations, Station IV recorded comparatively higher levels of bacterial population associated with fresh leaves in the range of 1.2 X 10^6 to $4.0x10^7$CFU/cm2 during the months of March, April and October '97. On the other hand Station V recorded low level of bacterial population. Of the Staions of Ernakulam Distriat Station I recorded relatively high levels of bacterial population.

3.4.1.4. Bacterial population associated with yellowing of leaves

Bacterial population associated with yellowing of leaves at the 5 stations of the study area varied, in general, from a minimum of 5.0 $x10^3$ CFU/cm2 in Station I (November'96) to a maximum of 1.56 x 10^9 CFU/cm2 in Station IV (November'96) (Table-13).

The bacterial population varied from 5.0 $x10^3$ CFU/cm2 (November'96) to 2.0 x 10^8 CFU/cm2 (July'97) in Station I; from 9.6 $x10^4$ CFU/cm2 (October'97) to 2.36 x 10^7 CFU/cm2 (June'97) in Station II; from 7.2 $x10^4$ CFU/cm2 (August'97) to 1.2 x 10^9 CFU/cm2 (December'96) in Station III; from 1.06 $x10^3$ CFU/cm2

Table 12. **Bacterial population associated with the Fresh leaves in Hybrid *Dendrobium* at the five stations of the study area during the period of study (expressed as no./cm/g).**

Month	Station I	Station II	Station III	Station IV	Station V
November'96	60	2,050	990	2,300	10
December'96	70	120	1,060	2,,030	8
January'97	180	150	820	40,000	4
February'97	56,000	380	60,000	4,000	6
March'97	48,000	260	26,000	1,200,000	19,000
April'97	12,000	950	16,000	5,730,000	75,000
May'97	28,000	1,020	112,000	341,000	15
June'97	16,000	11,800	8,000	16,000	32,000
July'97	2,660	49,000	26,000	600	22,000
August'97	390,000	82,000	30,000	960	75
September'97	42,000	2,080	17,000	26,000	13
October'97	360	780	60,000	40000,000	68

Table 13. Bacterial population associated with the yellowing of leaves in Hybrid *Dendrobium* at the five stations of the study area during the period of study (expressed as no./cm/g)

Month	Station I	Station II	Station III	Station IV	Station V
November'96	5000	0	14000	1560000000	40000000
December'96	3400000	245000	1.2E+09	0	102000
January'97	50000	298000	207000	10000	91000
February'97	78000	0	102000	295000	0
March'97	338000	0	0	15700000	0
April'97	167000	313000	80000	0	10800000
May'97	26400000	187000	93000	6300000	28200000
June'97	76000000	23600000	22000000	0	0
July'97	200000000	5000000	18000	15300000	480000
August'97	348000	13400000	72000	36900000	10800000
September'97	25000000	153000	62000	0	0
October'97	18000000	96000	0	1060	0

(October'97) to 1.56 x 10^9 CFU/cm2 (November'96) in Station IV; and from 9.1 x10^4 CFU/cm2 (January'97) to 4.0 x 10^7 CFU/cm2 (November'97) in Station V.

3.4.1.5. Bacterial population associated with brown dot of leaves

Bacterial population associated with brown dots in leaves at the different stations of the study area varied, in general, from a minimum of 4.0 x10^3 CFU/cm2 in Station II (December'96) to a maximum of 2.48 x 10^7 CFU/cm2 in Station III (August'97) (Table14).

The bacterial population varied from 5.0 x10^3 CFU/cm2 (December'96) to 1.79 x 10^5 CFU/cm2 (March'97) in Station I; from 4.0 x10^3 CFU/cm2 (October'97) to 1.03 x 10^7 CFU/cm2 (August'97) in Station II; and from 1.3 x10^4 CFU/cm2 (November'96) to 2.48 x 10^7 CFU/cm2 (August'97) in Station III. In Station IV this disease symptom was not observed throughout the period of study and in Station V the disease was observed only during January'97 and the bacterial population was recorded 1.6 x10^4 CFU/cm2.

Maximal bacterial population associated with brown dots was observed during the month of August'97 in both Stations II and III. Relatively, in all the 3 Stations of Ernakulam District this disease was observed during the month of December'96. While in Station I this disease was observed continuously during the period from December'96 to March'97, in Station III it occurred continuously during the period from November'96 to January'97. Whereas, in the case of Station IV the occurrence

Table14 .. Bacterial population associated with the brown dots in leaves in Hybrid *Dendrobium* at the five stations of the study area during the period of study (expressed as no./cm/g)

Month	Station I	Station II	Station III	Station IV	Station V
November'96	0	208000	13000	0	0
December'96	5000	4000	200000	0	0
January'97	53000	0	145000	0	16000
February'97	28000	0	0	0	0
March'97	179000	0	0	0	0
April'97	0	140000	0	0	0
May'97	0	238000	0	0	0
June'97	0	0	0	0	0
July'97	0	0	0	0	0
August'97	0	10300000	24800000	0	0
September'97	0	2700000	0	0	0
October'97	0	0	0	0	0

of this disease was discontinuous and occurred more in number during monsoon months (August and September'97).

3.4.1.6. Bacterial population associated with Leaf rot

Bacterial population associated with leaf rot, which was incurred only during few months at the different stations of the study area ,did not show much variation. Both minimum (3.5 $x10^5$ CFU/cm2 in August'97) and the maximum (1.03 x 10^9 CFU/cm2 in July'97)level of bacterial population were recorded in Station-I(Table-15).March'97 also recorded a high level of bacterial population($3.5x10^7$ CFU/cm2). In Station II, III, IV the bacterial population was observed as 1.75 $x10^8$ CFU/cm2 (December'96), 1.8 $x10^{47}$CFU/cm2 (August'97), and 2.8 $x10^8$ CFU/cm2 (February'97), respectively. In Station V this disease was not recorded.

3.4.1.7 Bacterial population associated with brown spots of flowers

Brown spot in flowers of the hybrid *Dendrobium* was recorded only during the months of January in Station I and during May'97 in Station III. Bacterial population associated with the diseased flowers was recorded as 2.8 $x10^5$ CFU/cm2 and 2.38 $x10^5$ CFU/cm2 , respectively for the months and Stations mentioned above.

3.4.1.8 Bacterial population associated with bud wilting

Bud wilting in flower stalks of the hybrid *Dendrobium* was recorded only in Station I during May'97 (3.98 x 10^9 CFU/gm) and in July'97(1.09 $x10^5$ CFU/gm), and in Station II during October'97(3.5 $x10^4$ CFU/gm).

Table15 .. Bacterial population associated with the Leaf rot In Hybrid *Dendrobium* at the five stations of the study area during the period of study (expressed as no./cm^2/g)

Month	Station I	Station II	Station III	Station IV	Station V
November'96	0	0	0	0	
December'96	0	1750000000	0	0	
January'97	0	0	0	0	
February'97	0	0	0	2800000000	
March'97	35000000	0	0	0	
April'97	0	0	0	0	
May'97	0	0	0	0	
June'97	0	0	0	0	
July'97	1.03E+09	0	0	0	
August'97	350000	0	18000000	0	
September'97	0	0	0	0	
October'97	0	0	0	0	

3.4.1.9. Bacterial population associated with dried stem

Dried stem in hybrid *Dendrobium* was recorded only in Station I during March'97with a bacterial population of 2.77 x 10^7 CFU/gm) and was not recorded elsewhere during the period of study.

3.4.1.10. Bacterial population associated with root rot

Root rot disease in hybrid *Dendrobium* was recorded only in Station I during the months of March'97 and July'97 with a bacterial population of 1.11 x 10^7 CFU/gm and 1.84 x 10^7 CFU/gm, respectively, and was not recorded elsewhere during the period of study.

3.4.1.11. Bacterial population associated with stem rot

Stem rot disease in hybrid *Dendrobium* was recorded only in Station I during the months of July, August and September'97 with a bacterial population of 2.47 x 10^9 CFU/gm, 2.58 x 10^9 and 2.07 x 10^7 CFU/gm, respectively, and was not recorded elsewhere during the period of study.

3.4.1.12. Bacterial population associated with flower drooping

Flower drooping in hybrid *Dendrobium* was recorded only in Station II during July'97with a bacterial population of 6.5 x 10^6 CFU/gm) and was not recorded elsewhere during the period of study.

3.4.2. Bacterial flora associated with the diseased hybrid *Dendrobium* plants in different Stations

Bacterial flora associated with the samples of diseased plants showed interesting observations, in all the selected Stations of study located in both the Ernakulam and Kanyakumari Districts, during the period of study. Results obtained for the bacterial flora associated with the samples are presented in Figures-13 to-17 and in Tables from 22-26.

Station I

Bacterial flora incurred at Station I associated with the diseased plants,included mainly species of, *Aeromonas, Alkaligenes, Erwinia, Flavobacterium, Pseudomonas, Vibrio and Xanthomonas*. Some isolates could not be placed under any known genera.

Among the different genera of bacteria incurred species of *Pseudomonas* was observed throughout the year and this genera was dominant among the flora during the period of study except for the months of December'96 and May'97. In general this genera was recorded in the range of 25.0%(May'97) to 58.33% (September'97).

Erwinia sp was observed during the period of study except in the months of September and october'97. This genera was the second largest flora dominant among the flora during the period of study except in December'96 (50%) and June'97 (40%), when it was the dominant group. In general this genera was recorded in the range of 14.29% (March'97) to 50.0% (September'97).

Figure - 13

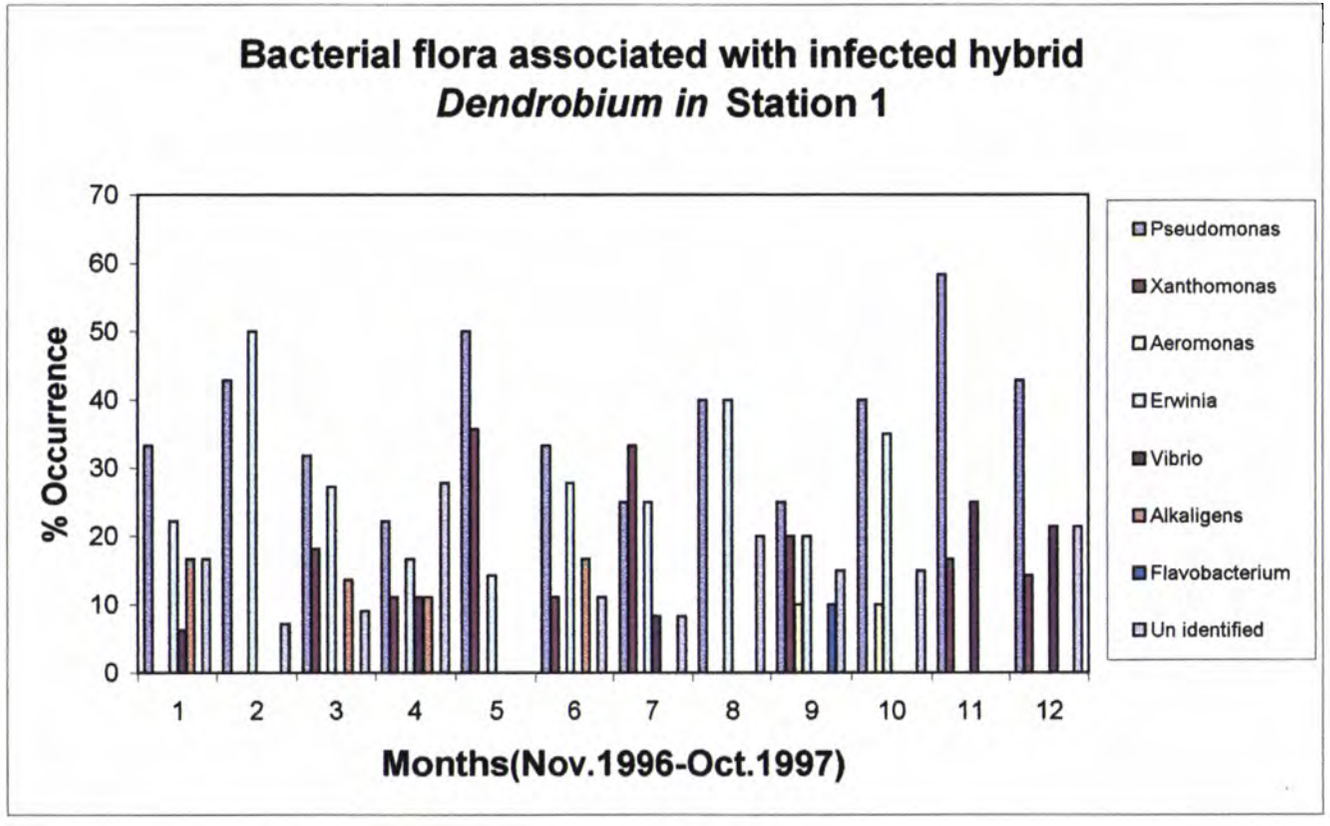

Bacterial flora associated with infected hybrid
Dendrobium in Station 1
% Occurrence
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
Months(Nov.1996-Oct.1997)
Pseudomonas
Xanthomonas
Aeromonas
Erwinia
Vibrio
Alkaligens
Flavobacterium
Un identified

Figure -14

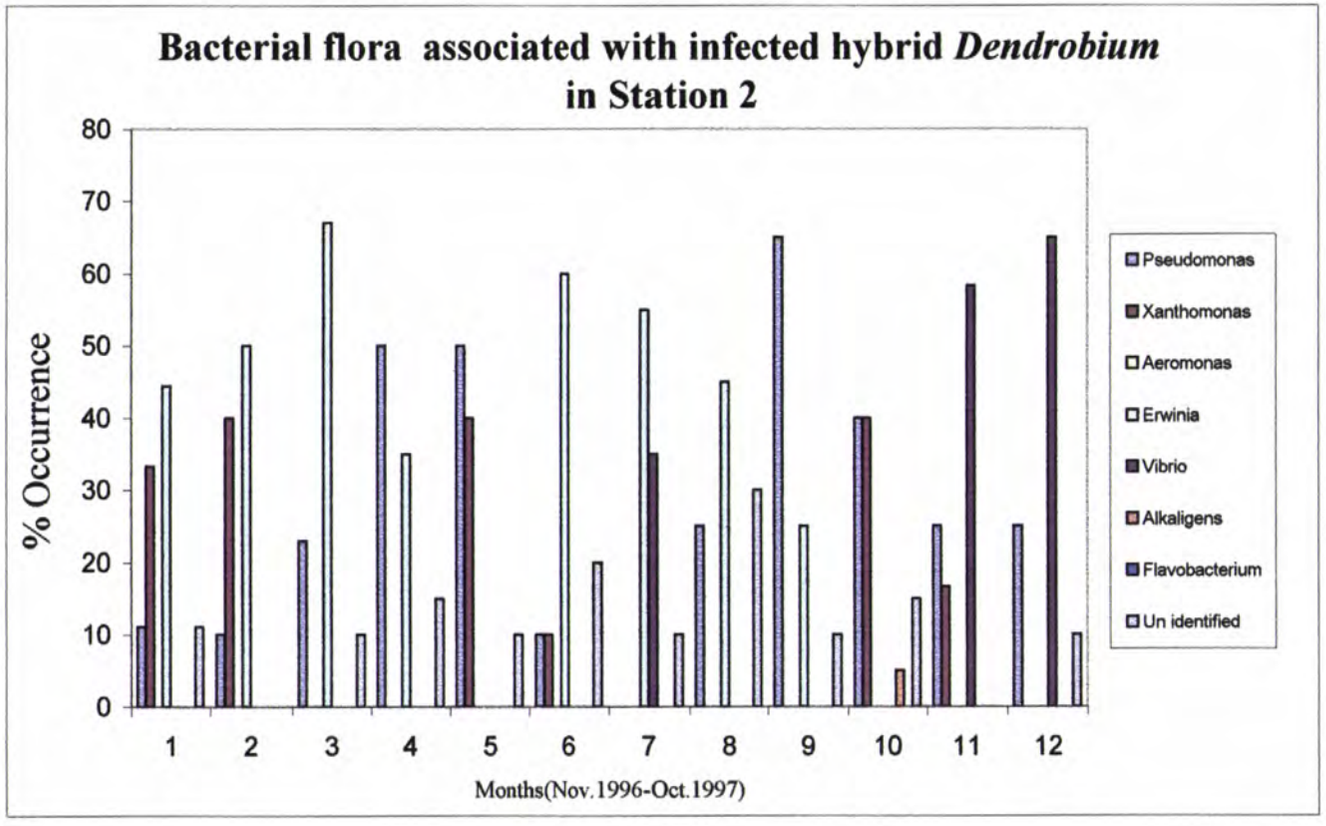

Bacterial flora associated with infected hybrid *Dendrobium* in Station 2
% Occurrence
0
10
20
30
40
50
60
70
80
1
2
3
4
5
6
7
8
9
10
11
12
Months(Nov.1996-Oct.1997)
Pseudomonas
Xanthomonas
Aeromonas
Erwinia
Vibrio
Alkaligens
Flavobacterium
Un identified

