MANAGEMENT OF DAMPING OFF AND IMPROVEMENT OF GROWTH IN CHILLI (*Capsicum annuum* L.) WITH NATIVE SPECIES OF ARBUSCULAR MYCORRHIZAE AND *Azospirillum*

BY

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THESIS
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DECLARATION

I hereby declare that this thesis entitled "**Management of damping off and improvement of growth in chilli (*Capsicum annuum* L.) with native species of arbuscular mycorrhizae and *Azospirillum***" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that this thesis entitled "**Management of damping off and improvement of growth in chilli (*Capsicum annuum* L.) with native species of arbuscular mycorrhizae and *Azospirillum***" is a record of research work done independently by Ms. Kavitha. K. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Appa, Amma and Thambi

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LIST OF ABBREVIATIONS

%	Per cent
µ	Micro
^{0}C	Degree Celsius
AMF	Arbuscular Mycorrhizal Fungi
ANOVA	Analysis of variance
CD	Critical difference
cm	Centimeter
CRD	Completely Randomized Design
DAS	Days after sowing
dil.	Dilute
et al	And others
Fig.	Figure
FYM	Farmyard manure
g	Gram
h	Hours
ha	Hectare
IAA	Indole Acetic Acid
K	Potassium
kg	Kilogram
m	Metre
M	Molar
mg	Milligram
ml	Millilitre
N	Nitrogen
NFB	Nitrogen free bromothylmol blue medium
nm	Nanometer
P	Phosphorus
PDA	Potato Dextrose Agar
POP	Package of Practices
t	Tonnes

Introduction

1. INTRODUCTION

Chilli is one of the most important solanaceous vegetables cultivated throughout India. It is an indispensable adjunct to the diet of people of this country. Chilli imparts pungency for culinary purpose and is used for seasoning and adding red colour. In fresh stage, it is a rich source of vitamin C and A. India is the largest producer of chilli in the world contributing 25 per cent of the total world production. On an average it occupies an area of 9.65 lakh hectares with a production of 10.74 lakh tonnes of dry chilly (Sasikumar and Sarma, 2000).

One of the reasons for low production is ascribed to the losses of the crop caused by various diseases. Among the fungal diseases, damping off incited by *Pythium aphanidermatum* (Edson) Fitz. in nurseries is a major constraint in chilli production causing even up to 90 per cent mortality (Sowmini, 1961). *Pythium* spp. are essentially soil-borne and are very difficult to manage. At present fungicides are used to manage the disease. But frequent and indiscriminate use of fungicides often lead to environmental pollution and development of fungicide resistance in pathogens. In this context, the relevance of management of such diseases by resorting to alternative ecofriendly strategies such as biocontrol becomes more prominent. In a sustainable management system, the deterrent property and growth enhancement of Arbuscular Mycorrhizal symbiosis could be exploited in association with other microbial inoculants such as *Azospirillum*.

The study on arbuscular mycorrhizal fungi association in plants is receiving considerable attention in recent years as mycorrhizal association

enables better plant growth by higher uptake of nutrients (Harley and Smith, 1983; Chandrashekara and Patil, 1997; Rajagopal and Ramarethinam, 1997) and reduce infection by soil-borne plant pathogens (Azcon-Aguilar and Barea, 1996; Quarles, 1999). Encouraging results have been obtained on the potential of AMF in promoting resistance against important plant pathogenic fungi including *Pythium* spp. (Hwang *et al.*, 1993; Sivaprasad, 1993; Thomas *et al.*, 1994; Odebode *et al.*, 1997; Joseph and Sivaprasad, 2000). The mechanism of mycorrhizae-induced tolerance in plants is mainly due to physiological and biochemical changes in host (Gianinazzi-Pearson *et al.*, 1996) and microbial changes in the mycorrhizosphere (Azcon-Aguilar and Bago, 1994). The desirable attributes of AMF on plant growth and health make them potential agents for biocontrol and as an useful biofertilizer. However no attempt was made so far to exploit the potential of AMF for the management of damping off in chilli.

Azospirillum spp., which are associative nitrogen fixing bacteria capable of fixing about 20-25 kg N ha^{-1} which improves the growth of crop plants and thereby indirectly reduces the harmful effects of pathogens. They are known to increase root development and biomass production through the production of plant growth hormones.

Both AMF and *Azospirillum* are ecofriendly microbes and can be exploited to the maximum for vegetable production. However, not much work has been done on the management of damping off and growth improvement in chilli with native species of AMF and *Azospirillum*. Hence the present investigation was undertaken to develop efficient native AMF and *Azospirillum* cultures for sustainable management of damping off and growth

improvement in chilli. The study was undertaken with major thrust on the following aspects.

- Isolation of the pathogens associated with damping off of chilli.
- Screening of native arbuscular mycorrhizal fungi for suppression of damping off.
- Standardisation of method of AMF inoculation and inoculum requirement.
- Isolation of native *Azospirillum* spp.
- Screening of native isolates of *Azospirillum* spp.
- Effect of interaction of AMF and *Azospirillum* on management of damping off and growth improvement in chilli.

Review of Literature

2. REVIEW OF LITERATURE

Chilli (*Capsicum annuum* L.) is one of the most important spice crops in India. It is affected by several diseases caused by soil-borne pathogens and among them damping off is the most devastating one. The most common fungi inciting damping off of seedlings are species of *Pythium, Phytophthora, Fusarium, Rhizoctonia,* etc. Among these fungi, several species of *Pythium* have been implicated as the most common causal agent of the disease. *Pythium aphanidermatum* (Edson) Fitz., a soil inhabiting pathogen is distributed worldwide and is a pathogen of more than 80 species of higher plants (Middleton, 1943). Gattani and Kaul (1951) identified *Pythium aphanidermatum* as the causal organism of damping off of tomato seedlings. Sowmini (1961) found that three isolates of *Pythium* could infect 20-day-old *Capsicum annuum* seedlings. *Pythium aphanidermatum* isolated from infected seedlings caused 90 per cent damping off in chilli. *Pythium filamentosa* and *Pythium aphanidermatum* isolated from soil recorded 80 and 75 per cent damping off, respectively (Devadoss, 1971). *Pythium aphanidermatum* also caused damping off in sugarbeet, radish, tobacco, papaya and various species of cucurbits (Bhargava, 1941).

At present, seed treatment and soil drenching with fungicides are the recommended practices for the management of the disease. However these methods are not widely practiced due to economic loss, residue problems etc., whereas biological control is simple, inexpensive and eco-friendly method for disease management. In the present study, attempts were made to evolve a

management strategy for damping off of chilli employing native arbuscular mycorrhizal fungi along with native species of *Azospirillum* for growth enhancement.

2.1 Arbuscular mycorrhizae and plant growth

Arbuscular mycorrhizal (AM) 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 beneficial effects of AMF associations in the host nutrition especially in the uptake of 'P' is well documented (Mosse, 1973; Harley and Smith, 1983; Joseph, 1997; Abdul-Khaliq *et al.*, 2001). Mycorrhizal infection enhances plant growth by increasing nutrient uptake either by increasing the absorbing surface and mobilizing 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; Abdul-Khaliq *et al.*, 2001). Further AM association increases the uptake of water (Safir *et al.*, 1971, 1972), reduce stress responses of plants to toxicity and drought (Atkinson and Davidson, 1972; Guttay, 1976), improve soil texture and reduce soil erosion (Clough and Sutton, 1978) and enhances revegetation of degraded soils like mine soils (Pfleger *et al.*, 1994). The potential of AM association in

suppressing soil-borne pathogens is the focal point of investigation of the present study.

2.1.1 AMF as biocontrol agent against soil-borne pathogens

Past and current research indicates that mycorrhizal fungi can deter or significantly reduce the effects of soil-borne pathogens on the host plant. Diseases caused by soil-borne fungi are influenced by the colonization of VA-mycorrhiza in the root system. In general mycorrhizal plants suffer less damage and the incidence of disease is reduced or pathogen development is inhibited (Azcon-Aguilar and Barea, 1996).

The first reported evidence of the effect of arbuscular mycorrhizal fungi in inducing disease resistance in plants was by Safir (1968) in onion against pink rot disease caused by *Pyrenochaeta terrestris* which was later confirmed by Becker (1976) with *Glomus fasciculatum* and *Glomus margarita*. The role of AMF in reducing wheat rot (*Urocystis tritici*) was emphasized by Khan and Khan (1974). The study on the influence of AMF (*Glomus 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 association significantly reduced the incidence and intensity of wilt of cucumber caused by *F. oxysporum* f.sp. *cucumerinum* (Dehne, 1977), tomato wilt incited by *F. oxysporum* f.sp. *lycopersici* (Dehne and Schonbeck, 1979), root rot of citrus incited by *Phytophthora parasitica* (Davis and Menge, 1980), *Thielaviopsis basicola* in cotton (Schonbeck and Dehne, 1977), *Rhizoctonia solani* in

poinsettia, *Macrophomina phaseoli* in soybean and *Cylindrocladium scoparium* in yellow poplar (Stewart and Pfleger, 1977).

The suppressive effect of *Glomus fasciculatum* and *G. mosseae* on *F. oxysporum* f.sp. *lycopersici* of tomato and *Rhizoctonia 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 effect of *Phytophthora megasperma* cv. *sojae.* Reduction of infection levels of *Thielaviopsis basicola* in *G. fasciculatum* inoculated sweet orange seedlings (Davis, 1980) and reduction of wilt incidence of *F. oxysporum* f.sp. *ciceri* in *G. fasciculatum* and *G. mosseae* inoculated chickpea (Jalali and Thareja, 1981) were the salient earlier documents of biocontrol using AMF.

Graham and Menge (1982) reported that take all disease of wheat caused by *Gaeumannomyces graminis* was suppressed by *G. fasciculatum* inoculation. The disease was favoured by 'P' deficiency and enhanced 'P' uptake of mycorrhizal fungi 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. Jalali (1983) observed significant suppression of *R. solani* and *Fusarium* sp. by mycorrhizal inoculation with potential strains of *Glomus* spp. in legumes. Inoculation of *G. mosseae* reduced the damage and infection of pathogenic root infecting fungi in soybean (Zambolin and Schenck, 1983).

Evidence from glasshouse pot 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). Infection of pea by the root rot pathogen (*Aphanomyces euteiches*) was suppressed by *G. fasciculatum* when the plants were preinoculated with AMF and the pathogen was inoculated after 2-4 weeks. The resistance induced was partially systemic (Rosendahl, 1985). Vrot and Grente (1985) reported the biological control of pink disease of chestnut incited by *Phytophthora cinnamomi* by the mycorrhizal symbiosis.

Caron *et al.* (1986) observed that *Glomus intraradices* significantly reduced the incidence and development of crown and root rot of tomato incited by *F. oxysporum* f.sp. *lycopersici*. Preinoculation of *G. fasciculatum* and *G. tenue* was found to suppress the fusarial wilt of *Albizia procera* and *Dalbergia sisoo* and reduced the pathogen population in the rhizosphere (Chakravarthy and Mishra, 1986). Graham (1986) also emphasized the importance of preinoculation with AMF for effective disease suppression. VA-mycorrhiza are capable of counteracting the populations of soil-borne pathogens of stem rot (*Sclerotium oryzae*) and sheath blight (*Rhizoctonia solani*) by inducing disease resistance potential of rice plants under irrigated condition (Gangopadhyay and Das, 1987). Jalali (1987) reported the significant reduction of root rot of mungbean by AMF inoculation. Biocontrol of fusarium wilt of tomato and pepper caused by *F. oxysporum* f.sp. *lycopersici* and *F. oxysporum* f.sp. *vasinfectum* were reported by Al-Momany and Al-Raddad (1988) using seven isolates of *Glomus* spp. These isolates reduced the disease in tomato and pepper at different intensities. Iqbal and Nasim (1988) studied the biological deterrent activity of AMF to damping off pathogen, *R. solani* and observed that cauliflower seedlings preinoculated

with AMF survived better and resisted damping off attack significantly. Inoculation of AMF inhibited the infection of *Macrophomina phaseoli* causing stunting root rot of rough lemon seedlings (Singh and Singh, 1989).

Chulan *et al.* (1990) reported that preinoculation of cocoa seedlings with mycorrhizal fungi significantly reduced the pathogenic infection of *Ganoderma pseudoferreum* which caused red root disease. Simultaneous inoculation of VA mycorrhizal fungi (*G. fasciculatum*) with the pathogen (*Sclerotium rolfsii*) reduced the incidence of disease in wheat (Harlapur *et al.*, 1990). In a study on the interaction of *G. mosseae* and root rot pathogen *Macrophomina phaseolina* in mungbean, Jalali *et al.* (1990) found that mycorrhizal inoculation significantly restricted the spread of the pathogen in host root tissues. Cassiolata and Melo (1991) investigated that tomato plants colonized by *G. leptotrichum* and *Acaulospora morrowae* showed greater disease resistance to damping off caused by *R. solani* than non mycorrhizal ones. Inoculation of AMF enhanced the nutrient uptake and reduced the wilt disease severity of cumin incited by *F. oxysporum* f.sp. *cumini* (Champawat, 1991). Hwang *et al.* (1992) found that alfalfa seedlings inoculated with AMF had significantly lower incidence of wilt caused by *Verticillium albo-atrum* and *F. oxysporum* f.sp. *medicagnis*.

The suppressive effect of AMF on foot rot of black pepper has been extensively studied. (Anandaraj *et al.*, 1993, Anandaraj and Sarma, 1994 and Sivaprasad *et al.*, 1995). When black pepper vines were inoculated with AMF, it suppressed the root pathogen (*Phytophthora capsici*). The root rot and subsequent foliar yellowing were also reduced. The inter relationship between AMF and phytotoxic micromycetes which are causative agents of

apple replant disease was investigated by Catska (1994). Inoculation of *G. fasciculatum* and *G. macrocarpum* suppressed the microorganism. In cardamom, the biocontrol of damping off caused by *F. moniliforme* and *R. solani* using AMF was investigated by Thomas *et al.* (1994). AMF inoculation not only reduced the severity of the disease but also increased plant growth characteristics and P uptake of roots.

Azhukal disease of cardamom caused by *Phytophthora nicotianae* was remarkably reduced in green house and field conditions and improved the growth conditions 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 *Acaulospora morrowea* was better for stimulative plant growth (Sivaprasad, 1995). Cordier *et al.* (1996) noticed that colonization of tomato roots with mycorrhizal fungi protected the root cortex from *Phytophthora nicotianae* var. *parasitica.* Increased plant resistance to *Phytophthora* was observed in tomato plants inoculated with *G. mosseae* (Trotta *et al.*, 1996). Reduction of fusarium root rot of black pepper from 50 to 80 per cent was obtained by mycorrhizal inoculation (Chu *et al.*, 1997). Inoculation of *G. intraradices* provided reduction of root and stem rot of cowpea caused by *Phytophthora vignae* (Fernando and Linderman, 1997). Kulkarni *et al.* (1997) observed that *G. fasciculatum* was found to be most effective in nullifying the effect of *Sclerotium rolfsii* affecting groundnut. In integrated management of root rot disease of safflower, inoculation of *G. fasciculatum* suppressed *Macrophomina phaseolina* infection (Prashanthi *et al.*, 1997).

Bodker *et al.* (1998) reported that AMF inoculation (*G. intraradices*) not only increased the P uptake but also reduced the disease severity of root rot of pear caused by *Aphanomyces euteiches*. Quarles (1999) reviewed the role of VA mycorrhiza in prevention of plant disease and the mechanism of protection. Peanut plants inoculated with *G. mosseae* had a lower incidence of root rot, decayed pods and death than non mycorrhizal ones. (Abdalla and Abdel-Fattah, 2000). *G. mosseae* inoculation earlier than the pathogen (*R. solani*, and *F. solani*) significantly increased the survival percentage (80.9 and 84.9 per cent) of *Acacia nilotica* and *Dalbergia sisoo* seedlings (Kaushik *et al.*, 2000). Matsubara *et al.* (2000) reported that seedlings of asparagus inoculated with AMF induced resistance to violet root rot caused by *Helicobasidium mompa*. Sivaprasad *et al.* (2000) studied the effect of inoculation of VAM fungi in black pepper cuttings on foot rot disease incidence and growth. Inoculation of *G. monosporum* gave absolute protection to 50 per cent of the plants. The bioprotection of pea roots against *Aphanomyces euteiches* by AMF (*G. mosseae*) was reported by Slezack *et al.* (2000).

2.1.1.1 AMF as biocontrol agent of *Pythium* incited disease

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 plant. The suppressive effect of *G. fasciculatum* and *G. mosseae* on *Pythium 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 was remarkably greater in soils containing moderate population of the pathogen.

Rosendahl and Rosendahl (1990) investigated the interaction between *Pythium ultimum*, *G. etunicatum* and *Glomus* spp. in cucumber seedlings. VAM inoculation before or at the same time of inoculation of pathogen increased the survival of seedlings. Rosendahl *et al.* (1992) observed that the presence of AMF (*Glomus* spp.) reduced the damping off caused by *Pythium ultimum* in vermiculite grown cucumber and the protective effect was present even if the pathogen was introduced simultaneously with AMF. Calvet *et al.* (1993) reported that mycorrhizal fungus inoculation protected the marigold plants from *P. ultimum*. Hwang *et al.* (1993) discussed the influence of AMF on growth stimulation of sainfoin in *Pythium* infested soils and found that *G. fasciculatum* and *G. intraradices* reduced the severity of damage. The interaction of AMF with *P. aphanidermatum*, the casual agent of rhizome rot of ginger was investigated by Rohini Iyer and Sundararaju (1993). The AMF association in ginger was found to reduce the disease incidence and suggested that prior inoculation with AMF was effective in ameliorating the deleterious effect of the pathogen. Similarly, Sivaprasad (1993) also obtained biocontrol of rhizome rot of ginger with *G. fasciculatum* and *G. mosseae*.

In *Tagetes patula*, *G. intraradices* reduced the infection by *P. ultimum* and the pathogen population was ten times lower in mycorrhizal plants than in controls (St.-Arnand *et al.*, 1994). Inoculation of cardamom with *G. fasciculatum* remarkably reduced the severity of damping off caused by *Pythium vexans* in

addition to increased plant growth due to high 'P' uptake (Thomas *et al.*, 1994). Joseph *et al.* (1995) studied the influence of mycorrhizal colonization in relation to natural incidence of ginger rhizome rot caused by *P. aphanidermatum*. They noticed that high AM colonization reduced the intensity of rhizome rot under natural conditions. Healthy plants showed higher mycorrhizal colonization than diseased ones. Odebode *et al.* (1997) reported that pepper seedlings simultaneously inoculated with AM fungus (*G. deserticola*) and pathogen (*P. aphanidermatum*) or dually inoculated with mycorrhizal fungi before pathogen, suppressed the disease symptom. Ratti *et al.* (1998) observed that treatment of *G. aggregatum* 15 days prior to *P. aphanidermatum* inoculation and simultaneous inoculation of these two, reduced lethal yellowing by 80 per cent and 60 per cent respectively, when compared with *P. aphanidermatum* treatment alone.

Joseph and Sivaprasad (2000) found that two isolates of *Glomus* sp. isolated from rhizosphere of ginger reduced the rhizome rot incidence of ginger (*P. aphanidermatum*) and pathogen build up and enhanced the growth and yield significantly.

2.2 Standardization of method of AMF inoculation

Various methods are used for the inoculation of arbuscular mycorrhizal fungi in the nursery and in the main field.

Successful application of vesicular arbuscular mycorrhizal fungal inoculum by seed treatment could be obtained in onion and tomato by Gaunt (1978). Multiseeded pellets of *Glomus* and *Gigaspora* spp. were used effectively in white clover (Powell, 1979). Placing cowpea seed and inoculum

(*G. mosseae*) in the same planting hole increased root infection, shoot yield, phosphorus content and grain yield (Islam and Ayanaba, 1981). The next best method of inoculation for cowpea was broadcasting. In green gram and blackgram both placement and seed treatment were equally effective in increasing growth.

Hayman *et al.* (1981) reported that when inoculation of red clover with *G. fasciculatum* and *G. cladonicum* was done, maximum colonization was obtained by placing inoculum along with the seeds in furrows (placement) followed by multiseeded pellets. Successful seed inoculations of *Glomus* spp. in sorghum, blackgram and acid lime were obtained by Shanmugam and Ramaraj (1982). Ferguson and Menge (1986) found that broadcasting, banding and drilling were more effective in the field inoculation of citrus seedlings with VAM fungus (*G. deserticola*) than seed inoculation or application of lyophilized roots to both direct seeded or transplanted citrus seedlings. Ramaraj and Shanmugam (1986) found that placement was the best method for *G. etunicatum* and *G. mosseae* in cowpea while in green gram, highest effect was obtained by seed treatment with *G. etunicatum* and *G. fasciculatum*. In blackgram, the growth response due to different methods of inoculation varied with VAM fungus species. However placement of *G. etunicatum* recorded highest shoot weight, root weight and pod weight.

Sukhada (1987) found that application of VA mycorrhiza at the rate of 1kg in rows 10 cm apart in raised nursery recorded 40 per cent colonization. Application of VA mycorrhizal cultures in small furrows made in the raised nursery beds recorded higher bulb yield of 20.52 t ha^{-1} in bellary onion (Gurubatham *et al.*, 1989). Petgen *et al.* (1998) observed that placement of

inoculum of AMF (*G. mosseae*) as deep band at a soil depth of 9-18 or 36-45 cm increased the growth and nutrient uptake of grapevine root stocks.

2.3 Standardisation of AMF inoculum requirement

Not much work has been done on the quantity of AMF inoculum to be applied to achieve satisfactory colonization. Anwar *et al.* (1979) reported that addition of 15 g AMF inoculum to 400 g of soil without mycorrhizae significantly increased the fresh weight, dry weight and mycorrhizal development but it did not significantly affect the height increase or root length of seedlings. Sukhada (1987) demonstrated that application of 1 kg of soil containing hyphae, vesicles, chlamydospores and arbuscules of *G. fasciculatum* in the nursery bed of 1 x 1.5m size in rows substantially increased the growth, nitrogen content, phosphorus content and yield of tomato. This recorded 40 per cent mycorrhizal colonization. Subbiah (1994) inoculated nursery beds of chilli and bellary onion with VAM fungi at the rate of 1 kg m^{-2}. Rajagopal and Ramarethinam (1997) in an interaction study between VAM and tea roots, satisfactory colonization was obtained when *G. fasciculatum* was applied at the rate of 2 x 10^4 cells pot^{-1}.

Balikai *et al.* (1998) obtained a survival percentage of 40.7 in mulberry cuttings when VAM (*G. fasciculatum*) was applied at the rate of 5 t ha^{-1}. Shrihari and Sreenivasa (1998) found that the best dose of *G. macrocarpum* was 50 and 60 g/10 kg of black clay and red sandy loam respectively for chilli Cv. Byadagi. Fernandez *et al.* (1999) used 10 per cent of total seed weight as the dose of mycorrhizal inoculation. He could obtain 15-30 per cent increase in grain yield with this dose.

2.4 *Azospirillum*- an associative diazotroph

Azospirillum is an associative nitrogen fixing bacterium which grows in association with various graminae and other host plants. They occur freely in rhizosphere also, but fix nitrogen only under microaerophilic condition. It was Beijerinck (1925) who described a vibroid soil bacterium that seemed to fix atmospheric nitrogen. It was initially called *Azotobacter spirillum* and later renamed as *Spirillum lipoferum*. Tarrand *et al.* (1978) grouped the strains under *S. lipoferum* into two groups (I and II) and assigned them to a new genus *Azospirillum*.

Azospirillium lipoferum is a very common soil and root inhabitant in tropics, subtropics and temperate regions of the world (Dobereiner and Day, 1976). Rao *et al* (1978) noted the presence of *Azospirillum* in different soil types like alluvial, laterite, pokkali and kari. Charyulu and Rao (1980) found that *Azospirillum* occurs in acid soils with pH as low as 3.2 and in alkaline and saline soils with pH of 8 to 8.8 (Purushothaman and Oblisami, 1985). The occurrence of *Azospirillum* in the roots of number of tropical forage grasses (Balandreau, 1975; Dobereiner and Day, 1976; Neves *et al.*, 1976), rice (Lakshmikumari *et al.*, 1976; Rao *et al.*, 1978), wheat (Neyra and Dobereiner, 1977), plantation crops (Govindan and Purushothaman, 1985) and cotton and soybean (Bashan, 1991) has been reported.

2.4.1 Morphology and Physiology of *Azospirillum*

Azospirillum is a gram negative, aerobic bacterium which has a typical curved rod shape, motile with polar flagellum and contains poly beta hydroxy

butyrate (PBH) granules and DNA base composition of 69-71 mole % G + C (Tarrand *et al.*, 1978).

