MANAGEMENT OF BACTERIAL BLIGHT OF ANTHURIUM (*Anthurium andreanum* Linden) USING BOTANICALS

SABITHA, S.R.

Thesis submitted in partial fulfilment of the requirement
for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2002

Department of Plant Pathology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM 695522

DECLARATION

I hereby declare that this thesis entitled "**Management of bacterial blight of anthurium (*Anthurium andreanum* Linden) using botanicals**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellayani,
12 -11-2002

Sabitha S.R
SABITHA, S.R.
(2000-11-02)

CERTIFICATE

s thesis entitled "**Management of bacterial blight of anthurium (*Anthurium andreanum* Linden) using botanicals**" is a record of research work done independently by Ms. Sabitha, S.R. (2000-11-02) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Vellayani,
12-11-2002

Dr. C.A. MARY
(Chairman, Advisory Committee)
Associate Professor
Department of Plant Pathology
College of Agriculture, Vellayani
Thiruvananthapuram.

Approved by :

Chairman :

Dr. C.A. MARY
Associate Professor,
Department of Plant Pathology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

31/12/02

Members :

Dr. C.K. PEETHAMBARAN
Professor and Head,
Department of Plant Pathology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

31/12/02

Dr. KAMALA NAYAR
Associate Professor,
Cropping Systems Research Centre,
Karamana.

31/12/02

Dr. K.B. SONI
Assistant Professor (Biotech.),
Plant Molecular Biology and
Biotechnology Centre,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

31/12/02

External Examiner :

Dr. RADHAKRISHNAN NAIR, R.
Senior Scientist
CTCRI, Sreekaryam
Thiruvananthapuram

31/12/2002

Dedicated to

My Beloved Parents

ACKNOWLEDGEMENT

First of all I bow my head before the God Almighty for giving me blessings, an optimistic mind and good luck for the successful completion of the thesis work.

I sincerely express my gratitude to Dr. C.A. Mary, Associate Professor of Department of Plant Pathology and Chairman of Advisory Committee for her sincere guidance, worthy suggestions, timely help and kind treatment extended at various stages of the work. Her unfailing interest and co-operation in the preparation of thesis is thankfully acknowledged.

I earnestly express my profound gratitude to Dr. C.K. Peethambaran, Professor and Head of Department of Plant Pathology for his constructive criticisms, timely advice, sincere support and valuable suggestions through out the course of this study. His sincere involvement in bringing out this thesis, in the present form, amidst his busy schedule is specially remembered.

I wish to extend my sincere thanks to Dr. Kamala Nayar for her generous assistance, co-operation and invaluable advice during this investigation.

I am glad to acknowledge Dr. K.B. Soni for her timely help and co-operation at all stages of this study.

I would specially like to mention the name of Dr. C. Gokulapalan for the assistance in taking photographs, affectionate suggestions, endless support, encouragement and for the keen interest in the submission of my thesis. So I avail this opportunity to convey my heartiest gratitude and indebtedness to him.

I would like to record my heartfelt thanks to Dr. V.K. Girija for the constant encouragement, timely help, moral support and valuable advices, which influenced me in proper planning and execution of thesis work.

I wish to place on record my heartiest gratitude to Dr. S.K. Nair, Dr. K. Umamaheswaran and Dr. P.J. Joseph for their concern and unforgettable help during the investigation.

I owe grateful thanks to Dr. A. Naseema, who provided me the necessary chemicals needed for the investigation.

I am deeply obliged to all the teaching and non teaching members of Department of Plant Pathology who helped me directly or indirectly during the investigation.

I thank C.E. Ajithkumar, Department of Statistics for the help extended in the statistical analysis of the data.

The wholehearted co-operation, ever willing help and support of seniors and juniors of Department of Plant Pathology particularly Rajkumar, Anoopsankar, praveenachechi, Pramod, Kavitha, Sindhu and Heera is thankfully acknowledged.

My heartiest thanks to Dhanya chechi for her endless support and timely advice throughout this study.

I have special pleasure and gratitude to my dearest friends and well wishers especially R.S. and G.V. for making this attempt a memorable one.

A note of love to all my colleagues and friends especially Sheena, Bindu, Preethichechi, Sreekalachechi, Sheeja Paul, Sindhu, L., M.S., K. Mathew, Saritha, Sreeja and to my classmates particularly Divya, Vrindachechi, Sundaramoorthy and Subramanyan for their unforgettable help.

I should like particularly to thank Ruby for the assistance rendered in conducting the survey and to Vyasan for arranging the planting materials needed for the investigation.

I acknowledge Biju. P. of ARDRA for prompt and timely help rendered in typing the thesis.

Financial assistance provided to me by STED is duly acknowledged.

At this moment I recall with love and gratitude for the constant encouragement and moral support given to me by Sajitha and Yasir Macha which cannot be expressed in words.

Besides all, the words fail to express by indebtedness to my beloved parents for their unbound love, prayers, blessings, constant encouragement, selfless help and mental support without which, I admit that I could not have been able to complete the research work in time.

Sabitha S.R

Sabitha, S.R.

LIST OF PLATES

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

°C	–	Degree Celsius
µl	–	Microlitre
µm	–	Micrometre
@	–	At the rate of
BX	–	Basal medium for Xanthomonads
CD	–	Critical difference
cm	–	Centimetre
cv	–	Cultivar
et al.	–	And others
Fig.	–	Figure
g	–	Gram
GA	–	Glucose Agar
GYA	–	Glucose Yeast Agar
h	–	Hour
kg	–	Kilogram
l	–	Litre
ml		Millilitre
mm	–	Millimetre
NA	–	Nutrient Agar
nm	–	Nanometre
PDA	–	Potato Dextrose Agar
PSA	–	Potato Sucrose Agar
pv.	–	Pathovar
sp.	–	Species
TTC	–	Tetrazolium chloride negative medium
viz.	–	Namely
Xad	–	*Xanthomonas axonopodis* pv. *dieffenbachiae*
YGCA	–	Yeast extract Glucose Chalk Agar

INTRODUCTION

1. INTRODUCTION

Anthurium (*Anthurium andreanum* Linden), a popular cut flower crop belonging to family Araceae, unquestionably occupies a place of pride among the cut flowers grown in Kerala. It is suitable for greenhouse and widely grown for its handsome foliage and fascinating coloured spathe. It provides a source of profound aesthetic pleasure to both growers and viewers. Due to change in social and cultural life style of people, it is in considerable demand both in domestic and export market. At present Kerala occupies an enviable position as a leading producer and exporter of Anthurium flowers. Its cultivation has very bright prospect in Kerala as the unique agroclimatic condition suitable for anthurium cultivation is prevalent in the state.

Several fungal and bacterial diseases affecting anthurium have been reported from different parts of the world. Among these, bacterial blight incited by *Xanthomonas axonopodis* pv. *dieffenbachiae* (McCulloch and Pirone) Vauterin *et al.* is the most serious one. The occurrence of this disease in Kerala was reported by Dhanya *et al.* (2000). The disease is prevalent in the anthurium gardens throughout the state. Both foliar and systemic infection of bacterial blight were reported, the systemic infection being more serious as it destroys the entire plant within days and causes heavy economic loss to the growers.

Use of chemicals, especially antibiotics is unavoidable in the integrated management of bacterial blight disease, since they provide a quick and reliable method of protecting the plant from this devastating disease. However, the ecofriendly approaches do not encourage the use of such synthetic chemicals, which pose problems of residual toxicity, environmental pollution, health hazards and development of resistance in the pathogen. Besides, these synthetic chemicals which are non-degradable in nature, disrupts Nature's equilibrium.

During recent years the use of botanicals is gaining importance in view of their economic and ecological preferences, like low cost, selective action, environmental safety, long lasting effect and easy biodegradability. Many plants provide rich store house of renewable biochemicals that could be exploited as natural sources of antimicrobial substances and hence called as "biocides". So in the present scenario, to facilitate the development and implementation of a balanced and sound policy of disease management, the use of plant products is of great importance, as it offers an ecofriendly means of disease management. Only limited work has been done on management of bacterial blight of anthurium using botanicals in Kerala. The present study envisages :

- Survey of bacterial blight disease of anthurium in Thiruvananthapuram district
- Isolation, pathogenicity test and characterization of the pathogen
- Symptomatology
- Host range of the pathogen
- Management of the disease using botanicals

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 BACTERIAL BLIGHT, DISTRIBUTION AND IMPORTANCE

Bacterial blight incited by *Xanthomonas axonopodis* pv. *dieffenbachiae* (McCulloch and Pirone) Vauterin *et al.* is a major problem in the production of ornamental aroids in Hawaii and Anthurium flowers in French Antilles (Rott and Prior, 1987; Natural, 1990; Vauterin *et al.*, 1995). McCulloch and Pirone (1939) first described the disease on dieffenbachiae and named the pathogen as *Bacterium Dieffenbachia.* Hayward (1972) from the islands of Kauai, Hawaii first reported bacterial blight of anthurium as a foliar disease. About 90 per cent of *Anthurium andreanum* cv. kansanko red were affected by bacterial blight. Two major outbreaks of this disease was reported from Hawaiian islands of Oahu and Hawaii in the 1971 and in 1980 (Nishijima and Fujiyama, 1985).

The disease has been reported worldwide from Venezuela (Guevara and Debrot, 1984), California (Cooksey (1985), Florida (Chase and Poole, 1986), French West Indies (Rott and Prior, 1987), Tahiti (Mu, 1990), Philippines (Natural, 1990), Jamaica (Young, 1990), Reunion Island (Soustrade *et al.*, 2000),

In India bacterial blight symptoms were noticed on Anthurium plants imported from Netherlands on three separate occasions (Satyanarayana *et al.*, 1998). Anthurium blight was reported from Kerala on varieties imported from abroad. The bacterium isolated from the infected anthurium plants was identified as *Xanthomonas* sp. by Das *et al.* (1996) and as *X. axonopodis* pv. *dieffenbachiae* by Dhanya *et al.* (2000).

As a consequence of this disease $ 5 million loss occurred in anthurium cut flower industry in Hawaii (Alvarez, 1988), resulting in reduction of anthurium farms by 24 per cent and flower sales by 22.8 per cent from 1984-1988 and the estimated average annual loss accounts to

$ 6387 per acre (Shehata *et al.*, 1990). The results of a survey conducted in 1989 in Tahiti by Mu (1990) showed that 60 per cent of the farms had plants with bacterial blight symptoms and 22 per cent of the plants were infected. Infection level in Jamaican anthurium farms varied from 25 to 100 per cent (Young, 1990).

2.2 SYMPTOMATOLOGY

According to Hayward (1972) the symptoms of bacterial blight incited by *X. axonopodis* pv. *dieffenbachiae* on anthurium were present on younger and older leaves. Extensive dark brown or black angular or irregular lesions with pale chlorotic halo were noticed on younger leaves, which extended along the midrib for several cm and between branch vein to fill intercoastal areas. On older leaves also angular pale brown necrotic one to three mm size spots surrounded by a marked chlorotic halo appeared. On spathe often black elongated spots extending along branch veins sometimes filling entire intercoastal areas were observed. However, contradictory to this Lipp (1992) observed that oldest and youngest leaves were least affected.

According to Guevara and Debrot (1984) symptoms of bacterial blight appeared as necrotic water soaked spots, some with chlorotic halo, mainly towards the margin of the leaves and spathes. Infection spread through petiole reaching the stems and roots and caused death of the plants. Cooksey (1985) noticed that symptoms of anthurium blight ranged from water soaked leaf spots with chlorotic halo's to severely blighted leaves and spathe. The disease was present on several cultivars. Leaf spot and blight symptoms were reproduced in Andrae's Flamingo flower in seven to fourteen days by injection or spray.

Natural *et al.* (1990) reported both systemic and foliar symptoms on anthurium plants. Foliar symptoms occurred on the undersurface of the leaf as minute water soaked spot about one to two mm diameter and later become necrotic and surrounded by a bright yellow water soaked border.

Occasionally leaf blighting was noticed. Systemic infection was noticed as yellowing of plants. The base of the infected petiole when cut lengthwise showed distinct brown spots or thin brown lines. Eventually as a result of systemic infection the leaf sheaths, newly emerging leaf and flower buds or the entire plant got killed. Local and systemic infection of anthurium by *X. campestris* pv. *dieffenbachiae* was also reported by Chase (1992), Kuehnle *et al.* (1995), Balakrishnan *et al.* (1996) and Das *et al.* (1996).

According to Dhanya (2000), Dhanya *et al.* (2000) and Soustrade *et al.* (2000) the diseased plants showed a variety of symptoms like blighting of leaf lamina, spathe and drying of spadix. In systemically infected plants, the infection started at the collar region and spread upwards and downwards killing the plant. Symptoms were initiated seven to ten days after artificial inoculation.

2.3 ISOLATION AND PROPERTIES OF THE PATHOGEN

Hayward (1972) reported the isolation method of *X. axonopodis* pv. *dieffenbachiae* from affected leaves and spathes. A compact coherent bacterial ooze was observed when the infected portions were observed under the microscope. It indicated the characteristic properties of genus *Xanthomonas*. About 5 mm square portion of lesion were suspended in sterile water and streaked out on two percent sucrose or peptone agar. A thin film of growth was evident in a pool of inoculum at 24 h and discrete colonies were visible at 48 h.

Natural *et al.* (1990) isolated the pathogen from advancing lesions of naturally infected leaves and streaked on potato dextrose peptone agar. Yellow colonies of *Xanthomonas* were developed after 72 h.

The pathogen was isolated from leaves, spathe, spadix and also from the base of the plant and streaked on potato sucrose peptone agar medium.

Smooth, round, glistening and slimy yellow coloured bacterial colonies were developed (Dhanya *et al.*, 2000).

The causal agent of bacterial blight of anthurium was first described as *X. campestris* pv. *dieffenbachiae* (McCulloch and Pirone) Dye by Dye (1980). The name was later changed to *X. axonopodis* pv. *dieffenbachiae* (McCulloch and Pirone) Vauterin *et al.* by Vauterin *et al.* (1995).

Guevara and Debrot (1984) described the morphology of *X. campestris* pv. *dieffenbachiae* isolated from *A. andreanum* as rod shaped 0.4 – 0.6 x 1.0 - 2.4 µm in size with a polar flagellum. According to Cooksey (1985) and Pohronezny *et al.* (1985) the pathogen was gram negative with a single polar flagellum.

The bacterium was positive for catalase, protein digestion, hydrolysis of starch, gelatin and aesculin, lipolysis of Tween 80 and produce acid from glucose, mannose and arabinose. It was negative for oxidase, urease, growth at 36°C, indole production and nitrate reduction (Cooksey, 1985).

Colonies of the pathogen were yellow and mucoid on nutrient agar and yeast extract dextrose calcium carbonate agar. The isolates were catalase positive, produced hydrogen sulphide from peptone, liquefied gelatin, hydrolyzed aesculin and grew well at 35°C. Marked proteolysis occurred in litmus milk. The isolates were oxidase negative and did not ferment glucose, indole was not produced, urease activity and reduction of nitrate was not observed. Asparagine was not utilized as a sole source of carbon and nitrogen. Most isolates produced acid from glucose, arabinose, cellobiose, fructose, galactose, maltose, mannose, raffinose, sucrose and trehalose, but failed to produce acid from glycerol, mannitol, dulcitol, erythritol, inositol, insulin, rhamnose, salicin and sorbitol (Pohronezny *et al.*, 1985).

According to Natural *et al.* (1990) size of the bacterium ranged from 0.4 – 0.8 x 1.5– 1.9 µm and was strictly aerobic. It grew well in potato dextrose petone agar and nutrient agar and were able to utilize dextrose, fructose, sucrose, glucose, galactose, maltose, lactose and arabinose oxidatively and produce hydrogen sulphide. It hydrolysed starch, Tween 80, gelatin and aesculin. The catalase test was positive however it was negative for nitrate test, Voges proskauer test to detect acetyl methyl carbinol, indole test and urease test.

The existence of two distinct biological types of *X. axonopodis* pv. *dieffenbachiae viz.*, the faster growing one which can digest starch and slower growing one which cannot digest starch were reported by Alvarez *et al.* (1990). Lee *et al.* (1992) while studying quinate or shikimate utilization in *X. campestris* observed that *X. campestris* pv. *dieffenbachiae* cannot utilized them as sole carbon source. Lipp *et al.* (1992) observed that 62 per cent anthurium strain did not hydrolyse starch as compared to seven per cent of strains from other hosts. Wells *et al.* (1993) in a study of cellular fatty acid composition of nine pathovars of *X. campestris*, classified *X. campestris* pv. *dieffenbachiae* as a separate group.

According to Dhanya *et al.* (2000) the bacterium produced a non water soluble yellow pigment on Yeast glucose chalk agar medium. Soustrade *et al.* (2000) reported that the bacterium responded positively to monoclonal antibodies raised against *X. axonopodis* pv. *dieffenbachiae* in an enzyme linked immunosorbent assay. Dhanya (2000) reported that the bacterium was aerobic. Positive results were noticed for production of hydrogen sulphide, ammonia, gelatin liquefaction and lipase production. Bacteria showed negative reaction for methyl red test, urease test, arginine hydrolase and tyrosinase activity. None of the isolates tested utilized asparagine as sole source of carbon and nitrogen and none were tolerant to six per cent sodium chloride. Variation was observed in starch hydrolysis, growth characters and virulence of the isolates.

2.4 CLIMATOLOGICAL FACTORS AND DISEASE DEVELOPMENT

Guevara and Debrot (1984) reported that incidence and spread of bacterial blight of *A. andreanum* in Venezuela was high in nursery where relative humidity was high. According to Natural *et al.* (1990) the incidence of bacterial blight was very severe in shade houses during summer when light intensity was unusually high and temperature was above 30°C. Alvarez *et al.* (1990) had shown that temperature above 29.5°C favour multiplication of *X. axonopodis* pv. *dieffenbachiae*. Influence of high temperature on disease incidence was also reported by Fukui *et al.* (1995).

2.5 HOST RANGE AND SYMPTOMATOLOGY

McFadden (1962) reported a pale yellow disease of *Aglaonema robelinii* incited by *X. axonopodis* pv. *dieffenbachiae* in Florida. Disease symptoms include diffused water soaked firm spots of one to five mm in diameter surrounded by a chlorotic halo.

Bacterial leaf blight of *Syngonium podophyllum* caused by *X. dieffenbachiae* was reported by Welburg (1969). The symptoms of the disease consisted of lesions along the margin extending sometimes towards the midrib. Newly incited lesions were dark green and water soaked gradually turning to yellow, brown and necrotic. Hayward (1972) observed *X. dieffenbachiae* from anthurium to be pathogenic on *Philodendron oxycardium* and *Dieffenbachia picta*. On *Philodendron* water soaked lesions of two to five mm size developed around the inoculated site within seven to 14 days. Dye and Lelliot (1974) reported *Dracaena fragrans* of Agavaceae family and *Aglaonema robelinii* of Araceae family as collateral hosts of *X. axonopodis* pv. *dieffenbachiae*.

Pohronezny *et al.* (1985) isolated *X. campestris* pv. *dieffenbachiae* from leaf spots of cocoyam. Symptoms were noticed as tiny water soaked spots usually more evident on the adaxial surface. It enlarged to necrotic

spots as large as two cm in diameter surrounded by prominent chlorotic halo. Pronounced water soaking continued on the adaxial surface. Although leaf spots were often delimited by veins, infection sometimes progressed into veins and proceeded basipetally resulting in streaks of infected tissues. Some lesions coalesced resulting in dead areas.

Bonner *et al.* (1987) reported *X. campestris* pv. *dieffenbachiae* the bacterial blight pathogen of aroids affected a broad range of ornamentals and edible aroids including *Anthurium*, *Aglaonema*, *Syngonium*, *Dieffenbachia*, *Epipremnum*, *Xanthosoma* and *Taro*. Natural *et al.* (1990) recorded sixteen alternate hosts of *X. campestris* pv. *dieffenbachiae*. The bacterium were able to infect various *Aglaonema* sp., *Dieffenbachia* sp. *Philodendron* sp. and *Syngonium* sp. The initial symptom in different hosts was water soaking of the infiltrated area three to four days after inoculation.

Lipp *et al.* (1992) observed the symptoms as a result of infection by *X. campestris* pv. *dieffenbachiae* on *Dieffenbachia*, *Anthurium* and *Syngonium*. The symptoms appeared within five to nine days of inoculation as small translucent water soaked pin point spots that later expanded and turned necrotic. The symptoms on *Epipremnum* and *Spathiphyllum* developed slowly forming only pin point, necrotic lesions. Symptoms on *Schefflera* began as small chlorotic pitted lesion which often expanded in size.

Chase *et al.* (1992) noticed that *X. axonopodis* pv. *dieffenbachiae* strains isolated from plants belonging to the genera of *Aglaonema*, *Anthurium*, *Colocasia*, *Dieffenbachia*, *Epipremnum*, *Philodendron*, *Syngonium* and *Xanthosoma* were more virulent on their host of origin than on other plants in pathogenicity and multiplication of bacterial population. According to Dhanya (2000) *Dieffenbachia* sp., *Philodendron oxycardium*, *Aglaonema robelinii*, *Colocasia esculenta* and *Syngonium podophyllum* were all hosts of *X. axonopodis* pv. *dieffenbachiae*.

Different species of *Xanthomonas* were reported to be pathogens of several ornamental plants.

The leaves of *Philodendron oxycardium* infected by *Xanthomonas* sp. exhibited water soaked to brownish narrow streaks along the margin of the leaf often near the apex. These elongated water soaked area extended from the leaf margin and radiated back to the mid vein. The lesions were frequently delimited by veins. There was no infection on stem and petiole (McFadden 1976). Shekhawat *et al.* (1980) found leaf spot of *Zinnia elegans* and *Primula denticulata* and *Pelargonium zonale* caused by *Xanthomonas* sp. Symptoms appeared as minute water soaked lesions on the adaxial surface of the leaf, which later enlarged two to three mm in diameter and turned necrotic and angular. The leaf tissues in the lesions became yellow, flaccid and finally withered.

Dickey and Zumoff (1987) identified the bacterial blight pathogen of *Syngonium podophyllum* as *X. campestris* pv. *syngonii*. The symptoms of the disease were most conspicuous on upper surface of leaf as watersoaked area. This extended gradually and affected older tissues, became light brown obviously necrotic and eventually dried and became papery. When the spread of lesions subsided a yellow border often developed between the necrotic area and surrounding unaffected areas. Finally unaffected areas became pale yellow, dried and the entire leaf shrivelled.

Chase and Jones (1987) reported bacterial leaf spot of *Sterilitzia reginae* (bird of paradise) caused by a strain of *X. campestris*. Angular, yellow to reddish brown lesions were found on all ages of leaves and petioles. Lesions were frequently boardered by leaf veins and remain less than one mm in diameter. Later the lesions coalesced to give leaves a blighted appearance.

Uddin and McCarter (1996) reported the angular leaf spot of *Hydrangea quercifolia* caused by a pathovar of *X. campestris*. Initial symptoms appeared on young unfolding leaves as irregular water soaked

spots of one to four mm that became angular and turned purple or dark brown as they enlarged. Coalescing lesions sometimes caused complete necrosis of older leaves. Phookan *et al.* (1996) noticed that leaf blight of *Colocasia esculenta* caused by *X. campestris* became prominent only seven to ten days after inoculation. The initial symptom appeared as water soaked interveinal lesions on the margin which gradually extended towards the centre and ultimately covered whole leaf lamina. In later stages, the infected lesions became dark brown in colour.

An outbreak of bacterial leaf spot on Zinnia (*Zinnia elegans*) by *X. campestris* pv. *zinniae* was reported by Catara and Sesto (1997), Hernandez and Trujillo (2000). Brown red angular leaf spot surrounded by a chlorotic halo was the typical symptom.

