MANAGEMENT OF BACTERIAL WILT OF SOLANACEOUS VEGETABLES USING MICROBIAL ANTAGONISTS

By

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THESIS

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Kerala Agricultural University

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DECLARATION

I hereby declare that this thesis entitled "**Management of bacterial wilt of solanaceous vegetables using microbial antagonists**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other university or society.

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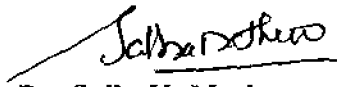
CERTIFICATE

Certified that the thesis entitled **"Management of bacterial wilt of solanaceous vegetables using microbial antagonists"** is a record of research work done independently by Ms. Manimala. R, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.



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Manimala.R

DEDICATED TO

MY LOVING
PARENTS
BROTHER
AND SISTER

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Introduction

1. INTRODUCTION

Our country has achieved self-sufficiency and good degree of stability of food production. This created an urgent need for providing health security to our population by supplying nutrition through balanced diet. We can grow vegetables all the year round. India shares 13% of the world output of vegetables from about 2% cropped area in the country. Vegetable production in Kerala is much less as compared to the neighbouring states. It is estimated that, about 60% of vegetable requirement of the state is met from outside sources and an amount of Rs.850 crores are spent yearly in this way (Gopalakrishnan, 1999). Pests and diseases are the major limiting factors in the vegetable production, because of the warm humid climatic conditions prevailing in Kerala. Among the diseases, bacterial wilt caused by *Ralstonia solanacearum* is a major constraint for the cultivation of solanaceous crops in Kerala and an yield loss upto 100 per cent have been reported in susceptible varieties from various parts of the state. Due to high variability strains of the pathogen, the differences in biological and physico chemical agroecosystem characteristics, bacterial wilt remains a serious and persistent one in Kerala.

Bacterial wilt is caused by a genetically diverse soil borne pathogen with a wide host range, and it is very difficult to control the disease once it is established in the field. Because of the systemic nature of pathogen it is very difficult and also it is costly, to control bacterial wilt with chemicals. However, breeding for host resistance has been predominantly used and has provided some substantial success in Kerala. But it may also fluctuate due to the oligogenic nature of the plant resistance, and to the great strain variation and aggressiveness of bacterial isolates from different locations. So alternate control measures such as biological control involving antagonists can be a potential mode to manage the disease.

Biocontrol potentially offers solution to many of the persistent problems in agriculture, including the problems of resource limitations, non-sustainable agricultural

systems and ever reliance of pesticides. And so, in the recent years, biocontrol is increasingly occupying the minds of scientists especially, the plant pathologists all over the world including India, to achieve the control of soil borne pathogens. Recognising the potentiality of this field and the importance of bacterial wilt in Kerala, priority was given to these aspects and the present investigation was carried out with the following objectives.

i) Isolation and maintenance of antagonistic organisms from Vellanikkara (Thrissur District) and Ozhalapathy (Palakkad District) areas

ii) *In vitro* evaluation of isolated organisms against *R. solanacearum*

iii) Identification of effective antagonists.

iv) Effect of microbial antagonist against *R. solanacearum* under pot culture studies

v) Management of bacterial wilt using effective antagonists under field condition.

Review of Literature

2. REVIEW OF LITERATURE

Bacterial wilt is a serious disease of agricultural crops in tropics, subtropics, and warm temperate regions of the world. The first report of bacterial wilt of solanaceous crops caused by *P.solanacearum* was that made by Burril (1890) in connection with an unidentified bacterial disease of potato in United States. Smith (1896) described the disease and causal agent and he was the first to report bacterial wilt in potato, tomato and brinjal. It attacked more than 200 plant species belonging to 33 families and the family Solanaceae has the largest number of hosts. (Kelman, 1953)

The bacterial wilt pathogen was first described as *Pseudomonas solanacearum* by Smith (1914). Later Yabuuchi *et al.* (1992) transferred several species of the rRNA homology group II *Pseudomonads*, including *P.solanacearum* to the genus *Burkholderia*. Sequencing of the 16s rRNA genes and polyphasic taxonomy revealed dichotomy among the species including in the genus *Burkholderia*. This phylogenic dichotomy had led to the proposal of the new genus, *Ralstonia* (Yabuuchi *et al.*, 1995).

In India, the occurrence of bacterial wilt of tomato was first reported by Hadayathulla and Saha (1941) from West Bengal and the bacterial wilt of brinjal was made by Das and Chattopadhyay (1955). They estimated that the average reduction in yield due to this disease was 54.6 to 62.3%. Bacterial wilt of chilli was first reported in India from Madhya Pradesh (ICAR, 1969).

2.1 THE PATHOGEN

Ralstonia solanacearum is non-spore forming, non-capsulate, gram negative, small rods with polar flagella. (Smith, 1896). Standford and Wolf (1917) observed that, the bacteria formed circular, glistening white colonies slightly raised with smooth margin and appeared within 36 – 48 h.

Kelman (1954) distinguished colony variants on Tetrazolium medium. The normal or wild type were irregularly round, entire, white or white with light pink centre and the mutant or butyrous type were round, translucent, smooth, deep red with a narrow light bluish margin.

An association between levels of virulence and colony morphology of the pathogen on TTC medium had also been demonstrated by various workers in Kerala. Paul (1998) isolated the pathogen from brinjal, chilli and tomato on TTC medium and found that all the isolates produced circular, smooth, raised, creamish white colonies with pink centre and entire margin. The fluidity was highest with brinjal isolate followed by tomato and lowest in chilli. However, Mathew *et al.* (2000) reported that, the slime production and fluidity were more in brinjal and chilli isolates compared to tomato.

James (2001) found that, all the three isolates of chilli, brinjal and tomato formed circular colony with entire margin on TTC medium. Brinjal isolate produced flat to slightly raised, low to high fluidal colony, while chilli isolate formed slightly raised, high to very high fluidal colony. Tomato isolates showed flat and very low, low and high colony characters.

Kelman (1954) found that, the wild type colonies are highly virulent and producing wilting in 14 days whereas the mutant type is either weakly pathogenic or non-pathogenic. James (2001) recorded virulence, based on Bacterial Wilt Index and aggressiveness, based on incubation period (IP) and latent period (LP 50). Among the isolates collected from Kumarakom, Ambalavayal and Vellanikkara, brinjal, chilli and tomato belonging to Kumarakom were found to be most virulent and aggressive. Mathew (2001) compared the virulence and aggressiveness of brinjal, chilli and tomato isolates of Vellanikkara, Mannuthy, Ozhalapathy and Chittoor areas and found that all isolates of *R.solanacearum* were aggressive and virulent except chilli isolate of Ozhalapathy However, Vellanikkara isolates were highly virulent and more aggressive than others.

The pathogen loses its virulence very rapidly in culture due to transformation to avirulent form. The virulence could be retained by preserving the cultures in mineral oil (Kelman and Jenson, 1951) or in sterile distilled water at room temperature. (He *et al*, 1983; Prior and Steva,1994; Mathew *et al*., 2000)

The aerobic nature of *P.solanacearum* was well established by many workers (Smith,1914; Labrousse,1932, Moraes, 1947 and Prior *et al*., 1990).However, Kelman and Jenson (1951) opined that, it could grow anaerobically. Devi (1978) also noticed both aerobic and anaerobic growth *of P.solanacearum*.

Biochemical studies conducted by various workers revealed that, different isolates of *R.solanacearum* were positive for solubility in 3% KOH, catalase, oxidase reaction, hydrolysis of Tween-80, gas production and negative for levan production and arginine dihydrolase. (He *et al*., 1983; Swanepoel and Young, 1988; Prior and Steva,1994; Jyothi, 1992; Paul,1998; Mathew *et al*., 2000).

Many workers have studied the cross infectivity of isolates of *P. solanacearum* from different host plants. 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. Devi (1978) observed that, the chilli strain of *P. solanacearum* caused high degree of wilting in tomato and eggplants and that the brinjal and tomato isolates were capable of cross infecting each other. Nayar (1982) showed that, tomato and brinjal isolates were capable of cross infecting each other and that chilli and ginger isolate caused wilting of their respective hosts only.Prior and Steva (1994) showed that, chilli isolate caused rapid wilting in tomato and eggplant. Jyothi, 1992; Paul, 1998; James. 2001 and Mathew (2001) also reported the cross inoculable nature of different solanaceous isolates of *R.solanacearum*.

The bacterial wilt pathogen *R.solanacearum* exhibits great degree of both phenotypic and genotypic diversity. Many workers attempted and grouped these isolates

into biotypes, varieties or races on the basis of difference in physiological characteristics (Kelman,1953; Buddenhagen and Kelman, 1964; Hayward,1964).

Buddenhagen *et al.* (1962) differentiated strains of *P.solanacearum* into three races, race-1 affecting tobacco, tomato, many solanaceous and other weeds, and certain diploid bananas; race -2 affecting triploid bananas, heliconia or both; race-3 affecting potato and tomato, but highly virulent on other solanaceous crops. Later, two new races were proposed affecting ginger and mulberry from Philippines and China respectively (Buddenhagen, 1986).

According to Persley *et al.* (1985) the bacterial wilt pathogen could be grouped into five races which differ in host ranges, geographic distribution and ability to survive under different environmental conditions. Samaddar *et al.* (1998) identified *R.solanacearum* affecting aubergine, tomato, potato and chilli collected from West Bengal as race-1. Paul (1998) reported that, among the three *R.solanacearum* from tomato, brinjal and chilli, the isolate obtained from chilli and tomato were characterised and identified as race-1 biovar III and that obtained from brinjal was identified as race 1 biovar V.

Mathew (2001) classified the isolates of *R.solanacearum* affecting solanaceous vegetables in Kerala as race 1 and biovars III, III A and V. James (2001) classified brinjal, chili isolates of Vellanikkara, brinjal and tomato isolates of both Kumarakom and Ambalavayal as biovar III and tomato isolate of Vellanikkara and chilli isolate of Kumarakom and Ambalavayal as biovar III A. She also grouped the isolates of Vellanikkara and Kumarakom into race 1 and Ambalavayal into race3.

2.2. ECOLOGY OF THE PATHOGEN

The ecology of the pathogen in infested soil is poorly understood. It is inferred that,

the primary inoculum came from the soil but there is no conclusive evidence that the pathogen is an ubiquitous inhabitant in the soil (Buddenhagen and Kelman,1964). Under natural conditions, the pathogen was able to survive saprophytically in the soil for as long as six years. (Chester, 1950)

Survival and dissemination of *R. solanacearum* is generally enhanced by high soil water content. Pereira and Normando (1993) observed that, the presence of a susceptible host and high soil humidity favoured the survival of bacteria, soil type did not affect *R.solanacearum* survival in those conditions.

P. solanacearum does not survive in the soil for prolonged periods because it is not a strong competitor. It does not survive in the soil itself but survive on or in plant roots. The bacterium appeared to survive by continuously infecting the roots of susceptible or carrier plants or by colonising the rhizosphere of non host plants (Sequeira, 1993). Silveira *et al.* (1996) reported that, multiplication and survival of the bacteria depends on its survival in weed hosts and cultivated crops.

Survival of *P. solanacearum* in the rhizosphere of beans and maize has been documented by Granada and Sequeira (1983) and reported long term survival of the bacterium with localized or systemic infection of plants did not express symptoms of bacterial wilt. Bekkum *et al.* (1997) observed the survival of *P. solanacearum* (race 3) in water, silt and soil and also on *Solanum dulcamara* as an alternative host.

2.3. FACTORS AFFECTING WILT INCIDENCE

The bacterium invaded plant vascular tissue from wounded roots or natural openings which occur after the emergence of secondary roots (Kelman and Sequeira 1965; Schmit (1978). The pathogen could enter into the uninjured roots also (Libman and Leach, 1964). They reported that, root contact with infected plants was not necessary for infection. Bacteria can enter at the points of origin of secondary roots.

Kuc (1964) stated that, disease resistance is not an absolute or static condition and depends on many factors. Expression of the biochemical potential, determined by the genetic component of the organism is influenced by a multitude of factors including mutation, growth regulators, temperature, moisture, day length, stage of development and nature of tissue.

Infection occurs at soil temperatures as low as 12.8°C but symptoms of wilt do not ordinarily become apparent at 12.8°C to 15.6°C (Vaughan, 1944). Acosta *et al.* (1964) noted that, tomato bacterial wilt infection was more severe during the summer at high soil temperature. Gallegly and Walker (1949) reported that, high moisture levels in soil affected the disease by favouring survival of bacterial in soil and thereby increasing capacity for infection. Kelman (1953) suggested that, high soil moisture levels usually favours the development of bacterial wilt. An increase in soil moisture from 50-100% of its water holding capacity and temperature from 21 to 35°C favoured the development of bacterial wilt of potato. (Hingorani *et al.*, 1956). Sabet and Baraket (1971) found that 90% of the soil WHC was optimal for bacterial wilt development in potato. Hiryati *et al.* (1983) noticed that, severity of diseases caused by *P. solanacearum* significantly increased with increased soil moisture from slightly above wilting point to slight below saturation point for each soil type tested.

Akiew (1985) recorded decrease in population of potato pathogen *P. solanacearum* with increase in air temperature and decrease in soil temperature. Ho (1988) reported that, high rainfall especially towards middle end of growing season favoured high bacterial wilt disease incidence in tomato.

Chupp and Sherf (1960) reported that, the infection occurs in dry soil and disease become serious in red laterite soil. A high wilt incidence was reported at pH 3.5 by Kelman and Cowling (1965). Shekhawat *et al.* (1978) observed that, the bacterial wilt

was more widespread in heavy and acidic soil. (pH 3.5 to 6.9) than in light and neutral (pH 6.5 to 7.5) to alkaline (pH 7.5 to 8.5) soils.

Bora *et al.* (1996) found that, the bacterial wilt incidence was significantly correlated with soil temperature, air temperature and total rain fall. Relative humidity had no correlation with incidence. They concluded that, soil temperature from 25-30°C accompanied by a maximum air temperature of 26-30°C and monthly rainfall ranges from 200-300 mm favoured bacterial growth and multiplication resulting in severe wilt incidence in tomato.

Koga *et al.* (1997) identified two types of suppressive soils for bacterial wilt of tobacco *ie.* loamy soil and sandy soil. A study conducted by Keshwal *et al.* (2000) showed that, sandyloam soil with a 27.3% field capacity (FC) and 34.6% water holding capacity (WHC) harboured minimum population of *R. solanacearum* PST_4 (21.77×10^{3}) inciting 32.2% wilt in tomato crop. Whereas, clay soil having 66% WHC and 36.2% FC harboured maximum population of pathogen, causing wilt incidence of 63%.

Winstead and Kelman (1952) suggested that, increased resistance in resistant lines was apparently associated with age rather than plant size. Bell (1981) stated that, each plant part changes in its level of resistance with age. Resistance level in stem and root generally increases rapidly during the first two weeks of seedlings or when new shoots grows and slowly thereafter. Levels of resistance in leaves and fruits frequently decline with age. He also reported that, long photoperiods generally result in higher levels of resistance. Increasing the concentration of potassium and calcium enhance most often level of resistance while nitrogen decreases resistance.

2.4 MANAGEMENT OF BACTERIAL WILT DISEASE

2.4.1. Host resistance

The use of resistant variety is a simple, effective and economical means to control soil borne diseases. Breeding for host resistance against *Ralstonia solanacearum* have been conducted by a number of workers and numerous reports are also available on this field. But it is beyond the scope of present study to go through the extensive literature on this aspect. This review, is therefore, confined to the work done at Kerala Agricultural University.

Rahim and Samraj (1974) found that, variety 'Khandari' was highly resistant to bacterial wilt of chilli. Goth *et al.* (1983) reported that, KAU cluster (Manjari) was resistant to four race 1 isolates and one race 3 isolate of *P.soanacearum*. Peter *et al.* (1984) evaluated four Indian hot chillies (Pant C-1, KAU cluster, white Khandari and Chuna) along with six US cultivars for reaction to nine isolates of *R. solanacearum*.. Rajan (1985) reported LE - 79 (Sakthi) highly resistant to the bacterial wilt of tomato. Thomas (1985) also observed resistance in 'KAU Cluster' against bacterial wilt pathogen.

A study conducted at KAU, Vellanikkara revealed that, *Capsicum annuum* accessions CA 33 (KAU Cluster) and CA 219 were resistant to bacterial wilt disease (KAU, 1988). Gopalakrishnan and Peter (1991) evaluated 146 accessions of *Capsicum* sp for resistance to bacterial wilt in a wilt sick soil after artificial inoculation and found that, CA - 219 (Ujjwala) and CA - 33 (Manjari) were highly resistant with good dry chilli yield. Cluster fruited plants gave significantly better wilt resistance than solitary fruited types. Jyothy (1992) also reported the resistance of variety 'Manjari' against bacterial wilt of chilli. Sadhankumar (1995) evaluated 66 tomato genotypes against bacterial wilt for three seasons and revealed that, LE - 415, Sakthi and LE 79-5 were consistently

resistant to bacterial wilt. Singh (1996) obtained a brinjal hybrid 'Neelima' highly resistant to bacterial wilt.

From the trials conducted at AICVIP, Vellanikkara it was observed that, out of 29 brinjal genotypes screened against bacterial wilt, 10 genotypes such as SM 6-7 (Surya), SM 6-6 (Swetha), SM 141(Haritha), BWR-12 (Arka Nidhi), BWR-21 (Arka Keshav), BWR-54 (Arka Neelakanth), Pusa Purple Cluster, BB 60-C and BB-64 were resistant and high yielders. Similarly, out of 22 tomato genotypes evaluated against bacterial wilt, LE-79 (Sakthi), LE 79-5 (Mukthi), LE 415 and L 66 were found to be resistant at Vellanikkara condition (AICVIP, 2002).

2.4.2. Mechanism of disease resistance

Expression of resistance has been linked to the resistance of bacterial colonization within xylem tissues located in the stem. (Grimault *et al.*, 1994).

Anderson and Brodbeck (1989) reported that, the xylem fluid contains the lowest concentrations of solute of any plant tissue with total osmolality averaging 10 to 25mm. Aminoacids and organic acids may be determinant of resistance to bacterial wilt.

Suppression of disease with increased level of calcium have been reported by various workers .Corden (1965) explained that, suppression of plant disease is due to direct inhibition of pectolytic enzymes such as polygalacturonase by calcium. However, indirect inhibition of enzymes due to the strengthening of cell walls by calcium was also reported. (Bateman and Lumsden, 1965; Conway *et al.*, 1992).Raz and Fluhr (1992) opined that, the suppression was due to inhibition of ethylene production. Sadhankumar (1995) and Paul (1998) also observed that, calcium content was higher in resistant genotypes of solanaceous hosts.

2.4.3. Chemical control

The inhibitory effects of Streptomycin and Streptocyclin on *Pseudomonas* have been observed by many workers. (Rangarajan and Chakravarti, 1969; Shivappashetty and Rangaswami, 1971).Rahim (1972) and George (1973) obtained excellent field control of chilli bacterial wilt by spraying the foliage with Streptomycin and Streptocycline or by soil drenching with chestnut compound.

Several antibiotics like Oxytetracycline, Tetracycline, Penicillin –G, Streptomycin were reported to inhibit the pathogen (Goorani *et al.*, 1978). 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. Ishikawa *et al.* (1996) noticed that, foliar sprays of Validamycin A at 250 µ g/ml 5 days before and 2 days after inoculation, reduced the wilt incidence of tomato.Said *et al.* (1996) applied 2.5 and 5.0 ml of aspirin or salicylic acid and observed, significant reduction in disease incidence and increase in the number of flowers and other different growth parameters in tomato. Dhital *et al.* (1997) found that, use of Stable Bleaching Powder (SBP) at the rate of 25 kg/ha is more effective and suitable for the control of bacterial wilt in potato both under green house and field conditions.

Yamada *et al.* (1997) reported that, Dazomet combined with soil solarization gave better control of tomato bacterial wilt. Paul (1998) obtained good inhibition and suppression of growth of *R. solanacearum* of tomato with Oxytetracycline and Streptomycin sulphate. Mazumder (1998) carried out field trials to control. *R. solanacearum* on tomato cv. Pusa Ruby and found that 200 µg/ml of Streptomycin treatment was the most effective giving a disease control of 79% with maximum yields of 274.6 q/ha. He also noted that, 10 g and 5 g/litre of bleaching powder could also effectively reduce disease incidence and increase the yield.

A perusal on literature revealed that, the reports on fungicidal toxicity on *R.solanacearum* is meagre and scanty. However, attempt has been made to include some of the available literature on the fungicidal action on bacterial wilt pathogen.

Severin and Kupferberg (1977) reported that. Bordeaux mixture. Copper oxychloride and Kocide were effective in controlling bacterial blight of walnut. Inhibitory action of Dithiocarbamate fungicides like Nabam (Dithane A - 40). Maneb (Dithane M - 22) and Dithane M - 45 on bacterial wilt pathogen was studied by Goorani *et al.* (1978). Leandro and Zak (1983) observed the inhibitory effect of Captan. Maneb. Mancozeb and Thiram on *R. solanacearum* . Jyothi (1992) reported that, among the three fungicides, Thiride, Blue copper and Bordeaux mixture tested, Bordeaux mixture recorded the maximum inhibition of *R.solanacearum.* Inhibition of *R.solanacearum* by copper hydroxide (kocide) 0.15% was reported by Akbar (2002).

2.4.4. Soil amendment

An application of urea @ 1000 kg/ha has been found to be effective in reducing the wilt incidence (Kelman, 1953). Jayaprakash (1977) observed a reduction in percentage of tomato wilted plants in plots amended with oil cakes, saw dust, cashew shell powder, coconut pith, oil palm seed waste and various crop residues. Devi (1978) reported that, application of soil amendment sawdust plus urea combined with agrimycin-100 spray reduced the wilt incidence to 26 per cent in tomato.

When brinjal seedlings were dipped in solution containing asafoetida, turmeric powder and water at the ratio of 1:5:10, significant protection against bacterial wilt was obtained (Bhattacharya *et al.*,1994;Pun and Das, 1997). Miah *et al.* (1995) observed that, amendments to soil with soybean husk, rice husk + urea and recommended dose of fertilizers effectively prevented both the progress and incidence of bacterial wilt in a susceptible variety of tomato.

Hanudin (1997) reported that, amending soil using 428 kg ha^{-1} urea + 5000 kg ha^{-1} Calcium oxide at 3 week prior to transplanting raised soil pH and was effective in suppressing bacterial wilt in the rigosol and alluvial soil. Hanudin and Machmud (1997) conducted studies with KNO3, NaNO3, KCl and NaCl and found that, NaNO3 was most effective in reducing the bacterial population to 65% of that in the control.

Kelaniyangoda (1997) reported that, bacterial wilt incidence was reduced to 21% both in tomato and potato when sunnhemp, CaO and urea were incorporated into the plots. Michel *et al.* (1997) observed significant reduction in bacterial wilt when soil amendment of urea (200 kg) and CaO (5000 kg/ha) was applied before planting. African or French marigolds (*Tagetus erecta/planta*) when planted with a susceptible tobacco cultivar was able to reduce the pathogen population as well prevent the plants from developing symptom (Terblanche and Villiers, 1997).

2.4.5. Biological control

Biocontrol of plant pathogen is becoming an important component of plant disease management. In view of the hazardous impact of pesticides and other agrochemicals on the ecosystem, biocontrol of plant diseases as an alternate strategy has received increasing attention in recent years. A search on literature showed that, the effect of microbial antagonists like bacteria and actinomycetes on *R.solanacearum* have been well studied by various workers. However, the informations on fungal antagonists activity on the pathogen are much lacking.

Opina and Valdez (1987) studied the effect of *Pseudomonas fluorescens* and *Bacillus polymyxa* on bacterial wilt pathogen of tomato and brinjal. He observed that, in brinjal, both organisms significantly reduced the incidence of wilt, when seedlings were dipped in the suspension than when the suspension is drenched at the base of seedlings. He also noted that, *P.fluorescens* showed better antagonism than *B.polymyxa*.

Anuratha and Gnanamanickam (1990) evaluated the strain pfcp of *P.fluorescens* and strains B33 and B36 of *Bacillus* spp. against bacterial wilt of banana, egg plant and tomato under green house and field conditions. Protection upto 50, 61 and 95% and 50, 49 and 36% were obtained for banana, eggplant and tomato in green house and field respectively.

Furuya *et al.* (1991) observed the different antibiotic activities of strain of *P.glumae* against *R.solanacearum* depending upon type of media used. All strains of *P.glumae* formed growth inhibition zones around their colonies on the lawn of *R.solanacearum* when TTC media was used. They also found that, dipping the roots of tomato seedlings in the bacterial suspension of 10^{10} cfu/ml for 24 h showed highest suppression of disease and also suggested that some mechanisms other than antibiotic productivity were involved in the suppression of the disease.

Phae *et al.* (1992) brought down the percentage bacterial wilt incidence to one-third of the control in tomato by pouring cultural suspension of *B.subtilis* NB 22 isolated from a compost into a heavily infested soils. Suresh and Ravi (1992) observed inhibition zones when *P.purpurescens* was tested on *P.solanacearum* and acids from their inhibitor.

Arwiyanto *et al.* (1994) were able to suppress the development of bacterial wilt in tomato under green house condition by applying the spontaneous avirulent mutant of strain str-10 of *R.solanacearum* isolated from *Strelitzia reginae*. Protection was more pronounced at low temperature (18-25°) and was lost at high temperature (18-25°C). The reason for suppression by the strain may be due to induced resistance. Hanudin and Machmud (1994) showed the effect of *P.fluorescens* against *P.solanacearum*.

Piexoto *et al.* (1995 a) reported that, some mutants and wild isolate of *P.aeruginosa* inhibited *R.solanacearum* in both King's B and nutrient yeast extract dextrose agar media with maximum zones of 8.1 and 14.3 mm respectively. However, no significant reduction on disease incidence was obtained with mutants against tomato

bacterial wilt under green house condition when drenching the seedbed substrate with the antagonists 5 days before planting.

Peixoto *et al.* (1995 b) followed two methods of application of antagonist *P.aeruginosa ie.* drenching of seedlings in the nursery 5 days before transplanting (M_1) and drenching of seed bed substrate 5 days before sowing (M_2) and M_2 was found to be the best application method, and the isolates FR 6, FR 48 and TR 25 induced DSR (Disease Severity Reduction) values of 44.0, 35.4 and 31.1% respectively. They also suggested that, the most efficient method for inducing growth promotion of tomato seedlings is combined bacterisation of seed and seed bed substrate.

Silveira *et al.* (1995) tested, 30 isolates of *Bacillus* spp. obtained from the rhizoplane of several crops, against *R.solanacearum* for their antagonistic activity under green house condition. Two methods of application were tried, including irrigation of the bed substrate with bacterial suspension 5 days before seeding and seed bacterization 16 h before seeding. The isolates BA-24 (*B. coagulans*) BA-46 (*B.megaterium*) and BA-3 (*B.cereus*) applied to the substrate showed lower BWI giving 54.6, 46.6 and 42.6% of disease control respectively. BA-24 and BA-46 were also found to promote growth parameters.

Abyad *et al.* (1996) isolated *S.pulcher, S.canescens* and *S.citrofluorescens* and found antagonistic against *R.solanacearum* in liquid media. Ciampi *et al.* (1996) noticed that, siderophore like compounds produced by isolate of *P.fluorescens* was responsible for the inhibition of *R.solanacearum* and also observed that, synthesis of the pigment is dependant on Fe^{3+} levels in the culture medium and the siderophores increased when iron concentration is limited.

Jianhua *et al.* (1996) dipped tomato seedlings in 55 selected bacterial suspensions for 12 h before planting in green house. He found that, roots were colonized by only 22 strains and the population of 17 inhibitory strains were higher than those of all virulent

strains. The population of 10 avirulent bacteriocin producing *R.solanacearum* strains were only < 10^4 cfu/groot. So he concluded that, *in vitro* inhibition test combined with assays of root colonization, provide an effective means of screening for bacterial antagonists against plant diseases.

Shanshoury *et al.* (1996) identified two *Streptomyces* spp. controlling tomato wilt pathogen. *F.oxysporum f* sp. *lycopersici* was inhibited by *S.corchorusii* and *P. solanacearum* by *S. mutabilis* under *in vitro* condition. Silveira *et al.* (1996) evaluated a number of microorganisms including *Streptomyces griseochemogenus, S.griseus, P.fluorescens, T.pseudokoningii, P.aeruginosa, B.coagulans, B.megaterium* and *B.cereus* against *R.solanacearum* and were found effective under *in vitro* condition.

Alice and Carlos (1996) observed that, treatment with avirulent mutant of *R.solanacearum* or fluorescent *Pseudomonas* isolated from potato rhizosphere did not show any significant difference in disease severity when compared to non treated control both in green house and field trials. Furuya *et al.* (1997) reported that, the strain ATCC 7700 of *P.aeruginosa* was highly antagonistic to *R.solanacearum* under *in vitro* condition and root dipping of tomato with *P.aeruginosa* suspension of 10^{10} cfu/ml at the time of transplanting in wilt sick soil increased the percentage of seedling survival. He also observed that, pre treating of tomato roots with killed cells of *P. aeruginosa* gave protection and stating that, mechanism of induced resistance and infection sites competition were involved in suppression of the disease other than antibiotic protection.

Karuna *et al.* (1997) tried seed bacterization followed by root dipping the Pusa Ruby seedling with *P.fluorescens, P.aeruginosa* and *B.subtilis* and found that, *P.fluorescens* was most effective in reducing the incidence of wilt by 50% under field condition. Singh (1997) reported 64% control of bacterial wilt in tomato cv. Pusa Ruby when heat killed cells of *R.solanacearum* were used. Sunaina *et al.* (1997) found that, *B.subtilis* ($S_{11}B_5$), *B.cereus* (B_4) and an avirulent strains of *P.solanacearum* exhibited antagonism against potato bacterial wilt pathogen both under laboratory and glass house

condition. In field, seed potato bacterized with B_4 and B_5 brought down the wilt incidence to nearly one third and one fourth compared to control. Substantial increase in per cent yield of 22.72, 46.72 and 62.67 were also obtained as compared to the control.

Strains of fluorescent *Pseudomonads* isolated form healthy eggplant roots inhibited the growth of *R.solanacearum* on King's B medium and PDA medium. Strain FPP_5 and FPP_3 increased the ration of plant height, total weight and root weight 10.0-25.5%, 12.7-37.8% and 2.1-71.3% respectively. However, some strains of fluorescent *Pseudomonads* inhibited the above growth parameters. (Yungchun *et al* , 1997). Guijing *et al.* (1998) observed that, conidial extracts of *Trichoderma koningii* BY-88 completely inhibited the growth of *R.solanacearum* and recovery rates of the antibiotic substance extracted by alcohol and NaoH were 0.89% and 0.82% respectively.

Out of 190 actinomycetes isolated from the rhizosphere, rhizoplane and root tissue of tomato, 18 actinomycetes showed cent percent control of *R.solanacearum*. The most efficient method of application was by dipping the seeds in a propagules suspension of the actinomycetes (Moura *et al.*,1998).*Bacillus subtilis, Pseudomonas* spp. and *P.cepacia* had highly inhibitory effect against *R.solanacearum* on culture medium and significant reductions in wilting of tomato was also noticed in greenhouse tests (Abdalla *et al.*,1999).Chun *et al.* (1999) opined that, an virulent and bacteriocinogenic strain Tms of *R.solanacearum* had an inhibitory effect on the tomato virulent strain.

Anith *et al.* (2000) observed that, seed treatment with *P.fluorescens* strain EM 85 along with soil solarization decreased the wilt incidence in ginger to 7.42% and increased the yield to 29.42 t/ha compared to 19.51 t/ha in control plots. Das *et al.* (2000) evaluated few established biocontrol agents for their inhibition action against *R.solanacearum*, using agar plate assays in dual culture. The bacterial antagonists *P.fluorescens* showed maximum inhibition of the pathogen (33.30mm), followed by *Aspergillus terreus* (23.1 mm), *Trichoderma harzianum* (14.3 mm), *B.subtilis* (13.6 mm), *Gliocladium virens* (13.3 mm), *T.koningii* (12.00 mm) and *T.viride* (9.96 mm). Out of

three best antagonists tested in tomato variety Pusa.Ruby, *P.fluorescens* applied 14 days prior to inoculation showed the least disease incidence and highest yield of 12.81 q/ha.

