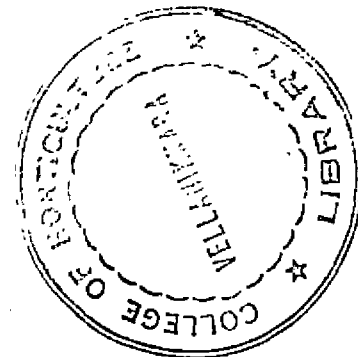


**STUDIES ON THE EFFECT OF AGE OF PLANT
AND HOST NUTRITION ON BACTERIAL LEAF
BLIGHT OF RICE AND ITS CONTROL**

By
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THESIS
submitted in partial fulfilment of the requirement
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1985

DECLARATION

I hereby declare that this thesis entitled "Studies on the effect of age of plant and host nutrition on bacterial leaf blight of rice and its control" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.



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C E R T I F I C A T E

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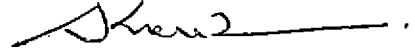
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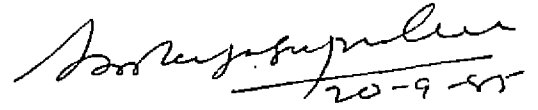
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INTRODUCTION

INTRODUCTION

Rice is a crop of considerable economic importance for a country like India. There are a large number of varieties differing in plant type, photosensitivity, yield and tolerance to various environmental conditions. It is subjected to several pests and diseases of fungal, bacterial and viral origin that bring about significant reduction in the annual net yield of this crop. Among the bacterial diseases, the leaf blight caused by Xanthomonas campestris pv oryzae is the most severe one. This was first reported from Japan in 1884 and from India in the year 1959. In Kerala this disease was first observed from Palghat and it now occurs in almost all the major rice growing tracts of the State belonging to Palghat, Kottayam, Alleppey and Pathanamthitta districts. In fact, in Kuttanad, this disease has become a major yield limiting factor during the additional crop season and especially so when there is intermittent rainfall during the crop period. However, an effective control measure for this disease is lacking even today.

Work is in progress in several laboratories in our country as well as outside to understand the nature of the pathogen, varietal susceptibility to bacterial leaf blight, effect of age of plant and host nutrition on disease incidence, ability of the pathogen to survive in various infected materials and to evolve an effective control measure for this most important bacterial disease of rice. The present study is also more or less on the same lines since information pertaining to specific regions will be more useful in solving some of the problems faced locally with regard to the onset and spread of this disease. Taking this fact into consideration, the present investigation was planned with the following main objectives.

1. To study the effect of age of plant on the incidence of bacterial leaf blight in T(N)1, Jaya and IR-20.
2. To study the effect of different levels of NPK and certain minor elements such as Zn, Mn, B on the incidence of bacterial leaf blight in T(N)1.

3. To study the ability of the pathogen to survive in soil, crop refuse, plant debris and seeds.
4. To evolve a suitable control measure for this most important bacterial disease of rice in the State.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Bacterial leaf blight of rice was first reported from Japan in 1884. Later it was reported from a number of other Asian countries like Korea (Takeuchi, 1930), Taiwan (Hashioka, 1951), Indonesia (Goto et al. 1955), Thailand (Jalevicharana, 1958), India (Srinivasan et al. 1959 and Bhapkar et al. 1960) Sri Lanka (Seneviratne, 1962), Pakistan (Mew and Majid, 1977) and Philippines (Anon, 1983). In India, this disease was first noticed during 1951 in the Khopoli area of Maharashtra State (Srinivasan et al. 1959 and Bhapkar et al. 1960). Subsequently it was reported from Kashmir (Kaul, 1959), Andhra Pradesh (Srivastava and Rao, 1964), Uttarpradesh (Pavgi et al., 1964), Tamilnadu (Soumini Rajagopalan et al. 1969), Bihar (Mahmood and Singh 1970) and Punjab (Sahu et al. 1982).

In Kerala, bacterial leaf blight of rice was first observed in the Palghat district during 1976. According to a study conducted by Mary and James Mathew (1980), it now occurs in almost all the major rice growing districts of the State such as Alleppey, Kottayam,

Pathanamthitta and Palghat. In Kuttanad this has in fact become a critical yield limiting factor during the additional crop season.

DESCRIPTION OF THE PATHOGEN

Takaishi in 1909 observed that the turbid dew drops obtained from bacterial leaf blight affected rice plants consisted of a mass of bacteria which could reproduce the disease when inoculated into healthy plants. Bokura (1911) could isolate a bacterium called Bacillus oryzae from such diseased leaves. Ishiyama (1922) reported that bacterial leaf blight was caused by a kind of rod shaped bacterium and he named it as Pseudomonas oryzae Uyeda et Ishiyama. This was subsequently changed to Xanthomonas oryzae (Uyeda and Ishiyama) Dowson by Breed et al. (1957). According to the current nomenclature the pathogen is renamed as Xanthomonas campestris pv oryzae (Dye et al. 1980).

Ishiyama during his study of the pathogen in 1922 described it as an aerobic, rod shaped, gram negative, nonspore forming bacterium with monotrichous flagellation. Breed et al. (1957) reported the pathogen as a rod shaped bacterium with a size ranging from 0.5

to 0.80 μ x 1.0 to 2.0 μ and producing smooth, circular glistening colonies of wax yellow colour on nutrient agar medium. Similar characters were also described by Yoshimura and Tahara (1960) and Chakravarti and Rangarajan (1967).

Misukami (1956) first described the symptoms of bacterial leaf blight as water soaked lesions which appeared along the margin of the upper leaves. These gradually enlarged along the veins and turned yellow in colour. As the disease progressed these lesions become white or greyish white in colour followed by withering of the infected parts. During the seedling stage, the infected leaves rolled completely and turned yellow before getting dried. This type of symptom was first observed in Indonesia as "Kresek" by Reitsma and Schure in 1950. Studies conducted at the International Rice Research Institute, Philippines, have also revealed that the Kresek phase of the disease initiated one or two weeks after transplanting as greyish discolouration of leaves, followed by the rolling of leaves along the mid rib (Anon 1964).

Singh and Saksena (1968) described the symptoms at the early stages of plant growth as yellowing of

leaves, poor root growth, less tillering and stunted growth. At flowering stage blighting appeared on the edges of leaves cross wise with a wavy margin.

Drivastava and Rao (1966) reported that the typical symptoms of bacterial leaf blight were visible one week after artificial inoculation as water soaked lesions on both the margins of leaves. These in turn became yellow and finally changed to straw coloured lesions. The disease later extended to leaf sheaths and clumps resulting in the death of the tiller or the whole clump. Yoshimura (1963) described the symptoms of bacterial leaf blight on grains as white water soaked spots which gradually turned to grey or yellowish white in colour at the ripening stage. Singh and Saksena (1968) reported poor grain filling and complete prevention of earhead development in severe areas of bacterial leaf blight affected crops.

AGE OF THE PLANTS AND SUSCEPTIBILITY TO BACTERIAL LEAF BLIGHT

Tagami et al. (1964) observed that severe epidemics of bacterial leaf blight occurred in paddy fields when the infected seedlings were transplanted. Seneviratne (1962) reported that bacterial leaf blight was wide

spread on rice in Ceylon and the symptoms were best seen at the flag leaf stage. Devadath and Padmanabhan (1969) studied the response of 20 rice varieties to nine isolates of Xanthomonas oryzae by the multiple pin prick inoculation method at seedling and flag leaf stages. Lesion spread was greater at the flag leaf stage in all the varieties except Chinsurah and Kaohsiung. Mahmood and Singh (1970) reported that the severity of infection increased significantly with increasing age of the seedlings. Fifty days old seedlings showed maximum infection than that of 40, 30 and 20 days old seedlings. According to Devadath and Rao (1973) varieties such as Karuna, IR-20 and IR-9 showed more susceptibility to bacterial leaf blight at flowering stage than at tillering and maximum tillering stages. Premalatha Dath and Padmanabhan (1973) reported that when the older leaves and the very young unfolded leaves were artificially inoculated with Xanthomonas oryzae at the maximum tillering stage, the older leaves first showed the symptoms, followed by the younger leaves. According to Ho and Lim (1976) in the rice variety Jaya, maximum disease intensity was noticed in 50 to 67 days old plants, corresponding to the maximum tillering and booting stages. A disease intensity of 74 to 98 per cent was noticed during this period.

Hsieh and Chang (1977) reported that young plants were more susceptible to kresek than older ones. Maw et al. (1979) also studied the kresek symptom of bacterial leaf blight of rice and found that it was less frequent when older plants were infected by the pathogen. Srinivasan (1982) reported that kresek appeared in plants at different ages but the intensity and lag period varied. Kresek appeared after 30 days of transplanting in 12 to 40 days old seedlings while the symptoms developed with a lag period in 40, 45 and 50 days old seedling.

EFFECT OF HOST NUTRITION ON THE INCIDENCE OF BACTERIAL LEAF BLIGHT

Mahmood and Singh (1970) found that the disease intensity due to bacterial leaf blight increased with increasing levels of nitrogen. Umagupta and Padmanabhan (1972) conducted a nutritional experiment using 0, 40, 80 and 120 kg/ha of nitrogen and found that with the increase in the level of nitrogen the disease intensity also increased. Singh and Kodgal (1976) reported that bacterial leaf blight increased with increasing levels of nitrogen upto 200 kg/ha but no disease was recorded at 50 kg/ha or with no nitrogen. According to Devadath and Rao (1979)

and Haidu et al. (1979) there was a significant difference in lesion length in susceptible cultivars with increase in nitrogen content. Reddy et al. (1979) conducted a field study using two rice cultivars to compare the effect of various levels of nitrogen in both yield and infection by Xanthomonas oryzae. They found that high nitrogen application (100 kg/ha) increased the leaf blight and reduced the yield in susceptible cultivars. Choi et al. (1980) reported that the incidence of blight increased with an increase in nitrogen regardless of the planting density.

Devadath (1969) found that at different levels of nitrogen used, the addition of potassium over 60 kg/ha was associated with a lower incidence of bacterial leaf blight. The application of nitrogen and potassium at 160 kg/ha resulted in reduced lesion length whereas nitrogen and potassium at 60 and 180 kg/ha respectively lead to an increase in infection. Studies conducted at the Central Rice Research Station, Cutback, showed that in variety T(N)1, nitrogen and potassium when applied at the rate of 60 kg/ha reduced the incidence of bacterial leaf blight (Anon, 1970). However, Ranga Reddy and Sridhar (1973) have reported that the application of

different levels of potassium had no effect on the development of this disease in the highly susceptible variety, T(N)1 although it reduced the incidence in the less susceptible variety such as IR-8.

Kim and Cho (1970) reported that nitrogen application was positively correlated with lesion development irrespective of the application of phosphorus and potassium. Padmanabhan (1973), Mukhopadhyay and Dasgupta (1980) conducted experiments using various combinations of nitrogen, phosphorus and potassium in order to study their effect on the incidence of bacterial leaf blight. They found that with an increase in the nitrogen level there was an increase in the lesion development and phosphorus did not influence the development of disease symptoms. Mohanty et al. (1983) reported that nitrogen at 60 and 120 mg/l of the culture solution and phosphorus at 25 mg/l enhanced the susceptibility of rice to Xanthomonas oryzae, where as potassium at 160 mg/l although reduced the susceptibility of intermediate cultivars like IR-8, it had no effect on a susceptible variety, like T(N)1.

Tagami and Mizukumi (1962) found that higher doses of silicate and magnesium increased the severity of bacterial

leaf blight in T(N)1. Mohanty and Reddy (1978) studied the influence of molybdenum, manganese and copper on the incidence of bacterial leaf blight. They found that as the concentration of copper increased from 10 to 100 ppm there was a decrease in disease intensity. However, there was no effect due to manganese and molybdenum. Mohanty and Reddy (1982) also studied the effect of calcium and magnesium at 10, 20, 40, 60, 80 and 100 ppm on the occurrence of bacterial leaf blight in T(N)1, IR-8 and Malagkit sung-song. They observed that increased supply of calcium reduced the susceptibility of plants to Xanthomonas oryzae while magnesium markedly enhanced the susceptibility.

EFFECT OF WEATHER ON THE INCIDENCE OF BACTERIAL LEAF BLIGHT

Soga (1918) observed that an endemic area to bacterial leaf blight was one with acidic soil, poor drainage, relatively high underground water level and frequent flooding. Kuwazuka (1942) concluded that the disease was more prevalent in areas with more than 20 mm of rainfall in July and an annual mean temperature of 14°C and above. According to Goto et al. (1955) high rainfall, little sunshine, strong wind and temperature

of 22 to 26°C favoured the out break of bacterial leaf blight. Studies conducted at the International Rice Research Institute, Philippines, have shown that a temperature range of 25 to 35°C was most favourable for the development of bacterial leaf blight (Anon 1974). Reddy and Pillai (1974) reported that a well distributed rainfall and a relative humidity of 90 per cent and above for 15 h per day favoured the onset of bacterial leaf blight. According to Mohiuddin (1977) if there were more than 27 rainy days during August, September and October, then, there was more incidence of bacterial leaf blight. Srinivasan and Singh (1983) reported that a combination of weather conditions like maximum temperature of 30 to 35°C, minimum temperature of 24 to 26°C, relative humidity of 64 to 68 per cent and a heavy well distributed rainfall associated with short sunny days favoured a severe outbreak of bacterial leaf blight of rice.

SURVIVAL OF XANTHOMONAS CAMPESTRIS PV ORYZAE IN VARIOUS MATERIALS

Hashioka (1951) reported that bacterial leaf blight resulted from seed beds where, Xanthomonas over winter in soil from a heavily infected preceding rice crop.

Chattopadhyay and Mukherjee (1974) studied the survival of Xanthomonas oryzae in soil by dilution plate technique and found that the pathogen survive^v for seven days in unsterilized soil and for one month in sterilized soil. Singh (1971) reported that Xanthomonas oryzae did not survive in unsterilized soil for a week or over summer in the field in manure or in compost pits. Mary and James Mathew (1980) conducted an in vitro study for the survival of Xanthomonas oryzae in artificially inoculated unsterilized and sterilized soil. They found that the pathogen could survive only for seven days in infected soil.

Srivastava and Rao (1968) reported that under the conditions prevailing in North India, infected straw or stubbles did not serve as an effective source of primary inoculum for the spread of bacterial leaf blight. A similar observation was also made by Chattopadhyay and Mukherjee (1974) who found that the infectivity was low in dry tissues of leaves, stubbles and dead roots. Nwigwe (1975) noted that Xanthomonas oryzae survived at least for five days on the leaf surface and the longevity was greatly determined by environmental conditions, especially the temperature. According to Chattopadhyay

and Mukherjee (1975) the residue from harvested crops provided the inoculum for the subsequent crop, particularly when two crops were raised in a year. According to Mary and James Mathew (1980) the Pathogen could survive only for 25 days in infected plant debris and crop refuse. Murthy and Devadath (1981) gave evidence that volunteer plants may be the source of primary inoculum for the next crop, especially under low land conditions in double cropped areas. Trimurthy et al. (1982) suggested that the pathogen survived on diseased stubbles after harvest on soil surface for 190 and for 130 days when buried in the soil. Flooding of infected materials reduced greatly the survival period.

According to Fang et al. (1956) Xanthomonas oryzae was present in the endosperm of the grain and the percentage of infected seeds varied from one to ten. Srivastava and Rao (1954) reported success in the seed transmission of bacterial leaf blight both under laboratory and field conditions. Smachit and Ou (1970) made attempts to demonstrate the seed transmission of the disease without any success. They concluded that only from the severely inoculated seeds, the seedlings became infected. According to Chattopadhyay and Mukherjee (1971) in naturally infected seeds, Xanthomonas oryzae survived

for 30 to 60 days after harvest depending on the variety. Singh and Rao (1977) demonstrated the presence of Xanthomonas oryzae in and on the seeds for a period upto 11 months after harvest. Kauffman and Reddy (1975) reported that the presence of the pathogen could be detected in infected seeds by the concentrated suspension method and the phage technique upto two months after harvest. According to Mary and James Mathew (1980) Xanthomonas oryzae survived for 90 days in infected seed materials. Raina et al. (1981) reported that the bacteria over winter to the next crop season through infected seeds and in the rhizosphere of wheat and other non host plants. Singh et al. (1981) observed that the bacterium could survive for about ten months at room temperature and the seeds retained enough infectivity till the next season to cause an epidemic under favourable conditions. Pal et al. (1982) studied the survival of Xanthomonas oryzae on varieties like Pusa 33 and T(N)1 and found that about 35 to 36 per cent of the infection was noticed when the seeds were stored in plastic jars at room temperature. However, the bacterium was not detected after three months of storage.