Figure - 15

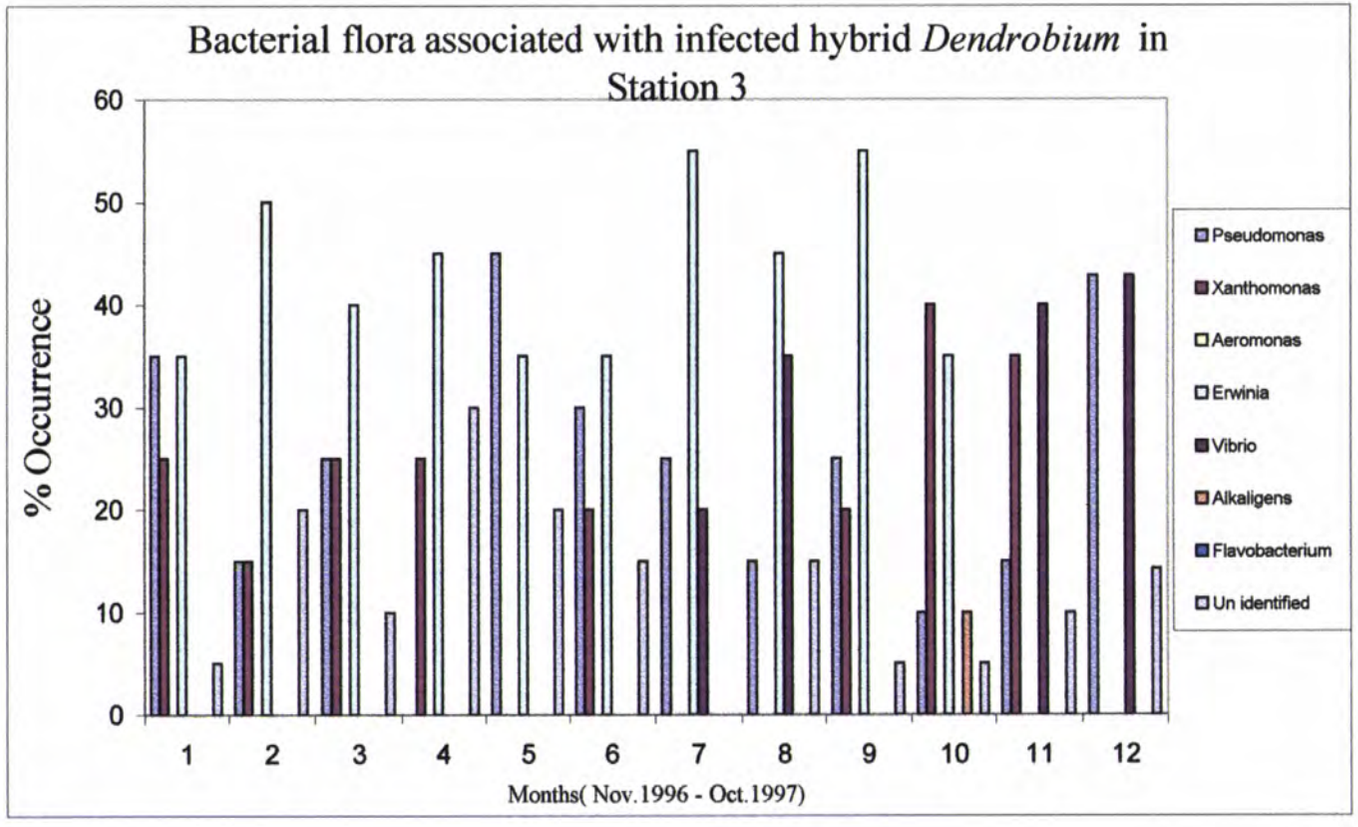

Bacterial flora associated with infected hybrid *Dendrobium* in Station 3
% Occurrence
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
Months(Nov.1996 - Oct.1997)
Pseudomonas
Xanthomonas
Aeromonas
Erwinia
Vibrio
Alkaligens
Flavobacterium
Un identified

Figure -16

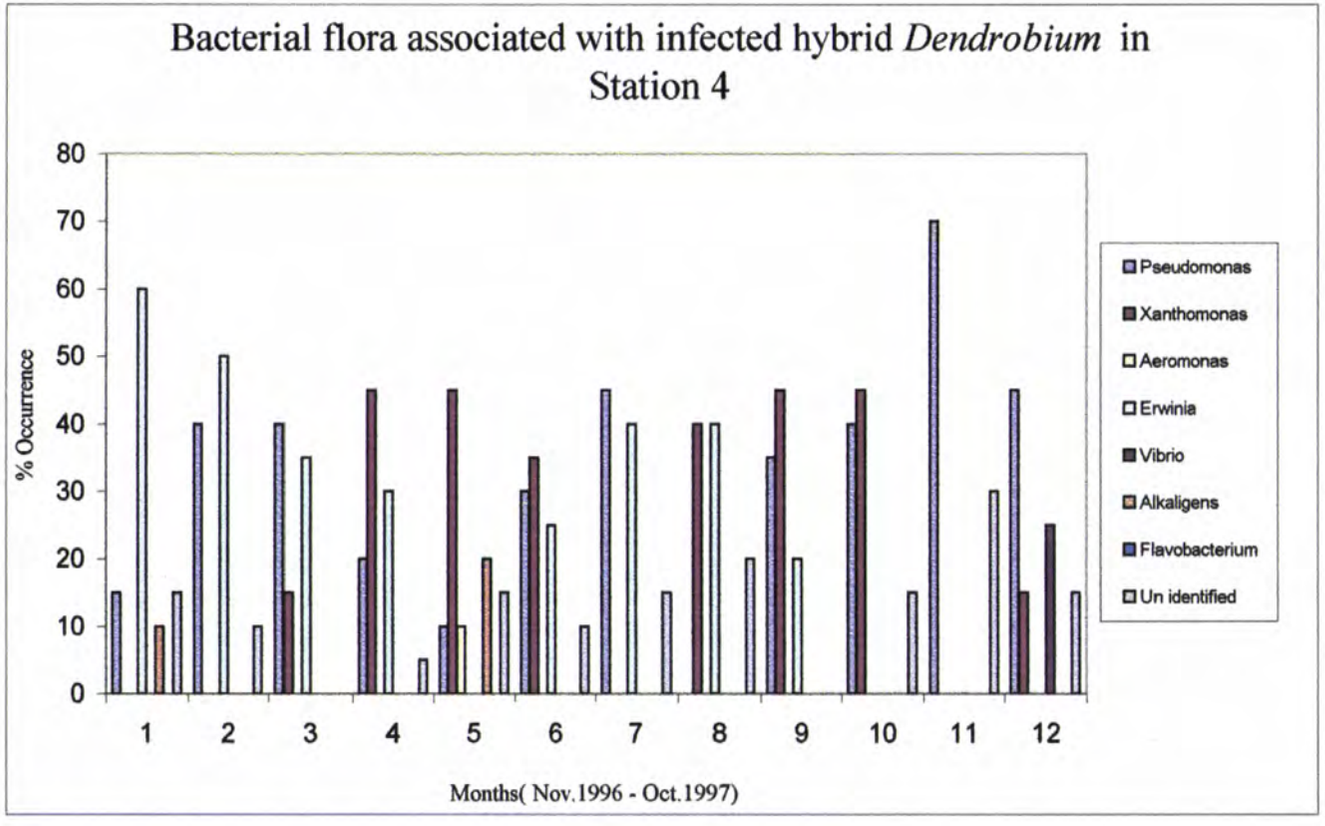

Bacterial flora associated with infected hybrid *Dendrobium* in Station 4
% Occurrence
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
Months(Nov.1996 - Oct.1997)
Pseudomonas
Xanthomonas
Aeromonas
Erwinia
Vibrio
Alkaligens
Flavobacterium
Un identified

Figure -17

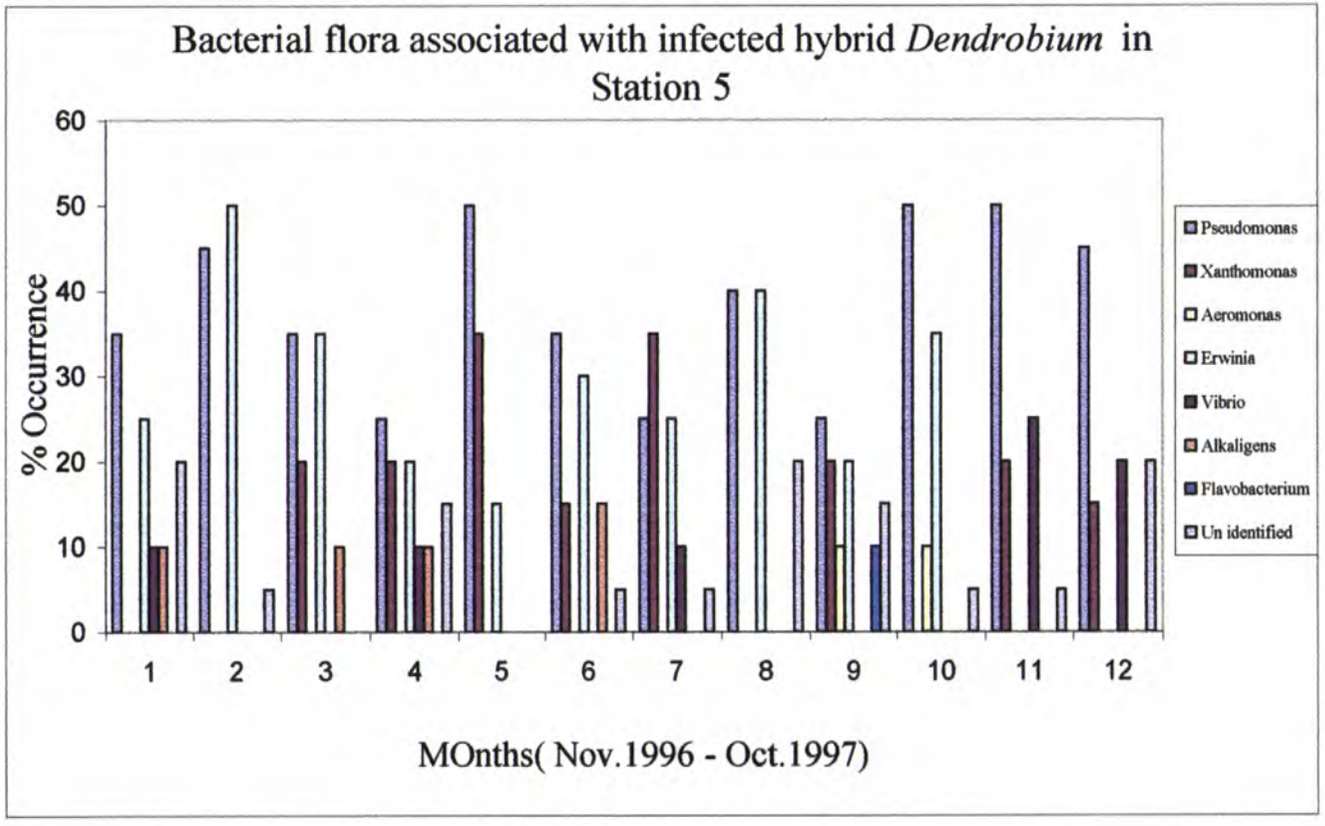

Bacterial flora associated with infected hybrid *Dendrobium* in Station 5
% Occurrence
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
MOnths(Nov.1996 - Oct.1997)
Pseudomonas
Xanthomonas
Aeromonas
Erwinia
Vibrio
Alkaligens
Flavobacterium
Un identified

Table. 22. Percent distribution of bacterial flora associatd with diseased samples air, water and fresh leaves in Station I

Disease	Pseudo monas	Erwini a	Xanth omona s	Vibrio	Alcali genes	Aero monas	Flavo bacteri um	Unide ntified
Y.L	30	25	20	-	-	10	-	15
B.DL	25	45	-	-	-	-	-	30
LR	15	35	15	-	-	25	-	10
DS	-	30	50	-	-	-	-	20
DR	30	20	20	-	-	10	-	20
SR	35	25	20	-	-	-	-	20
BW	25	35	-	-	-	15	-	25
FD	-	-	-	-	-	-	-	-
BS	-	70	-	-	-	-	-	30
A	35	30	15	-	-	-	-	20
W	40	30	15	-	-	5	-	10
FL	20	30	20	5	5	5	5	10

Table. 23. Percent distribution of bacterial flora associatd with diseased samples air, water and fresh leaves in Station II

Disease	Pseudo monas	Erwinia	Xanthomonas	Vibrio	Alcaligenes	Aeromonas	Flavobacterium	Unidentified
Y.L	20	35	15	5	5	5	-	15
B.DL	-	50	30	-	-	-	-	20
LR	30	45	-	-	-	-	-	25
BW	-	50	-	30	-	-	-	20
FD	70	-	-	-	-	-	-	30
A	30	30	20	-	-	10	-	10
W	35	30	15	5	-	5	-	10
FL	35	30	15	10	-	-	-	10

Table. 24. Percent distribution of bacterial flora associatd with diseased samples air, water and fresh leaves in Station III

Disease	Pseudo monas	Erwinia	Xanthomonas	Vibrio	Alcaligenes	Aeromonas	Flavobacterium	Unidentified
Y.L	20	35	25	5	5	-	-	10
B.DL	20	35	35	-	-	-	-	10
LR	-	40	30	-	-	-	-	30
BS	-	35	30	15	-	-	-	20
A	25	25	25	-	-	-	-	25
W	30	-	30	20	5	5	-	10
FL	25	35	15	5	-	5	-	15

Table. 25. Percent distribution of bacterial flora associatd with diseased samples air, water and fresh leaves in Station IV

Disease	Pseudo monas	Erwinia	Xanthomonas	Vibrio	Alcaligenes	Aeromonas	Flavobacterium	Unidentified
Y.L	40	30	-	-	5	-	-	25
LR	45	-	-	-	15	15	-	25
A	20	20	30	-	-	-	-	30
W	30	25	-	5	5	5	-	30
FL	25	25	25	-	-	-	-	25

Table. 26. Percent distribution of bacterial flora associatd with diseased samples air, water and fresh leaves in Station V

Disease	Pseudo monas	Erwinia	Xanthomonas	Vibrio	Alcaligenes	Aeromonas	Flavobacterium	Unidentified
Y.L	20	40	20	-	-	-	-	20
B.DL	-	80	-	-	-	-	-	20
A	25	30	25	-	-	-	-	20
W	35	-	20	5	5	5	-	30
FL	30	40	30	-	-	-	-	-

Xanthomonas sp was observed during the period of study except in the months of November and December'96, and June and August'97. This genera was dominant among the flora during the May'97 (33.33%). In general this genera was recorded in the range of 11.11% (February and April'97) to 35.71% (May'97).

Vibrio sp was observed only during the months of November'96, and February, May, September and October'97. This genera was comparatively less in number except during September'97 (25%) and October'97 (21.43%) when it formed the second dominant group among the flora. In general this genera was recorded in the range of 6.17% (November '96) to 25.0 % (September'97).

Alkaligens sp was observed only during the months of November'96, and January, February, and April'97. In general this genera was comparatively less in number and was recorded in the range of 11.11% (February'97) to 16.67 % (November'96 and April'97).

