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MANAGEMENT OF *Phytophthora* DISEASE IN BLACK PEPPER NURSERY

By

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THESIS

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Faculty of Agriculture

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2003

DECLARATION

I hereby declare that the thesis entitled **"Management of *Phytophthora* disease in black pepper nursery"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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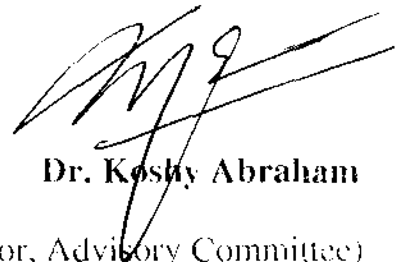

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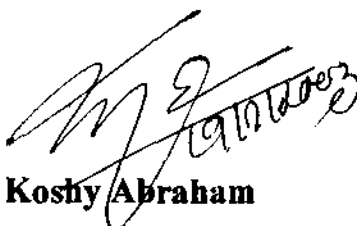

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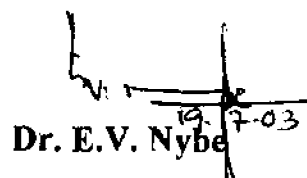

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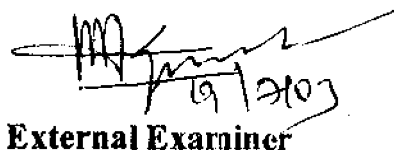

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Dedicated to my beloved parents

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Introduction

1. INTRODUCTION

Black pepper (*Piper nigrum* L.), popularly known as "King of Spices" is a native of Malabar coast of Western ghats of India. It is one of the important spice crops fetching an annual export earning of Rs. 4165.22 million to the Country (Rajan and Sarma, 2000). India, a leading producer and exporter of black pepper, has a unique position in spice trade and contributes to the major share of total world production with a production staggering around 60-65 thousand tonnes per year (Peter and Nybe, 2003). In India, more than 90 per cent of the area under black pepper is confined to Kerala state. Inspite of this, the productivity of black pepper realized in the state is not in any way near to the potential yielded level expressed by the crop. One of the major factors attributed to this low productivity is the high incidence of the devastating disease, the foot rot of black pepper.

Phytophthora capsici Leonian emend A. Alizadeh and P.H. Tsao, the foot rot pathogen, is very serious in all black pepper growing areas of the state and take a heavy toll of the crop every year (Sarma *et al.*, 1994 and Anandaraj and Sarma, 1998). This disease continues to be the major production constraint in all the pepper growing countries. The annual crop loss due to *Phytophthora* foot rot on global scale is estimated to be around 4.5-7.5 million dollars (Rajan *et al.*, 2002). The crop loss due to foot rot of pepper is reported to range upto 30 per cent (Samraj and Jose, 1966 and Nambiar and Sarma, 1977). Crop loss due to foot rot during 1982-1986 was estimated as 3.4 and 9.4 per cent in two major pepper growing districts (Kozhikode and Kannur) of Kerala (Anandaraj *et al.*, 1989a). Irrespective of the age of the plant, the fungus infects roots, stem, leaves and all other parts and causes severe crop loss (Plate 1).

Black pepper is propagated mainly through stem cuttings raised in nursery. One of the important factors, which limit the production of quality cuttings of black pepper in the nursery, is the occurrence of diseases. Among the various diseases, *Phytophthora* rot incited by *P. capsici* is the most serious one. This disease assumes serious proportions during South West monsoon and if proper and timely management practices are not adopted, cent per cent mortality of cuttings may occur. At times, this soil borne pathogen

Plate.1. Symptoms of *Phytophthora* rot in black pepper nursery

is inadvertently carried from nursery to the main field, if apparently healthy cuttings from infected nursery are used for planting.

As in the case of many other soil borne diseases, *Phytophthora* rot in black pepper nursery is also not amenable to a single method of control. Hence, an integrated approach involving cultural, biological and chemical methods are essential. Use of disease free planting material, solarization of potting mixture, addition of AM fungi and biocontrol agents like *Trichoderma* spp., regulation of aeration and shade and prophylactic application of fungicides for the management of this disease has been well documented (Anandaraj and Sarma, 1994). However, at times these measures may not provide desired level of management of the disease. Main reasons behind this failure are selection of biocontrol agents suitable for particular locality, inadequate and untimely cultural control operations and the improper use of fungicides incompatible with the antagonists.

Considering the above facts, the present study was undertaken on the management of *Phytophthora* rot of black pepper in the nursery with the following objectives:

- Isolation of the pathogen
- Isolation of antagonists of *P.capsici* from black pepper nurseries from Thrissur district
- Assessment of comparative efficacy of selected antagonists with *T.harzianum* to *P.capsici*
- Assessment of level of compatibility of selected antagonists and *T.harzianum* to common fungicides, insecticides and fertilizers used in pepper gardens
- Evaluation of antagonists in black pepper nursery

Review of literature

2. REVIEW OF LITERATURE

Phytophthora disease in black pepper nursery, incited by the ubiquitous soil borne fungus, *Phytophthora capsici*, poses serious threat in the production of quality planting material. This disease causes extensive damage to black pepper cuttings in the nursery as well as to pepper vines in the main field. *Phytophthora* rot disease in the main field is known as *Phytophthora* foot rot or quick wilt. Leafmans (1934) recorded vine death of about 10 per cent in West Borneo. Harper (1974) reported an yield loss of 50 per cent in Indonesia due to this disease. In India, forty to fifty per cent destruction of pepper vines due to the epidemic of the disease was recorded during 1967-68 (Dewaard, 1979). Sastry (1982) and Dutta (1984) recorded heavy incidence of the disease in Uttara Kannada and Shimoga districts of Karnataka causing 100 per cent death of vines in some gardens.

Crop loss to the tune of 25-30 per cent vine death has been reported from Kerala (Nambiar and Sarma, 1977). A survey conducted at Kozhikode and Kannur districts of Kerala, revealed that foot rot incidence ranged from 3.7 and 9.4 per cent resulting in an annual loss of 119 and 905 tonnes of black pepper respectively (Balakrishnan *et al.*, 1986). There are instances of complete wipe out of pepper plantations due to the disease in many areas of Kerala particularly in Idukki and Waynad districts. Incidence of *Phytophthora* rot is more pronounced in rooted cuttings raised in conventional nursery. It was estimated that about 123 million rooted cuttings were required to replant or gap fill 50 per cent of the total area under pepper in India (Sarma *et al.*, 1988). If the disease is unchecked in the nursery, it would result in the complete destruction of cuttings and further, any latent infection of the cuttings from the nursery would spread the disease in the field resulting in the introduction and establishment of the dreaded pathogen in the main field.

Sudden collapse and death of pepper vines was first reported from Lampung, Indonesia in 1885. In India, occurrence of the disease was reported as early as 1902 (Barber, 1902) from Waynad regions of Kerala. Butler (1906) coined the term "wilt" for

the disease of black pepper where there was a rapid death of the plant. Rao (1929) isolated *Phytophthora* sp. from diseased black pepper from Karnataka. Later, Muller (1936) reported a similar type of disease from Dutch East Indies caused by *Phytophthora palmivora* var. *piperis* and he coined the term "foot rot". Samraj and Jose (1966) gave the first authentic report on the *Phytophthora* wilt in black pepper from Kerala. They established the pathogenicity of *Phytophthora* in black pepper and adopted the identification as *P. palmivora* var. *piperina*. Nambiar and Sarma (1977) referred the disease as quick wilt disease of black pepper based on the sudden wilting and death of the vine. However, the terminology of the disease has been changed to *Phytophthora* foot rot.

2.1 CAUSAL ORGANISM

Rao (1929) and Leafmans (1934) identified the pathogen only up to the generic level. Muller (1936) first identified the *Phytophthora* isolates from black pepper as *P. palmivora* and added a varietal epithet 'piperis' on the grounds of its pathogenicity to *Piper nigrum*. But later, various other workers treated it as *P. palmivora* without giving any importance to the variety (Holliday and Mowat, 1963; Turner, 1969; Alconero *et al.*, 1972 and Nambiar and Sarma, 1976).

In the Cocoa *Phytophthora* workshop held in 1976 at Rothamsted Experimental Station, England, it was agreed that there were three and possibly more distinct forms like MF_1, MF_2, MF_3 and MF_4, "other types" and "pepper forms" and that the black pepper isolates of *P. palmivora* should be described as a separate species. Since then, the foot rot pathogen of black pepper was designated as *P. palmivora* MF_4 that exhibit different degrees of morphological variation (Sarma *et al.*, 1980 and Tsao *et al.*, 1985)

Certain non- piper isolates of the so called *P. palmivora* MF_4 were later renamed as *P.capsici* by Kunimoto *et al.* (1976) and some black pepper isolates were likewise identified as *P. capsici* (Kasim, 1978). Brasier and Griffin (1979) noted that *Phytophthora* isolates from black pepper closely resembled *P. palmivora* MF_4 in sporangial shape (fan shaped/ umbellate), sporangial pedicel type (extremely long pedicels), double septate

sporangia, oogonial morphology and their ability to respond to *Trichoderma* and therefore, suggested both black pepper and MF_4 forms as one and the same species. Indeed, many morphological similarities exist between *P.capsici* and the so called *P.palmivora* and therefore, *Piper nigrum* isolates formerly thought to be MF_4 were considered to be *P. capsici* by Kaosiri *et al.* (1978) and Sarma *et al.* (1980).

Detailed studies have been carried out on the variability and biology of the organism (Sastry and Hegde, 1987a and Santhakumari, 1987). The fungus often exhibited umbellate sporangial ontogeny with caducous sporangia with long pedicels. Sporangial shape varied from ovoid to pyriform with a tapering base. The fungus grew luxuriantly at 25-30 °C on carrot agar medium. Sporangial production was abundant under continuous light and zoospores germinated within 15-20 minutes after encystment.

Tsao and Alizadeh (1988) merged the two species (MF_4 isolates and *P.capsici*) into a single species and were designated as *Phytophthora capsici* Leonian emend A. Alizadeh and P.H. Tsao. Some of the important characteristic features of *P.capsici* as given by Tsao and Alizadeh (1988) and Zentmeyer (1988) are given in Table 1.

2.2 EPIDEMIOLOGY AND DISEASE SPREAD

Many workers carried out various studies, which lead to a better understanding of the epidemiology and spread of foot rot of black pepper.

The pathogen, *P.palmivora* infecting various crops is most active in warm tropical regions receiving higher amounts of rainfall (Coleman, 1910; Sundararaman and Ramakrishnan, 1924 and Rao, 1927). Muller (1936) reported that the chief source of infection of *Phytophthora* on black pepper was contaminated and diseased plant refuse. He also reported that the symptom of the disease was more pronounced under high relative humidity (91-99 per cent) and low temperature (19-23°C) and the pathogen remained inactive when the season was dry. Holliday and Mowat (1963), Samraj and Jose (1966) and Nambiar and Sarma (1976) also made similar observations.

Table 2.1. Characteristic features of *P.capsici*

SL.No.	Characters	Description
I	Colony morphology	petalloid pattern with diffuse edge; uniform dense aerial mycelium over entire colony on CA medium.
II	Sporangial characters	
1	Shape	spherical, ovoid, obovoid, ellipsoidal, fusiform, pyriform, rounded/tapered base.
2	Papilla	prominent occasionally
3	Sporangial size	40-52 x 20-31µm
4	L/B ratio	1.6-2.0
5	Caducity	caducous
6	Pedicel type	narrow , long and not occluded
7	Pedicel length	20-150µm, sometimes upto 250µm
8	Ontogeny	umbellate / irregular
III	Chlamydospores	rarely produced.

Tsao and Alizadeh (1988)

Zentmeyer (1988)

Transmission of the pathogen through rain and wind were reported by many workers (Muller, 1936; Holliday and Mowat, 1963 and Anon., 1965). Holliday and Mowat (1963) also observed that the spread of the disease was rapid in Sarawak due to continuous wet season coupled with application of large amounts of organic fertilizers. According to them infection started from fine roots, supporting the fact that the disease was soil borne.

Nambiar and Sarma (1977) reported that spread of the disease mainly occured through soil and water. Mammootty and Pillai (1981) noticed rotting of black pepper cuttings in the nursery under cloudy atmospheric conditions with higher atmospheric temperature and humidity. Das and Cheeran (1985) opined that arecanut, rubber, cocoa, coconut and cardamom harbouring *P. palmivora* served as collateral hosts for black pepper infection. According to Ramachandran *et al.* (1986), the spatial distribution of *Phytophthora* propagules in a plantation showed that inoculum was more upto 30 cm from the base and in upper layers of soil. It decreased with increase in depth and distance from the base of the vine.

Sastry and Hegde (1987 b) observed that wilt of pepper was favoured by high rainfall and high relative humidity. Nair *et al.* (1988) opined that a positive significant correlation was there between weekly incidence of disease and relative humidity, rainfall and number of rainy days, while the maximum temperature and bright sunshine hours showed significant negative correlation with disease in a pure black pepper plantation.

In addition, Ramachandran *et al.* (1990) observed that the initial symptom appeared on tender leaves and terminal portions of runner shoots creeping on the ground, which indicated that the soil was the primary source of inoculum. Planting of such rooted cuttings raised from runner shoots lead to gradual inoculum built up from its early stage of the growth.

Matsuda *et al.* (1994) opined that outbreaks of pepper root rot were enhanced by long periods of cloudy, humid weather with occasional rainfall. Anandaraj (1997) also noticed that infected plant debris in the soil and infected and dried up vines in the gardens

appear to be the primary source of inoculum and also opined that cummulative feeder root rot reaches the main root system ultimately culminating in foot rot.

Mammootty (2003) reported that daily rainfall of 15.8-23.0 mm, temperature range of 23-30 °C, relative humidity of 80-99 per cent and sunshine hours 2.8-3.5 hours per day favoured the initiation and spread of infection. He also found that the pathogen survived in infested plant debris and even in soil upto 19-24 months in the absence of host plant.

2.3 SYMPTOMATOLOGY

Many workers studied the symptomatology on *Phytophthora* foot rot disease of black pepper.

According to Butler (1906), the first visible symptom was the starved appearance of the vines and this was attributed to the loss of turgidity in leaves and leaf stalks resulting in drooping. Muller (1936) observed that all parts of the plant at all stages of growth were susceptible to the disease and according to him typical symptoms like leaf rot, collar rot and root rot are generally observed in a foot rot infected plant. He also observed inconspicuous greyish brown lesions near the tip and margin of lower leaves.

Samraj and Jose (1966) observed that infection occurred at a height of 25 cm above the soil level. They also found that leaves turn pale and flaccid and ultimately the plant died. Holliday and Mowat (1963) and Turner (1969) from Sarawak and Nambiar and Sarma (1976) from India observed zonate lesions with fimbriate margins on the infected leaves. In addition, Turner (1969) observed rapid development of symptoms within 36-48 h of leaf inoculation. Lee (1973) reported vascular browning at the points beyond the site of infection.

Appearance of water soaked lesions on the leaves and stems of infected vines and brown discolouration of infected fine roots were reported by Mammootty *et al.* (1980). They also observed flaccidity of younger and mature leaves followed by yellowing of

younger leaves. Mammootty and Pillai (1981) observed two types of rotting symptoms in pepper nursery. In one case, decaying starts at the lower cut end which gradually spreads upwards and ultimately the plant dies, whereas in the second type, rotting starts at the soil surface level and spreads both upwards and downwards.

Sarma and Nambiar (1982) noticed acropetal and basipetal advance of the pathogen in black pepper vines infected at the collar region. Discolouration of vascular bundles was also observed. According to Ramachandran *et al.* (1986), infections at the base (collar and foot) and roots were more destructive as they lead to outright death of vines. Sarma *et al.* (1988) treated root and stem infection of vines as most fatal as the infected vines succumbed within 10-20 days. Anandaraj and Sarma (1995) reported that being a soil borne pathogen, the fungus gained entry into main roots through fine roots and reached the foot and collar of the vine and culminated into foot rot. Further, rotting of roots impeded transportation of water and minerals thus brought physiological drought in plants.

2.4 MANAGEMENT OF THE DISEASE

2.4.1 **Chemical control**

Several workers studied the effect of plant protection chemicals and fertilizers against the pathogen. *P.capsici.*

2.4.1.1 ***Fungicides***

According to Turner (1969), Ferbam and Anthracol gave best results as protectants and were comparable to copper fungicides and that Brunolex and Nectryl were very effective for soil drenching against *P. palmivora* on black pepper. Turner (1970) also reported that laboratory screening of fungicides for use as soil drench against foot rot of black pepper showed that Vapam, Tillex and Dazomet to be the most effective.

Kheng (1971) observed the effectiveness of difolatan as leaf protectant and soil drench followed by Uspulum and Kocide 101 against *P. palmivora* on black pepper. According to the Anon. (1972), difolatan and Bordeaux mixture controlled *P. palmivora* of black pepper in the field. Turner (1973) elucidated the *in vitro* effect of Vapam, Tillex, Dazomet and Shell SD-345 followed by Verdasan and Nectryl against the sporangial production of *P. palmivora*. Filani (1976) noticed that the growth of *P. palmivora* was restricted by cuprous oxide, copper sulphate, copper oxychloride, copper hydroxide and copper carbonate at all concentrations tested and that total inhibition was produced by cuprous oxide at 200-250 ppm. Mammootty (1978) observed reduced growth of *Phytophthora* causing foot rot of black pepper in media incorporated with Dithane M-45 and Dithane Z-78.

A significant reduction in infection of black pepper was obtained after treatment with Actidion, difolatan, copper oxy chloride, Bordeaux mixture and DOWCOW-269 against foot rot infection. (Anon., 1977). Kueh and Khew (1980) reported that difolatan, copper oxychloride, Vapam, Verdasan, ethridiazole, cycloheximide and DOWCOW-269 were very effective for decreasing the rate of infection caused by *Phytophthora* in black pepper. The effectiveness of Bordeaux mixture as drench and spray for the control of nursery diseases of black pepper was well established by Mammootty *et al.* (1980).

Bruck *et al.* (1980) reported that the effect of metalaxyl on *Phytophthora* spp. was by blocking the formation of secondary haustoria and mycelial growth inside the leaf, lesion formation and sporulation. Kueh (1982) noticed the effectiveness of Ridomil (metalaxyl) followed by RE-26940, DOW-4408, Previcur N and Captafol in the control of *Phytophthora* foot rot of black pepper. Prophylactic spraying of Bordeaux mixture (1per cent) followed by soil drenching with Emisan (0.1per cent) coupled with foliar application of Difolatan (0.1per cent) at weekly intervals effectively controlled the rotting disease in pepper nurseries (Mammootty and Pillai, 1981).

Prophylactic application of Bordeaux paste to the collar, once during May- June period and foliage spray and soil drenching with Bordeaux mixture (1per cent) or 0.2 per

cent copper oxychloride twice as a pre monsoon and post monsoon treatment reduced the disease incidence (Sarma *et al.*, 1987). In addition, they also reported that spraying the cuttings in the whole nursery with Bordeaux mixture (1per cent) or copper oxychloride (0.2per cent) or prophylactic spray with Ridomil–Ziram (2.5ml l^{-1}) at monthly intervals gave good control of *Phytophthora* rot in nursery. Besides, metalaxyl was found compatible and synergistic with insecticides like endosulfan and quinalphos, the pesticides used in black pepper pest control.

According to Ramachandran and Sarma (1985), the best control of *P. palmivora* MF_4 on *Piper nigrum* was obtained with Ridomil compared to Ethridiazole (Terrazol) and fosetyl aluminium (Aliette). Sastry and Hegde (1987) also reported similar observations. Kasim (1986) obtained lowest disease index on infected black pepper with Ridomil followed by Aliette and Dithane M-45.

Ramachandran *et al.* (1988) noticed the sensitiveness of different isolates of *Phytophthora* to metalaxyl from different host plants including black pepper. Ramachandran *et al.* (1990) opined that it was not hazardous to use Metalaxyl in pepper plantations as detectable levels of metalaxyl were not observed in dried black pepper berries from vines treated four and six months after fungicide application.

Malebennur *et al.* (1991) stated that percentage disease incidence was lower in black pepper vines treated with Bordeaux mixture - copper oxychloride or Bordeaux mixture - metalaxyl or Bordeaux mixture - Captafol. Nair and Sasikumaran (1991) and Mammootty *et al.* (1991) reported that Bordeaux mixture gave the best control against foot rot incidence of black pepper followed by metalaxyl, copper oxychloride and Captafol.

Reduction in the incidence of foot rot of black pepper with Bordeaux mixture pasting, spraying and drenching (BMPSD) was noticed by Nair *et al.* (1993). However, Sarma (1994) opined that soil drenching with copper oxychloride and Bordeaux mixture spraying alternated with Ridomil were effective in reducing foot rot infection. Soil drenching with a mixture of metalaxyl, mancozeb and benomyl also gave good control of

the disease (Matsuda *et al*., 1994). Sarma *et al*. (1994) also reported that metalaxyl - ziram was found effective in checking infection and fosetyl Al was the next best.

Aerial spraying and soil drenching of potassium phosphonate (Akomin-40) gave maximum reduction of foliar and root infection (Veena and Sarma, 2000). They noticed absence of phytotoxicity on black pepper even at 4000ppm of Akomin-40.

The studies conducted at Kerala Agricultural University revealed the effectiveness of Bordeaux mixture, Akomin, Difolatan and Validamycin as drench and spray in checking the incidence and severity of *Phytophthora* rot in black pepper nursery. Further, it was reported that fungicides *viz*., Bordeaux mixture, Agallol, Bayer-5072, Thiride and Dithane M-45, checked the growth of the fungus *in vitro* as well as in the nursery. Control of rot disease in pepper nursery with Ridomil, Cuman L, Kitazin, Thiride, Bayer-5072 and Emisan was also reported (KAU, 2000).

Veena *et al*. (2002) noticed that the sporulation of *P. capsici* of black pepper was the most sensitive stage to potassium phosphonate and that the mycelial growth was least affected. Spraying Bordeaux mixture and drenching the basin with either Bordeaux mixture or copper oxychloride was advocated against the foot rot incidence of black pepper (Mammootty, 2003). Alternate application of potassium phosphonate and metalaxyl was also recommended.

There are many reports on the chemical control of various other species of *Phytophthora* on different crops. Wilson *et al*. (1974) reported complete inhibition of mycelial growth of *Phytophthora* species causing leaf and capsule rot of cardamom with Ceresan wet, Difolatan, Dithane C-90, Dithane M-45, Kocide, Miltox and Thiride.

Figueiredo and Lellis (1981) reported the effect of copper oxy chloride in inhibiting the growth of *P. palmivora* under *in vitro* conditions. According to Coffey and Bower (1984), *P. palmivora* was very sensitive to metalaxyl even at lower concentration of 0.1µg ml^{-1}. Tey and Wood (1984) reported that among the eight fungicides tested *in vitro*

against *P.palmivora*, mancozeb and cycloheximide were highly toxic at low concentration. Among the different fungicides tested *in vitro*, Raghu and Chandramohanan (1993) found that Ridomil MZ, Foltaf and Captaf inhibited *P.palmivora* infection on the detached cocoa pods.

The *in vitro* studies conducted by Khan *et al.* (1996) revealed that metalaxyl followed by Captan and copper oxychloride inhibited the mycelial growth of *P.cactorum*. Foliar sprays of partially neutralized phosphonic acid substantially reduced tuber infection by *P. infestans* causing potato late blight. Johnson and Palaniswami (1999) observed that Ridomil in *in vitro* could effectively control *P.palmivora* causing cassava tuber rot.

Jiazhuang and Yan (2000) could control papaya epidemic disease caused by *P. palmivora* and *P. capsici* by spraying metalaxyl. According to Mahanty (2000), fosetyl Al was the best in inhibiting the germination of sporangia of *P. parasitica*, which caused foot rot in betel vine, while mancozeb and chlorothalonil were the best in inhibiting the growth of mycelial cultures.

May and Kimati (2000) found that metalaxyl was the most efficient fungicide for inhibiting the mycelial growth of *P. parasitica*. Several reports in early years indicated that application of high concentration (>1000 ppm) of neutralized phosphorous acid and its salts (phoshonite and phosphonate) could directly protect the plants by inhibition and interference of the mycelial growth and sporangial production of *Phytophthora* species and other members of Oomycetes (Ann, 2001).

2.4.1.2 ***Fertilizers***

It is found that not much work has been conducted on the *in vitro* effect of fertilizers against *Phytophthora* spp. However, Lilly and Barnett (1951) reported that in general nitrate nitrogen favoured the mycelial growth of many fungi. Cameron and Milbrath (1965) and Pal (1974) reported that ammonium nitrate acted as the best nitrogen source for the growth of *Phytophthora* spp.

According to Jain *et al.* (1982), among the inorganic nitrogen salts, ammonium nitrate supported the growth of *P. parasitica* var. *nicotianae* followed by ammonium sulphate and ammonium chloride. Jayasekhar and Muthusamy (2000) studied the effect of six nitrogen sources *viz.*, ammonium sulphate, ammonium chloride, ammonium nitrate, sodium nitrate, potassium nitrate and peptone on growth of *P. capsici*, the causal agent of foot rot of black pepper. They found that ammonium nitrate was the best nitrogen source for the growth of isolates that recorded three fold increase of mycelial dry weight, which was closely followed by ammonium sulphate and ammonium chloride.

2.4.2 **Biological control**

According to Baker and Cook (1974), biological control is defined as "the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of environmental, host or antagonist or by mass introduction of one or more antagonists". Biological control, using microorganisms against the plant pathogens is an effective and alternative tool in managing the disease and is gaining importance in recent years. It aims at biological destruction of soil borne pathogens without impairing ecological balance. Although concerted efforts on biocontrol of pathogens were made since 1930's (Weindling, 1932), pragmatic approach to tackle the problem was made only in recent years.

Sarma *et al.* (1991) emphasized the need for biological control by manipulating the microbial status of soil suppressive to the pathogen. Baker and Cook (1974) stated that "antagonistic potential resides in every soil microorganism and antagonists should be sought in the rhizosphere rather than in the soil mass as their effective activity will be probably on the root surface"

A number of fungi belonging to the genera *Talaromyces*, *Pencillium* were found antagonistic to *P.palmivora* of black pepper (Dutta, 1984). Also, *Coniothyrium*, *Gliocadium*, *Trichoderma*, *Latesuria*, *Sporodesmium*, *Aspergillus* and *Fusarium* and

several bacteria and actinomycetes are known for their potential biocontrol activities against soil borne pathogens including several species of *Phytophthora* (Malajezuk, 1983; Adams, 1990 and Naik and Sen, 1992).

Biological control, especially using fungal antagonists against fungal pathogens has gained considerable attention and appears to be promising as a viable supplement or alternative to chemical control (Natarajan and Manibhushanarao, 1996). A combination of fungal antagonists like *Aspergillus* spp. and *Penicillium* spp. and several rhizosphere bacteria like fluorescent *Pseudomonads* isolated from different pepper growing regions were found to delay foliar infection and provided prolonged protection to black pepper against *P.capsici* (Jubina and Girija, 1997 and 1998).

2.4.2.1 ***Trichoderma* spp.**

Among the antagonists, *Trichoderma* plays a vital role in plant disease management and has opened new vistas for the commercialization. Several studies suggested the potential use of *Trichoderma* as an effective biocontrol agent against *Phytophthora* diseases of crop plants. The genus *Trichoderma* has been demonstrated to be a potential biocontrol agent against plant pathogenic fungi (Liu and Baker, 1980).

2.4.2.1.1 ***In vitro* antagonism of *Trichoderma* spp.**

Antibiotics produced by *Trichoderma* spp. have long been reported to be involved in biocontrol activities (Weindling, 1934). Pyke and Dietz (1966) described dermadine, a major volatile antibiotic produced by *Trichoderma*. Dennis and Webster (1971) stated that the inhibitory action of antagonists against the pathogen *in vitro* might be due to the production of inhibitory volatile metabolites. Reeves and Jackson (1972) and Brasier (1975) reported that *Trichoderma* spp. induced development of sex organs in normally sterile isolates of *Phytophthora* spp.

A number of parasitic fungi capable of penetrating thick walls of both chlamydospores and oospores have been identified (Snahe *et al.*, 1977). The mechanisms by which *Trichoderma* spp. control disease included competitive saprophytic ability, antibiotic production, direct parasitism and lysis (Ayers and Adams, 1981 and Bell *et al.* 1982). Antagonistic activity of the organism was mainly attributed to mycoparasitism, antibiosis and predation. Mycoparasitism by enzymatic lysis of pathogenic fungal hyphae through the production of enzymes like β - (1-3) glucanase, chitinase, cellulase and protease has been reported by Elad *et al.* (1983). Cristinzio (1987) noticed potential antagonism of *Trichoderma* species on *P.capsici* in capsicum *in vitro*. Mycoparasitism by *T. harzianum* combined with the production of cell wall degrading enzymes (Ridout *et al.*, 1986) and volatile alkyl pyrones, antibiotics (Claydon *et al.*, 1987) has also been documented.

Vinod (1988), Gokulapalan (1989) and Mukherjee *et al.* (1989) observed that between fungal antagonists and the pathogen, the parasitic hyphae of the fungal antagonists overgrew the pathogens at several places and ran closely along with host hyphae. Adams (1990) suggested that biocontrol methods using *Trichoderma* spp. could be made successful with at least 10^5 propagules per gram soil. Cates (1990) visualized large scale use of *Trichoderma* as a biological fungicide in control of plant diseases. Georgieva (1991) reported that soil application of *T.viride* (2 g / vine) to black pepper at the time of transplanting was highly effective than metalaxyl compounds against *Phytophthora* root rot.

In vitro study by D'Ercole *et al.* (1993) revealed that *T. harzianum* and *T. viride* produced volatile compounds like caprilic, caprinic, capronic acid, ethylene and formic aldehydes. Harman *et al.* (1993) purified chitinolytic enzymes produced by *T. harzianum*. Faull *et al.* (1994) observed production of homothalin II and isonitrile antibiotic by mutant strain of *T. harzianum*. According to Kausalya and Jeyarajan (1994), *Trichoderma* spp. can survive for long periods in soil only in the presence of food base in the form of organic substrates like farmyard manure, wheat bran, rice bran and groundnut shell.

Thomas *et al.* (1996) observed various antagonistic mechanisms of *Trichoderma* spp. and concluded *Trichoderma* as a potential bioagent against *Phytophthora* of small cardamom. Antagonistic potentiality of *Trichoderma* mutants when grown in dual culture against root rot and wilt causing pathogens was estimated by using a modified antagonistic index by Kasinathan (1998).

Sivasithamparam and Ghisalberti (1998) listed 43 substances produced by *Trichoderma* spp. that have antibiotic activity and of these alkyl pyrones, isonitriles, polyketides, peptaibols, diketopiperazines, sesquiterpenes and steroids have frequently been associated with biocontrol activity of some species and strains of *Trichoderma*. Bhai (2000) observed that *Trichoderma* spp. overgrew the colony of *Phytophthora* and parasitized the hyphae when both were grown on agar media. She also observed hyphal lysis, penetration and coiling of the parasite besides the production of volatile compounds.

2.4.2.1.2 ***In vivo antagonism of Trichoderma* spp.**

Sarma *et al.* (1994) noticed predominance of *Trichoderma* spp. antagonistic against *P.capsici* in black pepper. Anandaraj and Sarma (1994 and 1995) recorded potential biocontrol activity of *T. hamatum* and *Gliocadium virens* both under green house and field conditions. They also reported the disease suppressive role of *Trichoderma* spp. against *P. capsici*.

Sivaprasad (1997) reported enhanced crop protection against *P. capsici* from dual inoculation of VAM and *Trichoderma* spp. in black pepper. Robert (1998) elucidated the effectiveness of AMF isolates Pi-11, *Aspergillus* sp. and *T. viride* in reducing the symptom development in black pepper by *P. capsici*. Sodsa-art and Soytong (1999) noticed that a mixture of *Trichoderma* and *Chaetomium* mycofungicides significantly controlled the root and stem rot of black pepper caused by *P. palmivora*.

Elad *et al.* (1999) observed that some *Trichoderma* strains clearly are potent inducers of SAR-like responses which may be indicated by defense responses like

production of chitinase and peroxidases in both root and leaf tissue of treated plants. According to Harman (2000), *Trichoderma* spp. exhibited two types of mechanisms like rhizosphere competence, which is the ability of the microorganisms to grow and function in the developing rhizosphere and induced systemic acquired resistance (SAR), which gave long term protection at a substantial distance from the infection court.

Joe (2000) revealed the efficacy of *Trichoderma* mixed with compost in controlling *Phytophthora* sp. in black pepper. According to Mahanty *et al.* (2000), although biological control approach was not better than chemical control in terms of per cent disease incidence caused by *Phytophthora* in betel vine, the use of highly effective isolate of *T.harzianum* was much safer and gave equivalent yield in terms of number and weight of leaves produced.

Patel and Anahosur (2001) revealed that mode of antagonism of *T.harzianum* was not necessarily similar towards all host fungi. *T. harzianum* and *Alcaligenes* sp. strain AMB-8 applied alone or in combination significantly reduced the incidence of *P. capsici* in black pepper nursery (Anith and Das, 2001). According to Rajan *et al.* (2002), *T. virens* (TV-12) and *T. harzianum* (T-harz-26) were found more effective to control the foot rot disease and the isolate T. harz-26 was most adaptive to the rhizosphere of black pepper when compared to other isolates.

The isolates of *Trichoderma viz., T. virens, T. aureoviride, T. harzianum, T. pseudokoningii, T. polysporum, T. longibrachiatum* and *T. koningii* were able to inhibit, overgrow and lyse the mycelia of *P. capsici* of black pepper to varying degree (Saju, *et al.*, 2002). He also reported that percentage inhibition of foot rot by different species of *Trichoderma* varied from 20-81 per cent. Sarma (2003) noticed the disease suppression of root rot of black pepper caused by *P. capsici* through soil application of *T. harzianum*. Same effect was also noticed with the application of VAM.

2.4.2.1.3 ***Compatibility of Trichoderma* spp. *with fungicides***

According to Papavizas (1985), integrated approach can be successful only if antagonists are compatible with fungicides and biopesticides. Integration of effective antagonists with fungicides is important for the management of diseases. It is felt that, if the pesticide tolerant strains of *Trichoderma* spp. are identified then it may be possible to use them with lower concentration of desired fungicide against the pathogen. For such treatments, *Trichoderma isolates* may serve as an additive component in IDM system.

Several workers reported that some strains of *T. harzianum* Rifai were tolerant to fungicides and were used for the integrated control of plant diseases (Henis *et al*., 1979; Papavizas and Lewis, 1981; Papavizas, 1982; Upadhyay and Mukhopadhyay, 1986). The fungicides *viz*., chlorothalonil, MBC. Captan and Captafol were found as tolerant for *T. harzianum* even at higher concentrations (upto 2000 μgml^{-1}) in spore germination tests (Moity *et al*., 1982; Papavizas *et al*., 1982).

Metalaxyl (0.1per cent) and carbendazim (0.0065per cent) were seemed to be safe tolerance limit (ED_{50}) for *T.harzianum*. Similar results were obtained for Metalaxyl (Mukhopadhyay *et al*., 1986, Mukherjee *et al*., 1989) and for carbendazim and benomyl (Papavizas *et al*., 1982 and Viji *et al*., 1997).The compatibility of fungicides such as Captan, chlorothalonil. PCNB, Chloroneb, metalaxyl and potassium phosphonate to *Trichoderma* spp. has been reported by Moity *et al*., (1982); Wongwathanarat and Sivasithamparan, (1991) and Rajan and Sarma, (1997). Captan was found fungicidal to *T. viride* while, it had little effect on *T. harzianum* (Krishnamoorthy and Bhaskaran, 1994). They also observed that in the copper oxychloride poisoned medium, *T. harzianum* showed normal growth and sporulation while, *T. viride* did not sporulate.

Mondal *et al*. (1995) found that the mycelial growth of all the *Trichoderma* spp. was arrested to a greater extent with the addition of 200 and 500 ppm Carbendazim and tebuconazole in culture medium. *T. koningii*, *T. harzianum* and *T. lignosum* were compatible with carboxin at 200 and 500 ppm concentration. Sharma and Mishra (1995)

reported that fungicides like metalaxyl, chlorothalonil and Captafol showed little inhibition to *T. harzianum*, while Thiram was highly inhibitory even at low concentrations.

Singh *et al.* (1995) screened several isolates of *Trichoderma* spp. (*T. harzianum*, *T. reesei* and *T. koningii*) against common fungicides like Captaf, DithaneM-45 and Thiram and they found that the growth of *T. harzianum* was inhibited to an extent of 94.5 per cent at 500 ppm of Captaf, 63 per cent with 500 ppm of Dithane M-45 respectively after 3 days of incubation. The 200ppm concentration of Thiram completely inhibited the growth of the isolates, while the growth of *T.viride* and *T.koningii* were found comparatively compatible for all the fungicides tested.

Shanmugham (1996) found that Bordeaux mixture completely inhibited the growth of *T. viride*. May and Kimati (2000) reported that among several contact and systemic fungicides tested, metalaxyl at all tested rates (1, 10,100 μg l^{-1}) did not interfere with the mycelial growth of *Trichoderma*. According to Akbari and Parakhia (2001), thiran, mancozeb, tridemorph, metalaxyl MZ and fosetyl- Al were found non- inhibitory to *T. harzianum*, *T. viride* and *G.virens* at all concentrations tested. Chlorothalonil was found inhibitory to *T. harzianum* and *T. viride* but not to *G. virens*. Carbendazim inhibited the growth of antagonists at all concentrations tested.

Among two systemic and six non-systemic fungicides tested, tolerance of *T. harzianum* for metalaxyl was seven times higher than Carbendazim (Sharma *et al.*, 2001). They also reported that mancozeb and copper oxychloride inhibited maximum growth at 785 and 805 μg ml^{-1} respectively. Also, the concentration of 169, 375 and 625 μg ml^{-1} of Captan, Captafol and chlorothalonil seems to be a safer tolerance limit for the biocontrol agents. However, there was an increasing trend of inhibition at higher concentration of the fungicides tested.

2.4.2.1.4 *Compatibility of Trichoderma* spp. *with insecticides*

In the integrated disease management programme (IDM) chemicals are applied along with biocontrol agents. According to Sarma *et al*., (1996 b) and Eapen and Ramana (1996) Phorate was applied to control nematodes whereas antagonistic *Trichoderma* spp. was added to soil to prevent population build up of *P. capsici*. Hence, *Trichoderma* spp. need to be pesticide resistant/ tolerant for use in such programmes.

Very few reports are available on the compatibility of insecticides with *Trichoderma* spp. Sharma and Mishra (1995) studied the compatibility of the biocontrol agent, *T. harzianum*, utilized for the management of *Phytophthora* foot rot of black pepper (*Piper nigrum*) with aldicarb, phorate and carbofuran applied for the management of nematodes and mealy bugs. The study indicated that these insecticides were less toxic. Jebakumar *et al*. (2000) reported that phorate and chlorpyriphos could be safely applied with *T. harzianum* for the management of *Phytophthora* foot rot, nematodes and mealy bugs on black pepper.

Sushir and Pandey (2001) opined that among the four insecticides tested *in vitro*, chlorpyriphos (Durmet 20 EC) was found more safer as it has no adverse effect on radial growth upto 2000µl ml $^{-1}$ concentration, whereas endosulfan (Thiodan 35 EC) and triazophos (Hostathion 40 EC) were found to be more toxic even at 50µl ml $^{-1}$ which showed growth inhibition of 55.55 and 57.77 per cent respectively. However, growth inhibition of 40 per cent was observed with dimethoate (Rogor 30 EC) at 125µl ml $^{-1}$.

Trichoderma spp. being compatible with potassium phosphonate and chlorpyriphos indicated their potential for IDM with dual mode of action in suppressing both pathogenic fungi and plant parasitic nematodes and with growth promotion in black pepper (Sarma, 2003).

2.4.2.1.5 ***Compatibility of Trichoderma* spp. *with fertilizers***

The interaction of growth of *T. harzianum* with soil application of nutrients in the form of fertilizers has not been investigated clearly yet. Though *Trichoderma* spp. were seemed to be a versatile class of fungi, capable of utilizing a wide range of nitrogen sources (Danielson and Davey, 1973), the specific reports about the relative efficacy of various nitrogen sources are inadequate. According to them nitrogenous fertilizers may have definite influence on the population and activity of *Trichoderma* propagules introduced to soil.

