## MANAGEMENT OF BACTERIAL WILT OF GINGER (Zingiber officinale Rosc) INCITED BY Pseudomonas solanaceasum (SMITH) SMITH

Ву

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#### **THESIS**

Submitted in partial fulfilment of the requirement for the degree

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#### DECLARATION

I hereby declare that this thesis entitled "Management of bacterial wilt of ginger (Zingiber officinale Rosc) incited by Pseudomonas solanacearum (Smith) Smith" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed that basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellanikkara

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#### CERTIFICATE

Certified that this thesis entitled "Management of bacterial wilt of ginger (Zingiber officinale Rosc) incited by Pseudomonas solanacearum (Smith) Smith" is a record of research work done independently by Miss.Alli Rani,G. 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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We, the undersigned members of the Advisory Committee of Miss.Alli Rani,G. a candidate for the degree of Master of Science in Agriculture, agree that the thesis entitled "Management of bacterial wilt of ginger (Zingiber officinale Rosc) incited by Pseudomonas solanacearum (Smith) Smith" may be submitted by Miss.Alli Rani,G. in partial fulfilment of the requirement of the degree.

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To my parents

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## Introduction

#### INTRODUCTION

Ginger (Zingiber officinale Rosc.) belonging to the family Zingiberaceae is an important commercial spice crop esteemed for its aroma, flavour and pungency. The aromatic rhizomes of ginger find application as beverage in perfumery and in pharmaceutical industry. At present ginger ranks fourth among the important spices of India, standing next to pepper, cardamom and turmeric.

In India, its cultivation is distributed in the tropical and subtropical regions. India is also the largest producer and exporter of ginger in the world, contributing to about 50 per cent of world production and export. Indian ginger is considered to be of high quality. In India, it is grown over an area of 53,300 hectares distributed mainly in the States of Kerala, Karnataka, Madhya Pradesh, Himachal Pradesh, Orissa, West Bengal, Andhra Pradesh and Assam. The annual production of ginger in the country is 1,56,000 tonnes. Kerala accounts for 70 per cent of total ginger production and is the renowned centre for quality ginger.

A number of pathogens are reported to infect ginger causing several diseases and crop losses. Well known among them are the soft rot disease (Pythium spp.),

bacterial wilt (Pseudomonas solanacearum), yellows disease (Fusarium spp.) and leaf spot (Phyllosticta zingiberi).

Dake and Edison (1984) conducted the survey for disease incidence in major ginger growing areas of Kerala during 1984-1985 and reported that the incidence of bacterial wilt in Wyanad, Pathanamthitta, Kottayam and Thrissur districts had increased since 1978. The disease incidence due to P. solanacearum, Pythium spp. and Fusarium spp. were 29.61, 21.61 and 8.84 per cent respectively. Among them, the bacterial wilt caused by P. solanacearum (Smith) has proved to be one of the most destructive diseases of the crop which can inflict total crop losses (Plate 1).

As early as in 1953, this disease was reported from Mauritius and later other ginger growing areas of the world, such as Queensland, Malaya, Hawaii, Philippines and Malaysia. In India, it was reported during monsoon months of 1978, in the Ambalavayal and Sultan's Battery areas of Kozhikode district and Adipparamba areas of Thiruvananthapuram district in Kerala (Mathew et al., 1979).

The bacterial wilt disease incited by Pseudomonas solanacearum is one of the most destructive pathogens in the warm tropical regions of the world. The agroclimatic conditions of Kerala are quite conducive for development



of the bacterial wilt disease of many crop plants especially on ginger. Since the ginger bacterial wilt pathogen is primary seed and soil borne, the chances of spread of the disease to other disease free areas are quite high, which in turn may cause heavy crop losses. At present there is no viable management practice to reduce the crop losses. Considering the serious nature of the disease and the potential danger it can cause to ginger cultivation, the present study was undertaken on the following aspects:

- i. Characterization and identification of the pathogen
- ii. In vitro and in vivo evaluation of common antibiotics, fungicides, botanicals and other materials against bacterial wilt pathogen
- iii. To study the role of weather factors on the incidence of bacterial wilt disease of ginger
- iv. To study the changes in the rhizosphere microflora due to application of antibiotics, fungicides and botanicals.

# 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 eggplant (*Solanum melongena*).

The earliest report of Pseudomonas solanacearum causing wilt of ginger (Zingiber officinale Rosc.) was made by Orian (1953) from Mauritius. Ishii and Aragaki (1963) noticed the disease from Hawaii. Jamil (1964) observed the disease in Malaya. Hayward et al. (1967) and Pegg et al. (1974) reported heavy losses in Queensland due to bacterial wilt of ginger. In Philippines, this disease was observed by Zehr (1969). Chew (1969) recognised bacterial wilt as the greatest problem of ginger production in Malaysia. Sarma et al. (1978) and Mathew et al. (1979) reported the bacterial wilt of ginger caused by Pseudomonas solanacearum for the first time in India. From Srilanka, Gunawardena et al. (1980) noticed the occurrence of this disease.

Severe losses were reported in ginger planted in Southern parts of Thailand due to the bacterial wilt and identified the pathogen as Pseudomonas solanacearum

(Titatarn and Tananuson, 1981). Choi and Han (1990) observed that the ginger disease caused by *Erwinia* spp. be termed as "bacterial soft rot" and that caused by the *Pseudomonas* spp. be as "bacterial rhizome rot" in Korea.

#### 2.1 The Pathogen

The shape and size of the bacterium that cause wilt disease was first described by Smith (1896), as non-spore forming, non-capsulated, gram negative small rod with polar flagella. Okabe (1937) studied the colony morphology of potato brown rot pathogen and described four colony types viz., F = wild types which are fluidal, irregular milky colony, readily isolated from the lesions; OP = opalescent, circular, homogenous; C = circular, light brownish, striate; SS = pale, fluorite green with cream coloured centre. The last three variants developed spontaneously in the tissue of diseased host from advanced stage of the disease.

Kelman (1954) reported that colony morphology was related to degree of virulence. He distinguished colony variants on Tetrazolium chloride (TTC) medium. These were the normal or wild type which were irregularly round, entire white colony with pink centre and the mutant or butyrous type which were round, translucent, smooth, deep red with narrow bluish border. Culture from butyrous and

red colonies were either weakly pathogenic or non-pathogenic, whereas cultures from white colonies with pink centre were highly pathogenic.

Hodgkiss (1964) reported that the bacterium P. solanacearum was a gram-negative motile rod with 1-4 polar flagella and colonies on solid media were usually small round, slightly raised, glistening white and smooth, 3-5 mm in diameter and appeared within 36-48 h at 28°C. Buchanan and Gibbons (1974) reported that atleast two types of colonies were produced by the pathogen on complex media, one type was smooth, fluidal, elevated and the others were somewhat rough, dry and flat. Some strains produced a diffusible brown pigment on complex media.

Mathew et al. (1979) observed that on TZC medium, the isolate of ginger wilt bacterium produced pink centred colonies. Mathew and Nayar (1983) found that brinjal isolate of P. solanacearum on TZC medium yielded circular, smooth, greyish white colonies with light pink centre. Similar growth character of P. solanacearum on TZC medium were observed by other workers also (Samuel, 1980; Swanepoel and Young, 1988; Prior and Steva, 1990 and Jyothi, 1992).

The pathogen lost its virulence very rapidly in culture due to transformation to avirulent forms and the

virulence could be maintained by storing in potato dextrose agar slant culture under sterile mineral oil at 25°C (Kelman and Jensen, 1951) or in sterile distilled water (Kelman and Person, 1961).

Quinon et al. (1964) reported that different strains of P. solanacearum produce brown pigment adundantly in culture media or diseased tissue and did not ferment lactose. Krieg and Holt (1984) reported that some strains of pathogen produced a diffusible pigment in complex media but the pigment had not been chemically identified. But the absence of pigment production by bacterium either on Yeast Glucose Chalk Agar or King's medium had been observed by many workers (Hayward, 1964; El-Halaly et al., 1969; Devi, 1978; Samuel, 1980; Nayar, 1982; Swanepoel and Young, 1988; Jyothi, 1992).

The aerobic nature of the *P. solanacearum* was observed by many workers (Labrousse, 1932; Moraes, 1947 and Samuel, 1980). Kelman and Jensen (1951) reported that it could grow in anaerobic condition also. Buchanan and Gibbons (1974) stated that strains of *P. solanacearum* could be grown under anaerobic condition in media containing nitrate and an appropriate carbon source. Devi (1978)

and Jyothi (1992) reported that the isolate of *P. solana-cearum* could grow both under aerobic and anaerobic conditions.

Hayward (1964) studied the characteristics of P. solanacearum and reported that on agar medium containing tyrosine a diffusible brown pigment was produced, the intensity of which might vary between isolates. He observed that none of the isolates produced a green fluorescent pigment on King's medium.

Catalase and oxidase were produced by the organism and citrate but not melonate was utilised as the sole source of carbon. Nitrate reduction and ammonia production were positive. It did not hydrolyse soluble starch or produce indole. Characters with slight variations were observed by many workers while studying the biochemical and physiological characters of strains of *P. solanacearum* from tomato, brinjal, chilli and ginger (Rath and Addy, 1977; Devi, 1978; Mathew et al., 1979; Samuel, 1980; He et al., 1983; Psallidas, 1985; Swanepoel and Young, 1988; Prior and Steva, 1990).

Palleroni and Duodoroff (1971) reported that none of the strains of *P. solanacearum* decomposed arginine

either aerobically or anaerobically and hydrolysed gelatin. Liberation of hydrogen sulphide was observed by Devi (1978) and Jyothi (1992).

Hayward (1964) further observed that acid was produced oxidatively from glucose, sucrose, fructose, glycerol and meso-inositol within 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. Similar results were obtained by many workers while studying the utilization of a number of carbon/organic compounds by P. solanacearum with or without acid production (Devi, 1978; Mathew et al., 1979; Samuel, 1980; Nayar, 1982; He et al., 1983; Psallidas, 1985; Lallmohamad et al., 1988; Swanepoel and Young, 1988; Prior and Steva, 1990).

#### 2.1.1 Cross inoculation studies

Cross infectivity of isolates of *P. solanacearum* from different host plants were studied by many workers.

Orian (1953) reported that the ginger wilt bacterium produced typical wilt on tomato. Buddenhagen et al.

(1962) have reported that *P. solanacearum* from many solanaceous plants like tobacco, tomato and brinjal were capable of cross infecting each other. Ishii and Aragaki (1963) observed that ginger wilt pathogen was weakly virulent to tomato, chilli and eggplant.

Quinon et al. (1964) observed that the ginger strain failed to bring about a wilt in tomato, tobacco and peanut, whereas the tomato isolate wilted tomato and peanut but not ginger and tobacco. Zehr (1970) isolated a strain of the pathogen from ginger which was virulent to tomato but avirulent to potato and eggplant. He also observed that the isolates from these host plants were not virulent to ginger on artificial inoculation.

Lum (1973) isolated a weakly virulent form of *P. solanacearum* from ginger which did not produce any symptoms on tomato, tobacco and groundnut. Pegg et al. (1974) reported that biotype 3 and 4 of the pathogen were responsible for the wilt of ginger, out of which biotype 3 also caused typical wilt of tomato.

Samuel (1980) found that ginger isolate caused wilting in tomato, but tomato isolate failed to infect ginger. Ren and Fang (1981) found that isolates of ginger pathogen were not pathogenic to tomato and tomato isolates of the pathogen were only weakly pathogenic to ginger.

Nayar (1982) showed that tomato and brinjal isolates were capable of cross infecting each other and that chilli and ginger isolates caused wilting in their respective hosts only.

He et al. (1983) reported that ginger strain caused varying degree of wilting in tomato, eggplant and chilli and the chilli strain caused wilting in eggplant, tomato and chilli and the tomato strain and an eggplant strain caused wilting in tomato and eggplant and no wilting in chilli while, another strain from tomato caused wilting in tomato, eggplant and chilli. Velupillai and Stall (1984) found that the strain of P. solanacearum from tomato, brinjal, potato and sunflower were all of race 1 and pathogenic to brinjal, chilli, potato and tomato. Some were pathogenic to sunflower and ginger but did not infect groundnut.

He (1985) observed that the ginger strain caused varying degree of wilting on tomato, eggplant, ginger and chilli. The chilli isolate however did not cause wilting on ginger, though highly virulent to tomato and eggplant. Tomato and eggplant isolates were capable of cross infecting each other and these isolates caused no wilting on ginger.

Jyothi (1992) observed that chilli isolates caused

typical wilting on tomato, brinjal and ginger plants. Conversely the isolates from tomato, brinjal and ginger caused rapid and typical wilting on chilli. He further observed that isolates from tomato and brinjal were capable of cross infecting each other and isolates from tomato and brinjal did not cause any wilting on ginger and ginger isolates did not cause any infection on tomato and brinjal.

#### 2.2 Management of the disease

#### 2.2.1 In vitro sensitivity to antibiotics

Attempts have been made by many scientists to test the *in vitro* sensitivity of *P. solanacearum* to antibiotics.

Moorgan and Goodman (1955) reported that low concentration of Aureomycin and Terramycin effectively inhibited P. solanacearum. Hidaka and Murano (1956) found that Streptomycin at 0.3  $\mu$ g per ml of water inhibited and 5  $\mu$ g per ml killed the pathogen at once.

Foucart and Delcamb (1960) tested various chemicals in *in vitro* and found that Actinomycin and Chloromycetin gave promising results. Campacci et al. (1962) reported that among the various chemicals tested, the bacterium was sensitive to Agristrep (Streptomycin),

Penicillin-G-potassic, Penicillin procain, Dihydrostreptomycin sulphate and Erythromycin.

Chakravarti and Rangarajan (1966) reported that Streptocycline could control the pathogen in *in vitro*. Goorani et al. (1972) could inhibit the pathogen by Ampicillin, Chloramphenicol, Kenamycin, Oxytetracycline, Tetracycline, Penicillin-GandStreptomycin. Mondal and Mukherjee (1978) observed that Ampicillin, Streptomycin and Novabiocin at 500 ppm were promising against the pathogen in *in vitro*. Samuel (1980) found that ginger pathogen was sensitive to Ambistryn-S, Agrimycin-100, Chloromycetin, Terramycin and Streptocycline at 250 ppm but Ampicillin, Paushamycin at 250 ppm were ineffective.

Nayar (1982) found that Chloromycetin and Terramycin were most effective in inhibiting bacterial wilt pathogen of brinjal. He et al. (1983) reported that all the strains of P. solanacearum from China showed susceptibility to Streptomycin but were resistant to Penicillin, Viomycin and Chloramphenicol. Farag et al. (1986) observed that both virulent and avirulent forms of the pathogen were sensitive to Streptomycin and Dihydrostreptomycin. Gunawan (1988) tested various concentrations of Agrimycin-100 between 25 and 600 ppm and found that greatest inhibition of the pathogen occurred with 425 ppm.

Gunawan (1989) found that optimum concentration for suppression of bacterial multiplication in *in vitro* were 175 and 450 ppm of Streptomycin sulphate. Prior and Steva (1990) studied the sensitivity of some reference strains of *P. solanacearum* from French West Indies to selected antibiotics and found that chilli isolate showed varying sensitivity to Penicillin-G, Ampicillin, Erythromycin, Chloramphenicol and Rifampicin.

Jyothi (1992) tested five antibiotics against chilli wilt pathogen in *in vitro* and found that Chloromycetin and Streptocycline exhibited the maximum inhibition of the bacterium and that were significantly superior to Tetracycline, Terramycin and Ambistryn-S. Among the different concentrations of antibiotics tested, Streptocycline at 1000 ppm exerted the maximum inhibition of the bacterium.

There were only few reports to show the *in vitro* sensitivity of *P. solanacearum* to fungicides.

Goorani et al. (1978) reported that Nabam (Dithane A-40), Maneb (Dithane M-22) and Dithane M-45 inhibited P. solanacearum. Grinepadeze et al. (1978) conducted in vitro trials with 21 fungicides against the bacterial disease of mulberry caused by Pseudomonas mori and found that Chino-

sol, Zineb and Polychomphenthiuram showed high inhibitory activity. Leandro and Zak (1983) observed that Captan, Maneb, Mancozeb and Thiram at 1000 and 10000 ppm inhibited P. solanacearum in in vitro.

Lu et al. (1988) evaluated the effect of Methyl isothiocyanate (MITO) against nine soil borne fungal and bacterial pathogens by exposing them to the fumigant in a polypropylene bottle and found that P. solanacearum and Agrobacterium tumefaciens were killed at 2 mg per litre of water for 24 h and 1 mg per litre for 96 h. Jyothi (1992) tested various fungicides against P. solanacearum in in vitro and observed that Bordeaux mixture one per cent exerted the maximum inhibition of the pathogen followed by Thiride and Foltaf and Dithane M-45 failed to inhibit the growth of bacteria.

