MANAGEMENT OF RHIZOME ROT AND ROOT-KNOT OF GINGER (*Zingiber officinale* R.) USING V. A. MYCORRHIZAL FUNGI AND ANTAGONISTS

By

P. J. JOSEPH

THESIS
SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE
DOCTOR OF PHILOSOPHY
FACULTY OF AGRICULTURE
KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI
THIRUVANANTHAPURAM

1997

DECLARATION

I hereby declare that this thesis entitled "Management of rhizome rot and root-knot of ginger (*Zingiber officinale* R.) using V. A. Mycorrhizal fungi 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.

Vellayani
Date :

P. J. JOSEPH

CERTIFICATE

Certified that this thesis "Management of rhizome rot and root-knot of ginger (*Zingiber officinale* R.) using V. A. Mycorrhizal fungi and antagonists" is a record of research work done independently by Mr. P. J. JOSEPH (92-21-13) 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.

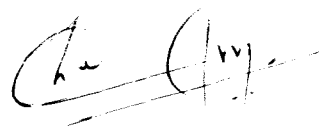
Dr. P. SIVAPRASAD,
Chairman, Advisory Committee,
Assoc. Professor of Agrl. Microbiology,
Department of Plant Pathology,
Kerala Agricultural University,
Thiruvananthapuram

Vellayani
Date :

APPROVED BY:

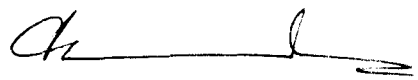
CHAIRMAN

Dr. P. SIVAPRASAD



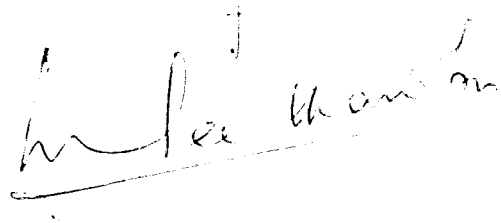
MEMBERS

1. Dr. P. KARUNAKARAN



2. Dr. S. RAMACHANDRAN NAIR

3. Dr. C. K. PEETHAMBARAN



4. Dr. M.S. SHEELA

EXTERNAL EXAMINER

ACKNOWLEDGEMENT

I recall with deep sense of gratitude my fervent indebtedness to the following persons / institutions in making this investigation fruitful.

I am most grateful to Dr. P. Sivaprasad, Associate Professor of Agrl. Microbiology, Chairman, Advisory Committee, for his inspiring guidance, constant encouragement and sustained interest evinced during the entire course of this investigation and for his untiring suggestions and help in the preparation of the thesis.

My profound gratitude is placed on record to Dr. P. Karunakaran, Professor and Head, Department of Plant Pathology, Dr. S. Ramachandran Nair, Professor and Head, Department of Horticulture, Dr. C. K. Peethambaran, Associate Professor of Plant Pathology and Dr. M. S. Sheela, Associate Professor of Agrl. Entomology as members of the Advisory Committee for the encouragement extended during the course of the study and pertinent suggestions in the preparation of the thesis.

I am extremely thankful to Dr. S. Balakrishnan, Professor of Plant Pathology for his valuable suggestions, critical comments and constructive perusal of the manuscript.

I wish to express my sincere gratitude and obligation to Dr. P. B. Gopinath, Professor of Agrl. Entomology and Dr. Arthur Jacob. J., Associate Professor of Entomology for the close association, unfailing interest, timely help and moral support extended to me during the study.

I thankfully acknowledge the timely help received from Dr. C. Gokulapalan, Associate Professor of Plant Pathology,

Dr. A. Nazeema, Associate Professor of Plant Pathology, Dr. P. Rajendran, Associate Professor of Soil Science and Agri. Chemistry and Dr. Thomas Biju Mathew, Asst. Professor of Agrl. Entomology during the course of the investigation.

I accost my sincere thanks to Sri. C. P. Robert, Ph.D. Scholar, Smt. Lekha K. S., Research Associate and Sarita V. Elizabeth, M.Sc. (Ag) student for the help rendered at one or other time of the study.

My colleagues, P. G. Students and non-teaching staff in the Department of Plant Pathology have been very helpful during the course of this study, their goodwill and support are gratefully acknowledged.

I acknowledge the services rendered by Shri. C. E. Ajith Kumar, Junior Programmer , Dept. of Agrl. Statistics during the statistical analysis.

My sincere thanks are due to Shri. K. Chandhrakumar, for neat type-setting of the manuscript and ARDRA Computers for the documentation of figures.

I am grateful to the Department of Biotechnology, Government of India for funding the project and providing Research Fellowship and to Kerala Agricultural University for the facilities provided for the study.

My thanks are also due to many others who have been helpful to me sometime or other during the period of study.

P. J. JOSEPH

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS USED

Ai	-	Antagonistic isolate
ai	-	active ingredient
AMF	-	Arbuscular mycorrhizal fungi
Ca	-	Calcium
CD	-	Critical Difference
cfu	-	colony forming units
cm	-	centimeter
CRD	-	Completely Randomized Design
Cu	-	Copper
DAP	-	Days after planting
Fe	-	Iron
g	-	gram
ha	-	hectare
K	-	Potassium
kg	-	kilogram
Mg	-	Magnesium
Mi	-	Mycorrhizal isolate
Mn	-	Manganese
µm	-	micrometer
P	-	Phosphorus
Zn	-	Zinc

INTRODUCTION

1. INTRODUCTION

Ginger (*Zingiber officinale* R.) is an important spice crop of India. Other countries cultivating ginger are China, Jamaica, Nigeria, Sierra Leone, Thailand and Australia. India is the largest producer and exporter of dry ginger of commerce. It is exported mostly to Europe, America and East African countries. In India, ginger is cultivated in 62009 hectares with a production of 1,86,050 tonnes of dry ginger (Directorate of Economics and Statistics, 1996). A major portion of it is consumed domestically in culinary preparations, extraction of oleoresins and for medicinal purposes. Kerala accounts for about 30-34 per cent of total production in India. It is largely cultivated as a commercial crop along the Western Ghats and is also an important component of the homestead gardens of Kerala.

Although ginger is affected by a number of diseases (Sharma and Jain, 1977), rhizome rot/soft rot incited by *Pythium aphanidermatum* (Edson)Fitz. (Mitra and Subramonian, 1928) has been identified as the major production constraint in all ginger growing tracts of Kerala (Iyer, 1987; Sarma, 1994) causing heavy economic loss ranging from 50-80 per cent (Butler, 1918; Joshi and Sharma, 1982). The pathogen is soilborne and inoculum present in the soil serves as the primary source of infection (Sarma, 1994). Further, frequent

infestation of ginger by *Meloidogyne incognita* is reported to favour the incidence of the disease (Dohroo *et al.*, 1987).

The present management practices to tackle the disease mainly focus on applying systemic and non-systemic fungicides. However, its constant use, which brings about serious environmental and ecological problems, is being viewed seriously the world over. Besides, ginger being an export oriented crop, the residual toxicity and connected problems are matters of major concern. The increasing awareness about the environmental consequences of fungicide applications and the growing interest for pesticide free agricultural products prompted scientists to explore alternate strategies of disease management. Many success stories of biological control, inconsistent performance of pesticides and the prohibitive investment associated with finding new pesticides further accelerated the interest in biological control. Biological control of plant pathogens by microorgranisms and induced systemic resistance in plants are two most talked about strategies. 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 is attempted.

The study on the AMF (VAMF) association in plants is receiving considerable attention in recent years as mycorrhizal

associations enable better plant growth by higher uptake of nutrients (Harley and Smith, 1983) and reduce infection by soilborne plant pathogens (Dehne, 1982; Azcon-Aguilar and Barea, 1996). Encouraging results have been obtained on the potential value of AMF in promoting plant disease resistance against important plant pathogenic fungi including *Pythium* spp. (Hwang *et al.*, 1993; Sivaprasad, 1993). The mechanism of mycorrhiza induced tolerance in plants is explained as mainly due to physiological and biochemical changes in the host (Gianinazzi-Pearson *et al.*, 1996) and microbial changes in the mycorrhizosphere (Azcon-Aguilar and Bargo, 1994). The function of AMF hyphae is analogous to plant root hair and helps the plant to acquire less mobile soil nutrients such as phosphorus, zinc and calcium. The desirable attributes of AMF on plant growth and health make them potential candidates for biocontrol and as useful biofertilizers. However, except the preliminary report, (Sivaprasad, 1993), no serious attempt was made to exploit the potential of AMF on rhizome rot management and growth improvement of ginger.

Since rhizome rot of ginger is soilborne, the resident flora may play a major role in keeping the population of the pathogen at low level in the natural system. Many potential mycoparasites with antibiosis property such as species of *Trichoderma*, *Gliocladium* and *Aspergillus* have been successfully employed for the biocontrol of soilborne plant

pathogens (Papavizas, 1985; Migheli *et al.*, 1993). It is reported that *Trichoderma spp.* can be effectively used as biocontrol agents for the management of rhizome rot of ginger (Usman *et al.*, 1996).

It has been aruged that native microbial inoculants adapted well to the existing environmental conditions, will be more competetive and exhibit better multiplication, persistance and activity in the soil than the introduced antagonists (Hadar *et al.*, 1984). Hence antagonists developed from native soil are more desirable for biocontrol.

The microbial interactions in the mycorrhizosphere are stimulatory (Clavet *et al.*, 1992) or inhibitory (McAllister *et al.*, 1994a,b). Instances of reports of positive intraction of AMF with antagonistic fungi and their cumulative effect on plant growth and disease suppression are increasing (Calvet *et al.*, 1993., Kumar *et al.*, 1993). However, no work has been done in this direction for the management of rhizome rot of ginger. The present investigation is designed to develop a sustainable management strategy of rhizome rot of ginger using native AMF and antagonists with major thrust on the following aspects:

* Identify AMF species able to suppress rhizome rot of ginger

* Develop and test native AMF against rhizome rot in comparison with recognised species

* Characterisation of native AMF associated with ginger genotypes in different soils

* Isolate and develop native antagonistic fungi capable of inhibiting *P. aphanidermatum*

* Test the combined use of selected AMF and antagonists in suppressing rhizome rot of ginger in green house and field

* Evolve a suitable AMF inoculation technique for rapid host colonization

* Test the effect of plant protection chemicals on AMF and antagonists

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Ginger (*Zingiber officinale* R.) is one of the most important seasonal spice crops of India. It is affected by several diseases of which rhizome rot/soft rot is the most devastating one (Joshi and Sharma, 1982; Iyer, 1987; Sarma, 1994). Several species of *Pythium* have been implicated as the causal organism of the disease. However, *Pythium aphanidermatum* (Edson) Fitz. (Mitra and Subramonian, 1928) has been found to be the most predominant species causing rhizome rot in Kerala (Joshi and Sharma, 1982; RAU, 1993; Sarma, 1994). Varying degrees of association of *Meloidogyne incognita* was noticed in the root system of ginger (RAU, 1993) and increased rhizome rot incidence was reported in association with *M. incognita* (Dohroo *et al.*, 1987).

Soil application of neem cake (Sadananthan and Iyer, 1986), fungicidal seed treatment (Dake *et al.*, 1989) and soil drenching are the recommended practices for the disease control. However, these methods are not widely practiced due to economic considerations, residue problems and practical difficulties whereas biological control is simple, inexpensive and ecofriendly method for disease management. In the present investigation attempts were made to evolve a biocontrol management strategy of rhizome rot using native arbuscular mycorrhizal fungi (AMF) and antagonists.

2.1 Arbuscular mycorrhizae and plant growth

The term 'mycorrhiza', which means 'fungus root' was first coined more than a century ago by Frank (1885), who suggested mycorrhizal fungus absorbs water and nutrients from the soil and translocates it to the tree which in turn supplies simple carbohydrates to the fungus. However, the real significance of the symbiotic association in plant growth and health was recognised only after 1950's. It has been accepted that mycorrhizal associations are so prevalent that the non-mycorrhizal plant is an exception rather than the rule. Mycorrhizae are broadly grouped into ectomycorrhizae, endomycorrhizae and ectendomycorrhizae. Among them endomycorrhizae are more predominant and economically important in cultivated crops. In endomycorrhizae, arbuscular mycorrhizae are often referred to as "universal plant symbiont" and are exceptionally common among terrestrial plants. It has been estimated that about 90 per cent of approximately 231,000 species of angiosperms form this symbiosis despite there being only approximately 120 described species of arbuscular mycorrhizal fungi (Koide and Schreiner, 1992).

Arbuscular mycorrhizal fungal (AMF) associations in plants are formed by a group of Zygomycetous fungi belonging to the order Glomales (Morton and Benny, 1990., Rosendahl *et al.*, 1994). They form chlamydospores or azygospores in

the rhizosphere, rhizoplane and sometimes in the feeder root tissues. The fungal hyphae invade only the root cortex leaving the apical meristem and vascular cylinder fungus free.

The beneficial effect of AMF associations in the host nutrition especially in the uptake of 'P' is well documented (Gerdemann 1968, Mosse, 1973, Harley and Smith, 1983). Mycorrhizal infection enhances plant growth by increasing nutrient uptake either by increasing the absorbing surface and mobilising sparingly available nutrient sources or by secretion of ectoenzymes (Rhodes, 1980; Bolan *et al*. 1987). AM plants absorb and accumulate more 'P' than non AM plants either due to more efficient translocation coupled with better exploration of soluble 'P' by the fungal hyphae or due to solubilization of insoluble 'P' by the AMF. The role of AMF in the uptake of other nutrients, *viz*., Cu, Zn, Ca, K, Fe, Mn is also well documented. (Marschner and Dell, 1994). Further, arbuscular mycorrhizal associations increase the uptake of water (Safir *et al*., 1971; 1972) reduce stress responses of plants to toxicity and drought (Atkinson and Davison, 1972; Guttay, 1976), improve soil texture and reduce soil erosion (Clough and Sutton, 1978), enhance revegetation of degraded soils like mine spoils (Pfleger *et al*., 1994), facilitate early establishment of nursery plants and enhances *ex vitro* establishment of micropropagated plantlets (Sivaprasad *et al*., 1995a). The potential of arbuscular mycorrhizal associations

in inducing resistance/tolerance of plants against soilborne diseases is the focal point of investigation in the present study.

2.2 The arbuscular mycorhizas as potential biocontrol agents of soilborne diseases

Terrestrial plants are dependent on the intense biological activity that sourrounds the root system for their nutrition, growth and health. In the rhizosphere, AMF occupy a unique ecological position and have a competitive advantage over other microorganisms as they are partly inside and partly outside the host (Read *et al.*, 1985). The primary effects of interaction between AMF and microorganisms in the rhizosphere are the result of altered host physiology due to the symbiosis (Mamata Sarma and Mukerji, 1992). Changes in phosphate nutrition due to AM symbiosis alter the membrane permeability and thus root exudation which in turn result in changed microflora equilibrium (Ratnayake *et al.*, 1978). Pathogenic fungi, bacteria and nematodes could directly interact with AMF in the mycorrhizosphere.

In one of the earlier studies, it has been demonstrated that mycorrhizal roots of yellow birch (*Betula lutea*) was predominated by saprophytic fungi such as *Trichoderma*, *Penicillium* and *Paecilomyces* species while non mycorrhizal roots had *Pythium*, *Fusarium* and *Cylindrocarpon*

(Katznelson, *et al.*, 1962). The modified mycorrhizosphere organisms may interact with pathogens so as to inhibit their growth and reproduction. Similarly the saprophytic fungi in and on mycorrhizal tomato roots were found increased and incidence of pathogenic *Pythium* and *Fusarium* decreased (Schenck, 1981).

The first reported evidence of the effect of AMF in inducing disease resistance in plants was made by Safir (1968) in onion against pink rot caused by *Pyrenocheta terrestris* and was later confirmed by Becker (1976) with *Glomus fasciculatum* and *G. margarita*. A detailed investigation on the possible interaction of AMF and *Thielaviopsis basicola* which causes root rot in tobacco and alfalfa, revealed that inoculation of *Glomus mosseae* increased the host resistance to the disease and inhibited the production and germination of chlamydospores of the pathogen (Baltruschat and Schonbeck, 1972a and b).

The role of AMF in reducing wheat rot (*Urocystis tritici* was emphasised by Khan and Khan (1974). In another study in the biocontrol of root rot of strawberry, Nemec (1974) observed higher endogone sporulation in the rhizosphere of healthy strawberry roots than diseased ones and added that as roots aged or were diseased, the endogone reacted by producing numerous spores in the rhizosphere. Similar suppression of disease in strawberry by *Cylindrocarpon destructans* using *G. fasciculatum* was also reported (Paget, 1975). The study on

the influence of AMF (*G. mosseae*) on vascular wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* showed that the disease was reduced when plants were preinoculated with the AMF and the spread of the pathogen in the host was arrested (Dehne and Schoenbeck, 1975). Similarly, AM associations significantly reduced the incidence and intensity of wilt of cucumber (Dehne, 1977) and tomato incited by *F. oxysporum* (Dehne and Schonbeck, 1979a), root rot of citrus incited by *Phytophthora parasitica* (Schenck *et al.*, 1977; Davis and Menge, 1980), *T. basicola* in cotton (Schonbeck and Dehne, 1977) and *Rhizoctonia solani* in poinsettia, *Macrophomina phaseolina* in soybean and *Cylindrocladium scoparium* in yellow poplar (Stewart and Pfledger, 1977).

The suppressive effect of *G. fasciculatum* and *G. mosseae* on *F. oxysproum* f.sp. *lycopersici* of tomato and *R. solani* of poinsettia was demonstrated by Schenck and Kellam (1978). Woodhead (1978) found that association of *G. calidonius* and *G. etunicatum* with soybean in steamed soil reduced the harmful effects of *P. megasperma* c.v. *sojae*. Reduction of infection level of *T. basicola* in *G. fasciculatum* inoculated sweet orange seedlings (Davis, 1980) and reduction of wilt incidence by *F. oxysporum f.sp. ciceri* in *G. fasciculatum* and *G. mosseae* inoculated chickpea (Jalali and Thareja, 1981) are the salient earlier documents of biocontrol using AMF.

In 'lawson pine' (*Chamecyparis lawsoniana*), the interaction of AM association and root rot pathogen, (*Phytophthora cinnamomi*) reduced infection and development of the pathogen (Baertschi *et al.*, 1981). The influence of AMF on disease incidence and development was critically analysed by Dehne (1982) and suggested that the mycorrhizal root tissues exhibited less susceptibility to parasites. He further pointed out that in direct competition with pathogen in a living host cell, mycorrhizal fungi may be more successful due to the high degree of susceptibility. Take all disease of wheat (*Gaeumanomyces graminis*) was suppressed by *G. fasciculatum* (Graham and Menge, 1982). The disease was favoured by 'P' deficiency and enhanced 'P' uptake by mycorrhizal wheat helped in counteracting the effect of take all fungus.

In peanuts *G. fasciculatum* was found to provide resistance against *Sclerotium rolfsii* (Krishna and Bagyaraj, 1983). The pathogen produced less sclerotia in mycorrhizal roots and AMF colonization and spore production were reduced by the pathogen. Jalali (1983) observed significant suppression of *R. solani* and *Fusarium* spp. by mycorrhizal inoculation with potential strains of *Glomus* spp. in legumes. Evidence from green house experiments suggested that *Gigaspora calospora* exerted an inhibitory effect on the development of pigeon pea blight incited by *Phytophthora drechsleri* f.sp. *cajani* (Bisht *et al.*, 1985). Melo *et al.* (1985) recognised the specificity

of interaction between different AMF and the aubergine wilt pathogen (*Verticillium albo-atrum*). *Gigaspora* spp. reduced wilt incidence while *Glomus* spp. increased severity through increased plant vigour.

Groundnut plants inoculated with *G. mosseae* alone exhibited greater AMF colonization than that recorded in *R. solani* and *F. solani* + AMF inoculated plants (Parvathi *et al.*, 1985). However, when the pathogens were inoculated separately after AMF inoculation, the mycorrhizal development was unaffected. Infection of pea by the root rot pathogen (*Aphanomyces euteiches*) was suppressed by *G. fasciculatum* when the plants were inoculated with the pathogen 2-4 weeks after inoculation with AMF while the pathogen was not affected by AMF when both the fungi were inoculated at the same time and the resistance induced was partially systemic (Rosendahl, 1985). Perrin (1985) suggested that the biocontrol potential of AMF is related to the type of host, mycorrhizal fungi, the pathogen and soil conditions.

In another important study, Caron *et al.* (1986a) obtained equally significant reduction in the incidence and development of crown and root rot of tomato incited by *F. oxysporum* f.sp. *radices-lycopersici*. When AMF was preinoculated, simultaneously inoculated with pathogen or 4 weeks after pathogen inoculation, there was significant

reduction on population of pathogen and root necrosis. Preinoculation with *G. fasciculatum* and *G. tenue* was found to suppress the fusarial wilt of *Cassia tora*, *Albizia procera* and *Dalbergia sissoo* and reduced the pathogen population in the rhizosphere (Chakravarthy and Misra, 1986a, b). Graham (1986) also emphasised the importance of preinoculation with AMF for effective disease suppression.

Biocontrol of Fusarium wilt of tomato and pepper caused by *F. oxysporum* f.sp. *lycopersici* and *F. oxysporum* f.sp. *vasinfectum* was investigated by Al-Momany and Al-Raddad (1988) using seven isolates of *Glomus* spp. The isolates reduced the disease in tomato and pepper at different rates. In sweet orange seedlings, Graham and Egel (1988) observed that AMF did not increase resistance/tolerance to Phytophthora root-rot unless AMF conferred a 'P' nutritional advantage. The significance of 'P' nutrition in AMF-soilborne plant pathogen interactions were further highlighted by Smith (1988). He also suggested that pre-colonization of plants with AMF 2-4 weeks before pathogen inoculation is desirable for the fungus to colonize the roots before challenged with the pathogen.

In Pakistan, Iqbal and Nasim (1988) studied the biological deterrent activity of AMF to damping off (*R. solani*) and observed that cauliflower seedlings preinoculated with AMF survived better and resisted damping off attack significantly.

Sampangiramaiah and Bagyaraj (1989) reviewed the interactions of root diseases and mycorrhizae and suggested that the potential use of mycorrhiza for disease control could be exploited in the field by a clear understanding of the defence mechanisms and conditions favouring protective effect.

Jalali *et al.* (1990) studied the interaction of *G. mosseae* and root rot pathogen *Macrophomina phaseolina* in mungbean and indicated that mycorrhizal inoculation significantly restricted the spread of the pathogen in the root tissue and increased dry matter production and nutrient uptake of N, P and K. The disease incidence was 13.3% in mycorrhizal plants while it was as high as 77.9 per cent in control plants.

Biocontrol of soilborne diseases with AMF and charcoal compost was investigated by Kobayashi (1990). Inoculation of AMF alone induced weak response but combined use of AMF and charcoal compost drastically reduced damping off in cucumber (*R. solani*). The principle of cross protection using avirulent strains of AMF was suggested by Vidhyashekaran (1990) and opined that such a strain need not always nutritionally be as effective as others but may synthesise inhibitory substances in the roots in increased quantity.

The influence of *G. fasciculatum* on important diseases of maize like maize leaf blight (*Helminthosporium maydis*), seedling blight and stalk rot (*F. moniliforme*) and

Acremonium stalk rot (*Acremonium kiliense*) was studied by Chabbra *et al*. (1992). Mycorrhizal plants exhibited complete resistance to *F. moniliforme* while there was no effect of mycorrhizal treatment on leaf blight or Acremonium stalk rot. Hwang *et al*. (1992) showed that inoculation of *Glomus* spp., *G. fasiculatum* and *G. mosseae* significantly enhanced shoot dry weight and had lower incidence and propagule number of two wilt pathogens, *viz*., *Verticillium albo-atrum* and *F. oxysporum* f.sp. *medicaginis* affecting alfalfa.

The suppressive effect of AMF on foot rot of black pepper was amply demonstrated (Anandaraj *et al*., 1993; Anandaraj and Sarma, 1994; Sivaprasad *et al*., 1995b). Black pepper vines were inoculated with AMF and subsequently challenged with *Phytophthora capsici*, *Rhadopholus similis* and *M. incognita*. Besides enhancing growth AMF association suppressed the root pathogens. The root damage caused by pathogens was compensated by enhanced rooting. The root rot and subsequent foliar yellowing were also remarkably reduced (Sivaprasad *et al*. 1995b).

The interrelationships between AMF and phytotoxic micromycetes which are the causative agents of apple replant disease were investigated (Catska, 1994). Inoculation of *G. fasciculatum* and *G. macrocarpum* suppressed the microorganisms and increased the population of *Azospirillum*.

It was suggested that use of AMF can replace chemical treatment of soil against apple replant disease. In cardamom, the biocontrol of damping off caused by *F. moniliforme* and *R. solani* using AMF was investigated (Thomas *et al.*, 1994). AMF inoculation not only reduced severity of disease but also increased plant growth characteristics and 'P' uptake by roots. Azhukal disease of cardamom caused by *Phytophthora nicotianae* was remarkably reduced in the green house and field conditions and improved growth and general condition of the plant due to AMF inoculation at the secondary nursery planting time. Of the different AMF tested *G. mosseae* was most effective for disease control while *A. morroweae* was better for stimulating plant growth (Sivaprasad, 1995).

Increased damage and disease incidence due to mycorrhizal associations are not uncommon. Ross (1972) observed increased damage and higher disease incidence by *Phytophthora megasperma* c.v. *sojae* in mycorrhizal soybean and found that 88 per cent of root rot susceptible plants infected with the pathogen and *Endogone* sp developed internal stem discolouration and 33 per cent of plants died due to root-rot while in plants with pathogen alone only 17 per cent plants developed the symptoms and none died.

In another study Chou and Schmithenner (1974) also showed that there was no disease reduction by *Endogone mosseae*

and *Rhizobium japonicum* in soybean infected by *P. ultimum*. The study indicated that the symbiosis neither dispose the host to infection nor enhance the severity of root rot. Mycorrhizal development was also not affected by the presence of the pathogen. Greater disease incidence and severity due to AM associations were also reported by Davis *et al.*, (1978) in avocado; alfalfa and citrus by *Phytophthora cinnamomi*, *P. megasperma* and *P. parasitica* respectively. They observed that the effect of AM associations was negated in the presence of the pathogen.

In cotton, *Verticillium* wilt was more severe in AMF inoculated plants (Davis *et al.*, 1979) and the reasons attributed were increased avenues for penetration of the pathogen due to rupturing the cortex by abundant chlamydospores of AMF, reduced concentration of K in mycorrhizal plants and enhanced growth of the plant due to better nutrient status.

Mcgraw and Schenck (1981) also reported increased disease incidence in mycorrhizal tomato due to wilt (*F. oxysporum* f.sp. *lycopersici*). There was no effect due to AMF inoculation in soybean against disease development by *F. solani*, *R. solani* and *M. phaseolina* (Zambolin and Schenck, 1983) and in tomato against verticillium wilt (Baath and Hayman, 1983).

2.2.1 AMF as biocontrol agents of Pythium incited diseases

In one of the early attempts, Stewart and Pfleger (1977) reported that damage due to *Pythium ultimum* in poinsettia was significantly reduced in mycorrhizal plants. They further suggested that once the AM association is established, it can deter the pathogen infection and development in the host root. The suppressive effect of *G. fasciculatum* and *G. mosseae* on *P. ultimum* in poinsettia was also demonstrated by Schenck and Kellam (1978). The interaction of *G. fasciculatum* and *P. ultimum* on green house grown poinsettia was analysed by Kaye *et al.* (1984) at different population levels of the pathogen and found that mycorrhizal colonization, plant height and foliar 'P' content were remarkably greater in soils containing moderate population of the pathogen.

Kobayashi (1990) investigated the possibility of biocontrol of damping off of cucumber seedlings caused by *P. splendens* and observed that inoculation of AMF alone induced weak resistance but the combined use of AMF and charcoal compost drastically reduced the disease on 2-3 week old seedlings.

Rosendahl *et al.* (1992) observed that the presence of AMF (*Glomus* spp.) reduced the damping off caused by *P. ultimum* in vermiculite grown cucumber and the protective effect was

present even if the pathogen was introduced simultaneously with AMF. Hwang *et al.*, (1993) discussed the influence of AMF on growth stimulation of saifoin in *Pythium* infected soils and found that *G. fasciculatum* and *G. intraradices* reduced the severity of damage. In *Tagetes patula* also, the infection of *P. ultimum* and *G. intraradices* tended to reduce the infection and the pathogen population was ten times lower in mycorrhizal plants (St-Arnaud *et al.*, 1994) irrespective of 'P' content. Inoculation of cardamom with *G. fasciculatum* remarkably reduced the severity of damping off caused by *Pythium vexans* in addition to increased plant growth and high 'P' uptake (Thomas *et al.*, 1994).

2.2.2 AMF as biocontrol agent of ginger rhizome rot

The interaction of AMF with *P. aphanidermatum*, the causal agent of rhizome rot of ginger and *M. incognita* causing root-knot of ginger was investigated by Rohini Iyer and Sundararaju (1993). The AMF, *viz.*, *G. multicaule* and *G. fasciculatum* significantly enhanced growth of ginger whereas the pathogens suppressed it. The AMF association was found to reduce the disease incidence and suggested that prior inoculation with AMF was effective in ameliorating the deleterious effect of the pathogen. Biocontrol of rhizome rot of ginger with *G. fasciculatum* and *G.* mosseae was also reported from College of Agriculture, Vellayani, Trivandrum (Sivaprasad, 1993).

2.3 Plant parasitic nematode management using AMF

One of the earliest studies of interaction was in tobacco between *Heterodera solanacearum* and *Gigaspora gigantea* by Fox and Spasoff (1972). They observed that nematode and AMF suppressed the population of each other due to mutual antagonism. The antagonistic effect of AMF on nematode was first experimentally proved by Baltruschat *et al*. (1973). They indicated that *Endogone mosseae* had deleterious effect on *Meloidogyne indica* in tobacco. In a survey of soybeans in Florida, Schenck and Kinloch (1974) found that the spore counts of AMF were consistently low when associated with high population of root-knot nematodes. In a subsequent study Schenck *et al*. (1975) showed that nematodes at low inoculum level (500 larvae/pot) stimulated sporulation of AMF while higher population (5000 larvae/pot) reduced it. They opined that the effect of a given interaction was strongly influenced by nematode inoculum level, cultivar resistance and the specificity of fungal symbionts.

The antagonistic effect of *G. mosseae* on population of *M. incognita* and *M. hapla* was convincingly demonstrated by Sikora and Schonbeck (1975). They noticed significant reduction (75 per cent) of nematode population in mycorrhizal tobacco, oats and tomato plants. The interaction between *M. arenaria* and *G. fasciculatum* resulted in stimulated growth

in dually inoculated grapes and reduced the deleterious effects of nematodes (Atilano *et al.*, 1976). Hussey and Roncadori (1977) investigated the interaction of migratory endoparasitic nematode, *Pratylenchus brachyurus* and *Gigaspora margarita* on cotton and observed the suppression of population of nematodes in the roots without affecting sporulation and colonization of AMF. Roncadori and Hussey (1977) studied the interaction of *G. margarita* with *M. incognita* in nematode susceptible and resistant cotton cultivars and found that the beneficial effect of AMF offset the damage of nematode in susceptible cultivars. The growth reduction due to nematode was about 30 per cent in non mycorrhizal plants but only 10 per cent in mycorrhizal plants.

Kellam and Schenck (1977, 1980) showed that gall formation by *M. incognita* in soybean was reduced only in root portions mycorrhized with *G. macrocarpum* suggesting a direct short range effect and also observed that the yield and root weight were increased in dually inoculated plants and had significantly fewer galls/g root than inoculations with nematode alone.

Bagyaraj *et al.* (1979) observed that nematode inoculation enhanced infection and sporulation of AMF in tomato whereas AMF significantly reduced the number and size of nematode galls, improved plant growth and higher 'P' content

and postulated that the AM induced physiological changes in plants limited nematode population and development. Hussey and Roncadori (1982) opined that the ability of AM plants to grow well despite nematode infection is the principal effect of mycorrhizal fungi and the effect of the tripartite interaction may vary depending on the members involved.

Preinoculation of tomato with AMF did not alter the *M. incognita* infection regardless of soil 'P' level whereas plants grown in high 'P' soil favoured nematode infection and decreased AMF colonization (Thomson-Cason *et al.*, 1983). The AMF root colonization and sporulation of **G.** *fasciculatum* were reduced due to *P. penetrans* on *Phaseolus vulgaris* (Elliot *et al.* 1984). The plant growth and yield were also reduced due to nematode attack.

The effect of spore density of AMF (*G. fasciculatum*) on nematodes was investigated by Saleh and Sikora (1984) and found that less number of spores (30/plant) did not affect nematode population, while higher spore load (480/plant) increased AM colonization and greatly suppressed egg production and nematode densities. Higher levels of phenolics which frequently have been implicated in nematode resistance have been reported in AMF plants in several instances (Krishna and Bagyaraj, 1984).

Increased resistance against nematodes and suppression of nematode population have been observed in

mycorrhizal soybean (Francl and Dropkin, 1985), tomato and white clover (Cooper and Grandison, 1986) and alfalfa (Grandison and Cooper, 1986). The susceptibility of mycorrhizal and non mycorrhizal 'P' fertilized cotton were compared by Smith (1987, 1988) and showed that the latter were more suceptible to nematode attack indicating the likely involvement of factors other than 'P' nutrition.

Prior inoculation of cardamom with *G. margarita* and *G. fasciculatum* was effective in ameliorating the deleterious effect of root-knot nematode and provided vigorous healthy seedlings (Thomas *et al.*, 1989). Similar results were also obtained for AMF in common beans against *M. incognita* (Osman *et al.*, 1990) and in tomato against *M. javanica* (Singh *et al.*, 1990b).. Sivaprasad *et al.* (1990) observed that the deleterious effect of nematodes was made insignificant due to AM associations in cowpea and galling, root-knot index and nematode population were reduced considerably. The suppressive effect of AMF (*G. macrocarpum*) on the population and development of *M. incognita* in cowpea (Ahmed and Alsayed, 1991) was attributed to change in root exudates causing fewer nematodes attracted to the host.

In ginger, the suppressive effect of AMF on root-knot nematode was proved by Rohini Iyer and Sundararaju (1993). In tomato and blackgram, the interaction of AMF and *M. incognita* increased biomass production and yield and suppressed nematode

population when plants were preinoculated with AMF (Sundarababu *et al.*, 1993).

The combined effect of two biocontrol agents (*G. mosseae* and *Paecilomyces lilacinus*) together or separately in the presence of layer manure completely inhibited infestation by *M. javonica* (Al-Raddad, 1995). *G. margarita* and *G. etunicatum* with *M. incognita* at different 'P' levels in peanut resulted in increased plant tolerance to the nematode and offset growth reduction by the nematode at lower 'P' levels (Carling *et al.*, 1995).

2.4 Combined effect of AMF and antagonists on plant diseases

Saprophytic fungi are integral component of the soil rhizosphere and they have been little studied with regard to interaction with AMF. The results of interactions between saprophytic fungi/antagonists and AMF differ widely even when the same genus is involved. *Trichoderma* spp. have been found to have both antagonistic (Cook and Baker, 1983; Wyss *et al.*, 1992) and stimulatory effects on AMF (Calvet *et al.*, 1992, 1993). Sylvia and Schenck (1983) reported general inhibitory effect of some contaminating fungi including *Trichoderma* spp. on spore germination of three *Glomus* spp. The effect of selected *Trichoderma* spp. used in the biocontrol of plant disease was found to be antagonistic to AMF as well, according to Cook and Baker (1983).

In an axenic growing medium, it was found that there was increase in the mycelial infection of lucerne (*Medicago sativa* L.) by *G. mosseae* when microorganisms were introduced into the medium (Azcon-Aguilar and Barea, 1985; De Oliveira and Garbaye, 1989). One of the earliest studies of the combined effect of AMF and antagonists by Kohl and Schlosser (1989) showed that infection and colonization of maize roots by *G. etunicatum* was almost unaffected by strains of *T. hamatum* and *T. harzianum*. They suggested that combined application of AMF and *Trichoderma* spp. should be feasible to promote plant health. The study of interaction between AM and two phosphate solubilizing fungi in finger millet showed that AMF and *Aspergillus niger* produced a synergistic effect on growth and nutrient uptake of the plant (Gopalakrishnan *et al.*, 1990).

The effect of another wellknown antagonist, *Gliocladium virens*, on colonization of cucumber by *Glomus. etunicatum* and *G. mosseae* was investigated by Paulitz and Linderman (1991). The results showed no detrimental effect of antagonist on AMF and they were compatible if applied as dual inoculum. The interaction between AMF and saprophytic *Trichoderma* spp. was tested under *in vitro* conditions (Calvet *et al.*, 1992). The germination rate of resting spores and the development of AMF mycelium were stimulated by the presence of *Trichoderma* spp. due to production of volatile compounds released to the growing media and suggested the possibility of

using such antagonists along with AMF for better plant growth and health.

Commercial biocontrol agents, *T. harzianum* and *Streptomyces griseoviridis* significantly suppressed the mycorrhiza formation by *G. mosseae* in soybean (Wyss *et al.*, 1992). Interestingly, *T. harzianum* alone and in combination with AMF induced accumulation of large quantity of glyceollin (phytoalexin) similarly as pathogen does; probably due to elicitation of plant defense mechanisms.

A very convincing study on the combined effect of AMF and antagonists was conducted by Kumar *et al.* (1993). They tested mixed biocontrol cultures comprising of *G. epigaeus* and *T. viride* singly and in combination against three wheat root rot pathogens, *viz. Bipolaris sorokiniana*, *F. avanaceum* and *F. javanicum* in green house. The mixed cultures protected wheat plants against all the three pathogens. The population of *T. viride* was higher while pathogen population was lesser and obtained higher plant height,weight and root length.

The study of the tripartite interactions of AMF-antagonist-pathogen in a given host system involving mycorrhizal fungus *G. mosseae*, pathogen, *P. ultimum* and antagonist, *T. aureoviride* in the rhizosphere of marigold (*Tagetes erecta*) by Calvet *et al.* (1993) revealed that combined effect of AMF and antagonist had a synergistic effect on growth

of marigold, AM colonization and reduction of the pathogen population. But McAlister *et al*. (1994a,b) examined the effect of *T. koningii* and *F. solani* on maize and lettuce with or without *G. mosseae* and observed that while plant dry weight of non AM plants was unaffected by the presence of saprophytes, *T. koningii* decreased plant weight in AM plants when inoculated before or at the same time as *G. mosseae*. Conversely, the population of saprophyte was considerably reduced when *G. mosseae* was inoculated two weeks before inoculation of the pathogen.

The effect of dual inoculation of wheat with phosphatase producing fungus (PPF) *Aspergillus fumigatus* and *G. mosseae* was studied by Tarafdar and Marschner (1995) and found that the seed inoculation with *A. fumigatus* or soil inoculation with AMF increased shoot and root weight, root length and shoot concentration of P, K and Mg. The results clearly indicated that organic P when added in the form of Na phytate was effectively used by AMF and organic 'P' can be increased by simultaneous inoculation with *A. fumigatus*. So in general the prophylactic property of AMF could be exploited in association with other rhizosphere microbial antagonists especially with *Trichoderma* and *Gliocladium* (Linderman, 1994; Barea *et al*., 1996).

2.5 Mechanism of biocontrol by AMF

The study of possible role of AM symbiosis in protection against plant diseases began in the 1970's and a great deal of information has been generated on the subject, but still very little is known about the underlying mechanisms (Hooker *et al.*, 1994; Linderman, 1994). Biocontrol of plant diseases may be strongly influenced by AMF by one or more of the mechanisms briefly illustrated as hereunder.

2.5.1 Improved host nutrition

The most obvious contribution of AM symbiosis to reduce the root diseases is the increased nutrient uptake particularly 'P' and other minerals resulting in more vigorous plants better able to resist/tolerate root diseases (Davis, 1980; Graham and Menge, 1982). Some reports indicated that AMF or added 'P' increased disease incidence (Davis *et al.*, 1979). In an attempt to clarify the confusion about the role of nutrition on disease incidence, Graham and Egel (1988) showed no difference between Phytophthora root rot levels on AMF and non AMF citrus seedlings fertilized to be of equal size and 'P' content. Similar experiments conducted by Caron *et al.* (1986a, b) suggested mechanisms of disease suppression other than enhanced 'P' uptake in mycorrhizal plants. AMF which were tolerant to 'P' reduced nematode effects even under high 'P'

conditions which indicated that non 'P' mediated mechanisms are involved, probably physiological changes in the roots (Smith, 1987).

2.5.2 Root damage compensation

It has been suggested that AM fungi increase host resistance to pathogen attack by compensating for the loss of root biomass or function caused by pathogens (Linderman, 1994; Cordier *et al.*, 1996). This could be an indirect effect through the conservation of root system function both by fungal hyphae growing out into the soil thereby increasing the absorbing surface and by the maintenance of root cell activity through arbuscule formation (Cordier *et al.*, 1996).

2.5.3 Competition for host photosynthates

It has been proposed that AMF and root pathogens depend on photosynthates of the host for their growth and they compete for the carbon compounds that reach the roots (Smith, 1987; Linderman, 1994). Nematode pathogens require host nutrients for reproduction and development and direct competition with AMF has been hypothesized as a mechanism for inhibition (Dehne, 1982; Smith, 1988). There is little evidence to support this hypothesis. AMF appear to more than make up for their nutrient needs by enhancing photosynthates without limiting nutrient supply to root pathogens (Linderman, 1994).

2.5.4 Competition for infection/colonization sites

Fungal root pathogens and AMF, although colonize the same root tissues, usually develop in different root cortical cells which indicated some sort of competition (Dehne, 1982). Depending on the pathogen, both localised and non localised competion could exist. Reports of Jalali and Jalali (1991) indicated localised effect while Dehne (1982) and Smith (1987) suggested that the exact protection cannot be explained by a localized mechanism alone. It has been shown that *Phytophthora* development is reduced in AMF colonized and adjacent regions of AM root system. In AMF colonised cells pathogen cannot penetrate arbuscule containing cells (Cordier *et al.*, 1996). It is a clear evidence of localised competition.

2.5.5 Anatomical and morphological changes in the root system

Dehne and Schonbeck (1979b) showed increased lignification of tomato and cucumber root cells of the endodermis in AM plants and speculated that such responses accounted for reduced Fusarium wilt. Becker (1976) showed similar effect on pink rot of onion. Atkinson *et al.* (1994) demonstrated that AMF colonisation induced remarkable changes in the root system morphology as well as in the meristematic and nuclear activities of root cells. The most frequent effect of AM colonization was increased root branching which resulted in relatively larger proportion of higher order roots in the root system (Hooker *et al.*, 1994).

2.5.6 Microbial changes in the mycorrhizosphere

Microbial changes occur in the rhizosphere mainly due to changes in the root exudation pattern as influenced by the changed host physiology of AM plants (Azcon-Aguilar and Bago, 1994). It results in qualitative and quantitative alterations in microbial populations in the rhizosphere. AM establishment can change both total population and specific functional groups of organisms in rhizosphere soil (Meyer and Linderman, 1986). They also found that the number of sporangia and zoospores formed by cultures of *P. cinnamomi* was reduced by the application of extracts of rhizosphere soil of AM plants. Similarly Caron (1989) demonstrated a reduction in Fusarium populations in soil surrounding mycorrhizal tomato roots.

Generally microbial antagonists of fungal pathogens, either fungi or plant growth promoting rhizobacteria do not antagonise AM fungi, instead, they improve the development of mycosymbiont and facilitate AM formation (Barea *et al.*, 1996). This has been shown particularly for *Trichoderma* spp. (Calvet *et al.*, 1992; 1993).

2.5.7 Activation of plant defense mechanisms

Activation of specific plant defense mechanisms in response to AMF colonization is the basis of protective capacity of AMF (Azcon-Aguilar and Barea, 1996). The

elicitation of specific plant defense reactions prepare the plant to guard against root pathogen attack (Gianinazzi-Pearson *et al.*, 1996).

Accumulation of amino acids, arginine and citrulline had been proposed to be the mechanism of disease suppression by AMF (Baltruschat and Schonbeck, 1975) in tobacco root rot caused by *T. basicola*. Arginine was shown to suppress the growth and sporulation of the pathogen. Accumulation of arginine in other AM plants like tomato, bean and corn was also reported by Dehne and Schonbeck (1979a). The reason for the accumulation was reported to be due to the interruption of ornithine cycle by some unknown mechanism of AMF. The Electrophoresis studies conducted by Gianinazzi-Pearson and Gianinazzi (1995) revealed that the host plants produced a number of new proteins (endomycorrhizins) in resposne to AMF colonization.

Selvaraj and Subramonian (1990) observed significant enhancement of phenolic content of AM sesamum plants in sterilized soils. Histochemical studies revealed accumulation of different types of lipids and phenolic compounds in AM structures particularly neutral lipids and catechol tannins in vesicles. Increased levels of phenolics have also been reported in mycorrhizal plants by other workers (Krishna and Bagyaraj, 1984; Sivaprasad *et al.*, 1995a). Dehne (1982)

reported increased lignification of root endodermal cells induced by AM colonization which makes penetration of pathogens in the root tissues more difficult.

Benhamou *et al.* (1994) suggested that the weak responses of AM infection with regard to activities like lignification, production of phytoalexins and peroxidase and expression of genes coding for PR proteins could sensitize the root to pathogens and enhance defence mechanisms to subsequent pathogen infection. They compared the responses of AM and non AM transformed carrot roots to infection by *F. oxysporum f. sp. chrysanthemi*. In mycorrhizal roots, pathogen growth was restricted to epidermis and cortical tissues whereas, in non mycorrhizal roots the pathogen developed further, infecting even the vascular stele. The pathogen hyphae in AM roots exhibited a high level of structural disorganization probably due to phenolics and hydrolytic enzymes like chitinase unlike in non AM roots. This suggests activation of plant defence responses by mycorrhiza formation provides some protection against the pathogen.

2.6 Effect of pesticides on AMF

The action of pesticides on infection, colonization and spore production of AMF depends greatly on the dose and type of pesticides, species of the fungus and the host plant. Conflicting reports are available on the effect of various

pesticides on AMF. Sreenivasa and Bagyaraj (1989) found that the *G. fasciculatum* inoculum production was greatly affected by the fungicides, nematicides and insecticides at the recommended dose but at half the recommended dose captan and carbofuran significantly increased AMF colonization and spore production.

The effect of fungicides on mycorrhizal wheat depended greatly on the AMF species and the type of fungicide (Dodd and Jeffries, 1989). While the field application of Bavistin prevented germination of *G. monosporum* and *G. mosseae*, the germination of *G. geosporium* was unaffected. Tilt inhibited spore germination less than Bavistin whereas Calixin increased the AMF infection resulting in greater yields and foliar 'P' levels.

Singh *et al.* (1990a) observed that out of the ten fungicides tested seed treatment with mancozeb, Sulfex and Vitavax significantly reduced the spore population and AMF colonization in rough lemon seedlings. Singh *et al.*, 1990c) obtained considerable inhibition of AMF development in pigeon pea due to seed treatment with Bavistin, captafol and thiram. However, other workers (Vyas *et al.* 1990; Tiwari and Shukla, 1991) could not observe any adverse effect of carbendazim and thiram on AMF colonization in soybean.

The reduction in AMF colonization and symbiotic effectiveness with increasing concentration of thiram was also reported by Habte and Manjunath (1992) in *Leucaena*

leucocephala. Vijayalakshmi and Rao (1993) studied the effect of insecticides and observed that monocrotophos at 5-50 ppm stimulated while dimethoate and cypermethrin reduced AMF colonization. Dichlorvos stimulated AMF colonization at lower concentration but was inhibitory at 50 ppm. Endosulfan significantly inhibited AMF infection even at 5 ppm. The effect of pesticide drenching of Bavistin, Cuman, COC, sulphur, carbofuran and chlordane at recommended rates initially reduced the AMF colonization and spore production in pigeon pea and cowpea but later a slow recovery was noticed (Udiyan *et al.*, 1995).

The effect of soil application of carbofuran at recommended dose (2 kg/ha) enhanced AMF colonization and sporulation of *G. clarum* in groundnut (Venkateswarlu *et al.*, 1995) whereas application at 5 kg/ha inhibited AMF infection. Vyas and Vyas (1995) observed that carbendazim, fosetyl-Al, mancozeb and thiram enhanced growth promoting effect, mycorrhizal infection and spore production whereas, triadimefon adversely affected growth of AMF. The potential merit of Ridomil 72 WP application in stimulating the colonization and spore production of *Sclerocystis coremioides* was highlighted by Robert *et al.* (1995).

2.7 Antagonistic fungi for biocontrol of plant diseases

Biological control is a basic component in the integrated disease management systems. One of the earliest

mentions of biological control of plant diseases was by Sanford (1926) who reported reduction of disease by addition of green manures. Sanford and Broadfoot (1931) provided experimental evidences supporting the finding of Sanford and first used the terms 'biological control' and 'suppressive effect' in Plant Pathology.

