STANDARDIZATION OF MASS PRODUCTION TECHNIQUE FOR VA MYCORRHIZA

By

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1999

Dedicated

to

My Beloved Parents

DECLARATION

I hereby declare that this thesis entitled "Standardization of mass production technique for VA mycorrhiza" 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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CONTENTS

		Page No.
1. INTRODUCTION	.....	1 – 3
2. REVIEW OF LITERATURE	.....	4 - 20
3. MATERIALS AND METHODS	.....	21 - 32
4. RESULTS	.....	33 - 69
5. DISCUSSION	.....	70 - 93
6. SUMMARY	.....	94 - 96
REFERENCES	.....	i - xviii
APPENDICES	.....	I - IV
ABSTRACT	.....	

LIST OF TABLES

Sl. No.	Title	Page No.
1a.	Effect of different substrates on growth characteristics of mycorrhizal sorghum plants (three factor interactions)	34
1b.	Effect of different substrates on growth characteristics of mycorrhizal sorghum plants (two factor interactions)	35
1c.	Effect of different substrates on growth characteristics mycorrhizal sorghum plants (main effects)	36
2a.	Effect of different substrates on fresh and dry weights of shoots and roots of mycorrhizal sorghum plants (three factor interactions)	38
2b.	Effect of different substrates on fresh and dry weights of shoots and roots of mycorrhizal sorghum plants (two factor interactions)	39
2c.	Effect of different substrates on fresh and dry weights of shoots and roots of mycorrhizal sorghum plants (main effects)	40
3a.	Effect of different substrates on mycorrhizal root colonization, intensity and spore production in sorghum plants (three factor interactions)	42
3b.	Effect of different substrates on mycorrhizal root colonization and spore production in sorghum plants (two factor interactions)	43
3c.	Effect of different substrates on mycorrhizal root colonization and spore production in sorghum plants (main effects)	46
4	Growth characteristics of host plants inoculated with AMF	47
5	Biomass production by different host plants inoculated with AMF	49
6	AMF colonization, intensity and spore production in different host plants	51
7	Effect of foliar application of growth regulators on growth characteristics of mycorrhizal sorghum plants	53

Sl. No.	Title	Page No.
8	Effect of foliar application of growth regulators on fresh and dry weights of shoots and roots of mycorrhizal sorghum plants	55
9	Effect of foliar application of growth regulators on AMF colonization, intensity and spore production in sorghum plants	57
10	Effect of foliar application of stress inducers on plant growth characteristics of mycorrhizal sorghum plants	58
11	Effect of foliar application of stress inducers on fresh and dry weights of shoots and roots of mycorrhizal sorghum plants	60
12	Effect of foliar application of stress inducers on AMF colonization, intensity and spore production in sorghum plants	61
13	Effect of plant protectants on plant growth characteristics of mycorrhizal sorghum plants	63
14	Effect of plant protectants on fresh and dry weights of shoots and roots of mycorrhizal sorghum plants	65
15	Effect of plant protectants on AMF colonization, intensity and spore production in sorghum plants	66
16	Effect of plant protectants on the associated microflora in the rhizosphere of mycorrhizal sorghum plants	68

LIST OF FIGURES

Sl. No.	Title	Page No.
1.	Effect of different substrates on AMF spore production in sorghum plants (three factor interaction)	73
2.	Effect of growth regulators on number of leaves of mycorrhizal sorghum plants	77
3.	Effect of growth regulators on plant height of mycorrhizal sorghum plants	78
4.	Effect of growth regulators on AMF colonization in sorghum plants	80
5.	Effect of growth regulators on AMF spore production in sorghum plants	81
6.	Effect of stress inducers on AMF colonization in sorghum plants	84
7.	Effect of stress inducers on AMF spore production in sorghum plants	85
8.	Effect of plant protectants on growth characteristics of mycorrhizal sorghum plants	88
9.	Effect of plant protectants on AMF spore production in sorghum plants	90
10.	Effect of plant protectants on the associated microflora in the rhizosphere of mycorrhizal sorghum plants	92

LIST OF PLATES

Sl. No.	Title	Between Pages
1.	Increase in root volume of mycorrhizal sorghum due to the effect of perlite 20 per cent	40-41
2.	Increase in root volume of mycorrhizal sorghum due to the effect of perlite 20 per cent	40-41
3.	Root development in different mycorrhizal host plants	50-51
4.	Root development in different mycorrhizal hosts in combination with stylosanthes	50-51
5.	Effect of humic acid 3000 µl l^{-1} on growth of mycorrhizal sorghum	54-55
6.	Effect of humic acid 1000 µl l^{-1} on root development in mycorrhizal sorghum	55-56
7.	Influence of water stress on growth of mycorrhizal sorghum	59-60
8.	Effect of streptomycin 2000 mg l^{-1} on growth of mycorrhizal sorghum	64-65

