INTEGRATED MANAGEMENT OF BACTERIAL WILT OF TOMATO CAUSED BY *Ralstonia solanacearum* (Smith) Yabuuchi *et al.*

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By

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THESIS

Submitted in partial fulfilment of the
requirement for the degree

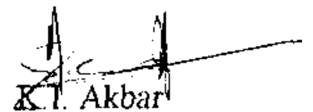
Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University

Department of Plant Pathology
COLLEGE OF HORTICULTURE
Vellanikkara, Thrissur
Kerala
2002

DECLARATION

I hereby declare that the thesis entitled **"Integrated management of bacterial wilt of tomato caused by *R. solanacearum* (Smith) Yabuuchi *et al.*"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.



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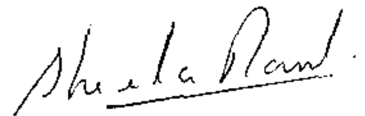

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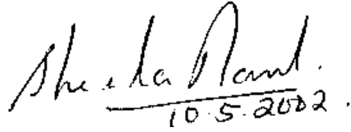

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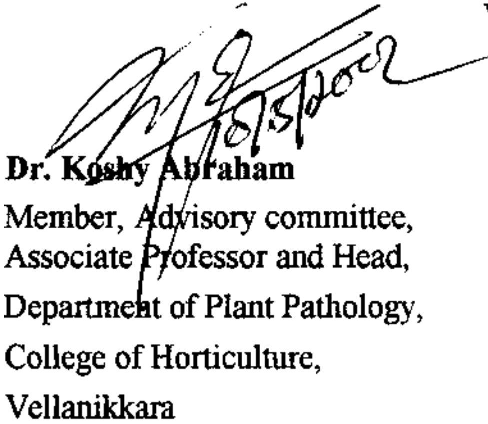

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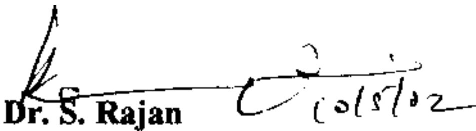

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Dedicated to

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Introduction

INTRODUCTION

Tomato is a well known and a very popular vegetable grown successfully under a wide range of environmental conditions. As a vegetable it has varied use, fruits can be taken in raw form when it is ripe or is used to make many other products like soup, pickles, sauce, ketch up etc. It has many medicinal uses and also has a very good nutritional value. In India 4.21 lakh hectare have been cultivated with tomato with a production of 72.71 lakh tones during 2000- 2001.Losses due to the bacterial wilt of tomato varies from 20 to 100 per cent

Bacterial wilt of tomato incited by *Ralstonia solanacearum*(Smith) Yabuuchi *et al.,* is the most devastating disease limiting tomato production in humid tropical region. *R. solanacearum* , a soil inhabiting bacterium consists of many races or strains with a wide host range and due to its persistence in soil it is considered very difficult to control. The disease could not be controlled even after more than 100 years of its occurrence. However breeding for resistance has been predominantly used as the most effective way of management of this disease but is handicapped by its complex interaction with soil temperature, nematode population, latent infection and the presence of highly virulent tropical strains of the pathogen.

The variability of the pathogen and the diversity of crops and cropping practices throughout the tropics have prevented the application of a single strategy for control of bacterial wilt. The use of chemicals /antibiotics for the control of this disease was not long lasting, rather their indiscriminate use can result in their accumulation in soil to toxic levels. This has also resulted in the emergence of resistant population of pathogens besides having an adverse effect on the non target beneficial organisms.

The greatest reduction in the incidence of disease was observed with the use of a resistant cultivar, followed by crop rotation. In many vegetables growing areas of our state much of the available land is already infested. This is particularly true in

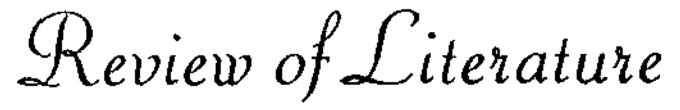
small and marginal holdings where land scarcity restricts crop rotation and several susceptible food crops may be produced on a regular basis.

The achieved levels of resistance to be successful must usually be accompanied by an association of cultural practices that reduce inoculum potential, minimise development of the field level epidemic and avoid or reduce spread of the pathogen to other sites. Such locally chosen factors constitute integrated disease management. Integrated disease management is in fact an ecological approach among all beneficial, biological and physical forms in the ecosystem to maintain plant health equilibrium.

Integrated disease management involves the simultaneous manipulation of a number of available strategies of reducing a plant disease with least damage to the environment. The strategy of integrated disease management comprises of the cultivation of resistant/tolerant cultivars, adoption of agronomic practices resulting in less disease, preserving and promoting the activities of natural antagonists and the use of chemical pesticides wherever necessary to reduce pathogen population to non damaging levels (Fadeev and Novozhilov 1987; Kendriek, 1988).

In view of this, the following aspects were taken up for the present study.

- Isolation and characterisation of the pathogen from four different agro climatic regions.
- Isolation and characterisation of antagonistic bacterial strains from each location.
- Evaluation of the different management strategies by pot culture study to screen out the most effective components.
- Integration of the selected components from the pot culture experiment to draw out the most efficient combinations so as to derive an eco friendly, management strategy against bacterial wilt of tomato for sustainable tomato production.


Review of Literature

2. REVIEW OF LITERATURE

In the year 1896 E.F. Smith described *Pseudomonas solanacearum* as the casual agent of bacterial wilt of potato and tomato. In india occurrence of bacterial wilt of tomato was first reported by Hedayathullah and Saha (1941) from West Bengal. However this disease still remain without a long lasting remedy. In view of the importance of bacterial wilt disease caused by *Ralstonia solanacearum* (Yabuuchi *et al.*, 1995) and the depth of information published in the past century on this disease, its causal agent and management practices a thorough review has been presented.

More than 100 years had elapsed since E.F. Smith described *Pseudomonas solanacearum* as the causal agent of bacterial wilt of potato and tomato (Smith, 1896). To the latest, the bacterium has been renamed as *Ralstonia solanacearum* (Yabuuchi *et al.*, 1995) based on sequencing of 16S rRNA genes and polyphasic taxonomy which led to the proposal of genus *Ralstonia.*

In Kerala review on various aspect of bacterial wilt of solanaceous crops were made by Rahim (1972), George (1973), Devi (1978), Celine (1981), Nayar (1982), Peter *et al.* (1984), Rajan (1985), Gopalakrishnan and Peter (1991), Markose (1996) Singh (1996) and Paul (1998).

2.1 Pathogen

Typical *Ralstonia solanacearum* colonies are identified with bold, irregular, fluidal morphologies and with characteristic tetrazolium red centers and creamy white margins.

Shape and size of the bacterium was first delineated by Smith (1896), as non-spore forming, non-capsulate, gram-negative, small rods with polar flagella.

Ralstonia solanacearum would be identified as a non fluorescent Pseudomonas and with experience confined by typical colony characteristics with the use of simpler or complex selective media (Kelman, 1954, Granada and Sequerria, 1983, Hayward, 1991 and Engelbrecht, 1994) provides a simpler and convenient way of confirming identity of isolates.

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 Jensen (1951) opined that it could grow anaerobically. While studying the utilization of carbon sources by isolates of *P. solanacearum*, Devi (1978) noticed both aerobic and anaerobic growth.

Smith (1896) opined that a temperature range of 35 to 37°C was most favourable for growth of *P. solanacearum* in culture. Kelman (1953) stated that relatively high temperature was required for rapid multiplication and growth of *P.solanacearum*. Devi (1978) found that the bacterial pathogen from different host range including chilli, brinjal and tomato preferred a temperature range of 30 to 35°C. Nayar (1982) observed that isolates from brinjal recorded maximum temperature of 35°C for growth but it did not grow at 10°C and 50°C. Jyothi (1992) reported that isolates from chilli, brinjal and tomato recorded growth at 30°C and no isolates of *R. solanacearum* grow beyond 55°C. Slight growth was observed at 40 to 45°C.

Fahy and Llyod (*1983)* suggested a sequence of tests for the identification of fluorescent pseudomonas, includes growth at temperature higher than 41°C fluorescence pigment production on Kings-B Agar (KBA) medium, oxidase activity, arginine dehydrolase, nitrate reduction, gelatin hydrolysis, levan production and utilization of carbon source.

John *et al.* (1994) identified *Pseudomonas aeruginosa* based on production of fluorescent diffusible pigment (blue green). In a few strains reddish brown pigment was obscured. The bacterial strain can grow at 41°C and not at 4°C.

P.aeruginosa produced negative reaction to levan and starch hydrolysis but produce positive reaction to Arginine hydrolase, gelatin liquifaction, denitrification and nitrate reduction.

Hayward (1964) studied the characteristics of *P. solanacearum* and reported that on tyrosine medium a diffusible brown pigment was produced and the intensity of this might vary between isolates. He further observed that one of the isolates produced a green fluorescent pigment on Kings medium. Catalase and oxidase were produced by the organism and citrate but not melonate was utilized as sole source of carbon. Nitrate reduction and ammonia production were positive. It did not hydrolyse soluble starch or produce indole. An alkaline reaction was produced in litmus milk and growth occurred in 0.5 and 1 per cent sodium chloride broth. Slight gelatin liquefaction took place on prolonged incubation. Similar characters with slight variations were observed by many workers while studying the biochemical and Physiological characters of strains of *P. solanacearum* from tomato (Devi, 1978, He *et al*. 1983; Psallidas, 1985; Swanepoel and Young, 1988; Prior and Steva, 1990).

However an acidic reaction of the isolates of the pathogen in milk was observed by Samuel (1980) Nayar (1982) and Paul (1998). They also observed positive urease activity and levan production. Paul (1998) also reported negative lipolytic activity for *R. solanacearum*.

Starr and Weiss (1943) reported that *P. solanacearum* could utilize asparagines as a sole source of carbon and nitrogen. Palleroni and Duodoroff (1971) considered the inability to hydrolyse arginine as a negative nutritional character of *P. solanacearum*. Nayar (1982) and Jyothi (1992) also observed negative arginine hydrolase activity.

Liberation of hydrogen sulphide and negative MR and VP tests by isolates of *P. solanacearum* were observed by many workers (Devi, 1978; He *et al*. 1983; Jyothi, 1992).

A large number of organic carbon compounds like glucose, ribose, fructose, sucrose, galactose, dextrose, lactose, maltose, mannose, xylose, trehalose, arabinose, cellobiose, mannitol, glycerol, dulcitol, sorbitol, inositol, dextrin, pectin and sodium acetate were reported to be utilized by *P. solanacearum* with or without acid production (Hayward, 1964; He *et al.*, 1983; Prior and Steva, 1990 and Paul, 1998).

The bacterial wilt pathogen (*Ralstonia solanacearum*) exhibits great degree of both phenotypic and genotypic diversity. The existence of variation among the isolates of *P. solanacearum* has been well demonstrated (Smith, 1896; Kelman, 1954; Buddenhagen and Kelman, 1964; Addy *et al.*, 1980).

Several attempts have been made to group *P. solanacearum* isolate into races biotypes or varieties on the basis of differences in physiological characteristics and pathogenicity (Kelman 1953; Kelman and Persoon, 1961; Buddenhagen *et al.*, 1962; Buddenhagen and Kelman, 1964; Hayward, 1964).

Buddenhagen *et al.* (1962) differentiated strains of *P. solanacearum* into three races, race-1 affecting tobacco, tomato, many other solanaceous crops and other weeds, certain diploid bananas, race-2 affecting triploid bananas, heliconia or both, race–3 affecting potato and tomato, but highly virulent on other solanaceous crops.

Buddenhagen (1985) proposed two races, race-4 affecting ginger and race-5 on mulberry from Philippines and China.

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.

Race-1 (Solanaceous strain): Wide host range, distributed throughout the lowlands of tropics and subtropics

Race-2 (Musaceous strain): Restricted to musa, and few perennial hosts initially limited to American tropics now spread to Asia

Race-3 (Potato strain): Strain is restricted to Potato and few alternate hosts in the tropic and sub tropics

Race-4 (Ginger strain): On ginger reported from Philippines.

Race-5 (Mulberry strain): It is reported from China on host mulberry.

Hayward (1964) classified a collection of 185 isolates of *P.solanacearum* into 4 biotypes, based on the capacity to oxidize three disaccharides (lactose, maltose, cellobiose) and 3 hexose alcohols (mannitol, sorbitol, dulcitol). Isolate of biotype-1 oxidized neither group; biotype-2 only disaccharides, biotype-3 both the groups and biotype-4 only hexose alcohols. Biotype-2 appeared to have a restricted host range and it was solely obtained from two host plants, potato and tomato whereas the other biotypes were obtained from many families in addition to Solanaceae.

It was well established that biovar III is the dominant strain of *P.solanacearum* affecting solanaceous crops (Hayward, 1964; Hayward *et al*., 1967; He *et al*., 1983; Prior and Steva, 1990).

In Kerala, Devi (1978) compared twenty six different isolates of *P.solanacearum* from tomato brinjal and chillies and grouped them into 12 pathogroups under race-1 and biotype III.

He *et al*. (1983) obtained a series of isolates from China which oxidized mannitol but not sorbitol or dulcitol, and these were designated as biovar V.

Kumar *et al*., (1993) differentiated twelve isolates of *P. solanacearum* from solanaceous hosts into biovars following Haywards classification. All the isolates from tomato, potato, aubergine and bell pepper (Capsicum) were identified as biovar III or a sub-type in biovar III, an isolate from chilli that differs from other was tentatively identified as biovar V.

Hayward (1994) differentiated biovar III of *P. solanacearum* from biovar V based on its ability to utilize the sugar alcohols, sorbitol and dulcitol.

Paul (1998) also reported that among the three *R. solanacearum* from tomato, brinjal and chilli, the isolate obtained from chilli and tomato were characterized and identified as race I biovar III and that obtained from brinjal was identified as race I biovar V.

2.2 *In vitro* sensitivity to chemicals

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

Hidaka and Murano (1956), Rangaswami (1957) found that Streptomycin at 0.3 μg per ml. of water inhibited and 5/ug per ml killed the pathogen at once. Campacci *et al.* (1962) reported that among various chemicals tested the bacterium was sensitive to Agristrep, Streptomycin, Penicillin-G-potassic, Penicillin procain, Dihydro streptomycin sulphate and Erythromycin. Streptocycline was found to give good control of *P. solanacearum in vitro*. The inhibitory effects of Streptomycin and Streptocycline on *Pseudomonas* and *Xanthomonas* have been observed by many workers (Rangarajan and Chakravarti, 1969; Shivappashetty and Rangaswami, 1971). Several antibiotics like Oxytetracycline, Tetracycline, Penicillin-G, Streptomycin were reported to inhibit the pathogen (Goorani *et al.*, 1978). Mondal and Mukherjee (1978) observed that Ampicillin, Streptomycin at 500 ppm each were of promise against the pathogen *in vitro*. He *et al.*, (1983) reported that all the strains of *P. solanacearum* from China showed susceptibility to Streptomycin, but were resistant to Penicillin, Viomycin and Chloramphenicol.

Farag *et al.* (1986) also observed that both virulent and avirulent forms of the pathogen were sensitive to Streptomycin and Dihydrostreptomycin.

Gunawan (1989) found that optimum concentration for suppression of bacterial multiplication in vitro were 175 and 450 ppm of Streptomycin sulphate.

In lamberg, Indonesia tomato crop were sprayed with 200 ppm streptomycin sulphate / oxytetracycline at intervals of 4, 7, 10 and 15 days of planting. The disease

incidence was evaluated each week from 59 to 77 days after planting. The best treatment of antibiotic was on four and seven days intervals (Hsu and Chang, 1989).

Paul (1998) has reported that Oxytetracycline and Streptomycin sulphate gave good inhibition and suppression of growth of *Ralstonia solanaecearum* from tomato.

Report on fungicidal toxicity on *R. solanaceaum* are very few. Effectiveness of Bordeaux mixture, Copper oxychloride and Kocide in controlling bacterial blight of walnut was reported by Servin and Kupferberg(1977).Inhibitory actions of Dithiocarbomate fungicide like Nabam(Dithane A 40),Maneb (Dithane M 22) and Dithane M 45 was studied by Goorani. *et al*,(1978).Growth inhibitory effect of Captan, Maneb,Mancozeb and Thiram on *P. solanacearum* was reported by Leandro and Zak(1983). Jyothi (1992) reported that among the three fungicidestested(Thiride, Bluecopper and Bordeaux mixture) Bordeauxmixture recorded the maximum inhibition against *R. solanacearum*,

2.3 Host resistance

The most effective way of control of *R. solanacearum* is the development of resistant varieties (Kelman, 1953). Grimault and Prior (1994) ranked 10 tomato cultivars from highly susceptible to totally resistant and no significant difference was observed in bacterial population in wilting plants, regardless of the cultivar.

Rajan (1985) reported variety "Sakthi" with high resistance against the bacterial wilt disease of tomato and recommended for cultivation. Sadhankumar (1995) evaluated 66 tomato genotypes against bacterial wilt for three seasons and revealed that Sakthi and LE 79-5 were consistently resistant to bacterial wilt.

Breeding for host resistance to *R. solanacearum* has been extensively studied, but it may be difficult because the pathogen variability introduction of contaminated materials in disease free area and biovar difference with their reaction towards resistant genotype. Plant breeding for resistance became very

laborious that each biovar shows resistance to one clone and susceptible to other (Martin *et al.*, 1996).

Peter *et al.* (1997) reported variety Sakthi as a resistant (survival 80%) and LE79-5 as a highly resistant tomato variety. The variety Sakthi and LE-79-5 now recommended for growing under warm humid tropics condition.

2.4 Biological control

Disease management using chemical is made difficult due to the location of the pathogen inside the xylem and its survival at the depth in the soil. Although considered as efficient this kind of control is not commercially feasible in the field.

Anuratha and Gnanamanikam (1990) reported that *Pseudomonas fluorescens* could protect tomato plants from wilt upto 95 per cent in green house and 36 per cent in field, protection afforded by *B. subtilis* strain was lower. In a field trial in Karnataka, seed dressing with bacterinol-100 and/or *Pseudomonas fluorescens* was effective for the control of bacterial wilt caused by *P. solanacearum* and increased yield (Rao, 1990).

Treatment of tomato seedlings with antibiotic producing *P. glumae* prior to planting in pots suppressed the severity of wilt incidence by challenge inoculation with *P. solanacearum*. Disease suppression was most effective when the seedlings were dipped in suspension of *P. glumae* (10^{10} cfu per ml) for 24 h. Pre-treatment with non antibiotic producing *P. glumae* or heat killed *P. glumae* cells also reduced disease incidence suggesting that mechanism other than antibiotic production were involved in disease suppression (Furuya *et al.*, 1991).

Phae *et al.* (1992) showed that bacterial wilt caused by *P. solanacearum* was suppressed when culture suspensions of *Bacillus subtlis* strain NB22, was poured into heavily infested soil.

El-Abyad *et al.* (1993) tried two species of Streptomyces (*Streptomyces pulcheror, S. citriflorescence*) to evaluate their effectiveness against *P.solanacearum* in *in vitro* studies and showed that 80 per cent concentration of the culture filtrate of either *Streptomyces pulcheror or S. citrifloresces* was detrimental to the populations of *P. solanacearum*. *In vivo* study showed that the seed coating treatment was the most effective in controlling the pathogen at 42 and 63 days after sowing. The result also revealed that seed coating with antagonistic Streptomyces species significantly improved tomato growth.

Hanudin and Machmud (1994) tested the effect of *Pseudomonas fluorescens* against the bacterial wilt caused by *P. solanacearum* (*R. solanacearum*) and reported some control of the disease.

Wei-ChunMei *et al.*, (1994) reported that cells of the antagonistic strain 90B 4-2-2 could multiply in rhizosphere soil around tomato plant, as well as penetrated the roots and moving up into stems and leaves. Suspensions of strain 90B 4-2-2 accelerated the germination of tomato seeds and increased seedling vigour when seeds were soaked in a suspension of 2 x 10^9 c.f.u./ml for 30 min and the seedling bed soil was drenched with a 125 ml suspension containing 5 x 10^{10} c.f.u./ml/m-2. SEM studies showed that cells of the antagonist colonized thin-walled cells of the phloem of stems or roots of tomato plants susceptible to Pseudomonas *solanacearum* (*Ralstonia solanacearum*)

Several microorganisms including *Streptomyces griseochromogeonus, S.griseus, P. fluorescence, Trichoderma pseudokoiingii, P. aeruginosa, Bacillus coagulans, B. megatermis* and *B. cereus* have been evaluated against *R.solanacearum* (Silveria *et al.*, 1996).

Toyota and Kimura (1996) recorded the effect of prior colonization of a sterile clay loam soil by individual soil bacteria (YUIRif43, a rifampicin – resistant derivative of a bacterial wilt pathogen from tomato) on the subsequent growth of *Ralstonia solanacearum*. It was reported that there was competition for nutrient and other ecological niches in soil. When *P. solanacearum* and individual soil bacteria

were simultaneously inoculated into sterile soil there was a significant reduction in the growth of *P. solanacearum* in the presence of the more effective inoculant strain. It is suggested that the effective strains were mainly present in larger pores while the less effective ones resided in the smaller pores.

Ciampi *et al.* (1997) developed a biological method to control bacterial wilt and to prevent latent infection in soil based on a selected antagonist strain BC8 of *P.fluorescens*. The strain caused strong inhibition of *R. solanacearum* in *in vitro* plate assays and prevented root and plant infection in growth chambers. The studies showed that protection can be achieved even in soil infested with 10^6 cfu of *R.solanacearum*/pot. Under well defined culture media BC8 was able to produce several metabolites among which a siderophore like substance was present in large amounts (more than 500 mg/litre). Based on the results of the studies new strategies to protect potato plants from the pathogen are being developed including the use of compounds stimulating over production of inhibitory metabolite synthesized by BC8.

Furuya *et al.* (1997) reported that numerous strains of Pseudomonads formed growth inhibition zones around their colonies which had been placed on lawns of phytopathogenic bacteria and fungi as indicators. Strains ATCC 7700 of *P.aeruginosa* was highly antagonistic *in vitro* to various plant phytopathogenic bacteria and fungi, especially *R. solanacearum* causal organism of tomato bacterial wilt. Treatment of tomato root with *P. aeruginosa* at the time of transplanting in *R.solanacearum* infected soil increased percentage of seedling survival. Soaking the root system of tomato seedlings in bacterial suspensions of 10^{10} cfu/ml resulted in the highest suppressiveness. Pre-treating tomato roots with killed cells of *P.aeruginosa* also gave protection suggesting that mechanism other than antibiotic production such as induced resistance and infection sites competition were involved in suppression of the disease.

Karuna et al., 1997 identified strains of boicontrol agent *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Bacillus subtilis* which inhibited the growth of *Ralstonia solanacearum* under *invitro* conditions. Bacteriasation of

tomato seeds (cv. Pusa Ruby) with antagonistic bacteria was found to be effective in controlling wilt in seedling raised in soil infested with *R. solanacearum*, in a nursery.

Mulya *et al.* (199[illegible]) reported that *Pseudomonas fluorescens* (PfG 32) isolates from rhizosphere of onion actively suppressed the occurrence of bacterial wilt diseases of tomato in vermiculate amended natural soil and produced antibiotic substance(s) and siderophores.

Abdulla *et al.* (1999) on a study conducted in the laboratory showed that use of antagonistic bacterial *B. subtilis* (BI), *Pseudomonas spp. Pseudomonas cepacia* (*Burkholderia cepacia*) had highly inhibitory effects against *R.solanacearum* on culture medium, significant reduction in wilt incidence was achieved in green house tests when antagonist bacteria were applied to tomato seedlings, which were then infested with *R. solanacearum*.

2.5 AM fungi inoculation

Halos and Zorilla (1979) reported a decrease in bacterial wilt incidence when the tomato roots were prior inoculated with *Glomus mossae*.

Biological control of soil borne disease with VAM fungi and charcoal compost was reported by Kobayashi (1991). They reported that vesicular arbuscular mycorrhiza inoculation alone induced weak resistance to disease, but combined effect of VAM and charcoal compost reduced bacterial wilt caused by *P.solanacearum* in green house. However in the open field where there was a high density of pathogen propagules, biological control had only a weak effect and integrated control was essential to express the disease resistance.

Suresh and Rai (1991) reported that extracts from mycorrhizal tomato roots infected with *Glomus fasciculatum* reduced pathogen (*R. solanacearum*) population in nutrient broth while nitrogen fixing bacteria (Azotobactor), *P. putida* and *P.fluorescens* were in effective.

Praveenkumar and Bagyaraj (1998) carried out a study to test the effect of plant growth promoting rhizomicroorganisms, *Bacillus coagulans*, *Pseudomonas fluorescens* and *Trichoderma harzianum* on the arbuscular mycorrhizal (AM) fungus, *Glomus mossae*. These organisms were either inoculated singly or in various combinations with *Glomus mossae*. The mycorrhizal parameters were enhanced when both the bacteria were inoculated together with *Glomus mossae*.

Gong-Ming Qin (1999) in a field and nursery trial established in China to determine the efficacy of antagonism of eight ectomycorrhizal isolates of four species (*Pistolithus tintorius*, *Scleroderma polyrhizum*, *Laccaria laccata*, *Hebloma westraliense*) to bacterial wilt pathogen *pseudomonas solanacearum* (*R.solanacearum*) on eucalyptus. The result showed that these ectomycorrhizal fungi were effectively controlled the occurrence and development of bacterial wilt disease. The disease rate of mycorrhizal seedling were reduced by 40.0 to 72.78 per cent in the *nursery and 20 to38.9 in the field compared with these uninoculated seedlings*. The height and basal diameter growth of trees in the field trial were enhanced by 11.67 to 59.70 per cent respectively in inoculated plants.

2.6 Plant extract

Ahmed and El-Shazle (1987) reported that toxic action of aqueous extracts of *Medicago hispida* (*M. polymorpha*), *Melilotus* spp., *Cornopus squantitus* and *Anagallis arvensis* and *Ammi majus* against *P. solanacearum*, *B. subtilis*, *E.carotovora* and *E. carotovora* sub *atroseptica*. Among these *P. solanacearum* was the most sensitive bacterium.

Hanudin (1987) reported that the extracts of garlic, shallot and *Tagetes erecta* suppressed the bacterial wilt disease in tests on inoculated plants.

Garlic bulb as a material for suppressing the incidence of bacterial wilt (*R.solanacearum*) on tomato was reported by Hutagalung (1988). Bacterial wilt of tomato was decreased by adding 10 ml suspension of 35 gm garlic bulb per 77 ml

sterile water or 6 gm of ground garlic bulb to the rhizosphere and this treatment resulted in normal plant growth and increased fruit size and weight.

Bora (1995) reported the effect of mixture of asafoetida, turmeric powder and water (ATW) at a ratio of 1 g:5 g:10 liter to control *Pseudomonas solanacearum* (*Ralstonia solanacearum*) causing wilt on tomatoes. ATW inhibited growth of *R.solanacearum in vitro.* Soil drenching (3 treatments at 15, 30 and 45 days after transplanting) was the most effective treatment for maximum disease control.

Singh (1997) reported that various botanicals viz. garlic (Allium cactum), Neem *(Azardiacta indica), Pongamia (P. glabra)*, Castor (*Ricinus communis)* and heat killed *R. solanacearum* were effective in *in vitro* and in field against *R.solanacearum*. Best control of bacterial wilt (64 per cent) in the field was obtained with 10 per cent garlic extract.

Yu Jing Quan (1999) observed a decrease in soil population of *R.solanacearum* when Chinese chive (*Allium tuberosum*)was grown in the field. Root exudates of Chinese chive collected with continuously trapping system were found inhibitory to the multiplication of *R. solanacearum*.

