MANAGEMENT OF FOOT ROT OF BLACK PEPPER (*Piper nigrum* L.) WITH MYCOINOCULANT ENRICHED VERMICOMPOST

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DECLARATION

I hereby declare that this thesis entitled "**Management of foot rot of black pepper (*Piper nigrum* L.) with mycoinoculant enriched vermicompost**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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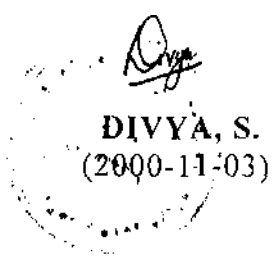

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CERTIFICATE

Certified that this thesis entitled "**Management of foot rot of black pepper (*Piper nigrum* L.) with mycoinoculant enriched vermicompost**" is a record of research work done independently by Ms. Divya. S. (2000-11-03) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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LIST OF ABBREVIATIONS

%	per cent
µl	Micro litre
µm	Micro metre
µg	Micro gram
@	At the rate of
^{0}C	Degree Celsius
AMF	Arbuscular Mycorrhizal Fungi
CD	Critical difference
cfu	Colony forming units
cm	Centimetre
COC	Copper oxychloride
CRD	Completely Randomised Design
cv.	Cultivar
DAI	Days after inoculation
et al.	And others
Fig.	Figure
FYM	Farmyard manure
g	Gram
h	Hour
ha	Hectare
K	Potassium
kDa	Kilo Dalton
kg	Kilogram
l	Litre
M	Molar
mg	Milligram
ml	Millilitre
N	Normal
NC	Neem cake
nm	Nanometer
OD	Ortho dihydroxy
P	Phosphorus
PAL	Phenylalanine ammonia lyase
ppm	Parts per million
pv.	Pathovar
rpm	Rotations per minute
SDS	Sodium Dodecyl Sulphate –
PAGE	Poly Acrylamide Gel Electrophoresis
sp.	Species
t	Tonnes
V	Volt
VC	Vermicompost

INTRODUCTION

1. INTRODUCTION

Black pepper (*Piper nigrum* L.) is one of the most important spice crops of Kerala with probable origin in the natural evergreen forest ecosystem of Western Ghats. The dried berries of this plant constitute the black pepper of commercial importance. Kerala accounts for nearly 90 per cent of the area and production in India. It is a major source of income and employment for rural households in the state where more than 2.5 lakh farm families are involved in pepper cultivation. The annual production of black pepper in India is around 75,000 t from an area of 2.38 lakh hectares. Out of this, the contribution from the State is 47,000 t from an area of 1.98 lakh hectares (Farm Guide, 2002). However, the productivity of pepper in India is only 315 kg ha^{-1} as against 4079 kg ha^{-1} in Thailand (Sivaraman *et al.*, 2002). One of the major reasons attributed to this is the high incidence of foot rot or quick wilt disease especially in Kerala. On a global scale the yield loss due to this disease is estimated to be around 4.5 to 7.5 million US dollars per annum (de Waard, 1979). This disease, caused by *Phytophthora capsici* Leonian, is also a serious problem in many other pepper growing countries such as Indonesia, Malaysia, Brazil, Thailand and Madagascar (de Waard, 1986; Sarma *et al.*, 1991; Mehrotra and Aggarwal, 2001).

Appropriate phytosanitation measures in pepper plantations, pre-monsoon painting of collar region of pepper vines with bordeaux paste, soil drenching and spraying the plants with one per cent bordeaux mixture are some of the commonly recommended plant protection measures to control this disease. It is also found that some of the systemic fungicides such as Metalaxyl (Ridomil), Fosetyl-Al (Aliette) and Ethazole (Terrazole) are also very effective for the control of foot rot of pepper. Eventhough the efficacy of systemic fungicides is well established, they are not widely used because of the residual problem in the products to be

exported to other countries. As a result, the concept of an integrated disease management practice is being introduced nowadays for the management of foot rot of pepper. It has also become an accepted component of organic farming.

Trichoderma and arbuscular mycorrhiza are the two most commonly used biocontrol agents for the management of foot rot of pepper. Talc based inoculum of *Trichoderma* and soil based granular inoculum of arbuscular mycorrhiza are now commercially available for this purpose. However, the cost of these products has become a limiting factor for their large scale application by small and marginal farmers. A solution to this problem is to encourage the farmers to mass produce these mycoinoculants by procuring good quality inoculum from a recognized agency. This will invariably require a cheap source of locally available organic carrier material for mass multiplication. One of the carrier materials with such a potential is vermicompost. The farmer can produce sufficient quantity of vermicompost either by recycling household or farm waste or produce the same on a collective basis as part of group farming activity.

The main objective of the present investigation was to study the utility of vermicompost as a carrier material for mass production of *Trichoderma* and arbuscular mycorrhiza and then use the mycoinoculant enriched vermicompost for the control of foot rot of pepper. In the first part, the efficacy of vermicompost as a carrier material for *Trichoderma* and arbuscular mycorrhiza was tested either by using vermicompost alone or in combination with neem cake or farmyard manure. The best combination of carrier material was then selected for a detailed study on changes in host physiology with respect to induced phenolics and defense related enzymes and pathogenesis related proteins after infection by *Phytophthora capsici*. These were done as per the following technical programme.

1. Testing the efficacy of vermicompost alone or in combination with farmyard manure or neem cake as carrier material for mass production for *Trichoderma harzianum* Rifai and *Glomus fasciculatum* (Thaxter) Gerd. & Trappe.
2. Use of mycoinoculant enriched vermicompost for biocontrol of foot rot of pepper.
3. Studies on changes in host physiology with respect to induced phenolics, defense related enzymes and pathogenesis related proteins after inoculation with *Phytophthora capsici* and application of biocontrol agents.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Foot rot is one of the most important diseases of black pepper in Kerala. Balakrishnan *et al.* (1986) and Anandaraj *et al.* (1988) reported vine death to the extent of 3.7 and 9.4 per cent from Calicut and Cannanore districts of Kerala with an annual loss of 119 and 905 t respectively. The first authentic account of sudden collapse and death of pepper vines was reported from Lampung, Indonesia in 1885 (Chattopadhyay and Maiti, 1990). In India, this disease was known as early as in 1902 when severe vine deaths were noticed in Wynad. This was investigated by Barber (1902, 1903, 1905) and Butler (1906,1918). Although the isolation of *Phytophthora* was reported from Mysore area by Venkata Rao (1929), it was Samraj and Jose (1966) who first identified the pathogen as *Phytophthora* sp. Subsequently, Sarma *et al.* (1982a) identified it as *P. palmivora* MF4. Tsao and Alizadeh (1988) after a detailed study of large number of *Phytophthora* isolates from pepper collected throughout the world finally identified the causal organism as *P. capsici*.

2.1 PATHOGEN

The growth of *P. capsici* on carrot agar medium was characterized by petalloid type colonies with maximum growth at 25-30°C. Chlamydospore production frequently occurred in old cultures. The sporangiophores were either of unbelloid or sympodial type (Mehrotra and Aggarwal, 2001). The sporangia were ellipsoidal with tapering base, ovoid or even fusiform with pedicel length of 6.7 to 12.5 µm. The length and breadth of the sporangium varied from 24.60 x 16.34 µm. According to Sarma *et al.* (1982b) 58.5 per cent of the sporangia observed showed a L/B ratio of 2.0 to 2.5 while 41.4 per cent showed a L/B ratio of 1.5 to 1.8. The diameter of the oospore varied from 20.7 to 29.9 µm.

2.2 SYMPTOMATOLOGY

A detailed symptomatology of foot rot of pepper was described by Sarma and Nambiar (1982). They observed that the pathogen could infect almost all parts of the plant including the stem, leaves, roots and spikes which caused collar rot, leaf rot, root rot, aerial vine death or spike shedding. The initial symptom of leaf infection appeared as water soaked lesions which rapidly extended to large dark brown spots with fimbriate margin and sometimes with concentric zonations and greyish centre. The tender leaves were more susceptible than mature leaves and irrespective of the type of lesion, heavy defoliation occurred due to foliar infection. The infection of the collar region occurred either in the collar region or just above or below the ground level with vascular discolouration, flaccidity of leaves, defoliation and breaking of the stem at nodal regions and spike shedding. Sarma *et al.* (1991) further observed that under stimulated field conditions, feeder root infection also led to collar rot and death of vines. Aerial infection of the stem occurred even at a height of four meters. This resulted in wet rotting of the stem which progressed both upwards and downwards from the initial point of infection. The direct infection of the spikes on the other hand, resulted in spike shedding.

2.3 CONTROL OF FOOT ROT DISEASE

Nambiar and Sarma (1976) stressed the importance of adopting phytosanitary measures under field conditions to reduce the inoculum potential of the pathogen in the soil to check disease severity. These included isolation of infected plants from healthy vines, better drainage facility and burning infected pits or drenching them with bordeaux mixture before replanting. An integrated disease management practice involving cultural, chemical and biological methods and breeding resistant varieties were also recommended by Sarma *et al.* (1988) for the control of foot rot of pepper. Periodical lopping of the branches of live supporting standards and shade trees in plantations to reduce humidity, tying of runner shoots to

main bush before the start of the monsoon season, covering the basins with thick dried leaf mulch to reduce soil splash and to prevent the new runner shoots from coming in contact with the soil and careful use of disinfected implements to avoid root injury and infection are some of the other measures commonly recommended to prevent the spread of this disease in pepper plantations.

Mammootty *et al.* (1979), Sasikumaran *et al.* (1981), Sarma and Nambiar (1982) and Sarma and Ramachandran (1984) observed that pre-monsoon painting of collar region with bordeaux paste, spraying the foliage and drenching the soil with one per cent bordeaux mixture once during May-June and again in July-August were very effective for the control of foot rot of pepper. The use of systemic fungicides such as Metalaxyl (Ridomil), Fosetyl-Al (Aliette) and Ethazole (Terrazole) both as foliar spray and as soil drench were also recommended by Ramachandran *et al.* (1982) and Ramachandran and Sarma (1985) for the control of this disease.

2.4 BIOCONTROL AGENTS

Cristinzio (1987) reported the antagonistic property of *Trichoderma* and *Chaetomium* spp. against *P. capsici* under *in vitro* conditions. Tsao *et al.* (1988) found that soil application of these antagonists to sick soils was effective for the biocontrol of *Phytophthora* spp. An integrated approach for the management of foot rot of black pepper involving the use of fungal antagonists like *Gliocladium virens* and *Trichoderma* spp. to soil along with foliar spray of bordeaux mixture, metalaxyl or phosphoric acid was recommended by Anandaraj and Sarma (1994a, 1995). Such management practices with *T. harzianum* were found to reduce disease incidence from 25 per cent to 15 per cent under field conditions (Sarma *et al.*, 1996a). Jebakumar *et al.* (2000) reported that *T. harzianum* utilized for the management of *Phytophthora* foot rot of black pepper was compatible with phorate and chlorphyriphos applied for the management of nematodes

and mealy bugs. The combination of *T. harzianum* and *G. virens* applied at the rate of 30 g per pot (Rajan and Sarma, 2000) and *T. harzianum* and *Alcaligenes* sp. applied alone or in combination (Anith and Manomohandas, 2001) were also found effective in reducing the incidence of *P. capsici* induced foot rot and nursery rot disease in black pepper. Similar results were also reported from other crops such as cardamom and betel vine. Bhai *et al.* (1993) observed that by using either *T. viride* or *T. harzianum* in nursery beds of cardamom one week prior to inoculation of *P. meadii*, resulted in significant reduction in seedling mortality. Bhai *et al.* (2000) also reported that the application of *T. harzianum* initially multiplied in coffee husk and farmyard manure in the ratio 1 : 1 and applied at the rate of one kilogram twice a year was effective in reducing the soil population of *Phytophthora* and thereby the incidence of capsule rot in cardamom by about 83 per cent. Similarly, Chaurasia and Bhatt (2000) reported that the use of *T. harzianum* at the rate of 500 kg ha^{-1} at quarterly intervals resulted in significant reduction in the intensity of foot and leaf rot of betel vine. However, Mahanty *et al.* (2000) observed that the biological control of foot rot of betel vine caused by *P. parasitica* var. *piperina* with *T. harzianum* was not as effective as chemical control in terms of reduction of disease index.

Ganesan *et al.* (2000) found that the application of *Trichoderma* significantly increased root dry weight in pepper cuttings when compared to untreated control. They further suggested that such a beneficial effect was due to enhanced nutrient uptake by inoculated plants. Anith and Manomohandas (2001) reported that the combined application of *T. harzianum* and *Alcaligenes* also resulted in enhanced shoot weight in pepper.

The use of different types of carrier materials has been recommended for mass production of *Trichoderma* spp. These included diatomaceous earth impregnated with 10 per cent molasses (Backman and Kabana,

1975), wheat bran and peat (Sivan *et al.*, 1984), wheat bran and saw dust (Elad *et al.*, 1986 and Dohroo, 2001), farmyard manure, gobar gas slurry, press mud and paddy chaff (Kousalya and Jeyarajan, 1988), talc (Jeyarajan *et al.*, 1994), gypsum (Renganathan *et al.*, 1995), vermiculite and wheat bran (Vidya, 1995), poultry manure and coffee fruit skin – biogas slurry (Sawant and Sawant, 1996), cowdung and neem cake (Sivaprasad, 1998), tea waste (Prakash *et al.*, 1999), shelled maize cob (Gandhikumar and Ranganathan, 2000), decomposed coffee husk (Ganeshan *et al.*, 2000), coconut water and coirpith (Kumar *et al.*, 2000), sugarcane straw and tea waste with two per cent molasses (Singh *et al.*, 2000) and farmyard manure and tapioca thippi (Umamaheswari and Ramakrishnan, 2000).

The successful use of the earthworm, *Eudrilus eugeniae* for vermicompost production in Kerala was first reported by Jiji *et al.* (1995) and Prabhakumari *et al.* (1995). Edwards (1982) found that the physical structure of vermicompost produced from organic waste depended on the original material from which it was produced. But the final product was always a finely powdered, peat-like material with excellent structure, porosity, aeration, drainage and moisture holding capacity. Rouelle and Randriamamonjizaka (1983) reported that vermicompost could supply the full requirement of trace elements and P and to some extent the initial requirement of K for plant growth. Bano *et al.* (1987) compared the nutrient status of vermicompost with organic manures. They found that the percentage of N in vermicompost was same as that of other organic manures.

The beneficial effect of arbuscular mycorrhizal association on plant growth in black pepper was reported by Manjuanth and Bagyaraj (1982), Ramesh (1982) and Sivaprasad (1995). Anandaraj *et al.* (1993) and Dare (1996) observed that the use of this endomycorrhiza as a biocontrol agent significantly reduced foot rot incidence in pepper. Anandaraj and Sarma (1994b) suggested that the suppressive effect of arbuscular mycorrhizal

fungi was due to enhanced root regeneration, nutrient uptake and altered host physiology in mycorrhizal plants. Similar observation was also made by Sarma *et al.* (1996b) and Thanuja (1999). They also reported considerable reduction in root rot, foliar yellowing and defoliation in black pepper due to mycorrhizal symbiosis under field conditions. Sivaprasad *et al.* (1995) got a negative correlation between foot rot incidence and percentage of mycorrhizal infection and spore count in black pepper mycorrhizosphere.

Anandaraj *et al.* (1996) emphasized the need for using native isolates of arbuscular mycorrhiza for reducing the incidence of foot rot in black pepper. Sivaprasad *et al.* (1997) and Robert (1998) found that plants inoculated with *G. monosporum* had no mortality till 40 day and that in inoculated plants there was also an increase in biomass production. A similar result was also reported by Thanuja and Hegde (2001) in pepper after inoculation with *G. fasciculatum*. The successful use of arbuscular mycorrhiza for the control of *Phytophthora* diseases was also reported from other crops such as papaya (Ramirez, 1974), citrus (Schenk *et al.*, 1977; Davis and Menge, 1980), soybean (Woodhead, 1978), pine (Baertschi *et al.*, 1981), sweet orange (Graham and Egel, 1988) and cardamom (Sivaprasad, 1995).

Bagyaraj and Manjunath (1980) suggested the culturing of arbuscular mycorrhizal fungi in highly susceptible trap plants in disinfected soil as a method for mass production of mycorrhizal inoculum. Hayman (1982) recommended the use of onion, black pepper, citrus, sorghum, maize and *Stylosanthes* for this purpose. Santhanakrishnan *et al.* (1995) used a mixture of vermiculite and sterilized soil in the ratio of 10 : 1 for mass production of arbuscular mycorrhiza with maize as host plant.

Kohl and Schlosser (1989) reported that infection and colonization of maize roots by *G. etunicatum* was unaffected by strains of *T. hamatum* and *T. harzianum*. They further observed that the combined application of

the two biocontrol agents significantly improved plant growth. Paulitz and Linderman (1991) got a compatible interaction in cucumber between the fungal antagonist, *Gliocladium virens* and *Glomus etunicatum* and *G. mosseae*. Calvet *et al.* (1992) found that *Trichoderma* spp. stimulated the germination of arbuscular mycorrhizal spores by producing volatile compounds and that it could be used along with AMF to ensure better plant growth. Kumar *et al.* (1993) observed that wheat plants were protected from infection by the root rot pathogens such as *Bipolaris sorokiniana, Fusarium avanaceum* and *F. javanicum* by combined inoculation of *G. epigaeus* and *T. viride*. Joseph (1998) reported that the incidence and intensity of rhizome rot of ginger caused by *Pythium aphanidermatum* was significantly reduced both in green house and field trials when native isolates of AMF (*Glomus* spp.) and antagonists (*T. viride* and *Aspergillus fumigatus*) were used at the time of planting.

Solarization of nursery mixture and subsequent fortification with vesicular arbuscular mycorrhiza, *T. harzianum* and *Gliocladium virens* were found highly effective in producing disease free planting materials in black pepper (Sarma and Anandaraj, 1996). An enhanced crop protection against *P. capsici* due to dual inoculation of arbuscular mycorrhizal fungi and the fungal antagonist, *Trichoderma* sp. was also reported by Robert (1998) and Sivaprasad (1998). Similarly, Kandiannan *et al.* (2000) observed that the combined inoculation of VAM, *Azospirillum* and phosphobacteria improved plant height, leaf area, biomass and dry matter production and nutrient content in black pepper.

2.5 PHENOL AND OD PHENOL CONTENT

Walker and Link (1935) first reported that the resistance of onion varieties to *Colletotrichum circinans* was due to the accumulation of flavones, anthocyanins and simple phenolics such as protocatechuic acid and catechol in the dead outer scales. A similar observation was made in apple by Martin *et al.* (1957) with respect to mildew fungus *Podosphaera*

leucotricha. Higher amount of chlorogenic acid was reported by Lee and Le Tourneau (1958) in potato roots resistant to *Verticillium* wilt. Patil *et al.* (1962) also showed that young potato roots that were resistant to *Verticillium* wilt had high levels of phenolics till five weeks after sprouting. Later, the chlorogenic acid content was found to decrease continuously which resulted in increased susceptibility to infection. The accumulation of phenolic compounds due to infection by pathogens was also reported in many other crops such as ragi (Vidhyasekaran, 1974), mung (Arora and Bajaj, 1978; Arora, 1983), tea (Borah *et al.*, 1978) and rice (Chattopadhyay and Bera, 1980). Beckman (2000) explained the physiological aspect of disease resistance and phenol accumulation as due to rapid oxidation of phenolic compounds which resulted in lignification and suberization of cells and cell death that sealed off further infection at the site of cellular penetration by the pathogen. The accumulation of such phenolic compounds was also found to take place at faster rate in resistant cultivars of many fruits and vegetables (Kuc, 1964), mung (Arora and Bajaj, 1978; Arora, 1983), wheat (Arora and Wagle, 1985), sorghum (Patil *et al.*, 1985) and cotton (Bashan, 1986).

Jeun and Hwang (1991) studied the phenolic content of *Capsicum* plants with respect to age related resistance to *P. capsici*. They reported an increase in resistance with age in both the susceptible and resistant cultivars. High phenol content was reported in mycorrhizal pepper plants by Sivaprasad *et al.* (2000).

Johnson and Schaal (1957) compared the phenolic content of scab resistant and susceptible potato tubers of different maturities. They found that there existed a correlation between OD phenol content and disease resistance. Vidhyasekaran (1973) got a direct correlation between OD phenol content and grape vine resistance to *Gloeosporium ampelophagum*. Such results were also reported from many other crops such as rice (Kasirajan, 1975; Parameswaran, 1979; Sundaram, 1980; Sathiyanathan

and Vidhyasekaran, 1981; Zuber and Rao, 1984), wheat (Vijayakumar and Rao, 1980) and cluster bean (Lodha *et al.*, 1993; Sindhan *et al.*, 1996).

2.6 DEFENSE RELATED ENZYMES

2.6.1 Peroxidase

Bonner (1950) reported that plant peroxidase was one of the key enzymes involved in disease resistance as it played an important role in the biosynthesis of lignin and oxidation of many mono and diphenolic compounds and aromatic amines to highly toxic quinones in the presence of hydrogen peroxide. The enzyme itself was found to be toxic to many pathogens by Macko *et al.* (1968) and Urs and Dunleavy, (1974). Kosuge (1969) observed that peroxidase activity was frequently increased in plants infected by pathogens and that its activity was closely correlated with disease resistance. Friend and Thornton (1974) reported that the peroxidase activity in potato tubers inoculated with *P. infestans* increased after inoculation in both resistant and susceptible varieties. Such alterations in enzyme activity was also reported in other crops such as oats (Yamamoto *et al.*, 1978), cucumber (Hammerschmidt *et al.*, 1982), greengram (Arora and Bajaj, 1984), cassava (Pereira *et al.*, 2000) and pea (Guleria *et al.*, 2001).

Rahayuningsih (1990) studied the peroxidase activities of five *P. nigrum* genotypes and one *P. hirsutum* genotype in relation to resistance to *P. palmivora*. He found that *P. hirsutum* had high peroxidase activity and was highly resistant to *P. palmivora*, while *P. nigrum* cv. Lampum Daun Lebar, which had low peroxidase activity was highly susceptible to the pathogen. Lizzy and Coulomb (1991) reported enhanced peroxidase and catalase activities in *Capsicum annuum* infected by *P. capsici*.

Alcazar *et al.* (1995) got both quantitative and qualitative changes in isoperoxidase activity in susceptible and resistant cultivars of *Capsicum*

annuum L. to *P. capsici*. The peroxidase activity was determined in the intercellular fluid and cytosolic fractions of the necrotic, healthy and intermediate zones of stem, six days after inoculation. The activity of susceptible cultivars increased from 4.7 to 12.9 units while in the resistant cultivar there was an increase from 5.7 to 662 units. An additional acidic isoperoxidase band was also obtained in the resistant cultivar.

