ETIOLOGY OF THE BACTERIAL WILT OF GINGER INCITED BY *Pseudomonas solanacearum* E. F. SMITH AND ITS CONTROL

BY

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THESIS

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DECLARATION

I hereby declare that this thesis entitled " Etiology of the bacterial wilt of ginger incited by Pseudomonas solanacearum E.F. Smith and its control " is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, followship or other similar title of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "Etiology of the bacterial wilt of ginger incited by *Pseudomonas solanacearum* E.F. Smith and its control" is a record of research work done independently by Kum. Mary Kutty Samuel under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Introduction

INTRODUCTION

Ginger (Zingiber officinale Rosc.) constitutes one of the five most important major spices of India. It is of great economic significance to India, and especially to Kerala, being a produce which earns foreign exchange. Apart from being used as a spice in homes and in processed foods, ginger finds its use as a constituent in soft drinks, alcoholic beverages and medicines. Ginger oil is used as flavourant in food, for pharmaceutical uses, and in perfumery.

India has been producing and exporting ginger from very ancient times and still enjoys the unique position of being the largest producer and exporter in the world. India contributes 50 per cent of the ginger production of the world, and Indian ginger is considered to be of high quality. It is grown in India over an area of 26,830 hectares distributed mainly in the States of Kerala, Karnataka, Madhya Pradesh, Himachal Pradesh, Orissa and West Bengal. The annual production of ginger in the country is 43,550 tonnes of which about 70 per cent is from Kerala State alone.

As in the case of other crops, ginger is also prone to a number of diseases, among which the bacterial wilt

incited by Pseudomonas solanacearum E.F. Smith is of recent occurrence in India. This disease has proved to be one of the most destructive diseases of the crop which can inflict total crop losses.

As early as 1953, this disease was reported from Mauritius, and later from other ginger growing areas of the world such as Queensland, Malaya, Philippines and Brazil. In Kerala it was first observed during the monsoon months of 1976, in the Ambalavayal and Sultan's Battery areas of Calicut district and Adippukamba area of Trivandrum district. In these areas, the disease was seen to occur in epiphytotic proportions, resulting in very severe crop losses.

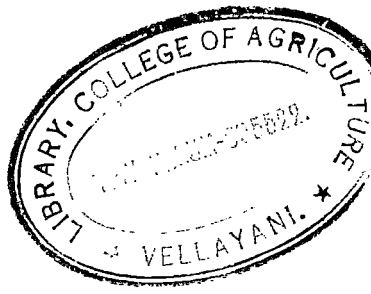
The causal agent of the disease, Pseudomonas solanacearum is one of the most destructive pathogens in the warm, tropical regions of the world. The agro-climatic conditions of Kerala are quite conducive for the large scale development of the bacterial wilt of ginger. The disease being primarily seed-borne, under such conditions, the chances of spread of the disease to other ginger growing areas of the State are also quite high.

Taking into consideration the potential crop losses the disease can cause to ginger cultivation in Kerala and the lack of earlier detailed studies on the disease, investigations were taken up on the following aspects:-

1. Symptomatology of the disease
2. Characterisation and identity of the pathogen
3. Survival of the organism
4. Screening of antibiotics under *in vitro* conditions and *in vivo* control of the disease using the most effective ones
5. Role and association of root-knot nematodes in the disease
6. Toxigenicity of the pathogen

The results of the above studies are presented in this thesis.

Review of Literature



REVIEW OF LITERATURE

The bacterial wilt disease of solanaceous crops caused by Pseudomonas solanacearum was first described by Smith (1896), who reported it on potato (Solanum tuberosum), tomato (Lycopersicon esculentum) and egg plant (Solanum melongena).

The earliest report of Pseudomonas solanacearum causing wilt of edible ginger (Zingiber officinale Rosc.) was made by Orian (1953) from Mauritius. Ishii and Aragaki (1963) reported the disease from Hawaii, which was previously unreported in the western hemisphere. Subsequently Jamil (1964) observed the disease in Malaya. Hayward et al.(1967) and Pegg et al.(1974) reported heavy losses in Queensland due to ginger wilt. Zehr (1969) observed the disease in Philippines. It was reported by Chew (1969) that bacterial wilt has been recognized as the greatest problem in ginger production in peat soils in Malaysia. Recently, Sarma et al.(1978) and Mathew et al.(1979) reported the disease from India.

SYMPTOMATOLOGY

Ishii and Aragaki (1963) has described the symptoms of bacterial wilt of ginger in detail. The initial symptoms

were a slight yellowing and wilting of the lowermost leaves. As the wilt progressed upward, it affected the younger leaves, followed by complete yellowing and browning of the entire shoot. They observed that under favourable conditions for disease development, the entire shoot became flaccid and wilted with little or no visible yellowing. Young succulent shoots frequently became soft and completely rotted, and the diseased shoot broke off from the underground rhizome at the soil line. On splitting the shoot longitudinally, vascular discolouration was observed. The underground parts were also completely infected. The infected rhizomes in early stages of infection showed translucent water soaked lesions which were localised first, but later involved major part of the rhizome. In advanced stages, the bacteria broke out into pockets within the rhizome, causing extensive browning and breakdown of tissues with the production of an offensive odour unlike that of natural ginger.

It was observed by the above authors that unlike the solanaceous hosts of Pseudomonas solanacearum, rapid wilt did not usually occur in the case of ginger. The infected

plant was stunted, yellowed, and the lower leaves remained dried for an extended period of time, until the plant was finally killed.

Begg et al.(1974) also described the symptoms of the disease as observed by Ishii and Aregaki (1963).

Sarma et al.(1978) described the initial symptoms as water soaked linear streaks on the collar region of the pseudostems. Mathew et al.(1979) reported that the first symptom of the disease was loss of turgidity of the leaves, followed by their rolling and yellowing. They observed that when infected portions of the plant were kept in water, milky bacterial ooze was obtained.

CAUSAL PATHOGEN

In general, the bacterium Pseudomonas solanacearum was a gram negative, motile rod with 1-4 polar flagella (Hodgkiss,1964). Colonies on solid media were usually small, round, slightly raised, glistening white and smooth, 3-5 mm in diameter and appeared within 36 - 48 hr at 28°C. Buchanan and Gibbons (1974) reported that at least two types of colonies were produced by the pathogen

on complex media, one type was smooth, fluidal, elevated, and the other was somewhat rough, dry and flat. Some strains produced a diffusible brown pigment on complex media.

Hayward (1964) studied the characteristics of Pseudomonas solanacearum and reported that on agar medium containing tyrosine, a diffusible brown pigment was produced, the intensity of which might vary between isolates. He further observed that none of the isolates studied produced a green fluorescent pigment on King's medium. Acid was produced oxidatively from glucose, sucrose, fructose, glycerol and meso-inositol in 2 - 6 days at 25°C. Partial reaction was noticed in galactose medium and no acid was produced from arabinose, lactose, maltose, cellobiose, mannitol, sorbitol, dulcitol, inulin, raffinose, salicin, meso-erythritol, dextrin, rhamnose, melibiose, amygdalin or sorbose in 21 days. Catalase and oxidase were produced by the organism and citrate but not malonate was utilized as the sole source of carbon. Nitrate reduction and ammonia production were positive. It did not hydrolyse soluble starch or produce

indole. An alkaline reaction was produced in litmus milk and growth occurred in 0.5 and 1 per cent but not in 2 per cent sodium chloride broth. Growth of the bacterium occurred at 37°C, but not at 40°C and slight gelatin liquefaction took place on prolonged incubation (2-4 weeks). Tween-80 was hydrolysed by the pathogen (4-6 days). Okabe and Cote (1954) found that only virulent, fluidal isolates of the pathogen could liquefy gelatin. With respect to catalase activity, Abo-El-Dahab and El-Gazzani (1972) reported that it was greater in the resting cell suspension of a virulent isolate than in an avirulent isolate of the pathogen.

Xanthomonas solanacearum was a complex species consisting of several races differing in many characters. Kelman (1954) reported that the colony morphology was related to virulence. He distinguished colony variants on Tetrazolium chloride (TTC) medium. There were the normal or wild type colonies which were irregularly round, entire, white or white with light pink centre and the mutant or butyrous type which were round, translucent, smooth, deep red with a narrow bluish border. Culture from the butyrous type caused little or no damage, whereas those from the normal type were very injurious to hosts when inoculated. Quinon et al.(1964) reported that

strains of Pseudomonas solanacearum which produced brown pigment abundantly in media and diseased tissues did not ferment lactose.

Based on the capacity to oxidise 3 disaccharides (lactose, maltose and cellobiose) and 3 hexose alcohols (mannitol, sorbitol and dulcitol), Hayward (1964) classified a collection of 165 isolates of Pseudomonas solanacearum into four biotypes. Isolates of biotype-1 oxidised neither group, biotype-2 only the disaccharides, biotype-3 both the groups, and biotype-4 only the hexose alcohols. Biotype-2 appeared to have a restricted host range and it was obtained solely from two host plants, potato and tomato, whereas the other biotypes were obtained from many families in addition to Solanaceae.

The pathogen lost its virulence very rapidly in culture due to transformation to avirulent forms and the virulence could be maintained by storing Potato dextrose agar slant cultures under sterile mineral oil at 25°C (Kelman and Jenson, 1951) or in sterile distilled water (Kelman and Jenson, 1961).

Klement (1963) described a method for the rapid detection of pathogenicity in phytopathogenic Pseudomonads.

He reported that only pathogenic bacteria produced necrotic lesions within 24 - 48 hr of infiltration of the bacteria on tobacco leaves.

In the past, only very little work has been done on the characterization of the ginger isolate of Pseudomonas solanacearum. However, Ishii and Aragaki (1963) reported that morphologically the bacterium was a gram negative rod with single polar flagellum. Colonies on nutrient agar were dull greyish white, which became quite watery or fluidal and showed distinct chromogenesis, producing a brown pigment in the media.

Quinon (1963) reported that the thermal death point of the pathogen was 52°C. Quinon et al., (1964) observed that it grew best in in vitro at 34°C. They compared the colonin production of isolates of Pseudomonas solanacearum from tomato, ginger and bird of paradise, and found that the ginger isolate was intermediate among the three.

Mathew et al.(1979) conducted studies on the bacterium and observed that on Potato dextrose agar the colonies were small, circular, white, smooth and slimy; on Tetrazolium chloride agar pink centred colonies were obtained. The

organism utilised ordinary carbon compounds like glucose, sucrose and dextrose. It did not produce hydrogen sulphide or indole, but produced ammonia in traces. Milk was slightly curdled with production of acid. Growth was slightly inhibited in 2 per cent sodium chloride broth.

Orian (1953) reported that the ginger wilt bacterium produced typical wilt of tomato. Zehr (1970) isolated a strain of the pathogen from ginger which was virulent to tomato but avirulent to potato and egg plant. He also observed that the isolates from these host plants were not virulent to ginger an artificial inoculation. Lum (1973) isolated a weakly virulent form of Pseudomonas solanacearum from ginger which did not produce symptoms on tomato, tobacco and groundnut. Pegg et al.(1974) reported that biotypes 3 and 4 of the pathogen were responsible for the wilt of ginger, out of which biotype-3 also caused typical wilt of tomato.

SURVIVAL OF THE PATHOGEN

Long periods of survival of Pseudomonas solanacearum in soil has been reported by several workers. Martin (1939)

studied the survival of the pathogen and reported that it was carried in the soil. Miller et al.(1941) found that the organism persisted in the soil of plots formerly occupied by its various hosts. Survival of race 1 of Pseudomonas solanacearum in bare-fallowed field plots for 4 years was reported by Smith (1944). Das and Chattopadhyay (1956) could observe that this pathogen infecting brinjal survived for 16 months in infested soil. It was reported by Robinson and Ramos (1964) that contaminated soils served as source of inoculum for Pseudomonas solanacearum. Dukes et al.(1965) reported that race 3 of the pathogen could survive in soil for a period of 1 year after cultivation and clearing of tomato. The organism was also able to remain endemic in the soil and become pathogenic in the presence of suitable hosts (Seneviratne, 1969). Potato wilt pathogen was reported to survive for 4 years in a heavily infested field (Graham,1978)

Infected plant debris has been reported to serve as a source of survival of the pathogen. Das and Chattopadhyay (1956) reported that Pseudomonas solanacearum survived in infested plant remains of brinjal for a period of 9 months. Lloyd (1978) suggested that contaminated debris of potato was important in the carry over

of the pathogen between successive crop plantings. Graham et al.(1979) reported that infected debris collected from a heavily infested field of potato and mixed with non-infested soil, remained so for a period of 32 weeks. They suggested that infested debris could serve as a short term survival site for Pseudomonas solanacearum in the field.

Infected seed materials also can serve as a source of survival and primary inoculum of the pathogen as was evident from the reports of earlier workers. Robinson and Jones (1964) have reported that infected seed tubers of potato could serve as source of inoculum of Pseudomonas solanacearum. Nikitina and Kersakov (1978) observed that the organism could survive for a period of 2-3 years in seeds of soybean. Lloyd (1978) and Graham et al. (1979) reported that latently infected seed tubers could serve as a long term sheltered site for the survival of the pathogen.

Detailed studies have not been conducted so far on the survival of the ginger wilt pathogen. However, Pegg and Moffett (1971) reported that biotypes 3 and 4 of Pseudomonas solanacearum causing wilt of ginger survived a 20 month period of severe drought in Queensland. Further,

Jose et al.(1974) observed that the pathogen could survive in infected seed rhizomes of ginger. When these diseased rhizomes rotted, the organism survived in the soil and attacked the next crop. In addition, the pathogen was able to infect a wide range of weeds in which it could survive from season to season. Mathew et al.(1979) have suggested that infected seed rhizomes of ginger could serve as primary inoculum of the pathogen.

CONTROL OF THE DISEASE BY CHEMICALS

Attempts have been made by many scientists to test the *in vitro* sensitivity of *Pseudomonas solanacearum* to chemicals. Hoagon and Goodman (1955) reported that low concentrations of oxytetracycline and terramycin effectively inhibited the pathogen. According to Hidaka and Murano (1956), streptomycin at 0.5 µg per ml of water inhibited the pathogen and 5 µg per ml killed the pathogen at once. Foucart and Delcambe (1960) tested various chemicals in *in vitro* and found that actinomycin and chloramycetin gave promising results in inhibiting the pathogen. Campaced et al.(1962) reported that among the various chemicals tested, the bacterium was most sensitive to agristep (streptomycin).

penicillin-G-potassic, penicillin procain, dihydro-streptomycin sulphate and erythromycin.

Chakravarti and Rangarajan (1966) reported that streptocycline could control Pseudomonas solanacearum in in vitro. Goorani et al.(1978) could inhibit the pathogen by ampicillin, chloramphenicol, kanamycin, oxytetracycline, tetracycline, penicillin-G, streptomycin, nabam (dithane-A-40), maneb (dithane M-22), dithane M-45 and the insecticide chlorothion. Mondal and Mukherjee (1978) reported that ampicillin, streptomycin and novobiocin at 500 ppm, each were of promise against the pathogen in in vitro.

Hidaka and Murano (1956) conducted studies on the control of Pseudomonas solanacearum in in vivo by surface absorption of streptomycin. One spray containing 110 µg per ml of water given the day before inoculation and a second spray 5 days after inoculation could control tobacco wilt. Kotsuo and Kawatsu (1957) reported that the pathogen inoculated in broad bean was inhibited by streptomycin at 500 µg per ml and 1000 µg per ml of water. Foucart (1959) recommended antibiotic spray as a curative treatment against the pathogen infecting potato. Dutta and Vasan (1969) could obtain good control of bacterial wilt

of egg plant by treating seedlings for 30 minutes before planting with streptocycline at 1 g in 40 litres of water.

Shetty and Rangaswami (1969) obtained control of brown rot of potato with C_6, an antibiotic similar to erythromycin at 500 ppm and 1000 ppm. Fairly high dosage of 2 amino-1, 3,4-thiodiazole (ADTA) could control bacterial wilt of tomato. Rahim (1972) and George (1975) reported that spraying streptomycin or streptocycline or drenching with chaghnut compound was effective for control of bacterial wilt of chillies in Kerala. Mondal and Mukherjee (1978) reported that inoculated tomato plants were protected against the pathogen by 6-amino penicillic acid and streptomycin sulphate. Mishra and Ghosh (1978) observed that the systemic fungicide Bavistin sprayed at 0.07 per cent effectively reduced the incidence of wilt of jute caused by Pseudomonas solanacearum.

Detailed studies have not been conducted on the chemical control of bacterial wilt of ginger. Preliminary studies conducted by Ishii and Aragaki (1965) indicated that good control of this disease could be obtained by soil fumigation with methyl bromide at the rate of 3 lb per 100 sq.ft of soil. Fegg et al.(1974) and Sarma et al.(1978) have

observed that chemical methods have not been successful in controlling bacterial wilt of ginger.

