ETIOLOGY AND MANAGEMENT OF BACTERIAL BLIGHT OF ANTHURIUM (*Anthurium andreanum* Linden)

BY

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THESIS
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DECLARATION

I hereby declare that this thesis entitled "**Etiology and management of bacterial blight of anthurium (*Anthurium andreanum* Linden)**" 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,

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CERTIFICATE

Certified that this thesis entitled **"Etiology and management of bacterial blight of anthurium (*Anthurium andreanum* Linden)"** is a record of research work done independently by Ms. Dhanya. M. K. 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.

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Dedicated

To

Pappa and Mummy

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INTRODUCTION

1. INTRODUCTION

Anthurium is an important cut-flower crop of Kerala. The most popular species, *Anthurium andreanum* Linden (Oil cloth flower, tail flower or painters palette flower) is a native of South West Columbia. It is the largest genus of the monocot family Araceae with more than 700 species. Anthurium is relatively easy to grow and under proper conditions it produce long lasting flowers all the year round. Its habitat extends from Central South America to Central Mexico and the Caribbean Islands. Anthuriums also grow very well in many parts of India, including Kerala. The agro-climatic conditions in Kerala are very suitable for its cultivation and the cut-flowers have a good market within the state and abroad. A plant produces a minimum of five flowers per year. From a small backyard consisting of 300 plants maintained in an area of 0.1 hectare, an annual income of Rs. 7500 can be obtained.

Anthurium is affected by a number of diseases of which, the most serious one is the bacterial blight incited by *Xanthomonas axonopodis* pv. *dieffenbachiae*. It was first reported on anthurium in Brazil in 1960. In Hawaii at first, in 1970 it was reported as a foliar disease. However, this disease is not restricted to leaves and spathes, as the pathogen can advance systemically and kill the susceptible plants. In Kerala, this disease was first observed in 2000 causing considerable economic loss to the farmers (Dhanya *et al.*, 2000).

Since very little work has been done on the etiology and control of this disease in Kerala, the present study was undertaken with the following objectives.

(1) Isolation of the causal organism of bacterial blight from infected anthurium plants and proving its pathogenicity.

(2) Characterization and identification of the pathogen.

(3) Detailed study on development of symptoms of disease.

(4) Study on host range of the pathogen.

(5) Study on mode of survival of the pathogen.

(6) Management of the disease.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 History, geographical distribution and losses

Bacterial blight of anthurium incited by *Xanthomonas axonopodis* pv. *dieffenbachiae* (Mc Culloch and Pirone) Vauterin *et al.*(1995) is one of the major diseases of anthurium. The disease was first reported from Brazil in 1960 and from Hawaii in 1970 (Nishijima, 1988).

Currently it has been reported from Maui, Florida, California, Jamaica, Thahiti, Philippines and almost all anthurium growing countries of the world.

Hayward (1972) reported that at one location of the island on Kauai in Hawaii about 90 per cent of *Anthurium andreanum* cv. Kansanko red were affected by bacterial blight incited by *Xanthomonas axonopodis* pv. *dieffenbachiae*.

The disease was prevalent in Jamaica since 1985 and in some instances it has been so severe that it led to abandoning of the crop on a farm (Young, 1990).

Sathyanarayana *et al.* (1998) reported that on three occassions *Anthurium andreanum* plants imported to India had typical bacterial blight symptom.

The occurrence of bacterial blight of *Anthurium andreanum* has also been reported from Kerala by Dhanya *et al.* (2000).

2.2 Symptomatology

2.2.1 Symptoms on *Anthurium andreanum* (Linden)

The symptoms of bacterial blight incited by *Xanthomonas axonopodis* pv. *dieffenbachiae* on *Anthurium andreanum* cv Kansako red were present on younger and older leaves. On the older leaves, angular pale brown necrotic

spots 1-3 mm in size occurred, which were surrounded by a marked chlorotic halo. On younger leaves, lesions were dark brown and black and more extensive. They were sometimes surrounded by a pale chlorotic halo. Lesions were variable in size, often angular and vein delimited or irregular and extending along the midrib for several cm. Blackened lesions on younger leaves often extended from midrib along and between branch vein to fill intercoastal areas. Similar symptoms were observed on spathe, where black elongated spots extending along branch veins sometimes filling entire intercoastal areas and the symptoms typical of the original infection were reproduced by spray inoculation of wounded leaves (Hayward, 1972).

Guevara *et al.* (1984) reported that symptoms of bacterial blight on *Anthurium andreanum* plants started as necrotic water soaked spots, some with a chlorotic halo mainly towards margin of leaves and spathes. Infection spread through petiole, reaching the stems and roots and caused plant death.

Majority of anthurium samples in Jamaica infected by *Xanthomonas axonopodis* pv. *dieffenbachiae* were systemically infected while in the remaining samples the pathogen was isolated only from leaf blade (Chase, 1990).

According to Dhanya *et al.* (2000) initial symptoms of bacterial blight of anthurium appeared on leaf lamina as small-scattered angular water soaked spots near leaf margins. These spots enlarged and gradually became necrotic with an yellow halo. The blighted leaves were shed. The symptoms were also noticed as irregular brown spot on the spathe as well as on spadix. Systemically infected plants wilted within a week after the appearance of the initial symptom.

2.2.2 Symptoms on other hosts

The symptoms produced on other hosts by *Xanthomonas axonopodis* pv. *dieffenbachiae*, the incitant of bacterial blight of anthurium were described by some workers.

On *Syngonium podophyllum*, *Xanthomonas axonopodis* pv. *dieffenbachiae* produced spots which were water soaked, firm and irregular. Affected areas turned yellow, brown, brittle and torn (Mc Fadden, 1962). He also reported that several leaf spots of 1-5 mm in diameter with a chlorotic halo developed on *Aglaonema robelinii* plants infected by *Xanthomonas axonopodis* pv. *dieffenbachiae*.

Phookan *et al.* (1996) reported that symptoms of bacterial leaf blight of *Colocasia esculenta* became visible seven to ten days after inoculation.

Catara and Sesto (1997) described the symptoms of bacterial leaf spot of Zinnia as brown red angular leaf spot surrounded by a chlorotic halo.

The symptoms of blight on potted begonias caused by *Xanthomonas campestris* pv. *begoniae* were described by Grijalba *et al.* (1998) as several yellowish water soaked spots on leaf margins, that were not limited by veins. The spots were more easily seen on underside of leaves. Tiny dark spots appeared inside the yellowish ones and gradually enlarged and coalesced forming large necrotic lesions with a yellow halo. Finally the entire leaf died. Stems, petioles and main veins were water soaked and discharged a whitish fluid when cut. As the disease progressed the plant became less turgescent and died.

2.3 Isolation and properties of the pathogen

Hayward (1972) reported the method of isolation of *Xanthomonas axonopodis* pv. *dieffenbachiae* from affected leaves and spathes. Portions of diseased material were cut from affected leaves and spathes, mounted in water and observed microscopically. A compact coherent bacterial ooze was observed which is characteristic of infection on leaves caused by members of genus *Xanthomonas*. Portions of lesion about five mm square were suspended in sterile water and streaked out on two per cent 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.

The causal organism of bacterial blight of anthurium was first described as *Xanthomonas campestris* pv. *dieffenbachiae* (Mc Culloch and Pirone) Dye (1980). This was later changed to *Xanthomonas axonopodis* pv. *dieffenbachiae* (Mc Culloch and Pirone) Vauterin *et al.* (1995).

Hayward (1972) reported that the pathogen *Xanthomonas axonopodis* pv. *dieffenbachiae* was actively motile, Gram negative, produced yellow growth in two per cent peptone sucrose agar. Based on physiological test, the bacterium was strictly aerobic, produced acid oxidatively from arabinose, cellobiose, fructose, galactose, glucose, glycerol, lactose, maltose, mannose, mannitol, raffinose, sucrose and trehalose within 4-14 days of incubation. No acid was produced from dulcitol, Inositol, Salicin and sorbitol, whereas starch, esculin, Tween 80 and gelatin were actively hydrolysed. He also found that growth of *Xanthomonas axonopodis* pv. *dieffenbachiae* occurred in media containing two and three per cent sodium chloride with little or no growth at four per cent or higher concentration. He also observed that no diffusible

pigment was produced by the bacterium on any of the agar media used. Sodium acetate, sodium malonate and sodium citrate act as the sole source of carbon in inorganically defined medium, whereas adipase and tartarate were not utilized. He also reported that production of nitrite from nitrate was absent for this bacterium. Production of Arginin dihydrolase, acetamide diamidation and production of 3-ketolactose from lactose were all negative.

Guevara and Debrot (1984) reported that *Xanthomonas axonopodis* pv. *dieffenbachiae* was rod shaped, 1.0 - 2.4 μm x 0.4-0.6 μm in size and some with a polar flagellum.

Alvarez *et al.* (1990) identified the existence of two distinct biological types of *Xanthomonas axonopodis* pv. *dieffenbachiae*, the faster growing one which can digest starch and the slower growing ones which cannot.

Pohronezny *et al.* (1990) reported that *Xanthomonas axonopodis* pv. *dieffenbachiae* remained viable across a wide range of temperature (27-57^{0}C) and pH values of 5.0 - 8.8.

2.4 Characters of Xanthomonads

Breed *et al.* (1957) reported that the bacterium *Xanthomonas betlicola* produced circular, smooth, glistening and waxy yellow colonies on nutrient agar medium. The bacterium liquefied gelatin. It had slightly acidic reaction in milk. The bacterium digested casein, but did not produce indole. It produced hydrogen sulphide, did not produce nitrite from nitrate. Negative results were recorded for methyl red and Voges Praskauer tests. The bacterium had no growth in synthetic asparagine medium. It produced acid but no gas from

glucose, lactose and sucrose. Salicin not utilized by this bacterium. Starch was hydrolysed, and tolerance to salt was upto three per cent.

Goto and Okabe (1959) reported that cellulase was produced by xanthomonads.

Starr and Stephens (1964) found that the unique yellow carotenoid pigment present in *Xanthomonas* spp. was sufficient to permit its recognition by simple screening procedure.

Starr *et al.* (1977) reported that the cell pigments produced by *Xanthomonas* spp. were isolated, characterized and named as xanthomonadins. The ability to form these distinctive pigments was a useful chemo taxonomic marker for the genus, since they were not known to be produced by taxonomically or ecologically adjacent bacteria.

Mathew *et al.* (1978) reported that starch hydrolysis of the pathogen *Xanthomonas betlicola* was strong and positive. Catalase activity was also positive. It produced hydrogen sulphide, but did not reduce nitrate. The bacterium produced acid in lactose, sucrose, xylose, glucose, fructose and not utilized dulcitol, inulin, salicin and inositol.

Souw and Demain (1979) reported that of the carbon sources tested using a chemically defined medium, a four per cent sucrose or glucose medium yielded the highest xanthan titres. They also reported that concentration of nitrogen higher than optimum level inhibit xanthan production.

During subculture on potato sucrose agar (PSA) *Xanthomonas campestris* pv. *oryzae* colony was waxy yellow (Choi *et al.*, 1981).

Chase (1988) reported the effect of temperature on growth of *Xanthomonas campestris* pv. *syngonii* isolated from *Syngonium podophyllum* and *Xanthomonas campestris* pv. *dieffenbachiae* isolated from *Anthurium andreanum* under *in vitro* conditions. He reported that growth of *Xanthomonas campestris* pv. *syngonii* occurred between 18-34^{0}C (optimum 22^{0}C).

Hilderbrand *et al.* (1993) reported that a total of 143 carbon sources was used in nutritional screening by 88 strains of Xanthomonads from 39 different *Xanthomonas campestris* pv. *dieffenbachiae.* Cellobiose, fructose, fumerate, glucose, L-malate and succinate supported the growth of all strains except *Xanthomonas albineans,* whereas 92 substrate were not utilized by any strain. Substrate utilization pattern appeared sufficiently uniform among the various genomic groups within *Xanthomonas* to allow the differentiation. But the utilization pattern of substrate differed substantially among pathovar that comprise the group. Substrate utilization was useful for distinguishing pathovars within genomic group. According to them, the pattern of carbon utilization were confirmed with biological tests, but there were exception. The biological tests also revealed some difference in carbon utilization not detected by standard test of carbon substrate. It was concluded that nutritional screening had promise for identifying genomic group.

2.5 Host range of the pathogen

Ark (1950) reported that host range of *Xanthomonas axonopodis* pv. *dieffenbachiae* include *Dieffenbachia* sp. Schott.

Mc Fadden (1962) reported a leaf spot of *Aglaonema robelinii* by *Xanthomonas axonopodis* pv. *dieffenbachiae.*

Anthurium sp. Schott as specific host of *Xanthomonas axonopodis* pv. *dieffenbachiae* was reported by Robbs (1965).

Hayward (1972) identified *Dieffenbachia maculata* (Lodd) G. Don, *Philodendron scandens* and aroids as hosts of *Xanthomonas axonopodis* pv. *dieffenbachiae*.

Dracaena fragrans of Agavaceae family and *Agaloenema robelinii* of Araceae family as collateral hosts of *Xanthomonas axonopodis* pv. *dieffenbachiae* were reported by Dye and Lelliot (1974).

Chase *et al.* (1992) observed that *Xanthomonas 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 pathogenisity tests and multiplication of bacterial population in leaf tissue for each strain was greatest in their hosts although strains also grew to significant levels in other hosts.

A new leaf blight of *Colocasia esculenta* by *Xanthomonas campestris* was reported by Phookan *et al.* (1996). Grijalba *et al.* (1998) reported a blight of potted begonias (Begonia tuber hybrida) by *Xanthomonas campestris* pv. *begoniae*.

2.6 Survival

Rattink (1979) reported that spread of the bacterial blight disease of anthurium was by drops of water, by direct contact and by propagating knife.

Monllor (1986) reported that crop residues in the field acted as the source of inoculum for the pathogen *Xanthomonas sagittifolium* causing bacterial spot of cocoyam.

Degaonkar and Kirtivar (1997) reported that in the case of bacterial blight of begonia by *Xanthomonas begoniae*, the bacteria remained viable and virulent for 17 months in naturally dried begonia leaves stored in glass tube at room temperature.

Sachin and Miller (1998) reported that primary source of pathogen of bacterial leaf spot of lettuce incited by *Xanthomonas axonopodis* pv. *vitians* was contaminated seed and infected plant debris in the field.

2.7 Weather factors and disease development

Severe outbreak of bacterial blight of anthurium was reported from Kauai, where warm humid conditions exists (Hayward, 1972).

Rattink (1979) reported that in *Xanthomonas begoniae*, infection developed better and symptoms were more pronounced when plants were grown in a warm, moist shaded glass house.

Guevara and Debrot (1984) reported that incidence and spread of bacterial blight of *Anthurium andreanum* Linden in Venenzuela was high in nursery where relative humidity was high. The pathogen *Xanthomonas axonopodis* pv. *dieffenbachiae* remained viable across a wide range of temperature (27-57^{0}C) (Webb *et al.*, 1987).

Namasivayam and Hedge (1971) found that in the case of black rot of crucifer (*Zinnia elegans*) cv. Peterpan by *Xanthomonas campestris* pv. *zinniae*, infection was recorded on 40 per cent of cultivated plants under conditions of high humidity.

2.8 Management

Very few work has been done on the management of bacterial blight of anthurium incited by *Xanthomonas axonopodis* pv. *dieffenbachiae* under *in vitro* and *in vivo* conditions.

