MANAGEMENT OF FOOT ROT OF BLACK PEPPER (*Piper nigrum* L.) WITH VA MYCORRHIZA AND ANTAGONISTS

By

CHRISTIN P. ROBERT

THESIS
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Dedicated

to

the Sacred Memory

of

Late Samuel appachan

and

Late Prof. Abdul Hameed

DECLARATION

I hereby declare that this thesis entitled "*Management of foot rot of black pepper (Piper nigrum L.) with VA mycorrhiza and antagonists*" 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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4-1-1999

CHRISTIN P. ROBERT

CERTIFICATE

Certified that this thesis entitled "*Management of foot rot of black pepper (Piper nigrum L.) with VA mycorrhiza and antagonists*" is a record of research work done independently by Mr. Christin P. Robert (93-21-15) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Vellayani
~~-07-~~ 1998
4-1-1999

Dr. P. Sivaprasad
Associate Professor (Ag. Micro biology)
Department of Plant Pathology,
College of Agriculture, Vellayani

APPROVED BY

Chairman

DR. P. SIVAPRASAD

Members

DR. S. BALAKRISHNAN

DR. G. SREEKANTAN NAIR

DR. P. RAJENDRAN

DR. M. VIJAYAN

External examiner

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INTRODUCTION

INTRODUCTION

Black pepper (*Piper nigrum* L.) popularly known as 'king of spices' is the most important spice crop of India. It is a native of Malabar coast of Westernghats of India and accounts for an annual export earning of Rs.226 crores. India, a leading producer and exporter of black pepper, contributes about 20 per cent of total world production with an area of 1,833,400 hectares (Sarma and Anandaraj, 1996) with an annual production of 46,100 tonnes. Kerala accounts for 90 per cent of total area under pepper in the country. Inspite of steady increase in area under black pepper cultivation in Kerala, there is no corresponding increase in production. The average yield in the state is far from satisfactory (314 kg ha^{-1}). The productivity of pepper in India (240 kg ha^{-1}) is only 6 per cent of that of Indonesia (4060 kg ha^{-1}). The reason for such a poor productivity can be attributed to various factors including diseases, pests and nutrition.

Of the 17 diseases reported in black pepper (Sarma *et al.*, 1991), foot rot incited by *Phytophthora capsici* Leonian emend A. Alizadeh and P.H. Tsao (Tsao, 1991) has been identified as the major production constraint in India. The fungus, irrespective of the age of the plant, infects roots, stem as well as leaves of black pepper and causes a loss of

30 to 100 per cent vines (Dutta, 1984). Annual yield loss of 905 and 119 tonnes of black pepper has been reported from Kannur and Kozhikode districts (Balakrishnan *et al.*, 1986, Anandaraj *et al.*, 1989).

The present management practices against the disease are mainly prophylactic by applying chemical fungicides (Bordeaux mixture 1%, copper oxychloride 0.2%). However, the constant use of chemical fungicides bringing about many environmental and ecological problems needs to be viewed seriously. Besides, black pepper being an export oriented crop, the residual toxicity and connected problems are matters of major concern. The increasing awareness of the possible deleterious effect of fungicides on the ecosystem and the growing interest in pesticide free agricultural products have created much enthusiasm among the scientists on the biological control of plant pathogenic fungi. The success of biological control in many diseases, frequent failure of pesticides and the difficulties and cost involvement associated with finding new pesticides furthered the interest in biological control. Reports are available on the successful suppression of foot rot pathogen of black pepper by different antagonistic soil heterotrophic fungi (Anandaraj and Sarma, 1994b; Sivaprasad, 1995; Sarma *et al.*, 1996). In the present study the concept to combine the biocontrol agents with different modes of suppression, viz., Arbuscular Mycorrhizal Fungi (AMF) and

antagonistic fungi to maximize the effectiveness of the system was attempted.

Arbuscular Mycorrhizal Fungi (AMF) are receiving considerable attention in the recent years because mycorrhizal plants have several advantages over non—mycorrhizal plants. Mycorrhizal association enables better plant growth and reduces the infection caused by many soilborne plant pathogens (Mamta Sharma and Mukerji, 1992). In fact the possibility of using mycorrhizal system to suppress soilborne pathogens and to promote plant growth is under vigorous exploration. Encouraging results have been reported on the AMF - root pathogen interaction and the potential of AMF in suppressing many important pathogenic species including *Phytophthora* (Davis and Menge, 1980; Sivaprasad *et al*., 1993; Sivaprasad, 1995). Mycorrhiza induced tolerance to soilborne pathogens is explained to be due to the physiological and biochemical changes occurring in the host (Mosse, 1973; Barea, 1992; Blee and Anderson, 1996) and also to better host nutrition (Azcon-Aguilar and Barea, 1997). Mycorrhizal hypha functions as analogous to plant root hair and helps the plant to acquire soil nutrients, especially less mobile elements such as P, Cu and Zn. Further, it improves the important physiological traits related to plant growth and biomass production. The desirable characters of AMF make it a potential biological means for controlling soilborne diseases as well as a useful

biofertilizer. However, except the preliminary reports, no serious attempt has been made to exploit the potential of AMF to control the foot rot disease and growth improvement.

Since the disease is soilborne, antagonistic microorganisms may play a major role in keeping the population of pathogen at low levels. In nature, many fungi are known to grow on other fungi and is generally named as mycoparasites. Many potential mycoparasites exhibit antibiosis by producing inhibitory metabolites. Species of *Trichoderma*, *Aspergillus* and *Gliocladium* are found successful in the control of many soilborne pathogens (Shukla and Dwivedi, 1979; Claydon *et al*., 1987; Lin *et al*., 1994). It is reported that foot rot pathogen is also very well repressed by species of such antagonists (Sarma *et al*., 1996).

It is well established that the microbial inoculants adapted to soil environmental conditions of the introduced system will be more competitive and exhibit better multiplication, persistence and activity. In this point of view the organism developed from native soil will be more desirable. The microbial interactions, including AMF, in the rhizosphere are either stimulatory or inhibitory (Linderman, 1988). Reports are available on the positive influence of AMF with antagonistic organisms and cumulative effect on disease suppression (Calvet *et al*., 1992; Calvet *et al*., 1993). However, no detailed work has been done

in this direction for foot rot disease management. The present investigation designed to develop a management strategy using native AMF and antagonists was attempted for the biocontrol of foot rot in green house and field with major emphasis on the following aspects:

1. Testing the influence of native AMF isolates against foot rot incidence and intensity, nutrient uptake and growth of black pepper

2. Characterization of AMF associated with black pepper genotypes in different soils

3. Isolation and development of fungal antagonists against *Phytophthora capsici* through *in vitro* and *in vivo* screening

4. Evaluation of AMF and antagonists interaction on incidence and intensity of foot rot in black pepper

5. Development of a technique for AMF inoculation to pepper vines in established plantations

6. Development of foot rot management strategy for established plantations using selected AMF and antagonists

7. Analysis of chemical and biochemical changes induced by AMF colonization in black pepper

REVIEW OF LITERATURE

REVIEW OF LITERATURE

'Foot rot' of black pepper caused by *Phytophthora capsici* is a devastating disease causing serious economic loss in the principal pepper growing countries such as India, Indonesia, Malaysia and Brazil (de Waard, 1986; Sarma *et al*., 1991). No precise data are available on crop losses due to foot rot incidence. However, crop loss on global scale is estimated to be about 4.5 to 7.5 million dollars (de Waard, 1979). In India, crop loss to the tune of 25-30 per cent vine death has been reported from Kerala (Nambiar and Sarma, 1977). The survey conducted in Kozhikode and Kannur districts of Kerala recorded foot rot incidence of 3.7 and 9.4 per cent, resulting in an annual loss of 119 and 905 tonnes of black pepper respectively (Balakrishnan *et al*,. 1986; Anandaraj *et al*., 1989). There are instances of complete wipe out of pepper plantations due to the disease in many areas of Kerala particularly in Idukki and Wayanad districts.

In India, the occurrence of the disease was known since 1902 (Barbar, 1902). Although Venkata Rao (1929) isolated *Phytophthora* sp. from diseased black pepper in Karnataka, the first authentic report on the *Phytophthora* wilt in black pepper was by Samraj and Jose (1966) from Kerala. The 'foot rot' pathogen, earlier described as *Phytophthora palmivora*

MF_4 (Sarma *et al*., 1982; Tsao *et al*., 1985) has been confirmed as *Phytophthora capsici* Leonian emend A. Alizadeh and P.H. Tsao (Tsao, 1991).

Foot rot disease management in black pepper is mainly based on chemical and cultural methods (KAU, 1996). The serious health and environmental problems associated with chemical methods triggered world wide interest in biological control. Antagonistic fungi especially species of *Trichoderma* were found successful for the biological control of root diseases of many crop plants. However, attempt made to utilise such biocontrol agents including AMF and their interaction with antagonists for the management of foot rot of black pepper is meagre (Sivaprasad, 1998). Arbuscular mycorrhizal fungi, in addition to their role as a deterrent to root pathogens, are also well recognized for their symbiotic effect on plant growth and development.

2.1 Effect of AMF on plant growth

Mosse (1957) demonstrated for the first time the improved P uptake and growth of apple due to mycorrhizal inoculation and observed that mycorrhizal apple also absorbed more P, K, Fe and Cu than non mycorrhizal plants. Later, Baylis (1959) confirmed the enhanced uptake of phosphorus by mycorrhizal plants. Since then the beneficial effect of arbuscular mycorrhizal association on soil nutrient uptake and

plant growth has been reported in most crop plants (Gerdemann, 1968; Abbott *et al*. 1984; Dehne and Backhous, 1986; O'keefe and Sylvia, 1990; Barea, 1992). Improved plant growth in most cases was related to the increased uptake of micro and macro nutrients, phosphorus in particular, from 'P' deficient soils. The mycorrhizal growth enhancement has been attributed to the increased nutrient uptake achieved by increasing the surface area of absorption mobilising sparingly available nutrient sources and by secretion of ecto enzymes (Sanders and Thinker, 1971; Rhodes, 1980).

Daft and Nicolson (1966) studied the response of tobacco, tomato and maize to mycorrhizal inoculation and noticed remarkable increase in growth and biomass production with AMF colonization. However, the response was dependent on soil nutrient status and intensity of AMF colonization in the root. Low P level favoured the growth and mycorrhizal development. Enhanced growth and yield have been reported in mycorrhizal soybean plants with greater accumulation of N, P, Ca, Cu and Mn in the foliage than non mycorrhizal plants (Ross and Harper, 1970; Ross, 1971). Hayman and Mosse (1971) reported significant increase in shoot dry weight of onion (nineteen fold) due to mycorrhizal inoculation in P deficient soil. A complete correction of Zn deficiency was possible in peach plants with mycorrhizal treatment (Glimore, 1971). Mycorrhizal seedlings of maize when planted in the field showed improved

growth compared to non-mycorrhizal control. Further, there was positive correlation between spore number in the soil and extent of root colonization and growth response of the host (Khan, 1972). Ross and Gilliam (1973) evaluated seed yield of mycorrhizal soybean in P deficient soil and noticed increased plant growth and yield. Mycorrhizal peach seedlings were reported to extract more Zn and P from soil (La-Rue *et al*. 1975). Increase in shoot and root dry weight in cowpea, tomato and maize showed a positive relationship with mycorrhizal infection (Sanni, 1976). Amees and Linderman (1978) noticed higher root volumes with higher per cent mycorrhizal colonization in easter lilly in soils of low P availability.

Bagyaraj and Manjunath (1980) found significant increase in root and shoot weight of cotton, cowpea and finger millet inoculated with *Glomus fasciculatum*. The effect was attributed to increased uptake of P and Zn due to mycorrhizal colonization. However, no difference in Mn content over uninoculated plants was noticed. Onion seedlings inoculated with *G. macrocarpus*, *G. mosseae* and *Sclerocystis rubiformis* produced significantly higher plant growth than in non-mycorrhizal control (Clarke and Mosse, 1981). Consistent results on growth and yield of different plants inoculated with various mycorrhizal isolates have also been reported (Krishna, 1981; Marschner and Dell, 1994). The well developed

mycorrhizal root system with an altered cellular process absorb more nutrients and water by expanded absorptive capacity which in turn results in improved growth and yield of plants (Graham *et al*., 1981; Reid, 1984, Hooker *et al*., 1994).

Hayman (1982) studied the physiology of arbuscular mycorrhizal symbiosis and observed that the mycorrhizal fungi reach beyond the depletion zone and translocate nutrient directly to the root cortex. Jarrell and Beverly (1985) reported dilution of toxic metal concentration in mycorrhizal plants due to enhanced biomass production which is attributed to the reason for reduced concentration of Mn, in mycorrhizal soybean (Bethlenfalvay and Franson, 1989). Studies on the uptake of immobile nutrients and micronutrients in mycorrhizal *Leucaena leucocephala* (Sivaprasad *et al*., 1993: Manjunath and Habte, 1988). Cocoa (Sivaprasad *et al*., 1984), soybean (Pacovasky, 1986), Cassava and sweet potato (Sivaprasad *et al*., 1989; Sivaprasad *et al*., 1990a), rice (Sivaprasad *et al*., 1990b) and cashew (Sivaprasad *et al*., 1992) revealed enhanced uptake, plant growth and biomass production due to mycorrhizal root colonization. Barea (1992) that increased level of soluble P in soil is determinent of mycorrhizal development in the root.

Tarafdar and Marschner (1994) studied phosphatase activity in the rhizosphere and hyphosphere of mycorrhizal wheat plants supplied with inorganic and organic P sources. According to them the enhanced plant growth in wheat with mycorrhizal association was not only due to improved P uptake but also the better availability of other elements like K, Zn, Cu, S, Al, Mn, Mg, Fe etc. Mungbean cultivars dually inoculated with *G. fasciculatum* and *Rhizobium* sp. increased plant growth due to enhanced uptake of K, Fe, Zn and Cu in addition to P. (Panwar and Thakur, 1994).

Sulochana *et al.* (1995) observed enhanced growth, yield and biomass production in cassava inoculated with mixed inoculum of *G. fasciculatum* and *G. etunicatum*. According to Sivaprasad (1995) nursery inoculation of cardamom seedlings with *Acaulospora morrowea* and *G. mosseae* significantly increased tillering and growth in the nursery and field. Similarly, inoculation with AMF fungi at the time of planting significantly increase the growth, biomass production and rhizome yield of ginger and turmeric in the green house and field in oxisol soil with medium P level (Sivaprasad, 1995; Joseph and Sivaprasad, 1997a).

In addition to growth enhancement effect AMF have been recognised as efficient biocontrol agent of several soilborne plant pathogens (Dehne, 1982; Jalali and Jalali, 1991; Azcon-Aguilar and Barea, 1997). Other principal benefits

of arbuscular mycorrhizal symbiosis are reduced transplant shock (Schenck and Kellam, 1978), tolerance to abiotic and biotic stresses (Lavy, *et al.*, 1983; Sieverding 1991; Barea and Jeffries, 1995), increased photosynthetic efficiency (Schoenbeck 1979 and Sivaprasad, 1983), enhanced growth hormone activities (Sivaprasad (1983), improved water relation (Panwar, 1993).

2.2 AMF as biocontrol agent

As early as 1956 Garret reported that the damage caused by pathogens in root tissue is relatively high when the AMF structures on plant roots are absent. However serious studies on arbuscular mycorrhizal symbiosis and its possible protective role against plant pathogens began only in the late 1960's (Safir 1968).

Ross (1972) noticed internal stem discolouration on 88 per cent of susceptible soybean plants grown in sandy loam soils infested by *Phytophthora megasperma* and chlamydosporic species of *Endogone*. The mortality due to the root rot was 33 per cent. In pots, when *Phytophthora* was inoculated alone, 17 per cent of plants developed internal stem discolouration without any mortality. However, even the disease tolerant cultivars showed more severity of disease when inoculated with *Endogone*. AMF induced suppression of disease incited by species of *Phytophthora* in papaya (Ramirez, 1974).

The effectiveness of biocontrol agents including AMF fungi depends on the virulence and inoculum potential of the pathogen in the soil. Baltruschat *et al*. (1973) reported that high inoculum density of *Thielaviopsis basicola* in the rhizosphere of mycorrhizal cotton plants rendered mycorrhizal fungi ineffective for biocontrol. Seedlings inoculated with *Glomus fasciculatum* showed significant reduction in the root colonization by *Cylindro carpon destructans* in strawberry (Peget, 1975). Similarly, mycorrhizal cotton and poinsettia seedlings exhibited less damage due to *T. basicola* (Schoenbeck and Dehne, 1977), *Pythium ultimum* and *Rhizoctonia solani* (Stewart and Pfleger, 1977). Root colonization of AM fungi suppressed the oospores of *Phytophthora* sp. on citrus (Davis *et al*., 1978; Davis and Menge, 1980).

Ames and Linderman (1978) noticed reduced crop damage in mycorrhizal easter lilly by *F. oxysporum* and attributed enhanced phosphorus nutrition. Similar observations on suppression of *Phytophthora* root rot in alfalfa and citrus have also been reported (Davis *et al*., 1978). They also reported little or no resistance to *Phytophthora parasitica* on citrus plants pre inoculated with *G. fasciculatum* and noticed increased damage in mycorrhizal avacado seedlings by *P. cinnamomi* and in cotton by wilt pathogen *Verticillium* sp. (Davis *et al*., 1979); Davis and Menge (1980) reasoned the

improved phosphorus nutrition by *G. fasciculatum* inoculation as a factor responsible for increased *Verticillium* wilt in cotton and *Phytophthora* root rot in citrus.

Since both AMF and plant pathogens are soilborne, the competition for space in the host tissue can influence the development of pathogen and mycorrhizal fungi as well. Davis and Menge (1981) studied the interaction effect of nine different AMF symbionts with *Phytophthora* root rot in citrus and noticed that *G. fasciculatum* and *G. constrictum* were more effective for disease suppression. They further observed that competition occurs between *G. fasciculatum* and *Phytophthora parasitica* for infection site on citrus root. Similar competition for space between arbuscular-mycorrhizal fungi and *Fusarium avenacearum* was also noticed in clover (Dehne, 1982).

Take all disease suppression in wheat by AMF was attributed to enhanced macro and micro nutrient uptake (Reis *et al.*, 1981). Although there are numerous reports on arbuscular mycorrhiza mediated disease suppression in crop plants, the mycorrhizal symbionts have not been recorded to interact with pathogens through antagonism, antibiosis or predation (Baker and Cook, 1982). Several other reports demonstrated the mycorrhiza mediated plant disease suppression particularly by enhanced nutrient uptake and other physiological changes in host plant which ameliorate the effect

exerted by soil fungal pathogens such as species of *Verticillium* in crysanthemum (Pegg and Jouglaekha, 1981) *Gaeumannomyces graminis* causing take all disease in wheat (Graham and Menge, 1982) *Fusarium solani* in soybean (Zambolim and Schenck, 1983), *Pythium ultimum* disease in poinsettia (Kaye *et al.*, 1984) and *Sclerotium rolfsii* of pea nut (Krishna and Bagyaraj, 1983).

Improved phosphorus nutrition, increased root growth and subsequent reduction in root exudation (Graham *et al.*, 1981; Smith *et al.*, 1986) due to AMF colonization are known to be the basis for reduced root infection by pathogenic fungi (Mamata Sharma and Mukherji, (1992). The disease suppressive activity in soybean and poinsettia was attributed to such improved 'P' nutrition by mycorrhizal symbionts (Zambolim and Schenck, 1983; Kaye *et al.*, 1984). However no impact of enhanced 'P' uptake by mycorrhizal tomato plants in suppressing verticillium wilt was noticed by Baath and Hayman (1983). Inoculation with *Verticillium alboatrum* decreased plant growth compared to noninoculated controls irrespective of mycorrhizal status of the plant. It is generally noticed that pathogenic interference in plant root tissue mostly occur where arbuscular mycorrhizal fungal structures do not occur (Harley and Smith, 1983; Linderman, 1985).

Zambolim and Schenck (1983) studied the root infecting fungi on soybean seedlings inoculated with

G. mosseae. There was no significant alteration in the per cent root infection or number of sclerotia produced by the pathogen (*Macrophomina phaseolina*) per gram of soil. However, the crop yield was higher than uninoculated control, indicating positive influence of arbuscular mycorrhizal symbionts without affecting the incidence of the pathogen in host. Interaction between *G. intraradices* and *Fusarium oxysporum* f.sp. *radicis lycopersici* in tomatoes showed that root colonization by *G. intraradices* was not affected by *F. oxysporum* inoculation. Roots colonized with *G. intraradices* also exhibited reduction in rot suppression due to *Fusarium* infection (Caron *et al.*, 1986). Several root invading pathogens are known to exert deficiencies of certain essential elements mainly Ca, Zn, Cu, Mn and S in host plants while, arbuscular mycorrhizal formation ameliorates such deficiencies. Pacovasky (1986) reported alleviated Zn deficiency by AMF in tomato which was evidenced with pathogenic invasion.

AMF mediated quantitative and qualitative shift in the microbial population in the mycorrhizosphere also contributes to disease suppression in soil. Mayer and Linderman (1986) studied the selective influence of mycorrhizal formation by *G. fasciculatum* on actinomycetes and certain rhizosphere bacteria. They noticed that change in soil microbial population stimulates certain microbiota that are antagonistic to root pathogens. Application of extract of

rhizosphere soil from mycorrhizal citrus plants reduced sporangial as well as zoospore production in *Phytophthora cinnamomi*. Secilia and Bagyaraj (1987) isolated more antagonistic actinomyces from the rhizosphere of different arbuscular mycorrhizal plants than non-mycorrhizal controls. Interaction of mycorrhizal fungi with several soil borne plant pathogens and other organisms also revealed that enhanced phosphorus uptake in mycorrhizal plants reduced membrane permeability resuling in lower root exudation. The quantitative and qualitative change in root exudates altered the rhizosphere activity of soil micro organisms including plant pathogens (Graham, 1988). Reduction in *Fusarium* population was noticed in the mycorrhizosphere of tomato plants (Caron, 1989).

The role of arbuscular mycorrhizal symbionts in the biological control of plant diseases has been reviewed by Jalali and Chand (1988) with particular emphasis on the influence of mycorrhizal fungi on disease incidence and development. They concluded that AMF is a potential biocontrol agent for the management of root diseases of crop plants. Chabbra *et al*. (1992) studied the influence of *G. fasciculatum* on maize leaf blight (*Helminthosporium maydis*), seedling blight and stalk rot (*F. moniliforme*) and *Acremonium* stalk rot (*Acremonium kiliense*) and noticed complete resistance of mycorrhizal maize plants to *F. moniliforme*.

The altered physiology of mycorrhizal plants play a decisive role in the root exudation pattern which in turn influence the rhizosphere flora (Azcon-aguilar and Bago 1994; Amora-Lazcano *et al.*, 1998). Volpin *et al.* (1994) emphasised that mycorrhiza mediated direct defence mechanism in the alfalfa roots protects the plants against the pathogens, while Gianinazzi-pearson *et al.* (1994) were of the view that only weak activation of plant defence mechanism occurred during mycorrhizal formation.

Joseph *et al.* (1995) studied the influence of mycorrhizal colonisation in relation to natural incidence of ginger rhizome rot (*Pythium aphanidermatum*). They noticed that higher arbuscular mycorrhizal colonization reduced the intensity of rhizome rot under natural conditions. Healthy plants showed higher mycorrhizal colonisation than in diseased ones. Sivaprasad (1995) studied azhukal disease (*Phytophthora meadii*) development in cardamom seedlings pre inoculated with different mycorrhizal fungi. On transplanting to the field of heavy pathogenic infection, complete disease suppression was recorded in seedlings inoculated with *G. mosseae* even after one year. They also observed varied degrees of rhizome rot development in ginger when inoculated with different arbuscular

mycorrhizal fungi. Plant mortality was reduced with *G. mosseae* (17%) and *G. fasciculatum*. (23%) over control (47%) under field conditions.

Cordier *et al*. (1996) studied colonisation of root tissues of mycorrhizal tomatoes by *Phytophthora nicotianae* and revealed that the pathogen related loss of root biomas and function was compensated by mycorrhizal fungi. Development of *P. nicotianae* was negated by induction of resistance away from the point of mycorrhizal colonization in the root tissues. Blee and Anderson (1996) noticed initial suppression of host reaction as a signal for induction of such symbiotic interaction in *Phaseolus vulgaris* inoculated with *Glomus intraradices*. Azcon-Aguilar and Barea (1997) reviewed the work on arbuscular mycorrhizal symbiont induced plant defense mechanisms in achieving disease suppression in various host plants, and suggested that primary access of mycorrhizal fungi to host root system coupled with high demand for carbon compounds may inhibit the growth of the pathogen and thus providing protection against the invading pathogens.

2.3 AMF Association in black pepper

The occurrence of arbuscular mycorrhizal association in black pepper has been reported by many workers (Ramesh, 1982; Manjunath and Bagyaraj, 1982). Shivashankar and Rohini Iyer (1988) demonstrated positive effect of AMF (*Glomus fasciculatum*) colonisation on the growth, phosphorus uptake and

nitrate reductase activity in black pepper. The shoot dry weight was significantly increased resulting in the significant reduction of root-shoot ratio. The growth promotion of black pepper with AMF association was noticed by Bopaiah and Abdul Khader (1989). Improved growth of mycorrhizal black pepper cuttings through elevated P uptake was also reported by Sivaprasad (1995). *Glomus fasciculatum* was effective in promoting the rooting and growth of black pepper cultivar karimunda. The mycorrhizal plants showed higher establishment rate of 98 per cent in the field (Anandaraj and Sarma, 1994a). Sivaprasad (1995) reported *G. monosporum* as the most promising arbuscular mycorrhizal fungus in promoting establishment and growth of black pepper under green house and field conditions.

Sarma *et al*. (1991) noticed low K, Ca and Mg level in foot rot infested black pepper tissues and suggested the application of Ca, Mg and K fertilizer to suppress the foot rot disease development. Suppressionn of foot rot, caused by *P. capsici*, in black pepper with *Glomus fasciculatum* was reported for the first time by Anandaraj *et al*. (1993). Subsequent reports further established the suppressive effect of arbuscular mycorrhizal fungi on foot rot incidence, enhanced root regeneration, nutrient uptake and altered physiology in mycorrhizal plants which is thought to be a possible reason for ameliorating foot rot incidence by *P. capsici* (Anandaraj and Sarma, 1994a; 1994b).

Sivaprasad *et al*. (1995a) noticed negative relationship between foot rot incidence and per cent mycorrhizal root colonisation and spore count in black pepper mycorrhizosphere. Arbuscular mycorrhizal colonization in black pepper roots was decreased with increased disease intensity under natural conditions. According to Sivaprasad (1995) considerable reduction in foot rot incidence could be achieved with native AMF isolates which are more adopted to local soil conditions. Inoculation with effective native isolates enabled plants to escape from the disease during the most favourable period for pathogenic attack. He further added that, although none of the mycorrhizal cultures tested could provide absolute resistance, *Glomus monosporum*, *G. fasciculatum* and *G. etunicatum* were found to be more effective with less mortality rate of 25, 50 and 25 per cent respectively, as against 75 per cent for non-mycorrhizal control plants in the field. AMF inoculated black pepper cuttings when planted in a diseased field, there was significant reduction of disease incidence in mycorrhizal plants (16.5 per cent) as compared to control (28.5 per cent) (DARE, 1996).

Sarma *et al*. (1996) observed enhanced rooting in black pepper cuttings by different mycorrhizal fungi and suggested that mycorrhizal development compensate the root damage by foot rot pathogen (*P. capsici*). 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*. (1997) observed that incidence of foot rot was significantly less in pepper plants pre-inoculated with *Glomus monosporum*. Further, the P and phenol content were enhanced in the mycorrhizal plants. However, extensive information on improved nutrition particularly that of micronutrients and altered physiology brought about by AMF association in black pepper is scanty.

2.4 Effect of soil and plant genotypes on AMF distribution

Wide variation in the qualitative and quantitative distribution of AMF symbionts in relation to soil and host genotypes have been reported. Potty (1990) conducted a detailed survey on AMF association in tuber crops grown in different soil types. There was wide variation in spore population and AMF colonization with soil and host genotypes. Highest spore load was recorded in alluvial soils followed by sandy and sandy loam soils Species of arbuscular mycorrhizal fungi belonging to genera *Glomus* and *Gigaspora* were most predominant in the rhizosphere of tuber crops. Studies on the mycorrhizal status of plants grown in on overburden soil at opencast coal mine sites showed the predominance of several species of *Glomus* including *G. ambissporum*, *G. margiratum*, *Acaulospora scorbiculata* and *Scutellispora calospora* (Mehrotra, 1995).

Studies on the characterisation of AMF symbionts associated with black pepper showed that *Glomus fasciulatum* is the most predominant arbuscular mycorrhizal fungi in organically rich forest soils of Kerala (Lekha *et al.*, 1995). Presence of *Sclerocystis coremioides* and *S. clavispora* have also been recorded. Survey carried out by Sarma *et al.* (1996) in Karnataka and Kerala states revealed associations of *Glomus fasciculatum*, *G. microcarpum* and *Gigaspora gigantea* in black pepper. Similarly the influence of host genotype and soil types on AMF association in spice crops (cardamom, pepper, ginger and turmeric) also has been reported (Sivaprasad, 1995).

2.5 Fungal antagonists as plant growth stimulants

Reports are available on the stimulatory effect of biocontrol agents in promoting plant growth when used as either seed treatment or soil application. Lindsey (1967) reported that microorganisms could induce growth of higher plants under gnotobiotic conditions. Plant growth responses are expected when the roots and rootlets are maintained in a state of health necessary for the uptake of nutrients (Bezdicek and Power, 1983). Beneficial microrganisms are strong competitors for one or more nutrients on the root surface and able to inhibit the pathogen directly by producing antibiotics, which enables them to provide consistent root protection and thus, the plant growth improvement (Cook and Baker, 1989).

Raw or steamed soil colonized with *T. harzianum* hastened flowering of periwinkle and increased the number of blooms produced per plant and biomass production in chrysanthemum (Chang *et al.*, 1986). According to Windham *et al.* (1986) *Trichoderma* spp. produced a growth regulating factor that increased the rate of emergence of tomato and tobacco seedlings. Report on the promotion of radish growth in raw soil by application of *Trichoderma harzianum* in the form of conidia or in peat bran culture formulation was proposed by Baker (1989). Shoot and root growth two commercial maize hybrids grown in *T. harzianum* infested soil was found to be better than that grown in uninfested field (Windham *et al.*, 1989).

Vrang *et al.* (1990) noticed increase in growth and yield of potato when the seed tubers were inoculated with *Trichoderma* spp. Early flowering of potato was also achieved with *Trichoderma viride* treatment (Lilyiona, 1991). She also noticed increased plant height, biomass production and grain yield in paddy seedlings when treated with culture of *T. harzianum* at the time of transplanting. Lynch *et al.* (1991) reported that some strains of *Trichoderma* induced seedling emergence of lettuce and produced larger plants. In legume seed treatment with *T. viride* and *Bacillus subtilis* along with *Rhizobium* spp. increased nodulation and plant growth characteristics (Sridhar *et al.* (1992). Reports on the effects of biocontrol agents on growth of black pepper are scanty.

According to Sarma *et al*. (1996) solarised nursery mixture fortified with mycorrhizal propagules in combination with a mixture of *Trichoderma* spp and *Gliocladium* sp yielded healthy and robust rooted cuttings of black pepper in the nursery.

2.6 Fungal antagonists for root disease management

Biological methods of disease control are mainly aimed at biological destruction of soilborne pathogens without impairing ecological balance. Although, concerted efforts on biocontrol of root pathogen were made since 1930's (Weindling, 1932), pragmatic approach to tackle the problem was made only in recent years. The mechanisms involved in the suppression of the pathogens through 'antagonism' differ among various biocontrol organisms (Parkinson and Waid, 1960; Mukhopadhyay, 1994).

Use of antagonistic fungal combinations would be more promsing for achieving better plant disease control. Welvaert (1961) reported positive effect on the use of combination of *Trichoderma lignorum* and *Penicillium commune* against *Fusarium oxysporum* f. sp. *melonis*. Seed inoculation with combinations of diverse organisms such as *T. viride*, *P. frequetans*, *Aspergillus* sp. and *Bacillus subtilis* are reported to control seedling blight and damping off caused by *Fusarium* spp., *Rhizoctonia solani* and *Pythium* spp. on cereals, sugarbeet and mustard (Baker, 1968; Baker and Cook (1974). The biocontrol

activity of the antagonists are invariably associated with their ability of suppression or antagonism of vegetative growth of pathogens. Dennis and Webster (1971a, 1971b) identified acetaldehyde as an inhibitory metabolite of *Trichoderma viride* and recorded production of a chloroform soluble non-volatile antibiotic from *Trichoderma* spp.

Several studies suggested the potential use of *Trichoderma* spp. as an effective biocontrol agent against *Phytophthora* diseases of crop plants. A number of *Trichoderma* spp are also known to induce development of sex organs in normally sterile isolates of *Phytophthora* spp. (Brasier, 1971; Reeves and Jackson, 1972). This phenomena was reported to be associated with the suppression of vegetative growth of *Phytophthora* spp. either by volatile or soluble metabolites or by mycoparasitism (Brasier, 1975a, 1975b). Studies on *P. cinnamomi* in eucalyptus and avacado made by Malajczuk (1979) reaffirmed the role of *Trichoderma* spp. in the oospore formation and lysis of hyphae of the pathogen. Besides *Trichoderma* spp. number of fungi belonging to the genera of *Coniothyrium*, *Gliocladium*, *Latesaria*, *Penicillium*, *Sporodesmium*, *Aspergillus* and *Fusarium* and several bacteria and actinomycetes are known for their potential biocontrol activities against soilborne pathogens including several species of *Phytophthora* (Malajczuk, 1983; Adams, 1990; Naik and Sen, 1992).

Several *Aspergillus* spp are known to produce inhibitory substances which play a role in soil fungistasis (Johri and Singh, 1975). Such substances have also been isolated from the culture filtrates of *A. niger*, *A. flavus* and *A. candidus* (Shukla and Dwivedi, 1979).

A number of parasitic fungi capable of penetrating thick walls of both chlamydospores and oospore of *Phytophthora* spp. have been identified (Snahe *et al.*, 1977). It was noticed that Oomycetes, Hyphomycetes and Chytridiomycetes colonized on oospores of *P. megasperma* var *sojae* and *P. cactorum*. Bora (1977) demonstrated greatest antagonism of *A. niger* on *F. solani*, *R. solani* and *Alternaria* spp. both *in vitro* and *in vivo*. Huang (1978) studied antagonistic activity of *Gliocladium catanulatum* against *Sclerotinia sclerotiorum* and *Fusarium* sp. The antagonism was attributed to antibiosis and hyperparasitism.

The biocontrol potential of *T. viride*, *P. funiculosum*, *A. terreus* and *A. flavus* against *F. oxysporum* f. sp. *vasinfectum*, the cotton wilt pathogen, was studied by Tashlieva (1980). The antagonistic activity of the organism was mainly attributed to mycorparasitism and predation. Production of antibiotic 'Trichodermin' by *T. viride* was demonstrated to protect cucumber against *Fusarium oxysporum* f. sp. *lycopersici* (Kudryavtseva, 1980). Marois *et al.* (1981)

reported potential efficiency of the conidial suspension of *T. harzianum*, *P. funiculosum* and *A. ochraceous* in controlling tomato crown rot pathogen (*F. oxysporum* f. sp. *radicis-lycopersici* under field condition. The successful biocontrol of pre and post emergence damping off of tomato incited by *F. oxysporum*, *P. parasitica* and *P. debarianum* was made possible with the application of *T. viride*, *Streptomyces griseus* and *Bascillus subtilis* (Ye Shia *et al.*, 1981). Incorporation of *T. viride* in seedling flats protected seedlings from root rot pathogens (Padmanabhan and Alexander, 1983). Upadhyay and Mukhopadhyay (1983) reported production of antibiotics by *T. harzianum* inhibitory to the growth of *Sclerotium rolfsii* ranging from 5.67 to 50.67 per cent.

Mycoparasitism by enzymatic lysis of pathogenic fungal hyphae through the production of Beta-(1-3) glucanase, chitinase, cellulase and protease has been reported (Elad *et al.*, 1983). Mycoparasitism by *T. harzianum* combined with the production of cell wall degrading enzymes (Ridout *et al.*, 1986) and volatile 'alkyl pyrones' antibiotics (Claydon *et al.*, 1987) has also been documented. Faull *et al.* (1994) observed production of Homothallin II, an isonitrile antibiotic, by a mutant strain of *T. harzianum*. Lin *et al* (1994) isolated Tricholin, an effective inhibitor of protein synthesis from *T. viride* which expressed antagonism towards *R. solani*.

Pythium nunn as a potential mycoparasite of a number of soilborne pathogens was reported for the first time from Colarado by Lifshitz and co-workers (1984a). The mycoparasite parasitized the hyphae of *Phytophthora parasitica* and *P. cinnamomi* through the formation of appresoria like structures (Lifshitz *et al.*, 1984b). Papavizas (1985) reported that species of *Gliocladium* possessed antagonistic property against soilborne pathogens. Mycelial preparations of *Trichoderma* spp. and *G. virens* reduced survival of *R. solani* by 50 per cent (Lewis and Papavizas, 1985). Direct penetration and utilisation of protoplasmic content of hyphae of *S. rolfsii* by *T. harzianum* was observed by Upadhyay and Mukhopadhyay (1986). According to Mihuata-Grimm and Rowe (1986) the antagonistic activity of *Trichoderma* spp. was highly variable. They noticed that only 15 per cent of the isolates tested were effective in controlling *Rhizoctonia* damping off in lettuce. The seedlings, when inoculated with *R. solani* and *T. harzianum* the latter reduced the damping off incited by the former. The antagonism was correlated to the production of volatile and non volatile antibiotics by *T. harzianum*.

Cristinzio (1987) noticed potential antagonism of *Trichoderma* sp. on *Phytophthora capsici, in vitro*. Parasitism of *T. harzianum* on *R. solani* coiling by coiling around and lysing the host hyphae has been recorded (Wilson *et al.*, 1988).

Adams (1990) suggested that biocontrol methods using antagonistic fungal species like *Trichoderma* spp. could be made successful with atleast 10^5 propagules g^{-1} soil. According to him the parasitic activity of various clones of antagonistic fungi in natural soils vary with their competetive saprophytic ability. Tsao (1990) conducted green house studies to evaluate the biocontrol activity of several species of *Aspergillus*, *Penicillium* and *Trichoderma* and noticed effective antagonism by the fungal isolates especially in reducing the severity of Azalea root rot (*P. cinnamomi*, *P. parasitica*) and citrus root rot (*P. citrophthora* or *P. parasitica*). Inwang and Chamswarng (1990) evaluated biocontrol efficacy of several species of *Penicillium*, *Trichoderma* and *Bacillus* against *Sclerotium rolfsii* causing stem rot of tomato. All the isolates inhibited mycelial growth of the pathogen *in vitro*. In green house studies, incorporation of minced sorghum seed inoculum of either *T. harzianum* or *T. viride* along with *S. rolfsii* reduced stem rot by 99.4 and 98.8 per cent respectively. Bopaiah *et al.* (1991) noticed growth inhibition of *P. areci* by *Aspergillus fumigatus*, *A. niger* and *P. islandicum*.

Pre-colonization of bean seeds with *Trichoderma* spp, for 24 hours gave 100 per cent protection against *Pythium* sp Cotes *et al.* (1992). The effect was attributed to competition between *Trichoderma* and *Pythium* for site nutrients and

attractive substances, and possible release of toxic substances by *Trichoderma* species. Marchetti *et al*. (1992) reported strong antagonism of *Trichoderma* spp. against *P. ultimum* and *R. solani*. The sensitivity of these pathogens was correlated to the maximum rate and extent of carbondioxide released by *Trichoderma*. Both the pathogens with slow rate of carbondioxide production were most sensitive to the antagonist. Mukherjee and Sen (1992) noticed antagonistic activity of *Aspergillus fumigatus* and *A. terreus* in reducing seedling blight of jute. The culture filtrate of *A. fumigatus* alone was found to be effective in inhibiting the growth and sclerotial germination of the pathogen (*Macrophomina phaseolina*).

Rathore *et al*. (1992) noticed production of volatile and non-volatile substances by *T. viride* which inhibited the growth of ginger rhizome rot pathogens viz., *Pythium myriotylum* and *Fusarium solani* by 70 and 10 per cent respectively. Uphadhyay (1992) investigated antagonistic activity of certain microorganisms and found that *Bacillus subtilis*, *Cephalasporium roseo-griseum* and *T. harzianum* were highly effective in suppressing the population of *Fusarium udum* in soil and root region of pigeon pea. The effect was also correlated with elevated level of soil fungistasis by these antagonists. Strong fungistatic effect against *F. udum* was also recorded with other microorganisms such as

Aspergillus fumigatus, *Cladosporium cladosporioides*, *Papulaspora* sp and *Penicillum decumbers*.

Benhamou and Chet (1993) demonstrated coiling of *T. harzianum* around *R. solani* as an early event preceding hyphal damage of the pathogen. Chitin breakdown was also noticed due to production of chitinase by the antagonist. *In vitro* study conducted to determine the volatile compounds produced by different isolates of *T. harzianum* and *T. viride* revealed that presence of caprilic, caprinic and capronic acids, ethylene and formic aldehydes in different quantities by the isolates (D'ercole *et al.*, 1993). Further, they found that the isolates with more ethylene and formic aldehyde production *in vitro* gave better control of *R. solani* and *P. ultimum* in tomato and pepper respectively. Harman *et al.* (1993) purified the chitinolytic enzymes produced by *T. harzianum*. The enzymes included N-acetyl-beta-D-glucosaminidases, chitin 1,4-beta-chitobiosidases and endo chitinase.

Abada (1994) recorded the biocontrol potential of *Trichoderma harzianum* in reducing damping off and root rot of sugarbeet both under pot culture and field conditions. Anandaraj and Sarma (1994b) evaluated several antagonistic fungal isolates against *Phytophthora capsici*, foot rot pathogen of black pepper and recorded potential biocontrol activity of *Trichoderma hamatum* and *Gliocladium*

virens both under green house and field conditions. Horvath *et al.* (1995) studied the production of soluble antifungal metabolites by *T. harzianum* and observed trichorzianines, produced during conidiogenesis was responsible for the biocontrol.

Sankar and Jayarajan (1996) studied the compatibility of antagonists, such as *T. viride*, *G. virens* and *Bacillus subtilis* combinations in controlling root rot of sesamum (*Macrophomina phaseolina*). The combined application of all the three antagonists resulted in least incidence of root rot as against individual antagonists. Sarma *et al.* (1996) evaluated antagonistic potential of several species of *Trichoderma* and *Gliocladium virens* against foot rot pathogen of black pepper. Among several fungi isolated from rhisozphere species of *Aspergillus*, *Penicillium* and *Verticillium* were found to be inhibitory to the growth of *Phytophthora* on dual culture *in vitro* (Veena, 1996). Sarma and Anandaraj (1996) noticed healthy black pepper vines in the presence of *Phytophthora* in silent valley soils of Western Ghats of Kerala suggesting the co-existance of host and pathogen in undisturbed ecosystem. Joseph and Sivaprasad (1997b) isolated fungal antagonists from ginger rhizosphere and noticed that among the isolates identified *Aspergillus fumigatus* and *Trichoderma viride* significantly reduced the rhizome rot incidence and population build up of *Pythium aphanidermatum*. Similar results of reduced

disease incidence with native isolates of *Trichoderma* spp. on dual inoculation was also obtained against foot and leaf rot of betel vine (Chaurasia and Bhatt, 1997), sheath blight of rice (Mishra and Sinha, 1997), root diseases of coffee (Nirmala Kannan *et al*., 1997), collar rot of pigeon pea (Robert and Saha, 1997) and capsule rot pathogen (*Phytophthora meadii* in cardamom (Suseela Bhai *et al*., 1997).

2.7 Interaction between AMF and antagonists

Arbuscular mycorrhizal fungal interaction with other soil microorganisms in the rhizosphere of crop plants are either synergestic or inhibitory to plant growth. The growth stimulatory substances produced as a result of microbial interaction stimulated mycorrhiza formation and rooting of host plants. Sylvia and Schenck (1983) recorded inhibitory effect of certain fungi including *Trichoderma* sp. on spore germination of *Glomus* spp. Linderman (1988) correlated response of mycorrhizal plants to mycorrhizosphere and observed the microbial community stimulated the development of arbuscular mycorrhizal fungal hyphae and rhizomorphs and suppressed the growth of soilborne pathogens.