Aeromonas sp was observed (at 10% level)only during the months of July'97 and August'97. *Flavobacterium* sp was observed (at 10% level)only during July'97.

Results presented in table 21indicate that *Pseudomonas* was dominat(30%) in yellow leaves, in root rot (30%), stem rot(35% air(35%0 and water(40%). Whereas *Erwinia* was dominant in brown dot leaves(45%), leaf rot (35%); bud wilting(35%) brown spot(70%) and fresh leaves(30%). *Xanthomonas* was also observed to be a dominat flora in Dried stem(50%).

Station II

Bacterial flora incurred at Station II associated with the diseased plants, included mainly species of, *Alkaligenes, Erwinia, Pseudomonas, Vibrio and Xanthomonas*. Some isolates could not be placed under any known genera.

Among the different genera of bacteria incurred species of *Pseudomonas* was observed throughout the year of study except in the month of May'97. In general this genera was recorded in the range of 10.0% (December'96 and April'97) to 65.0 % (July'97). This genera was dominant among the flora during the period of study during the months of February, March, July and August'97. Further, this genera recorded about 50% of the flora during the months of February and March'97.

Erwinia sp was observed during the period of study except in the months of March, August, September and october'97. This genera was recorded in the range of 25.0%(July'97) to 67.0% (January'97). This genera was the dominant group among the flora in the months of November, December'96(50%), January(67%), April(60%) May(55%) and June'97 during the period of study, except in February'97 and July'97, when it was the second dominant group. Further, it was observed in general as an important flora in this station during the period of its occurrence.

Xanthomonas sp was observed only during six months of the period of study. It was present in the months of November and December'96, and March, April August and September'97. This genera was dominant among the flora during the August'97 and was the second dominant during the month of November and

December'96. In general this genera varied between 10.0% (April'97) and 40.0% (December'96, March and August'97).

Vibrio sp was observed only during the months of May, September and October'97. This genera was comparatively higher in number when it made its occurrence and varied between 35.0% (May'97) to 65.0 % (October'97). Further, this formed the dominant group among the flora during the months of September and October'97. *Alkaligens* sp was observed only during the month of August'97(5%).

Results presented in table 22 indicate that *Pseudomonas* was dominat in only in air (30%), water(35%) and fresh leaves(35%). Whereas *Erwinia* was dominant in diseased samples, particularly in yellow leaves(35%), brown dot leaves(50%), leaf rot(45%);and bud wilting(50%).

Station III

Bacterial flora incurred at Station III associated with the diseased plants, included mainly species of *Alkaligenes, Erwinia, Pseudomonas, Vibrio and Xanthomonas*. Some isolates could not be placed under any known genera.

Among the different genera of bacteria incurred species of *Pseudomonas* was observed throughout the year of study except in the month of February'97. In general this genera was recorded in the range of 10.0% (August'97) to 45.0 % (March'97). This genera was dominant among the flora during the period of study during the months of November'96, March, October'97. Further, this genera recorded about 50% of the flora during the months of February and March'97.

Erwinia sp was observed during the period of study except in the months of September and october'97. This genera was recorded in the range of 15.0%(December'96) to 40.0% (August'97). This genera was the dominant group among the flora during all the months of its occurrence except during March and August'97, when it was second dominant. Further, it was observed in general as an important flora in this station during the period of its occurrence.

Xanthomonas sp was observed during eight months of the period of study except in the months March, May, June and October'97. This genera was dominant among the flora during August'97 and was the second dominant during the rest of the period of its occurrence. In general this genera varied between 15.0% (December'96) and 40.0% (August'97). In terms of percentage of occurrence this group also was an important group.

Vibrio sp was observed only during the months of May, June, September and October'97. This genera was comparatively higher in number when it made its occurrence and varied between 20.0% (May'97) to 42.86 % (October'97). Further, this formed the dominant group among the flora during the months of September and October'97. *Alkaligens* sp was observed only during the month of August'97 (10%).

Results presented in table 23 indicate that *Pseudomonas* was, dominant in air (35%) and water(30%). Whereas *Erwinia* was dominant in yellow leaves (35%), brown dot leaves (35%), leaf rot (40 %); and brown spot (35%).

Station IV

Bacterial flora incurred at Station IV associated with the diseased plants, included mainly species of *Aeromonas, Alkaligenes, Erwinia, Pseudomonas, Vibrio and Xanthomonas*. Some isolates could not be placed under any known genera.

Among the different genera of bacteria incurred species of *Pseudomonas* was observed throughout the year of study except in the month of June'97. In general this genera varied from a minimum of 10.0% (March'97) to a maximum of 70.0 % (September'97). This genera was dominant among the flora during the months of January, May, September, and October'97. Further, this genera was recorded at considerable level during the period of study.

Erwinia sp was observed during the period of study except in the months of March, August, September and october'97. This genera varied from 20.0% in July'97 to 60.0% in November'96). This genera was the dominant group among the flora in the months of November, December'96, and June'97. It was the second dominant group during January, February and May'97. Further, it was observed in general as an important flora in this station during the period of its occurrence.

Xanthomonas sp was observed during the entire period of study except in the months of November and December'96, and May and September'97. In general this genera varied between 15.0% (January and October'97) and 45.0 % (February, March, July and August'97). This genera was dominant among the flora during February to April, and June toAugust'97).

Vibrio sp, and *Aeromonas* sp were observed only during the months of October'97(25.0%), March'97(10.0%) respectively. *Alcaligens* sp was observed only during the month of November'96(10%) and March'97(20.0%).

Results presented in table 24 indicate that *Pseudomonas* was dominat in yellow leaves(40%), leaf rot(45%) , water(30%) and fresh leaves(25%). Whereas *Xanthomonas* was observed to be a dominant flora in air (30%).

Station V

Bacterial flora incurred at Station V associated with the diseased plants, included mainly species *Aeromonas, Alcaligenes, Erwinia, Flavobacterium, Pseudomonas, Vibrio and Xanthomonas.* Some isolates could not be placed under any known genera.

Among the different genera of bacteria incurred species of *Pseudomonas* was observed throughout the year of study. In general this genera varied from 25.0% (February and July'97) to 50.0 % (March, August and September'97). This genera was dominant among the flora during the entire period of study except during the months of December'96 and May'97 when it was second dominant group.

Erwinia sp was observed during the entire period of study except in the months of September and October'97. In general this genera varied from 15.0%(March'97) to 50.0% (December'96). This genera was the dominant group among the flora in the months of December'96, January and June'97, and it was the second dominant group during February, April, May, July, and August'97. Further, it

was observed in general as an important flora in this station during the period of its occurrence.

Xanthomonas sp was observed only during eight months of the period of study and was not inccurred during the months of November and December'96, and June and August'97. In general this genera varied between 15.0% (April and October'97) and 35.0% (March and May'97).This genera was dominant among the flora during the May'97 and was the second dominant during the month of February, March, and July'97.

Vibrio sp was observed during the months of November'96, February, May, September and October'97. In general this genera varied between 10.0% (November'96, February and May'97) to 25.0 % (September'97). This genera formed the second dominant group among the flora during the months of September and October'97.

Alcaligens sp was observed during the months of November'96 (10.0%), January(10.0%), February(10.0%), and April'97(15.0%). *Aeromonas* sp was observed during July(10.0%) and August'97(10.0%). *Flavobacterium* was recorded only in July'97(10.0%).

Results presented in table 25 indicate that *Erwinia* was the dominant flora in yellow leaves(40%), brown dot leaves(80%0, air(30%) and fresh leaves(40%). Whereas *Pseudomonas sp* was dominant in water(35%)

3.4.3 Fungal population associated with air, water, fresh and diseased hybrid *Dendrobium* plants in different Stations

3.4.3.1 Fungal population of atmospheric microbial flora

Fungal population of atmospheric microbial flora at the 5 different stations of the study area showed very less population (Table-16). In general the population varied from a minimum of 1 CFU in Station III (January'97) to a maximum of 26 CFU in Station IV (March'97). Particularly the fungal population varied from 3 CFU (January'97) to 16 CFU(June'97) in Station I. Comparatively a higher level of fungal counts(15CFU) were recorded during May'97 and October'97 in this Station I. The fungal population varied from 2 CFU (May'97) to 8 CFU(October'97) in Station II; from 1 CFU (January'97) to a maximum of 6 CFU(November'96 and September'97) in Station III; from 2 CFU (May'97) to 26 CFU (Marh'97) in Station IV. Comparatively a high level of 23 CFU was recorded during October'97 in this Station. In Station V the fungal population varied from 2 CFU (February'97) to 16 CFU (April and June'97). In general the fungal population of atmospheric flora was comparatively high in Stations IV and V and lesser in Station I, II and III.

3.4.3.2 Fungal population of water

Fungal population of water used for irrigation at the 5 different stations of the study area. In general the population varied from a minimum of 10 CFU/ml in Station I (December'96) to a maximum of 1.0 $x10^7$ CFU/ml in Station IV (March'97) (Table17). Particularly the Fungal population varied from 10 CFU/ml in (December'96) to 1.3 x 10^3CFU/ml (April'97) in Station I; from 10 CFU/ml

Table .16. Fungal population in the atmosphere(air) at the five stations of the study area during the period of study (expressed in cm)

Month	Station I	Station II	Station III	Station IV	Station V
November'96	5	3	6	12	12
December'96	6	0	2	16	4
January'97	3	3	1	8	3
February'97	4	0	3	6	2
March'97	0	0	2	26	13
April'97	0	3	4	18	16
May'97	15	2	2	2	11
June'97	16	6	4	3	16
July'97	10	0	3	6	11
August'97	8	0	2	8	15
September'97	6	0	6	16	12
October'97	15	8	4	23	10

Table 17.. Fungal population in the water used at the five stations of the study area during the period of study (expressed in cm)

Month	Station I	Station II	Station III	Station IV	Station V
November'96	20	40	60	560	0
December'96	10	0	0	0	0
January'97	0	40	0	0	0
February'97	0	0	70	1000	0
March'97	0	10	0	10000000	3000
April'97	1000	0	40	0	0
May'97	0	0	0	0	0
June'97	0	20	0	0	30000
July'97	120	0	10	500	4000
August'97	40	20	10	0	50000
September'97	30	20	30	0	120
October'97	60	30	60	500	220

(March'97) to 40 CFU/ml (November'96 and January'97) in Station II; from 10 CFU/ml (July and August'97) to 70 CFU/ml(February'97) in Station III; Comparatively a higher level of 60 CFU/ml was also recorded during the months of November'96 and October'97). In Station IV the fungal population varied from $5.0x10^2$ CFU/ml (July and October'97) to$1.0x10^7$ CFU/ml (March'97) and in Station V it varied from $1.2x10^2$ CFU/ml (September'97) to 5.0×10^4 CFU (August'97). In general the fungal population of water was comparatively high in Stations IV and V and lesser in Station I, II and III.

3.4.3.3. Fungal population of fresh leaves

Fungal population of fresh leaves of the hybrid *Dendrobium* at the 5 stations of the study area showed wide variation from a minimum of 20 CFU/cm^2 in Station IV (August'97) to a maximum of 1.5×10^6 CFU/cm^2 in Station III (June'97) (Table-18).

Particularly the fungal population varied from 2.9×10^2 CFU/cm^2 (September'97) to 4.0×10^5CFU/cm^2 (July'97) in Station I; from 60 CFU/cm^2 (January'97) to 1.27×10^5CFU/cm^2 (August'97) in Station II; from 40 CFU/cm^2 (February'97) to 1.5×10^6 CFU/cm^2 (June'97) in Station III; from 20 CFU/cm^2 (August'97) to $2.0x10^5$ CFU/cm^2 (March'97) in Station IV; and from $1.2x10^2$ CFU/cm^2 (April'97) to 1.02×10^5 CFU/cm^2 (May'97) in Station V. Among the 5 Stations, Station IV recorded comparatively higher levels of fungal population associated with fresh leaves in the range of $X\ 10^5$ during the months of March, May and October '97. On the other hand Station III, in spite of a maximum number during

Table .18 Fungal population in the fresh leaves of hybrid *Dendrobium* at the five stations of the study area during the period of study (expressed in cm)

Month	Station I	Station II	Station III	Station IV	Station V
November'96	0	4,460	930	12,000	18,000
December'96	7,000	740	17,000	32,000	0
January'97	0	60	0	80	0
February'97	38,000	210	40	11,000	0
March'97	3,000	260	80	200,000	0
April'97	0	25,000	120	9,000	120
May'97	0	720	60	122,000	102,000
June'97	62,000	660	1,500,000	60,000	66,000
July'97	400,000	19,900	10,000	200	85,000
August'97	70,000	127,000	60	20	1,080
September'97	290	1100	120	26,000	150
October'97	0	980	22,000	132,000	28,000

June'97, generally recorded low level of fungal population during other period. Of the Stations of Ernakulam District Station I recorded relatively high levels of fungal population, particularly during rainy season.

3.4.3.4. Fungal population associated with yellowing of leaves

Fungal population associated with yellowing of leaves at the 5 different stations of the study area varied, in general, from a minimum of 2.0 $x10^3$ CFU/cm^2 in Station V (January'97) to a maximum of 1.6 x 10^8 CFU/cm^2 in Station I (June'97) (Table-19).

Particularly the Fungal population varied from 3.0 $x10^3$ CFU/cm^2 (March'97) to 1.6 x 10^8 CFU/cm^2 (June'97) in Station I; from 1.5 $x10^4$ CFU/cm^2 (July'97) to 2.36 x 10^7 CFU/cm^2 (June'97) in Station II; from 4.0 $x10^3$ CFU/cm^2 m (January'97) to a maximum of 5.0 x 10^5 CFU/cm^2 (December'96) in Station III; from 3.0 $x10^3$ CFU/cm^2 (January'97) to 7.0 x 10^5 CFU/cm (February'97) in Station IV; from 2.0 $x10^3$ CFU/cm^2 (January'97) to 6.2 x 10^6CFU/cm^2 (June'97) in Station V. Comparatively the fungal population associated with yellowing of leaves was high in Station I, II and V during the rainy season (June-August) and Stations III and IV recorded lesser fungal population.

3.4.3.5.Fungal population associated with brown dot of leaves

Fungal population associated with brown dots in leaves at the different stations of the study area varied, in general, from a minimum of 4.0 $x10^3$ CFU/cm^2 in Station V(January'97 only occurrence during the period of study) to a maximum of 2.8 x 10^8 CFU/cm^2 in Station I (December'97) (Table-20). In Station I apart from the maximal

Table 19 **Fungal population in the yellowing of leaves of hybrid *Dendrobium* at the five stations of the study area during the period of study (expressed in cm)**

Month	Station I	Station II	Station III	Station IV	Station V
November'96	49000	0	87000	0	132000
December'96	420000	286000	500000	0	43000
January'97	185000	10000000	4000	3000	2000
February'97	0	0	8000	700000	0
March'97	3000	0	0	1000	0
April'97	36000	58000	19000	0	0
May'97	90000	15200000	39000	53000	2800000
June'97	160000000	23600000	0	0	6200000
July'97	200000	15000	0	23000	3300000
August'97	1700000	172000	15000	19000	68000
September'97	75000	143000	19000	0	0
October'97	16000	108000	0	80000	0

Table 20.. Fungal population in the brown dots in leaves of hybrid *Dendrobium* at the five stations of the study area during the period of study (expressed in cm)

Month	Station I	Station II	Station III	Station IV	Station V
November'96	0	22000	50000	0	0
December'96	2800000 00	26000	400000	0	0
January'97	0	0	0	0	4000
February'97	10000	0	0	0	0
March'97	43000	0	0	0	0
April'97	0	18000	0	0	0
May'97	0	55000	0	0	0
June'97	0	0	0	0	0
July'97	1700000	0	0	0	0
August'97	0	154000	0	0	0
September'97	0	84000	0	0	0
October'97	0	0	0	0	0

population recorded as mentioned above, fungal population was also recorded during the months of February(1.0×10^4 CFU/cm^2) March(4.3×10^4 CFU/cm^2) and July'97(1.7×10^6 CFU/cm^2). Station II recorded this disease, relatively, during several months, in a discontinuous fashion spread over the entire period of study and the fungal population varied from 1.8×10^4 CFU/cm^2 (April'97) to 1.54×10^5 CFU/cm^2 (August'97). In Station III the fungal population associated with the disease was recorded only during the months of November'96(5.0×10^4CFU/cm^2) and in December'96(4.0×10^5CFU/cm^2). In Station IV this disease symptom was not observed throughout the period of study.