Blight of potted begonias (Begonia tuber hybrida) by *X. campestris* pv. *begonia* started as several yellowish water soaked spots on leaf margins. These spots were easily seen on under side of leaves. Tiny dark spots gradually enlarged and coalesced forming large necrotic lesions with an yellow halo. Finally the entire leaf, stems, petioles and main veins became water soaked and the plant became less turgid and collapsed (Grijalba *et al.*, 1998).

Madusmitha *et al.* (1999) reported *Xanthomonas* infection on *Aglaonema commutatum* and *Scindapus aureus*. In *Aglaonema* the initial symptoms were noticed on the upper surface of leaves as small pale green oval water soaked areas which after coalescence covered entire leaf and finally caused the death of the plant. In case of *Scindapus* the disease started at the margin of the leaf as pale water soaked areas which further spread resulting in leaf necrosis.

2.6 MANAGEMENT

2.6.1 *In vitro*

Mishenkova *et al.* (1983) reported that plants belonging to Asteraceae and Labiata possessed potent inhibitors against phytopathogenic bacteria *viz.*,

X. campestris pv. *phaseoli, Pseudomonas syringae* and *Corynebacterium michiganensis* pv. *michiganensis*. Similarly nineteen species of *Tabernaemontana* were found to possess antimicrobial activity against *Agrobacterium tumefaciens, Aspergillus niger* and *Candida albicans* (Beek *et al.*, 1984) . Aqueous extracts of *Allium sativum* and *Allium uncinatus* inhibited *X. oryzae* pv. *oryzae* causal agent of bacterial blight of rice under *in vitro* conditions (Grainge *et al.*, 1985).

Tewari (1986) reported the fungicidal and bactericidal properties of *Aegle marmelos, Ocimum sanctum, Nyctanthes arbortristis, Piper betle* and *Citrus limonia.* According to Dhaliwal *et al.* (1990) plant extracts of rice cv. TKM6 inhibited the growth of *X. campestris* pv. *oryzae, Erwinia carotovora* f. sp. *chrysanthemi* and *Pseudomonas solanacearum.* Thakur *et al.* (1991) reported that water or alcohol extracts of certain medicinal plants inhibited *X. campestris* pv. *malvacearum.* Of the nine plants tested pomegranate and *Datura metel* showed best antibacterial activity.

The antibacterial property of garlic (*Allium sativum* L) against *X. campestris* pv. *vesicatoria,* the leaf spot pathogen of tomato were evaluated by Mangamma and Sreeramulu (1991) by *in vitro* by paper disc method. Of the three concentrations tried, 30g/100 ml resulted in larger inhibition zone. Of the different solvent extraction, ethyl acetate was found to be the most efficient. The antimicrobial activity of garlic extract has been partly attributed to the presence of sulphur containing compounds.

The inhibitory effect of gum exudates of cashew tree on growth of bacteria and fungi was reported by Marques *et al.* (1992). Srinivasachary (1995) found that *Ocimum* extract was more effective in inhibiting the growth of *X. campestris* pv. *moricola* isolated from mulberry followed by *Bursara, Citronella* and *Cinnamomum.* Shah *et al.* (1997) conducted a laboratory assay to evaluate different extracts against *Xanthomonads.* It was observed that neem, garlic, ginger, onion and tulsi extracts inhibited the growth of the test bacteria. Mehndi and opium were effective at higher

concentrations while safeda and pipal did not inhibit the growth of the test bacterium.

The aqueous extracts of *Allium cepa, Allium sativum* and *Euphorbia tirucalli* and *Piper betle* showed antibacterial property against *X. campestris* pv. *campestris*, *Ralstonia solanacearum* and *Erwinia carotovora* pv. *carotovora* (Lirio *et al.*, 1998). Bora and Jaya (1999) reported that twelve out of thirty plant extracts @ 20 per cent were effective in inhibiting the growth of *X. campestris* pv. *citri* and *X. campestris* pv. *betlicola* by producing significant degree of inhibition zone.

Sharma and Mehta (1999) reported that extracts from 22 plant species were assayed *in vitro* by paper disc method for their antibacterial activities against *X. campestris* pv. *campestris*. Among the various leaf extracts *Prosopis julifera* L. was the most inhibitory followed by *Allium sativum* L., *Vitis quadranqularis* Wall, *Curcuma longa* L., *Ocimum sanctum* L. and *Eucalyptus citridora* Hock. Satish and Raveesha (2000) screened the water extracts of leaves of thirty different plant species under *in vitro* condition for the antibacterial activity against three species of *Xanthomonas* by cup plate and disc diffusion method. Extract of *Lawsonia inermis, Oxalis corniculata, Prosopis julifera* and *Punica granatum* showed significant antibacterial activity. But when subjected to solvent extraction using petroleum ether, benzene, chloroform and methanol, methanol extract of *Prosopis julifera* showed significant inhibitory effect.

Girgune *et al.* (1980) reported that essential oil from rhizome of *Valeriana wallichi* possess antifungal and antibacterial activity. Banerjee *et al.* (1982) reported the antimicrobial activity of the essential oil of *Curcuma amada*. The oil inhibits growth of plant pathogens *viz.*, *Erwnia carotovora, Pseudomonas solanacearum, X. citri* and *X. malvacearum* and human pathogens like *Bacillus subtilis, Salmonella* sp. etc. The oil retained activity upto a dilution of 1 : 1000. Garg and Kasera (1984) reported that leaf oil from *Anacardium occidentale* showed antibacterial

activity against *Pseudomonas mangiferae* causing leaf spot in mango and *X. campestris* causing black rot of crucifers.

Essential oil from *Mentha piperita* inhibited the growth of *X. campestris* at 10^{-1} dilution under *in vitro* condition (Maiti *et al.*, 1985). Garg and Dengre (1986) reported that essential oil derived from *Tagetes erecta* was effective against four gram positive and fifteen gram negative pathogenic bacteria. The oil has exhibited better activity at dilution 1 : 50 followed by 1 : 100 and moderate inhibitory effect at 1 : 2000 against *X. campestris*.

Antibacterial effect of essential oil obtained from the flower petals of *Rosa damascena* was evaluated by *in vitro* agar diffusion and volatility method against *X. axonopodis* pv. *vesicatoria*. It exhibited antibacterial effect and was used as a potential control agent in the management of disease caused by *X. axonopodis* pv. *vesicatoria* (Basim and Basim, 2001). Baswa *et al.* (2001) observed that karanj and neem seed oil possess antibacterial activity. It was observed that 57.14 per cent and 42 per cent of the pathogens were inhibited at 500 µl/ml. The activity was mainly attributed to the inhibition of cell membrane synthesis.

2.6.2 *In vivo*

Hanudin (1987) reported that extract of garlic, shallot and *Tagetes erecta* suppressed the bacterial wilt disease caused by *Pseudomonas solanacearum* on inoculated plants. Bacterial wilt of tomatoes was decreased by adding a 10 ml suspension of 35 g garlic bulb per 77 ml sterile water or six gram of ground garlic bulb to the rhizosphere. Normal plant growth and increase in fruit weight was observed (Hutagalung, 1988).

Eswaramurthy *et al.* (1993) reported that use of neem cake could reduce the bacterial blight of rice caused by *X. oryzae* pv. *oryzae* and citrus canker caused by *X. axonopodis* pv. *citri*.

According to Gangopadhyay (1998) none of the turmeric treated rice plants developed disease symptoms. Seed treatment with turmeric powder impregnated with sodium bicarbonate in the proportion 10 : 1 at the rate of 1 g kg^{-1} seed was found to control soil borne disease of rice. Foliar spray of the mixture at the rate of 1 g l^{-1} reduced the incidence of bacterial blight of rice.

Hulloli *et al.* (1998) reported the antibacterial property of certain neem products like plantolyte and agricare. These neem based formulations acted synergistically with antibiotics and or fungicides and also reduced their hazardous effect. The neem formulations alone could control cotyledonary infection or in combination with antibiotics at very low doses, it reduced the symptom markedly. The leaf extracts of *Psidium guajava, Datura stramonium, Leucas indica* and *Allium sativum* @ 20 per cent were evaluated *in vivo* by spraying on crop foliage before inoculation with the pathogen. The extract of *Leucas indica* was found effective in suppressing *X. campestris* pv. *citri* and *X. campestris* pv. *betlicola* and demonstrated 78.46 per cent and 77.8 per cent disease control over checks (Bora and Jaya, 1999).

According to Dhanya (2000) bacterial blight of anthurium incited by *X. axonopodis* pv. *dieffenbachiae* can be controlled by spraying 0.15 per cent turmeric powder + sodium bicarbonate in 10 : 1 proportion in the initial stage of infection by five sprayings at one week interval. Sateesh (2001) reported that leaf extracts of *Zizyphus jujuba, Datura metel* and *Ipomeae carnae* showed direct inhibition on growth of *X. oryzae* pv. *oryzae.* The performance of leaf extract was better in pre inoculation spraying than in post inoculation spraying in reducing the bacterial blight incidence in rice plants under greenhouse condition.

Very good control of soft rot of potato incited by *X. campestris* pv. *campestris* was achieved with the application of five per cent emulsion of *Madhuca* and *Cymbopogon* (Mukherjee and Biswas (1981). Mukherjee and Biswas (1984) reported that application of five per cent emulsion of *Elaeis* and *Hydrocarpus* was very effective for controlling citrus canker and black vein of cabbage.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 SURVEY OF BACTERIAL BLIGHT OF ANTHURIUM IN THIRUVANANTHAPURAM DISTRICT

Survey was conducted in different localities of Thiruvananthapuram district to assess the disease intensity and varietal reaction of bacterial blight of anthurium. Thirty anthurium growers from different localities of Thiruvananthapuram district were randomly selected for the study. For assessing the disease intensity 0-5 scale (Dhanya, 2000) was used. Reaction of different varieties of anthurium to this disease, the cultural and managing practices followed by the farmers and the host plants having similar disease symptoms were studied. Disease specimens were collected from severely affected garden for further study.

3.2 ISOLATION AND PATHOGENICITY OF DIFFERENT ISOLATES

The disease affected parts of the collected specimens were subjected to ooze test to find out the presence of bacterium. The infected portion with profuse ooze was selected, cut in to bits and surface sterilized with 0.1 per cent $HgCl_2$ solution. These bits were then washed in three changes of sterile distilled water and placed in a drop of sterile distilled water on a sterile glass slide. The bits were then teased apart using sterilized blade and forceps and kept for one minute to allow the bacterial ooze to mix with water. The bacterial suspension thus obtained was streaked on Potato Sucrose Agar (PSA) medium to get well isolated colonies of the bacterium.

Composition of PSA medium

Potato	-	300g
Na_2 H PO_4	-	2.0g
Ca NO_3	-	0.5 g
Peptone	-	5.0g
Sucrose	-	20.0g
Agar agar	-	20.0g
Distilled water	-	1000 ml
pH	-	6.8-7.0

The plates were incubated for 24 h at room temperature. Single colonies were selected on the basis of their colour, shape and slime production. The culture was further purified by repeated streaking on PSA medium. The pure cultures thus obtained were then tested for pathogenicity. For this a thick suspension of 24 h old growth of each isolate was prepared separately. The isolates were artificially inoculated on leaves and at the collar region by giving pinpricks and rubbing with cotton dipped in the bacterial suspension. The inoculated plants were then maintained under high humidity by giving frequent water sprays and covering with polythene bags to create a favourable micro climatic conditions to initiate infection.

When the artificially inoculated plants developed symptoms of the disease, reisolation was done as per the procedure described. Single colonies thus obtained were compared with that of original isolate. The pure cultures were maintained on PSA medium for further characterization work. The details of the bacterial isolates used in the study are given in the Table 1.

Table 1 Details of isolates collected from survey

Isolate member	Locality from where the diseased specimen was collected	Cultivar of anthurium	Nature of infection
Xad 1	Palayam	Liver red	Foliar
Xad 2	Agriculture College, Vellayani	Kalympong red	Systemic
Xad 3	Sreekaryam	Honey moon red	Foliar
Xad 4	Nalanchira	Lady Jane	Foliar
Xad 5	Kuravankonam	Lima white	Foliar
Xad 6	Kawdiar	Honduras	Foliar
Xad 7	Peroorkada	Liver red	Foliar
Xad 8	Kazhakuttam	Hawaiin red	Systemic
Xad 9	Vandithadom	Tropical	Systemic
Xad 10	PTP Nagar	Cancan	Systemic

3.3 SYMPTOMATOLOGY

Naturally infected anthurium plants were observed for the symptoms of bacterial blight. Artificially inoculated plants under controlled conditions were also closely observed and the symptoms were recorded.

3.4 CHARACTERIZATION AND IDENTIFICATION OF THE PATHOGEN

Characterization and identification of different isolates of the pathogen were done according to the methods recommended in the Manual of Microbiological methods, published by the Society of American

Bacteriologists (S.A.B., 1957) and the methods prescribed by Dye (1962) with modification.

3.4.1 Cultural Characters

3.4.1.1 Morphological Characters

Colony characters and cell morphology were studied from a 24 h old culture of the bacterium grown on PSA medium. Colony characters were studied by streaking a loopful of 24 h old growth of the bacterium on solidified PSA medium. For studying the cell morphology, the cells were stained for gram reaction and observed the slides under oil immersion objective of a microscope.

3.4.1.2 Growth of Different Isolates of the Bacterium on PSA

Nature of growth, colour, shape, extent of growth, type of margin and slime production of the bacterial colonies were studied on PSA. A loopful of the dilute suspension of each isolate was streaked on PSA in triplicate, kept for incubation for 24 h at room temperature and observations were taken.

3.4.1.3 Growth of Different Isolates of the Bacterium on Potato Sucrose Broth

Potato sucrose broth was used for studying the growth of different isolates of the bacterium in liquid medium. Five ml of the sterilized broth was taken in a test tube and inoculated with a loopful of 24 h. old growth of the bacterium. Three replications were kept for each isolate. The inoculated broth was shaken daily. Uninoculated broth served as control. Optical density of the broth was measured 24, 48 and 72h after inoculation with different isolates using a spectrophotometer at 510 nm wavelength. Uninoculated broth was used as blank.

3.4.1.4 Pigment Production

Production of water insoluble and soluble pigments was tested in Yeast extract Glucose Chalk Agar medium (YGCA) and King's B medium respectively. 24h old culture of the bacterium was streaked on test tube slants of these media in triplicate for each isolate. Observations were recorded periodically on production of non water soluble yellow pigment on YGCA and fluorescent pigment on King's B medium.

3.4.1.5 Oxygen Requirement

To determine whether the bacterium was aerobic or anaerobic, Nutrient Agar (Containing 0.005% bromocresol purple) columns in test tubes were inoculated by stabbing with different isolates of the bacterium using a straight inoculation needle. To create an anaerobic condition, agar surface was covered with sterile liquid paraffin oil to a depth of one centimeter in one set of tubes. The agar surface in the other set of tubes were kept open without paraffin oil. Three replication were maintained for each isolate. Both sets of tubes with paraffin oil and without paraffin oil were incubated at room temperature and observations were recorded. Yellowing of the medium from the top was the indication of positive reaction.

Composition of Nutrient Agar Medium

Peptone	-	10.0 g
Beef extract	-	5.0 g
Agar agar	-	20.0 g
Distilled water	-	1000 ml
pH	-	6.8

3.4.2 Physiological Characters

All the isolates of the bacterium were compared for their physiological properties. The tests were performed in triplicate and the observations were recorded in comparison with uninoculated control.

3.4.2.1 Mode of Utilization of Glucose

Hugh and Leifson's (1953) method modified by Hayward (1964) was used to determine whether the bacterium utilized glucose only under aerobic conditions or both under aerobic and anaerobic conditions.

Composition of the medium

Peptone	-	1.0 g
$NH_4 H_2 PO_4$	-	1.0 g
KCl	-	0.2 g
$Mg SO_4. 7H_2O$	-	0.2 g
Bromothymol blue	-	0.03 g
Agar agar	-	3.0 g
Distilled water	-	1000 ml
pH	-	7.0

To the above medium one per cent glucose was added. The medium was dispensed in test tubes upto 4 cm and sterilized by tyndallization and inoculated by stabbing with bacterial growth. In one set of the tubes, the medium was sealed with one cm layer of sterile liquid paraffin and the other set without liquid paraffin. The tubes were incubated at room temperature and observations were taken at regular intervals upto 15 days.

3.4.2.2 Utilization of Organic Acids

Sodium salt of four organic acids *viz.*, sodium acetate, sodium benzoate, sodium citrate and sodium formate were used for this study. One per cent of the sodium salt of organic acids was added to the basal medium for Xanthomonads with bromothymol blue as indicator. Slants were inoculated in triplicate with the bacterium and incubated at room temperature. Uninoculated control was also maintained. Observations were recorded at regular intervals. Colour change of the medium from green to blue was positive indication of utilization of organic acids.

3.4.2.3 Starch Hydrolysis

The ability of the bacterium to hydrolyse starch was tested using starch medium containing 0.2 per cent soluble starch.

Composition of the medium

Peptone	-	10.0 g
Beef extract	-	5.0 g
Starch (soluble)	-	2.0 g
Agar agar	-	20.0 g
Distilled water	-	1000ml
pH	-	7.0

Bacterial culture of 24 h old growth was spot inoculated on the medium contained in the plates. After four days of incubation, hydrolysis was tested by pouring Lugol's iodine over the media. A colourless or reddish brown zone around the bacterial growth in contrast to the blue background of the medium was indicative of positive starch hydrolysis.

3.4.2.4 Production of Hydrogen Sulphide

The ability of the bacterium to produce hydrogen sulphide was tested using peptone water medium.

Composition of the medium

Peptone	-	10.0 g
NaCl	-	5.0 g
Distilled water	-	1000ml
pH	-	7.0

Five ml of the medium was dispensed in test tubes and autoclaved. Lead acetate paper strips of 5 x 50 mm size were prepared by soaking them in super saturated solution of lead acetate. The strips were dried, autoclaved and dried again. The tubes were inoculated with different isolates of the bacterium and the lead acetate strips were inserted aseptically between the plug and inner wall of the tube, hanging just above

the broth. The tubes were inoculated with 24 h old culture of bacterium and incubated at room temperature and observations were recorded up to 14 days at regular intervals. Blackening of the lead acetate impregnated strips indicated liberation of hydrogen sulphide.

3.4.2.5 Methyl Red and Voges Praskauer tests (MR and VP tests)

Methyl Red test was conducted to detect the production of acid from glucose and the Voges Praskauer test was conducted to find out whether glucose had fermented and produced neutral compounds. For both the tests Methyl Red broth was used.

Composition of Methyl Red broth

Proteose peptone	-	5.0 g
Glucose	-	5.0 g
$K_2H\ PO_4$	-	5.0 g
Distilled water	-	1000 ml
pH	-	7.0

The medium was dispensed in five ml aliquots in test tubes and sterilized by steaming for 30 minutes for three successive days. Two sets of tubes were inoculated with 48 h old culture of the isolates of the bacterium for MR and VP tests separately. The tubes were incubated for seven days at room temperature.

For MR test few drops of methyl red (0.1 g dissolved in 300 ml of 95 per cent ethyl alcohol and diluted to 500 ml with distilled water) was added to the culture tubes. A distinct red colour indicated positive methyl red reaction and yellow colour with methyl red indicator regarded as negative reaction.

For VP test 0.6 ml of alpha-napthol solution (5 per cent in 95 per cent alcohol) and 0.2 ml of 40 per cent aqueous solution of KOH was added to one ml of the culture. The mixture was shaken for few minutes

and allowed to stand for two hours. A crimson or ruby colour indicated positive VP test.

3.4.2.6 Gelatin Liquefaction

Nutrient Gelatin medium was used and stab method was employed for the test.

Composition of Nutrient Gelatin medium

Peptone	-	10.0 g
Beef extract	-	5.0 g
Gelatin	-	120.0 g
Distilled water	-	1000 ml
pH	-	7.0

Gelatin was mixed together with all the other ingredients and heated over a water bath until the gelatin was dissolved. The medium was dispensed in test tubes to a depth of about five cm and sterilized at 0.51 kg cm^{-2} pressure for 20 minutes. The sterile condition of the medium was checked by observing it for two days. Properly sterilized gelatin columns were inoculated by stabbing a straight inoculation needle charged with 24 h old culture of the bacterium. The tubes were incubated at room temperature and observed for the liquefaction of the gel column at regular intervals up to one month. Liquefaction was confirmed by placing the gel columns under refrigerated condition for five minutes.

3.4.2.7 Production of Indole

Tryptone broth medium was used for this test.

Composition of the medium

Tryptone or casein digest	-	10.0 g
NaCl	-	5.0 g
Distilled water	-	1000 ml
pH	-	7.0

The medium was dispensed in tubes and autoclaved. Gnezda oxalic acid strips were used for detecting indole production. Filter paper strips of size 5 x 50 mm were soaked in warm saturated solution of oxalic acid and cooled. When the strips got covered with oxalic acid crystals they were dried at room temperature and used without sterilizing.

The tryptone broth tubes were inoculated with isolates of the bacterium and oxalic acid strips were inserted into the tube by the side of the plug, incubated at room temperature and observed regularly for 14 days. Change in colour of oxalic acid crystals on test strip to pink or red indicated indole production.

3.4.2.8 Urease Production

The medium of Christensen's urea agar (Christensen, 1946) was used for this test.

Composition of the medium

Peptone	-	1.0 g
NaCl	-	5.0 g
KH_2PO_4	-	2.0 g
Glucose	-	1.0 g
Agar agar	-	2.0 g
Phenol red		
(0.2 per cent solution)	-	6.0ml
Distilled water	-	1000ml
pH	-	6.8

Ninety ml aliquots of the medium was dispensed in 250 ml conical flasks and autoclaved. To each flask 10 ml of 20 per cent urea solution sterilized by filtration was added and dispensed five ml quantities in tubes to prepare slants. The slants were inoculated with the test culture and observations were recorded for 15 days at regular intervals. Colour change of the medium from yellow to red was positive indication of urease activity.

3.4.2.9 Catalase Test

To assess the production of catalase enzyme by the bacterium, a loopful of 24 h old culture of different isolates of bacterium was smeared on the glass slide and covered with a few drops of 20 Volume hydrogen peroxide. The production of gas bubbles was indicative of catalase positive reaction.

3.4.2.10 Action on Milk

Both unskimmed and skimmed milk were used in this test. A 1:3 dilution of skimmed milk was prepared in water and bromocresol purple was added to give a final concentration of 0.002 per cent, when a light blue colour was obtained (Clark and Lubs, 1917). Unskimmed milk (containing approximately three per cent butter fat) was also diluted with water and bromocresol purple was added as above. The milk medium was then dispensed in five ml quantities in test tubes and sterilized by steaming for 30 minutes for three successive days in a pressure cooker. The medium was inoculated with a loopful of 24 h old test bacterium and incubated at room temperature and observed at regular intervals upto 30 days. Acid reaction was indicated by a colour change from blue to yellow and change to violet colour indicated alkaline reaction.

3.4.2.11 Utilization of Asparagine as sole Source of Carbon and Nitrogen

The test was performed using Dye's medium (Dye, 1966).

Composition of the medium

Solution 1.	$K_2H\ PO_4$	–	8.0 g
	$KH_2\ PO_4$	–	2.0 g
	Distilled water	–	100ml
Solution 2.	$Mg\ SO_4.\ 7H_2O$	–	2.0 g
	$Fe\ SO_4$	–	0.5 g
	NaCl	–	1.0 g
	$MnSO_4$	–	0.02 g
	$H_2\ SO_4$	–	1 drop
	Distilled water	–	100ml

Solution 3. Na_2MoO_4 – 0.02 g
Distilled water – 100 ml

Solution 4. $CuSO_4$ saturated solution in 100 ml distilled water.