2.7 Soil amendments

Prior and Beramis (1990) studied the effect of soil amendments with 750 units of N/ha with urea or organic matter (soybean flour, sewage sludge) in ferollic soil inoculated with *P. solanacearum*. Three successive crops of a highly susceptible cultivar (Floridal) increased the mortality in control soil (14.24 and 43 per cent). In soil amended with organic matter particularly sewage sludge no crop died in 2nd and 3rd crop and soil amended with urea remained stable (22.15 and 26 per cent).

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 (Miah *et al.*, 1995).

Dhital (1997) conducted a study on management of bacterial wilt on potato caused by *Ralstonia solanaecerum* by amending infested soils with stable bleaching powder (SBP) and a mixture of urea and lime (urea-lime) at different concentrations and combinations under glasshouse and in field conditions in Nepal. The soil infested with *R. solanaecerum* was treated before planting with stable bleaching powder at 25 kg/ha provided effective disease management in both glass house and in field condition. Also resulted lowest population count of 3.01 and 2.0 cfu/g dry soil at 120 days after amendments in glasshouse and field respectively. The result indicated that 25 kg stable bleaching powder was more effective than the other treatments for control of bacterial wilt. Alternatively soil amendmends with 428kg/ha urea and 5 tone/ha can be used for effectively control bacterial wilts.

Amending soil using 428 kg ha^{-1} urea + 5000 kg ha^{-1} calcium oxide at 3 week prior to transplanting raised soil pH for each weekly observations and was effective in suppressing bacterial wilt in greenhouse in the rigosol and alluvial soil (Hanudin 1997).

Michel *et al*, (1997) reported that at AVRDC Taiwan inter cropping tomato with cowpea (*Vigna unguiculata*) planted within the row significantly reduced bacterial wilt. At the same site, however, a preplanting soil amendment consisting of urea (200 kg/ha N) and CaO (5000 kg/ha) significantly reduced the pathogen population and tomato bacterial wilt ($P<0.001$). In comparison with a non-amended control, the addition of CaO alone significantly reduced the *R.solanacearum* population in AVRDC soil ($P<0.05$) but the reduction was significantly greater when the complete soil amendment was added. In contrast, urea alone did not affect the survival of *R. solanacearum* in the soil. The suppressive effect of the soil amendment on the *R. solanacearum* during the transformation of urea in the presence of CaO.

Yuan – Litte *et al.* (1997) recorded good suppressive effect of white mud soil in Yichun, Jiangxi, China on *P. solanacearum* the main physico-chemical properties of the white mud soil was that it had a calcium concentration 2 to 7 times than that

of susceptible soils and had a pH more than 7.2, while the susceptible soil had a pH less than 7.

Shiomi *et al.* (1999) compared bacterial community structures in the rhizoplanes of tomato plants grown in soils suppressive and conducive towards bacterial wilt in Japan to evaluate the biological factors contributing to suppressiveness of the soil in three treated Mutsumi soil (suppressive soil). The result indicted that the naturally existing population of microorganisms in Mutsumi soil was significantly able to reduce the severity of bacterial wilt of tomato plants. During *in vitro* assays, rhizo plane bacteria in Mutsumi soil grew more vigourously on pectin, one of the main components of root exudate of tomato, compared with those in Yamadi soils. The result imply that it is difficult for pathogen to dominate in diversified rhizo bacterial situations that thrive in pectin.

2.8 Soil solarization

Solarisation was effective during summer in Japan for solanaceous strains of the pathogen (Akiew and Trevorrow - 1994).

Chellemi *et al.* (1994) examined the effect of soil solarization in tomato genotype against bacterial wilt in North Florida. Maximum soil temperature achieved under solarization treatment using a photo selective polythene mulch were 49.5, 46.00 and 40.5°C at depth of 5, 15 and 25 cm respectively. Soil solarization did not reduce the incidence of bacterial wilt in a susceptible cultivar "solarset".

Vilasini (1996) could not notice a significant reduction in Pseudomonas population in 30 days solarised plot, but a marginal reduction was noticed in 45 days solarised plot.

Anith *et al.*, (2000) showed that soil solarization after irrigation, 45 days prior to planting, is found to be effective in reducing bacterial wilt disease incidence in ginger in a wilt stick field in Wayanad district of Kerala. The maximum mean difference in temperature taken at 14.00 h. was 12.2°C in plots mulched after

irrigation. Significant reduction in the soil microbial population was observed during solarization. Seed treatment with *Pseudomonas fluorescens* strain EM85 along with solarization decreased the wilt incidence and increased the yield. Soil amendment with neem cake before solarization provide no additional advantage in controlling the disease.

2.9 Integrated disease management

The system of delivering bacterial antagonist in an amended soil proved to be much more efficient biological control of the pathogen. Seed potato tubers were treated with a selected antagonistic bacterial isolates (BC8) and coated with $CaCO_3$. The isolate BC8 caused strong inhibition of *P. solanacearum* in both *in vitro* assays and growth chamber conditions. The above treatment ensured colonization of the antagonistic isolate BC8 in 80 per cent of the tuber assayed from plant growing in the naturally infested soil and were found to be free of *P. solanacearum*. It was established that the pathogen was still present in the soil after two years of non potato cropping. The system of delivering bacteria in an amendment proved to be much more efficient in the biological control of *P. solanacearum* than just coating in sweet potato tubers with the antagonistic isolate BC8 (Caimpi-pannol *et al.*, 1989).

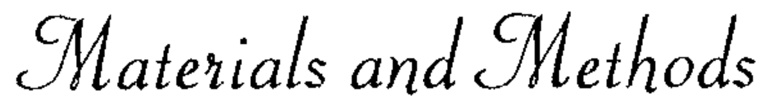
Effect of bactericide 'Terlai' and *P. fluorescens* on bacterial wilt on tomato was tried by Hanudin and Machmud (1994) in Indonesia, they revealed that both treatments and combined treatment gave some control of the disease.

Fortnum and Martin (1997) conducted experiments for the control of bacterial wilt and successful disease management was achieved with a combination of crop rotation, host resistance and multi purpose fungicides.

Yamada *et al.* (1997) reported dazoment combined with soil solarization gave better control of tomato bacterial wilt (*Ralstonia solanacearum*) to methyl bromide. One month after treatment, residues of dazomet in the soil were reduced to 0.2 per cent of that immediately after treatment.

Hasan and Abdulla (1999) reported that development of bacterial wilt (*R.solanacearum*) on aubergine grown in plots treated with combination of effective microorganism (beneficial soil microorganism) and Ca $(NO_3)_2$ was significantly reduced compared with plots with no treatment or with treatment of effective soil microorganism or calcium nitrate alone.

Sood *et al.* (2002) suggested the role of various components for the integrated management of bacterial wilt of tomato (BWT), these include host resistance, biocontrol with antagonists (*P. fluorescens* and *Bacillus cereus*) and AM fungus (*G. mossae* and *G. fasciculatum*), cultural methods like adjusting time of sowing/date of transplanting, soil amendments with sodium nitrate or potassium nitrate and soil solarazation for 8 and 10 weeks during March to June.



Materials and Methods

3. MATERIALS AND METHODS

The present study was undertaken during 1999-2000 at the college of Horticulture, Vellanikkara, Trichur, Kerala. The details of the present studies are elaborated under the following headings.

3.1 Isolation and characterization of *R. solanacearum* and antagonistic bacteria

3.1.1 Isolation of *R. solanacearum*

Tomato plants which showed typical wilt symptom were collected from the vegetable fields of four locations representing different agro-climatic zones namely College of Agriculture, Vellayani (South zone), College of Horticulture, Vellanikkara (Central zone), College of Agriculture, Pilicode (North zone) and Rice Research Station, Moncompu (Problem zone). Fresh stem bits from samples were surface sterilized, washed twice in sterile distilled water and used for isolation in *Triphenyl tetrazolium chloride agar medium* containing 0.005 per cent 2,4,5 triphenyl tetrazolium chloride.

Triphenyl Tetrazolium Chloride medium (TZC)

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

Circular, fluidal, slimy, white or creamy white colonies with light pink centers which characterise virulent colonies of *R. solanacearum* were selected after incubation at 30 ± 2°C for 24 to 48 h and were then purified by repeated streaking on the same medium (Kelman, 1954). Stock suspensions were maintained by keeping 2 or 3 loopful of bacteria from the pure culture in test tubes containing 5 ml of sterile distilled water. The suspensions were stored at 5°C under refrigeration and

also under room temperature. The stock cultures were also maintained in peptone casamino acid agar slants and stored at 5°C under refrigeration. The cultures were tested periodically for virulence and purified by streaking on TZC medium.

3.2 Pathogenicity tests of *R. solanacearum* on host plants

Pathogenicity of the different bacterial isolates was tested on tomato by using suspensions prepared from 24-48 h old the bacterial growth from peptone casamino acid agar slant cultures in sterile distilled water. The optical density (OD) of the suspension was adjusted to 0.5 which was equivalent to 10^7 cfu per ml. Cross inoculation studies were also conducted on other solanaceous vegetables like brinjal and chilli.

Thirty days old vigorously growing tomato, brinjal and chilli plants were used for inoculation of the bacterial isolates. The seedlings were inoculated by root dip method (Winstead and Kelman, 1952) and then transplanted in pots. The pathogen was reisolated from the artificially inoculated wilted plants and its characteristics compared with the original isolates of the pathogen. The pure culture of the isolates were maintained in peptone casamino acid (PCA) slants at 5°C in refrigerated condition and in sterile distilled water at room temperature. Cultures were tested periodically for virulence and purity by streaking on TZC medium.

3.3 Isolation of bacterial biocontrol agents

Soil samples were collected from the rhizosphere of healthy tomato plants from the four different locations for the isolation of bacterial antagonist (fluorescent pseudomonads). Soil samples were collected from the field where the pathogen is known to be present but disease occurrence is low (Baker and Cook, 1974).

The standard dilution plate technique as described by Dhingra and Sinclair (1995) was adopted for isolation of antagonistic bacteria. The media used for isolation is King's-B (King *et al.*, 1954).

King's B medium

Proteose peptone	20.0 g
$MgSO_4 7H_2O$	1.5 g
K_2HPO_4	1.5 g
Glycerol	10 ml
Distilled water	1000 ml
pH	7.2 – 7.4

3.3.1 Purification of fluorescent Pseudomonads (antagonist)

Dilutions of 10^{-5} and 10^{-6} were spread in the agar media (King's-B) and incubated for 36–48 h and bacterial colonies fluorescing under UV light of wavelength of 254 nm were selected. They were purified by repeated streaking to get single colonies. The pure culture of the isolate was maintained in King's B slants at 5°C in refrigerated condition and sterile distilled water at room temperature and further tested for antagonism.

3.3.2 Testing for antagonistic ability

The fluorescent bacteria purified as above were tested for antagonistic ability against *R. solanacearum* as described in Dhingra and Sinchlair (1995).

a) Dual culture method

Suspensions of *R. solanacearum* in sterile water was spread on TZC agar plates, using a glass spreader. The fluorescent bacterium was spotted at the center and the plate incubated for 36 – 48 h, after which, observation on inhibition zone was made.

b) Cross streaking method

Here both pathogen and fluorescent bacteria were streaked parallel and across the agar plate containing TZC agar medium and observed for antagonistic activity of the fluorescent bacteria against *R. solanacearum*.

3.4 Characterisation of the pathogen and antagonist

Characterisation of the four different isolates of the pathogen and antagonist bacterium 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 McCance, 1966). Before each test a loopful of the bacterial suspension from the stock culture was transferred to TZC agar plates for *R. solanacearum* and to King's 'B' agar plates for *antagonist bacterium* and incubated at room temperature for 24 to 48 h. The resulting bacterial growth was used for each study. Tests were conducted in *triplicates and incubated at* 30 ± 2°C.

3.4.1 *Cultural* characters

3.4.1 (i) Morphology

The morphology was studied using 24 h old culture of the bacterium.

Hucker's modification of gram staining was employed to study the gram reaction of the bacterial isolates (Hucker and Conn, 1923).

3.4.1(ii) Colony characteristics of different bacterial isolates of the pathogen and fluorescent bacterium

Colony characteristics of the different bacterial isolates were studied in peptone casamino acid medium for *R. solanacearum* and in King's 'B' medium for fluorescent bacterium.

A sterile loop charged with dilute suspension of each bacterial isolate was streaked on respective medium. The colony characteristics were studied after 24 to 48 h of incubation.

3.4.1 (iii) Pigment production

Production of water insoluble pigment by the four isolates and the fluorescent bacterium was tested after incubation for 48 h on Yeast Glucose Chalk Agar medium.

Yeast Glucose Chalk Agar medium

Yeast extract	- 10.0 g
Glucose	- 10.0 g
Chalk	- 20.0 g
Agar agar	- 20.0 g
Distilled water	- 1000.0 ml
PH	- 7.2

Production of water soluble pigment was studied using King's B medium.

The test cultures were spot inoculated on the sterilized medium in petri plates, incubated for 48 h and examined for pigmentation around the colonies.

3.4.2 Physiological characters

3.4.2 (i) Oxygen requirements

Nutrient Glucose Agar (containing 0.005 per cent Bromocresol purple) columns in test tubes were inoculated with each bacterial isolate by stabbing with a sterile inoculation needle. The agar surface of one set of tubes was covered with sterile liquid paraffin to a depth of 1 cm. The tubes were incubated and observations on colour change of the medium was recorded at 48 h intervals for 7 days.

3.4.2 (ii) Growth of pathogen and antagonist at different temperature

Growth of pathogenic bacterium and antagonistic bacterial isolates were studied using peptone water maintained at 4°, 20°, 40° and 60°C. The growth was recorded after 24, 36, 48 and 72 h of intervals.

3.4.3 Biochemical characters

3.4.3 (i) Production of levan

Peptone beef extract medium containing 5 per cent sucrose was used for this test.

Peptone	- 10.0 g
Beef extract	- 5.0 g
Sucrose	- 50.0 g
Agar agar	- 20.0 g
Distilled water	- 1000.0 ml

Dilute suspension of the bacterial isolates were streaked over the sterilized medium in petriplates and growth characters were observed after 48 h. Presence of large, white, domed and mucoid colonies characterized the production of levan from sucrose.

3.4.3 (ii) Starch hydrolysis

Nutrient agar containing 0.2 percent soluble starch was employed for this test.

Peptone	- 10.0 g
Beef extract	- 5.0 g
Starch (Soluble)	- 2.0 g
Agar agar	- 20.0 g
Distilled water	- 1000.0 ml
PH	- 7.0

The test isolates were spot inoculated on the medium poured in sterilized petriplates. Starch hydrolysis was tested after 4 days 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 starch hydrolysis.

3.4.3(iii) Tyrosinase activity

Dyes medium (Dye, 1962) was used for detection of tyrosinase.

$NH_4H_2PO_4$	- 0.5 g
K_2HPO_4	- 0.5 g
$MgSO_4.7H_2O$	- 0.2 g
NaCl	- 5.0 g
Yeast extract	- 5.0 g
Tyrosine	- 0.5 g
Agar agar	- 20.0 g
Distilled water	- 1000.0 ml
pH	- 6.8 to 7.0

The medium was dispensed in test tubes, autoclaved and slants were prepared. Slants were inoculated with the different bacterial isolates and incubated. Melanin production was estimated as high, medium or low based on the intensity of brown discolouration developed in the medium after 48 to 72 h of growth of bacteria.

3.4.3(iv) Production of indole

Tryptophan broth medium was employed for this test.

Tryptophan (Casein digest)	- 10.0 g
NaCl	- 5.0 g
Distilled water	- 1000.0 ml
pH	- 7.0

The medium was dispensed in test tubes and autoclaved. Whatman No. 1 filter paper strips. (5 x 50 mm) soaked in warm saturated solution of oxalic acid

were used as indicator paper. The strips became covered with oxalic acid crystals, dried at room temperature and used without sterilizing.

The tubes were inoculated with the test isolates and an oxalic acid strip was inserted into each tube by the side of the plug. They were incubated for 14 days. A change in colour of the oxalic acid crystals to pink or red denoted the production of indole.

3.4.3 (v) Methyl red and Voges – Proskaeur tests

Methyl red broth was used as the basal medium for both MR and VP tests.

Proteose peptone	- 5.0 g
Glucose	- 5.0 g
K_2HPO_4	- 5.0 g
Distilled water	- 1000.0 ml
pH	- 7.0

The medium was dispensed in test tubes in 5 ml aliquots and sterilized by tyndalisation. Two sets of tubes were inoculated with 48 h old culture of the isolates for MR and VP tests respectively. The tubes were incubated for seven days.

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

For VP test 0.6 ml of alpha naphthol solution (5 per cent in 95 per cent alcohol) and 0.2 ml of 40 per cent aqueous solution of KOH was added to one ml of the culture. The mixture was shaken for few minutes and allowed to stand for two h. A crimson or Ruby colour indicated positive VP test.

3.4.3 (vi) Production of ammonia

Peptone water medium was used for this test.

Peptone	- 10.0 g
NaCl	- 5.0 g
Distilled water	- 1000.0 g
pH	- 7.0

The bacterial cultures were inoculated in tubes containing sterilized peptone water medium and incubated for 48 h. The accumulation of ammonia was detected using Nessler's reagent which gave a brown to yellow precipitate with ammonia.

3.4.3 (vii) Action on milk

The bacterial isolates can cause changes in milk and the changes can best be detected in bromocresol purple milk. Both skimmed and unskimmed milk were used. A 1:3 dilution of skimmed milk was prepared in water and bromocresol purple was added to give a final concentration of 0.002 per cent when a light blue colour was obtained (Clark and Lubs, 1917). Unskimmed milk (containing approximately 3 per cent fat) was also diluted with water and the indicator was added as above. The milk medium was then dispensed in 5 ml aliquots in test tubes and sterilized by tyndalization. The medium was inoculated with a loopful of 48 h old growth of each bacterial isolate and incubated. Observations were recorded periodically for 30 days, for acidic or alkaline reaction, curdling and peptonization. The milk changed from light blue to yellow in acidic reaction and to violet in alkaline reaction. Curdling was indicated by the heterogenous clumps due to precipitation of casein. Peptonization was indicated by the partial clearing of milk.

3.4.3 (viii) Gelatin liquefaction

Nutrient gelatin medium was used in this test.

Peptone	- 10.0 g
Beef extract	- 5.0 g
Gelatin	- 120.0 g
Distilled water	- 1000.0 ml
pH	- 7.0

The medium was dispensed in test tubes to a depth of 4 cm and autoclaved. Forty eight hour old cultures of each isolate was stab inoculated into the sterilized gelatin columns. The tubes were incubated and observed for the liquefaction of the gelatin column at regular intervals for 30 days.

3.4.3 (ix) Sodium chloride tolerance test

Peptone water with different concentrations of sodium chloride namely 1, 2, 3, 4 and 5 per cent were used for this test. The isolates were inoculated into the tube containing different concentrations of sodium chloride and observed for growth for 48 to 72 h of incubation.

3.4.3 (x) Oxidase test

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

3.4.3 (xi) Catalase test

Smears of 24 h old bacterial isolates were prepared on clean glass slide and covered with a few drops of 20 volume hydrogen peroxide. Effervescens indicate the presence of catalase in the culture.

3.4.3 (xii) Denitrification

Medium composition for denitrification.

$K_2HPO_4.3H2O$	-0.8 g
KH_2PO_4	-0.2 g
$CaCl_2$	-0.1 g
$MgSO_4.7H_2O$	-0.5 g
$(NH_4)_2 SO_4$	-1.5 g
Yeast extract	-3.0 g
Glycerine	-10.0 g
Agar	-1.0 g
Distilled water	-1000 ml
pH	-7.2

Ten ml of the medium was sterilized in test tubes at 121°C for 15 min. For anaerobic sealing, 3 ml of 1 per cent water agar was poured on top of the semisolid medium and incubated.

3.4.3 (xiii) Urease test

Christensen's urea agar (Christensen, 1946) was used in this test. Ninety ml aliquots of the medium was dispensed in 250 ml conical flasks and autoclaved. To each flask 10 ml of 20 per cent sterilized urea solution was added and dispensed in sterilized test tubes in 5 ml quantities and slants were prepared. The tubes were inoculated as for slant culture and observations recorded periodically. A change in colour of the medium from yellow to pink or red indicated urease production.

Composition of medium

Peptone	-1.0 g
NaCl	-5.0 g
KH_2PO_4	- 2.0 g
Glucose	- 1.0 g
Phenol red (0.2 per cent solution)	- 6.0 ml
Distilled water	-1000.0 ml
pH	- 6.8

3.4.3 (xiv) Lipolytic activity

The medium of Sierra (Sierra, 1957) was employed in this test. Ninety-nine ml aliquots of the medium was dispensed in flasks, autoclaved and 1 ml of tween 80 (Oleic acid ester) was added to each flask and mixed thoroughly. Then the medium was poured into sterile petridishes, spot inoculated with the test bacteria, and incubated for 15 days. Observations were taken at regular intervals during incubation. Opaque zones produced around the bacterial growth was indicative of lipase production.

Composition of the media for the test.

Peptone	- 10.0 g
NaCl	- 5.0 g
$CaCl_2.7H_2O$	- 0.1 g
Agar agar	- 20.0 g
Distilled water	- 1000.0 ml
pH	- 7.0

3.4.3 (xv) Production of hydrogen sulphide

Peptone water containing 1 per cent casamino acid was used for testing the ability of the bacterial isolates to produce hydrogen sulphide.

Peptone	- 10.0 g
NaCl	- 5.0 g
Casamino acid	- 10.0 g
Distilled water	- 1000.0 ml
pH	- 7.0

The medium was dispensed in 5 ml quantities in test tubes and autoclaved. The indicator papers were prepared by cutting strips of Whatman No. 1 filter paper. These strips were soaked in warm saturated solution of lead acetate, dried, sterilized

and then again dried. The bacterial isolates were inoculated into the test tubes and the indicator papers were inserted aseptically into the tubes between the plug and the glass with the lower end of the strip just above the broth. The tubes were incubated and observations were recorded at regular intervals upto 14 days. Liberation of hydrogen sulphide caused blackening of the lead acetate paper strip.

3.4.3 (xvi) Arginine hydrolase test

Thornley's semi solid Arginine medium (Thornley, 1960) was used for this test.

Peptone	- 1.0 g
$2HPO_4$	- 0.3 g
NaCl	- 5.0 g
Agar agar	- 3.0 g
Phenol red	- 0.01 g
L-arginine mono hydrochloride	- 10. 0 g
Distilled water	- 1000.0 ml
pH	- 7.2

Five ml aliquot of each of the semi solid medium was dispensed in test tubes, autoclaved, cooled and stab inoculated with the test isolates. The surface of the medium was sealed with sterile paraffin oil to a depth of one cm. The tubes were incubated and observations recorded for seven days at regular intervals. Hydrolysis of arginine was indicated by a change in colour of the medium to red.

3.4.3 (xvii)Utilisation of asparagines as sole source of carbon and nitrogen

Dye's medium was used for this test (Dye 1966).

Solution No- 1.

K_2HPO_4	- 8.0 g
KH_2PO_4	- 2.0 g
Distilled water	- 1000.0 ml

Solution No.2

$MgSO_4.7H_2O$	- 2.0 g
$FeSO_4$	- 0.5 g
NaCl	- 1.0 g
$MnSO_4$	- 0.2 g
H_2SO_4	- 1 drop
Distilled water	– 1000.0 ml

Solution No.3

$NaMoO_4$	- 0.02 g
Distilled water	- 1000.00 ml

Solution No.4

Copper sulphate – saturated solution in distilled water.

Ten ml of each solution was mixed in the order of 3,4,2 and 1 and filtered. Ninety ml of distilled water and 2.0 g of L-asparagine was added, dispensed in 5 ml aliquots in test tubes and sterilized by autoclaving, The bacterial isolates were inoculated into the medium, incubated and examined for growth. Positive growth meant that asparagine was utilized as sole source of carbon and nitrogen.

3.4.3 (xviii) *Utilisation of carbohydrates*

The following carbohydrates were used for this test.

Monosaccharides		Disaccharides	Sugar alcohols
Pentoses	Arabinose	Sucrose	Mannitol
		Lactose	Inositol
Hexoses	Fructose	Cellobiose	Sorbitol
	Mannose	Maltose	Dulcitol
	Galactose		

An aliquot of 90 ml each of the basal medium was dispensed in 250 ml flasks and sterilized by autoclaving. Ten per cent solutions of the sugars and sugar alcohols were prepared in sterile distilled water and sterilized by tyndalisation. Ten

ml each of the sterile solution was added to 90 ml aliquots of the melted medium to obtain a carbohydrate concentration of 1 per cent and dispensed in sterile test tubes to a depth of 4 cm. The medium was stab inoculated with the bacterial isolates and the tests were done in duplicate. In one set of tubes the medium was sealed with sterilized liquid paraffin to a depth of 1 cm. The inoculated tubes were incubated and observations recorded at regular intervals upto a period of 30 days. Change in colour of the medium to yellow indicated positive utilization of the carbon compounds with the production of acid and gas.

The basal medium used in this test was Hayward's semi solid medium (Hayward, 1964).

Peptone	-1.0 g
$NH_4H_2PO_4$	- 1.0 g
KCl	- 0.2 g
$MgSO_4.7H_2O$	- 0.2 g
Bromothymol blue	- 0.03 g
Agar agar	- 3.0 g
Distilled water	- 1000.0 ml
pH	- 7.2

3.4.3 (xix) Utilisation of organic acids

Hayward's semi solid medium was employed in this test also. Sodium salts of organic acids namely acetate, citrate and benzoate were added to the medium to obtain a concentration of one per cent. The medium was dispensed in test tubes and autoclaved. A loopful of each test isolate was inoculated into the medium and observations were recorded for 30 days.

3.5 Sensitivity to antibiotics

Sensitivity of the isolates of the pathogen (*R. solanacearum*) and *antagonistic bacterum to antibiotic was determined by paper disc method. The*

antibiotics used were Ampicillin, Streptomycin sulphate, Kanamycin, Oxytetracycline, Chloramphenicol and Rifampicin at 50 mgl^{-1}, 100 mgl^{-1} and 200 mgl^{-1} concentrations.

Twenty four h old cultures of bacterial isolates were utilized for making lawns of bacteria in TZC for pathogen and King's B for fluorescent bacterium in this test. Sterile antibiotic discs were spot inoculated in the media TZC and King's B for pathogen and antagonistic bacterium respectively. Sterilised paper discs dipped in distilled water were used as control. The test was performed in triplicate and inhibition zone was measured after 48 h.

3.6 Effect of seed treatment with fluorescent bacterium and antibiotic on the germination of tomato seed.

In order to study the effect of seed treatment with antagonistic bacterium and antibiotic (Streptomycin sulphate 500 mgl^{-1}) a study was conducted using seeds of two varieties of tomato, a bacterial wilt resistant variety- Sakthi and susceptible variety Pusa Ruby. Germination studies were conducted following Blotter method and Roll towel method (ISTA, 1966).

3.6.1 Blotter method

Seeds were treated by soaking the seeds in antagonistic bacterial suspension (10^7 cfu per ml) and in Streptomycin sulphate solution (500 mgl^{-1}) for 30 min, drained and used. Untreated seeds soaked in distilled water were used as control. Hundred seeds from each treatment were placed in sterile petri dishes at the rate of 25 seeds per dish lined with sterilized moistened filter paper with three replications. These petri dishes were incubated at room temperature. Observations were recorded on germination percentage, length of shoot and root.

