EFFECT OF 2-Bromo-2-nitro propane-1, 3-diol AND COWDUNG EXTRACT ON GROWTH AND VIRULENCE OF *Xanthomonas campestris pv. oryzae*

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1991

Dedicated to my
beloved
father, mother and
brother

DECLARATION

I hereby declare that this thesis entitled "Effect of 2-Bromo-2-nitro propane-1,3-diol and cowdung extract on growth and virulence of Xanthomonas campestris pv. oryzae" 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 any similar title of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "Effect of 2-Bromo-2-nitro propane-1.3-diol and cowdung extract on growth and virulence of *Xanthomonas campestris* pv. *oryzae*" is a record of the research work done independently by Sri. Sreekumar, C.T. under my guidance and supervision and that it has not previously formed the basis for the award of any Degree, Diploma, Fellowship or Associateship to him.

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CONTENTS

LIST OF TABLES

(contd.)

LIST OF TABLES (Contd.)

LIST OF ILLUSTRATIONS

LIST OF PLATES

INTRODUCTION

INTRODUCTION

Bacterial blight caused by *Xanthomonas campestris* pv. *oryzae* is an important disease of rice in many countries, particularly in South East Asia. In India this disease was first reported by Srinivasan *et al*. in 1959. It is a serious problem in Kerala especially in Kuttanad where this disease is found to recur almost every year during the additional crop season. Many methods of control like spraying infected plants with antibiotics and application of stable bleaching powder to soil are usually recommended. However, recently, in some of the isolates of this pathogen from Kuttanad, a tendency for greater tolerance towards streptocycline, the most widely used antibiotic for the control of bacterial blight,was observed affecting considerably, the effective control of this disease in the region. Hence, there is an urgent need for developing an efficient alternative method for the control of bacterial blight of rice in Kuttanad.

Spraying infected plants with a new class of synthetic organic bactericide called bactrinol-100 (2 bromo-2-nitro-propane-1, 3-diol) and spraying with fresh cowdung extract are some of the alternative methods successfully tried in many places for the control of bacterial blight of rice.

This infact has opened a way for the control of plant diseases of bacterial origin without using any antibiotics. This is also important from another point of view in that there is a rethinking now-a-days about the safety of using antibiotics for the control of plant diseases since most of the phytoantibiotic formulations available today in the market are usually combinations of streptomycin and oxytetracycline hydrochloride in varying proportions. As these antibiotics are also used for the control of human diseases, the application of the same for plant disease control is likely to cause the emergence of resistant strains of bacteria, causing disease in man. Under such circumstances it will be essential to develop suitable alternative methods for the control of plant bacterial diseases which will either minimise or totally eliminate the use of antibiotics.

In the present investigation, the efficacy of two of the alternative methods available today for the control of bacterial blight of rice, the use of bactrinol-100 and fresh cowdung extract, was compared with that of antibiotics both under pot as well as field conditions, with the following technical programme.

1. Isolation of *X. campestris* pv. *oryzae* from a bacterial blight infected rice field in Kuttanad and testing its virulence using a susceptible rice variety T(N)1.

2. *In vitro* screening of the pathogen for sensitivity towards antibiotics, bactrinol-100 and cowdung extract.

3. Control of bacterial blight under pot culture conditions using antibiotics, bactrinol-100 and cowdung extract.

4. Control of bacterial blight under field conditions using antibiotics, bactrinol-100 and cowdung extract.

5. Elucidation of factors responsible for the control of bacterial blight by using cowdung extract.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Bacterial blight of rice caused by Xanthomonas campestris pv. oryzae (Dye et al., 1980) is an important rice disease in India and in many other rice growing countries of the world. This disease was first reported from Japan in 1884 and later from Korea (Takeuchi, 1930), Taiwan (Hashioka, 1951), Indonesia (Goto et al.,1955), Thailand (Jalavicharana, 1958), India (Srinivasan et al., 1959 and Bhapkar et al., 1960), Sri Lanka (Seneviratne, 1962), Pakistan (Mew and Majiid, 1977) and Philippines (Anon, 1973). In India, bacterial blight was first noticed during 1951 in the Khopli 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), Uttar Pradesh (Pavgi et al., 1964), Tamilnadu (Soumini Rajagopalan et al., 1969), Bihar (Mahmood and Singh, 1970) and Punjab (Sahu et al., 1982).

In Kerala, bacterial blight of rice was first observed in Palghat district during 1976. According to a study conducted by Mary and James Mathew (1980), it occurred in almost all the rice growing districts of the state, comprising of Alapuzha, Kottayam, Pathanamthitta and Palghat. Bacterial blight is infact a crucial yield limiting factor for rice cultivation in Kuttanad during the additional crop season.

THE PATHOGEN

Takaishi in 1909 observed that the turbid dew drops obtained from bacterial blight affected 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 blight was caused by a kind of rod shaped bacterium and named it Pseudomonas oryzae Uyeda and Ishiyama. This was subsequently renamed as Xanthomonas oryzae (Uyeda and Ishiyama) Dowson by Breed et al.(1957). According to current nomenclature, the pathogen is identified 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, non spore forming bacterium with monotrichous flagellation. Breed et al. (1957) reported the pathogen as rod shaped bacterium of size ranging from 0.5 to 0.8 x 1.0 to 2.0 μm which produced smooth, circular, glistening colonies of yellow colour on nutrient agar medium. Similar characters were also described by Yoshimura and Tahara (1960) and Chakravarti and Rangarajan (1967).

Mizukami (1956) first described the symptoms of bacterial blight as water soaked lesions which appeared along the margin of the upper leaves. These gradually enlarged along the veins and later turned yellow in colour. As the disease progressed, the lesions became 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 (1950). Studies conducted at the International Rice Research Institute, Philippines have also revealed that the kresek phase of the disease started one or two weeks after transplanting as greyish discolouration of leaves followed by the rolling of leaves along the midrib (Anon, 1964). Srinivasan and Singh (1983) reported that kresek (wilt) phase of bacterial blight caused by Xanthomonas campestris pv. oryzae was one of the major factors limiting rice cultivation where the pathogen was uniformly distributed. Besides, in their trial on different inoculation techniques for inducing kresek, they found that crown inoculation with needle pricking caused the highest kresek infection.

Srivastava and Rao (1966) reported that the typical symptoms of bacterial blight which appeared one week after artificial inoculation as water soaked lesions on both margins of the leaves later extended to leaf sheaths and clumps resulting in the death of the tiller or the whole clump. Singh and Saksena (1968) observed poor root growth in plants infected during the early stages of growth. They further reported poor grain filling and complete prevention of earhead development in severely affected plants.

Shukla (1981) found that 60 to 65 per cent leaf infection during the initial tillering to maximum tillering stage in PR 106 rice variety resulted in reduction in the number of tillers, ear bearing tillers and yield per plant by about 69, 60 and 58 per cent respectively. Sharma and Kaul (1984) also analysed the yield loss due to bacterial blight of rice. In pathogenicity tests with Xanthomonas campestris pv. oryzae on two susceptible and two resistant rice cultivars, all yield components were found to be adversely affected. Yield loss due to reduction in productive tiller number was however high for susceptible varieties.

Soga (1918) observed that an endemic area to bacterial blight was one with acidic soil, poor drainage, relatively high under ground water level and frequent flooding. Kuwazuka (1942) concluded that the disease was most prevalent in areas with more than 20 mm of rainfall in July and an annual mean temperature of 14^{o}C and above. According to Goto et al.(1955) high rainfall, little sunshine, strong wind and temperature of 22 to 26°C favoured the disease outbreak of bacterial 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 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 outbreak of bacterial blight. According to Mohiuddin (1977) if there were more than 27 rainy days during August, September and October, then, there was greater incidence of bacterial 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 occurrence of bacterial blight of rice. A similar observation was also made by Nair and

Sreelatha (1988). They could co-relate the recurrence of bacterial blight in Kuttanad during the additional crop season with the specific weather conditions prevailing there, during that period.

Many reports are already published on the extent of damage caused by Xanthomonas campestris pv. oryzae on growth and yield of rice. Ishiyama (1922) observed a reduction of 20 to 30 per cent 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. Ikeno (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 blight was 33.1 per cent in T(N)1, 46.8 per cent in Tainan-S and 74.9 per cent in LT-8 under field conditions (Anon, 1967).

In India, considerable loss in yield due to bacterial 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 blight with an yield loss upto 60 per cent. Ray and Sengupta (1970) studied the incidence of bacterial 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 measureable 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 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 a highly susceptible rice variety, Karuna, 10 per cent in moderately susceptible

IR-8 and insignificant loss in relatively resistant variety IR-22 during the monsoon season under field conditions. The influence of Xanthomonas oryzae on the yield component of 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 42 per cent in Sona. The loss in yield due to the disease at 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 in Punjab.

CONTROL OF BACTERIAL BLIGHT OF RICE

Control by using antibiotics.

Swarup et al.(1965) suggested that penicillin G (100 ppm) dithane M-22 (200 ppm) and mercuric chloride (100 ppm) gave maximum inhibition against Xanthomonas campestris pv. oryzae under in vitro conditions. Desai et al.(1967) reported that Xanthomonas sp. was inhibited by streptocycline, tetracycline, oxytetracycline and chloramphenicol at 500 ppm concentration. 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, Shetty and Rangaswami (1968) found that repeated application of streptocycline induced the development of resistant strains of *Xanthomonas*. A similar observation was made recently by Nair *et al.* (1990). Devadath *et al.*(1971) made a comparative study on the cultural, physiological and biochemical characters of streptomycin resistant (at 3000 ppm) and streptomycin sensitive isolates of *Xanthomonas oryzae*. The streptomycin resistant isolates grew slower and the sensitive ones grew faster with the production of yellow pigment.

Singh *et al.* (1977) reported that agrimycin was not effective in controlling this disease. Chauhan and Vaishnav (1980) reported that streptocycline was superior to agrimycin in controlling bacterial blight based on field and laboratory studies. A similar observation was also made by Sreelatha (1985). Balaraman and Soumini Rajagopalan (1978) conducted pot culture experiment for the control of bacterial blight and found that erythromycin was highly effective when three sprays were given at 15 d interval. This was followed by treatments with 1000 ppm of chloramphenicol and terramycin. Chen *et al*. (1980) purified an antibiotic identical with formycin produced by *Nocardia* sp. which showed a curative effect against *Xanthomonas oryzae*. Singh *et al*. (1977) found that terramycin, brestanol, agrimycin-100 and fytolan gave effective control of the disease.

Chauhan and Vaishnav (1980) observed that the best method for the control of *Xanthomonas oryzae* was the application of streptocycline along with copper containing compounds. Mary and James Mathew (1983) observed that penicillin at 500 ppm was inhibitory for the growth of *Xanthomonas oryzae* under *in vitro* conditions. Penicillin or agrimycin-100 (250 ppm) applied as post-inoculation sprays, were equally effective in reducing the disease intensity in rice. Durgapal (1983) found that the most suitable means for controlling Xanthomonas oryzae in infected nurseries was to submerge the seedlings for 24 h in 500 ppm agrimycin -100, dicrystin-S or streptocycline before transplanting. Swain et al. (1985) conducted field trials to determine the efficacy of certain chemicals in the control of bacterial blight. They observed that seed treatment with plantomycin (0.03%) showed the highest efficacy in controlling bacterial blight when it was used alone or in combination with carbendazim (0.3%). Chandrasekharan and Vidhyasekharan (1988) reported that chloramphenicol is superior to streptomycin, oxytetracycline, streptocycline and their various combinations.

Control by using fungicides.

Copper and mercuric compounds were tested earlier to control bacterial blight of rice. Hashioka (1951) found that spraying with bordeaux mixture partly controlled bacterial blight if applied before the typhoon. However it was not effective enough for practical use because of the copper sensitiveness of many rice varieties. Jain et al. (1965) reported that spraying with coppesan (copper oxychloride) at the rate of 2.8 kg/ha reduced infection by Xanthomonas oryzae. In 1966 they found that dipping of seeds for 8 h 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 blight. Pal and Singh (1978) observed that brestanol was translocated from roots to leaves through stem and leaf sheath which was sufficient to inhibit the growth of Xanthomonas campestris pv. 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 of that of the control. Deiveegasundaram et al. (1977) found that different concentrations (250, 500 and 1000 ppm) of various fungicides such as thiram, captan, 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 blight followed by agrimycin-500. Verma et al. (1980) reported that dithane C-90 and fytolan both at 0.3 per cent concentration reduced the bacterial blight of rice. Durgapal 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*.

Other methods for the control of bacterial blight of rice.

Bacteriophage specific for *Xanthomonas oryzae* was first isolated by Yoshii et al. (1953). Wakimoto (1954) made a detailed study of the phage and named it as *Xanthomonas oryzae* sp. bacteriophage. He further used this phage for the quantitative determination of *Xanthomonas* in soil and irrigation water. Investigations on phage population in the irrigation water running through the canals and rivers were subsequently used for forecasting the occurrence of bacterial blight in large areas, which was important for using chemicals for protection against this diseases (Anon, 1967). Nilpanit _et al_. (1984) isolated bacteriophage strains of _Xanthomonas campestris_ from parts of Thailand. He observed that the bacteriophage technique can be used to forecast bacterial blight outbreak. Phage population determined by plaque counting also gave an indication of the population density of the host bacterium, _Xanthomonas campestris_ in irrigation water.

Extensive research has been done by several workers, to evolve varieties resistant to bacterial blight. Reddy (1965) found 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 leaf blight, IR-5 and IR-8 were moderately susceptible and BRT, N-136 were resistant. Rice varieties like IR-20 and IR-22 were earlier found to be resistant to bacterial 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 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 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.

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. Palaniswami and Ahmed (1979) observed that application of stable bleaching powder containing 35 per cent chlorine was not effective in controlling the incidence of bacterial blight when applied at a concentration of 5 ppm to soil, 24 h before sowing. At higher concentrations it was found to be phytotoxic and seriously reduced the yield of the crop. However, Sivaswamy and Mahadevan (1986) reported that eventhough Xanthomonas campestris pv. oryzae survived in infected soil, its population was reduced considerably, by the addition of bleaching powder. Naidu et al. (1980) found that mixing of urea with neem cake or coal tar increased the incidence of bacterial blight. Mondal and Miah (1985) observed that plants grown in soil with 183 ppm K were more resistant to Xanthomonas campestris pv oryzae and yielded 16 per cent more than those grown with 100 ppm K.

Pandey and Iswaran (1982) found that in the presence of nitrogen fixing bacteria, invasion of Xanthomonas campestris pv. oryzae into rice leaves was enhanced. This suggested that certain metabolites liberated by these bacteria contributed to the process of invasion.

Sakthivel et al (1986) isolated several strains of Pseudomonas fluorescens from plant rhizosphere and identified them as biotypes C and G. These siderophore producing strains showed antagonism under in vitro tests to several plant pathogens including Fusarium oxysporum f. sp cubense, Rhizoctonia solani, Acrocylindrium oryzae and Xanthomonas campestris pv.

oryzae. Selected strains of Pseudomonas fluorescens used for bacterisation of rice and cotton seeds, enhanced plant growth by 12-27 per cent in rice. Anuratha and Gnanamanickam (1987) studied the effect of Pseudomonas fluorescens (biotype III) on Xanthomonas oryzae. Pseudomonas fluorescens (10^8 cfu/ml) was mixed with an one per cent solution of sterile carboxymethyl cellulose and powdered vermiculite and dried overnight at room temperature (28°C). Rice seeds coated with this mixture showed 40 to 60 per cent reduction in bacterial blight severity. Sivamani et al. (1987) observed that a rice strain of Pseudomonas fluorescens caused maximum inhibition of Xanthomonas oryzae in plate tests. Based on the results obtained, they suggested that native strains of Pseudomonas fluorescens could be used as a bio-control agent against bacterial blight pathogen.

Lin et al.(1981) suggested that bacterial 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 nursery bed itself. Padmanabhan (1983) advocated an integrated approach based on host tolerance, judicious use of fertilizers, adoption of appropriate agronomic practices direct control with biocides and use of biological antagonists to minimize losses caused by bacterial blight.

Gossele *et al*.(1984) studied the effect of 235 organic and inorganic compounds against *Xanthomonas oryzae* of which the most active were zinc oxide, 8-hydroxyquinoline and methyl glyoxal (all inhibitory at concentrations between 0.0001 and 0.005 per cent) and a cadmium salt, O-phenanthroline, CGA 78039 and formaldehyde (0.0001 to 0.0005 per cent). Salts of copper, nickel and cobalt were inhibitory for all concentrations between 0.0001 and 0.005 per cent. Hoa *et al*.(1984) observed that spraying with ammonium sulphate 5 d before glass house inoculation decreased the intensity of bacterial blight in moderately resistant and susceptible cultivars.

Takahi and Shirahagen (1985) reported the development of a new systemic bactericide namely, techloftalam for the control of bacterial blight. It did not kill the bacteria but was very effective in inhibiting its multiplication in rice plants. Natarajan and Lalitha-kumari (1989) observed that bactrinol-100 gave good control of bacterial blight when compared to other chemicals. Bactrinol-100 gave about 85 per cent disease reduction even in artificially inoculated plants than other chemicals. Moreoever, the grain weight, yield, straw weight and height of tillers were found to increase significantly by spraying with bactrinol-100. Besides killing the pathogen directly, the chemical was also found to stimulate the host defence mechanism by altering its metabolic activity.

Mary et al.(1986)observed that a foliar spray of cowdung extract (20 g/l) controlled bacterial blight equivalent to that given by penicillin (100 ppm), paushamycin (250 ppm) and streptomycin (100 ppm).

MATERIALS AND METHODS

MATERIALS AND METHODS

The study on the effect of 2 bromo-2-nitro-propane-1, 3-diol (Bactrinol-100) and cowdung extract on growth and virulence of <u>Xanthomonas</u> <u>campestris</u> pv. <u>oryzae</u> causing bacterial blight (BB) of rice was conducted at the College of Agriculture, Vellayani, Trivandrum during 1988-89. The field experiments to evaluate the comparative efficacy of certain commonly used antibiotics, bactrinol-100 and cowdung extract were laid out at Ulloor Seed farm of the Department of Agriculture, Government of Kerala and in a farmer's field at Nedumudy in Kuttanad. The following rice varieties were used for the present investigation.

Sl. No.	Variety	Duration	Susceptibility to bacterial blight	Experiment
1	T(N)1	110 - 115	Highly susceptible	Chemical control of BB under pot culture conditions.
2	Jyothi	120 - 125	Susceptible	Chemical control of BB under conditions at Ulloor seed farm Trivandrum.
3	Culture-153	120 - 125	Susceptible	Chemical control of BB under field conditions at Nedumudi, Kuttanad

1. THE PATHOGEN

A culture of _Xanthomonas campestris_ pv. _oryzae_ was initially isolated from a bacterial blight affected rice field in Kuttanad. A portion of the infected leaf showing profuse bacterial ooze was cut into small bits and surface sterilized with 0.1% mercuric chloride solution for one minute in a sterile petri plate and washed thoroughly with four changes of sterile water. The leaf bits were then teased apart to get a dense bacterial suspension. A loopful of the same was streaked aseptically on potato sucrose peptone agar medium of following composition and incubated for 48 h at room temperature.

Potato sucrose peptone agar medium (PSPA)

KH_2PO_4	- 0.2g
Na_2HPO_4	- 0.5g
$Ca(NO_3)_2$	- 0.5g
$FeSO_4$	- 0.05g
Kcl	- 0.05g
Peptone	- 2.0g

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

Typical colonies of Xanthomonas campestris pv. oryzae which appeared on these plates were selected and checked for purity once by streaking on PSPA medium and by Gram staining.

Procedure for Gram staining (Hucker, 1927)

A thin smear of 24 h old broth culture of the pathogen was prepared, heat fixed and stained with ammonium oxalate crystal violet for one minute. The slides were gently washed in tap water and treated with iodine solution for one minute. These were then washed in tap water and decolourised with 95 per cent ethyl alcohol for 30 seconds with gentle agitation. The slides were counter stained with safranin for one minute, washed again in tap water, dried and examined under oil-immersion objective of a microscope. The composition of different stains and reagents used were as follows.

<u>Ammonium oxalate crystal violet</u>

Solution A

Crystal violet	-0.2g
Ethy alcohol (95%)	-20 ml

Solution B

Ammonium oxalate	- 0.8g
Distilled water	- 80 ml

<u>Gram's modification of Lugol's solution</u>

Iodine	- 1.0g
Potassium iodide	- 2.0g
Distilled water	- 300 ml

<u>Counter stain</u>

Safranin (2.5% solution in 95% ethanol)	- 10 ml
Distilled water	- 100 ml

The culture was also tested once for virulence by inoculating healthy seedlings of T(N)1. Artificial inoculation was done by clipping young leaf tips with a pair of scissors dipped in 48 h old suspension of the pathogen in distilled water (grown on potato sucrose peptone agar medium) and by spraying the same suspension over the entire leaf surface. The plants were covered with polythene bags to maintain a high level of humidity necessary for infection. The symptoms of bacterial blight appeared seven days after inoculation as straw coloured lesions, on both margins of the leaves. The pathogen was then re-isolated by the procedure described earlier. A pure culture of the same was maintained on PSPA medium for further studies.

