STUDY OF BACTERIAL LEAF SPOT OF BETEL VINE- BIOCHEMICAL CHANGES AND CONTROL

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Study of bacterial leaf spot of betel vine - biochemical changes and control" 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.

Vellayani,
10-4 -1986

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CERTIFICATE

Certified that this thesis entitled "Study of bacterial leaf spot of betel vine - biochemical changes and control" is a record of research work done independently by Sri. Koshy Abraham, under my guidance and supervision and that it has not previously formed the basis of award of any degree, diploma, fellowship or associateship to him.

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INTRODUCTION

Betel vine (*Piper betle* L.), a native of South East Asia, is one of the important commercial crops grown in India, Sri Lanka and Bangladesh. The leaves of this crop which have high commercial value are being used mainly for chewing along with dry raw or processed arecanut. The area under this crop in Kerala State is about 1000 ha. In the State, cultivation of this crop is mainly concentrated in Malappuram, Quilon and Trivandrum districts. Since the leaves are of economic importance, the factors which influence the out turn of superior quality foliage are of paramount importance in the cultivation of this crop.

The research work carried out in the country on the agrotechniques and crop protection aspects of this valuable crop is quite scanty. Of late, there have been alarming reports of decline in betel vine cultivation in the country due to the heavy incidence of serious diseases. In many cases, the plantations have been completely destroyed resulting in substantial econonic losses.

Among the various diseases of betel vine, the bacterial leaf spot is the most important one in Kerala.

This disease affects the leaves and stem and occurs in very severe proportions in almost all betel vine gardens in the State. Though, the incidence of this major disease has been on record since the beginning of this century, the causal bacterium of this disease was first characterised and identified as Xanthomonas betlicola by Patel et al. (1951) from the former Bombay State. Subsequently, Dye et al. (1980) renamed the pathogen as X. campestris pv. betlicola (Patel, Kulkarni and Dhande) Dye. In Kerala, the occurrence of the disease in severe proportions has been reported by Mathew et al. (1978a).

This disease is prevalent throughout the year, becoming very severe during rainy seasons. The conditions which are congenial for the growth of betel vine crop are also found to be quite ideal for the rapid flare up of this disease. It has been observed that the pathogen causing leaf spot of betel vine can also infect black pepper, a commercial crop of great importance in Kerala. In homestead farming situation, black pepper and betel vine are grown either in the same holdings or these two crops frequently occur in close proximity. In such situations the potential hazards due to cross infection of the black pepper plants with the pathogen are formidable.

Only very little information is available on the various aspects of the pathogen, host-parasite interactions

and disease management. Specific recommendations on the management of the disease are not available at present. In view of the serious nature of the disease of betel vine and the potential danger that it poses to black pepper, studies were undertaken on the various aspects of the pathogen, symptomatology, histopathology, host range, post infectional biochemical changes in resistant and susceptible cultivars of betel vine and the effect of host nutrition on disease development. Screening of betel vine cultivars for host resistance was attempted in the present studies to identify the promising ones with resistance reaction. Work on chemical control of the disease was also carried out to develop chemical methods of containing the disease.

Review of Literature

REVIEW OF LITERATURE

The existence of bacterial leaf spot disease of betel vine (Piper betle L.) has been recorded by several workers since the early part of this century. In India, Hutchinson (1925) first observed the occurrence of a bacterial disease in betel vine from Bengal. Subsequently, the disease was reported from Assam (Mitra, 1928), Nagapur (Nirula, 1931), Central provinces of India (Asthana and Mahmud, 1944), Allahabad (Vestal, 1946), former Bombay State (Patel et al., 1951), Jabalpur (Singh and Chand, 1971) and Kerala (Mathew et al., 1978a).

Though bacterial leaf spot of betel vine was observed in Ceylon for a long time, it became destructive only since 1921 (Raghunathan, 1926; Park, 1934). This disease was also reported from Malaya (Thompson, 1928) and Mauritius (Shepherd, 1931).

2.1 The pathogen

Hutchinson (1925) proved the pathogenicity of a bacterium causing leaf spot of betel vine. Raghunathan (1926; 1928) named the causal agent as Bacterium betle and reported that the bacterium was in the form of

cylindrical rods, cells 0.5 x 1.5 - 2.5 /u , non-motile, without spores and capsule, honey yellow, slimy, viscid in mass and gram negative.

Patel et al. (1951) named the bacterium causing leaf spot of betel vine as Xanthomonas betlicola Patel, Kulkarni and Dhande. Dye et al. (1980) renamed the bacterium as Xanthomonas campestris pv. betlicola (Patel, Kulkarni and Dhande) Dye.

The cultural and biochemical characters of the bacterium were studied by many workers (Patel et al., 1951; 1953; Breed et al., 1957; Mathew et al., 1978a, b; 1979a; Abraham, 1980; Abraham and Mathew, 1983). They reported that the pathogen was a slender rod, single or in pairs, gram negative, motile with one or two flagella, capsulated, not acid fast, aerobic, cells 1.6 x 0.9 /u and readily stained with common dyes. The growth of the bacterium in Nutrient Agar was poor. In Nutrient Agar, the colonies were round with entire margins, umbonate and yellow in colour. The temperature requirement of the bacterium was found to be 26 to 30°C with the optimum growth at 28°C and thermal death point at 51°C. The bacterium utilized glucose oxidatively, hydrolysed starch, casein and arginine, reduced litmus, produced hydrogen sulphide, ammonia and lipases, liquified

gelatin and Loeffers blood serum, milk was turned alkaline, did not reduce nitrate and failed to produce indole and tyrosinase. It was positive in catalase test and negative in MR and VP, and Kovac's oxidase tests. The bacterium showed slight or no growth in synthetic asparagine medium. Good growth of the bacterium was observed in Uschinsky's solution but slight growth in Fermi's and Cohn's solutions. The bacterium produced acid but no gas from glucose, dextrose, lactose, xylose, fructose, sucrose, galactose, mannose and maltose, and did not utilize inositol, adonitol, dulcitol, inulin, salicin and ribose.

Abraham and Mathew (1983) reported that all the ten isolates of X. campestris pv. betlicola behaved alike in all the physiological and biochemical characters studied. Khatri et al., (1983c) observed the active motility of the cultures of X. campestris pv. betlicola at temperatures 25 and 27°C and between pH 6 and 7.

2.2 Symptomatology

The symptomatology of bacterial leaf spot disease of betel vine caused by X. betlicola was described by Patel et al. (1953). The symptom first appeared as minute water soaked spot on the lower surface of the

leaves between veins. After about ten days the spots became visible on the upper surface as dark brown round to angular areas surrounded by a yellow halo. The individual spots which initially measured 5 mm or less later coalesced to form brown to black areas of 1 to 2.5 cm in diameter. Some times, the dead portions of the infected leaves fell off leaving holes. The lesions, when marginal often resulted in deformities and cracking of tissues. Bacterial ooze of appreciable amount was also observed in the infected portions under humid condition. Infection was occasionally seen on petiole and stem, though leaves and leaf margins were the common sites of infection. Severe cases of infection resulted in defoliation.

Singh and Chand (1971) from Jabalpur reported that the symptom of the disease developed in the form of a small speck which later enlarged to form 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 leaf surface was almost covered with large spots and patches.

Mathew et al. (1978a) and Abraham (1980) observed these typical symptoms as described by Patel et al. (1953). In addition to these symptoms on the leaves,

Abraham (1980) reported that stem infection was very common in disease affected plants. The stem infection developed as small dark brown lesion surrounded by greenish yellow halo. Later, such lesions coalesced and formed large patches resulting in the death of plants.

Three different types of symptom namely, leaf spot, leaf blight and stem canker on betel vine plants due to infection by X. campestris pv. betlicola were noticed at Jabalpur (Jain et al., 1982).

2.3 Histopathology

Mirula (1944) studied the histopathology of stored betel vine leaves infected by a bacterium and observed that the bacterium gained entry through the cut ends of the petiole. The phloem tissues were involved at the early stage and later destroyed. The bacterium disorganised the xylem and mesophyll cells at the advanced stage of infection.

Asthana and Mahmud (1945) observed that in the early stage of infection in betel vine, the bacterial leaf spot pathogen was confined to the epidermis, but subsequently, it invaded the spongy and palisade parenchyma cells. The attacked parenchyma cells then

slightly enlarged, assumed dark yellow colour and disintegrated. Kotwal (1978) studied the histopathology of stem canker and bacterial wilt of betel vine caused by Xanthomonas sp. and noticed the presence of bacteria in vascular bundles and parenchymatous cells. Histopathological studies conducted at Jabalpur indicated that the bacterial leaf spot pathogen attacked the xylem vessels. Collenchymatous as well as parenchymatous cells were also infected by the pathogen (Jain et al., 1982).

2.4 Host range of the pathogen

Patel et al. (1953) and Buchanan and Gibbons (1974) reported that X. betlicola was pathogenic to Piper betle, Piper longum and Piper hockeri. Breed et al. (1957) noted that X. betlicola attacked the members of the family Piperaceae. Mathew et al. (1978b; 1979a) reported that Piper nigrum acts as a host of X. betlicola. Abraham (1980) observed that none of the weeds seen in betel vine gardens was infected by the bacterium.

2.5 Pathophysiology

One of the main areas of interest in host-parasite relationship is the patho-physiological studies concerning changes in the metabolism of the host as caused by parasite infection. The changes in phenolics,

carbohydrates, proteins, cell wall constituents, respiratory pattern and enzyme activities of plants infected with pathogens have received much attention (Hussain and Kelman, 1959; Farkas and Kiraly, 1962; Goodman et al., 1967).

2.5.1 Changes in phenolics

The metabolic changes occuring in diseased plants frequently lead to an accumulation of aromatic, especially phenolic compounds. This accumulation is often particularly striking in resistant and hypersensitive reactions. The role of phenolic compounds on the resistant mechanism of host plants against invading pathogen has been well recognised in a number of host-parasite interactions (Farkas and Kiraly, 1962; Kuc, 1964; Rohringer and Samborski, 1967; Sridhar and Mahadevan, 1968; Rao and Zuber, 1978).

The involvement of phenolics in plant diseases caused by bacteria has been observed less frequently (Goodman et al., 1967). The quantitative changes in phenolics in betel vine plants due to infection by X. campestris pv. betlicola have not yet been studied.

Easwaran (1967) observed that inoculation of sorghum plants with X. Vubrisorghi increased the total phenols in both less susceptible and susceptible varieties.

Prasad et al. (1972) reported that the rice variety resistant to bacterial leaf blight pathogen contained increased quantities of total and ortho-dihydric phenols than moderately resistant and susceptible ones. Inoculation of plants with X. oryzae resulted in an increase in total and ortho-dihydric phenols in all the varieties but it was conspicuous in the resistant variety. Sridhar and Ou (1974) reported that blast resistant rice variety contained higher levels of total and ortho-dihydric phenols. Tripathi and Chiranjeevi (1976) noticed higher levels of total phenols in the leaves of sorghum plants infected with zonate leaf spot pathogen. Jalali et al. (1976) did not observe any significant differences in the total phenols of cotton cultivars resistant and susceptible to bacterial blight. They also found that leucoanthocyanin was present in much greater quantity in resistant than susceptible cultivar. Avila et al. (1981) noticed more anthocyanin content in Macrophomina phaseolina resistant cultivars of Phaseolus vulgaris than other susceptible ones. Thind et al. (1982) observed an increased level of total phenols in moderately resistant variety of chilli and a decrease in the susceptible one due to infection by X. campestris pv. vesicatoria. Khatri et al. (1983a) reported the decrease in total and ortho-dihydric

phenols in soybean leaves infected with X. campestris pv. phaseoli sojense and also in french bean leaves infected with X. campestris pv. phaseoli.

Vyas and Chile (1980a, b) ascertained the role of phenolics in Phytophthora incidence of betel vine and found that the least susceptible Madrasi variety possessed the maximum amount of total phenols whereas the most susceptible Kapoori pan leaves contained the least.

2.5.2 Changes in chlorophylls

Photosynthesis is a basic function of green plants that enable them to transform light energy into chemical energy which they utilise in the cell activities. In leaf spot, blight and other kind of diseases, there is destruction of leaf tissues and obviously photosynthesis is reduced because of the reduction of the photosynthetic surface of plants (Agrios, 1978).

Studies conducted at Jabalpur on the effect of X. campestris pv. betlicola infection on the photosynthetic pigments of betel vine plants showed greater loss of chlorophyll 'a' than chlorophyll 'b' in infected leaves (Jain et al., 1982).

Marimuthu (1978) noted a decrease in chlorophyll pigments in susceptible green gram variety inoculated with X. phaseoli. Dagar et al. (1979) reported more than 80 per cent reduction in total chlorophyll in lemon varieties infected with X. citri. Gupta et al. (1983) noticed that the loss of chlorophyll increased with the progress in disease development in bacterial blight infected cow pea leaves. Rapid loss of chlorophyll in pepper leaves inoculated with X. campestris pv. vesicatoria was reported by Stall and Hall (1984).

2.5.3 Changes in sugars

The changes in sugar content in betel vine cultivars as influenced by X. campestris pv. betlicola infection has not been studied so far. However, the changes in carbohydrate metabolism in other host plants due to diseases has been studied by several workers.

A decrease in reducing sugar content was noticed in the susceptible variety of gingelly (Sesamum indicum L.) infected with Pseudomonas sesami, while the resistant variety showed an increase (Thomas and Orellana, 1962). Easwaran (1967) observed a high quantity of reducing sugar in sorghum variety most susceptible to bacterial disease caused by X. rubrisorghi than in the moderately susceptible one. Inoculation with the pathogen led to

a decrease in the sugar level in both the resistant and susceptible varieties. Sinclair et al. (1970) noted an increase in carbohydrate in pepper leaves inoculated with X. vesicatoria. Prasad et al. (1972) reported that the bacterial leaf blight resistant rice variety contained lesser quantities of reducing, non-reducing and total sugars than the susceptible and moderately susceptible varieties. Inoculation with the pathogen resulted in a general reduction in reducing and total sugars and an increase in non-reducing sugar. Vidhyasekaran (1974) found that the finger millet leaves which were susceptible to Helminthosporium nodulosum contained less total sugar than the resistant ones.

Reddy and Sridhar (1975) noted higher levels of reducing and non-reducing sugars in leaves of bacterial leaf blight susceptible rice variety than less susceptible ones. The decrease in reducing and non-reducing sugars in rice leaves infected with X. oryzae was also reported (Moses et al., 1976). Deiveekasundram and Prasad (1980) observed less reducing sugar in bacterial leaf blight resistant rice variety compared to the highly susceptible variety. On the other hand, the non-reducing and total sugars were more in the resistant than the susceptible variety. Marimuthu and Kandaswamy (1981) attributed the susceptibility of green gram variety to X. campestris pv.

phaseoli to the high level of reducing sugar and the resistance to low glucose level in the leaves. Khatri et al (1983a) noticed a decrease in total soluble sugar in infected leaves than normal ones in soybean infected by X. campestris pv. phaseoli sojense and french bean infected by X. campestris pv. phaseoli. Infected leaves of soybean exhibited a decrease while french bean showed an increase in non-reducing and reducing sugars compared to the healthy ones.

2.5.4 Changes in nitrogen contents

Alteration of nitrogen metabolism in plants in response to pathogenic invasion has been reported by several workers (Shaw, 1963; Kiraly and Farkas, 1959; Uritani, 1963; Van Andal, 1966). Enhanced protein synthesis was demonstrated by Lee (1952) in tissues infected by bacterial pathogen. Wolf and Wolf (1955) noticed significantly more quantities of total nitrogen in tobacco plants infected with the bacterial wilt pathogen. Nayudu and Walker (1961) reported a decrease in amino acid content in tomato plant infected with the bacterial leaf spot pathogen. Patel and Walker (1963) observed no appreciable changes in amino acid content in the resistant varieties of bean plants infected with the halo blight bacterium. Sinclair et al. (1970)

noticed an increase in amino acid and protein contents in pepper leaves infected with X. vesicatoria.

Prasad et al. (1972) observed that rice variety susceptible to bacterial leaf blight contained greater amounts of amino nitrogen, total nitrogen and crude protein than the moderately susceptible and resistant varieties. Inoculation of the pathogen resulted in a significant increase in amino nitrogen, total nitrogen and crude protein in all these varieties and the increase was more pronounced in the susceptible one. Reddy and Sridhar (1975) found that the leaves of highly susceptible rice variety to X. oryzae contained higher concentration of amino acids than less susceptible one. Karwasra and Chand (1981) noticed higher level of amino acids in healthy leaves of guar (Cyamopsis tetragonoloba) than in leaves infected with X. cyamopsidis. Singh and Saksena (1983) found that powdery mildew resistant pea variety contained less amount of total nitrogen and crude protein compared with the leaves of the susceptible one. Marimuthu and Kandaswamy (1983) reported that the moderately resistant mung line to bacterial leaf blight pathogen contained significantly greater amounts of amino nitrogen than the susceptible line. Upon inoculation, the moderately resistant variety registered a

progressive increase in the level of amino nitrogen while the susceptible one recorded a reduction.

Chile and Vyas (1983) studied the free amino acids in relation to Phytophthora leaf rot pathogenesis in Piper betle and found that the amount of free amino acids in uninoculated leaves was more in the less susceptible Madrasi variety than in the highly susceptible Kapoori variety. However, the changes in nitrogen metabolism in betel vine was not studied so far in relation to X. campestris pv. betlicola infection.

2.5.5. Changes in mineral metabolism

Khatri et al. (1983b) studied the ionic imbalance in the betel vine leaves infected with X. campestris pv. betlicola and suggested that the ionic imbalance was due to the altered permeability of host cells by some toxins. The analysis of leaked out electrolyte showed more of potassium than sodium ions.

Sinclair et al. (1970) noticed an increase in magnesium, potassium and sodium contents in pepper leaves infected with X. vesicatoria. However, there was only very little change in the manganese, calcium, copper and iron contents. Philip and Devadath (1981) found that bacterial leaf blight infected rice leaves contained

higher levels of iron and manganese and lower amounts of potassium and phosphorus. Singh and Saksena (1983) recorded higher amounts of potassium, calcium and magnesium in the pea cultivars resistant to the powdery mildew disease than in susceptible ones. The differences in phosphorus and sulphur contents in the resistant and the susceptible cultivars were insignificant.

2.5.6 Changes in phylloplane microflora

In nature, interactions are known to take place between pathogenic and saprophytic microbes as well as among the pathogens themselves. Blakeman and Brodie (1976) studied the inhibition of pathogen by epiphytic bacteria of aerial plant surface and reported that the production of acid, competition for nutrients and stimulation of host defense helped the plant in fighting the pathogen. Shekhawat and Chakravarthi (1977) reported the seasonal variation in the microbial populations on the surface of chilli leaves infected with X. vesicatoria. Philip and Devadath (1980) noticed some difference in the phylloplane fungal and bacterial flora of rice varieties tolerant and susceptible to the bacterial leaf blight disease.

2.6 Effect of host nutrition on the disease development

There are no studies on the effect of host nutrition on the development of bacterial leaf spot of betel vine. But, there are reports about the influence of nitrogen, phosphorus and potassium on the development of other bacterial diseases. The nitrogen application was positively correlated with lesion development of bacterial leaf blight of rice regardless of phosphorus and potassium levels (Kum and Cho, 1970). They also found that phosphorus and potassium twice the normal levels tended to stimulate lesion development. Reddy and Sridhar (1975) noticed that increase in potassium supply had no effect on the bacterial leaf blight in highly susceptible rice variety. Ho and Lim (1978) observed that nitrogen application affected the bacterial leaf blight of rice significantly, while phosphorus and potassium had very little or no effect. Subramanian et al. (1982) did not find any significant difference in mean disease incidence of bacterial blight of cotton at different levels of nitrogen.

The effect of manuring on the development of different fungal diseases of betel vine, was studied by some workers. Chowdhury (1944) reported that the percentage of mortality of the betel vine plants due to

infection by Rhizoctonia solani was not influenced by the nature of the fertilizers used. Subramanian and Rao (1970) noted that application of super phosphate reduced the mortality of betel vine plants infected with Phytophthora nicotiana var. parasitica. Thyagarajan et al. (1972) reported that application of farm yard manure in combination with super phosphate reduced the wilt of betel vine caused by P. nicotiana var. parasitica, while application of potassium had the reverse effect. It was reported from Jabalpur that betel vine plants receiving more phosphorus than nitrogen and potassium showed less mortality due to the foot rot and wilt pathogen (Jain et al., 1982).

2.7 Disease control with chemicals

In addition to the use of resistant varieties, crop rotation and sanitation, the control of bacterial diseases can be obtained to some extent by spraying several times with chemicals such as Bordeaux mixture, other copper compounds, Zineb, antibiotics such as Streptocycline and Tetracycline (Agrios, 1978).

For satisfactory control of bacterial leaf spot of betel vine, drenching and spraying of Bordeaux mixture once in every two months was recommended (Asthana and Mahmud, 1945). Thirumalachar et al. (1956) reported

the *in vitro* effectiveness of Terramycin, Chloromycetin and Dihydrostreptomycin against *X. betlicola*. Seneviratne and De (1963) noticed the promising effect of Phytomycin and Agrimycin-100 in controlling the bacterial leaf spot of betel vine. The utility of Streptomycin, Tetracycline and Streptocycline spray in controlling the bacterial leaf spot of betel vine was reported by Nema *et al*. (1975). The efficacy of Streptomycin sulphate, Chlorotetracycline, Oxytetracycline, Tetracycline and Agrimycin-100 in inhibiting the growth of *X. betlicola* was also reported by Kotwal (1978). Mathew *et al*. (1979a, b) tested the *in vitro* sensitivity of six antibiotics against *X. betlicola* and found that Chloramphenicol at 500 ppm exerted the maximum inhibition. Gupta (1981) reported the inhibitory effect of Streptomycin sulphate, Tetracycline, Oxytetracycline and Vitavax against this leaf spot pathogen. Jain and Nayak (1981) recommended the treatment of betel vine cuttings in solutions of Bordeaux mixture and Streptocycline for 20 minutes and foliar spray of Streptocycline soon after symptom expression for the control of bacterial leaf spot disease. Abraham and Mathew (1982) reported that Chloramphenicol and Terramycin were more inhibitory to *X. campestris* pv. *betlicola* than Agrimycin-100, Streptocycline and Streptomycin. They also observed that none of the antibiotics tested gave

an absolute control of the disease in field. However, Terramycin 500 ppm gave considerable control of the disease. *In vitro* studies conducted at Jabalpur showed that Streptocycline, Agrimycin-100 and Paushamycin gave maximum inhibition to *X. campestris* pv. *betlicola*. In field trial using seven antibiotics and Bordeaux mixture, it was found that the combination of one per cent Bordeaux mixture with Agrimycin-100 was the most effective in controlling the disease than antibiotics alone (Jain *et al*., 1982). Tripathi *et al*. (1984) evaluated the efficiency of five antibiotics, two phenols and a naphthaquinone for their *in vitro* toxicity against *X. campestris* pv. *betlicola* and found that Streptomycin was the most effective. In the field Streptomycin gave good control of the disease.

Attempts to control many bacterial plant diseases by fungicides were tried by several workers. Miller (1970) reported that a mixture of basic copper sulphate, Maneb and Agristep gave good control of bacterial leaf spot of Ixora. Sood *et al*. (1976) evaluated the *in vitro* efficacy of Dithane M-45, Dithane Z-78, Bavistin, Blitox, Benlate and the combination of Streptocycline with copper sulphate along with three antibiotics against *X. phaseoli* causing leaf spot of mung and reported that all the chemicals tested were effective in inhibiting the growth of the bacterium. The effectiveness of

Bordeaux mixture, copper oxychloride and Kocide in controlling the bacterial blight of walnut was recorded by Severin and Kupferberg (1977). Chauhan and Vaishnav (1980) noticed the in vitro and in vivo efficacy and Streptocycline and copper compounds against X. oryzae. Strider (1980) reported that weekly foliar application of Captan controlled the spread of bacterial leaf spot of zinnia. Parson and Edgington (1980) observed the efficacy of copper and dithiocarbamate fungicides and their combination in the control of bacterial speck of tomato. Sharma et al. (1982) reported that among the [redacted] various treatments the combination of Streptocycline and copper sulphate was most effective against X. campestris pv. vesicatoria in in vitro. Krishna and Nema (1983) noted that combination of Bordeaux mixture with Streptocycline gave maximum control of citrus canker followed by Streptocycline. Combination of Bordeaux mixture with Plantomycin also gave good control of this disease.

2.8 Screening for host resistance

Asthana and Mahmud (1945) stated that the Kapoori variety of betel vine was the most susceptible to bacterial leaf spot in central provinces of India. Patel et al. (1951, 1953) reported that bacterial leaf spot of betel vine was quite common in gardens of former

Bombay state and all the varieties were equally susceptible to this disease to varying degrees. Singh and Chand (1971) and Karbhari (1976) noticed the wide spread prevalence of this disease in Jabalpur and they individually reported that the variety - Bangla was the most susceptible one.

Abraham and Mathew (1981) reported that of the seven cultivars of betel vine screened against X. campestris pv. betlicola, none was resistant. However, the cultivars Tulasivettila and Karilanchikarpuran were less susceptible to the disease. Studies conducted at Jabalpur showed that the Desi Bangla and Bilhari varieties were highly susceptible to X. campestris pv. betlicola whereas, the cultivar Bangla obtained from Mandsaur was resistant (Jain et al., 1982).

Materials and Methods

MATERIALS AND METHODS

3.1 The pathogen

3.1.1 Isolation

Infected betel vine leaves were collected from severely disease affected betel vine gardens near the College of Agriculture, Vellayani. These leaves were subjected to the ooze test to confirm the presence of bacteria. The diseased leaves with profuse bacterial ooze were selected and the infected areas were cut into bits and surface sterilized with 0.1 per cent mercuric chloride solution for one minute. These bits were washed in three changes of sterile water and placed on a sterile glass slide with one or two drops of sterile water. These bits were teased apart to get the bacterial suspension. The suspension was streaked on petri plates having Potato Sucrose Peptone Agar (PSPA) medium (Appendix-I) to obtain well isolated colonies of the pathogen.

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.

Similarly isolation of the bacteria from infected betel vine stem was also carried out.

3.1.2 Pathogenicity test

Pathogenicity of the bacterium was proved by inoculating a thick suspension of 48 h old culture on betel vine plants. The suspension of the bacterium was prepared in sterile distilled water. Inoculation was done by smearing a thick suspension of the bacteria on both surface of the pin pricked betel vine leaves by means of a cotton swab. Stem inoculation was also carried out by smearing the bacterial suspension on the betel vine stem by a cotton swab without any injury. This method of inoculation was carried out throughout the period of study, unless otherwise mentioned. Inoculated plants were kept under shade and sufficient humidity was maintained.

The pathogen was re-isolated from artificially inoculated plant by the method already described. Morphological characters of the re-isolated colonies like their colour, fluidity and slime were compared with that of the original isolates to ensure their identity. Stock cultures of the bacterium were maintained in PSPA medium under refrigerated condition and also by periodic sub-culturing at room temperature.

Four isolates of the pathogen were collected from different betel vine growing areas of Kerala and the details of the different isolates are as follows:

Isolates	Cultivar of betel vine from which isolation was made	Location of collection
Xcb-1	Cheelanthikarpuran	Neyyatinkara, Trivandrum District
Xcb-2	Cheelanthikarpuran	Instructional Farm, College of Agriculture, Vellayani, Trivandrum District
Xcb-3	Tulasivettila Type II	Muttakkad, Trivandrum District
Xcb-4	Pozhikodi	Manjeri, Malappuram District

3.1.3 Cultural and biochemical characters of different isolates of the pathogen

The cultural and biochemical characters of different isolates of the bacterium such as colony character, gram reaction, pigment production, oxygen requirement, mode of utilization of glucose, starch hydrolysis, hydrogen sulphide production, catalase test, MR and VP tests, gelatin liquefaction, nitrate reduction, indole production, lipolytic activity, tyrosinase activity and arginine hydrolase test were studied following the methods recommended in the Manual of

Microbiological Methods, published by the Society of American Bacteriologists (1957) and those suggested by Dye (1962).

In addition to this, the effect of different levels of pH, temperature, nitrogen and carbon sources including amino acids on the growth of the isolates of the bacterium were also studied.

3.1.4 Effect of pH on the growth of different isolates of the pathogen

The basal medium for Xanthomonads (Dye, 1962) (Appendix-II) was used to study the growth of the isolates at different levels of pH. Fifty ml of the broth with different levels of pH, namely, 4, 5, 5.5, 6, 6.5, 6.8, 7, 7.5, 8, 9, 10 and 10.5 were dispensed in 100 ml conical flasks separately and sterilized by autoclaving. These flasks were inoculated with one ml of standardised suspension of the isolates of the bacterium and incubated at room temperature with periodic shaking. Three replications were maintained for each isolate and each level of pH and the uninoculated broth served as control. Growth of the bacterium was read in Spectronic-20 colorimeter at 640 nm after 96 h and the optical density was calculated from the per cent light transmission.

3.1.5 Effect of temperature on the growth of different isolates of the pathogen

The basal medium for Xanthomonads (Dye, 1962) was used for studying the growth of the bacterial isolates at different temperatures. Fifty ml of the liquid medium was dispensed in 100 ml conical flask and such flasks were sterilised by autoclaving. These flasks were inoculated with one ml of standardised suspension of the bacterial isolates and incubated at different temperatures of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 and 55°C with periodic shaking. Three replications were maintained for each isolate and temperature. After 96 h, the growth of the bacterium was measured in Spectronic-20 colorimeter at 640 nm against an appropriate control. From the per cent light transmission the optical density was calculated.

3.1.6 Effect of carbon sources on the growth of different isolates of the pathogen

The broth of the synthetic medium (Dye, 1962) was used for this study. Cellulose, cellobiose, dextrose, dulcitol, galactose, glucose, inositol, lactose, mannose, mannitol, melicitose, raffinose, starch and sucrose were used for the study on equivalent weight basis. Fifty ml of the medium containing each of the above carbon sources

was dispensed in 100 ml conical flask and sterilized by autoclaving. The flasks were inoculated with one ml of standardised suspension of the bacterial isolates and incubated at room temperature with periodic shaking. Three replications were maintained for each isolate and carbon source. After 96 h, growth of the bacterium was read at 640 nm in Spectronic-20 colorimeter against an appropriate control. Optical density was calculated from the per cent light transmission.

3.1.7 Effect of nitrogen sources on the growth of different isolates of the pathogen

The composition of the medium used for this study is given in Appendix-III.

Ammonium nitrate, asparagine, potassium nitrate, sodium nitrate and sodium nitrite were used as the nitrogen sources on equivalent weight basis. Fifty ml of the nitrogen source containing medium was dispensed in 100 ml conical flask and sterilized by autoclaving. The flasks were inoculated with one ml of standardised suspension of the bacterial isolates and incubated at room temperature with periodic shaking. Three replications were maintained for each isolate and nitrogen source. After 96 h, the growth of the bacterium was

read at 640 nm in Spectronic-20 colorimeter against an appropriate control. Optical density was calculated from the per cent light transmission.

3.1.8 Effect of amino acids on the growth of different isolates of the pathogen

Fifty ml of the synthetic medium (Dye, 1962) was dispensed in 100 ml conical flask. The amino acids arginine, cysteine, glutamic acid, glycine, histidine, lysine, methionine, proline, threonine and tyrosine at 0.1, 0.01 and 0.001 molar concentrations were incorporated into the medium. Water insoluble amino acids were first dissolved in minimum quantity of either 80 per cent ethanol or dilute hydrochloric acid (0.01N) and added to the medium. The final pH of the medium was adjusted to 7.0 and sterilized by autoclaving. Three replications were maintained. The flasks were inoculated with one ml of standardised suspension of bacterial isolates and incubated at room temperature with periodic shaking. The growth of the bacterium was read after 96 h in Spectronic-20 colorimeter at 640 nm against an appropriate control. The optical density was calculated from the per cent light transmission.

3.2 Symptomatology of bacterial leaf spot of betel vine

A detailed study was conducted on the symptomatology of the disease both under natural and artificial conditions. Symptoms of the disease under natural condition were studied in a betel vine garden severely infected with the pathogen. For studying the symptoms of the disease under artificial condition the cuttings of the cultivar Cheelanthikarpuran were raised in earthen pots and inoculated with the pathogen. After inoculation, the plants were kept under shade and sufficient humidity was maintained. The symptom development was studied by daily observing the inoculated plants upto a period of one month.

3.3 Histopathology

To study the histopathology of betel vine leaves infected with the pathogen, naturally infected leaves showing different stages of infection were cut into small pieces and fixed in formalin-acetic acid alcohol mixture (FAA) for one week. These tissues were dehydrated in tertiary butyl alcohol series and embedded in paraffin wax with ceresin (Jensen, 1962). They were then sectioned at 10 µ with a rotary hand microtome. The sections were affixed on the slides by Haupt's adhesive (Johanson, 1940) and stained with

hematoxylin and orange G (Johanson, 1940) using the schedule of Nelson and Dicky (1966) (Appendix-IV). Similarly sections of healthy leaves were also taken for comparison.

3.4 Host range of the pathogen

The following plants belonging to the family Piperaceae were used for the study.

Host	Location of collection
Piper nigrum L.	College of Agriculture, Vellayani, Trivandrum District
Piper longum L.	Konni, Pathanamthitta District
Piper attenuatum L.	Kerala University Campus, Karyavattom, Trivandrum District
Piper sp. Type I	Silent Valley, Palghat District
Piper sp. Type II	Moonnar, Iddikki District
Piper sp. Type III	Silent Valley, Palghat District
Piper sp. Type IV	Ponmudi, Trivandrum District
Peperomia pellucida WP&K	College of Agriculture, Vellayani, Trivandrum District
Peperomia sp. Type I	Government Museum, Trivandrum, Trivandrum District

The cuttings of the plants were raised in earthen pots and inoculated with the suspension of the bacterium after giving pin pricks. The inoculated plants were covered with polythene bags and kept in shade to provide sufficient humidity and these were observed for disease development up to a period of one month. Plants showing symptoms of the disease were subjected to ooze test to confirm the presence of bacteria.

The host plants which took up infection were selected and again inoculated with the suspension of the pathogen and the symptomatology was studied in detail up to a period of one month.

3.5 Biochemical changes in resistant and susceptible cultivars of betel vine as influenced by *X. campestris* pv. *betlicola* inoculation

An attempt was made in the present investigation to study the biochemical changes in resistant and susceptible cultivars of betel vine due to infection by *X. campestris* pv. *betlicola*. Based on the study on the screening of betel vine cultivars for host resistance against the pathogen, one each from resistant and susceptible cultivars was selected and grown in earthen pots containing potting mixture.

When the plants were five monthsold, they were artificially inoculated with suspension of the bacterium. For biochemical analysis, leaf samples of same age were taken from healthy and inoculated plants of both resistant and susceptible cultivars after 0, 3, 6, 9, 12 and 15 days of inoculation. Three replications were maintained for all the analyses.