CONTROL OF BACTERIAL LEAF BLIGHT OF RICE

Several reports have been published on the damage of rice caused by Xanthomonas campestris pv oryzae. Ishiyama (1922) reported a reduction of 20 to 30 per cent in yield when the infection was moderate and over 30 per cent when it was severe. The weight of 1000 grains of unhulled rice was also reduced by the disease. Ikano (1958) reported that the percentage of husked, sterile, unfilled grains showed an increase in diseased plants. Studies conducted at International Rice Research Institute, Philippines have revealed that the average loss in yield due to bacterial leaf blight was 33.1 per cent in T(N)1, 46.8 per cent in Tainan 8 and 74.89 per cent in IR 8 under field conditions (Anon 1967).

In India considerable loss in yield due to bacterial leaf blight has been reported by many workers. Srivastava et al. (1966) calculated that almost one million hectares of rice crop in India was affected by bacterial leaf blight with an yield loss upto 60 per cent. Ray and Sengupta (1970) studied the incidence of bacterial leaf blight in Tripura on T(N)1 and observed that in summer rice from December to April, the disease severity

was mild so that there was no measurable yield loss. However in transplanted, winter and autumn crops from May to September and July to December respectively, the intensity of infection was so severe that there was considerable yield loss. According to Rao and Kauffman (1971) during the monsoon season, bacterial leaf blight was more severe on dwarf varieties like T(N)1 and Jaya with an yield loss as high as 50 per cent. Mohiuddin et al. (1977) reported that infection of Co-33 variety of rice at the flag leaf stage resulted in 30 to 40 per cent loss in yield. Rao and Kauffman (1977) observed a potential grain loss of 56 per cent from Andhra Pradesh in highly susceptible Karuna, 10 per cent in moderately susceptible IR-8 and in significant loss in relatively resistant IR-22 during the monsoon season under field conditions. The influence of Xanthomonas oryzae on the yield component of the rice cultivars such as Karuna, Sona and T(N)1 was also studied by Reddy et al. (1978) at the Central Rice Research Institute, Cuttack. They found that when the crop was infected at the panicle initiation stage, the yield reduction was about 72.7 per cent in Karuna and 43 per cent in Sona. The loss in yield due to the disease at the flowering stage was only 25 to 28 per cent in Sona and T(N)1. According to

Raina et al. (1981) an yield loss of 60 to 70 per cent was caused in 1980 due to this disease in T(N)1 at Punjab.

Several chemicals have been tried for the control of bacterial leaf blight of rice caused by Xanthomonas campestris pv oryzae. Earlier, copper and mercuric compounds were tested to control this disease. Hashioka (1951) found that spraying with bordeaux mixture partly controlled the bacterial leaf blight if applied before the typhoon. However it was not effective enough for practical use because of the copper sensitivities of many rice varieties. Jain et al. (1965) reported that spraying with coppesan (copper oxychloride) at 2.8 kg/ha reduced the infection by Xanthomonas oryzae.

Swarup et al. (1965) suggested that penicillin G (100 ppm), dithane-M 22 (200 ppm) and $HgCl_2$ (1000 ppm) gave maximum inhibition against Xanthomonas oryzae under in vitro conditions. Deasi et al. (1967) reported that Xanthomonas sp. was inhibited by streptocycline, tetracycline, oxytetracycline and chloramphenicol at 500 ppm concentration. Shetty and Rangaswami (1968) studied the translocation of streptocycline from treated seeds by examining the plumule and radicle by the seeded

agar plate technique. They found that repeated application of streptomycin induced the development of resistant strains of Xanthomonas.

Pal and Das (1968) observed that spraying with agrimycin at the rate of 15 g/112 l of water completely checked the growth of Xanthomonas oryzae. However, Singh et al. (1977) reported that agrimycin and streptomycin were not effective in controlling this disease. Balaraman and Soumini Rajagopalan (1978) conducted a pot culture experiment for the control of bacterial leaf blight and found that erythromycin was highly effective when three sprays were given at 15 days interval. This was followed by the treatments with 1000 ppm of chloramphenicol and terramycin. Chen et al. (1980) purified an antibiotic identical with foraycin produced by Nocardia sp. which showed a curative effect against Xanthomonas oryzae.

Inderawati and Heitefuss (1977) observed that on agar medium containing 10 µg/ml of the commercial formulation of propanil, the growth of Xanthomonas oryzae was reduced by 50 per cent to that of the control. Deiveega sundaram et al. (1977) found that different concentrations (250, 500 and 1000 ppm) of various fungicides such as thiram, ceptan, vitavax and difoltan were effective in

inhibiting the growth of Xanthomonas oryzae. According to a field experiment conducted by Krishnappa and Singh (1978) TF 130 gave maximum control of bacterial leaf blight followed by agrimycin 500. Chauhan and Vaishnao (1980) observed that the best method for the control of Xanthomonas oryzae was the application of streptocycline along with copper containing compounds. Verma et al. (1980) reported that dithene C-90 and fytolan both at 0.3 per cent concentration reduced the bacterial leaf blight of rice.

Sulaiman and Ahamed (1966) recommended various control measures for bacterial leaf blight such as the burning of crop residue in the field and seed treatment with organomercuric fungicides. Jain et al. (1966) observed that the dipping of seeds for eight hours in 0.1 per cent ceresan wet and streptocycline at 0.3 g in 12.5 l of water had significant effect in controlling the initial infection and spread of bacterial leaf blight. Singh et al. (1980) found that terramycin, brestanol, agrimycin at 500 ppm and a combination of agrimycin 100 and fytolan gave effective control of the blight phase of the disease. Durgopal et al. (1981) reported that on agar medium thiram effectively inhibited the growth of Xanthomonas oryzae at 250 ppm. Thiram along with agrimycin

100 and streptomycin were also highly effective against Xanthomonas oryzae.

Jain et al. (1965) conducted an experiment to assess the relative effectiveness of various cultural and chemical treatments in controlling bacterial leaf blight of rice. They found that rubbing was not sufficient for the control of bacterial leaf blight. However, the application of plant protection chemicals like agalol-3, coppasan brought considerable reduction in the bacterial leaf blight infection. Singh and Nene (1967) suggested that Xanthomonas oryzae could be eradicated from rice seeds by soaking the seeds in water at room temperature for 12 h followed by hot water treatment at 53°C for 30 minutes. Naidu et al. (1980) found that mixing of urea with neemcake or coaltar increased the incidence of bacterial leaf blight of rice. Lin et al. (1981) suggested that bacterial leaf blight can be controlled only by an integrated method consisting of growing resistant cultivars, raising nursery beds in disease free plots with good drainage and by spraying the seedlings in epidemic areas once or twice with phenazine in the nurserybed itself. Dutta and Rafey (1982) reported that pruning the tip of seedlings before transplanting enhanced the incidence of bacterial leaf blight.

Bacteriophage specific for Xanthomonas oryzae was first isolated by Yoshi et al. (1953). Wakimoto (1954) made a detailed study of the phage and named it as Xanthomonas oryzae Op. bacteriophage. He further used this phage for the quantitative determination of Xanthomonas in soil and irrigation water. Investigations on the phage population in the irrigation water running along the canals and rivers were subsequently used for forecasting the occurrence of bacterial leaf blight in large areas, which was important for using chemicals for protection against this disease (Anon, 1967).

Many workers have also conducted research for evolving varieties resistant to bacterial leaf blight. Reddy (1965) observed that among the 16 varieties of rice tried against Xanthomonas oryzae, W.5296 was relatively resistant. Mahmood and Singh (1970) reported that while varieties such as T(N)1, T-65 and Padma were highly susceptible to bacterial leaf blight, IR-5 and IR-8 were moderately susceptible and BRT, M 136 were resistant. Rice varieties like IR-20 and IR-22 were earlier found to be resistant to bacterial leaf blight (Anon, 1970). Later it was found that these varieties also became susceptible to a virulent strain of Xanthomonas oryzae (Anon, 1973).

Dath et al. (1977) found that the build up of bacterial leaf blight was greater in highly susceptible varieties like T(N)1, than in moderately susceptible varieties. Wu et al. (1981) classified rice varieties resistant to bacterial leaf blight into three categories such as broad spectrum resistant varieties like IR-20, IR-8, non-broad spectrum resistant varieties like Zenith, Tetep, Co-22 and non-resistant variety like Tadakan.

MATERIALS AND METHODS

MATERIALS AND METHODS

A study on the effect of age of plants and host nutrition on the incidence of bacterial leaf blight (BLB) of rice caused by Xanthomonas campestris pv oryzae was conducted at the College of Agriculture, Vellayani, Trivandrum during 1983-84. The field experiment to evaluate the comparative efficacy of different control measures for bacterial leaf blight was laid out in a farmer's field at Kinakary in Kuttanad. The following rice varieties were used for the present investigation.

Sl. No. Variety	Duration	Degree of susceptibility to bacterial leaf blight	Experimental purpose
1. T(N)1	110-115	Highly susceptible	a) Effect of age of plants and host nutrition on the incidence of bacterial leaf blight. b) survival of the pathogen in various materials.

Sl. No.	Variety	Duration	Degree of susceptibility to bacterial leaf blight	Experimental purpose
2.	Jaya	120-125	Susceptible	Effect of age of plants on the incidence of bacterial leaf blight.
3.	Culture-4	120-125	Susceptible	Chemical control of bacterial leaf blight under field conditions.
4.	IR-20	120-125	Moderately resistant	Effect of age of plants on the incidence of bacterial leaf blight.

I. THE PATHOGEN

A virulent strain of Xanthomonas campestris pv oryzae maintained at the Department of Plant Pathology, College of Agriculture, Vellayani was used for the present study. The culture was however, tested once for its virulence by inoculating healthy seedlings of T(N)1. Artificial inoculation was done by clipping the tip of young leaves with a pair of scissor dipped in 48 h old suspension of the pathogen grown on potato sucrose peptone agar medium of following composition and by spraying the same suspension over the entire leaf surface.

Composition of potato sucrose peptone agar medium

KH_2PO_4	-	0.2 g
Na_2HPO_4	-	0.5 g
$\text{Ca}(\text{NO}_3)_2$	-	0.5 g
FeSO_4	-	0.05g
Kcl	-	0.05g
Peptone	-	2.0 g
Sucrose	-	20.0 g
Agar	-	20.0 g
Potato	-	300.0 g
Distilled water-		1000 ml
pH	-	7.0

The plants were covered with polythene bags for two days in order to maintain a high level of humidity required for infection. The pathogen was then reisolated from a portion of the infected leaf showing profuse ooze. This was initially cut into small bits and surface sterilized with 0.1 per cent mercuric chloride solution for one minute in a sterile petri-plate. These were washed thoroughly in three changes of sterile distilled water and teased a part to get a dense bacterial suspension. A loopful of this suspension was streaked aseptically on potato sucrose peptone agar medium. The plates were

incubated for 48 h at room temperature. Typical colonies of Xanthomonas campestris pv oryzae, which appeared on these plates were selected. They were checked for purity by streaking once on potato sucrose peptone agar medium and by gram staining (Hucker, 1927). A pure culture of this bacterium was then maintained on the same medium for further studies.

2. EFFECT OF AGE OF PLANTS ON THE INCIDENCE OF BACTERIAL LEAF BLIGHT IN T(N)1, JAYA AND IR-20.

A pot culture experiment was conducted using three different varieties of rice such as T(N)1, Jaya and IR-20 in order to assess the relative susceptibility of these varieties to bacterial leaf blight at four critical stages of plant growth. These were the seedling, maximum tillering, panicle initiation and early flowering stages. Three replications were maintained for each treatment in earthen pots of size 14" x 14". The plants were artificially inoculated at the chosen stage of growth with a dense suspension of the pathogen (5×10^9 cells/ml) grown in the basal medium for Xanthomonads supplemented with 20 g/l of sucrose.

Composition of the modified basal medium for Xanthomonads

NH ₄ H ₂ PO ₄	- 0.5 g
K ₂ HPO ₄	- 0.5 g
Mg SO ₄	- 0.2 g
NaCl	- 5.0 g
Yeast extract	- 1.0 g
Sucrose	- 20.0 g
Distilled water	- 1000 ml
pH	- 6.8

Artificial inoculation was done by the method described earlier. Observations on the onset of symptoms, disease intensity and susceptibility to bacterial leaf blight at different stages of plant growth were taken. The Disease intensity was recorded on the 14th day after inoculation as per the standard score chart prepared by the International Rice Research Institute, Philippines (Anon 1976).

$$\text{Disease intensity} = \frac{\text{Sum of individual rating}}{\text{Total No. of plants observed}} \times \frac{100}{\text{maximum disease score}}$$

3. EFFECT OF HOST NUTRITION ON THE INCIDENCE OF BACTERIAL LEAF BLIGHT IN T(N)1.

A pot culture experiment was conducted for studying the effect of varying levels of NPK and certain minor elements such as Zn, Mn and B on the incidence of bacterial leaf blight in T(N)1. Soil for this purpose was collected from the Instructional farm of College of Agriculture, Vellayani. It was analysed for its NPK content by the following standard procedures.

Estimation of total nitrogen by micro kjeldahl method

One gram of air dried soil passing through 0.25 mm sieve was digested with an equal quantity of digestion mixture consisting of potassium sulphate, copper sulphate and selenium powder in the ratio of 10:1:0.1 and 10 ml of concentrated sulphuric acid by heating for 30 minutes in a Kjeldahl flask. The clear digestate was allowed to cool down to room temperature before the volume was made upto 50 ml in a volumetric flask. Ten milli litre of this solution was then steam distilled with an equal volume of 50 per cent sodium hydroxide solution in a markham still. The ammonia distilled out was collected in 10 ml of four per cent boric acid mixed indicator solution of following composition.

Preparation of boric acid indicator solution.

A. Boric acid solution

Boric acid - 40.0 g
 Distilled water - 1000 ml

B. Mixed indicator solution

Bromocresol green - 0.5 g
 Methyl red - 0.1 g
 Ethanol (95%) - 100 ml

Add five milli litre of solution B to solution A.

The amount of ammoniacal nitrogen in the distillate was determined by titration against 0.01 N hydrochloric acid. From the titre value, the percentage of nitrogen and organic carbon in the soil sample was calculated.

$$\text{A. Percentage of nitrogen in the soil sample} = \frac{V \times N \times V_1 \times 0.014}{V_2 \times W} \times 100$$

V - titre value - blank value

V₁ - total volume of sample made up

V₂ - volume of sample distilled

N - normality of HCl.

W - weight of soil sample

B. Percentage of organic carbon } Percentage nitrogen
 in the soil sample) in the soil sample x 10

Determination of Phosphorus by Bray's extraction method

Five gram of air dried soil passing through 0.25 mm sieve was mixed with 50 ml of Bray's solution of following composition in a glass stoppered bottle.

Bray's solution

Ammonium fluoride	- 1.11 g
Hydrochloric acid (6 N)	- 4.16 ml
Distilled water	- 1000 ml

The mixture was shaken for five minutes before the supernatant was filtered through a moist Whatman No.42 filter paper. Five milli litre of this filtrate was then transferred to a 50 ml volumetric flask to which 5 ml of ammonium molybdate reagent and 1 ml of stannous chloride solution of following composition were added.

Ammonium molybdate reagent

Dissolve 15 g of ammonium molybdate in 300 ml of warm distilled water. Add 350 ml of 10 N HCl to this solution with constant stirring and dilute the resulting solution

with distilled water to 1000 ml in a volumetric flask.

Stannous chloride solution

Stannous chloride - 10 g
 Concentrated hydrochloric acid - 25 ml

Dilute the above solution by pipetting one milli litre into 30 ml of freshly prepared distilled water.

After ten minutes, the intensity of blue colour developed was measured in a Klett summerson photo electric colorimeter (Arthur H. Thomas Company, U.S.A.) at 600 nm using the red filter. A standard curve was also prepared in a similar manner using 0.04, 0.08, 0.12, 0.16, 0.2 ppm solution of potassium dihydrogen phosphate. The concentration of phosphorus in the filtrate was determined from the standard curve and the quantity of phosphorus (kg/ha) in the entire soil sample was then calculated as follows:

$$\text{Phosphorus content of the soil sample (kg/ha)} = \frac{P \times V_1 \times V_3}{W \times V_2} \times 2.24$$

P - ppm concentration of phosphorus in the filtrate

V₁ - volume of extractant (Bray's solution)

V₂ - Aliquot of soil extract used

V₃ - volume to which the aliquot is made up

W - weight of soil

Determination of potassium by Flame ionization technique

Ten gram of air dried soil passing through 0.25 mm sieve was mixed with 25 ml of neutral ammonium acetate solution of following composition and allowed to stand for 24 h at room temperature.

Neutral ammonium acetate solution

Add 57 ml of glacial acetic acid to 800 ml of distilled water. Neutralize this solution with ammonium hydroxide to pH 7 and dilute to one litre.

The supernatant was filtered through a moist Whatman No.42 filter paper. Ten milli litre of this filtrate was transferred to a 100 ml volumetric flask and the volume was made up with neutral ammonium acetate solution. The amount of potassium in the filtrate was then determined with the help of a flame ionization photometer (Evans electroselenium Ltd., England). A standard curve was also prepared in a similar manner using 1.25, 2.5, 3.75, 5 and 6.25 ppm solution of potassium chloride. The concentration of potassium in the filtrate was determined from the standard curve and the quantity of potassium (kg/ha) in the entire soil sample was then calculated as follows:

$$\text{Potassium content of the soil sample (kg/ha)} = \frac{P \times V}{W} \times 2.24$$

P - Concentration of potassium in the extract (in ppm)

V - Total volume made up

W - Weight of soil sample

NPK recommendation based on soil analysis

The NPK recommendation based on soil analysis and the package of practices recommendation of Kerala Agricultural University (1982) for a medium-duration high yielding rice variety is given below.