2. CHEMICAL CONTROL OF BACTERIAL BLIGHT OF RICE

2.1 Effect of different antibiotics, bactrinol-100 and cowdung extract on growth of *Xanthomonas campestris* pv. *oryzae* under *in vitro* conditions

The effect of two antibiotics, terramycin and streptocycline, a synthetic organic compound bactrinol-100 and cowdung extract on growth of *Xanthomonas campestris* pv. *oryzae* was studied under *in vitro* conditions using potato sucrose peptone agar medium. Sterile filter paper discs of 10 mm diameter were dipped in appropriate concentration (indicated below) of terramycin, strepto-

Concentration of antibiotics, bactrinol-100 and cowdung extract used

Sl. No.	Test substance	Manufacturing company.	Active ingredient	Concentration.	Diluent used
A.	Antibiotics				
1.	Terramycin	Pfizer Ltd.,Thane Bombay.	Oxy-tetracycline Hydrochloride	100 ppm 250 " 500 "	Sterilised Distilled water
2.	Streptocycline	Hindusthan Antibiotics Ltd.,Pimpri, Poona.	Streptomycin sulphate 90% and Tetracycline hydrochloride 10%	100 " 250 " 500 "	"
B.	Bactrinol-100	Merlin Laboratories Madras.	2-bromo-2-nitro-propane 1,3-diol	100 " 250 " 500 "	"
C.	Cowdung extract*				
1.	Normal extract	-	-	20 g/l 50 " 100 "	"
2.	Sterilized extract	-	-	20 g/l 50 " 100 "	"
3.	Heated extract	-	-	20 g/l 50 " 100 "	"

* 2, 5 and 10 g of fresh cowdung was mixed thoroughly in 100 ml of sterile distilled water and filtered through Whatmann No.1 filter paper to obtain cowdung extract of concentrations 20, 50 and 100 g/l respectively. One set of this extract was sterilized through sintered glass filter (G-5), while another set was subjected to a mild heat treatment of 60°C for 15 min in a water bath, to detect the presence of any heat labile antibacterial factor in cowdung extract.

cycline ,bactrinol-100 and cowdung extract and placed aseptically in the centre of each plate containing PSPA medium pre-seeded with a 48 h old virulent culture of _Xanthomonas campestris_ pv. _oryzae_. Three replications were maintained for each treatment. The mean zone of growth inhibition was recorded at 24 h interval for three days.

2.2. Effect of spraying with different antibiotics, bactrinol-100 and cowdung extract on control of bacterial blight under pot culture conditions

A pot culture experiment was conducted using the rice variety T(N)1 to study the extent of control of bacterial blight by spraying with different concentrations of terramycin, streptocycline, bactrinol-100 (100, 250 and 500 ppm each) and normal cowdung extract (20, 50 and 100 g/l). Three replications were maintained for each treatment in earthen pots of 30 cm diameter filled with 15 kg of unsterilized paddy soil. NPK fertilizers were applied at the rate of 90, 45 and 45 kg/h in the form of urea, super phosphate and muriate of potash as per the Package of Practices Recommendations of Kerala Agricultural University (1986). The plants were artificially inoculated with a virulent culture of *Xanthomonas campestris* pv. *oryzae* at maximum tillering stage by the method described earlier. Inoculated plants were covered with polythene bags for three days to maintain a high level of humidity required for infection. The disease intensity was scored 15 d after inoculation with the pathogen, based on a standard score chart prepared by the International Rice Research Institute of Manila, Philippines.

Disease score

0 - no blighting of leaves
1 - less than 1 per cent of the leaf area blighted
3 - 1 to 5 per cent of the leaf area blighted
5 - 6 to 25 per cent of the leaf area blighted
7 - 26 to 50 per cent of the leaf area blighted
9 - 51 to 100 per cent of the leaf area blighted

The disease intensity was calculated as follows:

$$\text{Disease intensity} = \frac{\text{Sum of individual ratings}}{\text{Total number of plants observed}} \times \frac{100}{\text{Maximum disease score}}$$

After disease assessment, the plants were sprayed with different concentrations of terramycin, streptocycline, bactrinol-100 and normal cowdung extract. Any reduction in disease intensity was recorded 15 d after spraying by the method described earlier.

2.3 Effect of spraying with different antibiotics, bactrinol-100 and cowdung extract on control of bacterial blight under field conditions.

Two separate field experiments were conducted at two locations to study the effect of spraying with different antibiotics, bactrinol-100 and cowdung extract on control of bacterial blight. The first experiment was laid out at Ulloor seed farm of the Department of Agriculture, Government of Kerala, during May, 1988 and the second experiment was laid out in a farmer's field at Nedumudy in Kuttanad during the additional crop season of July, 1989.

2.3.1. Field experiment at Ulloor seed farm, Trivandrum

The rice variety Jyothi was used for this experiment laid out in RBD with three replications each in individual plots of 3 x 3 m size. NPK fertilizers were added at the rate of 90, 30 and 20 kg/h respectively based on soil test data. Organic manure in the form of cowdung was added at the rate of 5 t/h at the time of first ploughing. Half the dose of nitrogen and potassium and the full dose of phosphorus in the form of urea, muriate of potash and super phosphate were applied at the time of final ploughing. The remaining quantity of nitrogen and potassium were added as top dressing at panicle initiation stage. A natural incidence of bacterial blight was observed in this field just prior to panicle initiation stage during the month of June 1988. An area of approximately, 360 m^2, where the disease incidence was found to be more or less uniform was selected for conducting the field experiment. The initial disease intensity was scored at this stage by the method described earlier. The plants were then sprayed with 100, 250 and 500 ppm each of terramycin, streptocycline and bactrinol-100 and 20, 50 and 100 g/l of normal cowdung extract. The control plots were maintained without any spray treatment.

Any reduction in disease intensity was recorded 15 d later when the plants were once again sprayed with the same concentrations of antibiotics, bactrinol-100 and cowdung extract. Fifteen days after second spray, the disease intensity was once again recorded. At the time of harvest, the different yield parameters specified below were taken.

1. Grain and straw yield

Each plot was harvested individually and the grains were separated from the straw. The grain and straw were dried in the sun for three days to remove any excess of moisture and then weighed to determine the final grain and straw yield.

2. Thousand grain weight

Thousand fully formed grains were counted out and the weight was determined.

3. Chaff percentage

The main culm panicles from 12 randomly selected hills were separated, threshed and the number of filled grains (f), number of unfilled grains (u) and the weight of filled grains (w) were determined. The rest of the panicles from all 12 hills were also threshed and the number of unfilled grains (U) and the weight of filled grains (W) were assessed. From the above data, the percentage of unfilled grains were worked out using the formula of Gomez (1972).

$$\text{Percentage of unfilled grains} = \frac{U + u}{f\ (W + w)/\ W + U + u} \times 100$$

2.3b <u>Field experiment at Nedumudi, Kuttanad</u>

The rice variety, Culture-153 was used for this experiment. NPK fertilizers were added at the rate of 90, 45 and 45 kg/h respectively. Half the dose 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/h in two split doses, 350 kg as basal dose at the time of final ploughing and 250 kg as top dressing, one month after transplanting. A natural incidence of bacterial blight was observed in the field during the peak tillering stage in August, 1989. An area of approximately 360 m^2 where the incidence of bacterial blight was found to be more or less uniform was selected for conducting the field experiment. The design of experiment, treatments used and the various observations taken were same as that of the previous field experiment.

3. EFFECT OF SOME BACTERIAL AND FUNGAL ISOLATES FROM COWDUNG EXTRACT ON GROWTH OF *Xanthomonas campestris* pv. *oryzae*

3.1 Isolation of antagonistic bacteria and fungi

One gram of fresh cowdung was added to 100 ml of sterile distilled water and thoroughly mixed using a mechanical shaker for 20 minutes. Serial dilutions to the order of 10^{-10} were prepared aseptically for the isolation of antagonistic bacteria and fungi present in cowdung extract using nutrient agar and Martin's rose bengal agar medium respectively.

Nutrient agar medium

Peptone	- 5.0 g
Beef extract	- 3.0 g
Agar	- 20.0 g
Distilled water	- 1000 ml
pH	- 6.8

<u>Martin's rose bengal agar medium</u>

Dextrose	- 10.0 g
Peptone	- 5.0 g
KH_2PO_4	- 1.0 g
$MgSO_4$	- 0.5 g
Rose bengal	- 33.0 mg
Agar	- 20.0 g
Distilled water	- 1000 ml
Streptomycin	- 1% solution (0.3 ml/100 ml of sterilized medium)

Dilutions ranging from 10^{-6} to 10^{-10} were used for the isolation of fungi and bacteria by routine pour plate technique. Five replications were maintained for each dilution. The plates were incubated at room temperature for 48 and 72 h for bacteria and fungi respectively. Dominant bacterial and fungal colonies were then isolated and transferred to nutrient agar and potato dextrose agar slants for further studies.

<u>Potato dextrose agar medium</u>

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

3.2a Effect of bacterial isolates on growth of *Xanthomonas campestris* pv. *oryzae*

Sterilized potato sucrose peptone agar medium (20 ml) was initially poured into sterilized petri plates and allowed to solidify. A distinct straight line streak of *Xanthomonas campestris* pv. *oryzae* was made on each plate under aseptic conditions. The test bacterial culture was then streaked parallel to the streak of the pathogen at a distance of 1 cm. The plates were incubated at room temperature for 72 h and observations for any antagonistic effect of the test organism on growth of the pathogen was recorded daily for three days. Three replications were maintained for each bacterial isolate.

3.2b Effect of fungal isolates on growth of *Xanthomonas campestris* pv. *oryzae*

Each fungal isolate was initially grown separately on potato dextrose agar medium for 72 hours. Using a sterilized cork borer of 5 mm diameter, circular discs of fungal growth were cut out and placed aseptically on potato sucrose peptone agar medium pre-seeded with a 48 h old virulent culture of *Xanthomonas campestris* pv. *oryzae*. The plates were incubated at room temperature for 96 h and observations for any antagonistic effect of the test organism on growth of the pathogen was recorded daily for four days. Three replications were maintained for each organism.

4. ECONOMIC BENEFITS OF CONTROLLING BACTERIAL BLIGHT OF RICE UNDER FIELD CONDITIONS

The economic return was calculated on the basis that the current cost of paddy cultivation is Rs.7000/- per hectare, the present market rates for one kilogram of grain and straw are Rs.3.00 and Rs.1.50 respectively, the cost per gram of terramycin, streptocycline and bactrinol-100 is Rs.3.20, Re.0.63 and Rs.2.50 respectively and that cowdung is available free of cost.

Net return per rupee invested was worked out by the formula,

$$\text{Net return per rupee invested} = \frac{X - Y}{Y}$$

where X = value of the product (grain/straw)

Y = total cost of production including treatments.

5. STATISTICAL METHODS OF ANALYSIS

The data on various observations were analysed by the methods described by Snedecor and Cochran (1967) and their significance was tested by 'F' test (Cochran and Cox, 1965).

RESULTS

RESULTS

1. THE PATHOGEN

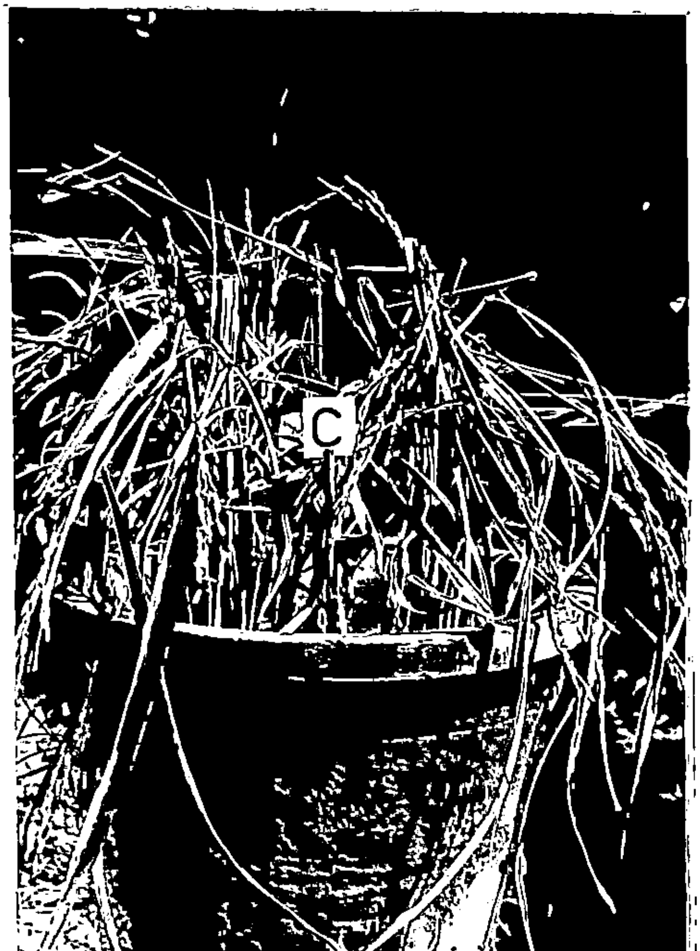
The culture of _Xanthomonas campestris_ pv. _oryzae_ isolated from a bacterial blight affected rice field in Kuttanad was tested once for virulence by inoculating healthy seedlings of T(N)1 under pot culture conditions. Typical symptoms of bacterial blight appeared 7 d after inoculation as straw coloured lesions on both margins of infected leaves (Plate 1). The pathogen was reisolated on potato sucrose-peptone agar medium, where it produced the characteristic yellow slimy colonies of _X. campestris_ pv. _oryzae_. The culture was gram negative without any contamination. It was maintained on PSPA medium for further studies.

2. CHEMICAL CONTROL OF BACTERIAL BLIGHT OF RICE

2.1 Effect of different antibiotics, bactrinol-100 and cowdung extract on growth of _X. campestris_ pv. _oryzae_ under _in vitro_ conditions

The growth inhibition of _X. campestris_ pv. _oryzae_ on PSPA medium was in proportion to the concentration of terramycin, streptocycline and bactrinol-100 used

Plate 1. Typical symptoms of bacterial blight in T(N)1 rice variety under artificial conditions.


C

during this investigation (Table 1 and Plates 2 and 3). The zone of growth inhibition of 15.3 mm was maximum with the use of terramycin 500 ppm followed by terramycin 250 ppm (13.3 mm) and bactrinol-100 500 ppm (10.7 mm) (Fig.1). The inhibitory effect of terramycin at 500 ppm on growth of the pathogen was significantly higher than all other treatments (Plate 5). However, none of the three forms of cowdung extract tested produced a typical growth inhibition zone as observed in the case of both the antibiotics and bactrinol-100 (Plate 4).

2.2 Effect of spraying with antibiotics, bactrinol-100 and cowdung extract on control of bacterial blight in T(N)1 under pot culture conditions

The different concentrations of terramycin, streptocycline, bactrinol-100 and cowdung extract tried earlier for any inhibitory effect on growth of the pathogen were used to test their relative efficacy to control bacterial blight in T(N)1 under pot culture conditions. The reduction in disease intensity in plants sprayed with various concentrations of these chemicals and cowdung extract

Table 1. Growth inhibition (mm)* of *X. campestris* pv. *oryzae* due to antibiotics, bactrinol-100 and cowdung extract under *in vitro* conditions

Chemicals used	Concentration (ppm)		
	100	250	500
Terramycin	9.80	13.30	15.30
Streptocycline	2.70	3.80	5.00
Bactrinol-100	5.00	7.70	10.70
Cowdung extract	20 g/l	50 g/l	100 g/l
Normal extract	-	-	-
Sterilized extract	-	-	-
Heated extract	-	-	-

- Absence of growth inhibition

1. CD (0.05) for comparison between treatments = 0.83
2. CD -do- levels of terramycin = 0.92
3. CD -do- " streptocycline = 0.68
4. CD -do- " bactrinol -100 = 0.75

* Mean of three replications

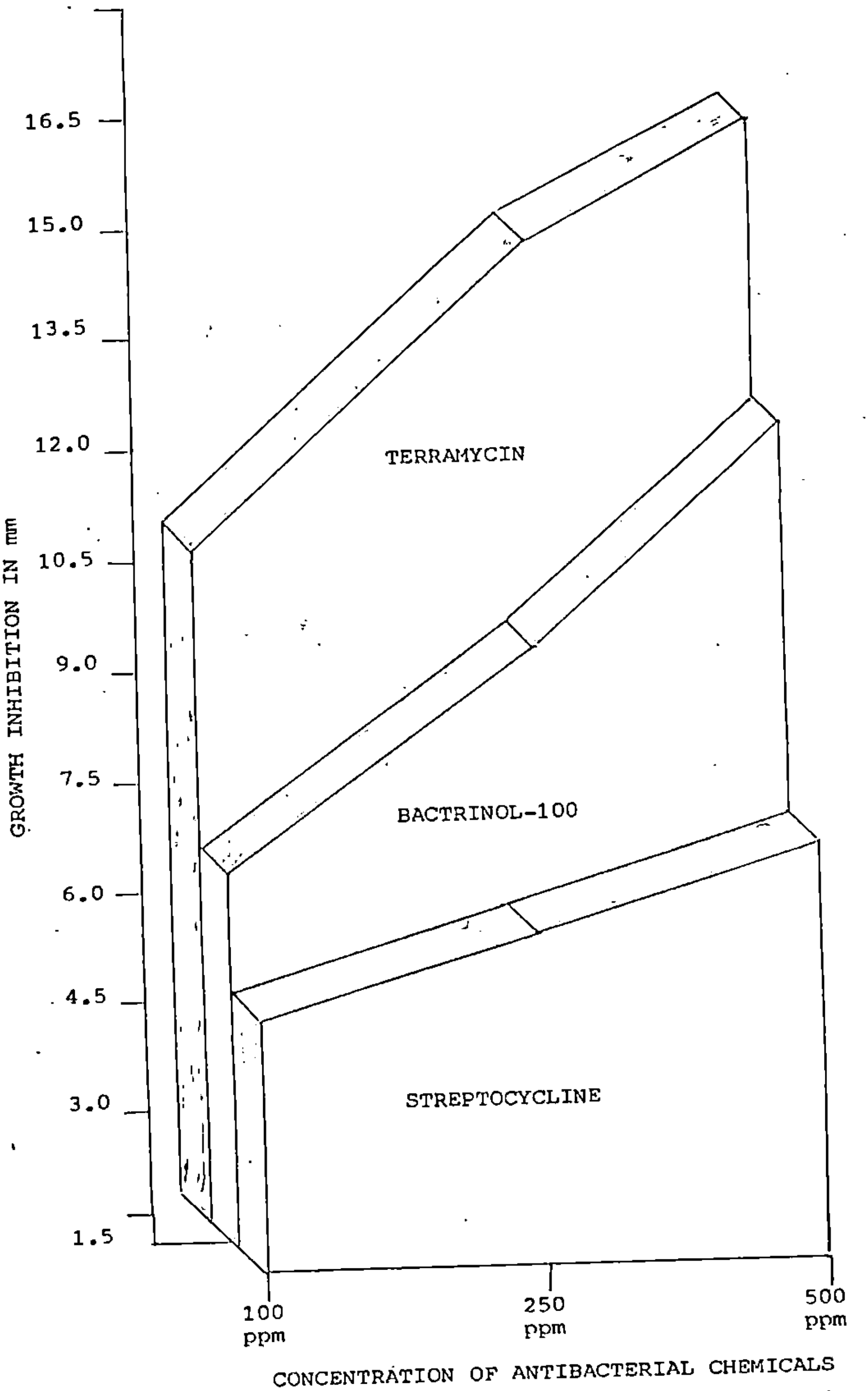


Fig.1 Growth inhibition of *Xanthomonas campestris* pv. *oryzae* due to terramycin, streptocycline and bactrinol-100 under *in vitro* conditions.