2.6.2 Polyphenol Oxidase

The role of polyphenol oxidase, a copper containing enzyme which oxidized phenolics to highly toxic quinones in plant disease resistance was reported earlier by Umaerus (1959); Fehrmann and Dimond (1967); Maxwell and Bateman (1967); Hanusova (1969); Kosuge (1969); Maraite (1973); Uritani (1976); Yamamoto *et al.* (1978); Hammerschmidt *et al.* (1982); Tayal *et al.* (1984) and Arora and Wagle (1985). Rao *et al.* (1988) explained that the increase in polyphenol oxidase activity was due to activation of latent host enzyme.

Nema (1991) got increased polyphenol oxidase activity in leaf tissue of betel vine infected with *Xanthomonas campestris* pv. *betlicola*. Avdiushko *et al.* (1993) also got enhanced polyphenol oxidase activity in cucumber leaves in the vicinity of lesions caused either by *Colletotrichum lagenarium* or tobacco nerosis virus. Similar results were reported from other crops such as groundnut infected by *Puccinia arachidis* (Velazhahan and Vidhyasekaran, 1994), Brassica infected by *Alternaria brassicae* (Gupta *et al.*, 1995), potato infected by *Fusarium sambucinum* (Ray and Hammerschmidt, 1998), cucumber after bacterization with *Pseudomonas corrugata* strain B (Chen *et al.*, 2000), wheat after inoculation with *Neovossia indica* (Gogoi *et al.*, 2000) and moringa in response to infection by *Rhizopus stolonifer* (Rahman *et al.*, 2001). However, Vidhyasekaran *et al.* (1973); Pollock and Drysdale (1976) and Nadolny and Sequeira (1980) did not get any correlation between polyphenol oxidase activity and disease resistance.

2.6.3 Phenylalanine Ammonia Lyase (PAL)

Friend *et al.* (1973) found that phenylalanine ammonia lyase activity involved in the biosynthesis of phytoalexins significantly increased in potato varieties resistant to *P. infestans*. Dixon and Fuller (1976) got a positive correlation between increased PAL activity and biosynthesis of phaseollin, the phytoalexin of bean. Patridge and Keen (1977) reported that in soybean var. Harosoy 63 inoculated with *P. megasperma* var. *sojae*, PAL activity increased after six hours and that this increase continued until 15 h, reaching a specific activity 55 times greater than at zero time. This was followed by a decrease and the basal enzyme level was attained with in 30 hours. Moesta and Grisebach (1982) observed that L-2, amino oxy-3-phenyl propionic acid (L-AoPP), an inhibitor of phenylalanine ammonia lyase, at concentrations of 500 μ mol completely reverted the resistant interaction between Harosoy 63 soybean seedlings and race one of *P. megasperma* var *sojae* into a susceptible one. Jebakumar *et al.* (2001) studied the activity of PAL in both leaf and root tissues of three pepper varieties, tolerant (P 24) and susceptible (Panniyoor and Subhakara), in healthy and *Phytophthora capsici* infected tissues. It was found that infection by *Phytophthora* enhanced the enzyme activity and among the three varieties studied, the *Phytophthora* tolerant P 24 variety expressed maximum PAL activity.

2.7 PATHOGENESIS RELATED PROTEINS (PR PROTEINS)

The induction of the pathogenesis related protein, β-1,3-glucanase was first reported in almond seeds by Grassman *et al.* (1934). Later, it was reported from many monocotyledonous and dicotyledonous plants by Netzer *et al.* (1979); Shivaraj and Pattabiraman (1981); Carr and Klessig (1989) and Jondle *et al.* (1989). Schroder *et al.* (1992) observed that infection of potato leaves with *P. infestans* resulted in a co-ordinated induction of β-1,3-glucanase and chitinase activity in compatible as well as incompatible reactions.

Enkerli *et al.* (1993) reported that systemic resistance was induced in tomato due to infection by *P. infestans*. The level of this resistance increased with changes in acid soluble proteins and chitinase activity. However, β-1,3-glucanase activity was not induced systemically. Wubben *et al.* (1993) observed that infection of tomato by *Cladosporium fulvum* induced the production of β-1,3-glucanase, chitinase and PR-1b proteins near the stomata in the lower epidermis. Manandhar *et al.* (1999) found that in rice plants, the transcripts for the pathogenesis related proteins, PR-1, PR-2, PR-3, PR-4 and PR-5 accumulated moderately in response to a virulent isolate of *Pyricularia oryzae* (*Magnaporthe grisea*) and lightly in response to an avirulent isolate of *P. oryzae*.

O' Garro and Charlemange (1994) compared the response of pepper leaves and flowers to infection with *Xanthomonas campestris* pv. *vesicatoria* and found that the bacterial growth was drastically restricted in flowers but not in leaf tissue. Eventhough Chitinase and β-1,3-glucanase activities were coordinately induced in infected leaf and flower tissue, these were considerably earlier in the flower tissue. Kim and Hwang (1994) reported that infection by *P. capsici* induced the synthesis and accumulation of β-1,3-glucanase and chitinase in the stem of *Capsicum annuum*, soon after inoculation. Two acidic isoforms of glucanase were also detected on PAGE gels as GA_1 and GA_2.

Dassi *et al.* (1998) observed a bioprotective effect in tomato plants due to root colonization by *G. mosseae* against *P. parasitca*. The PR protein expression was enhanced by the pathogen, while only a weak response was detected in *G. mosseae* colonized roots. Lee *et al.* (2000) observed that the induction of chitinase mRNA started as early as six hours after inoculation with *P. capsici* in pepper stems. This was usually localized in the vascular tissues and their expression was restricted in the phloem related cells. The activity of β-1,3-glucanase in both leaf and root tissues of three black pepper varieties both tolerant (P-24) and susceptible

(Panniyoor and Subhakara) was determined in healthy and *P. capsici* infected tissues by Jebakumar *et al.* (2001). They found that infection by the pathogen generally enhanced the enzyme activity and SDS-PAGE study revealed the induction of PR-proteins in infected tissues. Further, western blotting with anti tobacco β-1,3-glucanase antibody confirmed the presence of these isoforms in leaf extract. Among the three varieties studied, the *Phytophthora* tolerant P-24 had the maximum expression of defense related proteins.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation on "Management of foot rot of black pepper (*Piper nigrum* L) with mycoinoculant enriched vermicompost" was done at College of Agriculture, Vellayani during 2001-2002 as part of a research project funded by Science Technology and Environment Department, Government of Kerala. Three month old cuttings of pepper var. Karimunda procured from the Instructional Farm of the College were used for this study.

3.1 ISOLATION OF PATHOGEN

The pathogen was isolated from foot rot affected pepper plants (var. Karimunda) collected from the District Agricultural Farm of the Department of Agriculture, Peringamala during the South - West monsoon season of 2001. Small pieces of infected leaf and vine were initially surface sterilized with 0.1 per cent mercuric chloride solution and then repeatedly washed with sterile water. These were aseptically transferred to sterile carrot agar medium (Appendix I.A) supplemented with 30 ppm of Sporidex DS (Cephalexin, Ranbaxy Laboratories Ltd.) in Petri dishes and then incubated at room temperature, for three days. Visible growth of mycelium from infected leaf and vine bits were aseptically transferred to sterile slants of carrot agar medium and maintained in a refrigerator for further studies.

3.1.1 Testing of Pathogenicity

Pathogenicity of the fungus was tested by artificial inoculation on three month old rooted cuttings of pepper var. Karimunda maintained in poly bags. For this, the isolate was initially grown on oat meal agar medium (Appendix. I.B) in sterile Petri dishes for five days at room temperature. Sporangia production was induced by incubating the above Petri dishes in a refrigerator for 24 hours. Five cm discs cut from this growth was used for inoculating both the stem and leaves of healthy pepper plants. Stem inoculation was done by placing the mycelial disc

of the pathogen at the collar region after giving mild pin pricks and then covering with a piece of moist cotton. Leaf inoculation was done on the second and third leaf from the tip of the vine. Before inoculation, the selected leaves were washed with sterile water to remove adhering dust particles. The mycelial disc was placed on the upper surface of the leaf after giving mild pin pricks. These were then covered with moist cotton to initiate leaf infection. The inoculated plants were irrigated regularly and covered with polythene bags to maintain high relative humidity. Three plants were maintained along with appropriate control without pathogen inoculation. Observations on the date of onset of disease symptom both on the stem as well as on the leaves and its further spread for a period of one week were taken before reisolating the pathogen on carrot agar medium for further studies.

3.1.2 Identification of Pathogen

The pathogen was identified on the basis of cultural and morphological characters. These were compared with that of the type culture maintained in the Department of Plant Pathology, College of Agriculture, Vellayani.

3.1.3 Mass Production

Mass production of the pathogen was done on sterile oat meal agar medium for plant inoculation and on sterile sand - oat meal mixture for soil application. Two hundred and fifty grams of sand and oats in the ratio of 9 : 1(v/v) was taken in one litre conical flask and sterilized in an autoclave at 1.05 kg cm^{-2} for 20 minutes at 121.5^{0}C for two consecutive days. The flasks were inoculated with 10 mm mycelial disc of the pathogen grown on oat meal agar medium and then incubated at room temperature for nine days for mycelial proliferation.

3.2 BIOCONTROL AGENTS

The bio control agents selected for this study were *Trichoderma harzianum* and *Glomus fasciculatum*. Commercially used talc based inoculum of *T. harzianum*

and soil based granular inoculum of *G. fasciculatum* were obtained from the Department of Plant Pathology, College of Agriculture, Vellayani.

3.3 CARRIER MATERIALS

Vermicompost and farmyard manure based carrier materials were used for mass production of both the bio control agents. These were procured from the Instructional Farm of College of Agriculture, Vellayani and were used either as such or in suitable combination with neem cake or farmyard manure as described below.

1. Vermicompost alone (VC)
2. Vermicompost + farmyard manure (1:1) (VC + FYM)
3. Vermicompost + neem cake (5:1) (VC + NC)
4. Farmyard manure + neem cake (10:1) (FYM + NC)

3.3.1 Enrichment of Carrier Material with Biocontrol Agents

3.3.1.1 Trichoderma harzianum

Mass production of *T. harzianum* was done on each of the above selected carrier material. For this, 250 g of carrier material was initially taken in one litre conical flask and then sterilized after moistening with water in an autoclave at 1.05 kg cm^{-2} for 20 minutes at 121.5°C for two consecutive days. After cooling to room temperature, each flask was inoculated with talc based inoculum of *T. harzianum* @ 50 g/kg carrier material. These were incubated at room temperature for 15 days and the population of *T. harzianum* in each carrier material was estimated by serial dilution and plating technique using Rose bengal agar medium (Appendix. I.C)

3.3.1.2 Glomus fasciculatum

Mass production of *G. fasciculatum* was also done in different carrier materials as mentioned under 3.3 after mixing with sand in the ratio of 10:1 and guinea grass (*Panicum maximum*) as host plant. It was raised in large earthen pots of 30 cm diameter containing eight kg of appropriate carrier material and sand

mixture. Fifty grams of granular inoculum was placed at five cm depth before sowing the guinea grass seeds obtained from the Department of Agronomy, College of Agriculture, Vellayani. The plants were grown for 50 days with regular irrigation. A granular inoculum of *G. fasciculatum* was prepared by chopping the infected grass roots into small pieces of approximately one cm size and mixing the same with spore enriched carrier material used for raising the plant. Percentage of mycorrhizal infection of the inoculum was determined by staining with 0.05 per cent trypan blue (Phillips and Hayman, 1970). One hundred root bits of approximately one cm length (in triplicate) from each sample were examined for this purpose. The root bits were initially washed with tap water and then softened with 10 per cent KOH solution for 30 minutes at 90°C in a water bath. The alkali was poured off and after rinsing with tap water for four times, the root samples were acidified by immersing it in two per cent HCl for five minutes. The acid was decanted and the root bits were stained with 0.05 per cent trypan blue in lacto phenol by boiling for 10 minutes. The excess stain was poured off and root bits were destained overnight with fresh lacto phenol before observing under a microscope for the presence of typical arbuscular mycorrhizal colonization. The result was expressed as percentage of mycorrhizal infection.

3.4 USE OF MYCOINOCULANT ENRICHED VERMICOMPOST FOR THE MANAGEMENT OF FOOT ROT OF PEPPER

The efficacy of three different combinations of vermicompost, vermicompost alone (VC), VC + FYM (1:1) and VC + NC (5:1) along with FYM + NC (10:1) as carrier material for mass production of *T. harzianum* and *G. fasciculatum* were tested by studying the extent of disease control achieved in pepper var. Karimunda infected with the pathogen.

Design : CRD

Treatments : 24

Replication : 4

3.4.1 Treatment Combinations

3.4.1.1 T. harzianum (T) as Biocontrol Agent along with Pathogen (P) Inoculation

1. VC + T + P
2. VC + FYM + T + P
3. VC + NC + T + P
4. FYM + NC + T + P

3.4.1.2 G. fasciculatum (G) as Biocontrol Agent along with Pathogen Inoculation

1. VC + G + P
2. VC + FYM + G + P
3. VC + NC + G + P
4. FYM + NC + G + P

3.4.1.3 Combined Inoculation of T. harzianum and G. fasciculatum (1:1) along with Pathogen Inoculation

1. VC + T + G + P
2. VC + FYM + T + G + P
3. VC + NC + T + G + P
4. FYM + NC + T + G + P

3.4.1.4 Chemical Control with 0.3% Copper Oxychloride (COC) along with Pathogen Inoculation

1. VC + COC + P
2. VC + FYM + COC + P
3 VC + NC + COC + P
4. FYM + NC + COC + P

3.4.1.5 Control with Pathogen Inoculation Alone

1. VC + P
2. VC + FYM + P
3. VC + NC + P
4. FYM + NC + P

3.4.1.6 Control without Pathogen Inoculation

1. VC
2. VC + FYM
3. VC + NC
4. FYM + NC

Three month old rooted cuttings of pepper var. Karimunda were initially raised in solarized potting mixture of soil, cow dung and sand in the ratio of 3:2:1. The solarization of moistened potting mixture (1.5 kg each) was done in sealed polythene bags of 100 gauge thickness by exposure to sunlight for 14 days. The bags were overturned once in two days for maximum heat conduction during solarization. Biocontrol agents were incorporated in the form of enriched carrier material @ 50 g/kg potting mixture at the time of replanting the rooted cuttings of pepper in solarized potting mixture. The pathogen inoculation was done 15 days after the application of bio control agents. For this, the inoculum was raised aseptically in sand - oat meal mixture in the ratio of 9:1(v/v) and applied @10 g/kg potting mixture after raking the soil around the plants. In addition, the plants were sprayed with an aqueous suspension of ground mycelium of the pathogen. Small bits of the seven day old mycelium grown on oat meal agar medium were placed in the collar region of each inoculated plant after giving mild pinpricks and then covered with moistened cotton to initiate stem infection. In plants treated with copper oxychloride, soil drenching (@ 2.5 l m^{-2}) and spraying with 0.3 per cent COC was done along with pathogen inoculation. The control plants were also treated in a similar manner except for pathogen inoculation. All the plants were maintained under high humidity by regular irrigation, sprinkling with water

and covering with polythene bags. Observations were taken seven and fourteen days after pathogen inoculation.

3.4.2 Observation - Seven Days after Pathogen Inoculation

The data on total number of leaves, number of infected leaves, plant height and lesion length were taken by standard procedures.

3.4.2.1 Foliar Infection

Percentage of foliar infection was calculated by using the formula

$$\text{Percentage of foliar infection} = \frac{\text{Number of infected leaves}}{\text{Total number of leaves}} \times 100$$

3.4.2.2 Disease Index

The intensity of foliar infection was calculated by using the score chart of Mayee and Datar (1986). The individual leaves in each plant were scored by assigning scores of 0-4 for infection (Plate 1) as indicated below.

0 - no infection

1 - Lesion covering up to 25 per cent of leaf area

2 - Lesion covering 26 - 50 per cent of leaf area

3 - Lesion covering 51 - 75 per cent of leaf area

4 - Lesion covering > 75 per cent of leaf area

The disease index was calculated using the formula of Mayee and Datar (1986)

$$\text{Disease Index (DI)} = \frac{\text{Sum of grades of each leaf}}{\text{Total number of leaves assessed X Maximum grade}} \times 100$$

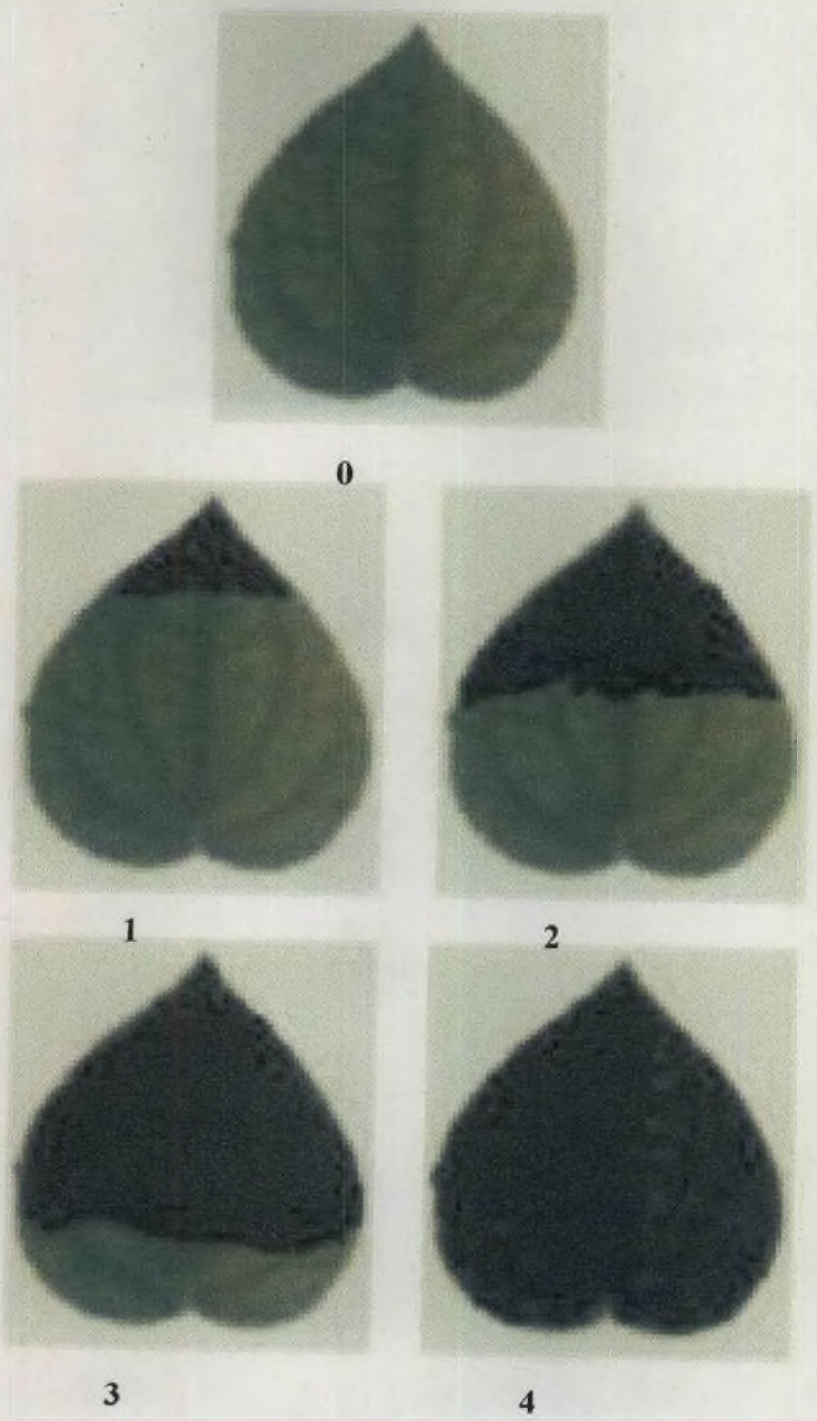


Plate 1. Score chart for foliar infection by *P. capsici*

3.4.2.3 Stem Infection

The percentage of stem infection was calculated as follows.

$$\text{Percentage of stem infection} = \frac{\text{Lesion length (cm)}}{\text{Plant height (cm)}} \times 100$$

3.4.3 Observation - Fourteen Days after Pathogen Inoculation

In addition to above observations, the following observations were also recorded 14 days after inoculation (DAI) of the pathogen.

3.4.3.1 Mortality

$$\text{Percentage of mortality of plants} = \frac{\text{Number of plants killed in each treatment}}{\text{Total number of plants in each treatment}} \times 100$$

3.4.3.2 Fresh Weight of Shoot

The fresh weight of shoot (g) was taken in an electronic single pan balance immediately after depotting the plants.

3.4.3.3 Dry Weight of Shoot

The dry weight of shoot (g) was taken after drying the samples to a constant weight at 60°C in a drying oven.

3.4.3.4 Fresh Weight of Roots

The fresh weight of roots (g) was taken in an electronic single pan balance immediately after depotting the plants.

3.4.3.5 Dry Weight of Roots

The dry weight of roots (g) was taken after drying root samples to a constant weight at 60°C in a drying oven.

3.5 ESTIMATION OF PHENOLS AND DEFENCE RELATED ENZYMES

The best combination of carrier material (VC + NC) and biocontrol agents with respect to plant growth and disease control was used for this study. The raising of rooted cuttings of pepper var. Karimunda in polybags, application of biocontrol agents, soil drenching and spraying with copper oxychloride and inoculation with *P. capsici* were done as per the methods described earlier. Samples were collected one, five and ten days after inoculation of the pathogen.

Design : CRD

Treatments : 8

Replication : 3x3

3.5.1 Treatment Combinations

1. VC + NC + T
2. VC + NC + T + P
3. VC + NC + G
4. VC + NC + G + P
5. VC + NC + COC
6. VC + NC + COC + P
7. VC + NC
8. VC + NC + P

3.5.2 Estimation of Total Phenol (Bray and Thorpe, 1954)

One gram each of healthy and infected leaf sample was ground in 10 ml of 80 per cent ethanol using a pestle and mortar. The homogenate was centrifuged (Hettich, EBA 12/12 R) at 10,000 rpm for 20 minutes. The supernatant was saved and the residue was re-extracted with five times the volume of 80 per cent ethanol and re-centrifuged. The supernatant was collected and pooled together before evaporating to dryness. The resulting residue was dissolved in five ml of distilled water. An aliquot of 0.1 ml of this sample was pipetted into a test tube and the volume was made up to three ml with distilled water. Folin-Ciocalteau reagent

(0.5 ml) and two ml of 20 per cent sodium carbonate solution were added to each tube after three minutes. The reaction mixture was thoroughly mixed and kept in boiling water for one minute. After cooling, the absorbance was measured at 650 nm against a reagent blank using a UV-VIS spectrophotometer (Systronics UV-VIS spectrophotometer 118). A standard curve was prepared using different concentrations of catechol (10, 20, 30, 40, 50, 60 and 70 µg) and phenol content was expressed as catechol equivalent per gram fresh weight of leaf.