Ten ml each of the four solutions were mixed with each other in the order 3, 4, 2, 1. This was then filtered and 960 ml of distilled water and 2g of L Asparagine were added. The medium was dispensed in five ml quantities in tubes and autoclaved. The tubes were inoculated with 24 h old culture of different isolates of the bacterium, incubated and examined for growth. Growth of the bacterium in the medium was indicative of the utilization of asparagine.

3.4.2.12 Growth at Six per cent Sodium Chloride

Peptone water with six per cent sodium chloride was used for the test.

Composition of the medium

Peptone	-	1.0 g
NaCl	-	6.0 g
Distilled water	-	100 ml

The medium was dispensed in tubes, autoclaved and inoculated with different isolates of the bacterium, incubated and observations recorded.

3.4.2.13 Lipolytic Activity

The medium of Sierra (1957) was used for this test.

Composition of the medium

Peptone	-	10.0 g
Na Cl	-	5.0 g
$Ca\ Cl_2.\ H_2O$	-	0.1 g
Agar agar	-	20.0 g
Distilled water	-	1000 ml
pH	-	7.0

Ninety nine ml of the medium was taken in flasks, autoclaved and cooled to 45^0C. One ml of Tween 80 (Oleic acid ester) was added to this medium and thoroughly mixed. The medium was poured in sterile petridishes and test bacterium was spot inoculated. The plates were incubated and observed at regular intervals for seven days. Opaque zone around the bacterial growth was indicative of positive lipase production.

3.4.2.14 Tyrosinase Activity

Dye's (1962) medium was employed for the test.

Composition of the medium

$NH_4H_2PO_4$	-	0.5 g
K_2HPO_4	-	0.5 g
$MgSO_4.7H_2O$	-	0.2 g
NaCl	-	5.0 g
Yeast extract	-	5.0 g
Tyrosine	-	0.5 g
Agar agar	-	20.0 g
Distilled water	-	1000ml
pH	-	6.8 –7.0

The medium was dispensed in tubes, autoclaved and slants were prepared. The slants were inoculated with different isolates of the bacterium and incubated. Browning of the medium indicated tyrosinase activity.

3.4.2.15 Arginine Hydrolase Test

Thornley's (1960) medium was used for the purpose.

Composition of the medium

Peptone	-	1.0 g
NaCl	-	5.0 g
K_2HPO_4	-	0.3 g
Agar agar	-	3.0 g
Phenol red	-	0.01 g

L Arginine	-	1.0 g
Distilled water	-	1000 ml
pH	-	7.2

The medium was dispensed in five ml quantities in test tubes and autoclaved. The tubes were stab inoculated with 24 h old culture of the bacterium and covered with sterile liquid paraffin to a depth of one cm, incubated for seven days and observed daily. A change in the colour of the medium to red indicated arginine hydrolase activity.

3.4.2.16 Production of Ammonia

The production of ammonia was detected by using Nessler's reagent which gave an yellow to brown precipitate with ammonia. The test culture was grown in autoclaved peptone water, in test tubes.

Composition of the medium

Bacteriological peptone	:	10.0 g
NaCl	:	5.0 g
Casamino acid	:	10.0 g
Distilled water	:	1000 ml
pH	:	7.0

After incubation for 48 h the reagent was added to the tubes and precipitate developed was noticed.

3.5 STUDIES ON SELECTED ISOLATE

Isolate Xad 10 which was found to be the most virulent among the ten isolates was selected for further studies.

3.5.1 Growth of Xad 10 on Solid Media

Nature of growth, colour, shape, extent of growth, type of margin and slime production by the isolate Xad 10 was studied on eight different solid media. A loopful of the dilute suspension of the bacterium was

streaked on different solid media in triplicate and kept for incubation a room temperature. Observations were recorded after 24 h incubation.

The following media were used :

1. Potato Sucrose Agar (PSA)
2. Nutrient Agar (NA)
3. Basal medium for Xanthomonads (BX)
4. Tetrazolium Chloride negative medium (TTC)
5. Potato Dextrose Agar (PDA)
6. Yeast extract Glucose Chalk Agar (YGCA)
7. Glucose Agar (GA)
8. Glucose Yeast extract Agar (GYA)

Composition of the media :

1. **Potato Sucrose Agar (PSA)** – Given above
2. **Nutrient Agar** – Given above
3. **Basal medium for Xanthomonads**

$NH_4H_2PO_4$	-	0.5 g
K_2HPO_4	-	0.5 g
$MgSO_4.7H_2O$	-	0.2 g
NaCl	-	5.0 g
Yeast extract	-	1.0 g
Agar agar	-	20.0 g
Distilled water	-	1000 ml
pH	-	6.8

4. **Tetrazolium Chloride negative media**

Peptone	-	10.0 g
Casamino acid	-	1.0 g
Glucose	-	5.0 g

Agar agar	-	20.0 g
Distilled water	-	1000 ml
pH	-	6.8

5. **Potato Dextrose Agar**

Potato	-	200 g
Dextrose	-	20.0 g
Agar agar	-	20.0 g
Distilled water	-	1000 ml
pH	-	6.8

6. **Yeast extract Glucose Chalk Agar** – given above

Yeast extract	-	10.0 g
Glucose	-	10.0 g
Chalk ($CaCO_3$)	-	20.0 g
Agar agar	-	20.0 g
Distilled water	-	1000 ml
pH	-	7.2

7. **Glucose Agar :**

Beef extract	-	5.0 g
Peptone	-	5.0 g
Glucose	-	10.0 g
Agar agar	-	20.0 g
Distilled water	-	1000 ml
pH	-	6.8

8. **Glucose Yeast extract Agar**

Yeast extract	-	5.0 g
Peptone	-	5.0 g
Glucose	-	10.0 g

Agar agar	-	20.0 g
Distilled water	-	1000 ml
pH	-	6.8

3.5.2 Utilization of Carbon Sources

Carbon utilization by isolate Xad 10 was studied as indicated by acid production (Dye, 1962) on agar slants of basal media for Xanthomonads. Ten carbon sources, *viz.*, maltose, inositol, galactose, dextrose, lactose, xylose, dulcitol, glucose, sucrose and fructose were used for the study. The carbon compound to be tested was added to the medium at one per cent concentration and 0.7 ml of five per cent alcoholic solution of bromocresol purple was added to get a reddish violet colour. The medium was sterilized by tyndallization and the slants were inoculated with 24 h old culture of the bacterium in triplicate and incubated at room temperature. Periodic observations were recorded upto 30 days. The change in colour of the medium from reddish violet to yellow indicated the production of acid. The test was done in triplicate.

The utilization of carbon compounds by isolate Xad 10 in broth of the basal media for Xanthomonads was studied using spectrophotometer. The carbon compound to be tested was added to the medium at one per cent concentration. The medium was sterilized by tynadllization and the broth was inoculated with 24 h old culture of the bacterium and incubated at room temperature for 24 h. Growth in each medium was recorded using spectrophotometer at 510 nm wave length. Higher absorbance indicated more growth in the medium which indicated more utilization of that particular carbon source.

3.6 HOST RANGE AND SYMPTOMATOLOGY

Some ornamental plants and some plants belonging to Araceae family were used for the study. Plants selected for the study are given in the Table 2.

Table 2 Plants used for host range study

Sl. No.	Host	Family
1.	*Aglaonema* sp.	Araceae
2.	*Alocasia sanderiana*	Araceae
3.	*Anthurium ornatum*	Araceae
4.	*Dieffenbachia maculata*	Araceae
5.	*Dieffenbachia* sp.	Araceae
6.	*Dieffenbachia* sp.	Araceae
7.	*Philodendron eichleri*	Araceae
8.	*Philodendron* sp.	Araceae
9.	*Philodendron* sp.	Araceae
10.	*Philodendron* sp.	Araceae
11.	*Colocasia esculenta*	Araceae
12.	*Xanthosoma sagittaefolium*	Araceae
13.	*Syngonium podophyllum*	Araceae
14.	*Orchid* sp.	Orchidaceae
15.	*Orchid* sp.	Orchidaceae
16.	*Heliconia rostrata*	Musaceae
17.	*Sterilitzia reginae*	Musaceae
18.	*Dracaena sanderiana*	Liliaceae
19.	*Dracaena* sp.	Liliaceae
20.	*Maranta arundinaceae*	Marantaceae

The plants were collected and planted in pots. The potted plants were inoculated as described under 3.2. Three replications were maintained for each host plant tested. The plants were observed for development of symptoms.

3.7 MANAGEMENT

3.7.1 *In vitro* Studies

Preliminary screening was conducted for antibacterial activity against bacterial blight pathogen using the botanicals listed in Table 3.

Table 3 Botanicals and the parts used for preparation of extract

Sl. No.	Botanical name	Common name	Family	Part used
1.	*Allium sativum* (Linn.)	Garlic	Liliaceae	Bulb
2.	*Allium cepa* (Linn.)	Onion	Liliaceae	Bulb
3.	*Tagetes erecta* (Linn.)	Marigold	Compositae	Leaves
4.	*Ocimum sanctum* (Linn.)	Thulsi	Lamiaceae	Leaves
5.	*Lawsonia inermis* (Linn.)	Mylangi	Lythraceae	Leaves
6.	*Tabernaemontana coronaria* (Linn.)	Nanthiyarvattom	Apocynaceae	Leaves
7.	*Centella asiatica* (Linn.)	Kudangal	Umbelliferae	Leaves
8.	*Coleus aromaticus* (Benth.)	Panikoorka	Lamiaceae	Leaves
9.	*Azadirachta indica* (A. Juss)	Neem	Meliaceae	Seed oil

Table 3 Continued

Sl. No.	Botanical name	Common name	Family	Part used
10.	*Cocos nucifera* (Linn.)	Coconut	Palmae	Seed oil
11.	*Ricinus communis* (Linn.)	Avanakku	Euphorbiaceae	Seed oil
12.	*Pongamia glabra* (Vent.)	Ungu	Leguminosae	Seed oil
13.	*Hydnocarpus wightiana* (Blume.)	Marotty	Flacourtiaceae	Seed oil
14.	Neem cake extract			
15.	Turmeric + Sodium bicarbonate (10 : 1) mixture			

3.7.1.1 Preparation of Plant Extracts

Fresh plant parts were used for the preparation of extract. They were first washed in running tap water followed by sterile distilled water. Plant parts were ground using a mortar and pestle and the crude extract was collected by filtering through two layers of muslin cloth.

2 : 1 dilution of the plant extract was prepared by the following procedure. Plant parts were ground in sterile distilled water in the proportion two parts plant material and one part water using a mortar and pestle. This was filtered through muslin cloth and the extract was collected to get the 2 : 1 dilution of the plant extract.

1 : 1 dilution (100 per cent) of the plant extract was prepared by grinding plant parts in sterile distilled water in the proportion 1 : 1 w/v and filtering through muslin cloth. This extract was further diluted by

adding half the quantity of sterile distilled water to get the 1 : 2 dilution (50 per cent) of the plant extract.

3.7.1.2 Preparation of Neem Cake Extract

For preparation of water soluble fractions of neem cake extract, 100 g of neem cake was mixed in 250 ml of distilled water and left for three hours. This was filtered through a layer of muslin cloth. The filtrate was again filtered through four layers of muslin cloth to remove all crude materials and using distilled water the final volume was made up to 250 ml. Three dilutions *viz.*, two per cent, one per cent and 0.5 per cent were prepared from the crude extract.

3.7.1.3 Preparation of Turmeric + Sodium Bicarbonate Mixture

For preparation of crude extract of turmeric + sodium bicarbonate mixture, 100 g of the mixture (10 parts turmeric + 1 part sodium bicarbonate) was mixed with 250 ml of distilled water and left for three hours. This was filtered through double layers of muslin cloth and using distilled water the final volume was made up to 250 ml. Three dilutions *viz.*, two per cent, one per cent and 0.5 per cent were prepared from the crude extract using distilled water.

3.7.1.4 Preparation of Oils for Screening

Neem oil, coconut oil, marotty oil, castor oil and pongamia oil were assayed for their antibacterial activity. Pure oils and different dilutions using Tween 80 as solvent *viz.*, two per cent (1 part oil and 50 part solvent), one per cent (1 part oil and 100 part solvent) and 0.5 per cent (1 part oil and 200 part solvent) were prepared and they were tested for antibacterial activity.

3.7.1.1.1 Antibacterial Effect of Botanicals Against *X. axonopodis* pv. *dieffenbachiae*

The filter paper disc agar diffusion technique (Maruzzella *et al.*, 1958) was followed for the assay. The study was conducted as two

separate experiment in completely randomised factorial design with three replications. The antibacterial activity of eight plant extracts *viz.*, *Allium sativum*, *Tagetes erecta*, *Coleus aromaticus*, *Lawsonia inermis*, *Tabernaemontana coronaria*, *Allium cepa*, *Centella asiatica*, *Ocimum sanctum* and four doses (Crude extract, 2:1, 1:1, 1:2 dilution) were evaluated in the first experiment. Streptocycline 100 ppm was used as the check.

3.7.1.1.2 Antibacterial Effect of Oils and Plant Products Against *X. axonopodis* pv. *dieffenbachiae*

The antibacterial activity of five oils *viz.*, Marotty oil, Neem oil, Castor oil, Coconut oil, Pongamia oil and two plant products *viz.*, Neem cake extract, Turmeric + Sodium bicarbonate (10 : 1 mixture) and four doses (Crude, 2 per cent, 1 per cent, 0.5 per cent) were evaluated in the second experiment. Streptocycline 100 ppm was used as the check.

PSA medium was sterilized in 250 ml flask and 20 ml of the media was poured under aseptic conditions to each petriplate. To prepare bacteria seeded petridishes, two ml of 24 h old growth of the bacterium in potato sucrose broth was added to the media in petridishes and mixed thoroughly by gentle rotation of the plates. Filter paper discs of 5 mm diameter impregnated with the respective treatments were placed in the centre of the bacteria seeded solidified media in petriplates. Three replications were maintained for each treatment. The petridishes were incubated at room temperature and observations were recorded at 24, 48 and 72 hours. The antibacterial efficacy was measured in terms of inhibitory zone developed around the filter paper disc.

3.7.2 *In vivo* Studies

The best five botanicals and their two doses were selected from the *in vitro* screening studies and their efficacy was tested against bacterial blight disease under *in vivo* conditions.

A pot culture experiment was laid out in completely randomised design with twelve treatments and three replications. Seven month old Cancan, a hybrid variety of anthurium, highly susceptible to bacterial blight disease was selected for the study. The following were the twelve treatments used.

1. *Allium sativum* (Linn.)	Crude extract
2. *Allium sativum* (Linn.)	2 : 1 per cent concentration
3. *Tagetes erecta* (Linn.)	Crude extract
4. *Tagetes erecta* (Linn.)	2 : 1 per cent concentration
5. Neem oil	2 per cent concentration
6. Neem oil	1 per cent concentration
7. Coconut oil	2 per cent concentration
8. Coconut oil	1 per cent concentration
9. Neem cake extract	Crude extract
10.Neem cake extract	2 per cent concentration
11. Streptocycline	100 ppm
12. Control	

Two pre inoculation sprayings and two post inoculation sprayings at five days and two days before artificial inoculation and two days and five days after the artificial inoculation were given.

Artificial inoculation of the plants was done as described under 3.2.

The observations were recorded on per cent disease infection and per cent reduction over control before the first and the second post inoculation

sprayings and also one week and two weeks after the post inoculation sprayings as per the disease score chart developed by Dhanya (2000).

Descriptive keys for scoring bacterial blight of Anthurium

Score	Percentage of infection	Description of the disease development
0	0	No infection
1	1-5	Lesion at pinpricks
2	6-25	Lesion at pinpricks along with yellowing of 1-2 leaves
3	26-50	Lesion of size 1.2 x 0.5 cm along with yellowing of 1-2 leaves
4	51-75	Yellowing of all the leaves with blackening of petioles
5	76-100	Complete death of plant

The data were statistically analysed and interpreted. The treatments for the second experiment on grown up plants of flowering size was fixed based on the results obtained from this experiment.

The second experiment was laid out in completely randomised design. The most effective dose of the two botanicals *viz.*, the crude extract of neem cake and *Tagetes erecta* were selected from the first experiment and compared with streptocycline 100 ppm. Plants with water spray served as the control. There were four treatments and five replications. Two pre inoculation sprayings and two post inoculation sprayings at five days and two days before the artificial inoculation and

two days and five days after the artificial inoculation were given in this experiment also. Observations on per cent disease infection and per cent reduction over control were recorded before the first and second post inoculation sprayings and also one week and two weeks after the post inoculation sprayings. Isolation were carried out from different parts of the sprayed plants to confirm the presence or absence of the pathogen in the plant system. The data were statistically analysed and interpreted.

RESULTS

4. RESULTS

4.1 SURVEY OF THE BACTERIAL BLIGHT OF ANTHURIUM IN THIRUVANANTHAPURAM DISTRICT

Survey on disease intensity and varietal reaction of bacterial blight of anthurium was conducted in different localities of Thiruvananthapuram district. The information on disease intensity and varietal reaction of bacterial blight were collected from among thirty gardens randomly selected for the study. The disease intensity is presented in Table 4 as mean disease score. Among the thirty gardens only two gardens had plants with more than 75 per cent disease infection. Infection upto 75 per cent was recorded for five gardens. In most of the anthurium gardens (Thirteen) disease infection ranged from 26-50 per cent. Only four gardens out of 30 gardens surveyed had plants free from bacterial blight. It was observed that red varieties of anthurium especially Cancan, Tinora, Tropical, Honduras and Hawaiian red were more susceptible to both foliar and systemic infection. The white varieties, Lima white and Acropolis were found commonly affected by foliar infection. Pink varieties like Lady Jane exhibited tolerance to bacterial blight. Orange, cream and double colour varieties of anthurium were also seen affected by bacterial blight disease, it was neither completely resistant or tolerant. The cultural and management practices followed by the farmers had a profound influence in reducing the disease incidence. It was evident from the survey that 13.3 per cent of the growers were not adopting proper cultural practices. About 70 per cent of the gardens surveyed were under proper care and management. Some foliage ornamental plants grown in the anthurium garden *viz.*, *Dieffenbachia* sp., *Philodendron* sp., *Caladium* sp., *Alocasia* sp. and *calathium* sp. served as alternate host of the pathogen. The survey indicated that the disease was more severe in humid weather condition.

Table 4 Distribution of bacterial blight disease among anthurium gardens in Thiruvananthapuram district

Sl. No.	Disease score	Per cent infection	Number of infected gardens surveyed	Distribution (per cent)
1	0	0	4	13.33
2	1	1-5	0	0.00
3	2	6-25	6	20.00
4	3	26-50	13	43.33
5	4	51-75	5	16.67
6	5	76-100	2	6.67

4.2 ISOLATION OF THE PATHOGEN

Diseased specimen collected from different locations of Thiruvananthapuram district were isolated. Isolation of pathogen yielded yellow, circular, slimy, glistening colonies with entire margin on PSA. *A. andreanum* var. Cancan artificially inoculated with the bacterial isolates on the leaves as well as on collar region developed symptoms within two weeks. Reisolation from such infected plants yielded colonies resembling the original isolates of the bacterium. Six isolates Xad 1, Xad 3, Xad 4, Xad 5, Xad 6 and Xad 7 from foliar infection and four isolates Xad 2, Xad 8, Xad 9 and Xad 10 from systemic infection were selected based on the virulence of the isolates for further studies.

4.3 SYMPTOMATOLOGY

4.3.1 Foliar symptoms

Initial symptoms appeared as scattered minute water soaked spots of one to two mm diameter on the leaf lamina. The water soaking was more pronounced at the lower surface of the leaf lamina compared to the upper

surface. Some times water soaked spots were minute and crowded. As these spots grew older, they turned dark brown with a distinct yellow halo around the spots (Plate 1). These spots later coalesced and formed large patches. Along the margin of the leaf lamina also small water soaked spots were formed which coalesced and blighted the entire margin. The blighted portions were dark brown to greyish brown with yellow halo. When the major portion of the leaf lamina got blighted, defoliation occurred. The leaf infection sometimes extended along the midrib for several centimetres. Sometimes the foliar infection spread through the petiole reaching the stem and root, resulting in systemic infection.

Symptoms of bacterial blight infection were also noticed on spathe as well as on spadix. On the spathe the symptoms initiated as minute translucent water soaked specks, which later turned dark brown and were delimited from the healthy portion by a wavy margin. The necrotic brown lesion gradually enlarged covering a major portion of the spathe. Eventually the affected portion of the tissues was dried up and the spathe got distorted (Plate 2).

On the spadix water soaked lesion developed first and later coalesced to form bigger patches. Severely affected spadix later got shrivelled.

4.3.2 Systemic Symptoms

Systemic infection was noticed as yellowing of leaves, which later turned light brown and gradually dried up. The petioles of the infected leaves could be easily pulled out from the base. The base of the petiole and collar region showed brownish water soaked lesion. Later the collar region also rotted. The rotting was also extended to the root region. The newly emerged leaves showed water soaking and failed to develop properly. Within one or two weeks the entire plant was killed (Plate 3).

Plate 1. Foliar infection of bacterial blight.

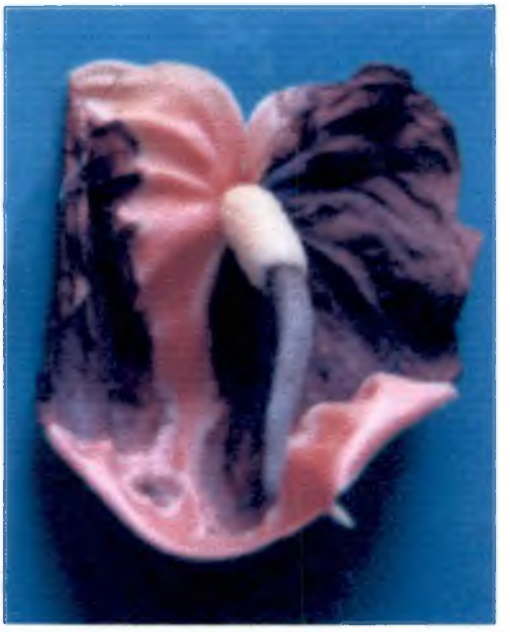


Plate 2. Bacterial blight infection on spathe and spadix

A. On leaf

B. On petiole

C. On collar region

D. On roots

Plate 3. Symptoms of systemic infection of bacterial blight

The isolates collected from the foliar infection *viz.*, Xad 1, 3, 4, 5, 6 and 7 when inoculated on leaf lamina of anthurium plants produced typical foliar blight symptoms within seven days, but it failed to produce systemic infection when inoculated on the collar region.

The isolates collected from the systemic infection when inoculated on both leaf lamina and collar region produced both foliar and systemic infection.

Variations in symptom production on artificial inoculation with different isolates of *X. axonopodis* pv. *dieffenbachiae* were studied. The different isolates showed differences in the type of symptoms such as the size of the initial lesion, yellow halo around the spots, severity of marginal and petiole infection, foliar yellowing and rotting of roots (Table 5).

4.4 CHARACTERIZATION AND IDENTIFICATION OF THE PATHOGEN

4.4.1 Cultural Characters

4.4.1.1 Morphological Characters

The bacterium was a gram negative short rod with rounded ends. All the isolates gave rise to yellow, circular, slimy, smooth, glistening and convex mucoid colonies with entire margin on PSA. Slight differences were noticed in the yellow colour produced between the isolates. The colonies of the isolates Xad 3, 4 and 8 were pale yellow while light yellow colour was observed for the colonies of Xad 10. The isolates were of creamy colour for Xad 9 and light yellow for Xad 1, 5 and 7. The rest of the isolates were deep yellow (Table 6).