Singh *et al.* (2000) reported increased yield and inhibition of wilt incidence in potato variety Kufri Jyothi when potato tubers were dipped in *Bacillus* spp. suspension before planting. In the study conducted by Jianhua *et al.* (2001) they observed that, six bacterial strains exhibited inhibition zone larger than 1 cm on artificial media against pepper bacterial wilt pathogen. They also found that, disease incidence was reduced by 37-73% at 40 days after treatment and the yield at ripeness was increased by 19-79% under field condition.

Kumar and Sood (2001) were able to reduce bacterial wilt incidence of tomato to 65.9 and 71.6% in 8 and 10 weeks solarised plots. They also observed the significant reduction in wilt incidence due to incorporation of antagonistic rhizobaceteria (*P.fluorescens* and *B.cereus*) in soil prior to solarisation as the population of the antagonist was considerably enhanced after solarisation and also increased the vigour of the plants. Akbar (2002) reported that, seed treatment + soil drenching with *P. aeruginosa* reduced the wilt incidence to 11.1 per cent in variety Pusa Ruby under pot culture experiments.

Materials and Methods

3. MATERIALS AND METHODS

The present study was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara. Microorganisms were isolated from rhizosphere soils collected from Vellanikkara (high wilt incidence area) having a soil type of laterite loam with a soil pH of 6.5-6.9 and a soil temperature of 44.4-45.6°C and Ozhalapathy (low wilt incidence area) having black soil with a pH of 7.4-7.7 and soil temperature of 26.6-27.7°C. The field experiment on the management of bacterial wilt disease was laid out in the wilt sick plots of Department of Olericulture, during May-August, 2002 using the susceptible varieties of brinjal (Pusa Purple Long), chilli (Pusa Jwala) and tomato (Pusa Ruby)

3.1. COLLECTION OF BACTERIAL WILT INFECTED PLANT SAMPLES.

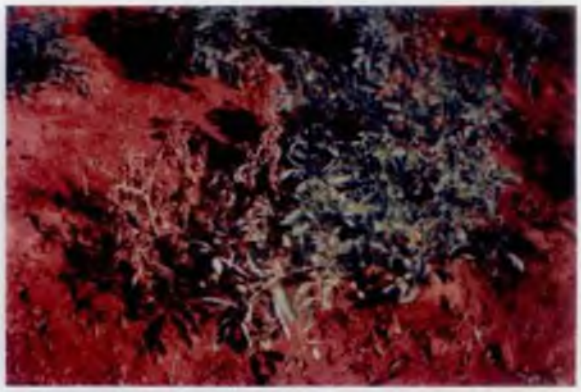
Wilted plant samples of brinjal, chilli and tomato were collected from Vellanikkara and Ozhalapathy. Samples were brought to the laboratory, washed under tap water to remove soil particles, air dried and then subjected to ooze test. The plants showing streaming out of ooze from the cut ends were separated and used for the isolation of the pathogen and for grouping the isolates.

3.2. ISOLATION OF THE PATHOGEN

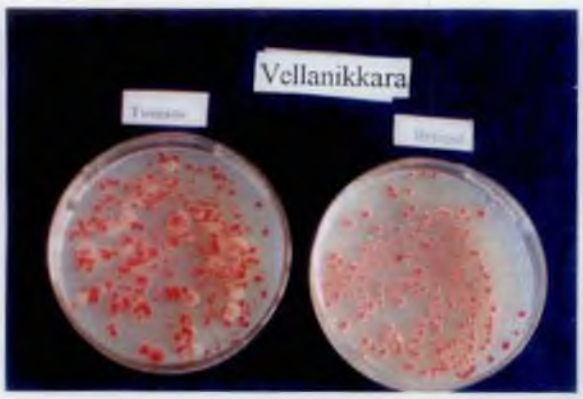
Basal stem portions of 10-15 mm length were cut with a sterile blade after surface sterilization with 70% ethyl alcohol and placed in test tubes containing five ml sterile water. When the water became turbid, a loopful of the suspension was streaked on Triphenyl Tetrazolium Chloride (TTC) medium (Kelman,1954) and the plates were incubated at 28 ± 2°C for 48 h to get well isolated colonies of bacteria (Plate.1) Composition of TTC medium is given in Appendix.I.

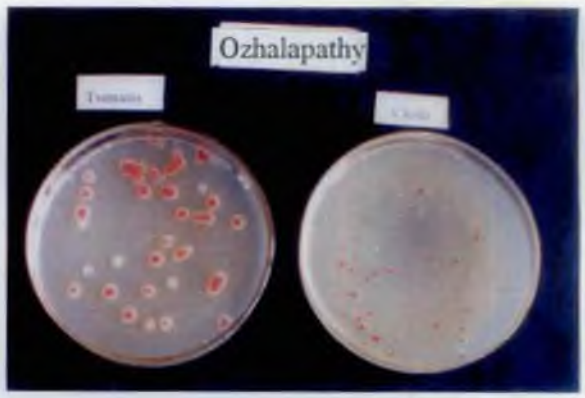
PLATE 1

Bacterial wilt of Brinjal



Bacterial wilt of Tomato





Colonies of *R. solanacearum* isolates on TZC meidum

3.3. PURIFICATION AND PRESERVATION OF *R. solanacearum* ISOLATES

Pin pointed, slimy fluidal cream coloured colonies with light pink centre were selected from TTC medium and then purified by repeated streaking on Nutrient Agar medium (NA).Three to five characteristic single colonies were picked and transferred into five ml sterile distilled water in screw capped glass vials and stored at room temperature for subsequent use.

3.4. PATHOGENICITY TEST

Fresh bacterial ooze was obtained from brinjal, chilli and tomato infected plants and adjusted to the concentration of OD_{600} nm = 0.3. Susceptible varieties of brinjal (Pusa Purple Long), chilli (Pusa Jwala) and tomato (Pusa Ruby) were inoculated with the isolates by applying stem puncturing and leaf clipping methods. Inoculated plants were observed daily for the symptom appearance upto 14 days.

3.5. ISOLATION AND MAINTENANCE OF ANTAGONISTIC MICROORGANISMS

3.5.1. Collection of soil sample

Rhizosphere soil samples were collected from different healthy solanaceous crops grown adjacent to wilted plants, from Vellanikkara and Ozhalapathy areas during early fruiting stage. Soil samples were also collected from the rhizosphere of bacterial wilt resistant genotypes of brinjal, chilli and tomato plants released from Kerala Agricultural University. An additional collection was also made from solarised beds and forest area.

3.5.2. Enumeration of *R.solanacearum* population in wilt sick soil

Soil samples were collected from the wilt sick plots of Vellanikkara and

Ozhalapathy locations and the pathogen was isolated by serial dilution technique on TTC medium. The population of the pathogen was determined as number of colony forming units per gram (cfu $^{-1}$gm) of dry soil.

3.5.3. Determination of soil pH and soil temperatures in different locations

The pH of the soils of the two locations was determined using the standard methods. The soil temperatures of these two locations were also recorded at the time of soil sampling.

3.5.4. Isolation of fungi, bacteria and actinomycetes

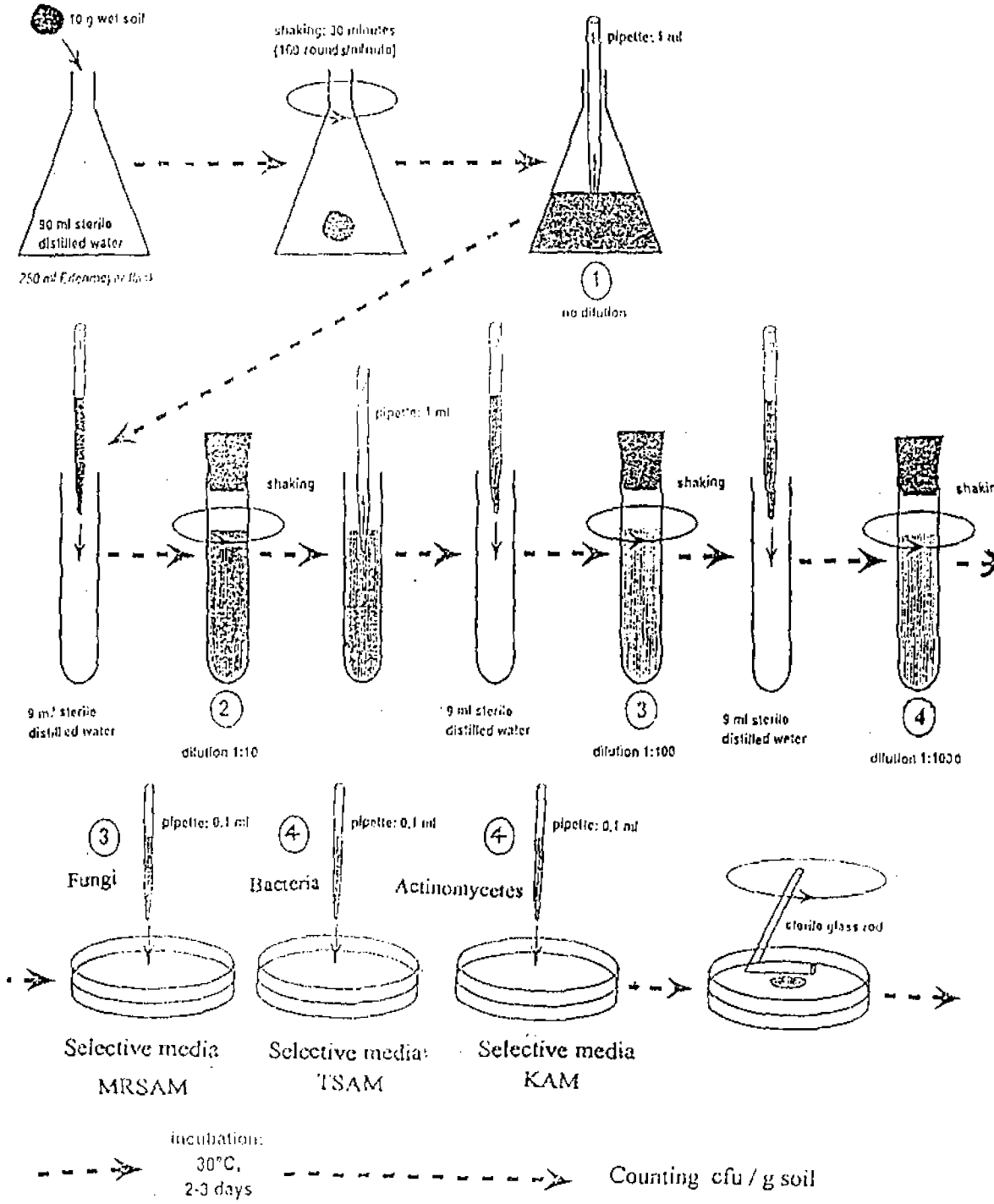
The microorganisms were isolated by serial dilution technique suggested by Johnson and Curl (1972). 10 g of soil sample was added to 100 ml sterile distilled water in 250 ml conical flasks and shaken for 30 minutes in orbital shaker (160 rounds/min.). One ml of this soil dilution was then transferred to test tube containing nine ml sterile distilled water to get 10^{-2} dilution. Likewise 10^{-3} and 10^{-4} dilutions were also prepared (Fig.1).

The fungi were isolated by plating 0.1 ml of the 10^{-3} dilutions of soil in a petridish containing 20 ml of solidified Martin Rose Bengal Streptomycin Agar media (MRBA) (Martin, 1950). The bacteria and the actinomycetes were isolated by plating 0.1 ml of 10^{-4} dilution on Thornton's Agar media (TAM) and Kenknight's Agar Media (KAM) respectively. The plates were incubated for 72 h, 48 h, and one week respectively. The colonies developed at the end of incubation period were counted and expressed as cfu $^{-1}$gm of soil. The composition of the media is given in Appendix II

3.5.5. Maintenance and preservation of isolated microorganisms

The isolated organisms were transferred into test tubes containing Potato Dextrose Agar (PDA), NA and KAM for maintaining fungi, bacteria and actinomycetes

Fig.1 . Isolation of microorganisms from soil.



respectively. The sealed and labelled cultures were stored in the refrigerator for further use.

3.6. *IN VITRO* EVALUATION OF ANTAGONISTS AGAINST THE ISOLATES OF *R.solanacearum*

The microorganisms isolated from the rhizosphere soils of solanaceous crops like brinjal, chilli and tomato (both susceptible and resistant varieties) from Vellanikkara and Ozhalapathy areas, from solarised soils and forest soils were tested for their antagonistic reactions against different isolates of *R. solanacearum*.

3.6.1. Preparation of cultures of test organism

Different isolates of *R . solanacearum* collected from Vellanikkara and Ozhalapathy were streaked on TTC medium from the stock culture stored in sterile water and incubated for 48 h at room temperature 28 ± 2°C. A single fluidal pink headed colony was selected and multiplied on NA medium. Bacterial suspensions of these different isolates were prepared by adjusting the concentration to 10^8cfu/ml. 0.1 ml of these suspensions were spread on appropriate agar medium plates using a glass spreader to prepare the bacterial lawn for all the experiments.

3.6.2. Preparation of culture media

The culture medium which favours the growth of antagonists as well as the pathogen was used. For fungi and actinomycetes, PDA and for bacteria NA media were used.

3.7. EVALUATION OF FUNGAL ANTAGONISTS AGAINST THE ISOLATES OF *R..solanacearum*

3.7.1.Standardisation of method for the evaluation of fungal antagonists

To standardize the best method for the evaluation of fungal antagonists, two methods were tried, using three Vellanikkara isolates of *R.solanacearum* and 12 fungal organisms.

i) Streak method: A four mm disc of the candidate organism was placed at centre of the mediated plate and the test pathogens were streaked simultaneously on both sides of it.

ii) Spread plate method : In this case, both simultaneous antagonism and deferred antagonism methods were tried. Four mm disc of candidate organism was placed at the centre of seeded agar plates on the same day as well as after an incubation of 24 h. Simultaneous antagonism method was found to be the best one and was selected for the further studies.

3.7.2. *In vitro* evaluation of fungal antagonists against the isolates of *R.solanacearum*

About 90 fungal cultures isolated from rhizosphere soil were screened against different isolates of *R.solanacearum* collected from Vellanikkara and Ozhalapathy.

For the initial screening, different isolates of test pathogens were seeded separately on PDA and four different candidate organisms were placed simultaneously at four corners of the culture plates at equidistant points. The organisms showing antagonistic reactions were selected and maintained for further studies.

The promising antagonists selected in the initial screening were then tested individually for its antagonistic property. For this, four mm disc of the potential antagonists selected were placed individually at the centre of the plates seeded separately with six different test pathogens. Three plates kept for each pathogen and each antagonist. The dishes with antagonistic fungi alone served as control and the observations were taken

till the control plates were fully covered with fungal growth. The commercial antagonists *viz. Trichoderma viride, T. harzianum, Aspergillus niger* were also tested and compared. The type of antagonism was recorded using a 1-5 scale score chart and per cent inhibition as well as Antagonism Index (AI) was calculated using the following formula (Kasinathan,1998)

$$AI = PI \times TIME \times CB \times IZ.$$

$$PI = \text{Per cent Inhibition} = \frac{C-T}{C} \times 100$$

Where C = growth of test pathogen (mm)

T = growth of test pathogen (mm) in the presence of the antagonistic strain.

CB = Colonization behaviour

TIME = Time taken by the antagonist / pathogen to overgrow after the contact of antagonist / both.

I Z = Inhibition zone (mm)

3.7.3 Evaluation of antagonism by mutant and hybrid of *Trichoderma spp.* against *R.solanacearum* under *in vitro* condition.

The two fungal antagonists, *Trichoderma viride* and *T. pseudokoningii* which were found effective against all six isolates of the pathogen were selected for this study.

1) Induction of mutation by U.V. light.

96 h old culture of *Trichoderma viride, T. pseudokoningii* were exposed to U.V. light for 15 minutes, along with the commercial antagonist *T.harzianum.* Four mm disc of these mutant antagonists were placed at the centre of the bacterial lawns of different isolates and the antagonistic reactions were observed.

2) Interspecific hybridization of *Trichoderma* spp.

Four mm discs cut from seven day old culture of *T. pseudokoningii* and *T.viride* were placed at two opposite ends in petridishes mediated with PDA. These cultures were incubated at room temperature till the hypha of the two species met and paired. Four mm disc was cut from the interaction point of the paired culture and tested for its antagonistic activity against different isolates of *R. solanacearum*. U.V. exposed mutant of this hybrid was also tested for its antagonistic property.

3.7.4. Standardisation of method for the evaluation of bacterial antagonists

1. Cross streaking method:

a) Simultaneous antagonism: Both test and the indicator organisms were streaked perpendicular to each other on the plates having nutrient agar medium. The plates were observed daily for the lysis at the juncture of the pathogen and the antagonist.

b) Deferred antagonism: Test antagonists were streaked on diametrically at the middle of dishes containing NA/King's B medium, and incubated for 24 h and challenged by cross streaking of three isolates of *R..solanacearum*. Lysis at the juncture of the pathogen and antagonist were observed.

2. Point inoculation of indicators:

a) Simultaneous antagonism: A loopful of the indicator organism was spotted at the centre of the plates seeded with test organism. Similarly, in another method 0.01 ml of the suspension of indicator organism was spotted at the centre of the bacterial lawn. Plates were observed daily for the lysis of the pathogen.

b) Deferred antagonism : In this method a loopful / 0.01 ml suspension of indicator organism was spotted at the centre of the 24 h old culture plates of test pathogen and observed for lysis.

Simultaneous antagonism by point inoculation with loopful of bacteria was found to be the best method and this was followed for further experiments.

3.7.4.1. In vitro evaluation of bacterial antagonists against the isolates of R.solanacearum

About 87 bacterial isolates were screened against, Vellanikkara isolates of *R. solanacearum*. For the initial evaluation, different isolates of *R.solanacearum* were grown separately in the form of a lawn on the surface of NA medium. A loopful of four different indicator organisms were spotted at four equidistant points of two cm from the plate periphery. Plates were observed for the inhibition of the pathogen. The organisms that showed antagonistic reactions were selected and maintained for further studies.

Bacterial isolates, which showed antagonistic reactions in the initial screening were tested individually. The potential antagonists selected were spotted at the centre of the lawn of the target organism. Three replications were maintained for each pathogen and each antagonist. Plates with pathogen alone served as control. Commercial antagonists such as *Pseudomonas fluorescens and Bacillus subtilis* were also tested and compared. The petridishes were incubated for 48 h and the diameter of inhibition zone was measured and AI was calculated using the formula:

$$PI = \frac{C-T}{C} \times 100$$ (Dennis and Webster,1971)

Where C = growth of test pathogen (mm)

T = growth of test pathogen (mm) in the presence of the antagonistic strain

AI = PI x IZ.

3.7.4.2. Evaluation of attenuated (heat killed)cells, mutant and avirulent strains of R.solanacearum against the isolates of the pathogen

Attenuated (heat killed) cells, mutant and avirulent strains of *R.solanacearum* were obtained by the following methods.

i) Attenuated (heat killed) cells of *R.solanacearum*

R.solanacearum isolate obtained from tomato plants was kept in hot water bath at 100°C for 5,10 and 15 minutes to get heat killed cells of *R..solanacearum*

ii) Mutant of *R.solanacearum*

48 h old culture of *R.solanacearum* of tomato isolate was exposed to UV light for five,10 and 15 minutes.

iii) Avirulent strain of *R.solanacearum*

An avirulent strain was obtained from tomato isolate of *R.solanacearum* by spontaneous mutation in the culture.

These heat killed, mutant and avirulent cells of *R.solanacearum* were tested against the isolates of the pathogen for their antagonistic activity.

3.7.5. Evaluation of actinomycetes antagonists against Vellanikkara isolates of *R.solanacearum*

About 57 actinomycetes were tested against three isolates of *R.solanacearum*. For initial screening, four mm disc of four different test antagonists were placed at four corners of the culture plates at equidistance and noted for its antagonistic reaction. The promising antagonists were selected and tested individually in three replications. Inhibition zone was measured and AI was calculated using the formula.

$$AI = PI \times IZ$$

3.7.6. *In vitro* evaluation of culture filtrates of antagonists against the isolates of *R.solanacearum*

The effect of culture filtrates of most effective fungal and bacterial antagonists were tested against six isolates of *R.solanacearum* by filter paper disc method. Five mm size disc was cut from four-day-old culture of potential fungal antagonists and placed in 100 ml conical flask containing 25 ml Potato Dextrose broth and incubated for seven days at the room temperature 26 ± 2°C. Similarly, a loopful of bacteria from 48 h old culture was inoculated into Nutrient broth, and incubated for four days. The culture filtrates of the antagonists were filtered twice through sterilized double layered filter paper under aseptic condition. Filter paper disc of one cm diameter were autoclaved, dried and soaked in the culture filtrate, then shaken thoroughly to remove excess filtrate and placed on the seeded medium, two cm from the periphery of the plate. Four discs were placed in a single plate and the disc dipped in sterile distilled water served as control. After an incubation of 24 and 48 h, the inhibition zone around the filter paper disc were measured.The culture filtrates of commercial fungal (*T.viride, T.harzianum, A.niger*) and bacterial (*P.fluorescens, B.subtilis*) antagonists were also tested and compared.

3.8. IDENTIFICATION OF ANTAGONISTS

3.8.1. Identification of fungal antagonists

The fungal antagonists which were found most effective in *in vitro* studies were identified, based on cultural and morphological characters. The morphological characters were studied by slide culture technique. The cultural characters like colour, growth, texture of the colonies and the morphological characters like type, shape and size of the hyphae, conidiophore, conidia etc. were recorded and compared with the original characters of the fungi described by various workers.

3.8.2. Characterisation of the bacterial antagonist

Characterisation of the different cultures of the antagonistic bacteria was done according to the methods recommended in the Manual of Microbiological Methods published by the society of American Bacteriologist (1957) and Laboratory Methods in Microbiology (Harrigan and Mc Cance,1966). For each test, 24 - 48 h old cultures were used.Tests were conducted in triplicate and incubated at 28 ± 2°C.
Composition of media used for various tests are given in Appendix III

3.8.2.(i)Morphological characters

24 h old culture of the bacterium was used for the morphological studies.

Hucker's modification of Gram staining was employed to study the Gram reaction (Hucker and Conn, 1923) and the shape of the bacterium was identified under oil immersion objective of the microscope.

3.8.3. Cultural characters

3.8.3.(i) Growth on solid media

The bacterial cultures were streaked on Nutrient Agar medium in Petriplates and after an incubation period of 24 h, the colonies were observed for its shape, elevation and margin.

3.8.3.(ii) Growth in liquid media

48 h old culture was inoculated into the test tubes containing five ml of Nutrient broth and observed for its nature of growth from 48 to 72 h of incubation.

3.8.3.(iii) Agar stroke

A loopful of 48h old bacterial culture was stabbed inoculated into the sterilized agar columns. The tubes were incubated and observed for the growth characteristics after 48h.

3.8.3.1 Biochemical tests for Gram negative bacteria

(i) KOH test

A loopful each of the bacterial culture was put on a clear glass slide. One drop of three per cent KOH solution was placed over it and thoroughly mixed with the help of a needle. Bacterial chromosomes came out as thin threads, indicated gram negative bacteria.

(ii) Pigment production

The bacterial cultures were streaked on King's A medium incubated at room temperature for 48 h and pigmentation of the colony was observed. Bacterial cultures were streaked on King's B medium containing 0.1 per cent tyrosine and incubated at room temperature for 48 h and zone of fluorescent pigmentation around the colonies was observed.

(iii) Anaerobic growth

Nutrient Glucose Agar (containing 0.005 per cent Bromocresol purple) columns in test tubes were inoculated with each bacterial culture by stabbing with a sterile inoculation needle. The surface of medium was covered with sterile 1% agar to a depth of one cm. The tubes were incubated and observations on colour change of the medium was recorded at 48 h intervals for eight days.

(iv) Oxidase test

The 24 h old bacterial cultures were spot inoculated on oxidase disc and change in colour of the disc from white to purple or blue was observed.

(v) Levan formation from sucrose

The bacterial cultures were streaked on the sterilized peptone beef extract containing 5% sucrose and growth characters were observed after 48 h. Presence of large, white, domed and mucoid colonies characterized the production of levan from sucrose. (Hayward, 1964).

(vi) **Starch hydrolysis**

Nutrient agar containing 0.2 per cent soluble starch was used. The test cultures were spotted on the mediated Petriplates. Starch hydrolysis was tested after 48 h of incubation by flooding the agar surface with Lugol's iodine solution. A colourless zone around the bacterial growth in contrast to the blue back ground of the medium, indicated positive reaction.

(vii) **Lipase test**

Bacterial cultures were streaked on the Sierra's medium containing 10% Tween-80 and incubated for seven days. Dishes were examined daily for the presence of a dense precipitate around the bacterial growth which is indicative of lipid hydrolysis.

(viii) **Growth at 4° and 41° C**

The bacterial cultures were streaked on NA medium and incubated at 4°C as in 41°C and observed for its growth after 24 and 48 h.

(ix) **Denitrification test**

Bacterial cultures were stabbed into the VMS medium and sealed with three ml of 1% molten agar at 45°C and examined daily for the production of gas under the seal. (Hayward *et al.*, 1990).

(x) **Arginine dihydrolase reaction**

The bacterial cultures were stabbed into the semi-solid medium of Thornley (1960) and the tubes were sealed with three ml of 1% molten agar at 45°C. The tubes were incubated at 28°C for 7 days and any colour change, indicative of change in pH, under the agar seal was observed.

3.8.3.2 Biochemical tests for Gram positive bacteria

(i) **Catalase Test**

Smears of 24 h old bacterial cultures were prepared on clean glass slide and

covered with a few drops of three per cent hydrogen peroxide. Effervescence indicated the presence of catalase in the culture.

(ii) **Anaerobic growth**

Test was carried out as explained in 3.8.3.1 (iii).

(iii) **Voges-Proskaeur test**

48 h old cultures were inoculated into five ml of the medium dispersed in test tubes. After an incubation period of seven days, 0.6 ml of alpha naphthol solution (5 per cent in 95 per cent alcohol) and 0.2 ml of 40 per cent aqueous solution of KOH were added to one ml of the culture. The mixture was shaken for few minutes and allowed to stand for two hours. A crimson or ruby colour indicated positive VP test.

(iv) **Acid/gas from glucose**

Different cultures of the bacteria were inoculated into Hayward's semisolid basal medium and tubes were sealed with three ml of 1% molten agar at 45°C. The tubes were observed for the colour change and gas production under agar seal after 24 h, 48 h and seven days.

(v) **Growth in NaCl**

The cultures were inoculated into the tube containing Nutrient broth supplemented with three, five and 17 per cent concentrations of sodium chloride and observed daily for the growth for seven days.

(vi) **Growth at 4°C and 40°C**

The bacterial cultures were streaked on NA medium and incubated at 4°C and 40°C and observed for the growth after 24 h and 48 h

3.9 POT CULTURE STUDIES FOR THE EVALUATION OF POTENTIAL ANTAGONISTS AGAINST THE ISOLATES OF *R.solanacearum*

A pot culture experiment was conducted to find out the efficacy of antagonists against three Vellanikkara isolates of *R.solanacearum.*

Earthern pots of size 9" x 9" were filled with potting mixture containing soil collected from wilt sick plots, sand and cow dung at the ratio of 2:1:1. One part of the soil used was initially made sick by incorporating the bacterial ooze as well as the cut pieces of wilted plants and three isolates were incubated separately for two weeks. Further, to ensure the enough population of the pathogen, 30 ml of bacterial ooze suspension of different isolates were also poured separately into each pot. 30 day old seedlings were transplanted after adopting the different treatments and various methods of application as mentioned below. Observations were made on per cent disease incidence, days to wilt appearance and biometric characters like shoot length, root length , number of leaves, fresh weight, dry weight, days to flowering, days to harvesting and fruit weight/plant.

The susceptible varieties such as Pusa Purple Long, Pusa Jwala, Pusa Ruby of brinjal, chilli and tomato were used. The study was conducted in Completely Randomized Block Design (CRD) with three replications and 12 plants were kept for each treatment. The antagonists which were found most effective under *in vitro* studies were selected for the experiment. The antagonists were applied adopting different methods using concentrations of 10^6 spores/ml (fungi) and 10^8 cfu/ml (bacteria)

The methods are

i) Seed treatment (M_1): Seeds of Pusa Purple Long ,Pusa Jwala and Pusa Ruby were treated with potential antagonists and sown in nursery.

ii) Soil Drenching (M_2): 30 ml suspension of antagonists were prepared in sterile distilled water and poured into the pots 15 days before and 30 days after transplanting.

iii) Seed treatment + soil drenching (M_3): Combination of (i) and (ii) was followed.

iv) Root dipping (M_4): The seedlings were dipped in antagonistic suspensions for 2 h and then transplanted.

Details of treatments used in pot culture studies

Treatments	Antagonists used	Sources
T_1	*Trichoderma viride*	Ozhalapathy
T_2	*T.pseudokoningii*	Forest soil
T_3	*T.viride*	Vellanikkara
T_4	*T.virens*	Ozhalapathy
T_5	*Aspergillus niger*	Eruthyampathy
T_6	*A.viridi nutans*	Ozhalapathy
T_7	*Aspergillus sp*	Ozhalapathy
T_8	*Mucor sp*	Eruthyampathy
T_9	*Unidentified*	Vellanikkara
T_{10}	*Aspergillus sp*	Ozhalapathy
T_{11}	*Trichoderma sp*	Ozhalapathy
T_{12}	*Aspergillus sp*	Ozhalapathy
T_{13}	*Aspergillus sp*	Vellanikkara
T_{14}	*Aspergillus sp*	Vellanikkara
T_{15}	*Trichoderma sp*	Forest Soil
T_{16}	*Pseudomonas aeruginosa*	Ozhalapathy
T_{17}	*Bacillus subtilis*	Vellanikkara
T_{18}	*B. cereus*	Vellanikkara
T_{19}	*B. polymyxa*	Ozhalapathy
T_{20}	Unidentified	Vellanikkara
T_{21}	*Avirulent R. solanacearum*	Vellanikkara
T_{22}	*Mutant of R. solanacearum*	Vellanikkara
T_{23}	*T.viride*	Commercial
T_{24}	*A. niger*	Commercial

T_{25}	*P fluorescens*	Commercial
T_{26}	control	

3.10 EVALUATION OF PROMISING ANTAGONISTS AGAINST *R.solanacearum* UNDER FIELD CONDITION

In order to find out the effective antagonists in reducing the severity of bacterial wilt, under natural condition, a field experiment was laid out in the wilt sick plots of Department of Olericulture, Vellanikkara.

Crop	Variety	Spacing
Brinjal	Pusa Purple Long	60 x 75 cm
Chilli	Pusa Jwala	45 x 45 cm
Tomato	Pusa Ruby	60 x 60 cm

Design: RBD

Plot Size: 3 x 3 m

Replication : 2

Treatment : 11

The antagonists which were found effective against all the three isolates of *R. solanacearum* in pot culture studies were selected for the field studies and the various antagonists used are listed below.

Treatments	Antagonists used	Source
T_1	*T. viride*	Ozhalapathy
T_2	*T.pseudokoningii*	Forest soil
T_3	*T.viride*	Vellanikkara

T_4	*A.niger*	Eruthyampathy
T_5	*P. aeruginosa*	Ozhalapathy
T_6	*B. subtilis*	Vellanikkara
T_7	*T.viride*	Commercial
T_8	*A. niger*	Commercial
T_9	*P.fluorescens*	Commercial
T_{10}	*B. subtilis*	Commercial
T_{11}	Control	

The field was prepared thoroughly and ridges and furrows were taken as per the spacing mentioned above. 30 day old seedlings were transplanted with 25 plants per treatment in tomato, 20 in brinjal and 36 in chilli. The crops received cultural and manurial practices as recommended by package of practices, KAU (1996). The treatments were followed as per the details mentioned earlier. Antagonists were applied by seed treatment + soil drenching and root dipping methods. Observations on per cent wilt incidence was noted at fortnightly intervals. Biometric observations of the seedlings, yield data and enumeration of rhizosphere microflora at 30, 60 and 90 DAP were also recorded.

Similarly, another field experiment was also conducted using a moderately resistant variety of tomato, Mukthi ,to study the integrated effect in the management of bacterial wilt pathogen. Observations on per cent wilt incidence, biometric characters of the seedlings and yield were recorded. Wilt severity was also recorded using 0-5 scale score chart and the wilt index was calculated using the following formula. (Winstead and Kelman ,1952)

$$\text{Per cent Bacterial Wilt Index} = \frac{\text{Sum of all readings per plot}}{\text{Number of plants per plot} \times 5} \times 100$$

Results

4. RESULTS

Investigation on the effect of various microbial antagonists against the bacterial wilt pathogen, *R. solanacearum* of brinjal, chilli and tomato was carried out under laboratory and natural conditions and the results are presented below.