3.4.3.6.Fungal population associated with leaf rot

Fungal population associated with leaf rot, which was incurred only during few months, at the different stations of the study area ,did not show much variation. In Station I the fungal population associated with the disease, which occurred during the months of March'97 and August'97 was 7.6×10^4 CFU/cm^2 and 8.0×10^5 CFU/cm^2, respectively. In Station II, III, IV the fungal population was observed as 2.01×10^5 CFU/cm^2 (December'96), 3.2×10^5 CFU/cm^2 (August'97), and 7.0×10^3 CFU/cm^2 (February'97), respectively. In Station V this disease was not recorded.

3.4.3.7 Fungal population associated with brown spots of flowers

Brown spot in flowers of the hybrid *Dendrobium* was recorded only during the months of January'97 in Station I and during May'97 in Station III. Fungal population associated with the diseased flowers was recorded as 9.1×10^4 CFU/cm^2 and 2.74×10^5 CFU/cm^2, respectively for the months and Stations mentioned above.

Table .21. Fungal population in the Leaf rot of hybrid *Dendrobium* at the five stations of the study area during the period of study (expressed in cm)

Month	Station I	Station II	Station III	Station IV	Station V
November'96	0	0	0	0	
December'96	0	201000	0	0	
January'97	0	0	0	0	
February'97	0	0	0	7000	
March'97	76000	0	0	0	
April'97	0	0	0	0	
May'97	0	0	0	0	
June'97	0	0	0	0	
July'97	0	0	0	0	
August'97	800000	0	320000	0	
September'97	0	0	0	0	
October'97	0	0	0	0	

3.4.3.8. Fungal population associated with bud wilting

Bud wilting in flower stalks of the hybrid *Dendrobium* was recorded only in Station I during May'97(1.46 x 10^5 CFU) and in July'97(3.0 x10^4 CFU), and in Station II during October'97(3.6 x10^4 CFU).

3.4.3.9. Fungal population associated with dried stem

Dried stem in hybrid *Dendrobium* was recorded only in Station I during March'97, July'97 and August'97 with a Fungal population of 5.2 x 10^4 CFU/g , 8.0 x 10^5 CFU/g and 5.7x 10^6 CFU/g, respectively and was not recorded elsewhere during the period of study.

3.4.3.10. Fungal population associated with root rot

Root rot disease in hybrid *Dendrobium* was recorded only in Station I during the months of March'97 and July'97 with a bacterial population of 1.2 x 10^4 CFU/g and 2.5 x 10^6 CFU/g, respectively, and was not recorded elsewhere during the period of study.

3.4.3.11. Fungal population associated with flower drooping

Flower drooping in hybrid Dendrobium was recorded only in Station II during July'97with a Fungal population of 6.4 x 10^6 CFU) and was not recorded elsewhere during the period of study.

3.5. Statistical analyses

Statistical analyses of the data obtained for the different variables were carried out using SPSS soft ware package. Correlation coefficient analysis, Multiple regression, analysis of variance and Multiple Linear regression Model were done for the variables including bacterial and fungal population associated with the disease symptoms, air, water, environmental parameters and water characteristics, for all the Stations.

3.5.1. Correlation coefficient analysis

Correlation coefficient analyses was carried out for all the variables analysed, during the period of study, in order to evaluate the interrelationships between them. However, correlation coefficients obtained for the microbial population(bacterial and fungal) associated with the disease symptoms and other variables, alone are presented here for consideration and discussion in this thesis, since the primary objective was to understood the interrelationship between microbial population associated with disease and environmental parameters. Correlation coefficients presented in Table 27 indicated that irrespective both negative and positive correlation between the bacterial population associated with yellowing of leaves and other environmental variables, significant negative correlation exist between bacterial population and pH, at Stations I and II; between bacterial population and Total alkalinity and hardness at Stations I; and between bacterial population, and hardness at Stations II. Whereas a significant positive correlation was recorded between bacterial population, and rainfall at Station I; was recorded between bacterial population, and aerial bacterial population at

Station II; and between Bacterial population, and akalinity and hardness at Station III. At all other instances, irrespective of the stations and variables there was no significant correlation whether it was positive or negative.

Correlation coefficients presented in Table 28 indicated that irrespective of both negative and positive correlation between the fungal population associated with yellowing of leaves and other environmental variables, significant positive correlation exist between bacterial population and pH and Total alkalinity only at Stations IV; and at all other instances, irrespective of the stations and variables there was no significant correlation whether it was positive or negative.

Correlation coefficients presented in Table 29 evince that despite the fact that there exist both negative and positive correlation between the bacterial population associated with brown dots in leaves and other environmental variables, significant positive correlation exist between bacterial population of brown dots and bacterial population in water at Stations I; between bacterial population and Total alkalinity at Station II; and between bacterial population, and humidity and pH at Station III; and between bacterial population, and aerial bacterial population at Station V. On the other hand a significant negative correlation exit between Bacterial population, and maximum atmosphere temperature at Station II. At all other instances, irrespective of the stations and variables there was no significant correlation whether it was positive or negative.

Data presented in Table 30 for Correlation coefficients indicated that irrespective both negative and positive correlation between the fungal population

Table. 27 Correlation Coefficient of bacterial population associated with Yellowing of leaves of *Dendrobium* hybrid at the different Stations

Variable	Air (B.p)	Water (B.p)	Tem (max)	Tem (min)	Humidity	Rain fall	pH value	T.Alkalinity	Hardness
Station.-I	0.3573	-0.1175	-0.3547	-0.1338	0.4132	**0.7601**	**-0.9473**	**-0.8296**	**-0.9765**
Station-II	**0.6577**	-0.0317	-0.1974	0.0915	0.4171	0.3161	**-0.6446**	-0.0516	**-0.6322**
Station III	- 0.0812	-0.1242	-0.2499	0.0915	0.2700	-0.0387	0.0561	**0.5205**	**0.5039**
Station IV	- 0.3840	-0.0981	-0.1718	0.2976	0.2601	0.0411	-0.0442	-0.0351	0.0019
Station-V	0.0436	-0.1664	0.1710	0.0579	0.0739	-0.0178	0.2139	0.0027	0.0008

Table. 28 Correlation Coefficient of Fungal population associated with Yellowing of leaves of *Dendrobium* hybrid at the 5 Stations

Variable	Air (F.p)	Water (F.p)	Tem (max)	Tem (min)	Humidity	Rain fall	pH value	T.Alkalinity	Hardness
Station.-I	0.4871	-0.1198	0.1258	0.1670	0.1466	-0.0199	0.0473	0.1493	-0.1212
Station-II	0.4295	0.1015	0.2740	0.3052	-0.0246	-0.2633	-0.4816	-0.0330	-0.4696
Station -III	-0.1594	-0.2077	-0.2531	0.1263	0.2330	-0.0851	0.0176	0.4771	0.4490
Station -IV	-0.2377	-0.1142	0.0193	0.3083	-0.0449	-0.2840	**0.6863**	**0.9007**	0.4800
Station-V	0.3567	0.3174	0.0643	-0.0695	0.4565	-0.1225	-0.2722	-0.0179	0.1783

Table. 29. Correlation Coefficient of bacterial population associated with Brown dots in leaves of Dendrobium hybrid at the different Stations

Variable	Air (B.p)	Water (B.p)	Tem (max)	Tem (min)	Humidity	Rain fall	pH value	T.Alkalinity	Hardness
Station.-I	-0.3100	**0.7528**	0.4448	0.0930	-0.3054	-0.1626	0.1864	0.0933	0.1499
Station-II	-0.2483	0.2318	**-0.5123**	-0.2365	0.4946	0.4422	0.3062	**0.5078**	0.2447
Station - III	-0.0977	-0.1482	-0.4502	0.0699	**0.5358**	0.4403	**0.6743**	0.3246	0.3840
Station - IV	*	*	*	*	*	*	*	*	*
Station-V	**0.7107**	-0.0969	0.1401	0.3045	-0.3979	-0.2591	-0.4605	-0.2819	-0.3046

* Bacterial population was not detected in the samples.

Table. 30 Correlation Coefficient of Fungal population associated with Brown dots in leaves of *Dendrobium* hybrid at the 5 Stations

Variable	Air (F.p)	Water (F.p)	Tem (max)	Tem (min)	Humidity	Rain fall	pH value	T.Alkalinity	Hardness
Station.-I	-0.0740	-0.1073	-0.2861	-0.3693	0.2948	-0.0393	0.1239	0.0086	0.2064
Station-II	-0.3722	0.0313	-0.4618	-0.1144	0.3486	0.3236	0.4499	**0.5693**	0.4031
Station -III	-0.1787	-0.2162	-0.2690	0.0976	0.2634	-0.0611	0.0389	**0.5025**	0.4789
Station -IV	*	*	*	*	*	*	*	*	*
Station-V	-0.4777	-0.1439	0.1401	0.3045	-0.3979	-0.2591	-0.4605	-0.2819	-0.3046

* Fungal population was not detected in the samples

associated with brown dots of leaves and other environmental variables, a significant positive correlation exist only between bacterial population, and Total alkainity at Stations II and II. At all other instances, irrespective of the stations and variables there was no significant correlation whether it was positive or negative.

Correlation coefficients presented in Table 31 indicated that irrespective both negative and positive correlation between the bacterial population associated with leaf rot and other environmental variables, significant positive correlation exist between bacterial population, and rainfall, at Stations I; between bacterial population, and humidity and pH at Station III. At all other instances, irrespective of the stations and variables there was no significant correlation whether it was positive or negative.

Correlation coefficients presented in Table 32 indicated that irrespective both negative and positive correlation between the fungal population associated with leaf rot and other environmental variables, a significant positive correlation was recorded between bacterial population, and humidity and pH at Station III; and between bacterial population, and pH, Total alkalinity abd Hardness at Station IV. At all other instances, irrespective of the stations and variables there was no significant correlation whether it was positive or negative.

Data presented in Table 33 for Correlation coefficients indicated that irrespective of both negative and positive correlation between the bacterial population associated with dried stem and other environmental variables, a significant positive correlation was recorded between bacterial population of dried stem, and bacterial

Table. 31 Correlation Coefficient of bacterial population associated with Leaf ~~et~~ of *Dendrobium* hybrid at the 5 Stations

Variable	Air (B.p)	Water (B.p)	Tem (max)	Tem (min)	Humidity	Rain fall	pH value	T.Alkalinity	Hardness
Station.-I	0.3193	0.0318	-0.3948	-0.1813	0.3366	**0.7302**	-0.9498	-0.8291	-0.9554
Stat- II	-0.1846	-0.1993	-0.2485	-0.1249	0.2748	-0.0373	0.1281	0.0308	0.1364
Stat -III	-0.0959	-0.1487	-0.4472	0.0688	**0.5327**	0.4417	**0.6740**	0.3215	0.3796
Stat -IV	-0.1630	-0.0361	0.0133	0.3855	-0.0837	-0.2987	0.7006	0.9050	0.5300
Station-V	*	*	*	*	*	*	*	*	*

* This disease symptom was not detected in the samples.

Table. 32 Correlation Coefficient of Fungal population associated with ~~Brown~~ ~~dots~~ in leaf of *Dendrobium* hybrid at the 5 Stations

Variable	Air (F.p)	Water (F.p)	Tem (max)	Tem (min)	Humidity	Rain fall	pH value	T.Alkalinity	Hardness
Station.-I	-0.0017	-0.0856	-0.4111	0.1029	0.3242	-0.1754	0.1342	0.0448	0.0956
Stat- II	-0.2449	-0.3015	-0.2485	-0.1249	0.2748	-0.0373	0.1281	0.0308	0.1364
Stat -III	-0.2456	-0.1532	-0.4472	0.0688	**0.5327**	0.4417	**0.6740**	0.3215	0.3796
Stat-IV	-0.1630	-0.0361	0.0133	0.3855	-0.0837	-0.2987	**0.7006**	**0.9050**	**0.5300**
Station-V	*	*	*	*	*	*	*	*	*

* * This disease symptom was not detected in the samples

population in water; and a significant negative correlation exist between fungal population and maximum temperature of atmosphere at Station I.

Correlation coefficients presented in Table 34 indicated that irrespective both negative and positive correlation between the bacterial population associated with flower drooping and other environmental variables, a significant positive correlation exist between bacterial population and rainfall at Station II. On the other hand a significant negative correlation was observed between bacterial population, and pH, Total alkalinity and hardness of water at Station II.

It could be seen from the data on Correlation coefficients presented in Table 35 and 36, respectively on the interrelationship of bacterial and fungal population associated with Bud wilting, and other environmental variables, that there was no significant correlation among the variables, at the stations I and II, irrespective of the positive or negative nature.

It is inferred from the data on Correlation coefficients presented in Table 37 and 38, respectively on the interrelationship of bacterial and fungal population associated with Brown spots on flowers, and other environmental variables, that there was no significant correlation among the variables, at the stations I and III, irrespective of the positive or negative nature.

Data on presented in Table 39 indicated that, the fungal populaion associated with root rot in Station I, both bacterial and fungal population showed a significant negative correlation with pH, Total alkalinity and hardness. Similarly both bacterial and fungal population demonstrated a significant positive correlation with rainfall.

Table-33 Correlation Coefficient of bacterial and Fungal population associated With Dried stem condition in *Dendrobium* hybrid at Station-I (This disease symptom was not incurred at other stations)

Variable	Air (B.p/F. p	Water (B.p/F. p	Tem (max)	Tem (min)	Humidity	Rain fall	pH value	T.Alkalinity	Hardness
Bact.popul	-0.2730	**0.8390**	0.4218	0.2565	-0.2090	-0.0637	0.1296	0.0407	0.0869
Fung.popu	0.0569	-0.0733	**-0.5083**	0.0491	0.3932	-0.0683	-0.0117	-0.0729	-0.0463

Table. 34 Correlation Coefficient of bacterial and Fungal population associated with Flower drooping in *Dendrobium* hybrid at Station-II (This disease symptom was not incurred at other stations)

Variable	Air (B/F.p)	Water (B/F.p)	Tem (max)	Tem (min)	Humidity	Rain fall	pH value	T.Alkalinity	Hardness
Bact.popul	0.4577	-0.0009	-0.3694	-0.2676	0.4318	**0.8009**	**-0.5602**	**-0.8251**	**-0.6391**
Fung.popu	-0.2449	-0.3015	-0.3694	-0.2676	0.4318	**0.8009**	**-0.5602**	**-0.8251**	**-0.6391**

Table. 35.Correlation Coefficient of bacterial population associated with Bud wilting condition in *Dendrobium* hybrid at Station-I and II

(This disease symptom was not incurred at other stations)

Variable	Air (B.p)	Water (B.p)	Tem (max)	Tem (min)	Humidity	Rain fall	pH value	T.Alkalinity	Hardness
Station-1	-0.3822	-0.1620	0.3388	0.3903	-0.1588	0.1736	0.0963	0.3395	0.0869
Station-II	-0.0217	0.1445	-0.1338	0.0688	-0.0484	-0.1885	-0.1634	-0.1839	-0.2464

Table. 36. Correlation Coefficient of Fungal population associated with Bud wilting condition in *Dendrobium* hybrid at Station-I and II

(This disease symptom was not incurred at other stations)

Variable	Air (F.p)	Water .F.p)	Tem (max)	Tem (min)	Humidity	Rain fall	pH value	T.Alkalinity	Hardness
Station-1	0.4597	-0.1151	0.2544	0.3505	-0.0882	-0.0234	-0.0990	0.1689	-0.1093
Station-II	0.2611	0.1036	0.1142	-0.0535	-0.1439	-0.0436	0.1521	0.0308	0.1364

