# STUDIES ON THE BACTERIAL LEAF SPOT OF BETEL VINE INCITED BY *Xanthomonas betlicola* PATEL *et al.*


By

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THESIS

Submitted in partial fulfilment of the
requirement for the degree
MASTER OF SCIENCE IN AGRICULTURE
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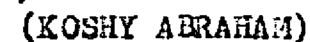
Vellayani - Trivandrum


1980

DECLARATION

I hereby declare that this thesis entitled "Studies on the bacterial leaf spot of betel vine incited by Xanthomonas betlicola Patel et al." 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 to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.



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CERTIFICATE

Certified that this thesis, entitled "Studies on the bacterial leaf spot of betel vine incited by *Xanthomonas betlicola* Patel *et al*." is a record of research work done independently by Shri. KOSHY ABRAHAM, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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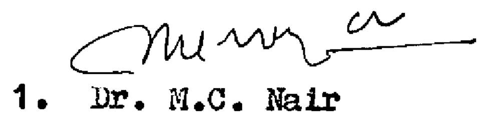

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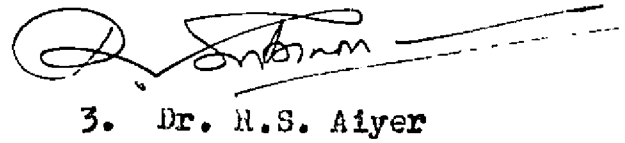

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## ACKNOWLEDGEMENTS

The author wishes to place on record his deep sense of gratitude to,

Dr. James Mathew, Associate Professor of Plant Pathology, for his sincere and invaluable guidance, critical suggestions and constant encouragement throughout the course of this study and in the preparation of the thesis,

Dr. M.C. Nair, Associate Professor of Plant Pathology, Dr. S.K. Nair, Associate Professor of Microbiology and Dr. R.S. Aiyer, Professor of Agricultural Chemistry, Members of the Advisory Committee, for helpful suggestions and criticism during the investigation and in the preparation of the thesis,

Dr. K.I. Wilson (formerly Associate Professor of Plant Pathology), Expert in Plant disease, Division of Plant Pathology, AbuGhraib, Iraq, for his valuable advice, encouragement and help,

Shri. E.J. Thomas, Professor of Agricultural Statistics and Shri. M.P. Abdurazak, Assistant Professor of Agricultural Statistics for the help in the analysis of data,

Kerala Agricultural University for the award of fellowship for the first year and Merit Scholarship for the second year,

and the staff and students of the Department of Plant Pathology for their whole hearted co-operation and assistance.

KOSHY ABRAHAM

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# Introduction

# INTRODUCTION

The cultivation of betel vine is a very profitable proposition as it gives a steady income throughout the year. The leaves of betel vine, commonly known as 'Vettila', find its use among quite a large number of people in Kerala State and in other parts of India as a chewing ingredient.

In Kerala, betelvine is cultivated in an area of about 2592 hectares. The major area under this crop comprising of about 1480 hectares is concentrated mainly in the Districts of Trivandrum, Quilon and Malappuram. As against the products of many cultivated crop plants, it is the leaves that are used in the case of betel vine, and hence, it is but natural that the vegetative yield and quality are the important factors in deciding the value of this crop.

Of the several diseases of betel vine, bacterial leaf spot caused by Xanthomonas betlicola is the most important and serious one in Kerala State. This disease severely affects the leaves and to a lesser extent the stem, thereby reducing both the yield and quality of the leaves considerably. Quite often the disease is seen in serious proportion in the betel vine gardens as a factor limiting production of quality leaves.

The first report on the occurrence of this disease in India was from Bombay State (Patel *et al*., 1951). The occurrence and severity of this disease in Kerala was reported by James Mathew *et al*. (1978a).

This disease is found to be prevalent in the betel vine growing areas of the State throughout the year, the severity being more during rainy seasons. The agro-climatic condition of Kerala with more number of rainy days, high humidity and low temperature, is very conducive for the disease to flare up in severe proportion which results in extensive crop losses. Another alarming observation is that the very same pathogen can infect pepper vine (*Piper nigrum* L. ) (James Mathew *et al*., 1978b, 1979a) an important cash crop of Kerala, which also emphasises the importance of studying the various aspects of this disease in detail.

Thus, in view of the severe crop losses due to this disease on betel vine, and the potential damage and havoc that it can cause to pepper vine, studies were undertaken on the symptomatology, variations in symptom on inoculation of different isolates of the pathogen, characterization and identification of the bacterium, host range and survival of the pathogen, screening of betel vine cultivars for host resistance and both *in vitro* and *in vivo* control of the pathogen with antibiotics. The results are presented in this thesis.

# Review of Literature

REVIEW OF LITERATURE

Bacterial leaf spot disease of betel vine (*Piper betle* L.) is known to have been in existence for over a century in North India. In Ceylon the occurrence of a bacterial disease was reported by Raghunathan (1926, 1928). Asthana and Mahmud (1944, 1945) and Asthana (1947) described bacterial leaf spot disease on *Piper betle* L. from North India. Patel *et al*. (1951, 1953) reported the disease from several parts of Bombay State. From Jabalpur (Madhya Pradesh) bacterial leaf spot on betel vine was reported by Singh and Chand (1971 a). James Mathew *et al*. (1978 a) reported the occurrence of bacterial leaf spot disease of betel vine from Kerala.

Considerable damage was first noted in Ceylon since 1921, though the disease was recorded in 1896 (Raghunathan, 1926). Heavy annual losses has been recorded in the Central Province of Berar, N. India in 1944 (Asthana and Mahmud, 1944). Considerable yield losses has also been reported from several parts of Bombay State (Patel *et al*., 1953), from Jabalpur (Singh and Chand, 1971 a) and from Kerala (James Mathew *et al*., 1978 a).

In India bacterial leaf spot of betel vine caused by *Xanthomonas betlicola*, Patel *et al*. was first reported from Bombay State by Patel *et al*. (1951). Subsequently

the disease was reported from Jabalpur (Singh and Chand, 1971a) and from Kerala (James Mathew et al., 1978 a).

Singh and Chand (1971 b) reported a fungus-bacterium complex disease due to *Colletotrichum capsici* and *Xanthomonas betlicola* on pan (*Piper betle* L.) from Jabalpur.

SYMPTOMATOLOGY

Patel et al. (1953) described the symptoms of the disease in detail. The symptoms of the disease first appeared as minute water soaked spot on the lower side of the leaves between the veins. After about 10 days, the spots became visible on the upper surface as dark, round to angular areas, surrounded by yellow zone or halo. The yellow zone on the upper surface corresponds to the water soaked area on the lower surface. The spots which in the beginning measure 5 mm or less, when numerous, coalesce forming brown to black areas of half to one inch in diameter. Sometimes, the dead portions of the infected leaves fall out leaving holes. The lesions when marginal often results in deformities and cracking of tissues. Heavily infected leaves were distinctly yellow. Under humid conditions there occurred an appreciable amount of bacterial gummy ooze. Infection sometimes occurred on petiole and stem though leaf and leaf edges form common place of infection. Defoliation occurred when infection became severe.

Singh and Chand (1971 a) from Jabalpur reported the symptoms of the disease. The symptom in the beginning appeared in the form of small speck, enlarged and formed almost circular dark brown spot surrounded by yellow lining. Often they coalesced to form patches invading larger areas of the leaves. In the advanced stages the leaves surface were almost entirely covered with bigger spots and patches.

James Mathew _et al_. (1978 a) had observed these typical symptoms as described by Patel _et al_. (1953). They observed that in advance stages of infection defoliation invariably occurred. They had also noted that symptoms of the disease varied with varieties.

Variations in symptom production

There were no relevant studies on the variations in symptom production due to different isolates of the bacterial leaf spot pathogen _Xanthomonas betlicola_. But in other diseases certain studies were made on the variation in symptom production due to isolates. Devadath and Padmanabhan (1969) studied the reaction of 20 rice varieties to 9 isolates of _Xanthomonas oryzae_ and observed the differential reaction of the varieties with isolates, especially in the seedling stage, thus establishing the existence of specialization in pathogenicity. Devadath (1970) reported that the virulent isolate 'E' of _X. oryzae_ produced the largest lesion and the less virulent 'F' the shortest on the rice variety Taichung Native-1. Maraite and Meyer (1975)

suggested that strains of different pathogenicity may exist in X. manihotis. Vakili (1977) studied about Xanthomonas strain causing bacterial blight and pustules of edible legumes in Pueto Rico and reported that all Xanthomonas isolates collected from fields of cow pea were pathogenic on both cow pea and bean (Phaseolus vulgaris) as were most bean isolates. A few bean isolates were pathogenic to bean alone. A variable range of pathogenicity was found among isolates from pustules on bean, P. coccineas, P. lunatus and soyabean but no bacterial pustules were found on cow pea. All isolates showed a variable range of pathogenicity.

PATHOGEN

The causal bacterium of the bacterial leaf spot of betel vine (Piper betle L.) was named as Xanthomonas betlicola Patel et al. (1951),(Breed et al., 1957).

Isolation and properties of the bacterium

Patel et al. (1953) isolated the pathogen by pour plate method.

Patel et al. (1951) studied the morphological and cultural characters of the bacterium and reported that the pathogen is a slender rod, single or in pairs, gram negative, motile, capsulated, no spores or involution forms, not acid fast and aerobic. On Potato Dextrose Agar the colonies of the bacterium were baryta yellow with lobate margins and striations at periphery, measuring 11 mm after 7 days.

On nutrient broth good cloudy yellow growth was observed. Copious growth was observed on potato cylinders. They reported that the optimum temperature for growth of the bacterium was 25 - 28°C.

Patel *et al.* (1953) also reported the same morphological and cultural characters which were described in their earlier studies. They also observed that the bacterium is motile by 1 or 2 polar flagella. On Potato Dextrose Agar the average dimensions of the bacteria, varying in age from 1 to 2 weeks, were 1.6 x 0.9 $\mu$. It readily stains with common dyes. The growth of the bacterium in Nutrient Agar plates were poor, flat, glistening and colonies measure 4 mm in 4 days. In Nutrient Dextrose Agar Plates, the colonies were round with entire margins, umbonate, 7 mm in four days, colour Naples yellow, striations starting from the centre. On potato cylinders, the growth of the bacterium was copious but slightly shining, covering the entire surface, colour was yellow, cylinder tunning orange buff. Thermal death point at 51°C.

Breed *et al.* (1957) and James Mathew *et al.* (1978 a) also reported the similar morphological and cultural characters of the bacterium which were described by Patel *et al.* (1951, 1953). James Mathew *et al.* (1978 a) also reported that the temperature range of the bacterium was 26 - 30°C, with a temperature optimum 28°C.

Physiological characters of the bacterium were studied by Patel et al. (1951) and reported that the pathogen liquified gelatin, hydrolysed starch and casein, reduced litmus, produced ammonia and hydrogen sulphide. They noted that the bacterium produced acid but no gas in dextrose, lactose and sucrose but no growth in salicin. The pathogen did not reduced nitrate and no indole was produced. MR & VP test were negative, no growth in synthetic asparagine medium and NaCl tolerant upto 3 per cent. They noted that the bacterium was lypolytic and liquified Loeffer's blood serum.

Patel et al. (1953) observed the same results of their earlier studies. They also noted that the bacterium produced acid but no gas in maltose. Good growth of the bacterium was observed in Uschinsky's solution but slight growth in Fermi's and Cohn's solution. In synthetic asparagine medium slight growth was observed and sodium chloride tolerance upto 2 per cent.

Breed et al. (1957) reported that the bacterium liquified gelatin, in milk litmus reduced, casein digested. Loeffer's blood serum liquified, Indole not produced, $H_2S$ produced, Nitrite not produced from nitrate, Methyl red negative, acetyl methyl cabinol not produced. No growth of the bacterium in synthetic asparagine medium. It produced acid but no gas from glucose, lactose and sucrose and salicin not attacked. Starch hydrolysed and salt

tolerant upto 3 per cent.

James Mathew et al. (1978a, 1978b, 1979a) reported that starch hydrolysis of the pathogen Xanthomonas betlicola was strong and positive, catalase activity positive and Kovac's oxidase test negative. It produced $H_2S$ but did not reduce nitrate to nitrite. The bacterium produced acid in lactose, sucrose, xylose, glucose, fructose and not utilized adonitol, dulcitol, inulin, salicin and inositol.

HOST RANGE

Patel et al. (1953) reported that Xanthomonas betlicola was pathogenic on Piper betle, Piper longum and Piper hookeri. Breed et al. (1957) noted that the pathogen X. betlicola attack members of the family Piperaceae. Buchanan and Gibbons (1974) reported that the host of X. betlicola include Piper betle, Piper longum and Piper hookeri. James Mathew et al. (1978b, 1979a) reported that X. betlicola was pathogenic on Piper nigrum.