4.4.1.2 Growth of Different Isolates of the Bacterium on PSA

The growth of ten different isolates were tested on PSA medium (Table 6). All the ten isolates tested produced yellow, circular, slimy, smooth, glistening, convex colonies with entire margin on PSA medium. Variations were noticed in the yellow colour produced by the different isolates.

Table 5. Symptom production on inoculation with different isolates of *Xanthomonas axonopodis* pv. *dieffenbachiae* on anthurium

Sl. No.	Symptoms	Xad 1	Xad 2	Xad 3	Xad 4	Xad 5	Xad 6	Xad 7	Xad 8	Xad 9	Xad 10
1.	Incubation period in days	6	4	5	7	8	7	6	4	5	3
2.	Initial water soaked lesions	Minute irregular	Minute irregular	Minute angular	Minute irregular	Minute angular	Large angular	Minute irregular	Large irregular	Minute irregular	Large angular
3.	Yellow halo surrounding	Small	Large	Small	Very small	Small	Small	Large	Small	Small	Large
4.	Size of lesions developed	Small	Large	Small	Small	Large	Large	Small	Very large	Large	Very large
5.	Marginal infection	Mild	Severe	Mild	Mild	Mild	Mild	Mild	Severe	Severe	Severe
6.	Petiole infection	Absent	Present, irregular water soaked lesion developed at base	Absent	Absent	Absent	Absent	Absent	Present, water soaked lesion developed at base	Present, water soaked lesion developed at base and later extended upward	Present, water soaked lesion developed at base and later extend upward
7.	Yellowing	Absent	Present	Absent	Absent	Absent	Absent	Absent	Present	Present	Present
8.	Defoliation	Absent	Present, when the petiole got infected	Absent	Absent	Absent	Absent	Absent	Present, when the petiole got infected	Present, when the petiole got infected	Severe defoliation due to petiole infection
9.	Rotting of roots	Absent	Present	Absent	Absent	Absent	Absent	Absent	Present	Present	Present

Table 6. Growth of different isolates of the bacterium on PSA

Sl. No.	Isolates	Nature of colony and colour	Growth and slime production
1	Xad 1	Yellow, smooth, circular, convex, glistening colonies with entire margin.	Gr +++ Sl +++
2	Xad 2	Deep yellow, smooth, circular, convex, glistening colonies with entire margin.	Gr +++ Sl ++
3	Xad 3	Pale yellow, smooth, circular, glistening colonies with entire margin.	Gr ++++ Sl ++
4	Xad 4	Pale yellow, smooth, circular, glistening colonies with entire margin.	Gr ++ Sl ++
5	Xad 5	Yellow, smooth, circular, convex, glistening colonies with entire margin.	Gr +++ Sl ++
6	Xad 6	Deep yellow, circular, smooth, glistening colonies with entire margin.	Gr ++ Sl ++
7	Xad 7	Yellow, circular, smooth, convex, glistening colonies with entire margin.	Gr +++ Sl ++
8	Xad 8	Pale yellow, circular, smooth, convex, glistening colonies with entire margin.	Gr +++ Sl +++
9	Xad 9	Creamy yellow, circular, smooth, convex, glistening colonies with entire margin.	Gr ++ Sl ++
10	Xad 10	Light yellow, circular, smooth, convex, glistening colonies with entire margin	Gr ++++ Sl ++++

Gr – Growth
Sl – Slime

++++ - Excellent
+++ - Good
++ - Moderate
+ - Slight

The isolates Xad 3 and Xad 10 had excellent growth on PSA, while Xad 1, Xad 2, Xad 5, Xad 7 and Xad 8 had good growth. Moderate growth was noticed for the isolates Xad 4, Xad 6 and Xad 9 on PSA. Excellent slime production was noticed for the isolate Xad 10. The isolates Xad 1 and Xad 8 had good amount of slime production. Slime production was moderate for the rest of the isolates *viz.*, Xad 2, Xad 3, Xad 4, Xad 5, Xad 6, Xad 7 and Xad 9.

4.4.1.3 Growth of Different Isolates of Bacterium on Potato Sucrose Broth

The growth of ten different isolates of bacterium was studied on potato sucrose broth. Growth was measured as change in the optical density of medium after 24, 48 and 72 h in comparison with control (Table 7 and Fig. 1).

Observations recorded after 24 h showed that Xad 4, Xad 10, Xad 6 and Xad 5 had the maximum growth. These four isolates did not differ significantly from one another. This was followed by Xad 9 and Xad 1, Xad 3, Xad 7 and Xad 8 which were statistically on par and recorded significantly lower growth than the above isolates. The least growth was recorded by the isolate Xad 2 (OD = 0.964).

After 48 h of incubation maximum growth was recorded by the isolates Xad 10, Xad 6 and Xad 9. This was followed by the isolate Xad 1 which recorded significantly lower growth than the above isolates and was statistically on par with Xad 5. Growth of isolates Xad 5, Xad 8 and Xad 4 were lower than all the above isolates and were statistically on par. The isolate Xad 2 recorded the lowest growth (OD=1.058).

At the end of 72 h among the ten isolates maximum growth was recorded by the isolate Xad 10 (OD = 1.823). Next best growth was recorded by the isolate Xad 4. The isolates Xad 9 and Xad 8 were on par and recorded the least growth.

Table 7. Growth of different isolates of the bacterium on potato sucrose broth

Sl. No.	Isolate	Optical density at 510 nm		
		24 h	48 h	72 h
1.	Xad 1	1.124 (1.060)	1.517 (1.232)	0.977 (0.988)
2.	Xad 2	0.964 (0.982)	1.058 (1.029)	0.933 (0.966)
3.	Xad 3	1.096 (1.047)	1.148 (1.071)	0.878 (0.937)
4.	Xad 4	1.267 (1.126)	1.354 (1.640)	1.509 (1.229)
5.	Xad 5	1.220 (1.105)	1.439 (1.200)	0.847 (0.920)
6.	Xad 6	1.231 (1.110)	1.695 (1.302)	0.986 (0.992)
7.	Xad 7	1.085 (1.042)	1.214 (1.102)	0.784 (0.884)
8.	Xad 8	1.067 (1.033)	1.384 (1.176)	0.675 (0.822)
9.	Xad 9	1.136 (1.066)	1.675 (1.294)	0.596 (0.772)
10.	Xad 10	1.249 (1.118)	1.723 (1.313)	1.823 (1.350)

CD for treatment (0.05 level) : 0.051
Figures in parenthesis are square root transformed values

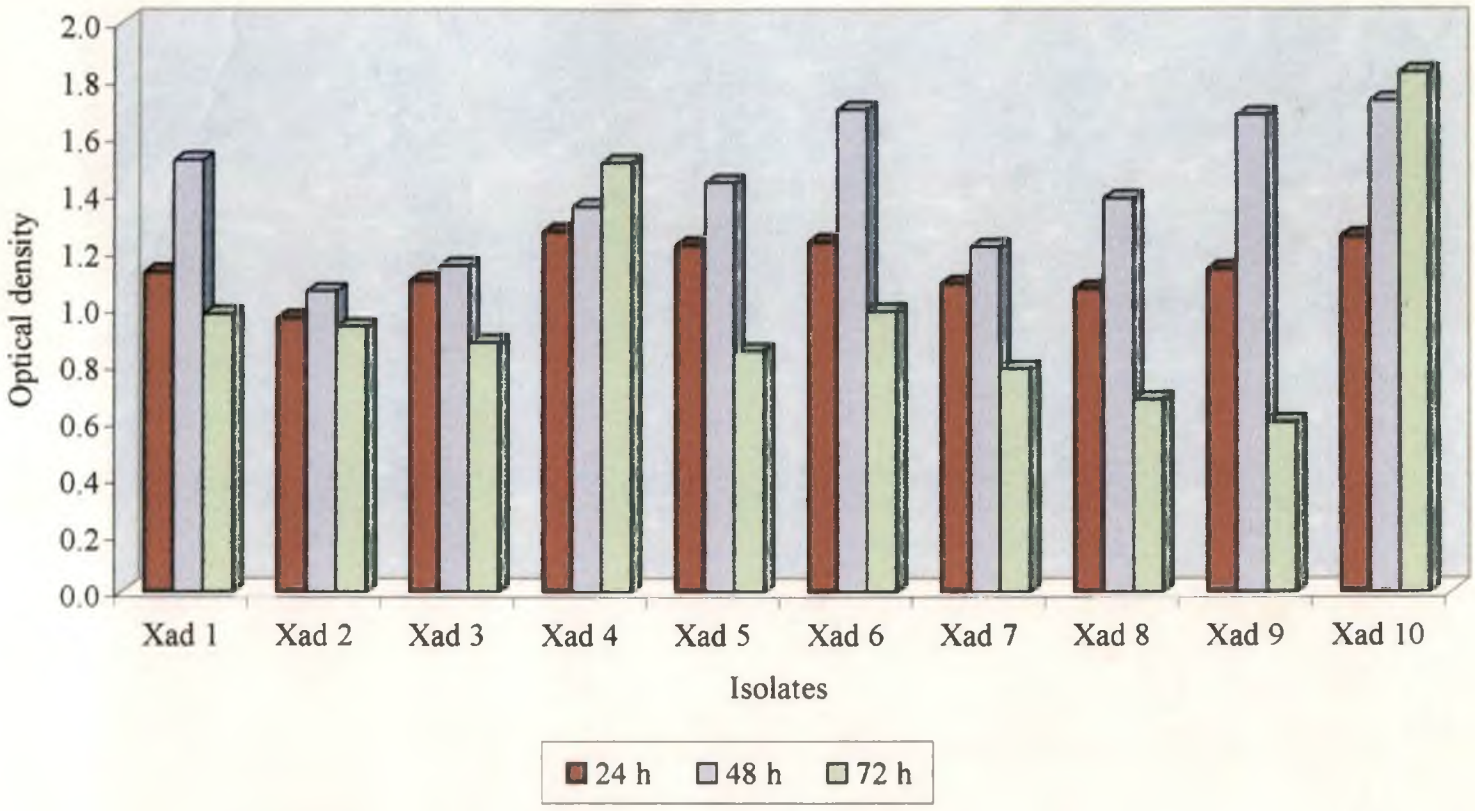


Fig. 1 Growth of different isolates of the bacterium on potato sucrose broth

All the isolates attained their maximum growth within a period of 48 h after incubation except Xad 4 and Xad 10. Among the ten isolates studied growth of Xad 4 and Xad 10 was found increasing even after a period of 72 h indicating that these isolates required much longer period of incubation for attaining maximum growth.

4.4.1.4 Pigment Production

Non water soluble yellow pigment on Yeast Glucose Chalk Agar medium was produced by all the isolates of the bacterium. None of the isolates produced water soluble fluorescent pigments on King's B medium (Table 8).

4.4.1.5 Oxygen Requirement

All the isolates of the bacterium were found to be aerobic, since the growth and change of blue colour of the Nutrient Agar medium containing 0.005 per cent bromocresol purple to yellow was observed only in case of tubes without paraffin sealing (Table 8).

4.4.2 Physiological Characters

4.4.2.1 Mode of Utilization of Glucose

All the isolates of the bacterium were found to utilize glucose oxidatively since the medium in open tubes turned yellow from the top with lack of colour change in the paraffin sealed tubes (Table 8).

4.4.2.2 Utilization of Organic Acids

All the isolates of the bacterium utilized sodium acetate and sodium citrate as the source of carbon as evidenced by the change of colour of the slants from green to blue. Sodium benzoate and sodium formate were not utilized as the source of carbon by any of the isolates of the bacterium (Table 8).

Table 8. Cultural, morphological and physiological characters of different isolates of *Xanthomonas axonopodis* pv. *dieffenbachiae*

Sl. No.	Characters studied	Isolates									
		Xad 1	Xad 2	Xad 3	Xad 4	Xad 5	Xad 6	Xad 7	Xad 8	Xad 9	Xad 10
1	Gram reaction	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve
2	Pigment production										
	a. YGCA (non water soluble)	+	+	+	+	+	+	+	+	+	+
	b. King's B (water soluble)	–	–	–	–	–	–	–	–	–	–
3.	Oxygen requirement	+	+	+	+	+	+	+	+	+	+
4.	Mode of utilization of glucose										
	a. Aerobic	+	+	+	+	+	+	+	+	+	+
	b. Anaerobic	–	–	–	–	–	–	–	–	–	–
5.	Utilization of organic acids										
	a. Sodium citrate	+	+	+	+	+	+	+	+	+	+
	b. Sodium acetate	+	+	+	+	+	+	+	+	+	+
	c. Sodium benzoate	–	–	–	–	–	–	–	–	–	–
	d. Sodium formate	–	–	–	–	–	–	–	–	–	–
6.	Starch hydrolysis	+	+	–	–	+	+	+	–	+	+
7.	Production of Hydrogen sulphide	+	+	+	+	+	+	+	+	+	+
8.	Methy Red and Voges Praskauer tests	–	–	–	–	–	–	–	–	–	–
9.	Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+
10.	Production of indole	–	–	–	–	–	–	–	–	–	–
11.	Urease production	–	–	–	–	–	–	–	–	–	–
12.	Catalase test	+	+	+	+	+	+	+	+	+	+
13.	Action on milk	AL	AL	AL	AL	AL	AL	AL	AL	AL	AL
14.	Utilization of asparagine as sole source of C and N	–	–	–	–	–	–	–	–	–	–
15.	Growth at six per cent NaCl	–	–	–	–	–	–	–	–	–	–
16.	Lipolytic activity	+	+	+	+	+	+	+	+	+	+
17.	Tyrosinase activity	–	–	–	–	–	–	–	–	–	–
18.	Arginine hydrolase test	+	+	+	+	+	+	+	+	+	+
19.	Production of ammonia	+	+	+	+	+	+	+	+	+	+

–Ve : negative, – : Negative reaction, + : Positive reaction, AL : Alkaline reaction

4.4.2.3 Starch Hydrolysis

All the isolates of the bacterium except Xad 3, Xad 4 and Xad 8 were found to hydrolyse starch as indicated by colourless zone around the bacterial growth in contrast to the blue background of the medium (Table 8).

4.4.2.4 Production of Hydrogen Sulphide

Liberation of hydrogen sulphide was observed in all the isolates of the bacterium. This was indicated by the blackening of lead acetate test strip (Table 8).

4.4.2.5 Methyl Red and Voges Praskauer Tests

All the isolates of the bacterium gave negative methyl red test as evidenced by the absence of development of distinct red colour in the culture tube when few drops of methyl red solution was added.

Similarly the Voges Praskauer test was also negative for all the isolates as indicated by the absence of a crimson or ruby colour by the addition of 0.6 ml alpha-napthol and 0.2 ml of 40 per cent aqueous solution of KOH in the culture tube (Table 8).

4.4.2.6 Gelatin Liquefaction

There was liquefaction of gel column in the tubes which were inoculated with isolates of the bacterium within 10 to 15 days, which indicated that all isolates of the bacterium liquefied gelatin (Table 8).

4.4.2.7 Production of Indole

The oxalic acid crystals on the test strip did not turn pink or red which indicated that none of the isolates produced indole (Table 8).

4.4.2.8 Urease Production

All the ten isolates gave negative urease test, for there was no change in colour of the medium from yellow to red (Table 8).

4.4.2.9 Catalase Test

Catalase positive reaction was shown by all the different isolates of the bacterium (Table 8).

4.4.2.10 Action on Milk

All the isolates of the bacterium turned the milk alkaline as evidenced by the colour change from blue to violet (Table 8).

4.4.2.11 Utilization of Asparagine as sole Source of Carbon and Nitrogen

None of the isolates utilized asparagine as the sole source of carbon and nitrogen as indicated by the absence of growth of isolates of the bacterium in the organic salt solution containing 0.2 per cent asparagines (Table 8).

4.4.2.12 Growth at Six per cent Sodium Chloride

The different isolates of the bacterium did not grow when inoculated in broth containing six per cent NaCl (Table 8).

4.4.2.13 Lipolytic Activity

Opaque zone around the bacterial growth indicated lipase production and so all the isolates of the bacterium were found to produce lipase (Table 8).

4.4.2.14 Tyrosinase Activity

Absence of dark brown pigment in the media inoculated with isolates of the bacterium indicated that all isolates were tyrosinase negative (Table 8).

4.4.2.15 Arginine Hydrolase Activity

All the isolates of the bacterium were arginine hydrolase positive as indicated by slight variation in the change of colour of the medium to red (Table 8).

4.4.2.16 Production of Ammonia

All the isolates of the bacterium produced ammonia which was detected by the formation of yellow to brown precipitate (Table 8).

4.5 STUDIES USING ISOLATE Xad 10

The isolate Xad 10 was found to be the most virulent among the ten isolates studied, hence further studies were conducted using this. This isolate produced systemic infection on *A. andreanum* and killed the plant within 13 days of artificial inoculation.

4.5.1 Growth of Xad 10 on Different Solid Media

The growth of the bacterial isolate Xad 10 on eight different solid media were studied and the results are presented in Table 9.

The isolate Xad 10 produced yellow, glistening, circular smooth convex colonies, with entire margin in all the media tested. The colonies were seen glistening on PSA and NA. On PSA and NA it produced light yellow and pale yellow pigments while in the remaining media the bacterium produced yellow pigment. Of the eight solid media tested, excellent growth was seen on PSA. The growth on NA and YGCA was found to be good. Moderate growth was noticed on BX, TZ, PDA and GYA. Only slight growth was observed in GA.

Excellent slime production was observed on PSA. Amount of slime production was good in NA. It was absent in GA and GYA.

4.5.2 Utilization of Carbon Source

Carbon utilization by isolate Xad 10 was studied in agar slant of BX and the results are presented in Table 10.

Of the 10 carbon sources tested, the isolate Xad 10 produced acid in Maltose, Dextrose, Galactose, Glucose, Sucrose, Fructose, Lactose and Xylose as indicated by change of colour of medium from reddish violet to yellow. In Maltose and Xylose acid production was noticed from the

Table 9. Growth of isolate Xad 10 on different solid media

Sl. No.	Medium	Nature of colony and colour	Growth and slime production
1	PSA	Light yellow, circular, smooth, convex, glistening colonies with entire margin.	Gr ++++ Sl ++++
2	BX	Yellow, circular, smooth, convex colonies with entire margin.	Gr ++ Sl ++
3	TZ	Yellow, circular, smooth. convex colonies with entire margin.	Gr ++ Sl +
4	YGCA	Yellow, circular, smooth, convex colonies with entire margin.	Gr +++ Sl ++
5	PDA	Yellow, circular, smooth, convex colonies with entire margin.	Gr ++ Sl +
6	GA	Yellow, circular, smooth, convex colonies with entire margin.	Gr + Sl –
7	GYA	Yellow, circular, smooth, convex colonies with entire margin.	Gr ++ Sl –
8	NA	Pale yellow, circular, smooth, convex, glistening colonies with entire margin.	Gr +++ Sl +++

Gr – Growth
Sl – Slime

++++ - Excellent
+++ - Good
++ - Moderate
+ - Slight

Table 10. Utilization of carbon sources by the isolate Xad 10 on agar slants and broth of basal medium for Xanthomonads

Sl. No.	Carbon sources	Acid production on agar slants	Time (days) when the colour change was noticed	Optical density in broth at 510 nm*
1.	Maltose	Positive	4	0.594
2.	Dextrose	Positive	3	0.634
3	Galactose	Positive	3	0.907
.4.	Glucose	Positive	3	0.974
5.	Sucrose	Positive	3	1.070
6.	Fructose	Positive	3	0.740
7.	Inositol	Negative	Unchanged	0.330
8.	Lactose	Positive	3	0.671
9.	Xylose	Positive	4	0.727
10.	Dulcitol	Negative	Unchanged	0.146

*Mean of three replications
CD for treatments (0.05 level) = 0.126

fourth day of inoculation while in others acid production started from the third day of inoculation. There was no change in the colour of the medium from reddish violet to yellow in the case of tubes containing Inositol and Dulcitol which indicated negative utilization of these sugars by the isolate Xad 10.

Carbon utilization by the isolate Xad 10 was also studied in broth of BX using ten carbon sources. The results are presented in the Table 10 and Fig. 2.

Among the ten carbon sources used in the study, the best c sources for Xad 10 were sucrose (OD = 1.070) and glucose (OD = 0.974) while those carbon sources which failed to produce acid on agar slants (inositol and dulcitol) were also utilised by the isolate as is evidenced by low optical density. Least utilized carbon source by the isolate Xad 10 was dulcitol (OD = 0.146).

4.6 HOST RANGE AND SYMPTOMATOLOGY

Plants with similar taxonomic affinity to anthurium as well as other ornamental plants seen in the anthurium garden were artificially inoculated with the test bacterium for studying the host range of the pathogen (Table 11 and Plate 4).

The time taken for symptom development after artificial inoculation on 20 different test plants showed variations. The plants belonging to Araceae and Marantaceae exhibited symptoms within six days, while those belonging to Orchidaceae took seven days for symptom expression. Plants belonging to Musaceae took nine days. The maximum time taken for symptom expression was in plants belonging to family Liliaceae. Among the plants belonging to family Araceae *Aglaonema* sp. produced the symptom within four days.