In general, nitrate nitrogen is most favourable for mycelial growth of many fungi (Lilly and Barnett, 1951). Kaufman and Williams (1965) and Rajan and Singh (1974) reported that nitrogen significantly influenced fungi antagonistic to soil borne plant pathogens. They also reported that application of fertilizers increased the population levels of soil saprophytes leading to increased antibiosis and competition.

Danielson and Davey (1973) reported that ammonium nitrate was found as the best nitrogen source for three species of *Trichoderma* tested. Neelamegam (1992) observed better growth of *T. viride* when ammoniacal form of nitrogen was incorporated into the medium. Krishnamoorthy and Bhaskaran (1993) noticed a significant increase in population of *T. viride* in soil following application of nitrogen and phosphate fertilizers.

Sharma and Mishra (1995) reported urea as a good source of nitrogen for *T. harzianum*, which was not only supportive, but also stimulatory to the growth and sporulation followed by ammonium sulphate. In addition to this, muriate of potash was appreciably tolerated by the bioagent, whereas zinc sulphate was highly toxic to the fungus. Jayaraj (1995) also supported these findings. Minerals like Mg, P and K are essential for sporulation of *Trichoderma* spp. (Jackson *et al.*, 1991).

Jayaraj and Ramabadran (1997) studied the influence of three nitrogenous fertilizers (urea, ammonium sulphate and ammonium chloride) on survival and competitive saprophytic ability of *T. harzianum*. It was found that all the nitrogenous fertilizers

favoured the growth and survival of *T. harzianum* in soil. Ammonium sulphate enhanced the growth and survival of *T. harzianum* to the maximum extent, followed by urea and ammonium chloride.

Jayaraj and Ramabadran (1998) also evaluated the efficacy of various nitrogen salts on the *in vitro* growth, sporulation and production of cellulase and antifungal substances of *T. harzianum*. They reported that among the various nitrogen sources, ammonium nitrate, ammonium sulphate and sodium nitrate recorded the maximum growth, sporulation, production of cellulases and antifungal substances, while urea and calcium nitrate recorded the least growth i.e., the addition of inorganic forms of nitrogen increased the production of fungal biomass.

2.4.2.1.6 ***Role of Trichoderma* spp. *as plant growth regulators***

Lindsey (1967) reported that microorganisms could induce growth of higher plants. Several reports are available on the stimulating effect of biocontrol agents in promoting plant growth when used as either seed treatment or soil application. Chang *et al.* (1986) and Mukhopadhyay (1988) demonstrated increased growth response of several crop plants in the presence of biological control agents, which may be caused by a direct effect on the plant as a biofertilizer or by control of some undiagonised plant pathogens. According to Windham *et al.* (1986) *Trichoderma* spp. produced growth regulatory factors that increased the rate of emergence of tomato and tobacco seedlings. Shoots grown in *T. harzianum* infested soil were found to be better than that grown in uninfected field (Windham *et al.*, 1989). Vrang *et al* (1990) noticed increase in growth and yield of potato, when the seed tubers were inoculated with *Trichoderma* spp.

Lynch *et al.* (1991) reported that some strains of *Trichoderma* induced seedling emergence of lettuce and produced larger plants. Lilyiona (1991) observed early flowering of potato was with *T. viride*. Seed treatment with *T. viride* and *Bacillus subtilis* along with *Rhizobium* spp. increased nodulation and plant growth characteristics in legumes (Sridhar

et al., 1992). Biological seed treatment not only reduced the disease but also increased plant stand (Mukhopadhyay, 1995).

Very few reports are available on the effect of biocontrol agents on growth of black pepper. According to Sarma *et al.* (1996 a), solarized nursery mixture fortified with mycorrhizal propagules in combination with a mixture of *Trichoderma* spp. and *Gliocladium* sp. yielded healthy and robust rooted cuttings of black pepper in the nursery. Treatment with *T. harzianum* under greenhouse conditions significantly increased root length, root dry weight, plant height, leaf number, leaf dry weight, leaf area, stem diameter and flower number per plant compared to the non-inoculated treatment of *Capsicum annuum* (Cruz and Cistierna, 1998). Seed germination, plant height, dry weight of roots, shoots and grain yield of soybean was found significantly maximum in plants raised from *T. harzianum* treated seeds (Dutta and Das, 1999).

The ability of *Trichoderma* spp. to increase the rate of plant growth and development, including their ability to cause the production of more robust roots has been reported by Binimol (2000) and Harman (2000). Lisha *et al.* (2002) noticed that the *Trichoderma* isolates obtained from black pepper rhizosphere from silent valley showed growth promotion to the tune of 55-116 per cent as compared to control. Rajan *et al.* (2002) reported that the isolate of *T. harzianum* (T. harz-26) efficiently proliferated in soil and gave good protection to the root system against *P. capsici* for a long time.

2.4.3 Integrated management of *Phytophthora* rot in black pepper

According to Papavizas (1985). in the integrated management of diseases, a combination of cultural, chemical and biological control measures is adopted besides growing of disease tolerant lines. In view of the insensitivity of the bioagents to some chemicals, biological treatment was integrated with suitable fungicides. Such treatments were found highly effective and resulted in enhanced crop performance when compared to biological or chemical treatment alone (Mukhopadhyay *et al.*, 1986 and Upadhyay and

Mukhopadhyay, 1986). In addition, soil solarization was found effective to control soil borne pathogens (Raj and Kapoor, 1993).

Katan *et al.* (1976) and Katan (1981) studied the effect of soil solarization for controlling various soil borne diseases. Sarma *et al.* (1988) reported that raising rooted cuttings in fumigated nursery mixture from the runner shoots collected from disease free gardens or raising single noded cuttings through bamboo method of multiplication would ensure disease free planting material. In addition, soil amendments like cotton seed and groundnut meal suppressed *P.palmivora* of black pepper. Anandaraj *et al.* (1989 b) suggested that application of Bordeaux mixture along with other cultural practices were very effective in managing the incidence of foot rot disease of black pepper. Sadanandan (1989) observed that application of neem cake reduced the disease incidence.

Moens and Ben-aicha (1990) and Satour *et al* (1991) reported the effectiveness of soil solarization in controlling *Phytophthora* disease in tomato and capsicum. The efficacy of soil solarization in suppressing soil borne plant pathogens was well established by Katan and DeVay (1991). Hartz *et al.* (1993) observed significant reduction in the population of *P. cactorum* and *P. citricola* by solarization. Solarization along with application 0.2 per cent of neem cake showed minimum infection in the pepper nursery followed by plant protection measures as per Package of Practices of KAU (KAU, 1994).

Sarma *et al.* (1994) and Anandaraj and Sarma (1995, 1998) suggested spraying of Bordeaux mixture and soil drenching with copper oxychloride (0.2per cent) and foliar spray of Ridomil / potassium phosphonate (0.3per cent) during the monsoon period and application of bio control agents such as *Trichoderma*, *Gliocladium* and VAM to prevent population build up of the *P.capsici* of black pepper along with phytosanitation measures. Sarma *et al.* (1994) reported that soil temperature of solarised bed rose to as high as 50°C, which was about 10 to 12 °C higher than non-solarised soil. They also reported the efficacy of soil solarization alone or in combination with biocontrol agents in managing *P. capsici*.

The incidence of foot rot of black pepper was reduced by Bordeaux mixture pasting, spraying and drenching (BMPSD) + neem seed cake. BMPSD +lime and BMPSD + neem seed cake+ lime (Nair *et al.*, 1993). Soil solarization amended with neem cake combined with foliar spray of metalaxyl recorded 81.3-95.5 per cent control of black shank disease of tobacco incited by *P. parasitica* var. *nicotianae* (Wajid *et al.*, 1995). Yucel (1995) observed that solarization alone or in combination with methyl bromide was effective in controlling blight of capsicum by *P. capsici*.

The incidence of *Phytophthora* foot rot was reduced in treatment combination of cultural practices + soil application of phorate + neem cake + four rounds of Bordeaux mixture (spray and drench) + application of Bordeaux paste + second round of Akomin (spray and drench) and third round of Ridomil MZ-72 WP (spray and drench) (Malebennur, *et al.* 1991 and Lokesh and Gangadharappa, 1995). Anandaraj *et al.* (1996) studied the suppressive effects of VAM on root damage caused by *P.capsici* and nematodes in black pepper and reported that the extent of root damage and foliar yellowing was less in VAM inoculated plants and provided better root protection against these plant pathogens. Sivaprasad (1997) also supported the benefits of VAM in reducing foot rot of black pepper.

Sarma *et al.* (1996 a, b) opined that IDM, with *T. harzianum* as a component for the management of foot rot, reduced the disease incidence from 25 per cent to 15 per cent in the field. They also reported that biocontrol agents were compatible with metalaxyl and potassium phosphonate and could be used for the management of *Phytophthora* disease in pepper. Increased growth response of ginger plants and significant yield increase were obtained through solarization (Vilasini, 1996). Hegde and Anahosur (1998) reported that integrated management of foot rot of black pepper included application of neem cake + *T. harzianum* + metalaxyl + garlic and mustard seed extract + mulching of the wet soil with transparent polythene sheets during hot summer.

The incidence of *Phytophthora* foot rot was the least in vines treated with potassium phosphonate (0.3per cent spray twice in the season) and *T. viride* (50 g/vine) along with 5 kg of FYM to basins of vine (AICRPS, 2000). It was also found that in the second trial

conducted by AICRPS, lowest disease incidence was noticed in the treatment receiving dipping in *T. harzianum* followed by treatments receiving Bordeaux mixture (1per cent) through spray and drench.

In the third disease management trial by AICRPS (2000), the most effective treatment against leaf and branch infection of black pepper was spraying and drenching with Akomin (potassium phosphonate), followed by application of biocontrol agents. However, in the case of death of vines due to wilt, Bordeaux mixture (1per cent) spraying and drenching with 0.2 per cent copper oxychloride were found to be effective. Forty-five days solarised, *T. viride* incorporated Fytolan drenched treatment was highly effective and there was cent per cent control of the disease (Binimol, 2000).

According to the annual report of AICRPS (2001). metalaxyl gold + *Trichoderma* was found most effective against the disease. The spraying and drenching of Ridomil and application of biocontrol agent and the combination of these two were also found effective in controlling the disease and they were on par with the spraying of Akomin and its combination with bioagent. Solarization of potting mixture and inoculation of beneficial organisms such as VAM and *Trichoderma* were adopted for producing disease free planting material. Besides enhancing plant growth, it also offered protection against nursery disease of black pepper (Binimol, 2000 and Mammootty, 2003).

Materials and Methods

3. MATERIALS AND METHODS

3.1 ISOLATION OF THE PATHOGEN

The pathogen causing *Phytophthora* rot disease of black pepper nursery was isolated from naturally infected plants from Thrissur district. The infected leaves were collected and cut into small bits with both healthy and infected portions and were surface sterilized with 0.1 per cent mercuric chloride solution for one minute and then repeatedly washed in two to three changes of sterile water. These sterilized bits were then placed on carrot agar (Appendix I) in sterile Petridishes and incubated at room temperature. When the fungal growth was visible, mycelial bits were transferred to carrot agar slants. The isolate was purified by single hyphal tip method. The cultural and morphological characters of the isolate were studied.

3.2 PATHOGENICITY

The pathogenicity of the isolate obtained was proved by following Koch's postulates. Mycelial discs of the pathogen from seven day old culture grown on carrot agar were inoculated on the lower surface of the leaves of pepper variety, Panniyur-1 after giving pinpricks. The humidity was provided by placing moist cotton over it. The inoculated leaves were placed in polythene bags and incubated. Observations were recorded on the development of typical symptoms of the disease. The pathogen was re-isolated from the artificially inoculated leaves and compared with the original isolate. This isolate maintained on carrot agar slants was used throughout the course of study.

3.3 ISOLATION OF RHIZOSPHERE MICROFLORA

The soil samples were collected during the month of June 2001 from the rhizosphere of healthy pepper cuttings adjacent to the infected ones. The samples were

collected from pepper nurseries raised in Government farms (Seed farms) at Chelakkara, Pazhayannur, Mannuthy and Pananchery of Thrissur district. The samples were pooled separately, shade dried and the total microflora were quantitatively estimated by serial dilution plate technique (Johnson and Curl, 1972). Martins Rose Bengal Streptomycin Agar, Thornton's standardized Agar and Kenknights Agar media were used for estimating fungi, bacteria and actinomycetes (Appendix II) at dilutions of 10^{-2}, 10^{-4} and 10^{-4} respectively.

Fungi - The fungal colonies developed on dilution plates were transferred to Potato Dextrose Agar medium (PDA) (Appendix I). Pure cultures of fungi obtained by single hyphal tip isolation method were maintained in PDA. An attempt was made to identify the fungi upto the generic level.

Bacteria – The bacterial colonies developed in the dilution plates were streaked on Nutrient Agar (NA) (Appendix I) and single colony isolation was made. The pure cultures were maintained on NA slants.

Actinomycetes – The single colonies of actinomycetes developed on Kenknights Agar were transferred to test tube slants of the same medium and maintained in pure culture.

3.4 GROWTH OF RHIZOSPHERE MICROFLORA AND PATHOGEN

3.4.1 Fungi

For measuring the growth rate of rhizosphere fungi, 8 mm disc from actively growing zone of each fungal isolate was transferred to the centre of Petridish containing PDA medium. The plates were incubated at room temperature and diameter of the fungal colonies were measured at intervals of 24 h until the fungal growth covered the entire Petridish to know their respective growth rates.

3.4.2 Bacteria

For estimating the growth rate of bacterial isolates, the bacteria were streaked on NA plates and measurement of growth of single colonies developed were recorded for five days at 24 h intervals.

3.4.3 Actinomycetes

Actinomycetes were grown on Kenknights Agar as described in section 3.4.1. Observations on growth were taken at intervals of 24 h for a period of 14 days.

3.4.4 Pathogen

For estimating the growth rate of *Phytophthora capsici*, the pathogen was grown on PDA by employing the method as in fungi and measurements were taken for seven days at an interval of 24 h.

3.5 *In vitro* SCREENING OF RHIZOSPHERE MICROFLORA FOR THEIR ANTAGONISTIC PROPERTIES AGAINST *P. capsici*

3.5.1 Fungi

3.5.1.1 *Screening of rhizosphere fungal isolates*

Twenty fungal isolates were tested for their antagonistic effect against *P.capsici* by dual culture method outlined by Skidmore and Dickinson (1976). The organisms were inoculated as dual cultures after giving due consideration for the growth rate of both the pathogen and the potential antagonists. Mycelial disc (8 mm) of the pathogen from seven day old culture grown on carrot agar was inoculated aseptically on one side of a Petridish and incubated at room temperature for 48 h. After this, 8 mm mycelial disc of the fungal isolates was inoculated in the same PDA plate, 3.5 cm away from the pathogen disc and

incubated. Three replications were maintained for each isolate. The pathogen and the fungal isolate grown in monoculture served as control.

The growth measurements were taken at regular intervals after 24 h of inoculation of the antagonists upto five days. The nature of reaction of the antagonist on the pathogen was recorded. The standard culture of *Trichoderma harzianum* was also tested for its antagonism to *P. capsici*. The per cent inhibition of mycelial growth of the pathogen (I) was calculated using the formula suggested by Vincent (1927).

$$I = \frac{C - T}{C} \times 100$$

where C - Growth of the pathogen in control (mm)

T - Growth of the pathogen in dual culture (mm)

The nature of antagonistic reaction of rhizosphere fungi tested against *P.capsici* was assessed by following the method of Purkayastha and Bhattacharya (1982) and assigned to four categories.

Homogenous	: Free intermingling of hyphae
Overgrowth	: *P.capsici* overgrown by test organism
Cessation of growth	: Cessation of growth at the line of contact
Aversion	: Development of clear zone of inhibition

3.5.1.2. ***Selection of efficient antagonists***

For selecting the most efficient isolates of rhizosphere fungi, which showed antagonism against *P.capsici*, a modified antagonistic index (AI) suggested by Kasinathan (1998) was employed. 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 antagonism index was calculated which is the product of PI, CB, SOOP and IZ.

$$AI = PI \times CB \times SOOP \times IZ$$

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

- Per cent inhibition: It was worked out as mentioned in 3.5.1.1
- Colonization behaviour of antagonist on pathogen (CB):

For studying CB, the description suggested by Bell *et al* (1982) for *Trichoderma* was followed with slight modification, the details of which are furnished in Table 3.1.

Table 3.1. Score chart of colonization behaviour of antagonist on pathogen

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

Bell *et al*. (1982) considered an isolate of *Trichoderma* to be antagonistic to the pathogen, if the mean score for a given comparison was greater than or equal to 3 but not highly antagonistic, if the score was between 1-2.

- Speed of overgrowth on pathogen (SOOP):

The score for time taken by the antagonist/pathogen to overgrow after the contact of antagonist/both is given in Table 3.2.

Table 3.2. 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 after 48 h
4	Antagonist completely overgrew on pathogen within 24 – 48 h.

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

- Inhibition zone (IZ):

For comparing the inhibition zone (IZ) produced by fungal antagonist, the following scores given in Table 3.3 were employed.

Table 3.3. Score chart of inhibition zone

Score	Inhibition Zone (IZ)
1	No IZ
2	1.0-2.5 mm
3	2.6-5.0 mm
4	>5 mm

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

3.5.2 Bacteria

3.5.2.1 *Screening of rhizosphere bacterial isolates*

Twenty bacterial isolates were tested for their antagonistic effect against *P. capsici* by dual culture method (Utkhede and Rahe, 1983). Potato Dextrose Agar (PDA) was allowed to solidify in sterilized Petridishes. Mycelial disc of 8 mm size of the pathogen was inoculated at the centre of the Petridish 48 h prior to inoculation of the bacteria. The bacterial isolates were inoculated as a line of streak on either side of the pathogen leaving 2.25 cm from the edge of the Petridish. Plates with *P. capsici* alone served as control. Three replications were maintained for each bacterial isolate. Observations on growth of the pathogen were taken at regular intervals upto five days. The per cent inhibition of mycelial growth of the pathogen was calculated as mentioned in 3.5.1.1.

3.5.3 Actinomycetes

3.5.3.1 *Screening of rhizosphere actinomycetes*

In the case of actinomycetes, five isolates were tested for their antagonistic effect by dual culture method. The isolates were inoculated two days prior to inoculation of the pathogen. The growth of antagonist and pathogen was recorded at 24 h intervals upto 14 days. Replications and control were maintained as in the case of fungal isolates.

3.6 IDENTIFICATION OF EFFICIENT ANTAGONISTS

An attempt was made to identify the two efficient antagonists, which showed high antagonistic index. For this, the cultural characters of the two antagonists like growth, colony colour and pigmentation were studied by growing them in Petridishes containing PDA. Morphological characters were studied by slide culture technique (Riddel, 1950).

Observations were made on the type of branching pattern of conidiophore, length and breadth of phialide and shape, size and colour of spore.

3.7 MECHANISM OF ANTAGONISM OF SELECTED FUNGAL ANTAGONISTS ON *P.capsici*

To study the mechanism of antagonism of two selected fungal antagonists on *P. capsici*, the dual culture technique of Dennis and Webster (1971) was used. In 90 mm sterile Petridishes, sterile PDA was poured and allowed to solidify. Sterilized cellophane discs of 90 mm diameter were placed over this so as to lie flat on the medium using a pair of sterile forceps. An agar disc of 8 mm diameter containing the mycelium of *P.capsici* taken from an actively growing culture of the fungus was inoculated at one end of the Petridish 48 h prior to inoculation of the antagonists, which was placed two cm away from the pathogen. The plates were incubated at room temperature and observations were taken at regular intervals until there was some hyphal contact. Microscopic observation for hyphal interaction was taken by cutting out one sq.cm. portion of cellophane containing intermingling hyphal growth of antagonist and pathogen and mounting in cotton blue lacto phenol. Photomicrographs of mycoparasitism exhibited by the antagonists were taken.

3.8 COMPATIBILITY OF SELECTED ANTAGONISTS AND *T.harzianum* TO COMMON FUNGICIDES, INSECTICIDES AND FERTILIZERS

The *in vitro* compatibility of the selected antagonists and the standard culture of *T. harzianum* to fungicides, insecticides and fertilizers commonly used in pepper gardens were studied by Poison Food Technique (Riker and Riker, 1936).

3.8.1 Fungicides

The following fungicides given in Table 3.4 were used for *in vitro* evaluation.

Table 3.4. Fungicides used for *in vitro* evaluation against antagonists

SL. No.	Chemical name	Trade name	Concentration (per cent)
1	Mancozeb	Indofil M- 45	0.2, 0.3, 0.4
2	Captan	Captaf	0.1, 0.2, 0.3
3	Propineb	Anthracol	0.05, 0.1, 0.15
4	Metalaxyl + Mancozeb	Ridomil MZ	0.2, 0.3, 0.4
5	Potassium phosphonate	Akomin – 40	0.2, 0.3, 0.4
6	Copper oxychloride	Fytolan	0.2, 0.3, 0.4
7	Copper hydroxide	Kocide	0.1, 0.2, 0.3
8	$CuSO_4$ + lime	Bordeaux mixture	0.5, 1.0, 1.5
9	Chlorothalonil	Kavach	0.2,0.3,0.4

3.8.2 Insecticides

The insecticides used for *in vitro* evaluation are presented in Table 3.5.

Table 3.5 Insecticides used for *in vitro* evaluation against antagonists

SL.No.	Chemical name	Trade name	Concentration (per cent/ kg a.i ha^{-1})
1	Endosulfan	Nagsulfon 35 EC	0.04, 0.05, 0.06
2	Monocrotophos	Nuvacron 36 EC	0.04, 0.05, 0.06
3	Chlorpyriphos	Durlax 20 EC	0.01, 0.02, 0.03
4	Dimethoate	Rogor 30 EC	0.04, 0.05, 0.06
5	Quinalphos	Ekalux 25 EC	0.03, 0.04, 0.05
6	Cypermethrin	Biloyp 10 EC	0.01, 0.02, 0.03
7	Phorate	Phorate 10 G	1.0, 1.5, 2.0 kg a.i ha^{-1}
8	Carbofuran	Furadan 3 G	0.5, 0.75, 1.0 kg a.i ha^{-1}

3.8.3 Fertilizers

The following fertilizers depicted in Table 3.6 were used for *in vitro* evaluation.

Table 3.6. Fertilizers used for *in vitro* evaluation against antagonists

SL.No.	Name	Concentration (per cent)
1	Urea	1.0, 1.5, 2.0
2	Rajphos	2.0, 2.5, 3.0
3	Muriate of potash	2.0, 2.5, 3.0
4	Ammonium sulphate	2.0,2.5,3.0
5	Factomphos	2.0, 2.5, 3.0

The quantity of fungicides, insecticides and fertilizers needed to get the desired concentration was added to 100 ml sterilized, molten PDA medium, mixed well and poured into sterilized Petridishes at the rate of 15 ml per plate. To avoid contamination, the fertilizers were exposed to U.V light in the laminar flow for a period of 45 min before adding into the medium. After solidification of the medium, mycelial discs of 8 mm diameter from actively growing antagonists were cut and placed at the centre of each Petridish. Control consisted of PDA medium alone inoculated with the antagonist. Three replications were maintained for each concentration of the chemicals. The inoculated Petridishes were incubated at room temperature and the observations on the growth and sporulation of the antagonist 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 3.5.1.1.

3.9 EVALUATION OF FUNGICIDES, INSECTICIDES AND FERTILIZERS AGAINST THE PATHOGEN

A similar method as mentioned in section 3.8 was followed for testing the effect of fungicides, insecticides and fertilizers on growth of the pathogen, *P.capsici*. All the fungicides, insecticides and fertilizers used for *in vitro* testing against the antagonists were taken for this study also. Three replications were maintained in each case and observations were recorded until the control plates showed full growth. The per cent inhibition of mycelial growth of the pathogen as given in 3.5.1.1 was also calculated.

3.10 MANAGEMENT OF *Phytophthora* DISEASE IN BLACK PEPPER NURSERY

An experiment was laid out to study the efficacy of two selected antagonists and standard culture of *T.harzianum* against *Phytophthora* rot disease in pepper nursery. The experiment was carried out during February – August 2002 at CCRP farm at College of Horticulture, Vellanikkara. The details of the experiment are as follows:

Design	: CRD
Replications	: 3
Treatments	: 20
Number of bags / replication	: 40
Number of plants / bag	: 4
Variety	: Panniyur I

The treatment details (Fig.3.1) are presented in Table 3.7.

Table 3.7. Details of various treatments

	Treatment
T_1	Control
T_2	Disease control as per PoP of KAU
T_3	*Trichoderma harzianum* alone
T_4	Selected antagonist from Thrissur (*Trichoderma viride*)
T_5	Selected antagonist from Thrissur (*Trichoderma longibrachiatum*)
T_6	Soil solarization for 30 days + *T. harzianum*
T_7	Soil solarization for 30 days + *T.viride*
T_8	Soil solarization for 30 days +*T.longibrachiatum*
T_9	*T. harzianum* + Ridomil MZ (1.25 g l^{-1})
T_{10}	*T.viride* + Ridomil MZ (1.25g l^{-1})
T_{11}	*T.longibrachiatum* + Ridomil MZ (1.25 g l^{-1})
T_{12}	Soil solarization for 30 days + *T.harzianum* + Ridomil MZ (1.25 g l^{-1})
T_{13}	Soil solarization for 30 days + *T.viride*+ Ridomil MZ (1.25 g l^{-1})
T_{14}	Soil solarisation for 30 days + *T.longibrachiatum* + Ridomil MZ (1.25 g l^{-1})
T_{15}	*T. harzianum* + Potassium phosphonate (3 ml l^{-1})
T_{16}	*T.viride* + Potassium phosphonate (3 ml l^{-1})
T_{17}	*T.longibrachiatum* + Potassium phosphonate (3 ml l^{-1})
T_{18}	Soil solarisation for 30 days + *T. harzianum* + Pot. phosphonate (3 ml l^{-1})
T_{19}	Soil solarisation for 30 days + *T.viride* + Potassium phosphonate (3ml l^{-1})
T_{20}	Soil solarisation for 30 days + *T.longibrachiatum* + Pot. phosphonate (3 ml l^{-1})

3.10.1 Preparation of potting mixture and soil solarization

The potting mixture consisting of sand : soil : cowdung in the ratio of 1:1:1 was made into a raised bed of height 25 cm and size 3 m x 1 m. The bed was levelled and watered sufficiently with a rose can. The potting mixture was then mulched with 150 gauge transparent polyethylene sheet. The sides of the sheet were covered with soil to keep the

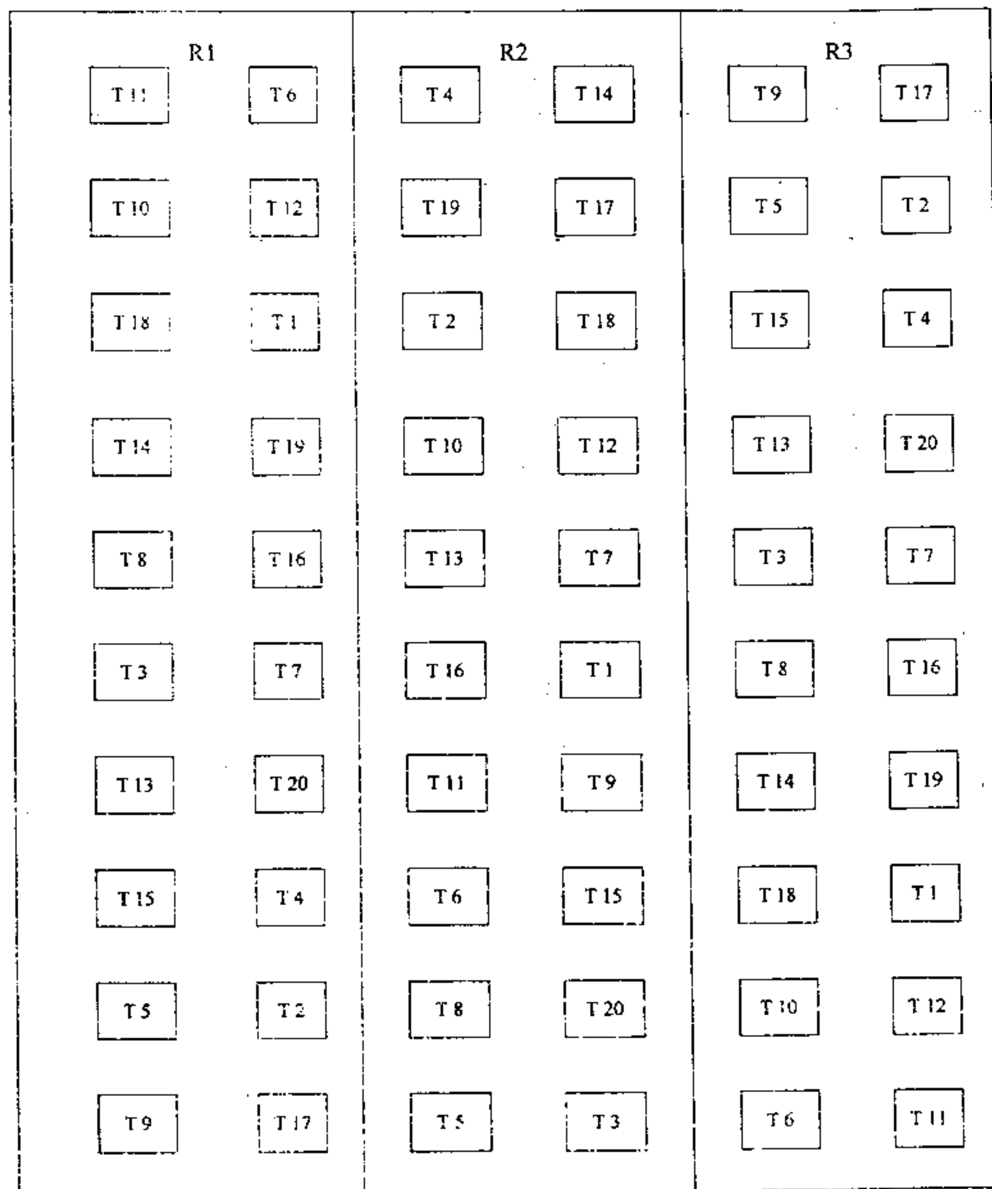


Fig. 3.1. Layout of the nursery experiment

T_1 Control

T_2 Disease control as per POP of KAU

T_3 *Trichoderma harzianaum* alone

T_4 Selected antagonist from Thrissur (*T. viride*)

T_5 Selected antagonist from Thrissur (*T. longibrachiatum*)

T_6 Soil sol. for 30 days +*T. harzianum*

T_7 Soil sol .for 30 days +*T. viride*

T_8 Soil sol.30 days + *T. longibrachiatum*

T_9 *T. harzianaum* +Ridomil MZ

T_{10} *T. viride*+ Ridomil MZ

T_{11} *T. longibrachiatum* + Ridomil MZ

T_{12} Soil sol. for 30 days +*T. harzianum*+Ridomil MZ

T_{13} Soil sol. for 30 days + *T. viride* +Ridomil MZ

T_{14} Soil sol. for 30 days + *T. longibrachiatum* + R id MZ

T_{15} *T. harzianum* +Pot. phosphonate

T_{16} *T. viride* + Pot. phosphonate

T_{17} *T. longibrachiatum* + Pot. phosphonate

T_{18} Soil sol. for 30 days +*T. harzianum*+ Pot.phosphonate

T_{19} Soil sol for 30 days +*T. viride*+ Pot.phosphonate

T_{20} Soil sol for 30 days +*T. longibrachiatum* + Pot.phosphonate

sheet in position. Care was taken to keep the sheet in close contact with the potting mixture. The polyethylene sheet was removed 30 days after solarization.

3.10.2 Soil temperature

Soil temperature of solarized and non-solarized soil at a depth of 5 cm, 10 cm and 15 cm were recorded. For this soil thermometers were installed by making a hole in the centre of the bed at a depth of 5 cm, 10 cm and 15 cm. Soil temperature was recorded daily at 8:30 a.m. and 2:30 p.m. for entire 30 days. The microbial load was estimated before solarization and immediately after removal of polyethylene sheet.

3.10.3 Soil inoculation of antagonists and AM fungi

Polyethylene sheets were removed from the potting mixture 30 days after solarization and both the solarized and non-solarized potting mixture was filled in polyethylene bags of size of 10 cm x 15 cm according to the treatments. After filling both solarized and non-solarized soil in polybags, mass multiplied *T. harzianum* and selected antagonists (*T.viride* and *T.longibrachiatum*) grown on rice bran were incorporated @ 4g bag^{-1} depending upon various treatments. Also AM fungi recommended for black pepper were given as a common treatment at the rate of 20g bag^{-1} except in T_1 (Control) and T_2 (POP, KAU).

3.10.4 Planting

The nursery was raised in a permanent nursery structure fitted with a shade net that allowed 50 per cent light infiltration. Cuttings of pepper variety Panniyur –1 obtained from the pepper garden of the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara were used for the experiment. Two node cuttings were planted @ 4 per polybag and mulched with leaves. All the cultural operations were carried out as per the Package of Practices Recommendations, 'Crops' 1996 (KAU, 1996). The initial spraying of fungicides was given at the onset of monsoon during the month of June and

repeated five times at fortnightly intervals as per the treatments. In addition, during the second week of July, the inoculum of the pathogen was spread uniformly to all the treatments.

3.11 OBSERVATIONS RECORDED

Observations on sprouting percentage, total soil microflora, disease incidence and severity and other growth parameters at different intervals were recorded.

3.11.1 Sprouting percentage

The number of cuttings sprouted in each treatment was counted 20, 30 and 45 days after planting (DAP) to calculate the sprouting percentage. Also observations on the number of cuttings rotten and those which remained dormant were taken at 45 DAP.

3.11.2 Per cent disease incidence

The incidence of *Phytophthora* rot in the nursery was recorded at weekly intervals starting from fourteen weeks after planting (WAP) for four times by periodic observations and the per cent disease incidence was calculated as follows:

$$\text{Per cent disease incidence} = \frac{\text{Number of cuttings infected}}{\text{Total number of sprouted cuttings}} \times 100$$

3.11.3 Per cent disease severity

The severity of disease on the leaves was recorded using a score chart based on 0–5 scale as shown in Table 3.8.

Table 3.8. Score chart for severity of disease on leaves

Score	Description
0	No leaves infected
1	Lesions covering < 10 per cent leaf area
2	Lesions covering >10 < 25 per cent leaf area
3	Lesions covering > 25 < 50 per cent leaf area
4	Lesions covering > 50 < 75 per cent leaf area
5	Lesions covering > 75per cent leaf area

Five polybags were randomly selected from each treatment and the severity was assessed at weekly intervals from the fourteenth week after planting (WAP) upto 4 weeks. Per cent disease severity was calculated using the formula suggested by Wheeler (1969).

$$\text{Per cent Disease Severity (PDS)} = \frac{\text{Sum of all numerical ratings} \times 100}{\text{Total number of leaves observed} \times \text{Maximum disease score}}$$

3.11.4 Per cent mortality of pepper cuttings

Per cent of mortality of cuttings was recorded at nineteenth WAP. The percentage of mortality of pepper cuttings was calculated using the following formula:

$$\text{Per cent Mortality} = \frac{\text{Number of cuttings dead} \times 100}{\text{Total number of sprouted cuttings}}$$

3.11.5 Estimation of soil microflora

Soil samples were collected from different treatments one, two and three months after planting. Population of fungi, bacteria and actinomycetes in different treatments were estimated by serial dilution plate technique as mentioned in 3.2.

3.11.6 Biometric observations

Five polybags each containing four cuttings was randomly tagged in each replication of different treatments for recording biometric observations. The following observations were taken at 45, 60 and 90 DAP.

- Height of plants – Distance from the base of the cutting to the growing point was taken as the height of the plant.
- Number of leaves per plant – Number of leaves was recorded by counting the number of fully opened leaves of the plant.

3.12 STATISTICAL ANALYSIS

Analysis of variance was performed on the data collected in various experiments using the statistical package MSTAT (Freed, 1986). Multiple comparison among treatment means was done using DMRT.

Results

4. RESULTS

The results of the studies carried out to evaluate the efficacy of selected native antagonists alone or in combination with fungicides for the management of *Phytophthora* rot disease in black pepper nursery are presented in this chapter.

4.1 ISOLATION OF THE PATHOGEN

The fungal pathogen causing *Phytophthora* disease in black pepper nursery was isolated from the naturally infected cuttings collected from Thrissur district. The fungus was purified by single hyphal tip method and maintained on potato dextrose agar (PDA) and carrot agar slants (CA) by periodic subculturing.

4.2 PATHOGENICITY

Pathogenicity of the isolate was tested on leaves of black pepper variety Panniyur-1. On artificial inoculation of the leaves, pale water soaked lesions appeared within a period of 48 h. which later turned black. The lesions gradually enlarged covering large areas of the leaves. The isolation of the pathogen from artificially inoculated leaves yielded the same organism.

4.3 CULTURAL AND MORPHOLOGICAL CHARACTERS OF THE PATHOGEN

The isolated fungal pathogen was subjected to cultural and morphological studies. Pure culture of the fungus on CA was slightly petalloid with uniformly dense cotton wool-like aerial mycelium over entire colony, rarely stellate or radiating and more dense in the central portion (Plate 2). Hyphae fine, non-septate, smooth upto 8.5μm wide. Sporangial arrangement umbellate, sometimes irregular. The number of sporangia arising from a common point was often 4-10 and sometimes more per umbel (Plate 3b). Sporangia elongated, base usually tapered towards the stalk (>15μm stalk length), papillate, caduceus, 45-52 x 18-25 μm with L/B ratio of 1.9, some upto 2.3μm. Sporangium occurred in various shapes from subspherical, ovoid, obovoid,

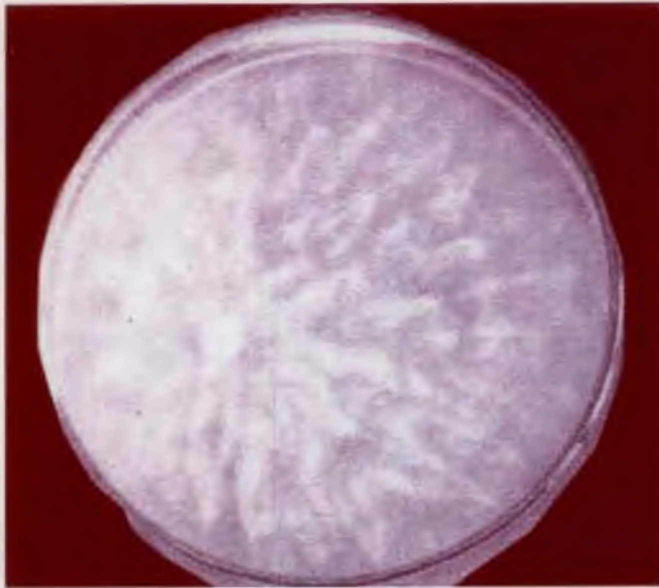


Plate. 2. Culture of *Phytophthora capsici*

Plate 3. Mycelium with sporangia of *Phytophthora capsici*

a. Different shapes of mature sporangia

b. Sporangial ontogeny

ellipsoid, pyriform or sometimes irregularly elongated (Plate 3a). Based on these characters the pathogen was identified as *Phytophthora capsici* Leonian amend A. Alizadeh and P.H.Tsao

4.4 COLLECTION OF SOIL SAMPLES AND ISOLATION OF MICROFLORA FROM THE RHIZOSPHERE

Soil samples were collected from pepper nurseries raised in Government farms at Chelakkara, Pazhayannur, Mannuthy and Pananchery areas of Thrissur district. The samples were collected from the rhizosphere of healthy pepper cuttings from diseased nurseries. Soil microorganisms *viz.*, fungi, bacteria and actinomycetes were isolated from these samples by serial dilution plate technique and the results are presented in Table 4.1.