Table. 37 Correlation Coefficient of bacterial population associated with Brown spot on flower condition in *Dendrobium* hybrid at Station-I and III (This disease symptom was not incurred at other stations)

Variable	Air (B.p)	Water (B.p)	Tem (max)	Tem (min)	Humidity	Rain fall	pH value	T.Alkalinily	Hardness
Station-1	-0.0546	-0.1717	0.0899	-0.3680	-0.4096	-0.2185	-0.1463	0.0136	0.1286
Stat-III	-0.2074	-0.1423	0.3362	0.1483	-0.2069	-0.2011	-0.2694	-0.2850	-0.3266

Table. 38 Correlation Coefficient of Fungal population associated with Brown spot on flowers in *Dendrobium* hybrid at Station-I and III (This disease symptom was not incurred at other stations)

Variable	Air (F.p)	Water .F.p)	Tem (max)	Tem (min)	Humidity	Rain fall	pH value	T.Alkalinily	Hardness
Station-1	-0.2431	-0.1185	0.0899	-0.3680	-0.4096	-0.2185	-0.1463	0.0136	0.1286
Stat-III	-0.2456	-0.2680	0.3362	0.1483	-0.2069	-0.2011	-0.2694	-0.2850	-0.3266

Table. 39. Correlation Coefficient of bacterial and Fungal population associated with Root rot in *Dendrobium* hybrid at Station-I

(This disease symptom was not incurred at other stations)

Variable	Air (B/F.p	Water (B/F.p	Tem (max)	Tem (min)	Humidity	Rain fall	pH value	T.Alkalinity	Hardness
Bact.popul	0.1455	0.4548	-0.1371	-0.0311	0.1935	**0.6180**	**-0.7800**	**-0.7177**	**-0.8068**
Fung.popu	0.1477	0.0142	-0.4062	0.1884	0.3419	**0.7306**	**-0.9516**	**-0.8285**	**-0.9560**

Table. 40 Correlation Coefficient of bacterial population associated with Stem rot in *Dendrobium* hybrid at Station-I

(This disease symptom was not incurred at other stations)

Variable	Air (B/F.p	Water (B/F.p	Tem (max)	Tem (min)	Humidity	Rain fall	pH value	T.Alkalinity	Hardness
Bact.popul	**0.5682**	-0.0867	**-0.6394**	-0.0796	**0.5110**	0.4060	**-0.5981**	**-0.5701**	**-0.6275**
Fung.popu	*	*	*	*	*	*	*	*	*

* Fungall population was not detected in the samples

From the Data on Correlation coefficients presented in Table 40 in is inferred that the bacterial population associated with Stem rot has a significant negative correlation with maximum temperature of atmosphere, pH, Total alkalinity and hardness; and a significant positive correlation with bacterial population of air and humidity.

3.5.2 Muliple Regression Analyses

In this statistical analyses, the degree of relation, whether the variables are independent or dependent, among the different variables and the bacterial / fungal populations associated with a said disease, was evaluated by conducting a multiple regression.

Data presented in Table 41 very clearly evidence that in general the bacterial population associated with different disease symptoms had a high degree of relation ship with most of the variables analysed during the period of study, at all the Stations under study.

Thus it may be seen from the Table that, the bacterial population associated with the yellowing of leaves had relatively a very high degree of relation with all other variables, at Station I (R square value 0.99837) and Station II(R square value 0.98168) compared to Stations III and V. Whereas in Station IV, there was a less degree of relation, indicating independent nature of variables at that Stations.

The bacterial population associated with the brown dots in leaves had a high degree of relation with all other variables, at all the stations excepting Station IV,

where there was a less degree of relation, Particularly Station III showed a high degree of relation (R square value 0.99481) compared to other stations.

The bacterial population associated with the water soaked leaves also had a high degree of relation with all other variables, at all the stations excepting Station V, where there was a less degree of relation, analyzed during the period of study as evidenced from the data presented in Table. Particularly Station III showed a high degree of relation (R square value 0.99464) compared to other stations.

The bacterial population associated with the bud wilting generally showed less degree of relation with all other variables, at both the stations I and II when incurred during the period of study. The bacterial population associated with the brown spots in flowers, which was recorded at stations I (R square vale 0.91210) and III, generally showed high degree of relation with all other variables, at both the stations.

The bacterial population associated with the water soaked stem, and dead root which were recorded at stations I (R square vale 0.94025 and 0.92565 respectively), and flower drooping,(R square value 0.99243) recorded at station II, showed high degree of relation with all other variables.

R squares values presented in Table 42 very clearly testify that, in general, the fungal population associated with different disease symptoms had a high degree of relation ship with most of the variables analysed during the period of study, at all the Stations under study.

Thus it may be seen from the Table that, the fungal population associated with the yellowing of leaves had relatively a very high degree of relation with all other variables, at Station V (R square value 0.96354) and Station IV(R square value 0.90084) compared to Stations 1 , II and III.

The fungal population associated with the brown dots in leaves had a high degree of relation with all other variables, at station II (R square value0.92943) followed by stations III. Whereas at Stations I and V, there was less degree of relation.

The fungal population associated with the water soaked leaves also had a high degree of relation with all other variables, at the stations III and IV(R square values 0.98493 and 0.95802 respectively) compared to stations I and II

The fungal population associated with the bud wilting showed high degree of relation with all other variables, at both the stations I(R square value 0.74272) and II(R square value 0.95589) when incurred during the period of study. The fungal population associated with the brown spots in flowers, showed high degree of relation with all other variables, at stations. I (R square vale 0.95464) while it recorded a less degree of relation with other variables at station III.

The fungal population associated with the dried stem and dead root which were recorded at stations I (R square vale 0.85371 and 0.98345 respectively), and flower drooping,(R square value 0.95973) recorded at station II, showed high degree of relation with all other variables.

3.5.3 Multiple Regression Model

Multiple regression analyses carried out using the data generated during the period of study, enabled us to derive Multiple Regression Model for prediction of a microbial population associated with any disease symptom. The following variables X_1.....X_9 were used for modeling.

X_1- Bacterial Population in Air

X_2- Bacterial Population in Water

X_3- Temperature of Atmosphere-maximum

X_4- Temperature of Atmosphere-minimum

X_5- Humidity

X_6- Rainfall

X_7- pH

X_8- Total Alkalinity

X_9- Hardness

Y= Bacterial /Fungal population associated with the disease symptom of interest

The model equations obtained based on computer analysis of data using SPSS package for various diseases, for each station is given below. → p 69

Table. 41. Multiple regression analyses carried out for evaluating the degree of relationship between the bacterial population associated with various diseases, and other variables, at different stations

Disease	Station	R Square value	Multiple R
Yellow Leaves	I	0.99837	0.99919
	II	0.98168	0.99080
	III	0.71712	0.84683
	IV	0.54912	0.74103
	V	0.70256	0.83819
Brown dots in leaves	I	0.89817	0.94772
	II	0.96524	0.98246
	III	0.99481	0.99740
	IV	*	*
	V	0.97548	0.98766
Leaf rot**	I	0.97186	0.98583
	II	0.74947	0.86572
	III	0.99464	0.99732
	IV	0.95802	0.97878
Bud wilting**	I	0.63709	0.79818
	II	0.45706	0.67606
Brown spot on flowers**	I	0.91210	0.95504
	III	0.71505	0.84560
Dried stem**	I	0.91179	0.95488

Stem rot**	I	0.94025	0.96967
Root rot**	I	0.92565	0.96211
Flower drooping**	II	0.99243	0.99621

- * Bacterial population not detected in the sample
- ** Disease sample not available in other Stations

Table. 42. Multiple regression analyses carried out for evaluating the degree of relationship between the Fungal population associated with various diseases, and other variables, at different stations

Disease	Station	R Square value	Multiple R
Yellow Leaves	I	0.86802	0.93168
	II	0.84894	0.92138
	III	0.75712	0.87013
	IV	0.90084	0.94913
	V	0.96354	0.98160
Brown dots in leaves	I	0.43218	0.65740
	II	0.92943	0.96407
	III	0.79109	0.88943
	IV	*	*
	V	0.60043	0.77488

Leaf rot**	I	0.83297	0.91267
	II	0.71153	0.84352
	III	0.98493	0.99244
	IV	0.95802	0.97878
Bud wilting**	I	0.74273	0.86182
	II	0.95589	0.97769
Brown spot on flowers**	I	0.95464	0.97706
	III	0.34994	0.59156
Dried stem**	I	0.85371	0.92396
Stem rot**	I	*	*
Root rot**	I	0.98345	0.99169
Flower drooping**	II	0.95973	0.97966

- * Fungal population not detected in the sample
- ** Disease sample not available in other Stations

3.5.3.1. Bacterial population

Yellow leaf

Station –I

$Y=-382641.6002x_1+-21980.76377\ x_2 + 21858221.471x_3+9227058.8447x_4 +3516490.2590\ x_5+114523.02079x_6+34953407.692x_7+-383550.7453x_8+-2648487.146x_9+ -1209465982$

Station –II

$Y=42424.601253x_1+5582.169380x_2+-188249.8932x_3+4530250.4813x_4 +564357.73449\ x_5 + -4194.173474\ x_6 + 33342506.0796\ x_7 + 217204.64258\ x_8 + -264200.5943\ x_9 + - 185485978.2$

Station –III

$Y = -50534681.46\ x_1 + -5586.721149\ x_2 + -343585593.3\ x_3 + -67708214.86\ x_4 + -60012339.92\ x_5 + 1194232.3146\ x_6 + -1637951381\ x_7 + 8212188.0975\ x_8 + 852382.2786\ x_9 + 30738632870.$

Station –IV

$Y = 250749446.168\ x_1 + -8780.530377\ x_2 + 573852667.69\ x_3 + 269194286.29\ x_4 + 5299667.8412\ x_5 + 17486428.848\ x_6 + -4051019185\ x_7 + 12895498.875\ x_8 + -6017232.766\ x_9 + 5659690185$

Station –V

$Y = 1219969.4271x_1 + -311.358605x_2 + 20938462.470\ x_3 + 6510423.0043\ x_4 + 121202.83466\ x_5 + 400344.77625\ x_6 + 9901469.064\ x_7 + -1051898.050\ x_8 + 1905761.5917\ x_9 + -1513752048$

2.Brown Dots (B.P)

Station- I

$Y = 2880.88x_1 + 58.64x_2 + 19450.0x_3 + -63286.73\ x_4 + 1650.89x_5 + -47.497x_6 + 119604.33x_7 + 237.40x_8 + -3444.99\ x_9 + -111109.6178$

Station- II

$Y = -50373.45\ x_1 + 4107.16x_2 + 1677601.57x_3 + 656322x_4 + 384043.82x_5 + 59212.75x_6 + -1783707.918x_7 + 96709.2\ x_8 + -5725.699\ x_9 + -100478753.1$

Station- III

$Y = 243127.33\ x_1 + -18.51\ x_2 + 970564.12\ x_3 + 1232858.89\ x_4 + 447282.69\ x_5 + -4754.089\ x_6 + 37978791.486\ x_7 + -92283.88\ x_8 + 38796.05\ x_9 + -396842985.9$

Station- IV

No Sample.

Station- V

$Y = 616.66\ x_1 + -0.8291\ x_2 + -1089.09\ x_3 + 1329.35\ x_4 + -44.395\ x_5 + 53.31\ x_6 + -15292.83\ x_7 + 13.999\ x_8 + 89.82\ x_9 + 106920.232$

3.Leaf rot (B.P)

Station-I

$Y = -2784007.86\ x_1 + 72469.61\ x_2 + -98701533\ x_3 + -33110965.25\ x_4 + -14867459\ x_5 + -61068\ x_6 + -721152654.0\ x_7 + 1531656.667\ x_8 + -8350592.63\ x_9 + 11391427017$

Station-II

$Y = 15811754.72\ x_1 + -178632.63\ x_2 + -626328944.9\ x_3 + 914645353.44\ x_4 + 105388714.2\ x_5 + -20408340.1\ x_6 + 3324228772.8\ x_7 + -24384222.54\ x_8 + 12011751.76\ x_9 + -34521070914$

Station-III

$Y = 187010.36\ x_1 + -11.656\ x_2 + 760489.377\ x_3 + 907383.34\ x_4 + 330928.08\ x_5 + -3179.34\ x_6 + 27817037.54\ x_7 + -67794.39\ x_8 + 28.20.33\ x_9 + -292763122.1$

Station-IV

$Y = -21869764.12\ x_1 + 17404.6\ x_2 + -682330399.8\ x_3 + -18904010\ x_4 + 32695142.99\ x_5 + -12122560.72\ x_6 + 4035626592.3\ x_7 + -3843681.75\ x_8 + 11721762.279\ x_9 + -13074644555$

Station –V

No Sample

4.Dried Stem. B.P.

Station-I

$Y = 445467.32\ x_1 + 8570.99\ x_2 + 4083533.75\ x_3 + -5328679.42\ x_4 + 747217.68\ x_5 + 4278.41\ x_6 + 12891037.47\ x_7 + 8229.30\ x_8 + -239650.10\ x_9 + -165127191.1$

5.Bud Wilting

Station I

$Y = -115922379.2\ x_1 + -849823.6849\ x_2 + 289881811.46\ x_3 + 1156368968.6\ x_4 + 64060881.758\ x_5 + -242639.5343\ x_6 + -4654331374\ x_7 + -824851.5616\ x_8 + 100484521.43\ x_9 + -7152919094$

Station-II

$Y = -2960.50\ x_1 + -0.3851\ x_2 + -18294.87\ x_3 + -3935.60\ x_4 + -3324.85\ x_5 + 30.03\ x_6 + -31713.65\ x_7 + 366.56\ x_8 + -464.93\ x_9 + 1244268.9275$

6.Stem rot

Station-I

$Y = 32771918.22\ x_1 + 405074.01\ x_2 + -835918711.3\ x_3 + 394150989.75\ x_4 + -30100622.7\ x_5 + -10917489.2\ x_6 + -388512414.6\ x_7 + 4364679.7\ x_8 + -61222516.28\ x_9 + 24929652706$

7.Dead roots

Station-1

$Y=118621.62130x_1+4535.100317x_2+-217600.4830x_3+-2607647.505x_4$ $+6947.359555x_5 + 553.431713x_6+ -8011314.492x_7+ 30471.894049x_8+$ $239697.1081x_9+141061393.38$

8.Brown Spot on flower

Station-I

$Y=-1796.226250x_1+ -13.920185x_2+ -26439.06031x_3+ -100228.6803x_4+-$ $15573.29828x_5 + -300.369797x_6+ 531734.69092x_7+ -252.502043x_8+$ $12828.74629x_9+ 981482.69869$

Station-III

$Y = 12847.55\ x_1 + -1.64\ x_2 + 112626.76\ x_3 + 17495.85\ x_4 + 31911.68\ x_5 + -1641.74$ $x_6 + 184609.36\ x_7 + -2383.50\ x_8 + 1417.94\ x_9 + -8086601.695$

9.Flower drooping

Station-II

$Y = -19029.54555x_1 + -1967.181580x_2 + -599187.8215x_3 + -997141.0599x_4 + -$ $273190.9194x_5 + 25265.439707\ x_6 + -764220.8510x_7 + -25399.12871x_8 +$ $14602.69127x_9 + 78337388.$

3.5.3.2. Fungal Population

1.Yellow Leaf

Station-I

$Y = 4555916.8518\ x_1 + 79823.934546\ x_2 + 29851366.683\ x_3 + -43951455.9\ x_4 + 6653363.3506\ x_5 + -324271.1878\ x_6 + 466441865.65\ x_7 + -297495.3793\ x_8 + 10379445.11\ x_9 + -3711936401$

Station-II

$Y = -152270.6497\ x_1 + 380803.83148\ x_2 + -4496395.395\ x_3 + 14600943.110\ x_4 + 1346947.5819\ x_5 + 225603.9749\ x_6 + 30919020.873\ x_7 + -124872.5203\ x_8 + 420057.0009\ x_9 + -525515719.3$

Station-III

$Y = 34908.62\ x_1 + -3503.96\ x_2 + -38792.0\ x_3 + -1878.57\ x_4 + -10125.78\ x_5 + -434.3\ x_6 + -385140.81\ x_7 + 1021.2\ x_8 + 2707.88\ x_9 + 5003810.478$

Station-IV

$Y = -11562.61992\ x_1 + 0.028918\ x_2 + -92893.20409\ x_3 + -31142.64826\ x_4 + -810.896103\ x_5 + -1576.002983\ x_6 + 264864.96967\ x_7 + 1849.382230\ x_8 + 314.113135\ x_9 + 1468964.9526$

Station-V

$Y = 353621.56\ x_1 + -39.698\ x_2 + -1044220.61\ x_3 + 243761.31\ x_4 + 280136.34\ x_5 + -64837.52\ x_6 + -8716844.01\ x_7 + -9109.97\ x_8 + 27961.39\ x_9 + 70073425.89$

2.Brown dots on leaves

Station-I

$Y = -808886.2781\ x_1 + 159337.68120\ x_2 + 37469996.935\ x_3 + -58819940.50\ x_4 + 15462007.360\ x_5 + 424920.5288\ x_6 + 6836702.6441 x_7 + -513403.8446\ x_8 + 3459015.9312\ x_9 + -1121869178$

Station-II

$Y = -343.797763 x_1 + -2250.46841 x_2 + -12911.17442 x_3 + -14870.95951 x_4 + 6104.030342 x_5 + 683.809451 x_6 + 121890.17467 x_7 + 2482.238295 x_8 + 2154.653565 x_9 + 153416.38338$

Station-III

$Y = 29258.404593 x_1 + -2928.938484 x_2 + -28650.07578 x_3 + -2514.397542 x_4 + -7206.742772 x_5 + -313.675797 x_6 + -329500.5234 x_7 + 881.154263 x_8 + 2225.093065 x_9 + 4025016.0300$

Station-IV

No fungal population.