3.6.2 Roll towel method

The towel papers were soaked and washed in water. the extra moisture from the paper was removed by pressing the soaked paper by hand and holding it in plastic trays in slanting position. Two layers of wet paper towels were used as substratum for keeping the seeds. The treated seeds (as in 3.6.1) were arranged in the paper with proper spacing. Hundred seeds were kept for each treatment in three replications. One layer of wet towel paper was kept over the seed and rolled up to the bottom edge of the towel paper or rolled firmly from left to right and then secured with a rubber band at the center. The prepared rolls were kept upright in a tray so that the bottom edge touches the water in the tray. Observations were recorded on germination percentage, shoot and root length after 10 days when the seedlings emerged out from the roll.

3.6.3 Pot culture method

A pot culture study was also taken up to compare the effectiveness of seed treatment with antagonist, antibiotic and AM fungus infection on seed germination and seedling vigour. The seed treatment was given as per 3.6.1. The AM fungus inoculation was done by thoroughly mixing 50g culture/pot of size 35 cm.

3.7 Pot culture experiment

With an objective to select effective treatments for bacterial wilt management a pot culture study was conducted in completely randomized block design (CRD) involving both Sakthi and Pusa Ruby (resistant and susceptible) varieties. Three replications were maintained for each treatment. The experiment was conducted during August 1999 to January 2000 using the wilt sick soils of vegetable field of the College of Horticulture, Vellanikkara. Thirty days old seedlings were transplanted into pot size of 22 cm. Cultural practices as per the package of practices recommendations for crop (KAU, 1996) was followed.

The treatments included are:

T_1- Soil solarisation with 150 gauge polythene sheets for 30 days

T_2- Application of calcium oxide @ 35 g per plant one week before planting

T_3 - Application of neem cake @ 45 g per plant one week before planting

T_4 - Application of urea alone @ 15.28 g per plant one week before planting

T_5- Calcium oxide @ 150 g and urea 15.28 g per plant one week before planting

T_6- AM Fungus inoculation - *Glomus fasciculatum* at the time of sowing

T_7- Seed treatment with *P aeruginosa* at the time of sowing

T_8- Drenching with *P aeruginosa* at the time of planting and after 30 DAP

T_9- Seed treatment and drenching with *P aeruginosa* at the time of planting and 30 DAP

T_{10}- Drenching with garlic extract 10 per cent at the time of planting and 30 DAP

T_{11}- Drenching with Bordeaux mixture 1 per cent at the time of planting and 30 DAP

T_{12}- Seed treatment with Streptomycine sulphate 500 mgl^{-1}

T_{13}- Spraying with Streptomycine sulphate 500 mgl^{-1} at 30 DAP

T_{14}- Control

3.7 (i) Soil solarisation

The wilt sick soils in the vegetable plot of the College of Horticulture is used for solarisation. Plot size of 10 m x 5 m was tilled to fine tilth and stubbles were cleared and clodes were broken, field has been levelled properly. Raised beds of 10 m x 5 m size prepared and covered with white polythene sheets of 150 gauge thickness, after drenching the soil with water @ 5 litres per m^2. The sheet has to be in close contact with bed so as to prevent the formation of air pockets between polythene sheet and bed. The edges of the polythene sheet all around the bed tightened by putting soil over it to keep the sheet in position. Soil thermometers were installed at 5 cm and 10 cm depth on the bed to record the daily temperatures at 08:00 h and 14:00 h.

3.7 (ii) Seed treatment with bacterial antagonist

Seeds of Sakthi and Pusa Ruby were treated with antagonistic bacterial isolate. Seeds were soaked in antagonistic bacterial suspension (10^7 cfuml^{-1}) for 30 min dried in shade for 2 h and sown in the nursery.

3.7 (iii) Drenching with bacterial antagonist suspension

Three hundred ml of antagonistic bacterial suspension (10^7 cfu per ml) was used for drenching in each treatment.

3.7 (iv) Seed treatment with antibiotics

A solution of Streptomycine sulphate 500 mgl^{-1} was used for treating seeds. Seeds were soaked in the antibiotic solutions for 30 min, dried in shade for 2 h and sown in the nursery.

3.7 (v) AM fungus inoculation

The soil used for raising nursery was thoroughly mixed with AM fungus (*Glomus fasciculatum*) culture. Fifty gram culture was used for pot size of 35 cm.

3.7 (vi) Drenching with garlic extracts

One kg peeled garlic bulb was ground well to a paste form and thoroughly mixed with 10 l of water to prepare 10 per cent garlic extract. Three hundred ml of the extract thus prepared was used for drenching each plant.

3.7 (vii) Observations recorded

The following observations were recorded from the pot culture experiments.

(a) Percentage wilt incidence at 20, 40 and 60 DAP

(b) Enumeration of rhizosphere microflora at 20, 40 and 60 DAP

Population of fungi, bacteria, actinomycetes, *R. solanacearum* and *P.aeruginosa* in the rhizosphere soil collected from different treatments were estimated by serial dilution techniques. Special media like Martin Rose Bengal Streptomycin Agar, Thorntons Agar, Kenkinghts Agar, Tetrazolium chloride Agar and Kings-B Agar and dilutions of 10^{-3}, 10^{-5}, 10^{-4}, 10^{-3} and 10^{-3} for estimating fungi, bacteria, actinomycetes, *R. solanacearum* and *P. aeruginosa* respectively were used.

(c) Estimation of percentage of AM fungus infection at 60 DAP

Root samples of tomato plants were stained for AM fungus infection by the method of Philips and Hayman (1970) and the percentage of infection was estimated.

$$\text{AM Fungi infection percentage} = \frac{\text{No. of positive root segment observed}}{\text{No.of root segment observed}} \times 100$$

(d) Biometric observations (wet weight)

(e) Yield

3.8 *In vitro* sensitivity to fungicides/plant extract

The study was undertaken to test the *in vitro* sensitivity of the fungicides to *R. solanacearum* and fluorescent bacterium selected for main field experiment and also to select the most effective dose of copper hydroxide 77 per cent (Kocide). The treatments were 10 per cent garlic extracts, 1 per cent Bordeaux mixture, Copper hydroxide 77 per cent (Kocide) 0.15 per cent, 0.2 per cent and 0.25 per cent. Copper hydroxide 77 per cent (Kocide) was included in the study to test its effectiveness as bactericide and to use it as a substitute for Bordeaux mixture. Bordeaux mixture 1 per cent was found effective against bacteria due to its colloidal nature it is not recommend for soil drenching.

The bacterial suspension was spread on TZC agar medium for *R.solanacearum* and King's 'B' for fluorescent bacterium so as to get a uniform

bacterial growth. Filter paper disc of 5 mm dipped in fungicides and plant extract was kept at the center of the dish and incubated. Observations were recorded for zone of inhibition in each dish after seven days.

3.9 Field experiments

Field experiments were laid out during September 2000 to January 2001 in wilt sick soil of College of Horticulture, Vellanikkara to find out the best combinations of the selected components from the pot culture experiments for the management of bacterial wilt of tomato. Based on pot culture experimental results a two stage management practice was tried, one as seed treatment (nursery stage) and the other soil drenching treatments with bactericides and soil solarization along with soil amendments with CaO + urea (main field). The cultivation practices as per the Package of Practices Recommendations for crop 1996 of the Kerala Agricultural University (1996) were followed.

Design	: Two factor RBD
Replications	: 3
Variety	: Sakthi
Plot size	: 4m × 3m
No. plants per plot	: 20
Total number of treatment combinations	: 15

Nursery treatment

S_0 – Untreated seeds

S_1 – Seed treatment with *P aeruginosa* at the time of sowing in the nursery

S_2 – Nursery soil treatment with AM fungus(*G. fasciculatum*) @ 50 g per pot

Treatment in the main field

T_0 – Control

T_1 – Drenching with 1 per cent Bordeaux mixture at the time of planting and 30 DAP

T_2 – Drenching with 10 per cent garlic extract at the time of planting and 30 DAP

T_3 – Solarization for 30 days and application of urea (15.28 g per plant) + calcium oxide (35 g per plant) at 30 DAP

T_4 – Drenching with Copper hydroxide 77 per cent (Kocide) 0.15 per cent at the time of planting and 30 DAP

Treatment combinations

S_1T_1	S_2T_1	S_0T_1
S_1T_2	S_2T_2	S_0T_2
S_1T_3	S_2T_3	S_0T_3
S_1T_4	S_2T_4	S_0T_4
S_1T_0	S_2T_0	S_0T_0

3.9.1 Observations recorded

1. Percentage wilt incidence at 15 days interval (15, 30, 45, 60 and 75 DAP)
2. Enumeration of rhizosphere microflora (fungi, bacteria, actinomycetes, *R.solanacearum* and *P. aeruginos*) at 30 and 60 DAP
3. Biometric observations of the plant at 30 DAP (Plant height, root length, number of leaves, wet weight, dry weight of plant)
4. Estimation of percentage of AM fungi infection at 60 DAP
5. Yield

3.10 Statistical analysis

Data generated were statistically analysed using the statistical package MSTAT (Feed, 1986) are presented in the result. Multiple comparison among treatments were done sing DMRT.

Results

4. RESULTS

Investigations on various aspects related to the integrated management of bacterial wilt of tomato such as cultural, biological and chemical methods were carried out and results are presented.

4.1 Isolation and characterization of *R. solanacearum* and antagonistic bacteria

4.1.1 Isolation of *R. solanacearum*

Tomato plants showing typical wilt symptoms were collected from four different locations viz., College of Agriculture, Vellayani (South zone), College of Horticulture, Vellanikkara (Central zone), College of Agriculture, Pilicode (North zone), Rice Research Station, Moncompu (Problem zone).

Isolation of the pathogenic bacterium was done in TZC agar medium and yielded typical colonies identified based on shape, size and colour as circular, fluidal, slimy white with light pink center. The cultures were purified by repeated cycles of streaking. Single colonies were selected, stored in TZC slants and in sterile distilled water.

4.2 Pathogenicity test of *R. solanacearum* on host plants

Isolates of *R. solanacearum* from four locations were inoculated by root dip method on tomato and other solanaceous crops viz., chilli and brinjal. Tomato plants inoculated at six to seven leaf stage wilted within eight days of inoculation but brinjal and chilli plants wilted in nine and eleven days after inoculation respectively. The bacteria was reisolated from all the wilted plants.

4.3 Isolation of antagonistic bacteria

Isolation of antagonistic bacteria were tried from the rhizophere soil samples collected from the four different locations. Among the four locations the maximum soil samples were collected from the central zone followed by south zone. (Table1) Out of the 29 fluorescent bacteria obtained maximum number was from central zone. When the flourescent bacterial isolates were tested for antagonistic ability only one isolate was showing effective antagonism against *R. solanacearum* , it was

obtained from central zone. Effective antagonism could not be detected from the Pseudomonads obtained from soil samples of other locations.

The isolated antagonistic bacteria have been identified as a fluorescent one producing spreading white to dirty white colonies with abundant bluish green fluorescent water soluble pigments in King's B medium. The colour of the culture changed to brown as it becomes older.

4.3.1 Purification of fluorescent Pseudomonad (antagonist)

Purification of the fluorescent pseudomonad was done on King's B medium by repeated streaking. Single colonies which showed slimy, irregular, white to dirty white colour and which produced abundant water soluble pigments were selected. The fluorescence of the bacteria has been tested under UV light of wavelength 254 nm and revealed as water soluble bluish green in colour. The colour of the culture turned brown as it became older. The pure culture was stored in slants of King's B medium at 5°C and in sterile distilled water at room temperature.

4.3.2. Testing for antagonistic ability

A clear zone of inhibition of *R. solanacearum* was shown in dual culture method by *P. aeruginosa* within 36 h after inoculation. By 60 h, *P. aeruginosa* overgrows *R. solanacearum* and completely covers the petri dish. Clear inhibition zone was produced in the cross streaking methods also (Plate 1).

4.4 Characterisation of the pathogen and antagonistic bacteria

Cultural, morphological, physiological and biochemical characters of the four isolates of *R. solanacearum* and the fluorescent Pseudomonad are presented in Table 2 and 3.

4.4.1 Cultural characters

4.4.1(i) Morphology

The isolates of the pathogen from four different locations were Gram negative, motile, short rods. The antagonistic bacteria was also Gram negative short rods with poor motility.

Plate 1. Dual culture method to test antagonism of *P. aeruginosa* against *R.solanacearum* at different intervals

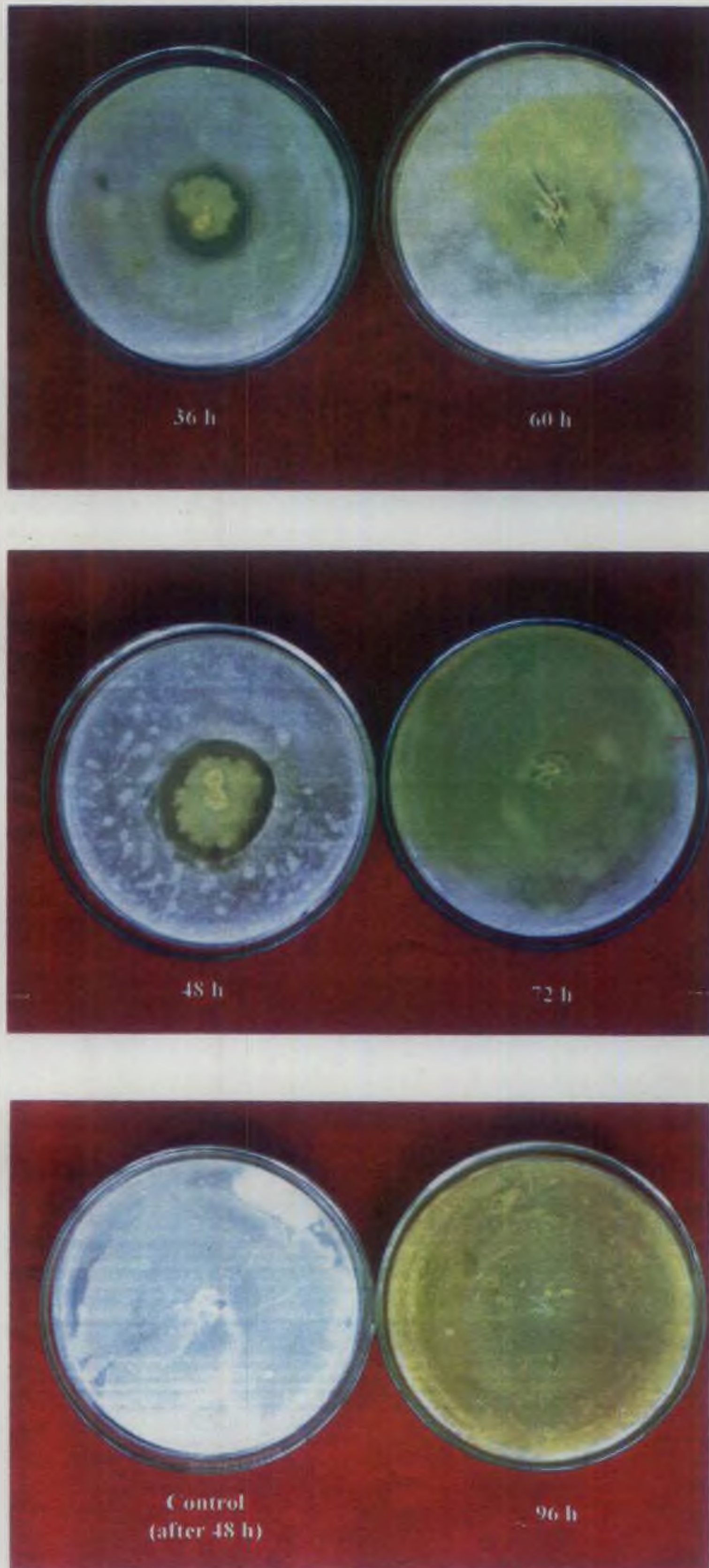


Table 1. Details of antagonistic bacteria isolated from different zones

Location (zone)	No. of soil samples drawn and screened	No. of fluorescent bacteria obtained	No. of bacteria showing antagonism against *R. solanacearum*	Remarks
1. South zone	18	06	--	No antagonist obtained
2. Central zone	56	19	1	Fluorescent antagonist obtained
3. North zone	10	12	--	No antagonist obtained
4. Problem zone	49	02	--	No antagonist obtained

Table 2. Comparison of colony characters of the isolates of *R. solanacearum* on TZC media.

Location of isolates	Nature & colour of colony	Growth, slime and fluidity	
South zone	Circular, smooth, creamy- white colony with light pink centre, convex with entire margine.	GR Sl Fl	++ + +
Central zone	Circular, smooth, creamy- white colony with light pink centre, convex with entire margine.	GR Sl Fl	+++ +++ ++
North zone	Circular, smooth, creamy- white colony with light pink centre, convex with entire margine.	GR Sl Fl	+++ +++ +
Problem zone	Circular, smooth, creamy- white colony with light pink centre, convex with entire margine.	GR Sl Fl	+++ +++ +++

CR – Growth Sl - Slime Fl – Fluidity +++ Good ++Moderate + Slight

4.4.1(ii) Colony characteristics of different isolates of *R. solanacearum* on TZC and fluorescent bacteria on King's B medum.

The colony characters of the four isolates of the pathogen on TZC medium were compared in Table 2. Within 24 h after inoculation all the four isolates produced circular, smooth, raised, creamy white colonies with light pink center and with entire margin.

The isolates from the central, north and the problem zone produced abundant growth fluidity and slime compared to the isolate from the south zone. The isolate from south zone showed poor growth, sliminess and fluidity compared to others. The fluidity was highest in the isolate from the problem zone followed by the isolate from the central zone. The antagonistic bacterium produced spreading white to dirty white colonies, within 24-48 h after inoculation.

4.4.1(iii) Pigment production

All the four isolates of *R. solanacearum* failed to produce water soluble pigments in King's B media and water insoluble pigments in glucose chalk agar medium. In the case of antagonistic bacterium it produced bluish green water soluble diffusible fluorescent pigments in King's B medium and a non fluorescent brown pigment as the culture become older. But no pigment production was noticed in glucose chalk agar medium (Table 3).

4.4.2 Physiological characters

4.4.2(i) Oxygen requirements

The colour of the nutrient glucose agar medium changed from blue to yellow in all the test tubes inoculated with bacterial isolates indicating the aerobic nature of the pathogenic bacteria as well as the antagonistic bacterium. The bacterial isolate also grew under anaerobic condition.

Table 3. Characterization of *R. solanacearum* and fluorescent pseudomonad

Sl. No.	Characters studied	*R. solanacearum* isolates				fluores cent pseudo monad
		Cent-ral zone	South zone	Prob-lem zone	Nort h zone	
A	**Morphological characters**					
1.	Gram staining	-ve	-ve	-ve	-ve	-ve
2.	Pigment Production					
	i) Water soluble					
	a) fluorescent	-ve	-ve	-ve	-ve	+ve
	b) Non fluorescent	-ve	-ve	-ve	-ve	+ve
	ii) Water insoluble	-ve	-ve	-ve	-ve	-ve
B	**Physiological characters**					
3.	Oxygen requirement					
	Aerobic	+ve	+ve	+ve	+ve	+ve
	Anaerobic	+ve	-ve	+ve	+ve	+ve
4.	Growth at different temperatures					
	4°C	+ve	+ve	+ve	+ve	+ve
	10°C	+ve	+ve	+ve	+ve	+ve
	20°C	+ve	+ve	+ve	+ve	+ve
	30°C	+ve	+ve	+ve	+ve	+ve
	40°C	+ve	+ve	+ve	+ve	+ve
	50°C	-ve	-ve	-ve	-ve	+ve
	60°C	-ve	-ve	-ve	-ve	-ve
C.	**Biochemical characters**					
5.	Production of levan	+ve	+ve	+ve	+ve	-ve
6.	Starch hydrolysis	+ve	+ve	+ve	+ve	+ve
7.	Tyrosinase activity	+ve	+ve	+ve	+ve	+ve
8.	Production of indole	-ve	-ve	-ve	-ve	-ve
9.	MR & VP test	-ve	-ve	-ve	-ve	-ve
10.	Production of ammonia	+ve	+ve	+ve	+ve	+ve
11.	Action on milk	+ve	+ve	+ve	+ve	+ve
12.	Gelatin liquifaction	-ve	-ve	-ve	-ve	-ve
13.	Sodium chloride tolerance test					
	NaCl 1%	+ve	+ve	+ve	+ve	+ve
	NaCl 2%	+ve	+ve	+ve	+ve	-ve
	NaCl 3%	-ve	-ve	-ve	-ve	-ve
	NaCl 4%	-ve	-ve	-ve	-ve	-ve
14.	Oxidase test	+ve	+ve	+ve	+ve	+ve
15.	Catalase test	+ve	+ve	+ve	+ve	+ve
16.	Denitrification	-ve	-ve	-ve	-ve	+ve
17.	Urease test	+ve	+ve	+ve	+ve	+ve
18.	Lipolytic activity	+ve	+ve	+ve	+ve	+ve
19.	Production of hydrogen sulphide	-ve	-ve	-ve	-ve	-ve
20.	Arginine hydrolase	-ve	-ve	-ve	-ve	-ve
21	Utilisation of aspargine	-ve	-ve	-ve	-ve	-ve

Table 3 contd...

22.	<u>Utilisation of carbohydrate</u>					
	Arabinose	+ve	+ve	+ve	+ve	+ve
	Fructose	+ve	+ve	+ve	+ve	+ve
	Mannose	-ve	-ve	-ve	-ve	-ve
	Galactose	+ve	+ve	+ve	+ve	+ve
	Sucrose	+ve	+ve	+ve	+ve	+ve
	Lactose	-ve	-ve	-ve	-ve	-ve
	Cellobiose	+ve	+ve	+ve	+ve	-ve
	Maltose	+ve	+ve	+ve	+ve	-ve
	Mannitol	+ve	+ve	+ve	+ve	+ve
	Sorbitol	+ve	+ve	+ve	+ve	-ve
	Inositol	+ve	+ve	+ve	+ve	+ve
	Dulsitol	+ve	+ve	+ve	+ve	-ve
	Innulin	-ve	-ve	-ve	-ve	-ve
23	Utilization of organic acids					
	Na acetate	-ve	-ve	-ve	-ve	-ve
	Na citrate	-ve	-ve	-ve	-ve	-ve
	Na benzoate	-ve	-ve	-ve	-ve	-ve

4.4.2 (ii) Growth of *R. solanacearum* and antagonistic bacterium at different temperature.

Growth of *R. solanacearum* and antagonistic bacterum was very meager at 4°C. The result showed that the antagonistic bacterium could tolerate wide range of temperature compared to the pathogenic bacterium. At 4°C growth of antagonistic fluorescent pseudomonad was less conspicuous than the *R. solanacearum*. At temperature beyond 40°C the growth of *R. solanacearum* was not prominent, but growth of fluorescent pseudomonad was noticed even at 50°C. Both pathogen as well as antagonistic bacterial isolates failed to produce any growth at 60°C.

4.4.3 Biochemical characters

4.4.3 (i) Production of levan

All the four pathogenic isolates produced large white doomed and mucoid colonies indicating high levan production in peptone beef extract medium containing 5 per cent sucrose. Largest colonies were produced by the isolate from problem zone. The antagonistic bacterium failed to produce levan.

4.4.3 (ii) Starch hydrolysis

The ability of all the four isolate of *R. solanacearum* and fluorescent Pseudomonad to hydrolyse starch was evidenced from the appearance of a colourless zone in contrast to the blue back ground of the medium around the bacterial growth on addition of iodine solution.

4.4.3 (iii) Tyrosinase activity

Pathogenic bacterial isolates developed a slight brown discolouration in the medium which indicated that they possessed low tyrosinase activity. The antagonistic bacterium when inoculated into the medium developed a dark brown discolouration indicating a high tyrosinase activity.

4.4.3 (iv) Production of indole

The colour of the oxalic acid crystals on the indicator strips did not changed to pink or red which showed absence of indole production in all the pathogenic isolates as well as the antagonistic bacterium.

4.4.3 (v) Methyl red and voges proskaeur test

All bacterial isolates tested could not change the colour of the medium on addition of 0.6 ml alpha naphthole and 0.2 ml of 40 per cent aqueous KOH solution in culture tube indicating the negative MR&VP reaction.

4.4.3 (vi) Production of ammonia

Addition of nesseler's reagent to the peptone water inoculated with bacterial isolates produced yellow precipitate. indicate ammonia production.

4.4.3 (vii) Action on milk

The *R. solanacearum* isolate showed a highly acidic reaction by clearing the milk and turning the colour from blue (bromocresole purple) to yellow. However fluorescent Pseudomonad (antagonist) has less acidic reaction and colour change was very meager.

4.4.3 (viii) Gelatin liquefaction

Neither the isolates of *R. solanacearum* nor antagonistic bacterium were capable of liquefying gelatin even after one month of observation.

4.4.3 (ix) Sodium chloride tolerance test

The isolates of pathogenic bacterium tolerated sodium chloride concentration upto 2 per cent. But antagonistic bacterium could tolerate only 1 per cent sodium chloride concentration.

4.4.3 (x) Oxidase test

The test was performed using oxidase disc. On touching with culture of isolates of pathogen and antagonistic bacterium. the disc changed its colour to deep purple blue reveled as oxidase positive reaction.

4.4.3 (xi) Catalase test

Positive catalase activity was shown by all the bacterial isolates by the production of effervescence up on addition of a few drops of 20 volume hydrogen peroxide solution to the culture smeared on a clean glass slide.

4.4.3 (xii) Denitrification

The isolates of *R. solanacearum* showed negative reaction as the test tubes inoculated with pathogen did not change the colour of the media to pink and no gas production or growth of bacterium noticed. The antagonistic bacteria showed slight colour change towards pink, and little gas production noticed in sealed tubes.

4.4.3 (xiii) Urease test

Different bacterial isolates tested gave a positive reaction in urease test indicated by the colour change of Christensen's Urea agar from yellow to pink within two days of inoculation.

4.4.3 (xiv) Lipolytic activity

The four isolates of *R. solanacearum* and antagonistic bacterium exhibited lipolytic activity as they produce opaque zone around bacterial growth.

4.4.3 (xv) Production of hydrogen sulphide

All the bacterial isolates failed to produce hydrogen sulphide up on inoculation in peptone water medium even after 14 days of incubation.

4.4.3 (xvi) Arginine hydrolase test

The four pathogenic bacterial isolates as well as the antagonistic isolate failed to gave a pink colouration to the medium indicating their inability to hydrolyse arginine.

4.4.3 (xvii) Utilisation of asparagine as sole source of carbon and nitrogen

None of the bacterial isolates tested was capable of utilizing asparagine as sole source of carbon and nitrogen.

4.4.3(xviii) Utilization of carbohydrates

The four pathogenic bacterial isolates and the antagonistic bacteria gave acidic reaction in most of the sugars tested. In the case of monosaccharides (arabinose, fructose & galactose) the colour of the media changed to yellow within 72 h after inoculation whereas all the bacterial isolates failed to utilise mannose as carbon source.

In the case of disaccharides the pathogenic isolates were able to utilize sucrose, cellobiose and maltose, they were not able to utilise lactose as carbon source. The antagonistic bacterium utilised sucrose but failed to utilise the other disaccharides like lactose, cellobiose and maltose as their carbon source.

The pathogenic isolates utilised sorbitol, dulcitol, manitol, and inositol as their carbon source. Slight variations were noticed between the pathogenic bacterial isolates in its ability to utilise mannitol as evidenced by the change in intensity of colour. The antagonistic bacterial isolate was also able to utilise inositol and

mannitol but not sorbitol and dulcitol as their carbon source as indicated by acidic reaction in the media.

4.4.3 (xix) Utilization of organic acids

All the bacterial isolates tested (both pathogenic and antagonistic) failed to utilise the organic acids sodium acetate, sodium citrate and sodium benzoate as their carbon source.

Pathogenicity and characterization studies revealed that the four isolates belong to *R. solanacearum* race 1 biovar 3.

