MAJOR DISEASES OF KACHOLAM [*Kaempferia galanga* L.] AND THEIR MANAGEMENT

By

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THESIS

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DECLARATION

I, Priya K (2002-11-15) hereby declare that this thesis entitled '**Major diseases of Kacholam [*Kaempferia galanga* L.] and their management** ' is a bonafied record of research work done by me during the course of research and this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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PRIYA K

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Introduction

1. INTRODUCTION

India is a varietal emporium of medicinal plants. The geographical location of Kerala is congenial for the cultivation of a majority of commercially important medicinal plants. The plant *Kaempferia galanga* L. popularly known as Kacholam is an important medicinal herb belonging to family Zingiberaceae. The genus *Kaempferia* is believed to have its origin in South East Asia, probably in Burma, and is distributed in the tropics, and subtropics of Asia and Africa (Holtum, 1950). Of the 55 species reported in the genus, only 10 are known to occur in India among which, *K. galanga* L. and *K. rotunda* L. are of economic importance.

Kacholam is an attractive rhizomatous herb and the economic part is the underground rhizome. The rhizomes possess stimulant, expectorant, diuretic and carminative properties. Kacholam can cure skin disorders, piles, oedema, fever, epilepsy, splenic disorders and asthma. They promote and improve digestion and can also cure inflammatory wounds. Apart from rhizomes, extract of whole plant is also used in some tonics (Brown, 1941; Aiyer and Kolammal, 1964).

Recently, it is noticed that this plant is affected by bacterial and fungal diseases, which cause severe damage to leaves and to rhizomes, thereby reducing yield from the crop, especially the oil yield.

Varma (1991) reported the incidence of leaf spot disease by *Colletotrichum gloeosporioides* from different locations in Kerala State. Dake and Manoj (1994) first reported the incidence of bacterial wilt by *Ralstonia solanacearum* (*Pseudomonas solanacearum*) from Kozhikode district of Kerala. Sarma and Kumar

(1997) also reported a bacterial isolate from *Kaempferia galanga* L. and identified it as biovar 2 of *Ralstonia solanacearum.*

The present study is aimed to identify the diseases of Kacholam occurring in Kerala as well as to evolve suitable effective management practices. There were reports on chemical control of diseases in Kacholam. Even though this ensures maximum and quick control in minimum possible time, they have adverse effect on yield and quality of oil. Certain chemicals affect the biosynthetic pathway of essential oils thereby reducing the quality of oil. The cultivation of this crop is to meet the requirement of essential oils and oleoresins in which besides quantity, quality also plays an important role. Sustainable agriculture necessitates the use of eco-friendly technologies for the production as well as protection of crops. So for disease management the use of eco-friendly measures like bioagents and organic amendments etc should be given more importance.

The effect of various organic amendments in reducing the severity of diseases caused by soil borne pathogens has been widely observed. Although several organic materials have been found useful in controlling diseases caused by fungi and nematodes, much work has not been carried out against diseases of bacterial origin.

So as to evolve an eco-friendly management of various diseases, the role of biocontrol agents, organic manures and composts are to be utilized well along with appreciable doses of chemicals. With due consideration of above facts, the study entitled " Major diseases of Kacholam [*Kaempferia galanga* L.] and their management ''was taken up with the following objectives.

1) Survey on various diseases of Kacholam in Ernakulam and Thrissur districts.
2) To study etiology and symptomatology of diseases of Kacholam.
3) Screening for host resistance of Kacholam genotypes against bacterial and fungal diseases.
4) Eco-friendly management of diseases, using antagonistic microorganisms, organic manures, composts and their effect on soil microflora

Review of Literature

2. REVIEW OF LITERATURE

Kacholam is an important medicinal plant grown in tropic and subtropics regions of India. Recently there has been increasing demand for cultivation of this plant to meet the requirement of oils, oleoresins and for use in ayurvedic preparations. Large-scale cultivation is carried out by farmers and firms as main crop and as intercrop in coconut garden. A perusal of literature revealed that no major studies are carried out for identification and control of diseases in this crop, even though, a few diseases, which includes both bacterial, and fungal diseases are reported.

2.1 PATHOGEN

2.1.1 Bacterial diseases

A perusal of literature did not reveal much work on bacterial disease of Kacholam. However the incidence of bacterial wilt of Kacholam caused by *Pseudomonas solanacearum* was first reported by Dake and Manoj (1994) from Kozhikode district of Kerala. Later on Sarma and Kumar (1997) also reported the same disease in *Kaempferia galanga* L. as caused by *Ralstonia solanacearum*.

Bacterial wilt was first reported from Italy in 1882 (Walker, 1952). Smith (1896) first described the disease and its causal agent and reported its occurrence in potato, tomato and brinjal. Later the disease appeared also in South Africa in 1914 on potato (Doidge, 1914). The disease was first reported from India on tomato by Hedayathullah and Saha (1941) from West Bengal. In India Mathew *et al*. (1979)

made the first authentic report of the disease as incited by *P. solanacearum* on ginger, which comes under the same family Zingiberaceae to which Kacholam belongs.

2.1.2 Fungal diseases

Varma (1991) first made the studies on fungal diseases of Kacholam. He reported one leaf spot disease caused by *Colletotrichum gloeosporioides* (Penz.) Sacc. from different locations in Kerala State. He also reported another disease, thread blight caused by *Rhizoctonia solani* Kuhn.

In India, Sydow (1913) first recorded the fruit rot of chilli at Madras presidency and reported the causal organism as *Vermicularia capsici* (Syn. *C. capsici* (Syd.) Butler and Bisby). Rai and Chohan (1966) reported that *C. capsici* was severe on chilli fruits in Punjab and it over wintered in plant debris in field and caused 10-30 per cent reduction in yield. Laxminarayana and Reddy (1976) explained the symptoms produced by *Colletotrichum gloeosporioides* on fruits of ivy gourd.

2.2 SYMTOMATOLOGY

2.2.1 Bacterial diseases

Dake and Manoj (1994) reported wilt symptoms in Kacholam in Kozhikode district of Kerala. Initial symptoms appeared as water soaked lesions on petioles and spread to all leaves resulting in death of plant. The rhizomes exuded ooze when cut opened. The rhizomes and roots of the affected plant exhibited varying degrees of rotting.

A bacterial wilt of ginger caused by *P.solanacearum* E.F.Smith was recorded from Queens land by Hayward *et al., (*1967) and has also been reported from Hawaii, Mauritius and Malaysia. The first symptoms of the disease are yellowing and wilting of lower leaves, which quickly spread upwards. In advanced stages the base of pseudostem becomes water soaked, readily breaking from the rhizome. The vascular tissue becomes dark brown or black. The cut pseudostem and rhizome give a white, milky exudates.

2.2.2 Fungal diseases

Only a single leaf spot disease has been reported from Kacholam as caused by *Colletotrichum gloeosporioides* (Varma, 1991). The initial symptoms on the leaves were small, irregular to circular pale green water soaked spots. The well developed spots were circular to oval or sometimes irregular in shape, 0.5-2.5 cm, holonecrotic region papery white with raised dark brown outer margin and a golden yellow halo. In severe infections, spots coalesced to affect major portion of leaf lamina giving a blighted appearance.

Subramanian *et al*. (1971) reported that the disease by *Colletotrichum capsici* first appeared when the chilli crop was in flowering stage. In severe cases, it infects the fruits and branches causing fruit rot and dieback respectively. Laxminarayana and Reddy (1976) explained the symptoms produced by *C. gloeosporioides* on fruits of ivygourd as water soaked areas with tissue disintegration and depression in the center of the spots with light brown margin. Varma (1991) reported anthracnose disease in *Piper longum*, caused by *C. gloeosporioides*. Viral symptoms appeared in the form of minute, chlorotic specks on the upper surface of leaves, soon the center of the spot became brown, with diffusing margin and a lemon yellow halo. Spots become

irregular in shape, measured 0.5-1.5 cm, adjacent spots coalesced, leaves turned yellow and defoliate.

2.3 PATHOGENICITY

2.3.1 Bacterial pathogen

To test the pathogenicity of *Ralstonia solanacearum*, several inoculation techniques have been attempted by earlier workers. Winstead and Kelman (1952) tested four different inoculation methods to test the pathogenicity of *R. solanacearum* strains infecting solanaceaous crops. Among the different methods used like stem puncturing, root injury, root dipping and soil drenching, they found stem puncturing to be most effective method. This method was later followed by several other workers (Hussain and Kelman, 1958; He *et al*., 1983 and Prior and Steva, 1990). Several workers also followed root inoculation (Khan *et al*., 1979; Swanepoel and Young, 1988; Paul, 1998 and James, 2001). Mathew (2001) and James (2001) found leaf clipping as effective method to inoculate chilli and tomato. Sambasivam (2003) reported, pseudostem injection as the best method for inoculation in ginger.

2.3.2 Fungal pathogens

The mechanisms by which *Colletotrichum* species penetrate plant surface are through different modes viz., through stomata, wounds, and by direct penetration of the cuticular barrier.

The importance and relevance of wounding is provided by Boher *et al*. (1983), who showed that intact stems of cassava were resistant to *C. gloeosporioides*.

Infection was achieved by inoculating the surface of stems, which had been pierced with a hot needle. Dickman and Patil (1986) showed that cutinase-deficient mutants of *C. gloeosporioides* were not pathogenic when placed on surfaces of intact papaya fruit, but when fruits were wounded normal lesions were produced.

2.4 IDENTIFICATION OF PATHOGENS

E.F. Smith described the bacterium as *Pseudomonas solanacearum.* Later Yabuuchi *et al*. (1992) transferred several species of the rRNA homology group II Pseudomonads, including *Pseudomonas solanacearum* to the genus *Burkholderia*. Sequencing of the 16S rRNA genes and poly phasic taxonomy revealed dichotomy among the species included in the genus *Burkholderia*. This phytogenetic dichotony had led to the new genus, *Ralstonia* (Yabuuchi *et al*., 1995).

2.4.1 Characterisation of the bacterial pathogen

2.4.1.1 Cultural and morphological characterisation

The first report on shape and size of *P. solanacearum* was made by Smith (1896) as non-spore forming, non-capsulate, gram negative, small rods with polar flagella. All strains of *R. solanacearum* so far reported were found to be Gram negative, motile and rod shaped (He *et al*., 1983; Swanepoel and Young, 1988; Paul, 1998). Variability in colony, morphology was described by Okabe (1949), while making a special reference to pathogenicity.

Khan *et al*. (1979) made a comparative study on the morphological and cultural characters of the isolates of *P. solanacearum* obtained from brinjal, chilli, tomato and potato in TZC medium. The colonies of the isolates from chilli and potato

were found fluidal, slightly convex, with slight pinkish center, whereas that from brinjal and tomato were highly fluidal, convex to flat with pink center.

Similar studies were also done by other workers (Paul, 1998; Mathew *et al*., 2000 and James, 2001). Sambasivam (2003) reported that isolates from ginger produced creamy white colours after 24 h and later by 48 h a light pink colour is developed at the center.

2.4.1.2 Biochemical characters

Determination of the gram reaction by staining is the essential first step in characterisation and classification of bacteria from any source. Suslow *et al*. (1982) developed a simple test, i.e. solubility in 3 per cent KOH solution and its result correlated very well with the staining reaction.

Catalase activity with *P. solanacearum* was detected by Kovacs in (1956). Based on arginine dihydrolase reaction, Thornley (1960) differentiated *Pseudomonas* from other gram-negative bacteria. He clearly distinguished those species, which are arginine dihydrolase positive (*P. aeruginosa* and *P. fluorescens*) and those unable to produce ammonia from arginine under anaerobic conditions (*P. syringae* and *P. solanacearum*). Based on the biochemical tests carried out by many workers, different isolates of *R. solanacearum* were identified as oxidase catalase, lipase positive, arginine dihydrolase negative (He *et al*., 1983; Swanepoel and Young, 1988; Prior and Steva, 1990; Jyothi, 1992; Paul, 1998; Mathew *et al*., 2000).

Hayward (1964) reported the production of levan (poly-fructose) by some fluorescent Pseudomonads and *P. solanacearum* did not produce levan. He also studied the characteristics of *P. solanacearum* and reported that slight gelatin

liquefaction took place on prolonged incubation. However, He *et al.* (1983) found that most of the strains of *P. solanacearum* from China produced levan. Similarly, the isolates obtained from, bringal, chilli and tomato produced levan in the peptone beef extract medium containing five per cent sucrose (Paul, 1998).

Liberation of hydrogen sulphide was observed by many workers (Devi, 1978; He *et al*., 1983; Jyothi, 1992). Samuel (1980) Nayar (1982) and Paul (1998), observed positive urease activity and levan production.

2.4.1.3 Race and biovar identification

The *Ralstonia solanacearum* isolates were characterised into different races based on their host reactions and grouping into biovars based on the utilisation of disaccharides and hexose alcohols.

The isolates were grouped into races based on the hypersensitivity reaction of bacterial isolates on capsicum as detailed by Lozano and Sequeira (1970).

Race 1: Dark brown necrosis by 36h with yellow zone around the edges, by 60h darkening of vein and veinlets, by 8 to 10 days systemic infection, within 20 days the plant die.
Race 2: by 10 to 12 h the leaf area will be water soaked with slightly yellowing, 60h the affected area becomes papery and will dry up.

Race 3 : Upto 48h symptomless, by 72h yellowing of infected area and limited lesion size.

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.

1. Solanaceous strain (Race – 1): Wide host range, distributed through- out the lowlands of tropics and subtropics
2. Musaceous strain (Race – 2): Restricted to Musa, and a few perennial hosts initially limited to American tropics, now spreading to Asia
3. Potato strain (Race – 3): Restricted to potato and a few alternative hosts in the tropics and subtropics
4. Ginger strain (Race – 4): From the Philippines
5. Mulberry strain (Race - 5): from China

Hayward (1964) classified the isolates of *P. solanacearum* from different host plants and different parts of the world into four biotypes based on the capacity to utilize disaccharides viz., lactose, maltose, and cellobiose and hexose alcohols viz., mannitol, sorbitol and dulcitol. Biotype I oxidised neither group, biotype 2 only disaccharides, biotype 3 both the groups and biotype 4 only hexose alcohols. Later He *et al*. (1983) identified certain isolates from China, which oxidised mannitol, but not sorbitol or dulcitol and designated them as biotype 5. Most of the *R. solanacearum* strains effecting solanaceous crops belong to biovar III (Hayward 1964; He *et al*., 1983; Prior and Steva, 1990; Paul, 1998; Mathew *et al*., 2000)

.

In Kerala Devi (1978) classified all the isolates obtained from tomato, brinjal and chillies as biovar IV. Jyothi (1992) characterised *P. solanacearum* from ginger also, apart from brinjal, chilli and tomato and reported that they belonged to biovar III.

The 12 isolates of *P. solanacearum* affecting solanaceous vegetables were collected from Himachal Pradesh and among these, chilli isolate was identified as biovar V, five of the six tomato isolates, one of the three brinjal isolates and potato isolate belonged to biovar III group. Other four isolates of brinjal and tomato differed from others in its inability to utilize/oxidise dulcitol and was considered as a sub-type in biovar III and designated as biovar III A (Kumar *et al*., 1993). Sarma and Kumar (1997) reported the bacterial isolate from *Kaempferia galanga* L. as biovar 2 of *R. solanacearum.* Paul (1998) identified certain tomato and chilli isolates as biovar III and those from brinjal as biovar V. Mathew *et al*. (2000) and Mathew (2001) reported biovar III, III A, and V infecting brinjal, chilli and tomato. James (2001) reported biovar III infecting tomato, brinjal and chilli and biovar III A infecting chilli and tomato. Mathew *et al*. (2002) identified biovar III and III A infecting ginger. Sambasivam (2003) also reported biovar III A infecting ginger.

2.4.1.4 Cross inoculation studies

Ishii and Aragaki (1963) reported the ginger isolates were capable of causing wilt on chilli. He *et al*. (1983) studied cross inoculation of strains from six major hosts, viz., brinjal, tomato, capsicum, potato, peanut and tobacco. Rapid wilting was noticed on eggplants on inoculation of all strains. Tomato was susceptible to all strains except that obtained from mulberry. Potato was also susceptible to most strains but the rapidity of wilting varied. Capsicum, peanut and tobacco showed marked differences in their reactions to specific strains.

Swanepoel and Young (1988) studied the pathogenicity of South African *P. solanacearum* strains on potato, tomato, tobacco, egg plant, capsicum, peanut, sunflowers and large thorn apple. Potato strains were virulent to all hosts except

sunflower, tobacco and peanut. Strains from tobacco and tomato were virulent to all the eight hosts.

In India Patel *et al*. (1952) and Hingorani *et al*. (1956) reported that the potato isolates could not infect tobacco and chilli plants. Devi (1978) observed that chilli strain caused high degree of wilting in tomato and brinjal and that of brinjal and tomato isolates were capable of cross infecting each other. Jyothi (1992) reported that the isolates of chilli caused high degree of wilting on ginger. Rani (1994) found that ginger isolates produced typical wilting in chilli, low incidence on tomato and no symptom on brinjal. Paul (1998) found that tomato, brinjal and chilli isolates were cross infectable producing symptoms at varying intensities. Similar results were also obtained by James (2001).

2.4.1.5 In vitro sensitivity of R.solanacearum to chemicals

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

Campacci *et al*. (1962) reported that among the various chemicals tested the bacterium was sensitive to Agristrep, Streptomycin, Penicillin, Streptomycine sulphate and Erythomycin. 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 a virulent, form of the pathogen were sensitive to Streptomycin. Gunawan (1989) found that optimum concentration for suppression of bacterial multiplication *in vitro* were 175 and 450 ppm of Streptomycin sulphate. Paul (1998) has reported that Oxytetracycline and Streptomycin sulphate gave good inhibition and suppression of growth of *Ralstonia solanacearum* from tomato.

Sambasivam (2003) observed that all the isolates of *R. solanacearum* were found to be resistant to Ampicillin and Rifampicin upto a concentration of 100 μm/ml. They were highly sensitive to Chlorampherical at 150 μg/ml and Kanamycin at 50μg/ml.

2.4.2 Fungal pathogen

2.4.2.1 Morphology of Colletotrichum spp.

Morphology of *Colletotrichum capsici* was studied by Chowdhury (1957) in chilli. The mycelium consist of septate, inter and intracellular hyphae. Acervuli and stroma on the stem are hemispherical and 7-120 microns in diameter. Setae are scattered, dark brown with light brown tip. They are several septate and upto 150 microns long. Conidiophores are septate and unbranched. Conidia in mass appear pinkish. Individually they are hyaline, unicellular, curved with narrow ends (fusaroid) and measure 17-28 x 3-4 microns.

Varma (1991) studied morphology of *C. gloeosporioides* on *Kaempferia galanga.* Acervuli was dark brown, globose, partially sunken in the epidermal layer, 70-100 µm diameter, setae dark brown with slightly swollen base, tapering to apex, 30-75 x 6-10 µm. Conidiophore plialidic unicellular, in groups, hyaline, cylindrical to fusiform, 9-14 x 3-5 µm. Conidia enteroblastic, cylindrical with obtuse ends, smooth walled with mucilaginous coating, hyaline, aseptate, 1-2 guttulate with granular protoplasm, 9-15 x 4-6 µm. He also observed anthracnose in *Piper longum* caused by *C. gloeosporioides*. Acervuli is scattered, circular, dark brown and measured 65-110 µm. Setae 32-80 x 5-7 µm, conidia hyaline, cylindrical to elliptic with both ends obtuse, base truncate, unicellular, 8-17 x 2-5 µm.

Davis (2003) studied the morphological characters of *Colletotrichum gloeosporioides* causing leaf spot in ivy gourd. Hyphae was branched, hyaline, with 3.8 m width and septate at an interval of 11.6 – 19.4 µ m. Conidia hyaline, cylindrical with both ends round, aseptate, oil globules present, 11.7- 15.6 µm x 3.9µm.

2.5 SCREENING OF GERMPLASM

2.5.1 **Bacterial pathogen**

Screening against bacterial wilt disease of solanaceous vegetables has been conducted by Peter *et al*. (1984), Gopalakrishnan and Peter (1991), Sadhankumar (1995) and AICVIP (2002). Out of 43 varieties/ lines, each of tomato, brinjal and chilli screened against *R.solanacearum,* only 6 tomato, 8 brinjal and 8 chilli lines were scored resistant (Paul, 1998).

2.5.2 Fungal pathogen

William (1977) screened 5000 cowpea lines against important bacterial and fungal diseases including *C. gloeosporioides* under field conditions and found that 16 cowpea lines showed multiple resistance to disease. Sohi and Rawal (1983) screened 141 cowpea varieties against *C. lindemuthuianum* and found 21 varieties as resistant to the pathogen.

Out of 34 turmeric accessions screened by Raigarh center all were found affected by *Colletotrichum* leaf spot diseases and only five were found to be moderately resistant. (AICRPS, 1999). Out of the 51 germploasm accessions screened, only cultivar Kohinur and G.L. Puram were found resistant to *C. capsici.*

Davis (2003) observed that out of the nineteen-ivygourd genotypes screened for host resistance against different leaf spot diseases, eight were found highly resistant, and two were moderately resistant.

2.6 CULTURE FILTRATES OF ANTAGONISTS ON PATHOGENS

2.6.1 Bacterial pathogen

Phae *et al*. (1992) observed the suppressiveness expressed by the culture filtrate of *Bacillus subtilis* against *R. solanacearum*, which was by the extracellular production of iturino. Manimala (2003) observed that among the bacterial culture filtrates tested, commercial culture of *B. subtilis* was found to be more effective followed by that isolated *B. subtilis* from Vellanikkara. With regard to fungal culture filtrates, better inhibitory effect on *R. solanacearum* was observed with *T. virens* and *T. pseudokoningii*.

2.6.2 Fungal pathogen

The treatment of the mycelial disc of the fungus with cultures filtrate of *P. fluorescens* for 15 min. time interval recorded maximum inhibitory effect with complete inhibition (100 per cent), on the mycelial growth of *Alternaria chlamydospora* showing 0.00 mm colony diameter as against 88.76 mm in the control, followed by culture filtrate of *T. viride*, which recorded 25.88 mm of the mycelial growth accounting for 70.10 per cent reduction over control (Vincent, 1927)

Dennis and Webster (1986) showed inhibitory action of culture filtrates of *Trichoderma* against *Macrophomina phaseolina*. Rajasab and Saraswathi (1999) observed that the sterilized culture filtrates of *A. niger* had no effect on *Sphacelotheca cruenta* and *Sporisorium sorghii* pathogens on sorghum. This suggest that the secondary metabolites produced by *A. niger* are heat sensitive and are involved in the suppression of pathogen activity under temperature range of 10 – 25^0 C and become inactive at higher temperature range (30^0 and above).

2.7 DISEASE MANAGEMENT

2.7.1 Biological control

2.7.1.1 Bacterial pathogen

Opina and Valdez (1987) studied the effect of *Pseudomonas fluorescens* and *Bacillus polymyxa* on bacterial wilt pathogen of tomato and brinjal. He observed that, in brinjal both organisms significantly reduced the incidence of wilt, when seedlings were dipped in the suspension than when the suspension is

drenched at the base of seedlings. He also noted that *P. fluorescens* showed better antagonism than *B. polymyxa*.

Anuradha and Gnanamanickam (1990) evaluated the strain Pf cp of *P. fluorescens* and strains B 33 and B 36 of *Bacillus* spp. against bacterial wilt of banana, egg plant and tomato under green house and field conditions. Protection upto 50, 61 and 91per cent and 50. 49 and 36 per cent were obtained for banana, eggplant, and tomato in green house and field respectively.

Suresh and Ravi (1992) observed inhibition zones when *P. purpurescens* was tested on *P. solanacearum*. Hanudin and Machmud (1994) showed the effect of *P. fluorescens* against *P. solanacearum*. Ciampi *et al*. (1996) noticed that, siderophore like compounds produced by isolate of *P. fluorescens* was responsible for the inhibition of *R. solanacearum* and also observed that, synthesis of the pigment is dependent on Fe^{3+} levels in the culture medium and the siderophores increased when iron concentration is limited. Silveira *et al*. (1996) evaluated a number of microorganisms including *Streptomyces griseochemogenus, S. griseus, P. fluorescens, T. pseudokoningii, P. aeruginosa, B. coagulens, B. megaterium* and *B. cereus* against *R. solanacerum* and were effective under *in vitro* condition.

Karuna *et al*. (1997) tried seed bacterization followed by root dipping the Pusa Ruby seedlings with *P. fluorescens, P. aeruginosa* and *B. subtilis* and found that *P. fluorescens* was most effective in reducing the incidence of wilt by 50 per cent under field condition.

Guijing *et al*. (1998) observed that conidial extracts of *Trichoderma koningii* B4-88 completely inhibited the growth of *R. solanacearum* and recovery rates of the antibiotic substance extracted by alcohol and NaOH were 0.89 per cent

and 0.82 per cent respectively. *Bacillus subtilis, Pseudomonas* spp. and *P. cepacia* had highly inhibitory effect against *R. solanacearum* on culture medium and significant reduction in wilting of tomato was also noticed in green house tests (Abdalla *et al*., 1999).

Anith *et al*. (2000) observed that, seed treatment with *P. fluorescens* strain EM 85 along with soil solarization decreased the wilt incidence in ginger to 7.42 per cent and increased the yield to 29.42 t/ha compared to 19.5 t/ha in control plots. Das *et al*. (2000) evaluated few established biocontrol agents for their inhibition action against *R. solanacearum*, using agar plate assays in dual culture. The bacterial antagonists *P. fluorescens* showed maximum inhibition of the pathogen (33.30 mm), followed by *Aspergillus terreus* (23.1 mm), *Trichoderma harzianum* (14.3 mm), *B. subtilis* (13.6 mm), *Gliochadium virens* (13.3 mm), *T. koningii* (12.00 mm) and *T. viride* (9.96 mm). Out of three best antagonists tested in tomato variety Pusa Ruby, *P. fluorescens* applied 14 days prior to inoculation showed the least disease incidence and highest yield of 12.81 q/ha.

Kumar and Sood (2001) were able to reduce bacterial wilt incidence of tomato to 65.9 and 71.6 per cent in 8 and 10 weeks solarized plots. They also observed the significant reduction in wilt incidence due to incorporation of antagonistic rhizobacteria (*P. fluorescens* and *B. cereus*) in soil prior to solarisation as the population of the antagonist was considerably enhanced after solarisation and also increased the vigour of the plants.

Manimala (2003) observed that among *Trichoderma* spp., *T. viride* and *T. pseudokoningii* were the promising ones by recording a maximum antagonism index value of 6000 and showed complete inhibition of all the six isolates of

R. solanacearum by its lysis and over growth type of antagonistic property. Among the *Aspergillus* spp., *A. niger* showed better antagonism against five isolates of the pathogen. Among the different bacterial isolates *P. aeruginosa* showed the maximum inhibitory effect against *R. solanacearum* with an AI value of 133.3 followed by the commercial *P. fluorescens* (AI value 111.1).

2.7.1.2 Fungal pathogen

Dennis and Webster (1971) assessed the effectiveness of volatile and non volatile produced by *T. viride* on two ginger rot pathogen. Volatile substances produced by *T. viride* seemed to be effective against *P. myriotylum* as the growth of this pathogen was completely inhibited by these substances (Rathore *et al.* 1990).

Gogoi and Roy (1996) reported that *Aspergillus teneus* reduced incidence of sheath blight caused by *Rhizoctonia solani* on rice cv. IR 50 in pot tests. PGPR treatments resulted in significant protection from naturally occurring anthracnose by *Colletotrichum orbiculare* in cucumber (Wei *et al*., 1991). The maximum growth of inhibition of pathogen was exerted by *S. cerevisae* followed by *T. viride, T. hamatum, T. pileatus* and *T. harzianum*. Among the bacterial antagonists, *B. subtilis*, followed by *P. fluorescens* exerted maximum inhibition on mycelial growth of *C. capsici* (Jeyalakshmi *et al*., 1998).

Kumar (1999) observed the effectiveness of *T. viride* against anthracnose disease of vegetable cowpea caused by *C. lindemuthianum*. Rajasab and Saraswathi (1999) observed that *Aspergillus niger* changed the colony colour in sorghum pathogens. *Sphacelotheca cruenta* from cream yellow to red and also suppressed formation of secondary sporidia.

The antagonists *T. harzianum* followed by *T. viride* was effective against *Alternaria solani* causing leaf blight disease in tomato (Babu *et al*., 2000).

Davis (2003) reported that *T. viride* and *T. harzianum*, followed by *A. niger* were effective against *Cercospora coccinae*, *Colletotrichum gloeosporioides* and *Alternaria alternata*, the leaf spot pathogens of ivygourd. *P. fluorescens* also was effective and recorded more than 50 per cent reduction on the growth of pathogens.

2.7.2 Composts in disease control

2.7.2.1 Bacterial pathogen

Composts and water extracts of composts are highly effective in many cases for the control of plant disease (Hoitink and Fahy, 1986; Zhang *et al*., 1998).