Kokoskov (1992) tested efficiency of 7 bactericides, 2 fungicides and 1 antibiotic (Streptomycin) against Erwinia, Pseudomonas, Xanthomonas, Corynebacterium, Agrobacterium and Flavobacterium in in vitro and reported that S-0208 was the most active followed by Mancozeb, Copper oxychloride, Streptomycin and Copper hydroxide. The fungicides Aliette and Ridomil WP were ineffective.

There were only few reports to show the in vitro

sensivitity of P. solanacearum to botanicals and others.

Hanudin and Djatrika (1986) evaluated the extracts from onion and garlic bulbs, roots and stems of Crotalaria sp. and Tagetes sp. and pawpaw leaves for suppression of P. solanacearum and reported that extracts from garlic bulb inhibited the bacterial growth. Hutagalung (1988) reported that bacterial wilt of tomatoes was decreased by adding a 10 ml suspension of 35 g of garlic bulb per 77 ml sterile water or 6 g of ground garlic bulb to the rhizosphere. Dhaliwal et al. (1990) reported that plant extracts of rice cv. TKM 6 inhibited growth of P. solanacearum. There were no reports for in vitro sensitivity of P. solanacearum to Kerosene and Cowdung.

#### 2.2.2 Field experiment on management of the disease

Hidaka and Murano (1956) conducted studies on the control of tobacco bacterial wilt disease caused by P. solanacearum in in vivo by surface absorption of Streptomycin. One spray containing 100  $\mu$ g per ml of water given one day before inoculation and a second spray 5 days after inoculation controlled the disease. Yamane and Komatsu (1957) reported that the pathogen inoculated on broad bean was inhibited by Streptomycin at 500  $\mu$ g and 1000  $\mu$ g per ml of water.

Ishii and Aragaki (1963) reported that good control of bacterial wilt of ginger could be obtained by soil fumigation with Methyl bromide at the rate of 3 lb per 100 sq.ft. of soil. Dutta and Verma (1969) showed that seedling treatment with Streptocycline at 1 g in 40 litres of water for 30 minutes could control the bacterial wilt of eggplant. Shetty and Rangaswami (1969) noticed the control of brown rot pathogen with C-6 an antibiotic similar to Erythromycin.

Rahim (1972) and George (1973) obtained excellent field control of bacterial wilt of chillies by foliar spraying with Streptomycin and Streptocycline or by soil drenching with Cheshunt compound. Devi (1978) found that soil amendment and antibiotic spray had significant influence in reducing the disease incidence. But none of them was found to be absolutely effective in checking the disease and the comparatively effective treatments were saw dust and Urea plus Agrimycin-100 spray.

Sarma et al. (1978) observed that Agrimycin-100, Streptocycline, Bordeaux mixiture and Bleaching powder did not check the spread of bacterial wilt of ginger. Samuel (1980) noticed that Ambistryn-S and Agrimycin-100 could effectively control the ginger bacterial wilt.

Kishun (1981) reported that soil application of

bleaching powder before transplanting of tomato gave the best control of *P. solanacearum*. Sitaramiah and Sinha (1983) evaluated five antibiotics against bacterial wilt of brinjal and found that Penicillin and Agrimycin-100 were consistently superior over other antibiotics against the pathogen. Foliar application of Agrimycin-100, Chloramphenicol and Streptomycin sulphate one day before bacterial inoculation was effective while, Penicillin and Tetracycline did not inhibit the wilt bacterium.

Ojha et al. (1986) obtained complete control of ginger bacterial wilt caused by P. solanacearum by rhizome treatment with Emisan-6 0.2 per cent plus Plantomycin 0.05 per cent for 50 minutes in addition to three sprayings, first at 30 days after planting followed by spraying at 15 days interval. They also reported that Plantomycin alone and in combination with Blitox-50 were less effective in controlling the disease. Dake et al. (1986) found that seed treatment with 200 ppm Streptocycline kept the ginger bacterial wilt disease under check for 3 months but subsequently succumbed to the disease. Soil treatment with Bordeaux mixiture or Bleaching powder was partly effective against the disease but uneconomical.

Bazzi and Calzolari (1986) found that industrial Bordeaux mixture and Copper Oxychloride were more effec-

tive than Copper hydroxide and Kasugamycin for lettuce bacterial wilt caused by *P. cichorii*. Gunawan (1988) tested the effect of spraying intervals of 200 ppm of Streptomycin sulphate and Oxytetracycline for the control of bacterial wilt of tomato and found that spraying at four and seven days intervals gave highest degree of disease control. Ho (1988) reported that bleaching powder and Jeypine were most effective than other chemicals tested in reducing the incidence of *P. solanacearum* in tomato. Kishun and Chand (1988) observed that application of bleaching powder 15 kg/ha was effective against bacterial wilt of tomato.

Gunawan (1989) reported that Agrimycin-100 at 200 ppm gave effective control of bacterial wilt of tomato. Verma and Shekhawat (1991) observed that soil application of bleaching powder in furrows at 50 g/m² controlled the potato bacterial wilt by 68.4 per cent while, Copper sulphate plus bleaching powder at 15 g/m² reduced wilt by more or less 50 per cent. Jyothi (1992) conducted a field experiment on the management of bacterial wilt of chillies and found that plants that received cowdung as one of the major treatments recorded comparatively low percentage of wilt incidence. Among the minor treatments, plants that received two applications of Streptocycline 1000 ppm also showed comparatively less wilt incidence.

### 2.3 Effect of environmental factors in relation to disease incidence and severity of *P. solanacearum*

Vaughan (1944) reported that tomato bacterial wilt symptom did not develop at soil temperature below 21°C. Disease incidence increased with increase in soil temperature. Gallegly and Walker (1949) reported that soil temperature plays an important role in development of bacterial wilt of tomato. An increase in soil moisture from 50 to 100 per cent of its water holding capacity and temperature from 21 to 37.5°C favoured the development of bacterial wilt of potato (Hingorani et al., 1956).

Sabet and Baraket (1971) found that 90 per cent of the soil water holding capacity was optimal for bacterial wilt development in potato. Acosta (1964) reported that tomato bacterial wilt infection was more severe during the summer at high soil temperature.

Krausz and Thurston (1975) showed that temperature of 32°C in controlled environment chamber significantly increased the severity of bacterial wilt in two tomato lines resistant to *P. solanacearum*. Mew and Ho (1977) found that resistance to bacterial wilt caused by *P. solanacearum* in one tomato cultivar was influenced by

changes in soil temperature. Mathew et al. (1979) reported that rainfall and relative humidity plays a definite role in spread and development of bacterial wilt of ginger. The maximum disease incidence was noticed during August-September when the crop was about 3-4 months old coinciding with south west monsoon ensuring high soil moisture, relative humidity and low temperature.

Hiryati et al. (1983) tested the effect of soil types and moisture levels on bacterial wilt disease of groundnut and reported that severity of *P. solanacearum* significantly increased with increased soil moisture. Soil type alone and in combination with varying soil moisture levels had a significant effect on the severity of bacterial wilt of groundnut.

Akiew (1985) found that population of potato pathogen *P. solanacearum* decreased sharply with increase in air temperature and with decrease in soil temperature. Ho (1988) reported that high rainfall towards the middle end of the growing season favoured bacterial wilt in tomato. Jyothi (1992) observed no significant correlation between environmental factors and wilt incidence of chilli for all varieties except one variety, which showed positive correlation between soil moisture and wilt incidence.

# 2.4 Effect of antibiotics, fungicides and botanicals on changes in the soil and rhizosphere microflora

There were no exhaustive previous study on the effect of antibiotics, fungicides and water extract of botanicals on rhizosphere microflora of ginger. However, there were reports on other crops which showed that spraying of Streptocycline and Streptomycin had reduced the population of Pseudomonas and Xanthomonas (Desai et al., 1967; Dath and Devadath, 1969; Rahim, 1972; George, 1973). Devi and Samraj (1962) observed that foliar spraying of Streptomycin was readily absorbed and translocated to various tissues of tomato plant. A marked decrease in the bacterial population was also noticed as a result of antibiotic spray. Reddy (1968) tested rhizosphere effect of rice seedlings to Agrimycin-100 and reported that rhizosphere microflora viz., fungi, bacteria and actinomycetes were reduced at lower concentrations, whereas, higher concentrations stimulated the rhizosphere population than in the untreated control.

Devi (1978) reported that spraying the plant with antibiotics was ineffective in decreasing the rhizosphere population and the pathogen *P. solanacearum*. Jeyaprakash (1978) observed that application of Streptomycin, Streptocycline, Cheshunt compound and Bordeaux mixture reduced

the wilt incidence of tomato and rhizosphere population of *P. solanacearum*. Dublish (1986) observed that spraying the plants with Urea and Chloramphenicol increased the number of fungi in rhizosphere soil whereas, Streptomycin decreased the fungal population in rhizosphere soil. In general, a reduction of bacterial population in soil by application of antibiotics such as Streptomycin, Terramycin, Ambistryn-S, Streptocycline, Chloromycetin has been reported by several workers (Nakas and Klein, 1980; Sah et al., 1985; Elias et al., 1987). Anderson and Domsch (1973) and Nakas and Klein (1980) reported that Oxytetracycline and Streptocycline had effectively reduced the actinomycetes population.

# Materials and Methods

#### MATERIALS AND METHODS

# 3.1 Isolation of the ginger bacterial wilt pathogen

Ginger plants showing the symptoms of bacterial wilt disease were collected from farmers field at Ambalavayal. The rhizomes and pseudostem of diseased plants thus collected were subjected to ooze test to confirm the presence of bacteria. Such pieces with profuse bacterial ooze were selected for isolation of the pathogen. They were cut into small bits and surface sterilized with 0.1 per cent mercuric chloride solution for one minute and then washed in three changes of sterile distilled water. These bits were placed on a sterilized glass slide with few drops of sterile distilled water, then they were teased apart to obtain a bacterial suspension. The suspension was streaked on Triphenyl Tetrazolium Chloride Agar (TZC) medium to get well isolated colonies of the bacterium (Kelman, 1954).

Composition of Triphenyl Tetrazolium Chloride Agar (TZC) 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

рН

- 6.8

The inoculated plates were incubated at room temperature for 48 h. Characteristic light pink centered slimy, fluidal colonies were selected and were purified by repeated streaking on TZC medium.

# 3.1.2 Pathogenicity test on ginger

Pathogenicity of the bacterium was established by inoculating a thick suspension of 48 h old culture grown on Peptone Dextrose Agar. The suspension of the bacterium was prepared in sterile distilled water. Vigorously growing young two month old plants were used for inoculation. The bacterial suspension was inoculated into leaf axil and basal portion of the pseudostem with a 5 ml syringe and 25 gauge hypodermic needle. Sterile cotton dipped in the bacterial suspension was also placed on the injured portion. The inoculated plants were observed periodically for symptom development. The pathogen was re-isolated from the artificially inoculated plants by the method already described.

Morphological characters of the re-isolated colonies of the bacterium were compared with that of the original isolate to ensure their identity. Stock cultures were

maintained at room temperature as well as at 10°C by storing two or three loopful of the culture of the bacterium in 5 ml of sterile distilled water in test tubes. Similarly stock cultures were also maintained in Potato Dextrose Agar slants both at room temperature as well as in refrigerated condition. Cultures were tested periodically for virulence and purify by streaking on TZC medium.

#### 3.2 Characterization of the pathogen

Characterization of the isolate of bacterium was done according to the methods recommended in the "Manual of Microbiological Methods", published by the Society of American Bacteriologists (1957) and "Laboratory Methods in Microbiology" (Harrigan and Mc Canco, 1966).

#### 3.2.1 Cultural characters

#### 3.2.1.1 Morphology

The colony morphology of the bacterium was studied from 24-48 h old culture of the pathogen grown on Peptone Casamino Acid (PCA) medium. The gram reaction of the isolate of the bacterium was studied using Hucker's modified method of gram staining (Hucker and Conn, 1923).

#### 3.2.1.2 Growth of the bacterium on TZC medium

The isolate of the bacterium was studied for its

colony growth, colour, shape, type of margin, consistency, extent of growth, slime production and fluidity on TZC medium. A loopful of dilute suspension of the bacterium prepared in sterile distilled water was streaked on the TZC medium in petridishes. Three replications were maintained and those were incubated at room temperature. Observations were taken after 24, 48, 72 and 96 h of incubation.

#### 3.2.1.3 Potato soft rot test

The method suggested by Lelliott et al. (1966) was employed for the test and 7-8 mm thick slices of washed, peeled and alcohol flamed potato tubers were placed in sterile petriplates. The surface of slices were immediately covered with sterile distilled water, till the slices were half immersed. A loopful of 48 h old growth of the bacterium was placed in a nitch made at centre of each slice. The slices were watched for rotting. Uninoculated slices served as control.

# 3.2.1.4 Pigment production

Production of water insoluble pigment by the bacterium was studied on Yeast Glucose Chalk Agar (YGCA) medium. Observation was recorded after 48 h of incubation. Composition of the medium:

Yeast extract	-	10.0	g
Glucose	-	10.0	g
CaCo <sub>3</sub>	<b>-</b> ,	20.0	g
Agar agar	-	20.0	g
Distilled water	-	1000	ml
рН	_	7.2	

Production of water soluble pigment was studied on King's medium (King et al., 1954).

# Composition of the medium:

Peptone	_	20.0	g
Glycerine	-	10.0	ml
K <sub>2</sub> HPO <sub>4</sub>	-	1.5	g
$Mgso_4.7H_2O$	-	1.5	g
Agar agar	-	20.0	g
Distilled water	-	1000	ml
рН	_	7.2	

The culture of bacterium was spot inoculated in the medium poured in petriplates, incubated for 48 h and examined for zone of pigmentation around the colonies.

# 3.2.1.5 Oxygen requirements

Nutrient Dextrose Agar (containing 0.05 per cent bromocresol purple) columns in test tubes were inoculated in duplicates with isolate of the bacterium using a

straight inoculation needle. The agar surface of one set of tubes was covered with sterile liquid paraffin to a depth of 1 cm. The tubes were incubated and observations were recorded periodically.

#### 3.2.2 Physiological characters

#### 3.2.2.1 Starch hydrolysis

The ability of the bacterial isolate to hydrolyse starch was studied using Nutrient Agar medium containing 0.2 per cent soluble starch (Anon, 1957).

# Composition of the medium

Peptone - 10.0 g

Beef extract - 5.0 g

Starch (soluble) - 2.0 g

Agar agar - 20.0 g

Distilled water - 1000 ml

pH - 7.0

The isolate was spot inoculated on the medium poured in plates. After four days of incubation, starch hydrolysis was assessed by pouring Lugol's iodine over the medium. A colourless or reddish brown zone around the bacterial growth indicated positive starch hydrolysis compared to the blue background of the medium.

# 3.2.2.2 Tyrosinase activity

Dye's medium (Dye, 1962) was used for the study. Composition of the medium:

 $NH_4 H_2PO_4$ -0.5 g $K_2HPo_4$ -0.5 g $MgSO_4.7H_2O$ -0.2 gNaCl - 5.0 g Yeast extract - 5.0 g Tyrosine -0.5 qAgar agar - 20.0 g Distilled water - 1000 ml Нq - 7.0

The medium was dispensed in test tubes, autoclaved and slants were prepared. The slants were inoculated with the culture of the bacterium and incubated for 48-72 h. Browning of the medium indicated positive result for tyrosinase activity.

#### 3.2.2.3 Production of indole

Tryptophan broth was employed for the test. Composition of the medium:

Tryptophan or casein digest - 10.0 g

NaCl - 5.0 g

Distilled water - 1000 ml

pH - 7.0

The medium was poured in test tubes and autoclaved. Whatman No.1 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 these were dried at room temperature and used
without sterilization. The test tubes were inoculated with
the culture of bacterium and oxalic acid strips were
inserted into the test tubes by the side of the plug. The
tubes were incubated and observed for 14 days. Change in
colour of the oxalic acid crystals to pink or red indicated as production of indole.

# 3.2.2.4 Production of hydrogen sulphide

The ability of the bacterial isolate to liberate hydrogen sulphide was detected using Peptone water medium with one per cent Casamino acid.

# Composition of the medium:

Peptone - 10.0 g

Nacl - 5.0 g

Casamino acid - 10.0 g

Distilled water - 1000 ml

pH - 7.0

Five ml of the medium was dispensed in test tubes and autoclaved. Whatman No.1 filter paper strips of  $5 \times 50$  mm size were cut and soaked in warm saturated lead acetate

solution. The test strips were dried, autoclaved and again dried at 60°C. The tubes were inoculated with the bacterial isolate and the strips were inserted as ptically by the side of the plug. The tubes were incubated at room temperature and observations were recorded at regular intervals for a period of 14 days. Liberation of hydrogen sulphide was indicated by the blackening of the test strip.