Based on the results of the characterization studies, growth characteristics and pigment production the antagonistic bacterium was tentatively identified as *Pseudomonas aeruginosa.*

4.5 Sensitivity to antibiotics

Data on the *in vitro* sensitivity of pathogenic and antagonistic bacterial isolates to six antibiotics at different concentrations are given in Table 4.

Among the antibiotics tried Oxytetracycline, Chloramphenicol and Rifampicin were not able to inhibit the growth of both type of bacteria at different concentrations. When the mean diameter of zone of inhibition were compared, both the bacterium were sensitive to Kanamycin at different concentrations tried and the antagonistic bacteria was more sensitive to Kanamycin at 50 mgl^{-1} and 100 mgl^{-1} compared to *R. solanacearum*. At 200 mgl^{-1} concentration *R. solanacearum* was more sensitive (96 mm) compared to antagonistic bacterium (65 mm).

In the case of Streptomycine sulphate *R. solanacearum* was sensitive at 100 and 200 mgl^{-1} concentrations, the antagonistic bacterium showed sensitivity only at 200 mgl^{-1} concentration. At 50 mgl^{-1} concentration the bacterial isolates were insensitive to Streptomycine sulphate giving maximum growth. Similarly antibiotic

Table 4. Sensitivity of *R. solanacearum* and *P. aeruginosa* to antibiotics

Antibiotics	concentrations (mgl^{-1})	Zone of inhibition (mm)	
		R. solanacearum	*P. aeruginosa*
Kanamycin	50	25.0	40.0
	100	36.0	51.0
	200	96.0	65.0
Streptomycine suplhate	50	0.00	0.00
	100	10.0	0.00
	200	15.0	14.0
Ampicillin	50	0.00	0.00
	100	25.0	0.00
	200	30.0	0.00
Oxytetracycline	50	0.00	0.00
	100	0.00	0.00
	200	0.00	0.00
Chloramphenicol	50	0.00	0.00
	100	0.00	0.00
	200	0.00	0.00
Rifampicin	50	0.00	0.00
	100	0.00	0.00
	200	0.00	0.00

Ampicillin was effective against *R. solanacearum* at 100 and 200 mgl^{-1} concentrations, the antagonistic bacterium was resistant at all the concentrations tried.

4.6 Effect of seed treatment with *P. aeruginosa* and antibiotic on germination of tomato seeds

The effect of seed treatment with *P. aeruginosa* (antagonist) and Streptomycin sulphate 500 mgl^{-1} on germination percentage and growth characteristics of seedlings were compared for both Pusa Ruby and Sakthi varieties of tomato and data are presented in Table 5.

Maximum germination was given by Roll towel method compared to blotter method. Among the treated seeds, seed treatment with antagonistic bacterium was found to be better, as it gave earlier germination by one day compared to the seeds treated with antibiotic and control in both the varieties.

4.6.1. Blotter method

In blotter method maximum germination was recorded in antagonistic seed treatment (84 per cent) in Pusa Ruby whereas in variety Sakthi the maximum germination obtained in Streptomycin sulphate treated seeds (89.36 per cent).

4.6.2.Roll towel method

In roll towel method also earlier germination was noticed in seeds treated with antagonistic bacterium for both the varieties. Germination percentage was also higher in the same treatment in both the varieties followed by seed treatment with antibiotics. Germination percentage increased by 8.34 and 6.33 per cent in varieties Pusa Ruby and Sakthi respectively compared to control. The growth characteristics of the seedlings of *P. aeruginosa* treated seeds were found to be much superior compared to other treatments in both the varieties. In the case of *P. aeruginosa* seed treatment, the root length and shoot length in Pusa Ruby were 12.7 cm and 8.5 cm respectively whereas in Sakthi it was 20.4 cm and 9.3 cm respectively.

Table 5. Effect of seed treatment with *P. aeruginosa* and antibiotics on germination percentage and plant growth characters.

Treatments	Blotter method		Roll towel method					
	Pusa Ruby	Sakthi	Pusa Ruby			Sakthi		
	Germination percentage	Germination percentage	Germination percentage	Root length (cm)	Shoot length (cm)	Germination percentage	Root length (cm)	Shoot length (cm)
P. aeruginosa	84.00	82.00	93.00	12.7	8.5	85.66	20.4	9.3
Streptomycine sulphate 500 mgl^{-1}	77.33	89.36	87.66	8.3	7.2	81.67	9.7	8.3
Control	72.67	88.36	84.66	8.2	7.7	79.33	11.3	7.6

4.6.3 Pot culture method

Pot culture study was taken up to compare the effect of beneficial agents namely *P. aeruginosa* and AM fungus and antibiotics on seed germination and seedling vigour on soil medium. Here also seed treatment with *P. aeruginosa* improved germination percentage and other plant growth characteristics in both varieties (Table 6).

Maximum germination was recorded in the case of *P. aeruginosa* treated seeds in both the varieties Pusa Ruby and Sakthi followed by seeds sown in AM fungus inoculated soil. Germination percentage increased by 18.25 per cent due to *P.aeruginosa* seed treatment in the variety Pusa Ruby and by 9 per cent in Sakthi when compared to control. In control percentage germination recorded were 70.75 and 76 respectively for varieties Pusa Ruby and Sakthi. The maximum height of the plant (11 cm) and length of root (10 cm) were observed in seedlings of variety Sakthi after seed treatment with *P.aeruginosa*. AM fungus inoculated soil also gave a better plant vigour compared to control in both the varieties. The number of leaves was maximum (6) in seeds treated with *P.aeruginosa* and AM fungus inoculation in both the varieties. However the antibiotic seed treatment was better to control but not to bio agents, *P.aeruginosa* and AM fungus (Fig.1).

4.7 Pot culture study

A pot culture study was taken up with 14 treatments in three replications using susceptible variety Pusa Ruby and resistant variety Sakthi (Plate 2).

4.7.1 Effect of solarisation on soil temperature

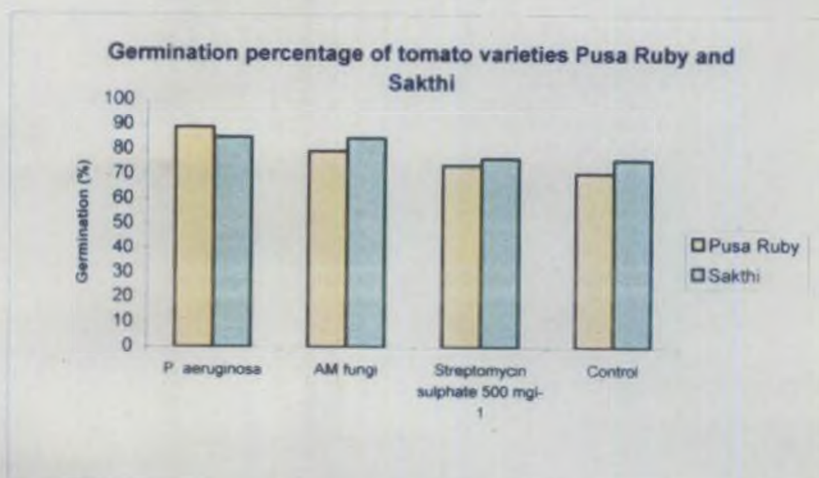
Data on soil temperature, in the morning (08.00h) and in the evening (14.00h) of non solarized and solarized soils, maximum and minimum atmospheric temperature were recorded and presented in Table 7.

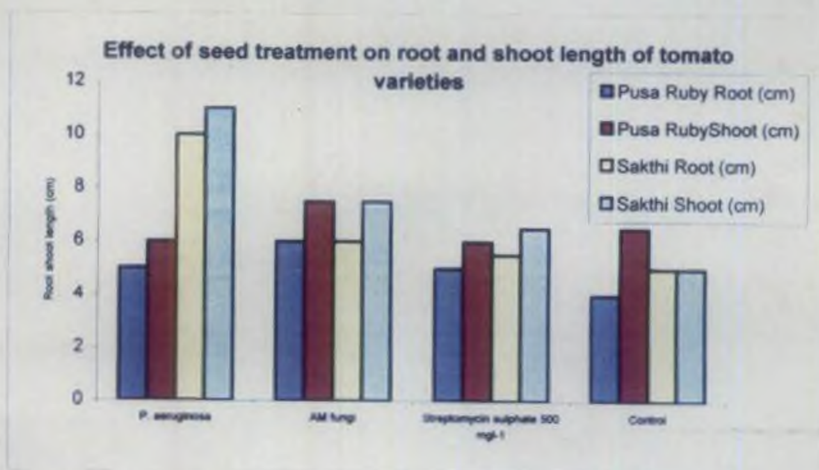
Plate 2. Pot culture experiment

Table 6. Effect of seed treatment with *P. aeruginosa,* antibiotics and AM fungus soil inoculation on germination percentage and plant growth characters at 30 DAP

Treatments	Pusa Ruby				Sakthi			
	Germination percent-age	Length of shoot (cm)	Length of root (cm)	No. of leaves	Germination percent-age	Length of shoot (cm)	Length of root (cm)	No. of leaves
P. aeruginosa	89.00	9.0	6.0	5.0	85.0	11.0	10.0	6.0
G. fasciculatum	79.25	7.5	7.5	6.0	84.5	7.5	6.0	6.0
Streptomycin sulphate 500 mgl^{-1}	73.70	6.0	6.0	5.0	76.5	6.5	5.5	2.0
Control	70.75	6.5	5.0	4.0	76.0	5.0	5.0	4.0

Fig. 1. Effect of seed treatment with *P. aeruginosa*, antibiotics and AM fungi soil inoculation on germination percentage and plant growth characters at 30 DAP





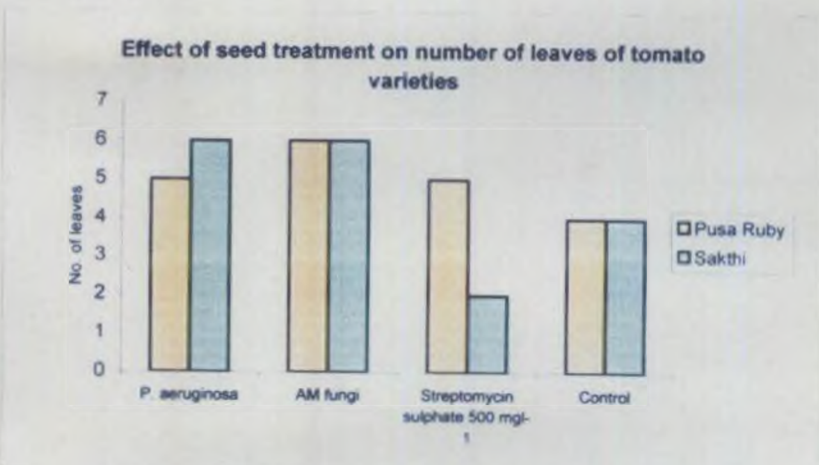


Table 7. Details of atmospheric and soil temperature from 12-12-1999 to 10-1-2000

Date	Atmospheric temperature (°C)		Soil temperature of non solarised soil (°C)				Soil temperature of solarised soil (°C)			
			At 5 cm depth		At 10 cm depth		At 5 cm depth		At 10 cm depth	
	Minimum	Maximum	08.00 h	14.00 h	08.00 h	14.00 h	08.00 h	14.00 h	08.00 h	14.00 h
11-12-99	20.0	32.6	25.0	40.0	25.0	37.0	26.0	36.0	26.0	34.5
12-12-99	22.4	31.8	25.4	34.5	26.1	36.1	27.0	38.0	25.5	35.6
13-12-99	23.0	32.0	27.0	43.0	27.0	37.6	28.5	46.0	25.5	39.0
14-12-99	24.5	28.0	26.0	43.0	26.0	36.0	28.5	38.5	27.0	35.5
15-12-99	21.8	32.0	27.0	43.0	26.0	38.0	28.0	37.5	28.0	39.5
16-12-99	23.0	32.5	27.0	43.1	26.0	38.0	29.0	42.0	28.0	37.5
17-12-99	21.0	31.6	24.4	37.2	24.8	30.5	27.0	43.0	29.5	39.5
18-12-99	22.9	32.6	24.9	34.5	25.2	30.5	26.5	37.0	25.5	39.5
19-12-99	23.9	32.2	25.2	37.5	25.6	33.2	27.5	39.0	26.4	40.0
20-12-99	23.0	31.0	24.5	36.0	26.1	34.0	27.0	39.5	26.0	39.0
21-12-99	23.4	30.0	25.3	38.0	25.1	33.0	26.0	38.5	25.5	38.5
22-12-99	22.1	30.6	26.7	32.2	26.4	38.3	28.5	41.5	27.0	37.5
23-12-99	24.4	29.8	26.0	42.0	25.4	36.5	26.4	38.4	26.0	38.0
24-12-99	24.0	31.6	26.8	42.0	25.5	38.0	26.5	39.0	25.2	39.2
25-12-99	24.5	32.0	26.0	42.0	27.0	38.0	29.0	35.0	29.5	33.5
26-12-99	23.5	31.4	26.6	42.0	26.4	38.0	28.9	46.0	28.0	34.6
27-12-99	22.8	32.8	27.0	41.0	27.0	36.5	29.0	44.0	27.5	38.5
28-12-99	24.0	32.0	25.7	41.5	26.7	37.5	30.0	45.0	29.0	41.5
29-12-99	23.1	31.8	25.8	42.3	26.4	37.7	31.0	47.0	30.0	42.0
30-12-99	21.4	31.4	25.2	39.0	25.2	39.0	31.0	50.0	29.5	44.0
31-12-99	22.8	31.8	25.2	38.0	26.1	36.6	31.5	51.0	30.0	43.0
01-01-00	23.6	31.8	26.0	37.0	26.0	37.0	31.0	52.0	30.0	43.0
02-01-00	23.3	31.6	26.0	26.5	26.0	36.5	31.6	52.5	30.0	44.0
03-01-00	23.4	31.4	26.0	38.0	27.4	38.0	32.0	52.0	29.5	44.0
04-01-00	23.6	32.0	27.4	38.5	27.6	38.5	32.5	53.0	30.5	46.0
05-01-00	24.6	32.5	27.0	38.5	29.0	38.5	33.0	53.5	31.5	46.0
06-01-00	25.6	32.7	27.6	39.5	28.5	39.5	34.0	54.0	32.5	49.0
07-01-00	25.7	33.3	29.0	38.5	29.0	38.5	34.0	54.0	33.5	48.0
08-01-00	25.6	32.2	28.5	38 0	28.5	38.0	33.5	53.5	32.5	48.0
09-01-00	25.6	31.8	28.7	34.5	28.7	34.5	33.0	52.0	32.0	46.0
10-01-00	25.6	32.5	29.3	32.5	29.3	32.5	33.0	54.5	31.0	49.0

Temperature variation during the period of solarization for atmosphere ranged from 20°C to 33.3°C and the soil temperature at 5 cm depth in non solarized soil was from 24.4°C to 43.1°C. The temperature corresponding to solarized soils was 26°C to54.5°C. The soil temperature at 10 cm in non solarized soil ranged from 24.8°C to 39.5°C and in solarized soil, the values were 25.2°C to 49°C.

Maximum temperature variation observed during a day for non-solarized soil at 5cm was 17°C and the minimum was 3.2°C and for solarized soil corresponding temperature was 21.5°C and 6°C.

The maximum variation in temperature in non solarized soil at 10 cm depth during the period under observation was 13.8°C while in solarized soil it was 18°C and minimum temperature recorded was 3.2°C in non-solarised soil and for solarized soil the value was 8.5°C.

4.7.2. Effect of soil solarization on microflora

The data on soil microbial population before and after solarization are presented in Table 8. There was a general decrease in microbial population upon solarization except *R. solanacearum* population. The mean fungal population before solarization (54.6 cfu) has came down to 12.66 cfu after solarization. The corresponding value in non-solarized soil after 30 days of solarization period was 31.33 cfu. The fungal population reduced by 18.67 cfu due to solarization and a decrease of 23.27 cfu in the non-solarized soil compared to the same soil before solarization. Similarly in the case of bacterial count also there was a decrease in the population from 123.6 cfu to 11.33 cfu due to solarization. When the bacterial population of solarized and non-solarized soil was compared the solarized soil gave a population of 11.33 cfu with a decrease of 96.67 cfu. In the non-solarized soil the bacterial population decreased to 108 cfu when checked after solarisation period. The actinomycete population was highest in soil before solarization (56 cfu), reduced to 11.33 cfu after one month of solarization, the value corresponding in non-

Table 8. Effect of solarisation on microbial population in soil (cfu g^{-1} soil) before planting.

Time of sampling	Fungi ($\times 10^3$)	Bacteria ($\times 10^5$)	Actino-mycetes ($\times 10^4$)	*R. solana-cearum* ($\times 10^3$)	fluorescent Pseudomonads ($\times 10^3$)
Before solarization	54.60	123.60	56.00	60.00	13.00
Solarised soil (after 30 days)	12.66	11.33	11.33	96.33	2.00
Non solarised soil (after 30 days)	31.33	108.00	15.00	226.60	0.00

solarized soil was 15 cfu. Decrease in actinomycetes population due to solarisation was only 3.67cfu.

Almost the same trend noticed in the case of fluorescent bacteria with respect to solarized soil and before solarisation. The population of fluorescent bacteria before solarization was 13 cfu, and decreased to 2 cfu in solarized soil, whereas in non-solarized soil the fluorescent bacterial colony was not noticed after the period of solarisation. The *R. solanacearum* population showed a different trend, the population increased from 60 cfu before solarsation to 96.33 cfu after solarization, an increase of 36.33 cfu. Corresponding value in non-solarized soil was 226.6 cfu .

4.7.3 Effect of treatments on rhizosphere microflora

The effect of different treatments on the populations of fungi, bacteria, actinomycetes, *R. solanacearum* and *P. aeruginosa* were studied at 20, 40 and 60 days after planting in varieties Pusa Ruby and Sakthi.

4.7.3.(i) Effect of treatments on rhizosphere microflora at 20 DAP

Rhizosphere population of fungi, bacteria, actinomycetes, *R.solanacearum* and *P. aeruginosa* were recorded at 20 DAP in both the varieties Pusa Ruby and Sakthi (Table 9a and 9b).

In the rhizosphere of variety Pusa Ruby, the fungal population was significantly highest in the control (36.66 cfu) and lowest in one per cent bordeaux mixture drenched pots (6 cfu). Similarly the mean bacterial count was also maximum in control pots (77 cfu) and minimum in CaO treated pots (10.5 cfu) whereas the population of actinomycete was maximum in CaO treated pots (62.0 cfu) and the minimum (2.33 cfu) in garlic drenched and antibiotic sprayed pots. *R.solanacearum* population was also highest in CaO treatment and was lowest in *P.aeruginosa* treated and AM fungus inoculated pots followed by one per cent bordeaux mixture drenched pots. *P. aeruginosa* count was also maximum in pots

Table 9a. Effect of different treatments on the rhizosphere microflora (cfu/g^{-1} soil) of tomato (var. Pusa Ruby) at 20 DAP

Treatments	Fungi ($\times 10^3$)	Bacteria ($\times 10^5$)	Actino-mycetes ($\times 10^4$)	*R.solanacearum* ($\times 10^3$)	*P.aeruginosa* ($\times 10^3$)
T_1 - Soil solarisation with 150 gauge polythene sheets for 30 days	17.50^{bc}	28.25^{bc}	20.25^{b}	5.33^{a}	11.00^{bcd}
T_2 - Application of calcium oxide @ 35 g per plant one week before planting	9.00^{cde}	10.50^{c}	62.00^{a}	7.66^{a}	0.00^{d}
T_3 - Application of neem cake @ 45 g per plant one week before planting	7.33^{de}	64.00^{ab}	27.00^{ab}	4.00^{a}	0.00^{d}
T_4 - Application of urea alone @ 15.28 g per plant one week before planting	7.66^{de}	43.33^{abc}	11.33^{b}	1.33^{a}	0.00^{d}
T_5 - Calcium oxide @ 150 g and urea 15.28 g per plant one week before planting	15.33^{bcd}	17.00^{c}	9.66^{b}	3.00^{a}	0.00^{d}
T_6 - AM fungi inoculation - *Glomus fasciculatum* at the time of sowing	18.33^{b}	32.66^{bc}	12.00^{b}	1.00^{a}	21.00^{b}
T_7 - Seed treatment with *P. aeruginosa* at the time of sowing	10.33^{bcde}	34.33^{abc}	3.66^{b}	1.00^{a}	43.33^{a}
T_8 - Drenching with *P. aeruginosa* at the time of planting and after 30 DAP	10.33^{bcde}	21.66^{bc}	10.66^{b}	3.00^{a}	47.00^{a}
T_9 - Seed treatment and drenching with *P. aeruginosa* at the time of planting and 30 DAP	7.33^{de}	35.00^{abc}	4.66^{b}	1.00^{a}	15.00^{bc}
T_{10} - Drenching with garlic extract 10 per cent at the time of planting and 30 DAP	8.33^{de}	27.00^{bc}	2.33^{b}	2.00^{a}	4.00^{cd}
T_{11} - Drenching with Bordeaux mixture one per cent at the time of planting and 30 DAP	6.00^{e}	40.00^{abc}	2.66^{b}	1.33^{a}	4.00^{cd}
T_{12} - Seed treatment with Streptomycin sulphate 500 mgl^{-1}	6.66^{de}	25.33^{bc}	10.00^{b}	3.00^{a}	0.00^{d}
T_{13} - Spraying with Streptomycin sulphate 500 mgl^{-1} at 30 DAP	12.00^{bcde}	28.66^{bc}	2.33^{b}	2.60^{a}	5.33^{cd}
T_{14} – Control	36.66^{a}	77.00^{a}	11.66^{b}	4.33^{a}	9.00^{bcd}

Table 9b. Effect of different treatments on the rhizosphere microflora (cfu/g^{-1} soil) of tomato (var. Sakthi) at 20 DAP

Treatments	Fungi ($\times 10^3$)	Bacteria ($\times 10^5$)	Actino-mycetes ($\times 10^4$)	*R.solanacearum* ($\times 10^3$)	*P.aeruginosa* ($\times 10^3$)
T_1	18.33^{bc}	34.33^{bcd}	5.33^{bcd}	5.77^{ab}	10.00^{ab}
T_2	10.00^{bcd}	6.66^{d}	73.66^{a}	6.68^{ab}	1.00^{b}
T_3	9.00^{bcd}	138.7^{a}	9.66^{bcd}	8.26^{a}	0.00^{b}
T_4	9.33^{bcd}	39.0^{bcd}	4.33^{bcd}	3.46^{bc}	0.00^{b}
T_5	20.33^{ab}	27.0^{bcd}	3.00^{cd}	5.81^{ab}	1.00^{b}
T_6	30.33^{a}	38.0^{bcd}	7.33^{bcd}	3.20^{bc}	15.00^{ab}
T_7	7.33^{cd}	75.33^{ab}	5.00^{bcd}	7.22^{ab}	20.00^{ab}
T_8	7.33^{cd}	76.00^{b}	18.00^{bc}	8.22^{a}	17.00^{ab}
T_9	4.66^{d}	61.00^{bc}	9.66^{bcd}	0.70^{c}	23.00^{a}
T_{10}	5.33^{d}	50.00^{bcd}	5.00^{bcd}	4.26^{abc}	10.00^{ab}
T_{11}	16.33^{bcd}	57.33^{bc}	4.33^{bcd}	5.20^{ab}	0.00^{b}
T_{12}	5.00^{d}	34.00^{bcd}	20.66^{b}	4.34^{abc}	0.00^{b}
T_{13}	13.66^{bcd}	25.33^{cd}	1.00^{d}	4.99^{abc}	0.00^{b}
T_{14}	30.33^{a}	72.00^{bc}	5.33^{bcd}	6.05^{ab}	7.00^{ab}

which were inoculated with the same bacterium and was absent in treatments which received CaO, neem cake, urea, CaO + urea and in antibiotic seed treatments.

In variety Sakthi the fungal population was lowest (4.66 cfu) in T_9 where *P.aeruginosa* seed treatment and drenching was given and significantly highest in control (30.33 cfu) and.AM fungi inoculated pots In the case of bacterial population lowest mean count of 6.66 cfu was noticed in the treatment where applications of CaO 35 g per plant was given, one week before planting and the highest population noticed in T_3 (138.7 cfu) where neem cake was applied. There were not much variation between treatments in actinomycetes population in rhizosphere soil. However highest count was observed in CaO applied pots (73.66 cfu) and the lowest count in T_{13} (1 cfu) where neem cake was applied.

Considerable variation of pathogenic bacterium (*R. solanacearum*) was noticed in rhizosphere soil of different treatments. Pathogenic bacterial count was lowest in T_9 (0.70 cfu) where antagonistic seed treatment and drenching was given. This was followed by AM fungus inoculation and control. Pathogenic bacterial count was highest (8.26 cfu) in neem cake applied soil.

With regard to the *P. aeruginosa* population the same trend as noticed in variety Pusa Ruby were recorded in the variety Sakthi. Highest fluorescent pseudomonad population was obtained from *P. aeruginosa* treated pot which received both seed treated and soil drenching.Fluorescent pseudomonad populationm was absent in CaO, neem cake and chemicals applied pots.

4.7.3(ii) Effect of treatments on the rhizosphere microflora at 40 DAP.

In the variety Pusa Ruby the fungal population showed significant difference between treatments (Table 10a and b). Fungal population was lowest in AM fungus treatment followed by streptomycine 500 mgl^{-1}. Fungal population was highest in control (19 cfu) followed by *P. aeruginosa* seed treatment and drenching (18.33 cfu).Bacterial population was minimum in garlic 10 per cent drenched pots (25 cfu) and maximum in urea applied treatment (77.33 cfu).