From the data, it was evident that the rhizosphere population of microbes varied with different locations. In general, the bacterial population was found to be the maximum followed by fungi and actinomycetes. The population of fungi showed variations among the different rhizosphere soil of pepper nurseries. The highest count of fungi (31.17×10^2 cfu g^{-1}soil) was obtained from the soils of Pazhayannur nursery followed by pepper nursery of Chelakkara (21.50×10^2 cfu g^{-1}soil). The least count of 10.67×10^2 cfu g^{-1}soil was observed in Pananchery nursery. Similarly, the highest population of bacteria (23.33×10^4 cfu g^{-1} soil) was recorded in Pazhayannur nursery that was closely followed by Chelakkara nursery (22×10^4 cfu g^{-1} soil) and the least population was recorded in Pananchery nursery (12×10^4 cfu g^{-1} soil). The population of actinomycetes varied with different locations with a maximum population of 15×10^4 cfu g^{-1} soil in Pazhayannur nursery and the least count of 3.67×10^4 cfu g^{-1} soil from Pananchery nursery.

Based on cultural characters of the rhizosphere microbes isolated, the representative colonies were subcultured and used for further studies. Thus, 22 fungi, 20 bacteria and five actinomycetes were selected. Attempts to identify the rhizosphere fungi upto their generic level based on the morphological characters revealed that, out of the different fungal isolates, nine belonged to the genus *Trichoderma*, three to the

Table 4.1. Rhizosphere microflora from black pepper nurseries at different locations of Thrissur district

Locations	Fungi ($x10^2$ cfu g^{-1})	Bacteria ($x10^4$ cfu g^{-1})	Actinomycetes ($x10^4$ cfu g^{-1})
Chelakkara	21.50	22.00	14.67
Pazhayannur	31.17	23.33	15.00
Mannuthy	14.35	17.67	6.34
Pananchery	10.67	12.00	3.67

genus *Aspergillus*, two each to *Rhizopus* and *Penicillium* and the rest of the cultures remained unidentified.

4.5 GROWTH OF RHIZOSPHERE MICROFLORA AND THE PATHOGEN

The growth rate of different rhizosphere microflora which comprised of 22 isolates of fungi, 20 isolates of bacteria and five isolates of actinomycetes obtained from different locations were studied. In addition to this, the growth of the pathogen, *P. capsici* and the standard culture of *Trichoderma harzianum* were also studied.

4.5.1 Fungi

The growth of the fungi studied including the pathogen, varied widely as shown in Table 4.2. Among the different fungal isolates, those which belonged to the genus *Trichoderma* (1F, 2F, 3F, 10F, 21F, 22F, 33F, 34F, 35F) and *Rhizopus* (14F, 28F) were very fast growing and covered the entire Petridish within a period of three to four days. The standard culture of *T.harzianum* also attained full growth within three days. The growth rate of different *Aspergillus* spp. was found highly varying. The isolate 36F (*Aspergillus* sp.) took 12 days to complete the full growth whereas isolate 20F (*Aspergillus niger*) and 17F (*Aspergillus* sp.) took only seven days. However, the *Penicillium* isolates, 13F and 25F were very slow growing covering 62 mm and 60 mm respectively even at 12 days after inoculation. The unidentified fungi (9F, 10F, 15F, 16F and 39 F) showed varying growth rate and took four to seven days to attain 90 mm growth in Petridishes.

The pathogen *P. capsici* attained full growth within a period of seven days.

4.5.2 Bacteria

The growth of bacterial isolates was studied and the results are presented in Table 4.3. Out of the 20 isolates, three isolates (4B, 9B, 18B) exhibited fast spreading growth and covered the entire Petridish within 72 -- 120 h of inoculation. The remaining ones showed variation in their growth with the maximum diameter of colonies of 12 mm by isolates 2B, 13B and 21B followed by 10B with a diameter of

Table 4.2. Growth of fungal isolates

Sl.No.	Fungal isolates	*Mean growth in diameter (mm)											
		Days after inoculation (DAI)											
		1	2	3	4	5	6	7	8	9	10	11	12
1	1F (*Trichoderma* sp.)	17	42	80	90								
2	2F (*Trichoderma* sp.)	24	36	65	90								
3	3F (*Trichoderma* sp.)	44	64	90									
4	4F (unidentified)	25	64	84	90								
5	9F (unidentified)	8	21	40	54	72	82	90					
6	10F (*Trichoderma* sp.)	45	66	90									
7	13F (*Penicillium* sp.)	9	13	16	19	30	34	41	47	50	56	58	62
8	14F (*Rhizopus* sp.)	9	30	90									
9	15F (unidentified)	24	41	70	90								
10	16F (unidentified)	25	44	64	90								
11	17F (*Aspergillus* sp.)	15	30	43	55	70	85	90					
12	20F (*Aspergillus* niger)	17	34	52	60	75	84	90					
13	21F (*Trichoderma* sp.)	21	50	75	90								
14	22F (*Trichoderma* sp.)	30	80	90									
15	25F (*Penicillium* sp.)	9	15	19	24	28	34	39	41	46	51	55	60
16	28F (*Rhizopus* sp.)	20	53	90									
17	33F (*Trichoderma* sp.)	24	72	90									
18	34F (*Trichoderma* sp.)	40	65	90									
19	35F (*Trichoderma* sp.)	36	67	90									
20	36F (*Aspergillus* sp.)	14	22	31	38	46	53	60	64	69	73	81	90
21	38F (unidentified)	30	49	74	90								
22	39F (unidentified)	8	19	33	55	69	79	90					
23	*T. harzianum* (Std. culture)	24	73	90									
24	*Phytophthora capsici*	17	36	50	63	79	84	90					

* Mean of three replications. 8mm growth indicates the diameter of original disc.

Table 4.3. Growth of bacterial isolates

Sl. No.	Bacterial Isolate	* Mean growth (mm)					Remarks
		Hours of incubation					
		24	48	72	96	120	
1	IB	0.5	1.0	1.0	1.5	2.0	Circular
2	2B	3.0	7.0	8.0	10.0	12.0	Circular
3	3B	1.0	4.0	6.0	7.0	8.0	Circular
4	4B	22.5	45.0	67.5	67.5	90.0	Spreading
5	5B	1.0	4.0	5.0	6.0	6.0	Circular
6	6B	1.0	2.0	2.5	3.0	4.0	Circular
7	7B	0.5	1.0	2.0	2.5	3.0	Circular
8	8B	2.0	3.0	4.0	5.0	5.0	Circular
9	9B	22.5	45.0	67.5	90.0	90.0	Spreading
10	10B	3.0	6.0	8.0	9.0	10.0	Circular
11	11B	1.0	2.0	3.0	4.0	4.5	Circular
12	13B	4.0	8.0	9.5	11.0	12.0	Circular
13	14B	1.0	2.0	3.0	4.0	5.0	Circular
14	15B	3.0	6.0	7.0	8.0	9.0	Circular
15	16B	1.0	4.0	5.0	5.5	6.0	Circular
16	17B	3.0	4.0	5.0	5.5	6.0	Circular
17	18B	22.5	67.5	90.0	90.0	90.0	Spreading
18	19B	1.0	2.0	2.0	2.5	3.0	Circular
19	20B	1.0	6.0	7.0	8.0	9.0	Circular
20	21B	4.0	8.0	9.0	11.0	12.0	Circular

* Mean of three replications

10 mm after 120 h of inoculation. The least growth of 2 mm after 120 h of inoculation was recorded by the isolate 1B.

4.5.3 Actinomycetes

The growth of actinomycetes was studied. All of them were very slow growing and recorded only 28-55 mm growth even at 12 days after inoculation (Table 4.4).

4.6 *In vitro* SCREENING OF RHIZOSPHERE MICROFLORA FOR THEIR ANTAGONISTIC PROPERTIES AGAINST THE PATHOGEN

4.6.1 Fungi

4.6.1.1 *Screening of fungal isolates*

The twenty-two rhizosphere fungal isolates and the standard culture of *T. harzianum* were screened for their antagonistic effect against *P. capsici* as described in the chapter material and methods. The antagonistic reactions of the organism in dual culture and per cent inhibition of the pathogen were recorded.

From the data (Table 4.5 and 4.6), it was evident that all the fungal isolates tested were antagonistic against the pathogen. Out of the 23 isolates including *T. harzianum*, 13 of them showed cent per cent inhibition on the growth of *P.capsici*. They included 10 isolates of *Trichoderma* spp. (1F, 2F, 3F, 10F, 21F, 22F, 33F, 34F, 35F) and two isolates of *Rhizopus* sp. (14F and 28F) and one unidentified culture (16 F). The rest of the fungal isolates showed a per cent inhibition ranging from 37.78 to 77.78 on the seventh day of incubation.

It was noticed that among the fungal isolates which showed 100 per cent inhibition, all the isolates of *Trichoderma* and *Rhizopus* overgrew the pathogen. However, the isolate 22F showed a slight aversion initially as they showed a clear zone of inhibition between the paired organisms and later completely overgrew the pathogen resulting in death and disintegration of the organism. Several isolates like 9F, 17 F, 20F, and 36 F exhibited a cessation of growth at the point of

Table 4.4. Growth of actinomycete isolates

Sl.No.	Actinomycete Isolates	*Mean growth diameter (mm) Days after inoculation (DAI)											
		1	2	3	4	5	6	7	8	9	10	11	12
1	1A	8	8	10	11	14	15	17	20	22	24	25	28
2	2A	8	8	9	10	17	22	25	29	32	33	34	35
3	5A	8	8	9	10	15	20	24	31	38	46	52	55
4	7A	8	8	11	13	16	19	20	22	25	27	29	30
5	8A	8	8	12	12	18	22	27	32	36	41	45	50

* Mean of three replications
8mm growth indicates the diameter of original disc

Table 4.5. Growth of *P. capsici* and fungal isolates in dual culture

Time of inoculation of pathogen	Fungal Isolate	*Mean radial growth (mm)													
		Days after inoculation (DAI)													
		1		2		3		4		5		6		7	
		A	P	A	P	A	P	A	P	A	P	A	P	A	P
2 days prior to Antagonists	1F (*Trichoderma* sp.)	11	25	25	14	52.5	5	62	0	-	-	-	-	-	-
,,	2F (*Trichoderma* sp.)	12	25	27	11	53	9	62	0	-	-	-	-	-	-
,,	3F (*Trichoderma* sp.)	12	24	25	13	51	8	62	0	-	-	-	-	-	-
,,	4F (unidentified)	10	24	13	25	13	26	14	26	14	25	14	25	14	25
,,	9F (unidentified)	8	21	10	28	12	25	18	20	27	10	27	10	27	10
,,	10F (*Trichoderma* sp.)	10	25	25	13	53	10	62	0	-	-	-	-	-	-
,,	14F (*Rhizopus* sp.)	8	27	10	26	18	22	55	0	-	-	-	-	-	-
,,	15F (unidentified)	8	25	8	25	10	23	14	21	18	15	18	15	18	15
,,	16F (unidentified)	10	27	12	27	44	10	62	0	-	-	-	-	-	-
,,	17F (*Aspergillus* sp.)	8	25	15	21	17	19	19	17	19	17	19	17	19	17
,,	20F (*Aspergillus niger*)	8	24	13	18	16	18	20	15	20	15	20	15	20	15
,,	21F (*Trichoderma* sp.)	14	21	28	8	53	4	62	0	-	-	-	-	-	-
,,	22F (*Trichoderma* sp.)	11	26	21	13	60	0	-	-	-	-	-	-	-	-
,,	28F (*Rhizopus* sp.)	10	22	15	22	21	18	52	0	-	-	-	-	-	-
,,	33F (*Trichoderma* sp.)	15	25	26	10	51	6	62	0	-	-	-	-	-	-
,,	34F (*Trichoderma* sp.)	12	24	25	9	65	0	-	-	-	-	-	-	-	-
,,	35F (*Trichoderma* sp.)	12	26	25	12	55	8	62	0	-	-	-	-	-	-
,,	36F (*Aspergillus* sp.)	8	22	13	20	18	18	22	15	25	10	25	10	25	10
,,	38F (unidentified)	9	23	13	24	15	25	10	26	10	26	10	26	10	26
,,	39F (unidentified)	8	24	8	24	10	26	10	27	10	27	10	27	10	27
,,	*T. harzianum*	13	24	28	10	63	0	-	-	-	-	-	-	-	-
2 days after Antagonists	13F (*Penicillium* sp.)	15	17	19	22	21	25	21	28	21	26	22	27	22	28
,,	25F (*Penicillium* sp.)	18	15	20	24	20	28	22	27	22	27	22	27	23	27

*Mean of 3 replications, 8mm growth indicates the diameter of original disc. A- Antagonist, P- Pathogen

Table 4.6. ***In vitro*** **screening of fungal isolates against** ***P.capsici***

Sl.No.	Fungal isolates	Per cent inhibition (PI)		
		4 th Day of incubation	7 th Day of incubation	Antagonistic reaction
1	1F (*Trichoderma* sp.)	100	100	B
2	2F (*Trichoderma* sp.)	100	100	B
3	3F (*Trichoderma* sp.)	100	100	B
4	4F (unidentified)	42.22	44.44	A
5	9F (unidentified)	55.55	77.78	C
6	10F (*Trichoderma* sp.)	100	100	B
7	13F (*Penicillium* sp.)	37.78	37.78	A
8	14F (*Rhizopus* sp.)	100	100	B
9	15F (unidentified)	53.33	66.67	A
10	16F (unidentified)	100	100	B
11	17F (*Aspergillus* sp.)	62.22	62.22	C
12	20F (*Aspergillus niger*)	66.67	66.67	C
13	21F (*Trichoderma* sp.)	100	100	B
14	22F (*Trichoderma* sp.)	100	100	D & B
15	25F (*Penicillium* sp.)	44.44	44.44	A
16	28F (*Rhizopus* sp.)	100	100	B
17	33F (*Trichoderma* sp.)	100	100	B
18	34F (*Trichoderma* sp.)	100	100	B
19	35F (*Trichoderma* sp.)	100	100	B
20	36F (*Aspergillus* sp.)	66.67	77.78	C
21	38F (unidentified)	42.22	42.22	A
22	39F (unidentified)	42.22	42.22	D
23	*T. harzianum*	100	100	B

A-Homogenous
B- Overgrowth
C-Cessation of growth
D-Aversion

contact with the pathogen. The species of *Pencillium* (13F and 25F) intermingled freely with *P. capsici* without showing any signs of interaction. The isolate 36F also showed a slight aversion towards the pathogen.

4.6.1.2 *Selection of efficient antagonists*

From among the different fungal isolates, the most efficient isolates were selected by employing the method of Kasinathan (1998). For this a modified antagonistic index (AI) was calculated as described in materials and methods which is the product of colonization behaviour (CB), speed of overgrowth on pathogen (SOOP), per cent inhibition (PI) and inhibition zone (IZ).

It was noticed that the fungal isolates showed an AI ranging from 106.66 to 3000 (Table 4.7). The highest AI of 3000 was registered by the isolate 22 F (*Trichoderma* sp.) followed by 34 F (*Trichoderma* sp.) and *T.harzianum* which recorded an AI of 1500 (Plate 4) . About ten isolates showed AI of 1200. The least AI of 106.66 was shown by the isolate 16F.

4.6.2 Bacteria

4.6.2.1 Screening of bacterial isolates

The bacterial isolates obtained were evaluated against *P.capsici* by dual culture experiment. Among the 20 bacterial isolates, only four of them showed inhibition of *P.capsici* (Table 4.8). The isolates *viz.*, 20B, 15B, 9B and 2B showed a clear zone of inhibition demarcating the growth of pathogen and the bacteria. All of them showed a per cent inhibition of 70 and above. Among them, the maximum inhibition of mycelial growth was recorded with the isolate 9B followed by 2B and the minimum with 15B.

4.6.3 Actinomycetes

4.6.3.1 *Screening of actinomycete isolates*

Soil actinomycetes were screened for their antagonism using the dual culture technique. The pathogen was inoculated eight days after the antagonist due to very slow growth of actinomycetes. The study revealed that none of the actinomycetes was

Table 4.7. Selection of efficient fungal antagonists

Sl.No.	Fungal isolate	PI	CB	SOOP	IZ	AI
1	1F (*Trichoderma* sp.)	100	4	3	1	1200
2	2F (*Trichoderma* sp.)	100	4	3	1	1200
3	3F (*Trichoderma* sp.)	100	4	3	1	1200
4	4F (unidentified)	42.22	2	2	1	168.88
5	9F (unidentified)	55.55	3	2	1	333.3
6	10F (*Trichoderma* sp.)	100	4	3	1	1200
7	13F (*Penicillium* sp.)	37.78	2	2	1	151.12
8	14F (*Rhizopus* sp.)	100	4	3	1	1200
9	15F (unidentified)	53.33	1	2	1	106.66
10	16F (unidentified)	100	4	3	1	1200
11	17F (*Aspergillus* sp.)	62.22	3	2	1	373.32
12	20F (*Aspergillus niger*)	66.67	3	2	1	400.02
13	21F (*Trichoderma* sp.)	100	4	3	1	1200
14	22F (*Trichoderma* sp.)	100	5	3	2	3000
15	25F (*Penicillium* sp.)	44.44	2	2	1	177.76
16	28F (*Rhizopus* sp.)	100	4	3	1	1200
17	33F (*Trichoderma* sp.)	100	4	3	1	1200
18	34F (*Trichoderma* sp.)	100	5	3	1	1500
19	35F (*Trichoderma* sp.)	100	4	3	1	1200
20	36F (*Aspergillus* sp.)	66.67	3	2	1	400.02
21	38F (unidentified)	42.22	2	2	1	168.88
22	39F (unidentified)	42.22	2	2	3	168.88
23	*T. harzianum*	100	5	3	1	1500

PI - Per cent inhibition
CB - Colonization behaviour
SOOP - Speed of overgrowth on pathogen (h)
IZ - Inhibition zone (mm)
AI - Antagonistic index

Table 4.8. *In vitro* screening of bacterial antagonists against *P. capsici*

Sl. No.	Bacterial isolate	Per cent inhibition (PI)	
		3rd day of incubation	5th day of incubation
1	15B	65.82	70.0
2	9B	74.68	77.78
3	2B	72.15	75.56
4	20B	70.89	74.44

antagonistic to the pathogen as evidenced by the full growth of the pathogen in the Petridishes.

4.7 IDENTIFICATION OF EFFICIENT ANTAGONISTS

The two *Trichoderma* spp. (34 F and 22 F) that showed a high antagonistic index of 1500 and 3000 respectively were selected and identified based on the cultural and morphological characters (Fig. 4.1a). Photomicrographs of the standard culture of *T.harzianum* were also taken (Plate 6a).

4.7.1 *Trichoderma* sp. (Isolate 22 F)

Colonies smooth, translucent, pale yellow radiating from the centre of the fungal disc (Plate 6b). The conidial areas gradually change their colour from olive green to bright yellow. Pigments secreted into the medium, so that the reverse of the colony show lemon yellow at first and later turn dark brown after four days. Mycelium septate, smooth walled and hyaline. Conidiophore branching simple. Phialides formed singly or alternately along the main branch. Phialides 6.9–11.5 x 2.3–2.5μm, bottle shaped abruptly attenuate towards their short conical apices (Fig. 4.1c). Phialospores 3.6–4.5μm, ellipsoidal, subglobose or globose, smooth walled and pale green. Chlamydospores numerous, globose, smooth walled and hyaline. Based on these characters, this isolate was identified as ***Trichoderma longibrachiatum* Rifai** aggr. (Rifai, 1964).

4.7.2 *Trichoderma* sp. (Isolate 34 F)

Colonies form smooth surfaced, watery white and sparse mycelial mat, which later become hairy from the formation of loose scanty aerial hyphae, which make the colonies, appear somewhat whitish. At maturity, conidial areas dark green (Plate 6c), whilst the reverse side remains uncoloured. A typical 'coconut odour' is emitted by older culture. Mycelium hyaline, smooth walled, septate and much branched. Conidiophores with dendroid branching system. Phialides in false or irregular whorls mostly with less than four phialides (8–14 x 2.4–3μm) in each whorl (Fig 4.1b). Phialospores subglobose, globose or short obovoid, 3.45–5.75 μm, surface minutely

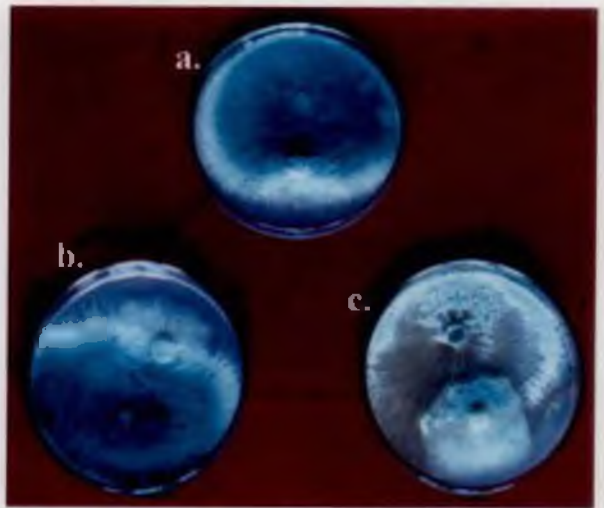
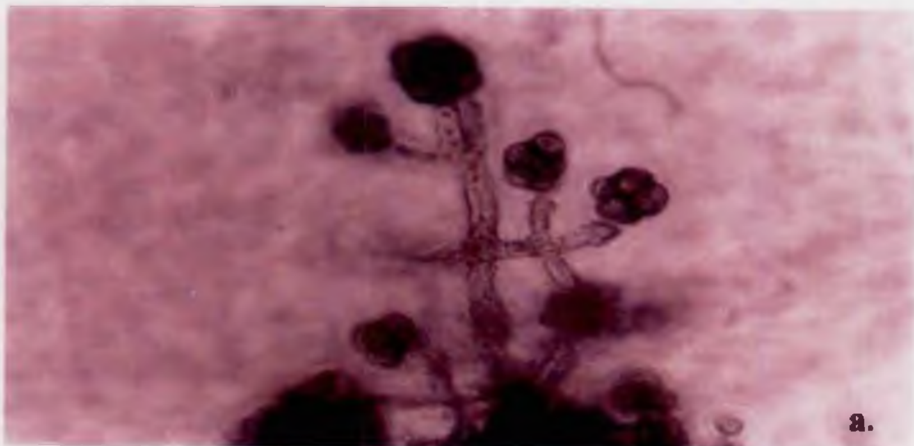


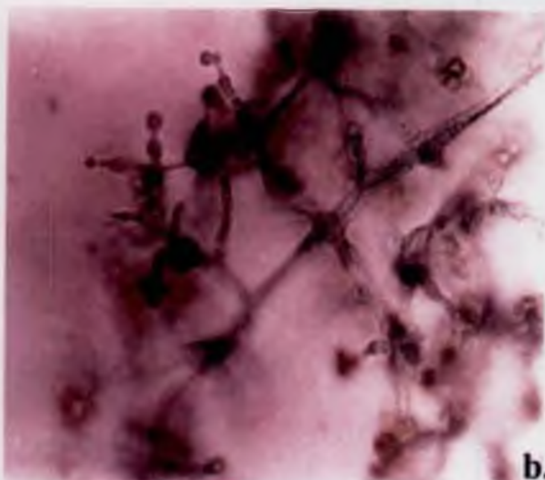
Plate 4. Cultures of *Trichoderma* spp.

a. *T. harzianum*
b. *T. viride*
c. *T. longibrachiatum*

Plate 5. ***Trichoderma* spp. and *P. capsici* in dual culture**

a. *T. harzianum*
b. *T. viride*
c. *T. longibrachiatum*





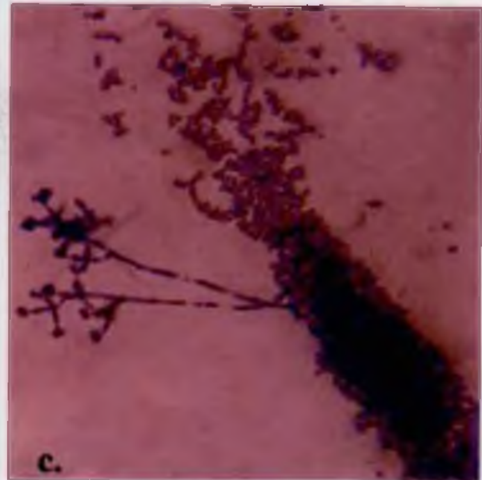
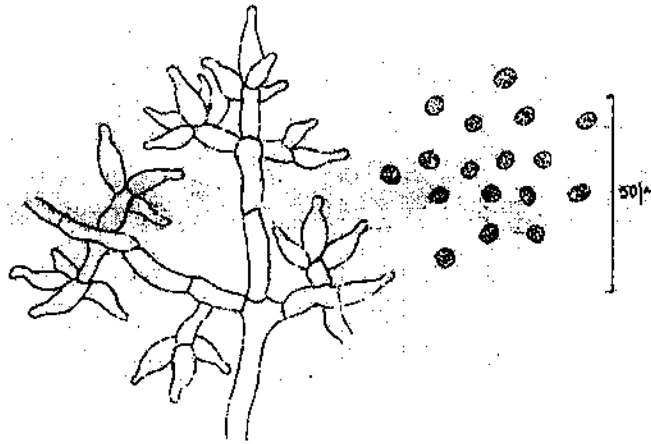
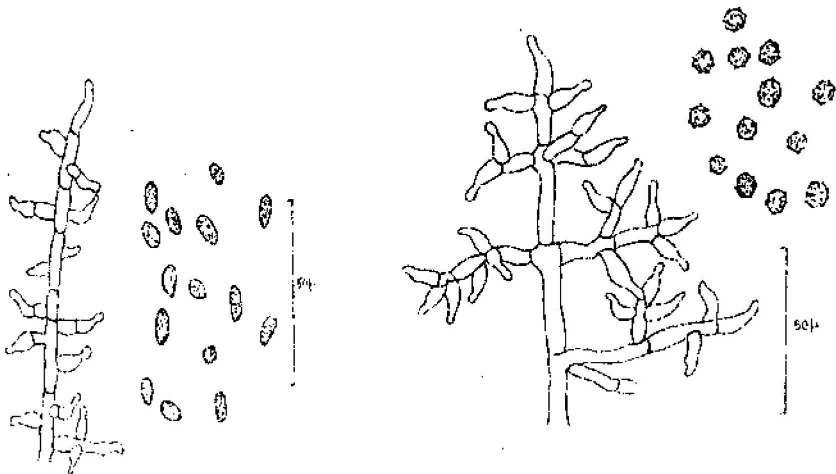


Plate 6. Photomicrographs of selected fungal antagonists and *T. harzianum*

a. *T. harzianum* (100 X) **b.** *T. viride* (45 X) **c.** *T. longibrachiatum* (45 X)



a. Trichoderma harzianum



c. Trichoderma longibrachiatum

b. Trichoderma viride

Fig. 4.1. Conidiophores and phialospores of *Trichoderma* spp.

rough walled (spiny). Chlamydospores globose, rarely ellipsoidal, hyaline, smooth walled. Based on these characters, the isolate was identified as ***Trichoderma viride* Pers. ex S.F. Gray aggr.** (Webster, 1964).

4.8 MECHANISM OF ANTAGONISM OF SELECTED FUNGAL ANTAGONISTS ON *P. capsici*

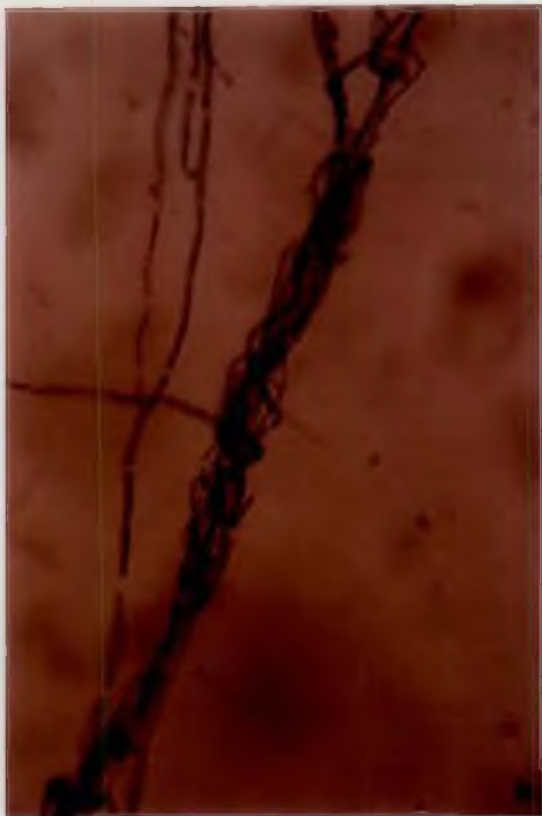
The standard culture of ***T.harzianum*** and the selected antagonist *T.viride* (34F), proved to be efficient parasites of *P.capsici*. The main pathogenic hyphae were found tightly held by coiling slender hyphae of the antagonists (Plate 7a , 8a). In addition, the parasitic hyphae of the antagonists penetrated the host hyphae at several points and grew alongside the inner cavity of the host hyphae. Besides overgrowing and coiling, the antagonists also caused disintegration of the pathogenic hyphae (Plate 7b). It was observed that though *T.longibrachiatum* (22F) caused coiling and penetration of host hyphae, the degree of antagonistic action was comparatively lesser than the other two antagonists (Plate 8b).

4.9 COMPATIBILITY OF SELECTED ANTAGONISTS AND *T.harzianum* TO COMMON FUNGICIDES, INSECTICIDES AND FERTILIZERS USED IN PEPPER GARDENS

Different fungicides, insecticides and fertilizers commonly used in pepper gardens were evaluated at various concentrations to know their compatibility with the standard culture of *T harzianum* and the selected antagonists (*T.viride and T.longibrachiatum*). The results are reported herein.

4.9.1 Fungicides

Nine fungicides *viz.*, Indofil M-45, Ridomil MZ, Akomin–40, Captaf, Kavach, Anthracol, Bordeaux mixture (BM), Fytolan and Kocide, each at three different concentrations, were evaluated to study the compatibility of these chemicals to the selected antagonists and *T.harzianum*. The results are presented in Table 4.9.



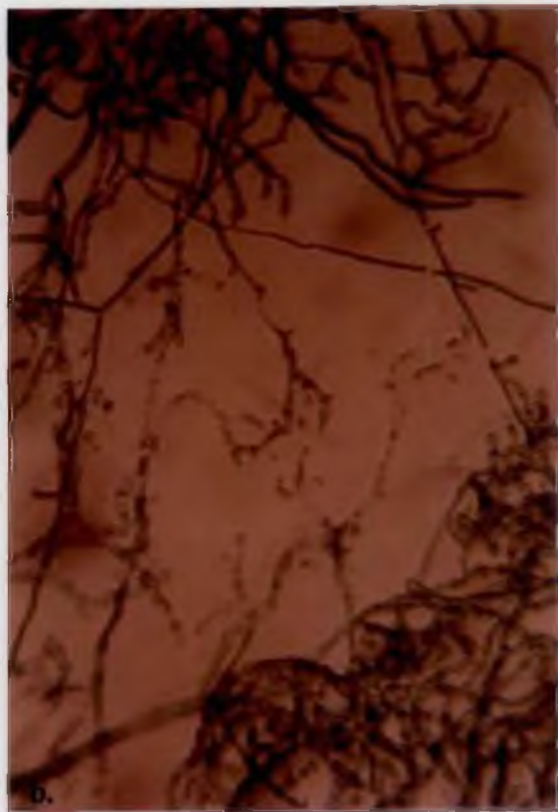


Plate 7. Mycoparasitism of *P.capsici* by *T. harzianum*
a. Coiling b. Disintegration

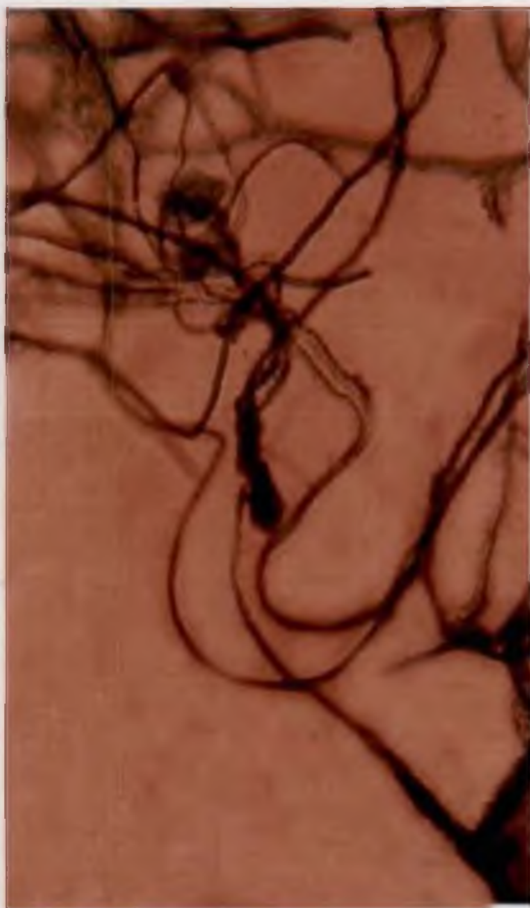


Plate 8. Mycoparasitism of *P. capsici* by selected fungal antagonists
a. Coiling of *T. viride* b. Coiling of *T.longibrachiatum*

Among the nine fungicides tested, Bordeaux mixture at all concentrations completely inhibited the growth of all the antagonists, while the remaining fungicides at different concentrations showed varying percentage of inhibition. In general, it was noticed that as the concentration of fungicides increased, there was an increased inhibition of the antagonist. It was observed that all the three species of *Trichoderma* proved to be compatible with Ridomil MZ, Akomin-40, Indofil M-45 and Anthracol as they showed comparatively good growth in the poisoned media. The least growth of the antagonist was observed in media containing Kocide, closely followed by Captaf, Kavach and Fytolan.

Results revealed that the response of *T.harzianum* to different fungicides at various concentrations differed significantly (Fig. 4.2). It was observed that the fungus showed the least inhibition with 0.2 per cent Indofil M-45 with a per cent inhibition of 13.33. This was closely followed by Akomin-40 (0.2per cent) and Anthracol (0.05per cent), which recorded a per cent inhibition of 23.33. Cent per cent inhibition of the fungus was recorded with BM at all concentrations. Fytolan at all concentrations and Ridomil MZ at 0.4 per cent concentration were on par with each other and recorded per cent inhibition ranging from 43 to 48 per cent respectively. The response of *T.harzianum* to Captaf, Kavach and Kocide was very poor indicating their incompatibility. It showed a per cent inhibition of 82.22 at 0.3 per cent concentration of Captaf, 77.78 per cent at 0.3 and 0.4 per cent concentrations of Kavach and 80 per cent at 0.3 per cent concentration of Kocide.

The response of *T.viride* also differed significantly to various fungicides (Fig. 4.3). It recorded a per cent inhibition of 11.11 with Indofil M-45 (0.2per cent) and Ridomil MZ (0.2per cent) which were closely followed by Akomin-40 (0.2per cent) with a per cent inhibition of 15.56. Cent per cent inhibition was exhibited with the fungicide BM at all concentrations followed by Captaf (0.3per cent), Kocide (0.3per cent) and Kavach (0.3 and 0.4per cent). Anthracol at 0.15 per cent and Fytolan at all concentrations were on par with each other and recorded a per cent inhibition upto 45 per cent.

In the case of *T.longibrachiatum* to different fungicides at varied concentrations, it was observed that the fungus was partially compatible with

Table 4.9. Compatibility of *Trichoderma* spp. with fungicides

Sl.No.	Fungicides	Concentration (per cent)	*T. harzianum*		*T.viride*		*T.longibrachiatum*	
			*Mean diameter of colony (mm)	Per cent inhibition over control	*Mean diameter of colony (mm)	Per cent inhibition over control	*Meandiameter of colony (mm)	Per cent inhibition over control
1	Indofil M-45	0.2	78 b	13.33	80 b	11.11	74 c	17.78
		0.3	70 c	22.22	68 d	24.44	60 f	33.33
		0.4	58 g	35.56	60 e	33.33	52 g	42.22
2	Ridomil MZ	0.2	62 f	31.11	80 b	11.11	70 d	22.22
		0.3	56 h	37.78	75 c	16.67	68 d	24.44
		0.4	51 i	43.33	62 e	31.11	54 g	40.00
3	Akomin-40	0.2	69 c	23.33	76 c	15.56	81 b	10.00
		0.3	66 de	26.67	60 e	33.33	71 d	21.11
		0.4	50 i	44.44	51 g	43.33	60 f	33.33
4	Captaf	0.1	24 k	73.33	22 h	75.56	22 i	75.56
		0.2	18 l m	80.00	11 ij	87.78	14 j	84.44
		0.3	16 m	82.22	9 j	90.00	13 j	85.56
5	Kavach	0.2	31 j	65.56	20 h	77.78	13 j	85.56
		0.3	20 l	77.78	19 h	78.89	9 k	90.00
		0.4	20 l	77.78	12 i	86.67	0k	100
6	Anthracol	0.05	69 c	23.33	60 e	33.33	70 d	22.22
		0.1	66 de	26.67	56 f	37.78	64 e	28.89
		0.15	63 ef	30.00	50 g	44.44	42 h	53.33
7	Bordeaux mixture	0.5	0 n	100	0 k	100	0 k	100
		1.0	0 n	100	0 k	100	0 k	100
		1.5	0 n	100	0 k	100	0 k	100
8	Fytolan	0.2	49 i	45.56	50 g	44.44	29 i	67.78
		0.3	47 i	47.78	50 g	44.44	25 i	72.22
		0.4	47 i	47.78	45 g	45.00	24 i	73.33
9	Kocide	0.1	21 l	76.67	24 h	73.33	12 j	86.67
		0.2	19 lm	78.89	19 h	78.89	11 j	87.78
		0.3	18 lm	80.00	14 ij	84.44	0k	100
10	Control		90 a		90 a		90 a	

* Mean of three replications. In each column figures followed by same letter donot differ significantly according to DMRT.