NPK content of the soil	NPK recommendation based on soil analysis (kg/ha)	NPK recommendation based on package of practices (kg/ha)
0.77 (percentage organic carbon)	87.5	90
14.78 (kg/ha of P)	40	45
44.80 (kg/ha of K)	56.5	45

* Recommendation of the soil testing laboratory of the Department of Agriculture at Pattambi.

As there was not much variation between the two recommendations, the latter was chosen for the present study. The different levels (kg/ha) of nitrogen, phos-

phorus and potassium tested were as follows:

$n_0p_1k_0$	$n_0p_1k_1$	$n_0p_1k_2$	$n_0p_1k_3$		
$n_0p_2k_0$	$n_0p_2k_1$	$n_0p_2k_2$	$n_0p_2k_3$		
$n_0p_3k_0$	$n_0p_3k_1$	$n_0p_3k_2$	$n_0p_3k_3$		
$n_1p_1k_0$	$n_1p_1k_1$	$n_1p_1k_2$	$n_1p_1k_3$		
$n_1p_2k_0$	$n_1p_2k_1$	$n_1p_2k_2$	$n_1p_2k_3$		
$n_1p_3k_0$	$n_1p_3k_1$	$n_1p_3k_2$	$n_1p_3k_3$		
$n_2p_1k_0$	$n_2p_1k_1$	$n_2p_1k_2$	$n_2p_1k_3$		
$n_2p_2k_0$	$n_2p_2k_1$	$n_2p_2k_2$	$n_2p_2k_3$		
$n_2p_3k_0$	$n_2p_3k_1$	(Recommended level) $n_2p_3k_2$	$n_2p_3k_3$		
$n_3p_1k_0$	$n_3p_1k_1$	$n_3p_1k_2$	$n_3p_1k_3$		
$n_3p_2k_0$	$n_3p_2k_1$	$n_3p_2k_2$	$n_3p_2k_3$		
$n_3p_3k_0$	$n_3p_3k_1$	$n_3p_3k_2$	$n_3p_3k_3$		
n_0	- 0	p_1	- 22.5	k_0	- 0
n_1	- 45	p_2	- 45	k_1	- 22.5
n_2	- 90	p_3	- 90	k_2	- 45
n_3	- 135			k_3	- 90

Since phosphorus is reported to have no effect on the incidence of bacterial leaf blight in rice, only three levels of phosphorus were used for the present investigation.

Nitrogen and potassium in the form of urea and muriate of potash were applied in two equal split doses as basal dose, at the time of transplanting and as top dressing at the time of tillering. The entire quantity of phosphorus as superphosphate was added as basal dose at the time of transplanting. The individual effect of three minor elements such as Zinc, manganese and boron on the incidence of bacterial leaf blight was also studied. These were added at the time of transplanting as zinc sulphate, manganese dioxide and borax at the rate of 20 kg/ha along with the normal recommendation of NPK for T(N)1.

Twentyone day old seedlings of T(N)1 raised in pots without the addition of any fertilizer were used for the transplanting purpose. These were artificially inoculated at the maximum tillering stage with a dense suspension of the pathogen (5×10^9 cells/ml) by the method described earlier. Three replications were maintained for each treatment. Observations on the onset of symptoms and disease intensity were recorded on the 14th day after inoculation with the pathogen.

4. SURVIVAL OF XANTHOMONAS CAMPESTRIS pv ORYZAE IN VARIOUS MATERIALS

1. Soil

A pot culture experiment was conducted in order to study the survival of Xanthomonas campestris pv oryzae in soil. Earthen pots of size 14" x 14" wa filled with 10 kg. of soil, collected from a previous pot culture experiment where there was a natural incidence of bacterial leaf blight. Disease free seeds of T(N)1 were used for this experiment by soaking seeds in water at room temperature for 12 h followed by hot water treatment at 53°C for 30 minute (Singh and Nene, 1967). Twenty such seeds were sown in pots at three days interval for one month from the date of harvest of the previous infected crop. Observations on the percentage of plants wilted due to bacterial leaf blight were taken for one month.

2. Plant debris and crop refuse

The survival of Xanthomonas campestris pv oryzae in infected plant debris and crop refuse was studied by using respectively the infected straw and crop refuse collected after the harvest of a previous diseased crop. In both the cases, the pots were initially filled with

nine kilogram of soil followed by one inch layer of chopped infected straw or crop refuse and one inch layer of fresh soil. Twenty disease free seeds of T(N)1 were sown in three pots at weekly intervals for two months from the date of harvest of the previous infected crop. Observations on the percentage of plants wilted due to bacterial leaf blight were taken for two months.

3. Infected seed material

Seeds from bacterial leaf blight affected T(N)1 panicles were used for this purpose. These were stored at room temperature in plastic containers for further studies. The survival of the pathogen was tested by plating aseptically 15 infected seeds on potato sucrose peptone agar medium, with and without dehusking the infected seeds. The planting was done at 15 days interval for 105 days. Observation on the percentage of seeds infected with Xanthomonas campestris pv oryzae was taken after incubation at room temperature for 2 days in each case.

Artificial inoculation of disease free seeds of T(N)1 was done by soaking the seeds of 12 h in a dense suspension (5×10^9 cells/ml) of Xanthomonas campestris pv oryzae. These were then air dried after draining off the

excess of bacterial suspension and stored at room temperature in plastic containers for further studies. The survival of the pathogen in artificially infected seeds was also studied by the same method described earlier.

5. CHEMICAL CONTROL OF BACTERIAL LEAF BLIGHT OF RICE

A. In vitro evaluation of antibiotics and fungicide against Xanthomonas campestris pv oryzae

Sterile filter paper discs of ten millimeter diameter were initially dipped in the appropriate concentration of various antibiotics and fungicides either alone or in suitable combinations as indicated below. These were air dried and placed aseptically in the centre of potato sucrose peptone agar medium preseeded with a 48 h old virulent culture of Xanthomonas campestris pv oryzae. Three replications were maintained for each treatment. Observation on the mean zone of growth inhibition was recorded after 48 h of incubation at room temperature.

Different concentrations and combinations of antibiotics and fungicides tested.

Sl. Antibiotic/ No. fungicide	Manufacturing company.	Active ingredient	Concentra- tion (ppm)	Diluent for stock solution
A. <u>Pure antibiotics</u>				
1. Ambistrin	Sarabai chemicals	Streptomycin sulphate	50, 100, 250, 500	Sterile distilled water
2. Chloromycetin	Piya chemicals	Chloramphenicol	50, 100, 250, 500	-do-
3. Chlorostrep	-do-	Chloramphenicol and streptomycin	50, 100, 250, 500	-do-
4. Penicillin	Hindustan anti-biotics	Procaine penicillin	50, 100, 250, 500	-do-
5. Terramycin	Pfizer	Oxytetra-cycline hydrochloride	50, 100, 250, 500	1:1 alcohol water mixture.
B. <u>Commercial preparation of Antibiotics</u>				
1. Paushamycin	Paushak Ltd. Cochin	Streptomycin 15% and Oxytetracycline 5%	50, 100, 250, 500	Sterile distilled water
2. Plantomycin	Aries Agro-Vet Industries Pv Ltd.	Streptomycin sulphate 9% and Tetracycline hydroxide 1%	50, 100, 250, 500	-do-
3. Streptocycline	Hindustan Antibiotics Ltd.	Streptomycin sulphate 90% and Tetracycline hydroxide 10%	50, 100, 250, 500	-do-

Sl. No.	Antibiotic/ fungicide	Manufacturing company.	Active ingredient	Concentration (ppm)	Diluent for stock solution
1.	Bavistin	BASF India Ltd.	50% carbendazim.	500,750,1000,1500	Sterile distilled water
2.	Dithane M 45	Indofil chemicals Ltd.	Co-ordination product of zinc ion and maneb	3000,3500,4000, 4500	-do-
3.	Hinosan	Bayer (India) Ltd.	50% ethyl S-S diethyl diphenyl dithio phosphate	500,750,1000,1500	-do-
4.	Kitazin	Pesticides India	48% Diisopropyl benzylthiophosphate	500,750,1000,1500	-do-
<u>D. Combination of antibiotics and fungicide</u>					
1.	Streptocycline and Dithane M 45	-	-	500 + 4500 500 + 4000 250 + 4500 250 + 4000	-do- -do- -do- -do-
2.	Terramycin and Dithane M 45	-	-	500 + 4500 500 + 4000 250 + 4500 250 + 4000	-do- -do- -do- -do-

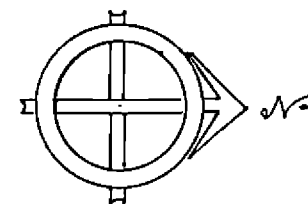
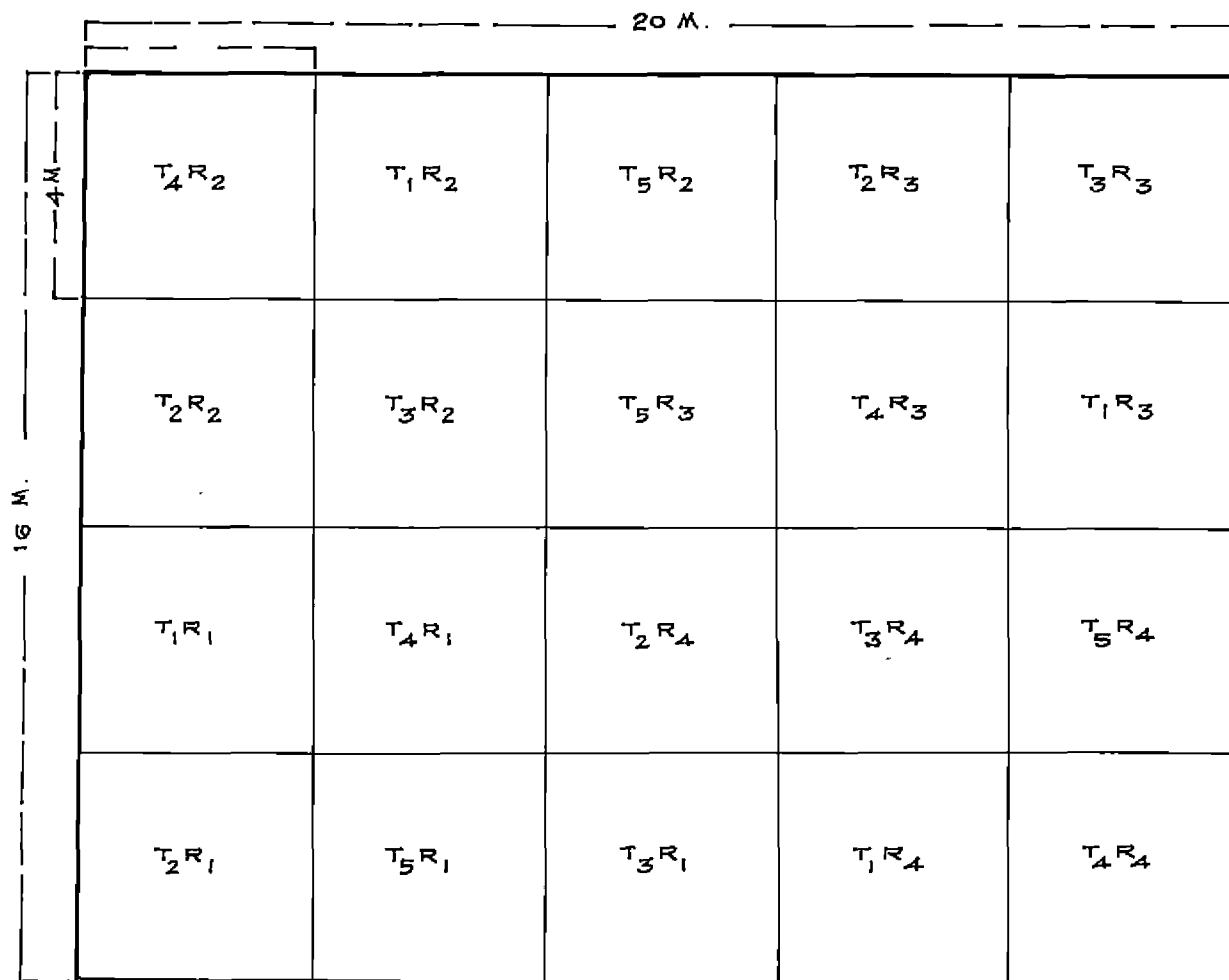
B. In vivo evaluation of antibiotics and fungicides against Xanthomonas campestris pv oryzae under field conditions

The antibiotics, fungicide and their combinations which were found to be more effective against Xanthomonas campestris pv oryzae under in vitro conditions were further used to study the control of bacterial leaf blight under field conditions. The experiment was laid out in a farmer's field at Kinakary in Kuttanad using the rice variety Culture-4 in a randomised block design (Fig.1)

NPK fertilizers were added at the rate of 90, 45 and 45 kg/ha respectively. Half the doses of nitrogen and potassium and the full dose of phosphorus were applied at the time of final ploughing. The remaining quantity of nitrogen and potassium were added as top dressing at panicle initiation stage. Lime was added at the rate of 600 kg/ha in two split doses of 350 kg as basal dose at the time of final ploughing and 250 kg as top dressing one month after sowing.

A natural incidence of bacterial leaf blight with more or less uniform disease intensity was noticed in the above field at the late panicle initiation stage

FIG. 1. LAY OUT OF THE FIELD EXPERIMENT



- T_1 TERRAMYCIN (500ppm)
- T_2 DITHANE M 45 (4500ppm)
- T_3 STREPTOCYCLINE (500ppm)
- T_4 DITHANE M 45 AND STREPTOCYCLINE (4500 AND 500ppm)
- T_5 CONTROL

during the additional crop season of the year 1984. The different control measures as per the treatment combinations specified in Figure I were taken on the 14th day after the onset of symptoms. The antibiotics and fungicide were sprayed once by using a knapsack sprayer. Control plots were sprayed with an equal volume (3.2 litre) of tap water. The disease intensity was recorded both before and after 14 days of spraying with various chemical agents. The yield data was taken at the time of harvest. Following additional observations on the maximum and minimum temperature, relative humidity and rainfall at Moncompu during the period of this experiment were recorded. Information on the incidence of bacterial leaf blight for the two major crop seasons at Kuttanad from the year 1979 along with the weather data for the same period were also collected.

RESULTS

RESULTS

1. THE PATHOGEN

Typical symptoms of bacterial leaf blight were observed in T(N)1 after seventh day of artificial inoculation with the stock culture of Xanthomonas campestris pv. oryzae. The pathogen was reisolated from infected leaves on potato sucrose peptone agar medium where it produced the characteristic yellow, slimy, colonies of Xanthomonas after 48 h of incubation at room temperature. The culture was gram negative without any contamination.

2. EFFECT OF AGE OF PLANT ON THE INCIDENCE OF BACTERIAL LEAF BLIGHT IN T(N)1, JAYA AND IR-20

The onset of symptoms of bacterial leaf blight in T(N)1, Jaya and IR-20 were observed uniformly on the fifth day after inoculation at seedling, maximum tillering and panicle initiation stages. However, at flowering stage, the symptoms appeared only on the seventh day after inoculation with the pathogen.

Significant differences between the varieties were observed with regard to their susceptibility to Xanthomonas campestris pv. oryzae. T(N)1 was found to be the most susceptible variety followed by Jaya and IR-20. The

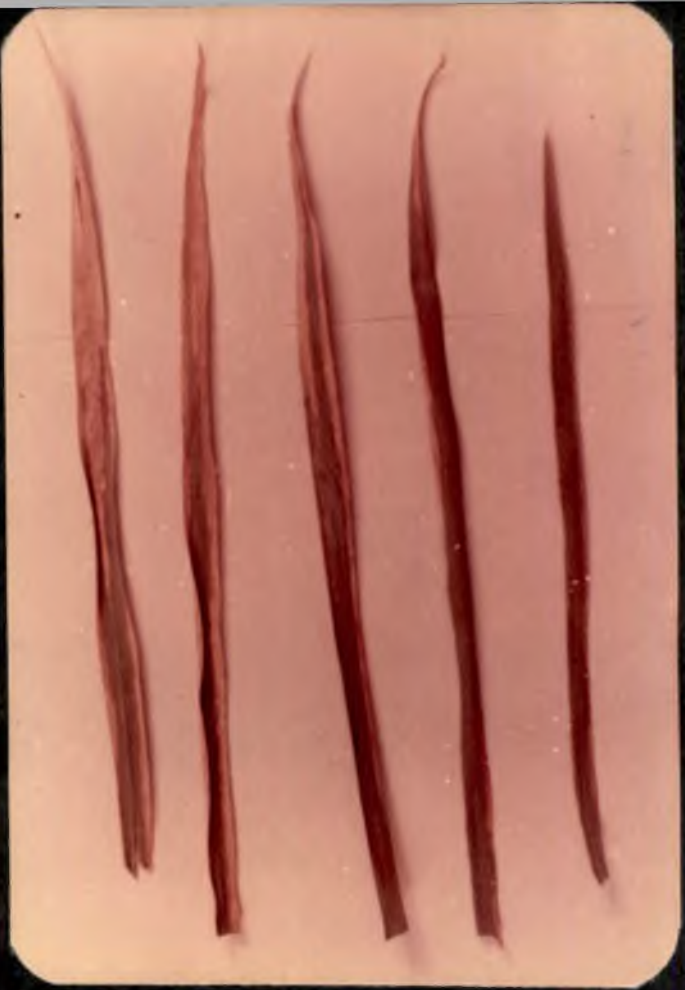


Plate I: Various stages of foliar symptoms
of bacterial leaf blight.

average disease intensity was 39.45, 34.79 and 23.05 respectively (Table 1).