Many microorganisms, *Bacillus* spp., *Enterobacter* spp., *Flavobacterium balustinum*, *Pseudomonas* spp., *Streptomyces* spp. and other bacterial and fungal strains have been identified as biocontrol agents in composts (Chung and Hoitink, 1990).

Jayaprakash (1977) observed that a reduction in percentage of tomato wilted plants in pots amended with oil cakes, saw dust, cashew shell powder, coconut pith, oil palm seed waste and various crop residues. Root diseases of capsicum and black gram reduced significantly, by the application of decomposed coir pith (Mani and Marimuthu, 1994).

Coirpith can also be enriched with cultures of beneficial microorganisms, such as *Trichoderma*, *Azotobacter* and Phosphate solubilizer

(Moorthy and Rao, 1997), so that the compost could serve as a biofertilizer and biopesticide. Jyothi (1992) conducted a field experiment on the management of bacterial wilt of chillies and found that plants that receive cowdung as one of the major treatments recorded comparatively low percentage of wilt incidence.

2.7.2.2 Fungal pathogen

In the mature compost *Rhizoctonia solani* sclerotia are killed by *Trichoderma*, so that biological control prevails (Nelson *et al*., 1983). Coirpith reduced damping off caused by *Rhizoctonia solani* and *Fusarium equiseti*, when used in potting mixture for raising eucalyptus seedlings (Marimuthu and Nagarajan, 1993).

The effect of decomposed coir pith, fungicides and biocontrol agents on damping off of chillies and dry root rot of black gram were studied. Among this decomposed coirpith was comparable with *T. hamatum* and *T. viride* - 2 which recorded 16.3 and 11.5 per cent post emergence damping off in chillies, whereas decomposed coirpith alone and in combination with *T. harzianum*-2 increased per cent survival of plants (Mani and Marimuthu, 1994).

A biocontrol agent-fortified compost mix, suppressive to several diseases by soil borne pathogens, induced systemic acquired resistance (SAR) in cucumber against anthracnose caused by *Colletotrichum orbiculare* (Zhang *et al*., 1998). Neelamegam and Govindarajalu (2002) observed that *T. viride* alone or in combination with farmyard manure (FYM) were effective in controlling the damping off of tomato. The combined treatment of *T. viride* and FYM showed better result in controlling the disease with concomitant increase in seedling length and biomass of tomato.

2.7.3 Chemical control

2.7.3.1 Bacterial pathogen

Severin and Kupferberg (1977) reported that, Bordeaux mixture, copper oxychloride and Kocide were effective in controlling bacterial blight of walnut. Goorani *et al*. (1978) reported that Nabam (Dithane A-40), Maneb (Dithane H-22) and Dithane M-45 inhibited *P. solanacearum*. Grinepadeze *et al*. (1978) conducted *in vitro* trials with 21 fungicides against bacterial disease of mulberry caused by *Pseudomonas mori* and found that chinosol, zineb and polychomphenthiuram showed high inhibitory activity.

Bazzi and Calzolari (1986) found that industrial Bordeaux mixture and copper oxychloride were more effective than copper hydroxide and Kasugamycin for lettuce bacterial wilt by *P. cichorii*.

A study was conducted for testing the sensitivity of common antibiotics, fungicides, botanicals and others against *R. solanacearum* under *in vitro* and *in vivo* conditions. Of the two fungicides tested Bordeaux mixture one per cent gave maximum inhibition of the bacterium. Field experiment on the management of bacterial wilt of ginger revealed that none of the treatments gave absolute control of the disease. However, plots treated with Ambistryn-S and Bordeaux mixture had minimum wilt incidence than other treatments (KAU, 2001).

Inhibition of *R. solanacearum* by copper hydroxide (Kocide) 0.15 per cent was reported by Akbar (2002).

2.7.3.2 *Fungal pathogen*

Mali and Joi (1985) reported that difolatan, thiram and carboxin were most effective against colony growth and sporulation of *Alternaria alternata, C. capsici, Curvularia calavata* and *Macrophomina phaseolina*.

When 11 fungicides were tested against chilli fruit rot caused by *C. capsici* and *Alternaria solani*, Foltaf (0.2 per cent captafol) gave the most effective control followed by Fytolan (0.25 per cent) and Bavistin (0.1 per cent) (Jayasekhar *et al*., 1987).

During 1979-80 trial, Bordeaux mixture (0.8 per cent), Dithane 2-78 (0.3 per cent), Cuman-L (0.3 per cent) and Blitox (0.3 per cent) appeared next in order of efficacy against anthracnose while during 1980-81 season, the effect of Bordeaux mixture and Cumen-L was almost on par with each other (Thind and Jhooty,1987).

Davis (2003) reported that copper oxychloride and copper hydroxide were found to be effective both in pot culture and field experiment against leaf spot diseases of ivygourd.

2.8 TOTAL PHENOL

2.8.1 Bacterial pathogen

A wide range of chemicals possessing an aromatic ring bearing a hydroxyl substituent called phenolics substances show antifungal, antibacterial and antiviral activities. Phenolics in high concentrations are toxic to plant cell themselves (Tepper and Anderson, 1984). Hence phenolics will be normally present in small quantities only in plants and these quantities may not be sufficient to suppress the

development of pathogens. But in many plant pathogen interactions, the synthesis of phenolics is activated after infection and high amount of phenolics synthesized rapidly suppress the pathogen development (Vidyasekaran, 1990).

Protective role of phenolics against bacterial wilt disease was reported by many scientists (Rajan, 1985 and Sadhankumar, 1995) in tomato. Gopinath and Madalageri (1986) and Sadhankumar (1995) indicated a high significant correlation of phenol with resistance and suggested a possible role of phenols in the mechanism of wilt resistance in brinjal and tomato respectively. But Sitaramaiah and Sinha (1983) and Geetha (1989) were unable to correlate the total phenol content to resistance/susceptibility to bacterial wilt in brinjal. However, Kuc (1964) and Rajan (1985) observed a negative correlation between resistance and total phenol content in tomato and they suggested that the lower levels of phenolics in the roots of the resistant line might be due to the increased rate of oxidation of phenolics.

Rajan (1985) reported that after artificial inoculation, total phenols were higher in roots and shoots of susceptible variety of tomato, whereas OD phenol content was increased and remained at a higher level in resistant variety. Markose (1996) reported that the resistant variety Ujwala exhibited significant increase in total phenol content in roots.

Paul (1998) observed that a high total phenol content was noticed in the susceptible varieties of tomato compared to resistant genotypes under healthy condition. In brinjal and chilli a reverse trend was noticed.

2.8.2 Fungal pathogen

Phenolics play a key role in Fusarium wilt resistance in tomato. Tomato plants inoculated with *Fusarium oxysporum* f.sp. *lycopersici* synthesized increased amount, both of total and orthodihydroxy phenols. These compounds were synthesized rapidly in resistant than in susceptible plants. Peroxidase and polyphenol oxidase are capable of oxidising phenolic compounds and oxidized phenolics are more toxic to fungi. (Matta *et al*., 1967).

Materials and Methods

3. MATERIALS AND METHODS

The present study on 'Major diseases of Kacholam [*Kaempferia galanga* L.] and their management" was conducted in the Department of Plant Pathology and in the field of AICRP on Medicinal and Aromatic plants, College of Horticulture, Vellanikkara, Thrissur during the period from June 2003 to August 2004.

The details of the materials used and the techniques adopted for the investigation are described below.

3.1 SURVEY AND COLLECTION OF DISEASED SAMPLES

Survey was conducted in the farmer's field at Asamannur and Koothattukulam area and also at the Aromatic and Medicinal Plants Research Station at Odakkali in Ernakulam district and farmers field at Varikulam, Chuvannamannu, Pattikkad area and research field of AICRP on Medicinal and Aromatic plants at College of Horticulture, Vellanikkara of Thrissur district to study the occurrence of diseases of Kacholam. The incidence and severity of different diseases were recorded and samples were collected for experiment studies.

3.2 ISOLATION OF PATHOGENS ASSOCIATED WITH THE DISEASES

Plants showing different types of symptoms were collected separately from the surveyed areas and brought to the laboratory. Samples were then washed under tap water. The wilted plants were subjected to ooze test to confirm the presence of bacteria. The infected rhizomes were cut into small bits, surface sterilized and placed on a clean glass slide with a drop of sterile water. The bacterial ooze streaming out

from xylem vessels were streaked on Triphenyl Tetrazolium chloride (TZC)medium and incubated for 48h at room temperature.

Isolation of different pathogens associated with fungal foliage diseases were done by taking small bits of infected leaves along with some healthy areas, surface sterilized with one per cent sodium hypochlorite, and then washed in three changes of sterile distilled water and planted in Potato Dextrose Agar (PDA) medium. This was then incubated for 4-5 days under room temperature. The composition of different media used for the isolation is given in Appendix I.

3.2.1 Purification of pathogens

Circular, fluidal, slimy, white or creamy white colonies with light pink centers which characterize virulent colonies of *R. solanacearum* were selected after incubation at 30±2 ^{0}C for 24 to 48 h and were then purified by repeated streaking on the TZC medium. Permanent stock cultures of the bacterial pathogens were maintained in vials by preparing bacterial suspensions containing 2 to 3 loopful of pure culture of bacteria in 5ml of sterile distilled water. The suspensions were stored at 5^{0}C under refrigeration and also under room temperature. The stock cultures were also maintained in peptone casamino acid agar slants and stored at 5^{0}C under refrigeration. The cultures were tested periodically for virulence and purified by streaking on TZC medium.

The fungi grown on the PDA medium was purified by hyphal tip method and sub cultured and maintained on PDA slants, for further investigations.

3.3 PATHOGENICITY

3.3.1 Bacterial wilt pathogen

Plants with 4-5 leaves were planted in polybags containing sterilized soil. The inoculum having a concentration of 10^8 cfu/ml was obtained by collecting bacterial ooze from wilted plants in 100 ml of sterile water. The ooze was used as the standard inoculum. Four different methods viz., rhizome treatment, leaf axil application, leaf clipping and soil drenching were tried.

Rhizome treatment - Dipping the rhizome of healthy plants for 30 minutes in bacterial inoculum and then planting in the same polybag.

Leaf axil application - Applying bacterial inoculum using sterile cotton at the leaf axil area, after giving pinpricks.

Leaf clipping - Using scissors dipped in bacterial inoculum.

Soil drenching - About 20 ml of bacterial inoculum was poured into rhizosphere region of healthy plants.

Three plants were used for each treatment and plants inoculated with sterile water served as control. The inoculated plants were kept under humid chamber and observed daily for the appearance of wilt symptoms. The pathogen was re-isolated from artificially inoculated wilted plants and its characteristics compared with the original isolates of pathogen.

3.3.2 Fungal pathogen

Healthy leaves collected from field were washed under tap water and then surface sterilized with 70 per cent ethyl alcohol. The leaves were inoculated

separately on both sides with 6mm mycelial disc of fungal isolates and also by spraying spore suspension of pathogen having a concentration of 10^6 spores\ml using an atomizer, with and without giving pinprick. Leaves inoculated with sterile water served as control.

3.4 SYMPTOMATOLOGY

Symptoms produced by the different bacterial and fungal pathogens were studied in detail both under natural and artificial conditions.

3.5 HOST RANGE STUDIES

Host range studies were conducted by cross inoculating bacterial isolate on tomato, brinjal and chilli by root dip method. For each crop, ten inoculated plants and one control were maintained. The roots were dipped in bacterial inoculum for 30 minutes and were planted in polybags. Observations on wilt incidence were recorded.

3.6 HYPERSENSITIVITY REACTION

Hypersensitivity reaction of Capsicum to different isolates of *R.solanacearum* was studied. Thirty-day-old Capsicum plants grown in plastic cups were used. About 0.5 ml of standard bacterial inoculum was infiltered into the intercostal region on the under surface of the Capsicum leaves using plastic disposable syringe without needles. The inoculated plants were observed for symptoms on the infiltrated areas. The isolates were grouped into races based on the criteria of Lozano and Sequeira (1970), as detailed below.

Race 1: Dark brown necrosis by 36h with yellow zone around the edges, by 60h darkening of vein and veinlets, by 8 to 10 days systemic infection, within 20 days the plant die.

Race 2: By 10 to 12 h the leaf area will be water soaked with slight yellowing, the affected area becomes papery and dry up after 60h.

Race 3: Upto 48h symptomless, by 72h yellowing of infected area with limited lesion size.

3.7 IDENTIFICATION OF PATHOGENS

The pathogens associated with the diseases were identified based on cultural, morphological and biochemical characters.

3.7.1 Characterization of bacterial isolates

The two bacterial isolates collected from Vellanikkara and Odakkali were compared with a ginger isolate for characterization studies. Before each test a loopful of bacterial suspension from stock culture was transferred to TZC agar plates and incubated at room temperature for 24 to 48 h. The resulting bacterial growth was used for each study.

3.7.1.1 Cultural characters

3.7.1.1.1 Staining reaction

R. solanacearum comes under the group of Gram negative bacteria. The isolates were stained to confirm its negative reaction to gram staining.

3.7.1.1.2 Colony characteristics of bacterial isolates

Colony characteristics of the three bacterial isolates were studied in TZC medium. Dilute suspension of bacterial isolates was streaked on the medium and characteristics were studied after 24 to 48h of incubation.

3.7.1.2 Biochemical characterization

3.7.1.2.1 Catalase test

A few drops of three per cent hydrogen peroxide were placed at the center of the sterile glass slide and a loopful of bacterial inoculum was agitated in the solution. Formation of effervescence indicated the positive reaction (Cappucino and Sherman, 1992)

3.7.1.2.2 Starch hydrolysis

Nutrient agar containing 0.2 per cent soluble starch was employed for this test. The test isolates were spot inoculated on the medium poured in sterilized Petri plates. 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 , indicated positive starch hydrolysis

3.7.1.2.3 ***Arginine dihydrolase reaction***

For this test Thornley's semi solid medium (Appendix I) (Thornley, 1960) was used. About 5ml quantities of the medium were dispensed in 15ml capacity screw capped test tubes and sterilized in autoclave at 121^0C . The medium was

inoculated by stabbing with a loopful of bacterial isolate. The surface of the medium was sealed with 3ml molten one per cent agar cooled to 45^0C and the tubes were incubated for seven days at room temperature. Any colour change of the medium from to pink or red was recorded at regular intervals for a period of seven days.

3.7.1.2.4 Production of levan

Peptone beef extract medium (Appendix I) containing 5 per cent sucrose was used for this test. Dilute suspension of the bacterial isolates was streaked over the sterilized medium in Petri plates and growth characters were observed after 48h. Presence of large, white domed and mucoid colonies, characterized the production of levan from sucrose

3.7.1.2.5 Urease test

Christensens urea agar (Appendix I) (Christensen, 1946) was used in this test. Ninety ml aliquots of the medium were dispensed in 250ml 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 test tubes were inoculated with isolates and observations recorded periodically. A change in colour of medium from yellow to pink or red indicated urease production.

3.7.1.2.6 Production of hydrogen sulphide

The medium SIM agar with peptone and sodium thiosulphate (Appendix I) was used for testing the ability of the bacterial isolates to produce hydrogen sulphide. The medium was dispensed in 5ml quantities in test tubes and autoclaved. The tubes were inoculated by stab inoculation and observations were recorded periodically. A

change in colour of medium along with formation of black precipitate shows hydrogen sulphide production.

3.7.1.2.7 Utilization of disaccharides and hexose alcohols

The isolates of *R. solanacearum* were categorized into biovars based on their ability to utilize disaccharides (lactose, maltose and cellobiose) and hexose alcohols(mannitol, sorbitol and dulcitol) as a source of carbon and energy (Hayward 1964, He *et al*., 1983). Haywards semi solid medium (Appendix I) was used for this test (Hayward, 1964). After dissolving the constituents of the medium, the pH of the medium was adjusted to neutral to get olivaceous green colour. Then the medium was sterilized. Solutions of disaccharides and hexose alcohols were prepared by dissolving 1g each in 10ml of sterile water and were filter sterilized. This solution was added to 90ml basal medium and mixed well. About 5ml of this was taken in sterile screw capped tubes and allowed to set at room temperature. A control was maintained for each isolate without the addition of sugar solution, to the basal medium. Fifty micro litre of bacterial suspension of the isolates were prepared by dispensing single colony in sterilized water and was used for inoculation. The tubes were incubated at room temperature. Observations on change in colour of medium were recorded periodically for one month.

3.7.1.3 Sensitivity to antibiotics

Sensitivity of the two isolates of bacterium to three antibiotics was determined by paper disc assay method. The antibiotics used were Chloramphenicol, Ampicillin and streptomycin sulphate each at 100, 250 and 500ppm concentrations respectively.

Sterilized filter paper discs of 10mm diameter soaked in the antibiotics solution were placed aseptically on Peptone Casamino acid agar medium seeded with 24 h old culture of the bacterial isolates. Sterilized filter paper discs dipped in sterile water were kept as control. The tests were performed in triplicates. After incubation for 48h, diameter of the zones of inhibition of growth around the discs was measured.

3.7.2 Cultural and morphological characters of fungal pathogens

Cultural characters of the pathogens such as rate of growth, colour and formation of fruiting bodies in the PDA medium were studied.

Morphological characters of pathogens from the naturally infected plants as well as from the pure culture were studied. Size of hyphae, conidia, conidiophores and fruiting bodies of pathogens were recorded by using micrometry. These characters were compared with the descriptions of IMI for comparing the pathogens.

3.8 SCREENING OF GEOGRAPHICAL TYPES FOR HOST RESISTANCE AGAINST DISEASES OF KACHOLAM.

3.8.1 Reactions of different geographical types to bacterial wilt disease

Twelve geographical types of Kacholam available at the AICRP on Medicinal and Aromatic plants were evaluated for their relative resistance/ tolerance to bacterial wilt in a wilt sick field during July to December 2003. Twenty plants from each geographical types were planted in two rows of 10 each. Wilt incidence was recorded at monthly intervals and wilt percentage of each geographical type was calculated. The disease reactions were scored according to Mew and Ho (1976) as follows.

R - Resistant < 20 per cent plants wilted

MR- Moderately resistant > 20 to < 40 per cent plants wilted

MS- Moderately susceptible > 40 to < 60 per cent plants wilted

S – Susceptible > 60 per cent plants wilted

3.8.2 Reactions of different geographical types to leaf spot pathogens

Twelve geographical types of Kacholam available at the AICRP on Medicinal and Aromatic plants at College of Horticulture were screened for resistance to leaf spot diseases by recording per cent disease incidence and severity**.** Twenty plants from each geographical type were planted in two rows of 10 each. Observations were recorded on per cent disease incidence and per cent disease severity.

3.8.2.1 Assessment of disease severity

Disease severity was calculated by selecting twenty plants from a bed.

Score chart for leaf spot diseases

Grade	Description
0	No symptoms
1	<10 per cent leaf area infected
2	11 –25 per cent leaf area infected
3	26-50 per cent of leaf area infected
4	51-75 per cent of leaf area infected
5	> 75 per cent of leaf area infected

Per cent disease severity was calculated using the formula suggested by Wheeler (1969)

$$\text{Per cent disease severity (PDS)} = \frac{\text{Sum of all numerical ratings x 100}}{\text{Total number of leaves observed x maximum disease grade}}$$

From per cent disease incidence and per cent disease severity, Co-efficient of Infection (CI value) was calculated using the formula suggested by Datar and Mayer (1981).

$$\text{Co-efficient of Infection} = \frac{\text{Per cent disease incidence x Per cent disease severity}}{100}$$

Based on the CI value, the geographical types were categorised into five groups as shown below

CI value	Category
0-4	Highly resistant (HR)
4.1-9	Resistant (R)
9.1-19	Moderately resistant (MR)
19.1-39	Moderately susceptible (MS)
39.1-69	Susceptible (S)
69.1-100	Highly susceptible (HS)

3.9 *In vitro* EVALUATION OF BIOAGENTS AND FUNGICIDES AGAINST PATHOGENS

The effectiveness of antagonistic organisms and fungicides against bacterial wilt pathogens and leaf spot pathogens were tested under laboratory conditions. The efficiency of antagonistic organisms was evaluated using dual culture method as suggested by Skidmore and Dickinson (1976). The fungicides against fungal pathogens were evaluated by Poison- Food Technique (Zentmeyer, 1955) and paper disc assay method against bacterial pathogens. The commercial preparations of *T. viride, A. niger* and *P. fluorescens* were used for *in vitro* evaluation

3.9.1 *In vitro* evaluation of *P. flourescens* against *R.solanacearum*

The following methods of inoculation were adopted for testing the effect of *P.fluorescens* against *R.solanacearum*. Both the antagonist and the pathogen were inoculated on the same day. Three replications were kept.

3.9.1.1 Cross streaking method

Both the test and indicator organism were streaked perpendicular to each other on the plates having nutrient agar medium. The plates were observed daily for the lysis at the juncture of the pathogen and the antagonists.

3.9.1.2 Point inoculation of the antagonistic organism

A loopful of antagonistic organism was spotted at the center of the plates seeded with test organism. Plates were observed upto 48 h and diameter of inhibition zone was recorded.

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

The following methods of inoculation were adopted for testing the effect of fungal antagonists against bacterial pathogen. Three replications were maintained.

3.9.2.1 Streaking on both sides

A mycelial disc of antagonists of 6mm size was inoculated at the centre of Petri dish, with PDA medium.. The bacterial pathogen was inoculated as a line of streak on either side of the pathogen on the same day.

3.9.2.2 Streaking on one side

A mycelial disc of antagonists of 6mm size was inoculated at the centre of one half of Petri dish. On the centre of other half of same Petri dish, the bacterial pathogen was streaked.

The Petri dishes were incubated at room temperature and observations on growth of antagonists were recorded upto when the growth in the control plates fully covered the medium.

The per cent inhibition of growth over control was calculated by the formula suggested by Vincent (1927).

$$\text{Per cent inhibition of growth} = \frac{C\text{-}T \times 100}{C}$$

C = Growth of fungus in control (mm)

T = Growth of fungus in treatment (mm)

3.9.3 *In vitro* evaluation of *P. fluorescens* against fungal pathogens

The following methods of inoculation were adopted for testing the effect of *P. fluorescens* against fungal pathogens.

3.9.3.1 Streaking on both sides

A mycelial disc of pathogen of 6mm size was inoculated at the center of Petri dish with PDA medium. The bacterial isolate was inoculated as a line of streak on either side of pathogen on the same day.

3.9.3.2 Streaking on one side

A mycelial disc of pathogen of 6mm size was inoculated in the center of half of Petri dish with PDA medium. The bacterial isolate was inoculated as a line of streak at the center of other half of Petri dish. The Petri dishes were incubated at room temperature and observations on growth of pathogen were taken at regular intervals. The per cent inhibition of mycelial growth of pathogen over control was calculated as mentioned in 3.9.2.2

3.9.4 *In vitro* evaluation of fungal antagonists against fungal pathogens

A mycelial disc of six mm diameter was cut from actively growing culture of the fungal pathogens and placed in the center of one half of the Petri dish with PDA medium. The fungal antagonist was similarly transferred and placed at the center of the other half of the same Petri dish on the same day. Five replications were maintained for each and the pathogen and antagonists grown as monoculture served as control. The growth measurements were taken at regular intervals after 24h of inoculation. The per cent

inhibition of mycelial growth of pathogen over control was calculated as mentioned in 3.9.2.2

A modified method *i.e.* antagonistic index (AI) was suggested by Kasinathan (1998) for evaluating the efficiency of antagonists. For this four criteria viz., per cent inhibition of the pathogen (PI), colonization behaviour of the antagonist on the pathogen (CB), speed of overgrowth on the pathogen (SOOP) and width of inhibition zone (IZ) were taken into consideration. Thus the antagonistic index is calculated which is the product of PI, CB, SOOP and IZ.

AI= PI x CB x SOOP x IZ

The various criteria for arriving at antagonistic index were worked out as follows.

Score chart of colonization behaviour of antagonist on pathogen, Bell *et al* (1982)

Antagonism score	Description
1	Pathogen partially/completely overgrew the antagonist strain or colonized one third of medium surface and antagonist covered one third of medium surface
2	Pathogen/antagonist colonized one half of the medium surface and neither dominated each other
3	Initiation of overgrowth of antagonist on pathogen
4	Overgrowth of antagonist on pathogen upto two third of the medium surface
5	Complete overgrowth on pathogen and covered the entire medium surface

Score chart of speed of overgrowth on pathogen

Score	Description
1	Pathogen overgrown on antagonist strains
2	Neither antagonist /pathogen overgrew on each other
3	Antagonist completely overgrew on pathogen on pathogen after 48h
4	Antagonist completely overgrew on pathogen within 24 - 48h

The mean score of a given comparison falling between classes 3-4 were antagonistic. If score falls between 1-2, then they were not antagonistic.

Score chart for inhibition zone

Score	Inhibition Zone (IZ)
1	No IZ
2	1.0-2.5 mm
3	2.6-5 mm
4	>5 mm

For isolates, which do not produce any inhibition zone, a weighted value of one was given uniformly.

3.10 EFFECT OF VOLATILE METABOLITES PRODUCED BY FUNGAL ANTAGONISTS ON LEAF SPOT PATHOGENS

To study the effect of volatile metabolites released by the fungal antagonists (*Trichoderma viride* and *Aspergillus niger*) against leaf spot pathogens, discs of 6mm diameter were excised with a cork borer from the leading edge of an actively growing culture of the pathogen and were placed at the center in Petri dishes. Discs from the each

actively growing antagonists were excised and were placed similarly in another Petri dish with PDA medium. The plates containing the pathogens were placed over the plates containing the antagonists, after removing the lids of the plates. The two plates were held together by adhesive tapes. For each pathogen, three replicate plates for each antagonist were maintained. The control plates include the pathogen paired with uninoculated plates with PDA medium alone. The plates were incubated and diameter of growth of colony of the pathogens was recorded upto when the control plates were fully covered with fungal growth.

3.11 In vitro EVALUATION OF CULTURE FILTRATES OF ANTAGONISTS AGAINST ISOLATES OF *R. solanacearum*

The effect of culture filtrates of the bacterial antagonist *(P. fluorescens*) and fungal antagonists (*Trichoderma viride* and *A. niger*) were tested against the two isolates of *R.solanacearum* by filter paper disc method. A loopful of bacterial antagonist from 48h old culture was inoculated into Nutrient Agar broth, and incubated for four days. Similarly a six mm size disc was cut from four day old culture of potential fungal antagonists and placed in 100ml conical flasks containing 25 ml Potato Dextrose broth and incubated for seven days at the room temperature. The culture filtrates were filtered twice through double layered filter paper and was sterilized. Filter paper discs of five mm diameter were autoclaved, dried and soaked in the culture filtrate, drained the excess filtrate and placed on the medium seeded with *R. solanacearum* at two centimeter from the periphery of the plate. Two discs were placed in a single plate and the disc dipped in sterile distilled water served as control. Three replications were kept. After incubation of 24 and 48h, the inhibition zone around the filter paper disc was measured.

For *in vitro* evaluation of culture filtrates against the fungal pathogens, 100 ml of PDA was taken in 250ml conical flask and sterilized at 1.05 kg/cm^2 pressure for 20

minutes. One ml of sterilized culture filtrates were mixed with the media and poured into sterilized Petri dishes@ 20ml per plate. Mycelial discs of 6mm diameter were cut from actively growing seven day old culture of fungus placed at the center of each Petri dish containing medium with filtrates. Three replications were maintained for each treatment.. Media without culture filtrates served as control. The inoculated Petri dishes were incubated at 28± 2^0c. The diameter of fungal colony was recorded upto and when growth in the control plates fully covered the medium. The per cent inhibition of mycelial growth of pathogen over control was calculated as mentioned in 3.9.2.2

3.12 In vitro EVALUATION OF FUNGICIDES AGAINST BACTERIAL AND FUNGAL PATHOGENS.

For *in vitro* evaluation of three copper fungicides namely, copper hydroxide (Kocide101 77WP), Copper oxychloride (Fytolan 50 WDP) each at 0.1,0.2 and 0.3 and Bordeaux mixture at 0.5,1.0 and1.5 per cent concentrations, against *R. solanacearum,* the bacterial isolates were seeded on Nutrient Agar medium (Appendix I). Two sterilized paper discs dipped in the fungicides were placed at equidistant in each Petri plate. Three replications were maintained for each concentration of fungicides. Observations on zone of inhibition were recorded 48h after inoculation. Paper discs dipped in sterile water served as control.