3.5.3 Estimation of Ortho Dihydroxy Phenol (Johnson and Schaal, 1957)

Three grams each of healthy and infected leaf sample were boiled for five minutes in 12 ml of 80 per cent ethanol. After cooling, the tissue was ground using a pestle and mortar and the homogenate was centrifuged (Hettich, EBA 12/12 R) at 10,000 rpm for 20 minutes. The supernatant was saved and the residue was re extracted with 80 per cent ethanol and centrifuged again. The supernatant was collected, pooled and the final volume was adjusted to 15 ml with 80 per cent ethanol. One ml of 0.5 N HCl, one ml of Arnon's reagent (Appendix II.D) and two ml of IN NaOH were added to one ml of the extract and the volume was made up to 25 ml with 80 per cent ethanol. The absorbance was measured at 540 nm in a UV-VIS spectrophotometer (Systronics UV-VIS Spectrophotometer 118). A standard curve was prepared using different concentrations of catechol (30, 40, 50, 60, 70 and 80 µg) and ortho dihydroxy phenol content was expressed as catechol equivalents per gram fresh weight of leaf.

3.5.4 Estimation of Peroxidase Activity (Srivastava, 1987)

Two hundred mg each of healthy and infected leaf samples were initially homogenised in one ml of 0.1 M sodium phosphate buffer (pH-6.5) (Appendix.II.B) to which a pinch of polyvinyl pyrrolidone (PVP) was added. The homogenization was done at 4°C using a precooled mortar and pestle. The homogenate was filtered through muslin cloth and then centrifuged (Hettich, EBA

12/12 R) at 5000 rpm for 15 minutes at 4°C. The supernatant was used as enzyme extract for the assay of peroxidase activity.

The reaction mixture consisting of one ml of 0.05M pyrogallol and 50 µl of enzyme extract was taken in both the reference as well as the sample cuvette of a UV-VIS spectrophotometer (Systronics UV-VIS spectrophotometer 118). The enzyme reaction was started by adding one ml of one percent hydrogen peroxide into the sample cuvette and change in absorbance was measured at 420 nm at 30 seconds interval up to 180 seconds. Three replications were maintained for each sample. Peroxidase activity was expressed as change in absorbance per minute per gram fresh weight of leaf.

3.5.5 Estimation of Polyphenol Oxidase Activity (Mayer et al., 1965)

The enzyme extract from both healthy and infected leaves was prepared as per the procedure described under 3.5.4. The reaction mixture contained one ml of 0.1M sodium phosphate buffer (pH 6.5) and 50 µl of enzyme extract. The reaction was initiated by adding one ml of 0.01M catechol and change in absorbance was measured in a UV-VIS spectrophotometer (Systronics UV-VIS spectrophotometer 118) at 495 nm. Polyphenol oxidase activity was expressed as change in absorbance of the reaction mixture per minute per gram fresh weight of leaf.

3.5.6 Estimation of Phenylalanine Ammonia Lyase (PAL) Activity (Dickerson *et al.*, 1984)

The enzyme extract was prepared by homogenizing one gram each of healthy and infected leaf samples in five ml of 0.1M sodium borate buffer (pH 8.8) containing a pinch of PVP using a chilled pestle and mortar. The homogenate was centrifuged (Hettich, EBA 12/12 R) at 10,000 rpm for 10 minutes at 4°C. The supernatant was used for the assay of PAL activity. The reaction mixture contained three ml of 0.1M sodium borate buffer (pH 8.8) (Appendix II.C) 0.2 ml enzyme extract and 0.1 ml of 12 Mm L-phenylalanine prepared in the same buffer.

The blank contained three ml of 0.1M sodium borate buffer (pH 8.8) and 0.2 ml enzyme extract. The reaction mixtures were incubated at 40°C for 30 minutes. It was stopped by adding 0.2 ml of 3N hydrochloric acid (HCl) and the absorbance was read at 290 nm in a UV-VIS spectrophotometer (Systronics UV-VIS spectrophotometer 118). A standard curve was prepared using different concentrations of cinnamic acid (10, 20, 30, 40, 50 and 60 µg) and PAL activity was expressed as microgram of cinnamic acid produced per minute per gram fresh weight of leaf.

3.6 PROTEIN PROFILE OF HEALTHY AND INFECTED LEAVES (Laemelli, 1970)

3.6.1 Sample Preparation

Five hundred milligram each of healthy and infected leaf samples from treatment combinations described under 3.5.1 were homogenized in 200 µl of cold denaturing solution (Appendix II E) at 4°C to which a pinch of PVP was added. The homogenate was centrifuged (Hettich, EBA 12/12R) at 14,000 rpm for 5 minutes at 4°C. The supernatant was mixed with equal volume of sample buffer and boiled at 95°C for four minutes in a water bath to ensure complete reaction between proteins and sodium dodecyl sulphate. These samples were used for sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE). Samples collected one day after inoculation of pathogen alone were used for the study.

3.6.2 Protein Markers

Protein molecular weight markers (Bangalore Genei Pvt. Ltd.) comprising of Insulin, α and β chains (3 k Da), Aprotinin (6.5 k Da), Lysozyme (14.3 k Da), Soyabean trypsin Inhibitor (20.1 k Da), Carbonic anhydrase (29.0 k Da), Ovalbumin (43 k Da), Bovine serum albumin (66 k Da), Phosphorylase b (97.4 k Da) and myosin (rabbit muscle) (205 k Da), were used along with different samples for identification of PR proteins.

3.6.3 Reagents

3.6.3.1 Acrylamide Stock (30%)

Acrylamide	-	29.2	g
Bis - acrylamide	-	0.8	g
Double distilled water	-	100.0	ml

3.6.3.2 Separating (Resolving) Gel Buffer Stock

1.5 M Tris - HCl pH8.8.

Tris - base (18.15 g) was dissolved in approximately 50 ml of double distilled water. The pH was adjusted to 8.8 with 6N HCl and the volume was made upto 100 ml with double distilled water and stored at 4°C.

3.6.3.3 Stacking Gel Buffer Stock

0.5M Tris - HCl pH 6.8

Tris-base (6.0 g) was dissolved in approximately 60 ml of double distilled water and adjusted the pH to 6.8 with 6N HCl. The volume was made upto 100 ml with double distilled water and stored at 4°C

3.6.3.4 Polymerising Agents

Ammonium persulphate (APS) 10 per cent (freshly prepared before use) and N, N, N', N' – tetramethyl ethylenediamine (TEMED) (fresh from the refrigerator) were used.

3.6.3.5 Electrode (Running) Buffer pH 8.3

Tris-base	-	3.0	g
Glycine	-	14.4	g
SDS	-	1.0	g
Double distilled water	-	1.0	l

3.6.3.6 Sample Buffer (SDS-reducing buffer)

Double distilled water	-	2.6 ml
0.5 M Tris-HCl (pH 6.8)	-	1.0 ml
2- Mercaptoethanol	-	0.8 ml
Glycerol	-	1.6 ml
SDS 20% (w/v)	-	1.6 ml
Bromo phenol blue (0.5%)	-	0.4 ml

3.6.3.7 Staining Solution

Coomassie brilliant blue R 250	-	0.1 g
Methanol	-	40.0 ml
Acetic acid	-	10.0 ml
Double distilled water	-	50.0 ml

3.6.3.8 Destaining Solution

Methanol	-	40.0 ml
Acetic acid	-	10.0 ml
Double distilled water	-	50.0 ml

3.6.4 Procedure for SDS - PAGE

The separating gel was first casted followed by stacking gel by mixing various solutions as indicated below.

3.6.4.1 Preparation of Separating Gel (12%)

Double distilled water	-	6.7 ml
1.5 M Tris-HCl, pH 8.8	-	5.0 ml
SDS 10%	-	0.2 ml
Acrylamide stock	-	8.0 ml

The above solution was mixed well and degassed for three minutes before the following were added.

APS 10% (freshly prepared)	-	0.10 ml
TEMED	-	0.01 ml

The separating gel was mixed well and poured immediately between glass plates. Water was layered over the polymerizing solution to quicken the polymerization process.

3.6.4.2 Preparation of Stacking Gel (4%)

Double distilled water	-	6.1 ml
0.5 M Tris-HCl, pH 6.8	-	2.5 ml
SDS 10%	-	0.1 ml
Acrylamide stock	-	1.3 ml

The solution was mixed well, degassed and the following were added.

APS 10 % (freshly prepared)	-	0.05 ml
TEMED	-	0.01 ml

The water layered over the separating gel was removed and washed with little electrode buffer. Stacking gel was poured over the polymerized separating gel, after keeping the comb in position.

3.6.5 Electrophoretic Separation of Samples

After polymerisation, the samples were loaded into each well. The protein concentration was adjusted in each sample to a strength of 50 µg of protein estimated by Bradford method (1976). The electrophoresis was initially done at 100 V till the dye reached the separating gel. Then the voltage was increased to 200 V and continued till the dye reached the bottom of the gel. The gel was carefully separated and incubated in the staining solution for over night. It was then destained with gentle shaking using the destaining solution till the protein

bands became clear. The gel was transferred to a transparent polypropylene cover and then scanned by using a scanner with the help of a computer.

3.7 STATISTICAL ANALYSIS

The statistical analysis of the data was done by the methods described by Snedecor and Cochran (1967).

RESULTS

4. RESULTS

4.1 PATHOGEN

The pathogen causing foot rot of pepper was isolated during the South West monsoon season from naturally infected pepper plants and maintained on carrot agar medium. On this medium, the isolate produced petalloid type colonies which covered a petridish of 9 cm diameter in five to seven days. The width of the mycelium was in the range of 3.3 to 5.8 µm while the size of individual sporangium varied from 29.7 to 52.8 x 16.5 to 23.1 µm. The sporangia were either papillate or spherical to irregular in shape (Plate 2). Based on the above characters which were compared with the type culture available in the Department of Plant Pathology, College of Agriculture, Vellayani and ability of the isolate to produce typical symptoms of foot rot disease in pepper plants, the pathogen was identified as *Phytophthora capsici* Leonian.

The pathogenicity of *P. capsici* was tested in three month old rooted cuttings of pepper var. Karimunda. On artificial inoculation of the leaf, pale water soaked lesions appeared within 48 h which later turned dark brown to black in colour (Plate 3a). These lesions with fimbriate margins gradually coalesced covering large area of the leaf resulting in defoliation. Similarly, in the collar region of the stem where the isolate was inoculated, the initial symptom appeared as a water soaked lesion within 72 hours. It subsequently turned dark brown to black colour as the infection progressed (Plate 3b). Finally the entire plant was defoliated and completely dried by about three weeks. The pathogen was reisolated from both the infected leaf and stem. The culture was mass produced either on oat meal agar medium or on sand oat meal mixture for further studies.

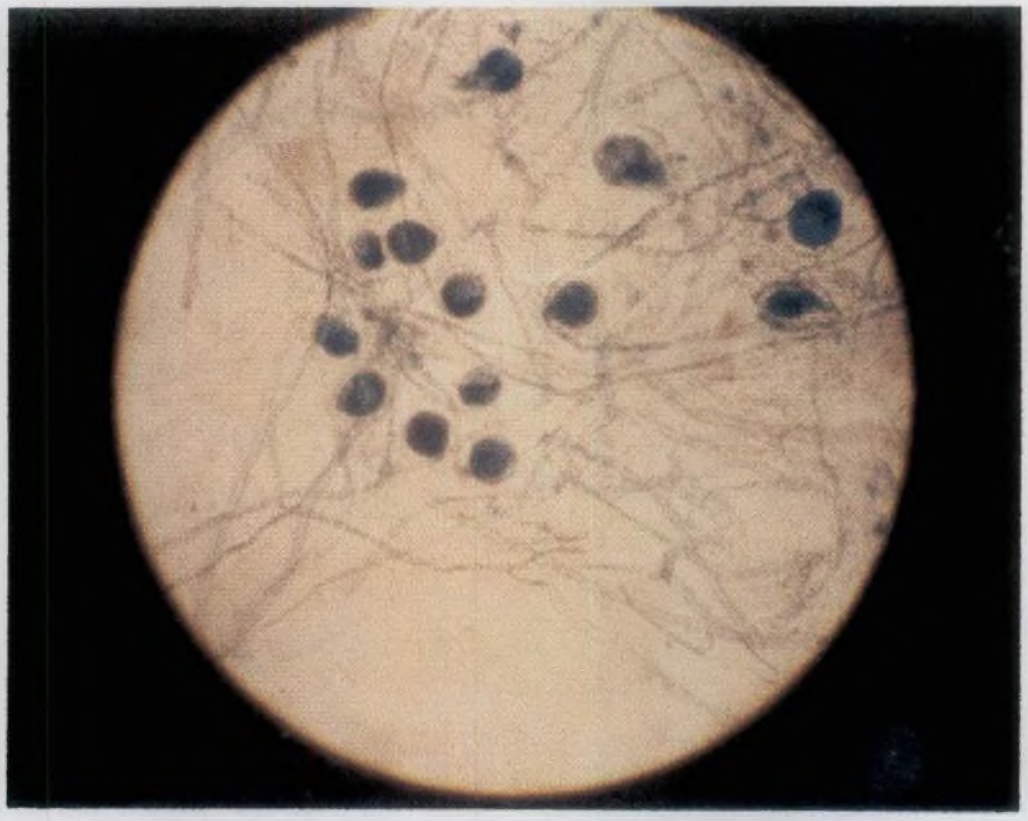
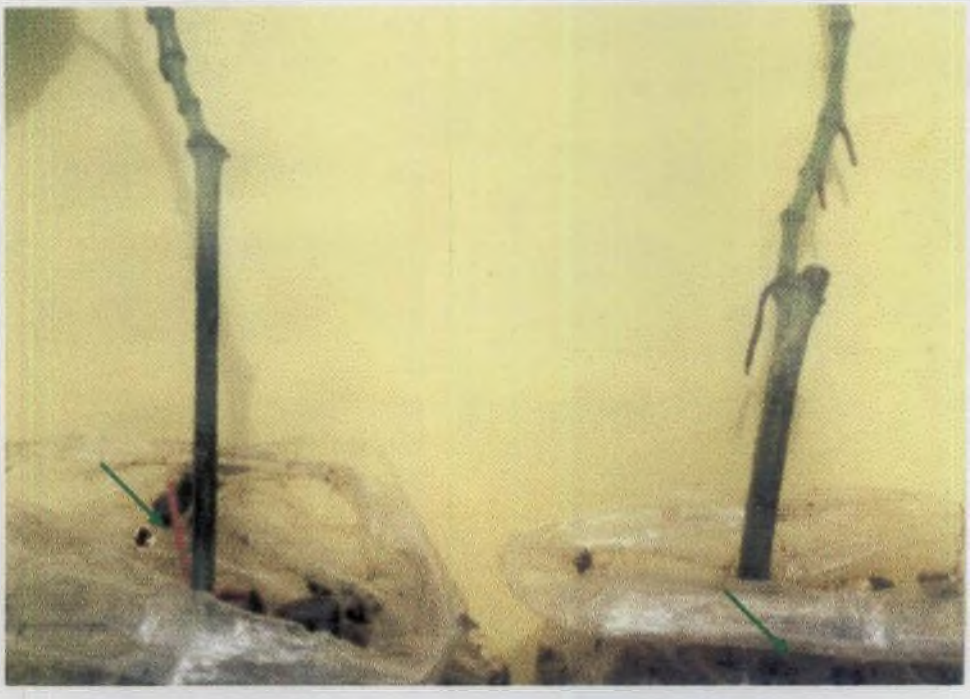


Plate 2. Sporangia of *Phytophthora capsici*

A. Foliar infection



B. Stem infection

Plate 3. Symptoms of foot rot of pepper var. Karimunda after inoculation with *P. capsici*

4.2 BIOCONTROL AGENTS

The two biocontrol agents, *Trichoderma harzianum* and *Glomus fasciculatum* selected for the present investigation were mass produced in different combinations of vermicompost such as vermicompost alone (VC), vermicompost + farmyard manure (VC + FYM), vermicompost + neem cake (VC + NC) and farmyard manure + neem cake (FYM + NC).

4.2.1 *Trichoderma harzianum*

There were significant differences between treatments in the population of *T. harzianum* in various carrier materials. The number of colony forming units estimated 15 DAI were maximum in the treatment combination of VC + NC. These were 72.87 x 10^6 when compared to 1.49 x 10^6 in vermicompost alone (Table 1, Fig. 1, Plate 4). In other combination of carrier materials such as VC + FYM and FYM + NC, the population of this biocontrol agent varied from 4.29 to 21.27 x 10^6 respectively.

4.2.2 *Glomus fasciculatum*

Root samples of guinea grass grown in different carrier materials for 50 days were examined for colonization by arbuscular mycorrhizal fungi. There was no significant difference between treatments in the percentage of mycorrhizal infection. The percentage infection ranged form 39.98 (FYM + NC) to 37.99 (VC alone) (Table 2, Fig. 2).

4.3 USE OF MYCOINOCULANT ENRICHED VERMICOMPOST FOR MANAGEMENT OF FOOT ROT OF PEPPER

4.3.1 Seven Days After Pathogen Inoculation

4.3.1.1 Total Number of Leaves

The influence of different carrier materials and control measures on the total number of leaves formed in pepper var. Karimunda after inoculation with *P. capsici* was not significant (Table 3). The treatment mean for different carrier materials varied from 5.83 (FYM + NC) to 7.88

Table 1. Population of *Trichoderma harzianum** (15 DAI) in different carrier materials

Carrier materials	Number of cfu g^{-1} (x 10^6)
VC alone	1.49
	(1.58)
VC + FYM (1 : 1)	4.29
	(2.30)
VC + NC (5 : 1)	72.87
	(8.59)
FYM + NC (10 : 1)	21.27
	(4.72)
CD (0.05)	0.749

*Values in parenthesis after square root transformation

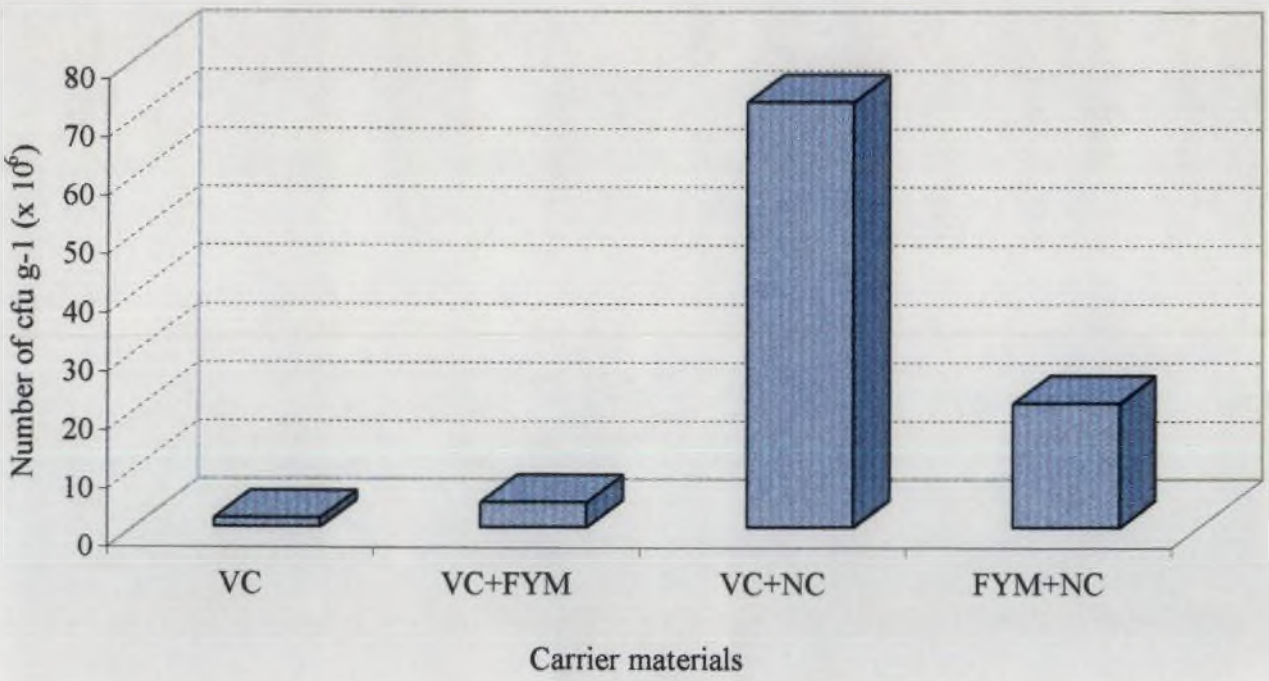


FIg. 1 Population of Trichoderma harzianum (15 DAI) in different carrier materials

Plate 4. Growth of *Trichoderma harzianum* in Rose bengal agar

Table 2. Percentage of mycorrhizal infection* (50 DAI) in different carrier materials

Carrier materials	Percentage of mycorrhizal infection
VC alone	37.99 (6.24)
VC + FYM (1 : 1)	38.95 (6.32)
VC + NC (5 : 1)	39.93 (6.40)
FYM + NC (10 : 1)	39.98 (6.40)
CD (0.05)	NS

*Values in parenthesis after square root transformation

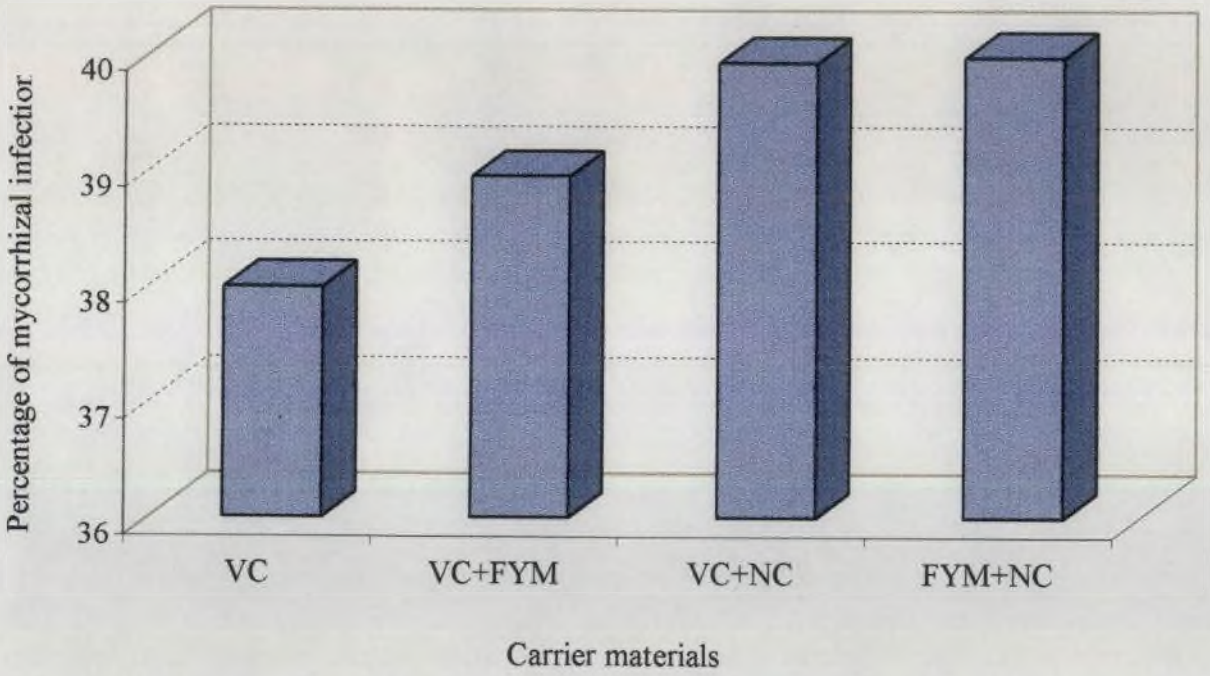


Fig. 2 Percentage of mycorrhizal infection (50 DAI) in different carrier materials

Table 3 Influence of vermicompost based mycoinoculants on total number of leaves in pepper var. Karimunda 7 DAI with *P. capsici*

Treatments (A)	Carrier materials (B)				Mean-A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	8.75	6.00	7.50	6.75	7.25
G. fasciculatum	10.00	8.25	8.75	4.75	7.94
T. harzianum + *G. fasciculatum*	6.00	6.25	7.00	6.00	6.31
COC (0.3 %)	7.50	7.25	7.50	7.00	7.31
Control (pathogen alone)	4.50	3.75	4.50	5.75	4.63
Control (without pathogen)	10.50	7.25	5.00	4.75	6.88
Mean - B	7.88	6.46	6.71	5.83	

CD (0.05)-A NS; CD-B NS; CD-AB NS

(VC alone) and for the biocontrol agents this varied from 6.31 (*T. harzianum* + *G. fasciculatum*) to 7.94 (*G. fasciculatum*). The average number of leaves in the control treatments with and without pathogen inoculation were 4.63 and 6.88 respectively.