Plate 2. Growth inhibition of _X. campestris_ pv. _oryzae_ by terramycin under _in vitro_ conditions

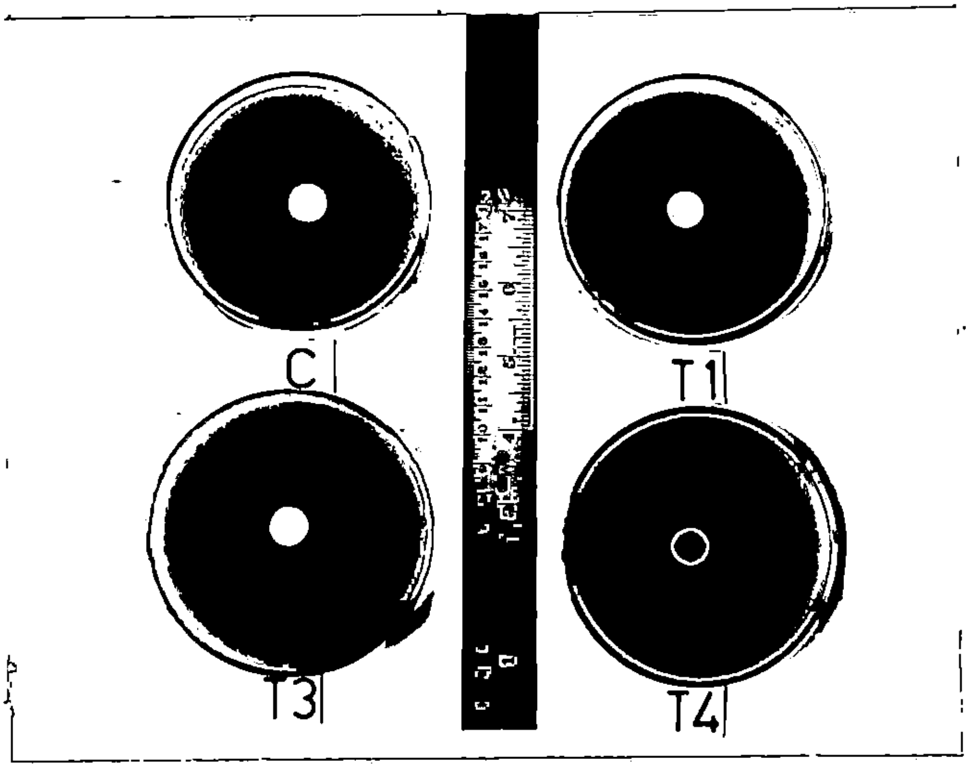
KEY

C	- Control		
T_1	- Terramycin	100	ppm
T_3	- "	250	ppm
T_4	- "	500	ppm

Plate 3. Growth inhibition of _X. campestris_ pv. _oryzae_ by bactrinol-100 under _in vitro_ conditions.

KEY

C	- Control		
B_1	- Bactrinol-100	100	ppm
B_3	- "	250	ppm
B_4	- "	500	ppm


C
T1
T3
T4

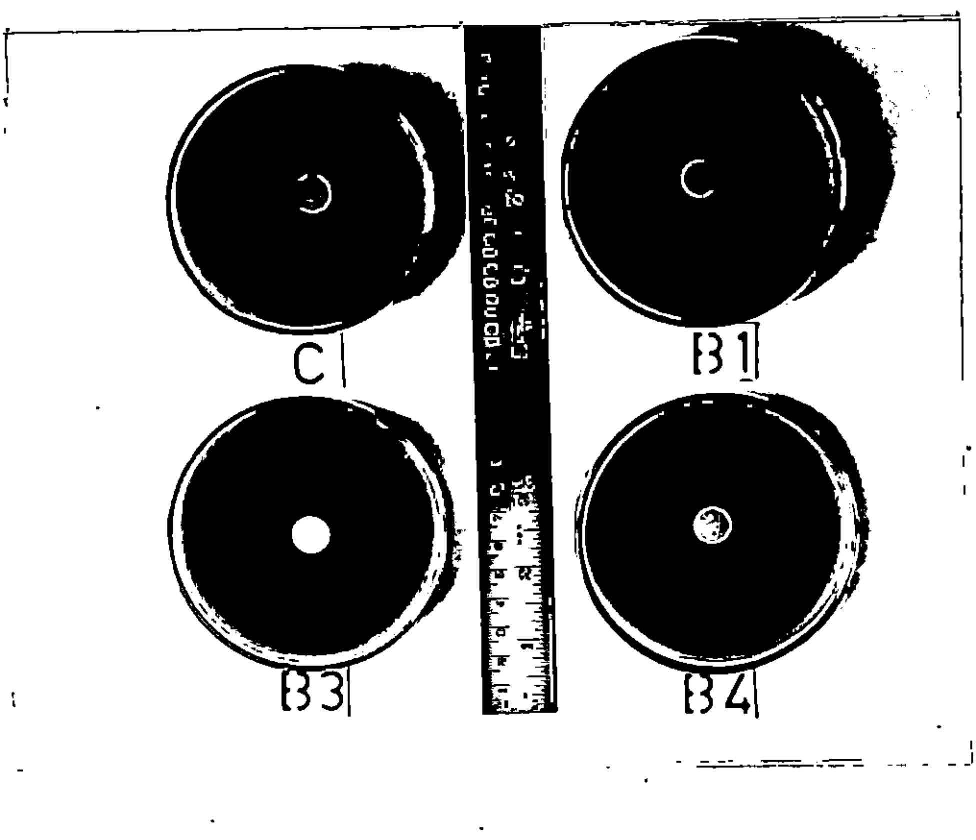

C
B1
B3
B4

Plate 4. Effect of cowdung extract on growth of *X. campestris* pv. *oryzae* under *in vitro* conditions.

KEY

C - Control

U_3 - Unsterilized extract (100g/l)

H_3 - Heated extract (100 g/l)

F_3 - Filtered extract (100 g/l)

Plate 5. Growth inhibition of *X. campestris* pv. *oryzae* by terramycin, streptocycline, bactrinol-100 and cowdung extract under *in vitro* conditions.

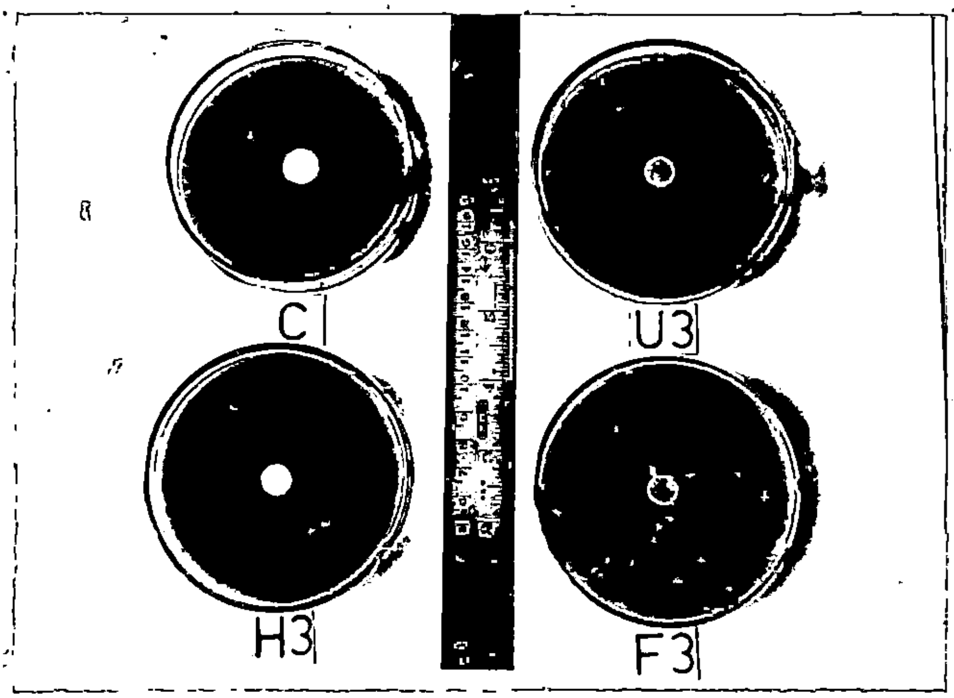
KEY

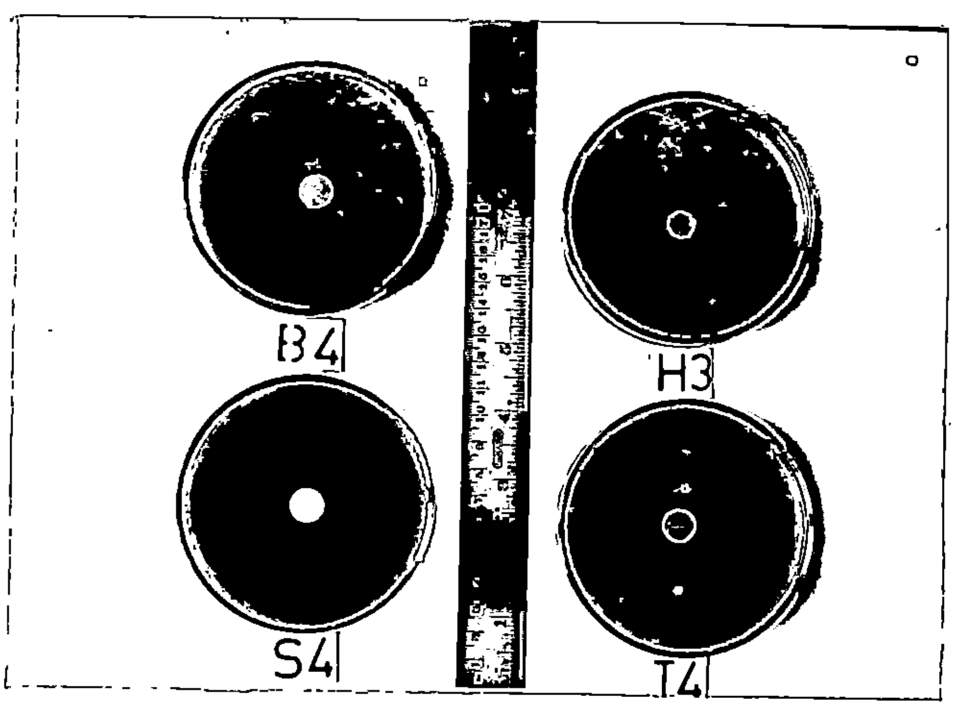
B_4 - Bactrinol-100 500 ppm

H_3 - Heated extract 100g/l

S_4 - Streptocycline 500 ppm

T_4 - Terramycin - 500 ppm


C
U3
H3
F3


B4
H3
S4
T4

was significant when compared to the control treatment. However, this effect was maximum after spraying with 500 ppm terramycin, followed by 500 ppm bactrinol-100. The disease intensity in these plants, 15 d after spraying with the above concentration of terramycin and bactrinol-100 were 46.98 and 53.19 respectively (Table 2 and Fig.2). This corresponded to a reduction of 42.24 and 34.61 per cent in disease intensity when compared to the control treatment where during this period, there was a significant increase in disease intensity by 19.05 per cent.

In general, higher the concentration of each chemical tried, better was the control of bacterial blight in T(N)1, except in plants sprayed with cowdung extract. Here, the reduction in disease intensity was more or less uniform (Table 2). Besides, the extent of disease control achieved after spraying with different concentrations of cowdung extract was better than some of the chemical treatments like terramycin 100 ppm, streptocycline at 100, 250 and 500 ppm and bactrinol-100 at 100 and 250 ppm.

Table 2. Effect of spraying with antibiotics, bactrinol-100 and cowdung extract on control of bacterial blight in T(N)1 under pot culture conditions

Antibiotics/ cowdung extract	Concen- tration	Disease intensity*		% redu- ction over control
		Before spraying	After spraying	
Terramycin	100 ppm	66.37	65.77	19.14
	250 "	66.45	58.86	27.64
	500 "	65.11	46.98	42.24
Streptocycline	100 "	66.96	72.47	10.91
	250 "	64.22	68.07	16.31
	500 "	65.09	63.15	22.36
Bactrinol-100	100 "	65.35	68.33	16.00
	250 "	64.59	64.50	20.70
	500 "	67.56	53.19	34.61
Cowdung extract	20 g/l	68.01	59.51	26.84
	50 "	65.38	60.74	25.33
	100 "	65.45	59.50	26.85
Control		68.32	81.34	(+19.05)
CD(0.05)			2.67	

* Mean of three replications

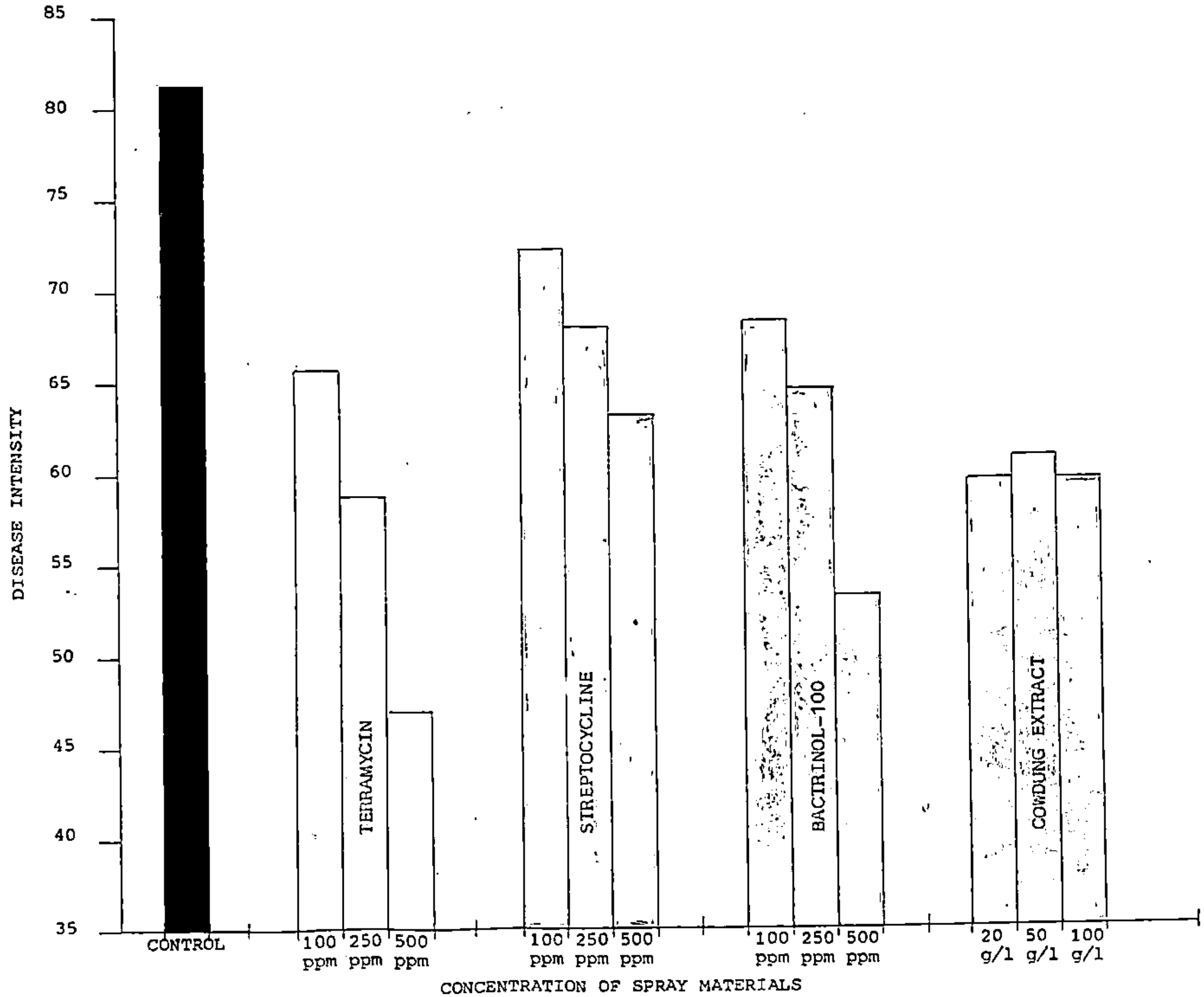


Fig.2 Effect of spraying with terramycin, streptocycline, bactrinol-100 and cowdung extract on control of bacterial blight in TN(1) rice variety under pot culture conditions.

2.3 Effect of spraying with antibiotics, bactrinol-100 and cowdung extract on control of bacterial blight in Jyothi rice variety under field conditions

The reduction in disease intensity in plants sprayed with different concentrations of antibiotics, bactrinol-100 and cowdung extract was significant when compared to the control treatment. However, this effect was maximum after spraying with 500 ppm terramycin, followed by 500 ppm bactrinol-100, which was statistically on par with the above treatment (Fig.3). The disease intensity in these plants 15 d after first spray with the above concentrations of terramycin and bactrinol-100 was 27.39 and 31.30 respectively (Table 3). This corresponded to a reduction of 45.56 and 37.79 per cent in disease intensity when compared to the control treatment, where during this period, there was a significant increase in disease intensity by nearly 16.35 per cent.

A further reduction in disease intensity was observed after the second spray and this followed a similar pattern as that of first spray with different

Table 3. Effect of spraying with antibiotics, bactrinol-100 and cowdung extract on control of bacterial blight in Jyothi rice variety

Antibiotics/ cowdung extract	Concen-tration	Disease intensity*			% reduction over control	
		Before spray	After Ist spray	After IInd spray	After Ist spray	After IInd spray
Terramycin	100 ppm	45.73	43.98	42.28	12.58	28.46
	250 "	42.82	40.31	34.25	19.88	42.05
	500 "	44.32	27.39	21.15	45.56	64.21
Streptocycline	100 "	44.48	46.14	46.01	8.29	22.15
	250 "	40.86	43.28	39.40	13.97	33.33
	500 "	45.67	34.60	28.27	31.23	52.17
Bactrinol-100	100 "	43.43	44.66	44.15	11.23	25.30
	250 "	45.39	40.69	35.74	19.12	39.53
	500 "	45.88	31.30	26.61	37.79	54.98
Cowdung extract	20 g/l	44.38	40.13	34.22	20.24	42.10
	50 "	42.79	40.03	33.56	20.43	43.22
	100 "	46.00	39.74	33.20	21.01	43.82
Control		43.24	50.31	59.10	(+ 16.35)	(+ 17.47)
CD(0.05)			5.16	3.35		

* Mean of three replications

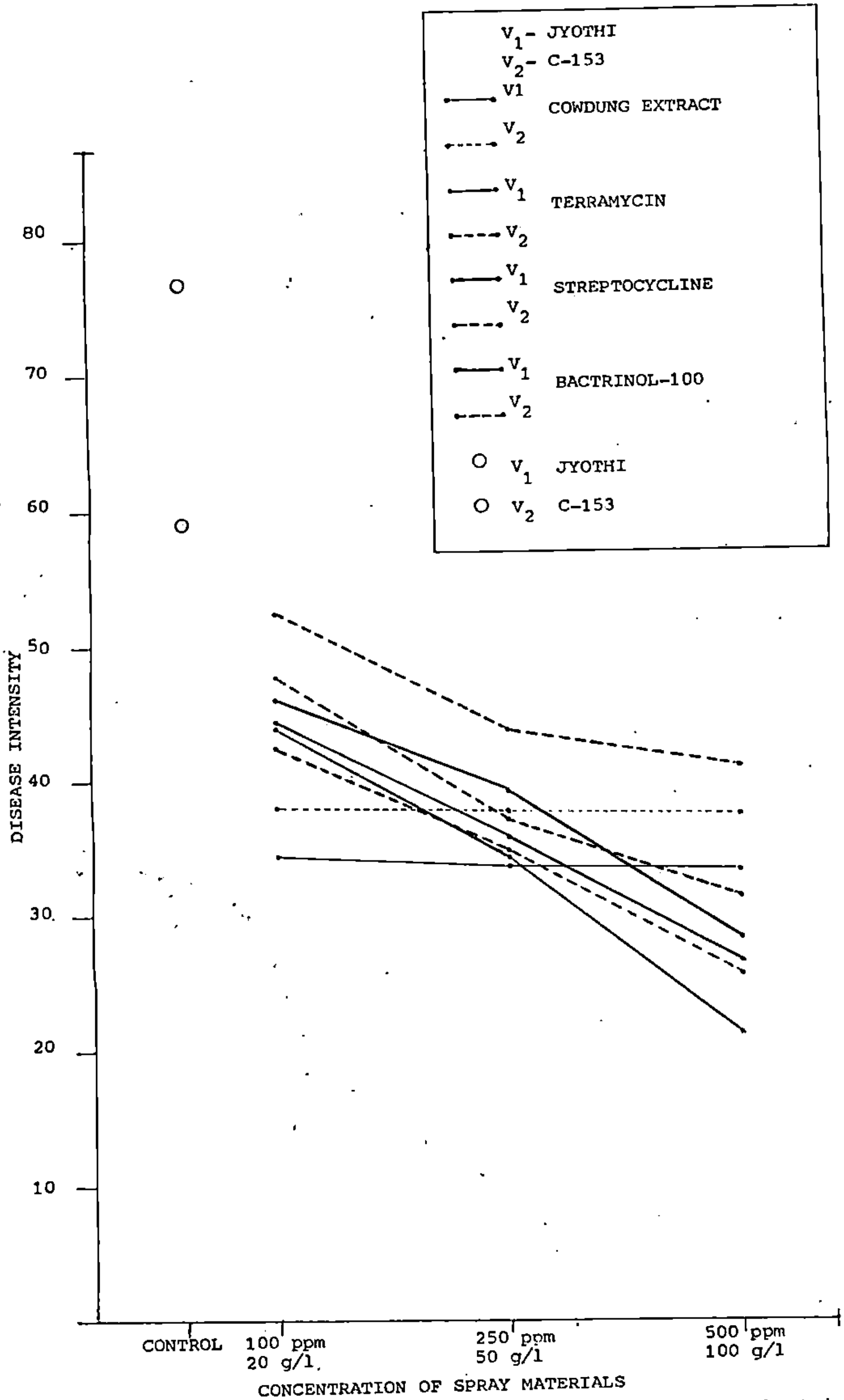


Fig.3 Effect of spraying with terramycin, streptocycline, bactrinol-100 and cowdung extract on control of bacterial blight in Jyothi and C-153 rice varieties under field conditions.

antibiotics, bactrinol-100 and cowdung extract (Table 3). The overall reduction in disease intensity was maximum in plants sprayed with 500 ppm terramycin. This was significantly better than all other treatments and corresponded to an actual reduction of 64.21 per cent in disease intensity in comparison with the control treatment. As observed earlier, the effect of bactrinol-100 was not on par with above treatment. In general, higher the concentration of each chemical sprayed, better was the control of bacterial blight under field conditions. However, with cowdung extract such an effect was only marginal since the level of disease control achieved with different concentrations was more or less uniform (Table 3). Further, the extent of disease control obtained by spraying cowdung extract, especially after the second spray, was significantly higher than some of the chemical treatments, like 100 ppm each of terramycin and bactrinol-100 and 100 and 250 ppm of streptocycline.