For *in vitro* evaluation of fungicides namely, copper hydroxide (Kocide 101 77WP), Copper oxychloride (Fytolan 50 WDP) each at 0.1,0.2 and 0.3 and Bordeaux mixture at 0.5,1.0 and 1.5 per cent concentrations against the fungal pathogens, 100 ml of PDA was taken in 250ml conical flask and sterilized at 1.05 kg/cm^2 pressure for 20 minutes. Required quantity of chemicals were mixed with the media to get desired concentrations and poured into sterilized Petri dishes@ 25ml per plate. Mycelial discs of 6mm diameter were cut from actively growing seven day old culture of both the fungi

and placed at the center of each Petri dish containing poisoned medium. Three replications were maintained for each treatment.. Media without fungicide served as control. The inoculated Petri dishes were incubated at 28±2^{0}c. The diameter of fungal colony was recorded upto and when growth in the control plates fully covered the medium. The per cent inhibition of mycelial growth of pathogen over control was calculated as mentioned in 3.9.2.2

3.13 COMPATIBILITY OF ANTAGONISTS AGAINST COPPER FUNGICIDES

The *in vitro* compatibility of antagonists *(T. viride, A. niger and P. fluorescens)* to fungicides namely, copper hydroxide (Kocide101 77WP), Copper oxychloride (Fytolan 50 WDP) each at 0.1,0.2 and 0.3 and Bordeaux mixture at 0.5,1.0 and 2.0 per cent concentrations, were studied by Poison Food Technique.

To study the compatibility of *P. fluorescens* against all the three fungicides, the bacterium was seeded on Nutrient Agar medium. Two sterilized paper discs dipped in the fungicides were placed at equidistant in each Petri plate. Three replications were maintained for each concentration of fungicides. Observations on inhibition zone were recorded 48h after inoculation. Paper discs dipped in sterile water served as control.

The fungal antagonists used were *Trichoderma viride* and *Aspergillus niger*. the quantity of fungicides needed to get the desired concentration was added to 100ml sterilized, molten PDA medium, mixed well and poured into sterilized Petri dishes at the rate of 20 ml per plate. After solidification of the medium, mycelial discs of six mm diameter from actively growing antagonists were cut and placed at the center of each Petri dish. Control consisted of PDA medium alone inoculated with the antagonist. Three replications were maintained for each concentration of the chemicals. The inoculated Petri dishes were incubated at room temperature and the observations on the growth of the antagonists were taken when the control dishes showed full growth. The per cent

inhibition of mycelial growth of antagonists was also calculated using the formula given in 3.9.2.2

3.14 FIELD EVALUATION ON MANAGEMENT OF BACTERIAL WILT DISEASE

The three antagonists tested in the laboratory were evaluated in combination with different composts, in order to find out the effectiveness of the antagonists and composts in reducing the severity of bacterial wilt disease under natural conditions. A field experiment was laid out in the wilt sick field of the AICRP on Medicinal and Aromatic plants, College of Horticulture, Vellanikkara. The details of the field experiment were as follows.

Design - RBD

Treatments - 13 (60 plants\ treatment)

Replications- 3

Plot size- 2mx1m

Variety- Peechi

T.No.	Treatments
T1	Coirpith compost + *T. viride*
T2	Coirpith compost + *P.fluorescens*
T3	Coirpith compost + *A. niger*
T4	Coirpith compost alone
T5	Vermicompost + *T. viride*
T6	Vermicompost + *P.fluorescens*
T7	Vermicompost + *A. niger*
T8	Vermicompost alone
T9	Farmyard manure + *T. viride*
T10	Farmyard manure + *P. fluorescens*
T11	Farmyard manure + *A. niger*
T12	Bordeaux mixture (1 per cent)
T13	Control

Land was ploughed thoroughly and beds of 2 x 1m sizes were prepared. Finger rhizomes were treated with antagonists by soaking them in antagonists solutions for 30 minutes and planted at the rate of two fingers per pit. Farmyard manure or coir pith compost or vermicompost @ (20t ha^{-1}) were applied in each pit and were mixed with soil before planting of rhizome bits.

The antagonists were given at the recommended dosages,10^6 spores per ml for fungal antagonists, 10^8 cfu per ml for bacterial antagonists and Bordeaux mixture (1 per cent) as three applications first as rhizome treatment during July at the planting, second and third applications as soil drenching (100ml per plant) during September and November.

3.15 BIOMETRIC OBSERVATIONS

Observations were recorded on disease incidence and severity for leaf spot pathogens from randomly selected labelled plants and per cent wilt incidence per bed for bacterial wilt pathogen. Various biometric observations were also recorded.

3.15.1 Germination percentage

Total number of plants germinated was recorded at weekly intervals for a period of 45 days after planting (DAP) and the germination percentage calculated.

3.15.2 Number of leaves produced per plant

Total number of leaves produced per plant was recorded at monthly intervals for a period of three months.

3.15.3 Fresh and dry weight of leaves

Fresh and dry weight of leaves was recorded from the samples taken 45 and 90 DAP.

3.15.4 Fresh and dry weight of rhizomes

Fresh and dry weights of rhizomes were recorded from the three plant samples taken on 45 and 90 DAP and also for the healthy and diseased plants separately, at the time of harvest

3.16 CHEMICAL ANALYSIS

3.16.1 Estimation of total phenols

Alcoholic extracts of rhizomes were prepared by homogenising 0.2 grams o rhizomes in a mortar and pestle with 10 times volume of 80 per cent ethanol. Th homogenized material was centrifuged at 10,000 rpm for 20 minutes.

Total phenols was estimated by Folins Ciocalteu method (Mahadevan and Sridhar, 1982). The intensity of colour developed was read at 650 nm in a spectrophotometer. The total phenol content was calculated from a standard curve of catechol and was expressed as mg g^{-1} of fresh weight of sample.

3.16.2 Essential oil in rhizomes

The essential oil in dried rhizomes of sample plants from different treatments was estimated by steam distillation, adopting Clevenger trap method as per AOAC (1965) and expressed in percentage.

3.16.2.1 *In vitro evaluation of essential oils against pathogens*

Oils were extracted from the rhizomes of Kacholam by hydro distillation using Clevenger's apparatus. The oils thus obtained were evaluated at 0.2, 0.5 and 1 per cent concentrations by poisoned food technique against bacterial and fungal pathogens.

3.17 ESTIMATION OF MICROBIAL POPULATION

For understanding the effect of biological factors on incidence of bacterial wilt disease and on imparting bacterial wilt resistance in Kacholam, the rhizosphere populations of *Trichoderma, Aspergillus,* Actinomycetes, virulent *R. solanacearum,* fluorescent pseudomonads and *Azospirillum* were estimated both in healthy plants as well as in the diseased plants for each treatment using dilution plate technique. The dilutions used were 10^{-3} for *Aspergillus niger, Trichoderma viride*, and *Azospirillum* colonies and 10^{-4} for fluorescent pseudomonads*, Ralstonia solanacearum* and actinomycetes.

The media used were Martins Rose Bengal Streptomycine Agar for *Trichoderma*, Potato Dextrose Agar for *Aspergillus niger*, TZC medium for *Ralstonia solanacearum,* Kenknights medium for actinomycetes, Okons medium for *Azospirillum,* and Kings B medium for *Pseudomonas fluorescens*. (Appendix I).

3.18 WILT INCIDENCE

Wilt incidence were recorded at 1, 2, 3 and 4 MAP.

3.19 DISEASE SEVERITY AND INTENSITY OF LEAF SPOT DISEASES

The disease incidence and severity was recorded 3 MAP.

3.20 STATISTICAL ANALYSIS

Analysis of variance was performed on the data collected in various experiments using the statistical package MSTATC (Freed, 1986). Multiple comparisons among treatment means were done using DMRT. Correlation studies were also carried out, to find out the influence of soil microbial population on yield and per centage of wilt incidence.

Results

4. RESULTS

Investigations on various aspects of major diseases of Kacholam such as etiology, symptomatology, and development of ecofriendly management strategy, were carried out and the results were presented below.

4.1 SURVEY ON OCCURRENCE OF DISEASES OF KACHOLAM

Results of the survey conducted on the occurrence of diseases at farmers' fields and research station of Ernakulam and Thrissur districts are furnished in Table 1. A wilt disease of Kacholam of bacterial origin was noticed at AMPRS research station Odakkali, at farmers field Chuvannamannu and at the AICRP on Medicinal and Aromatic plants at Vellanikkara. The wilt incidence recorded were 19,25 and 18 per cent at Odakkali, Chuvannamannu and Vellanikkara respectively. Leaf spot and leaf blight diseases were noticed at farmers' fields at Asamannur and Koothattukulam with the per cent disease severity of 7.99 and 5.5 respectively. In Thrissur district, leaf spot disease with 4.17 per cent disease severity was observed at farmers field at Varikkulam, where as no diseases was recorded from the farmers fields at Pattikad. In the AICRP on Medicinal and Aromatic plants at Vellanikkara, leaf spot was observed having a disease severity of 3.1 per cent.

4.2 ISOLATION OF PATHOGENS ASSOCIATED WITH DIFFERENT DISEASES

The diseased specimen with typical symptoms produced by the pathogens were collected from the farmers field at Asamannur and Koothattukulam and also at the Aromatic and Medicinal Plants Research Station, at Odakkali in Ernakulam District. The diseased specimens were also collected from the farmer's field at

Table 1. Intensity and severity of diseases of Kacholam in various locations

Sl no	Locations	Disease	Per cent disease incidence (PDI)	Per cent disease severity (PDS)	Wilt percentage
Ernakulam district					
1	AMPRS (Odakkali)	Wilt	-	-	19.09
2	FF - Asamannur	Leaf spot	25	7.99	-
3	FF - (Koothattukulam)	Blight	16	5.5	
Thrissur district					
1	FF - Varikulam	Leaf spot	35	4.17	-
2	FF - Pattikkad	-	-	-	-
3	FF - Chuvvannamannu	Wilt	-	-	25
4	AICRP - Vellanikkara	Wilt & Leaf spot	24	3.12	18

FF- Farmers field
PDI - per cent disease incidence
PDS - Per cent disease severity

Varikulam, Pattikad and Chuvannamannu and also from the AICRP on Medicinal and Aromatic plants at Vellanikkara, in Trichur district.

4.2.1 Isolation of pathogens associated with wilt diseases

Kacholam plants showing typical wilt/ rotting symptoms were collected from two locations viz., Vellanikkara and Odakkali.

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

4.2.2. Isolation of pathogens associated with fungal diseases

For the isolation of pathogens causing diseases, specimens were collected from different locations. The isolation yielded two types of fungal colonies on PDA medium and was found belonging to *Colletotrichum* spp. All the fungi were identified upto species level based on cultural and morphological characters described later in this chapter.

4.3 PATHOGENICITY

The bacterial isolates obtained from the two locations were inoculated on healthy plants by rhizome dip method. Kacholam plants wilted in ten to twelve days after inoculation. The bacteria were re-isolated from the wilted plants and the colonies showed typical characters on TZC medium.

On artificial inoculation of two different fungal pathogens on leaves, typical oval spots with an yellow halo were developed in the case of pathogen isolated from specimens collected from Odakkali within four to five days of inoculation. The spots later on coalesced and developed into blights within a period of 8-9 days after inoculation. In case of the pathogen isolated from specimens collected from Koothattukulam, small yellow water soaked irregular small specks were seen initially within three days of inoculation. The specks later increased in size turned to dark brown and resulted in blightening of whole leaves within 6-7 days. Re-isolation of the pathogen from the infected portions showed typical original type of fungal colonies on PDA medium.

4.4 METHODS OF INOCULATION

Among the four different inoculation methods tested for the bacterial pathogen, only rhizome dip method was found to be effective. Wilting started 10 days after inoculation and all the plants produced wilt symptoms within a period of 11-15days. Other methods viz. leaf axil application, soil drenching and leaf clipping failed to produce wilt symptoms.

Three different methods of inoculation were tried for fungal pathogens. In case of pathogen isolated from specimens collected from Odakkali, all the inoculation methods were able to produce typical oval spots with an yellow halo. The spots later coalesced resulting in the blightening of the leaves.

In case of the other fungal pathogen isolated from specimens collected from Koothattukulam spraying spore solution after giving pin pricks alone could produce typical water soaked yellow specks which later produced blightening symptoms.

4.5 SYMPTOMATOLOGY

Symptomatology of three different diseases caused by the different pathogens *viz*., *Ralstonia solanacearum, Colletotricum gloeoporioides* and *Colletotrichum capsici* collected from different locations were studied under natural infection and under artificial inoculation. Type of symptoms varied depending on pathogens, but no variation in symptoms was noticed between different locations (Plate. 1)

4.5.1 Symptomatology of bacterial wilt disease

Disease usually appears when the crop was in the 3-4-leaf stage (1 month old). External symptoms produced by the pathogen, includes yellowing and rotting of leaves, which starts from the basal portion of the leaf and extends upward. The leaves appeared water soaked. This rotting symptoms starts from lower leaves and proceeds to younger leaves. Rotting was noticed only in wet weather, when there was high soil moisture. Variation in symptom expressions was noticed as soil moisture decreases.

During dry season or in dry areas, the leaves were seen rolled inwards with blightening symptoms along the margin of leaves, which proceeds towards the midrib. Finally the plants wilt completely leading to the death of plant. Rotting of rhizomes was also noticed, but it was confirmed to the surface of the rhizome. Inner flesh was not at all affected during the initial stages. But as the crop matures, the diseased rhizomes were found to be crinkled.

Under artificial inoculation it was found that symptoms produced by the pathogens were almost similar to those produced in natural conditions with only slight variations. The plants were planted in sterile soil. Instead of rotting only wilting symptoms were seen. Initially one or two leaves started wilting, and leaves were

found to roll inwards. On later stage whole plant wilts. Sudden wilting of plants as in case of natural infection was not observed.

4.5.2 Symtomatology of leaf spot caused by *C. gloeosporioides*

Symptoms started as small irregular sunken water soaked lesions of size 0.5-3cm, with definite golden yellow halo. Center of lesions were brownish initially and lesions were mostly seen along the margin of the leaves. Lesions spread rapidly leading to complete blightening of leaves.

Under artificial conditions small water soaked yellow specks were formed. The specks later advanced, turned dark brown resulting in blightening of whole leaf.

4.5.3 *Symtomatology of leaf spot caused by* C. capsici

Infection started as small oval light brown sunken spots of size 0.5-1.25 cm. No distinct yellow halo was noticed. As the spots mature black acervuli were seen in concentric circles in the center of the spots. Many spots coalesce to form blight. On advanced stages central necrotic region of these spots dried, and were blown away resulting in shot hole symptom. Infection spreads rapidly during wet season producing 5 to 10 spots on a single leaf.

Under artificial conditions, definite water soaked spots were formed with light brown center and yellow halo. No black coloured concentric sporulation was observed. The yellow halo later advances resulting in blightening of whole leaf

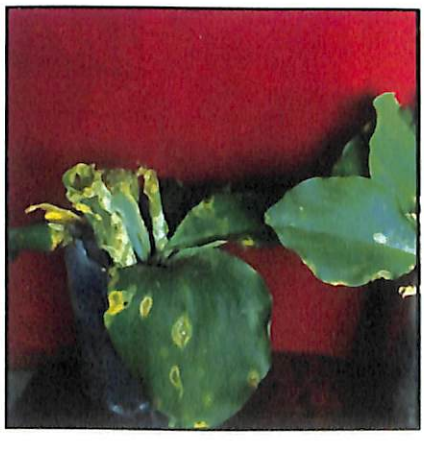
A. Bacterial wilt

Infection on leaves

Infection on rhizomes

B. Leaf blight

Colletotrichum gloeosporioides



Colletotrichum capsici

Plate 1. Symptomatology of diseases of Kacholam

4.6 HOST RANGE STUDIES

In order to study the host range, the bacterial isolates from Kacholam were inoculated on solanaceous plants. From the results it was evident that the isolates produced wilt symptoms on tomato within 5-6 days recording 100 per cent wilt incidence. In the case of brinjal, it took more days, 10-14 days for the appearance of wilt symptoms with 80 per cent wilt incidence. However, no wilt symptoms were noticed in chilli even after 30 days of inoculation.

Table 2. Host range studies of bacterial isolates

Sl No	Host plant tested	Incubation Period (Days)	Per cent wilt index
1	Tomato	5 - 6	100
2	Brinjal	10 – 14	80
3	Chilli	-	-

4.6 HYPERSENSITIVITY REACTION OF THE ISOLATES

Dark brown necrosis by 36h with yellow zone around the edges, by 60h darkening of vein and veinlets, by 8 to 10 days systemic infection, within 20 days the plant die. Based on the above criteria it was found that the isolates belonged to race 1.

4.7 IDENTIFICATION OF PATHOGEN ASSOCIATED WITH WILTING OF PLANTS

Cultural, morphological and biochemical characters of the three isolates of bacterial pathogen were studied.

4.8.1 Cultural Characters

4.8.1.1 Staining reaction

The isolates of the pathogen from two different locations were found to be gram negative, short rods.

4.8.1.2 Colony characteristics of the three bacterial isolates on TZC medium.

The colony characters of the three bacterial isolates on TZC medium were compared 48h after inoculation. All the isolates produced circular, smooth, raised, creamy white colonies with light pink center and with entire margin. The isolates from Vellanikkara produced abundant growth, fluidity and slime compared to the other two isolates. (Table 3).

4.8.2 Biochemical Characterization

Biochemical characters of the three isolates of bacterial pathogen were studied and presented in Table 4.

4.8.2.1 Production of levan

All the three isolates produced large white doomed and mucoid colonies indicating high levan production in peptone beef extract medium with five per cent sucrose.

Table 3. Comparison of colony characters of the isolates on TZC medium

Isolates	Nature and colour of colony	Growth, slime and fluidity
Vellanikkara	Circular, smooth, creamy- white colony with light pink center, convex with entire margin	GR- + + + SL - + + + FL - + + +
Odakkali	Circular, smooth, creamy- white colony with light pink center, convex with entire margin	GR- + + + SL - + + FL - + +
Ginger isolate	Circular, smooth, creamy- white colony with light pink center, convex with entire margin	GR- + + + SL - + + FL - + +

GR - Growth + + + Good
SL – Slime + + Moderate
FL - Fluidity + Slight

4.8.2.2 Starch hydrolysis

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

4.8.2.3 Gelatin liquefaction

All the three isolates were not capable of liquefying gelatin.

4.8.2.4 Catalase test

Positive catalase activity was shown by the three bacterial isolates by the production of effervescence upon addition of a few drops of hydrogen peroxide.

4.8.2.5 Urease test

Different bacterial isolates tested, gave a positive reaction in urease test as indicated by the colour change of Christensen's Urea Agar from yellow to pink within five days of inoculation. Vellanikkara isolates gave result within two days, where as Odakkali and ginger isolates took four days to give positive result.

4.8.2.6 Production of hydrogen sulphide

All the bacterial isolates gave black precipitate, which indicated the production of hydrogen sulphide. Vellanikkara and ginger isolate gave result within 4 days whereas Odakkali isolate took 6 days for positive result.

Table 4. Characterization of isolates

Sl No	Characters	Vellanikkara isolate	Odakkali isolate	Ginger isolate
	Morphological			
1	Gram staining	-	-	-
	Biochemical			
2	Levan production	+	+	+
3	Starch hydrolysis	+	+	+
4	Gelatin liquefaction	-	-	-
5	Catalase test	+	+	+
6	Urease test	+	+	+
7	Production of H2S	+	+	+
8	Arginine hydrolase	+	+	+
Utilisation of carbohydrates				
1	Lactose	+	+	+
2	Maltose	+	+	+
3	Cellobiose	+	+	+
4	Sorbitol	+	+	+
5	Mannitol	+	+	+
6	Dulcitol	+	-	+

+Positive
-Negative

Arginine dihydrolase test

All the isolates gave positive result by giving a pink colouration to the medium. Vellanikkara isolate was faster in giving the result within 2 days and Odakkali and ginger isolate took 4 and 5 days respectively.

4.8.2.7 Utilization of carbohydrates

The two isolates from Kacholam and one from ginger gave acidic reaction for the sugars tested, lactose, maltose and cellobiose. The colour of media changed to yellow in the case of all the isolates. Vellanikkara isolate gave positive result within 3 days after inoculation and utilized all the three sugars and hexose alcohols within 72h of inoculation.

The two isolates except the Odakkali isolate utilized all the hexose alcohols. Odakkali isolate did not utilize dulcitol. Both Odakkali and ginger isolate took more than a week compared to Vellanikkara isolate. Based on these results, Vellanikkara isolates from Kacholam were grouped under biovar III, and Odakkali isolate under biovar III A.

4.8.3 Sensitivity to antibiotics

Data on the *in vitro* sensitivity of the two bacterial isolates to three antibiotics at different concentrations were given in Table 5.Among the antibiotics tried all were found to inhibit the growth of both the isolates at different concentrations. In general it was noticed that as the concentration of antibiotics increased, there was an increased inhibition of the isolates.

Table 5. Sensitivity *of R.solanacearum* to antibiotics

Sl. No.	Antibiotics	Concentration (mg l^{-1})	Zone of inhibition (mm)	
			R.solanacearum	
			Vellanikkara	Odakkali
1	Chloramphenicol	100	8.00 (2.94)^{bc}	2.67(1.65) $^{d e}$
		250	13.50(3.77)^{a}	11.67(3.48)^{b}
		500	14.50(3.87) ^{a}	16.67(4.14) ^{a}
2	Ampicillin	100	1.33(1.29)^{e}	2.00(1.58)^{ef}
		250	6.00 (2.29)^{cd}	2.00(1.58) ^{ef}
		500	11.33(3.44) ^{ab}	3.00(1.86) $^{d e}$
3	Streptomycin	100	1.00 (1.23) ^{e}	1.00 (1.23)^{f}
		250	2.66 (1.77) $^{d e}$	3.67 (2.03) ^{cd}
		500	3.00(1.76) $^{d e}$	5.00 (2.35) ^{c}

Mean of three replications
In each column figures followed by same letter do not differ significantly
Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

Among the different concentrations of antibiotics tested, Chloramphenicol 250 mg l^{-1} and 500 mg l^{-1} was significantly superior to all others except Ampicillin 500 mg l^{-1} in inhibiting the growth of Vellanikkara isolate. Streptomycin sulphate was the least effective giving minimum zone of inhibition.

Similarly Chloramphenicol at 250 mg l^{-1} and 500 mg l^{-1} was significantly superior to all others in inhibiting the growth of Odakkali isolate. But here the isolate was least sensitive to Ampicillin.

4.9 CULTURAL AND MORPHOLOGICAL CHARACTERS OF THE FUNGAL PATHOGENS

Cultural and morphological characters of two pathogens (Plate.2) in PDA medium were studied in detail and based on that these organisms were identified. Details of morphological characters were given in Table 6.

4.9.1 Colletotrichum spp.

Potato Dextrose Agar medium was used for studying the characters of fungal pathogens. The fungal pathogen isolated from leaf blight symptoms from Koothattukulam, produced a fluffy white mycelial growth initially and attained a colony diameter of 9 cm in 8 days at room temperature. Later the colony colour changes to greyish white and the reverse side of the colony became unevenly greyish or darker, especially with age. Orange coloured spore mass was observed in the colony only when there is a shortage of nutrients.

Hyphae branched, hyaline with 3.9 µm width and septate at an interval of 11.6 –19.4 µm. Conidia hyaline, cylindrical with both ends round, septate, oil globules present, 8.3 – 15.64 µm x 3.6 µ m. Setae was absent.

Based on the characters, the organism was identified as *Colletotrichum gloeosporioides* (Penz.) Sacc.

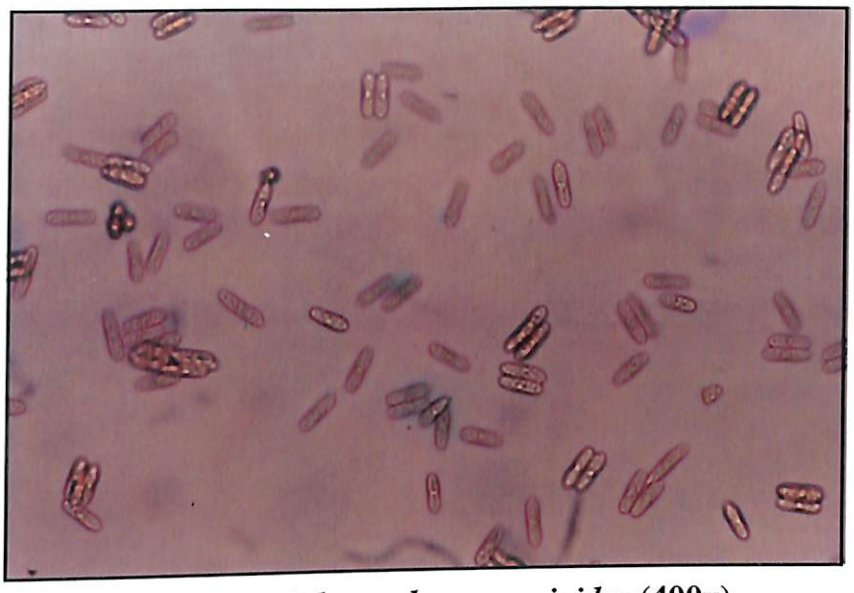
The fungal pathogen isolated from the leaf spot obtained from Asamannur showed slower growth. Took about 9-10 days to attain 9cm diameter at room temperature. Initially the colony was white and fluffy at the starting point. Later it turned to dark grey and then to black. The reverse side of the colony was dark in colour. Setae abundant, dark in colour, with 1-2 septa, swollen at base and tapering at the apex. Pinkish to orange coloured sporulation were seen all over the surface of fungal growth as small droplets.

Hyphae branched, hyaline with 3.9 µm width and septate at an interval of 21.4µm. Conidia hyaline, falcate, fusiform, 18-23 x 3.5-4µm gradually tapered towards each end, aseptate and oil globules present.

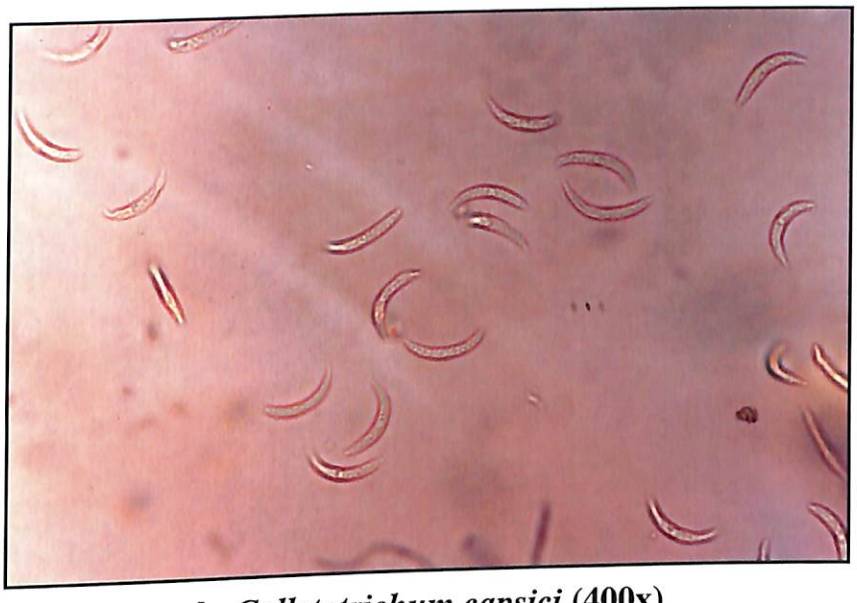
Based on the characters the organism was identified as *Colletotrichum capsici* (Syd.) Butler and Bisby.

Table 6. Morphological characters of fungal pathogen

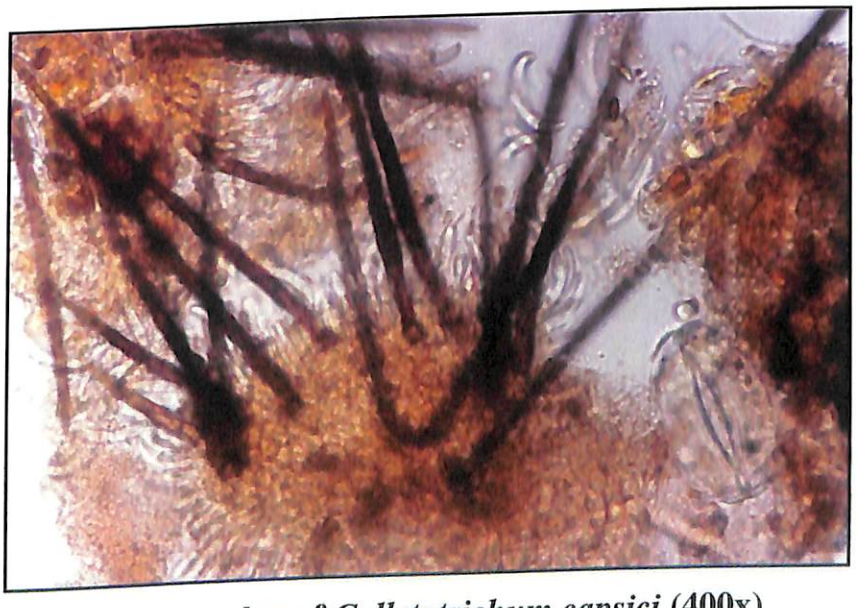
Organism	Hypha		Conidia	
	Width (µm)	Distance between two septa (µm)	Length (µm)	Width (µm)
Colletotrichum gloeosporioides	3.8	11.6-19.4	8.3-15.6	3.6
Colletotrichum capsici	3.8	21.40	16.97	3.5



a. *Colletotrichum gloeosoporioides* (400x)



b. *Colletotrichum capsici* (400x)



c. Acervulus of *Colletotrichum capsici* (400x)

Plate 2. Morphological characters of the pathogen

4.10 SCREENING OF GEOGRAPHICAL TYPES / GEOGRAPHICAL TYPES FOR HOST RESISTANCE AGAINST DISEASES OF KACHOLAM

4.10.1 Evaluation of Kacholam geographical types for resistance against bacterial wilt

The incidence of bacterial wilt among the 12 Kacholam geographical types ranged from 0 to 70 per cent. Among the 12 geographical types no wilt incidence were noticed in two geographical types namely Echippara and Chittoor and incidence of bacterial wilt was below 20 per cent in geographical types Vellanikkara, Peechi and Kuzhalmandam and hence categorized as resistant. The wilt incidence ranged from 20 to 40 per cent in geographical types like Kollamkodu, Varandarapally, Kuravilangadu and Palakkadu, and were grouped as moderately resistant. The two geographical types Koothattukulam and Alapuzha were considered as moderately susceptible as the incidence of bacterial wilt was more than 40 per cent, but below 60 per cent. The geographical type Thodupuzha gave maximum wilt incidence (70 per cent) and was considered susceptible to bacterial wilt. (Table 7).