SURVIVAL OF THE PATHOGEN

Patel et al. (1953) observed that bacterial leaf spot of betel vine is common throughout the year, but it became serious during the rainy weather (June to September) when there is low temperature and high humidity. The survival of the bacterium Xanthomonas betlicola has not been studied in detail by earlier workers. Brinkerhoff and Fink (1964) studied the survival and infectivity of Xanthomonas malvacearum in cotton plant debris and soil and reported that the

aqueous suspensions of finely ground, overwintered carpels of cotton balls from standing stalks and the soil surface induced bacterial blight symptoms when used to inoculate seedlings of susceptible varieties. They noted that viability of the pathogen depend on the extent of the decomposition of the debris, being lost after the plant tissues were broken down. *X. malvacearum* survived for 8 days in water suspension in both moist and air dried soil at 21 - 30°C and could thus probably be disseminated in dust. Singh (1971) reported that *X. oryzae* did not survive in unsterilized soil for a week or over summer in the field, in manure or in compost, which are therefore unlikely to be the source of infection for the next crop. Cho and Yoo (1977) conducted studies on the disease caused by *Pseudomonas glycine* and *Xanthomonas phaseoli* var. *sojense* on soyabean and observed that *X. phaseoli* var. *sojense* survived for 4 days and *P. glycine* for 30 days in sterile soil. Schuster (1977) reported that *P. glycine* and *X. phaseoli* var. *sojense* but not *P. tabaci* over winter in pathogenic form in the field in Neb. The survival of the pathogen was favoured when plant debris were kept on the soil surface rather than incorporated in the soil. Survival was also favoured when infected material was maintained under dry condition. Pure culture of the bacteria did not survive under dry or moist condition in either sterilized or non-sterilized soil.

VARIETAL SCREENING FOR DISEASE RESISTANCE

Patel et al. (1951) reported that bacterial leaf spot of betel vine was quite common in betel vine gardens of Bombay State. Patel et al. (1953) also observed that all varieties of betel vine namely Kali or Black, Pandhari or White, Velchi or small were equally susceptible to the disease to varying degrees of intensity. Breed et al. (1957) noted that the pathogen was a habitant of Piper betle. Singh and Chand (1971a) reported the wide spread prevalence of the bacterial leaf spot disease in different betel vine gardens on all varieties in Jabalpur. They also observed that Bangla variety of betel vine was most susceptible.

There are no reports of well conducted experiments on the resistance of betel vine varieties and cultivars against the leaf spot pathogen Xanthomonas betlicola. But similar types of works were conducted for other bacterial disease. Klement and Kapeller (1967) in field inoculation test with Xanthomonas vesicatoria on 4 vars and 10 strains of Capsicum annum observed that none of them were resistant to the pathogen, although a few were less susceptible. Strider (1976) noted that all the capsicum cvs tested for resistance to X. vesicatoria were susceptible. Das (1977) observed that of the 286 rice cultivars tested for resistance to bacterial leaf streak disease, none of them were proved to be resistant to X. translucens f. sp. oryzicola but some were moderately so. Sundaresh et al. (1978) noted that

none of the varieties tested for resistance to bacterial pustules diseases of soyabean were completely resistant to *X. phaseoli* var. *sojense* but several showed some resistance.

ANTIBIOTICS SCREENING AGAINST THE PATHOGEN

Thirumalachar *et al.* (1956) tested the *in vitro* effect of Terramycin and Chloromycetin (Chloramphenicol) at 60 $\mu$ gm per ml, sodium penicillin G 50 units and dihydrostreptomycin (20 $\mu$gm) by the cup assay method against 32 spp. of *Xanthomonas* occurring in India. They reported that all the antibiotics except penicillin were more or less effective against every one of the pathogen which included *X. alfalfa* on lucern, *X. betlicola* on *Piper betle*, *X. cajani* on Pigeon pea, *X. campestris* on cabbage, *X. cassiae* on *Cassia tora*, *X. citri* on lime, *X. malvacearum* on cotton, *X. phaseoli* var. *sojense* on soyabean, *X. recinicola* on *Ricinus communis*, *X. vesicatoria* on chilli and *X. vignicola* on cow pea.

James Mathew *et al.* (1979a, 1979b) reported that of the 6 antibiotics tested for the sensitivity to *Xanthomonas betlicola* by the standard filter paper disc method, chloramphenicol at 500 ppm concentration exerted maximum inhibition of the growth. Ampicillin was not at all effective against the bacterium.

There are no adequate earlier work on the control of this disease by antibiotics. Yamamoto and Kusaka (1965)

reported that the 8 strains of X. oryzae varied in susceptibility to streptomycin but showed little difference in reaction to penicillin, cellocidin, chloramphenicol and 7 other antibiotics. Verma et al.(1975) reported that in lab test busan, streptomycin sulphate and streptocycline inhibited growth of X. malvacearum at 2.5 ppm. They also observed that the isolates could not be grouped into well differenciated types on the basis of their relative sensitivity to chemicals.

Arene (1977) studied the effectiveness of Agrimycin-500 for the control of cassava bacterial blight in the field and reported that incidence and severity of the disease was considerably reduced by foliar application at 800 g/100 lit. water.

Dhanvantari et al. (1978) studied the effectiveness of antibiotics for control of bacterial spot of peach in South Western Ontario and noted that field experiment showed that both oxytetracyclin hydrochloride (OTC) and streptomycin sulphate significantly reduced defoliation and leaf infection due to Xanthomonas pruni. Fruit infection was similarly reduced by OTC but data for streptomycin were limited by low incidence of bacterial spot in the dry summer.

# Materials and Methods

## MATERIALS AND METHODS

### ISOLATION AND PATHOGENICITY OF DIFFERENT BACTERIAL ISOLATES

Infected Betelvine leaves were collected from severely disease affected gardens of Trivandrum and Alleppey District of Kerala State. These leaves were subjected to ooze test to find out the presence of bacteria. The diseased leaves with profuse ooze were selected, the infected areas were cut into bits, surface sterilized serially with rectified spirit and in 0.1 per cent mercuric chloride solution. These bits were then washed in three changes of sterilized distilled water. These were then placed in a sterilized glass slide in a drop of sterile distilled water and teased apart in order to get a bacterial suspension. The suspension was streaked on Potato Sucrose Peptone Agar (PSPA) medium to get well isolated colonies of bacterium.

Composition of PSPA medium

| | |
|---|---|
| $KH_2PO_4$ | - 0.2 g |
| $Na_2HPO_4$ | - 0.5 g |
| $Ca(NO_3)_2$ | - 0.5 g |
| $FeSO_4$ | - 0.05 g |
| KCl | - 0.05 g |
| Peptone | - 2.0 g |
| Sucrose | - 20.0 g |

| | |
|---|---|
| Potato | - 300 g |
| Agar agar | - 20.0 g |
| Distilled water | - 1000 ml |
| pH | - 7.0 |

The plates were incubated for 48 h at room temperature. Characteristic single colonies were selected on the basis of their colour, fluidity and slime and purified by repeated streaking on PSPA medium. These were then tested for their pathogenicity. A thick suspension of 24 h old cultures of different isolates were inoculated on betelvine plants by giving pin pricks on leaves and then smearing the culture on both the surface of the leaves by means of a cotton dipped in the bacterial suspension. The inoculated plants were covered with polythene covers and kept under shade. Stock cultures were maintained in Yeast Glucose Chalk Agar (YGCA) medium.

The pathogen was reisolted from artificially inoculated plants by using the above method. Single colonies of such isolates were also compared with that of the original isolates for their typical morphological characteristics. The details of the bacterial isolates used in the study are given below.

| Isolate Number | Locality from where the diseased specimen was collected | Cultivar of betelvine from which isolation was made | Year of isolation |
|---|---|---|---|
| Xb-1 | Vellayani, Trivandrum District | Chelanthivella | 1977 |
| Xb-2 | Kallara, do | Karilanchi-Karpuran | 1977 |
| Xb-3 | Mannamthala do | Pramuttan | 1977 |
| Xb-4 | Mavelikkara, Alleppey District | Pramuttan | 1977 |
| Xb-5 | Aruvikkara, Trivandrum District | Chelanthi Karpuran | 1978 |
| Xb-6 | Vandithadam, do | Pannivella | 1978 |
| Xb-7 | Palappur, do | Malamkodi | 1978 |
| Xb-8 | Muttakadu do | Chelanthi Karpuran | 1978 |
| Xb-9 | Near Vellayani Kayal, Trivandrum District | Tulasivettila | 1978 |
| Xb-10 | Agricultural College Campus, Trivandrum District | Chelanthivella | 1978 |

SYMPTOMATOLOGY

Symptoms of the disease were studied under natural conditions. Detailed studies were also conducted under controlled conditions. Variations in symptoms of the disease on inoculation of the different isolates of the bacterium were studied under artificial conditions. Chelanthivella, a local

betelvine cultivar in Trivandrum district was used for the study. A thick suspension of the 24 - 48 h old culture of the different isolates of the bacterium was inoculated on three months old betelvine plant by smearing the inoculum on both surface of the leaf lamina by a cotton dipped in the suspension after giving pin pricks on leaves. The inoculated plants were kept under shade and sufficient humidity was maintained. Observations on the variation of symptoms of the disease were taken for a period of one month after inoculation.

CHARACTERIZATION AND IDENTIFICATION OF THE PATHOGEN

Characterization and identification of the different isolates of the pathogen were performed according to the methods recommended in the Manual of Microbiological Methods, published by the Society of American Bacteriologists (Anon, 1957) and the methods prescribed by Dye (1962).

A. Cultural characters.

(1) Morphology

The colony morphology was studied from 24 - 48 h old culture of the bacterium grown on PSPA medium. The cells were stained for gram reaction of the organism.

(2) Growth of the bacterium on different solid media

Nature of growth, colour, size, shape, consistency, extent of growth, type of margin, slime production and

fluidity of the bacterial colonies of isolate Xb-1 were studied on eight solid media. Observations were taken after 24 h, 48 h, 72 h and 96 h. The following media were used.

1. Potato Sucrose Peptone Agar (PSPA)
2. Nutrient Agar (NA)
3. Basal medium for Xanthomonads (BX)
4. Tetrazolium chloride medium (without tetrazolium chloride (TZC)
5. Potato Dextrose Agar (PDA)
6. Yeast Glucose Chalk Agar (YGCA)
7. Glucose Agar (GA)
8. Glucose Yeast Extract Agar (GYEA)

Composition of the medium

1. Potato Sucrose Peptone Agar
2. Nutrient Agar

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

3. Basal medium for Xanthomonads

| | | |
|---|---|---|
| $NH_4H_2PO_4$ | - | 0.5 g |
| $K_2HPO_4$ | - | 0.5 g |
| $MgSO_4 \cdot 7H_2O$ | - | 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 Agar

| | | |
|---|---|---|
| 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

| | | |
|---|---|---|
| Dextrose | - | 20.0 g |
| Potato | - | 200.0 g |
| Agar agar | - | 20.0 g |
| Distilled water | - | 1000 ml |
| pH | - | 6.8 |

6. Yeast Glucose Chalk Agar

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

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 |

A loopful of the dilute suspension of the bacterium was streaked on different media in triplicate and incubated at room temperature. Observations were taken at intervals of 24 h, 48 h, 72 h and 96 h.

3. Growth of different isolates of the bacterium on solid medium (PSPA medium)

The isolates were compared for their colony growth, colour, shape, size, type of margin, consistency, extent of growth, slime production and fluidity on PSPA medium. A loopful of the dilute suspension of the isolates of the bacterium was streaked over the medium in petri plates. Three plates were maintained for each isolates and they were incubated at room temperature. Observations were taken at intervals of 24 h, 48 h, 72 h and 96 h.

4. Growth of different isolates of bacterium in liquid medium (PSP broth)

For studying the growth of different isolates of bacterium in liquid medium, Potato Sucrose Peptone broth was used. Forty ml of sterilized broth in 100 ml pyrex conical flask was inoculated with one ml of 24 h old bacterial culture grown in PSPA slant using a sterilized pipette. Uninoculated controls were also maintained. The inoculated medium were shaken daily. Optical density of the broth culture was measured at 24 h intervals for 3 successive days using a spectrophotometer at 510 wave length and uninoculated broth as blank.

5. Pigment production

Production of non-water soluble pigment by different isolates of the bacterium was tested on Yeast Glucose Chalk Agar (YGCA) medium. Forty eight hour old slant culture on the above medium was used for this purpose.

6. Oxygen requirement

Nutrient Agar (containing 0.005% bromocresol purple) columns in tubes were inoculated in duplicate by stabbing with different culture of the bacterium using a straight inoculation needle. The agar surface in one tube was covered with sterile liquid paraffin oil to a depth of one centimeter. The tubes were incubated at room temperature and observations were recorded.

B. Physiological characters.

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

1. Mode of utilization of glucose

To determine whether the isolates of the bacterium utilize glucose only under aerobic condition or both under aerobic and anaerobic condition the following modifications of Hugh and Leifson's (1953) method by Hayward (1964) was followed.

Basal medium

| | | |
|---|---|---|
| Peptone | - | 1.0 g |
| $NH_4H_2PO_4$ | - | 1.0 g |
| KCl | - | 0.2 g |
| $MgSO_4 \cdot 7H_2O$ | - | 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 upto 4 cm and sterilized by tyndallization and inoculated in duplicate by stabbing with a straight inoculation needle charged with bacterial growth. In one of the tube, the medium was sealed with 1 cm

layer of sterilised liquid paraffin. The tubes were incubated at room temperature and observations were taken at regular intervals upto 15 days.

2. Utilization of Organic Acids

Sodium salts of three organic acids, sodium acetate, sodium benzoate and sodium citrate, were used for the study. One per cent of the sodium salt of organic acids was added to the basal medium for Xanthomonads with bromothymol blue as indicator. Different isolates of the bacterium were inoculated in slants in triplicate and incubated at room temperature. Uninoculated controls were also maintained. Observations were recorded at regular intervals.

3. Starch hydrolysis

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

Composition of the medium

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

The isolates of the bacterium were spot inoculated on the medium in plates. After four days of incubation,

hydrolysis was tested by pouring Lugol's Iodine over the plate. A colourless zone around the bacterial growth indicated positive starch hydrolysis, compared to the blue back ground of the medium.