ROLE AND ASSOCIATION OF ROOT-KNOT NEMATODES

Kunger (1901) presented evidence to show that root-knot nematodes provided root wounds necessary for infection by Pseudomonas solanacearum. Numerous investigators have noted a correlation between high wilt incidence and heavy root-knot nematode infestation in the field (Kelman,1953). Lucas et al.(1955) reported that moderately wilt resistant tobacco plants of the variety Dixie Bright 101, growing in soils to which both Pseudomonas solanacearum and Meloidogyne incognita acrita had been added, developed wilt symptoms sooner and more extensively, than those in soil infested with the bacterial pathogen alone. These authors have concluded that the role of the nematodes might be mainly that of providing wounds through which the bacterium entered. Fitcher (1963) also supported this view.

Libman et al.(1964) conducted green-house experiments and reported that Meloidogyne hapla increased the

incidence and severity of tomato wilt caused by Pseudomonas solanacearum. Halim (1966) observed that Meloidogyne incognita acrita predisposed certain varieties of tomato to bacterial wilt by interfering with potassium nutrition.

Johnson and Powel (1969) have reported that in the case of bacterial wilt susceptible tobacco plants, the presence of Meloidogyne incognita greatly increased the wilt severity. If the plants were inoculated with the nematodes 3 or 4 weeks prior to inoculation with Pseudomonas solanacearum, about 50 per cent more disease developed than if the pathogens were added simultaneously.

Mukaidono and Sakaegawa (1972) conducted studies on the influence of Meloidogyne incognita on the occurrence of Granville wilt of tobacco, and put forward several findings. A simultaneous inoculation of the nematode and the bacterium hastened the occurrence and furthered the progress of the disease, compared with bacterial inoculation alone or with artificial wounding. Furthermore, when the bacterial inoculum was kept constant, the disease became more severe as the number of nematodes increased. Inoculation of a low bacterial population caused the disease only when

the nematodes were applied simultaneously with the bacteria.

Reddy et al.(1979) suggested that root-knot nematodes were probably responsible for breaking bacterial wilt resistance in Pusa Purple Cluster variety of brinjal, by acting as a modifier of plant tissue in such a way that it became more suitable for bacterial colonization.

TOXIGENICITY OF THE BACTERIUM

The mechanism by which vascular pathogens cause wilting in plants has been a controversial subject for a number of years (Husain and Kelman, 1958). Hutchinson (1913) based on his studies with Pseudomonas solanacearum, reported that wilting was due to the formation of a systemic toxin that altered cellular permeability. His paper is frequently cited as the first report that attributed pathological wilting of plants to the formation of a systemic toxin by a vascular pathogen.

Hutchinson's concept of toxigenic wilting was supported by Kunz (1952). He obtained two toxic fractions from 2-5 months old beef broth culture of the bacterium. He suggested that the first substance was a bacterial slime

(a polypeptide complex) that caused wilting by its physical action in plugging the water conducting tissue of the plant. The second toxic substance was considered to be a plasma poison that caused wilting by destruction of the semipermeable property of the plasma membrane. Dunn also suggested that the bacterial slime probably was converted to toxic substances by the action of the enzymes from the host plant.

Pectinolytic and cellulolytic enzymes were reported in tomato plants infected by Pseudomonas solanacearum (Kingstead and Walker, 1954; Husain and Kelman, 1957). These were found to be absent in healthy plants. This indicated that the parenchymatous breakdown in infected plants could be attributed to the production of these enzymes by the pathogen.

Husain and Kelman (1958) made detailed studies on the mechanism of wilting by Pseudomonas solanacearum. They observed that culture filtrates of slime forming virulent strains of the bacterium contained a heat-stable polysaccharide that played the primary role in wilting. These culture filtrates were found to contain heat-

constitive cellulase as well as pectic enzymes. Heating these filtrates slightly reduced their ability to wilt tomato cuttings.

Tomato cuttings placed in the culture filtrate of the highly virulent strain of the bacterium showed a pronounced softening of the stem immersed in the solution. Cuttings placed in heated culture filtrates showed no evidence of maceration of tissues. When the cuttings were transferred to water, those cuttings that had been placed in non-heated culture filtrates recovered only partially while those transferred from heated culture filtrates recovered completely (Husain and Kelman, 1958).

It was observed by the above workers that the heat-stable viscous material precipitated from the culture filtrate of the pathogenic strain was a complex polysaccharide with glucose as the main component. Aqueous solution of this polysaccharide also caused wilting of tomato cuttings. Excised leaves from these cuttings recovered turgor when placed in water.

According to Waine (1960), extracellular hydrolytic enzymes and toxins produced by Pseudomonas solanacearum

affected the structural integrity and essential physiological process of host tissues respectively. Gowda et al.(1977) studied the biological properties of a toxic compound isolated from Pseudomonas solanacearum. They conducted tests of partially purified toxin on various host plants and found that it was non-specific. Cuttings of host plants including Lycopersicon esculentum, Capsicum annum, Nicotiana tabacum and Solanum melongena when treated with 0.2 per cent aqueous toxin solution wilted, whereas plants kept in distilled water as control did not wilt.

Materials and Methods

MATERIALS AND METHODS

ISOLATION AND PATHOGENICITY OF DIFFERENT BACTERIAL ISOLATES

Healthy seed rhizomes of the ginger variety Rio-De-Janeiro were brought from the Horticultural Research Station, Ambalavayal and raised in pots. This variety was solely used in this study, unless otherwise stated.

Naturally wilted ginger plants of the above mentioned variety were collected from the disease affected areas of Ambalavayal in Calicut district and Adipperamba in Trivandrum district. The pathogen was isolated using the method similar to that of Ishii and Aragaki (1963). The discoloured portions of rhizome and pseudostem were cut and ooze test was done to ensure the presence of bacteria. Such pieces giving profuse ooze were selected for isolation of the bacterium. These were surface sterilized in 0.1 per cent mercuric chloride solution for one minute and serially passed through three changes of sterile distilled water. Two or three pieces of the material were transferred to a sterilized glass slide and a few drops of sterile distilled water were added to it. The pieces were teased apart using a pair of sterilized forceps to get a bacterial suspension. The

bacterium was isolated from the suspension by streaking over Tetrazolium chloride (TTC) medium. The plates were incubated at 30°C for 48 hr. Characteristic isolated, single colonies of Pseudomonas solanacearum were selected as described by Kelman (1954). These were again purified by streaking over the same medium.

Composition of TTC medium (Kelman, 1954)

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

Hundred ml portion of the medium was poured in 250 ml conical flasks and sterilised by autoclaving at 15 lb pressure for 20 minutes. One per cent solution of 2,3,5 - triphenyl tetrazolium chloride (TTC) was prepared in distilled water, sterilised by autoclaving for eight minutes and stored in the dark. Before plating the medium, 0.5 ml of this solution was aseptically transferred to the medium in each flask using a sterilised pipette.

to give a final concentration of 0.005 per cent.

Pathogenicity tests were conducted with the suspension of the bacteria in sterile distilled water with O.D. of 0.5. This was prepared from 24-48 hr old cultures of the bacterium grown on PDA. Vigorously growing 2 months old ginger plants were used for inoculation. The bacterial suspension was inoculated into the leaf axils and basal part of the pseudostem with a 5 ml syringe and 24 gauge hypodermic needle. Cotton dipped in the bacterial suspension was placed at the injured portions. In some cases, the roots of the plants were injured with a spatula, and the soil was drenched with the bacterial suspension (Ishii and Aragaki, 1965). The inoculated plants were maintained under high humidity conditions.

The inoculated plants were observed for symptom development. The pathogen was reisolated from the artificially inoculated plants, and the single colonies were compared with that of the original cultures.

Stock cultures of the isolates were maintained according to the method described by Kelman and Jensen (1951). Virulent, isolated, single colonies of the bacterium on

KNO medium were selected and streaked on Potato dextrose agar (PDA) slants. After 48 hr sterile mineral oil (Squibb, medicinal grade) was added aseptically to each tube to a level of 1 cm above the upper edge of the agar slant. The cultures were then stored at 30° ± 2°C.

Four isolates of the bacterium were used in the present study, the details of which are given below:

Isolate Number	Locality from which the diseased specimen was collected	Cultivar from which isolation was made	Year of isolation
Po-1	Ambalavayal, Calicut district	Rio-De-Janeiro	1978
Po-2	Adipparamba, Trivandrum district	Rio-De-Janeiro	1978
Po-3	Vellayani, Trivandrum district	Rio-De-Janeiro	1979
Po-4	Ambalavayal, Calicut district	Rio-De-Janeiro	1979

SYMPTOMATOLOGY

Symptoms of the disease were studied under natural and artificially inoculated conditions. The four

isolates of the bacterium were inoculated on healthy ginger plants to study the development of symptoms. Artificial inoculations were made as in the case of pathogenicity tests and the sequence of symptom development was closely observed.

CHARACTERIZATION OF THE PATHOGEN

The different isolates of the bacterium were characterized and identified following the methods recommended in the 'Manual of Microbiological Methods' (Anon., 1957) and those described by Hayward (1964) and Dye (1962). Tests were conducted in triplicates and incubation was carried out at 30° ± 2°C.

A. Cultural characters

1. Morphology

Morphology of the bacterial isolates was studied using 24 hr old cultures grown on Peptone casamino acid medium. Gram reaction was also studied.

2. Comparison of the growth characters of the different isolates on Peptone casamino acid medium

A loopful of the dilute suspension of the bacterial isolates was streaked over Peptone casamino acid medium

poured in plates. Growth characters were studied after 24 hr and 48 hr of incubation.

9. Pigment production

Production of water insoluble pigment was tested on Yeast glucose chalk agar (YGCA) medium. 48 hr old growth of the isolates was tested for pigment production.

Production of water soluble pigment was studied on King's medium (King et al., 1954) the composition of which is:

Peptone	-	20.0g
Glycerine	-	10 ml
$K_2H\ PO_4$	-	1.5g
$Mg\ SO_4 \cdot 7H_2O$	-	1.5g
Agar agar	-	20.0g
Distilled water	-	1000 ml
pH	-	7.0

The test cultures were spot inoculated on the medium poured in petri plates, incubated for 48 hr and examined for zone of pigmentation around the colonies.

4. Oxygen requirement

Nutrient dextrose agar (containing 0.005 per cent bromocresol purple) columns in test tubes were inoculated with the culture of each bacterial isolate by stabbing with a straight inoculation needle. The agar surface of one set of tubes were covered with sterile liquid paraffin to a depth of 1 cm. The tubes were incubated and observations were recorded periodically.

5. Physiological characters

The four bacterial isolates were compared for their physiological properties. In each case observations were compared with uninoculated control.

i. Mode of utilisation of glucose

To study the mode of utilisation of glucose, the method of Hugh and Leifson (1953), modified by Hayward (1964) was followed:

Basal medium

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

One per cent glucose was added to the above medium and dispensed in tubes upto a depth of 4 cm, and sterilised by tyndallization. They were inoculated by stabbing with a straight inoculation needle charged with bacterial growth. In one set of tubes, the medium was sealed with 1 cm layer of sterile liquid paraffin. The tubes were incubated and observations were recorded at regular intervals for 15 days.

2. Starch hydrolysis

Ability of the bacterial isolates to hydrolyse starch was studied using Nutrient agar containing 0.2 per cent soluble starch (Anon., 1957).

Composition

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

The isolates were spot inoculated on the medium poured in plates. After 4 days of incubation, starch

hydrolysis was tested by pouring Lugol's iodine over the plates. A colourless zone around the bacterial growth indicated positive starch hydrolysis, compared to the blue background of the medium.

3. Production of levan

Production of levan, indicated by the presence of large, white, domed and mucoid colonies was detected on Peptone beef extract medium with 5 per cent sucrose (Anon., 1957). The medium was sterilized by tyndallization. Dilute suspensions of the isolates were streaked over the medium and observed after 48 hr.

Composition of the medium

Peptone	-	10.0g
Beef extract	-	5.0g
Sucrose	-	50.0g
Agar agar	-	20.0g
Distilled water	-	1000 ml
pH	-	7.0

4. Production of hydrogen sulphide

The ability of the bacterial isolates to liberate hydrogen sulphide was tested using Peptone water medium

with 1 per cent cysteine acid (Anon., 1957).

Composition

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

Five ml aliquots of the medium were dispensed in test tubes and autoclaved. Whatman No.1 filter paper strips of 5 x 50 mm size were cut and soaked in warm, saturated solution of lead acetate. The strips were dried, autoclaved and again dried. The tubes were inoculated in triplicate with the different isolates of the bacterium and the strips were inserted aseptically by the side of the plug in the tubes and suspended over the broth. The tubes were incubated and observations recorded at regular intervals upto 14 days. Liberation of hydrogen sulphide was indicated by the blackening of the test strips.

5. Methyl Red and Vogen - Proskauer tests (M.R. and V.P. tests)

Methyl red broth was used for both tests.

Decel medium for M.R. and V.P. Tests (Anon., 1957)

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

Five ml aliquots of the medium were dispensed in test tubes and sterilised by tyndallization. Two sets of tubes were inoculated with 48 hr old cultures of the isolates for M.R. and V.P. tests respectively. The tubes were incubated for 7 days.

For M.R. test a few drops of 0.02 per cent methyl red in 50 per cent alcohol was added to the culture tubes. A distinct red colour indicated positive methyl red reaction.

For V.P. test 0.6 ml of alpha-naphthol solution (5 per cent in 95 per cent alcohol) and 0.2 ml of 40 per cent aqueous solution of KOH was added to 1 ml of the culture. The mixture was shaken for few minutes and allowed to stand for 2 hr. A crimson or ruby colour indicated positive V.P. test.

6. Reduction of nitrate

Nitrate broth (Anon., 1957) was used for the test.

Composition

Peptone	-	10.0g
Beef extract	-	5.0g
KNO_3 (nitrite free)	-	1.0g
Distilled water	-	1000 ml
pH	-	7.0

Five ml columns of the broth taken in test tubes were autoclaved, cooled and inoculated with the test cultures. Few drops of sulphanilic acid (0.8 per cent in 5 N acetic acid) and dimethyl alpha-naphthyl-amine (0.5 per cent in 5 N acetic acid) were added to the nitrate broth culture and observations recorded for 15 days at regular intervals (Wallace and Reeve, 1927; Mittaler, 1930). Distinct pink or red discolouration of the medium indicated the presence of nitrite. If there is no colour change of the medium it suggested that nitrate was present as such or has been reduced to ammonia and free nitrogen. To confirm this, few

zinc crystals were added to the above broth - reagent mixture and agitated for few minutes. Change in colour of the broth to pink or red indicated the presence of nitrate. Absence of colour in either of the above two tests would mean that nitrate was reduced to ammonia and/or free nitrogen.

7. Gelatin liquefaction

Nutrient gelatin medium was used for this test.

Composition

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

The medium was dispensed in test tubes to a depth of 4 cm and sterilised at 10 lb pressure for 20 minutes. The medium was observed for 2 days to check its sterile condition. Forty eight hr old cultures of the isolates of the bacterium were stab inoculated in the properly sterilized gelatin column. The tubes were incubated and

were observed for the liquefaction of the gel column at regular intervals upto 1 month.

9. Production of indole

Tryptophan broth medium (Anon., 1957) was used for this test.

Composition

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

The medium was dispensed in tubes and autoclaved. Filter paper strips of size 5 x 50 mm were soaked in warm saturated solution of oxalic acid and cooled. When the strips got covered with oxalic acid crystals, they were dried at room temperature and used without sterilising.

The tubes were inoculated with the isolates in triplicate and oxalic acid strips were inserted into the tubes by the side of the plug. The tubes were incubated for 14 days. Change in colour of the oxalic acid crystals to pink or red indicated indole production.

9. Catalase test

To assess the production of catalase enzyme by the isolates, a loopful of 24 hr old culture of each was smeared on a glass slide and covered with few drops of 20 volume Analar hydrogen peroxide solution. The production of gas bubbles indicated catalase positive reaction.

10. Action on milk

Action of the bacterial isolates on milk was tested in bromocresol purple milk. Both skimmed and unskimmed milk were used. A 1:3 dilution of skimmed milk was prepared in water and bromocresol purple was added to give a final concentration of 0.002 per cent, when a light blue colour was obtained (Clark and Lubs, 1917). Unskimmed milk (containing approximately 3 per cent fat) was also diluted with water and bromocresol purple was added as above. The milk medium was then dispensed in 5 ml aliquots in test tubes and sterilised by tyndallisation. The medium was inoculated with a loopful of 48 hr old test bacterium and incubated. Observations were recorded periodically for 30 days, for acidic or alkaline reaction, curdling and peptonisation. Change of the light blue

colour of the medium to yellow indicated acid reaction and to violet indicated alkaline reaction. Curdling was indicated by the heterogenous clumps due to precipitation of casein. Peptonisation was indicated by the partial clearing of milk.