Table 1. Effect of age of plants on the incidence of bacterial leaf blight in T(M)1, Jaya and IR-20

Rice Vari- ety	Disease intensity at different stages of plant growth*				Mean disease intensity for each variety
	Seedling stage	Maximum tiller- ing stage	Panicle initiation stage	Flower- ing stage	
T(M)1	33.58	52.41	40.55	31.24	39.45
Jaya	35.00	37.48	36.32	30.37	34.79
IR-20	24.81	27.69	21.45	18.25	23.05

* Mean of three replications

C.D. for comparison between varieties = 4.548

C.D. for comparison between stages = 5.293

Among the four different stages of plant growth tested for susceptibility to bacterial leaf blight, maximum tillering stage was found to be the most susceptible stage in all the three varieties (Table 1, Plate 2, 3 & 4). The extent of infection was higher at seedling stage in IR-20 and at panicle initiation stage in T(M)1 and Jaya (Figure 2, Plate 5, 6 & 7). However at flowering stage the disease intensity was uniformly low in all the three varieties (Table 1).



Plate II : Intensity of bacterial leaf blight in T(N)1 at maximum tillering stage.



Plate IV : Intensity of bacterial leaf blight in IR-20 at maximum tillering stage.



Plate V : Intensity of bacterial leaf blight
in T(M)1 at panicle initiation stage.

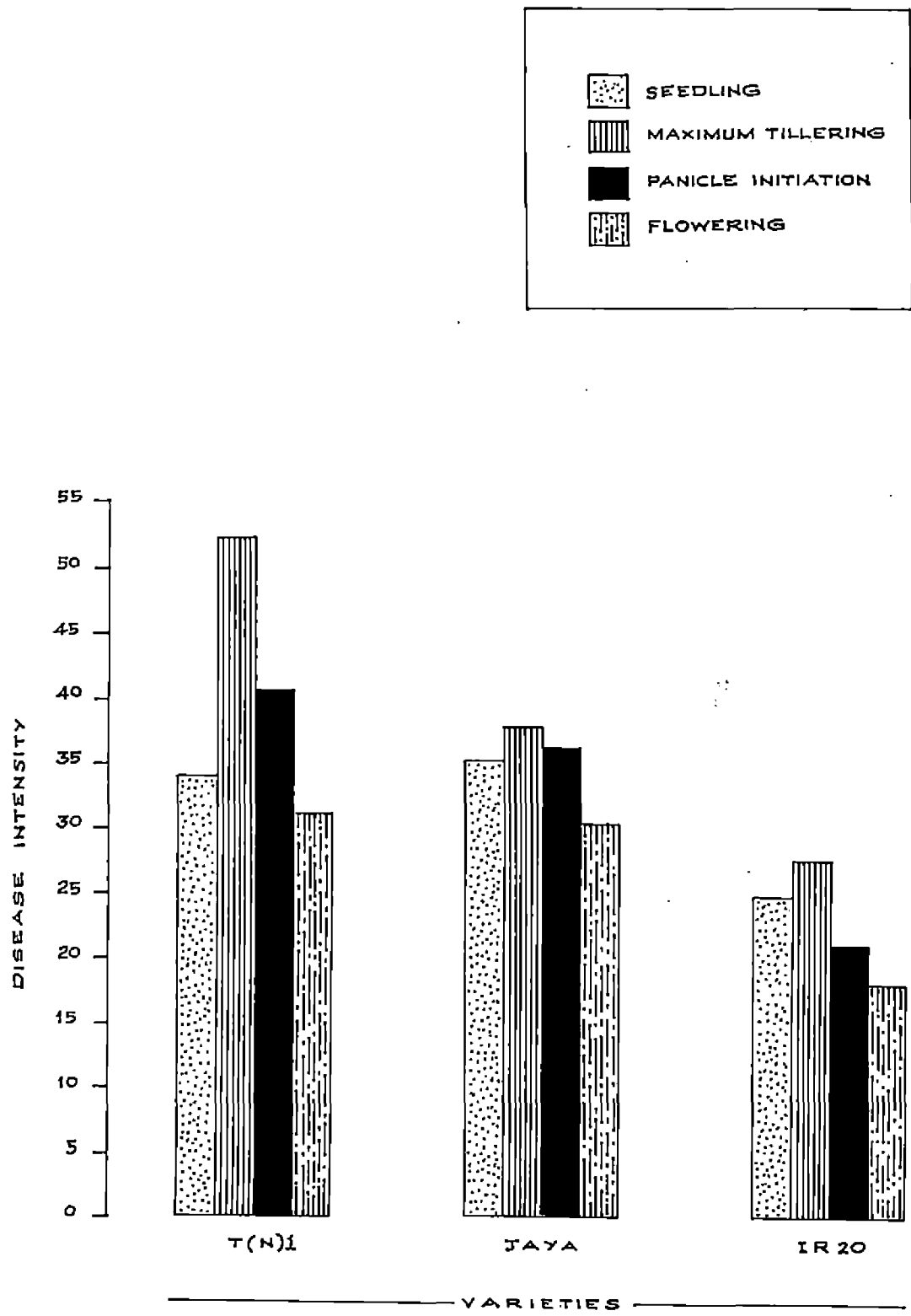


Plate VI : Intensity of bacterial leaf blight
in Java at panicle initiation stage.



Plate VII : Intensity of bacterial leaf blight
in IR-20 at panicle initiation stage.

FIG. 2. EFFECT OF AGE OF PLANTS ON THE INCIDENCE OF BLB IN T(N)1, JAYA AND IR 20.



3. EFFECT OF HOST NUTRITION ON THE INCIDENCE OF BACTERIAL LEAF BLIGHT IN T(N)1.

The data regarding the onset of symptoms and disease intensity are presented in Tables 2,3,4 and 5.

Table 2. Effect of varying levels of NPK on the onset of symptoms due to bacterial leaf blight in T(N)1.

Different levels of N and P	Levels of K/ the Day of onset of symptoms			
	K_0	K_1	K_2	K_3
n_0P_1	7	7	7	7
n_0P_2	8	7	7	7
n_0P_3	7	7	7	7
n_1P_1	7	7	7	7
n_1P_2	7	7	8	6
n_1P_3	7	8	7	7
n_2P_1	5	5	5	5
n_2P_2	6	5	5	5
n_2P_3	5	5	5	5
n_3P_1	7	5	5	5
n_3P_2	6	5	5	5
n_3P_3	5	5	5	5

Table 3. Effect of zinc, manganese and boron on the onset of symptoms due to bacterial leaf blight in T(N)1.

Treatment	Day of onset of symptoms
$n_2p_2k_2 + Zn$	5
$n_2p_2k_2 + Mn$	5
$n_2p_2k_2 + B$	5
$n_2p_2k_2 + Zn + Mn + B$	6

The onset of symptoms were either on the seventh or on the eighth day at lower levels of nitrogen n_0 and n_1 (45 kg/ha) irrespective of the different levels of potassium and phosphorus. However, with an increase in the level of nitrogen n_2 (90 kg/ ha) and n_3 (135 kg/ha) the typical symptoms of bacterial leaf blight were observed on the fifth day except in treatments like $n_2p_2k_0$, $n_3p_2k_0$ and $n_3p_1k_0$ where these symptoms appeared only on the sixth and seventh day respectively (Table 2). In pots supplemented with minor elements alone and in combination, the symptoms of bacterial leaf blight were noticed on the fifth and sixth day respectively after inoculation with the pathogen (Table 3).

Table 4. Effect of varying levels of NPK, Zn, Mn and B on disease* intensity due to bacterial leaf blight in T(N)1.

Treat- ment	Mean disease inten- sity	Treat- ment	Mean disease inten- sity	Treat- ment	Mean disease inten- sity	Treat- ment	Mean disease inten- sity	Treat- ment	Mean disease inten- sity
$n_0p_1k_0$	29.93	$n_1p_1k_0$	39.50	$n_2p_1k_0$	35.18	$n_3p_1k_0$	57.18	Zn	39.52
$n_0p_1k_1$	19.43	$n_1p_1k_1$	35.18	$n_2p_1k_1$	29.93	$n_3p_1k_1$	39.51	Mn	29.93
$n_0p_1k_2$	29.93	$n_1p_1k_2$	29.93	$n_2p_1k_2$	38.81	$n_3p_1k_2$	44.66	B	44.06
$n_0p_1k_3$	24.68	$n_1p_1k_3$	24.68	$n_2p_1k_3$	39.51	$n_3p_1k_3$	52.94	Zn+Mn+B	29.93
$n_0p_2k_0$	24.68	$n_1p_2k_0$	43.83	$n_2p_2k_0$	57.18	$n_3p_2k_0$	38.81		
$n_0p_2k_1$	35.18	$n_1p_2k_1$	34.27	$n_2p_2k_1$	34.25	$n_3p_2k_1$	34.26		
$n_0p_2k_2$	24.68	$n_1p_2k_2$	24.68	$n_2p_2k_2$	24.68	$n_3p_2k_2$	39.50		
$n_0p_2k_3$	24.68	$n_1p_2k_3$	24.68	$n_2p_2k_3$	24.68	$n_3p_2k_3$	44.06		
$n_0p_3k_0$	29.93	$n_1p_3k_0$	39.51	$n_2p_3k_0$	29.93	$n_3p_3k_0$	29.93		
$n_0p_3k_1$	29.93	$n_1p_3k_1$	29.93	$n_2p_3k_1$	19.43	$n_3p_3k_1$	29.93		
$n_0p_3k_2$	24.68	$n_1p_3k_2$	24.68	$n_2p_3k_2$	24.68	$n_3p_3k_2$	66.03		
$n_0p_3k_3$	19.43	$n_1p_3k_3$	24.68	$n_2p_3k_3$	19.43	$n_3p_3k_3$	48.16		

* Average of 3 replications

Table 5. Mean table for the interaction between different levels of NPK and disease intensity due to bacterial leaf blight in T(N)1.

	P ₁	P ₂	P ₃	K ₀	K ₁	K ₂	K ₃	Mean (N)
n ₀	25.99	27.30	25.99	28.18	28.18	26.43	22.93	26.43
n ₁	32.32	31.86	29.69	40.94	33.12	26.42	24.64	31.29
n ₂	35.85	35.19	23.37	40.76	29.87	29.39	27.67	31.47
n ₃	48.44	39.16	43.52	42.00	34.56	49.87	48.38	43.70
Mean p/k	35.65	33.39	30.64	37.97	30.94	33.03	30.97	
K ₀	40.47	41.18	32.32					
K ₁	31.01	34.49	27.31					
K ₂	35.68	28.39	35.02					
K ₃	35.45	29.53	27.92					

C.D. for comparison between N (or K) means - 5.319

C.D. for comparison between NK combination - 10.683

The study on the effect of varying levels of nitrogen, phosphorus and potassium and minor elements such as zinc, manganese and boron on disease intensity due to bacterial leaf blight in T(N)1 showed that the main effect of nitrogen and potassium was significant while that of phosphorus and minor elements like zinc, manganese and boron was insignificant (Tables 4 and 5). Pots which received zero nitrogen had the lowest disease intensity of 26.43 when compared to the disease intensity of 31.29, 31.47 and 43.70 respectively for treatments involving n_1 (45 kg/ha), n_2 (90 kg/ha) and n_3 (135 kg/ha) levels of nitrogen. A significant increase was observed with the use of 135 kg N/ha (Table 5). The application of potassium, in general, decreased the disease intensity. A significant decrease was obtained at k_1 (22.5 kg/ha) and k_3 (90 kg/ha) levels when compared to the K_0 level (Table 5). However, there was no significant difference in disease intensity, between the k_1 , k_2 and k_3 levels of potassium applied.

Only the n and k interaction was found to be significant. Since the effect of P was not significant, $n \times p$, $n \times p \times k$ and $p \times k$ interactions were also insignificant (Table 5). Pots which received different levels of potassium at zero level of nitrogen showed the least incidence

of bacterial leaf blight. In these treatments the disease intensity ranged from 22.93 to 28.18 (Table 5). But with the application of nitrogen at the rate of 45, 90 and 135 kg/ha the disease intensity increased significantly at k_0 level. However, this increase was not significant with the use of k_1 , k_2 and k_3 levels of potassium except in the case of n_3k_2 and n_3k_3 treatments where a significant increase in disease intensity was observed. In fact, the maximum disease intensity of 49.87 and 48.38 were recorded for these treatment combinations during this study (Table 5).

4. SURVIVAL OF XANTHOMONAS CAMPESTRIS pv. ORYZAE IN VARIOUS MATERIALS

1. Soil

The data based on the number of plants wilted after sowing disease free seeds of T(N)1 in soil is presented in Table 6 and Figure 3. The pathogen was found to survive in soil for about 9 days after harvest of the previous infected crop.

FIG. 3. SURVIVAL OF *Xanthomonas campestris* pv *oryzae*
IN NATURALLY INFECTED SOIL.

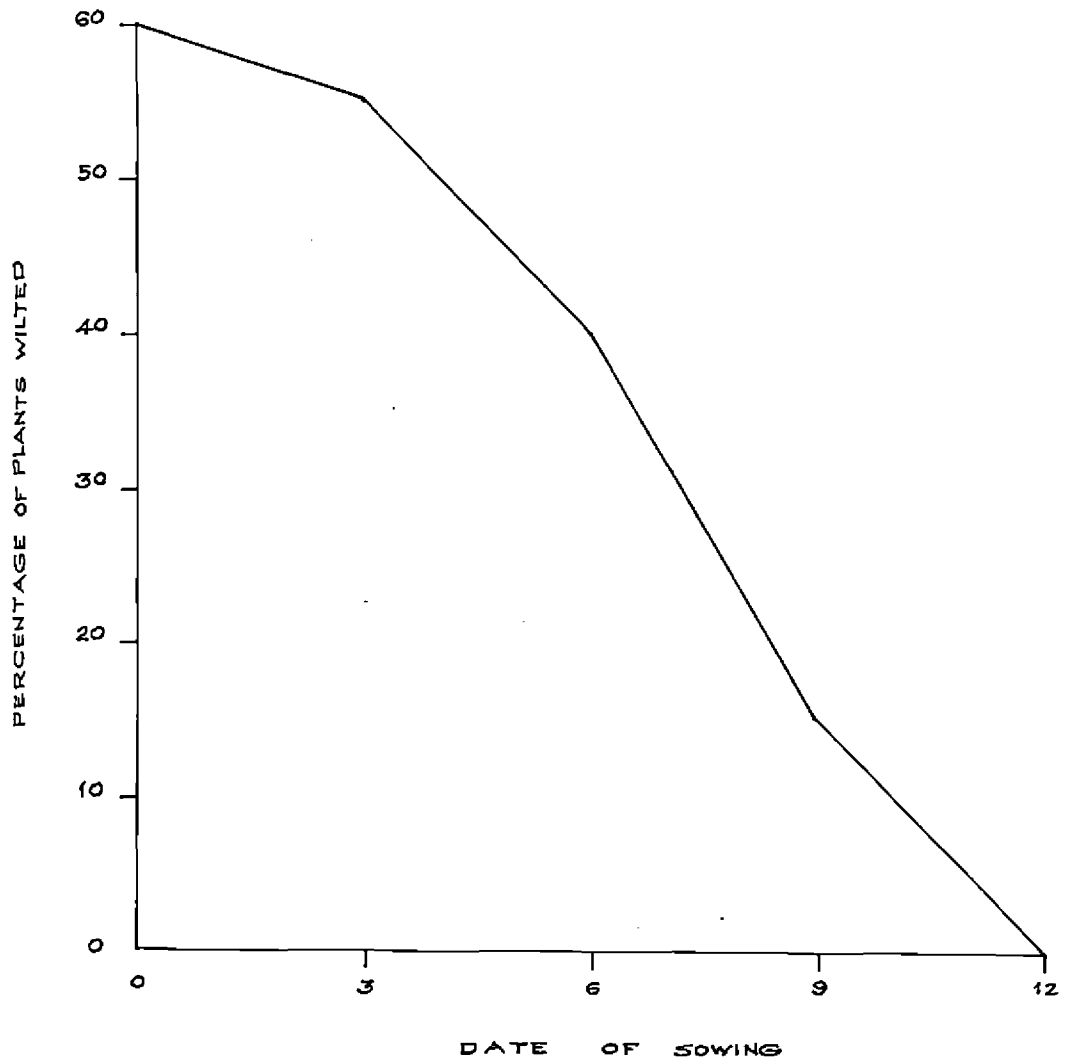


Table 6. Percentage of plants wilted due to bacterial leaf blight in soil.