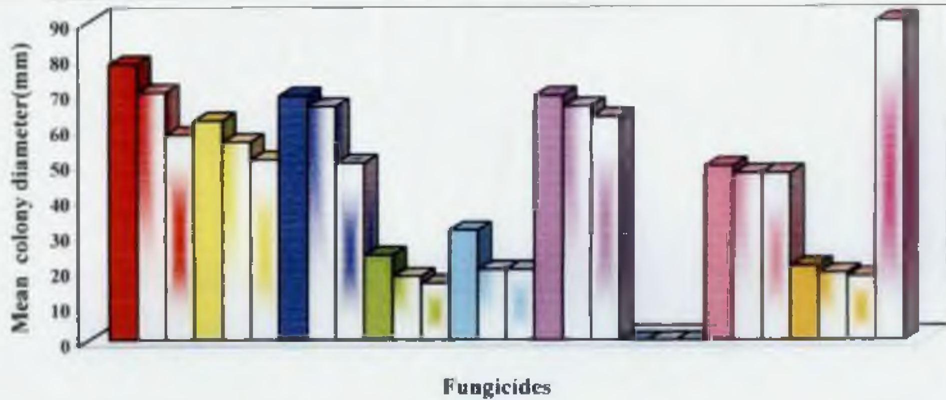


Fig. 4.2.Compatibility of *Trichoderma harzianum* with fungicides

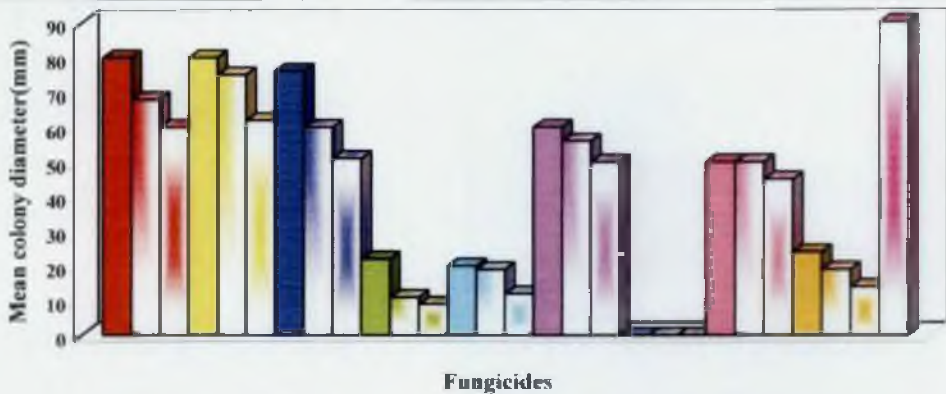


Fig. 4.3.Compatibility of *Trichoderma viride* with fungicides

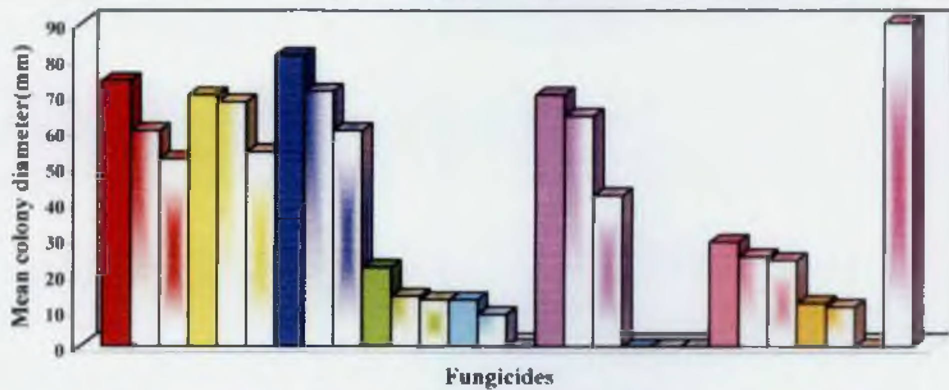
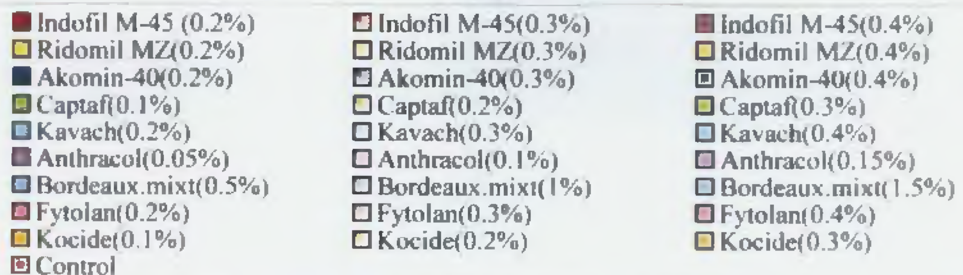


Fig. 4.4.Compatibility of *Trichoderma longibrachiatum* with fungicides



Akomin-40 at 0.2 per cent concentration followed by Indofil M–45 (0.2per cent) which showed a per cent inhibition of 10 and 17.78 respectively (Fig. 4.4). The fungus showed a per cent inhibition of 22.22 and 40.0 at 0.2 and 0.4 per cent of Ridomil MZ followed by 53.33 at 0.15 per cent of Anthracol. Bordeaux mixture at all concentrations and Kocide (0.3per cent) completely inhibited the antagonist and these were immediately followed by Kocide (0.2 and 0.1per cent), Captaf (0.3per cent) and Kavach (0.2per cent) with an inhibition per cent of 87.78, 86.67 and 85.56 respectively. It was also observed that Fytolan was more inhibitory to this fungus compared to the other two antagonists.

The sporulation of the various cultures of *Trichoderma* spp. in the fungicide incorporated media at their recommended dosage, which supported their growth was also studied and depicted in Table 4.10. It was noted that all the species of *Trichoderma* showed good sporulation with Indofil M–45 and Ridomil MZ. In Akomin–40 incorporated media, all fungal antagonists showed sparse conidial production. There was no sporulation of the three antagonists in media incorporated with Captaf, Kavach, Anthracol, Fytolan and Kocide except for *T.harzianum*, which showed sparse sporulation with Captaf and Fytolan.

4.9.2 Insecticides

The *in vitro* sensitivity of eight insecticides *viz.*, Monocrotophos, Chlorpyriphos, Endosulfan, Dimethoate, Quinalphos, Cypermethrin, Phorate and Carbofuran, each at three concentrations, was tested against the three *Trichoderma* spp.

It was evident from the data (Table 4.11) that the antagonists exhibited varying per cent of sensitivity to the different insecticides tested. All the three species of *Trichoderma* were found to be compatible with Phorate and Carbofuran at all concentrations tested. Monocrotophos and Quinalphos were incompatible with the antagonists showing complete inhibition of growth and the remaining insecticides recorded varying rates of inhibition at different concentrations.

Table 4.10. Effect of fungicides on the sporulation of *Trichoderma* spp.

Sl. No.	Fungicides	Concentration (per cent)	*T.harzianum*	*T.viride*	*T.longibrachiatum*
1	Indofil M-45	0.3	+++	+++	+++
2	Ridomil MZ	0.3	+++	+++	+++
3	Akomin-40	0.3	+	+	+
4	Captaf	0.2	+	-	-
5	Kavach	0.3	-	-	-
6	Anthracol	0.1	-	-	-
7	Fytolan	0.3	+	-	-
8	Kocide	0.2	-	-	-

Good : +++
Moderate : ++
Sparse : +
Absent : -

Considering the incompatibility of individual antagonists, it was observed that, *T. harzianum* was compatible with Phorate and Carbofuran at all concentrations as there was no inhibition on the growth of the fungus (Fig. 4.5). Chlorpyriphos at 0.01 per cent, recorded 50 per cent inhibition. However, its higher concentrations were incompatible. Contrary to this, the response of the fungus towards Monocrotophos and Quinalphos at all concentrations was very poor which recorded cent per cent inhibition. In addition to this, Endosulfan at all concentrations, Dimethoate at 0.06 per cent and Cypermethrin at 0.03 per cent recorded more than 80 per cent inhibition indicating the incompatibility of these insecticides to the antagonist.

Monocrotophos at all concentrations were completely inhibitory to the growth of *T.viride*, which was followed by Quinalphos that recorded an inhibition of 88.89 per cent at all concentrations (Fig. 4.6). More than 80 per cent inhibition was recorded with Endosulfan and Cypermethrin at all concentrations and Dimethoate at 0.06 per cent concentration respectively. Phorate and Carbofuran were found compatible with the fungus. Chlorpyriphos (0.01per cent) showed lesser compatibility than Phorate and Carbofuran, which recorded 34.44 per cent efficacy over control in inhibiting the fungus, while its higher concentrations showed more than 70 per cent inhibition.

The mycelial growth of *T.longibrachiatum* was completely arrested with Monocrotophos and Quinalphos at all concentrations (Fig. 4.7). Cypermethrin and Endosulfan at all concentrations were also found inhibitory to the antagonists as they showed more than 80 per cent inhibition. Phorate and Carbofuran at all concentrations were found compatible with the fungus. This was followed by Chlorpyriphos (0.01per cent), which recorded an inhibition percentage of 57.78, but their higher two concentrations showed 76.69 and 78.89 per cent inhibition respectively.

The sporulation of antagonists in various insecticides incorporated media was recorded and the results are given in Table 4.12. Good sporulation of the antagonists was noticed with Phorate and Carbofuran. Moderate sporulation was recorded with Cypermethrin and Chlorpyriphos by *T.viride* and *T.longibrachiatum*, whereas *T. harzianum* showed good sporulation with the latter. *T.viride* and *T.longibrachiatum* showed sparse conidial production in the media incorporated with Endosulfan.

Table 4.11. Compatibility of *Trichoderma* spp. with insecticides

Sl.No.	Insecticides	Concentration	*T. harzianum*		*T.viride*		*T.longibrachiatum*	
			*Mean colony diameter (mm)	Per cent inhibition over control	*Mean colony diameter (mm)	Per cent inhibition over control	*Mean colony diameter (mm)	Per cent inhibition over control
1	Monocrotophos	0.04%	0^{k}	100	0^{m}	100	0^{d}	100
		0.05%	0^{k}	100	0^{m}	100	0^{d}	100
		0.06%	0^{k}	100	0^{m}	100	0^{d}	100
2	Chlorpyriphos	0.01%	45^{b}	50.00	59^{b}	34.44	38^{b}	57.78
		0.02%	22^{de}	75.56	26^{c}	71.11	21^{d}	76.67
		0.03%	17^{gh}	81.11	23^{d}	74.44	19^{d}	78.89
3	Endosulfan	0.04%	13^{i}	85.56	15^{g}	83.33	10^{d}	88.89
		0.05%	13^{i}	85.56	13^{ijk}	85.56	9^{d}	90.00
		0.06%	11^{ij}	87.78	12^{jkl}	86.67	8^{d}	91.11
4	Dimethoate	0.04%	24^{c}	73.33	21^{e}	76.67	25^{cd}	72.22
		0.05%	24^{cd}	73.33	20^{e}	77.78	20^{d}	77.78
		0.06%	10^{j}	88.89	17^{f}	81.11	18^{d}	80.00
5	Quinalphos	0.03%	0^{k}	100	10^{kl}	88.89	0^{d}	100
		0.04%	0^{k}	100	10^{kl}	88.89	0^{d}	100
		0.05%	0^{k}	100	10^{kl}	88.89	0^{d}	100
6	Cypermethrin	0.01%	20^{ef}	77.78	16^{gh}	82.22	14^{d}	84.44
		0.02%	19^{fg}	78.89	14^{ghi}	84.44	12^{d}	86.67
		0.03%	17^{h}	81.11	13^{hij}	85.56	12^{d}	86.67
7	Phorate	1.0 kg a.i per ha	90^{a}	0	90^{a}	0	90^{a}	0
		1.5 kg a.i per ha	90^{a}	0	90^{a}	0	90^{a}	0
		2.0 kg a.i per ha	90^{a}	0	90^{a}	0	90^{a}	0
8	Carbofuran	0.5 kg a.i per ha	90^{a}	0	90^{a}	0	90^{a}	0
		0.75 kg a.i per ha	90^{a}	0	90^{a}	0	90^{a}	0
		1.0 kg a.i per ha	90^{a}	0	90^{a}	0	90^{a}	0
9	Control		90^{a}		90^{a}		90^{a}	

* Mean of three replications
In each column figures followed by same letter donot differ significantly according to DMRT.

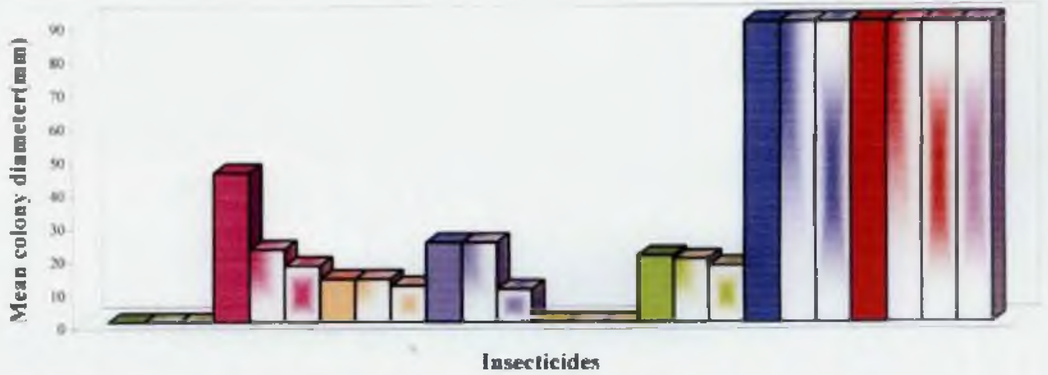


Fig. 4.5.Compatibility of *Trichoderma harzianum* with insecticides

Fig. 4.6.Compatibility of *Trichoderma viride* with insecticides

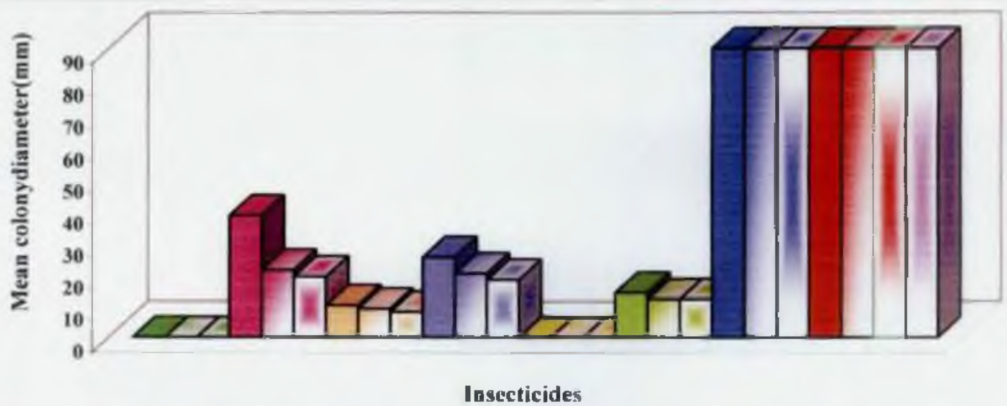


Fig. 4.7.Compatibility of *Trichoderma longibrachiatum* with insecticides

Monocrotophos 0.04%	Monocrotophos 0.05%	Monocrotophos 0.06%
Chlorpyriphos 0.01%	Chlorpyriphos 0.02%	Chlorpyriphos 0.03%
Endosulfan 0.04%	Endosulfan 0.05%	Endosulfan 0.06%
Dimethoate 0.04%	Dimethoate 0.05%	Dimethoate 0.06%
Quinalphos 0.03%	Quinalphos 0.04%	Quinalphos 0.05%
Cypermethrin 0.01%	Cypermethrin 0.02%	Cypermethrin 0.03%
Phorate 1.0 kg a.i per ha	Phorate 1.5 kg a.i per ha	Phorate 2.0 kg a.i per Ha
Carbofuran 0.5 kg a.i per ha.	Carbofuran 0.75 kg a.i per ha.	Carbofuran 1.0 kg a.i per ha
Control		

Table 4.12. Effect of insecticides on the sporulation of *Trichoderma* spp.

Sl. No.	Insecticides	Concentration	*T. harzianum*	*T.viride*	*T.longibrachiatum*
1	Chlorpyriphos	0.02%	+++	++	++
2	Endosulfan	0.05%	++	+	+
3	Rogor	0.05%	+++	+++	++
4	Cypermethrin	0.02%	+++	++	++
5	Phorate	1.5 kg a.i /ha	+++	+++	+++
6	Carbofuran	0.75 kg a.i /ha	+++	+++	+++

Good : +++
Moderate : ++
Sparse : +
Absent : -

T.harzianum also showed good sporulation with Dimethoate and moderate conidial production with Endosulfan.

4.9.3 Fertilizers

Fertilizers like urea, Rajphos, MoP, Factomphos and ammonium sulphate at various concentrations were evaluated for compatibility to the three antagonists. The study indicated that all the fertilizers, except Factomphos were compatible with the antagonists to various extents (Table 4.13).

T.harzianum proved to be compatible with MoP and ammonium sulphate (2 per cent) followed by Rajphos (2per cent) (Fig.4.8). The response of *T.harzianum* to higher concentrations of urea (1.5 and 2.0per cent) and Factomphos (2.5 and 3per cent) was comparatively poor. Factomphos (3per cent) recorded 54.4 per cent inhibition compared to control in inhibiting the fungus while urea 2 per cent recorded 42.2 per cent inhibition.

For *T.viride,* MoP at all concentrations were compatible (Fig. 4.9). The higher concentration (3per cent) of Rajphos and ammonium sulphate recorded a per cent inhibition of 22.1 and 21.3 respectively. Partial inhibition of mycelial growth of the fungus was noticed with the higher two concentrations (2per cent and 3per cent) of Factomphos, which recorded a per cent inhibition of 49.3 and 40.3 respectively. Urea was found inhibitory to the fungus at its higher concentration (1.5 and 2 per cent) as they recorded a per cent inhibition of 33.9 and 40.2 per cent.

In the case of *T.longibrachiatum*, full growth of the fungus was noticed with lower concentration of MoP and ammonium sulphate (Fig. 4.10). This was followed by urea (1per cent) and MoP (3per cent). However, the mycelial growth of the fungus was arrested to a greater extent with Factomphos at its higher two concentrations (2 and 3per cent), which recorded a per cent inhibition of 54.4 and 64.4 respectively. Rajphos at its higher concentration recorded an inhibition of 28.9 per cent.

Table 4.13. Compatibility of *Trichoderma* spp. with fertilizers

Sl. No.	Fertilizer	Concentration (per cent)	*T. harzianum*		*T.viride*		*T.longibrachiatum*	
			*Mean diameter of colony (mm)	Per cent inhibition over control	*Mean diameter of colony (mm)	Per cent inhibition over control	*Mean diameter of colony (mm)	Per cent inhibition over control
1	Urea	1.0	74.0^{d}	17.8	74.0^{d}	17.8	86.0^{b}	4.4
		1.5	70.0^{e}	22.2	59.5^{f}	33.9	71.0^{g}	21.1
		2.0	52.0^{h}	42.2	53.8^{g}	40.2	48.0^{j}	46.7
2	Rajphos	2.0	86.0^{b}	4.4	82.0^{c}	8.9	81.0^{d}	10.0
		2.5	80.0^{c}	11.1	74.5^{d}	17.2	79.0^{e}	12.2
		3.0	74.0^{d}	17.8	70.1^{e}	22.1	64.0^{h}	28.9
3	MOP	2.0	90.0^{a}	0	90.0^{a}	0	90.0^{a}	0
		2.5	79.0^{c}	12.2	90.0^{a}	0	90.0^{a}	0
		3.0	75.0^{d}	16.7	86.0^{b}	4.4	87.0^{b}	3.3
4	Factomphos	2.0	58.0^{f}	35.6	53.3^{g}	40.8	56.0^{i}	37.8
		2.5	55.0^{g}	38.9	49.3^{h}	45.2	41.0^{k}	54.4
		3.0	41.0^{i}	54.4	40.3^{i}	55.2	32.0^{l}	64.4
5	Amm.sulphate	2.0	88.0^{a}	2.2	87.5^{b}	2.8	90.0^{a}	0
		2.5	80.0^{c}	11.1	80.5^{c}	10.6	84.0^{c}	6.7
		3.0	74.0^{d}	17.8	70.8^{e}	21.3	73.0^{f}	18.9
6	Control		90^{a}		90^{a}		90^{a}	

* Mean of three replications.
In each column figures followed by same letter donot differ significantly according to DMRT.

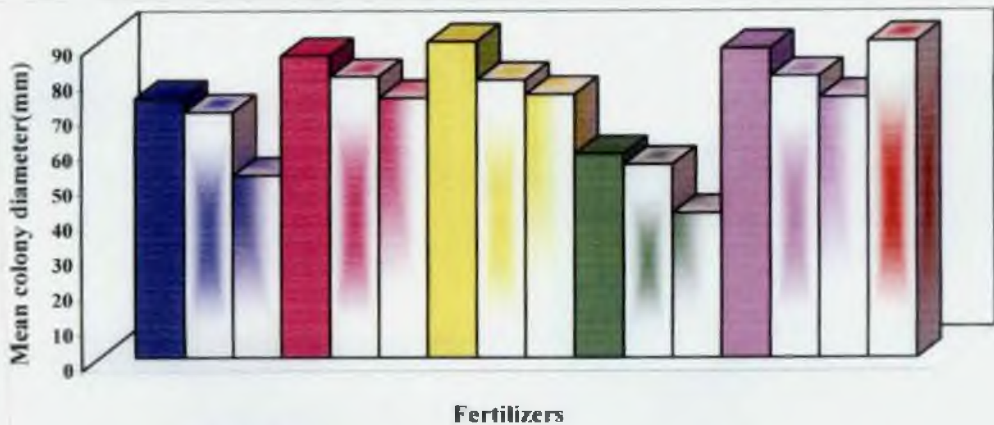


Fig. 4.8.Compatibility of *Trichoderma harzianum* with fertilizers

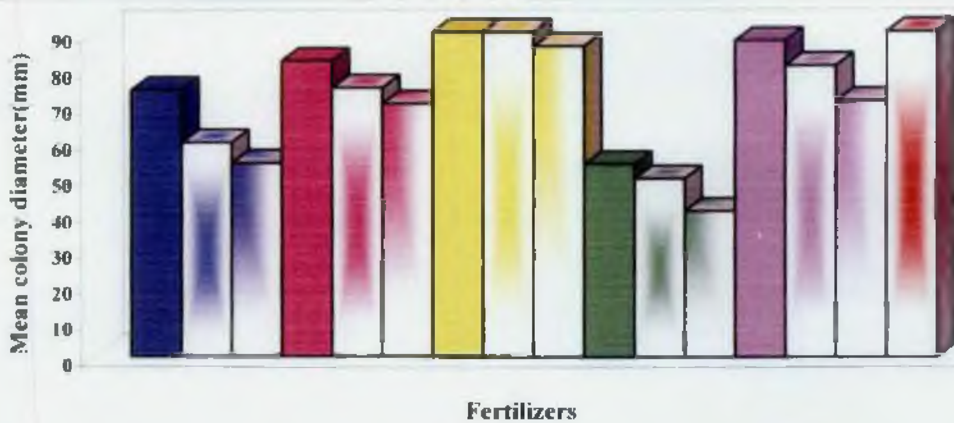


Fig. 4.9.Compatibility of *Trichoderma viride* with fertilizers

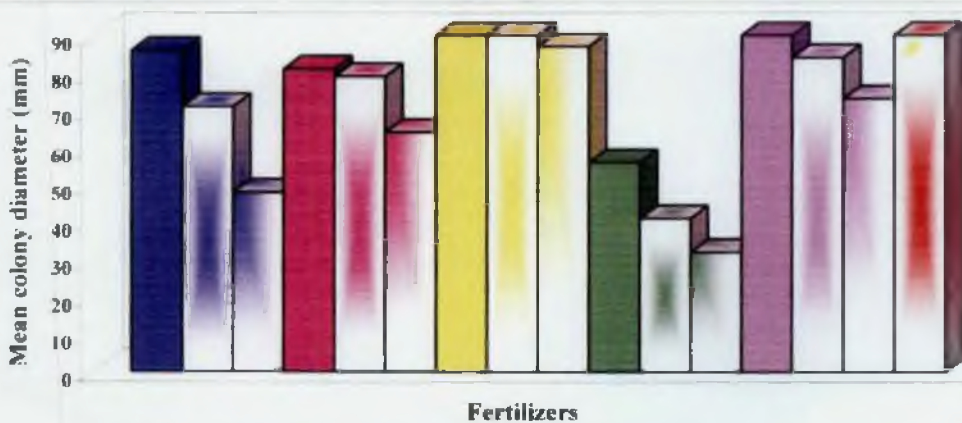


Fig. 4.10.Compatibility of *Trichoderma longibrachiatum* on fertilizers

- Urea 1%
- Urea 1.5 %
- Urea 2%
- Rajphos 2%
- Rajphos 2.5 %
- Rajphos 3 %
- MOP 2%
- MOP 2.5%
- MOP 3%
- Factomphos 2%
- Factomphos 2.5%
- Factomphos 3%
- Ammonium sulphate 2%
- Ammonium sulphate 2.5%
- Ammonium sulphate 3%
- Control

4.10 EVALUATION OF FUNGICIDES, INSECTICIDES AND FERTILIZERS AGAINST THE PATHOGEN

The *in vitro* sensitivity of the pathogen, *P.capsici*, to fungicides, insecticides and fertilizers commonly recommended for use in pepper gardens was tested.

4.10.1 Fungicides

The data on *in vitro* sensitivity of *P.capsici* to different fungicides are presented in Table 4.14. There was significant difference among the fungicides at all concentrations tested in inhibiting the growth of the pathogen (Fig. 4.11). Out of the nine fungicides tested, Indofil M- 45, Ridomil MZ, Captaf, BM, Fytolan and Kocide at all concentrations completely inhibited the growth of the pathogen. Though, the higher two concentrations of Akomin–40 (0.3per cent and 0.4per cent) showed complete inhibition of the growth of *P.capsici*, its lower concentration of 0.2 per cent permitted only slight growth and recorded 83.33 per cent efficacy over control in inhibiting the fungus. It was noticed that Kavach at all concentrations and Anthracol at 0.05 and 0.1 per cent concentrations recorded per cent inhibition between 60 to 75.56 respectively. However, the higher concentration of Anthracol (0.15per cent) completely inhibited the growth of the fungus.

4.10.2 Insecticides

The relative efficacy of eight different insecticides at varied concentrations on *in vitro* growth of *P.capsici* is presented in Table 4.15.

The insecticides *viz.*, Monocrotophos, Endosulfan, Quinalphos and Cypermethrin completely inhibited the mycelial growth of the fungus at all concentrations tested (Fig. 4.12). Chlorpyriphos, Dimethoate, Phorate and Carbofuran permitted slight growth of the fungus. Among insecticides, which supported growth of the pathogen, maximum mycelial growth of the fungus was observed at the lower concentration of Phorate (1kg a.i ha^{-1}) and Carbofuran (0.5 kg a.i ha^{-1}), which only recorded an inhibition of 68.89 and 70.0 per cent respectively. Chlorpyriphos and

Table 4.14. *In vitro* sensitivity of *P. capsici* to fungicides

Sl.No.	Fungicide	Concentration (per cent)	*P. capsici*	
			Mean diameter of colony (mm)	Per cent inhibition over control
1	Indofil M-45	0.2	0(0.707)^{d}	100
		0.3	0(0.707)^{d}	100
		0.4	0(0.707)^{d}	100
2	Ridomil MZ	0.2	0(0.707)^{d}	100
		0.3	0(0.707)^{d}	100
		0.4	0(0.707)^{d}	100
3	Akomin-40	0.2	15(3.89)^{cd}	83.33
		0.3	0(0.707)^{d}	100
		0.4	0(0.707)^{d}	100
4	Captaf	0.1	0(0.707)^{d}	100
		0.2	0(0.707)^{d}	100
		0.3	0(0.707)^{d}	100
5	Kavach	0.2	30(5.55)^{b}	66.67
		0.3	28(5.34)^{bc}	68.89
		0.4	22(4.71)^{bc}	75.56
6	Anthracol	0.05	36(5.96)^{b}	60.00
		0.1	27(5.21)^{bc}	70.00
		0.15	0(0.707)^{d}	100
7	Bordeaux mixture	0.5	0(0.707)^{d}	100
		1.0	0(0.707)^{d}	100
		1.5	0(0.707)^{d}	100
8	Fytolan	0.2	0(0.707)^{d}	100
		0.3	0(0.707)^{d}	100
		0.4	0(0.707)^{d}	100
9	Kocide	0.1	0(0.707)^{d}	100
		0.2	0(0.707)^{d}	100
		0.3	0(0.707)^{d}	100
10	Control		90(9.51)^{a}	

* Mean of three replications.
In each column figures followed by same letter donot differ significantly according to DMRT.
Figures in parantheses are $\sqrt{x+0.5}$ transformed values.

Table 4.15. ***In vitro*** **sensitivity of** ***P. capsici*** **to insecticides**

Sl.No.	Insecticides	Concentration (per cent)	*P.capsici*	
			*Average diameter of colony (mm)	Per cent inhibition over control
1	Monocrotophos	0.04	0^{k}	100
		0.05	0^{k}	100
		0.06	0^{k}	100
2	Chlorpyriphos	0.01	17^{f}	81.11
		0.02	13^{h}	85.56
		0.03	11^{ij}	87.78
3	Endosulfan	0.04	0^{k}	100
		0.05	0^{k}	100
		0.06	0^{k}	100
4	Dimethoate	0.04	15^{g}	83.33
		0.05	12^{i}	86.67
		0.06	10^{j}	88.89
5	Quinalphos	0.03	0^{k}	100
		0.04	0^{k}	100
		0.05	0^{k}	100
6	Cypermethrin	0.01	0^{k}	100
		0.02	0^{k}	100
		0.03	0^{k}	100
7	Phorate	1.0 kg a.i per ha	28^{b}	68.89
		1.5 kg a.i per ha	25^{cd}	72.22
		2.0 kg a.i per ha	19^{e}	78.89
8	Carbofuran	0.5 kg a.i per ha	27^{c}	70.00
		0.75 kg a.i per ha	25^{d}	72.22
		1.0 kg a.i per ha	25^{cd}	72.22
	Control		90^{a}	
	CD (0.05)		1.42	

* Mean of three replications

In each column figures followed by same letter donot differ significantly according to DMRT.

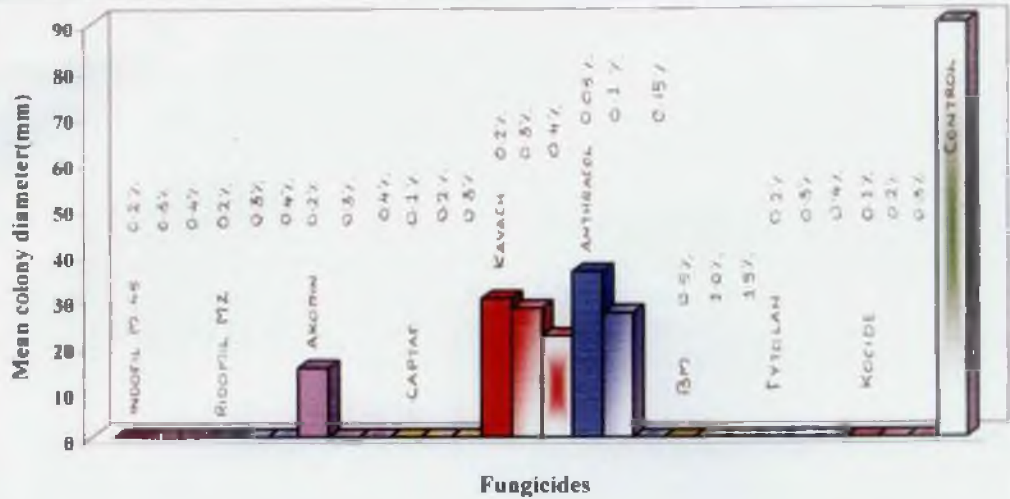


Fig. 4.11. Effect of fungicides on *Phytophthora capsici*

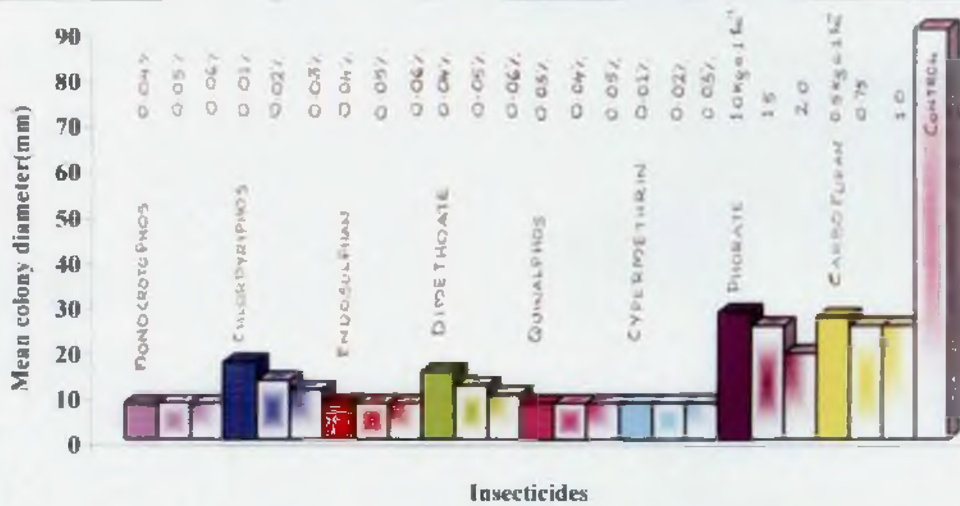


Fig. 4.12. Effect of insecticides on *Phytophthora capsici*

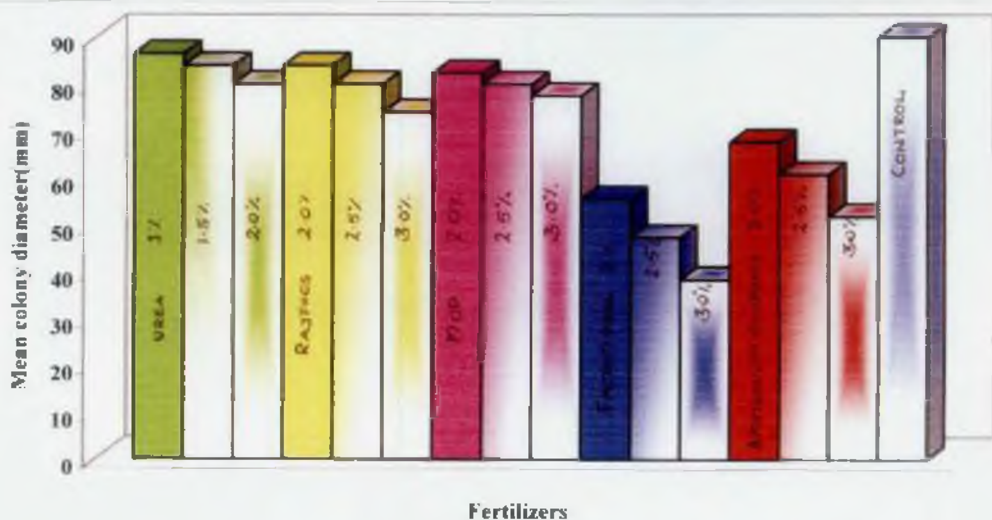


Fig. 4.13. Effect of fertilizers on *Phytophthora capsici*

Dimethoate at all concentrations recorded more than 80 per cent inhibition over control.

4.10.3 Fertilizers

The results furnished in Table 4.16 indicated significant effect on growth of the pathogen by the fertilizers tested *in vitro*.

In general, all the fertilizers except Factomphos, followed by ammonium sulphate supported comparatively good growth of the fungus (Fig. 4.13). Factomphos and ammonium sulphate at all concentrations had a significant effect in inhibiting the growth of the fungus. Among them, Factomphos at 3 per cent concentration recorded the maximum inhibition of 57.2 per cent, while the value recorded for ammonium sulphate (0.3per cent) was 42.2 per cent.

The higher concentration of urea (2per cent) and Rajphos (3per cent) recorded a per cent inhibition of 11.1 and 17.8 respectively while their lower concentrations, 1 and 1.5 per cent of urea and Rajphos 2 per cent were on par with each other and recorded only 3.9 and 6.7 per cent inhibition respectively. This was followed by the lower two concentrations of MoP (2 and 2.5per cent).

4.11 MANAGEMENT OF *Phytophthora* DISEASE IN BLACK PEPPER NURSERY

A nursery experiment (Plate 9a) was carried out to study the efficacy of the two selected antagonists *viz*., *T.viride* and *T.longibrachiatum* along with *T. harzianum* on the management of *Phytophthora* rot of black pepper nursery. The experiment was conducted as described in materials and methods.

4.11.1 Soil solarization

Temperature of the solarized and non-solarized potting mixture was recorded during the entire 30 days of soil solarization by installing soil thermometers at 5, 10

Table 4.16. ***In vitro*** **sensitivity of** ***P. capsici*** **to fertilizers**

Sl.No.	Fertilizer	Concentration (per cent)	*P. capsici*	
			*Mean diameter of colony (mm)	Per cent inhibition over control
1	Urea	1.0	86.5[b]	3.9
		1.5	84.0[b]	6.7
		2.0	80.0[c]	11.1
2	Rajphos	2.0	84.0[b]	6.7
		2.5	80.0[c]	11.1
		3.0	74.0[e]	17.8
3	MoP	2.0	82.4[c]	8.4
		2.5	80.0[c]	11.1
		3.0	77.6[d]	13.8
4	Factomphos	2.0	55.7[h]	38.1
		2.5	47.6[j]	47.1
		3.0	38.5[k]	57.2
5	Ammonium sulphate	2.0	67.8[f]	24.7
		2.5	61.0[g]	32.2
		3.0	52.0[i]	42.2
6	Control		90.0[a]	

* Mean of three replications

In each column figures followed by same letter donot differ significantly according to DMRT.

Plate 9. View of the nursery experiment
a. Before infection
b. After infection

and 15cm depth. Soil temperature at 8:30 am and 2:30 pm were recorded and are presented in Table 4.17. After covering the potting mixture with the polyethylene sheet, heat build up occurred within 24 – 48h.

It was observed that the soil temperature in the solarized potting mixture was always higher than that of the non-solarized. During solarization period, the soil temperature ranged from 47.5 to 58 ^{0}C at 5 cm depth, 35 to 46 ^{0}C at 10 cm depth and 37.5 to 45 ^{0}C at 15 cm depth in the solarized potting mixture at 2:30 pm, while the maximum temperature in the non-solarized potting mixture ranged from 33 to 38 ^{0}C, 31 to 33.5 ^{0}C and 24 to 25 ^{0}C at 5,10 and15 cm depth respectively. Further, it was noticed that, the maximum soil temperature of 58 ^{0}C at 5 cm depth in solarised soil was on 6-3-02, which was 23 ^{0}C higher than that of non-solarised soil.

The population count of soil microflora *viz.*, fungi, bacteria and actinomycetes was also recorded as mentioned in materials and methods. The soil microbial count was taken before and after solarization. It was observed that in general a significant reduction in the fungal population (27.33 x 10^2 cfu g^{-1}soil) was recorded immediately after solarization, compared to the count taken before solarization (34 x 10^2 cfu g^{-1} soil).

A significant reduction in the population of bacteria (123 x 10^4 cfu g^{-1} soil)) was recorded in the solarized soil on the day of removal of polyethylene sheet compared to the initial count (258.66 x 10^4 cfu g^{-1} soil) recorded before solarization.

The initial count of actinomycetes recorded before solarization in the potting mixture was 32.66 x 10^4 cfu g^{-1} of soil. The population of actinomycetes was reduced to 23.33 x 10^4 cfu g^{-1} in the solarized soil.

4.11.2 Effect of treatments on sprouting of black pepper cuttings

The number of sprouted pepper cuttings in each treatment was recorded 20, 30 and 45 days after planting (DAP) and sprouting percentage worked out.