4.3.1.2 Number of Infected Leaves

There was significant reduction in the number of infected leaves in treatments with biocontrol agents and copper oxychloride. The average number of infected leaves was only 0.06 after treatment with 0.3 per cent copper oxychloride (Table 4). Similarly with different biocontrol agents the number of infected leaves were 0.10, 0.10 and 0.21 with *G. fasciculatum*, *T. harzianum* + *G. fasciculatum* and *T. harzianum* respectively as compared to 1.92 in the control treatment with pathogen inoculation. The effect of different biocontrol agents was statistically on par with the chemical treatment. In the control treatment without pathogen inoculation, there was no leaf infection. The carrier materials as such had no significant effect in reducing leaf infection by *P. capsici*. Further, the interaction between different control measures and carrier materials was not significant.

4.3.1.3 Percentage of Foliar Infection

A significant reduction in the percentage of foliar infection was observed after the use of the biocontrol agents and copper oxychloride. The percentage of foliar infection was minimum (0.04) in the treatment with 0.3 per cent copper oxychloride (Table 5). The effect of different biocontrol agents was also significant and statistically on par with that of chemical control. Thus, in treatments with *T. harzianum* + *G. fasciculatum*, *G. fasciculatum* and *T. harzianum*, the percentage of foliar infection was only 0.18, 0.23 and 0.96 respectively as compared to 60.9 per cent in the control treatment with pathogen inoculation. The interaction between different control measures and carrier materials was not significant.

Table 4 Influence of vermicompost based mycoinoculants on number of infected leaves* in pepper var. Karimunda 7 DAI with *P. capsici*

Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	0.99 (1.41)	0 (1.00)	0 (1.00)	0 (1.00)	0.21 (1.10)
G. fasciculatum	0 (1.00)	0.22 (1.10)	0 (1.00)	0.22 (1.10)	0.10 (1.05)
T. harzianum + *G. fasciculatum*	0 (1.00)	0 (1.00)	0 (1.00)	0.40 (1.18)	0.10 (1.05)
COC (0.3 %)	0 (1.00)	0 (1.00)	0.22 (1.10)	0 (1.00)	0.06 (1.03)
Control (pathogen alone)	2.41 (1.85)	2.45 (1.86)	1.55 (1.60)	1.40 (1.55)	1.92 (1.71)
Control (without pathogen)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)
Mean - B	0.46 (1.21)	0.35 (1.16)	0.25 (1.12)	0.30 (1.14)	

*Values in parenthesis after $\sqrt{x + 1}$ transformation

CD (0.05) – A 0.179; CD-B NS; CD-AB NS

Table 5 Influence of vermicompost based mycoinoculants on percentage of foliar infection* in pepper var. Karimunda 7 DAI with *P. capsici*

Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	14.66 (22.50)	0 (0.00)	0 (0.00)	0 (0.00)	0.96 (5.63)
G. fasciculatum	0 (0.00)	0.72 (4.87)	0 (0.00)	1.10 (6.02)	0.23 (2.72)
T. harzianum + *G. fasciculatum*	0 (0.00)	0 (0.00)	0 (0.00)	2.90 (9.80)	0.18 (2.45)
COC (0.3 %)	0 (0.00)	0 (0.00)	0.65 (4.61)	0 (0.00)	0.04 (1.15)
Control (pathogen alone)	77.79 (61.86)	85.38 (67.49)	44.33 (41.73)	31.29 (33.99)	60.9 (51.27)
Control (without pathogen)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Mean - B	5.9 (14.06)	4.4 (12.06)	1.8 (7.72)	2.1 (8.30)	

*Values in parenthesis after angular transformation

CD (0.05) – A 11.897; CD-B NS; CD-AB NS

4.3.1.4 Disease Index

Significant reduction in the disease index was obtained with biocontrol agents and copper oxychloride. In plants treated with 0.3 per cent copper oxychloride, the mean disease index was only 0.01 (Table 6). In treatments with *T. harzianum* + *G. fasciculatum*, *G. fasciculatum* and *T. harzianum*, the disease index was 0.04, 0.10 and 0.96 respectively and these were on par with that of chemical control. Maximum disease index of 55.0 was in the control treatment with pathogen inoculation. The interaction between different control measures and carrier materials was however not significant.

4.3.1.5 Plant Height

There was significant increase in plant height in treatments with different control measures when compared to the control treatment with pathogen inoculation. The average plant height of 52.66 cm was maximum after treatment with 0.3 per cent copper oxychloride. At the same time, in the control treatment this was only 30.13 cm (Table 7). In treatments with biocontrol agents also, the plant heights were more and these were 45.69, 45.00 and 42.89 cm respectively with *G. fasciculatum*, *T. harzianum* + *G. fasciculatum* and *T. harzianum*. In the control treatment without pathogen inoculation the plant height was 39.81 cm. However, the interaction between different control measures and carrier materials was not significant.

4.3.1.6 Lesion Length

A significant reduction in lesion length due to infection by *P. capsici* in the collar region of pepper var. Karimunda was observed in treatments with different control measures. This reduction was maximum with 0.3 per cent copper oxychloride where the lesion length was only 0.39 cm as compared to 10.70 cm in the control treatment with pathogen inoculation (Table 8). In treatments with different biocontrol agents also the lesion

Table 6 Influence of vermicompost based mycoinoculants on disease index* in pepper var. Karimunda 7 DAI with *P. capsici*

Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	14.66 (22.50)	0 (0.00)	0 (0.00)	0 (0.00)	0.96 (5.63)
G. fasciculatum	0 (0.00)	0.53 (4.19)	0 (0.00)	0.26 (2.94)	0.10 (1.78)
T. harzianum + *G. fasciculatum*	0 (0.00)	0 (0.00)	0 (0.00)	0.65 (4.61)	0.04 (1.15)
COC (0.3 %)	0 (0.00)	0 (0.00)	0.16 (2.27)	0 (0.00)	0.01 (0.57)
Control (pathogen alone)	70.67 (57.19)	75.01 (59.98)	44.33 (41.73)	28.94 (32.53)	55.0 (47.86)
Control (without pathogen)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Mean - B	5.3 (13.28)	3.1 (10.70)	1.6 (7.33)	1.4 (6.68)	

*Values in parenthesis after angular transformation

CD (0.05) – A 11.597; CD-B NS; CD-AB NS

Table 7 Influence of vermicompost based mycoinoculants on plant height (cm) in pepper var. Karimunda 7 DAI with *P. capsici*

Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	36.00	36.83	50.38	48.38	42.89
G. fasciculatum	47.00	45.25	42.63	47.88	45.69
T. harzianum + *G. fasciculatum*	40.13	44.25	47.38	48.25	45.00
COC (0.3 %)	54.88	40.13	59.38	56.25	52.66
Control (pathogen alone)	30.25	30.38	29.75	30.13	30.13
Control (without pathogen)	56.25	39.13	34.63	29.25	39.81
Mean - B	44.08	39.33	44.02	43.35	

CD (0.05) – A 10.875; CD-B NS; CD-AB NS

Table 8 Influence of vermicompost based mycoinoculants on lesion length (cm)* in pepper var. Karimunda 7 DAI with *P. capsici*

Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	4.37 (2.32)	0.66 (1.29)	0.34 (1.16)	0.50 (1.22)	1.25 (1.50)
G. fasciculatum	0.82 (1.35)	2.14 (1.77)	0.62 (1.27)	2.28 (1.81)	1.40 (1.55)
T. harzianum + *G. fasciculatum*	1.30 (1.52)	0.69 (1.30)	0.63 (1.28)	1.10 (1.45)	0.93 (1.39)
COC (0.3 %)	0.46 (1.21)	0.25 (1.12)	0.47 (1.21)	0.37 (1.17)	0.39 (1.18)
Control (pathogen alone)	9.61 (3.26)	15.92 (4.11)	10.38 (3.37)	7.54 (2.92)	10.70 (3.42)
Control (without pathogen)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)
Mean - B	2.17 (1.78)	2.13 (1.77)	1.40 (1.55)	1.56 (1.60)	

*Values in parenthesis after $\sqrt{x + 1}$ transformation

CD (0.05) – A 0.572; CD-B NS; CD-AB NS

lengths were less and these were 0.93, 1.25 and 1.40 respectively with *T. harzianum* + *G. fasciculatum*, *T. harzianum* and *G. fasciculatum*. The effect of *T. harzianum* + *G. fasciculatum* in reducing the lesion length was also statistically on par with chemical control. However, the interaction between different control measures and carrier materials was not significant.

4.3.1.7 Percentage of Stem Infection

The percentage of stem infection was significantly reduced in treatments with biocontrol agents and copper oxychloride. It was least (0.81 per cent) after treatment with 0.3 per cent copper oxychloride when compared to 40.2 per cent in the control treatment with pathogen inoculation (Table 9). The effect of different biocontrol agents was also statistically on par with the chemical treatment. The average percentage of stem infection in treatments with *T. harzianum* + *G. fasciculatum*, *G. fasciculatum* and *T. harzianum* was 2.10, 2.80 and 2.90 respectively. But the interaction between different control measures and carrier materials was not significant.

4.3.2 Fourteen Days After Pathogen Inoculation

4.3.2.1 Number of Infected Leaves

A significant reduction in the number of infected leaves in treatments with biocontrol agents and copper oxychloride was observed 14 days after inoculation with *P. capsici*. The average number of infected leaves was only 0.10 after treatment with 0.3 per cent copper oxychloride (Table 10). Similarly, with different biocontrol agents, the number of infected leaves were 0.28, 0.49 and 0.80 respectively with *T. harzianum*, *G. fasciculatum* and *T. harzianum* + *G. fasciculatum* as compared to 3.20 in control treatment with pathogen inoculation. Besides, the treatment effect of *T. harzianum* was statistically on par with the chemical treatment. In the control treatment without pathogen inoculation there was no leaf

Table 9 Influence of vermicompost based mycoinoculants on percentage of stem infection* in pepper var. Karimunda 7 DAI with *P. capsici*

Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	13.06 (21.18)	1.80 (7.72)	0.60 (4.45)	0.94 (5.55)	2.90 (9.72)
G. fasciculatum	1.74 (7.57)	3.59 (10.91)	1.24 (6.38)	5.45 (13.49)	2.80 (9.59)
T. harzianum + *G. fasciculatum*	3.09 (10.12)	1.71 (7.51)	1.19 (6.25)	2.58 (9.23)	2.10 (8.28)
COC (0.3 %)	0.99 (5.72)	0.66 (4.66)	0.92 (5.50)	0.69 (4.75)	0.81 (5.16)
Control (pathogen alone)	38.34 (38.24)	55.06 (47.88)	42.60 (40.73)	25.86 (30.55)	40.2 (39.35)
Control (without pathogen)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Mean - B	5.7 (13.81)	5.2 (13.12)	3.3 (10.55)	3.4 (10.60)	

*Values in parenthesis after angular transformation

CD (0.05) – A 7.985; CD-B NS; CD-AB NS

Table 10 Influence of vermicompost based mycoinoculants on number of infected leaves* in pepper var. Karimunda 14 DAI with *P. capsici*

Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	1.30 (1.52)	0 (1.00)	0 (1.00)	0 (1.00)	0.28 (1.13)
G. fasciculatum	0 (1.00)	1.77 (1.66)	0 (1.00)	0.46 (1.21)	0.49 (1.22)
T. harzianum + *G. fasciculatum*	1.30 (1.52)	0.46 (1.21)	1.05 (1.43)	0.40 (1.18)	0.80 (1.34)
COC (0.3 %)	0 (1.00)	0.22 (1.10)	0.22 (1.10)	0 (1.00)	0.10 (1.05)
Control (pathogen alone)	2.41 (1.85)	3.64 (2.15)	3.49 (2.12)	3.39 (2.09)	3.20 (2.05)
Control (without pathogen)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)
Mean - B	0.72 (1.31)	0.85 (1.36)	0.64 (1.28)	0.56 (1.25)	

*Values in parenthesis after $\sqrt{x + 1}$ transformation

CD (0.05) – A 0.246; CD-B NS; CD-AB NS

infection. The interaction between different control measures and carrier materials was not significant.

4.3.2.2 Percentage of Foliar Infection

A significant reduction in the percentage of foliar infection was obtained after the use of both the biocontrol agents as well as copper oxychloride. The percentage of foliar infection of 0.22 was minimum in chemical control treatment (Table 11, Fig. 3). The effect of different biocontrol agents was also significant and statistically on par with the above treatment. Thus, in treatments with *T. harzianum*, *G. fasciculatum* and *T. harzianum* + *G. fasciculatum*, the percentage of foliar infection was only 1.4, 2.5 and 11.0 respectively as compared to 92.9 per cent in the control treatment with pathogen inoculation.

4.3.2.3 Disease Index

Significant reduction in disease index was obtained in treatments with different control measures. In plants treated with 0.3 per cent copper oxychloride, the mean disease index was only 0.05 (Table 12, Fig. 4, Plate 5-8). Similarly, in treatments with *T. harzianum, G. fasciculatum* and *T. harzianum* + *G. fasciculatum*, the disease index was 1.39, 1.68 and 8.72 respectively. The effect of different biocontrol agents was also statistically on par with that of chemical control. Maximum disease index of 89.54 was in the control treatment with pathogen inoculation.

4.3.2.4 Plant Height

There was significant increase in the plant height in treatments with different control measures. The average plant height of 54.59 cm was maximum after treatment with 0.3 per cent copper oxychloride compared to 30.22 cm in control treatment with pathogen (Table 13). In treatments with biocontrol agents also, the plant heights were more and these were 47.31, 46.91 and 44.69 cm respectively with *G. fasciculatum*, *T. harzianum* +

Table 11 Influence of vermicompost based mycoinoculants on percentage of foliar infection* in pepper var. Karimunda 14 DAI with *P. capsici*

Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	20.78 (27.11)	0 (0.00)	0 (0.00)	0 (0.00)	1.4 (6.78)
G. fasciculatum	0 (0.00)	17.79 (24.94)	0 (0.00)	3.77 (11.20)	2.5 (9.03)
T. harzianum + *G. fasciculatum*	12.07 (20.32)	21.59 (27.67)	11.20 (19.54)	2.90 (9.80)	11.0 (19.34)
COC (0.3 %)	0 (0.00)	1.10 (6.02)	0.65 (4.61)	0 (0.00)	0.22 (2.66)
Control (pathogen alone)	77.79 (61.86)	99.99 (90.00)	95.50 (77.72)	86.69 (68.58)	92.9 (74.54)
Control (without pathogen)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Mean - B	9.8 (18.21)	17.6 (24.77)	8.5 (16.98)	6.6 (14.93)	

*Values in parenthesis after angular transformation

CD (0.05) – A 13.638; CD-B NS; CD-AB NS

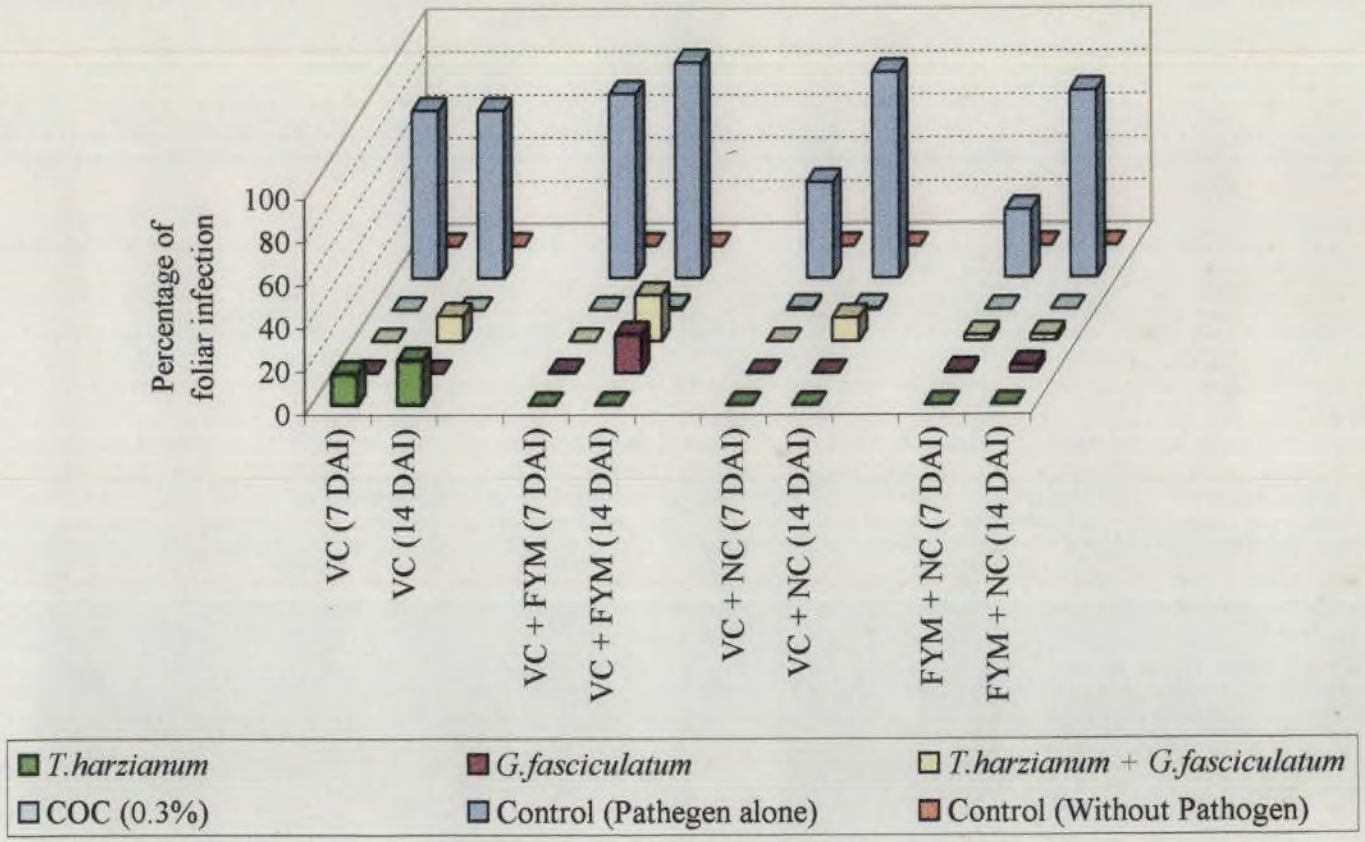


Fig. 3 Influence of vermicompost based mycoinoculants on percentage of foliar infection in pepper var. Karimunda at different days after inoculation with *P. capsici*

Table 12 Influence of vermicompost based mycoinoculants on disease index* in pepper var. Karimunda 14 DAI with *P. capsici*

Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	20.78 (27.11)	0 (0.00)	0 (0.00)	0 (0.00)	1.39 (6.78)
G. fasciculatum	0 (0.00)	16.90 (24.26)	0 (0.00)	0.92 (5.49)	1.68 (7.44)
T. harzianum + *G. fasciculatum*	8.47 (16.91)	21.59 (27.67)	11.20 (19.54)	0.65 (4.61)	8.72 (17.18)
COC (0.3 %)	0 (0.00)	0.26 (2.94)	0.16 (2.27)	0 (0.00)	0.05 (1.31)
Control (pathogen alone)	70.67 (57.19)	98.31 (82.49)	95.50 (77.72)	84.91 (67.11)	89.54 (71.13)
Control (without pathogen)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Mean - B	8.42 (16.87)	15.14 (22.90)	8.15 (16.59)	4.96 (12.87)	