INTRODUCTION

1. INTRODUCTION

Arbuscular mycorrhizae denote obligately symbiotic associations between certain zygomycetous soil fungi and higher plants in which the mycosymbiont is benefitted by obtaining its carbon requirements from the photosynthates of the host, which in turn function as additional absorbing surfaces of the host and supply less mobile nutrients, particularly phosphorus to the host plant with the help of extramatrical mycelium. This association is so prevalent in terrestrial plant community that "non-mycorrhizal plant is an exception rather than the rule". Arbuscular mycorrhizae impart enormous benefits to the plant community both in the natural ecosystem and in the different agricultural situations. It has been widely used as a bio-inoculant for sustaining the growth and health of cultivated crops (Harley and Smith, 1983; Rosendahl *et al.*, 1994). Its relevance in imparting stress tolerance to host plants, improving soil texture and reducing soil erosion, reclamation and revegetation of degraded soils and facilitating early establishment of nursery and micropropagated plants is also significant.

It is often considered that arbuscular mycorrhizal fungi (AMF) function as the link between plant and soil. This linkage is disrupted due to intervention brought about by intensive agricultural practices. For its restoration, efficient AMF inoculum has to be developed and introduced into the soil under different cropping situations. Arbuscular mycorrhizal technology is now considered to be a key component in sustainable agricultural systems world over. However, availability of quality inoculum is the major constraint in large scale adoption

the mycorrhizal activity or may not have any effect at all. Sufficient information is lacking on the potential use of these substances in AMF inoculum production.

The present investigation is taken up with the objective of tackling some of the daunting problems in AMF inoculum production in which major thrust is focussed on the following aspects :

- Selection of suitable substrate for AMF inoculum production
- Selection of suitable host plant for AMF inoculum production
- Effect of plant growth regulators on the mass inoculum production of AMF
- Effect of plant stress inducers on the mass inoculum production of AMF
- Effect of plant protectants on the mass inoculum production of AMF

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

'Mycorrhiza' is the symbiotic association between specific groups of soil fungi and higher plants. The term was first coined by Frank (1885) to describe this association in forest trees of the family, Cupuliferae. Mycorrhizal associations are broadly classified into three groups *viz.*, (i) Ectomycorrhizae (ii)Endomycorrhizae and (iii) Ectendomycorrhizae.

Arbuscular mycorrhizae (AM) which were previously described as vesicular arbuscular mycorrhizae (VAM) come under the group endomycorrhizae and are ubiquitous and often described as the "Universal plant symbionts". More than 90 per cent of the terrestrial plants form this symbiosis. AM associations are formed by a group of zygomycetous fungi belonging to the order, *Glomales* (Morton and Benny, 1990). The AM associations are receiving considerable attention in recent years since the symbiosis enables better plant growth by higher uptake of nutrients (Harley and Smith, 1983; Bolan *et al.*, 1987; O' keefe and Sylvia, 1990; Marschner and Dell, 1994). The other benefits imparted by AMF to host plants include (i) increased uptake of water (Safir *et al.*, 1971, 1972) (ii) reduced stress responses of plants to toxicity and drought (Guttay, 1976). (iii) improved soil texture and reduced soil erosion (Clough and Sutton, 1978) (iv) alteration in the production of plant growth regulators (Barea and Azcon-Aguilar, 1982; Miller *et al.*, 1987) (v) suppression of soil-borne pathogens (Dehne, 1982; Bagyaraj, 1984; Price *et al.*, 1990; Robert, 1998) (vi) enhanced revegetation of degraded soils (Pfleger *et al.*, 1994) (vii) early establishment of nursery seedlings and enhanced *ex vitro*

establishment of micropropagated plantlets (Sivaprasad *et al.*, 1995).

The scarcity of prime inoculum had always been a constraint for the wider adoption of AM technology by farmers (Ferguson and Woodhead, 1982), eventhough its potential use as biocontrol agent (Dehne, 1982) and biofertilizer (Marschner, 1995) is well recognised in sustainable agriculture. The major obstacle for mass production of inoculum is the obligate nature of the symbiont. Several problems confronted with the present system of inoculum production include high bulk density of the carrier medium, low level of inoculum production and low viability of mycorrhizal spores due to presence of antagonists.