2.3a Grain yield in bacterial blight affected Jyothi rice variety

The increase in grain yield in plants sprayed with different concentrations of antibiotics, bactrinol-100 and cowdung extract was significant when compared to the control treatment. However, this effect was maximum after spraying with 500 ppm of terramycin, where, an average yield of 4.04 kg/plot was obtained (Table 4 and Fig.4). This actually represented an yield increase of 159.54 per cent in comparison to the control treatment with an yield of only 1.56 kg/plot. The net grain yield from plants sprayed with 500 ppm of bactrinol-100 (4.00 kg/plot) and 50 and 100 g/l of cowdung extract (3.93 and 3.98 kg/plot respectively) were also significant and statistically on par with the above treatment. Further, it was observed that in plots sprayed with different concentrations of cowdung extract, the grain yield obtained (3.79, 3.93 and 3.98 kg/plot respectively for 20, 50 and 100 g/l of of cowdung extract) was significantly better than

Table 4. Grain yield in bacterial blight affected Jyothi rice variety after spraying with antibiotics, bactrinol-100 and cowdung extract

Antibiotics/ Cowdung extract	Concentration	Yield/ plot * (kg)	Yield (t/ha)	% increase over control
Terramycin	100 ppm	2.96	3.29	90.17
	250 "	3.59	3.99	130.64
	500 "	4.04	4.49	159.54
Streptocycline	100 "	2.68	2.98	72.25
	250 "	3.40	3.78	118.50
	500 "	3.90	4.34	150.87
Bactrinol-100	100 "	2.71	3.01	73.99
	250 "	3.44	3.83	121.39
	500 "	4.00	4.44	156.65
Cowdung extract	20 g/l	3.79	4.21	143.35
	50 "	3.93	4.36	152.02
	100 "	3.98	4.43	156.07
Control		1.56	1.73	
CD(0.05)		0.13		

* Mean of three replications

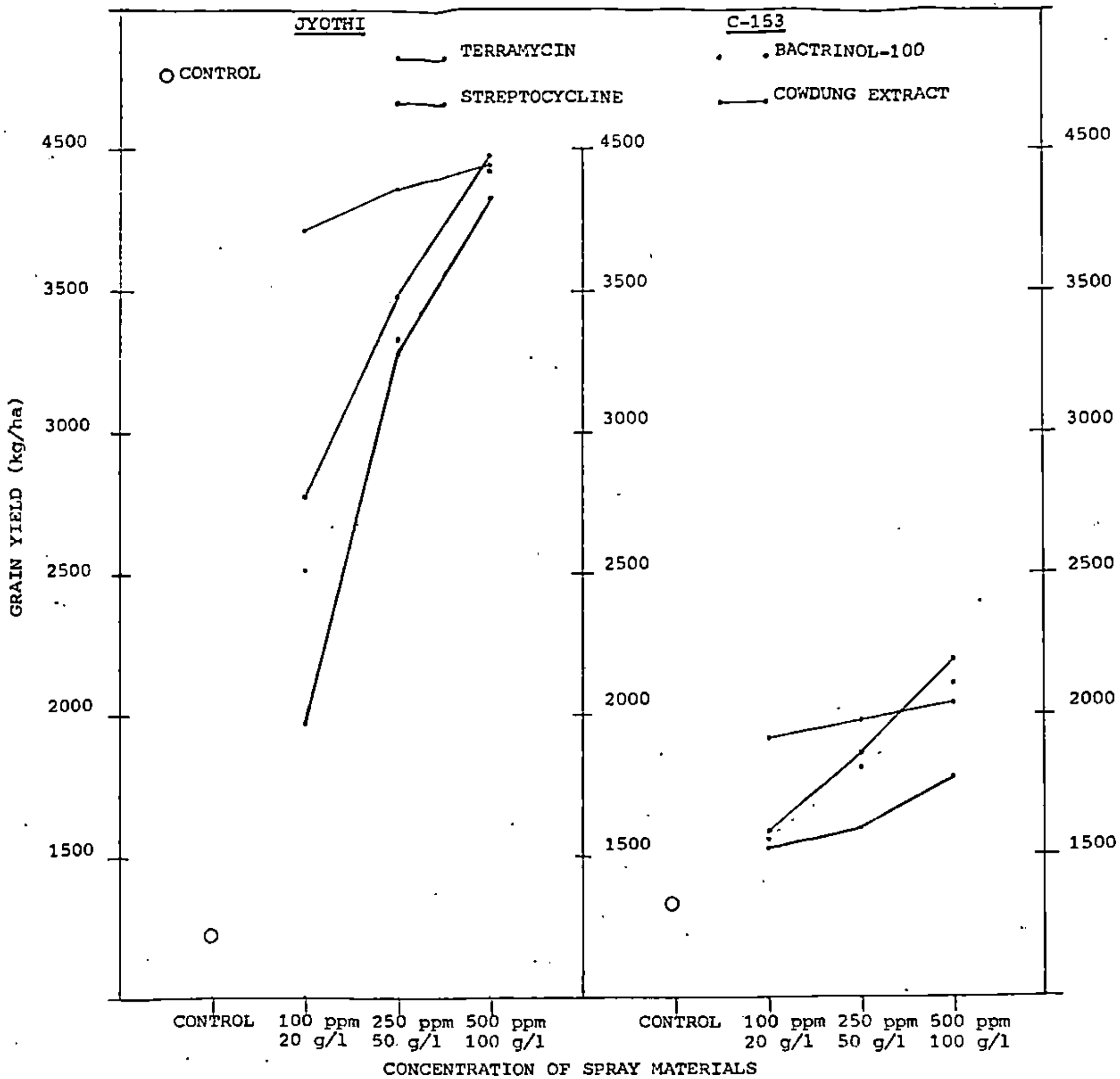


Fig.4 Grain yield in bacterial blight affected Jyothi and C-153 rice varieties after spraying under field conditions.

some of the chemical treatments like 100 and 250 ppm terramycin, bactrinol-100 and streptocycline.

2.3b <u>Thousand grain weight and number of chaffy grains in bacterial blight affected Jyothi rice variety</u>

The increase in thousand grain weight in plants sprayed with antibiotics, bactrinol-100 and cowdung extract was significant when compared to the control treatment. This effect was maximum after spraying with different concentrations of cowdung extract (20, 50 and 100 g/l) which increased the thousand grain weight by 8.82, 11.44 and 12.77 per cent respectively (Table 5). The spraying of infected plants with 500 ppm terramycin and bactrinol-100 also increased the thousand grain weight and these were on par with the treatment of 20 g/l cowdung extract.

The reduction in the number of chaffy grains per panicle in plants sprayed with different concentrations of antibiotics, bactrinol-100 and cowdung extract was significant when compared to the control treatment (Fig.5). However, this effect was maximum after

Table 5. Effect of spraying with antibiotics, bactrinol-100 and cowdung extract on thousand grain weight and the number of chaffy grains per panicle in bacterial blight affected Jyothi rice variety

Antibiotics/ Cowdung extract	Concentration	1000 grain* weight (g)	% increase over control.	No. of* chaffy grains	% reduction over control
Terramycin	100 ppm	24.85	6.88	30	28.57
	250 "	25.00	7.53	22	47.62
	500 "	25.21	8.43	16	61.90
Streptocycline	100 "	24.90	7.10	35	16.67
	250 "	24.45	5.16	32	23.81
	500 "	25.10	7.96	26	38.10
Bactrinol-100	100 "	25.05	7.74	29	30.95
	250 "	25.18	8.30	24	42.86
	500 "	25.72	10.62	20	52.38
Cowdung extract	20 g/l	25.30	8.82	25	40.48
	50 "	25.91	11.44	21	50.00
	100 "	26.15	12.77	22	47.62
Control		23.25		42	
CD(0.05)		0.12		6.17	

* Mean of three replications

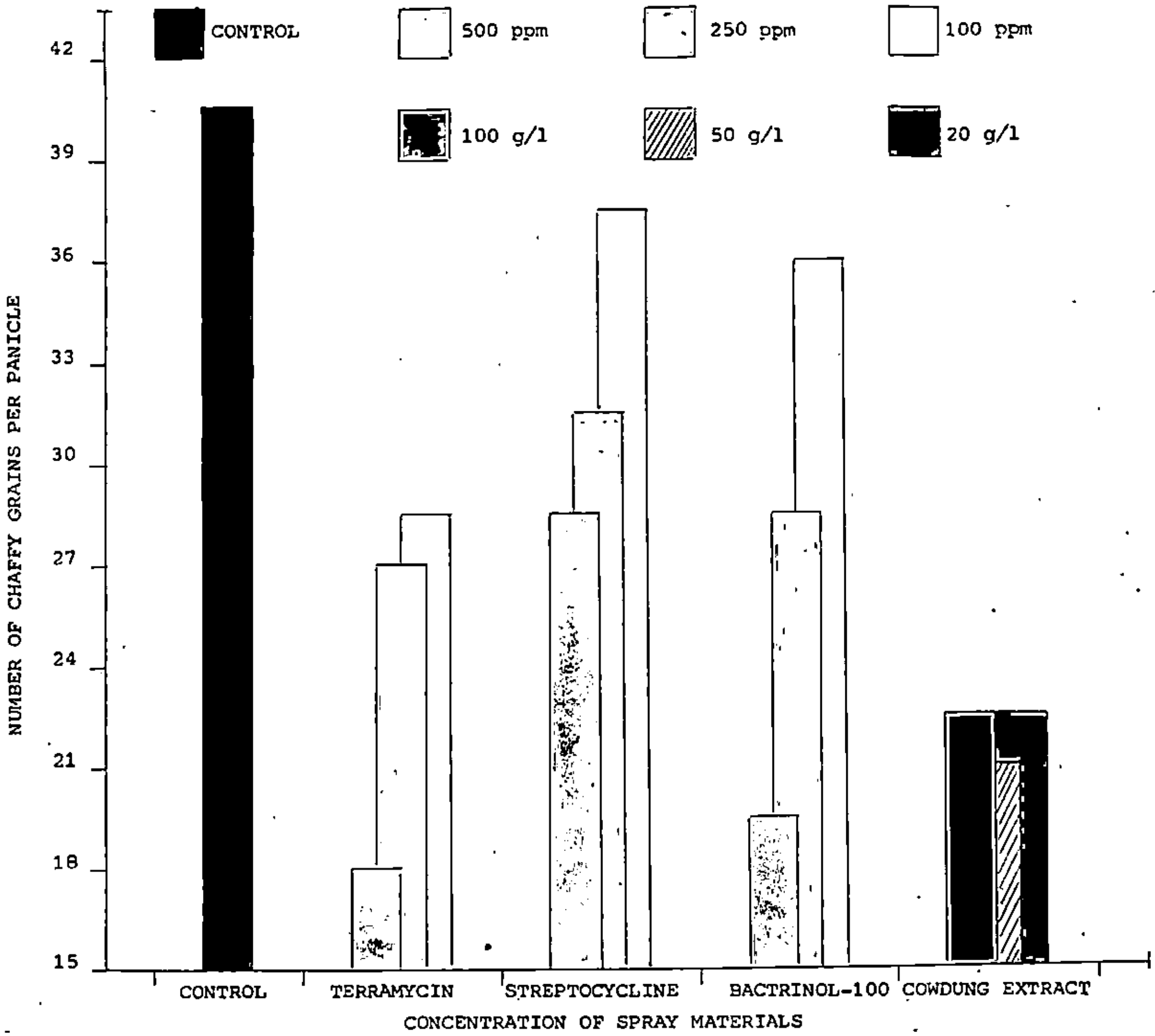


Fig.5 Effect of spraying with terramycin, streptocycline, bactrinol-100 and cowdung extract on the number of chaffy grains per panicle in bacterial blight affected Jyothi rice variety.

spraying with 500 ppm terramycin where, only 16 chaffy grains per panicle was observed (Table 5). This represented an actual reduction of 61.90 per cent in comparison to the control treatment with 42 chaffy grains per panicle. The reduction in the number of chaffy grains in treatments such as 500 ppm of bactrinol-100 (20 chaffy grains) and 50 and 100 g/l of cowdung extract (21 and 22 chaffy grains respectively) were also significant and statistically on par with that of terramycin 500 ppm.

2.3c Straw yield in bacterial blight affected Jyothi rice variety

The increase in straw yield from plants sprayed with different concentrations of antibiotics, bactrinol-100 and cowdung extract was significant when compared to the control treatment (Fig.6). Such an effect was found to be maximum after spraying with 100 g/l of cowdung extract, where an average straw yield of 8.16 kg/plot was obtained (Table 6). This represented an actual increase of 261.36 per cent in comparison to the control treatment with a straw yield of only 2.26 kg/plot.

Table 6. Effect of spraying with antibiotics, bactrinol-100 and cowdung extract on straw yield in bacterial blight affected Jyothi rice variety

Antibiotics/ Cowdung extract	Concen- tration	Yield/ plot* (kg)	Yield (t/ha)	% increase over control
Terramycin	100 ppm	5.26	5.85	133.07
	250 "	6.50	7.22	187.65
	500 "	8.08	8.98	257.77
Streptocycline	100 "	4.58	5.10	103.19
	250 "	6.12	6.80	170.92
	500 "	7.34	8.15	224.70
Bactrinol-100	100 "	4.83	5.36	113.55
	250 "	6.20	6.89	174.50
	500 "	7.83	8.71	247.01
Cowdung extract	20 g/l	7.54	8.38	233.87
	50 "	7.85	8.72	247.41
	100 "	8.16	9.07	261.36
Control		2.26	2.51	
CD(0.05)		0.11		

* Mean of three replications

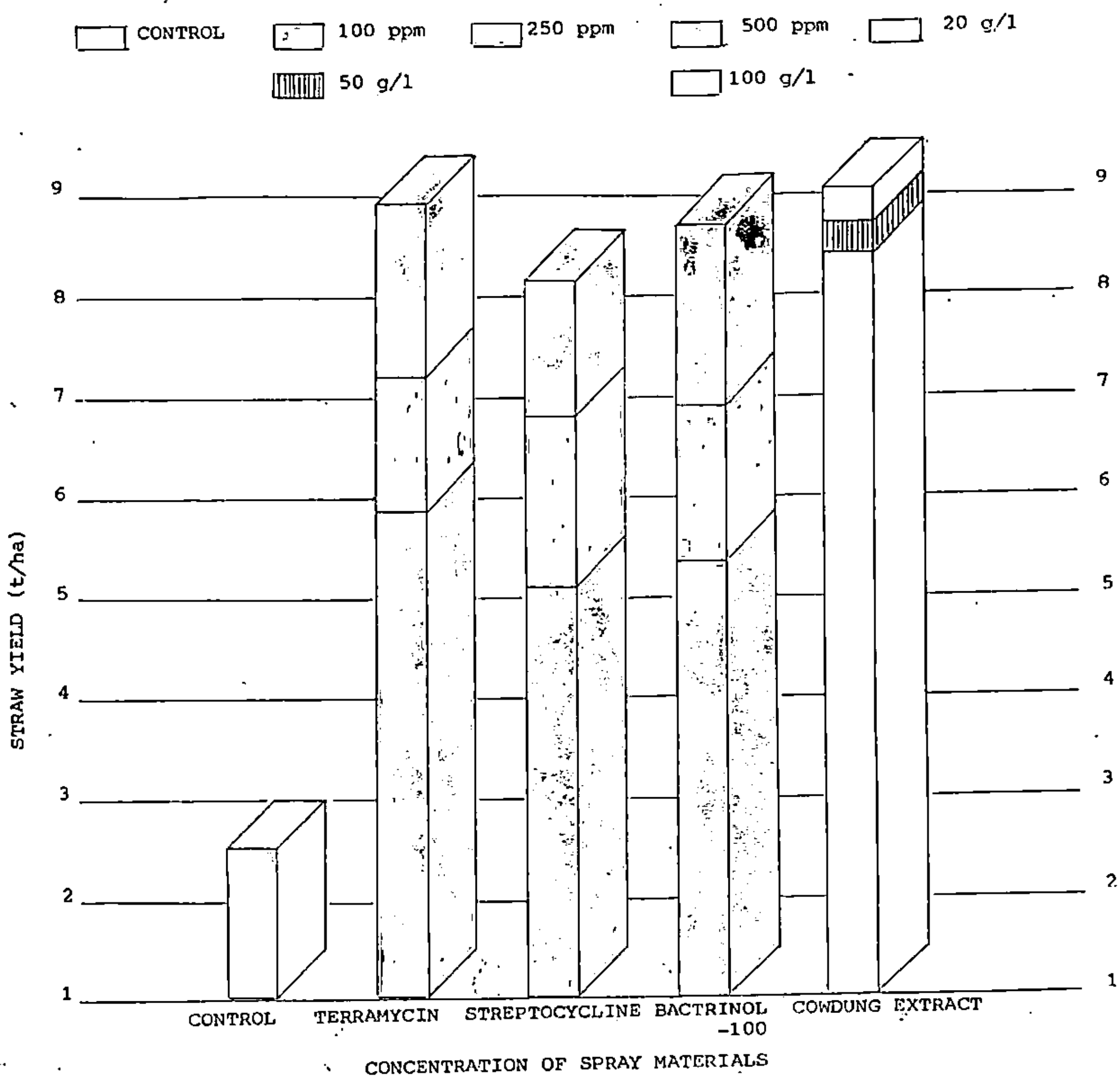


Fig. 6 Effect of spraying with terramycin, streptocycline, bactrinol-100 and cowdung extract on straw yield in bacterial blight affected Jyothi rice variety under field conditions.

The net straw yield from plants sprayed with 500 ppm terramycin (8.08 kg/plot) was also found to be significant and statistically on par with that of 100 g/l of cowdung extract. Further, it was observed that in plots sprayed with different concentrations of cowdung extract, the straw yield obtained was significantly better than most of the chemical treatments like 100 and 250 ppm terramycin and bactrinol-100 and 100, 250 and 500 ppm of streptocycline.

2.4 Effect of spraying with antibiotics, bactrinol-100 and cowdung extract on control of bacterial blight in C-153 rice variety under field conditions

The reduction in disease intensity in plants sprayed with different concentrations of antibiotics, bactrinol-100 and cowdung extract was significant when compared to the control treatment (Fig.3). However, this effect was maximum after spraying with 500 ppm terramycin (Table 7). The disease intensity in these plants 15 d after the first spray with the above concentrations of terramycin was 32.67. This corresponded to a reduction of 51.11 per cent in disease intensity when compared to the control treatment where, during this period, there was a significant increase in disease intensity by 22.76 per cent. As observed at Ulloor Seed farm, the effect of spraying with 500 ppm bactrinol-100 was not on par with that of 500 ppm terramycin.

A further reduction in disease intensity was observed after the second spray and this followed a similar pattern as that of the first spraying with different antibiotics, bactrinol-100 and cowdung extract (Table 7 and Plates, 6, 7, 8, 9, 10, 11 and 12). The overall reduction in disease intensity was maximum in plants sprayed with 500 ppm terramycin. This was significantly better than all other treatments and corresponded to an actual reduction of 66.55 per cent in comparison to the control treatment.

In general, higher the concentration of each chemical sprayed, better was the control of bacterial blight under field conditions. However, as observed in the earlier field experiment with cowdung extract,

Table 7. Effect of spraying with antibiotics, bactrinol-100 and cowdung extract on control of bacterial blight in C-153 rice variety

Antibiotics/ Cowdung extract	Concen- tration	Disease intensity*			% reduction over control	
		Before spray	After Ist spray	After IInd spray	After Ist spray	After IInd Spray
Terramycin	100 ppm	52.84	46.98	42.35	29.70	44.81
	250 "	54.21	42.33	34.66	36.66	54.83
	500 "	54.28	32.67	25.67	51.11	66.55
Streptocycline	100 "	54.42	54.37	52.37	18.64	31.75
	250 "	53.92	49.04	43.69	26.62	43.06
	500 "	54.60	45.98	41.00	31.20	46.57
Bactrinol-100	100 "	52.95	51.35	47.67	23.16	37.87
	250 "	56.09	43.31	37.01	35.19	51.77
	500 "	54.48	37.76	31.35	43.50	59.14
Cowdung extract	20 g/l	55.02	44.68	37.91	33.14	50.59
	50 "	54.57	45.38	37.69	32.10	50.88
	100 "	54.99	43.58	37.03	34.79	51.74
Control		54.44	66.83	76.73	(+22.76)	(+14.81)
CD(0.05)			4.03	4.42		

* Mean of three replications

Plate 6. Bacterial blight infected field of C-153 rice variety - control plots without treatment.