Mycelial growth and spore germination of *Glomus mosseae* was stimulated by the presence of *Trichoderma* spp. under auxenic conditions (Calvet *et al*., 1992). The effect was due to fungal exudates in the presence of moderate

concentration of carbondioxide. On the contrary, Wyss *et al.*, (1992) reported the inhibition of mycorrhizal colonization by saprophytic fungi. According to Calvet *et al.* (1993) the combined inoculation of *Trichoderma aureoviride* and *Glomus mosseae* resulted in synergestic effect on the growth of marigold (*Tegetes erecta*). The synergism between the fungi imparted host protection against *Pythium ultimum*. Mc Allister *et al.* (1994) noticed that the inoculation of maize plants with spores of *G. mosseae* decreased the population of *Trichoderma koningii* and *Fusarium solani*. *T. koningii* inhibited extra-matrical spore production of *Glomus mosseae*, and the effect was attributed to the production of certain soluble or volatile substances that are inhibitory to spore formation.

Dual inoculation of *Glomus mosseae* and *Aspergillus fumigatus* stimulated maximum mycorrhizal root colonization in wheat (*Triticum aestivum* L.) (Tarafdar and Marschner, 1995). Anandaraj *et al.* (1996) reported that field trials conducted with black pepper cuttings inoculated with arbuscular mycorrhizal fungi at the time of nursery planting and *Trichoderma harzianum* and *Gliocladium virens* added every year in the field reduced foot rot incidence. Microorganisms belonging to a wide range of taxonomic group are known to stimulate the establlisment and stability of mycorrhizal symbiosis (Singh, 1998).

2.8 AMF mediated biochemical changes in crop plants

Amino acids play a vital role in the synthesis of proteins, some of which are essential for the synthesis of phenolics and other molecules involved in plant disease resistance (Harborne, 1964; Emmanouil and Wood, 1981). The positive influence of mycorrhizal association in reducing onion pink rot caused by *Pyrenochaeta terrestris* was attributed to large amounts of reducing sugars in AMF colonized roots (Safir, 1968). Davis (1970) while studying non-pathogenic organisms associated with mycorrhizal fungi opined that AMF induced higher concentration of sugars in mycorrhizal plants were responsible for the inhibition and alteration of toxin producing ability of the pathogen. Baltruschat and Schoenbeck (1972) observed negative correlation between chlamydospore production in *Thielaviopsis basicola* and mycorrhizal colonization in the roots of tobacco and alfalfa seedlings. The root extract of mycorrhizal tobacco plants with higher arginine content reduced chlamydospore production in *Thielaviopsis basicola*. On the other hand, Dehne *et al*. (1978) suggested AMF associated blockage in the ornithine cycle as a reason for higher levels of arginine content in mycorrhizal plants. Higher rates of aminoacids and reducing sugars in the plant root exudates was also correlated with enhanced AMF colonization resulting in subsequent disease suppression (Ratnayaka *et al*., 1978; Graham *et al*., 1981).

The root invading organisms utilise the sugars in the host for their development. The sugar which forms a major source of energy for carbohydrate metabolism in host plants is thus altered due to pathogen invasion. Increased reducing and non reducing sugar content in blast suscceptible varieties of rice (Sridhar 1970; Prasad and Raghunathan, 1972) revealed increased disease development with enhanced sugar content. Hence, regulation of sugar content by mycorrhizal fungi in the host plants plays a decisive role in the suppression of pathogenic invasion. Krishna (1981) working on groundnut found decrease in the total and reducing sugar content in roots with enhanced mycorrhizal formation, and was correlated to mycorrhizal infection. Further, he noticed enhanced free amino nitrogen content in mycorrhizal ground nut. Azcon and Ocampo (1981) also recorded reduction in total and reducing sugar content in mycorrhizal wheat root which in turn suppressed infection by the plant pathogens. Sivaprasad (1983) working on mycorrhizal pigeon pea plants recorded inreased content of reducing sugars in plant top but not in roots. He also observed enhanced total sugar content on both parts after 25th day of plant growth. According to him increased carbondioxide fixation and carbohydate metabolism was attributed to this effect. He also noticed enhanced free amino nitrogen accumulation in Pigeon pea roots during mycorrhizal infection, which gradually decreased with mycorrhizal development. The

increase is attributed to mycorrhiza initiated assimilation of free aminoacids from the host cytoplasmic pool. Synthesis of new proteins or lytic enzymes in pea tissues on inoculation with mycorrhizal fungi hydrolised the polymers of fungal cell wall and was considered as a possible defence response to invasion by parasitic organisms (Mauch *et al.*, 1988). Arbuscular mycorrhizal fungal symbiosis in roots of leek (*Allium porrum*) plants induces peroxidase (Spanu and Bonfate - Fasolo, 1988) and chitinase (Spanu *et al.*, 1989.) activity during early stage of root colonization which are known to offer tolerance against pathogenic infection and multiplication.

Phenolic compounds in plants are considered to be preformed inhibitors or prohibitins of pathogens which play a significant role in disease resistance (Kairaly and Farkas, 1962; Mahadevan, 1970). An increased concentration of total phenols and orthodihydroxy (OD) phenols on peanut roots due to arbuscular mycorrhizal development was also recorded (Krishna 1981). Pot culture studies conducted by Krishna and Bagyaraj, (1983) revealed that inoculation of peanuts with *G. fasciculatum* conferred resistance against *Sclerotium rolfsii* attack which was related to the higher phenolic content in the host tissue. Number of sclerotia produced by *S. rolfsii* on mycorrhizal roots were much less. On the other hand mycorrhizal fungi reduced chlamydospore production and reduced

mycorrhizal colonization per cent in peanut roots. Morandi *et al.* (1984) reported increased level of isoflavanoids, a factor contributing to disease resistance and its accumulation in the roots of mycorrhizal soybean during initial stage of mycorrhizal colonization. However, at later stages of arbuscular mycorrhizal symbiosis, the concentration was lower than that induced by pathogens or elicitors. Histochemical studies on mycorrhizal plants showed enhanced phenolic accumulation on ground nut roots, at early stages of mycorrhizal formation (Krishna and Bagyaraj, 1986). However the phenolic concentration was reduced in the later stages of mycorrhizal development in host. The flavanoids present in plants have been reported to stimulate arbuscular mycorrhizal fungal development in the host roots (Becard *et al.*, 1982; Chabot *et al.*, 1992). Increased total phenol content in maize plants due to *G. fasciculatum* inoculation has also been reported (Chabra and Jalali, 1995). The total phenols and orthodihydroxy phenol content of tissue culture plantlets of jack was significantly increased due to AMF inoculation at the time of planting out of plantlets (Sivaprasad *et al.*, 1995b).

Chemical, physiological and morphological alteration due to AMF colonization in the host roots were correlated with induction of host resistance or tolerance against the pathogen (Mosse, 1973). She also noticed increased root chitinolytic

activity in mycorrhizal plants that facilitated digestion of arbuscules. This effect also resulted in the effective suppression of root pathogen (Dehne and Schoenbeck, 1978; Dehne *et al.*, 1978). Laimbais and Mehdy (1993) studied biochemical changes in mycorrhizal bean roots under different soil conditions. The transient increase in the chitinase and Beta-1-3-D gluconase activities in the roots were noticed only at initial stage of arbuscular mycorrhizal colonization, which was decreased well below the nonmycorrhizal control plants during later stage of mycorrhizal symbiosis. Similarly, studies on isoflavanoids accumulation in mycorrhizal roots of *Medicago truncatula* revealed that the level of phytoalexin (medicarpin) is high at early stage of mycorrhizal formation (Harrison and Dixson, 1993). First report on the effect of mycorrhizal inoculation on the host cytology due to pathogenic infection was proposed (Benhamou *et al.*, 1994). They studied, the stimulated plant defence reaction in mycorrhizal Ri T-DNA transformed carrot roots infected with *Fusarium oxysporum* under axenic system. The preinfection of carrot roots with *G. intraradices* increased protection against *F. oxysporum* and the mechanism was associated with accumulation of newly formed plant products at the site of fungal penetration which restricted pathogenic growth and penetration.

MATERIALS AND METHODS

MATERIALS AND METHODS

The investigation on the management of foot rot of black pepper with arbuscular mycorrhizal fungi (AMF) and antagonists was carried out at the Department of Plant Pathology, College of Agriculture, Vellayani, Kerala, India during 1994 to 1997. Black pepper variety Karimunda, a widely cultivated but most susceptible to foot rot disease was used for the investigation.

3.1 Isolation of pathogen

The pathogen (*Phytophthora capsici*) was isolated during July-September 1994 from infected vines and roots of black pepper and from sick soil collected from the rhizosphere of wilted vines. The specimens were collected from the severely affected areas of Idukki, Wayanad and Thiruvananthapuram districts of Kerala State. The pathogen was isolated following standard baiting and isolation technique (Ribeiro, 1978). The pathogenicity of each isolate was confirmed following Koch's postulate. The purified isolates were maintained on oat meal medium in BOD (27±1°C) with periodical subculturing.

3.1.1 Growth of *P. capsici* on solid medium

A culture disc of 4 mm diameter of *P. capsici* isolates from seven day old culture grown on modified carrot agar medium (Ribeiro, 1978) was placed at the centre of the petri dish containing 15 ml of the medium and incubated at 25±1°C. The mycelial growth of regular colonies were estimated by taking the means of the colony diameters taken in two directions at right angle to each other. In the case of irregular colonies the average of the longest and shortest linear growth were accounted.

3.1.2 Growth of *P. capsici* on liquid medium

The growth of *P. capsici* in liquid medium was tested by growing 4 mm culture disc of each isolate in 100 ml Erlenmeyer flask containing 25 ml Bartnicki Garcia's broth for 10 days (Ribeiro 1978) . The mycelial mats of the isolates were collected, washed and dried in hot air oven at 59-60°C and recorded the dry mycelial weight.

3.1.3 Mass multiplication of the pathogen

For all the pathological studies the mass culture of the pathogen was prepared in oats meal-sand medium (1:9 v/v). The medium was moistened sufficiently, filled in a conical flask and autoclaved at 1.02 kg cm^{2-1} for 2 hours inoculated with 6 mm disc of *P. capsici* and incubated at room temperature

for 15 days. The culture so multiplied was used for various experiments under the investigation.

3.1.4 Pathogen inoculation

Inoculation of the pathogen was done by placing the oatmeal-sand based inoculum in the root region of pepper cuttings at the rate of 5 g polythene bag^{-1} containing 2 kg of potting mixture. Inoculation in the field was given in the same way, after removing the surface layer of the soil to expose the roots. Each plant was inoculated and the roots were again covered with soil.

3.1.5 Testing the virulence of *P. capsici* isolates

Sixty days old rooted Karimunda cuttings raised in pots filled with sterilised soil were inoculated by mixing oatmeal-sand based inoculum of the pathogen (5 g kg^{-1} soil) uniformly around the pepper cuttings in the root region. The data on infection and mortality per cent were recorded on 15^{th}, 30^{th}, 45^{th}, 60^{th} and 75^{th} day of inoculation.

3.2 Arbuscular Mycorrhizal Fungal inoculum and inoculation

Guinea grass (*Panicum maximum* Jacq.) colonized with mycorrhizal fungus was grown in sterilised soil:sand mixture (2:1 v/v) for four months. The soil:sand mixture containing

mycorrhizal spores, colonized root segments and hyphae served as mycorrhizal inoculum. Whenever inoculation was given, 50 g of inoculum containing 350 spores was placed about 2 cm below the pepper cuttings at the time of planting.

3.3 Estimation of AMF colonisation

The mycorrhizal colonization per cent in the root samples of pepper was estimated following the procedure of Phillips and Hayman (1970). Per cent AMF colonisation was calculated using the following formula

$$\text{AMF colonisation (\%)} = \frac{\text{Number of root segments with mycorrhizal infection}}{\text{Number of root segments examined}} \times 100$$

3.4 Screening of AMF isolates on plant growth and foot rot incidence of black pepper in green house

3.4.1 Effect on plant growth

An experiment was conducted to evaluate the effect of AMF isolates on growth characteristics of black pepper. Seven native arbuscular mycorrhizal fungal isolates viz., Is-6, Pi-6, Pi-8, Ri-8, Pi-9, Ri-9, PI-11 along with three identified AMF species *Glomus fasciculatum*, *G. clarum* (ICRISAT, Hyderabad) and *Gigaspora margarita* (CTCRI, Trivandrum) were used for the study.

Amongst the native isolates, Is-6 (DBT project, KAU, Trivandrum), developed for the biocontrol of foot rot of black pepper, was used as a reference culture. The experiment was conducted in CRD with 4 replications and there were 11 treatments including a non-mycorrhizal control. Three rooted cuttings were maintained in each replication (polythene bags). Inoculation with AMF isolates was given at the time of planting as mentioned in item 3.2. Plant growth characteristics such as plant height and number of leaves were recorded on 3^{rd}, 5^{th} and 7^{th} month of plant growth. The root length and total plant dry weight were recorded after seven months of growth. Black pepper plants were carefully depotted and washed thoroughly and then recorded for root length. The plants were oven dried at 60°C until constant weight for recording the dry weight.

3.4.2 AMF isolates on nutrient uptake

Oven dried samples were powdered and used for the estimation of nutrient. The phosphorus (P) potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) content of shoot and root samples of black pepper plants were estimated following the standard procedure mentioned below:

Procedure followed for nutrient analysis

Nutrient	Estimation method	Reference
P	Vanedomolybdate yellow colour method	Jackson (1973)
K	Flame photometric method	Jackson (1973)
Ca, Mg, Cu, Fe, Mn and Zn.	Atomic absorption spectro photometric method	Lindsay and Norval, (1978)

3.4.3 Effect on foot rot incidence

The preliminary screening of AMF against foot rot incidence in black pepper was conducted in green house using sterilised soil in polythene bags. The experiment and the method of inoculation with AMF were same as mentioned in item 3.4.1. The plants were inoculated with pathogen on 120th day of planting.

The mycorrhizal colonization per cent was recorded at the time of inoculation with the pathogen and 30 days thereafter. The foot rot infection and mortality per cent were recorded on 15th, 30th, 45th, 60th and 75th day of *P. capsici* inoculation.

3.4.3.1 Estimation of foot rot infection and mortality

Plants were monitored regularly on foot rot infection and mortality after inoculation with pathogen. The total number of pepper cuttings infected and dead were recorded

separately and percentage was computed using the following formula

$$\text{Infection/ mortality (\%)} = \frac{\text{Total number of plants infected/dead}}{\text{Number of plants in each treatment}} \times 100$$

3.4.3.2 Estimation of root rot index

The root rot intensity of the pepper cuttings inoculated with *P. capsici* was scored on 60th day of pathogen inoculation using the following score chart.

Rating score (0 - 4)	Root rot (%)
0	No rot
1	1 - 25
2	26 - 50
3	51 - 75
4	76 - 100

The Root Rot Index (RI) was computed from the root rot intensity score using the following formula:

$$RI = \frac{\text{Total Root Rot Score}}{\text{Maximum score (4)} \times \text{Number of roots examined}}$$

The values, thus obtained, for root rot index was multiplied with hundred and expressed as root rot index (Singh, 1975).

3.5 Characterisation of native AMF associated with black pepper

Rhizosphere soil along with root samples of black pepper grown in laterite (typic plinthustult), forest(haplic argiustoll) and sandy (oxyaquic quartpsamment) soils of Kerala were collectd from different locations. AMF colonisation in black pepper genotypes grown in different soils were estimated following the procedure of Phillips and Hayman (1970). Arbuscular mycorrhizal fungal spores from different soil samples were extracted by wet sieving and decanting technique (Gerdemann and Nicholson, 1963). The spore characteristics were recorded using stereo and trinocular research microscopes and the identification of different AMF spores were done following the classifiction pattern proposed by Schenck and Perez (1988). The standard photographic slides developed by Hall and Abbott (1981) for the identification of different species of endogonaceae were used for comparison and identification

3.6 Isolation of fungal antagonists of *P. capsici*

Isolation of antagonistic microorganisms were made from different sources such as forest soils, decomposing organic matter, rhizosphere of healthy pepper vines in diseased plantations and vermicompost following the serial dilution plate technique (Waksman, 1922) using Martins Rosebengal agar. Samples from 10^4 and 10^6 dilutions were plated in the medium and incubated at room temperature for development of the

colonies. The colonies showing antagonistic nature were isolated, purified and maintained for further studies. The micro organisms isolated were subjected for different *in vitro* studies.

3.6.1 Testing of fungal antagonists against *P. capsici* in culture plate

The inhibition capacity of each microbial isolate was studied by dual culturing with *P. capsici* on carrot agar medium in petriplates. The mycelial disc (5 mm) of the pathogen from seven days old carrot agar culture was inoculated asceptically at the centre of petri dish and incubated at 28±1°C for 24 hours. After incubation, cut 5 mm size mycelial disc of the antagonists was placed at three corners of the plate. The observation on radial growth of *P. capsici* in the plate was recorded on fifth day of inoculation with the test organism. The *P. capsici* inoculated plate without test organism served as control. The Per cent Inhibition (PI) was calculated using the following formula (Kling storm and Johansson, 1973).

$$PI = \frac{(P.\ capsici \text{ growth in control}) - (P.\ capsici \text{ growth in treatment})}{(P.\ capsici \text{ growth in control})} \times 100$$

3.6.2 Effect of metabolites of fungal antagonists on growth of *P. capsici*

The metabolites of the antagonistic organism were prepared by inoculating 8 mm mycelial disc of respective test organism separately in a 100 ml flask containing 50 ml of Czapek's medium. The organism was allowed to grow in submerged conditions on a shaker for fifteen days under room temperature. The broth culture was initially filtered through Whatman No. 42 filter paper and then through millipore filter of 0.22 um pore size in order to achieve cell free filtrate containing metabolites of test organisms. Hundred ml Erylenmeyer flasks containing 20 ml of Bartnicki - Garcia's liquid medium (Ribeiro, 1978) inoculated with 5 mm mycelial disc of *P. capsici* were incorporated with 1 ml of cell free culture filtrate of the antagonistic organism. Flask inoculated with the pathogen without culture filtrate served as check. All the flasks were incubated at 27±1°C for 10 days. The mycelial production by the pathogen in different treatments and the per cent inhibition were recorded following the same method mentioned earlier.

3.6.3 *P. capsici* population in antagonists amended soil

Plastic pots of size 6.5" x 6.5" were used for the incubation studies to evaluate the effect of antagonists on the survival of *P. capsici* in the soil. The pot containing 1 kg of

sterile soil enriched with organic manure (2:1 v/v) was inoculated with the pathogen and antagonist isolates at the rate of 5 and 15 g respectively. Soil was provided with Karimunda shoot (5 g) bits as a food base for the pathogen. Adequate moisture was maintained for the development of the organisms. All the fifty isolates were included in the study. The population of *P capsici* and antagonists was estimated after 60 days of incubation. The medium used for the antagonist was Martin's Rosebengal while, modified Tsao's medium was used for the isolation of *P. capsici* (Ribeiro, 1978). Per cent Inhibition (PI) of the pathogenic population was calculated as follows:

$$PI = \frac{\text{(Population of pathogen in control)} - \text{(Population of pathogen in treatment)}}{\text{(Population of pathogen in control)}} \times 100$$

3.7 Mass multiplication of antagonistic isolates

Antagonistic isolates were mass multiplied on wheat bran-sand mixture (1:5 v/v) following the procedure described for the multiplication of pathogen.

3.8 Estimation of antagonistic population

Antagonistic population under different experiments were estimated by serial dilution method using Martin's Rosebengal agar medium. For the estimation of *Trichoderma* spp. selective medium was used (Elad and Chet, 1983).

3.9 Screening of fungal antagonists on plant growth and foot rot incidence of black pepper in green house

Based on the performance in the *in vitro* studies 24 isolates viz., A_1, A_{11}, A_{13}, A_{14}, A_{16}, A_{19}, A_{20}, A_{21}, A_{22}, A_{26}, A_{27}, A_{28}, A_{29}, A_{31}, A_{32}, A_{33}, A_{34}, A_{35}, A_{37}, A_{38}, A_{39}, A_{40}, A_{41}, and A_{42} were subjected for further screening in green house. Polythene bags of 15 x 10 cm size filled with 2 kg of sterilised potting mixture (soil:sand:cowdung = 2:1:1 v/v) were used for raising the pepper cuttings. The potting mixture was incorporated with different antagonists by mixing the mass multiplied inoculum with the top 1 to 2 cm layer soil at the rate of 30 g bag^{-1}. Five three noded cuttings of black pepper cultivar Karimunda were planted in each bag. Later, the plant population in the treatments was made uniform so as to have three cuttings in each replication (Polythene bag). There were 25 treatments including 24 isolates and control, in experiment conducted in CRD with four replications (Polythene bag) On 90^{th} day of planting, the plant growth and rhizosphere population of antagonists were recorded. On 120^{th} day of planting all the treatments were again inoculated with respective antagonist at the rate of 15 g bag^{-1}. The pathogen was also inoculated on 120^{th} day following the procedure described earlier. Treatment with pathogen alone served as control. The foot rot infection and mortality on the 30^{th}, 40^{th}, and 50^{th} and 60^{th} days of

P. capsici inoculation were recorded. The populations of the pathogen and antagonists were also recorded on 60th day of pathogen inoculation as described earlier.

3.10 Effect of dual inoculation of AMF and fungal antagonists on plant growth and foot rot incidence of black pepper in green house

Five cultures each of AMF (viz., Is-6, Pi-9, Pi-11, *Glomus fasciculatum* and *Gigaspora margarita*) and antagonists (viz., A_1, A_{13}, A_{21}, A_{22}, and A_{35}) were selected after the preliminary screening for testing their dual inoculation effect on foot rot incidence in black pepper.

The antagonists were incorporated in the unsterilised potting mixture (@ 1.5 kg per hundred kg) at the time of potting mixture preparation. Polybags filled with potting mixture so prepared were then planted with pepper cuttings. Inoculation with mycorrhizal isolates was given at the time of planting. The pathogen was inoculated 120th day of planting as mentioned earlier. All the possible combinations of AMF and antagonists, their single inoculation, *P. capsici* alone inoculation and chemical control practices using bordeaux mixture (1%) and copper oxychloride (0.2%) as per the package of practices recommendation (KAU, 1996) were included in the study. There were 38 treatments in the experiment conducted in CRD with three replications. Each replicate (polythene bags) had 4 plants.

The treatment details are as follows:

T_1 - A_1 x *G. margarita*	T_6 - A_{13} x *G. margarita*	T_{11} - A_{21} x *G.margarita*
T_2 - A_1 x Pi-9	T_7 - A_{13} x Pi-9	T_{12} - A_{21} x Pi-9
T_3 - A_1 x *G. fasciculatum*	T_8 - A_{13} x *G. fasciculatum*	T_{13} - A_{21} x *G. fasciculatum*
T_4 - A_1 x Is-6	T_9 - A_{13} x Is-6	T_{14} - A_{21} x Is-6
T_5 - A_1 x Pi-11	T_{10} - A_{13} x Pi-11	T_{15} - A_{21} x Pi-11
T_{16} - A_{22} x *G. margarita*	T_{21} - A_{35} x *G. margarita*	T_{26} - A_1
T_{17} - A_{22} x Pi-9	T_{22} - A_{35} x Pi-9	T_{27} - A_{13}
T_{18} - A_{22} x *G. fasciculatum*	T_{23} - A_{35} x *G. fasciculatum*	T_{28} - A_{21}
T_{19} - A_{22} x Is-6	T_{24} - A_{35} x Is-6	T_{29} - A_{22}
T_{20} - A_{22} x Pi-11	T_{25} - A_{35} x Pi-11	T_{30} - A_{35}

T_{31} - *G. margarita*	T_{36} - bordeaux mixture (BM)
T_{32} - Pi-9	T_{37} - Copper oxychloride (COC)
T_{33} - *G. fasciculatum*	T_{38} - Control (*P. capsici*)
T_{34} - Is-6	
T_{35} - Pi-11	

Observation on foot rot infection and mortality was recorded after 15^{th}, 30^{th}, 45^{th} and 60^{th} day of pathogen inoculation. Antagonistic population in different treatments were recorded on 100^{th} and 200^{th} day of incorporation in potting mixture. While the observation on AMF colonization in pepper roots were recorded on 45^{th}, 60^{th} 75^{th} and 120^{th} day of AMF inoculation. Separate set of plants (3 each) were also

maintained for each treatment excluding T_{36} and T_{37} to study the effect of dual inoculation on plant growth. Observations on plant height and number of leaves were recorded on 210th day of planting.

3.11 Effect of dual inoculation of AMF and fungal antagonists on plant growth and foot rot incidence of black pepper in field

Forty five days old rooted cuttings raised in polythene bags with desired combinations of AMF and antagonistic inoculation were transplanted to the field attached to the Instructional Farm, College of Agriculture, Vellyani, Thiruvananthapuram to evaluate the combined effect of biocontrol agents against foot rot incidence in black pepper. The treatment details were same as that of green house studies. Second inoculation with respective antagonist and AMF was given at the time of transplanting in the field. Recommended chemical control methods viz., bordeaux mixture (1%) and copper oxychloride (0.02%) were also included in the field study. The chemicals were applied at the time of planting in the nursery as well as in the main field as soil drench. The pathogen was introduced one month after transplanting following the inoculum preparation and inoculation as described earlier. There were 38 treatments in the experiment conducted in RBD with four replications. Each replications had 3 plants. AM colonisation (30th and 90th day).

Population of antagonist (30^{th} and 90^{th} day). per cent foot rot infection (15^{th}, 30^{th}, 60^{th}, 90^{th} day), plant mortality (30^{th}, 60^{th}, 90^{th}, 150^{th} day) and plant growth (150^{th} day) were recorded.

3.12 Identification of AMF and antagonists

The potential native isolates of both AMF and antagonists were subjected for identification. The AMF isolates were identified folowing the classification pattern proposed by Schenck and Perez (1988) and compared with the standard slide collection of (Hall and Abbott, 1981). The antagonists were identified (Domsch and Gams, 1980) and confirmed by Agharkar Research Institute, Pune.

3.13 Standardisation of AMF inoculation technique for established pepper plantations

3.13.1 AMF inoculation to black pepper in green house

Cowpea (*Vigna unguiculata* (L) Walp), green gram (*Vigna radiata* (L) Wilezek), Italian millet (*Setaria italica* (L) Beauv) and sorghum (*Sorghum bicolor* (L.) Moench were evaluated as carrier plant for introducing AMF in black pepper roots under green house condition. Black pepper cuttings were raised in pots of 30x30 cm size filled with sterilized soil. After forty five days of black pepper growth, top 1-2 cm layer of soil was removed uniformly from pots and a mixed inoculum of *Glomus fasciculatum* and Pi-11 (50 g) was spread around the

Plate 1. Method of AMF inoculation in established plantation

Plate 2. Multiplication of antagonists in cowdung-neemcake food base

plant. The seeds of the carrier plants were then sown over the inoculum layer and covered with soil. After germination five seedlings of each carrier plants were maintained in each pot around the pepper plants. . AMF inoculation pepper cuttings without carrier plants served as control. The experiment was conducted in CRD with 3 replications. The AMF colonization in the roots of carrier plants as well as black pepper was recorded on 30th 60th and 90th day of inoculation following the procedure of Phillips and Hayman, (1970). Root length and weight of carrier plants and pepper were also recorded.

3.13.2 AMF inoculation to established black pepper vines in the field

Green gram and sorghum were selected from pot studies were further tested as carrier plant in the field. Pepper vines of 8 years old were inoculated with mixed AMF inoculum of *G. fasciculatum* and Pi-11 at the rate of 400 g per vine. The *in situ* inoculation with AMF at the 'dibbling spots' made in a circle around the vines was followed for both green gram and sorghum (Plate 1). Seeds were sown over the inoculum and covered with soil. Two methods of inoculation without carrier plants viz., AMF inoculation as in dibbling spot and spreading around the vine and without carrier plants in both the cases served as two controls. There were 4 treatments in the experiment conducted in CRD with 3 replications. The observation on AMF root colonization in black pepper and carrier plant was recorded on 30th, 60th and 90th day of AMF inoculation.

3.14 Effect of AMF and antagonists on foot rot incidence of black pepper vines in the established plantation

Based on the performance in the green house and field antagonistic isolates A_{1},, A_{21}, and A_{22}, the AMF Is-6, Pi-11 and *Glomus fasciculatum* were chosen for further studies on established pepper vines in the field. The study was conducted in the pepper garden, at Cardamom Research Station, Pampadumpara, Idukki district, Kerala. The 8 year old black pepper vines which had disease incidence in the previous years, were selected for conducting the experiment. Antagonistic fungi multiplied in dry cowdung - neem cake food base (Sivaprasad *et al*. 1998) and soil based arbuscular mycorrhizal fungal inoculum were used. The antagonists inoculum (Plate 2) with 10^5 propagules g^{-1} at the rate of 1.5 kg $plant^{-1}$. AMF inoculation at the rate of 400 g per vine was given by *in situ* inoculation method using sorghum as carrier plant. The antagonists were applied on 15th May, 15th July, 15th August and 30th September, 1997. AMF inoculation was given on 15th May and 15th August. Recommended chemical method of foot rot disease management viz., bordeaux mixture (1%) and Copper oxychloride (0.2%) was maintained as check (KAU, 1996). The chemicals were applied as soil drenching during 15th May and 15th August. Food base with antagonists were also included as check and applied at the time of application of antagonistic treatments. There were 19 treatments with three replications in the experiment conducted in Randomised Block Design (RBD).

The treatment details are as follows:

T_1	- *G. fasciculatum* (*G.f.*)	T_4	- *A fumigatus* (*A.f.*)
T_2	- Is-6	T_5	- *A. sydowii* (*A.s.*)
T_3	- Pi-11	T_6	- *T. viride* (*T.v.*)
T_7	- *G. f.*	T_{10}	- Is-6 x *A.f.*
T_8	- *G. f.*	T_{11}	- Is-6 x *A.s.*
T_9	- *G. f*	T_{12}	- Is-6 x *T.v.*
T_{13}	- Pi-11 x *A.f.*	T_{16}	- bordeaux mixture (BM)
T_{14}	- Pi-11 x *A.s.*	T_{17}	- Copper oxychloride (COC)
T_{15}	- Pi-11 x *T.v.*	T_{18}	- Food base
		T_{19}	- Control

On 180th day the foliar yellowing and defoliation symptoms of foot rot were scored using the following chart.

Foliar yellowing/defoliation score (0 - 4)	Foliar yellowing/defoliation (%)
0	No incidence
1	1 - 25
2	26 - 50
3	51 - 75
4	76 - 100

Based on the score recorded for foliar yellowing and defoliation on 180th day, the per cent leaf yellowing and defoliation index were computed following the formula adopted for per cent root rot index. The intensity of leaf yellowing and defoliation were finally scored on the basis of the per cent index using the following chart:

Per cent foliar yellowing/ Defoliation index	Intensity score of Foliar yellowing (Fy)/ Defoliation (Df) (0 - 9)
No incidence	0
1 - 5	1
6 - 10	2
11 - 15	3
16 - 20	4
21 - 25	5
26 - 30	6
31 - 35	7
36 - 40	8
41 - 45	9

The mean of foliar yellowing and defoliation scores of each treatment was expressed as Foot rot disease Intensity Score (FIS)

$$FIS = \frac{Dy + Df}{2}$$

The observation on mycorrhizal colonization was recorded on 0, 90, 135 and 180th day of first inoculation. The population of antagonistic fungi and the resident soil fungi including the pathogen was recorded on 180th day.

3.15 Biochemical changes in black pepper due to AMF colonization

Five month old black pepper cuttings raised in sterilised soil pre-inoculated with selected AMF viz., Is-6, Pi-9, Pi-11, *Gigaspora margarita* and *Glomus fasciculatum* were used for biochemical analysis. Depoted pepper plants were washed in tap water and fresh plant top and root tissue samples were extracted using appropriate solvents as per the requirement of the analysis. The influence of mycorrhizal fungi on total free amino acids, total sugar, reducing sugar, protein, total phenols and orthodihydroxy (OD) phenol content, cellulase and chitinase activity were estimated the following the standard procedures and expressed on fresh weight basis.

Biochemical analysis in black pepper

Parameters	Estimation method	Reference
Total free amino acid	Colorimetry	Sadasivam and Manikam (1992)
Total sugar and Reducing sugar	Nelson-Somogi	Sadasivam and Manikam (1992)
Protein	Lowry's method	Sadasivam and Manikam (1992)
Total phenols	Folin ciocalteu method	Mahadevan and Sridhar (1974)
Orthodihydroxy (OD) phenol	Arnow's method	Mahadevan and Sridhar (1974)
Cellulase	Dinitro salicylic acid method	Sadasivam and Manikam (1992)
Chitinase	Colorimetry	Bollar and Mauch (1988)

3.16 Statistical analysis

The data generated under the investigation were analysed statistically following the analysis of variance (Panse and Sukhatme, 1978).

RESULTS

RESULTS

The salient findings of the investigation on "Management of foot rot of black pepper with VA mycorrhiza and antagonists" carried out during 1994-97 are presented in this chapter.

4.1 Isolation and characterisation of *Phytophthora capsici*

The isolates of the pathogen were subjected to morphological and cultural studies. They grew profusely on carrot agar at 27±1°C and the colony morphology varied with isolates. The sporangial arrangement of the isolates was generally umbellate. The sporangial shape varied within and between isolates and had long tapered base and caducus with length/breadth (L/B) ratio from 1.7 to 2.7 um. Morphological characters indicated that the isolates were *Phytophthora capsici* Leonian emend A. Alizadeh and P.H. Tsao.

4.1.1 Growth of *P. capsici* isolates on solid and liquid media

Growth rate of the seven isolates, identified as *P. capsici*, on carrot agar is presented in Table 1. All the isolates from Wayanad district showed relatively higher growth rate followed by Idukki isolates. Wayanad isolates showed colony diameter ranging from 75.33 - 77.16 mm on 7th day with

Table 1 Growth of *Phytophthora capsici* isolates in petriplates

Source	Colony diameter (mm) Days after inoculation 3	5	7	9	Dry mycelial weight (g)	Colony character
Idukki Dist.						
Pampadumpara	28.33	46.00	72.33	90.00	0.078	Irregular, fluffy, thin
Kattappana	31.00	51.43	73.80	90.00	0.069	Irregular, fluffy, thin
Thiruvananthapuram Dist.						
Vellayani	21.33	43.33	68.00	90.00	0.087	Irregular, fluffy, thick
Peringammala	22.33	41.33	66.66	90.00	0.100	Uniform, fluffy, thick
Wayanad Dist.						
Ambalavayal	38.33	50.66	77.16	90.00	0.072	Irregular,fluffy, thin
Pulpalley	35.66	47.66	75.33	90.00	0.081	Uniform, fluffy, thick
Mullamkolley	39.33	51.10	75.40	90.00	0.075	Uniform, fluffy, thick
CD (0.05)	3.10	4.02	5.22	-	0.016	

Table 2 Virulence of *Phytophthora* isolates in black pepper

Source	*Infection (%)					*Mortality (%)				
	Days after inoculation					Days after inoculation				
	15	30	45	60	75	15	30	45	60	75
Idukki Dist.										
Pampadumpara	10	30	70	90	100	-	10	30	70	90
Kattappana	-	30	40	70	80	-	-	20	30	50
Thiruvananthapuram Dist.										
Vellayani	-	10	30	40	40	-	-	10	30	40
Peringammala	20	60	90	100	100	-	20	60	90	100
Wayanad Dist.										
Ambalavayal	-	20	60	90	90	-	-	20	60	90
Pulpalley	10	20	30	70	90	-	-	10	30	70
Mullankolley	-	30	50	80	-	-	-	20	50	80

* Values are mean of 10 plants

uniform, fluffy and thick mycelia, while Vellayani and Peringammala isolates from Thiruvananthapuram were comparatively slow growing and recorded colony diameter of 68.00 and 66.66 mm, respectively. However, the isolate from Peringammala (0.100 g) and Vellayani (0.087 g) recorded significantly higher dry mycelial weight over other isolates (Table 1).

4.1.2 Testing the virulence of *P. capsici* isolates

All the *P. capsici* isolates tested on rooted black pepper plants incited the foot rot symptoms (Table 2). Infection occurred on the fine feeder roots initially and later spread to the main roots and collar region. In the collar region infection started as a wet slimy dark patch and as disease progressed rotting occurred with simultaneously yellowing of the leaves and subsequent defoliation. The infected vines succumbed to the disease from 30th day onwards. Peringammala isolate recorded consistently high disease intensity with 100 per cent infection and mortality by 75^{th} day. Isolates obtained from Pampadumpara and Ambalavayal had 90 per cent infection and mortality by 75^{th} day. The Peringammala isolate being the most virulent was selected for further studies.

Table 3 Effect of AMF isolates on growth characteristics of black pepper cuttings in green house

AMF isolate	Days after inoculation			
	90		150	
	Plant height (cm)	Leaves ($No.plant^{-1}$)	Plant height (cm)	Leaves ($No.plant^{-1}$)
Is-6	12.66	2.66	25.00	5.00
Pi-6	10.66	1.66	24.66	5.00
Pi-8	11.00	2.33	20.66	4.00
Ri-8	9.66	1.66	21.33	4.33
Pi-9	14.00	3.33	28.66	5.33
Ri-9	8.00	1.33	17.66	3.33
Pi-11	18.66	3.33	35.33	6.33
Glomus clarum	11.33	2.33	23.33	4.00
Glomus fasciculatum	13.00	3.00	27.00	5.33
Gigaspora margarita	14.66	3.33	29.00	6.33
Control	10.00	2.00	19.66	4.33
CD (0.05)	NS	NS	NS	NS

NS - Not significant

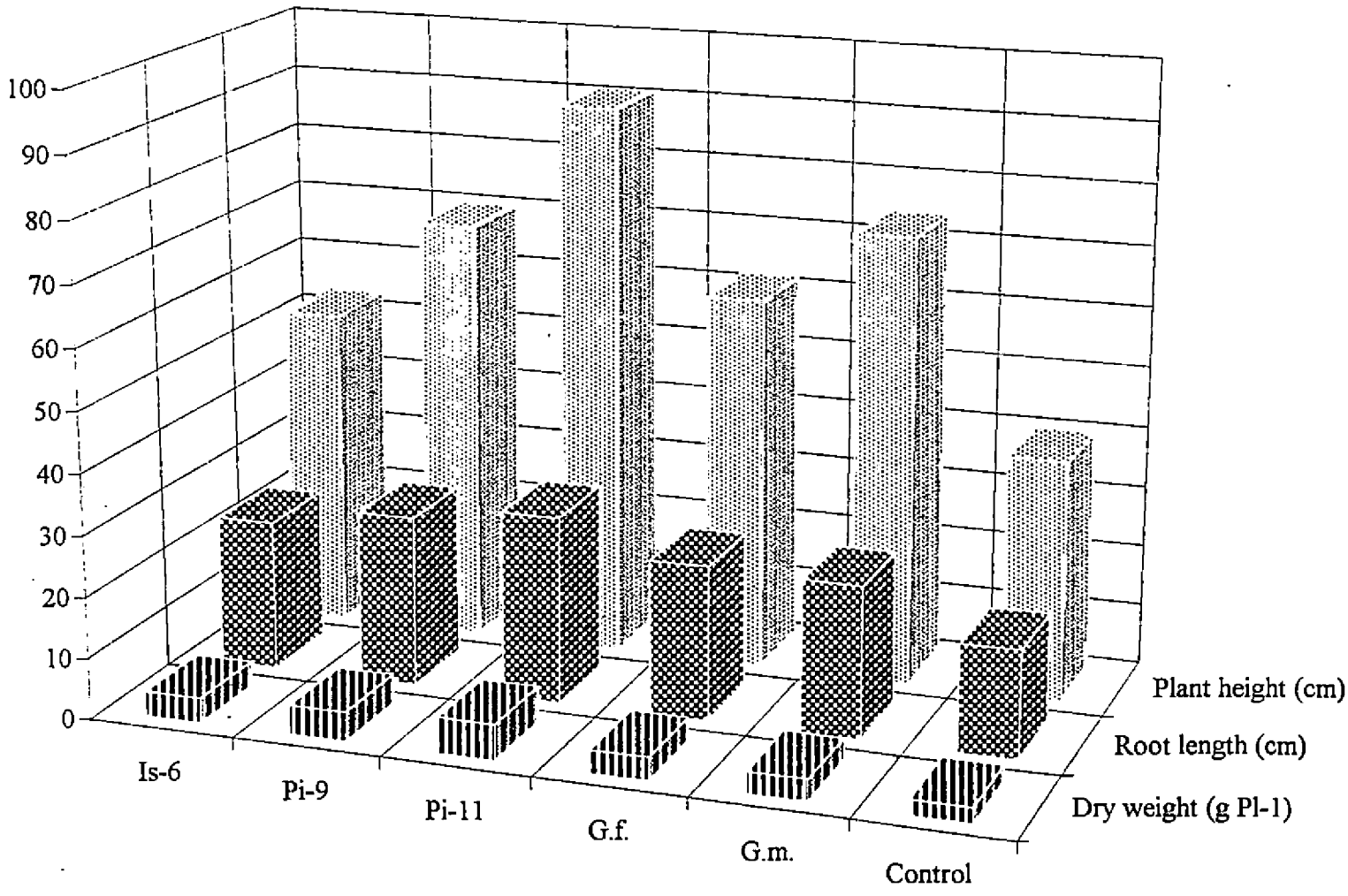


Fig. 1. Effect of selected AMF isolates on growth characteristics of black pepper in green house

Plate 3. Growth of black pepper cuttings inoculated with selected AMF in green house

4.2. Screening of AMF isolates on plant growth and foot rot incidence of black pepper in green house

4.2.1 Effect on growth

Influence of arbuscular mycorrhizal fungal isolates on the growth of black pepper cuttings was evaluated under green house conditions. The connected data are presented in Table 3 and 4. Observations on plant growth characters on 90^{th} and 150^{th} day of inoculation revealed that none of the AMF treatments significantly influenced the growth characteristics of black pepper cuttings (Table 3). However, AMF isolate Pi-11, *G. margarita* and Pi-9 recorded relatively higher plant growth. The observations made seven months after inoculation showed the significant influence of AMF on plant growth (Table 4). Isolate Pi-11 and *Gigaspora margarita* induced increased plant height of 90.66 and 73.66 cm respectively. (Fig. 1, Plate 3). Similarly Pi-9 and *Glomus fasciculatum* also enhanced plant height to 69.66 and 61.33 cm and was significantly higher than other treatments except recorded with Is-6 (52.66 cm).

Isolate Is-6 had given an exceptionally higher leaf number of 15 (Table 4). However, it was statistically on par with Pi-11 (14.33) and *G. margarita* (13.33) and differed significantly over other treatments. The treatments except isolate Pi-8 and Pi-9 recorded significantly higher root length over control (Table 4). Root length of 30.66 cm observed due to Pi-11 inoculation was the maximum.

O.K.

