ETIOLOGY AND CONTROL OF BACTERIAL LEAF BLIGHT OF RICE CAUSED BY *Xanthomonas oryzae* (UYEDA AND ISHIYAMA) DOWSON

BY

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ABSTRACT OF A THESIS

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DECLARATION

I hereby declare that this thesis entitled "Etiology and control of bacterial leaf blight of rice caused by Xanthomonas oryzae (Uyeda and Ishiyama) Dowson" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled "Etiology and control of bacterial leaf blight of rice caused by Xanthomonas oryzae (Uyeda and Ishiyama) Dowson" is a record of research work done independently by Kumari. C.A. MARY, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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INTRODUCTION

INTRODUCTION

Rice (Oryza sativa L.) ranks an important position among the numerous cereal crops of India. Being the staple food of a sizable section of the Indian population the crop receives much attention in all agricultural development and production programmes. India occupies first place among the rice growing countries of the world in coverage but falls second to China in production.

Though Kerala is not a major contributor to India's total rice production, the requirements of the States entire population, centers around this cereal crop. It is cultivated over an area of 840.37 thousand hectares and the annual production is 1294.64 thousand tonnes, the average production being 1541 kg/ha. Recent advances in agricultural technology has contributed towards increasing the rice production of Kerala considerably. Eventhen there is always a gap between the production and the requirements of the population. This is mainly due to unforeseen climatic calamities and pest and disease occurrences.

Of the several rice diseases, bacterial leaf blight incited by Xanthomonas oryzae (Uyeda and Ishiyama) Dowson has assumed importance in the recent past. This disease was first reported in India by Sreenivasan et al. (1959). Until the year 1963 this disease was of minor importance and was confined to certain areas of Maharashtra State. Now the

disease is found to occur all over the country on many of the exotic and indigenous rice varieties. The adoption of high yielding varieties, intensive and continuous cropping patterns and increased use of nitrogenous fertilizers have resulted in an increased incidence of this disease in all the rice growing tracts of our country inflicting heavy losses. In fact, this disease has become a serious constraint to rice production in the country. Several investigators have been working on the different aspects of this disease with the ultimate object of evolving a schedule of practical control measures of this disease.

In Kerala eventhough severe epiphytotics of this disease have not so far been reported, the disease is endemic in the major rice growing areas of Kuttanad and Palghat. Detailed studies on this disease or on the pathogen were not so far taken up in this State. In view of the potential crop losses the disease could cause in the State, investigations on this disease were thought to be worthwhile.

In the present study investigations were taken up on the characterisation and identification of the pathogen, survival of the pathogen in seed, plant debris and soil, effect of weather factors on the disease incidence, varietal screening for host resistance against the disease, *in vitro* and *in vivo* sensitivity of the pathogen to antibiotics. The results are presented and discussed in this thesis.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

HISTORY, GEOGRAPHICAL DISTRIBUTION AND LOSSES

Presently bacterial leaf blight of rice incited by Xanthomonas oryzae (Uyeda and Ishiyama) Dowson is one of the major rice diseases in India, in South East Asia and in other rice growing areas of the world. The disease was reported for the first time from Japan in 1884. Subsequently, it was reported from different Asian countries such as Korea, Indonesia, Taiwan, China, Thailand, Sri Lanka, Philippines, Pakistan, Malaysia, Cambodia and also from Australia, Central America and Latin America.

In India this disease was first reported from Maharashtra (Sreenivasan et al., 1959). Kaul (1959) stated that during August 1956 rice in Jammu Province was affected by bacterial leaf blight. An epidemic of bacterial leaf blight disease of rice broke out in the Shahabad district of Bihar in 1963 (Srivastava and Rao, 1963). Pavgi et al. (1964) reported that this disease was present in most rice fields in the Varanasi and neighbouring areas. A 1964 survey indicated that it was prevalent in Uttar Pradesh, Bihar, West Bengal, Orissa, North Madras and Maharashtra (Srivastava and Rao, 1966).

In Japan yield losses in severely diseased fields ranged between 20 and 30 per cent (Mizukami and Wakimoto, 1969) Ou (1973) reported that in Japan, 300,000 to 400,000 hectares

were damaged by this disease annually.

In India, the damage estimated in Maharashtra was 22.7 per cent costing Rupees 541,510 (Anon., 1964). Based on observations under natural conditions in Andhra Pradesh, Srivastava et al. (1966) estimated that the losses in grain yield ranged between 6 and 60 per cent.

SYMPTOMATOLOGY

A comprehensive review of the symptoms was published by Srivastava and Rao (1966). The symptoms of the disease became visible one week after inoculation and were characterised by linear straw coloured lesions rarely on one and generally on both margins of the leaves starting from the tip downwards. This was followed by drying of the leaf tip, inward rolling and twisting (somewhat spiral) of the infected portion of the leaf. The marginal blight extended rapidly to cover larger area of the leaf crosswise and lengthwise, usually leaving smaller greener areas in the centre, which also in course of time got blighted. In occasional cases, the linear stripes developed on the leaf lamina or along the midrib with or without the marginal stripes. These were yellowish in the beginning become straw coloured later. After blighting, the leaves unrolled and supported growth of sooty moulds. The disease extended to the leaf sheath and culms, killing the tiller or the whole clump. The glumes of the seeds were also infected, but the symptoms

were not well defined. The disease remained aggressive throughout the monsoon season at the end of which some new tillers were produced which were either mildly infected or remained green. Inoculations made on three to four weeks or older seedlings also produced the above symptoms, but seedlings younger than two weeks showed bleaching of the leaf tips followed by rapid wilting.

Singh and Sahsena (1968) described the symptoms of this disease. The disease affected the plant and caused leaf blighting at every stage of its development. Affected younger plants had poor root systems, less tillering and stunted growth. They either did not develop earheads or they developed fewer of them and contain less grain. Affected plants in fields with bad drainage and stagnant water usually collapsed completely. At flowering time, symptoms of this disease, particularly leaf blighting, manifested themselves so prominently. There was less area of leaf lamina in diseased leaves than in healthy leaves. The advancing edge of the marginal blight, which progressed rapidly to cover the larger area of the leaf crosswise and lengthwise, always showed wavy outline.

Ou (1973) reviewed the work of others and reported two additional types of symptoms occurring only in the tropics besides the leaf blight symptom. The kresek symptom characterised by withering of leaves and entire young plants, and the other symptom by the production of pale yellow leaves

in older plants.

Keku and Mori (1977) observed a browning reaction in rice plant tissues induced by Xanthomonas oryzae.

THE PATHOGEN

The causal organism was first described by Ishiyama (1922) who named it as Pseudomonas oryzae (Uyeda and Ishiyama). This was later changed to Bacterium oryzae (Uyeda and Ishiyama) Nakata and finally to Xanthomonas oryzae (Uyeda and Ishiyama) Dowson.

Properties of the pathogen

Ishiyama (1922) reported that the bacterium was a gram negative rod measuring 0.5 - 0.8 x 1-2 µm, motile with a single polar flagellum, capsulated and non spore forming.

Breed et al. (1957) reported that the bacterium was a gram negative rod measuring 0.5 - 0.8 x 1.0 - 2.0 µm, motile with a single polar flagellum. The colonies produced on nutrient agar were circular, smooth, glistening and wax-yellow. Optimum temperature for the growth of the bacterium ranged between 26°C and 30°C. It was strictly aerobic. It neither liquified gelatin nor hydrolysed starch. Slightly acidic reaction was observed in milk. The organism did not produce nitrites from nitrates. Produced hydrogen sulphide but no gas from glucose, lactose and sucrose.

A study on the physiological characters on rice bacterial blight pathogen in the Philippines showed that

starch was hydrolysed by the pathogen to a slight extent and gelatin was liquified(Fordesino, 1958).

Watanabe (1963) reported that physiologically the bacterium was more or less similar in general characteristics to other members of the genus except that it needed glutamic acid or cysteine as a nitrogen source, and did not utilise inorganic nitrogen.

Sulaiman and Ahmed (1965) reported that the organism was a short rod measuring 0.8 to 1.0 µm, motile by a single polar flagellum, gram negative, non acid fast and non spore former. It grew well only on protease peptone dextrose agar and its broth and also in yeast glucose chalk agar. The colonies were circular with entire margin, butyrous, bright and yellow in colour. The organism did not liquify gelatin, neither hydrolysed starch nor digested casein. Moreover, it did not produce nitrites, hydrogen sulphide and indole but changed litmus milk to pink with peptonisation. It produced faint acid without gas from glucose, starch and glycerol. It made moderate growth on potassium nitrate. The optimum temperature for the growth was 28-30°C and thermal death point was 52-53°C.

Reddy (1966) made a comparative study of Xanthomonas oryzae isolates occurring in India and reported that isolates from rice varieties T 141 and PTB 10 were morphologically and culturally similar to the original isolate of Xanthomonas oryzae but differed from other isolates in their ability to

hydrolyse starch and inability to reduce nitrate indicating that different strains of Xanthomonas oryzae occurred in India.

Chakravarti and Rangarajan (1967) studied the morphological, cultural, physiological and biochemical characters of the bacterium and reported that the organism was a gram negative short rod measuring 0.5 - 0.9 µm x 1.0 - 2.0 µm, motile with a single polar flagellum, encapsulated and non spore forming. Colonies on nutrient agar were yellow, smooth, slimy, circular and the margins were entire. In nutrient broth, yellow growth was observed with sedimentation and turbidity. On a potato plug copious slime was observed. Abundant slime was observed on slants of nutrient agar, potato dextrose agar and yeast extract dextrose agar media. The yellow pigment produced was soluble in alcohol but not in water. Optimum temperature for the growth of the bacterium was 28-30°C. The bacterium was strictly aerobic. It exhibited oxidative metabolism of glucose, produced catalase and liquified gelatin. Starch hydrolysis was positive and litmus milk was reduced turning the medium blue without coagulation or peptonization. Hydrogen sulphide and ammonia were produced from peptone water. Tests for indole, lipolytic activity and Methyl Red and the Voges-Proskauer test were negative. It produced acid but no gas from glucose, galactose, sucrose, xylose, maltose and lactose. Slight growth but no acid or gas was observed in inositol, glycerol, mannitol and sorbitol. Neither growth nor acid and gas were seen in

esculin and aesculin. The bacterium utilized the sodium salts of acetic acid and citric acid. Growth was observed in an inorganic basal medium containing different nitrogen sources. It did not reduce nitrates to nitrites. Neither urease nor tyrosinase was produced by the bacterium. It acted on uric acid, producing zones around the colonies within four days. It liquified potato extract gel containing two per cent carboxy methyl cellulose indicating the production of Cx-cellulose. Sodium polypectate gel was not liquified.

Mizuta (1953) studied the cultural characters of the bacterium and reported that the bacterium grew best on bouillon-glucose and potato sucrose agar at pH 4 - 8.8. Satisfactory growth was also observed with maltose as the sugar. According to Mizukami (1961) the optimum conditions for the growth of the bacterium in a potato artificial medium were pH 6.8 and temperature 20°C in the lag phase and 26-30°C in the growth phase and by the addition of $FeSO_4$ growth was increased.

SURVIVAL OF THE PATHOGEN IN SEED, PLANT DEBRIS AND SOIL

Several workers had demonstrated the seed infection and survival of the bacterium in the seeds for various lengths of time upto the next sowing season (Goto et al., 1953; Nakimoto, 1955; Seki and Mizukami, 1955; Fang et al., 1956; Srivastava and Rao, 1963; Yoshimura and Fagemi, 1967; Chakravarti and Rangarajan, 1968; Singh, 1972). Nakimoto (1954) reported that Xanthomonas oryzae survived on unhulled

grain for only 30 days. According to Fang et al. (1956) infected seeds usually produced diseased seedlings. Srivastava and Rao (1964a, 1964b, 1968) reported success in seed transmission both under laboratory and field conditions. According to Chattopadhyay and Mukherjee (1971) tests with seeds from naturally infected and apparently healthy plots showed that Xanthomonas oryzae survived 30-160 days after harvest, depending on the variety. No relationship was found between seed moisture content and survival of the pathogen. In a report by Iseka (1970) a conclusive evidence on the transmission of the disease through infected seeds was available. In an elaborate field experiment, Rao et al. (1971) had shown that infected seeds could lead to a diseased crop.

Attempts by some other workers (Ou and Ramchit, 1970) to demonstrate the transmission of the disease through infected seeds had not been successful. Kauffman and Reddy (1975) reported that viable bacteria occurred on infected seeds stored under natural conditions only for a period of two months, but after this time no bacteria could be detected by three different methods. Seed transmission was not observed in freshly harvested infected seeds grown under various conditions.

Hashioka (1951) observed that in the South East of Japan bacterial leaf blight infection came from the seed beds where the causal bacteria overwinter in great numbers in the

soil from any heavily infected preceding rice crop. Wakimoto (1956) reported that in Kyushu, Japan, Xanthomonas oryzae could overwinter not only in grain or straw under shelter, but also in the soil around the weed Leersia oryzoides var. japonica.

Singh (1971a) reported that Xanthomonas oryzae did not survive in unsterilized soil for a week or oversummer in the field, in manure or in compost which were therefore unlikely to be sources of infection for the next crop. In West Bengal the bacterium Xanthomonas oryzae survived for seven days in unsterilized soil and upto 30 days in sterilized soil (Chattopadhyay and Mukherjee, 1974). They also reported that pure cultures lasted longer than leaf inoculum in the soil. In general they survived in soil with 50 per cent moisture. According to Goto et al. (1953), Wakimoto (1956), Seki and Mizukami (1955), Inoue et al. (1957) and Yoshimura (1963) there was no positive evidence of perpetuation of the bacterium in soils of infected rice fields.

Several Japanese workers had reported perpetuation of the pathogen in the infected straw left over in the field or in the barn and also in the stubble (Ishiyama, 1928; Goto et al., 1953; Wakimoto, 1954; Wakimoto, 1956; Wakimoto and Temari, 1956; Inoue et al., 1957; Tagami et al., 1963; Yoshimura, 1963 ; Yoshimura and Tagami, 1967). Under the conditions in Northern India, these materials had not been found to serve as effective source of primary inoculum (Srivastava and Rao, 1968; Rao, 1970).

Chattopadhyay and Mukherjee (1975) reported that living post harvest rice plant tissues harboured a greater percentage of Xanthomonas oryzae infection (14.3 per cent) than dead tissues collected from harvested rice fields. They also reported that the residues from harvested crops provided inoculum for the following crop, particularly when two crops in a year were planted. Hsieh and Buddenhagen (1975) studied the survival of Xanthomonas oryzae in relation to substrate, temperature and humidity and reported that survival was longer at low than at higher relative humidity and temperatures, but higher temperatures with very low relative humidity also permitted long survival. They also reported that Xanthomonas oryzae survived for more than one month in warm (30°C), flooded or moist soil, or in leaves buried in rich soil. In ooze droplets, or in diseased leaves in air at warm temperatures, and 100 per cent relative humidity, the pathogen survived only 5 - 40 days. Lowering the relative humidity to 0 - 20 per cent lengthened such survival to almost one year. Nwigiwe (1975) reported that Xanthomonas oryzae survived for at least five days on the leaf surfaces of healthy susceptible rice cultivars JC-70 and IR 8. Tabei and Kanchit (1974) observed that bacteria liberated from infected rice stubble into irrigation water caused primary infection of bacterial leaf blight in Thailand.