KEY

C - Control

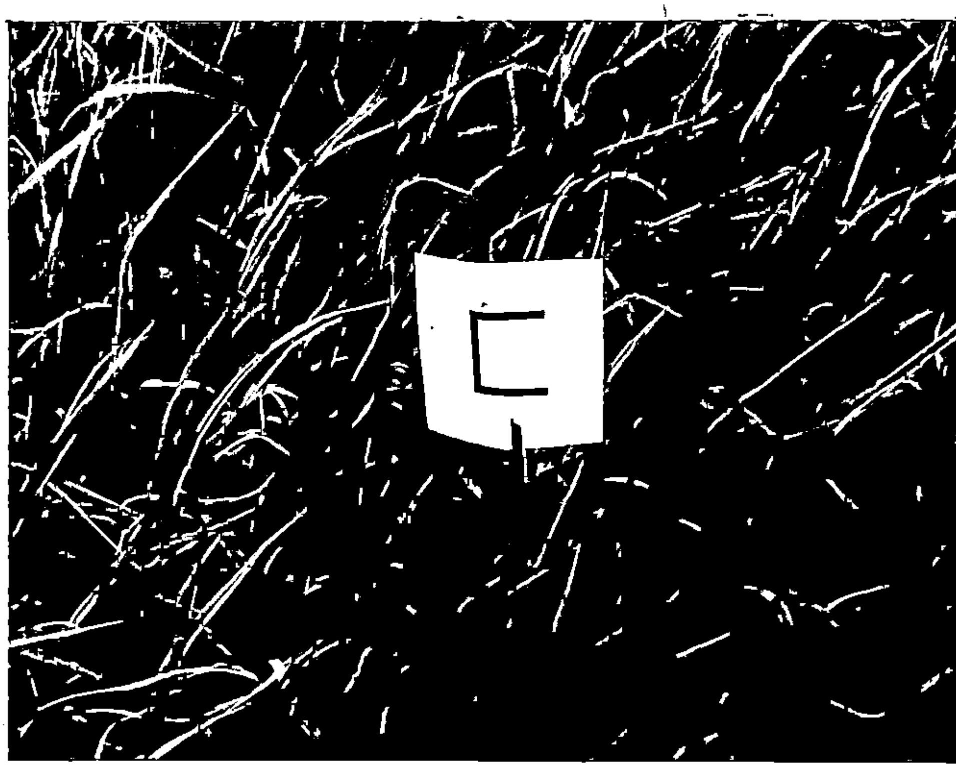


Plate 7. Effect of spraying with terramycin on control of bacterial blight in C-153 rice variety.

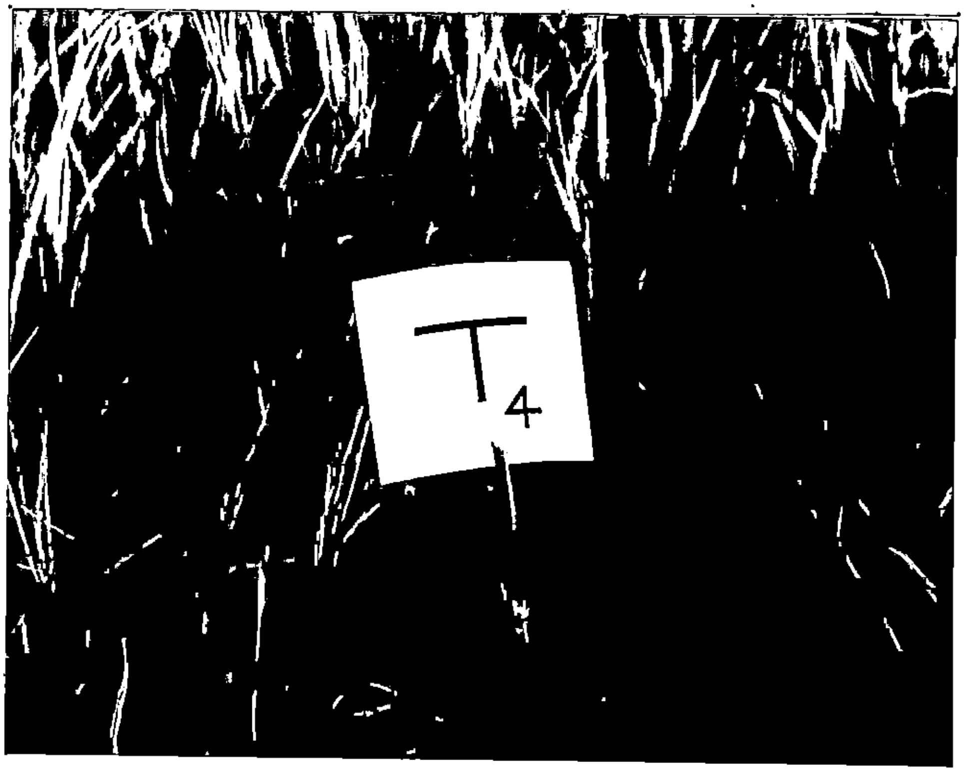
<u>KEY</u>

T_4 - Terramycin 500 ppm

Plate 8. Effect of spraying with streptocycline on control of bacterial blight in C-153 rice variety.

<u>KEY</u>

S_4 - Streptocycline 500 ppm


T4

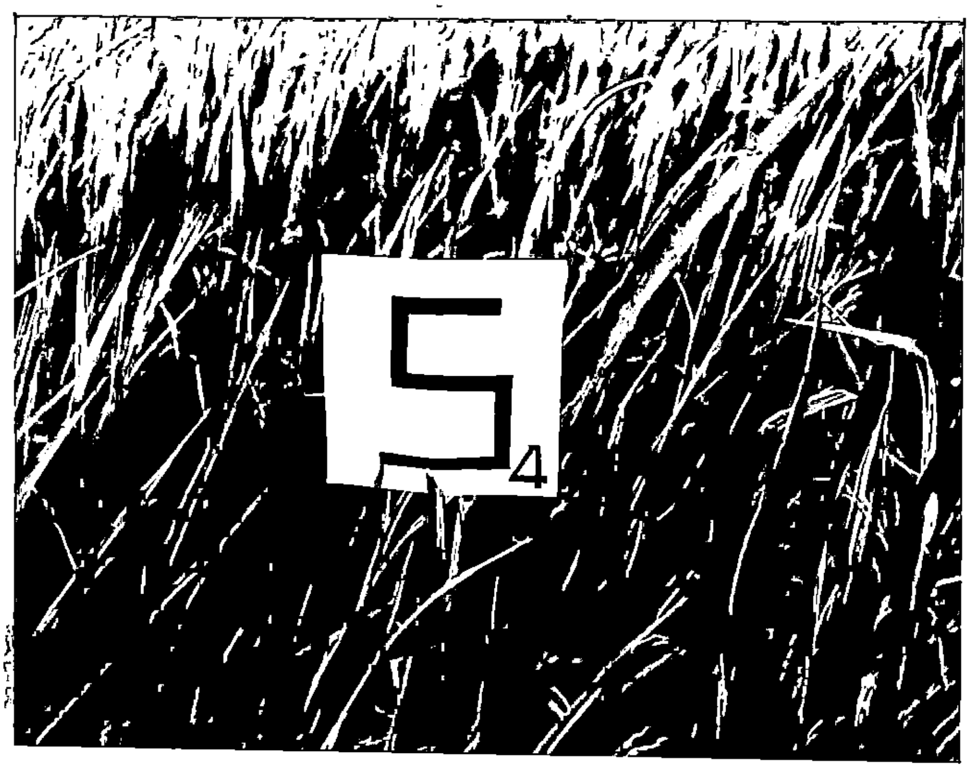

S4

Plate 9. Effect of spraying with bactrinol-100 on control of bacterial blight in C-153 rice variety.

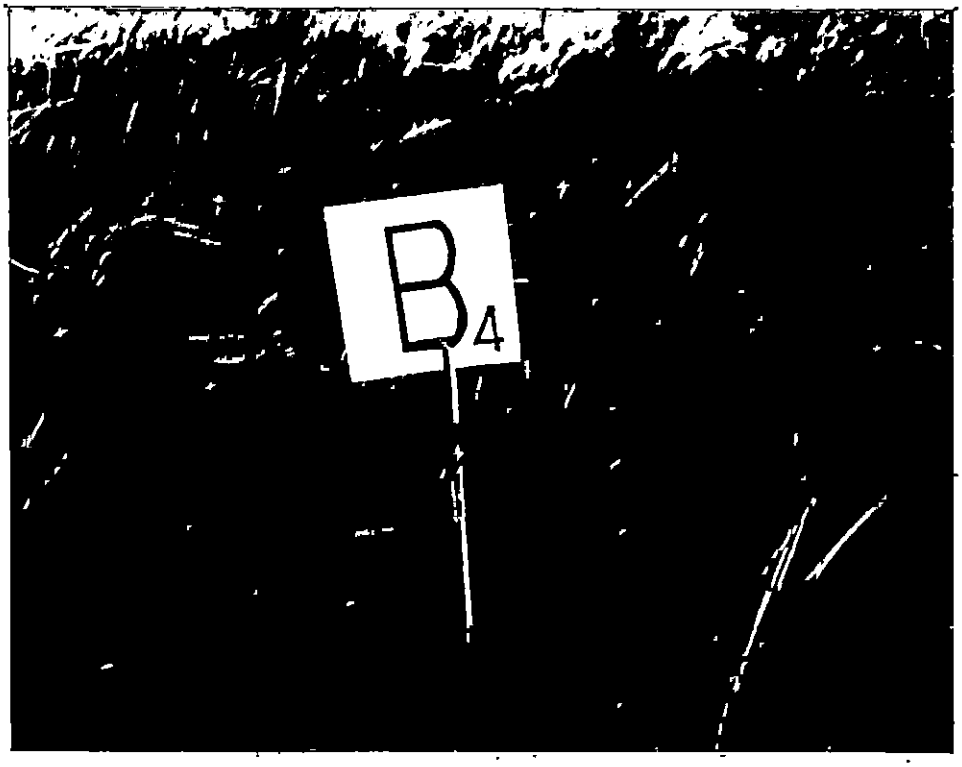
KEY

B_4 - Bactrinol-100 500 ppm

Plate 10. Effect of spraying with cowdung extract on control of bacterial blight in C-153 rice variety.

KEY

C_1 - Cowdung extract 20g/l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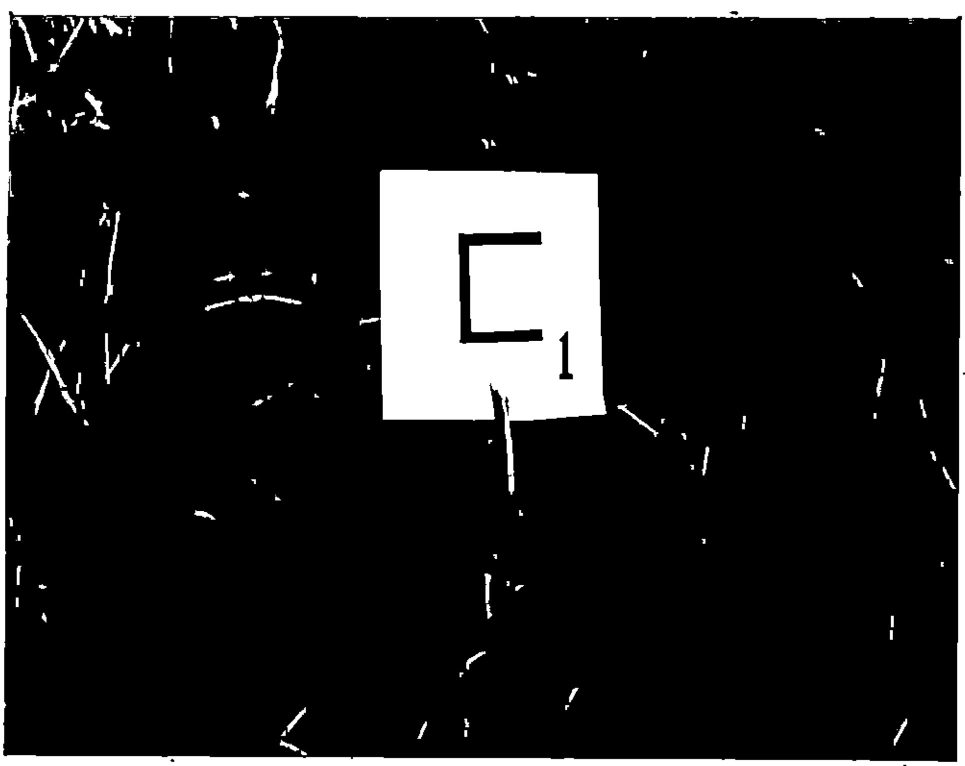

C1

Plate 11. Effect of spraying with cowdung extract (50 g/l) on control of bacterial blight in C-153 rice variety.

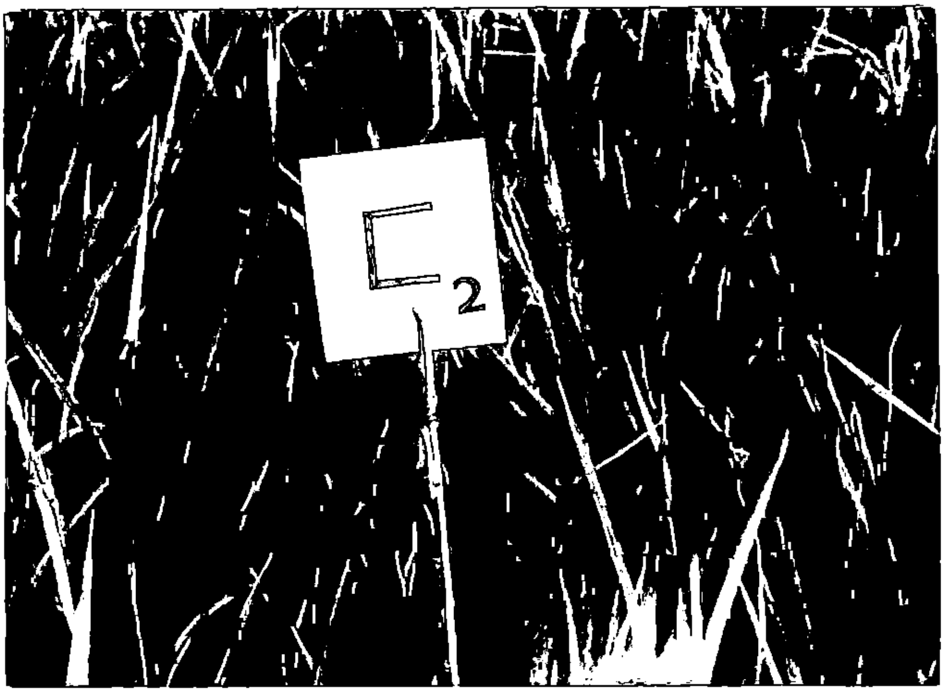
KEY

C_2 - Cowdung extract 50 g/l

Plate 12. Effect of spraying with cowdung extract (100 g/l) on control of bacterial blight in C-153 rice variety.

KEY

C_3 - Cowdung extract 100 g/l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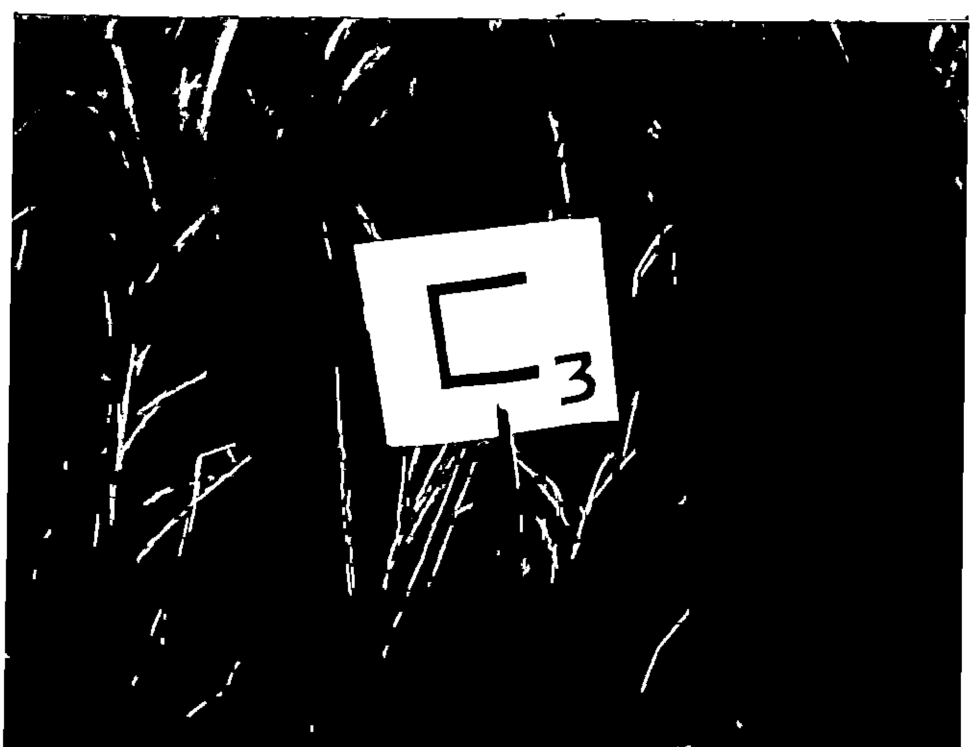

C3

such an effect was only marginal, since the level of disease control achieved after spraying with different concentrations was more or less uniform (Table 7). Further, the extent of disease control obtained, by spraying cowdung extract especially after the second spray, was significantly higher than some of the chemical treatments like 100 ppm bactrinol-100 and terramycin and 100 and 250 ppm of streptocycline.

2.4a <u>Grain yield in bacterial blight affected C-153 rice variety</u>

The increase in grain yield in plants sprayed with different concentrations of antibiotics, bactrinol-100 and cowdung extract was significant when compared to control treatment (Fig.4). However, this effect was maximum after spraying with 500 ppm terramycin, where an average yield of 2.43 kg/plot was obtained (Table 8). This actually represented an yield increase of 46.74 per cent in comparison with the control treatment with an yield of only 1.65 kg/plot. The net grain yield from plants sprayed with 500 ppm bactrinol-100 (2.36 kg/plot) was also significant and statistically on par with the

Table 8. Grain yield in bacterial blight affected C-153 rice variety after spraying with antibiotics, Bactrinol-100 and cowdung extract

Antibiotics/ Cowdung extract	Concen- traction	Yield/ plot* (kg)	Yield (t/ha)	% increase over control
Terramycin	100 ppm	1.87	2.08	13.04
	250 "	2.12	2.36	28.26
	500 "	2.43	2.70	46.74
Streptocycline	100 "	1.84	2.04	10.87
	250 "	1.89	2.10	14.13
	500 "	2.06	2.29	24.46
Bactrinol-100	100 "	1.87	2.07	12.50
	250 "	2.08	2.31	25.54
	500 "	2.36	2.62	42.39
Cowdung extract	20 g/l	2.18	2.42	31.52
	50 "	2.24	2.49	35.33
	100 "	2.30	2.55	38.59
Control		1.65	1.84	
CD(0.05)		0.12		

* Mean of three replications

above treatment. Further, it was observed that in plots sprayed with different concentrations of cow-dung extract, the grain yield obtained (2.18, 2.24 and 2.30 kg/plot respectively for 20, 50 and 100 g/l of cowdung extract) was significantly better than some of the chemical treatments like 100 ppm of terramycin and bactrinol-100 and 100, 250 and 500 ppm of streptocycline.

2.4b <u>Thousand grain weight and number of chaffy grains in bacterial blight affected C-153 rice variety</u>

The increase in thousand grain weight in plants sprayed with different concentrations of antibiotics, bactrinol-100 and cowdung extract was significant when compared to the control treatment (Table 9). This effect was maximum after spraying with 500 ppm bactrinol-100 (25.04 g). The increase in thousand grain weight after spraying with 250 ppm bactrinol-100 (24.85 g) was also significant and statistically on par with the above treatment.