4.10.2 Evaluation of Kacholam geographical types for resistance to leaf spot disease

Twelve Kacholam geographical types were screened against leaf spot diseases under natural conditions, as there was no incidence of leaf blight in Vellanikkara during the period under study. The results of the experiment were furnished in Table 8. During this season there was only a mild incidence of leaf spot disease caused by *C.capsici* irrespective of the geographical types. From the data, it was found that the geographical types did not show much variation in resistance to leaf spot disease. However, nine geographical types, Vellanikkara, Kollamkodu,

Table 7. Evaluation of Kacholam genotypes for resistance against bacterial wilt disease

Sl No	Genotypes	Wilting percentage	Disease reaction
1	Vellanikkara	10	R
2	Kollamkodu	20	MR
3	Echippara	0.0	R
4	Varadarappally	20	MR
5	Chittoor	0.0	R
6	Peechi	10	R
7	Kuravilangadu	30	MR
8	Thodupuzha	70	S
9	Koothattukulam	50	MS
10	Palakkadu	40	MS
11	Kuzhalmandam	10	R
12	Alapuzha	50	MS

R - Resistant - < 20 %
MR - Moderately Resistant - > 20 to < 40 %
MS - Moderately susceptible - > 40 to < 60 %
S - Susceptible - > 60 %

Table 8. Evaluation of Kacholam genotypes for resistance against leaf spot caused by *C. capsici*

Sl No	Genotypes	Per cent disease incidence	Per cent disease severity	Coefficient of infection	Disease reaction
1	Vellanikkara	14.40	24	3.45	HR
2	Kollamkodu	8.08	14	1.13	HR
3	Echippara	16.41	28	4.59	R
4	Varadarappally	16.93	16	2.70	HR
5	Chittoor	24.78	32	7.93	R
6	Peechi	23.0	28	6.44	R
7	Kuravilangadu	18.25	22	3.96	HR
8	Thodupuzha	4.00	10	0.40	HR
9	Koothattukulam	2.00	6.0	0.12	HR
10	Palakkadu	14.56	14	2.03	HR
11	Kuzhalmandam	13.0	20	2.60	HR
12	Alapuzha	6.30	20	1.26	HR

0 - 4 Highly Resistant (HR)
4.1- 9 Resistant (R)
9.1 – 19 Moderately Resistant (MR)
19.1 – 39 Moderately Susceptible (MS)
39.1 – 69 Susceptible (S)
69.1 – 100 Highly Susceptible (HS)

Varantharappally, Kuravilangadu, Thodupuzha, Koothattukulam, Palakkadu, Kuzhalmandam, and Alappuzha showed coefficient of infection (CI) value ranging from 0.12 to 3.96 and were found to be highly resistant to leaf spot disease. Among them Koothattukulam showed lowest CI value.

Three geographical types Echippara, Chittoor and Peechi gave resistance reaction giving a CI value of 4.59, 7.93 and 6.44 respectively. None were found to be moderately susceptible or susceptible to disease.

4.11 In vitro EVALUATION OF ANTAGONISTS AGAINST PATHOGENS

The antagonistic activity of two fungal and one bacterial antagonists was assessed against two fungal and bacterial pathogens as described in Materials and Methods.

4.11.1 ***In vitro* evaluation of *P.fluorescens* against isolates of *R.solanacearum***

Point inoculation of antagonist and cross streaking of pathogen and antagonist were done. The antagonists *P.fluorescens* was found to be equally effective against both the isolate when cross streaking was adopted. Formation of lysis at the juncture was noticed after 48 h of inoculation.

In the case of point inoculation, *P.fluorescens* gave an inhibition zone of 25 mm against Vellanikkara isolate, and a zone of 26 mm against Odakkali isolate.

4.11.2 ***In vitro*** **evaluation of fungal antagonists against** ***R. solanacearum***

The results of the experiment were presented in Table 9. It was found that both the fungal isolates *T.viride* and *A. niger* gave 100 per cent inhibition of *R. solanacearum*. Between the two antagonists, *T.viride* was found to be more effective, because of faster growth over the pathogen. *T.viride* took five days and *A.niger* took eight days for complete overgrowth of the pathogen. Sporulation was found more in case of both the antagonists over the area were pathogen was streaked.(Plate . 3)

4.11.3 ***In vitro*** **evaluation of** ***P.fluorescens*** **against fungal pathogens**

Among the two methods adopted (streaking on one side and streaking on both sides), streaking on both sides were found most effective in testing the antagonistic property of *P.fluorescens* against fungal pathogens. The rate of growth in monoculture of *C. capsici* was uniform as against *C.gloeosporioides.* In dual culture, rate of growth decreased from 2nd day onwards in the case of both the pathogens. (Table 10).

From the table it was found that *P. fluorescens* recorded 30.7 per cent inhibition on 5th day of incubation against *C. gloeosporioides* whereas, against *C. capsici* it was 38.8 per cent. An inhibition zone of 6 mm and 12 mm was developed between *P. fluorescens* and test organism as evidenced in case of *C. gloeosporioides* and *C. capsici*.

4.11.4 In vitro **evaluation of fungal antagonists against** ***C. gloeosporioides***

The efficiency of fungal antagonists against *C.gloeosporioides* was assessed by dual culture method and per cent inhibition of pathogen and antagonistic index were calculated. The results of experiment were presented in Table 11.

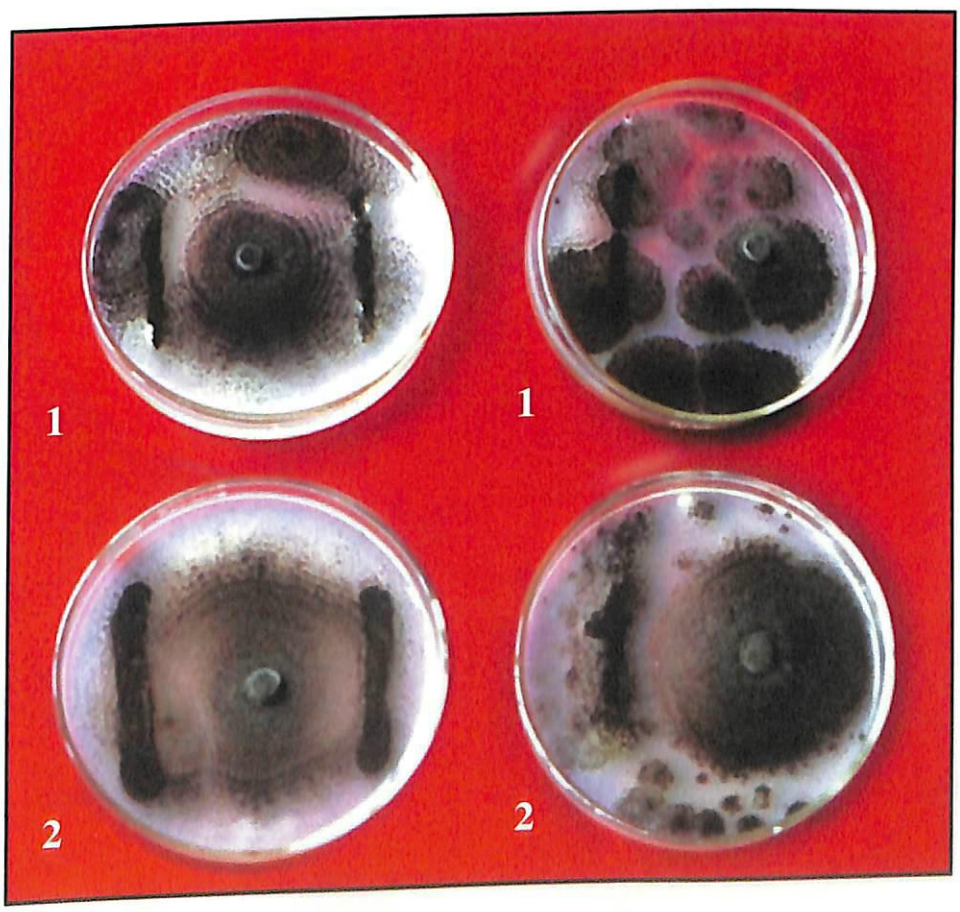
Table 9. *In vitro* evaluation of fungal antagonists against *R. solanacearum*

Pathogen	Antagonist	Days after incubation (Diameter in mm)									Per cent inhibition
			1	2	3	4	5	6	7	8	
Vellanikkara isolate	*T.viride*	D	21	36	57	72	90				100
		M	22	36	59	74	90				
	A.niger	D	11	23	33.6	42	53.3	67.3	74	90	100
		M	15	29	39	46	55	69	74	90	
Odakkali isolate	*T.viride*	D	22	36	58.3	74	90				100
		M	22	36	59	74	90				
	A.niger	D	11.5	27	32.3	42.3	54	67	73.3	90	100
		M	15	29	39	46	55	69	74	90	

D - Dual culture

M – Monoculture

1. *R. solanacearum* (O) x *T. viride*
2. *R. solanacearum* (V) x *T. viride*



1. *R. solanacearum* (O) x *A. niger*
2. *R. solanacearum* (V) x *A. niger*

Plate 3. ***In vitro*** **evaluation of fungal antagonists against** ***R. solanacearum***

Table 10 *In vitro* evaluation of *P.fluorescens* against fungal pathogens.

Pathogens	Days after incubation (Diameter in mm)						Per cent Inhibition
		1	2	3	4	5	5th day
Colletotrichum gloeosporioides	D	12.6	24.6	33.6	40.6	45	30.7
	M	15	22	33	49	65	
Colletotrichum capsici	D	12	20	24.6	30.3	30.6	38.8
	M	13	22	32	42	50	

Mean of three replications
D-Dual culture
M-monoculture

Table 11 *In vitro* evaluation of fungal antagonists against *C.gloeosporioides*

Antagonists		Days after inoculation (Diameter in mm)																		Antagonism Index	Type of Antagonism
		1		2		3		4		5		6		7		8		9			
		T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A		
T.viride	D	11.6	21	22	36	29.3	59	26.9	61.6	17.5	71	0	90							1500	I
	M	15	21	22	36	33	59	49	74	65	90	76	90	85	90	90	90				
A..niger	D	12	13	22	28	32	35	45	43	39	51	26	64	19	71	9	81	0	90	1500	I
	M	15	15	22	29	33	39	49	46	65	55	76	69	85	74	90	90	90	90		

T Test organism

A Antagonist

D Dual culture

M Mono culture

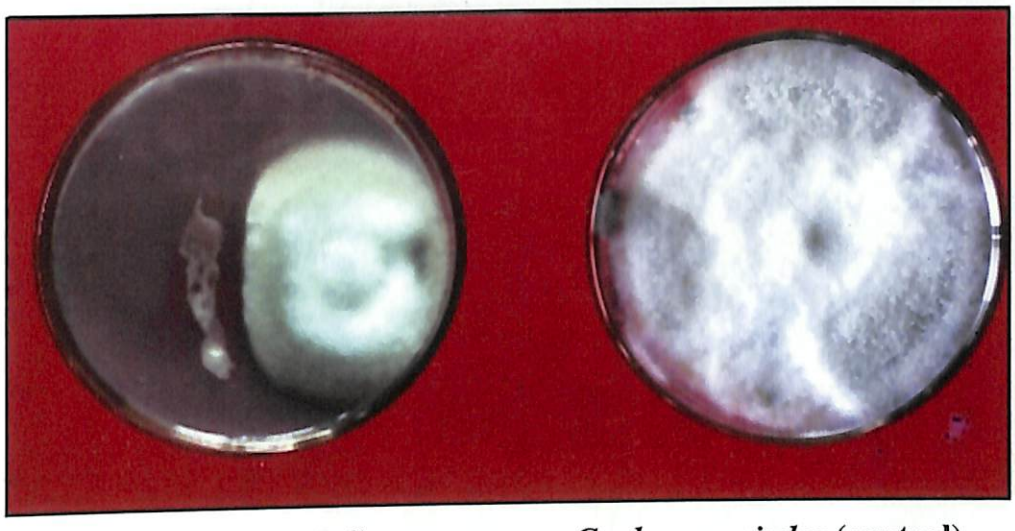
I Over growth

The initial growth rate of the test organism and antagonist was almost same in both mono and dual culture. But the growth of *T.viride* was faster as compared to pathogen. On the third day of inoculation in dual culture, growth of *T.viride* was 59 mm against 29.3 mm of pathogen, and *A. niger* showed 35 mm against 32 mm of pathogen. Four days after inoculation, *T. viride* started overgrowing pathogen. *A. niger* started overgrowing the pathogen after five days of inoculation. For *T. viride* over growth was completed by six days after inoculation and for *A. niger* it was completed by nine days after inoculation. In monoculture the antagonist *T. viride* took 5 days to cover the Petri dish in control and 8 days against test organism *C. gloeosporioides*. (Plate. 4)

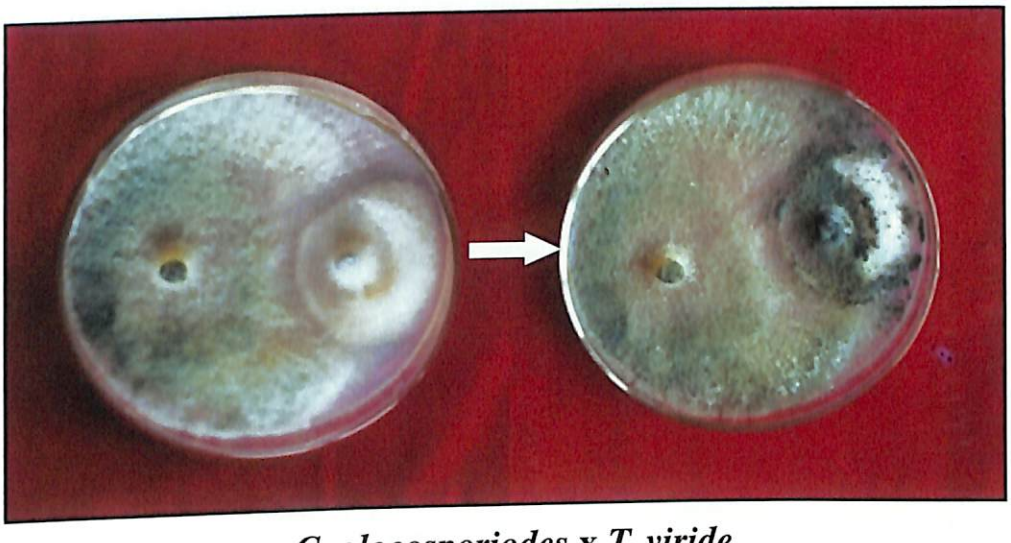
The type of antagonism noticed in *T. viride* and *A. niger* was over growth on the pathogens and both showed an antagonistic index of 1500.

4.11.5 *In vitro* evaluation of fungal antagonists against *C. capsici*

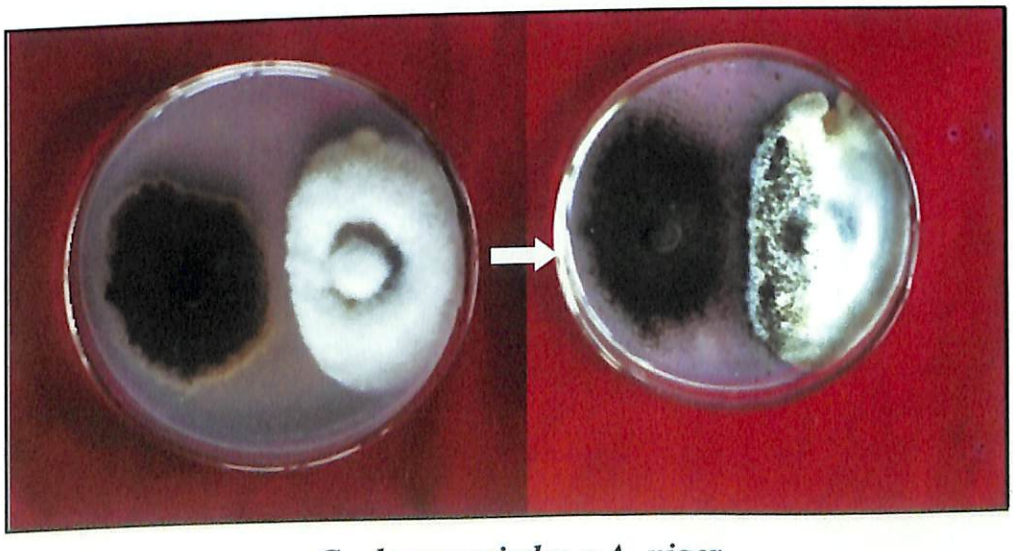
The growth rate of test organism and antagonists was almost same initially. In dual culture rate of growth of antagonist was faster compared to that of pathogen, as evidenced by the diameter of colony attained after three days of inoculation (Table 12). The measurement of *T. viride* showed 58 mm as against 31 mm of pathogen, and *A. niger* showed 39 mm against 28 mm of pathogen. Overgrowth on pathogen started four and six days after inoculation for *T. viride* and *A. niger* respectively. For *T.viride* overgrowth was completed by six days after inoculation and for *A. niger* it was completed by nine days after inoculation. The type of antagonism noticed in *T.viride* and *A. niger* was overgrowth on the pathogen and both showed on antagonistic index of 1500.(Plate. 5)



C. gloeosporiodes x *P. fluorescens* *C. gloeosporiodes* (control)



C. gloeosporiodes x *T. viride*



C. gloeosporiodes x *A. niger*

Plate 4. *In vitro* evaluation of antagonists against *C. gloeosporiodes*

Table 12 *In vitro* evaluation of fungal antagonists against *C.capsici*

Antagonists		Days after inoculation (Diameter in mm)																				Antago-nism Index	Type of Antagonism
		1		2		3		4		5		6		7		8		9		10			
		T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A		
T.viride	D	10.2	22	20	36	31	58	26.7	62.3	18	71	0	90									1500	I
	M	13	22	22	36	33	59	40	74	48	90	55	90	63	90	72	90	85	90	0	90		
A..niger	D	10.6	13.3	19.6	28.3	28	39	34.3	40	44	45	36	53	24	65	10	79	0	90	0	90	1500	I
	M	13	15	22	29	33	39	40	46	48	55	55	69	63	74	72	90	84	90	90	90		

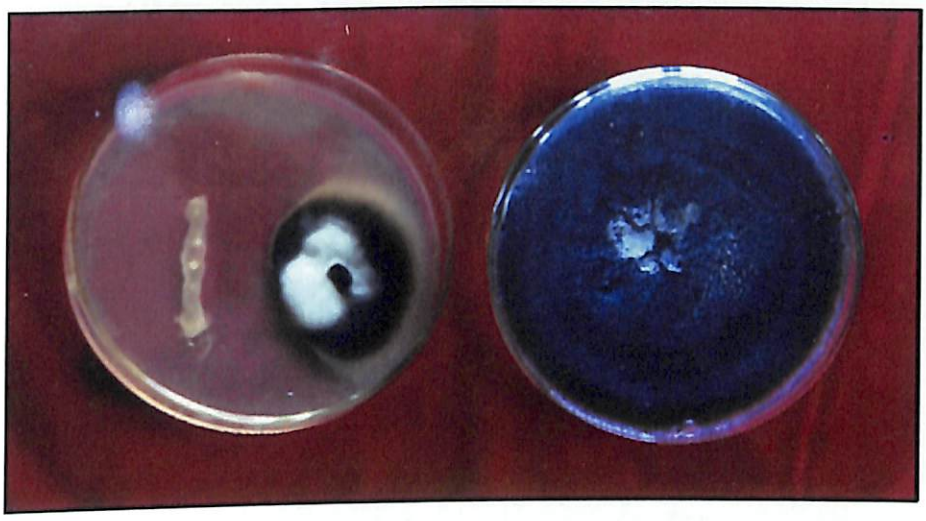
T Test organism

A Antagonist

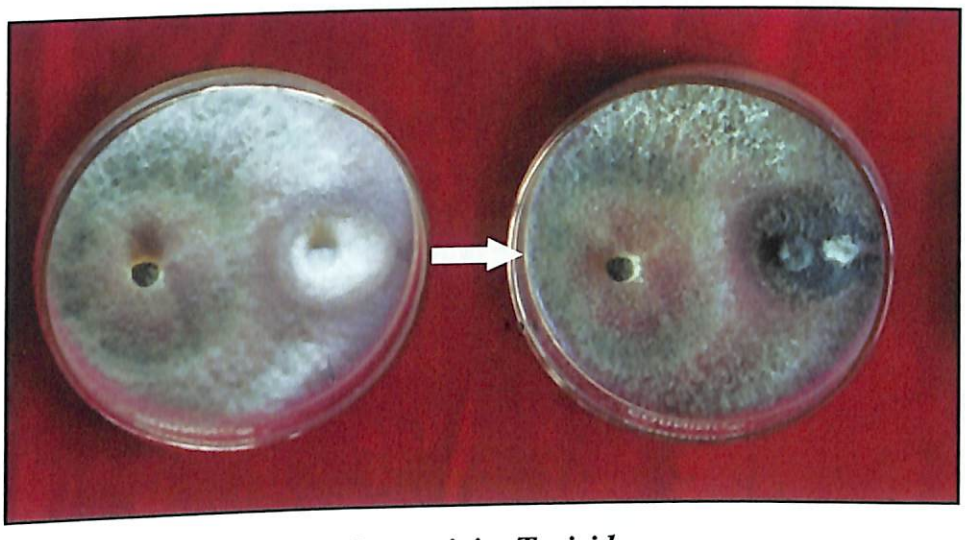
D Dual culture

M Mono culture

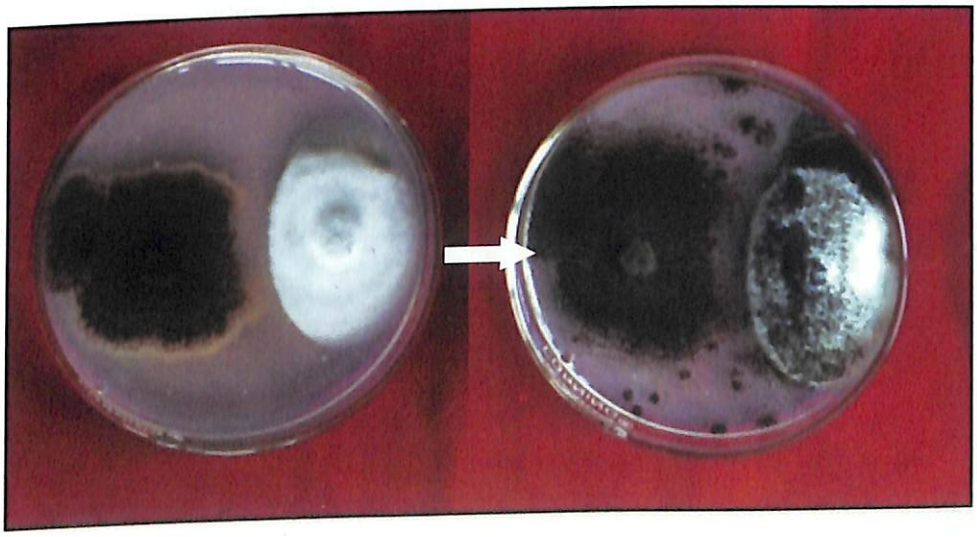
I Over growth



C. capsici x *P. fluorescens* **C. capsici (control)**



C. capsici x *T. viride*



C. capsici x *A. niger*

Plate 5. ***In vitro*** **evaluation of antagonists against** ***C. capsici***

PRODUCTION OF VOLATILES BY ANTAGONISTIC FUNGI

The effect of volatiles produced by *T.viride* and *A. niger* on *C. gloeosporioides* and *C. capsici* was studied. In the presence of volatiles produced by *T.viride* the growth of *C. gloeosporioides* and *C. capsici* was inhibited by 7 and 11.36 per cent respectively. The volatiles produced by *A.niger*, inhibited *C. gloeosporioides* and *C. capsici* by 3 and 5.6 per cent respectively. Volatiles produced by *T.viride* showed more inhibition than that of *A. niger* against both the pathogens (Table 13).

4.12 *In vitro* EVALUATION OF CULTURE FILTRATES OF FUNGAL AND BACTERIAL ANTAGONISTS AGAINST THE ISOLATES OF *R. solanacearum*

The effect of culture filtrates of *T.viride*, *A. niger* and *P. fluorescens*, was tested against two isolates of *R. solanacearum*. It was observed that culture filtrate of *P. fluorescens* was more effective followed by that of *A.niger*, and *T.viride.* Culture filtrates of *P. fluorescens* produced an inhibition zone of 11.5 mm and 9.5 mm against Odakkali and Vellanikkara isolates. Culture filtrates of *A.niger* gave an inhibition zone of 9 mm and 8 mm as against Vellanikkara and Odakkali isolates, whereas *T.viride* gave an inhibition zone of 6 and 11 mm respectively.

In general culture filtrate of *P.fluorescens* was more effective followed by *A. niger* against Vellanikkara isolates. But in the case of Odakkali isolates, culture filtrate *of P. fluorescens* followed by that of *T.viride* was found to be effective (Table 14)

Table 13. Effect of metabolites produced by fungal antagonists on *C.gloeosporioides* and *C.capsici*

Pathogen	Antagonists	Days after incubation (Diameter in mm)								Per cent inhibition over control
		T 1	T 2	T 3	T 4	T 5	T 6	T 7	T 8	
C.gloeosporioides	*T.viride*	13.6	21.6	32.3	44.3					7
	A.niger	13.3	22	33	43.6	63				3
	Control	15	22	36	48	65	76	84	90	
C.capsici	*T.viride*	11.3	19	28.6	36.3	39				11.36
	A.niger	11	18.3	27.6	35	42	50			5.6
	Control	13	20	30	38	44	53	61	73	

* Mean of three replications
T - Test organism

Table14. *In vitro* evaluation of culture filtrates of antagonists against isolates *R.solanacearum*

Culture filtrates		Zone of inhibition (mm)	
		Isolates	
		Vellanikkara	Odakkali
1	*A.niger*	9.0	8.0
2	*T.viride*	6.0	11.0
3	*P.fluorescens*	9.5	11.5

*Mean of three replications

In vitro EVALUATION OF COPPER FUNGICIDES AGAINST BACTERIAL AND FUNGAL PATHOGENS

4.14.1 *In vitro* evaluation of copper fungicides against isolates of *R. solanacearum*

Copper hydroxide and copper oxychloride at 3 doses namely 0.1, 0.2 and 0.3 per cent, and Bordeaux mixture at 0.5, 1 and 1.5 per cent were evaluated for their inhibitory effect against 2 isolates of *R. solanacearum* and *P. fluorescens* (Table 15). The two isolates of *R.solanacearum* differ in their sensitivity towards the different concentrations of fungicides.

There were significant differences among the fungicides and their concentrations in inhibiting the bacterial growth. All the three fungicides were significantly superior at their two higher concentrations compared to lowest concentrations against Vellanikkara isolate. Among the different treatments Kocide at 0.3 per cent was significantly superior to all other treatments against Odakkali isolate. The higher concentrations of all the fungicides were on par in inhibiting the growth of Odakkali isolate.

4.14.2 In vitro evaluation of copper fungicides against *C.gloeosporioides*

The data on *in vitro* sensitivity of *C. gloeosporioides* to different copper fungicides were presented in Table 16. There were significant differences among the fungicides at all concentrations tested in inhibiting the growth of the pathogen. Out of the three fungicides tested Bordeaux mixture at all concentrations recorded 100 per cent inhibition. Copper hydroxide and copper oxychloride showed maximum inhibition of 82.9 per cent and 75.5 per cent at 0.3 per cent concentration and least per cent inhibition of 50.7 per cent and 67.7 per cent at 0.1 per cent respectively.