#### 3.2.2.5 Nitrate reduction test

Nitrate broth medium was used for the test. Composition of the medium:

Peptone - 10.0 g

KNO<sub>3</sub> (Nitrite free) - 1.0 g

Beef extract - 5.0 g

Distilled water - 1000 ml

pH - 7.0

Five ml quantities of the broth were dispensed in test tubes, autoclaved, cooled and inoculated with the bacterium. Test for nitrate reduction was conducted at regular intervals upto 15 days. The test was performed by adding few drops of Griess Ilsovay's reagent consisting of sulphanilic acid (0.8 per cent in five molar acetic acid) and dimethyl - alphanaphthyl amine (0.5 per cent in five molar acetic acid) to the nitrate broth culture. Distinct

pink or red colouration of the broth indicates the presence of nitrate. If there is no colour change of the medium, it indicates that nitrate was present as such or reduced to ammonia and free nitrogen. To confirm this test, few zinc crystals were added to the broth reagent mixture and agitated for few minutes. The change in colour of the broth to pink or red indicated that nitrate was present without reduction. Absence of colour in either of the two tests would mean that nitrate was reduced to ammonia or free nitrogen.

#### 3.2.2.6 Production of ammonia

Peptone water medium was employed for this test.

Composition of the medium:

Peptone - 10.0 g

Distilled water - 1000 ml

pH - 7.0

The isolate of bacterium was inoculated in Peptone water medium and incubated for 48 h. The accumulation of ammonia was confirmed by using Nessler's reagent which gave a brown to yellow precipitate with ammonia.

#### 3.2.2.7 Gelatin liquefaction

Nutrient Agar medium containing 0.4 per cent gelatin was used for this test.

# Composition of the medium:

Peptone - 10.0 g

Beef extract - 5.0 g

Agar agar - 20.0 g

Distilled water - 1000 ml

pH - 6.8

The bacterium was spot inoculated in the medium in petriplates and incubated. After 42-78 h the agar surface was flooded with 0.2 per cent mercuric chloride solution in dilute hydrochloric acid (20 per cent) and allowed to act for few minutes to precipitate the gelatin in the medium (Smith et al., 1946). A clear zone surrounding the bacterial growth indicated positive gelatin utilization.

#### 3.2.2.8 Catalase test

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

#### 3.2.2.9 Arginine hydrolase test

Thornley's medium was employed for this test (Thornley, 1960).

## Composition of the medium:

Peptone - 1.0 g

 $K_2H PO_4 - 0.3 g$ 

NaCl - 5.0 g

Agar agar - 3.0 g

Phenol red - 0.01 g

L arginine monochloride - 1.0 g

Distilled water - 1000 ml

pH - 7.2

Five ml quantities of the medium was dispensed in test tubes, autoclaved, cooled and stab inoculated with the bacterial isolate. The surface of medium was covered with sterile liquid paraffin to a depth of 1 cm. The tubes were incubated for 7 days at 25-30°C and observations were recorded at regular intervals. A change in colour of the medium to red indicated arginine hydrolase activity.

#### 3.2.2.10 Production of levan

Peptone Beef extract medium with 5 per cent sucrose was used for this test.

# Composition of the mediumn:

Peptone - 10.0 g

Beef extract - 5.0 q

Sucrose - 50.0 g

Agar agar - 20.0 g

Distilled water - 1000 ml

pH - 7.0

The medium was sterilized by tyndallization. Dilute suspension of the bacterium was streaked over the medium and incubated for 48 h. Development of large, white, domed and mucoid colonies on the medium indicated production of levan.

#### 3.2.2.11 Utilisation of carbon compounds

Hayward's semisolid medium was used for this test (Hayward, 1964).

# Composition of the medium:

Peptone - 1.0 g

 $NH_4H_2 PO_4$  - 1.0 g

KC1 - 0.2 g

 $MgSO_4.7H_2O$  - 0.2 g

Bromothymol blue - 0.03 g

Agar agar - 30.0 g

Distilled water - 1000 ml

pH - 7.2

Nine carbon compounds viz., glucose, ribose, fructose, sucrose, dextrose, maltose, galactose, mannitol and dulcitol were tested individually for the utilization

by the bacterium. An aliquot of 90 ml of each of the basal medium was poured in 250 ml conical flask and autoclaved, 10 per cent solution of the carbon compounds were prepared in distilled water and steam sterilized. One ml each of the sterilized solution was added to 90 ml aliquotes of the melted medium and dispensed in sterilized test tubes to a depth of 4 cm. The medium was inoculated with bacterium. In one set of tubes containing the carbon compounds, the agar surface was covered with sterile paraffin to a depth of 1 cm. The inoculated tubes were incubated and observations were recorded at regular intervals upto a period of one month. Change in colour of the medium to yellow indicated positive utilization of the carbon compounds with acid production.

#### 3.2.2.12 Cross inoculation studies

Ginger, tomato, chilli and brinjal plants were used for the cross inoculation study. Bacterial coze was collected from the respective wilted host plants in sterile distilled water and the coze was cross inoculated with tomato, chilli, brinjal and ginger plants by the method suggested by Winstead and Kelman (1952) and observed for the symptom development. The identity of the bacterium from different host plants was confirmed by streaking the coze on TZC medium.

## 3.3 Management of the disease

3.3.1 In vitro evaluation of antibiotics, fungicides, botanicals and others against P. solanacearum

In vitro efficacy of antibiotics, fungicides, botanicals and others in inhibiting the growth of the bacterium was tested by the standard filter paper disc method. The details of the antibiotics, fungicides, botanicals and others used and their concentrations are given below.

	Antibiotics, fungicides, botanicals and others	Active ingredient/ method of preparat- ion	Concentrations	
1	2	3	4	
1	Ambistryn-S	Streptomycin sulphate	250, 500, 750, 1000 ppm	
2	Terramycin	Oxytetracycline hydrochloride	250, 500, 750, 1000 ppm	
3	Chloromycetin	Chloramphenicol	250, 500, 750, 1000 ppm	
4	Streptocycline	Streptomycin sulphate 90% and Tetracycline hydrochloride 10%	250, 500, 750, 1000 ppm	
5	Bordeaux mixture	Lime + Copper sulphate	0.5, 1, 1.5, 2%	
6	Calixin	Tridemomorph	0.05, 0.1, 0.15 0.2%	

Contd.

1	2	3	4
7	Water extract of garlic	Garlic bulb + water	10, 15, 25, 50 g/l
8	Water extract of Ocimum spp.	Ocimum spp + water	10, 15, 25, 50 g/l
9	Kerosene	Kerosene + water	10, 15, 25, 50 ml/l
10	Fresh cowdung	Cowdung + water	10, 15, 25 50 g/l

The different concentrations of antibiotics, fungicides, botanicals and others were prepared in sterile distilled water. The water extract of garlic and Ocimum spp. was prepared by crushing them using a mortar and pestle. The extracts were used after keeping them overnight. Sterile filter paper discs of 10 mm diameter were dipped in the prepared solution and placed over Peptone Casaminoacid Agar medium seeded with 24-48 h old culture of the bacterium. Sterile filter paper discs dipped in sterile water served as control. The test was done in triplicates and the plates were incubated at room temperature and observations were recorded after 48 h.

# 3.3.2 Field experiment on the management of bacterial wilt of ginger

A field experiment was conducted during May - December 1993 at the College of Horticulture, Vellanikkara

to locate an effective treatment which will reduce the economic loss due to the disease. The experiment was done in a field artificially made wilt sick. This was done by uniformly incorporating wilt sick soil and stubbles of diseased ginger plants collected from the farmers field of Ambalavayal in the beds before planting.

The cultural practices were given following the Package of Practice Recommendation (Kerala Agricultural University, 1989). The experiment was laid out in RBD with nine treatments in three replications. The details are given below:

Design - RBD

Treatments - 9

Replications - 3

Plot size - 1 x 1 m

Variety - Rio-de-Janeiro

Spacing - 20 x 20 cm

Date of planting - 18-5-1993

#### Treatments:

T<sub>1</sub> Ambistryn-S - 1000 ppm

 $T_2$  Chloromycetin - 1000 ppm

 $T_3$  Terramycin - 1000 ppm

T<sub>4</sub> Bordeaux mixture - 1%

 $T_5$  Streptocycline - 1000 ppm

 $T_6$  Water extract of Ocimum spp. - 50 g/l

 $T_7$  Water extract of garlic - 50 g/l

T<sub>8</sub> Calixin - 0.1%

T<sub>q</sub> Control

The treatments were given when the plants started showing symptom of bacterial wilt (70 days after planting). The treatments were given four times at an interval of ten days. The treatments were applied as soil drench as well as spray on the plants.

The incidence of bacterial wilt was recorded before application and further observations were recorded at ten days interval. Final observation was recorded 160 days after planting.

Population of fungi, bacteria, actinomycetes and the pathogen (P. solanacearum) in the rhizosphere of ginger plants before application and after each application of the treatments in the field were estimated by following serial dilution plate technique. Martin's rose bengal streptomycin agar medium, Soil extract agar, Kenknight agar and Triphenyltetrazolium chloride agar medium (Appendix-1, II, III) were used for estimating fungi, bacteria, actinomycetes and the pathogen (P. solanacearum) respectively.

# 3.3.3 Correlation studies on bacterial wilt incidence with weather parameters

In order to correlate the bacterial wilt incidence with environmental factors, observations on the maximum and minimum air and soil temperature, relative humidity and rainfall were recorded at weekly intervals for about 7 weeks. Wilt incidence were also recorded at weekly intervals in control plots only.

Observations on other diseases like damping-off, Phyllosticta leaf spot and growth parameters like number of tillers and height of plant were recorded at fortnightly intervals. In the case of Phyllosticta leaf spot the disease index was calculated based on the score card (Appendix-IV) suggested by Premanathan (1981).

# 3.3.4 Estimation of VA-mycorrhizal infection on ginger

In the field, due to severe attack of bacterial wilt, there was a limited number of plants per plot. So root samples were collected from bacterial wilt infected and healthy plants from different locations instead of collection from each treatments. The roots were stained for mycorrhizal infection by the method of Phillips and Hayman (1970) and the percentage of mycorrhizal infection was estimated.

The root bits were first washed in tap water to remove all adhering soil particles and cut into small bits of approximately one cm in length. The roots were transferred to clean labelled bottles and fixed with Formalin: Acetic acid: Alcohol mixture (FAA) (Appendix-V).

For staining, the root bits were initially softened by immersing in 10 per cent KOH at 90°C for one hour. After cooling the excess of alkali was removed by repeated rinsing with tap water and then acidified with 2 per cent HCL. The bits were stained with 0.05 per cent trypan blue in lactophenol by boiling for three minutes (Appendix-V).

The excess stain from the root tissue was removed by clearing overnight in fresh lactophenol. The root bits were examined for the typical VA-mycorrhizal infection under the light microscope. Each of the root bits were divided into four equal segments for recording the presence or absence of VA mycorrhiza in segmentwise manner. The percentage of VAM infection was calculated by the following formula:

% VAM infection = No. of positive root segments
No. of positive root segments
No. of root segments observed

3.3.5 In vitro effect of antibiotics, fungicides and botanicals on soil microflora in wilt sick soil

A study was conducted to confirm the results obtained in the field experiment on the changes in the microbial population due to application of antibiotics, fungicides and water extract of botanicals. The details of the treatment were same as that of field experiment on the management of the disease. Treatments were given four times at an interval of ten days. The changes in the population of fungi, bacteria, actinomycetes and the pathogen (P. solanacearum) were estimated before application and after each application of treatments by following serial dilution plate technique.

3.3.6 Pot culture experiment on the management of bacterial wilt of ginger

A pot culture experiment was also conducted simultaneously with the field experiment to study the effect of antibiotics, fungicides and botanicals in reducing the severity of bacterial wilt of ginger. The treatments given for the field experiment was used for this experiment also. The pots were filled with mixture of garden soil and powdered farm yard manure and ginger rhizomes were planted. After two month of planting vigorously growing plants were inoculated with the bacterial suspension as described earlier. The treatments were given as in the case of field

experiment, when the plants started showing symptoms of bacterial wilt. The incidence of bacterial wilt was recorded before application and further observations were recorded at ten days interval. Final observation was recorded 160 days after planting. Observations on other diseases like damping-off, leaf spot and growth parameters like number of tillers, height of plant and yield were recorded in pot culture experiment also.

# 3.4 Statistical analysis

All the data were analysed statistically using analysis of variance technique. A simple correlation was made between environmental factors with wilt incidence (Panse and Sukhatme, 1978).

# Results

#### RESULTS

# 4.1 Isolation of the pathogen and pathogenicity test on ginger

The bacterial wilt pathogen was isolated from newly wilted ginger plants on Triphenyl Tetrazolium Chloride Agar (TZC) medium. The culture was purified by repeated streaking on the same medium and Koch's postulates were proved with the isolate of the pathogen.

#### 4.2. Characterization of pathogen

The results of the cultural and physiological characters of the *P. solanacearum* are given in Table 1.

#### 4.2.1 Cultural characters

## 4.2.1.1 Morphology

The isolate of ginger wilt bacterium was gram negative, motile short rods.

#### 4.2.1.2 Growth of the bacterium on TZC medium

On TZC medium it produced circular, smooth, raised, greish white, fluidal and slimy colonies with light pink centre after 24-48 h of incubation.

#### 4.2.1.3 Potato soft rot test

The potato soft rot test was done as described in

materials and methods. The bacterium caused blackening and rotting of the potato slices within 48 h.

# 4.2.1.4 Pigment production

The isolate of the bacterium did not produce both water insoluble and soluble pigment on Yeast Glucose Chalk Agar and King's medium respectively.

# 4.2.1.5 Oxygen requirements

The bacterium grew and changed the colour of Nutrient Dextrose Agar medium with and without paraffin from purple to yellow indicating that the isolate could grow both in anaerobic and aerobic conditions respectively.

## 4.2.2 Physiological characters

#### 4.2.2.1 Starch hydrolysis

The test culture of the bacterium failed to hydrolyse the starch as indicated by the absence of colourless zone around the bacterial growth in contrast to the blue background of the medium.

# 4.2.2.2 Tyrosinase activity

The bacterial isolate produced a brown colouration in the medium which indicated tyrosinase activity.

## 4.2.2.3 Production of indole

The oxalic acid crystals on the test strips did not change into pink or red which indicated the absence of indole production by the bacterium.

#### 4.2.2.4 Production of hydrogen sulphide

The isolate of the bacterium had liberated hydrogen sulphide as evidenced by the blackening of the lead acetate test strips.

#### 4.2.2.5 Nitrate reduction test

The bacterium produced a red colouration in the nitrate broth upon addition of Griess Ilosvay's reagent consisting of sulphanilic acid showing that it was capable of reducing nitrate to nitrite.

#### 4.2.2.6 Production of ammonia

The bacterium produced a precipitate upon the addition of Nessler's reagent to Peptone water medium indicating that it was capable of producing ammonia.

## 4.2.2.7 Gelatin liguefaction

The bacterium failed to liquefy gelatin as indicated by the absence of clear zone surrounding the bacterial growth in the medium.

#### 4.2.2.8 Catalase reaction

Positive catalase reaction was produced by isolate of bacterium.

#### 4.2.2.9 Arginine hydrolase test

The isolate of bacterium failed to change Thornley semisolid Arginine medium to red which indicated its inability to hydrolyse arginine.

#### 4.2.2.10 Production of levan

Large, white, domed and mucoid colonies were produced by the bacterium on Peptone Beef extract medium Containing 5 per cent sucrose.

#### 4.2.2.11 Utilization of carbon compounds

The isolate of the bacterium utilized all the nine carbon compounds tested as evidenced by the change of colour of the medium to yellow.