2.8.1 *In vitro* experiments

Padhya *et al.* (1963) reported that by testing different species of *Xanthomonas* to different antibiotics, all were inhibited by streptomycin, tetracycline, oxytetracycline, neomycin, chloramphenicol and kanamycin. Penicillin (50 units / ml) was without effect.

De Azeredo *et al.* (1965) found that of five antibiotics added to a solid medium on which *Xanthomonas campestris* was grown, streptomycin had no effect. Aureomycin was most effective (0.3 µg/ml) followed by chloramphenicol 128 µg and penicillin 200 µg.

Sharma *et al.* (1981) found that under *in vitro* tests against *Xanthomonas campestris* pv. *vesicatoria*, streptocycline (streptomycin + chlor tetracycline) + $CuSO_4$ was most effective.

Sato (1983) found that control of *Xanthomonas campestris* pv. *dieffenbachiae* can be achieved by good management and the cautious use of streptomycin or oxytetracyline. The pathogen *Xanthomonas campestris* pv. *dieffenbachiae* was killed by exposure to oxytetracycline at 32 ppm under *in vitro* conditions (Nishijima, 1989).

Mahto *et al.* (1988) reported that the maximum inhibition to rice bacterial blight pathogen (*Xanthomonas campestris* pv. *oryzae*) under *in vitro* conditions was achieved with streptocycline among the two antibiotics and

eight fungicides tested. They also reported that streptocycline was inhibitory at 10,000 and 1000 ppm while streptomycin was inhibitory only at the two higher concentrations.

Jindal *et al.* (1989) while studying management of *Xanthomonas campestris* pv. *vignicola* from cowpea seeds found that of 10 compounds evaluated for control of *Xanthomonas campestris* pv. *vignicola* in paper disc agar diffusion test, agrimycin-100, streptocycline, streptomycin, agallol, captan and thiram inhibited bacterial growth. They also reported that streptocycline produced largest inhibition zone followed by streptomycin. Agallol, ceresan and captan 0.2 per cent were effective for control of *Xanthomonas campestris* pv. *vignicola* from cowpea seeds under *in vitro* conditions.

Barret and Casells (1994) reported *Xanthomonas campestris in vitro* growth was inhibited by streptocycline than by agrimycin or streptomycin. He also reported that tetracycline was inactivated in the presence of light.

Singh (1996) reported efficacy of antibiotics alone or in combination with bordeaux mixture and copper oxychloride in controlling bacterial leaf spot of *Piper betle* caused by *Xanthomonas campestris* pv. *betlicola.* He found that streptocycline 250 ppm was most effective in combination with bordeaux mixture and copper oxychloride followed by streptocycline alone.

Sakthivel and Gnanamanickam (1986) reported that *Pseudomonas fluorescens* isolated from rhizosphere were inhibitory to *Xanthomonas oryzae* pv. *oryzae* under *in vitro* conditions. Kalita *et al.* (1996) reported that *Pseudomonas fluorescens* isolated from phylloplane of lemon inhibited *in vitro* growth of *Xanthomonas campestris* pv. *citri.*

Schneider (1986) reported the antibacterial property of neem oil against *Staphylococcus aureus*.

Knauss (1972) reported resistance of *Xanthomonas axonopodis* pv. *dieffenbachiae* isolates to streptomycin. He found that the pathogen directly isolated from infected leaves on media containing streptomycin yielded vigorously growing colonies at all streptomycin concentration.

Nishijima and Fujiyama (1985) reported that the bacterium *Xanthomonas axonopodis* pv. *dieffenbachiae* causing foliar and systemic infection of anthurium appeared to be resistant to copper based preparation and these preparations were also phytotoxic to the plant.

2.8.2 *In vivo* experiments

Knauss (1972) found that foliage sprays of 200-400 ppm streptomycin during summer months at interval of four to seven days were ineffective against *Xanthomonas axonopodis* pv. *dieffenbachiae* isolates. He also reported that inoculation with resistant isolate on *Philodendron oxycardium* plants sprayed with 600 ppm streptomycin resulted in more severe disease than similar inoculation with streptomycin sensitive isolate.

Strider (1980) reported that in dense glass house planting the disease caused by *Xanthomonas campestris* pv. *zinniae* was decreased from hundred to two per cent when infected seeds were soaked for 30 minutes in 360 g a.i. captan l^{-1} of water containing Tween-80. They also reported that dry treatment with captan 50 WP was less effective (12.4 per cent diseased plants). In plant beds outside the glass house, the spread was decreased by weekly foliar application of captan 4.8 g a.i. l^{-1}. Thind *et al.* (1984) reported

that seed treatment with a mixture of captan (0.2 per cent) and streptocycline (100 ppm) eradicated *Xanthomonas campestris* pv. *vignae radiatae* from mungbean seed.

Sivamani *et al.* (1987) reported that *Pseudomonas fluorescens* was antagonistic to bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae*. *Pseudomonas fluorescens* treated rice plants recorded a substantial reduction of 40-60 per cent in bacterial blight severity (Anuratha and Gnanamanickam, 1987).

Jindal *et al.* (1989) evaluated combination of streptomycin at 100 mg/ml with the fungicides captan and thiram along with hot water and solar heat treatment under green house and field conditions against *Xanthomonas campestris* pv. *betlicola*.

Kamala (1996) reported that *Pseudomonas fluorescens* was an effective antagonist against the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae*. Application of *Pseudomonas fluorescens* by foliar spray at the rate of 10 g l^{-1} reduced the bacterial blight disease of rice.

Kalita *et al.* (1996) reported that when *Pseudomonas fluorescens* isolated from phylloplane of lemon were tested for their efficacy in control of citrus canker by applying them over crop foliage of Assam lemon, decreased citrus canker incidence occurred under field conditions.

Mary *et al.* (1986) reported that a foliar spray of cowdung extract 20 g l^{-1} controlled bacterial blight of rice equivalent to that given by penicillin (100 ppm), Paushamycin (250 ppm) and streptomycin (100 ppm). Sreekumar *et al.* (1990) reported that eventhough under *in vitro* conditions, cowdung extract failed to produce any typical growth inhibition zone, under pot culture

conditions cowdung extract was better than some of the chemical treatments like terramycin, streptocycline and Bactrinol-100. They also studied the effect of spraying with Bactrinol-100, oxytetracycline, streptocycline and cowdung extract on control of bacterial blight disease under field conditions. The reduction in disease intensity was maximum after spraying with terramycin followed by Bactrinol-100, streptocycline and cowdung extract. The grain yield was also maximum in plants sprayed with terramycin. However the increase in yield obtained by spraying with Bactrinol-100 and cowdung extract was higher than that of plants sprayed with streptocycline. Further the increase in thousand-grain weight and straw yield was maximum in plants sprayed with cowdung extract. Curative spraying with cowdung extract at the rate of 20 g l^{-1} was found very effective in reducing the per cent disease incidence and increasing significantly the grain and straw yield of bacterial blight affected paddy compared to streptocycline and Bactrinol-100 at 500 ppm (Mary, 1996).

Gangopadhyay (1998) reported that seed treatment with turmeric powder impregnated sodium bicarbonate in the proportion 10 : 1 at the rate of 1g kg^{-1} seed was found to control soil borne diseases of rice. Foliar spray of the mixture at the rate of 1 g l^{-1} of water at maximum tillering stage of the crop reduced the incidence of rice diseases.

Hulloli *et al.* (1998) reported the antibacterial property of certain neem products like plantolyte and agricare against *Xanthomonas axonopodis* pv. *malvacearum*. These neem formulations when applied along with antibiotics or fungicides acted synergistically and reduced the hazardous effect of fungicides and antibiotics.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Isolation of pathogen

The bacterial pathogen was initially isolated from infected anthurium samples collected from different parts of Thiruvananthapuram district, including the College of Agriculture, Vellayani. The affected parts were subjected to ooze test to find out the presence of bacterium. The infected portion with profuse ooze was selected, cut into 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	-	300 g
$Na_2 HPO_4$	-	2.0 g
$Ca NO_3$	-	0.5 g
Peptone	-	5.0 g
Sucrose	-	20.0 g
Agar agar	-	20.0 g
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 24h old growth of each isolate was prepared separately. They (Xad-1 to Xad-3) obtained from the leaf blight infection, (Table 1) were artificially inoculated on leaves of its own original host by giving pinpricks on the leaf lamina and rubbing with cotton dipped in the bacterial suspension. The isolates from systemically infected plants (Xad-4 to Xad-6) (Table 1) were artificially inoculated on the stem just above the soil 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 favourable microclimatic conditions to initiate infection.

When the artificially inoculated plants reproduced the symptoms of the disease, isolations were carried out from infected portions following the procedure described above. Single colonies thus obtained were compared with that of the original isolate. The pure cultures were maintained on PSA medium for further characterization work. The details of different bacterial isolates, are given below (Table 1).

3.2 Symptomatology

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

Table 1 Description of isolates collected

Isolates	Locality	Anthurium cv. used	Year of isolation
Xad-1	Agricultural College, Vellayani	Mauritius Orange	1998
Xad-2	Agricultural College, Vellayani	Kalympong Orange	1998
Xad-3	Palappur	Tropical	1998
Xad-4	Palayam	Inspiration	1998
Xad-5	Balaramapuram	Honeymoon Red	1999
Xad-6	Kattakkada	**Cancan**	2000

3.3 Characterization and identification of pathogen

Characterization and identification of the different isolates of the pathogen were done according to the methods recommended by "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.

3.3.1 Cultural characters

3.3.1.1 Morphological characters

Colony characters and cell morphology were studied from a 24h old culture of the bacterium grown on PSA medium. For studying colony characters, a loopful of 24h old growth of the bacterium was streaked on solidified PSA medium poured in sterilized petridishes. The growth of single colony was observed. Cell morphology was studied by Gram staining and observing the slides under oil immersion objective of a microscope.

3.3.1.2 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 and kept for incubation for 24 h at room temperature.

3.3.1.3 Growth of different isolates of bacterium on Potato Sucrose broth

For studying the growth of different isolates of the bacterium in liquid medium, Potato Sucrose broth was studied. Five ml sterilized broth of the medium taken in test tube was inoculated with one ml of 24-h-old bacterial

suspension grown on potato sucrose broth using a sterilized pipette. The tests were done for each isolate in triplicate. Uninoculated broth was also maintained. The inoculated medium was shaken daily. Optical density of the broth culture of each isolate was measured after 24, 48 and 72 h using a spectrophotometer at 510 nm wave length. Uninoculated broth was used as blank.

3.3.1.4 Pigment production

Production of water insoluble and soluble pigments was tested in Yeast extract Glucose Chalk Agar medium and King's medium respectively. 24-h-old culture was used for this purpose. The tests were performed in triplicate for all isolates and observations were recorded periodically.

Composition of Yeast extract Glucose Chalk Agar medium

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

3.3.1.5 Oxygen requirement

To determine whether the bacterium was aerobic or anaerobic, Nutrient Agar (NA) columns containing bromocresol purple in tubes were inoculated by stabbing with the 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 another set of tubes were kept open without paraffin oil. Both sets of tubes with paraffin oil and without paraffin oil were incubated at room temperature and observations were recorded.

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

3.3.2 Physiological characters

The tests were performed in triplicate and observations were made in comparison with uninoculated control.

3.3.2.1 Mode of utilization of glucose

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

Composition of the medium

Peptone	-	1.0 g
$NH_4 H_2 PO_4$	-	1.0 g
KCl	-	0.2 g
$MgSO_4 . 7H_2O$	-	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 tubes up to 4 cm and sterilized by tyndallization and inoculated in duplicate by stabbing with bacterial growth. In one of the tubes, the medium was sealed with one cm layer of sterile liquid paraffin. The tubes were incubated at room temperature and observations were taken at regular intervals up to 15 days.

3.3.2.2 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. Un inoculated controls were also maintained. Observations were recorded at regular intervals.

3.3.2.3 Starch hydrolysis

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

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	-	1000 ml
pH	-	7.0

Bacterial culture of 24-h-old was spot inoculated on the medium in plates. After four days of incubation, hydrolysis was tested by pouring Lugols iodine over the medium. 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.

3.3.2.4 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	-	1000 ml
pH	-	7.0

Five ml quantities 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 solutions of lead acetate. The strips were inserted aseptically between the plug and inner wall of the tube hanging just above the broth. The tubes inoculated with 24-h-old culture of bacterium were incubated at room temperature and observations were recorded upto 14 days at regular intervals. Blackening of the lead acetate impregnated strips indicated liberation of hydrogen sulphide.

3.3.2.5 Methyl Red test (MR test)

Methyl Red broth was used for the test.

Composition of the medium

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 tubes and sterilized by steaming for 30 minutes for three successive days. Two sets of tubes were inoculated with 24-h-old culture of the bacterium for MR test. The tubes were incubated for seven days at room temperature.

A 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 an yellow colour with the methyl red indicator regarded as negative reaction.

3.3.2.6 Gelatin liquefaction

Nutrient Gelatin medium was used for the purpose. Stab method was used for this test.

Composition of the 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 10 lbs pressure for 20 minutes. The sterile condition of the medium was checked by observing it for two days. Inoculated these properly sterilized gelatin columns by stabbing a straight inoculation needle charged with 24-h-old culture of the bacterium. The tubes were incubated and observed for the liquefaction of the gel column at regular intervals upto one month.

3.3.2.7 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 test 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 the bacterium in triplicate and oxalic acid strips were inserted into the tube by the side of the plug, incubated and observed regularly for 14 days. Change in colour of oxalic acid crystals on test strip to pink or red indicated indole production.

3.3.2.8 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	-	20 g
Phenol Red		
(0.2 per cent solution)	-	6.0 ml
Distilled water	-	1000 ml
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 in tubes in five ml quantities and slants were prepared. 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.

3.3.2.9 Catalase test

To assess the production of catalase enzyme by the bacterium, a loopful of 24-h-old culture of the 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.

3.3.2.10 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 aliquots in test tubes and sterilized by steaming for 10 minutes on three successive days in a Prestige pressure cooker. The medium was inoculated with a loopful of 24-h-old test bacteria and incubated. Observations were recorded for 30 days at regular intervals for acidic and alkaline reaction. Change of the light blue colour of the medium to yellow indicated acid reaction and to violet indicated reaction to be alkaline. Uninoculated control was kept as a reference.

3.3.2.11 Utilization of asparagine as sole source of carbon and nitrogen

The test was performed in the following 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	-	100 ml
Solution 2.	$MgSO_4.\ 7H_2O$	-	2.0 g
	$Fe\ SO_4$	-	0.5 g
	NaCl	-	1.0 g
	$MnSO_4$	-	0.02 g
	H_2SO_4	-	1 drop
	Distilled water	-	100 ml
Solution 3.	$Na_2Mo\ O_4$	-	0.02 g
	Distilled water	-	100 ml

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

Ten ml of each solution was mixed with each other in the order 3,4, 2, 1 filtered and added to 960 ml of distilled water and two gram of L-Asparagine. The medium was dispensed in five ml quantities in tubes and autoclaved. The tubes were inoculated with the 24-h-old culture of the bacterium, incubated and examined for growth. Growth of the bacterium in the medium was indicative of the utilization of asparagine.

3.3.2.12 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 24-h-old culture of the bacterium, incubated and observations recorded.