11. Tyrosinase activity

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

Composition

KH_2PO_4	-	0.0g
K_2HPO_4	-	0.5g
$MgSO_4 \cdot 7H_2O$	-	0.2g
NaCl	-	5.0g
Yeast extract	-	5.0g
Tyrosine	-	0.5g
Agar agar	-	20.0g
Distilled water	-	1000 ml
pH	-	7.0

The medium was dispensed in test tubes, autoclaved, and slants were prepared. They were inoculated with the four isolates and incubated.

Tyrosinase is the enzyme capable of converting the amino acid tyrosine to the brown pigment melanin. Melanin production was estimated as high, medium or low based on the intensity of the brown colouration observed after 48 - 72 hr growth of the bacterium.

12. Production of ammonia

The accumulation of ammonia was detected using Nessler's reagent which gives a brown precipitate with ammonia. The isolates were grown in autoclaved Peptone water medium in test tubes. After incubation for 48 hr the reagent was added to the tubes and the precipitate developed was noted.

13. Arginine hydrolase test

Medium used was Thornley's semisolid arginine medium (Thornley, 1960).

Composition

Peptone	-	1.0g
K_2HPO_4	-	0.3g
NaCl	-	5.0 g
Agar agar	-	3.0g
Phenol red	-	0.01g
L-arginine monochloride	-	10.0g
Distilled water	-	1000 ml
pH	-	7.2

Five ml aliquot each of the semi-solid medium was dispensed in test tubes, autoclaved, cooled and stab inoculated with the test cultures. Sterile liquid paraffin was layered over the medium to a depth of 1 cm. The tubes were incubated and observations recorded for 7 days at regular intervals. A change in the colour of the medium to red indicated arginine hydrolase activity.

14. Urease test

The medium of Christensen's urea agar (Christensen,1946) was used to test urease activity of the isolates.

Composition

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

Ninety ml aliquots of the medium were dispensed in 250 ml flasks and autoclaved. To each flask 10 ml of 20 per cent urea solution, sterilized by filtration was added

and dispensed in tubes in 5 ml quantities and slants were prepared. The plants were inoculated with the test cultures and observations were recorded periodically. Colour change of the medium from yellow to red indicated positive urease activity.

15. Utilisation of asparagine as the sole source of carbon and nitrogen

The test was performed in Dye's medium (Dye, 1966).

Solution 1

K_2HPO_4	- 5.0g
KH_2PO_4	- 2.0g
Distilled water	-1000 ml

Solution 2

$MgSO_4.7H_2O$	- 2.0g
$FeSO_4$	- 0.5g
NaCl	- 1.0g
$MnSO_4$	- 0.2g
H_2SO_4	- 1 drop
Distilled water	- 1000 ml

Solution 3

$Na_2 MoO_2$ - 0.02g

Distilled water - 100 ml

Solution 4

$CaSO_4$ - saturated solution in distilled water.

Ten ml of each solution was mixed in the order of 3,4,2 and 1 and filtered and 960 ml of distilled water and 2g of L-asparagine were added, dispensed in 5 ml aliquots in test tubes and sterilized by autoclaving. The different isolates of the bacterium were inoculated into the medium, incubated and examined for growth. Positive growth meant that asparagine was utilized as the sole source of carbon and nitrogen.

16. Sodium chloride tolerance test

Peptone water, with different concentrations of NaCl viz., 1 per cent, 2 per cent, 3 per cent and 5 per cent was used for the test.

The different isolates were inoculated into the medium containing the different concentrations of sodium chloride, incubated and observed for growth.

17 Utilization of carbon sources

The following carbon compounds were tested for utilization by the isolates as indicated by the production of acid:- Glucose, galactose, fructose, mannose, lactose, maltose, xylose, raffinose, ribose, dextrose, sucrose, sorbose, glycerol, salicin, cellulose, cellobiose, inositol, sorbitol, dulcitol and mannitol.

The medium of Ayers, Rupp and Johnson (1919) was utilized as the basal medium.

Composition

$NH_4 H_2PO_4$	- 1.0g
$MgSO_4 \cdot 7H_2O$	- 0.2g
KCl	- 0.2g
Agar agar	-20.0g
Bromocresol purple	- 0.7 ml of a 5 per cent alcholic solution
Distilled water	-1000 ml
pH	- 7.0

The sugar to be tested was added to the medium to make a concentration of 1 per cent. It was then dispensed in test tubes, sterilised by tyndallisation and slants

were prepared. A loopful of each test culture was streaked on the medium and observations were recorded for 1 month. Change of colour of the medium from reddish violet to yellow indicated utilization of the carbon compound.

18. Utilization of organic acids

The above medium of Ayers, Rupp and Johnson (1919) was employed here also.

Sodium salts of the organic acids viz., citrate, formate, acetate and benzoate were added to the medium to make a concentration of 1 per cent. The medium was autoclaved after dispensing in tubes and slants were prepared. A loopful of the test cultures was streaked on the medium and observed for 1 month.

19. Hypersensitive reaction on tobacco leaves

Dilute suspensions of 24 hr old cultures of the isolates containing approximately 10^7 cells per ml were injected into the intercelluler spaces of leaves of tobacco using a fine hypodermic needle (Klement, 1963). Observations were recorded for formation of necrotic spots upto a period of 72 hr.

20. Cross inoculations of Pseudomonas solanacearum from ginger and tomato

Suspension of 24-48 hr old culture of the ginger wilt bacterium was inoculated at the leaf axils of healthy tomato plants. Pseudomonas solanacearum isolated from naturally wilted tomato plants was inoculated on healthy ginger plants also.

21. Growth characters of the isolate Ps-1 on different solid media

Growth characters of the bacterial isolate Ps-1 were studied on eight solid media. The media employed for the study and their composition are given below:-

1. Peptone casamino acid (PCA) medium

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

2. Tetrazolium chloride (TTC) medium

Peptone	-	10.0g
Casamino acid	-	1.0g
Glucose	-	5.0g
Agar agar	-	20.0g
Distilled water	-	1000 ml
Triphenyl tetrazolium chloride	-	0.5 ml of 1 per cent solution added to 100 ml of the above medium.

3. Potato dextrose agar (PDA) medium

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

4. Nutrient agar (NA) medium

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

5. Glucose agar (GA) medium

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

6. Yeast glucose chalk agar (YGCA) medium

Yeast extract	-	10.0g
Glucose	-	10.0g
Chalk ($CaCO_3$)	-	20.0g
Agar agar	-	20.0g
Distilled water	-	1000 ml
pH	-	7.2

7. Glucose yeast extract agar (GYA) medium

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

C. Hagal medium for Xanthomonads (HX)

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

A loopful of the dilute suspension of 24 hr old bacterium was streaked over the media poured in plates. These plates were incubated and observations were recorded after 24 and 48 hr.

SURVIVAL OF THE PATHOGEN

Studies on the survival of the ginger wilt bacterium in three different sources such as infected seed rhizomes, soil and plant debris, were carried out in the laboratory as well as in the field.

For laboratory studies, infected seed rhizomes and sick soil were brought from severely wilt affected plots of

Ambalavayal. Fortnightly isolations were carried out from diseased rhizomes on TTC medium and the development of typical colonies of Pseudomonas solanacearum was observed. Such isolations were carried out for a period of 5 months.

Survival of the bacterium in sick soil was studied by serial dilution plating conducted at fortnightly intervals. One g of soil was put in 99 ml of sterile distilled water and serially diluted to 10^{-6}, from which one ml each was plated in 5 plates. The average colony count in the plates was recorded and the population of the pathogen per g dry weight of soil was calculated.

Sick soil was also kept in pots under field conditions and the pathogenic population was studied at fortnightly intervals as in the above case. These studies were carried out for a period of 10 months.

For studying the survival of the bacterium under field conditions, infected materials were brought from wilt affected plots of Adippaxamba.

Fifty pots were filled with equal quantities of sick and disease free soil. The sick soil was spread over disease free soil taken in pots, and again covered with

one inch layer of disease free soil. Healthy ginger rhizomes were cut and surface sterilized with Ambistrym-S at 100 ppm for 15 minutes. They were then washed in distilled water and planted in the pots with 4 pieces per pot.

Survival of the pathogen in infected plant debris in soil was also studied. Fifty pots were filled to about three-fourth with disease free soil. The diseased material was chopped into small pieces and spread over it and again covered with one inch layer of disease free soil. Healthy seed rhizomes were planted in these pots as in the above case.

The above plantings were carried out in the same pots during May 1978 and 1979. In order to study the survival of the ginger wilt bacterium in infected seed rhizomes, 4 pieces each, were planted in pots filled with disease free soil. Such plantings were carried out at weekly intervals for 5 months.

The pots were watered regularly and observed for wilt symptoms. Ooze test was also performed in each case of suspected wilt incidence. Sections of rhizomes and

pseudostems of diseased plants were placed in sterile water and examined for streaming of bacterial ooze, in order to confirm the incidence of the disease.

Percentage wilt incidence in infected soil and plant debris in soil in each pot was recorded during the two seasons. These values were transformed to angles and analysed using Student's 't' test.

CONTROL OF THE DISEASE USING ANTIBIOTICS

The *in vitro* sensitivity of the bacterium to different antibiotics was tested. The following antibiotics were used for the purpose:-

1. Ambistryn-S: Sarabhai Chemicals, Baroda (Streptomycin sulphate I.P.)
2. Agrimycin-100: Pfizer Limited, Thane-Belapur Road, Thane.
3. Chloromycetin: Parke Davis (India) Limited, Bombay (Chloramphenicol and Sodium succinate).
4. Terramycin: Pfizer Limited, Thane-Belapur road, Thane.
5. Streptocycline: Hindustan Antibiotics Limited, Pimpri, Poona, India.
6. Ampicillin: Ranbaxy Laboratories Limited, Okhla, New Delhi.
7. Paushamycin: Paushak Limited, Baroda (Streptomycin + Oxytetracycline).
8. Tetracycline: Indian Drugs and Pharmaceuticals Limited, New Delhi (Tetracycline hydrochloride I.P.

The antibiotics were prepared at concentrations of 250, 500, 1000 and 1500 ppm in sterile distilled water. Sterilised filter paper discs of 10 mm diameter were dipped in the appropriate solutions. These were placed aseptically over Peptone casamino acid (PCA) medium seeded with 24 hr old culture of the bacterium and poured in plates. Filter paper discs dipped in sterile distilled water were used as control. The test was done in triplicate. The plates were incubated at 30°C. The diameter of the zone of inhibition was measured after 24 and 48 hr.

An *in vitro* study with combination of antibiotics was also attempted. In this, two antibiotics at 250 ppm each were combined. The test was replicated five times. The combinations tested were:-

1. Ambistryn-S and Chloromycetin,
2. Agrimycin-100 and Ampicillin,
3. Terramycin and Ambistryn-S.

The method employed for this study was the same as above.

The *in vitro* trials were analysed using 'F' test and significant results were compared by working out the Critical Difference (C.D.).

An *in vivo* trial was also conducted with three antibiotics which were found the most effective in the *in vitro* study viz., Ambistryn-S, Agrimycin-100 and Chloromycetin.

For this experiment, artificially inoculated one month old ginger plants were used. A mixed inoculum prepared from the cultures of the four bacterial isolates was used for inoculation. The experiment was laid out with seven treatments and five replications. Each antibiotic at 500 ppm was applied as pre-and post-inoculation sprays in separate treatments. In the former case the antibiotic was sprayed five days prior to bacterial inoculation, whereas in the latter case, it was sprayed twice after bacterial inoculation at an interval of five days in between. In the control treatment, there was bacterial inoculation only. The treatments were:-

T_1 = Ambistryn-S pre-inoculation spray

T_2 = Ambistryn-S post-inoculation spray

T_3 = Agrimycin-100 pre-inoculation spray

T_4 = Agrimycin-100 post-inoculation spray

T_5 = Chloromycetin pre-inoculation spray

T_6 = Chloromycetin post-inoculation spray

T_7 = Control

The plants were given sufficient humidity and wilt incidence in each treatment was observed. The results were analysed by the method of Classificatory Analysis. The observations were classified into groups so that the mean sum of squares between groups was significantly higher than that within groups.

ROLE AND ASSOCIATION OF ROOT-KNOT NEMATODES IN THE DISEASE

An experiment was conducted to study the possible role and association of the root-knot nematodes Meloidogyne incognita in the incidence and development of bacterial wilt of ginger. In this, there were six treatments and six replications.

Accordingly, 36 pots were filled with soil and autoclaved at 15 lb pressure for 1 hr. One healthy seed piece was planted in each pot, after surface

sterilization in 100 ppm solution of Ambistryn-S.

When the ginger plants were 1 month old, bacterial and nematode inoculations were carried out. The treatments were as follows:-

T_1 = (Nematodes)	Nematode inoculation alone
T_2 = (Bacteria)	Bacterial inoculation alone
T_3 = (Bacteria + Nematodes)	Bacteria and nematodes inoculated simultaneously
T_4 = (Nematodes → Bacteria)	Nematodes inoculated two weeks prior to bacterial inoculation
T_5 = (Bacteria → Nematodes)	Bacteria inoculated two weeks prior to nematode inoculation
T_6 = (Control)	No inoculation of bacteria or nematodes

Nematode inoculum was prepared from egg masses of Meloidogyne incognita collected from infected roots of bhindi (Abelmoschus esculentus). These egg masses were allowed to hatch out and freshly hatched second stage larvae were used for inoculation. Two thousand larvae were inoculated into the soil near the root system of each ginger plant in the treatments T_1, T_3, T_4 and T_5.

Bacterial inoculation was done using a mixed inoculum prepared from all the four isolates of the bacterium. Forty eight hr old cultures were prepared on Peptone casamino acid medium and made into a suspension with O.D. of 0.5. Twenty ml of the above suspension was added to each pot in the treatments T_2, T_3, T_4 and T_5.

Sufficient humidity conditions were provided for the plants. Observations were recorded at regular intervals until all the plants in any one of treatments wilted completely. The percentage wilt incidence in each treatment was recorded and analysed by Classificatory Analysis.

The speed with which the plants in each treatment wilted after bacterial inoculation was also noted and analysed using 'F' test. The significant results were compared by working out the C.D.

TOXIGENICITY OF THE BACTERIUM

Attempts were made to study the toxigenicity of the ginger wilt bacterium, based on the methods followed by workers such as Alouf et al.(1970), Patil et al.(1972) and Gowda et al.(1977) with suitable modifications.

The bacterium was grown in broth of Peptone casamino acid medium. The broth was inoculated with a thick

suspension of 24 hr old culture of the bacterium and incubated at room temperature for five days, with intermittent shaking. At the end of the incubation period, the culture filtrate was prepared by passing the broth culture, first through coarse filter paper and then through sintered glass filter of Grade 5.

The culture filtrate was precipitated with acetone. The filtrate was mixed with three times the volume of the precipitant and kept overnight. The settled precipitate was decanted into a clean petri plate and allowed to evaporate to dryness.

The precipitate was dissolved in distilled water to make a concentration of 0.2 per cent. Healthy shoots of ginger plants were put in the solution for 48 hr and observed for possible sensitivity and symptom expression. Similarly, shoots of ginger kept in distilled water were also maintained as control.

In order to study the host specificity and sensitivity of the toxic metabolite, cuttings of plants such as Solanum melongena, Lycopersicon esculentum, Nicotiana tobacum and Capsicum annum were kept dipped in the same

aqueous solution of the metabolite. The shoots after keeping for 48 hr in the above solution were transferred to distilled water.

To study whether the compound was of [illegible] enzymatic nature or not, the aqueous toxic solution was autoclaved at 15 lb pressure for 20 minutes and again tested with ginger shoots for sensitivity.

Recovery of the toxic compound from the four isolates was compared under uniform conditions. Fifty ml each of Peptone casamino acid broth medium was taken in 4 conical flasks and autoclaved. Suspensions of 48 hr old cultures of the 4 isolates with O.D. of 0.5 were prepared. The flasks containing the broth were inoculated with 2 ml of the above bacterial suspension from a particular isolate. After five days, the recovery of the precipitate from each flask containing a particular isolate of the bacterium was compared.

Results

RESULTS

ISOLATION AND PATHOGENICITY OF THE BACTERIAL ISOLATES

Isolation of the bacterium on Tetrazolium chloride (TZC) medium yielded colonies which were round, fluidal, shiny and white with light pink centre. Healthy ginger plants inoculated with the bacterial isolates started developing symptoms within a week and completely wilted in 2-3 weeks. Bacteria isolated from such artificially inoculated plants developed colonies identical to that of the original isolates.