Preparation of alcohol extract

Leaf tissues of healthy and inoculated plants of both resistant and susceptible cultivars of betel vine were separately cut into small pieces and placed in boiling ethyl alcohol (10 ml for each g of tissue) for 10 minutes. The extract was cooled and the tissues were crushed thoroughly in a mortar and pestle. The contents were filtered through two layers of cheese cloth. The residue was re-extracted for 3 minutes in hot 80 per cent alcohol (3 ml for each g of tissue). The contents were cooled and filtered through cheese cloth. Both extracts were combined and again filtered through Whatman No.41 filter paper. The filtrate was concentrated and made to known volume (Mahadevan and Sridhar, 1982). The alcohol extract was used for the estimation of ortho-dihydric phenol, total phenols, anthocyanin, leucoanthocyanin and amino nitrogen.

3.5.1 Estimation of ortho-dihydric phenol

Estimation of ortho-dihydric phenol in leaf samples was carried out by the method described by Johnson and Schaal (1957). One ml of alcohol extract was pipetted into a test tube. To this, one ml of 0.5N HCl, one ml of Arnow's reagent (10 g $NaNO_2$ and 10 g Na_2MoO_4 dissolved in 100 ml distilled water), 10 ml of distilled water and 2 ml of 1 N NaOH were added. The solution was diluted to 50 ml and the absorbance of the pink solution was read in a colorimeter at 515 nm. All reagents except the ethanol extract served as blank. Catechol was used as the standard.

3.5.2 Estimation of total phenols

The total phenols was estimated by employing Folin-Ciocalteu reagent (Bray and Thorpe, 1954). One ml of alcohol extract was taken in a test tube. To this one ml of Folin-Ciocalteu reagent and 2 ml of 20 per cent sodium carbonate were added. The contents was shaken and heated for one minute on a boiling water bath and cooled in running water. The solution was diluted to 25 ml with distilled water and filtered. The intensity of blue colour developed was read at 650 nm in a colorimeter. The total phenol content in the sample was calculated from a standard curve prepared with catechol and was expressed as catechol equivalent.

3.5.3 Estimation of anthocyanin

The method described by Swain and Hillis (1959) was employed for the estimation of anthocyanin. One ml of the ethanol extract was taken in a test tube and 3 ml of HCl in aqueous methanol (0.5N HCl in 80-85 per cent methanol) was added followed by one ml of hydrogen peroxide reagent (1 ml of 30 per cent H_2O_2 mixed with 9 ml of methanolic HCl 5:1, 3N). The test tubes were kept in dark for 15 minutes and the absorbance was measured at 525 nm in Spectronic-20 colorimeter against the blank. The result was expressed as absorbance at 525 nm.

3.5.4 Estimation of leucoanthocyanin

One ml of the alcohol extract was taken in a test tube. The volume of the extract was reduced to 0.5 ml on a boiling water bath. To this, 0.5 ml distilled water and 10 ml of leucoanthocyanin reagent (25 ml Con. HCl diluted to 500 ml with n-butanol) was added and mixed. The tubes were heated on a water bath at 97 $\pm$ 1°C for 3 minutes without covering. Later, the tubes were covered with glass stopper and heated for 40 minutes. The tubes were cooled in running water. The blanks were maintained with the extract but without heating. The absorbance of the solution was

measured at 550 nm in Spectronic-20 colorimeter and result was expressed as absorbance at 550 nm (Mahadevan and Sridhar, 1982).

3.5.5 Estimation of chlorophylls

The chlorophyll content of the leaves was estimated by the method described by Arnon (1949). One gram of fresh leaf tissues was cut into small pieces and homogenised with 80 per cent acetone in a mortar with pestle. The homogenised material was filtered through a Buchner funnel using Whatman No.42 filter paper. The extraction was repeated three times with 80 per cent acetone. The filtrates were pooled together and the volume was made up to 100 ml in a volumetric flask. Five ml of the extract was transferred into a 50 ml volumetric flask and made up the volume with 80 per cent acetone. The absorbance of the extract was read at 645 and 663 nm in Spectronic-20 colorimeter. Chlorophyll contentswere calculated on fresh weight basis with the formulae given below:

$$\text{Total chlorophyll (mg/g)} = \frac{20.2\ A_{645} + 8.02\ A_{663}}{a \times 1000 \times W} \times V$$

$$\text{Chlorophyll 'a' (mg/g)} = \frac{12.7\ A_{663} - 2.69\ A_{645}}{a \times 1000 \times W} \times V$$

$$\text{Chlorophyll 'b' (mg/g)} = \frac{22.9\ A_{645} - 4.68\ A_{663}}{a \times 1000 \times W} \times V$$

where a = length of light path in the cell (1 cm)

V = volume of the extract

W = fresh weight of the sample in g

A = absorbance

3.5.6 Estimation of carbohydrate fractions

The carbohydrate fractions were estimated according to the methods suggested by Somogyi (1945) and Nath (1970).

Dried leaf samples (500 mg) were extracted in boiling 80 per cent ethanol for 30 minutes and the supernatent was collected. The residue was re-extracted serially with 60, 40 and 30 per cent ethanol and lastly in distilled water and the supernatents were collected. The supernatents were pooled together and the volume was reduced to 20 ml on a boiling water bath. To this, one ml of saturated lead acetate was added and filtered into a volumetric flask containing 3 ml of saturated Na_2HPO_4. The precipitate was washed and the volume was made up to 100 ml with distilled water. The extract in the volumetric flask was allowed to settle and the supernatent was used for the estimation of reducing, non-reducing and total sugars.

3.5.7 Estimation of reducing sugar

To 0.5 ml of the ethanol extract one ml of Somogyi's reagent (12 g of sodium potassium tartrate and 24 g of anhydrous Na_2CO_3 dissolved in 200 ml of distilled water, 4 g of $CuSO_4$ dissolved in 40 ml of H_2O and mixed the two solutions. In this combined solution, 16 g of $NaHCO_3$ was added and dissolved. Separately dissolved 180 g of anhydrous Na_2SO_4 in 500 ml hot water. Mixed the two solutions and made upto one litre) was added and heated on a boiling water bath for 12 minutes and cooled under running tap water. To this one ml of Nelson's reagent (25 g of ammonium molybdate dissolved in 450 ml of water and to this 21 ml of Con. H_2SO_4 was added and mixed with 3 g of sodium arsenate dissolved in 25 ml of water. The above solutions were mixed and kept in an incubator at 37°C for 24 to 48 h before use) was added and made up to 10 ml. The absorbance of the coloured solution was read at 530 nm in a colorimeter. The reagent blank consisted of distilled water instead of ethanol extract. Glucose was used as the standard.

3.5.8 Estimation of non-reducing and total sugars

To 5 ml of the alcohol extract 5 ml of 0.5N HCl was added and heated on a boiling water bath for 30 minutes

and cooled in running tap water and the volume was made up to 10 ml with distilled water. The contents were neutralized with 0.5N NaOH and then the volume was made up to 15 ml with 0.1N oxalic acid and the sugar content was estimated as in the case of reducing sugar. Blank contained distilled water instead of alcohol extract. Glucose was used as the standard. The difference between the total sugar and the reducing sugar estimated without hydrolysis corresponds to the non-reducing sugar.

3.5.9 Estimation of amino nitrogen

The amino nitrogen content was determined by employing the ninhydrin method (Moore and Stein, 1948). One ml of alcohol extract was pipetted into a test tube. The sample was neutralized with 0.1N NaOH using methyl red as indicator. To this, one ml of freshly prepared ninhydrin reagent (Dissolved separately 800 mg of hydrated stannous chloride in 500 ml of the citrate buffer of pH 5 and 20 g of re-crystallized ninhydrin in 500 ml of methyl cellosolve, and the two solutions were mixed) was added. The solution was mixed and heated on a boiling water bath for 20 minutes with a glass marble on the top of the test tube. Five ml of diluent solution (equal volume of glass distilled water and n-propanol) was added into the test tube while still in the water bath. Then the tubes were cooled in running tap water and mixed thoroughly. The content was diluted with diluent solution. The absorbance of

the purple colour developed was read at 570 nm in a colorimeter. Blank contained one ml of distilled water instead of alcohol extract. The amount of amino nitrogen present in the sample was calculated using a standard curve prepared with glycine.

3.5.10 Estimation of total nitrogen

The total nitrogen content in plant samples was determined by micro-kjeldahl digestion - distillation process as described by Jackson (1973). The total nitrogen content was expressed as percentage. The crude protein content was arrived by multiplying the total nitrogen content by the factor 6.25.

Preparation of triple acid extract

Triple acid extract was used for the determination of P, K, Ca, Mg and Na in the leaf samples (Johnson and Ulrich, 1959). For this, dried and powdered leaf samples each of one g were digested with triple acid mixture (9:2:1, Con. HNO_3: Con. H_2SO_4:Con. $HClO_3$) until clear. The digest was made up to 100 ml with distilled water, filtered and used for further analysis.

3.5.11 Estimation of phosphorus

From an aliquot of the triple acid extract of plant sample, phosphorus was determined by the

Vanadomolybdic yellow colour method in nitric acid system as described by Jackson (1973).

3.5.12 Estimation of potassium and sodium

Potassium and sodium contents in the triple acid extract were estimated using EEL flame photometer after making suitable dilutions.

3.5.13 Estimation of calcium and magnesium

Calcium and magnesium in the triple acid extract were determined in an Atomic Absorption Spectrophotometer. The concentrations of calcium and magnesium were worked out from standard curve prepared for each of the elements.

3.5.14 Enumeration of phylloplane microflora

Changes in the phylloplane microflora of betel vine cultivars as influenced by the inoculation of the pathogen were assessed. For this, leaf samples were taken from healthy and inoculated plants of resistant and susceptible cultivars after 0, 3, 6, 9, 12 and 15 days of inoculation. From the leaf samples, forty discs of 10 mm diameter were taken and placed into 100 ml sterile distilled water in a flask and shaken for 30 minutes. Sample dilutions of 10^{-1} for fungi,

10^{-4} for bacteria and actinomycetes were prepared. Kuster's agar, Nutrient agar and Potato dextrose agar media (Appendix-V) were used for the enumeration of actinomycetes, bacteria and fungi respectively. One ml of diluted suspension was transferred into a petri plate and then 15 ml of the appropriate medium, cooled just above the solidifying temperature was added. The petri plate was rotated by hand in a swirling motion so that the contents were evenly dispersed in the medium. Three replications were maintained. Plates were incubated at room temperature. Counting of the colonies of actinomycetes, bacteria and fungi was done from 48 h onwards to a period of one week and was expressed as the total number per cm^2.

3.6 Control of bacterial leaf spot of betel vine

The control of bacterial leaf spot of betel vine was attempted in the following lines:

Assessing the effect of host nutrition on the disease development,

Assessing the plant protection chemicals both in <u>in vitro</u> and <u>in vivo</u> for their efficacy in controlling the disease,

Screening of betel vine cultivars for resistance/ tolerance to the disease.

3.6.1 Effect of different levels of nitrogen, phosphorus and potassium on the development of bacterial leaf spot of betel vine

The investigation was carried out to assess the effect of four levels each of nitrogen, phosphorus and potassium on the development of bacterial leaf spot of betel vine. The experiment was laid out in a completely randomised factorial design with two replications. The treatment details are as follows:

Nitrogen

n_0 - no nitrogen

n_1 - 100 kg N/ha

n_2 - 150 kg N/ha

n_3 - 200 kg N/ha

Phosphorus

P_0 - no phosphorus

P_1 - 50 kg P_2O_5/ha

P_2 - 100 kg P_2O_5/ha

P_3 - 150 kg P_2O_5/ha

Potassium

k_0 - no potassium

k_1 - 50 kg K_2O/ha

k_2 - 100 kg K_2O/ha

k_3 - 150 kg K_2O/ha

Nitrogen, phosphorus and potassium were given to the plants in the form of urea, super phosphate and muriate of potash respectively. The cultivar Cheelanthikarpuran was used as a test crop for this experiment. The cuttings of plants were raised in earthen pots containing potting mixture. After establishment of the plantsnitrogen and potassium were applied in two split doses while phosphorus was applied as complete basal. Half dose of nitrogen, full dose of phosphorus and half dose of potassium were applied as first dose after establishment of the cuttings. The second dose of remaining nitrogen and potassium was given one month after first application. After two months of application of second dose, the plants were inoculated with the suspension of the pathogen. Observations on the disease score, percentage of stem infection and percentage of defoliation were recorded after ten and forty days of inoculation.

3.6.2 Chemical control of bacterial leaf spot of betel vine

The efficacy of antibiotics, fungicides and combination of antibiotics and Bordeaux mixture was tested both <u>in vitro</u> and <u>in vivo</u> for the control of bacterial leaf spot of betel vine.

3.6.2.1 In vitro evaluation of chemicals against the pathogen

In vitro efficacy of antibiotics, fungicides and the combination of antibiotics and Bordeaux mixture in inhibiting the growth of the bacterium was tested by the standard filter paper disc method. The details of the chemicals and their concentrations are given below:

Chemicals	Manufacturer	Active ingredient	Concentrations
Paushamycin	Paushak Ltd.	Streptomycin 15% and oxytetracycline 1.5%	100, 200, 300 ppm
Plantomycin	Aries Agro Vet Industries Pvt. Ltd.	Streptomycin sulphate 9% and tetracycline hydroxide	100, 200 300 ppm
Streptocycline	Hindustan Antibiotics Ltd.	Streptomycin sulphate 90% and tetracycline hydroxide 10%	100, 200 300 ppm
Blitox 50	Tata Fison Industries Ltd.	Copper oxychloride	1000, 2000, 3000 ppm
Bordeaux mixture	-	Copper sulphate + lime	0.5, 1%
Dithane M-45	Indofil Chemicals Ltd.	Co-ordination product of Zinc ion + maneb	1000, 2000, 3000 ppm

Chemicals	Manufacturer	Active ingredient	Concentrations
Dithane Z-78	Indofil Chemicals Ltd.	Zinc ethylene bis dithiocarbamate	1000, 2000 3000 ppm
Captaf 50 WP	Rallis India Ltd.	N-trichloro-methyl thio 4-cyclohexene-1, 2-dicarboximide	1000, 2000, 3000 ppm
Pausharnycin + Bordeaux mixture	-	-	100 ppm + 1% 200 ppm + 1% 300 ppm + 1%
Plantomycin + Bordeaux mixture	-	-	100 ppm + 1% 200 ppm + 1% 300 ppm + 1%
Streptocycline + Bordeaux mixture	-	-	100 ppm + 1% 200 ppm + 1% 300 ppm + 1%
Control	-	-	-

The different concentrations of the chemicals were prepared in sterile distilled water. Sterile filter paper discs of 10 mm diameter were dipped in the solutions and placed over PSPA medium seeded with 48 h old culture of the bacterium. The experiment was conducted with three replications. Observations on the zone of inhibition were recorded after 48 h.

3.6.2.2 Pot culture experiment on the chemical control of bacterial leaf spot of betel vine

The experiment was laid out in completely randomised

design with twelve treatments and four replications. The details of the treatments are furnished below:

Treatment	Chemical	Concentration
T_1	Paushamycin	200 ppm
T_2	Plantomycin	200 ppm
T_3	Streptocycline	200 ppm
T_4	Bordeaux mixture	1%
T_5	Blitox	3000 ppm
T_6	Captaf	3000 ppm
T_7	Dithane M-45	3000 ppm
T_8	Dithane Z-78	3000 ppm
T_9	Paushamycin + Bordeaux mixture	200 ppm + 1%
T_{10}	Plantomycin + Bordeaux mixture	200 ppm + 1%
T_{11}	Streptocycline + Bordeaux mixture	200 ppm + 1%
T_{12}	Control	-

The local cultivar Cheelanthikarpuran was used for this experiment. The cuttings of the plants were raised in earthen pots. The plants were inoculated with a suspension of the pathogen when they were five months old. At the onset of the disease on the seventh day after inoculation the chemicals were sprayed. Three

sprayings were given at an interval of fifteen days. Observations on the disease score, percentage of stem infection, percentage of defoliation, percentage of mortality and the disease score on new leaves formed after inoculation were recorded on the day of each spraying and also on the fifteenth day after the final spraying.

3.6.2.3 Field experiment on the chemical control of bacterial leaf spot of betel vine

The experiment was laid out in randomised block design in a farmer's field planted with cultivar Cheelanthikarpuran with twelve treatments and three replications at Vellayani, Trivandrum district. The treatments were same as that for the pot culture experiment.

Naturally infected plants were selected for this experiment. Three sprayings were given at an interval of fifteen days. Initial observations on the disease score and percentage of stem infection were recorded. Further observations on the disease score, percentage of stem infection, percentage of defoliation, percentage of mortality and the disease score on newly formed leaves after the start of chemical spraying were recorded on the fifteenth day after each spraying.

The residual toxicity of the above said chemicals was tested. For this, leaf discs of 10 mm diameter were taken from the treated plants at an interval of 24 h after spraying and placed in petri dishes containing PSPA medium seeded with X. campestris pv. betlicola. Four replications were maintained. Observations on the zone of inhibition due to the residual toxicity was recorded after 48 h.

3.6.3 Screening of betel vine cultivars for host resistance

The following ten cultivars of betel vine, collected from major betel vine growing areas of Kerala were screened for their resistance against the leaf spot pathogen.

	Cultivar	Location of collection
V_1	Cheelanthikarpuran	Near Vellayani Kayal, Trivandrum District
V_2	Tulasivettila Type I	Cannanore, Cannanore District
V_3	Aryan	Nedumangad, Trivandrum District
V_4	Arikodi	Konni, Pathanamthitta District
V_5	Pannivella	Kalliyoor, Trivandrum District

Cultivar		Location of collection
V_6	Njerukali	Neyyattinkara, Trivandrum District
V_7	Tulasivettila Type II	Muttakkad, Trivandrum District
V_8	Cheelanthikarpuran Chuvappu	Muttakkad, Trivandrum District
V_9	Pozhikodi	Manjeri, Malappuram District
V_{10}	Nadankodi	Malappuram, Malappuram District

The experiment was laid out in completely randomised design with eight replications. The plants were raised in earthen pots. When the plants attained the age of five months, they were inoculated with a mixed culture of all the isolates of the bacterium. The inoculated plants were kept under shade and sufficiently high humidity was maintained. Observations on the disease score, percentage of stem infection, percentage of defoliation and percentage of mortality were recorded after ten, twenty, thirty and forty days of inoculation. The intensity of the disease was assessed based on a newly developed scale.

3.6.3.1 Preparation of disease scale

In order to assess the disease intensity precisely, a new disease scale was developed on the basis of the extent of leaf area affected by the pathogen. For this, a number of infected leaves with different stages of infection were collected. The total leaf area and the area infected were calculated using standard graph paper method and grouped into various categories, based on the percentage of leaf area infected. Thus, a 0-9 scale was prepared. For facilitating the computations of complete defoliation and plant mortality two additional scales were provided. Accordingly the following new 0-11 scale was devised:

Disease scale	Percentage of leaf area infected
0	No infection
1	Less than 3
2	3 and less than 6
3	6 and less than 9
4	9 and less than 12
5	12 and less than 15
6	15 and less than 18
7	18 and less than 21
8	21 and less than 24

Disease scale	Percentage of leaf area infected
9	24 and more
10	Complete defoliation
11	Mortality of the plant

3.7 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.

Results

RESULTS

4.1 The pathogen

The bacterial pathogen Xanthomonas campestris pv. betlicola was isolated from infected leaves and stem of betel vine on Potato Sucrose Peptone Agar (PSPA) medium. It produced the characteristic yellow, circular and slimy colonies with entire margins. Four different isolates of the bacterium were used and they produced typical symptoms of the disease on the leaves and stem on artificial inoculation on the susceptible cultivar Cheelanthikarpuran. Re-isolation from artificially infected plants yielded the typical bacterial colonies resembling the original isolates. The cultural and biochemical characters of the different isolates studied are summarised in Table 1.

In addition to the above characters, detailed studies on the effect of different pH, temperature, carbon and nitrogen sources including amino acids on the growth of all the isolates were also carried out.

4.1.1 Effect of pH on the growth of different isolates of X. campestris pv. betlicola

The results of the study are presented in Table 2.

Table 1. Cultural and biochemical characters of different isolates of X.campestris pv. betlicola

Characters studied	Isolates			
	Xcb-1	Xcb-2	Xcb-3	Xcb-4
Gram reaction	-ve	-ve	-ve	-ve
Pigment production				
a) Non water soluble	+	+	+	+
b) Water soluble	-	-	-	-
Oxygen requirement	+	+	+	+
Mode of utilization of glucose				
a) Aerobic	+	+	+	+
b) Anaerobic	-	-	-	-
Starch hydrolysis	+	+	+	+
Hydrogen sulphide production	+	+	+	+
Catalase test	+	+	+	+
MR and VP tests	-	-	-	-
Gelatin liquefaction	+	+	+	+
Nitrate reduction	-	-	-	-
Indole production	-	-	-	-
Lipolytic activity	+	+	+	+
Tyrosinase activity	-	-	-	-
Arginine hydrolase activity	+	+	+	+

-ve - Gram negative
\+ - Positive reaction
\- - Negative reaction

Table 2. Effect of pH on the growth of different isolates of X. campestris pv. betlicola

pH	Optical density of isolates			
	Xcb-1	Xcb-2	Xcb-3	Xcb-4
4	0	0	0	0
5	0.118	0.110	0.125	0.133
5.5	0.141	0.139	0.153	0.155
6	0.189	0.155	0.157	0.159
6.5	0.270	0.192	0.189	0.192
6.8	0.325	0.215	0.221	0.215
7	0.263	0.201	0.178	0.222
7.5	0.217	0.135	0.133	0.181
8	0.178	0.116	0.119	0.131
9	0.086	0.047	0.037	0.056
10	0.054	0.039	0.029	0.049
10.5	0	0	0	0

(Mean of three replications)

Maximum growth of the isolates of the pathogen was observed between pH 6.5 and 7. Though, the isolates Xcb-1, Xcb-2 and Xcb-3 showed maximum growth at pH 6.8, the best pH for the isolate Xcb-4 was found to be 7. Growth of the isolates of the bacterium at pH 5, 8, 9 and 10 was very meagre. None of the bacterial isolates showed any growth at pH 4 and 10.5.

4.1.2 Effect of temperature on the growth of different isolates of X. campestris pv. betlicola

Among the different temperatures tested, all the isolates of the bacterium showed good growth at 25 and 30°C (Table 3). Maximum growth of the isolate Xcb-3 was at 25°C, while that of isolates Xcb-2 and Xcb-4 was at 30°C. The isolate Xcb-1 did not show much difference in growth at 25 and 30°C. Above 40 and at 5°C, the growth of the bacterial isolates was negligible and at 55°C all of them failed to grow.

4.1.3 Effect of carbon sources on the growth of different isolates of X. campestris pv. betlicola

The isolates of the bacterium showed varying growth rates in different carbon sources tested (Table 4). In general, all the tested carbon sources supported growth of the isolates. Starch incorporated medium supported

Table 3. Effect of temperature on the growth of different isolates of X. campestris pv. betlicola

Temperature °C	Optical density of isolates			
	Xcb-1	Xcb-2	Xcb-3	Xcb-4
5	0.074	0.095	0.061	0.064
10	0.131	0.144	0.131	0.163
15	0.154	0.161	0.141	0.171
20	0.164	0.194	0.189	0.206
25	0.305	0.238	0.243	0.237
30	0.304	0.295	0.223	0.293
35	0.143	0.145	0.136	0.135
40	0.057	0.091	0.069	0.046
45	0.033	0.051	0.036	0.038
50	0.021	0.021	0.018	0.019
55	0	0	0	0

(Mean of three replications)

Table 4. Effect of carbon sources on the growth of different isolates of X. campestris pv. betlicola

Carbon source	Optical density of isolates			
	Xcb-1	Xcb-2	Xcb-3	Xcb-4
Cellulose	0.165	0.166	0.135	0.174
Cellobiose	0.139	0.189	0.166	0.157
Dextrose	0.163	0.135	0.099	0.123
Dulcitol	0.183	0.187	0.164	0.199
Galactose	0.187	0.157	0.163	0.175
Glucose	0.108	0.131	0.103	0.135
Inositol	0.179	0.170	0.184	0.170
Lactose	0.121	0.189	0.166	0.157
Mannose	0.195	0.183	0.153	0.205
Mannitol	0.117	0.240	0.106	0.131
Melicitose	0.149	0.223	0.203	0.222
Raffinose	0.197	0.240	0.179	0.158
Starch	0.233	0.234	0.192	0.206
Sucrose	0.194	0.187	0.160	0.249

(Mean of three replications)

the maximum growth of the isolate Xcb-1, while the minimum growth was in glucose. Maximum growth of the isolate Xcb-2 was in raffinose and mannitol incorporated medium, whereas glucose and dextose containing medium showed poor growth. In the case of isolate Xcb-3, maximum growth was recorded in melicitose. Minimum growth of this isolate was observed in glucose and dextrose incorporated medium. The maximum growth of the isolate Xcb-4 was in medium containing sucrose, while the minimum growth was registered in the medium containing glucose and mannitol.

4.1.4 Effect of nitrogen sources on the growth of different isolates of *X. campestris* pv. *betlicola*

The bacterial isolates exhibited varying growth rates in different nitrogen sources tested (Table 5). All the isolates of the pathogen showed poor growth in $NaNO_2$ incorporated medium. Maximum growth of the isolate Xcb-1 was observed when NH_4NO_3 was used as the nitrogen source. The isolate Xcb-2 showed the maximum growth in asparagine incorporated medium followed by KNO_3 added medium. Asparagine supported the best growth of the isolate Xcb-3 also. Maximum growth of the isolate Xcb-4 was observed in KNO_3 incorporated medium.

Table 5. Effect of nitrogen sources on the growth of different isolates of X. campestris pv. betlicola

Nitrogen source	Optical density of isolates			
	Xcb-1	Xcb-2	Xcb-3	Xcb-4
$NaNO_3$	0.141	0.185	0.146	0.178
$NaNO_2$	0.097	0.094	0.083	0.116
KNO_3	0.157	0.203	0.168	0.243
NH_4NO_3	0.172	0.185	0.177	0.205
Asparagine	0.163	0.206	0.210	0.199

(Mean of three replications)

4.1.5 Effect of amino acids on the growth of different isolates of X. campestris pv. betlicola

The growth of the isolates of the pathogen in medium incorporated with different concentrations of amino acids was assessed and the related data are presented in Table 6. In general, a reduction in the growth of bacterial isolates was observed at higher concentrations of amino acids. However, difference in growth rate was also observed among the isolates at the various concentrations of the amino acids. Tyrosine, lysine, glycine, methionine, threonine, glutamic acid, cysteine and arginine supported the maximum growth when they were incorporated into the medium at 0.001 M concentration, while histidine and proline had a similar effect at 0.01 M concentration.

None of the isolates of the bacterium showed any growth at 0.1 M concentration of threonine. At 0.1 M concentration, methionine did not support the growth of the isolates Xcb-3 and Xcb-4 whereas the isolates Xcb-1 and Xcb-2 showed negligible growth. Tyrosine and proline at all the tested concentrations gave good growth of all the isolates while histidine at 0.001 M concentration supported poor growth compared to other concentrations.

Among the isolates, the isolate Xcb-1 showed the

Table 6. Effect of amino acids on the growth of different isolates of X. campestris pv. betlicola

Amino acid	Concentration (Molar)	Optical density of isolates			
		Xcb-1	Xcb-2	Xcb-3	Xcb-4
Arginine	0.1	0.004	0.001	0.001	0.006
	0.01	0.073	0.072	0.057	0.062
	0.001	0.208	0.127	0.058	0.141
Cysteine	0.1	0.003	0.040	0.003	0.062
	0.01	0.049	0.057	0.018	0.083
	0.001	0.237	0.170	0.110	0.155
Glutamic acid	0.1	0.013	0.004	0.003	0.013
	0.01	0.215	0.177	0.170	0.163
	0.001	0.239	0.187	0.183	0.181
Glycine	0.1	0.076	0.064	0.054	0.072
	0.01	0.102	0.114	0.091	0.110
	0.001	0.210	0.224	0.198	0.224
Histidine	0.1	0.170	0.132	0.119	0.125
	0.01	0.192	0.161	0.145	0.153
	0.001	0.088	0.106	0.084	0.088
Lysine	0.1	0.087	0.086	0.094	0.074
	0.01	0.207	0.201	0.187	0.176
	0.001	0.226	0.232	0.215	0.210
Methionine	0.1	0.001	0.003	0	0
	0.01	0.072	0.101	0.083	0.078
	0.001	0.187	0.201	0.205	0.192
Proline	0.1	0.163	0.161	0.165	0.168
	0.01	0.287	0.220	0.201	0.196
	0.001	0.234	0.181	0.170	0.194
Threonine	0.1	0	0	0	0
	0.01	0.086	0.081	0.073	0.093
	0.001	0.220	0.181	0.172	0.110
Tyrosine	0.1	0.219	0.182	0.176	0.163
	0.01	0.242	0.206	0.194	0.185
	0.001	0.284	0.222	0.222	0.211

(Mean of three replications)

maximum growth at 0.01 M concentration of proline, followed by 0.001 M concentration of tyrosine. Lysine, tyrosine and glycine each at 0.001 M concentration supported the maximum growth of isolates Xcb-2, Xcb-3 and Xcb-4 respectively.

4.2 Symptomatology of bacterial leaf spot of betel vine

The symptomatology of bacterial leaf spot of betel vine was studied in detail both under natural and artificial conditions.

Under natural condition infected betel vine plants showed different types of symptom (Plate I). The initial symptom of the disease appeared as minute water soaked lesions on the under surface of the leaf lamina (Plate II). These water soaked lesions were either scattered all over the leaf lamina (Plate III) or seen concentrated at certain areas. As the disease progressed, the lesions enlarged and became angular and delimited by veins (Plate IV). Sometimes, large patches of the lesions delimited by veins were seen on the leaf lamina. In certain cases, the lesions were small without any angularity. A chlorotic area appeared on the upper surface of the leaf corresponding to the water soaked lesions on the lower surface. Later, the centre of the spots turned dark brown with a distinct yellow halo (Plate V and VI). Sometimes, one or two spots were seen

Plate I Betel vine plant showing different symptoms of the disease

Plate II Initial water soaked lesions on betel vine leaf infected with X. campestris pv. betlicola

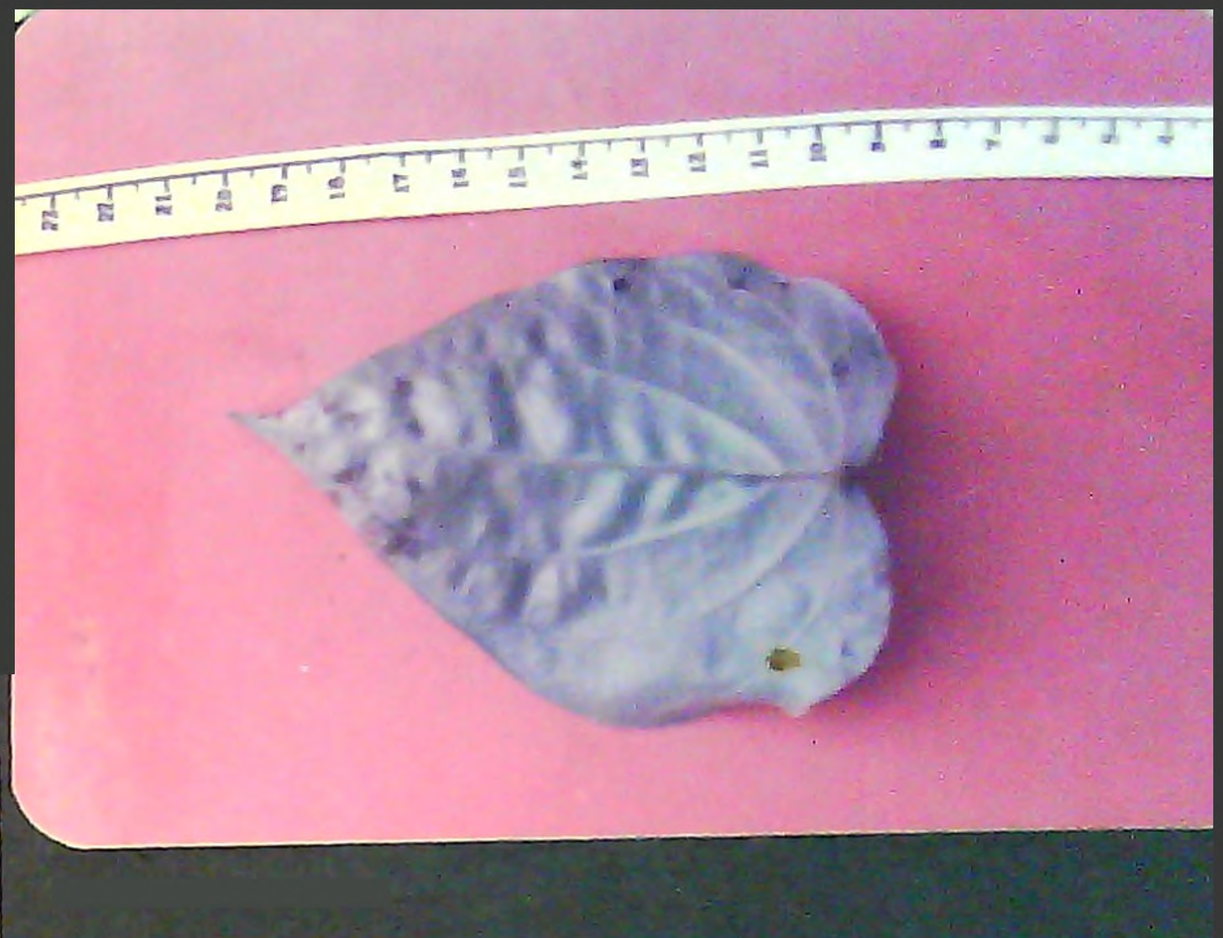
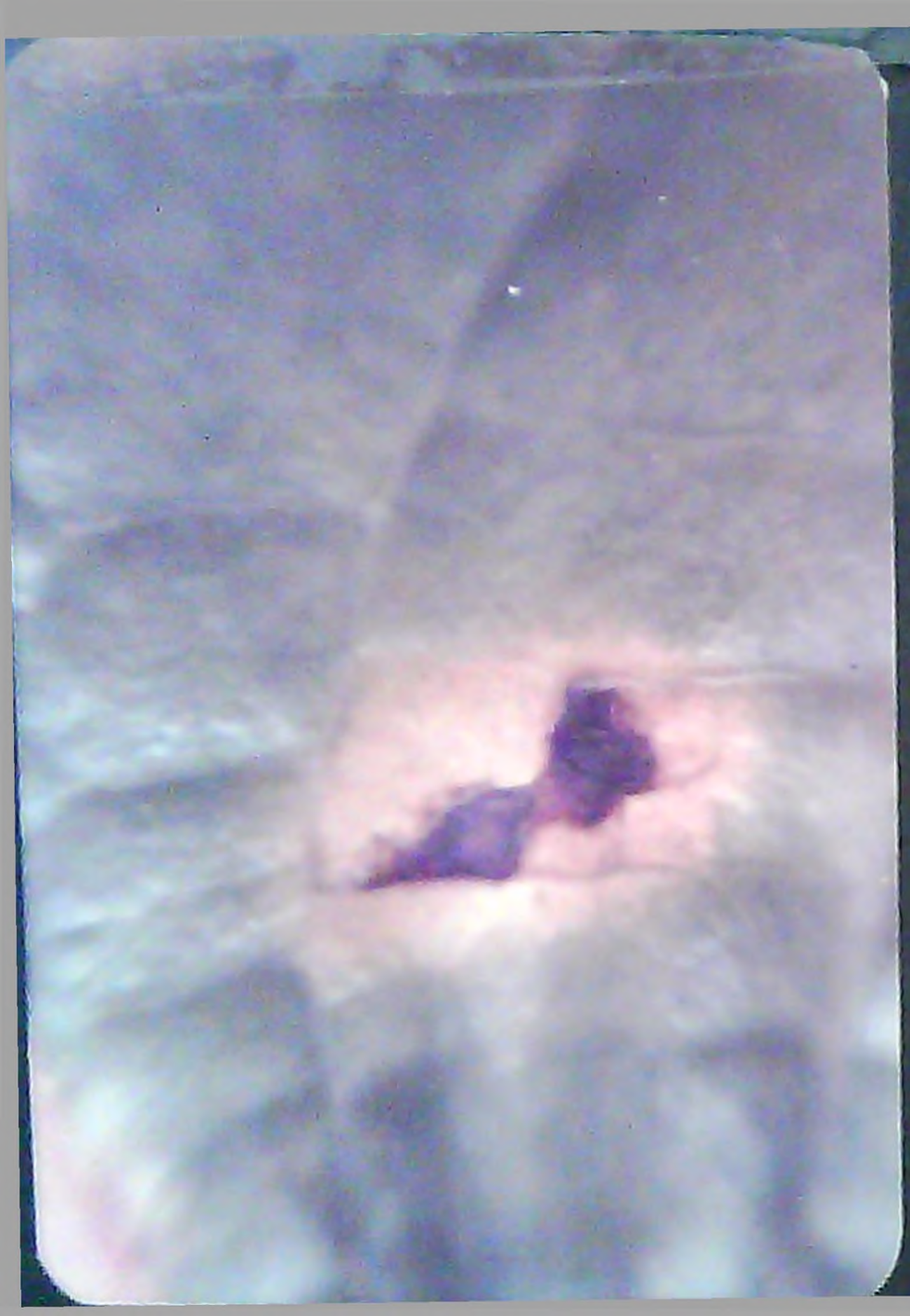


Plate III Scattered water soaked lesions on betel vine leaf infected with X. campestris pv. betlicol

Plate IV Angular lesions on betel vine leaf infected with X. campestris pv. betlicola (5·5X)

Plate V Dark brown spots on betel vine leaf infected with X. campestris pv. betlicola

Plate VI Dark brown spot with clear yellow halo on betel vine leaf infected with X. campestris pv. betlicola (5 5X)



on the leaf lamina and in other cases, scattered spots were observed all over the leaf. Ultimately, these resulted in defoliation. In certain cases, the centre of the spots fell off resulting in shot hole type symptom (Plate VII).