Date of sowing	Total number of seeds sown	Number of plants wilted	Percentage of plants wilted
0	20	12	60
3	20	11	55
6	20	8	40
9	20	3	15
12	20	0	0

When the seeds were sown on the same day after harvest of the previous infected crop, 60 per cent of the plants wilted due to bacterial leaf blight (Table 6). The percentage of plants infected on the 3rd and 6th day was also higher in the range of 40 to 55 per cent. However, there was considerable reduction in the number of plants affected on the ninth day and there was no incidence of bacterial leaf blight in seeds sown on the 12th day after harvest of the infected crop.

2. Plant debris and crop refuse

Xanthomonas campestris pv oryzae was found to survive in infected straw and crop refuse for about 28 days after harvest of the previous infected crop. The

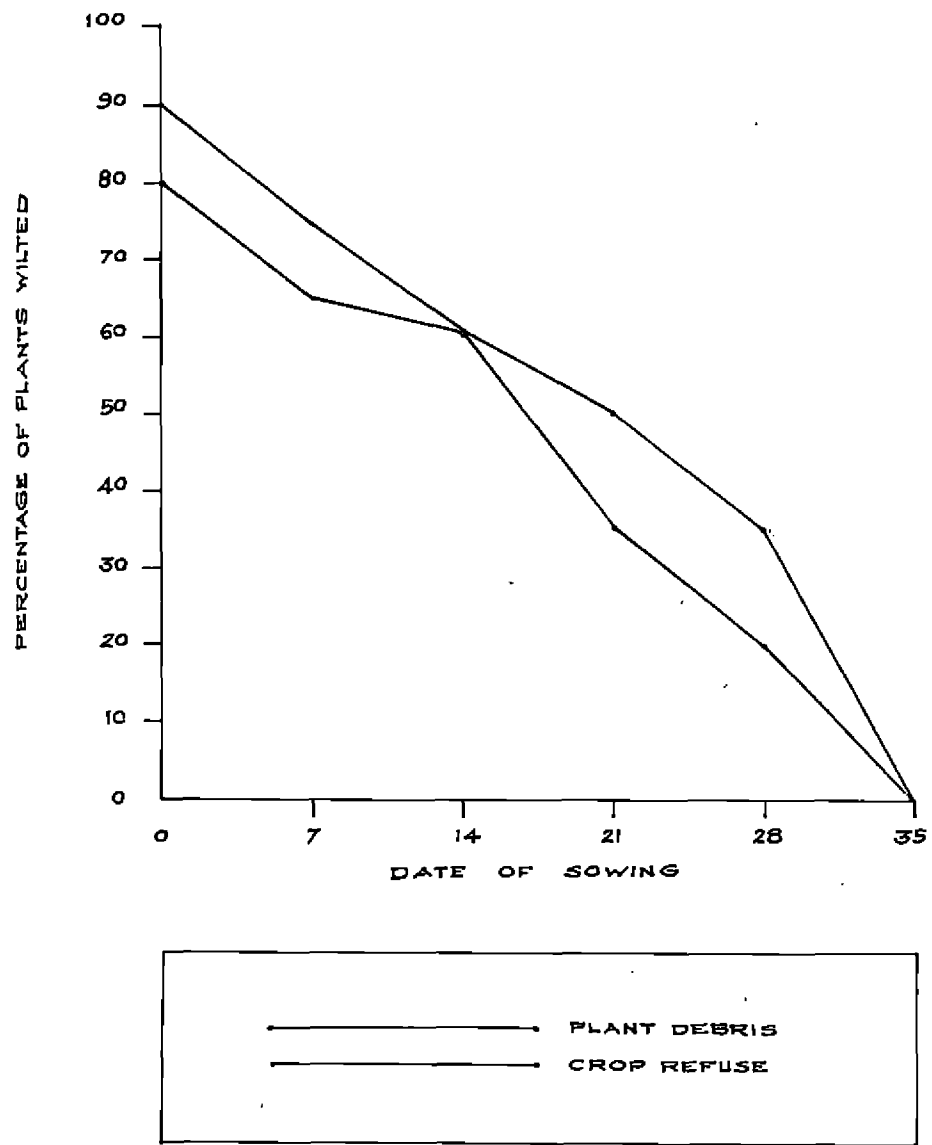
data based on the number of plants wilted after sowing disease free seeds of T(N)1 in soil supplemented with the infected straw and crop refuse is given in Table 7 and Figure 4.

Table 7. Percentage of plants wilted due to bacterial leaf blight in soil supplemented with infected plant debris and crop refuse

Infected material							
Plant debris				Crop refuse			
Date of sowing	Total No. of seeds sown	No. of plants wilted	Percentage of plants wilted	Date of sowing	Total No. of seeds sown	No. of plants wilted	Percentage of plants wilted
0	20	16	80	0	20	18	90
7	20	13	65	7	20	15	75
14	20	12	60	14	20	12	60
21	20	7	35	21	20	10	50
28	20	4	20	28	20	7	35
35	20	0	0	35	20	0	0

When seeds were sown on the same day after harvest of the previous infected crop, 80 to 90 per cent of the plants were wilted due to the incorporation of infected plant debris and crop refuse respectively (Table 7). The percentage of plants infected on the 7th, 14th and 21st day were also higher in both the cases. However,

FIG. 4. SURVIVAL OF *Xanthomonas campestris* pv *oryzae* IN INFECTED PLANT DEBRIS AND CROP REFUSE.



there was a progressive reduction in the number of plants affected on the 29th day and there was no incidence of bacterial leaf blight in seeds sown on the 35th day after harvest of the infected crop. The pathogen was found to survive relatively better in crop refuse than in plant debris.

3. Infected seed material

In infected seeds of T(N)1, the pathogen was found to survive for about 90 days. The data based on the percentage of seeds infected with Xanthomonas campestris pv. oryzae detected by plating on potato sucrose peptone agar medium is given in Table 3 and Figure 5. In general, a higher percentage of infection was noticed in the case of husked seeds. The pathogen could be detected for about 60 days in both naturally and artificially infected seeds.

The number of seeds infected on the 90th day was considerably low and on the 105th day no infection could be detected either in naturally or artificially infected seed material.

FIG. 5. SURVIVAL OF *Xanthomonas campestris* pv *oryzae* IN INFECTED SEED MATERIALS.

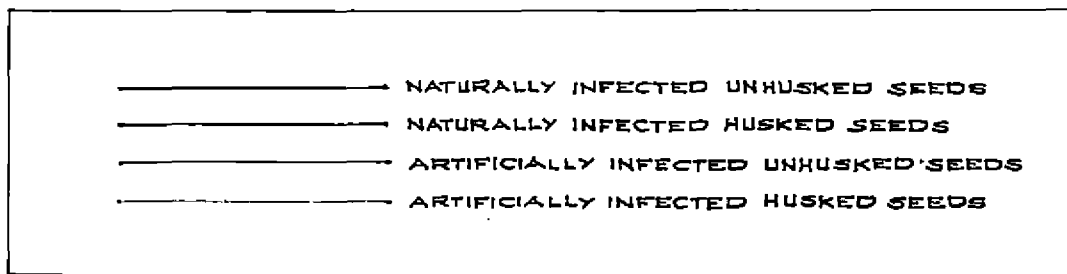
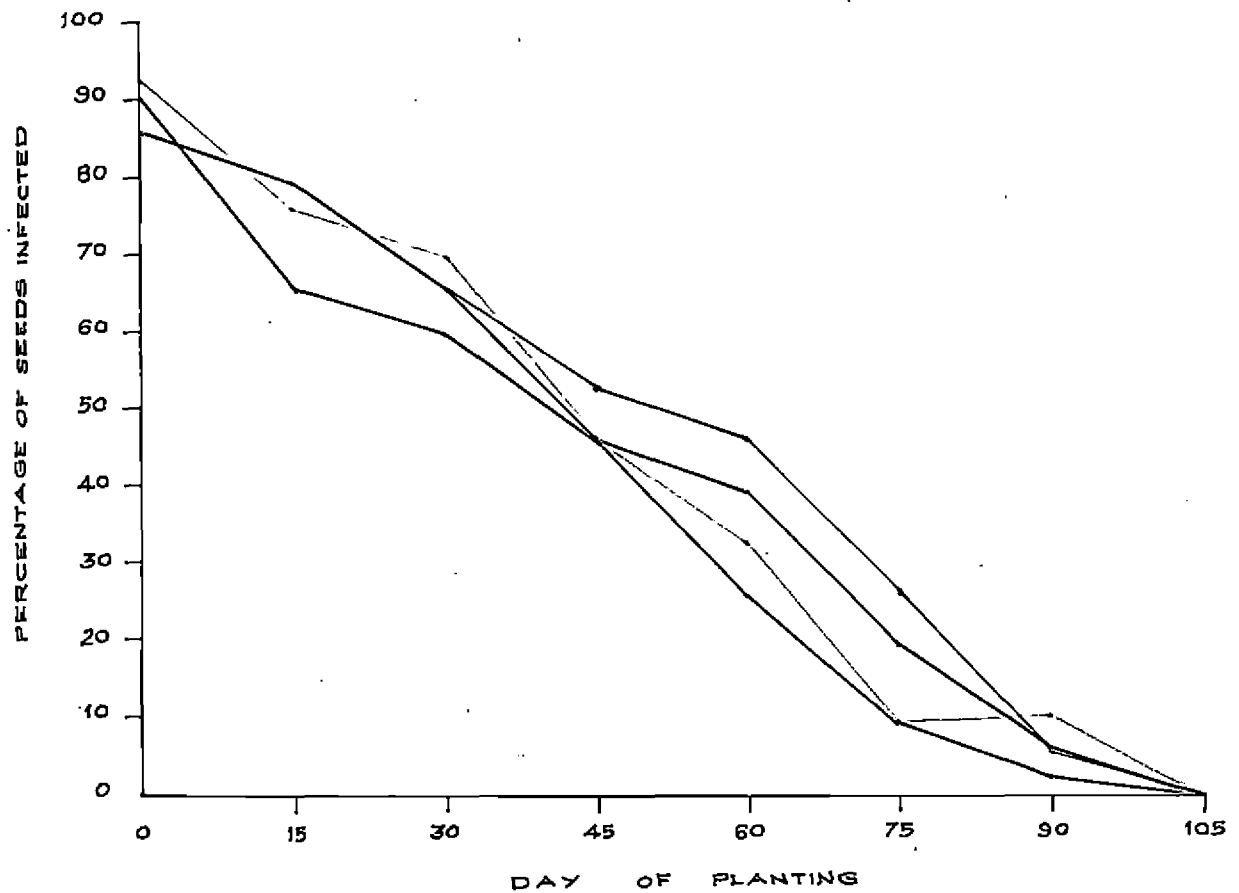


Table 8. Survival of Xanthomonas campestris pv. oryzae in infected seed material.

Treatment	Percentage of infected seeds at different days on plating on potato sucrose peptone agar medium.							
	0*	15	30	45	60	75	90	105
A. <u>Naturally infected seeds</u>								
Unhusked seeds	86.66	80	66.66	46.66	40	20	6.66	0
Husked seeds	86.66	80	66.66	53.33	46.66	26.66	6.66	0
B. <u>Artificially infected seeds</u>								
Unhusked seeds	90	66.66	60	46.66	26.66	10	3.3	0
Husked seeds	93.33	76.66	70	46.66	33.33	10	10	0

*0 - Day represents the day of harvest of the infected crop or the day of artificial inoculation of seeds with the pathogen.

5. CHEMICAL CONTROL OF BACTERIAL LEAF BLIGHT OF RICE

A. In vitro evaluation of antibiotics and fungicides against Xanthomonas campestris pv oryzaeTable 9. Growth inhibition (mm)^a of Xanthomonas campestris pv oryzae due to various antibiotics and fungicides under in vitro conditions

Nature of antibiotic/ fungicide	Concentration of antibiotic/fungicide (ppm)			
	50	100	250	500
A. <u>Pure antibiotics</u>				
1. Ambistrin	12.33	24.66	26.33	29.33
2. Chloromycetin	12.33	20.66	24.40	26.33
3. Chlorostrep	0	26.66	27	31.83
4. Penicillin	0	0	0	0
5. Terramycin	21	23	32.66	42.66
B. <u>Commercial preparation of antibiotics</u>				
1. Paushamycin	12	17	22.66	36
2. Plantomycin	11.66	20.33	23.33	25.66
3. Streptocycline	21.33	23.33	26.33	39
C. <u>Fungicides</u>				
	500	700	1000	1500
1. Bavistin	0	0	0	0
2. Hinosan	0	0	0	13.6
3. Kitazin	0	0	0	0
	3000	3500	4000	4500
4. Dithane M 45	0	14.66	21.60	26.30

* Mean of three replications

1.	CD for comparison of treatments A with B and C	= 0.0584
2.	-do- A with 4 of C	= 0.0876
3.	-do- between B with C	= 0.0923
4.	-do- between A/B/C	= 0.1131
5.	-do- between levels of A	= 0.1011
6.	-do- -do- B	= 0.1306
7.	-do- -do- C) except Dithane M-45)	= 0.1306
8.	-do- between levels of) Dithane M-45)	= 0.2262
9.	-do- between single treatments	= 0.2262

A progressive reduction in growth of Xanthomonas campestris pv oryzae was seen with an increase in the concentration of various antibiotics and fungicides except in the case of penicillin and fungicides like kitazin and bavistin. These chemicals did not show any inhibitory effect on the growth of the pathogen on potato sucrose peptone agar medium (Table 9). A similar result was also observed with chlorostrep at 50 ppm, hinosan at all the lower concentrations upto 1000 ppm and dithane M-45 at 3000 ppm.

Among the pure antibiotics, commercial preparation of antibiotics for plant protection and fungicides tested for any inhibitory effect on the growth of Xanthomonas campestris pv. oryzae, terramycin and streptocycline at 500 ppm and dithane M-45 at 4500 ppm produced the maximum zone of growth inhibition (Plate 8,9). These were respec-

Plate VIII

Growth inhibition (mm) of Xanthomonas campestris pv oryzae due to various antibiotics and fungicide.

1. Ambistrin	500 ppm
2. Chloromycetin	500 ppm
3. Chlorostrep	500 ppm
4. Terramycin	500 ppm
5. Dithane M-45	4500 ppm
C. Control	Distilled water

Plate IX

Growth inhibition (mm) of Xanthomonas campestris pv. oryzae due to various commercial preparation of antibiotics for plant protection.

A Flantomycin	500 ppm
B Paushamycin	500 ppm
C Streptocycline	500 ppm
D Control	Distilled water

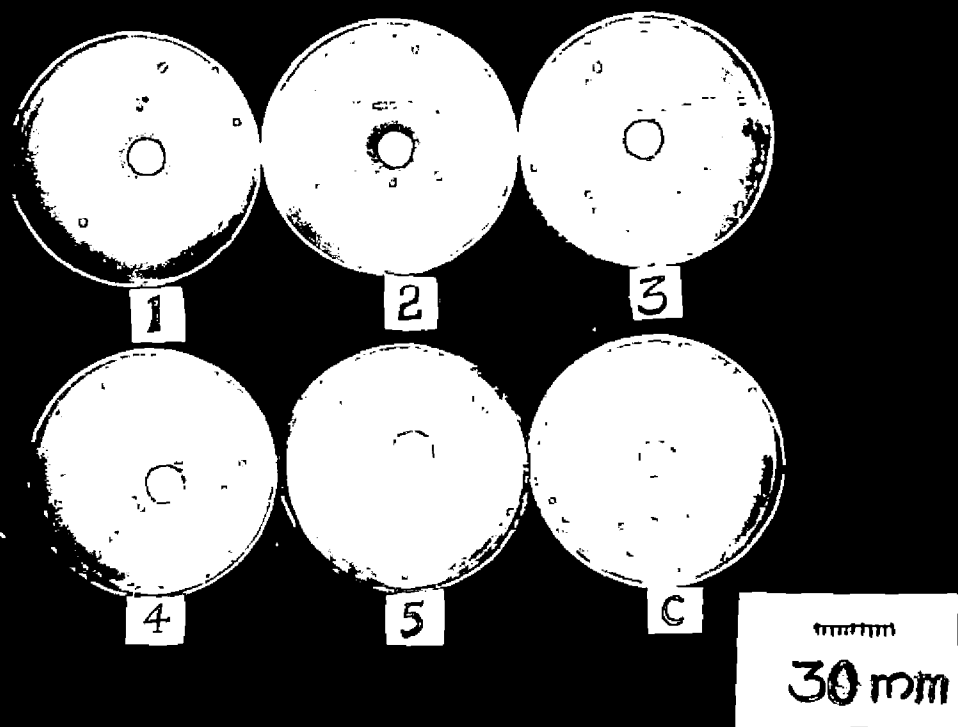


Plate VIII Growth inhibition (mm) of Xanthomonas campestris pv oryzae due to various antibiotics and fungicide.