4.1. PATHOGENICITY TEST

Inoculation of bacterial ooze obtained from the infected plants of brinjal, chilli and tomato on the respective hosts and other two solanaceous hosts produced wilt incidence within seven days. The pathogen was reisolated from all the wilted plants. Pathogenicity of the pathogen was thus established.

4.2. ENUMERATION OF *R. solanacearum* POPULATION IN THE SOILS OF DIFFERENT LOCATIONS

R. solanacearum populations in Vellanikkara and Ozhalapathy soils were estimated and it is observed that, among the two locations, Vellanikkara (high wilt incidence area) recorded for the maximum population with 1.8 – 6.3 x 10^7 cfu/g soil whereas in Ozhalapathy (low wilt incidence area) the population was low ranging from 1.01 – 3.6 x 10^7 cfu/g soil.

4.3. DETERMINATION OF SOIL pH AND SOIL TEMPERATURE IN DIFFERENT LOCATIONS

Variation in soil type was observed in two locations and also within locations based on up land and low land. In Vellanikkara, the main soil type was laterite loam where as low land had clayey type soil. Likewise, in Ozhalapathy, main soil type was black soil and in lowland, it was black clayey soil.

It is also observed that, there is a wide variation in the soil pH of the two locations. Even within the location also, pH varied in upland and lowland soils with a range of 6.5 – 6.9 and 6.1 – 6.4 respectively, indicating the acidic nature of soil. Whereas in Ozhalapathy, soil pH was high and varied from 7.4-7.7 in upland showing near alkaline nature of soil. However, the pH was slightly less in low land recording 6.0 – 6.6. It is also worthwhile to mention that, the disease was observed only in these low lands of Ozhalapathy.

With regard to the soil temperature, wide variation was observed in the two locations. The soil temperature was found to be high in Vellanikkara as compared to Ozhalapathy recording 44.4 – 45.6°C and 26.6 - 27.7°C respectively.

4.4. ISOLATION OF MICROFLORA FROM THE RHIZOSPHERE SOILS OF DIFFERENENT LOCATIONS

The microorganisms *viz* fungi, bacteria and actinomycetes were isolated from rhizosphere of the three solanaceous hosts of the two locations and the microbial population was estimated and presented in Table 1. It is observed that, among the two locations, the total microbial population was high in Ozhalapathy than Vellanikkara. Among the different microflora, fungi and actinomycetes were more predominant in Ozhalapathy recording 169 – 176 x 10^4 cfu/g and 79-133.6x10^5cfu/g soil respectively whereas in Vellanikkara, bacterial population was high, ranging from 39-103 x10^5 cfu/g soil. With respect to the crop, tomato crop harboured the maximum population of fungi whereas maximum population of actinomycetes was recorded in brinjal. With regard to bacterial population, maximum population was observed with chilli crop (103 x 10^5 cfu/g soil), that also in a resistant variety. As far as the varieties of the crops are considered, it is worthwhile to mention that, resistant varieties of the three crops recorded the better association of microflora than the susceptible ones. Additional collections of soil from solarised beds showed good population of bacteria (10 x 10^5cfu/g soil) and actinomycetes

Table 1: Quantitative estimation of rhizosphere microflora isolated from different hosts of Vellanikkara and Ozhalapathy areas

Sl.No.	Soil Samples	Fungi 10^4 cfu/g	Bacteria 10^5 cfu/g	Actinomycetes 10^5 cfu/g
I	**Vellanikkara**			
1.	**Brinjal**			
	Pusa Purple Long (SV)	10	2	2
	Swetha (RV)	8	24	21
	Surya (RV)	10	16	5
	Haritha (RV)	15	20	29
2.	**Chilli**			
	Pusa Jwala (SV)	4	7	38
	CA 578 (SV)	1	39	90
	Ujwala (RV)	9	25	18
	CA 572 (RV)	8	103	8
3.	**Tomato**			
	Pusa Ruby (SV)	14	7	15
	Sakthi(RV)	11	10	18
	Mukthi (RV)	36	11	29
	LE 415 (RV)	13	17	57
II	**Ozhalapathy**			
1.	**Brinjal**			
	Local variety	92.6	13.6	133.6
	Local variety	16	4	4
2.	**Chilli**			
	Local variety	8	2.3	79
3.	**Tomato**			
	Hybrid	169	32	16
	Local variety	176	1	5
III	**Vellanikkara**			
	Solarised soil	6	10	22
IV	**Nilambur**			
	Forest soil	37	10	6

RV-Resistant variety SV-Susceptible variety
Mean of three replications

(22 x 10^5 cfu/g) soil whereas soil from forest area showed more fungal population (37 x 10^5 cfu/g soil).Plate. 2

4.5. *IN VITRO* EVALUATION OF ANTAGONISTS AGAINST THE ISOLATES OF *R.solanacearum*.

4.5.1. *In vitro* evaluation of fungal antagonists against the isolates of *R.solanacearum*.

Fungi isolated from the rhizosphere soils of solanaceous hosts, solarised beds and forest areas were tested against different isolates of *R .solanacearum* for their antagonistic property (Plate.3).

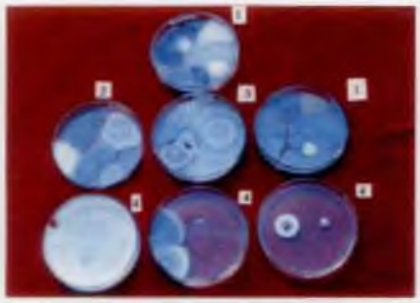
Among the 90 fungi screened against the six isolates of *R.solanacearum*, 23 fungi were found to exhibit antagonistic reactions to one or more isolates *of R.solanacearum*. The result of the experiment is presented in Table 2. It is found that, two fungal isolates, *Trichoderma viride* (F 30) isolated from chilli rhizosphere soil (Ozhalapathy) and *T. pseudokoningii* (F 143) isolated from forest soil were most effective against all six pathogens tested. They inhibited the pathogen completely, by the mechanism of either lysis and over growth or overgrowth alone recording the maximum Antagonism Index (AI) value of 6000.

Another *T.viride* (F 29) isolated from chilli plants (Vellanikkara) and *T.virens* (F 10) from tomato (Ozhalapathy) were also equally effective, which showed lysis and overgrowth type of reaction towards the pathogen and inhibited the growth of the pathogen to the higher extent recording an AI value of 1500-6000. In addition to these, *T.harzianum* (F140) isolated from tomato plants (Eruthyampathy) and *Trichoderma* sp. (F144) isolated from forest soil also showed AI value of 1500-4500 and 1500-3000 respectively and were found to suppress the pathogen's growth by their lysis and overgrowth antagonistic activity.

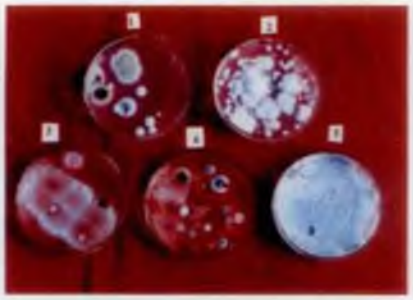
Plate 2

Microflora from the rhizosphere soil of different hosts

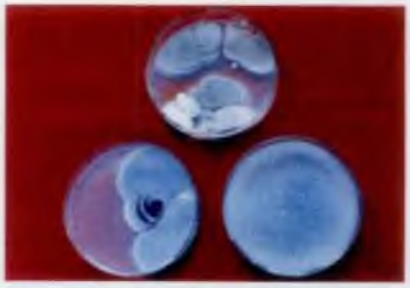
Fungi



1, 2 & 3. Brrinjal Vellanikkara (RV)
4. Chilli Vellanikkara (SV)

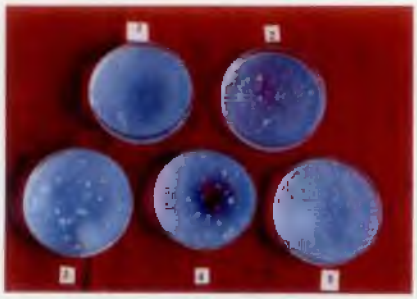


1. Brinjal Vellanikkara (SV)
2. Brinjal Ozhalapathy (LV)
3. Chilli Vellanikkara (SV)
4. Chilli Vellanikkara (RV)
5. Chilli Ozhalapathy (RV)

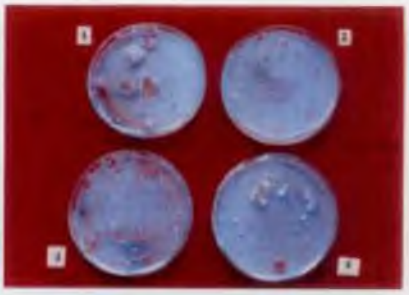


Solarised soil

Bacteria

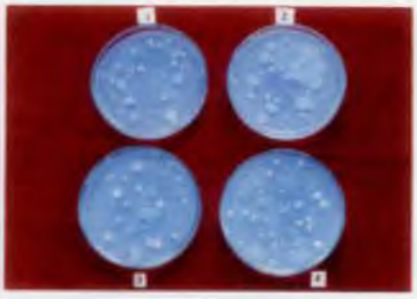


1. Tomato Vellanikkara (SV)
2 & 3. Tomato Vellanikkara (RV)
4. Brinjal Ozhalapathy (LV)
5. Solarised soil

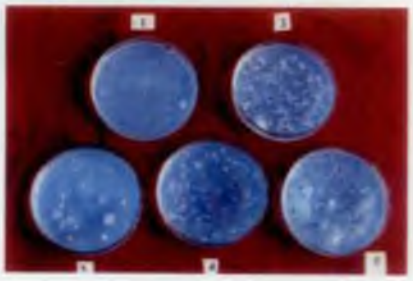


1. Brinjal Vellanikkara (SV)
2. Brinjal Ozhalapathy (LV)
3. Chilli Vellanikkara (SV)
4. Chilli Vellanikkara (RV)
5. Chilli Ozhalapathy (LV)

Actinomycetes



1. Chilli Vellanikkara (SV)
2. Solarised soil
3. Brinjal Vellanikkara (RV)
4. Brinjal Vellanikkara (RV)

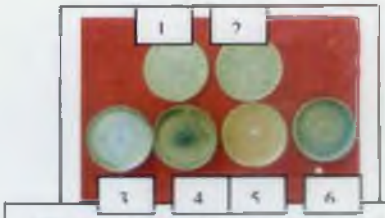


1. Brinjal Vellanikkara (SV)
2. Brinjal Ozhalapathy (LV)
3. Chilli Vellanikkara (SV)
4. Chilli Vellanikkara (RV)
5. Chilli Ozhalapathy (LV)

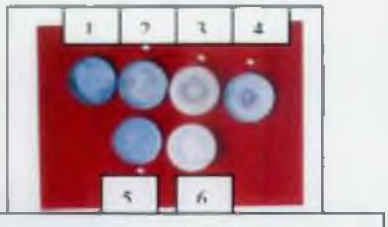
Plate. 3

In vitro evaluation of different fungal isolates against *R. solanacearum*

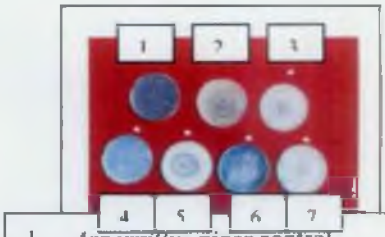
Trichoderma viride 1. control 2. on tomato Ozhalapathy 3. on chilli Ozhalapathy 4. on brinjal Ozhalapathy 5. on tomato Vellanikkara 6. on chilli Vellanikkara 7. on brinjal Vellanikkara



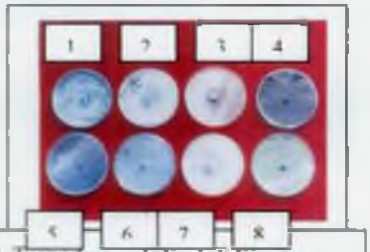
1.F32 on chilli Ozhalapathy 2. F32 control 3. *T. virens* on tomato Vellanikkara 4. . *T. virens* on chilli Vellanikkara 5. *T. virens* on tomato Ozhalapathy 6. *T. Virens* control



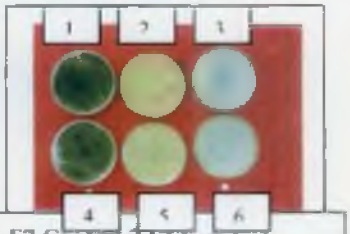
1.*Trichoderma viride* control
2.*T. viride* on chilli Ozhalapathy
3. *T. viride* on tomato Ozhalapathy
4. *T. viride* on chilli Vellanikkara
5. *Aspergillus viridi-nutans* control
6.*A. viridi- nutans* on tomato Ozhalapathy



1. *Aspergillus niger* control
2. *A. niger* on chilli Ozhalapathy
3. *A. niger* on chilli Vellanikkara
4. *Trichoderma viride* control
5. *T. viride on* chilli Ozhalapathy
6. *T. virens* control
7. *T. virens* on chilli Vellanikkara



1.*T.viride* on chilli 2.*T. Virens* on chilli 3. F17 on brinjal 4.F2 on chilli 5. F19 on chilli 6.F4 on chilli 7.*T. Viride* on chilli 8.F13 on chilli (Vellanikkara isolates)



1.F7 Control 2.F13 control 3. F33 control 4. F7 on chilli Ozhalapathy 5. F13 on chilli Vellanikkara
6. F33 on chilli Vellanikkara

Table 2. *In vitro* evaluation of fungal antagonists against the isolates of *R. solanacearum*

Sl. No.	Isolate No.	Location	Host	*R. Solanacearum* isolate	Antagonism index	Mechanism of antagonism	Fungus identified
1	F 32	Vellanikkara	Brinjal (SV)	Vellanikkara Chilli	 1500	 Overgrowth	*Aspergillus* sp.
2	F 33	Vellanikkara	Brinjal (SV)	Vellanikkara Chilli	 1500	 Overgrowth	*Aspergillus* sp.
3	F 47	Vellanikkara	Brinjal (RV)	Vellanikkara Brinjal Chilli Tomato Ozhalapathy Chilli	1500 1500 1500 1500	Overgrowth Overgrowth Overgrowth Overgrowth	Unidentified
4	F 58	Vellanikkara	Brinjal (RV)	Vellanikkara Chilli Ozhalapathy Brinjal Tomato	1500 1500 266.7	Overgrowth Overgrowth Overgrowth	*Aspergillus niger*
5	F 29	Vellanikkara	Chilli (SV)	Vellanikkara Chilli Tomato Ozhalapathy Chilli Tomato	6000 1500 1432.5 6000	Lysis and overgrowth Overgrowth Overgrowth Lysis and overgrowth	*Trichoderma viride*
6	F 96	Vellanikkara	Chilli (SV)	Vellanikkara Brinjal Chilli	 1500 1500	 Overgrowth Overgrowth	*Trichoderma* sp.
7	F 102	Vellanikkara	Chilli (RV)	Vellanikkara Chilli Ozhalapathy Brinjal Chilli Tomato	1500 3000 1500 800	Overgrowth Lysis and overgrowth Overgrowth Overgrowth	*Aspergillus niger*
8	F 105	Vellanikkara	Chilli (RV)	Vellanikkara Brinjal Chilli Tomato	 800 755 711	 Overgrowth Overgrowth Overgrowth	*Aspergillus flavus*
9	F 106	Vellanikkara	Chilli (RV)	Vellanikkara Brinjal Chilli Tomato	 264 999 264	 Overgrowth Overgrowth Overgrowth	*Aspergillus* sp.
10	F 72	Vellanikkara	Tomato (RV)	Vellanikkara Brinjal Chilli Tomato Ozhalapathy Chilli	 1500 1500 1500 1500	 Overgrowth Overgrowth Overgrowth Overgrowth	*Rhizopus* sp.

11	F 40	Vellanikkara	Solarized soil	Vellanikkara Brinjal Chilli Tomato Ozhalapathy Chilli	 1500 1500 1500 1500	 Overgrowth Overgrowth Overgrowth Overgrowth	*Aspergillus* sp.
12	F 50	Vellanikkara	Solarized soil	Vellanikkara Chilli Ozhalapathy Brinjal Chilli Tomato	 1500 1500 800 800	 Overgrowth Overgrowth Overgrowth Overgrowth	*Aspergillus* sp.
13	F 51	Vellanikkara	Solarized soil	Vellanikkara Chilli Ozhalapathy Brinjal Chilli Tomato	 1500 266.7 1500 400	 Overgrowth Overgrowth Overgrowth Overgrowth	*Aspergillus* sp.
14	F 133	Mannuthy	Brinjal (SV)	Vellanikkara Brinjal	 799.2	 Ovegrowth	*Aspergillus* sp.
15	F 30	Ozhalapathy	Chilli (LV)	Vellanikkara Brinjal Chilli Tomato Ozhalapathy Brinjal Chilli Tomato	 6000 6000 1500 6000 1500 6000	 Lysis and overgrowth Lysis and overgrowth Overgrowth Lysis and overgrowth Overgrowth Lysis and overgrowth	*Trichoderma viride*
16	F2	Ozhalapathy	Tomato (LV)	Vellanikkara Chilli Ozhalapathy Chilli	 1500 1500	 Overgrowth Overgrowth	*Aspergillus* sp.
17	F3	Ozhalapathy	Tomato (LV)	Ozhalapathy Chilli	 6000	Lysis and overgrowth	*Trichoderma viride*
18	F4	Ozhalapathy	Tomato (LV)	Ozhalapathy Tomato	 6000	Lysis and overgrowth	*Aspergillus viridi-nutans*
19	F7	Ozhalapathy	Tomato (LV)	Ozhalapathy Chilli	 6000	Lysis and overgrowth	*Aspergillus sp.*
20	F10	Ozhalapathy	Tomato (LV)	Vellanikkara Chilli Tomato Ozhalapathy Tomato	 1500 6000 6000	 Overgrowth Lysis and overgrowth Lysis and overgrowth	*Trichoderma virens*
21	F13	Ozhalapathy	Tomato (LV)	Vellanikkara Chilli	 1500	 Overgrowth	*Aspergillus* sp.
22	F 140	Eruthyampathy	Tomato (RV)	Vellanikkara Brinjal Chilli Ozhalapathy Brinjal Chilli	 1500 1500 4500 1500	 Overgrowth Overgrowth Lysis and overgrowth Overgrowth	*Trichoderma harzianum*

23	F 141	Eruthyampathy	Tomato (RV)	Vellanikkara Brinjal Tomato Ozhalapathy Brinjal Chilli Tomato	 1500 1500 3000 1500 1500	 Overgrowth Overgrowth Lysis and overgrowth Overgrowth Overgrowth	*Aspergillus niger*
24	F 142	Eruthyampathy	Tomato (RV)	Vellanikkara Brinjal Ozhalapathy Chilli Tomato	 1500 1500 1500	 Overgrowth Overgrowth Overgrowth	*Mucor* sp.
25	F 143	Nilambur	Forest soil	Vellanikkara Brinjal Chilli Tomato Ozhalapathy Brinjal Chilli Tomato	 1500 6000 6000 6000 1500 1500	 Overgrowth Lysis and overgrowth Lysis and overgrowth Lysis and overgrowth Overgrowth Overgrowth	*Trichoderma pseudokoningii*
26	F 144	Nilambur	Forest soil	Vellanikkara Chilli Ozhalapathy Brinjal Chilli Tomato	 1500 3000 1500 1500	 Overgrowth Lysis and overgrowth Overgrowth Overgrowth	*Trichoderma* sp.
27	F 145	Varanasi	solarised	Vellanikkara Brinjal Chilli Tomato Ozhalapathy Brinjal Chilli Tomato	 1500 1500 1500 1500 1500 1500	 Overgrowth Overgrowth Overgrowth Overgrowth Overgrowth Overgrowth	*Aspergillus niger*
28	F 146	IARI N.Delhi	Commercial	Vellanikkara Brinjal Chilli Tomato Ozhalapathy Brinjal Chilli Tomato	 1500 1500 1500 1500 1500 3000	 Overgrowth Overgrowth Overgrowth Overgrowth Overgrowth Lysis and Overgrowth	*Aspergillus niger*
29	F147	IISR Calicut	Commercial	Vellanikkara Brinjal Chilli Tomato Ozhalapathy Tomato Chilli	 1500 6000 6000 3000 3000	 Overgrowth Lysis and Overgrowth Lysis and Overgrowth Lysis and Overgrowth Lysis and Overgrowth	*Trichoderma harzianum*

30	F148	Biocontrol Lab, Thrissur	commercial	Vellanikkara Brinjal Chilli Tomato Ozhalapathy Tomato	 1500 6000 6000 4500	 Overgrowth Lysis and Overgrowth Lysis and Overgrowth Lysis and Overgrowth	*Trichoderma viride*
31	F149	*Trichoderma viride X Trichoderma pseudokoningii*	Interspecific hybrid species	Vellanikkara Brinjal Ozhalapathy Brinjal	 1500 1500	 Overgrowth Overgrowth	-
32	F150	*Trichoderma viride* (F 30)	mutant	Vellanikkara Brinjal Chilli	 6000 6000	 Lysis and overgrowth *Lysis and overgrowth*	-
33	F151	*T.pseudokoningii (F 143)*	mutant	Vellanikkara Chilli	 6000	 Lysis and overgrowth	-
34	F152	*T. harzianum* (commercial)	mutant	Vellanikkara Chilli	 1500	 *Overgrowth*	-
35	F153	*Interspecific hybrid T. viride x T. pseudokoningii*	Inter specific hybrid mutant	Vellanikkara Brinjal Chilli	 1500 1500	 Overgrowth *Overgrowth*	-

RV - Resistant variety SV - Susceptible variety LV - Local variety

Mean of three replications

Apart from *Trichoderma* spp., many *Aspergillus* spp. were found effective against different isolates of *R.solanacearum*. Majority of them showed overgrowth type of antagonism and a few with lysis and overgrowth mechanism.Among the *Aspergillus* spp., *A.niger* (F 141) isolated from the tomato plants (Eruthyampathy) was most effective against all isolates of *R.solanacearum* except chilli isolate of Vellanikkara. its main mechanism on the pathogen was complete overgrowth and however lysis and overgrowth type was observed on brinjal isolate of Ozhalapathy. It is also observed that, various other species of *Aspergillus* (F 40, F 50 and F 5 1) isolated from the solarised beds and *A.niger*(F 58 and F 102) from solanaceous hosts also showed antagonistic property against various isolates of the test organism. *A.viridi-nutans* isolated from tomato plants (Ozhalapathy) showed both lysis and overgrowth reaction against the tomato isolate of Ozhalapathy with the AI value of 6000. It is clear from the above facts that, fungi isolated from low wilt incidence area showed better antagonism with AI value ranging from 1500 – 6000.

On comparison with commercial antagonists, both *T.viride* and *T.harzianum* were found effective against four to five isolates of *R.solanacearum* with a good antagonistic property of both lysis and overgrowth and recording an AI value of 3000-6000. *A. niger* (AN-27) and *A.niger* (Varanasi) were also equally effective and showed overgrowth on all six isolates of the pathogen. On comparison with the above four commercial antagonists *Trichoderma* sp., were more effective than *A.niger* as it exhibited better antagonistic property with highest AI value (6000).

From the test conducted with an interspecific hybrid of *T.viride* x *T.pseudokoningii* it was also observed that, even though the interspecific hybrid inhibited two isolates of *R. solanacearum* with its overgrowth mechanism, but were not effective than the individual organisms in its antagonistic property as evident from the AI value of 1500 against the AI value of 6000. Likewise, the mutants of these *T.viride, T.pseudokoningii, T.harzianum* and interspecific hybrids also showed good antagonism against bacterial wilt pathogen, but were not superior than the original ones.

4.5.2. *In vitro* evaluation of bacterial antagonists against the isolates of *R.solanacearum*

Bacteria isolated from different rhizosphere soils were evaluated against three Vellanikkara isolates of *R.solanacearum* (Plate. 4a).

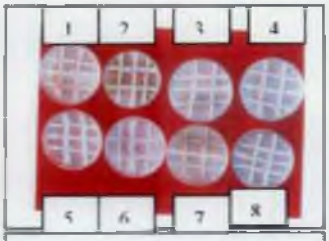
Out of 87 bacterial isolates evaluated, nine isolates showed antagonistic reaction by exhibiting the lysis of the test pathogen (Table 3). It is found that, maximum AI value (133.3) was recorded with *P.aeruginosa* (B 4) isolated from tomato plants collected from Ozhalapathy. *B.subtilis* (B 124) isolated from resistant brinjal genotypes (Vellanikkara) showed an AI value of 88.8 and 53.2 AI value on brinjal and chilli isolates respectively. In addition, *B.cereus* (B 125) and *P.fluorescens*(B 30) were also found to exhibit lytic activity on brinjal and chilli isolates of *R.solanacearum*. Other bacterial organisms were less effective as evident from their AI value (19.8 to 4.4). It is also noted that, bacteria isolated from the resistant variety of brinjal of Vellanikkara showed better antagonistic reaction as compared to other hosts. However, commercial antagonist, *P.fluorescens*, was also equally effective as isolated *B.subtilis* and inhibited the growth of the pathogens to the highest extent, recording an AI value of 111.1 against chilli isolate only. Commercial culture of *B.subtilis* also indicated good antagonistic property against all test isolates of *R.solanacearum* as evident by the AI value of 54.4 and 40.0 on brinjal and chilli isolates respectively.

The data also showed that, an avirulent form of *R.solanacearum* isolated from tomato was also effective against chilli by recording an AI value of 54.4. Whereas, mutant of *R.solanacearum* was found to be less effective on the pathogen as evident from the AI value of 17.7 on tomato and 1.11 on brinjal isolates. It is also observed that, the attenuated (heat killed) *R.solanacearum* showed no antagonistic property against the test pathogens.

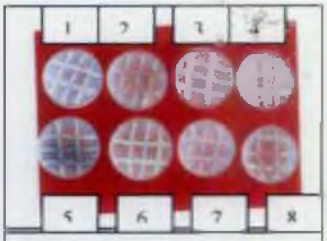
Plate. 4a

In vitro evaluation of bacteria & actinomycetes against *R. solanacearum*

Bacteria :Cross streaking method



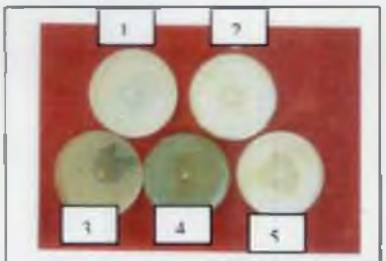
1. B14, 2. B13 , 3. B12, 4. B9, .5. B8, 6. B7, 7. B6, 8. B5 on Ozhalapathy brinjal , chilli & tomato isolates



1. B2, 2. B4 , 3. B7, 4. B8, .5. B9, 6. B12, 7. B13, 8. B14 on Vellanikkara brinjal , chilli & tomato isolates

Point Inoculation method

Bacteria



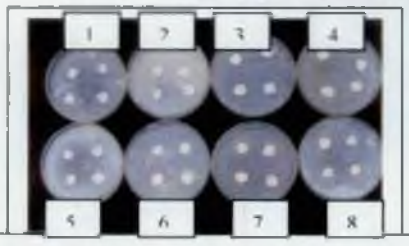
1. B19 on chilli 2. B4 on chilli 3. B125 on brinjal B124 on 4. brinjal 5. chilli

Actinomycete

A 25 against chilli isolate

Plate. 4b

In vitro evaluation of culture filtrates against *R. solanacearum* isolates



Bacillus subtilis on 1. Tomato Ozhalapathy 2. Chilli Ozhalapathy
B124 on 3. Chilli Ozhalapathy 4. Tomato Ozhalapathy
Pseudomonas fluorescens on 5. Chilli Vellanikkara 6. Tomato Ozhalapathy 7. Tomato Vellanikkara
8. B4 on Tomato Ozhalapathy

T.viride on 1,2&3. Tomato Ozhalapathy 4. Brinjal Ozhalapathy

Table 3. ***In vitro*** **evaluation of bacterial antagonists against the isolates of** ***R. solanacearum***

Sl. No.	Isolate No.	Location	Host	*R. solanacearum* isolate	Antagonism Index	Mechanism of antagonism
1	B 29	Vellanikkara	Brinjal (RV)	Vellanikkara Chilli	8.8	Lysis
2	B 30	Vellanikkara	Brinjal (RV)	Vellanikkara Brinjal Chilli	19.8 26.4	Lysis Lysis
3	B 32	Vellanikkara	Brinjal (RV)	Vellanikkara Chilli	8.8	Lysis
4	B124	Vellanikkara	Brinjal (RV)	Vellanikkara Brinjal Chilli	53.2 88.8	Lysis Lysis
5	B125	Vellanikkara	Brinjal (RV)	Vellanikkara Brinjal Chilli	44.4 33.3	Lysis Lysis
6	B 69	Vellanikkara	Chilli (RV)	Vellanikkara Tomato	8.8	Lysis
7	B 28	Vellanikkara	Solarized soil	Vellanikkara Brinjal	19.8	Lysis
8	B 19	Ozhalapathy	Chilli (LV)	Vellanikkara Chilli	88.8	Lysis
9	B 4	Ozhalapathy	Tomato(LV)	Vellanikkara Chilli	133.3	Lysis
10	B126	Biocontrol lab, Thrissur	commercial *Pseudomonas fluorescens*	Vellanikkara Brinjal Chilli Tomato	1.11 111.1 4.4	Lysis Lysis Lysis
11	B127	TNAU Coimbatore	commercial *Bacillus subtilis*	Vellanikkara Brinjal Chilli Tomato	54.4 40.0 1.11	Lysis Lysis Lysis
12	B128	Vellanikkara	Tomato (Mutant)	Vellanikkara Brinjal Tomato	1.11 17.7	Lysis Lysis
13	B129	Vellanikkara	Tomato (Avirulent)	Vellanikkara Chilli Tomato	54.4 4.44	Lysis Lysis

RV - Resistant variety; SV - Susceptibile variety; LV - Local variety

Mean of three replications

4.5.3. *In vitro* evaluation of actinomycete antagonists against Vellanikkara isolates of *R.solanacearum*

From a total of 57 actinomycetes screened, only four isolates of actinomycetes were found to show antagonistic activity against *R.solanacearum*. From the data presented in Table 4 it is found that, maximum AI value (283) was observed with the actinomycete isolated from brinjal rhizosphere soil of Ozhalapathy followed by the actinomycete isolated from chilli plants of Vellanikkara which showed an AI value of 66.6 on the same isolate. The actinomycete (A 34) isolated from solarised soil also showed inhibition of the pathogen with AI value of 39.6. It is also noted that, all these three actinomycetes showed antagonistic reaction only on the chilli isolate of the pathogen and exhibited lytic type of antagonism (Plate.4a).

4.5.4. *In vitro* evaluation of culture filtrates of antagonists against the isolates of *R.solanacearum*

Effect of culture filtrates of the antagonists against six different isolates of *R.solanacearum* was also studied and the results are furnished in Table 5.From the table, it is clear that, culture filtrates obtained from all the antagonists showed inhibition on one or more isolates of *R.solanacearum*. Among the bacterial filtrates tested, commercial *B.subtilis* showed maximum inhibition zone of 11.5 and 9.5 mm on tomato isolates of the pathogen collected from Vellanikkara and Ozhalapathy. *B.subtilis* (B 124) isolated from a resistant variety of brinjal (Vellanikkara) recorded 8 mm and 4.25 mm inhibition zone on tomato and chilli isolates of Ozhalapathy pathogen.Filtrates of *Pseudomonads* exhibited less inhibitory effect as compared to *Bacillus* sp. Commercial *P.fluorescens* showed a maximum inhibition zone of 3.75 mm on tomato isolate of Vellanikkara while *P.aeruginosa* recorded only 2.25 mm inhibition on tomato isolate of Ozhalapathy.