Table 4 Growth and biomass production of black pepper seven months after inoculation with AMF isolates

AMF isolates	Plant height (cm)	Leaves (No.plant^{-1})	Root length (cm)	Dry weight (g·plant^{-1})
Is-6	52.66	15.00	24.66	4.11
Pi-6	48.66	9.66	26.66	3.59
Pi-8	47.66	10.00	22.33	3.05
Ri-8	44.00	9.00	24.66	2.85
Pi-9	69.66	11.00	28.00	4.78
Ri-9	41.00	8.00	22.00	2.52
Pi-11	90.66	14.33	30.66	5.87
Glomus clarum	41.66	9.33	24.33	2.66
Glomus fasciculatum	61.33	11.00	25.33	3.75
Gigaspora margarita	73.66	13.33	25.00	3.54
Control	39.66	8.66	17.66	2.53
CD (0.05)	17.22	2.53	5.56	0.79

Biomass production comes under growth. Anyway, I retain original title

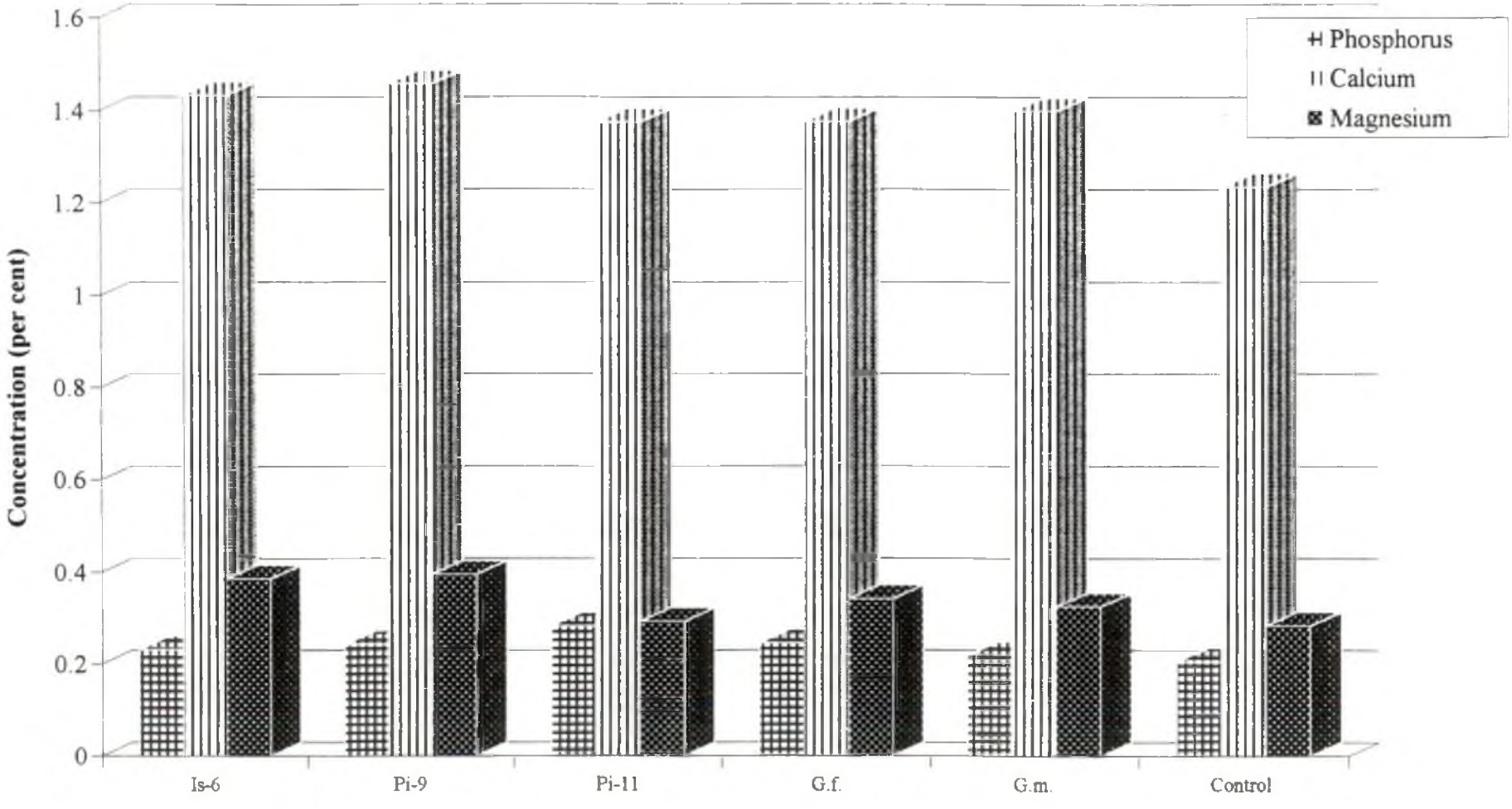


Fig. 2 Effect of AMF isolates on P, Ca and Mg concentration in black pepper

Table 5 Effect of AMF isolates on phosphorus and potash content of black pepper

AMF isolates	P content (%)	Total P_i (mg plant^{-1})	K content (%)	Total K (mg plant^{-1})
Is-6	0.23	9.58	2.69	110.07
Pi-6	0.22	7.64	2.68	96.17
Pi-8	0.21	6.28	2.54	76.99
Ri-8	0.21	5.87	2.45	69.02
Pi-9	0.24	11.54	2.56	122.01
Ri-9	0.21	5.21	2.46	61.78
Pi-11	0.28	16.62	2.55	149.69
Glomus clarum	0.21	7.06	2.88	95.83
Glomus fasciculatum	0.25	9.20	2.89	108.28
Gigaspora margarita	0.22	7.76	2.61	92.60
Control	0.21	5.19	2.57	65.11
CD (0.05)	0.03	1.47	0.20	16.64

Biomass production in black pepper was also significantly influenced by AMF isolates (Table 4) Isolate Pi-11 recorded 5.87 g dry weight, which was significantly higher than all other treatments (Fig.1). Other isolates Pi-9, Is-6, *G. fasciculatum*, Pi-6 and *G. margarita* also recorded significantly higher plant dry weight of 4.78, 4.11, 3.75, 3.59 and 3.54 g respectively. The control plant had 2.53 g.

4.2.2 AMF isolates on nutrient uptake

Effect of different AMF isolates on the uptake of P, K, Ca, Mg, Cu, Fe, Mn and Zn was analysed. It is clear from the data that the P concentration and total uptake in mycorrhizal pepper cuttings varied with different AM fungi (Table 5). Pi-9, Pi-11 and *G. fasciculatum* recorded significantly higher P concentration of 0.24, 0.28 and 0.25 per cent respectively (Fig. 2). The total uptake due to colonization by Pi-11 (16.62 mg), Pi-9 (11.54 mg), Is-6 (9.58 mg) and *Glomus fasciculatum* (9.20 mg) were also significantly higher than control plants (5.19 mg).

Significant increase in the total potassium accumulation in black pepper cuttings were recorded in most of the inoculation treatments (Table 5). *G. fasciculatum* recorded higher K concentration (2.89%), while total 'K' accumulation was more in Pi-11 inoculation (149.69 mg plant^{-1}). On the other

Table 6 Effect of AMF isolates on calcium and magnesium content of black pepper cuttings

AMF isolates	Ca content (%)	Total Ca (mg plant^{-1})	Mg content (%)	Total mg (mg plant^{-1})
Is-6	1.43	58.85	0.38	15.65
Pi-6	1.29	46.25	0.32	11.33
Pi-8	1.29	39.31	0.32	9.90
Ri-8	1.32	37.75	0.32	9.14
Pi-9	1.46	69.86	0.39	18.75
Ri-9	1.32	33.38	0.29	7.52
Pi-11	1.37	80.75	0.29	17.22
Glomus clarum	1.28	42.48	0.24	8.16
Glomus fasciculatum	1.38	51.52	0.34	12.87
Gigaspora margarita	1.39	49.57	0.33	11.59
Control	1.23	31.25	0.28	6.28
CD (0.05)	0.04	9.69	0.01	2.42

hand *G. clarum* that recorded higher 'K' concentration (2.88%) registered only a low level of total K content (95.83 mg plant^{-1}).

All the AMF isolates significantly increased Ca concentration in black pepper (Table 6). Isolate Pi-9 ranked top with 1.46 per cent concentration in the plant tissue. Isolate Pi-11 registered highest calcium uptake (80.75 mg) with 1.37 per cent concentration. Other cultures Is-6, *G. fasciculatum* and *Gigaspora margarita* also showed significantly higher Ca concentration and total uptake (Fig. 2). Regarding the Mg uptake, isolate Pi-9 and Is-6 showed significantly higher Mg concentration of 0.39 and 0.38 per cent respectively (Table 6, Fig. 2). The total Mg accumulation was 18.75 and 15.65 mg plant^{-1} respectively. Non-mycorrhizal plants had 6.28 mg plant^{-1}.

Regarding the copper uptake AMF isolate Pi-11 recorded 19.71 ppm concentration in plant tissue which was significantly higher than that in all other treatments (Table 7). Significant increase in total Cu uptake was evident in plants treated with Pi-11 (0.128 mg) and Pi-9 (0.085 mg) than in control (0.043 mg). The data on Fe content is given in Table 7. Isolate Pi-11 had significantly higher Fe concentration of 417.28 ppm and a total content of 2.446 mg plant^{-1}. *G.fasciculatum* also enhanced both Fe concentration (381.29 ppm)

Table 7 Effect of AMF isolates on copper, iron, manganese and zinc content of black pepper

AMF isolates	Cu content (ppm)	Total Cu (mg plant^{-1})	Fe content (ppm)	Total Fe (mg plant^{-1})	Mn content (ppm)	Total Mn (mg plant^{-1})	Zn content (ppm)	Total Zn (mg plant^{-1})
Is-6	16.27	0.067	328.64	1.348	58.24	0.239	37.76	0.155
Pi-6	16.19	0.057	316.04	1.135	52.29	0.186	38.43	0.137
Pi-8	16.12	0.049	329.81	1.006	48.62	0.148	40.01	0.121
Ri-8	16.74	0.047	346.46	0.977	48.66	0.140	39.08	0.111
Pi-9	17.82	0.085	307.06	1.471	63.08	0.301	33.48	0.158
Ri-9	16.65	0.041	262.23	0.660	51.12	0.127	27.77	0.069
Pi-11	19.71	0.128	417.28	2.446	52.54	0.308	37.23	0.217
Glomus clarum	15.19	0.049	300.88	0.997	57.12	0.176	25.08	0.082
Glomus fasciculatum	17.12	0.064	381.29	1.425	50.12	0.188	39.44	0.146
Gigaspora margarita	16.98	0.056	310.41	1.100	50.53	0.177	31.46	0.112
Control	17.19	0.043	296.27	0.749	41.29	0.104	24.44	0.066
CD (0.05)	1.63	0.011	19.75	0.229	6.58	0.048	2.83	0.023

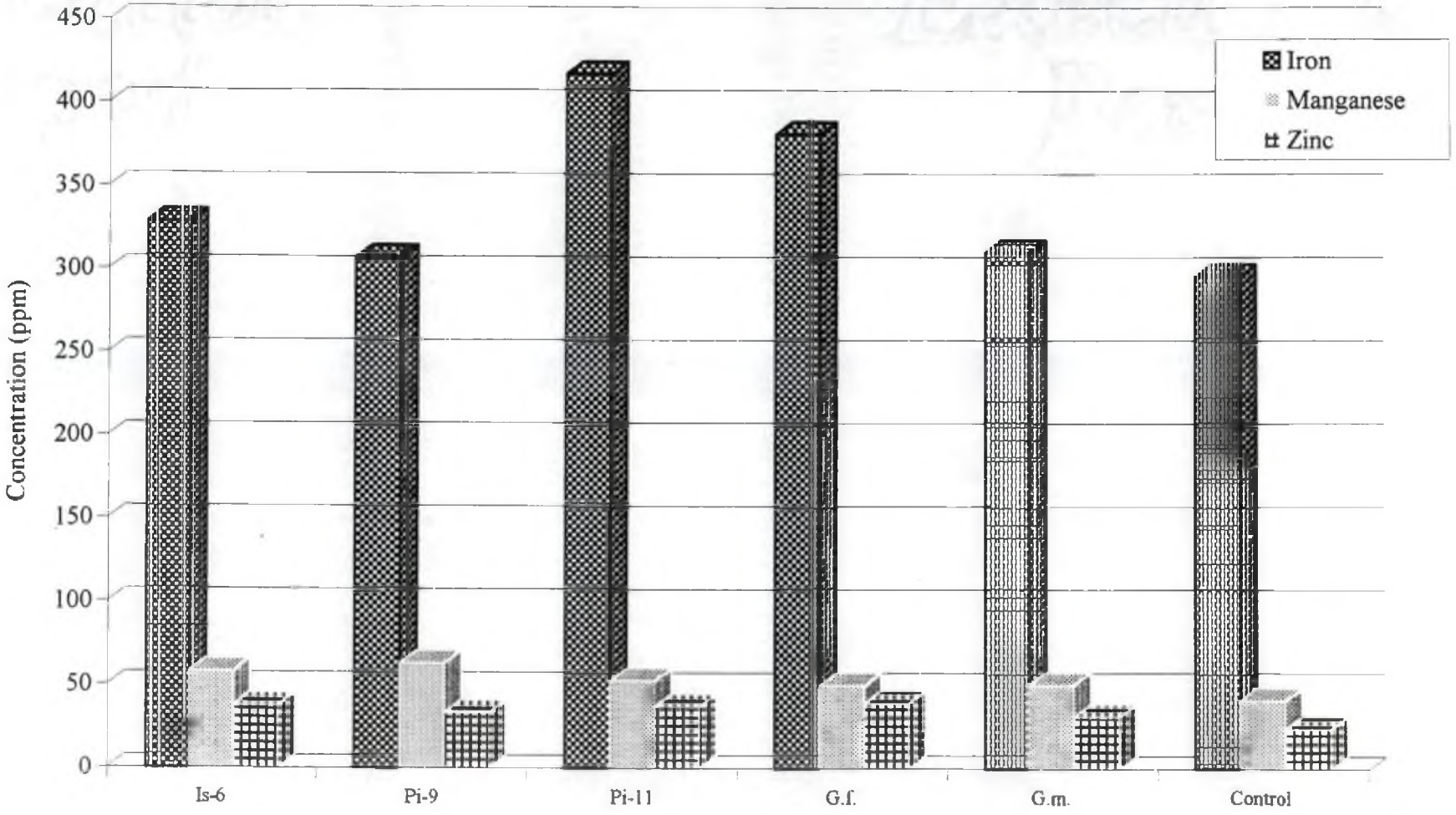


Fig. 3. Effect of AMF isolates on Fe, Mn and Zn concentration in black pepper

Table 8 Effect of AMF isolates on foot rot incidence (infection) in black pepper in green house

AMF isolates	Infection (%)					
	Days after *P. capsici* inoculation					
	15	30	45	60	75	Mean
Is-6	9.25 (17.70)	40.00 (39.21)	53.35 (46.90)	60.61 (51.12)	60.61 (51.12)	44.76 (41.21)
Pi-6	40.00 (39.21)	53.35 (46.90)	86.06 (68.05)	97.64 (81.14)	100.00 (90.00)	75.41 (65.06)
Pi-8	40.00 (39.21)	60.00 (50.75)	90.76 (72.27)	100.00 (90.00)	100.00 (90.00)	78.15 (68.45)
Ri-8	46.65 (43.06)	73.80 (59.19)	80.00 (63.41)	100.00 (90.00)	100.00 (90.00)	80.09 (69.13)
Pi-9	32.91 (34.99)	53.35 (46.90)	67.08 (54.97)	73.80 (59.19)	73.80 (59.19)	60.19 (51.05)
Ri-9	40.00 (39.21)	73.80 (59.19)	90.76 (72.27)	100.00 (90.00)	100.00 (90.00)	80.91 (70.14)
Pi-11	13.95 (21.92)	32.91 (34.99)	53.35 (46.90)	53.35 (46.90)	60.00 (50.75)	42.71 (40.29)
Glomus clarum	46.65 (43.36)	75.02 (59.99)	90.76 (72.27)	100.00 (90.00)	100.00 (90.00)	82.49 (71.06)
Glomus fasciculatum	2.37 (8.85)	20.00 (26.55)	46.65 (43.06)	53.35 (46.90)	53.35 (46.90)	35.14 (34.49)
Gigaspora margarita	26.20 (30.77)	60.00 (50.75)	73.80 (59.19)	90.76 (72.27)	100.00 (90.00)	70.15 (60.60)
Control (*P. capsici*)	46.65 (43.06)	73.80 (59.19)	90.76 (72.27)	100.00 (90.00)	100.00 (90.00)	82.24 (70.90)

Figures in parantheses are transformed values

	Treatment mean	Interaction
CD (0.05) for transformed means	9.21	NS

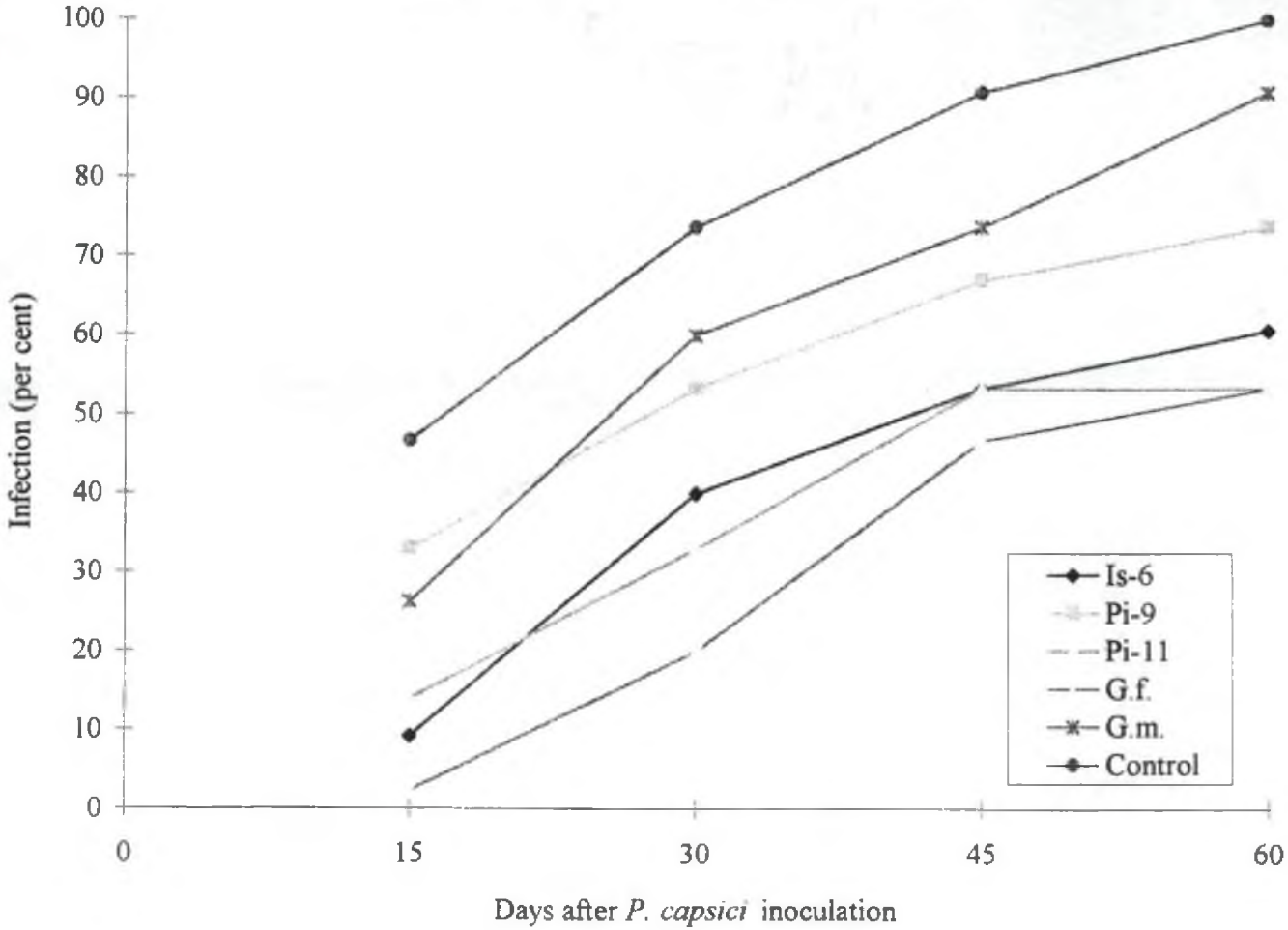


Fig. 4. Foot rot infection pattern in black pepper inoculated with AMF isolates in green house

and total uptake (1.425 mg) in pepper plants. Control plants recorded Fe concentration of 296.27 ppm and total content of 0.749 mg plant^{-1}.

Significant increase in both concentration and total uptake of Mn were noticed in the case of AMF cultures Pi-9 (63.08 ppm), Is-6 (58.24) and *G. clarum* (57.12). Total Mn content of 0.308 and 0.301 mg plant^{-1} were observed in Pi-11 and Pi-9 inoculated plants as against 0.104 mg recorded in control. The respective Mn concentration of pepper plants inoculated with these two isolates were 52.54 and 63.08 ppm as against 41.29 recorded for the control (Fig. 3).

With regard to the Zn uptake significant variation in concentration and total uptake was evident with different AMF treatments (Table 7, Fig. 3). AMF isolate Is-6, Pi-6, Pi-8, Ri-8, Pi-11 and *G. fasciculatum* showed significantly higher Zn concentration of 37.76, 38.43, 40.01, 39.08, 37.23 and 39.44 ppm respectively. The control plants recorded 24.44 ppm.

4.2.3 Effect on foot rot incidence

The native isolates of AMF (Is-6, Pi-6, Pi-8, Ri-8, Pi-9, Ri-9 and Pi-11) along with three identified cultures (*Glomus clarum*, *G. fasciculatum* and *Gigaspora margarita*) were screened against foot rot disease and the relevant data are presented in Table 8 and 9. The AMF tested against foot rot disease showed wide variation in foot rot infection (Table 8).

Table 9 Effect of AMF isolates on foot rot incidence (mortality) in black pepper in green house

AMF isolates	Mortality (%)										Mean
	Days after *P. capsici* inoculation										
	30		45		60		75		90		
Is-6	2.37	(8.85)	9.25	(17.70)	26.20	(30.77)	40.00	(39.21)	60.00	(50.75)	27.56 (29.46)
Pi-6	20.00	(26.55)	40.00	(39.21)	53.35	(46.90)	80.00	(63.41)	100.00	(90.00)	58.67 (53.22)
Pi-8	9.25	(17.70)	39.36	(38.84)	60.64	(51.12)	34.90	(76.92)	100.00	(90.00)	48.83 (54.92)
Ri-8	20.00	(26.55)	53.35	(46.90)	100.00	(90.00)	100.00	(90.00)	100.00	(90.00)	74.67 (68.69)
Pi-9	0.00	(0.00)	9.25	(17.70)	32.91	(34.99)	46.65	(43.06)	80.59	(63.83)	33.88 (31.92)
Ri-9	32.91	(34.99)	60.64	(51.12)	100.00	(90.00)	100.00	(90.00)	100.00	(90.00)	78.71 (71.22)
Pi-11	0.00	(0.00)	9.25	(17.70)	26.20	(30.77)	40.00	(39.21)	60.64	(51.12)	27.22 (27.76)
Glomus clarum	9.25	(17.70)	46.65	(43.06)	90.76	(72.47)	100.00	(90.00)	100.00	(90.00)	69.33 (62.61)
Glomus fasciculatum	0.00	(0.00)	2.37	(8.85)	20.00	(26.55)	26.20	(30.77)	53.35	(46.90)	20.38 (22.62)
Gigaspora margarita	20.00	(26.55)	46.65	(43.06)	60.60	(51.12)	80.59	(63.83)	100.00	(90.00)	61.57 (54.91)
Control (*P. capsici*)	26.20	(36.77)	73.80	(59.19)	100.00	(90.00)	100.00	(90.00)	100.00	(90.00)	80.00 (72.00)

Figures in parantheses are transformed values

	Treatment mean	Interaction
CD (0.05) for transformed means	10.51	12.64

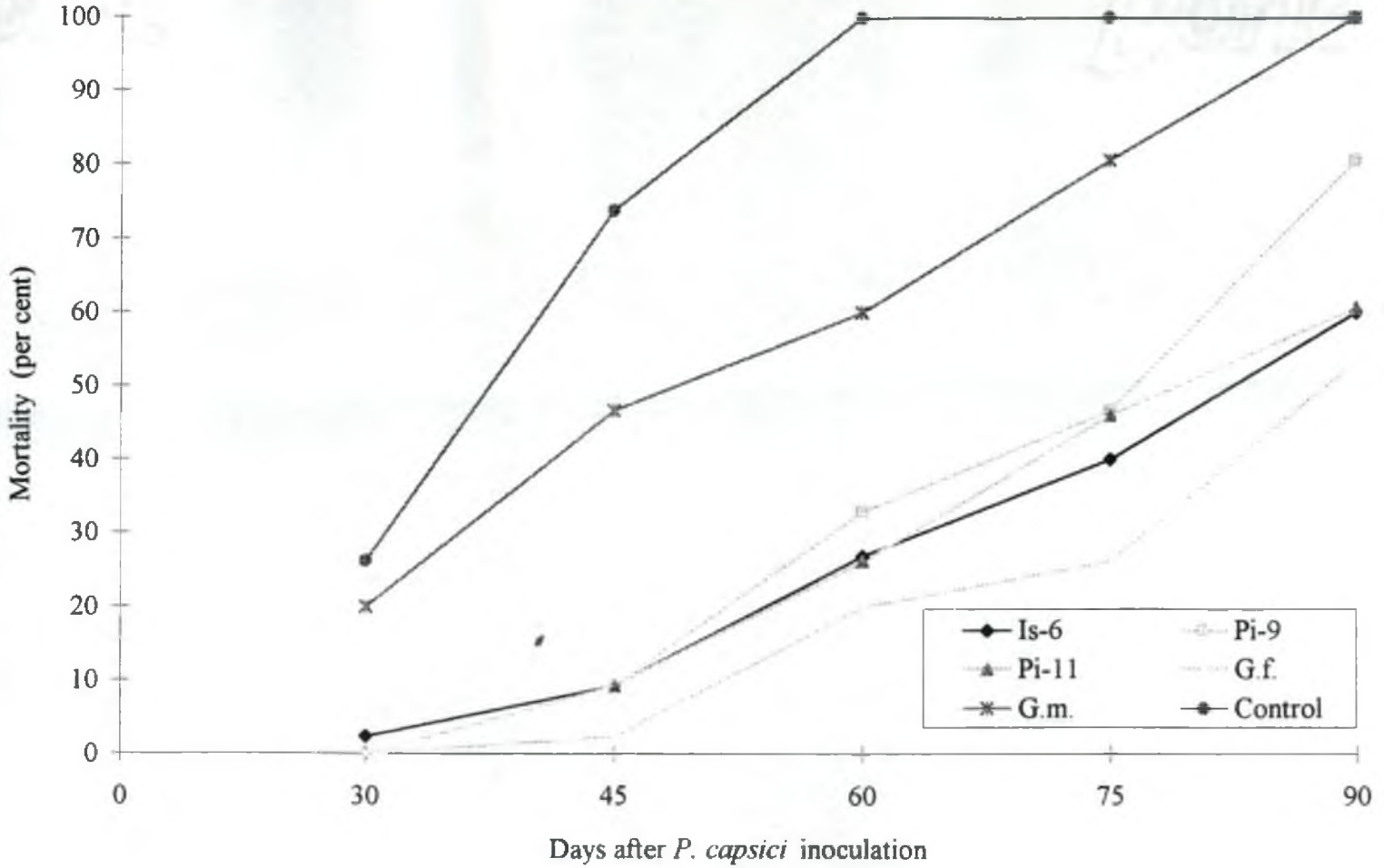


Fig. 5. Foot rot incidence (mortality) pattern in black pepper inoculated with AMF isolates in green house

Table 10 Effect of AMF inoculation on root rot index in black pepper in green house

AMF isolates	Root rot index	Root rot index (%)
Is-6	0.64	64.77
Pi-6	0.93	93.42
Pi-8	0.95	95.83
Ri-8	0.98	98.60
Pi-9	0.85	85.00
Ri-9	0.97	97.05
Pi-11	0.68	68.18
Glomus clarum	0.98	98.40
Glomus fasciculatum	0.62	62.50
Gigaspora margarita	0.95	95.00
Control (*P. capsici*)	0.98	98.60

Plate 4. Effect of selected AMF isolates on foot rot disease in green house

Plate 5. Suppression of root rot by AMF isolate Pi-11


CON
GF
15-6
Pi-11
Pi-9


CON[Pc]
Pi-11

The disease intensity was maximum on the 60th day of inoculation with the pathogen (Fig. 4). *G. fasciculatum* recorded lowest infection per cent of 53.35 followed by Pi-11 and Is-6 with 60.00 and 60.61 per cent respectively. Control plants showed 100 per cent infection by the 60th day. Treatment mean of different isolates showed that *G. fasciculatum*, Pi-11, Is-6, Pi-9 and *G. margarita* significantly reduced the per cent infection over control (Plate 4). Similar trend was noticed in plant mortality rate also (Table 9, Fig.5). *Glomus fasciculatum* recorded the lowest mortality of 53.35 per cent on 90th day of inoculation with the pathogen followed by Isolate Is-6 (60.00%) and Pi-11 (60.64%). This accounts for a reduction in mortality rate by 46.65, 40.00 and 39.36 per cent respectively for *G. fasciculatum*, Is-6 and Pi-11.

The data on per cent root rot index on the 60th day to *P. capsici* inoculation (Table 10) showed comparatively less root damage on inoculation with *G. fasciculatum* (62.50%). Is-6 (64.77%) and Pi-11 (68.18%) over un inoculated control which recorded 98.60 per cent (Table 10) The AMF isolate Pi-11 having recorded with higher root mass in black pepper cuttings also considerably lowered the root damage (Plate 5). Higher root rot index of 98.60, 98.40 and 97.05 per cent were observed with isolates Ri-8, *G. clarum* and Ri-9 respectively.

Table 11 Mycorrhizal colonization in black pepper inoculated with AMF isolates in green house

AMF isolate	AMF colonization (%)		
	Days after inoculation		
	*120	**150	Mean
Is-6	67.22 (55.05)	80.69 (63.90)	73.95 (59.48)
Pi-6	56.73 (48.85)	66.69 (54.73)	61.71 (51.79)
Pi-8	46.65 (43.06)	63.44 (52.77)	55.05 (47.92)
Ri-8	41.61 (40.15)	56.73 (48.85	49.17 (44.50)
Pi-9	63.63 (52.89)	77.17 (61.43)	70.40 (57.16)
Ri-9	38.23 (38.18)	48.31 (44.02)	43.27 (41.09)
Pi-11	73.48 (58.98)	85.95 (67.96)	79.72 (63.47)
Glomus clarum	48.31 (44.02)	61.72 (51.75)	55.02 (47.88)
Glomus fasciculatum	70.71 (57.21)	79.08 (62.76)	74.90 (59.99)
Gigaspora margarita	68.69 (55.95)	82.14 (64.97)	75.42 (60.46)
Control (*P. capsici*)	0.00 0.00	0.00 0.00	0.00 (0.00)

Figures in parantheses are transformed values
* At the time of *P. capsici* inoculation
** 30 days after *P. capsici* inoculation

	Treatment mean	Interaction
CD (0.05) for transformed means	9.74	NS

Table 12 AMF colonization in black pepper grown in different soil types

Soil type	Location	Cultivar	Colonization (%)
Forest (haplic argiustoll)	Mullamkolley	Karimunda	32.22
	Pampadumpara	Karimunda	51.66
	Peringammala	Panniyur-1	36.33
	Pulpalley	Panniyur-1	44.38
	Ranny	Karimunda	53.84
Laterite (typic plinthustult)	Chengannur	Local	58.82
	Kunnumpara	Karimunda	62.50
	Punkulam	Karimunda	46.66
	Suranad	Panniyur-1	60.00
	Vennikulam	Narayakody	61.11
Sandy (oxyaquic quartpsamment)	Karthikapally	Panniyur-1	78.57
	Karuvatta	Arimulaku	78.94
	Kayamkulam	Karimunda	66.66
	Keerikode	Local	83.33
	Muthukulam	Local	73.68
	Ochira	Local	80.00
	Trikkunnapuzha	Panniyur-1	73.33

AMF isolates showed significant difference in AMF colonization per cent in the pepper roots (Table 11). Maximum root colonization was attained with isolate Pi-11 (85.95%) followed by *G.margarita* (82.14%), Is-6 (80.69%), *G.fasciculatum* (79.08%) and Pi-9 (77.17%) is that 30th day after inoculation with the pathogen. The AMF cultures Ri-8, *G. clarum* and Pi-6 recorded relatively very low mycorrhizal colonization per cent (56.73 to 66.69). Is-6, Pi-9, Pi-11, *G. fasciculatum* and *Gigaspora margarita* were selected for further studies.

4.3 Characterisation of native AMF associated with black pepper

AMF colonization and the species associated with different cultivars grown in same and different soil types, viz., forest (haplic argiustoll), laterite (typic plinthustult) and sandy (oxyaquic quartpsamment) soils were studied and the results are presented in Table 12.

Of the three soil types surveyed the oxyaquic quartpsamment soils recorded higher root colonization ranging from 73.33 (Trikkunnapuzha) to 83.73 (Keerikode) per cent. Typic plinthustult soils recorded medium AMF colonization varying from 46.66 to 62.50 per cent, while in haplic argiustoll soils relatively lower colonization rates of 32.22 to 53.84 per cent were observed. There was variation in

Table 13 AMF associated with different pepper cultivars grown in different locations and solis

Soil type	Location	Cultivar	AM Fungi
Forest (haplic argiustoll)	Peringammala	Panniyur-1	*G. fasciculatum* *Glomus* spp *Sclerocystis* sp
	Pulpalely	Panniyur-1	*Glomus* spp
	Ranny	Karimunda	*Glomus* spp *Sclerocystis* sp.
Laterite (typic plinthustult)	Chengannur	Local	*Glomus* spp
	Punkulam	Karimunda	*Glomus* spp
	Suranad	Panniyur-1	*Glomus* spp
	Vennikulam	Narayakodi	*Glomus* spp *Sclerocystis* sp
Sandy (oxyaquic quartpsamment)	Karuvatta	Arimulaku	*Entrophosphora*/ *Gigaspora* spp *Glomus spp*
	Kayamkulam	Karimunda	*Gigaspora* spp *Glomus* pp
	Ochira	Local	*Glomus* spp
	Thrikkunnapuzha	Panniyur-1	*Acaulospora* sp *Gigaspora* sp *Glomus* spp

Table 14 AMF associated with black pepper cultivars grown in the same field

Cultivar*	AM Fungi
Karimunda	*Glomus fasciculatum*, *Gigaspora Sclerocystis coremioides*, *Glomus* sp. *G. macrocarpum* var. *macrocarpum*
Kottanadan	*S. clavispora*, *S. rubiformis*, *G. fasciculatum*, *Glomus* sp.
Kuthiravali	*S. coremioides*, *G. fasciculatum*, *G. multicaule*, *Glomus* sp.
Balankotta	*S. clavispora*, *S. caremioides*, *G. mosseae*, *G. fasciculatum*, *Glomus* sp.
Kalluvally	*G. clavispora*, *G. mosseae*, *Glomus* sp.
Punjarmunda	*S. clavispora*, *G. fasciculatum*, *G. invermaium*, *Glomus* sp.
Cheriyakaniyakadan	*S. clavispora*, *S. coremioides*, *G. fasciculatum*, *Glomus* sp.
Sullia	*S. clavispora*, *G. fasciculatum*, *Glomus* sp.
Doddia	*Acaulospora* sp., *S. clavispora*, *G. fasciculatum*
Cylon	*S. clavispora*, *S. coremioides*, *G. fasciculatum*
Narayakkodi	*G. fasciculatum*, *Glomus* sp
Padappan	*S. rubiformis*, *G. fasciculatum*, *Glomus* sp
Neelamundi	*S. coremioides*, *G. fasciculatum*
Perumkodi	*G. fasciculatum*, *S. coremioides*, *Glomus* sp
Uthirenkotta	*G. fasciculatum*, *S. coremioides*, *Glomus* sp
Tulakodi	*G. fasciculatum*, *Glomus* sp
Vellanamban	*G. fasciculatum*, *Glomus* sp

* Soil samples collected from Panniyur, Kannur district, Kerala

Plate 6. *Glomus* species (x 100) from forest soil

Plate 7. *Glomus* species (x 100) from forest soil

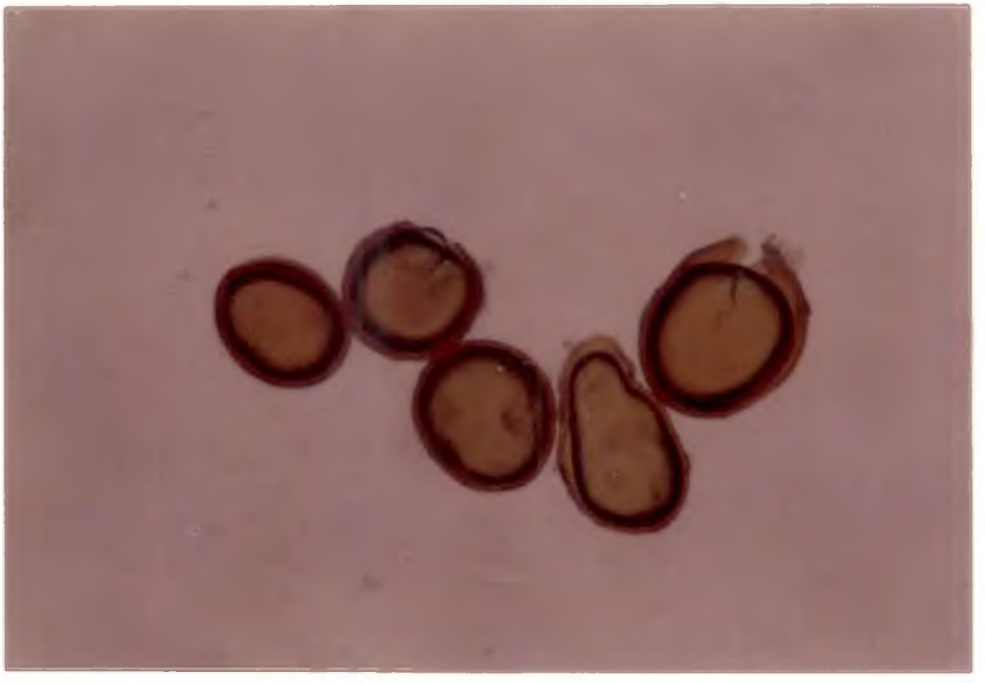
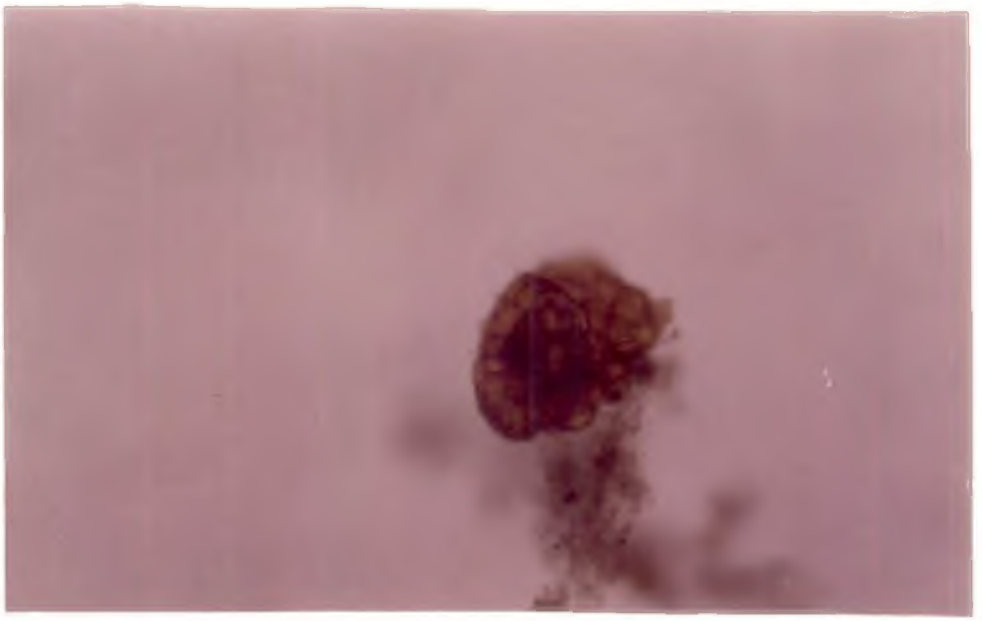
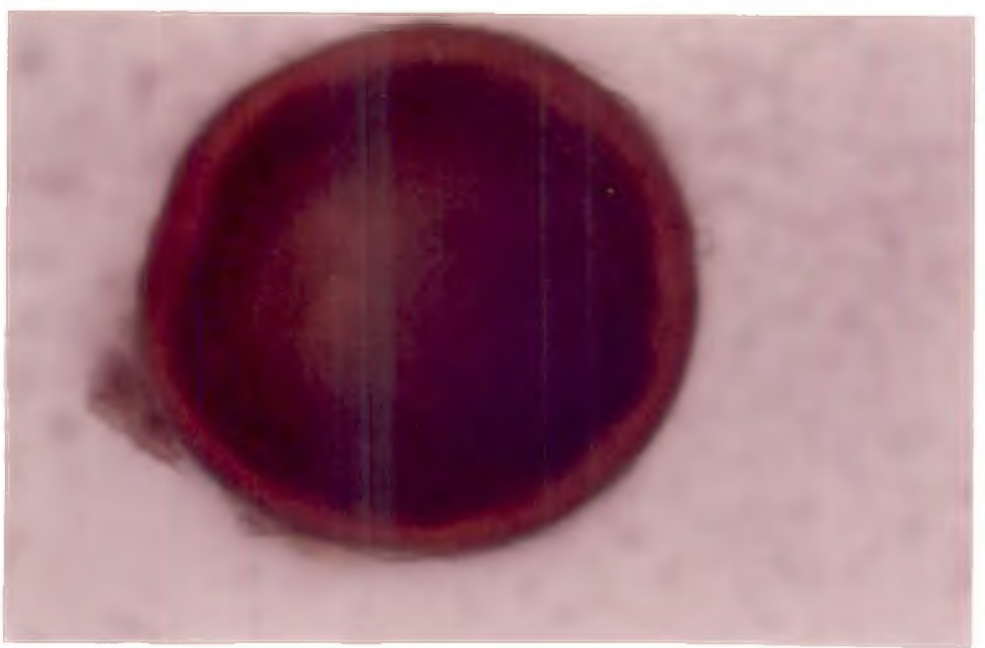


Plate 8. *Sclerocystis* species (x 100) from laterite soil

Plate 9. *Glomus* species (x 400) from laterite soil

Plate 10. *Glomus* species (x 400) from laterite soil





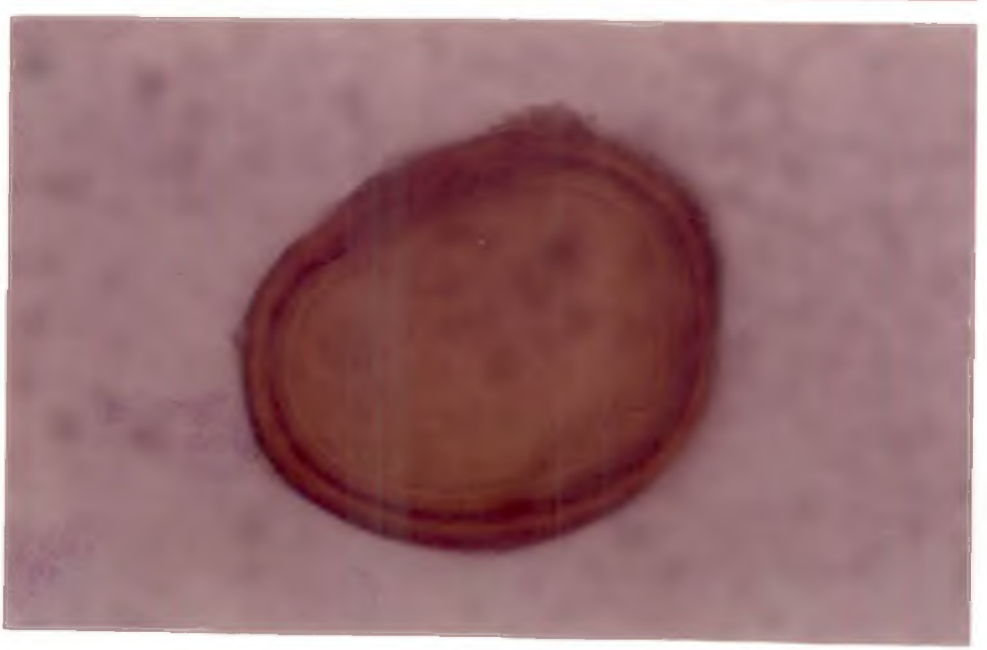
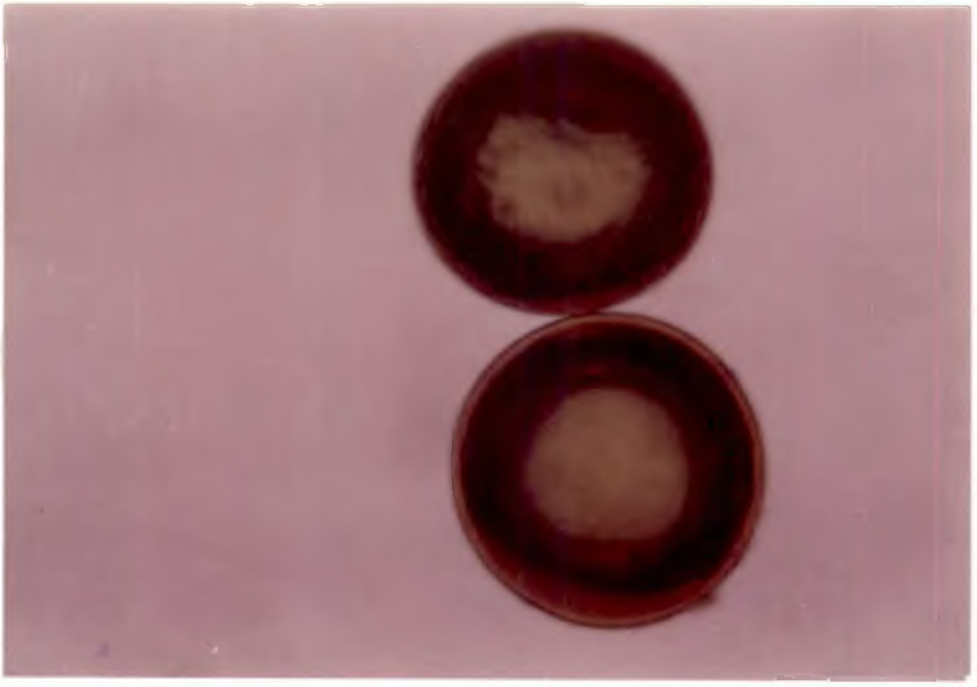


Plate 11. *Acaulospora* species (x 400) from sandy soil

Plate 12. *Gigaspora* species (x 100) from sandy soil



AMF root colonization with respect to pepper genotypes. In Karimunda and Panniyur-1 the colonization ranged from 46.66 to 66.66 and 36.33 to 73.33 per cent respectively. However, the data further revealed that root colonization was more influenced by soil types rather than host genotypes.