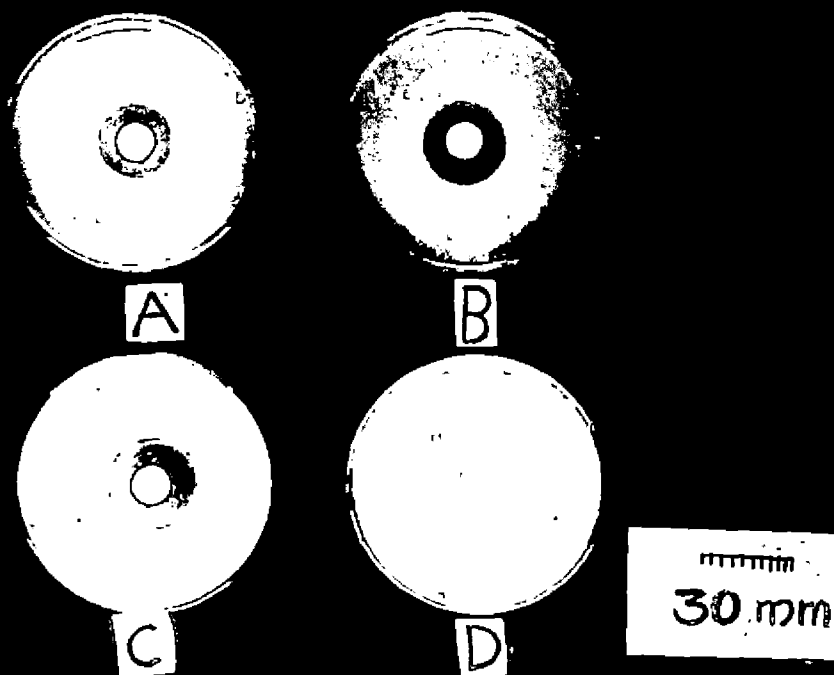


Plate IX Growth inhibition (mm) of Xanthomonas campestris pv oryzae due to various commercial preparation of antibiotics for plant protection.

tively 42.66, 39.0, 26.30 mm and were significantly higher than that obtained with the corresponding concentrations of all other antibiotics and fungicides tested (Table 9 and Figure 6).

Table 10. Growth inhibition of Xanthomonas campestris pv oryzae due to dithane M-45, streptocycline and terramycin under in vitro conditions.

Dithane M-45 (ppm)	Zone of growth inhibition (mm)*			
	Streptocycline (ppm)		Terramycin (ppm)	
	250	500	250	500
4000	26.07	29.68	20.67	25.81
4500	30	37.70	28.33	31.33

* Mean of three replication

C.D. for comparison between treatments = 0.33

Two different combinations of dithane M-45 at 4000 and 4500 ppm along with terramycin and streptocycline at 250 and 500 ppm were also tested for any increased inhibitory effect on the growth of the pathogen on potato sucrose peptone agar medium. In general, the inhibitory effect of dithane M-45 at 4000 and 4500 ppm with the above concentrations of terramycin and streptocycline, increased significantly when compared to the inhibition obtained with the use of dithane M-45 alone (Tables 9 and 10 and Plate 10). This increase was more with dithane M-45 at 4500 ppm, along

FIG. 6. GROWTH INHIBITION OF *Xanthomonas campestris pv oryzae* DUE TO VARIOUS ANTIBIOTICS AND FUNGICIDES UNDER *in vitro* CONDITIONS.

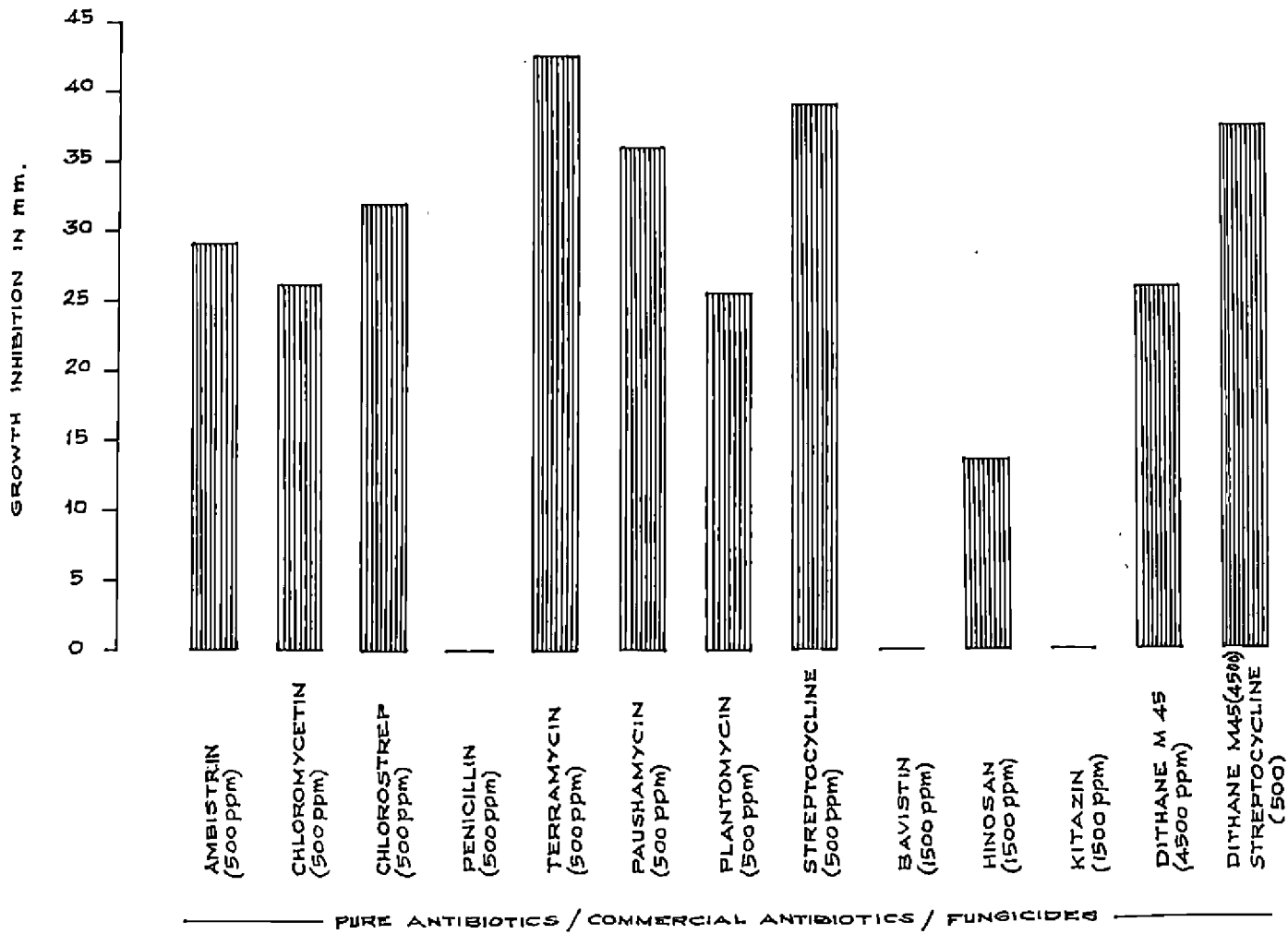


Plate X

Growth inhibition (mm) of Xanthomonas campestris
pv. oryzae due to dithane M-45 along with
streptocycline and terramycin.

A	- Terramycin	- 500 ppm
B	- Dithane M-45	- 4500 ppm
C	- Streptocycline	- 500 ppm
A+B	- Terramycin + Dithane M-45-	500 + 4500 ppm
B+C	- Dithane M-45 + Strepto- cycline	- 4500 + 500 ppm

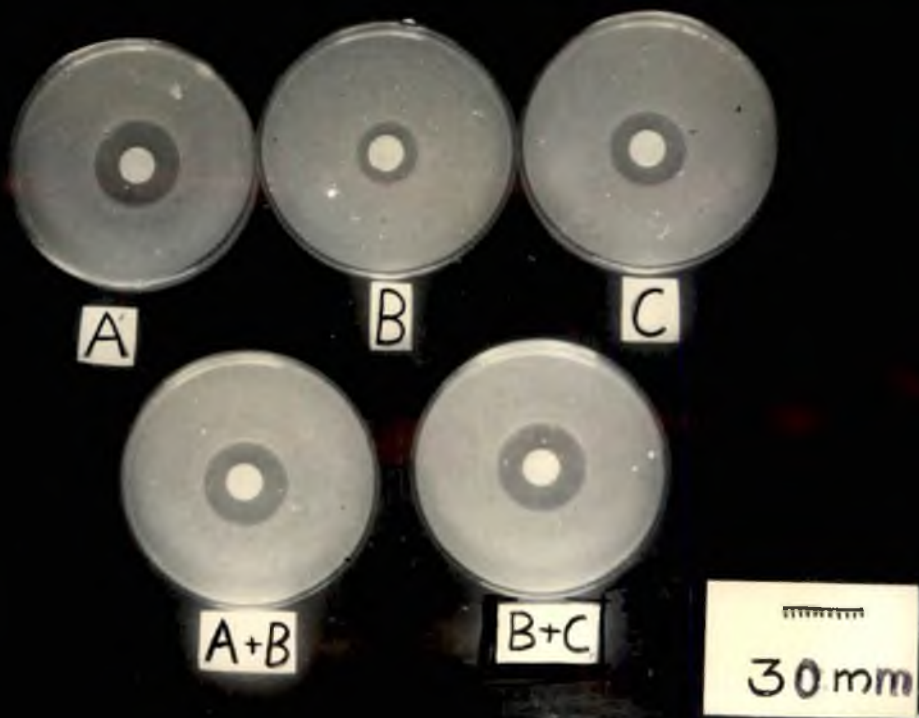


Plate X Growth inhibition (mm) of *Xanthomonas campestris* pv *oryzae* due to dithane M-45 along with streptomycin and terramycin.

with both the levels of streptomycin and terramycin. The maximum zone of growth inhibition of 37.70 mm was obtained with streptomycin at 500 ppm along with dithane M-45 4500 ppm. However, with dithane M-45 at 4000 ppm and terramycin at 250 ppm, the mean zone of growth inhibition was slightly less than that obtained with dithane M-45 alone at 4000 ppm concentration (Tables 9 and 10). Any additive effect due to this combination was noticed only with dithane M-45 at 4500 and streptomycin at 250 ppm. Here, the mean zone of growth inhibition obtained was higher than that of the same concentration of these chemicals tried alone (Tables 9 and 10).

B. In vivo evaluation of antibiotics and fungicides against Xanthomonas campestris pv oryzae under field conditions.

The antibiotics, fungicide and their combination which were found to be most effective against the pathogen under in vitro conditions were further used for its control under field conditions.

The disease intensity due to bacterial leaf blight in the experimental plots selected at Kinakary in Kuttanad during the additional crop season of 1984 was more or less uniform with an average intensity of 64.32 (Table 11).

Table 11. Disease intensity in culture-4 rice variety under field conditions before and after treatment with different antibiotics and fungicide

Antibiotics/ fungicide	Concen- tration (ppm)	Disease intensity*	
		Before treat- ment	After treat- ment
1. Terramycin	500	67.02	38.11
2. Dithane M-45	4500	62.11	75.70
3. Streptocycline	500	69.66	49.26
4. Dithane M-45 + Streptocycline	4500 + 500	62.39	60.44
5. Control		60.41	77.66
Mean disease intensity		64.32	-

* Mean of 4 replications

CD for comparison between treatments = 9.08

A significant reduction in the spread of bacterial leaf blight was observed in all the treated plots except where dithane M-45 alone was sprayed at 4500 ppm concentration (Plates 11,12,13 and 14). The disease intensity in terramycin, streptocycline and dithane M-45 and streptocycline sprayed plots on the 14th day after treatment was only 38.11, 49.26 and 60.44 respectively, when compared to the intensity of 77.66 of the control plots (Table 11 and Figure 7). In dithane M-45 alone treated plots, there was an increase in disease intensity from 62.11 to 75.70 during the same period.



Plate XI Effect of spraying with terramycin on the control of bacterial leaf blight under field condition.



Plate XII Effect of spraying with Githane M-45 on the control of bacterial leaf blight under field condition.



Plate XIII Effect of spraying with streptocycline on the control of bacterial leaf blight under field condition.



Plate XIV Effect of spraying with dithane M-45 and streptocycline on the control of bacterial leaf blight under field condition.

Plate XV Bacterial leaf blight infected plot
without any treatment.

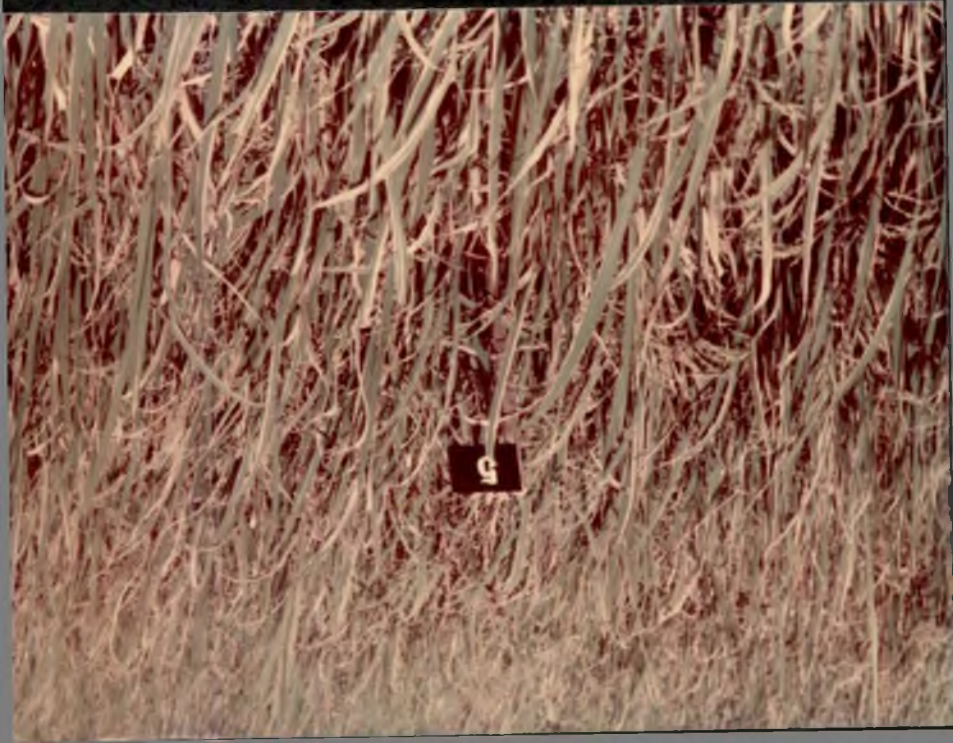
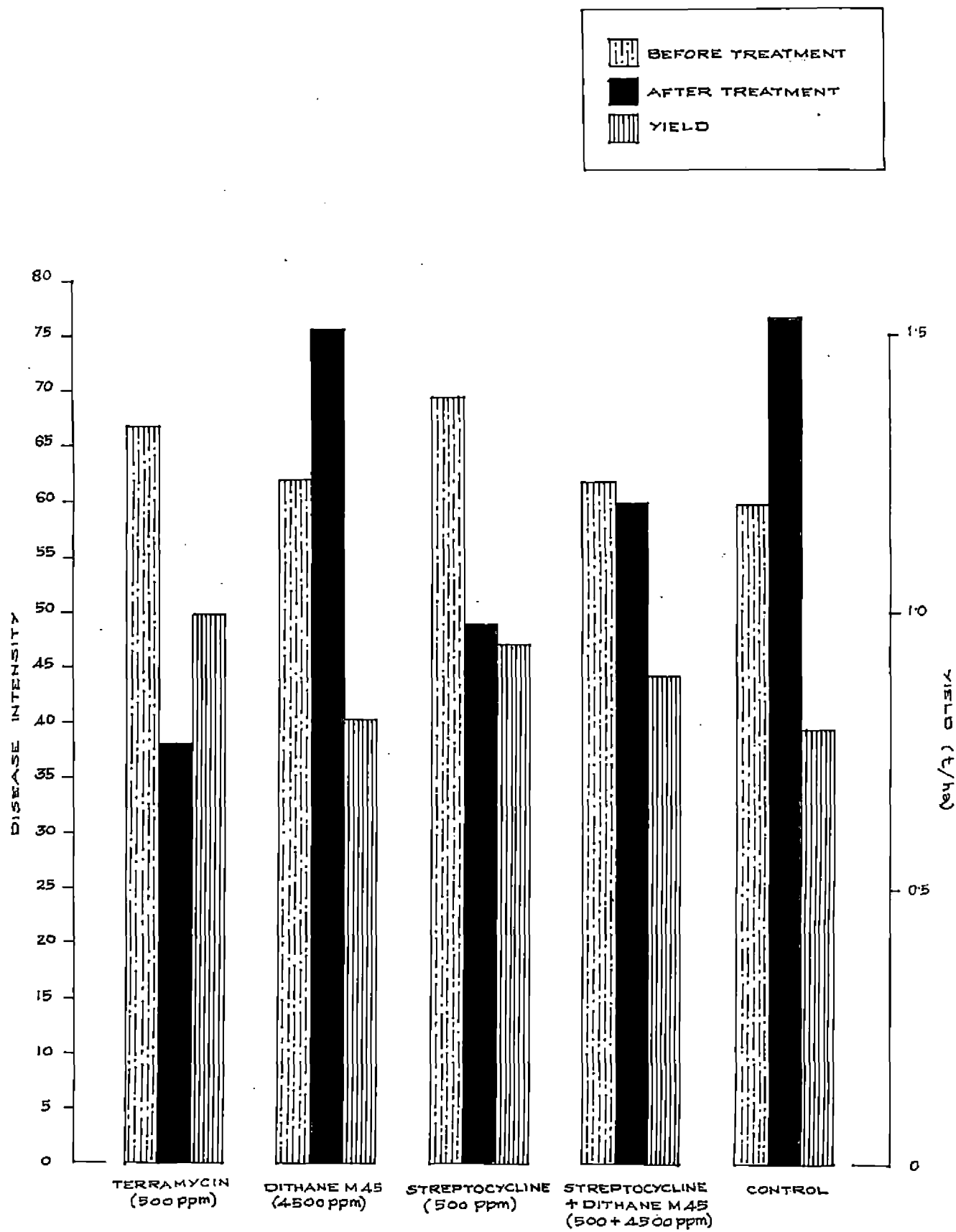


FIG. 7. EFFECT OF VARIOUS CHEMICAL TREATMENTS ON DISEASE INTENSITY AND YIELD OF C₄ VARIETY OF RICE AFFECTED WITH BACTERIAL LEAF BLIGHT.