*Values in parenthesis after angular transformation

CD (0.05) – A 13.748; CD-B NS; CD-AB NS

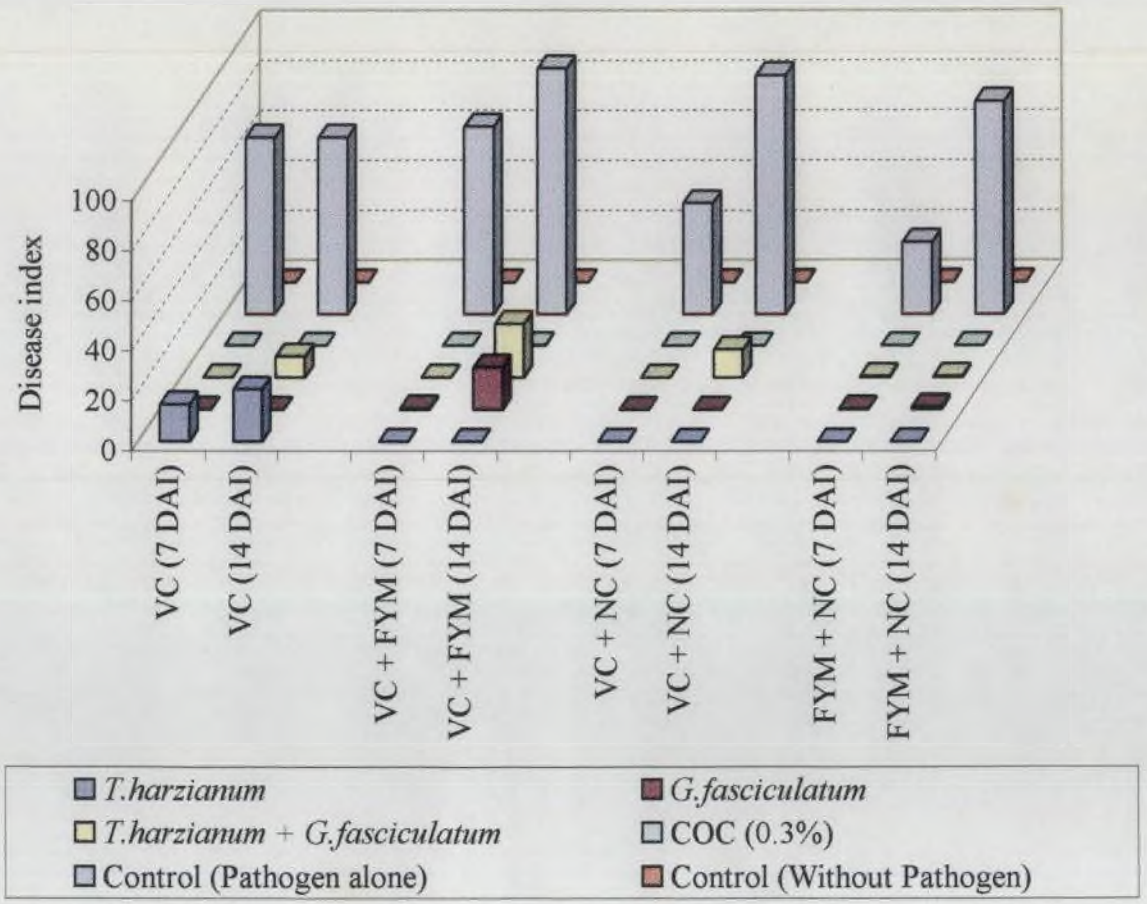
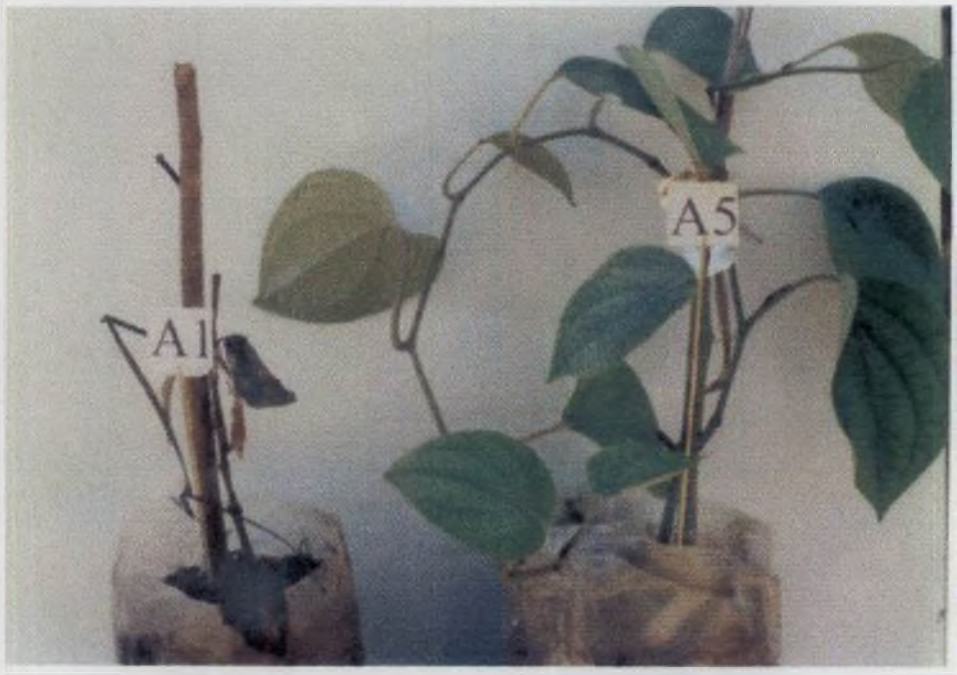


Fig. 4 Influence of vermicompost based mycoinoculants on disease index in pepper var. Karimunda at different days after inoculation with *P. capsici*

A. Control treatment

A – VC alone, A1 – VC + P



B. Treatment with 0.3 per cent COC

A1 – VC + P, A5 – VC + COC + P

Plate 5. Influence of VC based mycoinoculants on disease control in pepper var. Karimunda 14 DAI with *P. capsici*

C. Treatment with *T. harzianum*

A1 – VC + P, A2 – VC + T + P

D. Treatment with *G. fasciculatum*

A1 – VC + P, A3 – VC + G + P

E. Treatment with *T. harzianum* + *G. fasciculatum*

A1 – VC + P, A4 – VC + T + G + P

Plate 5. Continued

A. Control treatment

B – VC + FYM. B1 – VC + FYM + P

B. Treatment with 0.3 per cent COC

B1 – VC + FYM + P, B5 – VC + FYM + COC + P

Plate 6. Influence of VC + FYM based mycoinoculants on disease control in pepper var. Karimunda 14 DAI with *P. capsici*

C. Treatment with *T. harzianum*

B1 – VC + FYM + P,
B2 – VC + FYM + T + P

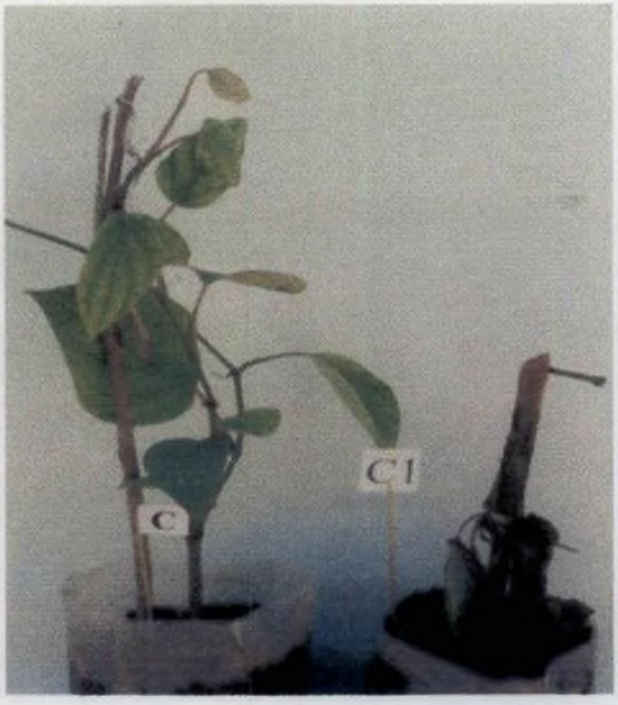
D. Treatment with *G. fasciculatum*

B1 – VC + FYM + P,
B3 – VC + FYM + G + P

E. Treatment with *T. harzianum* + *G. fasciculatum*

B1 – VC + FYM + P,
B4 – VC + FYM + T + G + P

Plate 6. Continued



A. Control treatment

C – VC + NC, C1 – VC + NC + P

B. Treatment with 0.3 per cent COC

C1 – VC + NC + P, C5 – VC + NC + COC + P

Plate 7. Influence of VC + NC based mycoinoculants on disease control in pepper var. Karimunda 14 DAI with *P. capsici*

C. Treatment with *T. harzianum*

C1 – VC + NC + P,
C2 – VC + NC + T + P

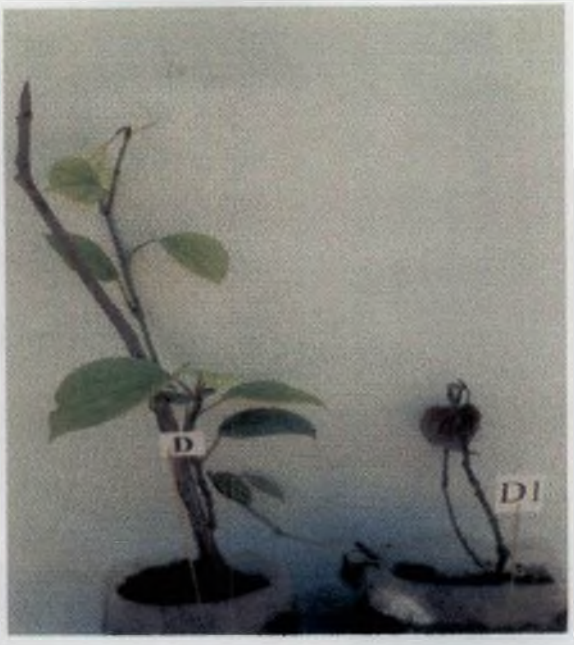
D. Treatment with *G. fasciculatum*

C1 – VC + NC + P,
C3 – VC + NC + G + P

E. Treatment with *T. harzianum* + *G. fasciculatum*

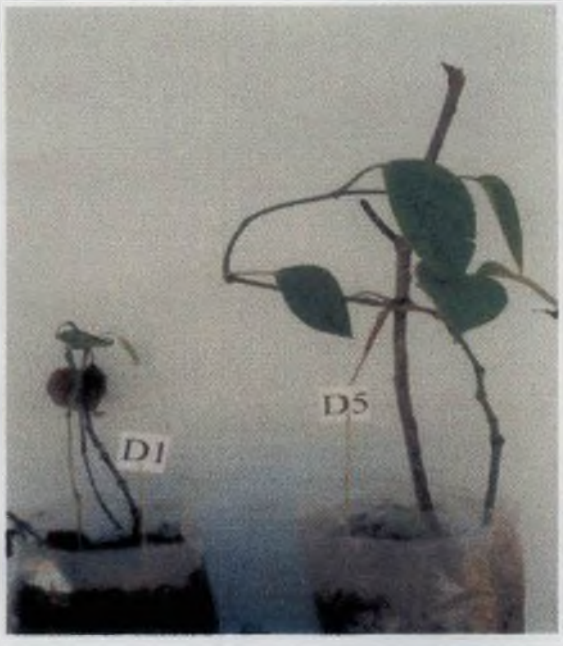
C1 – VC + NC + P,
C4 – VC + NC + T + G + P

Plate 7. Continued



A. Control treatment

D – FYM + NC, D1 – FYM + NC + P



B. Treatment with 0.3 per cent COC

D1 – FYM + NC + P, D5 – FYM + NC + COC + P

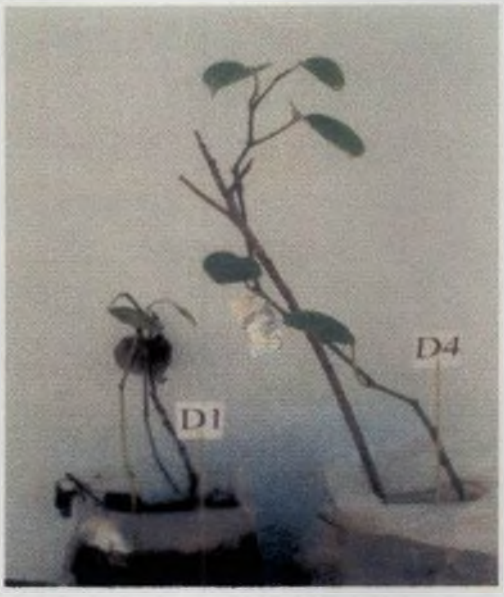
Plate 8. Influence of FYM + NC based mycoinoculants on disease control in pepper var. Karimunda 14 DAI with *P. capsici*

C. Treatment with *T. harzianum*

D1 – FYM + NC + P,
D2 – FYM + NC + T + P

D. Treatment with *G. fasciculatum*

D1 – FYM + NC + P,
D3 – FYM + NC + G + P



E. Treatment with *T. harzianum* + *G. fasciculatum*

D1 – FYM + NC + P,
D4 – FYM + NC + T + G + P

Plate 8. Continued

Table 13 Influence of vermicompost based mycoinoculants on plant height (cm) in pepper var. Karimunda 14 DAI with *P. capsici*

Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	37.25	39.00	52.13	50.38	44.69
G. fasciculatum	49.50	47.13	44.00	48.63	47.31
T. harzianum + *G. fasciculatum*	41.88	47.00	49.25	49.50	46.91
COC (0.3 %)	57.50	42.13	61.38	57.38	54.59
Control (pathogen alone)	30.25	30.50	29.75	30.38	30.22
Control (without pathogen)	58.63	39.50	36.00	31.00	41.28
Mean - B	45.83	40.88	45.42	44.54	

CD (0.05) – A 10.901; CD-B NS; CD-AB NS

G. fasciculatum and *T. harzianum*. In the control treatment without pathogen inoculation the plant height was 41.28 cm.

4.3.2.5 Lesion Length

A significant reduction in lesion length due to infection by *P. capsici* in the collar region of pepper var. Karimunda was observed in the treatments with different control measures. This reduction was maximum with 0.3 per cent copper oxychloride where the lesion length was only 0.72 cm as compared to 20.34 cm in the control treatment with pathogen inoculation (Table 14). In treatments with different biocontrol agents also the lesion lengths were less and these were 2.13, 2.65 and 3.20 respectively with *T. harzianum*, *T. harzianum* + *G. fasciculatum* and *G. fasciculatum*.

4.3.2.6 Percentage of Stem Infection

The percentage of stem infection was significantly reduced in treatments with biocontrol agents and copper oxychloride. It was least (1.3 per cent) after treatment with 0.3 per cent copper oxychloride compared to 87.6 per cent in the control treatment with pathogen inoculation (Table 15, Fig. 5). The effect of different biocontrol agents was also statistically on par with chemical treatment. The average percentage of stem infection in treatments with *T. harzianum* + *G. fasciculatum*, *T. harzianum* and *G. fasciculatum* was 5.9, 6.0 and 6.7 respectively.

4.3.2.7 Percentage of Mortality

The treatment effect of different control measures in reducing the percentage of mortality in pepper var. Karimunda infected with *P. capsici* was highly significant. There were no death of plants in treatments with *G. fasciculatum*, *T. harzianum* + *G. fasciculatum* and copper oxychloride (Table 16). However in the treatment with *T. harzianum* alone, a low mortality rate of 6.25 per cent was recorded. But it was insignificant when compared to the mortality rate of 62.5 per cent in the control treatment with pathogen inoculation. The influence of different carrier

Table 14 Influence of vermicompost based mycoinoculants on lesion length(cm)* in pepper var. Karimunda 14 DAI with *P. capsici*

Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	5.62 (2.57)	1.63 (1.62)	0.75 (1.32)	1.40 (1.55)	2.13 (1.77)
G. fasciculatum	0.98 (1.41)	5.95 (2.64)	1.27 (1.51)	6.06 (2.66)	3.20 (2.05)
T. harzianum + *G. fasciculatum*	6.27 (2.70)	0.87 (1.37)	1.09 (1.45)	3.47 (2.12)	2.65 (1.91)
COC (0.3 %)	1.06 (1.43)	0.49 (1.22)	0.92 (1.39)	0.42 (1.19)	0.72 (1.31)
Control (pathogen alone)	15.70 (4.09)	30.40 (5.60)	17.35 (4.28)	19.21 (4.50)	20.34 (4.62)
Control (without pathogen)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)
Mean - B	3.84 (2.20)	4.02 (2.24)	2.31 (1.82)	3.71 (2.17)	

*Values in parenthesis after $\sqrt{x + 1}$ transformation

CD (0.05) – A 0.767; CD-B NS; CD-AB NS

Table 15 Influence of vermicompost based mycoinoculants on percentage of stem infection* in pepper var. Karimunda 14 DAI with *P. capsici*

Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	23.57 (29.04)	4.23 (11.86)	1.31 (6.56)	2.61 (9.29)	6.0 (14.19)
G. fasciculatum	2.00 (8.14)	11.14 (19.49)	2.46 (9.01)	15.60 (23.25)	6.7 (14.97)
T. harzianum + *G. fasciculatum*	13.69 (21.71)	2.06 (8.24)	2.03 (8.20)	9.49 (17.93)	5.9 (14.02)
COC (0.3 %)	2.13 (8.39)	1.13 (6.10)	1.29 (6.52)	0.71 (4.82)	1.3 (6.46)
Control (pathogen alone)	75.46 (60.28)	99.99 (90.00)	79.31 (62.92)	81.16 (64.25)	87.6 (69.36)
Control (without pathogen)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Mean - B	13.2 (21.26)	14.8 (22.62)	7.2 (15.53)	11.6 (19.92)	

*Values in parenthesis after angular transformation

CD (0.05) – A 12.062; CD-B NS; CD-AB NS

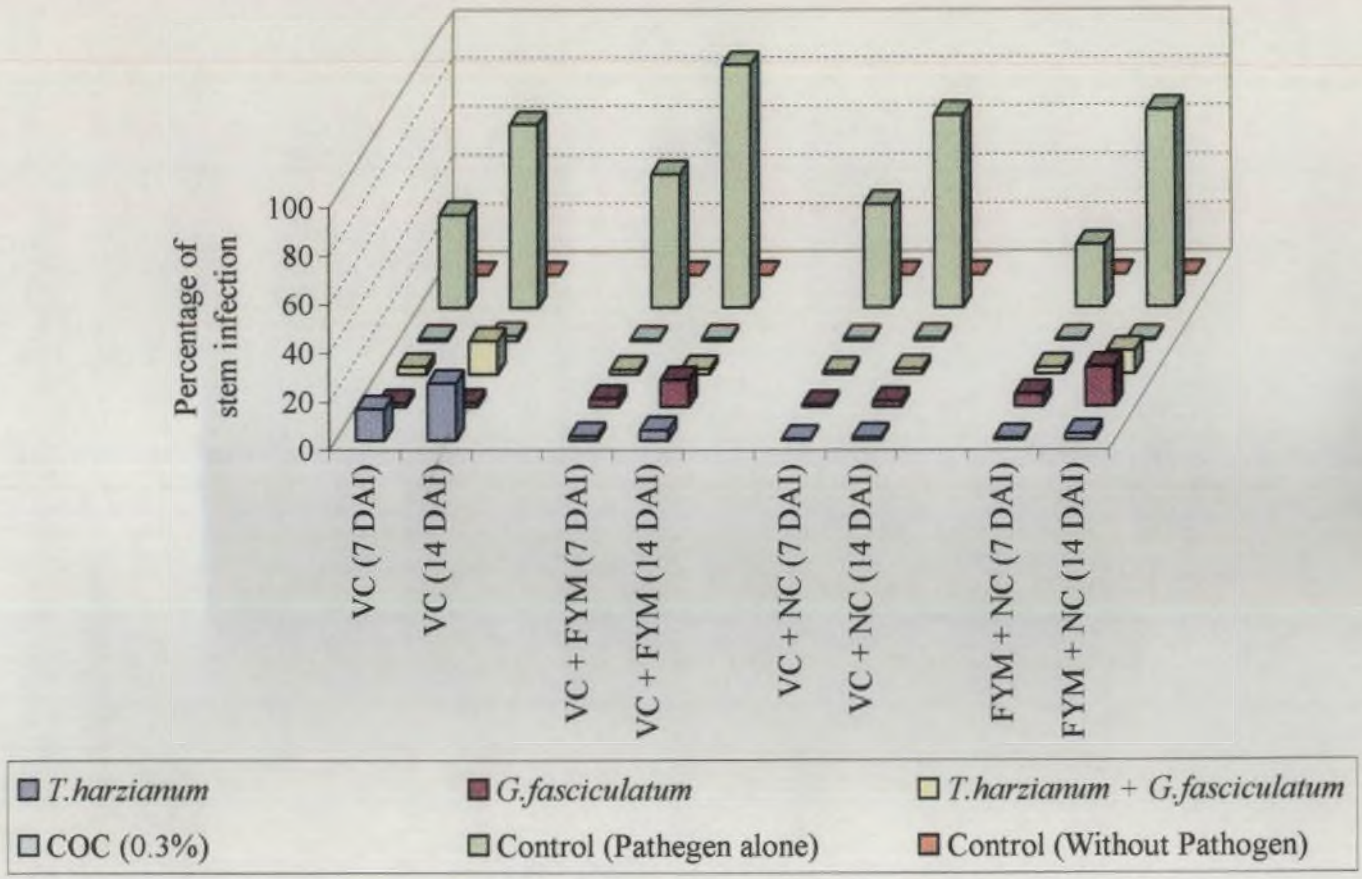


Fig. 5 Influence of vermicompost based mycoinoculants on percentage of stem infection in pepper var. Karimunda at different days after inoculation with P. capsici

Table 16 Influence of vermicompost based mycoinoculants on percentage of mortality in pepper var. Karimunda 14 DAI with *P. capsici*

Treatments	Total number of plants	Number of plants survived	Percentage of mortality
Influence of different control measures			
T. harzianum	16	15	6.25
G. fasciculatum	16	16	0
T. harzianum + *G. fasciculatum*	16	16	0
COC (0.3 %)	16	16	0
Control (pathogen alone)	16	6	62.5
Control (without pathogen)	16	16	0
$\chi^2 = 43.45^{**}$			
Influence of different carrier materials			
VC	24	21	12.5
VC + FYM	24	20	16.7
VC + NC	24	22	8.3
FYM + NC	24	22	8.3
$\chi^2 = 0.03$			

materials (Table 16) in reducing plant mortality after pathogen inoculation was not significant.