Singh (1971b) reported that Xanthomonas oryzae survived in sterile tap water and distilled water for more than twelve

months, but only 15 and 30 days in field and pond water, respectively.

Mohiuddin et al. (1979) was able to demonstrate the survival of the bacterium in the wheat rhizosphere through the winter season.

EFFECT OF WEATHER FACTORS ON THE DISEASE INCIDENCE

Our present knowledge of the relationship of environment to the disease appeared to be based on field observations rather than on any critical studies under controlled conditions. Goto et al. (1955) reported that a combination of rainy weather, stormy winds and a temperature of 22-26°C favoured the outbreaks of bacterial leaf blight disease.

Sulaiman and Ahamed (1965) reported that the critical conditions for the disease were found in the month of July when the maximum temperature was 29°C, minimum temperature was 24°C, rainfall was 1394.6 mm and relative humidity 91 per cent. In the month of August, the disease found its cardinal when maximum temperature was 27.6°C, minimum temperature was 23.4°C, rainfall was 1547.7 mm and relative humidity 95 per cent. The optimal of the disease was found in the month of September when the maximum temperature was 30°C, minimum temperature was 23.4°C, rainfall was 50.7 mm and relative humidity 93 per cent. Adverse conditions for disease development occurred in the month of October when maximum temperature was 34°C, minimum temperature was 22.4°C,

rainfall 52 mm and relative humidity was 91 per cent.

Studies conducted at the International Rice Research Institute showed that 25-35°C were the most favourable temperatures for the development of the disease (Amon., 1974).

Disease epidemics were favoured by low temperature, high relative humidity, heavy rainfall and insufficient day light (Lee, 1975). From the investigations of Nath and Padmanabhan (1976) it was found that inoculated rice plants growing under continuous shade developed significantly longer lesions than those under full day light. Rangareddy et al. (1977) reported that growing plants under low light intensity enhanced the tissue susceptibility of both the resistant and susceptible rice cultivars to bacterial leaf blight disease caused by Xanthomonas oryzae.

Mohiuddin et al. (1977) related the incidence of bacterial leaf blight to the amount of rainfall. From seven years observations they concluded that when there were more than 27 rainy days during August, September and October (total rainfall at least 200 mm) the disease showed the wilt phase, but in other years only the blight phase was evident.

EVALUATION OF RICE VARIETIES AND CULTIVARS FOR DISEASE RESISTANCE

Varietal evaluation for disease resistance was attempted by many workers and a lot of information was available about the mode and pattern of inheritance of the disease. Varying degrees of success have been accomplished

by different workers in the varietal resistance of bacterial leaf blight.

Goto (1965) reported that of the 18 wild rice species tested all of them were susceptible to the virulent Philippine isolates of Xanthomonas oryzae and some were highly resistant to the less virulent isolates. Reddy (1965) observed considerable variation amongst 16 rice varieties in their susceptibility to Xanthomonas oryzae, W.529 being relatively resistant. Report from the International Rice Research Institute showed that of the 3,676 varieties screened, 26 proved highly resistant to a virulent isolate of Xanthomonas oryzae (Anon., 1966). Srivastava et al. (1967) reported that of the 128 rice varieties tested with a mixed inoculum of five virulent isolates of the pathogen, only thirteen, including Tainan-3 and Chainung-242 were proved resistant and 11 including Taichung-65 were proved moderately resistant. Of the 109 exotic and indigenous rice varieties screened in two samples (50 in first sample and 59 in second sample) against Xanthomonas oryzae, seven in each sample were found resistant (Chattopadhyay et al., 1968).

Rice varieties IR 20 and IR 22 were more resistant than IR 8 to bacterial leaf blight (Anon., 1970). But later it was reported from the International Rice Research Institute that a virulent strain of Xanthomonas oryzae broke down the resistance of IR 20 and IR 26 (Anon., 1973). Pathak et al. (1973) also reported that IR 20 had varying degrees of

susceptibility to Xanthomonas oryzae.

Chien and Hung (1970) observed that of the 70 rice varieties tested against Xanthomonas oryzae none of them were either highly resistant or highly susceptible, thirteen were resistant (1-15 per cent infection) but most of them showed medium resistance (16-35 per cent infection). All the resistant varieties were japonica types and all the most susceptible varieties were indica types except Kaohsiungyu 637. Mahmood and Singh (1970) reported that when rice varieties were screened against the Xanthomonas oryzae isolates obtained from Sabour and Udaipur Taichung (Native)1, T65 and Padma were proved highly susceptible, IR 8, IR 5, IR 48 and CH-10 were proved moderately susceptible and BR 7 and N 136 were resistant.

When more than 8700 rice varieties were screened for resistance against the Philippine strains of bacterial blight pathogen, most of them were found to be susceptible and none of them were immune (Ou et al., 1971).

Ezuka and Horino (1976) reported that all the plants of the cultivar Wase Aikoku 3 were resistant and all those of Kinmaze were susceptible when inoculated either by cut and spray inoculation method and spray inoculation method or by the needle prick technique. Of the 6256 rice varieties from germ plasm collection when screened for resistance against the bacterial blight pathogen at the International Rice Research Institute about 555 entries were rated as

resistant and 124 were moderately resistant (Anon.,1957). John et al. (1970) screened 335 rice cultivar against Xanthomonas oryzae, of which, seven were proved resistant to the pathogen.

Ou et al. (1971) observed that varieties resistant at the seedling stage were also resistant at the flag leaf stage. Rao and Reddy (1975) compared the nursery screening with adult plant reactions and suggested that screening should be done on adult plants around the heading stage when the disease was more pronounced.

CONTROL

In vitro evaluation of different bacteriocides against the pathogen

Many chemicals including several antibiotic compounds had been tried for the control of the disease from time to time.

Wakimoto and Mukoo (1963) reported that chloramphenicol was best for disease control although 15 days incubation with 100 ppm was not completely lethal to the pathogen.

Okimoto and Misato (1963) reported that cellocidin was effective against Xanthomonas oryzae completely inhibiting the growth on shake cultures at four ppm when added six hours after inoculation.

According to Sekizawa et al. (1965) and Oda et al. (1966) phenazine derivatives both natural and synthetic were

active against Xanthomonas oryzae.

Suarup et al. (1965) reported that Penicillin G (100 ppm), Dithane M-22 (2000 ppm) and $HgCl_2$ (1000 ppm) gave maximum inhibition against Xanthomonas oryzae.

Studies on the bacteriostatic and bactericidal action of Streptocycline on Xanthomonas oryzae revealed that the antibiotic was inhibitory to the three isolates of the pathogen at 25-50 ppm and lethal at 50-100 ppm when incubated for 8-48 hour (Raj and Moniz, 1967; Shetty and Ranganswami, 1968). But Pal and Das (1968) observed that Streptocycline at three or four gram could not completely check the bacterial growth.

Raj and Moniz (1967) reported that among the seven fungicides tested against Xanthomonas oryzae, Shillagene gave the best control. Pal and Das (1968) observed that bacterial growth was completely checked by Agrimycin at 15 g/112 litre water. Jayaraman et al. (1970) reported that an erythromycin like antibiotic produced by Streptomyces griseoplanus was highly inhibitory to several bacterial plant pathogens including Xanthomonas oryzae. Kuseka (1971) observed that growth of Xanthomonas oryzae was inhibited at all stages by the antibiotic Aristeromycin. Yoneyama and Misato (1971) reported that growth of Xanthomonas oryzae in liquid medium was completely suppressed by 50 µg/ml of the fungicide (Na DNDO) when added immediately after inoculation and at midlog growth by about 50 per cent at 100 µg/ml.

According to Mondal and Mukherjee (1975) mercuric chloride, mercuric iodide, cobalt and cadmium nitrates were toxic to Xanthomonas oryzae and Xanthomonas citri in vitro at 0.01 µg. Some lead and organic compounds were effective only at higher concentrations.

Inderawati and Heitefuss (1977) observed that on agar media containing 10 µg/ml commercial formulation of propenil the growth of Xanthomonas oryzae was reduced to 50 per cent of the control. In vitro screening of antibiotics (500 ppm) and other drugs (500 ppm) had shown that antibiotics such as tetracyclines, ampicillin, streptomycin and novobiocin and drugs such as sulfacetamide, sulfadiazine, sulfadimidine were of promise against Xanthomonas oryzae (Mondal and Mukherjee, 1978). Nishikiori et al. (1978) reported that a polyene antibiotic aurantinum (KM-214) was active in vitro against gram positive bacteria and Xanthomonas oryzae.

In vivo evaluation of different bactericides against the pathogen

Sumina et al. (1963) tried 'folimycin' an antifungal antibiotic against Xanthomonas oryzae and found that it failed to control the bacterial blight of paddy.

Jain et al. (1965, 1966) reported that spraying with Coppesan (Cu oxychloride) at 2.0 kg/ha gave maximum protection and reduced infection of bacterial leaf blight, though the reduction was not reflected in increased yield. Verma et al. (1980) reported that Dithane C-90 and Fytolan

both at 0.3 per cent concentration reduced bacterial leaf blight.

Sekizawa et al. (1965) observed that on potted rice plants, leaf blight was controlled 93 per cent by 200 ppm phenazine and 83 per cent by 150 ppm. Oda et al. (1966) also reported that among the synthetic and natural derivatives of phenazine, phenazine 5-oxide was the most effective on potted rice plants against bacterial leaf blight.

Jain et al. (1966), Singh (1968), Padmanabhan (1969), Rajagopalan et al. (1969) and Jain (1970) reported Streptocycline to be effective against bacterial blight but Devdath and Prem Latha Dath (1970) and Verma et al. (1980) reported the same to be ineffective.

Padmanabhan and Jain (1966) and Chakraberti and Mathur (1970) found that chlorine at one ppm (2 kg bleaching powder/hectare) in the irrigation water gave good control of Xanthomonas oryzae. Okimoto (1968) reported that Xanthomonas oryzae was effectively controlled by sprays of cellocidin at 200 ppm.

Singh (1968) reported that Agrimycin-17 (1:2000) in water lowered Xanthomonas oryzae incidence on rice.

From the investigations with 48 chemicals Watanabe et al. (1970) reported that the best control of Xanthomonas oryzae was given by TF-128 three per cent dust in the irrigation water at 4 kg/10 acres, reducing losses in unhulled rice from 13 per cent to 5.6 per cent. The protective effect was

retained for more than two weeks.

Control of bacterial leaf blight by streptomycin sulphate was reported by Reddy and Reddy (1971).

Yokushiji and Wakao (1971) reported that 2 amino-1, 3,4-thiadiazole (ATDA) was effective against the disease when applied as a foliar spray or to irrigation water.

Ohmori et al. (1976a,b) reported that at extremely low dosage 1-methyl thio semi carbazide controlled Xanthomonas oryzae when applied to the roots, as a preventive or curative. It showed no control effect as a foliar spray and no activity against Xanthomonas oryzae on agar medium.

Inderawati and Heitefuss (1977) studied the effect of Simetryn and Nitrofen on disease severity and reported stronger activity than expected from the small direct action on the pathogen in culture.

From a field experiment conducted during kharif 1971 using six chemicals, Krishnappa and Singh (1978) reported that TF-130 gave maximum control followed by Agrimycin-500 both in reducing incidence and development of the disease.

MATERIALS AND METHODS

MATERIALS AND METHODS

ISOLATION AND PATHOGENICITY OF THE BACTERIUM

The bacterial isolate used in the present study was supplied by the Chairman of the Advisory Committee. The original isolate was collected from the severely infected rice fields of the Rice Research Station, Moncompu, in Alleppey District during 1976. The culture was inoculated on rice plants and infected leaves were subjected to ooze test (Srivastava and Rao, 1966) to find out the presence of bacteria. The diseased leaves with profuse ooze were selected, the infected areas were cut into bits, surface sterilised with 0.1 per cent mercuric chloride solution for about one minute. These bits were then washed in three changes of sterile distilled water. These were then placed on a sterile glass slide in a few drops of sterile distilled water and teased apart in order to get a bacterial suspension. The suspension was streaked on Potato Sucrose Peptone Agar medium to get well isolated colonies of the bacterium.

Composition of PSPA medium

KH_2PO_4	-	0.2 g
Na_2HPO_4	-	0.5 g
$Ca(NO_3)_2$	-	0.5 g
$FeSO_4$	-	0.05 g
KCl	-	0.05 g

Peptone	-	2.0 g
Sucrose	-	20.0 g
Potato	-	300.0 g
Agar agar	-	20.0 g
Distilled water	-	1000 ml
pH	-	7.0

The inoculated plates were incubated at room temperature for 48 hours. Characteristic single colonies were selected on the basis of their colour, fluidity and slime. The culture was purified by repeated streaking on PSPA medium. Pathogenicity of the isolate was tested by artificial inoculation. Clipping off the tips of leaves with scissors dipped in suspension of a 24 h old culture of the bacterium and also spraying the plants with the suspension. The inoculated plants were then kept under high humidity condition by covering with a polythene bag for disease development. The pathogen was reisolated from artificially inoculated plants using the above isolation procedure and was compared with that of the original isolate.

Stock cultures were maintained in Yeast extract Glucose Chalk Agar (YGCA) medium under sterile mineral oil. Only one isolate of the bacterium was used in these studies.

SYMPTOMATOLOGY

Symptoms of the disease were studied on artificially inoculated plants.

CHARACTERISATION OF THE PATHOGEN

The pathogen was characterised following the methods recommended in the Manual of Microbiological methods, published by the Society of American Bacteriologists (Anon., 1957), and the methods prescribed by Dye (1962) with modifications.

A. Cultural characters

(1) Morphology

Colony characters and cell morphology were studied from a 48 h old culture of the bacterium grown on PSPA medium. The cells were also stained for gram reaction.

(2) Growth of the bacterium on different solid media

Nature of growth, colour, size, shape, extent of growth, type of margin, slime production and fluidity of the bacterial colonies were studied on eight different solid media. A loopful of the dilute suspension of the bacterium was streaked on different media in triplicate and kept for incubation at room temperature. Observations were made after 24 h, 48 h, 72 h and 96 h of incubation. The following media were used.