AMF characterisation in black pepper with reference to different locations of same or different soil types revealed that, AMF species in the rhizosphere of black pepper was greatly influenced by soil types than plant geno types (Table 13).

Glomus spp were predominant in haplic argiustoll (Plate 6 and 7) oxyaquic quartpsamment and typic plinthustult (Plate 9 and 10) soils. Pepper roots in laterite soils were also frequently harboured by sclerocystis spp (Plate 8). However species of *Acaulospora* (Plate 11) and *Gigaspora* (Plate 12) were also frequently observed in oxyaquic quartpsamment soil.

Observation of the 17 cultivars grown in the same field showed that species of genus *Glomus* and *Sclerocystis* were the predominant AMF associated with black pepper grown in the typic plinthustult soils of Panniyur, Kannur district (Table 14). *Sclerocystis clavispora*, *S. coremioides* and *G. fasciculatum* were the most frequently noticed (Table 14). Except cultivar Kalluvally all the plants harboured

Table 15 AMF associated with different pepper cultivars grown in different soils

Cultivar	AM Fungi		
	Laterite soil (Kannur district)	Forest soil (Idukki district)	Forest soil (Wayanad district)
Panniyur 1	*G. fasciculatum* *G. multicaule* *Gigaspora* sp	*G. fasciculatum* *S. clavispora* *Glomus* sp	*Gigaspora nigra* *Glomus monosporum* *Gigaspora* sp
Uthiramkotta	*G. fasciculatum* *S. coremioides* *Glomus* sp	*G. fasciculatum* *S. clavispora* *Glomus* sp	*Gigaspora nigra* *Glomus* sp
Aimpiriyan	-	-	*Acaulospora* sp, *G. fasciculatum*, *Glomus* up
Wayanadan	-	-	*Acaulospora* sp, *G. fasciculatum*, *S. coremioides*, *S. clavispora*
Karimunda	*G. fasciculatum* *S. coremioides* *Sclerocystis* sp	*G. fasciculatum* *S. rubiformis* *Glomus* sp	*G. monosporum*, *G. fasciculatum*, *Gigaspora* sp.

Table 16 Effect of antagonistic fungal isolates on the growth of *P. capsici* *in vitro*

Source	Isolate No.	Colony diameter *P. capsici* (mm)	Growth inhibition(%)	Dry mycelial weight of *P. capsici* (g)
*Idukki district				
Balagram	A_1	8.33	80.17	0.039
	A_2	29.30	30.24	0.066
Pampadumpara	A_3	23.00	45.24	0.060
	A_4	36.60	12.86	0.073
Kallar	A_5	32.60	22.38	0.074
	A_6	21.00	50.00	0.058
Santhanpara	A_7	31.00	26.19	0.072
	A_8	33.33	20.64	0.071
Chathurangappara	A_9	30.66	27.00	0.068
	A_{10}	31.60	24.76	0.071
	A_{11}	11.33	73.02	0.063
Kattappana	A_{12}	28.60	31.90	0.071
	A_{13}	9.83	76.59	0.063
Mundieruma	A_{14}	10.83	74.21	0.053
	A_{15}	30.37	27.69	0.070
**Wayanad district				
Mullamkolly	A_{16}	11.83	71.83	0.065
	A_{17}	38.00	9.52	0.074
Ambalavayal	A_{18}	31.30	25.48	0.068
	A_{19}	15.33	63.50	0.056

Source	Isolate No.	Colony diameter *P. capsici* (mm)	Growth inhibition(%)	Dry mycelial weight of *P. capsici* (g)
*Puliyanmmala	A_{20}	13.33	68.26	0.065
	A_{21}	8.67	79.36	0.052
*Kochara	A_{22}	9.00	78.57	0.029
	A_{23}	38.30	8.81	0.073
**Ariapally	A_{24}	37.00	11.90	0.069
**Cheyambam	A_{25}	37.30	11.19	0.068
*Kailasappara	A_{26}	10.67	74.59	0.035
	A_{27}	12.16	71.05	0.045
*Puthanam	A_{28}	14.66	65.09	0.070
*Velumbanchola	A_{29}	9.83	76.59	0.061
Kannur district				
Kannavum	A_{30}	18.17	56.74	0.049
Peravur	A_{31}	12.17	71.02	0.048
Panniyur	A_{32}	16.60	60.48	0.046
	A_{33}	10.00	76.19	0.044
#Peringammala	A_{34}	12.67	69.83	0.051
**Pazhuppathoor	A_{35}	11.83	71.83	0.022
**Kolakappara	A_{36}	36.00	14.28	0.064
**Beenachi	A_{37}	12.30	70.71	0.048
**Thavinjal	A_{38}	13.30	68.33	0.060
#Thiruvananthapuram district				
Vellayani (Vermicompost)	A_{39}	16.67	60.31	0.036

Source	Isolate No.	Colony diameter *P. capsici* (mm)	Growth inhibition(%)	Dry mycelial weight of *P. capsici* (g)
Pattom (Vermicompost)	A_{40}	11.80	71.90	0.051
Vellanad (Vermicompost)	A_{41}	10.33	75.40	0.054
	A_{42}	12.50	70.24	0.057
Pathanamthitta district				
Ranni	A_{43}	35.00	16.67	0.070
	A_{44}	28.60	31.90	0.070
Elanthoor	A_{45}	28.25	32.74	0.068
	A_{46}	26.60	36.67	0.062
Ayroor	A_{47}	29.60	29.52	0.068
	A_{48}	29.33	30.17	0.066
***Sreekandapuram	A_{49}	20.33	51.59	0.063
#Vellayani	A_{50}	30.67	26.98	0.066
Control (*P. capsici*)		42.00	0.00	0.076
CD (0.05)		3.01	-	0.011

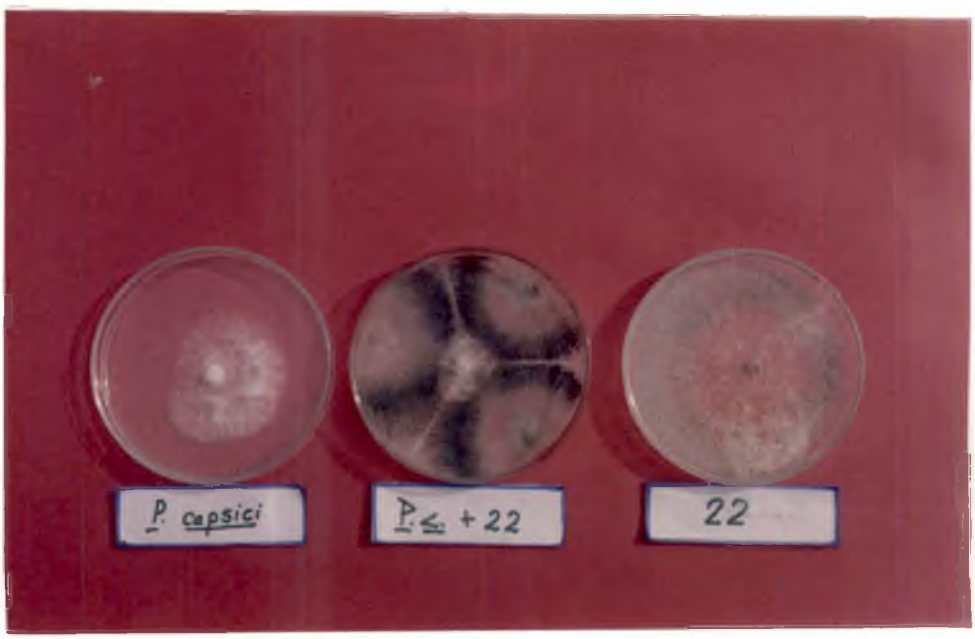
Plate 13. Growth inhibition of *P. capsici* by antagonist A_1

Plate 14. Growth inhibition of *P. capsici* by antagonist A_{21}

Plate 15. Growth inhibition of *P.capsici* by antagonist A_{22}


P. capsici
Pc +1
1


P. capsici
Pc +21
21


P. capsici
Pc +22
22

G. fasciculatum. The AMF species associated with pepper plants were, to a limited extent, influenced by host genotype (Table 14). Observation of different cultivars grown in different soils also indicated a clear influence of soil on AMF species in harbouring pepper cultivars (Table 15).

4.4 Isolation and testing of fungal antagonists against *P. capsici in vitro*

Fifty antagonistic fungi were isolated from 32 sources covering different locations and substrates. The details of source and the number assigned are given in Table 16.

4.4.1 Testing of fungal antagonists against *P. capsici* in culture plate

All the antagonistic isolates showed clear inhibitory effect on *P. capsici* on dual culturing in petri-plates (Table 16). However, there was wide variation among the isolates in their antagonistic ability. The growth suppression of *P. capsici* by isolates A_1 (Plate 13), A_{11}, A_{13}, A_{14}, A_{16}, A_{21} (Plate 14), A_{22} (Plate 15), A_{26}, A_{29}, A_{33}, A_{35}, A_{40} and A_{41} were statistically significant over control. Although all the fungal isolates established their antagonistic property, only 24 isolates were recorded inhibition over 60 per cent of *P. capsici* growth. The isolate A_1 exerted comparatively higher inhibition (80.17%) followed by A_{21} (79.36%) and A_{13} (76.59%).

Table 17 Effect of antagonists on survival of *P. capsici* in soil

Antagonist isolate No.	*Population		Reduction in *P. capsici* population
	Antagonists ($x10^5$ g^{-1})	*P. capsici* ($x10^3$ g^{-1})	(%)
A_1	7.75	0.50	89.47
A_2	2.75	3.00	36.84
A_3	2.00	1.50	68.42
A_4	2.75	3.75	21.05
A_5	1.00	3.00	36.84
A_6	3.50	3.50	26.31
A_7	2.75	3.50	26.31
A_8	4.00	2.00	57.89
A_9	2.75	3.75	21.05
A_{10}	1.50	4.00	15.78
A_{11}	2.50	2.00	57.89
A_{12}	3.50	2.50	47.36
A_{13}	2.75	2.50	47.36
A_{14}	1.00	1.75	63.15
A_{15}	2.75	3.00	36.84
A_{16}	4.50	1.50	68.42
A_{17}	2.00	3.75	21.05
A_{18}	2.75	3.00	36.84
A_{19}	1.00	2.50	47.36
A_{20}	2.00	1.75	64.37
A_{21}	1.75	1.00	78.94
A_{22}	13.75	0.75	84.21
A_{23}	1.00	3.50	26.31
A_{24}	2.50	4.00	15.78
A_{25}	2.75	3.75	21.05
A_{26}	11.00	1.50	68.42

Antagonist isolate No.	*Population		Reduction in *P. capsici* population
	Antagonists ($x10^5$ g^{-1})	*P. capsici* ($x10^3$ g^{-1})	(%)
A_{27}	3.75	1.50	68.42
A_{28}	2.50	2.75	42.10
A_{29}	7.75	1.50	68.42
A_{30}	1.75	2.75	42.10
A_{31}	6.75	0.50	89.47
A_{32}	11.00	0.75	84.91
A_{33}	12.00	0.33	93.05
A_{34}	2.50	1.75	63.15
A_{35}	4.50	0.50	89.47
A_{36}	1.50	3.00	36.84
A_{37}	3.00	0.50	89.47
A_{38}	1.50	2.75	42.10
A_{39}	3.00	2.50	47.36
A_{40}	4.75	1.50	68.42
A_{41}	3.50	1.75	63.15
A_{42}	3.50	0.50	89.47
A_{43}	1.75	2.75	42.10
A_{44}	2.50	2,00	36.84
A_{45}	2.00	2.75	42.10
A_{46}	3.50	2.50	47.36
A_{47}	1.50	2.75	42.10
A_{48}	1.00	2.75	42.10
A_{49}	2.75	3.00	36.84
A_{50}	1.75	2.50	47.36
Control (*P. capsici*)	-	4.75	0.00

Mean of 4 replications

4.4.2 Effect of metabolites of fungal antagonists on the growth of *P. capsici*

Incorporation of culture filtrates of antagonistic isolates in the medium showed a general reduction in the growth of *P. capsici* (Table 16). Isolate A_{35} exhibited highest inhibition followed by A_{22}, A_1, A_{26} and A_{39}. The dry mycelial weight of *P. capsici* in the medium amended with metabolites of A_{22} and A_{35} was 0.029 and 0.022 g respectively which accounted for 61.84 and 71.05 per cent growth inhibition over control. Similarly, culture filtrates of A_1, A_{26} and A_{39} also showed very effective inhibition and recorded relatively low mycelial production of 0.039, 0.035 and 0.036 g respectively. Control plates inoculated with *P. capsici* alone had 0.076 g.

4.4.3 *P. capsici* population in antagonists amended soil

The data pertaining to the effect of amending the soil with antagonistic organisms on the population dynamics of *P. capsici* is presented in Table 17. The soil amended with isolates A_1, A_{21}, A_{22}, A_{31}, A_{32}, A_{33}, A_{35}, A_{37} and A_{42} exhibited more than 75 per cent reduction in *P. capsici* population over control. There was a negative relationship between the population of the antagonist and the pathogen in the soil. Isolates A_{22}, A_{32} and A_{33}, which recorded a comparitively higher population of 13.75, 11 and 12 x 10^5 propagules g^{-1} soil

Table 18 Effect of antagonists on growth of Black pepper cuttings in green house

Antagonist Isolate No.	Plant height (cm)	Leaves (No. plant^{-1})
A_1	24.50	3.25
A_{11}	25.25	4.25
A_{13}	24.13	2.75
A_{14}	27.50	3.25
A_{16}	21.50	2.50
A_{19}	33.50	4.25
A_{20}	23.87	3.25
A_{21}	35.25	4.25
A_{22}	27.37	3.50
A_{26}	21.12	2.25
A_{27}	30.75	3.75
A_{28}	20.25	2.50
A_{29}	30.62	2.75
A_{31}	21.25	2.75
A_{32}	26.37	3.75
A_{33}	22.00	2.75
A_{34}	26.87	3.00
A_{35}	30.75	3.50
A_{37}	23.62	2.75
A_{38}	22.25	2.75
A_{39}	23.25	2.75
A_{40}	21.75	2.25
A_{41}	25.37	2.75
A_{42}	22.62	2.75
Control	21.00	2.75
CD (0.05)	NS	NS

NS - Not significant

Plate 16. Effect of selected antagonists on growth of black pepper in green house

Plate 17. Effect of selected antagonists on foot rot incidence in black pepper in green house


CON
A21
A19


CON
A1
A13
A21

respectively, were able to reduce the *P. capsici* population over 80 per cent. While isolate A_{26} with 11 x 10^5 propagules as an exception recorded only 68.42 per cent reduction in *P. capsici* population. Amendment with A_1,. A_{21}, A_{31}, A_{35}, A_{37} and A_{42} also showed comparitively low population of 7.5, 1.75, 6.75, 4.5, 3 and 3.05 x 10^5 propagules g^{-1} soil respectively. There was 4.75 x 10^3 *P. capsici* population in control.

4.5 Screening of fungal antagonists on plant growth and foot rot incidence of black pepper in green house

4.5.1 Effect on growth

The data on biometric observations recorded on 90^{th} day of planting did not show significant influence of antagonists on growth of black pepper (Table 18). However, the isolates A_{21} and A_{19} recorded relatively higher plant height of 35.25 and 33.50 cm, with an average leaf number of 4.25 each (Plate 16). The plant height and number of leaves for control plants were 21 cm and 2.75 respectively. Relatively better plant growth characteristics were also recorded in black pepper on inoculation with isolates A_{14}, A_{22}, A_{27}, A_{29} and A_{35} with plant height and leaf number ranging from 27.37 to 30.75 cm and 2.75 to 3.5 respectively (Table 18).

Table 19 Effect of antagonists against foot rot incidence (infection) in black pepper in green house

Antagonist isolate No.	Infection (%) Days after *P. capsici* inoculation					
	15	30	40	50	60	Mean
A_1	0.00 (0.00)	0.00 (0.00)	16.67 (24.08)	33.33 (35.25)	41.54 (40.12)	18.31 (19.89)
A_{11}	4.36 (12.04)	24.52 (29.67)	58.45 (49.85)	83.33 (65.88)	100.00 (90.00)	54.13 (49.49)
A_{13}	16.67 (24.08)	16.67 (24.08)	16.67 (24.08)	41.54 (40.11)	50.00 (44.98)	28.31 (31.47)
A_{14}	4.36 (12.04)	4.36 (12.04)	9.17 (17.62)	90.84 (72.36)	90.84 (72.36)	39.91 (37.28)
A_{16}	9.17 (17.62)	33.33 (35.25)	50.00 (44.98)	95.66 (77.94)	100.00 (90.00)	57.63 (53.16)
A_{19}	9.17 (17.62)	33.33 (35.25)	50.00 (44.98)	75.48 (60.30)	95.66 (77.93)	52.73 (47.22)
A_{20}	4.36 (12.04)	24.52 (29.67)	33.33 (35.24)	95.66 (77.93)	95.66 (77.93)	50.71 (46.57)
A_{21}	4.36 (12.04)	41.54 (40.12)	50.00 (44.98)	50.00 (44.98)	58.45 (49.85)	40.87 (38.39)
A_{22}	4.36 (12.04)	4.36 (12.04)	9.17 (17.62)	58.45 (49.85)	58.45 (49.85)	26.96 (28.28)
A_{26}	4.36 (12.04)	33.33 (35.25)	50.00 (44.98)	83.33 (65.88)	95.66 (77.93)	53.34 (47.22)
A_{27}	33.33 (35.25)	41.54 (40.12)	58.45 (49.85)	75.48 (60.30)	90.84 (72.36)	59.93 (51.57)
A_{28}	0.00 (0.00)	0.00 (0.00)	50.00 (44.98)	90.84 (72.36)	100.00 (90.00)	48.17 (41.47)
A_{29}	0.00 (0.00)	0.00 (0.00)	24.52 (29.67)	75.48 (60.30)	100.00 (90.00)	40.00 (36.00)

Antagonist isolate No.	Infection (%)									
	Days after *P. capsici* inoculation									
	15		30		40		50		60		Mean	
A_{31}	24.52	(29.67)	50.00	(44.98)	94.84	(72.36)	100.00	(90.00)	100.00	(90.00)	73.87	(65.40)
A_{32}	4.36	(12.04)	9.17	(17.62)	33.33	(35.25)	95.66	(77.94)	100.00	(90.00)	48.50	(66.57)
A_{33}	33.33	(35.25)	33.33	(35.25)	58.45	(49.85)	90.84	(72.36)	100.00	(90.00)	63.19	(56.54)
A_{34}	4.36	(12.04)	4.36	(12.04)	24.52	(29.67)	66.67	(54.71)	75.48	(60.37)	38.08	(33.75)
A_{35}	24.52	(29.67)	33.33	(35.25)	41.54	(40.12)	50.00	(44.98)	58.45	(49.85)	)41.57	(39.97
A_{37}	4.36	(12.04)	9.17	(17.62)	41.54	(40.12)	66.67	(54.71)	66.67	(54.71)	37.68	(35.84)
A_{38}	41.54	(40.12)	66.67	(54.71)	90.84	(72.36)	100.00	(90.00)	100.00	(90.00)	79.81	(69.44)
A_{39}	0.00	(0.00)	0.00	(0.00)	58.45	(49.85)	90.84	(72.36)	100.00	(90.00)	49.86	(42.44)
A_{40}	9.17	(17.62)	50.00	(44.98)	66.67	(54.71)	100.00	(90.00)	100.00	(90.00)	65.17	(59.49)
A_{41}	24.52	(29.67)	41.54	(40.12)	66.67	(54.71)	95.66	(77.93)	100.00	(90.00)	65.68	(58.49)
A_{42}	9.17	(17.62)	33.33	(35.25)	50.00	(44.98)	90.84	(72.36)	90.84	(72.36)	54.84	(48.51)
Control (*P. capsici*)	41.54	(40.12)	66.67	(54.71)	66.67	(54.71)	100.00	(90.00)	100.00	(90.00)	74.98	(65.91)

Figures in parantheses are transformed values

	Treatment mean	Interaction
CD (0.05) for transformed means	19.61	18.63

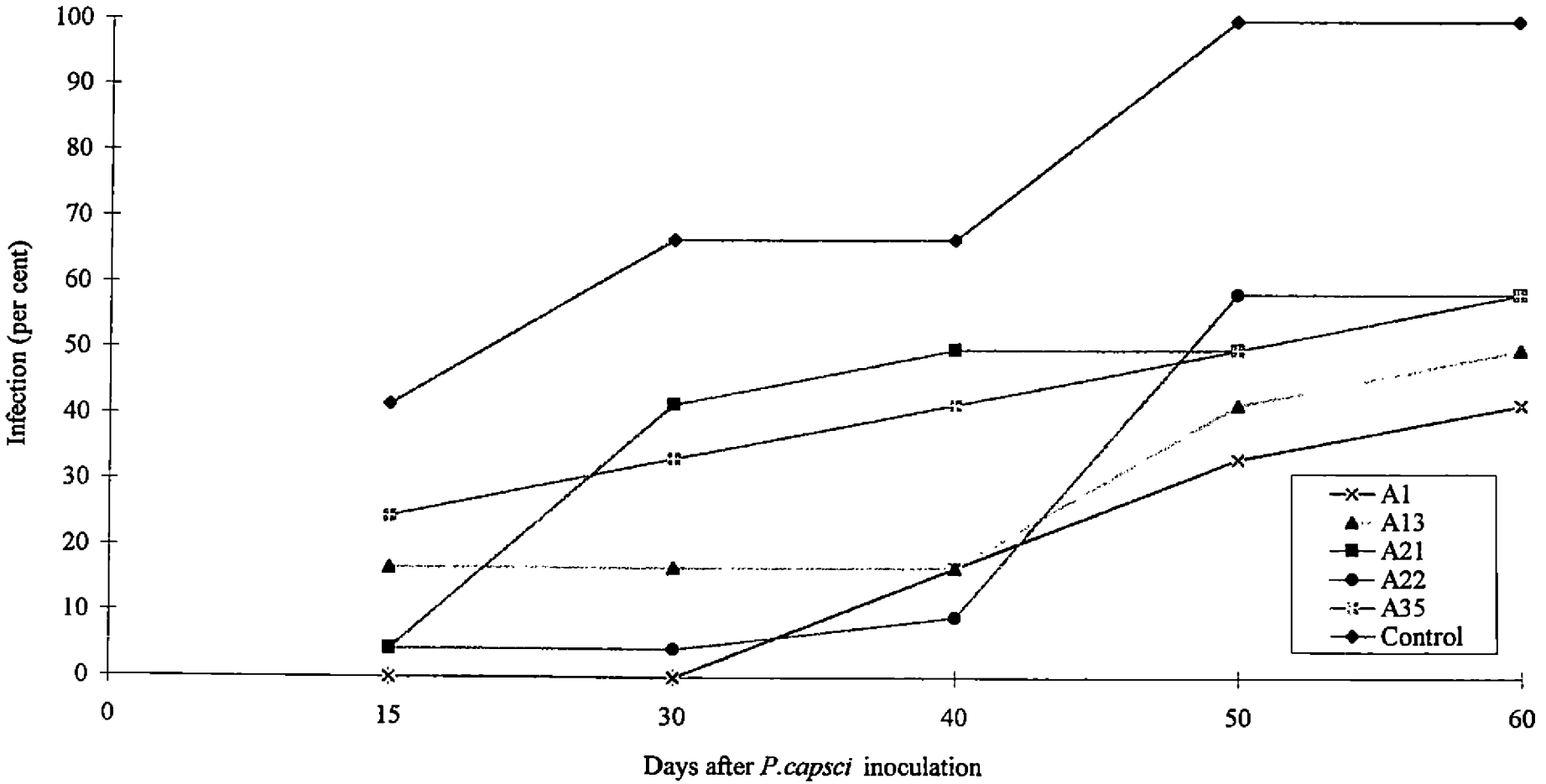


Fig. 6. Foot rot infection pattern in black pepper inoculated with antagonists in green house

4.5.2 Effect on foot rot incidence

The 24 fungal antagonists selected from *in vitro* studies were further tested against foot rot disease in black pepper under green house conditions. The data on per cent foot rot infection and mortality are presented in Table 19 and 20. There was no infection in A_1 treatment till 30th day of inoculation with *P. capsici*. Seven antagonists, viz., A_1, A_{13}, A_{21}, A_{22}, A_{34}, A_{35} and A_{37} brought about significant reduction in foot rot infection per cent over control (Table 19). Least incidence of 41.54 per cent was recorded with isolate A_1, followed by A_{13} with 50 per cent as against 100 per cent incidence observed in control on the 60th day of pathogen inoculation (Fig. 6, Plate 17). Antagonistic isolate A_{21}, A_{22} and A_{35} showed disease incidence of 58.45 per cent. Certain isolate (A_{11}, A_{16}, A_{28}, A_{29}, A_{31}, A_{32}, A_{33}, A_{38}, A_{39}, A_{40}, A_{41}) did not exhibit any disease suppression effect and recorded 100% infection as in the case of control.

Treatment mean of antagonistic isolates on infection per cent varied from 18.31 to 79.81. There was a gradual increase in foot rot incidence and reaching the maximum infection by 60th day (Table 19). The treatment mean computed for A_1, A_{13}, A_{14}, A_{21}, A_{22}, A_{27}, A_{34}, A_{35} and A_{39} were statistically significant in reducing the disease incidence.

Table 20 Effect of antagonists on foot rot incidence (mortality) in black pepper in green house

Antagonist isolate No.	Mortality (%) Days after *P. capsici* inoculation				
	30	40	50	60	Mean
A_1	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	33.33 (35.25)	8.33 (8.81)
A_{11}	4.36 (12.04)	9.17 (17.62)	58.45 (49.85)	90.84 (72.36)	40.70 (37.97)
A_{13}	9.17 (17.62)	9.17 (17.62)	33.33 (35.25)	41.54 (40.12)	23.30 (27.65)
A_{14}	4.36 (12.04)	4.36 (12.04)	24.52 (29.67)	75.48 (60.30)	27.18 (28.51)
A_{16}	9.17 (17.62)	9.17 (17.62)	50.00 (44.98)	95.66 (77.93)	41.00 (39.54)
A_{19}	9.17 (17.62)	9.17 (17.62)	50.00 (44.98)	75.48 (60.30)	35.96 (35.13)
A_{20}	4.36 (12.04)	24.52 (29.67)	58.45 (49.85)	90.84 (72.36)	44.54 (40.98)
A_{21}	4.36 (12.04)	41.54 (40.12)	50.00 (44.98)	50.00 (44.98)	36.47 (35.53)
A_{22}	4.36 (12.04)	4.36 (12.04)	24.52 (29.67)	58.45 (49.85)	22.92 (25.90)
A_{26}	4.36 (12.04)	9.17 (17.62)	33.33 (35.25)	75.48 (60.30)	30.59 (31.30)
A_{27}	33.00 (35.25)	41.54 (40.12)	50.00 (44.98)	75.48 (60.30)	50.01 (45.16)
A_{28}	0.00 (0.00)	0.00 (0.00)	50.00 (44.98)	90.94 (72.36)	35.23 (29.33)
A_{29}	0.00 (0.00)	0.00 (0.00)	24.52 (29.67)	75.48 (60.30)	25.00 (22.49)
A_{31}	24.52 (29.67)	58.45 (49.85)	66.67 (54.71)	100.00 (90.00)	62.41 (56.06)

Antagonist isolate No.	Mortality (%) Days after *P. capsici* inoculation				
	30	40	50	60	Mean
A_{32}	4.36 (12.04)	9.17 (17.62)	33.33 (35.25)	90.84 (72.36)	34.43 (34.32)
A_{33}	33.33 (35.25)	33.33 (35.25)	33.33 (35.25)	90.84 (72.36)	47.71 (44.53)
A_{34}	4.36 (12.04)	4.36 (12.04)	9.17 (17.62)	66.67 (54.71)	21.14 (24.11)
A_{35}	24.52 (29.67)	24.52 (29.67)	33.33 (35.25)	50.00 (44.98)	33.09 (34.89)
A_{37}	4.36 (12.04)	4.36 (12.04)	24.52 (29.67)	66.67 (54.71)	24.98 (27.12)
A_{38}	41.54 (40.12)	66.67 (54.71)	90.84 (72.36)	100.00 (90.00)	74.76 (64.30)
A_{39}	0.00 (0.00)	0.00 (0.00)	50.00 (44.98)	90.84 (72.36)	35.21 (29.33)
A_{40}	9.17 (17.62)	41.54 (40.12)	66.67 (54.71)	100.00 (90.00)	54.35 (50.61)
A_{41}	24.52 (29.67)	33.33 (35.25)	50.00 (44.98)	90.84 (72.36)	49.67 (45.56)
A_{42}	9.17 (17.62)	33.33 (35.25)	50.00 (44.98)	83.33 (65.88)	43.96 (40.93)
Control (*P. capsici*)	41.54 (40.12)	66.67 (54.71)	66.67 (54.71)	95.66 (77.93)	67.63 (56.87)

* Figures in parantheses are transformed values

	Treatment mean	Interaction
CD (0.05) for transformed means	19.67	NS

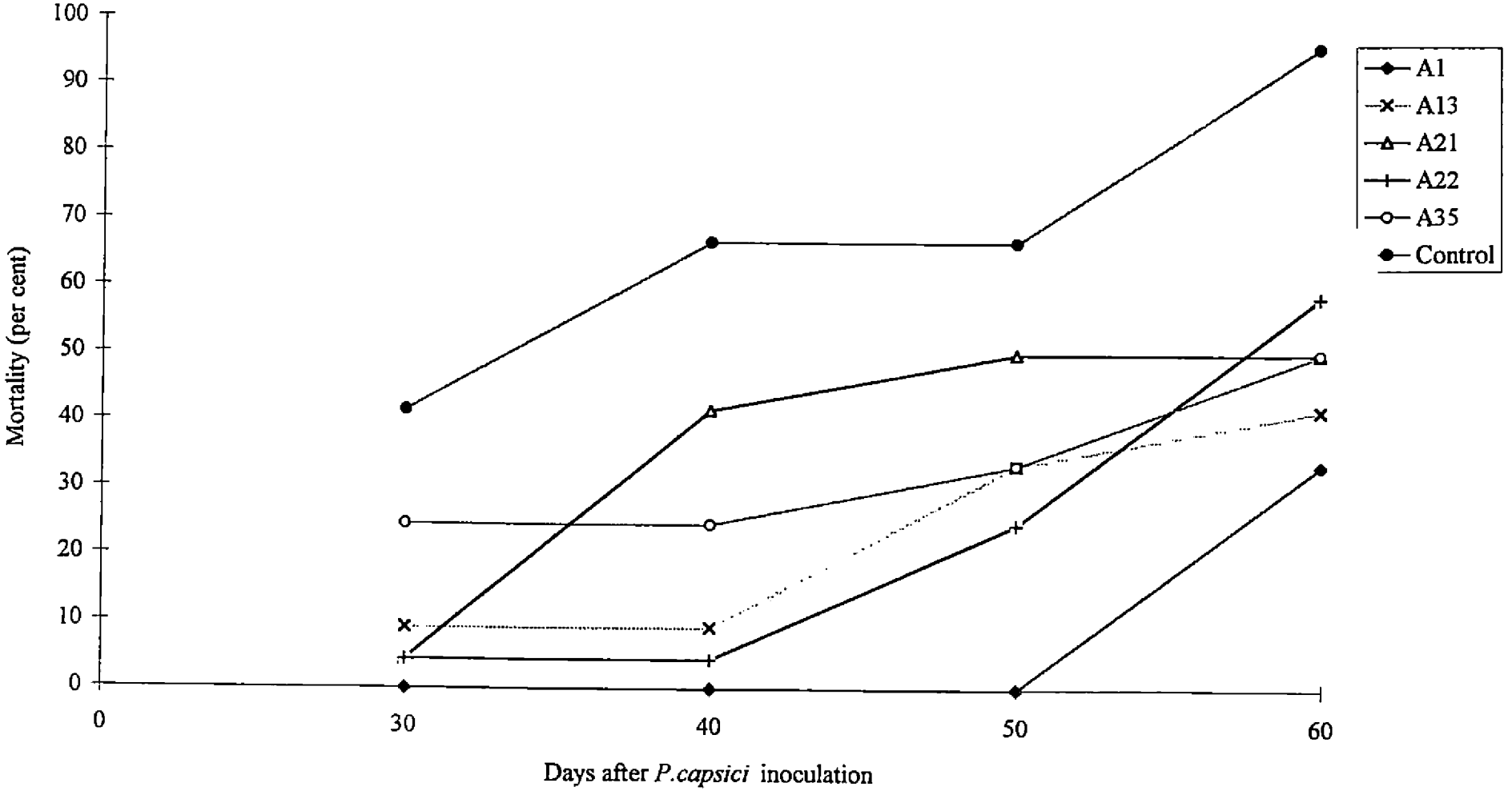


Fig. 7. Foot rot incidence (mortality) pattern in black pepper inoculated with antagonists in green house

Table 21 Population build up of *P. capsici* and antagonists in pepper rhizosphere in green house

Antagonist isolate No.	Population of fungi		
	*Antagonists ($x10^5$ g^{-1})	**P. capsici* ($x10^3$ g^{-1})	Reduction in *P. capsici* population (%)
A_1	5.50	0.50	86.67
A_{11}	3.75	2.75	26.67
A_{13}	3.50	1.50	60.00
A_{14}	1.00	1.71	53.33
A_{16}	4.75	2.00	46.67
A_{19}	1.75	1.50	50.00
A_{20}	2.00	1.75	53.33
A_{21}	2.75	1.75	53.33
A_{22}	12.50	0.50	86.67
A_{26}	1.75	1.50	60.00
A_{27}	3.75	1.00	73.33
A_{28}	2.50	1.50	60.00
A_{29}	4.50	2.76	26.67
A_{31}	2.75	2.50	33.33
A_{32}	8.75	2.75	26.67
A_{33}	12.75	1.50	60.00
A_{34}	2.50	1.75	53.33
A_{35}	4.75	0.50	86.67
A_{37}	5.50	1.50	60.00
A_{38}	2.75	3.00	20.00
A_{39}	1.75	1.75	53.33
A_{40}	2.00	2.50	33.33
A_{41}	1.75	1.75	53.33
A_{42}	3.75	2.00	46.67
Control (*P. capsici*)	0.00	3.75	0.00

Values are means of 4 replications

Table 22 Effect of dual inoculation of black pepper with AMF isolates on growth in green house

Treatment	Plant height (cm)	Leaves (No. plant^{-1})
A_1x *G. margarita*	36.67	7.33
A_1x Pi-9	62.67	10.67
A_1x *G. fasciculatum*	103.33	12.67
A_1x Is-6	79.00	11.00
A_1x Pi-11	86.00	13.67
A_{13}x *G. margarita*	55.67	9.00
A_{13}x Pi-9	50.67	6.33
A_{13}x *G. fasciculatum*	39.00	6.67
A_{13}x Is-6	73.00	10.67
A_{13}x Pi-11	60.33	9.00
A_{21}x *G. margarita*	72.33	10.33
A_{21}x Pi-9	56.67	7.67
A_{21}x *G. fasciculatum*	103.33	14.00
A_{21}x Is-6	78.67	16.00
A_{21}x Pi-11	79.00	11.00
A_{22} x *G. margarita*	61.67	11.00
A_{22} x Pi-9	58.33	8.67
A_{22} x *G. fasciculatum*	90.67	17.00
A_{22} x Is-6	63.67	15.00
A_{22} x Pi-11	65.00	11.67
A_{35} x *G. margarita*	88.33	10.33
A_{35} x Pi-9	78.67	10.00
A_{35} x *G. fasciculatum*	54.33	9.33
A_{35} x Is-6	70.67	12.67
A_{35} x Pi-11	95.67	16.33
A_1	66.67	10.33
A_{13}	50.50	8.67
A_{21}	71.00	12.00
A_{22}	61.50	9.33
A_{35}	70.50	11.00
G. margarita	57.67	9.00
Pi-9	55.33	8.00
G. fasciculatum	70.50	12.00
IS-6	60.50	11.67
Pi-11	70.33	13.00
Control	48.33	8.00
CD (0.05)	16.01	4.24

Mean values computed for A_1, A_{13}, A_{22} and control were 18.31, 28.31, 26.96 and 74.98 per cent respectively.

Regarding the plant mortality A_1, A_{13}, A_{14}, A_{21}, A_{22}, A_{26}, A_{28}, A_{29}, A_{34}, A_{35}, A_{37} and A_{39} exhibited significant reduction in plant mortality over control (Table 20). Isolate A_1, A_{13}, A_{21}, A_{22} and A_{35} had the mortality of 33.33, 41.54, 50.00, 58.45 and 50.00 per cent respectively on 60th day as against 95.66 per cent in control. Isolates A_{31} A_{38} and A_{40} also showed 100 per cent mortality. A similar trend was observed in treatment means of various isolates with plant mortality of 8.33, 23.30, 35.96, 36.47, 22.92 and 33.09 per cent for effective isolate such as A_1, A_{13}, A_{19}, A_{21}, A_{22} and A_{35} respectively. Control had the treatment mean of 56.87. Isolate A_1 did not register any mortality till 50th day of pathogen inoculation (Fig. 7).

Although none of the antagonistic isolates could provide complete protection against the disease under green house conditions, the isolates A_1, A_{13}, A_{21}, A_{22} and A_{35} showed relatively better suppression of the disease and the mortality rate was minimum. A_1 was the most effective isolate.

The data pertaining to the population of antagonists and *P. capsici* are given in Table 21. There was negative relationship between the population of antagonists and that of *P. capsici*. All the antagonists reduced *P. capsici* population

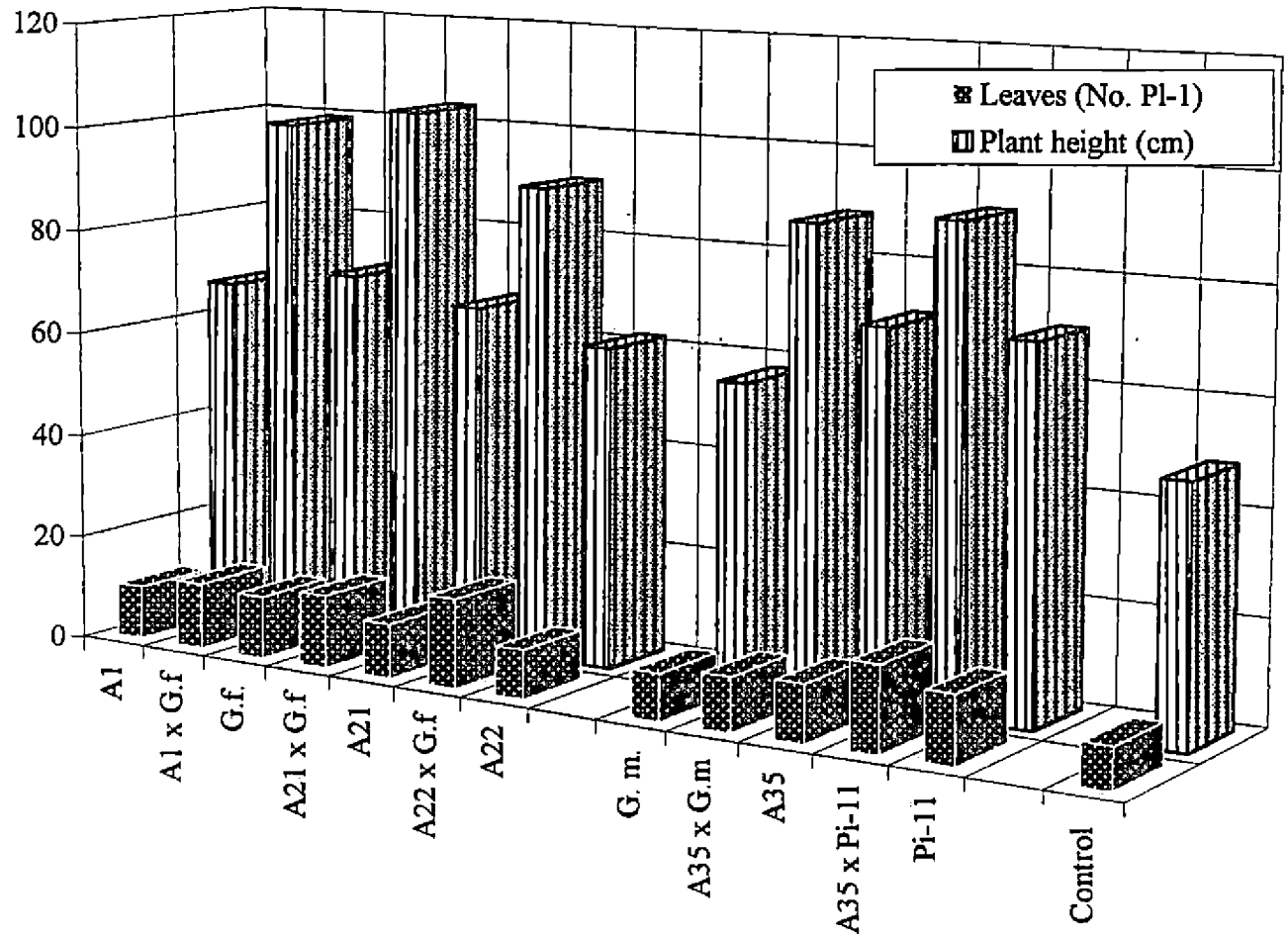


Fig. 8 Effect of AMF and antagonists on growth of black pepper in green house

to a greater extent as compared to control. The lowest *P. capsici* population of 0.50 x 10^3 was recorded with A_1, A_{22} and A_{35}. The control had 3.75 x 10^3 pathogen population. A_{13}, A_{19}, A_{21} and A_{27} showed the population of 1.50, 1.50, 1.75 and 1.00 x 10^3 respectively. Regarding the population of antagonists, isolates A_1, A_{22}, A_{32}, A_{33} and A_{37} showed a relatively higher population of 5.50, 12.50, 8.75, 12.75 and 5.50 x 10^5 respectively. A_{13} and A_{35} had the population of 3.50 and 4.75 x 10^5 A_{14}, A_{19}, A_{20}, A_{39}, and A_{41} recorded comparatively less population of 1.00 to 2.00 x 10^5. The highest reduction in pathogen population (86.67%) was achieved with A_1, A_{22} and A_{35}. Based on the performance under *in vitro* and green house conditions isolates A_1, A_{13}, A_{21}, A_{22} and A_{35} were selected for further studies.