In *Dieffenbachia maculata* and two other *Dieffenbachia* sp., *Syngonium podophyllum* and *Maranta arundinaceae*, the inoculated plants

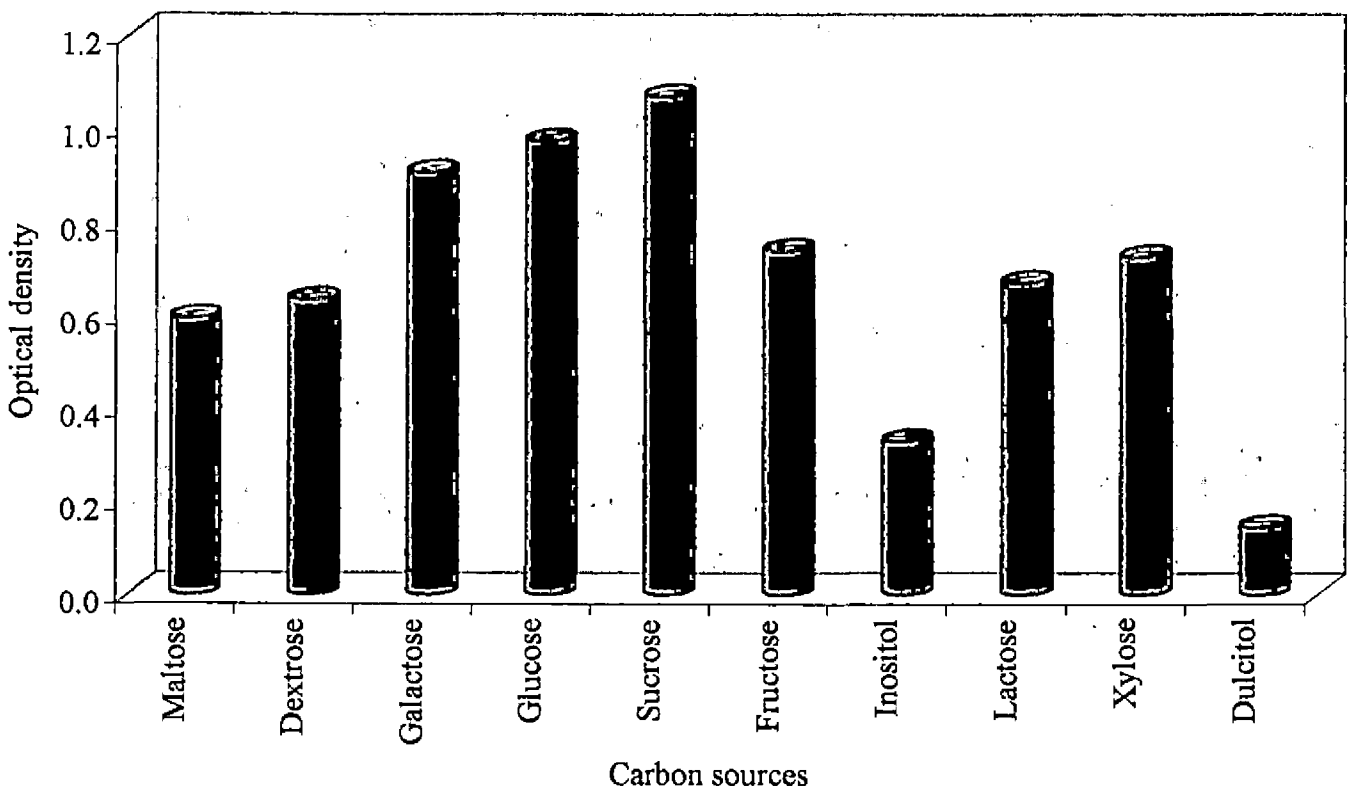


Fig. 2 Utilization of carbon sources by the isolate Xad 10 on broth of basal medium for Xanthomonads

Table 11. Host range of *Xanthomonas axonopodis* pv. *dieffenbachiae* causing bacterial blight of anthurium

Sl. No.	Plants	Reaction	Incubation period after artificial inoculation (in days)	Kind of symptoms
1	*Aglaonema* sp.	+	4	Yellowing and collapsing of the affected tissues
2	*Alocasia sanderiana*	+	5	Water soaked brown lesions surrounded by an yellow halo forming bigger patches
3	*Anthurium ornatum*	+	6	Water soaked brown necrotic spots surrounded by an yellow halo
4	*Colocasia esculenta*	+	6	Water soaked brown lesion resulted in shot hole
5	*Dieffenbachia maculata*	+	5	Water soaked spots later turn necrotic and became papery white with an yellow halo
6	*Dieffenbachia* sp.	+	5	Water soaked spots later turn necrotic and became papery white with an yellow halo
7	*Dieffenbachia* sp.	+	5	Water soaked light brown spots which later turned dark brown bigger patches
8	*Philodendron eichleri*	+	6	Circular to irregular water soaked spots which later turned dark brown and surrounded by an yellow halo
9	*Philodendron* sp.	+	6	Circular to irregular water soaked spots which later turned dark brown and surrounded by an yellow halo

Table 11. Continued

Sl. No.	Plants	Reaction	Incubation period after artificial inoculation (in days)	Kind of symptoms
10	*Philodendron domesticum*	+	6	Circular to irregular water soaked spots which later turned dark brown and surrounded by an yellow halo
11	*Philodendron* sp.	+	6	Circular to irregular water soaked spots which later turned dark brown and surrounded by an yellow halo
12	*Syngonium podophyllum*	+	5	Water soaked areas which later turned papery white and bordered by an yellow halo
13	*Xanthosoma sagittaefolium*	+	6	Water soaked spots which later turned black and resulted in shedding of affected tissue
14	Orchid sp.	+	7	Minute water soaked specks which enlarge and turn brown and later produced rotting symptoms
15	Orchid sp.	+	7	Minute water soaked specks which enlarge and turn brown and later produced rotting symptoms
16	*Strelitzia reginae*	+	9	Translucent spots later turning to bigger patches
17	*Maranta arundinaceae*	+	5	Water soaked spots later became necrotic and surrounded by an yellow halo
18	*Heliconia rostrata*	+	9	Translucent spots later turning to bigger patches
19	*Dracaena sanderiana*	+	12	Yellowing of the affected tissue
20	*Dracaena* sp.	+	10	Water soaking and shredding of leaves

A. *Dieffenbachia* sp.

B. *Dieffenbachia* sp.

C. *Dieffenbachia maculata*

D. *Syngonium podophyllum*

E. *Maranta arundinaceae*

Plate 4. Symptoms on host plants artificially inoculated with *Xanthomonas axonopodis* pv. *dieffenbachiae*

F. *Aglaonema* sp.

G. *Orchid* sp.

H. *Orchid* sp.

I. *Anthurium ornatum*

J. *Colocasia esculenta*

K. *Xanthosoma sagittaefolium* .

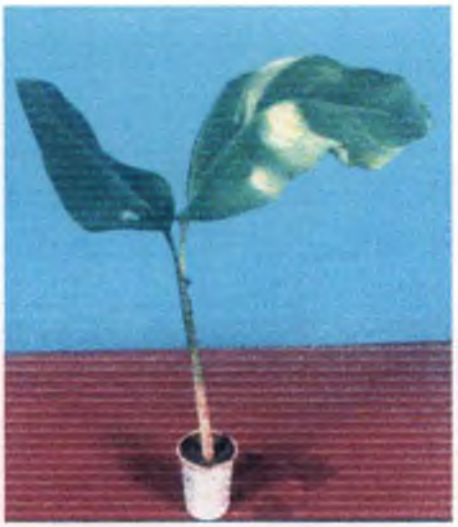
L. *Philodendron eichleri*.

M. *Philodendron* sp.

N. *Philodendron* sp.

O. *Philodendron* sp.

Plate 4. continued



P. *Heliconia rostrata* .

Q. *Sterilitzia reginae*

R. *Dracaena sanderiana*

S. *Alocasia sanderiana*

T. *Dracaena* sp.

Plate 4. continued

developed symptoms as irregular water soaked spots. The size of the spots varied from minute dot to 10 mm. Later these water soaked area became necrotic, papery white and were bordered by an yellow halo. The symptoms were more pronounced on upper surface of the leaf. But in one species of *Dieffenbachia* the symptom appeared as light brown water soaked area, which later enlarged and turned dark brown in colour covering a major portion of leaf lamina.

In *Aglaonema* species, the symptoms developed as yellowing of leaves. The affected portions later became flaccid and finally the affected tissue got collapsed.

Symptoms were initiated as minute water soaked specks which gradually enlarged and turned brown and were surrounded by an yellow halo. Water soaking was more pronounced on the undersurface of the leaf. As the disease advanced the leaves started rotting. Premature leaf fall was also noticed.

In aroids including *Anthurium ornatum, Colocasia esculenta* and *Xanthosoma sagittaefolium* initial symptoms were noticed as irregular water soaked spots of two to five mm diameter. Later these spots enlarged and turned black and necrotic. In *A. ornatum* the black necrotic area was surrounded by an yellow halo. Later blighting symptom developed. In *C. esculenta and X. sagittaefolium*, shot hole symptoms were produced as a result of shedding of central portion of the necrotic region.

All the four *Philodendron* spp. produced water soaked circular to irregular spots of varying size which later coalesced and turned dark brown with an yellow halo. As the disease progressed yellowing and defoliation was observed.

Symptoms on *Heliconia rostrata* and *Sterilitzia reginae* were developed minute translucent dots of one to two mm in size which later coalesced forming bigger patches. The affected tissue turned yellow in colour.

In *Dracaena sanderiana* symptoms appeared as yellowing of the inoculated region which later turned brown and finally the affected tissue dried up. But in another species of *Dracaena* the symptoms were noticed ten days after inoculation as water soaked necrotic spots which later coalesced forming larger patches. The water soaked area became flaccid and finally shredding of the affected tissues was noticed.

In *Alocasia sanderiana* symptoms developed as minute water soaked specks, which were light brown initially, gradually enlarged and turned dark brown.

4.7 MANAGEMENT

4.7.1 *In vitro* Studies

4.7.1.1 Antibacterial Effect of Botanicals Against X. axonopodis pv. dieffenbachiae

Antibacterial activity of aqueous extracts of eight different plant species were evaluated for their effectiveness in inhibiting the growth of *X. axonopodis* pv. *dieffenbachiae* under *in vitro* conditions (Table 12 and Fig. 3).

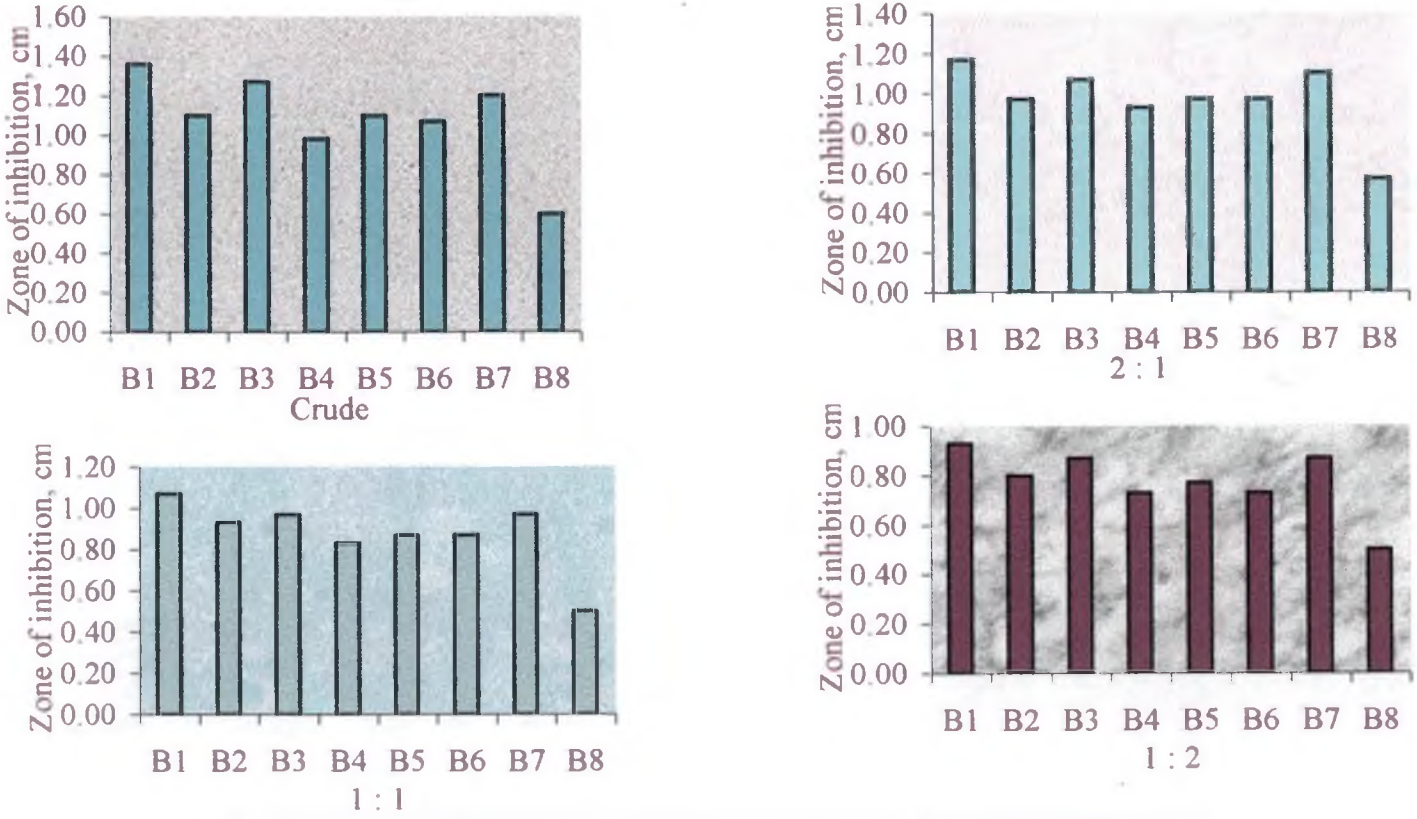
Among the eight different extracts tested, *Allium sativum* extract was found to be the best in inhibiting the growth of *X. axonopodis* pv. *dieffenbachiae*. This was followed by *Tagetes erecta* extract and *Centella asiatica* extract which were statistically on par and significantly inferior to *A. sativum* extract. Extract of *Allium cepa, Ocimum sanctum* and *Lawsonia inermis* were found inhibitory to the pathogen and were statistically on par but were inferior to all the above treatments. The lowest inhibitory response against the bacterium was exhibited by *Coleus aromaticus* and *Tabernaemontana coronaria* extracts.

The interaction between different doses and the botanicals was not statistically significant. So depending upon the botanical, the dose that gave higher response against the test pathogen was selected for further

Table 12. Effect of botanicals on the growth of *Xanthomonas axonopodis* pv. *dieffenbachiae*

Sl. No.	Treatments*	Diameter of inhibition zone (cm)				Mean
		Crude	2 : 1	1 : 1	1 : 2	
1	*Allium sativum*	1.36 (1.17)	1.17 (1.08)	1.07 (1.03)	0.93 (0.97)	1.128 (1.062)
2	*Allium cepa*	1.10 (1.05)	0.97 (0.98)	0.93 (0.97)	0.80 (0.90)	0.947 (0.973)
3	*Tagetes erecta*	1.27 (1.13)	1.07 (1.03)	0.97 (0.98)	0.87 (0.93)	1.036 (1.018)
4	*Tabernaemontana coronarea*	0.98 (0.99)	0.93 (0.96)	0.83 (0.91)	0.73 (0.86)	0.870 (0.933)
5	*Ocimum scantum*	1.10 (1.05)	0.97 (0.98)	0.87 (0.93)	0.77 (0.87)	0.919 (0.959)
6	*Lawsonia inermis*	1.07 (1.03)	0.97 (0.98)	0.87 (0.93)	0.73 (0.86)	0.903 (0.950)
7	*Centella asiatica*	1.20 (1.09)	1.10 (1.05)	0.97 (0.98)	0.87 (0.88)	1.000 (1.000)
8	*Coleus aromaticus*	0.60 (0.77)	0.57 (0.75)	0.50 (0.71)	0.50 (0.71)	0.540 (0.736)
	Mean	1.073 (1.036)	0.956 (0.978)	0.866 (0.931)	0.757 (0.870)	

CD (0.05 level) between treatments = 0.0249
CD (0.05 level) between doses = 0.017
Figures in parenthesis are square root transformed values
*Streprocycline 100 ppm check provided an inhibition zone of 1.70 cm



B1	*Allium sativum*	B5	*Ocimum scantum*
B2	*Allium cepa*	B6	*Lawsonia inermis*
B3	*Tagetes erecta*	B7	*Centella asiatica*
B4	*Tabernaemontana coronaria*	B8	*Coleus aromaticus*

Fig. 3 Effect of botanicals on the growth of *Xanthomonas axonopodis* pv. *dieffenbachiae* .

trials. As the concentration of crude extract was reduced the efficacy was also reduced.

Extracts of *A. sativum* and *T. erecta* were found to be the two best botanicals in inhibiting the growth of *X. axonopodis* pv. *dieffenbachiae* under *in vitro* condition. The two effective doses were crude extract and 2 : 1 dilution. The growth inhibition zones produced by crude extract of *A. sativum* were 1.36 cm and 1.17 cm while the growth inhibition zones produced by crude extract and 2 : 1 dilution of *T. erecta* were 1.27 cm and 1.07 cm respectively (Table 12). So these two botanicals and doses were selected for further evaluation under *in vivo* condition.

4.7.1.2 Antibacterial Effect of Oils and Two Plant Products Against X. axonopodis pv. dieffenbachiae

Antibacterial activity of five oils *viz.*, neem oil, coconut oil, marotty oil, pongamia oil, castor oil and plant products like neem cake extract and turmeric powder + sodium bicarbonate (10 : 1) mixture were evaluated against the test bacterium (Table 13 and Fig. 4).

Among the different treatments, inhibitory effect of neem oil was statistically on par with coconut oil and they were significantly better than other treatments. This was followed by neem cake extract. Marotty oil, turmeric + sodium bicarbonate (10 : 1) mixture and castor oil were inferior to all the above treatments and were statistically on par. The least response to the test pathogen was shown by pongamia oil.

The interaction between the doses and treatments was not statistically significant. Among the four doses of oils evaluated one and two per cent concentrations were found to be better in inhibiting the growth of *X. axonopodis* pv. *dieffenbachiae*. But for plant products *viz.*, neem cake extract and turmeric + sodium bicarbonate (10 : 1) mixture, crude extract was found to be the best followed by two per cent concentration in inhibiting the growth of *X. axonopodis* pv. *dieffenbachiae*.

Table 13. Effect of oils and plant products on growth of *Xanthomonas axonopodis* pv. *dieffenbachiae*

Sl. No.	Treatments*	Diameter of inhibition zone (cm)				Mean
		Crude / pure	2 per cent	1 per cent	0.5 per cent	
1	Neem cake extract	1.17 (1.08)	0.93 (0.97)	0.83 (0.91)	0.73 (0.86)	0.910 (0.954)
2	Neem oil	0.87 (0.93)	1.17 (1.07)	0.97 (0.98)	0.87 (0.93)	0.962 (0.981)
3	Coconut oil	0.83 (0.91)	1.07 (1.03)	0.97 (0.98)	0.83 (0.91)	0.922 (0.960)
4	Castor oil	0.73 (0.86)	0.83 (0.91)	0.77 (0.88)	0.70 (0.84)	0.757 (0.870)
5	Marotty oil	0.80 (0.89)	0.97 (0.98)	0.87 (0.93)	0.80 (0.90)	0.857 (0.926)
6	Pongamia oil	0.50 (0.71)	0.73 (0.86)	0.63 (0.79)	0.50 (0.71)	0.587 (0.766)
7	Turmeric + sodium bicarbonate	0.97 (0.98)	0.93 (0.97)	0.83 (0.91)	0.70 (0.84)	0.854 (0.924)
	Mean	0.826 (0.909)	0.943 (0.971)	0.834 (0.913)	0.728 (0.853)	

CD (0.05 level) between treatments = 0.024
CD (0.05 level) between doses = 0.018
Figures in parenthesis are square root transformed values
*Streptocycline 100 ppm check provided an inhibition zone of 1.70 cm

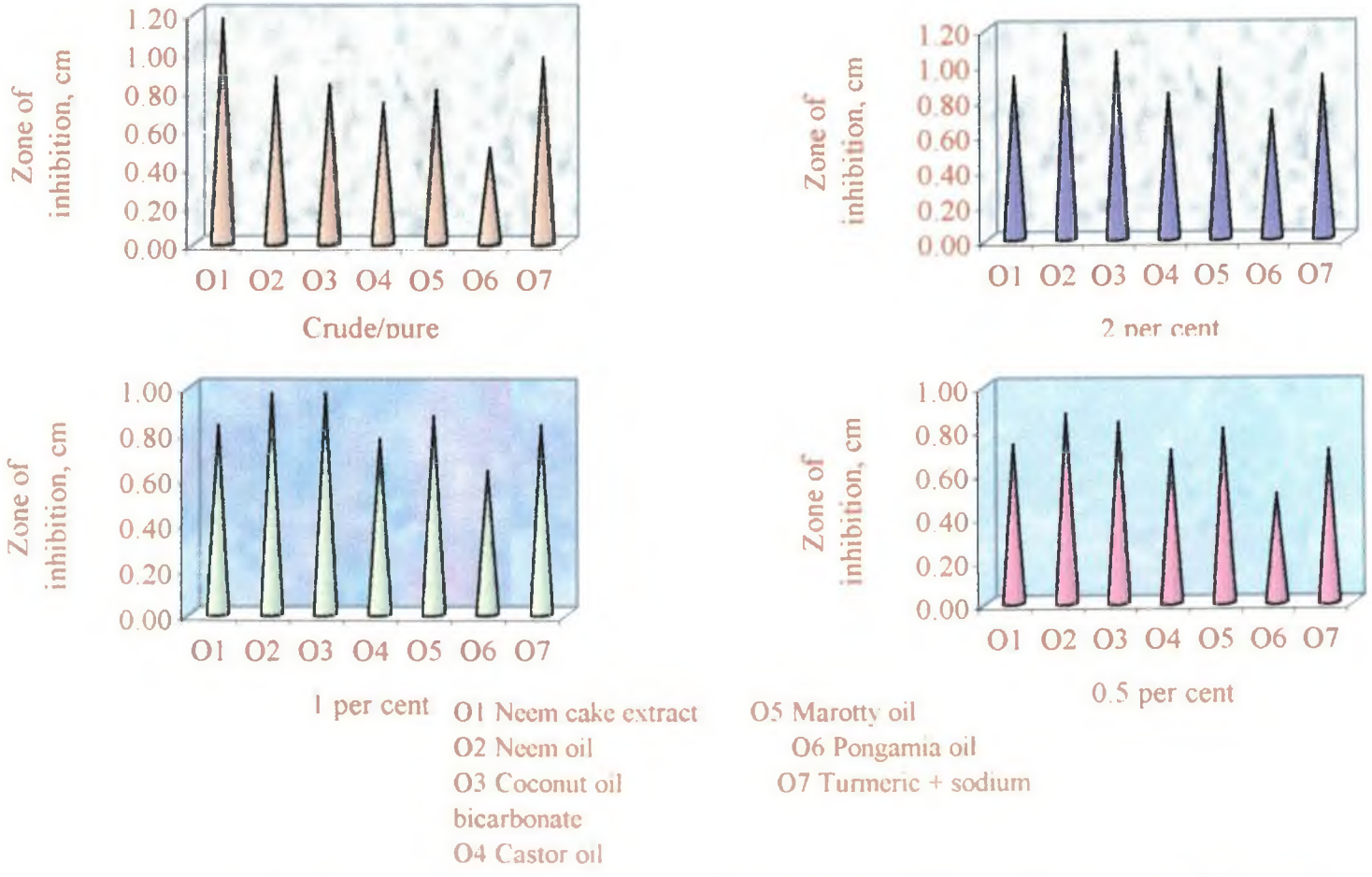


Fig 4 Effect of oils and plant products on growth of *Xanthomonas axonopodis* pv. *dieffenbachiae*

So two and one per cent concentrations of neem oil and coconut oil and crude and two per cent neem cake extract were selected for further evaluation under *in vivo* condition. The growth inhibition zones produced by neem and coconut oil at two per cent and one per cent concentration and were 1.17 cm, 0.97 cm, 1.07 cm and 0.97 cm respectively. The inhibition zones produced by crude extract and two per cent neem cake extract were 1.17 cm and 0.93 cm (Table 13).

The growth inhibition zone produced by 100 ppm streptocycline used as check was 1.7 cm.

4.7.2 *In vivo* Studies

4.7.2.1 Effect of Botanicals on Disease Infection on Seven Month Old Plants

Seven month old tissue culture plants of *A. andreanum* var. Cancan was used for the experiment. The plants were given two pre inoculation sprayings at five days and two days before artificial inoculation and two post inoculation sprayings at two days and five days after artificial inoculation with *X. axonopodis* pv. *dieffenbachiae*, the incitant of bacterial blight disease. Observations on per cent infection of the disease were recorded before the first and the second post inoculation spraying and also one week and two weeks after the second post inoculation spraying. The results of the study are presented in Table 14 and Plate 5.