The reduction in the number of chaffy grains per panicle in plants sprayed with different concentrations

Table 9. Effect of spraying with antibiotics, bactrinol-100 and cowdung extract on thousand grain weight and the number of chaffy grains per panicle in bacterial blight affected C-153 rice variety

Antibiotics/ Cowdung extract	Concen- tration	1000 grain* weight (g)	% increase over control	No. of* chaffy grains	% reduction over control
Terramycin	100 ppm	23.27	5.06	23	25.81
	250 "	22.85	3.16	22	29.03
	500 "	23.35	5.42	16	48.39
Streptocycline	100 "	22.95	3.61	29	6.45
	250 "	23.20	4.74	25	19.36
	500 "	23.15	4.52	23	25.81
Bactrinol-100	100 "	24.10	8.80	28	9.68
	250 "	24.85	12.19	23	25.81
	500 "	25.04	13.05	17	45.16
Cowdung extract	20 g/l	23.97	8.22	19	38.71
	50 "	23.95	8.13	18	41.94
	100 "	24.53	10.75	19	38.71
Control		22.15		31	
CD(0.05)		0.29		6.3	

* Mean of three replications

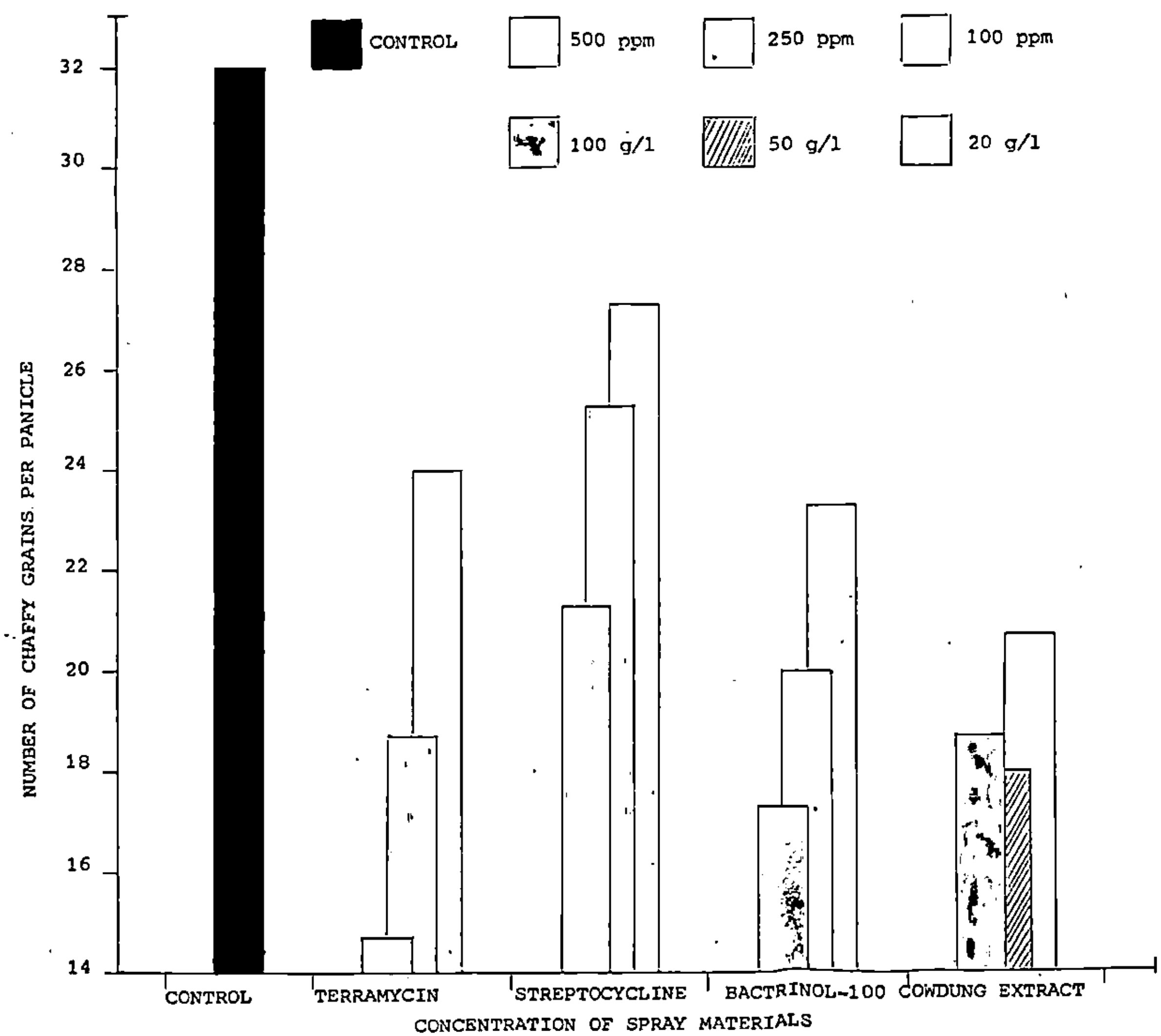


Fig. 7 Effect of spraying with terramycin, streptocycline, bactrinol-100 and cowdung extract on the number of chaffy grains per panicle in bacterial blight affected C-153 rice variety.

of antibiotics, bactrinol-100 and cowdung extract was significant when compared with the control treatment (Fig.7), with the exception of 100 and 250 ppm of streptocycline and 100 ppm of bactrinol-100. However, this effect was maximum after spraying with 500 ppm terramycin where only 16 chaffy grains per panicle was observed (Table 9). This represented an actual reduction of 48.39 per cent in comparison to the control treatment with 31 grains per panicle. The reduction in the number of chaffy grains present in treatments such as 250 ppm terramycin (22 chaffy grains) 20, 50 and 100 g/l of cowdung extract (19, 19 and 18 chaffy grains respectively) were also significant and statistically on par with that of terramycin 500 ppm.

2.4c <u>Straw yield in bacterial blight affected C-153 rice variety</u>

The increase in straw yield from plants sprayed with different concentrations of antibiotics, bactrinol-100 and cowdung extract was significant when compared with the control treatment (Fig.8). However, this effect was maximum after spraying with 500 ppm terramycin where an average straw yield of 3.36 kg/plot was obtained (Table 10). This represented an actual increase of 152.63 per cent in comparison to the control treatment with a straw yield of only 1.33 kg/plot. The net straw yield for plants sprayed with 500 ppm bactrinol-100 (3.12 kg/plot) and 50 and 100 g/l of cowdung extract (3.11 and 3.26 kg/plot respectively) were also significant and statistically on par with that of 500 ppm terramycin. Further, it was observed that in plants sprayed with different concentrations of cowdung extract, the straw yield obtained was significantly better than some of the chemical treatments like 100 ppm terramycin and bactrinol-100 and 100 and 250 ppm streptocycline.

3. EFFECT OF SOME BACTERIAL AND FUNGAL ISOLATES FROM COWDUNG EXTRACT ON GROWTH OF *X. campestris* pv. *oryzae*

Eleven bacterial and twelve fungal cultures initially isolated from cowdung extract by dilution pour plate technique were tested for any antagonistic effect on growth of the pathogen on PSPA medium.

Table 10. Effect of spraying with antibiotics, bactrinol-100 and cowdung extract on straw yield in bacterial blight affected C-153 rice variety

Antibiotics/ cowdung extract	Concen- tration	Yield/ plot* (kg)	Yield (t/ha)	% reduction over control
Terramycin	100 ppm	2.11	2.35	58.65
	250 "	2.73	3.03	105.26
	500 "	3.36	3.73	152.63
Streptocycline	100 "	1.86	2.07	39.85
	250 "	2.34	2.60	75.94
	500 "	2.62	2.91	96.99
Bactrinol-100	100 "	1.99	2.22	49.62
	250 "	2.66	2.96	100.00
	500 "	3.12	3.47	134.59
Cowdung extract	20 g/l	2.91	3.24	118.80
	50 "	3.11	3.46	133.84
	100 "	3.26	3.62	145.11
Control		1.33	1.47	
CD(0.05)		0.30		

* Mean of three replications

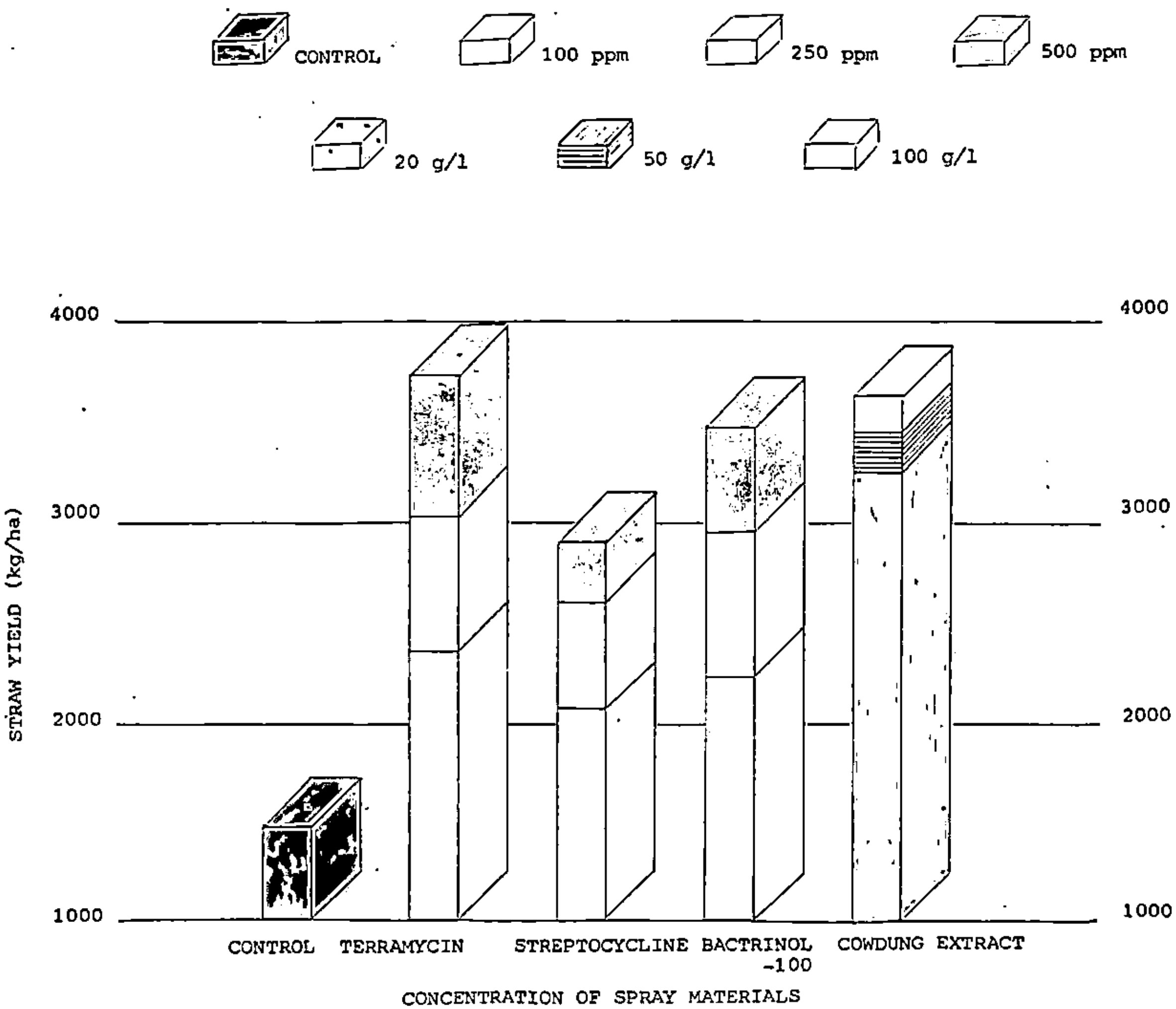


Fig.8 Effect of spraying with terramycin, streptocycline, bactrinol-100 and cowdung extract on straw yield in bacterial blight affected C-153 rice variety under field conditions.

Table 11. Effect of certain bacteria and fungi isolated from cowdung extract on growth of <u>X. campestris</u> pv. <u>oryzae</u>

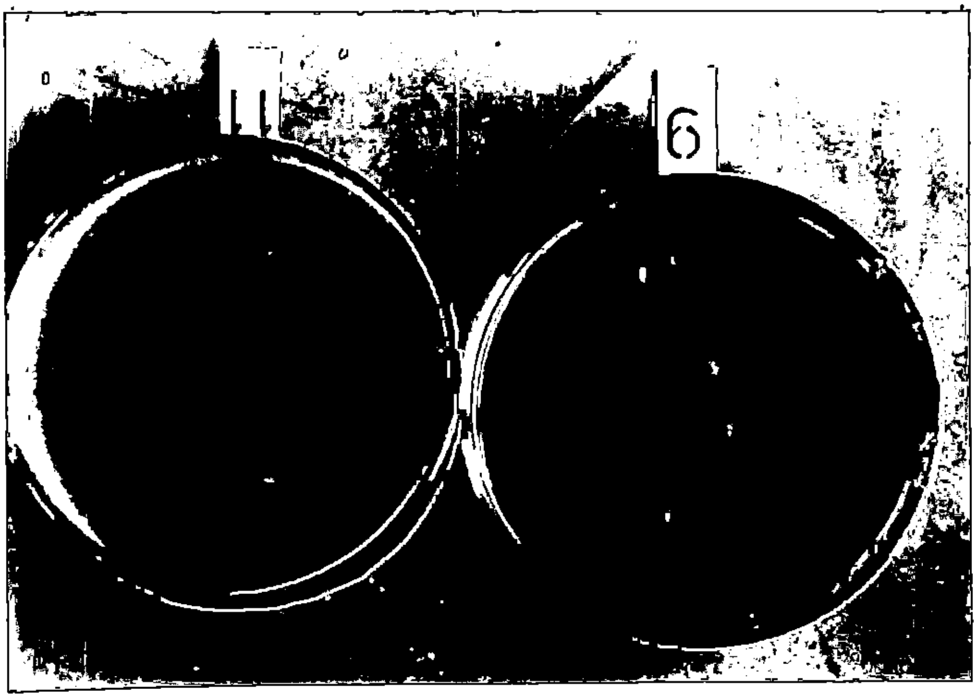
Bacterial/fungal isolates	Antagonistic reaction	
	Inhibition by antibiosis	Inhibition by over growth
A. <u>Bacterial isolates</u>		
1	Absent	Absent
2	Absent	Absent
3	Absent	Absent
4	Absent	Absent
5	Absent	Absent
6	Absent	Present
7	Absent	Absent
8	Absent	Absent
9	Absent	Absent
10	Absent	Present
11	Absent	Present
B. <u>Fungal isolates</u>		
1	Absent	Present
2	Absent	Absent
3	Absent	Present
4	Absent	Present
5	Absent	Present
6	Absent	Present
7	Absent	Absent
8	Absent	Absent
9	Absent	Absent
10	Absent	Absent
11	Absent	Present
12	Absent	Present

Plate 13. Effect of bacteria isolated from cowdung extract on growth of _X. campestris_ pv. _oryzae_.

KEY

Isolate 9 - No antagonism

Isolate 11- Antagonism by overgrowth


11
6

Among the different bacterial cultures isolated from cowdung extract, isolates 6, 10 and 11 exhibited antagonism through inhibition of the pathogen by overgrowth. In the case of fungi, isolates 1,3,4,5,6, 11 and 12 checked the development of the pathogen by overgrowth. The rest of the bacterial and fungal isolates failed to produce any antagonistic reaction and the pathogen grew unhindered.(Table 11 and Plate 13).

4. ECONOMIC BENEFITS OF CONTROLLING BACTERIAL BLIGHT OF RICE UNDER FIELD CONDITIONS

The relative economic benefits of controlling bacterial blight of rice by using antibiotics, bactrinol-100 and cowdung extract was also studied based on the data available from the field experiment conducted at Ulloor seed farm. It clearly showed that there can be a net loss of upto 72 paise for each rupee invested if this disease is not controlled in time. However, if suitable control measures are taken in time to check the spread of bacterial blight, there can be a net return of Rs.1.84 per rupee invested after spraying infected plants with 100 g/l of cowdung extract. The net return per rupee invested from treatments such as 50 g/l of cowdung extract, 500 ppm of bactrinol-100 and 20 g/l of cowdung extract were Rs.1.74 ,1.69 and 1.60 respectively. But the returns from plants sprayed with streptocycline was relatively low.

DISCUSSION

DISCUSSION

Bacterial blight caused by *Xanthomonas campestris* pv. *oryzae* is an important disease of rice particularly in Kuttanad during the additional crop season. Spraying infected plants with appropriate phytoantibiotics like streptocycline is usually recommended for the control of this disease (Desai *et al*. 1967, Pal and Das 1968, Chauhan and Vaishnav 1980, Sreelatha 1985). However, recently, it has been observed that an effective control of this disease is not often achieved in Kuttanad by using streptocycline alone (Nair *et al*. 1990). This appears to be due to the fact that the pathogen has become somewhat resistant to this antibiotic probably, because of its frequent use by the farmers. It is also debated now a days, whether it is desirable to use antibiotics for the control of plant diseases of bacterial origin as most of the phytoantibiotics available today in the market are combinations of streptomycin and oxy-tetracycline in various proportions. Since these antibiotics are also used for the treatment of human diseases, there is a possibility for the development of resistant strains of these pathogens due to exposure to such antibiotics. It is because of these reasons serious attention is being paid by several workers to develop an effective alternative method for the control of plant bacterial diseases.

Some of the promising methods developed with this objective for the control of bacterial blight of rice are the application of stable bleaching powder to soil (Palaniswamy and Ahmed 1979, Sivaswamy and Mahadevan 1986), spraying infected plants with cowdung extract (Mary et al. 1986) and the use of a new class of synthetic organic bactericide, namely bactrinol-100 (Natarajan and Lalithakumari 1989). In the present investigation, the efficacy of two of these methods, the use of bactrinol-100 and cowdung extract to control bacterial blight of rice was studied. The plan of implementation consisted of an initial isolation of the pathogen from a disease affected rice field in Kuttanad, testing its virulence, screening for sensitivity towards different concentrations of antibiotics, bactrinol-100 and cowdung extract and finally using the same for the control of the disease both under pot as well as field conditions.

The culture of Xanthomonas campestris pv. oryzae isolated from Nedumudi in Kuttanad during the additional crop season of 1988 was found to be very virulent. It produced typical symptoms of bacterial blight in T(N)1 7 d after artificial inoculation. The initial symptoms appeared as water soaked lesions which gradually enlarged along the veins and later turned into straw coloured lesions on both margins of the infected leaves (Plate 1). The pathogen was reisolated from such leaves on potato sucrose peptone agar medium and then tested for in vitro sensitivity against different concentrations of terramycin, streptocycline, bactrinol-100 and cowdung extract. The growth of X. campestris pv. oryzae was inhibited maximum by 500 ppm terramycin followed by 250 ppm terramycin and 500 ppm bactrinol-100 (Plates 2 and 3 and Fig.1). The zone of growth inhibition with these treatments were 15.3, 13.3 and 10.7 mm respectively (Table 1). The sensitivity of X. campestris pv. oryzae towards antibiotics like terramycin and streptocycline is already reported by Desai *et al*. (1967) and Sreelatha (1985). However, this culture was found to be less sensitive to streptocycline. The zone of growth inhibition obtained even with 500 ppm streptocycline was only 5 mm when compared to that of 15.3 mm produced by terramycin 500 ppm (Table 1). This indicated that probably, the particular isolate of the pathogen has become somewhat resistant to streptocycline. The chances for the same are quite evident from the fact that this culture was originally isolated from a region (Kuttanad) where there is an incidence of bacterial blight almost every year during the additional crop season and where it is a common practice of many farmers to spray infected plants with streptocycline to control the disease. A similar observation was made earlier by Shetty and Rangaswami (1968) and recently by Nair *et al*. (1990) when they reported that repeated application of streptocycline can induce the development of resistance in many strains of *Xanthomonas* spp.

It was also observed that none of the three forms of cowdung extract tried, the normal water extract, the filter sterilized extract and the extract initially heated up to 60°C for 15 m in a water bath, produced a typical growth inhibition zone similar to that of either the antibiotics or bactrinol-100. Here, the growth inhibition of the pathogen was mainly due to an over growth by the native microflora present in cowdung extract. The reason for testing the filtered and heated forms of cowdung extract was to detect the presence of any water soluble bactericidal factor in an aqueous extract of cowdung and also to find out its thermal susceptibility. However, the results obtained here indicated that such factors are probably absent in cowdung extract (Plate 4). Hence, only the normal water extract of cowdung was used for further studies.

The different concentrations of antibiotics, bactrinol-100 and cowdung extract used earlier for testing the *in vitro* sensitivity of the pathogen were also tried for their relative efficacy to control bacterial blight both under pot as well as field conditions. This was done so as to find out whether any of the lower concentrations of either the antibiotics, bactrinol-100 or cowdung extract can be used to control the disease, since under *in vivo* conditions, the growth and expression of virulence by the pathogen are under constant influence of various environmental factors. This infact is one of the ways to develop a method of disease control by using lower concentrations of either the antibiotics, other chemicals or cowdung extract.

The results of the pot culture experiment showed that the reduction in disease intensity in bacterial blight affected T(N)1 rice variety was maximum after spraying with 500 ppm terramycin followed by 500 ppm bactrinol-100. The disease intensity in these plants 15 d after spraying with the above concentrations of terramycin and bactrinol-100 were 46.98 and 53.19 respectively in comparison with that of 81.34 in the control treatment (Table 2 and Fig.2). This infact represented an actual reduction of 42.24 and 34.61 per cent respectively in disease intensity in treated plots. The efficiency recorded by terramycin and bactrinol-100 in controlling bacterial blight was almost similar to that reported earlier by Balaraman and Rajagopalan (1978) and Natarajan and Lalithakumari (1989). However, such an effect was not obtained even after spraying with 500 ppm of streptocycline. The probable reason for the same was discussed earlier.

