STUDIES ON THE BACTERIAL BLIGHT (WILT) OF TAPIOCA
INCITED BY *Xanthomonas manihotis* (ARTHAUD - BERTHET) STARR

BY

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THESIS

submitted in partial fulfilment of the
requirement for the degree
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DECLARATION

I hereby declare that this thesis entitled "Studies on the Bacterial Blight (Wilt) of Tapioca Incited by Xanthomonas manihotis (Arthaud - Berthet) Starr" is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.
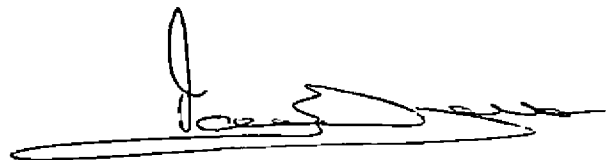
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Certified that this thesis, entitled "Studies on the Bacterial Blight (Wilt) of Tapioca Incited by Xanthomonas manihotis (Arthaud - Berthet) Starr" is a record of research work done independently by Shri. Mani T. Cherian, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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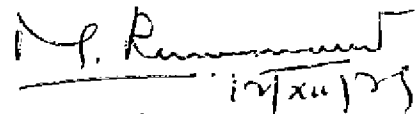
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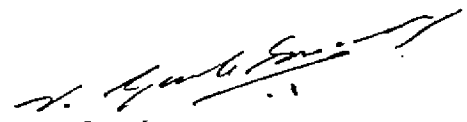
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MANI T. CHERIAN

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CIAT   Centro Internacional de Agricultura Tropical

IITA   International Institute of Tropical Agriculture

Introduction

INTRODUCTION

Tapioca widely known as cassava (_Manihot esculenta_ Crantz), is the major food crop of the middle and lower income class of people of Kerala State. This is now, and will be for some time to come, their primary source of food. With the limitations in land, labour and capital in the production of rice, more attention has been given to the production of tuber crops, with tapioca topping the list.

In India tapioca is cultivated in an area of 3,83,700 hectares with an annual production of 63,06,800 metric tonnes. Out of this, Kerala State alone accounts for 82 per cent of the area and 89 per cent of the production of the crop. Hence the importance of tapioca cultivation and its economic significance in Kerala State need not be over emphasised.

Of the several diseases of tapioca, bacterial blight (wilt) is the most important one, based on its nature of damage and extent of loss. Cassava bacterial blight is reported to be of very serious consequence in foreign countries like Brazil, Venezuela, Colombia and Africa. The disease was reported for the first time in Kerala during 1975 and also from Kanyakumari district of Tamil Nadu since 1976 (Nair, 1977, unpublished). This has become a serious concern to the tapioca growers of the State.

It is quite possible that the disease can flare up in epiphytotic proportions and can cause very heavy crop losses under congenial climatic conditions, because even total crop losses have been reported due to this disease in other countries (Costa, 1940; Lozano and Sequeira, 1974 a; Lozano, 1975).

In view of the potential crop losses, that the disease can cause and also of the economic significance of the disease to the tapioca growers of the state, investigations were taken up on its symptomatology, characterisation and identity of the pathogen, standardization of inoculation techniques, host range and survival of the pathogen, host nutrition in disease incidence and development, influence of age and HCN content on the disease, _in vitro_ sensitivity of the pathogen to antibiotics and toxigenicity of the bacterium. The results are presented in this thesis.

Review of Literature

REVIEW OF LITERATURE

HISTORY AND GEOGRAPHICAL DISTRIBUTION

The "Bacterial Blight (Wilt)" is the most important of several diseases reported on tapioca (Manihot esculenta Crantz). It was recorded for the first time in Brazil (Bondar, 1912; Costa, 1940) and has since then been reported from Colombia and Venezuela (Lozano, 1972; 1973 a; Lozano and Sequeira, 1974 a; 1974 b), Nigeria (Anon., 1972), Zaire (Maraite and Meyer, 1975) and has been observed in several other countries of tropical America and Africa.

Serious damage was first noted at Puli in Central Taiwan in 1963, though the disease probably existed before 1945 (Mau, 1951). Bacterial wilt of cassava has been causing severe reduction of tuber yield in the Savanna of southern part of Zaire since 1970. The disease is caused by Xanthomonas manihotis (Leu and Chen, 1972). In Africa, cassava bacterial blight (CBB) caused by X. manihotis was first reported in Nigeria (Williams et al., 1973) and subsequently in Zaire and in Cameroon (Terry and Ezhumah, 1974). Leu (1976) reported serious damage of cassava by bacterial blight which is known as "bacterial wilt" or "gumming disease".

This disease is now recognised as one of the most important factors limiting production, where in wet seasons, it can cause complete loss of yield (Costa, 1940; Drummond and Hipolito, 1941; Elliot, 1951; Pereira and Zagatto, 1967; Lozano, 1972; 1975; Lozano and Booth, 1974; Lozano and Sequeira, 1974 a). Lima (1944) reported that 90 per cent losses were recorded in the State of Santa Catarina.

In Kerala the disease has been reported by Paily et al. (1975) and Daniel et al. (1975).

SYMPTOMATOLOGY

The first symptoms of the disease in the field are loss of turgidity of leaves followed by their rapid wilting and shrivelling. Afterwards the base of the petiole collapse but the leaves generally remain attached to the stem for some time. Finally the unlignified tips of young branches die, and later new shoots appear at a lower level. Infection progress to the base of the stem also, causing wilt of the newly formed shoots. Because of these symptoms the disease is known in Zaire as the "Candle disease" (Maraite and Meyer, 1975).

In Colombia, the symptoms of the disease are characterised by angular leaf spotting, blight and wilting of leaves, die back and gum exudation of stem and vascular necrosis of stem and root. The leaf spots are water soaked initially, turning brown or dark brown, often with a yellowish

halo. Leaf spots often exude gum, that collects in droplets mostly along the lower surface of leaves along veins. Gum is also characteristically exuded from cracks, which often develop on the young infected stem and petioles (Lozano, 1975).

Nair (1977) had observed these typical symptoms, described by Maraite and Meyer (1975) and Lozano (1975), in his studies in Kerala, in all the susceptible varieties, except leaf spotting and gum exudation. This phenomenon of symptom variation has been reported by Maraite and Meyer (1975). Bondar (1939) reported that the symptoms differ between sweet and bitter varieties.

Pathogen

Nomenclature of the bacterium.

The causal bacterium was first named Bacillus manihotis Arthaud - Berthet (Bondar 1912), but was later renamed Phytomonas manihotis (Arthaud - Berthet and Bondar) Viegas (Viegas, 1940). However, Drummond and Hipolito (1941) found that some of the characteristics of the bacterium they isolated from cassava in Brazil were different from those of the species originally described by Bondar (1912). Burkholder (1942) concluded that the organism should be placed in the genus Phytomonas and the name Phytomonas manihotis was included in the Bergey's Manual (Breed et al., 1948).

Comparative studies of a new isolate with the strains of Burkholder and of Drummond and Hipolito were made by Amaral and Vasconcellos (1945). They concluded that all the three strains belonged to P. manihotis. Later, Starr (1946) changed the name to Xanthomonas manihotis (Arthaud - Berthet) Starr (Breed et al., 1957).

Isolation and properties of the bacterium

Lozano and Sequeira (1974 a) isolated the pathogen from diseased leaf and stem tissues, after making a suspension in sterile distilled water. A loopful of the suspension was streaked on Kelman's Tetrazolium Chloride (TZC) medium (Kelman, 1954), incubated at 30°C, and single colonies selected on the basis of colour and fluidity. The culture was stored in distilled water suspension.

Maraite and Meyer (1975) made isolations in a similar manner, after surface disinfection with 70 per cent ethanol, on Difco Nutrient Agar (NA) at 28°C. All isolates of similar colony appearance were chosen and stored on Commonwealth Mycological Institute's storage medium (Anon., 1968) and transferred every three months to fresh medium.

Leu (1976) isolated the pathogen on Potato Dextrose Agar (PDA) at 26 - 30°C from diseased tissues or gum substances.

Maraite and Meyer (1975) studied the cultural characters of the bacterium and reported that individual colonies of

purified isolates appeared on NA medium after 24 hours of incubation at 28°C. After 48 hours the colonies measured 1 mm in diameter. He observed that the growth on Difco PDA and Tryptone Soya Agar was faster than on NA. Leu (1976) had observed that the bacterium grew poorly on NA while the growth on PDA was profuse. In liquid medium the growth was membraneous and tended to be ring form when shaken and left standing overnight. Amaral (1942) reported that broth cultures (48 hr at 30°C) were characterised by dense turbidity and abundant viscid sediment. Nair (1977) studied the pathogenicity of the bacterium grown on different media. The colony growth, colour, shape, size, slime production and fluidity of the isolates on Nutrient Agar, Potato Dextrose Agar, Tetrazolium Chloride Agar, Potato Sucrose Peptone Agar and Host Extract media showed some differences. Growth on PDA was faster than on NA.

Maraite and Meyer (1975) observed that the colonies of _X. manihotis_ were white-grey to cream, raised, convex, smooth, shiny, with entire edges, initially hyaline, then opaque and turbid and of viscous consistency on NA. After 6 days incubation at 28°C, the colonies measured 14 mm on NA, 10 mm on PDA and 7 mm on Tryptone Soya Agar. Colonies on TZC medium were 8 mm in diameter after 6 days and were round, smooth, with a bright red centre and a narrow white edge. They resembled colony type B - 2 of weakly pathogenic mutants of _Pseudomonas solanacearum_ (E.F.Smith) E.F. Smith described by Kelman (1954). Leu (1976)

had observed that the colonies were circular, convex, entire
and filiform, with no odour, no fluorescence, milky white in
colour and sticky in texture on NA. On PDA the colonies were
milky white and mucoidal.

Braley (1965) and Stolp et al. (1965) reported that the
optimum temperature for the growth of the bacterium was higher
than that of most Xanthomonads. Leu (1976) observed that the
temperature range of the bacterium was 14 - 36°C with an
optimum at 30 - 34°C. Thermal death point was 52°C. Lozano
and Sequeira (1974 a) observed that the mass doubling time of
the CBB isolate 1. 23L in T2C liquid medium at 32°C was 46
minutes, at 30°C 47 minutes, at 28°C 55 minutes and at 34°C
57 minutes. The bacterium did not produce visible growth at
4°C or 39°C. Nair (1977) observed that the temperature range
for the growth of the bacterium was 20 to 32°C and profuse
growth was obtained at 30 to 32°C.

Lozano and Sequeira (1974 a) in their report on the
morphological characters indicated that the bacterium is a
slender gram negative rod, with a single polar flagellum and
numerous pili, non capsulated, non spore forming and was
either single or formed short chains of 3 to 4 cells. The
bacterium grew on ordinary culture media, did not produce
pigments and formed very slimy colonies on sucrose containing
media. The bacterium hydrolysed starch, liquified gelatin,
reduced litmus milk, produced levan, catalase, arginine

hydrolase and lipase but not hydrogen sulphide, indole, urease, tyrosinase or phenyl alanine deaminase. It grew on ordinary media containing sodium chloride or tetrazolium chloride at maximum concentrations of 2.5 and 0.2 per cent respectively. It utilised nitrate and ammonia as sources of nitrogen and most of the simple sugars as sources of carbon, but acid was not produced. Various amino acids and other organic acids were readily utilised. The physiological and biochemical characters of the 14 isolates studied by then were similar in nature. The bacterium grew well with weak acid production on basal medium containing D - mannose, D - glucose, L(-) fructose or melobiose, but grew poorly with slight acid production on basal medium containing L - rhamnose, D (-) ribose or maltose. The bacterium also grew well on basal medium containing (+) xylose or L (+) arabinose, but no acid was produced. The organism grew poorly on basal medium containing salicin, saccharin or lactose. The utilisation pattern of sucrose, cellobiose and trehalose by the isolates varied.

Biochemical characters were studied by Maraite and Meyer (1975) and they obtained similar results. They observed that the bacterium from 40 hr old cultures on HA were gram negative rods of 1.3 x 0.4 mμ (1.0 - 1.75 x 0.23 - 0.6), mostly single or in pairs with about 50 per cent motile cells

in 0.5 per cent glucose solution. The percentage of motile
cells decreased with age of the culture. Electron photo-
micrographs revealed the presence of a single polar flagellum
and numerous pili on the surface of the cells. Leu (1976)
had observed the morphological characters and reported that
the bacterium was a gram negative rod with round ends, a
single polar flagellum, no capsule and tending to become
gram positive when aged. Nair (1977) had studied the
physiological and biochemical characters of 8 isolates of the
bacterium and obtained similar results as the above workers.
He reported that the tests indicated the bacterium under
study to be X. manihotis (Arthaud - Berthet) Starr.

Pathogenic variability

As a result of the studies on the morphology, physiology,
serology and phage susceptibility of the isolates of the
bacterium from Colombia, Brazil and Venezuela, Lozano and
Sequeira (1974 a) concluded that they were sufficiently
different from X. manihotis to be considered as a separate
strain. They also reported that comparison with a type
culture of X. manihotis revealed differences in pathogenicity,
growth rate, serological characters and phage susceptibility.
Recently, however, comparative studies among different
American and African isolates of the organism revealed that
they possibly belong to the same bacterial species although
there are differences in virulence and a few physiological

characteristics (Lozano, 1975). The results of Maraite and
Meyer (1975) also suggested that strains of different
pathogenicity may exist within X. manihotis. Leu (1976)
reported that no visible mutants or changes in pathogenicity
were noted. Older (one month to several months) cultures
in the laboratory and subcultures (1 - 4 years) were all
found to induce the same degree of disease incidence as those
of freshly isolated cultures.

Serology and phage sensitivity

Serological reactions and phage susceptibility were
also studied by Lozano and Sequeira (1974 a). Out of the six
non pigmented Xanthomonads only X. manihotis induced slight
serological reaction after 15 days of incubation. Plaques
induced by Bdellovibrio sp. were 5 times larger than those
induced by the same phage on the same bacterial culture.

HOST RANGE

Xanthomonas manihotis has been reported to infect only
species and varieties of the genus Manihot (Bondar, 1915;
Amaral, 1942; Burkholder, 1942). Leu (1976) reported that
the cassava blight bacterium infects only cassava. Injection
of the bacterium near the apical meristem of the seedlings
of tomato, water-melon, lettuce, cucumber, sorghum and lima
bean and also into trees (Bischofia trifoliate, Codeaceum
variegatum and Euphorbia pulcherima) failed to result in

symptoms. No symptoms were also observed by pouring the
bacterial suspension into the injured roots of the seedlings
of the above mentioned plants. Arene (1974) also reported
that the cassava blight bacterium is host specific.