4.3.2.8 Fresh Weight of Shoot

The fresh weight of shoot was significantly high in treatments with different control measures. The average fresh weight of 20.06 g was maximum in the treatment with 0.3 per cent copper oxychloride as compared to 6.09 and 15.91 g respectively in control treatments with and without pathogen inoculation (Table 17). The fresh weights of shoot in treatments with *G. fasciculatum*, *T. harzianum* + *G. fasciculatum* and *T. harzianum* were 18.56, 17.31 and 16.69 g respectively. However, the interaction between different control measures and carrier materials was not significant.

4.3.2.9 Dry Weight of Shoot

The dry weight of shoot was significantly high in treatments with different control measures. The average dry weight of 4.0 g was maximum in the treatment with 0.3 per cent copper oxychloride as compared to 1.25 and 3.25 g respectively in control treatments with and without pathogen inoculation (Table 18). The dry weights of shoot in treatments with *G. fasciculatum*, *T. harzianum* + *G. fasciculatum* and *T. harzianum* were 3.66, 3.47 and 3.38 respectively. The treatment effect of biocontrol agents was statistically on par with chemical treatment.

4.3.2.10 Fresh Weight of Roots

The fresh weight of roots was significantly high in treatments with different control measures. However, unlike shoot fresh weight, the root biomass was maximum in treatments with *G. fasciculatum* either alone or in combination with *T. harzianum* (Table 19, Plate 9). In these treatments the fresh weights of roots were 5.97 and 5.56 g respectively. In the control treatment with pathogen inoculation this was only 2.66 g. The increase in root biomass in treatments with *T. harzianum* alone and copper

Table 17 Influence of vermicompost based mycoinoculants on fresh weight of shoot (g) in pepper var. Karimunda 14 DAI with *P. capsici*

Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	16.00	19.75	17.50	13.50	16.69
G. fasciculatum	25.00	16.25	19.75	13.25	18.56
T. harzianum + *G. fasciculatum*	13.00	17.50	20.75	18.00	17.31
COC (0.3 %)	17.00	17.75	22.00	23.50	20.06
Control (pathogen alone)	7.63	5.00	6.50	5.25	6.09
Control (without pathogen)	16.25	13.50	12.50	21.38	15.91
Mean - B	15.81	14.96	16.50	15.81	

CD (0.05) – A -6.386; CD-B NS; CD-AB NS

Table 18 Influence of vermicompost based mycoinoculants on dry weight of shoot (g) in pepper var. Karimunda 14 DAI with *P. capsici*

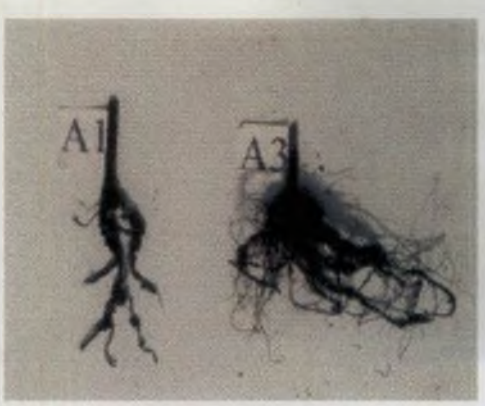
Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	3.25	4.00	3.50	2.75	3.38
G. fasciculatum	4.75	3.25	3.88	2.75	3.66
T. harzianum + *G. fasciculatum*	2.75	3.50	4.13	3.50	3.47
COC (0.3 %)	3.50	3.50	4.50	4.50	4.00
Control (pathogen alone)	1.63	1.00	1.25	1.13	1.25
Control (without pathogen)	3.38	2.75	2.63	4.25	3.25
Mean - B	3.21	3.00	3.31	3.15	

CD (0.05) – A 1.211; CD-B NS; CD-AB NS

Table 19 Influence of vermicompost based mycoinoculants on fresh weight of roots (g) in pepper var. Karimunda 14 DAI with *P. capsici*

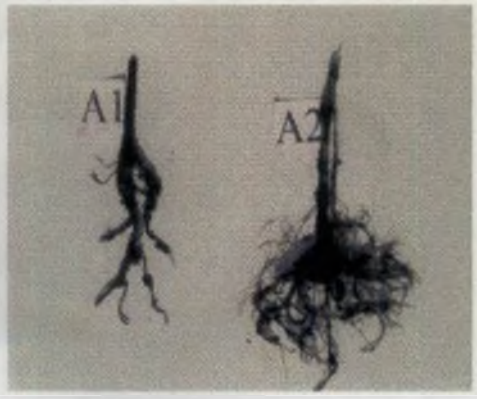
Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	5.50	5.00	3.75	2.75	4.25
G. fasciculatum	6.50	6.75	5.00	5.63	5.97
T. harzianum + *G. fasciculatum*	3.25	6.00	7.25	5.75	5.56
COC (0.3 %)	5.75	3.75	4.75	5.00	4.81
Control (pathogen alone)	3.25	2.25	2.63	2.50	2.66
Control (without pathogen)	4.25	4.75	3.63	4.00	4.16
Mean - B	4.75	4.75	4.50	4.27	

CD (0.05) – A 1.861; CD-B NS; CD-AB NS



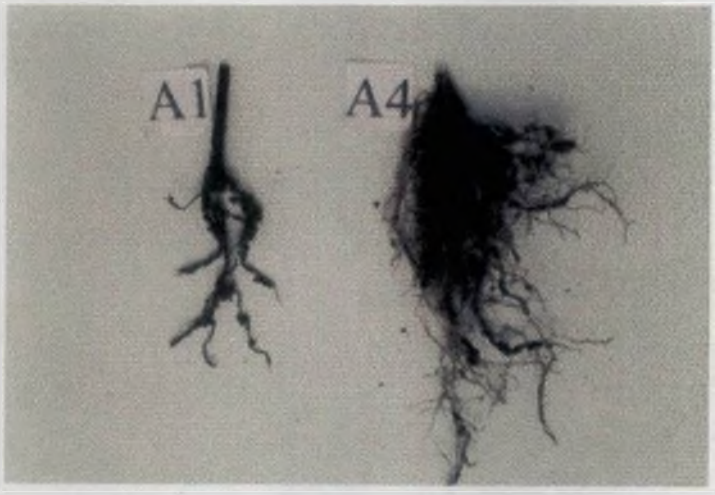
A. Treatment with *G. fasciculatum*

A1 – Carrier material + P,
A3 – Carrier material + G + P



B. Treatment with *T. harzianum*

A1 – Carrier material + P,
A2 – Carrier material + T + P



C. Treatment with *G. fasciculatum* + *T. harzianum*

A1 – Carrier material + P,
A4 – Carrier material + T + G + P

Plate 9. Influence of *G. fasciculatum* on root biomas in pepper var. Karimunda

oxychloride was statistically on par with the best treatment. These were 4.25 and 4.81 g respectively. Such an effect was also observed in the control treatment without pathogen inoculation. The interaction between different control measures and carrier materials and however not significant.

4.3.2.11 Dry Weight of Roots

The dry weight of roots was significantly high in treatments with different control measures. This was maximum in treatments with *G. fasciculatum* either alone or in combination with *T. harzianum*. In these treatments the dry weights of roots were 1.31 and 1.25 g respectively (Table 20). In the control treatment with pathogen inoculation this was only 0.69 g. The increase in root biomass in treatments with *T. harzianum* alone and copper oxychloride was statistically on par with the best treatment. These were 1.03 and 1.09 g respectively.

4.4 ESTIMATION OF PHENOLS AND DEFENCE RELATED ENZYMES

4.4.1 Total Phenol Content

The phenol content of plants was found to increase with pathogen inoculation especially after 10 days. The maximum phenol content of 4633 $\mu g\ g^{-1}$ fresh weight of leaf was in the treatment combination of VC + NC + G 10 DAI with *P. capsici* (Table 21, Fig. 6). The interaction between pathogen inoculation and disease status (A x B) was also significant. Thus, the mean phenol content of 3093.19 $\mu g\ g^{-1}$ in infected plants was significantly higher than that of 2264.58 $\mu g\ g^{-1}$ in healthy plants (Table 22). The day of estimation had also a significant effect on total phenol content. The total phenol content recorded one DAI (2082.50) was significantly lower than that recorded 10 DAI (3152.08). However, the interaction (Table 23) between pathogen inoculation and different control measures (A x C) was not significant.

Table 20 Influence of vermicompost based mycoinoculants on dry weight of roots (g) in pepper var. Karimunda 14 DAI with *P. capsici*

Treatments (A)	Carrier materials (B)				Mean- A
	VC	VC + FYM	VC + NC	FYM + NC	
T. harzianum	1.13	1.25	1.00	0.75	1.03
G. fasciculatum	1.25	1.50	1.25	1.25	1.31
T. harzianum + *G. fasciculatum*	0.88	1.13	1.63	1.38	1.25
COC (0.3 %)	1.25	1.00	1.00	1.13	1.09
Control (pathogen alone)	0.75	0.63	0.75	0.63	0.69
Control (without pathogen)	1.00	1.13	1.00	1.00	1.03
Mean - B	1.04	1.10	1.10	1.02	

CD (0.05) – A 0.325; CD-B NS; CD-AB NS

Table 21 Total phenol content of pepper var. Karimunda in response to inoculation with *P. capsici* and different control measures

P. capsici	Total phenol content, μg g^{-1} fresh weight of leaf							
	Control measures							
	Biocontrol agents				Chemical control		Control	
	VC+NC+T		VC+NC+G		VC+NC+COC		VC+NC	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
1 DAI	1775	2150	1740	2135	2205	1935	2205	2515
5 DAI	2450	3700	2383	3417	2100	3350	2100	2917
10 DAI	2700	4083	2583	4633	2467	3533	2467	2750
CD (0.05) – NS								

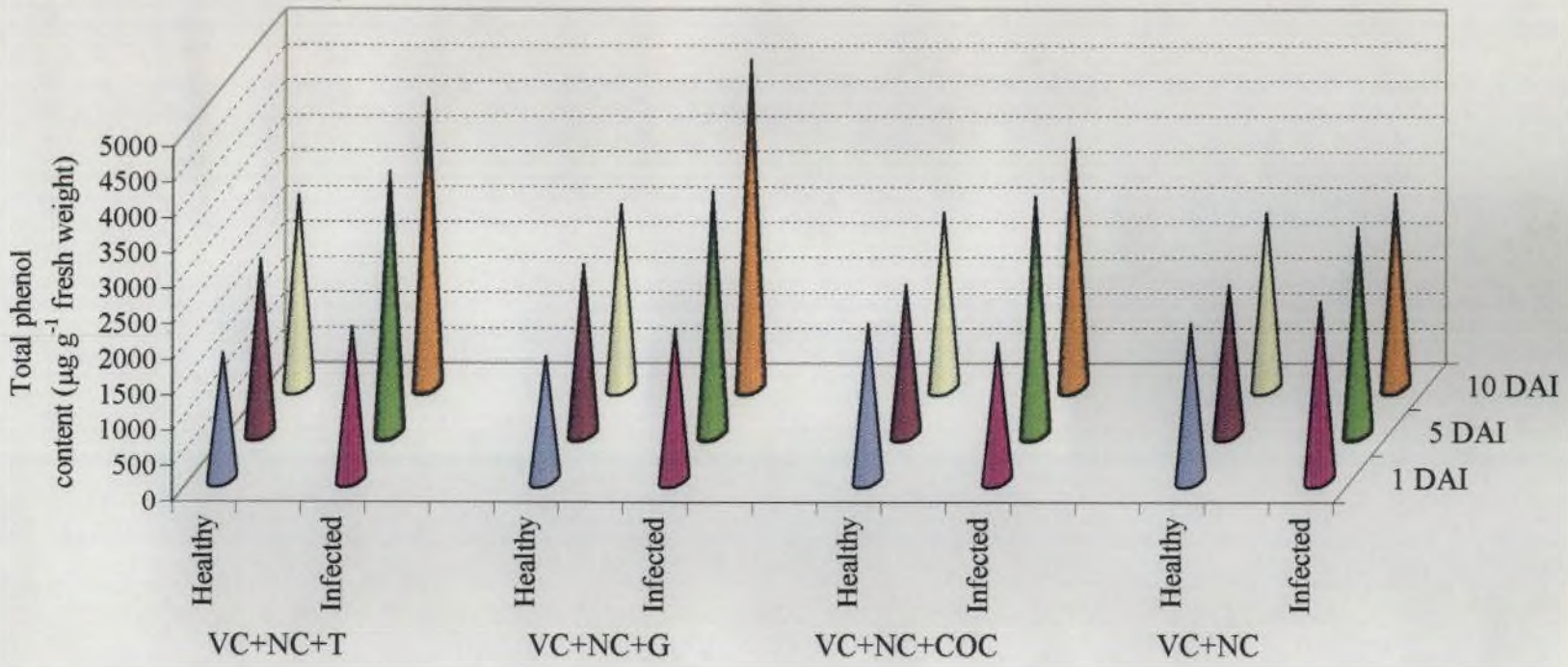


Fig. 6 Total phenol content in pepper var-Karimunda in response to inoculation with *P.capsici* and different control measures

Table 22 Interaction between pathogen inoculation and disease status on total phenol content in pepper var. Karimunda

P. capsici (A)	Total phenol content, µg g^{-1} fresh weight of leaf		Mean – A
	Disease status (B)		
	Healthy	Infected	
1 DAI	1981.25	2183.75	2082.50
5 DAI	2258.33	3345.83	2802.08
10 DAI	2554.17	3750.00	3152.08
Mean-B	2264.58	3093.19	
CD (0.05) A-381.92; B-311.83; AB-540.11			

Table 23 Interaction between pathogen inoculation and different control measures on total phenol content in pepper var. Karimunda

P. capsici (A)	Total phenol content, µg g^{-1} fresh weight of leaf				Mean-A
	Control measures (C)				
	Biocontrol agents		Chemical control	Control	
	VC+NC+T	VC+NC+G	VC+NC+COC	VC+NC	
1 DAI	1962.50	1937.50	2070	2360	2082.50
5 DAI	3075.00	2900.00	2725	2508.33	2802.08
10 DAI	3391.67	3608.33	3000	2608.33	3152.08
Mean –C	2809.72	2815.28	2598.83	2492.22	
CD (0.05) A – 381.92; C-NS; AC-NS					

4.4.2 OD Phenol Content

There was an increase in OD phenol content immediately after inoculation with *P. capsici*. The OD phenol content of 1334 μg g^{-1} fresh weight of leaf was maximum in the treatment combination of VC + NC + T 24 h after inoculation with pathogen (Table 24, Fig. 7). However, the interactions between pathogen inoculation and disease status (Table 25) and pathogen inoculation and different control measures (Table 26) were not significant.

4.4.3 Peroxidase Activity

The peroxidase activity of 28.78 g^{-1} fresh weigh of leaf was maximum in the treatment combination of VC + NC + G 10 DAI of pathogen (Table 27, Fig. 8). These observations, that the enzyme activity was more in infected plants and that in most of the treatments there was an increase in activity with the day of estimation after pathogen inoculation, were confirmed in the interaction studies between pathogen inoculation and disease status (Table 28) and pathogen inoculation and different control measures (Table 29). Eventhough, the A x B and A x C interactions were insignificant, the individual treatment effects were significant. Thus, the mean enzyme activity of 18.92 in infected plants was significantly high as compared to 10.84 in uninoculated plants (Table 28). Further, there was a progressive increase in enzyme activity with the day of estimation and it attained a peak level 10 DAI of the pathogen. It was 17.88 when compared to 11.68, 24 h after pathogen inoculation. Treatment with *G. fasciculatum* also resulted in maximum peroxidase activity. It was 18.60 as compared to 15.97 in the control (VC + NC) treatment (Table 29). But the mean enzyme activity of 12.72 and 12.23 in treatments with *T. harzianum* and COC was relatively low.

Table 24 OD phenol content of pepper var. Karimunda in response to inoculation with *P. capsici* and different control measures

P. capsici	OD phenol content, μg g^{-1} fresh weight of leaf							
	Control measures							
	Biocontrol agents				Chemical control		Control	
	VC+NC+T		VC+NC+G		VC+NC+COC		VC+NC	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
1 DAI	1222	1334	1111	1189	1066	1133	1066	1155
5 DAI	1061	1145	1022	1322	1017	1083	1017	1172
10 DAI	1050	1117	1072	1128	1061	1111	1061	1167
CD (0.05) – NS								

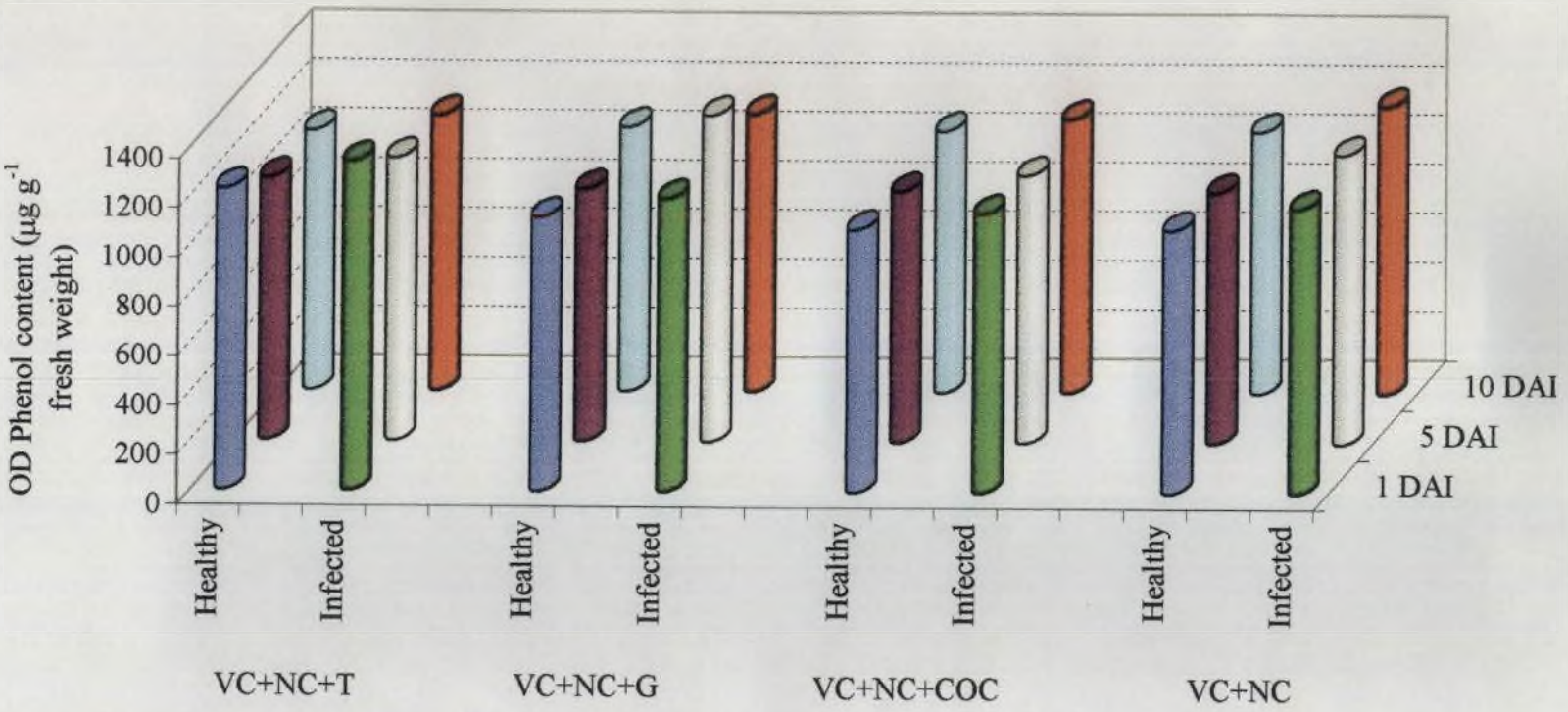


Fig. 7 OD phenol content in pepper var.Karimunda in response to inoculation with *P.capsici* and different control measures

Table 25 Interaction between pathogen inoculation and disease status on OD phenol content in pepper var. Karimunda

P. capsici (A)	OD phenol content, µg g^{-1} fresh weight of leaf		Mean – A
	Disease status (B)		
	Healthy	Infected	
1 DAI	1116.50	1202.83	1159.67
5 DAI	1029.17	1180.58	1104.88
10 DAI	1061.25	1130.50	1095.88
Mean-B	1068.97	1171.31	
CD (0.05) A-NS; B-47.02; AB-NS			

Table 26 Interaction between pathogen inoculation and different control measures on OD phenol content in pepper var. Karimunda

P. capsici (A)	OD phenol content, µg g^{-1} fresh weight of leaf				Mean-A
	Control measures (C)				
	Biocontrol agents		Chemical control	Control	
	VC+NC+T	VC+NC+G	VC+NC+COC	VC+NC	
1 DAI	1278	1150	1099.83	1110.83	1159.67
5 DAI	1103	1172.17	1050	1094.33	1104.88
10 DAI	1083.33	1100	1086.17	1114	1095.88
Mean –C	1154.78	1140.72	1078.67	1106.39	
CD (0.05) A – NS; C-NS; AC-NS					

Table 27 Peroxidase activity of pepper var. Karimunda in response to inoculation with *P. capsici* and different control measures

P. capsici	Peroxidase activity min^{-1} g^{-1} fresh weight of leaf							
	Control measures							
	Biocontrol agents				Chemical control		Control	
	VC+NC+T		VC+NC+G		VC+NC+COC		VC+NC	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
1 DAI	11.62	15.30	9.04	20.58	3.71	9.81	3.71	19.69
5 DAI	8.54	12.13	14.42	22.59	12.10	15.49	12.10	23.16
10 DAI	10.39	18.32	16.16	28.78	14.12	18.16	14.12	23.02
CD (0.05) - NS								