Table 15 *In vitro* effect of copper fungicides on *R.solanacearum*

Fungicides.	Concentration (per cent)	Inhibition zones (in mm)	
		R.solanacearum	
		Vellanikkara	Odakkali
Kocide	0.1	3.30(1.93)[b]	2.00(1.56) [b]
	0.2	7.70(2.83)[a]	3.00 (1.87) [ab]
	0.3	7.70(2.83) [a]	4.30(2.18) [a]
Fytolan	0.1	2.70(1.77) [b]	2.00(1.56) [b]
	0.2	6.30(2.60) [a]	3.30(1.95) [ab]
	0.3	7.00(2.74) [a]	3.30(1.95) [ab]
Bordeaux mixture	0.5	2.70(1.77) [b]	2.00(1.56) [b]
	1.0	7.30(2.76) [a]	2.60(1.74) [ab]
	1.5	7.30(2.76) [a]	3.00 (1.87) [ab]
control	-	0.00 (0.71) [c]	0.00 (0.71)[c]

Mean of three replications
In each column figures followed by same letter do not differ significantly
Figures in parenthesis are $\sqrt{x+0.5}$ transformed val

Table 16. *In vitro* evaluation of copper fungicides against *C.gloeosporioides*

Sl.No.	Fungicides	Concentration (per cent)	Mean diameter of colony (mm)	Per cent inhibition over control
1	Kocide	0.1	44.33(6.70)[b]	50.7
		0.2	33.33 (5.82)[c]	62.9
		0.3	15.33 (3.95)[f]	82.9
2	Fytolan	0.1	29.00 (5.43)[cd]	67.7
		0.2	25.67 (5.11)[de]	71.4
		0.3	22.00 (4.74)[e]	75.5
3	Bordeaux mixture	0.5	0.00 (0.71)[g]	100
		1.0	0.00 (0.71)[g]	100
		1.5	0.00 (0.71)[g]	100
	Control		90.00 (9.51)[a]	

* Mean of three replications
In each column figures followed by same letter do not differ significantly
Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

Table 17. *In vitro* evaluation of copper fungicides against *C.capsici*

Sl.No.	Fungicides	Concentration (percent)	Mean diameter of colony (mm)	Per cent inhibition over control
1	Kocide	0.1	34.67(5.93)[cd]	61.4
		0.2	31.33(5.63) [cd]	65.1
		0.3	27.67(5.31) [d]	69.2
2	Fytolan	0.1	52.00(7.22)[b]	42.2
		0.2	40.00(6.36)[c]	55.5
		0.3	39.33(6.30) [c]	56.3
3	Bordeaux mixture	0.5	0.00 (0.71) [e]	100
		1.0	0.00(0.71) [e]	100
		1.5	0.00(0.71) [e]	100
	Control		90.00 (9.51)[a]	

* Mean of three replications
In each column figures followed by same letter do not differ significantly
Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

Bordeaux mixture at all three concentrations was significantly superior, followed by copper hydroxide at 0.3 per cent and copper oxychloride at 0.3 per cent. The least inhibition was by copper hydroxide at 0.1 per cent concentration viz., 50.7 per cent

4.14.3 In vitro evaluation of Copper fungicides against *C.capsici*

The data on *in vitro* sensitivity of *C. capsici* to different copper fungicides was presented in Table 17. There was significant difference among fungicides at all concentrations tested in inhibiting the growth of the pathogen. Out of the three fungicides tested, Bordeaux mixture at all concentrations recorded 100 per cent inhibition. In general as the concentration of fungicides increased, the per cent inhibition over control was more. Copper hydroxide and copper oxychloride showed maximum inhibition of 69.2 per cent and 56.3 per cent at 0.3 per cent concentration. Copper oxychloride at 0.1 per cent was least effective, which gave an inhibition of 42.2 per cent.

4.13 COMPATIBILITY OF ANTAGONISTS TO COPPER FUNGICIDES

The three fungicides viz., copper hydroxide, copper oxychloride and Bordeaux mixture each at three concentrations, were evaluated to study the compatibility of these chemicals to *P.fluorescens, T.viride* and *A. niger*.

Among the three fungicides tested Bordeaux mixture at all concentrations completely inhibited the growth of both the antagonists, while the remaining fungicides at different concentration showed varying percentage of inhibition. In general it was noticed that as the concentration of fungicides increased, there was an increased inhibition of antagonist. Among the different concentrations of fungicides copper oxychloride at 0.1 and 0.2 per cent concentration gave a colony diameter of

Table 18. Compatability of antagonists to copper fungicides

Sl. No.	Fungicides	Concentration (per cent	*T.viride*		*A. niger*		*P. fluorescens*
			Mean diameter of colony (in mm)	Per cent inhibition	Mean diameter of colony (in mm)	Per cent inhibition	Zone of inhibition (mm)
1	Kocide	0.1	18.60 (4.37) ^{d}	79.2	72.30 (8.53)^{bc}	19.6	0.3(0.88) ^{b}
		0.2	18.00 (4.30)^{d}	80.0	70.60 (8.44) ^{bc}	21.4	1.0(1.23) ^{ab}
		0.3	16.00 (4.06) ^{d}	82.2	62.30 (7.93)^{c}	30.7	1.3(1.34) ^{ab}
2	Fytolan	0.1	78.30(8.88)^{b}	12.96	76.30 (8.76)^{b}	15.1	0.6(1.05) ^{ab}
		0.2	76.60 (8.78) ^{b}	14.8	53.00 (7.29)^{d}	41.1	1.0(1.24) ^{ab}
		0.3	57.30 (7.60)^{c}	36.3	51.66 (7.22) ^{d}	42.5	2.0(1.56) ^{ab}
3	Bordeaux mixture	0.5	0.00 (0.71) ^{e}	100	0.00(.71)^{e}	100	1.0(1.23) ^{ab}
		1.0	0.00(0.71) ^{e}	100	0.00 (.71)^{e}	100	1.6(1.46) ^{ab}
		1.5	0.00(0.71) ^{e}	100	0.00 (.71)^{e}	100	2.0(1.56) ^{a}
	Control		90.00 (9.51)^{a}		90.00 (9.51)^{a}		0(0.710) ^{c}

Mean of three replications
In each column figures followed by same letter do not differ significantly
Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

78.33 mm and 76.67 mm respectively for *T. viride* as against 90 mm in control. The least growth was noticed in media with copper hydroxide at all three concentrations.

The response of *A . niger* also differed significantly to various fungicides. Cent per cent inhibition was exhibited with the fungicide Bordeaux mixture at all concentrations. Copper oxychloride at 0.1 per cent and copper hydroxide at 0.1 per cent and 0.2 per cent were found to be significantly superior to others thus giving maximum growth of *A. niger*. Copper oxychloride was found to be less compatible than copper hydroxide at 0.3 per cent concentration giving a colony diameter of 51.6 mm and 62.3mm as against 90 mm in the control (Table 18).

None of the fungicides were found to be compatible with *P. fluorescens*. However, copper hydroxide was found to be less inhibitory against *P. fluorescens* compared to copper oxychloride and Bordeaux mixture.

4.14 BIOMETRIC OBSERVATIONS

4.14.1 Effect of different treatments on germination percentage

The number of plants germinated in each treatment was recorded at weekly intervals from second week of planting to a period of five weeks after planting and germination percentage worked out. The rhizomes started germination after one week of planting. The results of the study showed a significant difference in germination between various treatments only during third and fourth week after planting. (Table 19)

At 2 WAP, T_2 (Coirpith + *P. fluorescens*), T_5 (Vermi compost + *T.viride*) and T_9 (FYM + *T.viride*) recorded the maximum germination percentage. No

Plate 6. An overview of field experiment

Table 19. Effect of various treatments on germination percentage

Treatments	Germination percentage			
	2WAP	3WAP	4WAP	5WAP
T1	0 (.71)[a]	25.00(5.03)[abc]	95.00(9.77)[ab]	95.50(9.80))[a]
T2	1.60 (1.38) [a]	30.00(5.47)[a]	97.20 (9.89)[a]	96.60 (9.85)[a]
T3	0.50(0.96) [a]	24.40(4.94)[abc]	93.30 (9.68)[ab]	92.20 (9.62)[a]
T4	0.00 (0.71) [a]	28.80(5.34))[ab]	86.60 (9.34)[abc]	91.10 (9.56)[a]
T5	1.60(1.38) [a]	12.70(3.61)[bcd]	88.30 (9.42)[abc]	93.80 (9.72)[a]
T6	0.50(0.96) [a]	15.50(3.91)[abcd]	83.80(9.18)[abcd]	88.80 (9.45)[a]
T7	1.10(1.12) [a]	12.70(3.44)[cd]	90.00 (9.51)[ab]	89.40 (9.48)[a]
T8	0 (0.71) [a]	5.50(2.46)[de]	81.10 (9.03)[bcd]	88.30(9.42)[a]
T9	1.60 (1.38) [a]	13.30(3.43)[cd]	90.50 (9.53)[ab]	93.80 (9.71)[a]
T10	1.10(1.12) [a]	8.80(2.98)[d]	88.30 (9.41)[abc]	91.60 (9.59)[a]
T11	0.00 (0.71) [a]	11.60(3.44)[cd]	85.50 (9.27)[abc]	90.50 (9.53)[a]
T12	0.00 (0.71) [a]	0.50(0.96)[e]	71.60 (8.50)[d]	86.10 (9.30)[a]
T13	0.50 (0.96) [a]	5.00(2.33)[de]	75.00 (8.68) [cd]	85.00 (9.24)[a]

WAP - Weeks after planting

T1 - Coirpith compost + *T.viride*
T2 - Coirpith compost + *P.fluorescens*
T3 - Coirpith compost + *A. niger*
T4 - Coirpith compost alone
T5 - Vermicompost + *T.viride*
T6 - Vermicompost + *P.fluorescens*
T7 - Vermicompost + *A. niger*
T8 - Vermicompost alone
T9 - FYM+ *T.viride*
T10 - FYM+ *P.fluorescens*
T11 - FYM+ *A. niger*
T12 - Bordeaux mixture (1%)
T13 - FYM alone

Mean of three replications
In each column figures followed by same letter do not differ significantly
Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

germination was recorded in T_1 (Coirpith + *T.viride*), T_4 (Coirpith alone), T_8 (Vermicompost alone), T_{11} (FYM + *A.niger*) and T_{12} (Bordeaux mixture 1per cent).

Maximum germination percentage of 30 per cent was recorded in T_2 (Coirpith + *P.fluorescens*) at 3 WAP which was on par with T_4 (Coirpith alone), T_1 (Coirpith +*T.viride*), T_3 (Coirpith + *A.niger*) and T_6 (Vermicompost + *P.fluorescens*). Lowest germination percentage was noticed in T_{12} (Bordeaux mixture) followed by T_{13} (control) and T_8 (Vermicompost alone).

A significant difference in germination percentage was noticed 4WAP. Maximum sprouting percentage of 97.22 was noticed in T_2 (Coirpith +*P. fluorescens*) which was on par with all other treatments except T_8 (Vermicompost alone), T_{13} (Control) and T_{12} (Bordeaux mixture). T_{12} recorded lowest germination percentage (71.67).

At 5WAP, no significant difference was noticed among treatments. T_2 (Coirpith + *P. fluorescens)* recorded maximum percentage of 96.67 followed by T_1 (95.56) (Coirpith + *T. viride*). T_{13} (Control) recorded lowest percentage of 85 per cent, followed by T_{12} (Bordeaux mixture 1per cent) (86.11 per cent). In general germination percentage was high in plots treated with bioagents especially *P. fluorescens* and *T. viride* than plots without bioagents.(Plate .6)

4.14.2 Effect of different treatments on number of leaves

The data on the number of leaves at different intervals revealed a significant variation among the treatments only during 1MAP. Coirpith applied plots produced higher number of leaves especially during the early stages. At 1 MAP, maximum leaf number (16.42) was observed in T_4 (Coirpith alone) which was on par

Table 20. Effect of treatments on number of leaves

Treatments	Number of leaves per plant		
	1MAP	2MAP	3MAP
T1	12.23[ab]	14.85[a]	17.40[a]
T2	12.98[ab]	15.12[a]	19.66[a]
T3	13.44[ab]	16.83[a]	16.29[a]
T4	16.42[a]	20.29[a]	20.18[a]
T5	10.35[b]	17.83[a]	14.09[a]
T6	11.56[ab]	16.41[a]	17.37[a]
T7	11.40[ab]	14.64[a]	14.29[a]
T8	12.25[ab]	16.08[a]	16.88[a]
T9	10.98[ab]	14.71[a]	15.14[a]
T10	11.83[ab]	16.08[a]	16.87[a]
T11	13.08[ab]	16.96[a]	20.35[a]
T12	9.353[b]	14.62[a]	15.54[a]
T13	11.79[ab]	15.60[a]	17.41[a]

T1 - Coirpith compost + *T.viride*
T2 - Coirpith compost + *P.fluorescens*
T3 - Coirpith compost + *A. niger*
T4 - Coirpith compost alone
T5 - Vermicompost + *T.viride*
T6 - Vermicompost + *P.fluorescens*
T7 - Vermicompost + *A. niger*
T8 - Vermicompost alone
T9 - FYM+ *T.viride*
T10 - FYM+ *P.fluorescens*
T11 - FYM+ *A. niger*
T12 - Bordeaux mixture (1%)
T13 - FYM alone

Mean of three replications
In each column figures followed by same letter do not differ significantly

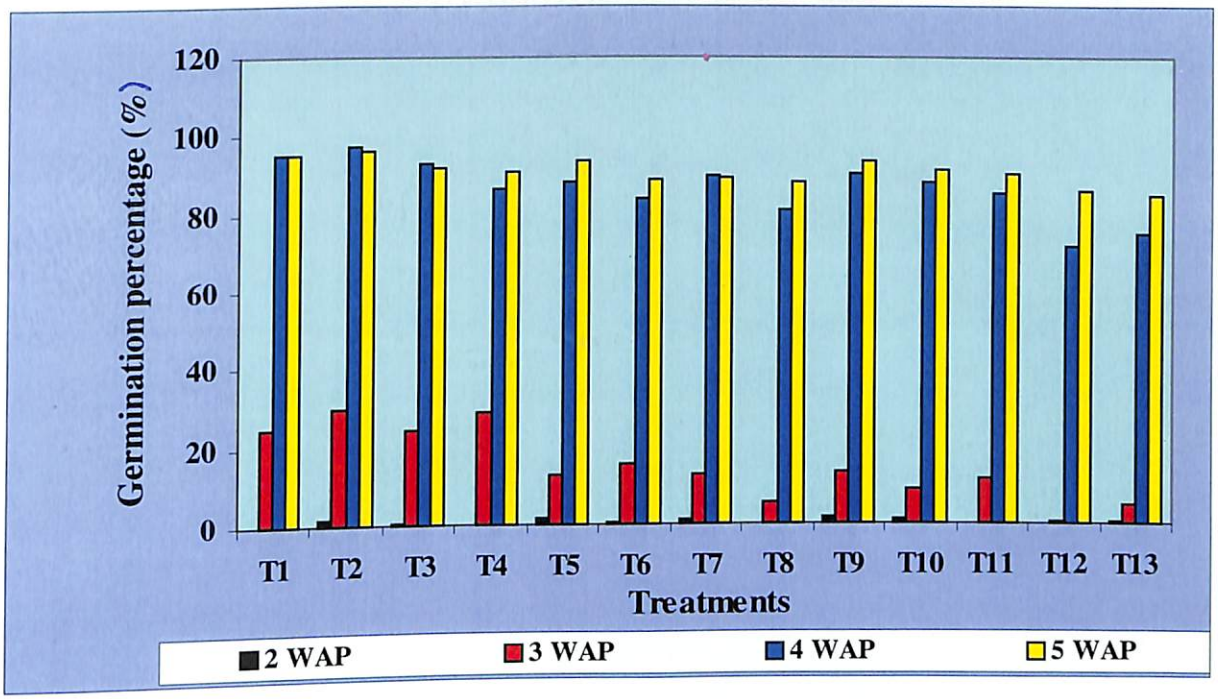


Fig 1. Effect of various treatments on percentage of germination

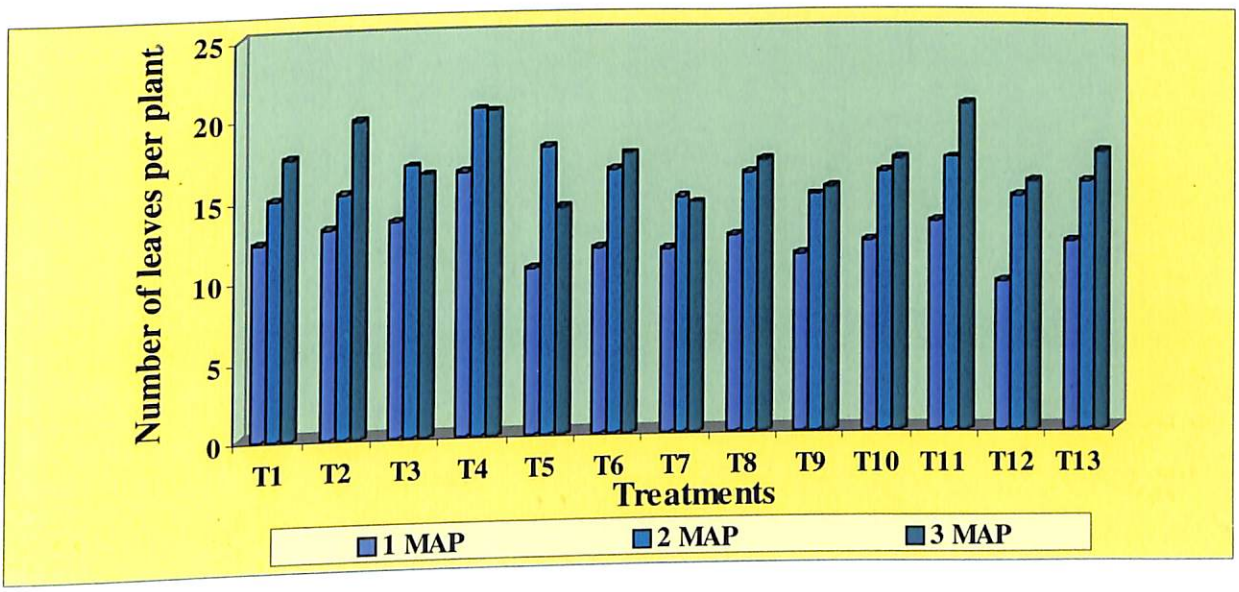


Fig 2. Effect of various treatments on number of leaves

T_1 - Coirpith compost + *T. viride*
T_2 - Coirpith compost + *P. fluorescens*
T_3 - Coirpith compost + *A. niger*
T_4 - Coirpith compost alone
T_5 - Vermicompost + *T. viride*
T_6 - Vermicompost + *P. fluorescens*
T_7 - Vermicompost + *A. niger*
T_8 - Vermicompost alone
T_9 - FYM+ *T.viride*
T_{10} - FYM+ *P. fluorescens*
T_{11} - FYM+ *A. niger*
T_{12} - Bordeaux mixture (1%)
T_{13} - FYM alone

with all the treatments except T_5 (Vermicompost + *T. viride*) and T_{12} (Bordeaux mixture 1per cent) which showed the least number.

Almost the same trend was noticed at 2MAP, with T_4 recording maximum leaf number (20.29) (Coirpith alone) and the least number was shown in T_{12} (Bordeaux mixture 1per cent) (14.62).

At 3 MAP T_{11} (FYM + *A. niger*) showed maximum leaf number (20.35) followed by T_4 (20.18) (Coir pith alone) and T_5 recorded (14.09) (Vermicompost + *T.viride*) the least number. In general when we take the coirpith treated plots alone or in combination it was found to produce maximum leaf number followed by FYM treated plots (Table 20).

4.14.3 Effect of treatments in leaf and rhizome yield at 45 and 90 DAP

4.14.3.1 Leaf yield

The data on fresh and dry weight of leaves showed a significant difference among treatments at 45 DAP and 90 DAP. As in the case of number of leaves, fresh weight of leaves was also high in coirpith-applied plots in combination with bioagents (Table 21).

At 45 DAP fresh weight of leaves was found to be maximum for T_2 (Coirpith + *P. fluorescens*) (16.88 g/plant) which was on par with T_{11} (FYM +*A.niger*), T_1 (Coirpith + *T.viride*) and T_6 (Vermicompost +*P.fluorescens*). The treatment T_5 (Vermicompost + *T. viride*) showed the least value.

Leaf fresh weight at 90 DAP showed that T_1 (Coirpith + *T. viride*) recorded maximum value (21.98 g/plant), which was on par with all other treatments except T_{11} (FYM + *A. niger*) and T_4 (Coirpith alone).

Leaf dry weight at 45 DAP showed that T_{11} (FYM + *A. niger*) (1.447 g/plant) recorded maximum value which was on par with all the other treatments except T_{10} (FYM + *P. fluorescens*) an T_5 (Vermicompost + *T. viride*) .The same trend as in the case of leaf fresh weight was noticed here.

The dry weight of leaves at 90 DAP showed that T_8 (Vermicompost alone) (1.980 g/plant) recorded the maximum value which was on par with all other treatments except T_{12} (Bordeaux mixture 1 per cent), T4 (Coirpith alone) and T_{11} (FYM + *A. niger*). In general it was noticed that plots treated with coirpith gave more leaf and rhizome yield at both 45 DAP and 90 DAP.

4.14.3.2 **Rhizome yield**

Significant variations among treatments were found in case of rhizome fresh weight and dry weight. At 45 DAP, T_{12} (Bordeaux mixture one per cent) (9.7 g/plant) recorded maximum fresh weight, which was on par with all other treatments except T_3 (Coirpith + *A.niger*), T_{13} (control), T_{10} (FYM + *P.fluorescens*) and T_8 (Vermicompost alone) (Table 21).

At 3 MAP, T_8 (FYM + *T. viride*) (18.90 g/plant) was the most effective in increasing the fresh weight of rhizomes, which was on par with all other treatments except T_{13} (control).

The dry weight of rhizome at 45 DAP, was also found to be maximum for T_{12} (Bordeaux mixture 1 per cent) (2.830 g/plant) and least for T_{13} (Control). At 3MAP,

Table 21. Effect of various treatments on leaf and rhizome yield at 45 and 90 DAP

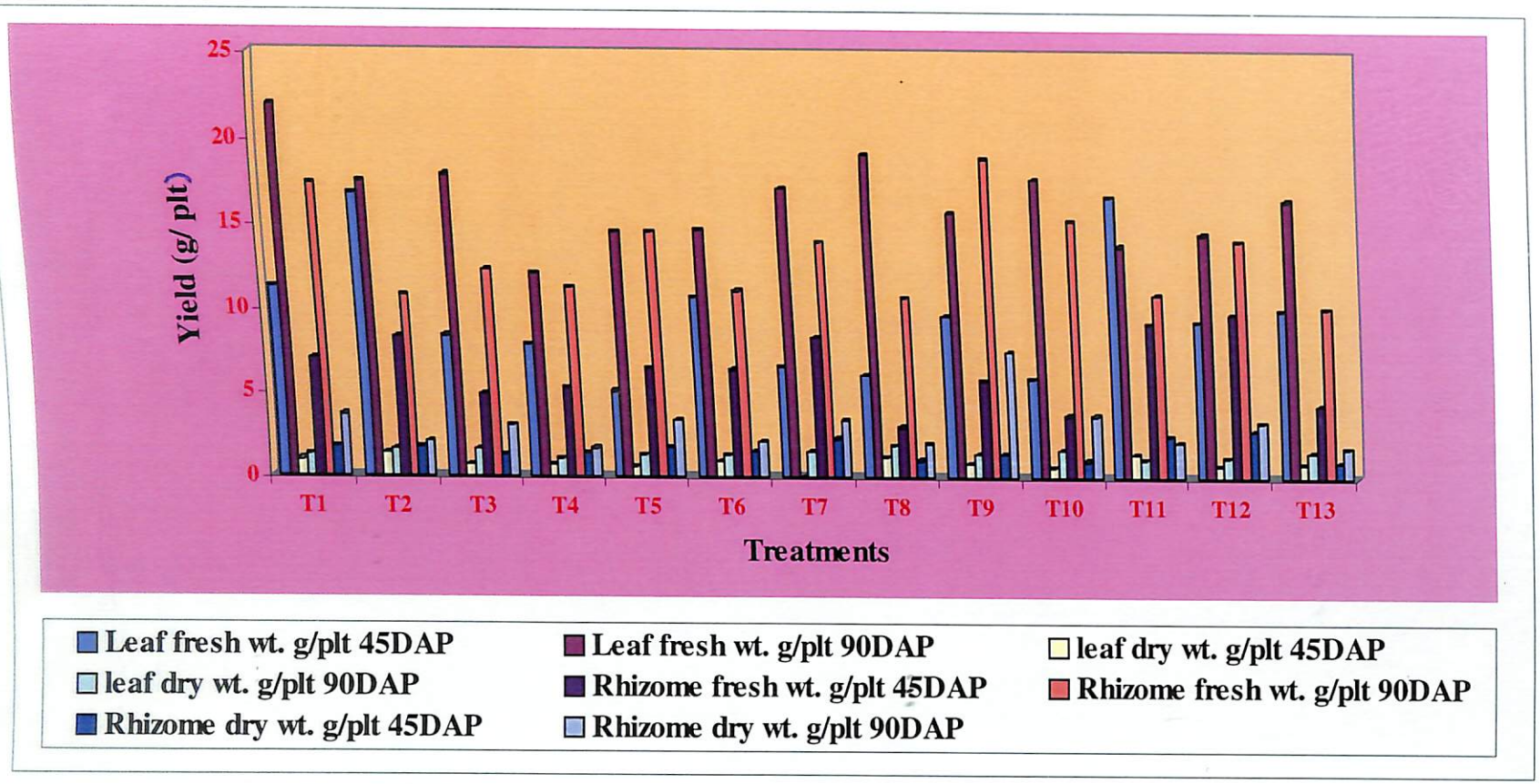
Treatments	Leaf fresh weight g/plant		Leaf dry weight g/plant		Rhizome fresh wt g/plant.		Rhizome dry wt. g/plant	
	45 DAP	90 DAP	45 DAP	90 DAP	45 DAP	90 DAP	45 DAP	90 DAP
T1	11.3^{ab}	21.9^{a}	1.0 ^{ab}	1.3 ^{ab}	7.0 ^{abcd}	17.4 ^{ab}	1.8^{abcd}	3.7^{b}
T2	16.8 ^{a}	17.5 ^{ab}	1.4 ^{a}	1.6 ^{ab}	8.3 ^{abc}	10.8 ^{ab}	1.8^{abcd}	2.1^{b}
T3	8.4 ^{b}	17.9 ^{ab}	0.7 ^{ab}	1.6 ^{ab}	4.9 ^{bcd}	12.3 ^{ab}	1.3^{cd}	3.1^{b}
T4	7.9 ^{b}	12.1 ^{b}	0.7 ^{ab}	1.1^{b}	5.3 ^{abcd}	11.3^{ab}	1.4^{cd}	1.7^{b}
T5	5.1 ^{b}	14.6 ^{ab}	0.6 ^{b}	1.3 ^{ab}	6.5^{abcd}	14.6 ^{ab}	1.8^{abcd}	3.4^{b}
T6	10.7^{ab}	14.7 ^{ab}	0.9 ^{ab}	1.3 ^{ab}	6.4 ^{abcd}	11.1 ^{ab}	1.5 ^{bcd}	2.1^{b}
T7	6.6 ^{b}	17.1 ^{ab}	0. ^{ab}	1.5 ^{ab}	8.3 ^{abc}	14.0 ^{ab}	2.3 ^{abc}	3.4^{b}
T8	6.1 ^{b}	19.1 ^{ab}	1.2 ^{ab}	1.9 ^{a}	3.0^{d}	10.7 ^{ab}	1.0^{d}	2.0^{b}
T9	9.6 ^{b}	15.7 ^{ab}	0.8 ^{ab}	1.4 ^{ab}	5.8^{abcd}	18.9 ^{a}	1.4 ^{bcd}	7.5 ^{a}
T10	5.9 ^{b}	17.7 ^{ab}	0.6^{b}	1.6 ^{ab}	3.7^{cd}	15.3 ^{ab}	1.0^{d}	3.7^{b}
T11	16.7 ^{a}	13.8 ^{b}	1.4 ^{a}	1.1^{b}	9.2 ^{ab}	10.9 ^{ab}	2.5 ^{ab}	2.1^{b}
T12	9.3 ^{b}	14.5 ^{ab}	0.7 ^{ab}	1.2^{b}	9.7 ^{a}	14.1 ^{ab}	2.8 ^{a}	3.3^{b}
T13	10.0 ^{b}	16.5 ^{ab}	0.8 ^{ab}	1.5 ^{ab}	4.3^{cd}	10.1^{b}	0.9^{d}	1.8^{b}

T1 - Coirpith compost + *T.viride*
T2 - Coirpith compost + *P.fluorescens*
T3 - Coirpith compost + *A. niger*
T4 - Coirpith compost alone
T5 - Vermicompost + *T.viride*
T6 - Vermicompost + *P.fluorescens*
T7 - Vermicompost + *A. niger*
T8 - Vermicompost alone
T9 - FYM+ *T.viride*
T10 - FYM+ *P.fluorescens*
T11 - FYM+ *A. niger*
T12 - Bordeaux mixture (1%)
T13 - FYM alone

DAP – Days after planting

Mean of three replications

In each column figures followed by same letter do not differ significantly



T_1 - Coirpith compost + *T. viride*
T_2 - Coirpith compost + *P. fluorescens*
T_3 - Coirpith compost + *A. niger*
T_4 - Coirpith compost alone
T_5 - Vermicompost + *T. viride*
T_6 - Vermicompost + *P. fluorescens*
T_7 - Vermicompost + *A. niger*
T_8 - Vermicompost alone
T_9 - FYM+ *T.viride*
T_{10} - FYM+ *P. fluorescens*
T_{11} - FYM+ *A. niger*
T_{12} - Bordeaux mixture (1%)
T_{13} - FYM alone

Fig 3. Effect of various treatments on leaf and rhizome yield at 45 and 90 DAP

T_9 (FYM + *T. viride*) recorded the maximum dry weight and T_4 (Coirpith) showed least value.