HOST PARASITE INTERACTION AND DISEASE DEVELOPMENT

Lozano and Sequeira (1974 a) used four different methods
of inoculation on 2 month old cassava plants of the variety
L. lanera, grown in sterilised sandy soils in pots. They
used a bacterial suspension containing $1 - 3 \times 10^9$ cells per
ml of sterile distilled water and kept the inoculated plants
in a growth chamber or green house at 25 to 30°C. Plants
which were sprayed with the cell suspension showed water
soaked angular spots on leaf lobes 8 - 10 days after inoculation.
Three days later tiny milky drops exuded from the spots. By
20 days after inoculation the spots had enlarged and coalesed,
covering a part or entire lobe of leaf lamina. Similar
results were obtained on the plants inoculated by the leaf
rubbing method. In plants inoculated by the stem puncture
method, wilting of leaves appeared near the point of inoculation, 5 - 6 days later. Afterwards additional leaves wilted.
The stem exuded gum profusely and by 20 days after
inoculation the entire plant had wilted. All the root
inoculated plants showed no noticeable symptoms of the
disease even after 4 months.

Leu (1976) had tried 4 methods of inoculation using a thick bacterial suspension on "Wu-Chi" the most widely cultivated variety. He observed that after the dipped cuttings sprouted to a height of 6 - 10 cm the leaves wilted with gum exudation and plantlets died in a few days. When plants were injected with bacterial suspension, gum exudation, indicative of infection, was noticed at the point of injection. Then the infection was seen to spread to the upper part of the stem and stipules. Intensity of infection was high and often reached cent per cent. After spraying, wilting and gum exudation of plants occurred in 10 to 20 days. About 10 per cent of inoculated plants left uncovered and 90 to 100 per cent of those kept covered with plastic bags died in a few days after inoculation. Water soaking was conspicuous when robust plants were sprayed and kept covered and not so when kept uncovered and even when young plants were kept covered after inoculation. Pouring bacterial suspension into the injured root caused wilting and death of plants 3 to 4 weeks after inoculation in summer. Leaf blades, cut with bacteria contaminated scissors, wilted and defoliated without gum exudation.

Nair (1977) tried 9 methods of inoculation and the plants showed wilting symptoms 4 to 7 days after inoculation. Of the 9 methods tested, the plants inoculated by smearing the lower surface of the leaves with injury developed wilting

symptoms first and wilted completely in 8 days. Next to wilt, were the plants inoculated with stem puncture, wilting completely in 10 days, followed by inoculation after nipping the growing bud, wilting in 12 days. Smear inoculation of the upper surface of leaves, lower surface of leaves and upper surface of leaves with injury, produced symptoms at a relatively slower rate with less severity, wilting completely in 15 to 20 days. Spray inoculation, set dipping and root inoculation methods were not successful and the plants did not produce any visible symptoms even after 2 months.

EPIDEMIOLOGY

Amaral (1945) suggested that the pathogen possibly spreads by the use of infected cuttings or through insects. Other workers (Carneiro, 1940; Drummond and Goncalves, 1939; 1943; Drummond and Hipolito, 1941; Lozano, 1972; Lozano and Sequeira, 1974 b) have suggested or demonstrated the role of infected cuttings in dissemination of the disease from season to season. Splashing rain and soil movement through cultural operations are also involved in dissemination over localised areas. That bacteria can penetrate the roots, was suggested by Amaral and Vasconcellos (1945), Pereira and Zagatto (1967) and Drummond and Hipolito (1941), but this is of minor importance because of the short survival of the pathogen in soil (Lozano, 1974).

The possible role of insects in the dissemination of bacteria was recently demonstrated at CIAT, in controlled experiments, and was shown to be as high as 100 per cent (Lozano, 1975). It was also revealed that insect transmission occurred only over short distances (Anon., 1972; Lozano, 1972; Lozano and Sequira, 1974 b).

Terry (1974) studied different factors affecting the incidence of cassava bacterial blight in Africa and reported that its severity varied with locality and climatic conditions. Factors that may affect its severity are soil type, cultural practices and varieties. Most serious outbreaks of the disease occur during the rainy seasons (Anon., 1973). Terry (1976) observed that there was a decrease in the angular leaf spots after the rains subsided in November. Maraite and Meyer (1975) also reported that the recent epidemics of wilt in Zaire could also be due to the conditions becoming particularly favourable. The severity varies greatly according to cultivar, climate and soil fertility, each of these factors being limiting.

Maraite and Meyer (1975) reported that, the repetition of the culture of a highly susceptible cultivar without rotation or fallow, leads to a decrease of the soil fertility which greatly increases the predisposition of cassava to wilt. Surveys conducted in Nigeria and Zaire (Ezhumah and Terry, 1974) suggested that CBB was more severe on cassava

planted on infertile soils. Glaser and Ogbogu (1974) also reported that the disease caused greater crop losses on sandy infertile soils and in fields under continuous cassava cultivation. They reported that there was significant difference in disease incidence between plants growing in water-logged soil at the bottom of the slope than in better drained soil further up the slope. Plants in water-logged soil were smaller and less vigorous, but there was less defoliation and death due to CBB.

The effect of soil type and the addition of NPK fertilizers on disease development was investigated experimentally by Terry (1976). The results suggested that the disease was more severe on plants grown in low nutrient soils. The indication that the addition of NPK fertilizers might decrease the number of plants killed by CBB had implication for disease control and efficient resistance screening and suggested that there was strong correlation between the vigour of the plant and resistance to CBB. Albuquerque (1963) had reported that the damage is really serious only when the crop is grown in poor leached soils.

The soil type and fertilizer levels were important factors affecting the severity of the disease although the nature of the effect was not known. Nutrients played an important part in the reduction in severity of many plant diseases but the mechanism of action was often obscure.

The effect of potassium and bacterial blight on starch yields of cassava cultivars was studied by Adenji and Obigbesan (1976) and Obigbesan and Matuluko (1976). The data collected by Adenji and Obigbesan (1976) revealed that only at 90 kg K_2O per hectare, there was no reduction in yield. The highest starch yields were produced at this K fertilizer level which suggested that the deliterious effect of CBB could be reduced.

During the dry season (November to March) dew deposits were an important source of leaf wetness and was probably a vital factor in producing some moisture for some bacteria to remain viable during the dry period (Rotem and Palti, 1969). Terry (1974) reported some epidemiological factors that may affect the survival and dissemination of the pathogen X. manihotis in pelletted bodies under natural field conditions. It was found that during periods of heavy early morning dew deposits, water droplets form around the bacterial exudation on the leaf surfaces. As solar radiation increases, these droplets evaporate leaving pelletted bodies containing up to 1.5×10^5 viable cells. X. manihotis cells from pellets remained viable for 14 months after they had been collected from infected plants (Anon., 1974).

Leu (1976) studied the ability of the causal bacterium in soil to induce the disease. The bacterial suspension was

poured and mixed with soil and infected plants were chopped
and mixed with soil. He showed that either 3 out of 9 plants
in the former and 2 out of 12 plants in the latter wilted
and died soon after cuttings were planted in the flat.
However, if cuttings were planted 1 or 2 weeks later, no
symptoms appeared. The incidence of the disease dropped
from 28.5 - 35 per cent when planting occurred immediately
after ploughing to zero per cent when the interval was 90 days
(Anon., 1973). Bondar (1915) reported that the bacterium
is found in cuttings stored for long periods. Lozano (1974)
suggested that because of the short survival of the pathogen
in soil, the possibility of the pathogen penetrating the
roots is rather limited.

CONTROL

The existence of varietal resistance to this disease
has been noted. Control by the use of cultivars resistant
to the bacteria was first suggested by Drummond and
Goncalves (1948), and numerous field resistant cultivars
has since then been reported (Carneiro, 1940; Drummond, 1946;
Drummond and Goncalves, 1939; 1948; 1953; Pereira and
Zagatto, 1967; Lozano and Sequeira, 1974 b; Agbo, 1974).
The studies conducted by Lozano and Sequeira (1974 b) also
revealed that three possible types of resistance exist in
different cultivars: one type apparently limits penetration,

another limits systemic invasion and the third type is
apparently based on hypersensitive response of the host.

For screening resistant varieties of the host,
cuttings were dipped in bacterial suspension and planted
(Leu and Chen, 1972; Leu, 1976). Lozano and Sequeira (1974 b)
tested 1,293 Colombian cultivars for resistance to infection
by artificial inoculation. The amount of leaf spotting
alone appeared to be as good an index of resistance as
all other characteristics combined. The use of resistant
cultivars remains the most promising method of control of
the disease in the tropics (Lozano, 1972; Lozano and Sequeira,
1974 b). Hahn et al. (1974) reported breeding for
resistance to CBB in Nigeria. The International Institute
of Tropical Agriculture has been involved in the research
for resistance to cassava bacterial blight in Africa.
Based on the results of these trials, it seems possible to
produce high yielding varieties with a high level of
resistance to cassava bacterial blight.

Maraite and Meyer (1975) reported in Zaire that,
besides measures to preserve or restore soil fertility and
the planting of healthy cuttings, selection of resistant
cultivars is the most effective method of control. The
introduction of an assortment of resistant cultivars is
advisable in order to reduce the danger of the appearance
of new bacterial strains infecting one particular resistant

cultivar. A combination of the use of resistant varieties and the use of bacteria free planting material appears to be the most promising means of controlling this important disease (Bondar, 1939; Castro et al., 1939; Drummond and Goncalves, 1939; 1948; Freire, 1963; Lozano, 1973 b; 1974; Lozano and Sequeira, 1974 b; Terry, 1976; 1977).

Nodu (1974) suggested some chemical methods of controlling CBB in Nigeria. He suggested spraying of foliage with dodine and Bordeaux mixture, use of paints to heal cut surfaces of cuttings, use of systemic fungicides such as benomyl and thiabendazole, application of methyl bromide and chloropicrin to the soil, use of streptomycin to prevent the dissemination of infection and control of insect vectors.

Arene (1974) reported preliminary evaluation of some fungicides for the control of the disease in Nigeria. Three fungicides (Dithane M-45, Bordeaux mixture - 5:5:50 and copper ammonium carbonate) at six concentrations of 0, 10, 100, 1000, 3000 and 6000 ppm were analysed to determine their effect in the control of CBB. Bordeaux mixture had no effect on the organism, in vitro, at any of the concentrations used. Dithane M-45 showed some bactericidal property as low as 10 ppm. Copper ammonium carbonate demonstrated bactericidal effect in vitro.

Materials and Methods

MATERIALS AND METHODS

ISOLATION AND PATHOGENICITY OF THE BACTERIUM

Healthy stems of the tapioca variety H-165, reported
to be susceptible, (Nair, 1977) were collected from the
Instructional Farm, College of Agriculture, Vellayani,
Trivandrum. This variety was solely used in this study
unless otherwise stated. The plants were raised in pots of
size 30 x 36 cm. Potting mixture consisting of garden soil
(red soil) and sand in the ratio 2:1 was used for all
purposes.

Naturally infected plants of the variety H-165 were
collected from the Instructional Farm, College of Agriculture,
Vellayani. The pathogen was isolated using methods similar
to that of Lozano and Sequeira (1974 a) and Maraite and
Meyer (1975). The diseased material with profuse ooze was
selected, surface sterilised in 0.1 per cent mercuric
chloride and in rectified spirit. The material was then
transferred to 10 ml sterile distilled water blanks in
tubes to get a bacterial suspension. The bacterium was
then isolated from the suspension by streaking over Potato
Sucrose Peptone Agar (PSPA) medium.

Composition of the medium

Potato   300 g

Peptone   2 g

Sucrose	20 g
KH_2PO_4	0.2 g
Na_2HPO_4	0.5 g
$Ca(NO_3)_2$	0.5 g
$FeSO_4$	0.05 g
KCl	0.05 g
Agar agar	20 g
Distilled water	1000 ml
pH	7.0

The inoculated plates were incubated at 30°C for 48 hours. Characteristic single colonies were selected on the basis of their colour, fluidity and slime. The culture was subjected to two cycles of purification using the same medium. Stock cultures were maintained in Yeast Glucose Chalk Agar (YGCA), and as suspension in sterile distilled water blanks stored at 4°C. These were subcultured at monthly intervals. Pathogenicity of the isolate was tested by placing a piece of cotton dipped in a thick suspension of a 24 hour old culture of the bacterium at the tip of the shoot after nipping the terminal bud (Bud nipping method) and keeping the plants under shade.

Growth and cultural characters of the bacterium

The growth of the bacterium was studied on eight solid culture media:-

1. Potato Sucrose Peptone Agar   (PSPA)

2. Nutrient Agar   (NA)

3. Basal medium for Xanthomonads   (BX)

4. Tetrazolium Chloride medium   (TZC) (without tetrazolium chloride)

5. Potato Dextrose Agar   (PDA)

6. Yeast Glucose Chalk Agar   (YGCA)

7. Glucose Agar   (GA)

8. Glucose Yeast Extract Agar   (GYEA)

Composition of the media

1. Potato Sucrose Peptone Agar

2. **Nutrient Agar**

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

3. **Basal medium for Xanthomonads**

$NH_4H_2PO_4$	0.5 g
K_2HPO_4	0.5 g
$MgSO_4$	0.2 g
NaCl	5.0 g
Yeast Extract	1.0 g
Agar agar	20.0 g
Distilled water	1000 ml
pH	6.8

4. <u>Tetrazolium Chloride Agar</u>

Peptone	10.0 g
Casamino acid	1.0 g
Glucose	5.0 g
Agar agar	20.0 g
Distilled water	1000 ml
pH	6.8

5. <u>Potato Dextrose Agar</u>

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

6. <u>Yeast Glucose Chalk Agar</u>

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

7. <u>Glucose Agar</u>

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

8. **Glucose Yeast Extract Agar**

Yeast extract	5.0 g
Peptone	5.0 g
Glucose	10.0 g
Agar agar	20.0 g
Distilled water	1000 ml
pH	6.8

A loopful of a dilute suspension of the bacterium was streaked over the medium in the plates. The plates were incubated at 30°C. Three plates were maintained for each medium. Daily observations were recorded. The colony morphology of the bacterium was recorded along with the observations on the growth over different media. The gram reaction of the bacterium was studied. Twenty four and 48 hour old cultures were stained and observed under the oil immersion objective.

For studying the growth of the bacterium in liquid media, broths of the above media, except Yeast Glucose Chalk Broth were used. Forty ml of the broth was prepared in 100 ml Pyrex conical flasks. One ml of the bacterial suspension from a 24 hr old culture was transferred into each flask containing the broth, using sterile mocro-pippette. Uninoculated controls were also maintained. The inoculated media were shaken for 6 hours daily. The optical density of the broth culture was measured at 24 hr intervals for

3 successive days and the observations were recorded. The observations were recorded using Spectronic 20 (photo-electric calorimeter - Bosch & Lamb.) using the uninoculated broths as blank.

PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF THE BACTERIAL ISOLATE

The physiological and biochemical properties of the bacterial isolate were studied according to the methods recommended in the Manual of Microbiological Methods published by the Society of American Bacteriologists (Anon., 1957) and the methods prescribed by Dye (1962). All the tests were made in triplicate and the observations were recorded in comparison with controls.

Temperature

For assessing the optimum temperature of growth of the bacterium, a dilute suspension was streaked over PSPA medium and incubated at 25, 30 and 35°C.

Starch hydrolysis

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

Composition of the medium

Peptone   10.0 g

Beef extract   5.0 g

Starch (soluble)	2.0 g
Agar agar	20.0 g
Distilled water	1000 ml

The bacterium was spot inoculated on the media in plates. After 4 days incubation, hydrolysis was tested by pouring Lugol's Iodine over the plate. A colourless brown zone around the bacterial growth indicated positive starch hydrolysis, compared to the blue background of the medium.

Hydrogen sulphide production

The production of hydrogen sulphide was tested using the peptone water medium.

Composition of the medium

Peptone	10.0 g
NaCl	5.0 g
Distilled water	1000 ml
pH	6.8

The medium was autoclaved in 5 ml quantities in pyrex test tubes. Lead acetate paper strips of size 5 x 50 mm were prepared by soaking them in super saturated solution of lead acetate, autoclaving and drying. The tubes were inoculated and the lead acetate strips were inserted aseptically by the side of the plug in the tube. The tubes were incubated at 30°C and observations recorded for 14 days. Blackening of the lead acetate strip was indicative of positive hydrogen sulphide production.

Nitrate reduction test

The nitrate broth was used to test the ability of the bacterium to reduce nitrate.

Composition of nitrate broth

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

The medium prepared in tubes were inoculated, incubated and tested for the reduction of nitrate at regular intervals for 15 days. The test was performed by adding a few drops of sulphanilic acid (0.8 per cent in 5 molar acetic acid) and dimethyl alpha naphthyl amine (0.5 per cent in 5 M acetic acid), to the nitrate broth culture. Few zinc crystalls were added to ensure whether the negative reaction was due to the reduction of nitrate beyond the nitrite level.

Catalase test

The Catalase activity of the bacterium was assessed. A loopful of the 24 hour old culture of the bacterium was smeared on a glass slide. The smear was covered with a drop of 20 vol: hydrogen peroxide. Active bubbling indicated catalase production.

Kovac's Oxidase test

Kovac's oxidase test was performed on the 48 hour old culture of the bacterium. A loopful of the culture was

streaked on a filter paper saturated with 1 per cent tetra-
methyl - para phenylene - diamine - dihydrochloride. The
appearance of a red or purple colour in 10 seconds indicated
positive reaction and in 10 to 60 seconds indicated a delayed
positive reaction.

Utilization of carbon sources

The following 10 carbon compounds were tested
individually for utilization by the baoterium as indicated
by acid production (Dye, 1962):

Xylose, Fructose, Glucose, Sucrose, Lactose, Inulin,

Salicin, Adonitol, Dulcitol and Inositol.

The production of acid was observed by using agar
slants of the basal medium for Xanthomonads (Dye, 1962). The
carbon compound to be tested was added to the medium at
1 per cent concentration and 0.7 ml of 5 per cent alcoholic
solution of bromocresol purple to get a reddish violet colour.
The medium was sterilised by tyndallisation and the slants
were inoculated and incubated at 30°C. Periodic observations
were recorded upto 28 days. The change in colour from
reddish violet to yellow indicated the production of acid.

STANDARDIZATION OF INOCULATION TECHNIQUES

Eleven different methods of artificial inoculation
were tried on 2 month old tapioca plants to standardize a
method.

1. (T_1) Inoculation after nipping the growing bud (bud nipping method)

2. (T_2) Inoculation after puncturing terminal 3 leaf axils.

3. (T_3) Inoculation after both nipping the growing bud and puncturing terminal 3 leaf axils

4. (T_4) Inoculation after puncturing lowermost 3 leaf axils

5. (T_5) Spray inoculation on top 3 leaves with injury

6. (T_6) Spray inoculation on top 3 leaves without injury

7. (T_7) Smear inoculation on the under surface of top 3 leaves with injury

8. (T_8) Smear inoculation on the under surface of top 3 leaves without injury

9. (T_9) Injection below the apical meristem

10. (T_{10}) Inoculation on the stem after scooping off a piece of the stem at the base

11. (T_{11}) Root inoculation after giving injury to the roots with a knife.

The experiment was laid out in a completely randomised design with 10 plants (replications) per treatment. Inoculation was done with a suspension of 24 hour old culture of the bacterium. The same suspension was used for inoculating the plants in all the treatments.

The disease was assessed using the criteria detailed below:

1. Percentage of leaves wilted

2. A disease scale with severity rating from 0 - 5 devised by Terry (1976).

    0 - No infection

    1 - Gum exudation

    2 - Few leaves wilted

    3 - Complete wilt and defoliation

    4 - Defoliation and die back

    5 - Complete death of the shoot

1. <u>Inoculation after nipping off the growing bud</u>

The growing terminal bud was nipped off and a piece of absorbant cotton dipped in the inoculum was placed over the injury.

2. <u>Inoculation after puncturing the terminal 3 leaf axils</u>

The axils of the fully opened 3 leaves at the top were punctured with entomological pins and cotton soaked in the inoculum was placed over the injury.

3. <u>Inoculation after both nipping the growing bud and puncturing terminal 3 leaf axils</u>

Cotton dipped in the inoculum was placed over the injured terminal bud and leaf axils.

4. <u>Inoculation after puncturing the lowermost 3 leaf axils</u>

Cotton dipped in the inoculum was placed over the injured leaf axils.

5. **Spray inoculation on top 3 leaves with injury**

The top 3 fully opened leaves were injured with entomological pins and the inoculum was sprayed on the leaves using an atomiser.

6. **Spray inoculation on top 3 leaves without injury**

The inoculum was sprayed on top 3 fully opened leaves using an atomiser.

7. **Smear inoculation on the under surface of the top 3 leaves with injury**

The lower surface of top 3 fully opened leaves were given injury with entomological pins. Cotton soaked in the inoculum was smeared on this surface.

8. **Smear inoculation on the under surface of the top 3 leaves without injury**

Cotton soaked in the inoculum was smeared on the lower surface of the top 3 fully opened leaves.

9. **Injection below the apical meristem**

The inoculum was injected into the axils of the top 3 fully opened leaves using a hypodermic syringe.

10. **Inoculation on the stem after scooping off a piece of the stem at the base**

A piece of the stem (about 2 cm long and 1 cm wide) of the originally planted set was scooped out from the side of the stem, near the middle, by means of a knife and a small piece of cotton dipped in the inoculum was placed at that region. The piece of stem was replaced and tied by means of a twine thread.

11. **Root inoculation after giving injury to the roots with a knife**

The roots of the growing plants were injured by piercing a knife into the soil in 3 places around and 1 inch away from the stem. A 50 ml aliquot of the bacterial suspension was used for inoculation.

The plants were inoculated during the month of June and placed in the open.

HOST RANGE OF THE PATHOGEN

Plants having similar taxonomic affinity were inoculated to study the host range of the bacterium. The following plants of the family Euphorbiaceae were inoculated.

1. *Euphorbia geniculata*, Ort

2. *E. hirta*, L.

3. *Phyllanthus neruri*, L.

4. *P. urinaria*, L.

5. *P. rotundifolia*, Klein

6. *Sebastiana chamaelea*, Muell.

7. *Ricinus communis*, L.

8. *Jatropha glandulifolia*, Roxb.

9. *J. curcas*, L.

10. *Hevea brasiliensis*, M. Arg.

These plants were collected when they sprouted with the onset of the monsoon and planted in pots. The plants were inoculated by the bud nipping method when they had

established and put forth new shoots.

SURVIVAL OF THE PATHOGEN

Detailed studies were conducted on the survival of the pathogen.

An attempt was made to establish the survival of the pathogen in infected planting material. Plants with mature stems were inoculated. When the wilting was complete the stems were collected and stored as usual planting material. Periodic isolations were made from these stems to observe how long the pathogen survived in the infected planting material.

Survival of the pathogen in infected plant debris and crop refuse in soil was also assessed. The diseased material was chopped into small pieces. Pots were filled three fourths with garden soil. A one inch layer of the material was spread over the soil in the pots, which was again covered with a one inch layer of the soil. Healthy tapioca sets were planted in these pots at an interval of 1 day for a period of 7 days; at 2 days interval for 8 days and at weekly interval twice. Ten disease free sets were planted each day. The disease incidence was recorded up to a period of 4 months from the first day of planting.

The survival of the pathogen in soil inoculated with the culture of the bacterium was studied. A 48 hour old

shake culture of the bacterium was made in Potato Sucrose
Peptone broth. Hundred ml aliquots were mixed with the top
one inch layer of the soil in pots. The pots were kept in
the open field under natural conditions. Planting was done in
these pots and periodic observations taken as in the previous
experiment.

HOST NUTRITION IN DISEASE DEVELOPMENT

An experiment was laid out to study the effect of host
nutrition in disease development. Pots were filled with 15 kg
of potting mixture. Three levels of nitrogen were given in
conjunction with lime. P_2O_5 and K_2O were given as recommended
in the Package of Practices Recommendations   of
the Kerala Agricultural University. Nutrients were supplied
at the rates calculated for 15 kg of soil on the basis that
the top 6 inch layer of soil per hectare weighs 2,250,000 kg.

Treatments

$N_{50} \times L_0$ -   (50 kg nitrogen without lime)

$N_{50} \times L_{1000}$ -   (50 kg nitrogen with 1000 kg lime)

$N_{100} \times L_0$ -   (100 kg nitrogen without lime)

$N_{100} \times L_{1000}$ -·   (100 kg nitrogen with 1000 kg lime)

$N_{150} \times L_0$ -   (150 kg nitrogen without lime)

$N_{150} \times L_{1000}$ -   (150 kg nitrogen with 1000 kg lime)

Lime was applied one week before planting and the
fertilizers were applied one month after planting. Five

months after planting, the plants were inoculated by the bud nipping method.

Observations were recorded as:

1. Percentage of leaves wilted

2. Disease index

The experiment was laid out in completely randomised design with 20 plants (replications) per treatment.

AGE OF THE PLANT ON DISEASE DEVELOPMENT

In another experiment the influence of stage of growth of the plant on disease incidence and development was studied. Sets were planted in pots and inoculations were done at 1 month stage to 9 month stage at monthly intervals.

Treatments

1. (T_1) 1 month old plants

2. (T_2) 2 month old plants

3. (T_3) 3 month old plants

4. (T_4) 4 month old plants

5. (T_5) 5 month old plants

6. (T_6) 6 month old plants

7. (T_7) 7 month old plants

8. (T_8) 8 month old plants

9. (T_9) 9 month old plants

The experiment was laid out in completely randomised design with 15 plants (replications) per treatment.

Observations were recorded as:

1. Percentage of leaves wilted

2. Disease index

Along with this experiment an attempt was also made to see if there is any correlation between the hydrocyanic acid content of the plant at the time of inoculation and susceptibility to the disease. For this the HCN content from the plant was analysed at 3 positions of the plant.

1. First fully opened leaf from the upper position of the plant

2. One leaf at random from the middle position of the plant

3. One leaf at random from the lower position of the plant.

The HCN content was analysed immediately before inoculation. The method followed was that of Indira and Sinha (1969). For the estimation of HCN 5 plants were chosen at random from each of the 9 treatments.

SENSITIVITY OF THE BACTERIUM TO ANTIBIOTICS

The _in vitro_ sensitivity of the bacterium to different antibiotics were tested. The following chemicals were used for the purpose.

1. Agrimycin 100   : Pfizer

                       (Streptomycin 15% and Terramycin 1.5%)

2. Ampicillin   : The Pharmaceutical and Chemical

                       Industries (Ampicillin Sodium)

                       "Synthocillin"

3. Streptomycin   : Merck Sharp and Dhome of India

                       Ltd. (Streptomycin Sulphate I.P)

                       "Merstrep".

4. Terramycin   : Pfizer (Oxytetracyclin)

5. Streptocycline   : Hindustan Antibiotics

6. Chloramphenicol   : Boots

                       "Chloromycetin"

The antibiotics were made at concentrations of 100, 250 and 500 ppm. Sterile filter paper discs of 10 mm diameter were dipped in the solutions and placed over PSPA medium seeded with 24 hour old bacterium. The test was conducted with 4 replications. Daily observations of the zone of inhibition, were recorded.

TOXIN STUDIES

An attempt was made to isolate a phytotoxin from the culture filtrate of the bacterium. The method followed was partly that of Alouf et al. (1970), Ludritz et al. (1971) and Patil et al. (1972).

The bacterium was grown in broth, containing potato, peptone and sucrose.

Potato	-	200 g
Peptone	-	5 g
Sucrose	-	20 g
Distilled water	-	1000 ml
pH	-	7.0

The broth was inoculated with a thick suspension of 24 hour old bacterium and maintained as still cultures for 15 days. After 15 days the purity of the broth culture was ensured by streaking a loopful of the culture over PSPA medium. The cell free extract of the culture was prepared by centrifuging at 6000 rpm for 30 minutes and filtering the supernatent through a cintered glass filter.

The cell free extract was inoculated on 1 month old tapioca plants by injection to the axils of the top 3 fully opened leaves. Blank media and distilled water were also injected in the same way, as controls. One month old plants were uprooted from pots without damaging the roots. These plants were kept dipped in the cell free extract in 250 ml beakers.

The cell free extract was tested for precipitation by adding ethanol, methanol and acetone. The extract was mixed with 3 times the precipitant. The precipitate was allowed

to settle, decanted into a clean petridish and dried in a
vacuum desiccator. The precipitate was dissolved in distilled
water to get a concentration of 100 ppm. This solution was
injected into the axils of the top 3 leaves.

STATISTICAL ANALYSIS

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

Results

RESULTS

Isolation and pathogenicity

Isolation of the pathogen on Potato Sucrose Peptone
Agar yielded white, circular and slimy colonies. The
artificially inoculated plants showed typical wilt symptoms
in 4 to 6 days after inoculation. Complete wilting of 90
per cent of the plants occurred in 8 to 10 days.

Symptomatology

The symptomatology of the disease was closely observed
during the study. The initial symptoms of the above ground
portions of the plants started as loss of turgidity of a
few tender leaves. Soon all the leaves wilted and shrivelled.
In very young plants the whole shoot died within two weeks,
whereas in mature plants only the green tips showed die back.
In such cases, after a short period, profuse axillary shoot
growth was observed. When these plants were retained for
some time, such shoots also wilted. In a few plants, only
gum exudations were seen on the stem. Initially cracks
appeared on the stem with the exudation of whitish gum
(Plate I). In monsoon seasons the gum turned spongy with
the absorption of moisture. Brownish black discolourations
of the stem were observed around the gum exuded cracks. When
pressure was applied to these discoloured areas it felt soft
to touch and further exuded gum. After some time one or two
leaves around the discoloured area defoliated.