4.6 Combined effect of dual inoculation of AMF and fungal antagonists on plant growth and foot rot incidence of black pepper in green house

4.6.1 Effect on growth

The dual inoculation effect on growth characteristics of black pepper in green house is given in Table 22. Antagonistic fungal isolates A_1 and A_{21} in combination with *G. fasciculatum* gave higher plant height of 98.33 and 103.33 cm respectively (Fig. 8). Combinations also yielded maximum leaf number of 12.67 and 14.00. *Glomus fasciculatum* in combination

with antagonistic isolate A_{22} also enhanced the plant height (90.67 cm) and leaf number (17.00). Combination of A_{35} and Pi-11 also resulted in the same plant height (90.67 cm) with leaf number of 16.33 leaves. Single inoculation of *G. fasciculatum* and Pi-11 recorded the plant height of 70.50 and 70.33 cm and leaf number of 12 and 13 respectively. Antagonists A_1, A_{21} and A_{35} recorded 66.67, 71.00 and 70.50 cm plant height and 10.33, 12.00 and 11.00 leaves respectively. The control plants recorded the plant height of 48.33 cm with 8 leaves. AMF cultures *G. fasiculatum*, Is-6 and Pi-11 in combination with antagonist A_1, A_{21}, A_{22} and A_{35} showed significant influence on growth of black pepper cuttings. The combination of A_1 x *G. margirata* showed lowest longest plant height (36.67 cm) and leaf number (7.33) than recorded for control.

4.6.2 Effect on foot rot incidence

The dual inoculation effect of AMF cultures Is-6, Pi-9, Pi-11, *Glomus fasciculatum* and *Gigaspora margarita* and antagonists A_1, A_{13}, A_{21}, A_{22} and A_{35} on foot rot was studied in green house. The results are presented in Table 23 and 24. The infection per cent recorded on 15^{th}, 30^{th}, 45^{th} and 60^{th} day indicated that the infection was maximum on 60^{th} day in all the treatments. However, the control plants showed high infection per cent of 88.31 on the 30^{th} day of *P. capsici*

inoculation (Table 23). Amongst the different combinations tested A_{22} x Is-6 and A_{22} x Pi-11 resulted in significantly lower disease incidence of 75.00 and 82.15 per cent infection on 60th day compared to other treatments including control (100.00%), bordeaux mixture (100.00%) and 97.00 per cent in copper oxychloride (Plate 18). The observation on the 45th day indicated significantly less infection per cent in bordeaux mixture (58.68) and copper oxychloride (41.32) treatment compared to control. On 45th day the rate of infection in A_{22}xIs-6 combination (50.00%) was on par with the chemical control. However, copper oxychloride application showed significant reduction than A_{22} x Pi-11 inoculation. Among the antagonists single inoculation with A_{21} showed significant decrease in plant infection on the 45th and 60th day. All other treatments with antagonists and AMF recorded more than 90.00 per cent infection on the 60th day. The infection per cent recorded on 60th day showed significant increase when A_{21} inoculated with *G. margarita*, Pi-9, *G. fasciculatum* and Pi-11 which resulted in 100.00 per cent infection. The combination of Is-6 and A_{22} reduced the disease incidence to 75.00 per cent infection over single inoculation with Is-6 (97.00%). The isolate Pi-11 on single inoculation recorded 97.00 per cent plant infection while the isolates on dual inoculation with antagonist A_{22} significantly reduced the infection per cent to 82.15.

Table 23 Effect of dual inoculation of black pepper with AMF and antagonistic isolates on foot rot infection in green house

Treatment	Infection (%)				
	Days after *P. capsici* inoculation				
	15	30	45	60	Mean
A_1 x *G. margarita*	41.32 (39.98)	93.32 (74.99)	97.00 (79.99)	97.00 (79.99)	82.16 (68.74)
A_1 x Pi-9	41.32 (39.98)	67.10 (54.98)	75.00 (59.97)	100.00 (90.00)	70.86 (61.23)
A_1 x *G. fasciculatum*	11.70 (19.99)	67.10 (54.98)	67.10 (54.98)	100.00 (90.00)	61.48 (54.99)
A_1 x Is-6	32.90 (34.98)	67.10 (54.98)	75.00 (59.97)	100.00 (90.00)	68.75 (59.98)
A_1 x Pi-11	41.32 (39.98)	67.10 (54.98)	67.10 (54.98)	100.00 (90.00)	68.88 (59.98)
A_{13} x *G. margarita*	32.90 (34.98)	88.31 (69.98)	97.00 (79.99)	100.00 (90.00)	79.55 (68.74)
A_{13} x Pi-9	88.31 (69.98)	88.31 (69.98)	97.00 (79.97)	100.00 (90.00)	93.41 (77.49)
A_{13} x *G. fasciculatum*	50.00 (44.98)	50.00 (44.98)	75.00 (59.99)	100.00 (90.00)	68.75 (59.98)
A_{13} x Is-6	41.32 (39.98)	50.00 (44.98)	88.31 (69.98)	100.00 (90.00)	69.91 (61.24)
A_{13} x Pi-11	50.00 (44.98)	82.15 (64.98)	82.15 (64.98)	100.00 (90.00)	78.58 (66.24)
A_{21} x *G. margarita*	50.00 (44.98)	75.00 (59.97)	82.15 (64.98)	100.00 (90.00)	76.79 (64.99)
A_{21} x Pi-9	50.00 (44.98)	82.15 (64.98)	88.31 (69.98)	100.00 (90.00)	80.12 (67.49)
A_{21} x *G. fasciculatum*	25.00 (29.99)	50.00 (44.98)	58.68 (49.97)	100.00 (90.00)	58.42 (53.74)
A_{21} x Is-6	32.90 (34.98)	41.32 (39.98)	75.00 (59.97)	97.00 (79.99)	61.56 (53.74)
A_{21} x Pi-11	58.68 (49.98)	67.10 (54.98)	67.10 (54.98)	100.00 (90.00)	73.22 (62.48)

Treatment	Infection (%)				
	Days after *P. capsici* inoculation				
	15	30	45	60	Mean
A_{22} x *G. margarita*	11.70 (19.99)	88.31 (69.98)	88.31 (69.98)	100.00 (90.00)	72.08 (62.49)
A_{22} x Pi-9	41.32 (39.98)	67.10 (54.98)	67.10 (54.98)	100.00 (90.00)	68.88 (59.98)
A_{22} x *G. fasciculatum*	11.70 (19.99)	67.10 (54.98)	88.31 (69.98)	97.00 (79.99)	66.03 (56.23)
A_{22} x Is-6	3.01 (10.00)	32.90 (34.58)	50.00 (44.98)	75.00 (59.97)	40.23 (37.48)
A_{22} x Pi-11	11.70 (19.99)	50.00 (44.98)	75.02 (59.99)	82.15 (64.98)	54.72 (47.49)
A_{35} x *G. margarita*	11.70 (19.99)	50.00 (44.98)	75.00 (59.97)	100.00 (90.00)	59.18 (53.74)
A_{35} x Pi-9	17.86 (24.99)	50.00 (44.98)	88.31 (69.98)	100.00 (90.00)	64.04 (57.49)
A_{35} x *G. fasciculatum*	25.00 (29.99)	75.00 (59.97)	75.00 (59.97)	100.00 (90.00)	68.75 (59.98)
A_{35} x Is-6	32.90 (34.98)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	83.23 (76.25)
A_{35} x Pi-11	32.90 (34.98)	97.00 (79.99)	100.00 (90.00)	100.00 (90.00)	82.48 (73.74)
A_1	32.90 (34.98)	75.00 (59.97)	82.15 (64.98)	100.00 (90.00)	72.51 (62.48)
A_{13}	58.68 (49.98)	75.00 (59.97)	88.31 (69.98)	100.00 (90.00)	80.50 (67.48)
A_{21}	32.90 (34.98)	67.10 (54.98)	75.00 (59.97)	88.31 (69.98)	65.83 (54.97)
A_{22}	11.70 (19.99)	67.10 (54.98)	75.00 (59.97)	97.00 (79.99)	62.70 (53.73)
A_{35}	41.32 (39.98)	82.15 (64.98)	88.31 (69.98)	100.00 (90.00)	77.95 (66.23)

Treatment	Infection (%)				
	Days after *P. capsici* inoculation				
	15	30	45	60	Mean
G. margarita	32.90 (34.98)	75.00 (59.97)	88.31 (69.98)	100.00 (90.00)	74.05 (63.73)
Pi-9	50.00 (44.98)	75.00 (59.97)	97.00 (79.99)	100.00 (90.00)	80.50 (68.73)
G. fasciculatum	25.00 (29.99)	67.10 (54.98)	75.00 (59.97)	100.00 (90.00)	66.78 (58.93)
Is-6	32.90 (34.98)	67.10 (54.98)	82.15 (64.98)	97.00 (79.99)	69.79 (58.73)
Pi-11	41.32 (39.98)	75.00 (59.97)	88.31 (69.98)	97.00 (79.99)	75.41 (62.48)
Bordeaux mixture	11.70 (19.99)	32.90 (34.98)	58.68 (49.98)	100.00 (90.00)	50.82 (48.74)
Copper oxychloride	11.70 (19.99)	32.90 (34.98)	41.32 (39.98)	97.00 (79.99)	45.73 (43.74)
Control (*P. capsici*)	41.32 (39.98)	88.31 (69.98)	100.00 (90.00)	100.00 (90.00)	82.41 (72.49)

Figures in parantheses are transformed values

	Treatment Mean	Interaction
CD (0.05) for transformed means	14.25	14.61

The treatment mean computed for the combined effect of A_{22} and Is-6 showed the lowest infection per cent of 40.23 as against 82.41 in control (Table 23). Significantly low treatment mean on foot rot infection was also recorded with A_{22} x *G. fasciculatum* (40.23), A_{22} x Pi-11 (54.72%), copper oxychloride (45.73) and bordeaux mixture (50.82). Antagonists A_{21} and A_{22} alone registered the mean infection per cent of 65.83 and 62.70 respectively.

The plant mortality due to the disease was recorded from 15th day of inoculation with the pathogen (Table 24). On the 15th day itself 25.00 per cent mortality was recorded in control. While antagonists A_{22} in combination with Pi-9, Is-6 and *G. fasciculatum* did not result in mortality. *G. margarita* and Pi-11 with A_{22} showed 3.01 per cent mortality. A_{22} on single inoculation also had only 3.01 per cent mortality. bordeaux mixture and copper oxychloride application also did not register any mortality on the 15th day. Similarly all the AMF in combination with A_{35} did not record death of plants on 15th day.

Antagonistic isolate A_{22} in combination with Is-6 recorded 3.01 per cent mortality on 30th day followed by its combination with *G. fasciculatum* (11.70%), Pi-11 (11.70%) and *G. margarita* (11.70%), while the mortality rate for Pi-11 inoculation was recorded 41.32 per cent plant mortality.

Table 24 Effect of dual inoculation of black pepper with AMF and antagonistic isolates on mortality due to foot root in green house

Treatment	Mortality (%)				
	Days after *P. capsici* inoculation				
	15	30	45	60	Mean
A_1 x *G. margarita*	0.00 (00.00)	41.32 (39.98)	58.68 (49.98)	93.32 (74.99)	48.33 (41.24)
A_1 x Pi-9	3.01 (10.00)	32.90 (34.98)	58.68 (49.98)	67.10 (54.98)	40.42 (37.48)
A_1 x *G. fasciculatum*	3.01 (10.00)	11.70 (19.99)	50.00 (44.98)	50.00 (44.98)	28.68 (27.49)
A_1 x Is-6	11.70 (9.99)	32.90 (34.98)	58.68 (49.98)	67.10 (54.98)	42.60 (39.98)
A_1 x Pi-11	0.00 (0.00)	32.90 (34.98)	58.68 (49.98)	58.68 (49.98)	37.50 (34.99)
A_{13} x *G. margarita*	0.00 (0.00)	32.90 (34.98)	75.00 (59.97)	88.31 (69.98)	49.05 (41.24)
A_{13} x Pi-9	11.70 (19.99)	88.31 (69.98)	88.31 (69.98)	97.00 (79.99)	71.33 (59.99)
A_{13} x *G. fasciculatum*	25.00 (29.99)	50.00 (44.98)	50.00 (44.98)	82.15 (64.99)	51.79 (46.23)
A_{13} x Is-6	11.70 (19.99)	32.90 (34.98)	50.00 (44.98)	82.15 (64.98)	44.19 (41.24)
A_{13} x Pi-11	25.00 (29.99)	41.32 (39.98)	58.68 (49.98)	67.10 (54.98)	48.02 (43.73)
A_{21} x *G. margarita*	0.00 (0.00)	50.00 (44.98)	58.68 (49.98)	58.68 (49.98)	41.84 (38.73)
A_{21} x Pi-9	11.70 (19.99)	50.00 (44.98)	58.68 (49.98)	82.15 (64.98)	50.63 (44.98)
A_{21} x *G. fasciculatum*	11.70 (19.99)	25.00 (29.99)	50.00 (44.98)	58.68 (49.98)	36.34 (36.23)
A_{21} x Is-6	11.70 (19.99)	32.90 (34.98)	50.00 (44.98)	82.15 (64.98)	44.19 (41.24)
A_{21} x Pi-11	17.86 (24.99)	58.68 (49.98)	67.10 (54.98)	67.10 (54.98)	52.69 (46.23)
A_{22} x *G. margarita*	3.01 (10.00)	11.70 (19.99)	58.68 (49.98)	93.32 (74.99)	41.68 (38.74)

Treatment	Mortality (%)				
	Days after *P. capsici* inoculation				
	15	30	45	60	Mean
A_{22}xPi-9	0.00 (0.00)	41.32 (39.98)	50.00 (44.98)	67.10 (54.98)	39.61 (34.99)
A_{22}x*G. fasciculatum*	0.00 (0.00)	11.70 (19.99)	67.10 (54.98)	75.02 (59.99)	38.46 (33.74)
A_{22}xIs-6	0.00 (00.00)	3.01 (10.00)	32.90 (34.98)	32.90 (34.98)	17.20 (19.19)
A_{22}xPi-11	3.01 (10.00)	11.70 (19.99)	67.10 (54.98)	67.10 (54.98)	37.22 (34.99)
A_{35}x*G. margarita*	0.00 (00.00)	11.70 (19.99)	67.10 (54.98)	67.10 (54.98)	36.48 (32.49)
A_{35}xPi-9	0.00 (00.00)	3.01 (10.00)	75.00 (59.97)	75.00 (59.97)	38.25 (32.49)
A_{35}x*G. fasciculatum*	0.00 (00.00)	25.00 (29.99)	32.90 (34.98)	82.15 (64.98)	35.01 (43.74)
A_{35}xIs-6	0.00 (00.00)	32.90 (34.98)	58.68 (49.98)	100.00 (90.00)	47.90 (43.74)
A_{35}xPi-11	0.00 (00.00)	32.90 (34.98)	75.00 (59.97)	97.00 (79.99)	51.22 (43.74)
A_{1}	3.01 (10.00)	32.90 (34.98)	58.68 (49.98)	75.00 (59.97)	42.40 (38.73)
A_{13}	11.70 (19.99)	50.00 (44.98)	67.10 (54.98)	88.31 (69.98)	52.28 (47.48)
A_{21}	11.70 (19.99)	41.32 (39.98)	58.68 (49.98)	75.00 (59.97)	46.68 (42.48)

Treatment	Mortality (%)				
	Days after *P. capsici* inoculation				
	15	30	45	60	Mean
A_{22}	3.01 (10.00)	11.70 (19.99)	58.68 (49.98)	75.00 (59.97)	37.10 (34.98)
A_{35}	0.00 (0.00)	25.00 (29.99)	67.10 (54.98)	82.15 (64.98)	43.56 (38.99)
G. margarita	3.01 (10.00)	32.90 (34.98)	67.10 (54.98)	88.31 (69.98)	47.83 (42.48)
Pi-9	3.01 (10.00)	50.00 (44.98)	75.00 (59.97)	82.15 (64.98)	52.54 (44.98)
G. fasciculatum	11.70 (19.99)	25.00 (29.99)	50.00 (44.98)	75.00 (59.97)	40.43 (38.73)
Is-6	11.70 (19.99)	32.90 (34.98)	50.00 (44.98)	75.00 (59.97)	42.40 (39.98)
Pi-11	3.01 (10.00)	58.68 (49.98)	67.10 (54.98)	75.00 (59.97)	50.95 (43.73)
Bordeaux mixture	0.00 (0.00)	11.70 (19.99)	32.90 (34.98)	32.90 (29.99)	19.37 (22.49)
Copper oxychloride	0.00 (0.00)	11.70 (19.99)	25.00 (29.99)	25.00 (34.98)	15.42 (19.99)
Control (*P. capsici*)	25.00 (29.99)	75.00 (59.97)	75.00 (59.97)	97.00 (79.99)	68.00 (57.48)

Figures in parantheses are transformed values

	Treatment mean	Interaction
CD (0.05) for transformed means	13.26	16.04

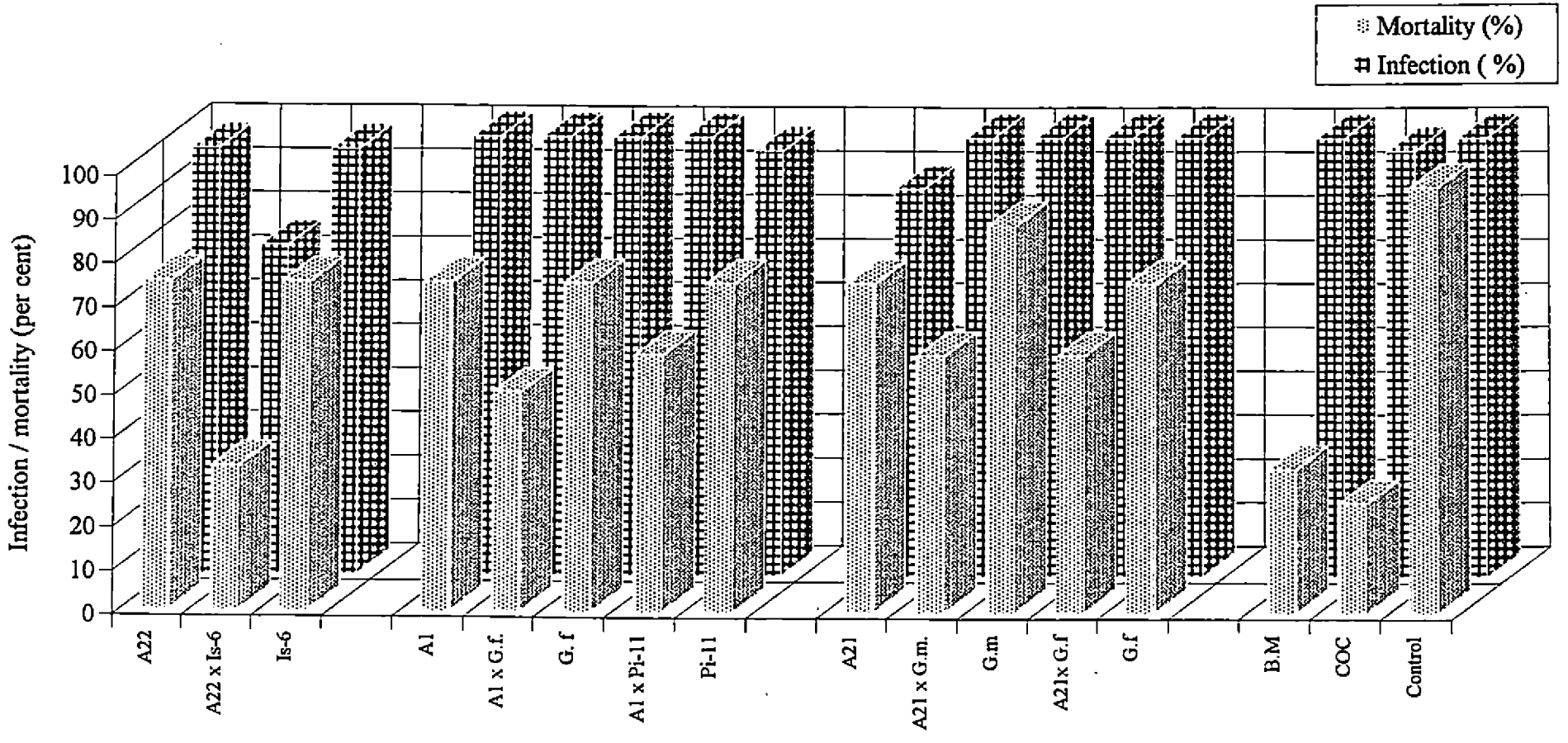


Fig. 9 Effect of AMF and antagonists on foot rot incidence in black pepper in green house

Plate 18. Foot rot incidence as influenced by dual inoculation of AMF and antagonists and their comparison with chemical fungicides (COC - copper oxychloride; BM - bordeaux mixture in green house)


A1xGF
A22xis6
CON
COC
BM

Antagonist A_{22} alone recorded 11.70 per cent mortality. All the mycorrhiza alone treatment had a higher mortality than dual inoculation with appropriate antagonists (25.00 to 58.68%). A_{35} in combination with AMF recorded higher mortality (11.70-32.90%). Chemicaly treated and control plants exhibited 11.70 and 75.00 per cent plant mortality respectively. The observations recorded on 45^{th} and 60^{th} day revealed the same trend with general increase in mortality rate (Table 24). Single inoculation of antagonist A_1, A_{21} and A_{22} and AMF isolate Pi-11 and *G. fasciculatum* showed significant reduction in plant mortality. Combination of A_{22} with Is-6 recorded lowest mortality of 32.90 per cent on 60th day, which was statistically on par with 32.90 and 25.00 recorded for bordeaux mixture and copper oxychloride respectively (Fig. 9). The control plants showed 97.00 per cent mortality. The dual inoculation of A_{35} and Is-6 recorded 100 per cent mortality, while, their individual inoculation showed 82.15 and 75.00 per cent respectively on 60th day. *G. fasciculatum* with A_1 and A_{21} recorded 50.00 and 58.68 per cent respectively, as against 75.00 per cent of *G. fasciculatum* alone inoculation.

The combination of A_{35} and Pi-11 increased the mortality to 97.00 per cent, while, it was 82.15 and 75.00 per cent for single inoculation of A_{35} and Pi-11. Antagonistic isolate A_1 and A_{22} with *G. margarita*, A_{13} with Pi-9, A_{35} with Is-6 and Pi-11 increased the plant mortality over their

Table 25 Antagonistic fungal population in pepper rhizosphere as influenced by dual inoculation with AMF isolates in green house

Treatment	Days after inoculation			
	100		200	
	Population			
	($x10^3$ g^{-1})	($x10^5$ g^{-1})	($x10^3$ g^{-1})	($x10^5$ g^{-1})
A_1 x *G. margarita*	9.25	3.00	5.00	1.75
A_1 x Pi-9	9.50	2.25	5.25	1.25
A_1 x *G. fasciculatum*	8.25	2.00	4.50	1.00
A_1 x Is-6	8.75	2.50	4.75	1.50
A_1 x Pi-11	9.00	2.75	6.00	2.00
A_{13} x *G. margarita*	2.50	1.00	1.75	0.75
A_{13} x Pi-9	2.25	1.25	1.50	1.00
A_{13} x *G. fasciculatua*	1.75	0.50	1.00	0.25
A_{13} x Is-6	2.00	0.75	1.50	0.50
A_{13} x Pi-11	2.75	0.75	2.00	0.50
A_{21} x *G. margarita*	9.75	1.50	6.75	1.00
A_{21} x Pi-9	8.00	1.25	5.50	0.75
A_{21} x *G. fasciculatum*	10.00	1.75	7.00	1.25
A_{21} x Is-6	9.50	1.25	6.50	0.75
A_{21} x Pi-11	9.25	1.50	6.25	1.00
A_{22} x *G. margarita*	9.25	4.75	6.25	3.25
A_{22} x Pi-9	8.50	3.50	5.75	2.50
A_{22} x *G. fasciculatum*	7.50	3.75	5.00	2.75
A_{22} x Is-6	9.00	4.50	6.00	3.25
A_{22} x Pi-11	9.25	4.00	6.00	3.00

Treatment	Days after inoculation			
	100		200	
	Population			
	($x10^3$ g^{-1})	($x10^5$ g^{-1})	($x10^3$ g^{-1})	($x10^5$ g^{-1})
A_{35} x *G. margarita*	3.50	2.00	2.50	1.25
A_{35} x Pi-9	2.25	1.75	1.50	1.25
A_{35} x *G. fasciculatum*	3.00	1.50	2.00	1.00
A_{35} x Is-6	3.25	1.00	2.25	0.75
A_{35} x Pi-11	2.75	1.25	2.00	0.75
A_1	9.50	2.75	5.25	2.00
A_{13}	2.00	0.75	1.50	1.00
A_{21}	10.00	1.50	6.75	2.50
A_{22}	7.50	3.75	5.25	3.00
A_{35}	3.00	1.25	2.50	1.25
CD (0.05)	2.00	0.92	1.27	1.16

Table 26 Effect of dual combinations of AMF with antagonistic fungi on mycorrhizal colonization in black pepper in green house

Treatment	AMF colonization (%)				
	Days after inoculation				Mean
	45	60	75	120	
A_1x *G. margarita*	11.57 (19.88)	38.22 (38.23)	50.00 (44.98)	76.99 (61.31)	44.20 (41.10)
A_1x Pi-9	18.86 (24.99)	63.40 (52.75)	70.28 (56.74)	73.98 (59.30)	56.63 (48.50)
A_1x *G. fasciculatum*	8.16 (16.59)	23.29 (28.84)	31.64 (34.22)	76.99 (61.33)	35.02 (35.24)
A_1x Is-6	11.57 (19.88)	31.64 (34.22)	46.59 (43.03)	71.89 (57.96)	40.42 (38.77)
A_1x Pi-11	23.29 (28.84)	71.89 (57.96)	86.08 (68.07)	89.81 (71.36)	67.77 (56.56)
A_{13}x *G. margarita*	10.00 (18.43)	26.52 (30.98)	34.94 (36.22)	68.49 (55.43)	34.99 (35.37)
A_{13}x Pi-9	6.49 (14.75)	16.60 (24.04)	23.29 (28.84)	63.35 (52.72)	27.43 (30.09)
A_{13}x *G. fasciculatum*	11.57 (19.88)	38.32 (38.23)	50.00 (44.98)	70.08 (56.82)	42.49 (32.98)
A_{13}x Is-6	10.00 (18.43)	29.92 (33.15)	44.98 (42.10)	76.71 (61.12)	40.40 (38.70)
A_{13}x Pi-11	13.24 (21.33)	46.65 (43.06)	58.39 (49.81)	82.14 (64.97)	50.10 (44.79)
A_{21}x *G. margarita*	18.12 (25.18)	65.23 (53.84)	82.14 (64.97)	83.40 (65.93)	62.22 (52.48)
A_{21}x Pi-9	8.16 (16.59)	25.00 (29.99)	31.64 (34.22)	65.05 (53.74)	32.46 (33.63)
A_{21}x *G. fasciculatum*	13.24 (21.33)	43.31 (41.14)	53.34 (46.89)	71.70 (57.84)	45.40 (41.80)
A_{21}x Is-6	18.27 (25.29)	50.00 (44.98)	58.49 (49.87)	81.88 (64.78)	52.16 (46.23)
A_{21}x Pi-11	11.57 (19.88)	31.64 (34.22)	43.27 (41.12)	73.56 (59.03)	40.01 (38.56)

Treatment	AMF colonization (%)				
	Days after inoculation				Mean
	45	60	75	120	
A_{22} x *G. margarita*	16.60 (24.04)	58.39 (49.81)	70.08 (56.82)	86.08 (68.07)	57.79 (49.68)
A_{22} x Pi-9	19.84 (26.44)	68.35 (55.75)	83.15 (65.75)	89.09 (70.66)	65.11 (54.65)
A_{22} x *G. fasciculatua*	18.20 (25.29)	61.96 (51.90)	70.08 (56.82)	80.69 (63.90)	57.73 (49.48)
A_{22} x Is-6	14.76 (22.59)	56.69 (48.83)	65.23 (53.84)	77.19 (61.43)	53.47 (46.67)
A_{22} x Pi-11	13.24 (21.33)	60.03 (50.77)	68.78 (56.01)	83.15 (65.74)	56.30 (48.46)
A_{35} x *G. margarita*	21.62 (27.70)	34.77 (36.12)	46.66 (43.07)	70.33 (56.97)	43.35 (40.97)
A_{35} x Pi-9	14.76 (22.59)	60.19 (50.86)	70.33 (56.97)	76.99 (61.33)	55.57 (47.93)
A_{35} x *G. fasiculatua*	8.16 (16.59)	31.51 (34.13)	41.62 (40.16)	71.78 (57.89)	38.27 (37.19)
A_{35} x Is-6	9.60 (18.04)	32.31 (35.24)	46.65 (43.06)	73.48 (58.98)	40.51 (38.83)
A_{35} x Pi-11	9.60 (18.04)	36.65 (37.24)	48.33 (44.02)	75.47 (60.29)	42.51 (39.90)
G. margarita	18.12 (25.18)	25.00 (29.99)	53.34 (46.89)	71.70 (59.84)	42.04 (39.97)
Pi-9	11.57 (19.88)	22.29 (28.84)	46.59 (43.03)	63.34 (59.72)	35.95 (37.87)
G. fasciculatua	14.76 (22.59)	26.52 (30.98)	58.49 (49.87)	73.98 (59.30)	43.44 (40.68)
Is-6	13.24 (21.33)	25.00 (29.99)	50.00 (44.98)	70.08 (56.82)	39.56 (38.28)
Pi-11	18.27 (25.29)	38.32 (38.23)	65.23 (53.84)	80.69 (62.90)	50.63 (45.31)
Bordeaux mixture	6.49 (14.75)	44.76 (22.59)	23.29 (28.84)	43.32 (41.15)	29.47 (26.83)
Copper oxychloride	8.16 (16.59)	11.57 (19.88)	26.44 (30.93)	51.72 (45.97)	24.47 (28.34)
Control	5.00 (12.91)	8.16 (16.59)	21.62 (27.70)	48.33 (44.92)	27.78 (25.31)

Figures in parantheses are transformed values

	Treatment mean	Interaction
CD (0.05) for transformed means	3.50	7.24

individual inoculation. Treatment mean computed for mortality in all the treatments, except single inoculation of A_{13} and its combination with *G. fasciculatum*, single inoculation of Pi-9 and the combination of A_{21} and Pi-11, were significantly less than that of *P. capsici* alone inoculated control.

The population of antagonists was not significantly influenced by interaction with different AMF isolates (Table 25). Antagonistic population on the 100th day of planting was maximum for isolate A_{21} (10.00×10^3) followed by A_1 (9.50×10^3). On 200th day after initial inoculation there was general reduction in the population of antagonists (Table 25). Antagonist A_{22} in combination with *G. margirata*, Is-6 and Pi-11 recorded population of 3.25, 3.25 and 3.00×10^5 respectively, as against 3×10^5 recorded for A_{22} alone inoculation. Similarly the population recorded for A_{21} (5.50 to 6.75×10^3) and A_1 4.50 to 6.00×10^3 in combination with different AMF tested were also on par with the population recorded for the single inoculation of these antagonists (5.25 and 6.75×10^3).

Interaction of different AMF isolates with A_{22} on 45th day showed a remarkable increase in AMF root colonization per cent in black pepper (Table 26, Fig. 10). AMF colonization on dual inoculation of *G. margarita*, Pi-9, *G. fasiculatum*, Is-6 and Pi-11 with A_{22} was 16.60, 19.84, 18.20, 14.76 and 13.24 per cent respectively. The root colonization for their

single inoculation was, 18.12, 11.57, 14.76, 13.24 and 18.27 per cent for respective AMF. The interaction of antagonist A_1 with AMF isolates showed both stimulatory and inhibitory effect on AMF colonization. Isolate A_1 on inoculation with Pi-11 recorded root colonization of 71.89 per cent as against 38.32 per cent observed in Pi-11 alone on 60th day.

The combination of *G. fasciculatum* with A_{22} recorded significantly high influence on the AMF colonization (80.69%) while it was reduced by antagonist A_1 (76.99%) and A_{13} (70.08%) The antagonists A_{21} and A_{22} were found beneficial to *G. margarita* root colonization. *G. margirata* and Pi-9 on single inoculation recorded 71.70 and 63.40 per cent mycorrhizal colonization on 120^{th} day (Table 26). While their interaction with antagonist A_{22} resulted in significantly higher colonization of 86.08 and 89.09 per cent respectively. Combination of A_{21} with Is-6 recorded 81.88 per cent colonization as against 70.08 per cent on single inoculation with Is-6.

Treatment mean computed for AMF root colonization was observed maximum with isolate Pi-11 x A_1 (67.77%) and was higher than single inoculation of black pepper cuttings with Pi-11. Treatment mean of Pi-11 on dual inoculation with A_{35} showed lower per cent root colonization (42.51%) over its single inoculation (50.63%). Similarly, treatment mean for

Table 27 Effect of dual inoculation of black pepper with AMF and fungal antagonists on growth in the field

Treatment	Plant height (cm)	Leaves (No.Plant^{-1})
A_1x *G. margarita*	43.00	6.33
A_1x Pi-9	41.33	7.67
A_1x *G. fasciculatum*	51.33	10.00
A_1x Is-6	47.33	9.67
A_1x Pi-11	59.00	9.67
A_{13}x *G. margarita*	41.57	9.33
A_{13}x Pi-9	49.17	8.67
A_{13}x *G. fasciculatum*	41.83	7.67
A_{13}x Is-6	51.17	8.00
A_{13}x Pi-11	53.67	10.00
A_{21}x *G. margarita*	40.00	6.00
A_{21}x Pi-9	42.00	6.33
A_{21}x *G. fasciculatum*	58.33	9.67
A_{21}x Is-6	61.00	7.33
A_{21}x Pi-11	56.67	11.00
A_{22} x *G. margarita*	39.17	6.33
A_{22} x Pi-9	41.33	8.00
A_{22} x *G. fasciculatum*	54.67	9.67
A_{22} x Is-6	42.33	6.33
A_{22} x Pi-11	45.00	8.33
A_{35} x *G. margarita*	41.00	8.00
A_{35} x Pi-9	40.83	7.33
A_{35} x *G. fasciculatum*	51.67	8.33
A_{35} x Is-6	42.00	7.67
A_{35} x Pi-11	40.67	9.00

Treatment	Plant height (cm)	Leaves ($No.Plant^{-1}$)
A_1	40.67	8.00
A_{13}	38.33	8.00
A_{21}	52.67	9.33
A_{22}	48.33	0.33
A_{35}	54.83	10.00
G. margarita	40.67	7.33
Pi-9	42.00	8.33
G. fasciculatum	49.50	9.00
Is-6	43.50	7.67
Pi-11	47.83	8.67
Bordeaux mixture	41.33	9.67
Copper oxychloride	42.83	8.67
Control	34.67	6.00
CD (0.05)	12.81	2.73

colonization by Is-6 (39.56%) was significantly increased with A_{21} (52.16%) and A_{22} (53.47%). The treatment means for different AMF isolates in combination with A_{21} and A_{22} were generally stimulatory.

4.7 Effect of dual inoculation of AMF and fungal antagonists on plant growth and foot rot incidence of black pepper in the field

4.7.1 Effect on growth

The data on biometric obsrvations on plant growth as influenced by AMF and antagonists are presented in Table 27. Maximum plant height of 61.00 cm with leaf number of 7.33 was recorded in the dual inoculation treatment of A_{21}. and Is-6 The combinations of A_1 x Pi- 11, A_{21} x *G. fasciculatum* and A_{21} x Pi-11 were also found to be enhancing plant growth, the value recorded for plant height were 59.00, 58.33 and 56.67 cm respectively. The individual use of AMF isolates Is-6, *G. fasciculatum* and Pi-11 resulted in plant height of 43.50, 49.50 and 47.83 cm with leaf number 7.67, 9.00 and 8.67 respectively. The single inoculation of antagonits A_1 and A_{21} recorded plant height of 40.67 and 52.67 cm and leaf number of 8.00 and 9.33. Amongst the antagonists, maximum plant height of 54.83 cm and leaf number of 10.00 was recored for A_{35} followed by A_{22} with 48.33 cm height and 10.33 leaves.

4.7.2 Effect on foot rot incidence

The potential arbuscular mycorrhizal fungi and antagonists tested under green house were simultaneously tested in the field (Table 28). There was no foot rot infection until the 15th day of *P. capsici* inoculation in the treatment receiving A_{21} and Pi-9 inoculation. However, infection among other treatments ranged from 9.17 to 50.00 per cent as against 41.54 per cent for the control. Combinations of A_{21} and Is-6, A_{22} and *G. fasciculatum*, Pi-9 alone and its dual inoculation with A_{35}, and single inoculation of A_{21} were on par with copper oxychloride which recorded least infection per cent of 9.17.

There was a general increase in the foot rot infection with time which attained the maximum intensity on 90th day of *P. capsici* inoculation. The treatments except single inoculation of *G. fasciculatum* or its combination with A_1 and A_{22}, Pi-11 with A_1 and A_{21}, A_{13} with Is-6 and Pi-11, A_{21} alone or on dual inoculation with Pi-9 and *G. fasciculatum* registered more than 80 per cent foot rot infection by 90th day of pathogen inocullattion. The copper oxychloride (COC) treatment remained with exceptionally low infection per cent of 59.68 than that of control (95.66). The combinations of A_1 x *G. fasciculatum*, A_1xPi-11, A_{13}xIs-6 and A_{21}xPi-11 recorded lower infection per cent of 67.84, 58.45, 66.67 and 67.67 per cent. The lowest foot rot infection of 58.45 per cent was recorded for A_1xPi-11 and A_{21}xIs-6, which was less than that achieved by copper oxychloride treatment (59.68%).