The results of the study shown in Table 4.18 revealed a significant difference of sprouting of cuttings among the various treatments at different intervals of

Table 4.17. Soil temperature at different depths during the solarization period

Date	Non solarized soil						Solarized soil					
	Soil Temperature °C at different soil depths						Soil Temperature °C at different soil depths					
	5 cm		10 cm		15 cm		5 cm		10 cm		15 cm	
	8.30 am	2.30 pm	8.30 am	2.30 pm	8.30 pm	2.30 pm	8.30 am	2.30 pm	8.30 am	2.30 pm	8.30 pm	2.30 pm
08-02-02	29.0	35.0	28.5	31.5	21.0	24.0	34.0	48.0	28.0	38.0	32.0	37.5
09-02-02	28.0	33.0	27.5	33.5	20.0	24.0	35.0	48.0	28.0	35.0	32.0	37.5
10-02-02	28.0	35.0	27.5	31.0	20.0	25.0	35.0	48.0	27.0	38.0	31.5	37.5
11-02-02	28.5	35.5	27.0	31.5	21.0	25.0	34.0	47.5	28.0	38.0	32.0	38.0
12-02-02	29.0	36.0	28.0	32.5	20.0	25.0	34.0	49.0	28.0	39.0	30.0	38.5
13-02-02	27.0	35.5	28.0	32.0	20.0	25.0	35.0	54.0	28.0	39.0	33.0	39.5
14-02-02	28.0	35.5	27.5	32.0	21.0	25.0	35.0	56.0	28.0	39.0	32.5	39.0
15-02-02	28.0	35.0	27.5	32.0	21.0	25.0	35.0	54.0	28.0	39.5	33.0	40.0
16-02-02	28.5	36.0	28.0	33.0	19.0	25.0	35.0	54.0	28.0	40.0	33.0	40.0
717-02-02	28.0	36.5	28.0	33.0	20.0	25.0	35.0	56.0	28.0	40.0	32.0	40.5
18-02-02	28.0	37.5	27.5	33.5	20.0	25.0	35.0	56.0	28.0	42.0	35.0	41.0
19-02-02	28.0	37.0	27.0	33.5	20.0	25.0	35.0	56.0	28.0	42.0	35.0	41.5
20-02-02	28.0	34.5	27.0	32.0	20.0	24.0	35.0	53.0	28.0	39.5	35.0	40.0
21-02-02	30.0	38.0	27.0	33.0	20.0	25.0	35.0	56.0	28.0	43.0	35.0	42.0
22-02-02	29.0	37.5	28.0	33.5	20.0	25.0	35.0	56.0	29.0	43.0	35.5	42.0
23-02-02	28.0	37.0	27.0	33.0	20.0	25.0	35.0	56.0	28.0	43.0	34.0	42.0
24-02-02	28.0	37.0	27.0	33.0	20.0	25 0	34.0	56.0	28.0	43.0	34.0	42.0
25-02-02	28.0	36.0	27.0	33.0	20.0	25.0	35.0	56.0	28.0	42.0	33.0	42.0
26-02-02	28.0	37.0	27.0	33.0	20.0	25.0	35.0	56.0	28.0	44.0	34.0	42.0
27-02-02	29.0	37.0	27.0	33.0	20.0	25.0	35.0	56.0	29.5	44.0	34.0	43.0
28-02-02	29.0	38.5	27.5	34.5	20.0	25.0	35.0	57.0	30.0	45.0	35.5	43.5
01-03-02	29.5	37.0	28.0	34.0	20.0	25.0	35.0	56.0	30.0	44.0	36.0	43.5
02-03-02	30.0	39.0	29.0	35.0	22.0	26.0	35.5	57.0	29.0	45.0	36.0	44.0
03-03-02	29.5	39.5	29.0	35.0	22.0	26.0	35.5	57.0	30.0	45.0	36.0	44.0
04-03-02	31.0	38.0	30.0	33.0	23.0	25.0	35.0	54.0	28.0	43.0	36.0	41.0
05-03-02	28.5	39.0	29.0	35.0	22.0	26.0	35.0	57.0	30.0	45.0	36.0	44.0
06-03-02	31.0	41.0	30.5	35.0	21.0	27.0	35.0	58.0	28.0	46.0	36.0	45.0
07-03-02	31.0	40.0	30.5	35.5	20.0	26.0	35.0	57.0	28.0	45.0	36.0	44.0
08-03-02	30.0	38.0	31.0	33.0	21.0	25.0	33.0	54.0	29.0	42.0	34.5	41.0
09-03-02	31.0	39.0	32.0	35.5	21.0	27.0	30.0	56.0	28.0	45.0	31.0	43.5

Table 4.18. Effect of various treatments on sprouting, dormancy and rotten percentage

Treatment	Sprouting (Per cent)			Dormancy (Per cent) (45 DAP)	Rotten (Per cent) (45 DAP)
	20 DAP	30 DAP	45 DAP		
T1	29.33 ^{cd}	59.33 ^{de}	75.67 ^{bcde}	20.52(4.55) ^{a}	3.81(1.90) ^{a}
T2	51.00 ^{abc}	78.67 ^{abcd}	86.67 ^{abcd}	10.32(3.21) ^{a}	3.37(1.88) ^{a}
T3	42:33 ^{abcd}	73.33 ^{abcde}	82.67 ^{abcde}	16.45(4.02) ^{a}	1.55(1.41) ^{a}
T4	36.00 ^{abcd}	66.67 ^{abcde}	77.00 ^{abcde}	18.5(4.23) ^{a}	4.49(2.16) ^{a}
T5	39.33 ^{abcd}	69.00 ^{abcde}	78.33 ^{abcde}	17.64(4.22) ^{a}	4.04(2.13) ^{a}
T6	47.33 ^{abcd}	81.67 ^{ab}	89.00 ^{abc}	9.37(3.13) ^{a}	1.72(1.45) ^{a}
T7	55.00 ^{ab}	81.00 ^{ab}	88.00 ^{abc}	11.08(3.32) ^{a}	0.92(1.15) ^{a}
T8	43.67 ^{abcd}	78.00 ^{abcd}	85.33 ^{abcde}	11.08(3.33) ^{a}	3.59(1.89) ^{a}
T9	40.00 ^{abcd}	67.67 ^{abcde}	76.33 ^{abcde}	17.73(4.25) ^{a}	5.95(2.37) ^{a}
T10	38.67 ^{abcd}	68.33 ^{abcde}	84.00 ^{abcde}	12.30(3.58) ^{a}	3.87(2.01) ^{a}
T11	25.00 ^{d}	57.67 ^{e}	73.00 ^{de}	21.15(4.63 ^{a}	5.80(2.26) ^{a}
T12	56.00 ^{ab}	80.33 ^{ab}	90.00 ^{ab}	8.07(2.91) ^{a}	1.91(1.49) ^{a}
T13	54.00 ^{ab}	82.67 ^{a}	89.67 ^{ab}	9.42(3.14) ^{a}	0.92(1.07) ^{a}
T14	46.67 ^{abcd}	74.33 ^{abcde}	84.00 ^{abcde}	13.28(3.52) ^{a}	3.48(1.92) ^{a}
T15	36.00 ^{abcd}	62.33 ^{bcde}	74.67 ^{cde}	22.63(4.79) ^{a}	2.54(1.73) ^{a}
T16	38.00 ^{abcd}	67.33 ^{abcde}	82.67 ^{abcde}	16.11(4.07) ^{a}	5.23(2.38) ^{a}
T17	32.00 ^{bcd}	60.33 ^{cde}	72.33 ^{e}	22.49(4.63) ^{a}	4.98(2.29) ^{a}
T18	54.33 ^{ab}	85.33 ^{a}	88.67 ^{abc}	8.78(2.99) ^{a}	2.59(1.73) ^{a}
T19	57.00 ^{a}	79.00 ^{abc}	89.33 ^{ab}	7.81(2.86) ^{a}	3.00(1.87) ^{a}
T20	54.67 ^{ab}	81.33 ^{ab}	90.33 ^{a}	12.05(3.29) ^{a}	0.95(1.15) ^{a}

T$_1$ Control

T$_2$ Disease control as per POP of KAU

T$_3$ *Trichoderma harzianaum* alone

T$_4$ Selected antagonist from Thrissur(*T. viride*)

T$_5$ Selected antagonist from Thrissur(*T.longibrachiatum*)

T$_6$ Soil sol. for 30 days +*T. harzianum*

T$_7$ Soil sol. for 30 days +*T. viride*

T$_8$ Soil sol. for 30 days + *T. longibrachiatum*

T$_9$ *T.harzianaum* + Ridomil MZ

T$_{10}$ *T. viride*+ Ridomil MZ

T$_{11}$ *T. longibrachiatum* + Ridomil MZ

T$_{12}$ Soil sol. for 30 days + *T. harzianum* +Ridomil MZ

T$_{13}$ Soil sol. for 30 days + *T. viride* + Ridomil MZ

T$_{14}$ Soil sol. for 30 days + *T. longibrachiatum* + Ridomil MZ

T$_{15}$ *T. harzianum* +Pot. phosphonate

T$_{16}$ *T. viride* + Pot. phosphonate

T$_{17}$ *T. longibrachiatum* + Pot. phosphonate

T$_{18}$ Soil sol. for 30 days + *T. harzianum*+ Pot.phosphonate

T$_{19}$ Soil sol for 30 days +*T.viride* + Pot.phosphonate

T$_{20}$ Soil sol for 30 days + *T. longibrachiatum* + Pot.phosphonate

In each column figures followed by same letter donot differ significantly according to DMRT.
Figures in paranthese are $\sqrt{x+0.5}$ transformed values.

observation. Maximum sprouting percentage of 57 was recorded in treatment T_{19} (Soil solarization + *T.viride* + Potassium phosphonate) 20 DAP which was on par with all other treatments except T_{11} (*T. longibrachiatum* + Ridomil MZ) and T_1 (Control). The least sprouting percentage of 25.0 was noticed in T_{11}. Control plants recorded a sprouting of 29.33 per cent.

A significant change of sprouting of pepper cuttings was noticed 30 DAP among the treatments. Maximum sprouting percentage of 85.33 followed by 82.67 was recorded in T_{18} (Soil solarization + *T. harzianum* + Potassium phosphonate) and T_{13} (Soil solarization + *T.viride* + Ridomil MZ), which were on par with other treatments except T_{15}, T_{17}, T_{11} and T_1. Minimum sprouting of 57.67 was recorded in T_{11} followed by T_1 (59.33).

It was evident from the data that 45 DAP, solarized potting mixture supplemented with *Trichoderma* spp. showed more than 84 per cent sprouting of pepper cuttings as noticed in T_6, T_7, T_8 ,T_{12} ,T_{13} ,T_{14} , T_{18}, T_{19} and T_{20}. In addition, plants raised as per PoP (T_2) also showed good sprouting. Out of the various treatments, highest sprouting percentage of 90.33 was observed in T_{20} (Soil solarization + *T.longibrachiatum* + Potassium phosphonate), which was on par with the remaining treatments except T_{17} (*T.longibrachiatum* + Potassium phosphonate), T_{15} (*T.harzianum* + Potassium phosphonate), T_{11} and T_1. The least percentage of sprouting was recorded in T_{17} (72.33) followed by T_{11} (73.0). The treatment T_{11} was statistically on par with $T_1$and T_{15}, which recorded a sprouting percentage of 75.67 and 74.67 respectively.

The observation on rotting of cuttings and those cuttings that remained green/dormant 45DAP was also recorded. Although there was no significant difference among the various treatments, a higher percentage of rotting of 5.95 was recorded in T_9 (*T.harzianum* + Ridomil MZ) and the least was shown by T_{13} (Soil solarization + *T.viride* + Ridomil MZ), which recorded a rotting percentage of 0.92. Maximum dormancy percentage of 22.63 was observed in T_{15} and the minimum of 7.81 was recorded in T_{19} (Soil solarization + *T.viride* + Potassium phosphonate).

4.11.3 Effect of various treatments on the incidence and severity of *Phytophthora* rot disease in black pepper nursery

With the onset of monsoon, six sprayings of fungicides were given on the naturally infected pepper cuttings at fortnightly intervals in respective treatments. Observations on the disease incidence and severity (Plate 9b) were recorded at weekly intervals for four weeks and the percentage efficiency of the treatments over control were calculated.

Data on disease incidence during fourteenth week after planting (WAP) revealed that there was no significant difference among the treatments (Table 4.19). However, the maximum percentage of disease incidence of 7.53 was recorded in T_1 (Control) followed by T_{12} (Soil solarization + *T.harzianum* + Ridomil MZ), which were on par with the remaining treatments. The least percentage of disease incidence (2.1) was observed in T_8 (Soil solarization + *T.longibrachiatum*) followed by T_3 (*T.harzianum* alone) (Fig. 4.14).

Observation on disease severity on fourteenth WAP, revealed that, the cuttings in treatment T_{12} (Soil solarization + *T.harzianum* + Ridomil MZ) recorded the minimum severity (4.53 per cent) and maximum percentage efficiency over control (90.34) followed by treatment T_{10} (*T.viride* + Ridomil MZ), T_9 (*T.harzianum* + Ridomil MZ) and T_{13} (Soil solarization + *T.viride* + Ridomil MZ) which were statistically on par with the remaining treatments except for T_1 (Control), T_2 (POP), T_3 (*T.harzianum* alone) and T_4 (*T.viride* alone). The highest PDS (46.93) was registered in the treatment T_1 (Control).

Statistical analysis of the data on disease incidence during fifteenth week after planting (Table 4.20) revealed no significant difference among the treatments. However, it revealed that the plants in T_1 (Control) recorded maximum disease incidence (27.89 per cent) followed by T_6 (Soil solarization + *T.harzianum*) (Fig. 4.15). The least percentage of disease incidence was noticed in T_{13} (Soil solarization + *T.viride* + Ridomil MZ)(7.83) and T_{14} (Soil solarization + *T.longibrachiatum* + Ridomil MZ)(7.83) followed by T_{12} (Soil solarization + *T.harzianum* + Ridomil MZ)(8.57). However, the cuttings in treatment T_{12} recorded the minimum PDS (12.8)

Table 4.19. Effect of various treatments on the incidence and severity of *Phytophthora* rot in pepper nursery (14 WAP)

Treatment	Fourteenth WAP		Per cent efficiency of the treatment over control	
	Per cent disease incidence	Per cent disease severity	Disease incidence	Disease severity
T1	7.53 (2.53) ^{a}	46.93(6.84) ^{a}	-	-
T2	3.53 (1.96) ^{a}	16.80 (4.12) ^{a}	53.08	64.20
T3	2.60 (1.73) ^{a}	19.73(3.90) ^{a}	65.47	57.95
T4	3.97 (1.84) ^{a}	26.40(4.78) ^{a}	47.32	43.75
T5	3.40 (1.92) ^{a}	21.07(4.27) ^{ab}	54.85	55.11
T6	5.53 (2.36) ^{a}	28.27(4.86) ^{ab}	26.52	39.77
T7	3.73 (2.04) ^{a}	14.13(3.22) ^{ab}	50.42	69.88
T8	2.10 (1.47) ^{a}	22.93(4.47) ^{ab}	72.11	51.13
T9	4.23 (2.15) ^{a}	8.27(2.80) ^{b}	43.78	82.39
T10	4.23 (2.14) ^{a}	8.00(2.76) ^{b}	43.78	82.95
T11	6.60 (2.43) ^{a}	18.67(3.83) ^{ab}	12.35	60.22
T12	7.33 (2.64) ^{a}	4.53(1.83) ^{b}	2.61	90.34
T13	5.73 (2.34) ^{a}	9.33(2.77) ^{b}	23.86	80.11
T14	6.00 (2.50) ^{a}	15.47(3.69) ^{ab}	20.32	67.04
T15	3.10 (1.90) ^{a}	15.40(3.93) ^{ab}	58.83	67.19
T16	3.93 (2.06) ^{a}	19.20(4.14) ^{ab}	47.76	59.09
T17	5.37 (2.34) ^{a}	14.93(3.88) ^{ab}	28.73	68.18
T18	5.20 (2.25) ^{a}	15.73(3.81) ^{ab}	30.94	66.47
T19	6.03 (2.50) ^{a}	15.47(3.92) ^{ab}	19.88	67.04
T20	7.00 (2.65) ^{a}	19.47(3.87) ^{ab}	7.04	58.52

T_1 Control
T_2 Disease control as per POP of KAU
T_3 *Trichoderma harzianaum* alone
T_4 Selected antagonist from Thrissur (*T. viride*)
T_5 Selected antagonist from Thrissur (*T.longibrachiatum*)
T_6 Soil sol. for 30 days +*T. harzianum*
T_7 Soil sol for 30 days +*T. viride*
T_8 Soil sol. for.30 days + *T. longibrachiatum*
T_9 *T.harzianaum* + Ridomil MZ
T_{10} *T. viride*+ Ridomil MZ
T_{11} *T. longibrachiatum* + Ridomil MZ
T_{12} Soil sol. for 30 days +*T. harzianum* +Ridomil MZ
T_{13} Soil sol. for 30 days + *T. viride* + Ridomil MZ
T_{14} Soil sol. for 30 days + *T. longibrachiatum* + Ridomil MZ
T_{15} *T. harzianum* +Pot. phosphonate
T_{16} *T. viride* + Pot. phosphonate
T_{17} *T. longibrachiatum* + Pot. phosphonate
T_{18} Soil sol. for 30 days +*T. harzianum*+ Pot.phosphonate
T_{19} Soil sol for 30 days +*T.viride*+ Pot.phosphonate
T_{20} Soil sol for 30 days +*T. longibrachiatum* + Pot.phosphonate

In each column figures followed by same letter donot differ significantly according to DMRT.
Figures in parantheses are $\sqrt{x+0.5}$ transformed values. (WAP:weeks after planting)

Table 4.20. Effect of various treatments on the incidence and severity of *Phytophthora* rot in pepper nursery (15 WAP)

Treatment	Fifteenth WAP		Per cent efficiency of the treatment over control	
	Per cent disease incidence	Per cent disease severity	Disease incidence	Disease severity
T1	27.87(5.11)[a]	69.87(8.38)[a]	-	-
T2	10.27(3.14)[a]	36.80(5.96)[ab]	63.16	47.33
T3	17.00(4.06)[a]	30.67(5.42)[ab]	39.00	56.11
T4	19.17(4.29)[a]	37.87(5.47)[ab]	31.23	45.80
T5	18.50(4.23)[a]	36.80(6.02)[ab]	33.62	47.33
T6	24.80(4.92)[a]	48.00(6.91)[ab]	11.02	31.30
T7	19.37(4.12)[a]	29.87(4.95)[ab]	30.51	57.25
T8	18.60(3.93)[a]	36.27(5.81)[ab]	33.26	48.09
T9	12.23(3.44)[a]	22.93(4.62)[ab]	56.11	67.18
T10	10.40(3.17)[a]	20.53(4.53)[ab]	62.68	70.61
T11	11.97(3.22)[a]	25.20(4.71)[ab]	57.06	63.93
T12	8.57 (3.01)[a]	12.80(3.62)[b]	69.26	81.68
T13	7.83 (2.86)[a]	23.73(4.65)[ab]	71.89	66.03
T14	7.83 (2.86)[a]	21.01(4.46)[ab]	71.89	69.85
T15	13.73(3.58)[a]	32.00(5.59)[ab]	50.72	54.20
T16	15.97(3.83)[a]	26.93(4.76)[ab]	42.71	61.45
T17	13.07(3.52)[a]	21.87(4.60)[ab]	53.12	68.70
T18	12.33(3.54)[a]	29.60(5.21)[ab]	55.75	57.64
T19	15.70(3.88)[a]	26.93(4.97)[ab]	43.67	61.45
T20	16.57(3.89)[a]	42.13(5.93)[ab]	40.56	39.70

T_1 Control
T_2 Disease control as per POP of KAU
T_3 *Trichoderma harzianum* alone
T_4 Selected antagonist from Thrissur (*T. viride*)
T_5 Selected antagonist from Thrissur (*T. longibrachiatum*)
T_6 Soil sol. for 30 days +*T. harzianum*
T_7 Soil sol. for 30 days +*T. viride*
T_8 Soil sol. for 30 days + *T. longibrachiatum*
T_9 *T. harzianum* + Ridomil MZ
T_{10} *T. viride*+ Ridomil MZ
T_{11} *T. longibrachiatum* + Ridomil MZ
T_{12} Soil sol. for 30 days +*T. harzianum* +Ridomil MZ
T_{13} Soil sol. for 30 days + *T. viride* + Ridomil MZ
T_{14} Soil sol. for 30 days + *T. longibrachiatum* + Ridomil MZ
T_{15} *T. harzianum* +Pot. phosphonate
T_{16} *T. viride* + Pot. phosphonate
T_{17} *T. longibrachiatum* + Pot. phosphonate
T_{18} Soil sol. for 30 days +*T. harzianum*+ Pot phosphonate
T_{19} Soil sol for 30 days +*T. viride*+ Pot.phosphonate
T_{20} Soil sol for 30 days +*T. longibrachiatum* + Pot.phosphonate

In each column figures followed by same letter donot differ significantly according to DMRT.
Figures in parantheses are $\sqrt{x+0.5}$ transformed values. (WAP: weeks after planting)

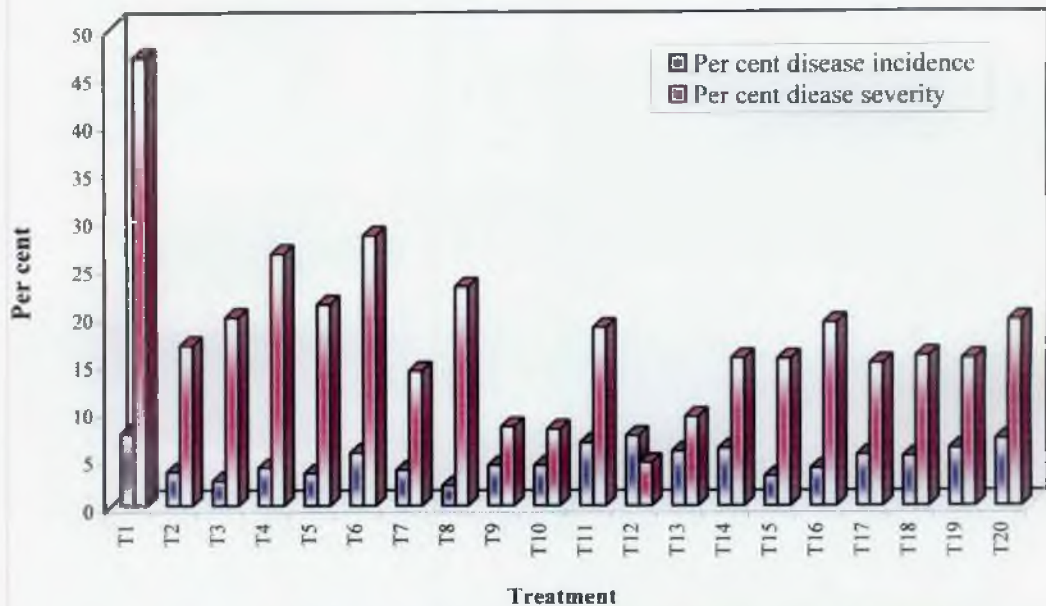


Fig. 4.14. Per cent disease incidence and severity 14 WAP

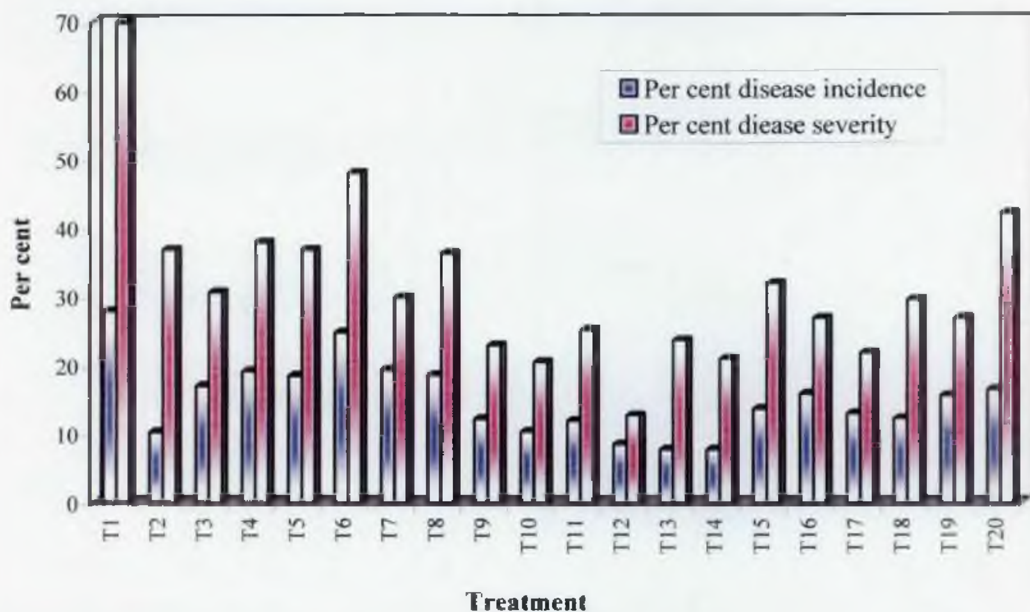


Fig. 4.15. Per cent disease incidence and severity 15 WAP

Table 4.21. Effect of various treatments on the incidence and severity of *Phytophthora* rot in pepper nursery (16 WAP)

Treatment	Sixteenth WAP		Per cent efficiency of the treatment over control	
	Per cent disease incidence	Per cent disease severity	Disease incidence	Disease severity
T1	38.23(6.10)^{a}	62.40(7.93)^{a}	-	-
T2	13.57(3.67)^{a}	23.47(4.65)^{b}	64.51	62.39
T3	30.63(5.44)^{a}	32.27(5.71)^{ab}	19.87	48.29
T4	33.77(5.71)^{a}	46.93(6.82)^{ab}	11.67	24.79
T5	29.57(5.36)^{a}	31.47(5.61)^{ab}	22.66	49.57
T6	33.40(5.79)^{a}	46.67(6.84)^{ab}	12.63	25.21
T7	25.53(4.94)^{a}	42.40(6.38)^{ab}	33.21	32.05
T8	34.63(5.80)^{a}	43.47(6.59)^{ab}	9.41	30.34
T9	18.13(4.17)^{a}	26.13(4.97)^{ab}	52.57	58.12
T10	15.37(3.77)^{a}	31.47(5.30)^{ab}	59.80	49.57
T11	14.67(3.75)^{a}	17.87(4.12)^{b}	61.64	71.37
T12	17.80(4.27)^{a}	34.13(5.84)^{ab}	53.44	45.30
T13	15.63(3.94)^{a}	20.00(4.33)^{b}	59.11	67.95
T14	14.60(3.78)^{a}	28.53(4.98)^{ab}	61.81	54.27
T15	18.23(4.26)^{a}	30.40(5.50)^{ab}	52.31	51.28
T16	21.13(4.46)^{a}	26.93(5.00)^{ab}	44.72	56.84
T17	19.17(4.33)^{a}	25.87(5.01)^{ab}	49.86	58.55
T18	21.70(4.68)^{a}	38.40(6.22)^{ab}	43.24	38.46
T19	20.17(4.43)^{a}	27.73(5.00)^{ab}	47.25	55.56
T20	24.50(4.85)^{a}	28.27(5.11)^{ab}	35.91	54.70

T_1	Control	T_{11}	*T. longibrachiatum* + Ridomil MZ
T_2	Disease control as per POP of KAU	T_{12}	Soil sol. for 30 days + *T. harzianum* + Ridomil MZ
T_3	*Trichoderma harzianaum* alone	T_{13}	Soil sol. for 30 days + *T. viride* + Ridomil MZ
T_4	Selected antagonist from Thrissur(*T. viride*)	T_{14}	Soil sol. for 30 days + *T. longibrachiatum* + Ridomil MZ
T_5	Selected antagonist from Thrissur(*T.longibrachiatum*)	T_{15}	*T. harzianum* +Pot. phosphonate
T_6	Soil sol. for 30 days +*T. harzianum*	T_{16}	*T. viride* + Pot. phosphonate
T_7	Soil sol. for 30 days +*T. viride*	T_{17}	*T. longibrachiatum* + Pot. phosphonate
T_8	Soil sol. for 30 days + *T. longibrachiatum*	T_{18}	Soil sol. for 30 days +*T. harzianum*+ Pot.phosphonate
T_9	*T.harzianaum* + Ridomil MZ	T_{19}	Soil sol for 30 days +*T viride*+ Pot.phosphonate
T_{10}	*T. viride*+ Ridomil MZ	T_{20}	Soil sol for 30 days +*T. longibrachiatum* + Pot.phosphonate

In each column figures followed by same letter donot differ significantly according to DMRT.
Figures in parantheses are $\sqrt{x+0.5}$ transformed values. (WAP:weeks after planting)

and showed the maximum percentage efficiency over control (81.68) in checking the severity of the disease. While, the plants in treatment T_1 (Control) recorded the maximum PDS of 69.87, which was on par with all other treatments except T_{12}.

Observations on disease incidence recorded during the sixteenth WAP showed no significant difference among the treatments (Table 4.21). It was noticed that the cuttings in various treatments recorded a per cent disease incidence ranging from a minimum 13.57 (T_2) to a maximum of 38.23 in T_1 (Fig. 4.16). However, there was significant difference among the treatments on the percentage of disease severity. The least disease severity was recorded in treatment T_{11} (*T.longibrachiatum* + Ridomil MZ) (17.87 per cent) closely followed by treatment T_{13} (Soil solarization + *T.viride* + Ridomil MZ)(20.0 per cent) and T_2 (POP) (23.47 per cent) which were on par with other treatments except T_1 (Control) which recorded the maximum disease severity (62.4 per cent). The treatment T_{11} showed more than 70 per cent efficiency in checking the severity of the disease.

At seventeenth WAP (Table 4.22), it was noticed that there was a significant difference among the treatments in the percentage of disease incidence and severity (Fig. 4.17). During the period of observation, the minimum disease incidence was observed in treatment T_2 (POP) (17.6 per cent) closely followed by T_{14} (Soil solarization + *T.longibrachiatum* + Ridomil MZ) (18.4 per cent) and T_{13} (Soil solarization + *T.viride* + Ridomil MZ) (18.43 per cent). All other treatments recorded comparatively higher disease incidence ranging from 21.8 in treatment T_2 to 58.4 in T_4 (*T.viride* alone). However, the maximum disease severity of 66.4 was recorded in the control treatment (T_1) and the minimum disease severity of 19.73 was recorded in T_{11} (*T.longibrachiatum* + Ridomil MZ) with maximum percentage efficiency of the treatment (70.28) followed by T_2, T_{13} and T_{14}.

The data on percentage of disease incidence and severity recorded during different intervals of observation were further subjected to pooled analysis (Fig. 4.18). The results (Table 4.23) of the analysis revealed significant difference among treatments on the incidence and severity of *Phytophthora* rot in black pepper nursery. The data revealed that the minimum disease incidence of 11.27 per cent was noticed in treatment T_2 (PoP) followed by T_{14} (Soil solarization + *T.longibrachiatum* + Ridomil

Table 4.22. Effect of various treatments on the incidence and severity of *Phytophthora* rot in pepper nursery (17 WAP)

Treatment	Seventeenth WAP		Per cent efficiency of the treatment over control	
	Per cent disease incidence	Per cent disease severity	Disease incidence	Disease severity
T1	53.07(7.22) ^{ab}	66.40(8.17) ^{a}	-	-
T2	17.67(4.24) ^{d}	22.93(4.82) ^{bc}	66.71	65.46
T3	45.30(6.69) ^{abcd}	40.53(6.39) ^{abc}	14.64	38.96
T4	58.47(7.44) ^{a}	34.13(5.85) ^{bc}	-10.17	48.59
T5	47.23(6.85) ^{abcd}	45.33(6.77) ^{ab}	11.00	31.73
T6	50.43(7.13) ^{abc}	44.53(6.71) ^{ab}	4.97	32.93
T7	38.57(5.23) ^{abcd}	38.13(6.17) ^{bc}	27.33	42.57
T8	42.77(6.51) ^{abcd}	37.33(6.14) ^{bc}	19.41	43.78
T9	22.80(4.63) ^{bcd}	23.73(4.78) ^{bc}	57.04	64.26
T10	21.93(4.50) ^{cd}	29.87(5.44) ^{bc}	58.67	55.02
T11	26.27(5.02) ^{abcd}	19.73(4.45) ^{c}	50.51	70.28
T12	21.80(4.70) ^{bcd}	29.87(5.41) ^{bc}	58.92	55.02
T13	18.43(4.28) ^{d}	24.53(4.83) ^{bc}	65.27	63.05
T14	18.40(4.18) ^{d}	24.80(4.82) ^{bc}	65.33	62.65
T15	29.63(5.37) ^{abcd}	28.00(5.30) ^{bc}	44.16	57.83
T16	26.07(4.95) ^{abcd}	27.47(5.07) ^{bc}	50.88	58.63
T17	29.37(5.26) ^{abcd}	29.07(5.37) ^{bc}	44.66	56.22
T18	30.27(5.53) ^{abcd}	35.47(6.00) ^{bc}	42.97	46.59
T19	26.27(5.15) ^{abcd}	26.13(5.00) ^{bc}	50.51	60.64
T20	38.73(6.08) ^{abcd}	36.53(6.00) ^{bc}	27.01	44.98

T_1 Control
T_2 Disease control as per POP of KAU
T_3 *Trichoderma harzianaum* alone
T_4 Selected antagonist from Thrissur (*T. viride*)
T_5 Selected antagonist from Thrissur (*T.longibrachiatum*)
T_6 Soil sol. for 30 days +*T. harzianum*
T_7 Soil sol. for 30 days +*T. viride*
T_8 Soil sol. for 30 days + *T. longibrachiatum*
T_9 *T.harzianaum* + Ridomil MZ
T_{10} *T. viride*+ Ridomil MZ
T_{11} *T. longibrachiatum* + Ridomil MZ
T_{12} Soil sol. for 30 days +*T. harzianum*+ Ridomil MZ
T_{13} Soil sol. for 30 days + *T. viride* + Ridomil MZ
T_{14} Soil sol. for 30 days + *T. longibrachiatum* + Ridomil MZ
T_{15} *T. harzianum* +Pot. phosphonate
T_{16} *T. viride* + Pot. phosphonate
T_{17} *T. longibrachiatum* + Pot. phosphonate
T_{18} Soil sol. for 30 days +*T. harzianum*+ Pot.phosphonate
T_{19} Soil sol for 30 days +*T.viride*+ Pot.phosphonate
T_{20} Soil sol for 30 days +*T. longibrachiatum* + Pot phosphonate

In each column figures followed by same letter donot differ significantly according to DMRT.
Figures in parantheses are $\sqrt{x+0.5}$ transformed values. (WAP:weeks after planting)

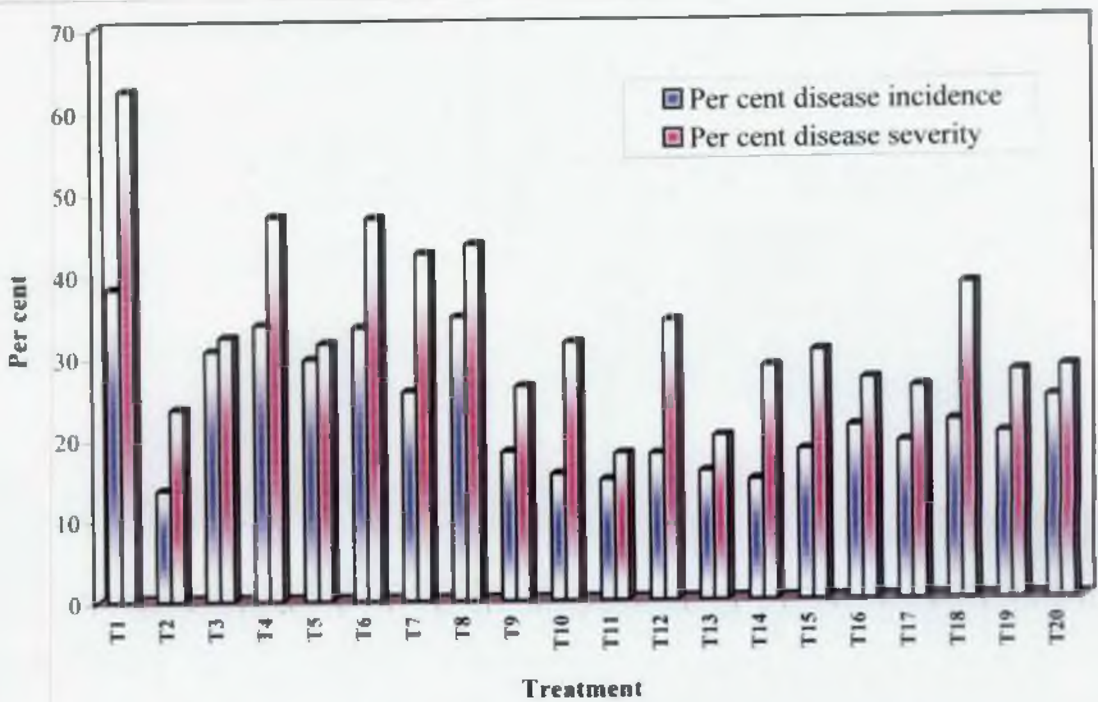


Fig. 4.16. Per cent disease incidence and severity 16 WAP

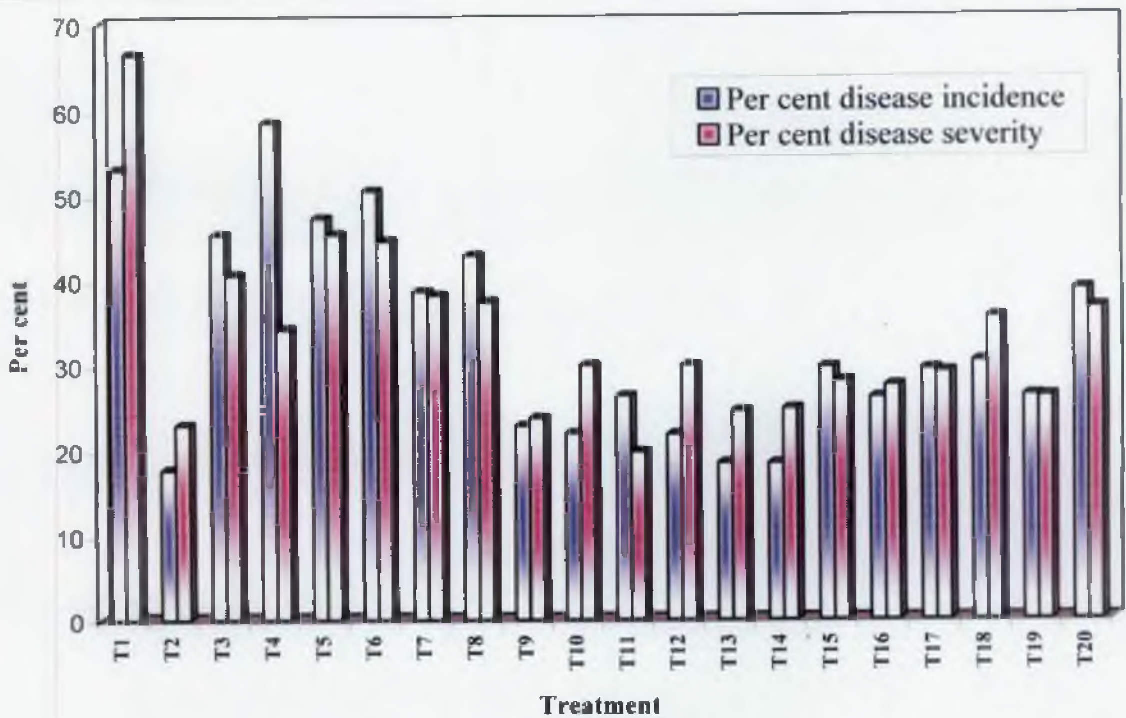


Fig. 4.17. Per cent disease incidence and severity 17 WAP

Table 4.23. Pooled analysis of various treatments on disease incidence and severity of *Phytophthora* rot in pepper nursery

Treatment	Pooled data		Per cent efficiency of the treatment over control	
	Per cent Disease incidence	Per cent Disease severity	Disease incidence	Disease severity
T1	31.70(5.55) ^{a}	61.40 ^{a}	-	-
T2	11.27(3.39) ^{b}	25.00 ^{bc}	64.45	59.19
T3	23.90(4.87) ^{ab}	30.80 ^{bc}	24.61	49.76
T4	28.83(5.26) ^{ab}	36.33 ^{bc}	9.05	40.76
T5	24.70(4.95) ^{ab}	33.67 ^{bc}	22.08	45.09
T6	28.53(5.36) ^{ab}	41.87 ^{bc}	10.00	31.76
T7	21.83(4.64) ^{ab}	31.13 ^{bc}	31.14	49.22
T8	24.53(4.92) ^{ab}	35.00 ^{bc}	22.62	42.93
T9	14.37(3.73) ^{ab}	20.27 ^{c}	54.67	66.88
T10	13.00(3.56) ^{ab}	22.47 ^{c}	58.99	63.30
T11	14.90(3.80) ^{ab}	20.37 ^{c}	53.00	66.72
T12	13.90(3.79) ^{ab}	20.33 ^{c}	56.15	66.78
T13	11.90(3.45) ^{ab}	19.40 ^{c}	62.46	68.29
T14	11.70(3.41) ^{b}	22.47 ^{c}	63.09	63.30
T15	16.17(3.99) ^{ab}	26.45 ^{bc}	48.99	56.83
T16	16.77(4.03) ^{ab}	25.13 ^{bc}	47.10	58.98
T17	16.77(4.07) ^{ab}	22.93 ^{c}	47.10	62.55
T18	17.37(4.22) ^{ab}	29.80 ^{bc}	45.21	51.38
T19	17.03(4.12) ^{ab}	24.07 ^{bc}	46.28	60.70
T20	21.70(4.60) ^{ab}	31.60 ^{bc}	31.55	48.46

T_1 Control
T_2 Disease control as per POP of KAU
T_3 *Trichoderma harzianaum* alone
T_4 Selected antagonist from Thrissur (*T. viride*)
T_5 Selected antagonist from Thrissur (*T.longibrachiatum*)
T_6 Soil sol. for 30 days +*T. harzianum*
T_7 Soil sol. for 30 days +*T. viride*
T_8 Soil sol. for 30 days + *T. longibrachiatum*
T_9 *T.harzianaum* + Ridomil MZ
T_{10} *T. viride*+ Ridomil MZ
T_{11} *T. longibrachiatum* + Ridomil MZ
T_{12} Soil sol. for 30 days +*T. harzianum*+ Ridomil MZ
T_{13} Soil sol. for 30 days + *T. viride* + Ridomil MZ
T_{14} Soil sol. for 30 days + *T. longibrachiatum* + Ridomil MZ
T_{15} *T. harzianum* +Pot. phosphonate
T_{16} *T. viride* + Pot. phosphonate
T_{17} *T. longibrachiatum* + Pot. phosphonate
T_{18} Soil sol. for 30 days +*T. harzianum*+ Pot.phosphonate
T_{19} Soil sol for 30 days +*T.viride*+ Pot.phosphonate
T_{20} Soil sol for 30 days +*T longibrachiatum* + Pot.phosphonate

In each column figures followed by same letter denot differ significantly according to DMRT.
Figures in parantheses are $\sqrt{x+0.5}$ transformed values. (WAP:weeks after planting)

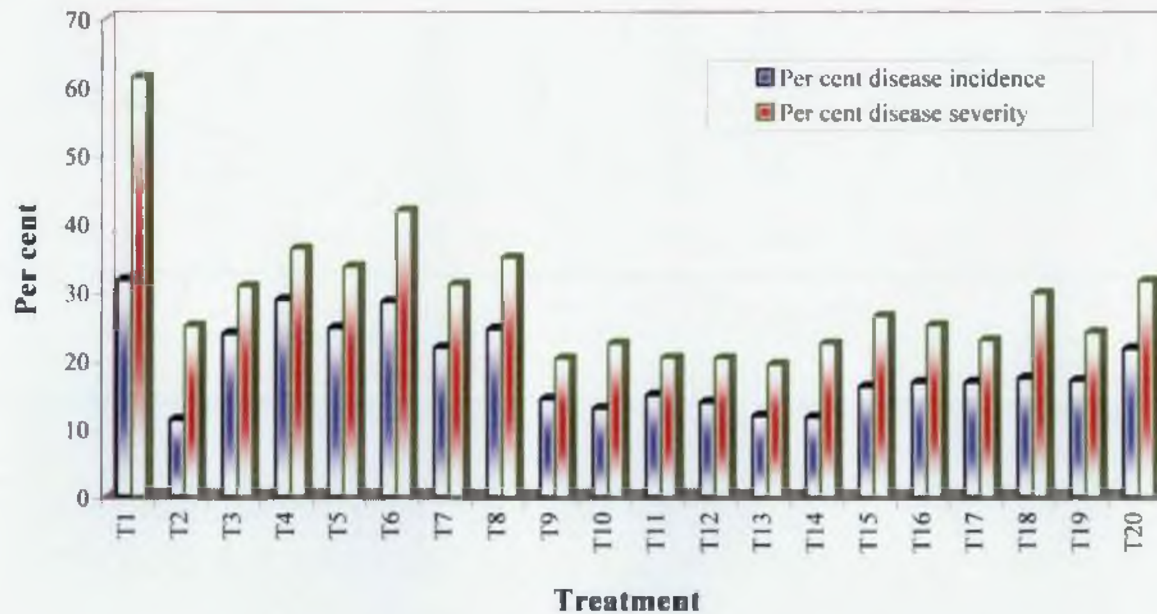


Fig. 4.18. Pooled Analysis of various treatments on the incidence and severity of *Phytophthora* rot disease in pepper nursery

MZ) (11.70 per cent) and T_{13} (Soil solarization + *T.viride* + Ridomil MZ) (11.9per cent). It was also observed that these treatments had more than 60 per cent efficiency in checking the disease incidence when compared to control. However, the minimum per cent disease severity (19.4 per cent) was observed in treatment T_{13} (Soil solarization + *T.viride* + Ridomil MZ) which was on par with all other treatments ,except T_1 (Control) which recorded the maximum disease severity (61.4 per cent).