Perhaps the earliest use of an antagonist, *Trichoderma* spp. in biocontrol was inadvertent when *Armillaria mellea* was controlled by soil treatment with carbondisulphide (Horne, 1914) which was later found to be due to enhanced mycoparasitism of the pathogen by *Trichoderma viride* (Bliss, 1951). Since the pioneering work of Weindling and Fawcett (1936) on the use of *Trichoderma* spp. to control damping off in citrus (*Rhizoctonia solani*), there is accumulating evidence to show that *Trichoderma* spp. which are easily isolated from soil and readily grown (Chet, 1987) are among the most promising biocontrol agents in terms of large scale applications (Benhamou and Chet, 1996).

The first field demonstration of effectiveness of mycoparasites on sclerotia of fungi was given by Tribe (1957) who showed that *Coniothyrium minitans* parsitized sclerotia of *Sclerotinia trifoliorum* in field soil killing 85-90 per cent of them in 11 weeks. Although there are a number of examples of fungi that parasitize plant pathogens. *Trichoderma* spp. and *Gliocladium virens* probably have been studied to the greatest

extent (Papavizas, 1985). Other potential mycoparasites are *Coniothyrium minitans* used against *Sclerotinia* spp., *Sclerotinia cepivorum*, *S. sclerotiorum* etc. (Turner and Tribe, 1975; Huang 1980), *Laetisaria arvalis* used against diseases caused by *Pythium ultimum* (Martin *et al.*, 1986), *Soridesmium sclerotivorum* against *Sclerotinia minor* in lettuce (Adams *et al.*, 1984), *Talaromyces flavus* against *R. solani* (McLaren *et al.*, 1983) and *Pythium nunn* and other related species against *Pythium ultimum*, *P. vexans* and *P. aphanidermatum* (Lifshitz *et al.*, 1984; Paulitz and Baker, 1987).

2.7.1 The potential of *Trichoderma* spp. as biocontrol agents

Trichoderma spp. have received considerable attention as possible biocontrol agents of soilborne plant pathogens and their recognized antagonistic potential has often been used as a means of *in vitro* screening for selecting the best biocontrol candidates (Chet and Inbar, 1994). Species of this genus occur worldwide and are considered to be very effective mycoparasities for biocontrol of a wide range of plant pathogens including *Armillaria mellea*, *Pythium* spp., *Phytophthora* spp., *R. solani*, *Sclerotium rolfsii*, *Sclerotinia* spp., *Heterobasidium annosum*, *Chondrostereum purpureum*, *Fusarium* spp., *Aphanomyces* spp. etc. The genus contains a wide range of mycoparasitic species which have been reported as biocontrol agents (Wells *et al.*, 1972; Chet and Baker, 1980; Papavizas, 1985). The desirable attributes of *Trichoderma* spp.

as effective biocontrol agents of soilborne plant pathogens are their ability to produce inoculum in excess and to survive, grow and proliferate after introduction to soil (Baker and Cook, 1974).

Much emphasis is now placed on the purposeful introduction of *Trichoderma* spp. and other specific biocontrol agents for biocontrol of soilborne of plant pathogens. Wells *et al*. (1972) probably were the first to report large scale use of *Trichoderma* preparations on solid media for field control of *S. rolfsii* in tomato. Successful control of *S. rolfsii* and *R. solani* by field inoculation with culture of *T. harzianum* have been shown by several workers (Backman and Rodriguez-Kabana, 1975; Elad *et al*., 1980; Elad *et al*., 1982a. *Trichoderma* spp. especially *T. harzianum* and *T. hamatum* have been shown to control *R. solani* on a variety of crops in green house (Chet and Baker, 1980, 1981; Harman *et al*. 1980, 1981) and in field studies (Elad *et al*., 1981, 1982b).

There are several other reports of successful use of *Trichoderma* spp. for the control of soilborne plant pathogens such as *T. harzianum* against *R. solani* in raddish and carnation (Henis *et al*., 1978), strawberry and tomato (Henis *et al*., 1979), *T. hamatum* and *Trichoderma* sp. against *R. solani* and *S. rolfsii* in corn, peas and soybeans (Kommedahl and Chang, 1975; Kommedahl *et al*., 1981) *T. harzianum* against *R. solani*

and *S. rolfsii* in bean, peanut and eggplant (Chet *et al.*, 1979), *T. harzianum* against *R. solani* in strawberry (Elad *et al.*, 1981) and tomato (Elad *et al.*, 1982a), *Trichoderma* spp. against *R. solani* in cotton (Elad *et al.*, 1982b) and *T. harzianum* against *F. oxysporum* in tomato (Sivan *et al.*, 1987).

Laboratory experiments (Elad *et al.*, 1983; Elad *et al.*, 1984) as well as field trials (Elad *et al.*, 1981) have shown convincingly that *Trichoderma* spp. displayed the ability to attack mycelium and sclerotia of *S. rolfsii* in reducing pathogen inoculum in the soil. No biocontrol agent had been used extensively for long periods partly because of large quantity of material required (Elad *et al.*, 1981) and also because of the variability in performance between locations, crops and seasons (Wells *et al.*, 1972; Kommedahl *et al.*, 1981).

Davet (1981) reported that the parasitic activity of various isolates of *Trichoderma* spp. in natural soil varied with their competitive saprophytic ability. Ahmed and Baker (1987) suggested that the amount of cellulase produced by an isolate is a measure of competitive saprophytic ability or rhizosphere competence of that isolate. It has been suggested by Hadar *et al.* (1984) that isolates of *Trichoderma* native to a soil may be better adapted to it than the introduced isolates and may be more able to coexist with native soil microflora.

A major problem of applying antagonists to soil is their inability to get established in the ecosystem and to overcome the resistance offered by soil microflora. Hence ability of an organism to suppress disease depends on factors other than presence of large number of propagules. Interactions of antagonists with resident flora and environment strongly affect their performance (Hadar *et al.*, 1984). Mihuta-Grimm and Rowe (1986) showed that the antagonistic activity of *Trichoderma* sp. is highly variable and only 15 per cent of the isolates tested was effective in controlling *R. solani* damping off in green house bioassay tests.

Pelleting cowpea seeds with *T. viride* either alone or in combination with carbendazim inhibited the growth of *Macrophomina phaseolina* and reduced post emergence damping off in pot experiments (Alagarswamy and Sivaprakasam, 1988). Dwivedi *et al.* (1993) reported that *T. viride* inhibited *F. oxysporum* f. sp. *lini* by 68.2 per cent. Root rot of avacado seedlings incited by *Phytophthora cinnamomi* was reduced by *T. hammatum* (Duvenhage and Kotze, 1993). The antagonistic activity of *T. harzianum* and *T. viride* was tested against *M. phaseolina* and found that both were effective but greater inhibition was shown by *T. viride* (Mahabirsingh and Manjumdar, 1995).

2.7.2 *Trichoderma* spp. as plant growth stimulants

In addition to their role as biocontrol agents *Trichoderma* spp. have been implicated to promote crop growth. Baker *et al.* (1984) first reported promotions of raddish growth in soil by application of *T. harzianum* and *T. viride* in the form of conidia or in peat-bran culture formulation.

Addition of *Trichoderma* spp. (*T. harzianum* and *T. koningii*) to autoclaved soil increased the rate of emergence and root and shoot dry weight of tomato and tobacco by 213-275 and 259-318 per cent respectively (Windham *et al.*, 1986). They further observed that *Trichoderma* spp. produced a growth regulating factor that increased the rate of seed germination and dry weight of shoots. Enhanced growth response by *Trichoderma* spp. have also been reported by several other workers (Baker *et al.*, 1986; Paulitz *et al.*, 1986; Windham *et al.*, 1989; Vrang *et al.*, 1990; Barnard and Davet, 1993; Inbar *et al.*, 1994; Mackenzie *et al.*, 1995). The mechanism of plant growth stimulation includes the effect as biofertilizers, biological control agents and plant growth hormone production (Lugtenberg *et al.*, 1991).

2.7.3 *Aspergillus* spp. as biocontrol agents

Although elaborate illustrations are lacking about the biocontrol potential of *Aspergillus* spp., few works amply

illustrated the antagonistic property of this widely distributed genus.

In one of the earliest mentions, Khare (1968) observed that *A. fumigatus* and *A. ochraceous* were antagonistic to *Phytophthora fragariae* both *in vitro* and *in vivo*. Incorporation of *A. niger* inoculum into the soil infested with *R. solani* reduced the incidence of collar rot under glass house and field conditions (Venketa Subbaiah and Safeeulla, 1984). Marrios *et al.* (1981) reported the potential of a multifungal conidial suspension including *A. ochraceous* in the biocontrol of tomato crown rot caused by *F. oxysporum* f. sp. *crysanthemi*.

Effect of *A. niger* and *A. fumigatus* obtained from the phylloplane of sorghum on the growth of *Phytophthora arecae* was investigated by Bopaiah *et al.* (1991) and found that the pathogen was inhibited by the antagonist in agar disc method due to antimicrobial compounds and competition for nutrients.

Mukherjee and Sen (1992) isolated *A. fumigatus* and *A. terreus* from soil and tested their antagonistic potential against *M. phaseolina*. The culture filtrate of *A. fumigatus* inhibited fungal growth and sclerotial germination of the pathogen. The antagonistic potential of *A. fumigatus* isolated from suppressive soils of *Pythium* and *Phytophthora* was found to be very effective in suppressing the pathogens in carnation (Migheli *et al.*, 1993).

When used to inoculate nursery planting medium, *A. candidus* reduced the root rot of avocado seedlings caused by *Phytophthora cinnamomi* (Duvenhage and Kotze, 1993). Cal *et al.*, (1994) observed that *A. nidulans* was capable of producing lytic enzymes and significantly reduced the disease incidence and severity by *F. oxysporum* f. sp. *lycopersici*. The inhibitory effect of *Aspergillus* spp. against *V. dahliae* by producing antibiotic substances was reported by Castrejon (1994).

2.8 Biocontrol of *Pythium* incited diseases by antagonists

One of the first attempts of direct application of biocontrol to plant pathogens was that of Harley (1921) who inoculated forest nursery soils with 13 antagonistic fungi against damping off caused by *Pythium debaryanum*.

Wright (1956) showed that inoculation of mustard seed with *T. viride* diminished 'damping off' caused by *Pythium* spp. Liu and Vaughm (1965) suceeded in controlling *Pythium ultimum* in table beet seedlings by coating seeds in a suspension of *T. harzianum* conidia. Reduction of tobacco damping off (*P. aphanidermatum*) by *T. harzianum* was reported by Fajola and Alasoadura (1975).

Chet and Baker (1980, 1981) showed that *T. hamatum* produced cellulases in addition to other cell wall degrading enzymes and thus protected the pea and raddish seedlings from

Pythium infection. Similarly seed treatment of pea and raddish with *T. hamatum* protected the seedlings from *P. ultimum* and *P. aphanidermatum* (Harman *et al.* 1980, 1981). Hadar *et al.* (1984) observed that *T. koningii* and *T. harzianum* protected peas and beans seeds against seed rots by *Pythium* spp. when applied either as seed coating or applied in gels used for fluid seed drilling. Isolates of *T. harzianum* (T-315) was found very effective against damping off (*P. aphanidermatum*) of peas, peppers, cucumbers and tomatoes (Sivan *et al.*, 1984).

Seedling root rot caused by *P. graminicola* in sugarcane did not occur when *T. viride* was incorporated in soil (Padmanabhan and Alexander, 1984, 1990). Other successful reports of biocontrol of *Pythium* incited diseases include that of Martin *et al.* (1986), Mukhopadyay and Chandra (1986) and Nagarajan and Reddy (1986). Reduction of disease incidence in barley, cucumber, pea, raddish and tomato incited by *P. ultimum* by rhizosphere mutants of *T. harzianum* (Ahmad and Baker, 1988) selected strains of *Trichoderma* spp. effective against damping off of sugar beet caused by *Pythium* spp. (Sawant and Mukhopadhyay, 1991) and damping off of capsicum caused by *P. aphanidermatum* by *T. harzianum* (Mani and Marimuthu, 1994) were also recorded.

2.9 Control of ginger rhizome rot with antagonistic fungi

In one of the early attempts Bharadwaj and Gupta (1987) studied *in vitro* antagonism of *Trichoderma* spp.

viz. T. viride, *T.harzianum* and *T. hamatum* against *P. aphanidermatum*, the causative agent of rhizome rot of ginger. They observed that all the *Trichoderma* spp. were antagonistic to the pathogen and suggested the use of *Trichoderma* spp. for checking the disease.

An antagonistic isolate, *Pythium acanthophoron* was reported as a mycoparasite on *Pythium myriotylum* by Lodha and Webster (1990). Rathore *et al*. (1992) studied the activity of volatile and non-volatile substances produced by *T. viride* on ginger rhizome rot pathogens and observed that the substances inhibited the growth of *P. myriotylum* and *F. solani* by 70 and 20 per cent respectively. In pot culture studies *T. viride*, *T. harzianum*, *T. aurioviride* and *G. virens* effectively suppressed the rhizome rot pathogen and increased yield of ginger (RAU, 1993).

Integrated application of biocontrol agents like *T. viride*, *T. harzianum*, *T. aurioviride*, *G. virens* and *P. acanthophoron* and organic amendments and rhizome dip with ridomil 72 WP (0.625 per cent), Bavistin (0.1 per cent) for 40 minutes were highly effective in reducing the rhizome rot incidence (RAU, 1993). In another trial, application of *T. viride*, *T. harzianum* and *G. virens* reduced the incidence of rhizome rot and increased the yield (NRCS, 1994)

In field evaluation of biocontrol agents isolated from ginger rhizospere soils such as *T. viride*, *T. harzianum*,

T. hamatum and *G. virens* conducted in *Pythium* sick soils for two years, it was found that *T. harzianum* and *T. hamatum* were more effective in controlling rhizome rot of ginger in both solarized and non solarized plots (Usman *et al.*, 1996).

2.10 Mechanism of biocontrol by antagonists

Much of the early work attributed the biological control acivity of antagonistic fungi to the production of antibiotics. The toxic metabolite production by *T. viride* was first reported by Weindling and Emerson (1936). Bruehl *et al.* (1969) showed that *Cephalosporium graminium* produces a wide spectrum of antibiotics in wheat straw that enables it to retain possession of the substrate for 2-3 years whereas non antibiotic producing mutants in straw were overgrown by saprophytes in a few months. Later, Denis and Webster (1971a,b) identified acetaldehyde as an inhibitory metabolite of *T. viride* and also the production of chloroform soluble non volatile antibiotics from different *Trichoderma* spp.

The properties of a successful antagonist include strong competitive ability, antibiotic production, direct parasitism and lysis which are needed for effective disease control (Ayers and Adams, 1981). Growth of mycelia of *Trichoderma* spp. coiled along and around hyphae of the host fungi (Chet *et al.*, 1981). Penetration of host mycelia may or may not occur, but susceptible hyphae vaculated, collapsed and

finally degenerated. The mycoparasite then grew on the hyphal content.

Elad *et al*. (1982a) showed that *T. harzianum* produced lytic extracellular β-1,3 glucanases and chitinase into the growth medium and even into soil that digest the cell walls of *S. rolfsii* and *R. solani* which are composed of β-1,3 glucans and chitin. Scanning electronmicroscopic and flouresent microscopic studies by Elad *et al*. (1983) also showed that *Trichoderma* spp. attached to the host fungus either by hyphal coils and hooks or appressoria and high β-1,3 glucanase and chitinase activities were detected in dual agar cultures. Several species of *Trichoderma* were tested on PDA against plant pathogenic fungi like *Pythium*, *Verticillium* and *Fusarium* by D'erole *et al*. (1984) and observed four types of antagonism, *viz*., relief formation in the contact zone between the fungi, lytic phenomenon, complete coverage of the pathogen by the antagonist and variable behaviour.

Although production of extracellular cell wall degrading enzymes such as β-1,3 glucanases, chitinases, proteases and lipases has been considered as the main mechanism involved in the biocontrol of fungal pathogens (Cherif and Benhamou, 1990), several workers in the last few years emphasized the role of antifungal molecules also in the antagonistic process of *T. hamatum* (Sivan *et al*., 1984),

T. harzianum (Claydon *et al.*, 1987) and *T. viride* (Dhedhi *et al.*, 1990), Production of isonitrile antibiotic by a mutant strain of *T. harzianum* (Graeme-Cook and Faull, 1991) and homothallin II, a broad spectrum antibiotic against *Pythium*, *R. solani* and *F. oxysporum* by a mutant strain of *T. harzianum* (Faull, 1994) are successful examples of antibiotic activity of biocontrol agents against soilborne plant pathogenic fungi.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Location and soil

All the green house experiments were conducted in the department of Plant Pathology and the field experiment in the garden land of Instructional Farm, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. Red laterite soil of the Instructional Farm, was used in all the trials.

The potting mixture used for the green house studies comprised of soil, sand and farmyard manure in the proportion of 2:2:1(w/w)

3.1.1 Soil sterilization

The sterilization of soil was done by autoclaving at 1.02 kg/cm^2 for one hour.

3.2 Planting material

Seed rhizome of ginger (*Zingiber officinale* R.) variety Rio-De-Janeiro obtained from the Department of Horticulture, College of Agriculture, Vellayani was used as the seed material for the study. The seed rhizome was disinfected by soaking in a solution of mancozeb (0.3 per cent) and Malathion (0.1 per cent) for 30 minutes. The soaked seed

rhizomes were spread in shade to drain excess water. The rhizome bits of 15-20 g each were planted at a depth of 4-5 cm with at least one viable healthy bud facing upwards.

3.3 Survey on AMF colonization and incidence of rhizome rot of ginger

A survey was undertaken during August-September of 1993 in the major ginger growing tracts of Idukki and Wayanad districts of Kerala. Althogether 42 locations (Table 1) were fixed in these areas in consultation with Agricultural Officers of the Department of Agriculture. Root samples and rhizosphere soil of healthy and rhizome rot affected ginger plants were collected from these locations and brought to the laboratory for estimation of spore count and per cent colonization of arbuscular mycorrhizal fungi (AMF). The intensity of rhizome rot of ginger was recorded plot wise by assigning grades, *viz.*, 0 - no disease; 1 - below 25 per cent plants infected; 2 - 26-50 per cent plants infected; 3 - 51-75 per cent plants infected and 4 - more than 75 per cent plants infected.

3.3.1 Estimation of mycorrhizal colonization percentage and intensity

The mycorrhizal colonization percent in the root samples of ginger was estimated following the procedure of

Phillips and Hayman (1970). The root samples were cleaned free of soil particles, cut into one cm bits and fixed in FAA (Formaldehyde - Acetic acid - Alcohol in 5:5:90 proportion) for one day. Roots were then autoclaved for hydrolysing with ten per cent potassium hydroxide solution at 1.02 kg /cm^2 for 15 minutes. The alkalinity of the samples was then neutralised with one per cent hydrochloric acid. Staining was performed by keeping the root bits in 0.05 per cent trypan blue solution in lactophenol reagent (Lactic acid 20 ml; Phenol 20 ml; Glycerol 40 ml and distilled water 40 ml). The stained root bits were arranged on a clean slide, covered with cover slips and scanned under compound microscope for the presence of mycelium, vesicles and arbuscules of the arbuscular mycorrhizal fungi (AMF). The AMF colonization percentage was calculated as given below:

$$\text{AMF colonization percentage} = \frac{\text{Number of root bits positive for AMF infection}}{\text{Total number of root bits investigated}} \times 100$$

A minimum of 24 root bits from each sample were scanned for estimating the AMF colonization percentage.

For estimating the intensity of colonization, one cm size root bits from each sample (10 nos.) were placed on a clean slide, the under surface of which was graduated into

square columns of 2 mm size in the form of a grating by drawing six length wise lines and sufficient number of cross lines across it. The portion of each root bit which falls in each column of the grating in a line (5 segments) was scanned in the microscope for observing mycorrhizal infection. Positive (+) or negative (-) signs were given for presence or absence of mycorrhizal infection in each segment. The total signs (positive and negative) of ten root bits were recorded and the per cent root segment AMF colonization was estimated using the formula:

$$\text{Per cent root segment AMF colonization} = \frac{\text{Total number of positive (+) signs for AMF infection}}{\text{Total number of all the segments scanned in the slide}} \times 100$$

Based on the root segment AMF colonization, the following intensity grades were assigned.

+	-	Less than 25 per cent root segment AMF colonization
++	-	25-50 per cent root segment AMF colonization
+++	-	51-75 per cent root segment AMF colonization
++++	-	Above 75 per cent root segment AMF colonization

3.3.2 Estimation of mycorrhizal spore count

Extramatrical chlamydospores produced by the mycorrhizal fungus was estimated following the wet sieving and decanting method (Gerdemann and Nicolson, 1963). Ten grams of

the rhizosphere soil was collected from each unit and made into a suspension in sufficient quantity of water. The soil suspension was then passed through a series of sieves ranging from 1000, 300, 250, 105 and 45 µm kept one below the other in the same order. Soil and spores collected at the bottom two sieves were transferred on a nylon mesh (45 µm) kept in a petriplate. The plate containing the nylon mesh with the spores were observed under stereomicroscope and recorded the total AMF spore count.

3.4 Isolation and purification of the pathogen

The pathogen from diseased ginger plants was isolated and brought into pure culture in PDA (Potato dextrose agar). Pathogenicity of the isolates was proved by artificially inoculating the culutre bits on healthy seedlings of ginger under aceptic conditions. Among the different isolates the most virulent isolate was obtained from Pannimattam, Idukki district, which was used for all the subsequent studies. The pathogenicity of the fungus was periodically tested on the host plant to ensure the virulence of the culture.

3.5 Symptomatology of rhizome rot of ginger

The manifestation of the symptoms of the disease was studied by observing the development of the disease in the field under natural conditions and by artificial inoculation of

ginger plants grown in green house by the most virulent isolate of the pathogen. The symptoms were recorded.

3.6 Estimation of *Pythium aphanidermatum* population

Fifty mg of the air dried and seived (200 mesh size) soil sample was sprinkled uniformly in sterile petriplates and approximately 20 ml of cooled selective medium (Peethambaran and Singh, 1977) was poured over it. The plates were rotated before solidification of the medium to ensure uniform distribution of the soil particles. The plates were incubated at 25-30°C for three days and the number of colonies formed were counted.

3.7 Mass multiplication of *Pythium aphanidermatum*

The pathogen was mass multiplied in sand oats medium and in ginger rhizome bits.

3.7.1 Sand oats medium

The medium was prepared by mixing washed fine white sand with oats meal in the ratio of 19:1(w|w).Sufficient quantity of water was sprinkled and incorporated to keep the medium moist. The mixture was sterilized in one litre conical flasks at 1.02 kg/cm^2 for one hour. Actively growing culture bits of the pathogen were aseptically introduced in to the flasks and incubated at 25-30°C for two weeks.

3.7.2 Ginger rhizome bits

Healthy, surface sterilized ginger rhizomes were cut into small bits and inoculated with seven-day-old culture of the pathogen and were kept in aseptic moist chamber at room temperature till the rhizome bits were completely covered by the fungal growth. These rhizome bits were then mixed with soil (1 kg rhizome bits and 2 kg soil) and used for pot/field inoculation.

3.8 Effect of different AMF species on growth and rhizome rot of ginger

Six recognized species of AMF obtained from the Mycorrhizae Laboratory of the Department of Plant Pathology, College of Agriculture, Vellayani were used for the preliminary study. The experiment was laid out in Completely Randomized Design with seven treatments and three replications under green house conditions in pots (20x30 cm) filled with sterilized potting mixture. The following were the different treatment combinations

T_1 - *Glomus fasciculatum* + *Pythium aphanidermatum*

T_2 - *G. etunicatum* + *P. aphanidermatum*

T_3 - *G. constrictum* + *P. aphanidermatum*

T_4 - *G. mosseae* + *P. aphanidermatum*

T_5 - *G. monosporum* + *P. aphanidermatum*

T_6 - *Acaulospora morroweae* + *P. aphanidermatum*

T_7 - No AMF + *P. aphanidermatum* (Control)

3.8.1 Mycorrhizal inoculum and inoculation

The inoculum of different species of AMF was maintained in *Panicum maximum* grown in earthen pots. The rhizosphere soil along with root bits, mycelial fragments and AMF spores served as the inoculum. Fifty g of inoculum carrying approximately 350 spores was incorporated to a depth of about 2 cm in the pit in the potting mixture just before planting ginger rhizome at the onset of monsoon.

3.8.2 Inoculation with the pathogen

Twenty-day-old culture of the pathogen, mass multiplied in sand oats medium was inoculated 45 days after planting at the rate of 30 g per pot. The pots were irrigated regularly so as to maintain a very high relative humidity for providing conducive environment for development of the disease. A booster dose of the pathogen in the form of rotten rhizome bits at the rate of 150 g per pot was given 90 days after planting.

3.8.3 Observation on plant growth characteristics

Observations on the important plant growth parameters such as number of leaves, number of tillers and plant height were recorded 30, 60, 90 and 120 days after planting.

3.8.4 Incidence and intensity of rhizome rot

The incidence and intensity of the disease were recorded as and when it appeared. The incidence and infection were determined by recording the number of tillers/hill exhibiting symptoms of the disease. Mortality rate was determined by counting the number of tillers/hill actually dried and dead. The disease severity was determined by assigning grades as detailed below.

0 - No disease

1 - Less than 10 per cent tillers were infected and/or dead.

3 - Between 10-25 per cent tillers were infected and/or dead.

5 - Between 26-50 per cent tillers were infected and/or dead.

7 - Between 51-75 per cent tillers were infected and/or dead.

9 - Above 75 per cent tillers were infected and/or dead.

3.8.5 Yield and dry matter production

Fresh weight of rhizome was recorded at the time of harvest after removing the adhering soil particles and roots. Plant top dry weight was determined after oven drying. The roots were thorougly washed in water for removing the adhering soil particles, oven dried and the root dry weight was determined.

3.8.6 Estimation of AMF colonization, spore count and pathogen population

The per cent AMF root colonization, intensity of colonization and spore count in the rhizosphere soil were estimated as per the procedures given under 3.3.1 and 3.3.2. The population of the pathogen in the rhizosphere soil was estimated as given under 3.6.

3.8.7 Estimation of nutrient content in plant tissues

The nutrient content of plant samples such as P, K, Ca, Mg, Cu, Fe, Zn and Mn were estimated by dry ashing followed by extraction in dilute hydrochloric acid as outlined by Piper (1960). AAS (Model Perkin Elmer PE 3030) was used for the estimation of micronutrients. Phosphorus was estimated colorimetrically by the vanedo-molybdate yellow colour method (Jackson, 1973) and potassium by flame emission method using flame photometer (Systronics model).

3.9 Root knot infestation in ginger as influenced by inoculation with different AMF species

Rootknot nematode infestation was reported to be a predisposing factor for rhizome rot incidence. Hence the effect of AMF colonization on root-knot nematode attack was studied. The experiment was laid out in Completely Randomised Design with seven treatments and three replications in green house. The different treatment combinations were:

T_1 - *Glomus fasciculatum* + *Meloidogyne incognita*

T_2 - *G. etunicatum* + *M. incognita*

T_3 - *G. constrictum* + *M. incognita*

T_4 - *G. mosseae* + *M. incognita*

T_5 - *G. monosporum* + *M. incognita*

T_6 - *A. morroweae* + *M. incognita*

T_7 - No AMF + *M. incognita*

3.9.1 Inoculation with AMF

Inoculation with different AMF species was done before planting rhizome as discussed under 3.8.1.

3.9.2 Inoculation with nematodes

Egg masses of *M. incognita* collected from pure culture plants were used for artificial inoculation. The egg masses were kept in tap water and the freshly hatched second stage larvae were used for inoculation at the rate of one larva per gram of soil 45 days after planting ginger rhizome bits. The larval suspension was poured into small wells made at the active root zone of the plants in pots which were then covered with fine sand and sealed by a thin film of water applied over it.

Observations on growth characteristics of the plants, rhizome yield, biomass production, mycorrhizal colonization, AMF spore count and analysis of nutrient content of plant tissues were recorded as mentioned under experiment 3.8.3 to 3.8.7.

3.9.3 Galling and nematode population

The population of nematodes in the rhizosphere soil was estimated following the Cobb's seiving and decanting technique. Hundred ml of the rhizosphere soil was processed for the study. The nematodes were extracted by this method and their population was estimated after clearing by modified Baermann's technique (petriplate method). The clear suspension thus obtained was used for the estimation. The population was assessed using a steroscopic microscope with 60 X magnification.

For the estimation of population in the root samples, 2 g of the root sample each was cut, washed and cut into small pieces of 2 - 3 cm length and placed in a wire mesh containing moistened double layered tissue paper kept over a petriplate with sterile water as done above. The petriplate was kept for 24-48 hours. Suspension in the petriplate was collected and the population of nematodes in the samples was estimated.

The root knot index (galling index) was computed using 1 - 5 scale as detailed below:

Scale 1 - 0 - 25 galls/root system

Scale 2 - 26 - 50 galls/root system

Scale 3 - 51 - 75 galls/root system

Scale 4 - 76 - 100 galls/root system

Scale 5 - Above 100 galls/root system

3.10 Isolation of native AMF

The spores of the native AMF in the rhizosphere soils of ginger brought from the various locations were isolated following the wet sieving and decanting technique described in para 3.3.2. The AMF spores collected in the nylon mesh were observed in stereomicroscope for viability. The viable spores were then retrieved and allowed to grow and colonize sorghum plants following the funnel technique.

The spores (3 to 4 nos) were isolated and transferred to a filter paper placed at the neck region of a funnel mounted on conical flask. From the tail of the funnel, a cotton wick was connected to the flask containing water for optimum moisture supply. Fine sterilized sand was applied over the spores and sorghum seeds were sown over it so that when the seeds germinated the radicle got infected by the appressorium of the germinating fungal spore. The seedlings with positive AMF infection were transferred to small plastic pots containing sterilized soil after three weeks. The plants were once again checked for AMF colonization and were then transferred to bigger plastic pots for proper growth, multiplication and colonization of AMF in the roots. The native AMF thus isolated were further maintained in larger pots in sterilized sand - soil medium using *Panicum maximum* as host for further studies. The list of native isolates thus developed is given in Table 12.

3.10.1 Native mycorrhizal inoculum and inoculation

The native isolates of AMF were maintained for 4-6 months in *Panicum maximum* grown in sterilized sand - soil mixture in earthen pots of size 20 x 30 cm for the proper colonization and sporulation. The soil along with root bits, mycelial fragments and AMF spores mixed together served as the AMF inoculum. For inoculation 50 gms of the inoculum containing approximately 350 spores was placed 2 cm below the seed rhizome in the pit and thoroughly incorporated with the soil over which the seed rhizomes were planted.

3.11 Characterisation of AMF associated with ginger

The rhizosphere soils of different cultivars of ginger grown in one soil type and in different soil types viz., laterite, sandy and forest, were collected from major ginger growing tracts of Kerala. The AMF spores from these soils were extracted by wet sieving and decanting technique and the spore characteristics were recorded using stereo and reserch microscopes. They were identified following the classification scheme proposed by Schenck and Perez (1988) and by comparing with the photographic slides developed for identification of species of Endogonaceae by Hall and Abbott (1981). Two of the selected native AMF were also identified following the same procedure.

3.12 Isolation of native antagonistic fungi

Fungi from the rhizosphere soil of healthy ginger plants in diseased plantations and from vermicompost were isolated following the serial dilution technique (Timonin, 1940) using Martin's rose bengal agar medium. Samples drawn from 10^6 and 10^4 dilutions were plated in the medium and incubated at room temperature for the development of colonies. The antagonistic fungi were isolated and brought to pure culture and used for further studies (Table 22).

3.13 Estimation of population of antagonists in the soil

Estimation of population of antagonistic fungi in the soil was made using Martin's rosebengal agar medium. For the estimation of *Trichoderma* spp. Trichoderma selective medium was used (Elad and Chet, 1983).

3.14 Mass multiplication of antagonistic fungi

Selected antagonistic fungi from the *in vitro* evaluation were mass multiplied in sand-oats/sand-wheat bran medium for further studies. The medium was prepared by mixing washed fine white sand with oats or wheat bran in the proportion of 9:1. Sufficient quantity of water was incorporated into it to keep the medium moist. The mixture was sterilised in one litre conical flasks at 1.02 kg/cm^2 for one hour. Actively growing culture discs of the respective antagonistic fungi were aseptically introduced into the flasks

and incubated at room temperature for two weeks. The inoculum so prepared was used for the soil inoculation.

3.15 Screening native AMF isolates against rhizome rot of ginger in green house

The native AMF isolates developed and maintained as described in para 3.10 and 3.10.1 were tested for their biocontrol potential against rhizome rot of ginger under green house condition. *G. constrictum*, which was found most effective in the preliminary screening, was used as reference culture. The experiment was laid out in CRD with 16 treatments and three replications. The following were the treatment details.

T_1 - Mi (Mycorrhizal isolate)-1 + *Pythium aphanidermatum*

T_2 - Mi - 2 + *P. aphanidermatum*

T_3 - Mi - 3 + *P. aphanidermatum*

T_4 - Mi - 4 + *P. aphanidermatum*

T_5 - Mi - 5 + *P. aphanidermatum*

T_6 - Mi - 6 + *P. aphanidermatum*

T_7 - Mi - 7 + *P. aphanidermatum*

T_8 - Mi - 8 + *P. aphanidermatum*

T_9 - Mi - 9 + *P. aphanidermatum*

T_{10} - Mi - 10 + *P. aphanidermatum*

T_{11} - Mi - 11 + *P. aphanidermatum*

T_{12} - Mi - 12 + *P. aphanidermatum*

T_{13} - Mi - 13 + *P. aphanidermatum*

T_{14} - Mi - 14 + *P. aphanidermatum*

T_{15} - *G. constrictum* + *P. aphanidermatum*

T_{16} - No AMF culture + *P. aphanidermatum*

Sterilized potting mixture was used for the study. Before planting ginger rhizome bits, the pots were inoculated with the respective AMF inoculum as described in 3.10.1. The rhizome bits were planted at the beginning of the monsoon season.

3.15.1 Inoculation with pathogen

Initial inoculation of *P. aphanidermatum* was given on 45th day of planting using 20-day-old culture, mass multiplied in sand-oats medium and second inoculation was given on 90th day using the pathogen mass cultured in ginger rhizome bits at the rate of 150 g/pot as was done in para 3.8.2.

Growth characteristics of the plant such as number of leaves, number of tillers and height of the plant were recorded on 30th, 60th, 90th and 120th days after planting. The plants were monitored continuously and recorded the incidence and intensity of the disease as mentioned in item 3.8.4. The rhizome yield, plant top and root dry weight, population of the pathogen in the rhizosphere, AMF colonization percentage and spore count and the nutrient content of plant tissue were

recorded as described in 3.8.5, 3.6, 3.3.1, 3.3.2 and 3.8.7 respectively.

3.16 *In vitro* screening of antagonistic fungal isolates against *P. aphanidermatum*

All the 28 isolates obtained from different locations of Kerala (Table 22) were screened along with four reference cultures *viz.*, *Trichoderma viride*, *T. harzianum*, *T. koningii*, *T. pseudokoningii* obtained from Tamil Nadu Agricultural University, Coimbatore. There were 32 treatments (28 isolates + 4 reference and control) in the experiment conducted *in vitro* with 5 replications in CRD. Dual plating technique was employed for testing the efficiency of these organisms in inhibiting the growth of *P. aphanidermatum*.

Observations on the growth of both pathogen and antagonist were taken every day. The plates were incubated for five days and the per cent inhibition, measurement of inhibition zone, degree of antagonism, pattern of growth and production of metabolites by the antagonists were also recorded. The per cent inhibition was calculated using the formula (Klingstrom and Johansson, 1973).

$$\text{Per cent inhibition} = \frac{\text{Growth of pathogen in the control} - \text{Growth of pathogen in the treatment}}{\text{Growth of pathogen in the control}} \times 100$$

Degree of antagonism was measured by assigning the following grades, depending on the extent of over growth, production of metabolite, the extent of lysis and production of inhibition zone.

\+ - low degree of antagonism

++ - medium degree of antagonism

+++ - high degree of antagonism

++++ - very high degree of antagonism

Isolates exhibiting per cent inhibition of 90 or above of the pathogen were selected for further studies. These antagonists were identified at Agarkar Research Institute, Pune (Table 27).

3.17 Screening of antagonistic fungal isolates against rhizome rot pathogen in green house

Antagonistic fungi which were found effective (more than 90 per cent inhibition) *in vitro* were subjected to further screening in green house using the four *Trichoderma* spp. *viz.*, *T. viride*, *T. koningii*, *T. pseudokoningii* and *T. harzianum* as reference.

The experiment was laid out in CRD with 16 treatments and 3 replications. The various treatment combinations are as follows:

T_1 - Ai (antagonistic fungal isolate)-30 + *P. aphanidermatum*

T_2 - *T. koningii* + *P. aphanidermatum*

T_3 - Ai-13 + *P. aphanidermatum*

T_4 - *T. viride* + *P. aphanidermatum*

T_5 - Ai-18 + *P. aphanidermatum*

T_6 - Ai-8 + *P. aphanidermatum*

T_7 - *T. harzianum* + *P. aphanidermatum*

T_8 - Ai-2 + *P. aphanidermatum*

T_9 - *T. pseudokoningii* + *P. aphanidermatum*

T_{10} - Ai-22 + *P. aphanidermatum*

T_{11} - Ai-7 + *P. aphanidermatum*

T_{12} - Ai-19 + *P. aphanidermatum*

T_{13} - Ai-12 + *P. aphanidermatum*

T_{14} - Ai-11 + *P. aphanidermatum*

T_{15} - Ai-6 + *P. aphanidermatum*

T_{16} - No antagonist + *P. aphanidermatum*

Sterilized potting mixture was used for the study. The respective antagonistic fungi were introduced into the pots before planting rhizome bits by applying 35 g each of the sand oats medium grown culture. For the rapid proliferation of the antagonistic fungi, 75 g of sterilized cowdung was also applied as food base to each pot and the fungal culture was thoroughly incorporated with the soil and cowdung. Ginger rhizome bits were planted at the beginning of the south west monsoon. Twenty day old culture of the pathogen (30g per plant)

was inoculated at the base of the plant 45 days after planting. The plants were maintained at very high relative humidity for providing conducive environment for the development of disease. A booster dose of the pathogen was given using rotten rhizome bits at the rate of 150 g per pot 90 days after planting.

Observations on growth characteristics, incidence of rhizome rot, yield and dry weight of plant tissue were recorded as described in 3.8.3, 3.8.4 and 3.8.5.

3.17.1 Estimation of population of antagonists and pathogen

The populations of the antagonistic fungi in the rhizosphere soil were determined following serial dilution technique using Martin's rose bengal agar medium and *Trichoderma* selective medium (Elad and Chet, 1983). Population of pathogen was estimated as described under 3.6.

3.18 Effect of dual inoculation of AMF and antagonists on growth and rhizome rot of ginger in green house

Two isolates each of AMF (Mi-1 and Mi-4) and antagonists (Ai-12 and Ai-13) which were found effective in the green house testing were subjected for further dual inoculation studies in green house. The experiment was laid out in CRD with 9 treatments and 3 replications. The treatment details are given hereunder.

T_1 - A0 x Mi-1 + *P. aphanidermatum*

T_2 - A0 x Mi-4 + *P. aphanidermatum*

T_3 - Ai-13 x M0 + *P. aphanidermatum*

T_4 - Ai-13 x Mi-1 + *P. aphanidermatum*

T_5 - Ai-13 x Mi-4 + *P. aphanidermatum*

T_6 - Ai-12 x M0 + *P. aphanidermatum*

T_7 - Ai-12 x Mi-1 + *P. aphanidermatum*

T_8 - Ai-12 x Mi-4 + *P. aphanidermatum*

T_9 - A0 x M0 + *P. aphanidermatum*

Sterilized potting mixture was used for the study. The plants in the various treatments were inoculated with AMF, antagonistic fungi and pathogen as detailed under 3.8.1., 3.17 and 3.8.2 sections respectively. Observations on growth characteristics, disease incidence and intensity, yield and dry weight of plant tissue were recorded as per descriptions in items 3.8.3., 3.8.4 and 3.8.5. Population of the pathogen was estimated as mentioned in para 3.6 and the antagonistic population as described in 3.13. Estimation of AMF colonization and spore count was done as given in para 3.3.1 and 3.3.2.

3.19 Field testing of dual inoculation effect of AMF and antagonistic fungi on growth and rhizome rot of ginger

The experiment was laid out in a 3^2 factorial RBD with nine treatments and three replications. The various treatment combinations are:

T_1 - A0 x Mi-1 + *P. aphanidermatum*

T_2 - A0 x Mi-4 + *P. aphanidermatum*

T_3 - Ai-13 x M0 + *P. aphanidermatum*

T_4 - Ai-13 x Mi-1 + *P. aphanidermatum*

T_5 - Ai-13 x Mi-4 + *P. aphanidermatum*

T_6 - Ai-12 x M0 + *P. aphanidermatum*

T_7 - Ai-12 x Mi-1 + *P. aphanidermatum*

T_8 - Ai-12 x Mi-4 + *P. aphanidermatum*

T_9 - A0 x M0 + *P. aphanidermatum*

Raised beds of 1 x 1 m size were taken at the beginning of south west monsoon during May. Pits of 5 cm deep at a spacing of 20x20 cm were taken in the raised beds. The inoculum of the selected AMF (50 g) was applied in pits and incorporated with the soil. The selected antagonistic fungi were mass multiplied in sand-wheatbran medium in conical flasks. For field application it was further mass cultured in powdered and sterilized cowdung - neem cake mixture (9:1) by mixing 50 g of the wheat bran culture per kg of the cowdung - neem cake mixture (Sivaprasad, 1995). The mixture was covered with polythene sheets and incubated under moist warm conditions for 10 days (Plate 1, 2). This was used as the antagonistic fungal inoculum. This inoculum was applied prior to planting rhizome bits at the rate of 60 g/pit. The plants in the field were maintained as per Package of Practices Recommendations of Kerala Agricultural University (1995). The plants were

Plate 1. Growth and sporulation of Ai-12 (*Trichoderma viride*) on cowdung - neem cake food base

Plate 2. Growth and sporulation of Ai-13 (*Aspergillus fumigatus*) on cowdung - neem cake food base


ISOLATE NO. 12.


ISOLATE NO 13

artificially inoculated with the pathogen 45 days after planting with sand -oats based culture at the rate of 30 g per plant. A second inoculation with rotten rhizome bits (150 g per plant) was done 90 days after planting.

Observations on the growth (3.8.3) yield and biomass production (3.8.5) and incidence of disease (3.8.4) were recorded. The AMF colonization, spore count, population of the pathogen and antagonists and the nutrient content of plant tissues were estimated as detailed in 3.3.1, 3.3.2, 3.6, 3.13 and 3.8.7 sections respectively.

3.20 Standardisation of mycorrhizal inoculation techniques for ginger

Different methods of inoculation were tested in green house to find out maximum AMF colonization in ginger. The experiment was laid out in CRD with 7 treatments and 4 replications to evaluate the different inoculation methods. The following were the different inoculation methods employed.

T_1 - Cowdung slurry + AMF inoculation

T_2 - Jaggery slurry + AMF inoculation

T_3 - Starch + AMF inoculation

T_4 - Wheat flour gum + AMF inoculation

T_5 - Gum arabic + AMF inoculation

T_6 - Soil inoculation with AMF

T_7 - Check (no AMF)

The AMF inoculum consisted of a mixture of the three recognized AMF species, *viz.*, *G. fasciculatum*, *G. etunicatum* and *G. mosseae*. The seed rhizome was dipped first in the respective thick slurry/adhesive and then rolled in the vermiculate based AMF inoculum and dried in shade for 30 minutes. Observations on the AMF colonization and intensity were recorded at monthly intervals for three months following standard procedures.

3.21 Effect of plant protection chemicals on selected AMF and antagonists

In order to estimate the sensitivity of selected AMF and antagonists to common plant protection chemicals, an experiment was laid out in CRD with 6 treatments and 4 replications in green house. The following are the treatment details.

T_1 - Soil drenching with 0.25 % thiride

T_2 - Soil drenching with 1% bordeaux mixture

T_3 - Soil drenching with 0.15 % Ekaulux

T_4 - Soil application with dimethoate (0.05%)

T_5 - Soil application with carbofuran (0.75 kg a.i/ha)

T_6 - No plant protection chemicals

The antagonist (Ai-12), mass multiplied in sand-wheat bran medium, was incorporated at the rate of 30 g/pit just before planting. Seventy five g of sterilized cowdung was also

incorporated in the pit along with the antagonists. The AMF (Mi-1) was inoculated by adding 50 g of the inoculum just before planting. Two applications of the pesticides were given, the first immediately after planting and the second after one month. Three months after planting the plants were pulled out and observations on colonization, intensity and spore count of AMF and population of the antagonist in the rhizosphere were recorded.

3.22 Statistical analysis

The data generated under the investigation were statistically analysed following analysis of variance (Snedecor and Cochran, 1962).

RESULTS

4. RESULTS

4.1 Natural AMF colonization of ginger and its influence on incidence of rhizome rot

The results of the survey revealed that ginger is a heavily mycorrhizal crop and the per cent root colonization and intensity varied widely with locality. Out of the thirteen samples drawn from healthy areas in Idukki district, seven showed a very heavy per cent AMF colonization of 80 or above and the mean per cent root colonization was 70.5 (Table-1). Nine samples (69%) recorded a very high intensity of colonization. Nine samples were collected from infected locations, out of which four samples showed very high per cent AMF colonization of above 80 and the mean colonization was 71.4 per cent. Only three samples (33.3 per cent) showed very high intensity of colonization. The intensity of colonization was relatively low in samples collected from diseased tracts.

The survey in Wayanad district indicated more convincing results (Table 2). Out of the 14 samples from healthy areas, as high as 13 samples (93 per cent) had a medium or high intensity of colonization and the mean AMF colonization was 69.6 per cent. Out of the 11 samples obtained from the infected areas, only four samples (36.4 per cent) had medium/ high intensity of colonization and the mean AMF colonization was 52.4 per cent.

Table 1 Relationship between AMF colonization and incidence of rhizome rot of ginger in Idukki district of Kerala

Location	AMF colonization (%)	Intensity of colonization	Healthy/ infected crop	Disease intensity
L_1	52.40	++	Infected	2
L_2	95.20	++++	Healthy	0
L_3	54.50	++	Healthy	0
L_4	54.50	+++	Infected	1
L_5	90.00	++++	Healthy	0
L_6	79.10	++++	Healthy	0
L_7	66.70	+++	Healthy	0
L_8	100.00	++++	Infected	2
L_9	47.10	++++	Healthy	0
L_{10}	95.20	++++	Infected	1
L_{11}	43.30	++	Infected	3
L_{12}	95.20	++++	Healthy	0
L_{13}	84.20	+++	Infected	2
L_{14}	66.70	+++	Healthy	0
L_{15}	66.70	+++	Infected	1
L_{16}	82.60	+++	Healthy	0
L_{17}	90.50	++++	Healthy	0
L_{18}	85.00	++++	Healthy	0
L_{19}	90.50	++++	Healthy	0
L_{20}	60.20	++	Infected	2
L_{21}	90.00	++++	Healthy	0
L_{22}	86.30	++++	Infected	1

Table 2 Relationship between AMF colonization and incidence of rhizome rot of ginger in Wayanad district of Kerala

Location	AMF colonization (%)	Intensity of colonization	Healthy/ infected crop	Disease intensity
L_1	34.80	+	Healthy	0
L_2	28.50	+	Infected	1
L_3	58.30	++	Healthy	0
L_4	66.70	++	Healthy	0
L_5	35.00	+	Infected	1
L_6	65.20	++	Healthy	0
L_7	77.20	+++	Infected	2
L_8	79.20	++	Healthy	0
L_9	29.10	+	Infected	1
L_{10}	89.50	+++	Healthy	0
L_{11}	47.80	+	Infected	1
L_{12}	86.90	++	Healthy	0
L_{13}	82.60	+++	Infected	1
L_{14}	90.90	++++	Healthy	0
L_{15}	30.40	+	Infected	1
L_{16}	63.60	++	Healthy	0
L_{17}	87.50	+++	Infected	1
L_{18}	90.90	+++	Healthy	0
L_{19}	63.60	++	Healthy	0
L_{20}	52.10	++	Healthy	0
L_{21}	34.70	+	Infected	1
L_{22}	62.50	++	Healthy	0
L_{23}	43.50	+	Infected	1
L_{24}	70.80	+++	Healthy	0
L_{25}	80.00	++	Infected	1

4.2 Isolation and purification of the pathogen

The causal organism of rhizome rot of ginger was isolated from the diseased plant parts and soil obtained from infected areas in major ginger growing tracts in potato dextrose agar (PDA) and brought to pure culture. Altogether six isolates were obtained from different locations. The isolate obtained from Pannimattam, Idukki district was the most virulent isolate based on its growth on artificial media and its ability to produce typical symptoms on artificial inoculation. This isolate was used for subsequent investigations. The pathogen causing rhizome rot of ginger was identified as *Pythium aphanidermatum*(Edson)Fitz. based on morphological and cultural characteristics.