Table 28 Effect of dual inoculation of black pepper with AMF and fungal antagonists on foot rot infection in the field

Treatment	Infection (%)				
	Days after *P. capsici* inoculation				Mean
	15	30	60	90	
A_1 x *G. margarita*	67.84 (55.43)	90.84 (72.36)	90.84 (72.36)	95.66 (77.93)	86.29 (69.52)
A_1 x Pi-9	50.00 (44.98)	50.00 (44.98)	60.67 (54.71)	90.84 (72.36)	62.88 (54.26)
A_1 x *G. fasciculatum*	16.67 (24.08)	41.54 (40.12)	50.00 (44.98)	67.84 (55.43)	44.01 (41.15)
A_1 x Is-6	33.33 (35.25)	33.33 (35.25)	58.45 (49.85)	83.33 (65.88)	52.11 (46.56)
A_1 x Pi-11	24.52 (29.67)	24.52 (29.67)	41.54 (40.12)	58.45 (49.85)	37.26 (37.32)
A_{13} x *G. margarita*	16.67 (24.08)	50.00 (44.98)	75.48 (60.30)	83.33 (65.88)	56.37 (48.81)
A_{13} x Pi-9	32.16 (34.53)	67.84 (55.43)	75.48 (60.30)	95.66 (77.93)	67.69 (57.05)
A_{13} x *G. fasciculatum*	50.00 (44.98)	58.45 (49.85)	66.67 (54.71)	83.33 (65.88)	64.61 (53.85)
A_{13} x Is-6	14.64 (22.49)	41.54 (40.12)	41.54 (40.12)	66.67 (54.71)	41.10 (39.36)
A_{13} x Pi-11	33.33 (35.25)	33.33 (35.25)	41.54 (40.12)	75.48 (60.30)	45.92 (42.73)
A_{21} x *G. margarita*	32.16 (34.53)	58.45 (49.85)	75.48 (60.30)	90.84 (72.36)	64.23 (54.26)
A_{21} x Pi-9	0.00 (0.00)	33.33 (35.25)	58.45 (49.85)	75.48 (60.30)	41.82 (36.35)
A_{21} x *G. fasciculatum*	24.52 (29.67)	41.54 (40.12)	50.00 (44.98)	67.84 (55.43)	45.98 (42.55)
A_{21} x Is-6	9.17 (17.62)	41.54 (40.12)	41.54 (40.12)	58.45 (49.85)	37.38 (36.93)
A_{21} x Pi-11	24.52 (29.67)	33.33 (35.25)	50.00 (44.98)	66.67 (54.71)	43.63 (41.15)

Treatment	Infection (%)				
	Days after *P. capsici* inoculation				Mean
	15	30	60	90	
A_{22} x *G. margarita*	24.52 (29.67)	75.48 (60.30)	75.48 (60.30)	90.84 (72.36)	66.58 (55.65)
A_{22} x Pi-9	32.16 (34.53)	58.45 (49.85)	75.48 (60.30)	83.83 (65.87)	62.48 (52.64)
A_{22} x *G. fasciculatum*	9.17 (17.62)	41.54 (40.12)	58.45 (49.85)	75.48 (60.30)	46.16 (41.97)
A_{22} x Is-6	33.33 (35.25)	41.54 (40.12)	75.48 (60.30)	90.84 (72.36)	60.30 (52.00)
A_{22} x Pi-11	32.16 (34.53)	58.45 (49.85)	75.48 (60.30)	83.33 (65.88)	62.35 (52.64)
A_{35} x *G. margarita*	41.54 (40.12)	50.00 (44.98)	58.45 (49.85)	90.84 (72.36)	60.21 (51.83)
A_{35} x Pi-9	9.17 (17.62)	85.38 (67.49)	90.80 (72.36)	95.66 (77.94)	70.25 (58.85)
A_{35}x *G. fasiculatum*	32.16 (34.53)	50.00 (44.98)	67.84 (55.43)	90.84 (72.36)	60.21 (51.83)
A_{35}x Is-6	24.53 (29.67)	75.48 (60.30)	75.48 (60.30)	95.66 (77.94)	67.79 (57.05)
A_{35}x Pi-11	32.16 (34.53)	41.54 (40.12)	67.84 (55.43)	85.38 (67.49)	56.73 (49.39)
A_1	24.52 (29.67)	33.33 (35.25)	58.45 (49.85)	90.84 (72.36)	51.79 (46.78)
A_{13}	41.54 (40.12)	58.45 (45.85)	75.48 (60.30)	90.84 (72.36)	66.58 (55.65)
A_{21}	9.17 (17.62)	24.52 (29.67)	41.54 (40.12)	75.48 (60.30)	37.68 (36.93)
A_{22}	16.67 (24.08)	33.33 (35.25)	58.45 (49.85)	90.84 (72.36)	49.82 (45.38)
A_{35}	41.54 (40.12)	41.54 (40.12)	58.45 (49.85)	90.84 (72.36)	58.09 (50.61)

Treatment	Infection (%)				
	Days after *P. capsici* inoculation				Mean
	15	30	60	90	
G. margarita	32.16 (34.53)	59.68 (50.56)	67.84 (55.43)	90.84 (72.36)	62.63 (53.22)
Pi-9	9.17 (17.62)	78.89 (62.62)	85.38 (67.50)	90.84 (72.36)	66.07 (55.02)
G. fasciculatum	16.67 (24.08)	41.54 (40.12)	50.00 (44.98)	75.48 (60.30)	45.92 (42.37)
Is-6	14.64 (22.49)	40.31 (39.40)	67.84 (55.43)	90.84 (72.36)	53.41 (47.42)
Pi-11	24.52 (29.67)	58.45 (49.85)	75.45 (60.30)	90.84 (72.36)	62.31 (53.04)
Bordeaux mixture	33.33 (35.25)	50.00 (44.98)	58.45 (49.85)	66.67 (54.71)	52.11 (46.20)
Copper oxychloride	9.17 (17.62)	33.33 (35.25)	50.00 (44.98)	59.68 (50.56)	38.04 (37.10)
Control (*P. capsici*)	41.54 (40.12)	67.84 (55.43)	90.84 (72.36)	95.66 (77.93)	73.97 (61.46)

Figures in parantheses are transformed values

	Treatment mean	Interaction
CD (0.05) for transformed means	16.78	NS

Table 29 Effect of dual inoculation of black pepper with AMF and fungal antagonists on plant mortality due to foot rot incidence in the field

Treatment	Mortality (%)				
	Days after *P. capsici* inoculation				Mean
	30	60	90	150	
A_1 x *G. margarita*	50.00 (44.98)	75.48 (60.30)	90.84 (72.36)	95.66 (77.93)	77.99 (63.89)
A_1 x Pi-9	33.33 (35.25)	50.00 (44.98)	58.45 (49.85)	90.84 (72.36)	58.16 (50.61)
A_1 x *G. fasciculatum*	16.67 (24.08)	41.51 (40.12)	50.00 (44.98)	66.67 (54.71)	43.71 (40.97)
A_1 x Is-6	33.33 (35.25)	33.33 (35.25)	50.00 (44.98)	83.33 (65.88)	50.00 (45.34)
A_1 x Pi-11	16.67 (24.08)	24.52 (29.67)	41.54 (40.12)	58.45 (49.85)	35.30 (35.93)
A_{13} x *G. margarita*	4.36 (12.04)	50.00 (44.98)	66.67 (54.71)	83.33 (65.88)	51.09 (44.40)
A_{13} x Pi-9	24.52 (29.67)	67.84 (55.43)	75.48 (60.30)	95.66 (77.93)	65.88 (55.83)
A_{13} x *G. fasciculatum*	33.33 (35.25)	58.45 (49.85)	66.67 (54.71)	83.33 (65.88)	60.45 (51.42)
A_{13} x Is-6	9.17 (17.62)	41.54 (40.12)	41.54 (40.12)	66.67 (54.71)	39.73 (38.14)
A_{13} x Pi-11	33.33 (35.25)	33.33 (35.25)	41.54 (40.12)	75.48 (60.30)	45.92 (42.73)
A_{21} x *G. margarita*	25.52 (29.67)	50.00 (44.98)	75.48 (60.30)	90.84 (72.36)	60.46 (51.83)
A_{21} x Pi-9	0.00 (0.00)	4.36 (12.04)	58.45 (49.85)	75.48 (60.30)	34.57 (30.55)
A_{21} x *G. fasciculatum*	24.52 (29.67)	41.54 (40.12)	50.00 (44.98)	67.84 (55.43)	45.98 (42.55)
A_{21} x Is-6	9.17 (17.62)	33.33 (35.25)	41.54 (40.12)	58.45 (49.85)	35.62 (35.71)
A_{21} x Pi-11	16.67 (24.08)	33.33 (35.25)	33.33 (35.25)	66.67 (54.71)	37.50 (37.32)

Treatment	Mortality (%)				
	Days after *P. capsici* inoculation				Mean
	30	60	90	150	
A_{22} x *G. margarita*	9.17 (17.62)	75.48 (60.30)	75.48 (60.30)	90.84 (72.36)	62.64 (52.64)
A_{22} x Pi-9	32.16 (34.53)	50.00 (44.98)	75.48 (60.30)	83.33 (65.88)	60.24 (51.42)
A_{22} x *G. fasciculatum*	9.17 (17.62)	41.54 (40.12)	50.00 (44.98)	75.48 (60.30)	44.05 (40.75)
A_{22} x Is-6	24.52 (29.67)	33.33 (35.25)	75.48 (60.30)	90.84 (72.36)	56.04 (49.39)
A_{22} x Pi-11	32.16 (34.53)	58.45 (49.88)	75.48 (60.30)	83.33 (65.87)	62.36 (52.64)
A_{35} x *G. margarita*	32.16 (34.53)	50.00 (44.98)	50.00 (44.98)	90.84 (72.36)	55.75 (49.21)
A_{35} x Pi-9	9.17 (17.62)	85.38 (67.49)	90.84 (72.36)	95.66 (77.93)	70.26 (58.85)
A_{35} x *G. fasiculatum*	24.52 (29.67)	41.54 (40.12)	67.84 (55.43)	90.84 (72.36)	59.19 (49.39)
A_{35} x Is-6	24.52 (29.67)	75.48 (60.30)	75.48 (60.30)	95.66 (77.93)	67.79 (57.05)
A_{35} x Pi-11	24.52 (29.67)	41.54 (40.12)	58.45 (49.85)	85.38 (67.49)	52.47 (46.78)
A_1	24.52 (29.67)	33.33 (35.25)	50.00 (60.30)	90.84 (72.36)	49.67 (45.56)
A_{13}	32.16 (34.53)	58.45 (49.85)	75.48 (44.98)	90.84 (72.36)	64.23 (54.26)
A_{21}	9.17 (17.62)	9.17 (17.62)	41.54 (40.12)	66.67 (34.71)	31.63 (32.52)
A_{22}	4.36 (12.04)	9.17 (17.62)	58.45 (49.85)	75.48 (60.30)	36.87 (34.95)
A_{35}	24.52 (29.67)	41.54 (40.12)	58.45 (49.85)	90.84 (72.36)	53.84 (48.00)

Treatment	Mortality (%)				Mean
	Days after *P. capsici* inoculation				
	30	60	90	150	
G. margarita	24.52 (29.67)	59.68 (50.56)	67.84 (55.43)	90.84 (72.36)	60.72 (52.00)
Pi-9	4.36 (12.04)	59.68 (50.56)	85.38 (67.49)	90.84 (72.36)	60.07 (50.61)
G. fasciculatum	16.67 (24.08)	41.54 (40.12)	50.00 (44.98)	75.48 (60.30)	45.92 (42.37)
Is-6	9.17 (17.62)	40.31 (39.40)	58.45 (49.85)	90.84 (72.36)	49.69 (44.81)
Pi-11	24.52 (29.67)	58.45 (49.85)	75.48 (60.30)	90.84 (72.36)	62.32 (53.04)
Bordeaux mixture	24.52 (29.67)	50.00 (44.98)	58.45 (49.85)	66.67 (54.71)	49.91 (44.80)
Copper oxychloride	9.17 (17.62)	33.33 (35.25)	50.00 (44.98)	59.68 (50.56)	38.05 (37.10)
Control (*P. capsici*)	33.33 (35.25)	67.84 (55.43)	90.84 (72.36)	95.66 (77.93)	71.92 (60.24)

Figures in parantheses are transformed values

	Treatment mean	Interaction
CD (0.05) for transformed means	14.25	NS

Data computed for treatment mean (Table 28) indicated significantly lower foot rot infection per cent with *G. fasciculatum* (45.92%) alone or in combination with A_1 (44.01%), A_{21} (45.98%) and A_{22} (46.16%) antagonist A_{21} alone (37.68%) or its combination with Is-6 (37.68%) and Pi-11 (43.63%) and other treatments such as A_1 x Pi-11 (37.26%), A_{13} x Is-6 (41.10%), A_{13}xPi-11 (45.92%), A_{21}xPi-9 (41.82) were also effective and on par with fungicidal application. Of the various treatments tested treatment mean of A_1 x *G. margarita* recorded highest infection per cent of 86.29 as against 51.79 and 62.63 per cent obtained for the single inoculation of A_1 and *G. margarita* respectively.

The data recorded on mortality rate of black pepper on 30, 60, 90 and 150th day of *P. capsici* inoculation revealed wide variation among the treatments (Table 29). Observations on the 30th day recorded no mortality with the dual inoculation of A_{21} and Pi-9, while the maximum mortality of 50.00 per cent was noticed with A_1 x *Gigaspora margarita* as against 33.33 per cent of control. Individual inoculation of AMF isolate Pi-9 and Is-6 resulted in lower mortality rates of 4.36 and 9.17 per cent respectively. Antagonists A_{22} (4.36) and A_{21} (9.17%) also recorded a very low mortality.

Mortality rates in all the treatments showed rapid increase from the 30th day onward and reached the maximum by 150th day of *P. capsici* inoculation (Table 29). AMF isolates

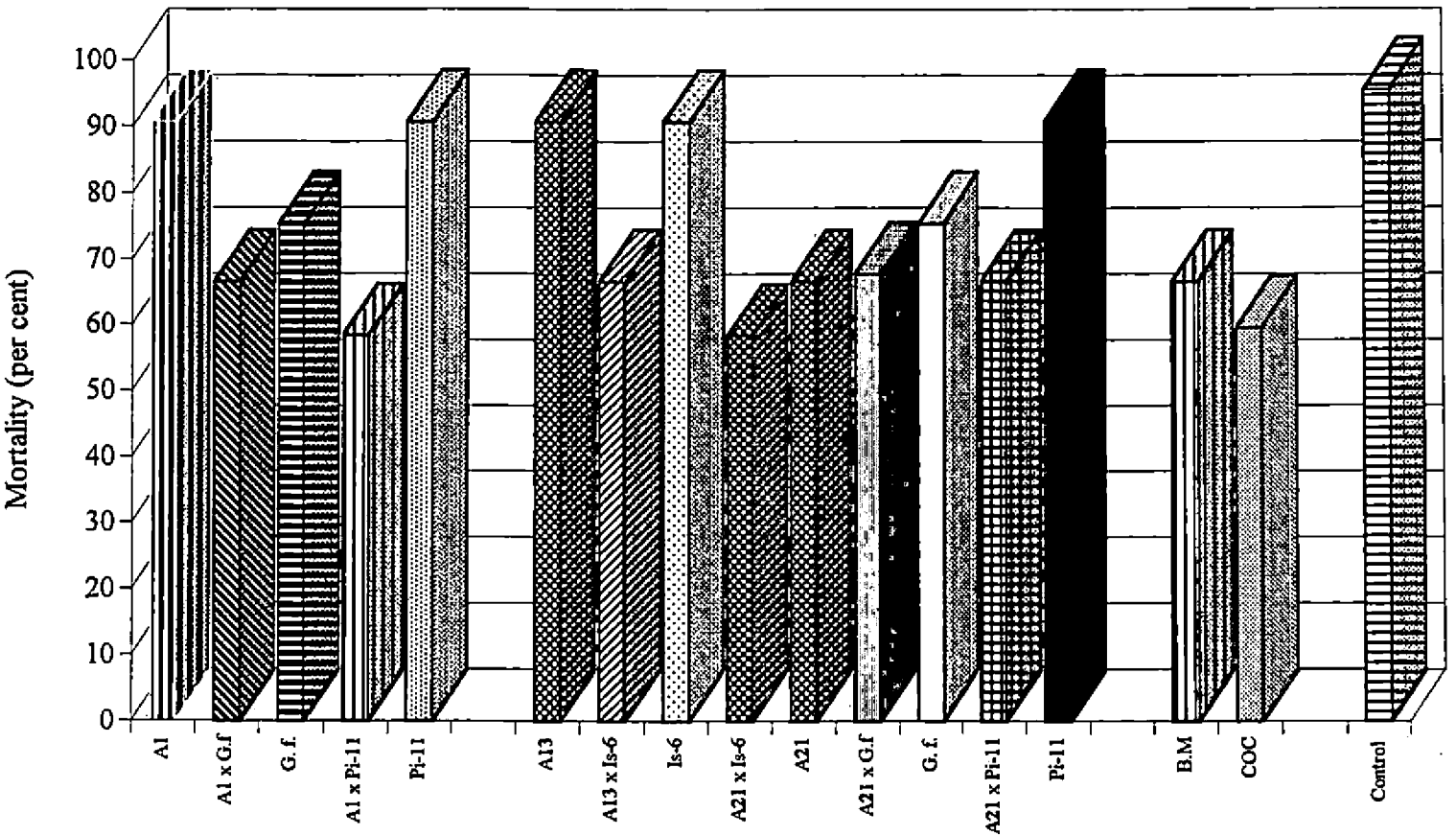


Fig. 10 Effect of AMF and antagonists on foot rot incidence (mortality) in black pepper in field

on individual inoculation registered more than 75 per cent plant mortality (Table 29). Antagonist A_{21} alone showed only 66.67 per cent plant mortality. Mortality observed in control on the 150th day was 95.66 per cent. Antagonistic isolates A_1 on co-inoculation with *G. fasciculatum* and Pi-11, A_{13} with Is-6 and A_{21} with *G. fasciculatum*, Is-6 and Pi-11 were recorded less than 70 per cent plant mortality. These treatments were comparable with copper oxychloride (59.68%) and bordeaux mixture (66.67%).

Lowest plant mortality of 58.45 per cent was recorded with the combinations of A_1xPi-11 and A_{21} x *G. fasciculatum* followed by A_1 x *G. fasciculatum* (Fig. 10). Combinations of A_{13} x Is-6 and A_{21} x Pi-11 recorded 66.67 per cent mortality on the 150th day of *P. capsici* inoculation. Single inoculation of *G. fasciculatum*, Is-6, A_{21} and A_{22} showed relatively less mortality on 30th, 60th, 90th day of inoculation with the pathogen (Fig. 10). This disease protection exhibited during the early stage of growth could not be sustained, as it is evidenced from higher mortality observed with the treatments on the 150th day of pathogen inoculation. Antagonists A_{13} and A_{35} on dual inoculation with *G. fasciclatum* increased the plant mortality to 83.33 and 90.84 per cent respectively. While the individual effect recorded for *G. fasciculatum*, A_{13} and A_{35} were 75.48, 90.84 and 90.84 per cent respectively. Similarly,

Table 30 Population of fungal antagonists in the pepper rhizosphere as influenced by dual inoculation with AMF in the field

Treatment	Population of antagonists ($x10^3$ g^{-1} soil)		
	Days after planting		Mean
	30	90	
A_1 x *G. margarita*	2.00	0.50	1.25
A_1 x Pi-9	1.00	0.75	0.87
A_1 x *G. fasciculatum*	1.00	0.25	0.62
A_1 x Is-6	0.50	0.50	0.50
A_1 x Pi-11	1.50	0.75	1.12
A_{13}x *G. margarita*	1.25	0.25	0.75
A_{13}x Pi-9	1.00	0.75	0.87
A_{13}x *G. fasciculatum*	1.00	0.25	0.62
A_{13}x Is-6	0.50	0.25	0.37
A_{13}x Pi-11	0.75	0.50	0.62
A_{21}x *G. margarita*	5.00	1.50	3.25
A_{21}x Pi-9	4.00	1.00	2.50
A_{21}x *G. fasciculatum*	4.50	1.75	3.12
A_{21}x Is-6	4.75	1.50	3.12
A_{21}x Pi-11	4.00	1.00	2.50

Treatment	Population of antagonists ($x10^3$ g^{-1} soil)		
	Days after planting		Mean
	30	90	
A_{22} x *G. margarita*	4.25	1.75	3.00
A_{22} x Pi-9	4.00	1.25	2.62
A_{22} x *G. fasciculatum*	3.50	1.50	2.50
A_{22} x Is-6	4.25	1.00	2.62
A_{22} x Pi-11	4.00	1.25	2.62
A_{35} x *G. margarita*	3.50	1.00	2.25
A_{35}x Pix9	4.00	1.25	2.62
A_{35}x *G. fasciculatum*	3.00	1.50	2.25
A_{35}x Is-6	3.25	1.00	2.12
A_{35}x Pi-11	3.50	1.25	2.37
A_1	1.50	1.00	1.25
A_{13}	1.00	0.75	0.87
A_{21}	4.25	1.50	2.87
A_{22}	3.75	1.50	2.62
A_{35}	4.00	1.25	2.62

	Treatment mean	Interaction
CD (0.05)	0.85	0.97

the dual inoculation of A_{21} with *G. margarita* (90.84%) and Pi-9 (75.48%) enhanced the plant mortality compared to inoculation of A_{21} alone (66.67%). The mean of per cent mortality (Table 29) for individual inoculation of A_{21} (31.63%), A_{22} (36.87%), *G. fasciculatum* (45.92%) and Is-6 (49.69%) was significantly less than that of control (71.92%). Among the combinations lowest treatment mean on plant mortality was recorded in the case of A_{21}xIs-6 (35.62%), followed by A_1xPi-11 (35.30) then and A_{21}xPi-11 (37.50%). The mean for individual isolate was 49.69, 62.32, 49.67 and 31.63 per cent for Is-6, Pi-11, A_1 and A_{21} respectively. Highest treatment mean on plant mortality was noticed with A_1 x *G. margarita* (77.99%).

The data on the population of antagonists showed significant variation (Table 30). Antagonists A_{21}, A_{22} and A_{35} and their combinations recorded significantly higher population over A_1, A_{13} and their combinations on the 30th day of planting. A_{21} on interaction with *G. fasciculatum* and Is-6 recorded population of 4.5 and 4.75 x 10^5 respectively. Similar trend was noticed in the case of A_{22} with Is-6 and *G. fasciculatum*. The population was significantly reduced by 90^{th} day. AMF isolates did not exhibit much effect on the population build up of antagonists.

Table 31 AMF colonization in black pepper as influenced by dual inoculation with antagonist in the field

Treatment	AMF colonization (%)		
	Days after planting		Mean
	30	90	
A_1 x *G. margarita*	29.92 (33.15)	65.05 (53.74)	47.48 (43.44)
A_1 x Pi-9	43.32 (41.15)	66.74 (54.76)	55.03 (47.95)
A_1 x *G. fasciculatum*	19.84 (26.44)	70.08 (56.82)	44.96 (41.63)
A_1 x Is-6	29.92 (33.15)	65.05 (53.74)	47.49 (43.44)
A_1 x Pi-11	55.02 (47.86)	77.17 (61.43)	66.10 (54.65)
A_{13}x *G. margarita*	21.62 (27.70)	66.82 (54.81)	44.22 (41.25)
A_{13}x Pi-9	13.24 (21.33)	65.54 (54.03)	39.39 (37.68)
A_{13}x *G. fasciculatum*	33.31 (35.24)	17.08 (56.82)	25.20 (46.03)
A_{13}x Is-6	29.92 (33.15)	73.56 (59.03)	51.74 (46.09)
A_{13}x Pi-11	16.60 (24.04)	71.89 (57.96)	44.25 (41.00)
A_{21}x *G. margarita*	50.00 (44.98)	75.11 (60.05)	62.56 (52.52)
A_{21}x Pi-9	19.84 (26.44)	68.49 (55.83)	44.17 (41.13)
A_{21}x *G. fasciculatum*	34.94 (36.22)	70.28 (56.94)	52.61 (46.58)
A_{21}x Is-6	36.65 (37.24)	73.56 (59.03)	55.11 (48.14)
A_{21}x Pi-11	28.30 (32.13)	68.35 (55.75)	48.33 (43.94)

.... contd.

If this Table is continued, mention.

Contd from previous continuation of Table 31

Treatment	AMF colonization (%)		
	Days after planting		Mean
	30	90	
A_{22} x *G. margarita*	41.66 (40.18)	71.78 (57.89)	56.72 (49.03)
A_{22} x Pi-9	51.67 (45.94)	75.11 (60.05)	63.39 (52.99)
A_{22} x *G. fasciculatum*	44.98 (42.10)	71.78 (57.89)	58.39 (49.99)
A_{22} x Is-6	24.89 (29.91)	68.49 (55.83)	46.69 (42.87)
A_{22} x Pi-11	44.98 (42.10)	70.33 (56.97)	57.66 (49.54)
A_{35} x *G. margarita*	28.30 (32.13)	60.00 (50.75)	44.15 (41.48)
A_{35} x Pix9	45.00 (42.11)	66.67 (54.73)	55.84 (48.42)
A_{35} x *G. fasciculatum*	26.63 (31.06)	68.49 (55.83)	47.56 (43.44)
A_{35} x Is-6	30.00 (32.20)	61.68 (51.73)	45.84 (42.47)
A_{35} x Pi-11	31.64 (34.22)	71.89 (57.96)	51.77 (46.09)
A_1	20.00 (26.55)	41.66 (40.18)	30.83 (33.37)
A_{13}	15.00 (22.77)	26.63 (30.06)	20.82 (26.92)
A_{21}	23.01 (28.65)	38.32 (38.23)	30.67 (33.44)
A_{22}	18.27 (25.29)	39.96 (39.19)	28.12 (32.24)
A_{35}	16.60 (24.04)	33.26 (35.20)	24.93 (29.62)
G. margarita	38.32 (38.23)	55.02 (47.86)	46.67 (43.04)
Pi-9	24.89 (29.91)	51.67 (45.94)	38.28 (37.93)
G. fasciculatum	26.44 (30.93)	63.58 (52.86)	45.01 (41.90)
Is-6	28.30 (32.13)	53.41 (46.93)	40.86 (39.53)
Pi-11	23.29 (28.84)	58.39 (49.81)	40.84 (39.33)
Bordeaux mixture	19.84 (26.44)	33.17 (35.15)	26.51 (30.80)
Copper oxychloride	15.00 (22.78)	37.96 (39.19)	26.48 (30.99)
Control (*P. capsici*)	21.62 (27.70)	31.51 (34.13)	26.57 (30.92)

Figures in parantheses are transformed values

	Treatment mean	Interaction
CD (0.05) for transformed means	4.28	5.62

Mycorrhizal colonization recorded on the 30th day after planting showed that dual inoculation of AMF isolates with antagonists generally increased the mycorrhizal colonization in black pepper (Table 31). AMF isolate Pi-9 with A_1, A_{22} and A_{35}, Pi-11 with A_1, A_{22} and A_{35}; *G. fasciculatum* with A_{13}, A_{21} and A_{22}; *G. margarita* with A_{21}, A_{22} and Is-6 with A_{21} significantly enhanced the colonization per cent. The observation on 90th day revealed significant increase in mycorrhizal colonization due to introduced AMF (Table 31). Highest mycorrhizal colonization of 71.17 per cent was obtained with the combination of A_1xPi-11 followed by A_{22}xPi-9 (75.11%). Root colonization per cent of 58.39 and 51.67% was recorded for single inoculation of Pi-11 and Pi-9 respectively. Mycorrhizal colonization by all the combinations of *G. fasciculatum* with antagonists remained on par with its single inoculation (63.58%). All the antagonists except A_{35} significantly enhanced root colonization by *G. margarita*, Is-6 and Pi-11.

4.8 Identification of AMF and fungal Antagonists

The AMF isolates Pi-11 and Is-6 were identified as species belonging to the genus *Glomus* the antagonistic isolates A_1, A_{13}, A_{21}, A_{22} and A_{35} were identified and confirmed as *Aspergillus fumigatus* Fres., *Fusarium oxysporum* Schlecht. ex Fr. *Aspergillus sydowii* (Bain. & Sart.) Thom. & Church, *Trichoderma viride* Pers. ex Gray. and *Gliomastix murorum* (Corda) Hughes.

Table 32 AMF colonization in black pepper due to inoculation through carrier plants in greenhouse

Treatment	*AMF colonization (%)					
	Days after inoculation					
	30		60		90	
	BP	CP	BP	CP	BP	CP
BP+CW	25.00	50.00	48.33	71.66	71.66	78.33
BP+GG	71.66	78.33	85.00	86.66	88.33	90.00
BP+IM	16.66	33.33	43.33	56.66	53.33	66.66
BP+SM	33.33	53.33	76.66	78.33	83.33	86.66
BP Control	6.66	-	21.66	-	31.66	-

*Mean of 60 root bits
CP - Carrier plant; CW - Cowpea; GG - Green gram;
IM - Italian millet; SM - Sorghum; BP - Black pepper

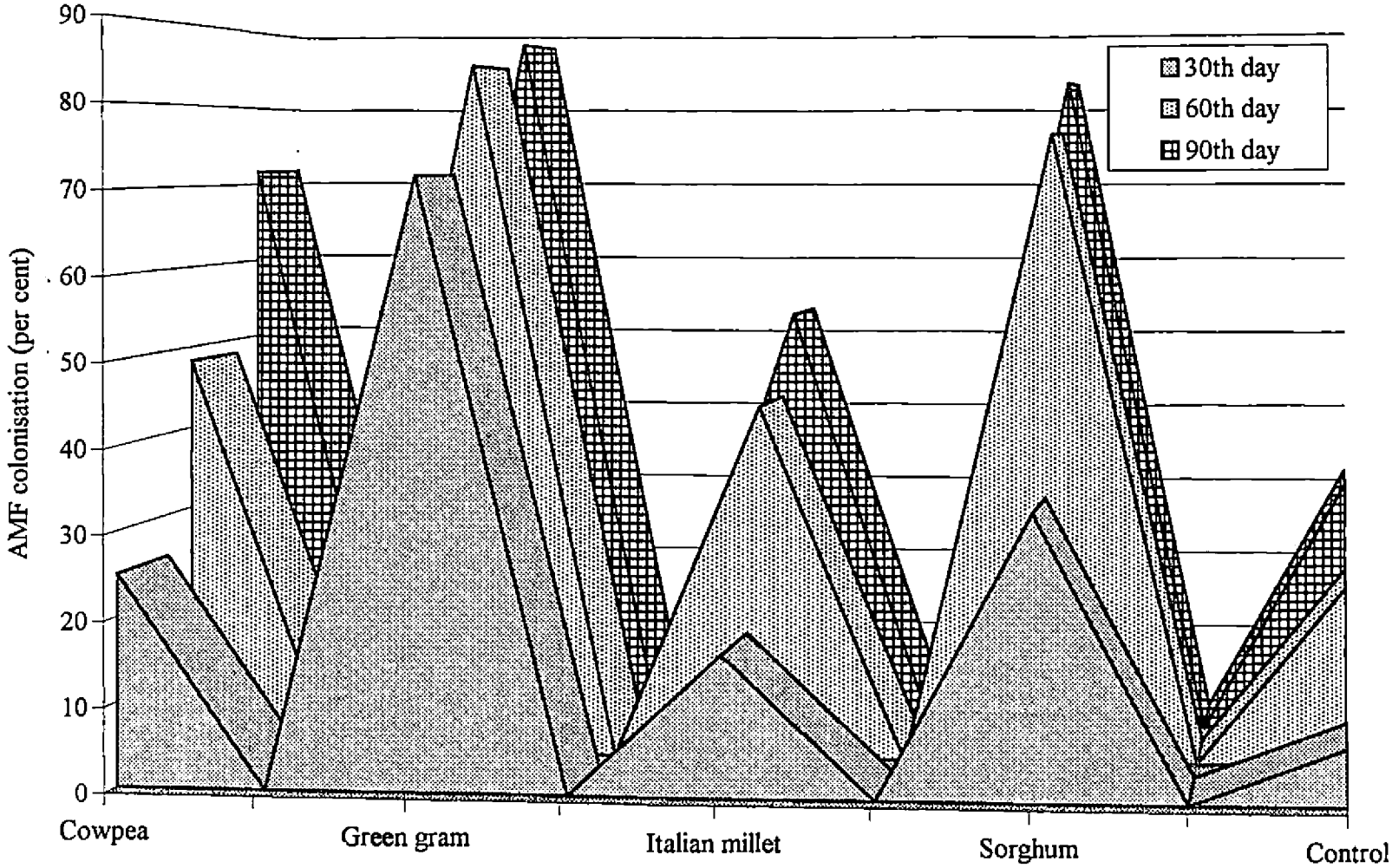


Fig. 11 AMF colonization pattern in black pepper inoculated with AMF through carrier plants in green house

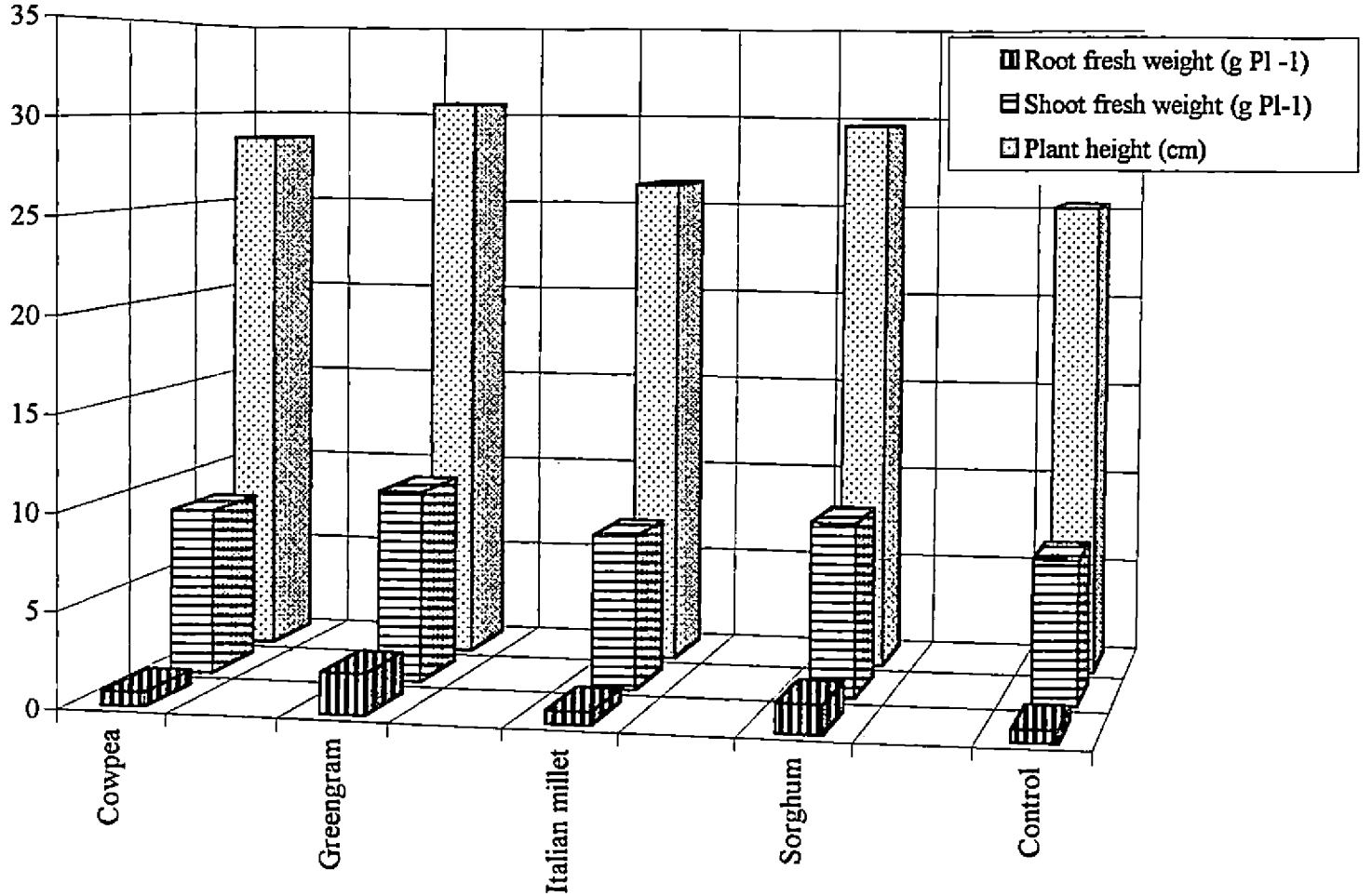


Fig. 12 Growth of black pepper on inoculation with AMF thorugh carrier plants in green house

Table 33 Growth characteristics of black pepper and carrier plants in the carrier plant based AMF inoculation in green house

Treatment	Black pepper (BP)					Carrier plant	
	Plant height (cm)	Leaves (No.plant^{-1})	Shoot fresh weight (g plant^{-1})	Root fresh weight (g plant^{-1})	Root length (cm)	Root fresh weight (g plant^{-1})	Root length (cm)
Black pepper + Cowpea	28.66	3.00	8.75	0.73	14.33	0.59	20.33
Black pepper + Green gram	30.66	4.00	10.11	2.12	21.33	1.64	21.00
Black pepper + Italian millet	26.33	2.66	8.12	0.67	15.66	0.32	15.33
Black pepper + Sorghum	29.66	3.33	9.15	1.51	24.00	2.21	21.00
Black pepper (Control)	25.33	2.33	7.56	0.65	10.00	-	-
CD (0.05)	3.41	0.81	1.64	8.49	8.49	0.73	NS

NS - Not significant

Plate 19. Root growth of carrier plants used for AMF inoculation (G - green gram; S - sorghum; C - cowpea; I - Italian millet)

Plate 20. Root growth in black pepper as influenced by AMF inoculation through carrier plants (1 - green gram, 2 - sorghum, 3- cowpea, 4 - Italian millet)


G
S
C
I


CON
1
2
3
4

4.9 Standardization of AMF inoculation technique for established pepper plantations

Amongst the different carrier plants tested in green house, the pepper plants inoculated through green gram recorded the maximum colonization of 88.33 per cent followed by sorghum with 83.33 per cent (Table 32, Fig. 11). Roots of green gram (90.00%) and sorghum (86.66%) also had remarkably higher AMF colonization. AMF colonization in pepper inoculated through carrier plants were 71.66 and 53.33 per cent for cowpea and italian millet. Higher root fresh weight of 1.64 and 2.21 g plant^{-1} were recorded for green gram and sorghum respectively (Fig. 12, Plate 19). Pepper cuttings inoculated with AMF culture using green gram and sorghum as carrier plants recorded higher plant height of 30.66 and 29.66 cm with fresh shoot weight of 10.11 and 9.15 g and root weight of 2.12 and 1.51g per plant respectively (Table 33). Significantly higher root length of 21.33 and 24.00 cm was recorded in black pepper inoculated with AMF using green gram and sorghum as carrier plants respectively (Plate 20). The uninoculated control plants showed 25.33 cm height with shoot weight of 7.56 g and the root weight of 0.656 g plant^{-1} (Fig. 12).

Although green gram ensured maximum root colonization in pots, the field studies indicated that it was inferior to sorghum as carrier plant for introducing AMF in to the pepper

Table 34 AMF colonization in black pepper due to inoculation using carrier plants in the field

Treatment	*AMF colonization (%)						
	Days after inoculation						
	0	30		60		90	
	BP	BP	CP	BP	CP	BP	CP
T_1 (BP+GG)	51.66	58.33	65.00	61.66	73.33	66.66	78.33
T_2 (BP+SM)	53.33	63.33	65.00	73.33	75.00	78.33	81.66
T_3 BP (Control)	46.66	48.33		50.00		51.66	
T_4 BP (Control)	46.66	48.33		51.66		53.33	

*Mean of 60 root bits

GG - Greengram SM - Sorghum

BP - Black pepper CP - Carrier plant

T_1, T_2 - Mixed AMF inocula (*G. fasciculatum* + Pi-11) applied to 'dibbling spot'

T_3 - Spreading inoculum in the basin without carrier plant

T_4 - AMF inoculation in 'dibbling spots' without carrier plant

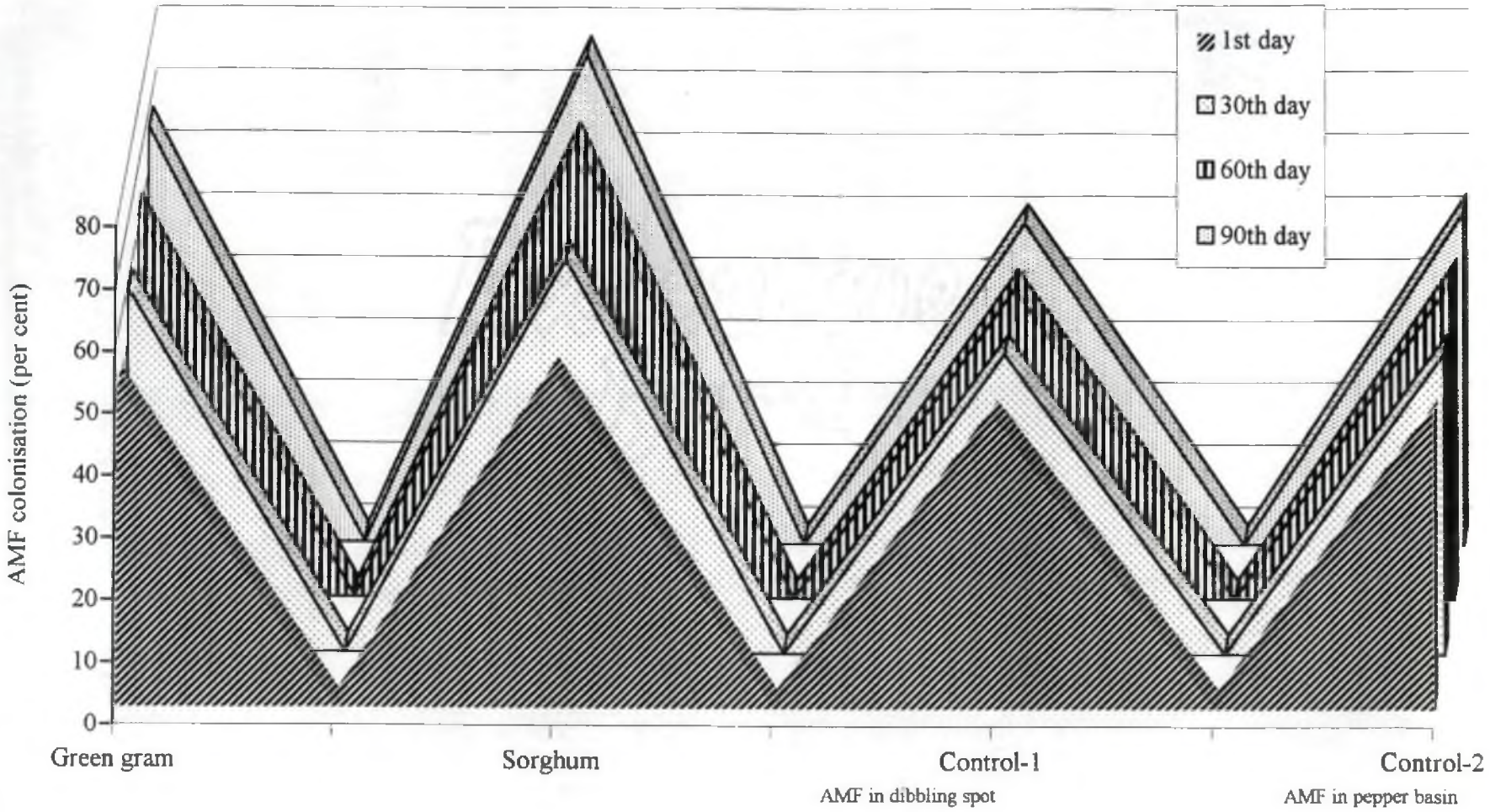


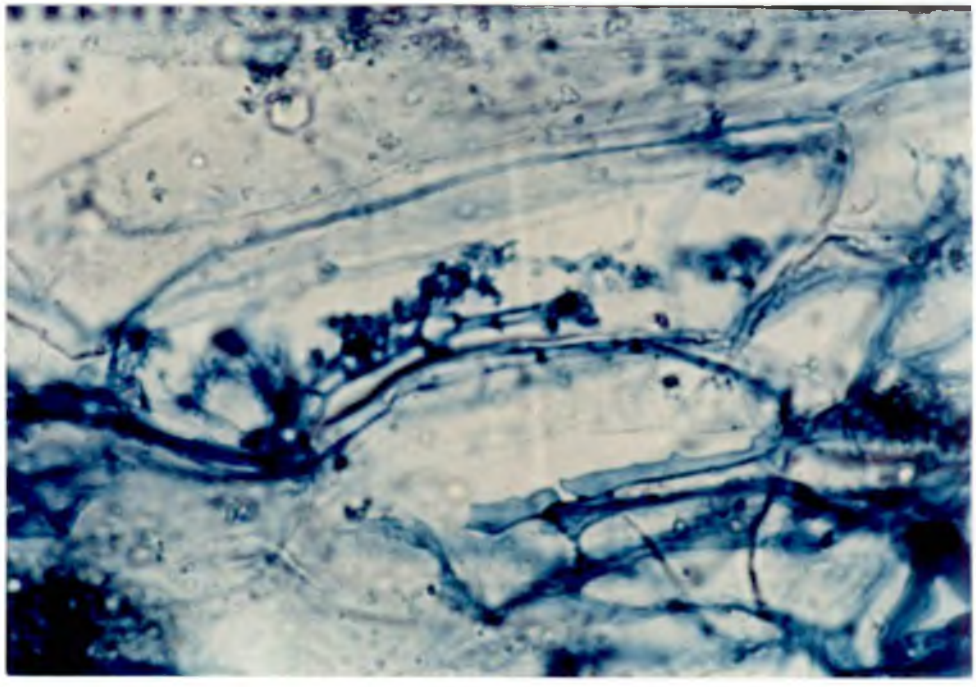
Fig. 13 AMF colonization pattern in black pepper inoculated with AMF through carrier plants in established plantation

Plate 21. Growth of carrier plant (Sorghum) around the pepper vines

Plate 22. Growth of carrier plant (green gram) around the pepper vines

Plate 23. Arbuscules in root cortical cells

Plate 24. Diagrammatic representation of AMF transfer from carrier plant roots to pepper roots



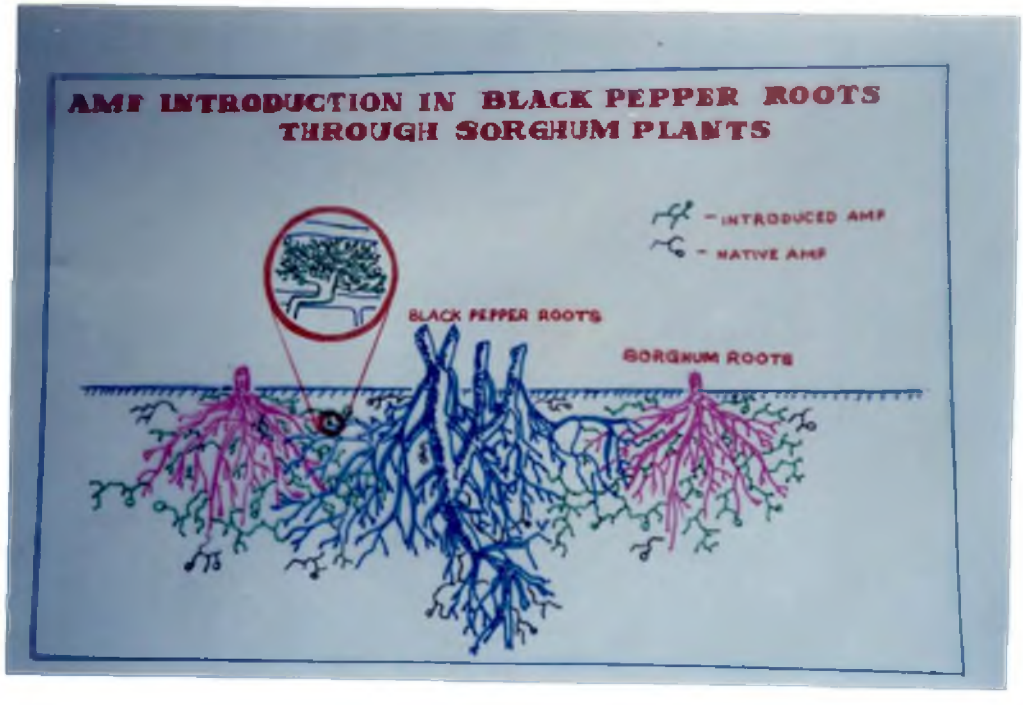

AMF INTRODUCTION IN BLACK PEPPER ROOTS
THROUGH SORGHUM PLANTS
- INTRODUCED AMF
- NATIVE AMF
BLACK PEPPER ROOTS
SORGHUM ROOTS

Plate 25. Foot rot symptom in black pepper in established plantation

rhizosphere (Table 34). Pepper vines inoculated with AMF using sorghum as carrier plant consistently recorded high AMF colonization with a maximum of 78.33 per cent on the 90th day of inoculation as against 66.66 per cent and 51.66 to 53.66 per cent recorded for green gram and control plants respectively (Fig. 13). Different AMF inoculation methods tested with out carrier plants did not influence mycorrhizal colonization in pepper roots. The sorghum plants (Plate 21) grown as AMF carrier plant in the pepper basin showed significantly higher root length (20.04 cm) and root mass (1.86 g) over green gram (Plate 22) which recorded 10.50 cm and 0.79 g respectively. Presence of vesicles and arbuscules was noticed in the root cortical cells (Plate 23 and 24).

4.10 Effect of AMF and antagonists on foot rot incidence of black pepper vines in the established plantation

Based on the result of the dual inoculation studies conducted in green house and in the field, AMF cultures Pi-11, Is-6 and *Glomus fasciculatum* and antagonists viz., *Aspergillus fumigatus*, *Aspergillus sydowii* and *Trichoderma viride* were further tested on pepper vines in the established plantation.