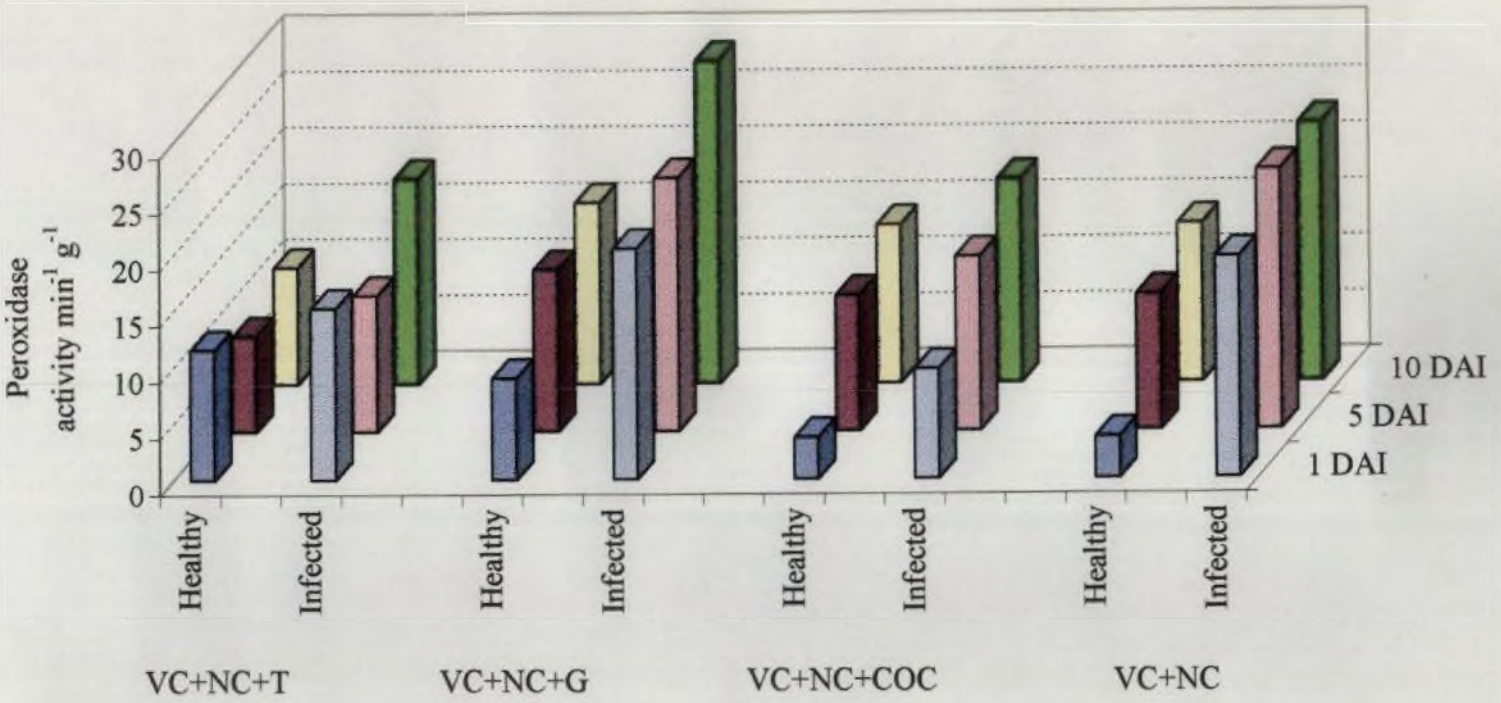


Fig. 8 Peroxidase activity in pepper var. Karimunda in response to inoculation with *P.capsici* and different control mesures

Table 28 Interaction between pathogen inoculation and disease status on peroxidase activity in pepper var. Karimunda

P. capsici (A)	Peroxidase activity min^{-1} g^{-1} fresh weight of leaf		Mean – A
	Disease status (B)		
	Healthy	Infected	
1 DAI	7.02	16.35	11.68
5 DAI	11.79	18.34	15.07
10 DAI	13.70	22.07	17.88
Mean-B	10.84	18.92	
CD (0.05) A-3.78; B-3.09; AB-NS			

Table 29 Interaction between pathogen inoculation and different control measures on peroxidase activity in pepper var. Karimunda

P. capsici (A)	Peroxidase activity min^{-1} g^{-1} fresh weight of leaf				Mean-A
	Control measures (C)				
	Biocontrol agent		Chemical control	Control	
	VC+NC+T	VC+NC+G	VC+NC+COC	VC+NC	
1 DAI	13.46	14.81	6.76	11.70	11.68
5 DAI	10.33	18.51	13.80	17.63	15.07
10 DAI	14.36	22.47	16.14	18.57	17.88
Mean-C	12.72	18.60	12.23	15.97	
CD (0.05) A – 3.78; C – 4.37; AC – NS					

4.4.4 Polyphenol Oxidase Activity

An early stimulation of polyphenol oxidase activity was observed in most of the treatments especially after pathogen inoculation. The mean enzyme activity of 9.70 g^{-1} fresh weight of leaf obtained after 24 h was maximum in the treatment combination of VC+NC+G (Table 30, Fig. 9). Eventhough the interaction between pathogen inoculation and disease status (AxB) and pathogen inoculation and different control measures (AxC) were insignificant, the individual treatment effects were significant. Thus, the mean polyphenol oxidase activity of 6.67 g^{-1} fresh weight of leaf was significantly high in infected plants when compared to 4.46 in healthy plants (Table 31). Further it was maximum (7.53) 24 h after pathogen inoculation. As regard to the effect of different control measures, the mean enzyme activity was more in treatment with *G. fasciculatum* (Table 32). The activity of 6.63 in VC + NC + G was significantly high when compared to the chemical treatment of VC + NC + COC, where it was only 4.59. The polyphenol oxidase activities of 5.24 and 5.80 in VC + NC + T and VC + NC were statistically on par with the VC + NC + G treatment.

4.4.5 Phenylalanine Ammonia Lyase (PAL) Activity

The maximum enzyme activity of 48.69 $\mu g\ g^{-1}\ min^{-1}$ was in the treatment combination of VC + NC + G 10 DAI of pathogen (Table 33, Fig. 10). The interactions between pathogen inoculation and disease status (A x B) and pathogen inoculation and different control measures (A x C) were significant. Thus, the mean enzyme activity of 29.31 was significantly high in infected plants when compared to 22.49 in healthy plants (Table 34). Similarly, the enzyme activity of 29.88 with *T. harzianum* and 28.20 with *G. fasciculatum* were significantly high when compared to either the control treatment (22.76) or the chemical treatment (22.76) with 0.3 per cent copper oxychloride (Table 35). The treatment effects of the two biocontrol agents were also statistically on par with

Table 30 Polyphenol oxidase activity of pepper var. Karimunda in response to inoculation with *P. capsici* and different control measures

P. capsici	Polyphenol oxidase activity min^{-1} g^{-1} fresh weight of leaf							
	Control measures							
	Biocontrol agents				Chemical control		Control	
	VC+NC+T		VC+NC+G		VC+NC+COC		VC+NC	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
1 DAI	3.52	8.38	5.26	9.70	4.09	4.03	4.09	8.02
5 DAI	2.74	6.34	4.52	6.01	3.70	4.01	3.70	5.74
10 DAI	4.23	6.25	5.58	8.72	6.03	5.68	6.03	7.23
CD (0.05) - NS								

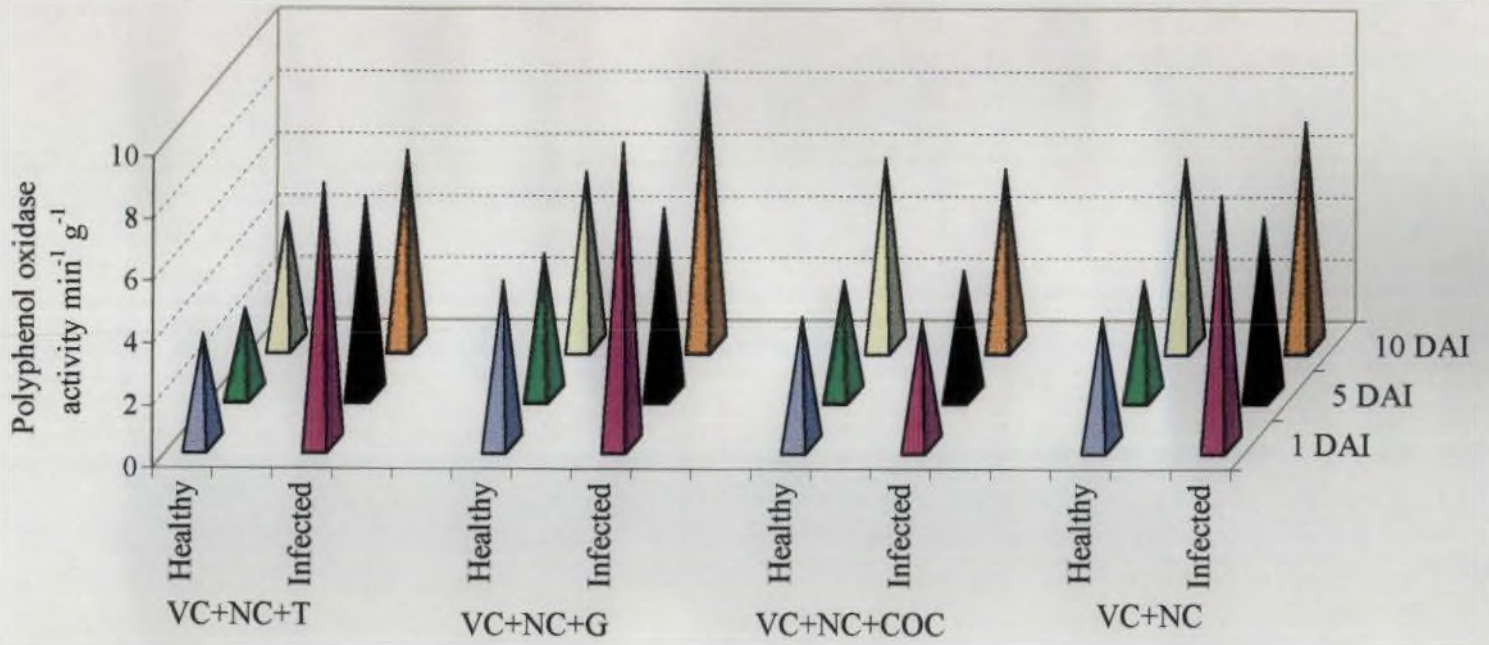


Fig. 9 Polyphenol oxidase activity in pepper var. Karimunda in response to inoculation with *P.capsici* and different control measures.

Table 31 Interaction between pathogen inoculation and disease status on polyphenol oxidase activity in pepper var. Karimunda

P. capsici (A)	Polyphenol oxidase activity min^{-1} g^{-1} fresh weight of leaf		Mean – A
	Disease status (B)		
	Healthy	Infected	
1 DAI	4.24	7.53	5.88
5 DAI	3.66	5.52	4.59
10 DAI	5.47	6.97	6.22
Mean-B	4.46	6.67	
CD (0.05) A-1.24; B-1.02; AB-NS			

Table 32 Interaction between pathogen inoculation and different control measures on polyphenol oxidase activity in pepper var. Karimunda

P. capsici (A)	Polyphenol oxidase activity min^{-1} g^{-1} fresh weight of leaf				Mean-A
	Control measures (C)				
	Biocontrol agents		Chemical control	Control	
	VC+NC+T	VC+NC+G	VC+NC+COC	VC+NC	
1 DAI	5.95	7.48	4.06	6.05	5.88
5 DAI	4.54	5.27	3.85	4.72	4.59
10 DAI	5.24	7.15	5.86	6.63	6.22
Mean –C	5.24	6.63	4.59	5.80	
CD (0.05) – A – 1.24 C-1.44 AC-NS					

Table 33 Phenylalanine ammonia lyase activity of pepper var. Karimunda in response to inoculation with *P. capsici* and different control measures

P. capsici	Phenylalanine ammonia lyase activity, μg g^{-1} min^{-1}							
	Control measures							
	Biocontrol agents				Chemical control		Control	
	VC+NC+T		VC+NC+G		VC+NC+COC		VC+NC	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
1 DAI	30.57	33.90	17.43	22.55	24.35	20.34	24.35	28.64
5 DAI	23.66	30.57	24.35	30.44	19.92	26.84	19.92	21.86
10 DAI	20.89	39.71	25.73	48.69	19.37	25.73	19.37	22.41
CD (0.05) – NS								

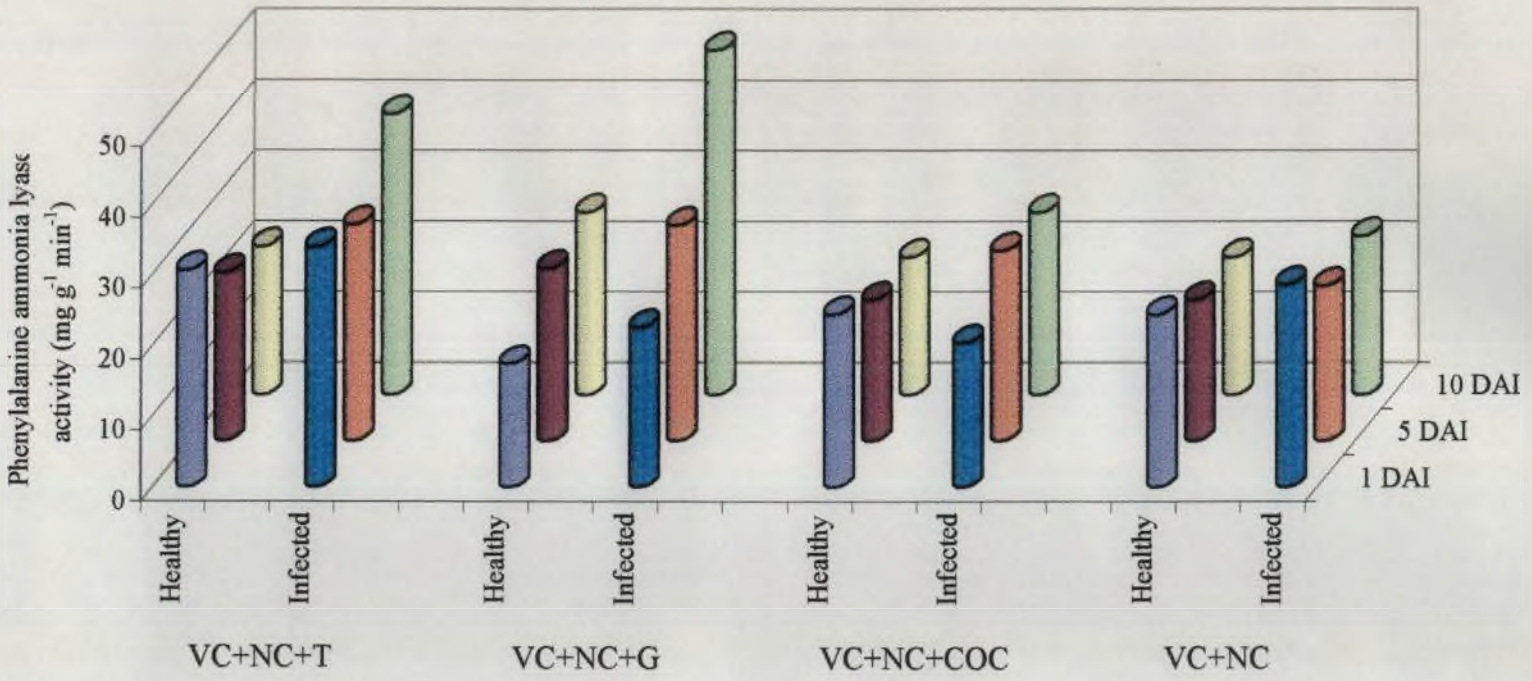


Fig. 10 Phenylalanine amonia lyase activity in pepper var. Karimunda in response to incoculation with *P. capsici* and different control measures

Table 34 Interaction between pathogen inoculation and disease status on phenylalanine ammonia lyase activity in pepper var. Karimunda

P. capsici (A)	Phenylalanine ammonia lyase activity, μg g^{-1} min^{-1}		Mean – A
	Disease status (B)		
	Healthy	Infected	
1 DAI	24.18	26.36	25.27
5 DAI	21.96	27.43	24.70
10 DAI	21.34	34.14	27.74
Mean-B	22.49	29.31	
CD (0.05) A-NS; B-2.47; AB-4.28			

Table 35 Interaction between pathogen inoculation and different control measures on phenylalanine ammonia lyase activity in pepper var. Karimunda

P. capsici (A)	Phenylalanine ammonia lyase activity, μg g^{-1} min^{-1}				Mean-A
	Control measures (C)				
	Biocontrol agents		Chemical control	Control	
	VC+NC+T	VC+NC+G	VC+NC+COC	VC+NC	
1 DAI	32.24	19.99	22.35	26.50	25.27
5 DAI	27.12	27.39	23.38	20.89	24.70
10 DAI	30.30	37.21	22.55	20.89	27.74
Mean –C	29.88	28.20	22.76	22.76	
CD (0.05) – A – NS; C-3.49; AC-6.05					

each other. However, unlike peroxidase and polyphenol activities, the day of estimation had no significant effect on PAL activity in pepper var. Karimunda.

4.6 PROTEIN PROFILE OF HEALTHY AND INFECTED LEAVES

Protein profile of the leaf extracts from eight treatments, VC + NC + T + P, VC + NC + T, VC + NC + G + P, VC + NC + G, VC + NC + P, VC + NC, VC + NC + COC + P, VC + NC + COC was studied by SDS-PAGE technique. It showed differences between infected and healthy plants in the total number and intensity of protein bands (Plate 10). The additional protein bands with molecular weights of 78 kDa and 28 kDa were observed in pepper var. Karimunda after infection with *P. capsici*. These were absent in treatments without pathogen inoculation and also in chemical control.

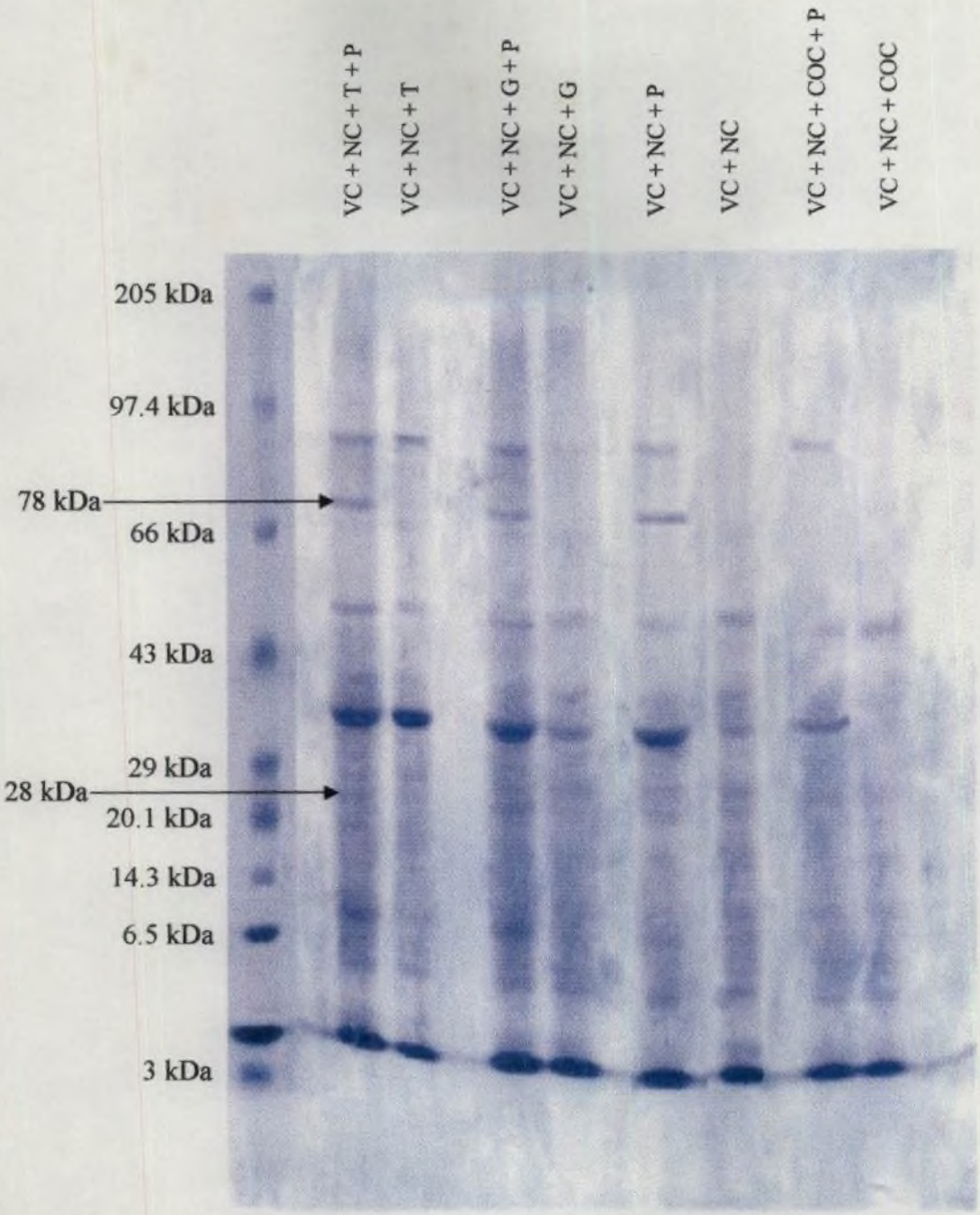


Plate 10. Protein profile of healthy and infected leaves of pepper var. Karimunda 1 DAI of *P. capsici*

DISCUSSION

5. DISCUSSION

The foot rot of pepper caused by *Phytophthora capsici* is a major problem in many pepper growing countries. A combination of factors such as lack of resistant host varieties, susceptibility of all plant parts to infection and the highly conducive microclimatic conditions existing in pepper plantations favouring rapid spread of the pathogen make it a difficult task to control this disease effectively. Application of bordeaux mixture either as soil drench or foliar spray, applying bordeaux paste in the collar region of existing vines before the onset of monsoon season and the use of systemic fungicides such as Metalaxyl are some of the commonly used methods for the management of foot rot of pepper. However, due to the necessity to produce pesticide free pepper especially for export purpose, it has become essential to evolve safe and ecofriendly methods for the control of this disease. One of these approaches apart from strict adherence to phytosanitary procedures in pepper plantations, is to use specific biocontrol agents such as *Trichoderma* and arbuscular mycorrhiza. The use of these mycoinoculants is becoming very popular nowadays among the farmers. However, one of the major constraints observed here is the relatively high cost of these products for large scale field application. A solution to this problem is to encourage the farmers to mass produce these mycoinoculants. But this will require a cheap source of good quality organic carrier material. An ideal carrier material for this purpose will be vermicompost. The main objective of the present study was to explore the possibility of using vermicompost as a carrier material for mass production of *Trichoderma harzianum* and *Glomus fasciculatum* and to test its efficacy for the biological control of foot rot of pepper. The study on changes in host physiology with respect to phenol content, defense related enzymes and pathogenesis related proteins due to

inoculation with *P. capsici* and application of biocontrol agents also formed a part of this investigation.