4. Production of Hydrogen sulphide

The ability of the bacterium to liberate 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 dried, autoclaved and again dried. The tubes were inoculated in triplicates with different isolates of the bacterium and the lead acetate strips were inserted aseptically by the side of the plug in the tube. The tubes were incubated at room temperature and observations were recorded at regular intervals upto 14 days. Blackening of test strip indicate liberation of hydrogen sulphide.

5. Methyl Red and Voges - Praskauer tests (MR & VP tests)

Methyl red broth was used for both tests.

Composition of methyl red broth

| | | |
|---|---|---|
| Proteose peptone | - | 5.0 g |
| Glucose | - | 5.0 g |
| $K_2HPO_4$ | - | 5.0 g |
| Distilled water | - | 1000 ml |
| pH | - | 7.0 |

Five ml quantities of the medium was dispensed in tubes and sterilized by steaming for 30 minutes for 3 successive days. Two sets of tubes were inoculated with 48 h old cultures of the isolates of the bacterium for MR & VP tests separately. The tubes were incubated for 7 days at room temperature.

For MR test few drops of 0.02 per cent methyl red in 50 per cent alcohol was added to the culture tubes. A distinct red colour indicated positive methyl red reaction.

For VP test 0.6 ml of alpha-naphthol solution (5 per cent in 95 per cent alcohol) and 0.2 ml of 40 per cent aqueous solution of KOH was added to 1 ml of the culture. The mixture was shaken for few minutes and allowed to stand for 2 h. A crimson or ruby colour indicated positive VP test.

6. Gelatin liquefaction

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

Composition of Nutrient gelatin medium

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

Gelatin was mixed together with the other ingredients by heating over a water bath. The medium was dispensed in test tubes to a depth of 4 cm and sterilized at 10 lbs pressure for 20 minutes. The sterility of the medium was checked by observing it for 2 days. Forty eight hour old culture of the isolates of the bacterium was stab inoculated in the properly sterilized gelatin column. The tubes were incubated and observed for the liquefaction of the gel column at regular intervals upto one month.

7. Production of Indole

Tryptophan broth medium was used for this test.

Composition of the medium

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

The medium was dispersed 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 tubes were inoculated with isolates of 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 the oxalic acid crystals on test strip to pink or red indicates indole production.

8. <u>Nitrate reduction test</u>

For studying the ability of the bacterium to reduce nitrate, nitrate broth medium was used.

<u>Composition of nitrate broth</u>

| | | |
|---|---|---|
| $KNO_3$ (Nitrite free) | - | 1.0 g |
| Peptone | - | 10.0 g |
| Beef extract | - | 5.0 g |
| Distilled water | - | 1000 ml |

The medium was dispensed in tubes, autoclaved and inoculated with different isolates of bacterium, incubated and tested for the reduction of nitrate at regular intervals upto 15 days. The tests was performed by adding few drops of sulphanilic acid (0.8 per cent in one molar acetic acid) and dimethyl alpha-naphthyl amine (0.5 per cent in 5 molar acetic acid), to the nitrate broth culture. If no pink or red colour developed it, indicates that nitrate was present

as such or reduced to ammonia and free nitrogen. Few zinc crystals were added to ensure whether the negative reaction was due to the reduction of nitrate beyond the nitrite level. If the broth become pink or red it indicates that the nitrate was present without reduction.

9. Catalase test

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

10. Action on milk

Action of the bacterium on milk was detected in bromocresol purple milk.

| | | |
|---|---|---|
| Skimmed milk | - | 750 ml |
| Distilled water | - | 250 ml |
| Bromocresol purple | - | 0.02 g |

Milk medium was dispensed in 5 ml quantitis in tubes and sterilized by steaming for 30 minutes for 3 successive days. The medium was inoculated with 48 h old isolates of the bacterium and incubated at room temperature and observed at regular intervals upto 30 days. If the milk changed from blue to yellow, the reaction was acidic and it was alkaline if it changed to violet. Peptonization was indicated by clearing of milk slowly.

11. <u>Utilization of asparagine as sole source of carbon and nitrogen</u>

The test was performed in the following medium (Dye, 1966).

Solution 1 — $K_2HPO_4$ - 3.0 g, $KH_2PO_4$ - 2.0 g, Distilled water - 100 ml

Solution 2 — $MgSO_4.7H_2O$ - 2.0 g, $FeSO_4$ - 0.5 g, NaCl - 1.0 g, $MnSO_4$ - 0.02 g, $H_2SO_4$ - 1 drop, Distilled water - 100 ml

Solution 3 — $Na_2MoO_4$ - 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 960 ml of distilled water and 2 g of L-asparagine. The medium was dispensed in 5 ml quantities in tubes and autoclaved. The tubes were inoculated with different isolates of the bacterium, incubated and examined for growth. Growth of the bacterium in the medium was indicative of the utilization of asparagine.

12. <u>Growth at six per cent sodium chloride</u>

Peptone water with 6 per cent sodium chloride was used for the test.

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

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

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 |
| $CaCl_2.1H_2O$ | - | 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°C. One ml of Tween-80 (Oleic acid ester) was added to the medium and thoroughly mixed. The medium was poured in sterile petridishes and test bacterium was spot inoculated on the medium. The plates were incubated and observed at regular intervals for 15 days. Opaque zone around the bacterial growth was indicative of positive lipase production.

14. Tyrosinase activity

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

Composition of the medium

| | |
|---|---|
| $NH_4H_2PO_4$ | - 0.5 g |
| $K_2HPO_4$ | - 0.5 g |
| $MgSO_4 \cdot 7H_2O$ | - 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 different isolates of the bacterium and incubated. Browning of the medium indicated tyrosinase activity.

15. Arginine hydrolase test

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

Composition of the medium

| | |
|---|---|
| Peptone | - 1.0 g |
| NaCl | - 5.0 g |
| $K_2HPO_4$ | - 0.3 g |
| Agar agar | - 3.0 g |
| Phenol red | - 0.01 g |
| L-arginine | - 1.0 g |
| Distilled water | - 1000 ml |
| pH | - 7.2 |

The medium was dispensed in 5 ml quantities in test tubes and autoclaved. The tubes were stab inoculated with 48 h old culture of isolates of the bacterium and covered with sterile liquid paraffin to a depth of one centimeter. Incubated for 7 days and observed daily. A change of the colour of the medium to red indicated arginine hydrolase activity.

16. Utilization of carbon sources

The following 15 carbon compounds were tested individually for utilization by the isolates of the bacterium as indicated by acid production (Dye, 1962). Galactose, Mannose, Xylose, Inositol, Dulcitol, Amygdalin, Fructose, Ribose, Glucose, Maltose, Lactose, Salicin, Sucrose, Raffinose, Dextrose. The production of acid was observed by using agar slants of the basal medium for Xanthomonads (Dye, 1962). The carbon compound to be tested was added to the medium at one per cent concentration and 0.7 ml of 5 per cent alcoholic solution of bromocresol purple to get a reddish violet colour. The medium was sterilized by tyndallization and the slants were inoculated with isolates of the bacterium in triplicates and incubated at room temperature. Periodic observations were recorded upto 28 days. The change in colour of the medium from reddish violet to yellow indicated the production of acid.

HOST RANGE

Plants with similar taxonomic affinity and the weeds that are commonly present in betelvine gardens were inoculated for studying the host range of the pathogen.

*Piper nigrum* L., *Piper longum* L., *Peperomia pellucida* WB. and K. belonging the family Piperaceae, *Ageratum conyzoides* L., *Eupatorium odoratum* L. belonging the family Compositae and *Brachiaria ramosa* (Griseb) Stapf. belonging the family Graminae were used for the study.

The plants were collected and planted in pots. The plants were inoculated after giving pin pricks on the leaves and then smearing on both the surface of leaves with cotton dipped in a suspension of 24 h old bacterial culture of isolate Xb-1.

SURVIVAL OF THE PATHOGEN

Survival of the pathogen in the infected vines and leaves were studied by periodic isolation using the special media for Xanthomonas (Kado and Heskett, 1970).

Composition of the medium

| | | |
|---|---|---|
| Cellobiose | - | 10.0 g |
| $K_2HPO_4$ | - | 3.0 g |
| $NaH_2PO_4$ | - | 1.0 g |
| $MgSO_4 \cdot 7H_2O$ | - | 0.5 g |
| Agar agar | - | 15.0 g |
| Distilled water | - | 1000 ml |

Infected leaves and vine were collected and placed in pots filled with soil. After ten days, the samples of infected leaves and vines were weighed and homogenized in normal saline. Three 1 ml samples of homogenised material were diluted in normal saline and 0.1 ml samples were spread on the media in petridishes, incubated and observed for the development of typical bacterial colonies. In vitro survival of the pathogen in soil was also studied using special medium for Xanthomonas. Unsterilized fresh soil was mixed with the bacterium as follows. 10 g of soil was added to 1.0 ml of dilute suspension of the bacterium. The soil slurry was diluted with 9.0 ml of normal saline then mixed mechanically for 10 minutes. After 10 days the suspension was diluted 1:10 in saline and 0.1 ml portion was plated on the medium in petridishes, incubated and observed for the development of typical bacterial colonies.

SCREENING OF BETELVINE CULTIVARS FOR HOST RESISTANCE AGAINST THE PATHOGEN

Seven locally recognised cultivars of betelvine were screened for host resistance against the pathogen. They were Chelanthivella ($T_1$), Pramuttan ($T_2$), Malamkodi ($T_3$), Pannivella ($T_4$), Chelanthikarpuran ($T_5$), Tulasivettila ($T_6$) and Karilanchikarpuran ($T_7$). The experiment was laid out in completely randomised design (CRD) with 5 replication.

Two month old plants were inoculated with a mixed culture of all isolates of the bacterium. The technique adopted was to smear a sterile water suspension of the mixed culture of pathogen on both the surfaces of pin pricked leaves. The plants were kept under shade and were well irrigated to ensure high humidity in the micro-environment of the plant. Observations on disease development was taken for a period of 30 days. Grading of the intensity of the disease was carried out on a scale specially prepared. Rate of defoliation was also included as a criterion for evaluating the intensity of the disease.

Preparation of disease scale

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 proportion of the area effected by the leaf spot. The number of spots, marginal infection and shot hole type symptoms were also taken into account for devising the scale. Based on this, the scale has been devised from 0-5 (Fig.1 to 6).

| Disease scale | Percentage of leaf area infected |
|---|---|
| 0 | 0 |
| 1 | 12 |
| 2 | 13 - 25 |
| 3 | 26 - 50 |

DISEASE SCALE OF BACTERIAL LEAF SPOT OF BETELVINE

FIG.1

(0) NO INFECTION

FIG.2

(1.) 12 % LEAF AREA AFFECTED

CONTD.

DISEASE SCALE OF BACTERIAL LEAF SPOT OF BETEL VINE

FIG. 3

(2) 13 % TO 25 % LEAF AREA AFFECTED

FIG. 4

(3) 26 % TO 50 % LEAF AREA AFFECTED

CONTD.

DISEASE SCALE OF BACTERIAL LEAF SPOT OF BETEL VINE

FIG. 5

FIG. 6

(4) 51 % TO 75 % LEAF AREA AFFECTED

(5) > 76 % LEAF AREA INFECTED

| | |
|---|---|
| 4 | 51 - 75 |
| 5 | 76 and above |

SENSITIVITY OF THE BACTERIUM TO ANTIBIOTICS

The *in vitro* sensitivity of the bacterium to different antibiotics were tested. The following antibiotics were used for the study.

| | | |
|---|---|---|
| 1. | Agrimycin-100 | Pfizer (Streptomycin 15% and Terramycin 1.5%) |
| 2. | Ampicillin | Hoechst (Alborcellin 500) |
| 3. | Streptocycline | Hindustan Antibiotics |
| 4. | Terramycin | Pfizer (Oxytetracyclin) |
| 5. | Streptomycin | Sarabai Chemicals (Ambistryn-S, Streptomycin sulphate) |
| 6. | Chloramphenicol | Piya Chemicals (Chloromycetin) |

Solutions of the antibiotics were prepared at concentrations of 100, 250 and 500 ppm. Sterile filter paper disc of 10 mm diameter were dipped in the solutions and placed over PSPA medium seeded with 24 - 48 h old culture of the bacterial isolate (Kb-1). The test was conducted with three replications. Observations on the zone of inhibition were recorded after 24 h and 48 h.

SENSITIVITY OF THE DIFFERENT ISOLATES OF THE BACTERIUM TO TERRAMYCIN AND CHLORAMPHENICOL

The *in vitro* sensitivity of the different isolates of the bacterium to Terramycin and Chloramphenicol were

also studied. 100, 250 and 500 ppm concentrations of the antibiotics were prepared and sterile filter paper discs of 10 mm diameter were dipped in the solutions and placed over PSPA medium seeded with 24 - 48 h old culture of the different isolates of the bacterium. Three replications were maintained and observations on the zone of inhibition were recorded after 24h and 48 h.

SENSITIVITY OF THE MIXTURE OF ALL THE ISOLATES OF THE BACTERIUM TO ANTIBIOTICS

The aforementioned antibiotics with the three earlier mentioned concentrations were used for this study also. The sterile filter paper disc of 10 mm diameter were dipped in the solution of the antibioticsand placed over PSPA medium seeded with a mixture of 24 - 48 h old culture of the ten different isolates of the bacterium. Three replications were maintained and observation of the zone of inhibition were recorded after 24 h and 48 h.