Table 10a. Effect of different treatments on the rhizosphere microflora (cfu/g^{-1} soil) of tomato (var.Pusa Ruby) at 40 DAP

Treatment	Fungi ($\times10^3$)	Bacteria ($\times10^5$)	Actino-mycetes ($\times10^4$)	*R.solanacearum* ($\times10^3$)	*P.aeruginosa* ($\times10^3$)
T_1	16.33^{ab}	40.66^{ab}	19.0^{abcd}	6.28^{a}	0.00^{e}
T_2	12.00^{abc}	65.66^{ab}	9.33^{d}	4.26^{abc}	2.33^{cde}
T_3	10.66^{abc}	41.00^{ab}	18.66^{abcd}	3.10^{c}	1.00^{de}
T_4	10.33^{abc}	77.33^{a}	16.33^{bcd}	3.63^{bc}	6.00^{cde}
T_5	12.00^{abc}	63.00^{ab}	19.66^{bcd}	4.60^{abc}	2.30^{cde}
T_6	5.33^{c}	61.33^{ab}	12.33^{cd}	5.76^{ab}	7.00^{bcde}
T_7	17.00^{a}	46.66^{ab}	31.0^{a}	4.69^{abc}	16.00^{a}
T_8	12.66^{abc}	29.33^{b}	17.00^{bcd}	4.49^{abc}	7.66^{bcde}
T_9	18.33^{a}	41.00^{ab}	10.33^{d}	4.99^{abc}	9.66^{abc}
T_{10}	12.66^{abc}	25.00^{b}	28.00^{ab}	4.12^{abc}	8.00^{abcd}
T_{11}	8.00^{bc}	56.33^{ab}	13.33^{cd}	4.99^{abc}	8.66^{abcd}
T_{12}	6.66^{c}	36.00^{ab}	22.33^{abcd}	5.24^{ab}	6.33^{cde}
T_{13}	10.66^{abc}	46.60^{ab}	17.66^{bcd}	4.56^{abc}	9.00^{abcd}
T_{14}	19.00^{a}	48.00^{ab}	25.33^{abc}	5.24^{ab}	15.00^{ab}

Table 10b. Effect of different treatments on the rhizosphere microflora (cfu/g^{-1} soil) of tomato (var. Sakthi) at 40 DAP

Treatment	Fungi ($\times10^3$)	Bacteria ($\times10^5$)	Actino-mycetes ($\times10^4$)	*R.solanacearum* ($\times10^3$)	*P.aeruginosa* ($\times10^3$)
T_1	12.6^{bc}	38.33^{a}	6.33^{cd}	6.51^{ab}	6.00^{bc}
T_2	12.00^{bc}	31.00^{a}	12.33^{abcd}	5.30^{b}	4.33^{bc}
T_3	10.00^{c}	53.33^{a}	5.66^{d}	7.15^{ab}	1.33^{c}
T_4	22.00^{ab}	49.66^{a}	9.33^{bcd}	7.56^{ab}	8.66^{bc}
T_5	15.00^{abc}	26.33^{a}	20.00^{abc}	8.22^{a}	3.33^{bc}
T_6	9.00^{c}	32.66^{a}	22.66^{ab}	7.46^{ab}	2.00^{c}
T_7	9.66^{c}	27.33^{a}	17.33^{abcd}	8.19^{a}	13.33^{ab}
T_8	11.00^{c}	27.66^{a}	17.33^{abcd}	7.69^{ab}	1.66^{c}
T_9	22.66^{ab}	40.00^{a}	17.66^{abcd}	6.07^{ab}	20.66^{a}
T_{10}	15.00^{abc}	26.00^{a}	19.33^{abcd}	6.19^{ab}	1.66^{c}
T_{11}	12.66^{bc}	37.33^{a}	14.66^{abcd}	7.09^{ab}	6.00^{bc}
T_{12}	10.33^{c}	30.33^{a}	15.66^{abcd}	8.08^{a}	2.66^{bc}
T_{13}	9.66^{c}	25.33^{a}	24.0^{a}	6.34^{ab}	2.00^{c}
T_{14}	24.33^{a}	35.0^{a}	17.66^{abcd}	7.08^{ab}	10.66^{bc}

The actinomycete count was lowest in CaO treatment (9.33 cfu) followed by T_6 (12.33 cfu) where AM fungus inoculated seedlings were used for planting and was significantly different from other treatments. Highest population count was obtained from T_7 where the *P. aeruginosa* seed treatment was administered (31 cfu).

Population count of pathogenic bacterium did not show much variation, maximum count of 6.28 cfu and minimum count of 3.10 cfu were recorded in T_1 (solarized soil) and T_3 (neem cake) respectively. The treatments with beneficial agents could not provide a reduction in pathogenic bacterial population in the rhizosphere soil.

Significant difference between treatments was obtained in *P. aeruginosa* population. The count was lowest in solarized soil and also in neem cake application. The highest count of *P. aeruginosa* was recorded in T_7 (16 cfu) where *P.aeruginosa* itself was used as seed treatment.

In the variety Sakthi slight variation was noticed between the treatments in the count of fungal population. The fungal population count was lowest in AM fungus inoculated pots and highest in control. In other treatments fungal count varied from 79.66 to 22.66 cfu. Significant variations were not obtained between treatments in the case of bacterial population at 40 DAP. The bacterial population was highest in neem cake treatment (53.33 cfu) and the lowest in antibiotic treatment (25.33 cfu).

Counts of actinomycetes also did not show any significant difference between treatments. However the lowest population (5.66 cfu) was noticed in neem cake applied pots. The highest actinomycetes population was recorded in pots with antibiotic streptomycine 500 mgl^{-1} as the treatment.

Pathogenic bacterial population did not differ significantly between treatments at 40 DAP. The lowest count of *R. solanacearum* population was noticed in CaO treatment with 5.30 cfu followed by *P. aeuroginosa* drenched pots and garlic 10 per cent drenched treatment. The highest count of *R. solanacearum* was in CaO and urea together applied pots (8.22 cfu).

Significant variation was noticed between treatments in the population of *P.aeruginosa* at 40 DAP. The *P. aeruginosa* population was highest (20.66 cfu) in pots which obtained antagonistic seed treatment and drenching, this was closely followed by *P. aeruginosa* seed treatment with 13.33 cfu. The lowest population count was recorded in neem cake amended pots (1.33 cfu) and followed by garlic 10 per cent drenched pots (1.66 cfu). In all other treatments the population count varied from 2.00 cfu to 10.66 cfu.

4.7.3 (iii) Effect of treatments on the rhizosphere microflora at 60 DAP

At 60 DAP rhizosphere soil microflora showed considerable variation in both the varieties and data are presented in Table 11a and b. At 60 days after planting in the variety Pusa Ruby significant difference was obtained between treatments in the different types of microbial population except in actinomycetes and *P. aeruginosa* population.

While comparing the count of fungi in the rhizosphere highest number of colony forming units was found in *P. aeruginosa* drenched pots (24.33 cfu) and the lowest in CaO + urea treatment (6 cfu). Control plots gave a population count of 14 cfu.

The bacterial population was highest in the control treatment and the lowest in antibiotic sprayed treatments. But the counts of actinomycete was maximum in AM fungus inoculated pots and minimum in one per cent bordeaux mixture drenched pots.

The pathogenic bacterium *R. solanacearum* population was maximum in soil with antibiotic seed treatment and minimum in *P. aeruginosa* treatments. The *P.aeruginosa* colonies were more obtained in pots with solarised soil and in *P.aeruginosa* inoculated treatments. Maximum population (48.0 cfu) was noticed in *P. aeruginosa* seed treatment together with drenching.

In the variety Sakthi the fungal population was lowest in CaO applied treatment (3.66 cfu)and highest in control(27.66 cfu). Bacterial count was lowest in antogonist drenched pot (11 cfu) and the highest in neem cake treated pot (116.60 cfu)

Table11a. Effect of different treatments on the rhizosphere microflora (cfu g^{-1} soil) of tomato var. Pusa Ruby at 60 DAP

Treatment	Fungi ($\times 10^3$)	Bacteria ($\times 10^5$)	Actino-mycetes ($\times 10^4$)	*R.solanacearum* ($\times 10^3$)	*P.aeruginosa* ($\times 10^3$)
T_1	11.33^{cde}	41.33^{cd}	1.66^{c}	4.88^{bc}	16.33^{c}
T_2	8.66^{e}	42.00^{cd}	3.00^{abc}	7.53^{abc}	2.30^{c}
T_3	13.33^{bcde}	60.33^{bcd}	2.00^{c}	7.96^{ab}	3.00^{c}
T_4	9.66^{de}	98.66^{abc}	2.33^{bc}	6.10^{bc}	4.40^{c}
T_5	6.00^{e}	101.60^{ab}	5.66^{ab}	6.85^{abc}	4.00^{c}
T_6	21.33^{ab}	48.00^{bcd}	6.00^{a}	5.56^{bc}	9.50^{c}
T_7	8.00^{e}	39.33^{cd}	1.30^{c}	4.84^{bc}	37.60^{ab}
T_8	24.33^{a}	77.60^{abcd}	2.00^{c}	4.64^{c}	34.30^{b}
T_9	20.33^{abc}	79.30^{abcd}	2.00^{c}	5.96^{bc}	48.00^{a}
T_{10}	11.00^{cde}	63.60^{bcd}	3.33^{abc}	6.49^{abc}	3.00^{c}
T_{11}	9.33^{de}	40.00^{cd}	0.66^{c}	6.73^{abc}	6.00^{c}
T_{12}	18.66^{abcd}	31.60^{d}	2.66^{abc}	9.43^{a}	6.40^{c}
T_{13}	20.66^{abc}	64.30^{bcd}	3.33^{abc}	7.40^{abc}	4.30^{c}
T_{14}	14.00^{bcde}	122.60^{a}	2.33^{bc}	5.03^{bc}	10.00^{c}

Table11b. Effect of different treatments on the rhizosphere microflora (cfu g^{-1} soil) of tomato var. Sakthi at 60 DAP

Treatment	Fungi ($\times 10^3$)	Bacteria ($\times 10^5$)	Actino-mycetes ($\times 10^4$)	*R.solanacearum* ($\times 10^3$)	*P.aeruginosa* ($\times 10^3$)
T_1	26.00^{a}	109.00^{b}	1.33^{c}	8.98^{ab}	32.00^{bc}
T_2	3.66^{d}	95.60^{bc}	2.60^{abc}	11.66^{a}	8.00^{cd}
T_3	19.33^{abc}	116.60^{b}	2.00^{bc}	9.11^{ab}	4.00^{d}
T_4	19.66^{abc}	69.33^{bcd}	4.33^{abc}	6.73^{b}	5.63^{d}
T_5	13.00^{bcd}	88.60^{a}	6.33^{abc}	8.27^{ab}	8.30^{cd}
T_6	26.33^{a}	78.00^{a}	4.66^{abc}	6.79^{b}	10.30^{cd}
T_7	9.66^{cd}	96.33^{bc}	1.66^{c}	5.16^{b}	37.60^{ab}
T_8	5.33^{d}	11.00^{b}	3.33^{abc}	9.12^{ab}	42.30^{ab}
T_9	4.66^{d}	112.00^{b}	2.66^{abc}	4.63^{b}	48.00^{a}
T_{10}	13.33^{bcd}	43.66^{cd}	8.33^{a}	5.40^{b}	5.24^{d}
T_{11}	20.00^{abc}	55.33^{bcd}	8.00^{ab}	11.53^{a}	6.50^{d}
T_{12}	23.00^{ab}	44.33^{cd}	1.00^{c}	9.33^{ab}	3.46^{d}
T_{13}	21.00^{abc}	29.00^{d}	5.00^{abc}	4.84^{b}	5.30^{d}
T_{14}	27.66^{a}	93.00^{bc}	2.33^{abc}	9.15^{ab}	9.50^{cd}

When the mean counts of actinomycetes were compared it was found that the Streptomycin sulphate seed treatment gave the minimum count and the maximum in garlic 10 per cent drenched treatment. In all the other treatments the count varied from 1.33 to 8.00 cfu.

The *R. solanacearum* population was comparatively higher in the rhizosphere soil of the resistant variety sakthi than that of susceptible variety Pusa Ruby. The pathogen population was minimum (4.63 cfu) in *P. aeruginosa* seed treated and drenched pots and maximum (11.66 cfu) in CaO amended pots.

The population of *P. aeruginosa* followed the same trend as in variety Pusa Ruby, the highest (48 cfu) in pot which received both *P. aeruginosa* seed treatment and drenching.

4.7.3 (iv) Effect of different treatments on the wilt incidence and on the population of *R. solanacearum*

The different management strategies were screened for microbial population and wilt incidence in pot culture for variety Pusa Ruby and Sakthi. Observations were recorded on percentage wilt incidence at 20, 40 and 60 DAP. The data on percentage wilt incidence and population of *R.solanacearum* in the rhizosphere soils of plants with different treatments are presented in Table 12a and 12b.

In Pusa Ruby, at 20 DAP the pathogenic bacterial population in the rhizosphere soil did not show much variation between treatments, the variation noticed was only 6.66cfu. The *R. solanacearum* population was lowest in pot treated with *P. aeruginosa* and highest in treatment which received CaO. The percentage wilt incidence at 20 DAP did not show much variation between the treatment. The lowest percentage wilt incidence (7.40) was recorded in T_7 (seed treatment with *P. aeruginosa*), T_{10} (garlic 10 per cent drenching), T_3 (neem cake application) and T_{11} (Bordeaux mixture 1 per cent drenching) and highest (30.77) in T_5 (urea + CaO) followed by T_1 (soil solarization). In the control only 18.5 per cent incidence was noticed.

Table 12a. Effect of different treatments on percentage wilt incidence and population of *R. solanacearum* (cfu g^{-1} soil) at different intervals in Pusa Ruby

Treatments	*Ralstonia solanacearum* ($\times 10^3$)			Wilt incidence (%)		
	20 DAP	40 DAP	60 DAP	20 DAP	40 DAP	60 DAP
T_1	5.33^{a}	6.28^{a}	4.88^{bc}	29.62^{a}	48.14^{a}	66.67^{a}
T_2	7.66^{a}	4.26^{abc}	7.53^{abc}	18.51^{a}	33.33^{abc}	51.85^{ab}
T_3	4.00^{a}	3.10^{c}	7.96^{ab}	7.40^{a}	18.51^{bcd}	18.51^{de}
T_4	1.33^{a}	3.63^{bc}	6.10^{bc}	11.11^{a}	14.81^{cd}	48.15^{ab}
T_5	3.00^{a}	4.60^{abc}	6.85^{abc}	30.77^{a}	40.74^{ab}	37.03^{bcde}
T_6	1.00^{a}	5.76^{ab}	5.56^{bc}	18.51^{a}	25.92^{abcd}	25.93^{bcde}
T_7	1.00^{a}	4.69^{abc}	4.84^{bc}	7.40^{a}	18.51^{bcd}	44.44^{abcd}
T_8	3.66^{a}	4.49^{abc}	4.64^{c}	18.51^{a}	29.62^{abcd}	22.22^{cde}
T_9	1.00^{a}	4.99^{abc}	5.964^{bc}	11.11^{a}	18.51^{bcd}	11.11^{e}
T_{10}	2.00^{a}	4.12^{abc}	6.49^{abc}	7.40^{a}	7.40^{d}	33.33^{bcde}
T_{11}	1.33^{a}	4.99^{abc}	6.73^{abc}	7.40^{a}	18.51^{bcd}	33.33^{bcde}
T_{12}	3.00^{a}	5.24^{ab}	9.43^{a}	11.11^{a}	22.22^{bcd}	37.03^{bcde}
T_{13}	2.66^{a}	4.56^{abc}	7.40^{abc}	14.81^{a}	25.93^{abcd}	37.03^{bcde}
T_{14}	4.33^{a}	5.24^{ab}	5.03^{bc}	18.51^{a}	37.03^{abc}	44.44^{abcd}

Table 12b. Effect of different treatments on percentage wilt incidence and population of *R. solanacearum* (cfu g^{-1} soil) at different intervals in Sakthi

Treatments	*Ralstonia solanacearum* ($\times 10^3$)			Wilt incidence (%)		
	20 DAP	40 DAP	60 DAP	20 DAP	40 DAP	60 DAP
T_1	5.77^{ab}	6.51^{ab}	8.98^{ab}	14.81^{ab}	29.63^{ab}	29.62^{abc}
T_2	6.68^{ab}	5.30^{b}	11.66^{a}	11.11^{ab}	22.22^{abc}	33.33^{ab}
T_3	8.26^{a}	7.15^{ab}	9.11^{ab}	7.40^{ab}	18.51^{abc}	22.22^{abcd}
T_4	3.46^{bc}	7.56^{ab}	6.73^{b}	18.51^{a}	33.33^{a}	44.44^{a}
T_5	5.81^{b}	8.22^{a}	8.27^{ab}	11.11^{ab}	14.81^{abc}	22.22^{bcd}
T_6	3.20^{bc}	7.46^{ab}	6.79^{b}	3.70^{ab}	3.70^{c}	7.40^{cd}
T_7	7.22^{ab}	8.19^{a}	5.16^{b}	0.00^{b}	3.70^{c}	3.40^{d}
T_8	8.22^{a}	7.69^{ab}	9.12^{ab}	3.70^{ab}	7.40^{bc}	7.40^{cd}
T_9	0.70^{c}	6.87^{ab}	4.63^{b}	7.40^{ab}	11.11^{bc}	11.11^{bcd}
T_{10}	4.26^{abc}	6.19^{ab}	5.40^{b}	11.11^{ab}	14.81^{abc}	14.81^{bcd}
T_{11}	5.20^{ab}	7.09^{ab}	11.53^{a}	11.11^{ab}	11.11^{bc}	18.51^{bcd}
T_{12}	4.34^{abc}	8.08^{a}	9.33^{ab}	7.40^{ab}	11.11^{bc}	18.51^{bcd}
T_{13}	4.99^{abc}	6.34^{b}	4.84^{b}	14.81^{ab}	22.22^{abc}	25.92^{abcd}
T_{14}	6.05^{ab}	6.87^{ab}	9.15^{ab}	11.11^{ab}	25.93^{abc}	33.33^{ab}

At 40 DAP the data on population of *R. solanacearum* showed only slight variation (3.10 to 6.28 cfu). Pathogenic bacterial population was minimum in neem cake amendment (3.1 cfu) followed by urea application and 10 per cent garlic drenching. The highest *R. solanacearum* population was noticed in solarized soil with a count of 6.28 cfu followed by AM fungus inoculation and control. Significant difference was not obtained between treatments in percentage wilt incidence, the wilt percentage varied from 7.40 to 48.14. The lowest wilt incidence was recorded in pots with garlic drenching followed by pots with urea application, *P. aeruginosa* treatments, one per cent bordeaux mixture drenching and neem cake application. Highest wilt incidence was noticed in the pots which received solarisation followed by urea + CaO application and in control. The trend of *R. solanacearum* population in rhizosphere soils has reflected in wilt incidence except in the case of AM fungus inoculated treatment.

The treatment effect on *R. solanacearum* population and wilt incidence differed significantly between treatments at 60 DAP. *R. solanacearum* population was lowest in treatment which received *P. aeruginosa* drenching (4.64 cfu) followed by *P. aeruginosa* seed treatment and treatment receiving solarized soil. The population of *R. solanacearum* was highest in Streptomycin sulphate seed treatment (9.43 cfu) followed by neem cake applied pot and CaO applied treatment. When the wilt incidence was compared *P. aeruginosa* seed treatment and drenching gave minimum disease incidence (11.11 per cent) followed by treatment which received neem cake application. The wilt incidence was maximum in solarized soil (66.67 per cent) followed by CaO treated pot. The variation in wilt incidence was by 55.56 per cent and in control the wilt incidence was 44.44 per cent.

Significant difference was obtained between treatments on *R.solanacearum* population in the rhizosphere soil of variety Sakthi at 20 DAP. Among the treatment pathogenic population was lowest in *P. aeruginosa* seed treatment along with drenching followed by AM fungus inoculated and urea treated pots, and these were significantly superior to other treatments. The population was highest in neem cake applied pots followed by *P. aeruginosa* soil drenched pot. The variation in *R.solanacearum* population ranges from 0.70 to 8.26 cfu. In the case of wilt

incidence the treatments did not differ significantly. However, the wilt incidence was absent in *P. aeruginosa* seed treated pots and was superior to all other treatment. The highest wilt incidence recorded in urea applied pots with 18.51 per cent incidence.

The population of pathogenic bacterium in the rhizosphere soil did not differ significantly between treatments at 40 DAP. Lowest population was noticed in CaO treatment (5.3 cfu) followed by *P. aeruginosa* seed treatment and drenching, then in garlic drenched pots. Population was maximum in CaO + urea treatment (8.22 cfu) followed by *P. aeruginosa* seed treatment. Similarly wilt incidence also did not differ significantly between treatments, however wilt incidence was lowest in *P.aeruginosa* seed treated and AM fungus inoculated treatments even though the pathogen population was comparatively higher in the rhizosphere soils of these treatments. Maximum wilt incidence was noticed in urea applied pot followed by solarisation treatment. In control 25.93 per cent incidence was recorded. Whereas variations in wilt incidence from T_7 (lowest) to T_4 (highest) ranged from 3.72 to 33.33 per cent.

As plants were in the flowering stage significant difference was obtained between treatments in *R. solanacearum* population as well as in percentage wilt incidence at 60 DAP.

The *R. solanacearum* population was lowest in *P. aeruginosa* seed treatment and drenching followed by antibiotic spray and in garlic drenched pot. The above treatments did not differ significantly. The highest *R. solanacearum* population was noticed in CaO applied treatments (11.66 cfu) followed by the plants which received bordeaux mixture one per cent drenching. In control treatment *R. solanacearum* population observed was 9.15 cfu. Similar trend was also noticed in the case of percentage wilt incidence at 60 DAP. The percentage wilt incidence was lowest in *P.aeruginosa* seed treatment (3.40 per cent) and was significantly superior to other treatments. This was followed by AM fungus inoculation and *P. aeruginosa* drenching (7.40 per cent). The wilt incidence was maximum (44.44 per cent) in pots

which received urea treatment @ 15.28 g per plant. This is followed by the control and CaO treatment.

In order to select the best treatment components for the main field trial the effect of different treatments on wilt incidence of both the varieties were compared together at 20, 40 and 60 DAP and are presented in Table 13. The resistant variety Sakthi was found to be much superior to the susceptible variety Pusa Ruby in reducing wilt incidence even without any treatment. But when the treatments were given the resistant mechanism of the variety Sakthi was enhanced considerably. At 20 DAP the treatments 10 per cent garlic drenching, *P. aeruginosa* seed treatment, one per cent bordeaux mixture drenching and neem cake application were giving lesser disease incidence in the variety Pusa Ruby. Among the treatments, variety Sakthi which received antagonistic seed treatments proved to the best without any disease incidence followed by the same variety with antagonistic drenching, and then AM fungus application. The wilt incidence was maximum in the variety Pusa Ruby with treatment CaO and urea followed by the same variety planted in solarized soil.

A similar comparison of the treatments of the two varieties was done at 40 DAP. Significant difference was not obtained between treatments. However, as in 20 DAP here also minimum wilt incidence was recorded in variety Sakthi with antagonistic seed treatment followed by AM fungus inoculated pots in the same variety. Garlic 10 per cent drenching was found to be effective in checking wilt incidence in Pusa Ruby and Sakthi. Highest wilt incidence was noticed in the variety Pusa Ruby with solarization treatment followed by CaO and urea amendment and then in control.

At 60 DAP significant difference was obtained between two varieties in percentage wilt incidence. The disease was minimum in antagonistic seed treatment in variety Sakthi with 3.4 per cent wilt incidence, followed by the AM fungus application and antagonistic seed treatment with drenching for the same variety. Garlic 10 per cent drenching was found effective in both varieties Pusa Ruby and Sakthi. Garlic treatment in Pusa Ruby could induce lesser wilt incidence and was on par with other effective treatments in the resistant variety Sakthi.

Table 13. Comparison of treatments on wilt incidence of Pusa Ruby and Sakthi varieties

Treatments	Wilt (%) 20 DAP	Wilt (%) 40 DAP	Wilt (%) 60 DAP
T_1 - Soil solarisation with 150 gauge polythene sheets for 30 days	29.63^{a}	48.14^{a}	66.67^{a}
T_2 - Application of calcium oxide @ 35 g per plant one week before planting	18.52^{ab}	33.33^{abcd}	51.85^{ab}
T_3 - Application of neem cake @ 45 g per plant one week before planting	7.40^{b}	18.52^{bddef}	18.51^{efgh}
T_4 - Application of urea alone @ 15.28 g per plant one week before planting	11.11^{ab}	14.81^{cdef}	48.15^{efgh}
T_5 - Calcium oxide @ 150 g and urea 15.28 g per plant one week before planting	30.77^{a}	40.74^{ab}	37.03^{abc}
T_6 - AM Fungi - *Glomus fasciculatum* at the time of sowing	18.52^{ab}	25.92^{abcdef}	25.93^{bcde}
T_7 - Seed treatment with antagonist at the time of sowing	7.40^{b}	16.52^{bcdef}	44.44^{bcd}
T_8 - Drenching with antagonist at the time of planting and after 30 DAP	18.52^{ab}	7.40^{abcde}	22.22^{defgh}
T_9 - Seed treatment and drenching with antagonist at the time of planting and 30 DAP	11.11^{ab}	18.52^{bcdef}	11.11^{fgh}
T_{10} - Drenching with garlic extract 10 per cent at the time of planting and 30 DAP	7.40^{b}	7.40^{ef}	33.33^{bcdef}
T_{11} - Drenching with Bordeaux mixture one per cent at the time of planting and 30 DAP	7.40^{b}	18.52^{bcdef}	33.33^{bcdef}
T_{12} - Seed treatment with Streptomycine sulphate 500 mgl^{-1}	11.11^{ab}	22.22^{bcdef}	37.03^{bcdef}
T_{13} - Spraying with Streptomycine sulphate 500 mgl^{-1} at 30 DAP	14.81^{ab}	25.92^{abcdef}	37.03^{bcde}
T_{14} - Control	18.52^{ab}	37.03^{abc}	44.44^{bcd}
T_{15} - Soil solarisation with 150 gauge polythene sheets for 30 days	14.81^{ab}	29.63^{abcde}	29.62^{bcdefg}
T_{16} - Application of calcium oxide @ 35 g per plant one week before planting	11.11^{ab}	22.22^{bcdef}	33.33^{bcdef}
T_{17} - Application of neem cake @ 45 g per plant one week before planting	7.40^{b}	18.52^{bcdef}	22.22^{defgh}
T_{18} - Application of urea alone @ 15.28 g per plant one week before planting	18.52^{ab}	33.33^{abcd}	44.44^{bcd}
T_{19} - Calcium oxide @ 150 g and urea 15.28 g per plant one week before planting	11.11^{ab}	14.81^{cdef}	22.22^{defgh}
T_{20} - AM Fungi - *Glomus fasciculatum* at the time of sowing	3.70^{b}	3.70^{f}	7.40^{gh}
T_{21} - Seed treatment with antagonist at the time of sowing	0.00^{b}	3.70^{f}	3.40^{h}
T_{22} - Drenching with antagonist at the time of planting and after 30 DAP	3.70^{b}	7.40^{ef}	7.40^{gh}
T_{23} - Seed treatment and drenching with antagonist at the time of planting and 30 DAP	7.40^{b}	11.11^{def}	11.11^{fgh}
T_{24} - Drenching with garlic extract 10 per cent at the time of planting and 30 DAP	11.11^{ab}	14.81^{cdef}	14.81^{efgh}
T_{25} - Drenching with Bordeaux mixture one per cent at the time of planting and 30 DAP	11.11^{ab}	11.11^{def}	18.51^{efgh}
T_{26} - Seed treatment with Streptomycine sulphate 500 mgl^{-1}	7.40^{b}	11.11^{def}	18.51^{efgh}
T_{27} - Spraying with Streptomycine sulphate 500 mgl^{-1} at 30 DAP	14.81^{ab}	22.22^{bcdef}	25.92^{cdefgh}
T_{28} - Control	11.11^{ab}	25.92^{abcdef}	33.33^{bcdef}

T_1 to T_{14} – Variety Pusa Ruby T_{15} – T_{28} Variety Sakthi

Based on the results of pot culture experiment at 20, 40 and 60 DAP, treatments were chosen and integrated for the field experiments.

4.7.3 (v)Effect of different treatments on AM fungus infection, plant growth and yield

The inoculation of AM fungus (*Glomus fasciculatum*) was made by incorporating 50 g AM fungusculture in each pot. A week after the emergence the roots were examined and found infected by AM fungus. These infected seedlings were used as treatments to evaluate the efficiency of AM fungus in checking the wilt incidence. AM fungi infection was also noticed in non- inoculated seedlings but the percentage infection was much higher in inoculated seedlings (Table14a and b).

In Pusa Ruby, among the different treatments tried the AM fungi infection was maximum in AM fungus inoculated seedlings (12.42 per cent), followed by *P.aeruginosa* treatments. The AM fungus infection was lowest in plants drenched with one per cent bordeaux mixture followed by urea application and Streptomycin sulphate seed treatment, in control AM fungus infection of 3.78 per cent was recorded. The treatments, which received *P. aeruginosa* and AM fungus inoculation recorded higher percentage infection compared to the chemical treated seedlings.

When the plant growth was compared, significant difference was not obtained between treatments. However *P. aeruginosa* seed treatment gave highest wet weight of 71.73 g per plant, followed by *P. aeruginosa* drenching (65.57gm per plant). The AM fungus inoculated seedlings gave 63.70g per plant wet weight, lowest was recorded in T_4 where urea (15.28 g/plant) was applied followed by Streptomycine sulphate seed treatment (36.53 g per plant).