The foliar yellowing and defoliation symptoms (Plate 25) which were taken as indices of foot rot incidence, varied with different treatments (Table 35). Of the AMF tested Pi-11 recorded the least per cent foliar yellowing (9.52%) and

Table 35 Effect of AMF and ıtagonistic fungal inoculation on foot rot incidence ın established black pepper vines

Treatment	Per cent ıncidence index				Foot rot Disease intensity score
	Foliar yellowing	Disease score (0-9)	Defoli- ation	Disease score (0-9)	
G. f. x *T. v.*	0.00	0	14.28	3	1.5
G. f. x *A. f.*	14.28	3	19.04	4	3.5
G. f. x *A. s.*	19.04	4	23.80	5	4.5
Is-6 x *T. v.*	9.52	2	9.52	2	2.0
Is-6 x *A. f.*	19.04	4	9.52	2	3.0
Is-6 x *A. s.*	0.00	0	19.04	4	2.0
Pi-11 x *T. v.*	33.33	7	28.57	6	6.5
Pi-11 x *A. f.*	23.80	5	14.28	3	4.0
Pi-11 x *A. s.*	0.00	0	0.00	0	0.0
G. f.	9.52	2	38.09	8	5.0
Is-6	19.04	4	23.80	5	4.5
Pi-11	9.52	2	4.76	1	1.5
T. v.	28.57	6	9.52	2	4.0
A. f.	14.28	3	28.57	6	4.5
A. s.	19.04	4	4.76	1	2.5
Bordeaux mixture	19.04	4	14.28	3	3.5
Copper oxychloride	9.52	2	19.04	4	3.0
Food base	19.04	4	9.52	2	3.0
Control	42.85	9	23.80	5	7.0

G.f.-*Glomus fasciculatum* *T.v.*-*Trichoderma viride*
A.f.-*Aspergillus fumigatus* *A.s.*-*Aspergillus sydowii*

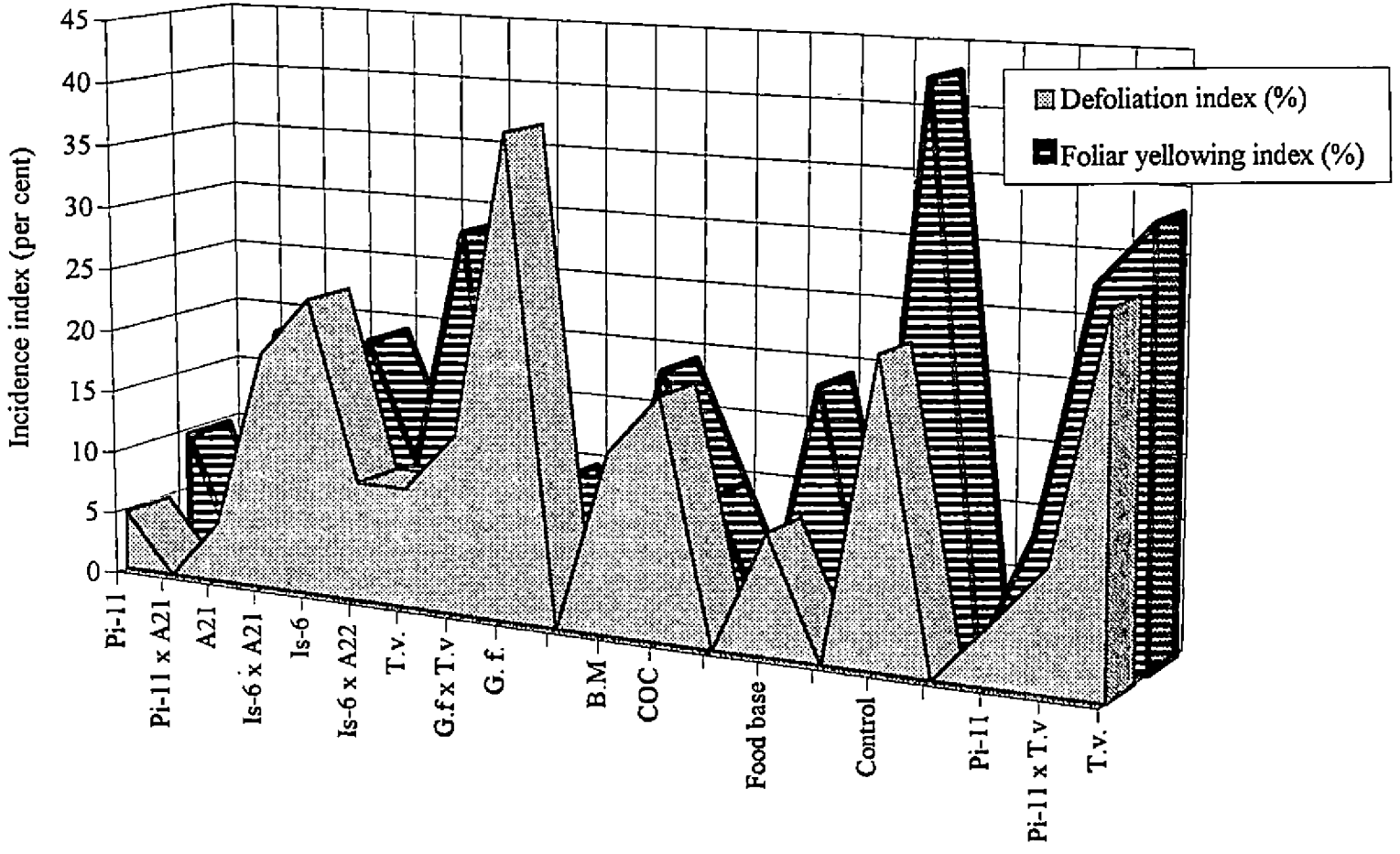


Fig. 14 Effect of AMF and antagonists on foot rot incidence in established black pepper vines

defoliation (4.76%) index. The antagonists were not as effective as AMF on single inoculation in suppressing the disease (Table 35). Isolate Pi-11 in combination with *A. sydowii* recorded no foliar yellowing whereas its combination with *T. viride* and *A. fumigatus* increased the foliar yellowing index from 9.52 per cent (for Pi-11 alone) to 23.80 and 33.33 per cent respectively. Similarly, *G. fasciculatum* showed per cent foliar yellowing index of 9.52 on single inoculation and in combination with *T. viride* no yellowing was noticed. Whereas *G. fasciculatum* in combination with *A. fumigatus* and *A. sydowii* increased the disease. In the case of Is-6 the foliar yellowing was reduced on dual inoculation with *T. viride* (9.52%) and *A. sydowii* (0.00%) than its single inoculation (19.04%). The reduction in foliar yellowing was comparable with the disease control achieved by the application of copper oxychloride (9.52%). Untreated control plants showed 42.86 per cent foliar yellowing.

Amongst single inoculation treatments Pi-11 and *A. sydowii* recorded less than 5 per cent defoliation (Fig. 14). Although Is-6 registered 23.80 per cent defoliation index on single inoculation, the dual inoculation with *T. viride* (9.52%) and *A. fumigatus* (9.52%) remarkably reduced defoliation. *G. fasciculatum* with 38.09 per cent defoliation on single inoculation, reduced the incidence to 14.28 per cent when inoculated in combination with *T viride*. A reduced defoliation

Table 36 Antagonistic fungal population as influenced by dual inoculation with AMF isolates in the field

Treatment	Population of fungi ($x10^3$ g^{-1})	
	*Soil fungi	Antagonists
G.f. x *T.v.*	4.94	14.33
G.f. x *A.f.*	4.06	29.67
G.f. x *A.s.*	4.86	34.33
Is-6 x *T.v.*	7.91	13.33
Is-6 x *A.f.*	6.00	27.67
Is-6 x *A.s.*	4.09	31.00
Pi-11 x *T.v.*	7.32	15.67
Pi-11 x *A.f.*	5.42	30.67
Pi-11 x *A.s.*	5.77	31.00
G. f.	11.52	0.00
Is-6	13.86	0.00
Pi-11	11.19	0.00
T. v.	3.62	14.67
A. *f.*	5.41	30.33
A. s.	6.57	35.67
Bordeaux mixture	28.19	0.00
Copper oxychloride	31.52	0.00
Food base	33.86	0.00
Control	14.86	0.00
CD (0.05)	8.01	

* Adjusted means to the regression of corresponding antagonistic population (co-variate)

G.f. - *Glomus fasciculatum*; *T.v.* - *Trichoderma viride*
A.f. - *Aspergillus fumigatus*; *A.s.* - *Aspergillus sydowii*

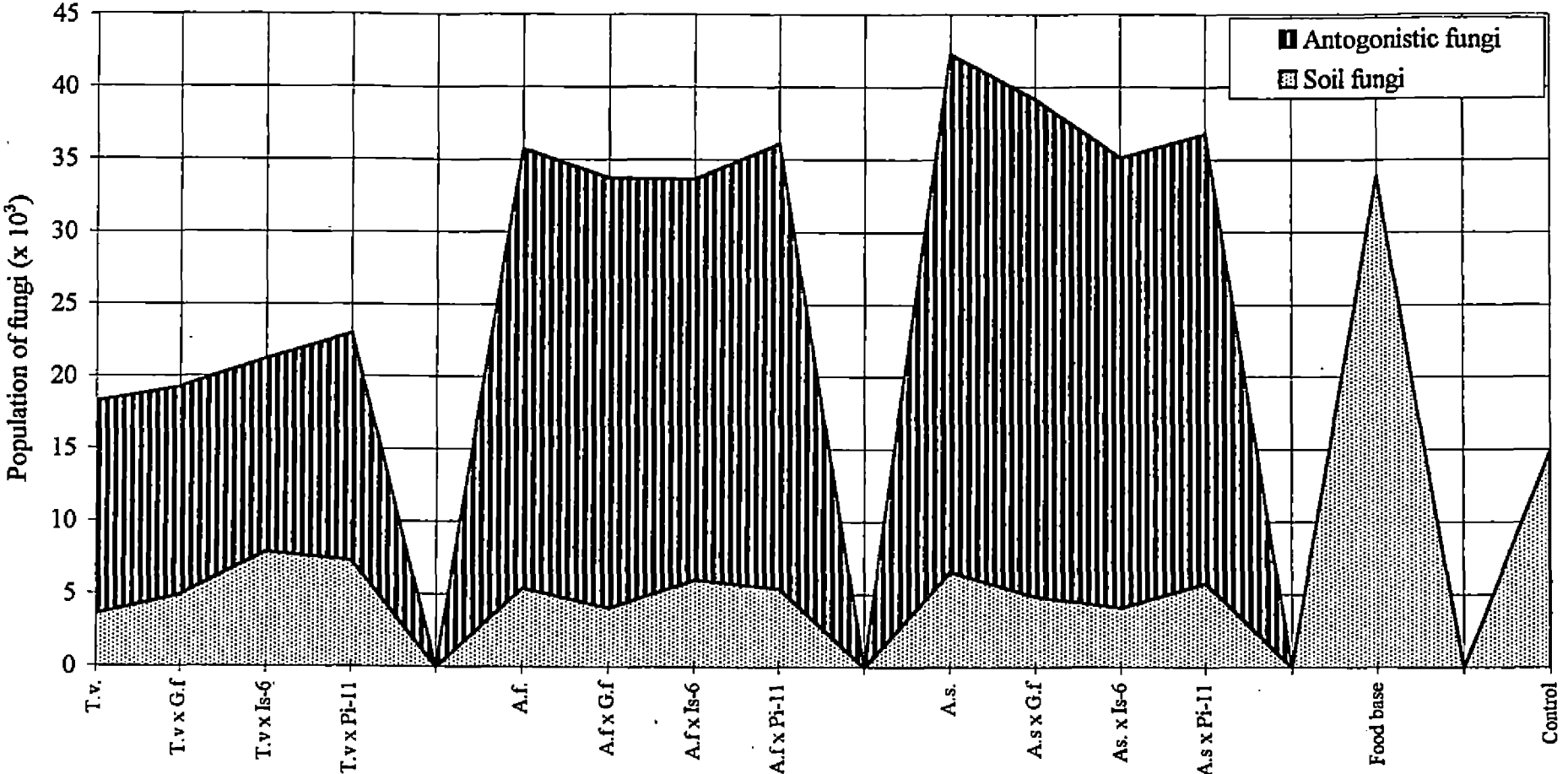


Fig. 15 Population of antagonists and soil fungi in established black pepper rhizosphere as influenced by AMF and antagonists inoculation

observed with the effective biocontrol combinations were comparable with the disease control obtained by bordeaux mixture (14.28%) and copper oxychloride (19.04%). The untreated control resulted 23.80 per cent defoliation. The foot rot disease intensity score computed from per cent leaf yellowing and defoliation score remarkably varied with the treatments (Table 35). From the data it is evident that combination of Pi-11 x *A. sydowii* which recorded the score of 'zero' was most effective of all the treatments under the trial (Fig. 14). The AMF isolate Pi-11 alone recorded the score of 1.5 and ranked second along with *G. fasciculatum* x *T. viride* combination The data also shows effectiveness of Is-6 in combination with the *T. viride* and *A. sydowii* (score 2). *A. sydowii* alone was also effective against foot rot with the intensity score of 2.5. The combination of Is-6 and *A. fumigatus* with the score of 3 was also on par with chemical methods using copper oxychloride (3.0).

Studies on the relationship between the population of introduced antagonists and other rhizosphere soil fungi showed negative relationship (Table 36). Lowest population of soil fungi (3.62×10^3 g^{-1}) was recorded on inoculation with *T. viride* alone. The population of *T. viride* was 14.67×10^3 g^{-1} soil. Significant reduction in soil fungal population was also recorded with *A. fumigatus*, *A. sydowii* and their combinations with all AMF (*G. fasciculatum*, Is-6, Pi-11) cultures (Fig. 15).

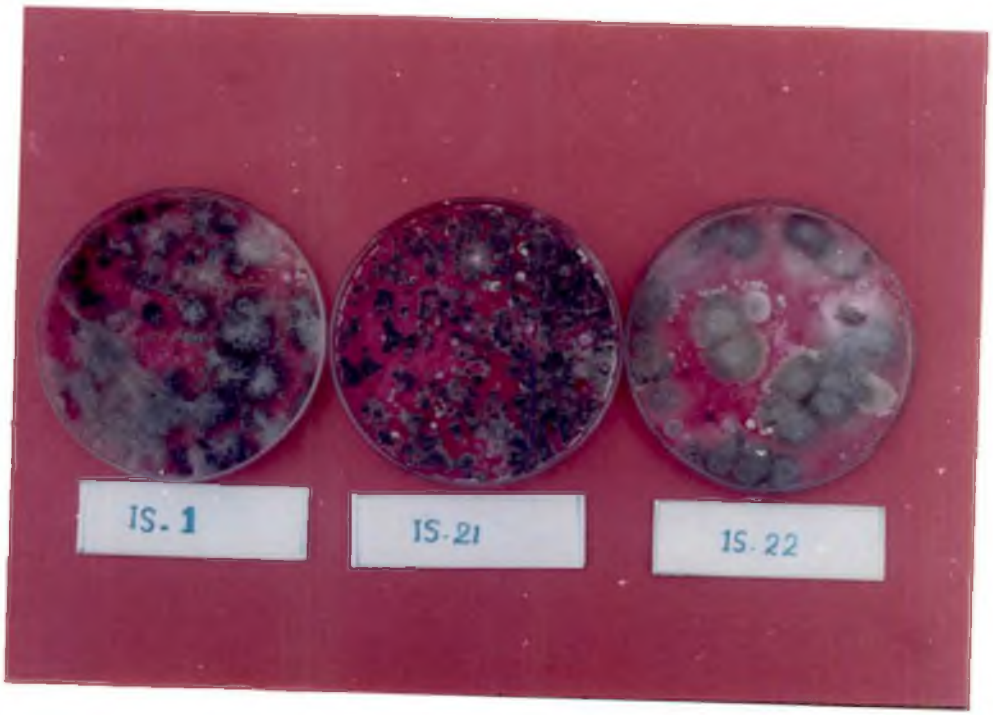
Table 37 Effect of AMF and antagonistic fungi inoculation on mycorrhizal colonization in established black pepper vines

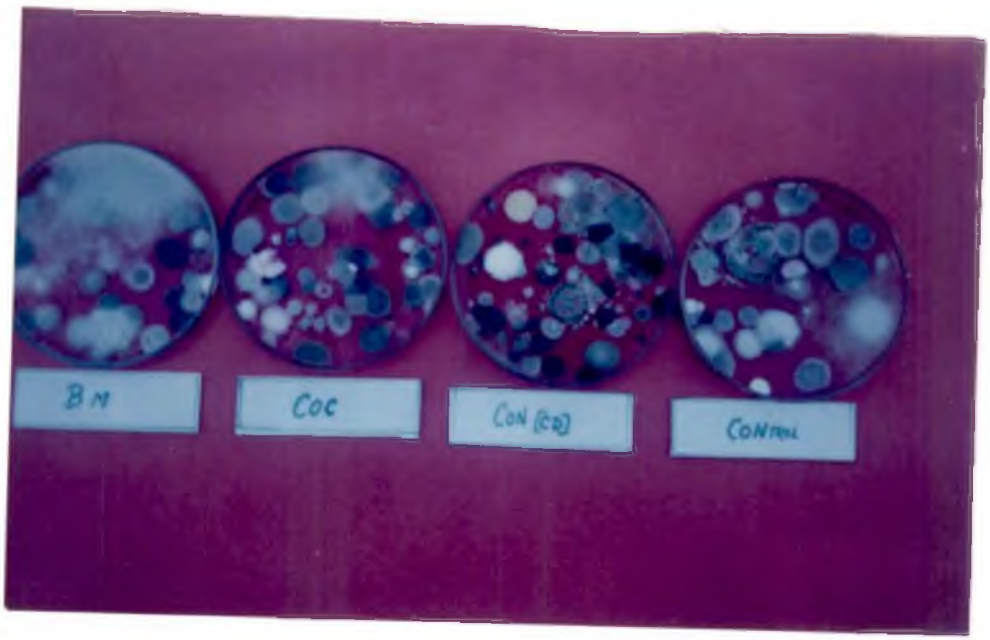
Treatment	AMF colonization (%)			
	Days after inoculation			
	0	90	120	180
G.f.xT.v.	53.35	57.85	64.47	69.93
G.fxA.f.	48.89	55.65	62.25	71.34
G.f.xA.s.	44.43	51.11	57.79	66.78
Is-6x*T.v.*	53.35	57.79	62.25	66.78
Is-6x*A.f.*	40.00	46.65	55.65	68.93
Is-6x*A.s.*	48.89	53.35	62.25	71.34
Pi-11x*T.v.*	51.11	55.65	62.31	66.67
Pi-11x*A.f.*	42.21	48.89	55.65	68.93
Pi-11x*A.s.*	46.65	55.65	64.47	73.51
G. f.	46.65	53.42	66.95	71.96
Is-6	42.14	46.65	57.85	64.47
Pi-11	48.89	53.33	60.06	71.96
T. v.	53.35	55.65	57.85	55.65
A. *f.*	51.11	57.85	55.65	57.85
A. s.	46.67	48.89	51.11	53.35
Bordeaux mixture	46.65	48.89	51.11	53.35
Copper oxychloride	44.43	51.11	53.35	55.65
Food base	48.89	53.35	51.11	48.89
Control	53.35	55.65	51.11	55.65

G.f. - *Glomus fasciculatum* *T.v.* - *Trichoderma viride*
A.f. - *Aspergillus fumigatus* *A.s.* - *Aspergillus sydowii*

Plate 26. Antagonists colonies from pepper rhizosphere inoculated with AMF and antagonists (IS.1 - *Aspergillus fumigatus*; IS.21 - *Aspergillus sydowii*; IS.22 - *Trichoderma viride*)

Plate 27. Fungal colonies in the rhizosphere of control plants (BM - bordeaux mixture; COC - Copper oxychloride; CON(CD) - Food base; Control - Control)


IS. 1
IS. 21
IS. 22


BM
COC
CON (CO)
CONTROL

However, the effect of introduced AMF on population of antagonists was not much evident. Inoculation of *A. sydowii* in different treatments recorded highest population of 31.00 to 35.67 x $10^3 . g^{-1}$ soil (Plate 26). Chemical fungicides recorded significantly higher population of resident rhizosphere soil fungi than biocontrol treatments (28.19 to 33.86 x 10^3 g^{-1}). Highest soil fungal population of 33.86 and 31.52 x$10^3 . g^{-1}$ was recorded for food base (cowdung + neem cake) and copper oxychlordie respectively (Plate 27) while untreated control recorded rhizosphere fungal population of 14.86 x$10^3 . g^{-1}$ soil.

The colonization due to native AMF at the time of inoculation with AMF cultures using sorghum carrier plant ranged from 42.14 to 53.35 per cent (Table 37). Subsequent increase in colonization was observed with AMF inoculation. The increase was more evident by 120^{th} day of AMF inoculation. *G. fasciculatum* recorded the higher colonization of 66.95 per cent on the 120^{th} day followed by Pi-11 (60.06%) and Is-6 (57.85%). Application of chemical fungicides did not show any significant effect on mycorrhizal colonization in the roots of established pepper vines. Colonization of 53.65 per cent was recorded in bordeaux mixture treatment as against 55.65 per cent of untreated control. All the combinations of AMF isolates with antagonists showed comparatively high root colonization than their single inoculation. Dual inoculation of Is-6 with antagonists markedly increased AMF colonization

Table 38 Effect of AMF inoculation on total free amino acid in black pepper

AMF isolate	Total free Amino acid (ug Leucine equivalent g^{-1})	
	Top	Root
Is-6	536.66	266.66
Pi-9	446.66	343.33
Pi-11	513.33	233.33
Glomus fasciculatum	433.33	176.66
Gigaspora margarita	453.33	290.00
Control	256.66	370.00
CD (0.05)	NS	NS

(66.78 to 71.34%) than single inoculation (64.47%). Considerable increase in AMF colonization over natural colonization was noticed with the combinations of Is-6 x *A. fumigatus* (68.93%) and Pi-11 with *A. sydowii* (73.51%) and *A. fumigatus* (68.93%).

4.11 Biochemical changes in black pepper due to AMF colonization

Effect of AMF inoculation on the biochemical changes in black pepper tissue was investigated. The AMF colonisation did not exert much influence on the total free amino acid content (Table 38). Highest amino acid content in plant top was recorded with Is-6 (536.66 ug g^{-1} fresh tissue) followed by Pi-11 (513.33 ug). In the AMF isolate Pi-9, *G. fasciculatum* and *Gigaspora margarita* inoculated pepper plants the free amino acid content was 446.66, 443.33 and 453.33 ug g^{-1} respectively. Control had 256.60 ug g^{-1} in plant top. All the AMF isolates recorded low amount of free amino acid in the root tissue compared to control plants. Among the AMF tested, Pi-9 and *G. margarita* showed maximum free amino acid content of 343.33, 290.00 ug g^{-1}. in the root followed by Is-6 with 266.66 ug g^{-1}. The lowest amount was recorded on inoculation with *G. fasciculatum* (176.66 ug). Roots of control plants contained 370 ug g^{-1} free amino acid (Table 38).

Table 39 Effect of AMF inoculation on total sugar, reducing sugar and protein content in black pepper

AMF isolate	Total sugar (mg glucose.g^{-1})		Reducing sugar (mg glucose.g^{-1})		Protein (%)	
	Top	Root	Top	Root	Top	Root
Is-6	5.33	2.67	2.17	1.67	1.46	1.43
Pi-9	3.58	2.83	1.67	2.33	1.45	1.35
Pi-11	3.67	3.00	2.83	2.00	1.35	1.25
Glomus fasciculatum	3.83	2.17	2.67	1.00	1.54	1.51
Gigaspora margarita	5.00	2.92	1.92	2.67	1.43	1.26
Control	5.67	2.25	3.67	0.75	1.67	1.25
CD (0.05)	NS	NS	NS	0.60	NS	NS

Amongst AMF tested, Is-6 showed higher total sugar content of 5.33 mg g^{-1} fresh plant tissue followed by *G. margarita* with 5.00 mg. Pi-9 and Pi-11 had 3.58 and 3.67 mg as against 5.67 mg of the control plants (Table 39). The total sugar content in roots did not differ significantly with the treatments. Highest total sugar content of 3.00 mg was observed in isolate Pi-11, followed by *G. margarita* (2.92 mg) and Pi-9 with 2.83 mg. *G. fasciculatum* recorded root sugar content of 2.17 mg, whereas the value for the control plants was 2.25 mg. The reducing sugar content in the plant top of mycorrhizal black pepper was not significantly influenced by AMF symbionts (Table 39). All the mycorrhizal plants showed comparatively lower reducing sugar content ranging from 1.67 (Pi-9) to 2.83 mg (Pi-11) than that was present in control plant (3.67 mg). However, there was significant variation in the reducing sugar content in the roots. All the cultures except *G. fasciculatum* (1.00 mg) showed significantly higher reducing sugar content in pepper roots. The highest concentration of reducing sugar in the roots was recorded with *G. margarita* (2.67 mg) followed by Pi-9 (2.33 mg) and Pi-11 (2.00 mg). Uninoculated control plants recorded 0.75 mg.

Protein content of the plant top of black pepper was not significantly influenced by AMF inoculation (Table 39). AMF *G. fasciculatum* and Is-6 recorded relatively higher protein content of 1.54 and 1.46 per cent g^{-1} fresh tissue respectively

Table 40 Effect of AMF inoculation on total phenols and orthodihydroxy phenol content in black pepper

AMF isolate	Total phenols ($ug \cdot g^{-1}$)		Orthodihydroxy phenol ($ug\ g^{-1}$)	
	Top	Root	Top	Root
IS-6	695.27	369.13	206.8	69.9
Pi-9	621.47	305.9	181.23	43.6
Pi-11	590.67	236.93	202.27	69.3
Glomus fasciculatum	811.97	350.73	245.87	64.3
Gigaspora margarita	633.73	277.17	211.2	50.5
Control (None)	1009.1	312.77	372.27	67.87
CD (0.05)	202.50	NS	NS	NS

in the plant top. Isolate Pi-11 recorded lowest protein (1.34%). Uninoculated control had 1.67 per cent protein. Regarding protein content of root, relatively higher values were noticed with different AMF cultures (Table 39). *G. fasciculatum* recorded highest protein content (1.51%) followed by Is-6 (1.43%) and Pi-9 (1.35%). The lowest protein content was recorded with Pi-11 (1.25%) and control.

The data on the phenol content in the plant top showed significant variation (Table 40). All the mycorrhizal treatments showed relatively less total phenol content in plant top compared to control plants. Among the AMF cultures *G. fasiculatum* had higher plant top phenol concentration of 811.97 ug. While the isolates Is-6 and *G. margarita* recorded 695.27 and 633.73 ug g^{-1} fresh root tissue respectively. Lowest phenol content was recorded in Pi-9 (621.47 ug). The control plants had a concentration of 1009.10 ug total phenol. There was no significant difference in root phenol concentration among the treatments (Table 40). However, higher phenol concentration of 369.13 ug g^{-1} fresh tissue was recorded in the case of Is-6. *G. fasciculatum* and Pi-11 recorded the phenol content of 350.73 and 305.90 ug respectively in the roots of pepper plants. Lowest concentration was recorded in relation to Pi-9 (305.93 ug) as against 312. 77 ug recorded for control plants.

Table 41 Effect of AMF inoculation on cellulase and chitinase activity in black pepper

AMF isolate	Cellulase activity (ug glucose released min^{-1} mg^{-1} protein)		Chitinase activity (nM N-acetyl glucosamine h^{-1} g^{-1})	
	Top	Root	Top	Root
Is-6	0.527	0.103	129.00	67.43
Pi-9	0.188	0.153	70.36	41.04
Pi-11	0.241	0.101	52.77	76.23
Glomus fasciculatum	0.286	0.141	29.31	46.90
Gigaspora margarita	0.068	0.230	25.90	36.37
Control	0.107	0.106	40.88	43.98
CD (0.05)	0.034	0.015	-	-

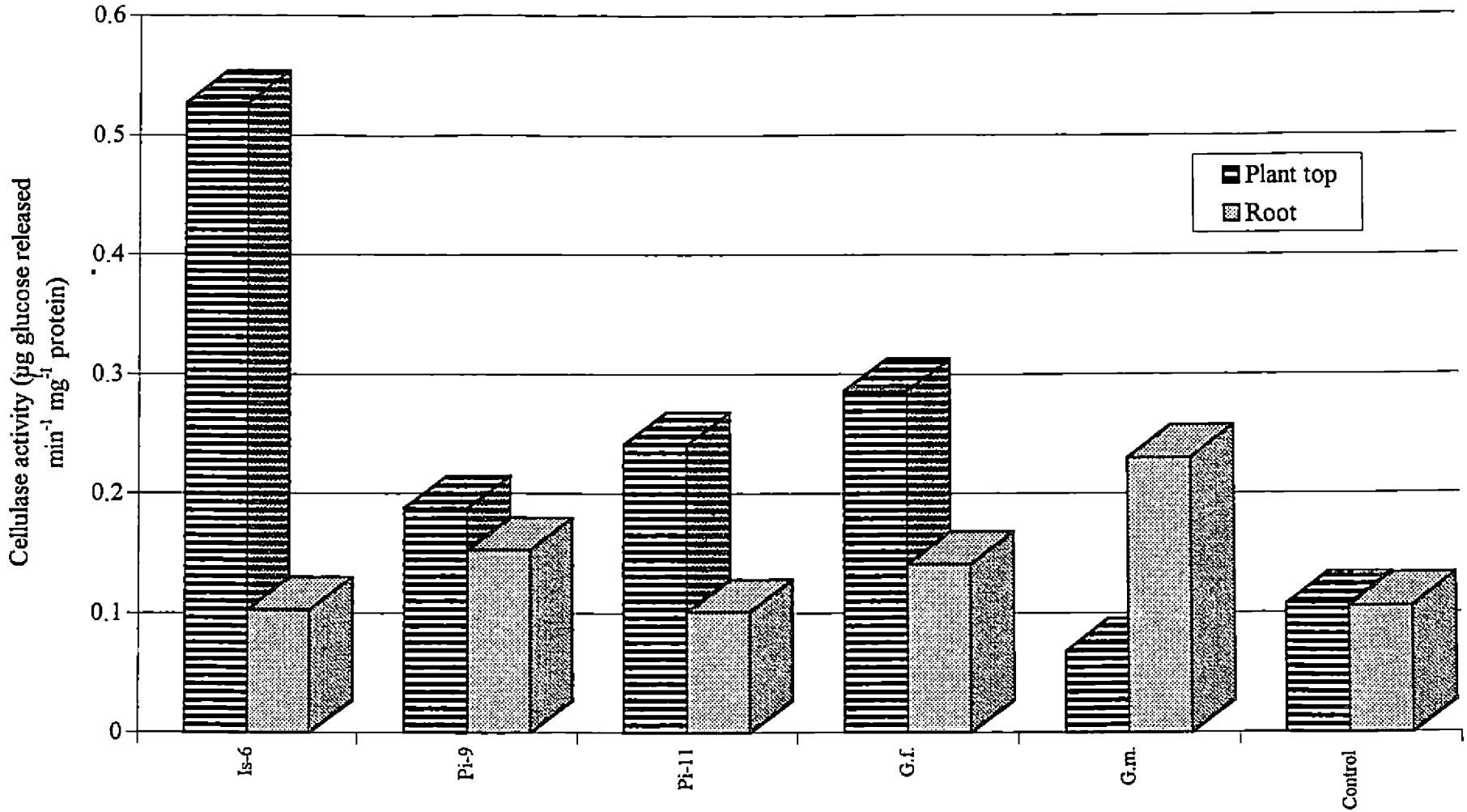


Fig. 16 Effect of AMF inoculation on cellulase activity in black pepper

Orthodihydroxy (OD) phenol content in plant top and root of mycorrhizal and non-mycorrhizal pepper plants were found to be statistically on par (Table 40). Relatively, lower amounts of OD phenol content in the plant top were observed as a result of AMF inoculation. *G. fasciculatum* recorded 245.87 ug OD phenol, while the isolate Pi-9 had the lowest OD phenol content of 181.23 ug g^{-1} plant tisue. Control plants were noted to have 272.27 ug OD phenol content. A slight increase in root OD phenol concentration due to AMF inoculation is evident from the analytical data (Table 40). Higher OD phenol content in pepper roots was noticed on inoculation with Is-6 and Pi-11 with value of 69.90 and 69.30 ug g^{-1} root tissue respectively. The lowest concentration was recorded in Pi-9 inoculation(43.60). Non mycorrhizal control plants had 67.87 ug OD phenol content in pepper roots.

The data pertaining to the effect of mycorrhizal inoculation on cellulase activity in black pepper showed significant variation with AMF cultures (Table 41). Significantly higher cellulase activity both in plant top and root tissue were noticed upon inoculation AMF isolates (Fig. 16). Highest cellulase activity in plant top was recorded with Is-6 (0.527 ug glucose released min^{-1}, mg^{-1} protein) followed by *G. fasiculatum* (0.286). *G. margarita* (0.068) was responsible for significantly lower cellulase activity in plant top than that found in uninoculated control

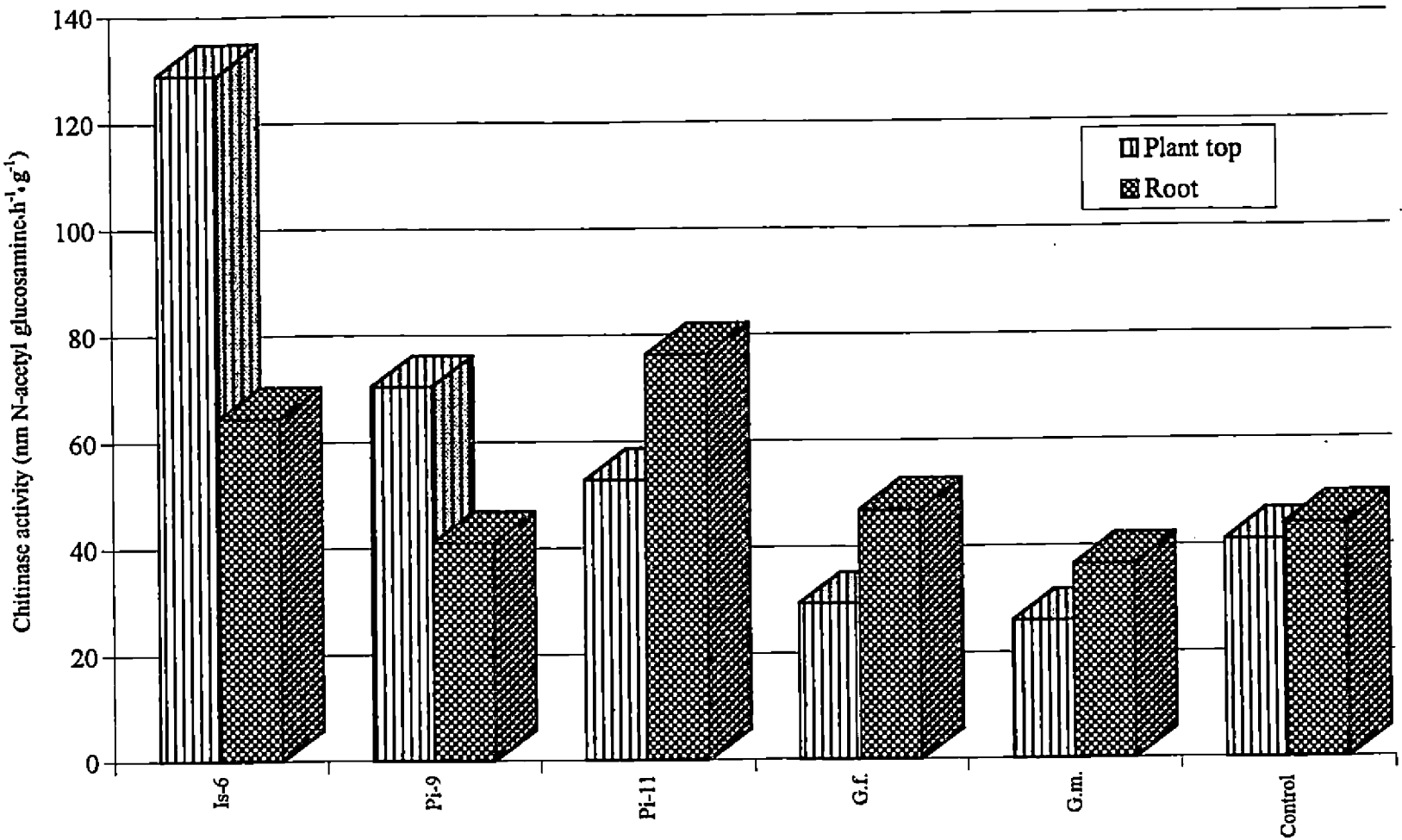


Fig. 17 Effect of AMF inoculation on chitinase activity in black pepper

(0.107 ug glucose released min $^{-1}$ mg^{-1} protein). With regard to the root cellulase activity *Gigaspora margarita* was found to encourage higher cellulase activity (0.230) as compared to control (0.106) while Is-6 and Pi-11 recorded lower root cellulase activity of 0.103 and 0.101 mg glucose released min^{-1} mg^{-1} protein. *Glomus fasciculatum* had cellulase activity of 0.141 ug glucose released min^{-1} mg^{-1} protein in black pepper roots.

Mycorrhizal isolates showed varied amount of chitinase activity in black pepper (Table 41). AMF isolate Is-6 recorded maximum chitinase activity of 129.00 nM N-acetyl glucosamine h^{-1} g^{-1} in plant top followed by Pi-9 (70.36) and Pi-11 (52.77) comparatively lower chitinase activity in plant top with *G. margarita* (25.90) and *G. fasciculatum* (29.1) was observed. The root chitinase activity was maximum on inoculation with Pi-11 (76.23) and Is-6 (67.43) (Fig. 17). *Gigaspora margarita* had relatively low activity of 36.37. Control plants registered 43.98 chitinase activity. *G. fasciculatum* and Pi-9 recorded chitinase activity of 46.90 and 41.04 respectively in the roots.

DISCUSSION

DISCUSSION

The present investigation was undertaken to develop a viable biocontrol strategy for the management of foot rot disease of black pepper. The causal organism of the disease was isolated from pepper growing tracts of Kerala. The morphological studies confirmed the isolates as *Phytophthora capsici* Leonian emend A. Alizadeh and P.H. Tsao, the foot rot pathogen of black pepper (Tsao, 1991). These isolates showed wide variation in the growth rate and pattern in carrot agar (Table 1). This variation may be due to the difference in the inherent ability of the pathogen to utilize the nutrients available in the medium (Danielson and Davey, 1973). The pathogenicity test indicated marked variation among the isolates in symptom development (Table 2). Peringammala isolate developed foot rot symptoms as early as on the 15th day of inoculation of the pathogen and registered 100 per cent mortality of plants by 75th day. Since the environment and host factors are uniform, the high disease intensity and mortality noted with the isolate indicated its highly virulent nature. This isolate was used for the studies under the investigation.

Positive influence of arbuscular mycorrhizal fungi (AMF) on plant growth promotion, biomass production and protection against root diseases is well recognised in most of

the plant species (Daft and Nicolson, 1966; Daft and Okusanya, 1973; Bagyaraj and Manjunath, 1980; Dehne, 1982; Bagyaraj, 1984; Caron, 1989; Sivaprasad *et al*., 1993; Sulochana *et al*., 1995; Sivaprasad, 1995). The present study conducted on growth of black pepper cuttings preinoculated with various AMF cultures showed no significant influence on growth till 150th day of planting (Table 3). However, isolate Pi-11, *G. margarita* and Pi-9 recorded higher in plant height and leaf number over other treatments. All the AMF cultures tested showed significant influence on black pepper growth and biomass production by seventh month. Pi-11, *Gigaspora margarita* and Pi-9 were more effective and recorded significant increase in plant height over all other treatments. Such variation among AMF cultures in stimulating plant growth is observed in crop plants (Bagyaraj and Manjunath, 1980; Jensen, 1982; Sulochana *et al*., 1995) including black pepper (Shivashankar and Rohini Iyer, 1988; Sivaprasad *et al*. 1993; Anandaraj and Sarma, 1994a; Sivaprasad, 1995). Regarding the plant dry weight, the native isolates in general and Pi-11, Pi-9 and Is-6 in particular were more effective and recorded significantly higher plant dry weight (Table 4). The increase in plant growth and biomass production is related to several factors. The beneficial effects of AMF such as increased phytohormone production, photsynthetic efficiency (Shoenbeck, 1979; Sivaprasad, 1983) and uptake of nutrients and water (Bagyaraj and Manjunath, 1980; Davis *et al*., 1992; 1993; Panwar, 1993) are known to

influence growth and biomass production of the host plant. The higher growth and biomass production achieved with the isolates in the present study may be attributed to these factors. Enhanced root elongation was also apparent with native isolates (Fig. 1). Well developed mycorrhizal root system alters the cellular process and absorb more nutrient and water by expanded absorptive capacity which result in improved growth and biomass production by the plants (Reid, 1984; Hooker *et al*., 1994).

Analysis of nutrient content in mycorrhizal black pepper showed wide variation with the AMF cultures (Table 5, 6 and 7). Maximum uptake and translocation of phosphorus was evident with isolate Pi-11 followed by Pi-9 and *Glomus fasciculatum* (Table 5, Fig. 2). Is-6, a native isolate, also enhanced the P uptake. The increased 'P' uptake in mycorrhizal plants generally related to the mobilization of the sparingly available nutrient from areas beyond the depletion zone (Hayman, 1982) through increased surface area of absorption (Reid, 1984) and also by improving the availability by secretion of ectoenzymes (Sanders and Thinker, 1971; Rhodes, 1980). The native isolates are more adapted to the soil conditions and therefore, resulted in better mycelial spread both in root and soil. This enables better exploration of soil for nutrient and its transport to root cortex (Wood, 1992; Sivaprasad, 1998). The per cent K content and total uptake were significantly

increased with native isolates Pi-11, Pi-9 and Is-6 and *Glomus clarum* (Fig. 2). The available reports are suggestive of positive influence of AMF on the uptake of K and, hence, on plant growth and disease tolerance (Bethlenfalvay *et al.*, 1988; Tarafdar and Marschner, 1994; Panwar and Thakur, 1994). Although there is no specific mechanism ascribed to higher uptake of the soluble nutrient, it could be due to improved root development and nutrient uptake system conferred by AMF colonization.

The Ca content was also significantly enhanced through AMF isolates Pi-9, Pi-11, Is-6 and *G. fasciculatum* (Table 6, Fig. 2). Enhanced growth and biomass due to mycorrhizal association aiding in Ca uptake have been reported (Ross and Harper, 1970; Ross, 1971; Rhodes and Gerdemann, 1975; 1978). All the AMF cultures showed significantly higher Mg concentration in host tissue. Maximum concentration and uptake was recorded with the inoculation of Pi-9 and Is-6 (Fig. 2). The relatively low concentration of Mg with Pi-11 was mainly due to dilution effect brought about by higher biomass production and it was also apparent from the enhanced total Mg uptake recorded with the isolate. Direct evidence of Mg uptake and transport by AMF is limited (Kothari *et al.*, 1990). However, the enhanced uptake of Mg consequent to AMF colonization has been observed in most mycorrhizal experiments with different plant species (Tarafdar and Marschner, 1994).

The enhanced uptake of Fe, Mn and Zn due to AMF has been reported by many workers (Pacovsky, 1986; Manjunath and Habte, 1988; Panwar and Thakur, 1994; Tarafdar and Marschner, 1994). In the present study also the Fe, Mn and Zn uptake was influenced by AMF colonization particularly Pi-11, Is-6 and *G. fasciculatum*. There was a notable increase in the Mn content of Pi-9 and Is-6 inoculated plant (Fig. 3). This may be due to the fact that AMF association favoured better uptake of the element, while the biomass production was not commensurate with the uptake to bring about dilution effect. The low concentration noticed with higher uptake in AMF Pi-11 support the view. It is reported that the dilution effect is one of the means of AMF protection against toxic metal (Bethlenfalvay and Franson, 1989).