A significant increase in yield over the control plots was obtained only in the case of terramycin and streptomycin treated plots (Table 12). These two treatments gave a net yield of 1.05 and 0.95 t/ha respectively when compared to the average yield 0.79 t of the control plots (Table 12). The increase in yield due to the combination of dithane M-45 and streptomycin was also higher. However, such an increase obtained due to dithane M-45 alone was only marginal to the extent of 2.42 per cent.

Table 12. Effect of various chemical treatments on the yield of Culture-4 rice variety affected with bacterial leaf blight.

Treatment	Yield t /ha	Increase in yield with respect to control plots	Percen- tage increase in yield
Terramycin (500 ppm)	1.0539	0.2634	24.99
Dithane M-45 (4500 ppm)	0.8101	0.0196	2.42
Streptomycin (500 ppm)	0.9451	0.1546	16.36
Dithane M-45 + streptomycin (4500 + 500 ppm)	0.8975	0.107	11.92
Control	0.7905	-	-

C.D. for comparison between treatments: 0.145

6. EFFECT OF WEATHER ON THE INCIDENCE OF BACTERIAL LEAF BLIGHT DURING DIFFERENT CROP SEASONS

In general, the incidence of bacterial leaf blight was observed only during the additional crop season when the relative humidity and rainfall was considerably higher than that of the Punja season. The average relative humidity and rainfall for the period from 1979 to 1983 was 75.80 per cent and 477.91 mm respectively during the additional crop season while these were 59.2 per cent and 118.15 mm respectively for the same period during the Punja season (Table 13). The average maximum and minimum temperature for these two seasons was 31.26 and 34.01°C and 24.53 and 24.04°C respectively.

Table 14. Weather data for the additional crop season of 1984

Month	Maximum temp. °C	Minimum temp. °C	Relative humidity (%)	Rainfall (mm)
June	29	23	89	379
July	30	24	90	380
August	30	24	91	383
September	31	21	90	350
Average	29.6	23.2	90	374.5

Table 13. Effect of weather on the incidence of bacterial leaf blight

Major crop season										
Additional crop (June-September)						Punja crop (October-January)				
Year	Maximum temp. °C	Minimum temp °C	Relative humidity (%)	Rainfall (mm)	Incidence of BLB	Maximum temp. °C	Minimum temp °C	Relative humidity (%)	Rainfall (mm)	Incidence of BLB
1979	32.02	24.52	77.53	474.85	+	34.48	23.97	64.5	154.98	-
1980	31.94	24.53	73.75	411.33	+	34.23	23.87	60.75	93.95	-
1981	30.9	23.78	81.63	476.83	+	33.67	24.19	55.5	143.75	-
1982	31.77	24.72	73.25	529.75	+	35	24.28	57.75	85.9	-
1983	29.67	25.1	72.83	497.08	+	32.67	23.89	57.50	107.2	-
Average	31.26	24.53	75.80	477.91		34.01	24.04	59.2	118.15	

The average relative humidity and rainfall during the present investigation ^{was} also higher, in the range of 90 per cent and 374.5 mm (Table 14) respectively.

During this period, a natural incidence of bacterial leaf blight was observed in culture-4 rice variety at Kainakary block. This area was subsequently selected for the in vivo evaluation of selected antibiotics and fungicide for the control of bacterial leaf blight under field conditions.

DISCUSSION

DISCUSSION

One of the most important factors that bring about considerable reduction in the yield of rice in Kerala, especially during the additional crop season at Kuttanad is the high incidence of bacterial leaf blight due to Xanthomonas campestris pv. oryzae. An effective control measure for this disease is lacking even today. Taking these facts into consideration, the present investigation was taken up with the following main objectives.

- (i) To find out the relative susceptibility of three commonly cultivated varieties of rice, T(N)1, Jaya and IR-20 to bacterial leaf blight.
- (ii) To study the effect of different levels of NPK and certain minor elements such as Zn, Mn, B on the incidence of bacterial leaf blight in T(N)1.
- (iii) To understand the ability of the pathogen to survive in various materials; ~~and~~
- (iv) To evolve a suitable control measure for this most devastating bacterial disease of rice in the State.

A virulent strain of Xanthomonas campestris pv. oryzae maintained at the Department of Plant Pathology, College of Agriculture, Vellayani was used for this investigation. This culture was tested once for its virulence in a bacterial leaf blight susceptible variety of rice, T(N)1, from which it was reisolated for further studies.

During the first part of this investigation, the relative susceptibility of three varieties of rice, T(N)1, Jaya and IR-20 to Xanthomonas campestris pv. oryzae at four different stages of plant growth was studied. In all these varieties, the onset of symptoms was uniformly on the fifth day at seedling, maximum tillering and panicle initiation stages, while at flowering stage, the symptoms appeared only on the seventh day after inoculation with the pathogen.

T(N)1 was found to be highly susceptible to bacterial leaf blight with a mean disease intensity of 39.45 followed by Jaya and IR-20. IR-20 had the least mean disease intensity of 23.05 for all the stages of plant growth studied together. This indicates that T(N)1 is the most susceptible variety to bacterial leaf blight and that IR-20 may be regarded as a relatively resistant variety to this disease. Similar observations

were also made earlier by Mahmood and Singh (1970) for rice varieties such as T(N)1, T65, Padma, IR-8 and IR-5, Dath et al. (1977) for T(N)1 and for varieties like IR-20 and IR-22 (Annon 1970, 1973).

Among the four critical stages of plant growth studied for their susceptibility to bacterial leaf blight, the maximum tillering stage was found to be most vulnerable stage to Xanthomonas in all the three varieties. The plants were comparatively more resistant at the flowering stage in T(N)1 and Jaya, while in IR-20 such a resistance was observed only at the panicle initiation stage. Several other workers have also studied the relative susceptibility of different varieties of rice at various stages of plant growth to bacterial leaf blight. However, these reports are not in agreement with one another with regard to their increased or decreased susceptibility to bacterial leaf blight at any one particular stage of plant growth. Thus Devadath and Padmanabhan (1969) based on their study using 20 different varieties of rice reported that the flag leaf stage was the most susceptible stage in all the varieties studied to bacterial leaf blight. Mahmood and Singh (1970), Ho and Lim (1976) have reported that the maximum tillering stage was the most susceptible stage in the rice variety Jaya, to Xanthomonas oryzae. A similar result was also observed during this

study as well. The flowering stage was found to be the most susceptible stage of plant growth to bacterial leaf blight in varieties such as Karuna, IR-20 and IR-8 by Devadath and Rao (1973). However, during the present investigation, in all the three varieties of rice studied, including IR-20, the flowering stage was found to be the least susceptible stage to bacterial leaf blight even after artificial inoculation with a virulent culture of Xanthomonas campestris pv. oryzae. The reason for this apparent lack of agreement with all these results may be due to the fact that these are the results reported from experiments conducted at different places with entirely different environmental conditions. It could also probably involve the comparison of experimental data based on the natural incidence of bacterial leaf blight with that resulting from the artificial inoculation of the pathogen either under green house or under field conditions. Even then, it appears to be reasonable to conclude that in general, the maximum tillering and panicle initiation stages of plant growth are the most susceptible stages to bacterial leaf blight due to Xanthomonas campestris pv. oryzae.

The effect of three major elements such as nitrogen, phosphorus and potassium and three minor elements such as zinc, manganese and boron on the incidence of bacterial leaf blight in T(N)1 was also studied. The plants were

uniformly inoculated with a virulent culture of Xanthomonas campestris pv. oryzae at the maximum tillering stage.

The onset of symptoms were either on the seventh or on the eighth day at lower levels of nitrogen, n_0 and n_1 (45 kg/ha) irrespective of the different levels of potassium and phosphorus. However, with an increase in the level of nitrogen, n_2 (90 kg/ha) and n_3 (135 kg/ha) the typical symptoms of bacterial leaf blight were observed on the fifth day except in treatments like $n_2p_2k_0$, $n_3p_2k_0$ and $n_3p_1k_0$ where these symptoms appeared only on the sixth and seventh day respectively. In pots supplemented with minor elements alone and in combination, the symptoms of bacterial leaf blight were noticed on the fifth and sixth day respectively after inoculation with the pathogen. The early appearance of these symptoms in pots supplemented with the higher doses of nitrogen can be due to the increased succulent nature of various plant tissues at higher levels of nitrogen. This can also be the reason for the early appearance of the symptoms either on the fifth or on the sixth day in pots supplemented with zinc, manganese and boron along with normal level of NPK for T(N)1.

The study on the effect of varying levels of nitrogen, phosphorus and potassium and zinc, manganese and boron on disease intensity due to bacterial leaf blight in T(N)1 showed that the main effect of nitrogen and potassium was significant while that of phosphorus and minor elements such as zinc, manganese and boron were insignificant. Pots which received zero nitrogen had the lowest disease intensity of 26.43 when compared to the disease intensity of 31.29, 31.47 and 43.70 respectively for treatments involving n_1 (45 kg/ha), n_2 (90 kg/ha) and n_3 (135 kg/ha) levels of nitrogen. A significant increase was observed with the use of 135 kg N/ha. The application of potassium, in general decreased the disease intensity. A significant decrease was obtained at k_1 (22.5 kg/ha) and k_3 (90 kg/ha) levels when compared to the k_0 level. However, there was no significant difference in disease intensity between the k_1 , k_2 and k_3 levels of potassium applied. These observations are in agreement with the studies conducted earlier by several other workers such as Devadath (1969), Umagupte and Padmanabhan (1972), Reddy *et al.* (1979), Choi *et al.*, (1980). The insignificant effect of phosphorus and minor elements on the incidence of bacterial leaf blight is also reported earlier by Kim

and Cho (1970), Mohanty and Reddy (1978).

Only the N and K interaction was found to be significant. Since the effect of P was not significant, N x P and N x P x K, P x K interactions were also insignificant. Pots which received different levels of potassium at zero level of nitrogen showed the least incidence of bacterial leaf blight. In these treatments the disease intensity ranged from 22.93 to 28.18. But with the application of nitrogen at the rate of 45, 90 and 135 kg/ha, the disease intensity increased significantly at k_0 level. However, this increase was not significant with the use of k_1 , k_2 and k_3 levels of potassium except in the case of n_3k_2 and n_3k_3 treatments where a significant increase in disease intensity was observed. In fact the maximum disease intensity of 49.87 and 48.38 were recorded for these treatment combinations during this study. This observed contradiction regarding the effect of potassium may be a phenomenon associated with highly bacterial leaf blight susceptible varieties of rice like T(N)1. A similar observation was also made by Ranga Reddy and Sridhar (1973). They observed that an increase in the levels of potassium did not bring about an expected decrease in the disease intensity due to bacterial leaf blight in T(N)1.

In soil, where a bacterial leaf blight affected crop was previously raised, Xanthomonas campestris pv oryzae was found to survive for about nine days. About 60 per cent of the plants were infected when disease free seeds of T(N)1 were sown in such a soil on the same day after harvest of the previous infected crop. However, the number of plants affected decreased gradually on the third, sixth and ninth day of sowing and there was complete absence of any disease symptoms in plants sown on the 12th day. A significant incidence of bacterial leaf blight was observed only upto the sixth day of sowing in soil. This indicates that the maximum number of days, the pathogen can survive in soil is only for about six or seven days. A similar observation was also made earlier by Singh (1971), Chattopadhyay and Mukherjee (1974), Mary and James Mathew (1980). This observation is important because of the fact that the time lag between two successive crops of rice in same field at Kuttanad is usually more than ten days and since the pathogen can survive in the soil only for a maximum period of nine days, one may rule out the possibility of a previously infected field serving as a source of inoculum for the next crop to be raised in the same area. However, it should be pointed out that this

conclusion is made mainly on the basis of taking soil as a lone factor for the survival and spread of bacterial leaf blight of rice. Therefore it is essential to study how the pathogen is going to survive in infected plant debris, crop refuse and seed material.

In infected plant debris and crop refuse, Xanthomonas campestris pv. oryzae was found to survive for about 28 days. However, out of these two infected materials, the incorporation of infected crop refuse in disease free soil was found to affect more number of plants with bacterial leaf blight than the plant debris. This shows that crop refuse can serve as a better source of inoculum for the pathogen than the plant debris, although the extent of survival of the pathogen in both the infected materials was more or less same. These observations are not in agreement with that of Trimarty et al. (1982). They have shown that Xanthomonas oryzae could survive in infected stubbles after harvest for about 190 days on soil surface and for 130 days when buried in the soil. However, Mary and James Mathew (1980) have shown that Xanthomonas campestris pv oryzae could survive only for about 28 days in crop refuse and plant debris. The non-identical environmental conditions of the present investigation

with that of Trimurthy et al. (1982) and the influence of the same on the survival of the pathogen may be a possible reason for the observed results here.

In infected seed material, the pathogen was found to survive for about 90 days with a gradual decline after 60 days. Similar results were also reported earlier by Chattopadhyay and Mukherjee (1975), Mary and James Mathew (1980) and Pal et al. (1982). However, Singh and Rao (1977) have shown that Xanthomonas campestris pv. oryzae could survive at room temperature in infected seed material for about 11 months and that the infected seed material can cause an epidemic of bacterial leaf blight during the next season under favourable conditions. Therefore one has to take into consideration this as a possible factor for the recurrence of bacterial leaf blight in an endemic area, especially if there is a possibility for sowing contaminated or mixed seed material during the following crop season. But this does not seem to be true at least in Kuttanad, where the general practice is to use seeds of the main or the punja crop for the additional crop and vice versa, wherever possible. Under this circumstances, one could normally expect an incidence of bacterial leaf

blight during the main crop or the punja season since there is every possibility for the use of some of bacterial leaf blight affected seed material collected from previous additional crop, when normally the disease occurs in Kuttanad. But the observations on the incidence of bacterial leaf blight for the last five years shows that there is practically no incidence of this disease in Kuttanad during the Punja season. At the same time during the additional crop season, when practically disease free seed material from the Punja crop will be used as seed material, there is always the incidence of bacterial leaf blight. This proves beyond doubt that the infected seed material in a normal cropping practice, will not serve as a source of inoculum for bacterial leaf blight, unless one uses it for raising a crop immediately after the harvest of a previous infected crop. Therefore, under normal circumstances what can be the predisposing factor for the incidence of bacterial leaf blight in Kuttanad that predominantly occurs only during the additional crop season?. It appears that a favourable environmental condition is essential for the wide spread occurrence of bacterial leaf blight even if the inoculum level is low. In the absence of a favourable environ-

mental condition for disease development, as it normally occurs during the Punja season in Kuttanad, the disease may not occur even ^{when} if there is a potentially ^{heavy load.} higher source of inoculum. This hypothesis appears to be true from the observations made during the present investigation.