4.11.4 Effect of treatments on mortality of pepper cuttings due to *P. capsici*

The effect of various treatments on mortality of pepper cuttings was recorded nineteenth WAP and are presented in Table 4.24.

A considerable variation in the mortality of pepper cuttings was noticed among the treatments. It was noticed that the maximum mortality (87.2 per cent) occured in control T_1, which was on par with treatment T_3 (*T.harzianum* alone). The cuttings in treatment T_{11} (*T.longibrachiatum* + Ridomil MZ) recorded the least mortality per cent (53.37) and was found superior to other treatments followed by T_{14} (Soil solarization + *T.longibrachiatum* + Ridomil MZ), T_2 (POP) and T_{13} (Soil solarization + *T.viride* + Ridomil MZ). It was observed that T_{11} recorded the maximum per cent efficiency of the treatment over control (38.80per cent).

4.11.5 Effect of treatments on the population of soil microflora

The effect of various treatments on the population of soil microflora *viz*., fungi. bacteria and actinomycetes in the potting mixture was estimated one, two and three months after planting.

4.11.5.1 *Fungi*

The data (Table 4.25) on the total fungal population showed significant fluctuation among the treatments at different intervals of observation. In general, it was observed that the total fungal population was comparatively higher after two months of planting. Comparatively lesser number of fungal propagules was recorded during the last observation taken three MAP. The total fungal population one MAP

Table 4.24. Effect of treatment on mortality of pepper cuttings due to *P.capsici*

Treatment	Per cent mortality	Per cent efficiency of the treatment over control
T1	87.20^{a}	-
T2	56.43^{ef}	35.29
T3	86.93^{ab}	0.31
T4	82.77^{abcd}	5.08
T5	86.13^{ab}	1.23
T6	85.00^{abc}	2.52
T7	84.40^{abcd}	3.21
T8	85.33^{ab}	2.14
T9	61.60^{ef}	29.36
T10	62.60^{cdef}	28.21
T11	53.37^{f}	38.80
T12	62.33^{def}	28.52
T13	57.00^{ef}	34.63
T14	56.43^{f}	35.29
T15	64.47^{bcdef}	26.07
T16	79.30^{abcd}	9.06
T17	60.77^{ef}	30.31
T18	77.67^{abcdef}	10.93
T19	71.07^{bcdef}	18.50
T20	76.40^{abcdef}	12.39

T_1 Control
T_2 Disease control as per POP of KAU
T_3 *Trichoderma harzianaum* alone
T_4 Selected antagonist from Thrissur(*T. viride*)
T_5 Selected antagonist from Thrissur(*T.longibrachiatum*)
T_6 Soil sol. for 30 days +*T. harzianum*
T_7 Soil sol. for 30 days +*T. viride*
T_8 Soil sol. for 30 days + *T. longibrachiatum*
T_9 *T harzianaum* + Ridomil MZ
T_{10} *T. viride*+ Ridomil MZ
T_{11} *T. longibrachiatum* + Ridomil MZ
T_{12} Soil sol. for 30 days +*T. harzianum*+ Ridomil MZ
T_{13} Soil sol. for 30 days + *T. viride* + Ridomil MZ
T_{14} Soil sol. for 30 days + *T. longibrachiatum* + Ridomil MZ
T_{15} *T. harzianum* +Pot. phosphonate
T_{16} *T. viride* + Pot. phosphonate
T_{17} *T. longibrachiatum* + Pot. phosphonate
T_{18} Soil sol. for 30 days +*T. harzianum*+ Pot.phosphonate
T_{19} Soil sol for 30 days +*T.viride* + Pot.phosphonate
T_{20} Soil sol for 30 days +*T. longibrachiatum* + Pot.phosphonate

In each column figures followed by same letter donot differ significantly according to DMRT.
WAP:weeks after planting

Table 4.25. Effect of treatments on the total fungal population of soil

Treatment	Fungal count ($x10^2$ cfu g^{-1} soil)		
	1 MAP	2 MAP	3 MAP
T1	13.33(3.61) ^{ab}	10.00(3.17) ^{c}	24.67(4.85) ^{a}
T2	14.00(3.76) ^{ab}	23.33(4.84) ^{ab}	15.00(3.76) ^{ab}
T3	7.33(2.78) ^{b}	12.67(3.57) ^{abc}	6.00(2.52) ^{b}
T4	8.67(2.98) ^{ab}	14.33(3.82) ^{abc}	11.00(3.38) ^{b}
T5	13.33(3.68) ^{ab}	15.33(3.95) ^{abc}	6.33(2.59) ^{b}
T6	26.67(4.66) ^{a}	14.33(3.81) ^{abc}	6.67(2.67) ^{b}
T7	9.67(3.19) ^{ab}	25.33(4.96) ^{a}	13.00(3.49) ^{ab}
T8	7.67(2.81) ^{b}	17.33(4.17) ^{abc}	9.00(3.06) ^{b}
T9	12.33(3.52) ^{ab}	11.67(3.48) ^{abc}	8.00(2.88) ^{b}
T10	11.00(3.30) ^{ab}	13.33(3.64) ^{abc}	9.67(3.11) ^{b}
T11	18.67(4.38) ^{ab}	16.00(4.05) ^{abc}	11.00(3.39) ^{b}
T12	18.00(4.24) ^{ab}	11.00(3.36) ^{bc}	7.67(2.83) ^{b}
T13	13.67(3.76) ^{ab}	11.33(3.43) ^{bc}	8.33(2.92) ^{b}
T14	16.00(4.05) ^{ab}	13.00(3.63) ^{abc}	12.33(3.48) ^{ab}
T15	9.67(3.17) ^{ab}	12.33(3.50) ^{abc}	9.00(3.04) ^{b}
T16	14.67(3.88) ^{ab}	12.00(3.50) ^{abc}	8.67(2.99) ^{b}
T17	15.33(3.97) ^{ab}	17.33(4.17) ^{abc}	12.33(3.58) ^{ab}
T18	14.33(3.80) ^{ab}	17.00(4.05) ^{abc}	10.33(3.23) ^{b}
T19	16.00(4.03) ^{ab}	11.67(3.48) ^{abc}	10.33(3.27) ^{b}
T20	16.67(4.08) ^{ab}	8.67(3.02) ^{c}	11.33(3.44) ^{ab}

T_1	Control	T_{11}	*T. longibrachiatum* + Ridomil MZ
T_2	Disease control as per POP of KAU	T_{12}	Soil sol. for 30 days + *T. harzianum* + Ridomil MZ
T_3	*Trichoderma harzianaum* alone	T_{13}	Soil sol. for 30 days + *T. viride* + Ridomil MZ
T_4	Selected antagonist from Thrissur(*T. viride*)	T_{14}	Soil sol. for 30 days + *T. longibrachiatum* + Ridomil MZ
T_5	Selected antagonist from Thrissur(*T. longibrachiatum*)	T_{15}	*T. harzianum* +Pot. phosphonate
T_6	Soil sol. for 30 days +*T. harzianum*	T_{16}	*T. viride* + Pot. phosphonate
T_7	Soil sol. for 30 days +*T. viride*	T_{17}	*T. longibrachiatum* + Pot. phosphonate
T_8	Soil sol. for 30 days + *T. longibrachiatum*	T_{18}	Soil sol for 30 days +*T. harzianum*+ Pot phosphonate
T_9	*T. harzianaum* + Ridomil MZ	T_{19}	Soil sol for 30 days +*T. viride*+ Pot phosphonate
T_{10}	*T. viride*+ Ridomil MZ	T_{20}	Soil sol for 30 days + *T. longibrachiatum* + Pot phosphonate

MAP: months after planting

In each column figures followed by same letter donot differ significantly according to DMRT.

Figures in parantheses are $\sqrt{x+0.5}$ transformed values.

was minimum in treatment T_3 (*T.harzianum* alone) followed by T_8 (Soil solarization + *T.longibrachiatum*) and the maximum was recorded in T_6 (Soil solarization + *T.harzianum*). However, after two MAP, the highest population (25.33) was observed in T_7 (Soil solarization + *T.viride*) and the least (8.67) in T_{20} (Soil solarization + *T.longibrachiatum* + Potassium phosphonate) and T_1 (Control) (10.0).At three MAP, it was observed there was a built up of fungal propagules in the control plots (24.67)which recorded the maximum fungal population.

4.11.5.2 *Bacteria*

A considerable variation in the population of bacteria was also noticed at different intervals of observation (Table 4.26).

One MAP, the highest count of 187.67 x 10^4 cfu g^{-1} soil was noticed in the treatment T_{14} (Soil solarization + *T.longibrachiatum* + Ridomil MZ) and the least count of 15.67 x 10^4 cfu g^{-1} soil was observed in T_6 (Soil solarization + *T.harzianum*). At the end of two months, T_4 (*T.viride* alone) recorded the highest population of 122.33x 10^4cfu g^{-1} soil, which was on par with T_9, T_6, T_{10}, T_{11}, T_{16} and the least count of 25.33x10^4 cfu g^{-1} soil was recorded in T_{15} (*T.harzianum* + Potassium phosphonate).

A gradual increase in the population of bacteria was observed at the end of three months after planting. T_{11} recorded the highest count of 153x 10^4 cfu g^{-1} soils and the least was observed in T_{14} (55.67x 10^4 cfu g^{-1} soil).

4.11.5.3 *Actinomycetes*

It was observed (Table 4.27) that there was no significant difference in the population of actinomycetes among the treatments one and two months after planting. However, the highest population of 61.67x10^4cfu g^{-1}soil was observed in T_{14} (Soil solarization + *T.longibrachiatum* + Ridomil MZ) one MAP and the least was observed in T_6 (Soil solarization + *T.harzianum*) with a count of 10.0x10^4cfu g^{-1}soil.

After two months, there was a general increase in the population of actinomycetes compared to the previous periods of enumeration. The maximum count of 39.33 x 10^4 cfu g^{-1}soil was noticed in T_6 and the least of 9.33 was observed in T_{14}.

Table 4.26. Effect of treatments on the total bacterial population of soil

Treatments	Bacterial count ($x10^4$ cfu g^{-1} soil)		
	1 MAP	2 MAP	3 MAP
T1	47.00 (6.31)^{bcdef}	34.00(5.71)^{cd}	102.33(9.82)
T2	85.67(7.87)^{abcdef}	40.67(6.22)^{cd}	98.33(9.76)
T3	19.00(4.37)^{ef}	45.00(6.72)^{bcd}	100.67(9.97)
T4	48.67(5.98)^{bcdef}	122.33(10.87)^{a}	108.67(10.30)
T5	57.00(7.39)^{abcdef}	48.67(6.94)^{bcd}	64(8.00)
T6	15.67(3.86)^{f}	82.00(9.04)^{abc}	68.33(7.97)
T7	31.33(4.84)^{def}	49.33(6.67)^{bcd}	90.67(9.43)
T8	39.00(6.05)^{bcdef}	39.67(6.13)^{cd}	58.00(7.65)
T9	32.67(5.57)^{cdef}	111.33(10.39)^{ab}	87.67(9.23)
T10	26.67(5.02)^{def}	82.00(9.06)^{abc}	124.00(10.97)
T11	143.33(11.70)^{abc}	70.33(8.32)^{abcd}	153.00(12.22)
T12	104.00(10.14)^{abcdef}	35.33(5.95)^{cd}	77.00(8.71)
T13	117.67(10.72)^{abcde}	55.67(7.02)^{bcd}	82.67(9.07)
T14	187.67(13.18)^{a}	46.33(6.81)^{bcd}	55.67(7.43)
T15	56.00(7.43)^{abcdef}	25.33(4.83)^{d}	103.33(10.12)
T16	108.00(10.27)^{abcdef}	69.67(8.21)^{abcd}	81.00(8.98)
T17	127.33(11.19)^{abcd}	49.33(6.90)^{bcd}	61.00(7.84)
T18	176(12.30)^{ab}	39.67(6.27)^{cd}	60.00(7.77)
T19	97.33(9.20)^{abcdef}	37.33(5.73)^{cd}	87.67(9.36)
T20	78.67(8.30)^{abcdef}	46.67(6.34)^{cd}	84.33(9.18)

T_1 Control
T_2 Disease control as per POP of KAU
T_3 *Trichoderma harzianaum* alone
T_4 Selected antagonist from Thrissur (*T. viride*)
T_5 Selected antagonist from Thrissur (*T.longibrachiatum*)
T_6 Soil sol. for 30 days +*T. harzianum*
T_7 Soil sol.for 30 days +*T. viride*
T_8 Soil sol.30 days + *T. longibrachiatum*
T_9 *T.harzianaum* + Ridomil MZ
T_{10} *T. viride*+ Ridomil MZ
T_{11} *T. longibrachiatum* + Ridomil MZ
T_{12} Soil sol. for 30 days +*T. harzianum*+ Ridomil MZ
T_{13} Soil sol. for 30 days + *T. viride* + Ridomil MZ
T_{14} Soil sol. for 30 days + *T. longibrachiatum* + Ridomil MZ
T_{15} *T. harzianum* +Pot. phosphonate
T_{16} *T. viride* + Pot. phosphonate
T_{17} *T. longibrachiatum* + Pot. phosphonate
T_{18} Soil sol. for 30 days +*T. harzianum*+ Pot.phosphonate
T_{19} Soil sol for 30 days +*T.viride*+ Pot.phosphonate
T_{20} Soil sol for 30 days +*T. longibrachiatum* + Pot phosphonate

In each column figures followed by same letter donot differ significantly according to DMRT.
Figures in parantheses are $\sqrt{x+0.5}$ transformed values. (MAP: months after planting)

Table 4.27. Effect of various treatments on the total population of actinomycetes

Treatment	Actinomycetal count ($x10^4 g^{-1}$ cfu)		
	1 MAP	2 MAP	3 MAP
T1	13.33(3.62)^{a}	23.33(4.21)^{a}	53.00(7.30)^{ab}
T2	20.00(4.24)^{a}	19.33(4.25)^{a}	52.67(7.23)^{ab}
T3	21.67(4.34)^{a}	22.33(4.09)^{a}	64.67(7.99)^{ab}
T4	26.33(4.82)^{a}	21.00(3.81)^{a}	68.00(8.24)^{ab}
T5	12.33(3.58)^{a}	23.33(4.69)^{a}	45.67(6.64)^{ab}
T6	10.00(3.16)^{a}	39.33(6.04)^{a}	49.33(6.95)^{ab}
T7	10.67(3.26)^{a}	31.33(5.48)^{a}	52.33(7.21)^{ab}
T8	15.67(3.89)^{a}	24.67(4.68)^{a}	50.00(7.08)^{ab}
T9	19.00(4.24)^{a}	23.67(4.27)^{a}	66.00(7.86)^{ab}
T10	17.00(4.04)^{a}	25.33(4.53)^{a}	71.00(8.39)^{ab}
T11	38.00(5.65)^{a}	38.00(5.72)^{a}	77.00(8.80)^{a}
T12	23.33(4.44)^{a}	19.00(4.36)^{a}	64.67(7.90)^{ab}
T13	55.00(6.47)^{a}	24.00(4.53)^{a}	77.67(8.73)^{ab}
T14	61.67(6.19)^{a}	9.33(3.08)^{a}	54.67(7.42)^{ab}
T15	47.33(5.83)^{a}	13.00(3.60)^{a}	68.00(8.25)^{ab}
T16	50.33(6.26)^{a}	14.00(3.76)^{a}	61.33(7.77)^{ab}
T17	51.33(7.14)^{a}	16.00(3.92)^{a}	73.33(8.56)^{ab}
T18	23.33(4.54)^{a}	25.33(4.73)^{a}	37.00(6.06)^{b}
T19	34.67(5.62)^{a}	18.67(4.00)^{a}	55.33(7.43)^{ab}
T20	20.67(4.36)^{a}	11.33(3.29)^{a}	53.00(7.22)^{ab}

T_1 Control
T_2 Disease control as per POP of KAU
T_3 *Trichoderma harzianaum* alone
T_4 Selected antagonist from Thrissur (*T. viride*)
T_5 Selected antagonist from Thrissur (*T.longibrachiatum*)
T_6 Soil sol. for 30 days +*T. harzianum*
T_7 Soil sol.for 30 days +*T. viride*
T_8 Soil sol.30 days + *T. longibrachiatum*
T_9 *T.harzianum* + Ridomil MZ
T_{10} *T. viride*+ Ridomil MZ
T_{11} *T. longibrachiatum* + Ridomil MZ
T_{12} Soil sol. for 30 days + *T. harzianum*
T_{13} Soil sol. for 30 days + *T. viride* + Ridomil MZ
T_{14} Soil sol. for 30 days + *T. longibrachiatum* + Ridomil MZ
T_{15} *T. harzianum* +Pot. phosphonate
T_{16} *T. viride* + Pot. phosphonate
T_{17} *T. longibrachiatum* + Pot. phosphonate
T_{18} Soil sol. for 30 days +*T. harzianum*+ Pot.phosphonate
T_{19} Soil sol for 30 days +*T.viride*+ Pot.phosphonate
T_{20} Soil sol for 30 days +*T. longibrachiatum* + Pot phosphonate

MAP: months after planting.

In each column figures followed by same letters donot differ significantly according to DMRT.Figures in parantheses are $\sqrt{x+0.5}$ transformed values.

However, after three months, a significant difference was noticed among the treatments with a maximum count of 77.0x 10^4cfug^{-1}soil in T_{11} (*T.longibrachiatum* + Ridomil MZ) and the least count was observed in T_{18} (Soil solarization + *T.harzianum* + Potassium phosphonate)(37.0x10^4cfug^{-1}soil).

4.11.6 *Biometric observations*

Biometric observations like height and number of leaves per plant were taken 45,60 and 90 DAP to know whether the treatments had an effect in the growth of the plants.

4.11.6.1 *Height of the plant*

The observations (Table 4.28) recorded on the height of plants at different intervals revealed a significant variation among the treatments. It was observed that at all intervals, the cuttings in treatment T_7 (Soil solarization +*T.viride*) recorded the maximum height. This was followed by T_{14} (Soil solarization + *T.longibrachiatum* + Ridomil MZ), which recorded a plant height of 16.73 cm 90 DAP. Plants under control had a height of 12.47cm (90 DAP).

4.11.6.2 *Number of leaves*

The data (Table 4.29) on the number of leaves at 45 DAP recorded the maximum value (6.33) in T_7 (Soil solarization +*T.viride*). However, there was no significant difference among the treatments.

At 60 and 90 DAP, there were significant differences on the number of leaves per plant. The maximum leaf number (8.67) was observed in 30 days solarised, *T.viride* incorporated treatment T_7 (60 DAP) that was significantly superior to all other treatments.

At 90 DAP, T_8 (Soil solarization +*T.longibrachiatum*) was the most effective in increasing the leaf number (20.67), which was significantly superior to the control T_1 (13.33) followed by T_2 (PoP) (11.67).

Table 4.28. Effect of various treatments on the height of pepper plants

Treatment	Height of the plant (cm)		
	45 DAP	60 DAP	90 DAP
T1	12.33 ^{ab}	12.47 ^{bc}	13.60 ^{bc}
T2	10.93 ^{bc}	13.27 ^{bc}	13.77 ^{bc}
T3	11.70 ^{ab}	13.17 ^{bc}	15.00 ^{abc}
T4	12.27 ^{ab}	13.93 ^{abc}	15.70 ^{ab}
T5	8.10 ^{c}	12.87 ^{bc}	13.57 ^{bc}
T6	12.20 ^{ab}	13.90 ^{abc}	14.33 ^{abc}
T7	14.50 ^{a}	16.53 ^{a}	19.00 ^{a}
T8	13.33 ^{ab}	14.83 ^{abc}	16.03 ^{ab}
T9	11.03 ^{bc}	12.67 ^{bc}	13.30 ^{bc}
T10	11.87 ^{ab}	13.77 ^{bc}	14.43 ^{abc}
T11	11.30 ^{ab}	12.83 ^{bc}	13.23 ^{bc}
T12	12.13 ^{ab}	15.00 ^{ab}	15.57 ^{ab}
T13	12.67 ^{ab}	15.23 ^{ab}	15.87 ^{ab}
T14	11.90 ^{ab}	13.73 ^{bc}	16.73 ^{ab}
T15	12.03 ^{ab}	13.77 ^{bc}	14.30 ^{abc}
T16	11.13 ^{abc}	13.30 ^{bc}	13.90 ^{bc}
T17	10.33 ^{bc}	12.03 ^{c}	12.83 ^{bc}
T18	12.27 ^{ab}	13.53 ^{bc}	15.43 ^{abc}
T19	12.63 ^{ab}	14.97 ^{ab}	16.30 ^{ab}
T20	11.90 ^{ab}	14.00 ^{abc}	14.93 ^{c}

T_1 Control
T_2 Disease control as per POP of KAU
T_3 *Trichoderma harzianaum* alone
T_4 Selected antagonist from Thrissur (*T. viride*)
T_5 Selected antagonist from Thrissur (*T.longibrachiatum*)
T_6 Soil sol. for 30 days +*T. harzianum*
T_7 Soil sol. for 30 days +*T. viride*
T_8 Soil sol. for 30 days + *T. longibrachiatum*
T_9 *T.harzianaum* + Ridomil MZ
T_{10} *T. viride*+ Ridomil MZ
T_{11} *T. longibrachiatum* + Ridomil MZ
T_{12} Soil sol. for 30 days +*T. harzianum*+ Ridomil MZ
T_{13} Soil sol. for 30 days + *T. viride* + Ridomil MZ
T_{14} Soil sol. for 30 days + *T. longibrachiatum* + Ridomil MZ
T_{15} *T. harzianum* +Pot. phosphonate
T_{16} *T. viride* + Pot. phosphonate
T_{17} *T. longibrachiatum* + Pot. phosphonate
T_{18} Soil sol. for 30 days +*T. harzianum*+ Pot.phosphonate
T_{19} Soil sol for 30 days +*T.viride*+ Pot.phosphonate
T_{20} Soil sol for 30 days +*T. longibrachiatum* + Pot.phosphonate

MAP: months after planting
In each column figures followed by same letters donot differ significantly according to DMRT

Table 4.29. Effect of treatments on number of leaves of pepper plants

Treatment	No. of leaves per plant		
	45 DAP	60 DAP	90 DAP
T1	5.00^{a}	6.67^{abcd}	13.33^{cd}
T2	4.33^{a}	6.33^{bcd}	11.67^{d}
T3	5.33^{a}	6.33^{bcd}	18.00^{abc}
T4	5.67^{a}	6.67^{abcd}	15.67^{abcd}
T5	4.67^{a}	6.00^{bcd}	13.67^{cd}
T6	5.00^{a}	7.33^{abcd}	16.33^{abcd}
T7	6.33^{a}	8.67^{a}	18.33^{abc}
T8	5.67^{a}	7.33^{abcd}	20.67^{a}
T9	4.67^{a}	5.67^{cd}	15.67^{abcd}
T10	4.33^{a}	6.00^{bcd}	16.33^{abcd}
T11	4.33^{a}	5.33^{d}	13.67^{cd}
T12	5.67^{a}	8.00^{ab}	17.67^{abc}
T13	5.33^{a}	8.00^{ab}	19.67^{ab}
T14	5.33^{a}	7.67^{abc}	16.67^{abcd}
T15	4.33^{a}	6.33^{bcd}	15.67^{abcd}
T16	4.67^{a}	6.00^{bcd}	15.33^{bcd}
T17	4.33^{a}	6.00^{bcd}	13.67^{cd}
T18	5.33^{a}	6.67^{abcd}	15.33^{bcd}
T19	5.33^{a}	7.67^{abc}	19.67^{ab}
T20	5.33^{a}	8.00^{ab}	18.33^{abc}

T_1	Control	T_{11}	*T. longibrachiatum* + Ridomil MZ
T_2	Disease control as per POP of KAU	T_{12}	Soil sol. for 30 days +*T. harzianum*+ Ridomil MZ
T_3	*Trichoderma harzianaum* alone	T_{13}	Soil sol. for 30 days + *T. viride* + Ridomil MZ
T_4	Selected antagonist from Thrissur(*T. viride*)	T_{14}	Soil sol. for 30 days + *T. longibrachiatum* + Ridomil MZ
T_5	Selected antagonist from Thrissur(*T.longibrachiotum*)	T_{15}	*T. harzianum* +Pot. phosphonate
T_6	Soil sol. for 30 days +*T. harzianum*	T_{16}	*T. viride* + Pot. phosphonate
T_7	Soil sol.for 30 days +*T. viride*	T_{17}	*T. longibrachiatum* + Pot. phosphonate
T_8	Soil sol.30 days + *T. longibrachiatum*	T_{18}	Soil sol. for 30 days +*T. harzianum*+ Pot.phosphonate
T_9	*T.harzianaum* + Ridomil MZ	T_{19}	Soil sol for 30 days +*T.viride*+ Pot phosphonate
T_{10}	*T. viride*+ Ridomil MZ	T_{20}	Soil sol for 30 days +*T. longibrachiatum* + Pot phosphonate

MAP: months after planting

In each column figures followed by same letters donot differ significantly according to DMRT

Discussion

5. DISCUSSION

Phytophthora disease is one of the major constraints in the production of quality rooted cuttings of black pepper. This disease, incited by the soil borne fungus *Phytophthora capsici*, causes extensive damage in black pepper nurseries. Further, it has been observed that if cuttings from infected nurseries are used for planting, the pathogen is unknowingly introduced to the main field, resulting in severe occurrence of *Phytophthora* foot rot in pepper plantations. Though, reports of successful control of this disease by integrating various cultural, biological and chemical methods are available and being practiced, in certain periods, *Phytophthora* rot disease assumes serious proportions in the black pepper nurseries especially during heavy monsoon periods. In view of the serious nature of this disease in the nursery and the potential threat it poses in the main field, the present study was undertaken for the effective management of *Phytophthora* rot in the pepper nursery using selected native antagonists alone or in combination with fungicides.

The occurrence of *Phytophthora* foot rot or quick wilt of pepper was reported by many workers from various pepper growing areas of the world (Coleman, 1910; Barbar, 1902; Butler, 1906; Rao, 1929; Leefmans, 1934; Muller, 1936; Samraj and Jose, 1966; Harper, 1974; Nambiar and Sarma, 1977; Dewaard, 1979; Sastry, 1982; Dutta, 1984 and Anandaraj *et al*., 1989a). Mammootty and Pillai (1981) and Sarma *et al*. (1988) observed extensive damage in black pepper nursery due to *Phytophthora* rot. In the present investigation, the causal organism of the disease was isolated from infected plants of black pepper nurseries of Thrissur district and the pathogenicity of the isolate was established. The cultural and morphological characters of the pathogen causing *Phytophthora* rot of black pepper nursery were studied which included colony and sporangial characters like sporangial ontogeny, size, shape, L/B ratio and pedicel length. The fungus produced uniformly dense cotton wool like aerial mycelium with petalloid pattern on carrot agar medium. The mycelium was coenocytic, producing sporangia in an umbellate fashion. Sporangia papillate, caducous, long stalked (>15μm), ovoid to pyriform, 45-52 x 18-25 μm with an L/B ratio of 1.9 to 2.3. The characters studied were almost in conformity with those reported by many workers for *Phytophthora capsici* (Kaosiri *et al*., 1978; Sarma *et al*., 1980; Zentmeyer, 1988;

Sastry and Hegde, 1987 b; Santhakumari, 1987 and Tsao and Alizadeh, 1988). Hence, based on the cultural and morphological characters coupled with pathogenicity on black pepper, the isolate was identified as *Phytophthora capsici* Leonian emend A. Alizadeh and P.H. Tsao (Tsao, 1991).

Success in control of *Phytophthora* rot in black pepper nurseries with chemical fungicides has been reported by several workers (Mammootty *et al.* 1980; Mammootty and Pillai, 1981; Ramachandran and Sarma, 1985; Nair and Sasikumaran, 1991; Veena and Sarma, 2000). However, the constant use of chemical fungicides may bring about many ecological problems including the development of resistant strains of the pathogens. The increasing awareness of the possible deleterious effects of chemical fungicides in the ecosystem have created interest among the scientists for locating biological control agents against soil borne diseases and foot rot disease of black pepper is no exception to this. It is very well established that due to the variability existing in the pathogen, it is ideal to select and develop native antagonists which would be more adapted to the soil conditions and having more competitive saprophytic ability than introduced cultures (Papavizas and Lewis, 1981).

Cook and Baker (1983) stated that, the starting point for biological control is often the isolation of potential antagonists from root environment where disease is lacking such as suppressive soil or from healthy plants in diseased fields. Further Baker and Cook (1974) opined that antagonists should be sought in the rhizosphere rather than in the soil mass as their effective activity will be on the root surface. Hence, in the present study, an attempt has been made to select antagonistic microbes prevalent in the rhizosphere of different pepper nurseries of Thrissur district. For this, the soil samples were collected from the rhizosphere of healthy plants from diseased nurseries and the quantitative estimation was carried out. Results of the study revealed more abundance of soil bacteria, actinomycetes and fungi in that order in different pepper nurseries. In addition, variation in the population of these microbes was also noticed in different nurseries. Jeyarajan *et al.* (1994) observed that in *Phytophthora* suppressive soils, the population of bacteria and actinomycetes was 30 and 35 times more than that of conducive soils. Jubina and Girija (1998), while studying the microflora of rhizosphere soil of black pepper, also observed the abundance of soil

bacteria. The variation in the population of soil microbes of different nurseries might be due to the varied type of soil and climatic conditions prevailing in the nurseries.

After the quantitative estimation of rhizosphere microflora, representative cultures of fungi, bacteria and actinomycetes were selected based on cultural characteristics of the colonies. So from the soil microbes, 22 isolates of fungi, 20 isolates of bacteria and five isolates of actinomycetes were selected for further studies. The antagonistic action of these organisms against *P. capsici* was estimated by dual culture method (Skidmore and Dickinson, 1976; Utkhede and Rahe, 1983) after giving due consideration to the growth rate of the soil microbes and the pathogen. Further the efficacy of the isolates was compared with the standard culture of *T. harzianum* specific against *Phytophthora* foot rot pathogen of black pepper.

The results of the study revealed that, all fungal isolates including *T. harzianum* tested were antagonistic to *P. capsici* in varying proportions. Among them, 13 isolates showed cent per cent inhibition on the growth of the pathogen. While, others recorded a per cent inhibition ranging from 37.78 to 77.78. It was also observed that the majority of the isolates screened, over grew the pathogen within a short period of time while, the remaining ones showed cessation, homogenous and avertion of growth as reported by Purkayastha and Bhattacharya (1982). Rajan *et al.* (2002) studied the antagonistic reaction of different *Trichoderma* isolates obtained from the rhizosphere of black pepper and reported varying percentage of inhibition of growth of the pathogen. Further, it is also pertinent to note that among the 13 fungal isolates that showed 100 per cent inhibition, 10 of them belonged to the genus *Trichoderma* and the rest two to *Rhizopus* spp. and one remained unidentified. Predominance of *Trichoderma* spp. in the rhizosphere soil of crop plants and its potential as effective biocontrol agent has been established by many workers (Mukhopadhyay, 1995; Harman, 2000 and Doraiswamy *et al.*, 2003). Out of the 20 bacteria tested, only five were antagonistic to the pathogen with a per cent inhibition ranging from 70 to 77.78, while none of the actinomycetes showed antagonistic action against the fungus. Similar results were observed by Gokulapalan (1986) and Jubina and Girija (1998). Among the microflora, the fungal isolates exhibited promising effect against the pathogen and hence these were subjected to further studies.

For the selection of the most efficient fungal antagonists from among the various isolates studied, the method suggested by Kasinathan (1998) was employed. For this, the antagonism index (AI) of the organism was worked out which he considered as pertinent in the selection of the most efficient antagonists *in vitro*. The AI of the organism was arrived at by taking into consideration the various parameters like per cent inhibition, colonization behaviour of the antagonist, speed of over growth on pathogen and inhibition zone. The study revealed that AI of the fungi tested ranged from 106.66 to 3000. Thirteen isolates which recorded cent per cent inhibition of growth of the pathogen showed an AI of 1200 and above. The maximum AI of 3000 was exhibited by isolate 22 F (*Trichoderma* sp.) and the next higher value of 1500 was recorded with isolate 34 F (*Trichoderma* sp.) and *T.harzianum*. So the native antagonists that showed an AI of 1500 (34 F) and above (22 F) were selected for further studies. The standard culture of *T. harzianum*, which recorded an AI of 1500, was also employed as comparison.

The two promising native isolates (22 F and 34 F) were identified at species level by studying the cultural and morphological characters. The isolate 22 F produced lemon yellow pigments in culture. The conidial areas were olive green to bright yellow. The mycelium septate, smooth walled and hyaline with simple conidiophore branching. Bottle shaped phialides of 6.9–11.5 x 2.3–2.5μm were formed singly or alternately along the main branch with phialospores of 3.6–4.5μm, ellipsoidal, subglobose or globose, smooth walled and pale green. These characters were almost similar to those reported by Rifai (1964) for ***Trichoderma longibrachiatum*** and hence it was identified as ***Trichoderma longibrachiatum* Rifai aggr.**

The other isolate 34F produced whitish colonies, which on maturity turned dark green. The mycelium septate, smooth walled and hyaline with dendroid conidiophore branching. Phialides formed in false or irregular whorls mostly with less than four phialides (8–14 x 2.4–3μm) in each whorl. Phialospores subglobose, globose or short obovoid, 3.45–5.75 μm with a spiny wall. These cultural and morphological characters were in conformity with that reported by Webster (1964) for ***Trichoderma viride*** and hence this isolate was identified as ***Trichoderma viride* Pers. ex S.F. Gray aggr.**

It is well known that the antagonistic organisms bring out action on the pathogen by various mechanisms thereby reducing the incidence and severity of the disease. According to Harman (2000), there are numerous mechanisms like mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, induced resistance, solubilization and sequestration of inorganic nutrients and inactivation of pathogenic enzymes. Also, it is likely that other mechanisms may be present but yet to be discovered. Hence, investigations were carried out to find the mode of action of selected antagonists on *P.capsici* in comparison with that of *T. harzianum*. It was observed that all the three antagonists were fast growing and overgrew the slow growing *P.capsici* colony within three days and this type of growth was comparable to that reported by Vinod (1988) and Mukherjee *et al.* (1989) in *Pythium* sp by *T.harzianum* and Thomas *et al.* (1996) and Bhai *et al.* (2000) in the case of *Phytophthora* sp. by different species of *Trichoderma*. In addition, the inhibitory effect of these fungi against *P. capsici* may be attributed to various reasons like competition, antibiosis or the production of volatile compounds by the bioagents as suggested by Dennis and Webster (1971). A slight zone of inhibition was initially observed at the point of contact between *T.longibrachiatum* and *P.capsici*, which may be due to the production of inhibitory metabolites (Elad *et al.*, 1983).

The hyphal interference between the antagonists and the pathogen was investigated by the method suggested by Dennis and Webster (1971). On microscopic observation of the intermingling growth of the organisms on cellophane paper, it was found that the interaction of the organisms lead to mycoparasitism in the form of hyphal coiling, penetration and disintegration of the host hyphae leading to death of the pathogen. Similar observations were reported by Bell *et al.* (1982), D 'Ercole *et al.*, (1984) and Harman (2000) by *Trichoderma* spp. on various plant pathogens. According to Bell *et al.* (1982), the hyphae of *Trichoderma* penetrated the mycelium of pathogens and grew luxuriantly within it and the pathogenic hyphae were severely ruptured at the points of contact with hyphae of *Trichoderma* ultimately leading to hyphal lysis. The role of cell wall degrading enzymes in this type of antagonistic action produced by the antagonists was well elucidated (Elad *et al.*, 1983; Rideout *et al.*, 1986; Claydon *et al.*, 1987). So the *Trichoderma* spp. used in the study is found to have effective parasitic effect on the pathogen in addition to some degree of antibiosis

and these fungi could be utilised for the management of *Phytophthora* rot in black pepper nursery.

According to Sarma and Anandaraj (1998), for the effective management of *Phytophthora* foot rot of black pepper, an integrated approach involving cultural, chemical and biological measures are to be adopted. As pepper is a remunerative crop, farmers are adopting all available methods to increase the productivity of the crop where plant protection measures play an important role. Hence, while adopting integrated disease management practice using antagonists, it is imperative that pesticides including fungicides, insecticides and nematicides and even fertilizers commonly used in black pepper must be compatible with the biocontrol agents and further care must be taken to select a suitable combination. So in the present study, the compatibility of the two selected antagonists and *T. harzianum* with commonly used fungicides, insecticides and fertilizers were evaluated.