Table 4. *In vitro* evaluation of actinomycetes against the isolates of *R. solanacearum*

Sl. No.	Isolate No.	Location	Host	*R. solanacearum* isolate	Antagonism index	Mechanism of antagonism
1	A 38	Vellanikkara	Chilli (RV)	Vellanikkara Chilli	66.6	Lysis
2	A 40	Vellanikkara	Chilli (RV)	Vellanikkara Chilli	17.6	Lysis
3	A 34	Vellanikkara	Solarized soil	Vellanikkara Chilli	39.6	Lysis
4	A 25	Ozhalapathy	Brinjal(LV)	Vellanikkara Chilli	283	Lysis

RV - Resistant variety SV - Susceptible variety LV - Local variety
Mean of three replications

Table 5. ***In vitro*** **evaluation of culture filtrates of selected antagonists against the isolates of** ***R. solanacearum***

Isolate No.	Antagonists Used	Inhibition zone (mm)					
		Vellanikkara isolates			Ozhalapathy isolates		
		Brinjal	Chilli	Tomato	Brinjal	Chilli	Tomato
Bacteria							
B4	*Pseudomonas aeruginosa.*	0.25	0	0.25	0	0	2.25
B124	*Bacillus subtilis.*	0.75	0	0	0.75	4.25	8.00
Commercial	*Pseudomonas fluorescens*	0	0.25	3.75	0	0	7.50
Commercial	*Bacillus subtilis*	0	1.5	11.5	0.25	0.25	9.50
Fungi							
F10	*Trichoderma virens*	0	0	5.25	0	0	2
F 29	*Trichoderma viride*	0.5	0	0	0	1.25	1.75
F 30	*Trichoderma viride*	0	0.5	1.25	3.25	1.0	3
F 140	*Trichoderma* sp.	0	0	0.5	0	1.0	1.0
F 143	*Trichoderma pseudokoningii*	1.5	0	4.25	0	1.0	1.25
Commercial	*Trichoderma harzianum*	0	0	1.0	0	1.5	1.0
Commercial	*Trichoderma viride*	0	0	0.25	0	0	4.75
Commercial	*Aspergillus niger* (AN 27)	0	0	1.25	0	1.0	2.25
Control		0	0	0	0	0	0

Mean of three replications

Among the different fungal cultures tested, culture filtrates of *T.virens*, *T.pseudokoningii* and *T.viride* (Ozhalapathy) showed good inhibitory effect on both Vellanikkara and Ozhalapathy isolates of the pathogen. Eventhough, the maximum inhibition zone of 5.25 mm and 4.25 mm were observed with *T.virens* and *T.pseudokoningii*, they were found effective only against the tomato pathogen. Whereas, *T.viride* was found to inhibit all the isolates of *R.solanacearum* except the brinjal isolate of Vellanikkara, but showed poor inhibition zone, ranging from 0.5 to 3.25 mm. It is also noted that, the filtrates of commercial cultures of *T. viride*, *T.harzianum* and *A.niger* also showed some inhibitory effect on the bacteria mainly on tomato isolate of the pathogen. However, the culture filtrates of the isolated fungi were more effective than the commercial ones (Plate 4b).

4.6. IDENTIFICATION OF ANTAGONISTS

4.6.1 Identification of fungal antagonists

Nine fungal antagonists which where found effective against *R.solanacearum* were identified based on the following cultural and morphological characters which are listed below.

1. Isolate No. F30, F 29, F 3

Cultural characters

Colonies : Fast growing, smooth surface, become hairy and colour change from whitish green to dark green.

Morphological characters

Mycelium : Hyaline, smooth, septate, branched and of 2-4 μm wide.

Conidiophores : Long and slender without sterile hyphae , side branches long, arise in compact tuft and all branches stand at wide angle.

Phialides : Slender, not crowded, 8-12 μm long, arise in groups of more

than 2-3 number. They are curved, pin shaped, narrower at the base widening above the middle and attenuated into long neck.

Phialospores : Globose, rough walled and of the size 2-4 μm.

Based on the above characters the isolates are identified as ***Trichoderma viride*** (Plate.5d & Fig. 2)

2. Isolate No. F-143

Cultural characters

Colonies : Grow fairly rapidly with very poor aerial growth. Conidial areas change in colour from white to greenish white to bright green. Pigments were secreted into the medium so that, the reverse of the medium was yellowish.

Morphological characters

Mycelium : Septate, smooth, colourless and 2-3 μm wide.

Conidiophores : Long ,slender, without sterile hyphae, side branches long,branching more complicated, loosely tuft and appear hairy at maturity.

Phialides : Pin shaped, narrower at base than middle attenuated distinctly, spindle shaped 8-10 μm in size, false whorals.

Phialospores : Short, smooth, mostly oblong or ellipsoidal with a size of 2.8-4.8μm.

Based on the above characters the fungus is identified as ***T. pseudokoningii*** (Plate.5c & Fig. 3)

3. Isolate No. F-140

Cultural characters

Colonies : Grow rapidly, white green to bright green and dull green.

Fig 2. Trichoderma viride

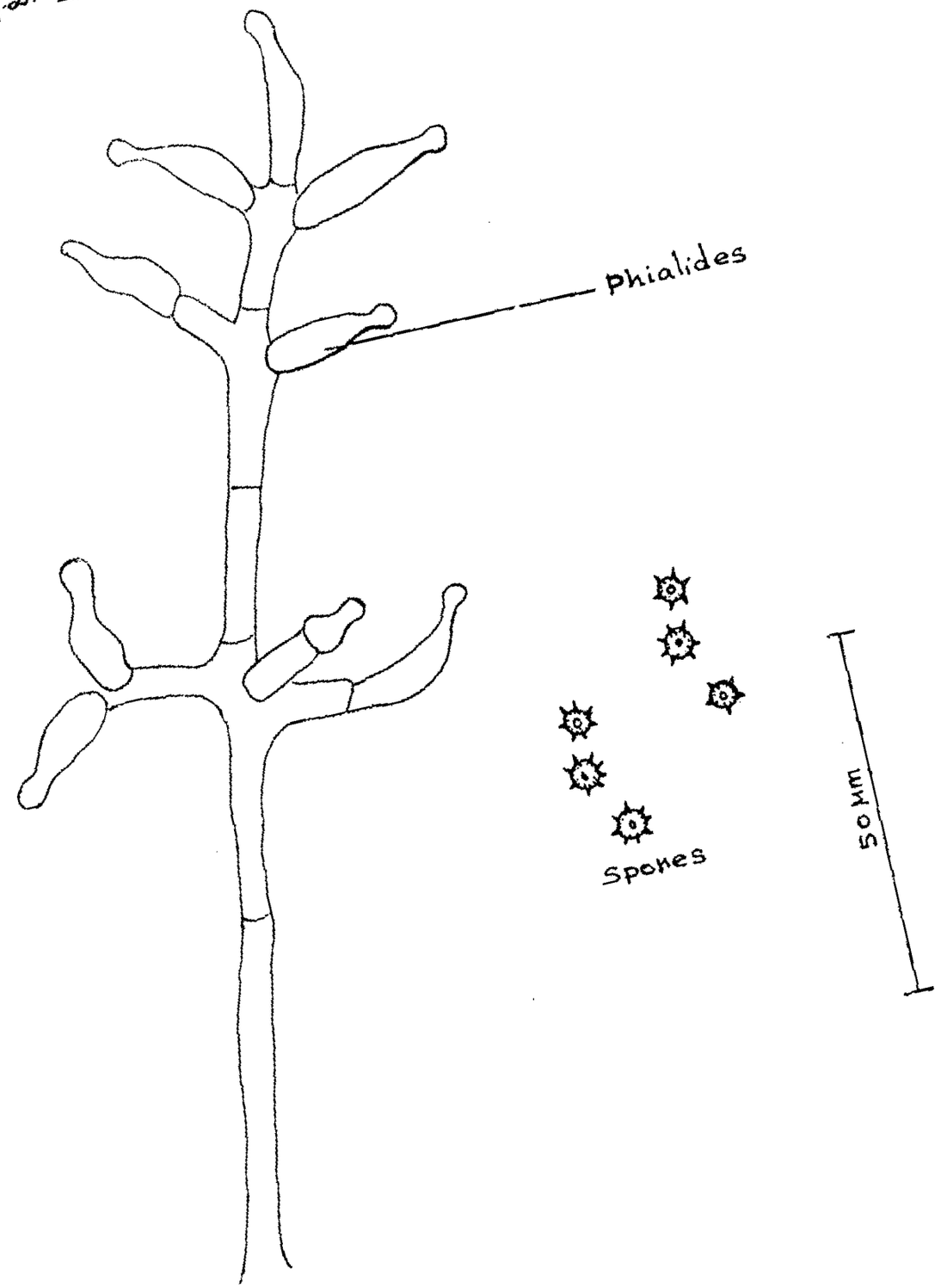
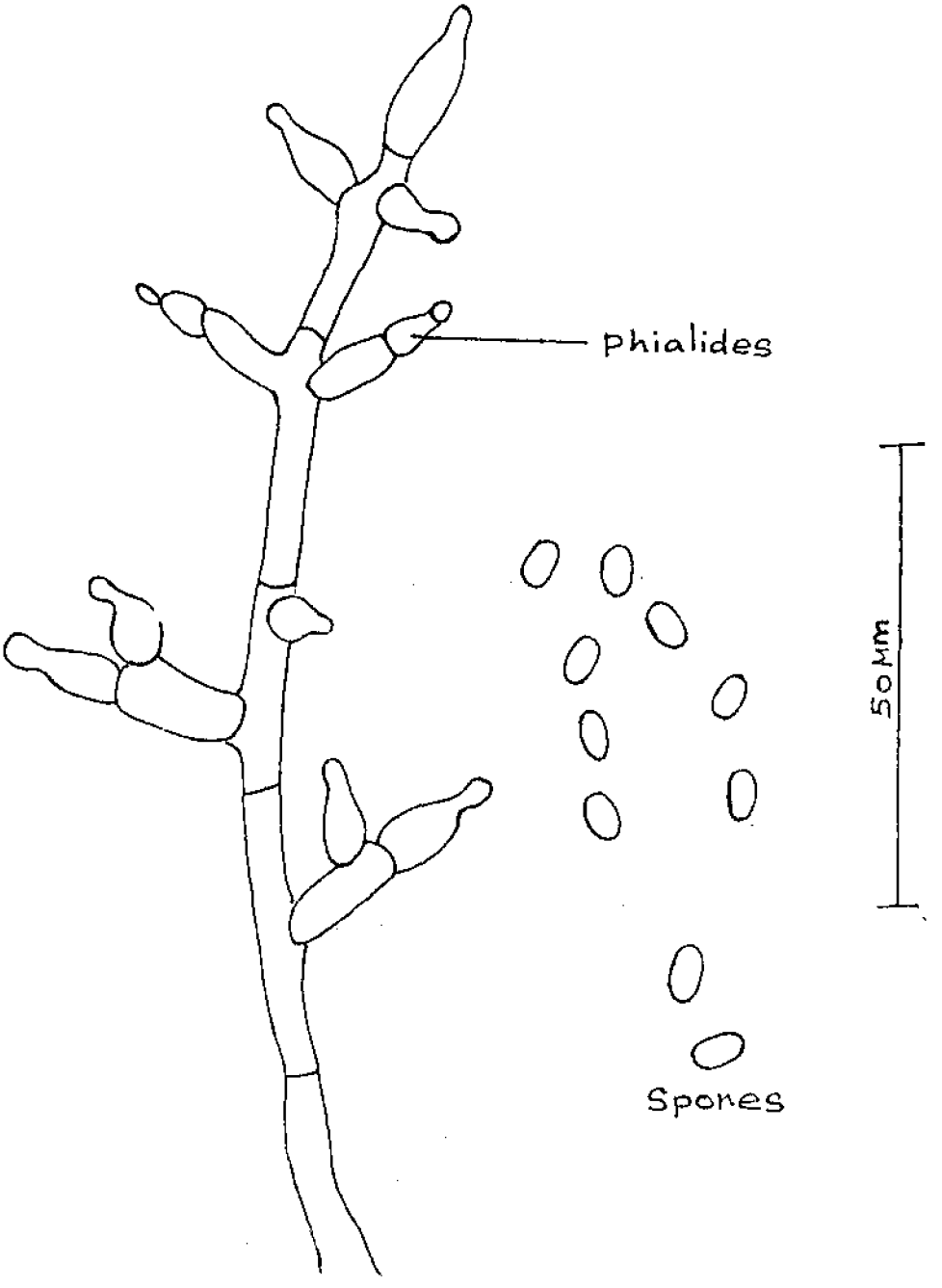


Fig. 3 · Trichoderma pseudokoningii



Morphological characters

Mycelium : Hyaline, septate, smooth of 2-3μm wide.

Conidiophores : Loose tuft, main branch produced numerous side branches specially lower portion.

Phialides : Arise in false verticils upto five short,skittle shaped, narrow at the base, attenuated abruptly, sharp pointed neck, 10-12 μm long

Phialospores : accumulated at the tip of phialides, subglobose, short obovoid,often with broad truncate base,smooth, pale green, much darker in mass 3-4 μm in size.

Based on the above characters the fungus is identified as ***Trichoderma harzianum*** **(ITCC No. 5493.03)**(Plate.5a & Fig. 4)

4. Isolate No.F-10

Cultural characters

Colonies :Fast growing and green coloured .

Morphological characters

Mycelium : septate, smooth, colourless and 2-4μm wide.

Conidiophores : Long, slender, without sterile hyphae, side branches are long.

Phialides : 6-12μm long,pin shaped , narrow at the base, attenuated into long neck. Bear a large drop of green conidia on each whorl.

Phialospores : Short- ellipsoidal, smooth walled, rather large and of the size 3-5 μm.

Based on the above characters the fungus is identified as ***T. virens***) (Plate.5b& Fig. 5)

5. Isolate No. F-141

Cultural characters

Colonies : Fast growing with white mycelium and heavily sporulating in

Fig. 4. Trichoderma harzianum

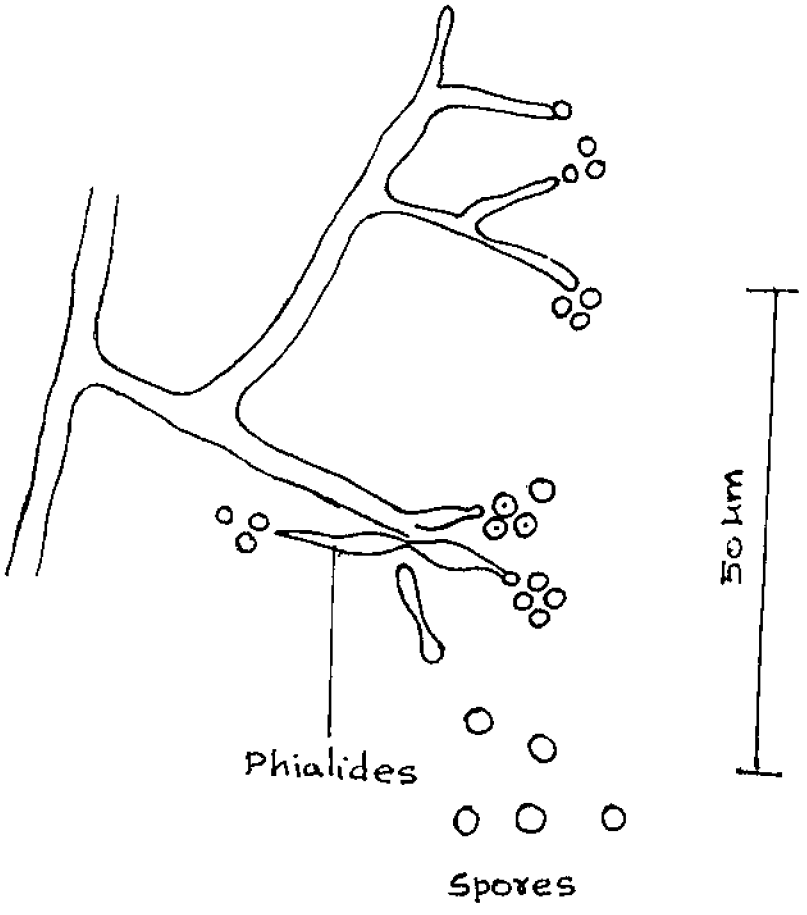
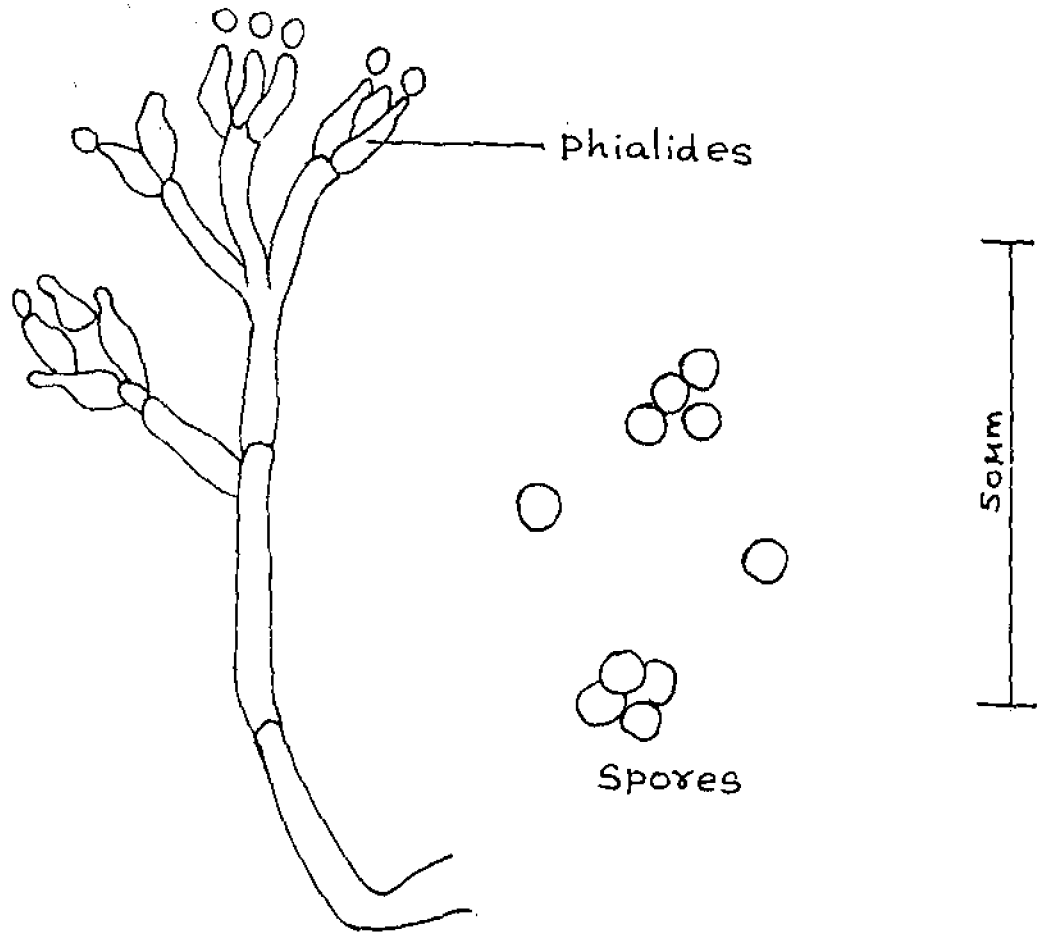


Fig. 5. Trichoderma virens



deep brownish black colour, conidial heads are large, globose and the reverse is colourless .

Morphological characters

Conidiophores : Colourless, walls smooth, thick and of 240-320 x 16 μm size.

Vesicle : Globose (diameter 48 μ), sterigmata biseriate, primary branches 24-32 x 5.2-5.8 μm, secondaries – 7.2-10 x 3-3.2 μm.

Conidia : Globose - 3-5.0 μm, brown, irregularly roughened.

Based on the above characters the fungus is identified as ***Aspergillus niger*** (Plate.5f, 5g & Fig. 6).

6. Isolate No.4

Cultural characters

Colonies : Colonies are sage green to poir green coloured, Reverse is colourless. Become 3.5 to 4 cm in diameter within 14 days at 25°C with centres raised.

Morphological characters

Mycelium : Septate, smooth , 6-8 μm wide

Conidiophore : 68 μm long.

Vesicle : Flask shaped (diameter: 20μm), green pigmented, typically fertile over the upper 3/4th part.

Sterigmata : Single series, borne on the uppermost surface of the vesicle only, comparatively few in number and of the size 6-7 x 2-2.2μm.

Conidia : Globose, smooth but delicately roughened, pale green and of 2.0-2.4μm diameter.

Based on the above characters the fungus is identified as ***Aspergillus viridi-nutans*** (Plate5h & Fig. 7).

Fig. 6. Aspergillus niger

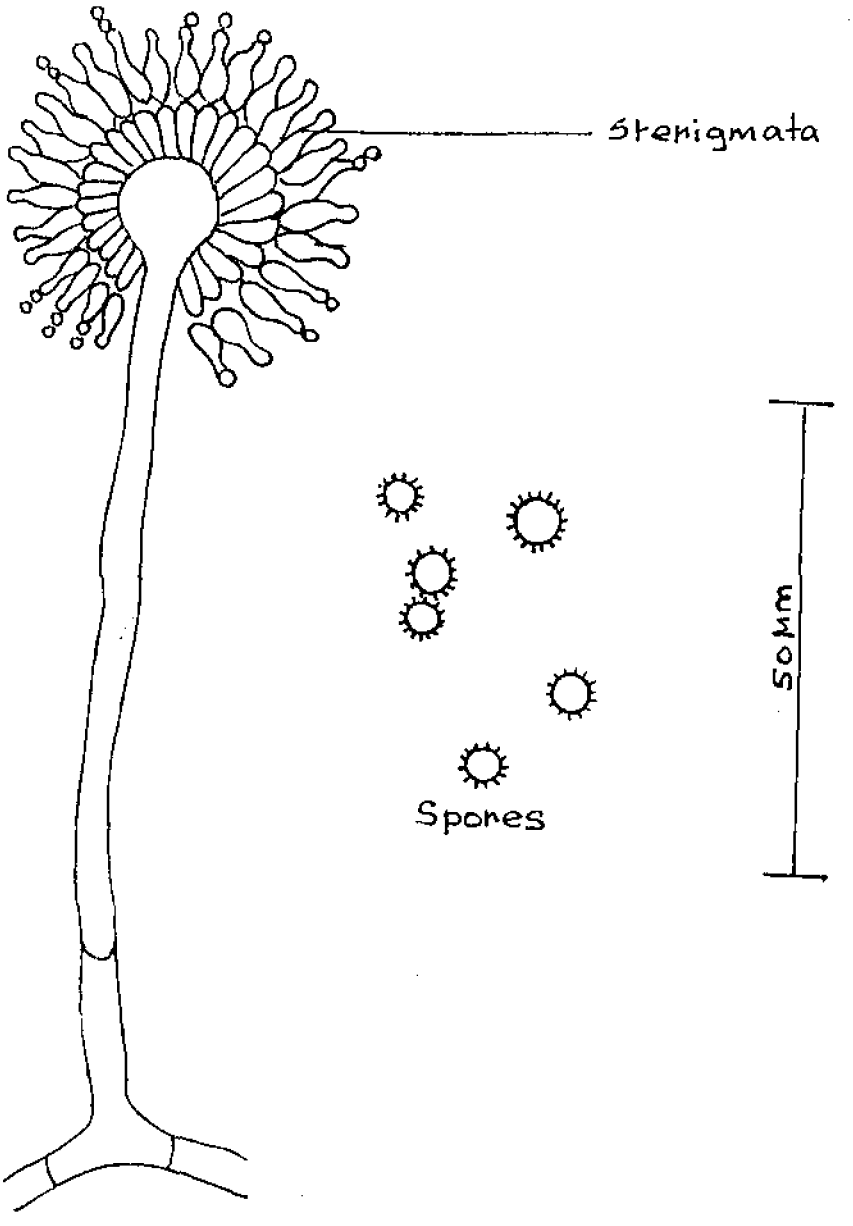


Fig. 7. Aspengillus viridi-nutans

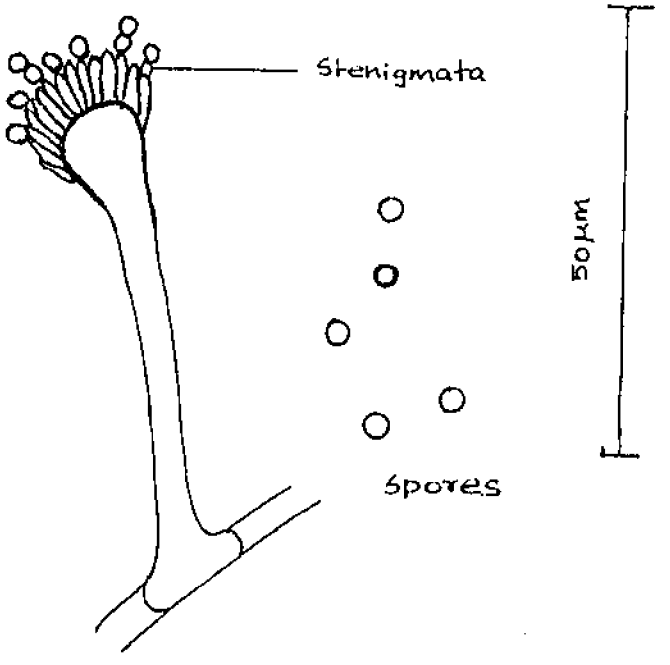


Plate 5
Identification of fungal antagonists

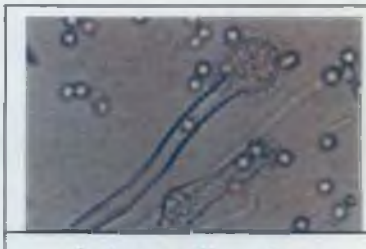
a. *Trichoderma harzianum.*

b. *Trichoderma virens*

c. *Trichoderma pseudokoningii*

d. *Trichoderma viride*

e. *Mucor* sp.



f. *Aspergillus niger*

g. *Aspergillus*

h. *Aspergillus viridi-nutans*

7. Isolate No.142

Cultural characters

Colonies : Fast growing and white coloured .

Morphological characters

Mycelia : Aseptate
Sporangiophores : Tall and short, branched in monopodial fashion
Sporangia : Hyaline and round, columella is globose
Sporangiospores : Smooth, hyaline and globose.

Based on the above characters the fungus is identified as ***Mucor*** sp (Plate.5e & Fig.8).

4.6.2. Characterisation of bacterial antagonists

Cultural, morphological and biochemical characters of isolates are presented in the Table 6,7 and 8 (Plate. 6)

4.6.2.1 Biochemical tests for gram negative bacteria

(i) KOH test

The bacterial antagonistic organisms B 4 and B 30 showed positive reactions by forming thick thread which is the further confirmation of gram negative bacteria.

(ii) Pigment production

Isolate B 4 produced pinkish brown pigment on King's A medium. On King's B medium, B 30 was found to produce (greenish yellow) fluorescence when observed under UV light.

Fig.8. Mucor Sp.

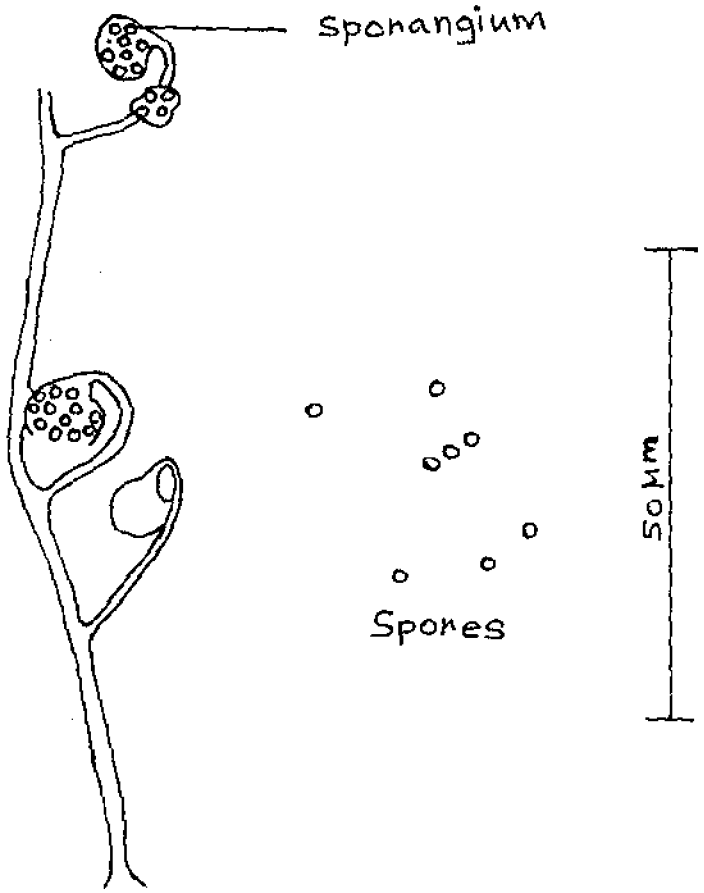


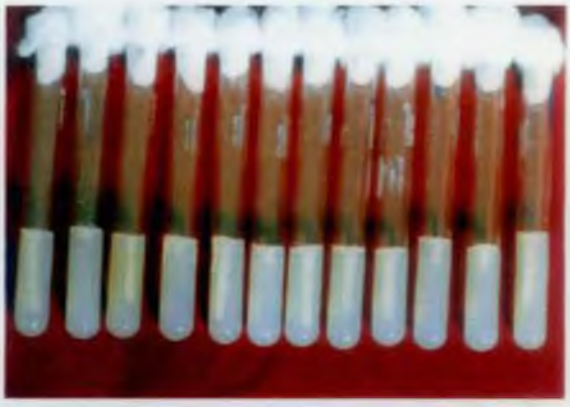
Table 6. Tests for the identification of bacteria

Sl.No.	Bacterial isolates	Gram staining	Solubility in 3% KOH	Form	Elevation	Margin	Growth in liquid media	Agar stroke
1	B 4	-	+	Circular	Flat	Entire	At bottom	Effuse
2	B 19	+	-	Circular	Convex	Entire	At bottom	Echinulate
3	B 28	+	-	Punctiform	Flat	Entire	At bottom	Filiform
4	B 29	+	-	Circular	Convex	Entire	At bottom	Filiform
5	B 30	-	+	Circular	Convex	Entire	At bottom	Beaded
6	B 32	+	-	Circular	Flat	Entire	On top and membranous	Filiform
7	B 56	+	-	Circular	Flat	Entire	At bottom	Echinulate
8	B 124	+	-	Circular	Flat	Curled	At bottom and flocculent	Echinulate
9	B 125	+	-	Circular	Convex	Entire	On top and membranous	Echinulate

+ positive; - negative

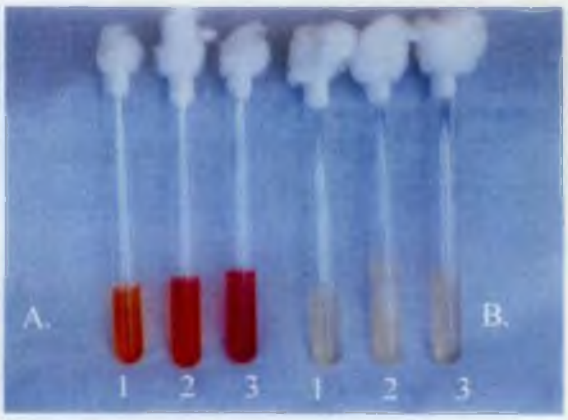
Plate 6
Biochemical tests for identification of bacteria

Growth of different bacterial isolates in liquid media



Agar stroke test

1. Lipase activity B4
2. Lipase activity B30
3. Levan production B4
4. Levan production B30



A Arginine dihydrolase reaction
B. Denitrification reaction
1. Control
2. B4
3. B30

(iii) Anaerobic growth

Both B 4 and B 30 showed positive reaction by changing colour of the medium, blue to yellow.

(iv) Oxidase test

The test was performed using oxidase disc. On touching with the culture of the isolates B 4 and B 30, the disc changed its colour to deep purple blue which revealed as oxidase positive reaction.

(v) Levan from sucrose

Both the isolates failed to produce white domed and mucoid colonies indicating negative for levan production.

(vi) Starch hydrolysis

None of the isolates were able to hydrolyse starch.

(vii) Lipase test

White dense precipitate could be seen around the isolates streaked on Tween 80 agar, indicating positive reaction by both the isolates.

(viii) Growth at 4° and 41°C

B 4 streaked on NA medium failed to grow under 4°C but growth was seen at 41°C, whereas, B 30 was able to grow at both 4° and 41°C.

(ix) Denitrification

Isolate B 4, changed the colour of the media to pink and showed positive reaction for gas production. B 30 did not change the colour of the media and also did not produce gas.

(x) Arginine dihydrolase

The isolates gave pink colourisation to the medium indicating their ability to hydrolyse arginine.