4.7.2.1.1 Effect of pre Inoculation Sprayings on Disease Infection

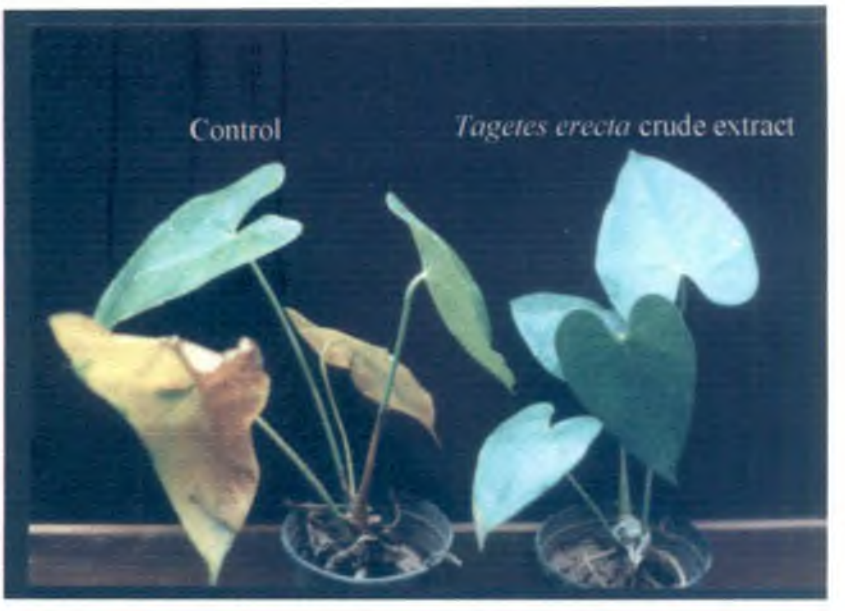
Two pre inoculation sprayings were given with the following botanicals *viz.*, *A. sativum* (crude extract and 2 : 1 dilution), *T. erecta* (crude extract and 2 : 1 dilution), neem oil (two per cent and one per cent), coconut oil (two per cent and one per cent) neemcake (crude extract and two per cent) and streptocycline 100 ppm. The plants sprayed with water served as the control. All the plants which were given preventive sprays developed symptoms of the disease after artificial inoculation, but the

Table 14. Effect of botanicals on bacterial blight of Anthurium (seven month old plants)

Sl. No.	Treatments	Per cent infection of bacterial blight							
		Before first post inoculation spraying	Reduction over control	Before second post inoculation spraying	Reduction over control	One week after last spraying	Reduction over control	Two weeks after last spraying	Reduction over control
1	*Allium sativum* (crude)	9.40 (3.23)	85.46	30.86 (5.64)	58.47	50.77 (7.19)	44.55	60.77 (7.80)	37.25
2	*Allium sativum* (2 : 1 dilution)	22.90 (4.89)	64.63	35.61 (6.05)	52.08	63.06 (8.00)	31.13	71.00 (8.32)	26.68
3	*Tagetes erecta* (crude)	3.13 (2.04)	95.17	5.74 (2.60)	92.28	12.91 (3.72)	85.90	14.05 (3.74)	85.49
4	*Tagetes erecta* (2 : 1 dilution)	11.91 (3.59)	81.67	14.22 (3.90)	80.86	21.93 (4.79)	76.05	27.93 (5.28)	71.16
5	Neem oil (2 per cent)	10.15 (3.34)	84.32	22.82 (4.88)	69.29	39.96 (6.40)	56.36	50.17 (7.07)	48.19
6	Neem oil (one per cent)	11.57 (3.55)	82.13	25.70 (5.16)	65.42	43.70 (6.69)	52.27	51.00 (7.14)	47.34
7	Coconut oil (two per cent)	7.68 (2.95)	88.14	13.14 (3.76)	82.32	26.26 (5.22)	71.32	31.52 (5.61)	67.45
8	Coconut oil (one per cent)	9.36 (3.22)	85.54	13.98 (3.87)	81.19	30.58 (5.62)	66.60	42.13 (6.49)	56.49
9	Neem cake extract (crude)	1.20 (1.48)	98.15	3.62 (7.15)	95.19	9.64 (3.26)	90.01	12.91 (3.72)	86.67
10	Neem cake extract (2 per cent)	5.66 (2.58)	91.26	7.23 (2.87)	90.27	14.54 (3.94)	84.12	19.01 (4.36)	80.36
11	Streptocycline	12.03 (4.23)	73.85	12.20 (3.63)	83.58	13.48 (3.81)	85.27	15.18 (3.97)	84.32
12	Control	64.75 (8.11)		74.34 (8.68)		91.56 (9.62)		96.84 (9.84)	
	CD (0.05) level	1.54		1.90		1.45		1.50	

Figures in parenthesis are square root transformed values

A. Effect of spraying crude extract of neem cake



B. Effect of spraying crude extract of *Tagetes erecta*

Plate 5. Management of bacterial blight of anthurium (seven month old plants)

intensity of symptom was less in treated plants compared to control. The untreated control plants exhibited systemic symptoms within three days after artificial inoculation.

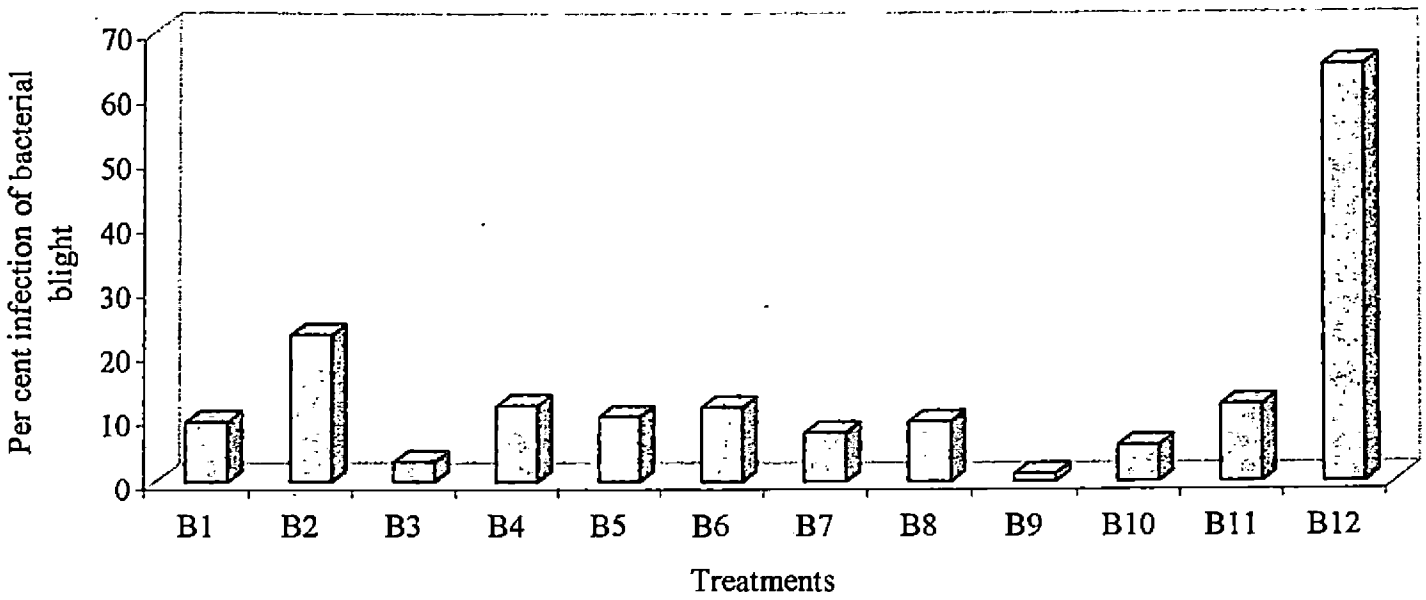
The initial observation on per cent disease infection of bacterial blight on anthurium plants was taken on the day of first post inoculation spraying *i.e.*, two days after artificial inoculation (Table 14 and Fig. 5). All the treated plants had bacterial blight infection ranging from 3.13 to 22.90 per cent compared to 64.75 per cent in control.

The disease reduction was maximum in the case of plants which received two pre inoculation sprayings of crude extract of neem cake (98.15 per cent). This was followed by two pre inoculation sprayings of crude extract of *T. erecta* (95.17 per cent), two per cent neem cake extract (91.26 per cent) and two per cent coconut oil (88.14 per cent).

4.7.2.1.2 Effect of Botanicals on Disease Infection After the First Post Inoculation Spray

The per cent infection of bacterial blight was recorded after the first post inoculation spraying. The results revealed that the effect of two pre inoculation and one post inoculation spraying with crude extract of neem cake and *T. erecta*, two per cent neem cake extract, streptocycline 100 ppm, two and one per cent coconut oil were statistically on par and were superior to rest of the treatments in reducing the bacterial blight disease on anthurium.

The control plants exhibited 74.34 per cent disease infection (Table 14). This was significantly more than all the other treatments. Among the other treatments crude extract of neem cake (3.62) and *T. erecta* (5.74), two per cent neem cake extract (7.23), streptocycline 100 ppm (12.20), two and one per cent coconut oil (13.14 and 13.98) were all on par and exhibited low per cent disease infection.



B1 *Allium sativum*(crude)
B2 *Allium sativum*(2 : 1 dilution)
B3 *Tagetes erecta*(crude)
B4 *Tagetes erecta*(2 : 1 dilution)
B5 Neem oil (2 per cent)
B6 Neem oil (one per cent)
B7 Coconut oil (two per cent)
B8 Coconut oil (one per cent)
B9 Neem cake extract (crude)
B10 Neem cake extract (2 : 1 dilution)
B11 Streptocycline
B12 Control

Fig. 5 Effect of spraying botanicals on bacterial blight of anthurium (seven month old plants) after two pre inoculation sprays

The extend of disease reduction was maximum (95.19 per cent) when crude extract of neem cake was sprayed. This was followed by crude extract of *T. erecta*, two per cent concentration of neem cake extract, 100 ppm streptocycline, two per cent and one per cent coconut oil, where the disease reduction over control were 92.28, 90.27, 83.58, 82.32 and 81.19 per cent respectively.

4.7.2.1.3 Effect of Pre Inoculation and Post Inoculation Spraying on Disease Infection

The per cent infection of bacterial blight was recorded one week after the second post inoculation spraying. The results showed that plants receiving two pre inoculation and two post inoculation applications of crude extract of neem cake and *T. erecta*, streptocycline 100 ppm and two per cent neem cake extract were statistically on par and superior to all other treatments in reducing the bacterial blight infection. The per cent disease infection recorded after four sprayings was 9.64 in the case of crude extract of neem cake. This was followed by crude extract of *T. erecta* (12.91), streptocycline 100 ppm (13.48) and two per cent neem cake extract (14.54). All the plants receiving other treatments possessed more than 15 per cent disease infection after four sprayings. The control plants showed 91.56 per cent disease infection. The extent of disease reduction over control was 90.01 per cent after four sprayings of crude extract of neem cake. This was closely followed by four spraying of crude extract of *T. erecta* (85.90 per cent), streptocycline 100 ppm (85.27 per cent) and two per cent neem cake extract (84.12) (Table 14).

4.7.2.1.4 Effect of Botanicals on Disease Infection Two Weeks After Fourth Spray

The per cent infection of bacterial blight was recorded two weeks after fourth spraying. The results revealed that plants which received four sprayings with crude extract of neem cake and *T. erecta*, streptocycline

100 ppm and two per cent neem cake extract were statistically on par and were superior to all the other botanicals tested (Table 14 and Fig. 6).

Two weeks after the last spraying, the lowest infection of 12.91 per cent was recorded by plants sprayed with crude extract of neem cake. This was closely followed by the treatments, crude extract of *T. erecta*, streptocycline 100 ppm and two per cent neem cake extract, where the per cent disease infection were 14.05, 15.18 and 19.01 per cent respectively. All the plants receiving other botanicals possessed more than 25 per cent infection two weeks after the fourth spraying. The control plants showed 96.84 per cent infection.

The extent of disease reduction over control was maximum (86.67 per cent) in the case of crude extract of neem cake followed by crude extract of *T. erecta* (85.49 per cent), 100 ppm streptocycline (84.32 per cent) and two per cent neem cake extract (80.36 per cent).

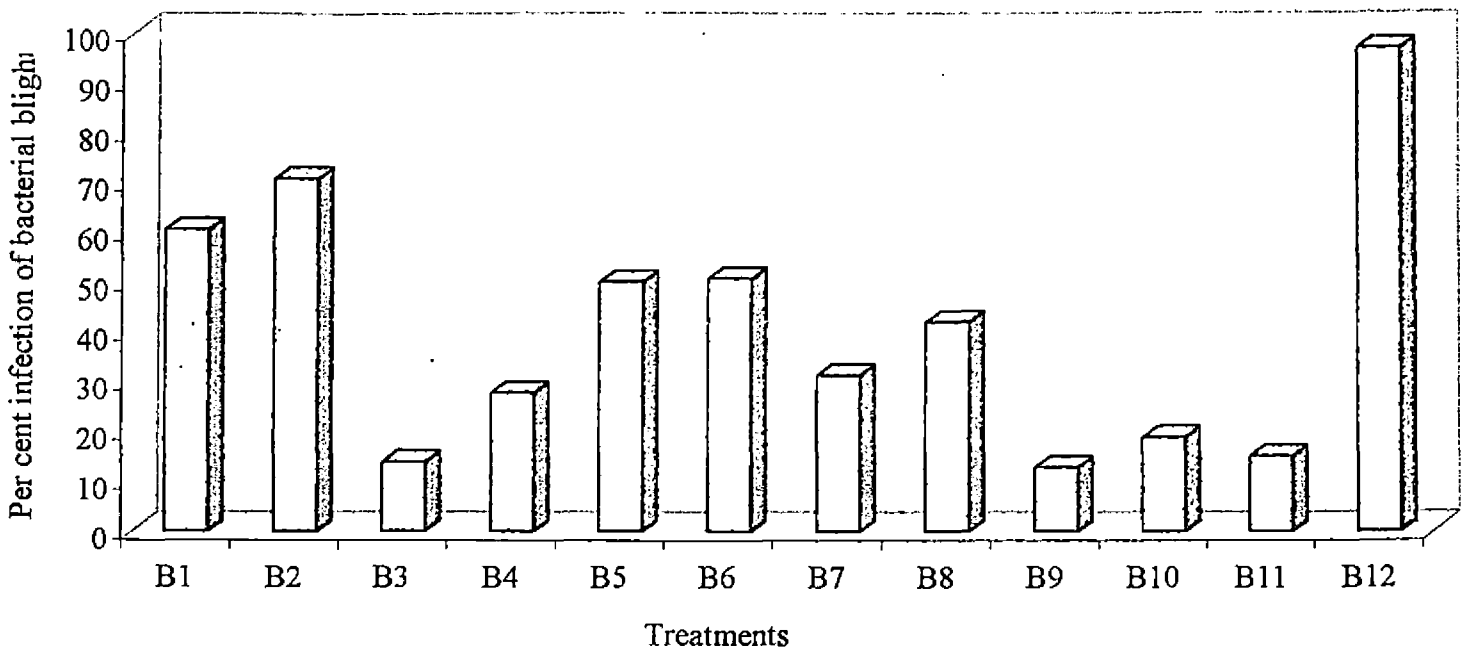
Based on this experiment the crude extract of neem cake and that of *T. erecta*, where the reduction of disease over control was more than 85 per cent were selected for further evaluation using grown up plants of flowering size.

In streptocycline treated plants the rate of development of the disease was less compared to botanicals. The per cent disease infection of this treatment was 12.03 three days after inoculation and at the end of two weeks after last spraying, the per cent disease infection was up to 15.18 *i.e.*, there was an increase of 3.15 per cent compared to per cent disease infection in neem cake extract (1.20 to 12.91) and *T. erecta* (3.13 to 14.05) were 11.71 per cent and 10.92 per cent respectively.

4.7.2.2 Effect of Botanicals on Disease Infection on Flowering Plants

4.7.2.2.1 Effect of Pre Inoculation Spraying on Disease Infection

All the plants developed symptoms after inoculation. But the intensity of symptom development was very less in those plants receiving



B1 *Allium sativum* (crude)
B2 *Allium sativum* (2 : 1 dilution)
B3 *Tagetes erecta* (crude)
B4 *Tagetes erecta* (2 : 1 dilution)
B5 Neem oil (2 per cent)
B6 Neem oil (one per cent)
B7 Coconut oil (two per cent)
B8 Coconut oil (one per cent)
B9 Neem cake extract (crude)
B10 Neem cake extract (2 : 1 dilution)
B11 Streptocycline
B12 Control

Fig. 6 Effect of spraying botanicals on bacterial blight of anthurium (seven month old plants) two weeks after last spray

treatments compared to that of control. The control plants exhibited systemic symptoms earlier than treated plants (Table 15 and Fig. 7).

The initial observation on per cent disease infection of bacterial blight on anthurium plants was recorded on the day of first post inoculation spray. All the treated plants developed bacterial blight infection ranging from 0.67 to 9.17 per cent compared to 32.17 per cent disease infection in control.

The results revealed that the per cent disease infection before post inoculation application of crude extract of neem cake was statistically on par with crude extract of *T. erecta* and was significantly less than other treatments. This was followed by the application of streptocycline 100 ppm.

The per cent reduction of bacterial blight disease over control are presented in Table 15. The extent of disease reduction (97.92 per cent) was maximum for those plants receiving two pre inoculation sprayings of crude extract of neem cake. This was followed by crude extract of *T. erecta* and streptocycline 100 ppm where the per cent disease reduction over the control were 90.24 and 69.79 respectively.

4.7.2.2.2 Effect of Post Inoculation Spraying on Disease Infection

The per cent infection of bacterial blight was recorded before the second post inoculation spraying and one week and two weeks after the post inoculation sprayings.

The results revealed that per cent disease infection recorded before the second post inoculation spraying was least in plants treated with crude extract of neem cake and *T. erecta* and they were statistically on par (Table 15 and Plate 6). This was followed by the application of streptocycline 100 ppm. The same trend was observed in the results recorded one week and two weeks after last spraying.

Table 15. Effect of botanicals on bacterial blight of Anthurium (flowering plants)

Sl. No.	Treatments	Per cent infection of bacterial blight							
		Before first post inoculation spraying	Reduction over control	Before second post inoculation spraying	Reduction over control	One week after last spraying	Reduction over control	Two weeks after last spraying	Reduction over control
1	*Tagetes erecta* (crude)	3.14 (2.04)	90.24	3.71 (2.17)	92.34	7.58 (2.93)	89.18	10.26 (3.20)	88.66
2	Neem cake extract (crude)	0.67 (1.29)	97.92	1.99 (1.73)	95.89	3.32 (2.08)	95.27	5.14 (2.27)	94.32
3	Streptocycline (100 ppm)	9.17 (3.27)	69.79	9.72 (2.98)	83.76	9.87 (3.29)	87.82	12.37 (3.52)	86.30
4	Control	32.17 (5.76)		48.45 (7.03)		70.13 (8.27)		90.50 (9.50)	
	CD (0.05 level)	1.90		1.23		1.16		1.21	

Figures in parenthesis are square root transformed values

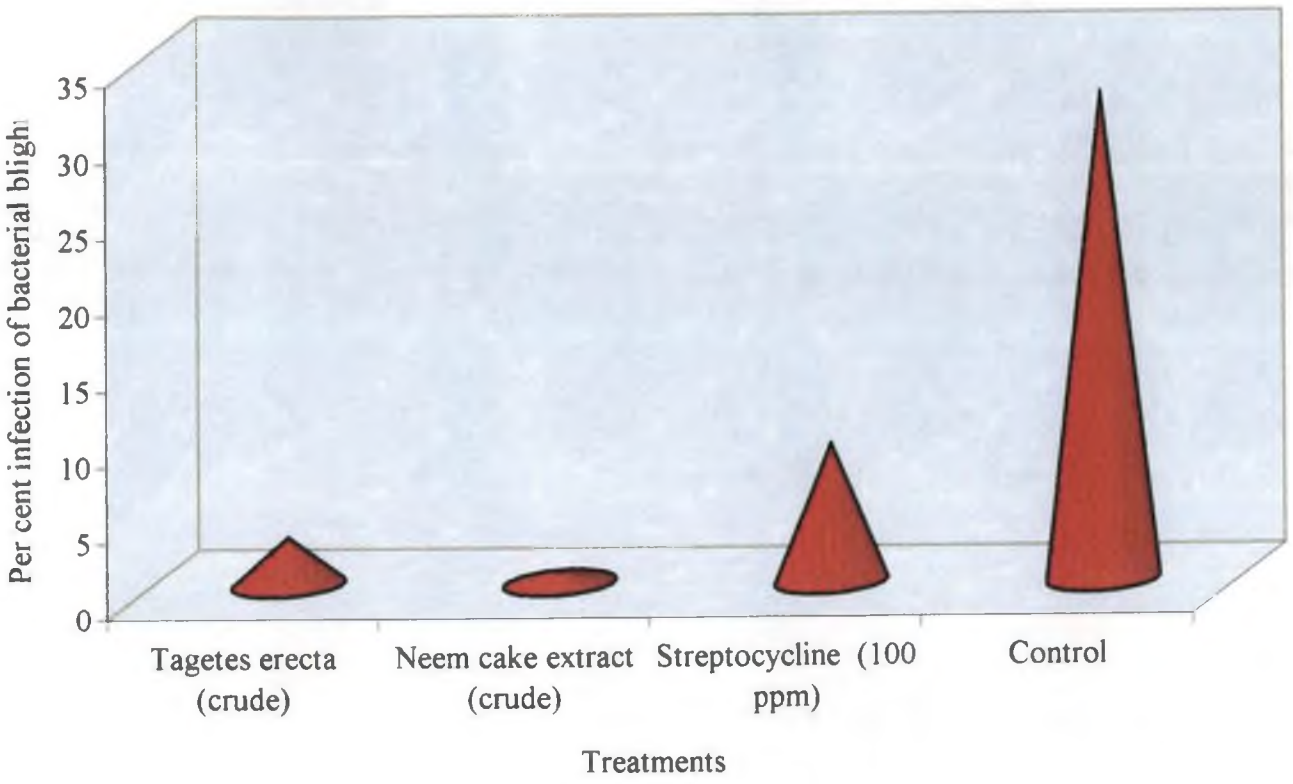
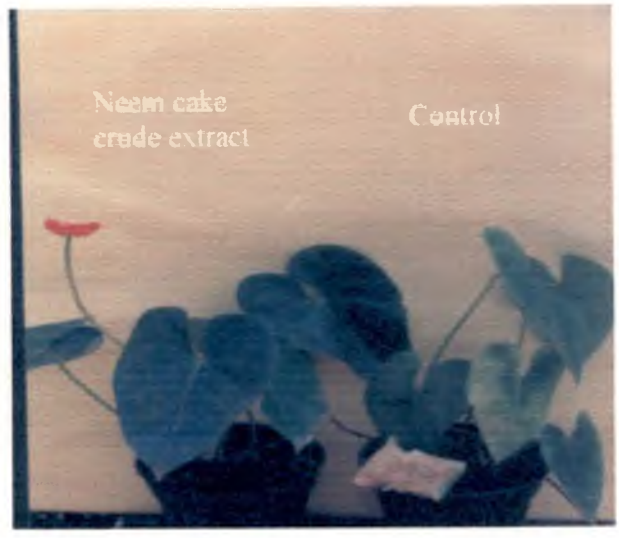


Fig. 7 Effect of spraying botanicals on bacterial blight of anthurium (flowering plants) after two pre inoculation sprays



A. Effect of spraying crude extract of neem cake

B. Effect of spraying crude extract of *Tagetes erecta*

Plate 6. Management of bacterial blight of anthurium (grown up plants)

The per cent infection recorded before the second post inoculation spraying and one week and two weeks after the last spraying of crude extract of neem cake were 1.99, 3.32 and 5.14 per cent respectively. While 3.71, 7.58 and 10.26 per cent infection was recorded for the treatment with crude extract of *T. erecta*. The per cent infection recorded for the application of 100 ppm streptocycline were 9.72, 9.87 and 12.37 per cent respectively. The control plants recorded 48.45, 70.13 and 90.50 per cent infection before the second post inoculation spraying and one week and two weeks after last spraying.