2.1 Inoculum production

Soil based inoculum is commonly used as the standard inoculum of mycorrhizal fungi. Introduction of AMF into plant production systems requires large scale production of the inoculum which can easily be cultured, transported and applied. Different methods of inoculum production have been suggested by several workers.

Manjunath and Bagyaraj (1981) observed that inoculation with extramatrical chlamydospores increased the dry matter and 'P' content of Onion. Biermann and Linderman (1983) found that the roots of subterranean clover and geranium colonized by vesicle forming mycorrhizal fungi like *Glomus* spp. and *Acaulospora spinosa* were highly infective, while root pieces colonized by non-vesicular AMF (*Gigaspora margarita)* were not infective. The use of chlamydospores, infected root fragments and infested soil or culture

substrate as inoculum increased the propagule number in the pot culture of AMF (Menge, 1983, 1984; Boudarga and Dexheimer, 1990).

Sylvia and Hubbel (1986) observed that the colonization and sporulation of *Glomus mosseae* in *Paspalum notatum* were very high in aeroponic system. But Nadarajah and Nawawi (1990) suggested that soil inoculum of *Glomus fasicul· um* associated with maize was more effective for root colonization and spore production than spore inoculum, external hyphae, chopped roots or vesicles from roots. *In vitro* mass production of *Glomus intraradices* and *Glomus versiforme* was achieved in petridishes using mycorrhizal root segment inoculum associated with Ri T-DNA transformed carrot roots by Vestberg and Uosukainen (1992) and Declerck *et al.* (1996). Singh and Deepa (1994) observed that the incorporation of bacteriological media such as Okon's media, Jensen's media and Pikovskaya's media and NPI fertilizer solution enhanced the formation of spores in AMF inoculum. The long range storage of mycorrhizal culture on roots of pot-grown sorghum was made possible by storing at 4^0C without losing infectivity (CMCC, 1996). Vilarino and Sainz (1997) treated the spores of *Glomus mosseae* with 50 per cent sucrose and found that the treatment increased spore germination and inoculum potential of the AMF.

2.2 Use of different substrates for inoculum production

Menge (1984) opined that it is advantageous to produce AMF in partially or completely artificial growth medium of less weight than in soil substrate. Jarstfer and Sylvia (1992) observed that soil less media were more

uniform in composition, weighed less, held the desired water and facilitated better aeration than soil.

2.2.1 Clay - based substrates

Plenchette *et al.* (1982) recommended the use of calcined montmorillonite clay as substrate in pot cultures of different endomycorrhizal fungi. Expanded clay particles were highly efficient as mycorrhizal substrates and led to intensive mycorrhizal formation in maize (Dehne and Backhans, 1986). Further, Baltruschat (1987) demonstrated that expanded clay could be used as a carrier for AMF spores to be used for crops like maize on a large scale. When tomato plants were inoculated with *Glomus* sp., the yield was found to be more when raised in clay and slate substrate than in wood based substrate (Schnitzler and Michalky, 1996). Recent works showed that mycorrhizal inoculum produced on expanded clay contained high levels of spores and fungal mycelium when inoculated on several hosts (Aboul - Nasr, 1997).

2.2.2 Organic substrates

Sylvia (1984) suggested that an ideal substrate for AMF mass production is preferred to have low organic matter and nutrient contents. Aziz and Habte (1988) also showed that high levels of organic residue in the substrate was detrimental to mycorrhizal symbiosis in *Leucaena leucocephala*.

In contrary, there are several reports of the successful use of organic substrates for mass production of AMF. Vestberg (1992) showed that AMF infection and sporulation on strawberry, arctic bramble and maize were more

rapid in sand fertilized with bone meal than in richer peat-based substrates. The germination and infectivity of *Glomus mosseae* in organic substrates like composted pine bark, sphagnum peat and composted olive pumice were investigated by Calvet *et al.* (1993). They found that the number of vegetative spores were higher in both composts than in peat. Coconut husk as growing medium produced superior growth of mycorrhizal sour orange than sawdust (Jean and Becerril, 1994). Ryan *et al.* (1994) found that AMF colonization of wheat was higher on a farm managed in organic manner than that operated in a conventional manner.