IN VIVO SCREENING OF ANTIBIOTICS AGAINST THE PATHOGEN

In order to assess the efficacy of the antibiotics against the disease and their field performance, an experiment was laid out in completely randomised design (CRD) with 7 treatments and 4 replications. Naturally infected plants were used for spraying the antibiotics. Two concentrations, 250 ppm and 500 ppm of three antibiotics namely

Chloramphenicol, Terramycin and Agrimycin-100 were used for the purpose. Details of the treatments are given below.

Treatments

| | | | |
|---|---|---|---|
| 1. | $T_1$ | Chloramphenicol | - 500 ppm |
| 2. | $T_2$ | Chloramphenicol | - 250 ppm |
| 3. | $T_3$ | Terramycin | - 500 ppm |
| 4. | $T_4$ | Terramycin | - 250 ppm |
| 5. | $T_5$ | Agrimycin-100 | - 500 ppm |
| 6. | $T_6$ | Agrimycin-100 | - 250 ppm |
| 7. | $T_7$ | Control | |

Two spraying were given at an interval of one week. Observations on the disease severity and intensity were recorded on the day of each spraying and 7 days after the second spraying.

STATISTICAL ANALYSIS

Data relating to different experiments were analysed statistically following the method of Snedecor and Cochran (1967). 'F' test was carried out by analysis of variance method and significant results were compared by working out the critical difference. The data on the percentage of leaves defoliated and percentage of survival of leaves were analysed after angular transformation of the data.

# Results

# RESULTS

## Isolation and pathogenicity of bacterial isolates.

Isolation of the bacteria on Potato Sucrose Peptone Agar yielded yellow, circular and slimy colonies with entire margin. The betelvine plants artificially inoculated with the bacterial isolates started developing typical leaf spot symptoms after five days. Reisolation from such infected plants yielded colonies resembling the original isolates of the bacterium.

## Symptomatology.

Initial symptoms of the disease appeared as minute water soaked lesions on the leaf lamina. The water soaked lesions were scattered all over the leaf lamina or seen crowded at certain portions. There was no uniformity in the localization of the appearance of such lesions on the leaf lamina. However at a later stage these lesions enlarged and became angular and delimited by the veins (Plate-I). Sometimes the water soaked lesions were minute and in a crowded manner, without any angularity (Plate II). The water soaking was more pronounced at the lower surface of the leaf lamina compared to the upper surface and slight chlorosis may be seen on the upper surface. Sometimes bacterial exudation could also be observed on the infected lesions. As the lesions grew older, the water soaked spots

Plate I. Angular lesions on betel vine leaves due to *Xanthomonas betlicola*.

turned dark brown in colour with a distinct yellow halo. These lesions later coalesced to form large patches. Invariably margins of the leaves also got infected. Initially on the margins of the leaf small water soaked lesions were formed, later they coalesced to form patches all along the margins with dark brown centre and yellow halo, ultimately resulting in deformities (Plate III). At times as the infection progressed the centre of the dark brown spot broke off leaving a shot hole type symptom on the leaf lamina (Plate IV). When the major portion of the leaf lamina got infected, defoliation occurred. Middle aged and younger leaves were usually more susceptible to infection compared to older leaves in a vine.

Infection on the stem could also be noticed at times. On the stem, small spindle shaped dark brown lesions surrounded by greenish yellow halo developed at first. Later these lesions coalesced to form large patches covering the entire length of the internode of the stem (Plate V). Usually lower and middle portion of the stem were infected, with severe infection on the middle nodes of the stem. With such infection the stem broke off at the middle portions leading to severe damages of the vine. Sometimes petiole infection was also observed as dark brown lesion with greenish yellow halo.

Plate V. Stem infection on betel vine due to *Xanthomonas betlicola*

Plate VI. Typical colonies of *Xanthomonas betlicola* on PSPA medium

Plate III. Marginal lesions on betel vine leaves due to *Xanthomonas betlicola*

Plate IV. Shot hole type of symptom on betel vine leaves due to *Xanthomonas betlicola*.

Variations in symptom on inoculation with different isolates of the bacterium on betelvine cultivar Chelanthivella.

Under conditions of inoculation with different isolates of Xanthomonas betlicola on betelvine cultivar Chelanthivella, the variationsin symptoms were studied. The different isolates of the bacterium showed differences in the type symptoms such as the size of the initial water soaked lesions, angularity, bacterial exudation on the diseased area, yellow halo around the spots, size of the lesions developed, extent of marginal infection, development of shot hole type symptom, susceptibility to the leaves, defoliation and stem and petiole infection. The details are given in table 1.

Characterization and identification of the pathogen.

A. Cultural characters

1. Morphology

The bacterium was a gram negative slender rod with rounded ends. The bacterium gave rise to yellow, circular, slimy, fluidal, and convex colonies with entire margin on PSPA medium (Plate VI).

2. Growth of the bacterial isolate Xb-1 on different solid media

The growth of the bacterial isolate Xb-1 on eight different solid media were tested and the results are presented in table 2. Of the eight solid media tested

maximum growth was observed on Yeast Glucose Chalk Agar as indicated by the diameter of the single colonies. The amount of growth, slime and fluidity was more on YGCA. After 48 h the diameter of the colonies were 1.5 mm. The diameter of the colonies increased to 4 mm after 72 h. The colonies were circular, yellow, slimy, fluidal, and convex with entire margin. The diameter increased to 6 mm after 96 h. A non-water soluble yellow pigment appeared in the medium. Potato Sucrose Peptone Agar medium also supported good growth. The colonies were yellow, slimy, fluidal, circular, and convex with entire margin. The single colonies measured 3 mm after 72 h and 5 mm after 96 h. Growth of the bacterium in Potato Dextrose Agar and Glucose Agar was not abundant with less slime and fluidity. The colonies were yellow in colour. The diameter of the colonies were 1.5 mm and 1.0 mm in PDA and GA respectively after 96 h. In Glucose Yeast Extract Agar, and Tetrazolium Chloride Agar (without tetrazolium chloride) there was absolutely no growth of the bacterium even after 96 h. In the basal medium for Xanthomonads and Nutrient Agar very minute initials of the bacterial growth could be observed after 72 h and no further increase observed even after 96 h. The results of the study indicated that YGCA and PSPA were the best solid media for growth of this isolate of the bacterium.

Table 2. Comparison of growth characters of Xanthomonas betlicola (isolate Xb-1) on different solid media

| Medium | Nature of colony and colour | Growth, slime and fluidity | Diameter in mm after 24 h | 48 h | 72 h | 96 h |
|---|---|---|---|---|---|---|
| PSPA | Yellow, circular, convex with entire margin | Gr. + + +<br>Sl. + + +<br>Fl. + + + | - | 1 | 3 | 5 |
| NA | Very small initials yellow, circular with entire margin | Gr. +<br>Sl. -<br>Fl. - | - | - | - | - |
| BX | Very small initials yellow, circular with entire margin | Gr. +<br>Sl. -<br>Fl. - | - | - | - | - |
| TZ | No growth | | - | - | - | - |
| YGCA | Yellow, circular, convex with entire margin | Gr. + + + +<br>Sl. + + + +<br>Fl. + + + + | - | 1.5 | 4 | 6 |
| PDA | Yellow, circular convex with entire margin | Gr. +<br>Sl. +<br>Fl. + | - | - | - | 1.5 |
| GA | Light yellow, circular, convex with entire margin | Gr. +<br>Sl. +<br>Fl. + | - | - | - | 1 |
| GYEA | No growth | - | - | - | - | - |

Gr. - Growth
Sl. - Slime
Fl. - Fluidity

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

3. Growth of diffeaent isolates of the bacterium on solid medium (PSPA medium)

All the isolates of the bacterium gave rise to yellow, circular, and convex colonies with entire margin on PSPA medium. Isolates XB-1, Xb-7, Xb-9 and Xb-10 recorded maximum growth, slime production and fluidity and the diameter was increased to 5 mm after 96 h (Table 3). Isolate Xb-4 and Xb-6 were similar in terms of their growth, slime production and fluidity and their diameter increased to 4 mm after 96 h. Isolates Xb-2, Xb-3, Xb-5 and Xb-8, the diameter of the colonies increased to 3 mm after 96 h and were similar with respect to their slime production and fluidity. The colonies of the isolate Xb-2, Xb-3, Xb-4, Xb-6, Xb-7, Xb-8 and Xb-10 were evenly yellow in colour with a light yellow region at the margin, where as the colonies of the isolates Xb-1, Xb-5 and Xb-9 were deep yellow at the centre than the margin. There were slight variation in the amount of growth, slime production and fluidity between the isolates of the pathogen. None of the isolates showed growth within 24 h.

4. Growth of different isolates of the bacterium on Potato Sucrose Peptone broth

Growth was measured as the change in the optical density of the medium in comparison with uninoculated control. The results are presented in table 4. Observations after 24 h showed that there was not much growth in any of the

Table 3. Growth characters of different isolates of <u>Xanthomonas betlicola</u> on PSPA medium

| Isolate | Nature of colony and colour | Growth, slime and fluidity | Diameter in mm after 24 h | 48 h | 72 h | 96 h |
|---|---|---|---|---|---|---|
| Xb-1 | Deep yellow at centre, light yellow at margin, circular, convex with entire margin | Gr + + +<br>Sl + + +<br>Fl + + + | - | 1 | 3 | 5 |
| Xb-2 | Evenly yellow, circular, convex with entire margin | Gr + +<br>Sl + +<br>Fl + + | - | - | 2 | 3 |
| Xb-3 | Evenly yellow, circular, convex with entire margin | Gr + +<br>Sl + +<br>Fl + + | - | - | 2 | 3 |
| Xb-4 | Evenly yellow, circular, convex, with entire margin | Gr + + +<br>Sl + +<br>Fl + + | - | - | 2 | 4 |
| Xb-5 | Deep yellow at centre, light yellow at margin, circular, convex with entire margin | Gr + +<br>Sl + +<br>Fl + + | - | - | 1 | 3 |
| Xb-6 | Evenly yellow, circular, convex with entire margin | Gr + + +<br>Sl + +<br>Fl + + | - | 1 | 3 | 4 |
| Xb-7 | Evenly yellow, circular, convex with entire margin | Gr + + +<br>Sl + + +<br>Fl + + + | - | 1 | 2 | 5 |
| Xb-8 | Evenly yellow, circular, convex with entire margin | Gr + +<br>Sl + +<br>Fl + + | - | - | 1 | 3 |
| Xb-9 | Deep yellow at centre, light yellow at margin, circular, convex with entire margin | Gr + + +<br>Sl + + +<br>Fl + + + | - | 2 | 4 | 5 |
| Xb-10 | Evenly yellow, circular, convex with entire margin | Gr + + +<br>Sl + + +<br>Fl + + + | - | 1 | 3 | 5 |

Gr - Growth
Sl - Slime
Fl - Fluidity

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

Table 4. Growth of different isolates of *Xanthomonas betlicola* on PSP broth

| Isolate No. | Optical density after | | | |
|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 96 h |
| Xb-1 | 0.041 | 0.071 | 0.119 | 0.149 |
| Xb-2 | 0.009 | 0.046 | 0.125 | 0.108 |
| Xb-3 | 0.022 | 0.051 | 0.086 | 0.125 |
| Xb-4 | 0.009 | 0.041 | 0.181 | 0.161 |
| Xb-5 | 0.013 | 0.051 | 0.125 | 0.155 |
| Xb-6 | 0.041 | 0.092 | 0.208 | 0.119 |
| Xb-7 | 0.022 | 0.036 | 0.161 | 0.155 |
| Xb-8 | 0.022 | 0.066 | 0.201 | 0.168 |
| Xb-9 | 0.032 | 0.092 | 0.155 | 0.125 |
| Xb-10 | 0.022 | 0.071 | 0.137 | 0.086 |

isolates. Among the isolates Xb-1 and Xb-6 showed maximum growth, followed by Xb-9, Xb-3, Xb-7, Xb-8 and Xb-10. For isolates Xb-2, Xb-4 and Xb-5 the growth was very negligible. After 48 h maximum growth was observed for isolate Xb-6 and Xb-9. For isolate Xb-1 and Xb-10 the growth was same. Among the other isolates more growth was seen with isolate Xb-8, followed by Xb-3, Xb-5, Xb-2, Xb-4 and Xb-7. Observation after 72 h showed maximum growth for isolate Xb-6 and Xb-8, followed by isolates Xb-4, Xb-7, Xb-9, Xb-10,

Xb-2, Xb-5 and Xb-1. Isolate Xb-3 showed very little growth. After 96 h all isolates except Xb-1, Xb-3 and Xb-5 showed less amount of growth compared to 72 h growth.

5. Pigment production

A non-water soluble yellow pigment on Yeast Glucose Chalk Agar medium was produced by all the isolates of the bacterium.

6. Oxygen requirement

All isolates of the bacterium were found to be aerobic since the growth and change of blue colour of the Nutrient dextrose agar medium (containing 0.005% bromocresol purple) to yellow was observed only in case of tubes containing no liquid paraffin indicating the aerobic nature of the organism.

B. PHYSIOLOGICAL CHARACTERS

1. Mode of utilization of glucose.

All isolates of the bacterium were found to utilize glucose oxidatively (aerobically) since the medium in the open tubes turns yellow from the top.

2. Utilization of organic acids.

Of the three sodium salts of organic acids tested, all the isolates utilized sodium citrate and sodium acetate as the source of carbon as evidenced by the change of colour of the slants from green to blue. Sodium benzoate was not utilized as the source of carbon by any of the isolates of the bacterium.