4.6.2.2. ***Biochemical tests for Gram positive bacteria***

(i) Catalase test

The three isolates B 19, B 124 and B 125 produced effervescens when hydrogen peroxide was added to the culture indicating the presence of catalase.

(ii) Anaerobic growth

Isolate B 19 and B 125 indicated positive by changing colour of the medium blue into yellow. But B 124 isolate did not show any colour change.

(iii) Voges Proskaeur test

All three isolates were able to change the colour of the medium to crimson colour indicating positive for VP test.

(iv) Acid and gas from glucose

All the isolates changed the colour of the medium indicating positive reaction for acid from glucose. Gas bubbles below the sealed agar indicating positive reaction for gas production was noticed in isolate B 19

v) Hydrolysis of starch

The ability of all the three isolates to hydrolyse starch was evidenced from the appearance of a colourless zone in contrast to the blue background of the medium around the bacterial growth on addition of iodine solution.

(vi) Nitrate reduced to nitrite

Colour change of the medium to red indicated positive reaction for nitrite production by all the isolates B 19, B 124 and B 125.

(vii) Growth in NaCl

Isolate B 124 and B 125 were able to grow at 3% and 5% NaCl but failed to grow at 17% NaCl. However, isolate B 19 was unable to grow at any of the three concentrations.

(viii) Growth at 4° and 40°C

Isolate B 19 showed growth both at 4 and 40°C but isolates B 124 and B 125 were able to grow only at 40°C.

Bacterial antagonists were identified based on above cultural, morphological and biochemical characters

1. Isolate No. : B4

Cultural characters : Colonies were circular, flat with entire margin and the growth was effuse in agar stroke and was at the bottom in nutrient broth.

Morphological characters : Bacterium was short rods and Gram negative

Biochemical characters : Based on the biochemical characters given in Table 7 the bacterium is identified as *Pseudomonas aeruginosa*

2. Isolate No.B30

Cultural characters : Colonies are punctiform, convex with entire margin. Growth was beaded in agar stroke and was at the bottom in nutrient broth .

Morphological characters : Bacteria were short rods and Gram negative.

Biochemical characters : Based on the biochemical characters given in Table 7 the bacterium is identified as *Pseudomonas fluorescens*

3. Isolate No. : B124

Cultural characters : Colonies are circular, flat with curled margin and the echinulate in agar stroke. The growth was flocculent and was at the bottom in nutrient broth.

Morphological characters : Bacterium was short rods and Gram positive

Biochemical characters : Based on the biochemical characters given in Table 8 the bacterium is identified as *Bacillus subtilis*

Table 7. Biochemical tests for identification of Gram negative bacteria

Characteristics		B 4	B 30
Pigmentation	King's A	+	-
	King's B	+	+
Anaerobic growth		+	+
Oxidase		+	+
Levan formation from sucrose		-	-
Starch hydrolysis		-	-
Lipase (Tween 80 hydrolysis)		+	++
Growth at 4^0C		-	+
Growth at 41^0C		+	+
Denitrification		++	-
Arginine dihydrolase		++	+++

+ positive ; - negative; ++ moderate; +++ good

Table 8. Biochemical tests for identification of Gram positive bacteria

Characteristics		B 19	B 124	B 125
Catalase		+	+	+
Anaerobic growth		+	-	+
Voges- proskauer test		+	+	+
Acid from D-glucose		+++	+++	+++
Gas from glucose		+	-	-
Hydrolysis of starch		+	+	+
Nitrate reduced to nitrite		+	+	+
Growth in NaCl	3%	-	+	+
	5%	-	+	+
	17%	-	-	-
Growth at	4^0C	+	-	-
	40^0C	+	+	+

+ positive ; - negative; ++ moderate; +++ good

4. Isolate No.B125

Cultural characters : Colonies are circular, convex with entire margin. Growth was echinulate in agar stroke and membranous and was at the bottom in nutrient broth .

Morphological characters : Bacteria were short rods and were Gram positive.

Biochemical characters : Based on the biochemical characters given in Table 8 the bacterium is identified as *Bacillus cereus*

5. Isolate No. B19

Cultural characters : Colonies are circular, convex with entire margin. Growth was echinulate in agar stroke and was at the bottom in Nutrient broth .

Morphological characters : Bacteria were short rods and were Gram positive.

Biochemical characters : Based on the biochemical characters given in Table 8 the bacterium is identified as *Bacillus polymyxa*

4.7. POT CULTURE STUDIES FOR THE EVALUATION OF POTENTIAL ANTAGONISTS AGAINST VELLANIKKARA ISOLATES OF *R.solanacearum*

A pot culture experiment was conducted for the evaluation of selected antagonists from the *in vitro* studies against bacterial wilt disease. The susceptible varieties of brinjal, chilli and tomato were used for the study. Observations on the per cent wilt incidence, days to wilt appearance and biometric characters were recorded and the results are given in Table 9 to 16.

4.7.1. Effect of antagonists on per cent wilt incidence

The data on per cent wilt incidence with different treatments on brinjal, chilli and tomato are furnished in table 9,10 and 11. (Plate 7).

The data revealed that, all treatments were superior to control in reducing bacterial wilt incidence. However, some treatments were found to be more effective than others. In the case of brinjal (Table 9), treatment with *T. viride* (T1) and *T.pseudokoningii* (T2) showed least incidence of 16.65 and 18.73 respectively, which were on par with the treatments (T3, T16, T23, T25, T17 and T5) which showed incidence ranged from 20.81to 26.36 per cent.

In the case of chilli (Table 10) also, same trend was observed in which the treatments with *T.viride* (T1), *T.pseudokoningii* (T2), *P.aeruginosa* (T16) and commercial *P.fluorescens* (T25) recorded no wilt incidence and thereby gave complete protection against the disease in chilli. However, they were found to be on par with other treatments (T23, T3, T17 and T5) in which wilt incidence was low and ranged from 10.41 to 17.34 only.

The per cent disease incidence in tomato (Table 11) showed that, wilt incidence were more in tomato as compared to other two crops as the minimum wilt incidence recorded was 24.28 per cent, that also with *T. viride* (T1), *T.pseudokoningii* (T2) and commercial *P.fluorescens* (T25) . However, they showed on par effect, with the treatments (T16, T3, T17, T23, T24 and T5) in which, incidence were from 26.36 to 37.46 per cent.

As far as the three crops are considered, the treatments *T.viride* (T1), *T.pseudokoningii* (T2), *T.viride* (T3), *A.niger* (T5), *P.aeruginosa* (T16), *B.subtilis* (T17) commercial *T.viride* (T23) and commercial *P.fluorescens* (T25) were more effective in reducing the wilt incidence of brinjal, chilli and tomato. However, *T.viride* (T1) and *T.pseudokoningii* (T2) were most effective against the three pathogen. It is also noted that,

Plate 7

Effect of *Trichoderma* spp on bacterial wilt of brinjal

1. *Trichoderma viride* (Ozh)
2. *Trichoderma pseudokoningii*
3. *Trichoderma viride* (com)
4. control

Effect of *Trichoderma spp.* on bacterial wilt of chilli

1. *Trichoderma viride* (Ozh)
2. *Trichoderma pseudokoningii*
3. *Trichoderma viride* (com)
4. control

Effect of *Trichoderma spp.* on bacterial wilt of tomato

1. control
2. *Trichoderma viride* (Ozh)
3. *Trichoderma pseudokoningii*
4. *Trichoderma viride* (com)

Effect of different antagoneists on bacterial wilt of tomato variety mukthi under field condition

Table 9: Effect of different antagonists and methods of application on per cent wilt incidence in brinjal

Per cent wilt incidence																										
Treatments / Methods	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24	T25	T26	Mean
M1	24.97 (0.508)	24.97 (0.508)	24.97 (0.508)	47.17 (0.734)	36.07 (0.621)	55.5 (0.841)	100 (1.278)	100 (1.278)	100 (1.278)	74.95 (1.062)	36.07 (0.621)	100 (1.278)	74.95 (1.062)	83.31 (1.170)	55.5 (0.841)	27.75 (0.513)	24.97 (0.508)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	24.97 (0.508)	33.3 (0.615)	24.97 (0.508)	100 (1.278)	59.80 0.903
M2	24.97 (0.508)	24.97 (0.508)	36.07 (0.621)	55.5 (0.841)	36.07 (0.621)	66.6 (0.955)	100 (1.278)	100 (1.278)	100 (1.278)	72.21 (1.057)	36.07 (0.621)	100 (1.278)	83.31 (1.170)	100 (1.278)	74.95 (1.062)	36.07 (0.621)	33.3 (0.615)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	24.97 (0.508)	33.3 (0.615)	36.07 (0.621)	100 (1.278)	63.76 (0.949)
M3	0 (0.293)	0 (0.293)	16.65 (0.400)	24.97 (0.508)	16.65 (0.400)	55.5 (0.841)	100 (1.278)	100 (1.278)	100 (1.278)	52.75 (0.836)	44.4 (0.728)	100 (1.278)	83.85 (0.949)	83.85 (0.949)	55.5 (0.841)	16.65 (0.400)	16.65 (0.400)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	16.65 (0.400)	24.97 (0.508)	16.65 (0.400)	100 (1.278)	53.83 (0.835)
M4	0 (0.293)	16.65 (0.400)	16.65 (0.400)	36.07 (0.621)	16.65 (0.400)	55.5 (0.841)	100 (1.278)	100 (1.278)	100 (1.278)	63.85 (0.949)	24.97 (0.508)	100 (1.278)	83.87 (0.949)	74.95 (1.062)	55.5 (0.841)	16.65 (0.400)	16.65 (0.400)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	16.65 (0.4)	24.97 (0.508)	16.65 (0.400)	100 (1.278)	53.61 (0.834)
Average	16.65 (0.400)	18.73 (0.427)	23.59 (0.482)	40.93 (0.676)	26.36 (0.511)	52.72 (0.813)	100.0 (1.278)	100.0 (1.278)	100.0 (1.278)	65.94 (0.976)	42.33 (0.702)	100.0 (1.278)	71.49 (1.033)	78.44 (1.115)	60.36 (0.897)	22.89 (0.481)	24.28 (0.484)	100.0 (1.278)	100.0 (1.278	100.0 (1.278	100.0 (1.278	100.0 (1.278	20.81 (0.454)	29.13 (0.561)	23.59 (0.482)	100.0 (1.278)	57.75 (0.879)

M1-Seed treatment M2-Soil drenching M3-Seed treatment + soil drenching M4-Root dipping

Mean of three replications Figures in parenthesis are transformed values

CD for comparison of treatments between methods of application – NS

CD for comparison of treatments averaged over four methods – 0.1441

CD for comparison of methods of application averaged over 26 treatments NS

Table 10: Effect of different antagonists and methods of application on per cent wilt incidence in chilli

Per cent wilt incidence																										
Treatments / Methods	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24	T25	T26	Mean
M1	0.0 (0.293)	0.0 (0.293)	16.65 (0.4)	36.07 (0.621)	27.75 0.513)	66.6 (0.955)	77.73 (1.16)	100 (1.278)	100 (1.278)	44.4 (0.728)	44.4 (0.728)	77.73 (1.160)	36.07 (0.621)	55.5 (0.841)	44.4 (0.728)	0 (0.293)	16.65 (0.4)	33.3 0.615	44.4 (0.728)	100 (1.278)	100 (1.278)	100 (1.278)	16.65 (0.400)	24.97 (0.508)	0 (0.293)	100 (1.278)	43.88 (0.717)
M2	0.0 (0.293)	0.0 (0.293)	24.97 (0.508)	36.07 (0.621)	24.97 (0.508)	74.95 (1.062)	74.95 (1.062)	100 (1.278)	100 (1.278)	66.6 (0.955)	44.4 (0.752)	74.95 (1.062)	55.5 (0.841)	66.6 (0.955)	55.5 (0.841)	0 (0.293)	27.75 (0.513)	44.4 (0.728)	55.5 (0.841)	100 (1.278)	100 (1.278)	100 (1.278)	0 (0.293)	36.07 (0.621)	0 (0.293)	100 (1.278)	49.65 (0.777)
M3	0.0 (0.293)	0.0 (0.293)	0.0 (0.293)	16.65 (0.400)	0.0 (1.278)	44.4 (0.728)	55.5 (0.841)	100 (1.278)	100 (1.278)	33.3 (0.615)	24.97 (0.508)	55.5 (0.841)	24.97 (0.508)	24.97 (0.508)	33.3 (0.615)	0 (0.293)	0 (0.293)	24.97 (0.508)	36.07 (0.621)	44.4 (1.278)	100 (1.278)	100 (1.278)	0 (0.293)	0 (0.293)	0 (0.293)	100 (1.278)	34.81 (0.611)
M4	0 0.293)	0 (0.293)	0 (0.293)	16.65 (0.40)	0 (0.293)	55.5 (0.841)	55.5 (0.841)	100 (1.278)	100 (1.278)	44.4 (0.728)	24.97 (0.508)	55.5 (0.841)	24.97 (0.508)	24.97 (0.508)	33 3 (0 615)	0 (0.293)	0 (0.293)	24.97 (0.508)	36.07 (0.621)	100 (1.278)	100 (1.278)	100 (1.278)	0 (0.293)	0 (0.293)	0 (0.293)	100 (1.278)	35.98 (0.624)
Average	0 (0.293)	0 (0.293	14.75 (0.373)	26.36 (0.511)	17.34 (0.402)	60.36 (0.897)	65.92 (0.976	100.0 (1.278)	100.0 (1.278)	47.17 (0.757)	34.68 (0.618)	65.92 (0.976)	35.38 (0.619)	43.01 (0.703)	41.62 (0.700)	0.0 (0.293)	15.26 (0.375)	31.91 (0.590)	43.01 (0.703)	100.0 (1.278)	100.0 (1.278)	100.0 (1.278)	10.41 (0.320)	19.42 (0.429)	0.0 (0.293)	100.0 (1.278)	41.63 (0.682)

M1-Seed treatment M2-Soil drenching M3-Seed treatment + soil drenching M4-Root dipping

Mean of three replications

Figures in parenthesis are transformed values

CD for comparison of treatments between methods of application – NS

CD for comparison of treatments averaged over four methods – 0.1180

CD for comparison of methods of application averaged over 26 treatments 0.1196

Table 11: Effect of different antagonists and methods of application on per cent wilt incidence in tomato

Per cent wilt incidence																											
Treatments / Methods	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24	T25	T26	Mean
M1	27.75 (0.513)	27.75 (0.513)	36.07 (0.621)	44.4 (0.728)	47.17 (0.734)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	72.21 (1.057)	66.6 (0.955)	72.21 (1.057)	83.31 (1.170)	83.31 (1.170)	74.95 (1.062)	24.97 (0.508)	36.07 (0.621)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	27.75 (0.513)	44.4 (0.728)	24.97 (0.508)	100 (1.278)	64.94 (0.962)
M2	36.07 (0.621)	36.07 (0.621)	44.4 (0.728)	55.5 (0.841)	47.17 (0.734)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	74.95 (1.602)	100 (1.278)	72.21 (1.057)	72.21 (1.057)	83.33 (1.070)	36.07 (0.621)	47.17 (0734)	100 (1.278)	100 (1 278)	100 (1.278)	100 (1.278)	100 (1.278)	36.07 (0.621)	44.4 (0.728)	36.07 (0.621)	100 (1.278)	68.89 1.008)
M3	16.65 (0.400)	16.65 (0.400)	27.75 (0.513)	33.3 (0.615)	24.97 (0.508)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	52.75 (0.836)	74.95 (1.603)	52.75 (0.836)	24.97 (0.508)	52.75 (0.836)	74.95 (1.062)	63.85 (0.400)	55.5 (0.813)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	24.97 (0.508)	24.97 (0.508)	16.65 (0.400)	100 (1.278)	56.60 0.868
M4	16.65 (0.400)	16.65 (0.400)	27.75 (0.513)	44.4 (0.728)	36.07 (0.621)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	72.21 (1.057)	55.5 (0.841)	72.21 (1.057)	83.31 (1.170)	83.31 (1.170)	66.6 (0.955)	27.75 (0.513)	24.97 (0.508)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	100 (1.278)	36.07 (0.621)	36.07 (0.621)	16.65 (0.400)	100 (1.278)	61.20 0.920
Average	24.28 (0.484)	24.28 (0.484)	33.99 (0.594)	44.4 (0.728)	38.85 (0.649)	100.0 (1.278)	100.0 (1.278	100.0 (1.278	100.0 (1.278	72.21 (1.057)	68.36 (0.897)	72.21 (1.057)	78.44 (1.115)	75.67 (1.087)	70.09 (1.007)	26.36 (0.511)	33.99 (0.594)	100.0 (1.278)	100.0 (1.278	100.0 (1.278	100.0 (1.278	100.0 (1.278	37.46 (0.646)	37.46 (0.646)	24.28 (0.484)	100.0 (1.278)	62.91 0.955

M1-Seed treatment M2-Soil drenching M3-Seed treatment + soil drenching M4-Root dipping

Mean of three replications Figures in parenthesis are transformed values

CD for comparison of treatments between methods of application – NS

CD for comparison of treatments averaged over four methods – 0.1674

CD for comparison of methods of application averaged over 26 treatments NS

avirulent and mutant form of *R.solanacearum* that were found effective under *in vitro* showed cent per cent wilt incidence.

It is also observed from the data that, effect of 26 treatments in reducing the wilt incidence was found to be significantly superior under seed treatment and soil drenching method as well as in root dipping method in the case of chilli.

4.7.2. Effect of antagonists on days to wilt appearance

The observations recorded on the effect of different treatments on days to wilt appearance in brinjal, chilli and tomato are presented in Table 12,13 and 14.

The data on brinjal (Table 12) indicated that, treatments *T.*viride (T1) and commercial *T.viride* delayed the symptom to 114.16 and 112.58 days respectively. However, the other treatments (T2, T16, T25, T5, T3) were on par with the above treatments as the days to wilt appearance recorded were from 110.08 to 107.41 days.

From the Table 13 it is evident that, *T.viride* (T1), *T.pseudokoningii* (T2), *P.aeruginosa* (T16) and commercial *P.fluorescens* (T25) could delay the wilt appearance in chilli to a maximum of 120 days followed by the treatments T 23, T3, T17, T5, and T24 which were also found effective in delaying the wilt appearance where the days ranged from 118.33 to 112.5.

The data (Table 14) showed that, treatments with *T.viride* (109.75 days) and commercial *T.viride* (109.58 days) recorded late wilt appearance in tomato. However, they were also found to be on par with the treatments (T2, T3, T16, T5, T25) where 106 to 102.4 days were taken for the wilt appearance.

From the overall view, it is found that, the treatments with *T.viride* (T_1), *T.pseudokoningii* (T2), *T.viride* (T3), *A.niger* (T5), *P.aeruginosa* (T16), commercial

Table : 12 Effect of different antagonists and methods of application on wilt appearance in brinjal

Treatments / Methods	Days after sowing																								
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24	T25	T26	Mean
M1	110	103.33	105	91.33	103.33	88	55	53.66	50.33	65.33	96	69.66	62	59.33	60	112	104.33	56.33	51.33	51.33	50	51.33	111	92	107.33	50.33	77.64
M2	106.66	101.66	99.66	82.66	101.66	84.33	53	52.06	53	60	88	63.66	57.66	56.33	59.66	100	93.66	53	50	57	54	55.33	107.66	88.33	105.33	49.67	74.41
M3	120	120	113.33	106	116.66	97.66	56.33	54.66	50.33	70.33	106.66	72.66	66.33	65	76	115	112.66	58.33	52.33	58	55	56	116.67	104.66	114.66	53.66	84.19
M4	120	115.33	111.66	98.66	110	95	56.33	52.33	49	66.66	98.33	72	65.66	64	76.66	113.33	110.66	58	51	50	54	52	115	103.33	112.66	51	81.64
Average	114.16	110.08	107.41	94.66	107.91	91.25	55.16	53.33	50.66	65.58	97.25	69.5	62.91	61.16	70.33	110.83	105.33	56.41	51.16	54.08	53.25	53.66	112.53	97.08	110	51.16	97.47

M1-Seed treatment M2-Soil drenching M3-Seed treatment + soil drenching M4-Root dipping

Mean of three replications

CD for comparison of treatments between methods of application NS

CD for comparison of treatments averaged over four method 7.436

CD for comparison of methods of application averaged over 26 treatments 6.213

Table : 13 Effect of different antagonists and methods of application on wilt appearance in chilli

Days after sowing																										
Treatments / Methods	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24	T25	T26	Mean
M1	120.0	120.0	113.33	97.66	113.3	65	77.33	55	51.33	79	106.66	91.66	85.67	73	81	120.0	113.33	88	90	65.66	56.66	57.33	120	106.66	120.0	53.33	89.91
M2	120.0	120.0	108.33	97.66	106.66	62.66	68.66	54	55.66	64.33	94	73.66	65.66	71	75.33	120.0	108.33	83.66	83	66	54	53.66	113.33	103.33	120.0	52.66	84.91
M3	120.0	120.0	120	114	120	80	92.33	56.33	54.67	86.33	110.66	107.33	93.66	97	92.33	120.0	120	103.33	101	69.33	55	55.66	120	120	120.0	55	97.18
M4	120.0	120.0	120	113.33	120	96.66	90	53.33	54.66	81.33	111	102.33	94	94.67	89	120.0	120	106.66	99	88.33	57.66	54	120	120	120.0	53.33	96.89
Average	120.0	120.0	115.41	105.67	114.75	76.08	82.08	54.66	54.08	77.75	105.58	93.75	84.75	83.91	84.41	120.0	115.41	95.41	93.5	79.83	55.83	55.16	118.33	112.5	120.0	53.58	92.23

:d treatment M2-Soil drenching M3-Seed treatment + soil drenching M4-Root dipping
f three replications

comparison of treatments between methods of application – NS
comparison of treatments averaged over four methods 8.749
comparison of methods of application averaged over 26 treatments 5.671

Table : 14 Effect of different antagonists and methods of application on wilt appearance in tomato

Treatments / Methods	Days after sowing																									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24	T25	T26	Mean
M1	101.67	100	101.66	90.66	100	55.66	53.66	52.66	49.66	59.33	80	61.66	60.66	59.66	65.33	98.33	99	56.33	54.33	58	53	50.67	113.33	91.67	102.33	52	74.60
M2	108.33	98.33	91.66	80.33	98.33	53	51	50	51.33	56.33	77.33	57	58.66	56.33	62.33	101	94.33	54.66	52.66	56	43.3	44	103.33	86.66	98	49.33	70.52
M3	115	114.33	111	100	107.33	58	55.33	55.66	49.49	70.33	97	68.33	65	66.33	78	107.66	110.66	57	53.33	57.66	55.3	54.33	111.66	105	102.33	54.66	80.03
M4	114	113.33	111.66	97.66	103.33	58.66	54.66	52.33	50	59.33	95	66.66	64.66	62	77.66	107.33	99	56.6	52.66	50.66	51	52	110	101.66	107	48.66	77.60
Average	109.70	106.5	104	92.16	102.20	56.33	53.66	52.66	50.15	61.33	87.33	63.41	62.25	61.08	70.83	103.58	100.75	56.16	53.25	55.58	50.66	50.25	196.2	96.25	102.41	51.44	75.70

M1-Seed treatment M2-Soil drenching M3-Seed treatment + soil drenching M4-Root dipping

Mean of three replications

CD for comparisqn of treatments between methods of application NS

CD for comparison of treatments averaged over four method 8.367

CD for comparison of methods of application averaged over 26 treatments 4.260

T.viride (T23) and *P.fluorescens* (T25) were the best antagonistic organisms in delaying the wilt appearance in solanaceous vegetables.

From the data, it appears that, the seed treatment and soil drenching (M3) and root dipping method (M4) were significantly superior in delaying the wilt appearance in brinjal, chilli and tomato as far as mean effect of 26 treatments were concerned.

Summing up the findings in pot culture studies, it is noted that, *T.viride* (Ozhalapathy), *T.pseudokoningii*, *T.viride* (Vellanikkara), *A.niger* (Eruthyampathy), *P.aeruginosa*, commercial *T. viride* and commercial *P.fluorescens* were the promising antagonists in reducing the wilt incidence and delaying the wilt appearance. It is also observed that, pot culture studies have imparted better control of bacterial wilt pathogen using microbial antagonists.

It also indicates that, all methods of application were equally effective and were statistically on par. However, either root dipping or seed treatment and soil drenching methods gave maximum treatments in reducing the wilt incidence as well as for recording maximum days to wilt appearance. Hence, these two methods were adopted for field trial.

4.7.3. Effect of antagonists on biometric characters

In order to find out the effect of antagonists on increase in plant vigour and yield the various biometric characters were recorded and presented in Table 15 to 20.

Among various biometric characters, fresh weight, days to harvesting and fruit weight were found to be influenced by the method of application in brinjal (Table 15). It is evident that, in seed treatment method, treatment with commercial *T.viride* (T16) recorded for the maximum fresh weight, fruit weight and early harvesting. In soil drenching method, both commercial *T.viride* and commercial *P.fluorescens* showed maximum fresh weight. First harvesting was recorded by treatments with commercial *T.viride* (T16),

Table 15: Effect of different antagonists on biometric characters of brinjal under different methods of application

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (g)	Dry weight (g)	* Days to flowering	* Days to harvesting	Fruit wt/pt (g)
M_1T_1	29.17	14.83	28.67	19.07	7.6	61.67	104.67	29.0
M_1T_2	29.37	14.63	29.0	18.7	7.07	60.67	105.33	28.5
M_1T_3	28.93	12.53	27.0	18.7	7.27	63.67	105.67	28.23
M_1T_4	22.23	7.03	17.33	17.33	6.73	64.33	109.33	23.67
M_1T_5	25.17	10.73	23.0	17.47	6.77	63.33	105.33	28.5
M_1T_6	24.5	10.8	23.0	17.77	6.63	63.0	107.0	25.13
M_1T_7	14.6	5.8	5.33	2.53	1.17	0	0	0
M_1T_8	13.63	5.77	4.33	2.30	1.03	0	0	0
M_1T_9	27.67	12.17	26.67	18.0	7.03	62.0	104.33	26.3
M_1T_{10}	27.3	12.40	23.0	17.67	6.67	62.0	105.67	27.0
M_1T_{11}	15.06	5.67	4.67	2.6	1.17	0	0	0
M_1T_{12}	15.56	5.567	6.0	2.667	1.067	0	0	0
M_1T_{13}	14.23	5.27	5.3	6.37	1.13	0	0	0
M_1T_{14}	13.63	4.87	4.33	1.93	1.13	0	0	0
M_1T_{15}	12.37	7.87	5.67	1.87	0.90	0	0	0
M_1T_{16}	29.83	14.7	30.0	20.13	7.87	61.0	102.67	31.3
M_1T_{17}	25.47	10.27	22.33	17.67	6.43	62.33	105.0	27.43
M_1T_{18}	28.17	12.6	27.0	18.67	7.67	63.0	104.67	29.83
M_1T_{19}	13.87	6.23	10.33	1.63	1.17	0	0	0
M_2T_1	30.0	14.83	28.67	17.97	7.17	62.33	104.67	28.7
M_2T_2	27.97	13.3	28.67	17.53	6.57	61.0	105.33	28.0
M_2T_3	29.17	12.1	26.0	18.47	7.6	64.0	105.33	28.0
M_2T_4	22.63	9.33	17.33	17.37	6.6	64.67	108.67	24.4
M_2T_5	26.43	11.7	24.0	17.87	6.57	61.0	105.33	28.0
M_2T_6	24.5	11.77	23.33	17.17	6.63	63.33	108.0	24.73
M_2T_7	14.77	5.57	4.67	1.6	1.03	0	0	0

M_2T_8	13.63	5.5	4.33	2.23	1.07	0	0	0
M_2T_9	27.33	11.93	25.33	17.83	6.8	61.67	104.0	26.17
M_2T_{10}	26.67	12.33	24.67	17.9	6.7	63.33	105.67	26.87
M_2T_{11}	14.97	5.7	4.33	1.8	0.93	0	0	0
M_2T_{12}	14.97	5.27	5.67	1.8	1.07	0	0	0
M_2T_{13}	14.27	4.1	5.0	1.9	1.20	0	0	0
M_2T_{14}	14.03	4.8	5.0	1.77	1.03	0	0	0
M_2T_{15}	12.67	4.93	5.0	1.57	1.03	0	0	0
M_2T_{16}	30.5	15.17	30.67	19.13	7.93	61.67	104.0	29.5
M_2T_{17}	26.3	12.27	25.3	17.17	6.5	64.3	105.0	27.23
M_2T_{18}	29.5	13.1	28.67	18.83	7.13	62.33	104.0	29.33
M_2T_{19}	12.13	4.27	5.0	1.47	1.0	0	0	0
M_3T_1	30.17	14.57	3.33	18.03	7.03	62.33	104.33	29.0
M_3T_2	29.5	13.6	29.33	17.17	6.47	61.67	104.33	28.83
M_3T_3	28.83	13.03	26.33	17.8	7.4	64.67	104.67	26.97
M_3T_4	25.3	12.1	25.0	17.67	6.63	64.0	106.67	24.33
M_3T_5	28.1	13.57	24.67	17.3	6.63	64.67	104.67	26.63
M_3T_6	26.7	11.53	25.0	17.4	6.5	62.67	106.0	24.67
M_3T_7	15.36	5.73	5.0	2.17	1.03	62.0	0	0
M_3T_8	13.13	5.6	5.33	2.23	1.27	0	0	0
M_3T_9	27.13	12.4	27.0	17.8	6.8	0	104.67	27.33
M_3T_{10}	28.70	13.5	26.0	17.57	6.23	62.0	105.0	27.37
M_3T_{11}	15.03	5.33	5.33	2.2	0.967	0	0	0
M_3T_{12}	14.57	4.97	6.0	2.03	0.93	0	0	0
M_3T_{13}	14.43	4.5	5.33	2.13	1.07	0	0	0
M_3T_{14}	13.47	5.07	5.0	2.03	0.97	0	0	0
M_3T_{15}	12.63	4.83	5.33	1.8	1.13	0	0	0
M_3T_{16}	31.1	14.13	30.0	19.23	7.77	61.33	105.67	31.17
M_3T_{17}	27.03	13.37	24.67	17.3	6.7	63.33	105.0	25.7
M_3T_{18}	29.93	13.43	29.33	18.9	7.2	62.67	104.0	31.0
M_3T_{19}	11.67	3.9	5.0	1.6	0.9	0	0	0
M_4T_1	28.93	13.37	29.0	17.83	7.2	62.33	104.33	28.87
M_4T_2	29.03	13.5	28.33	17.6	7.0	61.67	104.3	28.97

M_4T_3	28.0	12.8	26.0	17.7	7.2	64.0	106.33	29.5
M_4T4	27.3	12.6	26.0	16.87	6.4	64.33	108.67	23.43
M_4T_5	27.03	12.43	25.67	16.7	6.77	63.33	104.67	28.43
M_4T_6	25.53	11.93	26.0	17.17	6.7	62.0	106.07	24.50
M_4T_7	18.53	7.97	11.67	2.23	1.0	0	0	0
M_4T_8	13.8	5.63	5.33	1.83	1.17	0	0	0
M_4T_9	28.1	12.77	26.0	17.43	7.1	61.67	104.0	27.37
M_4T_{10}	24.67	12.53	22.33	17.4	7.13	63.33	105.0	25.57
M_4T_{11}	15.07	15.6	5.0	2.23	1	0	0	0
M_4T_{12}	15.13	5.0	5.67	2.27	1.03	0	0	0
M_4T_{13}	14.13	5.0	4.67	2.1	0.93	0	0	0
M_4T_{14}	13.2	5.1	4.67	1.93	0.77	0	0	0
M_4T_{15}	12.67	5.3	5.33	1.9	0.87	0	0	0
M_4T_{16}	28.27	11.07	27.67	19.03	8.03	61.33	104.67	30.5
M_4T_{17}	25.33	12.4	21.67	16.87	7.03	63.67	105.33	27.97
M_4T_{18}	29.1	13.17	29.0	18.43	7.87	61.67	104.67	30.33
M_4T_{19}	11.77	3.93	4.67	1.43	0.63	0	0	0
CD(P=0.05)	NS	NS	NS	0.6095	NS	NS	1.2846	1.5197

* Days counted from the day of sowing from days to flowering and harvesting

Value '0' represents absence of observation due to wilting of plants.