4.14.4 Effect of different treatments on rhizome yield of healthy and diseased plants

The observations recorded on the fresh weight and dry weight of healthy and diseased rhizomes, sampled out at final harvest showed that there was significant difference among the treatments, except in case of fresh weight of diseased rhizomes. (Table 22)

The yield of healthy rhizome at harvest was higher in coirpith compost treated plots especially when in combination with bioagents. The fresh weight of healthy rhizomes was found to be significantly high for T_3 (Coirpith + *A. niger)* (18.68 g/plant) followed by T_1 (Coirpith + *T. viride*) and T_2 Coirpith + *P.fluorescens*). The T_{13} (control) recorded the lowest yield. The same trend was noticed incase of dry rhizome yield with T_3 showing maximum value and least for T_{13}.

The fresh weight of diseased rhizomes showed maximum value for T_9 (FYM + *T. viride*) (8.014 g/plant) and lowest value for T_{13} (Control). But no significant difference was noticed among the treatments. The same trend was seen in case of dry weight also with T_9 giving maximum value of (0.682 g/plant) and T_{13} the least value.

4.16.5 Effect of treatments on total rhizome yield and oil recovery

4.16.5.1 Total yield

The data on total fresh weight and dry weight of rhizome showed a significant difference among the treatments. The total yield obtained was comparatively lower

Table 22. Effect of treatments on rhizome yield of healthy and diseased plants

Treatments	Healthy rhizome		Diseased rhizome	
	Rhizome fresh wt (g / plt)	Rhizome dry wt (g / plt)	Rhizome fresh wt (g / plt)	Rhizome dry wt (g / plt)
T1	12.20^{ab}	5.79 ^{abc}	5.30 ^{a}	0.26^{c}
T2	10.25^{b}	6.18 ^{ab}	6.66 ^{a}	0.42 ^{abc}
T3	18.68 ^{a}	7.09 ^{a}	6.36 ^{a}	0.39 ^{abc}
T4	7.00^{b}	2.37^{d}	5.40 ^{a}	0.31^{bc}
T5	7.16^{b}	2.64^{cd}	5.96 ^{a}	0.36^{bc}
T6	8.15^{b}	3.63 ^{bcd}	6.58 ^{a}	0.40 ^{abc}
T7	8.93^{b}	3.85^{abcd}	7.45 ^{a}	0.60^{ab}
T8	5.76^{b}	1.73^{d}	4.47 ^{a}	0.23^{c}
T9	9.21^{b}	4.10 ^{abcd}	8.01 ^{a}	0.68^{a}
T10	6.60^{b}	2.20^{d}	5.67 ^{a}	0.36^{bc}
T11	7.61^{b}	3.18 ^{bcd}	6.03 ^{a}	0.390^{bc}
T12	10.08^{b}	4.26 ^{abcd}	6.62 ^{a}	0.40 ^{abc}
T13	5.43^{b}	1.22^{d}	4.14 ^{a}	0.19^{c}

T1 - Coirpith compost + *T.viride*
T2 - Coirpith compost + *P.fluorescens*
T3 - Coirpith compost + *A. niger*
T4 - Coirpith compost alone
T5 - Vermicompost + *T.viride*
T6 - Vermicompost + *P.fluorescens*
T7 - Vermicompost + *A. niger*
T8 - Vermicompost alone
T9 - FYM+ *T.viride*
T10 - FYM+ *P.fluorescens*
T11 - FYM+ *A. niger*
T12 - Bordeaux mixture (1%)
T13 - FYM alone

Mean of three replications
In each column figures followed by same letter do not differ significantly

irrespective of the treatments because of low rainfall at tuberwasation stage viz., three months after planting. At final harvest T_9 (FYM + *T. viride*) recorded the maximum fresh weight (1.3 t $ha^{-1)}$ followed by T_1 (Coirpith + *T.viride*) and T_3 (Coirpith + *A.niger*) and was significantly superior to control T_{13}, which recorded the lowest yield (Table 23)

Rhizome dry weight at harvest gave a significantly higher value for T_3 (Coirpith + *A. niger)* (0.65 t ha^{-1}) followed by T_9 (FYM + *T. viride)* and T_1 (Coirpith + *T. viride*). T_{13} (Control) (0.12 t ha^{-1}) recorded the least value.

4.16.5.2 Oil recovery

The data on percentage recovery of oil showed that there were no significant differences among the treatments. How ever the maximum percentage of recovery was noticed for T_1 (Coirpith Compost + *T. viride*) (1.58 per cent) and T_3 (Coirpith compost + *A. niger*). The treatment T_5 (Vermicompost + *T. viride*) showed the lowest percentage recovery of oil, followed by T_8 (1.16 per cent) (Vermicompost alone) (Table 23).

4.16.5.2.1 In vitro effect of essential oils against pathogens

The data on in vitro sensitivity of essential oils against *R. solanacerum* were presented in Table 24. It was compared with one per cent Bordeaux mixture and Streptomycin. It was found that oils gave an inhibition zone of 23.6 and 25mm as against Vellanikkara and Odakkali isolates. The inhibitory actions of oils were more than that of both Bordeaux mixture and Streptomycin sulphate.

Table 23. Effect of treatments on total yield and oil recovery

Treatments	Total rhizome yield (t ha $^{-1}$)		Percentage recovery of oil
	Rhizome fresh weight	Rhizome dry weight	
T1	1.32[a]	0.55 [ab]	1.58[a]
T2	0.88[abcd]	0.45 [abc]	1.36 [a]
T3	1.24 [ab]	0.65 [a]	1.58 [a]
T4	0.57[cd]	0.24[cd]	1.36 [a]
T5	0.57[cd]	0.24[cd]	1.11 [a]
T6	0.74 [abcd]	0.33[bcd]	1.33 [a]
T7	0.74[abcd]	0.29 [bcd]	1.50 [a]
T8	0.33[cd]	0.14[cd]	1.16[a]
T9	1.36 [a]	0.58[ab]	1.41 [a]
T10	0.68[bcd]	0.29 [bcd]	1.36 [a]
T11	0.94 [abc]	0.55 [ab]	1.20 [a]
T12	0.46[cd]	0.22[cd]	1.33 [a]
T13	0.28[d]	0.12[d]	1.50 [a]

T1 –Coirpith compost + T.Viride

T2 – Coirpith + P.fluorescens

T3 – Coirpith compost + A.niger

T4 – Coirpith compost alone

T5-Vermicompost +T.viride

T6- Vermicompost + p.fluorescens

T8-Vermicompost alone

T9-Vermicompost alone

T10 – FYM + P.fluorescens

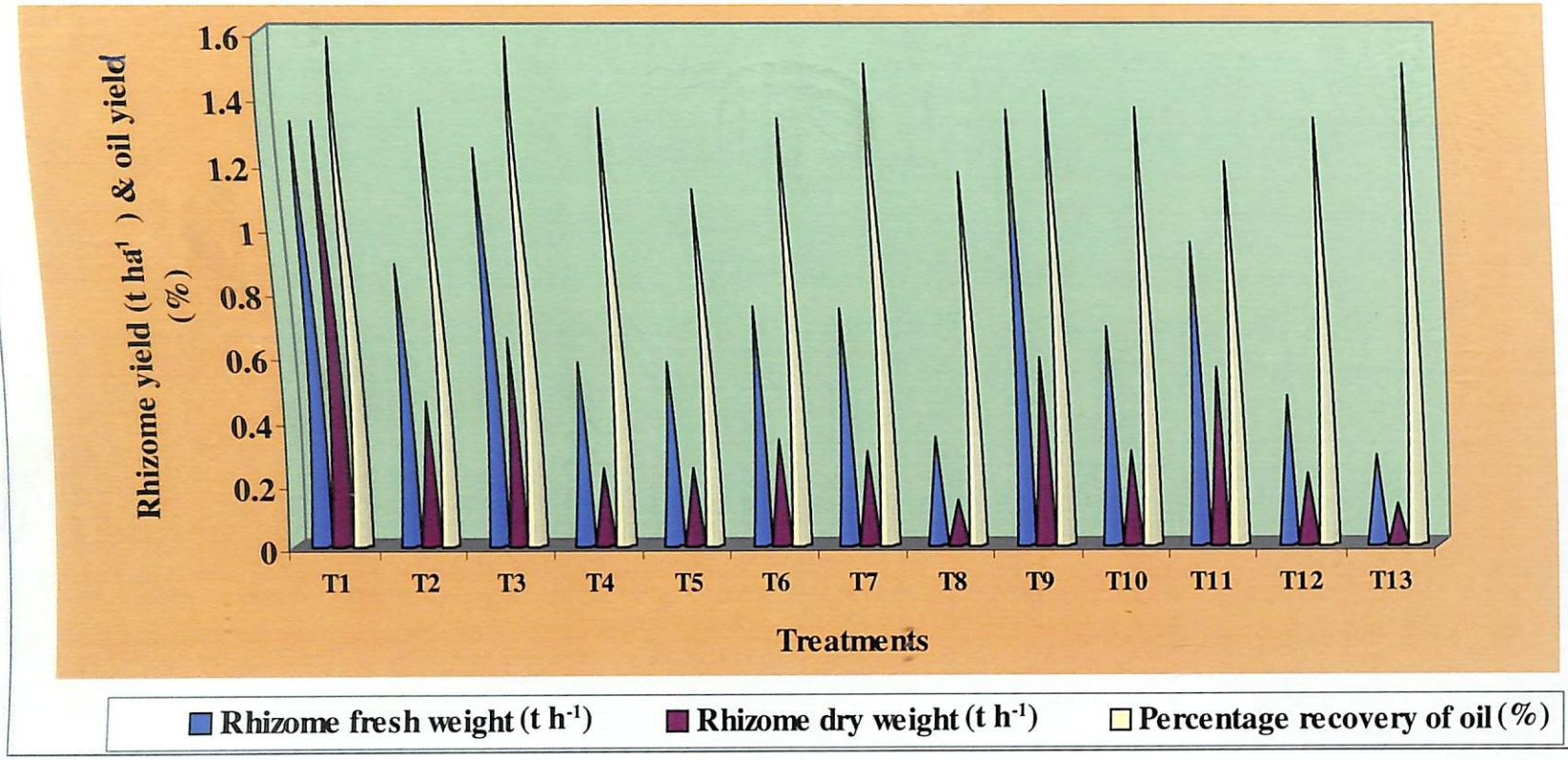
T11-FYM+A.niger

T12 – Bordeaux mixture (1%)

T13 – FYM alone

- Mean of three replications

  In each column figures followed by same letter do not differ significantly



T_1 - Coirpith compost + *T. viride*
T_2 - Coirpith compost + *P. fluorescens*
T_3 - Coirpith compost + *A. niger*
T_4 - Coirpith compost alone
T_5 - Vermicompost + *T. viride*
T_6 - Vermicompost + *P. fluorescens*
T_7 - Vermicompost + *A. niger*
T_8 - Vermicompost alone
T_9 - FYM+ *T.viride*
T_{10} - FYM+ *P. fluorescens*
T_{11} - FYM+ *A. niger*
T_{12} - Bordeaux mixture (1%)
T_{13} - FYM alone

Fig 4. Effect of treatments on rhizome yield and percentage of oil recovery

Table 24. In vitro essential oils against R.solanacearum

Sl.NO	Oil/chemicals	Concentration (percent)	Zone of inhibition (mm)	
			Vellanikkara	Odakkali
1	Oil	1	23.6	25
2	Boredeaux mixture	1	7.0	2.5
3	Streptomycin	1	12.6	13.3
4	Control		0.0	0.0

The oils showed complete inhibition of C.gloeosporioides and C. capcisi under in vitro condition at 0.5, 1.0 and 2 per cent concentration. Bordeaux mixture at one per cent concentration also gave cent percent inhibition of both fungal pathogens.

4.16.6 Total phenols

The total phenol content is diseased and healthy roots showed that there were significant fifferences among the various treatments. The total phenol content was significantly superior in T11 (FYM+ A.niger) (3.301 mg^{-1}) followed by T13 (control) and least for T10(FYM + A.niger) (3.301 mg g-1) followed by T 13 (Contro) and least for T10 (FYM+ p.fluorescens) (Table 25)

In diseased rhizomes the total phenol content was significantly superior in T1 (Coirpith +T.viride) (4.123 mg g-1) followed by T5 (Vermicompost + T.viride) and least was recorded in the case of T9 (FYM + T.viride) followed by T10 (FYM + p.fluorescens)

4.16.7 Effect of treatments on population of soil microflora

The effect of various treatments on the population of soil microflora, viz., Trichoderma, Aspergillus, actinomyces, Azospirillum, Ralstonia solancearum and

Table 25. Effect of treatments on phenol content of healthy and diseased rhizomes

Treatments	Total phenol (mg g^{-1})	
	Healthy rhizome	Diseased rhizome
T1	1.760^{bcd}	4.123 ^{a}
T2	1.355^{cd}	1.780^{d}
T3	0.7123^{d}	2.381^{cd}
T4	0.9556^{d}	1.632^{d}
T5	2.716^{abc}	3.587 ^{ab}
T6	2.661 ^{abc}	2.195^{cd}
T7	1.593 ^{bcd}	2.816^{bc}
T8	1.957 ^{abcd}	1.484^{d}
T9	1.253^{d}	-2.165^{f}
T10	-0.6443^{e}	0.4270^{e}
T11	3.301 ^{a}	3.187 ^{abc}
T12	1.090^{d}	2.167^{cd}
T13	2.761 ^{ab}	1.490^{d}

T1 - Coirpith compost + *T.viride*
T2 - Coirpith compost + *P.fluorescens*
T3 - Coirpith compost + *A. niger*
T4 - Coirpith compost alone
T5 - Vermicompost + *T.viride*
T6 - Vermicompost + *P.fluorescens*
T7 - Vermicompost + *A. niger*
T8 - Vermicompost alone
T9 - FYM+ *T.viride*
T10 - FYM+ *P.fluorescens*
T11 - FYM+ *A. niger*
T12-Bordeaux mixture (1%)
T13 - FYM alone

* Mean of three replications
In each column figures followed by same letter do not differ significantly

fluorescent pseudomonads were estimated before planting and at 45 DAP and 90 DAP. Rhizosphere soil from healthy plants was used for estimation at 45 DAP. At 90 DAP, soil from the rhizosphere of both healthy and also from rhizosphere of bacterial wilt affected plants were used for estimation.

4.16.7.1 Before planting

The mean population of *Trichoderma, Aspergillus*, actinomycetes, fluorescent pseudomonad, *Azospirillum and R. solanacearum* were found to be (1.0 cfu), (21 cfu), (1 cfu), (8.5 cfu), (90 cfu) and (9 cfu), respectively. The population of *Trichoderma* was lowest in the soil.

4.16.7.2 Effect of treatments on rhizosphere microbial population of healthy plants at 45 DAP.

Rhizosphere population of *Trichoderma, Aspergillus*, fluorescent pseudomonads, actinomycetes, *Azospirillum* and *R. solanacearum* were recorded at 45 DAP (Table 26).

Significant differences were shown between treatments in total *Trichoderma* count at 45 DAP. The total population was significantly higher in T_8 (Vermicompost) and T_9 (FYM + *T. viride*) (1.678 cfu) where as *Trichoderma* was absent in T_{13} (Control) (0cfu), T_{12} (Bordeaux mixture 1per cent) and T11 (FYM + *A. niger*).

A significant fluctuation in population was found between treatments at 45 DAP in case of *Aspergillus.* Maximum population was found in T_{11} (FYM + *A. niger*) (73 cfu) and minimum in T_{12} (Bordeaux mixture 1per cent) (2cfu) followed by T_6 (Vermicompost + *P. fluorescens*) (2.33 cfu).

Table 26. Effect of treatments on the healthy rhizosphere microbial population at 45 DAP

Treatment	Soil microbial population (cfu g^{-1})					
	Trichoderma (10^3)	*Aspergillus* (10^3)	Fluorescent Pseudomonas (10^4)	Actinomycetes (10^4)	*Azospirillum* (10^3)	*R.solanacearum* (10^4)
T1	2.33 (1.64)^{a}	5.00 (2.34)^{gh}	5.00 (2.32) ^{ab}	12.33(3.58)^{cd}	8.33(2.95) ^{ab}	8.33(2.92) ^{abc}
T2	2.00(1.56) ^{ab}	11.33(3.44)^{ef}	5.33(2.27) ^{ab}	15.33(3.98)^{bc}	14.00(3.81) ^{a}	6.00(2.54)^{bc}
T3	1.33(1.27) ^{ab}	22.33(4.78)^{cd}	4.33(1.97) ^{ab}	6.33(2.58)^{e}	11.33(3.31) ^{a}	9.67(3.14) ^{abc}
T4	1.00 (1.23) ^{abc}	24.00 (4.95)^{c}	1.33(1.34)^{b}	13.66(3.69)^{bcd}	15.00(3.93) ^{a}	10.67(3.34) ^{a}
T5	1.33 (1.34) ^{ab}	4.33(2.15)^{gh}	2.00(1.58)^{b}	16.00(4.03)^{bc}	4.33(1.97)^{bc}	8.33(2.97) ^{abc}
T6	1.00 (1.17) ^{abc}	2.33(1.68)^{h}	2.00 (1.47)^{b}	24.33(4.98) ^{a}	3.33(1.95)^{bc}	7.00(2.73) ^{abc}
T 7	0.66 (1.05)^{bc}	56.00 (7.45)^{b}	1.00 (1.23)^{b}	23.00(4.83) ^{a}	12.33(3.56) ^{a}	9.00(3.07) ^{abc}
T8	2.33 (1.68) ^{a}	4.00(2.11)^{gh}	7.00 (2.72) ^{a}	25.33(5.08) ^{a}	14.33(3.81) ^{a}	10.00(3.18) ^{ab}
T9	2.30(1.68) ^{a}	7.00(2.72)^{fg}	4.33 (2.20) ^{ab}	14.00(3.81)^{bc}	15.33(3.97) ^{a}	5.33(2.41)^{c}
T10	1.0 (1.26) ^{abc}	15.33(3.95)^{de}	7.33(2.78) ^{a}	12.33(3.58)^{cd}	2.33(1.68)^{c}	10.00 (3.23) ^{ab}
T11	0 (0.71)^{c}	73.00(8.56) ^{a}	2.33(1.54)^{b}	8.33(2.97)^{de}	2.00(1.47)^{c}	11.33(3.44) ^{a}
T12	0 (0.71)^{c}	2.00(1.47)^{h}	8.33(2.97) ^{a}	19.00(4.39) ^{ab}	16.33(4.09) ^{a}	9.33(3.14) ^{abc}
T13	0 (0.71)^{c}	17.00(4.18)^{cde}	4.00 (2.08) ^{ab}	13.00(3.67)^{bcd}	10.00(3.20) ^{a}	9.00(3.08) ^{ab}

T1-Coirpith compost + *T.viride*
T2 - Coirpith compost + *P.fluorescens*
T3 - Coirpith compost + *A. niger*
T4 - Coirpith compost alone
T5 - Vermicompost + *T.viride*
T6 - Vermicompost + *P.fluorescens*
T7 - Vermicompost + *A. niger*
T8 - Vermicompost alone
T9 - FYM+ *T.viride*
T10 - FYM+ *P.fluorescens*
T11 - FYM+ *A. niger*
T12 - Bordeaux mixture (1%)
T13 - FYM alone

* Mean of three replications
In each column figures followed by same letter do not differ significantly
Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

There was significant difference among the treatments with regard to the population of fluorescent pseudomonads. At 45 DAP, T_{12} (Bordeaux mixture 1per cent) recorded maximum count of 8.333 cfu, and least count was observed in T_7 (Vermicompost + *A. niger*) viz., (1cfu).

The population of actinomycetes also showed fluctuations with maximum value of 25.33 cfu in T_8 (Vermicompost alone) and least value in T_3 (Coirpith + *A. niger)* (6.33 cfu). Population was found to be high at 45 DAP than at 90 DAP.

Significant differences were observed in *Azospirillum* population with maximum value in T_{12} (Bordeaux mixture 1per cent) (16.333 cfu) and least in T_{11} (FYM + *A. niger)* (2cfu), followed by T_{10} (FYM + *P. fluorescens*) (2.33 cfu).

Population of *R. solanacearum* was maximum in T_{11} (FYM + *A .niger*) (11.33 cfu) which was on par with all other treatments except T_2 (Coirpith + *P.fluorescens*) (6cfu) and T_9 (FYM + *T.viride)* which showed the least population (5.33 cfu). The treatment T11 that showed maximum population showed less population of other microflora except *Aspergillus*.

4.16.7.3 Effect of treatments on rhizosphere microbial population of healthy plants at 90 DAP.

There was a general decrease in the rhizosphere microbial population (except for *R. solanacearum*) of healthy plants at 90 DAP. Significant differences were noticed in the microbial population at 90 DAP. Population of *Trichoderma* was found to be highest in T_2 (Coirpith compost + *P. fluorescens*) (3.333 cfu) and the lowest in T_{13} (Control) (0cfu), T_3 (Coirpith +*A.niger*), T_4 (Coirpith alone) and T_{10} (FYM + *P.fluorescens*) (Table 27).

Table 27. Effect of treatments on healthy rhizosphere microbial population at 90 DAP

Treatment	Soil microbial population (cfu g^{-1})					
	Trichoderma (10^3)	*Aspergillus* (10^3)	Fluorescent Pseudomonas (10^4)	Actinomycetes (10^4)	*Azospirillum* (10^3)	*R.solanacearum* (10^4)
T1	2.00(1.58)^{a}	4.00(2.12)^{bc}	2.00(1.58)^{cde}	22.33(4.76)^{a}	5.33(2.15)^{ab}	7.00(2.72)^{cde}
T2	3.33(1.77)^{a}	8.33(2.97)^{a}	5.33(2.40)^{bc}	13.33(3.59)^{ab}	6.00(2.54)^{ab}	14.33(3.83)^{a}
T3	0.00(0.71)^{b}	2.33(1.68)^{cd}	14.33(3.83)^{a}	14.33(3.85)^{ab}	7.00(2.67)^{ab}	3.00(1.86)^{e}
T4	0.00(0.71)^{b}	1.00(1.23)^{de}	3.00(1.86)^{bcd}	21.33(4.55)^{ab}	8.00(2.91)^{a}	7.00(2.69)^{cde}
T5	1.33(1.27)^{ab}	0.00(0.71)^{e}	1.00(1.17)^{de}	17.00(4.14)^{ab}	9.00(3.08)^{a}	12.67(3.52)^{abc}
T6	0.67(1.05)^{ab}	0.00(0.71)^{e}	5.00(2.28)^{bc}	19.00(4.39)^{ab}	2.00(1.56)^{bc}	4.67(2.27)^{de}
T 7	1.33(1.34)^{ab}	8.33(2.97)^{a}	5.33(2.38)^{bc}	12.33(3.58)^{ab}	5.33(2.27)^{ab}	11.67(3.48)^{abc}
T8	2.00(1.58)^{a}	0.66(1.05)^{e}	5.33(2.27)^{bc}	16.33(4.08)^{ab}	5.33(2.32)^{ab}	8.67(3.02)^{abcd}
T9	2.00(1.47)^{ab}	0.66(1.05)^{e}	7.00(2.67)^{b}	18.00(4.30)^{ab}	0.00(0.71)^{c}	10.67(3.34)^{abc}
T10	0.00(0.71)^{b}	0.66(1.05)^{e}	5.00(2.28)^{bc}	11.33(3.42)^{b}	4.33(2.19)^{ab}	14.34(3.85)^{a}
T11	0.66(1.05)^{ab}	8.00(2.91)^{a}	5.33(2.34)^{bc}	12.00(3.54)^{ab}	2.33(1.54)^{bc}	7.33(2.79)^{bcd}
T12	1.00(1.23)^{ab}	6.00(2.45)^{ab}	0.00(0.71)^{e}	4.00(1.92)^{c}	4.33(2.20)^{ab}	13.33(3.69)^{ab}
T13	0.00(0.71)^{b}	1.00(1.17)^{de}	7.00(2.74)^{b}	13.33(1.27)^{c}	6.00(2.53)^{ab}	14.33(3.81)^{a}

T1 - Coirpith compost + *T.viride*
T2 - Coirpith compost + *P.fluorescens*
T3 - Coirpith compost + *A. niger*
T4 - Coirpith compost alone
T5 - Vermicompost + *T.viride*
T6 - Vermicompost + *P.fluorescens*
T7 - Vermicompost + *A. niger*
T8 - Vermicompost alone
T9 - FYM+ *T.viride*
T10 - FYM+ *P.fluorescens*
T11 - FYM+ *A. niger*
T12 - Bordeaux mixture (1%)
T13 - FYM alone

* Mean of three replications
In each column figures followed by same letter do not differ significantly
Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

Population of *Aspergillus* was higher in T_2 (Coirpith + *P. fluorescens*) (8.333 cfu) and T_7 (Vermicompost + *A.niger)* and least in T_6 (Vermicompost + *P.fluorescens*) (0cfu) and T_5 (Vermicompost + *T.viride).*

A significantly higher population of fluorescent pseudomonads (14.33 cfu) was noticed in T_3 (Coirpith + *A. niger)* and T_{12} (Bordeaux mixture 1 per cent) recorded least count of 0 cfu.

Significantly higher actinomycetes population was recorded in T_1 (Coirpith + *T. viride*) (22.33 cfu) which was on par with all other treatments except T_{12} and T_{13} control (1.33 cfu).

Population of *Azospirillum* was maximum in T_5 (Vermicompost + *T. viride)* (9 cfu), and least in T_9 (FYM + *T. viride*) (0cfu).

Significant differences were obtained in *R. solanacearum* population also with highest in T_{10} (FYM + *P. fluorescens)* (14.33 cfu), T_2 and T_{13}. The least count of 3cfu was recorded inT_3.(Coirpith + *A.niger).*

4.16.7.4 Effect of treatments on diseased rhizosphere microbial population of diseased plants at 90 DAP

Trichoderma population was found to be comparatively less, than in case of healthy soil. Maximum population was recorded in T_6 (vermicompost + *P. fluorescens*) (7.333cfu) followed by T_9 (5.333 cfu), T_5 (Vermicompost + *T.viride*) and T_8 (2.333 cfu) (Vermicompost alone). In all other treatments population was nil. (Table 28).