2.2.3 Peat, perlite, vermiculite and soilrite as substrates

Caron and Parent (1988) found that peat-lite substrates increased the AMF colonization of leek plants. At the same time, Sreenivasa and Bagyaraj (1988 a) selected perlite - soilrite mix (1:1 v/v) as the best substrate for mass multiplication of *Glomus fasiculatum* on Rhodes grass. The dry matter yields of maize, cereals and lucerne were increased when selected AMF isolates grown in a peat - clay substrate with maize as host plant were used as inoculum (Hoflich and Glante, 1991). Zambolim *et al.* (1992) showed that various combinations of turf, vermiculite, soil and organic matter gave 100 per cent colonization of *Glomus etunicatum* on sorghum, while Mallesha *et al.* (1992) revealed that perlite-soilrite mix when used as a suitable carrier for dual inoculation of AMF and rhizobia resulted in superior growth of *Leucaena leucocephala*. A peat-based substrate low in phosphorus and with good aeration improved AMF spread and efficiency (Wang *et al.*, 1993). Niemira *et*

al. (1995) noticed that the yield of mycorrhizal potato was increased by 84 per cent when raised on peat based medium.

2.3 Selection of suitable host plant for AMF inoculum production

One of the early mentions of the mycorrhizal dependence of host plants was made by Nicolson (1960) who found that the AMF species influenced the sanddune grasses. Arbuscular mycorrhizal association induced by certain species of *Endogone* occurred in a wide range of plants in many habitats (Baylis, 1971). A few years later, Saif (1977) revealed that the stage of host development influenced AMF colonization and spore population in field grown vegetables. He recognised three phases of mycorrhizal root infection *viz.*, lag phase, a phase of rapid development and a constant phase. The spore population was maximum at final harvest of the crops.

Bagyaraj and Manjunath (1980) found that Guinea grass (*Panicum maximum*), was a better host for the maintenance and mass production of *Glomus fasciculatus* under tropical conditions since it was more susceptible to infection and higher spore production by AMF. In general, almost all vascular plant families except Cruciferae has AMF associations (Harely and Smith, 1983; Trappe, 1987). Another study conducted by Wood (1984) showed that large scale production of AMF inoculum is technically feasible through pot culture using an appropriate host. Hetrick *et al.* (1985) observed that the staining characteristics, pattern and morphology of mycorrhizal development of *Glomus epigeaeum* differed according to the host species. The sporulation of *Glomus fasiculatum* was significantly influenced by host plant, but that of *Glomus macrocarpus* and

G. mosseae had no effect (Hetrick and Bloom, 1986).

The pasture grass, *Setaria anceps* was found to be a suitable host for propagation of AMF under Malaysian conditions (Chulan and Omar, 1987). Sreenivasa and Bagyaraj (1988 b) selected Rhodes grass (*Chloris gayana*) as the most appropriate host for producing pot culture inoculum of *Glomus fasiculatum* on the basis of root colonization and spore production. *Leucaena leucocephala* was proved to be a plant of high mycorrhizal dependence in acid soils by Guzman-Plazola *et al.* (1988). Inoculation of *Glomus mosseae* resulted in largest growth response and spore production in grapevine and clover, while the AMF infection was low in meadow grass (Giovannetti *et al.*, 1988).

The effect of stage of growth of the host plants in AMF spore production was emphasised by Sreenivasa and Rajasekhara (1989) on wheat and by Jalaluddin and Anwar (1991) on rice and wheat. They observed a linear increase in spore production with the growth of the host achieving maximum at harvest. Singh and Tilak (1990) studied the response of different sorghum cultivars to inoculation with *Glomus versiforme* and observed that the cultivar PC-23 showed maximum mycorrhizal efficiency and 'P' uptake. In the studies conducted by Boyetchko and Tewari (1990), it was shown that infection levels of *Glomus dimorphicum* were low in different barley cultivars, but higher in beans, lucerne and onions and highest in red clover and maize roots. In the same way, the mycorrhizal colonization in cowpea was host-dependent and heritable (Mercy *et al.*, 1990).

Sorghum and maize produced more spores of *Glomus clarum* than

chickpea and the difference in spore numbers was influenced by infected root length and by the growing period (Simpson and Daft, 1990 a). Sanders and Fitter (1992) observed that AMF responded differently according to the host species and such differences could result in selection pressures which favour certain host fungus combinations. Isolates of indigenous AMF, *viz.*, *Glomus* sp., *G. geosporum* and *G. fasciculatum* showed a considerable amount of host preference in native grassland species (Dhillion, 1992). Singh (1992) inoculated *Glomus macrocarpum* on different forage grasses and observed that maximum infection was observed in *Stylosanthes hamata* and *S. guianensis*, while vesicle formation was greatest in *Pennisetum pedicellatum*, *P. parviflorum*, *Stylosanthes guianensis*, *S. humilis* and *S. hamata*. Jarstfer and Sylvia (1992) suggested that an ideal host should produce root mass quickly, tolerate high light conditions required for the fungal reproduction and become heavily colonized (>50 per cent of the root length).