Sugars in general are poor carbon substrates for *A. lipoferum*. It grows moderately in galactose or acetate but poorly in glucose or citrate. Optimal growth and nitrogenase activities are observed when *Azospirillum lipoferum* was grown on malate, succinate, lactate and pyruvate (Day and Dobereiner, 1976; Dobereiner and Day, 1976; Okon *et al.*, 1976). Dobereiner and Day (1976) showed that *A. lipoferum* can utilize glucose and sucrose as sole carbon source. But glucose and sucrose are poor carbon sources for *A. brasilense*. Tarrand *et al.* (1978) observed that *A. lipoferum* can grow on glucose where as *A. brasilense* cannot. They also studied the physiological tests like catalase activity, acid production from glucose, utilization of different carbon sources, pH requirement and salinity (1 per cent NaCl). Kundu and Krishna (1990) reported that among different carbon sources studied for the growth of *Azospirillum*, malate has been found the best recording 0.090 absorbance at 560 nm.

2.4.2 Nitrogen fixation by *Azospirillum*

The ability of *Azospirillum* to fix nitrogen has been confirmed by many workers using micro kjeldhal assay and acetylene reduction assay and ^{15}N isotope studies (Lakshmikumari *et al.*, 1976; Okon *et al.*, 1976, Depolli *et al.*, 1979).

Azospirillum fixes nitrogen only under microaerophilic conditions (Lakshmikumari *et al.*, 1976). Day and Dobereiner (1976) reported that efficiencies of nitrogen fixation on malate or glucose are similar and 52 mg

nitrogen per g of malate or glucose was fixed by *Spirillum lipoferum*. Dobereiner and Day (1976) could obtain nitrogen fixation values as high as 115 mg N g^{-1} of lactate. The nitrogen fixation of the bacteria was found to be in the range of 20-24 mg N g^{-1} of carbon source (Okon *et al.*, 1976). Nelson and Knowles (1978) observed that nitrogen fixation ranged between 4.7 and 28 mg N g^{-1} of malate. Subba Rao *et al.* (1980) used two strains of *A. brasilense* from rice which did not possess denitrifying character but fixed maximum amount of nitrogen of 28-36 mg N g^{-1} of calcium malate with a saving of 40 kg N ha^{-1} as inorganic nitrogen application.

Govindan (1982) reported that *Azospirillum* isolates varied considerably in their ability to fix nitrogen which ranged from 1.40 to 20.36 mg N g^{-1} of malate. The nitrogen fixing capacity of *Azospirillum* isolates varied from 9-18 mg g^{-1} of malate (Rai and Gaur, 1982). Dobereiner (1983) noticed that the efficiency of nitrogen fixation increased with increasing age of the culture. The nitrogen fixation was 92 mg N g^{-1} of lactate and 49 mg N g^{-1} of glucose for *A. brasilense* and *A. lipoferum*, respectively, in early stationary phase. Sasikumar (1996) observed that nitrogen fixing capacity of *Azospirillum* isolates showed wide variations i.e., between 11.20 and 21.28 mg N g^{-1} of malate. *A. lipoferum* had a higher average nitrogenous activity than *A. brasilense* both in NFB medium and in association with wheat roots (Han and New, 1998).

2.4.3 IAA production by *Azospirillum*

Research reports confirm that many *Azospirillum* strains produce plant hormones in broth cultures (Jjepkema and Burris, 1976 and Tien *et al.*, 1979). The production of phytohormones by the microorganism seems to play an

essential role in the *Azospirillum* plant interaction. These include IAA, gibberellin and cytokinin like substances (Tien *et al.*, 1979). The phytohormones induced root hair multiplication, shortening and thickening of roots in monoxenic cultures (Umali-Garcia, 1978).

Conversion of tryptophan to auxin by *A. brasilense* was reported by Reynders and Vlassak (1979). Tien *et al.* (1979) observed that indole-3-acetic acid and indole lactic acid were produced by *A. brasilense* from tryptophan and that IAA production increased from 1-100 μg ml^{-1} with increase in tryptophan concentration. Govindan (1982) found that *Azospirillum* in shake culture conditions produced maximum amount of IAA (1358.6 μg 100 ml^{-1}) compared to static culture (231.125 μg 100 ml^{-1}). Hartman *et al.* (1983) identified indole-3-pyruvic acid, indole-3-acetaldehyde and indole-3-acetic acid as the substances that are excreted by *A. brasilense* Sp7 strain. The isolates produced IAA in broth culture upto 16 μg ml^{-1}. Jain and Patriquin (1985) suggested that when tryptophan was added to the medium, the level of IAA was increased to 50 μg ml^{-1}. Crozier *et al.* (1988) obtained a maximum IAA of 26.1 μg ml^{-1} culture broth after salkowski assay. *Azospirillum* isolates from roots of cactaceae species produced, higher amount of IAA (36.5 – 77 μg ml^{-1}) whereas from opuntia roots produced low amounts of IAA (6.5 – 17.5 μg ml^{-1}) (Mascarua *et al.*, 1988).

Fallik *et al.* (1989) reported that IAA produced by *Azospirillum* in tryptophan free culture medium was 32-40 μg ml^{-1}. He also observed that the *Azospirillum* inoculated maize seedlings were found to have higher amount of free and bound IAA compared to control. Sangwan (1990) suggested that the

biomass of pearl millet was improved by *A. brasilense* strain particularly by the release of higher amount of phytohormones. Growth regulating substance ranging from 11-60.5 μ moles was produced by *A. brasilense* which improved the growth of pearl millet (Sangwan and Kundu, 1992). Andreeva *et al.* (1993) found that in the roots of legumes inoculated with *Azospirillum*, the IAA content was three fold higher than in uninoculated roots. Omay *et al.* (1993) demonstrated that in the presence of ammonium in the liquid culture solution, IAA released from bacterial cells was considerably higher in the corresponding nitrogen free medium. Baca *et al.* (1994) quantified IAA production which increased from 1.6 to 50 μg ml^{-1} with the addition of tryptophan to the medium.

Yamini Varma (1995) observed that the *Azospirillum* isolates from pepper produced maximum quantity of IAA equivalent to 69 μg ml^{-1}. Its production was maximum during second week of culture growth. Sasikumar (1996) in his study found that the production of IAA by the *Azospirillum* isolates ranged between 26 μg ml^{-1} to 55 μg ml^{-1} which led to better root elongation of rice seedlings. In addition to fixing nitrogen, strains of *A. brasilense* released plant growth regulating substances during late log phase (Kundu *et al.*, 1997). Dobbelaere *et al.* (1999) reported that IAA production by *Azospirillum* alters the root morphology in wheat.

2.4.4 Effect of inoculation of *Azospirillum* on plant growth

Inoculation with *Azospirillum* increased root length particularly of root elongation zone. Increased cell division in the meristematic region of inoculated plants resulted in better root elongation. Moreover the inoculated

plants showed increase in number of lateral roots with dense root hairs, root volume and root dry weight (Dewan and Subba Rao, 1979). Karthikeyan (1981) found that inoculation of *Azospirillum* increased the plant height and dry matter accumulation in rice. Inoculation with *A. brasilense* increased the growth, dry matter production, root growth, number of lateral roots and root hairs in pearl millet (Venkateswarlu and Rao, 1983). Govindan and Chandy (1985) found that inoculation of the diazotroph *Azospirillum* could induce rooting in pepper cuttings.

Inoculation of wheat seedlings with *Azospirillum* increased the total shoot and root dry weight, plant height, number of fertile tillers and grain yield than control treatments (Kapulnik *et al.*, 1985; Bashan, 1986). After a field experiment, Gopalaswamy and Vidhyasekaran (1987) confirmed that split application of *Azospirillum* through seed, seedlings and soil increased the plant height. Hadas and Okon (1987) observed significant increase in root length, top and root dry weight and total leaf area of tomato plants due to *Azospirillum* inoculation compared to uninoculated controls. Parvatham *et al.* (1989) investigated that inoculation of *Azospirillum* increased the root growth, root volume, plant height, girth and number of leaves of bhindi. Also *Azospirillum* treatment showed increase in total number of flowers and fruits which may be due to the increased activity of hormones produced by *Azospirillum.* Inoculation of chickpea seedlings with *A. brasilense* produced greater root and shoot dry weight and root length than the uninoculated plants (Gallo and Fabbri, 1990). Savalgi and Veena (1990) reported that inoculation of *Azospirillum* in sunflower seedlings resulted in increased lateral root

formation, number of root hairs, height of seedlings, root mass and shoot dry weight.

Govindaswamy *et al.* (1992) observed an increase in total grain yield and straw yield of rice due to *Azospirillum* inoculation and inoculation of *Azospirillum* in cabbage gave significantly higher yield than the untreated control (Jeevajothi *et al.*, 1993). Paramaguru and Natarajan (1993) suggested that seed treatment and soil application of *Azospirillum* combined with application of 56 kg N ha^{-1} was found to increase the plant height, number of primary branches $plant^{-1}$ and number of lateral roots $plant^{-1}$ under semidry condition. Inoculation of *Azospirillum* spp. in maize plants, showed a significant increase in shoot and root dry weight over the control (Fulchieri and Frioni, 1994). Significant increase in plant height, stem girth, root length and root weight was recorded by inoculation of *Azospirillum brasilense* in coffee seedlings (Swarupa, 1996). Rousta *et al.* (1998) reported that inoculation of *Azospirillum* in maize and wheat increased the height, shoot and root drymatter. Most of the native strains had better effect on different growth indices of inoculated plants than foreign strains. Jha and Mishra (1999) could obtain highest tuber yield of sweet potato when 40 kg N ha^{-1} was supplemented with 10 kg *Azospirillum* ha^{-1} as soil application. Seed inoculation of *A. brasilense* in wheat plants increased the leaf area, chlorophyll concentration, nitrate reductase activity, total biomass production and grain yield compared to untreated control (Panwar and Ompal Singh, 2000).

2.5 Synergistic effect of AMF and *Azospirillum* on plant growth

In general mycorrhizal inoculation together with diazotrophs gave better results than diazotrophic treatment alone. Application of VA-mycorrhiza alone or together with *Azospirillum* has been found to benefit crop growth and yield (Subba Rao *et al.*, 1985; Subbiah, 1994; Rajput, 1999).

Menze (1985) found that in *Paspalum notatum* and *Panicum virgatum*, interaction between *Azospirillum* and VA-mycorrhiza were efficient only at pH 6.5 and yield was higher than that of uninoculated plants. Pacovsky *et al.* (1985) reported that in sorghum inoculated with both the endophytes (*Azospirillum* and VAM), the presence of *A. brasilense* in the rhizosphere increased VAM colonization and biomass while nitrogen input due to *Azospirillum* decreased possibly due to competition for carbohydrates. Subba Rao *et al.* (1985) observed that soil inoculation with *G. mosseae* and *G. fasciculatum* in the presence of *A. brasilense* produced significantly higher dry matter production and grain yield in barley. Inoculation of sweet potato cuttings with VAM fungi (*G. fasciculatum* and *G. mosseae*) and *Azospirillum* significantly increased the growth, plant nitrogen and phosphorus content, tuber weight and starch content (Kandasamy *et al.*, 1988). Padma (1988) found that combined inoculation of VA mycorrhizal fungi (*Gigaspora calospora, Glomus margarita, G. mosseae, G. fasciculatum* and *A. brasilense*) enhanced the dry weight, nitrogen and phosphorus content of papaya plants. Dual inoculation of maize with *A. brasilense* and *G. fasciculatum* produced significant increase in plant growth, yield and dry matter (Sreeramulu *et al.*, 1988).

Al-Nahidh and Gomah (1991) observed that the shoot and spike dry weight of wheat was greatest with *Azospirillum* and VAM inoculation. Dual inoculation of *G. mosseae* and *A. brasilense* increased the plant dry weight and N content in *Sorghum bicolor* in pots (Subhashini and Potty, 1992). Likewise, combined inoculation of *Gigaspora margarita* and *A. brasilense* in coffee seedlings significantly increased the shoot length, root length and total dry weight of plants compared with uninoculated controls (Kumari and Balasubramanian, 1993). Subbiah (1994) reported that application of biofertilizers (VAM + *Azospirillum*) in combination with 75 per cent of recommended dose of nitrogen registered higher yield of chilli pods. Dual inoculation of VAM fungus *G. aggregatum* and *A. brasilense* increased the growth, yield and oil content of palmarosa significantly over uninoculated control (Neelima and Janardhanan, 1996). Murumkar and Patil (1996) found that combined inoculation of VAM fungi and a mixed diazotroph gave the highest yield of 19.1 t ha^{-1} in bell pepper.

Kennedy and Chellapillai (1998) found that combined inoculation of VAM fungi and *Azospirillum* showed increased height, total dry weight, VAM colonization and total N and P uptake in shola tree species. Sonawane and Konde (1998) observed that *Azospirillum* in combination with mixed VAM culture resulted in higher mycorrhizal colonization. Chezhiyan *et al.* (1999) reported that application of VAM and *Azospirillum* along with 75 per cent NPK was effective in increasing bunch weight of hill banana var. Virupakshi. Rajput (1999) also obtained 13 per cent increase in yield due to combined inoculation of *Azospirillum* and VAM in bajra.

Materials and Methods

3. MATERIALS AND METHODS

3.1 Isolation and purification of the pathogen

Damping off affected chilli seedlings collected from areas of Thiruvananthapuram district with high incidence of the disease were cut into small bits and surface sterilized in 0.1 per cent $HgCl_2$ for one minute. It was then washed in three changes of sterile water and placed aseptically in sterile petri dish containing potato dextrose agar (PDA) (Appendix I) and incubated for 48 h at room temperature (25 ^{0}C ± 4). The fungal mycelium growing out from the diseased bit was brought into pure culture in PDA slants. The pathogenicity of the isolates was proved by inoculating the culture bits on healthy seedlings of chilli under aseptic condition. The most virulent isolate was selected, its cultural characteristics and symptom development were studied and the identification of the pathogen was done. The pathogenicity of the fungus was periodically tested on the host plant to ensure the virulence of the culture.

3.1.1 Symptomatology of damping off of chilli

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 healthy chilli seedlings grown in greenhouse with the most virulent isolate of the pathogen. The symptom development was monitored.

3.1.2 Mass multiplication of the pathogen

The pathogen was mass multiplied in sand oat meal (19:1) medium.

3.1.3 Sand oat meal medium

The medium was prepared by mixing washed fine white sand with oat meal in the ratio 19 : 1 (w/w). Sufficient quantity of water was sprinkled and mixed thoroughly to keep the medium moist. The 250 g mixture was sterilized in one litre conical flask at 121 ^{0}C for one hour. Actively growing culture bits of the pathogen were aseptically transferred into the flask and incubated at 25-30^{0}C for 15 days. The culture so multiplied was used for inoculation in various experiments under investigation.

3.2 Arbuscular mycorrhizal fungi (AMF) inoculum multiplication and inoculation

Sorghum (*Sorghum vulgare* Pers.) colonized with different isolates of AM fungus was grown in sterilized soil : sand mixture (2 : 1 v/v) for four months. The soil : sand mixture containing mycorrhizal spores, colonized root segments and hyphae served as mycorrhizal inoculum. Inoculation was done by incorporating 50 g each of inoculum per pot with soil and thoroughly mixing it to a depth of 5 cm.

3.2.1 Screening of native AMF cultures for disease suppression and growth improvement in chilli

A pot culture experiment was conducted to evaluate the effect of nine local cultures of AMF, *viz.*, M_1, M_2, M_3, M_4, M_5, M_6, M_7, M_8, M_9 and one identified culture, *Glomus mosseae* (M_{10}) available in the Department of Plant Pathology, College of Agriculture, Vellayani on suppression of damping off and growth improvement in chilli. The variety Jwalamukhi was used for the experiment.

Potting mixture in the ratio of 1 : 1 : 1 (Sand : Soil : FYM) was used for the study. NPK fertilizers were applied as per POP recommendations for chilli (KAU, 1996) The experiment was conducted in completely randomised design with two replications and following twenty two treatments.

$T_1 - M_0P_0$	$T_{12} - M_5P_1$
$T_2 - M_0P_1$	$T_{13} - M_6P_0$
$T_3 - M_1P_0$	$T_{14} - M_6P_1$
$T_4 - M_1P_1$	$T_{15} - M_7P_0$
$T_5 - M_2P_0$	$T_{16} - M_7P_1$
$T_6 - M_2P_1$	$T_{17} - M_8P_0$
$T_7 - M_3P_0$	$T_{18} - M_8P_1$
$T_8 - M_3P_1$	$T_{19} - M_9P_0$
$T_9 - M_4P_0$	$T_{20} - M_9P_1$
$T_{10} - M_4P_1$	$T_{21} - M_{10}P_0$
$T_{11} - M_5P_0$	$T_{22} - M_{10}P_1$

M_0 – Control (without AMF)

M_1, M_2, M_3, M_4, M_5, M_6, M_7, M_8 and M_9 – Native cultures of AMF

M_{10} – Identified culture of *Glomus mosseae*

P_0 – Without inoculation of *Pythium aphanidermatum*

P_1 – With inoculation of *P. aphanidermatum*

3.2.1.1 Inoculation with the pathogen and incidence of damping off

Fifteen-day-old culture of the pathogen mass multiplied in sand oat meal medium was inoculated 10 days after transplanting at the rate of 30 g per pot. The pots were irrigated regularly so as to maintain a very high

humidity for providing conducive environment for the development of the disease and per cent disease incidence was recorded.

3.2.1.2 Biometric characteristics and yield

Observations on important plant growth parameters such as plant height, number of secondary branches, stem girth, root length and yield were recorded at the time of harvest. The dry weight of plants were determined after oven drying at 60 ^{0}C.

3.3 Standardization of method of AMF inoculation

The experiment was conducted to select the best method of application of AMF inoculum in the nursery. The AMF inoculum consisted of root bits, mycelial fragments and mycorrhizal spores. The inoculum was applied at the rate of 2 kg m^{-2}. The experiment was conducted in completely randomised design with six treatments and four replications. The treatments were

T_1 - Mixing with surface layer soil

T_2- Incorporating with organic manure

T_3- Mixing with seed

T_4- Spot inoculation

T_5-Inoculation in nursery furrows

T_6- Placing in the rootzone

Variety:Jwalamukhi

Mycorrhizal colonization percentage was recorded 20, 30, 40 and 50 days after inoculation.

3.3.1. Estimation of percentage mycorrhizal colonization

The percentage mycorrhizal colonization in the root samples of chilli 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. The roots were then autoclaved for hydrolyzing with 10 per cent potassium hydroxide solution at 1.02 kg cm^{-2} for 15 minutes. The alkalinity of the samples were then neutralized with one per cent hydrochloric acid. Staining was done by steaming 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) and destaining was done by adding lactophenol. 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 arbuscular mycorrhizal fungi (AMF). The AMF colonization percentage was calculated as given below.

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

A minimum of 50 root bits from each treatment were scanned for estimating the AMF colonization percentage.

3.4 Standardization of AMF inoculum requirement

An experiment was conducted to select the optimum quantity of AMF inoculum requirement for chilli nursery. The AMF inoculum consisted of

mycelial fragments, mycorrhizal spores, root bits etc. Based on the previous experiment, inoculation of AMF in the nursery furrows was found to be best and this method was followed with six different doses and the percentage colonization was recorded. The experiment was laid in completely randomized design with six treatments and three replications. The treatments were

T_1 - 0 g m^{-2}

T_2 - 200 g m^{-2}

T_3 - 400 g m^{-2}

T_4- 850 g m^{-2}

T_5- 1250 g m^{-2}

T_6- 1600 g m^{-2}

Variety: Jwalamukhi

Mycorrhizal colonization percentage on 20, 30, 40 and 50 days after inoculation was recorded as per the procedure described in 3.3.1

3.5 Isolation and purification of *Azospirillum* from chilli roots

The isolation of *Azospirillum* was done by following the method of Bulow and Dobereiner (1975). Chilli roots from different locations of Thiruvananthapuram district were collected for the isolation of native species of *Azospirillum*. The roots were cleaned to remove all adhering soil particles and other debris in clean tap water and later washed thoroughly with sterile water. The samples were then cut into pieces of 0.5cm length, surface sterilized with 80 per cent ethyl alcohol for 30 seconds and then washed repeatedly with sterile water. The surface sterilized bits were aseptically

transferred to test tubes containing semisolid nitrogen free bromothymol (NFB) blue medium (Appendix I). After 48-72 hours of incubation at 37^0C, appearance of characteristic pale dense pellicle just below the surface of the medium indicated the presence of *Azospirillum*. The colour of medium changed from yellowish green to blue. From the positive tubes a loopfull of the culture was transferred to fresh semisolid malate medium and purified by three serial transfers. Final purification was done by streaking purified cultures on potato infusion agar (Appendix I). Single dry wrinkled colonies that appeared on the plates were transferred to semisolid NFB after incubation for three days. The formation of white subsurface pellicle confirmed the presence of *Azospirillum*. The pure cultures were streaked finally on Okon's medium (Appendix I) and stored at 4^0C for further use. The *Azospirillum* isolates (Az) were given code numbers from 1-44.

3.5.1 Morphological characterization of *Azospirillum* isolates

The isolates of *Azospirillum* were grown in semisolid malate medium in screw capped vials. Gram's staining was done according to Hucker's modification (Frobischer, 1964). The shape and size of bacteria was studied using 72 hours old culture.

3.5.2 Estimation of *in vitro* nitrogen fixation

One hundred ml of NFB medium supplemented with 50 mg yeast extract was taken in 200 ml conical flask and 1ml of inoculum was added aseptically. After seven days of incubation, the medium was concentrated to five ml by keeping in sand bath and it was digested with 10 ml concentrated H_2SO_4 and digestion mixture. Digestion was carried out until the contents

were clear. After cooling, the aliquot was transferred to volumetric flask and the volume was made upto 100 ml. Ten ml of the aliquot was taken in the Kjeltec digestion tube and total nitrogen was estimated (Humphries, 1956). The results are expressed as mg of nitrogen fixed per gram of malate.

3.5.3 *In vitro* production of Indole Acetic Acid (IAA)

Nitrogen-free malate broth was prepared without addition of bromothymol blue indicator and 100 ml of the broth was taken in 250 ml flasks. After sterilization, freshly prepared filter sterilized L-Tryptophan solution was added so as to get a final concentration of 100 $\mu g\ ml^{-1}$. Fresh *Azospirllium* cultures were used to inoculate the flask and incubated for seven days in dark. The extraction and estimation of IAA from culture filtrate was done following the method of Chandramohan and Mahadevan (1968). The cultures after seven days incubation, were centrifuged to remove the bacterial cells and the supernatant was acidified to pH 3 with 1N HCI. IAA was extracted with equal volumes of diethyl ether in a separating funnel at 4^0C. The extraction was repeated thrice using 100 ml diethyl ether for each extraction. The diethyl ether extracts were pooled and ether was flash evaporated at 35-40^0C and the residue was dissolved in two ml methanol. IAA in the methanol fraction was quantitatively measured using Salper's reagent (Salper's reagent was prepared by taking 1 ml 0.5 M ferric chloride in 50 ml 30% perchloric acid). To 1.5 ml distilled water in a test tube, 0.5 ml methanol residue was mixed and four ml of Salper's reagent was rapidly added. The samples were incubated in dark for one hour for maximum colour development. The colour intensity was read in spectrophotometer at 535nm. The quantity of IAA in the culture filtrate was calculated from the standard curve.

3.5.3.1 Preparation of standard curve

A standard curve was prepared by using an aqueous solution of IAA (E-Merck India Pvt. Ltd, Bombay) of different concentrations such as 10, 20, 30, 40 and 50 μg ml^{-1}. To five ml of each of this dilution, 1.6 ml of methanol and 13.4 ml of Salper's reagent were added to get a final volume of 20 ml. These tubes were incubated in dark for 1 h at room temperature. The colour intensity was measured at 535 nm in spectrophotometer. The standard curve was prepared by plotting the OD values on Y-axis and concentration of IAA (μg ml^{-1}) on X- axis. The quantity of IAA produced by different isolates of *Azospirillum* was determined with the help of this standard curve. The final value was expressed as microgram of IAA per millilitre of culture broth.

3.5.4 *In vitro* screening of *Azospirillum* isolates for growth and biomass production in chilli

All the 44 isolates of *Azospirillum* were screened for growth and biomass production. The experiment was conducted in medium sized uniform cups filled with potting mixture consisting of farmyard manure, sand and soil in the ratio 1:1:1 under green house condition. The variety Jwalamukhi was used for the study. Chilli seeds were soaked in respective isolates of *Azospirillum* broth for 30 minutes prior to sowing and sown in cups. The experiment was conducted in CRD with three replications. The seedlings were grown for 40 days with regular irrigation. The chilli seedlings were carefully removed from the cups and biometric observations such as plant height, root length, dry weight of shoot and root were recorded after oven drying at 60 ^{0}C.

3.5.5 *In vivo* screening of selected *Azospirillum* isolates for growth, biomass production and yield

Based on *in vitro* screening, the native *Azospirillum* isolates Az-1, Az-2, Az-3, Az-24, Az-41 and reference culture Az-40 were selected for the study. The experiment was conducted to identify the best *Azospirillum* isolate for growth improvement in chilli. The experiment was conducted in completely randomised design with seven treatments and three replications. Chilli seeds were soaked in respective selected isolates of *Azospirillum* for 30 minutes prior to sowing. At the time of transplanting the chilli seedlings were given root dipping in the respective *Azospirillum* isolates and transplanted to pots containing potting mixture of sand, soil and FYM in the ratio of 1 : 1 : 1. The plants were irrigated regularly. Observations on important plant growth parameters such as plant height, stem girth, root length and yield were recorded at the time of harvest. The dry weight of shoot and roots were recorded after oven drying.