3.3.2.13 Lipolytic activity

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

Composition of the medium

Peptone	-	10.0 g
NaCl	-	5.0 g
$CaCl_2. H_2O$	-	0.1 g
Agar agar	-	20.0 g
Distilled water	-	1000 ml
pH	-	7.0

The medium was dispensed in 99 ml quantities in flasks autoclaved and cooled to 45^0C. One ml of Tween-80 (Oleic acid ester) was added to the medium and thoroughly mixed. The medium was poured in sterile petri dishes and test bacterium was spot inoculated on the medium. The plates were incubated and observed at regular intervals for seven days. Opaque zone around the bacterial growth was indicative of positive lipase production.

3.3.2.14 Tyrosinase activity

The following medium (Dye, 1962) was employed for the test.

Composition of the medium

$NH_4 H_2 PO_4$	-	0.5 g
$K_2H PO_4$	-	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	-	1000 ml
pH	-	6.8 - 7.0

The medium was dispensed in tubes, autoclaved and slants were prepared. The slants were inoculated with 24-h-old culture of the bacterium and incubated. Browning of the medium indicated tyrosinase activity.

3.3.2.15 Arginine hydrolase activity

The following medium was used for the purpose (Thornley, 1960).

Composition of the medium

Peptone	-	1.0 g
NaCl	-	5.0 g
$K_2H PO_4$	-	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 dispensed in five ml quantities in test 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 colour of the medium to red indicated arginine hydrolase activity.

3.3.2.16 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 noted.

3.4 Hyper sensitivity reaction on tobacco leaves

Dilute suspension of the bacterial growth was inoculated into the leaves of tobacco (*Nicotiana tobaccum*) plant using a hypodermic needle (Klement and Goodman, 1967). Observations were recorded for formation of necrotic spots after 24 h upto a period of 48 h.

3.5 Studies on selected isolate

Since isolate Xad-6 was found to be more virulent than the other five isolates, it was selected for further studies.

3.5.1 Growth of Xad-6 on different solid media

Nature of growth, colour, shape, extent of growth, type of margin and slime production by the isolate Xad-6 was studied on eight different solid media. A loopful of the dilute suspension of the bacterium was streaked on different media in triplicate and kept for incubation at 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

$NH_4 H_2 PO_4$	-	0.5 g
$K_2H PO_4$	-	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

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-6 was studied as indicated by acid production (Dye, 1962) on agar slants of basal media for xanthomonads. Eight carbon sources, viz., maltose, inositol, galactose, xylose, dulcitol, glucose, sucrose and fructose were tested. 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 utilization of carbon compounds by isolate Xad-6 in broth of the basal medium 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 tyndallization and the broth was inoculated with 24-h-old culture of the bacterium in triplicate and incubated at

room temperature for 24 h. Growth in each medium was recorded using Spectrophotometer at 510 nm wavelength. More absorbance indicated more growth in the medium which indicated more utilization of that particular carbon source.

3.6 Host range of the pathogen

Plants with similar taxonomic affinity were inoculated for studying the host range of the pathogen. *Dieffenbachia* sp. Schot, *Aglaonema robelinii, Colocasia esculenta, Syngonium podophyllum* and *Philodendron oxycardium* belonging to the family Araceae were used for the study.

The plants were collected and planted in pots. The potted plants were inoculated with 24-h-old growth of *Xanthomonas axonopodis* pv. *dieffenbachiae* after giving pinpricks on the leaves and smearing the bacterial suspension on both surfaces. Three replications were maintained for each host plant tested. Control plants were also maintained by giving pinpricks on the leaves and smearing the surface with cotton dipped in sterile distilled water. Humidity was maintained by covering the plants with polythene covers and giving water sprays frequently. The plants were observed for symptom development.

3.7 Survival of the pathogen

Studies were conducted on the survival of the pathogen in plant debris, soil and also in infected plant parts kept under refrigerated condition.

3.7.1 Survival in infected plant debris

The diseased materials were chopped into small pieces. Pots were filled three-forth with potting mixture of anthurium. Chopped material was spread over the potting mixture as a layer of one inch thickness and covered with another one inch layer of ordinary soil. Moist condition was maintained in the pots. Survival was studied by serial dilution of infected plant materials kept in the potting mixture. One g sample was collected and shaken well by keeping on a shaker for 30 minutes. This sample was serially diluted and 1 ml of the 10^{-6} dilution was plated in a sterile petridish. About 10 ml of PSA medium was poured over it. The plates were incubated at the room temperature and colonies resembling *Xanthomonas axonopodis* pv. *dieffenbachiae* were counted after 24 h. Isolations were carried out from the soil containing plant debris for a period of 2.5 months at 15 days interval.

3.7.2 Survival in soil

A 12 inch diameter pot containing potting mixture for anthurium was inoculated by 100 ml of a 24-h-old growth of the bacterium having OD of 1.209. Isolations at 15 days interval were carried out from the soil. One g of this soil was taken in 100 ml of sterile distilled water and shaken for 30 minutes. From that 10^{-8} dilution was prepared and 1 ml of the same was poured in sterile petridish. About 10 ml of PSA medium was poured over it under aseptic conditions and incubated at room temperature. After 24 h incubation number of colony forming units were counted. Isolations were carried out for a period of 2.5 months.

3.7.3 Survival inder refrigerated condition

Survival was studied in infected plant samples stored under refrigerated condition (4^0C). The plant samples were kept in polythene covers. One g sample was taken from this, chopped into very small pieces, placed in 100 ml of sterile distilled water in a 250 ml conical flask and shaken for 30 minutes. This sample was serially diluted and 10^{-6} dilution was prepared. One ml of this dilution was pipetted out into a sterile petridish and about 10 ml of PSA medium was poured over it under aseptic conditions. Colonies resembling *Xanthomonas axonopodis* pv. *dieffenbachiae* were counted after 24 h incubation. Isolations were carried out for a period of 2.5 months at 15 days interval.

3.8 Management

3.8.1 *In vitro* evaluation of different bactericides against *Xanthomonas axonopodis* pv. *dieffenbachiae*

3.8.1.1 *In vitro* sensitivity of the bacterium to antibiotics and a fungicide

The *in vitro* sensitivity of the bacterium to five different antibiotics and a fungicide were tested. The following were the treatments used for the study.

1. Oxytetracycline	Pfizer Limited (Terramycin)
2. Streptomycin	Sarabhai Chemicals (Ambistryn-S, Streptomycin Sulphate)
3. Chloramphenicol	Piya Chemicals (Chloromycetin)
4. Penicillin	Alembic Chemical Works Co. Ltd., Baroda (Fortified procaine penicillin injection I.P.)
5. Streptocycline	Hindustan antibiotics Ltd., Pimpri, Poona, India (Streptomycin 12 per cent + Chlorotetracycline hydrochloride 1.5 per cent)
6. Captan	

Solutions of antibiotics were prepared at 25, 50 and 100 ppm. Solutions of fungicide captan at 0.1, 0.2 and 0.3 per cent were also prepared. Sterile filter paper disc of 10 mm diameter was dipped in the appropriate solutions and placed over PSA medium seeded with 48-h-old culture of the bacterium. The test was conducted with three replications. Observations on the zone of inhibition were recorded after 48 h. Based on this study the best level of antibiotic and the fungicide was selected for field evaluation.

3.8.1.2 *In vitro* sensitivity of the bacterium to ecofriendly materials

Ecofriendly materials like turmeric powder + sodium bicarbonate (10 : 1 proportion) at 0.05, 0.1 and 0.15 per cent, neem oil at 1.4, 1.7 and 2.0 per cent, *Pseudomonas fluorescens* (proprietary product) at 0.5, 1.0 and 1.5 per cent and cowdung extract at 2.5, 5.0 and 7.5 per cent were tested under *in vitro* condition following the same method adopted for testing the effect of antibiotics and observations on zone of inhibition were recorded after 48 h.

3.8.2 Control of bacterial blight of anthurium under *in vivo* conditions

In order to assess the efficacy of some chemicals and ecofriendly materials against the disease and to assess their field performance, a pot culture experiment was laid out in the glass house of Department of Plant Pathology in completely randomised design with 15 treatments and three replications. Cancan, a hybrid variety of anthurium, highly susceptible to bacterial blight disease was selected for the study. Seven month old tissue cultured anthurium plants were artificially inoculated with 24-h-old bacterial

suspension by giving pinpricks at the collar region and swabbing with a cotton dipped in the bacterial suspension. The inoculated plants were covered with polythene covers and high humidity was maintained till the initiation of disease symptoms. The following 15 treatments, viz., streptocycline at 100 ppm, captan 0.3 per cent, turmeric powder + sodium bicarbonate in 10 : 1 proportion (0.05, 0.10 and 0.15 per cent), neem oil (1.4, 1.7 and 2.0 per cent), *Pseudomonas fluorescens* (proprietary product at 0.5, 1.00 and 1.5 per cent) and cowdung extract (2.5, 5.00 and 7.5 per cent)were given seven days after artificial inoculation.

The artificially inoculated plants were sprayed from seventh day onwards at weekly intervals using a hand sprayer.

Scoring of the disease was done using the disease scale developed for the purpose. Observations on per cent infection were recorded on the day of each spraying. The final observation was taken one week after fifth spray.

The plants were kept under observation after five sprayings for two months and further observations were recorded on the condition of the plant. Isolations were also carried out from foliar parts to confirm the presence or absence of the pathogen inside the host.

For assessing the disease intensity a scale was devised after careful study of the disease and disease development. The disease was studied in detail both under natural and artificial conditions of disease development. The extent of infection was estimated based on the parts of the plants affected. Size of the lesion, yellowing and drying of infected leaves were taken into account for devising the scale. Based on this a 0-5 scale has been devised (Plate 1).

Plate 1 Score chart for bacterial blight of anthurium

Disease scale	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 leaves with blackening of petioles
5	76 - 100	Complete death of the plant

3.9 Statistical analysis

The data generated from the experiment were subjected to analysis of variance (ANOVA) after appropriate transformations wherever needed.

RESULTS

4. RESULTS

4.1 Isolation of pathogen

Six bacterial isolates were collected from different parts of Thiruvananthapuram district and named as Xad-1 to Xad-6. Those isolates were artificially inoculated on the leaves as well as the collar region of *Anthurium andreanum* var. *Cancan*. The original symptoms on the host were reproduced by these isolates within one to two weeks.

4.2 Symptomatology

Both foliar and systemic infections were noticed in plants affected by bacterial blight.

4.2.1 Foliar infection

Older leaves of anthurium plants were more susceptible to foliar infection. The first indication of foliar infection was the appearance of irregular water soaked lesions of two to three mm in size on leaf margin and lamina. It was more pronounced at the lower surface of the leaf lamina. The lesions as they increased in size turned dark brown surrounded by a prominent yellow halo (Plate 2) and extended towards the midrib so that a considerable portion of the lamina got blighted. Spread of foliar infection through the petiole reaching the stem and roots caused systemic infection and resulted in plant death.

Plate 2 Foliar blight of anthurium

Symptoms were also initiated on spathe as well as spadix. On spathe the water soaked lesions of 2 mm increased in size and turned brown surrounded by light coloured wavy fringed margin of width 2 mm. The colour of the fringed margin varied (Plate 3) according to the colour of the spathe. The necrotic brownish area increased in size and spread to cover a major portion of spathe. Finally the affected portion dried out (Plate 4).

The water soaked lesion developed any where on spadix and later coalesced to form large dark brown patches. Sometimes it progressed upwards and downwards to cover the entire spadix. This spadix later shrunk, dried and remained in the center of the spathe as a black candle (Plate 4).

4.2.2 Systemic infection

Initial symptoms of disease appeared as water soaked lesions at the collar region of the plant. Later these lesions turned dark brown. With the development of disease, rotting of the collar region of the plant was observed. At the same time leaves started showing symptoms of browning and yellowing (Plate 5). The infection spread from the collar region through the petiole to the leaf base and to the leaf lamina as evidenced by the gradual dark brown discolouration of the petiole and yellowing of the lamina (Plate 6). In about two to three weeks after the appearance of first symptom, the whole plant died.

The isolates collected from bacterial blight affected anthurium plants when inoculated on leaf lamina of *Cancan* variety of anthurium produced necrotic leaf spots within five days of artificial inoculation. At first, the spots

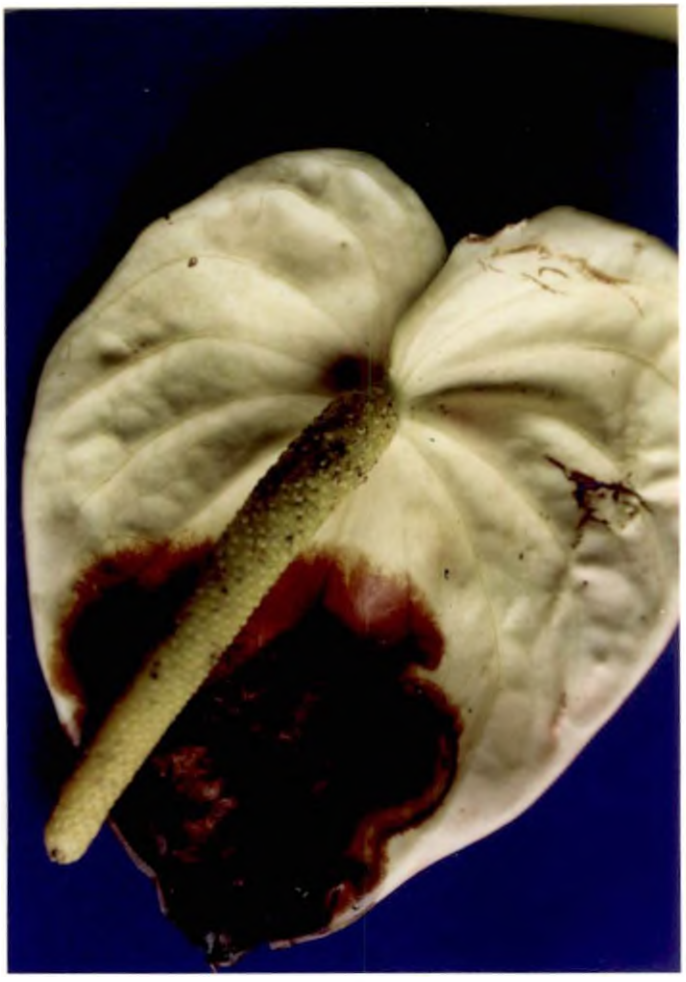


Plate 3 Symptoms on spathe

Plate 5 Bacterial blight of anthurium – systemic infection

Plate 6 Systemic infection – symptoms on the collar region and petiole

days. The adjacent spots coalesced and turned dark brown surrounded by a prominent yellow hallo.

The isolates collected from bacterial blight affected anthurium plants when inoculated on the collar region of the *Cancan* variety of anthurium by giving pinpricks developed dark brown lesion within seven days. They later coalesced and formed larger discoloured area. The lesions extended both upwards and downwards and caused yellowing and browning of the foliar parts and rotting of the root system (Plate 7). Rapid advancement of disease as well as earlier death of the inoculated plants were recorded in the case of isolate Xad-6.

4.3 Characterization and identification of pathogen

4.3.1 Cultural characters

4.3.1.1 Morphological characters

The bacterium was a Gram negative short rod. All isolates gave rise to yellow, circular, slimy and convex colonies with entire margin on PSA. A slight difference was observed in the yellow colour produced between isolates.

4.3.1.2 Growth of different isolates of the bacterium on PSA

The growth of six different isolates were tested on PSA medium (Table 2).