#### 4.2.2.12 Cross inoculation studies

Cross inoculation studies were conducted as described in the Materials and Methods and the results are presented in Table 2. The isolate of the bacterium from ginger, chilli, tomato and brinjal produced high wilt

Table 1. Cultural and physiological characters of the ginger isolate of <u>Pseudomonas solanacearum</u>

Sl.No.	Characters studied	Results		
	_			
1	Gram reaction			
2	Potato soft rot test	+		
3	Pigment production			
	a) Water soluble	-		
	b) Water insoluble	<b>-</b>		
4	Oxygen requirements			
	a) aerobic	+		
_	b) anaerobic	+		
5	Starch hydrolysis	<del>-</del>		
6	Tyrosinase activity	+		
7	Indole production	<del>-</del>		
8	Production of hydrogen sulphide	+		
9				
	11 Gelatin liquefaction			
12	Catalase reaction	+		
13	Arginine hydrolase test	<del>-</del>		
14	Levan production	+		
15	Utilization of carbon compounds			
	a) Glucose	+		
	b) Ribose	+		
	c) Fructose	+		
	d) Sucrose	+		
	e) Dextrose	+		
	f) Maltose	+		
	g) Galactose	+		
	h) Mannitol	+		
	i) Dulcitol	+		

<sup>+ =</sup> Positive reaction

<sup>- =</sup> Negative reaction



Table 2. Cross inoculation with the bacterial isolates from different host plants (ginger, brinjal, tomato and chilli)

Isolate	Relative	virulence	on different	hosts
Isolate	Ginger	Brinjal	Tomato	Chilli
Ginger	Н	0	L	Н
Brinjal	0	Н	L	Н
Tomato	0	L	Н	Н
Chilli	Н	Н	Н	Н

H - High (65-100% wilt incidence)

M - Medium (40-65% wilt incidence)

L - Low (below 40% wilt incidence) O - Nil

(Winstead and Kelman, 1952)

incidence on their respective hosts after 7-10 days of inoculation. The ginger isolate caused high wilt incidence on chilli, low wilt incidence on tomato and did not show any symptoms on brinjal. The chilli isolate of the bacterium caused high wilt incidence on tomato, ginger and brinjal. The isolate from brinjal produced high incidence of wilt on chilli, low incidence on tomato and did not produce any symptoms on ginger. The tomato isolate caused high wilt incidence on chilli, low incidence on brinjal and failed to produce symptoms on ginger. So the isolates of the pathogen from tomato and brinjal did not produce any symptoms on ginger.

#### 4.3 Management of the disease

4.3.1 In vitro evaluation of antibiotics, fungicides, botanicals and others against P. solanacearum

#### A. Antibiotics

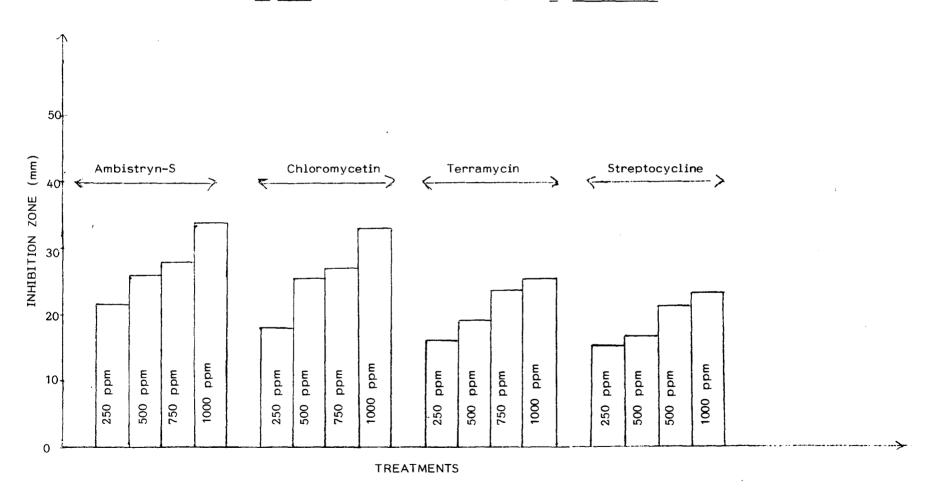
The *in vitro* efficacy of four antibiotics, viz., Ambistryn-S, Chloromycetin, Terramycin and Streptocycline each at 250, 500, 750 and 1000 ppm concentrations in inhibiting the growth of *P. solanacearum* were studied by standard filter paper disc method and the results are presented in Table 3 and Fig. 1. In general, higher concentrations of antibiotics exhibited maximum inhibition of the bacterium. Maximum inhibition of the bacterium was with Ambistryn-S at 1000 ppm followed by Chloromycetin at

Table 3. In vitro sensitivity of <u>Pseudomonas</u> solanacearum to antibiotics

sl.		Inhibition zone in mm			
No.	Antibiotics	250 ppm	500 ppm	750 ppm	1000 ppm
1	Ambistryn-S	21.66	26.33	28.67	34.00
2	Chloromycetin	18.67	25.83	27.67	33.00
3	Terramycin	16.17	19.67	23.50	25.67
4	Streptocycline	15.17	16.67	21.00	23.33

cD (0.05) for comparison between treatments = 0.906

Fig. 1. In vitro evaluation of antibiotics against P. solanacearum



1000 ppm and these were statistically superior to other concentrations of other antibiotics. The minimum inhibition of bacterium was noticed with 250 ppm of Streptocycline and found to be inferior to other antibiotics concentrations. Terramycin at 250 ppm was on par with Streptocycline at 500 ppm in inhibiting the growth of the bacterium. Ambistryn-S 250 ppm was significantly higher than Streptocycline at 750 ppm for its inhibition on bacterium. The inhibitory effect of Ambistryn-S and Chloromycetin at 500 ppm was on par with Terramycin at 1000 ppm. The inhibitory effect of Chloromycetin at 250 ppm was higher than Streptocycline at 500 ppm.

## B. Fungicides

Bordeaux mixture at 0.5, 1, 1.5 and 2 per cent and Calixin at 0.05, 6.1, 6.15 and 0.2 per cent were tested for its inhibitory effect on the growth of the bacterium in *in vitro* and the results are presented in Table 4 and Fig. 2. It is evident that Bordeaux mixture at one per cent concentration gave the maximum inhibition of bacterium than its other concentrations and Calixin at all concentrations tested. However, Bordeaux mixture at 0.5 per cent concentration gave better inhibitory effect than Calixin at all concentrations tested. The inhibitory effect of 2 per cent Bordeaux mixture was on par with 6.1

Table 4. <u>In vitro</u> sensitivity of <u>Pseudomonas</u> <u>solanacearum</u> to fungicides

a l		Inhibition zone in mm					
Sl.	Fungicides	0.5%	1.0%	1.5%	2.0%		
1	1 Bordeaux mixture	19.33	24.83	21.17	15.50		
		0.05%	0.1%	0.15%	0.2%		
2	Calixin	0	15.17	15.50	16.33		

CD (0.05) for comparison between treatments = 0.321

and 0.15 percentage of Calixin. Calixin 0.05 per cent did not give any inhibitory effect on P. solanacearum and found to be inferior than other treatments.

#### C. Botanicals and others

The effect of water extract of *Ocimum* spp., garlic and cowdung each at concentrations of 10, 15, 25 and 50 g/l and Kerosene at 10, 15, 25 and 50 ml/l in inhibiting the growth of the bacterium were studied and results are presented in Table 5 and Fig. 2.

Among the treatments, maximum inhibition of the bacterium was noticed in water extract of Ocimum spp. at 50 g/l followed by water extract of garlic at 50 g/l and these were statistically superior than other treatments. The inhibitory effect of water extract of Ocimum spp. at 15 g/l was on par with garlic at 15 g/l. Cowdung at 10, 15 and 25 g/l and Kerosene at 10 ml/l failed to inhibit the growth of P. solanacearum. The inhibitory effect of cowdung at 50 g/l was on par with Kerosene at 25 and 50 ml/l. Among the treatments, minimum inhibition of bacterium was observed with Kerosene at 15 ml/l and water extract of garlic at 10 g/l.

- 4.3.2 Field experiment on the management of bacterial wilt of ginger
  - A field experiment was conducted to find out the effect of

Table 5. <u>In vitro</u> sensitivity of <u>Pseudomonas</u> solanacearum to botanicals and others

	Botanicals and others	I:	nhibition	zone in m	mm
NO.	others	10 g/l	15 g/l	25 g/l	50 g/l
1	Water extract of Ocimum spp.	12.17	15.50	18.17	22.50
2	Water extract of garlic	11.17	15.50	16.83	21.66
3	Cowdung + water	0	0	0	13.13
		10 ml/l	15 ml/l	25 ml/l	50 ml/l
4	Kerosene + water	0	10.83	13.50	13.30

CD (0.05) for comparison between treatments = 0.768

Fig.2. In  $\underbrace{\frac{\text{P.}}{\text{P.}}}$  vitro evaluation of fungicides, botanicals and others against 60 (ww) 300Z NOILIBIHNI 20 Water extract of Calixin Ocimum spp. Cowdung + water 10 10 m1/1 m1/1m1/1m1/1m1/1g/1 g/1 m1/1 ml/1 10 g/1 g/1g/1 g/1 g/1 g/10.05% 0.15% 0.5% 1.5% 0.1% 0.2% 10 15 25 50 15 25 15 0 50 50 0 TREATMENTS

antibiotics, fungicides and water extract of botanicals on the management of bacterial wilt of ginger. The details of the treatments were given in Materials and Methods. Treatments were given four times at an interval of ten days starting from 70 days after planting. Observations on the percentage of wilt incidence were recorded before application and 10 days after each application of treatments. In addition to this the changes in population of the pathogen P. solanacearum and total microflora viz., bacteria, fungi and actinomycetes in the rhizosphere of ginger plants were also taken at different intervals.

From the data given in Table 6, it was evident that none of the treatments gave an absolute control of the bacterial wilt of ginger. However, observations on the percentage of wilt incidence ten days after first, second, third and fourth treatment application showed marked difference between treated and untreated control.

In the beginning the percentage of wilt incidence constantly increased in all the treatments with no significant difference between them ten days after first and second application. However, it was noticed that plots treated with Ambistryn-S, Bordeaux mixture and Streptocycline had the minimum wilt incidence than others ten days after first and second application. The maximum wilt

incidence was recorded in control plots. The plots treated with Ambistryn-S and Bordeaux mixture did not show further wilt incidence ten days after second application onwards. Ten days after third application the treatments showed significant difference. Plants in Ambistryn-S treated plots showed significantly less wilt incidence closely followed by Bordeaux mixture treated plots. The maximum wilt incidence was observed in control followed by Streptocycline treatment. Ten days after fourth application and 160 days after planting, increase in wilt incidence were not noticed in any of the treatments.

The percentage efficiency of the treatment over control in checking the wilt incidence was also worked out. In general, it was noticed that application of Ambistryn-S and Bordeaux mixture had more percentage of efficiency over control in reducing the wilt incidence.

The effect of antibiotics, fungicides and water extract of botanicals on the rhizosphere microflora viz., bacteria, fungi, actinomycetes and the pathogen *P. solanacearum* were assessed.

# a. The pathogen P. solanacearum

The statistical analysis of the data showed significant difference between the treatments on the popula-

Table 6. Field experiment on management of bacterial wilt of ginger with antibiotics, fungicides and botanicals: Percentage of wilt incidence at different intervals

Therefore		A	Wilt incid	dence (%)		
Treatments		after first	10 days after second application 90 DAP	after third application	after fourth	Final ob- servation
Tl Ambistryn-S	22.91	68.75	70.83	70.83	70.83	70.83
T2 Chloromycetin	31.41	70.83	79.16	81.25	81.25	81.25
T3 Terramycin	27.08	75.00	77.08	83.33	83.33	83.33
T4 Bordeaux mixture	31.25	70.00	75.00	75.00	75.00	75.00
T5 Streptocycline	29.16	72.91	75.00	91.66	91.66	91.66
T6 Water extract of Ocimum spp.	33.33	79.16	87.50	87.50	87.50	87.50
T7 Water extract of garlic	29.16	70.83	77.03	81.25	81.25	81.25
T8 Calixin	31.25	79.16	87.50	87.50	87.50	87.50
T9 Control	33.33	87.50	100.00	100.00	100.00	100.00
CD (0.05)	NS	NS	NS	11.853	11.853	11.853

DAP - Days after planting NS - Non significant

Contd.

Table 6. Continued

The	atments	Percentage efficiency of the treatment over control						
ire	астенся		10 days after second application 90 DAP		10 days after fourth application 110 DAP	Final ob- servation 160 DAP	Mean	
71	Ambistryn-S	21.43	19.05	29.17	29.17	29.17	25.60	
T2	Chloromycetin	19.05	9.53	18.75	18.75	18.75	16.97	
13	Terramycin	14.29	11.90	16.67	16.67	16.67	15.24	
Т4	Bordeaux mixture	20.00	14.28	25.00	25.00	25.00	21.87	
T5	Streptocycline	16.67	14.28	8.34	8.34	8.34	11.19	
ፐና	Water extract of Ocimum spp.	9.53	9.53	12.50	12.50	12.50	11.31	
Т7	Water extract of garlic	19.05	11.90	18.75	18.75	18.75	17.44	
тв	Calixin	9.53	9.53	12.50	12.50	12.50	11.31	

tion of *P. solanacearum* at different intervals of observation (Table 7). Among the treatments, minimum mean population of *P. solanacearum* was noticed in plots treated with Bordeaux mixture and Ambistryn-S ten days after first application. The maximum population was noticed in control plots.

The decreasing trend in the population of *P. solanacearum* was noticed in all the treated plots ten days after second application. Ten days after second and third application, plots treated with Ambistryn-S and Bordeaux mixture had minimum population of *P. solanacearum* than the other treatments. Similarly a reduction in the population of *P. solanacearum* was recorded in plots treated with Ambistryn-S, Bordeaux mixture and Chloromycetin ten days after fourth application and 160 days after planting.

From the data, it is evident that all the treatments except application of water extract of Ocimum spp. and garlic considerably reduced the population of the pathogen. In general, plots treated with Ambistryn-S effectively reduced the population of P. solanacearum which was followed by Bordeaux mixture and Chloromycetin treatments. The control plot had maximum P. solanacearum population than the other treatments.

Table 7. Mean population of  $\underline{P}$ . solanacearum in the rhizosphere of ginger plants in the field experiment (counts in  $10^{-6}$  dilution/g of soil)

Treatments	Before application (70 DAP)	10 days after first application (80 DAP)	10 days after second application (90 DAP)	10 days after third application (100 DAP)	10 days after fourth application (110 DAP)	Final observat- ion (160 DAP)
Tl Ambistryn-S	23.66	14.66	13.33	12.66	11.33	11.00
T2 Chloromycetin	20.66	18.66	16.66	16.66	15.66	14.00
T3 Terramycin	21.33	20.00	18.66	18.66	18.33	17.66
T4 Bordeaux mixture	22.00	14.33	13.66	13.33	13.33	13.00
T5 Streptocycline	20.00	20.33	19.00	18.66	18.33	19.33
T6 Water extract of Ocimum spp.	21.33	29.33	27.33	25.33	23.33	23.00
T7 Water extract of garlic	18.66	21.33	21.00	21.00	22.33	20.66
T8 Calixin	22.33	19.66	19.33	22.33	19.66	19.00
T9 Control	21.33	32.66	38.66	37.66	38.00	34.00
CD (0.05)	NS	3.110	1.649	1.682	1.580	0.870

DAP - Days after planting NS - Non significant

#### b. Bacteria

The changes in the mean population of total bacteria in different treatments are given in Table 8. Statistical analysis of data showed significant difference between treatments ten days after first, second, third and fourth application of treatments. As a result of first application, slight reduction in population of bacteria was observed in plots treated with Ambistryn-S, Chloromycetin, Terramycin, Bordeaux mixture, water extract of garlic and Streptocycline. The plots treated with Ambistryn-S and Bordeaux mixture had the minimum bacterial population ten days after second, third and fourth applications. The application of Chloromycetin, Streptocycline and Terramycin had also slightly reduced the population than the control.

The bacterial population was maximum in control Plots. Among the nine treatments, Ambistryn-S, Bordeaux mixture, Streptocycline and Chloromycetin had effectively reduced the bacterial population under field conditions.

#### c. Fungi

From the data given in the Table 9 it is evident that, the fungal population also differed significantly between the treatments like the other microorganisms.

Table 8. Mean population of total bacteria in the rhizosphere of ginger plants in the field experiment (counts in 10<sup>-6</sup> dilution/g of soil)

Treatments	Before application (70 DAP)	10 days after first application (80 DAP)	10 days after second application (90 DAP)	10 days after third application (100 DAP)	10 days after fourth application (110 DAP)	Final observat- ion (160 DAP)
T1 Ambistryn-S	17.00	15.00	12.66	12.33	11.66	11.33
T2 Chloromycetin	20.00	16.00	16.33	15.66	15.66	15.66
T3 Terramycin	20.66	19.00	17.66	17.00	17.33	17.00
T4 Bordeaux mixture	26.66	15.33	14.66	14.66	13.33	14.00
T5 Streptocycline	26.66	18.33	18.00	18.33	16.33	15.33
T6 Water extract of Ocimum spp.	22.33	25.66	22.66	22.66	22.66	20.66
T7 Water extract of garlic	20.00	17.00	19.66	18.00	19.00	19.66
T8 Calixin	19.00	20.66	21.66	21.00	20.33	18.66
T9 Control	22.33	29.66	32.33	39.66	33.33	32.33
CD (0.05)	NS	2.618	2.110	2.050	1.950	1.649

DAP - Days after planting NS - Non significant

After the first application, plots treated with Bordeaux mixture, Ambistryn-S, Terramycin, Calixin, water extract of garlic and Ocimum spp. had slightly reduced the fungal population. The plots treated with Bordeaux mixture and Ambistryn-S had minimum fungal population after second and third application of treatments. Here also the maximum fungal population was in control plots. However, ten days after fourth application, maximum fungal population was recorded in water extract of Ocimum spp. and minimum number of population was noticed in Bordeaux mixture applied plots.