1. Potato Sucrose Peptone Agar (PSPA)
2. Nutrient Agar (NA)
3. Basal medium for Xanthomonads (BX)
4. Tetrazolium chloride medium (without tetrazolium chloride) TTC

5. Potato Dextrose Agar (PDA)
6. Yeast extract Glucose Chalk Agar (YGCA)
7. Glucose Agar (GA)
8. Glucose Yeast extract Agar (GYA)

Composition of the media

1. Potato Sucrose Peptone Agar (Given above)
2. Nutrient Agar

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

3. Basal medium for Xanthomonads

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

4. Tetrazolium Chloride Agar

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

Agar agar - 20.0 g

Distilled water - 1000 ml

pH - 6.8

5. Potato Dextrose Agar

Dextrose - 20.0 g

Potato - 200.0 g

Agar agar - 20.0 g

Distilled water - 1000 ml

pH - 6.8

6. Yeast extract Glucose Chalk Agar

Yeast extract - 10.0 g

Glucose - 10.0 g

Chalk ($CaCO_3$) - 20.0 g

Agar agar - 20.0 g

Distilled water - 1000 ml

7. Glucose Agar

Beef extract - 5.0 g

Peptone - 5.0 g

Glucose - 10.0 g

Agar agar - 20.0 g

Distilled water - 1000 ml

pH - 6.8

8. Glucose Yeast extract Agar

Yeast extract - 5.0 g

Peptone - 5.0 g

Glucose	-	10.0 g
Agar agar	-	20.0 g
Distilled water	-	1000 ml
pH	-	6.8

3. Growth of the bacterium in different liquid media

For studying the growth of the bacterium in different liquid media, broth of the above mentioned eight media were used. Fifty ml sterilized broth of each medium taken in 100 ml pyrex conical flasks was inoculated with one ml of 24 h old bacterial suspension (with O.D. 0.60) grown on PSPA medium using a sterilized pipette. Tests were done in triplicates. Uninoculated controls were also maintained. The inoculated media were shaken daily. Optical density of the broth culture was measured after 24 h, 48 h and 72 h using a spectro - photometer (Spectronic 20 Bausch & Lomb) at 510 wave length. Uninoculated broth of the respective media were used as blank.

4. Pigment production

Production of non water soluble and water soluble pigments were tested in Yeast extract Glucose Chalk Agar medium and King's medium respectively. 48 h old culture was used for this purpose. The tests were performed in triplicate and observations were recorded periodically.

Composition of Yeast extract Glucose Chalk Agar medium

Yeast extract	-	10.0 g
Glucose	-	10.0 g

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

Composition of King's medium

Peptone	-	20.0 g
Glycerine	-	10 ml
K_2HPO_4	-	1.5 g
$MgSO_4.7H_2O$	-	1.5 g
Agar agar	-	20.0 g
Distilled water	-	1000 ml
pH	-	7.2

5. Oxygen requirement

To determine whether the bacterium was aerobic or anaerobic, Nutrient Agar (containing 0.005 per cent bromocresol purple) columns in tubes were inoculated in duplicate by stabbing a straight inoculation needle charged with the culture of the bacterium. The agar surface in one tube was covered with sterile paraffin oil to a depth of one cm. The tubes were incubated at room temperature and observations were recorded.

B. Physiological characters

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

1. Mode of utilisation of glucose

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

Basal medium

Peptone	-	1.0 g
$NH_4H_2PO_4$	-	1.0 g
KCl	-	0.2 g
$MgSO_4.7\ H_2O$	-	0.2 g
Bromothymol blue	-	0.03 g
Agar agar	-	3.0 g
Distilled water	-	1000 ml
pH	-	7.0

To the above medium one per cent glucose was added. The medium was dispensed in tubes upto four cm and sterilized by tyndallization and inoculated in duplicate by stabbing with a straight inoculation needle charged with bacterial growth. In one of the tubes, the medium was sealed with one cm layer of sterile liquid paraffin. The tubes were incubated at room temperature and observations were taken at regular intervals upto fifteen days.

2. Utilization of organic acids

Sodium salts of four organic acids: sodium acetate, sodium benzoate, sodium citrate and sodium formate were used for this study. One per cent of the sodium salt of organic

acids was added to the Basal medium for Xanthomonads with bromothymol blue as indicator. Slants were inoculated in triplicate with the bacterium and incubated at room temperature. Uninoculated controls were also maintained. Observations were recorded at regular intervals.

3. Starch hydrolysis

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

Composition of the medium

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

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

4. Production of hydrogen sulphide

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

Composition of the medium

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

Five ml quantities of the medium was dispensed in test tubes and autoclaved. Lead acetate paper strips of 5 x 50 mm size were prepared by soaking them in supersaturated solutions of lead acetate. The strips were dried, autoclaved and again dried. The tubes were inoculated in triplicate with 24 h old culture of the bacterium and lead acetate strips were inserted aseptically between the plug and innerwall of the tube hanging just above the broth. The tubes were incubated at room temperature and observations were recorded upto 14 days at regular intervals. Blackening of the lead acetate impregnated strips indicated liberation of hydrogen sulphide.

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

Methyl Red broth was used for both the tests.

Composition of Methyl Red broth

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

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

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

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

6. Gelatin liquefaction

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

Composition of the nutrient gelatin medium

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

Gelatin was mixed together with all the other ingredients and heated over a water bath until the gelatin

was dissolved. The medium was dispensed in test tubes to a depth of about four cm and sterilized at 10 lb pressure for 20 minutes. The sterile condition of the medium was checked by observing it for two days. Inoculated these properly sterilized gelatin columns by stabbing a straight inoculation needle charged with 48 h old culture of the bacterium. The tubes were incubated and observed for the liquifection of the gel column at regular intervals upto one month.

7. Production of indole

Tryptophan broth medium was used for this test.

Composition of the medium

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

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

The tryptophan broth tubes were inoculated with the bacterium in triplicate and oxalic acid strips were inserted into the tube by the side of the plug, incubated and observed regularly for 14 days. Change in colour of the oxalic acid

crystals on test strip to pink or red indicated indole production.

8. Nitrate reduction test

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

Composition of nitrate broth

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

The medium was dispensed in tubes, autoclaved and inoculated with the bacterium, incubated and tested for reduction of nitrate at regular intervals upto 15 days. The tests were performed by adding few drops of sulphanillic acid (0.8 per cent in 5 molar acetic acid) and dimethyl alpha-napthyl amine (0.5 per cent in 5 molar acetic acid), to the nitrate broth culture. If no pink or red colour developed it indicated that nitrate was present as such or reduced to ammonia and free nitrogen. Few zinc crystals were added to observe whether the negative reaction was due to the reduction of nitrate beyond the nitrite level. If the broth became pink or red it indicated that the nitrate was present without reduction.

9. Urease production

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

Composition of the medium

Peptone	-	1.0 g.
NaCl	-	5.0 g
KH_2PO_4	-	2.0 g
Glucose	-	1.0 g
Agar agar	-	20.0 g
Phenol red		
(0.2 per cent solution)	-	6.0 ml
Distilled water	-	1000 ml
pH	-	6.8

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

10. Catalase test

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

11. Action on milk

Both unskimmed and skimmed milk was used in this test. A 1:3 dilution of skimmed milk was prepared in water and bromocresol purple was added to give a final concentration of 0.002 per cent, when a light blue colour was obtained (Clark and Lubs, 1917). Unskimmed milk (containing approximately three per cent butter fat) was also diluted with water and bromo-cresol purple was added as above. The milk medium was then dispensed in five ml aliquots in test tubes and sterilized by steaming for 30 minutes on three successive days in Arnold steam steriliser. The medium was inoculated with a loopful of 48 h old test bacteria and incubated. Observations were recorded for 30 days at regular intervals for acidic or alkaline reaction, curdling and peptonization. Change of the light blue colour of the medium to yellow indicated acid reaction and to violet indicated reaction to be alkaline. Curdling was indicated by the heterogenous clumps formed due to precipitation of casein. Peptonization was indicated by partial clearing of the milk. Uninoculated control was kept as a reference.

12. Utilization of asparagine as sole source of carbon and nitrogen

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

Solution 1.	K_2HPO_4	- 8.0 g
	KH_2PO_4	- 2.0 g
	Distilled water	- 100 ml

Solution 2.	$MgSO_4.7\ H_2O$	-	2.0 g
	$FeSO_4$	-	0.5 g
	NaCl	-	1.0 g
	$MnSO_4$	-	0.02 g
	H_2SO_4	-	1 drop
	Distilled water	-	100 ml
Solution 3.	Na_2MoO_4	-	0.02 g
	Distilled water	-	100 ml
Solution 4.	$CuSO_4$ saturated solution in distilled water.		

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

15. Growth at six per cent sodium chloride

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

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

The medium was dispensed in tubes, autoclaved and inoculated with 24 h old culture of the bacterium, incubated and observations recorded.

14. Lipolytic activity

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

Composition of the medium

Peptone	-	10.0 g
NaCl	-	5.0 g
$CaCl_2.1H_2O$	-	0.1 g
Agar agar	-	20.0 g
Distilled water	-	1000 ml
pH	-	7.0

The medium was dispensed in 99 ml quantities in flasks autoclaved and cooled to 45°C. One ml of Tween-80 (Oleic acid ester) was added to the medium and thoroughly mixed. The medium was poured in sterile petri dishes and test bacterium was spot inoculated on the medium. The plates were incubated and observed at regular intervals for 15 days. Opaque zone around the bacterial growth was indicative of positive lipase production.

15. Tyrosinase activity

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

Composition of the medium

$NH_4 H_2PO_4$	-	0.5 g
K_2HPO_4	-	0.5 g
$MgSO_4.7 H_2O$	-	0.2 g

NaCl	-	5.0 g
Yeast extract	-	5.0 g
Tyrosine	-	0.5 g
Agar agar	-	20.0 g
Distilled water	-	1000 ml
pH	-	6.8 - 7.0

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

16. Arginine hydrolase test

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

Composition of the medium

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

The medium was dispensed in five ml quantities in test tubes and autoclaved. The tubes were stab inoculated with 48 h old culture of the bacterium and covered with

sterile liquid paraffin to a depth of one centimeter. Incubated for seven days and observed daily. A change of the colour of the medium to red indicated arginine hydrolase activity.

17. Production of ammonia

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

Composition of the medium

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

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

18. Utilization of carbon sources

The following 17 carbon compounds were tested individually for utilization by the bacterium as indicated by acid production (Dye, 1962). Fructose, lactose, mannose, cellulose, sorbose, maltose, inositol, galactose, xylose, gentiobiose, salicin, amygdalin, dulcitol, glucose, starch, cellobiose and sucrose.

The production of acid was observed by using agar slants of the Basal medium for Xanthomonads (Dye, 1962). The carbon compound to be tested was added to the medium at one per cent concentration and 0.7 ml of five per cent alcoholic solution of bromocresol purple to get a reddish violet colour. The medium was sterilized by tyndallization and the slants were inoculated with 24 h old culture of the bacterium in triplicates and incubated at room temperature. Periodic observations were recorded upto 30 days. The change in colour of the medium from reddish violet to yellow indicated the production of acid.

19. Hypersensitivity reaction on tobacco leaves

Dilute suspension of the bacterial growth was inoculated into the leaves of tobacco (Nicotiana tobaccum) plant using a hypodermic needle (Klement, 1963). Observations were recorded for formation of necrotic spots after 24 h upto a period of 72 h.

Survival of the pathogen

Detailed studies were conducted on the survival of the pathogen in seed, plant debris and soil.

1. Survival in seeds

Paddy seeds of the variety Jyothi, artificially inoculated by dipping in a thick bacterial suspension with 5×10^9 cells/ml for about 12 h, drained and dried under shade was used in this study. Seeds were stored under

laboratory conditions. Isolations of both husked and unhusked seeds were made on PSPA medium at an interval of 15 days by direct plating of surface sterilized and unsterilized seeds and observations were taken for a period of four months.

2. Survival in plant debris

Survival of the pathogen in infected plant debris and crop refuse in soil was also assessed. The diseased material was chopped into small pieces. Pots were filled three-fourths with soil from paddy field. A one inch layer of chopped material was spread over the soil in pots, which was again covered with a one inch layer of soil. The soil in pots were not allowed to dry. Treated disease free Jyothi seeds were sown in these pots at weekly intervals and observed for the development of disease symptoms.

In vitro studies on the survival of the pathogen in infected leaves were also conducted. Weekly isolations of the infected leaf sample kept in fridge and under laboratory conditions were made for a period of three months.

3. Survival in soil

In vitro studies were conducted on the survival of pathogen in unsterilized soil. Periodic isolations of the bacterium from sick soil were made on the special media for Xanthomonas (Kado and Heskett, 1970).

Composition of the medium

Cellobiose	-	10.0 g
K_2HPO_4	-	3.0 g
NaH_2PO_4	-	1.0 g
$MgSO_4.7H_2O$	-	0.3 g
Agar agar	-	15.0 g
Distilled water	-	1000 ml

Ten gram of peddy soil was added to 0.1 ml of dilute suspension of 24 h old growth of the bacterium. The soil slurry was diluted with 9.0 ml of normal saline, then mixed mechanically for ten minutes. After seven days the suspension was diluted 1:10 in saline and 0.1 ml portions were plated on the medium in petri dishes, incubated and observed for the development of typical bacterial colonies.

In vitro studies on the survival of the bacterium in sterilized soil was also conducted using special media for Xanthomonas.

In vivo studies were also conducted on the survival of the bacterium in soil. Forty eight hour old shake culture of the bacterium was made in Potato Peptone broth. Hundred ml aliquots were mixed with the top one inch layer of the rice soil in pots. The pots were kept in the open field under natural conditions. Weekly sowing was done in these pots and observations were taken at regular intervals upto a period of three months.

EFFECT OF WEATHER FACTORS ON THE DISEASE INCIDENCE AND DEVELOPMENT

Recording of weather data

The maximum and minimum temperatures and humidity were recorded using a Heavy Duty Whirling Hygrometer and the rainfall using a raingauge from the month of August 1979 to the end of July 1980.

Recording of disease development

Every month twenty day old Jyothi seedlings were artificially inoculated with 24 h old culture of the bacterium and kept under field conditions. Observations on the lesion lengthwere taken at the end of each month by measuring the length of lesion developed on each leaf and making an average.

EVALUATION OF RICE VARIETIES AND CULTIVARS FOR DISEASE RESISTANCE

The seeds of fifty high yielding and popular rice varieties collected from Rice Research Station, Pattambi and their reaction to infection with Xanthomonas oryzae was studied at the nursery and adult plant stage. They were Taichung (Native) 1, Satya, Suhasini, Soorya, Sona, Hema, Vijaya, Veni, Bhadra, Bluebonnet, Malinga, Sabari, Basumathi, IR 5, Rajeswari, PR 156, IR 32, IR 26, IR 20, IR 28, Jaya, Dee Geo Woo Gen, Bharathi, Ratna, Suma, Kumar, Pennai, Sakthi, Anupama, Pusa 33, Parijath, Rajendra, Krishna, Aswathi, Bhawani, H_4,

Cauvery, Kalinga I, Kalinga II, Karuna, Rohini, Bala, IR 8, Supriya, Kanchi, Triveni, Madhu, Padma and Annapoorna. The experiment (pot culture) was laid out in completely randomised design with eight replications.