Plate I. Gum exudation due to
          cassava bacterial blight

Plate II.   Vascular discolouration
            on infected stems of
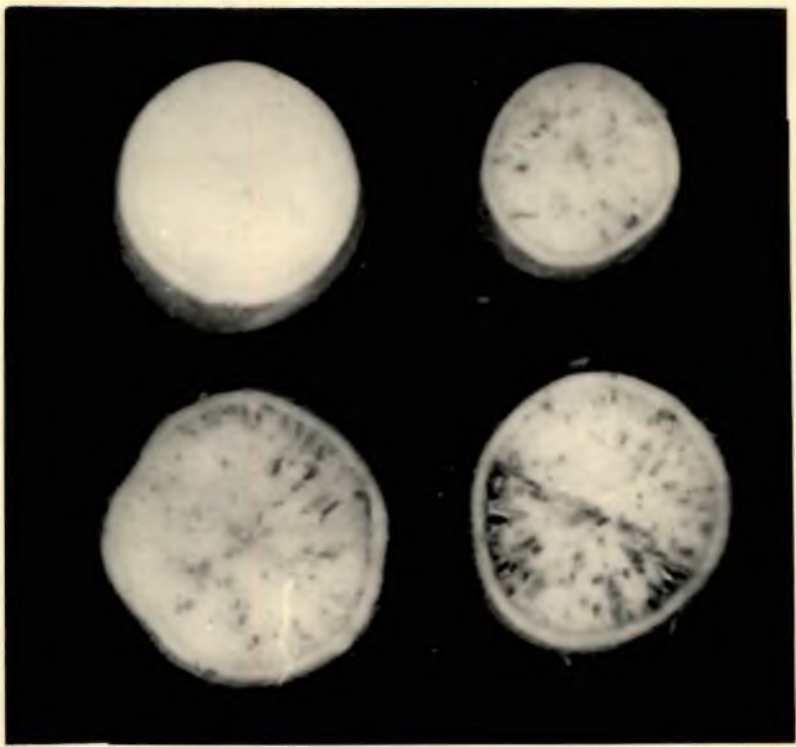   tapioca

Plate III.   Discolouration in
            infected stems

Plate IV.   Typical colonies
           of the pathogen on
           PSPA medium

Characteristic discolourations were also observed in the severely infected stem. When the severely infected stem was debarked the vascular system could be seen as brown or black strands running throughout the length of the stem (Plate II).

In the underground portions of the plant, the stem as well as the roots were found discoloured in a ring like pattern just below the rind. Damage was also observed in the tubers. Early infection showed brownish black ring like discolouration in the tubers (Plate III). In advanced infections the tubers were seen decayed, emitting a foul smell.

The morphology and growth of the bacterium

The bacterium gave rise to white, circular to oblong, raised colonies in PSPA medium with entire margin (Plate IV). The colonies were wet and slimy. The amount of growth, slime and fluidity were more on Yeast Glucose Chalk Agar. The colony characteristics were similar in Tetrazolium Chloride negative medium, Potato Dextrose Agar and Nutrient Agar. The amount of growth was comparatively less in these than in Yeast Glucose Chalk Agar and Potato Sucrose Peptone Agar. The 24 hr old colonies on Yeast Glucose Chalk Agar were hyaline and became more or less white in about 3 days. Similar appearance was also observed in Tetrazolium Chloride negative medium.

The 24 hr old bacterium was observed as a gram negative short rod with round ends. The cells appeared singly or in short chains of 3 to 4 cells. There was no change in the gram reaction at 48 hours.

The growth of the bacterium was tested in eight solid media and the results are presented in Table 1. Of the eight media tested the maximum growth was observed on Yeast Glucose Chalk Agar as evidenced by the diameter of the individual colonies. After 24 hours there were hyaline colonies of 3 mm diameter. By 48 hours the colonies were creamy white with a precipitated appearance. The diameter increased to 6 mm at 48 hours, 7.3 mm at 72 hours and 9 mm at 96 hours. By the fifth day a non-diffusible brown pigmentation was observed in the growth. Subsequently the colonies started to loose their slime and dry up.

Potato Suorose Peptone Agar and Tetrazolium Chloride Negative Agar medium supported good growth. The size of the colonies on Potato Sucrose Peptone Agar was larger than that in Tetrazolium Chloride negative medium. The growth in the latter had a precipitated appearance similar to that in Yeast Glucose Chalk Agar. The single colonies grew to 5.5 mm by 96 hours in Potato Sucrose Peptone Agar and 4.6 mm in Tetrazolium Chloride negative medium. The growth in PDA and NA were not so abundant as in the rest of the media, with less slime and less fluidity. The diameter of the colonies reached 3.5 mm in PDA after 96 hours and 2.5 mm in NA.

Table 1. Growth characters of Xanthomonas manihotis on different solid media

Medium	Nature of colony and colour	Growth, slime and fluidity	Diameter in mm			
			24	48	72	96
				hours		
PSPA	White, circular to oblong, raised colonies with entire margin	Gr + + + Sl + + + Fl + + +	-	2.7	4.5	5.5
NA	Bright white, circular raised colonies with entire margin	Gr + + Sl + Fl +	-	-	1.5	2.5
TZC	Dull white, circular to oblong to irregular, raised colonies	Gr + + + Sl + + Fl + +	-	1.6	3.2	4.6
PDA	Dull white, circular to oblong, raised colonies	Gr + + Sl + + Fl +	-	-	1.8	3.5
YGCA	Creamy white, oblong to irregular colonies initially hyaline and becoming white	Gr + + + + Sl + + + + Fl + + + +	3.0	6.0	7.3	9.0
GA	No growth	- - - - - -	-	-	-	-
GYEA	No growth	- - - - - -	-	-	-	-
BX	Very small initials	Gr + Sl - Fl -	-	-	-	-

+ + + +	Excellent	Gr	Growth
+ + +	Good	Sl	Slime
+ +	Moderate	Fl	Fluidity
+	Slight		
-	Nil		

In Glucose Agar and Glucose Yeast Extract Agar media there was absolutely no growth even after 96 hours. In the basal medium for Xanthomonads very minute initials could be observed after 48 hours. Even after 96 hours there was not much progress in growth.

Growth in liquid media

The bacterium was grown in seven broths. The growth was measured as the change in the Optical Density of the medium in comparison with the uninoculated medium. The results are presented in Table 2 and Figure 1.

Table 2. Growth of Xanthomonas manihotis in different liquid media

Media	Optical Density		
	24 hrs	48 hrs	72 hrs
PSPA	0.721	0.824	1.222
NA	0.796	0.921	1.097
TZC	0.041	0.745	0.921
PDA	0.081	0.168	0.229
GA	0.046	0.036	0.013
GYEA	0.036	0.268	0.319
BX	0.456	0.560	0.629

Observations after 24 hours showed that there was significant growth in Potato Sucrose Peptone Broth, Nutrient Broth and the Broth of the Basal medium for Xanthomonads, while the growth in the other four media were negligible.

After 48 hours the maximum growth was seen in Nutrient Broth
followed by Potato Sucrose Peptone Broth, Tetrazolium Chloride
Broth, Broth of the Basal medium for Xanthomonads and Glucose
Yeast Extract Broth. After 72 hours maximum growth was seen
in Potato Sucrose Peptone Broth followed by Nutrient Broth,
Tetrazolium Chloride Broth, Broth of the Basal medium for
Xanthomonads and Glucose Yeast Extract Broth. The growth in
Glucose Broth was found to be extremely poor.

Effect of temperature on growth

Of the three incubation temperatures tested 30°C was
found to be the best for obtaining maximum growth of the
bacterium as evidenced by visual observation of the size of
the colonies.

Physiological and biochemical properties of the bacterium

The bacterium hydrolysed starch feebly, produced
hydrogen sulphide and gave negative nitrate reduction test.
It was found to be catalase positive and oxidase negative.

Utilization of carbohydrates

Of the 10 carbon compounds tested, the bacterium
produced acid in xylose, fructose, glucose, sucrose and
lactose as indicated by the change of the medium from reddish
violet to yellow. In xylose and fructose acid production was
noticeable only from the 4th day of inoculation, while in the
latter three it was evident from the second day. There was
no change indicating utilization in adonitol, duloitol,

inulin and salicin. Acid production, indicative of utili-
zation was noticed in inositol.

Standardization of artificial inoculation techniques

Evaluation by the percentage of wilted leaves showed
that inoculation after nipping the terminal bud (T_1),
puncturing the lower most 3 leaf axils (T_4) and nipping the
bud and puncturing the upper most 3 leaf axils (T_3) were on
par and significantly better than the other techniques.
Further T_3 was on par with inoculation after puncturing the
upper most 3 leaf axils (T_2), the latter being on par with
injection below the apical meristem (T_9). There was no
significant difference between the other treatments.

Evaluation by the disease severity scale (Disease
Index) yielded more or less similar results (Table 3 and
Fig.2). T_1 and T_3 were on par and significantly better than
the other treatments. This was followed by T_4, T_2, T_9, T_7,
T_5, T_6, T_8, T_{10}, and T_{11} in the discending order.

Host range of the pathogen

None of the 10 plant species inoculated belonging
to the family Euphorbiaceae, showed any visible symptoms
even after one month. Parts of the plant near the point of
inoculation were subjected to ooze test. All these tests
gave negative results, indicating that the pathogen is
confined to the genus Manihot.

Table 3. Standardization of different methods of inoculation
of _Xanthomonas manihotis_ on tapioca

Treatments	Percentage of leaves wilted (in angles)	Disease index from 0 - 5
1	78.19	4.2
2	39.39	2.0
3	69.68	3.4
4	72.00	2.9
5	16.25	0.4
6	0.00	0.0
7	24.34	1.0
8	18.00	0.5
9	37.45	1.6
10	18.00	0.9
11	18.00	0.5

C.D. (0.01) percentage of leaves wilted $-$ 31.653

C.D. (0.01) disease index $-$ 0.937

1. $\overline{T_1 \quad T_4} \quad \overline{T_3 \quad T_2 \quad T_9} \quad \overline{T_5 \quad T_6 \quad T_7 \quad T_8 \quad T_{10} \quad T_{11}}$

2. $\overline{T_1 \quad T_3 \quad T_4} \quad \overline{T_2 \quad T_9 \quad T_7} \quad \overline{T_5 \quad T_6 \quad T_8 \quad T_{10} \quad T_{11}}$

Survival of pathogen

The survival of the pathogen in infected stem was assessed. It was observed that within one month after cutting and storing, the infected stems began to dry up fast from both ends. The pathogen could be isolated from the stems upto 3 weeks of storage.

Assessment of the survival of the pathogen in infected plant debris and crop refuse gave the following results. The maximum period of survival was noted in the 21st day's planting, where, one out of the 10 sets planted wilted 36 days after planting, i.e. 57 days after the incorporation of the debris in soil. The other records of wilt were in the 15th day planting and the 6th day planting where one plant each wilted out of the ten planted. The former wilted 22 days after planting and the latter wilted 28 days after planting. In these instances wilt was observed 37 days and 34 days after the incorporation of the plant debris and crop refuse in soil.

In the experiment conducted by adding the 48 hour old Potato Sucrose Peptone Broth culture in soil, it was noticed that the pathogen survived for a longer period. The maximum period of survival was observed in the planting on the eleventh day wherein one plant wilted 104 days after planting i.e. 115 days after incorporation of the culture in soil. The sets planted on the second day wilted 23 days later. This was found to be the shortest period taken for

the development of the disease under the conditions of this experiment. The results of this experiment are presented in Table 4.

Table 4. Survival of <u>Xanthomonas manihotis</u> in soil when 48 hour old culture was inoculated in the soil

Day of planting after the culture was inoculated in soil	Number of plants wilted out of ten	Interval between planting and symptom expression	Interval between the inoculation of culture and symptom expression
2n day	2	23, 25	25, 27
3rd day	1	26	29
4th day	2	23, 30	32, 34
5th day	2	20, 27	25, 32
6th day	1	29	35
7th day	1	34	41
11th day	2	31, 104	42, 115

<u>Effect of host nutrition on disease development</u>

Three levels of Nitrogen (50, 100 & 150 kg per ha) were tested for its effect on the development of the disease in conjunction with lime (L_0 & L_{1000}). The results are presented in Table 5 (percentage of wilted leaves) and Table 6 (disease index). It can be observed from the tables that in both the methods of evaluation, the maximum disease was recorded at 100 kg nitrogen in the presence of lime. However it was observed that the varying levels of nitrogen
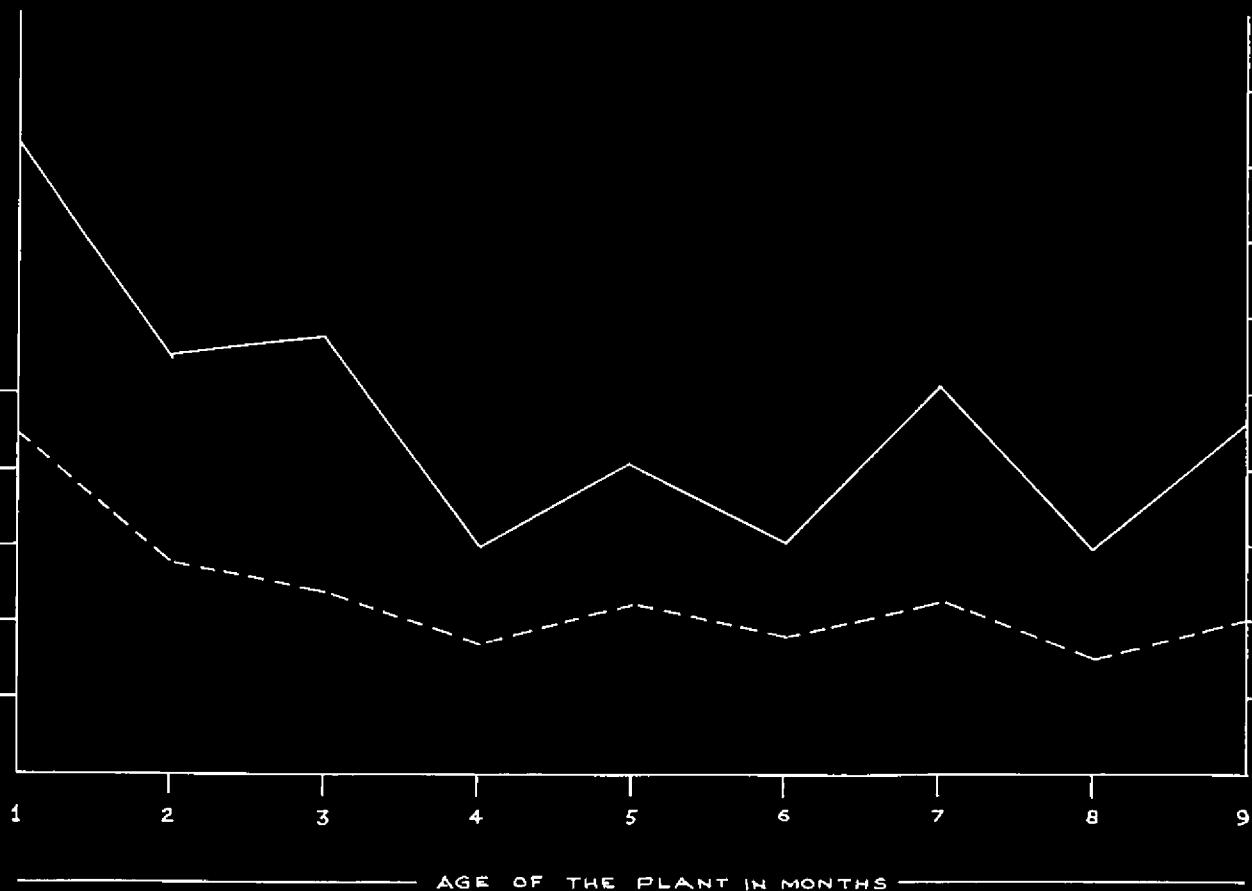
FIG. 3.   AGE OF TAPIOCA IN RELATION TO BACTERIAL BLIGHT INCIDENCE