M_1-seed treatment; M_2-soil drenching; M_3-Seed treatment + soil drenching and M_4-Root dipping

T1-*Trichoderma viride* T5-*A.niger* T9-*P.aeruginosa* T13-unidentified T17-*A.niger* (com)

T2-*T.pseudokoningii* T6-*A.viridi-nutans* T10-*B.subtilis* T14-mutant of *R.solanacearum* T18-*P.fluorescens* (com)

T3-*T.viride* T7-*Aspergillus* sp. T11-*B.cereus* T15-avirulent of *R.solanacearum* T19- control

T4-*T.virens* T8-*Mucor* sp. T12-*B.polymyxa* T16-*T.viride*(com)

commercial *A.niger* (T17) and maximum fruit weight was shown by treatments commercial *P. fluorescens* (T18), *T.pseudokoningii* (T2), *T.viride* (T3) and *A.niger* (T5). In seed treatment + soil drenching method, maximum fresh weight and fruit weight was recorded by treatments T16 and T18 and early harvesting was given by all treatments except treatments T4, T12, T16. In root dipping method, maximum fresh weight was shown by treatments T16 and T18 while fruit weight was increased to the maximum by treatments T16, T18 and T3. First harvesting was given by all treatments, except T3, T4 and T5.

From the data presented in Table 16 it is observed that, treatment with commercial *T.viride* (T16) showed significantly superior effect on all biometric characters. It recorded the maximum shoot length of 29.18 cm; root length of 13.77 cm; 28.0 number of leaves; fresh weight of 19.38 g; dry weight 7.9 g and fruit weight of 30.625 g. Days to flowering and harvesting was 61.33 and 104.25 days respectively.

From Table 17, it is clear that, only the days to flowering was affected by method of application and other characters were found to be non-significant in the case of chilli. In seed treatment method, treatments *T.viride* (T1) and commercial *T.viride* (T16) recorded first flowering. In soil drenching method, T1 showed the first flowering. In seed treatment + soil drenching method, *T.viride* (T1) and commercial *T.viride* differed significantly from other treatments by recording first flowering. In root dipping, treatment with commercial *T.viride* showed first flowering.

Among the treatments influencing growth parameters of chilli (Table 18) it is found that, treatment *T.viride* (T1) and commercial *T.viride* (T16) significantly increased all characters by recording maximum shoot length of 35.18, 34.73 cm; root length of 14.27, 14.29 cm; number of leaves of 112.5, 114.0; fresh weight of 16.65, 16.57 g; dry weight of 8.98, 9.13g; days to flowering as 60.83, 60.58;days to harvesting as 99.75, 98.33 and fruit weight of 27.48, 26.16g respectively.

Table 16: Effect of different antagonists on biometric characters of brinjal (mean of four methods)

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (g)	Dry weight (g)	* Days to flowering	* Days to harvesting	Fruit wt/pt (g)
T_1	29.57	14.4	29.17	18.22	7.25	62.17	104.5	28.89
T_2	28.97	13.76	28.83	17.75	6.78	61.25	104.83	28.58
T_3	28.93	12.62	26.33	18.17	7.37	63.92	105.5	28.28
T_4	24.36	10.27	21.42	17.31	6.60	64.5	108.33	23.96
T_5	26.68	11.86	24.33	17.38	6.68	63.17	105.0	27.08
T_6	25.31	11.51	6.67	17.51	6.62	62.58	106.92	24.76
T_7	15.82	6.27	4.83	2.13	1.06	0	0	0
T_8	13.55	5.63	26.25	2.15	1.13	0	0	0
T_9	27.56	12.32	24.0	17.77	6.93	61.83	104.25	0
T_{10}	26.84	12.44	4.83	17.63	6.68	63.08	105.33	26.7
T_{11}	15.03	5.58	5.83	2.21	1.02	0	0	0
T_{12}	15.06	5.2	5.08	2.09	1.03	0	0	0
T_{13}	14.27	4.72	4.75	2.13	1.08	0	0	0
T_{14}	13.58	4.96	5.33	1.92	0.98	0	0	0
T_{15}	12.58	5.73	28.0	1.78	0.98	0	0	0
T_{16}	29.18	13.77	25.0	19.38	7.9	61.33	104.25	30.63
T_{17}	26.78	12.08	28.5	17.25	6.67	63.67	105.08	27.08
T_{18}	29.2	13.08	28.5	18.71	7.47	62.42	104.42	30.13
T_{19}	13.1	4.59	6.25	1.53	0.93	0	0	0
CD(0.05)	1.678	1.08	2.4062	0.3048	0.2388	0.6522	0.6423	0.7599

* Days counted from the day of sowing for days to flowering and harvesting

T1-*Trichoderma viride* T5-*A.niger* T9-*P.aeruginosa* T13-unidentified T17-*A.niger* (com)
T2-*T.pseudokoningii* T6-*A.viridi-nutans* T10-*B.subtilis* T14-mutant of *R.solanacearum* T18-*P.fluorescens* (com)
T3-*T.viride* T7-*Aspergillus* sp. T11-*B.cereus* T15-avirulent of *R.solanacearum* T19- control
T4-*T.virens* T8-*Mucor* sp. T12-*B.polymyxa* T16-*T.viride*(com)

Table 17: Effect of different antagonists on biometric characters of chilli under different methods of application

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (g)	Dry weight (g)	* Days to flowering	* Days to harvesting	Fruit wt/pt (g)
M_1T_1	35.0	14.03	103.67	16.53	9.03	60.67	99.67	25.0
M_1T_2	35.63	14.0	106.33	16.1	8.97	61.67	104.0	25.63
M_1T_3	33.37	12.3	100.33	16.23	8.8	63.33	99.33	25.0
M_1T_4	31.27	7.1	84.33	10.33	7.17	61.33	109.33	19.1
M_1T_5	30.87	12.67	99.0	14.87	8.6	63.67	105.67	22.7
M_1T_6	29.57	7.73	92.33	11.4	6.93	67.0	106.0	20.3
M_1T_7	29.1	8.6	87.3	10.13	6.37	64.0	106.0	22.17
M_1T_8	15.77	5.8	10.67	1.47	0.8	0	0	0
M_1T_9	30.77	12.43	103.0	15.3	7.03	62.33	101.33	25.7
M_1T_{10}	32.5	12.8	92.67	15.63	7.03	63.0	102.33	23.5
M_1T_{11}	29.87	8.47	86.0	10.77	6.77	64.0	107.0	18.7
M_1T_{12}	28.0	8.13	82.33	10.93	6.63	64.67	106.0	18.5
M_1T_{13}	18.6	6.7	26.0	2.37	1.2	0	0	0
M_1T_{14}	15.1	5.97	12.67	1.83	0.90	0	0	0
M_1T_{15}	14.5	5.3	11.67	2.1	0.93	0	0	0
M_1T_{16}	34.5	14.1	116.67	16.43	9.17	60.67	98.0	25.27
M_1T_{17}	30.2	11.2	96.67	14.23	7.77	63.67	105.67	22.7
M_1T_{18}	34.43	12.77	101.67	16.37	8.43	62.33	100.0	27.1
M_1T_{19}	14.4	4.47	10.0	1.3	0.73	0	0	0
M_2T_1	34.77	13.93	113.0	16.57	9.03	61.0	101.0	25.9
M_2T_2	35.1	14.1	108.33	16.43	8.97	62.0	99.67	25.7
M_2T_3	33.3	12.17	99.33	16.47	8.8	63.67	99.67	23.3
M_2T_4	39.33	15.9	84.67	12.17	7.3	62.0	108.0	18.0
M_2T_5	30.67	12.3	91.67	14.87	8.6	64.67	104.67	21.67
M_2T_6	28.67	7.3	80.33	11.6	7.0	63.67	102.0	22.83

M_2T_7	39.17	5.7	82.0	11.77	4.7	64.33	108.67	21.73
M_2T_8	15.8	5.53	10.67	1.5	0.8	0	0	0
M_2T_9	29.93	12.57	96.67	15.23	6.9	62.67	104.0	24.43
M_2T_{10}	31.67	12.2	98.67	15.50	6.9	63.0	103.3	23.1
M_2T_{11}	22.77	7.27	84.67	11.67	6.53	64.67	105.67	19.53
M_2T_{12}	22.5	7.2	91.67	10.67	7.13	64.0	107.0	18.33
M_2T_{13}	15.2	6.3	14.33	1.03	0.7	0	0	0
M_2T_{14}	14.87	6.07	11.0	1.87	0.9	0	0	0
M_2T_{15}	15.0	5.63	12.33	2.03	0.93	0	0	0
M_2T_{16}	34.47	14.33	111.67	16.8	9.17	61.67	99.67	24.23
M_2T_{17}	26.5	10.97	103.33	13.33	7.1	64.67	104.67	21.67
M_2T_{18}	34.0	12.87	110.33	16.0	8.43	62.67	99.33	25.5
M_2T_{19}	14.3	4.6	11.33	1.27	0.73	0	0	0
M_3T_1	35.5	14.47	113.33	16.6	9.07	60.33	99.0	28.43
M_3T_2	35.33	14.33	104.33	16.63	9.13	61.67	100.0	26.53
M_3T_3	33.43	12.4	104.0	16.3	8.83	62.67	99.33	24.5
M_3T_4	29.17	8.07	77.67	11.3	6.4	63.0	108.0	18.0
M3T5	30.77	12.9	99.33	15.17	8.7	63.67	103.33	23.87
M_3T_6	28.4	8.87	89.67	11.1	7.13	64.67	113.33	22.9
M_3T_7	29.17	10.57	84.67	11.43	6.63	63.67	112.67	21.83
M_3T_8	16.4	5.1	11.67	1.37	1.07	0	0	0
M_3T_9	32.0	12.53	103.33	15.57	6.77	61.67	105.33	26.93
M_3T_{10}	31.83	12.6	88.67	15.27	6.97	62.33	105.0	24.1
M_3T_{11}	28.43	10.17	81.67	11.4	6.73	63.67	106.6	24.1
M_3T_{12}	28.93	8.87	89.0	11.03	6.53	64.0	106.67	19.78
M_3T_{13}	18.83	5.07	24.33	11.93	1.0	0	0	0
M_3T_{14}	15.33	5.47	13.0	1.5	0.9	0	0	0
M_3T_{15}	14.87	5.5	13.67	2.03	0.93	0	0	0
M_3T_{16}	35.1	14.53	115.0	16.57	9.33	59.33	98.67	27.37
M_3T_{17}	28.6	11.83	101.33	13.77	7.47	64.33	104.67	212.97

M_3T_{18}	34.53	12.83	114.33	16.47	7.93	61.67	100.0	27.37
M_3T_{19}	14.8	4.4	13.0	1.27	0.7	0	0	0
M_4T_1	35.47	14.63	120.0	16.9	9.0	61.33	99.33	28.33
M_4T_2	35.43	14.57	105.0	16.53	8.97	62.0	99.67	28.2
M_4T_3	33.9	12.27	105.33	15.97	8.6	63.0	100.67	24.47
M_4T_4	29.5	7.27	86.0	11.6	7.4	63.33	105.33	21.13
M_4T_5	30.37	12.3	103.0	14.63	8.73	64.33	105.0	23.17
M_4T_6	28.57	7.73	88.33	11.5	6.43	60.0	112.0	21.5
M_4T_7	29.17	8.97	85.67	11.23	6.17	64.33	112.0	21.33
M_4T_8	16.43	4.73	12.33	1.53	0.80	0	0	0
M_4T_9	34.2	12.63	105.0	14.97	7.0	62.67	105.33	26.83
M_4T_{10}	31.67	12.63	88.67	15.37	6.9	62.33	10533	23.1
M_4T_{11}	28.43	8.5	87.67	11.9	6.4	63.67	106.67	19.3
M_4T_{12}	27.5	9.03	86.67	11.27	6.23	64.33	106.0	17.47
M_4T_{13}	18.03	5.33	24.67	2.03	0.8	0.0	0.0	0.0
M_4T_{14}	15.1	5.37	13.67	1.83	0.9	0.0	0.0	0.0
M_4T_{15}	14.77	5.47	13.33	1.90	0.9	0.0	0.0	0.0
M_4T_{16}	34.87	14.2	112.67	16.47	9.07	60.67	99.0	27.77
M_4T_{17}	28.43	12.43	100.0	13.5	7.47	64.67	104.67	22.3
M_4T_{18}	34.33	13.0	108.33	16.23	7.8	61.67	101.0	27.53
M_4T_{19}	14.9	4.3	12.0	1.3	0.7	0	0	0
CD (P=0.05)	NS	NS	NS	NS	NS	1.955	NS	NS

* Days counted from the day of sowing from days to flowering and harvesting

Value '0' represents absence of observation due to wilting of plants.

M_1-seed treatment; M_2-soil drenching; M_3-Seed treatment + soil drenching and M_4-Root dipping

T1-*Trichoderma viride* T5-*A.niger* T9-*P.aeruginosa* T13-unidentified T17-*A.niger* (com)

T2-*T.pseudokoningii* T6-*A.viridi-nutans* T10-*B.subtilis* T14-mutant of *R.solanacearum* T18-*P.fluorescens* (com)

T3-*T.viride* T7-*Aspergillus* sp. T11-*B.cereus* T15-avirulent of *R.solanacearum* T19- control

T4-*T.virens* T8-*Mucor* sp. T12-*B.polymyxa* T16-*T.viride*(com)

Table 18: Effect of different antagonists on biometric characters of chilli (mean of four methods)

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (g)	Dry weight (g)	* Days to flowering	* Days to harvesting	Fruit wt/pt
T_1	35.18	14.27	112.5	16.65	8.98	60.83	99.75	27.48
T_2	35.37	14.25	106.0	16.42	9.01	61.83	100.83	26.36
T_3	33.5	12.41	102.25	16.24	8.82	63.17	99.75	24.33
T_4	29.81	7.1	83.66	11.35	7.07	62.42	107.67	19.21
T_5	30.67	12.54	98.25	14.88	8.65	64.08	104.67	22.85
T_6	28.8	7.9	87.67	11.4	6.86	63.83	108.33	21.88
T_7	29.8	8.48	83.92	11.14	5.97	64.08	102.17	21.77
T_8	29.15	5.29	11.33	1.47	0.9	0.0	0	0
T_9	16.1	12.54	102.0	15.27	6.92	62.33	104.0	25.97
T_{10}	31.73	12.55	92.17	15.44	6.95	62.67	104.0	23.45
T_{11}	28.79	8.6	85.0	10.43	6.61	64.0	106.0	19.35
T_{12}	28.33	8.3	87.41	10.97	6.63	64.5	106.33	18.28
T_{13}	18.33	5.9	19.3	1.09	.85	0.0	0	0
T_{14}	15.1	5.71	12.58	1.84	0.9	0.0	0	0
T_{15}	14.8	5.48	12.75	2.02	0.93	0.0	0	0
T_{16}	34.73	14.29	114.0	16.57	9.13	60.58	98.83	26.16
T_{17}	29.27	11.6	100.33	13.70	7.8	64.33	104.58	22.84
T_{18}	34.32	12.87	108.67	16.27	7.97	62.08	100.08	26.88
$T1_9$	14.6	4.44	11.58	1.28	0.71	0	0	0
CD (P=0.05)	0.7941	0.7417	6.647	0.825	0.7258	09777	6.303	1.3613

* Days counted from the day of sowing from days to flowering and harvesting

T1-*Trichoderma viride* T5-*A.niger* T9-*P.aeruginosa* T13-unidentified T17-*A.niger* (com)
T2-*T.pseudokoningii* T6-*A.viridi-nutans* T10-*B.subtilis* T14-mutant of *R.solanacearum* T18-*P.fluorescens* (com)
T3-*T.viride* T7-*Aspergillus* sp. T11-*B.cereus* T15-avirulent of *R.solanacearum* T19- control
T4-*T.virens* T8-*Mucor* sp. T12-*B.polymyxa* T16-*T.viride*(com)

The data (Table 19) indicates that, different treatments varied significantly with regard to specific method of application except in days to flowering and fruit weight in tomato. In seed treatment method, treatments *T.viride* (T1) and commercial *T.viride* (T16) showed highest shoot length and root length. Maximum number of leaves, fruit weight, dry weight and first harvesting was recorded by T16. In soil drenching method, treatments *T.viride* (T1), commercial *T.viride* (T16), commercial *P.fluorescens* (T18) were equally effective in increasing the root length. In remaining all characters, commercial *T.viride* was found to be significantly superior from others. In seed treatment + soil drenching method, commercial *T.viride* significantly influenced all biometric characters. In root dipping method, maximum shoot length as well as fruit weight was shown by treatments commercial *T.viride* and commercial *P.fluorescens*. Maximum root length, dry weight and early harvesting were all given by T16.

Overall performance of different treatments in four methods of application is given in Table 20. From the table, it is clear that, the treatment with commercial *T.viride* influenced all the plant growth characters significantly by recording, shoot length of 50.7 cm, root length of 15.2 cm, number of leaves as 29.0, fresh weight of 58.29 g, dry weight of 14.61g, days to flowering as 55.58, days to harvesting as 96.08 days and fruit weight of 65.09 g.

4.8. EVALUATION OF PROMISING ANTAGONISTS AGAINST *R.solanacearum* UNDER FIELD CONDITION

A field experiment was conducted to evaluate the performance of the promising antagonists under natural conditions. The experiment was laid out as per the details given in materials and methods and antagonists were applied by the root dipping as well as by seed treatment + soil drenching, the best methods selected from pot culture studies. Observations were recorded on wilt incidence at 15 days interval and also on biometric characters of the plants. The results of the experiments are summarised in Table 21 to 31.

Table 19: Effect of different antagonists on biometric characters of tomato under different methods of application

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (g)	Dry weight (g)	* Days to flowering	* Days to harvesting	Fruit wt/pt (g)
M_1T_1	49.16	14.8	26.33	52.1	14.33	56.66	93.33	60.4
M_1T_2	48.16	14.1	26.33	48.33	14.0	57.33	98.66	60.16
M_1T_3	46.26	14.1	25.33	49.2	13.56	57.66	101.0	56.46
M_1T_4	42.1	13.43	23.33	47.4	12.0	57.33	102	53.4
M_1T_5	44.1	13.26	24.33	44.36	12.1	57.66	104	56.76
M_1T_6	20.0	6.06	9.33	4.56	1.4	0	0	0
M_1T_7	17.33	6.0	9.0	3.3	1.36	0	0	0
M_1T_8	17.7	5.66	8.66	4.433	1.16	0	0	0
M_1T_9	44.43	13.26	24.66	44.8	12.7	57.6	102	53.33
M_1T_{10}	44.40	13.1	25.33	43.56	11.66	58.0	102	53.70
M_1T_{11}	15.96	5.8	7.66	4.1	1.1	0	0	0
M_1T_{12}	16.23	5.9	8.66	4.13	1.1	0	0	0
M_1T_{13}	18.0	5.33	7.66	4.5	1.06	0	0	0
M_1T_{14}	14.93	5.03	5.66	3.7	1.26	0	0	0
M_1T_{15}	15.16	5.1	4.66	3.33	1.1	0	0	0
M_1T_{16}	50.33	15.06	29.0	59.26	14.96	55.66	96.0	63.6
M_1T_{17}	44.1	13.26	24.33	44.36	12.1	57.66	104	56.76
M_1T_{18}	48.46	14.5	27.33	53.03	14.06	55.66	98	63.3
M_1T_{19}	14.96	4.33	5.0	3.1	0.96	0	0	0
M_2T_1	47.16	14.6	27.33	51.76	13.0	57.33	98.66	60.3
M_2T_2	44.23	14.03	26.66	48.4	12.46	58.33	98.66	60.0
M_2T_3	46.3	14.26	27.33	45.73	12.33	57.33	100.66	56.4
M_2T_4	42.76	13.4	24.33	41.56	11.96	58.33	102.66	55.4

M_2T_5	44.23	13.16	24.66	46.4	11.76	59.0	103.33	56.5
M_2T_6	16.56	6.1	5.66	4.33	1.17	0	0	0
M_2T_7	17.3	6.0	6.3	4.33	1.20	0	0	0
M_2T_8	16.57	5.6	7.0	3.97	1.2	0	0	0
M_2T_9	44.23	13.67	24.67	46.6	12.17	58.67	102.0	55.0
M_2T_{10}	44.43	13.27	24.67	46.1	11.9	58.33	102.0	54.13
M_2T_{11}	15.63	6.27	5.67	3.13	1.06	0	0	0
M_2T_{12}	15.17	5.77	5.33	3.23	1.0	0	0	0
M_2T_{13}	15.23	6.03	5.67	3.77	1.17	0	0	0
M_2T_{14}	14.3	5.37	5.67	3.06	0.93	0	0	0
M_2T_{15}	14.77	5.0	5.33	3.27	1.13	0	0	0
M_2T_{16}	50.9	15.06	28.67	59.27	14.27	55.67	96.67	63.16
M_2T_{17}	44.36	13.26	25.0	45.9	11.77	58.67	103.67	56.0
M_2T_{18}	48.47	14.57	27.67	43.7	13.7	56.3	98	63.0
M_2T_{19}	14.5	4.37	4.67	3.1	0.93	0	0	0
M_3T_1	48.93	14.6	27.33	54.7	13.77	57.33	98	60.37
M_3T_2	47.76	14.07	26.67	49.17	13.23	57.33	98	60.16
M_3T_3	45.96	13.6	26.33	47.5	12.27	58.0	100.33	57.47
M_3T_4	42.6	13.03	24.33	46.8	11.93	58.33	101.0	52.67
$M3T_5$	43.86	12.93	25.0	46.17	12.23	58.67	102.0	56.67
M_3T_6	18.0	6.13	6.33	3.97	0.97	0	0	0
M_3T_7	18.6	5.73	6.0	4.23	1.07	0	0	0
M_3T_8	15.9	5.7	6.33	3.53	0.97	0	0	0
M_3T_9	44.97	13.23	25.33	48.3	12.37	58.67	102.0	54.73
M_3T_{10}	75.27	13.23	25.67	47.3	12.0	58.67	102.0	53.33
M_3T_{11}	16.53	5.93	6.33	3.57	1.1	0	0	0
M_3T_{12}	16.63	5.77	5.33	3.93	1.17	0	0	0
M_3T_{13}	17.5	5.73	4.67	4.03	1.1	0	0	0
M_3T_{14}	15.43	5.07	5.0	3.77	1.03	0	0	0
M_3T_{15}	15.53	4.97	4.33	3.4	0.8	0	0	0

M_3T_{16}	51.13	15.13	29.33	59.77	14.67	55.33	95.67	66.4
M_3T_{17}	44.0	12.8	25.33	47.33	13.23	58.67	102.0	56.67
M_3T_{18}	49.4	14.57	28.33	55.63	13.97	56.0	98	63.67
M_3T_{19}	14.63	4.267	4.0	3.53	1.03	0	0	0
M_4T_1	48.10	13.97	23.33	53.4	13.77	56.67	98.33	59.97
M_4T_2	47.83	13.57	26.67	48.47	13.27	57.67	98.33	59.5
M_4T_3	45.87	13.63	26.0	49.3	12.37	57.67	100.67	54.97
M_4T_4	42.8	12.77	24.67	47.3	12.23	59.0	101.0	53.06
M_4T_5	44.03	12.57	25.33	44.83	12.47	59.33	102.33	57.5
M_4T_6	17.43	5.73	5.33	4.23	1.13	0	0	0
M_4T_7	17.07	5.8	4.67	4.2	1.23	0	0	0
M_4T_8	16.4	5.13	5.67	3.8	1.03	0	0	0
M_4T_9	45.06	13.77	25.33	48.0	12.17	58.33	101.33	52.83
M_4T_{10}	44.93	13.3	25.67	44.87	12.33	58..33	102.0	54.67
M_4T_{11}	16.53	4.97	5.33	3.87	1.17	0	0	0
M_4T_{12}	16.17	5.43	4.33	3.6	0.97	0	0	0
M_4T_{13}	16.9	4.83	4.67	3.23	0.93	0	0	0
M_4T_{14}	14.8	4.1	4.33	3.5	1.13	0	0	0
M_4T_{15}	15.23	4.2	4.33	3.63	1.03	0	0	0
M_4T_{16}	50.43	15.57	29.0	54.87	14.57	55.67	96.0	67.2
M_4T_{17}	44.17	12.73	25.67	43.57	12.57	58.67	102.3	55.83
M_4T_{18}	49.8	14.7	28.33	55.07	13.9	56.68	98.0	63.67
M_4T_{19}	14.0	3.93	4.0	3.37	0.93	0	0	0
CD(P=0.05)	1.276	0.5438	0.9975	2.7356	0.4900	NS	0.7404	NS

* Days counted from the day of sowing for days to flowering and harvesting

Value 'o' represents absence of observation due to wilting of plants.

M_1-seed treatment; M_2-soil drenching; M_3-Seed treatment + soil drenching and M_4-Root dipping

T1-*Trichoderma viride* T5-*A.niger* T9-*P.aeruginosa* T13-unidentified T17-*A.niger* (com)

T2-*T.pseudokoningii* T6-*A.viridi-nutans* T10-*B.subtilis* T14-mutant of *R.solanacearum* T18-*P.fluorescens* (com)

T3-*T.viride* T7-*Aspergillus* sp. T11-*B.cereus* T15-avirulent of *R.solanacearum* T19- control

T4-*T.virens* T8-*Mucor* sp. T12-*B.polymyxa* T16-*T.viride*(com)

From the Table 21 and 22, it is observed that, in the case of brinjal, no treatments could provide a better management of bacterial wilt disease as evident by the highest wilt incidence recorded at 60 days of planting. It is also noted that, a better management was noticed only up to 30 days in which T1 (*T.viride*) recorded 37.5 per cent against cent per cent in the check plot. The treatments T1, T2 and T5 which showed some performance till 30 days, have also recorded 97.5 per cent wilt incidence at 60 DAP.

From the Table 23 it appears that, the treatments differed significantly over control in the case of chilli.. Control plots showed cent per cent wilt incidence at 30 DAP, where as in treated plots, the maximum wilt incidence noticed was 47.15 per cent that was in plot treated with commercial *A.niger* (T8) by seed treatment + soil drenching method and 54.2 per cent incidence with commercial *B. subtilis* treatment (T10) by root dipping method. At the same time, minimum wilt incidence 29.15 and 38.85 per cent were observed with *T.viride* (T1) and *P.aeruginosa* (T5) in seed treatment and soil drenching method and root dipping methods respectively. The wilt incidence was found to increase subsequently as the days progressed.

From the data presented in Table 24 it is also evident that, as far as the wilt incidence is considered with different treatments, owest wilt incidence noticed in chilli was 52.75 per cent with *T. viride* (T1) per cent which cannot be considered as a very effective control. However, when compared to the check plot where cent per cent wilt incidence was noticed , it can be considered as a promising treatment in the management of bacterial wilt. Other treatments found effective was with *T.pseudokoningii* (T2) and *P.aeruginosa* (T5) which recorded 55.5 per cent wilt incidence. As far as the methods of application are considered, these lowest wilt incidence were recorded in seed + soil drenching method.

In the case of tomato (Table 25 and 26) also, same trend as in brinjal was noticed as the treatments did not have an impact on control of bacterial wilt. Eventhough, the

Table:21 Effect of different treatments and methods of application on per cent wilt incidence in brinjal at 15 – 45 days after planting

Treatments	Per cent wilt incidence		
	Days after transplanting (DAP)		
	15	30	45
M_1T_1	22.5	37.5	72.5
M_1T_2	27.5	42.5	72.5
M_1T_3	32.5	50.0	77.5
M_1T_4	42.5	62.5	82.5
M_1T_5	22.5	37.5	70.0
M_1T_6	32.5	52.5	75.0
M_1T_7	27.5	47.5	85.0
M_1T_8	37.5	72.5	92.5
M_1T_9	22.5	42.5	90.0
M_1T_{10}	30.0	52.5	87.5
M_1T_{11}	77.5	100.0	100.0
M_2T_1	25.0	72.5	97.5
M_2T_2	30.0	77.5	100.0
M_2T_3	50.0	77.5	95.0
M_2T_4	52.5	82.5	100.0
M_2T_5	37.5	75.0	97.5
M_2T_6	50.0	82.5	100.0
M_2T_7	50.0	77.5	97.5
M_2T_8	57.5	85.0	100.0
M_2T_9	45.0	75.0	100.0
M_2T_{10}	52.5	85.0	100.0
M_2T_{11}	87.5	100.0	100.0
CD (P=0.05)	9.994	9.005	12.159

M_1- Seed treatment + soil drenching
M_2- Root dipping
Mean of two replications

Table:22 Effect of different treatments and methods of application on per cent wilt incidence in brinjal under field condition at 60 days after planting

Treatments / Methods	Per cent wilt incidence										
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}
M_1	97.5	97.5	100.0	100.0	97.5	100.0	100.0	100.0	100.0	100.0	100.0
M_2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

CD(P=0.05) 4.527

M_1. Seed treatment and soil drenching
M_2 - Root dipping method
Mean of two replications

Table :23 Effect of different treatments and methods of application on per cent wilt incidence in chilli at 15-75 days after planting

Treatments	Per cent wilt incidence				
	Days after transplanting (DAP)				
	15	30	45	60	75
M_1T_1	22.2	29.15	36.05	43.0	52.75
M_1T_2	23.6	31.92	43.2	52.75	55.55
M_1T_3	26.1	31.9	40.2	48.6	55.55
M_1T_4	33.3	41.6	54.15	59.7	61.1
M_1T_5	19.4	31.93	41.6	51.4	55.5
M_1T_6	33.3	45.8	56.95	61.1	62.45
M_1T_7	27.75	38.8	44.4	52.75	56.9
M_1T_8	34.7	47.15	55.5	62.45	66.6
M_1T_9	24.95	36.05	45.8	51.40	56.9
M_1T_{10}	30.5	41.45	54.15	59.7	70.8
M_1T_{11}	58.3	100.0	100.0	100.0	100.0
M_2T_1	29.51	43.0	54.15	62.45	65.2
M_2T_2	34.7	43.0	55.5	62.7	66.6
M_2T_3	37.45	47.2	59.7	62.215	72.2
M_2T_4	37.45	53.9	65.25	72.2	72.2
M_2T_5	29.15	38.85	48.55	58.3	63.85
M_2T_6	45.8	52.75	63.85	72.2	76.35
M_2T_7	30.25	48.6	58.3	65.25	65.25
M_2T_8	45.8	52.75	62.45	66.6	73.55
M_2T_9	29.15	41.65	50.0	59.7	65.25
M_2T_{10}	38.85	54.2	59.75	72.2	72.2
M_2T_{11}	69.4	100.0	100.0	100.0	100.0
CD (P=0.05)	NS	NS	7.266	6.0776	NS

M1- Seed treatment + soil drenching M2- Root dipping
Mean of two replications

Table24. Effect of different treatments and methods of application on per cent wilt incidence in chilli underField condition at 90 days after planting

Treatments / Methods	Per cent wilt incidence										
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}
M_1	52.75	55.55	99.7	66.6	55.5	62.45	56.9	69.4	56.9	70.8	100.0
M_2	65.2	66.6	72.2	75.2	66.6	77.7	68.0	74.95	70.8	76.35	100.0

CD(P=0.05) 4.527

M_1- Seed treatment and soil drenching
M_2 - Root dipping method
Mean of two replications

Table :25 Effect of different treatments and methods of application on per cent wilt incidence in tomato at 15 – 30 days after planting

Treatments	Per cent wilt incidence	
	Days after transplanting (DAP)	
	15	30
M_1T_1	27.06	77.08
M_1T_2	33.33	77.08
M_1T_3	39.56	87.48
M_1T_4	39.55	93.74
M_1T_5	27.06	72.88
M_1T_6	35.4	83.3
M_1T_7	27.16	75.68
M_1T_8	43.75	95.74
M_1T_9	27.08	75.68
M_1T_{10}	43.71	85.4
M_1T_{11}	100.0	100.0
M_2T_1	43.75	91.66
M_2T_2	43.71	93.74
M_2T_3	45.8	97.91
M_2T_4	56.24	100
M_2T_5	43.75	91.66
M_2T_6	54.16	97.91
M_2T_7	41.6	95.83
M_2T_8	58.3	100
M_2T_9	45.8	93.71
M_2T_{10}	50.0	95.8
M_2T_{11}	100.0	100.0
CD (P=0.05)	NS	6.66

M_1 -Seed treatment + soil drenching
M_2-Root dipping

Table: 26 Effect of different treatments and methods of application on per cent wilt incidence in tomato under field condition at 45 days after planting

Treatments / Methods	Per cent wilt incidence										
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}
M_1	93.75	95.83	100.0	100.0	91.65	100.0	97.915	100.0	97.915	100.0	100.0
M_2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

CD(P=0.05) 4.527

M_1- Seed treatment and soil drenching
M_2 - Root dipping method
Mean of two replications

treatments T5 and T1 which showed some effectiveness at 15 DAP recording 27.06 per cent wilt incidence against cent per cent in control plots but recorded, 91.65 and 93.75 per cent wilt incidence at 45 DAP. Cent per cent wilt incidence was noticed in all treatments at 60 DAP. From the above findings, it is evident, that treatments with any antagonist in susceptible varieties, especially, Pusa Purple Long and Pusa Ruby cannot provide a control of bacterial wilt pathogen.