Plants at seedling stage (20 day old) and at the maximum tillering stage were inoculated with a 24 h old culture of the bacterium (10^9 cell/ml). The technique employed for inoculation was the standard clipping method combined with spraying. The plants after inoculation were kept under high humidity conditions and were well irrigated to ensure humidity in the microenvironment of the plants.

Readings were made upto 15 days after inoculation. Those varieties which did not show blight symptoms were again inoculated and observed. The disease intensity was graded using the standard scoring system developed by the International Rice Research Institute, Philippines (Anon., 1976).

IN VITRO SENSITIVITY OF THE BACTERIUM TO ANTIBIOTICS

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

1. Agrimycin-100 — Manufactured in India by Pfizer Limited, Thane-Belapur Road, Thane (Streptomycin 15 per cent + Terramycin 1.5 per cent).

2.	Ambistryn-S	Sarabhai Chemicals, Baroda (Streptomycin sulphate I.P.)
3.	Ampicillin	Ranbaxy Lab. Ltd., New Delhi (Ampicillin trihydrate).
4.	Chloromycetin	Parke Davis (India) Limited, Saki Naka, Bombay (Chloramphenicol and sodium succinate).
5.	Penicillin	Alembic Chemical Works Co. Ltd., Baroda (Fortified procaine penicillin injection I.P.
6.	Panchomycin	Panchak Ltd. Subsidiary of Alembic Chemical Works Co. Ltd. Alembic Road, Baroda (Streptomycin + Oxytetracycline).
7.	Streptocycline	Hindustan Antibiotics Ltd., Pimpri, Poona, India (Streptomycin 12 per cent + Chlorotetracycline hydrochloride 1.5 per cent).
8.	Terramycin	Pfizer Ltd., Thane-Belapur Road, Thane (Oxytetracycline Hydrochloride).
9.	Tetracycline	Indian drugs and Pharmaceuticals Ltd., New Delhi (Tetracycline hydrochloride I.P.).

Solutions of antibiotics were prepared at concentrations of 100, 250 and 500 ppm. Sterile filter paper discs of 10 mm diameter were dipped in the appropriate solutions and placed

over PSPA medium seeded with 46 h old culture of the bacterium. The test was conducted with three replications. Observations on the zone of inhibition were recorded after 48 hour.

CHEMICAL CONTROL OF BACTERIAL LEAF BLIGHT OF RICE USING ANTIBIOTIC SPRAYS

In order to assess the efficiency of antibiotics against the disease and their field performance, a pot culture experiment was laid out using completely randomised design with seven treatments and five replications. Rice variety Taichung (Native) 1, which was known to be highly susceptible to the bacterial leaf blight pathogen was selected for this study. The trial was conducted at the maximum tillering stage of the plants. All the plants were artificially inoculated with 24 h old bacterial suspension (10^9 cell/ml) by the standard clipping method combined with spraying.

Three antibiotics at 250 ppm concentration were used for spraying the infected plants. Pre and postinoculation treatments of the antibiotics were done separately. The details of the treatments are given below.

Treatments

Pre inoculation spray of the antibiotics	T_1	Agrimycin-100	- 250 ppm
	T_2	Terramycin	- 250 ppm
	T_3	Penicillin	- 250 ppm

Post inoculation spray of the antibiotics	T_4	Penicillin	- 250 ppm
	T_5	Terramycin	- 250 ppm
	T_6	Agrimycin-100	- 250 ppm
	T_7	Control	

The first three treatments were given eight days before inoculation (pre inoculation spray). Treatments 4, 5 and 6 were given on 8th and 16th day after inoculation (post inoculation spray). Control plants were sprayed with distilled water without antibiotics. Hand sprayer was used for spraying the plants.

Scoring of the disease was done using the disease scale prepared by the International Rice Research Institute, Philippines (Anon., 1976). Observations were recorded on the day of each spraying and eight days after the second post inoculation spraying.

STATISTICAL ANALYSIS

Data relating to different experiments were analysed statistically following the method of Snedecor and Cochran (1967). 'F' test was carried out by analysis of variance method and significant results were compared by working out the critical difference.

RESULTS

RESULTS

ISOLATION AND PATHOGENICITY OF THE BACTERIUM

Isolation of the bacteria on Potato Sucrose Peptone Agar media yielded yellow, circular and slimy colonies with entire margins. The artificially inoculated rice plants showed typical leaf blight symptoms seven to eight days after inoculation. Reisolation from such infected plants yielded colonies resembling the ones from the original isolate of the bacterium.

SYMPTOMATOLOGY

Initial symptoms started as watersoaked pale green linear lesions along the margins of the upper parts of leaf blades. The lesions enlarged and turned yellow within a few days followed by drying of leaf tips. Lesions appeared on one or both margins of the leaves. As the disease advanced, the lesions covered the entire leaf blade, usually leaving smaller green areas in the centre. The inner margin of the blighted patch in contact with the healthy portion of the leaf was wavy. In occassional cases, the linear lesions developed on the leaf lamina or along the midrib with or without marginal lesions. Theywere yellowish in the beginning became straw coloured later and supported growth of saprophytes (Plate I and II).

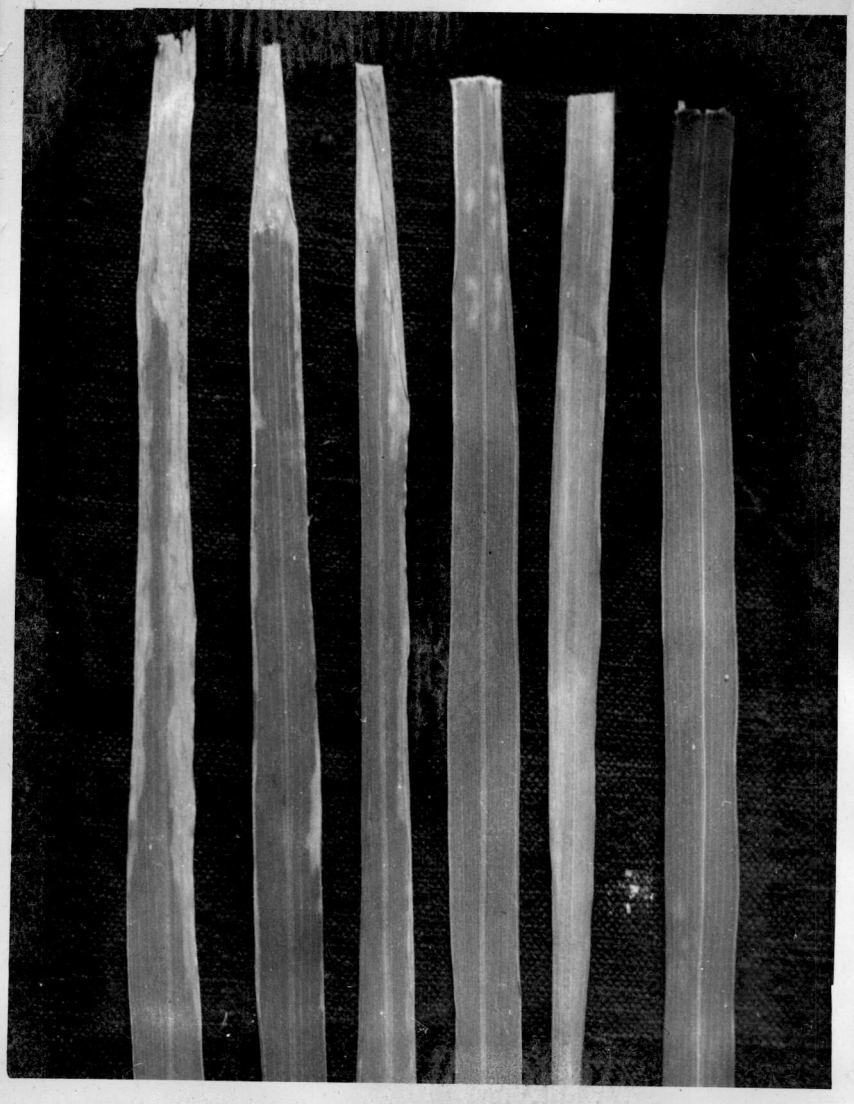

Plate I

Plate I & II. Foliar symptoms of bacterial leaf blight disease of rice

Plate II

CHARACTERISATION AND IDENTIFICATION OF THE PATHOGEN

A. Cultural characters

1. Morphology

The bacterium was a gram negative short rod. The bacterium gave rise to yellow, circular, slimy and convex colonies with entire margin on PSPA medium.

2. Growth of the bacterium on different solid media

The growth of the bacterium on eight different solid media were tested and the results are presented in table 1.

Among the eight different media, maximum growth was observed on GYA media after 96 h of incubation as indicated by the diameter of the colonies followed by GA, YGCA, PSPA, NA, EDA, TTC and BK. The maximum amount of sline was produced on GA medium closely followed by GYA, YGCA and PSPA. Least amount of growth, sline and fluidity were observed on Basal medium for Xanthomonads. The colonies produced on the eight different media were yellow, circular, smooth, slimy and convex with entire margins. In all the media except BK growth appeared in about 48 h of incubation and the colonies were moderately fluidal. The results of the study indicated that GYA and GA were the best solid media for culturing of this bacterium followed by YGCA and PSPA.

3. Growth of the bacterium in different liquid media

The growth of the bacterium in different liquid media was measured as the change in optical density of the medium in

Table 1. Comparison of growth charectere of Xanthomonas oryzae on different solid media.

Medium	Nature of colony and colour	Growth (Gr) slime (Sl) and fluidity (Fl)	Diameter of the bacterial colonies in mm after 24 h	48 h	72 h	96 h
PSPA	Yellow, circular, smooth and convex with entire margin	Gr + + + Sl + + + Fl + +	0	1.5	2.5	4.0
BX	Very small initials yellow, circular, smooth with entire margin	Gr + Sl + Fl +	0	0	0	0.25
TTC	Yellow, circular, smooth and convex with entire margin	Gr + + Sl + + Fl + +	0	1	1.6	3
GA	Yellow, circular, smooth and convex with entire margin	Gr + + + + Sl + + + + Fl + +	0	1.0	2.6	4.9
GYA	Yellow, circular, smooth and convex with entire margin	Gr + + + + Sl + + + Fl + +	0	1.5	3.1	5.5
YGCA	Yellow, circular, smooth and convex with entire margin	Gr + + + Sl + + + Fl + +	0	1.5	2.6	4.2
NA	Yellow, circular, smooth and convex with entire margin	Gr + + Sl + + Fl + +	0	1.2	2.3	3.4
PDA	Yellow, circular, smooth and convex with entire margin	Gr + + Sl + + Fl + +	0	1.0	1.9	3.1

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

comparison to control. The results are presented in table 2 and Fig.1.

Table 2. Comparison of growth of Xanthomonas oryzae in different liquid media after 24 h, 48 h and 72 h of incubation.

Medium	Optical density after		
	24 h	48 h	72 h
PSP broth	0.10	0.15	0.20
N broth	0.07	0.10	0.15
BX broth	0.05	0.09	0.13
TTC broth	0.06	0.09	0.14
PD broth	0.05	0.09	0.13
G broth	0.08	0.10	0.15
GY broth	0.10	0.12	0.17
YGC broth	0.15	0.20	0.22

After 72 h of incubation maximum growth was observed on YGC broth followed by PSP broth, GY broth, N broth, G broth, TTC broth, PD broth and BX broth. The amount of growth was almost equal in N broth and G broth. In PD broth and BX broth also the amount of growth was same. The increase in the amount of growth of the bacterium seemed to be directly proportional to the period of incubation for the first 72 h. The best liquid medium for the laboratory studies and mass culturing of the bacterium was YGC broth followed by PSP broth.

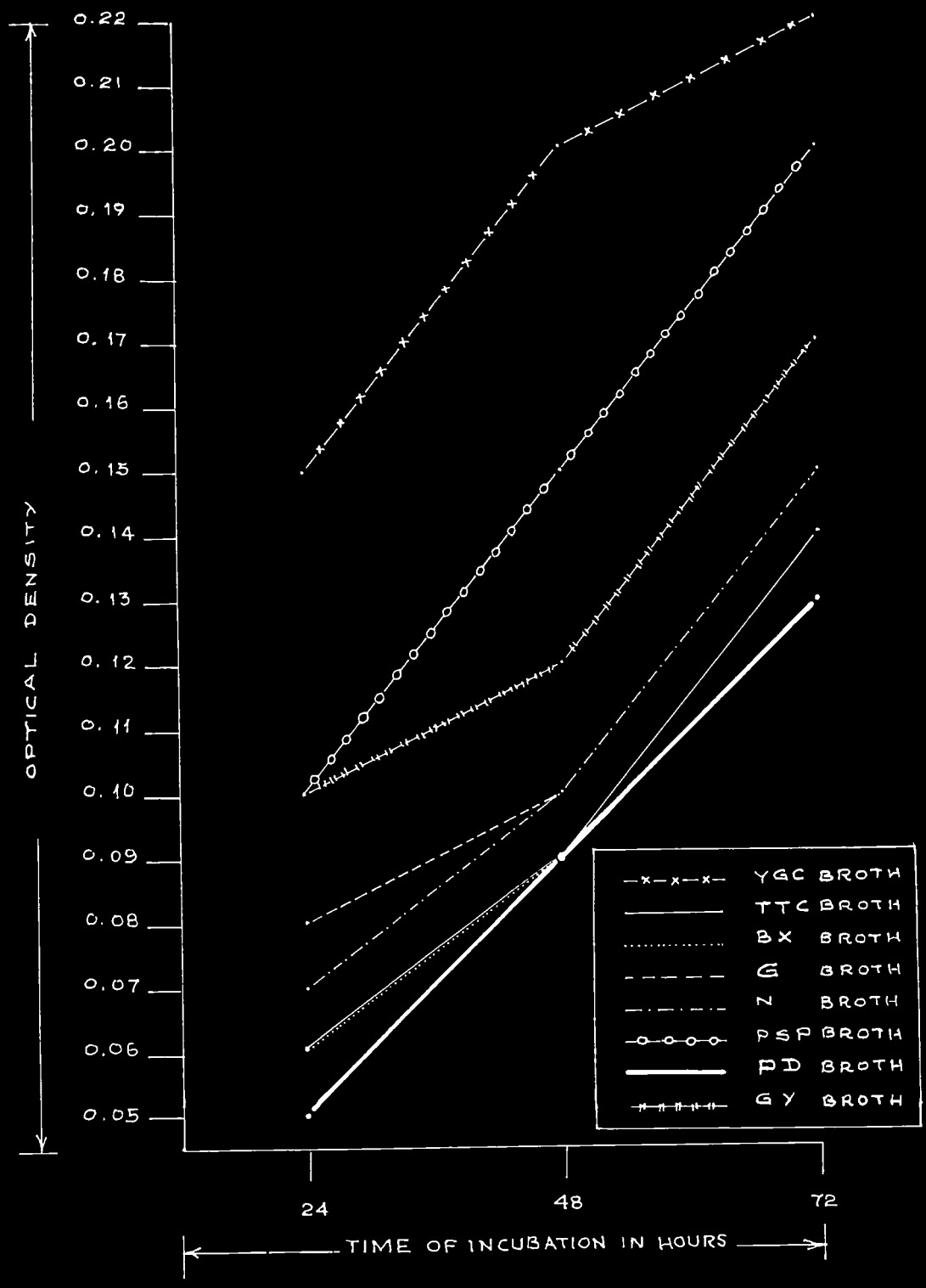

FIG: 1. GROWTH OF *Xanthomonas oryzae* IN DIFFERENT LIQUID MEDIA

4. Pigment production

A non water soluble yellow pigment was produced on Yeast Glucose Chalk Agar by the bacterium. No water soluble fluorescent pigment was produced by the bacterium on King's media.