3. Starch hydrolysis.

All the bacterial isolates were found to hydrolyse starch as indicated by colourless zone around the bacterial growth in contrast to the outer blue back ground of the medium.

4. Production of hydrogen sulphide.

Liberation of $H_2S$ within 14 days was observed for all the isolates of bacterium. The isolates Xb-2 and Xb-6 liberated $H_2S$ in less intensity as evidenced by less blackening of the lead acetate strip compared to other isolates.

5. MR and VP Tests

All isolates of the bacterium gave negative MR test as evidenced by the absence of development of distinct red colour in the culture tube when few drops of 0.02 per cent methyl red in 50 per cent alcohol was added.

Similarly the VP test was also negative for all isolates as indicated by the absence of a crimson or ruby colour on addition of 0.6 ml alpha-naphthol (5 per cent in 95 per cent alcohol) and 0.2 ml of 40 per cent aqueous solution of KOH in the culture tube.

6. Gelatin liquefaction

There was liquefaction of gel column in the tubes which were inoculated with isolates of the bacterium within one month, which indicated that all isolates of the bacterium liquified gelatin.

7. Production of Indole.

The oxalic acid crystals on the test strip did not turn pink or red which indicated that none of the isolates produced Indole.

8. Nitrate reduction.

Nitrate was not reduced by any of the bacterial isolates as evidenced by development of pink colour after addition of zinc.

9. Catalase test.

Catalase positive reaction was shown by the different isolates of the bacterium with slight variations in their intensity.

10. Action on milk.

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

11. Utilization of asparagine as sole source of Carbon and and Nitrogen

None of the isolates utilized asparagine as sole source of C and N as indicated by the absence of growth of isolates of the bacterium in the organic salt solution containing 0.2 per cent asparagine.

12. Growth in 6 per cent NaCl.

There was no growth of the isolates of the bacterium when inoculated on a media containing 6 per cent NaCl.

13. Lipolytic activity

Opaque zone around the bacterial growth indicated

lipase production and so all the isolates of the bacterium produced lipases.

14. Tyrosinase activity.

Absence of dark brown pigment in the media inoculated with isolates of bacterium indicated that all isolates were tyrosinase nagative.

15. Arginine hydrolase activity.

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

16. Utilization of carbon sources.

Of the 15 carbon compounds tested all isolates of the bacterium produced acid in Galactose, Mannose, Xylose, Fructose, Glucose, Maltose, Lactose, Sucrose, Dextrose as indicated by the change of colour of the medium from reddish violet to yellow. In Xylose acid production was noticed only from the 4th day of inoculation. In Lactose isolates Xb-1, Xb-3, Xb-4, Xb-5, Xb-7, Xb-8 and Xb-10 showed acid production from the second day of inoculation while others showed acid production on third day of inoculation. There was no change in the colour of the medium from reddish violet to yellow in tubes containing Inositol, Dulcitol, Amygdalin, Ribose, Salicin and Raffinose which indicated negative utilization of the above sugars.(Table.5)

Table 5. Summary of microscopical, biochemical and physiological characters of the isolates of Xanthomonas betlicola

| Sl. No. | Characters studied | Isolates | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Xb-1 | Xb-2 | Xb-3 | Xb-4 | Xb-5 | Xb-6 | Xb-7 | Xb-8 | Xb-9 | Xb-10 |
| 1. | Gram reaction | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |
| 2. | Pigment production | | | | | | | | | | |
| | (a) Non water soluble | + | + | + | + | + | + | + | + | + | + |
| | (b) Water soluble | - | - | - | - | - | - | - | - | - | - |
| 3. | Oxygen requirement | + | + | + | + | + | + | + | + | + | + |
| 4. | Mode of utilization of glucose | | | | | | | | | | |
| | (a) Aerobic | + | + | + | + | + | + | + | + | + | + |
| | (b) Anaerobic | - | - | - | - | - | - | - | - | - | - |
| 5. | Utilization of organic acids | | | | | | | | | | |
| | (a) Sodium citrate | + | + | + | + | + | + | + | + | + | + |
| | (b) Sodium acetate | + | + | + | + | + | + | + | + | + | + |
| | (c) Sodium benzoate | - | - | - | - | - | - | - | - | - | - |
| 6. | Starch hydrolysis | + | + | + | + | + | + | + | + | + | + |
| 7. | Production of hydrogen sulphide | + | + | + | + | + | + | + | + | + | + |
| 8. | MR & VP tests (Methyl red and Voges Praskauer Test) | - | - | - | - | - | - | - | - | - | - |
| 9. | Gelatin liquefaction | + | + | + | + | + | + | + | + | + | + |
| 10. | Production of Indole | - | - | - | - | - | - | - | - | - | - |

Table 5 contd...

| Sl. No. | Characters studied | Isolates | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Xb-1 | Xb-2 | Xb-3 | Xb-4 | Xb-5 | Xb-6 | Xb-7 | Xb-8 | Xb-9 | Xb-10 |
| 11. | Nitrate reduction | - | - | - | - | - | - | - | - | - | - |
| 12. | Catalase test | + | + | + | + | + | + | + | + | + | + |
| 13. | Action on milk (Acid or alkaline | AL | AL | AL | AL | AL | AL | AL | AL | AL | AL |
| 14. | Utilization of asparagine as sole source of C & N | - | - | - | - | - | - | - | - | - | - |
| 15. | Growth in 6% NACl | - | - | - | - | - | - | - | - | - | - |
| 16. | Lipolytic activity | + | + | + | + | + | + | + | + | + | + |
| 17. | Tyrosinase activity | - | - | - | - | - | - | - | - | - | - |
| 18. | Arginine hydrolase test | + | + | + | + | + | + | + | + | + | + |
| 19. | Utilization of carbon compounds with acid production | | | | | | | | | | |
| | 1. Galactose | + | + | + | + | + | + | + | + | + | + |
| | 2. Mannose | + | + | + | + | + | + | + | + | + | + |
| | 3. Xylose | +* | +* | +* | +* | +* | +* | +* | +* | +* | +* |
| | 4. Fructose | + | + | + | + | + | + | + | + | + | + |
| | 5. Glucose | + | + | + | + | + | + | + | + | + | + |
| | 6. Maltose | + | + | + | + | + | + | + | + | + | + |
| | 7. Lactose | + | +* | + | + | + | +* | + | + | +* | + |
| | 8. Sucrose | + | + | + | + | + | + | + | + | + | + |



continued..

Table 5 contd...

| Sl. No. | Characters studied | Isolates | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Xb-1 | Xb-2 | Xb-3 | Xb-4 | Xb-5 | Xb-6 | Xb-7 | Xb-8 | Xb-9 | Xb-10 |
| 9. | Dextrose | + | + | + | + | + | + | + | + | + | + |
| 10. | Inositol | - | - | - | - | - | - | - | - | - | - |
| 11. | Dulcitol | - | - | - | - | - | - | - | - | - | - |
| 12. | Amygdalin | - | - | - | - | - | - | - | - | - | - |
| 13. | Ribose | - | - | - | - | - | - | - | - | - | - |
| 14. | Salicin | - | - | - | - | - | - | - | - | - | - |
| 15. | Raffinose | - | - | - | - | - | - | - | - | - | - |

-ve - Gram negative
AL - Alkaline reaction
+ - Positive reaction
+* - Delayed positive reaction
- - Negative reaction

Host range of the pathogen

Plants with similar taxonomic affinity to betelvine as well as weeds commonly present in betelvine gardens were artificially inoculated with the bacterium for studying the host range of the pathogen. The following plants belonging to the family Piperaceae, *Piper nigrum* L. (Plate VII), *Piper longum* L. and *Peperomia pellucida* WB. & K. were found to develop symptoms of leaf spot disease after 1 - 2 weeks of inoculation. The disease affected portion of these plants were subjected to ooze test and confirmed the presence of bacteria. None of the weeds tested showed any visible symptoms of disease even after three weeks of inoculation. The inoculated portions of these plants were subjected to ooze test which gave negative results.

Survival of the pathogen

The survival of the pathogen in the infected vines and leaves were studied by periodic isolation using special media for Xanthomonas. Isolation of the pathogen after ten days from infected vines and leaves gave negative results similarly *in vitro* survival of the pathogen in soil was also assessed. Plating a dilute suspension of the inoculated soil slurry in normal saline after ten days for selective isolation of the bacterium in the special medium for Xanthomonas yielded some yellow colonies. But these colonies failed to gave any symptoms of the disease on betelvine plants upon inoculation.

Plate VII. Symptoms of bacterial leaf spot on pepper due to *Xanthomonas betlicola*

<u>Screening of betelvine cultivars for host resistance against the pathogen</u>

Of the seven cultivars of betelvine plants screened for host resistance against the pathogen, none of them was found to be resistant to the disease. In all cultivars the symptom development were initiated from 5 to 7 days after inoculation. The results are presented in table 6.

Table 6. Screening of betelvine cultivars for host resistance against *Xanthomonas betlioola*

| Treatments | Percentage of defoliation (in angles) | | | Disease grade of all leaves infected |
|---|---|---|---|---|
| | Within 10 days after inoculation | Within 20 days after inoculation | Within 30 days after inoculation | |
| $T_1$ | 0 | 47.33 | 74.31 | 3.34 |
| $T_2$ | 0 | 43.55 | 57.05 | 2.86 |
| $T_3$ | 0 | 55.94 | 75.00 | 3.14 |
| $T_4$ | 0 | 25.05 | 78.00 | 3.60 |
| $T_5$ | 0 | 40.16 | 61.03 | 3.46 |
| $T_6$ | 0 | 20.75 | 43.84 | 2.10 |
| $T_7$ | 0 | 10.13 | 43.84 | 2.56 |
| C.D.(0.05) | 0 | 25.0074 | 17.9928 | 0.4605 |

It was observed that maximum disease severity occurred on cultivar Pannivella ($T_4$) and minimum on Tulasivettila ($T_6$) when compared to others. There was absolutely no defoliation

within ten days after inoculation. Within a period of 20 days after inoculation minimum defoliation occurred on cultivars Karilanchikarpuran ($T_7$) and Tulasivettila ($T_6$) and maximum on Malamkodi ($T_3$). Observations within 30 days after inoculation showed lesser defoliation in the case of cultivars Karilanchikarpuran ($T_7$) and Tulasivettila ($T_6$) and maximum on Pannivella ($T_4$).

Evaluation by disease severity scale (disease scale) showed that the cultivar Tulasivettila ($T_6$) was significantly better than Malamkodi ($T_3$), Chelanthivella ($T_1$), Chelanthikarpuran ($T_5$) and Pannivella ($T_4$). The cultivar Pannivella ($T_4$) was on par with Chelanthikarpuran ($T_5$) and Chelanthivella ($T_1$) and significantly inferior to others. Statistical analysis on the percentage of defoliation within 20 days after inoculation showed significantly lesser defoliation in the case of cultivar Karilanchikarpuran ($T_7$) than other cultivars except Tulasivettila ($T_6$). The cultivar Tulasivettila ($T_6$) was also found to be on par with Pannivella ($T_4$), Chelanthikarpuran ($T_5$) and Pramuttan ($T_2$). Further the cultivar Malamkodi ($T_3$) was found to be on par with Chelanthivella ($T_1$), Pramuttan ($T_2$) and Chelanthikarpuran ($T_5$) and significantly inferior to other cultivars. Evaluation by the percentage of defoliation within 30 days after inoculation however revealed that the cultivars Karilanchikarpuran ($T_7$) and Tulasivettila ($T_6$) were found to be on par with Pramuttan ($T_2$) and

Chelanthikarpuran ($T_5$) and showed significantly lesser defoliation than other cultivars. Further the cultivar Pannivella ($T_4$) was on par with Malamkodi ($T_3$), Chelanthivella ($T_1$) and Chelanthikarpuran ($T_5$) and significantly inferior to all other cultivars.

In vitro sensitivity of the bacterium to antibiotics

The *in vitro* sensitivity of the bacterium to 6 antibiotics were tested and the results are presented in table 7, Plate VIII and Fig.7. Among the antibiotics tested Chloramphenicol was found to be significantly superior to all other antibiotics, which was closely followed by Terramycin.

Table 7. *In vitro* sensitivity of antibiotics at different concentrations to *Xanthomonas betlicola*

| Antibiotics | Inhibition zone in mm | | | |
|---|---|---|---|---|
| | 100 ppm | 250 ppm | 500 ppm | Mean |
| Agrimycin-100 | 19.33 | 25.33 | 29.00 | 24.55 |
| Ampicillin | 0.00 | 0.00 | 0.00 | 0.00 |
| Chloramphenicol | 28.00 | 30.33 | 36.33 | 31.55 |
| Streptomycin | 18.66 | 22.33 | 30.33 | 23.77 |
| Streptocycline | 19.33 | 24.33 | 27.66 | 23.77 |
| Terramycin | 26.33 | 28.66 | 33.00 | 29.33 |

C.D. (0.05) for comparison between antibiotics = 1.573
C.D. (0.05) for comparison between combination = 3.125

Agrimycin-100, Streptocycline and Streptomycin were on par and significantly inferior to Chloramphenicol and Terramycin.