All the six isolates tested produced yellow, circular, smooth and convex colonies with entire margin on PSA medium. Slight differences were noticed

Plate 7 Systemic infection – symptoms on roots

Table 2. Growth of different isolates of the bacterium on Potato Sucrose Agar

Sl. No.	Medium	Nature of colony and colour	Growth and slime production
1	Xad - 1	Deep yellow, circular, smooth, convex with entire margin	Gr. +++ Sl. +++
2	Xad - 2	Deep yellow, circular, smooth, convex with entire margin	Gr. ++ Sl. ++
3	Xad -3	Yellow, circular, smooth, convex with entire margin	Gr. +++ Sl. ++
4	Xad -4	Yellow, circular, smooth, convex with entire margin	Gr. ++ Sl. ++
5	Xad -5	Deep yellow, circular, smooth, convex with entire margin	Gr. +++ Sl. +++
6	Xad -6	Yellow, circular, smooth, convex with entire margin	Gr. +++ Sl. +++

Gr. Growth
Sl. Slime

+++ Good
++ Moderate
+ Slight

The isolates Xad–1, Xad–3, Xad–5 and Xad–6 had good growth, while the isolates Xad–2 and Xad–4 had only moderate growth.

All the isolates produced slime on PSA medium. Slime production was maximum for the isolates Xad–1, Xad–5 and Xad–6 and moderate for the isolates Xad–2, Xad–3 and Xad–4.

4.3.1.3 Growth of different isolates of bacterium on Potato Sucrose broth

The growth of six different isolates of bacterium was studied on Potato Sucrose broth. The data on optical density of the broth culture measured after 24, 48 and 72 h after incubation are presented in the Table 3.

Maximum growth was recorded by the isolate Xad-4 after an incubation period of 24 h (OD = 1.674). This was followed by isolates Xad-2 and Xad-3 which were statistically on par and significantly different from Xad-4 in their growth. Isolates Xad-6, Xad-5 and Xad-1 had significantly lower growth than isolate Xad-4, Xad-2 and Xad-3. The least growth was recorded by isolate Xad-1 (OD = 0.201).

Isolate Xad-6 recorded statistically significant growth (OD = 1.552) compared to other five isolates after an incubation period of 48 h. This was followed by isolates Xad-4, Xad-3, Xad-2 and Xad-1. The lowest growth was recorded by Xad-5 (OD = 0.270) which was statistically on par with Xad-1.

Among the six isolates studied, the isolate Xad-6 recorded (OD = 1.765) statistically significant growth after an incubation period of 72 h. This was followed by isolate Xad-2 and Xad-3 which were statistically on par. The growth of Xad-4 was significantly higher than the growth of isolate Xad-1

Table 3 Growth of different isolates of bacterium on Potato Sucrose broth

Sl No.	Isolates	Optical density* at 510 nm		
		Incubation period		
		24 hours	48 hours	72 hours
1	Xad - 1	0.201	0.310	0.153
2	Xad - 2	1.518	1.099	0.965
3	Xad - 3	1.493	1.308	0.979
4	Xad - 4	1.674	1.392	0.558
5	Xad - 5	0.261	0.270	0.195
6	Xad - 6	1.209	1.552	1.765

* Mean of three replications
CD for treatments (0.05 level) = 0.0546
CD for levels (0.05 level) = 0.0386

and Xad-5. Isolate Xad-5 recorded (OD = 0.195), the lowest growth which was statistically on par with Xad-1.

Isolates Xad-3 and Xad-4 attained maximum growth by about 24 h and after that the growth was declining (Fig. 1). Isolates Xad-1, Xad-2 and Xad-5 attained maximum growth within a period of 48 h and after that the growth was found to decline. Among the six isolates studied growth of isolate Xad-6 was found to be increasing even after a period of 72 h recording a growth curve different from others. In other words, to obtain maximum growth, the isolates Xad-6 required a much longer period i.e., more than 72 h, compared to all the other five isolates which required 48 to 72 h.

Taking into consideration the growth rate of the isolates 24 h after incubation, isolate Xad-4 was fast growing whereas Xad-2, Xad-3 and Xad-6 were moderate in their growth rate and Xad-5 and Xad-1 were slow growing.

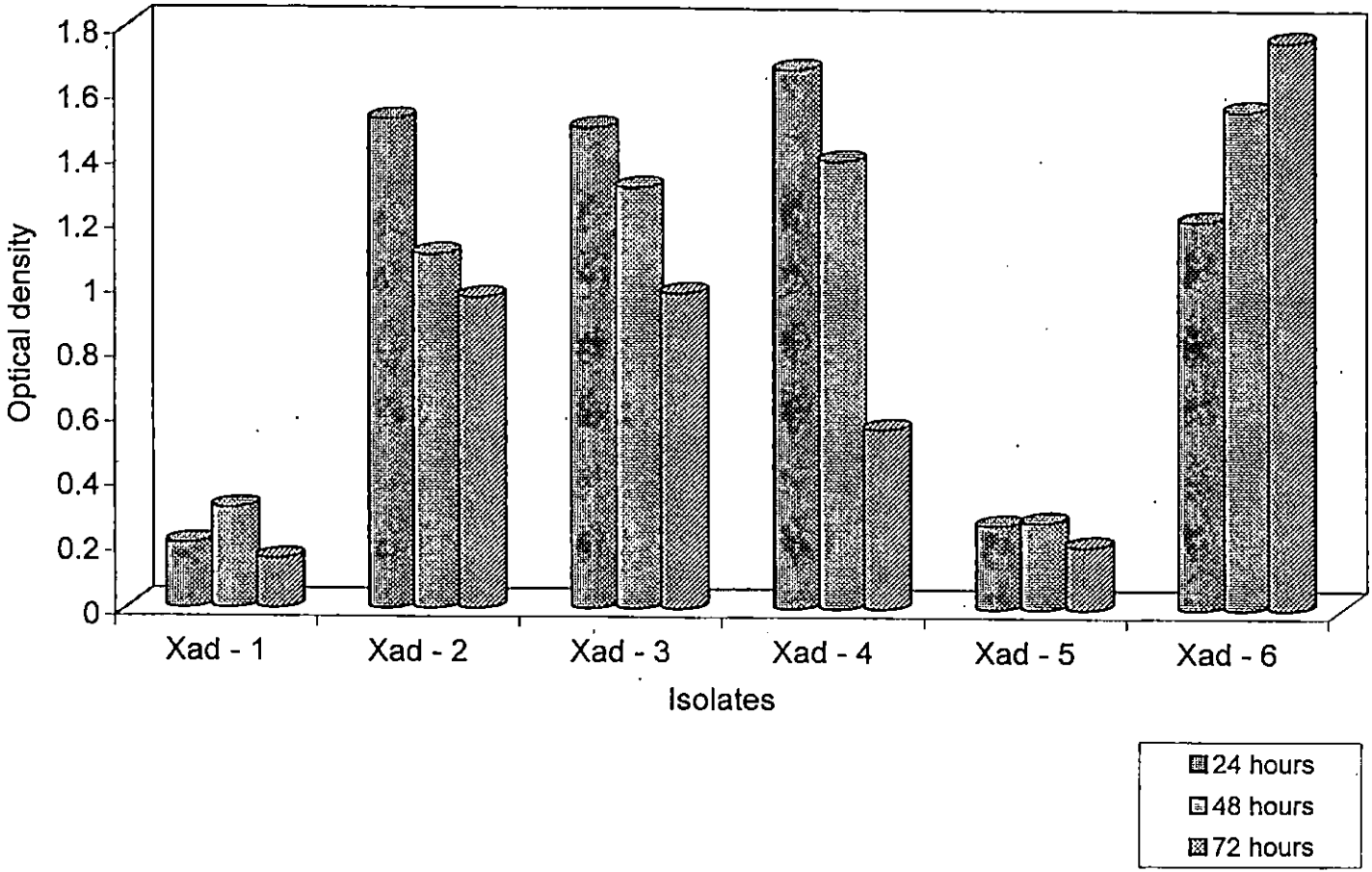
4.3.1.4 Pigment production

All the isolates of the bacterium produced a water insoluble yellow pigment on YGCA. None of the isolates produced water-soluble fluorescent pigments on King's medium.

4.3.1.5 Oxygen requirement

All the isolates of the bacterium were aerobic, since the growth and change of blue colour of the NA medium containing 0.005 per cent bromocresol purple to yellow was observed only in tubes without paraffin sealing.

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



4.3.2 Physiological characters

4.3.2.1 Mode of utilization of glucose

All 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.

4.3.2.2 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. None of the isolates tested utilized sodium benzoate and sodium formate as the source of carbon.

4.3.2.3 Starch hydrolysis

All the isolates except Xad–6 hydrolysed starch, since a colourless or reddish zone was observed around the bacterial growth in contrast with blue background of the medium.

4.3.2.4 Production of hydrogen sulphide

All the six isolates were found to liberate hydrogen sulphide. This was indicated by the blackening of the lead acetate test strip.

4.3.2.5 Methyl Red test (MR test)

All the six isolates gave negative MR test as evidenced by the development of yellow colour in the culture tube when a few drops of methyl red solution was added.

4.3.2.6 Gelatin liquefaction

Within 15 days of inoculation of bacterial isolates, the gel columns in the tubes were liquefied. All the isolates of the bacterium used in this study liquefied gelatin.

4.3.2.7 Production of Indole

The oxalic acid crystals on the test strip did not turn pink or red, which indicated the absence of indole production by the six isolates of the bacterium.

4.3.2.8 Urease production

All the six isolates gave negative urease test since there was no change in colour of the medium from yellow to red.

4.3.2.9 Catalase test

Catalase positive reaction was shown by the different isolates of the bacterium.

4.3.2.10 Action on milk

All isolates of the bacterium turned the milk alkaline as evidenced by the colour change of the medium from blue to violet.

4.3.2.11 Utilization of asparagine as sole source of carbon and nitrogen

None of the isolates utilized asparagine as sole source of carbon and nitrogen.

4.3.2.12 Growth at six per cent sodium chloride

There was no growth of the isolates of the bacterium when inoculated on a media containing six per cent sodium chloride.

4.3.2.13 Lipolytic activity

Opaque zone around the bacterial growth indicated lipase production and so all the isolates of the bacterium produced lipase.

4.3.2.14 Tyrosinase activity

Absence of a dark brown pigment in the medium inoculated with isolates of the bacterium indicated that all isolates were tyrosinase negative.

4.3.2.15 Arginine hydrolase activity

All isolates of the bacterium were arginine hydrolase positive as indicated by change in the colour of the medium to red.

4.3.2.16 Production of ammonia

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

4.4 Hyper sensitive reaction on tobacco leaves

Necrotic lesions were formed on tobacco leaves within a period of 48 hours after inoculation.

4.5 Studies on selected isolate

The isolate Xad-6 was more virulent than the other five isolate studied. It produced severe symptom earlier than other isolates and killed the plant (*Anthurium andreanum* var. Cancan) within 15 days from the initiation of disease symptom.

4.5.1 Growth of Xad-6 on different solid media

The growth of the bacterium on eight different solid media were tested and the results are presented in Table 4.

Of the eight solid media tested maximum growth was observed on PSA. The colonies were yellow, circular, smooth and convex with entire margin on all the media tested. Slight differences were observed in the yellow colour produced by the isolates in different media. Growth of the bacterium was moderate on NA, BX, TTC and in PDA. Only slight growth was observed in YGCA, GA, and GYA.

In case of slime production, it was maximum on PSA and NA. Slime production was moderate on BX, TTC and YGCA. Slime production was slight in PDA. It was absent in GA and GYA.

4.5.2 Utilization of carbon sources

Carbon utilization by isolate Xad-6 was studied in the agar slant of BX and the results are presented in Table 5. Xad-6 produced acid with six carbon sources, viz., maltose, galactose, xylose, glucose sucrose and fructose as indicated by the change of the colour of the medium from reddish violet to

Table 4 Growth of the isolate Xad-6 on different solid media

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

Gr. – Growth
Sl – Slime

+++ Good
++ Moderate
+ Slight
- Nil

yellow. Acid production was noticed from third day of incubation. No change in colour of the medium from reddish violet to yellow was observed in tubes containing dulcitol and inositol, which indicated negative utilization of these sugars by the isolate Xad-6.

Carbon utilization by isolate Xad-6 was also studied in broth of BX using eight carbon sources. The results are presented in Table 5 and Fig. 2.

Among the eight carbon sources used in the study, the best utilized carbon source by isolate Xad-6 was glucose (OD = 0.984). Utilization of glucose was significantly superior to all the other carbon sources studied. Next best utilized carbon source was galactose followed by sucrose and fructose which were statistically on par. Utilization of xylose and maltose were significantly on par, but their utilization was significantly lower than that of the above sources, but it was significantly higher than the utilization of inositol and dulcitol. Least utilized carbon source was dulcitol (OD = 0.156).

4.6 Host range of the pathogen

The following plants belonging to the family Araceae, viz., *Dieffenbachia* sp. Schott, *Aglaonema robelinii, Philodendron oxycardium*, *Colocasia esculenta* and *Syngonium podophyllum* were found to develop symptoms of leaf blight disease, one week after artificial inoculation. The disease affected portion of these plants were subjected to ooze test which confirmed the presence of pathogen.

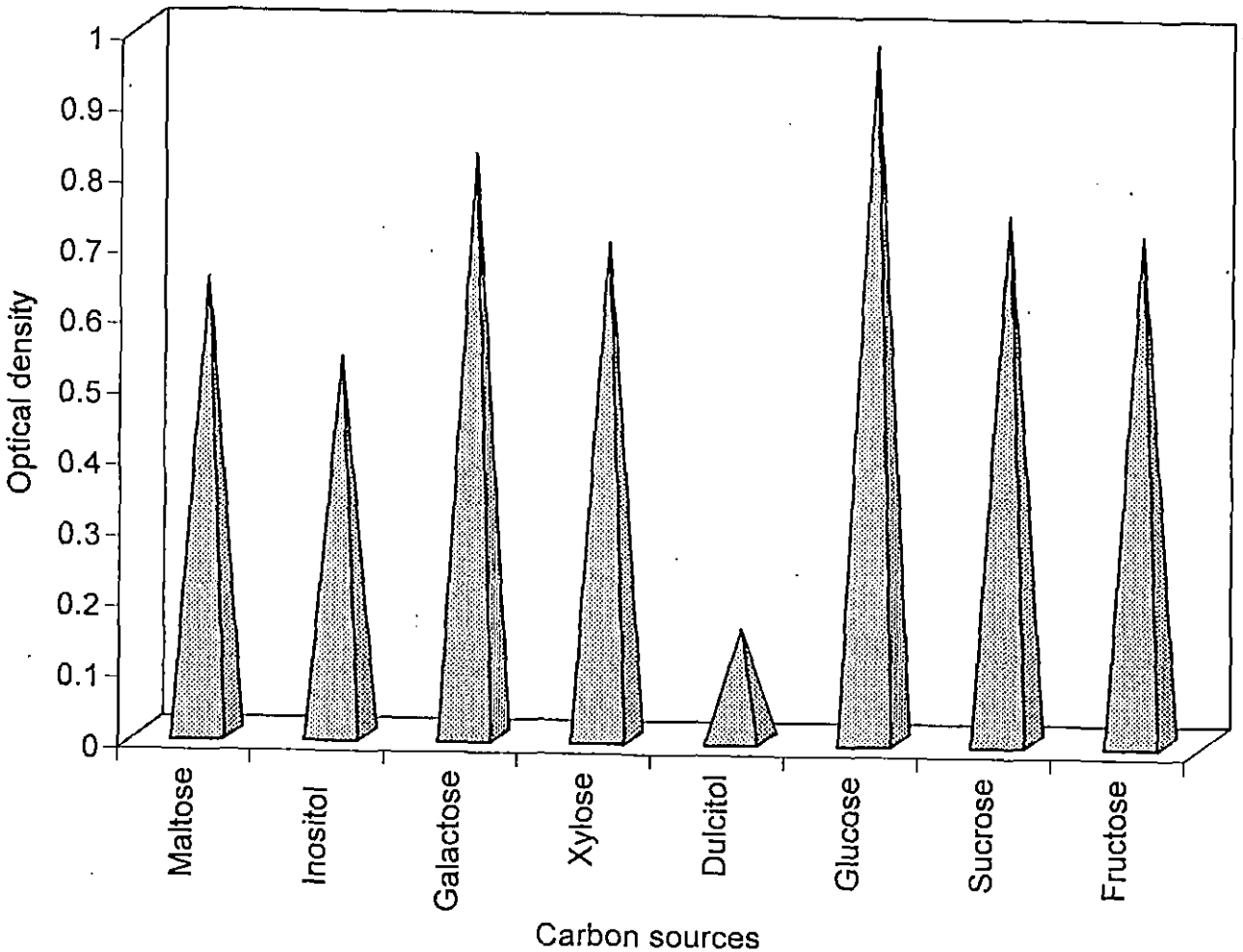
Table 5 Utilization of carbon sources by isolate Xad–6 on agar slants and broth of basal medium for xanthomonads