The margins of the leaves were also invariably infected. Lesions initiated as minute water soaked areas along the margin, later turned dark brown with a distinct yellow lining on the inner side (Plate VIII). Sometimes, the symptoms of the disease developed from the tip portion of the leaf downwards. Water soaked lesions, which developed initially at the leaf tip, later turned dark brown with a yellow lining (Plate IX). In certain cases, the leaf tip and marginal infections coalesced and large patches of blightened area were thus formed (Plate X). Often, middle aged and young leaves were severely infected as compared to old ones. Under high humid conditions, the bacterial ooze was invariably seen on the infected regions of the leaf. During dry condition, the dried up ooze formed a brownish gummy deposit on the infected portion.

The symptoms of the disease were also noticed on the stem. Initial symptom on the stem appeared as small dark brown lesions with a greenish yellow halo. Under

Plate VII Shot hole type symptom on betel vine leaves infected with X. campestris pv. betlcicola

Plate VIII Marginal infection on betel vine leaf infected with X. campestris pv.betlicola

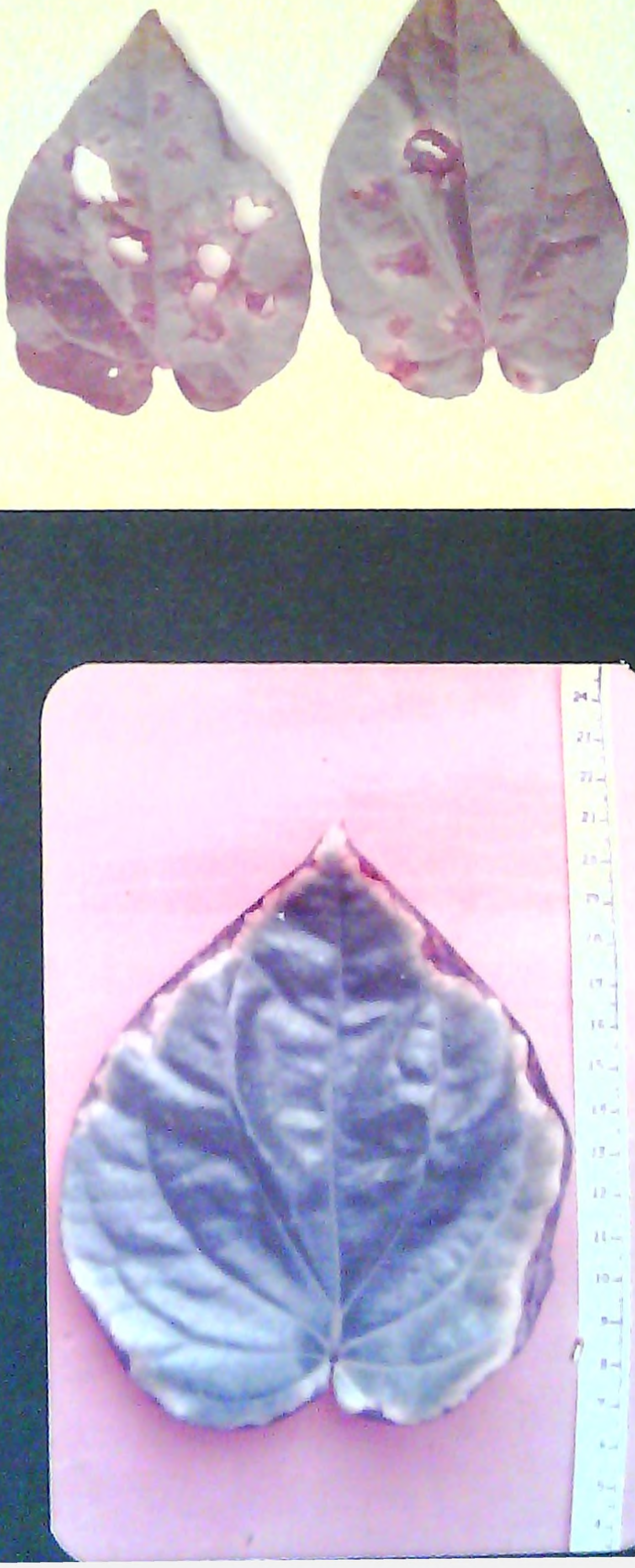


Plate IX Tip infection on betel vine leaf infected with *X. campestris* pv. *betlicola*

Plate X Blightening on betel vine leaf infected with *X. campestris* pv. *betlicola*

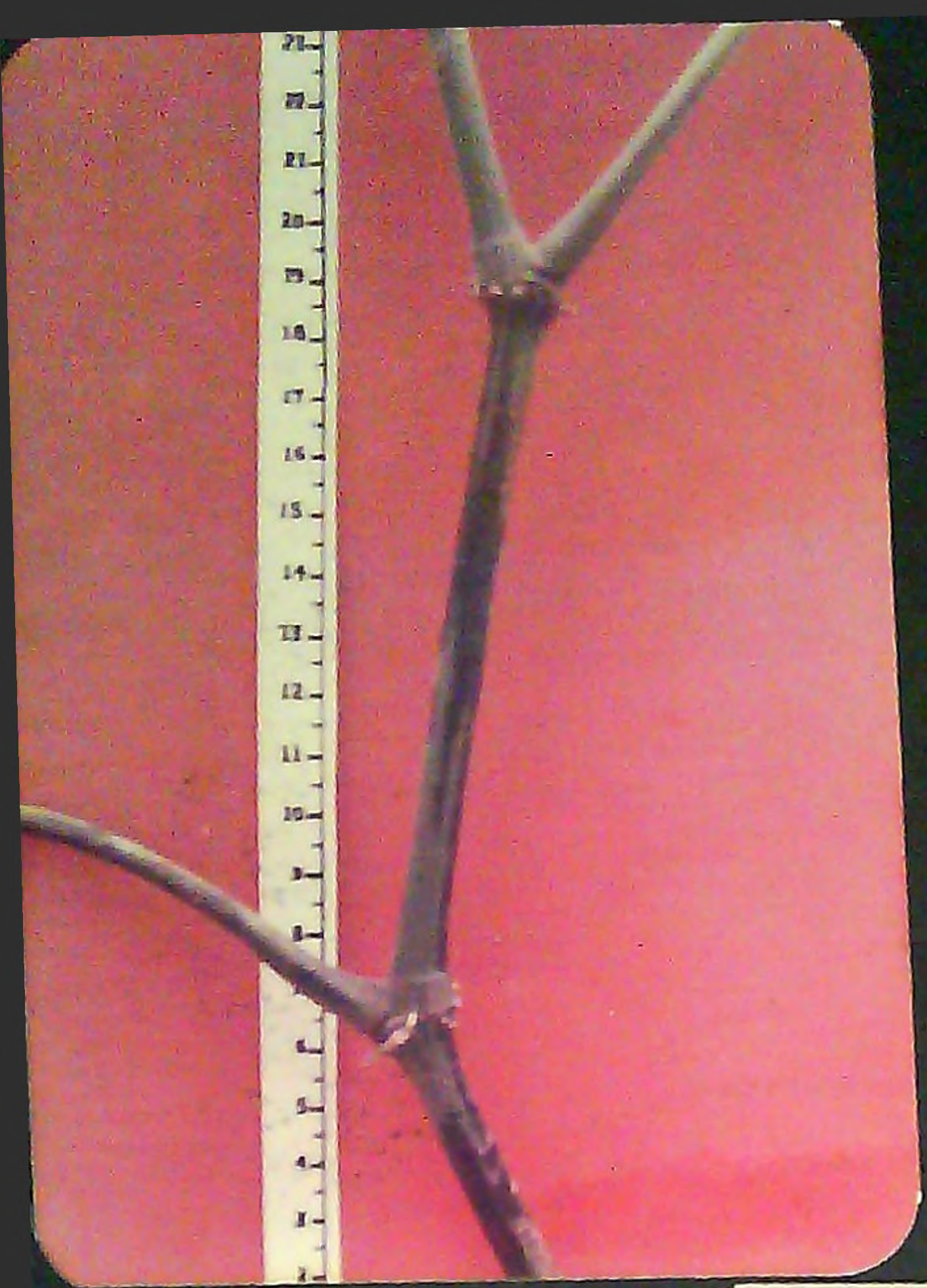
suitable climatic conditions, the lesions coalesced and formed large patches of dark areas covering the entire length of the internodes (Plate XI). In severe cases, the entire stem got infected (Plate XII). Under high humid condition, the bacterial exudations were found on the affected regions (Plate XIII). During dry period, the dried up exudates were seen as dark brown raised encrustations (Plate XIV). The infected portions broke away at the nodal region leading to the death of plants. When the severely infected stem portion was split up logitudinally, dark brown discolouration of the inner tissues was observed. Often, middle and lower portions of the stem were more prone to infection than the upper region.

Under artificial conditions, the symptom development was more or less similar as described above. Generally, under artificial conditions, the symptoms of the disease appeared within 5-7 days of inoculation and complete defoliation occurred within one month. The entire stem got infected due to artificial inoculation.

It was observed that even the presence of one or two spots on the leaves was sufficient to cause defoliation. The plants were killed even with a slight infection on the stem.

Plate XI Stem infection on betel vine due to X. campestris pv. betlicola

Plate XII Betel vine plant with severe stem infection



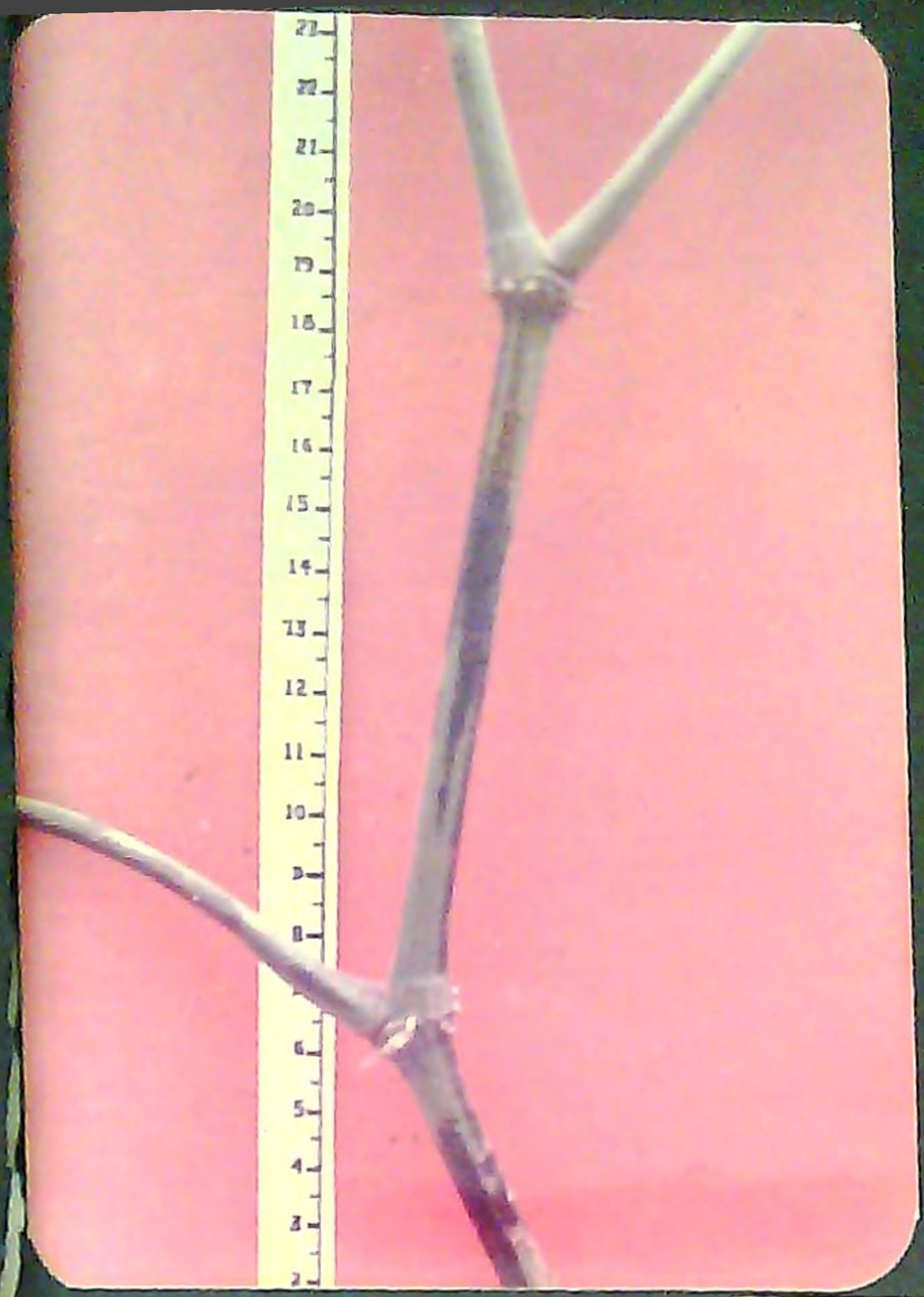


Plate XIII Bacterial exudations on betel vine stem infected with X. campestris pv. betlicola

Plate XIV Dried up bacterial exudates on betel vine stem (8 X)

4.3 Histopathology

To study the histopathology of betel vine leaves infected with X. campestris pv. betlicola, sections of naturally infected leaves showing different stages of infection were taken and they were compared with the healthy ones (Plate XV).

During the early stages of infection, water soaked lesions were produced. Sections of this area showed that the spongy parenchymatous cells were affected by the pathogen. The infected cells were chlorotic and in some areas slight browning was also observed (Plate XVI). The pathogen was also seen to affect the palisade parenchyma cells which were less compact than the healthy ones. The shape of both spongy and palisade parenchyma cells were distorted and the amount of chloroplasts showed marked reduction. The affected cells were generally seen above the stomata. During the early stages of infection, the epidermal cells were not damaged. As the disease progressed, the water soaked lesions turned dark brown with a yellow halo. Sections at this stage showed that all the cells except the upper epidermis were completely infected and their identity was lost (Plate XVII). The phloem cells were distorted and collapsed. The section through the yellow halo region showed a partial

Plate XV T.S. of healthy betel vine leaf (10 x 10X)

Plate XVI T.S. of betel vine leaf through the water soaked lesion (10 x 10X)

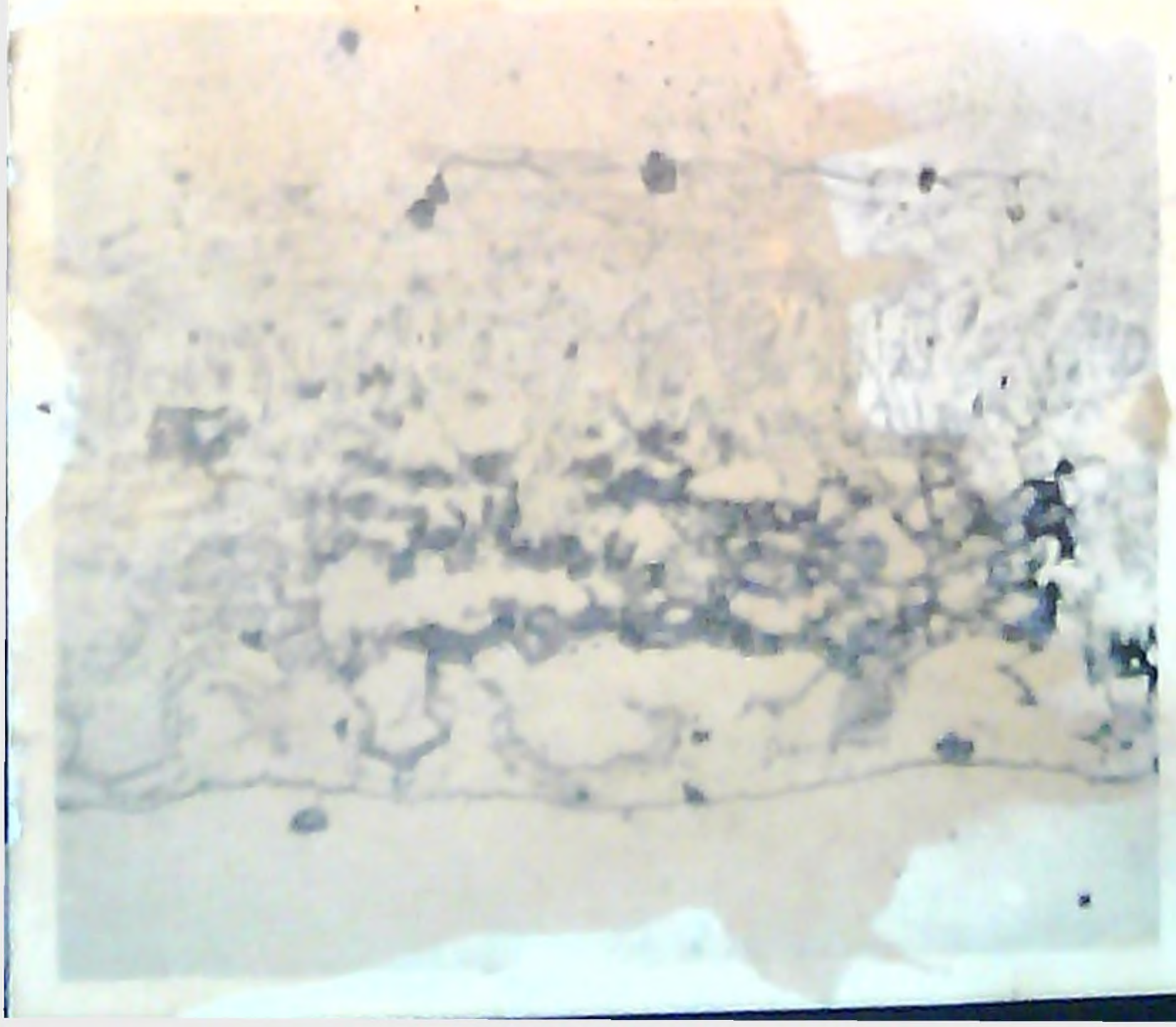


Plate XVII T.S. of betel vine leaf through dark brown lesion (10 x 10X)

Plate XVIII T.S. of betel vine leaf through yellow halo region (10 x 10X)

disintegration of all the cells except epidermis (Plate XVIII). During the advanced stages of infection, the lesions turned dark brown and dried up. The sections showed the complete disintegration and dehydration of all the cells, however, the xylem vessels were unaffected (Plate,XIX).

4.4 Host range of the pathogen X. campestris pv. betlicola

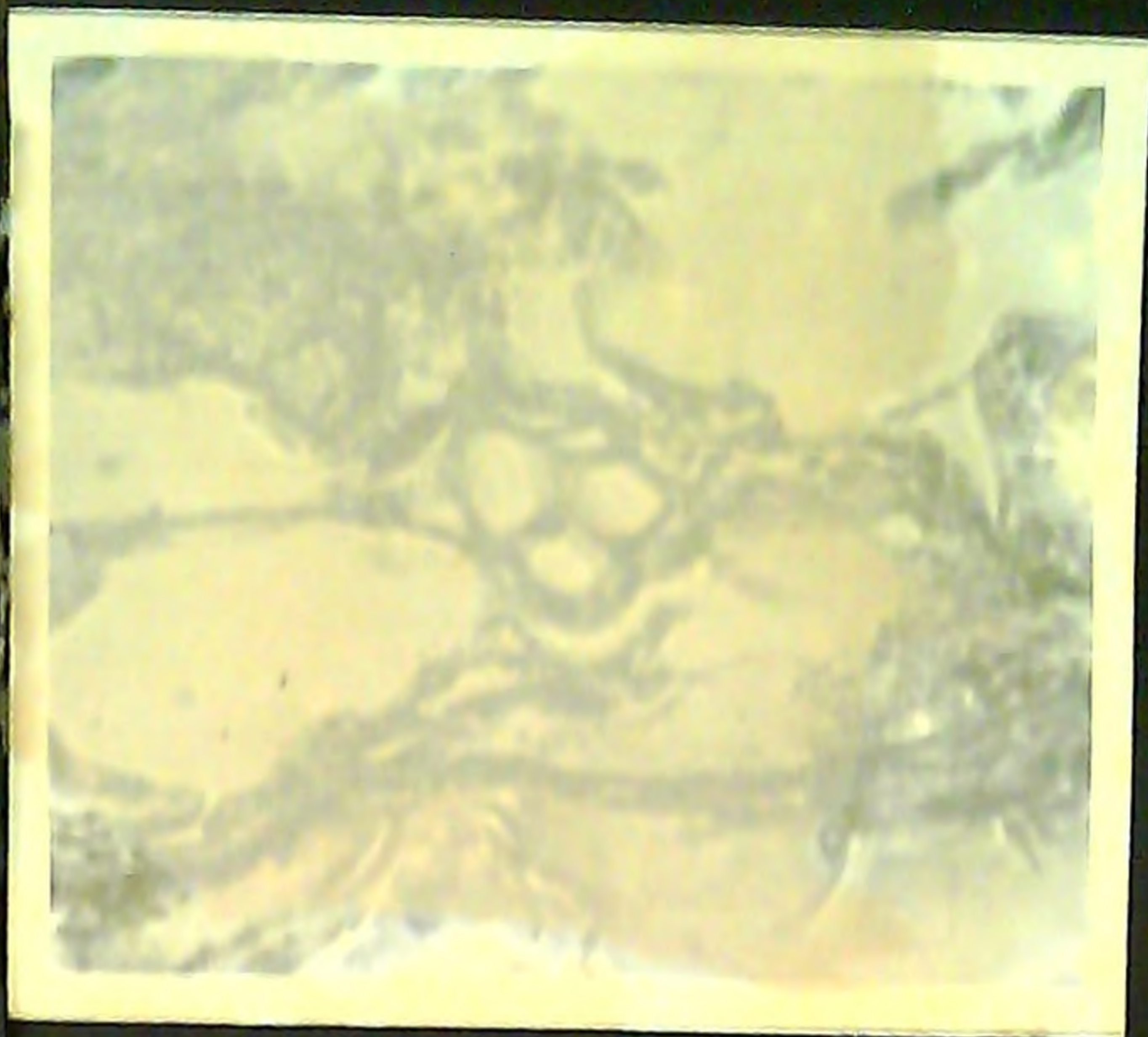
The present host range studies were confined only to the plants having taxonomic affinity to betel vine. The following plants belonging to the family Piperaceae, namely, Piper nigrum L, Piper longum L, Piper sp. Type I, Piper sp. Type II, Piper sp. Type III, Piper sp. Type IV and Peperomia sp. Type I showed symptoms of the disease within one week to three weeks after inoculation. However, Piper attenuatum L. and Peperomia pellucida WP&K did not show any symptoms even after repeated inoculation, revealing their immunity to the pathogen.

4.4.1 Symptomatology of the disease on other hosts

The different host plants showed slight variation in symptom expression. The symptoms on the other host plants are as follows:

Piper nigrum L.: The symptoms of the disease were initiated within 7-10 days of artificial inoculation.

leaf through adve
(40 x 10 X)



estlicola infectio

The initial symptom of the disease appeared as water soaked lesions on the under surface of the leaf lamina. A chlorotic area appeared on the upper surface of the leaf corresponding to the infected lower surface. Later, the centre of the spots turned dark brown with a distinct yellow halo (Plate XX). Margins of the leaf also got infected. As the disease advanced, a dark pigmentation often developed at the infection site, which eventually diffused into the surrounding area. Invariably, the presence of bacterial ooze was seen on the infected area during humid conditions and under dry conditions it dried up as a gum like encrustation. In severe cases of infection, defoliation was observed. Stem infection appeared as small dark patches with a greenish yellow halo, which were always mild.

Piper longum L.: The symptoms of the disease developed 10-15 days after inoculation. The initial symptom appeared around the pin pricked areas of the leaves as water soaked lesions. Later, the centre of the spots turned dark brown with a yellow halo (Plate XXI). Margins of the leaves were not usually infected. In severe cases of infection, defoliation occurred. Stem infection was rare and the overall development of the disease was very slow.

Plate XXI X. campestris pv. betlicola infection on Piper longum

Plate XXII X. campestris pv. betlicola infection on Piper sp. Type I

Piper sp. Type I : The symptoms of the disease appeared within 5-7 days of inoculation. It developed as minute water soaked lesion on the lower surface of the leaf lamina. Generally, the water soaked lesions were seen scattered all over the leaf. As the disease advanced, the lesions coalesced to form patches of water soaked lesions delimited by veins. Later, the centre of the spots turned dark brown with a clear yellow halo (Plate XXII) Leaf marginal infection was also common. Under high humid conditions, bacterial exudations were seen on the infected region and the exudates dried up to form dark brown gum like deposit during dry period. In severe cases of infection, defoliation occurred. Stem infection initiated as small dark brown spots with greenish yellow halo, later turning to large patches.

Piper sp. Type II : Symptoms of the disease were initiated within 7-10 days of inoculation as minute water soaked lesions around the pin pricked areas on the lower surface of the leaf lamina. Eventually, the centre of the lesions turned dark brown with a distinct yellow halo (Plate XXIII). As the disease advanced, the lesions coalesced and formed large dark brown areas. The infected leaves showed general chlorosis. Marginal infection on leaves was also noticed. Stem infection

Plate XXIII X. campestris pv. betlicola infection on Piper sp. Type II

Plate XXIV X. campestris pv. betlicola infection on Piper sp. Type III

developed as small dark areas surrounded by greenish yellow halo.

Piper sp. Type III : Artificially inoculated plants showed symptoms of the disease within 5-7 days. The initial symptom developed as minute water soaked lesions on the lower surface of the leaf lamina. As the lesions enlarged, they coalesced and formed large patches of water soaked areas delimited by veins. Bacterial exudations were seen on the under surface of the infected area. Later, the lesions turned dark brown with a yellow halo (Plate XXIV). Infected leaves showed general chlorosis and were shed within fifteen days of inoculation. Leaf margin and stem infections were also common. On the stem, the symptom developed as small dark brown spots with greenish yellow halo.

Piper sp. Type IV : The symptoms of the disease were initiated within 10-15 days of inoculation. The symptoms first developed as small water soaked area on the under surface of the leaves. Later, these spots turned dark brown with a yellow halo (Plate XXV). The spots later coalesced to form blightened area. Occurrence of leaf margin infection was also noticed. But stem infection was rare.

Plate XXV X. campestris pv. betlicola infection on Piper sp. Type IV

Plate XXVI X. campestris pv. betlicola infection on Peperomia sp. Type I

Peperomia sp. Type I : The symptoms of the disease appeared within 10-15 days of inoculation. Infection occurred around the pin pricked areas as small water soaked lesions. These spots later turned dark brown with a yellow halo (Plate XXVI). In advanced stages of infection the spots coalesced and formed patches of dark brown area. Leaf margin and stem infections were rare.

4.5 Biochemical changes in resistant and susceptible cultivars of betel vine as influenced by X. campestris pv. betlicola inoculation

The biochemical changes occurring in resistant and susceptible cultivars of betel vine due to infection by the bacterial leaf spot pathogen were assessed and the results are presented below:

4.5.1 Changes in ortho-dihydric phenol

The data on the changes in the ortho dihydric phenol are given in Table 7 and Fig.1.

The results indicated that significantly higher quantity of ortho-dihydric phenol was present in the resistant cultivar Poshikodi than in the susceptible cultivar Aryan. In general, inoculation with the pathogen

Table 7. Changes in ortho-dihydric phenol* content in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1) Healthy (H)	Pozhikodi (V_1) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy	Aryan (V_2) Healthy (H)	Aryan (V_2) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy
0	1.50	1.52	+1.33	1.38	1.40	+1.45
3	1.73	1.80	+4.05	1.37	1.42	+3.65
6	1.68	1.77	+5.36	1.52	1.47	-3.29
9	1.73	1.83	+5.78	1.78	1.77	-0.56
12	1.65	1.70	+3.03	1.65	1.45	-12.12
15	1.58	1.73	+9.49	1.53	1.62	+5.88

* Expressed as catechol equivalent in mg/g fresh tissue

Contd.

Table 7. (Contd.) Mean table for the changes in ortho-dihydric phenol content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	1.508	1.392	1.442	1.458	1.450
3	1.767	1.392	1.550	1.608	1.579
6	1.725	1.492	1.600	1.617	1.608
9	1.783	1.775	1.758	1.800	1.779
12	1.675	1.550	1.650	1.575	1.613
15	1.658	1.575	1.558	1.675	1.617
Mean	1.686	1.529	1.593	1.622	

	H	I
V_1	1.647	1.725
V_2	1.539	1.519

	CD (0.05)
Cultivar	- 0.0361
Treatment	- NS
Sampling time	- 0.0625
Cultivar x treatment	- 0.0510
Sampling time x cultivar	- 0.0886
Sampling line x treatment	- NS

NS - Non significant

resulted in an increase in the ortho-dihydric phenol in the resistant cultivar. But, in the susceptible cultivar it showed a varying trend. Inoculated plants of the cultivar Pozhikodi showed the maximum percentage increase of ortho-dihydric phenol over healthy ones on the fifteenth day after inoculation. In the cultivar Aryan, on the first, third and fifteenth day after inoculation, the infected plants showed an increase of ortho-dihydric phenol over healthy ones with the maximum percentage increase on the fifteenth day after inoculation. On other intervals a decrease was noticed with the maximum percentage decrease on the twelfth day after inoculation.

Sampling time exerted a significant effect on the ortho-dihydric phenol content. In both cultivars the maximum amount was in the samples taken on the ninth day after inoculation. The interaction effect of sampling time and inoculation was not significant.

4.5.2 Changes in total phenols

The results on the changes in total phenols in the leaves of betel vine cultivars are presented in Table 8 and Fig. 2.

Compared to the susceptible cultivar Aryan,

Table 8. Changes in total phenol content* in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1) Healthy (H)	Pozhikodi (V_1) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy	Aryan (V_2) Healthy (H)	Aryan (V_2) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy
0	2.50	2.53	+1.20	2.15	2.07	-3.72
3	2.75	3.30	+20.00	2.18	2.50	+14.68
6	3.00	3.85	+28.33	2.27	2.67	+17.62
9	2.67	3.30	+23.60	2.83	3.30	+16.61
12	2.07	2.58	+24.64	1.83	2.15	+17.49
15	1.90	2.50	+31.58	1.75	2.05	+17.14

* Expressed as catechol equivalent in mg/g fresh tissue

Contd.

Table 8. (Contd.) Mean table for the changes in total phenol content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	2.517	2.108	2.325	2.300	2.313
3	3.025	2.342	2.467	2.900	2.683
6	3.425	2.467	2.633	3.258	2.946
9	2.983	3.067	2.750	3.300	3.025
12	2.325	1.992	1.950	2.367	2.158
15	3.200	1.900	1.825	2.275	2.050
Mean	2.746	2.313	2.325	2.733	

	H	I
V_1	2.481	3.011
V_2	2.169	2.456

	CD (0.05)
Cultivar/treatment	- 0.0520
Sampling time	- 0.0900
Cultivar x treatment	- 0.0745
Sampling time x cultivar/ sampling time x treatment	- 0.1273

Fig-1. CHANGES IN ORTHO-DIHYDRIC PHENOL IN BETELVINE CULTIVARS AS INFLUENCED BY X. CAMPESTRIS PV. BETLICOLA INOCULATION.

——— V_1 H - RESISTANT CULTIVAR POZHIKODI - HEALTHY.
—·—·— V_1 I - " " " INOCULATED.