5. Oxygen requirement

The bacterium was found to be aerobic since the growth and change of blue colour of the nutrient dextrose agar medium (containing 0.005 per cent bromocresol purple) to yellow was observed only in the tubes without paraffin sealing.

B. Physiological characters

1. Mode of utilization of glucose

The bacterium was found to utilize glucose oxidatively since the medium in the open tubes turned yellow from the top and also the lack of colour change in the paraffin sealed tubes.

2. Utilization of organic acids

The bacterium utilized sodium citrate and sodium acetate as the source of carbon as evidenced by the change of colour of the slants from green to blue. Sodium benzoate and sodium formate were not utilized as the source of carbon by the bacterium.

3. Starch hydrolysis

The bacterium did not hydrolyse starch since no colourless or reddish zone was observed around the bacterial

growth in contrast to the blue back ground of the medium.

4. Production of hydrogen sulphide

The bacterium was found to liberate hydrogen sulphide as evidenced by the blackening of the lead acetate test strip.

5. Methyl Red and Voges-Proskauer tests

The bacterium gave negative MR test as evidenced by the absence of development of distinct red colour in the culture tubes when a few drops of 0.02 per cent Methyl Red in 50 per cent alcohol was added.

The VP test was also negative as indicated by the absence of development of crimson or ruby colour in the culture tubes when 0.6 ml of alpha naphthol solution (5 per cent in 95 per cent alcohol) and 0.2 ml of 40 per cent aqueous solution of KOH, were added to one ml of the culture and observed after two hours.

6. Gelatin liquifaction

There was no liquifaction of gel column in the tubes inoculated with the culture of the bacterium indicating that the bacterium did not liquify gelatin.

7. Production of indole

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

8. Nitrate reduction test

Nitrate was not reduced by the bacterium as evidenced by development of pink colour even after addition of zinc crystals.

9. Urease production

There was no change of colour of the medium from yellow to red which indicated negative urease production by the bacterium.

10. Catalase test

Production of gas bubbles when the bacterial smear on the glass slide was covered with 20 volume hydrogen peroxide indicated the positive catalase test by the bacterium.

11. Action on milk

The bacterium turned the milk alkaline as evidenced by the colour change from blue to violet.

12. Utilisation of asparagine as a sole source of carbon and nitrogen

The bacterium did not utilise asparagine as a sole source of carbon and nitrogen as evidenced by the absence of growth of the bacterium in organic salt solution containing 0.2 per cent asparagine.

13. Growth in six per cent sodium chloride

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

14. Lipolytic activity

No opaque zone around the bacterial growth indicated negative lipolytic activity.

15. Tyrosinase activity

The bacterium gave negative tyrosinase activity as evidenced by the absence of dark brown pigment in the medium.

16. Arginine hydrolase test

Absence of any change in colour of the medium to red indicated negative arginine hydrolase activity by the bacterium.

17. Production of ammonia

The bacterium produced ammonia which was detected by using Nessler's reagent which gave a brown to yellow precipitate with ammonia.

18. Utilisation of carbon sources

Of the 17 carbon compounds tested the bacterium produced acid in fructose, glucose, lactose, sucrose, starch, mannose, cellulose, maltose, galactose, xylose, cellobiose and sorbose as indicated by the change in colour of the medium from reddish violet to yellow. No change in colour of the medium from reddish violet to yellow was observed in tubes containing salicin, amygdalin, gentiobiose, dulcitol and inositol which indicated negative utilization of these sugars (Table 5).

19. Hypersensitive reaction on tobacco leaves

Necrotic lesions were formed on tobacco leaves within a period of 36 hours after inoculation (Plate.III).

Table 3. Summary of microscopical, biochemical and physiological characters of the bacterium Xanthomonas oryzae.

Sl.No.	Characters studied	Observations
1.	Gram reaction	-
2.	Pigment production	
	(a) Non water soluble	+
	(b) Water soluble	-
3.	Oxygen requirement	+
4.	Mode of utilization of glucose	
	(a) Aerobic	+
	(b) Anaerobic	-
5.	Utilization of organic acids	
	(a) Sodium citrate	+
	(b) Sodium acetate	+
	(c) Sodium benzoate	-
	(d) Sodium formate	-
6.	Starch hydrolysis	-
7.	Production of H_2S	+
8.	MR and VP test	
	(1) Methyl Red test	-
	(2) Voges-Proskauer test	-
9.	Gelatin liquifaction	-
10.	Production of indole	-
11.	Nitrate reduction	-
12.	Urease test	-
13.	Catalase test	+

(continued)

Table 3 continued

Sl.No.	Characters studied	Observations
14.	Action on milk (Acid or alkaline)	Alkaline
15.	Utilization of asperagine as a sole source of C & N	-
16.	Growth in 6 per cent NaCl	-
17.	Lipolytic activity	-
18.	Tyrosinase activity	-
19.	Arginine hydrolase test	-
20.	Production of ammonia	+
21.	Utilisation of carbon compounds with acid production	
	1. Fructose	+
	2. Lactose	+
	3. Mannose	+
	4. Cellulose	+
	5. Sorbose	+
	6. Maltose	+
	7. Inositol	-
	8. Galactose	+
	9. Xylose	+
	10. Gentiobiose	-
	11. Salicin	-
	12. Amygdalin	-
	13. Dulcitol	-
	14. Glucose	+
	15. Starch	+
	16. Cellobiose	+
	17. Sucrose	+
22.	Hypersensitive reaction on tobacco leaves	+

\+ Positive
\- Negative

Plate III. Hypersensitive reaction due to *Xanthomonas oryzae* on tobacco leaves

SURVIVAL OF THE PATHOGEN

Survival in seeds

The survival of the pathogen in artificially inoculated rice seeds, both husked and unhusked were conducted by periodic isolation of the pathogen. Seeds were used for isolation with and without surface sterilization. Pathogen isolated from surface sterilized and unhusked seeds, surface steriliged and husked seeds, and unsterilized and husked seeds were considered to be internally seed borne and from unsterilized and unhusked seeds were considered to be externally seed borne. The results are presented in table 4.

The pathogen was found to be both externally and internally seed borne under the present conditions of study and the internally seed borne nature was more pronounced than the external seed infection.

From the table 4 it could be seen that from surface sterilized and unhusked seeds the pathogen could be isolated only for a period of 75 days. But from surface steriliged and husked seeds and from unsterilized and husked seeds the pathogen could be obtained upto 90 days. From unsterilized and unhusked seeds the pathogen could be recovered only for a period of 75 days. It was also evident from the studies that the maximum possible period of seed transmission was 90 days and the percentage of seed transmission were 77.08 per cent, 37.5 per cent and 6.25 per cent respectively at the end of

Table 4. Survival of Xanthomonas oryzae in artificially inoculated rice seeds

Source	Number of days after artificial inoculation																			
	15			30			45			60			75			90			105		
	Number of seeds used for isolation	Number of seeds yielded the bacterium	Percentage of seed transmission	Number of seeds used for isolation	Number of seeds yielded the bacterium	Percentage of seed transmission	Number of seeds used for isolation	Number of seeds yielded the bacterium	Percentage of seed transmission	Number of seeds used for isolation	Number of seeds yielded the bacterium	Percentage of seed transmission	Number of seeds used for isolation	Number of seeds yielded the bacterium	Percentage of seed transmission	Number of seeds used for isolation	Number of seeds yielded the bacterium	Percentage of seed transmission	Number of seeds used for isolation	Number of seeds yielded the bacterium	Percentage of seed transmission
Surface sterilised and unhusked seeds	12	11	91.67	12	8	66.67	12	4	33.63	12	2	16.67	12	2	16.67	12	0	0	12	0	0
Surface sterilised and husked seeds	12	12	100.00	12	10	83.33	12	10	83.33	12	6	50.00	12	2	16.67	12	1	8.33	12	0	0
Unsterilised and husked seeds	12	12	100.00	12	10	83.33	12	8	66.67	12	7	58.33	12	4	33.33	12	2	16.67	12	0	0
Unsterilised and unhusked seeds	12	12	100.00	12	9	75.00	12	3	25.00	12	3	25.00	12	1	8.33	12	0	0	12	0	0
Percentage of seed transmission at the end of 30 days (Average)						77.08	Percentage of seed transmission at the end of 60 days (Average)					37.5	Percentage of seed transmission at the end of 90 days (Average)					6.25			

30, 60 and 90 days of artificial inoculation.

Survival in plant debris

Survival of the pathogen in infected plant debris in soil was also studied by weekly sowing of disease free rice seeds in pots. Symptoms of the disease was seen on plants raised from seeds sown upto the fourth week after incorporation of infected plant material in soil. Fifteen day old plants from fourth week sowing showed disease symptoms. Plants raised from fifth week sowing onwards did not show any symptoms of the disease. This showed that the maximum period of survival was about 28 days after incorporation of infected plant debris in soil. In vitro studies on the survival of the pathogen in infected leaves showed that from leaves kept in fridge the pathogen could be isolated upto a period of two and a half months from the day of storage and from leaves kept under laboratory conditions, only for a period of one week of storage.

Survival in soil

In vitro studies on the survival of the pathogen in artificially inoculated unsterilized and sterilized soil were conducted. Isolations of the bacterium after seven days of inoculation using special media for Xanthomonas gave negative result.

In vivo studies on the survival of the pathogen in unsterilized and inoculated soil were conducted by weekly sowing. Even the plants raised from the first week sowings

did not show any symptoms of the disease after a period of three months. The study was continued for a period of six weeks.

EFFECT OF WEATHER FACTORS ON THE DISEASE INCIDENCE AND DEVELOPMENT

The data on the disease incidence and the variation in the different environmental factors during the different periods of observations are presented in the table 5 and also in the Figs. 2 and 3.

Table 5. Effect of weather factors on the incidence and development of bacterial leaf blight disease of rice.

Months	Lesion length (cm)	Relative humidity (%)	Maximum temperature (°C)	Minimum temperature (°C)	Total rainfall (mm)
August '79	20.00	93.00	29.70	22.80	126
September '79	24.00	92.90	30.19	22.70	226
October '79	16.00	90.60	30.30	22.60	49
November '79	25.00	94.00	29.30	22.80	325
December '79	24.00	94.00	30.70	21.50	42
January '80	12.00	86.00	31.00	20.00	0
February '80	11.00	84.00	32.70	20.80	0
March '80	13.00	85.46	32.50	21.60	23
April '80	14.00	90.06	32.55	23.55	126
May '80	13.50	87.71	32.52	23.85	50
June '80	25.00	93.39	29.63	22.35	330
July '80	21.00	92.33	29.36	21.97	157

From the table it is seen that the maximum disease incidence and development as evidenced by lesion length was in the month of September and December. The minimum incidence and