Table 28. Effect of treatments on diseased rhizosphere microbial population at 90 DAP

Treatment	Soil microbial population (cfu g^{-1})					
	Trichoderma (10^3)	*Aspergillus* (10^3)	Fluorescent Pseudomonas (10^4)	Actinomycetes (10^4)	*Azospirillum* (10^3)	*R.solanacearum* (10^4)
T1	0.00(0.71)^{d}	7.00(2.72)^{d}	2.00(1.58)^{de}	3.00(1.86)^{d}	7.33(2.80)^{g}	11.00(3.38)^{abcd}
T2	0.00(0.71)^{d}	3.00(1.81)^{ef}	6.33(2.44)^{bcde}	21.33(4.66)^{a}	24.00(4.94)^{e}	9.33(3.12)^{d}
T3	0.00(0.71)^{d}	54.00(7.33)^{b}	12.33(3.32)^{abc}	19.33(4.45)^{a}	80.00(8.97)^{a}	9.00(3.08)^{d}
T4	0.00(0.71)^{d}	88.33(9.42)^{a}	4.00(2.12)^{cde}	17.33(4.22)^{a}	58.00(7.65)^{c}	14.00(3.80)^{abc}
T5	3.33(1.95)^{bc}	0.00(0.71)^{g}	1.00(1.23)^{e}	12.33(3.56)^{bc}	21.00(4.64)^{e}	14.67(3.86)^{ab}
T6	7.33(2.78)^{a}	19.00(4.42)^{c}	4.00(2.12)^{cde}	20.33(4.56)^{a}	32.00(5.69)^{d}	12.67(3.61)^{abcd}
T 7	0.00(0.71)^{d}	95.00(9.8)^{a}	1.00(1.17)^{e}	0.00(0.71)^{e}	16.00(4.06)^{f}	9.33(3.14)^{cd}
T8	2.33(1.54)^{c}	8.00(2.90)^{d}	20.00(4.50)^{a}	4.33(2.15)^{d}	59.33(7.74)^{c}	11.00(3.38)^{abcd}
T9	5.33(2.40)^{ab}	0.66(1.05)^{fg}	10.00(3.20)^{abc}	10.33(3.29)^{c}	33.00(5.78)^{d}	10.00(3.23)^{bcd}
T10	0.00(0.71)^{d}	1.33(1.27)^{fg}	15.00(3.74)^{ab}	6.00(2.46)^{d}	68.00(8.27)^{b}	15.33(3.97)^{a}
T11	0.00(0.71)^{d}	1.66(1.46)^{fg}	8.33(2.93)^{bcd}	9.33(3.13)^{c}	33.33(5.82)^{d}	13.33(3.71)^{abcd}
T12	0.00(0.71)^{d}	6.00(2.50)^{de}	3.33(1.95)^{cde}	16.00(4.06)^{ab}	24.33(4.98)^{e}	14.33(3.85)^{ab}
T13	3.33(1.95)^{bc}	3.00(1.86)^{ef}	2.33(1.54)^{de}	21.00(4.63)^{a}	15.33(3.94)^{f}	15.00(3.92)^{a}

T1 - Coirpith compost + *T.viride*
T2 - Coirpith compost + *P.fluorescens*
T3 - Coirpith compost + *A. niger*
T4 - Coirpith compost alone
T5 - Vermicompost + *T.viride*
T6 - Vermicompost + *P.fluorescens*
T7 - Vermicompost + *A. niger*
T8 - Vermicompost alone
T9 - FYM+ *T.viride*
T10 - FYM+ *P.fluorescens*
T11 - FYM+ *A. niger*
T12 - Bordeaux mixture (1%)
T13 - FYM alone

* Mean of three replications
In each column figures followed by same letter do not differ significantly
Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

Maximum population of *Aspergillus* was recorded in T_7 (Vermicompost + *A. niger)* (95 cfu) and minimum in T_5 (0cfu) (Vermicompost + *T. viride).*

Population of fluorescent pseudomonads was significantly higher in T_8 (Vermicompost alone)(20cfu) and least in T_7 (Vermicompost + *A.niger*) (1 cfu).

Maximum actinomycetes population was in T_2 (Coirpith + *P. fluorescens*) (21.33 cfu) and nil in T_7 (Vermicompost + *A. niger*).

Significantly higher *Azospirillum* population was noticed in T_3 (Coirpith + *A. niger)* (80 cfu) followed by T_{10} (FYM + *P. fluorescens*) (68 cfu) and least in T_1 (7.333 cfu) (Coirpith compost + *T. viride*).

Population of *R. solanacearum* was maximum in T_{10} (FYM + *P. fluorescens*) (15.33 cfu) followed by T_{13} (Control) (15 cfu) which was on par with all other treatments except T_9 (FYM + *T. viride*) (10cfu), T_7 (vermicompost + *A.niger*) (9.333 cfu), T_2 (Coirpith + *P.fluorescens*) (9.333 cfu) and T_3 (Coirpith + *A.niger*) (9 cfu). The least population was recorded in T_3.

4.16.8 Effect of different treatments on percentage wilt incidence

The observation on wilt incidence was started from 2 MAP itself, because of early appearance of wilt symptoms. The percentage wilt incidence was recorded at 1,2,3 and 4 MAP and data were presented in Table 29. Significant difference was obtained between treatments on wilt incidence at 1,3, and 4 MAP.

At 1 MAP, T_1 (Coirpith +*T. viride*) and (FYM + *T.viride*) T_9 was found to be the best treatment with minimum incidence of wilt (0 per cent). The highest

percentage was noticed in T_5 (Vermicompost + *T. viride*) giving 0.33 per cent wilt incidence.

At 2MAP, also the same trend as in 1 MAP was noticed. T_1 (Coir pith + *T. viride*) and T_9 (FYM + *T. viride*) recorded lowest wilt incidence of 0 per cent. The highest value was in T_5 (Vermicompost + *T. viride*) (7.220 per cent). No significant differences were noticed among the different treatments.

At 3 MAP T_9 (FYM + *T. viride*) (1.653) recorded the lowest percentage of wilt incidence followed by T_{12} (Bordeaux mixture 1 per cent) (1.667). The highest wilt incidence was noticed in T_4 (Coir pith alone) (7.767 per cent). At 4 MAP, also T_{12} (Bordeaux mixture 1per cent) recorded the lowest percentage of wilt incidence (3 per cent), followed by T_1 (Coirpith + *T. viride)* 6 per cent and T_9 (FYM + *T. viride*), (8.33 per cent). Maximum wilt incidence was noticed in T_2 (Coirpith + *P. fluorescens*) (27.33) followed by T_3 (Coir pith + *A. niger*) (26.0) and T_4 (Compost alone) (18.664).

Among the three composts Farmyard manure with various combinations of bioagents recorded least percentage of wilt incidence followed by coirpith compost. Vermicompost treated plots showed more wilt incidence during first three months after planting. At 4 MAP, Coirpith treated plots showed maximum wilt percentage followed by vermicompost treated plots.

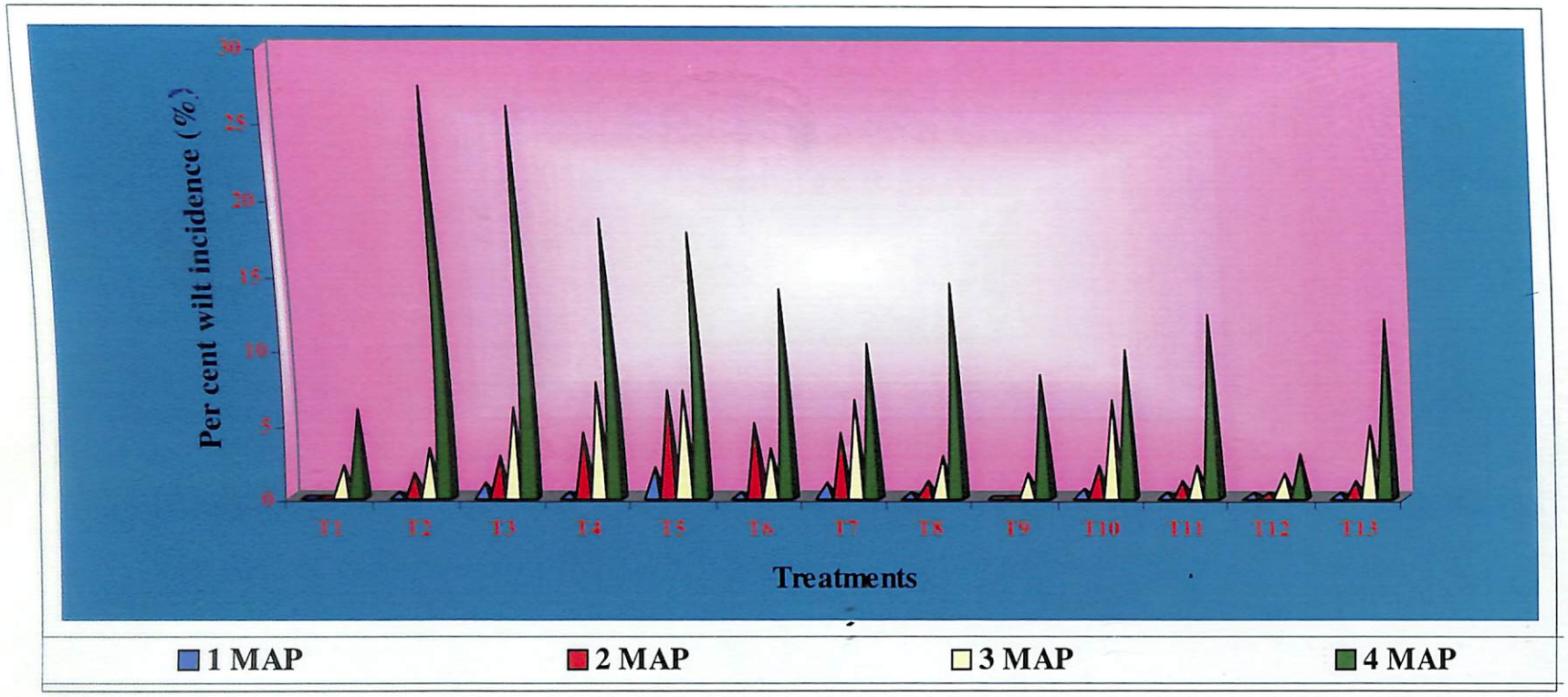
Among the various bioagents, *T. viride* treated plots recorded lowest wilt incidence. During 1 MAP and 3 MAP, *A. niger* treated plots recorded maximum wilt incidence. But at 2 MAP and 4 MAP, *P. fluorescens* treated plots recorded maximum wilt incidence. *T. viride* was found to be more effective than *P.fluorescens* and *A.niger*, with different combinations of compost.

Table 29. Effect of different treatments on wilt incidence at different intervals

Treatments	Wilt incidence (%)			
	1 MAP	2MAP	3MAP	4MAP
T1	0.00 (0.71)^{c}	0.00 (0.71)^{a}	2.20(1.54)^{bc}	6.00(2.51) ^{ab}
T2	0.33 (0.88)^{bc}	1.66 (1.25)^{a}	3.33 (1.55)^{bc}	27.33(4.80) ^{a}
T3	1.00 (1.23)^{ab}	2.77 (1.67) ^{a}	6.06 (2.28)^{abc}	26.00 (4.53) ^{ab}
T4	0.33 (0.88)^{bc}	4.44 (2.02) ^{a}	7.76 (2.74)^{a}	18.66 (4.30) ^{ab}
T5	2.00 (1.56)^{a}	7.22 (2.04) ^{a}	7.20(2.61)^{ab}	17.66 (4.24) ^{abc}
T6	0.33 (0.88)^{bc}	4.99 (1.97) ^{a}	3.33 (1.55)^{bc}	14.00 (3.60) ^{ab}
T7	1.00 (1.12)^{abc}	4.44(2.01) ^{a}	6.65 (2.63)^{ab}	10.33 (3.16) ^{ab}
T8	0.33(0.88)^{bc}	1.11 (1.12) ^{a}	2.77 (1.76)^{abc}	14.33 (3.78) ^{ab}
T9	0.00 (0.71)^{c}	0.00 (0.71)^{a}	1.65(1.38)^{c}	8.33 (2.88) ^{ab}
T10	0.66 (1.05) ^{bc}	2.2 (1.51) ^{a}	6.60 (2.67) ^{ab}	10.00 (3.20) ^{ab}
T11	0.33 (0.88)^{bc}	1.11 (1.12) ^{a}	2.22 (1.51) ^{bc}	12.30 (3.54) ^{ab}
T12	0.33 (0.88)^{bc}	0.33 (0.88)^{a}	1.66 (1.25)^{c}	3.00 (1.87)^{b}
T13	0.33 (0.88)^{bc}	1.11 (1.12) ^{a}	4.96(2.05) ^{abc}	12.00 (3.28) ^{ab}

T1 - Coirpith compost + *T.viride*
T2 - Coirpith compost + *P.fluorescens*
T3 - Coirpith compost + *A. niger*
T4 - Coirpith compost alone
T5 - Vermicompost + *T.viride*
T6 - Vermicompost + *P.fluorescens*
T7 - Vermicompost + *A. niger*
T8 - Vermicompost alone
T9 - FYM+ *T.viride*
T10 - FYM+ *P.fluorescens*
T11 - FYM+ *A. niger*
T12 - Bordeaux mixture (1%)
T13 - FYM alone

Mean of three replications
In each column figures followed by same letter do not differ significantly
MAP – Months after planting
Figures in parenthesis are √x+0.5 transformed values



T_1 - Coirpith compost + *T. viride*
T_2 - Coirpith compost + *P. fluorescens*
T_3 - Coirpith compost + *A. niger*
T_4 - Coirpith compost alone
T_5 - Vermicompost + *T. viride*
T_6 - Vermicompost + *P. fluorescens*
T_7 - Vermicompost + *A. niger*
T_8 - Vermicompost alone
T_9 - FYM+ *T.viride*
T_{10} - FYM+ *P. fluorescens*
T_{11} - FYM+ *A. niger*
T_{12} - Bordeaux mixture (1%)
T_{13} - FYM alone

Fig 5. Effect of different treatment combinations on wilt incidence at different intervals

4.16.9 Effect of treatments on intensity and severity of leaf spot disease.

The effectiveness of various treatments in controlling incidence and severity of leaf spot disease by *Colletotricium capsici* under natural condition was presented in Table 30. The incidence and severity of leaf spot was very less during the cropping period. The incidence was noticed only three months after planting. During the season, lowest disease incidence was noticed in T_{12} (Bordeaux mixture 1per cent) (3.3 per cent) followed by T9 (FYM + *T. viride*) (10.0 per cent). T_{11} (FYM + *A. niger)* showed maximum incidence, (70 per cent) followed by T_{13} (control) (36.67 per cent) and T_3 (Coirpith + *A.niger*)(36.67 per cent).

Lower severity was also noticed in T_{12} (Bordeaux mixture 1per cent) (0.80 per cent) and T_6 (Vermicompost + *P. fluorescens*) followed by T_9 (FYM + *T. viride*) (0.816 per cent). Maximum disease severity was noticed in T_3 (Coirpith + *A. niger*) (30.22 per cent) followed by T_4 (28.55 per cent) (Coirpith alone) and T_{13} control (28.37 per cent).

In general Bordeaux mixture treated plots showed less incidence and severity, followed by bioagents, *T. viride* and *P. fluorescens.*

4.17 CORRELATION STUDIES

Correlation of different microbial population in soil at 45 DAP and 90 DAP with per cent wilt incidence, fresh and dry weight of leaves, fresh and dry weight of rhizome and final rhizome yield were statistically analysed. Correlation of the soil microbial population as a whole with final rhizome yield and the beneficial microorganism with *R. solanacearum* were also analysed and the results were presented here.

Table 30. Effect of treatments on incidence and severity of leaf spot disease

Treatments	Per cent disease incidence	Per cent disease severity
T1	20.00 (4.28)[ab]	1.40(1.37) [a]
T2	23.33 (3.91) [ab]	2.25(1.48) [a]
T3	36.67(4.66) [ab]	30.23(3.77) [a]
T4	26.67(4.12) [ab]	28.55(3.67) [a]
T5	26.67(4.12) [ab]	3.16(1.76) [a]
T6	23.33(3.91) [ab]	0.80(1.11) [a]
T7	16.67(3.59) [ab]	1.28(1.25) [a]
T8	20.00(4.43) [ab]	1.35(1.35) [a]
T9	10.00(2.83) [ab]	0.817(1.11) [a]
T10	13.33(3.67) [ab]	1.20(1.303) [a]
T11	70.00 (8.02) [a]	3.20(1.80) [a]
T12	3.33(1.55)[b]	0.80(1.11) [a]
T13	36.67(4.66) [ab]	28.37(3.72) [a]

T1 - Coirpith compost + *T.viride*
T2 - Coirpith compost + *P.fluorescens*
T3 - Coirpith compost + *A. niger*
T4 - Coirpith compost alone
T5 - Vermicompost + *T.viride*
T6 - Vermicompost + *P.fluorescens*
T7 - Vermicompost + *A. niger*
T8 - Vermicompost alone
T9 - FYM+ *T.viride*
T10 - FYM+ *P.fluorescens*
T11 - FYM+ *A. niger*
T12 - Bordeaux mixture (1%)
T13 - FYM alone

Mean of three replications
In each column figures followed by same letter do not differ significantly
Figures in parenthesis are √x+0.5 transformed values

Correlation between different microbial population in soil and per cent wilt incidence

The results showed a positive correlation of the soil population of *Trichoderma, Aspergillus* and Actinomycetes with per cent wilt incidence at 45 DAP, where as at 90 DAP *Trichoderma* and *Aspergillus* recorded a negative correlation with per cent wilt incidence. The population of pseudomonads showed negative correlation with per cent wilt incidence both at 45 and 90 DAP. A positive correlation was observed between the microbial populations of *R. solanacearum* and per cent wilt incidence at 90 DAP.

4.17.1 Correlation between different microbial population in soil and fresh and dry weight of leaves.

At 45 and 90 DAP, *Trichoderma* and pseudomonads recorded a positive correlation with fresh weight of leaves where as in case of dry weight of leaves only the pseudomonads recorded a positive correlation

4.17.2 Correlation between different microbial population in soil and fresh and dry weight of rhizomes.

At 45 DAP, a positive correlation was observed in *Trichoderma* and actinomycetes with fresh weight of rhizomes, where as at 90 DAP, only actinomycetes showed a positive correlation with fresh weight of rhizomes. The dry weight of rhizomes showed positive correlation with soil population of *Trichoderma,* pseudomonads, actinomycetes and *Azospirillum* at 45 DAP. But no effect was recorded on dry weight of rhizome at 90 DAP. A negative correlation was observed between the population of *R. solanacearum* and rhizome fresh weights at 90 DAP.

4.17.3 Correlation of the microbial count as whole on final yield

Population of all the treated antagonists agents in soil was found to have positive influence on yield. The final fresh and dry weight of rhizome was found to increase as the population of *Trichoderma, Aspergillus* and *P. fluorescens* increased. The population of pathogen had a negative influence, i.e., as the population increased, yield was also low.

4.17.4 Correlation of bacterial microorganisms with R. solanacearum

At 45 DAP, as the population of *Aspergillus*, *P. fluorescens*, Actinomycetes and *Azospirillum* increases the population of pathogen *R. solanacearum* was found to decrease. *Trichoderma* did not have any influence in reducing the populations of *R. solanacearum* at 45 DAP.

At 90 DAP, *Trichoderma* and *Aspergillus* was found to have less influence in reducing the population of *R. solanacearum*. But *P. fluorescens* was effective in reducing the population of *R. solanacearum*. It was also noticed that as the population of *R. solanacearum* increased wilt also increased. So the pathogen population has direct influence on percentage of wilt incidence.

Discussion

5. DISCUSSION

Kacholam is an important medicinal cum aromatic herbaceous stemless plant grown in tropics and subtropics of India. Large-scale cultivation of this crop are carried out by farmers as a main or inter crop in coconut gardens. The crop when subjected to intensive cultivation suffers severe damage due to the incidence of various diseases, thereby affecting the quality and yield of the produce. Prolonged use of fungicides for the control of diseases is undesirable in medicinal plants, as it causes health hazards. Even though there were reports on various diseases in this crop (Varma, 1991; Dake and Manoj, 1994) a search on literature did not reveal much information on research conducted on different aspects of the diseases of Kacholam. In view of the above facts, an investigation was carried out to study various aspects of diseases of Kacholam, particularly the identification, symptomatology, host résistance and disease management which will add to our knowledge on the diseases of Kacholam.

A survey was conducted in the farmer's field at different locations of Ernakulam district and Thrissur district. A bacterial wilt and two fungal diseases were recorded from the districts. The incidence of bacterial wilt was noticed in areas wherever the area has been previously cultivated with Ginger or Kacholam. Isolation of pathogen from the wilted specimens collected from different places showed the association of a bacterium and two fungal species from foliage specimens.

The pathogenicity of these organisms were proved by artificial inoculation of Kacholam plants under *in vitro* and *in vivo* conditions. Among the different methods tried for inoculation, for bacterial pathogens, rhizome inoculation could only produce typical symptoms. Several workers have followed root inoculation (Khan *et. al*) 1979; Swanepoel and Young, 1988; Paul, 1998 and James, 2001) for bacterial wilt incidence on solanaceous crops.

The pathogens causing bacterial wilt and leaf spot diseases were reported on Kacholam by various workers. Dake and Manoj (1994) and Sarma and Kumar (1997) reported bacterial wilt by *Ralstonia solanacearum.* Varma (1991) reported the incidence of leaf spot disease on Kacholam by *C. gloeosporioides* from different districts in Kerala.

Symptomatological study is very much helpful for the correct identification of the disease and thereby help to adopt effective management practices. In the present study it was found that the symptoms produced by the same pathogen in all the locations were almost same. During wet season, the bacterial pathogen produced yellowing and rotting of leaves, which starts from basal portion of the plant and extends upwards towards the tip of the leaf. But during dry season, typical rolling and blightening of leaves from margin inwards were noticed. Only external rotting of rhizome is found when the crop is in early stages but the diseased rhizomes at harvest were found crinkled.

The rotting symptom seen in wet season may be due to the increased water soaking along with the attack by saprophytic organisms in the soil as the foliage of the plant is in touch with soil. The crinkling of rhizome, at harvest can be due to the poor translocation of nutrients to the rhizome due to bacterial infection. The symptoms observed are in confirmation with those of Dake and Manoj (1994). During artificial inoculation, no rotting was observed, only wilting was found.

In case of fungal diseases, leaf blight caused by *Colletotrichum gloeosporioides* produced initially a water soaked sunken lesions, which later turned to blight with definite yellow halo. Centre of spots were brownish. Varma (1991) also described similar symptoms on Kacholam caused by *C.gloeosporioides*. Under artificial condition small yellow specks were seen which later blightened the leaves completely. Another leaf spot caused by *Colletotrichum capsici* was also noticed. The infection starts as small sunken light spots, which later coalesce. No distinct yellow halo was noticed. Centre of spot

was light brown with black coloured fruiting bodies seen in concentric rings at the centre. Shot hole symptoms were seen in advanced stages. There is no report on leaf spot by *C. capsici* in Kacholam. Hence it is the first report of this pathogen in this host. Under artificial inoculation distinct yellow halo was seen but no fruiting bodies were observed at the center of the leaf spot.

The host range studies with the two bacterial isolates of Kacholam on tomato and brinjal produced wilt symptoms alike but not on chilli. Sambasivam (2003) also failed to obtain any wilting in chilli, when inoculated with ginger isolate. In this study race classification of *R. solanacearum* was done based on hypersensitivity reaction on Capsicum. The leaves of the Capsicum plants, infiltrated with bacterial ooze showed dark brown necrosis by 36h with yellow zone around the edges and within 20 days the plant die. The above results were in accordance with the descriptions of race 1 of *R. solanacearum* as suggested by Lozano and Sequiera (1970).

The cultural and morphological characters of the pathogens were studied. For this TZC medium were used for bacterial pathogen and PDA medium for fungal pathogen. All the isolates produced circular, smooth, raised, creamy white colonies with light pink center and with entire margin. The isolates from Vellanikkara produced abundant growth, fluidity and slime compared to the other two isolates. The same media was used by earlier workers for the isolation of the bacterium from various host plants and variation in fluidity was noticed by different bacterial isolate. Hussain and Kelman, 1958, Rani 1994, Paul, 1998; Mathew *et al*., 2000, James, 2001 and Sambasivam, 2003). Colony characters were related with pathogenicity by (Kelman, 1954). According to him highly pathogenic strains produced fluidal white colour with pink centre, while weekly pathogenic once produce butyrous red colonies.

The two bacterial isolates from Kacholam were compared with the bacterial isolate from ginger in biochemical studies. All the isolates gave similar

result in terms of biochemical tests. In most of the characters tested like gram reaction, levan production, production of hydrogen sulphide, urease activity, arginine dihydrolase activity, starch hydrolysis and catalase test, the isolates behaved alike viz., showed positive reaction except in case of gelatin hydrolysis, where the three isolates recorded negative reaction. Slight variations were found in the time taken by the isolates, Vellanikkara isolate gave result, within two to three days, whereas Odakkali and ginger isolates took more than four days.

In the biovar studies the Vellanikkara isolate utilized all sugars and were grouped as biovar 3, where as Odakkali isolate failed to utilize dulcitol, and hence grouped as biovar 3 A. Even though slight variations were seen in sugar utilization time, this was not taken into consideration for the grouping of the pathogen. The biovar grouping is based on a comparison of the studies conducted by He *et al*., (1983), Hayward (1964), Kumar *et al*., (1993) and Paul, (1998). Based on the above studies the Vellanikkara isolate has been identified as *R. solanacearum* race 1 biovar 3 and the Odakkali isolate has been identified as *R. solanacearum* biovar 3A. Mathew *et al*. (2002) identified biovar III and III A infecting ginger. Sambasivam (2003) also reported biovar III A infecting ginger.

The studies of *in vitro* sensitivity of the bacterial isolates to antibiotics revealed that both the isolates were sensitive to all the three antibiotics tested. Vellanikkara isolate was more sensitive to Chloramphenicol and Ampicillin, than Odakkali isolate. The sensitiveness of *R. solanacearum* isolates to Chloramphenicol were also reported by Sambasivam (2003) while screening isolate of ginger. Streptomycin sulphate was found to be more effective against Odakkali isolate than Vellanikkara isolate. The sensitivity of *R. solanacearum* to Ampicillin and streptomycin sulphate was reported by Mondal and Mukherjee (1978).

Based on the above studies the Vellanikkara isolate has been identified as *R. solanacearum* race 1 biovar 3 and the Odakkali isolate has been identified as *R. solanacearum* biovar 3A.

Ginger is an important crop in Odakkali area and can be seen grown as a pure crop. Kacholam in this area are seen grown as a pure crop immediately after ginger crop in garden land or are grown along with ginger as an intercrop. This may be reason why the Odakkali and ginger isolates behaved alike in their biochemical characteristics.

The cultural and morphological characters of fungal pathogens were studied. Potato dextrose agar was used for the study of *Colletotrichum* spp. Two species of *Colletotrichum* were isolated, one from Koothattukulam and other from Asamannur. The fungal pathogen isolated from Asamannur showed slower growth compared to other. It took 10 days to cover the 9cm diameter in Petri dish, where as the other species from Koothattukulam took only eight days. Species from Koothatukulam showing slightly faster growth was found to have fluffy growth, with light orange masses of spores only when stress is provided, by way of limited supply of nutrients. The conidia are hyaline, cylindrical with both ends round, septate, oil globules present, 8.3 – 15.64 µm x 3.6 µ m. Setae was absent. These characters were in confirmation with those described by Varma (1991). Thus the pathogen causing leaf blight with definite golden yellow halo was identified as *C. gloeosporioides* (Penz.) Sacc.

The fungal colony isolated from samples collected from Asamannur was greyish black with sparse growth and showed good sporulation as orange coloured droplets on the surface of medium. Setae were abundant in the old culture. . Conidia hyaline, falcate, fusiform, 18-23 x 3.5 - 4 µm, gradually tapered towards each end, aseptate, oil globules present. The morphological characters were found to be similar to that described by Chowdhury (1957). Thus the leaf spots with

brown margin and concentric sporulation were confirmed to be produced by *C. capsici* (Syd.) Butler and Bisby.

Resistance of host plants against pathogen is an important measure that can be exploited for disease management. Use of resistant or immune varieties is a simple and most effective method in controlling any disease. It is easy to select varieties by screening genotypes, which can be utilized in future as a source of resistance for development of resistant varieties. Development of resistant variety is the most effective method of control of bacterial wilt disease of any crops.

So twelve Kacholam geographical types, available in the AICRP on Medicinal and Aromatic plants were screened for host resistance against bacterial wilt and leaf spot disease under field condition. Geographical types Chittoor and Echippara did not show any wilt symptom. Vellanikkkara, Peechi and Kuzhalmandam geographical types had wilt incidence of < 20 per cent and were categorized as resistant. The accession Thodupuzha gave a wilt incidence of 70 per cent and was found to be susceptible. Vander Plank (1968) reported that the difference in disease resistance exhibited by the genotypes may be due to different types of interactions between pathogen and genotypes that has been affected. Many factors like insufficient inoculum load, absence of pathogenic races to that genotype, unfavourable environmental condition and nutrient status of the soil in which the crops are cultivated (Yarwood, 1978; Khan, 1989) are responsible for the resistant type of reaction shown by the genotype. These factors can be one of the reasons why, the geographical types collected from Thodupuzha showed maximum wilt incidence at Vellanikkara, eventhough the survey result showed no wilt incidence in the area from where it was collected.

When the geographical types were screened for resistance to leaf spot disease it was found that the genotypes did not show much variation in resistance. Nine genotypes were highly resistant with coefficient of infection value ranging from 0.12 to 3.96. The geographical type Koothattukulam was the most resistant

one. The Raigarh center screened 34 turmeric accessions against *Colletotrichum capsici* and were found that none was free from disease. (AICRPS, 1999). But in the same year out of the 51 germplasm accessions of turmeric screened cultivars Kohinur and G.L. Puram were resistant to *C. capsici* (AICRPS, 1999), at Dholi centre.