AGE OF THE PLANT IN MONTHS

Table 5. Influence of nutrition on disease development of tapioca blight (wilt) incited by Xanthomonas manihotis (Evaluation by percentage of wilt)

Treatment	Nitrogen kg/ha			Mean
	N_{50}	N_{100}	N_{150}	
Lime kg/ha				
L_0	24.263	31.932	30.037	28.761
L_{1000}	28.281	45.993	19.020	31.151
Mean	26.272	38.963	24.645	

Table 6. Influence of nutrition on disease development of tapioca blight (wilt) incited by Xanthomonas manihotis (Evaluation by disease index)

Treatment	Nitrogen kg/ha			Mean
	N_{50}	N_{100}	N_{150}	
Lime kg/ha				
L_0	1.20	1.90	1.65	2.375
L_{1000}	1.70	2.30	1.20	2.600
Mean	1.45	2.10	1.425	

and lime had no significant effect on the levels tested.

Influence of age of the plant on disease development

The influence of age of the plant on disease incidence and development was tested by inoculating 1 month old to 9 month old tapioca plants. The results are presented in Table 7 and Fig.3.

In both the methods of evaluation it was observed that as the age of the plant increased the disease development decreased. One month old plants (T_1) were observed to have significantly higher infection according to both the methods of evaluation. The 2 month (T_2), 3 month (T_3), 5 month (T_5), 7 month (T_7) and 9 month old plants (T_9) were on par showing significantly lower disease incidence and development than 1 month old plants and higher disease incidence and development than 4 month (T_4), 6 month (T_6) and 8 month old plants (T_8), according to the evaluation by the percentage of wilted leaves, and these two groups were on par.

In the evaluation using the disease index, 2 months, 3 months, 5 months, 7 months and 9 months old plants were on par and differed significantly from 1 month old plants showing lesser infection, and from 4 month, 6 month and 8 month old plants showing higher infection. Further the 4 months, 5 months, 6 months, 7 months, 8 months and 9 months old plants formed a group without significant difference between them but showing significantly lesser infection than the 1 month, 2 months and 3 months old plants.

Table 7. Influence of growth stages of tapioca plants on Bacterial Blight incidence and development

Treatment	Percentage of leaves wilted (in angles)	Disease index
1	82.62	4.53
2	55.19	2.87
3	57.23	2.47
4	29.95	1.73
5	41.36	2.27
6	30.19	1.80
7	51.36	2.33
8	29.75	1.53
9	45.74	2.07

1. C.D.(0.01) percentage of leaves wilted   -   21.85

2. C.D.(0.01) disease index   -   0.816

1. T_1 $\overline{T_2\ T_3\ T_7\ \overline{T_9\ T_5\ T_6\ T_4\ T_8}}$

2. T_1 $\overline{T_2\ T_3\ \underline{T_7\ T_5\ T_9}\ T_6\ T_4\ T_8}$

An attempt was also made to correlate the age of the plant with disease. It was observed that according to both the methods of evaluation, there was significant negative correlation between the age of the plant and the disease severity. According to the evaluation by the percentage of leaves wilted, the correlation was significant only at the 10 per cent level but was significant at 5 per cent level when evaluated on disease index basis.

	Correlation coefficient
Percentage wilt	0.6249
Disease index	0.7147
Critical value (0.1 level)	0.5822
(0.05 level)	0.6664

Another attempt was made to compare the hydrocyanic acid content (HCN) of the leaves at three positions on a plant and the infection by the pathogen to observe if there is any correlation between the two factors. Further the difference in HCN content between the different treatments was also compared as also the HCN content between the three positions of sampling. The results are presented in Table 8. The data revealed that there was no correlation between the HCN content of leaves at the time of inoculation and infection, in any of the three positions

Table 8. Comparison of HCN content in tapioca plants between 9 age groups and 3 positions of the plant

Treatments	HCN content at 3 positions μ gm per gm			Mean
	Upper	Middle	Lower	
1	302	233	230	255
2	244	199	168	203
3	301	246	117	221
4	212	176	171	186
5	344	247	221	271
6	195	191	155	180
7	278	224	237	246
8	241	243	174	219
9	187	172	298	219
Mean	256	215	197	

C.D. (0.05) for treatment — 56.76

C.D. (0.01) for position — 32.75

C.D. (0.01) for combinations — 98.25

(Table 9). The data also revealed that there was significant variation in the HCN content of the leaves over the different ages of the plant. But there was no regular pattern denoting the trend of HCN in the leaves. There was significant variation in the HON content due to the positions. HCN content was maximum in the upper leaves. This was significantly higher than those of the middle leaves and the lower leaves. There was no significant difference in the HCN content between the middle and lower leaves.

An attempt was also made to standardise a method of sampling the leaves of tapioca for estimation of HCN. The coefficient of variation of the HCN content in the three positions was assessed and the result is given below.

	Coefficient of variation
Upper position	36.9832
Middle position	29.0050
Lower position	45.7918

It was observed that the HCN content of the middle leaves is more consistant in sampling than those of the upper and lower leaves since the coefficient of variation is the least at this position.

Sensitivity of the bacterium to antibiotics

Six antibiotics were screened in the laboratory to judge the _in vitro_ sensitivity of the bacterium to antibiotics.

Table 9. Correlation coefficient of infection of tapioca by Xanthomonas manihotis with HCN content in 3 positions

Positions of leaves	Correlation coefficient in 2 evaluation methods	
	Percentage of wilt	Disease index
Upper	0.50249	0.47235
Middle	0.32485	0.27513
Lower	0.21276	0.19309

C.D.(0.05) level   - 0.6664

C.D.(0.01) level   - 0.5322

Table 10. In vitro sensitivity of antibiotics at different concentrations on Xanthomonas manihotis

Antibiotics	Inhibition zone in mm			Mean
	100 ppm	250 ppm	500 ppm	
Agrimycin - 100	18.00	22.00	23.50	21.17
Ampicillin	0.00	0.00	0.00	0.00
Streptomycin	20.00	21.75	24.00	21.92
Terramycin	14.25	16.75	17.00	16.00
Chloramphenicol	0.00	0.00	0.00	0.00
Streptocycline	19.50	22.00	24.00	21.83

C.D.(0.05) for comparison between antibiotics   - 1.303

C.D.(0.01) for comparison between antibiotics   - 2.258

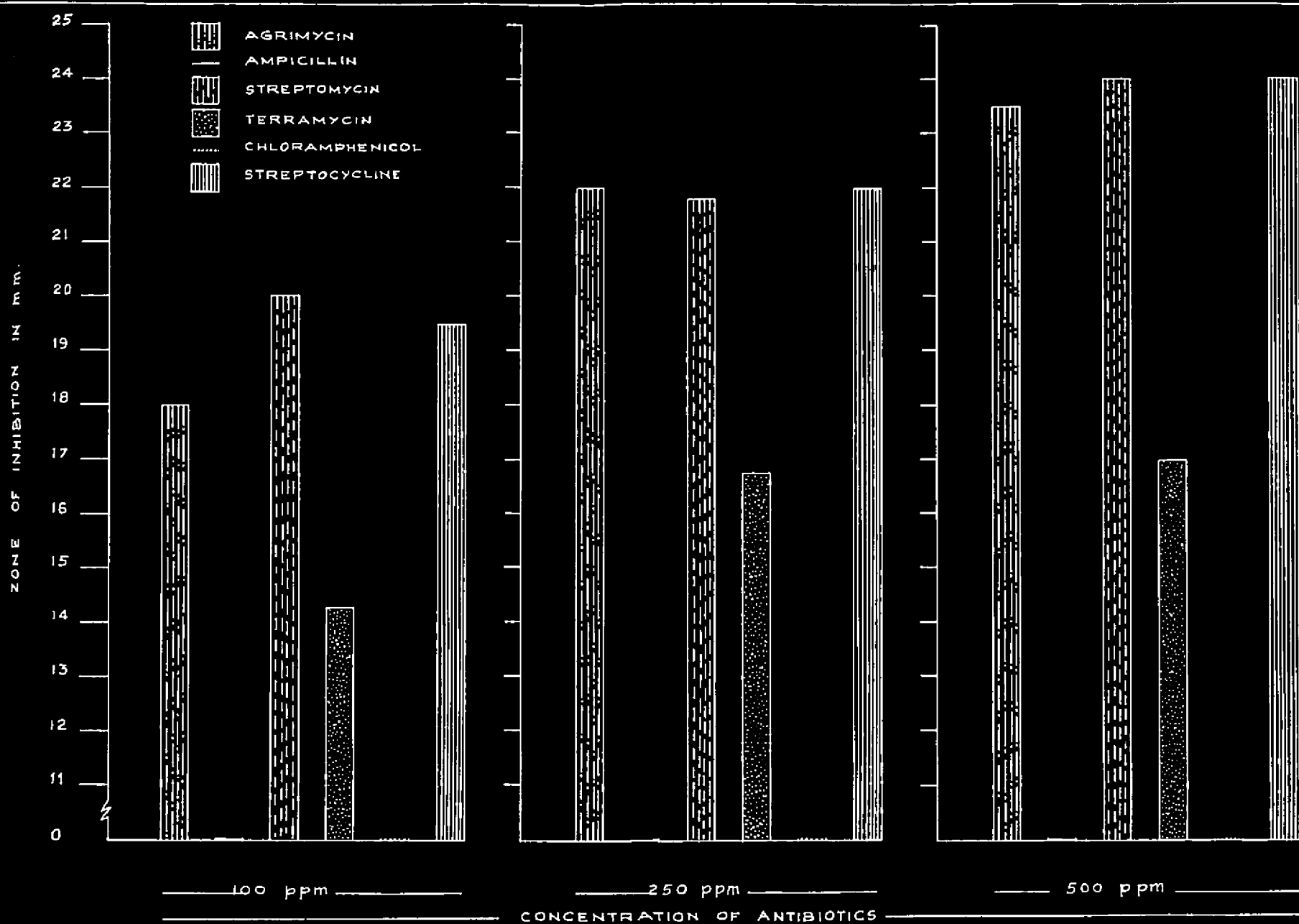C.D.(0.01) for comparison between combinations   - 3.002

FIG. 4 .   IN VITRO SENSITIVITY   OF   XANTHOMONAS MANIHOTIS TO ANTIBIOTICS.
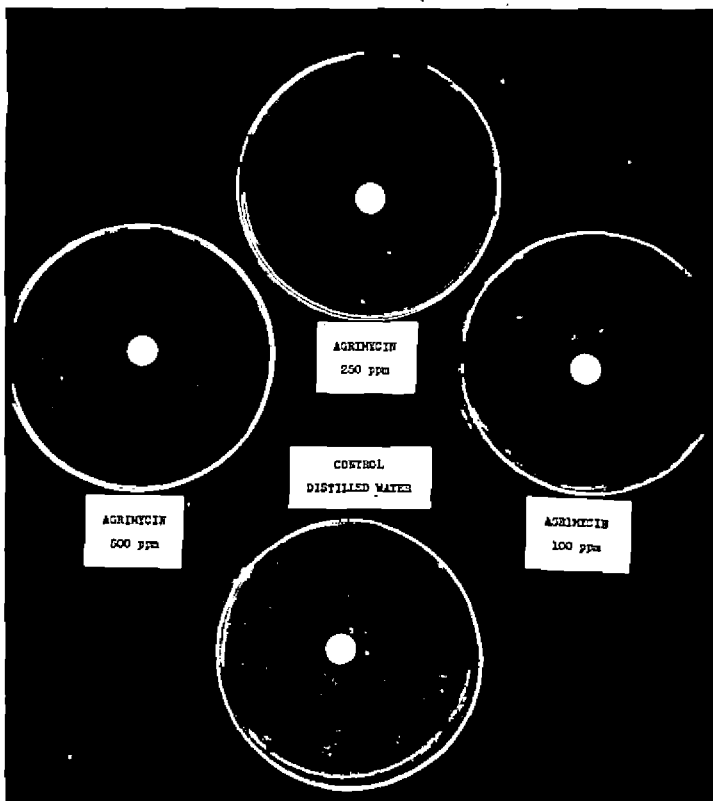
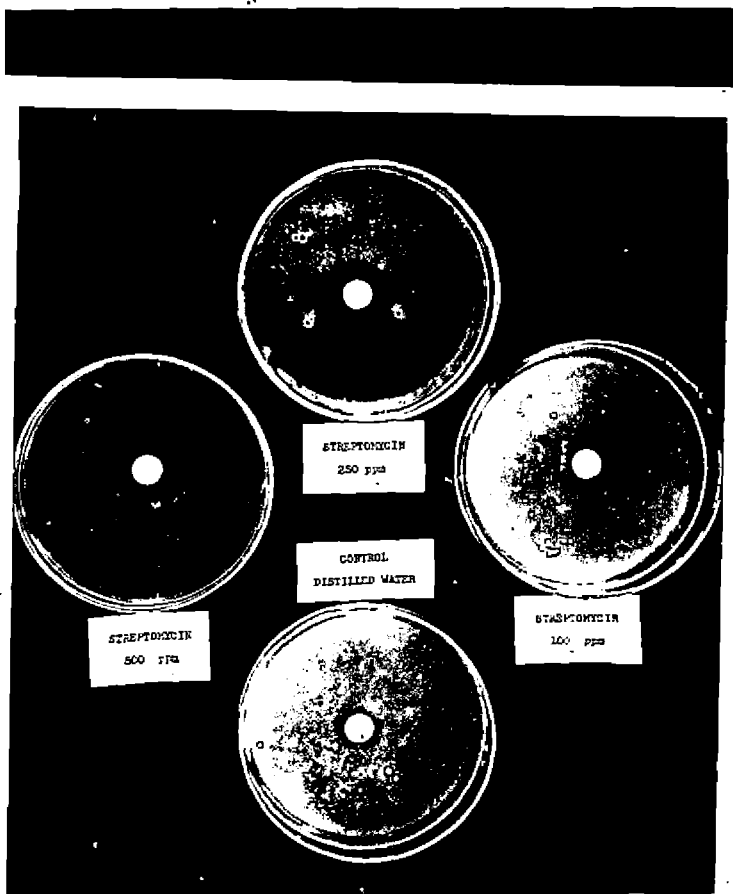Plate V. Inhibition zone with different concentrations of Agrimycin 100