SYMPTOMATOLOGY

Under natural conditions, the initial symptom of the disease was loss of turgidity of the leaves of the infected plant. This was followed by rolling of the leaflets, which became parallel. The lower leaflets of the older leaves developed an orange-yellow colour at the tips, which subsequently spread along their margins, leaving a band of the original green colour at the midrib. But later the midrib portion also became yellow. The yellowing progressed upwards, affecting the younger leaves and leaflets. This was followed by drooping and wilting of the plant

(Plates I and II). The basal portion of the pseudostem exhibited soft rot symptoms and the wilted shoots got detached from the rhizome at the soil line. On splitting the infected shoots longitudinally, vascular discolouration could be observed.

The rhizomes were also completely infected. Water soaked discoloured areas developed in localised parts of the rhizome, which later spread to the entire portion of it (Plate III). In advanced infections the entire rhizome became soft and completely rotted emitting a foul smell. When infected portions of the diseased plants were kept in a few drops of water, milky bacterial ooze was obtained.

On artificial inoculation, the plants showed a slight variation in the sequence of development of the initial foliar symptoms. The first visible symptom was the yellowing of the lower leaflets of the older leaves, starting from the tips and extending along the margins. After yellowing, the leaflets rolled up and became parallel, later they drooped and the whole plant wilted. In addition to the above mentioned symptoms, stunting was observed in artificially inoculated plants.

Plate I

Healthy and bacterial wilt affected ginger plants

A - Wilt affected plant
B - Healthy plant

Plate II
Different stages of bacterial wilt disease

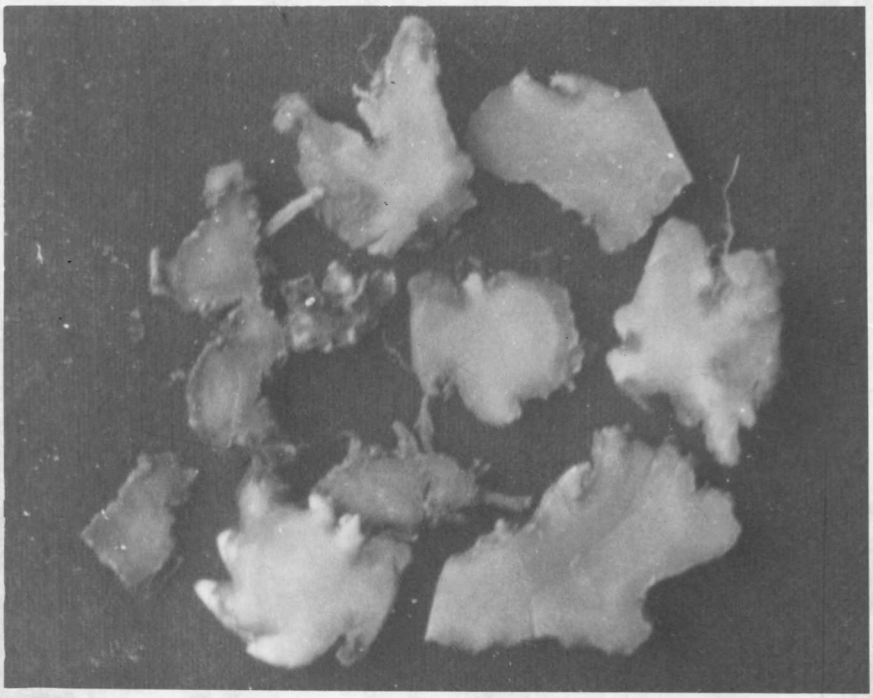


Plate III

Ginger rhizomes affected by bacterial wilt disease

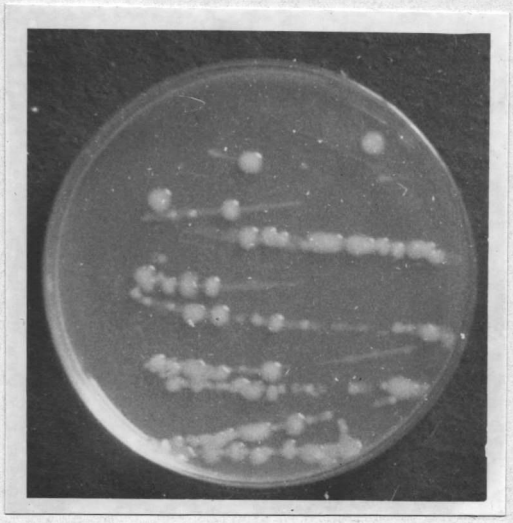


Plate IV

Typical colonies of *Pseudomonas solanacearum* from ginger on PCA medium

The four bacterial isolates on artificial inoculation produced no noticeable variations in symptom expression.

CHARACTERISATION AND IDENTITY OF THE PATHOGEN

A. Cultural characters

1. Morphology

Morphologically, the bacterial isolates were gram negative short rods.

2. Growth characters of the different bacterial isolates on solid medium (PCA medium)

A comparison of growth characters of the bacterial isolates on Peptone casamino acid medium is presented in Table 1.

All the bacterial isolates produced circular, smooth, shiny, raised and entire colonies on PCA medium within a period of 24 hr (Plate IV). Maximum growth was produced by Ps-1, as indicated by the average colony diameter of 10 isolated colonies. In this case, it was 3.0 mm after 24 hr and 5.8 mm after 48 hr. Colony colour of Ps-1 and Ps-4 was creamy white whereas that of the other two isolates was white. The isolates Ps-4 and Ps-1 produced larger and more fluidal colonies than Ps-2 and Ps-3. Slime production was

good in all the isolates.

Table 1

Comparison of growth characters of the different bacterial isolates on PCA medium

Isolates	Nature of colony and colour	Growth, slime and fluidity		Average diameter of 10 isolated colonies in mm after 24hr	48 hr
Po-1	Circular, smooth, shiny, raised, creamy white, with entire margin.	Gr Sl Fl	+++ +++ +++	3.0	5.8
Po-2	Circular, smooth, shiny, raised, white, with entire margin.	Gr Sl Fl	++ +++ ++	2.8	4.6
Po-3	Circular, smooth, shiny, raised, white, with entire margin.	Gr Sl Fl	++ +++ ++	2.5	4.7
Po-4	Circular, smooth, shiny, raised, creamy white, with entire margin.	Gr Sl Fl	+++ +++ +++	2.9	5.5

Gr - Growth
Sl - Slime
Fl - Fluidity

+++ - Good
++ - Moderate

3. Pigment production

None of the isolates produced water insoluble pigment on Yeast glucose chalk agar medium (YGCA). Similarly, no zone of pigmentation was noticed on King's medium, which indicated that none of them produced water soluble pigment also.

4. Oxygen requirement

Growth of the bacterium and subsequent change of the colour of the Nutrient dextrose agar medium (containing 0.005 per cent bromocresol purple) from blue to yellow was observed only in tubes containing no liquid paraffin. This indicated that the bacterial isolates were aerobic in nature.

B. Physiological characters

1. Mode of utilisation of glucose

Glucose was utilized oxidatively (aerobically) by the four isolates, since the medium in the tubes without liquid paraffin turned yellow from the top while that in the other tubes remained unchanged.

2. Starch hydrolysis

Upon the addition of Lugol's iodine, none of the isolates produced a colourless zone around the bacterial

growth, in contrast to the outer dark background of the medium. This indicated that they did not hydrolyse starch.

3. Levan production

All the isolates produced levan, as was evidenced by the development of large, white, domed and mucoid colonies on peptone beef extract medium with 5 per cent sucrose.

4. Production of hydrogen sulphide

Isolates Ps-1, Ps-3 and Ps-4 did not produce hydrogen sulphide whereas Ps-2 produced traces of it as indicated by the slight blackening of the lead acetate strips.

5. M.R. and V.P. tests

All the isolates gave negative M.R. test as indicated by the absence of development of distinct red colour in the culture tubes with the addition of a few drops of 0.02 per cent methyl red in 50 per cent alchohol.

V.P. test was found to be positive in the case of all the isolates. This was indicated by the presence of a crimson colour on addition of 0.6 ml of alpha-naphthol solution (5 per cent in 95 per cent alchohol) and 0.2 ml of 40 per cent aqueous solution of KOH to 1 ml of the culture.

6. Reduction of nitrate

Nitrate was reduced to nitrite by the four isolates of the bacterium. This was evident from the development of red colour when a few drops of sulphanilic acid (0.8 per cent in 5 N acetic acid) and dimethyl alpha-naphthyl amine (0.5 per cent in 5 N acetic acid) were added to the nitrate broth culture.

7. Gelatin liquefaction

Isolates Ps-1, Ps-3 and Ps-4 liquefied gelatin slowly, within a period of 1 month, whereas Ps-2 did not utilise gelatin at all.

8. Production of indole

None of the isolates produced indole, since the colour of the oxalic acid crystals on the test strips did not change to pink or red.

9. Catalase test

Catalase positive reaction was shown by the different isolates. This was indicated by the production of air bubbles upon the addition of a few drops of 20 volume Analar hydrogen peroxide solution to the culture of each isolate smeared on a glass slide.

10 Action on milk

The bacterial isolates showed similar type of action on skimmed and unskimmed milk. All the isolates turned milk slightly acidic but in the case of Pe-1 and Pe-2 peptonisation was also noticed.

11 Tyrosinase activity

All the isolates showed low tyrosinase activity, producing only small amounts of the brown pigment melanin.

12 Production of ammonia

With the addition of Nessler's reagent to the culture tubes the different bacterial isolates produced a brown precipitate, indicating the production of ammonia.

13 Arginine hydrolase test

All the isolates showed positive arginine hydrolase activity. This was observed from the change of the colour of Thornleys' semi-solid arginine medium to red.

14 Urease test

Positive urease activity was noticed in the case of the isolates Pe-1, Pe-2 and Pe-4 as indicated by the colour change of Christensen's urea agar from yellow to red. Pe-3 showed negative urease activity.

15. Utilisation of asparagine as the sole source of carbon and nitrogen

All the bacterial isolates produced growth in the organic salt solution containing 0.2 per cent asparagine. This indicated their ability to utilise asparagine as the sole source of carbon and nitrogen.

16. Sodium chloride tolerance test

The different isolates showed growth in Peptone water having sodium chloride concentration upto 2 per cent. Above this concentration, growth was inhibited.

17. Utilisation of carbon sources

The following carbon sources were found to be utilised by the four isolates, as indicated by the production of acid:- Glucose, galactose, fructose, mannose, lactose, maltose, xylose, raffinose, ribose, dextrose, sucrose, sorbose, glycerol, cellobiose, inositol, sorbitol, dulcitol and mannitol. Cellulose and salicin were not utilised by any of the isolates.

Acid production in the case of positive utilisation, was noticed within a period of 72 hr after bacterial

inoculation. It was indicated by the change of the colour of the medium from reddish violet to yellow.

18. Utilization of organic acids

Among the organic acids tested, formate, acetate and citrate were utilized by all the bacterial isolates, as evidenced by the colour change of the medium from reddish violet to yellow. Benzoate was not utilized by any of them.

19. Hypersensitive reaction on tobacco leaves

Dilute suspensions of 24 hr old cultures of the bacterial isolates when injected into the intercellular spaces of tobacco leaves produced necrotic lesions within a period of 48 hr. After about 18-24 hr of injection the infiltrated area became chlorotic, later that part became transparent and necrotic (Plate V).

A comparison of the cultural and physiological characters of the four isolates is presented in Table 2.

20. Cross inoculations of Pseudomonas solanacearum from ginger and tomato

Inoculation of the ginger isolate of Pseudomonas solanacearum on healthy tomato plants resulted in their

Plate V

Hypersensitive reaction of *Pseudomonas solanacearum* from ginger on tobacco leaf

Table 2

Comparison of the cultural and physiological characters of the different isolates of Pseudomonas solanacearum from ginger

Sl. No.	Characters studied	Isolates			
		Ps-1	Ps-2	Ps-3	Ps-4
1.	Gram reaction	-ve	-ve	-ve	-ve
2.	Pigment production				
	a) Water soluble	-	-	-	-
	b) Water insoluble	-	-	-	-
3.	Oxygen requirement	+	+	+	+
4.	Mode of utilization of glucose				
	a) Aerobic	+	+	+	+
	b) Anaerobic	-	-	-	-
5.	Starch hydrolysis	-	-	-	-
6.	Levan production	+	+	+	+
7.	Production of hydrogen sulphide	-	+	-	-
8.	M.R. test	-	-	-	-
9.	V.P. test	+	+	+	+
10.	Reduction of nitrate	+	+	+	+
11.	Gelatin liquefaction	+	-	+	+

Table 2 (Contd.)

Sl. No.	Characters studied	Isolates			
		Ps-1	Ps-2	Ps-3	Ps-4
12.	Indole production	-	-	-	-
13.	Catalase activity	+	+	+	+
14.	Action on milk				
	a) Skimmed	AP	AP	A	A
	b) Unskimmed	AP	AP	A	A
15.	Tyrosinase activity	+	+	+	+
16.	Production of ammonia	+	+	+	+
17.	Arginine hydrolase test	+	+	+	+
18.	Urease test	+	+	-	+
19.	Utilisation of asparagine as the sole source of carbon and nitrogen.	+	+	+	+
20.	Utilisation of carbon sources with acid production.				
	1. Dextrose	+	+	+	+
	2. Glucose	+	+	+	+
	3. Fructose	+	+	+	+
	4. Galactose	+	+	+	+
	5. Sucrose	+	+	+	+

Table 2 (Contd.)

Sl. No.	Characters studied	Isolates			
		Ps-1	Ps-2	Ps-3	Ps-4
6.	Sorbose	+	+	+	+
7.	Ribose	+	+	+	+
8.	Lactose	+	+	+	+
9.	Maltose	+	+	+	+
10.	Xylose	+	+	+	+
11.	Raffinose	+	+	+	+
12.	Ribose	+	+	+	+
13.	Cellobiose	+	+	+	+
14.	Inositol	+	+	+	+
15.	Sorbitol	+	+	+	+
16.	Mannitol	+	+	+	+
17.	Glycerol	+	+	+	+
18.	Dulcitol	+	+	+	+
19.	Cellulose	-	-	-	-
20.	Salicin	-	-	-	-
21.	Hypersensitive reaction on tobacco leaves	+	+	+	+

Table 2 (contd.)

Sl. No.	Characters studied	Isolates			
		Ps-1	Ps-2	Ps-3	Ps-4
22.	Sodium chloride tolerance at				
	a) 1%	+	+	+	+
	b) 2%	+	+	+	+
	c) 3% and above	-	-	-	-

-ve - Gram negative
\+ - Positive reaction
\- - Negative reaction
A - Acidic reaction
AP - Acidic reaction with peptonisation

complete wilt within a period of 10 days. Tomato isolate of the pathogen failed to cross infect healthy ginger plants on artificial inoculation.

21. Growth characters of the bacterial isolate Ps-1 on different solid media

Growth characters of the bacterial isolate Ps-1 on eight solid media were compared (Table 3). After 24 hr, well developed colonies were observed on all the media except the basal medium for Xanthomonads, on which very small initials were noticed. The bacterium produced circular, raised, shiny and smooth colonies with entire margin on all the eight media. The colony colour was creamy white except in the case of TTC. In this case, the colonies were white with light pink centre.

Maximum growth was observed on NA and PCA. After 24 hr of incubation, the average diameters of 10 isolated colonies on these media were 3.1 mm and 3 mm respectively. After 48 hr it was 6 mm in both cases. Growth was least on BX, while on CA, CYA and PDA the organism showed moderate growth. Good amount of slime was noticed on PCA, TTC, NA, CYA and PDA. The colonies on TTC, PCA, PDA and NA were more fluidal than the others.

Table 3

Comparison of growth characters of Pseudomonas solanacearum (Isolate Ps-1) from ginger on different solid media

Sl. No.	Media	Nature of colony and colour	Growth, slime and fluidity		Average diameter of 10 isolated colonies in mm after 24 hr	48 hr
1	PCA	Circular, smooth, raised, creamy white, with entire margin	Gr Sl Fl	+++ +++ +++	3.0	6.0
2	KBC	Circular smooth, raised, white with light pink centre, margin entire.	Gr Sl Fl	+++ +++ +++	2.8	5.7
3	Na	Circular, smooth, raised, creamy white, with entire margin.	Gr Sl Fl	+++ +++ +++	3.1	6.0
4	Ca	Circular, smooth, raised, creamy white, with entire margin.	Gr Sl Fl	++ ++ ++	2.3	5.3
5	CKA	Circular, smooth, raised, creamy white, with entire margin.	Gr Sl Fl	++ +++ ++	2.4	5.4
6	KCA	Circular, smooth, raised, creamy white, with entire margin.	Gr Sl Fl	+++ ++ ++	2.5	5.6
7	KPA	Circular, smooth, raised, creamy white, with entire margin.	Gr Sl Fl	++ +++ +++	2.1	4.8
8	KK	Circular, smooth, raised, creamy white, with entire margin.	Gr Sl Fl	+ very small initials ++ ++		3.0

Gr - Growth
Sl - Slime
Fl - Fluidity

+++ Good
++ Moderate
+ Slight

SURVIVAL OF THE PATHOGEN

The pathogen was found to survive for considerably long periods in infected seed rhizomes, soil and plant debris. It was indicated that these might serve as the initial inoculum for the disease from season to season.