In general, of the nine treatments, Bordeaux mixture had more ability to reduce the fungal population followed by Ambistryn-S and other treatments had only very little effect to reduce the fungal population.

### d. Actinomycetes

The mean population of actinomycetes in the rhizosphere of ginger plants in different treatments are given in Table 10. In contrast to the other microorganisms, the treatments did not reduce the population of actinomycetes in the field. However, significant difference was noticed among them ten days after first, second, third and fourth application of treatments. The treatments did not show a definite trend in increasing or

Table 9. Mean population of fungi in the rhizosphere of ginger plants in the field experiment (counts in  $10^{-3}$  dilution/g of soil)

		<del></del>	· · · · · · · · · · · · · · · · · · ·	·	~	
Treatments	Before application (70 DAP)	10 days after first application (80 DAP)	10 days after second application (90 DAP)	10 days after third application (100 DAP)	10 days after fourth application (110 DAP)	Final observat- ion (160 DAP)
T1 Ambistryn-S	25.33	21.00	18.66	18.66	19.66	17.33
T2 Chloromycetin	25.66	27.33	25.00	22.66	22.66	20.66
T3 Terramycin	23.00	20.66	21.00	21.33	20.66	18.66
T4 Bordeaux mixture	22.33	17.33	15.66	14.33	13.66	13.66
T5 Streptocycline	18.33	21.66	19.33	20.66	21.66	21.33
T6 Water extract of Ocimum spp.	26.00	19.00	20.33	20.66	23.66	21.33
T7 Water extract of garlic	23.00	20.66	23.33	22.33	21.00	22.33
T8 Calixin	24.00	17.66	19.33	18.33	19.00	20.00
T9 Control	22.33	26.33	24.00	23.00	22.66	20.00
CD (0.05)	NS	3.805	2.424	1.951	1.837	2.164

DAP - Days after planting NS - Non significant

Table 10. Mean population of actinomycetes in the rhizosphere of ginger plants in the field experiment (counts in 10<sup>-4</sup> dilution/g of soil)

Treatments	Before application (70 DAP)	10 days after first application (80 DAP)	10 days after second application (90 DAP)	10 days after third application (100 DAP)	10 days after fourth application (110 DAP)	Final observat- ion (160 DAP)
T1 Ambistryn-S	15.66	16.33	18.00	19.66	20.66	20.33
T2 Chloromycetin	13.66	14.00	18.33	21.00	22.66	22.00
T3 Terramycin	16.00	16.66	15.33	19.33	21.66	22.33
T4 Bordeaux mixture	16.00	17.33	18.66	19.66	22.00	21.00
T5 Streptocycline	13.33	18.00	16.33	18.00	19.00	18.66
T6 Water extract of Ocimum spp.	17.33	20.33	20.00	20.66	21.66	21.00
T7 Water extract of garlic	14.66	16.33	18.66	16.66	17.66	16.66
T8 Calixin	15.33	19.66	17.39	18.33	18.00	18.66
T9 Control	15.33	19.33	18.66	20.66	20.33	20.66
CD (0.05)	NS	2.819	2.097	1.923	1.714	3.805

DAP - Days after planting NS - Non significant

decreasing the population of actinomycetes. However, plots treated with water extract of garlic had minimum actinomycetes population. From the data, it is evident that plots treated with antibiotics and fungicides did not reduce the actinomycetes population to any remarkable extent.

4.3.3 In vitro effect of antibiotics, fungicides and water extract of botanicals on soil microbial populations in wilt sick soil

This study was conducted to confirm the results obtained in the field experiment on the changes in the microbial population due to applications of antibiotics, fungicides and water extract of botanicals. The changes in the soil microflora viz., bacteria, fungi, actinomycetes and pathogen *P. solanacearum* were estimated and results are given in the Tables 11, 12, 13 and 14 and Fig. 3.

### a) The pathogen P. solanacearum

The treatments differed significantly in reducing the population of *P. solanacearum* after first, second, third and fourth application of treatments. Of the nine treatments, soil treated with Ambistryn-S and Bordeaux mixture had effectively reduced the population of *P. solanacearum*. Chloromycetin, Streptocycline and Terramycin also moderately reduced the population of the pathogen. After the first application, Ambistryn-S and Calixin had

slightly reduced the population. The maximum population was recorded in control.

As a result of the second application, population of *P. solanacearum* was reduced to half in soil treated with Ambistryn-S and Bordeaux mixture compared to the previous observation. The soil treated with Terramycin, Streptocycline and water extract of garlic and *Ocimum* spp. were found to reduce the population of *P. solanacearum* slightly after second application. As a result of the third and fourth application of treatments, the maximum reduction of *P. solanacearum* was observed in Ambistryn-S and Bordeaux mixture and moderate reduction was noticed in Chloromycetin, Terramycin and Streptocycline treatments. However, other treatments like water extract of garlic, *Ocimum* spp. and Calixin also slightly reduced the population than control. The maximum population was observed in control only.

## b) Bacteria

The population of total bacteria in wilt sick soil under different treatments are presented in Table 12. Statistical analysis on the data showed significant difference among the treatments after first, second, third and fourth applications. The maximum reduction of total bacterial population was recorded in Ambistryn-S and

Table 11. Mean population of  $\underline{P}$ , solanacearum in wilt sick soil due to different treatments in  $\underline{in}$  vitro (counts in 10<sup>-6</sup> dilution/g of soil)

	- '	•	•		
Treatments	Before application	10 days after first application		10 days after third application	10 days after fourth application
T1 Ambistryn-S	32.00	31.66	14.66	4.66	4.33
T2 Chloromycetin	34.00	36.66	18.60	8.66	8.33
T3 Terramycin	33.33	33.66	20.00	12.66	10.66
T4 Bordeaux mixture	33.33	34.33	17.00	6.33	5.33
T5 Streptocycline	33.66	37.33	23.66	10.66	10.33
T6 Water extract of <u>Ocimum</u> spp.	32.00	35.33	29.33	16.00	18.00
T7 Water extract of garlic	33.66	36.00	28.33	20.66	20.00
T8 Calixin	34.00	33.33	32.66	26.66	24.66
T9 Control	34.33	38.66	37.00	35.00	35.55
CD (0.05)	NS	2.033	4.391	4.828	6.651

NS - Non significant

Streptocycline treatments. Bordeaux mixture, Chloromycetin and Terramycin also were found to reduce the bacterial population than the other treatments.

As a result of the first application all the treated soils had slightly reduced bacterial population than control. The soils treated with Ambistryn-S, Bordeaux mixture and Chloromycetin had effectively reduced bacterial population ten days after second application of treatments.

The minimum count of bacterial population was recorded in Ambistryn-S and Streptocycline treatments ten days after third and fourth application of chemicals. An increasing trend of bacterial population was noticed in control only. Pots treated with Chloromycetin, Terramycin and Bordeaux mixture had also reduced the population, whereas other treatments like Calixin, water extract of garlic and Ocimum spp. were found to reduce the population slightly.

## c. Fungi

The mean population of fungi in wilt sick soil in different treatments are presented in Table 13. There was a significant difference among the treatments at different

Table 12. Mean population of total bacteria in wilt sick soil due to different treatments in  $\underline{\text{in vitro}}$  (counts in  $10^{-6}$  dilution/g of soil)

<del></del>	•	,	•		•
Treatments	Before application	10 days after first application	10 days after second application	10 days after third application	10 days after fourth application
Tl Ambistryn-S	31.33	28.66	15.66	8.66	7.66
T2 Chloromycetin	30.66	30.33	16.66	12.33	11.33
T3 Terramycin	30.33	28.00	19.66	15.33	14.66
T4 Bordeaux mixture	31.33	29.33	16.66	12.00	10.66
T5 Streptocycline	31.00	27.33	20.00	10.00	9.33
T6 Water extract of Ocimum spp.	31.33	30.66	23.66	20.00	20.00
T7 Water extract of garlic	30.00	29.66	26.00	22.66	20.66
T8 Calixin	31.00	29.66	25.00	21.00	19.33
T9 Control	31.33	3 <b>2.</b> 66	33.66	32.00	34.33
CD (0.05)	NS	1.864	2.060	2.032	1.951

NS - Non significant

intervals of observation. Of the nine treatments, application of Bordeaux mixture and Calixin had effectively reduced the fungal population.

As a result of the first application, fungal population was slightly reduced in soils treated with Bordeaux mixture and Calixin treatments. After second application, a slight decrease in population was noticed in pots treated with Ambistryn-S, Terramycin, Bordeaux mixture, Calixin and water extract of garlic and Ocimum spp. The minimum fungal population was recorded in Bordeaux mixture and Calixin treatments after third and fourth applications. The other treatments have not reduced the fungal population to any remarkable degree and only slight difference was noticed before first application and after the fourth application of treatments.

#### d. Actinomycetes

The changes in the mean population of actinomycetes in wilt sick soil under different treatments are presented in Table 14. In contrast to the other microorganisms, the treatments were not effective in changing the actinomycetes population to any remarkable extent after the first and second application. It did not show any definite trend of decrease or increase of population, whereas after the third and fourth application, a slight

Table 13. Mean population of fungi in wilt sick soil due to different treatments in  $\underline{in}$  vitro (counts in  $10^{-3}$  dilution/g of soil)

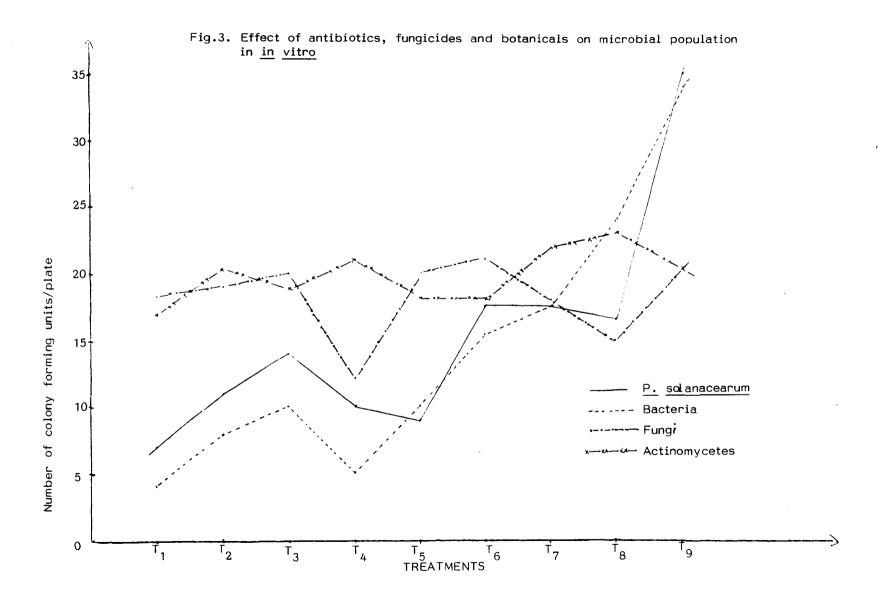
Treatments	Before application	10 days after first application	10 days after second application	10 days after third application	10 days after fourth application
Tl Ambistryn-S	20.00	21.33	18.66	19.33	18.66
T2 Chloromycetin	19.66	19.66	20.66	19.66	19.66
T3 Terramycin	21.00	23.33	21.33	18.66	20.00
T4 Bordeaux mixture	20.00	18.33	17.00	13.66	12.66
T5 Streptocycline	20.33	23.33	23.66	22.00	20.66
T6 Water extract of Ocimum spp.	20.66	20.00	19.33	20.00	21.33
T7 Water extract of garlic	20.00	22.33	20.00	17.66	18.33
T8 Calixin	19.66	18.66	17.33	16.00	15.00
T9 Control	20.66	22.33	22.00	21.66	20.66
CD (0.05)	NS	1.682	2.060	1.806	2.236

NS - Non significant

Table 14. Mean population of actinomycetes in wilt sick soil due to different treatments in in vitro (counts in 10<sup>-4</sup> dilution/g of soil)

<del></del>	<del></del>				
Treatments	Before application	10 days after first application	10 days after second application	10 days after third application	10 days after fourth application
T1 Ambistryn-S	19.00	18.66	17.33	16.66	17.33
T2 Chloromycetin	19.66	21.33	20.66	21.33	20.66
T3 Terramycin	21.00	19.66	18.33	17.33	19.33
T4 Bordeaux mixture	19.00	19.33	20.00	22.00	21.33
T5 Streptocycline	19.33	20.66	18.66	17.66	18.66
T6 Water extract of Ocimum spp.	20.66	20.33	19.66	19.33	18.66
T7 Water extract of garlic	20.66	18.66	17.66	23.33	22.66
T8 Calixin	19.66	21.33	19.33	22.66	23.00
T9 Control	20.66	20.66	18.66	19.33	20.00
CD (0.05)	NS	NS	NS	2.467	2.379

NS - Non significant



fluctuation was observed among the treatments. Of the nine treatments minimum population was recorded in Ambistryn-S, Streptocycline and water extract of *Ocimum* spp. treated soils. The maximum population was noticed in soil treated with Calixin and water extract of garlic.

# 4.3.4 Pot culture experiment on the management of bacterial wilt of ginger

A pot culture study was conducted simultaneously with the field experiment to find out the effect of antibiotics, fungicides and water extract of botanicals in reducing the bacterial wilt of ginger. The treatments given were the same as that of the field experiment. Observations on the percentage of wilt incidence were recorded on the day of each application and the results are given in Table 15.

The statistical analysis of the data on the percentage of wilt incidence showed marked difference among the treatments ten days after first, second, third and fourth application of treatments.

From the data, its is evident that the plants treated with Bordeaux mixture, Ambistryn-S, Terramycin, Chloromycetin and water extract of garlic treatments had minimum wilt incidence than the other treatments.

The plants treated with Bordeaux mixture had minimum percentage of wilt incidence followed by Ambistryn-S and Terramycin ten days after first application. As a result of second application, no further wilt incidence was noticed in pots treated with Bordeaux mixture, Ambistryn-S, Terramycin and Chloromycetin. Maximum percentage of wilt incidence was recorded in control. The plants treated with water extract of garlic did not develop further wilt incidence after third application. The maximum percentage of wilt incidence was noticed in control followed by Calixin, Streptocycline and water extract of Ocimum spp. treatments. After fourth application complete wilting was recorded in control, Streptocycline and Calixin treatments.

In general, of the nine treatments Bordeaux mixture treated plants had more percentage efficiency over control in reducing the wilt incidence followed by Ambistryn-S, Terramycin and Chloromycetin. The minimum percentage of efficiency over control and maximum percentage of wilt incidence was recorded in plants treated with Calixin, Streptocycline and water extract of Ocimum spp.

4.3.5 Correlation studies on bacterial wilt incidence with weather parameters

Correlation coefficients were made between percentage of

Table 15. Pot culture experiment on management of bacterial wilt of ginger with antibiotics fungicides and botanicals: Percentage of bacterial wilt incidence at different intervals

Theodone	Wilt incidence (%)									
Treatments	Before application		10 days after second application	after third		Final ob- servation				
Tl Ambistryn-S	19.91	24.07	24.07	24.07	24.07	24.07				
T2 Chloromycetin	19.09	27.42	27.42	27.42	27.42	27.42				
T3 Terramycin	17.42	25.76	25.76	25.76	25.76	25.76				
T4 Bordeaux mixture	22.05	22.05	22.05	22.05	22.05	22.05				
T5 Streptocycline	19.77	36.05	50.42	72.82	100.00	100.00				
T6 Water extract of Ocimum spp.	26.11	31.31	59.02	62.35	62.35	62.35				
T7 Water extract of garlic	20.62	27.35	30.13	30.13	30.13	30.13				
T8 Calixin	26.18	37.78	54.44	78.31	100.00	100.00				
T9 Control	24.59	34.92	72.69	100.00	100.00	100.00				
CD (0.05)	NS	12.827	15.872	15.644	14.347	14.347				

NS - Non significant

Contd.