The per cent reduction of bacterial blight disease over control was maximum for crude extract of neem cake where the disease reduction noticed before the second post inoculation spraying and one week and two weeks after the post inoculation sprayings were more than 94.32 per cent (Table 15 and Fig. 8). In the case of crude extract of *T. erecta* 92.34 per cent reduction was noticed over the control before the second post inoculation spraying. While the reduction over control ranged from 88.66 to 89.18 per cent, one week and two weeks after the post inoculation sprayings. Per cent reduction by streptocycline were between 83.76 to 86.30 during this period.

In streptocycline treated plants the rate of development of the disease was less compared to botanicals. The per cent disease infection of this treatment was 9.17 three days after inoculation and at the end of two weeks after last spraying, the per cent disease infection was up to 12.37 *i.e.*, there was an increase of 3.20 per cent compared to per cent disease infection in neem cake extract (0.67 to 5.14) and *T. erecta* (3.14 to 10.26) were 4.47 per cent and 7.12 per cent respectively.

None of the treatments were effective to give an absolute control against systemic infection of bacterial blight, but the crude extract of neem cake and *T. erecta* were effective in reducing the disease to an extent of 94.32 and 88.67 per cent respectively.

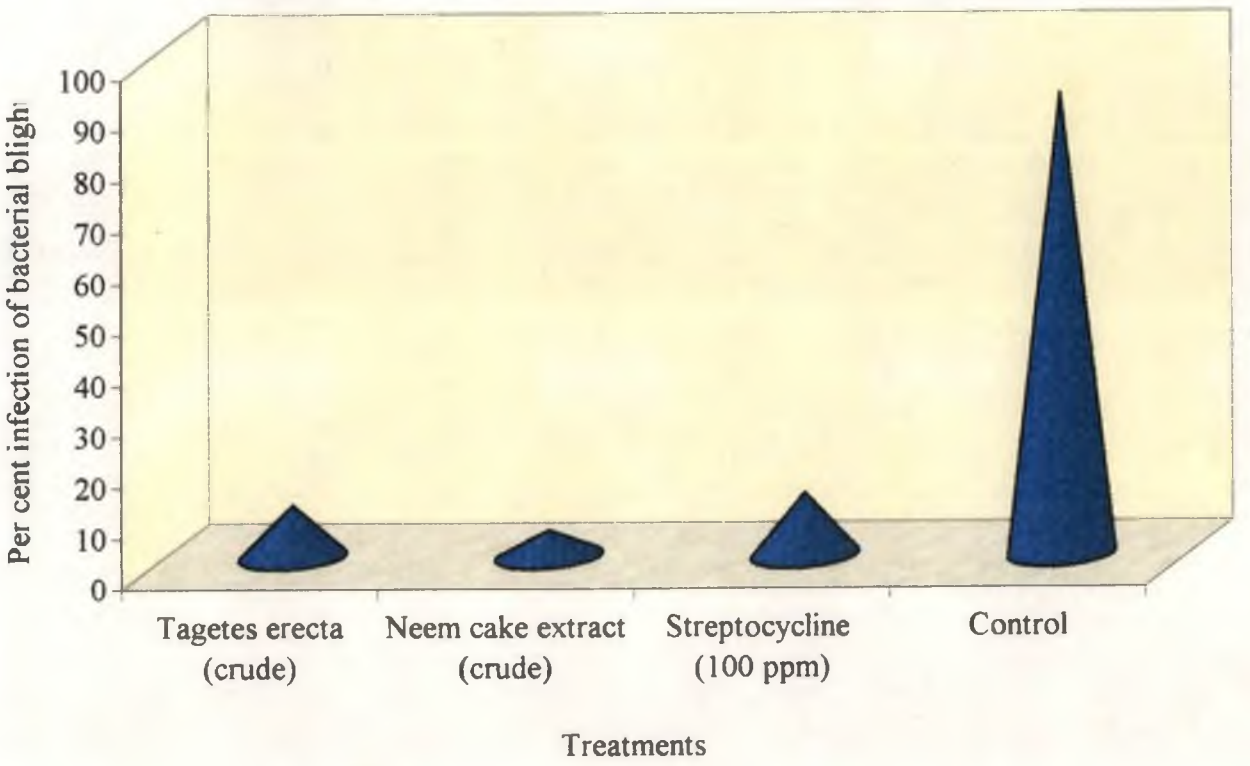


Fig. 8 Effect of spraying botanicals on bacterial blight of anthurium (flowering plants) two weeks after last spray

DISCUSSION

5. DISCUSSION

Anthurium (*Anthurium andreanum* Linden) is a valuable cut flower crop which has recently attained economic prominence in Kerala, offering great potential for floriculturist. The plants are in great demand among home gardeners and their cut flowers have good market within the state and abroad. Bacterial disease incited by *Xanthomonas axonopodis* pv. *dieffenbachiae* has been found to be a major constraint in the expansion and successful cultivation of anthurium. In recent years, consequent to the awareness on hazards of chemicals in controlling diseases, greater emphasis has been placed towards the development of botanicals.

In the present study, a survey was undertaken on the intensity of bacterial blight of anthurium in Thiruvananthapuram district, symptomatology of the disease, cultural and biochemical characterization of the pathogen, host range, screening of botanicals against the pathogen and management of the disease using botanicals.

Anthurium gardens from Thiruvananthapuram district were selected randomly and the intensity of the bacterial blight disease was recorded using the disease score chart developed by Dhanya (2000). Information on varietal reaction to the disease, manurial and cultural practices adopted, plant protection chemicals applied and the foliage and ornamental plants grown in and around anthurium gardens having similar disease symptoms were also collected.

The observation of the survey revealed that the disease intensity ranged from 0 to 90 per cent and in most of the gardens had plants with infection upto 50 per cent. A similar observation on survey conducted at Jamaica has been documented by Young (1990). He reported that disease infection varied from 25-100 per cent, thus causing heavy economic loss to the growers.

The most widely cultivated anthurium varieties by the growers in Thiruvananthapuram district are red varieties, especially Cancan, Honduras Hawaii, Tinora and Tropical. These varieties eventhough are highly susceptible to bacterial blight were cultivated due to consumer preference both locally and outside. Even though pink varieties showed a degree of tolerance it was not widely cultivated by growers as there is no market for this variety. Thus it was evident that considerable variation in varieties of anthurium to bacterial blight exist. Similar variations were observed by Natural *et al.* (1990). They observed that Nitta Exotic pink and orange to be resistant to infection.

Symptomatology of the disease, both under natural and artificial conditions were studied. On artificial inoculation all the general symptoms of the disease observed in naturally infected plants could be produced. Manifestation of symptoms commenced from three to seven days of inoculation. Further the symptoms of the disease observed under natural and artificial condition were almost similar to those described by Natural *et al.* (1990) and Dhanya (2000).

Naturally infected plants having foliar infection showed a variety of symptoms which were similar to those foliar symptoms described by Hayward (1972) in the first report on the incidence of bacterial blight of Anthurium from Hawaii. Originally he concluded bacterial blight of anthurium to be a foliar disease. Apart from foliar infection the bacterium also infected the petiole and rhizomes causing death of the plant. This type of systemic infection causes more economic loss as unlike in the case of foliar infection. These systemic symptoms are more common in red varieties.

When pathogen was inoculated on the leaves, the blight symptoms were primarily noticed on the foliage. When the base of the plants was inoculated with the pathogen systemic infection was noticed. Sometimes on inoculation of the leaves apart from developing foliar symptoms also

PSA (Table 6). Variations were observed in the growth of different isolates of the bacterium on potato sucrose broth (Table 7). All the isolates attained their maximum growth within a period of 48h except Xad 4 and Xad 10 and after that growth was found to decline. Among the ten isolates studied, the growth of isolate Xad 4 and Xad 10 was found to be increasing even after a period of 72h indicating that these isolates require much longer period of incubation when compared to rest of the isolates. Taking into consideration the nature of growth of the ten isolates, it was found that Xad 4 and Xad 10 were of faster growing type, Xad 2 was of slow growing type and rest of the isolates were of moderate growth. This result was in agreement with the reports of Natural *et al.* (1990). They reported that bacterium produce copious colonies in Potato Dextrose Agar and Nutrient Agar. Dhanya (2000) also reported maximum growth of the bacterium on PSA medium. The results of growth studies with the isolates of the bacterium revealed that PSA could be the best solid medium for routine laboratory test and mass culturing of the bacterium.

All the ten isolates of the bacterium studied, utilized glucose oxidatively, liquefied gelatin, hydrolysed arginine, produced hydrogen sulphide, lipase, ammonia and catalase. The biochemical tests were negative for indole production, Methyl Red and Voges Praskauer test. The bacterium failed to produce urease and tyrosinase. Milk was turned alkaline in reaction. None of the isolates utilized asparigine as the sole source of carbon and nitrogen. The different isolates of the bacterium utilized sodium salts of citric acid and acetic acid but not that of benzoic acid and formic acid and growth was inhibited at six per cent sodium chloride. The results were in agreement with the studies of earlier workers (Hayward, 1972; Cooksey, 1985; Pohronezny *et al.*, 1985; Dhanya, 2000).

In the present study, the isolates showed variable reaction to starch hydrolysis. This was in confirmity with the work of Bonner *et al.* (1987).

The most widely cultivated anthurium varieties by the growers in Thiruvananthapuram district are red varieties, especially Cancan, Honduras Hawaii, Tinora and Tropical. These varieties eventhough are highly susceptible to bacterial blight were cultivated due to consumer preference both locally and outside. Even though pink varieties showed a degree of tolerance it was not widely cultivated by growers as there is no market for this variety. Thus it was evident that considerable variation in varieties of anthurium to bacterial blight exist. Similar variations were observed by Natural *et al.* (1990). They observed that Nitta Exotic pink and orange to be resistant to infection.

Symptomatology of the disease, both under natural and artificial conditions were studied. On artificial inoculation all the general symptoms of the disease observed in naturally infected plants could be produced. Manifestation of symptoms commenced from three to seven days of inoculation. Further the symptoms of the disease observed under natural and artificial condition were almost similar to those described by Natural *et al.* (1990) and Dhanya (2000).

Naturally infected plants having foliar infection showed a variety of symptoms which were similar to those foliar symptoms described by Hayward (1972) in the first report on the incidence of bacterial blight of Anthurium from Hawaii. Originally he concluded bacterial blight of anthurium to be a foliar disease. Apart from foliar infection the bacterium also infected the petiole and rhizomes causing death of the plant. This type of systemic infection causes more economic loss as unlike in the case of foliar infection. These systemic symptoms are more common in red varieties.

When pathogen was inoculated on the leaves, the blight symptoms were primarily noticed on the foliage. When the base of the plants was inoculated with the pathogen systemic infection was noticed. Sometimes on inoculation of the leaves apart from developing foliar symptoms also

resulted systemic infection as was reported by Guevara and Debrot (1984) and Cooksey (1985). They observed that foliar symptoms spread through the petiole resulting in the systemic infection. In the present study systemic infection was found to develop after 13 days when pathogen was inoculated at base by providing injury. Similar observation were made by Chase (1990) and Dhanya (2000). Das *et al.* (1995) reported that death of 75 per cent plants occurred within a period of 15 days due to this disease. This variation observed may be due to the varietal character of the plant and also due to strain variations in the bacteria.

The variations in the symptom on anthurium plants by inoculating with different strains of the same pathogen revealed that the symptoms may differ in the size of the initial water soaked lesions on leaves, yellow halo surrounding the lesion, size of the developed lesion, marginal infection, petiole infection, yellowing of leaves, defoliation and rotting of roots. Effect of the strain variations of bacterial pathogen on symptom development have been studied by earlier workers on several diseases; Devadath and Padmanabhan (1969) in rice and Vakili (1977) in legumes studied bacterial blight infection with Xanthomonas isolates. However, the present study on this line is the first attempt on anthurium and its bacterial blight pathogen.

The morphological, cultural and physiological characters of the isolates were studied. All the isolates were aerobic, motile, gram negative rods. The colonies of the bacterium were yellow, circular, convex, slimy, smooth and glistening with entire margin on PSA medium. The organism produced a water insoluble yellow pigment on YGCA. These results were in confirmity with the observations of earlier workers (Hayward, 1972; Natural *et al.*, 1990; Dhanya, 2000).

The isolates Xad 3 and Xad 10 had excellent growth on PSA while the isolates, Xad 1, Xad 2, Xad 5, Xad 7 and Xad 8 had good growth. Moderate growth was noticed for the isolates Xad 4, Xad 6 and Xad 9 on

PSA (Table 6). Variations were observed in the growth of different isolates of the bacterium on potato sucrose broth (Table 7). All the isolates attained their maximum growth within a period of 48h except Xad 4 and Xad 10 and after that growth was found to decline. Among the ten isolates studied, the growth of isolate Xad 4 and Xad 10 was found to be increasing even after a period of 72h indicating that these isolates require much longer period of incubation when compared to rest of the isolates. Taking into consideration the nature of growth of the ten isolates, it was found that Xad 4 and Xad 10 were of faster growing type, Xad 2 was of slow growing type and rest of the isolates were of moderate growth. This result was in agreement with the reports of Natural *et al.* (1990). They reported that bacterium produce copious colonies in Potato Dextrose Agar and Nutrient Agar. Dhanya (2000) also reported maximum growth of the bacterium on PSA medium. The results of growth studies with the isolates of the bacterium revealed that PSA could be the best solid medium for routine laboratory test and mass culturing of the bacterium.

All the ten isolates of the bacterium studied, utilized glucose oxidatively, liquefied gelatin, hydrolysed arginine, produced hydrogen sulphide, lipase, ammonia and catalase. The biochemical tests were negative for indole production, Methyl Red and Voges Praskauer test. The bacterium failed to produce urease and tyrosinase. Milk was turned alkaline in reaction. None of the isolates utilized asparigine as the sole source of carbon and nitrogen. The different isolates of the bacterium utilized sodium salts of citric acid and acetic acid but not that of benzoic acid and formic acid and growth was inhibited at six per cent sodium chloride. The results were in agreement with the studies of earlier workers (Hayward, 1972; Cooksey, 1985; Pohronezny *et al.*, 1985; Dhanya, 2000).

In the present study, the isolates showed variable reaction to starch hydrolysis. This was in confirmity with the work of Bonner *et al.* (1987).

He reported that about 35 per cent of 435 pathogenic strains of *X. axonopodis* pv. *dieffenbachiae* tested failed to utilize starch. Variation in starch hydrolysis was also reported by Alvarez (1990), Lipp *et al.* (1992) and Dhanya (2000).

On the basis of morphological, physiological and biochemical characteristics, the isolates of the bacterium causing leaf spot/blight symptoms on anthurium in the present investigation was designated as *X. axonopodis* pv. *dieffenbachiae*. Earlier workers, McCulloch and Pirone (1939), Hayward (1972), Guevara and Debrot (1984), Cooksey (1985), Pohronezny *et al.* (1985), Chase *et al.* (1992). Natural *et al.* (1990) also designated the bacterium with these characteristics as *X. axonopodis* pv. *dieffenbachiae*.

The intensity of disease and extend of loss as a result of bacterial blight infection varied in different localities. This variation may be due to the presence of different strains of the bacterium possessing varying pathogenic ability. The bacteria which are present in the locality if is a virulent strain may cause severe infection while other strains which are less potent eventhough may cause disease but not cause destruction of the crop.

Among the ten isolates, the isolate Xad 10 was the most virulent. It produced systemic symptoms earlier than the other isolates and killed the plant (*Anthurium andreanum* var. Cancan) within 13 days from the initiation of disease symptoms.

The isolate Xad 10 was selected for further studies as it was the most virulent and it was grown on eight different solid media. On all the media the colonies produced were yellow, glistening, circular, smooth, convex with entire margin. Excellent growth was noticed on PSA. Growth on NA and YGCA was found to be good. Moderate growth was seen on BX, TZ, PDA and GYA. Slow growth was observed on GA. Slime production was

maximum on PSA, good on NA, moderate on BX and YGCA, slight on PDA and TZ and slime production was absent on GA and GYA (Table 9).

The results of the present study was also in agreement with the earlier workers (Hayward, 1972; Pohronezny *et al.*, 1985; Dhanya, 2000).

The isolate Xad 10 utilized sugars such as glucose, galactose, dextrose, lactose, maltose, xylose, sucrose and fructose with production of acid while it did not utilize inositol and dulcitol (Table 10). Among the carbon sources studied, the maximum utilization was noticed for sucrose which was statistically on par with utilization of glucose. Similar results were obtained by Hayward (1972) and Dhanya (2000).

Plants with similar taxonomic affinity to anthurium and other ornamental plants commonly seen in anthurium gardens were artificially inoculated with the culture of *X. axonopodis* pv. *dieffenbachiae* and the symptom development was observed. All the plants tested *viz.*, *Aglaonema* sp., *Alocasia sanderiana, Anthurium ornatum, Colocasia esculenta, Dieffenbachia maculata,* three other species of *Dieffenbachia, Philodendron eichleri,* three other *Philodendron* species, *Syngonium podophyllum, Xanthosoma sagittaefolium,* two orchid species, *Maranta arundinaceae, Sterilitzia reginae, Heliconia rostrata, Dracaena sanderiana* and another *Dracaena* species were infected by the bacterium under artificial conditions indicating that it has a broad host range. However the time taken for symptom development varied. All the aroids developed symptoms within five to six days on inoculation, while in *Orchid* spp. it took seven days and in *Maranta arundinaceae* only five days. *Sterilitzia reginae* and *Heliconia rostrata* developed symptoms on the ninth day of inoculation, while *Dracaena sanderiana* and *Dracaena* sp. took 12 and 10 days respectively to express symptom. In most of the tested plants water soaked lesions and brown necrotic spots were the characteristic symptoms noticed. Yellowing of the affected tissue and shredding of leaves were the other symptoms noticed (Table 11). However, McFadden (1962) observed

that among the aroids inoculated, only *Aglaonema* sp. and *Dieffenbachia* sp. were found susceptible. Welburg (1969) succeeded in infecting *Syngonium* sp. with the bacterial blight pathogen. The results of the present investigation were in agreement with reports of Hayward (1972) who succeded in infecting *Philodendron oxycardium* and *Dieffenbachia picta* with *X. axonopodis* pv. *dieffenbachiae*. Chase *et al.* (1992) reported *Syngonium podophyllum* and *Aglaonema commuttatum* also as host for *X. axonopodis* pv. *dieffenbachiae*. Lipp (1992) reported that anthurium strains were more virulent and had broad host range when compared to strains from other aroids namely *Syngonium*. The results of the present study on host range revealed that these plants could serve as potential source of inoculum. In most of the anthurium gardens several foliage and ornamental plants are also cultivated. The bacterial infections on these plants are not severe enough to take management practices by the growers. Thus these plants serve as a primary source of inoculum. As and when conditions became favourable they may attack anthurium plants and cause disease. Therefore for effective management of bacterial blight of anthurium, care should be taken to see that other foliage and ornamental plants grown in the gardens are also protected from infection by this bacterium.

A preliminary screening using plant extracts from *Allium sativum*, *A. cepa*, *Tagetes erecta*, *Ocimum sanctum*, *Lawsonia inermis*, *Tarbernaemontana coronaria*, *Centella asiatica* and *Coleus aromaticus* at four levels *viz.*, crude, 2 : 1, 1 : 1 and 1 : 2 dilution was conducted to find out the inhibitory effect of the botanicals against *X. axonopodis* pv. *dieffenbachiae*.

Among the various extracts tested, crude and 2 : 1 dilutions of *A. sativum* and *T. erecta* were found to be the two best in inhibiting the growth of *X. axonopodis* pv. *dieffenbachiae* under *in vitro* condition. The least response against the pathogen was exhibited by *Coleus aromaticus* extract.

Joseph (1997) reported garlic extract to be highly inhibitory to the growth of *X. axonopodis* pv. *dieffenbachiae* followed by *Ocimum* and *Citronella*. Inhibitory effect of garlic extract against various phytopathogenic bacteria has been well established by different workers (Grainage *et al.*, 1985; Shah *et al.*, 1997; Lirio, 1998).

In vitro studies conducted to test the efficacy of four oils and two plant products *viz.*, neem oil, coconut oil, castor oil, pongamia oil, marotty oil, neem cake extract and turmeric + sodium bicarbonate (10 : 1) mixture at four levels *viz.*, crude, two percent, one per cent and 0.5 per cent indicated that the two and one per cent concentration of neem and coconut oil were highly inhibitory to *X. axonopodis* pv. *dieffenbachiae*. This was followed by crude extract and two per cent concentration of neem cake extract.

Mishenkova *et al.* (1983) observed that essential oils extracted from *Calendula officinalis* and *Thymus serphyllum* were inhibitory to the growth of *Corynebacterium michiganensis* pv. *michiganensis* and *X. campestris* pv. *phaseoli*. Grag and Kasera (1984) reported the effect of essential oil extracted from *Annacardium occidentale* against four gram positive and eighteen gram negative bacteria including *Pseudomonas mangiferae* and *X. campestris*. Mohan and Moses (1990) reported that neem cake extract showed inhibitory effect towards *X. campestris citri*.

Streprocycline 100 ppm was used as check in all the *in vitro* screening trials. Inhibitory effect of the antibiotics on *X. axonopodis* pv. *dieffenbachiae* was comparatively greater than botanicals under *in vitro* condition. Similar observation was made earlier by Joseph (1997) and Dhanya (2000).

Based on this study crude extract and 2 : 1 dilutions of plant extracts and two per cent and one per cent concentration of oils and crude and two per cent extract of neem cake which gave higher response against the test pathogen and 100 ppm streptocycline were selected for field trial on seven

month old plants. These treatments were applied before and after inoculation.

Pre inoculation sprayings had reduced the disease infection. The disease reduction of bacterial blight of anthurium due to pre inoculation sprayings may be due to the induction of defense related proteins and enzymes as was observed by Sateesh (2001) working with leaf extracts of *Zizyphus jujuba, Datura metel* and *Ipomeae carnae* in controlling bacterial blight of rice. From the results on pre inoculation spraying it is clear that disease reduction of bacterial blight in anthurium is possible if the plants are sprayed with botanicals well before the host plant comes in contact with the pathogen.

Garlic extract was the most efficient botanical in inhibiting *X. axonopodis* pv. *dieffenbachiae* under *in vitro* condition. The inhibitory effect of garlic on microbes has been attributed to the presence of sulphur containing compounds (Mangamma and Sreeramulu, 1991). However, under field conditions, garlic extract performance was inferior to the other treatments. Unlike in the closed environment of an *in vitro* trial, the antimicrobial principles from garlic extract might have decomposed or got diluted due to the influence of external factors thereby reducing its antimicrobial property. From among the different botanicals tried on seven month old anthurium, two best botanical *viz.*, crude extract of *Allium sativum* and *Tagetes erecta* were tried on grown up plants.

The lowest disease infection (94.32 per cent less than control) was noticed on plants which received two pre inoculation and two post inoculation application of crude extract of neem cake. This was followed by treatment with crude extract of *T. erecta* which reduced the infection by 88.66 per cent over control after last spraying. The effect of streptocycline treatment was on par with that of *T. erecta*.

The antibiotics acts directly on the pathogen by inhibiting the protein synthesis resulting in death of the bacteria. Hence any further

increase in the disease after the application of the antibiotics may be nil or very gradual. On the other hand the inhibitory effect of botanicals is gradual as is evident from *in vivo* trials (Table 14 and Table 15) and the disease reducing ability may be due to its ability to induce systemic resistance in plants.