Station-V

$Y = -57.929754x_1 + -0.005518x_2 + -731.531116x_3 + 46.570643x_4 + -22.492158x_5 + -6.033031x_6 + -5820.146888x_7 + 37.675425x_8 + -47.452970x_9 + 65439.150292$

3. Leaf rot

Station-1

$Y = -8874.004586x_1 + -267.999225x_2 + -165909.4653x_3 + 283125.08076x_4 + 6892.213387x_5 + -3816.001341x_6 + 534377.31263x_7 + -1660.013208x_8 + 10802.68908x_9 + -5472359.689$

Station-lI

$Y=-70.885463x_1+2691.874134x_2 + -68309.34371x_3 + 155089.83619x_4 + 19187.714939x_5 + -2434.040839x_6 + 289703.96628x_7+-4102.946205x_8 + 450.505650x_9 + -5028299.312$

Station-III

$Y = -1728.047900x_1+111.940204x_2+ -6809.783302x_3 + 11825.860518x_4 + 300.616309x_5 + 250.063378x_6 + 450526.00969x_7 + -720.030644x_8 + 72.435524x_9 + -3620595.610$

Station-1V

$Y = -54.674410x_1 + 0.043512x_2 + -1705.825999x_3 + -47.260027x_4 + 81.737857x_5 + -30.306402x_6 + 10089.066481x_7 + -9.609204x_8 + 29.304406x_9 + -32686.61139$

Station-V

No fungal population.

4.Dried stem

Station-1

$Y = -50617.05740x_1 + -1681.169695x_2 + -1374269.042x_3 + 1936819.3105x_4 + 14497.360243x_5 + -26954.94021x_6 + 2770569.0299x_7 + -8532.821394x_8 + -79844.34024x_9 + -20128945.53$

5.Bud wilting

Station-1

$Y = 2739.379709x_1 + -93.509731x_2 + -21300.37732x_3 + 44209.846572x_4 + 7299.119731x_5 + 381.607010x_6 + -292826.0337x_7 + 696.534684x_8 + 4133.323376x_9 + 2238604.0454$

Station-II

$Y = 977.915356x_1 + -4.274969x_2 + 4954.882068x_3 + -2694.718415x_4 + 1276.733962x_5 + -14.603268x_6 + 30998.514361x_7 + -203.110557x_8 + 470.074160x_9 + -495731.0837$

6. Stem rot

No population

7.Root rot

Station-1

$Y = -29999.02441x1 + -422.274776x_2 + -160086.0567x_3 + 146867.10798x_4$

$+ -27349..04225x_5 + 540.850402x_6 + -1695597.223x_7$

$+ 2424.478417x_8 + -18441.32301x_9 + 18863086.868$

8. Brown spot on flower

Station-1

$Y = 456.713911x_1 + -30.074047x_2 + -15844.24466x_3 + -27703.24741x_4 + 7250.111973x_5 + -20.603933x_6 + 149531.19733x_7 + 29.333072x_8 + -3909.083990x_9 + 752479.80576$

Station-3

$Y= -7501.739880x_1 + -646.725476x_2 + 25948.424340x_3 + -241.289562x_4 + 2792.39921x_5 + -115.068002x_6 + 13184.566417x_7 + 307.264840x_8 + -185.258652x_9 + -1041388.328.$

9.Flower drooping

Station-2

$$Y = -81032.90744x_1 + 24014.762084x_2 + -262416.2091x_3 + 21675.230797x_4 + -21485.39707x_5 + 13510.224610x_6 + 345723.55110x_7 + -51056.23891x_8 + 3666.863176x_9 + 13237439.928.$$

DISCUSSION

4. Discussion

During the period of investigation, over one full year covering all seasons, hybrid *Dendrobium* under cultivation at two different districts, Ernakulam and Kanyakumari, showed various disease symptoms. Some of the disease symptoms were not so far described by previous investigators and hence it was very difficult to classify them under earlier reported disease symptoms. In general, the hybrid *Dendrobium* showed morphological changes in leaves, flowers, bud, stem and root, during certain period of study, which in due course of time after their first appearance, led to the loss of that portion of the plant and consequent loss in the flower productivity in the farm. Some diseases even led to the death of plants.

Data presented under results chapter very clearly testify that, on the whole, Kanyakumari District of Tamil Nadu recorded very less percentage of diseased plants in the study population during the period of study, compared to Ernakulam District located in Kerala, which recorded relatively a higher percentage of diseased plants. Among the five different stations, selected for monitoring the occurrence of various diseases in hybrid *Dendrobium* plants, Station I , located in Ernakulam District, showed comparatively larger population of infected plants (25.72% in July'97) and Station IV, located in Kanyakumari District of Tamil Nadu, showed comparatively lesser population of infected plants (1.2% February'97) during the period of study.

Even among the three stations selected in Ernakulam District, Station II and Station III recorded very less diseased population (4.64%,October'97 and 3.68%, June'97 respectively). On the other hand both stations selected in Nagercoil of

Kanyakumari District of Tamil Nadu showed only very less percentage of diseased plants.

These results suggest that the percentage of diseased plants in the population, under cultivation, is characteristic of the stations selected for the study, and largely governed by the geographical location of the station and other environmental factors. A cursory glance of the geographical map of South India would indicate the location of Ernakulam on the sea coast of Arabian sea and the location of Nagercoil, in Kanyakumari Dt (Fig.1) slightly interior to sea coast, in the main land, at the tip of peninsular India. These locations experience a different pattern of wind flow, rain fall, aerial microflora composition and quality of water used for irrigation of orchid plants, which could have influenced the occurrence of diseases.

During the course of study, in general, the percentage of infected plants in Station **I** were relatively more during the rainy season (south west monsoon period), *ie* during the months of June to September '97 compared to other seasons. In Station **II** , in general, hybrid *Dendrobium* plants were healthy, since only less than 5% of the total plants were infected by diseases during the entire period of study. Nevertheless, months of December'96, July'97 and October '97 witnessed relatively more percentages of diseases in the plant population compared to other months. In general, hybrid *Dendrobium* plants were healthy in Station **III,** and only less than 4% of the total plants were infected by diseases during the entire period of study. More plants were diseased during November'96 and June'97 compared to February'97, March'97 and October '97 which witnessed relatively less percentage of diseased

plants in the population. Station **IV,** , showed very less number of infected (less than 1.5%) hybrid *Dendrobium* plants during the entire period of study. Comparatively more plants were infected during February'97 than in other months. **Station V**, showed very less number of infected (less than 1.5%) hybrid *Dendrobium* plants during the entire period of study. More plants were infected during January'97, when compared to other months.

In general the hybrid orchids, in Station I, showed **eight** different disease symptoms which included **yellowing of leaves, brown dots on leaves, bud wilting, Root rot, dried stem, brown spot on flowers, Stem rot, and leaf rot,** during the course of the study. Among the different diseased conditions, yellowing of leaves, brown dots on leaves and root rot were observed among the diseased plants throughout the period of study. Among these three diseases the yellowing and brown dots in leaves were predominant. Whereas, the root rot disease was observed with a lesser population. Other disease conditions were observed only during certain period of the study. Altogether **five types** of diseases including **yellowing of leaves, brown dots on leaves, bud wilting , leaf rot and flower drooping,** were observed in Station II, during the period of study. Among these diseases, only yellowing of leaves was observed throughout the year. Altogether **five** types of diseases, including **yellowing of leaves, brown dot on leaves, brown spots on flower, bud wilting, and leaf rot,** were observed in hybrid *Dendrobium* in Station III during the period of study. Among these diseases only yellowing of leaves was observed throughout the year. Together **three** types of diseases, including **yellowing of leaves, brown dot on**

leaves, and leaf rot, in hybrid *Dendrobium* plants, were observed in Station IV, during the period of study. Among these diseases only yellowing of leaves was observed throughout the year. In Station-V only **two** types of disease, including **yellowing of leaves, and brown dot on leaves**, in hybrid *Dendrobium* plants, in, were observed during the period of study. Among these diseases only yellowing of leaves was observed throughout the year.

Yellowing of leaves in *Dendrobium* hybrids was observed throughout the year in all the Stations selected for Study, with a minimum of 2.4 % in March'97 to a maximum of 34.2% in June'97 in the plant population. The yellowing condition was predominant during June to September (31.8%), the rainy season in the west coast of India. Comparatively the incidence of this disease condition was predominant during the period from May'97 to September'97 in Station II. Yellowing of leaves, which was recorded throughout the year in Station III, showed fluctuation in their percent occurrence from minimum to maximum, over the period of year, unlike in other stations. Whereas Stations IV and V recorded maximum percent of disease populations in January'97. Results obtained in then present study suggest that yellowing of leaves is a disease common to hybrid *Dendrobium*, irrespective the geographical location of the orchid farm, and governed in general by the environmental conditions prevailing around the station of interest.

From the results it is inferred that Brown dots on leaves in *Dendrobium* hybrids is another major disease of concern in orchid farms as it was recorded in significant number both in Ernakulam and Nagercoil areas in all the stations except

station IV , during the period of study. It was generally not associated to rainy season, as it was recorded at low levels (0.6-0.8%) when present during rainy months, June and July'97 in all the stations. Interestingly this disease showed a progressive increase in the population during their occurrence.

Leaf rot in hybrid *Dendrobium* was observed in four stations except station V. This disease forms the third major disease among the disease recorded during the period of study in the orchid population which was incurred at certain period of the period of study alone. Except in station I, in other stations, this disease was recorded only once in the year.

From the data presented under results chapter it is inferred that other diseases including Bud wilting, Brown spot on flowers, flower drooping, Stem rot, Dried stem and Root rot are only seasonal diseases and governed primarily by the environmental factors prevailing at that stations and period of occurrence, since they were incurred in the hybrid *Dendrobium* populations only at certain months of the period of study and was not recorded through out the year.

Maximum Temperature of Atmosphere in the 5 stations did not show much variation during the period of study (Table-2) In general maximum atmosperic temperature varied between 28.0°C and 33.8°C for the stations indicating the characteristic ambient nature of temperature of atmosphere in the study area. These variations were not wide, and only at few occasions the temperature was above 30°C. However, higher temperatures around 33.0°C was observed with the stations located at Ernakulam District during summer compared to Kanyakumari Disrtrict. Usually

Ernakulam is very hot during summer compared to Nagercoil, although it is on sea coast. From the statistical analysis of correlation coefficient analysis, in general, excepting few occasions, maximum temperature was not observed as a major influencing factor on the microbial population associated with disease, since a significant negative correlation was recorded only with bacterial population of brown dot and stem rot in station II, and I, respectively, and with fungal population associated with dried stem at station I. Although not significant, the maximum temperature values had completely a negative correlation with bacterial population associated with yellow leaves in all stations. Whereas, in the case of fungal population associated with yellow leaves, except in station III, in all other stations there was a positive correlation with the maximum temperature, even though not significant. On the other hand, all other diseases, incurred in the stations during the period of study showed interestingly either positive or negative correlation for both bacterial and fungal populations with maximum temperature, except for brown dots on leaves and dried stem at station I. In fact whether it is bacteria or fungi, both as microbes, would show in general a similar response to variation in an environmental parameter such as atmospheric temperature. Whereas, in the present study, there were occasions when this was not exemplified. Of course since such cases did not record significant correlation emphasize may not be warranted. May be some other environmental factor influence this kind of result which need further detailed investigation.

Minimum Temperature of Atmosphere recorded in the 5 different stations, in general temperature, varied between 21.0°C and 27.6°C for the stations. Higher temperatures around 27.6° C was observed with the stations located at Kanyakumari Disrtrict during January and February'97 compared with Ernakulam District, which recorded very low temperatures around 24.0°C. Variations in the minimum temperatures were high in Kanyakumari District compared to Ernakulam District during the course of study. Statistical analyses did not show any significant correlation of minimum temperature with microbial population indicating minimal influence of lower temperatures on micobial populations associated with diseases, inspite of negative and positive correlation observed during the period of study.

Humidity of Atmosphere in the 5 different stations did not show much variation and presented very similar levels for the stations, during the period of study. In general humidity varied between 71% and 96% for the stations. Higher levels of humidity around 90% was observed with all the stations during July'97. Whereas, all the three stations of Ernakulam District recorded humidity levels above 90% during December'96 and August'97 also. Stations II and III recorded highest level of 96% humidity during August'97, while stations of Kanayakumari district recorded only 90% humidity as maximum compared to the stations of Ernakulam. Similarly lower levels of humidity (71% and 74% respectively for Kanyakumari and Ernakulam districts) were observed with all the stations during April'97. Humid warm atmosphere is most essential for the growth of most of the tropical orchids, which do not have well established root system. Humidity should be maintained not

less than 30% at night and 80% during day time. Humidity is an important factor that could influence variations in microbial population. Thus although not statistically significant , yet the bacterial population associated with yellow leaves showed a positive correlation with humidity at all the stations, in contrast to maximum atmospheric temperature. In general humidity showed a positive correlation with yellow leaves, brown dots on leaves, leaf rot, flower drooping, root rot and stem rot, except for bud wilting and brown spot on flowers, where the correlation was negative. However, a significant positive correlation recorded with bacterial and fungal populations associated with leaf rot, brown dot and stem rot indicate the strong influence of this parameter on the expression of disease in plant populations. Increase in humidity levels might have contributed to the rapid proliferation of microbial population in diseased plants, by providing conducive environment for infection.

Rainfall at the 5 different stations showed much variation during the period of study (Table- 5). In general, rainfall varied between 4 - 190cm in Ernakulam District and between 4 - 105 cm in Kanyakumari District during the period of study. High levels of rainfall around 168cm was observed during July'97 in the stations of Ernakulam District, and during September'97 in all the stations of Kanayakumari district. There was no rainfall during the months of January'97 and February'97 in Ernakulam District and during February and March'97 in Kanayakumari district.

Rainfall is an important factor that could influence variations in microbial population and outbreak diseases in any farm. Thus, although not statistically significant, yet the fungal population associated with yellow leaves showed a negative

correlation with rainfall at all the stations. In general, rainfall showed a significant positive correlation with bacterial population associated with yellow leaves at station I, leaf rot at station I and III, and for the bacterial and fungal populations associated with flower drooping at station II, and dead root at station I. Although not significant, the microbial population (bacterial and fungal) associated with dried stem and brown spots on flowers showed a negative correlation with rain fall.