Table 15. Continued

Treatments		Percentage efficiency of the treatment over control								
			10 days after second application 90 DAP		10 days after fourth application 110 DAP	Final ob- servation 160 DAP	Mean			
T1	Ambistryn-S	31.07	66.88	75.93	75.93	75.93	65.15			
T2	Chloromycetin	21.48	62.28	72.58	72.58	72.58	60.30			
Т3	Terramycin	26.23	64.56	74.24	74.24	74.24	62.70			
Т4	Bordeaux mixture	36.85	69.66	77.95	77.95	77.95	68.07			
<b>T</b> 5	Streptocycline	-3.23	30.64	27.18	0	0	10.92			
Т6	Water extract of Ocimum spp.	10.34	18.81	37.65	37.65	37.65	28.42			
<b>T</b> 7	Water extract of garlic	21.68	58.55	69.87	69.87	69.87	57.97			
T8	Calixin	-8.19	25.11	21.69	0	0	7.72			

wilt incidence in control plots of field experiment and meteorological factors such as maximum and minimum atmospheric temperature, maximum and minimum soil temperature, relative humidity and rainfall. The data were collected from Department of Agricultural Meteorology, College of Horticulture, Vellanikkara. The results are presented in Table 16 and Fig. 4 (Appendix-XIX). From the month of July to August, there was a steady increase in the percentage of wilt incidence and maximum wilt incidence of cent per cent was recorded during last week of August. A positive correlation between the wilt incidence and maximum air and soil temperatures were observed. The other parameters like relative humidity, rainfall, minimum air and soil temperature were not correlated with wilt incidence of ginger.

# 4.3.6 Estimation of VA-mycorrhizal infection on ginger

The colonization of VA-mycorrhiza was tested in healthy and bacterial wilt infected ginger roots. Ninety root bits each from healthy and infected plants were selected for the study. Out of this 29 and 14 root bits showed positive for mycorrhizal infection in healthy and infected roots respectively. The colonization of VA-mycorrhiza was greater in roots collected from healthy

Fig.4. Positive correlation between wilt incidence and maximum air and soil temperature

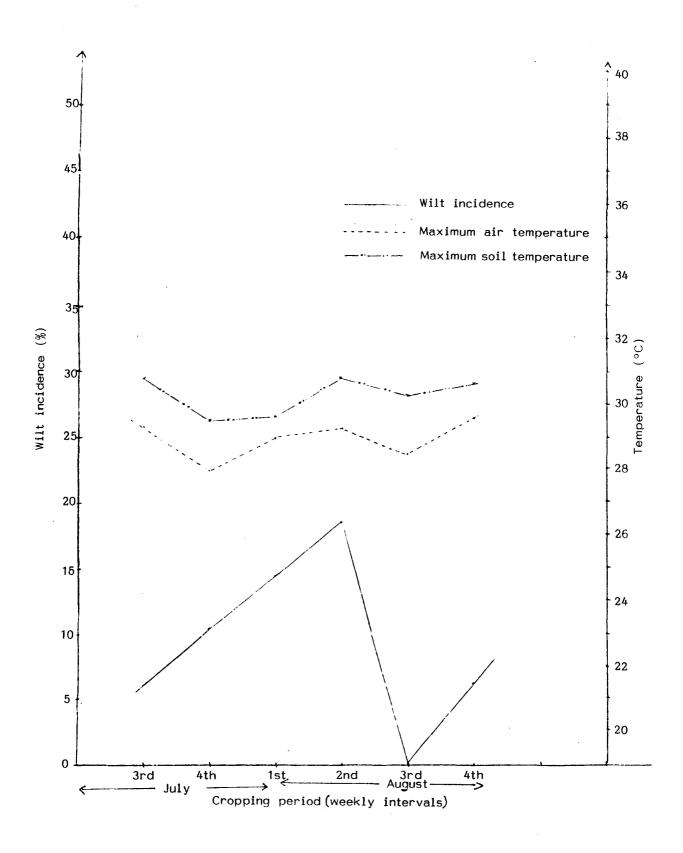


Table 16. Correlation coefficients between various environmental factors and per cent wilt incidence in control plot

Sl.		ion coefficients lt incidence
1	Maximum air temperature	0.818*
2	Minimum air temperature	0.370
3	Maximum soil temperature (2 pm)	0.812*
4	Minimum soil temperature (9 am)	0.160
5	Relative humidity	0.246
6	Rainfall	0.611
CD	(0.05) for comparison between variables	0.707
	•	

\* Significant at 5% level

ginger plants (32.2 per cent) than bacterial wilt infected plants (15.5 per cent).

# 4.3.7 Incidence of *Phyllosticta* leaf spot and rhizome rot in field and pot culture experiments

The disease index of Phyllosticta leaf spot before and after application of treatments were calculated in field and pot culture experiments and the data are given in Table 17 and 18. In the field experiment the disease index upto 100 days after planting was found to increase constantly in all treatments. However, statistical analysis of data showed significant difference among them after 115 days of planting. The leaf spot disease was more severe during second week to third week of August. In general, when the overall disease index was considered, it is evident that plants treated with water extract of Ocimum spp. Chloromycetin and Bordeaux mixture had minimum Phyllosticta leaf spot disease. But in the pot culture experiment, plants treated with Calixin and Bordeaux mixture had more efficiency in checking further increase and spread of the leaf spot disease, which was followed by Ambistryn-S and Chloromycetin.

In the field condition, rhizome rot disease was observed from last week of June to first week of July coinciding with south west monsoon. In order to control

Table 17. Disease index of Phyllosticta leaf spot in field experiment

Treatments	70 DAP 2nd fortnight of July	80 DAP 1st fortnight of Aug.	100 DAP 2nd fortnight of Aug.	115 DAP 1st fortnight of Sep.	130 DAP 2nd fortnight of Sep.	145 DAP 1st fortnight of Oct.	Mean
T1 Ambistryn-S	25.80	39.33	51.50	27.36	10.33	8.86	27.16
T2 Chloromycetin	25.00	33.76	45.50	11.46	11.03	8.86	22,60
T3 Terramycin	32.56	37.93	59.03	31.00	16.90	11.90	31.55
T4 Bordeaux mixture	28.56	33.80	40.00	20.80	12.63	7.46	23.87
T5 Streptocycline	29.90	40.36	50.63	19.83	14.10	8.83	27.27
T6 Water extract of Ocimum spp.	24.46	32.33	41.96	14.96	9.40	7.20	21.75
T7 Water extract of garlic	30.46	42.80	57.96	35.10	16.86	10.90	32.35
T8 Calixin	34.63	40.13	41.86	17.33	11.16	8.03	25.52
T9 Control	30.20	43.50	*	*	*	*	36.85
CD (0.05)	NS	NS	NS	21.446	17.754	17.695	

DAP - Days after planting \* - Complete wilting of plants NS - Non significant

Table 18. Disease index of Phyllosticta leaf spot in pot culture experiment

	Treatments	70 DAP 2nd fortnight of July	85 DAP 1st fortnight of Aug.	100 DAP 2nd fortnight of Aug.	115 DAP 1st fortnight of Sep.	130 DAP 2nd fortnight of Sep.	145 DAP 1st fortnight of Oct.	Mean
Т1	Ambistryn-S	0	1.02	2.16	3.30	1.76	1.80	1.67
Т2	Chloromycetin	0	1.27	2.76	3.06	1.71	1.80	1.76
Т3	Terramycin	0.40	2.06	6.03	5.00	2.92	2.38	3.12
<b>T4</b>	Bordeaux mixture	0	1.36	2.13	3.03	1.56	1.50	1.59
Т5	Streptocycline	0	1.54	3.12	*	. *	*	2.33
Т6	Water extract of Ocimum spp.	1.26	1.67	6.26	11.67	9.09	6.00	5.98
Т7	Water extract of garlic	0.47	1.11	3.00	9.50	7.60	6.27	4.73
Т8	Calixin	0	0.44	2.23	*	*	*	1.33
Т9	Control	0	2.00	*	*	*	*	2.00
CD	(0.05)	0.542	2.158	2.814	5.558	6.442	4.125	

DAP - Days after planting
\* - Complete wilting of plants

this disease, Dithane M-45 drenching was done in field. No further infection was noticed thereafter.

4.3.8 Effect of treatments on number of tillers and height of plant in field and pot culture experiments

Height of plant and number of tillers in different treatments under field and pot culture experiments are given in Table 19 and 20. The plant height did not differ significantly among treatments. However, plots treated with Ambistryn-S, Boardeaux mixture, Terramycin and Chloromycetin had maximum plant height than the other treatments. The minimum number of plants were observed in Streptocycline and water extract of *Ocimum* spp. treated plots 145 days after planting because more number of plants were severely infected by bacterial wilt. With regard to number of tillers, statistical analysis of data showed significant difference among treatments. The plots treated with Bordeaux mixture and Ambistryn-S had more number of tillers than the other treatments.

In the pot culture experiment, the plants treated with Ambistryn-S and Bordeaux mixture had more number of tillers and maximum plant height than the other treatments. Hundred per cent of wilting was noticed in plants treated with Streptocycline, Calixin and control 115 days after planting.

Table 19. Effect of treatments on height of plant and number of tillers in field condition

Treatments	Plant height (cm)						Number of tillers						
	70 DAP	80 DAP	100 DAP	115 DAP	130 DAP	145 DAP	70 DAP	85 DAP	100 DAP	115 DAP	130 DAP	145 DAP	
Tl Ambistryn-S	16.4	20.9	22.3	23.5	23.5	23.8	3.5	4.0	3.5	4.2	4.2	4.2	
T2 Chloromycetin	16.6	20.8	21.8	22.9	22.9	23.2	3.6	3.8	3.6	3.9	3.8	3.8	
T3 Terramycin	16.2	21.1	23.4	23.5	23.5	23.5	3.5	3.9	3.6	3.9	3.1	3.0	
T4 Bordeaux mixture	16.1	21.7	22.1	23.5	23.5	23.5	3.6	4.0	3.8	4.4	4.3	4.3	
T5 Streptocycline	16.2	20.6	21.5	21.2	21.5	21.15	3.5	3.7	3.2	3.0	2.9	2.7	
T6 Water extract of Ocimum spp.	15.8	20.6	22.0	22.0	22.0	22.0	3.2	3.8	3.3	3.0	2.9	2.9	
T7 Water extract of garlic	16.0	20.6	23.1	22.2	22.5	23.1	3.2	3.4	3.4	3.7	3.8	3.9	
T8 Calixin	16.3	20.4	22.6	22.4	22.4	22.5	3.4	3.8	3.4	3.2	3.2	3.2	
T9 Control	15.9	20.8	*	*	*	*	3.6	3.8	*	*	*	*	
CD (0.05)	NS	NS	NS	NS	NS	7.166	NS	NS	0.383	1.057	1.112	1.095	

DAP - Days after planting \* - Complete wilting of plants NS - Non significant

Table 20. Effect of treatments on height of plant and number of tillers in pot culture experiment

Treatments	Plant height (cm)							Number of tillers						
	70 DAP	85 DAP	100 DAP	115 DAP	130 DAP	145 DAP	70 DAP	85 DAP	100 DAP	115 DAP	130 DAP	145 DAP		
Tl Ambistryn-S	30.3	33.3	40.0	40.0	40.0	40.0	7.0	7.3	11.0	11.0	11.0	11.0		
T2 Chloromycetin	28.3	31.0	31.6	31.6	31.6	31.6	7.6	8.3	9.6	9.6	9.6	9.6		
T3 Terramycin	21.6	26.0	26.6	28.0	28.0	28.0	5.6	7.6	9.3	9.3	9.3	9.3		
T4 Bordeaux mixture	23.0	26.0	33.6	36.6	36.6	36.6	8.6	9.6	13.0	13.0	13.0	13.0		
T5 Streptocycline	30.0	35.6	36.0	*	*	*	7.0	7.0	8.6	*	*	*		
T6 Water extract of Ocimum spp.	30.6	32.3	32.3	32.3	32.3	32.3	5.3	6.0	9.0	7.3	5.3	5.3		
T7 Water extract of garlic	25.0	26.6	29.3	29.3	29.3	30.0	5.0	5.0	7.6	7.0	7.0	7.0		
T8 Calixin	29.6	32.3	32.3	*	*	*	7.0	7.0	8.0	*	*	*		
T9 Control	23.6	25.6	*	*	*	*	7.0	6.0	*	*	*	*		
CD (0.05)	NS	NS	NS 2	20.230	20.143	20.143	NS	NS	NS	7.126	7.034	6.932		

DAP - Days after planting
 \* - Complete wilting of plants
NS - Non significant

4.3.9 Effects of treatments on yield in pot culture experiment

The maximum yield per plant (131.66 g) was recorded in plants treated with Ambistryn-S followed by plants treated with Bordeaux mixture (121.00 g). The other treatments like water extract of garlic, Terramycin and Chloromycetin were inferior to Ambistryn-S and Boardeaux mixture treated plants. Because of complete wilting, yield could not be recorded in plants treated with Calixin, Streptocycline and control.

### Discussion

#### DISCUSSION

Bacterial wilt caused by Pseudomonas solanacearum (Smith) Smith is one of the most important and widespread bacterial disease of crops in tropics, subtropics and warm temperate regions of the world. Bacterial wilt caused by P. solanacearum (Smith) Smith of ginger is a destructive disease, which causes loss in yield as well as reduction in the quality and germinability of rhizomes. In India, eventhough few workers have tried for the control of bacterial wilt of ginger (Sarma et al., 1978; Samuel, 1980; Ojha et al., 1986; Dake et al., 1986), no absolute control has been obtained so far. In view of the serious onature of the disease the present study was undertaken to characterise the pathogen and to evolve a suitable management practice for the disease.

The bacterial wilt pathogen P. solanacearum was isolated from newly wilted ginger plants and its pathogenicity established.

The bacterium was gram negative, short rods. On Triphenyl Tetrazolium Chloride Agar (TZC) medium, it produced circular smooth, raised, fluidal and slimy colonies with a light pink centre. Similar growth characters of *P. solanacearum* from various crops has been observed by

several workers (Mathew et al., 1979; Samuel, 1980; He et al., 1983; Prior and Steva, 1990; Jyothi, 1992).

The isolate of *P. solanacearum* caused blackening and rotting, when inoculated on potato slices. The bacterium failed to produce any pigment either on Yeast Glucose Chalk Agar or King's medium and could grow both in aerobic and anaerobic conditions. These findings are in agreement with the earlier works of Samuel (1980) and Jyothi (1992). The bacterium was able to liberate hydrogen sulphide and failed to produce indole or hydrolyse starch and also showed positive reaction for nitrate reductase, catalase activity and levan production. Similar results were obtained by earlier workers (Devi, 1978; Mathew et al., 1979; Samuel, 1980; Jyothi, 1992).

Hayward (1964) considered production of ammonia and brown pigment on tyrosine medium as a characteristic feature of *P. solanacearum*. Similar results were also obtained in the present investigation. With regard to gelatin liquefaction, the bacterium was unable to liquefy gelatin. Very slow or no liquefaction was observed by many workers (Hayward, 1964; He et al., 1983; Prior and Steva, 1990; Jyothi, 1992).

The bacterium failed to hydrolyse arginine, which was in accordance with the findings of Palleroni and

Duodoroff (1971) and Jyothi (1992). The ability of P. solanacearum to utilize carbon compounds like glucose, ribose, fructose, sucrose, dextrose, maltose, galactose, mannitol and dulcitol as sole source of carbon, observed in this study was in confirmity with earlier findings of Hayward (1964), Mathew et al. (1979), Samuel (1980) and Jyothi (1992).

Based on the above studies on morphological, cultural, biochemical and physiological properties coupled with its pathogenicity trials, the isolate of the bacterium could be characterised and identified as *P. solanacearum* (Smith) Smith.

Cross inoculation studies showed variation in the different isolates of the bacterium in their ability to infect the different host plants. The ginger isolate caused typical wilting on chilli, low degree of wilting on tomato and did not show any symptom on brinjal. The isolate of chilli caused typical wilting on tomato, brinjal and ginger plants. The isolates from brinjal and tomato produced high degree of wilting on chilli and did not cause any wilting on ginger. The brinjal and tomato isolates were capable of cross infecting each other.

The ability of ginger isolate to cause wilting at various intensities on chilli was reported by Ishii and

Aragaki (1963), He et al. (1983) and Jyothi (1992). The isolate of ginger bacterium produced wilting on tomato at varying degree has been well documented by many workers (Ishii and Aragaki, 1963; Pegg et al., 1974; Samuel, 1980; He et al., 1983; He, 1985). Zehr (1970), Nayar (1982) and Jyothi (1992) reported that ginger isolate of P. solanacearum failed to cause wilting on brinjal. The ability of chilli isolate to cause typical wilting on tomato and eggplant have been reported by several workers (Devi, 1978; Prior and Steva, 1990; Jyothi, 1992). Jyothi (1992) reported that the isolate of chilli caused high degree of wilting on ginger.