In order to prove this, the weather data for the last five crop seasons in Kuttanad from 1979 to 1983 were examined for both the crop seasons along with the recorded incidence of bacterial leaf blight. The results were found to be quite interesting. In all these five years an incidence of bacterial leaf blight was recorded in Kuttanad only during the additional crop season and practically no incidence of this disease was noticed during the Punja or the main crop season. The average rainfall, relative humidity, maximum and minimum temperature for the period from 1979 to 1983 during this season (October to January) was 118.18 mm, 59.21 per cent 34.10 and 24.04°C respectively and for the additional crop season (June to October) these were 477.91 mm, 75.80 per cent, 31.26 and 24.53°C respectively. It is thus clear that the weather variations are quite distinct at least with regard to two important parameters such as the

rainfall and relative humidity. These were significantly higher during the additional crop season when only the incidence of bacterial leaf blight was consistently observed. This was also true for the additional crop season for the year 1984 when the present investigation was conducted. Therefore it appears to be reasonable to conclude that the incidence of bacterial leaf blight in Kuttanad is highly correlated with the weather conditions existing during the crop season. This is supported by the works of Reddy and Pillai (1974), Mohiuddin et al. (1977), Srinivasan and Singh (1983). They have, all reported that a weather condition involving a well distributed rainfall and high relative humidity during the crop season along with a maximum and minimum temperature of 30 to 35°C and 24 to 28°C respectively was highly favourable for the wide spread out break of bacterial leaf blight.

Different chemical agents have been tried by various workers for the control of bacterial leaf blight. Some of these chemicals tried earlier were fungicides especially the copper containing fungicides. However, with the advent of antibiotics, it became a general practice to use various antibiotics either in their pure form or

as their commercial preparation for plant protection purpose for the control of bacterial leaf blight of rice. During the present investigation also these three methods of control measures were used especially under field conditions.

During the first part of this study, five antibiotics such as ambistrin, chlorostrep, chloromycetin, penicillin and terramycin and three commercial preparations of antibiotics for plant protection, like paushamycin, plantomycin and streptocycline at concentrations ranging from 50 to 500 ppm and four fungicides such as hinosan, kitazin, bavistin and dithane M-45 at concentrations ranging from 500 to 4500 were used in order to study their inhibitory effect on the growth of Xanthomonas campestris pv. oryzae on potato sucrose peptone agar medium. The different concentrations of various antibiotics and fungicides were fixed as per their normal recommendations for the control of bacterial or fungal diseases of rice. It was found that terramycin, streptocycline and dithane M-45 were the most effective chemical agents against this pathogen under in vitro conditions. The zone of growth inhibition obtained

were respectively 42.66, 39.0 and 26.30 mm. However, for some unknown reason, no growth inhibition was observed with penicillin at any of the concentrations tried during this investigation.

In the study involving the combination of dithane M-45 at 4000 and 4500 ppm along with terramycin and streptocycline at 250 and 500 ppm concentrations, the treatments consisting of the higher level of this fungicide with either of the antibiotics gave the maximum zone of growth inhibition. However, an additive effect due to the combination of this fungicide along with either of the antibiotics tried was observed only with the combination of dithane M-45 at 4500 ppm and streptocycline at 250 ppm. Here the mean zone of growth inhibition noticed was higher than that obtained with the use of either of these chemicals alone.

Based on the above study, four treatments were subsequently selected for the control of bacterial leaf blight under field conditions. These were spraying with streptocycline and terramycin at 500 ppm, dithane M-45 at 4500 ppm and dithane M-45 along with streptocycline at 500 ppm respectively. The plants were sprayed on the 14th day after the appearance of the symptoms of bacterial leaf blight in a farmer's field at Kinakary in Kuttanad, during the additional crop season of 1984. An area of

320 sq.m with an average disease intensity of 64.32 was selected for this purpose. The observations were recorded 14 days after spraying with different chemical agents. A significant reduction in disease intensity when compared to the control plots was observed after spraying with terramycin and streptomycin. There was also a slight decrease in disease intensity by spraying with dithane M-45 along with streptomycin. However, there was no effect by spraying with dithane M-45 alone at 4500 ppm concentration. Here, the disease intensity in fact increased from that of the prespraying stage and was almost similar to that of the control plots.

The difference in the effectiveness of various treatments was also reflected in the net yield at the time of harvest. There was an increase in yield of about 25 per cent and 16.4 per cent in treatments involving the spraying of terramycin and streptomycin when compared to the control plots. The combination of dithane M-45 and streptomycin gave an increase in yield of about 12 per cent while that of dithane M-45 alone gave only a low increase in yield of about 2.5 per cent. The above observations on the control of

bacterial leaf blight is almost in agreement with that of Jain et al. (1966), Desai et al., (1967), Singh et al., (1980) and Durgapal et al. (1981) who have all reported the efficacy of various antibiotics such as streptocycline, oxytetracycline, chloramphenicol, agrimycin at concentrations from 100 to 500 ppm under various experimental conditions for the control of leaf blight of rice.

Therefore, for an effective control of bacterial leaf blight, one has to use an antibiotic, either in the form of terramycin or streptocycline. The use of any fungicide for this purpose does not seem to be advantageous, even when it is used in combination with an effective antibiotic. Another interesting result was that spraying of terramycin was the most effective treatment for the control of bacterial leaf blight in terms of yield and reduction of disease symptoms when compared to streptocycline, which is normally used for the control of bacterial leaf blight. Here one may raise the question, whether the terramycin ⁱⁿ its pure form, normally used for treatment of human diseases, can be used for a plant protection purpose by taking into

consideration its relatively higher cost (terracycline 500 mg - Rs.1.75 and streptocycline 500 mg - Rs.0.80) and the possibility for a rapid development of antibiotic resistant strains of Xanthomonas campestris pv. oryzae? However, the efficacy of streptocycline can be improved by changing the existing 9:1 ratio of streptomycin and oxytetracycline in the commercial preparation available today to a better formulation having a higher ratio of terracycline. This is further supported by the fact that under in vitro conditions terracycline was more effective than streptomycin in inhibiting the growth of Xanthomonas campestris pv. oryzae on potato sucrose peptone agar medium.

The net result will be a formulation of streptocycline with higher efficiency which can bring about an effective control of bacterial leaf blight under field conditions.

SUMMARY

SUMMARY

A study on the effect of age of plants and host nutrition on the incidence of bacterial leaf blight (BLB) of rice caused by Xanthomonas campestris pv oryzae was conducted at the College of Agriculture, Vellayani, Trivendrum during 1983-84. The field experiment to evaluate the comparative efficacy of different control measures of bacterial leaf blight was laid out in a farmers field at Kinakary in Kuttanad.

The effect of age of plants on the incidence of bacterial leaf blight was studied using three popular varieties of rice namely T(N)1, Jaya and IR-20 at four critical stages of plant growth such as seedling, maximum tillering, panicle initiation and early flowering stages. At flowering stage the onset of disease symptom was noticed only on the seventh day after inoculation with the pathogen while it was observed on the fifth day in all the three varieties for the remaining three stages of plant growth. T(N)1 was found to be the most susceptible variety followed by Jaya and IR-20. Among the four different stages of plant growth, the maximum tillering stage was found to be the most susceptible stage to bacterial leaf blight.

The study on the effect of host nutrition on the incidence of bacterial leaf blight in T(N)1, clearly indicated that when higher doses of nitrogen were added the onset of symptoms were earlier on the fifth day after inoculation with the pathogen while at lower levels the symptoms appeared only on the seventh day. In pots supplemented with minor nutrients like zinc, manganese boron the symptoms of bacterial leaf blight were noticed either on the fifth or on the sixth day. The main effect of nitrogen and potassium on the development of disease intensity was significant while the effect of phosphorus and minor elements was insignificant. The application of nitrogen in general increased the disease intensity while the application of potassium decreased the same. But at higher levels of nitrogen a similar positive effect of potassium on disease intensity was not observed.

The pathogen was found to survive for about nine days in soil. When the seeds were sown on the same day after harvest of the previous infected crop, 60 per cent of the plants were wilted due to bacterial leaf blight. The percentage of plants infected on the third and sixth day was also higher in the range of 40 to 55 per cent. However, there was considerable reduction in the number of plants affected on the ninth day and there was no incidence

of bacterial leaf blight in seeds sown on the 12th day after the harvest of the infected crop.

Xanthomonas campestris pv oryzae was found to survive for about 28 days in infected crop refuse and plant debris. When the seeds were sown on the same day after harvest of the previous infected crop, 80 to 90 per cent of the plants wilted due to the incorporation of bacterial leaf blight infected plant debris and crop refuse respectively. There was a progressive reduction in the number of plants affected on the 28th day and there was no incidence of bacterial leaf blight in seeds sown on the 35th day after harvest of the infected crop. When compared to both the types of infected material the pathogen was found to survive relatively better in crop refuse than in plant debris.

In infected seeds of T(N)1, the pathogen was found to survive for about 90 days. In general, a higher percentage of infection was noticed in the case of husked seeds. The pathogen could be detected for about 60 days in both naturally and artificially infected seeds.

In vitro screening of antibiotics revealed that among the pure antibiotics tested for any inhibitory effect on the growth of Xanthomonas campestris pv oryzae, terramycin

was the most effective followed by chlorostrep, chloromycetin and ambistrin. Penicillin was found to be ineffective against the pathogen during this study. Out of the three commercial preparations of antibiotics, commonly used for plant protection purpose, streptocycline was highly effective followed by paushamycin and plantomycin. Among the fungicides tested dithane M-45 was found to be better while two other fungicides tested, hinosan and kitazin did not show any inhibitory effect on the growth of this pathogen. Two different combinations of dithane M-45 at 4000 and 4500 ppm along with terramycin and streptocycline at 250 and 500 ppm respectively were also used. In this case maximum growth inhibition was obtained with streptocycline at 500 ppm along with dithane M-45 at 4500 ppm.

The antibiotics, fungicide and their combinations which were found to be most effective against Xanthomonas campestris pv oryzae under in vitro conditions were further used for the control of the pathogen under field conditions. This study was conducted in a farmer's field at Kainakary in Kuttanad. A significant reduction in the spread of bacterial leaf blight was observed in all the treated plots except where dithane M-45 alone was sprayed at 4500 ppm concentration. Terramycin (500 ppm)

was found to be highly effective followed by streptocycline at 500 ppm. A significant increase in yield over the control plots was obtained only in the case of terramycin and streptocycline treated plots.

The weather data collected for the two major crop seasons in Kuttanad for 1979 onwards and also the recorded incidence of bacterial leaf blight for both the crop seasons indicated that this disease occurred only during the additional crop season. This was found to be highly correlated with the high relative humidity and rainfall occurring during this period, when compared to the Punja season. This observation was further substantiated by the results obtained during this investigation as well.

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* Original not seen.

APPENDICES

APPENDIX I

Analysis of variance table

Effect of age of plants on the incidence of bacterial leaf blight in T(N)1, Jaya and IR-20.

Source	S.S	df	M.S.S.	F
Total	3464.04	35		
Between varieties	1713.13	2	856.56	29.39**
Between stages	731.38	3	243.79	8.37**
Varieties X Stages	320.10	6	53.35	1.83
Error	699.43	24	29.14	

* Significant at 0.05 level

** Significant at 0.01 level

APPENDIX- II

Analysis of variance table

Effect of varying levels of NPK, Zn, Mn, B on disease intensity due to bacterial leaf blight in T(N)1.

Source	S.S.	df	M.S.S.	F
Total	29980.04	155		
Treatments				
N	5862.2	3	1954.06	14.914**
P	604.33	2	302.16	2.306
NP	385.35	6	64.22	0.490
K	1188	3	396	3.022*
NK	2822.75	9	313.638	2.394*
PK	1051.86	6	175.31	1.338
NPK	3910.12	18	217.22	1.658
Minor nutrients	452.82	3	150.94	1.152
NPK vs minor nutrients	76.78	1	76.78	0.586
Error	13625.83	104	131.02	

* Significant at 0.05 level

** Significant at 0.01 level

APPENDIX- III
Analysis of Variance table

Growth inhibition of Xanthomonas campestris pv. oryzae (mm)
due to various antibiotics and fungicide under in vitro
conditions.

Source	S.S.	df	M.S.S.	F
Total	559.27	143		
Treatments	557.42	47		
1. Between treat- ments	269.77	3	89.9233	4683.00**
2. Between pure antibiotics	150.30	4	37.5750	1957.03**
3. Between levels of pure anti- biotics	35.01	3	11.6700	607.81**
4. Between levels within pure antibiotics	25.79	12	2.1492	111.93**
5. Between commer- cial preparations	3.72	2	1.8600	96.87**
6. Between levels of commercial pre- parations	16.96	3	5.6533	294.44**
7. Between levels within commer- cial preparations	2.12	6	0.3533	18.40**
8. Between fungicide except dithane M-45	4	2	2	104.16**
9. Between levels of fungicide except dithane M-45	6	3	2	104.16**
10. Between levels within fungicide	12	6	2	104.16**
11. Between levels of dithane M-45	31.75	3	10.5833	551.21
12. Error	1.85	96	0.0192	

* Significant at 0.05 level
** Significant at 0.01 level

APPENDIX- IV

Analysis of variance table

Growth inhibition of Xanthomonas campestris pv. oryzae due to the dithane M-45, streptocycline and terramycin under in vitro conditions.

Source	S.S.	df	M.S.S.	F
Total	4.468	23		
Treatment	4.432	7	0.633	275.217**
Error	0.0366	16	0.002	

* Significant at 0.05 level

** Significant at 0.01 level

APPENDIX-V

Analysis of variance table

Disease intensity under the field conditions after the treatments with antibiotics and fungicide

Source	S.S.	df	M.S.S.	F
Total	5038.770	19		
Treatment	4615.277	4	1153.819	33.239**
Block	6.942	3	2.314	0.0666
Error	416.551	12	34.712	

* Significant at 0.05 level

** Significant at 0.01 level

APPENDIX-VI

Effect of various chemical treatments on the yield of Culture-4 rice variety affected with bacterial leaf blight.

Source	S.S.	df	M.S.S.	F
Total	0.2947	19		
Treatment	0.1328	4	0.04570	5.005*
Block	0.0023	3	0.00076	0.083
Error	0.1096	12	0.00913	

* Significant at 0.05 level
 ** Significant at 0.01 level

**STUDIES ON THE EFFECT OF AGE OF PLANT
AND HOST NUTRITION ON BACTERIAL LEAF
BLIGHT OF RICE AND ITS CONTROL**

By
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ABSTRACT OF A THESIS
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ABSTRACT

A study on the effect of age of plants and host nutrition on the incidence of bacterial leaf blight of rice caused by Xanthomonas campestris pv. oryzae was conducted at the College of Agriculture, Vellayani, Trivandrum during 1983-84. The field experiment to evaluate the comparative efficacy of different control measures of bacterial leaf blight was laid out in a farmers' field at Kinakary in Kuttanad.

The effect of age of plants on the incidence of bacterial leaf blight was studied using three popular varieties of rice namely T(N)1, Jaya and IR-20 at four critical stages of plant growth such as seedling, maximum tillering, panicle initiation and early flowering stages. At flowering stage the onset of disease symptoms was noticed only on the seventh day while it was observed on the fifth day in all the three varieties for the remaining three stages of plant growth. T(N)1 was found to be the most susceptible variety to bacterial leaf blight followed by Jaya and IR-20. Among the four stages, maximum tillering stage was the most susceptible stage to bacterial leaf blight.

The study on the effect of host nutrition on the incidence of bacterial leaf blight indicated that when higher doses of nitrogen were added, the onset of symptoms were earlier on the fifth day after inoculation with the pathogen, while at lower levels, the symptoms appeared only on the seventh day. The main effect of nitrogen and potassium on the disease intensity was significant while the effect of phosphorus and minor element was insignificant. The application of nitrogen in general increased the disease intensity while the application of potassium decreased the same. But at higher levels of nitrogen a similar positive effect of potassium was not observed.

The pathogen was found to survive in infected seeds for a period of 90 days in infected plant debris and crop refuse for a period of 28 days and in soil for nine days.

In vitro sensitivity studies of the bacterium against pure antibiotics showed the pathogen was sensitive to terramycin, chlorostrep, chloromycetin and ambistrin. Terramycin (500 ppm) gave the maximum zone of inhibition followed by chlorostrep (500 ppm). Among the commercial preparation of antibiotics, streptocycline was highly effective followed by paushamycin and plantomycin. Out of the four fungicides tested, dithane M-45 was found to

be better. Two other fungicides tested hinosan and kitazin did not show any inhibitory effect on the growth of the pathogen. Two different combination of dithane M-45 along with terramycin and streptocycline were used. In this case maximum growth inhibition was obtained with streptocycline at 500 ppm along with dithane M-45 at 4500 ppm.

In vivo study using the antibiotics, fungicide and their combination on the control of the disease revealed that terramycin sprays offered better control of the disease than streptocycline sprays. A significant increase in yield over the control plot was obtained only in the case of terramycin and streptocycline treated plots.

The weather data collected for the two major crop seasons in Kuttanad for 1979 onwards and also the recorded incidence of bacterial leaf blight for both the crop seasons indicated that this disease occurred only during the additional crop season. This was found to be correlated with the high relative humidity and rainfall occurring during this period.