Studies on compatibility of *Trichoderma* spp. to different fungicides revealed that all the fungicides exerted varying levels of inhibition. A complete inhibition of the growth of all antagonists was observed with Bordeaux mixture and higher percentage of inhibition was recorded with Kocide, Captaf and Kavach, indicating incompatibility of these fungicides with the antagonists tested. The above finding on the effect of Bordeaux mixture is in agreement with that of Shanmugham (1996) who reported that Bordeaux mixture completely inhibited the growth of *T.viride*. Krishnamoorthy and Bhaskaran (1994) observed Captan as fungicidal to *T viride*, while, it had little effect on *T.harzianum*. Contrary to this, Singh *et al.* (1995) found higher per cent inhibition of growth of *T.harzianum* at 500 ppm of Captaf, which is in agreement with the present finding. The inhibitory effect of Kavach (Chlorothalonil) on *T. harzianum* and *T. viride* was noticed by Akbari and Parakhia (2001). On the contrary, there are many reports on the compatibility of *Trichoderma* spp. with Chlorothalonil (Moity *et al.* 1982; Wongwathanarat and Sivasithamparam, 1991; Rajan and Sarma, 1997). But Sharma and Mishra (1995) reported only slight inhibition of Kavach to *T.harzianum*. The variation in response of the *Trichoderma* spp. to the above fungicides observed during the study from those reported earlier might probably be due to the variation among isolates of the antagonists. A perusal of the literature revealed no reports of inhibitory effect of Kocide on *Trichoderma* spp.

However, it was observed that in media incorporated with Indofil M-45, Anthracol, Akomin and Ridomil MZ, there was good growth of all antagonists proving their compatibility. Similar results on the compatibility of *Trichoderma* spp. with Metalaxyl, Mancozeb and Potassium phosphonate have been noticed by many workers (Moiety *et al.*, 1982; Wongwathanarat and Sivasithamparam, 1991; Shanmugham 1996; Rajan and Sarma, 1997; May and Kimati, 2000; Akbari and Parakhia, 2001). Thus, the two selected antagonists and *T.harzianum* can be safely tried along with above fungicides in the integrated management of *Phytophthora* rot in black pepper nursery. Another point observed during the study was that *T. viride, T. longibrachiatum* and *T. harzianum* showed difference in their response to copper oxychloride fungicide, Fytolan. Earlier report suggested that copper oxychloride fungicides were not compatible with *Trichoderma* spp. (Sharma *et al.*, 2001).

Contrary to these observations, it was revealed that Fytolan exerted below 48 per cent inhibition to the growth of *T. viride* and *T. harzianum,* while, *T. longibrachiatum* recorded more than 70 per cent inhibition. According to Krishnamoorthy and Bhaskaran (1994), in copper oxychloride poisoned medium, *T.harzianum* showed normal growth, while, *T.viride* exhibited sparse mycelial growth. Shanmugham (1996) reported 56.66 per cent inhibition of *T.viride* with Fytolan. So, from the present finding, it would be concluded that *T. viride* and *T. harzianum* are partially compatible with copper oxychloride fungicide and hence, could be integrated together in the management of this disease in the nursery to a certain extent without much adverse effect on the antagonist.

The effect of various fungicides at their recommended dosage on the sporulation of *Trichoderma* spp. was studied. It was found that Indofil M-45 and Ridomil MZ supported the growth and showed good sporulation of all the three antagonists, while, sparse conidial production was observed with Akomin-40. Sporulation was absent in the case of all antagonists in media incorporated with Captaf, Kavach, Fytolan and Kocide except for *T.harzianum,* which showed sparse sporulation with Captaf and Fytolan. Different workers reported the tolerance of *T. harzianum* to metalaxyl in spore germination tests even at higher concentration (Moiety *et al.* 1982; Papavizas *et al.*, 1982; Sharma *et al.*, 2001). But, contradictory to

the results of the present study, they also reported that the fungal antagonists in sporulation tests tolerated Chlorothalonil (Kavach) and Captaf to some extent. The inability of *T.viride* to sporulate in Fytolan incorporated media was reported by Krishnamoorthy and Bhaskaran (1994). However, they reported that *T. harzianum* showed normal growth and sporulation with copper oxychloride, which was in non-confirmity with the present observation.

The *in vitro* compatibility of insecticides with the three antagonists were studied and it was found that Monocrotophos and Quinalphos showed 100 per cent inhibition on the growth of antagonists. However, Endosulfan, Dimethoate, Cypermethrin and higher concentration of Chlorpyriphos exerted more than 70 per cent inhibition. Thus, it could be concluded that these insecticides were incompatible with the antagonists. The lower concentration of Chlorpyriphos (0.01per cent) exerted a per cent inhibition ranging from 34.44 to 57.78 against the antagonist. However, Phorate and Carbofuran were compatible as evidenced by the luxuriant growth of antagonists in media incorporated with these systemic insecticides. Sharma and Mishra (1995) reported that Phorate and Carbofuran applied for the control of nematodes and mealy bugs in black pepper were found less toxic to *T.harzianum.*

According to Jebakumar *et al.* (2000), Phorate being a systemic pesticide has no effect on direct contact with *T.harzianum* whereas Chlorpyriphos being a contact insecticide has retarding effect on the growth of *T. harzianum* in *in vitro* studies. But when used in soil, no such inhibitory effect was noticed. This opinion is found reasonable in the case of Carbofuran also. Sushir and Pandey (2001) had conducted similar studies and reported that Chlorpyriphos (Durmet 20 EC) has no adverse effect on radial growth upto 2000 $\mu l\ ml^{-1}$ whereas Endosulfan (Thiodan 35 EC) was found more toxic even at 50 $\mu l\ ml^{-1}$ which showed growth inhibition of 55.55 per cent. The growth inhibition of 40 per cent was observed with Dimethoate (Rogor 30 EC) at 125 $\mu l\ ml^{-1}$. So, insecticides, which are being used for the control of nematodes and mealy bugs of black pepper, can be safely used along with the antagonists of *P.capsici.* However, Monocrotophos and Quinalphos that are very effective against pollu beetle on black pepper but incompatible with the antagonists should be applied with care. Every effort has to be made to prevent dripping of these insecticides to the basin of black pepper plants since it would have a deleterious effect on the antagonists.

The sporulation of antagonists in media incorporated with insecticides were studied and it was observed that all antagonists showed good sporulation with Phorate and Carbofuran, while, moderate sporulation was recorded with Cypermethrin and Chlorpyriphos by *T. viride* and *T. longibrachiatum*, whereas *T. harzianum* showed good sporulation with the latter as well as with Dimethoate. *T.viride* and *T. longibrachiatum* showed only sparse conidial production with Endosulfan while *T. harzianum* showed moderate sporulation. According to Jebakumar *et al.* (2000), Phorate at 6-36 ppm did not affect the sporulation of *T. harzianum* whereas Chlorpyriphos retarded sporulation of the antagonist.

In order to boost up the productivity of pepper cuttings, application of fertilizers is resorted to, in addition to the application of biocontrol agents and plant protection chemicals. So an *in vitro* evaluation was conducted to study the compatibility of biocontrol agents with fertilizers *viz.*, Urea, Rajphos, Muriate of potash, Ammonium sulphate and Factomphos. It was found that all the fertilizers except, Factomphos supported comparatively good growth of the antagonists. For all three antagonists, the higher concentration of Factomphos recorded a per cent inhibition ranging from 54.42 to 62.4 respectively. The response of the antagonists to the higher concentration of the Urea (2per cent) was comparatively poor and recorded a per cent inhibition ranging from 40.2 to 46.7. Rest of the fertilizers were compatible to all the three species of *Trichoderma* to varying extent.

A significant increase in the population of *T. viride* in soil was noticed by Krishnamoorthy and Bhaskaran (1993) following the application of nitrogen and phosphorous fertilizers. Jackson *et al.* (1991) also supported the present finding that Rajphos is compatible with the three species of *Trichoderma*, which may be attributed to the fact that phosphorous is essential for sporulation of *Trichoderma* spp. In addition, previous reports (Sharma and Mishra, 1995) are also in line with the present study that Urea is a good source of nitrogen for *T. harzianum* which was not only supportive but also stimulated the growth and sporulation and this was followed by Ammonium sulphate. Moreover, according to them, Muriate of Potash (MoP) was also tolerated by the bioagent. Partial inhibition of the antagonists observed in the case of Factomphos may be due to the presence of sulphur in the fertilizer as Sharma and Mishra (1995) reported that zinc sulphate was highly toxic to *T. harzianum*. But the

compatibility of *Trichoderma* spp. to Ammonium sulphate may be due to the presence of ammoniacal form of nitrogen that is more preferred and most favourable for mycelial growth of the three *Trichoderma* spp. (Neelamegam, 1992). Jayaraj and Ramabhadran (1997) also observed the enhancement of growth and survival of *T. harzianum* with Ammonium sulphate to the maximum extent followed by Urea and Ammonium chloride.

Another study was conducted to find out the *in vitro* inhibitory effect of fungicides, insecticides and fertilizers on the growth of *P.capsici*. The same chemicals, which were used for testing the compatibility with antagonists, were used in this study also. Results of the study showed that among the different fungicides tested, Bordeaux mixture, Fytolan, Kocide, Captan, Ridomil MZ and Indofil M-45 at different concentrations completely inhibited the growth of the pathogen. It was also noticed that the higher two concentrations of Akomin (0.2 per cent and 0.3per cent) and the 0.15 per cent concentration of Anthracol inhibited the fungus. The lower concentrations of Anthracol and Kavach recorded an inhibition ranging from 60 to 75 per cent. The *in vitro* inhibitory effect of Bordeaux mixture, Copper oxychloride, Copper hydroxide, Mancozeb, Metalaxyl and Anthracol against foot rot pathogen of black pepper was reported by many workers (Turner, 1969; Filani, 1976; Mammootty, 1978; Ramachandran and Sarma, 1985 and KAU, 2000).

Veena *et al.* (2002) observed the inhibitory action of Potassium phosphonate against *P. capsici* of black pepper and they further opined that compared to mycelial growth, sporulation stage is the most sensitive stage. There are no reports of the effect of Captan and Kavach on *P. capsici*. However, Khan *et al.* (1996) observed the inhibitory action of Captan against *Phytophthora* causing crown and root rot of apple and Mahanty *et al.* (2000) noticed the sensitiveness of Kavach against the pathogen causing *Phytophthora* foot rot of betel vine. Eventhough, Bordeaux mixture, copper oxychoride (Fytolan), Ridomil MZ and Potassium phosphonate are recommended to control *Phytophthora* rot in black pepper, the present *in vitro* study pointed out that Copper hydroxide (Kocide), Captan, Mancozeb and even Anthracol can also be used to control the pathogen, *P.capsici* of black pepper. However, the field efficacy of these fungicides has to be tested before recommending for large scale field application. Further, as evident in the case of lower concentration of Potassium phosphonate

(Akomin), the study also pointed out the importance of usage of correct dosage of fungicides, otherwise the proper control of the pathogen will not be possible.

Out of the eight insecticides tested *in vitro*, four of them *viz*., Monocrotophos, Quinalphos, Endosulfan and Cypermethrin at all concentrations inhibited the growth of the pathogen whereas Phorate, Carbofuran, Dimethoate and Chlorpyriphos exerted inhibition ranging from 68.89 to 88.89 per cent, thus indicating the deleterious effect of these insecticides on *P. capsici*. As many of these insecticides were regularly used to control insect pests of pepper, the present investigation revealed that the application of these insecticides had an indirect effect in checking the growth and multiplication of *P. capsici*.

The *in vitro* effect of common fertilizers applied in black pepper against the pathogen was assessed and it was observed that the fertilizers *viz*., Urea, Rajphos and MoP had not much inhibitory effect on growth of the fungus. These fertilizers showed a per cent inhibition ranging from 3.7 to 17.8. Factomphos and ammonium sulphate partially inhibited the mycelial growth of the fungus at all concentrations. The higher concentration of Factomphos (3 per cent) exerted more than 50 per cent inhibition over control. Not many studies were carried out on the growth of *P.capsici* in fertilizer incorporated media. However, Jayasekhar and Muthusamy (2000) observed that Ammonium sulphate supported the growth of *P.capsici*, which was contradictory to the present study as Ammonium sulphate showed an inhibition per cent ranging from 24.7 to 42.2. The reason attributed to this inhibition in growth of pathogen in Ammonium sulphate and Factomphos incorporated media may be due to the effect of sulphur present in these fungicides. Urea favoured the growth of the pathogen, which may be due to the fact that the pathogen utilizes or rather prefers ammoniacal form of nitrogen. Similar findings were reported by several workers (Cameron and Milbrath, 1965; Pal, 1974 and Jain *et al*, 1982). The pathogen must have utilized phosphorus of Rajphos and potassium of Muriate of potash for their growth thereby resulting in very less inhibition of growth.

Nowadays, the strategy for plant disease management has shifted from absolute and single method of control to integration of different approaches of disease management at economically acceptable level. But, eventhough, integrated approach

comprising of cultural, biological and chemical methods individually or in combination were reportedly found to be effective in combating the *Phytophthora* rot of black pepper nursery to a certain extent, an effective control of the disease is not obtained always. Reasons attributed to these are factors like macro and microclimatic conditions in and around the nursery, variation in the causative organism, improper and untimely application of chemicals, selection of bioagents not suited to the particular location and lack of adoption methods which hinder the growth, multiplication and infection of the pathogen. As selection and use of native isolates of antagonists is one of the important factors for the successful management of this soil borne disease, the present nursery experiment was undertaken to harness the effect of solarization and selected native fungal antagonists individually and or in combination with fungicides. The fungicides selected were reported to be effective against the pathogen as well as compatible with the bioagents. The experiment was laid out as delineated in the technical programme at the College of Horticulture, Vellanikkara. Potting mixture was solarized using 150 gauge polythene sheet for 30 days with an idea to eliminate the pathogen and pathogenic nematodes due to build up of soil temperature.

Observations on soil temperature recorded during the entire 30 days of solarization revealed a high build up of soil temperature in the solarized potting mixture compared to the non-solarized one. The maximum temperature build up occurred at 5 cm depth. Further, in solarized soil, the soil temperature at 5 cm depth was always 30°C and above at 8.30 a.m while at 2.30 p.m, at the same depth, the temperature for most of these days was above 50 °C which was 20-21°C higher than that recorded at 8.30 a.m. Such build up in soil temperature in solarized soil were reported by many workers (Katan *et al.*, 1976; Katan, 1981; Chandran, 1989; Vilasini, 1996; Binimol, 2000; Patel, 2001). They also opined that the increase in soil temperature have a deleterious effect on the microbial population including the pathogen, which ultimately lead to the less incidence of the disease. The result of estimation of the soil microflora before and after solarization was also in line with the above observation by these workers. It was noticed that after solarization there was a decrease in the population of bacteria, fungi and actinomycetes compared to the initial count.

The sprouting percentage of cuttings in different treatments was worked out at different intervals, which showed significant difference. After 45 DAP, in general, it was observed that the maximum sprouting percentage was noticed in treatments where solarized potting mixture and biocontrol agents were used. The maximum sprouting percentage was noticed in treatment T_{20} (Soil solarization + *T. longibrachiatum* + Pot. Phosphonate) immediately followed by T_{12} (Soil solarization + *T. harzianum* +Ridomil MZ) while, the least sprouting percentage was observed in T_{17} (*T. longibrachiatum* + Pot. Phosphonate). Further, comparatively lesser percentage of rotting was noticed in treatments where solarized potting mixture and antagonists were tried. Eventhough, there was no significant difference among the treatments, the minimum percentage of rotting was observed in T_7 (Soil solarization + *T. viride*) and T_{13} (Soil solarization + *T. viride* + Ridomil MZ) followed by T_{20}. The maximum rotting percentage of 5.95 was observed in T_9 (*T. harzianum* +Ridomil MZ) followed by T_{11} (*T. longibrachiatum* +Ridomil MZ). Similar findings were obtained by Binimol (2000). She noticed the effectiveness of soil solarization and addition of *Trichoderma* spp. in increasing the sprouting percentage and reducing pre-sprouting mortality of black pepper cuttings.

During the onset of monsoon, in June, prophylactic application of fungicides was given and was repeated at fortnightly intervals for five times. Observations on disease incidence and severity were recorded four times at weekly intervals starting from the third week of July. Results on the percentage incidence of disease recorded during the first three intervals did not show any significant difference among the treatments, while it was significant at 17 weeks after planting (WAP). However, data on the disease severity taken at all the four intervals showed significant differences, indicating the effect of treatments on the severity of the disease.

On the 14th WAP, the maximum disease incidence was noticed in plants in control (T_1) and minimum in T_8 (Soil solarization + *T. longibrachiatum*). However, disease severity was maximum in control and minimum in T_{12} (Soil solarization + *T.harzianum* + Ridomil MZ), followed by T_{10} (*T. viride* + Ridomil MZ), T_9 (*T. harzianum* + Ridomil MZ) and T_{13} (Soil solarization + *T.viride* + Ridomil MZ). The treatment T_{12} recorded an efficiency of more than 90 per cent over control. During the 15th WAP, again there was no significant difference among treatments on

disease incidence with maximum in T_1 (Control) and minimum in T_{13} (Soil solarization + *T.viride* + Ridomil MZ) and T_{14} (Soil solarization + *T.longibrachiatum* + Ridomil MZ). Here also control plants (T_1) recorded more disease severity and was on par with all treatments except T_{12} (Soil solarization + *T.harzianum* + Ridomil MZ) which recorded minimum value followed by T_{10} and T_{14}. On 16th WAP, there was no significant difference among the treatments, the control plants (T_1) recorded the maximum disease incidence and minimum in T_2 (PoP) followed by T_{14} and T_{11}. During this period, the minimum disease severity was recorded in T_{11} (*T.longibrachiatum* + Ridomil MZ), which also showed the maximum percentage efficiency over control followed by T_{13} and T_2. The data on the per cent disease incidence on 17th WAP showed significant difference with the minimum disease incidence in T_2 followed by T_{14}, T_{13} and T_{12}. All the other treatments recorded comparatively a higher disease incidence with a maximum T_4 (*T. viride* alone) followed by control (T_1). The minimum disease severity was recorded in T_{11} followed by T_2, T_9, T_{13} and T_{14}.

Further, the data obtained at different intervals of observation were subjected to pooled analysis to draw a meaningful conclusion. A significant difference among the treatments on the incidence and severity of *Phytophthora* rot in black pepper nursery was noticed. The minimum disease incidence was noticed in treatment T_2 (PoP) followed by T_{14} (Soil solarization + *T.longibrachiatum* + Ridomil MZ) and T_{13} (Soil solarization + *T.viride* + Ridomil MZ) recording more than 60 per cent efficiency over control. The least disease severity was in treatment T_{13} that was on par with all other treatments except control (T_1). It was also to be noticed that in Ridomil MZ sprayed plants, there was comparatively less disease severity. The per cent mortality of rooted pepper cuttings was also studied after 19th WAP. It was observed that the least percentage mortality of 53.37 was recorded in *T.longibrachiatum* incorporated Ridomil MZ sprayed treatment (T_{11}) followed by T_2, T_{14} and T_{13}. More than 85 per cent mortality was noticed in control (T_1).

The effect of Bordeaux mixture in reducing the incidence of *Phytophthora* rot in black pepper nursery as well as in the main field were reported by many workers (Mammootty *et al.*, 1980; Mammootty and Pillai, 1981 and Sarma *et al.*, 1987; Malebennur *et al.*, 1991and Nair *et al.*, 1993). There are many reports on the

effectiveness of soil solarization, biocontrol agents alone or in combination with fungicides like Metalaxyl or Potassium phosphonate in managing *P. capsici* infecting black pepper (Malebennur *et al.*, 1991; Sarma *et al.*, 1994; Anandaraj and Sarma. 1995, Lokesh and Gangadharappa, 1995; Hegde and Anahosur, 1998; AICRPS, 2000 and AICRPS, 2001). Further, the study also emphasized the importance of selection of efficient native isolates of antagonists *viz.*, *T.viride* and *T. longibrachiatum* in the management of the disease as evidenced by the positive response exerted by the native antagonists in reducing the disease incidence and severity compared to the standard culture of *T.harzianum*.

As a part of the nursery experiment, the population of soil microflora *viz.*, fungi, bacteria and actinomycetes in different treatments were estimated. The study indicated a fluctuation in the population of soil microbes in various treatments at different periods of observation. Similar findings were reported by (Stapleton and DeVay, 1982; Kurian, 1992; Vilasini, 1996; Raj *et al.*, 1997; Binimol, 2000).

The observation on height and number of leaves of pepper cuttings in different treatments were recorded. It was noticed that cuttings raised in solarised potting mixture incorporated with native antagonists, especially *T. viride*, exerted a significant effect in increasing the height of cuttings. With regard to total number of leaves, it was also observed that addition of native antagonists in solarised potting mixture had a positive effect in increasing the leaf production. These observations are in conformity with that reported by Binimol (2000). She reported that soil solarization and application of *T.viride* and *T.harzianum* had a positive effect in increasing the height and number of leaves in pepper cuttings. Effectiveness of solarization in enhancing the growth parameters in crop plants by making available the required nutrients have been reported by many workers (Chen and Katan, 1980; Vilasini, 1996; Binimol, 2000). There are many reports on the increased growth response of plants by soil application of *Trichoderma* spp., which may be due to the production of growth promoting substances by the antagonists (Chang *et al.*, 1986 and Windham *et al.*, 1986; Mukhopadhyay, 1994; Harman, 2000).

Thus, the nursery experiment revealed that, in general, soil solarization, incorporation of antagonists and application of Ridomil MZ had a favourable effect in

checking the *Phytophthora* rot in black pepper nursery and it is comparable with the recommended chemical control with one per cent Bordeaux mixture.

Summary

6. SUMMARY

Phytophthora rot is the most destructive disease of black pepper nursery inflicting heavy crop losses. Considering the seriousness of the disease, the present study was undertaken to isolate and select the efficient antagonists from black pepper nurseries and use them in the integrated management of the disease.

The pathogen was isolated from infected plants of black pepper nursery and its pathogenicity was established. Cultural and morphological characters of the pathogen were studied. On carrot agar, the fungus produced petalloid uniform dense aerial mycelium with fine, non-septate hyphae. Sporangia were borne in an umbellate fashion on an elongated pedicel of more than 15 µm length. Sporangia are papillate, ovoid to pyriform caducous with an L/B ratio of 1.9 to 2.3. Based on the cultural and morphological characters coupled with pathogenicity, the causal organism of *Phytophthora* rot of black pepper nursery was identified as *Phytophthora capsici* Leonian emend A. Alizadeh and P. H. Tsao.

Rhizosphere microflora from healthy pepper plants in diseased nurseries were isolated. A quantitative estimation of the rhizosphere microflora from different nurseries revealed more abundance of soil bacteria followed by fungi and actinomycetes. Based on cultural characters of the rhizosphere microflora, 22 fungi, 20 bacteria and five actinomycetes were selected for further studies.

Antagonistic action of these microflora against *P.capsici* was studied by dual culture method in comparison with that of standard culture of *T. harzianum*. The study revealed that all fungal isolates and five bacterial isolates were antagonistic towards the pathogen with varying degrees. Among the fungal isolates, 13 isolates including *T.harzianum* recorded cent per cent inhibition of *P.capsici*. From among the fungal isolates that showed antagonistic reaction in dual culture, further selection of efficient ones was carried out based on the antagonistic index (AI). The isolate 22 F (*Trichoderma* sp.) showed the maximum AI of 3000 followed by isolate 34 F (*Trichoderma* sp.) and *T. harzianum* with an AI of 1500 each. The cultural and morphological characters of the efficient native isolates (22 F and 34 F) were studied

and were identified as *Trichoderma longibrachiatum* Rifai aggr. and *Trichoderma viride* Pers. ex S.F. Gray aggr.

The compatibility of the selected antagonists and *T. harzianum* to nine common fungicides used in pepper gardens was assessed. It was found that Bordeaux mixture, Kocide, Captaf and Kavach were incompatible with the antagonists, while, Indofil M-45, Ridomil MZ, Akomin and Anthracol were compatible. However, Fytolan was partially compatible with *T. viride* and *T. harzianum* and incompatible with *T. longibrachiatum*.

The *in vitro* effects of eight insecticides towards the antagonists were tested. Phorate and Carbofuran at all concentrations supported good growth of the antagonists revealing their compatibility. Monocrotophos and Quinalphos exerted complete inhibition of the antagonists while Endosulfan, Dimethoate, Cypermethrin and higher concentration of Chlorpyriphos exerted higher percentage of inhibition indicating incompatibility of these insecticides to the antagonists.

The *in vitro* sensitivity of antagonists to the common fertilizers *viz*., urea, Rajphos, Muriate of Potash, ammonium sulphate and Factomphos were studied and it was found that all the fertilizers except Factomphos supported comparatively good growth of the antagonists. But the higher concentration of Urea was more inhibitory to the fungi than its lower two concentrations.

The effect of fungicides, insecticides and fertilizers used in pepper gardens against the pathogen *P.capsici* was evaluated *in vitro*. It was observed that Bordeaux mixture, Fytolan, Indofil M-45, Kocide, Ridomil MZ and Captaf at all concentrations and the higher two concentrations of Akomin–40 and Anthracol completely inhibited the growth of *P.capsici*. The lowest concentration of Akomin-40 and Kavach at different concentrations did not completely inhibited the pathogen.

Out of the eight insecticides tested *in vitro*, Monocrotophos, Quinalphos, Endosulfan and Cypermethrin at all concentrations completely inhibited the growth of *P.capsici* whereas Phorate, Carbofuran, Dimethoate and Chlorpyriphos exerted inhibition ranging from 65 to 90 per cent.

In the case of fertilizers, except for Factomphos and ammonium sulphate rest of the fertilizers *viz*., urea, Muriate of potash and Rajphos supported comparatively good growth of the pathogen *in vitro*.

A nursery experiment was conducted to study the effect of solarization, selected native antagonists *viz*., *T. viride* and *T. longibrachiatum* and the standard culture of *T. harzianum* alone or in combination with fungicides for the management of *Phytophthora* rot in black pepper nursery. The experiment was laid out at College of Horticulture with 20 different treatments and replicated thrice.

Soil temperature at different depths of solarized potting mixture was recorded for 30 days. There was a build up of temperature in the solarized potting mixture compared to the non-solarized ones and the build up was more pronounced at 5 cm depth. Solarization resulted in the reduction of fungi, bacteria and actinomycetes in the potting mixture.

A significant difference in the percentage of sprouting of pepper cuttings in different treatments was observed at various intervals. In general, it was observed that 45 DAP, more than 84 per cent of sprouting was noticed in treatments where solarized potting mixture and biocontrol agents were used, with the maximum sprouting per cent in T_{20} (Soil solarization + *T.longibrachiatum* + Pot. phosphonate), followed by T_{12} (Soil solarization + *T.harzianum* + Ridomil MZ). Plants raised as per PoP (T_2) also showed good sprouting percentage. No significant difference among the treatments was observed on the rotting of cuttings at 45 DAP with the maximum percentage of rotting in treatment T_9 (*T.harzianum* + Ridomil MZ) and the minimum in T_{13} (Soil solarization + *T.viride* + Ridomil MZ).

Prophylactic application of fungicides for the respective treatments was given at the onset of monsoon in June at fortnightly intervals for six times. Observations on the disease incidence and severity were recorded at weekly intervals for four times and it was found that there was no significant difference on disease incidence for the first three intervals but it was significant during the last observation. However, data on the disease severity at different intervals showed significant difference.

Analysis of the pooled data taken at different intervals of observation showed significant difference on the incidence and severity of the disease. The minimum disease incidence was noticed in treatment T_2 (PoP) followed by T_{14} (Soil solarization + *T.longibrachiatum* + Ridomil MZ) and T_{13} (Soil solarization + *T.viride* + Ridomil MZ) and these treatments recorded more than 60 per cent efficiency over control. The least percentage of disease severity was noticed in plants in treatment T_{13} that was on par with other treatments except control (T_1). The minimum mortality was observed in treatments incorporated with *T. longibrachiatum* and Ridomil MZ sprayed plants and this was closely followed by T_2, T_{14} and T_{13} and the maximum in control.

A fluctuation in the population of soil microflora in different treatments was observed. The cuttings raised in solarized potting mixture incorporated with antagonists especially *T. viride* had a significant effect in increasing the height of cuttings. Further, addition of native antagonists *viz.*, *T. viride* and *T. longibrachiatum* in solarized potting mixture had a significant effect in increasing the leaf number.

References

REFERENCES

Adams, P.G. 1990. The potential of mycoparasites for biological control of plant disease. *A. Rev. Phytopath.* 28: 59-72

AICRPS. 2000. Biological control of *Phytophthora* foot rot of black pepper – nursery trial. *Annual Report, 1999-2000.* All India Co-ordinated Research Project on Spices, IISR, Calicut, India, pp.30-33

AICRPS. 2001. Biological control of *Phytophthora* foot rot of black pepper – nursery trial. *Annual Report, 2000-2001.* All India Co-ordinated Research Project on Spices, IISR, Calicut, India, pp.34 -37

Akbari, L.F. and Parakhia, A.M. 2001. Effect of fungicides on fungal bioagents. (Abstr.). *J. Mycol. Pl. Path.* 31: 101

Alconero, R., Albuquerque, F.C., Almeyda, N. and Santiago, A.G. 1972. *Phytophthora* foot rot of black pepper in Brazil and Puerto Rico. *Phytopathology*. 62: 144-148

Anandaraj, M. 1997. Ecology of *Phytophthora capsici*, causal organism of foot rot of black pepper (*Piper nigrum* L.), Ph.D. thesis. University of Calicut, p.153

Anandaraj, M. and Sarma, Y.R. 1994. Biological control of black pepper diseases, *Indian Cocoa, Arecanut Spices J.* 18: pp.22-23

Anandaraj, M. and Sarma, Y.R. 1995. Diseases of black pepper (*Piper nigrum* L.) and their management. *J. Spic. Aromatic Crop.* 4: 17-23

Anandaraj, M. and Sarma, Y.R. 1998. *Phytophthora* infections in black pepper and its management. *Spice India.* 11: 20-23

Anandaraj, M., Abraham, J. and Balakrishnan, R. 1989 a. Crop loss due to foot rot disease of black pepper. *Indian Phytopath.* 42: 473-476

Anandaraj, M., Abraham, J., Sarma, Y.R. and Balakrishnan, R. 1989 b. Incidence of foot rot disease of black pepper (*Piper nigrum*) in Kerala in relation to cultivation practices. *Indian J. Agric. Sci.* 59: 751-753

Anandaraj, M., Ramana, K.V., Sarma, Y.R.and Nair, K.S.S. 1996. Suppressive effects of VAM on root damage caused by *Phytophthora capsici, Radopholus similis* and *Meloidogyne incognita* in black pepper. *Proceedings of the IUFRO Symposium. November 23-26, 1993* (eds. Sarma, J.K. and Varma, R.V.). Peechi, India. pp. 232-238

Anith, K.N. and Das, T.P.M. 2001. Combined application of *Trichoderma harzianum* and *Alcaligenes* sp. strain AMB-8 for controlling nursery rot disease of black pepper. *Indian Phytopath.* 54: 335-339

Ann, P.J. 2001. Control of plant diseases with non-pesticide compound - phosphorous acid. *Pl. Path. Bull.* 10:147-154

Anon. 1965. *Rep. Res. Brch. Dept. Agric., 1962-1963*. Plant Pathology Division, Sarawak, Malaysia

Anon. 1972. *Rep. Res. Brch. Dept. Agric., 1971*, Plant Pathology Division, Sarawak, Malaysia, p.191

Anon. 1977. *Rep. Res. Brch. Dept. Agric. 1975*, Plant Pathology Division, Sarawak, Malaysia

Ayers, W.A. and Adams, P.B. 1981. Mycoparasitism and its application of biological control of plant diseases. *Biological Control of Crop Production* (ed. Papavizas, G.C.). Allanhold, Osmun & Co., New Jersy, pp. 91-93

Baker, K.F. and Cook, R.J. 1974. *Biological Control of Plant Pathogens*. Freeman, W.H. & Co., San Francisco, p.433

Balakrishnan, R., Anandaraj, M., Nambiar, K.K.N., Sarma, Y.R., Brahma, R.N. and George, M.V. 1986. Estimates on the extent of loss due to quick wilt disease of black pepper (*Piper nigrum* L.) in Calicut district of Kerala. *J. Plantation Crops.* 14: 15-18

Barber, C.A. 1902. *Ann. Rep. for Pad. 1902.* Dept. Agric. Madras

Bell, D.K., Wells, H.D. and Markham, C.R. 1982. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathology.* 72: 379-382

Bhai, S.R. 2000. Perspective of biocontrol and its application in *Phytophthora* diseases. *Spice India.* 92 : 7-11

Binimol, K. S. 2000. Integrated management of *Phytophthora* rot in black pepper nursery. M.Sc. (Ag.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, p.101

Brasier, C.M. 1975. Stimulation of sex organ formation in *Phytophthora* by antagonistic species of *Trichoderma* - I. The effect *in vitro*. *New Phytol.* 74:183-194

Brasier, C.M. and Griffin, M.J. 1979. Taxonomy of *Phytophthora* on cocoa. *Trans. Br. Mycol. Soc.* 72: 111-143

Bruck, R.I., Fry, W.E. and Apple, A.E. 1980. Effect of metalaxyl, an acylalanine fungicide, on developmental stages of *Phytophthora infestans*. *Phytopathology.* 70:597-607

Butler, E.J. 1906. The wilt disease of pigeon pea and pepper. *Agric. J. India.* 1: 25-36

Cameron, H.R. and Milbrath, G. M.1965. Variability in the genus *Phytophthora* I. Effect of nitrogen source and pH on growth. *Phytopathology.* 55:653-657

Cates, D. 1990. Biological fungicide closer to market. *Ag. Consultant*, August 11

Chandran, C.R. 1989. Influence of soil solarization on soil microflora, plant growth and incidence of diseases. M.Sc. (Ag.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, p.97

Chang, Y.C., Baker, R., Kleifield, O. and Chet, I. 1986. Increased growth of plants in the presence of biological control agent *Trichoderma harzianum*. *Plant Disease.* 70: 145-148

Chen, Y. and Katan, J. 1980. Effect of solar heating of soils by transparent polythene mulching on their chemical properties. *Soil Sci.* 130:271-277

Claydon, N., Allan, M., Hanson, J.R. and Advent, A.G. 1987. Antifungal alkyl pyrones of *Trichoderma harzianum*. *Trans. Br. Mycol. Soc.* 88: 503-513

Coffey, M.D. and Bower, L.A. 1984. *In vitro* variability among isolates of six species of *Phytophthora* in response to metalaxyl. *Phytopathology*. 74: 502-506

Coleman, L.C. 1910. Diseases of the areca palm. I. Koleroga or rot disease. *Annual Mycology*. 8: 591-626

Cook, R. J. and Baker, K.F. 1983. *The Nature and Practice of Biological Control of Plant Pathogens*. American Phytopathological Society, St. Paul. MN, USA, p.539

Cristinzio, G. 1987. Studies on biological control of *Phytophthora capsici* on pepper. *Capsicum Newsl.* 6: 65

Cruz, A.M. and Cistierna, O.V. 1998. Integrated control of *Phytophthora capsici* in black pepper. I. Effect of antagonist fungi on plant growth. *Agricultura Tecnica*. 58:81-92

D' Ercole, N., Sportelli, M. and Nipoti, P. 1984. Different types of antagonism of *Trichoderma* sp. towards plant pathogenic soil fungi. *Informatore Fitopato logico*. 34: 43-47

D'Ercole, N., Nipoti, P., Manzati, D. and Di- Pillo, L. 1993. *In vitro* and *in vivo* activity of *Trichoderma* sp. against fungal diseases of vegetable seeds. *Colture Protette*. 2: 63-65

Danielson, R.M. and Davey, C.B. 1973. Carbon and nitrogen nutrition of *Trichoderma*. *Soil Biol. Biochem*. 5:510-515

Das, T.P.M. and Cheeran, A. 1985. Sporangial ontogeny of *Phytophthora palmivora* (Butl.) from black pepper (*Piper nigrum* L.). *Agric. Res. J. Kerala*. 23:129-134

Dennis, C. and Webster, J. 1971. Antagonistic properties of specific groups of *Trichoderma*. I. Production of volatile antibiotics. *Trans. Br. Mycol. Soc.* 57: 41-48

Dewaard, P.W.F. 1979. Evaluation of the results of research on eradication of *Phytophthora* foot rot of black pepper (*Piper nigrum* L.). *First meeting of the pepper community permanent panel on Techno economic studies, 31st January to 4th February, 1979* Cochin, India, pp. 1-47

Doraiswamy, S., Nakkeeran, S. and Chandrasekhar, G.2003. *Trichoderma viride*-Importance in plant disease control. *Microbial Inoculant Technology* (eds. Thangaraju, M., Prasad, G. and Govindarajan, K.). Centre of advanced studies in Agricultural Microbiology, TNAU, Coimbatore, pp. 222-232

Dutta, P.K. 1984. Studies on two *Phytophthora* diseases (Koleroga of arecanut and black pepper wilt) in Shimoga district, Karnataka State. Ph.D. thesis, University of Agricultural Sciences, Bangalore, Karnataka State, p.184

Dutta, P. and Das, B.C. 1999. Control of *Rhizoctonia solani* in soybean (*Glycine max*) by farmyard manure culture of *Trichoderma harzianum*. *Indian J. Agric. Sci.* 69: 596-598

Eapen, J. S. and Ramana, K.V. 1996. Biological control of plant parasitic nematodes of spices. *Biological Control in Spices.* (eds. Anandaraj, M. and Peter, K.V.). Indian Institute of Spices Research, Calicut, pp.20-32

Elad, Y., Chet, I., Boyk, P. and Henis, Y. 1983. Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii* - scanning electron microscopy and fluorescence microscopy. *Phytopathology.* 73: 85-88

Elad, Y., David, D.R., Levi, T., Kapat, A., Kirshner, B., Gurvin, E. and Levine, A. 1999. *Trichoderma harzianum* T-39 - Mechanisms of biocontrol of foliar pathogens. *Modern fungicides and antifungal compounds II.* (ed. Lyr, H.). Intercept Ltd., Andover, Hampshix, U.K. pp.459-467

Faull, J.L., Graeme-Cook, K.A. and Palkington, B.L. 1994. Production of an isonitrile antibiotic by U.V. induced mutant of *Trichoderma harzianum*. *Phytochemistry*. 36: 1273-1276

Figueiredo, J.J. and Lellis, W.T. 1981. The control of black pod of cocoa with copper fungicides sprayed in high and low volumes. *Revista Theobroma*. 11:31-38

Filani,G.A. 1976. Effects of different fungicidal copper compounds on *Phytophthora palmivora*. *Turnalba*. 26: 295-301

Freed, R. 1986. *MSTAT version 1.2*. Department of Crop and Soil Sciences, Michigan State University

Georgieva, O.1991. Antagonistic characters of *Trichoderma harzianum* towards *Verticillium dahliae* on pepper. *New Approaches in Biological Control of Soil borne Disease* (ed. Gensen, D.F.). American Phytopathological Society, pp.18-20

Gokulapalan, C. 1989. Effects of plant protection chemicals on foliar pathogens and phylloplane microflora of rice. Ph.D. thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, p.132

Harman, G.C. 2000. Myths and dogmas of biocontrol- changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Disease*. 84: 377-393

Harman, G.C., Hayer, C.K., Lorito, M Broadway, R.M., Di-Dietro, A., Peter bauer, C. and Transmo, A. 1993. Chitinolytic enzymes of *Trichoderma harzianum* purification of chitobasidase and endochitinase *Phytopathology*. 83:313-318

Harper, R.S. 1974. Pepper in Indonesia - Cultivation and major diseases. *World Crops* 26: 130-133

Hartz, T.K., De Vay, J.E. and Elmore, C.L. 1993. Solarization is an effective soil disinfection technique for strawberry production. *Hort. Sci.* 28: 104-16