The root colonization of AMF on sorghum, *Cassia mimosoides*, sesame and soyabean increased continuously until 50 days, but spore production was fluctuated after 30 days in pot cultures (Lee *et al.*, 1993). Al-Raddad (1995) reported that the type of crop and harvest date greatly influenced the spore size and root colonization of *Glomus mosseae*. Mehrotra (1996) recommended Paulownia as a suitable host for mass multiplication of *Glomus mosseae*. Sivaprasad (1998) selected *Setaria anceps* as a suitable host for mass production of *Glomus* sp. as the host plant supported the maximum sporulation of the AMF.

2.4 Effect of growth regulators on AMF colonization and spore production

In one of the early studies, Azcon *et al.* (1978) observed that the infection levels of *Glomus* sp. on lavender, tomato and lucerne were increased after treatment with bacterial cultures which were believed to have behaved as pure plant hormones. Arbuscular formation of *Glomus* sp. and *Gigaspora* sp. were increased in cowpea by application of 1000 ppm indole acetic acid (IAA) while vesicle formation was decreased (Gunze and Hennesy, 1980). Weritz *et al.* (1992) in a similar study, found that thidiazuron had enhanced infection by *Glomus etunicatum* on winter barely and monoculum spring barely. They concluded that growth regulators and mycorrhizae had a synergistic effect on grain yield. Application of indole butyric acid (IBA) increased the root and shoot growth of sour orange and carrizo citrange inoculated with *Glomus intraradices* (Dutra *et al.*, 1996).

Vallini *et al.* (1993) observed that colonization by *Glomus mosseae* was only slightly affected by increasing concentrations of humic substances to soil, while the hyphal growth was reduced. However, increasing doses of humic acids did not inhibit spore germination of *Glomus mosseae*.

Deleterious effect of growth regulators on AMF colonization was also observed in certain hosts. The suppressive effect of indole acetic acid on AMF infection in garlic was demonstrated by Firdaus *et al.* (1988). Mycorrhizal infection was found to be decreased with increase in temperature in IAA induced roots. The endogenous IBA concentration was not important for maize root colonization with *Glomus intraradices* (Ludwig-Muller *et al.*,

1997).

2.5 Effect of plant stress inducers on AMF colonization and spore production

Various environmental factors like soil moisture, temperature and pH influence the germination of spores of *Glomus epigaemus* (Daniels and Trappe, 1980). Bethlenfalvay *et al.* (1988) opined that stressed environments have varied effects on plant growth, endophyte development and soil stability.

2.5.1 Water stress and AMF colonization

Water stress has positive effects on AMF colonization. Iqbal and Tauqir (1982) reported that more AMF infections occurred in cereals under drier conditions (10-15 per cent soil moisture) than wet conditions (30-41 per cent soil moisture). Mahmood and Iqbal (1982) observed that mycorrhizal infection in rapeseed plants was negatively correlated with increase in soil moisture content. Sieverding and Toro (1988) also recognised the positive effect of water stress on mycorrhizal colonization and observed that *Entrophosphora colombiana*, *Glomus manihotis* and *G. occultum* increased cassava growth in water stressed plant only. Pai *et al.* (1990) recognised that maximum mycorrhizal colonization in cowpea occurred under severe water stress conditions with watering once in five days to field capacity. However, the infection levels of *Glomus clarum*, *G. monosporum* and *Acaulospora* sp. in maize and sorghum were not affected by water stress, both in total spore numbers and in terms of spores per gram plant weight (Simpson and Daft, 1990 b). Sivaprasad (1995) observed that the AMF spore production in sorghum was increased when

the water stress was given just before harvest of the crop.