The yield from the different treatments showed significant difference and highest yield (210.10 g) was obtained from garlic 10 per cent drenched pots, followed by CaO + urea and AM fungus inoculated pots. The yield was lowest in Streptomycin sulphate seed treatment.

Table 14a. Effect of different treatments on AM fungus infection, wet weight and yield of variety Pusa Ruby

Treatments	AM fungus infection (%)	Wet weight (g/plant)	Yield (g/plant)
T_1	4.93 (0.22)^{abc}	42.60^{bc}	180.36^{e}
T_2	2.55 (0.14)^{abc}	47.07^{abc}	156.70^{g}
T_3	255 (0.14)^{abc}	44.83^{abc}	167.03^{f}
T_4	1.40 (0.09)^{bc}	35.27^{c}	138.40^{h}
T_5	1.40 (0.14)^{abc}	61.97^{abc}	203.37^{b}
T_6	12.42 (0.34)^{a}	63.70^{abc}	190.26^{c}
T_7	11.11 (0.28)^{ab}	71.73^{a}	127.27^{j}
T_8	6.25 (0.22)^{abc}	65.57^{ab}	138.72^{h}
T_9	7.40 (0.27)^{abc}	58.82^{abc}	186.72^{d}
T_{10}	2.63 (0.12)^{abc}	53.43^{c}	210.10^{a}
T_{11}	0.25 (0.05)^{c}	45.60^{abc}	130.47^{i}
T_{12}	1.40 (0.09)^{bc}	36.53^{c}	70.20^{k}
T_{13}	3.78 (0.17)^{abc}	42.98^{abc}	138.38^{h}
T_{14}	3.78 (0.17)^{abc}	42.07^{bc}	129.86^{i}

Figures in the parenthesis arc sin transformed

Table 14b. Effect of different treatments on AM fungus infection, wet weight and yield of variety Sakthi

Treatments	AM fungus infection (%)	Wet weight (g/plant)	Yield (g/plant)
T_1	8.51 (0.44)^{ab}	30.46^{e}	162.30^{f}
T_2	3.78 (0.17)^{cd}	61.63^{bc}	190.66^{e}
T_3	2.55 (0.14)^{cd}	46.203^{cde}	151.33^{g}
T_4	0.25 (0.05)^{d}	33.78^{e}	131.05^{i}
T_5	1.40 (0.09)^{d}	71.66^{ab}	234.60^{b}
T_6	11.11 (0.32)^{a}	69.87^{ab}	299.36^{a}
T_7	7.40 (0.27)^{bc}	66.28^{ab}	191.90^{e}
T_8	3.7 (0.19)^{bcd}	45.87^{cde}	127.70^{i}
T_9	6.17 (0.24)^{bc}	53.62^{bcd}	205.66^{c}
T_{10}	7.40 (0.27)^{bc}	83.22^{a}	200.36^{d}
T_{11}	2.55 (0.14)^{cd}	33.54^{e}	136.71^{h}
T_{12}	0.25 (0.05)^{d}	31.39^{e}	65.51^{k}
T_{13}	1.40 (0.09)^{d}	31.97^{e}	123.86^{j}
T_{14}	1.40 (0.09)^{d}	35.97^{de}	124.91^{j}

Figures in the parenthesis arc sin transformed

In the variety Sakthi significant difference was obtained between treatments on AM fungi infection. The highest infection noticed in AM fungus inoculated pots followed by solarisation treated pots. The lowest infection was noticed in urea treated and Streptomycin sulphate seed treated pots.

The wet weight of the individual plant significantly differs between treatments and was highest in garlic 10 per cent drenched pot (83.22 g per plant). This was followed by treatments CaO + urea, AM fungus inoculation and *P.aeruginosa* seed treatment. The plant vigour was lowest in solarization treatment (30.46 g per plant).

The fruit production was maximum in AM fungus inoculated pots (299.36 g per plant) and was significantly superior to all other treatments, this was followed by CaO + urea, *P. aeruginosa* treatment and 10 per cent garlic drenched pots. Lowest yield was obtained in T_{12} (65.51 g per plant) which received antibiotic seed treatment, and this was followed by antibiotic spraying and control which were on par.

4.7.3 (vi) Effect of different treatment on wilt incidence and population of *R.solanacearum* and beneficial agents

The data on wilt incidence, population of *R. solanacearum*, *P. aeruginosa* and percentage AM fungi infection at 60 DAP and total yield with respect to variety Pusa Ruby and Sakthi were compared in Table 15a and b and Fig. 2 and 3.

The wilt incidence was highest in solarisation treatment followed by CaO application, CaO + urea and control, where as the population of pathogen was maximum in antibiotic seed treatment, neem cake application and CaO application. AM fungi infection was lowest in 1 per cent Bordeaux mixture drenching followed by urea application and CaO + urea treatment. Conversely the AM fungus infection and *P. aeruginosa* population was maximum in AM fungus inoculated pots and *P.aeruginosa* treatments, and *R. solanacearum* population and wilt incidence minimum in *P. aeruginosa* treatments.

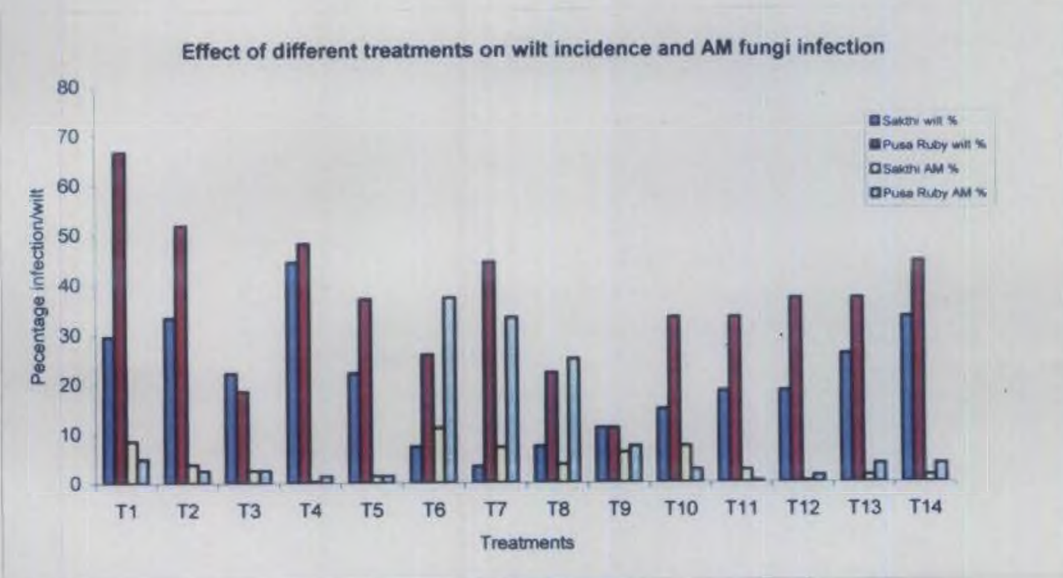
Table 15a. Effect of different treatments on wilt incidence and population of *R.solanacearum* and bioagents in respect of variety Pusa Ruby

Treatments	Wilt (%)	*R. solanacearum* cfu g^{-1} soil ($\times 10^3$)	*P. aeruginosa* cfu g^{-1} soil ($\times 10^3$)	AM fungus infection (%)	Yield (g/plant)
T_1	66.67^{a}	4.88^{bc}	16.33^{b}	$4.93\ (0.22)^{abc}$	180.36^{e}
T_2	51.85^{ab}	7.53^{c}	2.30^{c}	$2.55\ (0.14)^{abc}$	156.70^{g}
T_3	18.51^{de}	7.96^{ab}	3.00^{c}	$255\ (0.14)^{abc}$	167.03^{f}
T_4	48.15^{ab}	6.10^{bc}	4.40^{c}	$1.40\ (0.09)^{bc}$	138.40^{h}
T_5	37.03^{bcde}	6.85^{abc}	4.00^{c}	$1.40\ (0.14)^{abc}$	203.37^{b}
T_6	25.93^{bcde}	5.56^{bc}	9.50^{c}	$12.42\ (0.34)^{a}$	190.26^{c}
T_7	44.44^{abcd}	4.84^{bc}	37.60^{b}	$11.11\ (0.28)^{ab}$	127.27^{j}
T_8	22.22^{cde}	4.64^{c}	34.30^{ab}	$6.25\ (0.22)^{abc}$	138.72^{d}
T_9	11.11^{e}	5.96^{bc}	48.00^{a}	$7.40\ (0.27)^{abc}$	186.72^{d}
T_{10}	33.33^{bcde}	6.49^{abc}	3.00^{c}	$2.63\ (0.12)^{abc}$	210.05^{a}
T_{11}	33.33^{bcde}	6.73^{abc}	6.00^{c}	$0.25\ (0.05)^{c}$	130.47^{i}
T_{12}	37.03^{bcde}	9.43^{a}	6.40^{c}	$1.40\ (0.09)^{bc}$	70.20^{k}
T_{13}	37.03^{bcde}	7.40^{abc}	4.30^{c}	$3.78\ (0.17)^{abc}$	138.38^{h}
T_{14}	44.44^{abc}	5.03^{bc}	10.00^{c}	$3.78\ (0.17)^{abc}$	129.86^{i}

Table 15b. Effect of different treatments on wilt incidence and population of *R. solanacearum* and bioagents in respect of variety Sakthi

Treatments	Wilt (%)	*R. solanacearum* cfu g^{-1} soil ($\times 10^3$)	*P. aeruginosa* cfu g^{-1} soil ($\times 10^3$)	AM fungus infection (%)	Yield (g/plant)
T_1	29.62^{abc}	8.98^{ab}	32.00^{a}	$8.51(0.44)^{ab}$	162.53^{f}
T_2	33.33^{ab}	11.66^{a}	8.00^{ab}	$3.78(0.17)^{cd}$	190.66^{e}
T_3	22.22^{abcd}	9.11^{ab}	4.00^{ab}	$2.55(0.14)^{cd}$	151.33^{g}
T_4	44.44^{a}	6.73^{b}	5.63^{ab}	$0.25(0.05)^{d}$	131.05^{i}
T_5	22.22^{bcd}	8.27^{ab}	8.30^{ab}	$1.40(0.09)^{d}$	234.60^{b}
T_6	7.40^{cd}	6.79^{b}	10.30^{a}	$11.11(0.32)^{a}$	299.36^{a}
T_7	3.40^{d}	5.16^{b}	37.60^{a}	$7.40(0.27)^{bc}$	191.90^{e}
T_8	7.40^{cd}	9.12^{ab}	42.30^{a}	$3.7(0.19)^{bcd}$	127.70^{i}
T_9	11.11^{bcd}	4.63^{b}	48.00^{ab}	$6.17(0.24)^{bc}$	205.66^{c}
T_{10}	14.81^{bcd}	5.40^{b}	5.24^{ab}	$7.40(0.27)^{bc}$	200.36^{d}
T_{11}	18.51^{bcd}	11.53^{a}	6.50^{ab}	$2.55(0.14)^{cd}$	136.71^{h}
T_{12}	18.51^{bcd}	9.33^{ab}	3.46^{ab}	$0.25(0.05)^{d}$	65.51^{k}
T_{13}	25.92^{abc}	4.84^{b}	5.30^{ab}	$1.40(0.09)^{d}$	123.86^{i}
T_{14}	33.33^{ab}	9.15^{ab}	9.50^{ab}	$1.40(0.09)^{d}$	124.91^{i}

Fig. 2. Effect of different treatmentbon wilt incidence, AM fungi infection and pseudomonad population in variety Pusa Ruby and Sakti



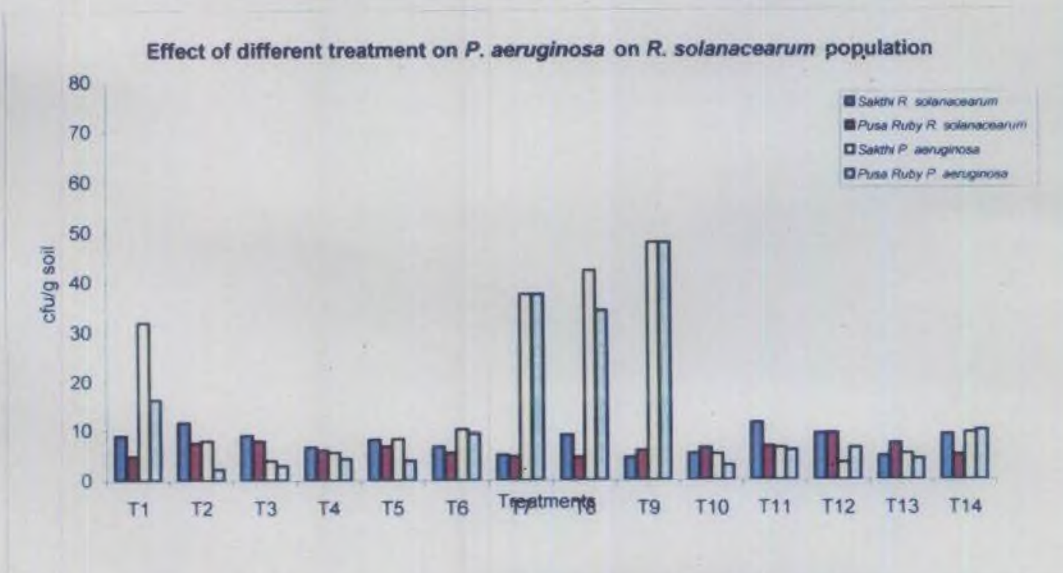
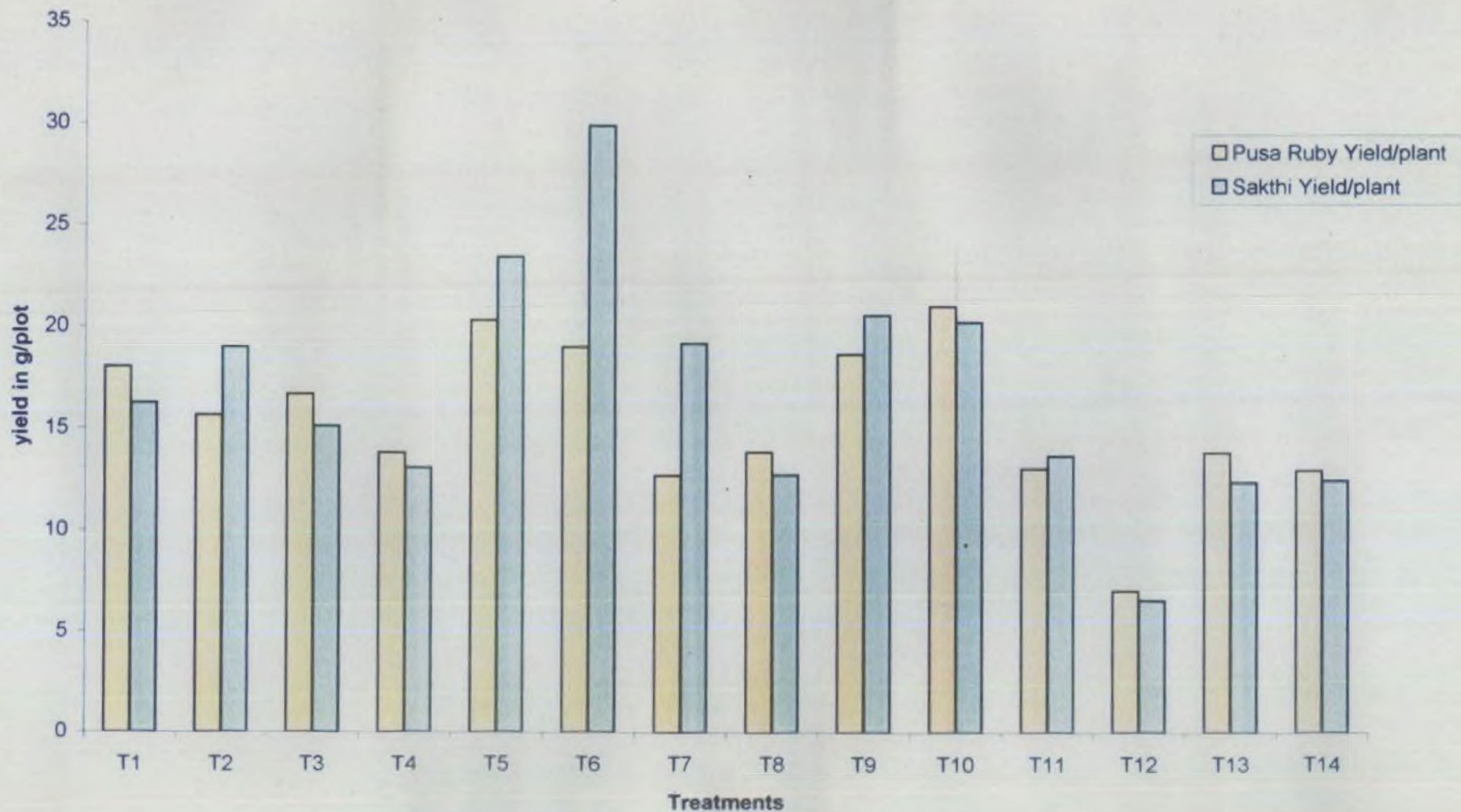


Fig. 3 . Effect of different treatments on yield (g/plot) in variety Pusa Ruby and Sakthi



A relationship was obtained between population of these organisms and wilt incidence, as the *P. aeruginosa* population and AM fungus infection increases there was a slight decrease in *Ralstonia solanacearum* population and wilt incidence. Even though slight variations were observed, a corresponding increase in yield was obtained only in AM fungus inoculated and *P. aeruginosa* seed treated and drenched pots.

In variety Sakthi, wilt incidence was highest in urea applied pot followed by control and CaO application (Table 15b). Also the population of *R. solanacearum* at 60 DAP was higher in pots which received CaO, 1 per cent bordeaux mixture, antibiotic seed treatment and control and a comparative decrease in AM fungus infection was noticed in the above treatments. Conversely the AM fungi infection was maximum AM fungus inoculated pots followed by solarisation treatment, *P.aeruginosa* inoculation and 10 per cent garlic extract drenching. The *P.aeruginosa* population was maximum in beneficial agents treated pots as well as in solarisation treatment. The *R. solanacearum* population was also minimum in *P. aeruginosa* and 10 per cent garlic extract treatments. Correspondingly reduction in wilt incidence noticed in bio agents treated pots. Yield for the variety was markedly high in AM fungus inoculated pots followed by CaO + urea treatment, *P. aeruginosa* treatment and 10 per cent garlic extract drenching.

4.8 *In* vitro effect of copper fungicides and garlic extract on *R.solanacearum* and *P. aeruginosa*

The copper hydroxide at three doses namely 0.15, 0.2 and 0.25 per cent, Bordeaux mixture 1 per cent and garlic extracts 10 per cent were evaluated for their inhibitory effect against *R. solanacearum* and *P. aeruginosa* (Table 16). Copper hydroxide 77% was included in the study as it can be used as a substitute for 1 per cent Bordeaux mixture. Out of the three concentrations of copper hydroxide tried the lowest concentration 0.15 per cent could produce an inhibitions zone of 0.75 cm but at 0.25 per cent concentration the inhibition zone obtained was 1.15 cm gave maximum inhibition (1.25 cm), followed by Bordeaux mixture 1 per cent and Copper hydroxide 0.25 per cent. When the inhibitory action against *P.aeruginosa*

Table 16. *In vitro* effect of copper fungicides and garlic extracts on *R. solanacearum* and *P. aeruginosa*

Bactericides	Inhibition zones (in cm)	
	R.solanacearum	*P. aeruginosa*
Copper hydroxide 0.15 percentage	0.75	0.00
Copper hydroxide 0.20 percentage	0.90	0.25
Copper hydroxide 0.25 percentage	1.15	1.60
Bordeaux mixture1 percentage	1.05	0.00
Garlic extract 10 percentage	1.25	0.00
Control	0.00	0.00

was evaluated the higher concentrations of copper hydroxide 0.2 per cent and 0.25 per cent produced inhibition zones of 0.25 cm and 1.60 cm respectively. All other treatments did not developed any inhibition zone under *in vitro* study. The lowest concentrations of copper hydroxide did not produce any inhibition zone against *P.aeruginosa* but could inhibit *R. solanacearum* considerably. Because of the selective property noticed the lowest concentration of Copper hydroxide (0.15 per cent) was selected for the main field trial (Plate 3).

4.9 Field Experiments

Based on the results of the pot culture experiments (Table 13) and *in vitro* studies (Table 16) the following treatments were selected for the main field trail (Plate 4).

Seed treatments

S_0 – Untreated seeds
S_1 – Seed treatment with *P. aeruginosa* at the time of sowing in the nursery
S_2 – Nursery soil treatment with AM fungus (*G. fasciculatum*) @ 50 g per pot

Treatments at the time of planting and 30 DAP.

T_0– Control
T_1– Drenching with one per cent Bordeaux mixture at the time of planting and 30 DAP
T_2– Drenching with 10 per cent garlic extract at the time of planting and 30 DAP
T_3– Solarization for 30 days and application of urea (15.28 g per plant) + calcium oxide (35 g per plant) at 30 DAP
T_4– Copper hydroxide 77 per cent (Kocide) 0.15 per cent at the time of planting and 30 DAP

Combinations of the above treatment were tried for effectiveness against bacterial wilt of tomato using the variety Sakthi. The resistant variety Sakthi was used since resistance is the most important factor in the management of the disease.

Plate 3. *In vitro* sensitivity of fungicide/plant extract on *R. solanacearum* and *P.aeruginosa*

Copper hydroxide 0.15 per cent

Copper hydroxide 0.20 per cent

Copper hydroxide 0.25 per cent

Bordeaux mixture 1 per cent

Garlic extract 10 per cent

Control plates

Seed treatment with biocontrol agents, soil drenching with bactericides and cultural method were selected and evaluated by integrating them. The resistant variety Sakthi was used since resistance is the most important mechanism for the management of the disease.

4.9.1 Field experiments – Effect of different treatments on rhizosphere microflora at 30 DAP.

Data on the rhizosphere microflora at 30 DAP were given in the (Table 17) .The result revealed that significant difference not obtained between treatments. The count of fungal population was lowest in treatment where soil drenching with 1 per cent Bordeaux mixture (S_0T_1) or Copper hydroxide (S_0T_4) (1.16 cfu) was given. The highest population was in AM fungus inoculation integrated with garlic drenching with 9.0 cfu (S_2T_2). When the mean data on bacterial population were compared it was found that bacterial population was lowest in control (S_0T_0) and highest in AM fungus integrated with Copper hydroxide (S_2T_4). In the case of actinomycetes population, count was lowest (3.0cfu) in *P. aeruginosa* seed treatment along with Copper hydroxide (S_1T_4) drenching and highest (16.33 cfu) in *P.aeruginosa* seed treatment with solarization and CaO+urea (S_1T_3). This was followed by S_2T_0 where AM fungus inoculation alone was given. AM fungus inoculation together with solarization (S_2T_3) and S_0T_4. In the case of pathogenic bacterial count, maximum population was noticed in AM fungus inoculation + drenching with 10 per cent garlic extract (S_2T_2) followed by S_1T_3 and S_1T_4. The population was lowest in *P. aeruginosa* seed treatment + drenching with 1 per cent Bordeaux mixture (S_1T_1) followed by *P. aeruginosa* seed treatment + drenching garlic extract 10 per cent (S_1T_2).

Fluorescent bacteria (*P. aeruginosa*) population in rhizosphere soil also showed no significant difference between the treatments. However *P. aeruginosa* population was more in *P. aeruginosa* seed treated plots with 19.60 cfu as highest in S_1T_1 and 2.3 cfu as the lowest in S_1T_3. The other treatments with AM fungus inoculation and no seed treatment gave meager counts of fluorescent bacteria or even absent.

Table 17. Effect of different treatment combinations on the rhizosphere microflora at 30 DAP

Treatments	Fungi cfu/g^{-1} soil ($\times 10^3$)	Bacteria cfu/g^{-1} soil ($\times 10^5$)	Actinomycets cfu/g^{-1} soil ($\times 10^1$)	*R. solanacearum* cfu/g^{-1} soil ($\times 10^3$)	*P. aeruginosa* cfu/g^{-1} soil ($\times 10^3$)
S_1T_1 – Seed treatment with *P. aeruginosa* + 1 % Bordeaux mixture at the time of planting and 30 DAP	2.16^{ab}	84.66^{ab}	10.83^{ab}	8.66^{a}	9.50^{b}
S_1T_2 – Seed treatment with *P. aeruginosa* + 10 % Garlic at the time of planting and 30 DAP	5.00^{ab}	88.00^{ab}	7.66^{ab}	13.00^{a}	3.60^{b}
S_1T_3 – Seed treatment with *P. aeruginosa* + solarised soil + CaO + Urea	2.66^{ab}	96.83^{ab}	16.33^{a}	78.16^{a}	2.30^{b}
S_1T_4 – Seed treatment with *P. aeruginosa* + 0.15 % copper hydroxide at the time of planting and 30 DAP	3.66^{ab}	46.83^{ab}	3.00^{b}	49.16^{a}	19.60^{a}
S_1T_0 – Seed treatment with *P. aeruginosa* alone	5.33^{ab}	36.83^{b}	9.16^{ab}	24.66^{a}	7.00^{b}
S_2T_1 – Soil application with *G. fasciculatum* + 1 % Bordeaux mixture at the time of planting and 30 DAP	2.66^{ab}	88.66^{ab}	7.63^{ab}	26.00^{a}	0.00^{b}
S_2T_2 – Soil application with *G. fasciculatum* + 10 % Garlic at the time of planting and 30 DAP	9.00^{a}	37.33^{b}	11.50^{ab}	89.16^{a}	0.00^{b}
S_2T_3 – Soil application with *G. fasciculatum* + solarised soil + CaO + Urea	4.83^{ab}	86.00^{ab}	12.66^{ab}	44.00^{a}	0.16^{b}
S_2T_4 – Soil application with *G. fasciculatum* + 0.15 % copper hydroxide at the time of planting and 30 DAP	5.50^{ab}	190.00^{a}	9.50^{ab}	21.5^{a}	0.00^{b}
S_2T_0 – Soil application with *G. fasciculatum*	2.50^{ab}	69.00^{ab}	14.66^{a}	14.6^{a}	0.50^{b}
S_0T_1 – Drenching with 1 % Bordeaux mixture at the time of planting and 30 DAP	1.16^{b}	115.16^{ab}	8.50^{ab}	49.00^{a}	0.00^{b}
S_0T_2 – Drenching with 10 % Garlic extract at the time of planting and 30 DAP	3.33^{ab}	150.00^{ab}	11.83^{ab}	21.50^{a}	1.00^{b}
S_0T_3 – Application of solarised soil at the time of planting + CaO + Urea at 30 DAP	2.33^{ab}	86.00^{ab}	10.00^{ab}	44.50^{a}	0.50^{b}
S_0T_4 – Drenching with copper hydroxide 0.15 % at the time of planting and 30 DAP	1.16^{b}	57.30^{ab}	12.16^{ab}	24.66^{a}	0.00^{b}
S_0T_0 – Control	4.66^{ab}	26.50^{b}	11.33^{ab}	28.83^{a}	0.00^{b}

Plate 4. Field experiment – a view

4.9.2 Field experiment – Effect of different treatments on rhizosphere microflora at 60 DAP

Rhizosphere microbial population at 60 DAP also did not show any significant difference between treatments (Table 18). However fungal population was minimum in plots with solarisation and addition of CaO + urea was given (S_0T_3) and maximum in treatment with 10 per cent garlic extract drenching (S_0T_2). In the case of bacterial population minimum colony forming units observed in S_1T_4 and maximum (95.33 cfu) in *P. aeruginosa* seed treatment alone (S_1T_0). In the control plot the count observed was 72.66 cfu. Actinomycetes population was lowest in AM fungus inoculation + Copper hydroxide drenching (S_2T_4) and highest in control (S_0T_0) followed by drenching with 10 percent garlic extract (S_0T_2).