Fortnightly isolations from infected rhizomes kept in the laboratory yielded typical colonies of Pseudomonas solanacearum on Tetrazolium chloride (TTC) medium. Such isolations were carried out for 5 months, during which period the pathogen was found to survive in the infected seed rhizomes. The seed-borne nature of the pathogen was evident from this observation.

Serial dilution plating was carried out on TTC medium from sick soil kept under laboratory and field conditions. Typical colonies of Pseudomonas solanacearum obtained at 10^{-6} dilution were counted and expressed as the bacterial population per g dry weight of soil (Table 4). In the case of soil kept under both conditions, a decline in the population of the bacterium was observed after the first two months. Even then, a high population was observed for the next eight months period of study also (Figure 1).

Table 4

In vitro studies on the survival of *Pseudomonas solanacearum* from ginger in sick soil

Replicates	Bacterial population per g dry weight of soil kept in the	
	Laboratory	Field
1	1.53×10^8	1.35×10^8
2	1.18×10^8	1.27×10^8
3	9.67×10^7	1.00×10^8
4	6.77×10^7	7.22×10^7
5	1.20×10^7	1.81×10^7
6	7.51×10^6	9.02×10^6
7	1.65×10^7	1.05×10^7
8	1.05×10^7	9.02×10^6
9	1.65×10^7	9.02×10^6
10	7.51×10^6	1.05×10^7
11	1.05×10^7	1.80×10^7
12	7.51×10^6	1.05×10^7
13	7.51×10^6	1.20×10^7
14	9.02×10^6	9.02×10^6
15	9.02×10^6	1.05×10^7
16	7.51×10^6	7.51×10^6
17	4.51×10^6	6.02×10^6
18	6.02×10^6	6.92×10^6
19	6.02×10^6	4.51×10^6
20	4.51×10^6	3.01×10^6

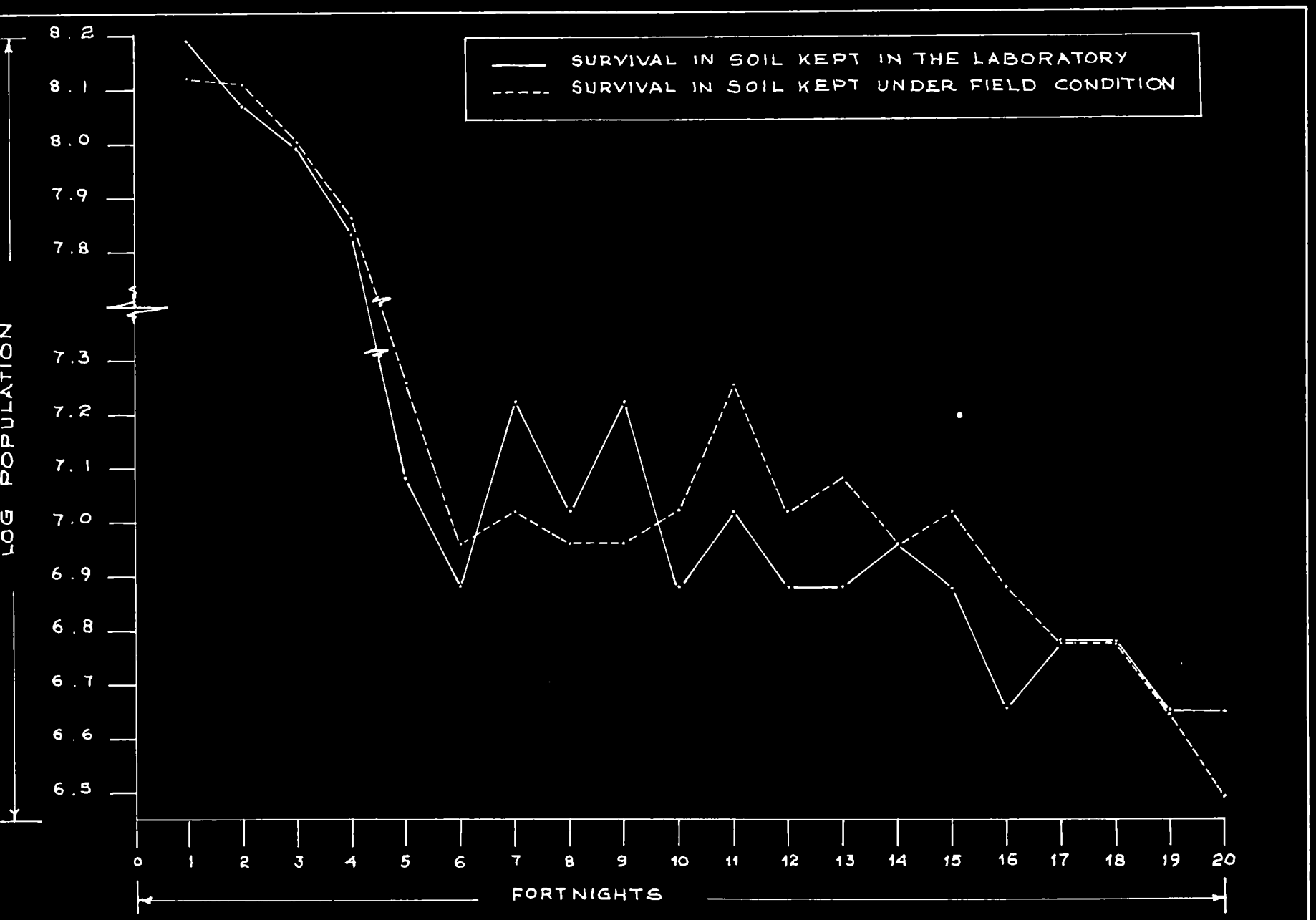


FIG: 1. SURVIVAL OF *Pseudomonas solanacearum* FROM GINGER IN SICK SOIL

Studies on the survival of the bacterium in sick soil and infected plant debris in pots were taken up for two successive seasons of 1978 and 1979. The observations on the incidence of wilt during the two seasons and the period of survival of the pathogen in both sources were recorded.

Statistical analysis showed that the incidence of wilt was significantly greater during the first season than the second in both these sources (Appendix I and II). Out of a total of 200 plants in each case, the number of plants wilted during the first season were 111 and 96 respectively in sick soil and infected plant debris. The respective percentages of wilt incidence were 55.5 and 48 (Table 5).

Table 5

Survival of the ginger wilt bacterium Pseudomonas solanacearum in sick soil and infected plant debris

Sl. No.	Sources	Percentage of wilt incidence during		Period of survival
		Season-1	Season-2	
1.	Sick soil in pots	55.5	37.0	22 months
2.	Infected plant debris mixed with soil in pots	48.0	23.0	20 months

The wilt percentages during the second season were 37 and 23 respectively in infected soil and plant debris. It was also observed that wilt incidence was greater in sick soil than plant debris during both the seasons studied.

The maximum period of survival of the pathogen in sick soil during the present study viz., the period between date of incorporation of sick soil and date of observation of the last incidence of wilt, was computed to be 22 months. Similarly, the maximum period of survival of the pathogen in infected plant debris was 20 months.

Weekly planting of severely infected seed rhizomes was carried out for 5 months. It was observed that these seed pieces showed poor germination and those which germinated also wilted ultimately. It could be assumed that infected seed rhizomes when planted reduced the percentage of germination considerably, apart from serving as a source of survival and primary inoculum of the pathogen.

CONTROL OF THE DISEASE USING ANTIBIOTICS

The *in vitro* sensitivity of the ginger wilt pathogen to 6 antibiotics was tested using the isolate Rc-1. The results obtained in the study are given in Table 6 and Figure.2.

Among the four concentrations tested, maximum inhibition was obtained at 1500 ppm. Maximum inhibition was noticed for Ambistryn-S, followed by Agrimycin-100 and Chloromycetin in that order. These antibiotics at 500 ppm also gave good inhibition of the pathogen (Plate VI).

Ampicillin and Paushamycin were ineffective at 250 ppm. Penicillin was the least effective in inhibiting the pathogen, to which it was sensitive only at 1500 ppm.

A comparison of the mean zone of inhibition of each antibiotic by statistical methods indicated that Ambistryn-S was on par with Agrimycin-100 and superior to the other antibiotics tested. Agrimycin-100 and Chloromycetin when compared were on par and superior to the others except Ambistryn-S. Streptocycline and Terramycin were on par and superior to Paushamycin, Ampicillin and Penicillin. Among these three, Penicillin was inferior to the other two.

Ambistryn-S, Agrimycin-100 and Chloromycetin at 500 ppm were superior to 250 ppm, but on par with 1000 ppm. Ambistryn-S and Agrimycin-100 at 1500 ppm were superior to 500 ppm, but Chloromycetin at these two concentrations was on par.

Table 6

In vitro sensitivity of Pseudomonas solanacearum from ginger to antibiotics

Sl. No.	Antibiotics	Inhibition zone in mm at 250 ppm	500 ppm	1000 ppm	1500 ppm	Mean
1.	A_1 - Ambistryn-S	25	33	39	40	34.5
2.	A_2 - Agrimycin-100	23	30	35	37	31.2
3.	A_3 - Chloromycetin	19	29	30	35	28.2
4.	A_4 - Terramycin	12	18	20	24	18.5
5.	A_5 - Streptocycline	15	17	20	25	19.2
6.	A_6 - Ampicillin	0	13	15	15	10.7
7.	A_7 - Penshamycin	0	14	19	21	13.5
8.	A_8 - Penicillin	0	0	0	15	3.7

C.D.(0.05) for comparison between antibiotics = 3.296

C.D.(0.05) for comparison between levels of antibiotics = 6.612

$\overline{A \quad A_2 \quad A_3} \quad \overline{A_5 \quad A_4} \quad \overline{A_7 \quad A_6 \quad A_8}$

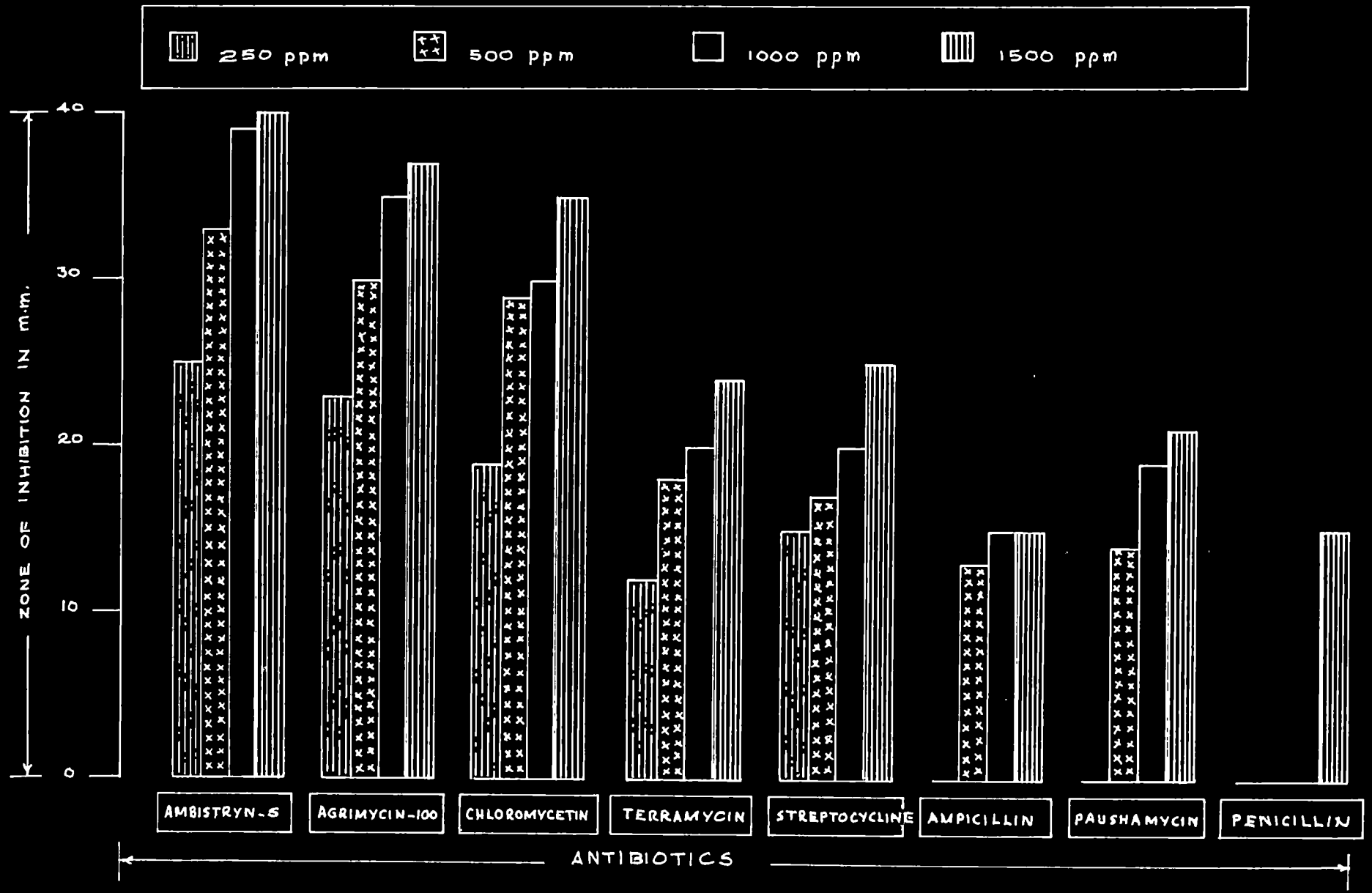


FIG: 2. *In vitro* SENSITIVITY OF *Pseudomonas solanacearum* FROM GINGER TO ANTIBIOTICS

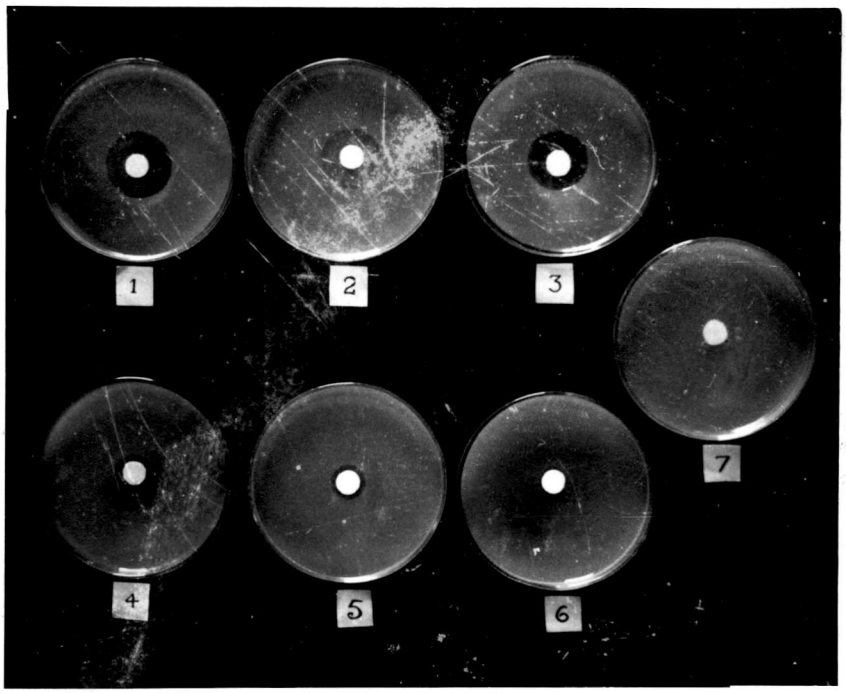


Plate VI
Comparison of the zone of inhibition of different antibiotics

1.	Ambistryn-S	500 ppm
2.	Agrimycin-100	500 ppm
3.	Chloromycetin	500 ppm
4.	Terramycin	500 ppm
5.	Streptocycline	500 ppm
6.	Penicillin	500 ppm
7.	Control	

An *in vitro* trial with 3 combinations of two antibiotics at 250 ppm each was also attempted. The results indicated that the combination of Ambistryn-S and Chloromycetin was superior to the other two. Combination of Terramycin and Ambistryn-S was superior to that of Agrimycin-100 and Ampicillin (Table 7). But there was no additive effect due to combination of antibiotics. The mean zone of inhibition in this case was even less than that of the individual antibiotics.