ate VI. Inhibition zone with different concentrations of Streptomycin
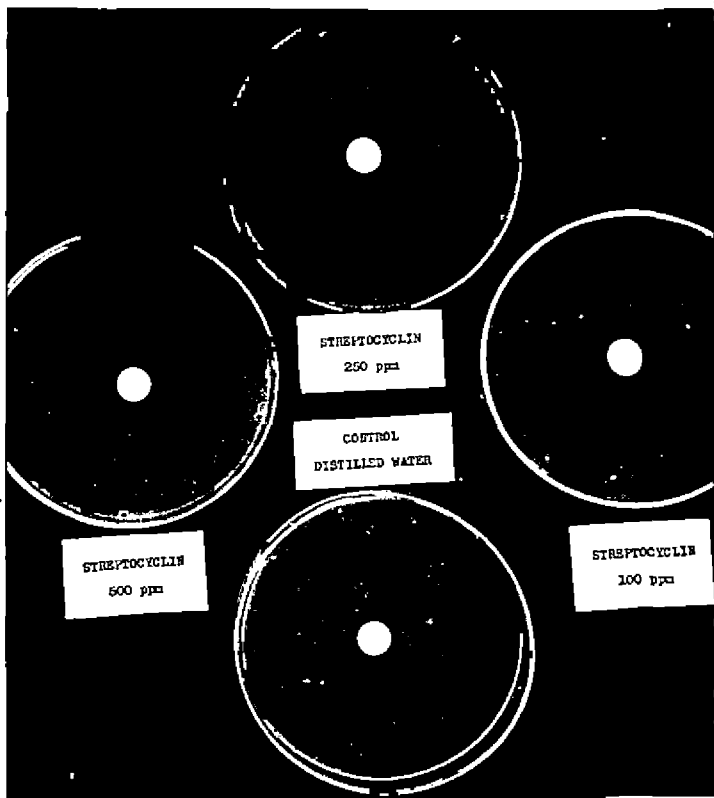
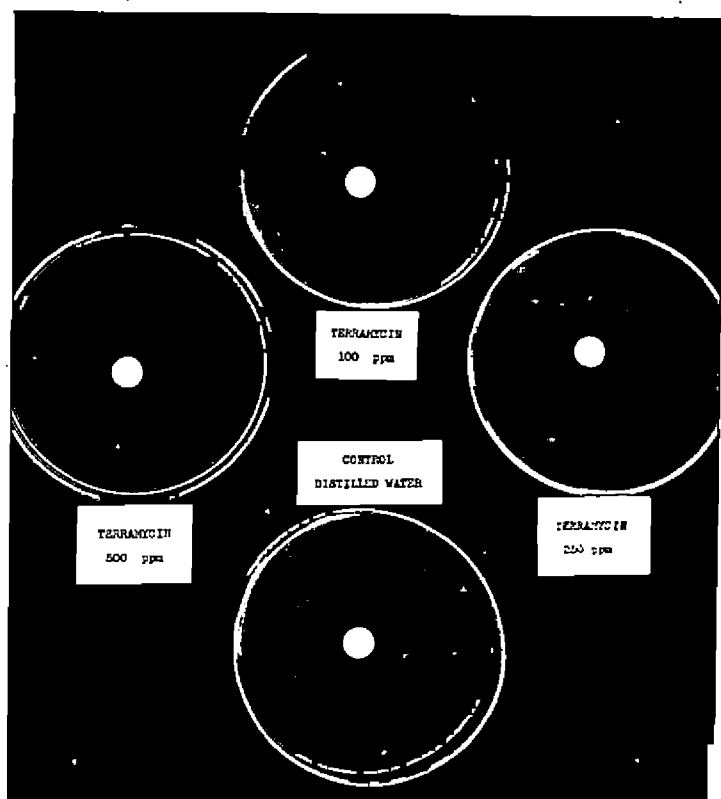Plate VII. Inhibition zone with different concentrations of Streptocycline

Plate VIII. Inhibition zone with different concentrations of Terramycin

The results are presented in Table 10 and Fig.4. Among the six antibiotics tested Agrimycin (Plate V), Streptomycin (Plate VI), and Streptocycline (Plate VII) were on par and significantly superior to Terramycin (Plate VIII). Ampicillin and Chloramphenicol were not effective (Plate IX) in controlling the bacterium at the concentrations tested. Agrimycin and Streptocycline at 250 ppm were found to be superior to 100 ppm but were on par with 500 ppm. Streptomycin and Terramycin at 500 ppm were significantly superior to the lower concentrations.

Toxin studies

Studies were conducted on the production of toxin in vitro by the pathogen. Injection of the cell free extract into the top three axils of the tapioca plants resulted in the drying up of the terminal leaves and bud. Tip burn and tip drying were the initial observations. Within two weeks of injection of the cell free extract, the top cluster of not fully opened leaves dried and twisted up. Later the next leaf down, one fully opened, also started curling up (Plate X), with discolouration and eventually dried up. These dried up leaves later defoliated. The growth of the terminal bud was arrested. In the control plants, where media and distilled water were injected no such symptoms were observed (Plate XI).

Uprooted 1 month old plants were kept dipped in the cell free extract as well as the media. Within two weeks
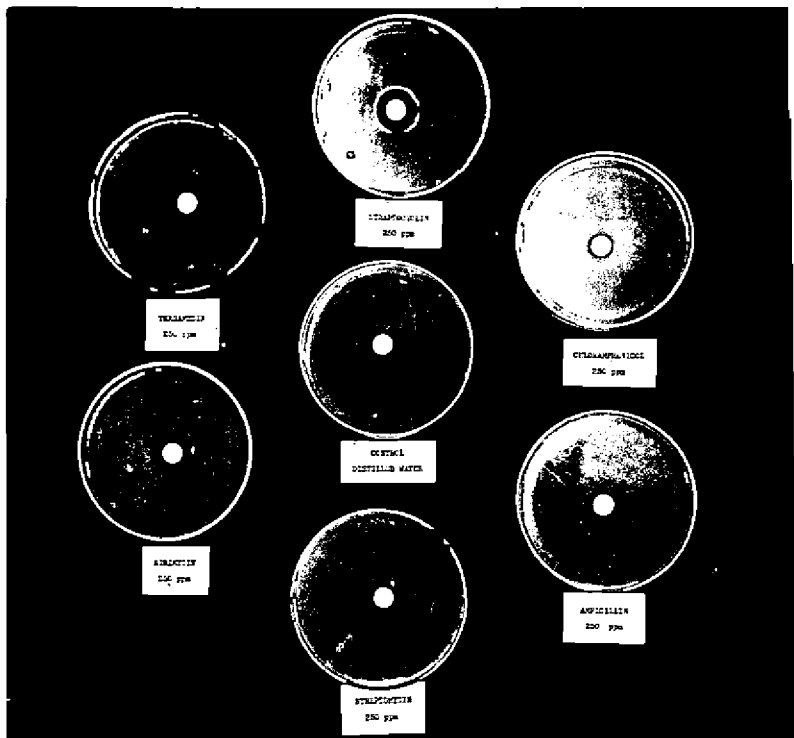
Plate IX. Comparison of the inhibition zones of the six antibiotics tested

Plate X. Tip burn and leaf curling, due to injection of the cell free extract of the bacterium

Plate XI.   Comparison between injection
of cell free extract and the
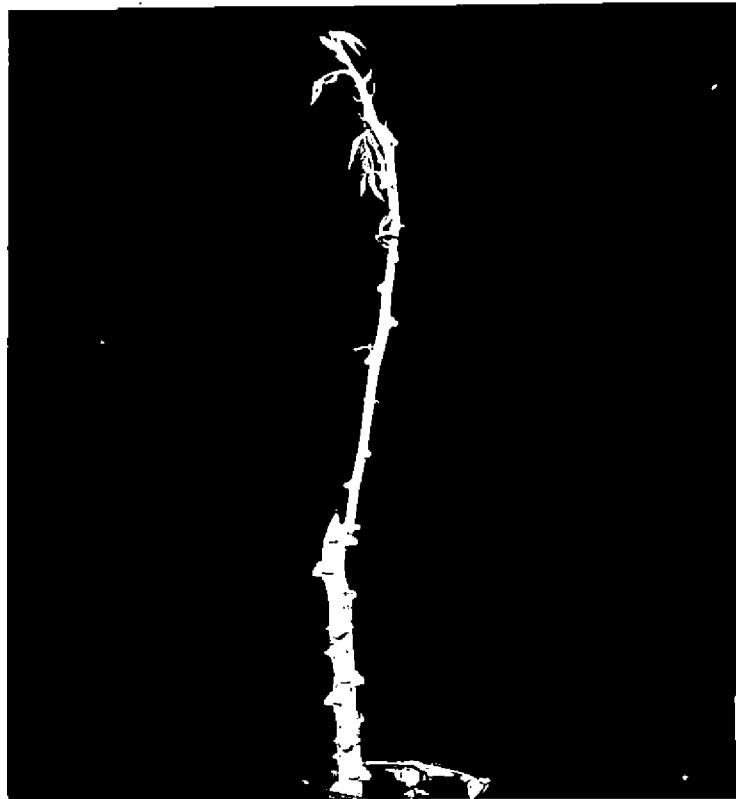growth medium of the bacterium

Plate XII.   Axillary shoot formation on
plant in toxin preparation

defoliation was observed in both cases. Peculiarly the plants kept in the toxin preparation started production of numerous axillary shoots (Plate XII).

The cell free extract was precipitated using ethanol, methanol and acetone. The maximum precipitation was observed in acetone. The precipitate dried in the vaccuum desicator was deep brown in colour. When the dried precipitate was scraped up it had a sticky consistancy. When the precipitate was reprecipitated in ethanol after dissolving in distilled water, the sticky nature disappeared. This was ash coloured and tended to crumble and powder when scraped up from the dish in which it was dried.

When a 100 ppm solution of the precipitate was injected to the terminal part of the growing shoot, it resulted in internodal elongation. The portion of the stem above the point of injection elongated to about 4 times the normal internodal length. In the control plants where distilled water was injected no such symptom was observed.

Discussion

DISCUSSION

The Bacterial Blight (Wilt) incited by *Xanthomonas manihotis* is one of the most serious diseases so far reported on tapioca. This disease is known to cause severe losses in several Latin American countries and Africa.

In Kerala the disease was first reported during the year 1975 from Trivandrum district (Paily et al., 1975; Daniel et al., 1975).

The aspects taken up for the present study were, symptomatology of the disease, characterization and identity of the pathogen, standardization of inoculation techniques, host range, survival of the pathogen, host nutrition in disease development, influence of age and HCN on the disease, *in vitro* sensitivity of the pathogen to antibiotics and toxin production by the pathogen.

Naturally infected plants showed a variety of symptoms. The initial symptoms were loss of turgidity of upper leaves, progressing downwards, followed by rapid wilting and shrivelling of leaves. The wilted leaves remain attached to the stem for some time and eventually fall off. The tip of immature stems showed die back, at times involving the whole shoot. Often, axillary shoots were produced from the green portions below the die back affected areas. These shoots also wilted later.

Maraite and Meyer described the disease as the 'Candle disease' of Zaire based on these symptoms.

The above symptoms had been already reported by Bondar (1912), Leu and Chen (1972), Lozano (1972), Lozano and Sequeira (1974 a), Lozano and Booth (1974) and Maraite and Meyer (1975). In addition, they reported the production of angular leaf spots and gum exudations on the stem and leaves.

In artificially inoculated plants most of these symptoms were produced with the exception of angular leaf spots. Profuse gum exudation was noted on plants from cracks in the stem. The exuded gum dried up and remained as brownish encrustations on the stem. A brownish black discolouration was observed around the point of gum exudation and frequently caused defoliation of one or two leaves. The discoloured areas were soft to touch and further exuded gum when pressed, which seemed to exude from some pockets in the stem.

Usually the symptoms were observed within 4 to 6 days after inoculation. Young plants wilted completely within 2 days of the observation of initial symptoms. In mature plants the progress of symptom expression was seen delayed, sometimes taking up to a week for complete wilting of the plants. Occassionally a few leaves on one side of the stem wilted, progressing downwards, and later spread to all the leaves around.

Nair (1977) did not observe gum exudation and leaf spotting. In the present study gum exudation was observed on the variety H-165.

The internal symptoms observed on peeling off the bark of the severely infected stem were characteristic brownish black streaks. Vascular discolouration could also be observed in sections of 3 to 4 months old plants. Cracks were frequently observed in infected tubers. In advanced cases of infection the tubers decayed and emitted a foul smell. This is in agreement with the observations of Nair (1977).

Maraite and Meyer (1975) reported the phenomenon of symptom variationto be due to the differences in the cultivar and variation in the pathogen due to climatic and soil factors. Bondar (1939) also reported that symptoms differ between sweet and bitter varieties.

The bacterium was isolated from infected plant materials and grown in culture media. The colonies were whitish to cream or grey, raised, fluidal, slimy and convex. The colonies were initially hyaline in Yeast Glucose Chalk Agar and then turned opaque. The bacterium was found to be gram negative short rods.

The growth of the bacterium in different media showed some variations. Out of the eight solid media used, maximum growth was obtained in Yeast Glucose Chalk Agar, with maximum

amount of slime and fluidity, followed by Potato Sucrose Peptone Agar, Tetrazolium Chloride negative medium, Potato Dextrose Agar and Nutrient Agar. The growth in the basal medium for Xanthomonads was very meagre. There was no growth in Glucose Agar and Glucose Yeast Extract Agar. Among the common media PDA was better than NA. This is in accordance with the findings of Maraite and Meyer (1975), Leu (1976) and Nair (1977). Dye (1962) had recorded that considerable variation could be expected in colonies produced by Xanthomonads and this may not be taken as a differentiating character.