Data obtained for the pH of the water used in the 5 stations indicate that the water was slightly alkaline in nature during certain months of study, and varied in general, between 7.30 and 8.71. pH influence the survival of microbial population in any environment and often promote floral succession. In the present study response to pH by the microbial population varied at different stations. The bacterial population showed a significant positive correlation with pH in the case of leaf rot at station III, while the fungal population showed significant positive correlation in the case of yellow leaves at station I, leaf rot at stations III and IV. Whereas a significant negative correlation with pH was recorded for the bacterial populations associated with yellow leaves in station I and II, both bacterial and fungal populations associated with flower drooping at station II, dead root and stem rot in station I. In general pH showed a positive correlation with both bacterial and fungal population associated with brown dot on leaves and leaf rot diseases compared to others. The present study suggest that influence of pH on the disease, and nature of interrelationship with microbial population is dependent on the nature of the disease, and the possible causative microorganism involved in the disease.

In general, total alkalinity varied between 32 ppm and 376ppm for the stations. Lowest and highest levels of alkalinity were recorded in Station I during July'97 and May'97 respectively, compared to other stations. Further Total alkalinity of water was high in Station I and Stations IV and V showed relatively lesser levels of total alkalinity, except for the months of February'97 (344ppm) and April'97(200ppm) at Station IV, and for the month of April'97(200 ppm) at Station V. The total alkalinity of water at Station III showed inconsistency and recorded fluctuations unlike in other Stations.

In the present study response to total alkalinity of water used for irrigation by the microbial population varied at different stations. The bacterial population showed a significant positive correlation with total alkalinity in the case of yellow leaves and brown dots on leaves at station III and II respectively, while the fungal population showed a significant positive correlation in the case of yellow leaves at station IV, brown dots on leaves at stations II and III, and leaf rot at stations IV Whereas a significant negative correlation with total alkalinity was recorded for the bacterial populations associated with yellow leaves in station I , leaf rot and stem rot at station I; both bacterial and fungal populations associated with flower drooping at station II, and dead root in station I. In general, total alkalinity showed a positive correlation with both bacterial and fungal population associated with brown dot on leaves and leaf rot diseases compared to others. The present study suggest that influence of total alkalinity on the disease, and nature of interrelationship with microbial population is

dependent on the nature of the disease and the possible causative microorganism involved in the disease, very similar to that of pH.

Hardness of water samples used in the 5 varied between 10 ppm and 140 ppm. Water samples of Station II recorded higher hardness compared to other stations. Whereas, water samples of Station V recorded relatively a lesser level of hardness during the period of study. The hardness of water at Station III showed inconsistency and recorded wide fluctuations unlike in other Stations. In the present study, response to hardness of water used for irrigation by the microbial population varied at different stations. The bacterial population showed a significant positive correlation with hardness in the case of yellow leaves and brown dots on leaves at station III , while the fungal population showed a significant positive correlation in the case of leaf rot at station IV. Whereas, a significant negative correlation with hardness was recorded for the bacterial populations associated with yellow leaves in station I and II, leaf rot and stem rot at station I; both bacterial and fungal populations associated with flower drooping at station II, and dead root in station I. In general hardness showed a positive correlation with both bacterial and fungal population associated with brown dot on leaves and leaf rot diseases compared to others. The present study indicate that hardness of water influence the disease, and nature of interrelationship with microbial population is dependent on the nature of the disease and the possible causative microorganism involved in the disease.

Bacterial population of atmospheric microbial flora at the 5 stations of the study area showed comparatively very less population and varied from 3 CFU to 82

CFU. In general the bacterial population of atmospheric flora was comparatively high in Stations IV and II and lesser in Station I, III and V. Fungal population of atmospheric microbial flora at the 5 stations of the study area showed very less population. In general the population varied from 1 CFU to 26 CFU In general the fungal population of atmospheric flora was comparatively high in Stations IV and V and lesser in Station I, II and III. A significant positive correlation was observed between aerial bacterial population and bacterial population associated with yellow leaves and stem rot at station II, and brown dots on leaves in station Other wise, although not significant statistically, there was a negative correlation , in general, with the bacterial and fungal population associated with brown dots on leaves, leaf rot, brown spots on flowers. Whereas yellow leaves bacterial population alone showed positive correlation at all stations. Similarly excepting few occasions fungal population associated with yellow leaves, dried stem and bud wilting showed positive correlation. This unusual trend of negative correlation could not be explained as the probable reason for such relation ship is not understood In fact since no similar studies have been reported it is very difficult make any comparison.

The airborne population of bacteria appears to be largely derived from the foliage of crop and wild plants. Sampling of bacterial cells from the air above crops has shown that approximately four times greater numbers of cells occurred over a crop of lucerne than over bare soil at the same time (Lindemann *et al.* 1982). This and other evidence suggests that plant foliage represents a major source of air borne bacteria, including pathogens.

Bacterial population of water used for irrigation varied from 6 CFU to $7.6x10^4$ CFU/ml. Whereas fungal population of water varied from 10 CFU/ml in Station I (December'96) to 1.0 $x10^7$ CFU/ml in Station IV (March'97) (Table..). In general the fungal population of water was comparatively high in Stations IV and V and lesser in Station I, II and III. A significant positive correlation was observed between aerial bacterial population and bacterial population associated with brown dots on leaves and dried stem at station I. Other wise, although not significant statistically, there was a negative correlation , in general, with the bacterial and fungal population associated with yellow leaves, brown dots on leaves, leaf rot, flower drooping, brown spots on flowers and dead stem.. This unusual trend of negative correlation could not be explained as the probable reason for such relation ship is not understood. Probably this might be due to the cumulative influence of several environmental factors on the proliferation of microbial population in diseased plants and may not be a direct influence of microbial population of water. In fact since no similar studies have been reported it is very difficult make any comparison.

Bacterial population of fresh leaves of the hybrid Dendrobium at the 5 stations of the study area showed wide variation from a minimum of 4 CFU in Station V (January'97) to a maximum of 4.0 $x10^7$ CFU in Station IV (October'97). Results presented in the study clearly indicated that stations of Nagercoil had more bacterial population, compared to Ernakulam, during the period from March, April and in Ocober'97, besides recording very high populations in the range of $x10^{7.}$ October is a period of North east monsoon and that might have influenced the bacterial population

at this place. Whereas Ernakulam stations showed comparatively higher bacterial populations during July to September'97, southwest monsoon period. Bacterial population associated with fresh leaves seems to be influenced by seasons and hence there is wide variation in the plants.

The aerial surfaces of plants are colonized by a characteristic micro flora consisting of bacteria, yeasts and filamentous fungi. Although representatives of each of these major groups may be found on leaves at any time of the year, there is an underlying seasonal succession (Blakeman 1985). As leaves open at the start of the growing season, the initial colonists are predominantly bacteria. Yeasts dominate during the middle of the growing season, followed by filamentous fungi, spores of which commence to germinate as leaves pass their peak of activity. Hyphae of filamentous fungi may later enter the tissues as minor pathogens causing yield losses (Dickinson 1981). Bacteria may not be entirely confined to the surface of the plant; internal populations may be present in intracellular spaces of sub stomatal chambers. Surface population of bacteria may become internal as a result of heavy rain inducing water-soaking of leaf tissue. This may be the cause of observed increases in out break of brown spot *(Pseudomonas syringae)* of been following heavy natural rain or simulated rain applied from a substantial height above the crop (Hirano & Upper 1988).

Fungal population of fresh leaves of the hybrid *Dendrobium* at the 5 stations of the study area showed wide variation from 20 CFU/cm^2 in Station IV (August'97) to 1.5 x10^6 CFU in Station III (June'97). Among the 5 Stations, Station IV recorded

comparatively higher levels of fungal population associated with fresh leaves in the range of X 10^5 On the other hand Station III, in spite of a maximum number during June'97, generally recorded low level of fungal population during other period. Of the Stations of Ernakulam District Station I recorded relatively high levels of fungal population, particularly during rainy season. It seems that variation in seasons and environmental conditions prevailing in the individual stations might influence the fungal population associated with fresh leaves.

Bacterial population associated with yellowing of leaves varied, in general, from 5.0 x10^3 CFU in Station I (November'96) to a 1.56 x 10^9 CFU in Station IV (November'96). In general bacterial population associated with yellowing of leaves was higher than what was recorded with fresh leaves. However, at certain instances such as in May and July'97 at station III, and in October'97 at station IV it was less than that of the fresh leaves. Perhaps during those months the fresh leaves population might not have harbored pathogens responsible for yellowing of leaves and could have been a different flora. Statistical analyses on correlation coefficients indicated that significant negative correlation exist between bacterial population and pH (at Stations I & II), Total alkalinity (Station I) and hardness(Stations I & II). Whereas a significant positive correlation was recorded between bacterial population, and rainfall (at Station I), aerial bacterial population(Station II), alkalinity and hardness(at Station III). At all other instances, irrespective of the stations and variables there was no significant correlation whether it was positive or negative. These results testify to

the fact that environmental variables do influence the intensity of disease in a said plant population.

Comparatively the fungal population associated with yellowing of leaves was high in Station I, II and V during the rainy season (June-August) and Stations III and IV recorded lesser fungal population. In general fungal population associated with yellowing of leaves was higher than what was recorded with fresh leaves. However at certain instances such as in July'97 at station I, and in March, May, August and October'97 at stationIV it was less compared to that of the fresh leaves. Perhaps during those months the fresh leaves population might not have harbored pathogens responsible for yellowing of leaves and could have been a different flora.Statistical evaluation of data indicated that, significant positive correlation exist between fungal population, and pH and Total alkalinity only at Stations IV; and at all other instances, irrespective of the stations and variables there was no significant correlation whether it was positive or negative.

These statistical evaluations suggest that rainfall and quality of water in terms of pH , alkalinity and hardness significantly influence the intensity and microbial populations associated with yellowing of leaves in Dendrobium population.

Bacterial population associated with brown dots in leaves varied, in general, from 4.0 $x10^3$ CFU in Station II (December'96) to a 2.48 x 10^7 CFU in Station III (August'97). In general bacterial population associated with yellowing of leaves was higher than what was recorded with fresh leaves. However in February'97 at station I it was less than that of the fresh leaves. Maximal bacterial population associated with

brown dots was observed during the month of August'97 in both Stations II and III. Relatively, in all the 3 Stations of Ernakulam District this disease was observed during the month of December'96. While in Station I this disease was observed continuously during the period from December'96 to March'97, in Station III it occurred continuously during the period from November'96 to January'97. Whereas, in the case of Station IV the occurrence of this disease was discontinuous and occurred more in number during monsoon months (August and September'97). Statistically a significant positive correlation exist between bacterial population of brown dots and bacterial population in water(Stations I); Total alkalinity(Station II) humidity and pH(Station III) and aerial bacterial population(Station V). On the other hand a significant negative correlation exit between Bacterial population, and maximum atmosphere temperature(Station II). Fungal population associated with brown dots in leaves at the different stations of the study area varied, in general, from 4.0 $x10^3$ CFU in Station V(January'97 only occurrence during the period of study) to a maximum of 2.8 x 10^8 CFU in Station I (December'97). In general fungal population associated with brown dots on leaves was higher than what was recorded with fresh leaves. However it was less than that of the fresh leaves during February'97 in Station I.

Statistically, a significant positive correlation exist only between fungal population, and Total alkainity at Stations III and V. At all other instances, irrespective of the stations and variables there was no significant correlation whether it was positive or negative.

These statistical evaluations suggest that humidity and quality of water in terms of pH, and alkalinity significantly influence the intensity and microbial populations associated with brown dots in leaves in *Dendrobium* population.

Bacterial population associated with leaf rot, which was incurred only during few months at the different stations of the study area ,did not show much variation. Both minimum (3.5 $x10^5$ CFU/cm in August'97) and the maximum (1.03 x 10^9 CFU in July'97) level of bacterial population were recorded in Station I (Table..). In Station V this disease was not recorded. In general bacterial population associated with leaf rot was higher than what was recorded with fresh leaves except in station I during August'97. Correlation coefficients obtained for statistical evaluation of data concerned indicated a significant positive correlation between bacterial population, and rainfall,(Station I); humidity and pH(Station III). Fungal population associated with leaf rot, which was incurred only during few months, at the different stations of the study area, did not show much variation and varied from 7.0 $x10^3$ CFU to 8.0 x 10^5 CFU/cm, In general fungal population associated with leaf rot was higher than what was recorded with fresh leaves except in station IV during February'97. Statistically a significant positive correlation was recorded between fungal population, and humidity(Station III), pH (Station III & IV) and Total alkalinity and Hardness (Station IV). At all other instances, irrespective of the stations and variables there was no significant correlation whether it was positive or negative.

Results of Statistical analyses very clearly evidence the significant influence of environmental parameters rainfall and humidity, and quality of water used for

irrigation namely pH, Total alkalinity and Hardness on the intensity of leaf rot disease and microbial population associated with the disease.

Other diseases including Bud wilting, Brown spot on flowers, flower drooping, Stem rot, Dried stem and Root rot were recorded in the hybrid *Dendrobium* populations only at certain months of the period of study and was not recorded through out the year. They harbored considerable level of microbial populations, both bacterial and fungal, when ever they were incurred, as it may be seen from the results presented in the previous chapter. In genera, bacterial and fungal population associated with Bud wilting, Brown spot on flowers, flower drooping, Stem rot, Dried stem and Root rot were higher than what was recorded with fresh leaves, except in July'97 in station I,when the fungal population was less for bud wilting. Perhaps during those months the fresh leaves population might not have harbored pathogens responsible for yellowing of leaves and could have been a different flora.

Statistical analyses revealed interesting observations with reference to the type of interrelationship between the microbial populations associated with the above said diseases and other variables. Thus a significant positive correlation was recorded between bacterial populations of dried stem, and that of water; and a significant negative correlation between fungal population and maximum temperature of atmosphere at Station I; a significant negative correlation between bacterial and fungal population of dead root with pH, Total alkalinity and hardness, and a significant positive correlation between bacterial and fungal population, and rainfall;

a significant negative correlation between bacterial population associated with stem rot and maximum temperature of atmosphere, pH, Total alkalinity and hardness; and a significant positive correlation with bacterial population of air and humidity; a significant positive correlation exist between the bacterial population associated with flower drooping and rainfall and a significant negative correlation with pH, Total alkalinity and hardness of water

Results of Statistical analyses very clearly evidence the significant influence of environmental parameters atmospheric temperature, rainfall and humidity, and quality of water used for irrigation namely pH, Total alkalinity and Hardness, and at few occasions even the microbial populations present in the atmosphere ands water used for irrigation on the intensity of these diseases and the microbial population associated with them.

Bacterial flora associated with the samples of diseased plants showed interesting observations, in all the selected Stations of study, located in both the Ernakulam and Kanyakumari Districts, during the period of study. In general, the bacterial flora was composed of species of *Aeromonas*, *Alkaligenes*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Vibrio* and *Xanthomonas* as major flora, at all the stations during the period of study. However, some isolates could not be placed under any known genera, probably, they need to be subjected to more number of biochemical tests to be classified to species and known genera. Due to lack of certain facilities the isolates were not identified to species level as well no attempt was done towards conducting Koch postulate studies to establish them as pathogens for any

specific disease that was incurred during the period of study. However, attempt has been made in this study to draw indirect conclusions on these bacterial flora as to their responsibility to be the causative organisms by virtue of their isolation from diseased samples in considerable level. Although this approach is not technically appropriate to make conclusions as pathogens, it is assumed that in diseased condition pathogenic organisms normally dominate the microbial population than the non pathogenic flora.

Results presented on the percent distribution of bacterial flora associated with air, water, fresh leaves and diseased samples, under the results chapter clearly indicate the close relationship between the major flora, species of *Pseudomonas*, *Erwinia* and *Xanthomonas*, and the various diseases recorded in the hybrid *Dendrobium* population at the different stations. *Pseudomonas* sp was observed to be a dominant flora associated with yellowing of leaves, Stem rot, flower drooping ,dead rot , and water and fresh leaves. *Erwinia* was associated as a dominant flora,with yellow leaves, bud wilting, leaf rot, brown dots on leaves and brown spots on flowers, besides air. Whereas *Xanthomonas* sp was dominant associated with dried stem and air. It may be noted that some diseases were associated with specific species of bacteria which showed dominance. This particular observation points out to a possible inference that such species might be responsible for the respective disease.