Table 7

In vitro sensitivity of *Pseudomonas solanacearum* from ginger to combinations of antibiotics

Sl. No.	Antibiotic combinations	Mean zone of inhibition in mm
1	T_1 - Ambistryn-S and Chloromycetin	25.0
2	T_2 - Agrimycin-100 and Ampicillin	15.0
3	T_3 - Terramycin and Ambistryn-S	22.5

C.D.(0.05) for comparison between the treatments = 1.6132

T_1 T_3 T_2

An *in vivo* trial was conducted with Ambistryn-S, Agrimycin-100 and Chloromycetin at 500 ppm. It was observed that none of these antibiotics could completely control the disease at the above concentration. The results of this study are presented in Table 8 and Figure 3.

Maximum control of the disease viz., 80 per cent was obtained by pre-inoculation spray of Ambistryn-S. When this antibiotic was applied as post-inoculation spray, the control was only 60 per cent. The same control was obtained by pre-inoculation sprays of Agrimycin-100 and Chloromycetin. By their post-inoculation sprays, the percentage of control was 40. In the control treatment receiving no antibiotic spray none of the plants remained healthy.

On statistical analysis, all the six treatments having antibiotic sprays were found to be superior to T_7 (Control). T_1 (Ambistryn-S pre-inoculation spray) was superior to all others. T_2 (post-inoculation spray of Ambistryn-S), T_3 and T_5 (pre-inoculation sprays of Agrimycin-100 and Chloromycetin respectively) were on par and superior to T_4 and T_6 (post-inoculation sprays of Agrimycin-100 and Chloromycetin respectively).

Table 8

In vivo control of bacterial wilt of ginger using antibiotics

Sl. No.	Treatments	Percentage of wilt incidence	Percentage of Control
1.	T_1 - Ambistryn-S pre-inoculation spray	20	80
2.	T_2 - Ambistryn-S post-inoculation spray	40	60
3.	T_3 - Agrimycin-100 pre-inoculation spray	40	60
4.	T_4 - Agrimycin-100 post-inoculation spray	60	40
5.	T_5 - Chloromycetin pre-inoculation spray	40	60
6.	T_6 - Chloromycetin post-inoculation spray	60	40
7.	T_7 - Control	100	0

T_1 $\overline{T_2\ T_3}$ T_5 $\overline{T_4\ T_6}$ T_7

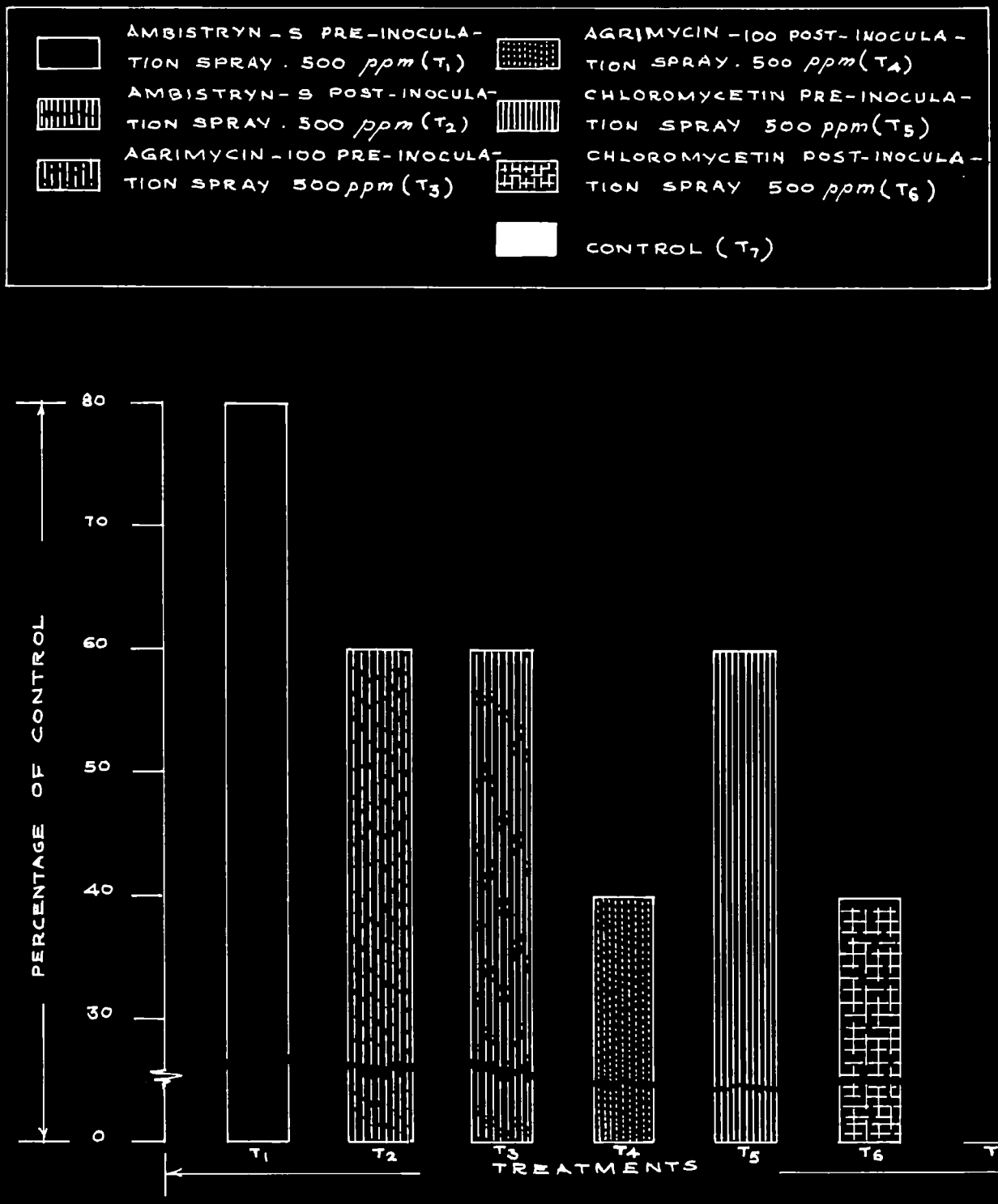


FIG: 3 . *In vivo* CONTROL OF BACTERIAL WILT OF GINGER USING ANTIBIOTICS

In the present study it was observed that pre-inoculation sprays of all the three antibiotics were superior to their post-inoculation sprays.

The three antibiotics were compared for their efficacy in controlling the disease. The results are given in Table 9. Ambistryn-S giving 70 per cent control of the disease was found to be superior to the other two antibiotics, Agrimycin-100 and Chloramycetin which gave only 50 per cent control.

Table 9

Comparison of the efficacy of Ambistryn-S, Agrimycin-100 and Chloramycetin in controlling bacterial wilt of ginger

Sl. No.	Antibiotics	Average percentage of control in pre-and post-inoculation spray treatments
1	A_1 - Ambistryn-S	70
2	A_2 - Agrimycin-100	50
3	A_3 - Chloramycetin	50

$A_1 \quad \overline{A_2 \quad A_3}$

The results obtained from the *in vivo* trial indicated that sprays of Ambistryn-S at 500 ppm gave significantly greater control of the disease than Agrimycin-100 and Chloromycetin at the same concentration.

ROLE AND ASSOCIATION OF ROOT-KNOT NEMATODES IN THE DISEASE

Studies were undertaken to assess the possible role and association of the root-knot nematodes *Meloidogyne incognita* in the incidence and development of bacterial wilt of ginger. The results are presented in Table 10 and Figure 4.

As is evident from the table given below, wilt incidence was maximum in T_4 in which nematode inoculation preceded bacterial inoculation. In this treatment 100 per cent wilt incidence was noticed. Next to T_4, wilt incidence was greatest in T_3 in which there was simultaneous inoculation of bacteria and nematodes. This was followed by T_5 where nematode inoculation succeeded bacterial inoculation. The wilt incidence in the above two treatments viz., T_3 and T_5 were 83.3 and 66.6 per cent respectively. In the case of T_2 in which bacteria alone were inoculated the wilt incidence was only 50 per cent. In T_1 and T_6 where there was no bacterial inoculation wilt incidence was nil.

Table 10

Influence of root-knot nematodes Meloidogyne incognita on the incidence of bacterial wilt of ginger

Sl. No.	Treatments	Percentage of wilt incidence
1.	T_1 - Nematodes	0
2.	T_2 - Bacteria	50.0
3.	T_3 - Bacteria + Nematodes	83.3
4.	T_4 - Nematodes → Bacteria	100.0
5.	T_5 - Bacteria → Nematodes	66.6
6.	T_6 - Control	0

T_4 T_3 T_5 T_2 $\overline{T_1 \quad T_6}$

Results obtained on statistical analysis showed that wilt incidence in the treatment T_4 (Nematodes → Bacteria) was significantly greater than T_3 (Bacteria + Nematodes), T_5 (Bacteria → Nematodes) and T_2 (Bacteria). Similarly, T_3 was superior to T_5 and T_2 and T_5 was superior to T_2. The treatments T_1 (Nematodes) and T_6 (Control) were on par and inferior to the other treatments.

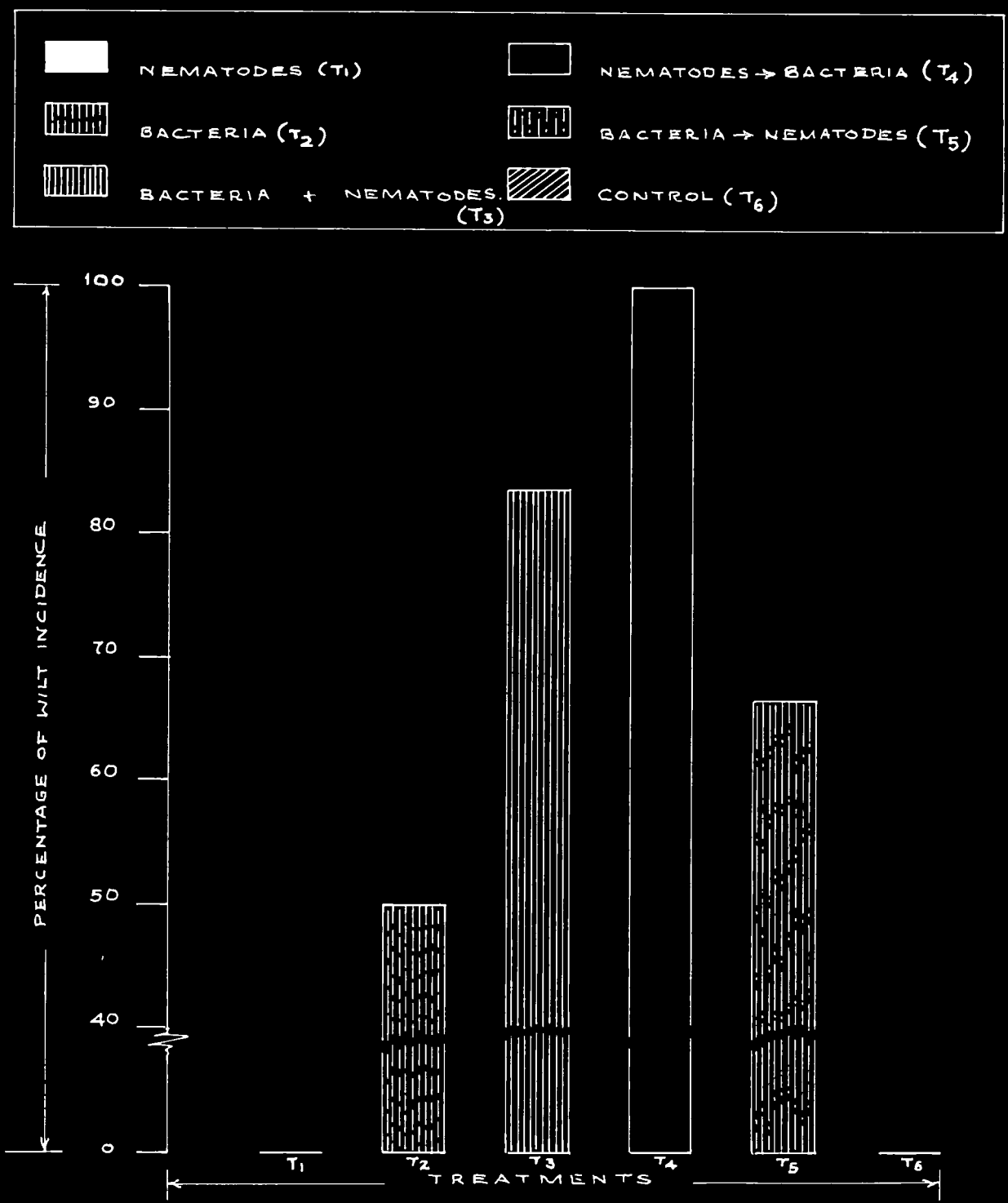


FIG: 4. INFLUENCE OF ROOT-KNOT NEMATODES *Meloidogyne incognita* ON THE INCIDENCE OF BACTERIAL WILT OF GINGER

It was also observed that the presence of root-knot nematodes could influence the speed of development of wilt. In the case of wilted plants in each treatment, the interval between bacterial inoculation and complete wilting was recorded. It was noticed that the average number of days taken by the plants in T_4 (Nematodes → Bacteria) for complete wilt was 15 (Table 11). Plants in T_3 (Bacteria + Nematodes) and T_5 (Bacteria → Nematodes) required an average of 25 days and 29 days respectively. The longest period of 32 days was taken by plants in T_2 (Bacteria).

On statistically analysing the number of days between bacterial inoculation and complete wilt in the case of wilted plants, T_4 (Nematodes→ Bacteria) took significantly lesser time than the others. Similarly, the time required by T_3 (Bacteria + Nematodes) was significantly less than T_5 (Bacteria → Nematodes) and T_2 (Bacteria). The latter two treatments were on par.

The results of the above studies indicated that the root-knot nematodes Meloidogyne incognita were having a positive role in the increased incidence and speed of development of ginger wilt caused by Pseudomonas

Table 11

Influence of root-knot nematodes Meloidogyne incognita on the speed of development of bacterial wilt of ginger.

Sl. No.	Treatments	Average number of days between bacterial inoculation and complete wilt (In the case of wilted plants)
1.	T_1 = Nematodes	--
2.	T_2 = Bacteria	32
3.	T_3 = Bacteria + Nematodes	25
4.	T_4 = Nematodes → Bacteria	15
5.	T_5 = Bacteria → Nematodes	29
6.	T_6 = Control	**

** = No incidence of wilt

$\overline{T_2 \quad T_5} \quad T_3 \quad T_4$

C.D. (0.05) for comparison between T_2 and T_4 = 3.25

C.D. (0.05) for comparison between T_2 and T_3 = 3.36

C.D. (0.05) for comparison between T_2 and T_5 = 3.51

C.D. (0.05) for comparison between T_4 and T_3 = 2.78

C.D. (0.05) for comparison between T_4 and T_5 = 2.97

C.D. (0.05) for comparison between T_5 and T_3 = 3.08

Meloidogyne. Incidence of wilt was found to be maximum when nematodes were already present in the soil at the time of inoculation of bacteria. Under the above circumstances, speed of wilt was double than when bacteria were inoculated into nematode free soil.

TOXIGENICITY OF THE PATHOGEN

A toxic compound was obtained from the culture filtrate of the ginger wilt bacterium by precipitation with acetone. The precipitate when allowed to dry by evaporation of the excess acetone and scraped out was a viscous, dark brown substance.