4.3 Symptomatology

Detailed symptomatology of the disease was studied on plants artificially inoculated with *P. aphanidermatum*. The pathogen produced distinct symptoms on leaves, leaf sheath, collar region, rhizomes and roots of plants depending on the stage of growth (Plate 3, 4).

The disease was destructive during the active tillering and later stages of growth of the plant when the monsoon was at its peak and the weather was most conducive for the development of disease. The first manifestation of the symptom appeared as yellowing of leaves which started from the

Plate 3. Typical symptoms of rhizome rot of ginger

Plate 4. Healthy and rhizome rot affected ginger plant

tip and progressed downwards along the margins while the central portion of the leaf lamina remained green. Gradually yellowing spread to the leaf sheath and other leaves. The discoloured leaves drooped, dried and hung down to the pseudostem. The entire shoot dried in severe cases.

Side by side with the appearance of leaf symptoms, the basal part of the pseudostem (collar region) showed pale and soft translucent water-soaked brown discolouration. The affected tillers broke off at the collar region by a gentle pull leaving the rhizome in the soil. Such affected tillers emitted foul smell at the point of breakage. Corresponding with these symptoms the disease developed in the rhizome and roots. As the pathogen proliferated in the parenchymatous tissues of rhizome, it became discoloured and gradually decomposed into a watery mass of putrefying tissues emitting foul smell enclosed by the tough rind of the rhizome. The fibrovascular tissues of rhizome were not affected by the pathogen which remained isolated among the decaying mass of rhizomes. Plants failed to produce rhizome when infected early. The roots of rhizomes were also infected and decayed.

4.4 Effect of different AMF species on growth and rhizome rot of ginger

Six AMF species were tested for their efficiency in promoting plant growth, yield and in suppressing rhizome rot of ginger.

4.4.1 Growth characteristics

Inoculation of *Glomus eutunicatum* produced significantly higher number of leaves (23.67) as compared to control (13.33) at 30 DAP (Table 3). However, the effect could not be observed during the subsequent growth stages. During the later stages of growth (120 DAP) *A. morroweae*, *G. fasciculatum* and *G. constrictum* produced more number of leaves of 119.69, 118.69 and 107.33 respectively as against 92.63 in control plants. More number of tillers of 12.7, 12 and 11.7 were produced by *A. morroweae*, *G. fasciculatum* and *G. constrictum*, respectively, as against 9.3 of control plants. With respect to height of plants, *G. etunicatum* consistently proved to be significantly superior in enhancing plant height throughout the growth stages while *G. fasciculatum*, *G. constrictum* and *G. mosseae* recorded significantly higher plant height from 60th day onwards. On 120 DAP, the maximum plant height of 36.33 cm was recorded in *G. mosseae* inoculation as against 25.67 cm in control plants (Plate 5).

4.4.2 Incidence and intensity of rhizome rot

The ability of AMF to suppress the disease incidence and intensity varied with the AMF species. The per cent tillers infected in *G. constrictum* (0.76) and *G. mosseae* (1.15) inoculated treatments were significantly low compared to control (24.23). No tiller was dead in the above two

Plate 5. Growth response of ginger inoculated with different AMF species


GnF
GnE
GnC
GnK
AT
GnT
CON

Table 3 Effect of different AMF species and *Pythium aphanidermatum* on growth characteristics of ginger

Treatment	Number of leaves Days after planting				Number of tillers Days after planting				Height of the plant Days after planting (cm)			
	30	60	90	120	30	60	90	120	30	60	90	120
G. fasciculatum	18.33	72.00	95.00	118.67	3.00	9.00	10.67	12.00	25.33	33.33	34.00	35.67
G. etunicatum	23.67	73.00	84.33	97.00	3.00	6.67	7.67	8.67	29.33	33.33	34.33	35.67
G. constrictum	20.67	64.33	86.00	107.33	3.33	6.00	8.33	11.67	26.67	31.67	32.67	33.67
G. mosseae	10.67	52.33	74.33	95.00	1.67	5.67	7.33	9.33	17.00	32.67	34.67	36.33
G. monosporum	13.67	51.67	77.67	102.00	1.67	6.33	8.33	10.33	16.67	27.67	29.67	32.00
A. morroweae	17.67	70.67	98.00	119.67	2.67	8.00	10.67	12.67	17.00	24.00	28.00	30.67
Control	13.33	53.00	73.33	92.33	2.00	6.33	8.00	9.33	14.33	21.83	24.67	25.67
CD (0.05)	8.96	NS	NS	NS	NS	NS	NS	NS	13.00	9.51	7.68	6.10

treatments while the per cent mortality in the control was 8.45 (Table 4). The disease intensity was also significantly reduced in *G. constrictum* (0.11) and *G. mosseae* (0.11) inoculated treatments than control (1.55). There was no significant difference between *G. constrictum* and *G. mosseae* inoculated treatments in any of the disease reducing parameters. But these two treatments were significantly superior in reducing the incidence and intensity of the disease when compared to *G. fasciculatum* and *G. etunicatum*. The *G. etunicatum* treatment recorded highest disease incidence and intensity over control.

4.4.3 Dry matter production and yield

Inoculation of *G. fasciculatum* gave a significantly higher yield of 160.00 g/plant as against 100.33 g of uninoculated control (Table 5). Although not significant, *G. constrictum* inoculated treatment also gave considerably higher yield of 154.00 g/plant.

Maximum plant top dry weight of 10.17 g was recorded for *G. monosporum* inoculated plants as compared to 8.97 g of control. *G. fasciculatum*, *G. constrictum* and *G. mosseae* inoculated treatments also gave remarkably higher values for plant top dry weight. All the AMF inoculated plants showed a general increase in plant dry weight. However, the treatment effects were not statistically significant (Table 5).

Table 4 Effect of different AMF species and *Pythium aphanidermatum* on the incidence of rhizome rot of ginger in green house

Treatments	Disease incidence		
	Infected tillers (%)	Tillers dead (%)	Intensity score (0-9 scale)
G. fasciculatum	24.72 (17.50)	13.80 (5.69)	8.56 (2.22)
G. etunicatum	28.90 (23.38)	19.19 (10.81)	8.73 (2.41)
G. constrictum	5.00 (0.76)	0.0 (0)	1.91 (0.11)
G. mosseae	6.14 (1.15)	0.0 (0)	1.91 (0.11)
G. monosporum	14.99 (6.70)	1.91 (0)	3.82 (0.65)
A. morroweae	23.00 (15.53)	16.02 (7.62)	8.45 (1.55)
Control	29.48 (24.23)	16.89 (8.45)	7.15 (1.55)
CD (P = 0.05)	17.49	7.13	0.94

Values in parenthesis denote transformed means

Table 5 Effect of different AMF species and pathogen inoculation on dry matter production and yield of ginger in green house

Treatment	Dry weight plant top ($g\ pl^{-1}$)	Dry weight of roots ($g\ pl^{-1}$)	Rhizome yield ($g\ pl^{-1}$)
G. fasciculatum	9.03	10.13	160.00
G. etunicatum	5.40	8.60	130.00
G. constrictum	9.03	10.20	154.00
G. mosseae	9.03	9.27	138.33
G. monosporum	10.17	12.53	115.83
A. morroweae	8.80	10.00	106.67
Control	8.97	8.73	100.33
CD (P = 0.05)	NS	NS	57.49

There was no significant difference among the treatments and control with regard to dry weight of roots. However, *G. monosporum* inoculated plants gave the highest value of 12.53 g which was followed by 10.20 g, 10.13 g and 10.00 g in *G. constrictum*, *G. fasciculatum* and *A. morroweae* inoculated treatments, respectively, whereas control treatment gave 8.7 g root dry weight (Table 5).

4.4.4 Mycorrhizal colonization, spore count and pathogen population

The data on the AMF colonization, spore count and pathogen population are presented in Table 6. Maximum per cent AMF root colonization of 95.8 was observed in *G. constrictum* inoculated plants followed by 91.7 per cent in *G. mosseae* and 87.5 per cent in *G. fasciculatum* inoculated plants. Very high intensity of colonization was also observed in these treatments. All the other treatments also had higher per cent root colonization and intensity. *G. mosseae* inoculated plants had the maximum AMF spore count of 52 numbers (Table 6) while 49 and 48 spores were retrieved from *G. monosporum* and *G. constrictum* inoculations respectively. *G. etunicatum* inoculated plants recorded the lowest AMF spore count of 36.

There was a general reduction in pathogen population due to AMF inoculation (Table 6). The least pathogen population of 28 cfu/50 mg soil was recorded in *G. constrictum*

Table 6 Effect of different AMF species and *Pythium aphanidermatum* on AMF colonization and pathogen population in ginger

Treatment	Per cent AMF colonization	Intensity of colonization	AMF spore count ($10g^{-1}$ soil)	Pathogen population (cfu/50mg soil)*
G. fasciculatum	87.5	++++	44	48
G. etunicatum	79.2	+++	36	67
G. constrictum	95.8	++++	48	28
G. mosseae	91.7	++++	52	36
G. monosporum	83.3	+++	49	54
A. morroweae	70.8	+++	41	59
Control	0	0	0	78

Mean of three replications

inoculated treatment as against 78 cfu in the control treatment. *G. mosseae* inoculated treatment also recorded a low pathogen population of 36 cfu. The reduction in pathogen population of *G. etunicatum* treatment was insignificant compared to control. However, the other treatments had a pathogen population less than that of control.

4.4.5 Nutrient content of plant tissues

Chemical analysis of plant top indicated that inoculation of ginger plants with different AMF species consistently increased phosphorus content compared to nonmycorrhizal plants (Table 7). Maximum phosphorus content was observed in plants inoculated with *A. morroweae* (463.8 ppm) followed by *G. mosseae* (452.8 ppm), *G. constrictum* (439.0 ppm) and *G. monosporum* (411 ppm) as against nonmycorrhizal control plants (241.7 ppm). With respect to plant top potassium content, none of the treatments demonstrated much variation. Maximum K content of 0.214 per cent was observed in plants inoculated with *G. mosseae* whereas in nonmycorrhizal plants the K content was 0.175 per cent (Table 7).

Estimation of micronutrients revealed that there was significant variation among various treatments (Table 7). *G. constrictum* inoculated treatment recorded the maximum Cu content of 1.6 ppm followed by 1.5 ppm in *G. mosseae* and 1.2 ppm in *G. monosporum* inoculated treatment which were all

Table 7 Effect of different AMF species and *Pythium aphanidermatum* on phosphorus, potassium and micronutrient content of plant top in ginger

Treatment	P (ppm)	K (%)	Cu (ppm)	Ca (ppm)	Mg (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)
G. fasciculatum	363.9	0.189	1.03	800.0	2249.1	22.8	21.8	149.2
G. etunicatum	347.2	0.192	1.00	661.7	2257.5	13.0	17.1	133.3
G. constrictum	439.0	0.204	1.60	965.8	2300.8	24.4	25.4	194.0
G. mosseae	452.8	0.214	1.50	559.2	2257.5	16.0	17.0	139.2
G. monosporum	411.1	0.198	1.20	603.3	2270.8	13.5	15.4	122.5
A. morroweae	463.8	0.204	1.10	790.8	2270.0	14.8	16.9	155.8
Control	241.7	0.175	0.95	605.8	2236.7	13.6	16.2	139.2
CD (P = 0.05)	88.94	NS	0.23	263.74	55.55	5.74	4.34	46.48

significantly higher than 0.95 ppm of control treatment. Maximum Ca, Mn and Zn content of 965.8 ppm, 24.4 ppm and 25.4 ppm were also observed in *G. constrictum* inoculated treatments which was closely followed by *G. fasciculatum* in which the values were 800 ppm, 22.8 ppm and 21.8 ppm, respectively. These values were significantly higher than the corresponding values of 605.8 ppm, 13.6 ppm and 16.2 ppm in control treatment. *G. constrictum* inoculated treatment recorded significantly higher Mg content (2300.8 ppm) than control (2236.7 ppm). Maximum Fe content of 194 ppm obtained in *G. constrictum* inoculated plants was significantly higher than other treatments and control. In general, study on the nutrient analysis of plant tissue indicated that *G. constrictum* recorded maximum nutrient content in the plant tissue.

4.5 Effect of AMF species on growth and root knot infestation in ginger

4.5.1 Growth characteristics

The data on the growth characteristics of plants inoculated with AMF and *Meloidogyne incognita* revealed that there was no significant difference between treatments and control with respect to the number of leaves and number of tillers produced by the plants throughout all the growth stages. However, the mycorrhizal plants had a general increase in growth characteristics particularly in *A. morroweae* (Table 8).

Table 8 Effect of different AMF species and *M. incognita* on growth characteristics of ginger

Treatments	Number of leaves Days after inoculation				Number of tillers Days after inoculation				Height of the plant Days after inoculation (cm)			
	30	60	90	120	30	60	90	120	30	60	90	120
G. fasciculatum + *M.i*	10.67	16.67	41.33	64.67	2.00	3.33	5.00	7.00	19.33	22.00	28.33	30.33
G. etunicatum + *M.i*	21.00	30.67	64.67	86.00	2.67	4.33	6.67	9.00	29.33	38.67	41.00	41.33
G. constrictum + *M.i*	17.67	25.00	64.67	85.67	2.00	4.00	7.33	9.33	22.00	26.67	33.00	35.00
G. mosseae + *M.i*	22.00	31.00	69.33	87.00	2.67	4.33	6.33	8.33	28.67	35.00	37.00	37.67
G. monosporum + *M.i*	18.67	26.67	64.33	86.33	2.33	3.67	8.00	10.00	16.00	19.00	28.67	31.33
A. morrowiae + *M.i*	25.33	34.33	90.00	102.33	2.67	4.33	9.67	11.00	21.00	28.33	35.00	35.67
Control + *M.i*	18.00	25.33	62.33	77.33	2.67	3.67	7.33	9.00	22.00	27.33	34.33	35.33
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	6.519	11.298	NS	NS

M.i - *Meloidogyne incognita*

4.5.2 Yield and Dry matter production

Although none of the dual inoculation treatments with AMF and *M. incognita* could significantly enhance the rhizome yield over inoculation with *M. incognita* alone, *A. morroweae* and *G. mosseae* along with *M. incognita* inoculated plants gave the best yields of 98.7 g and 97 g of rhizome than 70 g in the control (Table 9). The other AMF cultures did not induce any appreciable level of influence on the rhizome yield in the presence of *M. incognita*. Similarly, there was not any significant difference between treatments and control with regard to dry weight of plant top and roots (Table 9). *A. morroweae* proved its superiority over other cultures in the presence of *M. incognita* by giving highest dry weight of plant top (8.23 g) and roots (7.06 g) as against 6.70 g and 5.13 g of control.

4.5.3 Effect of mycorrhizal inoculation on AMF colonization, galling and nematode population

A higher mycorrhizal colonization per cent of 91.6 and 87.5 were recorded in *G. mosseae* and *A. morroweae* in the dual inoculation with *M. incognita* (Table 10). The intensity of colonization was also very high in these treatments. All the other AMF cultures also recorded high per cent colonization. A maximum AMF spore count of 60 could be retrieved from the *G. mosseae* treatment. The study indicated

Table 9 Effect of different AMF species and root-knot nematode on dry weight and yield of ginger

Treatment	Dry weight of plant top ($g\ pl^{-1}$)	Dry weight of roots ($g\ pl^{-1}$)	Rhizome yield ($g\ pl^{-1}$)
G. fasciculatum + *M.i*	6.20	4.83	50.43
G. etunicatum + *M.i*	6.43	5.70	83.33
G. constrictum + *M.i*	7.53	4.83	76.00
G. mosseae + *M.i*	6.80	6.23	97.00
G. monosporum + *M.i*	7.20	4.30	60.33
A. morroweae + *M.i*	8.23	7.06	98.67
Control + M.i	6.70	5.13	70.33
CD (P = 0.05)	NS	NS	NS

Mean of three replications
M.i - *Meloidogyne incognita*

Table 10 Effect of different AMF species and *M. incognita* on AMF colonization and nematode infestation

Treatment	Per cent AMF colonization	Intensity of colonization	AMF spore count ($10g^{-1}$ soil)	Root knot index (Scale)	Nematode population	
					Root (5^{-1} g)	Soil (100^{-1} ml)
G. fasciculatum + *M.i*	75.6	+++	31	3	8	43
G. etunicatum + *M.i*	66.4	+++	43	2	10	62
G. constrictum + *M.i*	71.5	+++	32	3	9	69
G. mosseae + *M.i*	91.6	++++	60	2	12	60
G. monosporum + *M.i*	69.8	+++	46	2	6	48
A. morroweae + *M.i*	87.5	++++	48	2	11	53
Control + M.I	0	0	0	3	19	137

Mean of three replications
M.i - *Meloidogyne incognita*

that there was not much variation in root knot index in dual inoculation and inoculation with *M. incognita* alone. Lower root knot index was recorded in *G. etunicatum*, *G. mosseae*, *G. monosporum* and *A. morroweae* inoculations. With regard to nematode population the mycorrhizal treatment had relatively low number of nematodes in the root and soil compared to control plants (Table 10). The numbers of nematodes recorded in the root tissues of ginger inoculated with *G. monosporum* (6), *G. fasciculatum* (8) and *A. morroweae* (11) were remarkably less than that of control which recorded a nematode population of 19. The populations of nematodes in the soil were also drastically reduced in *G. fasciculatum* (43) and *G. monosporum* (48) inoculated treatments unlike the control treatment (137). In all the mycorrhiza and nematode dual inoculation treatments, the nematode populations were comparatively less.

4.5.4 Nutrient content of plant tissue

All the AMF inoculated plants had higher P content than control. The P content was significantly higher in *G. constrictum* (297.2 ppm) and *G. mosseae* (308.2 ppm) inoculated treatments (Table 11). The per cent K content of 0.235 was found to be maximum in *G. mosseae* inoculated plants. However there was no significant difference between different treatments and control with regard to K content (Table 11).

Table 11 Effect of different AMF species and *M. incognita* on phosphorus, potassium and micronutrient content of plant top of ginger

Treatment	P (ppm)	K (%)	Cu (ppm)	Ca (ppm)	Mg (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)
G. fasciculatum + *M.i*	247.2	0.208	1.03	878.3	2248.3	14.0	17.6	163.3
G. etunicatum + *M.i*	219.5	0.190	1.09	910.8	2267.5	13.9	13.1	160.0
G. constrictum + *M.i*	297.2	0.170	1.13	1074.2	2237.5	10.9	21.8	193.0
G. mosseae + *M.i*	308.3	0.235	1.16	955.8	2226.0	14.4	40.0	185.9
G. monosporum + *M.i*	288.9	0.232	1.43	823.3	2203.3	11.1	23.7	132.3
A. morroweae + *M.i*	283.3	0.226	1.83	787.2	2215.8	12.2	17.6	120.0
Control + M.i	188.9	0.187	1.13	792.8	2259.5	12.0	30.2	158.2
CD (P = 0.05)	102.723	NS	0.354	NS	NS	NS	NS	NS

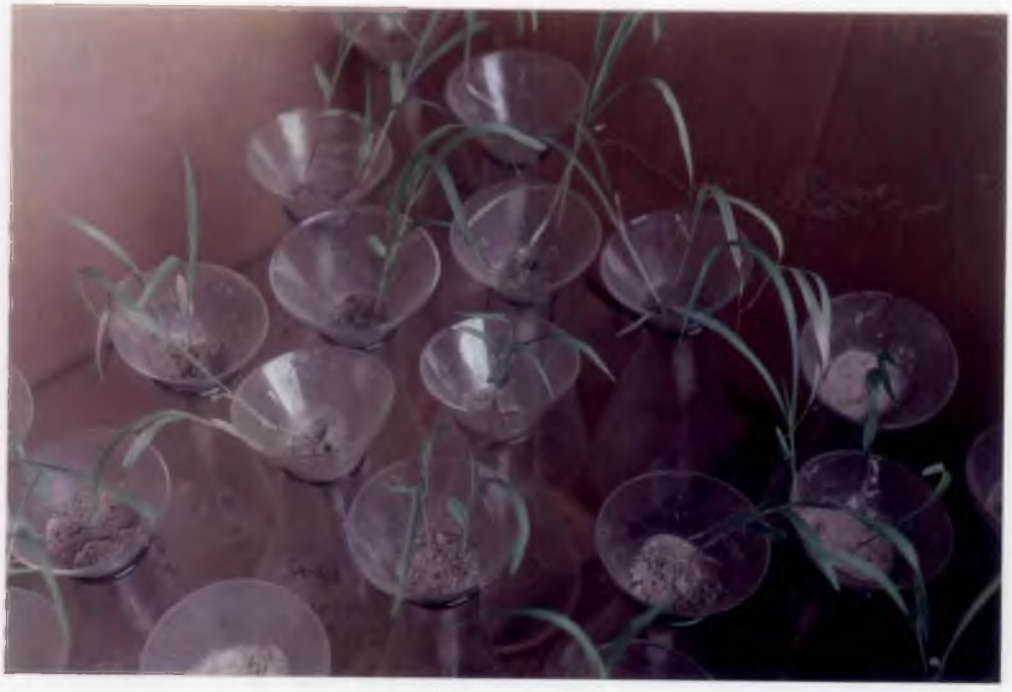
M.i - *Meloidogyne incognita*

Cu content of plant top was greatly influenced by AMF inoculation along with root knot nematode. Maximum Cu content of 1.8 ppm was observed in *A. morroweae* inoculated plants which was significantly higher than the other treatments and control. Ca content was found to be substantially higher in *G. constrictum*, *G. mosseae* and *G. etunicatum* inoculated plants with respective values of 1074 ppm, 955.8 ppm and 910.8 ppm while the non treated control plants recorded a Ca content of 792.8 ppm. Mycorrhizal inoculation along with root knot nematode did not influence the Mg content of plant top significantly. *G. mosseae* inoculated plants had the highest Zn content (14.4 ppm) which was closely followed in *G. fasciculatum* (14 ppm) and *G. etunicatum* (13.96 ppm) inoculated plants. However these values were not significantly different from that of non mycorrhizal *M. incognita* inoculated plants. AMF inoculation along with *M. incognita* did not exert any significant influence on the Mn and Fe content of the plant top. However *G. constrictum* and *G. mosseae* along with nematode inoculation recorded higher Fe content than the control.

4.6 Isolation and maintenance of native AMF

Arbuscular mycorrhizal fungal chlamydospores were isolated from the rhizosphere soil of healthy ginger plants from different locations of ginger growing tracts of Kerala by the funnel technique (Plate 6) and maintained in the green

Plate 6. Multiplication of AMF spore in sorghum by funnel technique



house in large pots for six months. Altogether there were 14 cultures (Table 12) and these cultures served as the native AMF inoculum for further studies.

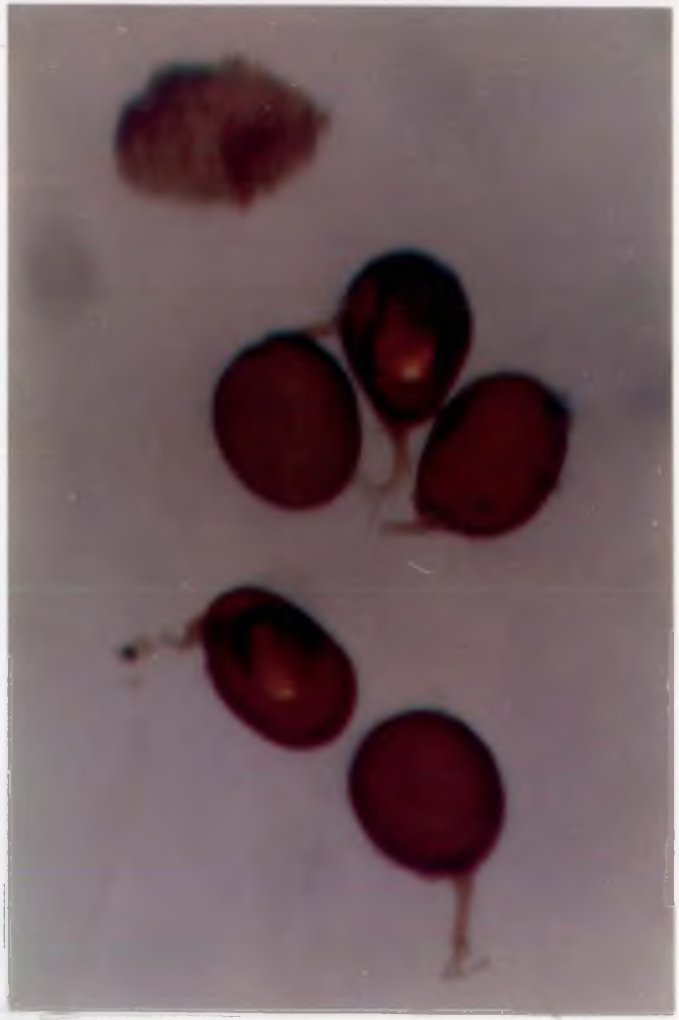
4.7 Characterisation of AMF associated with ginger

Out of the nine cultivars of ginger grown at Vellanikkara, Thrissur, all were found to be colonized with *Glomus* spp. and eight were particularly colonized with *G. fasciculatum* (Table 13). Other genera found associated with different cultivars were *Sclerocystis* and *Gigaspora* spp. (Plate 7-C) However, *Acaulospora* sp. was not observed in any of the cultivars grown.

Study of samples collected from different locations and soil types showed a clear variation in the AMF species associated with ginger with respect to soil type and location (Table 14). *Glomus* spp. particularly *G. fasciculatum* was observed as a predominent AMF in all the soil types (Plate 7-B, E). In sandy loam (Ernakulam district), species of *Gigaspora* and *Acaulospora* were more frequently observed (Plate 7-A). The genus *Acaulospora* was notably absent in lateritic soil. *Sclerocystis clavisporum* and *S. corrymioides* were mainly observed in forest soils while in other soils they were less frequently observed (Plate 7-D, F). In the forest soil of Pampadumpara, Idukki district, *G. convolutum* was frequently associated with ginger.

Plate 7. A - *Acaulospora* sp(×100)

Plate 7. B - *Glomus* sp(×100)



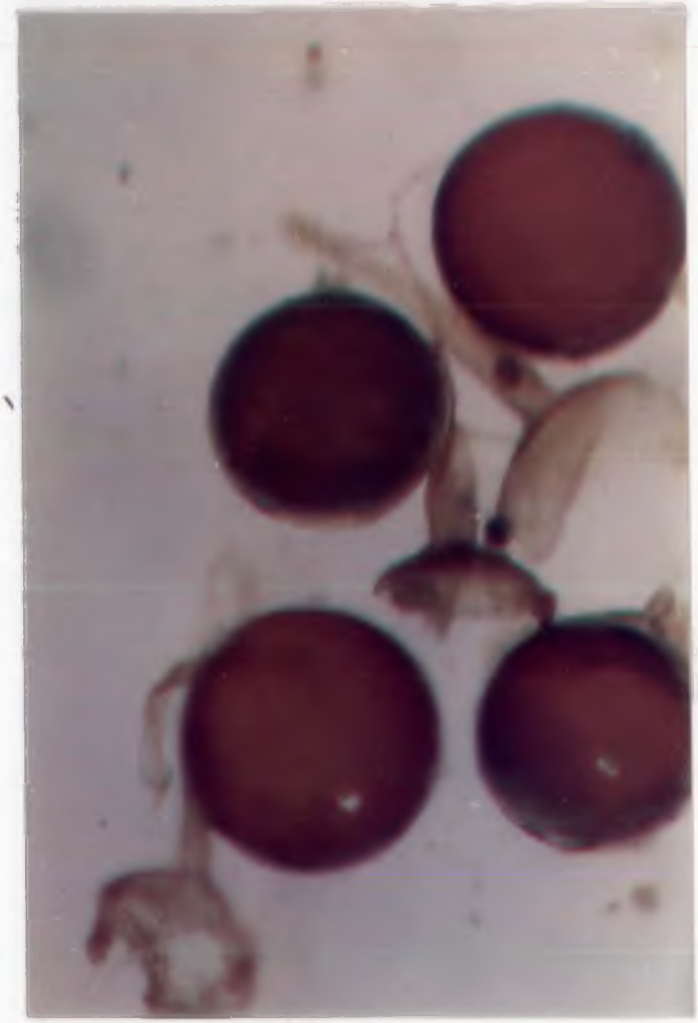
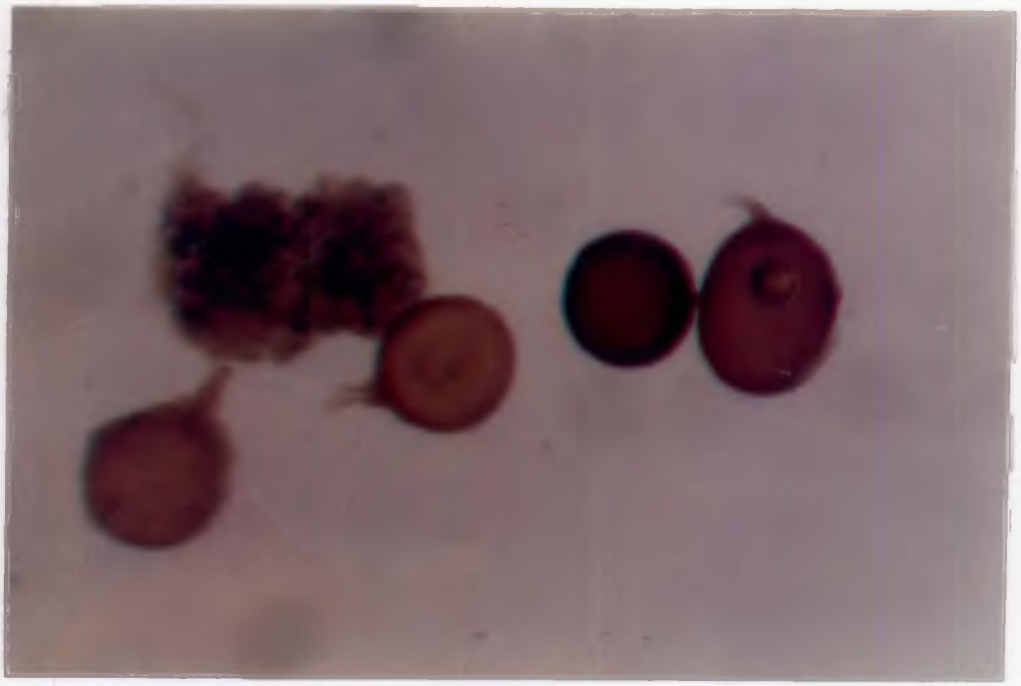


Plate 7. C - *Glomus* sp. and *Sclerocystis* sp (X100)

Plate 7. D - *Sclerocystis coremioides* (X100)



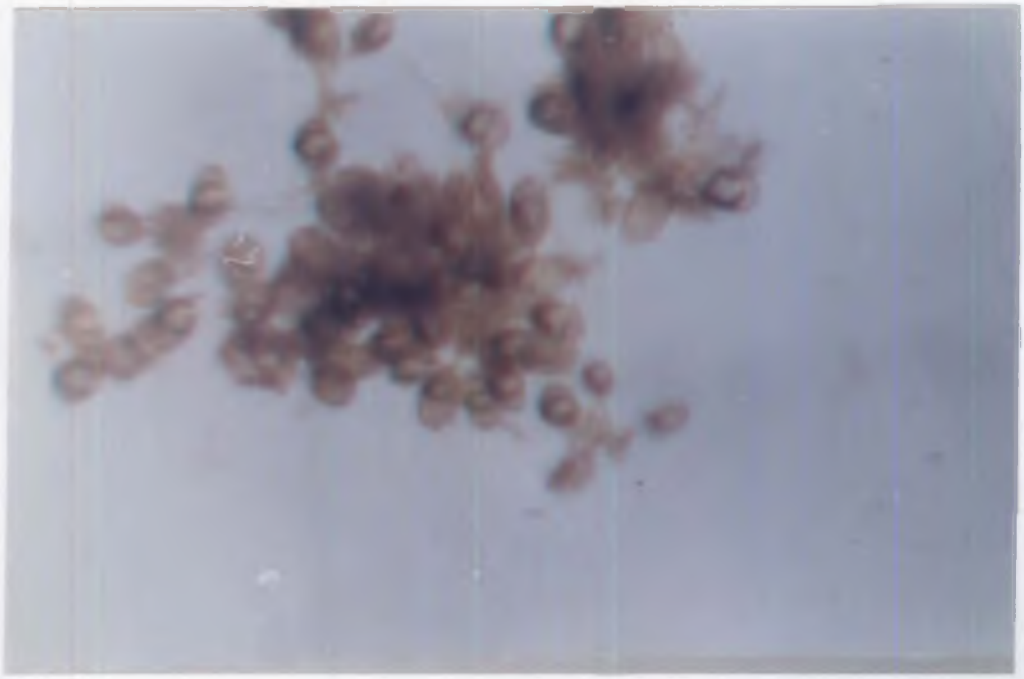


Plate 7. E - *Glomus* sp(X100)

Plate 7. F - *Sclerocystis clavispora* (X100)
(with broken sporocarp)

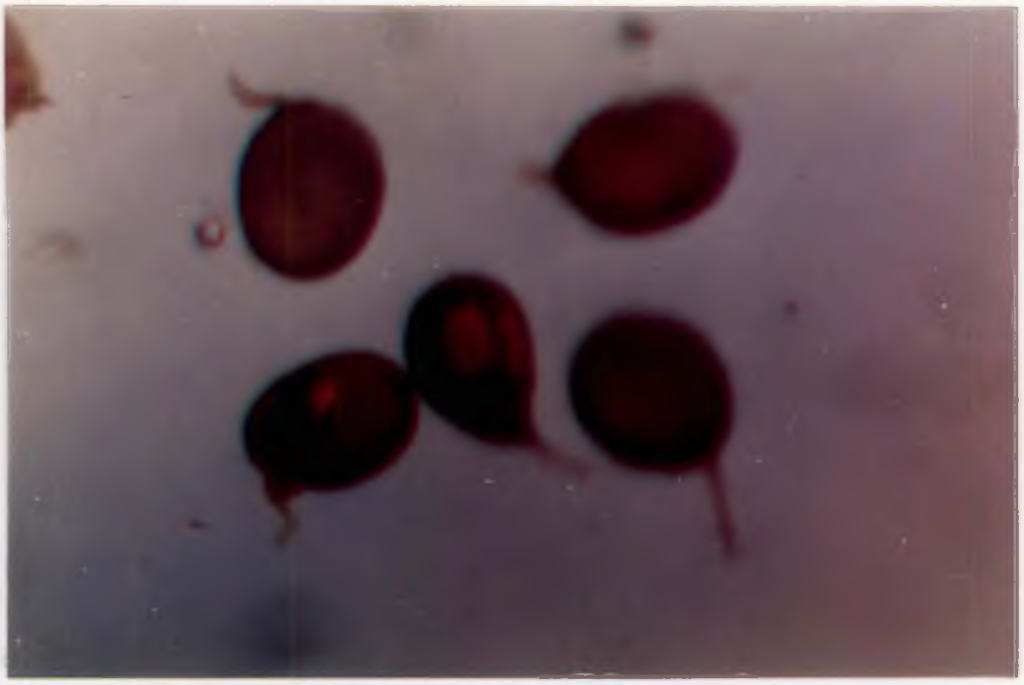


Table 12 Native AMF developed from rhizosphere of ginger

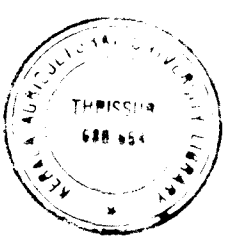
Sl. No.	Name of the isolate	Name of location from where obtained	Soil type
1.	Mi-1	Pulppally, Wayanad	Forest
2.	Mi-2	Odakkali, Ernakulam	Forest
3.	Mi-3	Vellanikkara, Thrissur	Laterite
4.	Mi-4	Adimali, Ernakulam	Forest
5.	Mi-5	Thrissur	Laterite
6.	Mi-6	Thodupuzha, Idukki	Laterite
7.	Mi-7	Ambalavayal, Wayanad	Forest
8.	Mi-8	Karimkunnam, Thodupuhza	Laterite
9.	Mi-9	Peringammala, Thiruvananthapuram	Forest
10.	Mi-10	Paika, Kottayam	Laterite
11.	Mi-11	Mananthavady, Wayanad	Forest
12.	Mi-12	Pannimattom, Idukki	Laterite
13.	Mi-13	Kumaramangalam, Idukki	Laterite
14.	Mi-14	Vaithiri, Wayanad	Forest

Table 13 AMF associated with ginger cultivars grown in the same field

Cultivar	Location	AMF species
Bajpai	Thrissur (Vellanikkara)	*Glomus fasciculatum*, *G. multicaule*, *G. mosseae*, *Glomus* sp.
Kuruppampady	Thrissur (Vellanikkara)	*Gigaspora nigra*, *Gigaspora* sp, *S. coremioides*, *scleroystis* sp., *Glomus* sp.
Wayandu local	Thrissur (Vellanikkara)	*G. fasciculatum*, *Glomus* sp., *Gigaspora nigra*, *Sclerocystics* sp.
Valluvanad	Thrissur (Vellanikkara)	*G. fasciculatum*, *Glomus mosseae*, *Glomus* sp., *Gigaspora* sp.
Jorhat	Thrissur (Vellanikkara)	*G. fasciculatum*, *S.coremioides*, *Glomus* sp., *Gigaspora* sp.
Bardwan	Thrissur (Vellanikkara)	*G. fasciculatum*, *G.monosporum*, *Glomus* sp., *Gigaspora migra*, *Gigaspora* sp.
Juggijan	Thrissur (Vellanikkara)	*G.fasciculatum*, *Glomus* sp., *Sclerocystis* sp., *Glomus* sp.
P.G.S. 35	Thrissur (Vellanikkara)	*G.fasciculatum*, *G.macrocarpum*, *Glomus* sp., *Gigaspora nigra* var. *geosporum*, *Gigaspora* sp.
Eranadan	Thrissur (Vellanikkara)	*G.fasciculatum*, *G.mosseae*, *Glomus* sp., *Sclerocystis* sp., *Gigaspora* sp.

Table 14 AMF associated with ginger grown in different locations

Location	Soil type	AMF species
Thrissur (Vellanikkara)	Laterite	*S.clavispora*, *G.fasciculatum*, *Glomus* sp.
Thiruvananthapuram (Vellayani)	Laterite	*Gigaspora nigra*, *Gigaspora* sp., *G.fasciculatum*, *G.etunicatum*, *Glomus* sp.
Kollam (Kundara)	Laterite	*Gigaspora* sp., *Glomus* sp.
Ernakulam (Odakkali)	Sandy loam	*Cultivar chinese*: *Gigaspora* sp., *Glomus* sp., *Acaulospora* sp.
Ernakulam (Odakkali)	Sandy loam	*Cultivar local* : *G. fasciculatum*, *G. etunicatum*, *Glomus* sp., *Gigaspora nigra*, *S. clavispora*, *Acaulospora* sp.
Ernakulam	Laterite	*Gigaspora nigra*, *G. fasciculatum*, *Glomus* sp.
Wayanad (Ambalawayal)	Forest soil	*G. fasciculatum*, *G. monosporum*, *Glomus* sp., *S. clavispora*, *Acaulospora* sp., *Gigaspora* sp.
Iudkki (Pampadumpara)	Forest soil	*G. fasciculatum*, *G.convulutum*, *Glomus* sp., *S. coremioides*, *G.macrocarpum* var. *geosporum*, *Gigaspora* sp.



4.8 Screening native AMF isolates for growth and disease suppression of rhizome rot of ginger

4.8.1 Effect of growth

Data on the effect of inoculation of different native AMF on the growth characteristics at various growth stages of ginger are presented in Table 15. At 30 DAP, Mi-11 (mycorrhizal isolate - 11) inoculated plants produced significantly higher number of leaves (25.7) over control treatment (15). The number of leaves produced on 60th day were 117.7, 115, 105, 102, 96 and 91 in the treatments inoculated with mycorrhizal isolates Mi-13, Mi-2, Mi-1, Mi-6, Mi-11 and Mi-3 respectively, whereas it was only 50 in the non mycorrhizal control. On 90 DAP, Mi-1, Mi-2 and Mi-13 inoculated plants were more stimulatory in producing significantly higher number of leaves of 173.7, 172.3 and 163 in comparison to 102.7 number of leaves in control treatment. Only two isolates, *viz.*, Mi-13 (213) and (Mi-1) (202) produced significantly higher number of leaves over control (131) on 120 DAP. Although the effect varied widely, inoculation with all the isolates recorded a higher leaf number over control.

Mycorrhizal inoculation did not influence the number of tillers produced at 30 DAP whereas, there was significant variation at 60 DAP. The number of tillers produced by Mi-13, Mi-11, Mi-2 and Mi-1 inoculated treatments were 11, 10.7, 10.3

Table 15 Effect of native AMF and *P. aphanidermatum* inoculation on growth of ginger in the green house

Treatment	Number of leaves Days after planting				Number of tillers Days after planting				Height of the plant (cm) Days after planting			
	30	60	90	120	30	60	90	120	30	60	90	120
Mi-1	23.7	105.7	173.7	202.7	5.0	10.0	14.0	15.3	20.3	34.7	46.8	54.3
Mi-2	24.3	115.0	172.3	184.7	5.0	10.3	13.0	13.7	20.7	32.7	43.7	52.7
Mi-3	23.3	91.0	134.3	156.7	4.7	8.7	11.0	11.7	27.0	42.7	55.0	58.3
Mi-4	15.7	83.7	127.7	153.7	3.3	8.0	10.3	12.7	21.3	34.7	47.0	53.7
Mi-5	13.3	81.7	140.0	159.0	3.7	7.7	10.3	11.0	21.0	35.3	49.0	54.7
Mi-6	17.7	102.0	139.7	159.0	3.7	9.7	12.3	14.0	28.0	40.3	49.7	53.3
Mi-7	13.7	60.0	117.0	133.7	2.7	6.0	8.7	8.7	19.3	37.3	49.0	55.0
Mi-8	16.0	80.0	128.7	160.3	2.7	8.3	11.0	11.7	18.0	35.3	45.7	49.3
Mi-9	12.0	68.0	114.7	153.3	3.7	7.7	10.7	12.0	17.3	36.0	48.3	50.7
Mi-10	16.3	80.7	140.3	157.7	3.0	9.7	13.0	13.3	24.0	34.7	43.3	46.3
Mi-11	25.7	96.0	153.7	185.3	4.3	10.7	13.7	15.7	24.0	39.0	47.7	52.0
Mi-12	16.7	81.7	119.0	153.0	2.7	8.3	11.3	13.3	24.0	35.0	46.7	50.7
Mi-13	22.3	117.7	163.3	213.3	4.3	11.0	14.0	17.3	21.7	34.0	41.7	48.0
Mi-14	18.3	74.0	117.3	146.0	3.7	8.0	10.7	11.7	25.3	38.3	48.3	51.7
G. constrictum	19.0	68.7	127.0	138.3	3.7	7.7	10.3	11.0	23.7	35.3	43.0	48.0
Control	15.0	50.0	102.7	131.0	3.3	6.3	9.3	10.7	15.7	32.0	42.3	44.3
CD (P=0.05)	9.73	36.44	53.22	56.85	NS	3.73	NS	5.61	9.46	7.23	10.01	11.69

and 10 respectively which were all significantly higher than 6.3 of non mycorrhizal plants (Table 15). Significantly higher number of tillers of 17.3 was produced only in Mi-13 inoculated plants at 120 DAP compared to 10.7 of control treatment. It was also observed that all the mycorrhizal isolates except Mi-7 produced higher number of tillers than the nonmycorrhizal control.

There was significant difference in the height of the plants due to inoculation with different mycorrhizal isolates. Inoculation with Mi-6, Mi-3 and Mi-14 produced significantly higher plant height of 28 cm, 27 cm and 25.3 cm respectively in contrast to 15.7 cm in the control plants at 30 DAP (Table 15). At 60 DAP, mycorrhizal treatments Mi-3 (42.7 cm) and Mi-6 (40.3 cm) produced significantly higher plant height over control (32 cm). Mi-3 inoculated treatment continued to produce higher plant height of 55 cm and 58.3 cm at subsequent growth stages (at 90 DAP and 120 DAP) also. The data revealed that all the mycorrhizal isolates produced better plant height than non mycorrhizal plants although there were wide variations in the individual responses to plant growth. The most significant effect, throughout the growing stages, was exhibited by Mi-3 inoculated treatment. In general, the growth stimulating effect was more pronounced with Mi-13 and Mi-1. The effect of four different AMF isolates on growth of ginger are presented (Plates 8, 9, 10, 11).

Plate 8. Effect of Mi-1 (T1) on growth of ginger

Plate 9. Effect of Mi-4 (T4) on growth of ginger


T1
CON


T4
CON

Plate 10. Effect of Mi-9 (T9) on growth of ginger

Plate 11. Effect of Mi-13 (T13) on growth of ginger


T5
CON


T1
CON

4.8.2 Incidence and intensity of rhizome rot

There was no disease incidence in Mi-4 inoculated plants throughout the growing season (Table 16). Mi-1 and Mi-9 recorded very low per cent disease incidence of 18.8 and 12.4 which were significantly lower than 83.1 per cent recorded in control treatment. Other native isolates recorded significantly low per cent disease incidence were Mi-2, Mi-5, Mi-3, Mi-6, *G. constrictum* and Mi-7 inoculated plants showing per cent disease incidence of 24.6, 24.9, 26.9, 32.5, 38.6 and 40.0 respectively. With regard to the plant mortality rate (per cent tillers dead) due to the disease, it was found that there was no dead tillers in Mi-4 treatment as there was no disease incidence. The mortality rate was significantly low in Mi-9 and Mi-1 inoculated treatments having per cent mortality of 10.1 and 15.9 respectively as against 69.1 per cent in non mycorrhizal control (Plate 9, 10). Other treatments which exhibited significantly low rate of mortality were Mi-2 (21.1), Mi-6 (22.8), Mi-3 (24.6), Mi-5 (24.9), *G. constrictum* (38.6) and Mi-7 (40.0).

In Mi-4 inoculated treatment there was no disease incidence and hence ranked least in the disease intensity score. The disease intensity score was 1.27 in Mi-9 inoculated treatment while in Mi-1, Mi-2, Mi-3, Mi-5, Mi-6, Mi-7 and *G. constrictum* inoculated treatments the scores were 1.66, 1.62, 1.60, 1.62, 1.80, 1.85 and 2.0 which were all

Table 16 Rhizome rot of ginger as influenced by native AMF and *P. aphanidermatum* inoculation in green house

Treatment	Infected tillers (%)	Dead tillers (%)	Intensity score (0-9 scale)
Mi-1	10.4 (18.8)	7.5 (15.9)	1.77 (1.66)
Mi-2	17.3 (24.6)	12.9 (21.1)	1.62 (1.62)
Mi-3	20.5 (26.9)	17.4 (24.6)	1.59 (1.60)
Mi-4	0 (0)	0 (0)	0 (1)
Mi-5	17.8 (24.9)	17.8 (24.9)	1.62 (1.62)
Mi-6	28.9 (32.5)	15.0 (22.8)	2.25 (1.80)
Mi-7	41.3 (40.0)	41.3 (40.0)	2.45 (1.85)
Mi-8	94.2 (76.1)	91.2 (72.7)	8.30 (3.05)
Mi-9	4.6 (12.4)	3.1 (10.1)	0.62 (1.27)
Mi-10	60.5 (51.0)	60.5 (51.0)	6.09 (2.66)
Mi-11	64.2 (53.2)	57.6 (49.3)	6.09 (2.66)
Mi-12	86.5 (68.4)	86.5 (68.4)	5.65 (2.57)
Mi-13	93.1 (74.4)	93.1 (74.4)	9.00 (3.16)
Mi-14	96.2 (78.8)	96.3 (78.8)	9.00 (3.16)
G. constrictum	38.9 (38.6)	38.9 (38.6)	3.00 (2.00)
Control	98.6 (83.1)	87.4 (69.1)	8.00 (3.00)
CD (P = 0.05)	35.53	36.14	0.91

Values in parenthesis denote transformed means

significantly less than the score value (3.0) in control. From the data on disease incidence and intensity it was evident that mycorrhizal isolates Mi-1, Mi-4 and MI-9 were significantly more effective in reducing incidence and intensity of rhizome rot (Plates 12, 13, 14) whereas disease intensity was maximum in Mi-13 isolate (Plate 15).