Sl. No.	Carbon source	Acid production on agar slants	Optical density in broth*
1	Maltose	Positive	0.647
2	Inositol	Negative	0.535
3	Galactose	Positive	0.826
4	Xylose	Positive	0.702
5	Dulcitol	Negative	0.156
6	Glucose	Positive	0.984
7	Sucrose	Positive	0.748
8	Fructose	Positive	0.719

* Mean of three replications

CD for treatments (0.05 level) = 0.117

Fig. 2 Utilization of carbon sources by isolate Xad-6 in broth of basal medium for xanthomonads



4.7 Survival of the pathogen

4.7.1 Survival in infected plant debris

Survival in plant debris was studied by serial dilution of the plant debris in soil at 15 days interval and the results are presented in Table 6 and Fig. 3. The population of the pathogen was 48 cfu/g sample after 15 days of storage. There was a gradual reduction in the number of cfu/g sample with increase in time of storage and the colony count came down to zero after 60 days indicating that the pathogen could survive for about 45 days in plant debris remaining in the soil under glass house condition.

4.7.2 Survival in soil

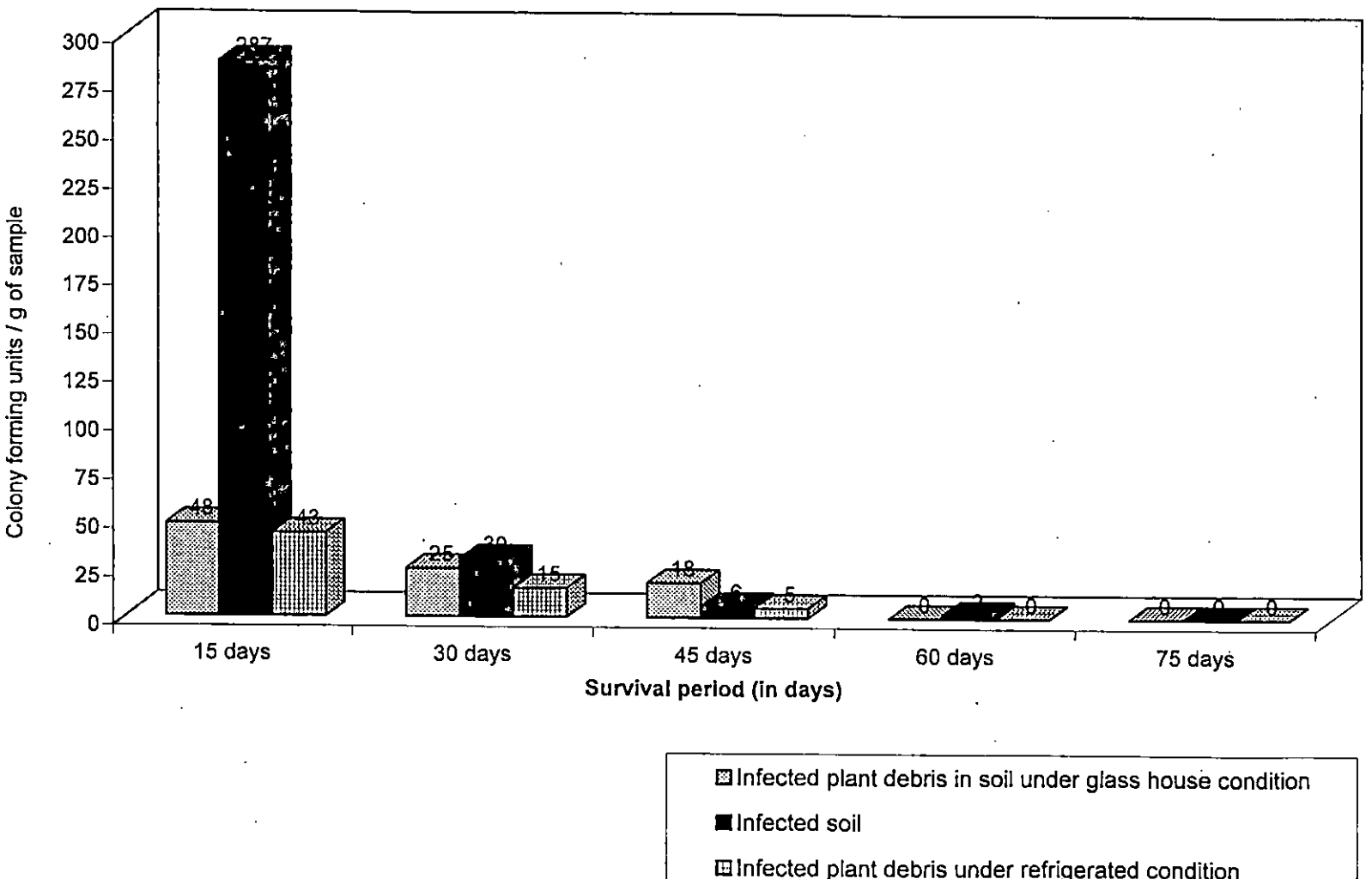
Survival of the pathogen in soil was studied by artificially inoculating the soil with 24-h-old bacterial suspension and periodical isolation of the pathogen from soil by serial dilution. The results of the study are presented in the Table 6 and Fig. 3. The colony count was very high i.e., 287 cfu/g soil, 15 days after inoculation of the soil. Ten times reduction in population was noticed after another 15 days of storage (30 cfu/g soil). Five times reduction in population was recorded after 45 days (6 cfu/g soil) and by 60 days of storage the population was reduced to two cfu/g soil. By about 75 days the population of the pathogen was reduced to zero. The study indicated that the pathogen could survive in soil for more than 60 days but not upto 75 days under glass house conditions.

Table 6 Survival of *Xanthomonas axonopodis* pv. *dieffenbachiae* in different sources

Source	Colony count (cfu / g)* of *Xanthomonas axonopodis* pv. *dieffenbachiae* at				
	15 days	30 days	45 days	60 days	75 days
Infected plant debris in soil under glass house condition	48	25	18	0	0
Infected soil	287	30	6	2	0
Infected plant debris under refrigerated condition	43	15	5	0	0

cfu = colony forming unit
* Average of three replications

Fig. 3 Survival of *Xanthomonas axonopodis* pv. *dieffenbachiae* in different sources



4.7.3 Survival under refrigerated conditions

The infected plant samples were kept in the refrigerator and isolations were carried out at 15 days interval by serial dilution. The results of the study are presented in Table 6 and Fig. 3. The colony count was 43 cfu/g at 15 days after storage. The reduction in population of the pathogen was three times, each at 30^{th} and 45^{th} day of storage, i.e., 15 and five cfu/g respectively. The colony count decreased to zero after sixty days of storage of plant sample under refrigerated condition indicated that the pathogen could survive in plant sample under refrigerated conditions for more than 45 days but not upto 60 days.

4.8 Management

4.8.1 *In vitro* evaluation of different bactericides against *Xanthomonas axonopodis* pv. *dieffenbachiae*

4.8.1.1 *In vitro* sensitivity of the bacterium to antibiotics and a fungicide

Five different antibiotics and a fungicide were evaluated for their effectiveness in inhibiting the growth of the pathogen *Xanthomonas axonopodis* pv. *dieffenbachiae* under *in vitro* conditions. The results are presented in Table 7.

Among the five different antibiotics and a fungicide tested, the treatments captan 0.3 per cent and streptocycline 100 ppm were found to be superior in inhibiting the growth of *Xanthomonas axonopodis* pv. *dieffenbachiae*. The growth inhibition zone recorded for these two treatments (captan 0.3 per cent and streptocycline 100 ppm) were 1.45 and 1.38 cm respectively, which were statistically on par. These were followed by

Table 7 ***In vitro*** **sensitivity of** ***Xanthomonas axonopodis*** **pv.** ***dieffenbachiae*** **to different levels of antibiotics and a fungicide**

Sl. No.	Antibiotics	Inhibition zone in cm *		
		25 ppm	50 ppm	100 ppm
1.	Oxytetracycline	1.23	1.23	1.33
2.	Penicillin	1.07	1.17	1.17
3.	Streptomycin	1.13	1.17	1.23
4.	Streptocycline	1.32	1.33	1.38
5.	Chloramphenicol	1.10	1.07	1.27
6.	Captan	Levels tested (per cent)		
		0.1	0.2	0.3
		1.00	1.20	1.45
7.	Control	0	0	0

* Mean of three replications
CD (0.05 level) = 0.10

streptocycline 50 ppm, oxytetracycline 100 ppm, streptocycline 25 ppm, chloramphenicol 100 ppm, streptomycin 100 ppm, oxytetracycline 25 ppm and oxytetracycline 50 ppm, where zone of inhibition were 1.33, 1.33, 1.32, 1.27, 1. 23, 1.23 and 1.23 respectively. All these treatments were statistically on par and significantly inferior to the most effective level of the antibiotic (streptocycline 100 ppm) and the fungicide (captan 0.3 per cent). The growth inhibition zone produced by captan 0.2 per cent, streptomycin 50 ppm, penicillin 100 and 50 ppm, streptomycin 25 ppm and chloramphenicol 25 ppm were 1.2, 1.17, 1.17, 1.17, 1.13 and 1.1 respectively and were inferior to all the above treatments. Among the antibiotics tested the lowest inhibitory effect against the bacterium was recorded by penicillin 25 ppm and among three different levels of fungicide the least effective was captan 0.1 per cent. Zone produced by streptomycin was found to decrease as the bacteria developed resistance and later grew in the zone.

4.8.1.2 *In vitro* sensitivity of the bacterium to ecofriendly materials

Some ecofriendly materials such as turmeric powder + sodium bicarbonate, neem oil, *Pseudomonas fluorescens* (proprietary product) and cowdung extract were tested against *Xanthomonas axonopodis* pv. *dieffenbachiae* under *in vitro* conditions. The results of the study are presented in the Table 8.

Statistical analysis was not possible on the data obtained under *in vitro* evaluation since there was no significant difference in growth inhibition zone produced by the three levels of both neem oil and turmeric powder + sodium bicarbonate (Table 8).

Table 8 *In vitro* sensitivity of *Xanthomonas axonopodis* pv. *dieffenbachiae* to ecofriendly materials

Sl. No.	Treatments	Growth* inhibition zone (cm)		
1.	Turmeric powder + sodium bicarbonate (10 : 1)	Levels tested (per cent)		
		0.05	0.10	0.15
		1.1	1.2	1.2
2.	Neem oil	Levels tested (per cent)		
		1.4	1.7	2.0
		1.00	1.00	1.10
3.	*Pseudomonas fluorsecens* (Proprietary product)	Levels tested (per cent)		
		0.5	1.0	1.5
		0	0	0
4.	Cowdung extract	Levels tested (per cent)		
		2.5	5.0	7.5
		0	0	0

* Mean of three replications

No growth inhibition zone was produced by *Pseudomonas fluorescens* (proprietary product) and cowdung extract at the three different levels tested.

So three levels of all the four treatments, viz., turmeric powder + sodium bicarbonate, neem oil, *Pseudomonas fluorescens* (proprietary product) and cowdung extract were further evaluated under field conditions.

4.8.2 Control of bacterial blight of anthurium under *in vivo* conditions

Anthurium andreanum var. *Cancan* highly susceptible to bacterial blight was used for the study. Seven-month-old tissue cultured plants were artificially inoculated with the bacterial blight pathogen *Xanthomonas axonopodis* pv. *dieffenbachiae*. When the symptoms were initiated seven days after inoculation, the following treatments were given by spraying at one week interval for five times. Observations on per cent infection of the disease were recorded before each spraying and one week after fifth spraying based on the score chart developed for the purpose as described in the materials and methods.

The per cent infection of bacterial blight on anthurium plants sprayed with chemicals and ecofriendly materials under glass house conditions and the per cent reduction of disease over control are presented in Table 9.

4.8.2.1 Effect of one round spraying against bacterial blight of anthurium

The initial scoring on per cent disease infection of bacterial blight on anthurium plants on the day of spraying and one week after spraying are presented in Table 9. All the plants had bacterial blight infection ranging from 4.33 to 5.00 per cent before the start of spraying. There was no

Table 9 Effect of spraying on bacterial blight of anthurium under *in vivo* condition

Sl. No.	Treatments	Per cent infection of bacterial blight after									
		One spray	Per cent reduction over control	Two spray	Per cent reduction over control	Three spray	Per cent reduction over control	Four spray	Per cent reduction over control	Five spray	Per cent reduction over control
1.	Streptocycline 100 ppm	12.92	47.86	10.52	18.52	8.13	66.56	1.91	95.20	0.00	100
2.	Captan 0.3 per cent	12.92	47.86	12.92	0.00	10.52	56.69	3.83	90.40	1.91	96.67
3.	*Pseudomonas fluorescens* (proprietary product) 0.5 per cent	10.52	0.00	17.46	35.19	19.62	19.24	39.98	0.00	64.31	12.42
4.	*Pseudomonas fluorescens* (proprietary product) 1.0 per cent	10.52	0.00	16.20	25.44	18.30	32.75	21.90	45.20	30.00	47.70
5.	*Pseudomonas fluorescens* (proprietary product) 1.5 per cent	12.92	47.86	16.59	28.45	14.75	39.28	9.97	75.00	1.91	96.67
6.	Neem oil 1.4 per cent	10.52	0.00	17.46	35.19	28.84	18.71	54.98	37.50	64.98	13.26
7.	Neem oil 1.7 per cent	12.92	47.86	16.20	25.45	12.92	46.84	1.91	95.20	4.31	92.49
8.	Neem oil 2.0 per cent	12.92	47.86	20.75	60.65	10.52	56.60	1.91	95.20	1.91	96.67

Table 9 contd...