For effective management of any disease sustainable eco-friendly measures like, the use of resistant varieties, cultural, and biological and chemical methods should be undertaken. Use of chemicals offer comparatively more effective and speedy action in reducing diseases. Many earlier reports revealed successful control of diseases by chemicals, but also gave a chance to develop resistant strains of pathogen and above all it has an adverse effect on oil yield and quality. So as an alterative, sustainable ecofriendly measure like use of antagonist, and soil amendments like compost etc can be tried. Many reports are there showing their effectiveness in controlling different pathogens. So as to compare their effectiveness copper fungicides, which were commonly used against bacterial and fungal pathogens, were also included as a standard check both in lab and field conditions.

Initially an *in vitro* evaluation study was conducted to find out the efficiency of antagonists and fungicides in checking the growth of the pathogen. The two different fungal antagonists and one bacterial antagonist were evaluated *in vitro* against *R. solanacearum*, *C. gloeosporioides* and *C. capsici*, the three pathogens isolated from Kacholam at different locations. Among the different methods tested cross streaking was effective in checking the growth of bacterial pathogen by the formation of lysis at the juncture. The present study showed that *P. fluorescens* was equally effective against both the bacterial isolates by giving good inhibition zones. The fluorescent pseudomonads may produce iron sequestering siderophores which also inhibit the growth of pathogen. Ciampi *et al.* (1996) observed that siderophores like compound produced by *P. fluorescens* was

responsible for inhibition of *R. solanacearum,* and also reported its synthesis was dependant on Fe^{3+} level on culture medium.

The fungal antagonists were also found effective against *R. solanacearum.* Among the fungal antagonists, *T. viride* was found to be more efficient than *A. niger* because of its faster growth over the pathogen. *T. viride* took five days and *A. niger* took eight days for over growth. Similar phenomenon like complete overgrowth of pathogen and lytic nature of *Trichoderma* were observed by D' Ercole *et al.* (1984). The possible reason for the lysis of pathogen by *Trichoderma* may be due to the production of certain inhibitory metaboilites. Weindling (1934) reported that biocontrol activity of strain of *Trichoderma virens* was due to production of a lethal principle, which he named as Gliotoxin. The mechanism of antagonism by antibiosis and by lytic enzyme of *T. pseudokoningi* was already reported by Gayathri and Murugesan (1994) and Silveira *et al.* (1996). How ever an interesting thing noticed was that, both the antagonist, produced maximum overgrowth and sporulation on the area where the pathogen was streaked. Sporulation of *T. viride* was less on Vellanikkara isolate but *A. niger* sporulated well on both the isolates. The antagonists might be utilizing the degraded products of the pathogen.

Among the different methods for the evaluation of antagonistic property of *P. fluorescens* against fungal pathogens, streaking on both sides of pathogen was found to be an effective method than one side streaking. From *in vitro* evaluation it was concluded that *P. fluorescens* was an efficient antagonist to both the pathogen which recorded more than 50 per cent reduction in growth. Fluorecent pseudomonads suppress the pathogen by various modes of action like antibiosis, lytic enzymes, siderophore production and degradation of toxins (Borowitz *et al.,* 1992). Srinivasan and Gunasekharan (1998) reported that *P. fluorescens* was effectively used for the management of leaf spot diseases of coconut palm by *C. gloeosporioides*. Similar reports were also given by Davis (2003) against *C. gloeosporioides* in ivygourd. Jeyalakshmi *et al*., (1998) reported that *Bacillus*

subtilis followed by *P. fluorescens* exerted maximum inhibition of *Colletotrichum capsici* in chilli.

Among the two antagonists tried *T. viride* was found to be more effective than *A. niger* because of its faster growth and complete over growth on the pathogen within six days after inoculation. But *A. niger* also showed overgrowth but not as fast as *Trichoderma* sp and completed its overgrowth in nine days. A slight decrease in rate of growth of *A. niger* was observed just before it starts its overgrowth. Both *T. viride* and *A. niger* recorded the AI value of 1500. Dense sporulation was seen over the pathogen after over growth in the case of *T. viride*. Efficiency of *T. viride* on *Colletotrichum capsici* was reported by Jeyalakhsmi *et al.* (1998) in chilli and Kumar (1999) on *C. lindemuthianum* in cow pea. Davis (2003) also showed the effectiveness of *T. viride, A. niger, Chaetomium globosum* and *P. fluorescens* on *C. gloeosporioides.* Howell (2003) reported that the enzymes chitinase or glucanase produced by the biocontrol agent are responsible for suppression of pathogen by breaking down the polysaccharides, chitan and β- glucans that are responsible for the rigidity of fungal cell wall, there by destroying the cell wall integrity.

The next aspect of investigation was to find out the effect of culture filtrates of the bacterial and fungal antagonists against bacterial and fungal pathogens. Culture filtrates of *P. fluorescens* followed by that of *A. niger* were effective against Vellanikkara isolate of *R. solanacearum*. But in the case of Odakkali isolate of *R. solanacearum*, culture filtrate of *P. fluorescens* followed by that of *T. viride* was found to be effective. Manimala (2003) also reported the effectiveness of culture filtrates of *B. subtilis* and *T.viride* on *R.solanacearum.*

With regard to fungal pathogen, the culture filtrate of both the fungal antagonists did not show any inhibitory action. This may be because of fact that culture filtrate of antagonists might be heat sensitive and inhibitory action might have lost during sterilization. But the culture filtrates of bacteria were found to

inhibit the growth of *C. gloeosporioides* by about 13 per cent. This may be because of the fact that the heat tolerant part may be active against pathogen. Rajasab and Saraswathi (1999) also observed similar results for sterilized culture filtrates of *A, niger*, which had no effect on *Sphacelotheca cruenta* and *Sporisorium sorghii* pathogens on sorghum. Dennis and Webster (1986) showed inhibitory action of culture filtrates of *Trichoderma* against *Macrophomina phaseolina*.

The ability of *T. viride* and *A. niger* to produce volatiles and their effect on fungal pathogens were assessed. It was noted that the volatiles produced by *T. viride* was more effective than that of *A. niger* against fungal pathogens. Volatiles produced by *T. viride* inhibited the growth by 7 and 11 per cent and volatiles produced by *A. niger* upto 3 and 5.6 per cent against *C. gloeosporioides* and *C. capsici* respectively. Even though the extent of inhibition is less compared to direct action, volatiles could also be one of the mechanisms of action of *T. viride* and *A. niger* against fungal pathogens. Dennis and Webster (1971) reported that species of *Trichoderma* have been demonstrated *in vitro* to act against fungal plant pathogens by producing both volatile and nonvolatile diffusible antibiotics. Rathore *et al.* (1990) observed that *T. viride* possessed volatile and non volatile substances against *P. myriotylum* and very little of *F. solani* the ginger rot pathogens. They also observed that diffusible substances inhibited oogonia formation and increased vacuolation and emptying of hyphae. Mumpani *et al.* (1998) observed that volatile metabolites produced *in vitro* by *T. harzianum* have fungistatic effect on growth of *Agaricus bisporus.* The present study showed that the action of *T. viride* and *A. niger* were not similar against *C. capsici* and *C. gloeosporiodes* of both the pathogens. So depending on strain of pathogen and antagonist, the activities of volatiles var. Similar results were obtained by Mumpani *et al.* (1998) were he found out that, out of the three biotypes of *T. harzianum*, Th 2 was stimulated by compounds produced by *A. bisporus* and *A. bisporus* was tolerant to toxins produced by Th 2, which resulted in simultaneous growth of both organisms initially. The present study

revealed that besides direct action, the antagonists had indirect activity, which also contributes in the suppression of pathogens.

In vitro evaluation of copper fungicides on *R. solanacearum* showed that, Vellanikkara isolate was more susceptible to the recommended doses of all three fungicides than Odakkali isolate. Among the three fungicides Vellanikkara isolate was more susceptible to Kocide, whereas Odakkali isolate was more susceptible to Fytolan at recommended dosage. Out of the antibiotics, fungicides and botanicals tested against *R. solanacearum*, Bordeaux mixture at one per cent gave maximum inhibition (KAU, 2001).

In vitro evaluation of fungal pathogens showed that complete inhibition was noticed against both *C. gloeosporioides* and *C. capsici* at all concentrations of Bordeaux mixture. Besides Bordeaux mixture, Fytolan was also found to be effective against *C. gloeosporioides* at the recommended doses. But against *C. capsici*, Kocide was found to be more effective than Fytolan at recommended dosages. Davis (2003) also reported the effectiveness of copper hydroxide and copper oxychloride against *C. gloeosporioides in* ivy gourd plants.

In vitro evaluation of compatibility of copper fungicides with *P. fluorescens* showed that none of the fungicides were compatible with it. However, Kocide was found to be less inhibitory than that of Fytolan and Bordeaux mixture at recommended doses. Akbar (2002) reported that copper hydroxide at 0.15 per cent was compatible with *P. aeruginosa.*

Compatibility of fungal antagonists namely *T. viride* and *A.niger* with copper fungicides were studied and found that Fytolan was more compatible than Kocide with *T.viride* whereas a reverse trend was noticed in the case of *A.niger*. Vijayaraghavan (2003) also reported that *T. viride* was more compatible with Fytolan, than Kocide and Bordeaux mixture. Results of present study showed that

none of the fungicides were compatible with any of the antagonists under *in vitro* evaluation.

When the effect of various treatments were studied in contributing to earliness in germination it was noticed that significant differences were found between treatments during third and fourth week of planting. From second to fifth week after planting (Coirpith compost + *P. fluorescencs*) treated plots showed maximum percentage of germination. Along with it plots treated with Vermicompost + *T. viride* and FYM + *T. viride* showed earliness in germination. It was interesting to note that, at 3 WAP all the coirpith compost treated plots showed maximum germination. The lowest germination percentage was recorded in control and Bordeaux mixture treated plots. As a whole the study revealed that bioagents treated plots had better percentage of germination especially with *P. fluorescencs* and *T. viride* treated plots in combination with composts. Among composts, coirpith compost was found to be the best. The water holding capacity of soil has been reported to increase when applied with decomposed coirpith compost. Nagarajan *et al* (1986). Ramamoorthy *et al* (1999) used the biocontrol agent *T. harzianum* for degrading coirpith compost and the compost as carrier for delivery of fungi. Cocopeat was reported to find it use in germination of seeds, nursery raising, rooting of cutting etc., (Bavappa and Gurusingha (1978). *Pseudomonas* spp. has also been found to degrade lignin of coir pith (Uma *et al*.1994). When coirpith compost is added to other media, it improves water holding capacity and CEC thereby influencing root growth, plant health and establishment. Because of all these characters, cocopeat has been found to be better than peat in supporting seed germination and early growth of plants. Cresswell (1997). Seed treatment with *Pseudomonas fluorescens* native isolate and strains of pf-1 recorded maximum germination 98.66% and 86.66%. Jayalakshmi *et al*. (2003). So as a whole we can say that water holding capacity of coirpith compost, its ability to support bioagents, growth enhancement activity of bioagents and better degradation of composts all together must have contributed in earliness in germination.

The effect of various treatments on number of leaves showed that significant differences were found only during one month after planting. During first two months coirpith compost alone applied plots showed maximum number of leaves, and Bordeaux mixture treated plots showed lowest number. But after three months of planting, along with coirpith compost alone plots, FYM + *A. niger* showed maximum leaf number. So study revealed that plots applied with coirpith compost showed maximum enhancement of growth with increase in leaf number and also germination. According to Verhagen and Papadopoulos, (1997). earliness in germination is also a factor that must have contributed in better leaf number and better biomass. The high CEC enables coirpith compost to retain large amount of nutrients and the adsorption complex has high contents of exchangeable K, Na, Ca and Mg. Awang *et al*. (1997) reported an increase in leaf number, plant height and flower number in case of anthurium. Vijayaraghavan (2003) reported that *T. viride* incorporated treatments produced maximum leaf number in pepper cuttings. Maheswarappa *et al.* (2000) found an increase in number of tillers, leaf number and leaf area in FYM + NPK, FYM and vermicompost treated plots than that of coirpith compost treated plots. Better growth in coirpith compost in the present study might be due to better availability of nutrients, because of the well decomposed nature of coirpith used in this study.

The studies on leaf yield and rhizome yield at 45 and 90 DAP, showed that farmyard manure + *A. niger* treated plots along with coirpith compost + *T. viride* and coir pith + *P.fluorescencs* showed a better leaf yield in terms of fresh weight and dry weight. In case of rhizome yield, FYM + *T. viride* and BM 1 per cent alone recorded maximum yield. In the case of Bordeaux mixture treated plots, there was no growth enhancement. But the better yield might be due to the effective control of wilt in field. Bora *et al*. (2000) studied the influence of soil amendment and antagonist on yield of tomato revealed that soil amendment with cowdung in combination with the antagonist *P. fluorescencs* produced maximum yield. According to Sen *et al.* (1998) the seed and soil treatments by Kalisena

increased the biomass of the seedlings by 45 per cent. In general, leaf yield was not found to contribute to rhizome yield.. This might be due to less rainfall available during the period viz., 3 MAP, the stage at which development of rhizomes starts.

The comparison of yield of both healthy and diseased plants revealed that yield of diseased rhizomes was less compared to that of healthy plants. Coirpith compost and FYM along with bioagents were found to give good result. Control plots recorded lowest yield. The result showed that wilt was found to have an advance effect on yield. The treatment effect on final yield also showed the same trend as showed earlier, with maximum yield in coirpith compost and FYM treated plots along with *T. viride*. But Maheswarappa *et al.* (2000) reported that FYM treated plots gave superior yield characters and yield than vermicompost and coirpith compost treated plots. Moorthy and Rao,(1997) reported that coirpith compost enriched with *Trichoderma, Azotobacter* and phosphate solubilizer could serve as a biofertilizer and biopesticide and were superior to all other treatments.

Eventhough there was no significant difference in percentage recovery of oil, coirpith compost applied plots recorded maximum recovery with bioagents *T. viride* and *A. niger* followed by FYM treated plots. Higher oil content in these treatments was mainly attributed to higher nutrient uptake and better growth of crop in these treatments, resulting in higher oil synthesis. According to Rajagopalan *et al.* (1989) the oil yield is usually influenced only by varietal charaters and also better growth conditions. Maheswarappa *et al.* (2000) also reported the presence of high oil content in FYM and vermicompost treated plots than in coirpith compost treated plots.

An investigation was carried out to study the effect of oils of Kacholam, on *R. solanacearum* and *C. gloeosporioides* and *C.capsici*. It was found that oils completely inhibited the growth of both fungal pathogens. Against both the isolates of *R. solanacearum* also it was equally effective. The antibacterial and

antifungal activity of roots and rhizome oils of *K. galanga* against *Colletotrichum* sp. *Alternaria* sp. *Fusarium oxysporum* and *Pseudomonas aeruginosa* was reported by Arambawela *et al*. (1997).

The estimation of phenols from diseased and healthy rhizome showed that phenol content was high in diseased plants than in healthy plants. It was interesting to note that *T. viride* applied plots showed higher amount of total phenol. Besides that *T. viride* applied plots also had a good influence on reduction of bacterial wilt. Harman (2000) reported that *Trichoderma* sp exhibit other characteristics during interaction with most plants that may contribute to disease resistance or tolerance. Yedidia *et al*. (1999) observed that *T. harziarum* treated cucumber seedlings had an increase in peroxidase activity, chitinase activity and deposition of callose enriched wall appositions on the inner surface of cell walls, that provides resistance to plants. Gopinath and Madalageri (1986) and Sadhankumar (1995) indicated a high significant concentration of phenol with resistance and suggested a possible role of phenols in the mechanism of wilt resistance in brinjal and tomato respectively.

The effect of antagonists treatment on soil microbial population were also studied. Significant difference was noticed between treatments in the case of microbial population at 45 DAP and 90 DAP, in rhizosphere of healthy plants. In general soil microbial population enhanced after planting at 45 DAP. But there was a decrease in the soil microbial population except for *R. solanacearum* in the rhizosphere of healthy plants at 90 DAP. Manimala (2003) observed the increase in population of *R solanacearum* with days after planting, in case of chilli, whereas in case of brinjal and tomato no noticeable change in population was observed after 60 days of planting. The increase in population during 90 DAP might be due to attraction of *R. solanacearum* to root exudates of Kacholam, especially during flowering stage.

The correlation studies conducted also revealed the influence of soil microbial population on yield. *Trichoderma* and Pseudomonads showed a positive correlation with leaf yield at 45 and 90 DAP. When the rhizome yield was compared with population of *Trichoderma,* pseudomonads actinomycetes and *Azospirillum,* a positive correlation was noticed only at 45 DAP. The population of microorganisms were found to influence the final yield also. As the population of *R. solanacearum* increased there was a rise in wilt incidence at 90 DAP and a reduction in final rhizome yield. Here we can conclude that in general, non pathogenic soil microbial population does have an influence on crop yield and also in reducing disease severity. So in addition to the natural microflora, addition of suitable antagonists to the rhizosphere of crop plants will definitely help in suppression of soil pathogens and thereby increasing the yield. So efficient delivery system is a must for each crop, for the supply of antagonists and also for its survival in the rhizosphere of the crop.

The next aspect to be discussed is the effect of coirpith compost and antagonists on wilt incidence and it is noticed that*, T. viride* was found to be the best antagonists, which besides having growth promotion also reduced wilt incidence. During early stages of crop, FYM and coirpith compost with *T. viride* was found to be effective in reducing wilt incidence. But later BM was found to be the best one followed by these two treatments. Beneficial effect of *T. viride*, in combination with different compost was pronounced from early stages of the crop itself. Coirpith compost enhanced growth, yield in combination with bio agents, but its effect on control of wilt was not as pronounced during later stages except with *T. viride.* This clearly indicates that this control might be due to the effect of *T. viride*. Bordeaux mixture recorded minimum wilt incidence in the later stages of the crop because of its bactericidal effect by the repeated applications up to 90 DAP. The efficiency of Bordeaux mixture in controlling bacterial wilt was reported by many workers. (Severin and Kupferberg,1977., KAU, 2001)

Besides the effect of antagonists on reducing wilt incidence, their effect on incidence of leaf spot disease were also studied. Similar to that of bacterial wilt, Bordeaux mixture was found to be the best treatment in reducing the leaf spot disease, followed by FYM and *T. viride.* Even though, *P. fluorescens* was found to be less effective in controlling bacterial wilt, it was better in reducing leaf spot disease than *A. niger* in combination with compost. Davis (2003) reported the inhibitory action of *P. fluorescens* against *C. gloeosporioides* recording more than 50 per cent reduction in growth.

The three bioagents tested could inhibit the growth of bacterial pathogen and two fungal pathogens under *in vitro* conditions, but the same result was not reflected under *in vivo* conditions except in the case of *T. viride.* The efficiency of *P. fluorescencs* was not reflected in field condition. This may be because of poor colonization of the bacteria on host rhizosphere, due to the various reasons like poor competitive ability, adverse soil environment, and also because of absence of suitable substrate to supply the nutritional requirements of the organism. So more work is needed to develop better delivery technique and to understand more about soil ecology, so that the antagonistic activity of the introduced bioagents could be enhanced.

In the present study, rhizome treatment + soil drenching of antagonists were adopted and were found to be an effective method for the delivery of antagonists to soil. Manimala (2003) observed that seed treatment, soil drenching, seed treatment + soil drenching, all were equally effective in reducing wilt incidence.

Among the different bioagents, *T. viride* was found to be the best bioagent, and coirpith compost the best soil amendment. Amendments of soil with coir pith + *T. viride* not only reduced the Kacholam diseases and increased the yield, but will also have an additive effect for the disposal of coir pith in coconut growing regions. Even though the other bioagents were found to be efficient

under *in vitro* conditions, they were less effective under *in vivo* conditions. This may be because of the soil conditions, competitions from other microrganisms, and other environmental factors. So as to enhance the effectiveness of these bioagents in field, further investigations should be carried out to find out the inhibitory factors of each isolate under field conditions and also to find out the ways of maintaining the population level of delivered antagonists in the rhizosphere. It is also necessary to find out the best combinations of bioagents and amendments suited for different crops and soil types. This is especially important in medicinal plants like Kacholam where quality of the rhizome could be maintained by resorting to ecofriendly management of important diseases.

Summary

6. SUMMARY

The present study on "Major diseases of Kacholam [*Kaempferia galanga* L.] and their management was carried out to study various aspects particulary, etiology, symptomatology, host resistance, and management of disease of kacholam.

A survey was conducted at three different locations *viz*., Aromatic and Medicinal plants research station at Odakkali, farmers field at Asamannur, Koothattukulam in Ernakulam district and at farmers field at Pattikad, Varikulam, Chuvannamannu and also at the AICRP on Medicinal and Aromatic plants garden at Vellanikkara. Three wilt symptoms and two different types of leaf spots were noticed and isolation of pathogen from infected plants showed the association of two different bacterial pathogens and fungal pathogens. The pathogenicity of these organisms was proved by artificial inoculation under *in vivo* and *in vitro* conditions. Based on cultural, morphological and biochemical characters, the bacterial pathogens were identified as *R. solanacearum* (Smith) Yabuuchi *et al*. Based on cultural, morphological characters, the fungal pathogens were identified as *Colletotrichum gloeosporioides* (Penz.) Sacc., and *C. capsici* (Syd.) Butler and Bisby.

Studies on symptomatology of the various symptoms collected, showed that wilt symptoms at different locations were same. But the different leaf spot symptoms varied. The leaf spot by *Colletotrichum* sp. from Koothattukulam produced irregular spots on younger and mature leaves, which were sunken with definite yellow halo. The leaf spot from Asamannur caused by *Colletotrichum* sp. produced circular to oval spots, with black coloured sporulations as concentric rings on the center.

The symptoms produced by the bacterial pathogen were rotting of leaves from petiole region upwards, and external rotting of rhizomes in wet

season. In dry season only wilt symptoms with inward rolling of leaves were observed.

Cultural, morphological and biochemical characters of bacterial pathogen, were studied and it was seen that, both the Odakkali and Vellanikkara isolates gave creamy white colonies with pick center, but with less fluidity in case of Odakkali isolates. The isolates showed positive test for solubility in KOH, production of hydrogen sulphide, urease production, arginine dihydrolase reaction, levan production but negative reaction for gelatin liquefaction. The Vellanikkara isolate utilized all sugars and were classified as biovar III and Odakkali did not utilize dulcitol and were categorized as biovar III A. The isolates were grouped into race 1 based on the hypersensitive reaction. In host range studies the isolates infected only tomato and brinjal. Both the isolates were more sensitive to chloramphenicol than Ampicillin and Streptomycin. Vellanikkara isolate was more sensitive then Odakkali isolate.

The cultural and morphological characters of the two fungal pathogens were also studied. Colonies of *Colletotrichum* sp. were greyish white with fluffy growth. Hyphae branched with 3.8 µm width and septate at an interval of 11.6 - 19.4 µm. Conidia measured 8.3 - 15.6 µm x 3.6 µm. Based on these characters the organism were identified as *Colletotrichum gloeosporioides* (Penz.) Sacc.

The colony obtained from the leaf spots from Odakkali was dark grey to black, with sparse growth. Setae abundant, with 1-2 septa, hyphae branched, 3.9 µm width, conidia falcate, fusiform, 18-23 x 3.5 - 4 µm. Based on these characters organism were identified as *Colletotrichum capsici* (Syd.) Butler and Bisby.

Among the twelve genotypes screened against both bacterial wilt and leaf spot diseases, Echippara and Chittoor were immune to bacterial wilt, but none were immune to leaf spot diseases. The accession Thodupuzha showed maximum

wilt incidence and was susceptible to wilt. But none were susceptible to leaf spot diseases, and about nine genotypes were highly resistant with a CI value ranging from 0.12 to 3.96.

For the management of the diseases, *in vitro* evaluation of antagonists and fungicides were done against both bacterial and fungal pathogens. Based on the result of the *in vitro* evaluation, all the three antagonists were selected for field evaluation. Among them *T. viride* was found to be more effective than *A. niger* because of its fast growth. Among the three copper fungicides Bordeaux mixture completely inhibited growth of all fungal pathogens. Fytolan was more effective than Kocide against Odakkali isolate and *C. gloeosporioides* whereas, Kocide was more effective against Vellanikkara isolate and *C. capsici*. When the compatibility of antagonists to fungicides was studied it was found that *P. fluorescens* and *A. niger* was more compatible to Kocide, but *T. viride* was more compatible to Fytolan.

When the effect of culture filtrates of antagonists were studied, it was found to inhibit the growth of bacterial isolates but less effective against fungal pathogens. The volatiles produced by fungal antagonists were found to inhibit growth of fungal pathogens but only to less extend.

Field experiment done to find out the effectiveness of antagonists and soil amendments against both bacterial and fungal pathogens and also for crop growth confirmed the efficiency of *T. viride* as the best antagonist and coirpith as the best soil amendment in providing better germination, leaf number, rhizome yield, and oil recovery. Farmyard manure treated plots was also on par with coirpith treated plots in increasing yield, and also for providing resistance to wilt and leaf spot. But Bordeaux mixture was found to be the best treatment against both bacterial wilt and leaf spot diseases.

The correlation studies of soil microbial population revealed that their population especially that of *Trichoderma* and fluorescent pseudomonads has a positive influence on yield. The population of *R. solanacearum* was also found to be a factor for wilt incidence. So we can conclude that soil microbial fluctuations do influence yield and disease severity.

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* Originals not seen

Appendix

APPENDIX-I

Composition of different media used for various studies

Triphenyl Tetrazolium Chloride (TZC) medium

TZC	: 1%
Peptone	: 10.0 g
Lasein hydrolysate	: 1.0 g
Glucose	: 5.0 g
Agar agar	: 20.0 g
Distilled water	: 1000 ml
pH	: 6.8

Martin Rose Bengal Streptomycin Agar (MRBA)

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

Thornley's medium

Peptone	: 20.0 g
$2HPO_4$	: 0.3 g
NaCl	: 5.0 g
Phenol red	: 0.01 g

L-arginine meno hydrochloride	: 10.0 g
Agar agar	: 15.0 g
Distilled water	: 1000 ml
pH	: 7.2

Hayward's semisolid medium

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

Nutrient Agar medium (NA)

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

King's B medium

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

pH : 7.2 – 7.4

Kenknight's Agar medium (KAM)

Glucose : 1.0 g

KH_2PO_4 : 0.1 g

$NaNO_3$: 0.1 g

KCl : 0.1 g

$MgSO_4.7H_2O$: 0.1 g

Agar agar : 20.0 g

Distilled water : 1000 ml

pH : 7.0

Potato Dextrose Agar (PDA)

Potato : 200 g

Dextrose : 20 g

Agar agar : 20.0 g

Distilled water : 1000 ml

pH : 7.0

Christensen's Urea agar

$NH_4 H_2 PO_4$: 0.5 g

K_2HPO_4 : 0.5 g

$MgSO_4.7H_2O$: 0.2 g

NaCl : 5g

Yeast extract : 1.0 g

Agar agar : 20.0 g

Phenol red : 0.012 g

Distilled water : 1000 ml

MAJOR DISEASES OF KACHOLAM [*Kaempferia galanga* L.] AND THEIR MANAGEMENT

By

PRIYA. K

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the
requirement for the degree of

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Faculty of Agriculture

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ABSTRACT

A study on "Major diseases of Kacholam [*Kaempferia galanga* L.] and their management" was conducted at College of Horticulture, Vellanikkara during 2003-2004. The study revealed that *Ralstonia solanacearum* (Smith) Yabuuchi *et al*., *Colletotrichum gloeosporioides* (Penz.) Sacc, *Colletotrichum capsici* (Syd.) Butler and Bisby were major pathogens in Kacholam. *Ralstonia solanacearum* was the major pathogen causing wilt in Kacholam. Among the 12 genotypes of kacholam tested two genotypes were immune to wilt and nine were highly resistant to leaf spot disease.

From the *in vitro* evaluation done to find out the efficacy of different antagonists, all the three antagonists *P. fluorescens, T. viride* and *A. niger* were found to be effective against both the pathogens. Among the fungicides tested, Bordeaux mixture was most effective against both the fungal pathogens. Against the bacterial pathogen Kocide followed by Bordeaux mixture was effective. None of the fungicides were fully compatible with the antagonists. But Kocide was more compatible with *A. niger* and *P. fluorescens* and Fytolan with *T. viride*. Studies on the effect of culture filtrates showed its effectivness only against bacterial pathogen. The inhibitory effect of volatiles of fungal antagonists or fungal pathogens was also revealed.

In the field experiment, *Trichoderma viride* was found to be the efficient antagonist. Among soil amendments, coirpith was found to be best for increasing germination, leaf number, rhizome yield and oil recovery. Farmyard manure treated plots was on par with that of coirpith treated plots with *T. viride* for giving maximum yield, and disease control. The study on the effect of soil microbial population revealed that the population does have an influence on yield and also on disease management. The correlation studies also showed that population of bacterial wilt pathogen is directly correlated with percentage wilt incidence.