4.8.3 Yield and dry matter production

The data on rhizome yield revealed that seven isolates gave significantly higher yield over control (Table 17). Maximum yield of 292.7g was recorded in Mi-4 inoculated treatment followed by Mi-1 with 271.7 g as against 117.3 g in the control. All mycorrhizal plants gave higher yields than non mycorrhizal control.

Plant top dry weight was also remarkably higher in all the mycorrhizal treatments except in Mi-14 inoculation. Maximum plant top dry weight of 25.7 g was recorded in Mi-13 inoculated plants which was closely followed by 25.4 g, 24.6 g, 23.5 g and 22.3 g in Mi-5, Mi-1, Mi-2, Mi-4 and Mi-11 treated plants respectively which were significantly higher than 15.4 g recorded in non mycorrhizal control (Table 17).

All the mycorrhizal isolates tested gave higher dry root weight than nonmycorrhizal control, twelve of which were statistically significant. Maximum root dry weight of 16.6 g was recorded with Mi-1 followed by 14.3 g and 13.5 g in Mi-4

Plate 12. Influence of Mi-1 (T1) on rhizome rot

Plate 13. Influence of Mi-4 (T4) on rhizome rot


T1


T4
CON

Plate 14. Influence of Mi-9 (T9) on rhizome rot

Plate 15. Increase in disease intensity as influenced by Mi-13 (T13)


T5
CON


T10
CON

Table 17 Effect of native AMF and *Pythium aphanidermatum* inoculation on dry weight and yield of ginger

Treatment	Plant top dry weight ($g\ pl^{-1}$)	Root dry weight ($g\ pl^{-1}$)	Rhizome yield ($g\ pl^{-1}$)
Mi-1	24.6	16.6	271.7
Mi-2	23.5	13.5	234.3
Mi-3	20.4	12.2	256.0
Mi-4	22.3	14.3	292.7
Mi-5	25.4	12.7	234.3
Mi-6	21.0	12.9	258.3
Mi-7	18.1	12.9	201.7
Mi-8	16.0	6.7	150.0
Mi-9	17.1	11.8	186.0
Mi-10	19.1	10.6	188.3
Mi-11	22.3	12.6	257.3
Mi-12	16.9	10.3	166.7
Mi-13	25.7	7.1	138.7
Mi-14	14.5	6.0	135.0
G. constrictum	18.2	12.8	216.0
Control	15.4	5.4	117.3
CD (P = 0.05)	6.109	4.758	110.755

Mean of three replications

and Mi-2 respectively as against 5.4 g of control (Table 17). There were significant differences between the different isolates in the root dry weight.

4.8.4 Pathogen population as influenced by AMF isolates

Higher root colonization of 95.2, 92.3, 91.7 and 90.9 per cent were recorded for inoculations with Mi-4, Mi-3, Mi-5 and Mi-1 respectively (Table 18). Mycorrhizal isolates Mi-12, Mi-14 and Mi-10 recorded relatively low colonization and intensity. The maximum spore count of 73 was recorded in the Mi-4 inoculation. All the other treatments also recorded higher AMF spore count.

A substantial reduction in pathogen population was noticed with Mi-4 and Mi-1 inoculations with 33 and 42 cfu/50 mg soil respectively as against 171 in non mycorrhizal control (Table 18). Other treatments with less pathogen population were those inoculated with Mi-5, Mi-3 and Mi-9. The mycorrhizal isolates Mi-10, Mi-12, Mi-13 and Mi-14 inoculated plants recorded very high pathogen populations of 159, 132, 141 and 189 cfu/50 mg soil respectively.

The mycorrhizal isolates, Mi-1 and Mi-4 which were found more effective for yield increase and rhizome rot suppression in the green house, were selected for further studies. Both these isolates were identified as *Glomus* spp. based on spore characteristics following the classification scheme as described in item 3.11 (Plates 16, 17).

Table 18 Effect of native AMF and rhizome rot pathogen inoculation on AMF colonization and pathogen population in ginger in green house

Treatment	AMF colonization (%)	Intensity of colonization	AMF spore count ($10g^{-1}$ soil)	Pathogen population (cfu/50mg soil)
Mi-1	90.9	++++	68	42
Mi-2	86.3	++++	59	75
Mi-3	92.3	++++	64	68
Mi-4	95.2	++++	73	33
Mi-5	91.7	++++	58	85
Mi-6	82.6	+++	49	112
Mi-7	84.2	+++	52	103
Mi-8	66.7	+++	38	106
Mi-9	85.0	++++	65	72
Mi-10	62.5	++	47	159
Mi-11	82.6	+++	51	102
Mi-12	58.3	++	42	136
Mi-13	89.1	++++	54	141
Mi-14	60.7	++	39	189
G. constrictum	80.0	++++	43	99
Control	0	0	0	171

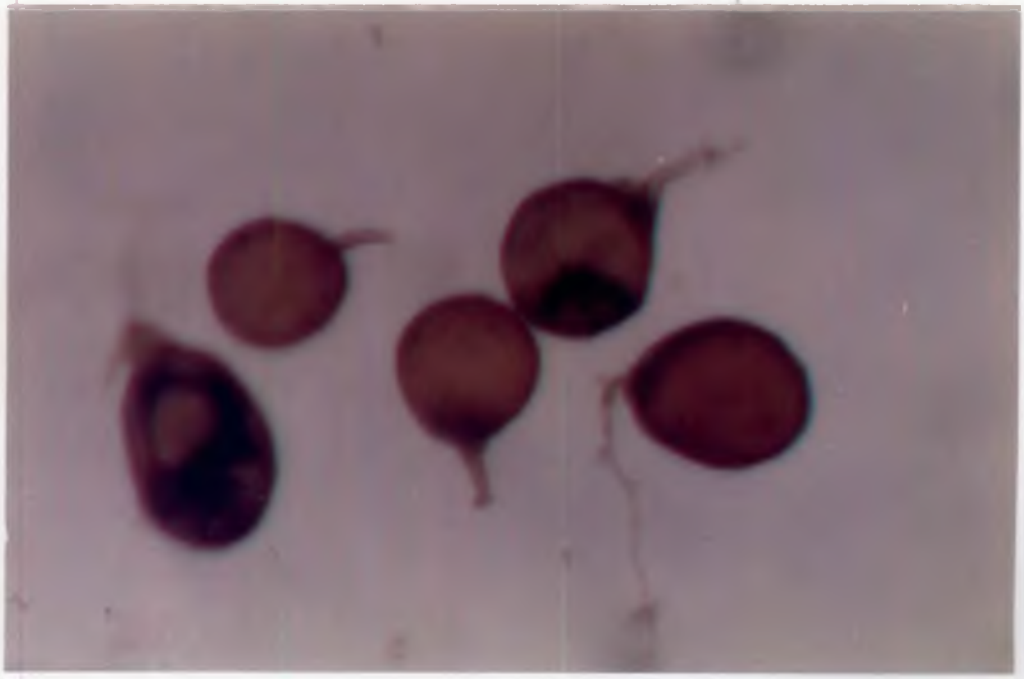
Values are means of three replications

Plate 16.

Mi-1 (*Glomus* sp.) isolated from ginger rhizosphere of Pulppally, Wayanad (x100)

Plate 17

Mi-4 (*Glomus* sp.) isolted from ginger rhizosphere of Adimali, Ernakulam (x100)



4.8.5 Analysis of nutrients

Maximum P content of 725.4 ppm and 691.6 ppm were observed in the plant top and roots of Mi-10 inoculated plants (Table 19). Inoculations with *G. constrictum*, Mi-12 and Mi-13 also exhibited high P contents in the plant top and roots (699.9 ppm and 625 ppm for *G. constrictum*, 658.1 ppm and 608.3 ppm for Mi-12 and 634.9 ppm and 664.8 ppm for Mi-13). All the other mycorrhizal treatments also had significantly higher P content in the plant top and roots compared to 325.3 ppm and 274.9 ppm of P in the control.

K content of plant top was maximum in *G. constrictum* inoculation with 0.317 per cent (Table 19). Other treatments with significantly higher K content were those inoculated with Mi-3, Mi-9, Mi-11, Mi-1, Mi-2 and Mi-10. The K content of all the other treatments were on par with that of control (0.192 per cent).

Data on the analysis of micronutrients (Table 20 and 21) showed that Mi-3 inoculated plants had the maximum Cu content of 2.1 ppm and 2.5 ppm in the plant top and roots respectively. Other treatments with significantly higher Cu content were those inoculated with Mi-7 (1.5 ppm), Mi-9 (1.7 ppm), Mi-12 (1.7 ppm), Mi-13 (1.5 ppm) and *G. constrictum* (1.6 ppm) in the plant top and Mi-5 (2.2 ppm), Mi-7 (2.2 ppm), Mi-8 (2.3 ppm) and Mi-9 (2.1 ppm) in the roots whereas Cu

Table 19 Effect of inoculation of native AMF and *Pythium aphanidermatum* on phosphorus and potassium content of ginger plant tissue

Treatment	P content (ppm)		K content (%)	
	plant top	roots	plant top	roots
Mi-1	624.7	566.5	0.265	0.214
Mi-2	591.7	518.3	0.265	0.221
Mi-3	473.6	489.7	0.279	0.189
Mi-4	608.1	584.0	0.239	0.186
Mi-5	533.2	584.7	0.204	0.171
Mi-6	509.1	466.6	0.203	0.239
Mi-7	466.7	458.3	0.207	0.193
Mi-8	577.2	599.9	0.194	0.221
Mi-9	558.8	500.0	0.279	0.175
Mi-10	725.4	691.6	0.252	0.218
Mi-11	601.0	616.6	0.279	0.225
Mi-12	658.1	608.3	0.221	0.225
Mi-13	634.9	664.8	0.209	0.212
Mi-14	533.2	466.7	0.186	0.194
G. constrictum	699.9	625.0	0.317	0.239
Control	325.3	274.9	0.192	0.168
CD (P=0.05)	73.63	63.97	0.055	0.045

Table 20 Effect of inoculation of native AMF and *Pythium aphanidermatum* on the micronutrient content of ginger plant tissue - plant top

Treatment	Cu (ppm)	Ca (ppm)	Mg (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)
Mi-1	1.2	598.7	2442.5	11.2	37.2	149.0
Mi-2	1.4	582.4	2420.0	8.2	26.5	103.0
Mi-3	2.1	505.5	2417.5	11.8	28.6	88.5
Mi-4	1.4	674.9	2412.5	11.4	27.4	157.5
Mi-5	1.4	472.8	2417.5	9.0	33.5	225.0
Mi-6	1.2	483.8	2370.0	9.1	31.4	105.3
Mi-7	1.5	516.6	2382.5	9.6	25.4	88.6
Mi-8	1.4	410.6	2362.5	10.6	20.1	86.7
Mi-9	1.7	578.2	2397.5	9.5	38.6	120.4
Mi-10	1.3	700.6	2390.0	10.3	28.0	118.5
Mi-11	1.3	653.2	2437.5	9.0	34.8	130.5
Mi-12	1.7	381.2	2405.0	7.9	19.0	167.5
Mi-13	1.5	374.2	2420.0	10.2	30.7	145.3
Mi-14	0.9	626.7	2355.0	8.9	26.1	171.2
G.constrictum	1.6	645.9	2367.5	15.5	28.4	151.6
Control	1.0	433.5	2330.0	9.3	30.2	142.3
CD (P=0.05)	0.49	86.42	55.91	1.34	8.15	28.24

Table 21 Effect of inoculation of native AMF and *P. aphanidermatum* on the micronutrient content of ginger roots

Treatment	Cu (ppm)	Ca (ppm)	Mg (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)
Mi-1	1.8	155.0	2275.0	9.4	25.1	162.5
Mi-2	2.0	197.5	2302.5	10.2	27.6	152.5
Mi-3	2.5	212.5	2312.5	9.3	24.6	145.0
Mi-4	1.8	222.5	2252.5	7.1	22.1	177.5
Mi-5	2.2	190.0	2270.0	7.1	32.3	157.5
Mi-6	1.7	205.0	2288.0	7.8	30.7	172.5
Mi-7	2.2	220.0	2287.3	8.6	22.0	145.0
Mi-8	2.3	288.3	2310.7	13.8	31.7	172.5
Mi-9	2.1	195.0	2253.0	10.2	23.1	242.5
Mi-10	1.6	185.0	2270.0	7.8	27.4	230.0
Mi-11	1.7	187.5	2282.5	7.5	30.4	225.0
Mi-12	1.7	207.5	2265.0	6.6	28.1	180.0
Mi-13	1.9	192.5	2265.0	8.2	29.6	210.0
Mi-14	1.3	202.5	2260.0	7.7	26.5	130.0
G.constrictum	1.8	290.0	2292.0	8.9	33.3	145.0
Control	1.4	115.0	2170.0	6.1	20.8	132.5
CD (P=0.05)	0.67	46.53	50.70	0.73	4.73	23.78

content in the control was 1.0 ppm and 1.7 ppm in plant top and roots respectively.

Ca content of plant top was maximum (700.6 ppm) in Mi-10 inoculation followed by Mi-4 (674.9 ppm) and Mi-11 (653.2 ppm). Ca content of root was highest in *G. constrictum* (290 ppm) followed by Mi-8 (288.3 ppm) and Mi-4 (222.5 ppm). Ca content of control plants was 433.5 ppm and 115 ppm in the plant top and roots respectively. Plant top Ca content was also significantly higher in Mi-1, Mi-2, Mi-9, Mi-14 and *G. constrictum* treatments. Ca content in root was significantly higher in all the mycorrhizal treatments except Mi-1.

There was significant variation in Mg content of plant top and roots due to inoculation with native mycorrhizal isolates. Maximum content of Mg in plant top was recorded in Mi-1 inoculation (2442.5 ppm) while in the roots it was in Mi-3 inoculated plants (2312.5 ppm). Plant top Mg content was also significantly higher in Mi-2, Mi-3, Mi-4, Mi-5 etc. inoculated treatments while Mg content of roots was significantly higher in all the mycorrhizal treatments.

Zn content of plant top was significantly higher in *G. constrictum* inoculated plants (15.5 ppm) in comparison to control (9.3 ppm) and other treatments. Inoculations with Mi-3, Mi-4, Mi-1 and Mi-8 also showed higher plant top Zn

content (11.8 ppm, 11.4 ppm, 11.2 ppm and 10.6 ppm respectively). All mycorrhizal inoculations except Mi-12 gave significantly higher root Zn content. Except in Mi-9 inoculated plants (38.6 ppm) there was no significant difference between treatments and control with regard to Mn content of plant top. Maximum root Mn content of 33.3 ppm was observed in *G. constrictum* as against 20.8 ppm in control. Mn content of roots was significantly higher in all the treatments except Mi-4, Mi-7 and Mi-9 inoculations.

Maximum Fe content of plant top was observed in Mi-5 inoculated plants (225 ppm) followed by Mi-14 (171.2 ppm) which were significantly higher than non mycorrhizal plants (142.3 ppm). But in all other treatments the Fe content was on par with control. In the roots, maximum Fe content was recorded in Mi-9 inoculated plants (242.5 ppm). Isolates Mi-10, Mi-11, Mi-13, Mi-1, Mi-4, Mi-5 etc. also recorded significantly higher Fe content than control (132.5 ppm).

4.9 Isolation of native antagonistic fungi

Antagonistic fungi in the rhizosphere soil of healthy ginger plants and vermiculite brought from different locations were isolated and brought to pure culture. Altogether 28 antagonistic fungal isolates were obtained (Table 22).

Table 22 *In vitro* evaluation of biocontrol efficiency of native antagonists against *Pythium aphanidermatum*

Isolate No.	Location from where obtained	Growth of Pythium in control (mm)	Growth of Pythium in treatment (mm)	Growth of antagonist (mm)	Per cent inhibition	Inhibition zone	Rating of inhibition	Remarks
Ai-1	Peringamala (Thiruvananthapuram)	90	29.5	60.5	67.2	-	++	-
Ai-2	Thiruvananthapuram (From vermicompost)	90	4.0	84.3	95.6	1.3	++++	Fully over grown (metabolites produced)
Ai-3	Thodupuzha (Idukki)	90	21.7	68.3	75.9	-	++	-
Ai-4	Kumaramangalam (Idukki)	90	26.0	64.0	71.1	-	++	-
Ai-5	Santhanpara (Idukki)	90	10.0	78.9	88.9	-	+++	-
Ai-6	Thalappuzha (Wayanad)	90	3.0	87.0	96.7	-	++++	Fully over grown metabolites produced
Ai-7	Balagram (Idukki)	90	6.0	84.0	93.3	-	++++	Fully over grown
Ai-8	Pannimattam (Idukki)	90	6.3	83.7	93.0	-	++++	Fully over grown
Ai-9	Kailasappara (Idukki)	90	24.0	66.0	73.3	-	++	-
Ai-10	Cheenikkuzhi (Idukki)	90	12.5	72.5	86.1	5.0	+++	Over grown

Table 22 contd.

Isolate No.	Location from where obtained	Growth of Pythium in control (mm)	Growth of Pythium in treatment (mm)	Growth of antagonist (mm)	Per cent inhibition	Inhibition zone	Rating of inhibition	Remarks
Ai-11	Pazhuppathur (Idukki)	90	3.7	86.3	95.9	-	++++	Fully over grown
Ai-12	Kailasappara (Idukki)	90	6.0	84.0	93.3	-	++++	Fully over grown
Ai-13	Udumbanchola (Idukki)	90	5.0	81.0	94.4	4.0	++++	Fully over grown
Ai-14	Kattappana (Idukki)	90	14.0	76.0	84.4	-	+++	Over grown
Ai-15	Cheeyambam (Wayanad)	90	19.0	70.2	78.9	0.8	+++	Over grown
Ai-16	Puliyanmala (Idukki)	90	26.5	63.5	70.6	-	++	Lysis, yellow metabolites produced
Ai-17	Adimali (Idukki)	90	35.0	55.0	61.1	-	+	Lysis
Ai-18	Kochara (Idukki)	90	0	90.0	100.0	-	++++	Very high over growth
Ai-19	Mananthody (Wayanad)	90	4.5	73.5	95.0	12.0	++++	Lysis
No-20	*Trichoderma koningii* (TNAU, Coimbatore)	90	2.0	88.0	97.8	-	++++	Complete lysis
No-21	*T. pseudokoningii* (TNAU, Coimbatore)	90	2.4	84.0	97.6	3.6	++++	Complete lysis

Table 22 contd.

Isolate No.	Location from where obtained	Growth of Pythium in control (mm)	Growth of Pythium in treatment (mm)	Growth of antagonist (mm)	Per cent inhibition	Inhibition zone	Rating of inhibition	Remarks
Ai-22	Pulppally (Wayanad)	90	9.0	81.0	90.0	-	++++	Complete lysis
No-23	*T. viride* (TNAU, Coimbatore)	90	0	90.0	100.0	-	++++	Complete lysis
No-24	*T. harzianum* (TNAU, Coimbatore)	90	2.2	87.8	97.6	-	++++	Complete lysis
Ai-25	Mitranikethan (Vermicompost)	90	13.0	77.0	85.5	-	+++	Over grown
Ai-26	Kalpetta (Wayanad)	90	41.0	49.0	54.4	-	+	-
Ai-27	Meenangadi (Wayanad)	90	29.0	61.0	67.8	-	+	-
Ai-28	Ambalavayal (Wayanad)	90	32.0	58.0	64.4	-	+	-
Ai-29	Vaduvanchal (Wayanad)	90	28.5	61.5	68.3	-	+	-
Ai-30	Vermicompost Mitranikethan	90	7.0	83.0	92.2	-	++++	Completely over grown
Ai-31	Nadavayal (Wayanad)	90	42.5	47.5	52.8	-	+	-
Ai-32	Karimkunnam (Idukki)	90	31.1	58.9	65.4	-	+	-

\+ - Low
++ - Medium
+++ - High
++++ - Very high

4.10 *In vitro* screening of antagonistic fungi against *P. aphanidermatum*

Results of the *in vitro* screening using 28 native antagonistic fungi in comparison with four *Trichoderma* cultures are presented in Table 22. Maximum per cent inhibition was exhibited by Ai-18 and *T. viride* (100 per cent). Eleven isolates and four *Trichoderma* cultures have exhibited per cent inhibition of 90 or above and very high intensity of inhibition. These isolates and cultures were selected for further screening in the green house against rhizome rot. *In vitro* growth inhibition of *P. aphanidermatum* by two antagonistic isolates and two reference cultures are presented (Plate 18, 19, 20, 21).

4.11 Effect of antagonistic fungal isolates on growth and rhizome rot of ginger

4.11.1 Growth characteristics

The effect of selected antagonistic fungal isolates from the *in vitro* study was further tested for the growth stimulating property in green house. Ai-12 inoculated plants produced the maximum number of leaves (54.7) followed by Ai-7 (46.7) and *T. viride* (45.7) as compared to uninoculated control (26.4) at 30 DAP. At subsequent stages of growth also (60, 90 and 120 DAP) these three treatments produced significantly higher number of leaves than control. The number

Plate 18.

In vitro inhibition of *P. aphanidermatum* by AI-12 (*T. viride*)

Plate 19.

In vitro inhibition of *P. aphanidermatum* by Ai-13 (*A. fumigatus*)


ISOLATE NO-12 Vs PYTHIUM APHANIDERMATUM
CONTROL


ISOLATE NO-13 Vs PYTHIUM APHANIDERMATUM
CONTROL

Plate 20.

In vitro inhibition of *P. aphanidermatum* by *T. viride* (reference culture)

Plate 21.

In vitro inhibition of *P. aphanidermatum* by *T. pseudokoningii* (reference culture)


TRICHODERMA VIRIDAE vs PYTHIUM APHANIDERMATUM
CONTROL


vs
CONTROL

of leaves produced by Ai-12, Ai-7 and *T. viride* inoculated plants at 120 DAP were 114.7, 114 and 99 respectively as against 74.3 in the control treatment. All the other treatments exerted less influence on growth (Table 23).

A similar trend was noticed with regard to the number of tillers. At all stages of growth, Ai-12, Ai-7 and *T. viride* inoculated plants produced more number of tillers. At 30 DAP and 60 DAP, the effect was significantly higher. Height of the plant was significantly more in the *T. harzianum* inoculated plants throughout the growth stages. Isolates, Ai-12 and Ai-13 had recorded a height of 41 and 42 cm respectively at 120 DAP as against 29 cm recorded for control plants. Most isolates exhibited considerable effect on plant height. There was wide variation on growth pattern of ginger inoculated with different antagonistic fungal isolates. Substantial increase in growth characteristics was recorded with Ai-12, Ai-7 and *T. viride* inoculations.

4.11.2 Incidence and intensity of rhizome rot

The per cent infected tillers were significantly low in Ai-12 (8.4) in comparison with uninoculated control (77.4) (Table 24). Per cent tillers infected were also considerably low in the Ai-13 (21.9) and Ai-6 (23.5) inoculations. With regard to per cent mortality (per cent tillers dead) Ai-12 (no mortality) and Ai-13 (8.0) inoculated plants recorded

Table 23 Effect of inoculation of antagonistic fungal isolates and *P. aphanidermatum* on growth characteristics of ginger in green house

Treatments	Number of leaves Days after inoculation				Number of tillers Days after inoculation				Height of the plant Days after inoculation (cm)			
	30	60	90	120	30	60	90	120	30	60	90	120
Ai-30	33.3	46.7	70.7	81.0	3.7	5.7	7.0	7.3	22.3	31.7	43.0	45.0
T. koningii	18.0	27.3	46.37	56.0	2.7	4.0	4.3	5.0	20.7	28.0	42.7	44.7
Ai-13	35.7	51.7	66.7	72.0	4.7	7.0	8.0	8.0	20.0	31.3	38.7	40.7
T. viride	45.7	57.3	85.5	99.0	6.0	8.7	10.0	10.03	26.3	37.0	42.0	42.7
Ai-18	15.7	23.7	39.7	51.3	2.3	3.7	5.7	6.00	14.7	23.3	28.3	29.0
Ai-8	42.0	56.3	75.3	91.3	4.7	6.7	7.0	7.00	24.0	35.7	38.3	39.7
T. harzianum	40.0	54.0	71.0	87.0	4.0	5.3	6.7	7.0	31.7	47.0	49.7	50.3
Ai-2	22.7	30.3	43.7	54.7	3.0	4.3	6.7	6.7	12.3	18.7	24.7	27.3
T. pseudokoningii	23.7	31.0	44.0	57.7	3.3	4.7	6.3	7.0	15.7	21.7	26.0	29.0
Ai-22	43.0	56.7	77.0	91.3	3.3	5.7	8.0	8.3	22.0	30.0	36.7	38.3
Ai-7	46.7	67.0	94.3	114.0	5.3	8.3	9.7	9.7	22.0	31.0	35.3	36.0
Ai-19	26.0	37.0	57.7	73.0	2.7	4.3	5.7	6.0	22.3	31.0	38.7	39.7
Ai-12	54.7	75.3	98.0	114.7	6.0	9.0	11.0	11.0	23.3	34.0	41.3	42.3
Ai-11	21.3	30.3	49.3	63.7	3.0	4.0	5.7	6.0	16.7	24.3	31.0	32.7
Ai-6	19.0	26.0	39.0	53.7	3.0	4.3	5.0	5.7	10.7	14.3	22.0	24.3
Control	26.4	38.0	61.7	74.3	2.7	4.3	6.7	7.3	14.7	22.0	26.7	29.3
CD (P = 0.05)	NS	NS	NS	NS	2.64	4.30	NS	NS	14.21	17.64	16.43	15.39

Table 24 Effect of inoculation of antagonists and *Pythium aphanidermatum* on rhizome rot of ginger in the green house

Treatment	Disease incidence		
	Infected tillers (%)	Dead tillers (%)	Intensity score (0-9 scale)
Ai-30	62.0 (51.9)	48.1 (43.9)	4.4 (2.3)
T. koningii	75.0 (60.0)	75.0 (60.0)	4.4 (2.3)
Ai-13	14.0 (21.9)	1.95 (8.0)	1.6 (1.6)
T. viride	99.9 (90.0)	99.1 (84.6)	9.0 (3.2)
Ai-18	41.3 (39.9)	41.3 (39.9)	3.2 (2.1)
Ai-8	49.2 (44.5)	25.0 (29.9)	4.0 (2.2)
T. harzianum	55.7 (48.2)	55.7 (48.0)	3.9 (2.2)
Ai-2	67.2 (55.0)	31.9 (34.2)	5.6 (2.6)
T. pseudokoningi	25.0 (30.0)	17.3 (24.6)	2.0 (1.7)
Ai-22	48.9 (44.4)	48.2 (44.4)	4.4 (2.3)
Ai-7	67.2 (55.1)	52.4 (46.4)	4.5 (2.3)
Ai-19	88.3 (70.0)	77.9 (61.9)	8.3 (3.1)
Ai-12	2.1 (8.4)	0.0 (0.0)	0.3 (1.1)
Ai-11	75.0 (60.0)	63.9 (53.1)	5.0 (2.4)
Ai-6	15.9 (23.5)	12.4 (20.6)	2.0 (1.7)
Control	95.3 (77.4)	72.7 (58.5)	7.6 (2.9)
CD (P=0.05)	61.635	48.170	1.537

Values given in parenthesis denote transformed means

significantly low mortality rate whereas in untreated control, the per cent tillers dead was as high as 58.5. The per cent tillers dead were also low in the Ai-6 inoculated treatment (20.6). The disease intensity score was significantly low in Ai-12 (1.1) in comparison with control (2.9). Score values of disease intensity were also comparatively low in Ai-13 and Ai-6 inoculations (1.6 and 1.7). The per cent tillers infected (90.0),dead (84.6) and disease intensity score (3.2) were the highest in *T. viride* inoculation (Plate 24). Thus Ai-12 and Ai-13 inoculations were more effective in reducing the incidence and intensity of rhizome rot (Plate 22, 23).

4.11.3 Dry matter production and yield

Maximum rhizome yield was recorded in *T. harzianum* (90 g) followed by Ai-12 (86.7 g) inoculated treatments as compared to control (58 g) (Table 25). The yield was highly variable among treatments and many treatments gave yields lower than control. Plant top dry weight was significantly higher in Ai-12 (12.3 g), Ai-22 (11.6 g) and Ai-7 (11.4 g) in comparison with untreated plants (6.1 g). Plant top dry weight was higher in all the treatments compared to control except Ai-19. Root dry weight was also considerably increased due to inoculation with antagonistic fungal isolates. It was significantly higher in Ai-7 inoculation (7 g) over control plants (2.4 g). Inoculation with Ai-12, Ai-8, Ai-13 and Ai-22 also gave higher

Plate 22. Influence of Ai-12 (*T. viride*) on rhizome rot

Plate 23. Influence of Ai-13 (*A. fumigatus*) on rhizome rot


CON
Ai-12


CON
Ai-13

Plate 24.

Increase in disease intensity due to *T. viride* (reference culture)


CON
T·V

Table 25 Effect of native antagonists and *P. aphanidermatum* inoculation on dry weight and yield of ginger in the green house

Treatment	Root Dry Weight ($g\ pl^{-1}$)	Plant Top dry weight ($g\ pl^{-1}$)	Rhizome yield ($g\ pl^{-1}$)
Ai-30	2.3	7.3	44.3*
T. koningii	2.4	8.4	31.7
Ai-13	4.5	7.2	36.3
T. viride	1.7	6.7	19.0
Ai-18	2.6	6.8	32.3
Ai-8	4.6	11.0	73.3
T. harzianum	3.9	10.2	90.0
Ai-2	5.0	9.5	38.7
T. pseudokoningi	3.2	6.6	23.7
Ai-22	4.5	11.6	73.3
Ai-7	7.0	11.4	67.7
Ai-19	2.4	4.6	43.3
Ai-12	6.3	12.3	86.7
Ai-11	2.8	6.3	31.7
Ai-6	2.5	7.3	53.3
Control	2.4	6.1	58.0
CD (P=0.05)	4.274	5.932	NS

Mean of three replications

dry root weights of 6.3 g, 4.6 g, 4.5 g and 4.5 g respectively. It is evident from the data that inoculation with Ai-12 recorded better yield and dry matter production.

4.11.4 Populations of antagonists and pathogen

The populations of the antagonists estimated from the antagonist incorporated pots revealed that except in Ai-13 and Ai-12 the populations were very low (Table 26). The population of antagonists in Ai-13 and Ai-12 inoculations were 75 cfu x 10^4/g and 104 cfux10^4/g soil respectively.

The pathogen population was lowest in Ai-12 inoculated treatment (26 cfu/50 mg soil) followed by Ai-13 (32 cfu) (Table 26). In control, the population was as high as 145 cfu/50 mg soil. In rest of the treatments, the pathogen population exhibited a highly varying trend. Treatments which recorded very high pathogen population were *T. viride* (136 cfu), *T. koningii* (128 cfu), Ai-19 (112 cfu) and Ai-18 (102 cfu) inoculations.

4.11.5 Identification of the selected antagonistic fungal isolates

The antagonistic fungal isolates used for the green house screening were identified at Agarkar Research Institute, Pune (Table 27). Ai-12 was identified as *Trichoderma viride* and Ai-13 as *Aspergillus fumigatus*.

Table 26 Effect of inoculation of native antagonists on pathogen population in the ginger rhizosphere

Treatment	Population of antagonist ($x10^4$/g soil)	Population of pathogen (cfu/50 mg soil)
Ai-30	29	91
T. koningii	16	128
Ai-13	75	32
T. viride	36	136
Ai-18	23	102
Ai-8	42	73
T. harzianum	47	86
Ai-2	19	107
T. pseudokoningi	32	59
Ai-22	41	66
Ai-7	11	97
Ai-19	17	112
Ai-12	103	26
Ai-11	35	84
Ai-6	48	47
Control	21	145

Values are mean of three replications

Table 27 List of antagonists identified and used for green house screening

Sl. No.	Isolate No.	Name of the fungus identified	Source from where obtained
1.	A1-30	*Aspergillus fumigatus*	Vermicompost, Mithranikethan.
2.	A1-13	*A. fumigatus*	Udumbanchola, Idukki
3.	A1-18	*Gliomastrix murorum*	Kochara, Idukki
4.	A1-1	*A. fumigatus*	Peringamala, Thiruvananthapuram
5.	A1-22	*Fusarium oxysporum*	Pulppally, Wayanad
6.	A1-19	*A. swidovii*	Mamanthody, Wayanad
7.	A1-12	*Trichoderma viride*	Kailasappara, Idukki
8.	A1-6	Not identified	Thodupuzha, Wayanad
9.	A1-7	*A. fumigatus*	Balagram, Idukki
10.	A1-8	*F. oxysporum*	Pannimattom, Idukki
11.	A1-11	*G. murorum*	Pazhuppathoor, Idukki

4.12 Effect of dual inoculation of native AMF and antagonists on growth of ginger and disease suppression in the green house

4.12.1 Growth characteristics

The results indicated that dual inoculation of Ai-12 with Mi-1 (92) and Mi-4 (103.7) produced significantly higher number of leaves (Table 28). The single inoculation of Ai12 (84.7) and the combined effect of Ai-13 x Mi-4 also substantially increased the number of leaves produced (83) over control (56). However, the individual effect of mycorrhizal isolates and antagonistic fungal isolates and combined effect of Ai-13xMi-1 did not exert significant effect on the number of leaves produced. The same trend was noticed in the case of number of tillers and height of plants. Maximum number of tillers (12) were produced by Ai-12 x Mi-4 followed by Ai-12 x Mi-1 (10.3), Ai-12 alone (10.0) and Ai-13 x Mi-4 (10) inoculations, all of which were significantly higher over control (6.7). Maximum plant height was recorded in Ai-12 x Mi-4 treatment (43.7 cm) followed by Ai-12 alone inoculation (43.3 cm). The main effect of Ai-12 was significantly higher in enhancing the plant growth characteristics while main effect of Mi-4 was significant in the number of tillers produced. The main effect of Mi-1 and Ai-13 were not significant in enhancing plant growth.

Table 28 Evaluation of combined effect of native AMF, antagonists and *P. aphanidermatum* on growth characteristics of ginger in the green house

Treatment	Number of leaves	Number of tillers	Height of plant
Ao x Mi-1	58.0	6.7	29.0
Ao x Mi-4	46.0	5.7	37.0
Ai 13 x M0	43.3	5.7	34.7
Ai-13 x Mi-1	59.0	6.7	33.3
Ai-13 x Mi-4	83.0	10.0	39.0
Ai-12 x M0	84.7	10.0	43.3
Ai-12 x Mi-1	92.0	10.3	40.3
Ai-12 x Mi-4	103.7	12.0	43.7
AOxMO	56.0	6.7	31.3
CD-T (P=0.05)	30.0	2.9	6.8
Main effect			
A0	53.3	6.3	32.4
Ai-13	61.8	7.4	35.7
Ai-12	93.4	10.8	42.4
M0	61.3	7.4	36.4
Mi-1	69.7	7.9	35.3
Mi-4	77.6	9.2	38.8
CD-A (P=0.05)	17.3	1.7	3.9
CD-M (P=0.05)	17.3	1.7	3.9

4.12.2 Incidence and intensity of rhizome rot

The combined inoculation of Ai-12 x Mi-1 (29.8%) and Ai-12 x Mi-4 (34.1%) were significantly effective and interacted synergistically in reducing the per cent tillers infected compared to control (67.6%) (Table 29). The single inoculations of Mi-1 (43.7), Mi-4 (41.7) and Ai-12 (42.8) also reduced the per cent tillers infected remarkably. In contrast, the single inoculation of Ai-13 (79.2) and its dual inoculation with Mi-1 (65.2) and Mi-4 (55.5) considerably increased the per cent tillers infected compared to single mycorrhizal inoculations. The main effect of Ai-12 significantly reduced the per cent tillers infected (35.0) over control (50.9) and Ai-13 (66.6). The main effect of Ai-13 significantly increased the disease. The main effect of mycorrhizal isolates significantly reduced the disease (46.2 for Mi-1 and 43.8 for Mi-4) over non mycorrhizal plants (63.2).

The per cent mortality rate of tillers was lowest in the combined treatment of Ai-12 x Mi-1 (22.4) followed by Ai-12 x Mi-4 (26.1) and single inoculations of Mi-4 (28.6) and Mi-1 (32.4) which were all significantly lower than uninoculated control (47.5). Maximum mortality rate of 48.2 per cent was observed in single inoculation of Ai-13 (Table 29). It was observed that dual inoculation of Ai-13 with mycorrhizal fungi (Ai-13 x Mi-1 and Ai-13 x Mi-4) reduced

Table 29 Effect of interaction of AMF, antagonists and *P. aphanidermatum* on rhizome rot of ginger in the green house

Treatment	Disease incidence		
	Infected tiller (%)	Dead tiller (%)	Intensity score (0-9 scale)
AOxMi-1	47.7 (43.7)	28.7 (32.4)	2.7 (9.5)
AOxMi-4	44.3 (41.7)	23.0 (28.6)	2.7 (9.5)
Ai 13 x MO	96.5 (79.2)	55.6 (48.2)	6.3 (14.5)
Ai-13 x Mi-1	82.4 (65.2)	45.8 (42.6)	5.5 (13.5)
Ai-13 x Mi-4	68.0 (55.5)	34.5 (36.0)	4.3 (11.9)
Ai-12 x MO	46.3 (42.8)	30.2 (33.3)	3.6 (11.0)
Ai-12 x Mi-1	24.7 (29.8)	14.5 (22.4)	1.5 (7.1)
Ai-12 x Mi-4	31.5 (34.1)	19.4 (26.1)	2.2 (8.6)
AOxMO	85.5 (67.6)	54.4 (47.5)	6.1 (14.3)
CD-T (P=0.05)	27.2	14.4	5.0
Main effect			
AO	50.9	36.2	11.1
Ai-13	66.6	42.3	13.3
Ai-12	35.6	27.3	8.9
MO	63.2	43.0	13.2
Mi-1	46.2	32.4	10.1
Mi-4	43.7	30.2	10.0
CD-A (P=0.05)	15.7	8.4	2.9
CD-M (P=0.05)	15.7	8.4	2.9

Values in parenthesis denote transformed means

the beneficial effect of mycorrhizal isolates in checking the mortality rate. The main effect of Ai-12 in reducing the mortality from 36.2 per cent in the control to 27.3 per cent was significantly high whereas the Ai-13 main effect increased the mortality. The mycorrhizal effects in reducing the mortality from 43.0 of control to 32.4 by Mi-1 and 30.2 by Mi-4 were statistically significant (Table 29). The disease intensity score was least (7.1) in the combined inoculation of Ai-12 x Mi-1 followed by Ai-12 x Mi-4 (8.6) which were significantly lower than control treatment (14.3). Other treatments with less disease intensity score were A0 x Mi-1, A0 x Mi-4 and Ai-12 x M0 (Table 29). The main effect of mycorrhizal isolates exerted a significant influence in reducing the disease intensity from 13.2 in the nonmycorrhizal plants to 10.1 in Mi-1 and 10.0 in Mi-4 inoculations. Ai-12 also remarkably reduced the disease intensity. However, Ai-13 inoculation increased the intensity.

4.12.3 Yield and dry matter production

Maximum rhizome yield of 95 g was obtained in Ai-12 x Mi-4 inoculation followed by A1-12 x M0 (92.3 g) and Ai-12 x Mi-1 (82 g) compared to 50 g in control (Table 30). The main effect of Ai-12 in enhancing the rhizome yield was significantly high (89.8) over control (54.7). A similar trend was observed with regard to dry weight of plant top and roots.

Table 30 Effect of selected native AMF, antagonists and *P. aphanidermatum* inoculation on dry weight and yield of ginger in the green house

Treatment	Plant top dry weight ($g\ pl^{-1}$)	Root dry weight ($g\ pl^{-1}$)	Rhizome yield ($g\ pl^{-1}$)
Ao x Mi-1	18.7	10.0	59.7
Ao x Mi-4	15.3	8.2	54.3
Ai 13 x M0	12.7	7.1	51.3
Ai-13 x Mi-1	17.3	10.0	53.3
Ai-13 x Mi-4	15.3	8.3	76.3
Ai-12 x M0	31.3	16.7	92.3
Ai-12 x Mi-1	28.0	15.5	82.0
Ai-12 x Mi-4	33.3	17.0	95.0
AOxMO	15.3	8.8	50.0
CD-T (P=0.05)	17.3	NS	NS
Main effect-			
A0	16.4	9.0	54.7
Ai-13	15.1	8.5	60.3
Ai-12	30.9	26.8	89.8
M0	19.8	10.9	64.6
Mi-1	21.3	11.2	65.0
Mi-4	21.3	22.3	75.2
CD-A (P=0.05)	9.9	NS	31.1
CD-M (P=0.05)	NS	NS	NS

A significantly high plant top dry weight of 33.3 g was recorded in Ai-12 x Mi-4 inoculation over control (15.3g). Higher plant top dry weight were also observed in Ai-12 x MO (31.3 g) and Ai-12 x Mi-1 (28 g). The main effect of antagonistic fungal isolate, Ai-12 was significant in obtaining higher plant top dry weight (30.9 g) over control (16.4 g). Root dry weight of 17 g was maximum in the combined inoculation of Ai-12 x Mi-4 as against 8.8 g of control. The main effect of antagonistic fungal isolates and mycorrhizal isolates were not significant in this respect.

4.12.4 AMF colonization and population of pathogen and antagonists

The tripartite interaction of AMF, antagonists and pathogen on AMF colonization and intensity (Table 31) showed that there were very high AMF colonization and intensity in the AMF inoculated treatments (Mi-1 - 86.9 per cent; Mi-4 - 82.6 per cent and combined inoculatiosn of Ai-12 x Mi-1 (90 per cent) and Ai-12 x Mi-4 (83 per cent). In contrast, when AMF isolates were combined with Ai-13 (Ai-13 x Mi-1 - 66.7 per cent and Ai-13 x Mi-4 - 63.7 per cent) the colonization and intensity were reduced.

The highest AMF spore count was obtained in combined treatment of Ai-12 x Mi-1 (71) and Ai-12 x Mi-4 (64) while in the combined treatment of Ai-13 x Mi-1 and Ai-13 x Mi-4 the

Table 31 Effect of selected AMF isolates, antagonists and *P. aphanidermatum* on AMF colonization and population of pathogen and antagonists in the ginger rhizosphere in the green house

Treatment	AMF colonization (%)	Intensity of colonization	AMF spore count ($10\ g^{-1}$ soil)	Pathogen population (cfu/50mg soil)	Antagonist population ($x10^4$/g soil)	
					Isolate No.12	Isolate No.13
Ao x Mi-1	86.9	++++	63	83	3	1
Ao x Mi-4	82.6	++++	52	75	2	3
Ai 13 x M0	0	0	0	112	2	58
Ai-13 x Mi-1	66.7	+++	51	86	3	46
Ai-13 x Mi-4	63.7	+++	43	118	5	51
Ai-12 x M0	0	0	0	39	89	5
Ai-12 x Mi-1	90.0	++++	71	40	96	3
Ai-12 x Mi-4	83.0	++++	64	32	110	7
A0xM0	0	0	0	136	3	5

spore counts are declined compared to single mycorrhizal inoculations.

The least pathogen population of 32 cfu/50 mg soil was observed in the Ai-12 x Mi-4 treatment as against 136 cfu in the non-treated control plants (Table 31). The combined inoculation of Ai-13 x Mi-4 increased the pathogen population from 75 in Mi-4 to 116. The population of antagonist, Ai-12 was very high in Ai-12 x MO,, Ai-12 x Mi-1 and Ai-12 x Mi-4 inoculations (89, 96 and 110 respectively) while the population of Ai-13 was comparatively high in Ai-13 x MO, Ai-13 x Mi-1 and Ai-13 x Mi-4 (58, 46 and 51 respectively). In all other treatments the population of the respective antagonists were very low.

4.13 Field evaluation of the combined effect of AMF and antagonists on growth, yield and suppression of rhizome rot of ginger

The combined effect of selected AMF and antagonists were tested under the field conditions for growth improvement and disease suppression.

4.13.1 Growth enhancement

The number of leaves produced were maximum (118.9) in the combined inoculation of Ai-12 x Mi-4 followed by Ai-12 x MO (118.2), AO x Mi-1 (117.8) and Ai-12 x Mi-1 (106.6) compared to

Table 32 Effect of selected AMF, antagonists and rhizome rot pathogen on growth characteristics of ginger in the field

Treatment	Number of leaves	Number of tillers	Height of the plant (cm)
AoxMi-1	117.8	8.4	46.4
AOxMi-4	96.0	8.3	51.9
Ai-13xMo	78.6	7.3	42.5
Ai-13xMi-1	83.8	8.6	45.1
Ai-13xMi-4	89.1	7.9	52.4
Ai-12xMo	118.2	9.6	45.4
Ai-12xMi-1	106.6	8.5	45.7
Ai-12xMi-4	118.9	11.9	42.6
AoxMo	86.0	7.9	36.1
CD-T (P=0.05)	NS	NS	14.5
Main effect			
A0	99.9	8.2	44.8
Ai-13	83.9	7.9	46.7
Ai-12	114.6	10.0	44.6
Mo	94.3	8.2	41.3
Mi-1	102.7	8.5	45.7
Mi-4	101.3	9.4	48.9
CD-A (P=0.05)	NS	NS	NS
CD-M (P=0.05)	NS	NS	NS

control (86). The individual effect of Mi-1 and Mi-4 was negated when these were inoculated along with Ai-13 and produced lesser number of leaves (Table 32).

Maximum number of tillers of 11.9 were produced in Ai-12 x Mi-4 inoculation. All the other treatments were on par with control. Plant height was significantly higher with AO x Mi-4 (51.9 cm) and Ai-13 x Mi-4 (52.4 cm) in comparison with control (36.1 cm). In the field, the main effect of antagonists and mycorrhizal isolates were not significant in enhancing plant growth characteristics.

4.13.2 Disease incidence and intensity

Tiller infection due to rhizome rot was lowest in mycorrhizal treatment AO x Mi-1 (27.1 per cent) followed by combined inoculation of Ai-12 x Mi-1 (28.7 per cent) (Table 33). Inoculation with AO x Mi-4 (31.1 per cent) and Ai-12 x Mi-4 (32.8 per cent) also significantly reduced the infection over control (47.1 per cent). Although inoculation of Ai-12 x MO exerted significant reduction in the per cent tillers infected (35.1), its interactive effect with AMF was not significant over their single inoculation. Antagonistic fungal isolate, Ai-13 did not reduce the tiller infection significantly (40.7 per cent) and its interaction with AMF isolates affected the disease reducing ability of Mi-1 and Mi-4 considerably. The main effects of antagonists were not

Table 33 Effect of native AMF, antagonists and *Pythium aphanidermatum* on rhizome rot incidence of ginger in the field

Treatment	Disease incidence		
	Infected tillers (%)	Dead tillers (%)	Disease intensity (0-9 scale)
AoxMi-1	20.7 (27.1)	13.1 (21.2)	1.5 (7.1)
AOxMi-4	26.7 (31.1)	20.0 (26.5)	2.0 (8.1)
Ai-13xMo	42.7 (40.7)	26.0 (30.6)	3.7 (11.2)
Ai-13xMi-1	50.6 (45.3)	28.2 (32.1)	3.7 (11.1)
Ai-13xMi-4	36.1 (36.9)	28.4 (32.1)	3.3 (10.5)
Ai-12xMo	33.1 (35.1)	22.1 (28.1)	2.8 (9.6)
Ai-12xMi-1	23.1 (28.7)	12.7 (20.8)	1.7 (7.6)
Ai-12xMi-4	29.4 (32.8)	15.1 (22.8)	2.3 (8.6)
AoxMo	53.8 (47.1)	37.0 (37.4)	4.3 (12.0)
CD-T (P=0.05)	9.93	4.50	2.18
Main effect			
A0	35.1	28.4	9.0
Ai-13	40.9	31.6	10.9
Ai-12	32.2	23.9	8.6
Mo	40.9	32.0	10.9
Mi-1	33.6	24.7	8.6
Mi-4	33.6	27.1	9.0
CD-T (P=0.05)	5.73	2.59	1.25
Main effect Ao	5.73	2.59	1.25

Values given in parenthesis denote transformed means

significant while the effects of AMF were highly significant in reducing the per cent infection. The per cent tillers infected due to the main effects of Mi-1 and Mi-4 were 33.69 and 33.60 respectively as against 40.99 in control (Table 33).