3.5.6 Physiological characterization of selected isolates of *Azospirillum*

Based on *in vitro* and *in vivo* screening the native *Azospirillum* isolates Az-1 and Az-2 along with the reference culture, Az-40 were selected and characterized by studying their utilization of different carbon sources, biotin requirement, acidification of peptone based glucose medium, catalase activity and pH sensitivity.

3.5.6.1 Utilization of different carbon sources

The selected native isolates of *Azospirillum* viz., Az-1 and Az-2 were tested for the utilization of three different carbon sources namely glucose, fructose and sucrose along with the reference culture Az-40. The selected *Azospirillum* cultures were grown in test tubes containing the above carbon sources with 0.3 per cent ammonium chloride. The tubes were inoculated with 0.1 ml of 72 h old broth culture of the respective *Azospirillum* isolates and incubated at 28 ± 1^{0}C in an incubator. Three replications were maintained for each treatment. The extent of growth was measured after 144 h of incubation using UV- visible spectrophotometer at 600nm.

3.5.6.2 Biotin requirement

The selected isolates of *Azospirillum* were grown in NFB malate broth with or without 0.04% biotin to study the biotin requirement. The tubes were inoculated with 0.1ml of 72 h old broth culture of the respective *Azospirillum* and incubated at 36±1^{0}C in an incubator. Three replications were maintained for each treatment. The extent of growth was measured after 144 h of incubation using UV-visible spectrophotometer at 600 nm.

3.5.6.3 Acidification of peptone based glucose medium

The medium with the following composition described by Kreig and Dobereiner (1984) was used to detect the production of acid by *Azospirillum* isolates.

Peptone	: 2 g
$MgSO_4$. 7 H_2O	: 1 g
$(NH_4)_2$ SO_4	: 1 g

$FeCl_3$. 6 H_2O : 0.002 g

$MnSO_4$. H_2O : 0.002 g

BTB in dil. KOH : 0.025 g

This was prepared in 950 ml distilled water and the pH was adjusted to 7.0 and sterilized by autoclaving. After cooling, 50 ml of 20% w/v filter sterilized glucose was added aseptically. The tubes were inoculated with 72 h old cultures of *Azospirillum*. The development of yellow colour after 48-96 h incubation indicated acidification.

3.5.6.4 Catalase activity

A loopful of 72 h old *Azospirillum* from semisolid malate medium was taken on a clean glass slide. One ml of hydrogen peroxide was poured over it. A brisk effervescence indicated the presence of catalase activity in the culture.

3.5.6.5 Growth in different pH

The selected *Azospirillum* cultures were grown in test tubes using malate broth containing 0.3 per cent NH_4Cl and with pH initially adjusted to 4, 5, 6, 7 and 8, respectively. The tubes were inoculated with 0.1 ml of 72 h broth culture of respective *Azospirillum* initially grown at pH 7 and incubated at 28 ± 1 ^{0}C in an incubator. Three replications were maintained for each treatment. The extent of growth was measured after 144 h of incubation using UV-visible spectrophotometer at 600 nm.

3.6 Dual inoculation of native AMF and *Azospirillum* on disease suppression and growth improvement in chilli

An experiment was conducted to evaluate the effect of the selected AMF cultures (M_8 and M_9) and *Azospirillum* isolates (Az-1 and Az-2) on disease suppression and growth improvement in chilli. The variety Jwalamuhki was used for the study. NPK fertilizers were applied as per POP recommendation for chilli (KAU, 1996). The experiment was laid out in completely randomised design with eighteen treatments and three replications. The following were the different treatment combinations.

$T_1 - M_0A_0P_0$	$T_{10} - M_8A_1P_1$
$T_2 - M_0A_0P_1$	$T_{11} - M_8A_2P_0$
$T_3 - M_0A_1P_0$	$T_{12} - M_8A_2P_1$
$T_4 - M_0A_1P_1$	$T_{13} - M_9A_0P_0$
$T_5 - M_0A_2P_0$	$T_{14} - M_9A_0P_1$
$T_6 - M_0A_2P_1$	$T_{15} - M_9A_1P_0$
$T_7 - M_8A_0P_0$	$T_{16} - M_9A_1P_1$
$T_8 - M_8A_0P_1$	$T_{17} - M_9A_2P_0$
$T_9 - M_8A_1P_0$	$T_{18} - M_9A_2P_1$

M_8 and M_9 - Selected native cultures of AMF

A_1 and A_2 - Selected native isolates of *Azospirillum* (Az-1 and Az-2)

P_0 - Without inoculation of *Pythium aphanidermatum*

P_1- With inoculation of *P. aphanidermatum*

3.6.1 Inoculation with the pathogen and incidence of damping off

Fifteen-day-old culture of the pathogen mass multiplied in sand oat meal medium was inoculated 10 days after transplanting at the rate of 30 g per pot. The pots were irrigated regularly so as to maintain high relative humidity for providing conducive environment for the development of the disease and per cent disease incidence was recorded.

3.6.2 Growth characters and yield

Observations on important plant growth parameters such as plant height, number of secondary branches, stem girth, root length and yield were recorded at the time of harvest. The dry weight of plants were determined after oven drying.

3.7 Statistical analysis

The data generated from the experiments were subjected to analysis of variance (ANOVA) after appropriate transformations wherever needed.

Results

4. RESULTS

4.1 Isolation and purification of the pathogen

Different isolates of the causal organism inciting damping off of chilli were isolated from highly infested areas of Thiruvananthapuram district in potato dextrose agar (PDA). The fungus was brought into pure culture (Plate 1) and pathogenicity was proved. The most virulent isolate causing damping off of chilli was selected and identified as *Pythium aphanidermatum* (Edson) Fitz. based on morphological and cultural characteristics. The hyphal width ranged between 3.3 to 6.6 µm in diameter. The sporangia consisted of terminal complexes of swollen hyphal branches varying from 50 to 200 µm in length and 4 to 10 µm in width. The oogonia are terminal, globose and smooth and antheridia are terminal or intercalary and are club shaped. The oospores are single, smooth and 17-19 µm in diameter.

4.1.1 Symptomatology of damping off of chilli

Detailed symptomatology was studied in plants artificially inoculated with *Pythium aphanidermatum*. In post emergence damping off, the infected seedlings turned pale green and brownish. Water soaked lesions were seen (Plate 2) at the basal portion of the stem. The infected tissues turned soft and water soaked. The lesions girdled the stem and finally the seedlings became constricted at the base and the plants collapsed (Plate 3).

Plate 1 Pure culture of *Pythium aphanidermatum* (Edson) Fitz.

Plate 2 Natural infection of damping off of chilli seedlings showing water soaked lesions

Plate 3 Damping off infected chilli seedlings compared with healthy seedlings

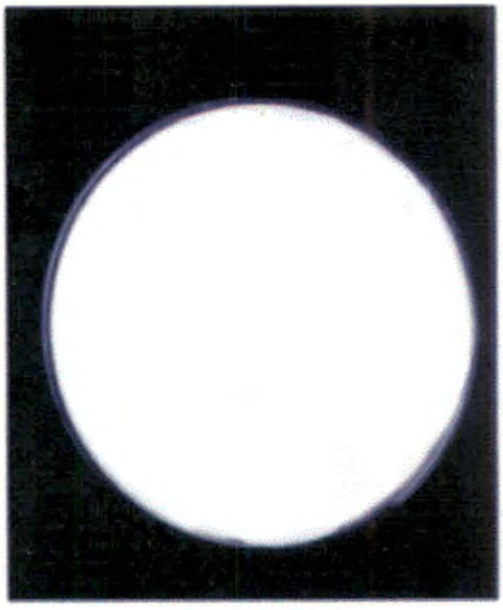


PLATE 1

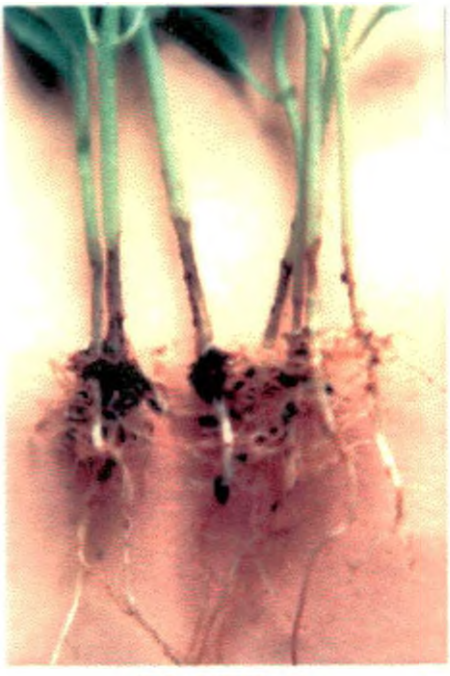


PLATE 2

PLATE 3

4.2 Arbuscular mycorrhizal fungi (AMF) inoculum multiplication and inoculation

Sorghum roots colonized by AMF grown in soil : sand mixture was used as the AMF inoculum throughout the study. The inoculum was applied at the rate of 50 g per pot.

4.2.1 Screening of native AMF cultures for disease suppression and growth improvement in chilli

Chilli plants preinoculated in the nursery with nine native AMF cultures along with one identified culture of *Glomus mosseae* were used for the study. The data on the per cent disease incidence and biometric characteristics are presented in Table 1 and 2.

4.2.1.1 Inoculation with the pathogen and incidence of damping off

The pathogen *P. aphanidermatum* mass multiplied in sand oats medium was applied at the rate of 30 g per pot and the per cent disease incidence was recorded. Chilli seedlings pre-inoculated with M_9 culture recorded no disease incidence (0.00) and was significantly superior in suppressing the pathogen (Plate 4) over other AMF cultures and control (Table 1). The next best culture was M_8 which recorded a disease incidence of 15.80 per cent as against 61.42 per cent noticed in uninoculated control (Plate 5). Seedlings pre-inoculated with *Glomus mosseae* (M_{10}) recorded a disease incidence of 30.58 per cent and was statistically on par with the other native AMF cultures such as M_6, M_4, M_5, M_7, M_3 and M_2 which recorded 30.73, 33.89, 35.40, 35.47, 38.29 and 41.38 per cent, respectively (Table 1).

Table 1. Effect of AMF inoculation on suppression of *Pythium aphanidermatum* infection in chilli

Treatments	Per cent disease incidence	Per cent reduction over control
M_0P_1	77.15 (61.42)	-
M_1P_1	47.78 (43.54)	38.46
M_2P_1	48.73 (41.38)	43.32
M_3P_1	38.42 (38.29)	50.20
M_4P_1	31.13 (33.89)	59.69
M_5P_1	33.59 (35.40)	56.46
M_6P_1	26.14 (30.73)	66.12
M_7P_1	33.69 (35.47)	56.33
M_8P_1	7.42 (**15.80**)	**90.38**
M_9P_1	0.00 (**0.00**)	**100.00**
$M_{10}P_1$	25.89 (30.58)	66.44
CD	10.611	-

Figures in parentheses are values after angular transformation

The disease reduction over control was 100 per cent in chilli seedlings pre-inoculated with culture M_9 followed by 90.38 per cent in seedlings pre-inoculated with culture M_8 (Table 1). These two native AMF cultures were selected for further studies.

4.2.1.2 Biometric characteristics and yield

The data on the effect of inoculation of different native AMF cultures and *Glomus mosseae* on the growth characteristics of chilli are presented in Table 2. Significant increase in height was observed in plants pre-inoculated with AMF. The treatment M_4P_0 recorded the maximum plant height of 68.33 cm. However this was statistically on par with treatments M_2P_0 (61.33 cm), M_3P_0 (60.83 cm), M_9P_0 (56.83 cm), M_5P_0 (55.50 cm) and M_6P_0 (55.17 cm) (Table 2). These treatments were significantly superior to the control (M_0P_1) which recorded 39.00 cm (Table 2).

The number of secondary branches was significantly higher in chilli plants pre-inoculated with AMF. It was maximum in the treatment $M_{10}P_0$ (36.67). This was statistically on par with other treatments such as M_9P_0 (35.83), $M_{10}P_1$ (33.75), M_7P_0 (32.50) and M_3P_1 (29.83). All these treatments were found to be significantly superior to the control which recorded 22.83 number of secondary branches (Table 2).

Significant increase in the root length was observed in chilli plants with AMF inoculation. The treatment M_2P_1 recorded the maximum root length of 24.25 cm (Table 2) and this was statistically on par with other treatments such as M_2P_0 (24.00 cm), M_6P_1 (23.96 cm), M_8P_1 (23.33 cm), M_3P_1 (23.17 cm), M_8P_0 (23.13 cm), M_9P_0 (23.11 cm), M_4P_1 (22.75 cm), M_9P_1 (22.58 cm),

Table 2. Effect of AMF inoculation on growth characteristics and yield in chilli

Treatments	Height (cm)	No. of secondary branches (no plant^{-1})	Stem girth (cm)	Root length (cm)	Dry weight (g plant^{-1})	Yield (g plant^{-1})
M_0P_0	37.67	17.67	2.96	18.33	8.75	32.50
M_0P_1	39.00	22.83	3.30	17.25	11.35	16.67
M_1P_0	49.67	16.62	3.10	19.10	15.00	34.79
M_1P_1	44.50	20.50	3.25	16.25	12.50	60.00
M_2P_0	61.33	26.50	3.88	24.00	15.00	48.33
M_2P_1	46.00	21.25	3.30	**24.25**	15.00	65.25
M_3P_0	60.83	24.83	3.50	23.33	19.17	65.83
M_3P_1	45.67	29.83	3.65	23.17	17.50	**109.17**
M_4P_0	**68.33**	22.67	3.11	19.57	19.16	43.33
M_4P_1	49.75	27.00	3.75	22.75	14.50	74.42
M_5P_0	55.50	21.13	2.80	19.25	12.50	62.50
M_5P_1	42.00	17.17	3.10	18.96	11.67	59.58
M_6P_0	55.17	26.21	3.00	21.75	15.00	40.42
M_6P_1	40.50	20.33	3.33	23.96	13.33	29.66
M_7P_0	47.50	32.50	3.42	20.33	15.00	15.00
M_7P_1	33.83	24.50	2.98	14.67	13.33	30.00
M_8P_0	45.46	23.46	2.65	23.13	22.45	50.42
M_8P_1	39.33	28.67	2.91	23.33	22.75	25.83
M_9P_0	56.83	35.83	3.75	23.11	20.99	50.83
M_9P_1	37.99	29.16	3.08	22.58	**27.50**	52.00
$M_{10}P_0$	50.49	**36.67**	3.47	20.15	14.67	64.17
$M_{10}P_1$	38.50	33.75	2.65	18.33	15.00	28.75
CD	13.490	7.471	NS	5.175	9.127	38.130

Plate 4 Effect of native AMF (M_9P_1) on suppression of damping off in chilli

1 – M_9P_1

2 – Control

Plate 5 Effect of native AMF (M_8P_1) on suppression of damping off in chilli

1 – M_8P_1

2 – Control

Plate 6 Nursery furrow method of application of AMF inoculum in chilli

PLATE 4

PLATE 5

PLATE 6

M_6P_0 (21.75 cm), M_7P_0 (20.33 cm), $M_{10}P_0$ (20.15 cm) and M_1P_0 (19.10 cm). These treatments were significantly superior to the control which recorded root length of 17.25 cm.

The dry weight of chilli plants was significantly higher in plants inoculated with AMF. Maximum dry weight of 27.50 g plant^{-1} was recorded in the treatment M_9P_1 (Table 2) which was statistically on par with other treatments such as M_8P_1, M_8P_0, M_9P_0, M_3P_0 and M_4P_0 which recorded 22.75, 22.45, 20.99, 19.17 and 19.16 g plant^{-1} respectively. The control plants recorded a dry weight of 11.35 g plant^{-1}.

Inoculation of AMF cultures resulted in significant increase in the yield of chilli plants. M_3P_1 recorded the highest yield of 109.17 g plant^{-1} as against control which recorded an yield of only 16.67 g plant^{-1} (Table 2). The other treatments M_4P_1, M_3P_0, M_2P_1 and $M_{10}P_0$ recorded 74.42, 65.83, 65.25 and 64.17 g plant^{-1} respectively (Table 2).

The observations on stem girth was not significant. However the maximum stem girth was recorded in M_2P_0 (3.88 cm) whereas in control, the stem girth was 3.30 cm (Table 2).

4.3 Standardisation of method of AMF inoculation

Different methods of AMF inoculation in the nursery were tried and the data on AMF colonization at different intervals, *viz.*, 20, 30, 40 and 50 days after sowing are presented in Table 3. The inoculum was applied at the rate of 2 kg m^{-2}. The application of AMF inoculum in the nursery furrows (Plate 6) gave maximum per cent colonization uniformly in all the intervals of observation.

Table 3. Effect of different methods of AMF inoculation on per cent mycorrhizal colonization in chilli

Treatments	Colonization (%)			
	20 DAS	30 DAS	40 DAS	50 DAS
T_1 (mixing with surface layer soil)	63.54 (52.83)	73.60 (59.06)	58.35 (49.81)	63.30 (52.69)
T_2 (incorporating with organic manure)	71.14 (57.49)	70.36 (56.99)	55.62 (48.22)	67.67 (55.33)
T_3 (mixing with seed)	78.95 (62.67)	54.29 (47.44)	68.21 (55.67)	68.47 (55.82)
T_4 (spot inoculation)	85.36 (67.47)	76.14 (60.74)	70.28 (56.95)	61.22 (51.47)
T_5 (inoculation in nursery furrow)	92.54 (**74.12**)	96.48 (**79.15**)	92.54 (**74.05**)	87.07 (**68.89**)
T_6 (placing in root zone)	70.43 (57.03)	66.24 (54.45)	70.19 (56.90)	54.02 (47.29)
CD	9.700	12.750	15.449	8.708

DAS – days after sowing
Figures in parentheses are values after angular transformation

On 20th day of observation, maximum AMF colonization of 74.12 per cent was recorded in the treatment consisting of application of inoculum in the nursery furrows (T_5). However, this was statistically on par with spot inoculation, which recorded 67.47 per cent colonization. The other treatments such as mixing with seed, incorporating with organic manure, placing in the root zone and mixing with surface layer soil recorded 62.67, 57.49, 57.03 and 52.83 per cent colonization, respectively (Table 3).

After 30 days of AMF inoculation, the per cent colonization was significantly superior in the treatment consisting of application of inoculum in nursery furrows (79.15) as against the other treatments (Table 3), which recorded a per cent colonization of 60.74 (spot inoculation), 59.06 (mixing with surface layer soil), 56.99 (incorporating with organic manure), 54.45 (placing in root zone) and 47.44 (mixing with seed).

At 40th day of observation, AMF inoculation in the nursery furrows (T_5) recorded the maximum per cent colonization of 74.05 which was significantly superior to all other treatments (Table 3). The other treatments such as spot inoculation, placing in root zone, mixing with seed, mixing with surface layer soil, incorporating with organic manure recorded 56.95, 56.90, 55.67, 49.81 and 48.22 per cent colonization, respectively.

The observation on 50th day also showed that application of AMF inoculum in the nursery furrows was significantly superior (68.89) to all the other methods of application such as mixing with seed, mixing with organic manure, mixing with surface layer soil, spot inoculation and placing in the root zone which recorded 55.82, 55.33, 52.69, 51.47 and 47.29 per cent colonization, respectively (Table 3).

Table 4. Effect of different doses of AMF inoculum on per cent mycorrhizal colonization in chilli

Treatments	Colonization (%)			
	20 DAS	30 DAS	40 DAS	50 DAS
T_1 (0 g m^{-2})	12.12 (20.37)	26.14 (30.60)	15.75 (23.34)	39.04 (38.65)
T_2 (200 g m^{-2})	21.34 (27.49)	34.55 (35.97)	56.73 (48.90)	61.97 (52.27)
T_3 (400 g m^{-2})	47.09 (43.32)	54.42 (42.59)	60.10 (50.82)	72.62 (58.54)
T_4 (850 g m^{-2})	62.28 (52.09)	80.55 (65.17)	77.96 (62.10)	86.45 (68.73)
T_5 (1250 g m^{-2})	62.69 (52.33)	87.31 (69.31)	83.00 (**65.74**)	86.42 (69.65)
T_6 (1600 g m^{-2})	72.99 (**58.67**)	89.68 (**71.40**)	80.75 (64.19)	91.67 (**73.62**)
CD	7.380	12.16	6.10	13.61

DAS – days after sowing
Figures in parentheses are values after angular transformation

4.4 Standardization of AMF inoculum requirement

Application of inoculum in nursery furrows which was found to be the best method was selected for this study. The data on the effect of different doses of AMF inoculum application in the nursery on the percentage mycorrhizal colonization at 20th, 30th, 40th and 50th day after sowing are presented in Table 4. Maximum per cent colonization was recorded in the treatment T_6 (1600 g m^{-2}) and this was statistically on par with T_5 (1250 g m^{-2}) and T_4 (850 g m^2).

On 20th day, the treatments T_6 (1600 g m^{-2}), T_5 (1250 g m^{-2}) and T_4 (850 g m^{-2}) recorded 58.67, 52.33 and 52.09 per cent colonization and were statistically on par and superior over the uninoculated control which recorded 20.37 per cent colonization (Table 4). The other treatments T_3 (400 g m^{-2}) and T_2 (200 g m^{-2}) recorded 43.32 and 27.49 per cent colonization, respectively.

After 30 days, the percentage colonization was maximum in the treatment T_6 which recorded 71.40. However this was statistically on par with T_5 and T_4 which recorded 69.31 and 65.17 per cent colonization, respectively. The other treatments T_3 and T_2 recorded 42.59 and 35.97 per cent colonization, respectively (Table 4).

On 40th day, maximum per cent colonization was recorded in the treatment T_5 followed by T_6 and T_4 with 65.74, 64.19 and 62.10 respectively (Table 4) and were statistically on par. At 50th day, maximum per cent colonization was recorded in the treatment T_6 with 73.62 (Table 4). However this was statistically on par with T_5, T_4, T_3 and T_2 which recorded 69.65,

68.73, 58.54 and 52.27 per cent respectively where as the control (T_1) showed 38.65 per cent colonization. Application of AMF inoculum at the rate of 850 g m^{-2} which recorded satisfactory colonization was selected as the economic dose.

4.5 Isolation and purification of *Azospirillum* from chilli roots

Azospirillum was isolated from chilli roots collected from chilli growing areas of Thiruvananthapuram district following the procedure described in 3.5. After 48-72 h of incubation at 37^0C, appearance of characteristic pale dense pellicle just below the surface of the medium indicated the presence of *Azospirillum*. The colour of medium changed from yellowish green to blue. From the positive tubes, a loopfull of the culture was transferred to fresh semisolid malate medium and it was further purified by three serial transfers. Final purification was done by streaking purified cultures on potato infusion agar. Single dry wrinkled colonies that appeared on the plates were transferred to semisolid NFB after 3 days incubation. The formation of white subsurface pellicle confirmed the presence of *Azospirillum*. In all, 44 isolates of *Azospirillum* were obtained.

4.5.1 Morphological characterization of *Azospirillum* isolates

The morphological characters of the isolates such as size, shape and presence of poly beta hydroxy granules were studied in 72 h old culture grown in semi solid malate medium. The isolates were small to medium sized rods. Eventhough polymorphism was noted, majority of the isolates were curved rods. The poly beta hydroxy granules were present in all the isolates.

Table 5. ***In vitro*** **nitrogen fixation and IAA production by native** ***Azospirillum*** **isolates**

Azospirillum isolates	Nitrogen fixation mg N fixed g^{-1} of malate	IAA production (µg ml^{-1})
Az-1	19.53	49.66
Az-2	**20.00**	**55.00**
Az-3	15.86	48.33
Az-4	12.27	36.33
Az-5	12.00	23.33
Az-6	14.43	21.00
Az-7	11.73	26.00
Az-8	13.53	26.00
Az-9	16.80	23.33
Az-10	11.20	22.00
Az-11	14.00	23.33
Az-12	12.30	23.00
Az-13	14.43	25.00
Az-14	11.93	21.00
Az-15	13.20	24.33
Az-16	12.83	24.00
Az-17	12.13	33.33
Az-18	16.46	23.67
Az-19	13.07	31.00
Az-20	12.80	29.33
Az-21	14.00	27.33
Az-22	16.80	30.33
Az-23	16.60	26.67
Az-24	19.20	48.00

Table 5. Contd...