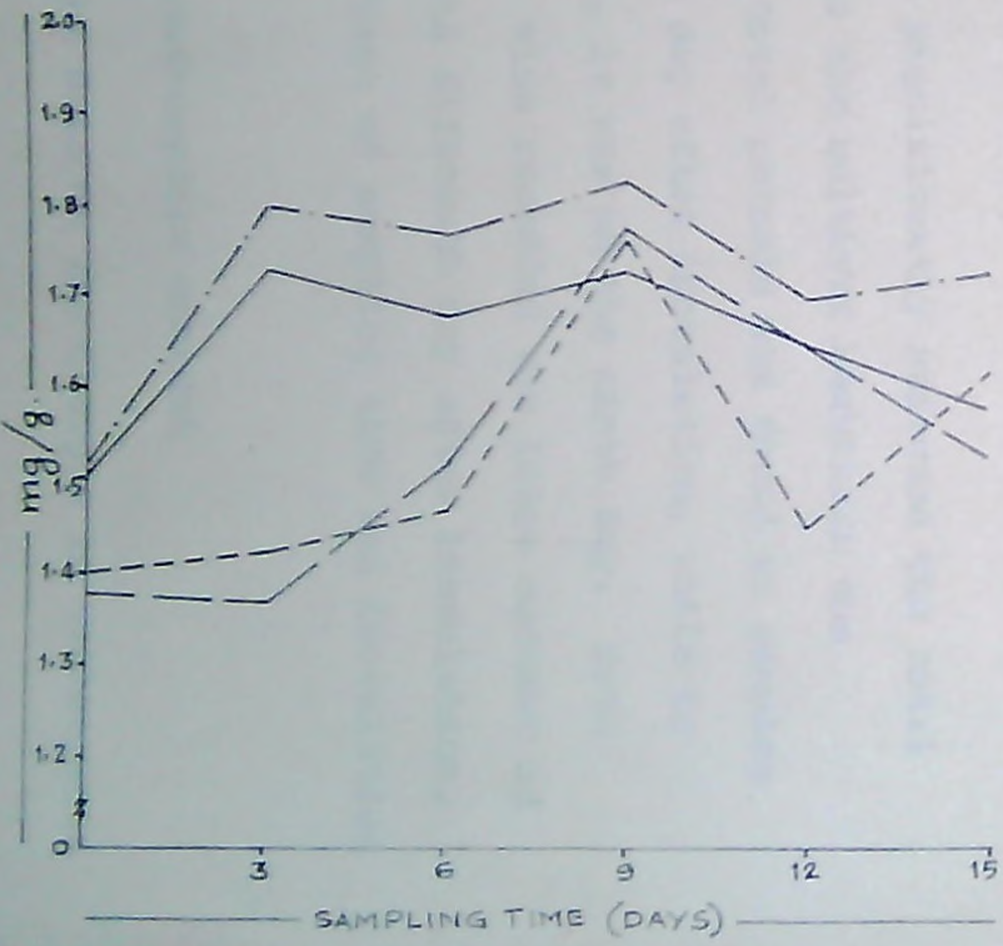
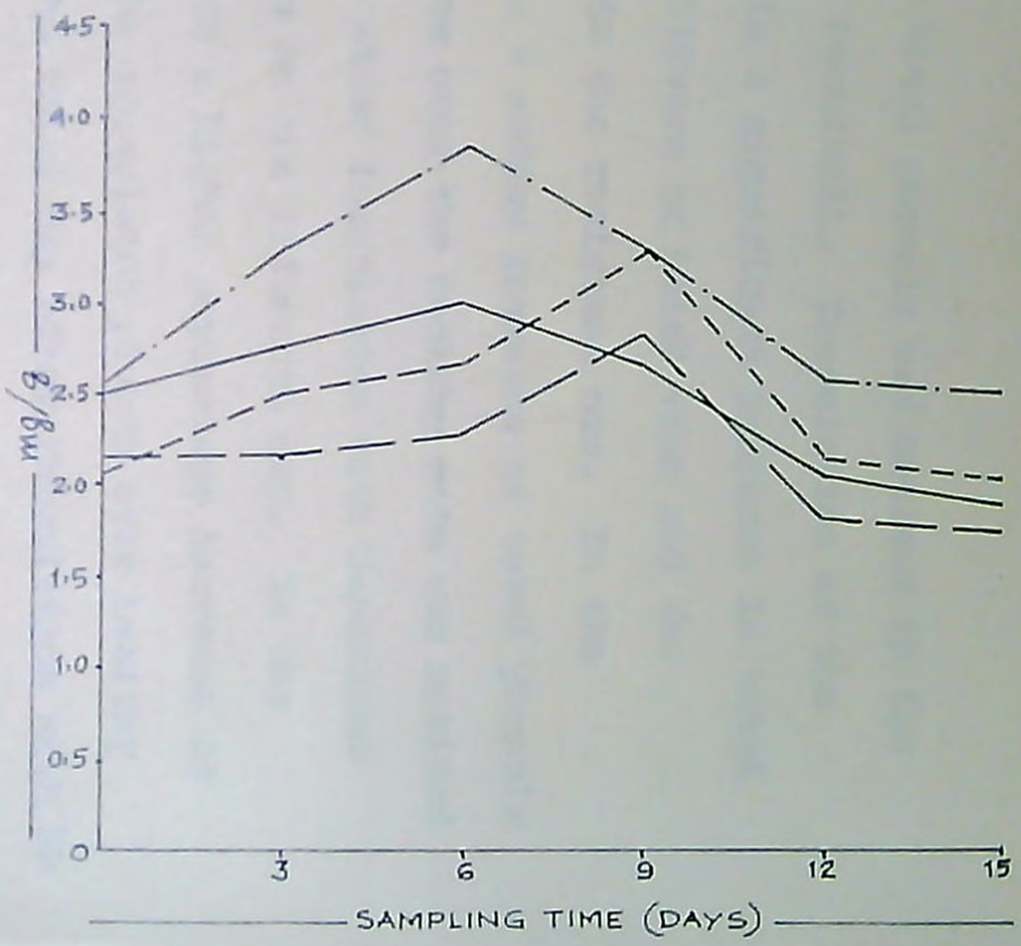


Fig-2. CHANGES IN TOTAL PHENOLS IN BETELVINE CULTIVARS AS INFLUENCED BY X. CAMPESTRIS PV. BETLICOLA INOCULATION.

— — — — V_2 H - SUSCEPTIBLE CULTIVAR ARYAN - HEALTHY.
— — — — — V_2 I - " " " INOCULATED.



significantly more total phenols was noticed in the resistant cultivar Pozhikodi. Inoculation of the pathogen resulted in a significant increase in total phenols in both cultivars of betel vine and the increase was more in the resistant one. In the resistant cultivar, a sudden increase of total phenols in inoçulated plants over the healthy ones was noticed from the third day after inoculation with themaximum percentage increase on the fifteenth day. In the susceptible cultivar a higher percentage increase of total phenols in the inoculated plants over healthy was noticed from the third day after inoculation with the maximum on the sixth day.

Sampling time significantly affected the total phenol content. In the cultivar Pozhikodi the maximum amount of total phenols was found in samples drawn on the sixth day after inoculation, while in the cultivar Aryan, it was on the ninth day. Both cultivars of betel vine recorded the least content of total phenols on the fifteenth day after inoculation. The interaction effect of sampling time and inoculation was significant.

4.5.3 Changes in anthocyanin content

Analysis of the data on the changes in anthocyanin

content revealed that the resistant cultivar Pozhikodi contained significantly higher quantity than the susceptible cultivar Aryan (Table 9). Inoculation of the bacterium resulted in a significant increase in the anthocyanin content in both cultivars of betel vine. In the resistant cultivar, an increase of anthocyanin content in inoculated plants over healthy ones was noticed from the day of inoculation onwards, reaching the maximum on the ninth day after which there was gradual decrease. In the susceptible cultivar, an increase in anthocyanin content in inoculated plants over healthy was noticed at all intervals of observation except on the day of inoculation with the maximum percentage increase on the sixth day.

Sampling time significantly affected the anthocyanin content. In the cultivar Pozhikodi the third day samples and in the cultivar Aryan the third and fifteenth day samples contained higher quantities of anthocyanin. The interaction effect of sampling time and inoculation was significant.

4.5.4 Changes in leucoanthocyanin content

The data on the changes of leucoanthocyanin content in betel vine cultivars as a result of inoculation the

Table 9. Changes in anthocyanin content* in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1) Healthy (H)	Pozhikodi (V_1) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy	Aryan (V_2) Healthy (H)	Aryan (V_2) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy
0	0.220	0.225	+2.27	0.235	0.230	-2.13
3	0.268	0.280	+4.48	0.236	0.271	+14.83
6	0.201	0.231	+14.93	0.153	0.220	+43.79
9	0.199	0.279	+40.20	0.145	0.185	+27.59
12	0.232	0.255	+9.91	0.177	0.235	+32.77
15	0.232	0.244	+5.17	0.210	0.295	+40.48

* Expressed as optical density at 525 nm

Contd.

Table 9. (Contd.) Mean table for the changes in anthocyanin content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	0.222	0.228	0.222	0.227	0.225
3	0.274	0.253	0.252	0.275	0.264
6	0.216	0.186	0.177	0.226	0.201
9	0.239	0.165	0.172	0.232	0.202
12	0.243	0.206	0.204	0.245	0.225
15	0.238	0.253	0.221	0.269	0.245
Mean	0.239	0.215	0.208	0.246	

	H	I
V_1	0.225	0.252
V_2	0.191	0.239

	CD (0.05)
Cultivar/treatment	- 0.0084
Sampling time	- 0.0150
Cultivar x treatment	- 0.0120
Sampling time x cultivar/ sampling time x treatment	- 0.0210

pathogen are given in Table 10.

It was found that significantly more amount of leucoanthocyanin was present in the susceptible cultivar Aryan than in the resistant cultivar Pozhikodi. In general, inoculation of the cultivars with the pathogen resulted in a significant increase in leucoanthocyanin content and the increase was more pronounced in the resistant cultivar. In the cultivar Pozhikodi a sudden and maximum percentage increase in leucoanthocyanin content in inoculated plants over healthy was observed on the third day after inoculation. But the inoculated plants of the cultivar Aryan showed the maximum increase of leucoanthocyanin over healthy on the sixth day after inoculation.

Sampling time significantly affected the leucoanthocyanin content. In the cultivar Pozhikodi, the maximum amount of leucoanthocyanin was observed in the samples drawn on the twelfth day after inoculation while in the cultivar Aryan it was on the fifteenth day. The interaction effect of sampling time and inoculation was significant.

4.5.5 Changes in total chlorophyll content

The results on the changes in total chlorophyll content in betel vine cultivars are presented in Table 11

Table 10. Changes in leucoanthocyanin content* in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1) Healthy (H)	Pozhikodi (V_1) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy	Aryan (V_2) Healthy (H)	Aryan (V_2) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy
0	0.021	0.021	0	0.044	0.044	0
3	0.027	0.074	+174.07	0.035	0.044	+25.71
6	0.025	0.066	+164.00	0.078	0.133	+70.51
9	0.108	0.144	+33.33	0.076	0.108	+42.11
12	0.118	0.149	+26.27	0.102	0.151	+48.04
15	0.108	0.149	+37.96	0.145	0.176	+21.38

* Expressed as optical density at 550 nm

Contd.

Table 10. (Contd.) Mean table for the changes in leucoanthocyanin content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	0.021	0.044	0.033	0.033	0.033
3	0.051	0.040	0.031	0.059	0.045
6	0.046	0.105	0.052	0.100	0.076
9	0.126	0.092	0.092	0.126	0.109
12	0.134	0.128	0.111	0.150	0.131
15	0.129	0.160	0.127	0.163	0.145
Mean	0.084	0.095	0.074	0.105	

	H	I
V_1	0.068	0.101
V_2	0.080	0.109

		CD (0.05)
Cultivar/treatment	-	0.0031
Sampling time	-	0.0050
Cultivar x treatment	-	NS
Sampling time x cultivar/ sampling time x treatment	-	0.0080

NS - Non significant

Table 11. Changes in total chlorophyll content* in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1) Healthy (H)	Pozhikodi (V_1) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy	Aryan (V_2) Healthy (H)	Aryan (V_2) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy
0	0.249	0.245	-1.61	0.244	0.241	-1.23
3	0.243	0.238	-2.06	0.229	0.209	-8.73
6	0.229	0.219	-4.37	0.214	0.170	-20.56
9	0.224	0.205	-8.48	0.209	0.129	-38.28
12	0.223	0.194	-13.00	0.201	0.089	-55.72
15	0.218	0.170	-22.02	0.200	0.073	-63.50

* Expressed as mg/g fresh tissue

Contd.

Table 11. (Contd.) Mean table for the changes in total chlorophyll content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	0.247	0.242	0.247	0.243	0.245
3	0.240	0.219	0.236	0.223	0.230
6	0.224	0.192	0.221	0.195	0.208
9	0.215	0.169	0.217	0.167	0.192
12	0.209	0.145	0.212	0.142	0.177
15	0.194	0.137	0.209	0.122	0.165
Mean	0.222	0.184	0.224	0.182	

	H	I
V_1	0.231	0.212
V_2	0.216	0.152

		CD (0.05)
Cultivar/treatment	-	0.0003
Sampling time	-	0.0005
Cultivar x treatment	-	0.0039
Sampling time x cultivar/ sampling time x treatment	-	0.0007

From the data it was evident that significantly more quantity of total chlorophyll was present in the resistant cultivar Pozhikodi than in the susceptible cultivar Aryan. Inoculation with the pathogen resulted in a significant reduction in total chlorophyll content in both cultivars of betel vine and the reduction was relatively more in the susceptible cultivar. In the cultivar Pozhikodi, the maximum percentage decrease in total chlorophyll in inoculated plants over uninoculated was observed on the fifteenth day after inoculation. Inoculated plants of the cultivar Aryan showed a higher percentage decrease in total chlorophyll over healthy ones from the sixth day after inoculation onwards, reaching the maximum on the fifteenth day.

Sampling time significantly influenced the total chlorophyll content in both cultivars, the minimum being found in samples taken on the fifteenth day after inoculation. The interaction effect of sampling time and inoculation was significant.

4.5.6 Changes in chlorophyll 'a' content

The resistant cultivar Pozhikodi contained significantly more amount of chlorophyll 'a' than the susceptible cultivar Aryan (Table 12). In both cultivars of betel vine, inoculation with the pathogen resulted in

Table 12. Changes in chlorophyll 'a' content* in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1) Healthy (H)	Pozhikodi (V_1) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy	Aryan (V_2) Healthy (H)	Aryan (V_2) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy
0	0.131	0.128	-2.29	0.138	0.136	-1.45
3	0.124	0.122	-1.61	0.135	0.121	-10.37
6	0.118	0.115	-2.54	0.129	0.110	-14.73
9	0.116	0.110	-5.17	0.121	0.095	-21.49
12	0.115	0.112	-2.61	0.123	0.064	-47.97
15	0.119	0.110	-7.56	0.123	0.054	-56.10

* Expressed as mg/g fresh tissue

Contd.

Table 12. (Contd.) Mean table for the changes in chlorophyll 'a' content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	0.130	0.137	0.135	0.132	0.133
3	0.123	0.128	0.130	0.121	0.126
6	0.117	0.120	0.123	0.113	0.118
9	0.114	0.107	0.119	0.103	0.111
12	0.114	0.094	0.119	0.090	0.105
15	0.115	0.088	0.120	0.078	0.100
Mean	0.118	0.112	0.124	0.106	

	H	I
V_1	0.121	0.116
V_2	0.128	0.097

	CD (0.05)
Cultivar/treatment	- 0.0009
Sampling time	- 0.0015
Cultivar x treatment	- 0.0013
Sampling time x cultivar/ sampling time x treatment	- 0.0022

a significant decrease in chlorophyll 'a' content and the reduction was more in the cultivar Aryan. In the cultivar Pozhikodi the maximum percentage decrease of chlorophyll 'a' content in inoculated plants over healthy was on the fifteenth day after inoculation. Comparatively higher percentage decrease of chlorophyll 'a' in infected plants of cultivar Aryan over healthy was noticed from the third day onwards, reaching the maximum on the fifteenth day.

In the cultivar Pozhikodi, samples taken on the ninth, twelfth and fifteenth day after inoculation contained significantly less amount of chlorophyll 'a' than the samples of other intervals. In the cultivar Aryan, the minimum chlorophyll 'a' content was recorded in samples taken on the fifteenth day after inoculation. The interaction effect of sampling time and inoculation was significant.

4.5.7 Changes in chlorophyll 'b' content

Significantly higher amount of chlorophyll 'b' was noticed in the cultivar Pozhikodi than in the cultivar Aryan (Table 13). Inoculation with the pathogen resulted in a significant reduction in chlorophyll 'b' content in both cultivars and the reduction was more in the susceptible cultivar Aryan. In the cultivars Pozhikodi and Aryan, the maximum percentage decrease of

Table 13. Changes in chlorophyll 'b' content* in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1) Healthy (H)	Pozhikodi (V_1) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy	Aryan (V_2) Healthy (H)	Aryan (V_2) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy
0	0.118	0.117	-0.85	0.106	0.104	-1.89
3	0.120	0.116	-3.33	0.094	0.088	-6.38
6	0.111	0.104	-6.31	0.085	0.060	-29.41
9	0.108	0.094	-12.96	0.088	0.034	-61.36
12	0.108	0.082	-24.07	0.078	0.024	-69.23
15	0.099	0.060	-39.39	0.077	0.019	-75.32

* Expressed as mg/g fresh tissue

Contd.

Table 13. (Contd.) Mean table for the changes in cholorophyll 'b' content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	0.118	0.105	0.112	0.111	0.111
3	0.118	0.091	0.107	0.102	0.105
6	0.108	0.073	0.098	0.082	0.090
9	0.101	0.061	0.096	0.064	0.081
12	0.090	0.051	0.090	0.051	0.071
15	0.080	0.047	0.088	0.038	0.063
Mean	0.102	0.071	0.099	0.075	

	H	I
V_1	0.110	0.095
V_2	0.088	0.054

		CD (0.05)
Cultivar/treatment	-	0.0005
Sampling time	-	0.0009
Cultivar x treatment	-	0.0007
Sampling time x cultivar/ sampling time x treatment	--	0.0013

was observed on the fifteenth day after inoculation. In both cultivars of betel vine, the fifteenth day samples contained the minimum amount of chlorophyll 'b'. The interaction of sampling time and inoculation was significant.

4.5.8 Changes in reducing sugar

Analysis of the data on the changes of reducing sugar indicated significant difference between the cultivars of betel vine (Table 14 and Fig. 4). The resistant cultivar Pozhikodi contained significantly more quantity of reducing sugar than the susceptible cultivar Aryan. In both cultivars of betel vine, inoculation with the pathogen resulted in a significant decrease in reducing sugar content and the decrease was more in the susceptible cultivar. In the cultivar Pozhikodi, a higher percentage decrease in reducing sugar was recorded on the twelfth and fifteenth days after inoculation with the maximum decrease on the fifteenth day. In the cultivar Aryan, a sharp decrease in the reducing sugar in inoculated plants over healthy was observed from the sixth day after inoculation onwards with the maximum percentage decrease on the twelfth day.

Sampling time significantly influenced the reducing

Table 14. Changes in reducing sugar content* in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1) Healthy (H)	Pozhikodi (V_1) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy	Aryan (V_2) Healthy (H)	Aryan (V_2) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy
0	18.42	18.23	-1.03	20.86	20.73	-0.62
3	19.06	18.53	-2.78	24.83	24.54	-1.17
6	18.27	15.71	-14.01	19.83	12.85	-35.20
9	19.25	17.42	-9.51	17.86	10.43	-41.60
12	18.73	11.74	-37.32	17.73	9.15	-48.39
15	18.23	10.53	-42.24	12.17	8.28	-31.96

* Expressed as glucose equivalent in mg/g dry weight

Contd.

Table 14. (Contd.) Mean table for the changes in reducing sugar content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	18.325	20.800	19.640	19.483	19.562
3	18.800	24.675	21.940	21.583	21.737
6	16.990	16.340	19.050	14.283	16.666
9	18.343	14.145	18.555	13.933	16.244
12	15.235	13.444	18.233	10.445	14.339
15	14.383	10.225	15.200	9.405	12.303
Mean	17.013	16.605	18.770	14.847	

	H	I
V_1	18.667	15.362
V_2	18.878	14.328

		CD (0.05)
Cultivar/treatment	-	0.0444
Sampling time	-	0.0770
Cultivar x treatment	-	0.0628
Sampling time x cultivar/ sampling time x treatment	-	0.1088

Fig-3. CHANGES IN TOTAL CHLOROPHYLL IN BETELVINE CULTIVARS AS INFLUENCED BY X. CAMPESTRIS PV. BETLICOLA INOCULATION.

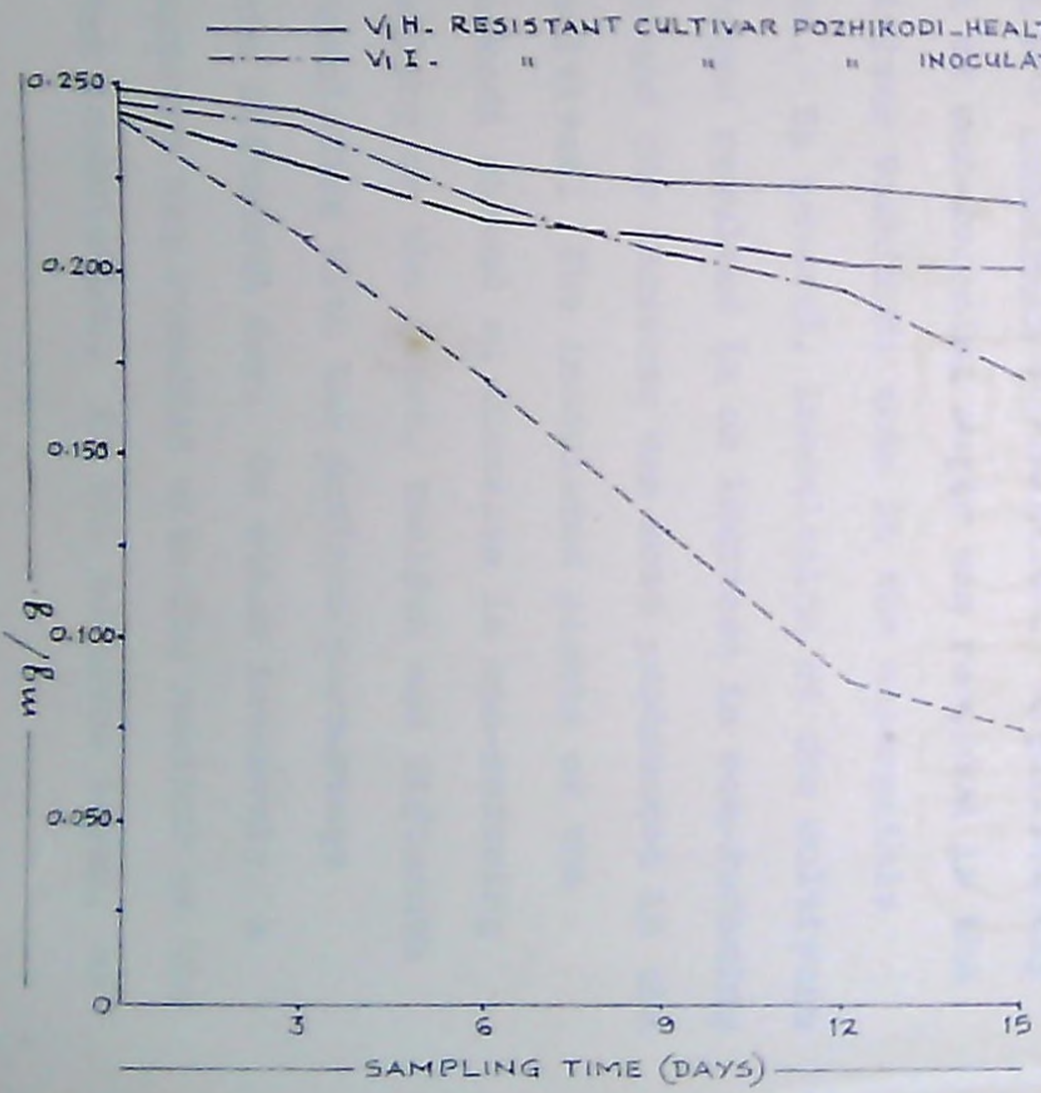
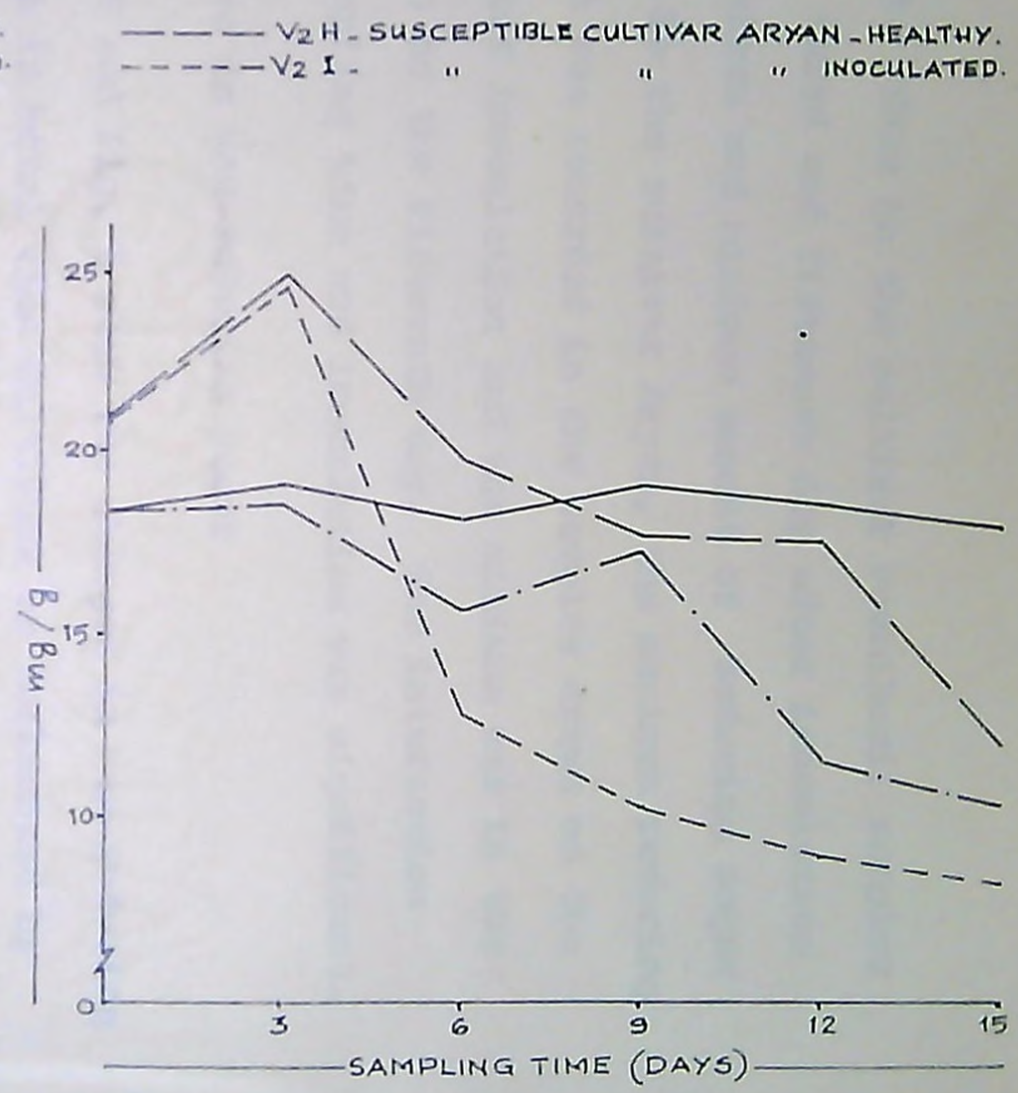


Fig-4. CHANGES IN REDUCING SUGAR IN BETELVINE CULTIVARS AS INFLUENCED BY X. CAMESTRIS PV. BETLICOLA INOCULATION.



sugar content. Thus in the cultivar Pozhikodi, samples drawn on the third and fifteenth day after inoculation contained maximum and minimum amount of reducing sugar respectively. In the cultivar Aryan, the maximum reducing sugar content was recorded in the samples drawn on the third day after inoculation and the minimum was in the samples drawn on the fifteenth day. The interaction effect of sampling time and inoculation was significant.

4.5.9 Changes in non-reducing sugar

Table 15 and Fig. 5 show the changes in non-reducing sugar content in betel vine cultivars as influenced by X. campestris pv. betlicola inoculation. Significantly higher amount of non-reducing sugar was recorded in the resistant cultivar Pozhikodi than in the susceptible cultivar Aryan. In general, inoculation of the cultivars with the pathogen resulted in an increase in non-reducing sugar content and the increase was more pronounced in the susceptible cultivar. The inoculated plants of the cultivar Pozhikodi showed an increase in non-reducing sugar over healthy on the first, twelfth and fifteenth days after inoculation with the maximum percentage increase on the fifteenth day. On other intervals, a percentage decrease was recorded with the maximum on the ninth day after inoculation. In the cultivar Aryan, an

Table 15. Changes in non-reducing sugar content* in leaves of betel vine cultivars as influenced by *X. campestris* pv. *betlicola* inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1)		Per cent increase(+) or decrease(-) over healthy	Aryan (V_2)		Per cent increase(+) or decrease(-) over healthy
	Healthy (H)	Inoculated (I)		Healthy (H)	Inoculated (I)	
0	10.01	10.15	+1.40	12.57	12.65	+0.64
3	11.97	11.85	-1.00	12.16	13.53	+11.27
6	13.11	12.02	-8.31	12.21	12.52	+2.54
9	16.81	15.28	-9.10	10.82	11.61	+7.30
12	13.98	14.95	+6.94	10.98	12.88	+17.30
15	13.80	16.16	+17.10	9.53	15.09	+58.34

* Expressed as glucose equivalent in mg/g dry weight

Contd.

Table 15. (Contd.) Mean table for the changes in non-reducing sugar content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	10.083	12.610	11.293	11.400	11.347
3	11.910	12.845	12.065	12.690	12.378
6	12.565	12.365	12.662	12.270	12.466
9	16.045	11.213	13.815	13.842	13.629
12	14.480	11.935	12.478	13.914	13.202
15	14.980	12.313	11.663	15.625	13.645
Mean	13.344	12.214	12.329	13.224	

	H	I
V_1	13.285	13.402
V_2	11.378	13.043

	CD (0.05)
Cultivar/treatment	- 0.0470
Sampling time	- 0.0815
Cultivar x treatment	- 0.0665
Sampling time x cultivar/ sampling time x treatment	- 0.1152

increase of non-reducing sugar in inoculated plants over uninoculated was noticed at all intervals of observation with the maximum percentage increase on the fifteenth day.

In the cultivar Pozhikodi, the maximum amount of non-reducing sugar was recorded in samples drawn on the ninth day after inoculation, while in the cultivar Aryan it was on the third day. The interaction effect of sampling time and inoculation was significant.

4.5.10 Changes in total sugar content

The results on the changes in total sugar content in leaves of betel vine cultivars are given in Table 16 and Fig. 6.

Significantly more quantity of total sugar was recorded in the resistant cultivar Pozhikodi than in the susceptible cultivar Aryan. In general, inoculation of the pathogen resulted in a decrease in total sugar content in both cultivars of betel vine. In the cultivar Pozhikodi, a decrease in total sugar content was observed in inoculated plants over uninoculated at all intervals of observation with the maximum percentage decrease on the twelfth day after inoculation. In the cultivar Aryan, a decrease of total sugar content in inoculated plants over healthy ones was noticed at different periods

Table 16. Changes in total sugar content* in leaves of betel vine cultivars as influenced by *X. campestris* pv. *betlicola* inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1)		Per cent increase(+) or decrease(-) over healthy	Aryan (V_2)		Per cent increase(+) or decrease(-) over healthy
	Healthy (H)	Inoculated (I)		Healthy (H)	Inoculated (I)	
0	28.43	28.38	-0.18	33.43	33.38	-0.15
3	31.03	30.38	-2.09	36.99	38.07	+2.92
6	31.38	27.73	-11.63	32.04	25.37	-20.82
9	36.06	32.72	-9.26	28.68	22.04	-23.15
12	31.71	26.69	-15.83	28.71	22.03	-23.27
15	32.03	26.69	-16.67	21.70	23.37	+7.70

* Expressed as glucose equivalent in mg/g dry weight

Contd.

Table 16. (Contd.) Mean table for the changes in total sugar content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	24.407	33.410	30.933	30.883	30.908
3	30.710	37.527	34.013	34.223	34.118
6	29.557	28.708	31.712	26.553	29.133
9	34.387	25.358	32.370	27.375	29.873
12	29.703	24.368	30.712	24.360	27.536
15	29.363	22.530	26.863	25.030	25.947
Mean	30.354	28.817	31.101	28.071	

	H	I
V_1	31.943	28.766
V_2	30.258	27.376

	CD (0.05)
Cultivar/treatment	0.0154
Sampling time	0.0267
Cultivar x treatment	0.0218
Sampling time x cultivar sampling time x treatment	0.0378

Fig.5. CHANGES IN NON-REDUCING SUGAR IN BETELVINE CULTIVARS AS INFLUENCED BY X. CAMPESTRIS PV. BETLICOLA INOCULATION.