Sl. No.	Treatments	Per cent infection of bacterial blight after									
		One spray	Per cent reduction over control	Two spray	Per cent reduction over control	Three spray	Per cent reduction over control	Four spray	Per cent reduction over control	Five spray	Per cent reduction over control
9.	Turmeric powder + sodium bicarbonate 0.05 per cent	10.52	0.00	17.46	35.19	25.24	3.89	34.99	12.50	12.92	77.49
10.	Turmeric powder + sodium bicarbonate 0.10 per cent	12.92	47.86	14.75	14.22	10.52	56.69	4.94	87.60	1.35	97.65
11.	Turmeric powder + sodium bicarbonate 0.15 per cent	12.92	47.86	12.92	0.00	6.64	72.68	1.91	95.20	0.00	100.00
12.	Cowdung extract 2.5 per cent	12.92	47.86	24.72	91.41	27.26	12.20	47.89	19.77	58.27	1.60
13.	Cowdung extract 5.0 per cent	12.92	47.86	25.07	94.10	25.07	3.21	25.01	37.45	23.28	59.42
14.	Cowdung extract 7.5 per cent	12.92	47.86	26.55	105.40	25.09	3.30	19.31	51.69	14.09	75.44
15.	Control	10.52		12.92		24.30		39.98		57.38	
CD for treatments (0.05 level)		3.99		8.36		11.94		22.44		31.79	

significant reduction in disease between the sprayed and unsprayed control plants one week after first round of spraying. This showed that one application of none of the treatments was effective to control the disease.

4.8.2.2 Effect of two rounds of spraying against bacterial blight of anthurium

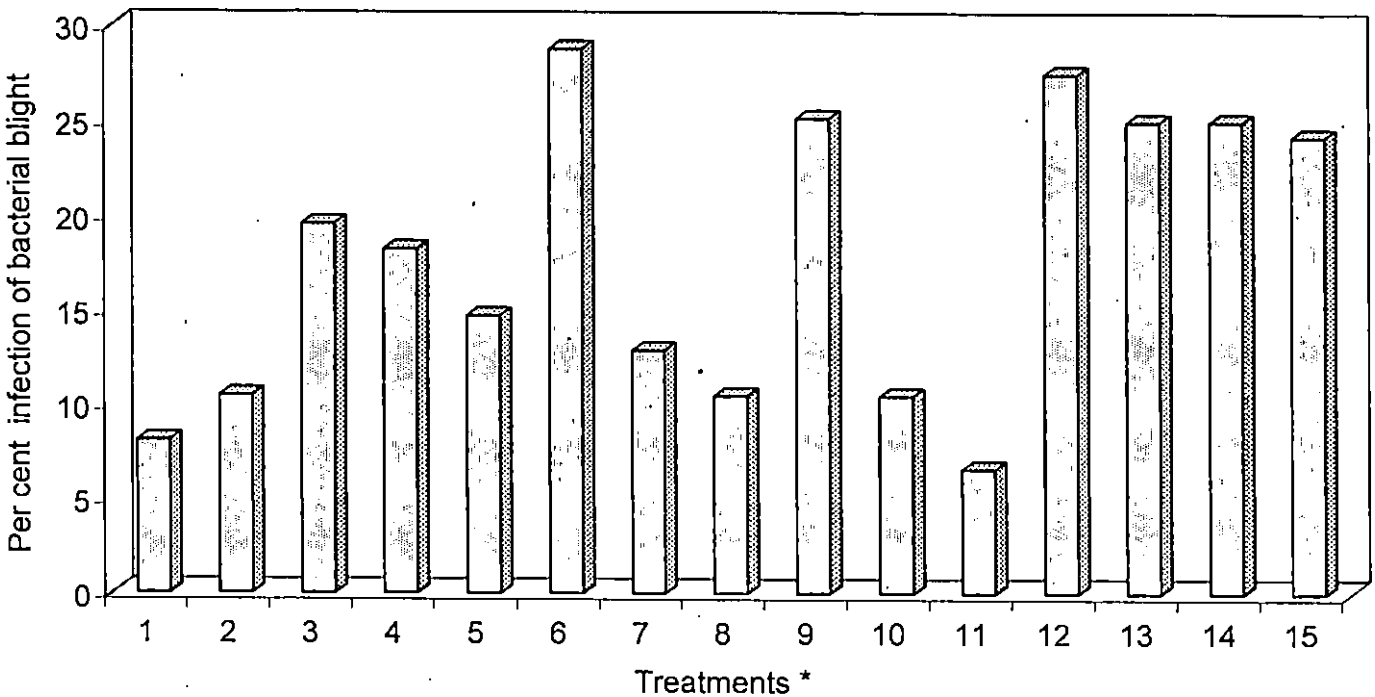
The per cent infection of bacterial blight was scored one week after second spraying. As evident from Table 9 two applications of various treatments were not enough to bring the disease under control as per cent infection in all the treatments including unsprayed control plants were statistically on par.

4.8.2.3 Effect of three rounds of spraying against bacterial blight of anthurium

The per cent infection of bacterial blight was scored one week after third spraying.

The data (Table 9 and Fig. 4) showed that the percentage of disease infection after three application of turmeric powder + sodium bicarbonate 0.15 per cent, streptocycline 100 ppm, turmeric powder + sodium bicarbonate 0.1 per cent, neem oil 2.0 per cent, captan 0.3 per cent, neem oil 1.7 per cent, *Pseudomonas fluorescens* (proprietary product) 1.5 per cent and 1 per cent were 6.64, 8.13, 10.52, 12.92, 10.52, 10.52, 14.75 and 18.30 respectively and were significantly superior in effectiveness to control the bacterial blight disease than the application of rest of the treatments like *Pseudomonas fluorescens* (proprietary product) 0.5 per cent, cowdung 7.5 per cent, cowdung 5.0 per cent, turmeric powder + sodium bicarbonate 0.05 per cent, cowdung 2.5 per cent, neem oil 1.4 per cent including the unsprayed control where the

Fig. 4 Effect of three rounds of spraying on bacterial blight of anthurium



*
1. Streptocycline 100 ppm
2. Captan 0.3 per cent
3. Pseudomonas fluorescens (proprietary product) 0.5 per cent
4. Pseudomonas fluorescens (proprietary product) 1.0 per cent
5. Pseudomonas fluorescens (proprietary product) 1.5 per cent
6. Neem oil 1.4 per cent
7. Neem oil 1.7 per cent
8. Neem oil 2.0 per cent
9. Turmeric in sodium bicarbonate 0.05 per cent
10. Turmeric in sodium bicarbonate 0.10 per cent
11. Turmeric in sodium bicarbonate 0.15 per cent
12. Cow dung extract 2.5 per cent
13. Cow dung extract 5.0 per cent
14. Cow dung extract 7.5 per cent
15. Control

percentage of disease were 19.62 per cent, 25.09 per cent, 25.07 per cent, 25.24 per cent, 27.26 per cent, 28.84 per cent and 24.30 respectively.

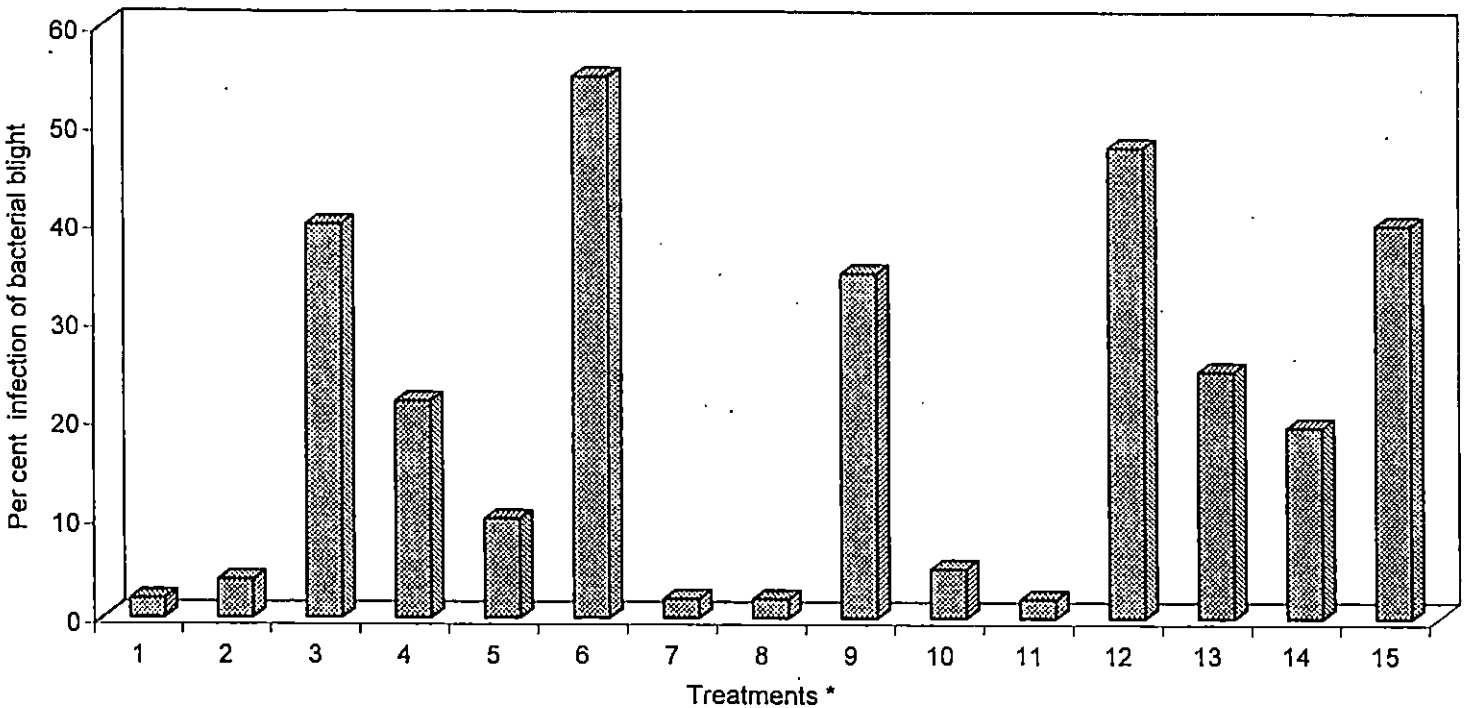
As evident from the results the lowest infection of 6.64 was recorded by plants receiving three application of turmeric powder + sodium bicarbonate 0.15 per cent, whereas unsprayed control plants developed 24.30 per cent disease (Fig. 4).

The per cent reduction of bacterial blight disease over control were worked out and presented in Table 9. The extent of disease reduction (72.68) was maximum in the case of three spraying of turmeric powder + sodium bicarbonate 0.15 per cent followed by streptocycline 100 ppm, turmeric powder + sodium bicarbonate 0.1 per cent, neem oil 2.0 per cent, captan 0.3 per cent, neem oil 1.7 per cent, *Pseudomonas fluorescens* (proprietary product) 1.5 and 1.0 per cent where the disease reduction over control were 66.56, 56.69, 46.84, 56.69, 56.60, 39.28, and 32.75 respectively. These treatments were statistically superior over control in their effectiveness to control bacterial blight of anthurium.

4.8.2.4 Effect of four rounds of spraying against bacterial blight of anthurium

The per cent infection of bacterial blight was scored one week after fourth spraying. The data (Table 9 and Fig. 5) showed that four applications of neem oil 2.0 per cent, neem oil 1.7 per cent, streptocycline 100 ppm, turmeric powder + sodium bicarbonate 0.15 per cent, captan 0.3 per cent, turmeric powder + sodium bicarbonate 0.1 per cent, *Pseudomonas fluorescens* (proprietary product) 1.5 per cent, cowdung extract 7.5 per cent, *Pseudomonas fluorescens* (proprietary product) 1.0 per cent were significantly superior in

Fig. 5 Effect of four rounds of spraying on bacterial blight of anthurium



*

1. Streptocycline 100 ppm
2. Captan 0.3 per cent
3. Pseudomonas fluorescens (proprietary product) 0.5 per cent
4. Pseudomonas fluorescens (proprietary product) 1.0 per cent
5. Pseudomonas fluorescens (proprietary product) 1.5 per cent
6. Neem oil 1.4 per cent
7. Neem oil 1.7 per cent
8. Neem oil 2.0 per cent
9. Turmeric in sodium bicarbonate 0.05 per cent
10. Turmeric in sodium bicarbonate 0.10 per cent
11. Turmeric in sodium bicarbonate 0.15 per cent
12. Cow dung extract 2.5 per cent
13. Cow dung extract 5.0 per cent
14. Cow dung extract 7.5 per cent
15. Control

effectiveness to control the bacterial blight disease than the application of cowdung 5 per cent, turmeric powder + sodium bicarbonate 0.05 per cent, *Pseudomonas fluorescens* (proprietary product) 0.5 per cent, cowdung extract 2.5 per cent and neem oil 1.4 per cent including unsprayed control.

The lowest infection of 1.91 was recorded by plants receiving four spraying of streptocycline 100 ppm, neem oil 1.7 per cent, neem oil 2.0 per cent, turmeric powder + sodium bicarbonate 0.15 per cent whereas the unsprayed control plants recorded 39.98 per cent infection. The infection recorded by captan 0.3 per cent, turmeric powder + sodium bicarbonate 0.1 per cent, *Pseudomonas fluorescens* (proprietary product) 1.5 per cent, cowdung extract 7.5 per cent and *Pseudomonas fluorescens* (proprietary product) 1.0 per cent were 3.83, 4.94, 9.97, 19.31 and 21.90 respectively which were also found considerably effective against the disease (Fig. 5).

The extent of disease reduction (95.20) was maximum in the case of spraying neem oil 2.0 and 1.7 per cent, streptocycline 100 ppm and turmeric powder + sodium bicarbonate 0.15 per cent. This was followed by captan 0.3 per cent, turmeric powder + sodium bicarbonate 0.1 per cent, *Pseudomonas fluorescens* (proprietary product) 1.5 per cent, cowdung 7.5 per cent and *Pseudomonas fluorescens* (proprietary product) 1.0 per cent where the disease reduction over control were 90.4, 87.6, 75.0, 51.69 and 45.2 respectively.

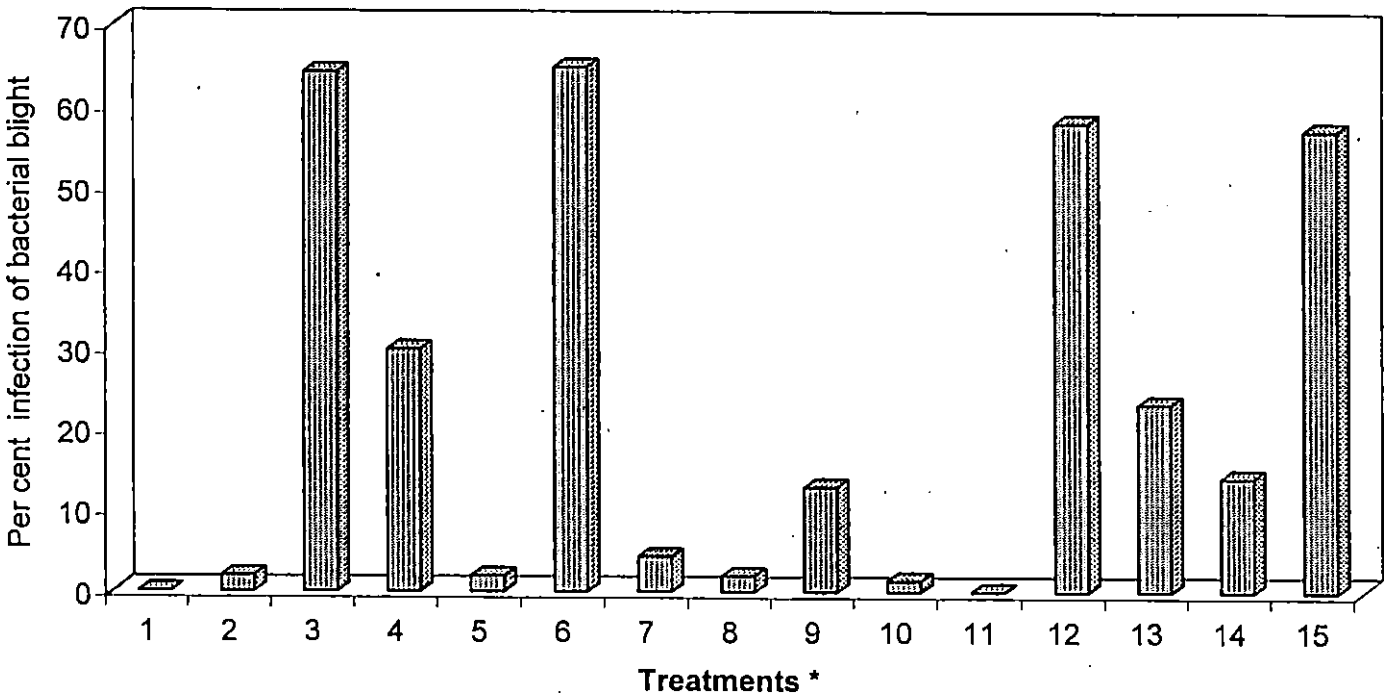
4.8.2.5 Effect of five rounds of spraying against bacterial blight of anthurium

The per cent infection of bacterial blight was scored one week after fifth spraying.