Azospirillum isolates	Nitrogen fixation mg N fixed g^{-1} of malate	IAA production (µg ml^{-1})
Az-25	17.20	26.67
Az-26	16.40	23.33
Az-27	12.13	27.33
Az-28	16.73	31.33
Az-29	14.37	31.67
Az-30	17.33	27.33
Az-31	15.47	30.00
Az-32	12.66	24.33
Az-33	14.93	24.00
Az-34	14.00	29.67
Az-35	16.20	39.33
Az-36	14.00	23.33
Az-37	15.86	25.67
Az-38	14.93	27.67
Az-39	14.93	30.67
Az-40	19.53	50.00
Az-41	18.40	48.66
Az-42	16.20	24.33
Az-43	16.80	26.67
Az-44	17.53	25.00
CD	2.354	5.786

All the isolates took the colour of the counterstain safranin in gram staining and appeared pink indicating that they are gram negative.

4.5.2 Estimation of *in vitro* nitrogen fixation

The *in vitro* nitrogen fixation by the isolates of *Azospirillum* ranged from 11.20-20.00 mg N g^{-1} malate (Table 5). The maximum nitrogen fixation of 20.00 mg N g^{-1} malate was recorded in Az-2. However this was statistically on par with Az-1, Az-40, Az-24 and Az-41 which recorded 19.53, 19.53. 19.20 and 18.40 mg N g^{-1} malate respectively.

4.5.3 *In vitro* production of Indole-Acetic Acid (IAA)

Indole acetic acid production by the different isolates of *Azospirillum* showed wide variations ranging from 21.00 to 55.00 µg ml^{-1} (Table 5). The isolate Az-2 recorded the maximum IAA production of 55.00 µg ml^{-1}. This was statistically on par with the IAA produced by the reference culture (Az-40) and Az-1 which recorded 50.00 µg ml^{-1} and 49.66 µg ml^{-1} respectively. The isolates Az-6 and Az-14, recorded the minimum IAA production of 21.00 µg ml^{-1} each.

4.5.4 *In vitro* screening of *Azospirillum* isolates for growth and biomass production in chilli

The influence of *Azospirillum* isolates on growth and biomass production of chilli seedlings was studied and the data are presented in the Table 6.

Out of the 44 isolates screened, 10 isolates of *Azospirillum* produced significant increase in height of chilli seedlings over uninoculated control

Table 6. Effect of inoculation of *Azospirillum* on plant growth characteristics in chilli under *in vitro* conditions

Azospirillum isolates	Height (cm)	Root length (cm)	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})
Az-1	10.50	8.60	0.116	0.045
Az-2	**11.70**	**9.33**	**0.169**	**0.080**
Az-3	10.73	8.10	0.108	0.039
Az-4	7.33	5.93	0.067	0.028
Az-5	7.43	4.93	0.059	0.029
Az-6	8.03	6.67	0.079	0.026
Az-7	7.50	4.67	0.079	0.019
Az-8	6.60	4.33	0.049	0.020
Az-9	7.60	6.07	0.070	0.038
Az-10	6.80	7.07	0.058	0.033
Az-11	9.07	6.73	0.074	0.025
Az-12	8.00	7.17	0.066	0.029
Az-13	7.50	6.67	0.054	0.035
Az-14	6.93	6.50	0.077	0.031
Az-15	8.83	7.17	0.085	0.027
Az-16	7.83	4.83	0.067	0.029
Az-17	7.00	5.17	0.064	0.022
Az-18	8.83	7.50	0.066	0.022
Az-19	7.67	6.73	0.049	0.024
Az-20	6.57	6.07	0.657	0.039
Az-21	6.93	5.07	0.072	0.026
Az-22	8.50	6.17	0.067	0.032
Az-23	7.83	7.73	0.071	0.026
Az-24	10.33	8.20	0.108	0.026

Table 6. Contd...

Azospirillum isolates	Height (cm)	Root length (cm)	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})
Az-25	9.00	6.83	0.090	0.025
Az-26	8.17	7.43	0.082	0.031
Az-27	8.70	7.23	0.084	0.030
Az-28	8.33	6.77	0.086	0.036
Az-29	9.50	6.33	0.076	0.021
Az-30	9.17	6.27	0.068	0.026
Az-31	7.67	5.73	0065	0.029
Az-32	9.00	5.90	0.091	0.023
Az-33	8.66	7.10	0.070	0.032
Az-34	8.30	7.50	0.095	0.030
Az-35	9.17	8.17	0.091	0.035
Az-36	8.00	5.77	0.093	0.030
Az-37	9.23	7.17	0.085	0.024
Az-38	8.17	5.90	0.055	0.023
Az-39	8.67	6.73	0.069	0.025
Az-40	11.00	8.50	0.166	0.057
Az-41	10.23	7.83	0.087	0.052
Az-42	7.93	6.50	0.088	0.025
Az-43	8.83	6.73	0.072	0.053
Az-44	8.50	7.73	0.084	0.022
Control	7.67	6.46	0.082	0.030
CD	1.455	1.660	0.0305	0.0225

Plate 7 Influence of Az-2 on growth of chilli seedlings under *in vitro* conditions

1 – Seed treatment with Az-2

2 – Control

Plate 8 Influence of Az-1 on growth of chilli seedlings under *in vitro* conditions.

1 – Seed treatment with Az-1

2 – Control

Plate 9 Influence of Az-2 on root elongation of chilli seedlings under *in vitro* conditions.

1 – Seed treatment with Az-2

2 – Control

Plate 10 Influence of Az-1 on root elongation of chilli seedlings under *in vitro* conditions

1 – Seed treatment with Az-1

2 – Control

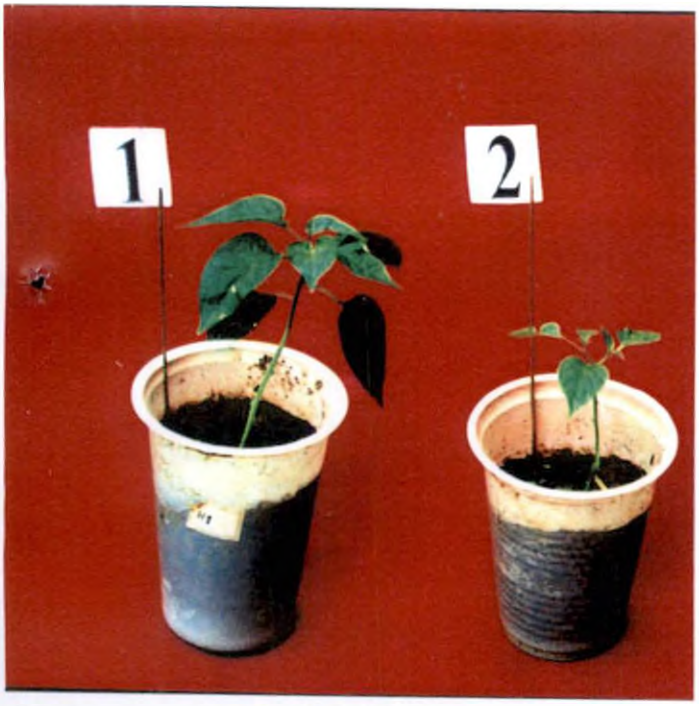


PLATE 7

PLATE 8

PLATE 9

PLATE 10

(Table 6). The maximum height of 11.70 cm was recorded in seedlings inoculated with the isolate Az-2 (Plate 7) and this was statistically on par with Az-40 (11.00 cm), Az-3 (10.73 cm), Az-1 (10.50 cm) (Plate 8) and Az-24 (10.33 cm) whereas the uninoculated control plants recorded a height of 7.67 cm.

Inoculation of five isolates of *Azospirillum* showed significant increase in root length over uninoculated control. The isolate Az-2 recorded the maximum root length of 9.33 cm (Plate 9). However this was statistically on par with Az-1 (8.60 cm) (Plate 10), Az-40 (8.50 cm), Az-24 (8.20 cm), Az-35 (8.17 cm), Az-3 (8.10 cm), Az-41 (7.83), Az-44 (7.73 cm) and Az-23 (7.73 cm) (Table 6). The uninoculated control plants recorded a root length of 6.46 cm.

Maximum dry weight of shoots (0.169 g plant^{-1}) was recorded in Az-2 inoculated seedlings. However, this was statistically on par with Az-40 which recorded a shoot dry weight of 0.166 g plant^{-1} as against 0.082 g plant^{-1} in the control plants (Table 6).

The dry weight of root was significantly superior in plants inoculated with Az-2 which recorded 0.080 g plant^{-1} compared to the other treatments including the uninoculated control which recorded a root dry weight of 0.030 g plant^{-1} (Table 6).

4.5.5 *In vivo* screening of selected *Azospirillum* isolates for growth, biomass production and yield

Based on *in vitro* nitrogen fixation, indole acetic acid production and growth and biomass production, six isolates of *Azospirillum* viz., Az-1, Az-2, Az-3, Az-24, Az-41 along with reference culture Az-40 were selected for *in vivo* screening. Chilli seeds were treated with selected isolates of

Azospirillum at sowing and seedlings were given root dip at the time of transplanting. The data on the effect of inoculation of selected *Azospirillum* isolates on growth, biomass production and yield in chilli are presented in Table 7.

The *Azospirillum* treated seeds germinated two days earlier than the uninoculated control. The treated seeds took five days for germination as against seven days taken for control (Plate 11).

Significant increase in the height of chilli plants were observed when treated with *Azospirillum*. Inoculation with Az-41 recorded the maximum plant height of 28.83 cm. However, this was statistically on par with the treatments such as Az-1 (27 cm) (Plate 12), Az-2 (27 cm) (Plate 13), Az-40 (25.66 cm) and Az-24 (25.5 cm). These treatments were found to be significantly superior to the uninoculated control which recorded a plant height of 18 cm (Table 7).

Azospirillum inoculated plants recorded a significant increase in root length. Maximum root length of 26.77 cm (Table 7) was obtained in plants treated with Az-1. However, this was statistically on par with Az-2 (26.08 cm) and Az-24 (26.00 cm) which were significantly superior to the uninoculated control (16.16 cm).

Maximum yield of 15.7 g plant^{-1} was recorded in plants inoculated with Az-40. However, this was statistically on par with Az-2 and Az-1 which recorded 14.0 g and 10.7 g plant^{-1} respectively (Table 7).

Significant increase in the shoot dry weight was recorded when treated with *Azospirillum*. Inoculation with Az-3 recorded maximum shoot dry

Table 7. Effect of inoculation of native *Azospirillum* on plant growth characteristics and yield in chilli under *in vivo* condition

Azospirillum isolates	Height (cm)	Root length (cm)	Stem girth (cm)	Yield (g plant^{-1})	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})
Az-1	27.00	**26.77**	1.8	10.7	4.1	1.8
Az-2	27.00	26.08	1.7	14.0	4.4	**2.0**
Az-3	21.80	18.83	1.5	8.3	**4.5**	1.8
Az-24	25.50	26.00	1.9	9.7	3.5	1.7
Az-40	25.66	25.00	1.6	**15.7**	4.2	1.8
Az-41	**28.83**	22.16	1.9	6.7	3.4	1.6
Control	18.00	16.16	1.1	5.0	1.6	0.6
CD	5.024	3.643	NS	5.112	1.49	0.485

Table 8. Effect of different carbon sources on growth of selected *Azospirillum* isolates

Azospirillum isolates	Growth (OD at 600 nm)		
	Az-1	Az-2	Az-40
Glucose	**0.937**	**0.592**	0.733
Fructose	0.561	0.485	0.632
Sucrose	0.759	0.410	0.592
CD	0.0887	0.1340	NS

Plate 11 Effect of *Azospirillum* on germination of chilli seeds

1 – Seed treatment with *Azospirillum*

2 – Control

Plate 12 Influence of Az-1 on growth characteristics and yield in chilli under *in vivo* conditions

1 – Control

2 – Seed treatment + seedling root dip with Az-1

Plate 13 Influence of Az-2 on growth characteristics and yield in chilli under *in vivo* conditions

1 – Control

2 – Seed treatment + seedling root dip with Az-2

PLATE 11

PLATE 12

PLATE 13

weight of 4.5 g plant^{-1} (Table 7) and was statistically on par with the other treatments such as Az-2 (4.4 g plant^{-1}), Az-40 (4.2 g plant^{-1}), Az-1 (4.1 g plant $^{-1}$), Az-24 (3.5 g plant^{-1}) and Az-41 (3.4 g plant^{-1}).

The root dry weight was also significantly superior in chilli plants inoculated with *Azospirillum*. Inoculation with Az-2 recorded the maximum root dry weight of 2.0 g plant^{-1} and was statistically on par with Az-1 (1.8 g plant^{-1}), Az-3 (1.8 g plant^{-1}), Az-40 (1.8 g plant^{-1}), Az-24 (1.7 g plant^{-1}) and Az-41 (1.6 g plant^{-1}) and were significantly superior to the uninoculated control (0.6 g plant^{-1}).

Observations on stem girth was not significant. However, the maximum stem girth was recorded in the treatment consisting of inoculation with Az-24 (1.9 cm) whereas in the control, the stem girth was 1.1 cm.

Based on *in vitro* and *in vivo* screening, native *Azospirillum* isolates viz., Az-1 and Az-2 which performed best were selected for further studies.

4.5.6 Physiological characterization of selected isolates of *Azospirillum*

Based on *in vitro* and *in vivo* screening, two native isolates of *Azospirillum* viz., Az-1 and Az-2 were selected for studying the utilization of different carbon sources, biotin requirement, acidification of peptone based glucose medium, catalase activity and pH sensitivity. Az-40 was used as reference culture. The data on the characterization studies are presented in Table 8, 9, 10 and 11.

Table 9. Effect of biotin on growth of selected *Azospirillum* isolates

Biotin requirement	Growth (OD at 600 nm)		
	Az-1	Az-2	Az-40
Biotin	0.424	0.602	0.633
Non-biotin	0.414	0.775	0.780
CD	NS	NS	NS

Table 10. Acidification and catalase activity of selected *Azospirillum* isolates

Azospirillum isolates	Acidification of peptone based glucose medium	Catalase activity
Az-1	+	+
Az-2	-	+
Az-40	-	+

Table 11. Effect of different pH on growth of selected *Azospirillum* isolates

pH	Growth (OD at 600 nm)		
	Az-1	Az-2	Az-40
pH4	0.254	0.028	0.013
pH5	0.452	0.065	0.083
pH6	0.465	**0.766**	0.510
pH7	**0.521**	0.621	**0.561**
pH8	0.317	0.610	0.366
CD	0.0793	0.0932	0.0349

4.5.6.1 Utilization of different carbon sources

The data on the utilization of different carbon sources by two selected native *Azospirillum* isolates, *viz.*, Az-1 and Az-2 along with the reference culture, Az-40 are presented in Table 8. In NFB medium, malate was substituted with either glucose, fructose or sucrose. The medium was inoculated with the selected *Azospirillum* isolates and growth was measured after 144 h of incubation.

When glucose was used as carbon source, the isolate Az-1 recorded a maximum OD value of 0.937. This was significantly different when fructose and sucrose were used as carbon source (Table 8). The isolate Az-2 recorded a maximum OD value of 0.592 when glucose was used as carbon source and this was statistically on par with fructose (0.485). Az-40 showed no significant difference in the growth between the different carbon sources.

4.5.6.2 Biotin requirement

The biotin requirement of the two selected isolates, *viz.*, Az-1 and Az-2 were studied and the data are presented in Table 9. There was no significant difference in the growth of the selected *Azospirillum* isolates when media was supplemented with or without biotin. However, the isolate Az-1 recorded a maximum OD value of 0.424 with biotin. Whereas the isolates Az-2 and Az-40 recorded a maximum OD value of 0.775 and 0.780 without biotin.

4.5.6.3 Acidification of peptone based glucose medium

The data on the acidification of peptone based glucose medium with the selected native isolates of *Azospirillum*, Az-1 and Az-2 along with reference culture, Az-40 are presented in Table 10. The isolate Az-1 acidified

the peptone based glucose medium by changing the colour from blue to yellow (+) whereas Az-1 and Az-40 did not cause any colour change (-).

4.5.6.4 Catalase activity

The catalase activity of the selected *Azospirillum* isolates are presented in Table 10. All the *Azospirillum* isolates produced brisk effervescence indicating positive catalase activity.

4.5.6.5 Growth in different pH

The effect of different pH levels on growth of two selected native *Azospirillum* isolates, Az-1 and Az-2 along with the reference culture (Az-40) was studied and the data are presented in Table 11.

The isolate Az-1 recorded a maximum OD value of 0.521 at pH 7 and this was statistically on par with the growth at pH 6 (0.465) and pH 5 (0.452) whereas the isolate Az-2 recorded a maximum OD value of 0.766 at pH 6 and this was significantly different from the growth of the isolate at the other four levels of pH (Table 11). The isolate Az-40 showed a maximum OD value of 0.561 at pH 7 and this was significantly different from the growth at pH 4, 5, 6 and 8.

4.6 Dual inoculation of native AMF and *Azospirillum* isolates on disease suppression and growth improvement in chilli

Chilli seedlings pre-inoculated with two selected native AMF cultures (M_8 and M_9) were used for the study. *Azospirillum* isolates viz., Az-1 and Az-2 were applied as seed treatment at sowing and seedling root dip at the time of transplanting. The pathogen *Pythium aphanidermatum*, mass multiplied in sand oats medium was inoculated 10 days after transplanting.

Table 12. Effect of combined inoculation of native AMF and *Azospirillum* on suppression of damping off in chilli

Treatments	Per cent disease incidence	Per cent reduction over control
$M_0A_0P_1$	80.83 (65.12)	-
$M_0A_1P_1$	54.16 (47.41)	33.00
$M_0A_2P_1$	37.50 (36.90)	53.61
$M_8A_0P_1$	41.58 (39.84)	48.56
$M_8A_1P_1$	41.66 (40.00)	48.46
$M_8A_2P_1$	23.15 (28.74)	71.36
$M_9A_0P_1$	14.29 (**22.27**)	**82.32**
$M_9A_1P_1$	31.47 (29.80)	61.07
$M_9A_2P_1$	22.03 (27.39)	72.75
CD	18.362	

Figures in parentheses are values after angular transformation

Data on per cent disease incidence, growth characters and yield were recorded and presented in Table 12 and 13.

4.6.1 Inoculation with the pathogen and incidence of damping off

Chilli seedlings pre-inoculated with native AMF ($M_9A_0P_1$) recorded the least per cent disease incidence of 22.27 (Plate 14) and this was significantly superior as against the control ($M_0A_0P_1$) which recorded 65.12 per cent disease incidence (Table 12). However this was statistically on par with $M_9A_2P_1$ (27.39), $M_8A_2P_1$ (28.74), $M_9A_1P_1$ (29.80), $M_0A_2P_1$ (36.90) and $M_8A_0P_1$ (39.84).

The treatment $M_9A_0P_1$ recorded 82.32 per cent disease reduction over control and $M_9A_2P_1$ recorded 72.75 per cent disease reduction.

4.6.2 Growth characters and yield

The data on the effect of dual inoculation of native AMF and *Azospirillum* on growth characteristics of chilli are presented in Table 13. Dual inoculation of AMF and *Azospirillum* resulted in significant increase in height of plants. The treatment $M_9A_2P_0$ recorded the maximum plant height of 44.00 cm (Plate 15). However, this was statistically on par with $M_8A_2P_0$ (43.00 cm), $M_8A_1P_0$ (39.16 cm) and $M_8A_0P_0$ (35.83 cm) as against the control $M_0A_0P_1$ which recorded 29.83 cm (Table 13).

The number of secondary branches was significantly higher in chilli plants inoculated with AMF and *Azospirillum*. It was maximum in the treatment $M_9A_2P_0$ (8.50). This was statistically on par with the other treatments $M_0A_2P_0$ (8.17), $M_8A_1P_0$ (6.17) compared to control, which recorded 2.50 (Table 13).

Table 13. Effect of combined inoculation of native AMF and *Azospirillum* on growth characters and yield in chilli

Treatment	Height (cm)	No. of secondary branches (no plant^{-1})	Stem girth (cm)	Root length (cm)	Dry weight of plant (g plant^{-1})	Yield (g plant^{-1})
$M_0A_0P_0$	28.67	5.17	3.08	13.50	10.83	26.66
$M_0A_0P_1$	29.83	2.50	2.67	11.22	7.33	23.33
$M_0A_1P_0$	40.17	4.50	3.08	19.83	13.67	53.33
$M_0A_1P_1$	25.83	5.67	2.82	14.94	5.67	26.66
$M_0A_2P_0$	31.67	8.17	3.13	23.08	13.50	18.33
$M_0A_2P_1$	24.17	6.50	2.62	15.58	7.67	30.00
$M_8A_0P_0$	35.83	5.67	2.87	**27.40**	13.00	48.33
$M_8A_0P_1$	27.33	4.67	2.95	15.22	7.67	25.00
$M_8A_1P_0$	39.16	6.17	3.15	18.42	12.17	21.67
$M_8A_1P_1$	29.16	6.00	3.05	15.71	7.67	41.67
$M_8A_2P_0$	43.00	7.83	3.25	21.27	13.83	35.00
$M_8A_2P_1$	24.50	5.83	2.80	14.22	6.83	26.67
$M_9A_0P_0$	32.17	5.83	2.67	20.22	12.50	58.33
$M_9A_0P_1$	32.50	5.00	3.08	16.53	7.43	53.33
$M_9A_1P_0$	31.67	5.17	3.40	16.44	12.93	36.67
$M_9A_1P_1$	30.17	5.83	3.23	15.07	8.83	23.33
$M_9A_2P_0$	**44.00**	**8.50**	3.53	24.58	**14.33**	**76.67**
$M_9A_2P_1$	31.50	4.50	2.75	14.33	7.33	36.66
CD	9.313	2.417	NS	9.876	4.083	24.019

Plate 14 **Influence of native AMF ($M_9A_0P_1$) on suppression of damping off in chilli**

1 – $M_9A_0P_1$

2 – Control

Plate 15 **Influence of native AMF and *Azospirillum* ($M_9A_2P_0$) on growth characteristics and yield in chilli**

1 – $M_9A_2P_0$

2 – Control

PLATE 14

PLATE 15

The root length was maximum (27.40 cm) in the treatment $M_8A_0P_0$ and this was statistically on par with $M_9A_2P_0$ (24.58 cm), $M_0A_2P_0$ (23.08 cm), $M_8A_2P_0$ (21.27 cm), $M_9A_0P_0$ (20.22 cm), $M_0A_1P_0$ (19.83 cm), $M_8A_1P_0$ (18.42 cm) as against the control (11.22 cm). Combined inoculation of AMF and *Azospirillum* significantly increased the dry weight of chilli plants. Maximum dry weight of 14.33 g plant^{-1} was recorded in $M_9A_2P_0$ (Table 13). However, this was statistically on par with $M_8A_2P_0$ (13.83 g plant^{-1}), $M_0A_1P_0$ (13.67 g plant^{-1}), $M_0A_2P_0$ (13.50 g plant^{-1}), $M_8A_0P_0$ (13.00 g plant^{-1}), $M_9A_1P_0$ (12.93 g plant^{-1}), $M_9A_0P_0$ (12.50 g plant^{-1}) and $M_8A_1P_0$ (12.17 g plant^{-1}) compared to the control (7.33 g plant^{-1}).

Dual inoculation of AMF and *Azospirillum* significantly increased the yield of chilli plants. $M_9A_2P_0$ recorded the highest yield of 76.67 g plant^{-1} and this was statistically on par with $M_9A_0P_0$ (58.33 g plant^{-1}), $M_9A_0P_1$ (53.33 g plant^{-1}) and $M_0A_1P_0$ (53.33 g plant^{-1}) whereas the control recorded an yield of 23.33 g plant^{-1}.

The observation on stem girth was not significant. However, maximum stem girth was recorded in $M_9A_2P_0$ (3.53 cm) whereas the control recorded a stem girth of 2.67 cm (Table 13).