The mortality rate of tillers was significantly less in all the treatments compared to the control plot (Table 33). The lowest per cent mortality was recorded in the dual inoculation of Ai-12 x Mi-1 (20.8) followed by AO x Mi-1 (21.2). Treatments, AI-12 x Mi-4 (22.8) and AO x Mi-4 (26.5) also reduced per cent tillers dead significantly over control plots (37.4). The AO x Mi-1 inoculation (21.2 per cent) was significantly more effective in reducing the mortality than AO x Mi-4 (26.5 per cent). The interactive effect of Ai-12 with AMF significantly reduced the mortality. The main effect of antagonistic fungal isolate, Ai-12 and mycorrhizal isolates, Mi-1 and Mi-4 were significantly high in reducing the mortality rate due to the disease incidence. The intensity of disease was significantly low in mycorrhizal treatments AOxMi-1 (7.1) and AO x Mi-4 (8.1), in the antagonistic fungal inoculations, Ai-12 x MO (9.6) and in their combined inoculations, *viz.*, Ai-12 x Mi-1 (7.6) and Ai-12 x Mi-4 (8.6) over control plot (12.0). However, the interactive effect of Ai-13 x Mi-1 and Ai-13 x Mi-4 increased the disease intensity (11.1 and 10.5) compared to the AMF alone inoculation (7.1 and 8.1). The main effect of AMF isolates were significant in

reducing the disease intensity while the main effect of antagonist, Ai-13 significantly increased the disease intensity.

4.13.3 Yield and dry matter production

Maximum yield was obtained in AO x Mi-1 inoculated plots (785 g) followed by Ai-12 x Mi-1 (740 g), AO x Mi-4 (708.3 g) and Ai-12 x Mi-4 (690 g) accounting for 42.5, 34.1, 28.3 and 25.1 per cent yield increase over control (551.7 g) (Table 34). The interactive effect of Ai-13 x Mi-1 and Ai-13 x Mi-4 resulted in decreased yield than the individual effect of AMF isolates (from 785 g to 625 g and from 708.3 g to 640 g).

Maximum plant top dry weight was observed in the combined inoculation of Ai-12 x Mi-4 (61.7 g) and Ai-12 x Mi-1 (61 g) as against 41.2 g in control treatment (Table 34). A substantially higher plant top dry weight was also obtained in AO x Mi-1, AO x Mi-4 and Ai-12 x MO inoculations. But the interactive effect of Ai-13 x Mi-1 and Ai-13 x Mi-4 reduced the plant top dry weight from 54.2 g to 45.7 g for Mi-1 and from 52.2 g to 46.7 g for Mi-4. Significantly higher root dry weights were observed in the combined inoculations of Ai-12 x Mi-4 (28.7 g) and Ai-12 x Mi-1 (27.3 g) and also in the AMF treatments, AO x Mi-1 (23.7 g) and AO x Mi-4 (26.8 g) as against 14.2 g in control plots. In contrast, the interactive

Table 34 Effect of selected AMF, antagonists and rhizome rot pathogen on dry weight and yield of ginger in the field

Treatment	Plant top dry weight ($g\ pl^{-1}$)	Root dry weight ($g\ pl^{-1}$)	Rhizome yield ($g\ pl^{-1}$)
AoxMi-1	54.2	23.7	785.0*
AOxMi-4	52.2	26.8	708.3
Ai-13xMo	38.2	12.7	625.0
Ai-13xMi-1	45.7	13.7	640.0
Ai-13xMi-4	46.7	12.7	610.0
Ai-12xMo	52.0	17.7	616.7
Ai-12xMi-1	61.0	27.3	740.0
Ai-12xMi-4	61.7	28.7	690.0
AoxMo	41.2	14.2	551.7
CD-T (P=0.05)	NS	6.6	NS
Main effect			
A0	49.2	21.6	681.7
Ai-13	43.5	13.0	625.0
Ai-12	58.2	24.7	682.2
Mo	43.8	14.8	597.8
Mi-1	53.6	21.7	721.7
Mi-4	53.5	22.7	669.4
CD-A (P=0.05)	NS	3.8	NS
CD-M (P=0.05)	NS	3.8	NS

*Total of four plants

effect of Ai-13 with Mi-1 and Mi-4 substantially reduced the dry root weight of Mi-1 from 23.7 g to 13.7 g and Mi-4 from 26.8 g to 12.7 g. Although the main effects of antagonists or mycorrhizal isolates were not significant with regard to yield and dry weight of plant top, the main effect of mycorrhizal isolates on root dry weight was significant and recorded 21.7 g and 22.7 g for Mi-1 and Mi-4 respectively as against 14.8 g of non mycorrhizal plants. The interactive effect of Ai-13 masked the beneficial effect of AMF on growth and yield attributes.

4.13.4 AMF colonization, population of pathogen and antagonists

Treatments with mycorrhizal isolates, *viz.*, AO x Mi-1 and AO x Mi-4 produced higer AMF root colonization of 77.2 and 72.7 per cent respectively (Table 35). Inoculation with Ai-13 decreased the colonization considerably, while Ai-12 did not show such deleterious effect. The natural AMF colonization in different treatments did not show much variation (30 - 40 per cent). The AMF spore count was maximum in the Ai-12 x Mi-1 (58) followed by AO x Mi-4 treatment (52). Inoculation with Ai-13 reduced the spore count of Mi-1 and Mi-4.

The pathogen population of 21 cfu/50 mg soil in the Ai-12 x Mi-1 inoculation was the lowest, followed by AO x Mi-1 (29 cfu), Ai-12 x Mi-4 (33 cfu) and AO x Mi-4 (37 cfu) treatments (Table 35). Maximum pathogen population of

Table 35 Effect of selected AMF, antagonists and *P. aphanidermatum* on AMF colonization and population of pathogen and antagonists in the ginger rhizosphere in the field

Treatment	AMF colonization (%)	Intensity of colonization	AMF spore count ($10\ g^{-1}$ soil)	Pathogen population (cfu/50mg soil)	Antagonist population	
					Isolate No.12 ($x10^4$/g soil)	Isolate No.13 ($x10^4$/g soil)
AoxMi-1	77.2	+++	46	29	12	2
AOxMi-4	72.7	+++	52	37	18	7
Ai-13xMo	41.7	++	23	62	8	36
Ai-13xMi-1	54.5	++	41	58	11	45
Ai-13xMi-4	63.6	+++	50	46	7	28
Ai-12xMo	30.4	+	26	38	72	7
Ai-12xMi-1	65.2	+++	58	21	81	10
Ai-12xMi-4	68.3	+++	49	33	79	8
AoxMo	34.7	+	24	83	24	4

Values are mean of three replications

83 cfu/50 mg soil was observed in control plot. Between the antagonistic fungal isolates, Ai-12 x MO inoculated plots harboured relatively less number of pathogen (38 cfu) compared to Ai-13 x MO (62 cfu). The population of antagonists, Ai-12 and Ai-13 were very high in the respective inoculated plots.

4.13.5 Analysis of nutrients

Estimation of P of plant top revealed that all the mycorrhizal fungi inoculated treatments had high P content (Table 36). Maximum P content was observed in AOxMi-4 (395 ppm) followed by Ai-12 x Mi-4 (384.9 ppm) inoculations which were significantly higher than the untreated control (286.1 ppm). The antagonists had no significant influence on P content of plant top. The P content of roots were significantly higher in all the mycorrhizal inoculations and the maximum P content was recorded in the combined inoculation of Ai-12 x Mi-1 (475 ppm) followed by Ai-12 x Mi-4 (461 ppm) (Table 36). The P content of control plot was 333.3 ppm. In the combined treatment of Ai-13 x Mi-1 and Ai-13 x Mi-4 P content was reduced compared to single inoculations of AMF. The antagonistic fungal isolates had no significant main effect in enhancing the P content of roots. Both the mycorrhizal isolates (Mi-1 and Mi-4) exerted significant main effect in enhancing the P content (451.8 and 448.1 ppm respectively) compared to control (354.6 ppm).

Table 36 Effect of selected AMF and antagonists on phosphorus and potassium content of plant tissue of ginger in the field

Treatment	P		K	
	Plant top (ppm)	Roots (ppm)	Plant top (%)	Roots (%)
Ao x Mi-1	327.8	447.2	0.201	0.199
Ao x Mi-2	395.0	452.8	0.244	0.221
Ai 13 x M0	288.9	358.3	0.215	0.211
Ai-13 x Mi-1	311.1	433.3	0.228	0.214
Ai-13 x Mi-4	316.0	430.5	0.238	0.220
Ai-12 x M0	263.9	372.2	0.199	0.175
Ai-12 x Mi-1	347.2	475.0	0.248	0.247
Ai-12 x Mi-4	384.9	461.1	0.231	0.217
AOxMO	286.1	333.3	0.178	0.176
CD-T (P = 0.05)	85.8	45.7	0.056	0.047
Main effect				
AO	336.3	411.1	0.208	0.199
Ai-13	305.5	407.4	0.227	0.215
Ai-12	331.9	436.1	0.226	0.213
M0	279.6	354.6	0.198	0.188
Mi-1	328.7	451.8	0.226	0.220
Mi-4	365.5	448.1	0.238	0.220
CD-A (P=0.05)	49.5	26.4	0.010	0.027
CD-M (P=0.05)	49.5	26.4	0.010	0.027

Maximum plant top K content of 0.248 per cent was recorded in Ai-12 x Mi-1 inoculation. K content was also significantly higher in the combined inoculation of Ai-13 x Mi-4 (0.238 per cent) and Ao x Mi-4 (0.244 per cent) in comparison with 0.178 per cent of control plot (Table 36). All the rest of the treatments were on par with the control. Between the antagonist inoculated plots, Ai-13 recorded higher K content (0.215 per cent) in comparison with Ai-12 inoculation (0.199 per cent). Between the AMF inoculated plots, Mi-4 recorded higher K content (0.244 per cent) than Mi-1 (0.201 per cent). The main effect of antagonistic fungal isolates, Ai-13 and Ai-12 and mycorrhizal isolates Mi-1 and Mi-4 were significant in enhancing the K concentration of plant top.

K content of root was significantly higher only in the combined inoculation of Ai-12 x Mi-4 (0.247 per cent) as against the control treatment (0.176 per cent). Root K content of Ai-13 inoculation (0.211 per cent) was higher than Ai-12 (0.175 per cent) inoculation (Table 36). Eventhough the antagonists did not exert any significant main effect in enhancing the root K content, the mycorrhizal isolates exerted significant effect. The K content due to mycorrhizal effects was 0.220 for both Mi-1 and Mi-4 isolates as against 0.188 of control.

Analysis of micronutrients of the plant top and roots indicated varying responses. Cu content in the plant top was

significantly higher in AO x Mi-4 (1.550 ppm) in comparison with control (1.146 ppm) and other treatments (Table 37). Ca content of plant top was on par with treatments and control. However, maximum Ca was recorded in the combined inoculation of Ai-12 x Mi-4 (908.5 ppm) followed by Ai-12 x Mi-1 (908 ppm) in comparison with control (700.6 ppm). The main effect of Ai-12 was significant in enhancing the plant top Ca content (788 ppm) over Ai-13 (638.9 ppm) whereas the main effects of mycorrhizal isolates, Mi-1 and Mi-4 were significantly higher in increasing the Ca content (767.3 and 760.1 ppm) over control (581.7 ppm).

With regard to Mg content there was no significant difference between the different treatments. All the treatments had lesser Mg content than the uninoculated control (Table 37). The main effect of antagonists and mycorrhizal isolates were not significant. Zn content of 14.03 ppm was maximum in the combined inoculation of Ai-12 x Mi-4 which was significantly higher than 9.8 ppm in control and antagonists inoculations of AI-12 and Ai-13. No significant difference could be observed due to the main effect of antagonists with regard to Zncontent of plant top. But the mycorrhizal main effects were significant in having higher Zn content (12.88 ppm for Mi-1 and 12 ppm for Mi-4 as against 9.06 ppm for control).

No significant difference could be observed in the Mn and Fe content of different treatments and control (Table 37).

Table 37 Effect of selected AMF and antagonists on micronutrient content of ginger plant top in the field

Treatment	Copper (ppm)	Calcium (ppm)	Magnisium (ppm)	Zinc (ppm)	Managnese (ppm)	Iron (ppm)
Ao x Mi-1	1.133	617.8	2155.8	12.73	31.13	60.6
Ao x Mi-4	1.550	727.8	2199.3	10.97	34.00	73.9
Ai 13 x M0	0.976	496.8	2161.5	8.53	24.06	99.6
Ai-13 x Mi-1	1.233	776.2	2190.8	13.40	23.56	112.5
Ai-13 x Mi-4	0.753	643.9	2142.7	11.00	32.13	75.8
Ai-12 x M0	1.090	547.5	2177.5	8.86	20.83	62.8
Ai-12 x Mi-1	1.166	908.0	2166.7	12.50	34.50	62.3
Ai-12 x Mi-4	0.876	908.5	2195.8	14.03	32.63	88.5
AOxMO	1.146	700.6	2216.7	9.80	30.76	95.6
CD-T (P=0.05)	0.311	NS	NS	3.94	NS	25.0
Main effect						
AO	1.270	682.1	2190.6	11.17	31.97	76.7
Ai-13	0.981	638.9	2165.0	10.97	26.59	95.9
Ai-12	1.040	788.0	2180.0	11.80	29.32	71.2
M0	1.073	581.7	2185.2	9.06	25.22	86.0
Mi-1	1.181	767.3	2171.1	12.88	29.73	78.4
Mi-4	1.060	760.1	2179.0	12.00	32.92	79.4
CD-A (P=0.05)	NS	NS	NS	NS	NS	14.4
CD-M (P=0.05)	NS	130.3	NS	2.28	NS	NS

However, more Mn content was observed in mycorrhizal isolates than control. Maximum Fe content was recorded in the Ai-13xMi-1 inoculated plots (112.5 ppm). Between the antagonistic fungal isolates, Fe content was significantly higher in the Ai-13 inoculation (99.6 ppm) than Ai-12 inoculation (62.8 ppm). The main effect due to antagonist, Ai-13 was significantly higher (95.98) over control (76.71). There was no significant main effect due to inoculation with mycorrhizal isolates.

Root Cu content was significantly higher in the AMF inoculated treatment of Ao x Mi-1 (1.21 ppm) and Ao x Mi-4 (1.24 ppm) and combined inoculation of Ai-13 x Mi-4 (1.16 ppm) and Ai-12 x Mi-4 (1.20 ppm) as against 0.843 ppm in the control treatment and against the antagonist inoculations of Ai-13 and Ai-12 (Table 38). The interactive effect of Ai-13 x Mi-1 and Ai-12 x Mi-1 tended to reduce the Cu content. The main effect of antagonists inoculation was not significant in enhancing root Cu content. Mycorrhizal main effects were highly significant in increasing the Cu content of roots (1.03 and 1.20) over non mycorrhizal control (0.81).

There was significantly higher Ca content in the combined inoculation of Ai-13 x Mi-1 (274.3 ppm), Ai-12 x Mi-1 (293.7 ppm) and Ai-12 x Mi-4 (291.6 ppm) in comparison with control (163.9 ppm) and antagonists inoculations of Ai-13 x MO and Ai-12 x MO (Table 38). There was no significant main

Table 38 Effect of selected AMF and antagonists on micronutrient content of ginger roots in the field

Treatment	Copper (ppm)	Calcium (ppm)	Magnisium (ppm)	Zinc (ppm)	Managnese (ppm)	Iron (ppm)
Ao x Mi-1	1.210	229.9	2061.7	7.27	18.7	103.2
Ao x Mi-4	1.243	250.8	2137.5	6.23	25.0	134.0
Ai 13 x M0	0.743	169.4	2069.2	3.57	16.5	93.5
Ai-13 x Mi-1	0.900	274.3	2111.7	7.97	18.0	98.7
Ai-13 x Mi-4	1.166	252.8	2097.5	8.10	11.8	73.8
Ai-12 x M0	0.846	160.8	2074.2	2.83	18.1	100.2
Ai-12 x Mi-1	0.993	293.7	2148.3	7.13	16.3	137.7
Ai-12 x Mi-4	1.203	291.5	2138.3	7.10	21.1	124.3
AOxMO	0.843	163.9	2090.0	2.90	18.9	114.9
CD-T (P=0.05)	0.200	98.1	NS	3.07	NS	NS
Main effect						
AO	1.090	214.9	2096.4	5.47	20.8	117.4
Ai-13	0.932	232.2	2092.8	6.54	15.5	88.7
Ai-12	1.014	248.7	2120.3	5.69	18.5	120.7
M0	0.813	164.7	2077.8	4.53	17.8	102.9
Mi-1	1.033	266.0	2107.2	6.02	17.7	113.2
Mi-4	1.204	265.1	2124.4	7.14	19.3	110.7
CD-A (P=0.05)	NS	NS	NS	NS	NS	NS
CD-M (P=0.05)	0.115	56.6	40.6	1.77	NS	NS

effect due to antagonists in the Ca content of roots. But the mycorrhizal inoculations exerted significant main effect in increasing Ca content (266 in Mi-1 and 265.07 in Mi-4 as against 164.7 in control). The data also indicated that Ca content of roots was increased due to the combined inoculation of AMF and antagonists than the single inoculations of AMF.

The Mg content of roots in all the treatments were on par and not significantly different from control (Table 38). However maximum Mg content of 2148.3 ppm was noticed in the dual inoculation of Ai-12 x Mi-1 as against 2090 ppm in control.

Root Zn content was significantly higher in all the single inoculations of AMF and all the combined inoculations. Maximum Zn content of 8.1 ppm was observed in the combined inoculation of Ai-13 x Mi-4 as against 2.9 ppm in the untreated control and 3.6 ppm in Ai-13 and 2.8 ppm in Ai-12 alone inoculations (Table 38). The main effect of antagonists was not significant in enhancing Zn content over control. The main effect of mycorrhizal isolate, Mi-4 significantly enhanced Zn content of roots (7.14) as against control (4.53). There was no significant difference between the different treatments and control with respect to Mn and Fe content of roots.

4.14 Standardization of mycorrhizal inoculation techniques for ginger

The data on the different AMF inoculation techniques for increasing the colonization are presented in Table 39. Maximum per cent AMF colonization of 70 was recorded in the starch treatment at 30 DAP which was closely followed with 56.8 per cent in the soil inoculation and 50 per cent in the jaggary slurry treatment as against 13.6 per cent in the non mycorrhizal control. At 60 and 90 DAP maximum AMF colonization of 85 per cent and 95 per cent were observed in the starch treatment as against 76.8 and 91 per cent in the normal soil inoculation method. In the non inoculated control, the per cent colonizations were 24.4 and 28 respectively. Very high intensity of colonization were also recorded in the starch and soil inoculations. The per cent AMF colonization was lowest in wheat gum (42) followed by gum arabic (55) treatments.

4.15 Effect of plant protection chemicals on AMF and antagonists

The highest per cent AMF colonization of 70.8 was recorded in the non treated mycorrhizal control plants (Table 40). Carbofuran, dimethoate and thiram treated plants also exhibited remarkably higher per cent AMF colonization of 66.7, 63.6 and 62.5 respectively. Bordeaux mixture recorded the lowest AMF colonization of 37.5 per cent. A similar trend was observed with respect to AMF spore count also. The spore counts

Table 39 Effect of different inoculation methods on per cent AMF colonization in ginger

Treatments	Days after planting					
	30		60		90	
	AMF colonization (%)	Intensity of colonization	AMF colonization (%)	Intensity of colonization	AMF colonization (%)	Intensity of colonization
Cowdung slurry +AMF	40.0	++	60.0	++	74.0	+++
Jaggary slurry +AMF	50.0	++	70.0	++	79.0	+++
Starch + AMF	70.0	+++	85.0	+++	95.0	++++
Wheat gum + AMF	25.4	+	35.4	+	42.0	++
Gum arabic + AMF	32.3	+	47.2	+	55.0	++
Soil inoculation+AMF	56.8	++	76.8	+++	91.0	++++
Control (No AMF)	13.6	+	24.4	+	28.0	+

Values are mean of four replications

Table 40 Effect of plant protection chemicals on AMF and antagonist

Treatments	Per cent AMF colonization	Intensity of colonization	AMF spore count ($10\ g^{-1}$ soil)	Population of the antagonist A1-12 ($\times 10^4$ soil)
Thiram (0.25%)	62.50	+++	47	62
Bordeaux mixture (1%)	37.50	+	28	35
Ekalux (0.15%)	58.30	++	39	68
Dimethoate (0.05%)	63.60	+++	52	65
Carbofuran (0.75 kg a.i/ha)	66.70	+++	49	71
Control (No pesticide)	70.80	+++	55	74

Values are mean of four replications

were drastically reduced in Bordeaux mixture and Ekalux treatments with 28 and 29 spores respectively as against 55 in the non treated control. Although slightly reduced, dimethoate, carbofuran and thiram treatments recorded spore counts of 52, 49 and 47 respectively.

The antagonistic fungal population was also drastically reduced in Bordeaux mixture treatment (35 cfu x $10^4/g$) compared to control (74 cfu x $10^4/g$). The population of antagonists in the other treatments were on par.

DISCUSSION

5. DISCUSSION

Rhizome rot is a devastating disease of ginger and various control measures including application of fungicides are not very successful in reducing the severity of the disease. The present investigation was intended to explore the potential of native arbuscular mycorrhizal fungi and antagonistic fungi for the management of rhizome rot of ginger and to analyse theircombined effect on nutrient uptake, growth and rhizome yield of ginger.

Apart from some preliminary reports (Iqbal and Nasim, 1991; Sivaprasad, 1993) no detailed investigation was undertaken on the possible role of AMF in the growth and health of this precious spice crop. The initial survey conducted in the major ginger growing tracts of Kerala revealed that ginger is a heavily mycorrhizal crop (Table 1 and 2). Further, it clearly indicated a definite negative relationship between AMF colonization and incidence and intensity of rhizome rot of ginger. The extent of colonization may be related to the inherent nature and mycorrhizal dependency of the host (Mosse, 1973) which are known to be influenced by the soil conditions particularly the nutrient status (Abbott *et al.*, 1984; Amijee *et al.*, 1989; Smith, 1988). The ability of mycorrhizal plants to reduce incidence of disease is well known (Dehne, 1982; Jalali and Jalali, 1991). The low rate of incidence of rhizome

rot in heavily colonized mycorrhizal ginger plants in the natural conditions is a reflection of this phenomenon.

The high incidence of disease in few heavily AMF colonized plants may be either due to the succulent growth of the plant by better nutrient absorption (Davis and Menge, 1980; Davis *et al.*, 1979; Smith, 1988) or due to negation of AMF effect by heavy population build up of the pathogen or due to the presence of deleterious microbes and absence of antagonists in the rhizosphere (Davis *et al.*, 1978; Linderman, 1994; Ross, 1972). The data further illustrated the influence of locations on the AMF colonization. This may probably be due to the variations in soil factors which are known to influence AMF colonization (Hayman, 1982; Bethlenfalvay *et al.*, 1985).

Based on the survey, a preliminary trial was conducted to test the efficacy of different AMF species in promoting plant growth, yield and reducing rhizome rot incidence. Observations on the growth characteristics, biomass production and yield showed that the plants responded differently to different AMF species (Fig. 2). *Glomus fasciculatum* and *G. constrictum* stimulated the production of more number of leaves and tillers while *G. fasciculatum* gave significantly higher yield over control which was followed by *G. constrictum*. But the highest plant dry weight was observed in *G. monosporum*, followed by *G. fasciculatum*. The better growth and rhizome yield of ginger due to inoculation with appropriate AMF has

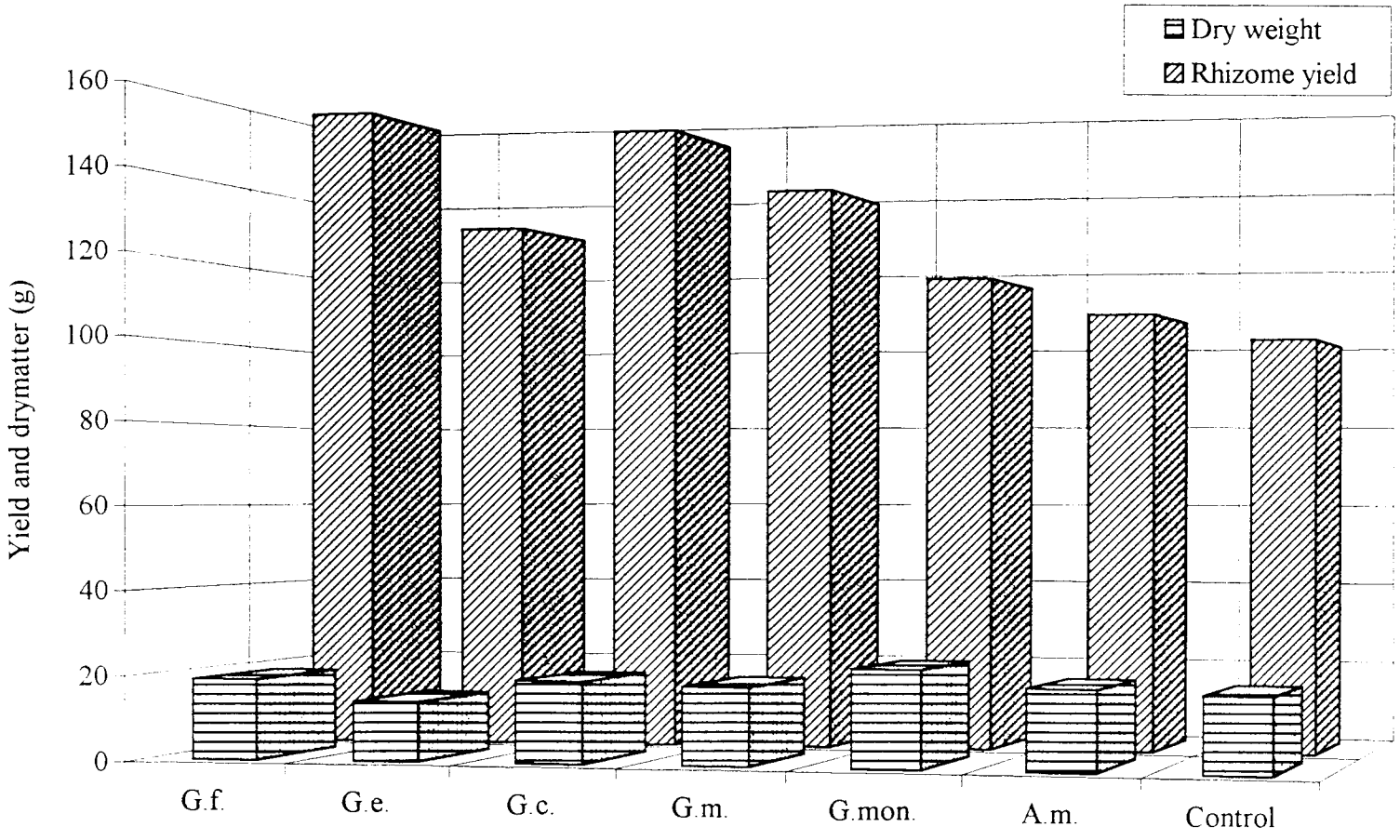


Fig. 2 Effect of different AMF species and *P. aphanidermatum* on drymatter production and yiled of ginger

been reported (Sivaprasad, 1993). The ability of AMF to stimulate growth and biomass production depends on mycorrhizal dependency of host, the endophyte species and soil fertility level (Harley and Smith, 1983; Hayman, 1982; Mosse, 1973). Hence, the variation observed amongst the AMF species to stimulate growth and biomass production might be a reflection of the inherent ability of the species tested. Such variation in the effectiveness of AMF has been observed in most plant species (Harley and Smith, 1983; Sanders *et al.*, 1977)

The influence of AMF on incidence and intensity of rhizome rot disease was studied in the present investigation using a virulent strain of the pathogen, *Pythium aphanidermatum*(Edson)Fitz.

The ability of AMF species to induce tolerance against rhizome rot also varied with AMF species. The disease incidence and intensity were significantly low in *G.constrictum* and *G. mosseae* inoculated treatments (Fig.1). Similar effect of *G. mosseae* on rhizome rot was observed by Sivaprasad (1993) and of *G. fasciculatum* by Rohini Iyer and Sundararaju (1993). It may be noted that eventhough *G.fasciculatum* and *G.etunicatum* were found to be effective for growth stimulation, their association recorded significantly high disease incidence and intensity; probably the succulent growth predisposed the plants to pathogen (Davis *et al.*, 1978; Davis *et al.*, 1979). It may also be due to differences in physiological and biochemical

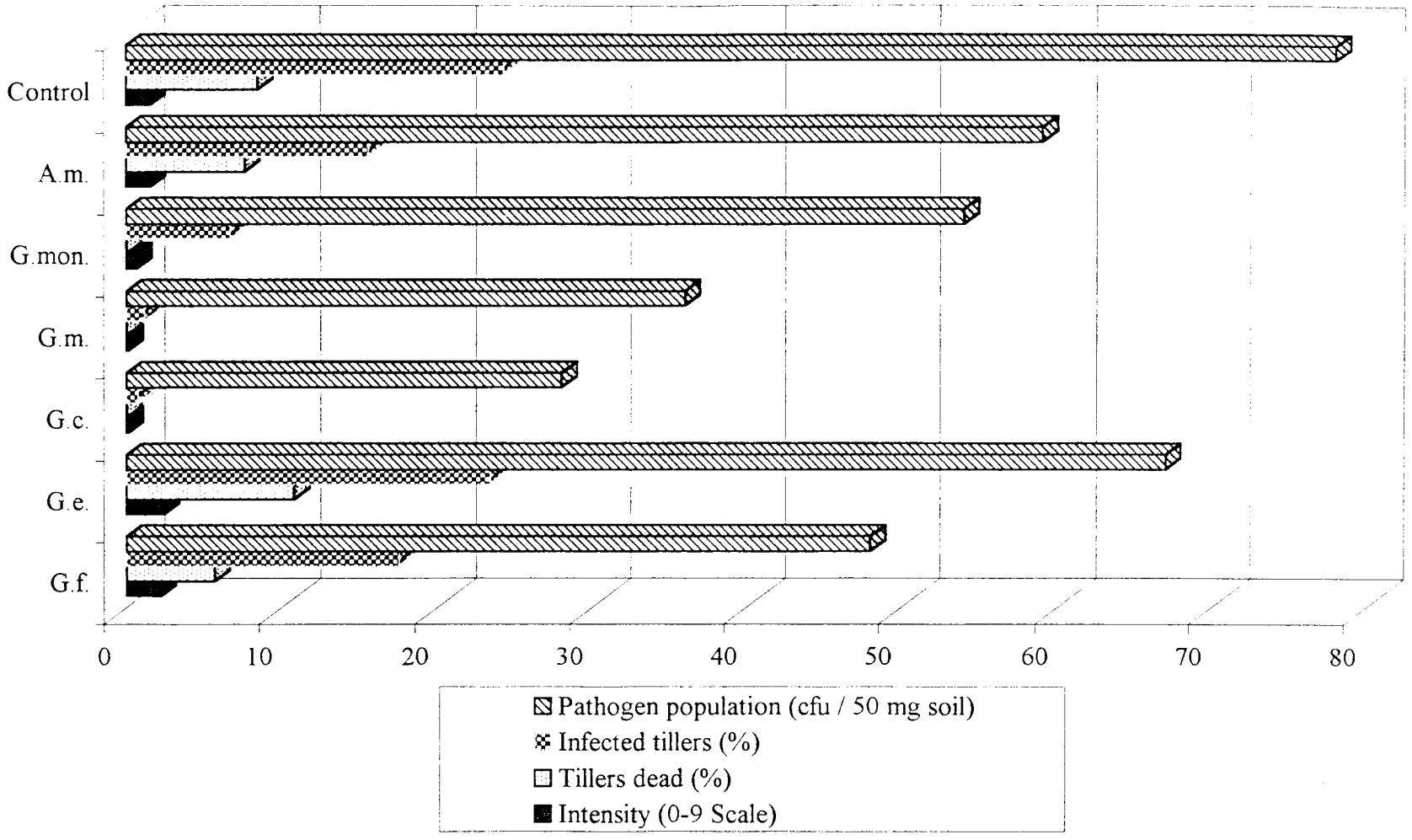


Fig. 1 Influence of different AMF species on rhizome rot of ginger and pathogen population

changes brought about by AMF species (Azcon-Aguilar and Barea, 1996) in plants.

Inoculation with AMF remarkably increases the mycorrhizal colonization and the spore production (Harlay and Smith, 1983; Mosse, 1973). Significantly higher AMF colonization and spore production observed in the present investigation in AMF inoculated ginger plants were in agreement with the above reports. Eventhough there was a general reduction in the pathogen population in mycorrhizal plants the least pathogen population was observed in plants inoculated with the *G. constrictum*. Ability to colonize the roots and suppress the population build up of pathogens have been considered to be desirable attributes of AMF for biocontrol (Dehne, 1982; Caron, 1989; Kaye *et al.*, 1984). In this point of view *G. constrictum*, which recorded high root colonization and low pathogen population and disease intensity is more desirable amongst AMF species tested in suppressing the disease.

The enhanced absorption of nutrients such as P, Ca, Cu, Zn, Fe by AMF is well documented (Barea and Jeffries, 1995; Marschner and Dell, 1994; Wellings *et al.*, 1991). Chemical analysis of plant samples indicated that P content was maximum in *Acaulospora morroweae* followed by *G. mosseae* and *G. constrictum* inoculated plants.. In the case of micronutrient content, *G. constrictum* showed significantly

higher values of Cu, Ca, Mg, Zn, Mn and Fe. The higher uptake of soil nutrients, particularly the less mobile nutrients, is attributed to greater volume of soil explored by the external AMF hyphae (Marschner and Dell, 1994; Jacobsen, 1995).

In the present study *G. constrictum* and *G. mosseae* were identified as the effective AMF species in reducing the incidence and intensity of rhizome rot while *G. fasciculatum* was the best AMF species in enhancing plant growth and yield. Considering the potential of *G. constrictum* in the suppression of disease and enhancement of growth and yield, it was used as the reference culture for further studies.

Root knot nematode infestation predisposes ginger plants to rhizome rot disease development (Dohroo *et al.*, 1987). The influence of AMF on *Meloidogyne incognita* infestation was tested. Among the different AMF species, *A. morroweae* produced better plant growth, yield and dry matter in the presence of *M. incognita* (Fig.3). The beneficial effect of AMF to offset the damage caused by nematodes are well documented (Roncadori and Hussey, 1977; Kellam and Schenck, 1980; Carling *et al.*, 1995). It has been indicated that the effect of a given interaction is strongly influenced by nematode inoculum level, cultivar resistance and the specific fungal symbiont (Schenck *et al.*, 1975). The study revealed that *A. morroweae* is a better biocontrol agent of *M. incognita* in ginger. The beneficial effect of *G. fasciculatum* has been reduced by the presence of

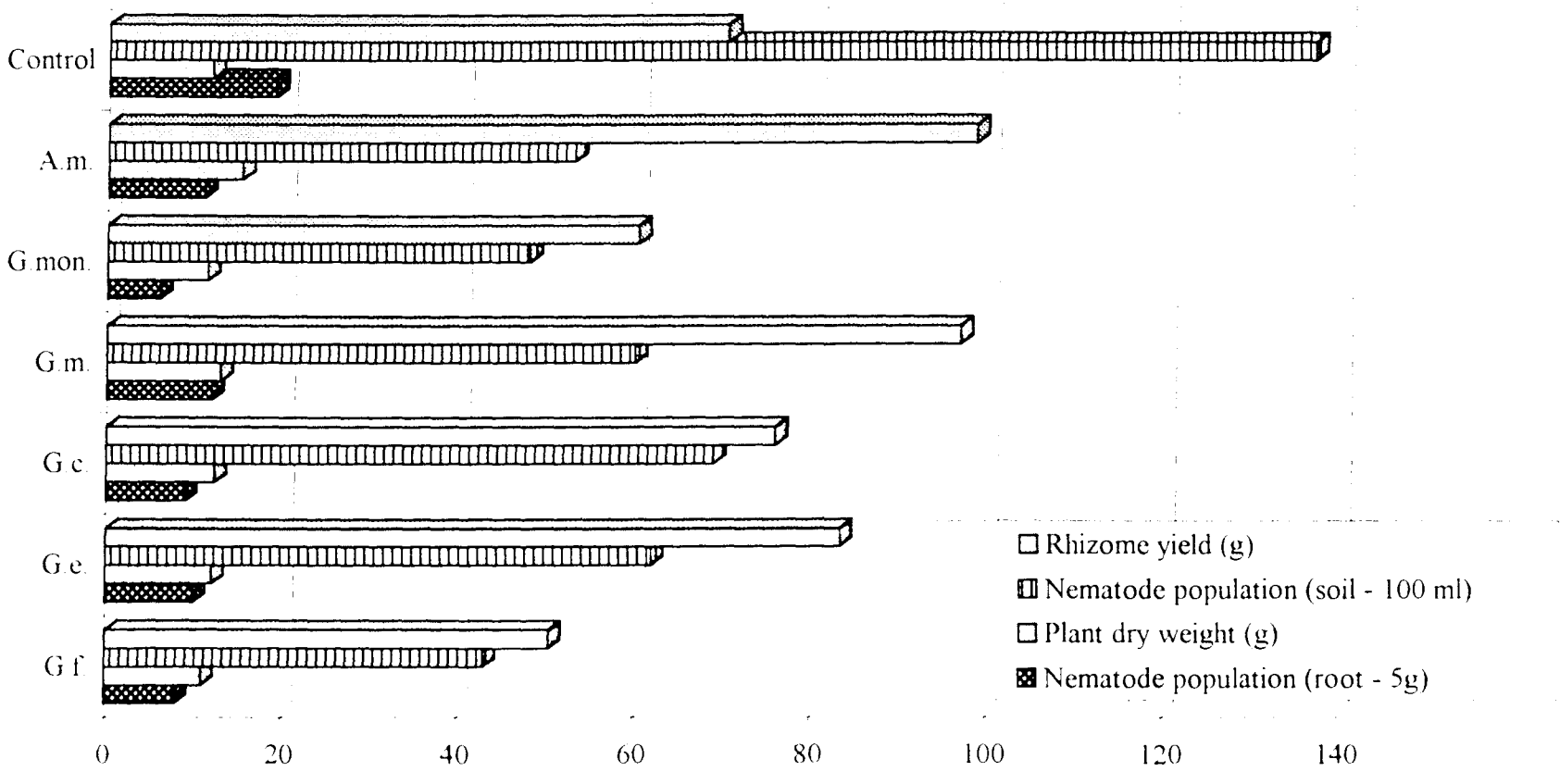


Fig. 3 Effect of different AMF species on drymatter and yield of ginger and nematode population

neamtode, indicating its failure to suppress the nematodes. Such negative effects are not uncommon (Atilano *et al.*, 1981; Elliot *et al.*, 1984). The AMF spore count was also lower in this treatment (Table 10). The nematode might have exerted a deleterious effect on the establishment of AMF in the host. Similar observation of negation of beneficial effect of *G. fasciculatum* and reduced colonization and sporulation were recorded in *Phaseolus vulgaris* due to *Pratylenchus penetrans* (Elliot *et al.*, 1984).

The populations of nematodes both inside the root and in the soil were significantly low in all the AMF inoculations, suggesting the inhibitory effect of AMF on nematode development (Fig.3). The AMF induced physiological changes in the plant may be a major factor limiting nematode multiplication (Bagyaraj *et al.*, 1979) or changes in the root exudates causing fewer nematodes attracted to the host (Ahmed and AlSayed, 1991).

It is well established that the root-knot nematode infestation seriously affects the root development and hence the soil nutrient uptake by the plants (Roncadori and Hussay 1977; Carling *et al.*, 1995). In the present investigation this effect was evident in both mycorrhizal and non-mycorrhizal plants. Eventhough higher P content was observed in all the AMF inoculated plants, significantly higher levels were observed only in *G. constrictum* and *G. mosseae* inoculations

indicating their ability to check the deleterious effect of root-knot nematode. The variation in the suppressive ability of AMF species on root knot nematode as observed in the present investigation has been reported in different crops and different conditions (Hussay and Roncadori, 1982).

There was no significant variation among all the AMF species in the K, Cu, Ca, Mg, Mn, Zn and Fe content of plants. The damage caused to the roots and AMF colonization by the nematode perhaps decreased the ability for increased absorption of nutrients by mycorrhizal plants. Such adverse effect of *M. incognita* infestation on nutrient uptake in the presence of AMF is observed (Elliot *et al.*, 1984; Thomson-Cason *et al.*, 1983).

In order to evaluate the influence of host genotypes on the species of AMF harboured in the ginger rhizosphere, different cultivars grown in the same field (lateritic soil) were subjected for characterization of associated AMF. The study indicated not much variation among the AMF species associated with different cultivars. However *Acaulospora* spp. stood as an exception with notable absence. This may be attributed to the soil factors, rather than genotype, as was evident from its occurrence in the ginger rhizosphere grown in sandy loam soil. The lateritic soil, where the genotypes were grown, differs in most of the soil characteristics from the low fertile sandy loam soil. This is in accordance with the

observation that soil factors exert much influence on the species of AMF association rather than host genotype (Bethlenfalvay *et al.*, 1985). The observations made with samples collectd from different locations and soil types further strengthened this view. For example, species of *Gigaspora* and *Acaulospora* were predominant in sandy loam whereas *Sclerocystis* spp. were more frequently observed in forest soils and *G. convolutum* in Pampadumpara of Idukki district. This clearly indicates the influence of soil types on the occurrence and development of AMF species in ginger rhizosphere.

All the fourteen native AMF isolates developed under the investigation were tested for their ability to induce growth and disease tolerance in ginger. There were wide variations among the mycorrhizal isolates in their ability to stimulate plant growth. The overall growth effect was highly significant in Mi-1 and Mi-13 inoculations. The study revealed that stimulation of plant growth is the inherent ability of AMF isolates in a given host-fungus - soil system: perhaps it is related to the ability of AMF hyphae to spread in the soil and tap the required nutrients beyond the depletion zone and make it available to the plant (Marschner and Dell, 1994).

All the native mycorrhizal isolates enhanced rhizome yield and plant dry weight (Fig.5). The enhanced growth of mycorrhizal plants is generally attributed to better uptake of

nutrients especially P (Harley and Smith, 1983; Gerdemann 1968; Mosse and Tinker, 1980). In the present study highest rhizome yield was recorded in Mi-4 followed by Mi-1 inoculations. The AMF induced growth improvement and resultant increase in economic yield have been reported in various plant species (Ross and Harper, 1970; Powell, 1981; Hwang *et al*., 1993).

The study on the incidence of the disease indicated that the ability of AMF to suppress the disease varied with isolates and as many as nine isolates significantly reduced the incidence and intensity of disease (Fig.4). Among them Mi-4, Mi-1 and Mi-9 were highly effective in reducing the incidence of thedisease. In Mi-4 inoculation no disease occurred while in Mi-1 and Mi-9 the disease was significantly less than many other mycorrhizal inoculations (Table 16).

Although Mi-9 did not show much effect on growth and yield, its potential to reduce disease incidence and intensity was very high. Likewise, in Mi-4 inoculation the effect on growth was not as pronounced as in Mi-1 or Mi-13, but it ranked top in its ability to check the disease throughout the period of observation. Surprisingly Mi-13, the most effective isolate for plant growth enhancement, was inefficient in checking the disease. Rhizome yield and root dry weight were very poor in this treatment due to very heavy disease incidence: perhaps the higher biomass production made the plant more succulent and

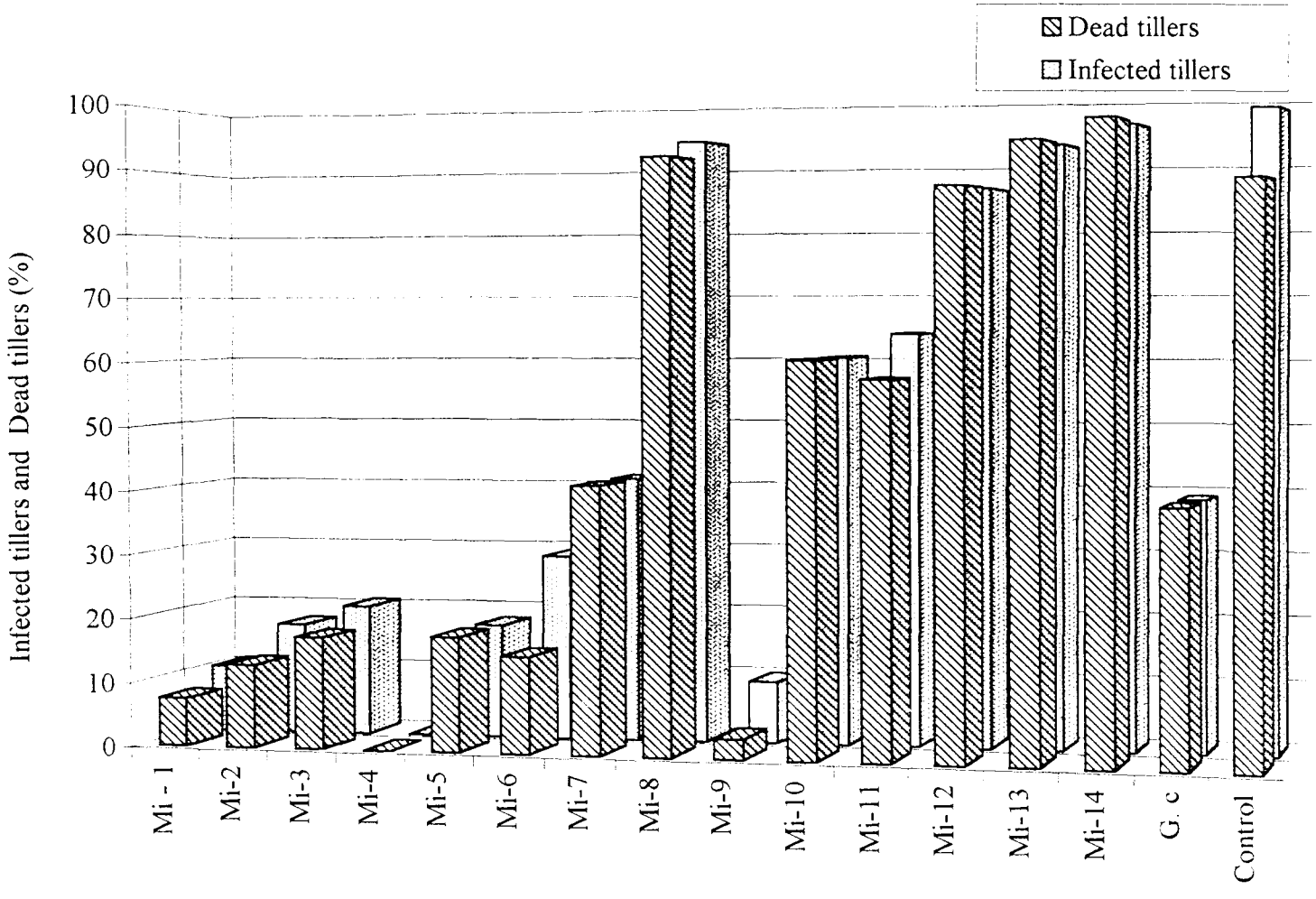


Fig. 4 Influence of native AMF on rhizome rot of ginger

vulnerable to the disease (Davis *et al.*, 1979; Davis and Menge, 1980).

Maximum stability in its potential to stimulate plant growth and suppression of rhizome rot was consistently expressed by the isolate, Mi-1. It vividly elucidates the fact that the ability of AMF for growth stimulation and disease suppression need not occur together in an isolate, rather they are independent traits. An isolate effective for growth enhancement and higher yield may be a poor bicontrol candidate (eg. Mi-13). Similarly, an AMF isolate having potential biocontrol efficiency need not induce significant growth enhancement (eg: Mi-9) (Vidhyasekaran, 1990). It is highly desirable if an isolate possessing both the qualities could be obtained. Mi-1 and Mi-4 have been identified as isolates possessing such characteristics.