The data (Table 9 and Fig. 6) showed that five application of turmeric powder + sodium bicarbonate 1.5 per cent, streptocycline 100 ppm, turmeric powder + sodium bicarbonate 1.0 per cent, *Pseudomonas fluorescens* (proprietary product) 1.5 per cent, neem oil 2.0 per cent, captan 0.3 per cent, neem oil 1.7 per cent, turmeric powder + sodium bicarbonate 0.05 per cent, cowdung 7.5 and 5.0 per cent and *Pseudomonas fluorescens* (proprietary product) 1.0 per cent were significantly superior in effectiveness to control the bacterial blight where the per cent disease recorded was nil in the case of turmeric powder + sodium bicarbonate (0.15 per cent) application and streptocycline 100 ppm and 1.35 per cent disease development in the case of turmeric powder + sodium bicarbonate 0.1 per cent and 1.91 per cent infection in the case of *Pseudomonas fluorescens* (proprietary product) 1.5 per cent, neem oil 2.0 per cent and captan 0.3 per cent. Turmeric powder impregnated in sodium bicarbonate 0.05 per cent, cowdung 7.5 per cent and 5.0 per cent, *Pseudomonas fluorescens* (proprietary product) 1.0 per cent though effective recorded more than ten per cent disease after five rounds of spraying. The application of cowdung 2.5 per cent, *Pseudomonas fluorescens* (proprietary product) 0.5 per cent and neem oil 1.4 per cent were not effective in management of the disease.

The absence of disease recorded by plants receiving five rounds of spraying with turmeric powder + sodium bicarbonate 0.15 per cent (Plate 8) and streptocycline 100 ppm (Plate 9) could be considered as the best treatment to control the bacterial blight of anthurium in the present study. Treatments like turmeric powder + sodium bicarbonate 0.1 per cent, *Pseudomonas fluorescens* (proprietary product) 1.5 per cent, neem oil 2.0 per cent, captan

Fig. 6 Effect of five rounds of spraying on bacterial blight of anthurium



*

1. Streptocycline 100 ppm
2. Captan 0.3 per cent
3. Pseudomonas fluorescens (proprietary product) 0.5 per cent
4. Pseudomonas fluorescens (proprietary product) 1.0 per cent
5. Pseudomonas fluorescens (proprietary product) 1.5 per cent
6. Neem oil 1.4 per cent
7. Neem oil 1.7 per cent
8. Neem oil 2.0 per cent
9. Turmeric in sodium bicarbonate 0.05 per cent
10. Turmeric in sodium bicarbonate 0.10 per cent
11. Turmeric in sodium bicarbonate 0.15 per cent
12. Cow dung extract 2.5 per cent
13. Cow dung extract 5.0 per cent
14. Cow dung extract 7.5 per cent
15. Control

Plate 8 Effect of five rounds of spraying with turmeric powder + sodium bicarbonate (0.15 per cent)

Plate 9 Effect of five rounds of spraying with streptocycline (100 ppm)

0.3 per cent and neem oil 1.7 per cent, where the disease incidence recorded were below 5.0 per cent could also be considered as good treatments to control bacterial blight disease.

The extent of reduction i.e., 100 per cent reduction (healthy plant) was by spraying turmeric powder + sodium bicarbonate at 0.15 per cent and streptocycline 100 ppm followed by turmeric powder + sodium bicarbonate 0.1 per cent, *Pseudomonas fluorescens* (proprietary product) 1.5 per cent, neem oil 2.0 per cent, captan 0.3 per cent and neem oil 1.7 per cent where the disease reduction over control were 97.65, 96.67, 96.67, 96.67, and 92.49 respectively (Table 9).

As the extent of control obtained was 100 per cent in the case of application of five rounds of spraying with turmeric powder + sodium bicarbonate 0.15 per cent and streptocycline 100 ppm and though the treatments like turmeric powder + sodium bicarbonate 0.1 per cent, *Pseudomonas fluorescens* (proprietary product) 1.5 per cent, neem oil 1.7 per cent and 2.0 per cent and captan 0.3 per cent offered significant disease reduction ranging from 97.65 to 92.49 per cent and the treatments like turmeric powder + sodium bicarbonate 0.05 per cent, cow dung 7.5 and 5.0 per cent and *Pseudomonas fluorescens* (proprietary product) 1.0 per cent offered 77.49 to 47.70 per cent disease reduction over control, we should not go in for those treatments which have not given 100 per cent control due to the systemic nature and possibility of multiplication of inoculum and further development of disease under favourable conditions.

DISCUSSION

5. DISCUSSION

Anthurium (*Anthurium andreanum* Linden) is a recently introduced cut-flower crop in Kerala. But its large-scale cultivation in the state is seriously affected due to the incidence of bacterial blight incited by *Xanthomonas axonopodis* pv. *dieffenbachiae*. This disease was first reported from Brazil in 1960. In India, the occurrence of the same was reported in imported anthurium plants by Sathyanarayana *et al.* (1998). Detailed investigations on the etiology and control of bacterial blight of anthurium are yet to be taken up in Kerala. It is under this circumstance, the present research project was taken up.

The diseased specimens were collected from Vellayani (Mauritius Orange and Kalympong Orange), Balaramapuram (Honeymoon Red), Palappur (Tropical), Kattakada (Cancan) and Palayam (Inspiration) of Thiruvananthapuram district. The specimens from Palayam, Kattakkada and Balaramapuram were systemically infected and the others were of foliar blight affected. Isolations were carried out by the method followed by Hayward (1972). Pure cultures were maintained on PSA medium. In addition to Thiruvananthapuram incidence of the disease was also observed in Kollam, Pathanamthitta and Thrissur districts of Kerala. In all these places heavy economic loss were reported due to the systemic nature of the disease.

Symptomatology of the disease both under natural and artificial conditions were studied. Naturally infected plants showed a variety of symptoms. The initial symptoms of the disease appeared as irregular water soaked lesions on leaf margins and lamina. They increased in size and turned

dark brown surrounded by a prominent yellow halo. When a considerable portion of the lamina got blighted, the leaves were shed. Symptoms were also present on spathe as well as on the spadix. On spathe, instead of an yellow halo, brownish black lesions with wavy fringed margin were formed. Affected spadix finally turned to a black candle. A similar observation was reported by Hayward (1972) with slight variation.

The systemic infection started as water soaked lesion at the collar region of the plant, leading to rotting of that portion. Leaves showed browning and yellowing. The infection spread from collar region through the petiole to the leaf base and to the leaf lamina. In about two to three weeks after the appearance of first symptoms, the whole plant died. The systemic phase of the disease observed during the present investigation has been reported earlier also by Chase (1990).

It was clear from the above study that two types of symptoms were manifested in bacterial blight of anthurium. The leaf infection leading to leaf blight and defoliation and the systemic infection resulting in the death of the whole plant. As in the case of other bacterial diseases like bacterial leaf spot of tapioca incited by *Xanthomonas axonopodis* pv. *manihotis* (Maraite and Meyer, 1975) and black arm of cotton incited by *Xanthomonas axonopodis* pv. *malvacearum* (Rangaswamy, 1985), primary infection and secondary infection may occur in the case of bacterial blight of anthurium. Here the primary infection leading to the systemic phase of the disease and could later spread upwards through the vascular system resulting in the browning and yellowing of the foliar parts and finally the death of the plant. In the case of secondary infection leaf blight symptoms alone were seen. Slowly, the pathogen may

spread downward through the petiole reaching the stem and roots leading to death of the plants. This observation was in confirmity with Guevara and Debrot (1984).

All the general symptoms of naturally infected plants could be produced on artificial inoculation of anthurium plants. Symptoms were initiated seven to ten days after inoculation. The symptoms observed both under natural and inoculated conditions were almost identical.

Pure cultures of all the six isolates of the pathogen were maintained on PSA slants, and their morphological, cultural and physiological characters were studied. All the isolates were aerobic, short, Gram negative and motile rods. Typical colonies on PSA medium were yellow, convex, slimy and circular with entire margin. They produced a water insoluble yellow pigment on Yeast Glucose Chalk Agar medium. These observations were in confirmity with that of Hayward (1972). Isolates Xad-1, Xad-3 and Xad-5 had good growth, while Xad-2 and Xad-4 had moderate growth. Slime production was maximum for the isolates Xad-1, Xad-5 and Xad-6 and moderate for Xad-2, Xad-3 and Xad-4 (Table 2).

Similarly, variations were also observed in the growth of different isolates of the bacterium on Potato Sucrose broth (Table 3 and Fig. 1). Isolates Xad-3 and Xad-4 attained maximum growth by about 24 h and after that, the growth was declining. Isolates Xad-1, Xad-2 and Xad-5 attained maximum growth within a period of 48 h and after that, the growth was found to decline. Among the six isolates studied, growth of isolate Xad-6 was found to be increasing even after a period of 72 h recording a growth curve different from others. In other words, to obtain maximum growth, the isolates Xad-6

required a much longer period of more than 72 h, compared to the remaining five isolates which required only 48 to 72 h. By taking into consideration the growth rate of the isolates 24 h after incubation isolate Xad-4 was faster growing whereas Xad-2, Xad-3 and Xad-6 were moderate in their growth rate and Xad-5 and Xad-1 were slow growing.

The growth of *Xanthomonas axonopodis* pv. *dieffenbachiae* on PSA medium and its broth has not been studied so far by earlier workers. However, similar type of work with *Xanthomonas betlicola* has been done by Abraham (1980). He reported that maximum growth of *Xanthomonas betlicola* was on Potato Sucrose Peptone Agar medium (PSPA) and YGCA medium. Patel *et al.* (1951, 1953) had reported that PDA supported maximum growth of the bacterium *Xanthomonas betlicola*, with poor growth in NA.

Dye (1962) observed that considerable variations could be expected in colonies produced by xanthomonads and in the extent of intra species and inter species variability in physiological characters.

The results of growth studies conducted with *Xanthomonas axonopodis* pv. *dieffenbachiae* in the present investigation indicated that for routine laboratory test and mass culturing of the bacterium, PSA was the best solid medium.

As respect to the physiological and biochemical properties of the bacterium, it utilized glucose oxidatively. In the present study, different isolates showed variable reaction to starch hydrolysis. The isolates of the bacterium liquefied gelatin, produced hydrogen sulphide, ammonia and catalase. The tests for indole, lipolytic activity and methyl red test were negative. Neither urease nor tyrosinase was produced by the bacterium. The

bacterium turned milk alkaline and utilized sodium salts of citric acid and acetic acid but not that of benzoic acid and formic acid.

These tests were not done so far by earlier workers on *Xanthomonas axonopodis* pv. *dieffenbachiae*. But the result of the present study are in agreement with work of Breed *et al.* (1957), Mathew *et al.* (1978) and Mary *et al.* (1986) on *Xanthomonas* sp. Alvarez *et al.* (1990) reported variation in starch hydrolysis by the bacterium *Xanthomonas*. The alkaline reaction of milk by *Xanthomonas* sp. was observed in the present study as against the acidic reaction reported by Breed *et al.* (1957), but our result was in agreement with that reported by Chakravarthi and Rangarajan (1967).

Based on the above observations, the organism causing bacterial blight of anthurium could be identified as *Xanthomonas axonopodis* pv. *dieffenbachiae* (Mc Culloch and Pirone) Vauterin *et al.* (1995). Earlier the name of this bacterium was *Xanthomonas campestris* pv. *dieffenbachiae* (Mc Culloch and Pirone) Dye (1980).

The isolate Xad-6 was more virulent than the other five isolate studied. It produced severe symptom earlier than other isolates and killed the plant (*Anthurium andreanum* var. Cancan) within 15 days from the initiation of disease symptom. Therefore, isolate Xad-6 was selected for further studies.

Growth of the isolate Xad-6 on eight different solid media were studied to find out the best medium suitable for its growth and to study the colony characters and slime production (Table 4). The colonies produced on all media were yellow, convex, slimy, smooth and circular with entire margin. Maximum growth was observed on PSA. Moderate growth was observed in

NA, BX, TTC and PDA. Amount of growth was slight in YGCA, GA and GYA.

In case of slime production, it was maximum on PSA and NA. Slime production was moderate on BX, TTC and YGCA. Slime production was slight in PDA and it was absent in GA and GYA.

Hayward (1972) reported that *Xanthomonas axonopodis* pv. *dieffenbachiae* produced yellow growth in two per cent Peptone Sucrose Agar. There has been no other earlier work on growth and slime production of *Xanthomonas axonopodis* pv. *dieffenbachiae*. Abraham (1980) reported that *Xanthomonas betlicola* produced circular, smooth, glistening and waxy yellow colonies on PSPA. Choi *et al.* (1981) reported that during sub culture on PSA, *Xanthomonas campestris* pv. *oryzae* colony was waxy yellow.

Of the eight carbon compounds tested maltose, galactose, xylose, glucose, sucrose and fructose were utilized by the isolate Xad-6 of bacteria as indicated by acid production (Table 5 and Fig. 2), whereas no acid production was reported with dulcitol and inositol. When the utilization of these carbon sources were tested by inoculating the broth (Table 5) with the isolate Xad-6, maximum utilization was for glucose and least was for dulcitol and inositol. This study was in agreement with the result obtained for Hayward (1972).

Another study carried out was host range of the pathogen. For this plants of Araceae family were artificially inoculated with *Xanthomonas axonopodis* pv. *dieffenbachiae* and observed for symptom development. The following plants, viz., *Dieffenbachia* sp., *Aglaonema robelinii*, *Philodendron oxycardium*, *Colocasia esculenta* and *Syngonium podophyllum* were found infected by the bacterium.

The results of the present study were in agreement with the observations of Ark (1950), Mc Fadden (1962), Hayward (1972), Chase *et al.* (1992) and Phookan *et al.* (1996). This finding is important since this included some of commonly grown foliage ornamentals raised in homestead gardens. Bacterial blight pathogen may perpetuate in these plants and may serve as a source of inoculum for the highly priced anthurium plants.