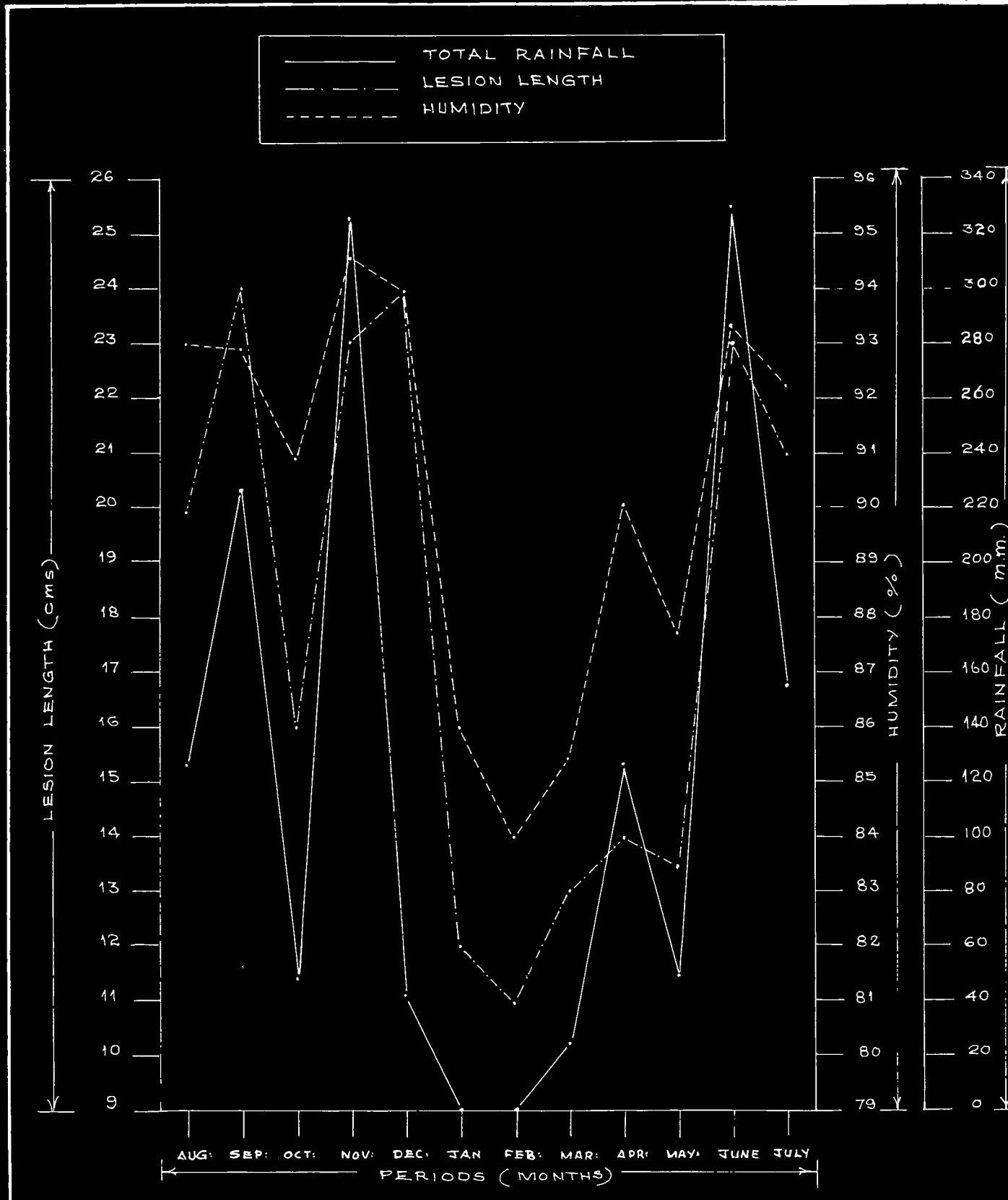

FIG: 2. EFFECT OF RELATIVE HUMIDITY AND TOTAL RAINFALL ON THE DEVELOPMENT OF BACTERIAL BLIGHT DISEASE OF RICE

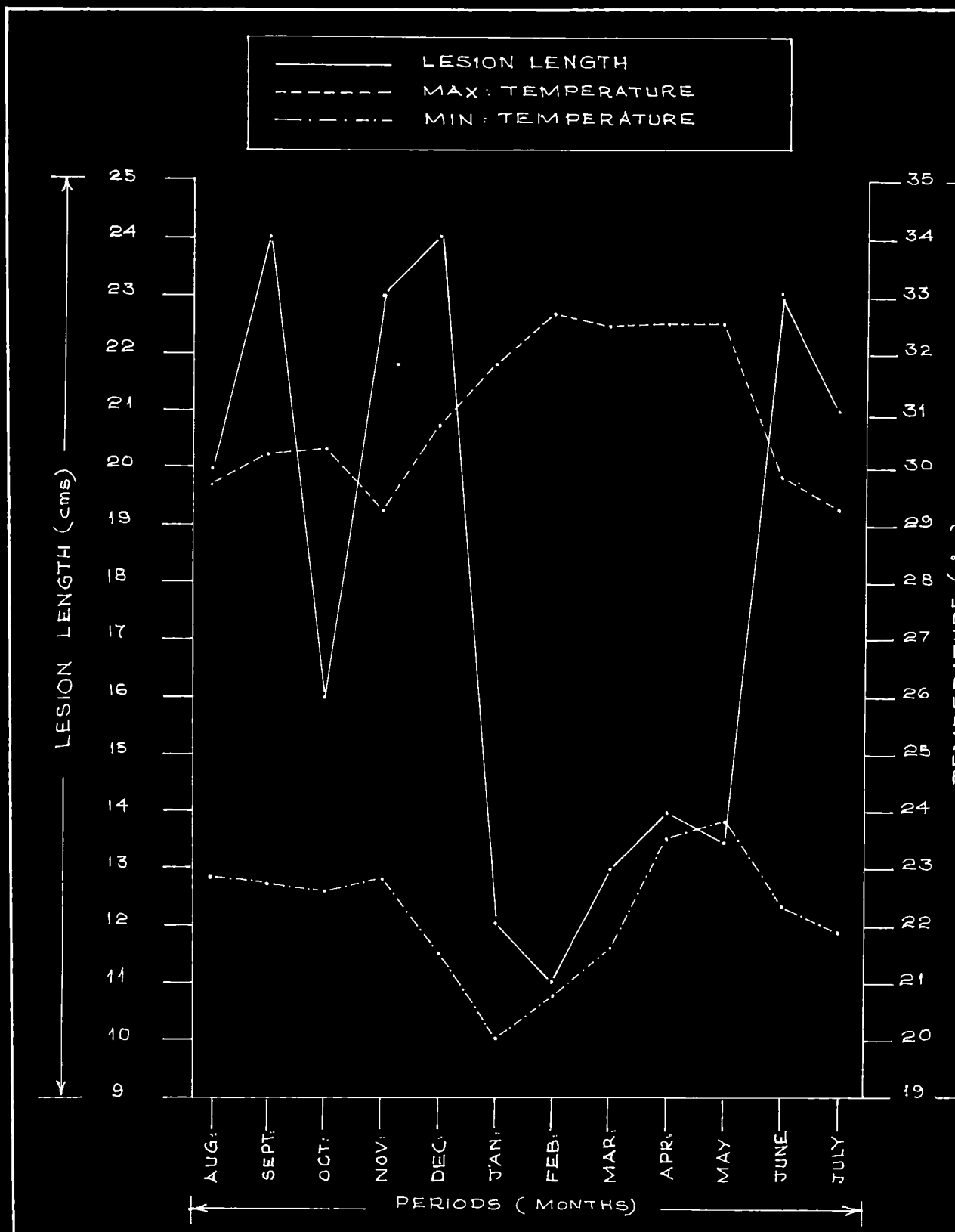

FIG: 3. EFFECT OF MAXIMUM AND MINIMUM TEMPERATURE ON THE DEVELOPMENT OF BACTERIAL BLIGHT DISEASE OF RICE

disease development was recorded in the month of February.

An attempt was made to correlate the development of disease with weather factors like relative humidity, maximum temperature, minimum temperature and total rainfall. The correlation coefficients between these weather factors and disease incidence are presented in table 6.

Table 6. Correlation coefficients of bacterial leaf blight disease incidence and development with weather factors like relative humidity, maximum temperature, minimum temperature and total rainfall.

Characters	Lesion length
Relative humidity	+0.9522**
Maximum temperature	-0.8458**
Minimum temperature	+0.2484
Total rainfall	+0.7357**

Critical value (0.1 level) = 0.4973
Critical value (0.5 level) = 0.5760

**Significant at both levels

Relative humidity and total rainfall were found to have a significant and positive correlation with disease development. Significant negative correlation was observed between disease development and maximum temperature. There was no significant correlation between disease development and minimum temperature both at 5 per cent and 10 per cent level. The multiple correlation coefficient calculated was found to be highly

significant (the square of multiple correlation coefficient (R^2) = 0.914). This explained that the disease incidence and development could be predicted with 91 per cent accuracy based on the above weather parameters provided the initial inoculum was present in the plant population.

EVALUATION OF RICE VARIETIES AND CULTIVARS FOR DISEASE RESISTANCE

Of the 50 high yielding rice varieties screened for host resistance against the pathogen Xanthomonas oryzae both at the seedling stage and adult plant stage, none of them were found to be resistant to the disease. The reaction of varieties at both stages are given in table 7.

Table 7. Reaction of fifty rice cultivars to the bacterial leaf blight pathogen Xanthomonas oryzae tested at the seedling stage and adult plant stage.

Sl.No.	Varieties	Disease grade	
		Seedling stage	Adult plant stage
1.	Taichung (Native)-1	9	9
2.	Suhasini	7	5
3.	Soorya	7	5
4.	Sona	3	5
5.	Hema	9	7
6.	Vijaya	5	5
7.	Vani	7	7
8.	Bhadra	7	5
9.	Blue bonnet	3	5

(continued)

Table 7 continued

Sl.No.	Varieties	Disease grade: Seedling stage	Disease grade: Adult plant stage
10.	Kalinga	3	5
11.	Saberi	5	5
12.	Basumathi	7	5
13.	Rajeswari	9	7
14.	PR 156	7	7
15.	IR 32	5	7
16.	IR 26	5	7
17.	IR 22	5	5
18.	IR 20	5	5
19.	IR 28	7	7
20.	IR 8	7	7
21.	Jaya	9	7
22.	Dee Geo Woo Gen	7	7
23.	Bharathi	7	7
24.	Ratna	7	7
25.	Suma	5	7
26.	Kumar	7	7
27.	Pennai	5	5
28.	Sakthi	9	7
29.	Anupama	5	5
30.	Puse 33	3	5
31.	Parijath	7	7
32.	Rajendra	7	7
33.	Krishna	7	7
34.	Aswathi	5	5
35.	Bhavani	5	5
36.	H4	7	5
37.	Cauvery	7	5
38.	Kalinga I	7	5
39.	Kalinga II	7	5

(continued)

Table 7 continued

Sl.No.	Varieties	Disease grade	
		Seedling stage	Adult plant stage
40.	Karuna	7	7
41.	Rohini	7	7
42.	Bala	5	5
43.	Supriya	7	5
44.	Kanchi	3	5
45.	Triveni	5	5
46.	Medha	5	5
47.	Padma	9	7
48.	Annapoorna	7	5
49.	Satya	5	5
50.	IR 5	3	5

IN VITRO EVALUATION OF DIFFERENT ANTIBIOTICS AGAINST THE PATHOGEN

The *in vitro* sensitivity of the bacterium to nine antibiotics were tested and the results are presented in table 8, Plate IV and Fig. 4.

Plate IV

Comparison between zone of inhibition of antibiotics tested against the pathogen *Xanthomonas oryzae*

1.	Penicillin	500 ppm
2.	Ampicillin	500 ppm
3.	Agrimycin-100	500 ppm
4.	Terramycin	500 ppm
5.	Tetracycline	500 ppm
6.	Ambistryn-S	500 ppm
7.	Chloromycetin	500 ppm
8.	Paushamycin	500 ppm
9.	Streptocycline	500 ppm
10.	Control	Distilled water

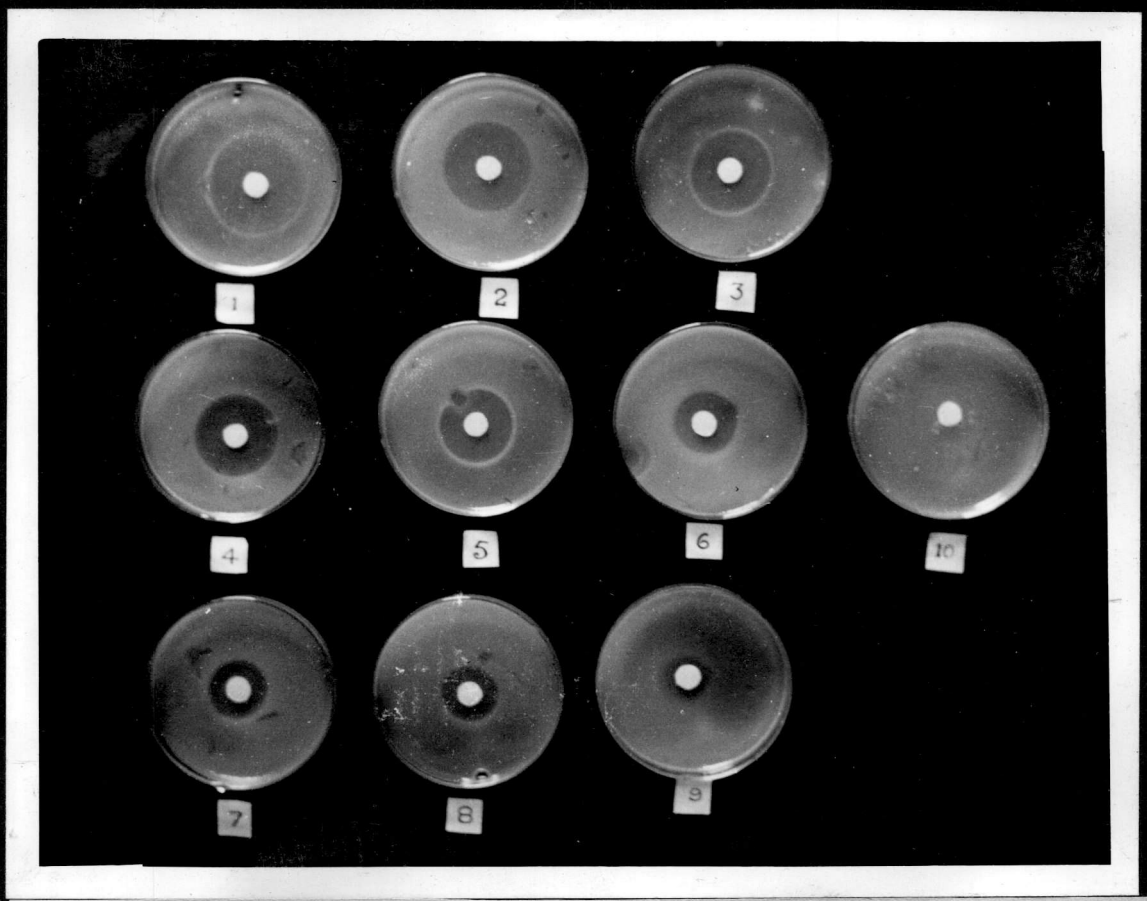

Plate IV. Comparison between zone of inhibition of antibiotics tested against the pathogen Xanthomonas oryzae

Table 8. *In vitro* sensitivity of antibiotics at different concentrations to *Xanthomonas oryzae*.

Antibiotics	Inhibition zone in mm			
	100 ppm	250 ppm	500 ppm	Mean
1. Chloromycetin	11.3	18.1	26.6	18.67
2. Terramycin	25.8	26.0	29.8	27.20
3. Penicillin	31.5	32.0	32.8	32.10
4. Tetracycline	24.5	25.0	29.4	26.30
5. Ampicillin	22.0	27.0	32.0	27.00
6. Ambistryn-S	10.0	22.0	27.0	19.67
7. Paushamycin	15.6	17.6	21.5	18.23
8. Agrimycin-100	20.0	24.6	30.9	25.17
9. Streptocycline	10.0	12.0	16.0	12.67

C.D. for comparison between antibiotics = 0.717
C.D. for comparison between combinations = 1.242

Among the nine antibiotics tested Penicillin was found to be superior to all other antibiotics. This was followed by Terramycin and Ampicillin which were on par. Ampicillin and Tetracycline were on par. But Terramycin was significantly superior to Tetracycline. Agrimycin-100 and Ambistryn-S were significantly different and inferior to Penicillin, Terramycin, Ampicillin, Tetracycline and superior to Chloromycetin, Paushamycin and Streptocycline. Chloromycetin and Paushamycin were on par and significantly superior to the least effective antibiotic Streptocycline.

In vitro sensitivity of Xanthomonas oryzae to antibiotics.