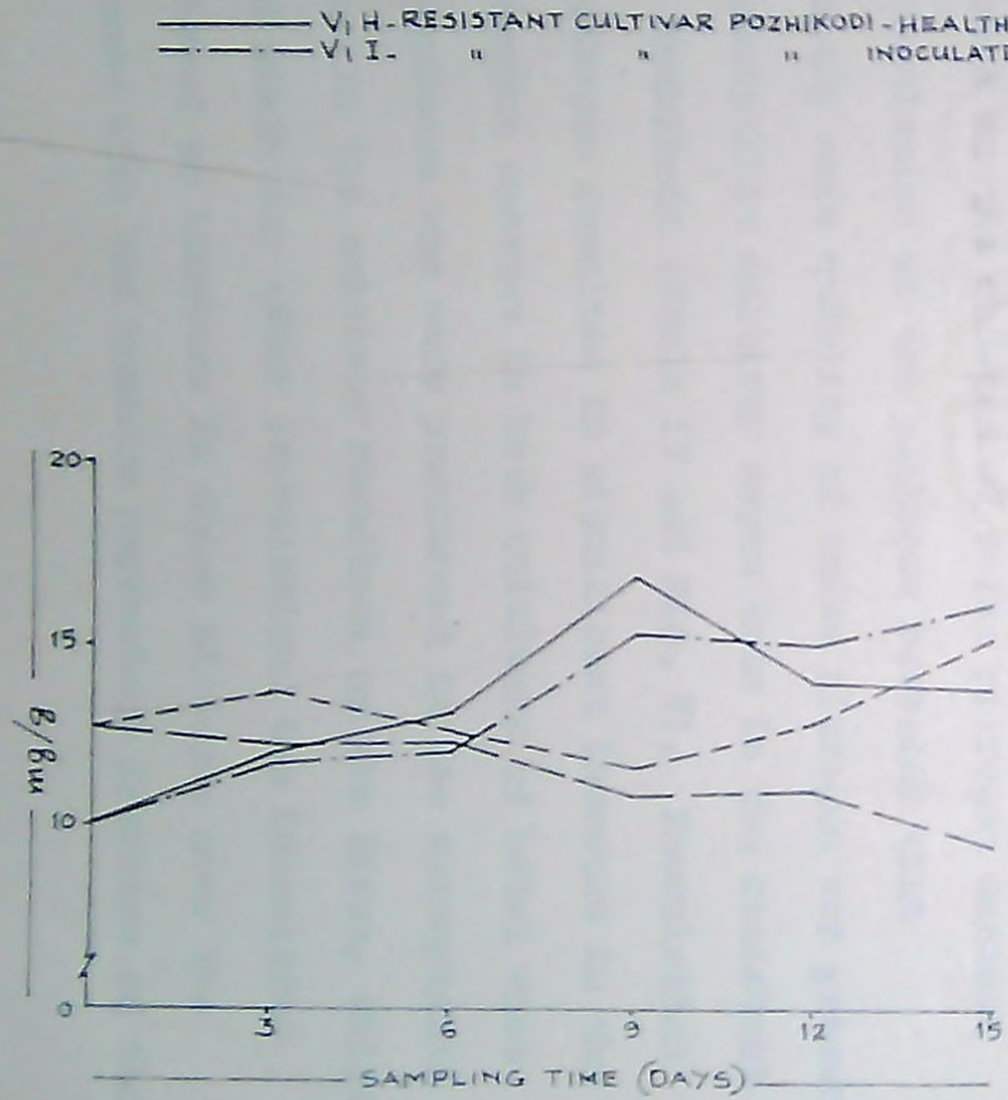
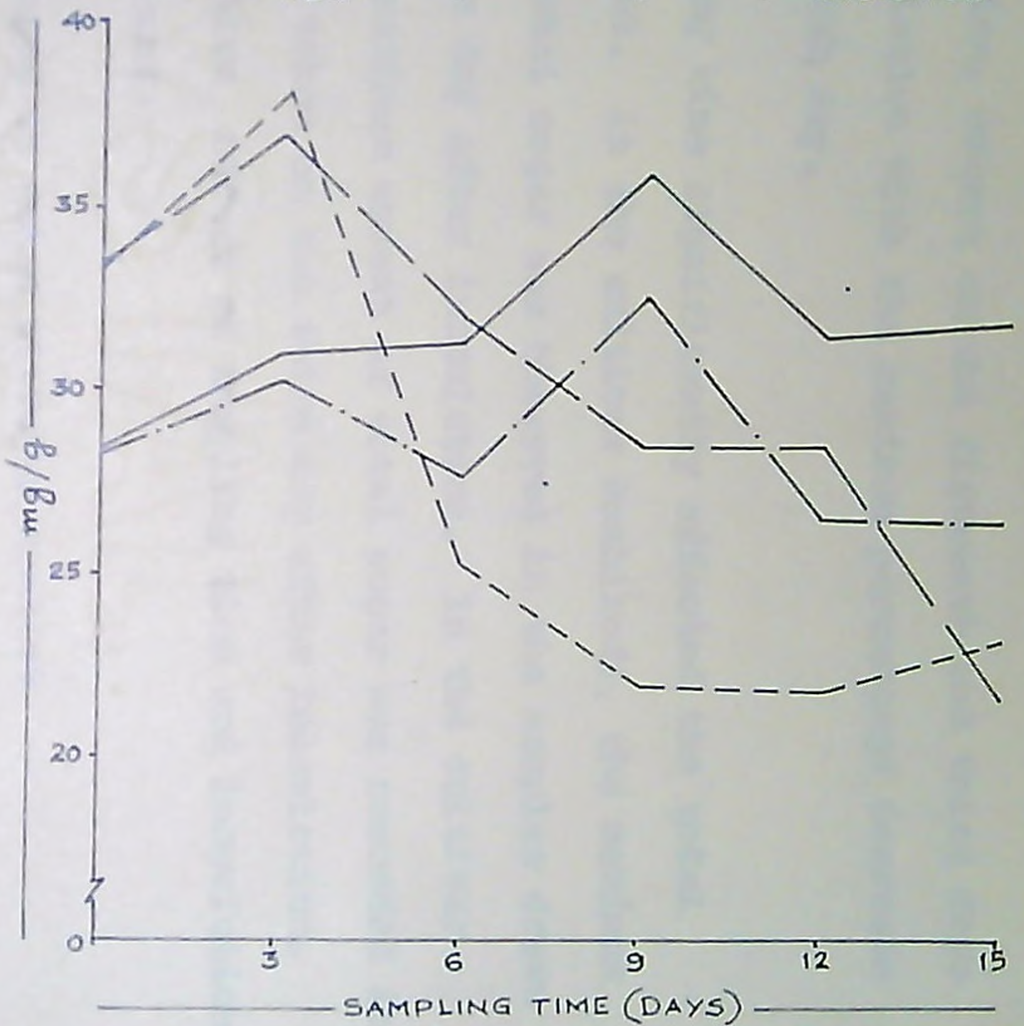


Fig.6. CHANGES IN TOTAL SUGAR IN BETELVINE CULTIVARS AS INFLUENCED BY X. CAMPESTRIS PV. BETLICOLA INOCULATION.



of observation, except on the fifteenth and third days after inoculation with the maximum percentage decrease on the twelfth day.

Sampling time significantly affected the total sugar content. In the cultivar Pozhikodi, the maximum amount of total sugar was observed in the samples drawn on the ninth day after inoculation. In the cultivar Aryan, the maximum amount of total sugar was recorded in the samples taken on the third day after inoculation. The interaction effect of sampling time and inoculation was significant.

4.5.11 Changes in amino nitrogen content

Results on the changes of amino nitrogen content due to inoculation of the pathogen revealed that significantly more quantity of amino nitrogen was present in the susceptible cultivar Aryan than in the resistant cultivar Pozhikodi (Table 17 and Fig. 7). Inoculation of the pathogen resulted in significant increase in the amino nitrogen content in both cultivars of betel vine and the increase was more pronounced in the susceptible cultivar. In the cultivar Pozhikodi, on the first, third, sixth and ninth day after inoculation, the inoculated plants showed an increase in amino nitrogen over the healthy ones with the maximum percentage increase on the

Table 17. Changes in amino nitrogen content in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1)		Per cent increase(+) or decrease(-) over healthy	Aryan (V_2)		Per cent increase(+) or decrease(-) over healthy
	Healthy (H)	Inoculated (I)		Healthy (H)	Inoculated (I)	
0	4.90	4.93	+0.61	6.57	6.53	-0.61
3	5.47	5.67	+3.66	6.87	7.57	+10.19
6	6.00	8.77	+46.17	6.90	8.93	+29.42
9	5.20	5.53	+6.35	7.07	9.73	+37.62
12	8.33	7.03	-15.61	6.63	8.83	+33.18
15	9.27	9.13	-1.51	9.73	9.57	-1.64

* Expressed as glycine equivalent in mg/g fresh tissues

Contd.

Table 17. (Contd.) Mean table for the changes in amino nitrogen content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	4.917	6.550	5.733	5.733	5.733
3	5.567	7.217	6.167	6.617	6.392
6	7.383	7.917	6.450	8.850	7.650
9	5.367	8.400	6.133	7.633	6.883
12	7.683	7.733	7.483	7.933	7.708
15	9.200	9.650	9.500	9.350	9.425
Mean	6.686	7.911	6.911	7.686	

	H	I
V_1	6.528	6.844
V_2	7.294	8.528

	CD (0.05)
Cultivar/treatment	- 0.1196
Sampling time	- 0.2071
Cultivar x treatment	- 0.1691
Sampling time x cultivar/ sampling time x treatment	- 0.2929

sixth day. At other intervals, there was a decrease in amino nitrogen content in the inoculated leaves over healthy with the maximum percentage decrease on the twelfth day. In the cultivar Aryan, except samples of the first and fifteenth day after inoculation, all other samples of inoculated plants showed an increase of amino nitrogen content over healthy with the maximum percentage increase on the ninth day.

Sampling time had a significant effect on the amino nitrogen content. In both cultivars of betel vine, the maximum amino nitrogen content was recorded in samples drawn on the fifteenth day after inoculation. Interaction effect of sampling time and inoculation was significant.

4.5.12 Changes in total nitrogen content

The results indicated that the susceptible cultivar Aryan contained significantly higher quantity of total nitrogen than the resistant cultivar Poozhikodi (Table 18 and Fig. 8). Inoculation of the pathogen resulted in an accumulation of total nitrogen in both cultivars of betel vine and the increase was more in the susceptible cultivar Aryan. In the cultivar Poozhikodi, the maximum percentage increase of total nitrogen in inoculated plants over uninoculated was on the twelfth day after inoculation. In the cultivar Aryan, a higher percentage

Table 18. Changes in total nitrogen content* in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1) Healthy (H)	Pozhikodi (V_1) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy	Aryan (V_2) Healthy (H)	Aryan (V_2) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy
0	1.274	1.286	+0.94	1.322	1.322	0
3	1.395	1.407	+0.86	1.492	1.553	+4.09
6	1.516	1.577	+4.02	1.553	1.735	+11.72
9	1.553	1.613	+3.86	1.516	1.759	+16.03
12	1.480	1.577	+6.55	1.589	1.685	+6.04
15	1.504	1.589	+5.65	1.625	1.698	+4.49

* Expressed as per cent dry weight

Contd.

Table 18. (Contd.) Mean table for the changes in total nitrogen content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	1.281	1.322	1.299	1.305	1.302
3	1.400	1.523	1.443	1.480	1.461
6	1.547	1.644	1.535	1.656	1.596
9	1.583	1.638	1.535	1.687	1.611
12	1.529	1.642	1.538	1.632	1.585
15	1.550	1.662	1.565	1.647	1.606
Mean	1.482	1.572	1.486	1.568	

	H	I
V_1	1.454	1.510
V_2	1.518	1.626

	CD (0.05)
Cultivar/treatment	- 0.0135
Sampling time	- 0.0234
Cultivar x treatment	- 0.0191
Sampling time x cultivar/ sampling time x treatment	- 0.0331

Fig-7. CHANGES IN AMINO NITROGEN IN BETELVINE CULTIVARS AS INFLUENCED BY X. CAMPESTRIS PV. BETLICOLA INOCULATION.

———— V_1 H - RESISTANT CULTIVAR POZHIKODI - HEALTHY.
—·—·— V_1 I - " " " INOCULATED.

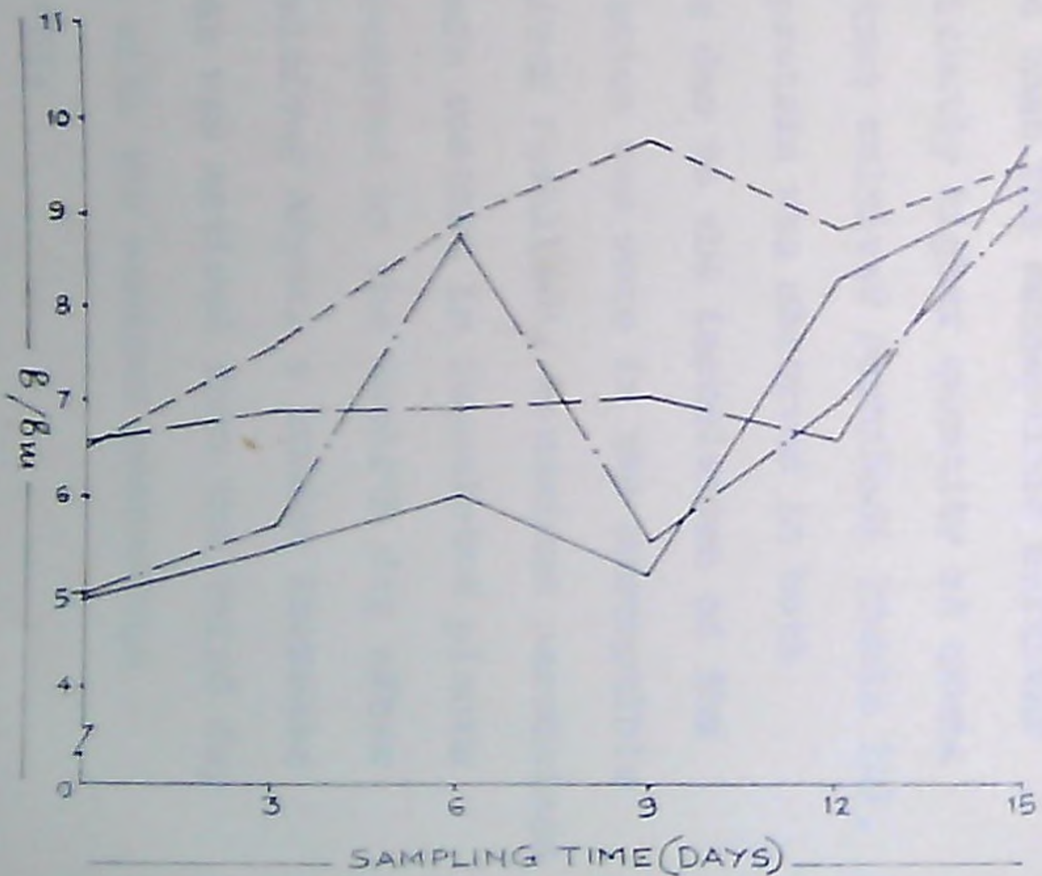
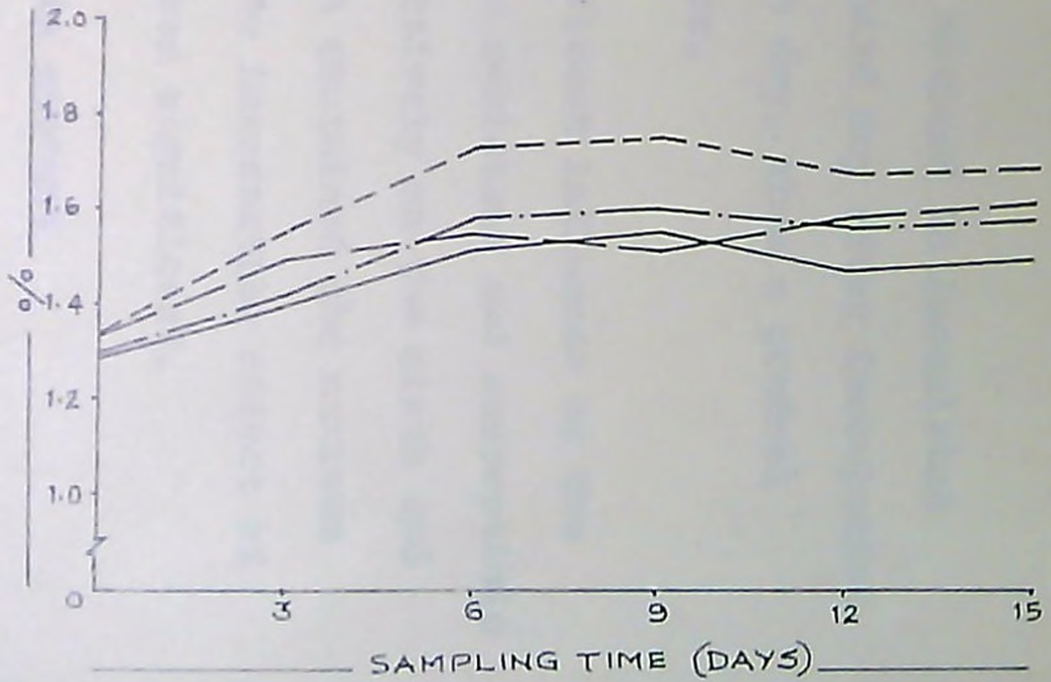


Fig-8. CHANGES IN TOTAL NITROGEN IN BETELVINE CULTIVARS AS INFLUENCED BY X. CAMPESTRIS PV. BETLICOLA INOCULATION.

— — — V_2 H . SUSCEPTIBLE CULTIVAR ARYAN - HEALTHY.
– – – – – V_2 I . " " " INOCULATED



increase of total nitrogen was noticed in inoculated plants over healthy from the third day after inoculation, reaching the maximum on the ninth day, then a gradual decrease was noticed there after.

Sampling time had a significant influence on the total nitrogen content. In the resistant and susceptible cultivars, samples taken respectively on the ninth and fifteenth day after inoculation contained the maximum quantity of total nitrogen. The interaction effect of sampling time and inoculation was significant.

4.5.13 Changes in crude protein content

The results showed that the susceptible cultivar Aryan contained significantly higher quantity of crude protein than the resistant cultivar Pozhikodi (Table 19). Accumulation of crude protein was observed in both cultivars of betel vine due to the inoculation of the pathogen. The accumulation was more in the susceptible cultivar. In the cultivar Pozhikodi, the maximum percentage increase of crude protein content in inoculated plants over healthy ones was observed on the twelfth day after inoculation. In the cultivar Aryan, a sudden increase of crude protein content was noticed from the third day of inoculation onwards with the maximum percentage increase on the ninth day.

Table 19. Changes in crude protein content* in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1)		Per cent increase(+) or decrease(-) over healthy	Aryan (V_2)		Per cent increase(+) or decrease(-) over healthy
	Healthy (H)	Inoculated (I)		Healthy (H)	Inoculated (I)	
0	7.967	8.042	+0.94	8.266	8.266	0
3	8.721	8.797	+0.87	9.327	9.707	+4.07
6	9.479	9.858	+4.00	9.707	10.840	+11.67
9	9.707	10.086	+3.90	9.479	10.996	+16.00
12	9.252	9.858	+6.55	9.934	10.541	+6.11
15	9.403	9.934	+5.64	10.162	10.616	+4.47

* Expressed as per cent dry weight

Contd.

Table 19. (Contd.) Mean table for the changes in crude protein content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	8.005	8.266	8.117	8.154	8.135
3	8.759	9.517	9.024	9.252	9.138
6	9.669	10.161	9.479	10.351	9.915
9	9.897	10.352	9.707	10.541	10.124
12	9.555	10.238	9.593	10.199	9.897
15	9.669	10.389	9.783	10.276	10.029
Mean	9.259	9.821	9.284	9.796	

	H	I
V_1	9.088	9.430
V_2	9.479	10.162

	CD (0.05)
Cultivar/treatment	- 0.0836
Sampling time	- 0.1448
Cultivar x treatment	- 0.1182
Sampling time x cultivar/ sampling time x treatment	- 0.2048

Sampling time significantly influenced the crude protein content. In the cultivar Pozhikodi, samples taken on the ninth day after inoculation had the maximum amount of crude protein while in the cultivar Aryan it was on the fifteenth day samples. The interaction effect of sampling time and inoculation was significant.

4.5.14 Changes in phosphorus content

The result of the study did not show significant differences in the phosphorus content between the cultivars (Table 20). In general, inoculation of the pathogen resulted in a decrease in phosphorus content in both cultivars of betel vine, but the decrease was significant only in the case of susceptible cultivar Aryan. In the resistant cultivar, the maximum percentage decrease in phosphorus content in inoculated plants over healthy was on the third day after inoculation but in the susceptible cultivar it was on the twelfth day.

Sampling time had a significant influence on the phosphorus content. In the cultivar Pozhikodi, the maximun amount of phosphorus was noticed in samples taken on the twelfth day after inoculation, while in the cultivar Aryan, it was in samples of the ninth day. The interaction effect of sampling time and inoculation was significant.

Table 20. Changes in phosphorus content* in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1)		Per cent increase(+) or decrease(-) over healthy	Aryan (V_2)		Per cent increase(+) or decrease(-) over healthy
	Healthy (H)	Inoculated (I)		Healthy (H)	Inoculated (I)	
0	0.369	0.359	-2.71	0.342	0.339	-0.88
3	0.364	0.338	-7.14	0.369	0.369	0
6	0.395	0.409	-3.54	0.397	0.390	-1.76
9	0.378	0.376	-0.53	0.438	0.430	-1.83
12	0.447	0.445	-0.45	0.430	0.378	-12.09
15	0.442	0.428	-3.17	0.435	0.390	-10.34

* Expressed as per cent dry weight

Contd.

Table 20. (Contd.) Mean table for the changes in phosphorus content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	0.364	0.342	0.356	0.350	0.353
3	0.351	0.369	0.367	0.354	0.360
6	0.402	0.394	0.397	0.400	0.398
9	0.377	0.435	0.408	0.404	0.406
12	0.446	0.405	0.439	0.412	0.426
15	0.436	0.413	0.440	0.410	0.425
Mean	0.396	0.393	0.401	0.388	

	H	I
V_1	0.400	0.393
V_2	0.402	0.383

	CD (0.05)
Cultivar	- NS
Treatment	- 0.0050
Sampling time	- 0.0090
Cultivar x treatment	- 0.0070
Sampling time x cultivar/ sampling time x treatment	- 0.0130

NS - Non significant

4.5.15 Changes in potassium content

In general, inoculation of the pathogen resulted in a reduction of potassium content in both the resistant and susceptible cultivars of betel vine (Table 21). In the resistant cultivar Pozhikodi, a decrease of potassium content was recorded in inoculated plants over uninoculated ones at different intervals of observation, with the maximum percentage decrease on the sixth day after inoculation. In the susceptible cultivar Aryan, inoculated plants showed a decrease in potassium content over uninoculated ones at all intervals of observation except on the third day. The maximum percentage decrease in potassium content in this cultivar was on the fifteenth day after inoculation.

Sampling time had a significant influence on the potassium content. In both cultivars, the maximum amount of potassium was noticed in samples drawn on the third day after inoculation and the minimum on the fifteenth day. The interaction effect of sampling time and inoculation was significant.

4.5.16 Changes in calcium content

Significantly higher quantity of calcium was observed in the resistant cultivar Pozhikodi than in the susceptible

Table 21. Changes in potassium content* in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1) Healthy (H)	Pozhikodi (V_1) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy	Aryan (V_2) Healthy (H)	Aryan (V_2) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy
0	3.84	3.78	-1.56	3.76	3.70	-1.60
3	4.85	4.53	-6.60	4.18	4.66	+11.48
6	4.00	3.68	-8.00	4.34	4.02	-7.37
9	3.97	3.94	-0.76	4.37	4.00	-8.47
12	4.05	3.81	-5.93	4.10	3.94	-3.90
15	3.76	3.54	-5.85	3.54	2.96	-16.38

* Expressed as per cent dry weight

Contd.

Table 21. (Contd.) Mean table for the changes in potassium content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	3.81	3.73	3.80	3.75	3.77
3	4.69	4.43	4.52	4.60	4.56
6	3.84	4.19	4.17	3.85	4.01
9	3.96	4.19	4.17	3.97	4.07
12	3.93	4.03	4.08	3.88	3.98
15	3.65	3.25	3.63	3.25	3.45
Mean	3.98	3.97	4.07	3.88	

	H	I
V_1	4.08	3.88
V_2	4.05	3.88

	CD (0.05)
Cultiver	- NS
Treatment	- 0.0060
Sampling time	- 0.0100
Cultivar x treatment	- NS
Sampling time x cultivar/ sampling time x treatment	- 0.0140

NS - Non significant

cultivar Aryan (Table 22). Inoculation of the pathogen resulted in an increase in calcium content in the resistant cultivar, while in the susceptible cultivar it led to a decrease. The inoculated plants of the cultivar Pozhikodi showed the maximum percentage increase of calcium over healthy ones on the twelfth day after inoculation. Inoculated plant of the cultivar Aryan on the first and third day of inoculation showed a percentage increase of calcium over healthy ones. At all other intervals, a percentage decrease was noticed with the maximum on the fifteenth day after inoculation.

Sampling time had a significant effect on the calcium content. In the cultivar Pozhikodi, samples taken on the day of inoculation showed the maximum calcium content while in the cultivar Aryan, the maximum amount was in the ninth day samples. The interaction effect of sampling time and inoculation was significant.

4.5.17 Changes in magnesium content

The results revealed that significantly higher quantity of magnesium was present in the resistant cultivar Pozhikodi than in the susceptible cultivar

Table 22. Changes in calcium content* in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1) Healthy (H)	Pozhikodi (V_1) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy	Aryan (V_2) Healthy (H)	Aryan (V_2) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy
0	1.128	1.148	+1.77	0.704	0.713	+1.28
3	0.928	0.985	+6.14	0.653	0.794	+21.59
6	0.782	0.873	+11.64	0.886	0.695	-21.56
9	0.658	0.808	+22.80	1.040	0.846	-18.65
12	0.886	1.123	+26.75	1.170	0.663	-43.33
15	1.027	1.151	+12.07	1.130	0.633	-43.98

* Expressed as per cent dry weight

Contd.

Table 22. (Contd.) Mean table for the changes in calcium content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	1.138	0.708	0.916	0.930	0.923
3	0.956	0.723	0.790	0.889	0.840
6	0.828	0.790	0.834	0.784	0.809
9	0.733	0.941	0.847	0.827	0.837
12	1.005	0.911	1.022	0.893	0.958
15	1.089	0.881	1.078	0.892	0.985
Mean	0.958	0.826	0.915	0.869	

	H	I
V_1	0.902	1.015
V_2	0.928	0.724

	CD (0.05)
Cultivar/treatment	- 0.0050
Sampling time	- 0.0090
Cultivar x treatment	- 0.0070
Sampling time x cultivar/ sampling time x treatment	- 0.0120

Aryan (Table 23). In the cultivar Pozhikodi, inoculation of the pathogen resulted in an increase in magnesium content while, in the cultivar Aryan, it led to a decrease. In the resistant cultivar, higher increase in magnesium in inoculated plants over healthy ones was noticed at all intervals of observation except on the third and fifteenth day after inoculation with the maximum percentage increase on the twelfth day. In the susceptible cultivar the maximum percentage decrease of magnesium content in inoculated plants over healthy was observed on the third day after inoculation.

Sampling time significantly influenced the magnesium content. In the cultivar Pozhikodi, the fifteenth day samples contained the maximum amount of magnesium, while in the cultivar Aryan it was in samples taken on the day of inoculation. The interaction effect of sampling time and inoculation was significant.

4.5.18 Changes in sodium content

In general, there was not much change in sodium content due to inoculation of the pathogen in both resistant and susceptible cultivars of betel vine (Table 24). However, it was found that the resistant cultivar Pozhikodi contained slightly higher amount of sodium than the susceptible cultivar Aryan. Inoculation

Table 23. Changes in magnesium* content in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1) Healthy (H)	Pozhikodi (V_1) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy	Aryan (V_2) Healthy (H)	Aryan (V_2) Inoculated (I)	Per cent increase(+) or decrease(-) over healthy
0	0.612	0.615	+0.49	0.641	0.644	+0.47
3	0.643	0.602	-6.38	0.643	0.469	-27.06
6	0.633	0.670	+5.85	0.570	0.422	-25.96
9	0.623	0.648	+4.01	0.644	0.491	-23.76
12	0.541	0.642	+18.67	0.569	0.543	-4.57
15	0.684	0.676	-1.17	0.514	0.469	-8.75

* Expressed as per cent dry weight

Contd.

Table 23. (Contd.) Mean table for the changes in magnesium content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	0.613	0.643	0.626	0.630	0.628
3	0.623	0.556	0.643	0.536	0.589
6	0.651	0.496	0.601	0.546	0.573
9	0.636	0.568	0.634	0.569	0.602
12	0.591	0.556	0.555	0.593	0.574
15	0.680	0.491	0.599	0.572	0.586
Mean	0.632	0.552	0.610	0.574	

	H	I
V_1	0.623	0.642
V_2	0.597	0.506

		CD (0.05)
Cultivar/treatment	-	0.0070
Sampling time	-	0.0013
Cultivar x treatment	-	0.0010
Sampling time x cultivar/ sampling time x treatment	-	0.0018

Table 24. Changes in sodium content* in leaves of betel vine cultivars as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation (T)	Pozhikodi (V_1)		Per cent increase(+) or decrease(-) over healthy	Aryan (V_2)		Per cent increase(+) or decrease(-) over healthy
	Healthy (H)	Inoculated (I)		Healthy (H)	Inoculated (I)	
0	0.176	0.176	0	0.173	0.173	0
3	0.176	0.173	-1.70	0.176	0.173	-1.70
6	0.173	0.176	+1.73	0.173	0.173	0
9	0.170	0.176	+3.53	0.173	0.173	0
12	0.173	0.173	0	0.173	0.173	0
15	0.176	0.173	-1.70	0.173	0.173	0

* Expressed as per cent dry weight

Contd.

Table 24. (Contd.) Mean table for the changes in sodium content in betel vine cultivars

T	V_1	V_2	H	I	Mean
0	0.176	0.173	0.175	0.175	0.175
3	0.175	0.175	0.176	0.173	0.175
6	0.175	0.173	0.173	0.175	0.174
9	0.173	0.173	0.172	0.175	0.173
12	0.173	0.172	0.172	0.173	0.173
15	0.173	0.173	0.173	0.173	0.173
Mean	0.174	0.173	0.174	0.174	

	H	I
V_1	0.174	0.175
V_2	0.173	0.173

	CD (0.05)
Cultivar/treatment	- NS
Sampling time	- NS
Cultivar x treatment	- NS
Sampling time x cultivar/ sampling time x treatment	- NS

NS - Non significant

of the cultivars with the pathogen did not alter the sodium content significantly.

In the cultivar Pozhikodi on the third and fifteenth day after inoculation a percentage decrease in sodium content in inoculated plants over healthy was noticed, while a percentage increase was noticed on the sixth and the ninth day after inoculation. In the cultivar Aryan a percentage decrease of sodium content in inoculated plants over uninoculated was observed on the third day after inoculation. At all other intervals there was no change in sodium content due to inoculation of the pathogen.

4.5.19 Changes in phylloplane microflora

The results on the changes in phylloplane microflora of the resistant and the susceptible cultivars of betel vine are presented in Tables 25a and 25b.

Fluctuation in the total phylloplane microflora was observed in inoculated and uninoculated leaves of both resistant and susceptible cultivars of betel vine. In general, inoculation of the cultivars with the pathogen resulted in an increase in the total phylloplane microflora. Fluctuations in the population of actinomycetes, bacteria and fungi were also noticed in inoculated and uninoculated

Table 25a. Changes in phylloplane microflora* of resistant betel vine cultivar Pozhikodi as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation	Actinomycetes		Bacteria		Fungi		Total	
	Healthy	Inoculated	Healthy	Inoculated	Healthy	Inoculated	Healthy	Inoculated
0	1804.67	1698.52	1167.73	849.26	1.06	0.37	2973.46	2548.15
3	4033.97	2760.08	1272.20	5520.17	3.29	2.44	5309.45	8282.69
6	5414.01	4033.97	5201.70	11040.34	5.94	1.38	10621.65	15075.69
9	530.78	2123.14	1273.89	5414.01	0.75	1.27	1805.42	7538.42
12	2016.99	5095.54	6263.27	5095.54	1.48	1.27	8281.74	10192.35
15	743.10	530.78	20382.17	6369.43	5.10	3.39	21130.37	6903.60

* Expressed as number per cm^2, (Average of three replications)

Table 25b. Changes in the phylloplane microflora* of the susceptible betel vine cultivar Aryan as influenced by X. campestris pv. betlicola inoculation

Sampling time in days after inoculation	Actinomycetes		Bacteria		Fungi		Total	
	Healthy	Inoculated	Healthy	Inoculated	Healthy	Inoculated	Healthy	Inoculated
0	955.41	2016.99	1166.04	4352.44	0.85	0.85	2122.30	6370.28
3	4033.97	530.78	5626.32	1378.36	1.91	1.71	9662.20	1910.31
6	1486.20	636.94	9872.61	8492.57	1.70	4.46	11360.51	9133.97
9	743.10	1804.67	743.10	6475.58	1.48	1.06	1487.68	8281.31
12	5944.80	4140.13	1482.83	15498.94	1.17	0.75	7428.80	19639.82
15	1167.73	2441.62	19320.59	36798.64	1.70	4.03	20490.02	39244.29

* Expressed as number per cm^2, (Average of three replications)

leaves of the resistant cultivar Poshikodi and the susceptible cultivar Aryan. The individual population of these microbes generally increased with the increase in sampling time. Observation made on the fifteenth day after inoculation revealed a decrease in the population of actinomycetes, bacteria and fungi in the inoculated leaves of the resistant cultivar compared to the uninoculated ones, while a reverse trend was noticed in the susceptible cultivar Aryan.

4.6 Control of bacterial leaf spot of betel vine

As part of studies to evolve a viable management/ control method against the bacterial leaf spot of betel vine, the effect of nitrogen, phosphorus and potassium on the development of the disease was assessed. *In vitro* and *in vivo* testing of plant protection chemicals against the pathogen and screening of betel vine cultivars for resistance/tolerance to the disease were also carried out in this study.

4.6.1 Effect of different levels of nitrogen, phosphorus and potassium on the development of bacterial leaf spot of betel vine

The results of the experiment on the effect of nitrogen, phosphorus and potassium on the development of

the bacterial leaf spot of betel vine are presented below:

Disease score

The effect of nitrogen, phosphorus and potassium on the disease score was not significant after ten and forty days of inoculation (Table 26a and 26b). However, plants receiving 100 kg N/ha and no phosphorus showed the minimum disease score after ten and forty days of inoculation. Plants receiving potassium showed comparatively less disease score after ten days of inoculation, whereas no noticeable difference was observed after forty days. The interaction effect of nitrogen, phosphorus and potassium was not significant.