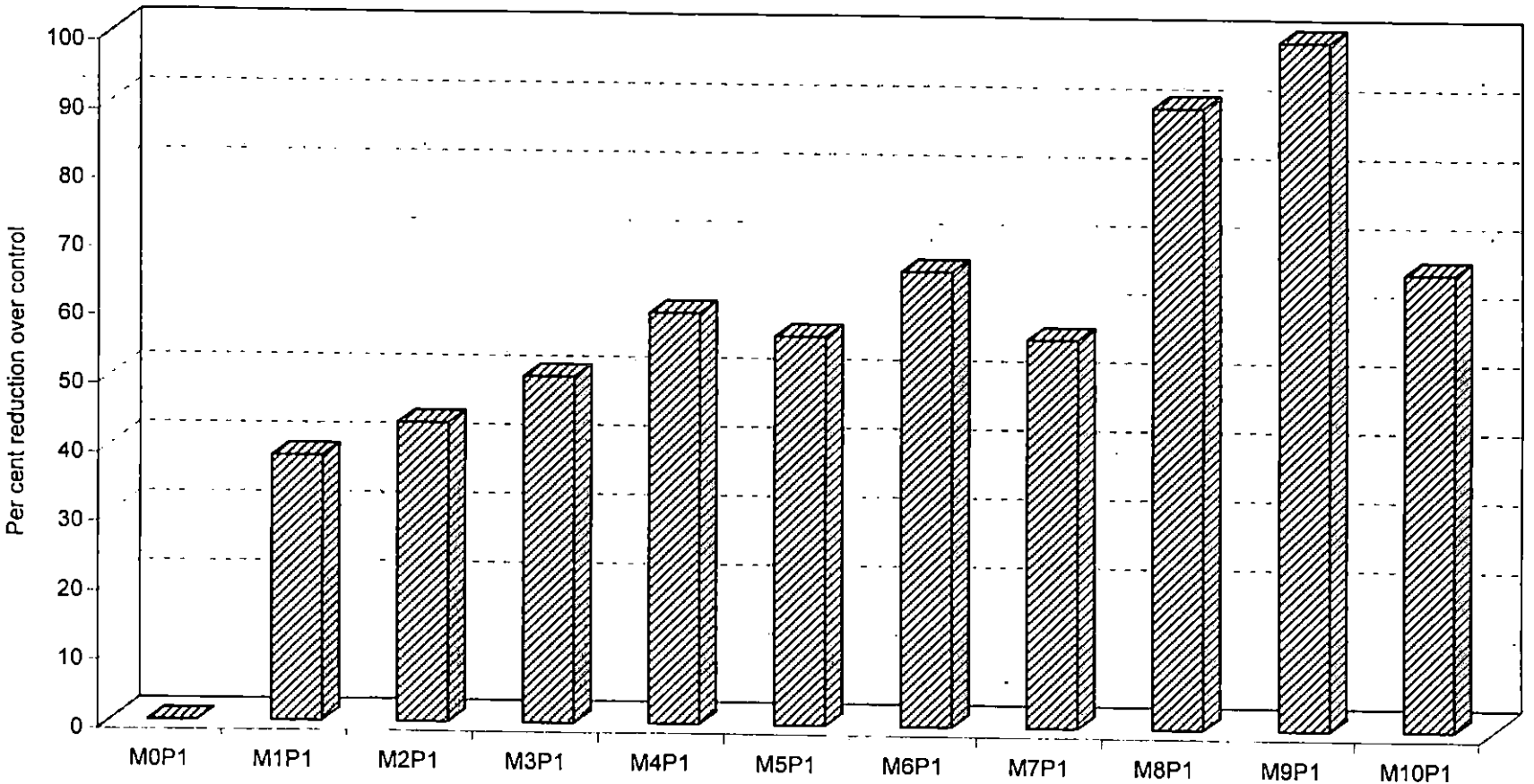
Discussion

5. DISCUSSION

Damping off is one of the most devastating diseases of chilli which causes serious damage to the crop both in the nursery and in main field. Management of *Pythium* damping off using fungicides has become less popular due to high cost, residual toxicity and environmental hazards. The present investigation was undertaken to explore the possibility of management of damping off and improvement of growth in chilli with native species of AMF and *Azospirillum*. The causal organism inciting damping off of chilli was isolated from diseased chilli seedlings collected from highly infested areas of Thiruvananthapuram district and the pathogenicity was proved. The most virulent isolate was selected and the morphological and cultural characteristics showed that the isolate was *Pythium aphanidermatum* (Edson) Fitz. as reported by Sowmini (1961).

Positive influence of AMF on plant growth promotion, biomass production and protection against root diseases is well recognized in most plant species (Jalali and Jalali, 1991; Sivaprasad, 1995; Sreeramulu and Bagyaraj, 1999). In the present study, a preliminary screening trial was conducted to identify efficient AMF cultures capable of suppressing damping off of chilli. Eventhough the ability of AMF to suppress the disease varied with different cultures, 100 per cent disease reduction was obtained in plants pre-inoculated with M_9 culture followed by 90.38 per cent with M_8 culture (Fig. 1). Among the cultures screened, M_8 and M_9 were highly effective in reducing the incidence of the disease (Table 1). The ability of AMF in

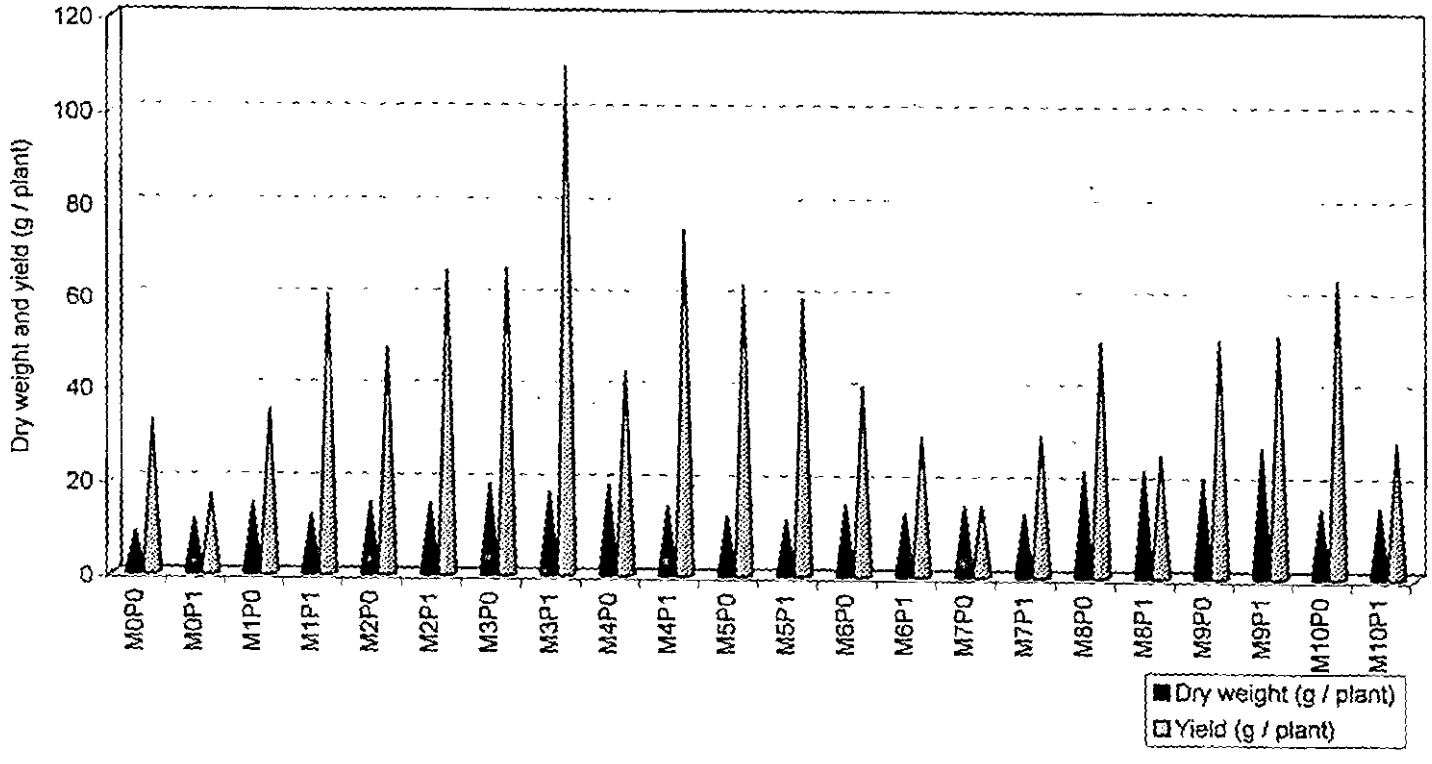
Fig. 1. Incidence of damping off in chilli as influenced by AMF



suppressing damping off has already been reported in crops like cucumber (Rosendahl and Rosendahl, 1990), ginger (Rohini Iyer and Sundararaju, 1993; Joseph, 1997), cardamom (Thomas *et al.*, 1994) and pepper (Odebode *et al.*, 1997). When plants were pre-inoculated with AMF, the spread of the pathogen in the host may be restricted (Dehne and Schoenbeck, 1975). The AMF induced resistance against fungal pathogens could also be attributed to factors such as improved nutrient uptake especially phosphorus (Ames and Linderman, 1978; Abdul-Khaliq *et al.*, 2001), competition with pathogen for space, nutrition and host photosynthate (Harley and Smith, 1983; Linderman, 1985), qualitative and quantitative shift in microbial population in the rhizosphere (Mayer and Linderman, 1986; Secilia and Bagyaraj, 1987; Graham, 1988) and altered physiology of the host that induces host defense mechanisms (Gianinazzi-Pearson *et al.*, 1996).

Variations were observed in the response of chilli plants to different AMF cultures on growth characteristics, biomass production and yield. In general, all the native AMF isolates enhanced growth, biomass and yield of chilli plants (Fig. 2). The enhanced growth can be attributed to better uptake of nutrients by AMF inoculated plants. Maximum root length and height were recorded in plants inoculated with M_2 culture whereas the biomass was higher in plants inoculated with M_9 and M_8 cultures (Table 2). The yield was significantly greater in plants inoculated with M_3 culture. Stimulation of plant growth is the inherent ability of AMF isolates which depends on the efficiency of hyphae to spread in the soil and absorb the nutrients beyond the depletion zone and make it available to the plants (Marschner and Dell, 1994). The increase in growth, biomass production and yield in AMF

Fig. 2. Effect of AMF on biomass production and yield of chilli

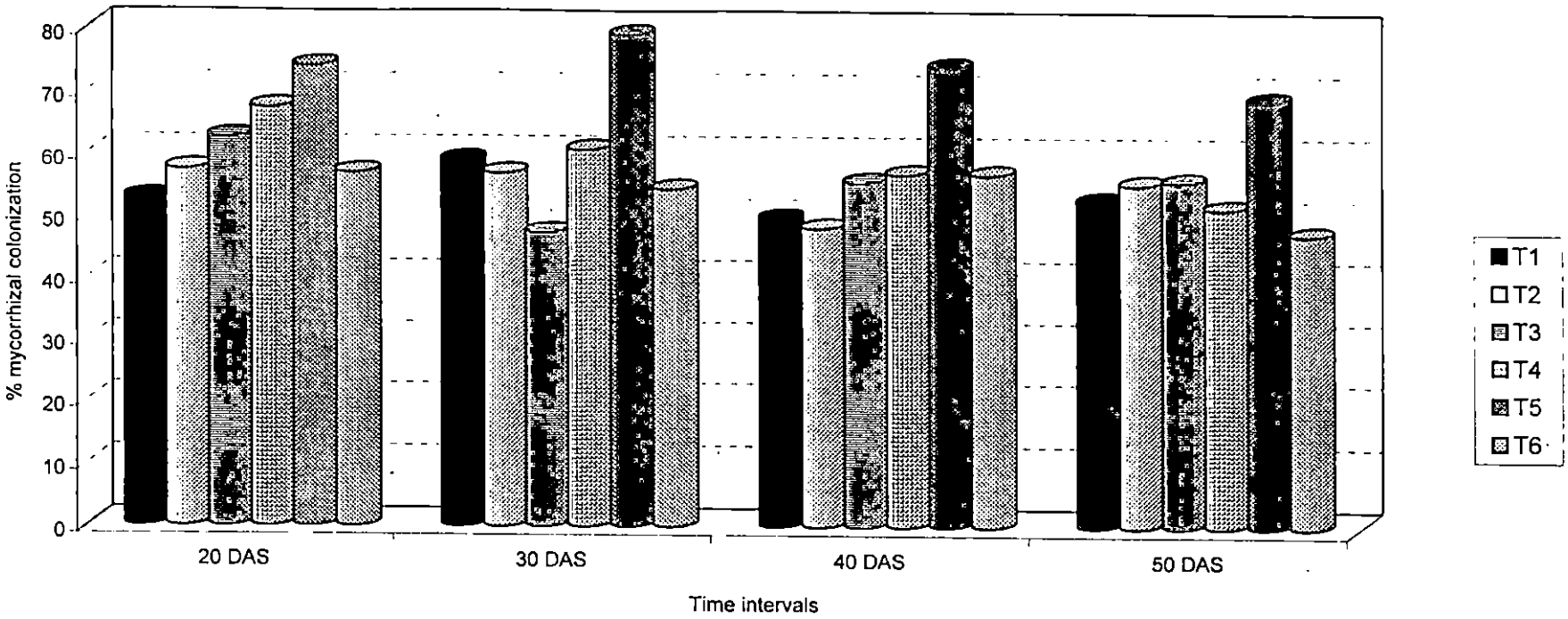


inoculated plants were reported earlier in chilli (Bagyaraj and Sreeramulu, 1982 and Sreeramulu and Bagyaraj, 1986), cassava (Sivaprasad *et al.*, 1990), tomato (Mallesha *et al.*, 1994), papaya (Balakrishna *et al.*, 1996), cowpea (Gupta *et al.*, 1999), tomato (Thippeswamy and Sreenivasa, 1999) cucumber (Cigsar *et al.*, 2000) and papaya (Johnkennedy and Rangarajan, 2001).

Thus, the cultures M_8 and M_9 were found to be highly effective in suppressing damping off incited by *Pythium*. Interestingly, another culture M_2 increased the root length and height of chilli plants whereas cultures M_8 and M_9 could enhance only the biomass production. But the yield was maximum in plants inoculated with culture M_3. Thus it is clear that the ability of AMF for growth stimulation and disease suppression are independent traits and they need not occur together in a single culture. A culture effective for growth enhancement and higher yield may be a poor biocontrol candidate. Similarly an AMF culture having potential biocontrol efficiency need not induce significant growth enhancement (Vidhyasekaran, 1990). It is highly desirable if a culture possessing both the qualities could be obtained. In the present investigation the native AMF cultures viz., M_8 and M_9 have been identified as effective cultures in suppressing damping off as well as increasing biomass production.

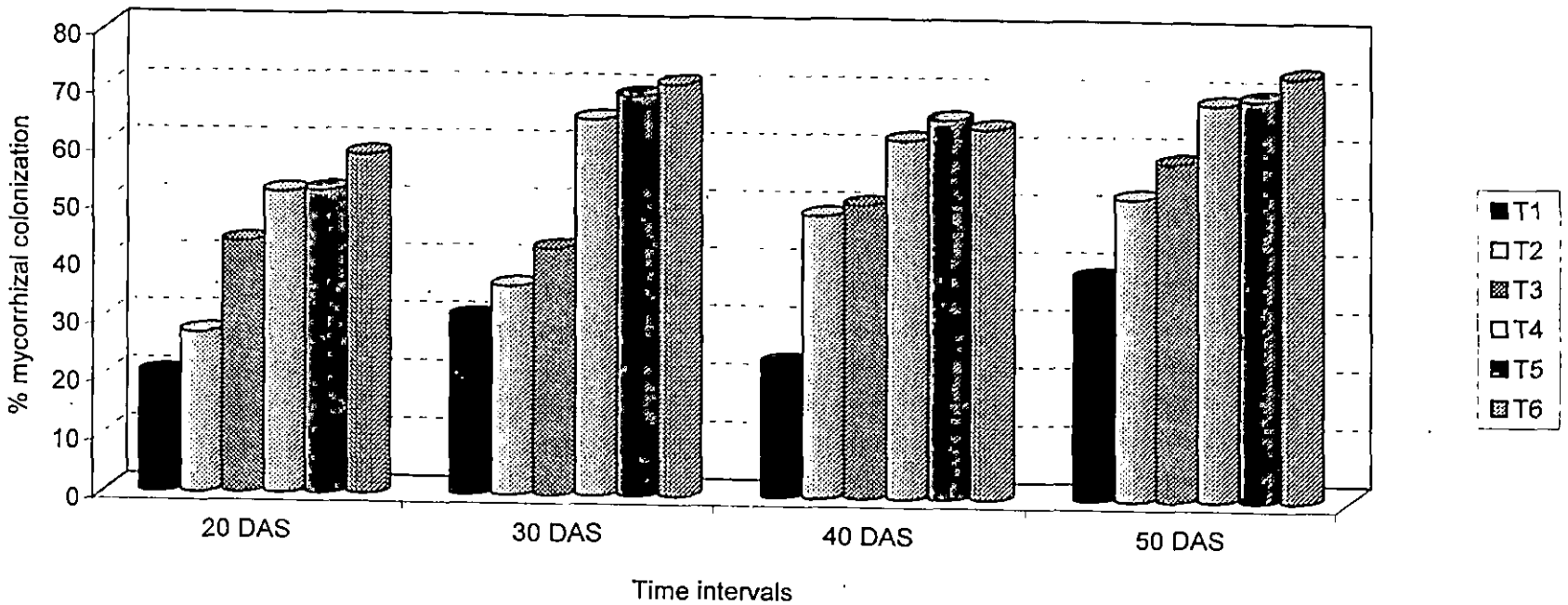
The study on standardisation of suitable method of inoculation of AMF in chilli nursery revealed that inoculation in nursery furrows (Fig. 3) recorded the maximum percentage colonization uniformly at different intervals of observation (Table 3). By placing AMF inoculum in nursery furrows along with the seeds, more root infection could be obtained. This may be due to direct contact of the inoculum with seed and emerging root. The results of

Fig.3. Effect of methods of inoculation on AMF colonization in chilli



T1 (mixing with surface layer soil)
T2 (incorporating with organic manure)
T3 (Mixing with seed)
T4 (Spot inoculation)
T5 (Inoculation in nursery furrow)
T6 (Placing in root zone)

Fig. 4. Effect of different doses of AMF inoculum on colonization in chilli



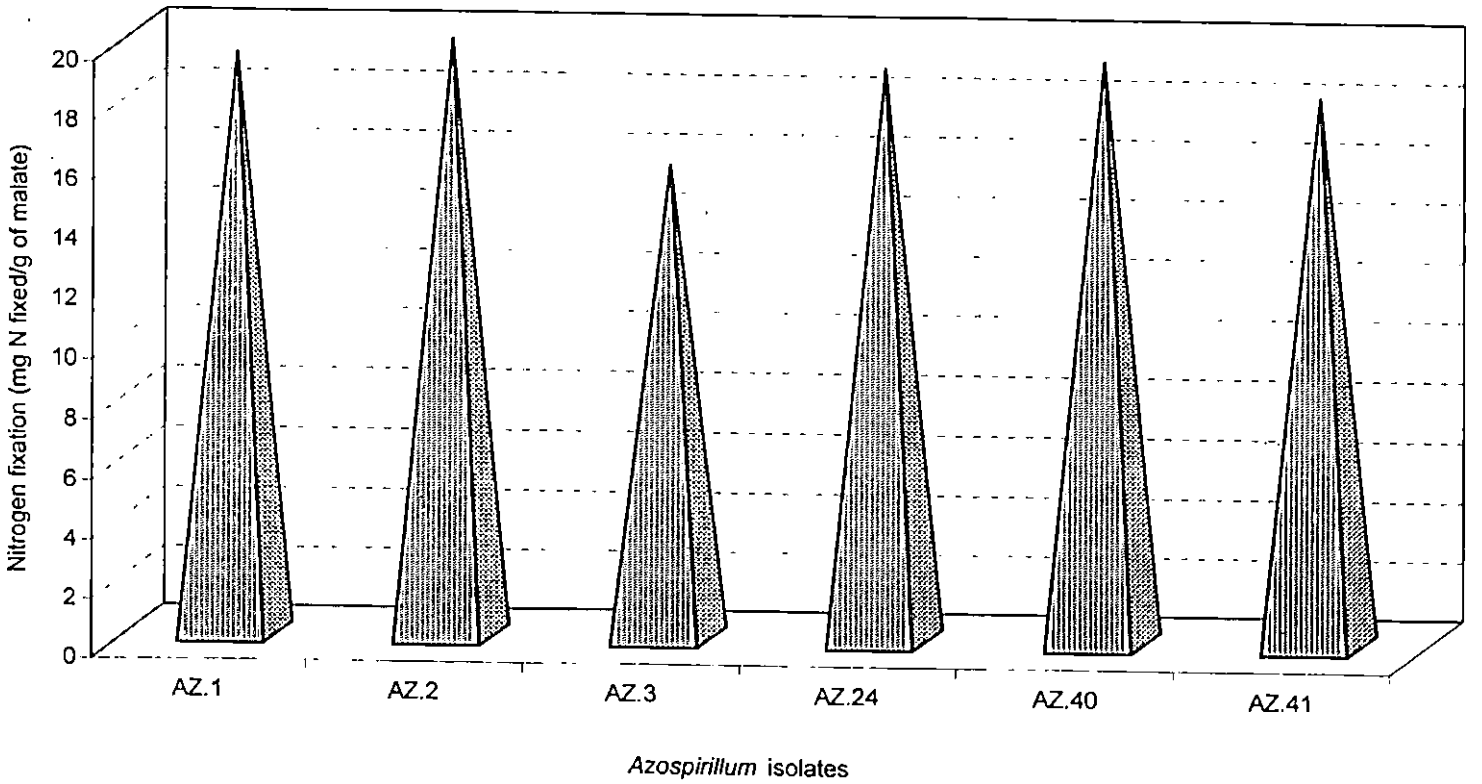
T1 (0 g m^{-2})
T2 (200 g m^{-2})
T3 (400 g m^{-2})
T4 (800 g m-2)
T5 (1250 g m-2)
T6 (1600 g m-2)

this experiment supports the view of Hayman *et al.* (1981) in red clover, Sukhada (1987) in tomato and Gurubathan *et al.* (1989) in bellary onion.

Application of AMF inoculum in nursery furrows was identified as the best method and this method was selected to standardize the quantity of AMF inoculum required for chilli nursery. Different doses of AMF inoculum application were tried (Table 4). Eventhough maximum colonization was obtained with 1600 g m^{-2} (T_6), the rate of 850 g m^{-2} was selected as the economic dose for achieving satisfactory colonization (Fig. 4). Similar results were obtained by Sukhada (1987) who could get 40 per cent colonization using 1 kg inoculum in tomato. Almost similar results were also reported by Subbiah (1994) in chilli and bellary onion and Shrihari and Sreenivasa (1998) in chilli.

The association of *Azospirillum* with crop plants such as maize, sorghum, sugarcane and forage grasses (Lakshmikumari *et al.*, 1976); wheat (Kavimandan *et al.*, 1978 and Padshetly *et al.*, 1986); pepper (Govindan and Chandy, 1985); bhindi (Parvatham *et al.*, 1989); barley (Lukin *et al.*, 1992); cabbage (Jeevajothi *et al.*, 1993) and chilli (Paramaguru and Nagarajan, 1993) has already been reported. In the present study an attempt was made to isolate *Azospirillum* from chilli roots collected from different locations of Thiruvananthapuram district. Forty four isolates were obtained and these isolates grew well in semisolid malate medium. This medium encouraged the growth of *Azospirillum* probably by providing microaerophilic conditions and thereby reduced oxygen concentration. The growth of *Azospirillum* occurred as a white undulating pellicle below the meniscus (Burlow and Dobereiner, 1975; Lakshmikumari *et al.*, 1976; Burris *et al.*, 1977). The purified isolates on transfer to semisolid medium formed the characteristic white undulating

Fig. 5. *In vitro* nitrogen fixation of selected *Azospirillum* isolates

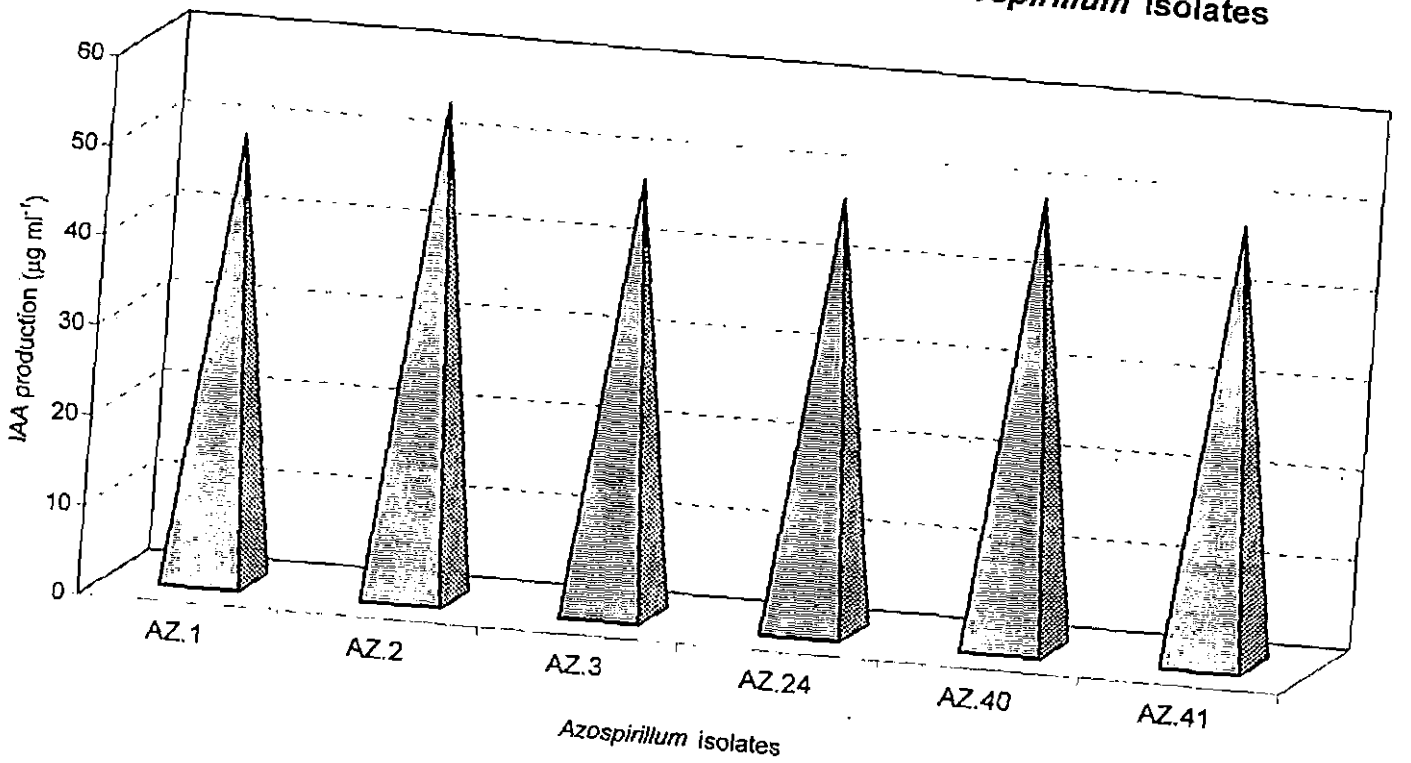


pellicle and the presence of *Azospirillum* was confirmed. The purified isolates were then subjected to morphological studies. All the isolates were gram negative, motile and contained poly beta hydroxy granules. They were short to medium sized slightly curved rods.