In general, higher the concentration of each chemical sprayed, better was the control of the disease. This showed that atleast in the case of antibiotics and bactrinol-100 the use of lower concentrations such as 100 or 250 ppm will not be sufficient to bring out a satisfactory control of the disease. At the same time, the results obtained after spraying with different concentrations of cowdung extract were somewhat different. Here, the reduction in disease intensity was more or less uniform. Thus, the disease intensity 15 d after spraying with 20, 50 and 100 g/l of cowdung extract was 59.51, 60.74 and 59.50 respectively (Table 2). This indicated that for plant protection purpose there is practically no need for using a higher concentration of cowdung extract. This may also be advantageous to the farmer from a practical point of view while preparing the spray solution.

The results of the field experiments conducted at Ulloor seed farm and in a farmer's field at Nedumudy only confirmed the observations made earlier during the pot culture experiment. But by including certain additional parameters like grain yield, increase in thousand grain weight, reduction in chaffy grains per panicle and straw yield in the observations recorded, it was possible to derive some more useful information from the field experiments. Unlike the pot trial, the infected plants were given two sprayings with terramycin, streptocycline, bactrinol-100 and cowdung extract at 15 d interval to study the extent of disease control. It was found that terramycin 500 ppm was again most effective in controlling bacterial blight at both locations. The reduction in disease intensity was nearly 64.21 per cent in Jyothi rice variety at

Ulloor (Table 3 and Fig.3) and 66.55 per cent in C-153 rice variety at Nedumudy (Table 7 and Fig.3). However, such an effect was not observed in the case of streptocycline. The relative efficacy of bactrinol-100 was next only to that of terramycin 500 ppm with an overall reduction in disease intensity by 54.98 and 59.14 per cent respectively at the above two locations (Tables 3 and 7). These results thus indicated that 500 ppm bactrinol-100 may be used as a substitute for antibiotics to control bacterial blight of rice. The possibility for the same has already been demonstrated by Natarajan and Lalithakumari (1989) in a pioneering work carried out in Tamil Nadu.

The extent of disease control in plants sprayed with different concentrations of cowdung extract was more or less uniform at both locations. Thus, after the second spray with 20, 50 and 100 g/l of cowdung extract, the disease intensity was only 34.22, 33.56 and 33.20 respectively at Ulloor and 37.91, 37.69 and 37.03 respectively at Nedumudi (Tables 3 and 7). These were even better than some of the chemical treatments like 100 ppm terramycin and bactrinol-100 and 100 and 250 ppm streptocycline. However, such reduction in disease intensity was not on par with that of either 500 ppm terramycin, or bactrinol-100. This showed that cowdung extract will be useful for the control of bacterial blight of rice only under certain situations like when there is a mild incidence of this disease. In areas where bacterial blight occur every year the farmers may even give a prophylactic spraying of plants with 20 g/l of cowdung extract which may reduce the severity of disease incidence.

The grain yield obtained from diseased plots only confirmed the relative efficacy of various treatments in controlling bacterial blight under field conditions. At both the locations, maximum grain yield from infected plants was obtained after spraying with 500 ppm terramycin. At Ulloor seed farm, the average grain yield was 4.04 kg/plot from Jyothi rice variety, which represented an actual yield increase of 159.54 per cent in comparison with the control treatment which yielded only 1.56 kg/plot

(Table 4 and Fig.4). The corresponding grain yield from C-153 rice variety at Nedumudy were 2.43 and 1.65 kg/plot respectively. The increase in grain yield from plants sprayed with 500 ppm bactrinol-100, 156.65 per cent at Ulloor and 42.39 per cent at Nedumudy were also significant and statistically on par with the 500 ppm terramycin treatment. A similar result was also obtained at Ulloor seed farm after spraying with 50 and 100 g/l cowdung extract (Table 4). In further support to the earlier observation, that atleast in certain locations, cowdung extract may be used for the control of bacterial blight of rice, it was found that even in plots sprayed with only 20 g/l of cowdung extract, the average yield obtained, 3.79 kg/plot at Ulloor and 2.18 kg/plot at Nedumudy were more than some of the chemical treatments like 100 and 250 ppm of terramycin, bactrinol-100 and streptocycline at both the locations (Tables 4 and 8). At Nedumudi it was even better than 500 ppm streptocycline.

In many earlier studies substantial yield loss due to bacterial blight has been reported, eventhough it varied from place to place and the variety under cultivation. For example, the International Rice Research Institute at Manila in Philippines reported an average loss of 33.10 per cent in T(N)1, 46.80 per cent in Tainan and 74.90 per cent in LT-8 variety due to bacterial blight under field conditions. In India, similar studies conducted by Reddy et al. (1978) and Raina et al.(1981) have reported yield losses ranging from 60 to 70 per cent in rice varieties like Karuna and T(N)1. The present investigation also indicated that significant yield losses in rice varieties such as Jyothi and C-153 can occur, if they are infected with *X. campestris* pv. *oryzae*. Assuming that the yield obtained from these varieties after treatment with 500 ppm terramycin was near to their normal yield potential, such losses due to bacterial blight, when untreated, can be to the order of 159.54 per cent in Jyothi and 46.74 per cent in C-153 rice varieties. Hence, it is essential to take appropriate control measures at the early stage itself to reduce subsequent yield loss.

The grain yield from disease affected plots was also found related to thousand grain weight and number of chaffy grains present per panicle (Ikeno, 1958). However, in plants sprayed with either bactrinol-100 or cowdung extract, the thousand grain weight was greatly increased. Thus after spraying with 500 ppm bactrinol-100, the increase in thousand grain weight was by about 10.62 and 13.05 per cent in Jyothi and C-153 varieties respectively when compared to the control treatments. An increase in thousand grain weight, by bactrinol-100 treatment has been reported earlier by Natarajan and Lalithakumari (1989). Similarly, the use of different concentrations of cowdung extract, 20,50 and 100 g/l, also resulted in an increase in thousand grain weight from diseased plots by 8.82, 11.44 and 12.77 per cent in Jyothi and 8.22, 8.13 and 10.75 per cent in C-153 rice varieties over their respective control treatments. It was further observed that spraying infected plants with 500 ppm terramycin resulted in significant reduction in the number of chaffy grains at both the locations. There were only 16 chaffy grains per panicle both at Ulloor and Nedumudy when compared to that of 42 and 31 respectively at these locations in the control treatment (Tables 5 and 9 and Fig. 5 and 7). The extent of reduction in the number of chaffy grains formed after spraying with 250 ppm terramycin, 500 ppm bactrinol-100 and 50 and 100 g/l of cowdung extract at Ulloor seed farm and 250 ppm of terramycin, 500 ppm of bactrinol-100 and all concentrations of cowdung extract at Nedumudy were also significant and statistically on par with the 500 ppm terramycin treatment. There was also considerable reduction in the formation of chaffy grains after spraying with cowdung extract. However, the reasons for the same and also the relative inefficacy of streptocycline to produce such an effect are to be investigated further.

The straw yield from diseased plants increased significantly in treated plots in relation to the control treatment at both the locations. At Ulloor seed farm, the straw yield of 8.16 kg/plot was maximum from plants sprayed with 100 g/l cowdung extract followed by 500 ppm terramycin treatment with a net straw yield of 8.08 kg/ plot (Table 6 and Fig.6). On the other hand, at Nedumudy, the straw yield of 3.36 kg/plot was maximum after spraying with 500 ppm terramycin (Table 10 and Fig. 8). The straw yield from three other treatments such as 500 ppm bactrinol-100 (3.12 kg/plot) and 50 and 100 g/l of cowdung extract (3.11 and 3.20 kg/plot respectively) were also significant and statistically on par with the above treatment (Table 10). This type of increase in straw yield from bacterial blight affected rice plants after taking appropriate measures to control the disease has been reported earlier also by Natarajan and Lalithakumari (1989). The straw yield from plants sprayed with even the lowest concentration of cowdung extract was better than some of the chemical treatments like 100 and 250 ppm of terra-mycine and bactrinol-100 and all concentrations of streptocycline at Ulloor seed farm and Nedumudi. This showed that cowdung extract spray can not only control bacterial blight to some extent, increase the thousand grain weight and reduce the number of chaffy grains per panicle, but it can also increase the straw yield from diseased plants. Hence, if the conditions stipulated earlier for the control of bacterial blight using cowdung extract are prevailing, then the farmer may try this method for controlling this disease.

In the last part of this investigation, an attempt was also made to find out the probable reason for the mode of control of bacterial blight by cowdung extract. In the earlier part of this study, it was found that apparently no soluble bactericidal factors are present as such in cowdung extract which can inhibit the growth of *X. campestris* pv. *oryzae* under *in vitro* conditions. The mode of inhibition appeared to be due to an over growth of the pathogen mainly by bacteria and probably by some fungi present in cowdung extract (Table 11 and Plate 13). Hence, under field conditions, if susceptible rice varieties are given a prophylactic spraying with cowdung extract, it is likely that some of these microflora may colonise the leaf surface. This may either reduce or eliminate subsequent leaf colonisation by the pathogen and thereby decrease the severity or even prevent the incidence of the disease itself. However, it will be essential to use fresh cowdung for preparing the spray solution to get such an effect since it alone will contain a very high population of native microflora. In the other situation, where cowdung extract is used for the control of the disease after the onset of infection by the pathogen, the mechanism of disease control has to be elucidated.

The relative economic benefits of controlling bacterial blight by using antibiotics, bactrinol-100 and cowdung extract was studied based on the data available from the field experiment conducted at Ulloor seed farm. It was found that it will be advantageous for the farmer if suitable control measures are taken in time to check the spread of this disease. But as observed there can be a net loss of upto 72 paise for each rupee invested for rice cultivation. At the same time it will be possible to get a satisfactory return from bacterial blight affected plants after spraying with either bactrinol-100 or cowdung extract. Since terramycin or oxy-tetracycline as such is not usually recommended for the control of bacterial blight and since it was included in the field study only as an experimental treatment, it was excluded from the study. In the remaining treatments, the economic return of Rs.1.84 was maximum after spraying infected plants with 100 g/l of cowdung extract followed by 50 g/l of cowdung extract (Rs.1.74), 500 ppm bactrinol-100 (Rs.1.69) and 20 g/l of cowdung extract (Rs.1.60). Among these, the maximum grain yield was after treatment with 500 ppm bactrinol-100. In other cases, the high return appeared to be due to an increase in straw yield. The net return from plants sprayed with streptocycline was comparatively low. Thus it appears that it will be advantageous to use either bactrinol-100 or cowdung extract for the control of bacterial blight of rice. However, this observation has to be confirmed by conducting further field trials before it can be recommended for the control of this rice disease in Kerala.

SUMMARY

Bacterial blight caused by *X. campestris* pv. *oryzae* is a major rice disease in many countries. In Kerala this disease is a serious problem for paddy cultivators in Kuttanad, especially during the additional crop season. The present investigation was taken up to assess the efficacy of two alternative control methods, spraying with bactrinol-100 and cowdung extract and to elucidate the factor present in cowdung extract, responsible for the control of bacterial blight pathogen.

The experiment was conducted in a three phased manner. The pathogen was isolated from an infected rice field in Kuttanad and subjected to *in vitro* antibiotic assay. The pathogen was then, inoculated in T(N)1 rice plants raised in pots, the disease was induced and the plants were sprayed with antibiotics, bactrinol-100 and cowdung extract and the efficacy of each spray material in reducing the disease intensity was assessed. The same spray materials were subsequently assessed for their efficacy in reducing the disease intensity under field conditions at two locations, Nedumudy and Ulloor seed farm.

Under *in vitro* conditions terramycin 500 ppm was found to be most efficient in checking the growth and virulence of the pathogen. This was followed by terramycin 250 ppm and bactrinol-100 500 ppm. But cowdung extract failed to produce a growth inhibition zone similar to that of antibiotics and bactrinol-100. Under pot culture conditions terramycin 500 ppm reduced the disease intensity to the maximum. This was followed by 500 ppm bactrinol-100. It was observed that higher the concentration of each chemical tried, better was the control of bacterial blight except for cowdung extract. The extent of disease control achieved by spraying with different concentrations of cowdung extract was found to be better than some of the chemical treatments like terramycin 100 ppm, streptocycline at 100, 250 and 500 ppm and bactrinol-100 at 100 and 250 ppm. Under field conditions 500 ppm terramycin was found to give maximum disease control at both the locations after the first spray. But this was on par with 500 ppm bactrinol-100 at Ulloor seed farm while it was not, at Nedumudy. Terramycin 500 ppm proved to be the best treatment after the second spray also, at both the locations.

The grain yield was found to be maximum after spraying with 500 ppm terramycin in Jyothi variety at Ulloor seed farm. The grain yield from plants sprayed with 500 ppm bactrinol-100 and 50 and 100 g/l of cowdung extract were also significant and statistically on par with the above treatment.

At Nedumudy C-153 rice plants sprayed with 500 ppm terramycin gave maximum yield. The net grain yield from plants sprayed with 500 ppm bactrinol-100 was found to be significant and statistically on par with the above treatment.

The increase in thousand grain weight was found to be maximum in plants sprayed with cowdung extract at Ulloor in Jyothi variety, while it was maximum in plants sprayed with 500 ppm bactrinol-100 at Nedumudy in C-153 variety. The maximum reduction in chaffy grains was achieved after spraying with 500 ppm terramycin at both the locations.

The increase in straw yield was maximum after spraying with 100 g/l of cowdung extract in Jyothi variety, but, this was on par with that of terramycin 500 ppm. In C-153 variety the maximum straw yield was obtained from plants sprayed with 500 ppm terramycin. But this was on par with that of 500 ppm bactrinol-100 and 50 and 100 g/l of cowdung extract.

The results obtained prove that significant yield lossess in rice varieties like Jyothi and C-153 can occur if infected with bacterial blight. Thus the need for taking appropriate control measures at the early stage of the disease is quite evident. The present investigation indicates that spraying cowdung extract would be very useful to control bacterial blight of rice, when the disease incidence is mild. The farmers can also be exhorted to give a prophylactic spraying of 20 g/l of cowdung extract which may reduce the severity of disease. These hypotheses can be proved conclusively only after conducting repeated multi-locational trials.

An attempt was also made to work out the probable reason for the mode of control of bacterial blight by cowdung extract. The mode of inhibition appeared to be due to an overgrowth of the pathogen mainly by bacteria and probably by some fungi present in cowdung extract.

Detailed laboratory studies should be conducted to prove this conclusively.

On working out the economic benefits of controlling bacterial blight it was observed that the economic return was maximum after spraying infected plants with 100 g/l of cowdung extract followed by 50 g/l of cowdung extract, 500 ppm of bactrinol-100 and 20 g/l of cowdung extract. Thus it also appears, that it will be economically advantageous to use, either bactrinol-100 or cowdung extract to control bacterial blight of rice.

REFERENCE

REFERENCES

Anonymous (1964). Systemic symptoms of bacterial leaf blight. Int. Rice Res. Inst. Annu. Rep. pp.152.

Anonymous (1967). Bacterial population in dry seasons in Central Luzon. Int. Rice Res. Inst. Annu.Rept. pp.93.

Anonymous (1970). Tech. Rept. Cent. Rice Res. Inst. Cuttack 191: 4.

Anonymous (1970). IR-20 and IR-22 new rice varieties. Systemic fungicide against blast. Int. Rice Res. Inst. Rept. 6: pp.1-4.

Anonymous (1973). Int. Rice Res. Inst. Annu. Rept. pp.266.

Anonymous (1974). Int. Rice Res. Inst. Annu. Rept. pp.384.

Anuratha, C.S. and Gnanamanickam, S.S. (1987). Suppression of development of bacterial blight symptoms by Pseudomonas fluorescens. Int. Rice Res. Newsl. 12: 1.

Balaraman, K. and Soumini Rajagopalan, C.K. (1978). Control of bacterial leaf blight of rice. Assay of chemicals.Madras Agric. J. 65: 463-469.

*Bhapkar, D.G., Kulkarni, N.S. and Chavan, V.M. (1960). Bacterial leaf blight of paddy. Poona Agric. Coll. Mag. 51: pp.36-46.

*Bokura, V. (1911). Bacterial leaf blight of rice. Teikoku. Nokaiho, 2: pp.62-66.

Breed, R.S., Murrary, K.G.D. and Smith, N.R. (1957). Bergey's Manual of Determinative Bacteriology. 7th ed. The Williams and Wilkins Co., Baltimore, U.S.A. pp.175.

Chakravarti, B.P. and Rangarajan, M. (1967). A virulent strain of Xanthomonas oryzae isolated from rice seeds in India. Phytopathology 57: pp.688-690.

Chandrasekharan, A. and Vidhyasekharan, P. (1988). Screening antibiotics for their control of bacterial blight. Int. Rice Res. Newsl. 13: pp.1.

Chauhan, H.L. and Vaishnav, M.V. (1980). Control of bacterial blight of rice caused by Xanthomonas oryzae. Indian J. of Mycol. and Plant Pathol. 10: pp.77-79.

*Chen, L.O., Guo, Y.F. and Ma, G.O. (1980). Isolation, purification and identification of the antibiotics 4-215. Actamicrobiologica sinica. 20: pp.94-99.

Cochran, W.G. and Cox, G.M. (1965). Experimental designs. John Wiley and sons Inc., New York.

*Dath, A.P., Padmanabhan, S.Y. and Devadath, S. (1977). The relation between certain host characters and bacterial blight incidence in rice. Proceedings of the Indian Acad. of Sci. 85: pp.301-309.

Deiveegasundaram, M., Kannaiyan, S. and Prasad, N.I. (1977). In vitro effect of certain fungicides and antibiotics against the bacterial leaf blight pathogen. Int. Rice Res. Newsl. 2: pp.5.

Desai, S.G., Patel, M.K. and Desai, M.V. (1967). In vitro activity of streptocycline against bacterial plant pathogens. Indian Phytopathol. 20: 296.

Devadath, S., Dath, A.P. and Umagupta (1971). Studies on the physiological and biochemical characters of streptomycin sensitive and resistant isolates of Xanthomonas oryzae. Cent. Rice Res. Inst. Cuttack. Annu. Rep. 142-143.

Durgapal, J.C., Singh, B. and Pandey, K.R. (1981). Efficacy of thiram against Xanthomonas oryzae in vitro. Indian J. of Agric. Sci. 51: 521-526.

Durgapal, J.C. (1983). Management of bacterial blight of rice by nursery treatment-Preliminary evaluation. Indian Phytopathol. 36:(1) 146-149.

Dye, D.W., Bradbury, J.F., Goto, M.G., Hayward, A.C., Lelljott, R.A. and Schroth, M.N. (1980). International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. Rev. Plant Pathol. 59: 153.

Gomez, K.A. (1972). Techniques for field experiments with rice. Int. Rice Res. Inst. Annu. Rept. pp.33.

Gossele, F., Swings, J., Ley, J. De. (1984). Effect of organic and inorganic chemicals on in vitro growth of Xanthomonas campestris pv. oryzae. Int. Rice Res. Newsl. 9: 1.

*Goto, K., Inoue, Y., Fakatsu, R. and Ohata, K. (1955). Field observations on the outbreak and fluctuation of severity of bacterial leaf blight of rice plant. Bull. Tokai-Kinki Agric. Expt. Sta. 2: 53-68.

*Hashioka, Y. (1951). Bacterial leaf blight of rice and its control. Agric. Hort. Tokyo. 26: 644-648.

Hoa, T.T.C., Binh, T.C., Kandaswamy, T.K., Luat, N.Van. (1984). Prophylactic chemical treatments for control of bacterial blight. Int. Rice Res. Newsl. 9: 3.

*Hucker, G.J. (1927). Further studies on method of gram staining. N.Y. Agrl. Exp. Sta. Tech. Bull: 128-130.

*Ikeno, S. (1958). Bacterial blight of rice and the damage in Niigata prefecture. Proc. Assoc. Plant Protec. 6: 13-14.

Inderawati, E. and Heitefuss, R. (1977). Effect of herbicides on diseases of rice (Oryzae sativa). Weed Sci. 25: 441-447.

*Ishiyama (1922). Studies on bacterial leaf blight. Rep. Agric. Exp. Sta. 45: 233-261.

Jain, S.S., Padmanabhan, S.Y. and Reddy, P.R. (1965). Control of bacterial leaf blight of rice, Xanthomonas oryzae (Uyda and Ishiyama) Dowson. Oryza 2: 145-152.

Jain, S.S., Reddy, R.P. and Padmanabhan, S.Y. (1966). Symposia on diseases of rice, maize, sorghum and millets. Bull. Indian Phytopathol. Sco. 3: 127.

*Jalavicharana (1958). Outbreaks and new records. FAO Pl. Prot. Bull. 6: 12-126.

*Kaul, T.N. (1959). Outbreaks and new records. FAO Plant Prot. Bull. 7: 54-55.

Kerala Agricultural University (1986). Package of Practices Recommendations pp 10-35.

Krishnappa, K. and Singh, R.A. (1978). Chemical control of bacterial leaf blight of rice. Mysore J. Agric. Sci. 11: 530-534.

*Kuwazuka, K. (1942). Bacterial leaf blight disease of rice. Nogyo. 168: 33-35.

Lin, S.H., Ho, B.L., Yu, Y.Y. (1981). Techniques of integrated control of rice bacterial leaf blight. Fukien Agric. Sci. and Tech. 4: 26-27.