Stem infection

The data on the percentage of stem infection after ten and forty days of inoculation are given in Table 27a and 27b. The application of nitrogen, phosphorus and potassium had no significant influence on the development of the stem infection at different intervals of observation. However, after ten and forty days of inoculation, comparatively minimum percentage of stem infection was observed in plants receiving 100 kg N/ha and 200 kg N/ha respectively. Minimum percentage of

Table 26a. Effect of different levels of N, P and K on the development of bacterial leaf spot of betel vine - Disease score ten days after inoculation

	n_0	n_1	n_2	n_3	Mean
P_0	3.61	4.17	3.96	3.77	3.88
P_1	4.92	4.46	4.75	5.03	4.79
P_2	4.88	4.91	5.70	4.86	5.09
P_3	6.20	4.97	6.05	5.11	5.58
k_0	5.51	4.67	5.36	5.03	5.14
k_1	4.25	4.95	4.19	4.15	4.38
k_2	5.68	3.62	5.95	4.26	4.88
k_3	4.17	5.26	4.96	5.32	4.93
Mean	4.90	4.63	5.11	4.69	

	k_0	k_1	k_2	k_3
P_0	4.29	3.46	4.64	3.12
P_1	5.18	4.62	4.42	4.95
P_2	4.75	4.11	5.95	5.54
P_3	6.36	5.35	4.51	6.10

CD (0.05) N, P and K - NS

CD (0.05) N x P, N x K and P x K - NS

NS - Non significant

Table 26b. Effect of different levels of N, P and K on the development of bacterial leaf spot of betel vine - Disease score forty days after inoculation

	n_0	n_1	n_2	n_3	Mean
P_0	9.91	9.71	10.02	10.00	9.91
P_1	10.23	10.13	10.00	10.25	10.15
P_2	10.00	9.91	10.13	10.00	10.00
P_3	10.25	10.00	10.25	10.00	10.15
k_0	9.76	9.98	10.13	10.13	10.00
k_1	10.25	10.00	10.13	10.00	10.09
k_2	10.13	9.91	9.89	10.13	10.01
k_3	10.25	9.86	10.25	10.00	10.09
Mean	10.10	9.94	10.10	10.06	

	k_0	k_1	k_2	k_3
P_0	9.89	10.00	9.89	9.86
P_1	10.11	10.13	10.25	10.13
P_2	10.00	10.00	9.91	10.13
P_3	10.00	10.25	10.00	10.25

CD (0.05) N, P and K - NS

CD (0.05) N x P, N x K and P x K - NS

NS - Non significant

Table 27a. Effect of different levels of N, P and K on the development of bacterial leaf spot of betel vine - Percentage of stem infection ten days after inoculation

	n_0	n_1	n_2	n_3	Mean
P_0	2.09	3.64	3.71	7.07	4.13
P_1	5.09	2.32	4.95	5.11	4.37
P_2	3.79	3.31	8.48	2.57	4.54
P_3	11.10	2.80	3.20	2.09	4.78
k_0	2.49	4.68	5.04	5.14	4.34
k_1	8.18	2.58	5.26	3.07	4.77
k_2	6.76	1.90	5.56	5.71	4.98
k_3	4.56	2.92	4.49	2.93	3.72
Mean	5.50	3.02	5.09	4.21	

	k_0	k_1	k_2	k_3
P_0	6.49	2.62	4.58	2.83
P_1	3.76	3.30	7.35	3.05
P_2	3.47	5.44	4.59	4.66
P_3	3.62	7.73	3.40	4.36

CD (0.05) N, P and K - NS

CD (0.05) N x P - 5.03

CD (0.05) N x K and P x K - NS

NS - Non significant

Table 27b. Effect of different levels of N, P and K on the development of bacterial leaf spot of betel vine - Percentage of stem infection forty days after inoculation

	n_0	n_1	n_2	n_3	Mean
P_0	36.13	26.08	60.87	41.20	41.07
P_1	58.94	44.85	36.07	44.64	46.13
P_2	28.85	36.07	48.40	27.98	35.32
P_3	58.50	35.45	42.64	28.27	41.21
k_0	36.20	46.48	39.15	40.95	40.70
k_1	51.01	27.01	49.92	36.80	41.18
k_2	47.22	30.21	50.33	39.29	41.76
k_3	47.99	38.75	48.57	25.05	40.09
Mean	45.61	35.61	46.99	35.52	

	k_0	k_1	k_2	k_3
P_0	45.28	38.54	47.30	33.15
P_1	48.78	38.17	56.15	41.46
P_2	32.96	34.21	35.44	38.69
P_3	35.82	53.83	28.16	47.06

CD (0.05) N, P and K - NS

CD (0.05) N x P, N x K and P x K - NS

NS - Non significant

stem infection ten days after inoculation was noticed in plants receiving no phosphorus, but after forty days the minimum stem infection was in plants supplied with 100 kg P_2O_5/ha. There was not much difference among the levels of potassium on the development of stem infection. However, plants receiving 150 kg K_2O/ha showed comparatively lower percentage of stem infection.

The interaction effect of nitrogen and phosphorus was significant after ten days of inoculation and after forty days it was not significant. The interaction effect of nitrogen and potassium, and phosphorus and potassium on the percentage of stem infection was not significant at all intervals.

Defoliation

Nitrogen and potassium had no significant effect in reducing the percentage of defoliation after ten and forty days of inoculation (Tables 28a and 28b). But after ten and forty days of inoculation, the minimum percentage of defoliation was observed when nitrogen was applied at the rate of 200 kg N/ha. Plants receiving potassium at the rate of 50 kg K_2O/ha and 150 kg K_2O/ha recorded the minimum percentage of defoliation after ten and forty days of inoculation respectively.

Table 28a. Effect of different levels of N, P and K on the development of bacterial leaf spot of betel vine - Percentage of defoliation ten days after inoculation

	n_0	n_1	n_2	n_3	Mean
P_0	17.56	22.06	20.94	16.82	19.34
P_1	39.06	23.50	29.10	26.16	29.46
P_2	34.25	37.30	41.45	38.57	37.89
P_3	57.29	35.36	47.07	31.35	42.77
k_0	41.45	29.71	39.46	23.22	33.46
k_1	25.00	34.90	20.66	22.58	25.79
k_2	46.19	14.63	48.19	23.03	33.01
k_3	35.52	38.96	30.24	44.08	37.20
Mean	37.04	29.55	34.64	28.23	

	k_0	k_1	k_2	k_3
P_0	21.08	12.64	28.69	14.96
P_1	29.54	27.46	30.64	30.18
P_2	32.28	26.68	45.07	47.52
P_3	50.94	36.35	27.64	56.15

CD (0.05) P - 14.62

CD (0.05) N and K - NS

CD (0.05) N x P, N x K and P x K - NS

NS - Non significant

Table 28b. Effect of different levels of N, P and K on the development of bacterial leaf spot of betel vine - Percentage of defoliation forty days after inoculation

	n_0	n_1	n_2	n_3	Mean
P_0	98.96	95.29	96.87	100.00	97.78
P_1	98.06	100.00	100.00	100.00	99.52
P_2	100.00	100.00	100.00	91.67	97.92
P_3	100.00	100.00	100.00	100.00	100.00
k_0	97.04	98.21	100.00	100.00	98.81
k_1	100.00	100.00	100.00	100.00	100.00
k_2	100.00	98.86	96.87	100.00	98.93
k_3	100.00	98.21	100.00	91.67	97.47
Mean	99.26	98.82	99.22	97.92	

	k_0	k_1	k_2	k_3
P_0	97.17	100.00	95.74	98.21
P_1	98.08	100.00	100.00	100.00
P_2	100.00	100.00	100.00	91.67
P_3	100.00	100.00	100.00	100.00

CD (0.05) N, P and K - NS

CD (0.05) N x P, N x K and P x K - 4.45

NS - Non significant

Significant difference in the percentage of defoliation among the levels of phosphorus was observed after ten days of inoculation, while it was not significant after forty days. Ten days after inoculation, plants not receiving phosphorus showed the lowest percentage of defoliation and this treatment was on par with that of 50 kg P_2O_5/ha and the latter being on par with that of other levels. After forty days of inoculation, the minimum percentage of defoliation was noticed in plants receiving no phosphorus.

The interaction effect of nitrogen and phosphorus, nitrogen and potassium, and phosphorus and potassium on the percentage of defoliation after ten days of inoculation was not significant, while it was significant after forty days. Nitrogen and phosphorus at 200 kg N/ha and 100 kg P_2O_5/ha when applied to plants showed the lowest percentage of defoliation and was superior to other levels except 100 kg N/ha with no phosphorus. Nitrogen and potassium at the rate of 200 kg N/ha and 150 kg K_2O/ha recorded the minimum percentage of defoliation and was superior to all other levels which were on par. Phosphorus and potassium at the rate of 100 kg P_2O_5/ha and 150 kg K_2O/ha showed the lowest percentage of defoliation and was on par with no phosphorus and 150 kg K_2O/ha and the latter was on par with other levels.

4.6.2 Chemical control of bacterial leaf spot of betel vine

4.6.2.1 *In vitro* evaluation of chemicals against *X. campestris* pv. *betlicola*

The present study was carried out to assess the efficacy of antibiotics, fungicides and the combination of antibiotics and Bordeaux mixture in inhibiting the growth of the pathogen *X. campestris* pv. *betlicola*.

In vitro effect of antibiotics on the growth of *X. campestris* pv. *betlicola*

Three antibiotics, namely Paushamycin, Plantomycin and Streptocycline at 100, 200 and 300 ppm were used to study their inhibitory effect on the growth of the bacterium. The results are presented in Table 29a & Fig.9. Among the antibiotics tested, Plantomycin was found to be significantly superior in inhibiting the growth of the bacterium followed by Streptocycline. Paushamycin recorded the least inhibitory effect. Higher the concentration of antibiotics, more was the inhibitory effect. No significant difference was noticed between antibiotics and their concentrations.

Table 29a **In vitro** sensitivity of X. **campestris** pv. **betlicola** to antibiotics

Antibiotics	Inhibition zone in mm			Mean
	100 ppm	200 ppm	300 ppm	
Paushamycin	19.00	20.33	23.33	20.89
Plantomycin	23.00	24.67	27.00	24.89
Streptocycline	20.33	23.33	25.33	23.00
Mean	20.78	22.78	25.22	

C.D. (0.05) Antibiotic/concentration - 1.011

C.D. (0.05) Antibiotic X concentration - N.S.

However, among the concentrations tested Plantomycin at 300 ppm gave the maximum inhibition followed by Streptocycline at 300 ppm.

In vitro effect of fungicides on the growth of X. campestris pv. betlicola

Blitox, Captaf, Dithane M-45 and Dithane Z-78, each at 1000, 2000 and 3000 ppm were screened for their inhibitory effect on the growth of the bacterium. Bordeaux mixture at 0.5 and one per cent concentrations were also tested for its inhibitory effect. The results are presented in Table 29b. and Fig. 9.

Of the four fungicides tested, Dithane M-45 recorded the maximum inhibition and was superior to Blitox, Captaf and Dithane Z-78 in that order. The inhibitory effect of Blitox and Captaf was on par but superior to Dithane Z-78. Higher the concentration of fungicidesmore was the inhibition. Dithane M-45 at 3000 ppm gave the maximum inhibition followed by Blitox 3000 ppm. Bordeaux mixture one per cent gave the maximum inhibition of the bacterium and was superior to 0.5 per cent. Bordeaux mixture was found to be superior in inhibiting the growth of the bacterium than other fungicides tested, all other fungicides showed relatively less efficacy.

Table 29b. *In vitro* sensitivity of *X. campestris* pv. *betlicola* to fungicides

Fungicides	Inhibition zone in mm			Mean
	1000 ppm	2000 ppm	3000 ppm	
Blitox	13.00	17.33	19.33	16.55
Captaf	14.33	16.67	18.33	16.44
Dithane M-45	15.67	18.00	20.33	18.00
Dithane Z-78	13.67	14.67	15.67	14.67
Mean	14.17	16.67	18.42	16.40

	Inhibition zone in mm		Mean
	0.5%	1.0%	
Bordeaux mixture	17.33	20.67	19.00

C.D. (0.05) Fungicide - 1.011
C.D. (0.05) Concentration - 0.876
C.D. (0.05) Fungicide x concentration - 1.751

C.D. (0.05) Bordeaux mixture with fungicide - 0.946
C.D. (0.05) Bordeaux mixture with antibiotic/ Bordeaux mixture + antibiotic - 0.960

In vitro effect of the combination of antibiotics with Bordeaux mixture on the growth of X. campestris pv. betlicola

Paushamycin, Plantomycin and Streptocycline each at 100, 200 and 300 ppm concentrationswas mixed with one per cent Bordeaux mixture separately and their inhibitory effect against the bacterium was tested. The data are presented in Table 29c and Fig. 9.

Analysis of the data revealed that the inhibitory effect on the bacterial growth was the highest in Plantomycin and Bordeaux mixture combination while that of Paushamycin and Bordeaux mixture and Streptocycline and Bordeaux mixture was on par. Higher the concentrations of antibioticswith one per cent Bordeaux mixture more was the inhibition. The combination of 300 ppm of Plantomycin with one per cent Bordeaux mixture recorded the maximum inhibition and was superior to all other combinations.

The results of the experiment revealed that generally, the combination of antibiotics with Bordeaux mixture was more inhibitory to the bacterium than the antibiotics alone. An additive effect was recorded when Paushamycin at all concentrations and Plantomycin at 200 and 300 ppm were combined with one per cent Bordeaux mixture. Such

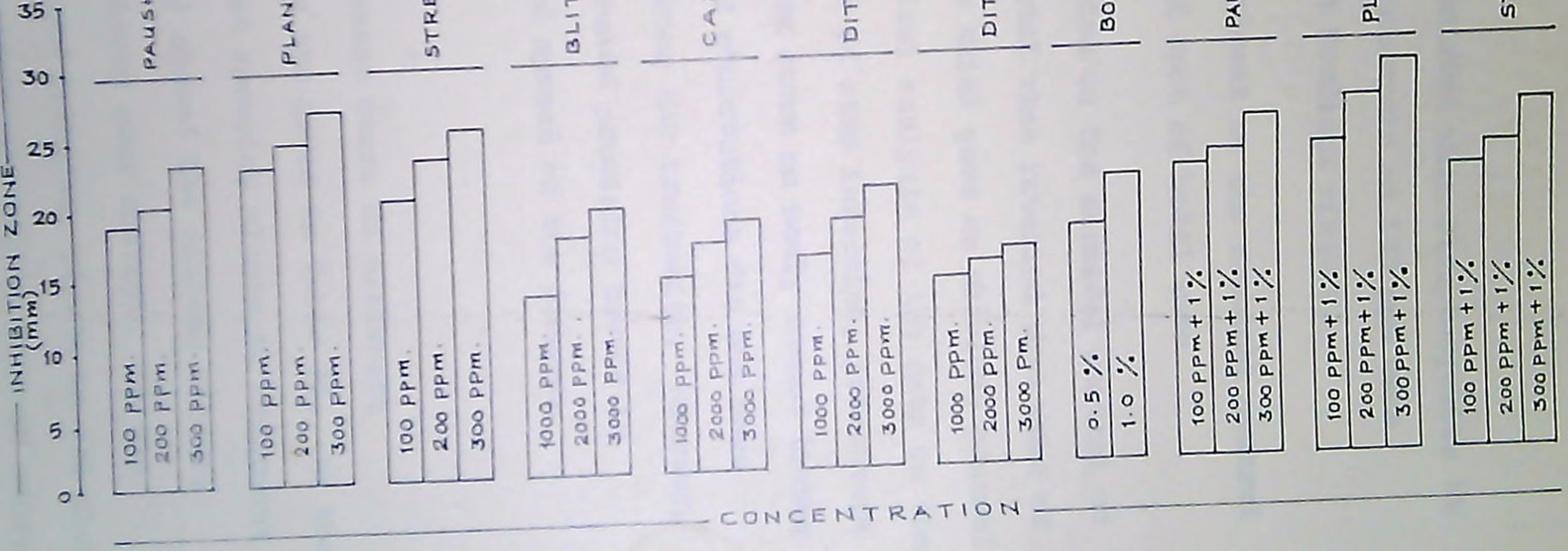
Table 29c. *In vitro* sensitivity of *X. campestris* pv. *betlicola* to the combination of antibiotics with Bordeaux mixture

Combination of chemicals	Inhibition zone in mm			Mean
	100 ppm +1%	200 ppm + 1%	300 ppm + 1%	
Paushamycin + Bordeaux mixture	21.33	22.33	24.67	22.78
Plantomycin + Bordeaux mixture	22.67	26.00	28.33	25.00
Streptocycline + Bordeaux mixture	20.83	22.33	25.33	22.76
Mean	20.78	23.55	26.11	

C.D. (0.05) Antibiotic + Bordeaux mixture/ concentration - 1.011

C.D. (0.05) Antibiotic + Bordeaux mixture x concentration - 1.751

FIG-9. IN VITRO EVALUATION OF CHEMICALS AGAINST X. CAMPESTRIS PV. BETLICOLA



additive effect was not observed when Streptocycline at all concentrations and Plantomycin at 100 ppm were combined with one per cent Bordeaux mixture.

4.6.2.2 Pot culture experiment on the chemical control of bacterial leaf spot of betel vine

The pot culture studies on the chemical control of bacterial leaf spot of betel vine revealed that none of the chemicals used in the trial gave an absolute control of the disease. Statistical analysis of the data on the disease score, percentage of stem infection, percentage of defoliation and disease score on newly formed leaves at different intervals of observations did not show significant difference among the treatments. However, some of the treatments showed promising trends in reducing the severity and spread of the disease.

Disease score

The data on the disease score at different intervals of observation are given in Table 30. On the fifteenth day after first spraying plants in treatment T_1 (Pausheamycin-200 ppm) showed the minimum disease score and maximum efficiency over control. On the fifteenth day after second spraying, plants in all the treatments recorded comparatively higher disease score.

Table 30. Pot culture experiment - Effect of chemical treatments on the control of bacterial leaf spot of betel vine - Disease score

Treatments	Disease score				Percentage efficiency of the treatment over control			
	On the day of first spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying	Mean
T_1 Paushamycin-200 ppm	1.06	8.03	10.75	11.00	18.48	2.27	0	6.92
T_2 Plantomycin-200 ppm	4.38	10.25	10.75	11.00	-4.06	2.27	0	-0.60
T_3 Streptocycline-200 ppm	2.39	9.96	10.25	10.75	-1.12	6.82	0	1.90
T_4 Bordeaux mixture-1%	3.10	10.50	10.50	10.50	-6.60	4.55	4.55	0.83
T_5 Blitox-3000 ppm	3.15	10.20	10.75	10.75	-3.55	2.27	2.27	0.33
T_6 Captaf-3000 ppm	1.67	9.31	10.75	11.00	5.48	2.27	0	2.58
T_7 Dithane M-45 - 3000 ppm	2.19	9.75	10.50	11.00	1.02	4.55	0	1.86
T_8 Dithane Z-78 - 3000 ppm	3.03	9.60	10.25	11.00	2.54	6.82	0	3.12
T_9 Paushamycin-200 ppm + Bordeaux mixture-1%	2.90	9.42	11.00	11.00	4.37	0	0	1.46
T_{10} Plantomycin-200 ppm + Bordeaux mixture-1%	2.78	9.63	10.50	10.75	2.23	4.55	2.27	3.02
T_{11} Streptocycline-200 ppm + Bordeaux mixture-1%	4.06	9.58	10.25	10.50	2.74	6.82	4.55	4.70
T_{12} Control	2.30	9.85	11.00	11.00	-	-	-	-
CD (0.05)	NS	NS	NS	NS				

NS - Non significant

Among the treatments, the treatments T_{11} (Streptocycline-200 ppm + Bordeaux mixture-1%), T_8 (Dithane Z-78) and T_3 (Streptocycline-200 ppm) showed the lowest disease score. None of the treatments showed any appreciable percentage efficiency over control in reducing the disease score. Observation on the fifteenth day after third spraying revealed that the minimum disease score was in treatments T_4 (Bordeaux mixture-1%) and T_{11} when compared with others. Here also, none of the treatments showed any appreciable percentage efficiency over control.

It was noticed that even though the plants sprayed with one per cent Bordeaux mixture showed a higher disease score at different intervals of observation, the disease score remained static through out the period of study revealing its efficacy in checking the further spread of the disease.

Stem infection

Results on the percentage of stem infection on the fifteenth day after first, second and third spraying are furnished in Table 31. Minimum percentage of stem infection on the fifteenth day after first spraying was in treatment T_{11} (Streptocycline-200 ppm + Bordeaux mixture-1%) and this treatment showed the maximum percentage efficiency over control. On the fifteenth day

Table 31. Pot culture experiment - Effect of chemical treatments on the control of bacterial leaf spot of betel vine - Percentage of stem infection

Treatments	Percentage of stem infection				Percentage efficiency of the treatment over control			
	On the day of first spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying	Mean
T_1 Paushamycin-200 ppm	2.63	52.61	78.10	100.00	21.08	21.90	0	14.33
T_2 Plantomycin-200 ppm	6.05	57.18	85.40	100.00	14.22	14.60	0	9.61
T_3 Streptocycline-200 ppm	6.86	62.69	65.68	88.60	5.96	34.32	11.40	17.23
T_4 Bordeaux mixture-1%	5.31	41.61	65.73	66.93	37.58	34.27	33.07	34.97
T_5 Blitox-3000 ppm	7.43	65.05	82.35	86.61	2.42	17.65	13.39	11.15
T_6 Captaf-3000 ppm	6.37	42.29	78.95	100.00	36.56	21.05	0	19.20
T_7 Dithane M-45 - 3000 ppm	12.13	36.71	69.61	100.00	44.93	30.39	0	25.11
T_8 Dithane Z-78 - 3000 ppm	4.41	57.82	70.04	100.00	13.26	29.96	0	14.41
T_9 Paushamycin-200 ppm + Bordeaux mixture-1%	10.22	54.17	100.00	100.00	18.74	0	0	6.25
T_{10} Plantomycin-200 ppm + Bordeaux mixture-1%	3.43	36.38	74.07	84.70	45.42	25.93	15.30	28.88
T_{11} Streptocycline-200 ppm + Bordeaux mixture-1%	4.22	29.45	62.75	79.40	55.82	37.25	20.60	37.89
T_{12} Control	7.98	66.66	100.00	100.00	-	-	-	-
CD (0.05)	NS	NS	NS	NS				

NS - Non significant

after second spraying also, the treatment T_{11} recorded the minimum percentage of stem infection and the maximum percentage efficiency over control and this was followed by the treatments T_3 (Streptocycline-200 ppm) and T_4 (Bordeaux mixture-1%). But, the minimum percentage of stem infection and the maximum percentage efficiency over control were observed with the treatment T_4 followed by the treatment T_{11} on the fifteenth day after third spraying.

It was also observed that the plants sprayed with one per cent Bordeaux mixture did not show any substantial increase in the percentage of stem infection during the last two observations revealing its superiority in checking the severity and further spread of stem infection.

Defoliation

The data on the percentage of defoliation at different intervals of observation are given in Table 32. Mean value of the percentage of defoliation on the fifteenth day after first spraying showed the least defoliation in treatment T_1 (Paushamycin-200 ppm) and also the maximum percentage efficiency over control. Plants in treatment T_2 (Plantomycin-200 ppm) recorded

Table 32. Pot culture experiment - Effect of chemical treatments on the control of bacterial leaf spot of betel vine - Percentage of defoliation

Treatments	Percentage of defoliation				Percentage efficiency of the treatment over control			
	On the day of first spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying	Mean
T_1 Paushamycin-200 ppm	0	70.83	100.00	100.00	25.44	0	0	8.48
T_2 Plantomycin-200 ppm	23.02	100.00	100.00	100.00	-5.26	0	0	-1.75
T_3 Streptocycline-200 ppm	3.57	96.43	100.00	100.00	-1.51	0	0	-0.50
T_4 Bordeaux mixture-1%	17.08	93.75	93.75	93.75	1.32	6.25	6.25	4.61
T_5 Blitox-3000 ppm	14.10	95.30	100.00	100.00	-0.32	0	0	-0.11
T_6 Captaf-3000 ppm	4.86	89.59	100.00	100.00	5.69	0	0	1.90
T_7 Dithane M-45 - 3000 ppm	7.50	95.00	100.00	100.00	0	0	0	0
T_8 Dithane Z-78 - 3000 ppm	16.79	94.72	100.00	100.00	0.29	0	0	0.10
T_9 Paushamycin-200 ppm + Bordeaux mixture-1%	6.35	90.87	100.00	100.00	4.35	0	0	1.45
T_{10} Plantomycin-200 ppm + Bordeaux mixture-1%	16.19	95.64	100.00	100.00	-0.67	0	0	-0.22
T_{11} Streptocycline-200 ppm + Bordeaux mixture-1%	19.38	95.83	100.00	100.00	-0.87	0	0	-0.29
T_{12} Control	8.33	95.00	100.00	100.00	-	-	-	-
CD (0.05)	NS	NS	NS	NS				

NS - Not significant

hundred per cent defoliation. After fifteenth day of second and third spraying, all treatments except T_4 (Bordeaux mixture-1%) showed hundred per cent defoliation.

Though, the percentage of defoliation on the fifteenth day after first spraying was high in plants treated with one per cent Bordeaux mixture, no further increase was noticed thereafter. This showed the effect of this treatment in checking further defoliation in diseased betel vine plants.

Disease score on new leaves

Data on the disease score on newly formed leaves after the start of chemical sprayings are given in Table 33. Plants receiving the treatment T_{11} (Streptocycline-200 ppm + Bordeaux mixture-1%) showed the lowest disease score on the fifteenth day after first spraying and the maximum efficiency over control. Fifteenth day after the second spraying, the treatment T_3 (Streptocycline-200 ppm) showed the minimum disease score and maximum efficiency over control. Observations on the fifteenth day after third spraying revealed that the treatment T_4 (Bordeaux mixture-1%) had the lowest

Table 33. Pot culture experiment - Effect of chemical treatments on the control of bacterial leaf spot of betel vine - Disease score on new leaves

Treatments	Disease score				Percentage efficiency of the treatment over control			
	On the day of first spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying	Mean
T_1 Paushamycin-200 ppm	0.63	5.73	10.75	11.00	-45.43	2.27	0	-14.39
T_2 Plantomycin-200 ppm	0.75	3.45	8.50	11.00	12.44	22.73	0	11.72
T_3 Streptocycline-200 ppm	0	3.40	5.48	9.63	13.71	50.18	12.45	25.45
T_4 Bordeaux mixture-1%	0.25	5.49	6.34	6.00	-39.34	42.36	45.45	16.16
T_5 Blitox-3000 ppm	0.38	5.97	8.60	9.19	-51.52	21.82	16.45	-4.42
T_6 Captaf-3000 ppm	0.13	5.67	8.45	11.00	-43.91	23.18	0	-6.91
T_7 Dithane M-45 - 3000 ppm	0	0.70	6.50	11.00	82.23	40.91	0	41.05
T_8 Dithane Z-78 - 3000 ppm	0	1.02	7.56	11.00	74.11	31.27	0	35.13
T_9 Paushamycin-200 ppm + Bordeaux mixture-1%	0.25	0.87	11.00	11.00	77.92	0	0	25.97
T_{10} Plantomycin-200 ppm + Bordeaux mixture-1%	0.38	3.22	5.88	8.52	18.27	46.55	22.55	29.12
T_{11} Streptocycline-200 ppm + Bordeaux mixture-1%	0	0.29	5.82	8.44	92.64	47.09	23.27	54.33
T_{12} Control	0	3.94	11.00	11.00	-	-	-	-
CD (0.05)	NS	NS	NS	NS				

NS - Non significant

disease score and maximum efficiency over control in checking the disease. Here also it was evident that even though the plants treated with one per cent Bordeaux mixture had a comparatively higher disease score, it remained at a constant level throughout the period of observation confirming the efficacy of this treatment in arresting further increase and spread of the disease.

Mortality

The data on the percentage of mortality on the fifteenth day after first, second and third spraying are furnished in Table 34. On the fifteenth day of first spraying no mortality of plants was observed in treatments T_7 (Dithane M-45-3000 ppm), T_8 (Dithane Z-78-3000 ppm), T_9 (Paushamycin-200 ppm + Bordeaux mixture-1%), T_{10} (Plantomycin-200 ppm + Bordeaux mixture-1%) and T_{11} (Streptocycline-200 ppm + Bordeaux mixture-1%). On the fifteenth day after second spraying, hundred per cent mortality of plants was observed in treatments T_9 and T_{12} (Control). Only 50 per cent mortality of plants was observed in treatment T_4 (Bordeaux mixture-1%) on the fifteenth day after third spraying and all other treatments showed comparatively higher percentage of mortality. Here also, the plants sprayed with one

Table 34. Pot culture experiment - Effect of chemical treatments on the control of bacterial leaf spot of betel vine - Percentage of mortality

Treatments		Percentage of mortality		
		15th day after first spraying	15th day after second spraying	15th day after third spraying
T_1	Pausharnycin-200 ppm	50	75	100
T_2	Plantomycin-200 ppm	25	75	100
T_3	Streptocycline-200 ppm	25	25	75
T_4	Bordeaux mixture-1%	25	50	50
T_5	Blitox - 3000 ppm	50	75	75
T_6	Captaf - 3000 ppm	25	75	100
T_7	Dithane M-45 - 3000 ppm	0	50	100
T_8	Dithane Z-78 - 3000 ppm	0	25	100
T_9	Pausharnycin-200 ppm + Bordeaux mixture-1%	0	100	100
T_{10}	Plantomycin-200 ppm + Bordeaux mixture-1%	0	50	75
T_{11}	Streptocycline-200 ppm + Bordeaux mixture-1%	0	50	75
T_{12}	Control	25	100	100

per cent Bordeaux mixture did not show any further increase in mortality on the fifteenth day after second and third spraying revealing its superiority over other treatments.

The results of the experiment thus revealed that application of one per cent Bordeaux mixture had an effect in checking the severity and further spread of bacterial leaf spot of betel vine.

4.6.2.3 Field experiment on the chemical control of bacterial leaf spot of betel vine

Out of the twelve treatments of antibiotics, fungicides and the combination of antibiotics and Bordeaux mixture, none of them gave an absolute control of the disease. Analysis of the data on the disease score, percentage of stem infection, percentage of defoliation at different intervals of observation did not show any significant difference among the treatments. Analysis of the data on the disease score on newly opened leaves after the start of chemical spraying showed no significant difference among the treatments on the fifteenth day after first and second spraying, but there was significant difference among treatments on the fifteenth day after third spraying. In general, it was noticed that certain treatments had marked efficacy in

checking the intensity and spread of the disease.

Disease score

The data on the disease score at different intervals of observation are presented in Table 35. Fifteenth day after first spraying, plants in treatment T_{10} (Plantomycin-200 ppm + Bordeaux mixture-1%) showed the minimum disease score and maximum percentage efficiency over control. This was followed by the treatments T_{11} and T_5 (Streptocycline-200 ppm + Bordeaux mixture-1% and Blitox-3000 ppm). Fifteenth day after second spraying, the treatment T_{10} showed the lowest disease score followed by the treatments T_4 (Bordeaux mixture-1%), T_3 (Streptocycline-200 ppm) and T_{11}. The former treatment showed highest percentage efficiency over control. Minimum disease score was observed in treatment T_{10} on the fifteenth day after third spraying, followed by the treatments T_{11} and T_4. More than twenty per cent efficiency over control in reducing the disease score was recorded by the treatments T_{10}, T_{11}, T_4, T_3 and T_2 (Plantomycin-200 ppm) with maximum efficiency for the former.

It was observed that the plants in treatment T_9 (Paushamycin-200 ppm + Bordeaux mixture-1%) showed more disease score than control on the fifteenth day

Table 35. Field experiment - Effect of chemical treatments on the control of bacterial leaf spot of betel vine - Disease score

Treatments		Disease score				Percentage efficiency of the treatment over control			
		On the day of first spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying	Mean
T_1	Paushamycin-200 ppm	0.92	5.02	7.17	7.79	-8.89	-11.51	4.88	-5.17
T_2	Plantomycin-200 ppm	1.10	4.41	5.58	6.54	4.34	13.22	20.15	12.57
T_3	Streptocycline-200 ppm	1.12	4.38	5.19	6.22	4.99	19.28	24.05	16.11
T_4	Bordeaux mixture-1%	0.80	4.03	5.10	5.84	12.58	20.68	28.69	20.65
T_5	Blitox-3000 ppm	0.87	3.92	5.94	6.85	14.97	7.62	16.36	12.98
T_6	Captaf-3000 ppm	0.91	6.40	7.49	8.76	-38.83	-16.49	-6.96	-20.76
T_7	Dithane M-45-3000 ppm	1.12	4.34	6.17	6.92	5.86	4.04	15.51	8.47
T_8	Dithane Z-78-3000 ppm	0.71	4.09	5.82	6.81	11.28	9.49	16.85	12.54
T_9	Paushamycin-200 ppm + Bordeaux mixture-1%	1.06	5.22	6.01	7.02	-13.23	6.53	14.29	2.53
T_{10}	Plantomycin-200 ppm + Bordeaux mixture-1%	0.71	2.89	4.40	4.83	37.31	31.57	41.03	36.63
T_{11}	Streptocycline-200 ppm + Bordeaux mixture-1%	0.95	3.64	5.21	5.79	21.04	18.97	29.30	23.10
T_{12}	Control	0.92	4.61	6.43	8.19	-	-	-	-
	CD (0.05)	NS	NS	NS	NS				

NS - Non significant

after first spraying. But, in the case of treatment T_1 (Paushamycin-200 ppm) this trend was observed upto the fifteenth day after second spraying. The plants in treatment T_6 (Captaf-3000 ppm) always showed more disease score than control at all intervals of observation.

Stem infection

Observations on the percentage of stem infection on the fifteenth day after first, second and third spraying showed marked difference between treated plants and control (Table 36).

It was observed that on the fifteenth day after first spraying there was no stem infection in plants in treatments T_9, T_{10} and T_{11} (Paushamycin-200 ppm + Bordeaux mixture-1%, Plantomycin-200 ppm + Bordeaux mixture-1% and Streptocycline-200 ppm + Bordeaux mixture-1%). Fifteenth day after second spraying absolutely no stem infection was noticed in plant receiving the treatment T_{11} while T_{12} (Control) showed the maximum percentage of stem infection. Comparatively low percentage of stem infection was noticed in treatments T_{10} and T_9 also. All the treatments showed more than sixty per cent efficiency over control in reducing the

Table 36. Field experiment - Effect of chemical treatments on the control of bacterial leaf spot of betel vine - Percentage of stem infection

Treatments	Percentage of stem infection				Percentage efficiency of the treatment over control			
	On the day of first spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying	Mean
T_1 Paushamycin-200 ppm	0.41	1.42	3.01	18.57	34.86	85.15	24.51	48.17
T_2 Plantomycin-200 ppm	1.70	2.53	2.60	3.04	-16.06	87.17	87.64	52.92
T_3 Streptocycline-200 ppm	0	1.97	1.99	2.43	9.63	90.18	90.12	63.31
T_4 Bordeaux mixture-1%	0.20	1.63	2.98	4.20	25.23	85.30	82.93	64.49
T_5 Blitox-3000 ppm	0.15	3.22	6.91	8.68	-47.71	65.91	64.72	27.64
T_6 Captaf-3000 ppm	0.15	2.16	2.36	4.32	0.92	88.36	82.44	57.24
T_7 Dithane M-45 - 3000 ppm	0.51	3.23	4.40	5.12	-48.17	78.30	79.19	36.44
T_8 Dithane Z-78 - 3000 ppm	0.78	1.69	3.38	4.93	22.48	83.33	79.96	61.92
T_9 Paushamycin-200 ppm + Bordeaux mixture-1%	0	0	0.71	1.22	100.00	96.50	95.04	97.18
T_{10} Plantomycin-200 ppm + Bordeaux mixture-1%	0	0	0.23	0.59	100.00	98.87	97.60	98.82
T_{11} Streptocycline-200 ppm + Bordeaux mixture-1%	0	0	0	0.59	100.00	100.00	97.60	99.20
T_{12} Control	0.64	2.18	20.27	24.60	-	-	-	-
CD (0.05)	NS	NS	NS	NS				

NS - Non significant

percentage of stem infection. Observation on the percentage of stem infection on the fifteenth day after third spraying showed the minimum infection in treatments T_{10} and T_{11} and the maximum in T_{12} and T_1 (Pausharnycin-200 ppm). All the treatments except T_1 recorded more than sixty per cent efficiency over control with the maximum for the treatments T_{10} and T_{11}.