A direct positive relationship of AMF colonization and spore count with growth stimulation and negative relationship with disease intensity was evident in the present study. The very high per cent AMF colonization and spore count might have rendered the plants more resistant to pathogen attack either by restricting the competition for colonization/ infection sites for favour of AMF, (Dehne, 1982; Jalali and Jalali, 1991; Cordier *et al.*, 1996) or by favouring the development of antagonists in the mycorrhizosphere, due to heavy

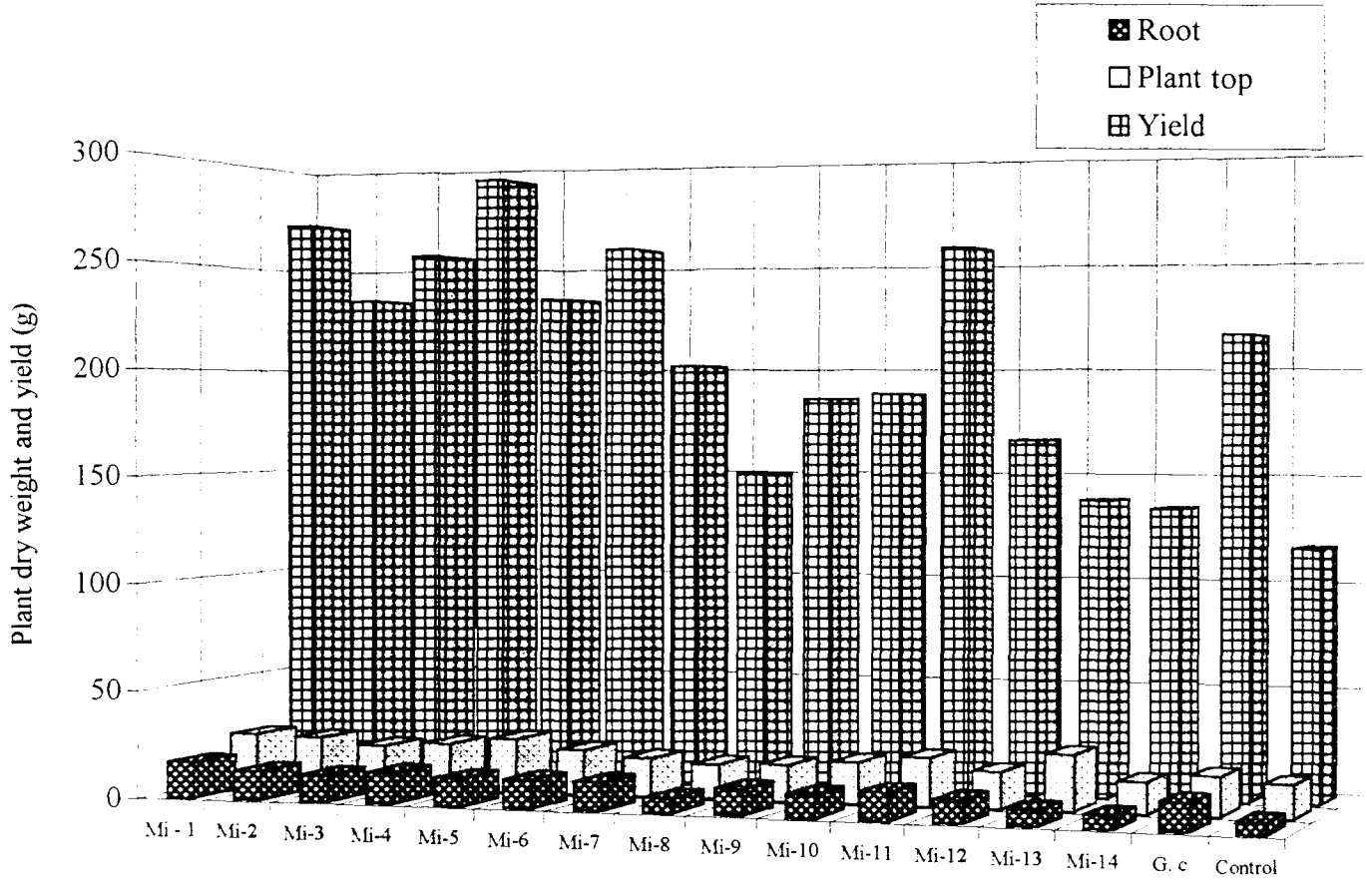


Fig. 5 Effect of native AMF and *P.aphanidermatum* on dry weight and yield of ginger

AMF colonization and spore production (Secilia and Bagyaraj, 1987; Meyer and Linderman, 1986).

A remarkably low pathogen population in all the mycorrhizal inoculations in which the disease incidence and intensity were significantly low indicated the potential of these AMF isolates in preventing the inoculum build up of the pathogen (Fig.6). Direct inhibitory effect of AMF on chlamydospores of the pathogen was reported in *Thielaviopsis basicola* (Baltruschat and Schonbeck, 1972a). However, the exact mechanism of this suppressive effect is not fully understood. Competition for infective sites (Linderman, 1985), microbial changes in the mycorrhizosphere due to altered root exudation (Azcon-Aguilar and Barea, 1996) altered host physiology due to activation of defence mechanisms (Azcon-Aguilar and Barea, 1996) etc. have been suggested as probable mechanisms. The mycosymbiont, which colonized the roots at the initial stages of plant growth, can subsequently deter the pathogen infection at later stages (Stewart and Pfleger, 1977; Dehne, 1982; Smith, 1988).

Analysis of the plant top and roots indicated very high P content in all the mycorrhizal plants irrespective of the isolates (Fig.7). This reflected the inherent ability of the AMF isolates for better exploration and absorption of soil P. The growth enhancement effect of AMF due to better

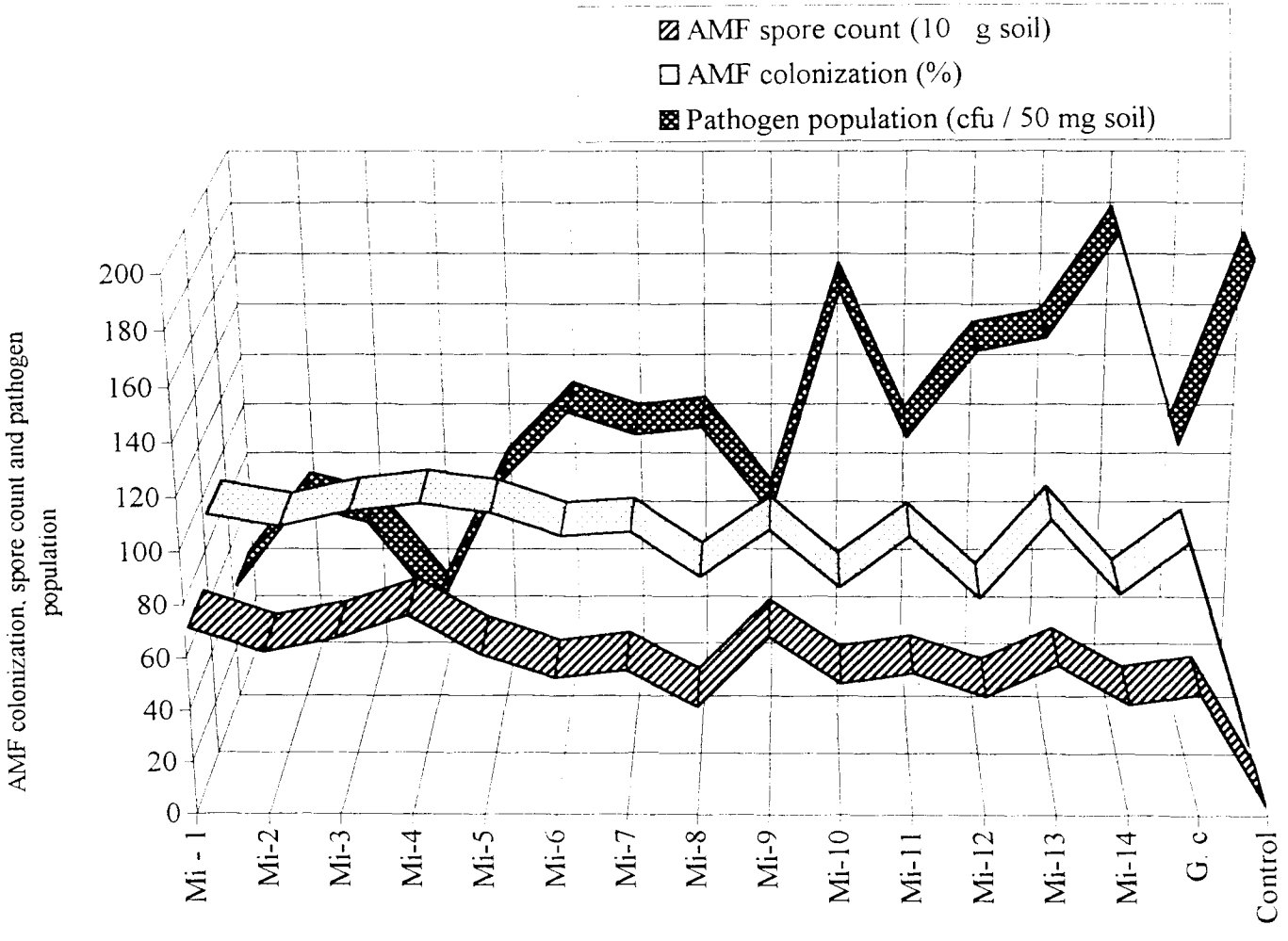


Fig. 6 Effect of native AMF and *P. aphanidermatum* on mycorrhizal colonization, spore count and pathogen population

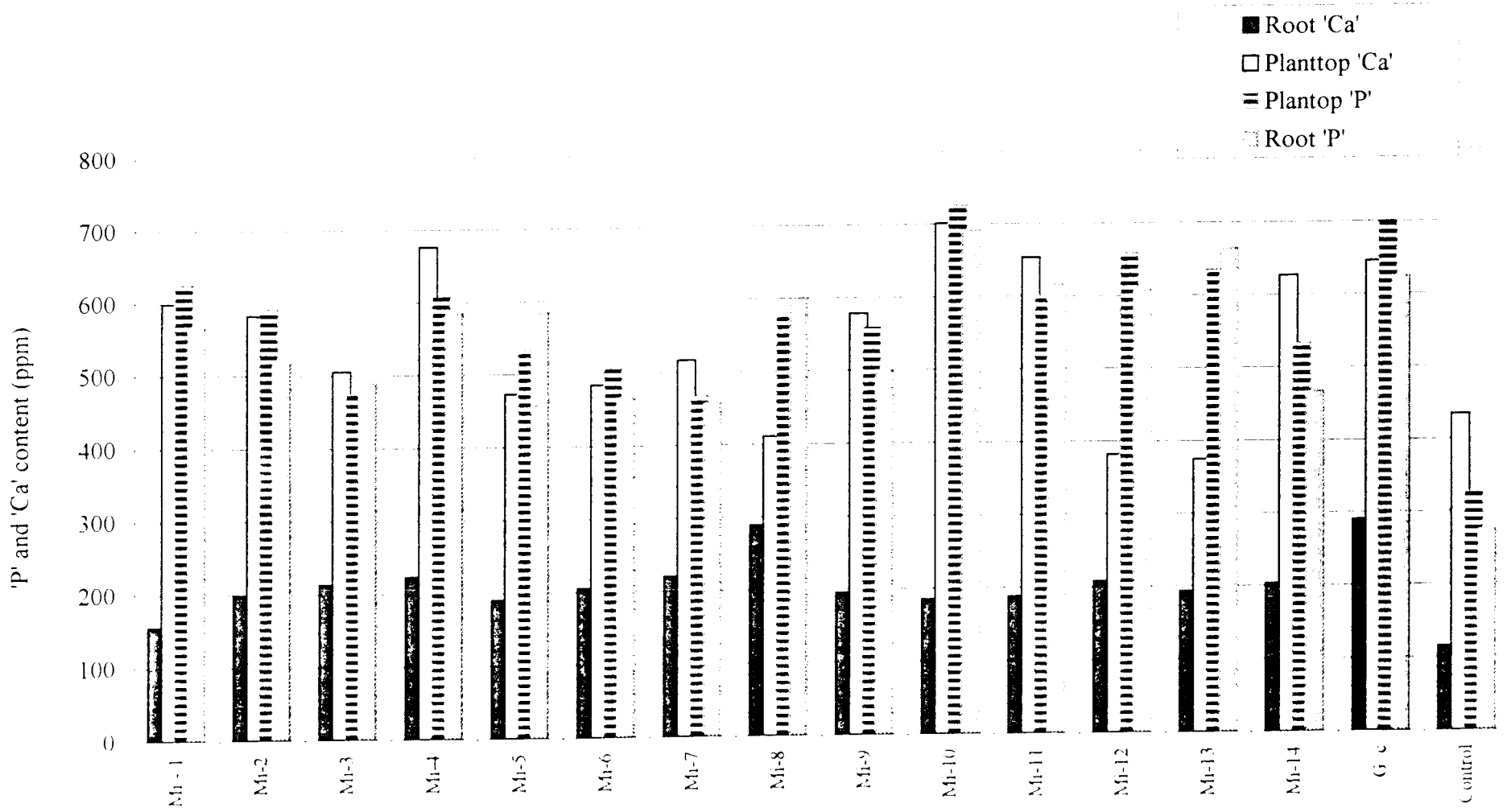


Fig. 7 Effect of native AMF on 'P' and 'Ca' content of ginger

absorption of soil P is well documented (Harley and Smith, 1983; Bolan *et al.*, 1987; Li *et al.*, 1991a). No positive relationship could be observed between the plant P content and relative incidence and intensity of disease, which signified that the AMF induced resistance is not conferred by better P nutrition. Experimental evidence with other crops are also available to support this view (Carron *et al.*, 1986a,b; Graham and Egel, 1988). Certain mycorrhizal isolates studied, recorded significantly higher K content (Table 19). However, no relationship could be observed between the relative K content and growth or disease incidence. Although there are a few reports of effects of AMF on higher K uptake (George *et al.*, 1992) these results are inconsistent and difficult to interpret (Sieverding and Toro, 1988).

Wide variations among mycorrhizal isolates were noticed with regard to the uptake of Ca, Mg, Cu, Zn, Mn and Fe. Significantly higher content of micronutrients could be observed in certain AMF isolates. All isolates recorded significantly higher Ca (Fig.7), Mg and Zn content in roots. The ability of AMF external hyphae to absorb and transport Ca and Zn to the host roots has been documented (Rhodes and Gerdeman, 1978; Kothari *et al.*, 1991). For Mg, direct experimental evidence for uptake and transport by AMF is sparse (Kothari *et al.*, 1990a).

Significantly higher Cu, Mn and Fe content could also be observed in many of the mycorrhizal inoculations. Information on better uptake of Mn in mycorrhizal plants is lacking. Similarly, not much information is available about the influence of AMF on Fe uptake and translocation, but Cu content has been found to be consistently high in mycorrhizal plants (Li *et al.*, 1991c). However, the role of these nutrients in suppressing disease needs further elaboration.

The potential of native AMF in enhancing plant growth and health is well understood. Similarly native antagonistic fungi are efficient biocontrol agents of many soil borne plant pathogens. With a view to explore the possibility of furtherence of the effect of AMF on plant growth and disease suppression of rhizome rot, native antagonistic fungi were isolated from the ginger rhizosphere and were evaluated *in vitro* for their antagonistic property against *P. aphanidermatum*. Of the 28 antagonistic fungal isolates tested along with four *Trichoderma* cultures 11 isolates exhibited more than 90 per cent inhibition (Table 22). The mechanism of inhibition may be by hyper parasitism (Chet *et al.*, 1981; Elad *et al.*, 1983), by production of cell wall degrading enzymes like cellulase and β-1,3 glucanase (Chet and Baker, 1980, 1981; Cherif and Benhamou, 1990) and/or production of inhibitory metabolites (Faull, 1994). Volatile and nonvolatile compounds produced by *T.viride* and *T. harzianum* have been

reported to be antagonistic to *P. aphanidermatum* in *in vitro* screening (Rathore *et al.*, 1992).

The isolates found effective under *in vitro* testing were further evaluated in the green house using the four *Trichoderma* cultures as reference. The effect of different antagonistic fungi on growth characteristics of ginger indicated remarkable increase by isolates, Ai-12, Ai-7 and *T. viride*. In contrast, Ai-18, Ai-6, Ai-2 etc. considerably reduced the growth characteristics. The effect of antagonistic fungi especially *Trichoderma* spp. on growth enhancement has been well illustrated (Baker *et al.*, 1984; 1986; Windham *et al.*, 1989). The probable mechanisms of growth promotion could be due to its effects as biofertilizer cum biocontrol agent and production of plant growth hormones (Lugtenberg *et al.*, 1991). However, how they reduce the plant growth is not well understood. Probably they might have affected the development of host root system and interfered with the absorption of nutrients or might have inhibited the development of other beneficial microorganisms in the rhizosphere.

The data on disease incidence indicated that Ai-12 significantly suppressed the disease in terms of incidence, mortality rate and intensity, followed by Ai-13 isolate (Fig.8). This can be attributed to the effective multiplication and persistence of these antagonists in the rhizosphere of ginger. They are better adapted to the soil conditions as

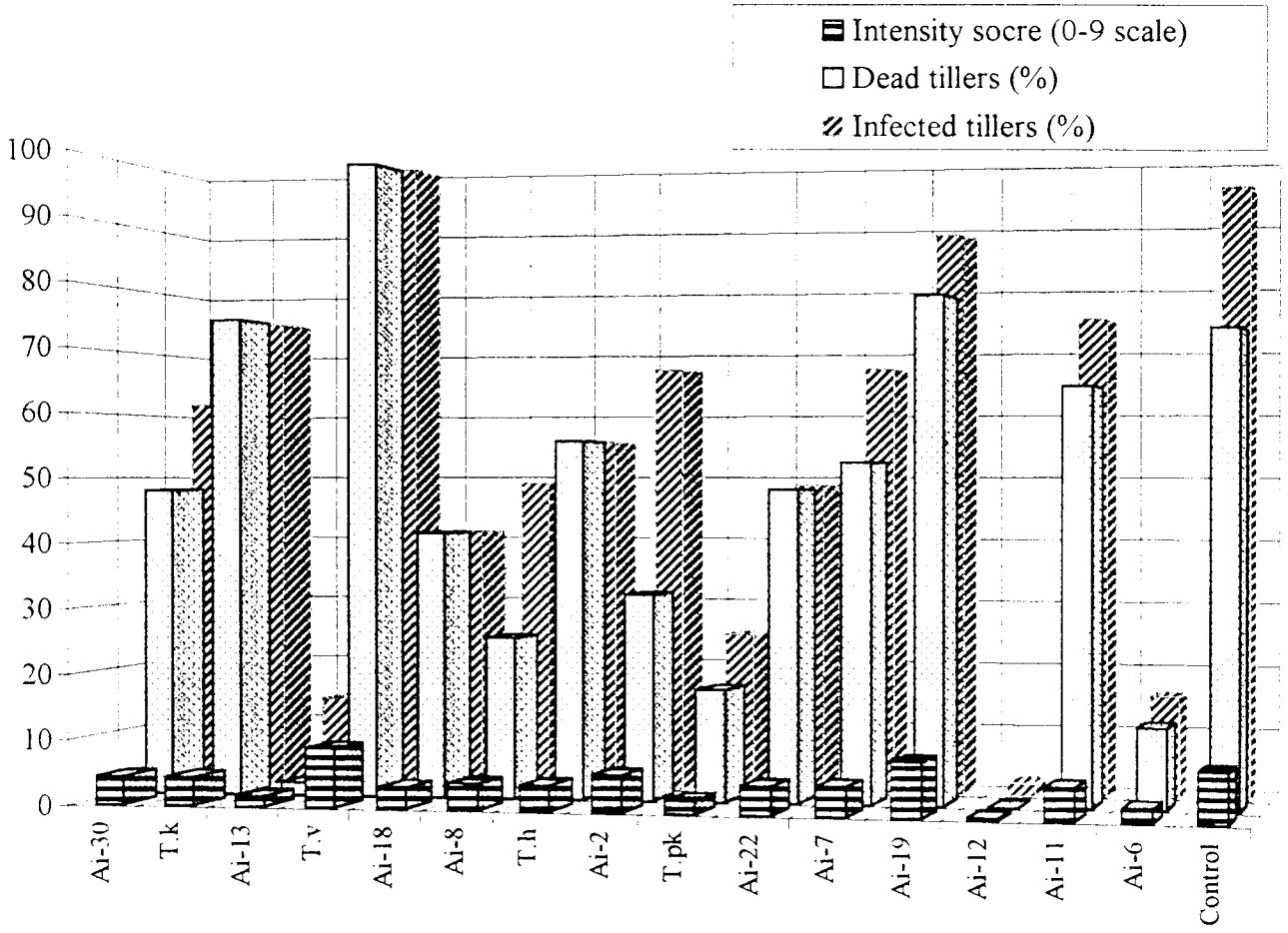


Fig. 8 Effect of native antagonists on rhizome rot of ginger in the green house

they were originally isolated and developed from native soil. It may also be due to the inherent antagonistic property of the isolates. Eventhough *T. viride* inoculation considerably enhanced plant growth, it was completely succumbed to the disease. *T viride* has been recognised as an efficient biocontrol agent of *P. aphanidermatum* (Rathore *et al.*, 1992; RAU, 1993). However, its failure in the present investigation may probably be due to its inability to multiply and persist in the introduced soil system. The low population of *T. viride* observed was the reflection of this fact (Table 26).

In most situations, the antagonists failed to maintain a high population level in the rhizosphere eventhough they were introduced with a food base. This could be one of the reasons for the poor performance of most of the antagonistic fungal isolates in the green house. The inability of most antagonists to maintain higher population levels might be either due to the unfavourable soil conditions or due to the unsuitability of the food base. The exceptions were Ai-12 and Ai-13 isolates which were able to maintain a high soil population level and consequently the pathogen population were suppressed to a low level, probably the heavy population of the antagonists exhibited better antagonistic activity (Fig. 10).

Substantial yield increase was noticed in plants inoculated with Ai-12 and *T. harzianum* inoculations whereas plant top dry weight and root dry weight were significantly

high in Ai-12 and Ai-7 inoculations (Fig.9). The overall effect of Ai-12 on yield and dry matter production was remarkably higher than other isolates. Although *T. viride* exhibited better plant growth characteristics, the yield and dry matter production were the least due to very heavy disease incidence and death of the plants (84.6 per cent mortality).

Isolate Ai-12 was found to be the most effective isolate for growth stimulation, yield and disease control while Ai-13 eventhough not very effective for growth and yield, was very effective in reducing the disease incidence and intensity. Thus, considering the superior effectiveness on growth, yield and disease suppression over other isolates, antagonistic fungal isolates Ai-12 and Ai-13 were selected for further testing. Ai-12 was identified as *T. viride*. The biocontrol potential of *T. viride* against several plant pathogens such as *Fusarium oxysporum* (Dwivedi *et al.*, 1993), *Macrophomina phaseolina* (Mahabir Singh and Majumdar, 1995), *Phytophthora capsici* (Anandaraj and Sarma, 1994) and *Pythium* spp. (Padmanabhan and Alexander, 1990) are well documented. *T. viride* has also been found to be a successful biocontrol agent of *P. aphanidermatum*, causative agent of rhizome rot of ginger (Dataran, 1988; Rathore *et al.*, 1992; RAU, 1993; NRCS, 1994). *T. viride* and other *Trichoderma* spp. isolated from ginger rhizosphere soils were found to be effective in controlling rhizome rot of ginger in the field conditions (Usman *et al.*, 1996).

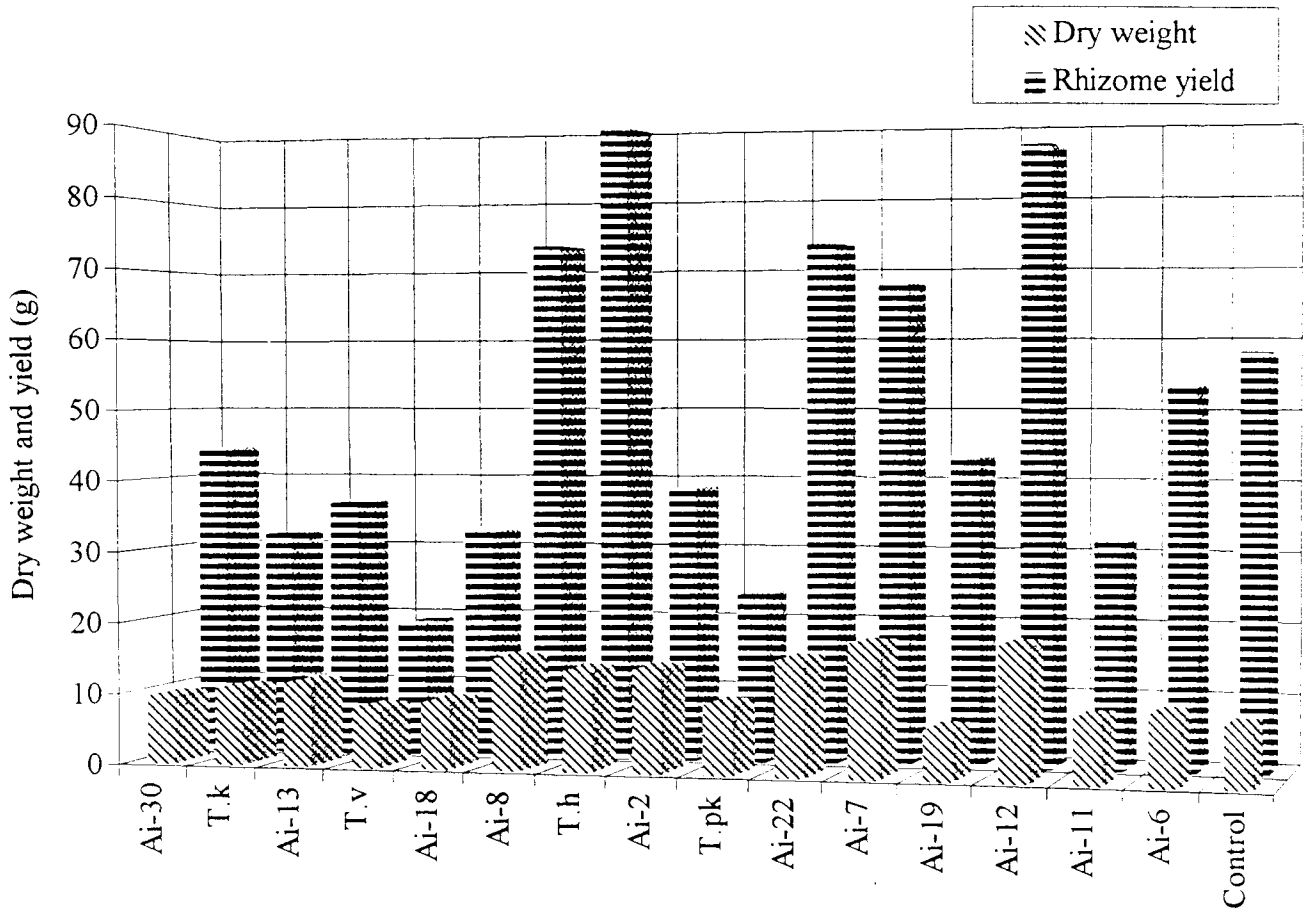


Fig. 9 Effect of native antagonists and *P. aphanidermatum* on dry weight and yield of ginger in the green house

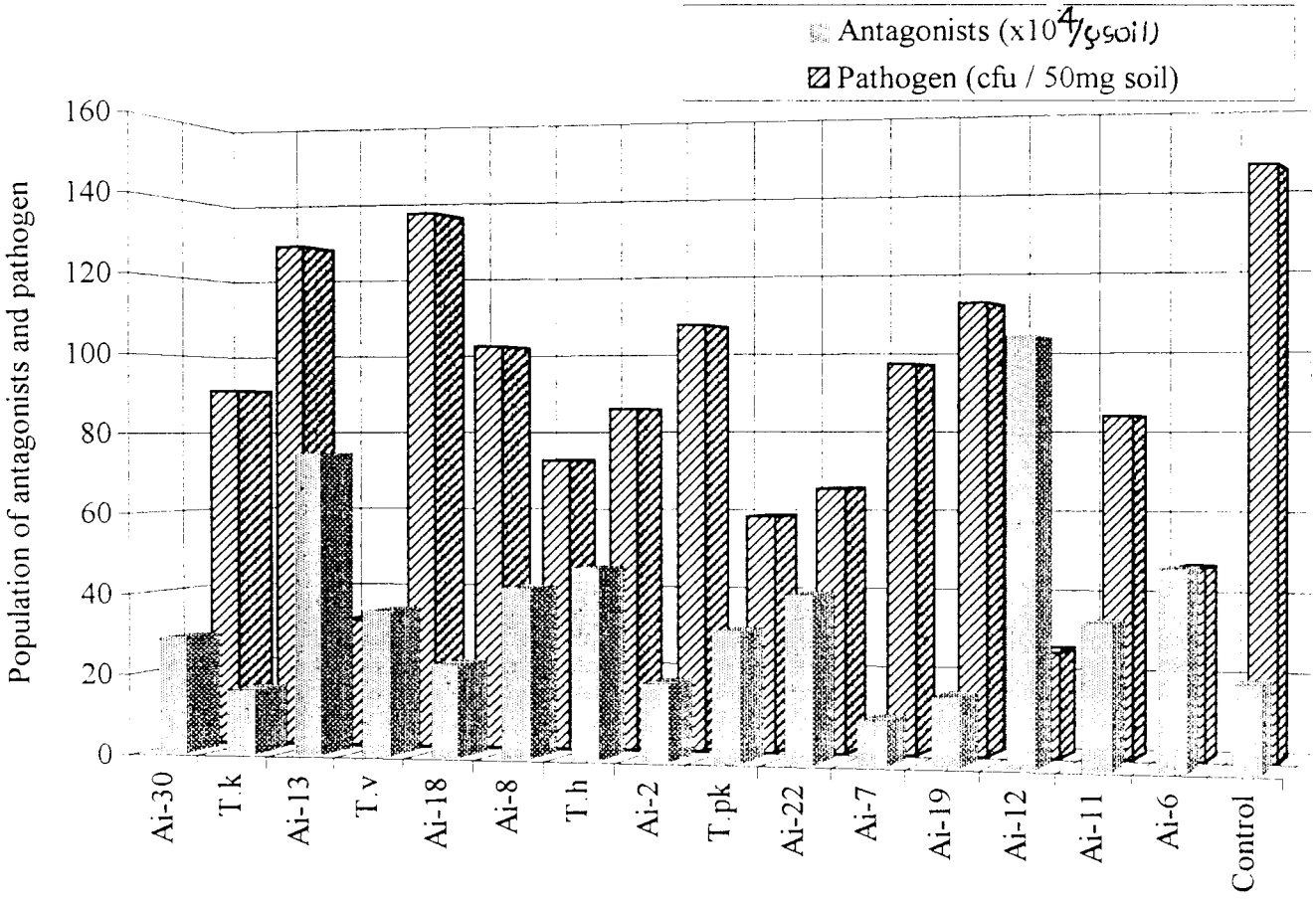


Fig. 10 Effect of antagonist isolates on *P. aphanidermatum* population in ginger rhizosphere

The antagonistic fungal isolate, Ai-13 was identified as *Aspergillus fumigatus*. *Aspergillus* spp. are effective biocontrol agents against several pathogens such as *Phytophthora arecae* (Duvenhage and Kotze, 1993), *M. phaseolina* (Mukherjee and Sen, 1992) and *Pythium* and *Phytophthora* spp. (Migheli *et al.*, 1993). However, the biocontrol potential of *Aspergillus fumigatus* against ginger rhizome rot pathogen has not been reported previously.

The selected isolates of AMF (Mi-1 and Mi-4) and antagonistic fungi (Ai-12 and Ai-13) were further tested for their combined effect in the green house. The results indicated that the interaction effect of Ai-12 with Mi-1 and Mi-4 were significant on plant growth characteristics. The combined effect was greater than the effect of single inoculations with the mycorrhizal isolates and the antagonistic isolates (Table 28). It indicated that the combined inoculation exerted a synergistic interaction to promote plant growth. Such synergistic effects on promotion of plant growth by interaction of *T. viride* and *G. epigaeus* in wheat (Kumar *et al.*, 1993) and *T. aureoviride* and *G. mosseae* in marigold (Calvet *et al.*, 1993) have been documented. However, no such synergistic interaction could be observed due to dual inoculation of Ai-13 with mycorrhizal isolates. Analysis of the main effect indicated that significant growth enhancement was achieved due to main effect of Ai-12 rather than the main

effect of mycorrhizal isolates. The antagonistic fungal isolate, Ai-12 stimulated plant growth probably by producing plant growth stimulating substances (Lugtenberg *et al.*, 1991) in the presence of AMF.

A similar pattern was observed with respect to yield and dry matter production. Interaction of Ai-12 with the mycorrhizal isolates gave remarkably high rhizome yield and plant dry weight (Fig.12). The effect of Ai-12, Mi-1 and Mi-4 on yield and dry matter production was remarkable which was further boosted by the interaction. Similar effects on yield was also reported by Kumar *et al.* (1993). Although Ai-13 x Mi-4 inoculation recorded better plant growth, it was not reflected on yield or dry matter production. This could be attributed to the heavy incidence of disease observed in the treatment. This further revealed that their interaction stimulated plant growth, but not the disease tolerance.

The disease incidence and intensity were significantly low in the combined inoculations of Ai-12 x Mi-1 and Ai-12 x Mi-4 signifying the potential of their interaction effect on disease suppression without any inhibitory effect on each other (Fig. 11). Similar results were obtained when mixed biocontrol cultures comprising of *G. epigaeus* and *T. viride* singly and in combination were tested against three wheat root rot pathogens viz., *Bipolaris sorokiniana*, *F. avanaceum* and *F. javanicum* (Kumar *et al.*, 1993). It may be noted that

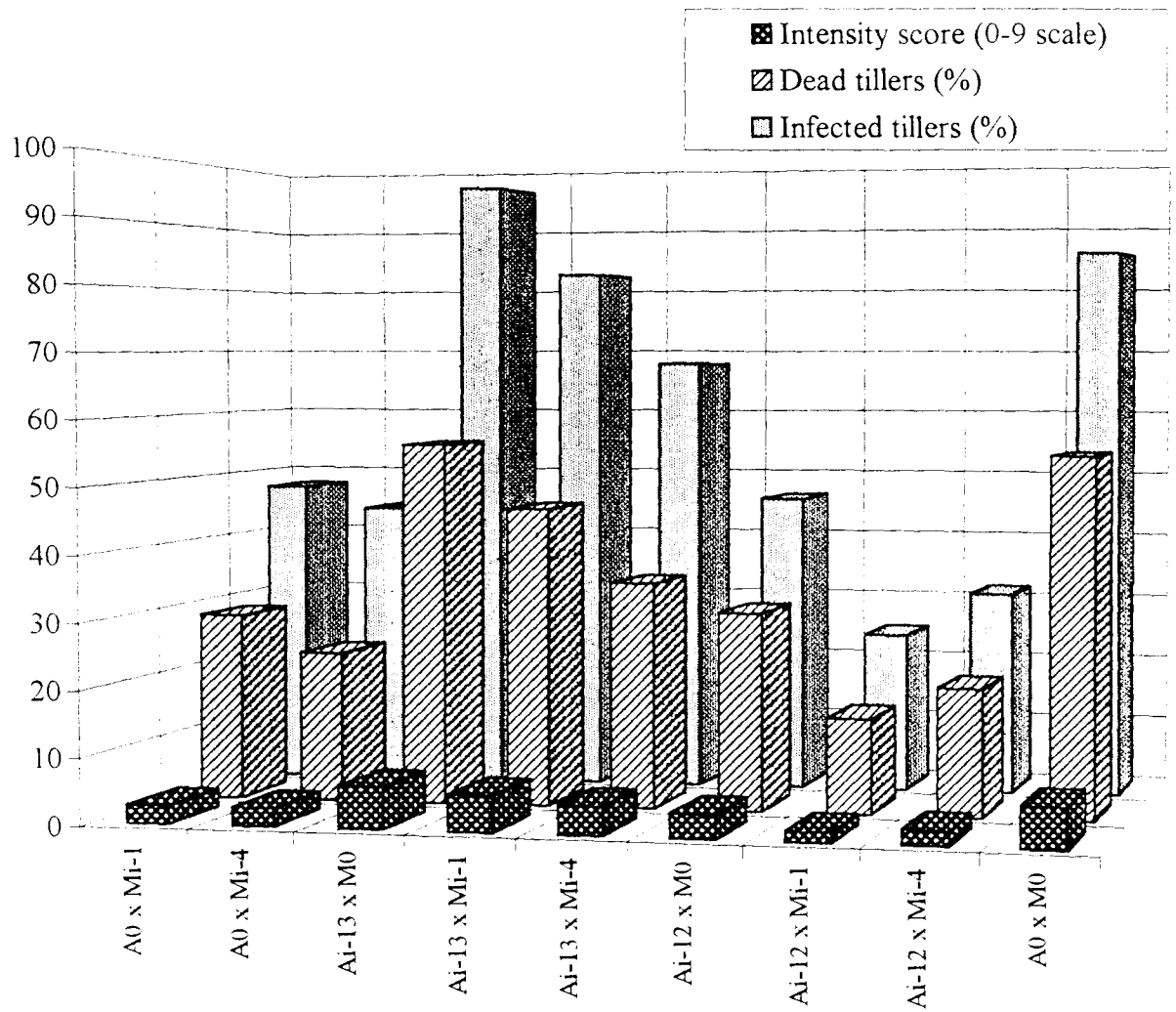


Fig. 11 Effect of interaction of AMF, antagonists and *P. aphanidermatum* on rhizome rot of ginger in green house

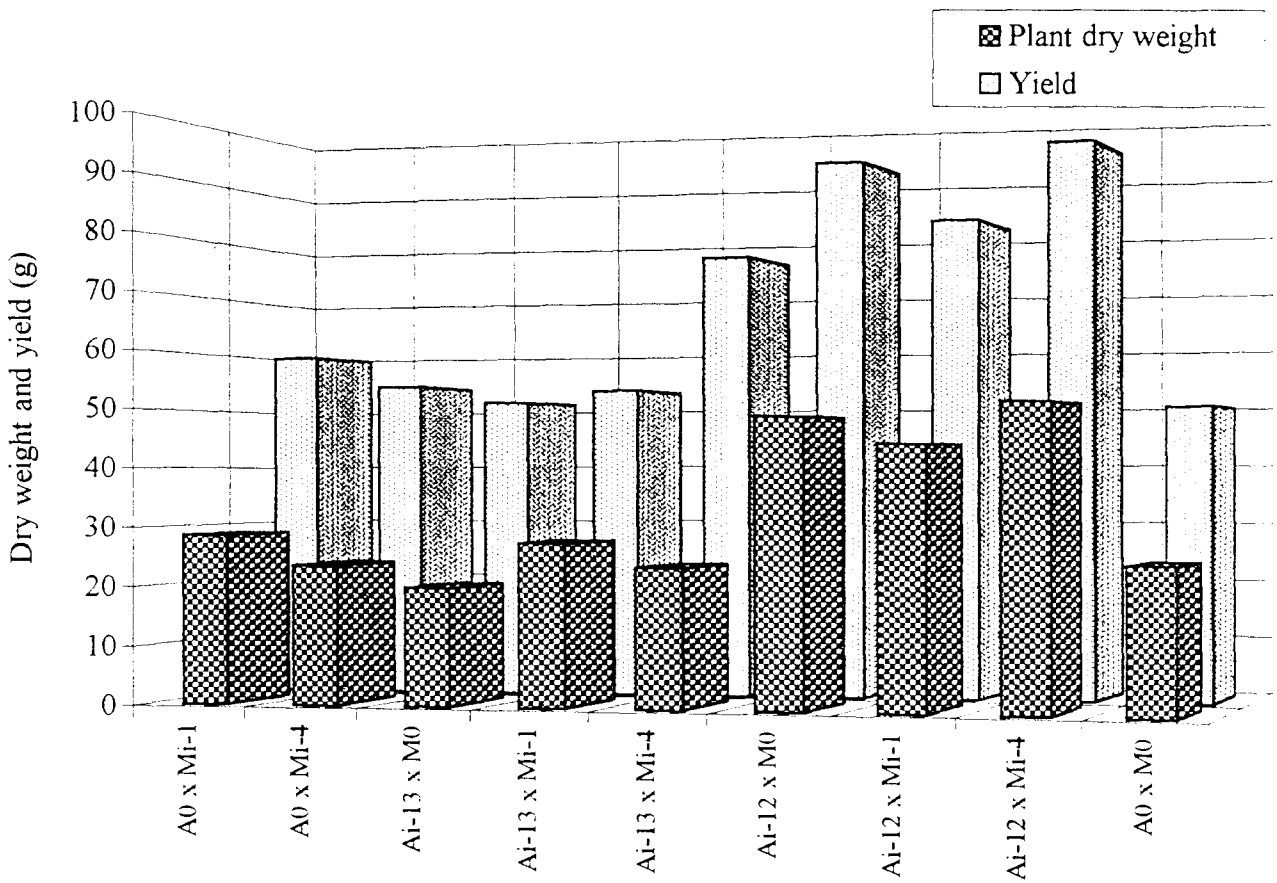


Fig. 12 Effect of selected AMF, antagonists and *P.aphanidermatum* on dry weight and yield of ginger in the green hosue

eventhough the single inoculation of either the mycorrhizal isolates, Mi-1 and Mi-4 or the antagonistic isolate Ai-12 could significantly reduce the incidence and intensity of the disease combined inoculation could further boost the disease reduction. Such phenomenon has been observed earlier by Calvet *et al*. (1992; 1993) in AMF - *Trichoderma* sp. interactions.

In contrast, the disease incidence was maximum in Ai-13 and the dual inoculation of Ai-13 with Mi-1 and Mi-4 reduced the disease suppressive ability of mycorrhizal isolates considerably. The antagonist, Ai-13 was inhibitory to AMF colonization and development as evident from the table 31. Such suppression of AMF development by antagonist has been reported by Wyss *et al*. (1992). It indicated the deleterious effect of Ai-13 on AMF development whereas Ai-12 did not reduce the normally high per cent colonization and spore production exhibited by Mi-1 and Mi-4 isolates. This signified the need for testing the compatibility and effectiveness of each biocontrol agent for dual applications in formulating integrated disease management strategies. Further, the study clearly shows the importance of having the appropriate isolates of AMF and antagonists to achieve effective biocontrol synergistically.

The pathogen population was consistently at lower level in the single inoculation of Ai-12 and its combination

with Mi-1 and Mi-4. It is well understood that the success of any antagonistic organism depends on its ability to multiply and persist in the soil conditions prevailing in the introduced field and to overcome the resistance offered by the soil microflora (Ahmed and Baker, 1987; Hader *et al.*, 1984). In the present investigation Ai-12 maintained a very high population in the rhizosphere. Perhaps this could have been one of the reasons for the effective suppression of the pathogen and successful control of the disease. The low pathogen population in dual inoculations of Ai-12, Mi-1 and Mi-4 might be due to the synergistic effect of AMF and antagonists in inhibiting the multiplication of the pathogen in the rhizosphere.

Generally, the pathogen population should have reached a minimum threshold level to incite a disease in a plant community. Once diseased, the population of the pathogen will continue to be very high in the rhizosphere. But application of an effective antagonistic fungus with high rhizosphere competence checks the multiplication and development of the pathogen in the rhizosphere by hyperparasitism, production of toxins and antibiosis (Inbar and Chet, 1994). Arbuscular mycorrhizal associations, on the other hand, induce tolerance to diseases in plants by enhancing defense mechanisms of the host through physiological means (Azcon-Aguilar and Barea, 1996). Further, the mycorrhizosphere of the mycorrhizal host generally does not favour

multiplication of the pathogen due to mycorrhiza induced changes in the root exudation pattern (Linderman, 1994). Thus, in dual inoculations, while the antagonist directly interact with the pathogen to reduce the disease, the AMF enhances the defence response of the host to fight against the disease. Hence the pathogen population may not reach the threshold level to incite the disease.

The population of pathogen was higher in Ai-13 inoculation as well as its combination with Mi-1 and Mi-4, indicating its inability to reduce the build up of the pathogen. The antagonist itself could not maintain a higher population level in the rhizosphere probably due to soil factors and/or interference from other rhizosphere microflora and lack of competitive saprophytic ability. This may also be a reason for the poor performance of the isolate in disease control.

The selected antagonists and mycorrhizal isolates (Ai-12, Ai-13, Mi-1 and Mi-4) were tested under the field conditions to evaluate the effect of dual inoculation on plant growth, yield and rhizome rot suppression in ginger. Single and dual inoculations of Ai-12, Mi-1 and Mi-4 registered remarkable increase in plant growth characteristics, plant dry weight and rhizome yield in the field. Maximum rhizome yield was with mycorrhizal isolate, Mi-1 (Fig. 14). This proves the effectiveness of native isolates particularly AMF in increasing

biomass production and yield of ginger in the field. The role of AMF as an effective biofertilizer has been well recognized (Harley and Smith, 1983; Marschner and Dell, 1994). The better growth characteristics observed in Ai-12 (*T. viride*) treatment could be attributed mainly to the production of growth promoting substances and other volatile compounds which resulted in overall improvement in growth and development of ginger plants (Lugtenberg *et al.*, 1991). Such positive growth stimulating effect of *Trichoderma* spp. has also been observed in other plant species (Baker *et al.*, 1984, 1986; Windham *et al.*, 1986, 1989; Bernard and Davet, 1993; Inbar *et al.*, 1994; Mackenzie *et al.*, 1995).

These native isolates may be better adapted to the field situations and exhibited their potential over other native flora to perform well in a diverse and complex soil environment and against competition from native AMF and antagonists. It has been suggested by Hadar *et al.* (1984) that isolate of *Trichoderma* native to a soil may be better adapted to it than the introduced isolates and may be more able to co-exist with native soil microflora.

Unlike the green house studies, no synergistic interaction between AMF and Ai-12 was noticed in the field on plant growth and yield. Probably, under the influence of complex soil environment, these isolates could not express their full potential due to interaction with and interference

from other rhizosphere microflora. Moreover, in the green house, the effectiveness of the isolates was tested under nutrient deficient sterile conditions, while in the field, all the package of practices recommendations of KAU were followed. It has been widely accepted that the beneficial effects of AMF are better expressed under nutrient deficient situations (Smith, 1988). Hence, it could also be a reason for the absence of significant synergistic interaction between these isolates in the field.

As was observed in the green house studies, single inoculation of Ai-13 or its combination with AMF did not show any significant effect on plant growth and yield of ginger in the field. Further, it should be emphasised that Ai-13 substantially reduced the AMF effect on plant growth and yield in dual inoculations. This clearly demonstrates the inhibitory effect of Ai-13 on the mycorrhizal isolates to exert its full potential on plant growth. This is further evident from the considerable decrease in per cent AMF colonization, intensity and AMF spore count noticed due to the dual inoculations (Table 35). Similar inhibitory effects of certain *Trichoderma* spp. on AMF development and sporulation have been established earlier (Wyss *et al.*, 1992; McAllister *et al.*, 1994a). However, this forms the first report of the inhibitory effect of *Aspergillus fumigatus* (Ai-13) on AMF development under field conditions.

It has been reported that phosphatase producing fungus, *A. fumigatus* along with *G. mosseae* enhanced organic P uptake and increased growth of wheat (Tarafdar and Marschner, 1995). *A. niger*, another phosphate solubilising fungus, and *G. fasciculatum* also produced synergistic interaction and improved the growth and nutrient uptake in finger millet (Gopalakrishnan *et al.*, 1990). But, in the present study, both in the green house and field, *A. fumigatus* (Ai-13) was found to be inhibitory to the development of mycorrhizal isolates and reduced their beneficial effects in dual inoculations. This vividly illustrates the need for selecting appropriate combination of AMF and antagonists in dual inoculation technology to achieve the full potential of the synergistic interaction on growth and disease control.