Hegde, S.G. and Anahosur, K.H. 1998. Integrated management of foot rot of black pepper. *Karnataka J. Agric. Sci.* 11:78-82

Henis,Y., Elad,Y., Chet,I. and Hadar, Y. 1979. Control of soil borne plant pathogenic fungi in carnation, strawberry and tomato by *Trichoderma harzianum*. *Phytopathology*. 69: 1031

Holliday, P. and Mowat, W.P. 1963. Foot rot of *Piper nigrum* L. (*Phytophthora palmivora*). *Phytopathology Paper*. CMI, Kew. 5:62

Jackson, A.M., Whipps, J.M. and Lynch, J.M. 1991. Effects of some carbon and nitrogen sources on spore germination, production of biomass and antifungal metabolites by species of *Trichoderma* and *Gliocladium* antagonism to *Sclerotium cepivorum*. *Biocontrol Sci. Technol.* 1: 43-61

Jain, M.P., Bhatnagar, M.K. and Jain, K.L. 1982. Nutritional and pathological studies on foot rot of brinjal caused by *Phytophthora nicotianae* var. *nicotianae*. *Proceedings of the Workshop on Phytophthora disease of Tropical Cultivated Plants* (ed. Nambiar, K.K.). CPCRI. Kasargode, Kerala, pp 109-117

Jayaraj, J. 1995. Studies on the biocontrol potential of *Trichoderma harzianum* Rifai. Ph. D. thesis. Annamalai University, Annamalainagar, India, p.214

Jayaraj, J. and Ramabadran, R. 1997. Effect of nitrogenous fertilizers on the survival and competitive saprophytic ability of *Trichoderma harzianum* in soil. *J. Mycol. Pl. Path.* 28:21-23

Jayaraj, J. and Ramabadran, R. 1998. Effect of certain nitrogenous sources on the *in vitro* growth, sporuiation and production of antifungal substances by *Trichoderma harzianum*. *J.Mycol. Pl. Path.* 28: 23-25

Jayasekhar, M. and Muthusamy, M. 2000. Effect of carbon and nitrogen sources on the growth of *Phytophthora capsici*. *Madras Agric. J.* 87: 482-483

Jebakumar R.S., Anandaraj, M. and Sarma, Y.R. 2000 Compatibility of Phorate and Chlorpyriphos with *Trichoderma harzianum* Rifai. applied for integrated disease management in black pepper (*Piper nigrum* L.). *J. Spic. Aromatic. Crop.* 9: 111-115

Jeyarajan, R., Ramakrishnan, G., and Sridhar, R. 1994. Biological control of soil borne diseases - Future prospects. *Crop Diseases- Innovative techniques and*

Management (eds. Sivaprakasam, K. and Seetharaman, K.). Kalyan Publishers, Ludhiana, pp.43-49

Jiazhuang, C. and Yan, W.X. 2000. Study on the occurrence of papaya epidemic disease and its pathogen identification. *South China Fruits*. 29:28

Joe. 2000. *Trichoderma* as a potential and inexpensive biofungicide for organic agriculture. *IFOAM 2000: The world grows organic*. (eds. Alfoldi, T., Joe, Y. Lockeretz, W. and Niggli, U.). Proceedings of the 13th International IFOAM Scientific Conference, 8-31 August 2000, Basel, Switzerland, p.117

Johnson, I. and Palaniswami, A. 1999. *Phyophthora* tuber rot of cassava – a new record in India. *J. Mycol. Pl. Path.* 29: 323-332

Johnson, L.F. and Curl, E.A. 1972. Isolation of groups of microorganisms from soil. *Methods for Research in Ecology of Soil-borne Plant Pathogens*. Burgess Publishing Co., New York, p.142

Jubina, P.A. and Girija V.K. 1997. Fungal antagonists from forest soils for the control of foot rot pathogen of black pepper. Abstract of papers of 23rd Annual meeting and Symposium of MSI on Fungi in Forest Ecosystem, 9-10 May 1997, KFRI, Peechi. *Abstract: 14*

Jubina, P.A. and Girija, V.K. 1998. Antagonistic rhizobacteria for management of *Phytophthora capsici*, the incitant of foot rot of black pepper. *J. Mycol. Pl. Path.* 28: 147-153

Kaosiri, T., Zentmeyer, G.A. and Erwin, D.C. 1978. Stalk length as a taxonomic criterion for *Phytophthora palmivora* isolates from Cacao. *Can. J. Bot.* 56: 1730-1738

Kasim, R. T. 1978. Inoculation method of pepper cuttings with *Phytophthora capsici*. *Pemberitan Lembaga Penelitian Tananan Industri*. 29:79-81

Kasim, R. T. 1986. The control of foot rot disease of pepper caused by *P. palmivora* using fungicides. *Edisi Khusus Penelitian Tanaman Rempondan Obat.* 11:63-66

Kasinathan, R. 1998. Studies on employing *Trichoderma* chitinases against plant pathogenic fungi. M. Sc. (Ag.) thesis, Tamil Nadu Agricultural University, Coimbatore, p.79

Katan, J. 1981. Solar heating (solarization) of soil for control of soil borne pests. *A. Rev. Phytopath.* 19: 211-236

Katan, J. and De Vay, J.E. 1991. Soil solarization: historical perspectives, principles and uses. *Soil Solarization* (eds. Katan, J. and De Vay, J.E.). CRC Press, Florida, pp. 23-37

Katan, J., Greenberger,A., Alon, H. and Grinstein, A. 1976. Solar heating by polyethylene mulching for the control of disease caused by soil-borne pathogens. *Phytopathology*. 66:683-688

KAU. 1994. *Research Report 1992-93*, Kerala Agricultural University, Vellanikkara, p.53

KAU. 1996. *Package of Practices Recommendations-Crops 96*. Kerala Agricultural University, Directorate of Extension, Mannuthy, Thrissur, India, p.267

KAU. 2000. Black pepper: *Three Decades of Spices Research at KAU* (ed. Nybe E.V.). Kerala Agricultural University Vellanikkara, Kerala, pp.1-36

Kaufman, D.D. and Williams, L.E. 1965. Influence of soil reaction and mineral fertilization on number and types of fungi antagonistic to four soil borne plant pathogens. *Phytopathology*. 55: 70-574

Kausalya, G. and Jeyarajan, R. 1994. . Biological control of soil borne diseases-future prospects. *Crop Diseases- Innovative Techniques and Management* (eds. Sivaprakasam, K. and Seetharaman, K.). Kalyan Publishers, Ludhiana, pp.43-49

Khan, J., Mohibullah, M. and Saljoqi, A.R. 1996. Effect of fungicides on the control of *Phytophthora* crown and root rot of apple trees. *Sarhad J. Agric.* 12: 319-324

Kheng, K.T. 1971. *Rep. Res. Brch. Den Agric* Plant Pathology Division, Sarawak, Malaysia. pp. 185-192

Krishnamoorthy, A.S. and Bhaskaran, R. 1993. Effect of chemical fertilizers and the antagonist *Trichoderma viride* on the control of damping off disease of tomato caused by *Pythium indicum*. *Crop Diseases- Innovative Techniques and Management* (eds. Sivaprakasam, K. and Seetharaman, K.). Kalyan Publishers, Ludhiana, pp.205-210

Krishnamoorthy, A.S. and Bhaskaran, R. 1994. Effect of some soil drenching fungicides on the growth and sporulation of *Trichoderma viride*, *Trichoderma harzianum* and *Laetisaria arvalis*. *Crop Diseases- Innovative Techniques and Management* (eds. Sivaprakasam, K. and Setharaman, K.). Kalyan Publishers, Ludhiana, pp.517-519

Kueh, T.K. 1982. Pepper (*Piper nigrum* L.). *A. Rep. Res. Brch. Dept. Agric., 1982.* Sarawak, Malaysia, pp.271-275

Kueh, T.K., Khew, K.L.1980. Evaluation of chemicals for the control of *Phytophthora* from *Piper nigrum*. *Malaysian Agric. J.* 52:263-272

Kunimoto, R.K., Aragaki, M., Hunter, J.E. and Ko, W.H. 1976 *Phytophthora capsici*, corrected name for the cause of *Phytophthora* blight of macadamia racemes. *Phytopathology*. 66:546-548

Kurain, P.S. 1992. Effect of solarization on damping off diseases of vegetables. M.Sc. (Ag.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, p.136

Leafmans, S. 1934. Diseases and pests of cultivated crops in the Dutch East Indies in 1931. *Meded. Inst. Voor. Plantenziekten*. 82: 92

Lee, B.S. 1973. The use of toxin for the screening of black pepper for foot rot resistance. *MARDI Res. Bull.* 1:10-14

Lilly, V.G. and Barnett, H.L. 1951. *Physiology of Fungi*. Mc.Graw Hill Book Co., New York and London

Lilyiona, K. 1991. Biology, growth stimulatory and biocontrol potentialities of *Trichoderma* spp. M.Sc. (Ag.) thesis, North Eastern Hill University, Shillong, Meghalaya, p.112

Lindsey, D.L. 1967. Growth of beans, tomatoes and corn under gnotobiotic conditions. *Phytopathology*. 57: 960

Lisha, K.P., Anandaraj, M., Paul, D., Jisha, P.J. and Sarma, Y.R. 2002 Evaluation of biocontrol agents obtained from Silent Valley biosphere reserve against *Phytophthora capsici*, the foot rot pathogen of black pepper (Abstr.). *Indian Phytopath.* 55:373

Liu, S. and Baker, R. 1980. Mechanism of biological control in soils suppressive to *Rhizoctonia solani*. *Phytopathology*. 70:404-412

Lokesh, M.S. and Gangadharappa, P.M. 1995. Management of *Phytophthora* foot rot and nematode diseases in black pepper (*Piper nigrum* L.). *J. Spic. Aromatic. Crop.* 4: 61-63

Lynch, J.M., Wilson, K.L., Ousley, M.A. and Whipps, J.M. 1991. Response of letuce to *Trichoderma* treatment. *Applied Microbiol.* 12: 59-61

Mahanty, B., Roy, J.K., Dasgupta, B. and Sen, C. 2000. Relative efficacy of promising fungicides and biocontrol agent *Trichoderma* in the management of foot rot of betelvine. *J. Plantation Crops.* 28: 179-184

Malajczuk, N. 1983. Microbial antagonism to *Phytophthora*. *Phytophthora - Its Biology, Taxonomy, Ecology and Pathology.* (eds. Erwin, D.C., Bartnick, Garcia and Tsao, P.H.). American Phytopathological Society, St. Paul, Minnesota, pp.197-218

Malebennur, N.S., Gangadharappa, P.M. and Hegde, H.G.1991. Chemical control of foot rot of black pepper caused by *Phytophthora capsici* (*P. palmivora*-MF_4). *Indian Cocoa, Arecanut Spices J.* 14: 148-149

Mammootty, K.P. 1978. Quick wilt disease of pepper (*Piper nigrum* L.) I. Symptomatological studies on the quick wilt disease of pepper. M.Sc. (Ag.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, p.87

Mammootty, K.P. 2003. Diseases and pest management in black pepper (*Piper nigrum* L.). *Paper presented in State level Seminar on Black Pepper held on 21st January 2003*. Asma towers, Mavoor road, Calicut. pp. 19-21

Mammootty, K.P. and Pillai, V.S. 1981. Studies on the chemical control of rotting disease of pepper cuttings in nursery. *Indian Phytopath.* 34: 240

Mammootty, K.P., Cheeran, A., Peethambaran, C.K. and Pillai, V.S. 1980. Symptomatological studies on the quick wilt of disease of pepper (*Piper nigrum* L.). *Proceedings of the Seminar on Diseases of Plantation Crops, 29th May 1980*, TNAU, Agricultural College and Research Institute, Madurai, pp.26-35

Mammootty, K.P., Das, T.P.M., Nair, S.S., Nair, P.K.U. and Cheeran A. 1991. Some aspects on epidemiology of *Phytophthora* foot rot disease of pepper. *Black Pepper Diseases* (eds. Sarma, Y.R. and Premkumar, T.). NRCS, Calicut, pp. 55-101

Matsuda, A., Hamada, M., and Gonzalez, J.L. 1994. Outbreak and control of foot rot on black pepper in Dominican Repablic. *Agrochemicals Japan.* pp.23-26

May, L.L. and Kimati, H. 2000. *Phytophthora parasitica* control with fungicides and effect of these products in the mycelial growth of *Trichoderma*. *Summa Phytopathologica.* 26: 52-57

Moeas, M. and Ben-aicha, B. 1990. Control of pepper wilt in Tuniasia. *Parasitica.* 46: 103-109

Moity, T.H. A, Papavizas, G.C. and Shatala, M.N. 1982. Induction of new isolates of *Trichoderma harzianum* tolerant to fungicides and their experimental use for control of white rot of onion. *Phytopathology.* 72: 396-400

Mondal, G., Srivastava, K.D. and Aggarwal, R. 1995. Antagonistic effect of *Trichoderma* spp. on *Ustilago segetum* var. *tritici* and their compatibility with fungicides and biocides. *Indian Phytopath.* 48: 466-470

Mukherjee, P.K., Upadhyay, J.P. and Mukopadhyay, A.N. 1989. Biological control of *Pythium* damping off of cauliflower with *Trichoderma harzianum*. *J. Biol. Control*. 3:119-124

Mukhopadhyay, A. N. 1988. Biological control of soil borne plant pathogens by *Trichoderma* spp. *Indian J. Mycol. Pl. Path.* 17: 1-9

Mukhopadhyay, A.N. 1994. Biocontrol of soil borne fungal plant pathogens- Current status, future propects and potential limitations. *Indian Phytopath*. 47. 119-126

Mukhopadhyay, A.N. 1995. Exploitation of *Gliocladium virens* and *Trichoderma harzianum* for biological seed treatment against soil borne diseases. *Indian J. Mycol. Pl. Path.* 25: 124

Mukhopadhyay, A.N., Brahmbhatt, A. and Patel, G.H. 1986. *Trichoderma harzianum* – a potential biocontrol agent for tobacco damping off. *Tobacco Res.* 12: 6-35

Muller, H.R.A. 1936. The *Phytophthora* foot rot of pepper (*Piper nigrum* L.) in the Dutch East Indies. *Meded. Inst. Piziekt. Batavia.* 88:73

Naik, M.K. and Sen, B. 1992. Biocontrol of plant diseases caused by *Fusarium* species. *Recent Developments in Biocontrol of Plant Diseases*. (eds. Mukherji, K.G., Tewari, J.P., Arora, D.K. and Saxena, G.). Aditya Books Pvt. Ltd., New Delhi, India, pp.37-51

Nair, P.K.U. and Sasikumaran, S. 1991. Effect of some fungicides on quick wilt (foot rot) disease of black pepper. *Indian Cocoa, Arecanut and Spices Journal*. 14: 95-96

Nair, P.K.U., Mammootty, K.P., Sasikumaran, S. and Pillai, V. S.1993. *Phytophthora* foot rot of black pepper (*Piper nigrum* L.) - a management study with organic soil amendments. *Indian Cocoa, Arecanut Spices J.* 17:1-2

Nair, P.K.U., Nair, S.S., Pillai, V.S. and Rao, G.S.L.H.V.P. 1988. Influence of weather on foot rot disease of black pepper. *Agrometeorology of Plantation Crops*. (eds. Rao, G.S.L.H.V.P. and Nair, R.R.). KAU, Trichur, pp.98-103

Nambiar, K.K.N. and Sarma, Y.R. 1976. Quick wilt (foot rot) disease of pepper (*Piper nigrum* L.). *Arecanut and Spices Bulletin*. 7: 89-91

Nambiar, K.K.N. and Sarma, Y.R. 1977. Wilt diseases of black pepper. *Journal of Plantation Crops*. 5: 92-103

Natarajan, K. and Manibhushanrao, K. 1996. Fungi as biocontrol agents against fungal plant pathogens. *Current Trend in Life Sciences: Recent Developments in Biocontrol of Plant Pathogens, Vol. XXI* (eds. Manibhushanrao, K. and Mahadevan, A.). Today and Tomorrows Printers and Publishers, New Delhi. India, pp: 83-91

Neelamegam, R. 1992. Integrated control of damping-off of tomato. Ph. D. thesis, Annamalai University, Annamalainagar, India, p.186

Pal, M. 1974. Studies on *Phytophthora* blight of pigeon pea. Ph. D. thesis. IARI. New Delhi. p.126

Papavizas, G.C. 1982. Survival of *Trichoderma harzianum* in soil in pea and bean rhizosphere. *Phytopathology*. 72: 121-125

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: Biology, ecology and potential for biocontrol. *A. Rev. Phytpathol*. 23: 23-54

Papavizas, G.C. and J.A. Lewis. 1981. *Biological Control in CropProduction*. Allanheld and Osmen, Totowa, New Jersey, pp. 305-322

Papavizas, G. C., Lewis, J.A. and Moity, T. H. A. 1982. Evaluation of new genotypes of *Trichoderma harzianum* for tolerance to benomyl and enhanced biocontrol capabilities. *Phytopathology*. 72:126-132

Patel, D.J. 2001. Soil solarization for the management of soil borne plant diseases. *J. Mycol. Pl. Path.* 31: 1-8

Patel, S.T. and Anahosur, K.H. 2001 Potential antagonism of *Trichoderma harzianum* against *Fusarium* spp., *Macrophomina phaseolina* and *Sclerotium rolfsii*. *J. Mycol. Pl. Path.* 31: 365

Peter, K. V. and Nybe, E. V. 2003. Black pepper cultivation in India – Opportunities and Challenges. *Papers presented in State level seminar on black pepper held on 21st January 2003*, Asma Towers, Mavoor Road, Calicut pp.1-10

Purakayastha, R.P. and Bhattacharya, B. 1982. Antagonism of microorganisms from jute phyllosphere towards *Colletotrichum corchori*. *Trans. Br. Mycol. Soc.* 78:504-513

Pyke, T.R. and Dietz, A. 1966. U-21, 963 - a new antibiotic I. Discovery and biological activity. *Appl. Microbiol.* 14:506

Raghu, P.A. and Chandramohanan, R. 1993. Effect of certain fungicides on *Phytophthora palmivora* (Butl.) Butl., the causal organism of black pod disease of cocoa. *Pestology.* 17:19-22

Raj, H. and Kapoor, I.J. 1993. Soil solarization for the control of tomato wilt pathogen (*Fusarium oxysporum* Schl.). *Z. Pflkrankh. Pflschutz.* 100:652-661

Raj, H., Bharadwaj, M.L. and Sharma, N.K. 1997. Soil solarization for the control of damping off of different vegetable crops in the nursery. *Indian Phytopath.* 50:524-528

Rajan, K.M. and Singh, R.S. 1974. Effect of fertilizers on population of *Pythium aphanidermatum*, associated soil microflora and seedling stand of tomato. *Indian Phytopath.* 27:62-69

Rajan, P.P. and Sarma, Y.R. 2000. Effect of organic soil amendments and chemical fertilizers on foot rot pathogen (*Phytophthora capsici*) of black pepper (*Piper nigrum* L.). *CAB International*, 2002, pp. 249-253

Rajan, P.P., Sarma, Y.R. and Anandaraj, M. 2002. Management of foot rot disease of black pepper with *Trichoderma* spp. *Indian Phytopath.* 55:34 -38

Rajan,P.P. and Sarma, Y.R. 1997. Compatibility of potassium phosphonate (Akomin-40) with different species of *Trichoderma* and *Gliocladium virens*. *Proceedings of the National Seminar on Biotechnology of Spices and Aromatic Plants* (eds. Edison, S., Ramana, K.V., Sasikumar, B., Babu, N. K. and Eapen, J.S.). Indian Society for Spices, Calicut, pp. 150-155

Ramachandran, N. and Sarma, Y.R. 1985. Efficacy of three systemic fungicides in controlling *Phytophthora* infections of black pepper. *Indian Phytopath.* 38:160-162

Ramachandran, N., Sarma, Y.R. and Anandaraj, M. 1988. Sensitivity of *Phytophthora* species affecting different plantation crops in Kerala to metalaxyl. *Indian Phytopath.* 41: 438-442

Ramachandran, N., Sarma, Y.R. and Anandaraj, M. 1990. Vertical progression and spread of *Phytophthora* leaf infection in black pepper in areca – black pepper mixed cropping system. *Indian Phytopath.* 43: 414-419

Ramachandran, N., Sarma, Y.R., Nambiar, K.K.N. 1986. Spatial distribution of *Phytophthora palmivora*-MF4 in the root zone of *Piper nigrum. Indian Phytopath.* 39: 414 – 417

Rao, M.K.V. 1927. *Report of the work done in the mycological section, 1925-1926.* Dep. Agric. Mysore, pp. 7-9

Rao, M.K.V. 1929. *Annual Report for the year 1927-28.* Dept. Agric. Mysore, pp. 19-22

Reeves, R.J. and Jackson, R.M. 1972. Induction of *Phytophthora cinnamomi* oospores in soil by *Trichoderma viride. Trans. Br. Mycol. Soc.* 59: 156-159

Riddle, R.W. 1950. Slide cultures. *Mycologia.* 42: 265-270

Rideout, C.J., Coley-Smith, J.R. and Cynch, J.M. 1986. Enzyme activity and electrophoretic profile of extracellular protein induced in *Trichoderma* spp. by wall of *Rhizoctonia solani. J. Gen. Microbiol.* 132: 181-187

Rifai, M.A. 1964. A reinvestigation of the taxonomy of the genus *Trichoderma* Pers.,M.Sc. thesis, University of Sheffield

Riker, A.J. and Riker, R.S 1936. *Introduction to research on plant diseases.* John Swift Co., St. Louis, Chicago, p.117

Robert, P.C. 1998. Management of foot rot of pepper with mycorrhiza and antagonists. Ph.D. thesis, Kerala Agricultural University, Vellanikkara Thrissur, p.227

Sadanandan, A. K. 1989. Management of wilt affected black pepper (*Piper nigrum* L.) garden. *J. Plantation Crops*. 16: 287-291

Saju, K.A., Anandaraj, M. and Sarma, Y.R. 2002. Evaluation of *Trichoderma* spp. for controlling foot rot of black pepper caused by *Phytophthora capsici* (Abstr.). *Indian Phytopath*. 55: 373

Samraj, J. and Jose, P.C. 1966. A *Phytophthora* wilt of pepper. *Sci. Cult* .32: 90-92

Santhakumari, P. 1987. Studies on the *Phytophthora* diseases of plantation crops. Ph. D. thesis, University of Agricultural Sciences, Dharwad, p.139

Sarma, Y. R., Kumar, P. T., Ramana K. V., Ramachandran, N. and Anandaraj, M. 1987. Disease and pest management in black pepper nurseries. *Indian Cocoa, Arecanut Spices J*. 11: 45-49

Sarma, Y.R. 1994. Management of *Phytophthora* foot rot and slow decline on black pepper. *The Planter's Chronicle*. 76 : 29-30

Sarma, Y.R. 2003. Global Scenario of disease and pest management in black pepper. *Paper presented in State level Seminar on Black Pepper held on 21st January 2003*, Asma Towers, Mavoor Road, Calicut, pp.11-15

Sarma, Y.R. and Anandraj, M. 1998. *Phytophthora* foot rot of black pepper. *Management of Threatening Plant Diseases of National Importance* (eds Agnihothri, V.P., Sarbhoy, A.K. and Singh, D.V.). Malhotra Publishing House, New Delhi, pp.237-248

Sarma, Y.R. and Nambiar, K.K.N. 1982. Foot rot disease of black pepper (*Piper nigrum* L.). *Proceedings of the Workshop on Phytophthora diseases of Tropical Cultivated Plants* (ed. Nambiar K.K.N.). CPCRI, Kasargode, Kerala, India, pp.209-224

Sarma, Y.R., Anandaraj, M. and Rajan, P.P. 1994. *Phytophthora* – A threat to black pepper, present status and future strategies of disease management. *Spice India.* 7: 10-13

Sarma, Y.R., Anandaraj, M. and Ramana, K.V. 1996a. Disease management in *Phytophthora* foot rot affected black pepper plantations. *Annual Report*, IISR. p. 63

Sarma, Y.R., Anandaraj, M. and Venugopalan, M.N. 1996b. Biological control of disease of spices. *Biological Control on Spices.* (eds. Anandaraj, M. and Peter, K.V.). Indian Institute of Spices Research, Calicut, p.42

Sarma, Y.R., Ramachandran, N. and Anandaraj, M. 1988. Integrated disease management of quick wilt (foot rot) of black pepper (*Piper nigrum* L.) caused by *Phytophthora palmivora* MF_4. *J. Coffee Res.* 18: 61-67

Sarma, Y.R., Ramachandran, N. and Anandaraj, M. 1991. Black pepper diseases in India. *Proceedings of the International Pepper Community Workshop on Joint Research for the control of black pepper diseases* (eds. Sarma, Y.R. and Premkumar, T.). NRCS, Calicut, p.75

Sarma, Y.R., Ramachandran,N. and Nambiar, K.K.N. 1980. Morphology of black pepper *Phytophthora* isolates from India. *Proceedings of the Workshop on Phytophthora Diseases of Tropical Cultivated Plants, September 19-23, 1980,* (ed. Nambiar, K.K.N.). CPCRI, Kasargode, 233-236

Sastry, M.N.L. 1982. Studies on species of *Phytophthora* affecting plantation crops in Karnataka with special reference to Koleroga of arecanut and wilt of black pepper. Ph.D. thesis, University of Agricultural Sciences, Bangalore, Karnataka State

Sastry, M.N.L. and Hegde, R.K. 1987a. Pathogenic variation in *Phytophthora* species affecting plantation crops. *Indian Phytopath.* 40: 365-369

Sastry, M.N.L. and Hegde, R.K. 1987b. Studies on *Phytophthora* species affecting plantation crops in Karnataka with special reference to Koleroga of arecanut and wilt of black pepper. *Plant Pathology Newsl.* 5:27

Satour, M.N., El-Sherif, E.M., El Ghareeb, L., El-Hazdad, A. and El-Wateil, H.R. 1991. Achievement of soil solarization in Egypt. *FAO Pl. Prod. Prot. Pap.* 109:200-212

Shanmugham, V. 1996. Biocontrol of rhizome rot of ginger (*Zingiber officinale*) by antagonistic microorganisms. M.Sc. (Ag.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, p.72

Sharma, S.D. and Mishra, A. 1995. Tolerance of *Trichoderma harzianum* to agrochemicals. *Abstract of Global Conference on Advances in Research on Plant disease and their management, Feb 12-17, 1995*. Rajasthan College of Agriculture, Udaipur, India, p.162

Sharma, S.D., Mishra, A., Pandey, R.N. and Patel, S.J. 2001. Sensitivity of *Trichoderma harzianum* to fungicides. *J. Mycol. Pl. Pathol.* 31: 251-253

Singh, R.S., Jindal, A., Singh, D. and Singh, T. 1995. Selection of *Trichoderma* isolates against common fungicides for their use in integrated plant disease management (Abstr.). *Indian J. Mycol. Pl. Path.* 25: 127

Sivaprasad, P. 1997. Management of root diseases of important spice crops of Kerala with VA mycorrhiza. *DBT Project Report*. Kerala Agricultural University, Thrissur, p.98

Sivasithamparam, K. and Ghisalberti, E.L. 1998. Secondary metabolism in *Trichoderma* and *Gliocladium*. *Trichoderma* and *Gliocladium, Vol. I.* (eds. Kubicek, C.P., G.E. Harman, Taylor and Francis). London, pp.139-191

Skidmore, A.M. and Dickinson, C.H. 1976. Colony interactions and hyphal interference between *Septoria nodorum* and phylloplane fungi. *Trans. Br. Mycol. Soc.* 66:57-64

Sneh, B., Humble, S.J., and Lockwood, J.L. 1977. Parasitism of oospores of *Phytophthora megasperma* var. *sojae*, *P. cactorum*, *Pythium* sp. and *Aphanomyces euteiches* in soil by oomycetes, chitridiomycetes, hyphomycetes, actinomycetes and bacteria. *Phytopathology* 67: 622-68

Sodsa-art, P. and Soytong, K. 1999. Biological control in the tropics: towards efficient biodiversity and bioresource management for effective biological control. *Proceedings of the Symposium on Biological Control in the Tropics held at MARDI training center, March 18-19, 1999* (eds. Hong, L.W., Sastroutomo, S. S., Caunter, I. G., Ali, J., Yeang, L.K. Vijaysegaran, S. and Sen, Y.H.). Serdang Malaysia

Sridhar, R., Radhakrishnan, G. and Jeyarajan. 1992. Studies on compatibility of *Rhizobium* with biocontrol agent *Bacillus subtilis* in urd bean. *J. Biol. Control.* 2:51-52

Stapleton, J.J. and De Vay, J.E. 1982. Effect of soil solarization on population of selected soil borne microorganisms and growth of deciduous fruit tree seedlings. *Phytopathology.* 72:323-326

Sundararaman, S. and Ramakrishnan, T.S. 1924. Mahali disease of coconuts in Malabar. *Mem. Dep. Agric. India Bot. Ser.* 13: 87-97

Sushir, A.M. and Pandey, R.N. 2001. Tolerance of *Trichoderma harzianum* Rifai to insecticides and weedicides (Abstr.). *J. Mycol. Pl. Path.* 31: 106

Tey, C. C. and Wood, R.K.S. 1984. Effect of various fungicides *in vitro* on *Phytophthora palmivora* from cocoa. *Trans.Br. Mycol. Soc.*80: 271-282

Thomas, J. Bhai, S. R., Vijayan, A.K. and Dhanapal, K. 1996. *Trichoderma* - A potential bioagent for control of soil borne diseases of small cardamom (*Elettaria cardamomum* Maton.). *Current Trend in Life Sciences Vol.XXI* (eds. Rao, K.M. and Mahadevan,K.). Today and Tomorrow's Printers and Publishers, New Delhi, pp.43-52

Tsao, P. H. 1991. The identities, nomenclature and taxonomy of *Phytophthora* isolates from black pepper. *Diseases of Black Pepper.* (eds. Sarma, Y. R. and Premkumar, T.) Proceedings of the International Pepper Community Workshop on Black pepper disease, NRCS, Calicut, Kerala, India. pp. 185-211

Tsao, P.H. and Alizadeh, A. 1988. Recent advances in the taxonomy and nomenclature of the so called *Phytophthora palmivora* MF4 occurring on cocoa and other tropical crops. *Proceedings of the tenth International Cocoa Research Conference, May 17-23,1987*, Santo Domingo, Dominican Republic, pp.441-445

Tsao, P.H., Sarma, Y.R., Kasim, R., Mustika, T. and Kueh, T.K. 1985. Variation in *Phytophthora palmivora* MF_4 (*P. capsici*) isolates from black pepper in India, Indonesia and Malaysia (Abstr.). *Phytopathology*. 75: 1315

Turner, G.J. 1969. Leaf lesions associated with foot rot of *Piper nigrum* and *Piper betle* caused by *Phytophthora palmivora*. *Trans. Br. Mycol. Soc*. 53: 407-415

Turner, G. J. 1970. *Rep. Res. Brch. Dep. Agric*. Plant Pathology Division, Sarawak, Malaysia, pp. 100-104

Turner, G.J. 1973. Effect of fungicides used as soil drenches in laboratory tests against *Phytophthora palmivora* from *Piper nigrum*. *Trans. Br. Mycol. Soc.* 61: 186-189

Upadhyay, J.P. and Mukhopadhyay, A.N. 1986. Biological control of *Sclerotium rolfsii* by *Trichoderma harzianum* in sugar beet. *Trop. Pest Mgmt*. 32: 215-220

Utkhede, R.S. and Rahe, J.E. 1983. Interactions of antagonist and pathogen in biological control of onion white rot. *Phytopathology*. 73: 890-893

Veena, S.S. and Sarma, Y.R. 2000. Uptake and persistence of potassium phosphonate and its protection against *Phytophthora capsici* in black pepper. *CAB International, 2002*,pp.243-248

Veena, S.S., Vijaya, P., Anandaraj, M. and Sarma, Y.R. 2002. Variability in sensitivity of *Phytophthora capsici* isolates to Potassium phosphonate (Abstr.). *Indian Phytopath*. 55.389

Viji, G.K., Rao, M. and Baby, U.I. 1997. Non-target effect of systemic fungicides on antagonistic microflora on *Rhizoctonia solani*. *Indian Phytopath*. 50: 324-328

Vilasini, T.N. 1996. Effectiveness of soil solarization for the control of soft rot disease in ginger. Ph.D. thesis, Kerala Agricultural University, Vellanikkara, Trichur, p.160

Vincent, J.M. 1927. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*. 159:850

Vraol, P. B. 1988. Antibiotic producing and antagonistic microorganisms in the forest soils of Kerala. M.Sc. (Ag.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala. p.159

Vrany, J., Crove, M. R., Fiker, A. and Dobias, K. 1990. Inoculation of potato with microorganisms under field conditions. I. Effect of plant growth, yield and physiological properties of microorganisms in potato and sugarbeet. *Folia Microbiologia* 35:326-335

Wajid, S.M.A., Sheroj, M. M. and Sreenivas, S. S. 1995. Seed bed solarization as a component of integrated disease management in FCV tobacco nurseries of Karnataka. *Tobacco Res.* 21:58-65

Webster, J. 1964. Culture studies on *Hypocrea* and *Trichoderma* I - Comparison of the perfect and imperfect stages of *H. gelatinosa*, *H. rufa* and *Hypocrea* sp. 1. *Trans. Br. Mycol. Soc.* 47:75-96

Weindling, R. 1932. *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology*. 22: 837-845

Weindling, R. 1934. Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. *Phytopathology*. 24: 1153-1179

Wheeler, B.E. J. 1969. *An Introduction of Plant Disease*. John Wiley and Sons Ltd, London. p.301

Wilson, K.I., Rahim, M.A. and Luke, P.L. 1974. *In vitro* evaluation of fungicides against Azhukal disease of cardamom. *Agri. Res. J. Kerala*. 12: 94-95

Windham, G.L., Windham, M.T. and Williams, W.P. 1989. Effect of *Trichoderma* spp. on maize growth and *Meloidogyne arenaria* reproduction. *Plant Disease.* 73: 493-495

Windham, M.T., Elad, Y. and Baker, R. 1986. A mechanism of increased plant growth induced by *Trichoderma* spp. *Phytopathology.* 76: 518-521

Wongwathanarat, P. and Sivasithamparam, K. 1991. Effect of phosphonate on the *Rhizosphere* microflora and the development of root rot (*Phytophthora cinnamomi*) in avocado seedlings. *Biol. Fert. Soils.* 11: 13-17

Yucel, S. 1995. A study on soil solarization combined with fumigant application to control *Phytophthora* crown blight (*Phytophthora capsici* Leonian) on peppers in the East Mediterranean region of Turkey. *Crop Prot.* 14: 453-655

Zentmeyer, G.A. 1988. Taxonomic relationships and distribution of species of *Phytophthora* causing black pod of cocoa. *Proceedings of the 10th International Cocoa Research Conference, May17-23, 1987*. Santo Domingo, Dominican Republic, pp.39-399

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Appendix

APPENDIX - I

MEDIA COMPOSITION

1. CARROT AGAR

Carrot	: 200 g
Agar	: 20.0 g
Distilled water	: 1000 ml

2. POTATO DEXTROSE AGAR

Potato	: 200 g
Dextrose	: 20.0 g
Agar	: 20.0 g
Distilled water	: 1000 ml

3. NUTRIENT AGAR MEDIUM

Glucose	: 5g
Peptone	: 5g
Beef extract	: 3g
NaCl	: 5g
Agar	: 20g
Distilledwater	: 1 litre
p^H	: 6.5 to 7.5

APPENDIX - II

MEDIA COMPOSITION

1. MARTIN'S ROSE BENGAL STREPTOMYCIN AGAR MEDIUM

Dextrose : 10g

Peptone : 5.0 g

KH_2PO_4 :1.0 g

$MgSO_4$: 0.5 g

Agar : 20 g

Rose Bengal : 0.03 g

Streptomycin : 30 mg (added aseptically to the sterilized medium)

Distilled water : 1000 ml

2. KEN KNIGHTS AGAR MEDIUM

Dextrose : 1g

KH_2PO_4 : 0.1 g

$NaNO_3$: 0.1 g

KCl : 0.1g

$MgSO_4$: 0.1g

Agar : 20g

Distilled water : 1000 ml

p^H : 7

3. THORNTON'S STANDARDISED AGAR

Mannitol	:	1.0 g
Asparagine	:	0.5 g
K_2HPO_4	:	1.0 g
KNO_3	:	0.5 g
$MgSO_4$	:	0.2 g
$CaCl_2$	:	0.1 g
NaCl	:	0.1 g
Ferric Chloride	:	0.002g
Agar	:	20.0 g
Distilled water	:	1000 ml
p^H	:	7.4

MANAGEMENT OF *Phytophthora* DISEASE IN BLACK PEPPER NURSERY

By

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ABSTRACT OF THE THESIS

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COLLEGE OF HORTICULTURE

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Abstract

ABSTRACT

Phytophthora rot is the serious disease of black pepper nursery. An investigation was carried out to isolate and select the efficient antagonists from black pepper nurseries and use them alone or in combination with fungicides in the integrated management of the disease. The experiment was laid out at CCRP farm at College of Horticulture, Vellanikkara.

The pathogen causing the disease was isolated and identified as *Phytophthora capsici* Leonian emend A. Alizadeh and P.H.Tsao based on the cultural and morphological characters. Quantitative estimation of rhizosphere microflora from different pepper nurseries yielded more soil bacteria followed by fungi and actinomycetes. All the 22 fungi, five out of 20 bacteria and none of the actinomycetes tested were antagonistic to *P. capsici*. Among the fungal isolates, 13 isolates including standard culture of *T. harzianum* recorded cent per cent inhibition of *P. capsici*. Further, selection of the efficient isolates was carried out based on the antagonistic index (AI). The isolates 22 F and 34 F recorded an AI of 3000 and 1500 respectively and these were identified as *Trichoderma longibrachiatum* and *Trichoderma viride*. The standard culture of *T. harzianum* also recorded an AI of 1500. The three antagonists were found parasitic on *P.capsici* as evidenced by excessive coiling, penetration and disintegration of the hyphae.

The fungicides *viz.*, Bordeaux mixture, Kocide, Captaf and Kavach were incompatible with the three antagonists, while, Indofil M-45, Ridomil MZ, Akomin and Anthracol were compatible. Fytolan showed partial compatibility with *T.viride* and *T. harzianum* but incompatible with *T. longibrachiatum*. Among the eight insecticides tested, Phorate and Carbofuran showed compatibility with the antagonists, whereas Monocrotophos, Quinalphos, Endosulfan, Dimethoate, Cypermethrin and higher concentration of Chlorpyriphos were incompatible. In general, fertilizers like Urea, Rajphos, Ammonium sulphate and Muriate of potash (MoP) were compatible with antagonists, while, Factomphos and higher concentration of Urea did not support good growth.

Bordeaux mixture, Fytolan, Kocide, Indofil M-45, Ridomil MZ and Captaf at all concentrations and higher concentration of Akomin-40 and Anthracol were inhibitory to *P. capsici*. The insecticides Phorate, Carbofuran and Chlorpyriphos showed comparatively good inhibitory effect against the pathogen but complete inhibition of pathogen was noticed with Monocrotophos, Endosulfan, Quinalphos, Dimethoate and Cypermethrin. The fertilizers *viz.*, urea, MoP, Rajphos supported growth of the pathogen while, Factomphos and ammonium sulphate exerted an inhibitory effect.

Solarization of potting mixture resulted in the build up of soil temperature and the build up was more in the upper layer of soil. Solarization of potting mixture and application of biocontrol agents had a positive effect in increasing the sprouting and reducing the pre-sprouting mortality of cuttings and is comparable to plants raised as per PoP. Observations on the incidence and severity of *Phytophthora* rot in black pepper showed that in general soil solarization, application of antagonists and spraying of Ridomil MZ had a favourable effect in checking the disease and the effect is almost similar to that of disease management as per PoP.

A variation in the population of soil microflora in different treatments was observed. The cuttings raised in solarized potting mixture incorporated with native antagonists had a significant effect in increasing the height and number of leaves.