Defoliation

In general, it was seen that the treatment T_{10} (Plantomycin-200 ppm + Bordeaux mixture-1%) had considerable efficiency in reducing the percentage of defoliation (Table 37). The percentage of defoliation on the fifteenth day after first spraying was minimum in treatments T_{10} and T_{11} (Streptocycline-200 ppm + Bordeaux mixture-1%). The treatment T_{10} recorded the maximum percentage efficiency over control followed by the treatment T_{11}. Fifteenth day after second spraying also the minimum percentage of defoliation was observed in treatment T_{10} and the maximum percentage efficiency over control. Mean value of the percentage of defoliation on the fifteenth day after third spraying revealed a reduction in the rate of defoliation of plants in treatment T_{10} and T_4 (Bordeaux mixture-1%) compared to others and former treatment had the maximum per cent efficiency over control.

Table 37. Field experiment - Effect of chemical treatments on the control of bacterial leaf spot of betel vine - Percentage of defoliation

	Treatments	Percentage of defoliation			Percentage efficiency of the treatment over control			
		15th day after first spraying	15th day after second spraying	15th day after third spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying	Mean
T_1	Paushamycin-200 ppm	41.61	63.10	66.59	-14.60	-17.13	15.32	-5.47
T_2	Plantomycin-200 ppm	35.28	44.24	56.18	2.84	17.88	28.56	16.43
T_3	Streptocycline-200 ppm	37.04	45.10	58.41	-2.01	16.28	25.72	13.33
T_4	Bordeaux mixture-1%	31.83	42.49	49.86	12.34	21.12	36.60	23.35
T_5	Blitox-3000 ppm	33.04	54.82	64.12	9.01	-1.76	18.46	8.57
T_6	Captaf-3000 ppm	54.76	68.82	83.11	-50.81	-27.75	-5.68	-28.08
T_7	Dithane M-45 - 3000 ppm	29.43	51.17	58.18	18.95	5.01	26.02	16.66
T_8	Dithane Z-78 - 3000 ppm	34.08	52.13	62.25	6.14	3.23	20.84	10.07
T_9	Paushamycin-200 ppm + Bordeaux mixture-1%	43.02	50.15	66.59	-18.48	6.91	15.32	1.25
T_{10}	Plantomycin-200 ppm + Bordeaux mixture-1%	22.77	38.77	41.68	37.29	28.03	47.00	37.44
T_{11}	Streptocycline-200 ppm + Bordeaux mixture	24.92	43.73	50.32	31.37	18.82	36.01	28.73
T_{12}	Control	36.31	53.87	78.64	-	-	-	-
	CD (0.05)	NS	NS	NS				

NS - Non significant

It was observed that the plants in treatment T_6 (Captaf-3000 ppm) at all intervals of observation showed a higher rate of defoliation compared to the control. The plants receiving the treatment T_1 (Paushamycin-200 ppm) recorded higher rate of defoliation during the first two intervals of observation, while those in treatments T_3(Streptocycline-200 ppm) and T_9 (Paushamycin-200 ppm + Bordeaux mixture 1%) showed it only on the fifteenth day after first spraying.

Disease score on new leaves

Data on the disease score on new leaves formed after the start of chemical treatments are presented in Table 38. There was no disease score on newly formed leaves on the fifteenth day after first spraying in plants receiving the treatment T_9, T_{10} and T_2 (Paushamycin-200 ppm + Bordeaux mixture-1%, Plantomycin-200 ppm + Bordeaux mixture-1% and Plantomycin-200 ppm). Only plants in treatment T_2 showed no disease incidence on new leaves on the fifteenth day after second spraying. All the treatments except T_1 (Paushamycin-200 ppm) showed high percentage efficiency over control in reducing the disease on new leaves. Fifteenth day after third spraying there was significant difference among the

Table 38. Field experiment - Effect of chemical treatments on the control of bacterial leaf spot of betel vine - Disease score on new leaves

	Treatments	Disease score			Percentage efficiency of the treatment over control			
		15th day after first spraying	15th day after second spraying	15ty day after third spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying	Mean
T_1	Paushamycin-200 ppm	0.21	2.18	3.09	-162.50	-6.34	20.16	-49.56
T_2	Plantomycin-200 ppm	0	0	0.04	100.00	100.00	98.97	99.66
T_3	Streptocycline-200 ppm	0.03	0.03	0.04	62.50	98.54	98.97	86.67
T_4	Bordeaux mixture-1%	0.08	0.21	0.29	0	89.76	92.51	60.76
T_5	Blitox-3000 ppm	0.17	0.27	0.28	-112.50	86.83	92.76	22.36
T_6	Captaf-3000 ppm	0.17	0.19	0.22	-112.50	90.73	94.32	24.18
T_7	Dithane M-45 - 3000 ppm	0.08	0.27	0.36	0	86.83	90.70	59.18
T_8	Dithane Z-78 - 3000 ppm	0.08	0.04	0.06	0	98.05	98.45	65.50
T_9	Paushamycin-200 ppm + Bordeaux mixture-1%	0	0.03	0.04	100.00	98.54	98.97	99.17
T_{10}	Plantomycin-200 ppm + Bordeaux mixture-1%	0	0.03	0.02	100.00	98.54	99.48	99.34
T_{11}	Streptocycline-200 ppm + Bordeaux mixture-1%	0.06	0.08	0.15	25.00	96.10	96.12	72.41
T_{12}	Control	0.08	2.05	3.87	-	-	-	-
	CD (0.05)	NS	NS	1.928				

NS - Non significant

treatments and it was observed that the maximum disease score on new leaves was on plants in treatment T_1 and T_{12} (Control) which were on par. All other treatments were on par with minimum disease score in treatment T_{10}.

Mortality

No mortality of plants was observed in all the treatments on the fifteenth day after first spraying (Table 39). Fifteenth day after second and third spraying, only the plants in treatments T_3 (Streptocycline-200 ppm) and T_{12} (Control)showed mortality.

From the field experiment on the chemical control of bacterial leaf spot of betel vine, it was thus clear that the combined application of Plantomycin-200 ppm and Bordeaux mixture one per cent exerted marked efficacy in checking the severity and spread of the disease. Comparatively good efficiency in checking the disease was also recorded by the treatments Streptocycline-200 ppm with Bordeaux mixture one per cent and Bordeaux mixture one per cent alone.

The residual effect of the above said chemicals were assessed <u>in vitro</u>. It was found that, there was very little inhibition in the growth of the bacterium around the leaf discs, suggesting that there was no appreciable

Table 39. Field experiment - Effect of chemical treatments on the control of bacterial leaf spot of betel vine - Percentage of mortality

Treatments		Percentage of mortality		
		15th day after first spraying	15th day after second spraying	15th day after third spraying
T_1	Paushamycin - 200 ppm	0	0	0
T_2	Plantomycin - 200 ppm	0	0	0
T_3	Streptocycline - 200 ppm	0	16.67	16.67
T_4	Bordeaux mixture - 1%	0	0	0
T_5	Blitox - 3000 ppm	0	0	0
T_6	Captaf - 3000 ppm	0	0	0
T_7	Dithane M-45 - 3000 ppm	0	0	0
T_8	Dithane Z-78 - 3000 ppm	0	0	0
T_9	Paushamycin - 200 ppm + Bordeaux mixture - 1%	0	0	0
T_{10}	Plantomycin - 200 ppm + Bordeaux mixture - 1%	0	0	0
T_{11}	Streptocycline - 200 ppm + Bordeaux mixture - 1%	0	0	0
T_{12}	Control	0	16.67	16.67

amount of residue left in the leaves even during the first sampling.

4.6.3 Screening of betel vine cultivars for host resistance against the pathogen X. campestris pv. betlicola

Of the ten cultivars of betel vine screened for host resistance against the pathogen X. campestris pv. betlicola none of them was found to be immune to the disease. But, the cultivars Tulasivettila Type I (V_2), Pozhikodi (V_9) and Nadankodi (V_{10}) were found to be resistant to the disease. In the resistant cultivars the disease development was very slow, the spots were comparatively smaller in size and fewer in number than the susceptible ones.

Disease score

Analysis of the data on the disease score after ten, twenty, thirty and forty days of inoculation revealed significant difference among the cultivars of betel vine (Table 40). The cultivars Tulasivettila Type I (V_2), Pozhikodi (V_9) and Nadankodi (V_{10}) showed minimum disease score at different intervals of observation compared to others.

Table 40. Screening of betel vine cultivars for host resistance against X. campestris pv. betlicola - Disease score

Cultivars		Disease score			
		Ten days after inoculation	Twenty days after inoculation	Thirty days after inoculation	Forty days after inoculation
V_1	Cheelanthikarpuran	3.55	9.30	10.38	10.38
V_2	Tulasivettila Type I	0.92	1.46	2.50	3.58
V_3	Aryan	3.95	7.37	10.88	10.88
V_4	Arikodi	3.22	8.57	10.63	10.63
V_5	Pannivella	6.12	9.59	9.38	10.63
V_6	Njarukali	4.05	9.81	10.25	10.38
V_7	Tulasivettila Type II	2.48	7.38	9.34	9.34
V_8	Cheelanthikarpuran Chuvappu	7.40	9.31	10.63	10.75
V_9	Pozhikodi	0.37	0.62	2.65	3.15
V_{10}	Nadankodi	0.75	1.45	2.56	2.95
	C.D. (0.05)	1.801	1.121	1.414	0.971

After ten days of inoculation, the cultivar Pozhikodi (V_9) showed the minimum disease score and was on par with Nadankodi (V_{10}) and Tulasivettila Type I (V_2). The cultivar Cheelanthikarpuran Chuvappu (V_8) showed the maximum disease score and was on par with Pennivella (V_5). Other cultivars were on par and inferior to Pozhikodi (V_9), Nadankodi (V_{10}) and Tulasivettila Type I (V_2).

Twenty days after inoculation, minimum disease score was observed in the cultivar Pozhikodi (V_9) and was on par with Nadankodi (V_{10}) and Tulasivettila Type I (V_2) and these were superior to other cultivars of betel vine. The cultivar Njarukali (V_6) showed the maximum disease score and was on par with Pennivella (V_5), Cheelanthikarpuran Chuvappu (V_8) and Cheelanthikarpuran (V_1).

Significantly lower disease score after thirty days of inoculation was observed in the cultivar Tulasivettila Type I (V_2) and was on par with Nadankodi (V_{10}) and Pozhikodi (V_9) and these were superior to others. The cultivar Aryan (V_3) exhibited the highest disease score and was on par with Arikodi (V_4), Cheelanthikarpuran Chuvappu (V_8), Cheelanthikarpuran (V_1) and Njarukali (V_6).

Forty days after inoculation minimum disease score was observed in the cultivar Nadankodi (V_{10}) and was

superior to all other cultivars except Tulasivettila Type I (V_2) and Pozhikodi (V_9) which were on par. Maximum disease score was recorded in the cultivar Aryan (V_3) and was on par with Cheelanthikarpuran (V_8), Arikodi (V_4), Pannivella (V_5), Cheelanthikarpuran (V_1) and Njarukali (V_6).

Stem infection

Analysis of the data on the percentage of stem infection at different intervals of observation showed significant difference among the cultivars of betel vine (Table 41).

Evaluation of the percentage of stem infection ten days after inoculation revealed that there was no stem infection in the cultivars Tulasivettila Type II (V_7), Pozhikodi (V_9) and Nadankodi (V_{10}), and these were superior to all others. Maximum percentage of stem infection was noticed in the cultivar Cheelanthikarpuran Chuvappu (V_8).

Twenty days after inoculation, lowest percentage of stem infection was recorded in the cultivar Pozhikodi (V_9) and was on par with Nadankodi (V_{10}), Tulasivettila Type I (V_2) and Tulasivettila Type II (V_7). The cultivar Cheelanthikarpuran Chuvappu (V_8) continued to record the

Table 41. Screening of betel vine cultivars for host resistance against X. campestris pv. betlicola - Percentage of stem infection

Cultivars		Percentage of stem infection			
		Ten days after inoculation	Twenty days after inoculation	Thirty days after inoculation	Forty days after inoculation
V_1	Cheelanthikarpuran	6.64	17.70	52.72	53.74
V_2	Tulasivettila Type I	0.05	1.19	2.73	4.94
V_3	Aryan	4.65	32.93	88.27	88.44
V_4	Arikodi	7.77	27.88	85.37	91.50
V_5	Pannivella	4.66	22.59	72.01	73.11
V_6	Njarukali	4.85	16.45	50.32	42.28
V_7	Tulasivettila Type II	0	2.26	15.57	17.28
V_8	Cheelanthikarpuran Chuvappu	28.88	59.76	78.35	84.67
V_9	Pozhikodi	0	0.31	0.79	1.17
V_{10}	Nadankodi	0	0.51	1.44	2.44
	C.D. (0.05)	14.208	14.692	29.537	28.078

maximum percentage of stem infection and was inferior to all other cultivars.

Minimum percentage of stem infection thirty days after inoculation was observed in the cultivar Pozhikodi (V_9) followed by Nadankodi (V_{10}), Tulasivettila Type I (V_2) and Tulasivettila Type II (V_7) and these were on par and superior to other cultivars. Highest percentage of stem infection was recorded in the cultivar Aryan (V_3) and was on par with Arikodi (V_4), Cheelanthikarpuran Chuvappu (V_8) and Pannivella (V_5).

The cultivar Pozhikodi (V_9) recorded the minimum percentage of stem infection forty days after inoculation also and was on par with Nadankodi (V_{10}), Tulasivettila Type I (V_2) and Tulasivettila Type II (V_7) and these were superior to others. Maximum percentage of stem infection was noticed in the cultivar Arikodi (V_4) which was on par with Aryan (V_3), Cheelanthikarpuran Chuvappu (V_8) and Pannivella (V_5).

Defoliation

The data on the percentage of defoliation at different intervals of observation are given in Table 42. The analysis of the data revealed significant difference among the cultivars on the percentage of defoliation after ten,

Table 42. Screening of betel vine cultivars for host resistance against X. campestris pv. betlicola - Percentage of defoliation

Cultivars		Percentage of defoliation			
		Ten days after inoculation	Twenty days after inoculation	Thirty days after inoculation	Forty days after inoculation
V_1	Cheelanthikarpuran	15.72	89.75	100.00	100.00
V_2	Tulasivettila Type I	0	3.89	23.52	29.40
V_3	Aryan	4.89	53.26	100.00	100.00
V_4	Arikodi	9.44	82.59	100.00	100.00
V_5	Pannivella	40.27	92.52	100.00	100.00
V_6	Njarukali	22.07	96.33	100.00	100.00
V_7	Tulasivettila Type II	4.06	61.19	89.87	100.00
V_8	Cheelanthikarpuran Chuvappu	57.33	84.55	100.00	100.00
V_9	Pozhikodi	0.70	0.70	19.37	21.42
V_{10}	Nadankodi	0	1.39	9.86	23.75
	C.D. (0.05)	20.644	15.900	11.593	13.498

twenty, thirty and forty days of inoculation. After ten days of inoculation, there was no defoliation in the cultivars Nadankodi (V_{10}) and Tulasivettila Type I (V_2) but these were on par with other cultivars except Njarukali (V_6), Pannivella (V_5) and Cheelanthikarpuran Chuvappu (V_8).

Twenty days after inoculation, minimum percentage of defoliation was observed in the cultivar Pozhikodi (V_9) and was on par with Nadankodi (V_{10}) and Tulasivettila Type I (V_2) and they were superior to all others. Maximum percentage of defoliation was recorded in cultivar Njarukali (V_6) and was on par with Pannivella (V_5), Cheelanthikarpuran (V_1) Cheelanthikarpuran Chuvappu (V_8) and Arikodi (V_4).

Thirty days after inoculation the minimum percentage of defoliation was observed in the cultivar Nadankodi (V_{10}) and was on par with Pozhikodi (V_9) and Tulasivettila Type I (V_2). These cultivars were significantly superior to all others. Hundered per cent defoliation was observed in the cultivars Cheelanthikarpuran (V_1) Aryan (V_3), Arikodi (V_4), Pannivella (V_5) Njarukali (V_6) and Cheelanthikarpuran Chuvappu (V_8) and they were on par with Tulasivettila Type II (V_7).

After forty days of inoculation, complete defoliation

was observed in all the cultivars of betel vine, except Pozhikodi (V_9), Nadankodi (V_{10}) and Tulasivettila Type I (V_2) and they were on par.

Mortality

The cultivars Tulasivettila Type I (V_2) Pozhikodi (V_9) and Nadankodi (V_{10}) did not show any mortality at different intervals of observation (Table 43). After ten and twenty days of inoculation, only the cultivar Cheelanthikarpuran Chuvappu (V_8) showed mortality. Among the seven cultivars showing mortality after thirty and forty days of inoculation, the cultivar Aryan had the maximum percentage of mortality, while the cultivar Tulasivettila Type II (V_7) showed comparatively less percentage of mortality.

The result of the study on the screening of betel vine cultivars for host resistance against the bacterial leaf spot pathogen was encouraging. The cultivars Pozhikodi (V_9), Nadankodi (V_{10}) and Tulasivettila Type I (V_2) possessed fairly high degree of resistance as they showed very low ratings of the disease score, stem infection, defoliation and mortality over a period of forty days of inoculation. Even though the cultivar Tulasivettila Type II (V_7) showed fairly high disease score and defoliation it had less percentage of stem infection and mortality compared to other susceptible cultivars.

Table 43. Screening of betel vine cultivars for host resistance against X. campestris pv. betlicola - Percentage of mortality

Cultivars		Percentage of mortality			
		Ten days after inoculation	Twenty days after inoculation	Thirty days after inoculation	Forty days after inoculation
V_1	Cheelanthikarpuran	0	0	37.50	37.50
V_2	Tulasivettila Type I	0	0	0	0
V_3	Aryan	0	0	87.50	87.50
V_4	Arikodi	0	0	62.50	75.00
V_5	Pannivella	0	0	62.50	62.50
V_6	Njarukali	0	0	25.00	37.50
V_7	Tulasivettila Type II	0	0	12.50	12.50
V_8	Cheelanthikarpuran Chuvappu	25.00	37.50	50.00	75.00
V_9	Pozhikodi	0	0	0	0
V_{10}	Nadankodi	0	0	0	0

Discussion

DISCUSSION

Bacterial leaf spot incited by Xanthomonas campestris pv. betlicola is one of the most serious diseases of betel vine in Kerala. Except a few preliminary studies, there has been no detailed systematic work on the various aspects of this disease. In view of the serious nature of the disease to betel vine in Kerala, the present investigation was carried out to understand the various aspects of the pathogen, host-parasite interaction and to evolve a viable management/control measure for this disease.

The bacterial pathogen X. campestris pv. betlicola was isolated from infected leaves and stem of betel vine and its pathogenicity was established by artificial inoculation in the susceptible cultivar Cheelanthikarpuran. Four isolates of the pathogen were obtained from different parts of Kerala. The cultural and biochemical characters of the pathogen studied were in conformity with those reported by Patel et al. (1951, (1953), Breed et al.(1957), Dye (1962), Mathew et al. (1978a, b; 1979a) and Abraham (1980). Based on the cultural and biochemical characters of the causal bacterium and its pathogenicity, the pathogen causing bacterial leaf spot of betel vine was identified as X. campestris pv. betlicola (Patel, Kulkarni and Dhande) Dye.

An attempt was also made to find out whether any variation existed among the different isolates of the pathogen. It was found that all the four isolates of the bacterium were alike in respect of all the cultural and biochemical characters studied (Table 1). Further, all of them produced almost identical symptoms of the disease on artificial inoculation. Abraham and Mathew (1983) had also made similar observation with ten different isolates of X. campestris pv. betlicola. All the isolates used in the present investigation showed good growth at pH 6.5 - 7.0, the optimal pH being 6.8 for isolates Xcb-1, Xcb-2 and Xcb-3 and 7.0 for the isolate Xcb-4 (Table 2). Khatri et al. (1983c) have reported that X. campestris pv. betlicola grows well between pH 6 and 7. It showed more active motility within the above range of pH than those grown at pH below 6 and above 7. The different isolates were mesophilic with a rapid growth rate at temperature 25 to 30°C (Table 3). The isolate Xcb-3 showed maximum growth at 25°C. For isolates Xcb-2 and Xcb-4 30°C was the optimum temperature, while Xcb-1 did not show much difference in growth between 25 and 30°C. Such slight variations in the optimum temperature requirement for the growth of X. campestris pv. betlicola have been reported by Breed et al. (1957), Mathew et al. (1978a) and Khatri et al. (1983c).

The bacterial isolates showed varying growth rates in the different carbon sources tested (Table 4). Starch, raffinose and mannitol, melicitose and starch, and sucrose incorporated medium supported the maximum growth of isolates Xcb-1, Xcb-2, Xcb-3 and Xcb-4 respectively. In general, glucose and dextrose did not support good growth of different isolates. There are no reports so far on the effect of different carbon sources in liquid medium on the growth of X. campestris pv. betlicola. However, Patel et al. (1951, 1953), Breed et al. (1957), Mathew et al. (1978a, b, 1979a) and Abraham (1980) reported that X. campestris pv. betlicola produced acid but no gas from glucose, dextrose, lactose, sucrose, xylose, fructose, galactose, mannose and maltose but did not utilise inositol, adonitol, dulicitol, inulin, salicin and ribose. Positive starch hydrolysis activity of the bacterium was also reported by them.

The bacterial isolates exhibited varying growth rate in different nitrogen sources (Table 5). All nitrogen sources tested, except $NaIO_3$ supported good growth of all isolates of the pathogen. Ammonium nitrate, asparagine and KNO_3, asparagine, and KNO_3 supported maximum growth of isolates Xcb-1, Xcb-2, Xcb-3 and Xcb-4 respectively. Patel et al. (1951), Mathew et al. (1978a, b; 1979a), Abraham (1980) and Abraham and Mathew (1983) reported that

X. campestris pv. betlicola did not utilise asparagine as a sole source of carbon and nitrogen. In the present study, all isolates of the pathogen showed good growth in liquid medium containing asparagine. However, Patel et al. (1953) noticed slight growth of the bacterium in asparagine. The presence of fairly good amount of asparagine in betel vine leaves has been reported (The Wealth of India, 1969). This may be the probable reason for the growth of the bacterial isolates in asparagine incorporated medium since they were adjusted to the condition in which asparagine was always present in fairly good amount.

In general, lower concentrations of amino acids supported good growth of different isolates of the pathogen (Table 6). Tyrosine, lysine, glycine, methionine, threonine, glutamic acid, cysteine and arginine at 0.001 M concentration supported good growth of all isolates while, histidine and proline had the similar effect at 0.01 M concentration. Proline at 0.01 M and tyrosine at 0.001 M concentrations supported maximum growth of the isolate Xcb-1. Isolates Xcb-2, Xcb-3 and Xcb-4 showed maximum growth at 0.001 M concentration of lysine, tyrosine and glycine respectively. The effect of amino acids on the growth of isolates of X. campestris pv. betlicola has not been studied so far. However, the result of this study was in agreement with the similar work on other bacterial

pathogens. It was reported that higher concentrations of amino acids generally caused a reduction in the growth of X. oryzae and that different concentrations had varying effect on its growth (Prasad et al., 1972). The differential effect of certain amino acids on the growth of X. malvacearum (Nayudu, 1972; Verma and Singh, 1974) and X. campestris pv. phaseoli (Marimuthu, 1983) have already been reported.

Thus, during this investigation no significant difference except some slight variations were observed among the four isolates of the pathogen in their cultural, biochemical and physiological characters and also in the symptom expression in the susceptible cultivar of betel vine. Dye (1962) has reported that physiological characters are of little value in distinguishing species because the extent of intra species variability in respect of physiological characters is as great as inter species variability.

Symptomatology of the disease both under natural and artificial conditions were studied. Naturally infected plants showed different types of symptom on leaves and stem. On the leaves, water soaked lesions, dark brown spots with yellow halo, marginal infection, leaf tip infection, shot hole type symptom, bacterial exudations and blightening (Plates I - X) were noticed. On the stem,

large dark brown lesions were observed in addition to bacterial exudations (Plates XI - XIV). Further, it was noticed that even the presence of one or two spots on the leaf lamina caused defoliation. Similarly, a slight infection on the stem was seen to cause mortality of plant. Thus, the disease was found to cause heavy damage even with very little incidence.

The symptoms produced on artificial inoculation were similar to that of naturally infected plants. The symptoms of the disease observed both under natural and artificial conditions were almost similar to those described by Patel et al. (1953), Mathew et al. (1978a) and Abraham (1980). However, Singh and Chand (1971) could not observe any leaf marginal infection, bacterial exudation, shot hole type symptom, leaf tip and stem infections in disease affected plants at Jabalpur. Three different types of symptom , namely, leaf spot, leaf blight and stem canker were reported from Jabalpur (Jain et al., 1982). But in the present study stem canker symptom was not observed. Instead, dark lesions on the stem were commonly found.

Histopathological studies revealed that X. campestris pv. betlicola is a parenchymatous pathogen. During the initial stages of infection only the parenchymatous cells were affected leaving the upper and lower epidermal cells

intact. Further, it is interesting to note that the infected parenchymatous cells were initially seen just above the stomata indicating that the entry of the pathogen is only through the natural openings (Plates XV and XVI). Karbhari (1976) also observed that X. campestris pv. betlicola enters through stomata on the leaf surfaces. In the advanced stages of infection the spongy and palisade parenchyma and lower epidermis cells were completely affected resulting in their disorganisation and disintegration (Plates XVII and XVIII). The disintegration of cells may be due to the production of pectic and cellulolytic enzymes of the pathogen (Khatri et al., 1983b) and the disorganisation of cells may be due to effect of some toxins. Disintegration of spongy and palisade parenchyma cells in leaves of betel vine due to bacterial leaf spot pathogen has also been reported by Asthana and Mahmud (1945), Kotwal (1978) and Jain et al. (1982). They also reported that the pathogen attacked the xylem vessels. But in the present study, it was found that the xylem vessels remained intact even in the advanced stages of infection (Plate XIX) again confirming the parenchymatous nature of the pathogen.

An important aspect in the perpetuation of a disease is the host range of the pathogen. In an earlier study, it was observed by Abraham (1980) that none of the weeds

commonly seen in betel vine gardens serve as alternate host of the pathogen. Therefore, a number of plants belonging to the family Piperaceae were screened for the susceptibility to X. campestris pv. betlicola. These were Piper nigrum, Piper longum, Piper attenuatum, Piper sp. Types I - IV, Peperomia pellucida and Peperomia sp Type I. It was found that except Piper attenuatum and Peperomia pellucida, all other plants showed symptoms of the disease on artificial inoculation (Plates XX - XXVI) Patel et al. (1953), Breed et al. (1957), Buchanan and Gibbons (1974) and Mathew et al. (1978b, 1979a) have reported that the host range of X. campestris pv. betlicola include various members of Piperaceae such as Piper longum, Piper betle, Piper hockeri, and Piper nigrum. The immunity of Piper attenuatum and Peperomia pellucida to this pathogen has not been reported earlier. The observation that the pathogen can also infect Piper nigrum is quite alarming, since black pepper is a major spice crop cultivated in homestead and other situation along with betel vine. An infection by X. campestris pv. betlicola in a betel vine plant can serve as a potential source of inoculum for inciting the disease in black pepper plants grown in adjacent plots or in the same garden as a mixed crop.

The seven other host plants of the pathogen showed some minor variation in symptom expression. All the

general symptoms of the disease described earlier were noticed in other host plants also.

An important aspect in understanding the host-parasite relationship is the study of biochemical changes occurring in host plants due to pathogenic infection. In this connection, a comparative study was carried out on the changes in the levels of phenols, chlorophylls, sugars, nitrogen and mineral contents of both resistant and susceptible cultivars of betel vine as influenced by X. campestris pv. betlicola inoculation.

It is an established fact that phenols and quinones - an oxidation product of the former and their derivatives are generally responsible for conferring resistance to plants against pathogenic infection (Farkas and Kiraly, 1962; Kuc, 1967). The result of the present investigation revealed that the phenolic make up of the two cultivars of betel vine varied considerably. The resistant cultivar Pozhikodi contained higher quantity of ortho-dihydric phenol than the susceptible cultivar Aryan (Table 7 and Fig. 1). In general, inoculation of the cultivars with the pathogen resulted in an increase in ortho-dihydric phenol. In the resistant cultivar Pozhikodi, inoculation with the pathogen resulted in an accumulation of ortho-dihydric phenol at all intervals of observation.

Prasad et al. (1972) found that bacterial leaf blight resistant rice variety had more ortho-dihydric phenol than the moderately susceptible and susceptible varieties. They also reported that inoculation of the pathogen resulted in an accumulation of ortho-dihydric phenol in all the three varieties but the increase was more in the resistant one. Sridhar and Ou (1974) had also reported similar results in the case of blast resistant rice variety. Even though, the susceptible cultivar showed a slight increase in the ortho-dihydric phenol at certain intervals upon inoculation the rate of increase was less compared to the resistant cultivar. It was also noticed that the susceptible cultivar at certain intervals showed a decrease in the ortho-dyhydric phenol content over the corresponding healthy one. Khatri et al. (1983a) also observed a decrease in the ortho-dihydric phenol in soybean and french bean infected with bacterial pathogens.

There was also significantly higher amount of total phenols in the resistant cultivar Pozhikodi than in the susceptible cultivar Aryan (Table 8 and Fig. 2). Inoculation of the cultivars with the pathogen resulted in an increase in total phenols and the increase was more pronounced in the resistant one. Vyas and Chile (1980a, b) reported that the Madrasi variety of betel vine least susceptible to Phytophthora infection possessed more

amount of total phenols than the most susceptible variety Kapoori. Prasad et al. (1972) detected more total phenol content in bacterial leaf blight resistant rice variety than the moderately resistant and susceptible varieties. An increase in total phenols in these rice varieties due to inoculation with the pathogen was also reported by them. Similar type of results were also reported by Easwaran (1967), Sridhar and Ou (1974) and Tripathi and Chiranjeevi (1976) in other host parasite interactions. Farkas and Kiraly (1962) stated that it is not merely the level of total phenols in the healthy tissue that determines the resistance, but also the rate at which the phenolic substance accumulate in the infected tissues determines the resistance. In the present study also, the resistant cultivar Pozhikodi showed higher rate of phenolic accumulation than the susceptible cultivar Aryan. This increased accumulation of phenols in infected tissues may be due to the higher enzymatic activity catalysing various steps in the biosynthesis of phenols (Goodman et al., 1967).

The resistant cultivar Pozhikodi contained greater amount of anthocyanin than the susceptible cultivar Aryan (Table 9). Inoculation with the pathogen resulted in a significant increase in anthocyanin content in both cultivars. Avila et al. (1981) reported that Phaseolus vulgaris cultivar resistant to Macrophomina phaseolina

contained more amount of anthocyanin than the susceptible cultivars. More amount of leucoanthocyanin content was found in the susceptible cultivar Aryan than resistant cultivar Pozhikodi (Table 10). Inoculation of the Pathogen led to an increase in leucoanthocyanin content in both cultivars but the rate of increase was more in the resistant cultivar than susceptible one. It is well established that lignification in plants is closely related with the presence of leucoanthocyanin. Lignification during infection is an important aspect of defense reaction in plants (Mahadevan and Sridhar, 1982). In the present investigation also, the resistant cultivar Pozhikodi accumulated more of leucoanthocyanin consequent of inoculation possibly resulting in increased lignification of cells leading to resistance.

Attack by phytobacterial pathogens frequently resulted in chlorotic symptom. The results of the study on the changes in chlorophylls revealed that the resistant cultivar Pozhikodi contained significantly higher amount of total chlorophyll as well as chlorophyll 'a' and 'b' than the susceptible cultivar Aryan (Table 11 - 13 and Fig. 3). Inoculation with the pathogen led to a significant reduction in the levels of total chlorophyll, chlorophyll 'a' and 'b' in both cultivars. But this reduction was more in the susceptible one. There was also

a gradual reduction in chlorophyll content of leaves as the disease progressed. The loss of chlorophylls in betel vine plants infected with bacterial leaf spot pathogen has already been reported from Jabalpur (Jain et al., 1982). A reduction in chlorophyll content due to pathogenic infection has been reported by many workers in the case of other host-parasite interactions (Marimuthu, 1978; Dager et al., 1979; Gupta et al., 1983; Stall and Hall, 1984). The reduction of chlorophyll pigment may be due to the production of toxic metabolites by the pathogen (Goodman et al., 1967) and/or due to the reduction in photosynthetic area through death of tissues (Agrios, 1978).

The level of carbohydrates in the plant tissues influence to a large extent the incidence and development of disease. The present study revealed that the resistant cultivar had more of reducing sugar than the susceptible one. Upon inoculation, the reducing sugar depletion was noticed in both cultivars, but it was more pronounced in the susceptible one (Table 14 and Fig. 4). Studies conducted with other host-parasite interactions also indicated a decrease in reducing sugar following infection (Easwaran, 1967, Prasad et al., 1972, Reddy and Sridhar, 1975, Moses et al., 1976, Delveekasundram and Prasad, 1980, Marimuthu and Kandaswamy, 1981, Khatri et al., 1983a).

The reduction in reducing sugar may be due to the decrease in photosynthetic activity and/or increase in respiration of the infected plant and by utilising a portion of sugar for the synthesis of polyphenols (Uritani, 1961).

Higher quantity of non-reducing sugar was also noticed in the resistant cultivar Pozhikodi than in the susceptible cultivar Aryan (Table 15 and Fig. 5). Inoculation of the cultivars with the pathogen caused an increase in non-reducing sugar content. But this increase was more in the susceptible cultivar. Deiveekasundram and Prasad (1980) also reported higher level of non-reducing sugar in bacterial leaf blight resistant rice variety than the susceptible one. Increase of non-reducing sugar following infection by X. oryzae in rice varieties (Prasad et al. 1972) and french bean inoculated with X. campestris pv. phaseoli (Khatri et al., 1983a) was also reported.