Significant reduction in disease incidence, with respect to per cent tillers infected and dead and intensity of disease, were recorded in both the single mycorrhizal inoculations and their dual inoculations with Ai-12 (Fig.13). It may be noted that in the green house study, the disease suppressive ability of mycorrhizal isolates and antagonistic fungus, Ai-12 were significantly and synergistically expressed only in their dual inoculations while in the field the disease suppressive ability was exhibited significantly in the single inoculation itself. No further synergestic interaction was demonstrated in reducing the disease in combined inoculations

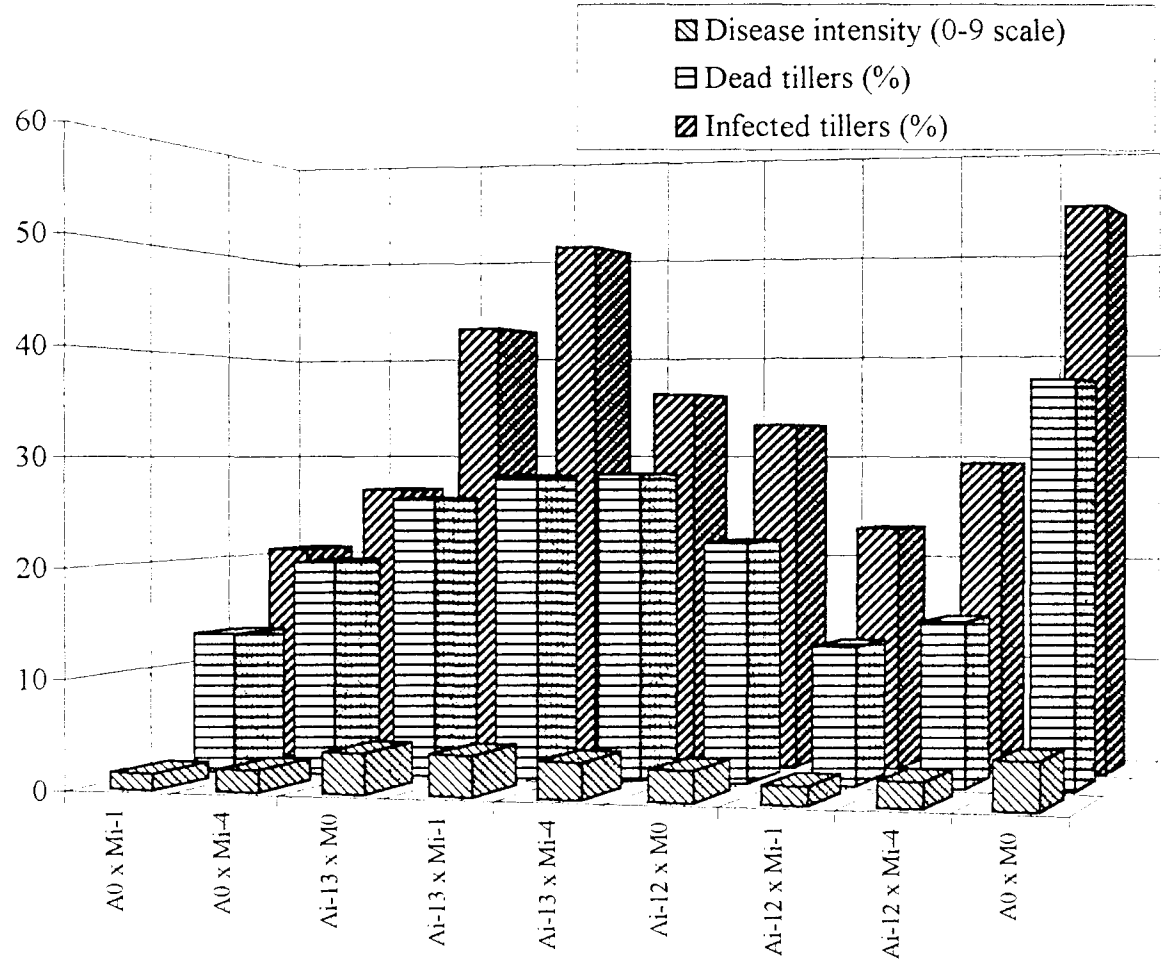


Fig. 13 Effect of native AMF and antagonists with *P. aphanidermatum* on rhizome rot of ginger in the field

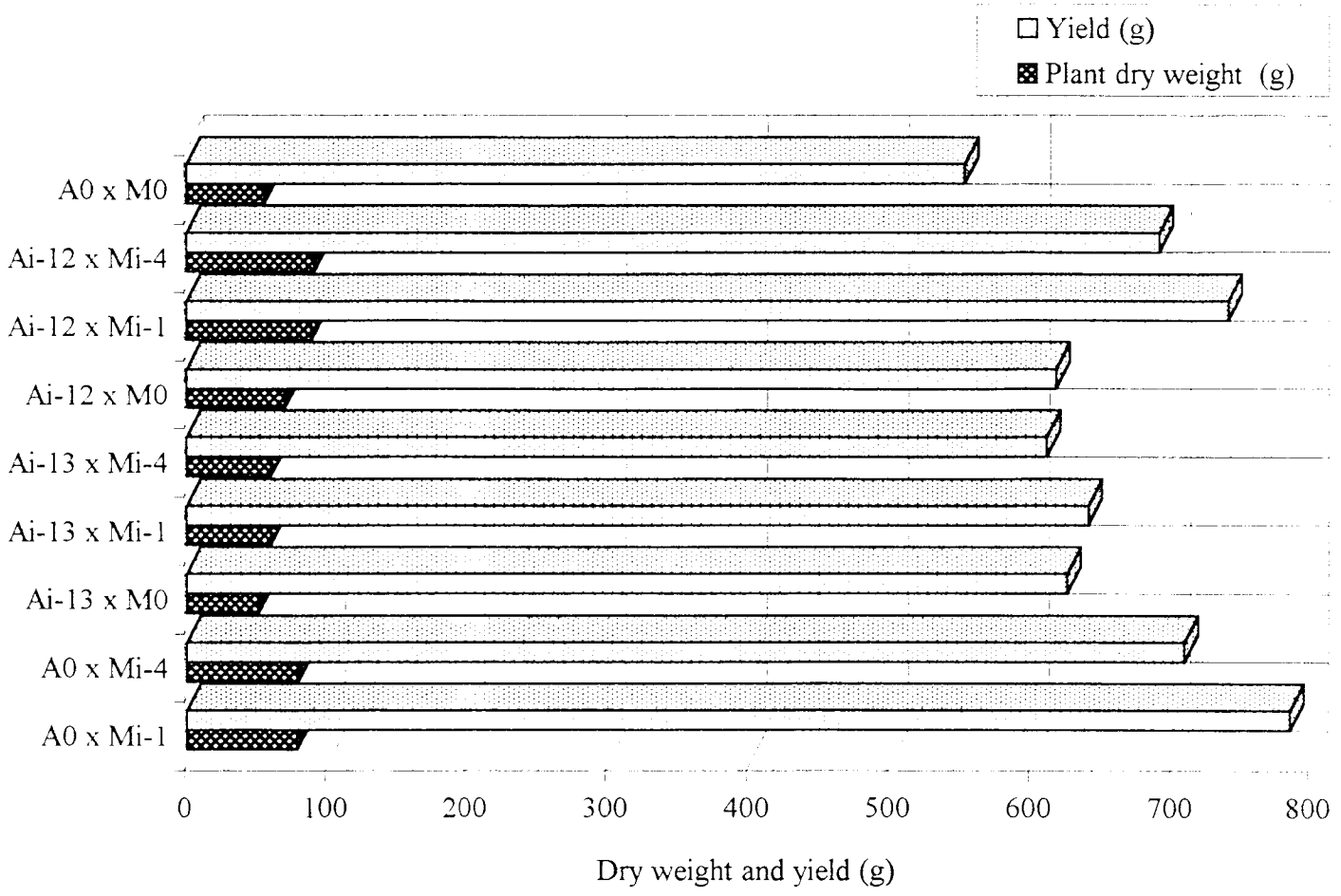


Fig. 14 Effect of inoculation of AMF, antagonists and *P.aphanidermatum* on dry weight and yield of ginger in the field

in the field. Since sterile soil was used in the green house studies, there was only limited microflora to interact with the introduced antagonist/AMF. Hence it could not express the full potential by interacting with native flora in the single inoculation whereas in the field a complex soil flora are present and the possibility for interaction with a specturm of organisms exists. It has been suggested that due to co-evolution of AMF with plants, they are highly rhizosphere competent and are compatible with such antagonists and even function in concert with them (Linderman, 1988). It is amply illustrated in many soils that establishment of mycorrhizal associations in certain hosts obligately requires the interaction with mycorrhization helper bacteria (MHB) and other antagonists (Garbaye, 1994; Barea *et al*., 1996).

Maximum reduction in disease incidence and intensity was achieved by Mi-1 isolate. It can be observed from the data (Table 33) that the disease reducing ability was expressed in the dual inoculation mainly due to the significant main effect of Mi-1 and Mi-4 and no significance was noticed in the main effect of Ai-12.

The decrease in the biocontrol efficiency of Mi-1 and Mi-4 upon dual inoculation with Ai-13 in the field is further proof of the inhibitory effect of Ai-13 on mycorrhizal isolates which was evident from the significant increase in disease recorded due to the main effect of Ai-13. So, in dual testing,

both in the green house and field, Ai-13 consistently proved to be incompatible with mycorrhizal isolates.

A high per cent AMF colonization, intensity and spore count could be observed in the single mycorrhizal inoculations and dual inoculation with Ai-12 in the field (Fig.15). It is a clear indication of the competitive ability of the mycorrhizal isolates for rapid infection and colonization and hence they proved to be superior bioinoculants for ginger, both as biofertiliser and biocontrol agent. However, it seemed that. their rapid infection and colonization capability was marred due to the interaction with antagonistic fungal isolate Ai-13 upon dual inoculation.

The population of the pathogen was remarkably low in the single mycorrhizal inoculations and dual inoculation with Ai-12 (Fig.15). The failure of the pathogen to build up a heavy inoculum density in the presence of mycorrhizal isolates and Ai-12 could be attributed either due to direct effect of inoculants or due to the tolerance conferred on the host by the AMF. The ability of *T. viride* (Ai-12) to produce hydrolytic enzymes like cellulase and β-1,3 glucanase has been attributed as the mechanism of biocontrol against cellulose containing pathogens (Chet and Baker, 1980, 1981; Harman *et al.* 1980). The cell wall of *Pythium* spp. is essentially composed of cellulose and glucan (Harman, *et al.*, 1980). Hence, the biocontrol efficiency of Ai-12 depended on its ability to produce these

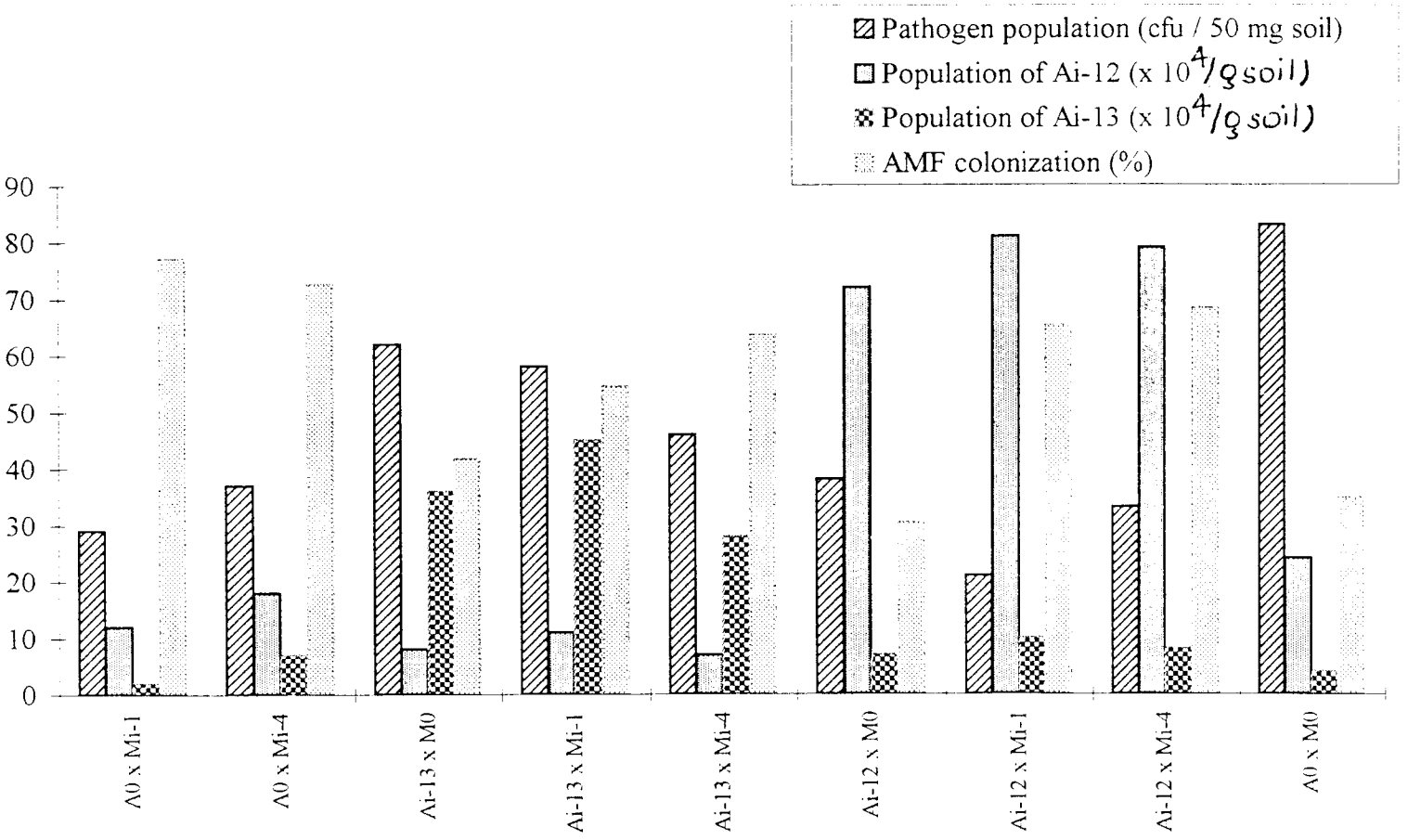


Fig. 15 Effect of selected AMF and antagonists on populations of pathogen and antagonists and AMF colonization in the field

enzymes to inhibit the pathogen. The biocontrol mechanism of mycorrhizal isolates has not been fully understood. The probable mechanisms suggested include competition for infection/ colonization sites (Dehne, 1982; Jalali and Jalali, 1991), root damage compensation (Cordier *et al.*, 1996), morphological changes in the host root (Atkinson *et al.*, 1994), microbial changes in the mycorrhizosphere (Meyer and Linderman, 1986; Barea *et al.*, 1996) and activation of specific plant defence responses (Azcon-Aguilar and Barea, 1996).

The populations of Ai-12 and Ai-13 were high only in treatments where they were inoculated signifying that these isolates got established in the field. Native isolates are known to get established in the field faster than introduced organisms (Hadar *et al.*, 1984). However, Ai-12 was found to be more successful for the establishment in the field.

All the mycorrhizal inoculations (both single and combined with Ai-13 and Ai-12) recorded significantly higher root P content under field conditions. However, plant top P content was significantly higher only in Mi-4 and Ai-12 x Mi-4 inoculations (Fig.16). The data clearly demonstrated the ability of both mycorrhizal isolates especially Mi-4 in increasing the P content of plant tissues significantly. It can also be inferred from the results (Table 36) that significantly higher P content of plant top and roots was attributable only to the main effect of mycorrhizal isolates

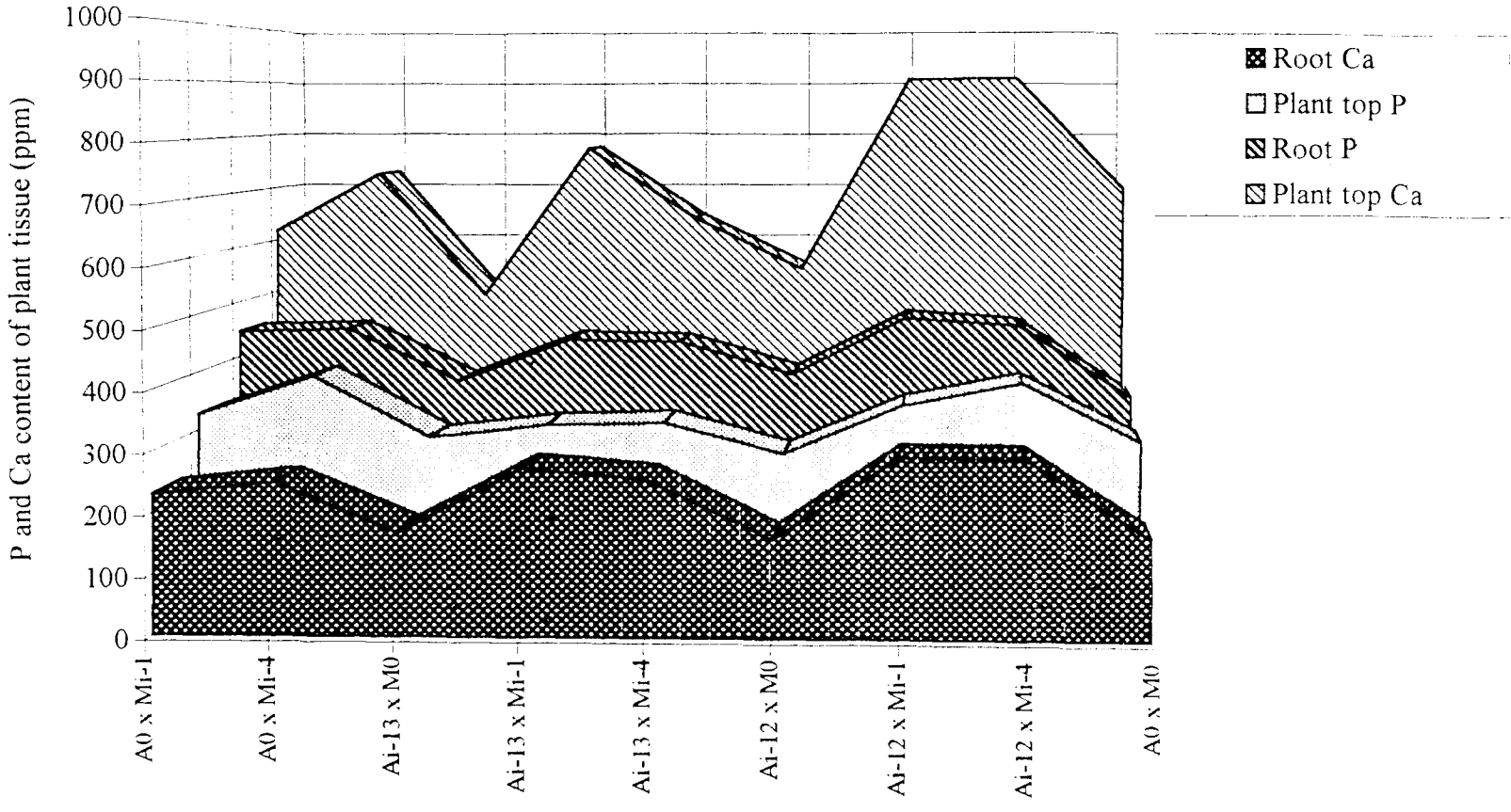


Fig. 16 P and Ca content of ginger as influenced by the interaction of AMF, antagonists and *P.aphanidermatum* in the field

and the antagonistic fungal isolates did not exert any effect on the P content of plant tissues. Higher P content is an indirect indication of higher P uptake by mycorrhizal isolates which is a well established phenomenon in mycorrhizal associations (Harlay and Smith, 1983; Marschner and Dell, 1994).

All the different inoculations recorded higher K content in plant tissues. The role of AMF in enhancing K content of plant tissues is not fully understood. Although there are several reports of varying K concentration in shoots (Mosse, 1973; Marschner and Dell, 1994) they are inconsistent and difficult to interpret (Sieverding and Toro, 1988). About 10 per cent of total K uptake in mycorrhizal coach grass is attributed to hyphal uptake and transport (George *et al*.1992). In the present study it can be inferred from the data that both the antagonistic and mycorrhizal isolates contributed for the significantly increased K content of plant tissues. Perhaps the better growth and root development facilitated by the AMF and antagonists and AMF hyphal K transport contributed much to the enhanced K content.

Significantly higher levels of Cu, Ca and Zn were recorded in the mycorrhizal and combined inoculations. In the roots significantly higher levels of Cu was recorded in only Mi-4 inoculation and Zn only in the combined inoculation of Ai-12 x Mi-4. There was not much variation with respect to

other nutrients (Table 37, 38). The increased uptake of micronutrients by AMF has been well documented for Ca (Rhodes and Gerdemann 1978) Zn (Kothari *et al*. 1991) and Cu (Li *et al*., 1991b). Generally, the better growth and root development due to the microbial inoculants result in better uptake of soil nutrients. However, there was not much variation in the Mg, Mn and Fe contents. A perusal of literature also showed that AMF did not exert much influence on the absorption of these nutrients. The enhanced uptake of diffusion limited micronutrients might make the plants more vigorous and equip them to resist or tolerate soilborne diseases (Davis, 1980; Linderman, 1994). Thus, better uptake of nutrients has also contributed in enhancing disease tolerance in plants.

The study on the standardization of different inoculation techniques of AMF in ginger revealed that starch inoculation was the best method for obtaining higher AMF colonization and intensity (Fig.17). The effect of starch inoculation was more pronounced during the initial stages of infection (Table 39) which facilitated rapid and early colonization in ginger. In addition to the firm adherence of the AMF inoculum on the planting material, due to the sticky nature of starch, it might have also stimulated the growth of beneficial microorganisms including MHB and other antagonists in the ginger rhizosphere by serving as source of nutrients. These microflora have been recognized as organisms in favoring

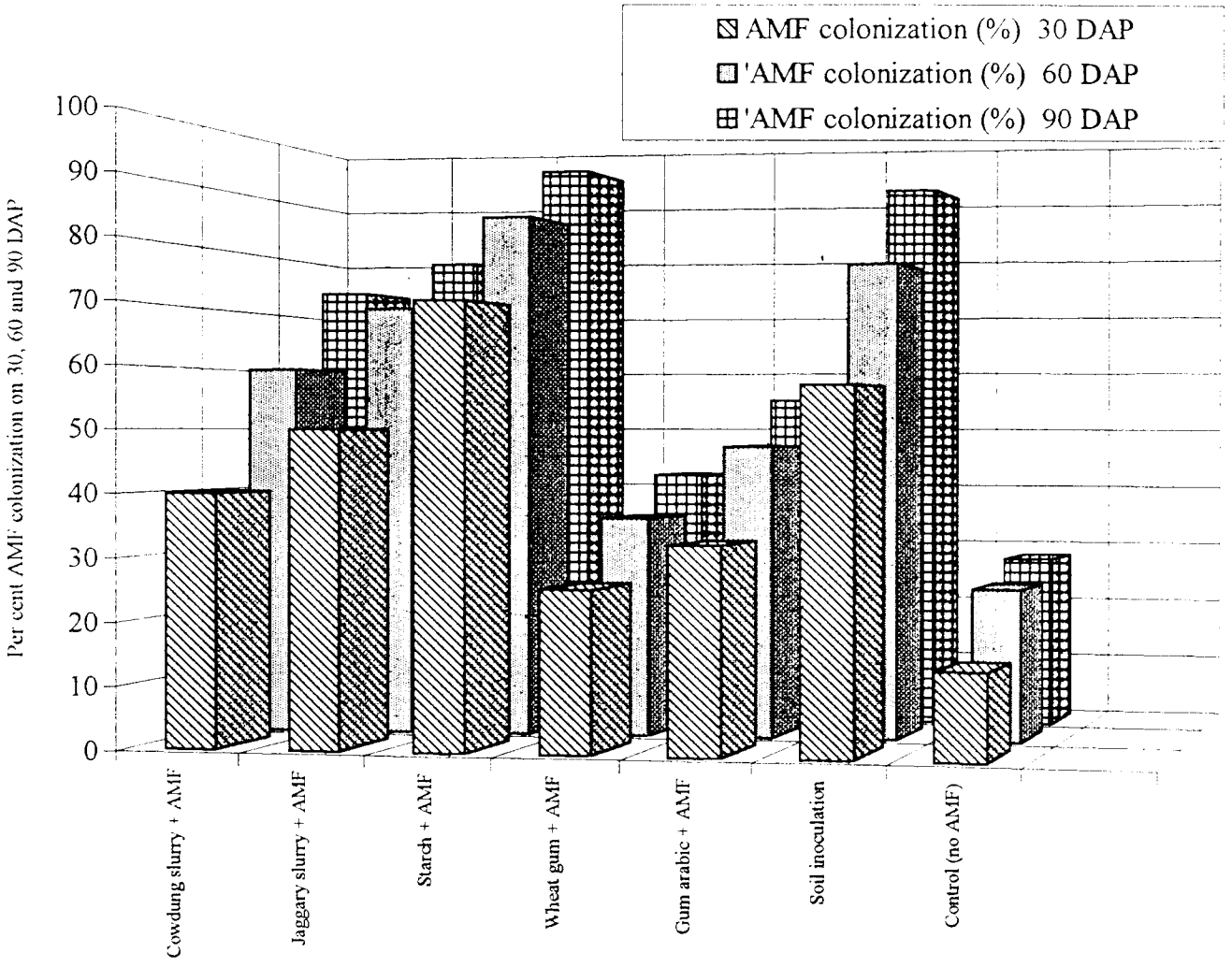


Fig. 17 Influence of inoculation methods on AMF colonization

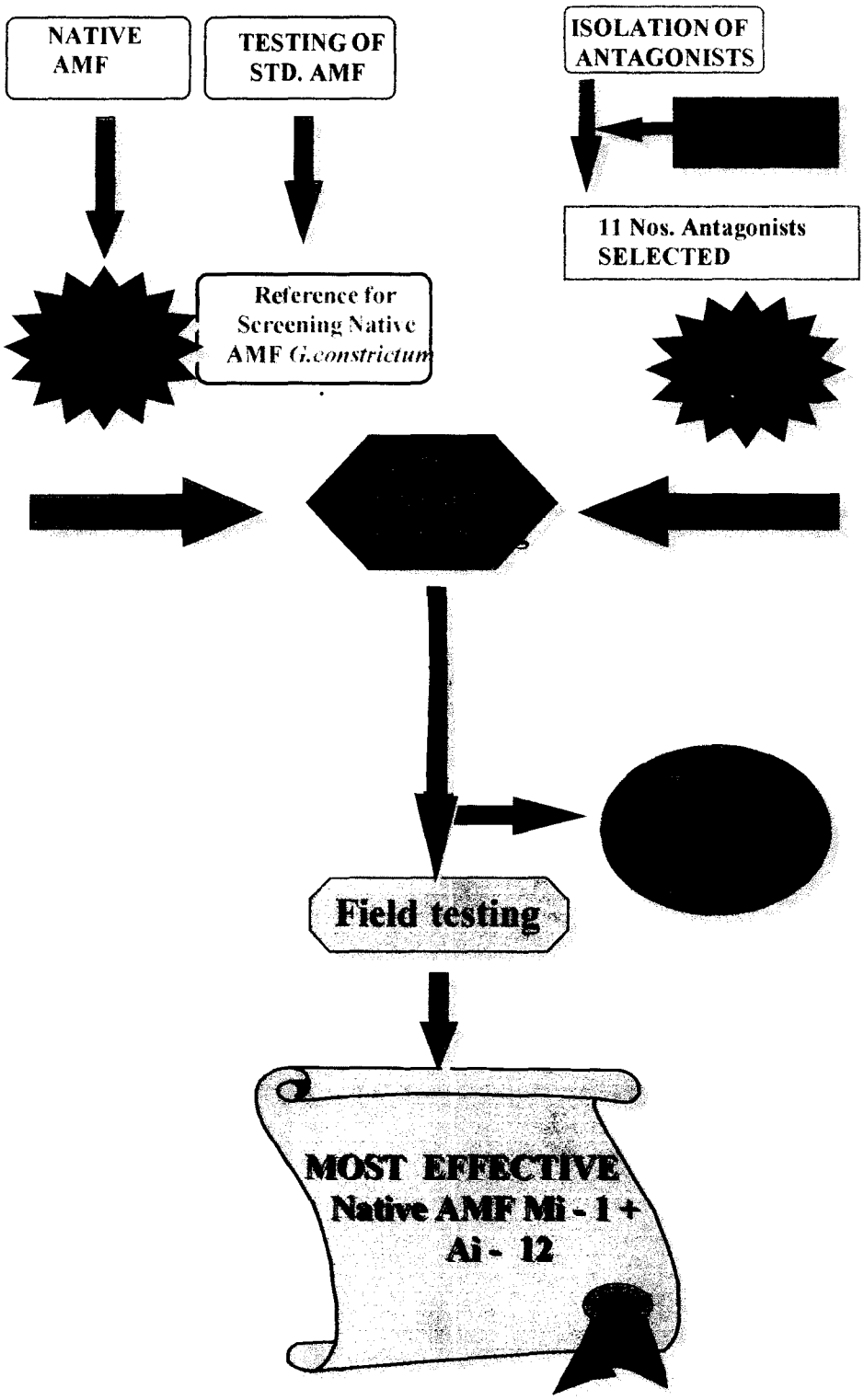
AMF development in different host plants (Garbaye, 1994; Barea *et al.*, 1996).

From the results of the study on the effect of plant protection chemicals on AMF and antagonists in the ginger rhizosphere it is observed that different chemicals exerted varying degrees of effects on these biocontrol agents (Table 40). The antagonist population and AMF colonization were least affected by carbofuran. Enhanced AMF colonization, as an effect of soil application of carbofuran at recommended dose have been reported by Venkateswarlu *et al.* (1995). Significant increase of AMF colonization and spore production at half the recommended dose of carbofuran have also been observed by Sreenivasa and Bagyaraj (1989). *Trichoderma* spp. are known to have greater tolerance for broad spectrum biocides than many other microorganisms and colonize the treated soil more rapdily than other soil competitors (Munnecke *et al.*, 1972). In fungicides, thiram was found to be less inhibitory to AMF and antagonist. There have been contradictory reports on the effect of thiram on AMF. Singh *et al.* (1990c) reported considerable inhibition of AMF development due to thiram treatment, whereas enhanced growth promoting effect, mycorrhizal infection and spore production due to thiram was observed by Vyas and Vyas (1995). Probably the effect of fungicides on the AMF development depends on the type and concentrations of the chemical, species of AMF and the prevailing soil conditions.

Thiram was also less inhibitory to the antagonist. Experiments conducted earlier showed that *Trichoderma* spp consistently survived and multiplied well in thiram treated soil (Richardson, 1954). The study revealed that in integrated disease management systems AMF and antagonists could be safely incorporated with selected biocides.

An effective management strategy of rhizome rot of ginger is evolved in the present investigation by combining native AMF (Mi-1 and Mi-4) and antagonistic fungi (Ai-12) for inoculation of seed rhizomes at planting time. The two biocontrol agents could be recommended for farmer adoption after final confirmatory trials in the farmers field. The study forms the first report of extensive work on the utilisation of native AMF, antagonist and their combined effect for the management of the rhizome rot and detailed documentation of native AMF associated with ginger.

SUMMARY DIAGRAM



SUMMARY

6. SUMMARY

Ginger is a major spice crop of India. The present investigation was undertaken to develop a biocontrol strategy for rhizome rot of ginger, the most destructive disease incited by *Pythium aphanidermatum* (Edson)Fitz. using native arbuscular mycorrhizal fungi (AMF) and antagonistic fungi from ginger rhizosphere.

The extensive survey conducted in the state revealed a definite negative relationship between AMF colonization and incidence and intensity of disease. Preliminary studies with six different AMF species indicated that *Glomus fasciculatum* and *G. constrictum* were more efficient in enhancing plant growth and yield, while *G. constrictum* and *G. mosseae* were superior in reducing incidence and intensity of rhizome rot. Nutrient content of plant tissue with respect to P, K, Cu, Ca, Mg, Zn, Mn and Fe were also higher in *G. constrictum* inoculation. Hence *G. constrictum*, which ranked top in reducing rhizome rot coupled with substantial growth stimulating property, was selected as reference culture for further studies. All the six AMF species tested substantially reduced the *Meloidogyne incognita* infestation and multiplication particularly the *G. fasciculatum* treatment. In the presence of nematode *Acaulospora morroweae* inoculation

recorded highest plant growth and yield, while *G. mosseae* treatment recorded the highest P, K, Zn and Mn content.

Characterization of native AMF associated with different ginger cultivars and that grown in different soil types indicated a clear influence of soil types on AMF association in ginger rather than genotypes. Although most of the genera were encountered, *Glomus* spp. were more frequent and *G. fasciulatum* was the most predominant species associated with ginger.

Selection was made among the fourteen native AMF isolates developed under the investigation for disease suppression and growth stimulation. Mycorrhizal isolates, Mi-1 and Mi-13 were more efficient for growth promotion while Mi-1 and Mi-4 (identified as *Glomus* spp.) were more effective for suppression of rhizome rot, promoting biomass production and yield. It was found that growth stimulation and disease suppression are independent traits of AMF and rarely occur together. Mi-1 and Mi-4 possessed these characteristics. All the native AMF recorded significantly higher phosphorus content and several of them with higher values of K, Cu, Ca, Mg, Zn, Mn and Fe content.

Twenty eight native antagonistic fungi were isolated from ginger rhizosphere and tested against *P. aphanidermatum in vitro*. The eleven isolates with 90 per cent or more growth

inhibition were selected and further screened in the green house. Isolate, Ai-12 was most effective for growth stimulation, higher yield and reduction of rhizome rot incidence. Isolate, Ai-13 with less effect on growth, also significantly reduced rhizome rot. Ai-12 was identified as *Trichoderma viride* and Ai-13 as *Aspergillus fumigatus*.

The combined effect of selected AMF (Mi-1 and Mi-4) and antagonists (Ai-12 and Ai-13) on growth, yield and rhizome rot of ginger was tested in the green house and field. In the green house, dual inoculation of Ai-12 along with Mi-1 and Mi-4 exhibited remarkable synergistic effect in enhancing biomass production and yield and in reducing the disease and pathogen build up. Ai-13 was inhibitory to AMF development, increased the disease and favoured pathogen build up.

In the field, single and dual inoculations of Ai-12, Mi-1 and Mi-4 increased plant growth and yield and significantly reduced rhizome rot. The inhibitory effect of Ai-13 on mycorrhizal development was evident in the field also. All the mycorrhizal inoculations enhanced P, K, Cu, Ca and Zn content of plant tissue. Maximum yield increase, disease suppression and nutrient uptake were recorded with Mi-1 inoculation.

Standardisation of AMF inoculation technique indicated that inoculation of AMF with starch coating of

rhizome was most effective for rapid and early colonization in ginger. Out of the two fungicides and three insecticides tested, thiram and carbofuran least affected AMF and antagonist.

The present investigation vividly highlights the potential prophylactic property of native AMF and antagonist in the management of rhizome rot of ginger apart from their cumulative effect on growth. The technology could be recommended for former adoption after final confirmatory trials in the farmers field. The study forms the first report of extensive work on the utilization of AMF, antagonists and their combined effect for the management of rhizome rot. It is also the first detailed documentation of native AMF associated with ginger.

REFERENCES

REFERENCES

Abbott, L.K., Robson, A.D. and De Boer, G. 1984. The effect of phosphorus on the formation of hyphae in soil by the vesicular arbuscular mycorrhizal fungus, *Glomus fasciculatum*. *New Phytol*. **97**: 437-446

Adams, P.B., Marios, J.J. and Ayers, W.A. 1984. Popopulation dynamics of the mycoparasite, *Sporidesmium sclerotivorum* and its host, *Sclerotinia minor* in soil. *Soil Biol. Biochem*. **16**: 627-633

Ahmed, J.S. and Baker, R. 1987. Competitive saprophytic ability and cellulolytic activity of rhizosphere competent mutants of *Trichoderma harzianum*. *Phytopathology* **77**: 358-362

Ahmed, J.S. and Baker, R. 1988. Implications of rhizosphere competence of *Trichoderma harzianum*. *Can. J. Microbiol*. **34**: 229-234

*Ahmed, S.S. and Alsayed, A.A. 1991. Interaction between the vesicular arbuscular mycorrhiza, *Glomus macrocarpus* and *Meloidogyne incognita* infecting cowpea. *Annals of Agricultural Science, Moshtohor*, **29**: 1765-1772

Alagarswamy, G. and Sivaprakasom, K. 1988 Effect of antagonists in combination with carbendazim against *Macrophomina phaseolina* infection in cowpea. *J. Biol. Control*. **2**: 123-125

*Al-Momany and AL-Raddad, A. M. 1988. Effect of vesicular arbuscular mycorrhizae on *Fusarium* wilt of tomato and pepper. *Alexandria J. Agric. Res*. **33**: 249-261

Al-Raddad, A.M. 1995. Interaction of *Glomus mosseae* and *Paecilomyces lilacinus* on *Meloidogyne javanica* of tomato. *Mycorrhiza* **5**: 233-236

Amijee, F., Tinker, P.B. and Stribley, D.P. 1989. The development of endomycorrhizal root systems. VII. A detailed study of effects of soil phosphorus on colonization. *New Phytol.* **111**: 435-446

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

Anandaraj, M., Ramana, K.V. and Sarma, Y.R. 1993. Suppressive effects of VAM on root pathogens of black pepper, a component of Western Ghats forest ecosystem. In : *IUFRO symposium* Nov. 1993. p. 64. Kerala Forest Research Institute, Peechi

Atilano, R.A., Menge, J.A. and Van Bundy, S.D. 1981. Interaction between *Meloidogyne arenaria* and *Glomus fasciculatum* in grape. *J. Nematol.* **13**: 52-57

Atilano, R.A., Rich, J.R., Ferris, H. and Menge, J.A. 1976. Effect of *Meloidogyne arenaria* on endomycorrhizal grape (*Vitis vinifera*) rooting. *J. Nematol.* **8**: 278

Atkinson, D. and Davison, A.W. 1972. The effects of phosphorus deficiency on water content and response to drought. *New Phytol.* **72**: 307-313

*Atkinson, D, Berta, G. and Hooker, J.E. 1994. Impact of mycorrhizal colonization on root architecture: root longevity and formation of growth regulators. In : *Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems*. Gianinazzi, S. and Schuepp, H. (eds.) pp. 89-99. Birkhauser, Basel

*Ayers, W.A. and Adams, P.B. 1981. Mycoparasitism and its application to biological control of plant diseases. In : *Biological control of crop production*. Papavizas, G.C. (ed.) pp. 91-103. *Beltsville Symp. Agric. Res.* **5**

*Azcon-Aguilar C. and Bago. B. 1994. Physiological characteristics of the host plant promoting an undisturbed functioning of the mycorrhizal symbiosis In : *Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems*. Ginaninazzi, S. Schuepp, H. (eds.) pp. 47-60. Birkhauser, Basel

Azcon-Aguilar, C. and Barea, J.M. 1985. Effect of soil microorganisms on formation of vesicular-arbuscular mycorrhizas. *Trans. Br. Mycol. Soc.* **84**: 536-537

Azcon-Aguilar, C. and Barea, J.M. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens - an overview of the mechanism involved. *Mycorrhiza* 6: 457-464

Baath, E. and Hayman, D.S. 1983. Plant growth responses to vesicular-arbuscular mycorrhiza XIV. Interactions with Verticillium wilt on tomato plant. *New phytol.* **95**: 419

Backman, P.A. and Rodriguez-Kabana, R. 1975. A system for the growth and delivery of biological control agents to the soil. *Phytopathology* **65**: 819-821

*Baertschi, H., Gianinazzi-Pearson, V. and Vegh, I. 1981. Vesicular arbuscular mycorrhiza and root-rot disease (*Phytophthora cinnamomi* Rands.) development in *Chamaecyparis lawsoniana* (Murr.)Parl. *Phytopathol. Z.* **102**: 213

Bagyaraj, D.J., Manjunath, A. and Reddy, D.D.R., 1979. Interaction of vesicular arbuscular mycorrhiza with root-knot nematodes in tomato. *Plant Soil* **51**: 397-403

Baker, K.F. and Cook, R.J. 1974. Biological control of Plant pathogens. Freeman, W.H. and Company, San Francisco. American Phytopathological Society, St.Paul, MN pp. 433

Baker, R., Elad, Y. and Chet, I. 1984. The controlled experiment in the scientific method with special emphasis on biological control. *Phytopathology* **74**: 1019-1021

*Baker, R., Paulitz, T., Windham, M.T. and Elad, Y. 1986. Enhancement of growth of ornamentals by a biological control agent. *Res. Bull. Colorado Green house Grow. Assoc.* p. 8

*Baltruschat, H. and Schonbeck, F. 1972a. Influence of endotrophic mycorrhiza on chlamydospore production of *Thielaviopsis basicola* in tobacco roots. *Phytopathol. Z.* **74**: 358

*Baltruschat, H. and Schonbeck, F. 1972b. The influence of endotrophic mycorrhiza on the infestation of tobacco by *Thielaviopsis basicola*. *Phytopathol. Z.* **74**: 172-188

*Baltruschat, H. and Schonbeck, F. 1975. The influence of endotrophic mycorrhiza on the infestation of tobacco by *Thielaviopsis basicola*. *Phytopathol. Z.* **84**: 172-188

*Baltruschat, H., Sikora, R.A. and Schonbeck, F. 1973. Effect of V.A mycorrhiza (*Endogone mosseae*) on the establishment of *Thielaviopsis basicola* and *Meloidiogyne incognita* in tobacco. *Abstr. 2nd Intern. Cong. Plant Pathol.* pp 661

Barea, J.M. and Jeffries, P. 1995. Arbuscular mycorrhizas in sustainable soil plant systems. In : *Mycorrhiza, structure, function, molecular biology and biotechnology*. Hock, B. and Varma, A. (eds.) pp. 521-559. Springer, Heidelberg

*Barea, J. M., Azcon-Aguilar, C. and Azcon, R. 1996. Interactions between mycorrhizal fungi and rhizosphere microorganisms within the context of sustainable soil-plant systems. In : *Multitrophic interactions in terrestrial systems*. Gange, A.C., Brown, V.K. (eds.) Blackwell, Oxford

Barnard, O. and Davet, P. 1993. Observations on some *Trichoderma* spp. strains that are simultaneously antagonistic to *Pythium ultimum* and growth stimulating. *Agronomie* **13**: 413-421

*Becker, W.N. 1976. Quantification of onion vesicular arbuscular mycorrhizae and their resistance to *Pyrenochaeta terrestris*. Ph.D. thesis, University of Illinois, Urbana

Benhamu, N. and Chet, I. 1996. Parasitism of sclerotia of *Sclerotium rolfsii* by *Trichoderma harzianum* : Ultrastructural and cytochemical aspects of interaction. *Phytopathology* **86**: 405-416

Benhamou, N., Fortin, J.A., Hamel, C., St. Arnaud, M. and Shatilla, A. 1994. Resistance response of mycorrhizal Ri T-DNA-transformed carrot roots to infection by *Fusarium oxysporum* f. sp. *chrysanthemi*. *Phytopathology* **84**: 958-968

*Bethlenfalvay, G.J., Ulrich, J.M. and Brown, M.S. 1985. Plant response to mycorrhizal fungi : host, endophyte and soil effects. *Soil. Sci. Soc. Am. J.* **49**: 1164-1168

Bharadwaj, S.S. and Gupta, P.K. 1987. *In vitro* antagonism of *Trichoderma* species against fungal pathogens associated with rhizome rot of ginger. *Indian J. Pl. Pathol.* **6**: 56-58

*Bisht, V.S., Krishna, K.R. and Nene, Y.L. 1985. Interaction between vesicular arbuscular mycorrhiza and *Phytophthora drechsleri* f sp *cajani*. *International pigeonpea news letter* **4**: 63-64

Bliss, D.E. 1951. The destruction of *Armillaria mellea* in citrus soils. *Phytopathology* **41**: 665-683

Bolan, N.S., Robson, A.D. and Barrow, N.J. 1987. Effects of vesicular-arbuscular mycorrhiza on the availability of iron phosphates to plants. *Plant Soil* **99**: 401-410

Bopaiah, B.M., Wani, S.P. and Rai, P.V. 1991. Interaction of leaf surface saprophytic fungi with pathogenic fungi *in vitro*. *Indian Phytopath.* **44**: 407-409

Bruehl, G.W., Millar, R.L. and Cunfer, B. 1969. Significance of antibiotic production by *Cephalosporium graminium* to its saprophytic survival. *Can. J. Plant Sci.* **49**: 235-246

Butler, E.J. 1918. Fungi and diseases in plants. Thacker Spink and Co., Culcutta

*Cal, A.De, Pascual, S. and Melgarejo, P. 1994. Lytic enzymes in the biological control of Fusarium wilt of tomato. In : *Brighten Crop Protection Conference. Pests and Disease* **3**: 1127-1132

Calvet, C., Barea, J.M. and Pera, J. 1992. *In vitro* interactions between the vesicular arbuscular mycorrhizal fungus *Glomus mosseae* and some saprophytic fungi isolated from organic substrates. *Soil Biol. Biochem.* **24**: 775-780

Calvet, C., Pera, J. and Barea, J.M. 1993. Growth response of marigold (*Tagetes erecta* L.) to inoculation with *Glomus mosseae*, *Trichoderma aureoviride* and *Pythium ultimum* in a peat-perlite mixture. *Plant Soil* **148**: 1-6

Carling, D.E., Roncadori, R.W. and Hussey, R.S. 1995. Interactions of arbuscular mycorrhizae, *Meloidogyne arenaria* and phosphorus fertilization on peanut. *Mycorrhiza* **5**: 9-13

Caron, M. 1989. Potential use of mycorrhizae in control of soil borne diseases. *Can. J. Plant Pathol.* **11**: 177-179

Caron, M., Fortin, J.A. and Richard, C. 1986a. Effect of inoculation sequence on the interaction between *Glomus intraradius* and *Fusarium oxysporum* f. sp. *radicis - lycopersici* in tomatoes. *Can. J. Plant Pathol.* **8**: 12-16

Caron, M., Fortin, J.A. and Richard, C. 1986b. Effect of phosphorus concentration and *Glomus intraradius* on Fusarium grown and root rot of tomatoes. *Phytopathology* **76**: 942-946

Castrejon, S.A. 1994. *In vitro* detection of fungi antagonistic to *Verticillium dahliae* Klbe race T-9. *ITEA Production Vegetal.* **90**: 129-131

*Catska, V. 1994. Interrelationships between vesicular arbuscular mycorrhizae and rhizosphere microflora in apple replant disease. *Biologia Plantarum* **36**: 99-104

Chabbra, M.L. Bhatnagar, M.K. and Sharma, M.P. 1992. Influence of a vesicular-arbuscular (VA) mycorrhizal fungus on important diseases of maize. *Indian Phytopath.* **45**: 235-236

*Chakravarthy, P. and Misra, P.R. 1986a. The influence of V.A. mycorrhizae on wilting of *Albizia procera* and *Dalbergia sissoo*. *Eur. J. For. Pathol.* **16**: 91-97

Chakravarthy, P. and Misra, P.R. 1986b. Influence of endotrophic mycorrhizae on the fusarial wilt of *Cassia tora* L. *J. Phytopathol.* **115**: 130-133

Cherif, M. and Benhemon, N. 1990. Cytochemical aspects of chitin breakdown during the parasitic action of *Trichoderma* sp. on *Fusarium oxysporum* f. sp. *radicis - lycoprsici*. *Phytopathology* **80**: 1406-1414

Chet, I. 1987. *Trichoderma* : Application, mode of action, and potential as a biocontrol agent of soilborne plant pathogenic fungi. In : *Innovative approaches to plant Disease control*. Chet, I (ed.) pp. 137-160. John Wiley and sons, Inc., New York

Chet, I. and Baker, R. 1980. Induction of suppressiveness to *Rhizoctonia solani* in soil. *Phytopathology* **70**:994-998

Chet, I. and Baker, R. 1981. Isolation and biocontrol potential of *Trichoderma hamatum* from soil naturally suppressive to *Rhizoctonia solani*. *Phytopathology* **71**: 286-290

Chet, I. and Inbar, J. 1994. Biological control of fungal pathogens. *Appl. Biochem. Biotechnol.* **48**: 37-43

Chet, I., Harman, Y.E. and Baker, R. 1981. *Trichoderma hamatum*. Its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. *Microb. Ecol.* **7**: 29-38

Chet, I., Hadar, Y., Elad, Y., Katan, J. and Henis, Y. 1979. Biological control of soil borne plant pathogens by *Trichoderma harzianum*. In : *Soilborne plant pathogens*, Schippers, B. and Gams, W. (eds.) pp. 585-591. Academic Press, London

Chou, L.G. and Schmitthenner, A.F. 1974. Effect of *Rhizobium japonicum* and *Endogone mosseae* on soybean root rot caused by *Pythium ultimum* and *Phytophthora megasperma* var. *sojae*. *Plant. Dis. Rep.* **58**: 221-225

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

Clough, K.S. and Sutton, J.C. 1978. Direct observation of fungal aggregates in sand dune soil. *Can. J. Microbiol.* **24**: 333-335

Cook, R.J. and Baker, K.F. 1983. The native and practice of biological control of plant pathogens. APS press, The American Phytopathological Society, St Paul, Minnesota 539 pp.

Cooper, K.M. and Grandison, G.S. 1986. Interaction of vesicular arbuscular mycorrhizal fungi and root knot nematodes on cultivars of tomato and white clover susceptible to *Meloidogyne hapla*. *Ann. Appl. Bio.* **108**: 555-565

*Cordier, C., Gianinazzi, S. and Gianinazzi-Pearson, V. 1996. Colonization patterns of root tissues by *Phytophthora nicotianae* var. *parasitica* related to reduced disease in mycorrhizal tomato. *Plant Soil* (in press)

Dake, G.N., Anandaraj, M., Raju, C.A. and Iyer, R. 1989. Storage method of ginger seed rhizomes. Annual Report, 1989. National Research Centre for Spices, Calicut, Kerala.