The extent of survival of the pathogen in infected plant debris, in soil and in infected plant sample stored under refrigerated condition was studied. Periodical isolation yielded the pathogen up to 45 days in plant debris stored in soil under glasshouse conditions and under refrigerated conditions. In infected soil under glasshouse conditions pathogen could be isolated upto 60 days (Table 6 and Fig. 3). This type of work has not been carried out so far by earlier workers on *Xanthomonas axonopodis* pv. *dieffenbachiae*.

Similar works were reported on the ornamentals and crop plants. Degaonkar and Kirtivar (1997) reported the presence of *Xanthomonas begoniae* causing bacterial blight of begonia, in virulent form in naturally dried begonia leaves stored in glass tube at room temperature for 17 months. Brinkerhoff and Pink (1964) reported that viability of *Xanthomonas malvacearum*, depend on the extent of decomposition of debris and the viability being lost after the plant tissues were broken down. Hsieh and Buddenhagen (1975) reported that the pathogen *Xanthomonas oryzae* survived in infected plant debris for a period of 28 days. He also reported that infected plant parts kept under refrigerated conditions could yield the pathogen on isolation for a period of 75 days. Monllor (1986) reported that source of inoculum of pathogen *Xanthomonas sagittifolium* causing bacterial

spot of cocoyam was crop residues in the field. Results of our study also was in agreement with this. Sachin and Miller (1998) again reported that infected plant debris present in the field act as source of inoculum for the pathogen *Xanthomonas axonopodis* pv. *vitians* causing bacterial leaf spot of lettuce. The difference in extent of survival recorded in infected plant debris in soil and in soil alone in the present study may be due to the higher population of pathogen initially present in soil.

The last part of the study was management of disease. In the first part of the management study, five different antibiotics, viz., oxytetracycline, chloramphenicol, penicillin, streptocycline, streptomycin and a fungicide captan were screened for the *in vitro* sensitivity of bacterium *Xanthomonas axonopodis* pv. *dieffenbachiae*.

Among the antibiotics studied streptocycline 100 ppm was found to show maximum growth inhibition zone (Table 7). This was statistically on par with captan 0.3 per cent. All the other antibiotics at different levels and the lower levels of streptocycline and captan were inferior to the above treatments in their growth inhibitory effect.

Inhibitory effect of antibiotic streptocycline, chloramphenicol and penicillin against *Xanthomonas axonopodis* pv. *dieffenbachiae* has not been reported by earlier workers but inhibitory effect of streptocycline against *Xanthomonas campestris* pv. *vesicatoria* (Sharma *et al.*, 1981), against *Xanthomonas oryzae* pv. *oryzae* (Mahto *et al.*, 1988), against *Xanthomonas campestris* pv. *vignicola* (Jindal *et al.*, 1989) and against *Xanthomonas campestris* pv. *betlicola* (Singh, 1996) had been reported earlier.

No previous reports are there about effectiveness of captan under *in vitro* conditions against this pathogen. But the bactericidal activity of captan 0.2 per cent against *Xanthomonas campestris* pv. *vignicola* has been reported by Jindal *et al.* (1989).

Nishijima (1989) reported the inhibitory effect of oxytetracycline at 32 ppm under *in vitro* condition against *Xanthomonas campestris* pv. *dieffenbachiae*. In another report it was said that tetracycline was inactivated in presence of light (Barret and Casells, 1994). Effectiveness of streptomycin against *Xanthomonas axonopodis* pv. *dieffenbachiae* was reported by Sato (1983) and Barret and Casells (1994). Resistance to streptomycin by *Xanthomonas axonopodis* pv. *dieffenbachiae* was reported by Knauss (1972). He found that the pathogen directly isolated from infected leaves on media containing streptomycin yielded vigorously growing colonies at all streptomycin concentration. In our study also we have observed that clear growth inhibition zone formed after 24 h incubation disappeared later, giving the clear evidence of build up of resistance by the bacterium *Xanthomonas axonopodis* pv. *dieffenbachiae* against the antibiotic streptomycin.

The most effective level of antibiotic (streptocycline at 100 ppm) and the fungicide (captan 0.3 per cent) were selected for the *in vivo* management studies.

In the second part of *in vitro* studies, effect of some ecofriendly materials such as neem oil, turmeric powder, cowdung extract and *Pseudomonas fluorescens* (proprietary product) at three different levels were tested against the bacteria *Xanthomonas axonopodis* pv. *dieffenbachiae*. Cowdung extract and *Pseudomonas fluorescens* (proprietary product) tested

against the bacteria failed to produce growth inhibition zone (Table 8). Antagonistic activity of *Pseudomonas fluorescens* and cowdung extract against *Xanthomonas axonopodis* pv. *dieffenbachiae* has not been reported earlier. Sakthivel and Gnanamanickam (1986) reported that *Pseudomonas fluorescens* isolated from rhizosphere were inhibitory to *Xanthomonas oryzae* pv. *oryzae* under *in vitro* conditions. Kalita *et al.* (1996) also reported effectiveness of *Pseudomonas fluorescens* isolated from phylloplane of lemon against *Xanthomonas campestris* pv. *citri*.

Sreekumar and Nair (1990) reported that under *in vitro* conditions cowdung extract failed to produce any typical growth inhibition zone against *Xanthomonas oryzae* pv. *oryzae*. Our observation on the effect of cowdung extract against *Xanthomonas axonopodis* pv. *dieffenbachiae* was on line with this.

In the present investigation, a proprietary product of *Pseudomonas fluorescens* was used for *in vitro* screening as against the pure cultures of *Pseudomonas fluorescens* used by earlier workers. That may be the reason for the failure of development in growth inhibition zone.

There are no earlier works on the effect of neem oil and turmeric powder against *Xanthomonas axonopodis* pv. *dieffenbachiae* under *in vitro* conditions. But under *in vivo* conditions there are reports on the antibacterial property of neem products against *Xanthomonas axonopodis* pv. *malvacearum* (Hulloli *et al.*, 1998).

Under *in vitro* conditions there was no significant difference between the three levels of neem oil and three levels of turmeric impregnated in

sodium bicarbonate tested against the bacterium (Table 8). So all the three levels were carried over to *in vivo* studies.

The final part of the investigation in the management of bacterial blight of anthurium was the *in vivo* studies. The treatments included were a fungicide captan 0.3 per cent, an antibiotic streptocycline at 100 ppm and some ecofriendly materials, viz., turmeric powder + sodium bicarbonate (10 : 1 proportion at 0.05, 0.1 and 0.15 per cent) *Pseudomonas fluorescens* 0.5 and 1.0 and 1.5 per cent, neem oil 1.4, 1.7 and 2.0 per cent and cowdung extract 2.5, 5.0 and 7.5 per cent.

The effect of spraying became visible only after three rounds of sprayings. After four rounds of sprayings (Table 9 and Fig. 4) the extent of disease reduction of 95.2 per cent was maximum in the case of spraying neem oil 1.7 and 2.0 per cent, streptocycline 100 ppm, turmeric powder + sodium bicarbonate 0.15 per cent followed by captan 0.3 per cent, turmeric powder + sodium bicarbonate 0.1 per cent, *Pseudomonas fluorescens* (proprietary product) 1.5 per cent, cowdung extract 7.5 per cent and *Pseudomonas fluorescens* (proprietary product) 1.0 per cent where the disease reduction over control were 90.4, 87.6, 75.0, 51.69 and 45.20 per cent respectively.

Final observations on disease management were made after five rounds of spray schedule. Absolute control of bacterial blight of anthurium (per cent disease infection zero) was obtained by 0.15 per cent turmeric powder + sodium bicarbonate (10 : 1 proportion) and 100 ppm streptocycline (Table 9).

Turmeric powder impregnated in sodium bicarbonate 0.1 per cent, proprietary product of *Pseudomonas fluorescens* 1.5 per cent, neem oil 1.7 and 2.0 per cent, captan 0.3 per cent gave more than 90 per cent control of the

disease. Turmeric powder impregnated in sodium bicarbonate 0.05 per cent, cowdung extract 7.5 and 5.0 per cent and *Pseudomonas fluorescens* (proprietary product) 1.0 per cent gave disease reduction over control within a range of 78 to 48 per cent.

Streptocycline, captan, neem oil, turmeric powder, *Pseudomonas fluorescens* and cowdung extract have not been used by earlier workers for control of bacterial blight of anthurium incited by *Xanthomonas axonopodis* pv. *dieffenbachiae*.

There are reports on the control of other bacterial diseases using these spray materials. Gangopadhyay (1998) reported effectiveness of turmeric powder + sodium bicarbonate as foliar spray @ 1 g per litre against rice diseases. Thind *et al.* (1984) reported the effectiveness of streptocycline 100 ppm against *Xanthomonas campestris* pv. *vignae* infecting mungbean. The effectiveness of streptocycline was also reported by Sharma *et al.* (1996) and Mahto *et al.* (1988), Jindal *et al.* (1989) and Singh (1996). Anuratha and Gnanamanickam (1987) and Kamala (1996) reported the effectiveness of *Pseudomonas fluorescens* to bacterial blight of rice. Effectiveness of *Pseudomonas fluorescens* against citrus canker under field condition was reported by Kalita *et al.* (1996). Cowdung extract was reported to control bacterial blight of rice (Sreekumar and Nair, 1990 and Mary, 1996).

Though the treatments like turmeric powder + sodium bicarbonate 0.1 per cent, *Pseudomonas fluorescens* (proprietary product) 1.5 per cent, neem oil 1.7 and 2.0 per cent and captan 0.3 per cent offered significant disease reduction ranging from 97.65 to 92.49 per cent and the treatments like turmeric powder + sodium bicarbonate 0.05 per cent, cowdung 7.5 and 5.0 per

cent and *Pseudomonas fluorescens* 1.0 per cent offered 77.49 to 47.7 per cent disease reduction over control, we should not go in for those treatments which have not given 100 per cent control due to the systemic nature and possibility of multiplication of inoculum and further development of disease under favourable conditions.

Among the two effective control strategies, it will be economically advantageous to choose the spray schedule with turmeric powder + sodium bicarbonate as it will enable to discontinue the present practices of spraying with antibiotics and fungicides for the control of this disease. Besides being ecofriendly, this technology can also be easily adapted by the farmers.

SUMMARY

6. SUMMARY

Bacterial blight incited by *Xanthomonas axonopodis* pv. *dieffenbachiae* is an important disease of anthurium (*Anthurium andreanum* Linden) in tropical and subtropical regions of the world including India. This disease has become a state wide problem in Kerala causing severe decline in production of cut flowers.

The present research project was taken up with the objective of isolation and characterization of the pathogen, studying symptomatology, host range, survival and management of the disease using both conventional and non-conventional methods.

The pathogen was isolated from infected sample collected from different parts of Thiruvananthapuram district and pathogenicity was proved. In all six isolates of the bacterium, three from foliar blighted plants and three from systemically infected plants, were isolated during this investigation.

The diseased plants showed a variety of symptoms like blighting of leaf lamina, spathe and drying of the spadix. In systemically infected plants, the infection started at the collar region and spread upwards and downwards killing the plant. All the general symptoms of naturally infected plants could be reproduced on artificial inoculation. Symptoms were initiated seven to ten days after inoculation.

The bacterium was a rod shaped, Gram negative and motile. The organism was aerobic, utilizing glucose oxidatively. It utilized both sodium acetate and sodium citrate as sources of carbon. All the isolates produced hydrogen sulphide and indole, liquefied gelatin and gave negative reaction for

MR test and urease test. Milk was turned alkaline and catalase was produced by the bacterium. None of the isolates utilized asparagine as sole source of carbon and nitrogen. All the six isolates produced lipase and ammonia. Arginin hydrolase and tyrosinase activity were negative. However, none of the isolates were tolerant to six per cent sodium chloride.

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

The most virulent isolate (Xad-6) was used for further studies. Among the eight different solid media tested, Potato Sucrose Agar was found to be the best media for growth of this bacterium. Glucose was found to be the best utilized carbon source for the bacterium.

Dieffenbachia sp., *Philodendron oxycardium, Aglaonema robelinii, Colocasia esculenta* and *Syngonium podophyllum* were found to be carriers of pathogen *Xanthomonas axonopodis* pv. *dieffenbachiae*.

The pathogen survived in infected plant debris kept in soil under glass house conditions and in refrigerated conditions for about 45 days and in infected soil for 60 days.

Of the five antibiotics and a fungicide tested against *Xanthomonas axonopodis* pv. *dieffenbachiae* under *in vitro* conditions, the antibiotic streptocycline @ 100 ppm and fungicide captan @ 0.3 per cent were found to be superior to all the other treatments. There was no significant difference

between levels of different ecofriendly materials tested under *in vitro* conditions against the bacterium. Cowdung extract and *Pseudomonas fluorescens* (proprietary product) failed to produce any growth inhibition zone against the pathogen.

Any significant level of disease control under *in vivo* conditions was apparent only from third schedule of spraying. After the fifth round of spraying, absolute control of the disease was obtained with 0.15 per cent turmeric powder + sodium bicarbonate in 10 : 1 proportion and also with 100 ppm streptocycline. The extent of disease control achieved after a schedule of five sprayings at one week interval was above 90 per cent in the case of turmeric powder + sodium bicarbonate 0.1 per cent, *Pseudomonas fluorescens* (proprietary product) 1.5 per cent, neem oil 1.7 and 2.0 per cent and captan 0.3 per cent.

The results obtained during this investigation in controlling the bacterial blight of anthurium (*Anthurium andreanum* Linden), by spraying with 0.15 per cent turmeric powder + sodium bicarbonate is quite significant as it will enable to discontinue the present practices of spraying with antibiotics and fungicides for the control of this disease. Besides being ecofriendly, this technology can also be easily adapted by the farmers.

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ETIOLOGY AND MANAGEMENT OF BACTERIAL BLIGHT OF ANTHURIUM (*Anthurium andreanum* Linden)

BY

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ABSTRACT OF THE THESIS
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ABSTRACT

Bacterial blight incited by *Xanthomonas axonopodis* pv. *dieffenbachiae* (Mc Culloch and Pirone) Vauterin *et al.* (1995) is a serious disease of anthurium (*Anthurium andreanum* Linden) causing heavy economic loss to growers in Kerala.

The diseased plants showed both foliar and systemic infections. The bacterium was a Gram negative, motile rod. Morphological, physiological and biochemical characters of the six isolates were same except for some characters like nature and rate of growth, virulence and hydrolysis of starch. The bacterium inciting this disease was identified as *Xanthomonas axonopodis* pv. *dieffenbachiae* based on morphological, physiological and biochemical characters coupled with pathogenicity.

Potato Sucrose Agar was found to be the best medium for growth and slime production of the bacterium.

Dieffenbachia sp., *Philodendron oxycardium, Aglaonema robelinii, Colocasia esculenta* and *Syngonium podophyllum* were carriers of *Xanthomonas axonopodis* pv. *dieffenbachiae*. The pathogen survived in infected plant debris kept in soil under glass house conditions and in refrigerated conditions for about 45 days and in soil for 60 days.

Under *in vitro* evaluation, 100 ppm streptocycline and 0.3 per cent captan were most effective in inhibiting the growth of the pathogen.

Under *in vivo* conditions, the relative efficiency of four ecofriendly management practices namely the application of turmeric powder + sodium bicarbonate, *Pseudomonas fluorescens* (proprietary product), neem oil and cowdung extract at three different levels were compared with that of spraying with 100 ppm streptocycline and 0.3 per cent captan. It was observed that the use of 0.15 per cent turmeric powder + sodium bicarbonate (10 : 1 proportion) was most effective and the extent of disease control achieved after a schedule of five sprayings at one week interval was same as that of 100 ppm streptocycline.