The isolates of *Azospirillum* were screened for *in vitro* nitrogen fixation. The reference culture (Az-40) was also used for the study. The *in vitro* nitrogen fixation by different isolates showed wide variations (Table 5). The isolate Az-2 recorded maximum nitrogen fixation of 20.00 mg N g^{-1} malate (Fig. 5) and the least amount of nitrogen fixation of 11.20 mg N g^{-1} malate was recorded by isolate Az-10. Perhaps this variation may be due to the difference in their inherent capacity to fix nitrogen. Many earlier researchers have reported that *in vitro* nitrogen fixation by *Azospirillum* is highly variable (Subha Rao *et al.*, 1980 and Purushothaman and Vijila, 1988). The quantity of nitrogen fixed by the isolates were similar to that obtained by many workers (Okon *et al.*, 1976; Govindan,1982 and Sasikumar, 1996).

The different isolates of *Azospirillum* were screened along with the reference culture (Az-40) for the production of indole acetic acid under *in vitro* conditions. Many research workers have reported the production of IAA by *Azospirillum* (Tien *et al.*, 1979; Hartman *et al.*, 1983; Baca *et al.*, 1994; Kundu *et al.*, 1997). The isolate Az-2 produced maximum amount of IAA (55.00 µg ml^{-1}) (Fig. 6) whereas isolates Az-14 and Az-6 produced the least amount of IAA (21.00 µg ml^{-1}) under *in vitro* conditions (Table 5). The variation in IAA production may be due to the difference in their inherent ability to produce phytohormones. Similar variations in the amount of IAA production by different isolates of *Azospirillum* has been observed earlier also

Fig. 6. *In vitro* IAA production of selected *Azospirillum* isolates

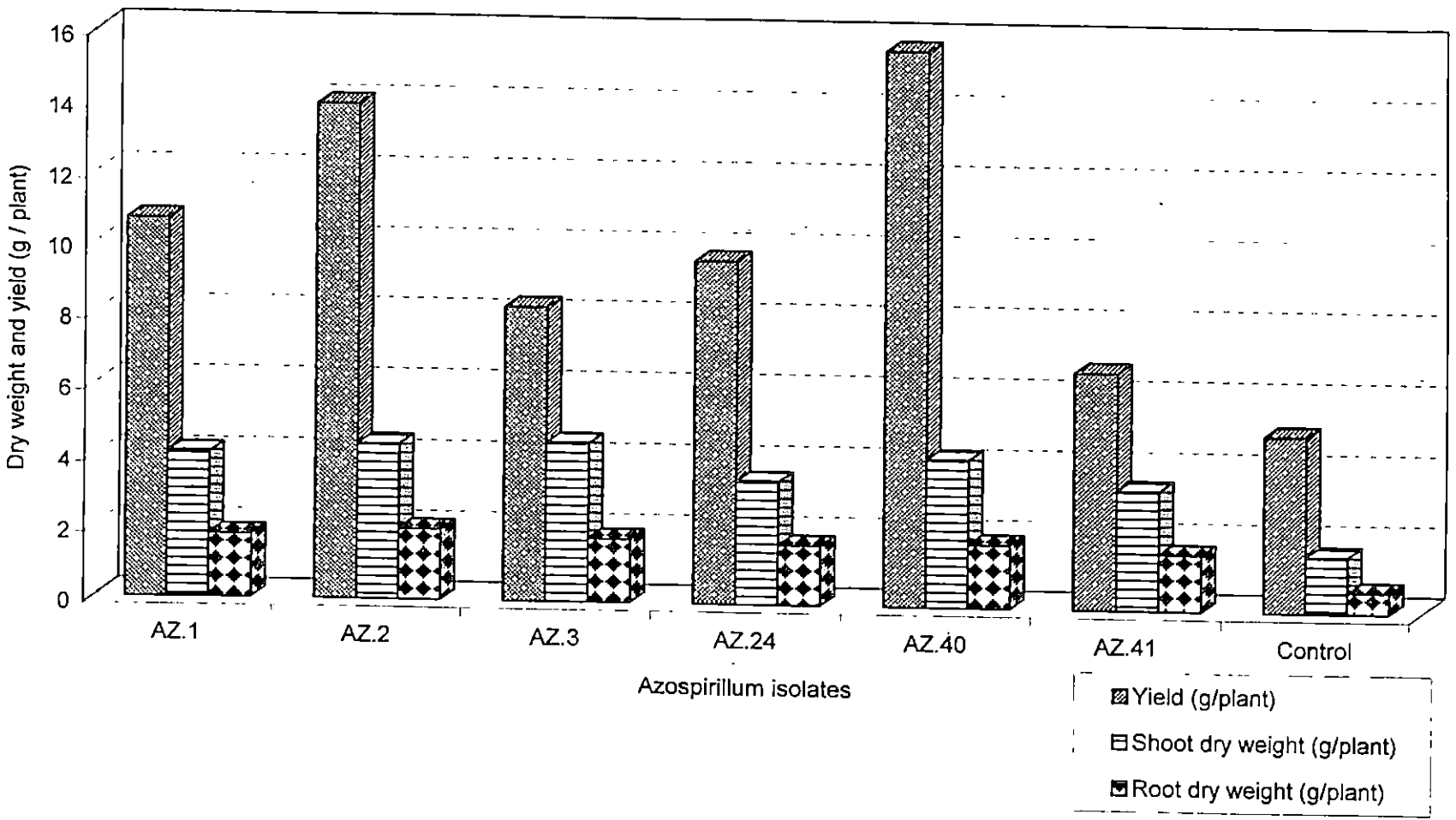


(Mascaru *et al.*, 1988; Baca *et al.*, 1994; Yamini Varma, 1995 and Sasikumar, 1996).

All the 44 isolates of *Azospirillum* along with the reference culture (Az-40) were subjected to *in vitro* screening to study their effect on growth and biomass production of chilli seedlings. The different isolates of *Azospirillum* were used for seed treatment of chilli at the time of sowing. Many earlier researchers have obtained increased growth and drymatter production due to *Azospirillum* inoculation in crops like pearl millet (Venkateswarlu and Rao, 1983), in wheat (Bashan, 1986) and in sunflower (Savalgi and Veena, 1990). The results of the present study revealed that out of 44 isolates screened, Az-1, Az-2, Az-3, Az-24, Az-40 and Az-41 recorded significant increase in seedling height and root length (Table 6). The dry weights of the shoot and root were higher in seedlings inoculated with Az-2. Such growth enhancement in chilli seedling may be due to the production of phytohormones, particularly IAA by the *Azospirillum* isolates. In a study with rice seedlings, Subramanian (1987) also obtained similar results.

Based on *in vitro* IAA production, nitrogen fixation, growth and biomass production of chilli seedlings, six best isolates of *Azospirillum* viz., Az-1, Az-2, Az-3, Az-24 and Az-41 including reference culture (Az-40) were selected for *in vivo* screening to study the effect of *Azospirillum* inoculation on growth, biomass production and yield in chilli. It was observed that the chilli seeds treated with *Azospirillum* germinated two days earlier compared to the control which took seven days for germination. All the six isolates of *Azospirillum* could produce significant increase in plant height, root length, dry weight of shoot and root (Fig. 7) when compared to uninoculated control

Fig. 7. Effect of selected *Azospirillum* isolates on biomass production and yield of chilli

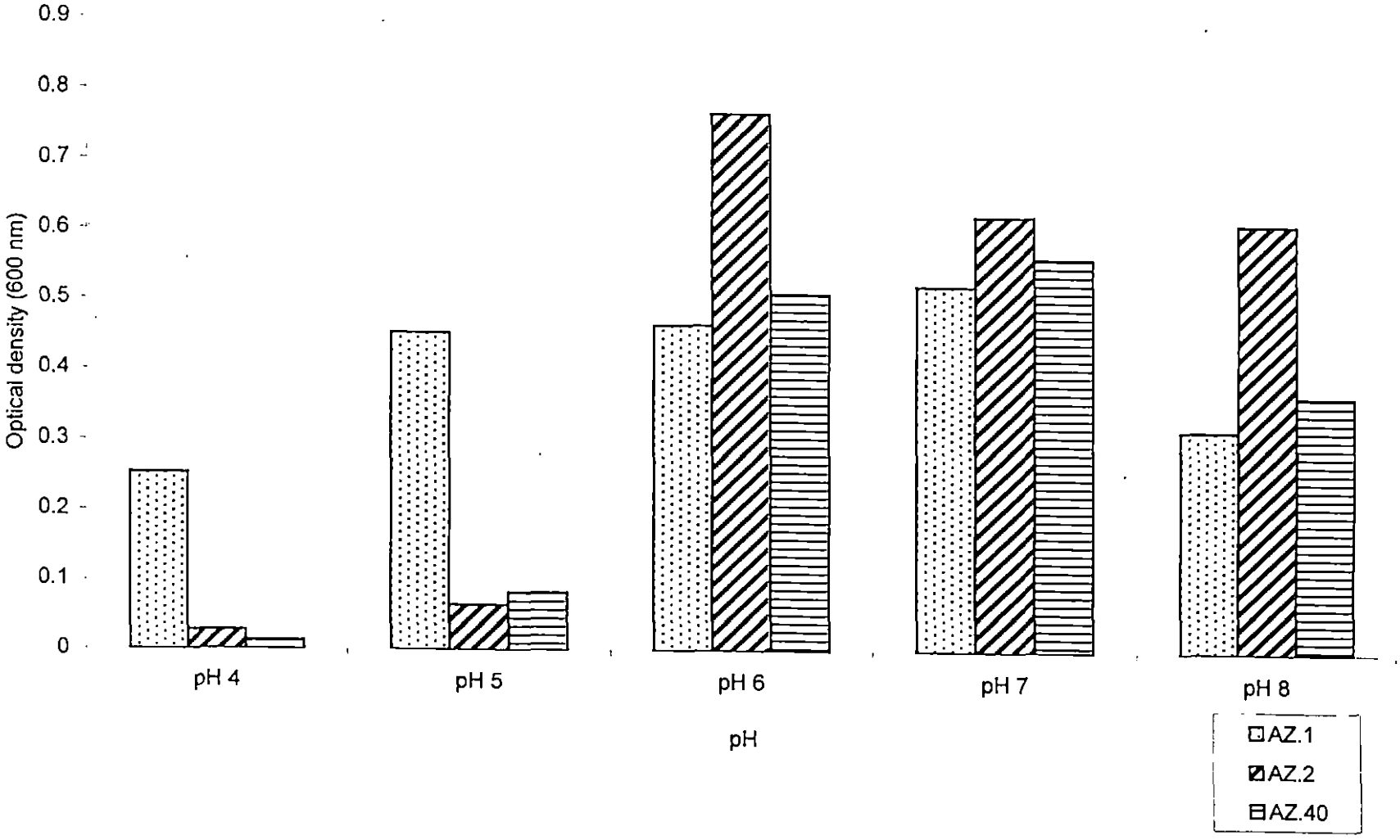


plants. As a result of phytohormone production there is increase in cell division of the meristematic region of inoculated plants which resulted in better root elongation, dense root hairs, root volume and root dry weight (Dewan and Subba Rao, 1979). These observations were in agreement with the findings of Hadas and Okon (1987) in tomato, Parvatham *et al.* (1989) in bhindi and Paramaguru and Natarajan (1993) in chilli. Among the six isolates tested, Az-1 and Az-2 which performed best under both *in vitro* and *in vivo* conditions were selected for further studies.

The selected isolates viz., Az-1 and Az-2 along with reference culture Az-40 were characterized by studying the utilization of different carbon sources, biotin requirement, acidification of peptone based glucose medium, catalase activity and pH sensitivity.

The utilization of different carbon sources by the selected *Azospirillum* isolates viz., Az-1 and Az-2 along with the reference culture Az-40 were studied under *in vitro* conditions. In NFB medium, malic acid was substituted with either glucose, fructose or sucrose and inoculated with selected cultures, incubated for 144 h before their growth was measured. The isolate Az-1 recorded a maximum growth of 0.937 when glucose was used as carbon source. The isolate Az-2 also showed a maximum growth of 0.592 with glucose, followed by fructose (0.485) and the least with sucrose (0.410). The reference culture (Az-40) had no preference for glucose, fructose or sucrose (Table 8). The present study revealed that the isolate Az-1 prefers glucose and isolate Az-2 grew well both in glucose and fructose medium. The results are in agreement with the findings of Dobereiner and Day (1976), Tarrand *et al.* (1978); Indira and Bagyaraj (1997) who reported that *A. lipoferum* is capable

Fig. 8. Effect of different pH on growth of *Azospirillum* isolates



of using glucose as a sole carbon source for growth in N free semisolid medium whereas *A. brasilense* is not capable of utilizing glucose as a sole carbon source for growth in N free semisolid medium.

When the medium was supplemented with or without biotin, there was no difference in the growth of *Azospirillum* isolate (Table 9). Eventhough there are earlier reports regarding the requirement of biotin for *Azospirillum* (Tarrand *et al.*, 1978) in the present investigation, the selected *Azospirillum* isolates did not show any preference for biotin.

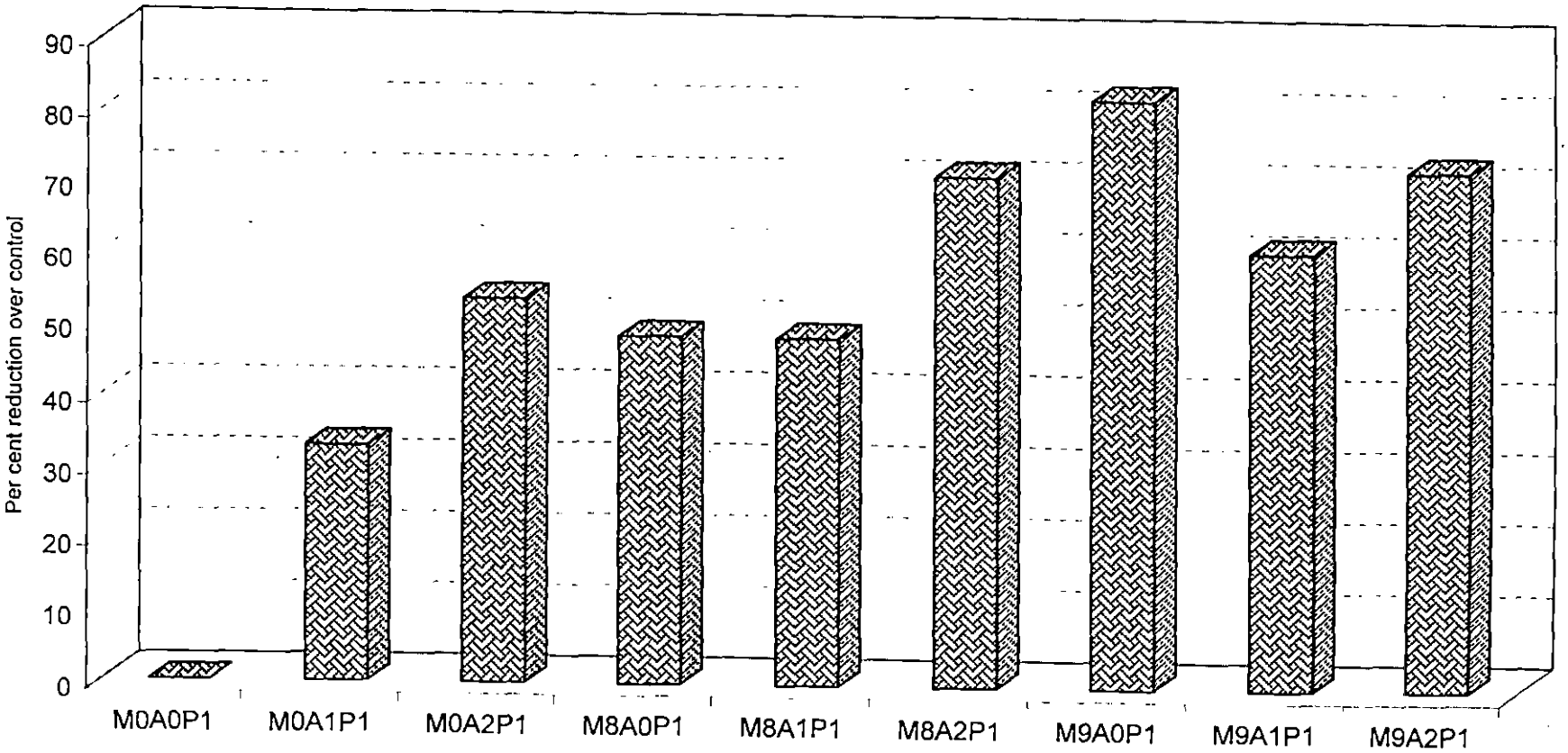
In the study on the acidification of peptone medium, it was observed that the isolate Az-1 produced acid in the peptone medium whereas isolates Az-2 and Az-40 did not produce acid in peptone medium. This acidification has been obtained earlier by Tarrand *et al.* (1978) and Indira and Bagyaraj (1997) with *A. lipoferum* which acidified the peptone medium whereas *A. brasilense* did not acidify the peptone medium.

With regard to catalase activity, all the selected *Azospirillum* isolates produced brisk effervescence when hydrogen peroxide was added. Similar positive catalase activity has been reported earlier by Sasikumar (1996).

From the physiological studies it can be presumed that the isolate Az-1 resembles the characters of *A. lipoferum* whereas isolate Az-2 has characters similar to *A. brasilense* (Tarrand *et al.*, 1978 and Indira and Bagyaraj (1997).

The pH sensitivity of *Azospirillum* isolates were tested under *in vitro* conditions using malate broth initially adjusted to different pH such as 4, 5, 6, 7 and 8. The isolate Az-1 grew well at pH 5, 6 and 7 whereas the isolate Az-2 and Az-40 grew well at pH 6 and 7. Eventhough the native *Azospirillum*

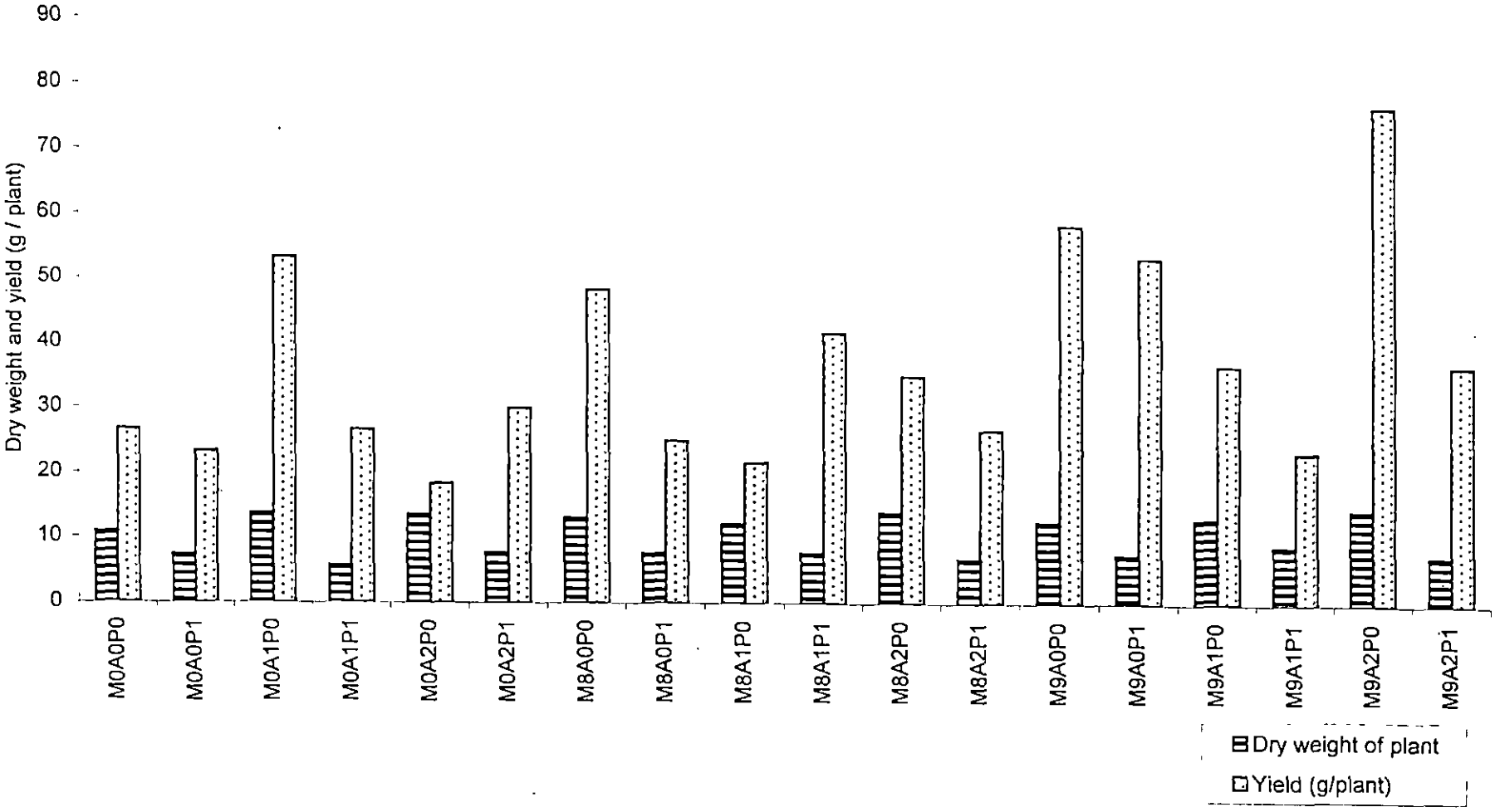
Fig. 9. Effect of dual inoculation of AMF and *Azospirillum* on suppression of damping off in chilli



isolates were obtained from chilli roots grown in acidic soils they could grow well at pH 5, 6 and 7. The ability of *Azospirillum* to grow in acidic (Charyulu and Rao, 1980), neutral (Indira and Bagyaraj, 1997) and alkaline soils (Purushothaman and Oblisami, 1985) has already been reported.

The selected native cultures of AMF viz., M_8 and M_9 and the native *Azospirillum* isolates viz., Az-1 and Az-2 were used to study the interaction effect of AMF, *Azospirillum* and *Pythium aphanidermatum* in chilli on suppression of damping off and improvement of growth, biomass production and yield in chilli under pot culture conditions. Mycorrhizal inoculation alone ($M_9A_0P_1$) could suppress the damping off by 82.32 per cent over control (Fig. 9). The ability of AMF to suppress the damping off may be due to the competition with pathogen for space, nutrition and host photosynthates (Harley and Smith, 1983; Linderman, 1985) or the alteration of the physiology of the host which induces host defence mechanisms (Gianinazzi Pearson *et al.*, 1996). Dual inoculation of AMF along with *Azospirillum* ($M_9A_2P_1$) could also reduce damping off by 72.75 per cent over control (Table 12). This may be due to effect of *Azospirillum* and AMF interaction which makes the plant healthier by way of enhanced uptake of nutrients and trigger of host defense mechanisms. Eventhough dual inoculation of AMF and *Azospirillum* could suppress the damping off, *Azospirillum* had no direct effect on disease suppression. No report is available on the interaction effect of AMF and *Azospirillum* on the suppression of damping off. However some work on interaction effect of AMF with other diazotrophs like *Rhizobium leguminosorum* has been reported by Dar *et al.* (1997) in common bean in

Fig. 10. Effect of dual inoculation of AMF and *Azospirillum* on biomass production and yield of chilli



reducing root rot pathogen (*Fusarium solani*) and Rabie (1998) in *Vicia fabae* in reducing *Botrytis fabae* infection.