Ability of the mycorrhizal fungi to suppress foot rot incidence and intensity varied with cultures. *Glomus fasciculatum* was most effective in reducing foot rot incidence and plant mortality (53.35%) under sterilized green house conditions (Fig. 4 and 5). Native isolates Is-6 and Pi-11 also reduced the mortality. The symptom development and mortality were at a slow pace in black pepper inoculated with *G. fasciculatum*, Is-6 and Pi-11 as compared to control. Other AMF cultures ((Pi-6, Pi-8, Pi-9 and Ri-9 and *Gigaspora margarita*) were not effective in suppressing the disease. Arbuscular mycorrhizal fungi induced tolerance against

infection by fungal pathogens including *Phytophthora capsici* in black pepper has been reported (Anandaraj and Sarma, 1994a; 1994b; Sivaprasad *et al*., 1995a). In the present investigation the variation observed with AMF cultures in suppressing the foot rot incidence can be attributed to the inherent ability of the organism to suppress the pathogen and to the degree of defence related physiological and biochemical changes occurring in the host due to AMF association (Mosse, 1973; Barea, 1992).

The AMF induced resistance against fungal pathogen is mainly attributed to factors such as improved nutrient uptake especially phosphorus (Ames and Linderman, 1978), competition with the pathogen for space, nutrition and host photosynthate (Harley and Smith, 1983; Linderman, 1985), qualitative and quantitative shift in the microbial population in the rhizospore (Mayer and Linderman, 1986; Secilia and Bagyaraj, 1987; Graham, 1988) and altered physiology of the host that induces host defence mechanism (Smith and Gianinazzi-pearson, 1988). In the present study improved plant nutrient uptake particularly P was recorded with AMF isolates, viz., Is-6, Pi-11 and *G. fasciculatum*. The enhanced 'P' concentration in plants is considered to retard the cell membrane permeability a result of which root exudation is declined (Graham *et al*., 1981), which may restrict the pathogen multiplication and invasion (Graham and Menge, 1982). The minor and micro-nutrients also play a vital role in imparting disease tolerance (Pacovasky, 1986; Hooker *et al*., 1994). Concentration and total

uptake of these elements were consistently higher in black pepper colonized by *G. fasciculatum*, Pi-11 and Is-6. Excessive concentration of calcium inactivates toxic action of phenolics and making plants more succeptible to pathogen invasion (Stoessl and Unwin, 1970) . This may be one of the reasons for making the AMF isolate Pi-9, which recorded consistently higher plant growth and nutrient uptake, less active against *P. capsici* invasion. However, optimum calcium levels leads to the formation of calcium pectate in the host cell wall by altering the host pectin metabolism which in turn retard the cell membrane permeability (Cook and Stall, 1971). The better disease tolerence exhibited by Pi-11, Is-6 and *G. fasciculatum* may also be due to these factors as they are having relatively less amount of Ca than Pi-9. Reduced nutrient availability might have reduced the multiplication and development of pathogen in the rhizosphere.

Enhanced Fe uptake and translocation make the plants more resistent to pathogen invasion, as the activation of enzymes required for the synthesis of defence related macromolecules are ascribed to Fe content (Brown and Swine burne, 1981). Iron mediated synthesis of phytoalexin with resultant induction of host defence mechanism has also been reported (Adikaram *et al.*, 1982). Hence, the enhanced uptake and accumulation of Fe by Is-6, Pi-11 and *G. fasciculatum* might have also contributed towards increasing the resistance

of black pepper against foot rot disease. The study also confirms earlier report of enhanced Fe uptake by mycorrhizal plants (Mosse, 1957; Tarafdar and Marschner, 1994).

AMF isolates Is-6, Pi-11 and *G. fasciculatum* showed relatively higher colonization. This could be attributed to the better infectivity of these AMF isolates and also to positive host genotype and microsymbiont interaction. The intense root colonization by AMF offers a direct competition for space against the pathogen (Davis and Menge, 1980; Linderman, 1985). Since, the microsymbionts already occupied the root tissue, the invasion and multiplication of the *P. capsici* is hindered. Perhaps, this could be the reason for low root rot index noted in pepper plants colonized by *G. fasciculatum*, Is-6 and Pi-11. Further, the well developed root system conferred by AMF association might have compensated the root damage caused by pathogenic infection and reduced deleterious effect (Thompson *et al.*, 1983; Cordier *et al.*, 1996). However, it is to be noticed that *Gigaspora margarita* with a higher root colonization showed higher foot rot intensity indicating that the root colonization alone cannot be considered as an index of disease suppression. There could be physiological, in addition to physical, barriers also.

There was qualitative and quantitative variation in the distribution of AMF in the pepper rhizosphere. Maximum AMF root colonization in black pepper by native AMF, irrespective of host genotype, was evident in oxyaquic quartipsamment (Table 12), while lower root colonization was noticed in highly fertile haplic argiustoll. Low nutrient availability, especially 'P', in sandy soils may be ascribed to the higher colonization (Hayman *et al.*, 1975; Liyanage, 1989). Predominance of *Glomus* spp. was evident in all soil types (Table 13). Besides *Glomus* spp. the frequent occurrence of *Acaulospora* and *Gigaspora* in sandy soils (oxyaquic quartipsamment) exhibited the clear influence of soil types on mycorrhizal association. Influence of host genotypes and soil types on AMF has been elucidated in tuber crops (Potty, 1990) and spice crops (Sivaprasad, 1995). AMF characterisation studies conducted in three districts (Idukki, Kannur, Wayanad) having different soil types depicted specific influence of soil types rather than genotypes in harbouring different AMF species (Table 15). *Glomus fasciculatum* was most predominant with all soil types, irrespective of host genotypes. Earlier work on AMF characterisation also showed frequent occurrence of *G. fasciculatum* in different soils of Kerala (Lekha *et al.*, 1995).

The success of biological control with antagonistic fungi depends on the availability of potential strains having high competetive saprophytic ability (Adams, 1990; Hooker *et al.*, 1994) with antagonistic activities like predation, parasitism or antibiosis (Parkinson and Waid, 1960; Mukhopadhyay, 1994). It is well established that native isolates, more adapted to the soil conditions, are always having more competetive saprophytic ability than introduced cultures (Papavizas and Lawis, 1981). Amongst the 50 native antagonistic isolates tested *in vitro* 24 isolates showed over 60 per cent suppression of growth of *P. capsici* either by over-growing or by production of toxic metabolites. Isolate A_1, A_{21}, A_{22} and A_{13} exerted maximum growth inhibition of *P. capsici* (Table 16). Colonies of *P. capsici* were over grown by these antagonists and on contact resulted in the lysis of the mycelial strands of the pathogen. Clear zone of inhibition was also recorded with isolates (A_{35}, A_{33} and A_1), indicating the possible production of inhibitory metabolites. The direct penetration and coiling of hyphae (Marchetti *et al.*, 1992) and enzymatic lysis of the pathogen are the major mechanisms in inhibition by antagonists (Elad *et al.*, 1983; Rideout *et al.*, 1986; Claydon *et al.*, 1987). In the present study the cell free culture filterate of A_{35}, A_{22}, A_1, A_{26} and A_{39} showed considerable inhibitory effect. The enzymes and inhibitor metabolites present in the culture filtrate are attributed to such inhibition (Elad *et al.*, 1983; Claydon *et al.*, 1987;

D'ercole *et al.*, 1993; Harman *et al.*, 1993; Faull *et al.*, 1994). Hence, it could be concluded that these isolates are also producing such metabolites inhibitory to *P. capsici*.

Remarkable suppression of the pathogen was observed in soil incubation studies also (Table 17). Isolates A_1 A_{21}, A_{22}, A_{31}, A_{32}, A_{33}, A_{35} and A_{42} exhibited over 75 per cent population inhibition of *P. capsici*. Possible release of inhibitory substances with elevated levels of fungistasis and the high concentration of carbondioxide accumulation in the soil, which is detrimental to the survival of the pathogen, as well as fast multiplication and population build up of antagonists (Marchetti *et al.*, 1992) may also have contributed to effective pathogenic repression (Johri and Singh, 1975; Cotes *et al.*, 1992). The higher population build up of the introduced antagonists (A_1, A_{22}, A_{32} and A_{33}) in the soil indicates their competitive ability to multiply and persist in the soil. Even at relatively low population, the antagonists A_{21} and A_{35} have considerably decreased the *P. capsici* population; probably the isolates are more potent than others but are not competitive enough to multiply and persist under the given soil conditions.

The plant growth stimulation due to the biocontrol agents including several species of *Trichoderma* and *Aspergillus* have been well documented (Windham *et al.*, 1989; Lynch *et al.*, 1991; Sankar and Jayarajan, 1996). In the present study

antagonistic isolates in general and the isolates A_{21}, A_{19}, A_{27}, A_{29} and A_{35} in particular showed remarkable increase in plant height (Table 18). It is reported to be due to the production of growth hormones (Kloepper and Schroton, 1981; Suslow, 1982; Windham *et al.*, 1986), release of certain enzymes like phosphatases (Tarafdar and Marschner, 1995) and stimulation of nutrient uptake.

With reference to the foot rot incidence and intensity, isolate A_1 was recorded as most effective one with remarkable reduction in plant mortality (Table 20). Other isolates A_{13}, A_{21}, A_{22} and A_{35} also considerably reduced the disease intensity (Fig. 7). This reflects the effectiveness of these antagonists to check the multiplication of and infection by the pathogen as there was no competition from native flora in the sterilized soil system used for the study. It was observed that antagonists with better growth stimulation (A_{19}, A_{27} and A_{29}) were not effective in disease suppression. These cultures were subjected for further testing along with selected AMF cultures.

Interaction between soil microorganisms in the rhizosphere would be either beneficial or detrimental to overall growth and development of plants. The interaction of selected AMF (Is-6, Pi-9, Pi-11, *G. fasciculatum* and *Gigaspora margarita*) and antagonists (A_1, A_{13}, A_{21}, A_{22} and A_{35}) on growth and disease tolerance of black pepper was tested in the

green house. Significant growth improvement was achieved with *G. fasciculatum* in combination with A_{21} A_1 and A_{22} over single inoculation of the cultures, indicating the synergestic interaction of AMF and antagonists (Table 22, Fig. 8). Plant growth stimulatory effect of the antagonists could be attributed to hormone production, and the ability to produce certain enzymes like phosphatase, as seen in *Aspergillus fumigatus*, which are known to have direct effect on plant growth (Tarafdar and Marschner, 1995). These effects in combination with mycorrhizal fungi induced elaborate nutrient uptake and the physiological and biochemical changes in the host might have favoured the faster growth and development of the plants. Combined inoculation of *Trichoderma aureoviride* and *Glomus mosseae* has shown synergistic effect on growth of marigold (Calvet *et al.*, 1993).

The maximum reduction in foot rot incidence and plant mortality was achieved with dual inoculation of A_{22}xIs-6 followed by A_1 x *G. fasciculatum* with plant mortality reduction of 66.08 and 45.45 per cent respectively, which was considerably higher than that obtained with the individual inoculation of these inoculants (Fig. 9). Further, both the combination did not record any mortality beyond 45th day of *P. capsici* inoculation. This unambiguously indicates the positive interaction between AMF and antagonists leading to protection of the host against the pathogen. Such synergistic

interactions have been reported by many workers (Calvet *et al.*, 1992; Calvet *et al.*, 1993; Tarafdar and Marschner, 1995). Beneficial microorganism are considered strong competitors of pathogens for one or more nutrients on the root surface and are able to inhibit the pathogens directly by producing antibiotics, which enable them to provide consistent root protection (Cook and Baker, 1989). The high antagonistic population and mycorrhizal colonization observed is an evidence for the synergistic interaction (Table 25 and 26). Enhanced root colonization by AMF may be due to positive interaction with antagonists leading to direct trophic effect, detoxification and release of certain stimulatory substances or indirect effect through action on the roots and antagonism to other microorganisms inhibiting the mycorrhizal development (Oliveira *et al.*, 1989). Stimulation of mycorrhizal spore germination and mycelial spread by the production of stimulatory substances by *Trichoderma* spp. have been observed (Davey, 1971; Linderman, 1988; Calvet *et al.*, 1992). The influence of interaction effect on the rhizosphere population of antagonists was however not substantial. This may be due to the inherent ability of the antagonists to multiply and persist in the rhizosphere which perhaps masked the influence of mycorrhizal fungi.

There was instances of increased incidence of foot rot and plant growth suppression due to the interaction of AMF and antagonits. Dual inoculation of A_1 x *G. margarita* significantly increased diseased incidence and intensity and reduced plant growth over single inoculation. This was a clear indication of the suppression of the individual effect of the inoculants by each other in the interaction system. Stimulated plant growth also, on the contrary, produced higher foot rot incidence and plant mortality. Combination of A_{35} x P1-11 and A_{35} x Is-6, which stimulated better plant growth, but was more vulnerable to *P. capsici* invasion. This may be due to the succulent plant growth with no concomitant effect on host defence mechanism. Hence, the microorganism with growth stimulation activity need not necessarily be a potential biocontrol agent. The result corroborates the earlier reports of improved plant nutrition leading to increased disease incidence (Atilano *et al.*, 1976; Davis *et al.*, 1978; Davis and Menge, 1981). Although *Gigaspora margarita* consistently showed higher colonization on single and dual inoculation, the pathogenic infection and mortality were always higher (Table 23 and 24). This vividly illustrates that the higher colonization and spread of mycorrhiza in the root cortex alone cannot be taken as a criterion for inducing disease tolerance. The chemical control methods using bordeaux mixture and copper oxychloride as recommended in the package of practice recommendation of Kerala Agricultural University (1996) substantially reduced the

mortality of pepper vines to about 30 per cent. The result obtained with Is-6 and A_{22} (32.90%) and *G. fasciculatum* x A_1 (50.00) are comparable with chemical control.

The effect of dual inoculation of AMF and antagonists on foot rot was tested under field conditions. Observation on growth showed significant increase with dual inoculations which indicate that the interaction is beneficial to the growth and development of pepper plants in the field (Table 27). It is noticed that the dual inoculations, viz., A_1xPi-11 and A_{21}xIs-6 were found most effective in reducing plant mortality and in improving the plant growth. The mortality rate recorded for the above combination was 58.45 per cent (Fig. 10). This was better than that recorded for bordeaux mixture (66.67%) and copper oxychloride (59.68%). The effective disease suppression by dual inoculation could be due to the induction of host defence mechanism and the higher suppression of the pathogen achieved with the synergistic interaction of the inoculants. Although A_{22} x Is-6 and A_1 x *G. fasciculatum* exhibited excellent disease control in the green house, their performance in the field was not appreciable. However, moderate disease suppression was achieved with A_1 x *G. fasciculatum* (66.67%). The A_{22} x Is-6 recorded 75.00 per cent mortality on 90^{th} day, while on 150^{th} day it was 90.84 per cent. This apparantly indicate the failure of the antagonist (A_{22}) in the field condition as the AMF Is-6 has performed well with other

antagonists A_1 and A_{21}. It is worthwhile to note that the single inoculation of AMF Pi-11 (90.00%) and Is-6 (90.00%) and antagonist A_{21} (66.67%) recorded higher disease intensity and mortality than the combination on 150th day in the field. Similarly A_1 with *G. fasciculatum* and Pi-11 induced better tolerance against the disease. Similar trend was also noticed with the isolates in improving plant growth characteristics. This clearly demonstrates the synergistic effect of AMF and antagonists on disease suppression and plant growth enhancement under field conditions. There is no record of work with dual inoculation for foot root disease management, however, results agree with the studies made on foot rot incidence and plant growth in black pepper with single inoculation of AMF and antagonists (Anandraj *et al*., 1994b; Anandaraj *et al*., 1996).

AMF isolates Pi-9 and *G. margarita* consistently recorded higher colonization in dual inoculation system over single inoculation in the field. However, this was not reflected on disease tolerance and plant growth characteristics indicating the ineffective nature of these AMF cultures. On the contrary, isolate Pi-11, Is-6 and *G. fasciculatum* with higher colonization showed better plant growth (Table 27) and disease suppression (Table 29). In the field study the A_1xPi-11 and A_{21} x Is-6 combinations were as good as the present recommended chemical control methods using bordeaux mixture and copper oxychloride (KAU, 1996).

The selected inoculants were identified on the basis of their morphological characters. AMF isolates Is-6 and Pi-11 were identified as *Glomus* spp. while the antagonists A_1, A_{13}, A_{21}, A_{22} and A_{35} as *Aspergillus fumigatus*, *Fusarium oxysporum*, *Aspergillus sydowii*, *Trichoderma viride* and *Gliomastix murorum* respectively, by Agharkar Research Institute, Pune (Maharashtra).

Although the pepper plants in the nursery and field can be inoculated with AMF cultures at the time of planting, there is no viable technique hitherto suggested to inoculate AMF to the root zone of already established pepper vines. The carrier plant based inoculation method followed in the present study was found successful. Inoculation through green gram recorded maximum AMF colonization (71.66 to 88.34%) followed by sorghum (33.33 to 83.33%) in pot studies (Fig. 11). Higher AMF colonization could be related with higher root mass of the carrier plants, as higher volume of root with intense colonization will introduce more AMF propagules into the rhizosphere (Sreenivasa and Bagyaraj, 1988). The coherent net work between roots of carrier plant and pepper cutting may also favour higher root colonization (Table 32). The present observation is in agreement with the report that the AMF spread from root to root of different plant species and transfer the nutrient (Barea, 1992). The variation observed in the colonization per cent of black pepper is probably due to difference in the intensity of

root colonization and root development of carrier plant. The colonization acquired through carrier plant has positively influenced the growth and biomass production of black pepper (Fig. 12).

In established pepper plantations sorghum was more effective than green gram with the colonization per cent of 78.33 and 66.66 per cent for sorghum and green gram, respectively (Table 34). The control had the colonization per cent of 51.66 to 53.33 per cent due to resident AMF. The better colonization through sorghum could be attributed to higher root length and mass with intense colonization than that recorded for green gram under field condition (Fig. 13). The higher root length and spread ensures better penetration of sorghum roots deep into the root zone of pepper and introduce higher amount of AMF propagules. On the contrary green gram root with less length and spread may fail to reach the root zone of black pepper. The conventional *in situ* inoculation around the plant basin did not show much effect and the colonization remained as low as that of uninoculated control (53%). This novel technology of AMF inoculation using sorghum as carrier plant is viable and can be practiced by farmers for inoculating the established plantations of pepper vines and other perennial crops with desirable AMF.

Irrespective of age of plants, the disease occurs in the existing pepper plantations and result in the serious economic loss (Balakrishnan *et al*., 1986; Anandaraj *et al*., 1989). The inoculants selected were further tested in an eight year old pepper plantation with earlier record of foot rot incidence. Foliar yellowing and defoliation are the most prominent and persistent symptom of foot rot in established plantations (Abraham *et al*., 1995). Hence the per cent foliar yellowing and defoliation index were computed for assessing the performance of the inoculants AMF and antagonist inoculation during pre, mid and post monsoon period yielded encouraging results. Of the different combinations evaluated Pi-11 x *Aspergillus sydowii*, *G. fasiculatum* x *T. viride* and Is-6 with *T. viride* and *A. sydowii* remarkably reduced the foot rot disease and recorded with mean disease score of 0.0, 1.5, 2.0 and 2.0 respectively, as against 7.0, 3.5 and 3.0 recorded for control, bordeaux mixture and copper oxychloride, respectively. As observed in the field trial AMF Pi-11, *G. fasciculatum*, Is-6 and antagonist, *A. sydowii* offered better resistance to disease (Fig. 14). It was also noticed that *Trichoderma viride*, which was less effective in the field, in combination with Is-6 and *G. fasciculatum* considerably suppressed disease with the mean disease score of less than that recorded for copper oxychloride and bordeaux mixture. The effect could be attributed to high multiplication and activity of *T. viride* ensured by cowdung neem cake food base (Fig. 15).

Analysis of the population of antagonists and soil fungi including pathogen showed that the resident soil fungal population was significantly reduced with application of antagonists (Table 36, Fig. 15). This indicates the inhibitory effect of antagonists on deleterious soil fungi including *P. capsici*. The antagonistic effect was further augmented with AMF and the dual inoculation reduced the rhizosphere fungal population to less than $6x10^3$. The influence of AMF on antagonistic population was not so evident; perhaps the effect was indistinguishable because of fast multiplication of the antagonists in the presence of cowdung-neem cake food base which is known for its stimulatory effect on multiplication and activity of species of *Trichoderma* and *Aspergillus* (Sivaprasad *et al.*, 1998). The stimulatory effect of the food base on the rhizosphere fungal population was evident from the increase in the population from $14.8x10^3$ observed in control to 33.86×10^3 in food base applied in pepper basins.

With regard to AMF colonization, irrespective of antagonist inoculation, all the established vines showed higher colonization over control (Table 37). However the colonization

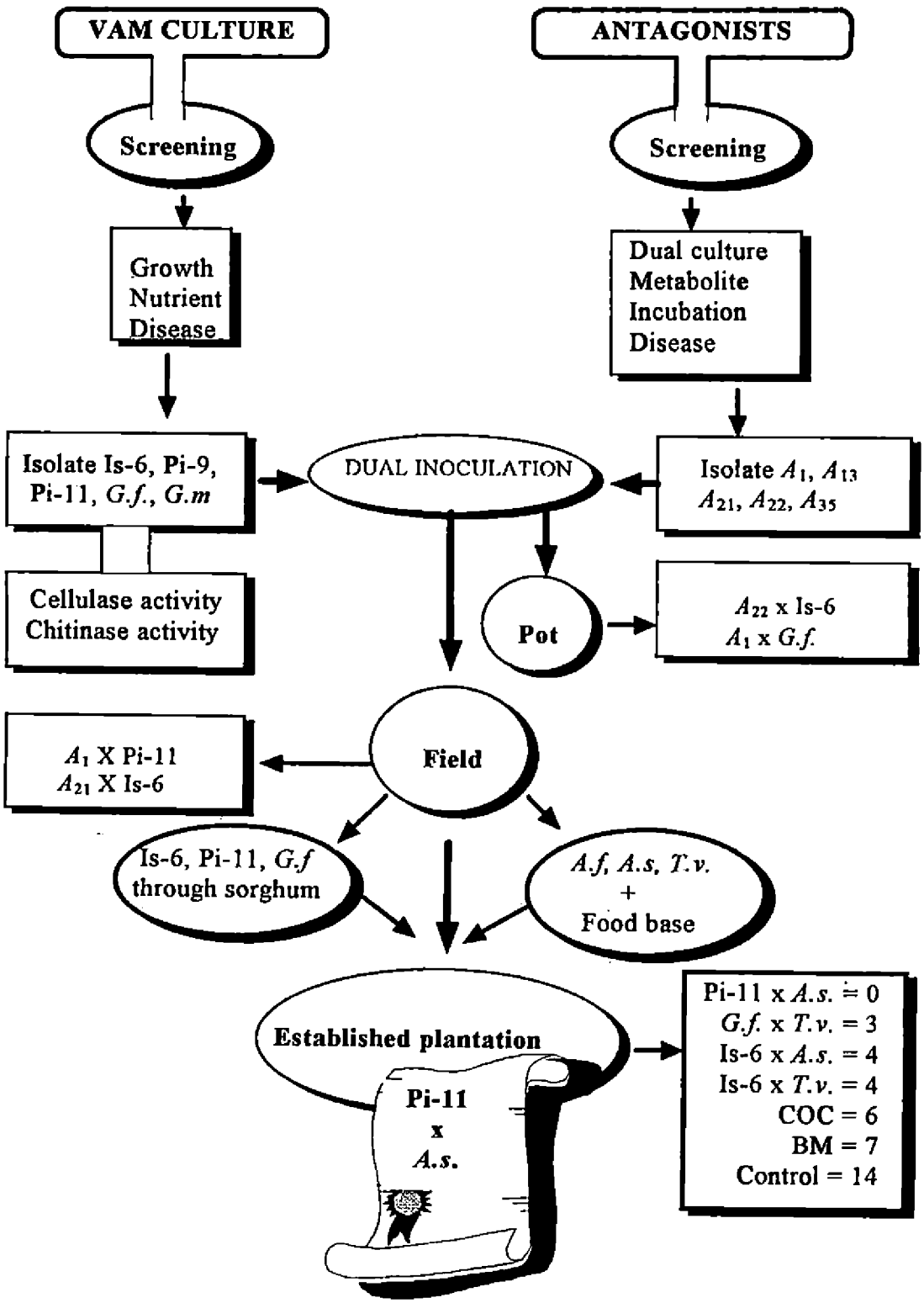


Fig. 18 Summary diagram of biocontrol strategy developed for foot rot of black pepper

was relatively low with a maximum of 73.51 per cent in Pi-11 x *A. sydowii* as against 46.65 per cent observed at the time of AMF inoculation.

The series of experiments conducted under the investigation vividly established the potential of combining AMF and antagonists in the biocontrol strategy for the management of foot rot of black pepper (Fig. 18). The combinations of Pi-11 x *A. sydowii*, *G. fasciculatum* x *T. viride* and Is-6 x *T. viride* or *A. sydowii* can be recommended for farmers level trial.

Amino acids have a role in the synthesis of proteins some of which are essential for the synthesis of phenolics and other molecules involved in the development of plant disease resistance (Harborne, 1964; Emmanouil and Wood, 1981). Analysis of free amino nitrogen content of plant tissue indicated remarkably high concentration in plant top due to AMF coloization (Table 38), while, it was consistently lower in roots. The decrease in total free amino acid concentration in AMF inoculated pepper roots may be attributed to assimilation by AMF (Sivaprasad, 1983). The higher amino acid content on plant top may be due to increased metabolic process in the host as a result of better nutrition and physiological traits conferred by AMF. Increase in free amino acid concentration in plants consequent to invasion by pathogen has also been

reported (Rohringer, 1957). However, the specific role of such a high levels of free aminonitrogen in the plant defense against the pathogen needs elaboration.

The total and reducing sugar content in plant top was consistently less with AMF colonization, while it was considerably higher in mycorrhizal roots (Table 39). This may be due to higher potassium content which leads to better transportation of simple sugars from plant top to root (Black, 1968). AMF induced higher concentration of sugar is considered responsible for the inhibition of toxin producing ability of the pathogen (Davis, 1970). Similarly, the relatively high protein content observed in the root tissue might have some role in inducing the disease resistance as it is reported that proteins are essential for the synthesis of phenolics and other molecules involved in the plant defense mechanism (Emmanouil and Wood 1981).

Accumulation of phenols are considered as passive or active defense response in plants (Nicholson, 1992). The phenol content in mycorrhizal plant tissue is known to increase during the initial stage and decline in the later phase of AMF colonization (Krishna and Bagyaraj, 1986). In the present investigation the total and orthodihydroxy (OD) phenol content in plant top was less on 150th day of AMF inoculation in comparison to that recorded for control (Table 40). This is

in agreement with the view that phenol concentration in mycorrhizal plants decreases after initial phase of clonization (Morandi *et al*. 1984). However, the total phenol content in root was higher with Is-6 and *G. fasciculatum* while the OD phenols did not show much variation. The observation indicates that the disease suppression observed with AMF isolates in black pepper was not specifically related to the change in the phenol content. Further, the low phenol content may be related to the suppression of host defense reaction for the induction of AMF symbiotic system (Blee and Anderson, 1996) which would further promote more responsive defense reaction against root pathogen (Azcon-Aguilar and Barea, 1997).

The activity of hydrolytic enzymes such as cellulase and chitinase were generally high in AMF colonised plant tissue (Table 41). However, there was much variation among the AMF cultures. Except *G. margarita* all the cultures recorded significantly higher cellulase activity in plant top, while, in the root the activity recorded for Is-6 and Pi-11 was on par with control (Fig. 16). The higher cellulase activity has much relevance with the biocontrol of *Phytophthora* as cellulose is a major constituent of the cell wall of the pathogen (Kinghorn *et al*., 1991). The relatively less activity recorded in the roots of Is-6 and Pi-11 inoculated plants in contrast to the very high activity noticed in the plant top may be attributed to the pre- occupation of enzymes on substrate in the pathogen.

This view becomes more relevant in the context that these isolates effectively suppressed the pathogen development and foot rot incidence. With regard to the chitinase activity it could be noticed that the AMF isolates (Is-6 and Pi-11) that effectively suppressed the disease had induced remarkably higher enzyme activity both in plant top and root (Fig. 17). However, this cannot be related to the success in biological control of foot rot disease, as chitin is not a major constituent of *Phytophthora* cell wall.

Mycorrhizal roots exhibit high chitinolytic activity and it is thought that the enzyme is produced by the host for the digestion of arbuscules (Dehne, 1977; Priestel, 1980). The higher levels of chitinase activity recorded with AMF isolates emphasises the possibility of utilising these isolates against other fungal pathogens having chitin as a major cell wall constituent in future investigations. Lytic enzymes that hydrolyse the polymers of fungal cell wall are known to be synthesized in plant tissue as a defence response to invasion by parasitic organisms (Mauch *et al.*, 1988). Mycorrhizal colonization also trigger the production of lytic enzymes such as chitinase and beta 1-3-D-gluconase (Spanu *et al.*, 1989; Laimbais and Mehdy, 1993) which are involved in the host defence mechanism. Hence, the high activity of defence related proteins recorded might have also contributed in inducing the disease resistance.

SUMMARY

SUMMARY

The present investigation entitled 'Management of foot rot of black pepper with VA mycorrhiza and antagonists' was conducted at College of Agriculture, Vellayani during 1994-97. The study was envisaged to develop a biocontrol strategy using native Arbuscular Mycorrhizal Fungi (AMF) and fungal antagonists for the foot rot of black pepper in the nursery and field.

The causal organism from different pepper growing tracts of Idukki, Wayanad and Thiruvananthapuram districts of Kerala was isolated, purified and based on morphological studies, identified as *Phytophthora capsici* Leonian emend A. Alizadeh and P.H. Tsao. The growth rate and virulence varied with isolate. The isolate obtained from Peringammala, Thiruvananthapuram district, showed maximum mycelial production on Bartnicki garcia's liquid medium and exhibited maximum virulence amongst the isolates in rooted pepper cuttings. This isolate was selected for further studies under the investigation.

Seven native AMF isolates, viz., Is-6, Pi-6, Pi-8, Ri-8, Pi-9, Ri-9, Pi-11 and three identified cultures such as *Glomus clarum*, *G. fasciculatum* and *Gigaspora margarita*) were evaluated for their effect on plant growth, nutrient uptake and

foot rot suppression in black pepper. The AMF cultures Pi-11, *Gigaspora margarita*, Pi-9, Is-6 and *G. fasciculatum* were very effective in enhancing plant growth and biomass production under green house condition. Analysis of the nutrient status indicated a nutritional advantage to black pepper due to AMF colonization. The effect was more pronounced with isolates Pi-11, Pi-9, Is-6 and *G. fasciculatum* which recorded significantly higher concentrations of P, K, Ca, Mg, Cu, Fe, Mn and Zn in plant tissue.

The AMF isolates Is-6, Pi-11 and *G. fasciculatum* exhibited significant effect in suppressing the foot rot disease. *Glomus fasciculatum* recorded lowest plant mortality (53.35%) on 90th day of inoculation with the pathogen followed by Is-6 (60.64%) and Pi-11 (60.64%). Comparatively low root damage was also recorded with these isolates. Although *Gigaspora margarita* exhibited higher root colonization (84.14%), it failed to protect the plants against the root damage and subsequent symptom development. It was generally observed that growth stimulation and foot rot repression due to AMF inoculation are two independent traits. However, isolate Pi-11 was an exception. Based on growth stimulation and foot rot suppression ability AMF isolates Is-6, Pi-9, Pi-11, *G. fasciculatum* and *G. margarita* were selected for further studies.

Studies on the AMF colonization in black pepper cultivars grown in different soils showed high variation among the soil types irrespective of host genotype. Maximum root colonization was evident in sandy (oxyaquic quartpsamment) soils. Laterite (typic plinthustult) soils exhibited medium colonization, while lowest colonization was noticed in nutrient rich forest ((haplic argiustoll) soils. Soil types showed a definite influence on AMF species harboured by pepper plants rather than host genotype. Species of *Glomus* and *Sclerocystis*, particularly the *G. fasciculatum*, were more common in the rhizosphere of black pepper. However, *Acaulospora* spp. and *Gigaspora* spp were also frequently observed in sandy soils.

Fifty fungal antagonists isolated from different sources were evaluated for their antagonistic property against *P. capsici in vitro*. Based on their ability to suppress *P. capsici* either through mycoparasitism, antibiosis and soil fungistasis, 24 isolates were selected for green house studies. Amongst the antagonists tested in green house, isolates A_{19} and A_{21} considerably enhanced plant growth. While, isolates A_1, A_{13}, A_{21}, A_{22}, and A_{35} were more effective in foot rot disease suppression. The result demonstrated that the plant growth stimulation and disease suppression traits are not interdependent. Isolates A_1, A_{13}, A_{21}, A_{22}, and A_{35} showed comparatively better population build up in the soil and

suppressed the *P. capsici* population considerably. However, reduction in the antagonists population due to seasonal variation was noticed.

The promising cultures of AMF (Is-6, Pi-9, Pi-11, *G. fasciculatum* and *G. margarita*) and fungal antagonists (A_1 A_{13}, A_{21}, A_{22}, and A_{35}) were further subjected for dual inoculation studies in green house and field. In green house the dual inoculation of *G. fasciculatum* with fungal antagonists A_1 or A_{21} significantly enhanced the plant height to 98.33 and 103.33 cm respectively. However, the interaction between *G. margarita* and A_1 was inhibitory (36.67 cm) compared to their individual effect. In general higher mycorrhizal colonization was noticed with synergistic combination of AMF and antagonists. However, the population of fungal antagonists was not evidently influenced by the interactions.

With regard to foot rot incidence, the dual inoculation of A_{22} x Is-6, A_1 x *G. fasciculatum* was very effective. A_{22} x Is-6 recorded lowest plant mortality of 32.90 per cent. The effective combinations also had higher antagonistic population and mycorrhizal development. The disease suppression achieved by dual inoculation was comparable with that of bordeaux mixture and copper oxychloride application.

The AMF and antagonists interactions also showed desirable effect on plant growth and foot root incidence in the field. Combination of A_{21} x Is-6 and A_1 x Pi-11 were highly effective in plant growth stimulation as well as disease suppression. The lowest foot rot infection of 58.45 per cent was recorded for A_1 x P-11 and A_{21} xIs-6 which was lower than that achieved by copper oxychloride treatment (59.68%). Regarding plant mortality, both the combination recorded less than 60 per cent as against 95.66 per cent recorded for control. The bordeaux mixture and copper oxychloride recorded 66.67 and 59.68 per cent mortality respectively. The AMF colonization and multiplication of the antagonists were also favoured by the dual inoculation.

The potential AMF isolates Is-6 and Pi-11 were identified as species of *Glomus* while, the antagonistic isolates A_1, A_{13}, A_{21}, A_{22}, and A_{35} were confirmed as *Aspergillus fumigatus* Fres., *Fusarium oxysporum* Schlecht. ex Fr. *Aspergillus sydowii* (Bain. & Sart.) Thom. & Church, *Trichoderma viride* Pers. ex Gray. and *Gliomastix murorum* (Corda) Hughes respectively.

The study was also directed towards developing AMF inoculation technique for established pepper vines using 'carrier plants'. Among the four 'carrier plants', viz., cowpea (*Vigna unguiculata* (L) Walp), green gram (*Vigna radiata* (L) Wilezek), Italian millet (*Setaria italica* (L) Beauv) and

sorghum (*Sorghum bicolor* (L.) Moench tested in green house, intense mycorrhizal colonization in pepper roots was achieved with green gram and sorghum. Increased growth and biomass production of black pepper was also noticed with higher colonization. In the field sorghum was most effective in imparting AMF colonization to pepper roots. Sorghum plants raised around the eight year old pepper vines with mixed AMF inocula (*G. fasciculatum* + Pi-11) resulted in 78.33 per cent colonization in black pepper roots as against 53.33 per cent colonization recorded without 'carrier plants' in control. The method was found successful to achieve AMF colonization by introduced AMF in pepper vines of established plantation.

Based on the performance in the green house and field AMF cultures Pi-11, Is-6 and *G. fasciculatum* and antagonists *Aspergillus fumigatus*, *A. sydowii* and *Trichoderma viride* were selected for further testing in established pepper plantation. The study was conducted in eight year old pepper vines. Antagonists were multiplied in cowdung neem cake food base prior to field application. Dual inoculation of Pi-11 x *A. sydowii*, *G. fasciculatum* x *T. viride*, Is-6 x *A. sydowii* and Is-6x*T. viride* were identified as potential combinations for foot rot management in established plantation. Foliar yellowing and defoliation score recorded for these treatments were considerably less than that recorded for bordeaux mixture (3 : 3) and copper oxychloride (3.0) application. The treatment

Pi-11 x *A. sydowii* was most effective and did not show any disease symptom which was followed by Is-6 x *T. viride* or *A. sydowii* with disease score of 2.0 as against 7.0 recorded for control. Irrespective of AMF and antagonists combinations, all the treatments showed significantly higher population build up by the antagonists. The neem cake - cowdung food base was found highly favourable for the multiplication and activity of the antagonists in the field. AMF colonization in pepper roots acquired through the 'carrier plant' based inoculation was appreciable.

Higher amino acid content was observed in AMF colonized plant top while the concentration was low in roots. Mycorrhizal roots showed higher total sugar and reducing sugar content. While, in plant top it was consistently less. Relatively high protein and total phenolic content recorded in root tissue particularly on inoculation with isolate Is-6 and *G. fasciculatum*. However, the orthodihydroxy phenol concentration in AMF inoculated plants did not show significant variation with that of uninoculated control. The cellulase and chitinase activity were generally high in AMF inoculated plant tissue. AMF isolate Is-6 recorded higher cellulase activity of 0.527 ug in plant top and 0.103 ug glucose released min^{-1} mg^{-1} protein in root. The chitinase activity in the root was maximum with Pi-11 (76.23 nM) followed

by Is-6 (67.43 nM). The control plants had 43.98 nM N-acetyl glucosamine h^{-1} g^{-1} activity in the root. The change in biochemical constituents in black pepper brought about by potential native AMF isolates Is-6 and Pi-11 were found to be effective in inducing resistance against the disease.

In the present investigation the combinations of native isolates of AMF and Antagonists, viz., Pi-11x*A. sydowii* and Is-6 with *T. viride* or *A. sydowii* were found very effective for the repression of foot rot disease in nursery and field. The disease control achieved by the inoculants was better than that recorded for bordeaux mixture and copper oxychloride. The present study established the desirability of selecting the potential native AMF and antagonists for foot rot disease management and growth improvement.

The extensive studies conducted to develop desirable combinations of native AMF and antagonists and their field testings against foot rot disease and AMF inoculation technique for established pepper vines are first records in this area of research.

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* Originals not seen

APPENDICES

APPENDIX - I

Composition of modified Tsao's medium

Oats meal	-	60 g
Benomyl (substitute for pimaricin)	-	10 ppm
Ampicillin (substitute for vancomycin)	-	200 ppm
PCNB	-	100 ppm
Rosebengal	-	40 mg
Agar	-	15 g
Distilled water	-	1000 ml

APPENDIX - II

Composition of *Trichoderma* selective medium

$MgSO_4.7H_2O$	-	0.2 g
KH_2PO_4	-	0.9 g
NH_4NO_3	-	1.0 g
KCl	-	0.15g
Glucose	-	0.3 g
Dexon	-	0.3 g
PCNB	-	0.2 g
Rose Bengal	-	0.15g
Chloramphenicol	-	0.25g
Agar agar	-	20.0 g
Water	-	1000 ml

MANAGEMENT OF FOOT ROT OF BLACK PEPPER (*Piper nigrum* L.) WITH VA MYCORRHIZA AND ANTAGONISTS

By

CHRISTIN P. ROBERT

ABSTRACT OF THESIS
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ABSTRACT

Extensive investigation was carried out to develop a native microbial inoculant based technology involving Arbuscular Mycorrhizal Fungi (AMF) and fungal antagonists for the foot rot disease management and growth improvement of black pepper in the nursery and field.

Phytophthora capsici Leonian emend A. Alizadeh and P.H. Tsao, the foot rot pathogen isolated from Peringammala, Thiruvananthapuram district was found most virulent isolate.

Seven native AMF cultures and fifty fungal antagonists were isolated from Kerala soils. AMF isolates were screened in the green house for plant growth improvement and disease tolerance in comparison with identified species-*Glomus fasciculatum*, *G. clarum* and *Gigaspora margarita*. Of the ten AMF tested isolates Is-6, Pi-11, Pi-9, *G. fasciculatum* and *Gigaspora margarita* were very effective in stimulating growth and nutrient (P, K, Ca, Mg, Cu, Fe, Mn and Zn) uptake of black pepper. Regarding the ability of AMF in reducing the foot rot incidence, *Glomus fasciculatum* recorded the lowest plant mortality and root rot index (53.35% and 62.50%) followed by Is-6 (60.00% and 64.77%) and Pi-11 (60.64% and 68.18%) as against 100 per cent mortality and 98.60 per cent root rot index noticed in control. The above five cultures were subjected for further studies.

Characterisation of AMF associated with different genotypes of black pepper grown in various soil types indicated the definite influence of soil type on AMF colonization. Sandy soil (oxyaquic quartpsamment) harboured maximum root colonization while forest soil (haplic argiustoll) had the lowest. Species of *Glomus* particularly *G. fasciculatum* was the predominant AMF associated with black pepper irrespective of soil type. As an exception *Acaulospora* and *Gigaspora* species were frequently noticed in sandy soils.

Based on the ability of the fungal antagonists to suppress *P. capsici in vitro* either through mycoparasitism, antibiosis or soil fungistasis, 24 isolates were selected for green house studies. In the further testing isolates A_1, A_{13}, A_{21}, A_{22} and A_{35} significantly reduced the foot rot infection and increased the plant growth. They showed better population build up in the soil and suppressed the *P. capsici* population considerably. These native antagonists were further tested in combination with selected AMF in the green house and field.

Under green house condition, combination of *G. fasciculatum* x A_1 or A_{21} showed significant influence on growth stimulation, while Is-6 x A_{22} recorded lowest mortality of 32.90 per cent due to foot rot incidence as against 97 per cent in control. The dual inoculation of Is-6 x A_{21} and Pi-11 x A_1 was highly effective in plant growth stimulation and disease suppression. Both the combination recorded less than

60 per cent infection and mortality due to the disease, while control showed 95.66 per cent infection and plant mortality. Bordeaux mixture and copper oxychloride recorded 66.67 and 59.68 per cent mortality respectively. AMF colonization and multiplication of antagonists were also favoured by dual inoculation.

The potential AMF isolates Is-6 and Pi-11 were identified as species of *Glomus* while, the antagonistic isolates A_1, A_{13}, A_{21}, A_{22}, and A_{35} were confirmed as *Aspergillus fumigatus* Fres., *Fusarium oxysporum* Schlecht. ex Fr. *Aspergillus sydowii* (Bain. & Sart.) Thom. & Church, *Trichoderma viride* Pers. ex Gray. and *Gliomastix murorum* (Corda) Hughes respectively.

A technique for AMF inoculation to established pepper vines was developed using 'carrier plants'. Raising sorghum with AMF inoculation around the pepper vines was found effective to achieve intense colonization in pepper roots by the introduced AMF in the field. This technique developed for the pepper vines may be tried for extending to other perennial crops for AMF inoculation.

Promising AMF cultures Pi-11, Is-6, *G. fasciculatum* and antagonists *Aspergillus fumigatus*, *A. sydowii*, *Trichoderma viride* were further tested on eight year old established pepper vines following 'carrier plant' based AMF inoculation and cowdung-neem cake based antagonist inoculation. The treatment

Pi-11 x *A. sydowii* was most effective with no symptom development, followed by Is-6 x *T. viride* or *A. sydowii* with disease score of 2.0 as against 7.0 recorded for control. The disease score for bordeaux mixture and copper oxychloride application was 3.5 and 3 respectively. Neem cake-cowdung food base was highly favourable for multiplication and activity of fungal antagonists.

The amino acids, total sugar and reducing sugar and total phenols and orthodihydroxy phenol content and activity of cellulase and chitinase were influenced by AMF colonization particularly by Is-6 and Pi-11. The positive change could be related with the relative disease tolerance recorded for various AMF isolates.

The development of native AMF and antagonists through extensive testing in the green house and field and also the technology of AMF inoculation for established pepper vines are the first record of work.