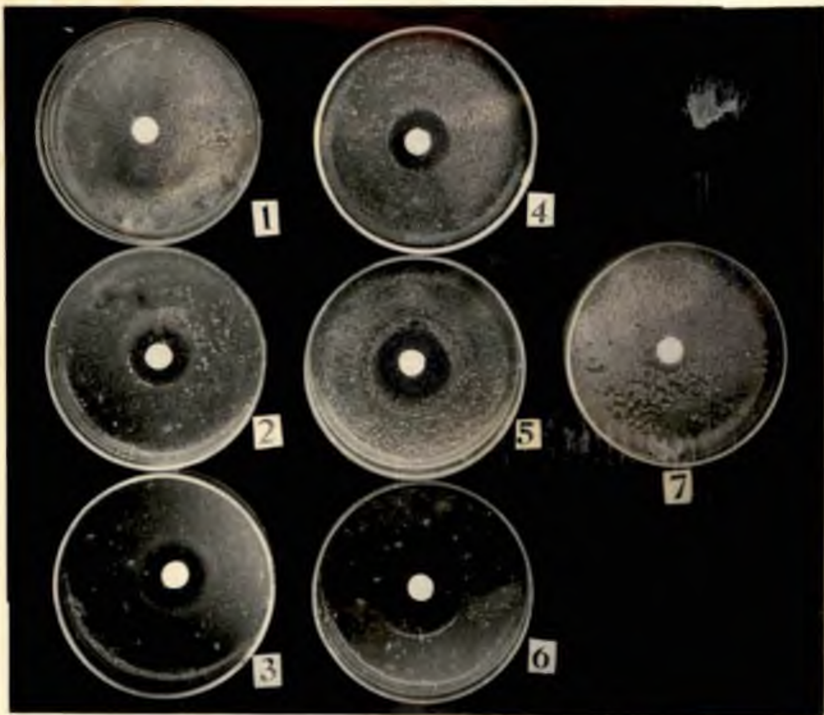


Plate VIII. Comparison between zone of inhibition of antibiotics tested.

1. Ampicillin - 500 ppm
2. Agrimycin-100 - 500 ppm
3. Streptocycline - 500 ppm
4. Streptomycin - 500 ppm
5. Terramycin - 500 ppm
6. Chloramphenicol - 500 ppm
7. Control - Distilled water

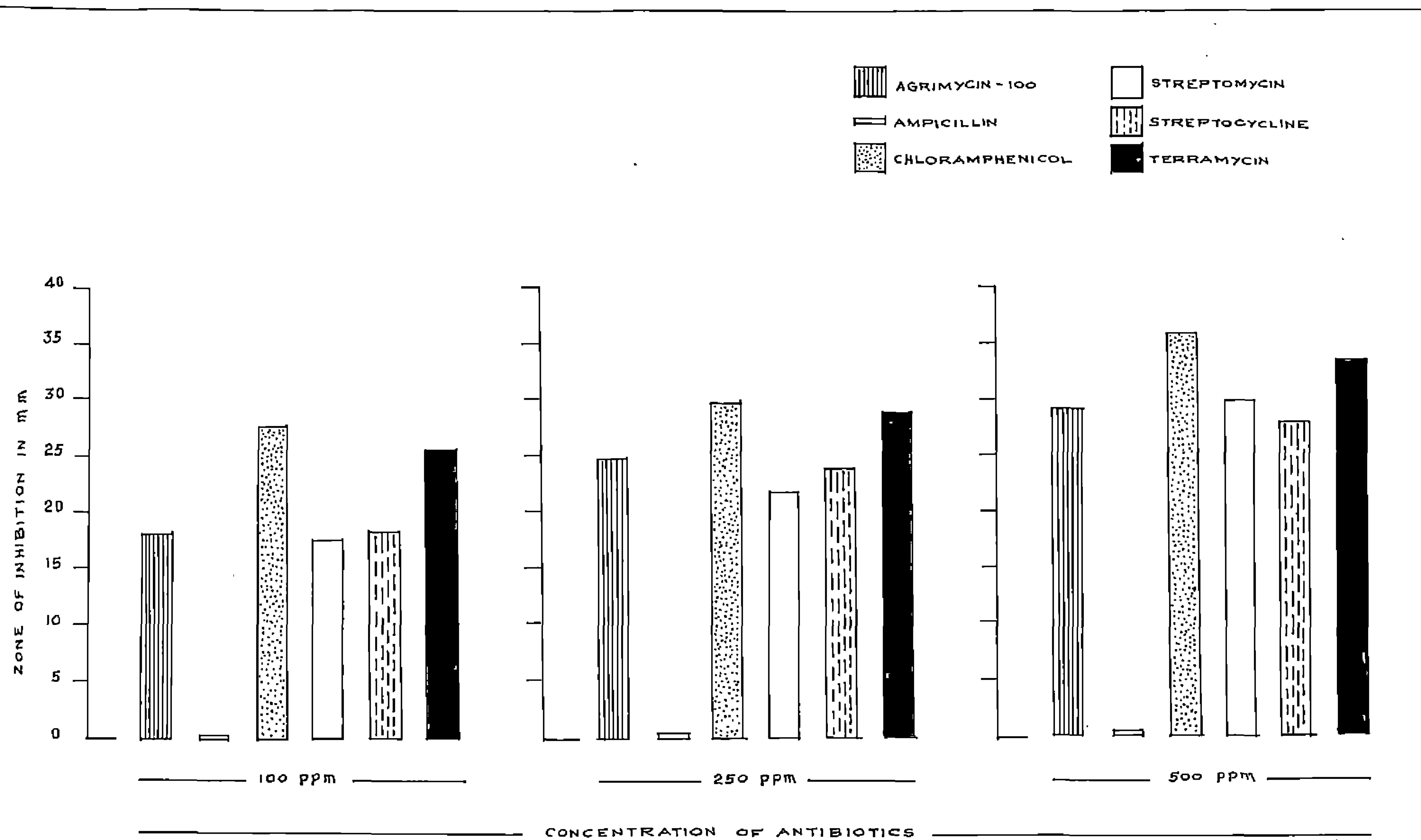


FIG. 7. *In vitro* SENSITIVITY OF XANTHOMONAS BETLICOLA TO ANTIBIOTICS.

Ampicillin was not effective against the bacterium at the concentrations tested. Chloramphenicol at 500 ppm was significantly superior to its lower concentrations and to all other antibiotics. Terramycin at 500 ppm was found to be on par with streptomycin 500 ppm and Chloramphenicol 250 ppm but was significantly superior to its lower concentrations and to other antibiotics except Chloramphenicol at 500 ppm. The higher concentrations of Streptocycline, Streptomycin and Agrimycin-100, were found to be significantly superior to their lower concentrations. Terramycin and Chloramphenicol at 250 ppm were on par with 100 ppm but was significantly inferior to 500 ppm.

<u>In vitro</u> sensitivity of the different bacterial isolates to Terramycin

Ten different isolates of the bacterium were tested in the laboratory to find out whether any difference in the <u>in vitro</u> sensitivity among the isolates to the various concentrations of Terramycin. The results are presented in table 8. Among the different isolates, isolate Xb-1 was most sensitive to Terramycin followed by Xb-9 and Xb-4. Isolate Xb-3 showed the least sensitivity to Terramycin. Evaluation by the zone of inhibition revealed that isolate Xb-1 was significantly more sensitive to Terramycin compared to other isolates. Sensitivity of isolates Xb-9 and Xb-4 to Terramycin were on par and significantly better than other isolates except Xb-1.

Table 8. *In vitro* sensitivity of Terramycin to different isolates of *Xanthomonas betlicola*

| Isolates | Inhibition zone in mm | | | |
|---|---|---|---|---|
| | 100 ppm | 250 ppm | 500 ppm | Mean |
| Xb-1 | 38.00 | 41.67 | 48.67 | 42.78 |
| Xb-2 | 23.00 | 29.00 | 32.33 | 28.11 |
| Xb-3 | 25.00 | 28.00 | 29.33 | 27.44 |
| Xb-4 | 30.33 | 37.00 | 39.00 | 35.44 |
| Xb-5 | 24.00 | 29.33 | 33.33 | 28.88 |
| Xb-6 | 27.00 | 29.33 | 34.67 | 30.33 |
| Xb-7 | 27.67 | 31.00 | 34.00 | 30.89 |
| Xb-8 | 27.33 | 29.33 | 32.33 | 29.66 |
| Xb-9 | 34.00 | 36.67 | 38.67 | 36.44 |
| Xb-10 | 26.00 | 30.33 | 31.00 | 29.11 |

C.D.(0.05) for comparison between isolates = 1.45
C.D.(0.05) for comparison between combination = 2.52

Isolate Xb-3 was significantly less sensitive to Terramycin compared to all other isolates and did not differ significantly with Xb-2 and Xb-5. For isolates Xb-3, Xb-4, Xb-9 and Xb-10 sensitivity at 250 ppm of Terramycin was found to be superior to 100 ppm but was on par with 500 ppm. Sensitivity to Terramycin at 250 ppm for isolates Xb-6 and Xb-8 were on par with 100 ppm but was inferior to 500 ppm. For isolates Xb-1,

Xb-2, Xb-5 and Xb-7 higher concentrations of Terramycin were significantly more sensitive than its lower concentrations.

*In vitro* sensitivity of the different bacterial isolates to Chloramphenicol

Sensitivity of the different isolates of the bacterium to the various concentrations of Chloramphenicol were tested in the laboratory. The results are presented in table 9.

Table 9. *In vitro* sensitivity of Chloramphenicol to different isolates of *Xanthomonas betlicola*

| Isolates | Inhibition zone in mm | | | |
|---|---|---|---|---|
| | 100 ppm | 250 ppm | 500 ppm | Mean |
| Xb-1 | 27.33 | 32.33 | 42.00 | 33.88 |
| Xb-2 | 25.00 | 32.67 | 45.67 | 34.44 |
| Xb-3 | 30.67 | 33.00 | 40.33 | 34.66 |
| Xb-4 | 30.67 | 38.67 | 43.67 | 37.67 |
| Xb-5 | 30.00 | 35.00 | 42.00 | 35.66 |
| Xb-6 | 26.33 | 35.33 | 40.33 | 33.99 |
| Xb-7 | 28.00 | 33.33 | 41.33 | 34.22 |
| Xb-8 | 25.00 | 33.67 | 41.00 | 33.22 |
| Xb-9 | 22.00 | 31.00 | 43.00 | 32.00 |
| Xb-10 | 27.67 | 35.67 | 42.00 | 35.11 |

C.D.(0.05) for comparison between isolates = 3.74

C.D.(0.05) for comparison between combination = 6.48

Among the different isolates tested, isolate Xb-4 was most sensitive to Chloramphenicol and the isolate Xb-9 the least. Evaluation by the zone of inhibition revealed that isolate Xb-4 was significantly more sensitive to Chloramphenicol compared to isolates Xb-9, Xb-8, Xb-6 and Xb-1 and was on par with other isolates. Chloramphenicol sensitivity of isolate Xb-9 was significantly less to isolate Xb-4 but did not differ significantly from other isolates. For isolates Xb-4, Xb-6 and Xb-10 sensitivity to Chloramphenicol at 250 ppm was found to be significantly superior to 100 ppm but was on par with 500 ppm. Sensitivity of Chloramphenicol at 250 ppm to isolates Xb-1, Xb-3, Xb-5 and Xb-7 were found to be on par with 100 ppm but differ significantly with 500 ppm. For isolates Xb-2, Xb-8 and Xb-9 sensitivity at higher concentrations of Chloramphenicol was found to be superior to lower concentrations.

<u>*In vitro* sensitivity of the mixture of isolates of the bacterium to antibiotics</u>

In order to assess the *in vitro* sensitivity of a mixture of all the 10 different isolates, six antibiotics were screened in the laboratory and the results are presented in table 10. Among the antibiotics tested Chloramphenicol was significantly superior to all other antibiotics.

Table 10. *In vitro* sensitivity of antibiotics to mixture of ten isolates of *Xanthomonas betlicola*

| Antibiotics | Inhibition zone in mm | | | |
|---|---|---|---|---|
| | 100 ppm | 250 ppm | 500 ppm | Mean |
| Agrimycin-100 | 18.33 | 22.00 | 23.33 | 21.22 |
| Ampicillin | 0.00 | 0.00 | 0.00 | 0.00 |
| Chloramphenicol | 25.33 | 32.00 | 38.66 | 31.99 |
| Streptomycin | 22.66 | 26.00 | 30.66 | 26.44 |
| Streptocycline | 22.33 | 25.66 | 31.00 | 26.33 |
| Terramycin | 22.00 | 29.66 | 34.33 | 28.66 |

C.D.(0.05)for comparison between antibiotics = 0.823
C.D.(0.05) for comparison between combination = 1.425

Ampicillin was not at all effective against the bacterium at the concentrations tested. Terramycin was found to be significantly better than other antibiotics except Chloramphenicol. Streptomycin and Streptocycline were found to be on par and significantly better than Agrimycin-100. Chloramphenicol at 500 ppm was significantly superior to its lower concentrations and to all other antibiotics. Terramycin at 500 ppm was significantly better than to its lower concentrations and to all other antibiotics except Chloramphenicol 500 ppm. Agrimycin-100 at 250 ppm was found to be superior to 100 ppm but was on par with 500 ppm.

For other antibiotics higher concentrations were significantly superior to their lower concentrations.

In vivo screening of antibiotics against the pathogen

Chloramphenicol, Terramycin and Agrimycin-100 at 500 ppm and 250 ppm concentrations were used for the *in vivo* testing against the disease. None of these treatments gave any absolute control to the disease. But there was significant decrease in disease intensity when compared with control. Similarly there was significant difference in the percentage of survival of diseased leaves when treated plants were compared with the control. There was no significant difference in the percentage of defoliation between treated plants and control one week after first spraying and one week after second spraying.