Dual inoculation of AMF and *Azospirillum* could also enhance growth, biomass production and yield in chilli (Fig. 10). The treatment $M_9A_2P_0$ recorded the maximum plant height (44.00 cm), number of branches (8.50 no plant^{-1}), yield (76.67 g plant^{-1}) and dry weight (14.33 g plant^{-1}) whereas the root length of 27.40 cm was maximum in $M_8A_0P_0$. This positive response of AMF and *Azospirillum* on growth and biomass production may be due to the enhanced uptake of nutrients especially phosphorus, potassium and zinc (Abdul-Khaliq *et al.*, 2001) by AMF and production of phytohormones by *Azospirillum* (Sangwan, 1990).

The increase in root length due to AMF inoculation may be to absorb the nutrients present in the soil and make it available to the plants. Similar reports of increase in growth, biomass production and yield due to dual inoculation of AMF and *Azospirillum* has already been reported by Subba Rao *et al.* (1985) in barley and Sreeramulu *et al.* (1988) in maize, Kumari and Balasubramani (1993) in coffee.

The present investigation emphasizes the importance of pre-inoculation of AMF in the chilli nursery as a prophylatic measure to prevent pathogen attack. The technology of combined inoculation of AMF and *Azospirillum* could be recommended for adoption by the vegetable farmers. This study forms the first report of its nature in vegetables. Eventhough the present study was carried out in chilli, the same cultures could be recommended for all transplanted solanaceous vegetables after confirming the results through field trials.

Summary

6. SUMMARY

Chilli (*Capsicum annuum* L.) is an important transplanted vegetable of Kerala. Damping off incited by *Pythium aphanidermatum* is one of the devastating diseases of chilli both in the nursery and in main field. The present study was taken up with the objective of management of damping off and improvement of growth in chilli with native species of arbuscular mycorrhizal fungi and *Azospirillum*.

The different isolates of the pathogen inciting damping off were isolated from diseased chilli seedlings collected from highly infested areas of Thiruvananthapuram district and pathogenicity was proved. The most virulent isolate was identified as *Pythium aphanidermatum* (Edson) Fitz. based on morphological and cultural characteristics.

Nine native AMF cultures along with one identified culture (*Glomus mosseae*) were screened for suppression of damping off and growth improvement in chilli. Chilli plants pre-inoculated with M_9 and M_8 culture recorded least disease incidence and this was significantly superior over the other AMF cultures. Also significant increase in plant height, number of secondary branches, root length, dry weight and yield were observed in chilli plants pre-inoculated with native AMF. Based on the screening trial, two best cultures of native AMF viz., M_8 and M_9 were selected for further studies.

Different methods of inoculation of AMF in the nursery such as mixing with surface layer soil, incorporating with organic manure, mixing with seed, spot inoculation, inoculation in nursery furrows and placing in root zones

were studied. Of the different methods tested, inoculation in nursery furrows recorded the maximum per cent colonization uniformly at different time intervals when compared to other methods.

Application of AMF inoculum in nursery furrows which was found to be best method was selected to standardize the quantity of inoculum required for chilli nursery. Different doses such as 0 g m^{-2}, 200 g m^{-2}, 400 g m^{-2}, 850 g m^{-2}, 1250 g m^{-2} and 1600 g m^{-2} were tried. Of the different doses of AMF inoculum studied, application at the rate of 850 g m^{-2}, which recorded satisfactory colonization, was selected as the economic dose for chilli nursery.

Forty four isolates of *Azospirillum* obtained from chilli roots collected from different chilli growing areas of Thiruvananthapuram district were screened for *in vitro* nitrogen fixation, IAA production and biomass production. The *Azospirillum* isolate Az-2 fixed maximum amount of nitrogen followed by Az-1, Az-40, Az-24 and Az-41. With regard to *in vitro* IAA production, the *Azospirillum* isolate Az-2 produced maximum IAA followed by Az-40 (reference culture) and Az-1.

The chilli seeds treated with the different native *Azospirillum* isolates were subjected to *in vitro* screening to study the effect on growth and biomass production in chilli seedlings. Significant increase in height, root length, dry weight of shoot and root were obtained with majority of *Azospirillum* isolates. Based on *in vitro* screening, the native *Azospirillum* isolates such as Az-1, Az-2, Az-3, Az-24 and Az-41 along with reference culture Az-40 were selected and subjected to *in vivo* screening for growth, biomass production and yield. Significant increase in height, root length, yield, shoot and root

dry weight were obtained in *Azospirillum* inoculated plants as against the control. Based on *in vitro* and *in vivo* screening, native *Azospirillum* isolates, Az-1 and Az-2 were selected for further studies.

The selected *Azospirillum* isolates viz., Az-1 and Az-2 along with reference culture Az-40 were characterized, by studying the utilization of different carbon sources, biotin requirement, acidification of peptone based glucose medium, catalase activity and growth at different pH. When glucose was used as carbon source, the isolate Az-1 showed maximum growth whereas isolate Az-2 showed maximum growth both in glucose and fructose. However the isolate Az-1 acidified the peptone based glucose medium whereas Az-2 and Az-40 did not acidify the peptone based glucose medium. The isolate Az-1 is related to *A. lipoferum* whereas Az-2 and Az-40 has the characters similar to *A. brasilense*. Regarding the growth of *Azospirillum* isolates in different pH, the isolate Az-1 grew well at pH 5, 6 and 7 whereas Az-2 and Az-40 grew well at pH 6 and 7.

Effect of combined inoculation of the selected native AMF cultures (M_8 and M_9) and *Azospirillum* isolates (Az-1 and Az-2) on damping off suppression and growth improvement in chilli was studied. Pre-inoculation of M_9 culture alone ($M_9A_0P_1$) recorded the least disease incidence. Eventhough dual inoculation of AMF and *Azospirillum* ($M_9A_2P_1$) could suppress the damping off, *Azospirillum* had no direct effect on disease suppression. However dual inoculation of AMF and *Azospirillum* ($M_9A_2P_0$) could significantly increase plant height, number of branches, dry weight of plants and yield. The AMF culture M_9 was found to be the best in suppressing damping off incited by *Pythium aphanidermatum* whereas for growth

improvement of chilli, dual inoculation of AMF culture M_9 along with *Azospirillum* (Az-2) was found to be the best.

The present investigation emphasizes the importance of pre-inoculation of AMF in the chilli nursery as a prophylatic measure to prevent pathogen attack. The technology of combined inoculation of AMF and *Azospirillum* could be recommended for adoption by the vegetable farmers. Eventhough the present study was carried out in chilli, the same cultures could be recommended for all transplanted solanaceous vegetables after confirming the results through field trials.

References

REFERENCES

Abdalla, M.E. and Abdel-Fattah, G.M. 2000. Influence of the endomycorrhizal fungus *Glomus mosseae* on the development of peanut pod rot disease in Egypt. *Mycorrhiza* **10** : 29-35

Abdul-Khaliq, Gupta, M.L. and Kumar, S. 2001. The effect of VAM fungi on growth of pepper mint. *Indian Phytopath.* **54** : 82-84

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-Nahidh, S.I. and Gomah, A.H.M. 1991. Response of wheat to dual inoculation with VA mycorrhiza and *Azospirillum* fertilized with NPK and irrigated with sewage effluent. *Arid Soil Research and Rehabilitation* **5** : 83-96

Ames, R.N. and Linderman, R.G. 1978. The growth of Easter lily (*Lilium longiflorum*) as influenced by vesicular arbuscular mycorrhizal fungi, *Fusarium oxysporum* and fertility level. *Can. J. Bot.* **56** : 2773

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, Kerala Forest Research Institute, Peechi, p. 64

Andreeva, I.N., Redkina, T.V. and Izmailov, S.F. 1993. The involvement of indole acetic acid in the stimulation of Rhizobium-legume symbiosis by *Azospirillum brasilense*. *Russian J. Plant Physiol.* **40** : 780-784

*Anwar, C., Djokosudardjo, S. and Hadi, S. 1979. The effect of method of inoculation with mycorrhizal fungi on the development of mycorrhizal and growth of *Pinus merkusii*. *Laporan Lembaga Penelitian Hutan* No. 325, p. 33

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

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 mycorrhiza on Sustainable Agriculture and Natural Ecosystems.* Gianinazzi, S. and Schuepp, H. (eds.). Birkhauser, Basel, pp. 47-60

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

Baca, B.E., Soto- Urzua, I., Xochichua- Corona, Y.G. and Cuervo- Garcia, A. 1994. Characterization of two aromatic aminoacid amino transferase and production of IAA in *Azospirillum* strains. *Soil Biol. Biochem.* **26** : 57-63

Bagyaraj D.J. and Sreeramulu, K.R.1982. Preinoculation with VA mycorrhiza improves growth and yield of chilli transplanted in the field and saves phosphatic fertilizer. *Plant Soil* **69** : 375-381

Balakrishna, R., Bagyaraj, D.J. and Mallesha, B.C. 1996. Selection of efficient VA mycorrhizal fungi for papaya. *Biological Agriculture and Horticulture* **13** : 1-6

*Balandreau, J. 1975. Measure de'lative nitrogenasique des microorganisms fixateurs libres d' azote de larhizosphere de quelques graminees *Rev. Ecol. Biol. Sol.* **12** : 273-290

Baldani, D. and Dobereiner, J. 1980. Host plant specificity in the infection of cereals with *Azospirillum* sp. *Soil Biol. Biochem.* **12** : 433-439

Balikai, R.A., Rajashekar, D.W. and Patil, G.M. 1998. Influence of FYM, Vermicompost, RDF, Mycorrhiza and their combinations on the establishment of mulberry cuttings in summer nursery. *Advances in Agricultural Research in India* **94** : 147-150

Bashan, Y. 1986. Enhancement of wheat root colonization and plant development by *Azospirillum brasilense* following temporary depression of rhizosphere microflora. *Appl. Environ. Microbiol.* **51** : 1067-1071

Bashan, Y. 1991. Airborne transmission of the rhizosphere bacterium *Azospirillum. Microbiol. Ecol.* **22** : 257-270

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

*Beijerinck, M.W. 1925. Uberein spirillum, Welchen freien Stickstoffbinden Kann? *Zentbl. Bakt. Parasitkde. Abt II* **63**: 352-359

Bhargava, K.S. 1941. *Pythium aphanidermatum* (Edson) Fitz. on *Carica papaya. Curr. Sci.* **32** : 226-227

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

Bodker, L., Kjoller, R. and Rosendahl, S. 1998. Effect of phosphate and the AMF (*Glomus intraradices*) on disease severity of root rot of peas (*Pisum sativum*) caused by *Aphanomyces euteiches. Mycorrhiza* **8** : 169-174

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

*Bulow, J.F.W., Van and Doberiener, J. 1975. Potential for nitrogen fixation in maize genotypes in Brazil. *Proc. Natl. Acad. Sci.*, USA **72** : 1115-1119

Burris, R.H., Okon, Y. and Albrecht, S.I. 1977. Physiological studies of *Spirilum lipoferum*. In : *Genetic Engineering for Nitrogen Fixation*, A. Hollaender (ed.). Plenum Press, New York, pp. 445-450

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 pert-perlite mixture. *Plant Soil* **148** : 1-6

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

*Cassiolata, A.M.R. and Melo, I.S. De. 1991. Interaction between *Rhizoctonia solani* and VAM fungus in tomato. *Summa Phytopathologia* **17** : 195-200

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

Chakravarthy, P. and Mishra, P.R. 1986. The influence of VA mycorrhiza on wilting of *Albizia procera* and *Dalbergia sisoo*. *Eur. J. For. Pathol.* **16** : 91-97

Champawat, R.S. 1991. Interaction between VA- mycorrhizal fungi and *Fusarium oxysporum* fsp. *cumini*- their effects on cumin. *Proc. Indian Natl. Sci. Acad. Part B. Biological Sciences* **57** : 59-62

Chandramohan, D. and Mahadevan. 1968. Indole acetic acid metabolism in soil. *Curr. Sci.* **37** : 112-113

Chandrashekara, C.P. and Patil, V.C. 1997. Influence of VAM inoculation and phosphorus on drymatter production and seed yield of two sunflower genotypes. *J. Oilseeds Res.* **14** : 210-215

Charyulu, P.B.B.N. and Rao, V.R. 1980. Influence of various soil factors on nitrogen fixation by *Azospirillum*. *Soil Biol. Biochem.* **12** : 343-346

Chezhiyan, N., Balasubramani, P., Harris, C.V. and Ananthan, M. 1999. Effect of inorganic fertilizers on growth and yield of hill banana var. Virupakshi. *South Indian Hort.* **47** : 161

*Chu, E.Y., Endo. T., Sten, R.L.B., Albuquesque, F.C. and De. 1997. Evaluation of AMF inoculation on the incidence of Fusarium root rot of black pepper. *Fitopatologia Brasileira* **22** (2) : 205-208

Chulan, A.H., Shaji, G.T. and Christine, Z.A. 1990. Interactions of mycorrhizal fungi with root pathogens of cocoa. In: *Current trends in mycorrhizal research, Proc. Natl. Con. Mycorrhiza*, Hisar, pp. 78-79

*Cigsar, S., Sari, N. and Ortas, I. 2000. The effects of VAM on the plant growth and nutrient uptake of cucumber. *Turkish Journal of Agriculture and Forestry* **24** : 571-578

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

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* **185** (2) : 223-232

Crozier, A., Arruda, P., Jasmin, J.M., Monteiro, A.M. and Sandberg, B. 1988. Analysis of indole-3-acetic acid and related indoles in culture medium from *Azospirillum lipoferum* and *Azospirillum brasilense*. *Appl. Environ. Microbiol.* **54** : 2833-2837

Dar, G.H., Zargar, M.Y. and Beigh, G.M. 1997. Biocontrol of Fusarium root rot in the common bean by using symbiotic *Glomus mosseae* and *Rhizobium leguminosorum. Microbial Ecology* **34** : 74-80

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

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

Day, J.M. and Dobereiner, J. 1976. Physiological aspects of nitrogen fixation by a *Spirillum* from Digitaria roots. *Soil Biol. Biochem.* **8** : 45-50

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

*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. 1979. The influence of endotrophic mycorrhiza on plant diseases I. Colonization of tomato plants by *Fusarium oxysposum* fsp. *lycopersici. Phytopath. Z.* **95**: 105-110

*Depolli, H., Bohlool, B.B and Dobereiner, J. 1979. *Int. Workshop on Associative N_2 Fixation Piracicatia*, Brazil.

Devadoss, R. 1971. Studies on damping off of vegetables. M.Sc (Ag) thesis, TNAU, Coimbatore, 123 pp.

Dewan, G.I. and Subba Rao, N.S. 1979. Seed inoculation with *Azospirillum brasilense* and *Azotobacter chroococcum* on the root biomass of rice (*Oryza sativa*). *Plant soil* **53** : 295-300

Dobbelaere, S. Eroonenborghs, A., Thys, A., Broek, A.V. and Vanderleyden, J. 1999. Phytostimulatory effect of *Azospirillum brasilense* wild type and mutant strain altered in IAA production on wheat. *Plant Soil* **212** : 155-164

*Dobereiner, J. 1983. Ten years of *Azospirillum*. *Azospirillum* II *Genetics, Physiology, Ecology*. Kling Muller, W. (ed.). Birkhanaser, Basel, pp. 9-23

*Dobereiner, J. and Day, J.M. 1976. Associative symbiosis in tropical grasses: Characterization of microorganisms and dinitrogen fixing sites. In: *Proc. 1st International symposium on nitrogen fixation*. W.E. Newton and C.J. Nyman (eds.) Washington State University Press, pp. 518-538

Fallik, E., Okon, Y., Epstein, E., Goldmen, A. and Fisher, M. 1989. Identification and qualification of IAA and IBA of *Azospirillum brasilense* inoculated maize roots. *Soil Biol. Biochem.* **21** : 169-189

*Ferguson, J.J. and Menge, J.A. 1986. Response of citrus seedlings to various field inoculation methods with *Glomus deserticola* in fumigated nursery soils. *Journal of American Society for Horticultural Science* **111** : 288-292

*Fernandez, F., Rodriguez, E.I. and Gomez, 1999. Characterization of the effectiveness of a new mycorrhizal inoculant in Poaceae. *Cultivos Tropicales* **20** : 9-14

Fernando, W.G.D. and Linderman, R.G. 1997. The effect of mycorrhiza (*Glomus intraradices*) colonization on the development of root and stem rot (*Phytophthora vignae*) of cowpea. *Journal of the National Science Council of Srilanka* **25** : 39-47

Frobischer, M. 1964. *Fundamentals of Microbiology*. V.R. Saunders Co., Philadelphia

Fulchieri, M. and Frioni. L. 1994. *Azospirillum* inoculation on maize: Effect on yield in a field experiment in Central Argentina. *Soil Biol. Biochem.* **26** : 921-923

Gallo, M. and Fabbri, P. 1990. Inoculation of *Azospirillum brasilense* on chickpea. *Symbiosis* (Rehovot) **9** : 283-287

Gangopadhyay, S. and Das, K.M. 1987. Control of soil borne diseases of rice through vesicular arbuscular mycorrhiza. *Proc. Mycorrhizae Round Table Workshop*, New Delhi, pp. 560-580

Gattani, M.L and Kaul, T.N. 1951. Damping off of tomato seedlings, its causes and control. *Indian Phytopath.* **4** : 157-161

Gaunt, R.E. 1978. Inoculation of vesicular arbuscular mycorrhizal fungi on onion and tomato seeds. *New Zealand J. Bot.* **16** : 69-77

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

Gopalaswamy, G. and Vidhyasekaran, P. 1987. Effect of method of applying *Azospirillum brasilense* on rice yield. *Int. Rice Res. Newsl.* **12** : 56

Govindan, 1982. Studies on biological nitrogen fixation by *Azospirillum* in pearl millet. M.Sc. (Ag). Thesis, Tamil Nadu Agricultural University, Coimbatore, 80 pp.

Govindan, H. and Purushothaman, D. 1984. Production of phytohormones by nitrogen fixing bacterium *Azospirillum*. *Agric. Res. J. Kerala* **22** : 133-138

Govindan, M. and Chandy, K.C. 1985. Utilization of the diazotroph *Azospirillum* for inducing rooting in pepper cuttings. *Curr. Sci.* **54** : 1186-1188

Govindaswamy, K.N., Lakshminarayan, T. and Subramanian, S. 1992. Effect of submergence timing and application of *Azospirillum* and K on direct seeded rice. *IRRN* **17** : 18

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

Graham, J.H. 1988. Interaction of mycorrhizal fungi with soil borne plant pathogen and other organism. *Phytopathology* **78** : 365

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

Gupta, P.P., Rakesh kumar and Jalali, B.L. 1999. Response of different vesicular arbuscular mycorrhizal fungi on cowpea. *Plant Dis. Res.* **14** : 25-30

Gurubatham, J., Thamburaj, S. and Kandasamy, D. 1989. Studies on the effect of biofertilizers on the bulb yield in Bellary onion. *South Indian Hort.* **37** : 150-153

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

Hadas, R. and Okon, Y. 1987. Effect of *Azospirillum brasilense* inoculation on root morphology and respiration in tomato seedlings. *Biol. Fertil. Soils.* **5** : 241-247

Han, S.O. and New, P.B. 1998. Variation in nitrogen fixing ability among natural isolates of *Azospirillum*. *Microbial Ecology* **36** : 193-201

Harlapur, S. I., Srikant Kulkarni., Hegale, R.K., Sreenivasa, M.N. and Rajashekara, E. 1990. Interaction between VAM and *Sclerotium rolfoii* in wheat. *Karnataka J. Agric. Sci.* **3** : 203-207

*Harley, J.L. and Smith, S.E. 1983. *Mycorrhizal Symbiosis*. Academic press, London

Hartman, A., Singh, M. and Kingmuller, W. 1983. Isolation and characterization of *Azospirillum* mutants excreting high amounts of indole acetic acid. *Can. J. Microbiol.* **29** : 916-923

Hayman, D.S., Morris, E.J. and Page R.J. 1981. Methods for inoculating field crops with mycorrhizal fungi. *Ann. Appl. Biol.* **99** : 247-253

Humphries, E.C. 1956. Mineral composition and ash analysis. In : *Modern Method of Plant Analysis* Vol. I. K. Peach and M.V. Tracey (eds.). Springer Verlag, Berlin, pp. 468-502

Hwang, S.F., Chakravarty, P. and Prevost, D. 1993. Effect of rhizobia, metalaxyl and VA 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 Chakravarthy, P. 1992. Effect of vesicular arbuscular mycorrhizal fungi on the development of Verticillium and Fusarium wilts of alfalfa. *Plant Dis.* **76** : 239-243

Indira, B.N. and Bagyaraj, D.J., 1997. Associative diazotrophs of sugarcane (*Saccharum officinarum* L.) cultivars. *Indian J. Microbiol.* **37** : 95-98

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

Islam, R. and Ayanaba, A. 1981. Growth and yield responses of cowpea and maize to inoculation with *Glomus mosseae* in sterilized soil under field conditions. *Plant Soil* **63** : 505 –510

Jain, D.K. and Patriquin, D.G. 1985. Characteristics of a substance produced by *Azospirillum* which causes branching of wheat root hairs. *Can. J. Microbiol.* **31** : 206-210

*Jalali, B.L. 1983. Biological suppression of root disease fungi by vesicular arbuscular mycorrhizal system. *Proc. 4th Int. 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. I. *Soil and Planta*. Arora, K., Rair, B., Mukerji, K.G. and Knudsen, G.R. (eds.). M. Dekker Inc., New York, pp. 131-154

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

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

Jalali, B.S. 1987. Vesicular arbuscular mycorrhiza. Progress and Projection. *Proc. Mycorrhiza Round table Workshop*, New Delhi, pp. 226-237

Jeevajothi, L., Mani, A.K., Pappiah, C.M. and Rajagopalan, R. 1993. Influence of N, P, K and *Azospirillum* on yield of cabbage. *South Indian Hort.* **41** : 270-273

Jha, R.K. and Mishra, S. 1999. Effect of *Azospirillum* inoculation on nitrogen fixation and growth of sweet potato. *J. Appl. Biol.* 9 : 49-51

Jjepkema, J.D. and Burris, R.H. 1976. Nitrogenase activity associated with some wisconsin prairie grasses. *Plant Soil* **45** : 81-94

Johnkennedy, Z. and Rangarajan, M. 2001. Biomass production, root colonization and phosphoatase activity of six VA-mycorrhizal fungi in papaya. *Indian Phytopath.* **54** (1) : 72-77

Joseph, P.J. 1997. Management of rhizome rot of ginger using VA mycorrhizae and antagonists. Ph.D. thesis, Kerala Agricultural University, Thrissur, 192 pp.

Joseph, P.J. and Sivaprasad, P. 2000. Biocontrol potential of arbuscular mycorrhizal fungal isolates against rhizome rot of ginger. *Proc. of Indian Phytopathological society- Golden Jubilee. International Conference on Integrated Plant Disease Management for Sustainable Agriculture*. Indian Phytopathological Society, p. 399.

Joseph, P.J., Sivaprasad, P., Lekha, K. and Vijayan, M. 1995. Vesicular-arbuscular mycorrhizal colonization in ginger and its influence on the natural incidence for rhizome rot. In: *Mycorrhizae; bio fertilizers for the future Proc. Third Nat. Conf. on Mycorrhiza.* Alok Adholeya and Sujan Singh (eds.) Tata Energy Research Institute, New Delhi, pp. 77-80

Kandasamy, D., Palanisamy, D. and Oblisamy, G. 1988. Screening of germplasm of sweet potato for VA mycorrhizal fungal occurrence and response of the crop to the inoculation of VAM fungi and *Azospirillum. J. Root Crops* **14** : 37-42

Kapulnik, Y., Gafny, R. and Okon, Y. 1985. Effect of *Azospirillum* sp. inoculation on root development and nitrate uptake in wheat in hydroponic systems. *Can. J. Bot.* **63** : 627-631

Karthikeyan, M. 1981. Effect of Azospirillum inoculation on rice under graded levels of nitrogen. M.Sc. (Ag.) thesis Tamil Nadu Agricultural University, Coimbatore, 93 pp.

KAU. 1996. *Package of Practices Recommendations.* Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, 266 pp.