As the results obtained from the above treatments could not provide any beneficial effect, another experiment was laid out using a moderately resistant variety of tomato (Mukthi) to improve the resistance mechanism with antagonist treatments. The results are presented in Table 27 (Plate 7).

From the data (Table 27) it is observed that, the treatment *T.viride* (T1) followed by treatments *T.pseudokoningii* (T2) and *P.aeruginosa* (T5) were effective in controlling the disease to minimum extent recording 50, 58.3, 58.3 per cent respectively at 75 DAP. Of these, *T.viride* was found to give the lowest wilt incidence at all intervals and recorded the minimum of 50 per cent disease incidence at 75 DAP against cent per cent wilt incidence in control plots. From the table it is also noted that, eventhough wilt incidence were 50 and 58.3 per cent in *T.viride* (T1) and *P.aeruginosa* (T5) treatments, wilt index recorded were only 33.3 per cent showing a good effect of the antagonists. So, it can be concluded that a combined effect of host resistance and antagonist are necessary to achieve better management of bacterial wilt in natural condition.

The effect of different treatments in influencing biometric characters and yield in brinjal is presented in Table 28.From the table it is clear that, at 30 DAP, among the treatments, *T.viride* (T1) showed the maximum plant height of 18 cm.

In the case of chilli (Table 29) all treatments were effective in enhancing the growth characters when compared to control at 30 DAP. Of these, the maximum height of 17.25 cm was recorded in treatment with commercial *T.viride* (T7). As far as the treated

Table:27 Effect of different treatment on per cent wilt incidence in tomato var. Mukthi under field condition

Treatments	Per cent wilt incidence					Per cent wilt index
	Days after transplanting (DAP)					
	15	30	45	60	75	
T_1	0.0 (0.206)	8.3 (0.313)	24.95(0.517)	41.65(0.700)	50.0(0.785)	33.3
T_2	0.0(0.206)	16.6 (0.420)	41.65(0.700)	58.3(0.870)	58.3(0.870)	36.65
T_3	16.65 (0.410)	33.3 (0.615)	50.0(0.785)	83.3(1.150)	83.3(1.150)	53.3
T_4	16.6 (0.420)	41.65(0.700)	58.3(0.870)	74.95(1.052)	91.65(1.257)	65.0
T_5	0.0(0.206)	24.95 (5.517)	41.65(0.700)	50.0(0.785)	58.3(0.870)	33.3
T_6	0.0(0.206)	8.3(0.313)	24.95(0.517)	50.0(0.785)	66.6(0.955)	34.95
T_7	8.3 (0.313)	33.3 (0.615)	41.65(0.700)	58.3(0.870)	74.95(1.052)	61.65
T_8	8.3 (0.313)	24.95 (0.517)	33.3(0.615)	58.3(0.870)	74.95(1.052)	53.3
T_9	8.3 (0.313)	41.65 (0.700)	58.3(0.870)	74.95(1.052)	91.65(1.257)	48.3
T_{10}	8.3 (0.313)	41.65 (0.700)	50.0(0.785)	74.95(1.052)	83.3(1.150)	59.95
T_{11}	16.6 (0.420)	41.65 (0.700)	74.95(1.052)	91.65(1.257)	100.0(1.365)	78.3
CD(P=0.05)	NS	0.245	NS	0.246	0.244	20.99

Mean of two replications
Figures in parenthesis are transformed values

Table:28 Effect of different treatments on biometric characters of brinjal under field condition

Treatments	Plant height (cm)		Number of leaves		Days to flowering	Days to harvesting	Number of fruits/plant	Fruit wt/pt(g)
	30DAP	60DAP	30DAP	60DAP				
T_1	18.0	38.4	9.5	38	53	85	3	100
T_2	17.0	37.0	9.0	35	54	85	3	100
T_3	16.85	-	9.0	-	55	-	-	-
T_4	16.1	-	9.0	-	57	-	-	-
T_5	17.05	37.5	8.5	36	52	-	-	100
T_6	16.6	-	8.5	-	53	-	-	-
T_7	17.95	-	10.0	-	53	-	-	-
T_8	15.95	-	8.5	-	57	-	-	-
T_9	17.65	-	9.5	-	52	-	-	-
T_{10}	16.5	-	8.5	-	56	-	-	-
T_{11}	16.0	-	8	-	-	-	-	-

'-' represents no observations due to wilting of plants
Mean of two replications

Table: 29 Effect of different treatments on biometric characters of chilli under field condition

Treatments	Plant height (cm)			Number of leaves			Days to flowering	Days to harvesting	Number of fruits/ plant	Fruit wt/pt(g)
	30DAP	60DAP	90DAP	30DAP	60DAP	90DAP				
M_1T_1	17.9	29.63	37.83	19.5	59.25	120.75	54.25	85.25	29.0	29.15
M_1T_2	17.83	29.08	37.53	19.25	59.0	112.25	55.0	85.0	27.5	27.95
M_1T_3	17.0	26.23	36.3	18.0	59.0	119.5	54.75	85.25	25.75	27.2
M_1T_4	15.9	25.1	35.4	16.75	55.25	107.0	55.25	85.5	23.5	24.98
M_1T_5	17.78	28.86	37.8	19.5	59.0	120.0	54.75	85.25	26.75	28.88
M_1T_6	16.02	25.9	34.25	17.25	55.5	109.75	55.75	88.25	23.0	25.03
M_1T_7	18.22	30.0	38.1	20.0	61.0	124.25	54.25	84.25	27.75	28.38
M_1T_8	15.9	24.95	33.9	17.25	54.5	104.25	55.5	84.75	23.25	25.13
M_1T_9	17.35	29.65	36.9	18.0	57.5	121.75	54.0	84.5	27.75	29.38
M_1T_{10}	16.5	27.83	36.08	17.5	56.5	102.5	56.25	85.5	24.5	26.68
M_1T_{11}	15.1	-	-	14.0	-	-	-	-	-	-
CD (P=0.05)	0.6572	0.6183	1.3219	0.9903	2.346	5.919	1.153	NS	2.1951	3.332

'-' represents no observations due to wilting of plants
Mean of two replications

plots are concerned, commercial *T.viride* recorded the maximum plant height (38.1cm), number of leaves (124.25) and commercial *P.fluorescens* showed early flowering (54.0 days) and maximum number of fruits (29.0). Both commercial *T.viride* and commercial *P.fluorescens* recorded highest fruit weight (29.38g).

In tomato (Table 30), among the treated plots, maximum plant height (28.6 cm) was recorded in treatment commercial *P.fluorescens* while maximum number of leaves (11.0) was noticed in *T.viride* (T_1) at 30 DAP.

In the case of moderately resistant variety Mukthi (Table 31) treatment T1 (*T.viride*) was found to enhance all the biometric characters and yield as compared to control as well as other treated plots. It recorded for the maximum height (57.3cm) of the plant, number of leaves (43.5) and also the highest yield (1705.0g).

Study on rhizosphere microflora population was conducted in chilli, brinjal and tomato at 30, 60 and 90 days of planting and the results are presented in Table 32 and 34.

From the Table 32 it is evident that, the microbial population varied among the treatments. However, no considerable effect was noticed on population with different methods of applications in brinjal. An increase in total microbial population were noticed with days after planting whereas in the case of *R. solanacearum* the population was found to increase upto 60 days of planting and found to decrease towards the fag end of the crop.

It is observed from Table 33 that, microbial population varied with different treatments and was also found increasing with days after planting in chilli. Maximum population of the microflora was observed at 90 DAP in commercial *P.fluorescens* treated plots (T9). And in the case of methods of application, considerable difference was noticed only in case of bacterial population, in which root dipping was found to enhance the population. In the case of *R.solanacearum* also, a tremendous increase in population was noticed with increase in days after planting.

Table:30 Effect of different treatments on biometric characters of tomato under field condition

Treatments	Plant height (cm) 30DAP	Number of leaves 30DAP	Days to flowering
T_1	28.5	11	52
T_2	56.2	10	54
T_3	24.1	9	54
T_4	26.5	9	56
T_5	24.0	9	56
T_6	22.6	8	57
T_7	28.5	9	53
T_8	28.0	9	57
T_9	28.6	10	49
T_{10}	23.5	8	53
T_{11}	-	-	-
CD(P=0.05)	2.459	1.545	3.708

'-' represents no observations due to wilting of plants

Table:31 Effect of different treatments on biometric characters in tomato var. Mukthi under field condition

Treatments	Plant height (cm)	Number of leaves	Days of flowering	Days of harvest	No. of fruits/pt	Fruit yield/pt (g)
T_1	57.3	43.5	49.0	82.5	53.150	1705.0
T_2	55.1	42.5	50.5	81.5	55.350	1671.0
T_3	48.1	32.0	53.0	84.0	45.55	1305.0
T_4	46.9	17.0	56.0	86.5	29.85	911.5
T_5	52.9	41.5	52.5	81.0	50.16	1557.5
T_6	53.35	42.5	52.0	82.5	49.3	1543.5
T_7	55.35	43.5	49.5	81.0	52.6	1695.0
T_8	49.65	30.5	53.0	84.5	39.55	1014.5
T_9	43.75	25.0	54.5	87.5	24.35	918.5
T_{10}	40.25	16.5	58.5	83.5	31.45	983.5
T_{11}	34.35	15.0	57.5	84.5	6.0	270.0
CD	6.478	4.00	NS	NS	7.05	126.5

Mean of two replications

Figures in parentheses are transformed values

Table:32 Effect of different treatments on microbial population in brinjal rhizosphere soil under field condition

Treatments	Days after transplanting (DAP)											
	Fungi (10^3 cfu/g)			Bacteria (10^5 cfu/g)			Actinomycetes (10^5 cfu/g)			R.solanacearum (10^4 cfu/g)		
	30	60	90	30	60	90	30	60	90	30	60	90
M_1T_1	3.5 (0.540)	4.5 (0.651)	9.5 (0.977)	14 (1.145)	24 (1.380)	31 (1.491)	14 (1.230)	17 (1.145)	23 (1.361)	11 (1.040)	25 (1.398)	18 (1.278)
M_1T_2	6.5 (0.812)	6.5 (0.812)	11.5 (1.060)	3.5 (0.540)	13 (1.113)	25 (1.398)	3.5 (0.540)	10 (0.998)	10 (0.998)	10 (0.998)	17 (1.230)	20.5 (1.312)
M_1T_3	3 (0.477)	1.2 (0.151)	7.5 (0.874)	11 (1.040)	27 (1.431)	34 (1.531)	4.5 (0.651)	11.5 (1.060)	21 (1.322)	10 (0.998)	21 (1.322)	21 (1.322)
M_1T_4	4.5 (0.651)	5.5 (0.739)	6.5 (0.812)	16 (1.203)	27 (1.431)	37.5 (1.574)	1.5 (0.151)	6.5 (0.812)	9.5 (0.977)	4.5 (0.651)	24.5 (1.389)	21.5 (1.332)
M_1T_5	2.5 (0.389)	5.5 (0.739)	7.5 (0.874)	20 (1.300)	19 (1.278)	39 (1.591)	21.5 (1.332)	12 (1.078)	10 (0.998)	4 (0.602)	36.5 (1.550)	19 (1.278)
M_1T_6	3.5 (0.540)	3.5 (0.540)	11 (1.040)	8 (0.900)	18 (1.255)	28 (1.447)	4.5 (0.651)	11 (1.040)	7 (0.841)	5.5 (0.732)	25 (1.398)	23 (1.361)
M_1T_7	1 (0.000)	3 (0.477)	8 (0.900)	7.5 (0.874)	27 (1.431)	36 (1.556)	2 (0.301)	7.5 (0.874)	11 (1.040)	3.5 (0.540)	33.5 (1.525)	38 (1.580)
M_1T_8	3 (0.477)	4.5 (0.651)	8 (0.903)	10 (0.998)	33 (1.518)	34.5 (1.537)	11 (1.040)	10 (0.998)	6 (0.778)	8.5 (0.929)	34.5 (1.538)	22 (1.342)
M_1T_9	1.5 (0.151)	3.5 (0.540)	9 (0.952)	12 (1.078)	32 (1.505)	43.5 (1.638)	1 (0.000)	9 (0.952)	15.5 (1.190)	9 (0.952)	17 (1.230)	18 (1.255)
M_1T_{10}	1 (0.000)	3 (0.477)	8 (0.900)	14.5 (1.161)	34 (1.531)	25.5 (1.406)	3 (0.452)	11 (1.040)	10 (1.000)	9 (0.954)	27.5 (1.439)	19 (1.278)
M_1T_{11}	3.5 (0.540)	4.5 (0.651)	10 (0.998)	19 (1.278)	19 (1.278)	44.5 (1.648)	20.5 (1.312)	15 (1.175)	11.5 (1.060)	15 (1.380)	24.5 (1.538)	24 (1.322)

M_2T_1	3.5 (0.3890)	4.5 (0.651)	10 (1.060)	19 (1.040)	19 (1.398)	44.5 (1.537)	20.5 (1.0400	15 (0.998)	11.5 (0.812)	24 (0.778)	34.5 (1.079)	21 (1.290)
M_2T_2	4.5 (0.651)	2.5 (0.389)	12 (1.078)	12 (1.078)	27 (1.431)	37 (1.568)	5.5 (0.739)	12.5 (1.097)	5.5 (0.739)	8.5 (0.929)	10.5 (1.021)	19.5 (1.290)
M_2T_3	3.5 (0.540)	1.5 (0.151)	1.5 (0.929)	8.5 (0.929)	18.5 (1.266)	39 (1.591)	6.5 (0.812)	16.5 (1.217)	10.5 (1.021)	11.5 (1.060)	22.5 (1.352)	29.5 (1.469)
M_2T_4	3 (0.477)	5.5 (0.739)	11 (1.040)	10 (0.998)	18.5 (1.267)	28 (1.447)	8 (0.903)	8.5 (0.929)	7.5 (0.874)	9 (0.952)	19 (1.278)	19 (1.278)
M_2T_5	4.5 (0.651)	7.5 (0.874)	14 (1.145)	14 (1.145)	20.5 (1.312)	32 (1.505)	2.5 (0.389)	13 (1.113)	21 (1.322)	10.5 (1.021)	8.5 (0.929)	21 (1.322)
M_2T_6	3.5 (0.540)	5.5 (0.739)	13.5 (1.130)	13 (1.113)	23.5 (1.371)	34 (1.531)	4.5 (0.651)	9 (0.954)	13 (1.1130	8.5 (0.929)	17.5 (1.243)	21 (1.322)
M_2T_7	1.5 (0.151)	7.5 (0.874)	10 (0.998)	11.5 (1.060)	18 (1.255)	58.5 (1.767)	19 (0.278)	7.5 (0.874)	16 (1.203)	7.5 (0.874)	31 (1.491)	18 (1.255)
M_2T_8	2 (0.301)	6.5 (0.812)	8.5 (0.929)	10 (0.998)	27 (1.431)	47 (1.672)	13 (1.113)	20 (1.300)	15 (1.175)	14 (1.145)	20.5 (1.312)	17.5 (1.243)
M_2T_9	4.5 (0.651)	7.5 (0.874)	6 (0.772)	13 (1.113)	30 (1.4770	40 (1.602)	2.5 (1.389)	21 (1.322)	28.5 (1.455)	12.5 (1.097)	28.5 (1.455)	20.5 (1.312)
M_2T_{10}	3.5 (0.540)	5.5 (0.739)	9 (0.952)	15 (1.175)	35.5 (1.550)	44 (1.643)	1 (0.000)	17 (1.230)	31 (1.491)	25 (1.398)	22.5 (1.352)	19.5 (1.290)
M_2T_{11}	2.5 (0.389)	8.5 (0.929)	14 (1.145)	17 (1.230)	29 (1.462)	59 (1.771)	17 (1.230)	20.5 (1.312)	30.5 (1.484)	23.5 (1.371)	20.5 (1.312)	18 (1.255)
CD (P=0.05)	0.2062	0.1846	0.0480	0.0342	0.0274	0.0250	0.1445	0.0607	0.0672	0.0637	0.0356	0.0300

Mean of two replications
M1 Seed treatment + soil drenching method
M2 Root dipping method
Figures in parenthesis are transformed values

Table:33 Effect of different treatments on microbial population in chilli rhizosphere soil under field condition

Treatments	Days after transplanting (DAP)											
	Fungi (10^3 cfu/g)			Bacteria (10^5 cfu/g)			Actinomycetes (10^5 cfu/g)			R.solanacearum (10^4 cfu/g)		
	30	60	90	30	60	90	30	60	90	30	60	90
M_1T_1	1.5 (0.151)	4.5 (0.651)	11 (1.040)	16 (1.203)	20.5 (1.312)	31 (1.491)	2.5 (0.389)	6 (0.772)	14 (1.145)	16 (1.203)	11.5 (1.060)	21.5 (1.332)
M_1T_2	3.5 (0.540)	6.5 (0.812)	12 (1.078)	11 (1.040)	18 (1.255)	28 (1.447)	12 (1.078)	19 (1.278)	22.5 (1.352)	17 (1.230)	8.5 (0.929)	25.5 (1.406)
M_1T_3	3 (0.477)	4.5 (0.651)	10 (0.998)	11 (1.040)	17 (1.230)	32 (1.505)	3.5 (0.540)	12.5 (1.097)	20.5 (1.312)	8.5 (0.929)	11 (1.040)	19.5 (1.290)
M_1T_4	4.5 (0.651)	6.5 (0.812)	11 (1.040)	11.5 (1.060)	24 (1.380)	33 (1.518)	5.5 (0.739)	7.5 (0.874)	17 (1.230)	21.5 (1.332)	29 (1.462)	25 (1.398)
M_1T_5	5 (0.690)	8 (0.900)	15 (1.175)	25 (1.398)	24 (1.380)	38 (1.580)	1.5 (0.151)	10 (0.998)	17.5 (1.243)	12 (1.078)	6.5 (0.812)	29 (1.462)
M_1T_6	5 (0.699)	8 (0.903)	8 (0.900)	9.5 (0.977)	15 (1.175)	27 (1.431)	5 (0.690)	13 (1.113)	21 (1.322)	15 (1.175)	11 (1.040)	21 (1.322)
M_1T_7	5 (0.389)	11.5 (1.060)	9.5 (0.977)	12 (1.078)	14 (1.145)	26 (1.415)	9 (0.952)	7 (0.841)	27 (1.431)	17 (1.230)	19 (1.278)	23.5 (1.371)
M_1T_8	8 (0.900)	9.5 (0.977)	11.5 (1.060)	17 (1.230)	17.5 (1.243)	35 (1.544)	11.5 (1.060)	12.5 (1.097)	30.5 (1.484)	13.5 (1.130)	30 (1.471)	21 (1.322)
M_1T_9	4.5 (0.651)	11.5 (1.060)	8 (0.900)	12 (1.078)	21 (1.322)	25.5 (1.406)	2.5 (0.389)	21.5 (1.322)	25 (1.398)	9 (0.952)	12 (1.078)	34.5 (1.537)
M_1T_{10}	2.5 (0.389)	10 (0.998)	9 (0.954)	12 (1.079)	23.5 (1.371)	41 (1.613)	21 (1.322)	17.5 (1.243)	21 (1.322)	11.5 (1.060)	13 (1.113)	28.5 (1.455)
M_1T_{11}	6.5 (0.812)	11 (1.040)	14 (1.145)	17 (1.230)	27 (1.431)	38 (1.580)	1.5 (0.151)	21 (1.322)	18.5 (1.266)	21 (1.322)	25 (1.398)	24.5 (1.388)

M_2T_1	1.5 (0.151)	5.5 (0.739)	9 (0.952)	21.5 (1.332)	17 (1.230)	42 (1.623)	6.5 (0.812)	12.5 (1.097)	17 (1.230)	9 (0.952)	11.5 (1.060)	22.5 (1.352)
M_2T_2	3 (0.477)	7 (0.841)	8.5 (0.929)	11.5 (1.040)	20.5 (1.312)	37 (1.568)	8 (0.900)	16 (1.203)	20 (1.300)	8 (0.900)	11 (1.040)	19.5 (1.289)
M_2T_3	5 (0.690)	7.5 (0.874)	10 (0.998)	17 (1.230	23 (1.361)	26 (1.415)	7 (0.841)	9.5 (0.977)	7.5 (0.874)	5.5 (0.739)	15 (1.175)	23.5 (1.371)
M_2T_4	4.5 (0.651)	3 (0.452)	10.5 (1.021)	14.5 (1.161)	15 (1.175)	35 (1.544)	11.5 (1.060)	20.5 (1.312)	10 (0.998)	13.5 (1.130)	17 (1.230)	29 (1.462)
M_2T_5	5.5 (0.739)	8 (0.900)	11.5 (1.060)	19 (1.278)	27 (1.431)	39 (1.591)	2.5 (0.389)	12.5 (1.097)	8 (0.903)	9 (0.952)	9 (0.952)	25 (1.398)
M_2T_6	3.5 (0.540)	11 (1.040)	12.5 (1.097)	18 (1.255)	25 (1.398)	36.5 (1.562)	4 (0.588)	14.5 (1.161)	6.5 (0.812)	9.5 (0.977)	20.5 (1.312)	18 (1.255)
M_2T_7	2.5 (0.389)	12 (1.078)	11.5 (1.057)	8 (1.900)	10.5 (1.021)	27.5 (1.439)	5.5 (0.739)	25 (1.398)	11.5 (1.060)	11.5 (1.060)	15 (1.175)	11.5 (1.080)
M_2T_8	5.5 (0.739)	8.5 (0.929)	8.5 (0.929)	20 (0.300)	16 (1.203)	28.5 (1.454)	11 (1.040)	21 (1.322)	12 (1.078)	10 (0.998)	17 (1.230)	16 (1.203)
M_2T_9	6 (0.772)	12 (1.078)	9.5 (0.977)	14 (1.145)	28.5 (1.455)	44.5 (1.648)	8 (0.900)	21.5 (1.332)	27 (1.431)	11 (1.040)	12.5 (1.097)	13 (1.113)
M_2T_{10}	3.5 (0.540)	9.5 (0.977)	11.5 (1.060)	16.5 (1.217)	18 (1.255)	34 (1.531)	1.5 (0.151)	18.5 (1.267)	20 (1.300)	12 (1.078)	15.5 (1.190)	14 (1.145)
M_2T_{11}	5.5 (0.739)	11 (1.040)	14.5 (1.161)	19 (1.278)	28 (1.447)	46 (1.663)	9 (0.952)	25.5 (1.406)	25.5 (1.406)	21 (1.322)	18.5 (1.267)	32 (1.505)
CD (0.05)	NS	0.138	0.0976	0.0708	0.0209	0.0268	0.2185	0.0870	0.0669	0.0958	0.0702	0.0551

Mean of two replications
M1 Seed treatment + soil drenching method
M2 Root dipping method
Figures in parenthesis are transformed values

In case of tomato, it is also noticed that (Table 34), total microflora population varied with different treatments. However, methods of application had no effect on population. It is also observed that, microbial population was found increasing with days after planting. But, the *R.solanacearum* population showed a trend of decreasing with days after planting.

Table:34 Effect of different treatments on microbial population in tomato rhizosphere soil under field condition

Treatments	Days after transplanting (DAP)											
	Fungi (10^3 cfu/g)			Bacteria (10^5 cfu/g)			Actinomycetes (10^5 cfu/g)			R.solanacearum (10^4 cfu/g)		
	30	60	90	30	60	90	30	60	90	30	60	90
M_1T_1	7.5 (0.874)	12 (1.078)	11 (1.040)	14.5 (1.161)	11.5 (1.060)	12.5 (1.097)	8 (0.900)	15 (1.175)	12.5 (1.097)	12 (1.078)	22 (1.342)	13.5 (1.130)
M_1T_2	7.5 (0.874)	12.5 (1.094)	13 (1.113)	20 (1.300)	27 (1.431)	25 (1.398)	7 (0.841)	27 (1.431)	15 (1.175)	10.5 (1.021)	18.5 (1.241)	15.5 (1.190)
M_1T_3	7 (0.841)	7.5 (0.874)	9 (0.952)	47 (1.672)	51 (1.707)	47 (1.672)	4.5 (0.651)	15.5 (1.190)	9 (0.954)	8 (0.900)	17.5 (1.243)	14 (1.145)
M_1T_4	3.5 (0.540)	5.5 (0.739)	20 (1.300)	25.5 (1.406)	25 (1.398)	35.5 (1.538)	6.5 (0.812)	20 (1.300)	20 (1.300)	13 (1.113)	21 (1.322)	13 91.113)
M_1T_5	4 (0.588)	3.5 (0.540)	8 (0.900)	17 (1.230)	32.5 (1.512)	42 (1.623)	11 (0.040)	23 (1.362)	29.5 (1.470)	14 (1.145)	15.5 (1.190)	26 (1.415)
M_1T_6	2 (0.301)	3.5 (0.540)	6 (0.772)	23 (1.361)	30.5 (1.484)	34 (1.531)	8 (1.900)	13 (1.113)	15.5 (1.190)	8.5 (0.929)	13 91.113)	12.5 (1.097)
M_1T_7	4.5 (0.651)	3 (0.477)	3.5 (0.540)	15 (1.175)	21.5 (1.332)	28 (1.447)	9.5 (0.977)	17 (1.230)	14.5 (1.161)	11.5 (1.060)	19 (1.278)	12.5 (1.097)
M_1T_8	3.5 (0.540)	1.5 (0.151)	4 (0.588)	32 (1.505)	27 (1.431)	25 (1.398)	10 (0.998)	21.5 (1.332)	7.5 (0.874)	9 (0.952)	16.5 (1.2170	14.5 (1.161)
M_1T_9	4.5 (0.651)	2 (0.301)	5 (0.690)	27 (1.431)	35 (1.544)	41 (1.613)	5 (0.690)	27 (1.431)	8 (0.900)	6 (0.778)	13 (1.113)	14 (1.146)
M_1T_{10}	1.5 (0.151)	3.5 (0.540)	5 (0.690)	28 (1.447)	36 (1.556)	50 (1.699)	20.5 (1.312)	30.5 (1.484)	15 (1.176)	6.5 (0.812)	11 (1.040)	12.5 (1.097)
M_1T_{11}	10 (0.998)	8 (0.900)	19 (1.278)	28 (1.447)	34 (1.531)	47 (1.672)	15 (1.175)	30.5 (1.484)	22 (1.342)	12.5 (1.097)	22 (1.342)	28 (1.447)
M_2T_1	4.5 (0.651)	7 (0.841)	10 (0.998)	15.5 (1.190)	17 (1.230)	20 (1.300)	10.5 (1.021)	21.5 (1.332)	17 (1.230)	9.5 (0.977)	21.5 (1.332)	20 (1.300)

M_2T_2	7 (0.841)	5 (0.690)	12 (1.078)	25 (1.398)	25.5 (1.406)	26 (1.415)	6.5 (0.812)	16.5 (1.217)	20.5 (1.312)	8 (0.900)	18 (1.255)	20 (1.300)
M_2T_3	8 (0.900)	6 (0.772)	9 (0.952)	32 (1.505)	29 (1.462)	22 (1.342)	13 (1.113)	21 (1.322)	34.5 (1.532)	17 (1.230)	27 (1.431)	19 (1.278)
M_2T_4	6 (0.772)	8 (0.900)	9 (0.952)	21 (1.322)	24 (1.380)	30 (1.447)	19 (1.278)	24 (1.380)	12.5 (1.097)	15 (1.175)	15.5 (1.190)	30 (1.477)
M_2T_5	5 (0.699)	7 (0.841)	6.5 (0.812)	28 (1.447)	36.5 (1.562)	37 (1.568)	3 (0.477)	13.5 (1.130)	5 (0.690)	19 (1.278)	18.5 (1.261)	19 (1.279)
M_2T_6	4.5 (0.651)	6 (0.772)	5 (0.690)	32 (1.505)	36 (1.556)	39.5 (1.597)	9 (0.952)	17 (1.230)	8.5 (0.929)	12 (1.078)	22 (1.267)	20 (1.300)
M_2T_7	3.5 (0.540)	7 (0.841)	17 (1.230)	24 (1.380)	23 (1.361)	28 (1.447)	6 (0.772)	26 (1.415)	23 (1.361)	7 (1.841)	11 (1.342)	17.5 (1.243)
M_2T_8	2.5 (0.389)	1.5 (0.151)	11 (1.040)	25.5 91.406)	25 (1.398)	29 (1.462)	13 (1.113)	22.5 (1.352)	19 (1.278)	9 (0.952)	11 (1.040)	19 (1.278)
M_2T_9	3.5 (0.540)	3.5 (0.540)	13 (1.113)	31.5 (1.498)	37 (1.568)	37 (1.568)	13.5 (1.130)	31.5 (1.498)	15.5 (1.190)	5.5 (0.739)	18 (1.255)	20.5 (1.312)
M_2T_{10}	9 (0.952)	5 (0.690)	9.5 (0.977)	28 (1.447)	34 (1.531)	38 (1.580)	5.5 (0.739)	11 (1.040)	17 (1.230)	7 (0.841)	14.5 (1.161)	23 (1.362)
M_2T_{11}	9.5 (0.972)	10 (0.998)	13 (1.113)	35.5 (1.550)	28 (1.447)	37 (1.568)	22.5 (1.352)	22 (1.342)	21 (1.322)	14.5 (0.161)	25.5 (1.406)	24.5 (1.389)
CD (0.05)	0.1988	0.3407	0.0433	0.0433	0.0327	0.1073	0.0581	0.0820	0.0687	0.0595	0.1961	0.0413

Mean of two replications
M1 Seed treatment + soil drenching method
M2 Root dipping method
Figures in parenthesis are transformed values

Discussion

5. DISCUSSION

Bacterial wilt caused by *R.solanacearum* is a globally distributed disease, affecting more than 200 plant species including many economic important families, particularly Solanaceae which includes tomato, brinjal and chillies. It has been ranked as most important disease because of its destructive economic impact. The warm humid tropical climate and acidic soils of Kerala are conducive for the development of bacterial wilt. Crop loss upto 100 per cent have been reported in the susceptible varieties of solanaceous crops. The causal bacterium, *Ralstonia solanacearum,* is soil borne and very difficult to control because of their multiplication, both phenotypic and genotypic diversity and continual persistence in soil. Though chemicals offer certain degree of protection against the pathogen, chemical control has its own constraints such as the cost factor, pollution etc. Breeding for resistance was considered as best control strategy which are also found fluctuating due to the extreme variability of the pathogen. So, there is a considerable interest in manipulating the soil community to achieve the biocontrol of this soil borne pathogen. During recent years, plant pathologist all over the world give emphasis on biocontrol methods in combating crop diseases and it is also becoming an important component of plant disease management.

A perusal of literature revealed that, much emphasis has not been given in Kerala so far, for the biocontrol of bacterial wilt pathogen using microbial antagonists. In view of the above facts and considering the importance of bacterial wilt disease in Kerala, the present investigation was carried out to study the effect of biocontrol of bacterial wilt, which will add to our knowledge especially about the control of the pathogen with microbial antagonists. Even though bacterial wilt disease is a serious problem all over Kerala, the present study has been limited to only two locations viz. Vellanikkara (Thrissur district) and Ozhalapathy (Palakkad district) representing high wilt incidence and low wilt incidence areas respectively. The attempt to estimate the population of *R.solanacearum* in

these areas revealed that Vellanikkara soil recorded highest population of the bacterium ranging from 1.8 to 6.3 x 10^7 cfu/g soil whereas in Ozhalapathy soil , the *R.solanacearum* population was only 1.01 – 3.6 x 10^7 cfu/g soil which indicate the main reason for the high and low incidence of bacterial wilt in these areas. Further, the studies on soil type, soil pH and soil temperature also provided enough explanation to support this factor. In Vellanikkara, the main soil type was laterite loam with soil temperature of 44.4 to 45.6°C and soil pH ranged from 6.5-6.9 whereas, in Ozhalapathy, the main soil type was black soil with a pH of 7.4 –7.7 and temperature ranging from 26.6 – 27.7°C. Hence, it is clear that, soil type as well as the acidic nature of the soil and high soil temperature are the factors which favour the growth of *R.solanacearum* population in Vellanikkara soil leading to high wilt incidence. This view is supported by the findings of Chupp and Sherf (1960) who reported that, bacterial wilt infection occurs in dry soil and the disease is serious in red laterite soil. Keshwal *et a*l. (2000) also observed that, clay soil harboured maximum population of bacterial wilt pathogen. Shekhawat *et al.* (1978) reported that, the bacterial wilt of potato was more widespread in heavy and acidic soil (pH 3.5 to 6.9) than in light and neutral (pH 6.5 to 7.5) to alkaline (pH 7.5 to 8.5) soils. Hingorani *et al.* (1956) noted that, increase in soil temperature from 21 to 35°C favoured the development of bacterial wilt of potato. Acosta *et al.* (1964) had also noticed that, tomato bacterial wilt infection was more severe during the summer at high soil temperature.