Statistical analysis on the percentage of defoliation one week after first spraying and one week after second spraying showed that there was no significant difference among treatments and control. There was considerable decrease in the percentage of defoliation on antibiotic treated plants one week after first spraying and one week after second spraying. It was observed that the percentage of defoliation one week after first spraying was less on plants receiving Terramycin spray. This was closely followed by those receiving Chloramphenicol spray. The comparison of the percentage of defoliation one week after second spraying

showed less defoliation on Chloramphenicol sprayed plants, followed by plants receiving Terramycin spray.

The percentage index of disease status one week after first spraying revealed that the plant receiving the treatment $T_3$ (Terramycin 500 ppm) showed less disease intensity and treatment $T_7$ (Control) showed maximum intensity (Table 11). Similarly one week after the second spraying of the antibiotics the percentage index of disease status was less on all antibiotics treated plants when compared to the control.

Statistical analysis of the percentage index of disease status one week after first spraying showed that $T_3$ (Terramycin 500 ppm) was significantly superior than the treatments $T_5$ and $T_7$ (Agrimycin 500 ppm and Control). Similarly evaluation of the percentage index of disease status one week after second spraying revealed that the treatment $T_7$ (Control) was significantly inferior to all other treatments which were all on par.

The percentage of survival of diseased leaves over two weeks was maximum on plants receiving Terramycin at 500 ppm spray and minimum on control. Statistical analysis showed that the treatment $T_3$ (Terramycin 500 ppm) was significantly superior to all other treatments except treatment $T_5$ (Agrimycin 500 ppm). Treatment $T_5$ (Agrimycin 500 ppm) was on par with $T_2$ (Chloramphenicol 250 ppm),

Table 11. *In vivo* screening of antibiotics at different concentrations against the pathogen

| Treatment | Percentage of defoliation (in angles) | | Percentage index of disease status | | Percentage of survival of diseased leaves over two weeks (in angles) |
|---|---|---|---|---|---|
| | One week after 1st spraying | One week after 2nd spraying | One week after 1st spraying | One week after 2nd spraying | |
| $T_1$ | 20.69 | 14.41 | 19.37 | 21.87 | 35.08 |
| $T_2$ | 18.95 | 25.26 | 25.43 | 48.17 | 45.32 |
| $T_3$ | 4.86 | 18.85 | 15.81 | 32.68 | 67.37 |
| $T_4$ | 15.36 | 27.47 | 18.52 | 28.35 | 41.35 |
| $T_5$ | 19.18 | 22.45 | 39.64 | 46.77 | 57.23 |
| $T_6$ | 24.00 | 30.08 | 28.14 | 38.95 | 38.31 |
| $T_7$ | 24.36 | 35.65 | 59.27 | 97.44 | 29.59 |
| C.D. | 0.00 | 0.00 | 20.93 (0.05 level) | 27.40 (0.05 level) | 21.80 (0.01 level) |

$T_4$ (Terramycin 250 ppm) and $T_6$ (Agrimycin 250 ppm). Treatment $T_7$ (Control) and $T_1$ (Chloramphenicol 500 ppm) were significantly inferior to treatment $T_3$ and $T_5$ (Terramycin 500 ppm and Agrimycin 500 ppm).

# Discussion

## DISCUSSION

The betel vine cultivation in Kerala is found to be very severely affected by the bacterial leaf spot disease incited by Xanthomonas betlicola causing severe crop losses. In other regions of India also this disease is known to cause extensive damage and was first reported from Bombay State during the year 1951 (Patel et al., 1951). Except for a few preliminary reports there has been no systematic work on the various aspects of this disease.

The aspects taken up for the present study were, symptomatology, variations in symptoms on inoculation of different isolates of the bacterium, characterization and identification, host range, and survival of the pathogen, screening betel vine cultivars for host resistance and both in vitro and in vivo screening of antibiotics against the pathogen.

Symptomatology of the disease under natural and artificial conditions were studied in detail. Naturally infected plants showed a variety of symptoms. The initial symptoms of the disease appeared as minute water soaked lesions on the leaf lamina delimited by veins. These lesions may be either angular or without any angularity. Bacterial exudations were invariably associated with the water soaked lesions. When these lesions grew older they became dark brown with a distinct yellow halo surrounding

them. Later such lesions coalesced to form large patches. At times shot hole type symptoms developed on the leaf lamina. Marginal infection was observed in most cases ultimately resulting in deformities. When major portions of leaf lamina got infected defoliation occurred. Stem and petiole infection was also observed. Both shot hole type symptoms and marginal lesions considerably decrease the quality and thus the markettability of the leaves.

On artificial inoculation all the general symptoms of the disease observed in naturally infected plants could be produced. Manifestation of symptom commenced from 5 to 7 days of inoculation. Further the symptoms of the disease observed both under field and inoculated conditions were almost similar to those described by Patel et al. (1953). However, Singh and Chand (1971a) in their studies reported from Jabalpur, did not observe any marginal infection, bacterial exudation, shot hole type symptoms and stem and petiole infection.

Minor variations in the nature of symptom produced on inoculation with different isolates of the bacterium were critically observed. The isolates of the bacterium were found to produce differences in the nature of the symptoms particularly, in the size of the initial water soaked lesions, angularity, gum production, yellow halo,

size of the spot developed, marginal infection, shot hole type symptom, susceptibility of leaves, defoliation and stem and petiole infection. Eventhough there is no previous work of this type on the symptoms of this disease, similar variations in symptoms due to the difference in the isolates of other inoculated bacterial pathogen has been reported. Devadath and Padmanabhan (1969) could observe differential reaction in rice with different isolates of Xanthomonas oryzae. Devadath (1970) also reported that there was difference in the lesion development on Taichung Native 1 with virulant and avirulant isolates of X. oryzae. Maraite and Meyer (1975) observed similar type of symptom variation with X. manihotis. Vakili (1977) reported the occurrence of variable range of pathogenicity with isolates of Xanthomonas strains causing bacterial blight and pustule disease of edible legumes. Thus in the present study the observed variation in the extent, nature and severity of the symptoms might be due to difference in the pathogenic isolates of the bacterium.

The growth of the bacterial isolate Xb-1 on different solid media showed some variations. Out of the eight solid media used, maximum growth was observed in Yeast Glucose Chalk Agar, followed by Potato Sucrose Peptone Agar, Potato Dextrose Agar and Glucose Agar. The growth of the bacterium in the basal medium for xanthomonads and

Nutrient Agar was very meagre. There was no growth in Glucose Yeast Extract Agar and Tetrazolium Chloride negative medium. YGCA and PSPA medium were found to be the best media for the growth of the bacterial isolate Xb-1. Patel et al. (1951, 1953) had recorded that PDA supported growth of the bacterium and growth was poor in NA. In the present study only slight growth was observed in PDA and in NA the growth was meagre. Dye (1962) had noted that considerable variation could be expected in colonies produced by Xanthomonads and this may not be taken as the only differentiating character.

The growth of the ten different isolates of the bacterium on PSPA medium showed some variations. Isolates Xb-1, Xb-7, Xb-9 and Xb-10 recorded maximum growth, slime production, fluidity followed by isolates Xb-4, and Xb-6. The growth, slime production and fluidity by isolates Xb-2, Xb-3, Xb-5, and Xb-8 were poor. Similarly variation in the growth of the different isolates of the bacterium on Potato Sucrose Peptone broth was also recorded. Growth of the different isolates of Xanthomonas betlicola on a single solid medium and its broth has not been studied so far by earlier workers. Patel et al. (1951), Breed et al. (1957) and James Mathew et al. (1978a) reported only a turbid yellow growth of the bacterium in nutrient broth.

All isolates of the bacterium were found to be aerobic and produced a non-water soluble yellow pigment on Yeast Glucose Chalk Agar medium.

With respect to the physiological and biochemical properties of the bacterial isolates, all isolates utilized glucose oxidatively, hydrolysed starch and arginine, produced hydrogen sulphide and lipases. The different isolates of the bacterium utilized sodium citrate, sodium acetate but not sodium benzoate as the sole source of carbon. The isolates of the bacterium liquified gelatin, did not reduce nitrates and failed to produce indole and tyrosinase. Milk was turned alkaline in reaction. None of the isolates utilized asparagine as the sole source of carbon and nitrogen. All of them gave negative MR & VP test. All isolates were found to be catalase positive and growth was inhibited at 6 per cent sodium chloride.

Out of the 15 carbon compounds tested, all isolates of the bacterium produced acid in Galactose, Mannose, Xylose, Fructose, Glucose, Maltose, Lactose, Sucrose and Dextrose. The bacterium did not produce acid in Inositol, Dulcitol, Amygdalin, Ribose, Salicin and Raffinose.

Similar results have been observed by Patel et al. (1951, 1953) and James Mathew et al. (1978a, 1978b, 1979a). The observations in the present study were in conformity with those reported by Breed et al. (1957) and Dye (1962).

Dye (1962) reported that physiological characters are of little value in distinguishing species because the extent of intra species variability in physiological characters is so great as inter species variability.

The present studies on the morphological, cultural, physiological and biochemical characters of the bacterial isolates coupled with its pathogenicity confirm the reports of Patel et al. (1951, 1953), Breed et al. (1957), Singh and Chand (1971a), Buchanan and Gibbons (1974) and James Mathew et al. (1978a, 1978b, 1979a) that the organism causing bacterial leaf spot of betel vine could be identified as *Xanthomonas betlicola*, Patel et al. Further in the present study ten different pathogenic isolates of the bacterium were employed and there was no indication of any physiological and biochemical variability existing in the causal bacterium. But the isolates on inoculation produced variation in symptom expression indicating difference among them. However, detailed studies with more number of isolates from different geographical regions are required before arriving at a definite conclusion on the variability of the pathogen.

Plants with similar taxonomic affinity to betel vine and weeds that are commonly present in the betel vine gardens were artificially inoculated to see if any of these plants took up infection. The following plants

Piper nigrum L, Piper longum L and Peperomia pellucida WB & K belonging to the family Piperaceae were found to be infected by the bacterium. None of the weeds tested were susceptible to the pathogen. These results were in agreement with the observations of Patel et al. (1953), Breed et al. (1957), Buchanan and Gibbons (1974) and James Mathew et al. (1978b, 1979a). The fact that the pathogen is able to infect pepper (Piper nigrum L.) is of concern to pepper cultivation and at this stage it is desirable that betel vine gardens are not located near pepper gardens.

Survival of the pathogen in the infected vines and leaves were studied. Repeated isolations from diseased leaves and vines after ten days did not yield the bacterium. Similarly isolations of the bacterium from soil after 10 days also gave negative result. No previous work was available on the survival of this organism. But there were reports of survival studies on other plant pathogenic Xanthomonads. Brinkerhoff and Fink (1964) reported that viability of X. malvacearum depend on the extent of decomposition of the debris, and the viability being lost after the plant tissues were broken down. Singh (1971) noted that X. oryzae did not survive in unsterilized soil for a week or over summer in the field. Cho and Yoo (1977) reported that X. phaseoli var. sojense survived for four days only in sterile soil. Schuster (1977)

recorded that pure cultures of *Pseudomonas glycine* and *X. phaseoli* var. *sojense* did not survive under dry or wet conditions in either sterilized or non-sterilized soil. In the absence of any earlier work on the survival of *X. betlicola* and in the light of the above reports of survival of other Xanthomonads, it is likely that the bacterial leaf spot pathogen of betel vine may not survive in leaf, vine or soil for long periods.

Further Patel *et al.* (1953) reported the prevalence of the disease throughout the year. However he had observed that it occurred in serious proportions during the rainy season characterised by low temperature and high humidity. Betel vine being a perenial crop, is able to continue the disease cycle transmitting the causative organism from diseased leaves to emerging leaves.

Of the seven cultivars of betel vine plants screened for host resistance against the pathogen, none of them were found to be absolutely resistant to the disease. Minimum disease severity was observed on cultivar Tulasivettila and maximum on cultivar Pannivella. Within a period of 20 days after inoculation less defoliation was noticed on cultivars Karilanchikarpuran and Tulasivettila and maximum on Malamkodi. Similarly within a period of 30 days after inoculation minimum defoliation occurred on cultivars Karilanchikarpuran and Tulasivettila and maximum on Pannivella. So it can be presumed that

eventhough all cultivars of betel vine were susceptible to the disease, cultivars Tulasivettila and Karilanchi-karpuran were less susceptible. Patel *et al.* (1953) reported from Bombay State that all varieties of betel vine were susceptible to the disease with varying degrees of intensity. Singh and Chand (1971a) observed that this disease was widely prevalent in betel vine gardens on all varieties in Jabalpur and the Bangla variety was the most susceptible. But these inferences were based mainly on field observation. Similar types of observations were made by Klement and Kapeller (1967) and Strider (1976) on *Capsicum annum* against *Xanthomonas vesicatoria*. Das (1977) could not locate a single resistant cultivar out of the 286 rice cultivars tested against bacterial leaf streak disease. Sundaresh *et al.* (1978) also could not get source of resistance against bacterial pustule disease of soyabean in their study. In general to get a cultivar with genetic resistance against a bacterial disease is considered to be difficult and the leaf spot disease of betel vine appears to be no exception to this.