The causal organism of foot rot of pepper was isolated from disease affected pepper plants collected from the District Agricultural Farm of Department of Agriculture, Peringamala. The pathogen was identified as *Phytophthora capsici* Leonian based on morphological and cultural characters besides its ability to induce typical symptoms of foot rot disease in inoculated pepper plants. The different characters were compared with the type culture of *P. capsici* maintained in the Department of Plant Pathology, College of Agriculture, Vellayani. The isolate produced petalloid type colonies on carrot agar medium. The sporangia were either papillate or spherical to irregular in shape (Plate 2) with size varying from 29.7 to 52.8 x 16.5 to 23.1 µm. These observations were in agreement with those reported earlier by Sarma *et al.* (1982) and Mehrotra and Aggarwal (2001). The symptoms produced by the isolate after inoculation of pepper var. Karimunda were also identical to those described earlier by Sarma and Nambiar (1982). On artificial inoculation of the leaf, pale water soaked lesions appeared within 48 hours which later turned dark brown to black in colour (Plate 3a). These lesions with fimbriate margins gradually coalesced covering large area of the leaf resulting in defoliation. In the collar region of the stem also where the isolate was inoculated, the initial symptom appeared as a water soaked lesion within 72 hours. It subsequently turned dark brown to black colour as the infection progressed (Plate 3b). Finally the entire plant was defoliated and completely dried by about three weeks. Once the pathogenicity of the isolate was proved it was used for further studies.

Trichoderma harzianum and *Glomus fasciculatum* were the two biocontrol agents selected for testing the possibility of using vermicompost as a carrier material for mass production. A talc based inoculum of *T. harzianum* and soil based granular inoculum of

G. fasciculatum were used for this purpose. These were mass produced either in vermicompost alone or in combination with FYM and neem cake in the ratio of 1 : 1 and 5 : 1 respectively. The efficacy of this system was compared with the standard carrier material of FYM + neem cake in the ratio 10 : 1 (Sivaprasad, 1998). Vermicompost was selected for this purpose because of its comparable nutrient quality with FYM in terms of organic matter content, micronutrient and P availability as reported earlier by Rouelle and Randriamanonjizaka (1983) and Bano *et al.* (1987).

The population of *T. harzianum* was maximum in the treatment combination of VC + NC. These were 72.87 x 10^6 when compared to 21.27 x 10^6 in FYM + NC (Table 1, Fig. 1, Plate 4). In other carrier materials like VC + FYM and vermicompost alone the population of *T. harzianum* were 4.29 x 10^6 and 1.49 x 10^6 respectively. This clearly showed that the combination of VC + NC was a better medium when compared to vermicompost alone or in combination with FYM, for the mass production of *Trichoderma*. The occurrence of such high population of *T. harzianum* has been reported earlier also in other carrier materials such as tea waste (82.9 x 10^6 cfu/g) by Prakash *et al.* (1999) and in shelled maize cob (28 x 10^6 cfu/g) by Gandhikumar and Ranganathan (2000).

Influence of vermicompost either alone or in combination with FYM or neem cake was not significant in the root colonization by *G. fasciculatum*. The percentage of mycorrhizal infection in guinea grass raised in different combination of carrier materials after 50 days varied from 37.99 in vermicompost alone to 39.98 in FYM + NC (Table 2, Fig. 2). In VC + NC and VC + FYM, these were 39.93 and 38.95 respectively. The lack of much variation between treatments in the percentage of mycorrhizal infection may be due to the fact that AMF being a symbiotic organism is solely depended on its host plant for carbon requirement and therefore variations in the nutrient content of the medium used for raising the host plant need not necessarily influence its level of root colonization.

The efficacy of vermicompost as a carrier material for biocontrol agents was further evaluated by using the same for the biological control of foot rot of pepper. For this, Karimunda, a susceptible pepper variety, was used and the disease was induced by inoculation with the isolate of *P. capsici* obtained during this investigation. The effect of both the carrier materials and biocontrol agents on plant growth was also studied. The different observations were taken seven and fourteen days after inoculation with pathogen. Plant biometric characters such as fresh and dry weight of shoot and roots and percentage mortality were taken only fourteen days after inoculation with the pathogens. Since most of the parameters studied whether related to disease control or plant growth showed an almost identical trend, the data are mainly interpreted based on the observations made fourteen days after inoculation of pathogen. The treatment effect of biocontrol agents were statistically on par with the chemical control using 0.3 per cent copper oxychloride.

A significant reduction in the percentage of foliar infection was obtained with the use of both the biocontrol agents. Thus, in treatments with *T. harzianum*, *G. fasciculatum* and *T. harzianum* + *G. fasciculatum*, the percentage of foliar infection was only 1.4, 2.5 and 11.0 respectively as compared to 92.9 per cent in the control treatment with pathogen inoculation (Table 11, Fig. 3). The treatment effect of these mycoinoculants was statistically on par with chemical control where the percentage of foliar infection was only 0.22. There was also significant reduction in disease index after the use of these biocontrol agents. The disease index of 1.39 was least with *T. harzianum* (Table 12, Fig. 4, Plate 5-8). These were 1.68 and 8.72 respectively in treatments with *G. fasciculatum* and *T. harzianum* + *G. fasciculatum* as against the maximum value of 89.54 in the control treatment with pathogen inoculation. However, the effect of different biocontrol agents was statistically on par with chemical control where the disease index was only 0.05. The stem infection was significantly reduced by using a combination of *T. harzianum* + *G. fasciculatum*.

In this treatment, the percentage of stem infection was only 5.9 as compared to 87.6 per cent in the control treatment with pathogen inoculation (Table 15, Fig. 5). The average percentage of stem infection in treatments with *T. harzianum* and *G. fasciculatum* alone was 6.0 and 6.7 respectively. The treatment effect of these biocontrol agents was statistically on par with chemical treatment where the stem infection was only 1.3 per cent. There was also significant difference in the percentage of mortality in pepper var. Karimunda infected with *P. capsici* (Table 16). There were no death of plants in treatments with *T. harzianum* + *G. fasciculatum* and *G. fasciculatum*. The fact that the use of *T. harzianum* either alone or in combination with *G. fasciculatum* resulted in significant reduction in leaf infection, disease index, stem infection and percentage mortality clearly indicated that it was a better biocontrol agent for the control of foot rot of pepper. This could be due to its already reported antagonistic effect on growth and proliferation of *P. capsici* in the plant rhizosphere. Similar results were reported earlier also both under *in vitro* condition by Cristinzio (1987) and under *in vivo* condition by Tsao *et al.* (1988). Further, a management practice using *Trichoderma* has been successfully used by Anandaraj and Sarma (1994, 1995) and Sarma *et al.* (1996) for the control of foot rot of pepper. The treatment effect of *G. fasciculatum* eventhough was not as effective as *T. harzianum* in items of disease control, its effect was also on par with chemical control. The reduction in foot rot incidence in pepper with arbuscular mycorrhizae has also been reported earlier by Anandaraj *et al.* (1993), Anandaraj and Sarma (1994), Sivaprasad *et al.* (1995) and Dare (1996).

The effect of *G. fasciculatum* was more pronounced on plant growth characters. The fresh and dry weight of shoot and roots were maximum in this treatment. The fresh weight of shoot in treatments with *G. fasciculatum*, *T. harzianum* + *G. fasciculatum* and *T. harzianum* were 18.56, 17.31 and 16.69 g respectively when compared to 6.09 and 15.91 g respectively in control treatments with and without pathogen inoculation (Table 17). The

average dry weight of shoot (3.66 g) was also more in the treatment with *G. fasciculatum* (Table 18). The root biomass was more in treatment with *G. fasciculatum* either alone or in combination with *T. harzianum*. In these treatments the fresh weights of roots were 5.97 and 5.56 g respectively when compared to 2.66 g in the control treatment with pathogen inoculation (Table 19, Plate 9). Similar trend was observed in the case of dry weight of roots also. The beneficial effect of arbuscular mycorrhizal association on plant growth in black pepper has already been reported by Manjunath and Bagyaraj (1982), Ramesh (1982) and Sivaprasad (1995). This may be the reason for the observed reduction in foliar infection, disease index, stem infection and percentage mortality after treatment with *G. fasciculatum*. Increase in root biomass could also be a favourable factor here as it will enable enhanced uptake of nutrients from the soil. Improved regeneration of roots in mycorrhizal pepper plants infected with *P. capsici* has been observed earlier also by Sarma *et al.* (1996) and Thanuja (1999).

It was further observed that eventhough the different types of carrier materials did not have any direct influence on disease control the fact that the use of both the mycoinoculants mass produced in these carrier materials resulted in significant reduction in plant mortality after infection with *P. capsici*, clearly indicated that vermicompost was a good carrier material. Further, among the different combinations of vermicompost used, VC + NC in the ratio 5 : 1 was found to be the best carrier material as it also supported better growth and proliferation of both *T. harzianum* and *G. fasciculatum*. Therefore, the combination of VC + NC can be recommended for mass production of these biocontrol agents.

The changes in host physiology due to infection by *P. capsici* and application of biocontrol agents was more apparent in pathogen inoculated plants. Thus, the mean phenol content of 3093.19 $\mu g\ g^{-1}$ fresh weight of leaf was significantly high in infected leaves when compared to that of

2264.58 μg g^{-1} in healthy leaves (Table 22). The day of estimation also had a significant effect on total phenol content. The phenol content recorded 10 DAI (3152.08 μg g^{-1}) was significantly higher than that obtained 1 DAI (2082.50 μg g^{-1}) of pathogen. The gradual increase in phenol content might be due to a susceptible interaction between the pathogen and the host. The accumulation of phenolic compounds due to infection by pathogens has been reported earlier also in many crops such as onion (Walker and Link, 1935), apple (Martin *et al.*, 1957), potato (Lee and Le Tourneau, 1958; Patil *et al.*, 1962), ragi (Vidhyasekaran, 1974), mung (Arora and Bajaj, 1978; Arora, 1983), tea (Borah *et al.*, 1978) and rice (Chattopadhyay and Bera, 1980). Further, it is likely that accumulation of such phenolic compounds take place at a faster rate in resistant cultivars. The work of Arora (1983), Arora and Wagle (1985), Patil *et al.* (1985) and Bashan (1986) are in support of this observation. An increase in phenol content due to inoculation with *G. fasciculatum* was also observed. Thus the phenol content of 4633 μg g^{-1} fresh weight of leaf was maximum in the treatment combination of VC + NC + G 10 DAI with *P. capsici* (Table 21, Fig. 6). Such an effect due to AM inoculation was reported earlier in pepper by Sivaprasad *et al.* (2000).

There was also an increase in ortho dihydroxy phenol content immediately after inoculation with *P. capsici*. The OD phenol content of 1334 μg g^{-1} fresh weight of leaf was maximum in the treatment combination of VC + NC + T 24 h after inoculation with pathogen (Table 24, Fig. 7). However, the interactions between pathogen inoculation and disease status (Table 25) and pathogen inoculation and different control measures (Table 26) were not significant eventhough a positive effect has been reported earlier by Vidhyasekaran (1981), Lodha *et al.* (1993) and Sindhan *et al.* (1996).

The changes in defence related enzymes such as peroxidase, polyphenol oxidase and phenylalanine ammonia lyase were also more in

pathogen inoculated plants. The mean peroxidase activity 18.92 in infected plants was significantly high when compared to 10.84 in uninoculated plants (Table 28). Such increase in enzyme activity due to pathogen inoculation has been reported earlier by Kosuge (1969), Yamamoto *et al.* (1978), Hammerschmidt *et al.* (1982), Arora and Bajaj (1984), Lizzi and Coulomb (1991) and Alcazar *et al.* (1995). There was also an increase in enzyme activity with the day of estimation and it was maximum 10 DAI of pathogen. It was 17.88 when compared to 11.68, 24 h after pathogen inoculation. Among the biocontrol agents, inoculation with *G. fasciculatum* also resulted in higher peroxidase activity. It was 18.60 as compared to 15.97 in the control (VC + NC) treatment (Table 29). However, the mean enzyme activity of 12.72 and 12.23 in treatments with *T. harzianum* and COC was relatively low. The effect of enhanced peroxidase activity might be due to its role in the biosynthesis of lignin and oxidation of many mono and diphenolic compounds and aromatic amines to highly toxic quinones in the presence of hydrogen peroxide (Bonner, 1950). Besides, the enzyme itself was reported to be toxic to many microorganisms (Macko *et al.*, 1968; Urs and Dunleavy, 1974).

Unlike peroxidase activity there was an early stimulation of polyphenol oxidase activity in most of the treatments especially after pathogen inoculation. The mean polyphenol oxidase activity of 6.67 g^{-1} fresh weight of leaf was significantly high in infected plants when compared to 4.46 in healthy plants (Table 31). Further, it was maximum (7.53) 24 h after pathogen inoculation. An early stimulation of polyphenol oxidase activity may be beneficial for inducing better host resistance to infection by a pathogen because of the known ability of this enzyme to oxidise phenolic compounds to more toxic quinones to which many pathogens are highly susceptible. Many early workers such as Umaerus (1959); Fehrmann and Dimond (1967); Maxwell and Bateman (1967); Hanusova (1969); Kosuge (1969); Maraite (1973); Uritani (1976); Yamamoto *et al.* (1978); Hammerschmidt *et al.* (1982); Tayal *et al.* (1984);

Arora and Wagle, 1985 could also get direct correlation between polyphenol oxidase activity and disease resistance in different crop plants.

The phenylalanine ammonia lyase (PAL) activity was also found to increase due to inoculation with *P. capsici*. Thus, the enzyme activity of 29.31 was significantly high in infected plants when compared to 22.49 in healthy plants (Table 34). Similar results were reported earlier in soybean Patridge and Keen (1977) and in pepper by Jebakumar *et al.* (2001). This increase in PAL activity was significant since it was always found associated with biosynthesis of phytoalexin in plant tissue conferring better resistance to infection by the pathogen (Friend, 1973). In addition to the pathogen, the biocontrol agents were also found to induce the production of this defence related enzyme. Thus, the enzyme activity of 29.88 with *T. harzianum* and 28.20 with *G. fasciculatum* were significantly high when compared to either the control treatment (22.76) or the chemical treatment (22.76) with 0.3 per cent copper oxychloride (Table 35). However, unlike peroxidase and polyphenol oxidase activities, the day of estimation had no significant effect on PAL activity.

The SDS PAGE analysis of soluble proteins in pepper var. Karimunda following inoculation with *P. capsici* clearly indicated the presence of two new pathogenesis related protein bands with molecular weights of 78 kDa and 28 kDa (Plate 10). Similar induction of PR proteins especially β1, 3 glucanase and chitinase has been reported by many earlier workers in potato (Schroder *et al.*, 1992), tomato (Enkerli *et al.*, 1993; Dassi *et al.*, 1998), *Capsicum annuum* (Kim and Hwang, 1994) and black pepper (Lee *et al.*, 2000; Jebakumar *et al.*, 2001). The protein band with molecular weight of 28 kDa belonged to the acidic PR proteins and is likely to be a polymer of acidic chitinase. Fritig *et al.* (1989) also reported the PR proteins with molecular weights 27.5 and 28.5 kDa belong to Group 3 P and Q respectively which is an acidic PR protein possessing chitinase activity. Taylor *et al.* (1990) reported the presence of several

acidic proteins which accumulated rapidly following inoculation with *P. infestans* in potato leaves and they found the molecular weights in the range of about 14-30 kDa. These findings were in accordance with the present investigation. However, the protein band with 78 kDa could be grouped along with unidentified PR proteins. Further studies would be required to identify the same. Further, the fact that these PR proteins were induced only in pathogen inoculated treatments also indicated that neither the biocontrol agents nor the carrier materials have got any significant role in induction of such pathogenesis related proteins.

SUMMARY

6. SUMMARY

The main objective of the present study was to explore the possibility of using vermicompost as a carrier material for mass production of biocontrol agents and to test its efficacy in controlling the foot rot disease of black pepper caused by *Phytophthora capsici.*

The pathogen causing foot rot of pepper was isolated from naturally infected plants collected from the District Agricultural Farm of the Department of Agriculture, Peringamala during the South West monsoon season and pathogenicity was proved. It was identified as *Phytophthora capsici* based on morphological and cultural characters besides its ability to induce typical symptoms of foot rot disease in a susceptible variety of pepper, Karimunda.

A talc based inoculum of *Trichoderma harzianum* and soil based granular inoculum of *Glomus fasciculatum* obtained from Department of Plant Pathology, College of Agriculture, Vellayani were used for the study. The primary inoculum of these biocontrol agents was mass produced in vermicompost either alone or in combination with farmyard manure or neem cake in the ratio 1 : 1 and 5 : 1 respectively and the efficacy was compared with the standard carrier material of farmyard manure and neem cake (10 : 1). Guinea grass was used as the host plant for mass production of *G. fasciculatum.*

The population of *T. harzianum* when estimated 15 DAI was maximum in the treatment combination of VC + NC. However, the influence of these carrier materials on percentage of mycorrhizal infection 50 DAI was not significant.

The efficacy of vermicompost as a carrier material for biocontrol agents was further tested by studying the extend of disease control achieved in pepper var. Karimunda inoculated with *P. capsici.* From the

observations recorded 7 DAI and 14 DAI with pathogen it was inferred that treatment effect was mainly significant for the biocontrol agents and carrier materials as scuh did not have a significant influence on disease control.

Among the biocontrol agents used disease control was maximum in treatments involving *T. harzianum*. Percentage of foliar infection and disease index were minimum in treatments with *T. harzianum* alone where as the percentage stem infection and percentage mortality were minimum in the treatment combination of *T. harzianum* + *G. fasciculatum*. The fresh weight and dry weight of shoot and root were maximum in treatments with *G. fasciculatum*. Since the disease control was more with *T. harzianum* and growth characters with *G. fasciculatum*, these were selected for further studies. In the second part of the present investigation, induction of plant phenolics, defence related enzymes and pathogenesis related proteins due to inoculation with *P. capsici* and application of biocontrol agents were studied.

Pathogen inoculation increased the phenol content of plants especially after 10 days and was maximum in the treatment combination of VC + NC + G. Higher phenol content was noticed in infected plants compared to healthy. The OD phenol content increased immediately after pathogen inoculation and was maximum in the treatment combination of VC + NC + T 24 h after pathogen inoculation.

Peroxidase activity in plants increased with the day of estimation after pathogen inoculation and a peak level was obtained after 10 days. The mean enzyme activity in infected plants was higher compared to uninoculated plants. Maximum peroxidase activity was recorded in treatments with *G.fasciculatum* .An early stimulation of polyphenol oxidase activity was pronounced after pathogen inoculation and it was more in treatments with *G. fasciculatum*.The phenylalanine ammonia lyase activity was significantly high in infected plants and had no relation with

the day of estimation.*T.harzianum* and *G.fasciculatum* treated plants had a high enzyme activity compared to copper oxychloride treated and control plants.

SDS-PAGE study indicated that protein profile of healthy and infected leaves of pepper one day after pathogen inoculation differed in the total number and intensity of the protein bands. In the infected leaves two additional protein bands with molecular weights of78kDa and 28 kDa were observed which were absent in healthy as well as copper oxychloride treated plants.

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7. REFERENCES

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APPENDICES

APPENDIX -I

A. Carrot agar medium

Carrot	-	200 g
Dextrose	-	20.0 g
Agar	-	20.0 g
Distilled water	-	1000 ml.

B. Oat meal agar medium

Oats	-	100 g
Agar	-	20.0 g
Distilled water	-	1000 ml

C. Rose bengal agar medium

Dextrose	-	10 g
Peptone	-	5 g
KH_2PO_4	-	1 g
$MgSO_4.7H2O$	-	0.5 g
Rose bengal	-	0.035 g
Agar	-	20 g
Distilled water	-	1000 ml
Streptomycin	-	30 mg

APPENDIX –II

Composition of reagents and buffers

A. Lacto phenol reagent

Lactic acid	-	20 ml
Phenol	-	20 ml
Glycerol	-	40 ml
Distilled water	-	20 ml

B. 0.1 M sodium phosphate buffer (pH 6.5)

Solution A : 3.12 g of $NaHPO_4$ in 100 ml

Solution B : 3.55g of Na_2HPO_4 in 100ml

68.5 ml of A was added to 31.5 ml of B and the final volume was made up to 200 ml.

C. 0.1 M Sodium borate buffer (pH 8.8)

A : 0.2 M solution of boric acid (12.4g in 1000 ml)

B : 0.05 M solution of borax (19.05g in 1000 ml)

50 ml of A was mixed with 30 ml of B and the final volume was made upto 200 ml

D. Arnon's reagent

Sodium molybdate	-	10 g
Sodium nitrite	-	10 g
distilled water	-	100 ml

E. Denaturing Solution

8 M Urea	-	48.05	g
50 mM $NaH_2PO_4.2H_2O$ (pH 8.0)	-	5.0	ml
10 mM Tris (pH 8.0)	-	1.0	ml
100 mM NaCl	-	0.58	g

Final volume was made upto 100 ml with double distilled water

MANAGEMENT OF FOOT ROT OF BLACK PEPPER (*Piper nigrum* L.) WITH MYCOINOCULANT ENRICHED VERMICOMPOST

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Abstract of the
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8. ABSTRACT

The study, "Management of foot rot of black pepper (*Piper nigrum* L.) with mycoinoculant enriched vermicompost" was done at the Department of Plant Pathology, College of Agriculture, Vellayani to explore the possibility of using vermicompost as a carrier material for mass production of biocontrol agents and to test its efficacy in controlling the disease. The talc based inoculum of *Trichoderma harzianum* and soil based granular inoculum of *Glomus fasciculatum* were mass produced in vermicompost either alone or in combination with farmyard manure or neem cake in the ratio 1 : 1 and 5 : 1 respectively and the efficacy was compared with the standard carrier material of FYM + neem cake (10 : 1). The population of *T. harzianum* 15 DAI was maximum in the treatment combination of VC + NC (5 : 1). However, the influence of these carrier materials on percentage of mycorrhizal infection 50 DAI was not statistically significant. Vermicompost as a carrier material for biocontrol agents was further tested in pepper var. Karimunda inoculated with *P. capsici*. Carrier materials as such had no significant influence on disease control. Reduction in foliar infection, disease index, stem infection and percentage mortality was observed in treatments with biocontrol agents. Disease control was maximum with the treatments involving *T. harzianum* while plant growth promotion was maximum with *G. fasciculatum*. The physiological changes in pepper plants after inoculation with *P. capsici* and biocontrol agents were studied. The phenol and OD phenol content was more in pathogen inoculated plants. The defence related enzymes peroxidase, polyphenol oxidase and phenylalanine ammonia lyase were enhanced with pathogen inoculation as well as with the application of biocontrol agents. SDS-PAGE analysis of proteins with samples extracted from plants one day after inoculation of pathogen showed the presence of two novel proteins with molecular weights of 78 kDa and 28 kDa in diseased samples which were absent in treatments without pathogen and also in plants treated with COC.