Kaushik, J.C., Paramod Dabas and Rakesh Kumar, 2000. Impact of *Glomus mosseae* inoculation on root pathogen in *Acacia nilotica* and *Dalbergia sisoo* seedlings. *Indian J. For.* **29** : 238-240

Kavimandan, S.K., Subba Rao, N.S. and Mohvir, A.V. 1978. Isolation of *Spirillum lipoferum* from stems of wheat and nitrogen fixation in enrichment cultures. *Curr. Sci.* **47** : 96-98

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

Kennedy, R.W. and Chellapillai, K.L. 1998. Synergistic effect of VAM, *Azospirillum* and phosphobacteria on growth response and nutrient uptake of shola tree species. *Indian J. For.* **21** : 308-312

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

Kreig, N.R. and Dobereiner, J. 1984. Genus *Azospirillum*. In : *Bergey's Manual of Systematic Bacteriology* Vol. I. Kreig, N.R., Williams and Wilkins (eds.). Baltimore, pp. 94-104

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

Kulkarni, S.A., Srikant Kulkarni and Sreenivasa, M.N. 1997. Interaction between vesicular arbuscular mycorrhiza and *Sclerotium rolfii* in groundnut. *Karnataka J. Agric. Sci.* **10** : 919-921

Kumari, S.M.P. and Balasubramanian, A. 1993. Effect of combined inoculation of VAM and *Azospirillum* on growth and nutrient uptake by coffee seedlings. *Indian Coffee* **67** : 5-11

Kundu, B.S. and Krishna, S. 1990. Carbon metabolism in *Azospirillum brasilense*. *Indian J. Microbiol.* **30** : 29-33

Kundu, B.S., Sangwan, P., Sharma, P.K. and Nandwal, A.S. 1997. Response of pearl millet to phytohormone produced by *Azospirillum brasilense*. *Indian J. Plant Physiol.* **2** : 101-104

Lakshmikumari, M., Kavimandan, S.K. and Subba Rao, N.S. 1976. Occurrence of nitrogen fixing *Spirillum* in roots of rice, sorghum, maize and other plants. *Indian. J. Exp. Biol.* **14** : 638-639

Lakshmikumari, M., Lakshmi, V., Nalini, P.A. and Subba Rao, N.S. 1980. Reactions of *Azospirillum* to certain dyes and other usefulness in enumeration of the organism. *Curr. Sci.* **49** : 438-439

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

Lukin, S.A., Kozhevin, P.A. and Zuyagintser, D.G. 1992. Spatial distribution of *Azospirillum* in the rhizosphere of barley. *Soil Fert.* **55** : 1033

Mallesha, B.C., Padmavathi Ravindra, T. and Bagyaraj, D.J. 1994. Response of tomato to different VA mycorrhizal fungi. *J. Soil Biol. Ecol.* **14** : 36-40

Marschner, H. and Dell, B. 1994. Nutrient uptake in mycorrhizal symbiosis. *Plant Soil* **159** : 89-102

Mascarua, Esparza, M.A., Villa-Gonzalez, R. and Caballero-Mellado, J. 1988. Acetylene reduction and indole acetic acid production by *Azospirillum* isolates from cactaceous plants. *Soil Fert.* **51** : 782

Matsubara, Y., Kayukawa, Y., Yano, M and Fukui, H. 2000. Tolerance of asparagus seedlings infected with AMF to violet root rot caused by *Helicobasidium mompa. Journal of the Japanese Society for Horticultural Science* **69** : 552-556

*Menze, H. 1985. Interactions between *Azospirillum* and VA mycorrhiza in Gramineae at different soil pH level. *Zeitschrift für Aucker-und Pflanzenbau* **155** : 232-237

Mayer, J.R. and Linderman, R.G. 1986. Selective influence on population of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizal formed by *Glomus fasciculatum. Soil Biol. Biochem.* **18** : 191-196

*Middleton, J.T. 1943. The taxonomy, host range and geographic distribution of the genus *Pythium. Mem. Torrey Bot. Club* **20** : 1-171

*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.* **11** : 171-196

Murumkar, D.R. and Patil, P.I. VAM-diazotrophs bell pepper symbiosis. *Journal of Maharashtra Agricultural Universities* **21** : 394

Neelima, R. and Janardhanan, K.K. 1996. Response of dual inoculation with VAM and *Azospirillum* on the yield and oil content of palmarosa. *Microbiol. Res.* **151** : 325-328

Nelson, L.M. and Knowles, R. 1978. Effect of oxygen and nitrate on nitrogen fixation and denitrification by *Azospirillum brasilense* grown in continuous culture. *Can. J. Microbiol.* **24** : 1395-1403

*Neves, M.C.P., Nery, M. and Day, J.M. 1976. Efeito da temperaturana fixaca de nitrogenio de estirpes de Spirillum sp. isolated de Degitoria sp e. milho. In : *Congresso Brasilferro de ciencias do solo 15,* Anain, *Campinas* sp. pp. 143-149

Neyra, C.A. and Dobereiner, J. 1977. Nitrogen fixation in grasses. In *Adv. Agron.* N.C. Brady (ed.). Academic Press, New York **29** : 1-38

Odebode, A.C., Ladoye, A.O. and Osonubi, O. 1997. Effect of *Pythium aphanidermatum* and arbuscular mycorrhizal fungus *Glomus deserticola* on disease severity and growth of pepper. *Int. J. Trop. Plant Dis.* **15** : 85-92

Okon, Y., Albrecht, S.L. and Burris, R.H. 1976. Carbon and ammonia metabolism of *Spirillum lipoferum*. *J. Bact.* **128** : 592-597

Okon, Y., Albrecht, S.L. and Burris, R.H. 1977. Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. *J. Appl. Environ. Microbiol.* **33** : 85-88

Omay, S.H., Schmidt, W.A., Martin, P. and Bangerth, F. 1993. Indole acetic acid production by the rhizosphere bacterium *Azospirillum brasilense* under *in vitro* condition. *Can. J. Microbiol.* **39** : 187-192

Pacovsky, R.S., Fuller, G. and Paul, E.A. 1985. Influence of soil in the interaction between endomycorrhiza and *Azospirillum* in sorghum. *Soil Biol. Biochem.* **17** : 525-531

Padma, T.M.R. 1988. Effect of interaction between VA-mycorrhizae and graded levels of phosphorus on the growth of papaya. M.Sc. (Ag.) thesis, Tamil Nadu Agricultural University, Coimbatore, 115 pp.

Padshetty, N.S., Ramaswamy, K. and Dayanand, A. 1986. Isolation and screening of native *Azospirillum* species and their effects on wheat. *Research and Development Reporter* **3** : 45-49

Panwar, J.D.S and Ompal Singh 2000 Response of *Azospirillum* and *Bacillus* on growth and yield of wheat under field conditions. *Indian J. Plant Physiol.* **5** : 108-110

Paramaguru, P. and Natarajan, S. 1993. Effect of *Azospirillum* on growth and yield of chilli grown under semidry condition. *South Indian Hort.* **41** : 80-83

Parvatham, A., Vijayan, K.P. Nazar, A. 1989. Effect of *Azospirillum* on growth and nutrient uptake of Pusa sawani bhindi. *South Indian Hort.* **37** : 227-229

Petgen, M., Schropp, A and George, E. Romheld, V. 1998. Influence of different inoculum places of mycorrhizal fungus *Glomus mosseae* on mycorrhizal colonization in grapevine rootstocks (*Vitis* sp.) *Vitis* **37** : 99-105

Pfleger, F.L., Stewart, E.L. and Noyd, R.K. 1994. Role of VAM fungi in mine land revegetation. In : *Mycorrhizae and Plant Health*. Pfleger, F.L. and Linderman, P.G. (eds.). APS, Minnesota, USA, pp. 47-81

Phillips, J.M. and Hayman, D.S. 1970. Improved procedure of clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **55** : 158-161

Powell, C.L.I. 1979. Inoculation of white clover and ryegrass seed with mycorrhizal fungi. *New Phytol.* **83** : 81-85

Prashanthi, S.K., Srikant Kulkarni and Sreenivasa, M.N. 1997. Integrated management of root rot disease of safflower caused by *Rhizoctonia bataticola. Environment and Ecology* **15** : 800-802

Purushothaman and Vijila, K. 1988. Isolation of *Azospirillum* strain resistant to high soil temperature. *Curr. Sci.* **57** : 962-963

Purushothaman, D. and Oblisami, G. 1985. Occurrence of *Azospirillum* in certain problem soils of Tamil Nadu. *Symp. Soil. Biology*. Haryana Agricultural University, Haryana, p. 120-128

Quarles, W. 1999. Plant disease biocontrol and VAM fungi. *IPM Practitioner* **21** : 1-9

Rabie, G.H. 1998. Induction of fungal disease resistance in *Vicia faba* by dual inoculation of *Rhizobium leguminosorum* and VAM fungi. *Mycopathologia* **141** : 159-166

Rai, S.N. and Gaur, A.C. 1982. Nitrogen fixation by *Azospirillum* spp. and effect of *Azospirillum lipoferum* on the yield and N-uptake of wheat Crop. *Plant Soil* **69** : 233-238

Rajagopal, B. and Ramarethinam, S. 1997. Interaction between vesicular arbuscular mycorrhiza and tea roots. *Planter's chronicle* **92** : 263-268

Rajput, R.L. 1999. Effect of biofertilizer application on the yield of bajra. *Bhartiya Krishi Anusandhan Patrika* **14** : 43-46

Ramaraj, B and Shanmugam, 1986. Growth responses of vesicular arbuscular mycorrhizae on pulses. *Madras Agric. J.* **73** : 32-35

Rao, V.R., Charyulu, P.B.B.N., Nayak, D.N and Ramakrishana, R. 1978. Nitrogen fixation by free living organisms in tropical rice soils. In : *Limitations and Potentials*. Plenum press, New York, pp. 354

Ratti, N., Alam, M., Sharma, S. and Janardhanan, K.K. 1998. Effects of *Glomus aggregatum* on lethal yellowing disease of Java Citronella caused by *Pythium aphanidermatum. Symbiosis* (Rehovot) **24**: 115-126

Reynders, L. and Vlassak, K. 1979. Conversion of tryptophon to Indole acetic acid by *Azospirillum* spp. *Soil Biol. Biochem.* **11** : 547-548

Rhodes, L.H. 1980. The use of mycorrhizae in crop production system. *Outlook Agriculture.* **10** : 275-281

Rohini Iyer and Sundararaju, P. 1993. Interaction of VA mycorrhiza with *Meloidogyne incognita* and *Pythium aphanidermatum* affecting ginger. *J. Plantn. Crops.* **21** : 30-34

Rosendahl, C.N., and Rosendahl, S. 1990. The role of vesicular-arbuscular mycorrhiza in controlling damping off and growth reduction in cucumber caused by *Pythium ultimum. Symbiosis* (Rehovot) **9** : 363-366

Rosendahl, S. 1985. Interaction between vesicular arbuscular mycorrhizal fungus, *Glomus fasciculatum* and *Aphanomyces euteiches* root rot of peas. *Phytopath. 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.). Birkhauser, Basel, p. 1-2

*Rosendahl, S., Rosendahl, C.N and Thingstrup, I. 1992. The use of vesicular arbuscular mycorrhizal fungi as a biocontrol agent. *Bulletin OILB/ SROP* **15** : 48-50

Rousta, M.J., Rastin, N.S. and Assadi, M.M. 1998. Occurrence and activity of *Azospirillum* in some soils of Iran. *Iranian J. Agric. Sci.* **29** : 285-298

*Safir, G. 1968. The influence of vesicular arbuscular mycorrhizae on the resistance of onion to *Pyrenochaeta terrestis*. 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

Sangwan and Kundu, B.S. 1992. Pearl Millet-*Azospirillum brasilense* interaction with reference to growth stimulating substances. *Indian J. Microbiol.* **32** : 405-410

*Sangwan, P. 1990. Interaction between *Azospirillum brasilense* and pearl millet with regard to growth promoting substance. M.Sc. (Ag.) thesis, Haryana Agricultural University, Hisar

Sasikumar, B. and Sarma, Y.R. 2000. Role of spices in national economy. *Indian Spices* **37** : 19-20

Sasikumar, S. 1996. Development of inoculant cultures of *Azospirillum* for rice in Kuttanad. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 98 pp.

Savalgi, V.P. and Veena, S. 1990. Diazotrophic rhizocoenosis of *Azospirillum brasilense* with the roots of sunflower. *Science and Culture* **56** : 125-127

*Schenck, N.C. and Kellam, M.K. 1978. The influence of vesicular arbuscular mycorrhiza on disease development. *Fla. Agric. Exp. Stn. Bull.* : 799

Schonbeck, F and Dehne, H. 1977. Damage to mycorrhizal and nonmycorrhizal cotton seedlings by *Thielaviopsis basicola. Plant Dis. Rep.* **61** : 266-268

Secilia, J. and Bagyaraj, D.J. 1987. Bacteria and actinomycetes associated with pot cultures of vesicular arbuscular mycorrhiza. *Can. J. Microbiol.* **33** : 1069-1073

*Shanmugam, N. and Ramaraj, B. 1982. Preliminary studies on the inoculation of seeds with vesicular arbuscular mycorrhizal fungi. *National Seminar on Seed Pathology,* Tamil Nadu Agricultural University, Coimbatore

Shrihari, P.C. and Sreenivasa, M.N. 1998. Optimum dose of arbuscular mycorrhizal inoculum for chilli in two soil types. *Karnataka J. Agric. Sci.* **11** : 696-700

Singh, R.S. and Singh, H., 1989. Influence of vesicular-arbuscular mycorrhizae on root infection of citrus seedlings with *Macrophomina phaseolina. Indian Phytopath.* **42** : 300

Sivaprasad, P. 1993. Management of root diseases of important spice crops of Kerala with VA mycorrhizae. Annual Report of DBT project - 1993. Kerala Agricultural University, Trichur, India

Sivaprasad, P. 1995. Management of root disease of important spice crops of Kerala with VA mycorrhiza, DBT Project Report, Kerala Agricultural University, Thrissur, India

Sivaprasad, P., Joseph, P.J. and Balakrishnan, S. 2000. Management of foot rot of black pepper with arbuscular mycorrhizal fungi. In : *Proc. of Indian Phytopathological society- Golden Jubilee. International Conference on Integrated Plant Disease Management for Sustainable Agriculture*. Indian Phytopathological Society, p. 341

Sivaprasad, P., Robert, C.P., Vijayan, M., and Joseph, P.J. 1995. Vesicular arbuscular mycorrhizal colonization in relation to foot rot disease intensity in black pepper. In : *Mycorrhizae biofertilizers for the future. Proc. Third Nat. Conf. on mycorrhiza.* Adholeya A, and Singh, S. (eds.) Tata Energy Research Institute, New Delhi, pp. 137-140

Sivaprasad, P., Sulochana, K.K. and Nair, S.K. 1990. Comparative efficiency of different VA mycorrhizal fungi on cassava. *J. Root Crops* **16** : 39-40

Slezack, S., Dumas-Gaudol., Paynot, M. and Gianinazzi, S. 2000. Is a fully established AM symbiosis required for bioprotection of *Pisum sativum* roots against *Aphanomyces euteiches* ? *Molecular Plant Microbe Interaction* **13** : 238-241

Sonawane, R.B. and Konde, B.K. 1998. Response of grape vine to dual inoculation of VAM fungi and diazotrophs. *J. Maharashtra Agricultural Universities* **22** : 356-357

Sowmini, R. 1961. Studies on Phycomycetes in Agricultural soils with special reference to Pythiaceae. M.Sc. (Ag.) thesis, University of Madras, 160 pp.

Sreeramulu, K.R. and Bagyaraj, D.J. 1986. Field response of chilli to VA mycorrhiza on black clayey soil. *Plant Soil* **93** : 299-302

Sreeramulu, K.R. and Bagyaraj, D.J. 1999. Screening of AMF in cardamom cv. Mysore. *J. Plantn. Crops* **27** : 207-211

Sreeramulu, K.R., Dyaladoss, D., Reddy, T.K.R. and Patil, R.H. 1988. Interaction effects of VA mycorrhiza and *Azospirillum* on growth and uptake of nutrients in maize. *Indian J. Microbiol.* **28** : 247-250

St. Arnand, M., Hamel, C., Canon, M. and Fortin, J.A. 1994. Inhibition of *Pythium ultimum* in roots of 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

Subba Rao, N.S., Tilak, K.V.B.R. and Singh, C.S. 1985. Effect of combined inoculation of *Azospirillum brasilense* and vesicular arbuscular mycorrhiza on pearl millet. *Plant Soil* : 283-286

Subba Rao, N.S., Tilak, K.V.B.R., Lakshmikumari, M. and Singh, C.S. 1980. *Azospirillum*, a new bacterial fertilizer for tropical crops. *Science Reporter, CSIR* **16** : 690-692

Subbiah, K. 1994. Effect of N, P and biofertilizers on yield and nutrient uptake in chilli and bellary onion. *Madras Agric. J.* **81** : 277-279

Subhashini, D.V. and Potty, V.P. 1992. Interaction between *Glomus constrictum* and *Sclerotium bataticola* in sorghum. In : *New Trends in Biotechnology.* Subba Rao, N.S., Balagopalan, C. and Ramakrishna, S.V.(eds.) Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, pp. 281-286

Subramonian, P. 1987. Studies on the effect of methods of *Azospirillum* inoculation and nitrogen levels on the yield of low land rice (IR-50). M.Sc. (Ag.) thesis, Tamil Nadu Agricultural University, Coimbatore. 105 pp.

Sukhada, M. 1987. Field response of tomato to inoculation with VA mycorrhizal fungus *Glomus fasciculatum* and *Azotobacter vinelandii. Plant Soil* **98** : 295-297

Swarupa, S.G. 1996. Study on the effect of biofertilizers on the growth of C x R coffee seedlings. *J. Coffee Res.* **26** : 62-66

Tarrand, J.J., Kreig, N.R. and Dobereiner, J. 1978. Taxonomic study of the *Spirillum lipoferum* group with description of a new genus *Azospirillum* gen. Nov. and two species *Azospirillum lipoferum* (Beijerinck) Comb. Nov. and *Azospirillum brasilense* sp. Nov. *Can. J. Microbiol.* **24** : 967-980

Thippeswamy, R. and Sreenivasa, M.N. 1999. Physiological nutritional and yield responses of tomato to the inoculation of *Glomus fasciculatum* under water stress condition. *J. of Maharashtra Agricultural Universities* **23** : 265-268

Thomas, L., Mallesha, B.C. and Bagyaraj, D.J. 1994. Biological control of damping off of cardamom by VA mycorrhizal fungus *Glomus fasciculatum. Microbiol. Res.* **149** : 413-417

Tien, T.M., Gaskins, M.H. and Hubbel, D.H. 1979. Plant growth susbstances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet. *Appl. Environ. Microbiol.* 37 : 1016-1024

Trotta, A., Varese, G.C., Gnavi, E., Fusconi, A., Sampo, S. and Berta, G. 1996. Interactions between the soil borne root pathogen *Phytophthora nicotianae* var. *parasitica* and the AMF *Glomus mosseae* in tomato plant. *Plant Soil* **185** : 199-209

Umali-Garcia, M. 1978. Process of infection of *Panicum maximum* by *Spirillum lipoferum*. *Ecol. Bull.* (Stockholm) **26** : 273-379

Venkateswarlu, B. and Rao, A.V. 1983. Response of pearl millet to inoculation with different srains of *Azospirillum brasilense*. *Plant Soil* **74** : 379-386

Vidhyasekaran, P. 1990. Interaction between plants and pathogens resulting in resistance to diseases. In : *Physiology of Disease Resistance in Field Crops*. Today and Tomorrow's Printers Publishers, New Delhi, pp. 1-10

Vort, F. and Grente, J. 1985. Research for a method of biological control of ink disease by using mycorrhizal symbiosis. *Eur. J. For. Pathol.* **15** : 379-383

*Woodhead, S.H. 1978. Mycorrhizal infection of soybean root as it influences *Phytophthora* root rot. Ph.D. diss. University of Illinois, Urbana

Yamini Varma, C.K. 1995. Effect of *Azospirillum* inoculation on establishment and growth of bush pepper. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 87 pp.

Zambolin, L. and Schenck, N.C. 1983. Reduction of the effect of pathogenic root infecting fungi on soybean by mycorrhizal fungus, *Glomus mosseae*. *Phytopathology* **73** : 1402-1405

*Original not seen

Appendix

APPENDIX – I

COMPOSITION OF DIFFERENT MEDIA

(a) Potato dextrose agar

Potato	:	200 g
Dextrose	:	20 g
Agar	:	20 g
Distilled water	:	1 litre

(b) Nitrogen free Bromothymol blue (NFB) medium

(Baldani and Dobereiner, 1980)

Malic acid	:	5 g
K_2HPO_4	:	0.5 g
$MgSO_4.\ 7H_2O$	:	0.2 g
NaCl	:	0.1 g
$CaCl_2$	:	0.02 g
Trace element solution	:	2 ml
Alcoholic solution of		
Bromothymol blue (5%)	:	2 ml
Fe EDTA (1.64 % w/v aqueous)	:	4 ml
Vitamin solution	:	4 ml
KOH	:	4 g
Agar	:	1.75 g
Distilled water	:	1 litre
pH	:	6.8

Trace element solution is prepared as follows

$Na_2M_0O_4.\ 2H_2O$	:	200 mg
$MnSO_4.\ H_2O$	:	250 mg
H_3BO_3	:	280 mg
$CuSO_4.\ 5H_2O$	:	8 mg
$ZnSO_4.\ 7H_2O$	:	24 mg
Distilled water	:	200 ml

Vitamin solution is prepared as follows

Biotin	:	10 mg
Pyridoxin	:	20 mg
Distilled water	:	100 ml

(c) Potato infusion agar (Baldani and Dobereiner, 1980)

Potato	:	200 g
Malic acid	:	2.5 g
KOH	:	2.0 g
Sucrose	:	2.5 g
Vitamin solution	:	1.0 ml
Agar	:	15 g

Washed potatoes were boiled for 30 minutes and the solution was filtered. Malic acid was dissolved in 50 ml H_2O. Two drops of bromothymol blue (0.5 % solution of ethanol) was added and pH was adjusted to 7.0 with KOH (green colour). Sucrose, vitamin solution and agar were added to the potato filtrate and made upto 1000 ml.

(d) Okon's media (Okon *et al.*, 1977, medium as modified by Lakshmikumari *et al.*, 1980)

K_2HPO_4	:	6.0 g
KH_2PO_4	:	4 g
$MgSO_4$	:	0.2 g
NaCl	:	0.1 g
$CaCl_2$	:	0.02 g
NH_4Cl	:	1.0 g
Malic acid	:	5.0 g
NaOH	:	3.0 g
Yeast extract	:	0.1 g
$Na_2M_0O_4$	:	0.002 g
$MnSO_4$	:	0.001 g
H_3BO_3	:	0.001 g
Cu $(NO_3)_2$	:	0.0004 g
$ZnSO_4$	:	0.002 g
$FeCl_3$	:	0.002 g
Bromothymol blue		
(0.5 % alcoholic solution)	:	2 ml
Distilled water	:	1 litre
pH	:	6.8

MANAGEMENT OF DAMPING OFF AND IMPROVEMENT OF GROWTH IN CHILLI (*Capsicum annuum* L.) WITH NATIVE SPECIES OF ARBUSCULAR MYCORRHIZAE AND *Azospirillum*

BY

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ABSTRACT OF THE THESIS
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ABSTRACT

Management of damping off, the most destructive disease of chilli both in nursery and main field incited by *Pythium aphanidermatum* (Edson) Fitz. using native AMF was attempted in the present investigation. Out of nine native AMF and one identified culture (*Glomus mosseae*) screened, the cultures M_8 and M_9 were found effective for suppression of damping off and growth improvement in chilli.

Application of AMF inoculum in the nursery furrows along with chilli seeds was very effective for rapid and easy colonization of AMF. Likewise application of AMF inoculum at the rate of 850 g m^{-2} was selected as the economic dose for achieving satisfactory colonization of AMF.

Azospirillum spp. were isolated from chilli roots collected from different locations of Thiruvananthapuram district. The *in vitro* nitrogen fixing capacity of the isolates ranged between 11.2 and 20 mg N g^{-1} of malate and IAA production between 21 and 55 µg ml^{-1}. Six best isolates which performed well under *in vitro* screening were selected and subjected to *in vivo* screening for growth, biomass production and yield in chilli. The isolates Az-1 and Az-2 which performed well both under *in vitro* and *in vivo* screening were selected for further studies. Based on the characterization studies it was found that the isolate Az-1 is related to *Azospirillum lipoferum* and Az-2 is similar to *Azospirillum brasilense*. The isolate Az-1 grew well at pH 5, 6 and 7 whereas the isolate Az-2 grew well at pH 6 and 7.

In the study on the interaction of native AMF and *Azospirillum* on damping off disease suppression, pre-inoculation of chilli seedlings with M_9 culture alone recorded the least disease incidence. Eventhough dual inoculation of AMF and *Azospirillum* could suppress the damping off, *Azospirillum* had no direct effect on disease suppression. However dual inoculation of AMF and *Azospirillum* ($M_9A_2P_0$) significantly increased the growth, biomass production and yield in chilli. The present study forms the first report of the synergistic effect of AMF and *Azospirillum* for the management of damping off and growth improvement in chilli.

The present investigation emphasizes the importance of pre-inoculation of AMF in the chilli nursery as a prophylatic measure to prevent pathogen attack. The technology of combined inoculation of AMF and *Azospirillum* could be recommended for adoption by the vegetable farmers. Eventhough the present study was carried out in chilli, the same cultures could be recommended for all transplanted solanaceous vegetables after confirming the results through field trials.