Out of the six antibiotics screened for the *in vitro* sensitivity of the bacterium, it was found that Chloramphenicol and Terramycin had better inhibitory effect over Agrimycin-100, Streptocycline, Streptomycin. Ampicillin was found to be ineffective upto 500 ppm. Chloramphenicol

at 500 ppm had better inhibitory effect than its lower concentrations and to all other antibiotics. Similar results were also obtained when the mixture of ten isolates of the bacterium was tested for the in vitro sensitivity against the six antibiotics. Thirumalachar et al. (1956) reported that Terramycin, Chloromycetin (Chloramphenicol) and Streptomycin had got inhibitory effect to X. betlicola on Piper betle. James Mathew et al. (1979a, 1979b) noted that of the six antibiotics tested in vitro against X. betlicola on Piper nigrum, Chloramphenicol at 500 ppm exerted maximum inhibition to growth and Ampicillin was not at all effective against the bacterium. Thus the present study indicates that Chloramphenicol at 500 ppm exerted maximum inhibition to growth of the bacterium in vitro.

The ten different isolates of the bacterium were tested in vitro to assess the difference in sensitivity to various concentrations of Terramycin and Chloramphenicol. It was found that the isolate Xb-1 was most sensitive to Terramycin and the isolate Xb-3 the least. Maximum sensitivity to Chloramphenicol was shown by isolate Xb-4 and the minimum by isolate Xb-9. The other isolates of the bacterium showed varying degrees of sensitivity to different concentrations of Terramycin and Chloramphenicol. Sensitivity of the different isolates of this bacterium against antibiotics were not studied so far. But similar types of results have been reported with other bacterial

pathogens. Yamamoto and Kusaka (1965) reported that the 8 strains of X. oryzae varied in susceptibility to Streptomycin but showed little difference in reaction to Penicillin, Cellocidin, Chloramphenicol and 7 other antibiotics. Verma et al. (1975) observed that the isolates of X. malvacearum could not be grouped into well differentiated types on the basis of their relative sensitivity. In the present study also the isolates of the bacterium showed variation in sensitivity to antibiotics.

Of the three antibiotics at two concentrations used for the in vivo screening, it was found that none of the treatments gave complete control of the disease. It was observed that one week after first spraying the percentage index of disease status was minimum on plants receiving Terramycin 500 ppm spray and maximum on control plants. Similarly the percentage index of disease status one week after second spraying was minimum on all antibiotics treated plants and maximum on control. It was also noted that those plants receiving Terramycin at 500 ppm spray, the percentage of survival of diseased leaves over two weeks after spraying was maximum while the survival of such leaves was a minimum on control plants.

In vivo control of bacterial leaf spot disease of betel vine by antibiotics has not been studied so far by earlier workers. In the case of other bacterial disease

similar studies were conducted. Arene (1977) noted that the incidence and severity of cassava bacterial blight in the field was significantly reduced by foliar application of Agrimycin-500. Dhanvantari *et al*. (1978) reported that both Oxytetracyclin hydrochloride (OTC) and Streptomycin sulphate significantly reduced defoliation and leaf infection of bacterial spot of peach due to *X. pruni* in South Western Ontaria. Fruit infection was similarly reduced by OTC.

In the present study the result of *in vitro* sensitivity of antibiotics against the bacterium showed that Chloramphenicol at 500 ppm was having maximum sensitivity. But in the field trial Terramycin 500 ppm gave the maximum effect against the disease. In the absence of resistant cultivars of betel vine, Terramycin at 500 ppm could be used for reducing the severity of the disease incidence in the betel vine gardens.

# *Summary*

## SUMMARY


The bacterial leaf spot incited by Xanthomonas betlicola Patel et al. is one of the most serious diseases on betel vine. This disease causes considerable reduction in the yield and quality of the leaves. In India, this leaf spot disease was first reported from Bombay State in the Year 1951.

In view of the severe crop losses due to this disease in the State, studies were undertaken on the symptomatology, variations in symptom production with different isolates of the pathogen, characterisation and identification of the bacterium, host range and survival of the pathogen, screening of betel vine cultivars for host resistance and both in vitro and in vivo sensitivity of the pathogen to antibiotics.

The causal bacterium was isolated from severely disease affected betel vine leaves. Ten pathogenic isolates of the bacterium from different localities were used in the present study.

Symptoms of the disease both under natural and artificial conditions were studied. The typical symptoms of the disease such as initial water soaked lesions, bacterial exudations, development of dark brown patches with yellow halo, shot hole type symptoms, defoliation, stem and petiole infection were observed.

Minor variations in the above symptom expression were observed on inoculation with the different isolates of the bacterium.

Growth of the bacterial isolate Xb-1 on eight solid media showed that Yeast Glucose Chalk Agar and Potato Sucrose Peptone Agar were the best solid media for routine laboratory studies. The different isolates of the bacterium showed some differences in growth on PSPA medium and its broth.

Morphological, physiological and biochemical characters of the different isolates studied were similar. Based on the above characters of the pathogen coupled with pathogenicity and symptom expression suggested the identity of the pathogen as *Xanthomonas betlicola* (Patel *et. al.*, 1951).

Plants belonging to the family Piperaceae such as *Piper nigrum* L, *Piper longum* L and *Peperomia pellucida* WB & K were found to take up infection upon artificial inoculation of the pathogen and the weeds tested did not take up infection. The fact that the pathogen attack *Piper nigrum* L is of economic concern to pepper cultivation in the State.

Repeated isolations of the bacterium from infected leaves, stem and soil did not yield any bacterial colonies, indicating that the pathogen may not survive for long periods in the above sources.

Out of the seven cultivars of betel vine tested for host resistance, none of them was found to be resistant to the disease. But Tulasivettila and Karilanchikarpuran were found to be less susceptible to the pathogen.

The bacterium was found to be sensitive to Chloramphenicol, Terramycin, Agrimycin-100, Streptocycline, and Streptomycin. Ampicillin was not at all effective against the bacterium. Chloramphenicol gave the maximum zone of inhibition followed by Terramycin. Similar results were also obtained when the mixture of ten isolates were tested for the sensitivity to the above six antibiotics.

Variations in sensitivity among the ten different isolates of the bacterium to Chloramphenicol and Terramycin were also noticed.

The *in vivo* screening of three antibiotics at two concentrations did not give any absolute control of the disease. But based on the percentage index of disease status and the percentage of survival of diseased leaves over two weeks after spraying, it was found that Terramycin at 500 ppm gave maximum effect in reducing the disease intensity.

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*Original not seen

# Appendices

## APPENDIX Ia

Analysis of variance table

Screening of betel vine cultivars for host resistance against *Xanthomonas betlicola*

Diseases grade of all leaves infected

| Source | Sum of square | df | Mean square | F at 0.05 level | Whether significant or not |
|---|---|---|---|---|---|
| Total | 12.187 | 34 | | | |
| Treatment | 8.647 | 6 | 1.441 | 11.399 | Significant |
| Error | 3.540 | 28 | 0.126 | | |

## APPENDIX Ib

Analysis of variance table

Screening of betel vine cultivars for host resistance against *Xanthomonas betlicola*

Percentage of defoliation within 20 days after inoculation (after angular transformation)

| Source | Sum of square | df | Mean square | F at 0.05 level | Whether significant or not |
|---|---|---|---|---|---|
| Total | 18488.761 | 34 | | | |
| Treatment | 8051.727 | 6 | 1341.954 | 3.60 | Significant |
| Error | 10437.033 | 28 | 372.751 | | |

## APPENDIX Ic

Analysis of variance table

Screening of betel vine cultivars for host resistance against *Xanthomonas betlicola*

Percentage of defoliation within 30 days after inoculation (after angular transformation)

| Source | Sum of square | df | Mean square | F at 0.05 level | Whether significant or not |
|---|---|---|---|---|---|
| Total | 11707.874 | 34 | | | |
| Treatment | 6304.862 | 6 | 1050.81 | 5.44 | Significant |
| Error | 5403.012 | 28 | 192.96 | | |

## APPENDIX II

Analysis of variance table

*In vitro* sensitivity of antibiotics at different concentrations to *Xanthomonas betlicola*

| Source | Sum of square | df | Mean square | F at 0.05 level | Whether significant or not |
|---|---|---|---|---|---|
| Total | 6545.5 | 53 | | | |
| Treatment | 6417.5 | 17 | 377.5 | 106.171 | Significant |
| Error | 128.0 | 36 | 3.5 | | |

## APPENDIX III

### Analysis of variance table

*In vitro* sensitivity of Terramycin to different isolates of *Xanthomonas betlicola*

| Source | Sum of square | df | Mean square | F at 0.05 level | Whether significant or not |
|---|---|---|---|---|---|
| Total | 2905.29 | 89 | | | |
| Treatment | 2762.62 | 29 | 95.26 | 40.03 | Significant |
| Error | 142.67 | 60 | 2.38 | | |

## APPENDIX IV

### Analysis of variance table

In vitro sensitivity of Chloramphenicol to different isolates of *Xanthomonas betlicola*

| Source | Sum of square | df | Mean square | F at 0.05 level | Whether significant or not |
|---|---|---|---|---|---|
| Total | 4680.49 | 89 | | | |
| Treatment | 3733.16 | 29 | 128.73 | 8.15 | Significant |
| Error | 947.33 | 60 | 15.79 | | |

## APPENDIX V

Analysis of variance table

*In vitro* sensitivity of antibiotics to mixture of ten isolates of *Xanthomonas betlicola*

| Source | Sum of square | df | Mean square | F at 0.05 level | Whether significant or not |
|---|---|---|---|---|---|
| Total | 6775.34 | 53 | | | |
| Treatment | 6748.67 | 17 | 396.98 | 536.45 | Significant |
| Error | 26.67 | 36 | 0.74 | | |

## APPENDIX VIa

Analysis of variance table

*In vivo* screening of antibiotics at different concentrations against the pathogen

Percentage index of disease status one week after first spraying

| Source | Sum of square | df | Mean square | F at 0.05 level | Whether significant or not |
|---|---|---|---|---|---|
| Total | 9927.25 | 27 | | | |
| Treatment | 5673.73 | 6 | 945.65 | 4.66 | Significant |
| Error | 4253.52 | 21 | 202.54 | | |

## APPENDIX VIb

Analysis of variance table

*In vivo* screening of antibiotics at different concentrations against the pathogen

Percentage index of disease status one week after second spraying

| Source | Sum of square | df | Mean square | F at 0.05 level | Whether significant or not |
|---|---|---|---|---|---|
| Total | 22344.45 | 27 | | | |
| Treatment | 15053.12 | 6 | 2508.85 | 7.22 | Significant |
| Error | 7291.33 | 21 | 347.21 | | |

## APPENDIX VIc

Analysis of variance table

*In vivo* screening of antibiotics at different concentrations against the pathogen

Percentage of defoliation one week after first spraying (After angular transformation)

| Source | Sum of square | df | Mean square | F at 0.05 level | Whether significant or not |
|---|---|---|---|---|---|
| Total | 6294.85 | 27 | | | |
| Treatment | 1060.97 | 6 | 176.82 | 0.709 | Not significant |
| Error | 5233.88 | 21 | 249.23 | | |

## APPENDIX VId

### Analysis of variance table

*In vivo* screening of antibiotics at different concentrations against the pathogen

Percentage of defoliation one week after second spraying (after angular transformation)

| Source | Sum of square | df | Mean square | F at 0.05 level | Whether significant or not |
|---|---|---|---|---|---|
| Total | 7909.40 | 27 | | | |
| Treatment | 1206.69 | 6 | 201.12 | 0.63 | Not significant |
| Error | 6702.71 | 21 | 319.18 | | |

## APPENDIX VIe

### Analysis of variance table

*In vivo* screening of antibiotics at different concentrations against the pathogen

Percentage of survival of diseased leaves over two weeks (after angular transformation)

| Source | Sum of square | df | Mean square | F at 0.01 level | Whether significant or not |
|---|---|---|---|---|---|
| Total | 10929.99 | 27 | | | |
| Treatment | 4175.12 | 6 | 695.85 | 2.16 | Significant |
| Error | 6754.87 | 21 | 321.60 | | |

# STUDIES ON THE BACTERIAL LEAF SPOT OF BETEL VINE INCITED BY *Xanthomonas betlicola* PATEL *et al.*

By

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ABSTRACT OF A THESIS
Submitted in partial fulfilment of the
requirement for the degree
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1980

ABSTRACT

The bacterial leaf spot of betel vine incited by Xanthomonas betlicola Patel et al. is one of the most serious diseases recorded on the plant and was first reported in the year 1951. The occurrence and severity of the disease in Kerala was reported from 1978 onwards. The symptoms of the disease is characterised by water soaked lesions, bacterial exudations, dark brown patches with yellow halo, marginal infection, shot hole type symptoms, defoliation, stem and petiole infection. Minor variations in the above symptoms were observed with different isolates of the pathogen.

Yeast Glucose Chalk Agar and Potato Sucrose Peptone Agar were found to be the best solid media for the growth of the bacterium. Slight variations in growth was observed among isolates when grown on PSPA medium.

The pathogen was found to be Xanthomonas betlicola from its morphological, physiological and biochemical characters coupled with pathogenicity. The pathogen infected other members of the family Piperaceae such as Piper nigrum, Piper longum, Peperomia pellucida.

Repeated isolations of the bacterium from infected leaves, stem and soil did not give any positive results indicating that the pathogen may not survive for long periods in the above sources.

None of the cultivars screened were absolutely resistant to the disease but Tulasivettila and

Karilanchikarpuran were less susceptible.

The bacterium was sensitive *in vitro* to Chloramphenicol, Terramycin, Agrimycin-100, Streptocycline and Streptomycin and not to Ampicillin. Differences in sensitivity among isolates to Chloramphenicol and Terramycin was also observed. Field trial of antibiotics against the disease did not give any complete control. But it has been observed that Terramycin at 500 ppm had some effect in reducing the disease severity.