The resistant cultivar Pozhikodi possessed more quantity of total sugar than the susceptible cultivar Aryan (Table 16 and Fig. 6). In general, inoculation of cultivars with the pathogen resulted in a decrease in total sugar content. Deiveekasundram and Prasad (1980) observed high level of total sugar in rice variety resistant to X. campestris pv. oryzae than susceptible one. Vidhyasekaran (1974) reported that finger millet leaves

susceptible to Helminthosporium nodulosum contained less total sugar than the resistant leaves. Decrease in total sugar due to pathogenic invasion in other crop plants has also been reported (Prasad et al., 1972, Khatri et al., 1983a). Sinclair et al. (1970) noticed an increase in carbohydrate level in chilli leaves infected with X. vesicatoria. The decrease in total sugard due to infection may be due to the preferential utilisation of sugars by the pathogen and/or due to the decrease in photosynthetic activity.

Tissues containing higher amount of soluble nitrogen are generally susceptible to pathogen. In the present investigation, the presence of significantly higher amount of amino nitrogen was noticed in the susceptible cultivar Aryan than resistant cultivar Pozhikodi (Table 17 and Fig. 7). In general, the infected leaves of both cultivars showed an increased amount of amino nitrogen compared to the healthy ones and the accumulation was more striking in the susceptible cultivar. However, inoculated plants of both cultivars at certain intervals showed a decrease in the amino nitrogen content compared to the uninoculated ones. Prasad et al. (1972) reported greater amounts of amino nitrogen in bacterial leaf blight susceptible rice variety than the moderately susceptible and resistant varieties and inoculation of

the pathogen resulted in its accumulation in all varieties. The increase in amino acids due to pathogenic invasion in other plants was reported by Sinclair et al. (1970) as well as by Reddy and Sridhar (1975). On the other hand, a decrease in amino acids in infected plants was also reported (Nayudu and Walker, 1961, Karwasra and Chand, 1981, Marimuthu and Kandaswamy, 1983) in other host plants infected with bacterial pathogens. Chile and Vyas (1983) noticed more amount of free amino acids in the healthy leaves of the Madrasi variety of betel vine which was less susceptible to Phytophthora infection than the highly susceptible Kapoori variety. The increase in amino nitrogen content following infection may be due to activated synthesis of amino acids by the pathogen or due to degradation of proteins in the cell by its proteolytic activity (McCombs and Winstead, 1964, Van Andal, 1966). The decrease in amino nitrogen in inoculated leaves may be due to increased utilisation by the pathogen for its cellular activities (Clifton, 1957, Fowdon, 1965). The high level of amino nitrogen in the tissues of susceptible cultivar may stimulate the growth of the pathogen as its nutritional requirements are effectively met under such condition.

Greater quantities of total nitrogen and crude protein were observed in the susceptible cultivar Aryen

than the resistant cultivar Pozhikodi (Tables 18, 19 & Fig. 8). Inoculation of cultivars with the pathogen significantly increased the total nitrogen and crude protein contents and the accumulation was more in the susceptible cultivar. Many experimental data on the pathophysiology supports the contention that nitrogen and protein content are higher in diseased plants than their healthy counterparts. (Lee, 1952; Wolf and Wolf, 1955; Sinclair et al., 1970; Prasad et al., 1972; Singh and Saksena, 1983). The increase in protein content following infection may be due to the formation of newer proteins or due to the activation of protein synthetic system of the plant due to inoculation (Prasad et al., 1972). The high level of nitrogen and crude protein in the susceptible cultivar may favour the growth of the pathogen as these constituents meet its nutritional requirement. Kirkham (1954) suggested that high level of nitrogen in the tissues enhances the breakdown of phenolics leading to decrease in resistance to invading pathogen.

No significant difference in phosphorus content was observed between cultivars of betel vine and the inoculation of the cultivars with the pathogen resulted in a decrease in its content and the decrease was significant only in the case of susceptible cultivar

Aryan (Table 20). The result was in agreement with that of Philip and Devadath (1981). They reported lower level of phosphorus in bacterial leaf blight infected rice plants as compared to the content of healthy plants. Singh and Saksena (1983) observed no significant difference in phosphorus content in the resistant and susceptible cultivars of pea to powdery mildew infection.

The potassium content of the resistant and susceptible cultivars of betel vine was found to be almost of the same order. Inoculation of the cultivars with the pathogen resulted in a reduction of potassium content in both cultivars of betel vine (Table 21). Khatri et al. (1983b) reported that the electrolyte which leaked out from betel vine leaves infected with X. campestris pv. betlicola contained more amount of potassium ions. They also reported that ionic imbalance occurred in betel vine leaves infected with X. campestris pv. betlicola and this may be due to altered permeability of host cells due to the action by some toxin. Philip and Devadath (1981) reported lower levels of potassium in rice leaves infected with X. campestris pv. oryzae. However, an increase in potassium content was observed in chilli leaves infected with X. vesicatoria (Sinclair et al., 1970).

Higher amount of calcium and magnesium was observed

in the resistant cultivar Pozhikodi than susceptible cultivar Aryan (Tables 22 and 23). Inoculation with the pathogen resulted in an increase in calcium and magnesium contents in the resistant cultivar Pozhikodi while in the susceptible cultivar it led to a decrease. Changes in calcium content due to pathogenic invasion in other plant diseases have also been reported. Sinclair et al. (1970) noticed little change in calcium content in chilli leaves infected with X. vesicatoria. Singh and Saksena (1983) reported higher amount of calcium and magnesium in pea cultivars resistant to powdery mildew than susceptible varieties.

There was not much change in sodium content due to inoculation of the pathogen in both cultivars of betel vine (Table 24). The resistant cultivar Pzohikodi possessed slightly higher amount of sodium than susceptible cultivar Aryan. Khatri et al. (1983b) observed that the electrolyte leaked out from betel vine leaves infected with X. campestris pv. betlicola contained only less amount of sodium ions.

Thus, from the above discussion on biochemical changes it can be presumed that, the resistant reaction exhibited by the cultivar Pozhikodi may be due to the higher content of ortho-dihydric and total phenols and

the lower amounts of amino nitrogen, total nitrogen and crude protein in the leaf tissues. Further, on inoculation with the pathogen there was a rapid rate of increase in the ortho-dihydric and total phenols in the resistant cultivar than the susceptible one. Though, more quantity of leucoanthocyanin was present in the susceptible cultivar, there was a sudden increase in its content in the resistant cultivar upon inoculation. It was also found that the rate of increase in amino-nitrogen, total nitrogen and crude protein after inoculation with the pathogen was less in the resistant cultivar compared to the susceptible one. Though, more amount of reducing sugar was present in the resistant cultivar, the rate of decrease following infection was less in the resistant cultivar, compared to the susceptible one.

Interactions are known to occur among the pathogens and saprophytes which are inhabiting on the surface of the leaves side by side. Furthemore, the varietal response also has profound influence on the phylloplane microflora. Philip and Devadath (1980) detected some differences in the phylloplane fungal and bacterial population of the bacterial leaf blight tolerant and susceptible varieties of rice.

Results of the present investigation on the phylloplane microflora of betel vine cultivars revealed that in general, inoculation of the pathogen resulted in an increase in the total microbial population in both the resistant and the susceptible cultivars. Generally, the population of actinomycetes, bacteria and fungi increased with the increase in sampling time. Shekhawat and Chakravarthi (1977) also reported the variation in microbial population on the surface of chilli leaves infected with X. vesicatoria due to time lag. It was interesting to note that the microbial population in the inoculated plants of the resistant cultivar Pozhikodi decreased on the fifteenth day of inoculation than the uninoculated plant while in the susceptible cultivar Aryan, the microbial population load showed a reversal. This may be due to the altered permeability of the susceptible host cells resulting in more electrolyte leakage which in turn may enhance the saprophytic activity. Khatri et al. (1983b) reported the altered permeability of host cells due to X. campestris pv.betlicola infection in betel vine.

It is well established that the most effective way of controlling bacterial plant disease is the use of resistant varieties, supplemented with proper cultural practices and chemical application. So the control/management of the

bacterial leaf spot of betel vine was approached from three different lines namely, effect of nutrients on the disease development, testing of chemicals for their efficacy against the pathogen and screening of betel vine cultivars for host resistance.

The present investigation revealed that application of nitrogen, phosphorus and potassium had no significant effect on the development of bacterial leaf spot of betel vine. However, some of the treatments showed some influence on the development of this disease. Ten days after inoculation plants receiving 100 kg N/ha showed the lowest disease score and percentage of sten infection, whereas the minimum percentage of defoliation was with 200 kg N/ha (Tables 26a, 27a and 28a). After ten days of inoculation, plants which received no phosphorus showed the lowest disease score, percentage of stem infection and defoliation. Plants supplied with 50 kg K_2O/ha showed the lowest disease score and percentage of defoliation ten days after inoculation while those receiving 150 kg K_2O/ha recorded the minimum percentage of stem infection.

After forty days of inoculation, the lowest disease score was found in plants which received 100 kg N/ha, while the minimum percentage of stem infection and defoliation were noticed with 200 kg N/ha (Tables 26b, 27b and 28b). The minimum disease score and percentage

of defoliation were recorded where phosphorus application was withheld while, in the case of stem infection minimum percentage infection was at 100 kg P_2O_5/ha. Potassium at the rate of 150 kg K_2O/ha showed the lowest percentage of stem infection and defoliation whereas zero level of potassium resulted in the lowest disease score after forty days of inoculation.

Similar results has been reported by several workers in the case of bacterial disease of various crops. Subramani)an *et al*. (1982) did not observe any difference in the mean disease incidence of bacterial blight of cotton with application of nitrogen. Similarly, Ho and Lim (1978) observed that application of phosphorus and potassium had little or no effect on the bacterial leaf blight of rice. Reddy and Sridhar (1975) noticed that the increased supply of potassium had no effect on the same disease. Chowdhury (1944) found that the nature of the fertilizers did not alter the incidence of *Rhizoctonia solani* in betel vine plants.

The *in vitro* and *in vivo* efficacy of antibiotics, fungicides and combinations of antibiotics and Bordeaux mixture in controlling the bacterial leaf spot pathogen was assessed.

Of the three antibiotics tested for the *in vitro*

sensitivity against the bacterium, Plantomycin had the maximum inhibitory effect followed by Streptocycline while Paushamycin recorded the least inhibition. Plantomycin at 300 ppm concentration showed the maximum inhibition (Table 29a). Among the fungicides tested, Dithane M-45 exerted the maximum inhibition of the bacterium and was superior to Blitox, Captaf and Dithane Z-78 in that order (Table 29b). Dithane M-45 at 3000 ppm gave the maximum inhibition followed by Blitox 3000 ppm concentration. One per cent Bordeaux mixture gave the maximum inhibition and was superior to its lower concentration and also to all other fungicides tested.

The combination of Plantomycin 300 ppm and one per cent Bordeaux mixture was found to be the best against the pathogen followed by the combination of Streptocycline 300 ppm and one per cent Bordeaux mixture (Table 29c). The former combination was more inhibitory to the bacterium than the other antibiotics and fungicides when tested individually. An additive effect was recorded only in the case of Paushamycin at all concentrations and Plantomycin at 200 and 300 ppm were mixed separately with one per cent Bordeaux mixture.

The *in vitro* effectiveness of Terramycin, Chloromycetin, Dihydrostreptomycin, Tetracycline, Streptocycline,

Streptomycin sulphate, Chlorotetracycline, Oxytetracycline, Agrimycin, Chloramphenicol, Paushamycin in inhibiting the growth of X. campestris pv. betlicola was reported by several workers (Thirumalachar et al., 1956; Nema et al., 1975; Kotwal; 1978, Mathew et al., 1979a, b; Gupta, 1981; Jain et al., 1982; Tripathi et al., 1984).

The inhibitory effect of fungicides and combination of antibiotics with one per cent Bordeaux mixture on the growth of this bacterium has not been studied so far. However, there are reports on the in vitro control of other bacterial pathogens with fungicides and combination of antibiotics with copper fungicides. Sood et al. (1976) reported the efficacy of Dithane M-45, Dithane Z-78, Bavistin, Blitox, Benomyl and the combination of Streptocycline and copper sulphate in inhibiting the growth of X. phaseoli. Chauhan and Vaishnav (1980) noticed the inhibitory effect of Streptocycline and copper compounds against X. campestris pv. oryzae. The inhibitory effect of the combination of Streptocycline and copper sulphate against X. campestris pv. vesicatoria was also recorded (Sharma et al., 1982).

In the pot culture experiment, the plants were artificially inoculated with the pathogen prior to the chemical treatment. In this experiment, none of the

treatments gave an absolute control of the disease. But it was noticed that one per cent Bordeaux mixture had an effect in checking the further spread of the disease as the plants in this treatment showed comparatively lower ratings of the disease score, percentage of stem infection, percentage of defoliation, disease score on new leaves and mortality at different intervals of observation (Tables 30 - 34). It was also noticed that though the ratings of the above parameters were high in one per cent Bordeaux mixture treated plants during the initial stages of observation, these remained static throughout the later stages thereby indicating that this particular treatment prevented the further advancement of the disease. The combination of Streptocycline 200 ppm and one per cent Bordeaux mixture also showed comparatively low disease score and stem infection.

In the field experiment also, none of the treatments gave absolute control of the disease. However, plants receiving the treatment combination of Plantomycin 200 ppm and one per cent Bordeaux mixture showed comparatively lower disease score, percentage of stem infection, percentage of defoliation and disease score on newly formed leaves than other treatments at different intervals of observation (Tables 35 - 39). The treatments,

Streptocycline 200 ppm with one per cent Bordeaux mixture, and one per cent Bordeaux mixture also showed an effect in checking the severity of the disease and spread of the pathogen.

In vivo control of bacterial leaf spot of betel vine with antibiotics and Bordeaux mixture was reported by several workers. Drenching and spraying of Bordeaux mixture for controlling bacterial leaf spot of betel vine was recommended by Asthana and Mahmud (1945). Jain and Nayak (1981) suggested the treatment of betel vine cuttings in solutions of Bordeaux mixture and Streptocycline for 20 minutes and foliar application of Streptocycline for the control of bacterial leaf spot disease. Abraham and Mathew (1982) reported that none of the antibiotics tested gave absolute control of bacterial leaf spot of betel vine, but Terramycin 500 ppm had some effect in reducing the disease severity. The superiority of the combination of Agrimycin-100 and one per cent Bordeaux mixture in controlling this disease was reported from Jabalpur (Jain et al., 1982). Tripathi et al. (1984) noticed the usefulness of Streptomycin in controlling the bacterial leaf spot of betel vine.

Successful control of many other bacterial plant diseases using fungicides and combination of antibiotics

and fungicides especially copper compounds has also been reported by many workers (Miller, 1970; Severin and Kupferberg, 1977; Strider, 1980; Parson and Edgington, 1980; Krishna and Nema, 1983).

The results of the present studies on the chemical control of bacterial leaf spot of betel vine revealed that none of the chemicals tried in pot culture and field experiments gave absolute control of the disease. In the pot culture experiment one per cent Bordeaux mixture and in field experiment the combination of Plantomycin 200 ppm and one per cent Bordeaux mixture exhibited comparatively good effect in checking the severity and spread of this disease. The superior inhibitory effect of these chemicals against the pathogen was also noticed in *in vitro* studies. In general, chemical control of bacterial plant diseases is found to be less successful and the bacterial leaf spot of betel vine is no exception to this.

An attempt was made to screen available betel vine cultivars for host resistance against *X. campestris* pv. *betlicola*. Of the ten cultivars of betel vine screened for host resistance against the pathogen, none of them was found to be immune to the disease. However, cultivars Poshikodi, Nadankodi and Tulasivettila Type I

showed fairly good resistant reaction as they showed comparatively lower rates of disease score, percentage of stem infection, percentage of defoliation compared to other cultivars tested. None of the plants of these cultivars showed any mortality even after forty days of inoculation. All other cultivars were susceptible to the disease with cultivar Aryan showing the highest susceptibility. Patel et al. (1953) found that all varieties of betel vine in former Bombay state were susceptible to bacterial leaf spot pathogen. Singh and Chand (1971) and Karbhari (1976) observed that all varieties of betel vine grown in Jabalpur were susceptible to X. campestris pv. betlicola, but among them the Bangala variety was relatively more susceptible. However, a report from Jabalpur suggested that the variety Bangala obtained from Mandsaur was resistant to the disease (Jain et al., 1982). Abraham and Mathew (1981) reported that none of the seven cultivars of betel vine screened by them was resistant to the bacterial leaf spot disease, but cultivars Tulasivettila and Karilanchikarpuran showed less degree of susceptibility.

The screening trial revealed that cultivars Poozhikodi, Nadenkodi and Tulasivettila Type I were resistant to bacterial leaf spot disease. The resistance reaction exhibited by these cultivars might be due to their biochemical make up which restricts disease development.

Summary

SUMMARY

The bacterial leaf spot incited by Xanthomonas campestris pv. betlicola is one of the most serious diseases of betel vine. Considering the seriousness of the disease, studies were undertaken on the different aspects of the disease and to find out a suitable control/management practice.

The causal bacterium was isolated from disease affected leaves and stem of betel vine and its pathogenicity was established. Four isolates of the pathogen were collected from different parts of Kerala. Based on the cultural and biochemical characters of the bacterium and its pathogenicity, the pathogen causing leaf spot of betel vine was identified as X. campestris pv. betlicola (Patel, Kulkarni and Dhande) Dye.

The four isolates of the pathogen were alike in respect of their cultural and biochemical characters and in the symptom expression on the susceptible cultivar. Maximum growth of the isolates was observed between pH 6 and 7 and temperature 25-30°C. The isolates of the pathogen showed slight variations in their preference to utilise different carbon and nitrogen sources including amino acids. In general, higher

concentrations of amino acids did not support the growth of the isolates.

Naturally infected betel vine showed different types of symptom on leaves and stem. On leaves, water soaked lesions, dark brown spots with yellow halo, shot hole type symptom, marginal and tip infections, bacterial exudations and blightening were observed. On stem, dark brown lesions were observed in addition to bacterial exudations. Further, it was observed that even the presence of one or two spotson leaves caused defoliation. Similarly, a slight infection on the stem caused mortality of plants.

Histopathological studies revealed that the pathogen is a parenchymatous one. The entry of the bacterium was found to be through natural openings. The pathogen affected the spongy and palisade parenchymatous cells resulting in their complete disintegration. However, the xylem vessels remained intact even in the advanced stages of infection.

Plants belonging to the family Piperaceae such as Piper nigrum, Piper longum, Piper sp. Types I-IV and Peperomia sp. Type I were found to be infected by the pathogen on artificial inoculation. However, Piper attenuatum and Peperomia pellucida did not show any

symptoms of the disease.

Biochemical changes in resistant and susceptible cultivars of betel vine as influenced by X. campestris pv. betlicola inoculation were assessed. The study revealed that the resistant cultivar Pozhikodi contained more quantities of ortho-dihydric and total phenols, anthocyanin, chlorophylls, reducing, non-reducing and total sugars, calcium and magnesium and lower contents of leucoanthocyanin, amino nitrogen, total nitrogen and crude protein compared to the susceptible cultivar Aryan. But there was not much difference between the cultivars in the phosphorus, potassium and sodium contents. Upon inoculation with the pathogen, pronounced increase in ortho-dihydric and total phenols, leucoanthocyanin was noticed in the resistant cultivar than the susceptible one while, the increase of anthocyanin, non-reducing sugar, amino nitrogen, total nitrogen and crude protein was more in the susceptible cultivar than the resistant one. The decrease in chlorophylls, reducing sugar and phosphorus contents following infection was more in the susceptible cultivar than the resistant one. The contents of calcium and magnesium increased in the resistant cultivar following infection while a reversal was noticed in the susceptible cultivar. Potassium contents of both cultivars decreased following infection by the pathogen but there was not much change in sodium content.

Fluctuation in phylloplane microbial population was observed in both cultivars of betel vine following inoculation of the pathogen. The individual population of actinomycetes, bacteria and fungi was also fluctuated.

Nitrogen, phosphorus and potassium had no significant effect on the development of the disease. However, certain treatments showed some effect in the disease development.

In vitro evaluation of chemicals against *X.campestris* pv. *betlicola* showed that among the antibiotics, Plantomycin exerted the maximun inhibition of the bacterium while, Dithane M-45 showed the maximum inhibition out of four fungicidal formulations tested. The efficacy of one per cent Bordeaux mixture was superior to other fungicide formulations. Among the combinations of antibiotics and Bordeaux mixture, Plantomycin 300 ppm with one per cent Bordeaux mixture exerted the maximum inhibition of the bacterium and was superior to other fungicides and antibiotics tested.

In *in vivo* chemical control of bacterial leaf spot of betel vine, none of the treatments gave absolute control of the disease. In the pot culture experiment, one per cent Bordeaux mixture and in the field experiment the combination of Plantomycin 200 ppm and

one per cent Bordeaux mixture showed some effect in checking the severity and spread of the disease as the plants receiving these treatments showed comparatively lower ratings of disease score, stem infection, defoliation, disease score on new leaves and mortality at different intervals of observation. The combination of Streptocycline 200 ppm and one per cent Bordeaux mixture had also showed some effect in checking the disease in both field and pot trials.

Screening of betel vine cultivars of host resistance against the pathogen indicated that, the cultivars Pozhikodi, Nadankodi and Tulasivettila Type I were resistant to the disease. These cultivars showed comparatively lower disease score, percentage of stem infection, percentage of defoliation at different intervals of observation. None of the plants of these cultivars showed mortality even after forty days of inoculation. All other cultivars were susceptible to the disease, the cultivar Aryan being the most susceptible one.

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Appendices

APPENDIX-I

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	- 200.0 g
Agar agar	- 20.0 g
Distilled water	- 1000 ml
pH	7

APPENDIX-II

Composition of basal medium for Xanthomonads (Dye, 1962)

$NH_4H_2PO_4$	- 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
Glucose	- 10.0 g
Distilled water	- 1000 ml
pH	6.8

APPENDIX-III

Composition of the medium

KH_2PO_4	- 0.2 g
Na_2HPO_4	- 0.5 g
$FeSO_4$	- 0.05 g
KCl	- 0.05 g
Peptone	- 2.0 g
Sucrose	- 20.0 g
Distilled water	- 1000 ml
pH 7	

APPENDIX-IV

Composition of FAA

C_2H_5OH	- 50.0 ml
CH_3COOH	- 5.0 ml
HCHO	- 10.0 ml
H_2O	- 35.0 ml

Tertiary butyl alcohol(TBA) series for dehydration (Jensen, 1962)

Solution No.	C_2H_5OH ml	TBA ml	Distilled H_2O	Time
1	50	10	40	3 h
2	50	20	30	Over night
3	50	35	15	3 h
4	45	55	-	3 h
5	25	75	-	Over night
6	-	100	-	1 h
7	-	100	-	1 h
8	-	100	-	1 h

Haupt's adhesive (Johanson, 1940)

Gelatin	- 1.0 g
Phenol crystals	- 2.0 g
Glycerin	- 15 ml
Distilled water	- 100 ml

Staining schedule, (Johanson, 1940, Nelson and Dickey, 1966)

	Time in minutes
Xylene	5
Xylene + absolute alcohol	5
Absolute alcohol	5
95% alcohol	5
70% alcohol	5
50% alcohol	5
Harris hematoxylin	5
Distilled water	Rinse to remove excess stain
Tap water with few drops of ammonia	5
	5
Tap water	5
50% alcohol	5
70% alcohol	5
95% alcohol	3
Orange G	
Equal amount of clove oil, absolute alcohol and xylene	Wash of the excess stain

	Time in minutes
Equal amount of clove oil, absolute alcohol and xylene	Agitate the slide for 15 seconds
Xylene + few drops of absolute alcohol	5
Xylene	5
Xylene	5
Mount in Canada balsam	

APPENDIX-V

Kusters Agar

Starch	- 10.0 g
Casein	- 0.3 g
$MgSO_4$	- 0.5 g
$FeSO_4$	- 0.1 g
KNO_3	- 2.0 g
NaCl	- 2.0 g
K_2HPO_4	- 0.5 g
$CaCO_3$	- 0.2 g
Agar agar	- 20.0 g
Distilled water	- 1000 ml
pH 6.8 - 7.0	

Nutrient agar

Beef extract	- 0.5 g
Peptone	- 10.0 g

Agar agar - 20.0 g

Distilled water - 1000 ml

pH 6.8

Potato Dextrose Agar

Potato - 200.0 g

Dextrose - 20.0 g

Agar agar - 20.0 g

Distilled water - 1000 ml

APPENDIX-VIa Analysis of Variance Table - Biochemical changes in resistant and susceptible cultivars of betel vine as influenced by *X. campestris* pv. *betlicola* inoculation

Source	df	Mean square								
		OD phenol	Total phenols	Anthocyanin	Leuco-anthocyanin	Total chlorophyll	Chlorophyll 'a'	Chlorophyll 'b'	Reducing sugar	Non-reducing sugar
Cultivar	1	0.44339*	3.37991*	0.00999*	0.00207*	0.0254252*	0.0006849*	0.0173911*	3.0839*	22.8418*
Treatment	1	0.01535	3.00119*	0.02554*	0.01717*	0.0313746*	0.0059047*	0.0104401*	278.0898*	14.5215*
Cultivar x treatment	1	0.04245*	0.26901*	0.00198*	0.00006	0.0090451*	0.0032262*	0.0016723*	7.1543*	10.9854*
Sampling time	5	0.13246*	2.05748*	0.00715*	0.02524*	0.0112676*	0.0019751*	0.0042663*	141.0820*	9.5615*
Cultivar x sampling time	5	0.05011*	0.38009*	0.00303*	0.00346*	0.0032815*	0.0005589*	0.0003094*	46.8801*	22.0418*
Treatment x sampling time	5	0.01188	0.15436*	0.00121*	0.00081*	0.0090451*	0.0006743*	0.0011553*	27.6145*	8.2168*
Cultivar x treatment x sampling time	5	0.00631	0.01044	0.00108*	0.00029*	0.0008957*	0.0004310*	0.0001326*	8.8820*	0.9559*
Error	48	0.00581	0.01201	0.00031	0.00004	0.0000003	0.0000035	0.0000013	0.0088	0.0098

* Significant at 5% level

APPENDIX-VIb Analysis of Variance Table - Biochemical changes in resistant and susceptible cultivars of betel vine as influenced by X. campestris pv. betlicola inoculation

Source	df	Mean square								
		Total sugar	Amino nitrogen	Total nitrogen	Crude protein	Phosphorus	Potassium	Calcium	Magnesium	Sodium
Cultivar	1	42.5625*	27.0117*	0.1452*	5.6762*	0.0002	0.0003	0.3149*	0.1177*	0.00001
Treatment	1	165.2265*	10.8115*	0.1206*	4.1145*	0.0030*	0.0598*	0.0372*	0.0226*	0.000004
Cultivar x treatment	1	0.3750*	3.7810*	0.0133*	0.5233*	0.0007*	0.0003	0.4528*	0.0546*	0.000004
Sampling time	5	96.0633*	19.8193*	0.1822*	7.1582*	0.0119*	0.1599*	0.0639*	0.0050*	0.000008
Cultivar x sampling time	5	123.0164*	3.6461*	0.0034*	0.1110*	0.0038*	0.0250*	0.1386*	0.0191*	0.000004
Treatment x sampling time	5	24.1398*	2.9002*	0.0086*	0.3289*	0.0005*	0.0091*	0.0311*	0.0079*	0.000001
Cultivar x treatment x sampling time	5	10.8250	2.0169*	0.0049*	0.1804*	0.0006*	0.0135*	0.0783*	0.0043*	0.000006
Error	48	0.0011	0.0636	0.0007	0.0309	0.0001	0.0015	0.0001	0.000002	0.00003

* Significant at 5% level

APPENDIX-VII Analysis of Variance Table - Effect of different levels of N, P and K on the development of bacterial leaf spot of betel vine

Source	df	Mean square					
		Disease score		Percentage of stem infection		Percentage of defoliation	
		10 days after inoculation	40 days after inoculation	10 days after inoculation	40 days after inoculation	10 days after inoculation	40 days after inoculation
N	3	1.5597	0.1839	38.4619	1239.1354	555.3750	12.4480
P	3	16.4377	0.4007	2.4369	624.6667	3379.0574*	40.2746
NP	9	1.3175	0.1013	69.8212*	1079.6945	347.8924	56.4651*
K	3	3.3060	0.0798	9.8471	16.1354	728.6094	34.4226
NK	9	4.3248	0.2016	23.0203	524.1649	954.7673	49.0858*
PK	9	4.1651	0.0716	28.7677	642.7656	626.1476	45.8705*
NPK	27	3.4947	0.1201	21.2186	293.8223	605.4196	41.7110
Error	64	7.3622	0.1980	25.2949	770.4356	854.9121	19.7880

* Significant at 5% level

APPENDIX-VIII

Analysis of Variance Table - *In vitro* evaluation of chemicals against *X. campestris* pv. *betlicola*

Source	df	Mean square
Antibiotic	2	36.0370*
Concentration	2	84.5930*
Antibiotic x concentration	4	0.6483
Fungicide	3	16.7687*
Concentration	2	54.7500*
Fungicide x concentration	6	2.7123*
Fungicide x Bordeaux mixture	1	34.3220*
Concentration of Bordeaux mixture	1	16.6670*
Antibiotic + Bordeaux mixture	2	15.5930*
Concentration	2	64.0370*
Antibiotic + Bordeaux mixture x concentration	4	5.0373*
Between chemicals	2	488.6450*
Error	64	1.1458

* Significant at 5% level

APPENDIX-IX **Analysis of Variance Table - Pot culture experiment on the chemical control of bacterial leaf spot of betel vine**

Observation	Source	df	Mean square			
			On the day of first spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying
Disease score	Treatment	11	3.4388	1.5725	0.2935	0.1572
	Error	36	2.3499	2.4676	0.2292	0.1181
Percentage of stem infection	Treatment	11	30.5944	623.6364	627.6904	483.1421
	Error	36	29.4679	917.1048	1045.0278	444.3099
Percentage of defoliation	Treatment	11	208.7885	217.9631	13.0199	13.0199
	Error	36	216.5573	269.4184	13.0208	13.0208
Disease score on new leaves	Treatment	11	0.2708	18.4632	17.1689	14.1389
	Error	36	0.2917	19.8009	19.6607	15.6973

APPENDIX-X Analysis of Variance Table - Field experiment on the chemical control of bacterial leaf spot of betel vine

Observation	Source	df	Mean square			
			On the day of first spraying	15th day after first spraying	15th day after second spraying	15th day after third spraying
Disease score	Block	2	0.2501*	3.4614	5.2365	4.2237
	Treatment	11	0.0625	2.2990	2.3064	3.5590
	Error	22	0.0645	1.2040	1.8187	1.6298
Percentage of stem infection	Block	2	0.1929	3.4155	96.8844	285.4859
	Treatment	11	0.7382	3.9850	88.7143	168.7643
	Error	22	0.7362	3.6131	67.6568	123.8432
Percentage of defoliation	Block	2	-	332.7148	613.9023	555.6328
	Treatment	11	-	218.1069	229.8395	411.9276
	Error	22	-	141.4572	221.4847	236.3722
Disease score on new leaves	Block	2	-	0.0027	3.4244	2.3471
	Treatment	11	-	0.0149	1.8467	5.1565*
	Error	22	-	0.0184	1.4492	1.2957

* Significant at 5% level

APPENDIX-XI Analysis of Variance Table - Screening of betel vine cultivars for host resistance against the pathogen *X. campestris* pv. *betlicola*

Observation	Source	df	Mean square			
			10 days after inoculation	20 days after inoculation	30 days after inoculation	40 days after inoculation
Disease score	Treatment	9	41.9320*	114.1020*	109.9820*	98.2600*
	Error	70	3.2460	1.2570	2.0010	0.9440
Percentage of stem infection	Treatment	9	599.9013*	2877.3660*	10609.457*	11194.170*
	Error	70	201.8635	215.8679	872.4592	788.3661
Percentage of defoliation	Treatment	9	3009.8601*	12806.915*	12403.830*	10570.743*
	Error	70	426.1662	252.7487	132.0884	182.2018

* Significant at 5% level

STUDY OF BACTERIAL LEAF SPOT OF BETEL VINE- BIOCHEMICAL CHANGES AND CONTROL

By

KOSHY ABRAHAM

ABSTRACT OF A THESIS

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ABSTRACT

The bacterial leaf spot is one of the most serious diseases of betel vine in Kerala. The bacterium causing this disease was identified as Xanthomonas campestris pv. betlicola based on the cultural and biochemical characters and its pathogenicity. The four isolates of the pathogen obtained from different parts of Kerala showed no significant difference except some slight variation in their cultural, biochemical and physiological characters and also in the symptom expression.

Different types of symptom were produced by the pathogen such as water soaked lesions, dark brown spots with yellow halo, shot hole type symptom , leaf marginal and tip infections, blightening, defoliation, stem infection, bacterial exudations and mortality of plants.

X. campestris pv. betlicola was found to be a parenchymatous pathogen, entering through the stomata. The pathogen disintegrated the spongy and palisade parenchymatous cells. However, the xylem vessels were not affected even in the advanced stages of infection.

The pathogen infected other members of the family Piperaceae such as Piper nigrum, Piper longum, Piper sp. Type I-IV and Peperomia sp. Type I, but did not infect

Piper attenuatum and *Peperomia pellucida* on artificial inoculation.

The resistant cultivar pozhikodi contained more amounts of *ortho*-dihydric phenol, total phenols, anthocyanin, chlorophylls, reducing, non-reducing and total sugars and lower contents of leucoanthocyanin, amino nitrogen, total nitrogen and crude protein than the susceptible cultivar Aryan. Not much difference in phosphorus, potassium and sodium contents was observed between the cultivars. Upon inoculation with the pathogen, pronounced increase in *ortho*-dihydric phenol, total phenols and leucoanthocyanin contents was noticed in the resistant cultivar than the susceptible one whereas, there was more increase in anthocyanin, non-reducing sugar, amino nitrogen, total nitrogen and crude protein in the susceptible cultivar compared to the resistant one. There was greater decrease of chlorophylls, reducing sugar, and phosphorus contents in the susceptible cultivar following infection than the resistant one. Calcium and magnesium contents increased in the resistant cultivar following infection while a reverse was noticed in the susceptible cultivar. Potassium content also decreased upon infection in both cultivars but there was not much difference in sodium content.

Fluctuation in the total phylloplane microflora was observed in both cultivars following inoculation. The individual population of actinomycetes, bacteria and fungi was also fluctuated.

Application of nitrogen, phosphorus and potassium had no significant effect on the development of bacterial leaf spot of betel vine.

The combination of Plantomycin and one per cent Bordeaux mixture was found to be more inhibitory to the bacterium than antibiotics and fungicides alone.

In in vivo chemical control, none of the treatments gave absolute control of the disease. However, in the pot culture experiment one per cent Bordeaux mixture and in the field experiment the combination of Plantomycin 200 ppm and one per cent Bordeaux mixture showed some effect in checking the severity and spread of the disease.

The cultivars Poozhikodi, Nadankodi and Tulasivettila Type I were found to be resistant to the disease and other cultivars tested were highly susceptible.