Healthy shoots of ginger kept dipped in a 0.2 per cent aqueous solution of the toxic compound were found to be sensitive to it. A close observation of the symptoms expressed by such shoots indicated that after about 12 hr of dipping in the solution, the leaflets lost turgor and they rolled up and became parallel. This was followed by their yellowing, which started from the tips and extended along the margins. The leaflets subsequently drooped and wilted (Plate VII). Complete wilting of the shoots occurred in 48 hr. All the above

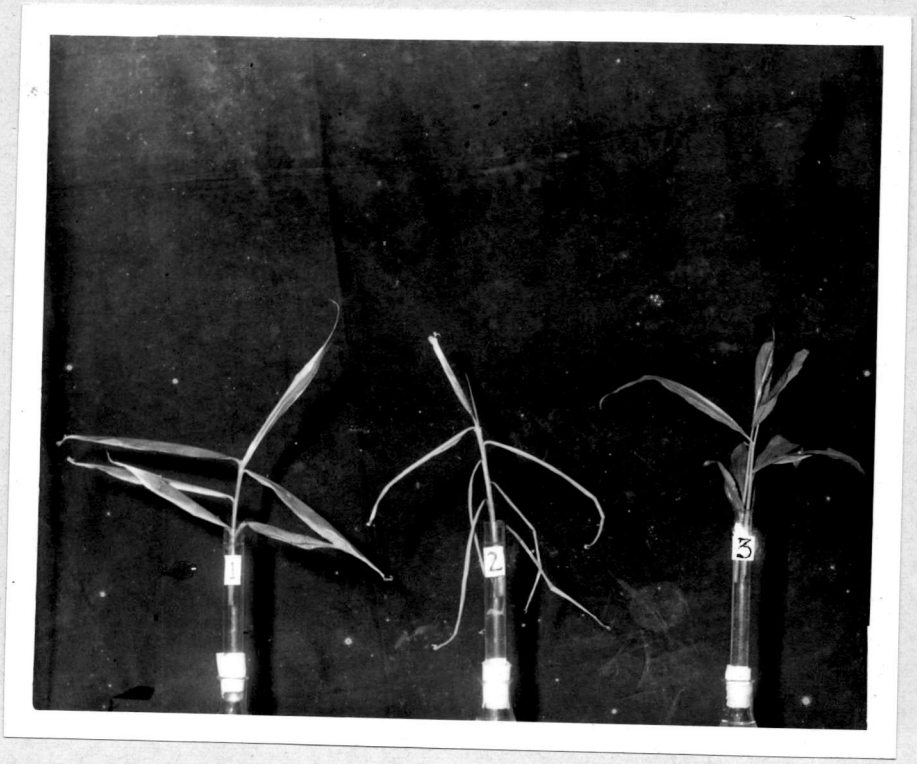


Plate VII

Effect of toxic metabolite on healthy ginger shoots

1,2 - Shoots kept in aqueous solution of the metabolite

3 - Shoots kept in distilled water

mentioned symptoms were first exhibited by the older leaflets but later progressed upwards, affecting the younger ones. The lower ends of the shoots kept immersed in the toxic solution showed maceration of tissues.

Shoots of Solanum melongena, Lycopersicon esculentum, Nicotiana tabacum and Capsicum annum were found to be sensitive to the toxic compound at the above concentration. They showed symptoms of loss of turgidity and wilting starting from the lower leaves onwards. Maceration of the tissues at the lower ends of the shoots was observed as in the case of ginger.

The above shoots were transferred to distilled water after 48 hr of keeping in the solution, and maintained for the same period. It was observed that all the shoots except that of ginger regained turgor. In the case of ginger shoots once the wilt symptoms were initiated it wilted completely, even after keeping in distilled water.

The aqueous solution of the toxic metabolite was autoclaved at 15 lb pressure for 20 minutes and again tested

with ginger shoots for sensitivity. It was noticed that the compound could reproduce the wilt symptoms as before, indicating that it was heat-stable and non-enzymatic.

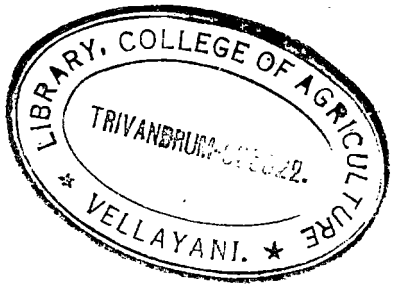
Recovery of the toxic compound from the four isolates was compared under uniform conditions (Table 12). Culture filtrate of the isolate Ps-1 yielded the maximum amount of precipitate which weighed 75 mg. This was followed by Ps-3, Ps-2 and Ps-4, giving 70, 60 and 50 mg respectively.

Table 12

A comparison of the recovery of the toxic compound from the four isolates of the ginger wilt bacterium after 5 days of incubation

Sl. No.	Isolates	Recovery of the toxic compound in mg
1	Ps-1	75
2	Ps-2	60
3	Ps-3	70
4	Ps-4	50

Discussion



DISCUSSION

The bacterial wilt of ginger, incited by Pseudomonas solanacearum, although of recent occurrence in Kerala, has proved to be a threat to the cultivation of ginger in the State. This disease is known to cause severe losses in the ginger growing areas of the world such as Mauritius, Malaya, Philippines, Hawaii and Queensland (Ishii and Arogaki, 1963; Chew, 1969; Pegg et al., 1974). In India it was first reported during 1978 from Sultan's Battery and Ambalavayal areas of Kerala State where it assumed very serious and alarming proportions (Sarma et al., 1979; Mathew et al., 1979). Since then the disease has spread to various ginger growing areas of the State.

The aspects taken up for the present study were, symptomatology of the disease, characterization and identity of the pathogen, its survival, control of the disease by chemicals, role and association of root-knot nematodes in the disease, and toxigenicity of the bacterium.

Symptoms of the disease were studied under natural and artificially inoculated conditions. The initial symptoms exhibited by naturally infected plants were

loss of turgidity and rolling of the leaves. The lower leaflets of the older leaves started yellowing from the tips, and the yellowing extended along the margins. It progressed upwards, affecting the upper leaves also. This was followed by drooping and wilting of the leaves, giving the plant a wilted appearance.

The basal portion of the pseudostem showed soft rot symptoms and the wilted shoot got detached from the rhizome at the soil line. Vascular discolouration of the infected shoots was also observed.

The initial infection of the rhizome was characterised by localised water soaked discoloured areas which later spread to the entire rhizome. In advanced stages of infection, the entire rhizome rotted, emitting a foul smell.

The infected portions when cut and kept in a few drops of water, milky bacterial ooze was observed.

The above symptoms have already been reported by Ishii and Aragaki (1963), Zehr (1969), Pegg et al.(1974), Sinha et al. (1978) and Mathew et al. (1979) with slight variations in the initial symptoms. Ishii and Aragaki (1963), Zehr (1969) and Pegg et al. (1974)

described the initial symptoms as yellowing and wilting of the lower leaves whereas Sarma et al.(1979) observed the earliest symptom as linear water soaked streaks on the collar region of the pseudostems. But Mathew et al.(1979) reported that the first symptom was loss of turgidity of the leaves. An additional symptom reported by Ishii and Azegami (1963) was stunting of the infected plants.

On artificial inoculation, all the symptoms observed under natural conditions were produced. But there was a slight variation in the sequence of the initial symptoms. Here, the first symptom noticed was a yellow discolouration of the lower leaflets, starting from the tips and extending along the margins. This was followed by rolling, drooping and wilting of the leaves. The inoculated plants were usually stunted than the healthy ones.

No noticeable changes in symptom expression were observed on artificial inoculation of the different isolates of the bacterium. Usually the first symptoms were observed within a week after inoculation of the bacterium and complete wilting of the plants occured within a period of 2-3 weeks.

In the present studies of the symptomatology of bacterial wilt of ginger, it could be observed that on the whole, the symptom picture was in agreement with that of earlier workers. The slight variations in the progress of expression of the initial symptoms might be due to the variation in the environmental conditions of study, in the variety of the host, or slight variations in the isolates of the bacterium.

Studies on the characterisation of the pathogen revealed that the bacterial isolates were similar in their morphology and gram reaction. All were gram negative, short rods. These characters were in conformity with those reported by Ishii and Aragaki (1963). The isolates produced circular, smooth, raised, creamy white or white colonies on Peptone casamino acid medium, which were slimy and fluidal. According to Kelman (1954), Okabe and Goto (1954) and Hussain and Kelman (1958), fluidal consistency of the colony was associated with high virulence.

None of the isolates produced water insoluble pigment on yeast glucose chalk agar medium. A similar result was observed by Rema Devi (1978) while studying the characters of 26 isolates of Pseudomonas solanacearum. On King's

medium water soluble pigment was not produced. This observation was in conformity with that of Hayward (1964). A study of the oxygen requirement of the bacterial isolates indicated that they were aerobic.

Majority of the physiological and biochemical properties studied were found to be in accordance with those described by Hayward (1964). However, with regard to certain characters, variations were noticed among the isolates. Variation among isolates of Pseudomonas solanacearum has been reported by many workers (Hayward, 1964; Buddenhagen and Kelman, 1964; Zehr, 1970).

The different isolates utilized glucose oxidatively, did not produce indole or hydrolyse starch, but produced ammonia. Mathew et al.(1979) have reported these characters of the ginger wilt bacterium. Nitrate reduction and positive catalase activity were observed in all the isolates. All the above reactions have been reported to be characteristic of Pseudomonas solanacearum (Hayward, 1964).

All the isolates produced small amounts of the brown pigment melanin on tyrosine medium. Hayward (1964)

reported that production of this pigment varied in intensity between isolates of Pseudomonas solanacearum. Quinon et al.(1964) compared the melanin production of isolates of Pseudomonas solanacearum from ginger, bird of paradise and tomato, and reported that the ginger isolate was intermediate among the three.

The isolates were positive to arginine hydrolase test, produced levan and grew well in asparagine medium. These characters were in conformity with those observed by Rama Devi (1978) while studying characters of Pseudomonas solanacearum. Growth was observed in concentrations of sodium chloride upto 2 per cent. Mathew et al. (1979) observed that growth of the ginger wilt bacterium was slightly inhibited at this concentration. However, Hayward (1964) while studying characteristics of different isolates of the bacterium observed that no growth was observed in the medium containing 2 per cent sodium chloride.

With respect to production of hydrogen sulphide, the isolate Ps-2 was found to vary from the others. It produced traces of it whereas the other isolates did not produce it. It has been reported by Mathew et al.(1979) that the ginger wilt bacterium did not produce hydrogen sulphide.

In the present study urease activity was positive in the case of all the isolates except Ps-3. All of them showed negative M.R. and positive V.P. tests. Slow gelatin liquefaction was observed in the isolates Ps-1, Ps-3 and Ps-4 while Ps-2 did not liquefy gelatin.

Similar type of reaction was exhibited by the isolates in skimmed and unskimmed milk. All the isolates showed acidic reaction in milk. In the case of isolates Ps-1 and Ps-2 there was peptonisation also. This observation was in accordance with that of Mathew et al.(1979). But isolates Ps-3 and Ps-4 did not peptonise milk.

Among the organic acids tested for utilization, citrate, acetate and formate were utilised, but benzoate was not utilised by the isolates. Citrate utilization by the ginger wilt bacterium was reported by Ishii and Aragaki (1963).

The isolates utilised a wide variety of carbon compounds viz., glucose, galactose, fructose, mannose, lactose, maltose, xylose, raffinose, ribose, dextrose, sucrose, glycerol, sorbose, cellobiose, inositol, sorbitol, dulcitol and mannitol. Cellulose and salicin were not utilised. It was observed that the organism oxidised the

3 disaccharides (lactose, maltose and cellobiose) and 3 hexose alcohols (mannitol, sorbitol and dulcitol) which according to Heyward (1964), were oxidised by members of biotype-3.

All the isolates produced necrotic lesions on tobacco leaves within 48 hr of injection of the bacterial suspension.

Inoculation of the ginger isolate of Pseudomonas solanacearum caused wilt of tomato plants, but tomato isolate of the organism did not cause wilt of ginger plants. Similar results have been reported by other workers. Orton (1953) and Zehr (1970) have reported that the ginger isolate of Pseudomonas solanacearum produced typical wilt of tomato. Zehr (1970) further observed that the isolate from tomato was not virulent to ginger on artificial inoculation.

The results of the present studies when viewed in the light of early reports on the characterisation of the bacterium seemed to be almost similar to that of Pseudomonas solanacearum, and as such the bacterial isolates used in this investigation could be characterized and identified as Pseudomonas solanacearum. Further, based on the capacity of the isolates to oxidise the 3 disaccharides

(maltose, lactose, and cellobiose) and 3 hexose alcohols (dulcitol, mannitol and sorbitol), they could be identified as biotype-3 of Hayward (1964). Apart from this, the pathogen was found to infect tomato plants on artificial inoculation. Pegg et al.(1974) have already reported that biotype-3 of Pseudomonas solanacearum causing ginger wilt was able to cause wilt of tomato plants. So the isolates used in the present study could be Pseudomonas solanacearum E.F. Smith, biotype-3 of Hayward. This view was supported by Somma et al.(1978) who have reported that the ginger wilt bacterium isolated from Ambalavayal has been identified as Pseudomonas solanacearum E.F. Smith, biotype-3 of Hayward.

However, there were slight variations among the isolates used in the present study with respect to a few physiological characters such as urease test, hydrogen sulphide production, gelatin liquefaction and action on milk. Detailed studies with more number of isolates from different areas are required to draw definite conclusions on the variability if any, of the bacterium.

A study of the growth characters of the isolate Ps-1 on different solid media indicated that on all the media

the colonies were circular, raised and entire with good or moderate slime and fluidity. Nutrient agar and Peptone casamino acid medium supported maximum growth of the organism while least growth was noticed on the basal medium for Xanthomonads.

In the absence of similar studies on the growth media of the bacterium it could be stated that Nutrient agar and Peptone casamino acid medium were the best solid media for the routine laboratory studies and mass multiplication of the bacterium.

Results of the studies on the survival of the ginger wilt pathogen in infected seed rhizomes, plant debris and sick soil indicated that it was able to survive for considerably long periods in these sources. Since the pathogen could be isolated for a period of five months from infected rhizomes, it can definitely serve as a source of primary inoculum of the disease for the next crop. When infected seed rhizomes were planted, these gave rise to infected plants. Similar reports regarding the seed-borne nature of the ginger wilt bacterium were made by Pegg et al.(1974) and Mathew et al.(1979). Survival of

Pseudomonas solanacearum in infected seed tubers of potato was reported by Robinson and Ramos (1964). In the case of soybean, Nikitina and Korsakov (1978) noticed that the pathogen could survive for a period of 2-3 years in its seeds. Lloyd (1978) suggested that long term survival of Pseudomonas solanacearum on potato was dependent on latently infected potato tubers.

Studies conducted on sick soil kept in the laboratory as well as field conditions indicated that the population of the pathogen remained high for the 10 months period of study. It indicated that the pathogen persisted in the soil for a long time. Similar observations were made by other workers such as Miller et al.(1940) who reported that Pseudomonas solanacearum persisted in soil formerly occupied by its various hosts. Tanaka (1979) observed that when a naturally infected field of tobacco was kept fallow, the soil population of Pseudomonas solanacearum remained high for 1 year and then decreased rapidly.

Survival of the pathogen in sick soil and infected plant debris in pots was studied during 2 successive seasons by observing the wilt incidence of ginger plants in them.

Incidence of wilt was greater during the first season than the second in the case of both sick soil and infected plant debris. In the present study the period of survival of the bacterium in sick soil was 22 months. Pegg and Moffett (1971) could also obtain similar results in their studies on the survival of the ginger wilt bacterium in Queensland.

Survival of Pseudomonas solanacearum in soil for different periods of time has been reported by various workers. A survival period of 4 years was reported by Smith (1944) and Graham (1978). Das and Chattopadhyay (1956) reported that the organism persisted in infested soil for 16 months in the case of bacterial wilt of brinjal. In the present study also, it was observed that infested soil can serve as a long term survival site for the pathogen.

During the present study, it was observed that the pathogen survived in infected debris of ginger for a period of 20 months. Survival of Pseudomonas solanacearum in debris of other crops has been reported by earlier workers. Das and Chattopadhyay (1956) reported its survival for 9 months in infested plant remains of brinjal. According to Lloyd (1978), infested crop residues were important in the survival of the pathogen between

successive crop plantings. Graham et al.(1979) observed that infested debris of potato when mixed with non-infested soil remained so for 32 weeks.

During the present study, the ginger wilt pathogen survived for a period of 5 months in seed rhizomes, 22 months in soil and 20 months in plant debris. Long periods of survival of Pseudomonas solanacearum, the incitant of bacterial wilt of various crop plants in different sources such as seed, soil and plant debris have been well documented by earlier workers.

Survival of the ginger wilt bacterium in seed rhizomes for 5 months would be quite a significant period, as the interval between two cropping seasons is more or less the same. Infected plant debris in soil and also sick soil could be the sources for long term survival of the bacterium. The fact that this pathogen could survive in these sources for considerably long periods, is an alarming observation of great epidemiological significance in the cultivation of ginger in the State.

Out of the 8 antibiotics tested for in vitro sensitivity of the pathogen, Ambistryn-S and Agrimycin-100 were the

most effective, followed by Chloromycetin. Of the 4 concentrations tested, maximum inhibition was obtained at 1500 ppm, but good effect was noticed from 500 ppm onwards. These 3 antibiotics have already been reported to be effective for the in vitro control of Pseudomonas solanacearum (Middleton and Murano, 1956; Foucart and Delcambe, 1960; Goszani et al., 1978).

Streptomycline and Terramycin were found to be superior to Kasugamycin, Ampicillin and Penicillin. Ampicillin was ineffective against the pathogen at 250 ppm. Penicillin had the least effect, inhibiting the pathogen only at 1500 ppm. Workers like Campacci et al.(1962) and Goszani et al.(1978) have found that Ampicillin and Penicillin were effective against Pseudomonas solanacearum. Contrary to these reports, these two antibiotics were found to have the least effect in the present study.

The antibiotic combinations tested were found to be inferior to the single antibiotics in inhibiting the pathogen in in vitro.