The isolates from brinjal and tomato were able to infect chilli has been observed by Velupillai and Stall (1984), Prior and Steva (1990) and Jyothi (1992). The inability of tomato and brinjal isolates to cause typical wilting on ginger have been observed by Quinon et al. (1964), Samuel (1980), He (1985) and Jyothi (1992). The isolates of tomato and brinjal were capable of cross infecting each other with varying intensities were documented by several workers (Devi, 1978; Nayar, 1982; He, 1985; Jyothi, 1992). In the present study on cross inoculation of isolate of ginger to other solanaceous plants, it was found to cross infect chilli and tomato. But Jyothi (1992) obtained an isolate of ginger to produce wilting on

chilli only. However, the results are not in full agreement with those of earlier workers. Further detailed studies with more number of isolates from all the hosts from different geographical areas are required to elucidate the fact.

The in vitro and in vivo efficacy of antibiotics, fungicides, water extract of botanicals and others in controlling the bacterial wilt of ginger were assessed.

In order to find out suitable treatments in reducing the severity of the disease, an in vitro study was conducted using antibiotics, fungicides, water extract of botanicals and others. Among the four antibiotics tested for in vitro sensitivity against P. solanacearum, Ambistryn-S and Chloromycetin at 1000 ppm exhibited maximum inhibition of the bacterium and these two were statistically superior to other antibiotics. The inhibitory effect of Terramycin was better than Streptocycline. The minimum inhibition of the bacterium was observed with 250 ppm of Streptocycline and found to be inferior than other antibiotics (Table 3). The inhibitory effect of Streptomycin, Chloromycetin, Terramycin, Streptocycline, Chlorotetracycline, Aureomycin, Dihydrostreptomycin sulphate, Agrimycin-100 on the growth of P. solanacearum from various host plants were well documented by several workers (Moorgan and Goodman, 1955; Foucart and Delcamb, 1960; Samuel, 1980; Nayar, 1982; Farag et al., 1986; Gunawan, 1989; Jyothi, 1992).

Of the two fungicides tested, one per cent Bordeaux mixture exhibited maximum inhibition of the bacterium than its other concentrations and calixin at all concentrations tested (Table 4). Jyothi (1992) tested various fungicides against P. solanacearum in in vitro and observed that Bordeaux mixture one per cent exhibited the maximum inhibition on the bacterium. Similar result was obtained in the present investigation also. Among the water extract of botanicals and other tested, water extract of Ocimum spp. and garlic at 50 g/l exhibited maximum inhibition of the bacterium and they were statistically superior to other treatments. Cowdung and Kerosene showed the least inhibition against the bacterium. The in vitro efficacy of extracts from garlic bulb in inhibiting the growth of P. solanacearum was reported by Hanudin and DjatYika (1986) and Hutagalung (1988). Similar results on the effect of garlic bulbs were obtained in the present study also. The inhibitory effect of water extract of Ocimum spp. on P. solanacearum in in vitro has not been Teported so far. In the present in vitro studies, it was observed that Ambistryn-S, Chloromycetin, Terramycin, Bordeaux mixture, water extract of Ocimum spp. and garlic

bulbs were the most effective treatments against the pathogen.

A field experiment was conducted in wilt sick field to find out the effect of antibiotics, fungicides and water extract of botanicals. These treatments were selected based on the *in vitro* sensitivity on *P. solanacearum* the incitant of the bacterial wilt of ginger. Results of the experiment showed that none of the treatments gave an absolute control of the disease. However, plots treated with Ambistryn-S 1000 ppm and Bordeaux mixture at one per cent had minimum wilt incidence than other treatments (Table 6). The plots treated with Ambistryn-S and Bordeaux mixture did not develop further wilt incidence after second application.

Sarma et al. (1978) observed that Agrimycin-100 1000 ppm, Streptocycline 1000 ppm, Bordeaux mixture one per cent and Bleaching powder one per cent did not check the spread of bacterial wilt of ginger. However, there were few reports which showed partial or complete control of bacterial wilt of ginger by chemicals. Dake et al. (1986) found that soil treatment with Bordeaux mixture or Bleaching powder was partly effective against the bacterial wilt of ginger. Samuel (1980) noticed that Ambistryn-S and Agrimycin-100 could effectively control the bacterial

wilt of ginger. The efficacy of water extract of botanicals against ginger wilt in *in vivo* has not been reported so far. From the results of the present study in *in vivo* revealed that Ambistryn-S 1000 ppm and Boardeaux mixture one per cent could be used to prevent the further spread of disease eventhough they could not completely control the disease.

The changes in the total rhizosphere microflora viz., bacteria, fungi, actinomycetes and the pathogen P. solanacearum as a result of treatments in field condition were also assessed. Among the treatments, Ambistryn-S, Bordeaux mixture and Chloromycetin had reduced the population of P. solanacearum. Ambistryn-S, Bordeaux mixture, Streptocycline and Chloromycetin had effectively reduced the bacterial population. With regard to fungal population, Bordeaux mixture treatment had more ability to reduce the fungal population followed by Ambistryn-S. In contrast to the other microorganisms, the treatments did not reduce the population of actinomycetes. An increasing trend of population was noticed in all the treatments. However, plots treated with garlic had minimum actinomycetes population than the other increatments.

The effect of treatments (those used in field experiment) on soil microflora viz., bacteria, fungi,

actinomycetes and pathogen *P. solanacearum* in wilt sick soil were estimated *in vitro*. The results of the study revealed that, Ambistryn-S and Bordeaux mixture effectively reduced the population of *P. solanacearum* in soil. The minimum bacterial population was recorded in soil treated with Ambistryn-S, Streptocycline, Bordeaux mixture, Chloromycetin and Terramycin. A drastic reduction of fungal population was recorded in Bordeaux mixture and Calixin treatments. With regard to actinomycetes population, it did not show any definite decreasing or increasing trend. However, the minimum count of actinomycetes population was noticed in Ambristyn-S, Streptocycline, water extract of *Ocimum* spp. treatments.

A perusal of literature revealed that there were no exhaustive previous study on the effect of antibiotics, fungicides and water extract of botanicals in soil, rhizosphere microflora of ginger. However, there were reports on other crops which showed that spraying of Streptocycline and Streptomycin had reduced the population of Pseudomonas and Xanthomonas (Desai et al., 1967; Dath and Devadath, 1969; Rahim, 1972; George, 1973). Jeyaprakash (1978) observed that application of Streptomycin, Streptocycline, Cheshunt compound and Bordeaux mixture had reduced the wilt incidence of tomato and rhizosphere population of P. solanacearum. In general, reduction of

bacterial population by spraying of antibiotics such as Streptomycin, Terramycin, Ambistryn-S, Streptocycline and Chloromycetin has been reported by several workers (Nakas and Klein, 1980; Shah et al., 1985; Elias et al., 1987).

Anderson and Domsch (1973) and Nakas and Klein (1980) reported that Oxytetracycline and Streptocycline had effectively reduced the actinomycetes population. But in the present study no such observation was noticed. The effect of Bordeaux mixture and calixin for reducing the fungal population has been observed by Zengin (1978) and Nair and Menon (1983). Similar results were obtained in the present study also.

The results of pot culture study on the management of bacterial wilt of ginger revealed that, plants treated with Bordeaux mixture, Ambistryn-S, Terramycin and Chloromycetin had minimum wilt incidence than the other treatments. The plants treated with Ambistryn-S, Terramycin and Chloromycetin did not develop further wilt incidence ten days after second application of treatments. But in the plants treated with Bordeaux mixture, the wilt incidence remained static throughout the period thereby indicating that Bordeaux mixture had ability to prevent the further spread and development of the disease. The control of bacterial wilt of ginger by spraying of chemicals has been

reported by Samuel (1980) and Dake et al. (1986) and Ohja et al. (1986). The results of pot culture study also revealed that Bordeaux mixture one per cent effectively controlled the further spread of the disease. The plants treated with Ambistryn-S, Terramycin and Chloromycetin also showed comparatively less wilt incidence and thus proved the ability of these chemicals to prevent the further spread of disease after second application. All the chemicals found to inhibit the pathogen under in vitro condition were not found to control the disease as well as the pathogen under field conditions.

The effect of treatments on the population of P. solanacearum in the field and in vitro is similar to that results obtained for the control of bacterial wilt of ginger in field and pot culture experiments. The maximum reduction in the population of P. solanacearum and minimum wilt incidence was found in Ambistryn-S and Bordeaux mixture treatments in all the above studies. From the results of the field experiment, pot culture study and studies on the rhizosphere microflora and soil microflora (wilt sick soil), when examined with the results of earlier workers, it was clear that Ambistryn-S 1000 ppm and Bordeaux mixture one per cent could control the spread of bacterial wilt as well as the pathogen P. solanacearum. In addition to this, the population of fungi and bacteria

were also found to decrease in plots treated with Ambistryn-S and Bordeaux mixture. Since Ambistryn-S is costly, the application of Bordeaux mixture at one per cent before incidence of ginger bacterial wilt could be recommended as a prophylatic soil drench and spray on plants for ginger cultivation which can reduce the wilt incidence and prevent the further spread of the disease in the field.

Correlation coefficients were made between environmental factors and per cent wilt incidence. Environmental factors play an important role in the development of plant disease or make the plant vulnerable to infection. There was a steady increase in the percentage of wilt incidence from the month of July to August and maximum wilt incidence was during last week of August. There was a positive correlation between wilt incidence and maximum air and soil temperature. A high temperature of 22 to 36°C favour rapid development of wilt disease of many crops has been observed by many workers (Vaughan, 1944; Gallegely and Walker, 1949; Hingorani et al., 1956). Acosta (1964) reported that infection by tomato pathogen P. solanacearum was more severe during summer at high soil temperature. Of the various environmental factors studied, only maximum air and soil temperatures were found to have a direct effect on disease incidence and this fact was supported by earlier findings. Further these two factors



of maximum air and soil temperatures has got a role in the epidemiology of the disease.

The colonization of VA-mycorrhiza was greater in roots collected from healthy ginger plants than bacterial wilt infected plants. Taber and Trappe (1982) and Stasz and Sakai (1984) could obtain hyphae, vesicles and spores of the Glomus type in ginger. It could be found sparingly in the rhizome tissue both above and below the scale like leaves, primary roots and smallest secondary roots. Halos and Zorilla (1979) reported that VA-mycorrhiza increased the growth and yield of tomato and reduced the infection of P. solanacearum. Suresh and Rai (1991) found that extracts from mycorrhizal tomato roots infected with Glomus fasciculatum reduced the population of P. solanacearum in nutrient broth. In the present study, the presence of VA-mycorrhiza in the healthy and infected root samples were compared. The colonization was found to be more in roots of healthy plants than in roots of bacterial wilt infected plants.

The presence of *Phyllosticta* leaf spot disease was also recorded during the cropping season. The *Phyllosticta* leaf spot was more during last week of July to first week of August. Among the treatments, Chloromycetin, Bordeaux mixture, (a)ixin and Ambistryn-S treatments recorded

minimum leaf spot disease in pot oculture and field experiments. Mailum and Divinagracia (1969) reported that leaf spot disease (*Phyllosticta zingiberi*) was prevalent from July to September, affecting the plants at all stages of development and causing serious growth reduction in the rainy season.

The efficiency of Bordeaux mixture one per cent for control of ginger leaf spot (*P. zingiberi*) was reported by Ramakrishnan (1942), Sohi et al. (1973) and Premanathan (1981). Similar results were also obtained in the present study. But there are no reports on control of leaf spot by spraying of antibiotics in the field. In general, more of healthy (wilt free) plants in field has minimum of *Phyllosticta* leaf spot disease.

The effect of treatments on height of plant and number of tillers both under field and pot culture experiments was also studied. Among the treatments, plots treated with Ambistryn-S, Bordeaux mixture, Terramycin and Chloromycetin had maximum plant height than the other treatments. More number of tillers was recorded in plots treated with Bordeaux mixture and Ambistryn-S in field condition. In the case of pot culture experiment also pots treated with Bordeaux mixture and Ambistryn-S had maximum plant height and tillers.

Ishii and Aragaki (1963) obtained more mean plant height and shoot numbers in treated plots (Methylbromide, D-D and steam sterilization) than untreated control (bacterial wilt infected plot). In general, chemical treated plots had more number of tillers and maximum plant height than untreated control.

Since there was severe incidence of bacterial wilt in the field, the yield data could not be recorded completely in the field condition. But in the pot culture experiment plants treated with Ambistryn-S and Bordeaux mixture had more yield (g)/plant than other treatments. In other treatments yield data could not be taken as the plants wilted partially or completely.

Ishii and Aragaki (1963) obtained minimum mean rhizome yield (g) in control (bacterial wilt infected plots) than treated plots. Indrasenan et al. (1982) tested reaction of different types of ginger varieties to bacterial wilt and found that highly susceptible or highly infected variety gave less yield than the other types. As a result of bacterial wilt infection, the yield might have been highly reduced in ginger.

From the present study on the management of bacterial wilt of ginger, the following facts emerged. Ambistryn-S 1000 ppm and Bordeaux mixture one per cent were better in the control of bacterial wilt of ginger. Apart from the control of disease, these chemicals stimulated better plant height, tiller counts and yield of ginger. Ambistryn-S being a costly antibiotic, application of Bordeaux mixture one per cent could be recommended as a prophylatic soil drench and spray before the incidence of disease, which can reduce the incidence and prevent the further spread of disease in field.

# Summary

#### SUMMARY

Bacterial wilt caused by Pseudomonas solanacearum (Smith) Smith is one of the most destructive diseases of ginger. Considering the seriousness of the disease, the present study was undertaken to characterise the pathogen and to evolve a suitable management practice for the disease.

The bacterial wilt pathogen was isolated from newly wilted ginger plants and its pathogenicity established. The bacterium produced circular, smooth, raised, fluidal and slimy colonies with a light pink centre. The bacterium did not produce any water soluble or water insoluble pigment and could grow both in aerobic and anaerobic conditions. The isolate of bacterium was able to liberate hydrogen sulphide and failed to produce indole or hydrolyse starch and also showed positive reaction for nitrate reduction and catalase activity. The bacterial isolate was able to produce levan, ammonia and brown pigment on tyrosine medium. The bacterium failed to hydrolyse arginine or liquify gelatin. All the carbon compounds tested viz., glucose, ribose, fructose, sucrose, dextrose, maltose, galactose, mannitol and dulcitol were utilized by the bacterium.

Cross inoculation studies were conducted using the isolates from ginger, chilli, tomato and brinjal. The isolate of ginger caused typical wilting on chilli, low incidence of wilting on tomato and did not show any symptom on brinjal. The chilli isolate caused typical wilting on tomato, brinjal and ginger. The isolate from tomato, brinjal and ginger produced typical wilting on chilli. The brinjal and tomato isolates were capable of cross infecting each other. The isolates from tomato and brinjal did not cause any wilting on ginger. Based on the above studies on morphological, biochemical and physiological properties coupled with pathogenecity trials, the isolate of the bacterium could be characterised and identified as P. solanacearum (Smith) Smith.

An in vitro study was conducted to find out the inhibitory effect of antibiotics, fungicides, botanicals and others on P. solanacearum. Among the four antibiotics tested, Ambistryn-S and Chloromycetin at 1000 ppm exhibited the maximum inhibition of the bacterium and they were statistically superior to other antibiotics. Of the two fungicides tested, Bordeaux mixture one per cent showed the maximum inhibition of the bacterium. Among the botanicals and others tested, water extract of Ocimum spp. and garlic at 50 g/l exhibited the maximum inhibition of the bacterium and they were significantly superior to other

treatments. Cowdung and kerosene showed the least inhibition of the bacterium.

The result of the field experiment on the management of bacterial wilt of ginger showed that none of the treatments gave an absolute control of the disease. However, plots treated with Ambistryn-S 1000 ppm and Bordeaux mixture one per cent had minimum wilt incidence than the other treatments and also they did not develop further wilt incidence after second application of treatments. The changes in the total rhizosphere microflora viz., bacteria, fungi, actinomycetes and the pathogen P. solanacearum as a result of treatments in field condition were assessed. Among the nine treatments, Ambistryn-S, Bordeaux mixture and Chloromycetin had effectively reduced the population of P. solanacearum and Ambistryn-S, Bordeaux mixture, Streptocycline and Chloromycetinham effectively reduced the bacterial population. The maximum reduction of fungal population was noticed in Bordeaux mixture and Ambistryn-S treated plots. With regard to actinomycetes population plots treated with garlic had minimum actinomycetes population than the other treatments.