1. Chloromycetin
2. Terramycin
3. Penicillin
4. Tetracycline
5. Ampicillin
6. Ambistryn-S
7. Panshemycin
8. Agrimycin-100
9. Streptocycline

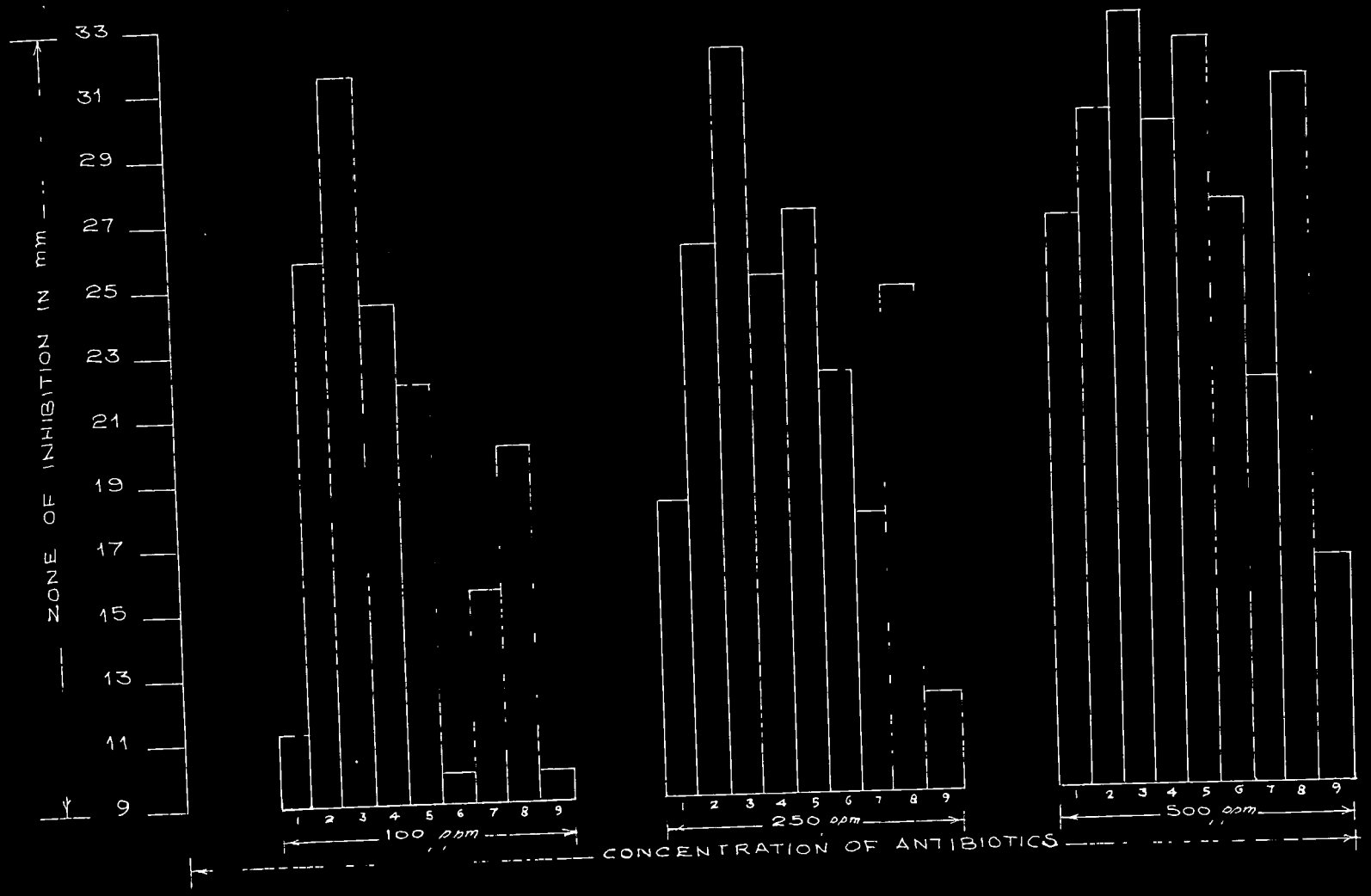

FIG: 4. *In vitro* SENSITIVITY OF *Xanthomonas oryzae* TO ANTIBIOTICS

A comparison between the different concentrations of antibiotics gave the following result. Penicillin 500 ppm concentration was not significantly different from its lower concentration 250 ppm. Penicillin 250 ppm was on par with its lower concentration 100 ppm. But Penicillin 500 ppm was significantly superior to its lower concentration of 100 ppm.

The higher concentration of 500 ppm of all the nine antibiotics tested were found to be significantly superior to their lower concentration of 100 ppm. The 250 ppm concentration of all the antibiotics except Penicillin, Terramycin and Tetracycline were superior to their lower concentration of 100 ppm.

CHEMICAL CONTROL OF BACTERIAL LEAF BLIGHT OF RICE USING ANTIBIOTIC SPRAYS

In this study 250 ppm of Agrimycin-100, Terramycin and Penicillin were used both as preinoculation and postinoculation sprays.

The effect of antibiotics on the intensity of the disease was taken two weeks after the preinoculation spray and one week and two weeks after the postinoculation sprays. The results are presented in table 9 and Fig.5.

The data taken two weeks after preinoculation spray showed that all the treatments were better than control. Statistical analysis of the data revealed that there was no significant difference between the treatments.

Table 9. Chemical control of bacterial leaf blight of rice using antibiotic sprays.

Treatment	Percentage index of disease status after		
	Preinoculation spray (in angles)	1st post-inoculation spray (in angles)	2nd post-inoculation spray (in angles)
T_1	17.43	25.33	36.48
T_2	17.80	27.93	36.39
T_3	17.53	26.69	36.84
T_4	19.46	25.47	33.08
T_5	19.46	25.07	34.13
T_6	19.46	25.34	33.68
T_7	19.46	28.64	41.38
C.D.(0.05 level)	0.556	0.857	0.941

The observations taken one week after first postinoculation spray showed that all the treatments were significantly superior to control. Agrimycin-100 pre and postinoculation sprays and Penicillin postinoculation spray were on par. They were significantly superior to Penicillin preinoculation spray and Terramycin postinoculation spray which were on par. Terramycin preinoculation spray though it was the least effective was significantly superior to control.

FIG 5. EFFECT OF ANTIBIOTICS ON THE INTENSITY OF BACTERIAL BLIGHT DISEASE OF RICE CAUSED BY *Xanthomonas oryzae*

The observations taken one week after second post-inoculation spray showed that all the treatments were significantly superior to control and all the postinoculation sprays were significantly superior to preinoculation sprays. Among the postinoculation sprays Penicillin and Agrimycin-100 were on par, Agrimycin-100 and Terramycin were on par but Penicillin was significantly superior to Terramycin.

DISCUSSION

DISCUSSION

Bacterial leaf blight of rice incited by Xanthomonas oryzae (Uyeda & Ishiyama) Dowson was first reported in India from Maharashtra by Sreenivasan et al. (1959). Until the year 1963 this disease was of minor importance and was confined to certain areas of Maharashtra State. Now probably with the adoption of High Yielding Varieties and intensive cultivation practices, the disease has become widespread in the rice growing areas of the country inflicting heavy losses. At present bacterial leaf blight of rice is one of the most important constraints to rice production in this country. Several investigations have been attempted on the different aspects of this disease by various workers from time to time with the object of controlling this serious malady.

The major aspects taken up for the present study were characterisation and identification of the pathogen, survival of the pathogen in seed, plant debris and soil, effect of weather factors on the incidence and development of the disease and varietal screening for host resistance. Further, studies on the in vitro sensitivity of the bacterium to different antibiotics and in vivo studies on the control of the disease has also been undertaken.

The bacterium was isolated and brought into pure culture. The morphological, cultural and physiological

characters were studied. The bacterium was a short gram negative motile rod. The colonies of the bacterium were yellow, convex, shiny and circular with entire margin.

The organism was found to be aerobic and produced a non-water soluble yellow pigment on yeast glucose chalk agar medium. With respect to the physiological and biochemical properties of the bacterium, it utilized glucose oxidatively. It neither hydrolysed starch nor liquified gelatin. It produced hydrogen sulphide, ammonia and catalase. Tests for indole, lipolytic activity, Methyl Red and Voges-Proskauer tests were negative. It did not reduce nitrate. Neither urease nor tyrosinase was produced by the bacterium. The bacterium turned milk alkaline without peptonization and utilised sodium salts of citric acid and acetic acid but not that of benzoic acid and formic acid. Growth of the organism was inhibited at six per cent sodium chloride.

Of the 17 carbon compounds tested the organism produced acid in presence of fructose, lactose, mannose, cellulose, sorbose, maltose, galactose, xylose, glucose, starch, cellobiose and sucrose indicating their utilisation. It did not produce acid in inositol, gentiobiose, salicin, amygdalin and dulcitol.

Similar results have been reported by Breed et al. (1957), Sulaiman and Ahamed (1965) and Chakravarti and Rangarajan (1967) with a few differences. An alkaline

reaction was observed in the present study as against the acidic reaction reported by Breed et al. (1957) and Sulaiman and Ahamed (1965). But the report of Chakraverti and Rangarajan (1967) was in agreement with the results of the present study. Similarly production of hydrogen sulphide, non hydrolysis of starch and nonliquification of gelatin observed in the present studies were also in agreement with the reports of Breed et al. (1957). Controversial reports on the above aspects were also made by Sulaiman and Ahamed (1965), Fordesimo (1958) and Chakraverti and Rangarajan (1967). However, Dye (1962) reported that physiological characters were of little value in distinguishing species, because the extent of intra species variability in physiological characters was so great as interspecies variability.

The pathogenicity of the bacterial isolate was proved on healthy, 25 day old rice plants of the variety Jyothi. Again, necrotic lesions on tobacco leaves were formed within a period of 36 hours after inoculation of the bacterial isolate indicated its pathogenicity.

The results of the present studies on the morphological, cultural, physiological and biochemical characters of the isolate of bacterium together with its pathogenicity when examined in the light of the reports of Breed et al. (1957), Sulaiman and Ahamed (1965) and Chakraverti and Rangarajan (1967) indicated that the organism could be identified as Xanthomonas oryzae (Uyeda and Ishiyama) Dowson. Detailed

studies were not conducted so far on the characterisation and identity of the bacterial isolate of the pathogen causing bacterial leaf blight of rice occurring in Kerala. In the present study the incitant of bacterial leaf blight of rice could be characterised and identified as Xanthomonas Oryzae (Uyeda and Ishiyama) Dowson.

The growth of the bacterium on eight different solid media were studied which showed some variations. Maximum growth was observed on GYA followed by GA, YGCA and PSPA. Amount of growth, slime and fluidity were less on Basal medium for Xanthomonads. The colonies produced on all media were yellow, convex, slimy, smooth and circular with entire margin.

Sulaiman and Ahamed (1965) reported that the organism grew well only on yeast glucose chalk agar and protease peptone dextrose agar. Similar to the result obtained in the present study, Breed et al. (1957) and Chakravarti and Rangarajan (1967) also reported that the colonies on nutrient agar were yellow, smooth, slimy, circular, glistening and with entire margins. Dye (1962) reported that considerable variations could be expected in colonies produced by Xanthomonads and this might not be taken as a differentiating character.

Growth of the bacterium in different liquid media was studied by spectrophotometric methods. In contrast to the

growth on different solid media, maximum growth was observed after 72 h of incubation in Yeast Glucose Chalk broth followed by PSP broth, GY broth, G broth and N broth, TTC broth, PD broth and BK broth. Chakraverti and Rangarajan (1967) observed an yellow growth of the bacterium with sedimentation and turbidity in nutrient broth.

The results of the present studies indicated that for routine laboratory studies and mass culturing of the bacterium GYA and GA to be the best solid media followed by PSPA and YGCA. The best liquid media were found to be Glucose Yeast extract Chalk broth and PSP broth.

Survival of the pathogen in artificially inoculated husked and unhusked seeds were studied. It was evident from the studies that the maximum possible period of survival of the bacterium in seeds was 90 days and the percentage of possible seed transmission were 77.08 per cent, 37.5 per cent and 6.25 per cent respectively at the end of 30, 60 and 90 days of artificial inoculation. The pathogen was found to be both externally and internally seed borne under the present conditions of study.

Survival of the bacterium in seeds for various periods of time upto next sowing season was reported by several workers (Goto et al., 1953; Fang et al., 1956; Srivastava and Rao, 1963). Nakimoto (1954) reported that Xanthomonas oryzae survived in unhulled grain for only 30 days. Chattopadhyay and Mukherjee (1971) reported that the pathogen

survived in seeds for 30-160 days after harvest. Kauffman and Reddy (1975) reported survival of the bacterium only for two months. Kauffman and Reddy (1975) reported that they could isolate Xanthomonas oryzae from 70 per cent of the naturally infected seeds one month after harvest and from 40 per cent at the end of two months. The bacterium could not be isolated at the end of three months. Misukami (1961) noted the presence of the bacterium in the husks of seeds. Fang et al. (1956) and Srivastava and Rao (1964a) reported that the bacterium was also present in the endosperm of the seeds.

Hsieh (1973) reported that the survival of the pathogen in seeds was dependent on temperature and relative humidity. This might be the probable reason for the divergence in the periods of survival observed by different workers. The pathogen might survive in seeds for longer periods under certain conditions of storage. The results of the present studies highlight the possibilities of seed transmission of the disease. The fact that the pathogen could be both internally and externally seed borne and viable in seeds for about three months was all the more alarming observation. In a continuous cropping pattern, the interval between two successive cropping seasons is less than this three months period, in which case the infected seeds can serve as a ready source of primary inoculum in initiating the disease.

Studies on the survival of the pathogen in infected leaves kept under laboratory conditions showed that the bacterium survived in infected leaves for only seven days. Hsieh and Buddenhagen (1975) reported that the pathogen survived for only 5-40 days in diseased leaves kept in air at warm temperatures and at 100 per cent relative humidity and lowering the relative humidity to 0-20 per cent lengthened such survival.

Weekly isolations of the pathogen from infected leaves kept in fridge yielded the pathogen for a period of 75 days. No previous work was available on this study.

Studies on the survival of the pathogen in infected plant debris showed that the pathogen survived in infected plant debris in moist soil for a period of 28 days. Hsieh and Buddenhagen (1975) observed that the pathogen survived for more than one month in warm (30°C) flooded or moist soil, or in leaves buried in soil.

In the present study negative results were obtained on the survival of the pathogen in artificially inoculated unsterilized and sterilized soils when isolated after seven days. This was in conformity with the findings of Singh (1971a), who reported that the bacterium did not survive in unsterilised soil for more than a week and at pH levels below seven the period of survival further deteriorated. Negative results of the survival of the pathogen in soil was also reported by Nakimoto (1956) and Seki and Hisukami (1955).

But Chattopadhyay and Mukherjee (1974) reported that in West Bengal Xanthomonas oryzae survived for seven days in unsterilized soil and upto 30 days in sterilized soil.

From the results of the present studies on the survival of the pathogen in seed, infected debris in soil and in soil it was observed that seed and infected debris in soil served as important sources of survival of the pathogen. The period of survival of 90 days in seed and 28 days in crop refuse in soil seemed to be a significant period. These two sources could readily contribute the primary inoculum for the succeeding crop. Infected seeds and infected plant debris could be considered as important sources of survival of the pathogen and it is likely that they could play an important role in the epidemiology of the disease under the existing cropping pattern and Agroclimatic conditions of Kerala State.

Effect of important weather factors on the disease incidence and development was studied for a period of one year during 1979-80. The disease incidence and development was maximum during September and December and the minimum incidence was during February. The relative humidity and total rainfall seemed to have a significant positive correlation with the disease incidence and development and the maximum temperature seemed to have a negative correlation. No correlation was found to exist between minimum temperature and disease incidence. The multiple correlation was worked out and found that the disease incidence and development

could be predicted with 91 per cent accuracy based on these weather parameters provided the initial inoculum was present in the plant population.