Isolation of the pathogen from wilted plants of brinjal, chilli and tomato from these two locations showed typical colonies of *R.solanacearum* with circular, smooth, raised, creamy white with pink centre and with entire margin on TZC medium. The inoculation with the bacterial suspensions of different isolates of *R.solanacearum* on the respective hosts established the pathogenicity of the organisms and the inoculation of the isolates on different hosts indicated the cross inoculable nature of pathogen. As the main aim of the present study was the management of bacterial wilt pathogen using microbial antagonists, attempt was made to isolate the microorganisms from the rhizosphere soil of healthy solanaceous plants of both susceptible and resistant varieties. Rhizosphere is the zone

where interactions between soil microorganisms and plants takes place. It is a region of intense microbial activity driven by root exudation (Bowen and Rovira, 1999). Beneficial free living bacteria can be found among them. (Chanway *et al.*1991). Additional care was taken to isolate the organisms from solarised and forest soils also.

As far as the locations were considered, maximum microbial population was found in Ozhalapathy area. The alkaline nature and low soil temperature might be favouring the microbial growth in this area. With respect to the varieties, resistant variety of different hosts showed high population than the susceptible ones. The possible explanation for such association of high microflora in the resistant genotype may be due to the high content of polysaccharides in the root exudates which favour the microbial growth or has got a direct action on the preferential colonization of microorganism. Paul (1998) also noticed increased population of microflora in the resistant genotypes of chilli, brinjal and tomato than the susceptible varieties.

Rhizpsphere microorganisms isolated were then tested against different isolates of *R.solanacesarum* for their antagonistic property under *in vitro* condition. The potential antagonists under *in vitro* studies were selected and screened for their performance in pot culture. Promising antagonists in this study were further tested for the management of bacterial wilt pathogen under field condition. The antagonists performed well in the final evaluation were selected and identified.

Fungal antagonists were identified based on cultural and morphological characters. The information on these characters were compared with the descriptions documented by various workers. For the identification of bacterial antagonists cultural and various biochemical characters were taken into consideration and were identified based on the key described by the earlier workers. Basic characters of the fungal and bacterial antagonists observed in the present studies were in agreement with the description of various workers. Accordingly, the potential fungal antagonists were identified as *Trichoderma viride* (F 30,

chilli ,Ozh), *T.pseudokoningii* (F 143, Forest soil), *T.viride* (F 29, chilli, Vka), *Aspergillus viridi-nutans* (F4, tomato, Ozh), *T.virens* (F 10,tomato,Ozh), *T.viride* (F3,tomato, Ozh), *T. harzianum* (F 140, tomato, Ery) *A.niger* (F 141, tomato, Ery), *Mucor sp.*(F 142, tomato, Ery) and the promising bacterial antagonists were *Pseudomonas aeruginosa* (B 4, tomato, Ozh) *P.fluorescens* (B 6, brinjal, Vka) *Bacillus subtilis* (B 124, brinjal, Vka) *B.cereus* (B 125, brinjal, Vka) and *B.polymyxa* (B 19, chilli, Ozh).

The organisms isolated from different rhizosphere soils were first screened for their antagonistic activity against *R.solanacearum* under *in vitro* condition. Out of 90 fungi isolated, 23 fungi showed antagonistic activity against the different isolates of test pathogens. Most of them were found belonged to the species of *Trichoderma* and *Aspergillus* of which *Trichoderma* sp. were more effective. Among *Trichoderma* spp., *T.viride* (Ozh) and *T.pseudokoningii* were the promising ones by recording a maximum Antagonism Index value of 6000 and showed complete inhibition of all the six isolates of *R.solanacearum* by its lysis and overgrowth type of antagonistic property. Similar phenomena like complete covering of pathogen and lytic nature of *Trichoderma* were observed by D'Ercole *et al.* (1984). The possible reason for the lysis of the pathogen by *T. pseudokoningii* may be due to the production of certain inhibitory metabolites as evidenced by the presence of deep yellow colour in the medium. There is report on the production of non-volatile sequisterpene like trichodermin, dermadin, trichoviridin and the production of β-(1-3) glucanase, chitinase and protease enzymes which are also capable of degrading the cellwalls of *R.solanacearum*. The mechanism of antagonisms by antibiosis and by the cellular lytic enzymes of *T.pseudokoningi* is already reported by Gayathri and Murugesan (1994). Similar to the result obtained in the present study, Silveira *et al.* (1996) also reported *in vitro* inhibition of *R.solanacearum* by *T.pseudokoningii*. In some cases, only the overgrowth of the fungus was observed without lysis, which indicate direct antagonism on the pathogen. Added to them, *T. virens* and various other species of *Trichoderma* including commercial ones also exhibited antagonism against the test organism. Das *et al.* (2000) reported the antagonistic activity of *T.viride, T.virens, T.harzianum* against

R.solanacearum under dual culture. It is interesting to note that, most of the *Trichoderma* isolated from Ozhalapathy areas were more antagonistic than Vellanikkara .

The next potential fungal antagonists were *Aspergillus* spp which showed antagonistic property against *R.solanacearum* mainly by its overgrowth mode of antagonism. Of these, *A. niger* showed better antagonism against five isolates of the pathogen. Das *et al.* (2000) observed the effect of *A.terreus* on *R.solanacearum*. A search through the relevant literature did not give any information about antagonistic property of the aforesaid organisms against *R.solanacearum*. It is also worthwhile to mention that, the fungal species showing effective antagonistic property were all isolated from the wilt suppressive region of Ozhalapathy and Eruthyampathy. This is in accordance with the findings of Weller (1988) who observed that, suppressive soils were the sources of useful microorganisms.

On comparison with commercially available fungal antagonists like *T.viride*, *T.harzianum* and *A.niger* also yielded good results against *R.solanacearum*. A study conducted to improve the antagonistic efficiency of the promising antagonists obtained in the present study by interspecific hybridization did not provide a better result than the individual culture. This might be due to the competition among the isolates and which might have nullified their individual effect. Likewise, mutants of different *Trichoderma* sp. did not show any superior effect than the original ones. However, it is a novel experiment with regard to the fungal antagonism, as these type studies are not conducted so far against the bacterial wilt pathogen.

Among the different bacterial organisms tested, only nine showed antagonistic activity by exhibiting the lysis of the test pathogen. Of them, *P.aeruginosa* showed the maximum inhibitory effect against *R.solanacearum* with an AI value of 133.3 followed by the commercial *P.fluorescens* (AI value 111.1). *B.subtilis* isolated from Vellanikkara also

showed inhibitory effect on chilli and brinjal isolates giving an AI value of 88.8 and 53.2 respectively. The lytic activity exhibited by bacterial antagonist is mainly due to their lytic enzymes. Moreover, the fluorescent *Pseudomonads* produce iron sequestering siderophore which can also inhibit the growth of pathogen. Siderophore like Pyochelin, Pyoverdine, Pseudobactin, ferribactin, ferrichrome and ferroxamine are secreted by *Pseudomonads*. *B.subtilis* produce antagonistic protein which are strongly inhibitory to bacteria.

In addition to these, *B.polymyxa*, *B.cereus and P.fluorescens* were also found exhibiting lysis of the test bacterium. The possibility of using *P.aeruginosa, B.subtilis, B.polymyxa, B.cereus, P.fluorescens* as bioagents was investigated with those results reported by Furuya *et al.* (1997). Akbar (2002) also observed the antagonistic activity of *P.aeruginosa* against *R.solanacearum* under *in vitro* condition. Sunaina *et al.* (1997) reported the antagonism against *R.solanacearum* of potato by *B.subtilis* and *B.cereus*. Opina and Valdez (1987) showed the existence of antagonistic activity of *P.fluorescens* and *B.polymyxa* against *R.solanacearum*. Ciampi *et al.* (1996) observed that, the siderophore like compound produced by *P.fluorescens* was responsible for inhibition of *R.solanacearum* and also reported its synthesis is dependant on Fe^{3+} levels in the culture medium. It is also interesting to note that, bacterial organisms isolated from the resistant varieties of brinjal (Vka) showed antagonistic reactions as compared to chilli and tomato.

The test conducted with an avirulent form of *R.solanacearum* exhibited better antagonism by recording an AI value of 54.4 on the virulent *R.solanacearum*. The present finding is in agreement with Chun *et al.* (1999) who also noticed that, avirulent strain of *R.solanacearum* is inhibitory to virulent strain of tomato. The mutant of *R.solanacearum* also showed its antagonistic property against two isolates of *R.solanacearum* supporting the findings of Arwiyanto *et al* . (1994). However, Alice and Carlos (1996) observed no inhibitory action of the mutant against *R.solanacearum*. In the present study, the attenuated or heat killed *R.solanacearum* failed to show antagonistic property. But the inhibitory

effect of heat killed cells of *R.solanacearum* on bacterial wilt pathogen was reported by Singh (1997).

Studies on the antagonistic effect of actinomycetes on *R.solanacearum* indicated that,out of 57 organisms tested, only four were inhibitory to the test pathogen. Among them, A 25 isolated from Ozhalapathy soil showed the highest Antagonism Index value of 283, with lysis type of antagonism, in which mechanism of antagonism worked will be the same as that of bacterial antagonists. Shanshoury *et al.* (1996) reported the *in vitro* inhibition of *P.solanacearum* by *Streptomyces mutabills*. Moura *et al.* (1998) also confirmed the antagonistic activity of actinomycetes against *R.solanacearum*.

The results obtained so far from the above studies indicate that, the organisms isolated from Ozhalapathy soils are best antagonists which once again confirms that the suppressive soils are the sources of useful microorganisms for controlling the bacterial wilt pathogen. It is interesting to note that, majority of the antagonists were most effective against chilli isolate of *R.solanacearum* as compared to other isolates and the tomato isolate showed least response to the microbial antagonists. It may be due to the virulence of strain variation among the different isolates and is beyond the scope of present study to go much deeper into this aspect.

The antagonistic property of bacteria as well as actinomycetes against *R.solanacearum* are well studied and documented by various workers. However, very few attempts have been reported on the fungal antagonistic activity against *R.solanacearum*. The present study reveals that, fungi are the most potential antagonists against *R.solanacearum* than that of the bacteria as reported by various other workers. So, the findings of this study may open up a new approach for the management of the bacterial wilt by biological means.

The next aspect of investigation was to find out the effect of culture filtrates of the potential bacterial and fungal antagonists to study the type of mechanism, whether the antibiosis or direct antagonism are working in these antagonists against *R.solanacearum*. Among the bacterial culture filtrates tested, *B.subtilis* (com) was found to be more effective followed by isolated *B.subtilis* from Vellanikkara. It may be due to the production of certain antibiotics. It is interesting to note that, eventhough the bacterial antagonist did not show good antagonistic reaction against tomato isolates in the bioassay studies, the culture filtrates of these antagonists showed maximum inhibition on tomato isolates of the pathogens indicating that, the main mechanisms of antagonism is by antibiosis. *B.subtilis* produces iturin, bacilycin and fengacin antibiotics which can inhibit various fungal and bacterial pathogens. Phae *et al.* (1992) also observed the suppressiveness expressed, by the culture filtrate of *B.subtilis* against *R.solanacearum*, was by the extracellular production of iturin.

With regard to fungal culture filtrates, better inhibitory effect on *R.solanacearum* was observed with *T.virens* and *T.pseudokoningii*. It is also noted that, the colour of culture filtrates of these antagonists were yellowish green and deep yellow respectively, indicating that the inhibitory action of the culture filtrate might be due to the production of inhibitory volatile metabolites. The culture filtrates of *T.harzianum* had the least inhibiting action indicating that it acts mainly by direct antagonism. On the other hand, *T.pseudokoningii* probably produces antibiotics alone, hence exhibited the maximum inhibition of the pathogen. One isolate of *T.viride* (Ozh) also showed some inhibitory action appended to have acted both by direct parasitism and antibiosis. Similar to this finding, Dennis and Webster (1986) also observed the inhibitory action of the culture filtrates of *Trichoderma* sp. against *Macrophomina phaseolina*. But the inhibitory effect of fungal culture filtrates on bacterial wilt pathogen is studied for the first time.

The next point of consideration was to find out the performance of the promising antagonists under pot culture condition. In pot culture studies, most of the *Trichoderma*

spp. *Aspergillus* spp. *Pseudomonas* sp and *Bacillus* spp. were effective in reducing the wilt incidence. In the case of chilli crop, *T.viride* (Ozh), *T.pseudokoningii*, *P.aeruginosa* showed complete protection against the wilt pathogen as no wilt incidence were noticed till the complete cropping period of 120 days. In brinjal, *T.viride* (Ozh) and *T.pseudokoningii* recorded the lowest wilt incidence of 16.65 and 18.73 per cent respectively in which 114 and 110 days were taken for the wilt appearance. In tomato also, *T.viride* (Ozh), *T.pseudokoningii*, *P.fluorescens* were most effective in reducing the wilt incidence to 24.28 per cent. It is also noted that, even though different bacterial antagonist were found effective in reducing the wilt incidence, mutant or the avirulent *P.solanacearum* have recorded cent per cent incidence showing its ineffectiveness. As a whole, *T. viride* (Ozh), *T.pseudokoningii* were the most effective antagonists for all the three crops. In addition to the above, *P.aeruginosa*, *B.subtilis*, *T.viride* (Vka), *A.niger* (Ery), *T.viride* (com) and *P.fluorescens* (com) were also found to give better control of bacterial wilt of chilli, brinjal and tomato. There are number of ways by which antagonists suppress the growth of pathogen and control the disease (Cook, 1990). Of which, production of antibiotics, volatile compounds, lytic enzymes, induction of host resistance and antagonistic proteins are important. *Trichoderma* species has been proved to be potential tools in the control of many plant pathogenic fungi by various workers. However, the information on its efficacy on bacterial pathogens especially in pot or field conditions are rather lacking. The possibility of using *B.subtilis* and *P.aeruginosa* as bioagents was investigated with promising results (Anuratha and Gunanamanickam, 1990; Piexoto, 1995a; Abdalla *et al.*, 1999; Akbar, 2002). Thus, our findings are in confirmity with the above results. As the pot culture studies are concerned, it is worthy to mention that, use of microbial antagonists is effective in the management of bacterial wilt pathogen, *R.solanacearum*. The possible factors attributed for the low wilt incidence in pots may be due to less competition, less root injury, controlled irrigation, low inoculum level. More over, the enhancement of soil temperature with irrigation in pot which is almost similar to that of solarised soil, and is always be more than that of the normal soil, (>10°C) which may be unfavourable for the multiplication and survival of pathogens that may also reduce the wilt incidence.

Among the various methods of applications such as seed treatment, soil drenching, seed treatment +soil drenching and root dipping adopted, all were found equally effective. However, seed treatment +soil drenching and root dipping alone provided the maximum reduction of the wilt incidence as well as in delaying the wilt appearance. The present finding is in agreement with Akbar (2002) who also reported the seed treatment + soil drenching of *P. aeruginosa* was more effective in reducing the wilt incidence than when it was applied alone. In addition,effective disease suppression by root dipping method was observed by several workers. (Opina and Valdez,1987; Furuya *et al.*,1991; Furuya *et al.*,1997) Karuna *et al.* (1997) observed that seed treatment and root dipping of Pusa Ruby seedling with *P.fluorescens* was effective in reducing the incidence of wilt by 50 per cent under field conditions. Sunaina *et al.* (1997) also noticed that, seed treatment with *B.cereus* and *B.subtilis* reduced wilt incidence, with increased yield in potato under field conditions.

For the better understanding of the establishment of the antagonists under natural condition, an investigation was conducted to study the efficacy of different antagonists under field condition, which has not provided any valuable information on the control of bacterial wilt pathogen. No satisfactory control was obtained with microbial treatments in susceptible varieties of brinjal and tomato except chilli. In case of chilli, even though the lowest wilt incidence noticed was 52.75 per cent with *T.viride* (Ozh) treatment, it can be considered as a good control compared to the cent per cent wilt incidence in control plot. Another important factor noticed is that, treatment with antagonists could delay the wilt appearance in plants which can also provide some yield instead of complete loss.

In reviewing the effect of antagonist *in toto* showed the drop in inhibition on pathogen when the study is gradually shifted from *in vitro* set up to field condition. There has been little evidence that, these biocontrol agents are effective against bacterial wilt

pathogen in the field. Anuratha and Gnanamanickam (1990) obtained 49 and 36 per cent protection against bacterial wilt in brinjal and tomato seedling using *P.fluorescens* in field condition.

As compared to pot culture condition, bacterial wilt incidence is always high in field condition. Root injury occur during the cultural practices facilitate easy entry of the pathogen, spread of the bacterium through irrigation water, high inoculum level in the soil are some of the factors that favour the wilt incidence in the field. With regard to the ineffectiveness of biological control using microbial antagonists, the main reason is that, under field condition the establishment of the antagonists might have been affected by the competition with other soil pathogens. Biological control depends upon the establishment and maintenance of a threshold population of antagonist in the soil. So, more work is needed to develop better delivery techniques and to understand more about the soil ecology so that, antagonist activity can be enhanced.

It is also understood that, management of bacterial wilt using biocontrol means in highly susceptible varieties are not feasible for bacterial hot spot soils of Kerala. The combination of host resistance with additional specific management practices are the cheapest strategies for integrated management of this disease and also one of the satisfactory methods from an ecological point of view.

Mukthi, a tomato variety considered as resistant to bacterial wilt in Kerala is found fluctuating due to the variability of the pathogen. So, an attempt was made to improve its resistance by an integrated approach with biocontrol antagonists which not only inhibit the pathogens but also induce systemic resistance against the pathogen. In the present investigation, a combination of host resistance and biocontrol with antagonists revealed a better control of *R.solanacearum* by reducing the wilt incidence. The wilt severity was also reduced considerably which led to the increase in the crop yield. This result confirm

the finding of Akbar (2002) that, biological control is effective only when combined with host resistance under Kerala condition. Furuya *et al.* (1997) noticed increase in survival of tomato seedling with *P.aeruginosa* in wilt sick soil and also observed that, mechanism of induced resistance and infection sites competition were involved in suppression of disease.

Apart from suppresion of the pathogens, certain biocontrol agents may contribute to the enhancement of crop vigour and thereby increase the crop yield. Among the promising fungal and bacterial antagonists, *Trichoderma* spp. were found to be most effective to enhance the biometric characters of the solanaceous crop in pots as well as in field conditions. Among the *Trichoderma* spp., *T.viride* was found to be the best in increasing the crop vigour and yield. Mechanism involved in this phenomenon might be due to the elimination of pathogens in the rhizosphere and production of growth promoting substance. The above results are in agreement with the findings of Krishnamoorthy (1987) and Neelamegam and Govindarajan (2002) who reported increased seedling vigour in tomato with *T.viride*. Early germination and enhanced growth of the plants in the presence of *T.harzianum* has been reported by Chang *et al.* (1986). Manomohandas and Sivaprakasam (1993) also observed enhanced growth vigour in chilli with *Trichoderma* sp. and seed treatment + soil application of *Trichoderma* was found to be the best one. Sivaprasad (1999) also reported the growth stimulatory effects of *Pseudomonads* isolated from Kerala soil and observed that, the organisms produce secondary metabolites and plant growth stimulatory hormones like IAA.

The next aspect to be discussed is the effect of antagonistic treatment on total population of soil microflora and it is noticed that, microbial population was found increasing subsequently with days after planting. In case of chilli and brinjal, an increase in total microbial population was noticed up to 90 days whereas in case of tomato, microbial population was found to decrease after 60 days of planting. With regard to *R.solanacearum*, an increase in population was noticed with days after planting in the case of chilli, whereas in the case of brinjal and tomato no noticeable change in population was

observed after 60 days of planting. Devi (1978) observed an increasing trend in bacteria and fungi but the actinomycetes population did not show any definite trend of increase or decrease when soil amendments were added to soils planted with tomato.

Summing up the findings so far, it was observed that, biocontrol agents were effective in inhibiting the pathogen in *in vitro* as well as in pot culture conditions. However, they were less effective under field condition. Hence, extensive research on ecological studies on both target pathogen and the biocontrol agent is needed to obtain economical control of disease.

Recalling back the results observed in the present study so far, it is also evident that, fungal antagonists especially *T.viride* (Ozh) and *T.pseudokoningii* played a vital role in the inhibition of bacterial wilt pathogen, *R.solanacearum*. Therefore, the exploitation of these bioagents for the management of bacterial wilt is highly promising.

Summary

Bacterial wilt caused by *R. solanacearum* is one of the major constraint in cultivation of brinjal, chilli and tomato in Kerala .It accounts for nearly 100 per cent of crop loss in the case of susceptible varieties. Soil borne nature, wide host range, variation in strains, make control measures less effective. In the light of hazards caused by the chemicals and economic threat posed on farmers, biological control especially with microbial antagonist is gaining importance. In this view, present investigation was carried out to evaluate the effect of microbial antagonists against the bacterial wilt pathogen *R. solanacearum*. The salient findings of the present study are summarized here.

1. *R.solanacearum* were isolated and the pathogenicity of organism was established on respective host as well as on other two solanaceous crops.
2. Maximum population of *R. solanacearum* (1.8-6.3 x 10^7 cfu/g) was recorded in Vellanikkara than Ozhalapathy (1.01-3.06 x 10^7 cfu/g).
3. Soil pH and soil temperature varied with the two locations, in which soil pH were 6.1-6.9 and 6.0-7.7 and soil temperature was 44.4-45.6 °C and 26.6-27.7 °C in Vellanikkara and Ozhalapathy respectively.
4. Among the two locations, the total microflora population was high in Ozhalapathy than Vellanikkara. Among the different microflora, fungi and actinomycetes were more predominant in Ozhalapathy while bacterial population was more in Vellanikkara soil. The resistant varieties of these crops recorded better association of microflora than the susceptible ones.
5. Organisms isolated from low wilt incidence area (Ozhalapathy) showed better antagonism than those isolated from high wilt incidence area (Vellanikkara).
6. Fungi were found more effective than bacterial and actinomycete antagonist.
7. The major fungal antagonists effective against *R. solanacearum* belonged to *Trichoderma* spp. and *Aspergillus* spp.
8. *T.viride* (Ozhalapathy),*T.pseudokoningii* (forest soil), *A.niger* (Eruthyampathy) were the most effective antagonists against *R.solanacearum* under *in vitro* condition.

9. Mutants and interspecific hybrid of *Trichoderma* spp. were also found effective.
10. Bacterial organisms isolated from KAU resistant genotypes of brinjal exhibited more antagonistic property and the isolates B4 (*P.aeruginosa*) and B124 (*B.subtilis*) were the most effective ones.
11. Avirulent and mutant strains of *R. solanacearum* also showed antagonistic property whereas, heat killed cells of *R. solanacearum* were ineffective.
12. The commercial antagonists viz.. *T. viride*, *A. niger* (Varanasi), *A niger* (AN-27), *T. harzianum*, *B. subtilis* and *P. fluorescens* were found effective against *R. solanacearum*
13. Actinomycetes isolated from brinjal (Ozhalapathy) showed maximum antagonism against *R. solanacearum*
14. In general, the antagonists were found most effective against chilli isolates of *R.solanacearum* rather than brinjal and tomato isolates.
15. Among the different culture filtrates tested, *T. virens* and *B. subtilis* (commercial) showed the maximum inhibition of the pathogen.
16. The most of the antagonists which were found effective in laboratory condition performed well under pot culture studies also. Among the fungi, *T.viride* (Ozhalapathy) *T.pseudokoningii* (forest soil), *A. niger* (Eruthyampathy) and bacterial antagonists *P.aeruginosa* (Ozhalapathy) and *B. subtilis* (Vellanikkara) were the promising antagonists against the bacterial wilt pathogens of chilli, brinjal and tomato.
17. All commercial antagonists were found equally effective as that of the isolated ones in pot culture studies also.
18. The mutant and avirulent strains of *R. solanacearum* which showed inhibition of *R. solanacearum* in laboratory conditions did not show antagonism in pot culture study.
19. Among different methods of application of antagonists, either seed treatment + soil drenching or root dipping method were effective in reducing the wilt incidence and in delaying the wilt appearance.
20. Under field conditions, the treatments with antagonist in highly susceptible varieties did not give a promising control of bacterial wilt. However, treatments

with *T.viride* (Ozhalapathy) and *T. pseudokoningii, P. aeruginosa* showed some effectiveness in chilli crop.

21. Combined effect of host resistance and antagonists were necessary to achieve better management of bacterial wilt in natural condition.
22. Field trial conducted with a moderately resistant variety of tomato, Mukthi, showed better control of bacterial wilt with various antagonists by increasing host resistance and *T.viride* (Ozhalapathy) was found to be the most effective.
23. With respect to the performance of antagonists in different studies, same trend was observed under *in vitro*, pot culture and field conditions.
24. The plant vigour and crop yield were enhanced by the application of antagonists and *T.viride* was best among them.
25. Total microbial population was found to increase with days after planting. However no noticeable change in population was observed after 60 days of planting in case of brinjal and tomato.

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* Originals not seen

Appendices

APPENDIX I

Composition of different media used for various studies

Triphenyl Tetrazolium Chloride (TTC) medium

Peptone	:	10.0 g
Casein hydrolysate	:	1 0 g
Glucose	:	5.0 g
Agar agar	:	20.0 g
Distilled water	:	1000 ml
pH	:	6.8
TTC	:	1 %

APPENDIX II

Martin Rose Bengal Streptomycin Agar (MRBA)

Agar agar	:	20.0 g
KH_2PO_4	:	1.0 g
$MgSO_4.7H_2O$	:	0.5 g
Peptone	:	5.0 g
Dextrose	:	10.0 g
Rosebengal	:	0.03 g
Distilled water	:	1000 ml
Streptomycin	:	30.0 mg

Thornton's Agar medium (TAM)

Mannitol	:	1.0 g
Asparagine	:	0.5 g
KH_2PO_4	:	1.0 g
KNO_3	:	0.5 g
$MgSO_4$	:	0.2 g
$CaCl_2$	:	0.1 g
NaCl	:	0.1 g
Ferricchloride	:	0.002 g
Agar agar	:	20.0 g
Distilled water	:	1000 ml
p^H	:	7.4

Kenknight's Agar medium (KAM)

Glucose	:	1.0 g
KH_2PO_4	:	0.1 g
NaNO3	:	0.1 g
KCl	:	0.1 g
MgSO4.7H2O	:	0.1 g
Agar agar	:	20.0 g
Distilled water	:	1000 ml
pH	:	7.0

Sierra's Medium

Peptone	:	10.0g
NaCl	:	5.0g
$CaCl_2.7H_2O$	:	0.1g
Agar agar	:	20.0g
Distilled water	:	1000ml
pH	:	7.0

Van den Mooter Succinate (VMS) medium

K_2HPO_4	:	0.5 g
KH_2PO_4	:	0.5 g
$K_2SO_4.7H_2O$	:	0.2 g
Sodium succinate	:	2.0 g
KNO_3	:	3.0 g
Yeast Extract	:	5.0 g
Distilled water	:	1000 ml
pH	:	6-7
Agar-agar	:	3.0 g

Thornley's medium

Peptone	:	20.0 g
$2HPO_4$	:	0.3 g
NaCl	:	5.0 g
Agar agar	:	15.0 g
Phenol red	:	0.01 g
L-arginine mono hydro		
Chloride	:	10.0g

APPENDIX III

Nutrient Agar Medium (NA)

Beef extract	:	1.0 g
Peptone	:	5.0 g
Sodium Chloride	:	5.0 g
Agar agar	:	15.0 g
Distilled water	:	1000 ml
pH	:	7.2-7.4

King's A medium

Peptone	:	20.0 g
Glycerol	:	10.0 ml
K_2SO_4	:	10.0 g
$MgCl_2$	:	1.4 g
Agar agar	:	15.0 g
Distilled water	:	1000 ml
pH	:	7.2

King's B medium

Peptone	:	20.0 g
Glycerol	:	10.0 ml
K_2HPO_4	:	10.0 g
$MgSO_4.7H_2O$	:	1.5 g
Agar agar	:	20.0 g
Distilled water	:	1000 ml

Sierra's Medium

Peptone	:	10.0g
NaCl	:	5.0g
$CaCl_2.7H_2O$	:	0.1g
Agar agar	:	20.0g
Distilled water	:	1000ml
pH	:	7.0

Van den Mooter Succinate (VMS) medium

K_2HPO_4	:	0.5 g
KH_2PO_4	:	0.5 g
$K_2SO_4.7H_2O$	:	0.2 g
Sodium succinate	:	2.0 g
KNO_3	:	3.0 g
Yeast Extract	:	5.0 g
Distilled water	:	1000 ml
pH	:	6-7
Agar-agar	:	3.0 g

Thornley's medium

Peptone	:	20.0 g
$2HPO_4$	:	0.3 g
NaCl	:	5.0 g
Agar agar	:	15.0 g
Phenol red	:	0.01 g
L-arginine mono hydro Chloride	:	10.0g

Distilled water	:	1000ml
pH	:	7.2

Hayward's semi-solid medium

$NH_4H_2PO_4$	:	1.0 g
KCl	:	0.2 g
$MgSO_4.7H_2O$	:	0.2 g
Bacto peptone	:	1.0 g
Bromothymol blue	:	0.08 g
Distilled water	:	1000 ml
pH	:	7.0-7.1
Agar-agar	:	3.0 g

Nutrient Glucose Agar medium (with Bromocresol purple)

Glucose	:	5.0 g
Peptone	:	10 g
Agar-agar	:	15.0 g
Distilled water	:	1000 ml
Bromocresol purple	:	0.005 %

MANAGEMENT OF BACTERIAL WILT OF SOLANACEOUS VEGETABLES USING MICROBIAL ANTAGONISTS

By

MANIMALA R.

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

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ABSTRACT

Studies on 'Management of bacterial wilt of solanaceous vegetables using microbial antagonists' was conducted at the College of Horticulture, Vellanikkara during 2000-2002.The major objective was to find out effective microbial antagonists against bacterial wilt of brinjal, chilli and tomato.

Estimation of population of *R. solanacearum* showed the maximum in Vellanikkara than Ozhalapathy. The total microflora was higher in Ozhalapathy than Vellanikkara and resistant varieties of the three crops recorded better association of microflora than susceptible ones.

In *in vitro* evaluation , rhizosphere organisms isolated from the low wilt incidence area (Ozhalapathy) exhibited better antagonism against *R. solanacearum* than those isolated from Vellanikkara. Among them, fungi were more effective than bacterial and actinomycete antagonists. The major fungal antagonists belonged to *Trichoderma* spp. and *Aspergillus* spp. *T.viride* (Ozhalapathy), *T. pseudokoningii* (forest soil) and *Aspergillus niger* (Eruthyampathy) were the most effective ones against *R. solanacearum*. Among the bacterial antagonists, *P. aeruginosa* and *B. subtilis* (Vellanikkara) were promising one. All commercial antagonists *viz. T. viride, A. niger* (Varanasi), *A. niger* (AN 27) *T. harzianum, B. subtilis* and *P. fluorescens* were also effective against *R. solanacearum.* Of the culture filtrates tested, *T. virens* and *B. subtilis* (commercial) showed the maximum inhibition of the pathogen. The antagonists which were found most effective in laboratory condition performed well in pot culture studies also. Among the different methods of application of antagonists adopted, either seed treatment + soil drenching or root dipping were effective in reducing the wilt incidence and in delaying the wilt appearance. Use of antagonists in highly susceptible varieties did not give a promising control of bacterial wilt under field condition. In field trial, using a moderately resistant variety Mukthi, antagonists showed some effect in controlling bacterial wilt indicating that, an integrated effect by combined use of host resistance and microbial antagonists can provide a better control of bacterial wilt pathogen in the field.