The *R. solanacearum* population was highest in S_1T_0, followed by S_2T_2. The population was minimum in S_1T_1 followed by S_0T_4. In control plot the pathogen population count was 6.67 cfu.

The population of *P. aeruginosa* was maximum in S_0T_0 (control), S_2T_1, S_2T_4, S_0T_1 i.e. Bordeaux mixture and Copper hydroxide drenched plots irrespective of seed treatment. All these treatments gave a population of 3.33 cfu. The population was minimum in S_2T_2 and S_2T_3 both AM fungus inoculated non chemical treatments.

The AM fungus infection was also recorded at flowering stage, significant difference was obtained between treatments in percentage AM fungus infection. The infection percentage was maximum in AM fungus inoculated plot (S_2T_0), followed by S_2T_2. These two treatments are significantly superior to all other treatments. The AM fungi infection was minimum in S_0T_4 followed by AM fungus inoculation and 1% Bordeaux mixture drenching (S_2T_1) was given. The AM fungus infection was found comparatively lesser in *P. aeruginosa* seed treated seedlings compared to AM fungus inoculated seedlings and even to seedlings without any seed treatments. This was evidenced by the infection percentage 25.33 in S_1T_0 and 44.33 per cent in S_2T_0 and 38.66 in S_0T_0.

Table 18. Effect of different treatment combinations on the rhizosphere microflora at 60 DAP

Treatments	Fungi cfu/g^{-1} soil ($\times 10^3$)	Bacteria cfu/g^{-1} soil ($\times 10^5$)	Actinomycets cfu/g^{-1} soil ($\times 10^4$)	*R. solanacearum* cfu/g^{-1} soil ($\times 10^3$)	*P. aeruginosa* cfu/g^{-1} soil ($\times 10^3$)	AM fungi infection (%)
S_1T_1	8.00^{a}	48.00^{ab}	11.66^{ab}	2.33^{a}	1.00^{a}	17.0^{cdef}
S_1T_2	9.66^{a}	67.66^{ab}	9.33^{ab}	7.00^{a}	1.66^{a}	16.0^{cdef}
S_1T_3	8.00^{a}	75.00^{ab}	4.33^{ab}	4.33^{a}	1.66^{a}	6.60^{ef}
S_1T_4	4.66^{a}	28.66^{b}	7.66^{ab}	7.00^{a}	2.33^{a}	8.00^{def}
S_1T_0	5.66^{a}	95.33^{a}	14.00^{ab}	11.66^{a}	3.00^{a}	5.33^{ef}
S_2T_1	5.66^{a}	30.33^{b}	8.00^{ab}	6.00^{a}	3.33^{a}	25.33^{bcd}
S_2T_2	8.66^{a}	66.33^{ab}	10.00^{ab}	11.33^{a}	0.66^{a}	44.00^{a}
S_2T_3	9.00^{a}	75.33^{ab}	6.33^{ab}	10.33^{a}	0.66^{a}	25.33^{bcd}
S_2T_4	4.66^{a}	51.00^{ab}	2.00^{b}	6.66^{a}	3.33^{a}	22.66^{bcde}
S_2T_0	6.66^{a}	36.66^{b}	13.66^{ab}	9.33^{a}	1.33^{a}	44.33^{a}
S_0T_1	6.33^{a}	67.00^{ab}	7.33^{ab}	11.00^{a}	3.33^{a}	11.66^{cdef}
S_0T_2	12.33^{a}	50.66^{ab}	15.00^{a}	9.00^{a}	2.00^{a}	14.66^{cdef}
S_0T_3	4.00^{a}	53.00^{ab}	10.00^{ab}	6.66^{a}	2.00^{a}	28.00^{abc}
S_0T_4	5.33^{a}	48.66^{ab}	8.33^{ab}	3.33^{a}	1.66^{a}	2.66^{f}
S_0T_0	4.33^{a}	72.66^{ab}	15.33^{a}	6.67^{a}	3.33^{a}	38.66^{ab}

4.9.3 Field experiment – Effect of different treatments on wilt incidence

The percentage wilt incidence in the main field was recorded at 15 , 30, 45, 60 and 75 DAP and data are presented in Table 19 and Fig. 6.

The wilt incidence increased in all the treatments at different intervals of observations. Significant difference was obtained between treatments on wilt incidence at 15 DAP and 75 DAP. At 15 DAP application of bactericides was found to be effective in checking disease incidence irrespective of seed treatment. The treatment S_1T_2 and S_0T_4 were found to be best treatment contributing with minimum disease incidence of 3.33 per cent, this was followed by S_1T_4 and these were significantly superior to other treatments. The highest percentage of wilt incidence was noticed in control giving 14.25 per cent wilt incidence.

There was no significant difference between treatments in percentage wilt incidence at 30 DAP. However, lowest wilt incidence was registered in S_1T_4 followed by S_1T_2. The highest value was recorded in AM fungus inoculation with Bordeaux mixture drenching followed by S_2T_2.

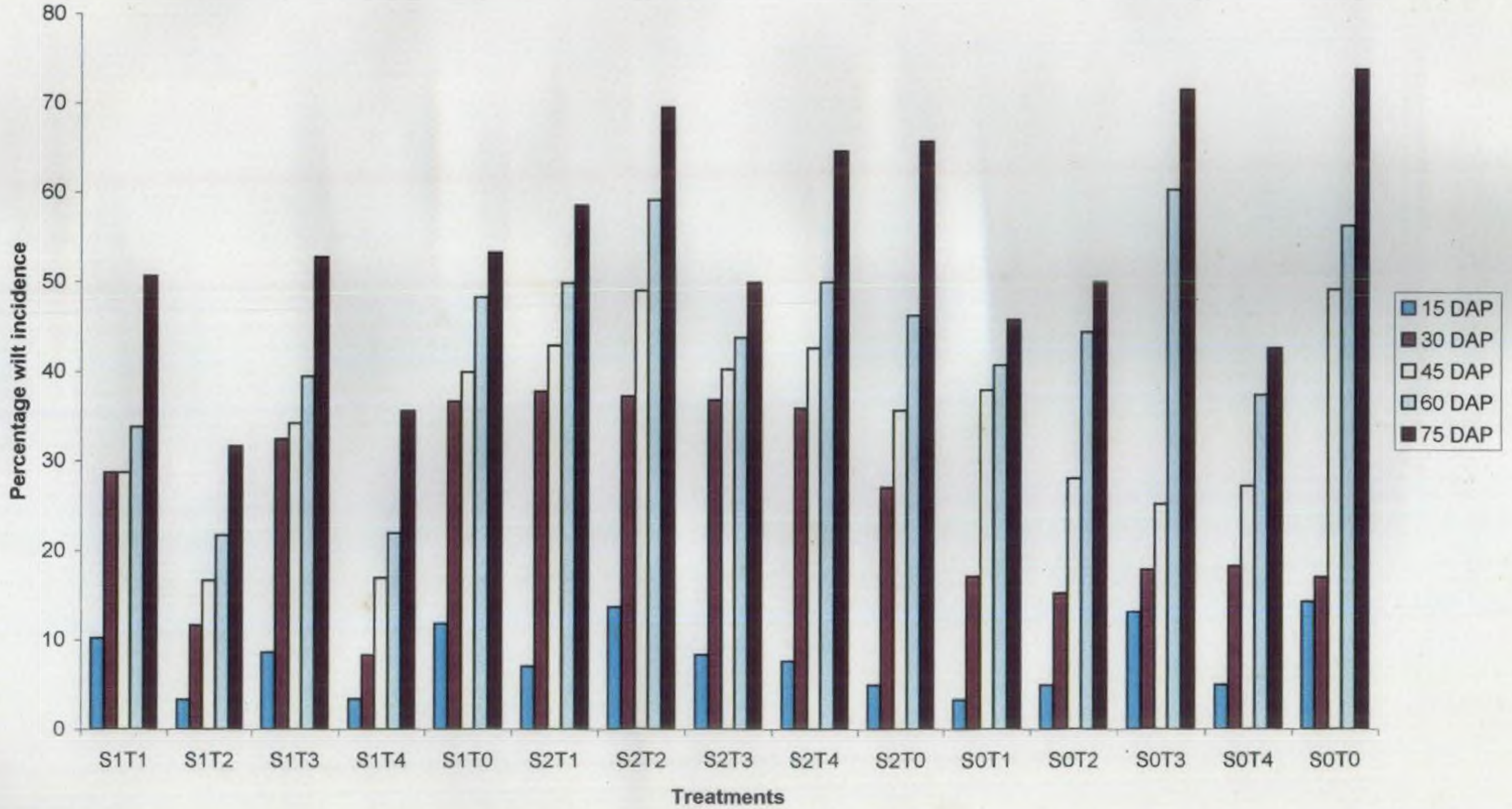
The rate of increase in wilt incidence from 30 to 45 DAP decreased when compared to 15 to 30 DAP. The data revealed that as in 15 DAP, here also at 45 DAP S_1T_2 and S_1T_4 were the most effective treatments giving minimum percentage wilt incidence. The highest wilt infection noticed in control plot with 49.14 per cent infection, this was immediately followed by S_2T_2.

Wilt percentage at 60 DAP also revealed the effectiveness of treatments S_1T_2 and S_1T_4 as the best combinations giving maximum reduction in wilt incidence with 21.67 and 21.93 per cent respectively. This was followed by S_1T_1 and S_0T_4. This observation also confirms the effectiveness of antagonistic action of *P.aeruginosa*, but combination of seed treatment with *P. aeruginosa* and a bactericide was found to be better than *P. aeruginosa* alone. Maximum wilt incidence was noticed in solarization treatment (S_0T_3) followed by S_2T_2 and S_0T_0.

Table 19. Effect of different treatment combinations on wilt incidence at different intervals

Treatments	Wilt incidence (%)				
	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP
S_1T_1	10.26^{abc}	28.68^{a}	28.68^{ab}	33.77^{ab}	50.67^{abcde}
S_1T_2	3.33^{c}	11.67^{a}	16.67^{b}	21.67^{b}	31.67^{e}
S_1T_3	8.59^{abc}	32.45^{a}	34.21^{ab}	39.47^{ab}	52.81^{abcde}
S_1T_4	3.42^{c}	8.33^{a}	16.93^{b}	21.93^{b}	35.61^{de}
S_1T_0	11.83^{abc}	36.67^{a}	40.00^{ab}	48.33^{ab}	53.33^{abcde}
S_2T_1	7.08^{abc}	37.79^{a}	42.95^{ab}	49.89^{ab}	58.60^{abcd}
S_2T_2	13.67^{ab}	37.27^{a}	49.03^{a}	59.20^{a}	69.56^{ab}
S_2T_3	8.33^{abc}	36.84^{a}	40.26^{ab}	43.77^{ab}	49.95^{abcde}
S_2T_4	7.62^{abc}	35.89^{a}	42.65^{ab}	49.99^{ab}	64.73^{abc}
S_2T_0	5.00^{bc}	27.05^{a}	35.67^{ab}	46.27^{ab}	65.78^{abc}
S_0T_1	3.33^{c}	17.11^{a}	37.94^{ab}	40.75^{ab}	45.81^{bcde}
S_0T_2	5.00^{abc}	15.26^{a}	28.05^{ab}	44.38^{ab}	49.98^{abcde}
S_0T_3	13.12^{ab}	17.88^{a}	25.17^{ab}	60.39^{a}	71.57^{a}
S_0T_4	5.00^{abc}	18.67^{a}	27.17^{ab}	37.36^{ab}	42.54^{cde}
S_0T_0	14.25^{a}	27.01^{a}	49.14^{a}	56.35^{a}	73.84^{a}

Fig. 4. Effect of different treatments on wilt incidence at 15, 30, 45, 60 and 75 DAP



Significant difference was obtained between treatments in checking the wilt incidence at 75 DAP. As in the previous observations the minimum wilt incidence was noticed in combinations of *P. aeruginosa* seed treatment and garlic drench (31.67 per cent), followed by S_1T_4 (35.61 per cent). The wilt incidence was maximum in control plot with 73.84 per cent, followed by solarization treatment (S_0T_3) with 71.57 per cent, and were significantly inferior to all other treatments. The AM fungus treatment was not giving any positive effect on wilt management.

4.9.4 Field experiment – Effect of treatments on AM fungus, infection plant growth and yield.

The effect of different treatments on different plant growth parameters viz., plant height, root length, number of leaves, wet weight, dry weight and yield were recorded and presented in Table 20.

The AM fungus infection was maximum in AM fungus inoculated plants irrespective of the treatment integrated with it. Percentage AM fungus infection was lowest in S_0T_4 giving only 2.66 per cent infection followed by S_2T_1 and S_1T_3 giving 5.33 and 6.60 per cent infection respectively. The AM fungus infection was maximum in S_2T_2 followed by S_2T_0 and were significantly superior to all other treatments. The control treatment also recorded a comparatively higher percentage of AM fungus infection (38.66). The *P. aeruginosa* bacteria treated seedlings has got comparatively lesser AM fungus infection.

The growth parameters namely, plant height, root length, number of leaves, wet weight and dry weight were recorded at 60 DAP. Significant difference was not obtained between treatments with regards to the height of the plant. The height of the plant was minimum in S_0T_3 (28.66 cm) followed by S_0T_1 (33.33 cm). The maximum plant height was noticed in S_1T_0 (56.00 cm), followed by S_1T_1 (49.00 cm). All other treatments did not show much variation in plant height.

When the mean wet weight of plants were compared significant difference was noticed between the treatments. The wet weight was minimum in S_0T_4 followed

Table 20. Effect of different treatment combinations on AM fungus infection plant growth and yield

Treatments	AM fungus infection (%)	Plant height (cm)	Plant wet weight (g)	Plant dry weight (g)	No. of leaves per plant	Root length (cm)	No. of fruits per plant	Yield (g) per plot
S_1T_1	17.0^{cdef}	49.33^{ab}	47.00^{bc}	8.60^{b}	22.67^{bcd}	11.00^{a}	184.00^{ab}	2556.66^{a}
S_1T_2	16.0^{cdef}	40.66^{ab}	51.82^{abc}	10.01^{ab}	34.33^{bc}	16.66^{a}	294.33^{a}	4616.66^{a}
S_1T_3	6.60^{ef}	43.00^{ab}	67.26^{abc}	18.34^{ab}	27.00^{bcd}	14.00^{a}	238.00^{ab}	3758.66^{a}
S_1T_4	8.00^{def}	43.00^{ab}	38.93^{bc}	10.14^{ab}	24.67^{bcd}	15.00^{a}	197.33^{ab}	2729.00^{a}
S_1T_0	5.33^{ef}	56.00^{a}	85.23^{ab}	19.33^{ab}	34.67^{ab}	17.66^{a}	86.66^{b}	1128.33^{a}
S_2T_1	25.33^{bcd}	45.80^{ab}	85.57^{ab}	22.45^{a}	36.00^{ab}	16.00^{a}	207.33^{ab}	3199.33^{a}
S_2T_2	44.00^{a}	48.66^{ab}	54.60^{abc}	13.14^{ab}	25.33^{bcd}	12.33^{a}	109.66^{ab}	1565.00^{a}
S_2T_3	25.33^{bcd}	47.33^{ab}	62.47^{abc}	14.76^{ab}	21.67^{bcd}	13.33^{a}	218.00^{ab}	3251.66^{a}
S_2T_4	22.66^{bcde}	42.33^{ab}	69.48^{abc}	14.67^{ab}	29.33^{abcd}	15.33^{a}	191.00^{ab}	3470.00^{a}
S_2T_0	44.33^{a}	48.33^{ab}	102.46^{a}	13.10^{ab}	43.00^{a}	14.00^{a}	138.33^{ab}	2345.66^{a}
S_0T_1	11.66^{cdef}	33.33^{b}	55.73^{abc}	14.59^{ab}	24.00^{bcd}	13.66^{a}	149.00^{ab}	2372.33^{a}
S_0T_2	14.66^{cdef}	44.66^{ab}	51.07^{abc}	15.00^{ab}	28.67^{abcd}	12.00^{a}	222.00^{ab}	3417.33^{a}
S_0T_3	28.00^{abc}	28.66^{b}	53.39^{abc}	13.36^{ab}	13.34^{d}	10.66^{a}	118.00^{ab}	2037.66^{a}
S_0T_4	2.66^{f}	43.33^{ab}	31.51^{c}	8.20^{b}	18.33^{cd}	11.66^{a}	209.00^{ab}	3015.00^{a}
S_0T_0	38.66^{ab}	47.33^{ab}	71.33^{abc}	20.02^{ab}	32.67^{abc}	13.66^{a}	256.33^{ab}	4160.33^{a}

by S_1T_1. The maximum wet weight was registered in S_2T_0 (102.46 g). This was followed by S_2T_1 and S_1T_0. Control plot also gave a wet weight of 71.33 g. All other treatments gave comparatively lesser wet weight. When the dry weight of plants were compared significant difference was not obtained between treatments. The dry weight was minimum in S_2T_4 (8.20g) and was maximum in S_2T_1 (22.45g). All other treatments did not show much variation.

Significant difference was obtained between treatments in number of leaves. The number of leaves was maximum in S_2T_0 (43.00) followed by S_2T_1 (36.00), then in S_1T_0 (34.60). The number of leaves were lowest in S_0T_3 (13.34) followed by S_0T_4 (18.30).

When the mean length of the root was compared, significant difference was not noticed between treatments. The maximum root length recorded in S_1T_0 (17.66 cm) followed by S_1T_2, measuring 16.66 cm. The root length was minimum in S_0T_3 (10.66 cm).

Observations on number of fruits as well as weight of the fruits were recorded. Significant difference was not obtained between treatments, when mean number of fruits were compared. The number of fruits were maximum in S_1T_2 (294.33). All the other treatments were inferior to control even though different trend was noticed in wilt incidence. The minimum yield was recorded in S_1T_0 (86.66). Same trend was noticed in the case of fruit weight also. Significant difference was not noticed between treatments. However, here also maximum weight of fruit was obtained in S_1T_2 (4616.7g).The yield was minimum in S_1T_0 (1128.33 g). Though the wilt incidence was higher in control it did not reflected in yield. The *P. aeruginosa* seed treated plots recorded a higher yield, compared to AM fungi inoculated plots.

4.9.5 Effect of different treatments combinations on beneficial agents, *R.solanacearum*, percentage wilt incidence and yield

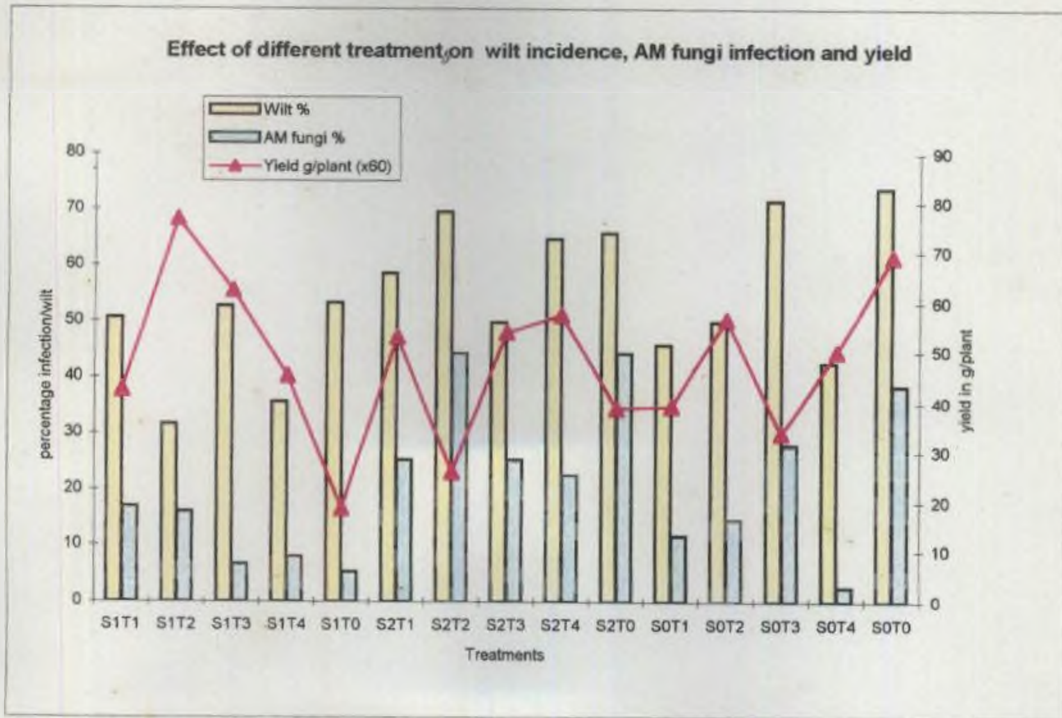
The effect of different treatments on total wilt incidence, population of *R.solanacearum*, *P. aeruginosa* and percentage AM fungus infection and yield were presented in Table 21 and Fig. 5.

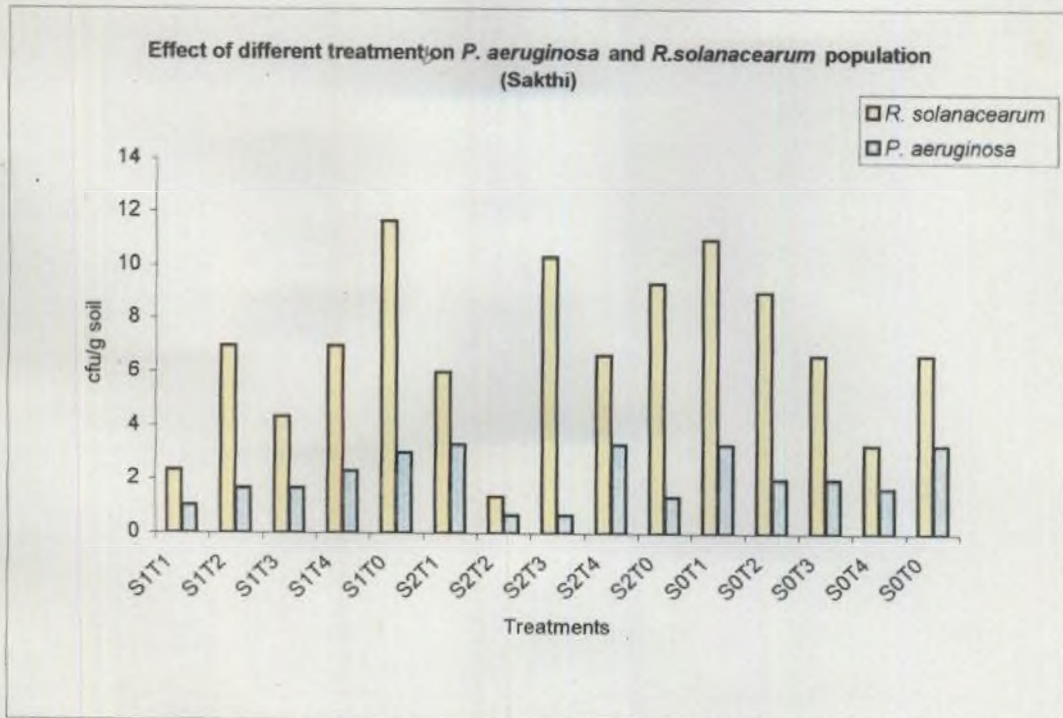
Percentage wilt incidence and population of *R. solanacearum* did not gave a positive relation. The wilt incidence was maximum in S_0T_0 and S_0T_3 though the *R.solanacearum* population was lesser. Similarly wilt incidence was lowest in S_1T_2, but the population of *R. solanacearum* was comparatively higher. The *P.aeruginosa* population also did not show any positive correlation with wilt incidence. The pseudomonads population, both *R. solanacerum* and *P. aeruginosa* was maximum in control plots, but the wilt incidence was higher. Higher AM fungus infection in the control plot has reflected in the yield obtained. The yield was maximum S_1T_2 where the wilt incidence was minimum and populations of *R. solanacearum, P. aeruginosa* and AM fungus infection moderate. The percentage AM fungus infection was lower in *P. aeruginosa* inoculated seedlings but *P. aeruginosa* population was higher with a low percentage wilt incidence and minimum pathogen population.

Table 21. Effect of different treatment combinations on wilt incidence, population of *R. solanacearum*, *P. aeruginosa* and AM fungus infection and yield

Treatments	Wilt incidence % (75 DAP)	*R. solanacearum* cfu g^{-1} soil ($\times 10^3$) (60 DAP)	*P. aeruginosa* cfu g^{-1} soil ($\times 10^3$) (60 DAP)	AM fungus infection (%) (60 DAP)	Yield (g/plot)
S_1T_1	50.67[abcde]	2.33[a]	1.00[a]	17.0[bcde]	2556.66[a]
S_1T_2	31.67[e]	7.00[a]	1.66[a]	16.0[bcde]	4616.66[a]
S_1T_3	52.81[abcde]	4.33[a]	1.66[a]	6.60[ef]	3758.66[a]
S_1T_4	35.61[de]	7.00[a]	2.33[a]	8.00[def]	2729.00[a]
S_1T_0	53.33[abcde]	11.66[a]	3.00[a]	5.33[def]	1128.33[a]
S_2T_1	58.60[abcd]	6.00[a]	3.33[a]	25.33[abcde]	3199.33[a]
S_2T_2	69.56[ab]	11.33[a]	0.66[a]	44.00[a]	1565.00[a]
S_2T_3	49.95[abcde]	10.33[a]	0.66[a]	25.33[abc]	3251.66[a]
S_2T_4	64.73[abc]	6.66[a]	3.33[a]	22.66[abcd]	3470.00[a]
S_2T_0	65.78[abc]	9.33[a]	1.33[a]	44.33[a]	2345.66[a]
S_0T_1	45.81[bcde]	11.00[a]	3.33[a]	11.66[cdef]	2372.33[a]
S_0T_2	49.98[abcde]	9.00[a]	2.00[a]	14.66	3417.33[a]
S_0T_3	71.57[a]	6.66[a]	2.00[a]	28.00[abc]	2037.66[a]
S_0T_4	42.54[cde]	3.33[a]	1.66[a]	2.66[f]	3015.00[a]
S_0T_0	73.84[a]	6.67[a]	3.33[a]	38.66[ab]	4160.33[a]

Fig. 5. Effect of different treatment on populationof pseudomonads, AM Fungi, wilt incidence and yield in the field





Discussion

5. DISCUSSION

Bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* is one of the major limitations in tomato production. This pathogen is a fastidious soil borne organism with substantial economic importance as it is a perennial problem in wilt sick soils. Moreover the pathogen shows great variation in its aggressiveness at varying degrees at different locations, thereby management of this disease become rather difficult. Eventhough various management practices were reported, no remedy lasted long due to various reasons such as pathogen diversity-both phenotypic and genotypic, and loss of host resistance. Hence integration of different management practices were tried to effectively manage the disease. In view of the above situations investigations were carried out to study the pathogen variability from different locations and to isolate potent antagonistic bacteria against the pathogen. Cultural practices like soil amendments, solarisation, use of bio-agents, chemicals like fungicides and plant extracts were integrated to evolve an eco-friendly, location specific management practice to combat the tomato bacterial wilt disease.

The pathogen *R. solanacearum* was isolated from the infected plant collected from four different agro-climatic locations of the state. All four isolates produced positive reaction to oxidase, catalase, utilization of sucrose, cellobiose, sorbitol and dulcitol, but failed to liquefy gelatin and hydrolyse arginine. The characterisation studies revealed that the four isolates belongs to *R. solanacearum* race 1 biovar 3. In the present study no variability could be detected between the isolates eventhough they were collected from varied agro climatic regions of Kerala. The results of the characterisation studies did not show much difference between isolates, only slight variations were noticed on colony characters like growth, slime production and fluidity. Pathogenicity and characterisation results have confirmed the bacteria as *Ralstonia solanacearum* race 1 biovar 3 (smith) Yabuuchi *et al.*, (1995). The biovar grouping is based on the study of He *et al.*, 1983 Kumar *et al.*, 1993, Hayward 1994 and Paul 1998.