Growth of the bacterium in liquid media was assessed by spectrophotometric methods. In contrast to the growth in solid media, the maximum amount of growth was observed in Nutrient broth at 24 and 48 hours after inoculation. After 24 hours there was significant growth in Nutrient broth, Potato Sucrose Peptone broth and the broth of the basal medium for Xanthomonads. At 72 hours of incubation the maximum growth was observed in Potato Sucrose Peptone broth followed by Nutrient broth, Tetrazolium Chloride negative broth and the broth of the basal medium for Xanthomonads.

For routine laboratory studies of the bacterium, Yest Glucose Chalk Agar and Potato Sucrose Peptone Agar were found to be the suitable solid media and Potato Sucrose Peptone broth and Nutrient broth suitable liquid media. Growth on different liquid media has not been studied by

earlier workers.

Among the three incubation temperatures tested 30°C was found to be the most suitable for obtaining maximum growth of the pathogen.

With regard to the physiological and biochemical properties of the bacterial isolate, it hydrolysed starch feebly, produced hydrogen sulphide, and reduced nitrate to free nitrogen or ammonia. The organism was found to be catalase positive and oxidase negative. Of the 10 carbon compounds tested, the bacterium produced acid in presence of xylose, fructose, glucose, sucrose, lactose and inositol indicating their utilisation. The acid production was delayed in xylose and fructose. The bacterium did not produce acid in adonitol, dulcitol, inulin and salicin.

Similar results have been obtained by Lozano and Sequeira (1974 a) and Leu (1976). The observations are in conformity with those reported by Breed et al. (1957); Dye (1962) and Buchanan et al. (1974). Dye (1962) reported that physiological characters are of little value in distinguishing species, because the extent of intra-species variability in physiological characters is so great as inter-species variability.

From the present studies, the pathogenic isolate of the bacterium could be characterised and the identity confirmed as Xanthomonas manihotis. This is more pertinent in the

context of a controversial report by Daniel et al. (1975) stating that the cassava blight bacterium resembles more to Pseudomonas solanacearum. Bradbury (1975) reported that the name appears in some disease lists but usually in lieu of Xanthomonas manihotis.

Because of the lack of formation of yellow pigment by the bacterium, one may question the inclusion of the bacterium under the genus Xanthomonas. Cassava blight bacterium possesses some of the characteristics of Xanthomonads (Braley, 1965; Stolp et al., 1965). A few non pigmented species other than Xanthomonas manihotis have been included in the genus Xanthomonas (Buchanan et al., 1974). Another difficulty in recognising Xanthomonads is the occurrence of albino strains. These do not fit into the genus Xanthomonas if pigmentation is taken as the most important character, but they fulfil all the other characters of Xanthomonads. In X. manihotis and X. pedalli only albino strains are known, whereas in X. ricinicola and X. uppali, both yellow and albino strains occur (Dye, 1962; 1963 a; 1966).

From the studies on the morphological, cultural, physiological and biochemical characters coupled with its pathogenicity and host specificity, viewed in the light of the reports by Drummond and Hipolito (1941), Amaral (1942; 1959), Starr (1946), Dye (1962; 1963 a; 1966), Lozano (1972; 1975),

Anon. (1973; 1974), Lozano and Sequeira (1974 a; 1974 b)
Lozano and Booth (1974), Buchanan et al. (1974), Maraite and
Meyer (1975) and Bradbury (1975), it is evident that the
pathogen inciting the 'Bacterial Blight (Wilt)' of tapioca is
an albino Xanthomonad, Xanthomonas manihotis.

Eleven inoculation methods were tested and the most
efficient method was inoculation by the 'Bud Nipping Method'.
Of the other methods tested, bud nipping and puncturing leaf
axils, puncturing lower leaf axils, puncturing upper leaf axils
and injection below the apical meristem also gave infection.
Among all the treatments, better infection was noted when the
inoculum was given after puncturing or causing injury to the
plant parts.

Lozano and Sequeira (1974 a) conducted similar experiments.
They got infection in 5 to 6 days in the case of stem puncture
method and only in 8 to 10 days in the case of spray inoculation
and leaf rubbing. Leu (1976) got cent per cent infection by
injecting the bacterial suspension below the apical meristem.
Nair (1977) had tried 9 methods of inoculation and recorded
that smearing the surface of leaves with injury gave the
fastest infection, followed by stem puncture and nipping the
bud.

In the present study of standardization of inoculation
techniques, inoculation by the 'Bud Nipping Method' was found

to be the best, and gave very good infection and disease development.

Common plants of similar taxonomic affinity, belonging to the family Euphorbiaceae were inoculated to see if any of these plants took up infection. The bacterium could not infect any of the plants tested. Bondar (1915), Amaral (1942), Burkholder (1942) and Leu (1976) also have reported that the bacterium causing the blight (wilt) of tapioca did not go outside the genus _Manihot_.

It was observed that the survival of the pathogen in infected stems is only for a short period if the stems are cut and stored. These infected stems dry up in 3 to 4 weeks on storage. But as live plants in the field the pathogen is found to survive as long as the plant remained alive. In the experiment conducted by the incorporation of infected plant material in soil, symptoms of wilting was observed 36 days after planting healthy stems: i.e. 57 days after incorporation of the infected plant material, which is the longest period recorded in this experiment. In a similar experiment conducted by the inoculation of the soil with the bacterial culture, wilting was noticed 104 days after planting the sets: i.e. 115 days after inoculating the soil with the culture.

Based on the above results it is indicated that the bacterium can survive in the soil and cause infection to

tapioca upto a period of 3 months. This is on the assumption that a minimum period of 22 days is necessary for symptom expression after initiation of infection by the bacterium, as evidenced by the results of the experiment conducted on this aspect.

Leu (1976) conducted experiments by incorporation of infected debris and also by mixing the bacterial culture in soil and observed that wilting symptoms appeared when sets were planted immediately but not when planted one or two months later.

Studies conducted in Nigeria revealed that the disease did not develop when tapioca sets were planted in a disease affected plot, 90 days after uprooting the infected plants and ploughing the field, whereas 28.5 to 35 per cent of the plants became infected when planting was done immediately after uprooting the plants in the infected field (Anon., 1974).

Studies on the effect of host nutrition on disease development and severity indicated that the disease development and severity cannot be correlated with nitrogen and lime at the levels tested. The observations made by Albuquerque (1963), Ezhumah and Terry (1974) Glaser and Ogbogu (1974) and Maraite and Meyer (1975) were generally on the line that the disease is more in infertile and sandy soils, and places where the same cultivar was repeated. Terry (1976) reported severe

disease incidence in low nutrient soils and that the addition of NPK fertilizers might decrease the number of plants killed by CBB. This indicated an inverse relationship with host nutrition. In the absence of detailed studies it may not be possible to draw definite conclusions from this study.

In the study of the influence of the age of the plant on the disease incidence, development and severity, it was noticed that infection was possible at all stages of growth of the plant. The disease development and severity was maximum in one month old plants followed by two and three month old plants, while the rest of the age groups did not follow a regular pattern. It was however indicated that there is a negative correlation between the age of the plant and disease development. This was significant at 5 per cent level when correlated with the disease index and significant at 10 per cent level when correlated with per cent wilt.

Maraite and Meyer (1975) had inoculated 17 month as well as 5 month old plants by stem and leaf inoculations and obtained infection in both cases. Eventhough all the age groups in the present study were found susceptible to infection by the pathogen, maximum damage was caused in the early stages of crop growth (1 to 3 months).

In the attempt to correlate the HCN content of leaves at different growth stages of the plant with the disease development,

it was observed that the HCN content of the leaves had no correlation with the disease development. The HCN content of tapioca plants in relation to bacterial wilt has not been studied so far.

Out of the six antibiotics screened for the in vitro sensitivity of the bacterium, it was found that Agrimycin 100, Streptocycline and Streptomycin had better inhibitory effect over Terramycin, while Ampicillin and Chloramphenicol were found to be ineffective upto 500 ppm. Agrimycin and Streptocycline had better effect at 250 ppm and Streptomycin and Terramycin at 500 ppm. Nodu (1974) felt that Streptomycin can be used to prevent dissemination of CBB.

From the present studies the indication was that Agrimycin 100 and Streptocycline at 250 ppm and Streptomycin and Terramycin at 500 ppm had equal in vitro sensitivity against the pathogen. However detailed studies are necessary to assess the efficiency of the antibiotics for the control of the disease on a field scale.

Studies conducted on the toxigenicity of the bacterium indicated that some toxic metabolite may be involved in the pathogenicity. Injection of the cell free extract of the bacterium produced tip burn and drying in tapioca plants. Defoliation and profuse axillary growth were produced in one month old plants when kept dipped in the cell free extract.

Similar symptoms are noticed in naturally infected plants also. Internodal elongation of the plants were produced when the toxin was injected through the tissues near the apical meristem.

From the above observations it is felt that some toxic metabolites may be involved in the pathogenicity of the bacterium and symptom expression.

Summary

SUMMARY

The Bacterial Blight of tapioca, popularly known as cassava bacterial blight, incited by <u>Xanthomonas manihotis</u> (Arthaud - Berthet) Starr, is perhaps the most serious of all the diseases reported on the crop. Infection of the plant results in the complete loss of yield, unlike the other diseases reported on the plant. The disease has been causing severe loss to the crop in the cassava growing tracts of the tropical belts of America, Africa and the Asian countries. The disease was reported from Trivandrum in 1975, since when, there has been reports of its sporadic occurrence from various tapioca growing tracts in the state.

In view of the potential losses the disease can cause to the crop, aspects such as, symptomatology of the disease, characterisation and identity of the pathogen, standardization of inoculation techniques, host range and survival of the pathogen, host nutrition on disease development, influence of age and HCN on the disease, <u>in vitro</u> sensitivity of the pathogen to antibiotics and toxigenicity of the bacterium were taken up for the study.

Most of the reported symptoms of the disease such as loss of turgidity, shrivelling and wilting of the leaves, gum exudations, die-back and death of the stem, axillary shoot growth, discolouration of the vascular system and root and

finally damage and decay of the tuber were observed in this study. For laboratory studies on the pathogen, Yeast Glucose Chalk Agar and Potato Sucrose Peptone Agar were found to be the best solid media and Nutrient broth and Potato Sucrose Peptone Broth the best liquid media.

Morpholigically the bacterium was a gram negative short rod, usually single or in short chains of 3 to 4 cells with maximum growth around 30°C. Physiological and biochemical studies showed that the bacterium produced catalase, hydrogen sulphide, gave negative nitrate reduction test and oxidase test and hydrolysed starch feebly. The bacterium produced acid, indicative of utilisation, from xylose, fructose, glucose, sucrose, lactose and inositol and not from adonitol dulcitol, inulin and salicin.

Studies on the morphological, physiological and biochemical characters of the pathogen coupled with its host specificity and symptom expression confirmed the identity of the pathogen as <u>Xanthomonas manihotis</u> (Arthaud - Berthet) Starr.

Of the eleven inoculation methods tested, inoculation after nipping the bud was found to be the best, giving the maximum infection. This method was followed in further studies.

Plants having similar growth and taxonomic affinity, of the family Euphorbiaceae, were tested to see, if any of these took up infection. These tests confirmed the reports that the pathogen does not go out side the genus <u>Manihot</u>,

and the host specificity of the pathogen.

The pathogen was found to survive for 3 weeks in infected stems, 41 days in infected plant debris in soil, 3 months in soil mixed with the pure culture of the bacterium and 3 weeks in soil from where the infected plants have been uprooted. The result, that the pathogen survives for considerable periods in infected material and soil has some epidemiological significance in the disease cycle.

The effect of different levels of nitrogen in conjunction with lime was not found to be significant on the disease incidence and disease development, though a higher severity was noted at 100 kg nitrogen per hectare in presence of lime.

The pathogen infected the plant at all stages of its growth. But the severity of the disease was much more from 1 to 3 months and decreased as the plant grew older. Though the HCN content of the plant varied significantly between different ages of the plant, it did not have any relation with the disease incidence and severity.

The pathogen was found to be sensitive to Agrimycin 100, Streptocycline, Streptomycin and Terramycin when screened in vitro. Further in vivo trials are necessary to assess the efficiency of the antibiotics for the control of the disease on a field scale.

The toxic metabolite produced by the pathogen is assumed to have some role in pathogenisis and symptom expression.

References

²S

*Adenji, M.O. and G.O.Obigbesan (1976). The effect of potassium nutrition on the bacterial wilt of cassava. Nigerian Journal of Plant Protection 2: 1-3.

*Agbo, F.M. (1974). Preliminary screening for bacterial blight resistance. Proceedings of the Federal Agricultural Research and Training Station, Umudike, Nigeria, 1974. 1st workshop on cassava bacterial blight in Nigeria. 52-56.

*Albuquerque, M. (1963). Diseases and pests of cassava. Revista Brasileira de Fertilizantes Insectidas e Racoes. 5(8): 32-36.

Alouf, Joseph, E. and Marcel Raynard (1970). Isolation and Purification of bacterial toxic proteins. In Microbial Toxins, A Comprehensive Treatice. Ed. Samuel, T. Ajil; Solomon Kadis and Thomas, C. Montie. Vol.I - Bacterial Protein Toxins. 119-182.

*Amaral, J.F. Do. (1942). A study of the causal agent of the cassava bacteriosis. Arq. Inst. Biol. 13: 119-126.

*Amaral, J.F. Do. (1945). Plant Vascular Diseases caused by bacteria. Biologico. 11(9): 250-253.

*Amaral, J.F. Do. and L.G.Do. Vasconcellos (1945). Novos estudo do agente etiologico da bacteriose da mandioca. Arq. Inst. Biol. 16: 361-368.

*Amaral, J.F. Do. (1958). Biochemical characters of Xanthomonas manihotis and Xanthomonas rubresubalbicans; and their position in Burkholder's key for this genus. Arq. Inst. Biol. 21: 66-72.

Anonymous (1957). Mannual of Microbiological Methods (Society of American Bacteriologists. Mc Graw Hill Book Co., Inc. New York, USA.

Anonymous (1968). Commonwealth Micological Institute (CMI) Kew. Plant Pathologist's Pocket Book. pp.120.

*Anonymous (1972). Centro Internacional de Agricultura Tropical. Ann. Rept. 1972. CIAT. Cali, Colombia. pp.192.

*Anonymous (1973). Centro Internacional de Agricultura Tropical. Ann. Rept. 1973. CIAT, Cali, Colombia. pp.254.

Anonymous (1973 b). Bacterial Wilt disease of cassava. Advisory Bulletin No.1. Federal Agricultural Research and Training Station, Umudike, Nigeria. pp.18.

Anonymous (1974). Root and Tuber Improvement programme. Annual Report. 1974. International Institute of Tropical Agriculture, Ibadan, Nigeria. 125-152.