Very few reports are available on the *in vivo* management of bacterial blight of anthurium using botanicals. Dhanya (2000) reported that bacterial blight incited by *X. axonopodis* pv. *dieffenbachiae* could be controlled by spraying 0.15 per cent turmeric powder + sodium bicarbonate in 10:1 proportion in the initial stage of infection by giving five spraying at one week interval. Hutagalung (1988) reported that the addition of 10 ml suspension of 35 g garlic bulb per 77 ml sterile water or 6 g of ground bulb to the rhizosphere decreased the bacterial wilt in tomatoes incited by *Ralstonia solanacearum*. There are reports on the bactericidal properties of neem products under *in vivo* condition. Hulloli *et al.* (1998) reported that neem based products, plantolyte (200 µg/ml) and agricare (200 µg/ml) were effective in managing cotyledonary infection of bacterial blight of cotton incited by *X. axonopodis* pv. *malvacearum*. The results of the present study with neem products were in agreement with this finding.

The results of our investigation revealed that botanicals like crude extract of neem cake and *T. erecta* could be an ideal substitute for antibiotics in the management of bacterial blight of anthurium. Besides their ecofriendly nature, they can be easily prepared by the farmers locally. Repeated application of botanicals neither build up resistance against the pathogen nor accumulate any toxic residues in the environment and it is cost effective compared to antibiotics and chemicals in managing the disease.

SUMMARY

6. SUMMARY

Anthurium (*Anthurium andreanum* Linden) is an important cut flower crop which has recently attained economic prominence in Kerala. The unique agroclimatic conditions in Kerala is ideally suitable for its cultivation. It suffers from a serious disease incited by *Xanthomonas axonopodis* pv. *dieffenbachiae* (McCulloch and Pirone) Vauterin *et al.*

Taking into consideration the severe crop loss due to this disease in the state, this work was undertaken to study the disease intensity, varietal reaction, symptomatology, host range and management using botanicals *viz.*, plant extracts, oils and plant products.

A survey on the disease intensity and varietal reaction was conducted in thirty anthurium gardens located in different parts of Thiruvananthapuram district. Most of the anthurium gardens had plants with disease infection upto 50 per cent. Among different varieties, the pink varieties exhibited tolerance to bacterial blight, while red varieties were susceptible to the disease. Ten isolates of the bacterium, six from foliar infection and four from systemically infected plants were collected and isolated. On PSA medium colonies of the pathogen were yellow, circular, slimy, glistening with entire margin.

The symptoms appeared on leaf margins as water soaked lesions which later enlarged and became brown with an yellow halo. These lesions coalesced and resulted in blighting of lamina and shedding of leaves. Occasionally infection spread through the petiole to the stem and became systemic. Necrotic spots were also visible on spathe and spadix. The collar region of the infected plants showed browning and rotting which leads to rotting of roots. Blighting as well as drying of spathe and spadix were also noticed. Symptom production on plants varied depending on the isolates.

The bacterium was aerobic, gram negative, motile rods. It used glucose oxidatively and could utilize both sodium acetate and citrate as source of carbon. All the isolates produced hydrogen sulphide, ammonia, liquefied gelatin and gave positive reaction for catalase test. The results were negative for indole production and tyrosinase activity. Milk was turned alkaline, hydrolysed arginine and produced lipase. It was negative for urease and MR and VP test. None of the isolates were tolerant to six per cent sodium chloride.

Among the isolates variations were observed in starch hydrolysis, growth characters and virulence. Based on morphological, physiological and biochemical characters coupled with pathogenicity and symptom development in anthurium, the isolated bacterium was identified as *X. axonopodis* pv. *dieffenbachiae*.

The most virulent isolate (Xad 10) was used for further studies. Among the eight different solid media tested, PSA was found to be the best medium for growth of the bacterium. Sucrose was the best utilized carbon source.

The studies conducted on host range under artificial conditions revealed that *X. axonopodis* pv. *dieffenbachiae* had a broad host range. Many aroids like *Aglaonema* sp., *Alocasia sanderiana, Anthurium ornatum, Colocasia esculenta, Dieffenbachia* spp., *Philodendron* spp., *Syngonium podophyllum,* and *Xanthosoma sagittaefolium* could act as alternate hosts of the bacterium. Besides the members of Araceae, the pathogen also infected other ornamental plants *viz.*, *Dracaena* spp., *Heliconia rostrata, Sterilitizia reginae, Maranta arundinaceae* and *Orchid* spp.

Studies under *in vitro* condition on growth inhibition by eight plant extracts, five oils and two plant products revealed that crude extract and 2 : 1 dilution of *Allium sativum* and *Tagetes erecta,* crude and two per cent extract of neem cake and two per cent and one per cent of neem oil and coconut oil were effective in inhibiting the test pathogen.

In vivo studies were carried out to test the efficacy of botanicals on seven month old tissue culture plants. Five botanicals *viz., A. sativum, T. erecta*, neem oil, coconut oil, neem cake extract, at two levels, streptocycline 100 ppm and control were the treatments included. Pre inoculation spraying showed a profound influence in reducing the disease. Among the treatments, the disease reduction over control was more than 85 per cent in the case of plants receiving two pre inoculation and two post inoculation application of crude extract of neem cake and *T. erecta*.

When crude extract of neem cake and *T. erecta,* streptocycline 100 ppm were tested on flowering plants with the same spray schedule, the crude extract of neem cake was found to be the best and the extent of disease control achieved was 94.32 per cent. The effect of *T. erecta* was on par with streptocycline 100 ppm where the extend of reduction over control were 88.66 and 86.30 per cent respectively.

The promising results of the present study indicate that botanicals *viz.*, crude extract of neem cake and *T. erecta* could be used as a substitute for antibiotics for reducing the severity of bacterial blight of Anthurium.

REFERENCES

7. REFERENCES

Alvarez, A.M. 1988. Anthurium blight. An overview. *Proceedings of the First Anthurium Blight Conference, May 12-14, 1988* (ed. Benedict, A.A.) Univ. of Hawaii, USA, pp. 39-40

Alvarez, A.M., Lipp, R.L., Norman, D. and Gladstone, L. 1990. Epidemiology and control of Anthurium blight. *Proceedings of the Third Anthurium Blight Conference, May 23-24, 1990* (ed. Alvarez, A.M.) Univ. of Hawaii, USA, pp. 27-30

Balakrishnan, S., Louis, V. and Gokulapalan, C. 1996. Disease of Anthurium. *Kisan Wld* 23 : 59

Banerjee, A., Kaul, V.K. and Nigam, S.S. 1982. Antimicrobial activity of the essential oil of *Curcuma amada.* Roxb. *Indian J. Microbiol.* 22 : 153-155

Basim, E. and Basim, H. 2001. Antibacterial effect of essential oil produced by *Rosa damascena* on *Xanthomonas axonopodis* pv. *vesicatoria. Phytopathology* 91 : 6

Baswa, M., Rath, C.C., Dash, S.K. and Mishra, R.K. 2001. Antibacterial activity of karanj (*Pongamia pinnata*) and neem (*Azadirachta indica*). Seed oil : a preliminary report. *Microbiol. Res.* 105 : 183-189

Beek, T.A., Deelder, A.M., Verpoorte, R. and Svendsen, A.B. 1984. Antimicrobial, antiamoebic and antiviral screening of some *Tabernaemontana* species. *Planta Medica.* 50 : 180-185

Bonner, R.L., Alvarez, A.M., Berestecky, J.M. and Benedict, A.A. 1987. Monoclonal antibodies used to characterize *Xanthomonas campestris* pv. *dieffenbachiae. Phytopathology* 77 : 1725

Bora, L.C. and Jaya, S. 1999. Efficacy of medicinal plant extracts in suppression of citrus canker (*Xanthomonas campestris* pv. *citri*) and bacterial leaf spot of betelvine (*X. campestris* pv. *betlicola*). *J. Mycol. Pl. Path.* 29 : 281

*Catara, V. and Sesto, F. 1997. Bacterial leaf spot of zinnia caused by *Xanthomonas campestris* pv. *zinniae*. *Informatore Fitopatologia* 47 : 62-64

Chase, A.R. 1990. Characterization of *Xanthomonads* from aroids. *Phytopathology* 80 : 754-759

Chase, A.R. and Jones, J.B. 1987. Leaf spot and blight of *Sterilitzia reginae* (Bird of paradise) caused by *Xanthomonas campestris*. *Pl. Dis.* 71 : 845-847

Chase, A.R. and Poole, R.T. 1986. Effects of host nutrition on growth and susceptibility of *Anthurium scherzeranum* to *Xanthomonas* leaf spot. *Nurserymen's Digest* 20 (6) : 58-59

Chase, A.R., Stall, R.E., Hodge, N.C. and Jones, J.B. 1992. Characterization of *Xanthomonas campestris* strains from aroids using physiological, pathological and fatty acid analysis. *Phytopathology* 82 : 754-759

Christensen, W.B. 1946. Urea decomposition as a means of differentiating *Proteus* and *Paracolon* cultures from each other and from *Salmonella* and *Shigella* types. *J. Bact.* 52 : 461

Clark, W.M. and Lubs, H.A. 1917. A substitute for litmus for use in milk cultures. *J. agric. Res.* 10 : 105-111

Cooksey, D.A. 1985. *Xanthomonas* blight of *Anthurium andreanum* in California. *Pl. Dis.* 69 : 727

Das, L., Peethambaran, C.K. and Nair, M.C. 1996. Bacterial blight of anthurium. *Kisan Wld* 23 : 15

Devadath, S. and Padmanabhan, S.V. 1969. A preliminary study on the variability of *Xanthomonas oryzae* on some rice varieties. *Pl. Dis. Reptr.* 53 : 145-148

Dhaliwal, G.S., Pathak, M.D. and Vega, C.R. 1990. Effect of a rice allelochemical on insect pest, predators and plant pathogens. *J. Insect Sci.* 32 : 136-140

Dhanya, M.K. 2000. Etiology and management of bacterial blight of anthurium (*Anthurium andreanum* Linden). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, p. 85

Dhanya, M.K., Mary, C.A. and Nair, S.K. 2000. Studies on bacterial blight of Anthurium (*Anthurium andreanum* Linden). *Proceedings of the Twelfth Kerala Science Congress, January 27-29, 2000* (ed. Das, M.R.). Kerala State Committee on Science, Technology and Environment, pp. 573-574

Dickey, R.S. and Zumoff, C.H. 1987. Bacterial leaf blight of *Syngonium* caused by a parthovar of *Xanthomonas campestris*. *Phytopathology* 77: 1257-1262

*Dye, D.W. 1962. The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. *N. Z. J. Sci.* 5 : 393-416

*Dye, D.W. 1966. Cultural and biochemical reactions of additional *Xanthomonas* sp. *N.Z. J. Sci.* 9 : 913-919

Dye, D.W. 1980. International standards on naming pathovars of phytopathogenic bacteria and a list of pathovar names and patho types. *Rev. Pl. Path.* 59 : 153-158

Dye, D.W. and Lelliot. 1974. The host range of *Xanthomonas* bacteria. *Bot. Rev.* 60 : 310

Eswaramurthy, S., Mariappan, V., Muthusamy, M., Alaginagalingam, M.N. and Subramanian, K.S. 1993. Efficacy of neem products in controlling bacterial blight of paddy. *Adv. Pl. Path.* 8 : 224-229

Fukui, R., Munui, H., Nelson, S.C. and Alvarez, A.M. 1995. Effect of temperature and nitrogen fertilization on infection process in leaves of anthurium inoculated with a bioengineered bioluminescent strain of *Xanthomonas campestris* pv. *dieffenbachiae*. *Phytopathology* 85 : 1164

Gangopadhyay, S. 1998. Turmeric (*Curcuma longa*) an ecofriendly ingredient to control soil borne diseases. *Curr. Sci.* 75 : 21-23

Garg, S.C. and Dengre, S.L. 1986. Antibacterial activity of essential oil of *Tagetes erecta* Linn. *Hindustan Antibiotics Bull.* 28 : 27-29

Garg, S.C. and Kasera, H.L. 1984. Antibacterial activity of the essential oil of *Anacardium occidentale* Linn. *Indian Perfumer* 28 : 95-97

Girgune, J.B., Jain, N.K. and Garg, B.D. 1980. Antimicrobial activity of the essential oil from *Valeriana wallichii* DC (Valerianaceae). *Indian J. Microbiol.* 20 : 142-143

Grainge, M., Berger, C. and Ahmed, S. 1985. Effect of extracts of *Atrabotrys uncinatus* and *Allium sativum* on *Xanthomonas campestris* pv. *oryzae*. *Curr. Sci.* 54 : 90

*Grijalba, P.E., Irigoyen, E.D., Rivera, M.C. and Wright, E.R. 1998. *Xanthomonas campestris* pv. *begoniae*. First report in Buenos Aires (Argentina) inoculum sources and preliminary chemical control tests. *Fitopatologia* 33 : 94-98

Guevara, M.Y. and Debrot, C.E. 1984. Bacterial blight of *Anthurium andreanum* Linden in Venezuela. *Agron. trop.* 34 : 153-160

*Hanudin, C. 1987. Controlling the incidence of the bacterial wilt (*Pseudomonas solanacearum* E. F Smith) on tomato plants by some extracts. *Bull. Penelitian Hortikultura* 15 : 60-66

Hayward, A.C. 1964. Characterization of *Pseudomonas solanacearum*. *J. appl. Bact.* 27 : 265-277

Hayward, A.C. 1972. A bacterial disease of anthurium in Hawaii. *Pl. Dis. Reptr.* 58: 876-888

Hernandez, Y. and Trujillo, G. 2000. *Xanthomonas campestris* pv. *zinniae* infecting zinnia plants (*Zinnia elegans* Jacq.) in Venezuela. *Rev. Microbiol.* 17 : 156-163

Hugh, R. and Leifson, E. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. *J. Bact.* 66 : 24-26

Hulloli, S.S., Singh, R.P. and Verma, J.P. 1998. Management of bacterial blight of cotton induced by *Xanthomonas axonopodis* pv. *malvacearum* with the use of neem based formulations. *Indian Phytopath.* 51 : 21-25

*Hutagalung, C. 1988. Garlic bulb as material for suppressing the incidence of bacterial wilt (*P. solanacearum*) on tomatoes. *Bull. Penelitian Hortikultura* 16 : 89-93

Joseph, S. 1997. Etiology, survival and chemical control of bacterial blight of Anthurium. M.Sc. (Ag.) thesis, University of Agricultural Sciences, Bangalore, p. 140

Kuehnle A.R., Chen, F. and Suggi, N. 1995. Novel approaches for genetic resistance to bacterial pathogens in flower crops. *HortScience* 30 : 456-460

Lee, Y.A., Hildebrand, D.C. and Schroth, M.N. 1992. Use of quinate metabolism as a phenotypic property to identify members of *Xanthomonas campestris* DNA homology group six. *Phytopathology* 82 : 971-973

Lipp, R.L., Alvarez, A.M., Benedict, A.A. and Berestecky, J. 1992. Use of monoclonal antibodies and pathogenicity test to characterize strains of *Xanthomonas campestris* pv. *dieffenbachiae* from Aroids. *Phytopathology* 82 : 677-682

Lirio, L.G., Hermano, M.L. and Fontanilla, Q.M. 1998. Antibacterial activity of medicinal plants from the Philippines. *Pharmaceut. Biol.* 36 : 357-359

Madusmitha, K., Bhagabati, K.N., Borah, P.K. and Bora, L.C. 1999. Some bacterial diseases of ornamental plants of Assam. *J. Mycol. Pl. Path.* 29 : 172-175

*Maiti, D., Kole, C.R. and Sen, C. 1985. Antimicrobial efficiency of some essential oils. *Z. Pflanzenkr. Pflanzensch.* 92 : 64-68

Mangamma, P. and Sreeramulu, A. 1991. Garlic extract inhibitory to growth of *Xanthomonas campestris* pv. *vesicatoria. Indian Phytopath.* 44 : 372-374

Marques, M.R., Albaquerque, E.B.M. and Xavier, P.J. 1992. Antimicrobial and insecticidal activities of cashew tree gum exudate. *Ann. appl. Biol.* 121 : 371-377

Maruzzella, J.C., Snood, V.K. and Henry, P.A. 1958. *In vitro* screening of phytopathogenic bacteria. *J. Am. Pharm. Ass.* 47 : 471

McCulloch, L. and Pirone, P.P. 1939. Bacterial leaf spot of *Dieffenbachia. Phytopathology* 29 : 956-962

McFadden, L.A. 1962. Two bacterial pathogens affecting leaves of *Aglaonema robelinii*. *Phytopathology* 52 : 20

McFadden, L.A. 1976. A Xanthomonas infection of *Philodendron oxycardium* leaves. *Phytopathology* 66 : 343

*Mishenkova, E.L., Petrenko, G.T., Evseenko, O.V. and Paulenko, L.A. 1983. Inhibition of the growth of phytopathogenic bacteria by preparation from higher plants. *Mikrobiologischeskii Zhurnal* 45 : 91-95

Mohan, C. and Moses, M. 1990. Effect of fifteen medicinal plant extract. *Indian J. agric. Sci.* 60 : 122-127

Mu, L. 1990. Anthurium culture and blight in Tahiti. *Proceedings of the Third Anthurium Blight Conference, May 23-24, 1990* (ed. Alvarez, A.M.). University of Hawaii, USA, pp. 37-38

Mukherjee, N. and Biswas, P. 1981. Control of bacterial diseaes of plants with crude extracts. *Madras agric. J.* 68 : 28

Mukherjee, N. and Biswas, P. 1984. Control of plant disease with plant oils – their problems and prospects. *J. agric. Sci.* 94 : 145-151

Natural, M.P. 1990. Anthurium blight in Philippines. *Proceedings of the Third Anthurium Blight Conference, May 23-25, 1990* (ed. Alvarez, A.M.). University of Hawaii, pp. 38

Natural, M.P., Tabo, G.A. and Telebanco, M.J.G. 1990. Bacterial blight of *Anthurium andreanum* Linden in the Philippines. *Philipp. Phytopath.* 26 : 25-35

*Nishijima, W. and Fujiyama, D. 1985. Guidelines for control of Anthurium bacterial blight. *Hawaii Coop. Ext. Serv. Instant Info.* 14 : 2

Phookan, A.K., Rachid, H.A., Rathaiah, Y., Bhagabati, K.N. and Roy, A.K. 1996. Bacterial leaf blight of colocasia in Assam – a new record from India. *Indian Phytopath.* 49 : 104-105

Pohronezny, K., Volin, R.B. and Danker, S.W. 1985. Bacterial leaf spot of cocoyam (*Xanthosoma caracu*) incited by *Xanthomonas campestris* pv. *dieffenbachiae* in Florida. *Pl. Dis.* 69 : 170-173

Robbs, C.F. 1965. The host range of *Xanthomonas* bacteria. *Bot. Rev.* 50 : 310

*Rott, P. and Prior, P. 1987. Un deperissement bacteriem de 1 anthurium provoque par *Xanthomonas campestris* pv. *dieffenbachiae* aux Antilles francaises. *Agron. trop.* 42 : 61-68

Sateesh, A.K. 2001. Purification and identification of antifungal and antibacterial principle from plant species against *Rhizoctonia solani* (khun) and *Xanthomonas oryzae* pv. *oryzae* (Uyeda and Ishiyama) Dye. M.Sc. (Ag.) thesis, Tamil Nadu Agricultural University, Coimbatore, p. 93

Satyanarayana, N., Reddy, O.R., Latha, S. and Rajak, R.L. 1998. Interception of *Xanthomonas campestris* pv. *dieffenbachiae* on anthurium plants from Netherlands. *Plant Dis.* 82 : 262

Satish, S. and Raveesha, K.A. 2000. *In vitro* screening of plant extracts for antibacterial activity against *Xanthomonas* species. *Proceedings of International Conference on Integrated Plant Disease Management for Sustainable Agriculture, November 11-15, 1997* (eds. Indian Phytopathological Society), IARI, New Delhi, pp. 389

Shah, R., Mali, B.L. and Bhatnagar, M.K. 1997. Efficacy of plant extracts against Xanthomonads. *Indian Phytopath.* 50 : 122

Sharma, P. and Mehta, B.P. 1999. Antibacterial activity of plant extracts to phytopathogenic *Xanthomonas campestris* pv. *campestris* causing black rot of cabbage. *J. Mycol. Pl. Path.* 30 : 111

Shehata, S.A., Nishimoto, M. and Hamilton, M. 1990. The impact of anthurium blight on the profitability of the industry. *Proceedings of the Third Anthurium Blight Conference, May 23-24, 1990* (ed. Alvarez, A.N.). University of Hawaii, USA, pp. 3-6

Shekhawat, G.S., Singh, R., Nanda, S. and Ansari, M.M. 1980. Bacterial diseases of ornamental plants in Simla hills. *Indian Phytopath.* 33 : 419-422

*Sierra, G. 1957. A simple method for detection of lipolytic activity of microorganism and some observations on the influence of the contact between cells and fatty substance. *Antonie-Van Leeuwenhock. J. Microbiol.* 23 : 15-22

Society of American Bacteriologists. 1957. *Manual of Microbiological Methods.* Mc Graw Hills Book Co., Inc., New York, USA, p. 315

Soustrade, I., Gagnevin, L. and Roumagnae, P. 2000. First report of anthurium blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* in Reunion Island. *Pl. Dis.* 84 : 1343

Srinivasachary. 1995. Etiology and ecology of bacterial blight of mulberry (*Morus alba* L.). M.Sc. (Ag.) thesis, University of Agricultural Sciences, Bangalore, p. 89

Tewari, S.N. 1986. Evaluation of some of the indigenous plants for anti fungal and antibacterial properties against major fungal and bacterial pathogens of rice. Ph.D. thesis, Utkal University, Bhuvaneswar, p. 142

Thakur, K.D., Khune, N.N. and Sabley, J.E. 1991. Efficacy of some plant extracts on inhibition of cotton pathogens. *Orissa J. agric. Res.* 4 : 90-94

Thornley, M. 1960. The differentiation of *Pseudomonas* from other Gram negative bacteria on the basis of arginine metabolism. *J. appl. Bact.* 23 : 37

Uddin, W. and McCarter, S.M. 1996. First report of oak leaf hydrangea bacterial leaf spot caused by a pathovar of *Xanthomonas campestris. Pl. Dis.* 80 : 599

Vakili, N.G. 1977. Pathogenicity of *Xanthomonas* strain causing bacterial blight and pustules of edible legumes in Pueto Rice. *Trop. Grain Legume Bull.* 8 : 33-38

Vauterin, L., Hoste, B., Kersters, K. and Swings, J. 1995. Reclassification of Xanthomonas. *Int. J. Systematic Bact.* 45 : 472-489

Welburg, C. 1969. Bacterial leaf blight of *Syngonium podophyllum. Phytopathology* 59 : 1056

Wells, J., Civerolo, E., Hartung, J. and Pohronezny, K. 1993. Cellular fatty acid composition of nine pathovars of *Xanthomonas campestris. J. Phytopath.* 138 : 125-136

Young, F. 1990. Anthurium blight in Jamaica. *Proceedings of the Third Anthurium Blight Conference, May 23-24, 1990* (ed. Alvarez, A.M.). University of Hawaii, USA, pp. 37-38

---

*Original not seen

MANAGEMENT OF BACTERIAL BLIGHT OF ANTHURIUM (*Anthurium andreanum* Linden) USING BOTANICALS

SABITHA, S.R.

Abstract of the
thesis submitted in partial fulfilment of the requirement
for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2002

Department of Plant Pathology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM 695522

extract, at two levels and 100 ppm streptocycline were tested on seven month old tissue culture plants. Pre and post inoculation sprayings with crude extract of neem cake and *T. erecta* reduced the disease by 85 per cent.

When the same spraying schedule with these two botanicals and 100 ppm streptocycline was given on flowering plants, crude extract of neem cake was the most effective followed by crude extract of *T. erecta*. The effect of streptocycline 100 ppm was on par with *T. erecta*. The study thus indicates that botanicals could be used as a substitute for antibiotics in the management of bacterial blight of anthurium.