Isolation of fluorescent bacterial strains was tried from the samples collected from these four locations. Effective antagonism was obtained from the sample collected from the central zone and was utilized for pot culture and field studies. The antagonostic bacterial strains produced slimy, irregular white to dirty white colonies and abundance of water soluble bluish green fluorescent pigment in King's B media. As the culture becomes older the bacteria produced non diffusible non fluorescent brown pigments. The antagonostic bacterial strain failed to produce levan but produced positive reactions to gelatin liquifaction, oxidase activity, catalase activity, denitrification, growth at 42°C, and the organism was tentatively identified as *Pseudomonas aeruginosa*. Diagnosis of the bacterial strain based on the above characters were made according to Fahy and Llyod (1983) and John *et al.* (1994). Later studies with the antagonistic bacterial strain revealed the antagonistic activity against the pathogenic bacteria *R. solanacearum* as described by Dhingra and Sinclair (1995).The *in vitro* antagonistic effect of *P. aeruginosa* was also reported by Karuna *et al.*, (1997).

The studies on the *in vitro* sensitivity to antibiotics has revealed that the bacterial strains were insensitive to Oxytetracycline, Chloramphenicol and Rifampicin. The insensitivity of *R. solanacearum* to Chloramphenicol was also reported by He *et al.*, (1983). But Goorani *et al.*, (1978) reported growth inhibition for the pathogen by the antibiotic Oxyteracycline. Both the bacteria were sensitive to Kanamycine, *P. aeruoginosa* was more sensitive to Kanamycin 50 mgl^{-1} compared to *R. solanacearum*. The pathogenic bacteria was found to be more sensitive to antibiotic Streptomycine sulphate even at 100 mgl^{-1} than *P. aeruginosa*, which is sensitive only at 200 mgl^{-1}. Sensitivity of Streptomycine sulphate to *R.solanacearum* was also reported by Campacci *et al.* (1962), Chakravarthy and Rangarajan (1966) and Paul (1998). The *R. solanacearum* was also sensitive to Ampicillin but not the antagonistic strain, *P. aeruginosa.* The sensitivity of Ampicillin and Streptomycin sulphate to *R. solanacearum* was also reported by Mondal and Mukherjee (1978) and Jyothi (1992).

Present study revealed that seed treatment with *P. aeruginosa* facilitates earliness in germination. Maximum germination percentage was obtained with the

P. aeruginosa seed treatment in both the varieties. This might be because of the plant growth promoting ability of *P. aeruginosa* in addition to its antagonistic ability against *R. solanacearum*, the wilt pathogen. Similar acceleration in the germination of tomato seeds and increase in seedling vigour, when the seeds soaked in a suspension of 2 x 10^9 cfu ml^{-1} of antagonistic strain 90B 4-2-2 for 30 min was reported by Wei-ChunMei *et al.*, (1994). When the plant growth responses were compared, in the seed treatment with antagonistic bacterium *P. aeruginosa* and antibiotic Streptomycine sulphate, an increased shoot and root length were obtained when compared to control in both the varieties. The increase was more pronounced in the resistant variety Sakthi compared to the susceptible variety Pusa Ruby. *P.aeruginosa* could increase plant growth, might be due to its ability to produce the plant growth regulating hormones in the rhizosphere soils as reported by Sobieszcanski *et al.* (1989) with the strains of *P. fluorescens*. The effectiveness of seed treatment with Streptomycine sulphate in enhancing the root length and root shoot ratio in the resistant tomato varieties compared to the susceptible variety was reported by (Mishra *et al.*, 1999). The effectiveness of the antagonistic seed treatment in improving the germination percentage and increasing the seedling vigour was also reported by Wei-ChunMei *et al.*, (1994).

Under *in vitro* condition and in pot culture the *P. aeruginosa* seed treatment as well as the AM fungus inoculation were found superior to antibiotic seed treatment and control in seed germination and in plant vigour. Similar findings on antagonistic bacteria was made by Wei-ChunMei *et al.*,(1994). Hedge and Rai (1984) reported that inoculation of tomato with AM fungus, *Glomus fasciculatum* reduced the damping off and increased the plant height and bio-mass.

Soil solarization by covering the moist soil with white transparent 150 gauge polythene sheets increased the soil temperature to 54.5°C and 49°C at 5 and 10 cm soil depth respectively. Similar results were observed by Anith *et al.* (2000) in the case of polythene mulching of wet soil for the control of bacterial wilt in ginger.

The soil microbial population namely fungal, bacterial and actinomycetes decreased due to solarization whereas pathogen and fluorescent bacterial population

increased after one month of solarization. In the nonsolarised soil the rate of increase was still higher after one month period. The increase of *R. solanacearum* population in solarised soil might be due to its ability to survive at higher temperature compared to other bacterial populations present in the soil. But the rate of increase in the *R.solanacearum* population was much higher in the nonsolarised soil compared to solarised soil after one month. This shows that solarisation for 30days has a weak effect in checking the *R. solanacearum* population and pathogen might be able to withstand temperature to some extend. Vilasini (1996) also could not notice a significant reduction in Pseudomonas population in 30 days solarised plot, but a marginal reduction was noticed in 45 days solarised plot.

Based on the available reports on the management of bacterial wilt of tomato the treatments were finalised for the pot culture study. The pot culture study was taken up to screen out the most effective components for management of bacterial wilt that can be used for integrated treatment combinations in the main field trial. The microbial count in the rhizosphere soil at 20, 40 and 60 DAP were assessed to test the effectiveness of treatments on the microbial population as well as percentage wilt incidence in both susceptible and resistant varieties under Vellanikkara condition.

At 20 DAP fungal population was minimum in Bordeaux mixture drenched pots and the bacterial population was minimum in lime applied pots in the variety Pusa Ruby. These results confirms the already known efficacy of the treatments, fungicides against fungi and higher pH against bacteria. The *R. solanacearum* population was managed best by the *P. aeruginosa* and AM fungus treatments in both the varieties. The efficiency of *P. aeruginosa* for the biological control of *R.solanacearum* was also reported by Silveria *et al.* (1996) and effectiveness of root extracts of *G. fasciculatum* infested roots against R. *solanacearum* in nutrient broth was reported by Suresh and Ravi (1991). In susceptible variety population of fluorescent bacteria was higher in antagonistic treatments and a decrease in pathogen population obtained, but the same trend for the treatment was not observed in percentage wilt incidence. However *P. aeruginosa* seed treatments recorded the minimum disease incidence.

In the resistant variety Sakthi, R. *solanacearum* populaion was lowest in *P.aeruginosa* treated (both seed treatment and drenching) plants and AM fungi infected plants. The wilt incidence was low in all the bio agent treated plants. From these results, it is clear that, even though beneficial agents could reduce the pathogen population as well as the disease incidence, their efficiency as a biocontrol agent could be better utilized in the management of the bacterial wilt disease by incorporating host resistance in the disease management scheme. Kobayashi (1991) reported that AM fungi alone could induce only weak resistance to disease and in open field, where there is high density of pathogen population, biocontrol agent had a weak effect and integrated control was essential to express the disease resistance. In the present study at 20 DAP, *P. aeruginosa* and AM fungi in combination with host resistance could reduce the disease incidence considerably.

A similar effect was not noticed at 40 DAP with regards to the population statistics of fluorescent bacteria *P. aeruginosa*, pathogenic bacteria *R. solanacearum* and percentage wilt incidence. In susceptible variety Pusa ruby, *R. solanacearum* population was lowest in neem cake and urea treatments, but the *P. aeruginosa* population was maximum in *P. aeruginosa* treated pots and the wilt incidence was minimum in garlic treatment as well as in *P. aeruginosa* treatments. But in variety Sakthi the fluorescent bacterial population was maximum in *P. aeruginosa treated* pots and the pathogenic bacterial population was minimum in garlic 10 per cent drenched pots as well as in lime amended pots. However the treatments receiving *P.aeruginosa* recorded minimum wilt percentage. The present results indicated that the resistance of the hosts enhanced by the bio agents reduced the disease incidence in the host rather than affecting the bacterial population in the soil. The improvement in the host resistance due to bio agent treatments was contributing to disease resistance rather than the control of pathogenic population in the rhizosphere soils. Furuya *et al.* (1997) reported the mechanism of induced resistance and infection sites competition in suppression of bacterial wilt disease of tomato when the root were pre-treated with *P. aeruginosa.*

In Pusa Ruby no definite pattern noticed with respect to the population of fungi, bacteria and *P. aeruginosa* at 60DAP. The *P. aeruginosa* population was

maximum in the beneficial agent treated pots with a corresponding decrease in *R.solanacearum* population. But the percentage wilt incidence was lminimum in garlic 10 per cent drenched pots. Whereas in variety Sakthi the *P. aeruginosa* treated pots showed lesser disease incidence with maximum fluorescent *P. aeruginosa* bacterial population and minimum *R. solanacearum* population. The garlic drenched plants also stood along with the bio agent treated plants with respect to lesser *R.solanacearum* as well as *P. aeruginosa* population and a corresponding lesser disease incidence. The effect of garlic extract in suppression of bacterial wilt of tomato was also noticed by Hutagalung (1988). Bordeaux mixture was also shown its effectiveness in reducing wilt incidence though less effective than bio control treatment. The AM fungus treatments although did not show any effect on pathogenic bacterial population, expressed a lesser disease incidence. This may be because of its ability to improve plant resistance by the absorption of nutrients from soil and made it available to host plant. Halos and Zorilla (1979) reported a decrease in bacterial wilt incidence when the tomato roots were prior inoculated with *Glomus mossae*.

The observation on pathogen population and wilt incidence at 20 days interval showed an increasing trend. The population of *R. solanaceous* do not show much variation at 20, 40 and 60 DAP. But the percentage wilt incidence showed an increasing trend and maximum wilt incidence was noticed at 60 DAP in both the varieties. The percentage wilt incidence as well as the rate of increase in wilt incidence was maximum in susceptible variety Pusa Ruby compared to the resistant variety Sakthi. The treatment of bio agent was more effective when integrated with host resistance. But in Pusa Ruby the *P. aeruginosa* seed treatment along with drenching gave considerable disease management.

In the present study the treatment effect was more pronounced at 20 DAP. At 40 days after planting, the higher *R. solanacearum* population in the rhizosphere soil has not contributed to wilt incidence. This might be because of the PGPR activity of the inoculated *P. aeruginosa* towards triggering the resistant mechanism of the host plants. Similar findings was also reported by Furuya *et al.*, (1997) with *P.aeruginosa* on *R. solanacearum*.

Garlic extract 10 per cent and the bactericides Bordeaux mixture one per cent and Copper hydroxide 0.15 per cent were sensitive to *R. solanacearum* but insensitive to *P. aeruginosa* and no inhibition zone was formed around it. Copper hydroxide was included in the experiment because of the colloidal nature of Bordeaux mixture which is expected to reduce its efficacy as soil drenching bactericide. The inhibitory effect of garlic on wilt incidence was reported by Hutagalung (1988) and Bordeaux mixture as bactericide on *R. solanacearum* was reported by Jyothi (1992).

In this study comparison on effectiveness of treatments on tomato wilt incidence was made, and it was found that Sakthi was very much superior to Pusa Ruby in reducing wilt incidence. At 60 DAP the variety Sakthi superseded over all the different treatments of Pusa Ruby except garlic 10 per cent drenching in reducing the wilt incidence. *P. aeruginosa* inoculated susceptible variety also showed lesser disease incidence. This might be due to the mechanism of antagonism other than the antibiotic production such as induced resistance, infestation and site competition. Similar findings were also reported by Furuya *et al.*, (1997) with *P.aeruginosa* on *R. solanaecearum*. The resistant variety Sakthi treated with bio agent, combined with garlic drenching or Bordeaux mixture drenching was found to be superior to all other treatments in reducing the wilt incidence.

The percentage AM fungi infection and the yield data along with wet weight of the plant showed positive effects. When percentage AM fungi infection was higher the yield also was increased. This may be because of absorption of minerals and nutrients by the AM fungi and making them available for the plant. Halos and Zorilla (1979) reported an increase in plant height and yield in addition to a decrease in bacterial wilt incidence when the tomato roots were prior inoculated with *Glomus mossae*.

In the main field, treatment combinations were tried based on the results of pot culture experiments to evolve a most effective ecofriendly scheme for bacterial wilt management of tomato. At 30 DAP, *R.solanacearum* population was minimum in *P. aeruginosa* seed treatment when integrated with Bordeaux mixture or garlic

extract 10 per cent. In the case of inorganic bactericides where Cu^{2+} is toxic agent, there is direct inhibition of bacterial growth, and chemically Kocide is copper hydroxide as reported by Hudson and Sigee (1991). According to them the treatment of bacterial cell with copper compound led to major changes in elemental composition and that these changes are consistent with a toxic effect at the cell surface, leading to large scale efflux of K^{+} and influx of Ca^{2+} and Cu^{2-}. The biocontrol agent existing in the natural environment multiply and compete with pathogenic organisms in soil and provide efficient management of the disease, if the rhizosphere is least disturbed.

At 60 days after planting percentage AM infection was higher in AM inoculated control as well as AM with garlic drenching. The fluorescent bacterial population was maximum in control and other fungicidal treatments, and pathogen population was minimum in one per cent bordeaux mixture and in Copper hydroxide drenched plots. But the wilt incidence was minimum in *P. aeruginosa* seed treatment when integrated with garlic and *P. aeruginosa* seed treatment when integrated with Copper hydroxide. Even though the pathogen population was high in the rhizoshere soil, its effect was not reflected in the wilt incidence. It indicated that in addition to the antagonistic activity of *P. aeruginosa,* it has got the mechanism for improving the resistance of the host plants.Similar reports were obtained by Wei-Chunmei *et al.* (1994) and Furya *et al.* (1997). Many bacterial antagonists (fluorescent *pseudomonas*) induced/triggered systemic resistance in host crop besides inhibiting the pathogen directly and thereby reducing the disease severity.

AM fungus infestation was maximum in AM fungus inoculated control plots, AM fungus inoculated garlic drenching and in control plot. The highest fresh weight was obtained from AM fungus inoculated control, but the wilt incidence was also higher in control plot. Maximum yield was noticed in *P. aeruginosa* inoculated and garlic drenched plot where the wilt incidence was minimum. Higher the AM fungi infection, the number of leaves, fresh weight and comparatively higher plant height were noticed in AM fungus inoculated treatment but were not reflected in yield. The decrease in yield may be because of increase in percentage wilt incidence.

When the wilt incidence at different intervals were compared, the percentage disease incidence showed an increasing trend. The rate of increase in wilt incidence was highest in the early stages and then in the flowering stage. The plants withstand bacterial infection upto flowering stage because of the resistant mechanism present in the variety Sakthi. The present findings are in line with the observations of Elphinstone and Aley (1992).

The percentage wilt incidence at 15 days intervals showed the effectiveness of *P. aeruginosa* seed treatment throughout the crop growth period. Seed treatment alone could not manage the wilt disease but when this was integrated with either garlic or Copper hydroxide, the treatment became most effective and significantly reduced the percentage wilt incidence. Hasan and Abdulla (1999) obtained the same result with beneficial soil micro organisms and $Ca(NO_2)_3$ which when applied separately were less effective than when they are applied in combination. The result of the study indicated that the bacterial disease of tomato could be effectively managed by integrating indigenous antagonistic activity of *P. aeruginosa* and selective bactericides like garlic extract or Copper hydroxide 0.15 per cent along with the host resistance of variety Sakthi.

Survival and establishment of an introduced biocontrol agent at the target site is an important factor determining the efficiency of the biological control. The selection of the bactericides to integrate with the beneficial antagonistic organisms should be specific to each location, so that the beneficial microflora remain harmless. In the present study *G. fasciculatum* infection in tomato roots was found to be very efficient in promoting the plant growth. But the disease management ability was more expressed in *P. aeruginosa*. Disease management efficiency, better plant vigour along with the increased yield could be brought about if the AM infection and infestation of *P. aeruginosa* could be incorporated simultaneously to increase the resistant mechanism of the host plant.

As it is a soil borne pathogen it is not possible to completely eradicate the organism from the soil. The soil factors conducive for the host is favourable for the pathogen also. Hence the only possible way out is Integrated Disease Management

for improving the general health of the plant by way of manipulating the rhizosphere environment at micro and macro level.

Since the antagonistic bacterium *P. aeruginosa* was isolated from the same location, the efficiency could be better exploited in deriving an ecofriendly location specific sustainable bacterial wilt management. Both AM fungus inoculation and *P.aeruginosa* treatment was effective in triggering the host resistance mechanism, the efficiency of reducing wilt incidence was better expressed at different stages of the crop. Hence the compatibility of AM fungus and *P. aeruginosa* has to be further evaluated for a sustainable disease management, which can ultimately contribute to higher yield.

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SUMMARY

A study on integrated disease management of bacterial wilt of tomato caused by *Ralstonia solanacearum* Smith (Yabuuchi *et al.*) was undertaken in the Department of Plant Pathology, College of Horticulture, Vellanikkara during the period 1998-2000. The objective of the study was to select major disease management components and to develop a strategy by integrating them to have a sustainable eco-friendly crop production. The salient findings of the study are summarized below.

1. The bacterial wilt pathogen *Ralstonia solanacearum was* isolated from wilted tomato plants collected from College of Agriculture, Vellayani (south zone), College of Horticulture, Vellanikkara (central zone), College of Agriculture, Pilicode (north zone) and Rice Research station, Moncompu (problem zone) on TZC agar medium and identified by the circular fluidal, slimywhite colony with light pink centre. Pathogencity test on tomato, brinjal and chilli were also conducted using these isolates.
2. Isolation of antagonistic bacteria was effected from soil samples collected from the central zone. The bacterium produced spreading white to dirty white colonies with abundant bluish green fluorescent water soluble pigment and a non diffusible non fluorescent brown pigment in King's B medium.
3. Cultural, physiological and biochemical characters of the four bacterial isolates revealed that the four pathogenic bacterium belongs to *Ralstonia solanacearum* race I biovar III (Smith) Yabuuchi *et al.* The antagonistic bacterium tentatively identified as *P. aeruginosa.*
4. When the sensitivity of the bacterial isolates to antibiotics were tested both bacteria were sensitive to Kanamycin but *P aeruginosa* was more sensitive compared to *R solanacearum* at 50 mgl^{-1}. In the case of Streptomycine sulphate and Ampicillin *R solanacearum* showed sensitivity at 100 and 200 mgl^{-1} concentration but the antagonistic bacterium was not inhibited at 100 mgl^{-1} concentration.

5. Seed treatment with *P aeruginosa* and Streptomycine sulphate gave a better germination for both the varieties but seed treatment with antagonistic bacterium gave earlier germination, maximum germination percentage and plant vigour compared to other treatment.
6. In pot culture study also the performance of *P aeruginosa* treatment was superior followed by AM fungus inoculation. However the antibiotic seed treatment was better to control but not to *P. aeruginosa* and AM fungus.
7. Soil solarisation for 30 days recorded a temperature range from 26 to 54.5°C at 5cm depth in solarised soil, whereas in non solarised soil it was 24.4 to 43.1°C.
8. A general decrease in soil microbial population was noticed due to solarisation for 30 days but an increase was noticed in the case of *R solanacearum* and *P.aeruginosa* populations.
9. A pot culture study was conducted using two varieties Pusa Ruby and Sakthi to evaluate 14 treatments. The rhizosphere soil microorganism population at 20DAP showed minimum fungal population in Bordeaux mixture one per cent drenched pots and the minimum bacterial population was in CaO treated pots. The counts of *P. aeruginosa* was maximum and *R. solanacearum* was minimum in pot inoculated with *P. aeruginosa.*
10. A different trend was noticed at 40DAP in Pusa Ruby. A minimum fungal and bacterial population was noticed in AM fungus treatment and garlic 10 per cent treatment respectively. *R. solanacearum* population and *P aeruginosa* was maximum in solarised treatment and the highest count of *P aeruginosa recorded* in treatment, which received *P. aeruginosa.* In the variety Sakthi also a similar trend was noticed in the case of fungal population and *P.aeruginosa* population. The bacterial population was lowest in antibiotic treatment and the lowest *R solanacearum* population observed in CaO treatment pots.
11. At 60 DAP a considerable variation was noticed in the rhizosphere soil microflora in both the varieties. The fungal population was minimum in CaO + urea treated pots and bacterial population minimum in antibiotic treatment. *R.solanacearum* population was minimum in *P aeruginosa* treatment but the same treatment gave a higher *P aeruginosa* population. In the variety Sakthi fungal population and bacterial population were the lowest in CaO and *P.aeruginosa* drenched treatment respectively. *R solanacearum* and

P.aeruginosa population counts were showing the same trend as in the case of Pusa Ruby.

12. In variety Pusa Ruby at 20 DAP the percentage wilt incidence and *R.solanacearum* population was lowest in *P. aeruginosa* seed treated pots .At 40DAP the lowest *R solanacearum population* was recorded in neem cake treatment but the lowest wilt percentage was in 10 per cent garlic treatment. Where as at 60 DAP both *R solanacearum* and wilt incidence were lowest in *P.aeruginosa* treatment.
13. In the variety Sakthi at 20 DAP both pathogen population and wilt incidence were lowest in *P. aeruginosa* seed treatments where as in 40 DAP the lowest pathogenic bacterium population obtained from CaO treatment pots and the lowest wilt incidence in *P aeruginosa* treatments. At 60 DAP *P aeruginosa* seed treatment was found to be better giving minimum *R solanacearum* population and the lowest wilt incidence.
14. The variety Sakthi was found to be much superior to pusa Ruby in reducing wilt incidence even without any treatment application in the pot culture studies, At 20 DAP 10 percent garlic drenching, *P. aeruginosa* seed treatment, 1 per cent Bordeaux mixture and neem cake application could give a lesser wilt incidence even in the variety Pusa Ruby. Among the different treatments the variety Sakthi which received *P. aeruginosa* seed treatment was proved to be the best without any disease incidence. A similar trend was noticed at 40 DAP also. At 60 DAP variety Sakthi which received antagonistic seed treatment recorded minimum wilt incidence.
15. In Pusa Ruby the AM fungus infection was higher in AM fungus treated and *P.aeruginosa* seed treated pots. These treatments gave a better plant vigour also. But the highest yield was reported in garlic 10 per cent drenched pots. The same trend was recorded in variety Sakthi but the plant vigour was better in garlic 10 per cent treatment and maximum yield was recorded in AM fungus inoculated pots.
16. In the variety pusa Ruby as the *P. aeruginosa* population and AM fungus population increased a slight decrease in pathogen population and wilt incidence were noticed, but corresponding yield increase was obtained only in AM fungus inoculation and *P. aeruginosa* treatments. Similarly in the variety

Sakthi pathogen population was minimum in *P. aeruginosa* and 10 per cent garlic treatment and reduction in wilt incidence in bioagent treated plots. The yield was markedly high in AM fungus infected pots. Based on the results of pot culture experiment the treatment S_1 (*P. aeruginosa* seed treated), S_2 (AM fungus inoculation) and S_0 (no seed treatment) were selected for nursery stage and T_1 (1 per cent Bordeaux mixture drenching), T_2 (10 per cent garlic drenching), T_3 (Solarised soil + Calcium oxide + urea), T_4 (Copper hydroxide 0.15 per cent drench) and T_0 (Control) were selected for treatment application at planting and at 30 DAP.

17. The bioassay of bactericides and botanicals revealed that Copper hydroxide at 0.15 per cent was equally good compared to all other treatments in inhibiting the growth of *R.solanacearum*. At the same time this treatment was not inhibited to *P.aeruginosa*.
18. In the main field trial at 30 DAP *R. solanacearum* population was minimum in *P. aeruginosa* seed treatment when integrated with Bordeaux mixture or garlic 10 per cent extract.
19. Rhizosphere microbial population at 60 DAP also did not show any significant difference between treatments. The *R. solanacearum* population was minimum in *P. aeruginosa* seed treatment when integrated with one per cent Bordeaux mixture The population of *P. aeruginosa* was maximum in control and copper fungicide treated plots and the AM fungs infection was highest in plots when AM fungus inoculation alone was given.
20. The percentage wilt incidence was recorded at 15, 30, 45, 60 and 75 DAP. At 15 DAP, *P. aeruginosa* seed treatment with garlic drench/Bordeaux mixture 1 per cent drenching were found to be the best treatments giving minimum wilt incidence.
21. At 30 DAP, *P. aeruginosa* seed treatment along with Copper hydroxide drenching was giving minimum wilt incidence, whereas in 45 DAP and 60 DAP, the same result as in 15 DAP, was giving minimum percentage wilt incidence. At 75 DAP, minimum wilt incidence was noticed in combination of *P. aeruginosa* seed treatment and garlic drench.
22. The AM fungus infection was maximum in AM fungus inoculated treatment. The plant height and root length were maximum in *P. aeruginosa* treated plot.

23. The treatments which received *P. aeruginosa* seed treatment along with Copper hydroxide drenching was found to be superior giving higher yield. But the wilt incidence though higher in control did not reflected in yield.
24. Percentage wilt incidence and populations of *R. solanacearum* and *P.aeruginosa* did not give a positive relation. But higher AM fungus infection in the control plot has reflected in the yield obtained.
25. The yield was maximum in plots which received combination of *P. aeruginosa* seed treatment and garlic drenching where the wilt incidence was minimum and the populations of *R. solanacearum*, *P. aeruginosa* and AM fungus infection moderate.

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INTEGRATED MANAGEMENT OF BACTERIAL WILT OF TOMATO CAUSED BY *Ralstonia solanacearum* (Smith) Yabuuchi *et al.*

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ABSTRACT OF THE THESIS

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ABSTRACT

Bacterial wilt of tomato caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* has continued to be a perennial problem due to its complex interaction with soil. Hence a study has been taken up to isolate a potent biocontrol organism and to develop a strategy by integrating the selected control methods so as to have a sustainable eco-friendly tomato production.

The pathogenic bacterium *R. solanacearum* was isolated from four different locations and the characterization studies revealed that the four isolates of the pathogen belong to *R. solanacearum* race1 biovars3. The antagonistic bacterium isolated from the central zone was capable of producing abundant bluish green diffusible fluorescent pigment and a non diffusible non fluorescent brown pigment in King's B medium. Based on the physiological and biochemical studies, this bacterium was tentatively identified as *Pseudomonas aeruginosa.*

Seed treatment with antagonistic bacterium facilitates earliness in germination and better plant vigour in both Pusa Ruby and Sakthi varieties compared to other treatments. Data on solarisation studies also showed that solarisation for 30 days has a weak effect in checking the *R. solanacearum* population.

In bioassay studies garlic 10 per cent and the Copper fungicides, Bordeaux mixture 1 per cent and Copper hydroxide 0.15 per cent were sensitive to *R.solanacearum* but insensitive to *P. aeruginosa.*

In pot culture studies, the seeds of the resistant variety Sakthi treated with beneficial agent, garlic drenching or Bordeaux mixture drenching were found to be superior to all other treatments in reducing the wilt incidence.

In the main field, treatment combinations were tried based on the results of pot culture experiments. The rate of increase in wilt incidence was highest in the early stages and at flowering stage. The plants withstand bacterial infection upto flowering stage because of the resistant mechanism present in the variety Sakthi. The result of the study indicated that the bacterial disease of tomato could be effectively managed by integrating indigenous antagonistic activity of *P. aeruginosa* and selective bactericides like garlic extract or Copper hydroxide 0.15 per cent along with the host resistance of variety Sakthi.