It may be noted that most of the reports on orchid diseases deal only with specific plant pathogen and the symptoms it cause in a said population, and no report is available which has been done to monitor and assess a diverse group of microbial

flora over a said period of study. Of course, most often a particular disease is been associated with a specific species by the earlier investigators. Hence it would be inappropriate to compare the present results with their observation as to the symptoms observed in the present study. However it is attempted here to suggest a possible relation between a particular genera and a disease. Thus possibly the species of *Pseudomonas*, *Xanthomonas* and *Erwinia* which are known to include certain specific species which are qualified as plant pathogens. So, there is a possibility that these flora which were isolated during the present study could be such species, although they need to be identified to species level for confirmation. Bacteria associated with aerial plant surfaces are mainly Gram-negative rods with a higher proportion of chromogenic forms than from other habitats. The most common genera are *Pseudomonas*, *Eerwinia*, and *Xanthomonas*, together with the less well defined group F*lavobacteria*.. Strains of *Ps. syringae* are the most widely distributed colonists on foliar surfaces; many of these are saprophytic but some are pathovars able to attack a specific host under appropriate conditions. Fluorescent *pseudomonads* also occur widely on aerial plant surfaces.. *Erwinia herbicola*, a yellowpigmented saprophyte, is present widely as an epiphyte, especially on fruit trees where it may often be associated with the fireblight pathogen, *E. amylovora*, with which it competes (Blakeman. 1985).

Hirano & Upper (1983) list 27 different bacterial pathogens which exist as epiphytes on over 30 hosts belonging to both temperate and tropical genera. Despite numerous pathovars being represented, these belong to only three bacterial genera, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. So, there is a possibility that these flora which were

isolated during the present study could be such species, although they need to be identified to species level for confirmation.

The black rot fungi, *Phytophthora cactorum* (Led. & Cohn) Schroet. and *Pythium ultimum* Trow., and bacterial brown spot caused by *Pseudomonas cattleyae* (Pavareno) Savulescu are organisms that can cause death (Burnett1986).

Bacterial soft rot is a common disease of *Dendrobium*, especially when the conditions are very wet. The casual organism was found to be *Pseudomonas gladiolii* (Ganapathi & Chinathambi 1988).

Disease organisms that attack orchid plants in the Northern Territory of Australia, are bacterial soft rot caused by possibly *Erwinia carotovora* on *Dendrobium, Oncidium,* a rapid collapse of bulb and stem tissues resulting in leaf loss. *Pseudomonas cattleyae* has been reported on *Dendrobium, Oncidium, Rhynchostylis* as small brown soft water soaked areas (John,1997). The most serious rots of *Dendrobium, Oncidium,* and *Phalenopsis* plants. in wet areas of Hawaii. Involved *Erwinia chrysanthemi* Burkholder, McFadden, and Dimock and *Pseudomonas gladioli* Severini pv. *gladioli* as pathogens in *Dendrobium* fields. (Uchida 1994).

In the present study, the degree of relation among the different variables and the bacterial / fungal populations associated with a said disease, whether the variables are independent or dependent, was evaluated by conducting a multiple regression. It is evident from the results that the bacterial population associated with different

disease symptoms had a high degree of relation ship with most of the variables analysed during the period of study, at all the Stations under study.

The bacterial population associated with the yellowing of leaves had relatively a very high degree of relation with all other variables, at Station I (R square value 0.99837) and Station II(R square value 0.98168) compared to Stations III and V. Whereas in Station IV, there was a less degree of relation, indicating independent nature of variables at that Stations. The fungal population associated with the yellowing of leaves had relatively a very high degree of relation with all other variables, at Station V (R square value 0.96354) and Station IV(R square value 0.90084) compared to Stations I , II and III.

The bacterial population associated with the brown dots in leaves had a high degree of relation with all other variables, at all the stations excepting Station IV, where there was a less degree of relation, Particularly Station III showed a high degree of relation (R square value 0.99481) compared to other stations. The fungal population associated with the brown dots in leaves had a high degree of relation with all other variables, at station II (R square value0.92943) followed by stations III. Whereas at Stations I and V, there was less degree of relation.

The bacterial population associated with the leaf rot also had a high degree of relation with all other variables, at all the stations excepting Station V, where there was a less degree of relation, Particularly Station III showed a high degree of relation (R square value 0.99464) compared to other stations. Similarly the fungal population associated with the leaf rot also had a high degree of relation with all other variables,

at the stations III and IV(R square values 0.98493 and 0.95802 respectively) compared to stations I and II

The bacterial population associated with the bud wilting generally showed less degree of relation with all other variables, at both the stations I and II. Whereas, the fungal population associated with the bud wilting showed high degree of relation with all other variables, at both the stations I(R square value 0.74272) and II(R square value 0.95589)

The bacterial population associated with the brown spots in flowers, which was recorded at stations I (R square vale 0.91210) and III, generally showed high degree of relation with all other variables, at both the stations. Similarly the fungal population associated with the brown spots in flowers, showed high degree of relation with all other variables, at stations. I (R square vale 0.95464) while it recorded a less degree of relation with other variables at station III.

The bacterial population associated with the stem rot, and dead root which were recorded at stations I (R square vale 0.94025 and 0.92565 respectively), and flower drooping,(R square value 0.99243) recorded at station II, showed high degree of relation with all other variables. The fungal population associated with the dried stem and dead root which were recorded at stations I (R square vale 0.85371 and 0.98345 respectively), and flower drooping,(R square value 0.95973) recorded at station II, showed high degree of relation with all other variables.

From the multiple regression analyses of the data obtained for the different variables from the 5 stations it is concluded that the environmental parameters such as

atmospheric temperature, humidity, rainfall, quality of water used in the different stations for irrigation of the *Dendrobium* hybrid plants significantly influence the outbreak of diseases in the plant population in a cumulative fashion as they are dependent among themselves in getting a disease expressed. Further the statistical modeling obtained base on the data suggest that the occurrence of these diseases could be predicted and appropriate control measures could be forecast

SUMMARY AND CONCLUSIONS

5. SUMMARY AND CONCLUSIONS`

The present study was conducted on *Dendrobium* hybrid orchid cultivated commercially, in Ernakulam,(Kerala) and Nagercoil in Kanyakumari District of TamilNadu, both situated on the west coast of India. Survey on microbial diseases associated with this *Dendrobium* over a period of one full year from November'96 to October'97 was conducted, at regular intervals of one month. Data on Atmospheric temperature(both maximum and minimum), rainfall, humidity,, quality of water- pH, total alkalinity and hardness; microbial population associated with air, water, fresh leaves and diseased leaves, flowers, bud, stem and root were analysed. Symptoms of diseases were assessed and classified in terms of the differences in the nature of symptoms, employing 5point score system.

Results obtained for the various analyses indicated that there is not much of variation in environmental parameters in the 5 stations selected for the study. Maximum temperature of atmosphere varied from 28°Cto 33.8°C and minimum temperature varied from 21°C to 27.6°C. Humidity varied from 71% to 91%. Rain fall varied from 4cm to 190 cm. pH of the water used was slightly alkailne and varied from pH 7.3 to 8.71 in all the stations. Total alkalinity of water varied from 32ppm to 376 ppm. Whereas hardness of water showed variation from 10ppm to 140 ppm. Water samples of station II recorded higher level of hardness compared to other stations.

Ernakulam area recorded relatively higher level of percentage of diseases in the plant population compared to Kanyakumari District. Among the three stations

selected in Ernakulam Dt. Station- I, recorded comparatively high percent of diseased plants and more number of diseases. Whereas both stations of Kanyakumari showed relatively a lesser disease population and less number of diseases.

In general the hybrid orchids, in Station I, showed **eight** different disease symptoms which included **yellowing of leaves, brown dots on leaves, bud wilting, Root rot, dried stem, brown spot on flowers, Stem rot, and leaf rot,** during the course of the study. Among the different diseased conditions, yellowing of leaves, brown dots on leaves and leaf rot, root rot were observed among the diseased plants throughout the period of study. Among these three disease conditions, the yellowing and brown dots in leaves were predominant. Whereas, the leaf rot and root rot condition was observed with a lesser population. Other disease conditions were observed only during certain period of the course of study.

Altogether **five types** of disease conditions, including **yellowing of leaves, brown dots on leaves, bud wilting , leaf rot and flower drooping,** were observed in Station II, during the period of study. Among these diseases only yellowing of leaves was observed throughout the year.

Altogether **four** types of diseases, including **yellowing of leaves, brown dot on leaves, brown spots on flower and leaf rot,** were observed in hybrid *Dendrobium* in Station III during the period of study. Among these diseases only yellowing of leaves was observed throughout the year.

Altogether **three** types of diseases, including **yellowing of leaves, brown dot on leaves, and leaf rot,** in hybrid *Dendrobium* plants, were observed in Station IV,

during the period of study. Among these diseases only yellowing of leaves was observed throughout the year.

Altogether only **two** types of disease, including **yellowing of leaves, and brown dot on leaves**, in hybrid *Dendrobium* plants, in Station V, were observed during the period of study. Among these diseases only yellowing of leaves was observed throughout the year.

Bacterial flora associated with the samples of diseased plants showed interesting observations, in all the selected Stations of study located in both the Ernakulam and Kanyakumari Districts, during the period of study. In general the bacterial flora was composed of species of *Aeromonas, Alkaligenes, Erwinia, Flavobacterium, Pseudomonas, Vibrio and Xanthomonas* as major flora, at all the stations during the period of study.

Results presented on the percent distribution of bacterial flora associated with air, water, fresh leaves and diseased samples, indicate the close relationship between the major flora, species of *Pseudomonas, Erwinia* and *Xanthomonas,* and the various diseases recorded in the hybrid *Dendrobium* population at the different stations. *Pseudomonas* sp was observed to be a dominant flora associated with yellowing of leaves, Stem rot, flower drooping ,root rot , and water and fresh leaves. *Erwinia* was associated as a dominant flora,with yellow leaves, bud wilting, leaf rot, brown dots on leaves and brown spots on flowers, besides air. Whereas *Xanthomonas* sp was dominant associated with dried stem and air. It may be noted that some diseases were associated with specific species of bacteria which showed dominance. This particular

observation points out to a possible inference that such species might be responsible for the respective disease.

Multiple regression analyses of the data obtained for the different variables from the 5 stations indicated that the environmental parameters such as atmospheric temperature, humidity, rainfall, quality of water used in the different stations for irrigation of the *Dendrobium* hybrid plants significantly influence the outbreak of diseases in the plant population in a cumulative fashion as they are dependent among themselves in getting a disease expressed.

Further the statistical modeling obtained based on the data suggest that the occurrence of these diseases could be predicted and appropriate control measures could be forecast.

References

Abraham, A., and Vatsala.P. 1981. Introduction to Orchids. Printed by the St. Josephs press, Trivandrum, South India.

Anonymous. 1989. Hawaii Agricultural and Food Products Export Directory. Hawaii Marketing Division, Honolulu.

Anonymous. 1991. Statistics of Hawaiian Agriculture, 1990. Hawai Agricultural Statistics Service, Honolulu.

Arditti, J. 1979. Aspects of orchid physiology 421-655: Advances of Botanical Research vol. 7 ed. H. W. Woolhouse.

Blakeman,J.P 1985 Ecological succession of leaf surface microorganism in relation to biological control. In *Biological Controlon the Phylloplane* ed. Windels, C.E. & Lindow, S.E. pp. 6-30. St Paul, Minnesota: The American Phytopathological Society.

Burnett, HarryC. 1986. Diseases caused by fungi and bacteria. Hand book on Orchid Pest and Diseases. American Orchid Society. 37-57

Burgess, P.J. 1989 Studies on the biology of soft-rot erwinias on potato leaves. Ph.D. Thesis. The Queens University of Belfast.

Dickinson, C.H.1981 Biology of *Alternaria alternata, Cladosporium cladsporioides* and *C. herbarum* in respect of their activity on green plants. In *Microbial Ecology of the Phylloplane* ed. Blakeman,J.P. pp. 169-184. London: Academic press.

Farr, D.F., Bills, G.F., Chamuris, G.P., and Rossman, A.Y. 1989. Fungi on plants and plants products in the United States. American Phytopathological Society, St. Paul,MN.

Frank,D.W. 1988. Controle of bacterial soft rot caused by *Pseudomonas cattleyae* in the culture of *Phalenopsis. Orchid Digest* 52 (2) 66 - 67 [En]

Ganapathi,A. & Chinnathambi, C. 1988. Bacterial and fungal diseases of Orchids in Malaysia. Paper presented at the seminar of the *7th ASEAN Orchid Congres*, Jakarta, 4-9 Nov 1988.

George, H.M. Lawrence. 1951. *Taxonomy of Vascular Plants*. Oxford & IBH Publishing Co. Pvt Ltd.

Guptha, A.K. 1994. International Trade in Orchids: Some Facts. *Souvenir & Abstracts of The Orchid Society of India*. Punjab University, Chandigarh. 37-39.

Hirano, S.S. & Upper, C.D. 1983 Ecology and epidemiology of foliar bacterial plant pathogens. *Annual Review of phytopathology* 21, 243-269.

Hirano, S.S. & Upper, C.D. 1988 Rain and the population dynamics of *Pseudomonas* syringae on snap been leaflets. In *abstracts of papers, proceedings of the Fifth Indernational Congress of plant pathology*, Kyoto, Japan, 20-27 *August1988* p. 86.

Hooker,J.D.1885. *The Flora of British India*, vol. 5 & 6. L. Reeve and Co. Ltd., Kent.

Ito,J.S. and Aragaki, M. 1977 *Botrytis* blossom blight of *Dendrobium. Phytopathology* 67:820-824

John, D. Duff. 1997. Orchid diseases of the Northern Territory. *Agnote* No 568 /13 September1997 Primary Industry and Fisheries of Australia.

Johnson, M., Babu, D.U.N., Latha,S., Muthaiyan, M. C. 1993. Inspection from imported orchid plants. Plant protection bulletin (Faridabad) 45 (2-3) -26 -27[En, 4ref.]

Kamjaipai, K. 1984. Diseases and pests of orchids. *Proc. Asia Pac. Orchid Conf.* 1st

Koay Sim Huat. !993. Overview of the Singapore Orchid Industry. *Malayan Orchid Review.* Vol. 27. 73-75.

Lindemann, J., Constantinidou, H.A., Barchett, W.R. & Upper, C.D. 1982 Plants as sources of airborne bacteria, including ice nucliation-active bactera. *Applied and Environmental Microbiology* 44, 1059-1063.

Miller, J. W. 1990. Bacterial brown spot of orchid caused by *Pseudomonas cattleyae* . *Plant pathology circular* (*Gainesville*) No.330, 2pp [En, 3 ref].

Polunin, N. 1959. *Circumpolar Arctic Flora.* 126-29

Stearn, W.T. 1960. Two thousand years of orchidology. Proc. of 3rd *World Orchid Conference*: 26-41.

Sutabutr, T. !980. Important diseases of orchid in Thailand and their control. Proc. World Orchid Conf. 9th.

Uchida, J.Y. 1994. Diseases of Orchids in Hawaii. Plant Disease. vol. 78. No. 3 pp 220- 224.

Uchida, J.Y. , and Aragaki, M. 1979. Etiology of necrotic flecks on Dendrobium blossoms. Phytopathology69:1115-1117.

Wanitprapha, K., Yokoyama, K.M., Nakamoto, S.T., Leonhardt, K.W., and **Halloran, J.M.** 1991. Dendrobium economic fact sheet No. 13 Hawaii Inst. Tropical Agricultural Human Resources.

Wey,G.C. 1991 Occurance and chemical control of *Phalenopsis* soft rot caused by *Erwinia chrysantheni. Report of the Taiwan Sugar Research Institute* No:133, 1 - 10 [ch,en, 9 ref]

Yam Tim Wing. 1993 Native orchids of Singapore and their conservation-2. Terrestrials. *Malayan Orchid Review* vol.27. pp.53-57 & 96.