*Arene, O.B. (1974). Preliminary evaluation of some fungicides for the control of bacterial blight disease of cassava caused by Xanthomonas manihotis (Arthaud - Berthet and Bondar) Burk. Proceedings of the Federal Agricultural Research and Training Station, Umudike, Nigeria. 1st workshop on cassava bacterial blight in Nigeria. 57-60.

*Bondar, G. (1912). A new bacterial disease of cassava stems. Characas e Quintaes. 5(4): 15-18.

*Bondar, G. (1915). A bacterial disease in cassava. Boletin de Agricultura (Brazil). 16: 513-524.

*Bondar, G. (1939). A bacteriosis of cassava. Campo. 10(119): 28-30.

*Braley, D.E. (1965). Replica and Shadowing Techniques. In Techniques for Electron Microscopy, 2nd ed. Blackwell Scientific Publication, London. pp.560.

Breed, R.S., E.G.D. Murray and A.P. Hitchens (1948). Bergey's Mannual of Determinative Bacteriology, 6th ed. The Williams and Wilkins Co., Baltimore, USA. pp.1929.

Breed, R.S., E.G.D. Murray and N.R.Smith (1957). Bergey's Mannual of Determinative Bacteriology, 7th ed. The Williams & Wilkins Co., Baltimore, USA. pp.1094.

Bradbury, J.F. (1975). Bacterial diseases of cassava. PANS 21(1): 44.

Buchanan, R.E. and N.E. Gibbons (1974). Bergey's Mannual of Determinative Bacteriology, 8th ed. The Williams and Wilkins Co., Baltimore, USA. pp.1268.

Burkholder, W.H. (1942). Three bacterial plant pathogens:
    Phytomonas caryophyll sp. n., Phytomonas allicola sp.n.
    and Phytomonas manihotis (Arthaud - Berthet and Bondar)
    Viegas. Phytopathology. 32(2): 141-149.

*Carneiro, J.G. (1940). Doenca bacteriana da mandioca.   Notas e
    Informacoes. Portario do Min. Agr. 573: 447.

*Castro, J.B. DE., R.D. Goncalves and E.S. Normanha (1939). A
    bacteriose da mandioca. Bahia Rural 6: 225-226.

*Costa, F. (1940). Regios infestadas pella bacteriose da mandioca.
    Notas e informacoes. Biologico. 6: 332.

Daniel, R.S., N.G. Nair and N. Hrishi. (1975). A new wilt
    disease of cassava. J. Root Crops 1(2): 81.

*Drummond, O.A. (1946). Diseases of cassava. Revista Ceres
    (Brazil) 7(37): 24-33.

*Drummond, O.A. and R.D. Goncalves (1948).   Bacteriosis of
    cassava. Biologico. 14: 145-146.

*Drummond, O.A. and R.D. Goncalves (1953). Bacteriosis in
    gauxipe cassava. Biologico. 19: 114-117.

*Drummond, O.A. and R.D. Goncalves (1939). Cassava bacteriosis
    in the valley of Parabia.   Biologico. 5: 117-118.

*Drummond, O.A. and O.Hipolito (1941). Cassava bacteriosis.
    Ceres (Brazil). 2: 281-307.

Dye, D.W. (1962). The inadequacy of the usual determinative
    tests for the identification of Xanthomonas spp.
    N. Z. J. Sci. 5: 393-416.

*Dye, D.W. (1963 a). Comparative study of additional Xanthomonas
    spp. N. Z. J. Sci. 6: 483-486.

*Dye, D.W. (1963 b). The taxonomic position of Xanthomonas
    stewartii (Erw. Smith, 1914) Dawson, 1939. N. Z. J.
    Sci. 6: 495-506.

*Dye, D.W. (1964). The taxonomic position of _Xanthomonas trifolio_ (Huss. 1907) James, 1955. _N. Z. J. Sci._ 7: 265-269.

Dye, D.W. (1966). Cultural and biochemical reactions of additional _Xanthomonas_ spp. _N. Z. J. Sci._ 9: 913-919.

Dye, D.W. and R.A. Lelliot (1974). _Bergey's Mannual of Determinative Bacteriology,_ 8th ed. Williams and Wilkins Co. Baltimore, USA. 243-244.

Elliot, C. (1951). _Mannual of Bacterial Plant Pathogens._ 2nd ed. Chronica Botanica Co., Waltham, Mass. pp.186.

Ezhumah, H.C. and E.R. Terry (1974). Cultural considerations in the control of cassava bacterial blight. _Proceedings of the Federal Agricultural Research and Training Station,_ Umudike, Nigeria. 1st Workshop on cassava bacterial blight in Nigeria. 25-32.

*Freire, J.R.J. (1963). Cassava bacteriosis. _Granja_ (Brazil). 18(189): 64.

Glaser, H.J. and F.C. Ogbogu (1974). Problems and experiences of plant protection extension in the east central states of Nigeria. _Proceedings of the Federal Agricultural Research and Training Station,_ Umudike Nigeria. 1st Workshop on cassava bacterial blight in Nigeria. 11-12.

Hahn, S.K., A.K.Howland and C.A. Okoli (1974). Breeding for resistance to cassava bacterial blight at IITA. _Proceedings of the Federal Agricultural Research and Training Station,_ Umudike, Nigeria. 1st Workshop on cassava bacterial blight in Nigeria. pp.11-14.

Indira, P. and S.K. Sinha (1969). Calorimetric method for estimation of HCN in tubers and leaves of cassava (_Manihot esculenta_ Crantz). _Indian J. Agric. Sci._ 39: 1021-1023.

Kelman, A. (1954). The relationship of pathogenicity in _Pseudomonas solanacearum_ to colony appearance. _Phytopathology._ 44: 693-695.

Leu, L.S. (1976). Cassava bacterial blight in Taiwan. Proceedings of the 4th symposium of the international society for tropical root crops. CIAT, Cali, Colombia, pp.175-179.

*Leu, L.S. and C.T.Chen (1972). Bacterial wilt of cassava (Manihot utilissima Phol.) caused by Xanthomonas manihotis (Arthaud - Berthet) Starr. Plant Protection Bullettin (Taiwan). 1(1): 17-26.

*Lima, A.D.F. (1944). Bitter and sweet cassava. Boletim do Minesterio da Agricultura. 33(12): 6-18.

*Lozano, J.C. (1972). Bacterial blight of cassava, Manihot esculenta Crantz, in Colombia: etiology, epidemiology and control. Ph.D. Thesis, University of Wisconsin, Madison, 114 pp.

Lozano, J.C. (1973 a). Cassava bacterial blight. CIAT, Information Bullettin No.2. pp.10.

Lozano, J.C. (1973 b). Bacterial blight of cassava in Central and South America: Etiology, Epidemiology and Control, 3rd International symposium on tropical root crops, Ibadan, Nigeria, CIAT, Palmira, Colombia, 1973. pp.19.

*Lozano, J.C. (1974). Bacterial wilt of Manihot esculenta Crantz in America: Etiology, epidemiology and control. Fitopatologia 9(2): 110-119.

Lozano, J.C. (1975). Bacterial blight of cassava. PANS 21(1): 28-43.

Lozano, J.C. and R.H. Booth (1974). Diseases of cassava (Manihot esculenta Crantz) PANS 20(1): 30-54.

Lozano, J.C. and L. Sequeira (1974 a). Bacterial blight of cassava in Colombia: Etiology. Phytopathology. 64(1): 74-82.

Lozano, J.C. and L. Sequeira (1974 b). Bacterial blight of cassava in Colombia: Epidemiology and Control. Phytopathology. 64(1): 83-88.

Ludritz Otto, Otto Westphal, Anne Marie Staub and Hiroshi Nikaido, (1971). Isolation and chemical and immunological characterisation of bacterial lipopolysaccharides. In Microbial Toxins: A Comprehensive Treatice, Vol. IV. Baoterial Endotoxins. Ed. George Weinbaum, Solomon Kadis and Samuel, T. Ajll. Academic Press. 145-224.

*Mau, S.C. (1951). Cassava in Taiwan. Taiwan Speoial Crops, No.8, Bank of Taiwan (In Chinese). Proceedings of the 4th symposium of the international society for tropical root crops, CIAT, Cali., Colombia, pp.80.

Maraite, H. and J.A. Meyer (1975). Xanthomonas manihotis (Arthaud - Berthet) Starr., Causal agent of bacterial wilt, blight and leaf spots of cassava in Zaire. PANS. 21(1·): 27-37.

Nair, Raveendran, R. (1977). Studies on the bacterial blight (wilt) of cassava in Kerala. M.Sc. (Ag.) Thesis, Kerala Agricultural University (Unpublished).

*Nodu, N. (1974). Chemical aspects of control of bacterial blight of cassava. Proceedings of the Federal Agricultural Research and Training Station, Umudike, Nigeria. 1st workshop on cassava bacterial blight in Nigeria. pp.22-24.

Obigbesan, G.O. and E.O. Matuluko (1976). Effect of potassium and bacterial blight on the yield and chemical composition of cassava cultivars. Proceedings of the 4th symposium of the International Society for tropical root crops. CIAT, Cali, Colombia. 185-187.

Paily, P.V., Devi, L.R. and M.R. Menon (1975). Bacterial wilt of oassava (Manihot esculenta Crantz) in Kerala. Agri. Res. J. Kerala. 13(1): 107-109.

*Palti, J. and Rotem (1973). Epidemiological limitations to the forecasting of downy mildews and late blight in Israel. Phyto Parasitica 2: 119-126.

*Pereira, A.L.G. and Zagatto (1967). Etiology of angular leaf spot on cassava leaves. Arq. Inst. Biol. Sao Paulo, 34: 153-160.

Patil, S.S., L.Q. Tam and P.E. Kolattukudy (1972). Isolation and mode of action of the toxin from Pseudomonas phaseolicola. In Phytotoxins in Plant Diseases. Ed. Wood, R.K.S., A. Ballio and A. Graniti. Academic Press. 365-372.

Rotem, J. and J. Palti (1969). Irrigation and plant diseases. Ann. Rev. Phytopath. 7: 267-288.

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

Starr, M.P. (1946). The nutrition of phytopathogenic bacteria. Minimal nutritive requirements of the genus Xanthomonas J. Bacteriol. 51: 131-143.

Stolp, H., M.P. Starr and N.L. Baigent (1965). Problems in speciation of phytopathogenic Pseudomonads and Xanthomonads. Ann. Rev. Phytopath. 3: 251-264.

*Terry, E.R. (1974). Some epidemiological factors affecting the survival and dissemination of Xanthomonas manihotis. Proceedings of the Federal Agricultural Research and Training Station, Umudike, Nigeria. 1st Workshop on cassava bacterial blight in Nigeria. 39-43.

Terry, E.R. (1976). Cassava bacterial blight in Africa. Proceedings of an inter-disciplinary workshop. In the international centre for the exchange and testing of cassava germ plasm in Africa. International Development Centre, Ottawa, Canada. 23-25.

*Terry, E.R. (1977). Fear of cassava bacterial blight in Africa makes control imperative. World crops and livestock. 29(3): 107-108.

Terry, E.R. and Ezhumah, H.C. (1974). Report on root crops disease survey and evaluation of production problems in Cameroon, 22-27th August, 1974. IITA, Ibdan, Nigeria. pp.4.

*Viegas, A.P. (1940). Mofo dos afideos e alericrodiedos. Revista de Agricultura, Piracicaba 15: 475-485.

Williams, R.J., S.D. Agboola and R.W. Schneider. (1973). Bacterial wilt of cassava in Nigeria. Plant. Disi Reptr. 57(10): 324-327.

*Original not seen

Appendices

APPENDIX I

Analysis of variance
Standardization of artificial inoculation techniques

Source	df	Mean square	
		Percentage of leaves wilted (in angles)	Disease index
Treatment	10	7016.1923**	19.1164**
Error	99	1277.7960	

**Significant at 0.01 level

APPENDIX II

Analysis of variance
Influence of nutrition on disease development

Source	df	Mean Square	
		Percentage of leaves wilted (in angles)	Disease index
Treatment	5	1647.88	3.5633
N	2	2457.99	5.8583
L	1	171.51	0.6756
N x L	2	1575.45	2.7249
Error	114	1340.22	2.9048

APPENDIX III

Analysis of variance

Influence of stage of growth on disease incidence

Source	df	Mean Square	
		Percentage of leaves wilted (in angles)	Disease index
tment	8	4432.62**	12.12**
r	126	870.43	1.29

**Significant at 0.01 level

APPENDIX IV

Analysis of variance

Comparison of HCN content between growth stages of the plant and leaf positions in a plant

Source	df	Mean Square
Treatment	8	13871.40*
Position	2	41399.02**
T x P interaction	16	9950.36
Error	108	6032.94

* Significant at 0.05 level

** Significant at 0.01 level

APPENDIX V

Analysis of variance

In vitro sensitivity of the bacterium to antibiotics

Source	df	Mean Square
Treatment	17	411.3962**
Antibiotics	5	1367.5472*
Between levels of Antibiotics		
Agrimycin	2	32.3333**
Ampicillin	2	0.0000
Streptomycin	2	16.0833*
Terramycin	2	9.2500*
Chloramphenicol	2	0.0000
Streptocycline	2	20.3333**

* Significant at 0.05 level

** Significant at 0.01 level

STUDIES ON THE BACTERIAL BLIGHT (WILT) OF TAPIOCA
INCITED BY *Xanthomonas manihotis* (ARTHAUD - BERTHET) STARR

BY

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ABSTRACT

The Bacterial Blight of tapioca, incited by Xanthomonas manihotis (Arthaud - Berthet) Starr, probably the most serious of all the diseases recorded on the plant, was reported sporadically from different parts of Kerala since 1975. The disease is characterised by loss of turgidity, shrivelling and wilting of leaves, gum exudation, die-back and death of shoots, vascular discolouration of stem and roots and damage and decay of tubers.

The pathogen was confirmed to be Xanthomonas manihotis from morphological, physiological and biochemical studies coupled with symptomatology and host specificity. The pathogenicity was confirmed on tapioca using different methods of artificial inoculation, of which inoculation after nipping the bud was found to be the best.

The pathogen was found to survive for 3 to 4 weeks in infected plant parts in soil, but it survived upto 3 months when the culture was inoculated in soil. X. manihotis was found to be host specific since it did not infect the other Euphorbiaceous plants inoculated. It is reported to be confined to the genus Manihot.

Nitrogen in conjunction with lime did not have much influence on the incidence and severity of the disease at the levels tested. The pathogen infected tapioca plants from

1 to 9 months of age decreasing in severity as the plant
grew. The HON content of the plant did not have any relation
with the disease.

The pathogen was inhibited _in vitro_ by Agrimycin-100,
Streptocycline, Streptomycin and Terramycin. The organism
is presumed to be toxigenic since the cell free extract
produced tip burn and arrest of growth of the terminal
leaves and bud and partly mimicked the symptoms of the
disease.