Mahmood, M. and Singh, M.N. (1970). Investigation on bacterial blight of paddy caused by Xanthomonas oryzae (Uyeda and Ishiyama)Dowson, Sci. Cult. 36: 170-171.

Mary, C.A. and James Mathew (1980). Etiology and control of bacterial leaf blight of rice caused by Xanthomonas oryzae (Uyeda and Ishiyama) Dowson. M.Sc. thesis Coll. Agric. Vellayani pp.59-62.

Mary, C.A., Mathew, J. (1983). Chemical control of bacterial leaf blight of rice using antibiotics. Agric. Res. J. Kerala. 21: 17-20.

Mary, C.A., Dev, V.P.S., Karunakaran, K. and Nair, N.R. (1986). Cowdung extract for controlling bacterial blight. Int. Rice Res. Newsl. 11: 2.

Mew, T.W. and Majiid, A. (1977). Bacterial blight of rice in Pakistan, Int. Rice Res. Newsl. 2: 5.

Mizukami, T. (1956). Studies on bacterial leaf blight of rice plant on the entrance and multiplicating portion of pathogen upon the rice plant leaves (Preliminary report). Sci. Bull. FAO Agric. 4: 169-175.

Mohiuddin, M.S., Verma, J.P. and Rao, Y.P. (1977). Development of different phases of bacterial blight of rice in relation to rainy days and rainfall. Indian Phytopathol. 30: 418-419.

Mondal, A.H., Miah, S.A. (1985). Effect of K on bacterial blight development. Int. Rice Res. Newsl. 10: 2.

Naidu, V.D., Reddy, A.P.K. and John, V.T. (1980). Influence of neem cake and coal tar coated urea on bacterial blight of rice. Int. Rice Res. Newsl. 55: 14.

Nair, S.K. and Sreelatha, K. (1988). Effect of high rainfall and relative humidity on the incidence of bacterial leaf blight of rice. Abst. III-4 of Third Indian Agrometeorological Congress, April 1988. Organised by Kerala Agric. Uty. and Indian Agrometeorological Society.

Nair, S.K., Sreelath, K., Remadevi, L. and Wilson, K.I. (1990). An alternative concept on antibiotic formulations used for the control of bacterial leaf blight of rice. Abst. National symposium on rice in wetland ecosystem. Organised by Kerala Agricultural University, December 19-21, (1990).

Natarajan, M.R. and Lalithakumari, D. (1989). Bactrinol-100 A novel agrobactericide for bacterial blight of paddy. Indian Phytopathol 19: 163.

Nilpanit, N., Srisantana, W., Kiatsuranoot, P., Phawichit,S. and Disthaporn, S. (1984). Bacteriophage strain of X. campestris pv. oryzae from parts of Thailand. Int. Rice Res. Newsl. 9 (6): 11-12.

Padmanabhan, S.Y. (1983). Integrated control of bacterial blight of rice. Oryza 20: 4 pp.188-194.

Pal, A. and Das, C.R. (1968). Preliminary experiments on the laboratory evaluation of some antibiotics against bacterial leaf blight of paddy caused by Xanthomonas oryzae (Uyeda and Ishiyama) Dowson. Indian J. Mycol. Res. 6: 78-80.

Pal, V. and Singh, R.A. (1978). Systemic bactericidal activity of brestanol against Xanthomonas oryzae in rice seedlings. Indian Phytopathol. 30: 1. 84-86.

Palaniswami, A. and Ahmed, N.J. (1979). Bleaching powder for bacterial blight control. Int. Rice Res. Newsl. 4: 3.

Pandey, K.R. and Iswaran, V. (1982). Influence of microbial fertilizers on bacterial blight of rice-II - Nitrogen content and extent of bacterial invasion of the rice leaves. Indian Phytopathol. 35(4):714-716.

Pavgi, M.S., Singh, R.A. and Dular, R. (1964). Bacterial leaf blight of rice in North India. Sci. Cult. 30: 406-407.

Raina, G.L., Sidhu, G.S. and Saini, P.K. (1981). Rice bacterial blight status in the Punjab. India. Int. Rice Res. Newsl. 6: 12.

Rao, P.S. and Kauffman, H.E. (1971). New Indian host of Xanthomonas oryzae incitant of bacterial leaf blight of rice. Curr. Sci. 40: 271-272.

Rao, P.S. and Kauffman, H.E. (1977). Potential yield losses in dwarf rice varieties due to bacterial blight in India. Phytopath. Z. 90: 281-284.

Ray, P.R. and Sengupta, T.K. (1970). A study on the extent of loss in yield in rice due to bacterial blight. Indian Phytopathol. 23: 713.

Reddy, P.R. (1965). Varietal susceptibility to the bacterial blight of rice. Oryza. 2: 153-154.

Reddy, A.P.K. and Pillai, K.G. (1974). Epidemiology of rice pathogen. Cent. Rice Res. Inst. Cuttack Annu. Rep. pp.244.

Reddy, A.P.K., Saxena, N.P. and Reddy, A.V.R. (1978). Analysis of yield due to incidence of bacterial blight diseases of rice. Indian Phytopathol. 31: 210-212.

*Reitsma, J. and Schure, P.S.J.(1950). 'Kresek' a bacterial disease of rice. Contr. Gen. Agri. Res. Sta. 117: 1-17.

Sahu, R.K., Srivastava, M.N., Sahu, V.N., Chaudhary, B.P. and Srivastava, P.S. (1982). Bacterial blight resistance under natural conditions. Int. Rice Res. Newsl. 7: 6.

Sakthivel, N., Sivamani, E., Unnamalai, N. and Gnanamanickam, S.S. (1986). Plant growth promoting rhizobacteria in enhancing plant growth and suppressing plant pathogens. Curr. Sci. 55: 1. pp.22-25.

*Seneviratne, S.N. (1962). Bacterial leaf blight of rice. Trop. Agric. 118: 123.

Sharma, K.K. and Kaul, M.L.H. (1984). Analysis of yield loss due to bacterial blight of rice. Sci. and Cult. 50(3): 102-104.

Shetty, K.S. and Rangaswami, G. (1968). Studies on the bacteriostatic and bactericidal action of streptocycline on Xanthomonas oryzae. Mysore J. Agric. Sci. 2: 1-5.

Shukla, S.N. and Gangopadhyay, S. (1981). Stomatal index and size of stomatal opening of rice cultivars varying in reaction to BLB. Proc. Indian Nat. Sci. Acad. 47(4): 557-559.

Shukhla, S.N. (1986). Reaction of rices to bacterial blight at different growth stages. Int. Rice Res. Newsl. 11: 2.

Singh, S.K. and Nene, Y.L. (1967). Eradication of seed borne inoculum of Xanthomonas oryzae by hot water treatment of paddy seeds. Plant Dis. Reptr. 51: 882-883.

Singh, R.N. and Saksena, H.K. (1968). Bacterial blight diseases of paddy - symptomatology. Plant Dis. Reptr. 52: 663-664.

Singh, N., Singh, H. and Chochan, J.S. (1977). Field efficiency of certain chemicals in the control of bacterial leaf blight of rice in Punjab. Int. Rice Res. Newsl. 2: 6.

Sivamani, E., Anuratha, C.S., Gnanamanickam, S.S. (1987). Toxicity of Pseudomonas fluorescens towards bacterial pathogens of banana (Pseudomonas solanacearum) and rice (Xanthomonas campestris pv. oryzae) Curr. Sci. 56: 11. pp.547-548.

Sivaswamy, S.N. and Mahadevan, A. (1986). Effect of stable bleaching powder on growth of Xanthomonas campestris pv. oryzae. Indian Phytopathol. 39: 1. pp. 32-36.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods. VIth edition. Oxford and IBH publishing Co., Calcutta, India.

*Soga, N. (1918). On the bacterial leaf blight diseases of rice in Kumamota prefecture. Byochuqi Zassi 5: 543-549.

Soumini Rajagopalan, C.K., Padmanabhan, C., Natarajan, R. and Venkata Rao, A. (1969). Effect of hot water treatment on the control of primary seed infection in rice caused by Xanthomonas oryzae. Madras Agric. J. 56:354-360.

Sreelatha, K. (1985). Studies on the effect of age of plant and host nutrition on bacterial leaf blight of rice and its control. M.Sc. Thesis. Coll. Agric. Vellayani.

Srinivasan, M.C., Thirumalachar, M.J., Patel, M.K.(1959). Bacterial blight of rice. Curr. Sci. 28: 469-470.

Srinivasan, N. and Singh, R.A. (1983). Role of meteorological factors and soil moisture on the epidemiology of the kresek phase of bacterial blight of rice. Indian J. Agric. Sci. 53: 233-239.

Srivastava, D.N. and Rao, Y.P. (1964). Paddy farmers should beware of bacterial blight. Indian Farming 13: 32-33.

Srivastava, D.N. and Rao, Y.P. (1966). Symptoms and diagnosis of the bacterial disease of rice. Curr. Sci. 35: 60-61.

Swain, N.C., Sahu, K.C. and Narain, A. (1985). Efficacy of certain chemicals on the control of bacterial blight of rice. Asian Farm Chem. 1: 4.

Swarup, J., Verma, S.C. and Chauhan, L.S. (1965). Bioassay of antibiotics, fungicides and antiseptic chemicals against Xanthomonas oryzae as a test organism. Lab. Dev. J. Sci. Tech. 3: 38-40.

*Takahi, Y. and Shirahagen, S. (1985). Techloftalam a new systemic bactericide for controlling rice bacterial leaf blight. Japan Pest. Info. 46: 25-30.

*Takaishi, M. (1909). First report, studies on bacterial leaf blight of rice. Dainihon Nokaiho. 340: 53-58.

*Takeuchi, H. (1930). On the occurrence of bacterial leaf blight of rice. Bull. Chosen Sotukuhu Agric. Expt. Sta. 5: 62-64.

Verma, M.L., Agarwal, K.C., Upadhyaya, A.R. (1980). Chemical control of bacterial blight and bacterial leaf sheath diseases of rice. Pesticides. 24: 27-28.

*Wakimoto, S. (1954). The determination of presence of Xanthomonas oryzae by the phage technique. Sci. Bull. Fac. Agric. Kyushu Univ. 14: 495-498.

Wu, S.Z., Hsu, S.M., Chen, F.K., Choi, L.C. and Liu, K.M. (1981). Varietal resistance to Xanthomonas campestris pv. oryzae in Guangdong China. Int. Rice Res. Newsl. 615.

*Yoshii, H., Yoshida, T. and Matsui, C. (1953). Isolation of Xanthomonas oryzae phage. Annu. Phytopathol. Soc. Jpn. 17: 77.

*Yoshimura, S. and Tahara, S.K. (1960). Morphology of bacterial leaf blight organism under (electron) microscope. Annu. Phytopath. Soc. Jpn. 26: 61

* Originals not seen

APPENDICES

APPENDIX I

ANOVA *In vitro* studies

Source	DF	S.S.	M.S.S.	F
Treatments	11	6.43	0.58	19.33**
A	2	3.89	1.94	64.66**
Between B_1	3	1.03	0.34	11.33**
Between B_2	3	1.32	0.44	14.67**
Between B_3	3	0.19	0.06	2.00
Error	24	0.82	0.03	

* Significant at 5% level

** Significant at 1% level

APPENDIX II

ANOVA Pot culture studies

Source	DF	S.S.	M.S.S.	F
Treatments	11	1605.23	145.93	57.57**
A	3	557.55	185.85	73.32**
Between B_1	2	371.98	185.99	73.37**
Between B_2	2	542.11	271.06	106.93**
Between B_3	2	130.52	65.26	25.75**
Between B_4	2	3.07	1.53	0.61
Treated Vs control	1	1061.69	1061.69	418.84**
Error	26	65.91	2.54	

APPENDIX III

ANOVA-Field studies using Jyothi rice variety

Disease intensity after first spray

Source	DF	S.S	M.S.S.	F
Replication	2	3.91	1.95	0.21
Treatments	11	1036.63	94.24	10.05**
TR	3	81.61	27.20	2.90*
Between B_1	2	282.19	141.10	15.04**
Between B_2	2	455.96	227.98	24.31**
Between B_3	2	216.64	108.32	11.55**
Between B_4	2	0.24	0.12	0.01
Treated Vs Control	1	332.55	332.55	35.45**
Error	24	225.11	9.38	

Disease intensity after second spray

Source	DF	S.S.	M.S.S	F
Replication	2	17.64	8.82	2.24
Treatments	11	1774.98	161.36	40.93**
TR	3	147.11	49.04	12.44**
Between B_1	2	461.74	230.87	58.55**
Between B_2	2	682.11	341.06	86.50**
Between B_3	2	482.44	241.22	61.18**
Between B_4	2	1.59	0.79	0.20
Treated Vs Control	1	1620.98	1620.98	411.12**
Error	24	94.63	3.94	

APPENDIX IV

ANOVA - Grain yield in Jyothi rice variety

Source	DF	S.S.	M.S.S.	F
Replication	2	4.13	2.06	3.61*
Treatments	11	8.37	0.76	1.33*
TR	3	1.79	0.60	1.05*
Between B_1	2	2.50	1.25	2.19*
Between B_2	2	1.77	0.88	1.54*
Between B_3	2	2.27	1.13	1.98*
Between B_4	2	5.55	2.77	4.86*
Treated Vs Control	1	10.92	10.92	19.16**
Error	24	13.68	0.57	

APPENDIX V

ANOVA - 1000 grain weight and number of chaffy grains in Jyothi rice variety.

1000 grain weight

Source	DF	S.S.	M.S.S.	F
Replication	2	9.77	4.88	488.00**
Treatments	11	7.55	0.69	69.00**
A	3	4.81	1.60	160.00**
Between B_1	2	0.73	0.37	37.00**
Between B_2	2	0.20	0.10	10.00**
Between B_3	2	0.67	0.33	33.00**
Between B_4	2	1.15	0.58	58.00**
Treated Vs Control	1	10.94	10.94	1094.00**
Error	24	0.11	0.01	

Number of chaffy grains

Source	DF	S.S.	M.S.S.	F
Replication	2	1.56	0.78	0.06
Treatments	11	984.22	89.48	6.67*
A	3	435.11	145.04	10.82**
Between B_1	2	130.67	65.33	4.87*
Between B_2	2	270.22	135.11	10.08**
Between B_3	2	120.67	60.33	4.50*
Between B_4	2	27.56	13.78	1.03
Treated Vs Control	1	789.88	789.88	58.91**
Error	24	321.78	13.41	

APPENDIX VI

ANOVA - Straw yield in Jyothi rice variety

Source	DF	S.S.	M.S.S.	F
Replication	2	3.32	1.66	4.26*
Treatments	11	55.35	5.03	12.90**
TR	3	17.74	5.91	15.15**
Between B_1	2	13.65	6.83	17.51**
Between B_2	2	11.94	5.97	15.31**
Between B_3	2	11.43	5.71	14.64**
Between B_4	2	0.60	0.30	0.77
Treated Vs Control	1	54.50	54.50	139.74**
Error	24	9.41	0.39	

APPENDIX VII

ANOVA - Disease intensity in C-153 rice variety

Disease intensity after first spray

Source	DF	S.S.	M.S.S.	F
Replication	2	35.62	17.81	3.13*
Treatments	11	1096.37	99.67	17.49**
TR	3	383.53	127.84	22.43**
Between B_1	2	279.86	139.93	24.55**
Between B_2	2	319.97	159.98	28.07**
Between B_3	2	108.06	54.03	9.48**
Between B_4	2	4.94	2.47	0.43
Treated Vs Control	1	1345.31	1345.31	236.02**
Error	24	136.88	5.70	

Disease intensity after second spray

Source	DF	S.S.	M.S.S.	F
Replication	2	24.12	12.06	1.75
Treatments	11	1670.95	151.90	22.05**
TR	3	627.55	209.19	30.36**
Between B_1	2	412.31	206.15	29.92**
Between B_2	2	418.01	209.00	30.33**
Between B_3	2	211.82	105.91	15.37**
Between B_4	2	1.27	0.63	0.09
Treated Vs Control	1	3935.83	3935.83	571.24**
Error	24	165.31	6.89	

APPENDIX VIII

ANOVA - Grain yield in C-153 rice variety

Source	DF	S.S.	M.S.S.	F
Replication	2	2.21	1.11	111.00**
Treatments	11	1.39	0.12	12.00**
TR	3	0.45	0.15	15.00**
Between B_1	2	0.36	0.18	18.00**
Between B_2	2	0.48	0.24	24.00**
Between B_3	2	0.08	0.04	4.00**
Between B_4	2	2.17	1.09	109.00**
Treated Vs Control	1	0.56	0.56	56.00**
Error	24	0.12	0.01	

APPENDIX IX

ANOVA - 1000 grain weight and number of chaffy grains in C-153 rice variety

1000 grain weight

Source	DF	S.S.	M.S.S.	F
Replication	2	0.03	0.02	0.66
Treatments	11	18.63	1.69	56.33**
A	3	15.95	5.32	177.33**
Between B_1	2	1.49	0.75	25.00**
Between B_2	2	0.43	0.22	7.33**
Between B_3	2	0.11	0.06	2.00
Between B_4	2	0.65	0.33	11.00**
Treated Vs Control	1	7.25	7.25	241.66**
Error	24	0.69	0.03	

Number of chaffy grains

Source	DF	S.S.	M.S.S.	F
Replication	2	51.17	25.58	1.83
Treatments	11	552.08	50.18	3.59
A	3	226.53	75.50	5.40**
Between B_1	2	181.56	90.78	6.49**
Between B_2	2	91.56	45.78	3.27
Between B_3	2	50.89	25.44	1.82
Between B_4	2	1.56	0.78	0.06
Treated Vs Control	1	254.33	254.33	18.19**
Error	24	335.50	13.98	

APPENDIX X

ANOVA - Straw yield in C-153 rice variety

Source	DF	S.S.	M.S.S.	F
Replication	2	3.46	1.73	57.67**
Treatments	11	8.48	0.77	25.66**
TR	3	3.14	1.05	35.00**
Between B_1	2	1.93	0.97	32.33**
Between B_2	2	2.33	1.17	39.00**
Between B_3	2	0.89	0.44	14.67**
Between B_4	2	0.19	0.10	3.33
Treated Vs Control	1	5.02	5.02	167.33**
Error	24	0.76	0.03	

EFFECT OF 2-Bromo-2-nitro propane-1, 3-diol AND COWDUNG EXTRACT ON GROWTH AND VIRULENCE OF *Xanthomonas campestris pv. oryzae*

By
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ABSTRACT OF A THESIS
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VELLAYANI, TRIVANDRUM

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ABSTRACT

The present investigation was taken up to assess the efficacy of two alternative control methods, spraying with bactrinol-100 and cowdung extract in controlling bacterial blight of rice. An attempt was also made to elucidate the factor present in cowdung extract responsible for the control of bacterial blight pathogen.

The pathogen was isolated from an infected rice field in Kuttanad and tested for sensitivity to antibiotics, bactrinol-100 and cowdung extract under *in vitro* conditions. Under *in vitro* conditions, terramycin 500 ppm was found to be most efficient in checking the growth and virulence of the pathogen followed by 250 ppm terramycin and 500 ppm bactrinol-100. Cowdung extract failed to produce a typical growth inhibition zone. Subsequently the pathogen was inoculated in T(N)1 rice plants raised in pots, the disease was induced and the plants were sprayed with antibiotics, bactrinol-100 and cowdung extract. Under pot culture conditions terramycin 500 ppm reduced the disease intensity to the maximum followed by 500 ppm bactrinol-100. Cowdung extract was found to be better than some of the chemical treatments

like terramycin 100 ppm, streptocycline 100, 250 and 500 ppm and bactrinol-100 at 100 and 250 ppm. Under field conditions 500 ppm terramycin gave maximum disease control at two locations after the first and second spray. The grain yield was found to be maximum after spraying with 500 ppm terramycin at both the locations. The increase in thousand grain weight was found to be maximum in plants sprayed with cowdung extract in Jyothi variety and in plants sprayed with 500 ppm bactrinol-100 in C-153 rice variety. Maximum reduction in chaffy grains was achieved after spraying with 500 ppm terramycin at both the locations. Straw yield was maximum in plants sprayed with 100 g/l of cowdung extract in Jyothi variety and in plants sprayed with 500 ppm terramycin in C-153 variety.

So it is evident that unless appropriate control measures for bacterial blight are taken in time, significant yield lossess can occur in rice varieties like Jyothi and C-153. Spraying cowdung extract may be very useful to control bacterial blight especially when the disease incidence is mild. It can also be given as a prophylactic spray which may reduce the severity of the disease. These hypotheses have to be proved conclusively by further field trials.

The mode of inhibition of X. campestris pv. oryzae appeared to be due to an overgrowth of the pathogen mainly by bacteria and probably by some fungi present in cowdung extract.

On working out the economic aspects of controlling bacterial blight it was observed that economic return was maximum after spraying infected plants with 100 g/l cowdung extract followed by 500 ppm bactrinol-100. So economically it appears, that the use of cowdung extract or bactrinol-100 would be ideal to control bacterial blight of rice.