When the field performance of the best three antibiotics was tested, it was observed that none of them could

offer an absolute control of the disease. But wilt incidence was considerably reduced in the antibiotic treated plants.

Maximum control of the disease was obtained by pre-inoculation spray of Ambistryn-S. This was followed by post-inoculation spray of the same antibiotic, and also by pre-inoculation sprays of Agrimycin-100 and Chloromycetin. Pre-inoculation sprays of all the three antibiotics were superior to their post inoculation sprays. This indicated that bacterial wilt of ginger can be controlled better if antibiotic spraying is resorted to, before wilt initiation.

Among the three antibiotics, Ambistryn-S was superior to the others. This result was concurrent to that of earlier workers. Hidaka and Murano (1956) reported that tobacco wilt caused by Pseudomonas solanacearum could be controlled by 2 sprays of streptomycin at 110ppm, one before and the other after inoculation of the pathogen. Yamane and Kokubun (1957) observed that this pathogen inoculated on broad bean was inhibited by the above antibiotic at 500 ppm. Further, it was reported by Mondal and Mukherjee (1976) that this antibiotic was effective in protecting tomato plants against the bacterial wilt pathogen.

With regard to control of bacterial wilt of ginger, Jeon et al.(1974) and Sasma et al.(1978) have reported that chemical methods have not been so far efficient. But Ichii and Aragaki (1965) could control this disease using methyl bromide at 3 lb per 100 sq. ft of soil. In the present study, about 70 per cent control of the disease could be obtained by sprays of Ambistryn-S at 500 ppm. Agrimycin-100 and Chloromycetin offered 50 per cent control. However, it is felt that more detailed studies are necessary on a field scale in order to arrive at definite conclusions on the efficacy of the antibiotics in controlling bacterial wilt of ginger.

Studies conducted on the role and association of root-knot nematodes indicated that they had a positive influence on the increased incidence and speed of development of the disease. Maximum incidence and speed of development of wilt were observed in plants receiving inoculation of nematodes prior to bacteria. This was followed by those receiving combined inoculation of the two organisms. On the whole, it was noticed that in the treatments where both bacteria and nematodes were inoculated, incidence and speed of wilt were greater than that in which

there were bacteria alone. These results highlight the possibility of a predisposing effect by the nematodes on the induction and speed of development of bacterial wilt of ginger.

Aggravation of bacterial wilt of other crops like brinjal, tomato and tobacco due to the influence of root-knot nematodes has been previously documented by earlier workers (Lucas et al., 1955; Litman et al., 1964; Johnson and Powel, 1969).

Johnson and Powel (1969) reported that when wilt susceptible tomato plants were inoculated with root-knot nematodes 3 or 4 weeks prior to bacteria, there was greater incidence of wilt than when the pathogens were added simultaneously. Further, Kukudome and Sakagawa (1972) observed that in the case of Granville wilt of tobacco, a simultaneous inoculation of Pseudomonas solanacearum and Meloidogyne incognita hastened the occurrence and furthered the progress of the disease, than inoculation with the bacterial pathogen alone.

The results obtained in the present studies were similar to that of previous workers. Since the incidence

and speed of wilt were highest when bacteria were added to soil already infected with the nematodes, it could be assumed that these nematodes provided wounds and thus predisposed the ginger plants to increased bacterial infection. Workers like Kuno et al.(1955); Pitcher (1963) and Libman et al.(1964) have arrived at a similar conclusion from their studies.

Further, another assumption that could be made from the present results is that root-knot nematodes probably modified the plant tissues and made it more suitable for bacterial colonization, as was put forward by Reddy et al.(1979) in the case of bacterial wilt of brinjal.

The results of the present investigation on the role and association of root-knot nematodes in the bacterial wilt of ginger, indicated that they have got a significant role in the increased rate of incidence and speed of development of the disease. The root-knot nematodes might be predisposing the ginger plants to increased infection and colonisation of the bacterial pathogen by providing wounds, modifying the plant tissues, or by reducing the vitality and vigour of the plants. This is all the more pertinent and important in view of the fact that root-knot nematodes are well distributed in the soils of Kerala. Under the above

circumstances it is quite likely that the chances of epiphytotics of ginger bacterial wilt is still greater due to the interaction of the two organisms. Since both the organisms are soil-borne and once the soil is infested by these organisms, it will be very difficult to eradicate them from the soil and therefore might pose a major threat to any attempt of field control of the disease.

Further, it would be worthwhile to carry out investigations on the soil transmission of the bacterial pathogen by the root-knot nematode which was not taken up during the course of the present study.

Studies conducted on the toxigenicity of the pathogen indicated that some toxic metabolite might be involved in its pathogenicity. A heat-stable, viscous material was obtained from the culture filtrates of the bacterial isolates by precipitation with acetone. Husain and Kelman (1958) have obtained such a substance from the culture filtrates of other pathogenic strains of Pseudomonas solanacearum.

The four isolates of the bacterium gave different amounts of the precipitate under uniform conditions. This might be due to the variations among the isolates, as indicated by the differences in certain physiological properties among them.

Aqueous solution of the toxic compound was found to cause irreversible wilting of ginger shoots. But in the case of tomato, brinjal, chillies and tobacco, wilting was reversible, as observed by Husain and Kelman (1958) in the case of tomato cuttings. So in the present study, the toxic compound was found to be specific to ginger.

The toxic metabolite obtained had a pronounced effect on the expression of the symptoms of the disease on ginger shoots. Wilt symptoms such as rolling, yellowing, drooping and wilting were produced by the toxic compound in that order.

From the results of the studies on the toxigenicity of the bacterium causing ginger wilt, it was evident that the heat-stable, specific, toxic substance produced by the pathogen might be involved in its pathogenicity and had a significant influence on the symptom expression of the disease.

Summary

SUMMARY

The bacterial wilt disease of ginger, caused by Pseudomonas solanacearum E.F. Smith is one of the most severe and economically important diseases so far reported on the crop. This disease was of unknown occurrence in India till 1973, when it was first reported from parts of Calicut and Trivandrum districts in Kerala, where it occurred in epiphytotic proportions (Sarma et al., 1978; Mathew et al., 1979). Subsequently it was noticed in other parts of the State also.

In view of the importance of the disease, and the havoc it can cause to ginger cultivation in the State, studies were undertaken on the symptomatology of the disease, characterisation and identity of the pathogen, its survival, in vitro and in vivo control of the pathogen using antibiotics, role and association of root-knot nematodes in the disease and toxigenicity of the bacterium.

Four pathogenic isolates of the bacterium from different localities were established and employed in the present investigations.

Symptoms of the disease were studied under natural and controlled conditions. There were no noticeable

differences in symptoms expressed by the different isolates of the bacterium on artificial inoculation. All the symptoms of the disease under natural conditions such as loss of turgidity of the leaves, rolling and yellowing of the leaflets, followed by drooping and wilting of the plant and rotting of the rhizome could be produced on artificial inoculation, although slight variation was noticed in the sequence of expression of the initial symptoms.

The isolates produced circular, creamy white or white, raised, entire, slimy and fluidal colonies on artificial media. On tetrazolium chloride medium, they yielded white colonies with light pink centre.

Morphologically, the isolates of the bacterium were gram negative, short rods. Physiological and biochemical studies indicated that they utilized glucose oxidatively, did not hydrolyse starch or produce indole, produced levan and ammonia, showed positive activity of catalase, tyrosinase and arginine hydrolase, reduced nitrate to nitrite, did not produce water soluble or insoluble pigments, grew well in asparagine medium, tolerated sodium chloride concentration

upto 2 per cent and produced hypersensitive reaction on tobacco leaves within 48 hr of infiltration. With respect to some characters such as action on milk, gelatin liquefaction, urease activity and hydrogen sulphide production, there were differences among the bacterial isolates.

Sodium salts of citric, formic and acetic acids were utilised by the isolates, but not that of benzoic acid. Acid production, indicative of utilization was observed in the case of glucose, galactose, fructose, mannose, inotose, maltose, xylose, raffinose, ribose, sorbose, dextrose, sucrose, glycerol, cellobiose, inositol, sorbitol, dulcitol and mannitol. Cellulose and salicin were not utilized by the isolates.

Based on the above characters coupled with its pathogenicity and symptom expression, the identity of the pathogen was established as Pseudomonas solanacearum E.F. Smith, biotype-3 of Hayward.

For laboratory studies and mass culturing of the bacterium, NA and PCA were found to be the best solid media.

The pathogen could survive for considerably long periods of time in infected seed rhizomes, plant debris

and clay soil. The periods of survival in these sources were 5, 20 and 22 months respectively. These results indicated that the pathogen could survive in the above sources and carry over the disease from season to season.

The organism was sensitive to Ambistryn-S, Agrimycin-100, Chloromycetin, Terramycin and Streptocycline at 250 ppm. Penicillin and Fuchsamycin were ineffective upto 500 ppm. Penicillin had the least effect, inhibiting the pathogen only at 1500 ppm. Ambistryn-S and Agrimycin-100 were the most effective antibiotics in the in vitro trial, followed by Chloromycetin.

None of the antibiotics tested in the field could give absolute control of the disease. But wilt incidence was lesser in the treatments receiving antibiotic sprays, than in the control. Pre-inoculation sprays offered better control than post-inoculation sprays. Ambistryn-S at 500 ppm gave 70 per cent control of the disease, whereas Agrimycin-100 and Chloromycetin at the same concentration could give only 50 per cent control.

Studies conducted on the role and association of root-knot nematodes Meloidogyne incognita revealed that

they had a positive influence in increasing the incidence and speed of development of the disease. Wilt was more accentuated when nematodes were also present in the soil than when there were bacteria alone. Incidence and speed of wilt were maximum when nematodes were already present in the soil at the time of bacterial inoculation. These results indicated the possibility that the root-knot nematodes were predisposing the ginger plants to increased bacterial infection.

A heat-stable viscous substance was obtained from the culture filtrate of the pathogen, which could produce wilt symptoms on healthy ginger shoots. It is therefore felt that it might be having a role in the pathogenicity of the bacterium and symptom expression of the disease.

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* Originals not seen

Appendices

APPENDIX - I(a)

Incidence of wilt in sick soil in pots during season-1

Pot No.	Percentage of wilted plants in each pot (in angles)	Pot No.	Percentage of wilted plants in each pot (in angles)	Pot No.	Percentage of wilted plants in each pot (in angles)
1	60	18	45	35	0
2	45	19	60	36	30
3	45	20	60	37	60
4	45	21	30	38	45
5	30	22	45	39	45
6	90	23	45	40	45
7	45	24	60	41	30
8	90	25	60	42	60
9	30	26	30	43	0
10	60	27	90	44	45
11	60	28	45	45	90
12	60	29	60	46	45
13	45	30	60	47	30
14	45	31	60	48	30
15	30	32	90	49	45
16	45	33	45	50	60
17	45	34	30		

APPENDIX - I(b)

Incidence of wilt in sick soil in pots during season-2

Pot No.	Percentage of wilted plants in each pot (in angles)	Pot No.	Percentage of wilted plants in each pot (in angles)	Pot No.	Percentage of wilted plants in each pot (in angles)
1	30	17	0	34	30
2	30	18	45	35	0
3	0	19	30	36	30
4	30	20	60	37	30
5	0	21	30	38	30
6	45	22	30	39	45
7	30	23	0	40	30
8	0	24	90	41	0
9	30	25	30	42	60
10	45	26	0	43	0
11	30	27	0	44	30
12	30	28	45	45	45
13	45	29	30	46	30
14	30	30	45	47	30
15	0	31	60	48	45
16	0	32	30	49	30
		33	0	50	0

$t_{(0.05)\ (n_1+n_2-2)}$ = 1.9828

Computed 't' value for comparing the wilt incidence in inoculated plant debris during both the seasons = 6.1290*

* Significant at 0.05 level.

APPENDIX II (a)

Incidence of wilt in infected plant debris in pots during season-1

Pot No.	Percentage of wilted plants in each pot (in angles)	Pot No.	Percentage of wilted plants in each pot (in angles)	Pot No.	Percentage of wilted plants in each pot (in angles)
1	30	19	60	37	60
2	0	20	60	38	45
3	30	21	45	39	30
4	60	22	90	40	30
5	45	23	45	41	0
6	45	24	45	42	45
7	60	25	60	43	30
8	60	26	60	44	0
9	30	27	45	45	45
10	45	28	45	46	30
11	30	29	30	47	30
12	60	30	30	48	45
13	60	31	45	49	30
14	30	32	30	50	0
15	30	33	45		
16	45	34	30		
17	45	35	30		
18	45	36	45		

APPENDIX-II(b)

Incidence of wilt in infected plant debris in pots during season-2

Pot No.	Percentage of wilted plants in each pot (in angles)	Pot No.	Percentage of wilted plants in each pot (in angles)	Pot No.	Percentage of wilted plants in each pot (in angles
1	90	18	30	35	30
2	0	19	0	36	30
3	45	20	45	37	0
4	30	21	30	38	30
5	30	22	45	39	45
6	45	23	30	40	30
7	60	24	45	41	0
8	30	25	0	42	30
9	0	26	0	43	30
10	0	27	45	44	0
11	0	28	30	45	45
12	30	29	0	46	0
13	30	30	0	47	45
14	0	31	30	48	30
15	30	32	0	49	0
16	0	33	30	50	0
17	45	34	0		

$t_{(0.05)}$ = 1.9828

$(n_1 + n_2 - 2)$

Computed 't' value for comparing the wilt incidence in infected plant debris during both the seasons = 4.9756*

* Significant at 0.05 level.

APPENDIX-III

Analysis of variance table

In vitro sensitivity of *Pseudomonas solanacearum* from ginger to antibiotics

Source	df	Sum of square	Mean square	F
Total	95	130.02	..	..
Treatments	31	119.54	3.8561	23.5415**
Antibiotics	7	95.9475	13.7068	83.6800**
Between levels of antibiotics				
Ambatrep-S	3	4.5900	1.5300	9.3407**
Agrimycin-100	3	3.5025	1.1675	7.1276**
Chloromycetin	3	4.0425	1.3475	8.2265**
Terramycin	3	2.2500	0.7500	4.5788**
Streptomycetine	3	1.7025	0.5675	3.4646*
Ampicillin	3	4.7025	1.5675	9.5696**
Penstrepcin	3	8.0700	2.6900	16.4224**
Penicillin	3	5.0625	1.6875	10.3022**
Error	64	10.4800	0.1638	..

** Significant at 0.01 level

* Significant at 0.01 level

APPENDIX-IV

Analysis of variance table

In vitro sensitivity of *Pseudomonas solanacearum* from ginger to combinations of antibiotics

Source	df	Sum of square	Mean square	F
Total	14	3.8733	0.2767	..
Treatments	2	3.7083	1.8541	134.3565*
Error	12	0.165	0.0138	..

* Significant at 0.05 level.

APPENDIX - V

Analysis of variance table

Influence of root-knot nematodes *Meloidogyne incognita* on the speed of development of bacterial wilt of ginger.

Source	df	Sum of square	Mean square	F
Total	17	845.612	49.74	..
Treatments	3	781.612	260.54	57.01*
Error	14	64.000	4.57	..

* Significant at 0.05 level.

ABSTRACT

The bacterial wilt of ginger incited by Pseudomonas solanacearum E.F. Smith, probably the most serious of all the diseases recorded on this crop was first reported from India in 1978. The symptoms of the disease include loss of turgidity of leaves, rolling and yellowing of the leaflets, drooping and wilting of the plant and rotting of the rhizome. No variations in symptoms were observed with the different isolates of the pathogen.

Nutrient agar and Peptone casamino acid were the best solid media for the growth of the bacterium. Slight variations in growth were observed among the isolates when grown on PCA medium.

The pathogen was identified to be Pseudomonas solanacearum E.F. Smith, biotype-3 of Hayward, from its morphological, physiological and biochemical characters coupled with pathogenicity.

Infected seed rhizomes, plant debris and soil were found to serve as sources of survival of the pathogen for considerably long periods of 5, 20 and 22 months respectively. Aubiotyn-S and Agrimycin-100 were the most effective antibiotics in inhibiting the pathogen under in vitro conditions. When these antibiotics were tested in the field,

none of them could absolutely control the disease. But wilt incidence was reduced due to antibiotic sprays. Control of the disease was greater when antibiotic spray was given prior to bacterial inoculation. Ambistryn-S at 500 ppm gave 70 per cent control of the disease. Agrimycin-100 and Chloramycetin applied at the same concentration offered 50 per cent control.

The root-knot nematodes Meloidogyne incognita were found to have a positive role in the increased incidence and speed of development of bacterial wilt of ginger. It was also indicated that these nematodes were predisposing the ginger plants to increased bacterial infection.

A heat-stable, specific toxic substance produced by the pathogen was observed to have a role in its pathogenicity and symptom expression of the disease.