Results of the study on the *in vitro* effect of treatments on the soil microflora and the pathogen P.

solanacearum revealed that Ambistryn-S and Bordeaux mixture had effectively reduced the population of *P. solanacearum*. There was maximum reduction of bacterial population in soil treated with Ambistryn-S, Streptocycline, Bordeaux mixture, Chloromycetin and Terramycin. The minimum fungal population was observed in Bordeaux mixture and Calixin treated soils. The soil treated with Ambistryn-S, Streptocycline and water extract of *Ocimum* spp. had minimum actinomycetes population.

The result of pot culture study on the management of bacterial wilt of ginger revealed that, plants treated with Bordeaux mixture, Ambistryn-S, Terramycin, Chloromycetin had minimum wilt incidence than other treatments. But in plants treated with Bordeaux mixture one per cent, the wilt incidence remained static throughout the period thereby indicating that Bordeaux mixture had ability to prevent the further spread and development of the disease. From the result of the studies on management of bacterial wilt under in vitro and in vivo conditions, it is clear that Ambistryn-S 1000 ppm and one per cent Bordeaux mixture were effective than other treatments.

Correlation studies were made between wilt incidence and environmental factors. A positive correlation

between maximum air and soil temperature with wilt incidence was observed. The other factors were not found to be correlated with bacterial wilt incidence.

The VA-mycorrhizal colonization was greater in roots of healthy plants than the roots of bacterial wilt infected plants.

The incidence of *Phyllosticta* leaf spot was recorded in different treatments both in field and pot culture experiments. Among the treatments, plants treated with Bordeaux mixture, Ambistryn-S, Calixin and Chloromycetin had minimum leaf spot disease than the other treatments.

The effect of treatments on plant height, number of tillers and yield of plant was also studied and the result indicated that plants treated with Ambistryn-S and Bordeaux mixture had more number of tillers, maximum plant height and better yield than other treatments.

Thus the present study revealed that one per cent Bordeaux mixture can be used as a prophylatic measure to reduce the incidence of bacterial wilt disease in ginger, and the same type of treatment can be recommended to conrol the further spread of the disease under field conditions. The use of water extracts of botanicals for the

control of the disease needs further detailed investigations.

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Appendices

#### APPENDIX-I

# Composition of the medium Martin's Rose bengal streptomycin agar medium (Martin, 1950)

Peptone	_	5.0	g
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Dextrose - 10.0 g

 $KH_2PO_4$  - 1.0 g

 $MgSO_4.7H_2O$  - 0.5 g

Agar agar - 15.0 g

Rose bengal - 1 part in 30,000 parts of the

medium

Distilled water - 1000 ml

Streptomycin - 30.0 mg

pH - 6.2

#### APPENDIX-II

Soil extract agar medium (Lochhead and Thaxton, 1952)

Soil extract - 100 ml

 $K_2HPO_4$  - 0.2 g

Agar agar - 15.0 g

Glucose - 1 g

Tap water - 900 ml

pH - 7 - 7.2

### APPENDIX-III

### Kenknight's agar medium

Glucose	-	1.0 g	
KH <sub>2</sub> PO <sub>4</sub>	-	0.1 g	
${\tt MgSO_4.7H_2O}$	-	0.1 g	
Na NO <sub>3</sub>	-	0.1 g	
Agar agar	-	15.0 g	
KCl	_	0.1 g	
Distilled water	-	1000 ml	
рН	-	6.8	

# APPENDIX-IV Score card for grading the disease intensity of Phyllosticta leaf spot of ginger (Premanathan, 1981)

<u>Grade</u>			% of infection
0			No infection
1			2% infection (1-3 spots)
2			5% infection (3-5 spots)
3			10% infection (6-9 spots)
4			11-20% infection (9 spots)
5			21-35% infection
6			36-60% infection
7			61-85% infection
8			85% infection
Disease	indov	=	Grade x No. of leaves infected
Disease	THUCK	_	Total No. of leaves x maximum disease score

### APPENDIX-V

## Preparation of Formalin : Acetic acid : Alcohol mixture (FAA)

Phillips and Hayman (1970)

Formalin (40%) - 5 ml

Glacial acetic acid - 5 ml

Ethanol 95% - 90 ml

Preparation of lactophenlol

Lactic acid - 10 ml

Phenol - 10 ml

Glycerol - 20 ml

Water - 20 ml

Preparation of Trypan blue

Trypan blue (Romali) - 50.0 mg

Lactophenol - 100 ml

APPENDIX-VI
Analysis of variance for in vitro sensitivity of P. solanacearum to antibiotics

		<del></del>		
Source	DF	SS	MSS	F
Total	47	1426.74		
Treatment	15	1417.24	94.4826	318.33**
Error	32	9.50	0.2968	
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APPENDIX-VII

Analysis of variance for in vitro sensitivity of P. solanacearum to fungicides

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Source	DF	SS	MSS	F
Total	20	253.24		
Treatment	5	250.07	41.678	184.09**
Error	14	3.17	0.2268	

<sup>\*\*</sup> Significant at 1 per cent level

Analysis of variance for in vitro sensitivity of P. solanacearum to botanicals and others

Source	DF	SS	MSS	F
Total	35	495.45		
Treatment	11	488.19	44.38	146.71**
Error	24	7.26	0.3025	

APPENDIX-IX
Abstract of ANOVA (M.S.S. values) for field experiment on management of bacterial wilt of ginger with antibiotics, fungicides and botanicls

Source	DF	70 DAP	80 DAP	90 DAP	100 DAP	110 DAP	160 DAP
Total	26						
Treatment	8	32.740	100.180	61.849	228.226**	213.411**	213.411**
Error	18	60.764	54.977	52.083	47.743	49.132	49.132

\*\*Significant at 1 per cent level DAP - DAys after planting

APPENDIX-X
Abstract of ANOVA (M.S.S. values) for mean population of pathogen P. solanacearum in rhizosphere of ginger plants in the field experiment

Source	DF	70 DAP	80 DAP	90 DAP	100 DAP	110 DAP	160 DAP
Total	26						
Treatment	8	6.065	111.917**	185.846**	169.676**	181.704**	139.093**
Error	18	7.926	3.296	0.926	0.963	0.852	0.259

APPENDIX-XI
Abstract of ANOVA (M.S.S. values) for mean population of total bacteria in rhizosphere of ginger plants in the field experiment

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Source	DF	70 DAP	80 DAP	90 DAP	100 DAP	110 DAP	160 DAP
Total	26				·		
Treatment	8	154.667**	74.250**	99.426**	193.731**	122.759**	108.370**
Error	18	4.926	2.333	1.519	1.444	1.296	0.926

<sup>\*\*</sup> Significant at 1 per cent level DAP - Days after planting

APPENDIX-XII
Abstract of ANOVA (M.S.S. values) for mean population of fungi in rhizosphere of of ginger plants in the field experiment

Source	DF	70 DAP	80 DAP	90 DAP	100 DAP	110 DAP	160 DAP
Total	26						
Treatment	8	18.083**	36 <sub>4</sub> 370**	26.148**	22.667**	26.259**	22.500**
Error	18	7.185	4.926	2.000	1.296	1.148	1.593

APPENDIX-XIII

Abstract of ANOVA (M.S.S. values) for mean population of actinomycetes in rhizosphere of ginger plants in the field experiment

Source	DF	70 DAP	80 DAP	90 DAP	100 DAP	110 DAP	160 DAP
Total	26						
Treatment	8	4.565*	12.000**	5.871**	6.167**	9.815**	10.009**
Error	18	2.037	2.704	1.497	1.259	1.000	1.556

<sup>\*</sup> Significant at 5 per cent level

<sup>\*\*</sup> Significang at 1 per cent level
DAP - Days after planting

APPENDIX-XIV
Abstract of ANOVA (M.S.S. values) for mean population of P. solanacearum in wilt sick soil due to different treatments in in vitro

Source	DF	Before application	10 days after first application	10 days after second application	10 days after third application	10 days after fourth application
Total	<b>2</b> 6					
Treatment	8	0.167	14.417**	176.815**	304.120**	312.256**
Error	18	0.926	1.407	6.556	7.926	15.037

APPENDIX-XV
Abstract of ANOVA (M.S.S. values) for mean population of fungi in wilt sick soil due to different treatments in <u>in vitro</u>

Source	DF	Before application	10 days after first application	10 days after second application	10 days after third application	10 days after fourth application	
Total	26						
Treatment	8	0.667	11.204**	14.250**	21.148**	25.250**	
Error	18	0.963	0.963	1.444	1.111	1.704	

<sup>\*\*</sup> Significant at 1 per cent level

APPENDIX-XVI
Abstract of ANOVA (M.S.S. values) for mean population of total bacteria in wilt sick soil due to different treatments in in vitro

Source	DF	Before application	10 days after first application	10 days after second application	10 days after third application	10 days after fourth application	
Total	26						
Treatment	8	0.917	7.620**	101.333**	167.009**	205.917**	
Error	18	0.963	1.185	1.444	1.407	1.296	

APPENDIX-XVII
Abstract of ANOVA (M.S.S. values) for mean population of actinomycetes in wilt sick soil due to different treatments in <u>in vitro</u>

Source	DF	Before application	10 days after first application	10 days after second application	10 days after third application	10 days after fourth application
Total	26					
Treatment	8	1.537	3.231**	3.565*	18.204**	10.926**
Error	18	1.481	1.333	1.741	2.074	1.926

<sup>\*</sup> Significant at 5 per cent level

<sup>\*\*</sup> Significant at 1 per cent level

APPENDIX-XVIII

Abstract of ANOVA (M.S.S. values) for pot culture experiment on management of bacterial wilt of ginger with antibiotics, fungicides and botanicals

		00 010		200 DID	110 DID	160 DID
Source	DF	80 DAP	90 DAP	100 DAP	110 DAP	160 DAP
Total	26					
Treatment	8	69.722	221.20	754.526*	5714.28**	5714.28**
Error	18	71.934	133.699	267.473	370.37	370.37

<sup>\*</sup> Significant at 5 per cent level

APPENDIX-XIX
Observation on the weather parameters during cropping period of ginger and per cent wilt incidence in control plot of field experiment

Period		Air temperature °C		Soil temperature °C		Relative - humidity	Rainfall (mm)	Wilt incidenc
(Weekly interval)	Maximum	Minimum	7 <b>AM</b>	2 PM	(%)	( mm )	Therdene	
July ·	- 3rd week	29.3	23.21	25.98	30.89	91.9	9.9	12.5
•	4th week	28.0	22.91	25.3	29.5	<b>87.1</b> 5	14.7	20.83
August -	- 1st week	29.0	23.85	25.9	29.6	85.5	3.7	29.17
-	2nd week	29.3	23.30	24.5	30.8	91.7	13.3	37.5
	3rd week	28.5	24.4	24.75	30.25	87.4	25.0	0
	4th week	29.6	24.0	26.0	30.73	84.14	11.5	12.5

<sup>\*\*</sup> Significant at 1 per cent level

APPENDIX-XX
Abstract of ANOVA (M.S.S. values) for disease index of <u>Phyllosticta</u> leaf spot in field experiment

Source	DF	70 DAP	85 DAP	100 DAP	115 DAP	130 DAP	145 DAP
Total	28						
Treatment	8	34.943	49.675	266.247	867.005**	1030.196**	1157.825**
Error	18	161.807	241.581	391.423	156.300	107.113	106.409

APPENDIX-XXI
Abstract of ANOVA (M.S.S. values) for disease index of Phyllosticta leaf spot in pot culture experiment

Source	DF	70 DAP	85 DAP	100 DAP	115 DAP	130 DAP	145 DAP
Total	26						
Treatment	8	0.554	0.771	9.842**	48.183**	62.382**	57.296**
Error	18	0.100	1.583	2.692	10.501	14.105	5.789

\*\*\* Significant at 1% level DAP - Days after planting

APPENDIX-XXIIa
Abstract of ANOVA (M.S.S. values) for effect of treatments on number of tillers in field experiment

Source	DF	70 DAP	85 DAP	100 DAP	115 DAP	130 DAP	145 DAP
Total	26			angka kaharat garanka mada dimensionen yang samirik ini sampan 19 4 - 1998 gamp (A)			
Treatment	8	0.433	0.021	0.183	1.811**	3.638**	4.689**
Error	18	0.155	0.083	0.050	0.323	0.421	0.408

APPENDIX-XXIIb
Abstract of ANOVA (M.S.S. values) for effect of treatments on height on plant in field experiment

Source	DF	70 DAP	85 DAP	100 DAP	115 DAP	130 DAP	145 DAP
Total	26						
Treatment	8	0.174	0.025	1.526	1.906	25.570	86.183**
Error	18	1.648	1.372	1.954	1.658	18.310	17.451

<sup>\*\*</sup> Significant at 1 per cent level DAP - Days after planting

APPENDIX-XXIIIa

Abstract of ANOVA (M.S.S. values) for effect of treatments on number of tillers in pot culture experiment

Source	DF	70 DAP	85 DAP	100 DAP	115 DAP	130 DAP	145 DAP
Total	28						
Treatment	8	7.787	8.500	17.426	49.666*	4.167	84.333**
Error	18	9.814	16.370	17.330	17.259	16.814	16.333

APPENDIX-XXIIIb

Abstract of ANOVA (M.S.S. values) for effect of treatments on height on plant in pot culture experiment

Source	DF	70 DAP	85 DAP	100 DAP	115 DAP	130 DAP	145 DAP
Total	28						
Treatment	8	56.250	43.833	0.128	214.35	945.477**	423.732**
Error	18	72.592	65.333	96.207	199.48	139.148	137.88

<sup>\*</sup> Significant at 5 per cent level

<sup>\*\*</sup> Significant at 1 per cent level DAP - Days after planting

# MANAGEMENT OF BACTERIAL WILT OF GINGER (Zingiber officinale Rosc) INCITED BY Pseudomonas solanacearum (SMITH) SMITH

Ву

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### ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

## Master of Science in Agriculture

Faculty of Agriculture
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#### ABSTRACT

Bacterial wilt is one of the most destructive disease of ginger in Kerala. The pathogen was isolated from wilted ginger plant and its pathogenicity established. The isolate of bacterium was characterised and identified as *Pseudomonas solanacearum* (Smith) Smith based on its morphological, cultural, biochemical and physiological characters coupled with its pathogenicity.

In vitro inhibitory effect of antibiotics, fungicides, botanicals and others against P. solanacearum was tested. Ambistryn-S and Chloromycetin 1000 ppm exhibited maximum inhibition of bacterium. Of the two fungicides tested Bordeaux mixture one per cent gave maximum inhibition of the bacterium. Among the botanicals and others tested, water extract of Ocimum spp. and garlic at 50 g/l exhibited maximum inhibition of the bacterium.

Field experiment on the management of bacterial wilt of ginger revealed that none of the treatments gave an absolute control of the disease. However, plots treated with Ambistryn-S and Bordeaux mixture had minimum wilt incidence than the other treatments. The changes in the total rhizosphere microflora and the pathogen *P. solanacearum* as a result of treatments in field condition were

assessed. Ambistryn-S, Bordeaux mixture and Chloromycetin had effectively reduced the population of P. solanacearum. Ambistryn-S, Bordeaux mixture, Streptocycline and Chloromycetin had reduced the bacterial population. The maximum reduction of fungal population was observed in Bordeaux mixture and Ambistryn-S treated plots. Actinomycetes population was minimum in plots treated with garlic. Results of the study on the in vitro effect of treatments on the soil microflora and the pathogen P. solanacearum revealed that Ambistryn-S and Bordeaux mixture had effectively reduced the P. solanacearum. There was a maximum reduction of bacterial population was recorded in soil treated with Ambistryn-S, Streptocycline, Bordeaux mixture, Chloromycetin and Terramycin. The soil treated with Bordeaux mixture and Calixin had minimum count of fungal population. Ambistryn-S, Streptocycline and water extract of Ocimum spp. treated soils had minimum count of actinomycetes.

The result of the pot culture study on the management of bacterial wilt of ginger revealed that plants treated with Bordeaux mixture, Ambistryn-S, Terramycin and Chloromycetin had minimum wilt incidence.

Correlation studies were made between environmental factors and wilt incidence. There was a positive

correlation between maximum air and soil temperature and wilt incidence. The colonization of VA-mycorrhiza was greater in roots of healthy plants than the roots of infected plants. The incidence of *Phyllosticta* leaf spot was minimum in plants treated with Bordeaux mixture, Ambistryn-S, Calixin and Chloromycetin both in pot culture and field experiments.

The effect of treatments on plant height, number of tillers and yield were also recorded. Bordeaux mixture and Ambistryn-S treated plants had maximum plant height, more number of tillers and better yield than the other treatments.

Thus the present study revealed that one per cent Bordeaux mixture can be used as a prephylatic measure to reduce the incidence of bacterial wilt of ginger. The use of water extracts of botanicals for the control of the disease needs further detailed investigations.