Mohiuddin et al. (1977) reported that rainfall was highly correlated with disease incidence. Sulaiman and Ahamed (1965) reported that the optimum conditions for disease development was found in the month of September when the maximum temperature was 30°C, minimum temperature was 23.4°C, rainfall was 60.7 mm and relative humidity 93 per cent. From the present studies on the effect of important weather parameters on disease incidence and development it appeared that the relative humidity and total rainfall were very much correlated with the disease incidence and development. The main rice growing periods in Kerala coincide with the monsoon seasons and the agroclimatic conditions during this periods are highly conducive for the epiphytotic development of bacterial leaf blight provided the initial inoculum is present. But invariably the disease seems to occur in an endemic pattern in the State probably due to the vagaries of the weather during the monsoon periods. More detailed and critical studies are however required to isolate some of the visible weather parameters or any other factors which are involved in the occurrence of the disease in an endemic pattern.

An attempt was made to evaluate 50 popular rice cultivars for their resistance against the pathogen. None of them were found to have any useful degree of resistance against the pathogen. Varieties were tested for resistance to the pathogen

both at the seedling and at the adult plant stage. Fifty per cent of the varieties tested showed the same reactions both at the seedling stage and at the adult plant stage.

Report from the International Rice Research Institute showed that, out of the 3676 rice varieties screened only 26 of them proved highly resistant to a virulent isolate of Xanthomonas oryzae (Anon., 1966). Srivastava et al. (1967) also reported that out of the 128 rice cultivars tested only 13 of them proved resistant and 11 of them proved moderately resistant. Chien and Hung (1970) observed that out of the 70 rice varieties tested none of them was highly resistant. Ou et al. (1971) reported that out of 8700 rice cultivars screened none of the varieties were immune. They also reported that the varieties resistant at the seedling stage were also resistant at the flag leaf stage. Similar result was obtained in the present study also. Rao and Reddy (1975) compared the nursery screening with adult plant reactions and suggested that screening should be done on adult plants around the heading stage when the disease was more pronounced.

It has been a general phenomenon that even the resistant varieties developed once, eventually become susceptible to the new virulent isolates of the pathogen (Fujii and Okada, 1967). In general, to get a cultiver with genetic resistance against a bacterial disease is considered to be a difficult task and the bacterial leaf blight of rice appeared to be no exception to this.

Of the nine antibiotics screened for the *in vitro* sensitivity of the bacterium, Penicillin gave the highest zone of inhibition followed by Terramycin and Ampicillin which were equally effective. This was closely followed by Tetracycline, Agrimycin-100, Ambistryn-S, Chloromycetin, Paushamycin and Streptocycline. Least effective among the nine was Streptocycline.

Swarup *et al.* (1965) reported that Penicillin G (100 ppm) gave maximum inhibition against *Xanthomonas oryzae*. Mondal and Mukherjee (1978) noted that antibiotics such as Tetracycline and Ampicillin were of promise against *Xanthomonas oryzae*. The present study also indicated that Penicillin (500 ppm) exerted maximum inhibition to the growth of the bacterium *in vitro*.

From the *in vivo* studies using three promising antibiotics both as pre and postinoculation sprays it was observed that none of the treatments gave any absolute control of the disease. But it was noted that the percentage disease status was minimum on all antibiotic sprayed plants and the maximum on control. It was further observed that post inoculation sprays offered better control of the disease than preinoculation sprays. Among the postinoculation sprays Penicillin 250 ppm and Agrimycin-100 at 250 ppm were equally effective. Singh (1968) also observed that Agrimycin-17 (1:2000) lowered bacterial leaf blight incidence on rice. The present recommendation in Kerala for the control of bacterial

leaf blight disease is also Agrimycin-100 250 ppm spray.

In the absence of resistant cultivars of rice, a 250 ppm spray of Penicillin or Agrimycin-100 could be used for reducing the severity of bacterial blight disease in rice fields. Agrimycin-100 being a commercial formulation for plant disease control, it could be used for the purpose with advantage.

SUMMARY

SUMMARY

The bacterial leaf blight of rice, incited by Xanthomonas oryzae (Uyeda and Ishiyama) Dowson is one of the serious diseases of economic importance in India and several other rice growing countries of the world. This disease was reported in India from Maharashtra by Sreenivasan et al. (1959). But this disease was considered to be of little importance until 1963, when a severe epiphytotic broke out in Bihar and other parts of North India. In severe cases of disease incidence, losses upto 60 per cent in grain yield was reported with this disease. Though serious epiphytotics were not so far been reported from Kerala the disease is considered to be endemic in the major rice growing areas of Kuttanad and Palghat.

In view of the potential crop losses the disease can cause in the State, studies were undertaken on the characterisation and identification of the pathogen, survival of the pathogen in seed, plant debris and soil, effect of weather factors on the disease incidence, varietal screening for host resistance, in vitro sensitivity of the bacterium to different antibiotics and in vivo studies on the control of the disease using antibiotics.

The causal organism was isolated from the infected rice leaves collected from the rice fields of the Rice Research Station, Moncompu.

Typical bacterial leaf blight lesions starting near the

leaf tip and progressing downwards through the leaf margins with wavy inner margins were observed in the present study.

Morphological studies showed that the organism was a gram negative short rod. Physiological and biochemical studies indicated that the bacterium utilized glucose oxidatively. It neither hydrolysed starch nor liquified gelatin but produced hydrogen sulphide, ammonia and catalase. Tests for indole, lipolytic activity, Methyl Red and Voges-Proskauer tests were negative. It did not reduce nitrate. Neither urease nor tyrosinase was produced by the bacterium. It turned milk alkaline without peptonization and utilized sodium salts of citric acid and acetic acid but not that of benzoic acid and formic acid. Growth of the organism was inhibited at six per cent sodium chloride. The bacterium produced acid indicative of its utilization from fructose, lactose, mannose, cellulose, sorbose, maltose, galactose, xylose, glucose, starch, cellobiose and sucrose and not from inositol, gentiobiose, salicin, amygdalin and dulcitol.

Studies on the morphological, physiological and biochemical characters of the pathogen along with its pathogenicity established the identity of the pathogen as Xanthomonas oryzae (Uyeda and Ishiyama) Dowson.

For laboratory studies and mass culturing of the pathogen, Glucose Yeast Extract Agar and Glucose Agar were found to be the best solid media and Glucose Yeast extract Chalk broth and Potato Peptone Sucrose broth the best liquid media.

Studies on the survival of the bacterium on different sources indicated that the pathogen was able to survive for a period of 90 days in infected seeds, 28 days in infected plant debris in soil, 75 days in infected leaves kept in fridge, seven days in infected leaves kept under laboratory conditions and less than one week in infected soil. This study indicated that the infected seeds and infected plant debris play an important role in the epidemiology of the disease under the existing agroclimatic conditions and cropping pattern of the State.

The effect of weather factors on the disease incidence and development was studied for a period of one year revealed that the disease incidence and development was maximum during September and December and minimum during February. The relative humidity and total rainfall seemed to have a significant positive correlation with disease incidence and development. The maximum temperature had got a negative correlation and the minimum temperature had no significant correlation with disease incidence and development.

From an evaluation of rice varieties and cultivars for disease resistance it was observed that out of the 50 rice varieties tested both at the seedling stage and adult plant stage, none of them had any useful degree of resistance against the pathogen. At both the growth stages of the rice plants, more than 50 per cent of the varieties showed the same type of reactions.

The results of the *in vitro* sensitivity of the pathogen to antibiotics showed that the organism was sensitive to Penicillin, Terramycin, Ampicillin, Tetracycline, Agrimycin-100, Ambistryn-S, Chloromycetin, Fenchamycin and Streptocycline at 100 ppm. Penicillin (500 ppm) gave the maximum zone of inhibition followed by Terramycin (500 ppm).

The *in vivo* studies using antibiotics revealed that none of the antibiotics gave any absolute control of the disease. But it was found that the percentage of disease status was minimum on all antibiotic sprayed plants and the maximum on control plants. It was also observed that post inoculation sprays offered better control of the disease than preinoculation sprays. Sprays of Penicillin (250 ppm) and Agrimycin-100 (250 ppm) were equally effective and gave maximum effect in lowering the disease intensity. Agrimycin-100, being a commercial preparation for plant disease control it would be better to use this antibiotic preparation against the disease.

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*Original not seen

APPENDICES

APPENDIX I

Analysis of variance table

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

Source	Total SS	df	Mean SS	F at 0.05 level	Whether significant or not
Total	3980.20	80			
Treatment	3948.96	26	151.88	262.53	Significant
Error	31.24	54	0.58		

APPENDIX II

In vivo studies using antibiotics on the control of bacterial leaf blight of rice.
Disease score on 6th day after inoculation

Treatments	Disease grade	Number of leaves in each grade out of 24 leaves examined				
		Replication I	Replication II	Replication III	Replication IV	Replication V
Treatment I (Agrimycin-100 250 ppm preinoculation spray)	0	5	4	5	3	6
	1	19	20	19	21	18
Treatment II (Terramycin 250 ppm preinoculation spray)	0	2	6	5	4	2
	1	22	18	19	20	22
Treatment III (Penicillin 250 ppm preinoculation spray)	0	3	4	6	4	5
	1	21	20	18	20	19
Treatment IV, V, VI & VIII (No spray)	1	24	24	24	24	24

APPENDIX III

In vivo studies using antibiotics on the control of bacterial leaf blight of rice. Disease score on 8th day after first postinoculation spray.

Treatments	Disease grade	Number of leaves in each grade out of 24 leaves examined				
		Replication I	Replication II	Replication III	Replication IV	Replication V
Treatment I (Agrimycin-100 250 ppm preinoculation spray)	1	15	18	17	16	15
	3	9	6	7	8	9
Treatment II (Terramycin 250 ppm preinoculation spray)	1	13	10	12	13	12
	3	11	13	12	11	12
Treatment III (Penicillin 250 ppm preinoculation spray)	1	15	14	13	15	14
	3	9	10	11	9	10
Treatment IV (Penicillin 250 ppm postinoculation spray)	1	15	16	15	17	17
	3	9	8	9	7	7
Treatment V (Terramycin 250 ppm postinoculation spray)	1	13	12	12	13	12
	3	11	10	12	11	10
Treatment VI (Agrimycin-100 250 ppm postinoculation spray)	1	15	17	16	17	16
	3	9	7	8	7	8
Control (No spray)	1	10	11	10	12	12
	3	13	13	13	10	12
	5				2	

APPENDIX IV

In vivo studies using antibiotics on the control of bacterial leaf blight of rice. Disease score on 8th day after second postinoculation spray.

Treatments	Disease grade	Number of leaves in each grade out of 24 leaves examined				
		Replication I	Replication II	Replication III	Replication IV	Replication V
Treatment I (Agrimycin 100 250 ppm preinoculation spray)	3	21	19	22	21	21
	5	3	5	2	3	3
Treatment II (Terramycin 250 ppm preinoculation spray)	3	23	21	22	23	21
	5	1	3	2	1	3
Treatment III (Penicillin 250 ppm preinoculation spray)	3	23	20	20	23	22
	5	1	3	3	1	2
	7		1			
Treatment IV (Penicillin 250 ppm postinoculation spray)	1	4	3	4	4	4
	3	20	21	20	20	20
Treatment V (Terramycin 250 ppm postinoculation spray)	1	5	4	5	4	5
	3	16	17	16	15	15
	5	3	3	3	5	4
Treatment VI (Agrimycin 100 250 ppm postinoculation spray)	1	2	3	5	4	4
	3	22	21	16	20	19
	5			3		1
Treatment VII (Control) (No spray)	3	16	15	13	13	13
	5	7	8	10	9	10
	7	1	1	1	2	1

APPENDIX V

Analysis of variance table

Chemical control of bacterial leaf blight of rice using antibiotic sprays. Percentage index of disease status (in angles) two weeks after preinoculation spreying.

Source	Sum of squares	df	Mean square	F at 0.05 level	Whether significant or not
Total	35.461	34			
Treatment	30.318	6	5.053	27.46	Significant
Error	5.143	28	0.184		

APPENDIX VI

Analysis of variance table

Chemical control of bacterial leaf blight of rice using antibiotic sprays.
Percentage index of disease status one week after first postinoculation spraying.

Source	Sum of squares	df	Mean square	F at 0.05 level	Whether significant or not
Total	65.413	34			
Treatments	53.155	6	8.859	20.23	Significant
Error	12.258	28	0.438		

APPENDIX VII

Analysis of variance table

Chemical control of bacterial leaf blight of rice using antibiotic sprays.
Percentage index of disease status one week after second postinoculation spraying.

Source	Sum of squares	df	Mean square	F at 0.05 level	Whether significant or not
Total	251.712	34			
Treatments	236.928	6	39.488	74.788	Significant
Error	14.784	28	0.528		

ABSTRACT

The bacterial leaf blight of rice, incited by Xanthomonas oryzae (Uyeda and Ishiyama) Dowson is one of the most serious disease of economic importance in India and several other rice growing countries of the world. This disease was first reported in India by Sreenivasan et al. (1959) from Maharashtra and a serious epiphytotic was reported by Srivastava and Rao (1963) from Bihar. In Kerala, eventhough severe epiphytotics of this disease have not been reported so far, the disease is endemic in the major rice growing areas of Kuttanad and Palghat.

The pathogen was identified as Xanthomonas oryzae (Uyeda and Ishiyama)Dowson based on its morphological, cultural, physiological and biochemical characters together with its pathogenicity.

For laboratory studies and mass culturing of the organism, Glucose Yeast extract Agar and Glucose Agar were found to be the best solid media and Glucose Yeast extract chalk broth and Potato Sucrose Peptone broth were the best liquid media.

The pathogen was found to survive in infected seeds for a period of 90 days, in infected debris in soil for a period of 28 days and in infected soil for less than a week indicating that the infected seeds and infected plant debris in soil play an important role in the epidemiology of the disease.

The effect of weather factors on the disease incidence and development was studied for a period of one year. The results of the study revealed that the disease incidence and development

was maximum during the months of September and December and the minimum during February. The relative humidity and total rainfall seemed to have a significant positive correlation with the disease incidence and development. But the maximum temperature had a negative correlation and the minimum temperature had no significant correlation.

Out of the 50 popular rice varieties screened for host resistance, none of them were found to have any useful degree of resistance against the pathogen and more than 50 per cent of the varieties tested gave the same reactions both at the seedling stage and at the adult plant stage.

In vitro sensitivity studies of the bacterium against common antibiotics showed that the pathogen was sensitive to Penicillin, Terramycin, Ampicillin, Tetracycline, Agrimycin-100, Ambistryn-S, Chloromycetin, Pseudomycin and Streptocycline at 100 ppm. Penicillin (500 ppm) gave the maximum zone of inhibition followed by Terramycin (500 ppm).

In vivo studies using antibiotics on the control of the disease revealed that postinoculation sprays offered better control of the disease than preinoculation sprays and sprays of Penicillin (250 ppm) and Agrimycin-100 (250 ppm) were equally effective in lowering the disease intensity. But Agrimycin-100, being a commercial preparation for plant disease control, it would be better to use this antibiotic against the disease.