*Dataran, 1988. Studies on the management of ginger rhizome rot. MSc. thesis, College of Agriculture, Rajasthan Agricultural University, Udaipur, Rajastan

Davet, P. 1981. Effects de quelques pesticides Sur la colonization d'um substrat parle *Trichoderma harzianum* Rifai en presence desautres champignons dusol. *Soil Biol. Biochem.* **13**: 513-517

Davis, R.M. 1980. Influence of *Glomus fasciculatus* on *Thielaviopsis basicola* root rot of citrus. *Plant Dis.* **64**: 839-840

Davis, R.M. and Menge, J.A. 1980. Influence of *Glomus fasciculatus* and soil phosphorus on Phytophthora root rot of citrus. *Phytopathology* **70**: 447-452

Davis, R.M., Menge, J.A. and Erwin, D. 1979. Influence of *Glomus fasciculatus* and soil phosphorus on Verticillium wilt of cotton. *Phytopathology* **69**: 453-456

Davis, R.M., Menge, J.A. and Zentmyer, G.A. 1978. Influence of vesicular arbuscular mycorrhizae on *Phytophthora* root rot of three crop plants. *Phytopathology* **68**: 1614-1617

*Dehne, H.W. 1977. Untersuchungen uber den Einfluss der endotrophen mycorrhiza auf die Fusarium-Welke an Tomato und Gurke. Dessertation, Univ. Bonn., W. Germany. 150 p

Dehne, H.W. 1982. Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. *Phytopathology* **72**: 1115-1119

*Dehne, H.W. and Schonbeck, F. 1975. The influence of endotrophic mycorrhiza on the *Fusarium* wilt of tomato. *Z. Pflanzenkr. Pflanzenschutz.* **82**: 630

Dehne, H.W. and Schonbeck, F. 1979a. The influence of endotrophic mycorrhiza on plant diseases. 1. Colonization of tomato plants by *Fusarium oxysporum* f. sp. *lycopersici*. *Phytopathol. Z* : **95**: 105-110

Dehne, H.W. and Schonbeck, F. 1979b. The influence of endotrophic mycorrhiza on plant disease. 2. Phenol metabolism and lignification. *Phytopathol. Z.* **95**: 210-216

Dennis, C. and Webster, J. 1971a. Antagonistic properties of species - groups of *Trichoderma* 1. Production of non volatile antibiotics. *Trans. Br. Mycol. Soc.* **57**:25-39

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

*De Oliveira, V.L. and Garbaye, J. 1989. Les microorganismes auxilliaires de l' establissement des symbioses mycorrhiziennes. *Eur. J. For. Pathol.* **19**: 54-64

*D'erole, N., Sporteth, M. and Nipoti, P. 1984. Different types of antagonism of *Trichoderma* sp. towards plant pathogenic soil fungi. *Informatore Fitopatologico* **39**:43-47

Dhedhi, B.M., Omgupta and Patel, V.A. 1990. Influence of metabolites of microorganisms on the growth of *Fusarium oxysporum* f.sp. *ciceri*. *Indian J. Mycol. Pl. Pathol.* **20**: 66-69

Directorate of Economics and Statistics. 1996. Directorate of Economics and Statistics, Government of India, New Delhi

Dodd, J.C. and Jeffries, P. 1989. Effect of fungicides on three vesicular arbucular mycorrhizal fungi associated with winter wheat (*Triticum aestivum* L.). *Biology and Fertility of soils* **7**: 120-128

Dohroo, N.P., Shyam, K.R. and Bharadwaj, S.S. 1987. Distribution, diagnosis and incidence of rhizome rot complex of ginger in Himachal Pradesh. *Indian J. Plant Pathol.* **5**: 24-25

*Duvenhage, J.A. and Kotze, J.M. 1993. Biocontrol of root rot of avocado seedlings. Year book-South Africa Avocado growers Association **16**: 70-72

Dwivedi, S.K., Ambasht, R.S. and Dwivedi, R.S. 1993. Toxicity of some antagonistic fungi on pathogenic fungi of two economic crops. *Natl. Acad. Sci. Lett.* **16**: 209-210

Elad, Y. and Chet, I. 1983. Improved selective medium for isolation of *Trichoderma* or *Fusarium* spp. *Phytoparasitica* **11**: 55-58

Elad, Y., Berak, R. and Chet, I. 1984. Parasitism of sclerotia of *Sclerotium rolfsii* by *Trichoderma harzianum*. *Soil Biol. Biochem.* **16**: 381-386

Elad, Y., Chet, I. and Kantan, J. 1980. *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. *Phytopathology* **70**: 119-121

Elad, Y., Chet, I. and Henis, Y. 1981. Biological control of *Rhizoctonia solani* in strawberry fields by *Trichoderma harzianum*. *Plant Soil* **60**: 245-254

Elad, Y., Kalfon, A. and Chet, I. 1982a. Control of *Rhizoctonia solani* in cotton by seed coating with *Trichoderma* spp. *Plant Soil* **66**: 279-281

Elad, Y., Hadar, Y., Chet, I. and Hinis, Y. 1982b. Prevention with *Trichoderma harzianum* Rifai. aggr., of reinfestation by *Sclerotium rolfsii* Sacc. and *Rhizoctonia solani* Kuhn of soil fumigated with methyl bromide and improvement of disease control in tomatoes and plants. *Crop Prot.* 1: 199-211

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

Elliot, A.P., Bird, G.W. and Safir, G.R. 1984. Joint influence of *Pratylenchus penetrans* (Nematoda) and *Glomus fasciculatum* (Phycomyceta) on the ontogeny of *Phaseolus vulgaris*. *Nematropica* **14**: 111-119

*Fajola and Alasoadura. 1975. Antagonistic effects of *Trichoderma harzianum* on *Pythium aphanidermatum*. *Mycopathologia* **57**: 47-52

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

Fox, J.A. and Spasoff, L. 1972. Interaction of *Heterodera solanacearum* and *Endogone gigantea* on tobacco. *J. Nematol.* **4**: 224-225

Francl, L. and Dropkin, V. 1985. *Glomus fasciculatum*, a weak pathogen of *Heterodera glycines*. *J. Nematol.* **17**: 470-475

*Frank, A.B. 1885. Ueber die auf Wurzelsymbiose beruhende Ernabrung gewisser Baume durch unterirdische pilze. *Berichte der Deutsche Botanische Gesellschaft* **3**: 128-145

Garbaye, J. 1994. Helper bacteria: a new diamension to the mycorrhizal symbiosis. *New Phytol.* **128**: 197-210

George, E., Hanssler, K.U., Vetterlim, D., Gorgus, E. and Marschner, H. 1992. Water and nutrient translocation by hyphae of *Glomus mosseae*. *Can. J. Bot.* **70**: 2130-2137

Gerdemann, J.W. 1968. Vesicular arbuscular mycorrhiza and plant growth. *Annu. Rev. Phytopathol.* **6**: 397-418

Gerdemann, J.W. and Nicolson, T.H. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* **46**: 235-244

Gianinazzi-Pearson, V. and Gianinazzi, S. 1995. Proteins and protein activities in endomycorrhizal symbiosis. In : *Mycorrhiza, structure, function, molecular biology and biotechnology*. Hock, B and Varma, A. (eds.) pp. 251-266. Springer, Heidelbert

Gianinazzi-Pearson, V., Dumas-Gaudot, E., Gollotte, A., Jahiri-Alaoui, A. and Gianinazzi, S. 1996. Cellular and molecular defence-related root responses to invasion by arbuscular mycorrhizal fungi. *New Phytol.* **133**: 45-57

Gopalakrishnan, M.N., Bagyaraj, D.J. and Vasanthakrishna, M. 1990. Interaction between *Glomus fasciculatum* and two phosphate solubilising fungi in finger millet. In : *Trends in Mycorrhizal Research - Proc. Nat. Conf.* on Mycorrhiza. Jalali, B.L. and Chand, H. (eds.) pp. 111-112. Hisar, India.

Graeme-Cook, K.A. and Faull, J.L. 1991. Effect of ultraviolet induced mutants of *Trichoderma harzianum* with altered antibiotic production on selected pathogens *in vitro*. *Can. J. Microbiol.* 37(9): 659-664

Graham, J.H. 1986. Citrus mycorrhizae : potential benefits and interactions with pathogens. *Hort. Sci.* 21: 1302-1306

Graham, J.H. and Egel, D.S. 1988. Phytophthora root rot development on mycorrhizal and phosphorus fertilized non-mycorrhizal sweet orange seedlings. *Plant Dis.* **72**: 611-614

Graham, J.H. and Menge, J.A. 1982. Influence of vesicular arbuscular mycorrhizas and soil phosphorus on take all disease of wheat. *Phytopathology* **72**: 95-98

Grandison, G.S. and Cooper, K.M. 1986. Interaction of vesicular-arbuscular mycorrhizae and cultivars of lucerne susceptible and resistant to *Meloidogyne hapla*. *J. Nematol.* **18**: 141-149

*Guttay, A.J.R. 1976. Impact of de-icing salts upon the endomycorrhizae of roadside sugar maple *Soil. Sci. Soc. Amer. J.* **40**: 952-954

Habte, M. and Manjunath, A. 1992. Initial and residual toxicity of soil applied thiram on the vesicular arbuscular mycorrhizal symbiosis. *Mycorrhiza* 2: 25-31

Hadar, Y., Harman, G.E. and Taylor, A.G. 1984. Evaluation of *Trichoderma koningii* and *T. harzianum* from New York soils for biological control of seed rot caused by *Pythium* spp. *Phytopathology* **74**: 106-110

Hall, I.R. and Abbott, L.K. 1981. Photographic slide collection illustrating features of the Endogonaceae. Technical Report No. 14, Agricultural Research Division, Ministry of Agriculture and Fisheries, Mosgiel, New Zealand

*Harley, C. 1921. Damping-off in fruit nurseries. U.S. *Dept. Agric. Bull.* **93**: 1-99

Harley, J.L. and Smith, S.E. 1983. Mycorrhizal symbiosis. Academic Press, London

Harman, G.E., Chet, I. and Baker, R. 1980. *Trichoderma hamatum* effects on seed and seedling disease induced in radish and pea by *Pythium* spp. and *Rhizoctonia solani*. *Phytopathology* 70: 1167-1172

Harman, G.E., Chet, I. and Baker, R. 1981. Factors affecting *Trichoderma hamatum* applied to seeds as biocontrol agents. *Phytopathology* **71**: 569-572

Hayman, D.S. 1982. Influence of soils and fertlity on activity and survival of vesicular-arbuscular mycorrhizal fungi. *Phytopathology* **72**: 1119-1125

Henis, Y., Ghaffer, A. and Baker, R. 1978. Integrated control of *Rhizoctonia solani* damping-off of raddish. Effect of successive plantings, PCNB and *Trichoderma harzianum* on pathogen and disease. *Phytopathology* **68**: 900-907

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

*Hooker, J.E., Jaizme-vega, M. and Atkinson, D. 1994. Biocontrol of plant pathogens using arbuscular mycorrhizal fungi. In : *Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems*. Gianinazzi, S. and Schuepp, H. (eds.). pp. 191-200. Birkhauser, Basel

*Horne, W.T. 1914. The oak fungus disease of fruit trees. *Calif. Comm. Hortic. Monthly Bull.* **3**: 275-282

Huang, H.C. 1980. Control of Sclerotinia wilt of sunflower by hyperparasites. *Can J. Plant Pathol.* **2**: 26-32

Hussey, R.S. and Roncadori, R.W. 1977. Interaction of *Pratylenchus brachyurus* and an endomycorrhizal fungus on cotton. *J. Nematol.* **9**: 270-271

Hussey, R.S. and Roncadori, R.W. 1982. Vesicular arbuscular mycorrhiza may limit nematode activity and improve plant growth. *Plant Dis.* **66**: 9-14

Hwang, S.F., Chakravarty, P. and Prevost, D. 1993. Effect of rhizobia, metalaxyl and V.A. mycorrhizal fungi on growth, nitrogen fixation and development of *Pythium* root rot of sainfoin. *Plant Dis.* **77**: 1093-1098

Hwang, S.F. Chang, K.F. and Chakravarty, P. 1992. Effect of vesicular-arbuscular mycorrhizal fungi on the development of Verticillium and Fusarium wilts of alfalfa. *Plant Dis.* **76**: 239-243

Inbar, J. and Chet, I. 1994. A newly isolated lectin from the plant pathogenic fungus *Sclerotium rolfsii*: Purification, characterisation and role in mycoparasitism. *Microbiology* **140**: 651-657

Inbar, J., Ambransky, M., Cohen, D. and Chet, I. 1994. Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. *Eur. J. Plant Pathol.* **10**: 337-346

*Iqbal, S.H. and Nasim, G. 1988. V.A. mycorrhiza as a deterrent to damping off caused by *Rhizoctonia solani* at different temperature regions. *Biologia Pakistan*, **34**: 215-221

*Iqbal, S.H. and Nasim, G. 1991. Are under ground non-root portions of tropical plants vesicular arbuscular mycorrhizal? *Trans. Mycol. Soc. Japan* 32: 467-476

Iyer, R., 1987. Disease of ginger. *Rev. Trop. Pl. Pathol.* **4**: 251-288

Jackson, M.L. 1973. Soil chemical analysis. Prentice Hall of India (Pvt.) Ltd., New Delhi, 2nd Edn.

Jacobsen, I. 1995. Transport of phosphorus and carbon in V.A. mycorrhizas. *Mycorrhiza* 5: 297-324

Jalali, B.L. 1983. Biological suppression of root disease fungi by vesicular-arbuscular mycorrhizal system. 4th *Intern. Congr. Plant Pathol. Melbourne* (Australia) August, 1983. **46**: 183

Jalali, B.L. and Jalali, I. 1991. Mycorrhiza in plant disease control. In : *Handbook of Applied Mycology. Vol. 1, Soil and Plants*. Arora, K., Rai, B., Mukerji, K.G. and Knudsen, G.R. (eds.) pp. 131-154. M. Dekker Inc., New York

Jalali, B.L. and Thareja, M.L. 1981. Suppression of fusarial wilt of chickpea in VA mycorrhiza inoculated soils. *Intern. Chickpea Newsl. ICRISAT*. **4**: 21-23

Jalali, B.L., Chhabra, M.L. and Singh, R.P. 1990. Interaction between vesicular-arbuscular mycorrhizal endophyte and *Macrophomina phaseolina* in mungbean. *Indian Phytopath*. **43**: 527-530

Joshi, L.K. and N.D. Sharma. 1982. Disease of ginger and turmeric. In : *Proceedings of the National Seminar on ginger and turmeric* April, 1980. Nair, M.K., Premkumar, T., Ravindran, P.N. and Sarma, Y.R. (eds.) pp. 131-154. Calicut, CPCRI, Kasargod, Kerala, India

KAU. 1993. *Package of Practices Recommendations*. Kerala Agricultural University, Vellanikkara, Trichur, Kerala

Kaye, J.W., Pfleger, F.L. and Steward, E.L. 1984. Interaction of *Glomus fasciculatum* and *Pythium ultimum* in green house grown poinsettia *Can. J. Bot.* **62**: 1575-1579

Katznelson, H., Rouatt, J.W. and Peterson, E.A. 1962. The rhizosphere effect of mycorrhizal and non-mycorrhizal roots of yellow birch seedlings. *Can. J. Bot.* **40**: 379-382

*Kellam, M.K. and Schenck, N.C. 1977. The effect of initial sequence on the interaction between *Glomus macrocarpus* and *Meloidoygne incognita* on soybean. In : *Proceedings of the 3rd NACOM*. p.3 Georgia, USA

Kellam, M.K. and Schenck, N.C. 1980. Interaction between a vesicular-arbuscular mycorrhizal fungus and root-knot nematode on soybean. *Phytopathology* **70**: 293-296

*Khan, S. and Khan, A.G. 1974. Role of VAM fungus in the resistance of wheat roots to infection by *Urocystis tritici*. *Proc. Pak. Sci. Cong.* **25**: 7

*Khare, M.N. 1968. The relationship of *Phytophthora fragariae* Hickman to the fungal flora in the rhizosphere of the strawberry plant. *Diss. Abstr.* **28**: 4836

Klingstrom, A.E. and Johansson, S.M. 1973. Antagonism of *Scytatidium* isolates against decay fungi. *Phytopathology* **63**: 473

Kobayashi, N. 1990. Biological control of soil borne diseases with VAM fungi and charcoal compost. In : *Biological control of plant diseases*. Proc. Int. Sem. Biol. control of plant diseases and virus vectors, Tsukuba, Japan, 1990. Komadan, H., Kiritani, K. and Bay-Peterson, J. (eds.) pp. 151-160. Taipei, Taiwan

*Kohl, J. and Schlosser, E. 1989. Effect of two *Trichoderma* spp. on the infection of maize roots by vesicular arbuscular mycorrhizae. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz* **96**: 439-443

Koide, R.T. and Schreiner, R.P. 1992. Regulation of the vesicular-arbuscular mycorrhizal symbiosis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **43**: 557-581

Kommedahl, T. and Chang, I. 1975. Biocontrol of corn root infection in the field by seed treatment with antagonists. *Phyopathology* **65**: 295-300

Kommedahl, T., Windels, C.E., Sarbini, Y. and Wiley, H.B. 1981. Variability in performance of biological and fungicidal seed treatments in corn, peas and soybeans. *Prot. Ecol.* **3**: 55-61

Kothari, S.K., Marschner, H. and Romheld, V. 1990. Direct and indirect effects of VA mycorrhiza and rhizosphere microorganisms on mineral nutrient acquisition by maize (*Zea mays* L) in a calcarious soil. *New Phytol.* **116**: 637-645

Kothari, S.K. Marschner, H. and Romheld, V. 1991. Contribution of VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcarious soil. *Plant Soil* **131**: 177-185

Krishna, K.R. and Bagyaraj, D.J. 1983. Interaction between *Glomus fasciculatum* and *Sclerotium rolfsii* in pea nuts. *Can. J. Bot.* **61**: 2349-2351

Krishna, K.R. and Bagyaraj, D.J. 1984. Phenols in mycorrhizal roots of *Arachis hypogaea*. *Experientia* **40**: 85-86

Kumar, C.P.C., Garibova, L.V., Vellikanov, L.L. and Durinina, E.P. 1993. Biocontrol of wheat root rots using mixed cultures of *Trichoderma viride* and *Glomus epigaeus*. *Indian J. Plant Prot.* **21**: 145-148

Lifshitz, R. Dapler, M., Elad, Y. and Baker, R. 1984. Hyphal interaction between mycoparasites, *Pythium nunn*, and several soil fungi. *Can. J. Microbiol.* **30**: 1482-87

*Linderman, R.G. 1985. Microbial interactions in the mycorrhizosphere. In : *Proc. 6th NACOM.* Malina, R. (ed.) pp. 177. *For. Res. Lab.*, Corvallis

Linderman, R.G. 1988. Mycorrhizal interactions with the rhizosphere microflora: The mycorrhizosphere effect. *Phytopathology* 78: 366-371

Linderman, R.G. 1994. Role of VAM fungi in biocontrol. In : *Mycorrhizae and plant health*. Pfleger, F.L., Linderman, R.G. (eds.) pp. 1-26. APS Press Minnisota, U.S.A.

LI X-L, George, E. and Murschner, H. 1991a. Extension of phosphorus depletion zone in V.A. mycorrhizal white clover in a calcarious soil. *Plant Soil* **136**: 41-48

LI X-L, Marschner, H. and George, E. 1991b. Acquisition of phosphorus and copper by VA mycorrhizal hyphae and root-to-shoot transport in white clover. *Plant Soil* **136**: 49-57

Liu, S.Y. and Vaughan, E.K. 1965. Control of *Pythium* infection in table beet seedlings by antagonistic micro-organisms. *Phytopathology* **55**: 986-989

Lodha, B.C. and Webster, J. 1990. *Pythium accanthophoron*, a mycoparasite rediscovered in India and Britan. *Mycol. Res.* **94**: 1006-1008

*Lugtenberg, B.J.J., Weyer, L.A., De and Bennet, J.W. 1991. Microbial stimulation of plant growth and protection from disease. *Current opinion in Biotechnology* 2: 457-464

Mackenzie, A.J., Starman, T.W. and Windham, M.T. 1995. Enhanced shoot and root growth of crysanthimum cuttings propagated with the fungus *Trichoderma harzianum*. *Hort. Sci.* 30: 496-498

Mahabirsingh and Majumdar, V.L. 1995. Antagonistic activity of *Trichoderma* sp. to *Macrophomina phaseolina* (Tassi)Goid *in vitro*. *Environ. Ecol.* 13: 481-482

Mamata Sharma and Mukherji, K.G. 1992. Mycorrhiza -Tool for biocontrol. In : *Recent developments in biocontrol of plant diseases*. Mukerji, K.G., Teware, J.P., Arora, D.K., Geeta Saxena (eds.) pp. 52-80. Aditya books Private Ltd., New Delhi, India

Mani, M.T. and Marimuthu, T. 1994. Effect of decomposed coirpith, fungicides and biocontrol agents on damping off of chillies and dry root rot of blackgram. *Indian J. Mycol. Pl. Pathol.* **24**: 20-23

Marios, J.J., Mitchell, D.J. and Sonoda, R.M. 1981. Biological control of Fusarium crown rot of tomato under field conditions. *Phytopathology* **71**: 1257-1260

Marschner, H. and Dell, B. 1994. Nutrient uptake in mycorrhizal symbiosis. *Plant Soil* **159**: 89-102

Martin, S.B., Abawi, G.S. and Hoch, H.C. 1986. The relationship of population densities of the antagonist, *Laetisaria arvalis* to seedling disease of table beet incited by *Pythium ultimum*. *Can J. Microbiol.* **32**: 156-159

Mc Allister, C.B., Garcia-Romera, I., Godeas, A. and Ocampo, J.A. 1994a. Interactions between *Trichoderma koningii*, *Fusarium solani* and *Glomus mosseae* : Effects on plant growth, arbuscular mycorrhizas and the saprophyte inoculants. *Soil Biol. Biochem.* **26**: 1363-1367

Mc Allister, C.B., Garcia-Romera, I., Godeas, A. and Ocampo, J.A. 1994b. *In vitro* interactions between *Trichoderma koningii*, *Fusarium solani* and *Glomus mosseae*. *Soil Biol. Biochem.* **26**: 1369-1374

Mc Graw, A.C. and Schenck, N.C. 1981. Effects of two species of vesicular-arbuscular mycorrhizal fungi on the development of Fusarium wilt of Tomato. *Phytopathology* **71**: 894

Mc Laren, D.L., Rimmer, S.R. and Huang, H.C. 1983. Biological control of Sclerotinia wilt of sunflowers by *Talaromyces flavus*. *Phytopathology* **73**: 822

*Melo, I.S., Costa, C.P. and Silveira, A.P.D. 1985. Effect of vesicular arbuscular mycorrhizas on aubergine wilt caused by *Verticillium albo-atrum* Reinke and Berth. *Summa Phytopathologica* **11**: 173-179

Meyer, J.R. and Linderman, R.G. 1986. Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. *Soil Biol. Biochem.* **18**: 191-196

*Migheli, Q., Mocioni, M., Varese, C. and Garibaldi, A. 1993. Evaluation of the activity of antagonistic microorganisms against *Phytophthora nicotianae* var *Parasitica* on carnation. *Difesa delle Piante* **16**: 3-10

Mihuta-Grimm, L. and Rowe, R.C. 1986. Trichoderma spp. biocontrol agents of Rhizoctonia damping off of radish in organic soil and comparison of four delivery systems. *Phytopathology* **76**: 306-312

*Mitra, M. and Subramonian, L.S. 1928. Fruit rot disease of cultivted cucurbitaceae caused by *Pythium aphanidermatum* (Edson)Fitz. *Mem. Dep. Agric. India (Bot Ser)*. **15**: 79-84

Morton, J.B. and Benny, G.L. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, Glominae and Gigasporinae and two new families, Acaulosporaceae and Gigasporaceae with an emendation of Glomaceae. *Mycotaxon* **37**: 471-491

Mosse, B. 1973. Advances in the study of vesicular-arbuscular mycorrhiza. *Annu. Rev. Phytopathol.* II. 171-196

Mukherjee, B. and Sen, C. 1992. *Aspergillus* and *Penicillium* species : potential agents for biocontrol of *Macrophomina phaseolina*. *Indian Phytopath.* **45**: 39-43

Mukhopadyay, A.N. and Chandra, 1986. Biocontrol of sugarbeet and tobacco damping off by *Trichoderma harzianum*. Abstr. of papers presented in seminar on Management of Soilborne diseases of crop plants. Jayarajan, R., Bhaskaran, R. and Shunmugam, N. (eds.) p. 34 Department of Plant Pathology, Centre for Plant Protection Studies, TNAU, Coimbatore

Munnecke, D.E., Hanssler, K.M., Vetterlion, D. Gorgus, E. and Marschner, H. 1992. Water and nutrient translocation by hyphae of *Glomus mosseae*. *Can. J. Bot.* 70: 2130-2137

Nagarajan, K. and Reddy, T.S.N. 1986. Role of *T. viride* and *T. harzianum* in the biocontrol of *P. aphanidermatum*. Abstr. of papers presented in seminar on Management of Soilborne diseases of crop plants. Jeyarajan, R., Bhaskaran, R. and Shanmugan, N. (eds.) p. 33 Department of Plant Pathology, Centre for Plant Protection Studies, TNAU, Coimbatore

Nemec, S. 1974. Population of Endogone in strawberry fields in relation to root rot infection. *Trans. Br. Mycol. Soc.* **62**: 45

NRCS. 1994. Final report of multilocational project on rhizome rot of ginger (ICAR Cess Fund Scheme), National Research Centre for Spices, Calicut, Kerala

*Osman, H.A., Korayem, A.M., Ameen, H.H. and Badreldin, S.M.S. 1990. Interaction of root-knot nematodes and mycorrhizal fungi on common bean, *Phaseolus vulgaris* L. *Anzeiger fur Schadlingskunde Pflanzenschutz Umeveltschutz* **63**: 129-131

Padmanaban, P. and Alexander, K.C. 1984. Biological control of cane seedling root rot. *Sugarcane* **1**: 11

Padmanaban, P. and Alexander, K.C. 1990. Biological control of sugarcane root rot disease. *J. Biol. Control*, **4**: 135-136

Paget, D.K. 1975. The effect of cylindrocarpon on plant growth response to vesicular arbuscular mycorrhizae. In *"Endomycorrhizas"* Sanders, F.E., Mosse, B. and Tinker,P.B. (eds.) pp. 593-606. Academic Press, London

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: Biology, ecology and potential for bicontrol. *Annu. Rev. Phytopathol.* **23**: 23-54

Parvathi, K., Venkateswarlu, K. and Rao, A.S. 1985. Influence of root infecting fungi on development of *Glomus mossae* in groundnut. *Curr. Sci.* **54**: 1006-1007

Paulitz, T.C. and Baker, R. 1987. Biological control of *Pythium* damping off of cucumbers with *Pythium nunn*: Population dynamics and disease suppression. *Phytopathology* **77**: 335-340

Paulitz, T.C. and Linderman, R.G. 1991. Lack of antagonism between the biocontrol agent, *Gliocladium virens* and vesicular arbuscular mycorrhizal fungi. *New phytol.* **117**: 303-308

Paulitz, T., Windham, M.G. and Baker, R 1986. Effect of peat vermiculite mixer containing *Trichoderma harzianum* on increased growth responses of radish. *J. Am. Soc. Hort. Sci.* **33**: 810-816

Peethambaran, C.K. and Singh, R.S. 1977. Survival of different structures of *Pythium* spp. in soil. *Indian Phytopath.* **30**: 347-352

*Perrin, R. 1985. The ability of mycorrhiza to protect plants against disease : Panaca or Chimera? *Annales des Sciences Forerstieres* **42**: 453-469

Pfleger, F.L., Stewart, E.L. and Noyd, R.K. 1994. Role of VAM fungi in mine land revegettion. In : *Mycorrhizae and Plant Health*. Pfleger, F.L. and Linderman, R.G. (eds.) pp. 47-81. APS, Minnesota, U.S.A.

Phillips, J.M. and Hayman, D.S. 1970. Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **55**: 158-161

Piper, C.S. 1966. Soil and plant analysis. Hans publishers, Bombay. pp. 368

Powell, C.L.L. 1981. Inoculation of barley with efficient mycorrhizal fungi stimulates seed yield. *Plant Soil*. **59**: 487-489

Rathore, V.R.S., Mathur, K. and Lodha, B.C. 1992. Activity of volatile and non-volatile substances produced by *Trichoderma viride* on ginger rhizome rot pathogen. *Indian Phytopath.* **45**: 253-254

Ratnayake, M., Leonard, R.T. and Menge, J.A. 1978. Root exudation in relation to supply of phosphorus and its possible relevence to mycorrhizal formation. *New Phytol.* **31**: 543-552

RAU. 1993. Multilocational project on rhizome rot of ginger (*Zingiber officinale* R.). Final report 1990 - 93, Department of Plant Pathology, Rajasthan Agricultural University, Udaipur. pp. 46

Read, D.J., Francis, R. and Finlay, R.D. 1985. Mycorrhizal mycelia and nutrient cycling in plant communities. In : *Ecological interactions in soil: Plants, microbes and animals*. Fitter, A.H. (ed.). British ecological society No.4, Blackwell Scientific publications, London

Rhodes, L.H. 1980. The use of mycorrhizae in crop production system. *Outlook Agriculture* **10**: 275-281

Rhodes, L.H. and Gerdemann, J.W. 1978. Translocation of calcium and phosphate by external hyphae of vesicular-arbuscular mycorrhiza. *Soil Sci.* **126**: 125

Richardson, L.T. 1954. The persistence of thiram in soil and its relationship to the microbiological balance and damping off control. *Can. J. Bot.* **32**: 339-346

Robert, C.P., Sivaprasad, P., Mammootty, K.P. and Lekha, K.S. 1995. Effect of fungicides on vesicular-arbuscular mycorrhizal fungi under natural conditions. In : *Mycorrhizae : bio fertilizers for the future*. Proc. 3rd Nat. Conf. on mycorrhiza. Adholeya, A. and Singh, S. (eds.) pp. 137-146. Tata Energy Research Institute, New Delhi

Rohini Iyer and Sundararaju, P. 1993. Interaction of V.A. Mycorrhiza with *Meloidogyne incognita* and *Pythium aphanidermatum* affecting ginger (*Zingiber officinale* Rose.) *J. Plant. Crops.* **21**: 30-34

Roncadori, R.W. and Hussey, R.S. 1977. Interaction of the endomycorrhizal fungus, *Gigaspora margarita* and root knot nematode on cotton. *Phytopathology* **67**: 1507-1511

*Rosendahl, S. 1985. Interactions between vesicular-arbuscular mycorrhizal fungus, *Glomus fasciculatum* and *Aphanomyces euteiches* root rot of peas. *Phytopathol. Z.* **114**: 31-40

Rosendahl, S., Dodd, J.C. and Walker, C. 1994. Taxonomy and phylogeny of the Glomales. In : *Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems*. Gianinazzi, S. and Schuepp, H. (eds.) pp. 1-12. Birkhauser, Basel

*Rosendahl, S., Rosendahl, C.N. and Thingstrup, I. 1992. The use of vesicular-arbuscular mycorrhizal (VAM) fungi as a biocontrol agent. *Bulletin* OILB/SROP. **15**: 48-50

Ross, J.P. 1972. Influence of endogens mycorrhiza on *Phytophthora* rot of soybean. *Phytopathology* **62**: 896-897

Ross, J.P. and Harper, P.A. 1970. Effect of endogone mycorrhiza in soybean yield. *Phytopathology* **60**: 1552-1556

Sadanandan, A.K. and Iyer, R. 1986. Effect of organic amendments on rhizome rot of ginger. *Indian Cocoa, Arecanut and Spices J.* **9**: 94-95

*Safir, G. 1968. The influence of vesicular-arbuscular mycorrhizae on the resistance of onion to *Pyrenochaeta terrestris*. M.S. thesis University of Illinois, Urbana

Safir, G.R., Boyer, J.S. and Gerdemann, J.W. 1971. Mycorrhizal enhancement of water transport in soybean. *Science* **172**: 581-583

Safir, G.R., Boyer, J.S. and Gerdemann, J.W. 1972. Nutrient status and mycorrhizal enhancement of water transport in soybean. *Plant Physiol.* **49**: 700-703

Saleh, H. and Sikora, R.A. 1984. Relationship between *Glomus fasciculatum* root colonization of cotton and its effect on *Meloidogyne incognita*. *Nematologica* **30**: 230-237

Sampangiramaiah, K. and Bagyaraj, D.J. 1989. Root diseases and mycorrhiza - a review. *J.Phytol. Res.* **2**: 1-6

Sanders, F.E., Tinker, P.B., Black, R.L.B. and Palmerley, S.M. 1977. The development of endomycorrhizal root systems: 1. Spread of infection and growth promoting effects with four species of vesicular-arbuscular endophyte. *New Phytol.* **78**: 553-558

Sanford, G.B. 1926. Some factors affecting the pathogenicity of *Actinomyces scabies*. *Phytopathology* **16**: 525-547

*Sanford, G.B. and Broadfoot, W.C. 1931. A note on the biological control of root rot of cereals. Studies on the effects of other soil inhabiting microorganisms on the virulence of *Ophiobolus graminis* Sacc. *Sci. Agric.* **11**: 460, 512-528

Sarma, Y.R. 1994. Rhizome rot diseases of ginger and turmeric. In : *Advances in Horticulture Vol. 10. Plantation and Spice Crops*. Part 2. Chadha, K.L. and Rethinam, P. (eds.) pp. 113-138 Malhotra Publishing House, New Delhi

Sawant, I.S. and Mukhopadhyay, A.N. 1991. Integration of metalaxyl with *Trichoderma harzianum* for the control of Pythium damping off of sugar beat *Indian Phytopath.* **43**: 535-541

Schenck, N.C. 1981. Can mycorrhiza control root disease? *Plant Dis.* **65**: 230-234

Schenck, N.C. and Kellam, M.K. 1978. The influence of vesicular-arbuscular mycorrhizae on disease development. *Fla. Agric. Exp. Stn. Bull.* 799

Schenck, N.C. and Kinloch, R.A. 1974. Pathogenic fungi, parasitic nematodes and endomycorrhizal fungi associated with soybean roots in Florida. *Plant Dis. Rep.* **58**: 169-173

Schenck, N.C. and Perez, Y. 1988. Manual for the identification of V.A. mycorrhizal fungi. Gainesville, F.L.: INVAM pp 241

Schenck, N.C., Kinloch, R.A. and Dickson. 1975. Interaction of endomycorrhizal fungi and root-knot nematode on soybean. In : *Endomycorrazhas*. Proc. Symp. University of Leeds, England. Sanders, F.E., Mosse, B. and Tinker, P.B. (eds.) pp. 607-617 Academic Press, New York

*Schenck, N.C., Ridings, W.H. and Cornell, J.A. 1977. Interaction of two vesicular-arbuscular mycorrhizal fungi and *Phytophthora parasitica* on two citrus root-stocks. (Abstr.). In : Proc. Third *North Am. Cong. Mycorrhizae*. Corvallis, Oregon

Schonbeck, F. and Dehne, H. 1977. Damage to mycorrhizal and non-mycorrhizal cotton seedlings by *Thielaviopsis basicola*. *Plant Dis. Rep.* **61**: 266-268

Secilia, J. and Bagyaraj, D.J. 1987. Bacteria and actinomycetes associated with pot culture of vesicular-arbuscular mycorrhizae. *Can. J. Microbiol.* **83**: 1069-1073

Selvaraj, T. and Subramanian, G. 1990. Phenols and lipids in mycorrhizal roots of *Sesamum indicum*. *Curr. Sci.* **59**: 471-473

Sharma, N.D. and Jain, A.C. 1977. A check list and selected bibliography of ginger diseases of the world. *PANS.* **23**: 474-481

*Sieverding, E. and Toro, S. 1988. Influence of soil water regime on V.A. mycorrhiza. V. Performance of different VAM fungal species with cassava. *J. Agron. Crops. Sci.* **161**: 322-332

Sikora, R.A. and Schonbeck, F. 1975. Effect of vesicular arbuscular mycorrhiza (*Glomus mosseae*) on the population dynamics of root-knot nematodes (*Meloidogyne incognita* and *Meloidogyne hapla*). *Eighth Int. Plant Prot. Congr.*, **5**: 158-166

Singh, R.S., Hardip Singh and Singh, O.S. 1990a. Effects of fungicides on vesicular arbuscular mycorrhizal association and plant growth response of citrus seedlings. In : *Trends in mycorrhizal research*. Proc. Nat. Conf. on Mycorrhiza. Jalali, B.L. and Chand, H. (eds.) Haryana Agricultural University, Hisar

Singh, Y.P., Singh, R.S. and Sitaramaiah,K. 1990b. Mechanism of resistance of mycorrhizal tomato against root-knot nematode. In : *Trends in Mycorrhizal Research*. Proc. Nat. Conf. on Mycorrhiza, 1990, Jalali, B.L. and Chand, H. (eds). Hisar, India.

Singh, Y.P., Chhabra, M.L. and Jalali, B.L. 1990c. Pesticides-Mycorrhizae interaction on the growth and development of pigeon pea (*C. cajan*) In : *Trends in Mycorrhizal Research*. Proc. Nat. Conf. on Mycorrhiza. Jalali, B.L. and Chand, H. (eds.) Haryana Agrl. University, Hisar

Sivan, A., Elad, Y. and Chet, I. 1984. Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum*. *Phytopathology* **74**: 498-501

Sivan, A., Ucko, O. and Chet, I. 1987. Biological control of Fusarium crown rot of tomato by *Trichoderma harzianum* under field conditions. *Plant Dis.* **71**: 587-592

Sivaprasad, P. 1993. Management of root diseases of important spice crops of Kerala with V.A. mycorrhiza. Annual Report of DBT Project, 1993. Kerala Agricultural University, Trichur, Kerala, India

Sivaprasad, P. 1995. Management of root diseases of important spice crops of Kerala with VA-mycorrhiza. DBT Project Report, 1995. Kerala Agricultural University, Trichur, Kerala, India

Sivaprasad, P., Jacob, A.J. and George, B. 1990. Root-knot nematode infestation and nodulation as influenced by V.A. mycorrhizal association in cowpea. *Indian J. Nematol.* **20**: 49-52

Sivaprasad, P., Ramesh, B., Mohanakumaran, N., Rajmohan, K. and Joseph, P.J. 1995a. Vesicular-arbuscular mycorrhizae for the establishment of tissue culture plantlets. In : *biofertilizers for the future*. Proc. 3rd Nat. Conf. on mycorrhiza. Adholeya, A. and Singh, S.(eds.) pp.281-283. Tata Energy Research Institute, New Delhi

Sivaprasad, P., Robert, C.P., Vijayan, M. and Joseph, P.J. 1995b. Vesicular arbuscular mycorrhizal colonization in relation to foot rot disease intensity in black pepper. In : *Mycorrhizae biofertilizers for the future*. Adholeya, A. and Singh, S. (eds.). pp. 137-146 Tata Energy Research Institute, New Delhi

Smith, G.S., 1987. Interactions of nematodes with mycorrhizal fungi. In : *vistas on Nematology*. Veech, J.A. and Dickson, D.W. (eds.) pp. 292-300 Society of Nematologists, INC, Hyattsville, Md

Smith, G.S. 1988. The role of phosphorus nutrition in interactions of vesicular arbuscular mycorrhizal fungi with soil borne nematodes and fungi. *Phytopathology* 78: 371-374

Snedecor, G.W. and Cochran, W.G. 1962. Statistical methods. Oxford and IBH publishing Co., New Delhi

Sreenivasa, M.N. and Bagyaraj, D.J. 1989. Use of pesticides for mass production of vesicular-arbuscular mycorrhizal inoculum. *Plant Soil* **119**: 127-132

St-Arnaud, M., Hamel, C., Caron, M. and Fortin, J.A. 1994. Inhibition of *Pythium ultimum* in roots and growth substrates of mycorrhizal *Tagetes patula* colonized with *Glomus intraradices*. *Can. J. Plant Pathol.* **16**: 187-194

Stewart, E.L. and Pfleger, F.L. 1977. Development of poinsettia as influenced by endomycorrhizae, fertilizer and root rot pathogens, *Pythium ultimum* and *Rhizoctonia solani*. *Flor. Rev.* 159-37

Sundarababu, R., Sankaranarayanan, C. and Vadivelu, S. 1993. Interaction of mycorrhiza species with *Meloidogyne incognita* on tomato. *Indian J. Nematol.* **23**: 121-123

Sylvia, D.M. and Schenck, N.C. 1983. Germination of chlamydospores of three *Glomus* species as affected by soil matric potential and fungal contamination. *Mycologia* **75**: 30-35

Tarafdar, J.C. and Marschner, M. 1995. Dual inoculation with *Aspergillus fumigatus* and *Glomus mosseae* enhances biomass production and nutrient uptake in wheat (*Triticum aestivum* L.) supplied with organic phosphorus as Na-Phytate. *Plant Soil* **173**: 97-102

Thomas, G, V., Sundararaju, P., Ali, S.S. and Ghai, S.K. 1989. Individual and interactive effects of VA mycorrhizal fungi and root knot nematode, *Meloidogyne incognita* on cardamom. *Trop. Agric.* (Trindad) **66**: 21-24

Thomas, L. Mallesha, B.C. and Bagyaraj, D.J. 1994. Biological control of damping off of cardamom by VA mycorrizhal fungus *Glomus fasciculatum*. *Microbiol. Res.* **149**: 413-417

Thomson-Cason, K.M., Hussey, R.S. and Roncadori, R.W. 1983. Interaction of vesicular-arbuscular mycorrhizal fungi and phosphorus with *Meloidogyne incognita* on tomato. *J. Nematol.* **15**: 410-417

Timonin, M. 1940. The interaction of higher plants and soil microorganisms. 1. Microbial population of seedlings of certain cultivated plants. *Can. J. Res.* **18**:307-317

Tiwari, P.K. and Shukla, B.N. 1991. Influence of pesticides on *Glomus fasciculatum* and growth, development and nutrient uptake by soybean. *Indian J. Mycol. Pl. Pathol.* **21**: 49-55

Tribe, H.T. 1957. On the parasitism of *Sclerotinia trifoliorum* by *Coniothyrium minitans*. *Trans. Br. Mycol. Soc.* **40**: 489-499

Turner, G.J. and Tribe, H.T. 1975. Preliminary field plot trials on biological control of *Sclerotinia trifoliorum* by *Coniothyrium minitans*. *Plant Pathol.* **24**: 109-113

Udiyan, K., Manian, S., Muthukumar, T. and Greep, S. 1995. Biostatic effect of fumigation and pesticide drenches on an endomycorrhizal-Rhizobium legume tripartite association under field conditions. *Biology and Fertility of soils*. **20**: 275-283

Usman, N.M., Balakrishnan, P. and Sarma, Y.R. 1996. Biocontrol of rhizome rot of ginger. *J. Plant. Crops* **24** (Suppl.): 184-191

Venkateswarlu, S.M., At Garni, S.M. and Daft, M.J. 1995. The impact of carbofuran soil application on growth and mycorrhizal colonization by *Glomus clarum* of groundnut. *Mycorrhiza*. **5**: 125-128

Venketasubbaiah, P. and Safeeulla, K.M. 1984. *Aspergillus niger* for biocontrol of *Rhizoctonia solani* on coffee seedlings. *Tropical Pest Management* **30**: 401-406

Vidhyasekaran, P. 1990. Interaction between plants and pathogens resulting in Resistance to Diseases. In : *Physiology of Disease Resistance in field crops*. pp. 1-10. Today and Tomorrow's printers publishers, New Delhi

*Vijayalakshmi, M. and Rao, A.S. 1993. Effects of six insecticides and one fungicide on the development of VAM fungi in peanut (*Arachis hypogaea* L.). *Zentralblatt fur Mikrobiologie* **14**: 60-65

*Vrang, J., Rasochova, M., Fiker, A. and Dobias, K. 1990. Inoculation of potatoes with microorganisms under field conditions I. Effect of plant growth, yield and physiological properties of tuber in potato and sugarbeat regions. *Folia Microbiologica* **35** : 326-335

Vyas, S.C., Aditya, Vyas, Mahijan, K.C. and Shroff, V.N. 1990. Effects of fungicide on mycorrhizal and rhizobial development of soybean. In : *Trends in Mycorrhizal Research*. Proc. Nat. Conf. on Myoorrhiza. Jalali, B.L. and Chand, H. (eds.) Haryana Agrl. University, Hisar, Inida

Vyas, S.C. and Vyas, S. 1995. Influence of pesticides on mycorrhizal infection and chlamydospores formation in soybean (*Glycine max*). In : *Mycorrhiza biofertilizers for the future*. Proc. third Nat. Conf. on Mycorrhiza. Adholeya, A. and Singh, S. (eds.) pp. 147-151 Tata Energy Research Institute, New Delhi

*Weindling, R. and Emerson, O.H. 1936. The isolation of a toxic substance from the culture filtrate of *Trichoderma*. *Phytopathology* **24**: 1068-1070

*Weindling, R. and Fawcett, H.S. 1936. Experiments in the control of Rhizoctonia damping off of citrus seedlings. *Hilgradia* **10**: 1-16

*Wellings, N.P., Wearing, A.H. and Thompson, J.P. 1991. Vesicular arbuscular mycorrhizae (VAM) improve phosphorus and zinc nutrition and growth of pigeon pea in a vertisol. *Australian J. Agri. Resh.* **42**: 835-845

Wells, H.D., Bell, D.K. and Jaworski, C.A. 1972. Efficacy of *Trichoderma harzianum* as a biological control for *Sclerotium rolfsii*. *Phytopathology* **62**: 442-447

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

Windham, B.L., Windhan, M.T. and Williams, W.P. 1989. Efficiency of *Trichoderma* spp. on maize growth and *Meloidogyne arenaria* reproduction. *Plant Dis.* **73**: 493-495

*Woodhead, S.H. 1978. Mycorrhizal infection of soybean roots as it influences Phytophthora root rot. Ph.D. diss. University of Illinois, Urbana

Wright, J.M. 1956. The production of antibiotics in soil. III Production of Gliotoxin in wheat straw buried in soil. *Ann. Appl. Biol.* **44**: 461-466

Wyss, P., Boller, T. and Wiemken, A. 1992. Testing the effect of biological control agents on the formation of vesicular arbuscular mycorrhiza. *Plant Soil* **147**: 159-162

Zambolim, L. and Schenck, N.C. 1983. Reduction of the effect of pathogenic root infecting fungi on soybean by the mycorrhizal fungus, *Glomus mosseae*. *Phytopathology* 73: 1402-1405

* Originals not seen

MANAGEMENT OF RHIZOME ROT AND ROOT-KNOT OF GINGER (*Zingiber officinale* R.) USING V. A. MYCORRHIZAL FUNGI AND ANTAGONISTS

By

P. J. JOSEPH

ABSTRACT OF THESIS
SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE
DOCTOR OF PHILOSOPHY
FACULTY OF AGRICULTURE
KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI
THIRUVANANTHAPURAM

1997

ABSTRACT

Management of rhizome rot, the most destructive disease of ginger incited by *Pythium aphanidermatum* (Edson)Fitz. using native arbuscular mycorrhizal fungi (AMF) and antagonists was attempted. The extensive survey in the ginger growing belts of the state revealed a definite negative relationship between AMF development and disease incidence. Based on preliminary evaluation with six AMF species, *Glomus constrictum* was selected as most efficient for disease suppresion, growth enhancement and yield and used as reference culture for the subsequent studies. The population of *Meloidogyne incognita*, a pre disposing factor for the disease, was reduced in the presence of all the six AMF species particularly with *G. fasciculatum*. Characterization studies of native AMF associated with ginger indicated that *Glomus* spp. were more frequent and *G. fasciculatum* was the most predominent species. Influence of soil types on the AMF occurrence in ginger was evident.

Out of the fourteen native AMF developed from ginger rhizosphere, Mi-1 and Mi-4, identified as *Glomus* species, were the most efficient isolates for rhizome rot suppression yield increase and growth enhancement in ginger. Eleven native antagonistic fungi, selected from *in vitro* evaluation, were screened in the green house and Ai-12, identified as

Trichoderma viride, was the most effective isolate for suppression of rhizome rot, growth enhancement and yield of ginger. Ai-13 isolate, identified as *Aspergillus fumigatus*, was also effective for disease suppression. The isolates, Mi-1, Mi-4, Ai-12 and Ai-13 were tested for their combined effect in the green house. Dual inoculation of Ai-12 with Mi-1 and Mi-4 resulted in remarkable synergistic interaction in reducing the disease and enhancing biomass and yield. Both single and dual inoculations of Ai-12, Mi-1 and Mi-4 significantly reduced the disease and enhanced plant growth and yield in the field also. Isolate Ai-13 inhibited AMF development and increased disease in the green house and field.

Application of AMF inoculum on ginger rhizome after starch coating was very effective for rapid and early colonization of AMF. Out of the various plant protection chemicals tested, thiram and carbofuran least affected the AMF and antagonist development.

An effective management strategy of rhizome rot of ginger is evolved in the present study by combining appropriate native AMF (Mi-1 and Mi-2) and antagonistic fungi (Ai-12) for inoculation of seed rhizomes at planting time. The present study forms the first report of detailed documentation of native AMF associated with ginger and extensive study on the utilisation of AMF, antagonists and their combined effect for the management of rhizome rot.

APPENDICES

APPENDIX - I

Composition of the *Pythium* selective medium used

Peptone	-	5.0 g
Dextrose	-	10.0 g
H_2 PO_4	-	1.0 g
Mg SO_4. 7 H_2O	-	0.5 g
Agar Agar	-	20.0 g
Rose bengal	-	1:30000 (50 ppm)
Benlate	-	20 ppm
Mycostatin	-	1000 ppm (100000 units per litre)
Dicrysticin	-	500 ppm
PCNB	-	500 ppm
Water	-	to match 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