There are also reports regarding the negative effects of water stress on AMF infection. Nelsen and Safir (1982) opined that non-saturated and non-stressed water conditions are needed for spore production in high and low 'P' conditions. Rain or irrigation before inoculation may stimulate germination of indigenous mycorrhizal fungi (Wilson, 1984). AMF colonization was found to be maximum when plants were grown under 24 hours saturation followed by 48 hours drainage. Cerligione *et al.* (1988) observed that there was a reduction in the per cent AMF colonization in little bluestem as the soil water availability was reduced. In the urd cultivars, T-9 and Bhadeli, AMF infection was affected by water stress (Kehri and Chandra, 1990). The laboratory studies conducted by Stevens and Peterson (1995) showed that colonization of *Glomus* spp. and *Gigaspora margarita* were higher in wet conditions than in dry conditions, whereas field studies showed high colonization in dry regions than in wet regions. The infectivity of mycorrhizal root fragments containing *Glomus invermaium* increased by alternate wetting and drying while that of *Scutella calospora* decreased (Braunberger *et al.*, 1996).

2.5.2 Ethrel and AMF

Wertiz *et al.* (1992) studied the interactions of growth regulators and AMF and observed that foliar application of ethephon had a positive effect on colonization of *Glomus etunicatum* in barley.

The application of ethrel can also inhibit the AMF infection. Azcon-Aguilar *et al.* (1981) reported that formation of *Glomus mosseae* in lucerne

and wheat was reduced when ethrel was sprayed on the foliage or applied to the rooting medium. Sivaprasad (1995) observed that AMF colonization and spore production in sorghum was stimulated by ethephon with the maximum at 200 ppm. In another study by Ishii *et al.* (1996), it was evident that a concentration of 0.01 to 0.1 ppm ethylene stimulated the spore germination and hyphal growth of AMF associated with trifoliate orange, but the hyphal growth was severely inhibited at 0.2 ppm and above.

2.5.3 Sodium chloride and AMF

Hirrel (1981) observed that the *in vitro* germination rates of azygospores of *Gigaspora margarita* were reduced more in the presence of chloride salts than sodium. The recovery of germinability by these spores was more rapid when the spores were previously exposed to sodium. It has been shown that sodium chloride stress could reduce dry matter accumulation of citrus seedlings, but no effect on mycorrhizal colonization (Duke *et al.*, 1986). However, Estaun (1990) showed that sodium chloride inhibited the *in vitro* germination and hyphal growth of *Glomus mosseae*.

2.6 Effect of plant protectants on AMF colonization and spore production

The effect of plant protection chemicals on AMF colonization and spore production vary with type and dosage of the chemical, species of the fungus and the host plant. Treatment of mycorrhizal inoculum with selective pesticides had been recommended by Menge (1983) to eliminate contamination and to increase its shelf-life.

2.6.1 Stimulation of AMF by fungicides

Sutton and Sheppard (1976) reported that AMF infection was stimulated in plant roots in soil drenched with 0.5 M captan. Spokes *et al.* (1981) observed that application of chloroneb to inoculum of *Glomus microcarpus* used with pot-grown lettuce stimulated mycorrhizal development.

The beneficial effects of application of metalaxyl on AMF colonization have been highlighted by several workers. Groth and Martinson (1983) observed that metalaxyl increased the infection of *Glomus fasiculatum* on soybean from 57 to 72 per cent while spore production was not significantly affected. In India, Sreenivasa and Bagyaraj (1989) studied the effect of captan and carbofuran at half the recommended dose and found that the chemical increased the root colonization, chlamydospore number and inoculum potential of *Glomus fasciculatus*. An increase in root colonization of *Glomus intraradices* was observed by Afek *et al.* (1990) in cotton, onion and pepper. The effects of triadimefon and pyrazophos on AMF of flax and barely were studied by Alten *et al.* (1993) and found that there was an enhancement in the infection. Application of metalaxyl resulted in maximum colonization of *Glomus* sp. in maize and was recommended for routine incorporation to open pot cultures of AMF on maize (Seymour *et al.*, 1994).

The treatment of carbendazim, fosetyl-al, mancozeb and thiram enhanced growth promoting effect, mycorrhizal infection and spore production of AMF in soybean (Vyas and Vyas, 1995). The potential merit of ridomil 72 WP application in stimulating the colonization and sporulation of *Selerocystis coremioides* in black

pepper was highlighted by Robert *et al.* (1995). Drenching with fytolon supported intense root colonization and high spore density of AMF on tree seedlings (Udaiyan *et al.*, 1996). Shetty and Magu (1997) also found that soil incorporation of metalaxyl significantly increased root colonization of *Glomus fasciculatum* associated with wheat.

2.6.2 Inhibition of AMF by fungicides

Several fungicides are detrimental to the AMF. Early works conducted by Norman and Linn (1969) showed that arasan, botran, lanstan, terrachlor, captan, mylone and vapam restricted mycorrhizal development of *Endogone fasciculata* on corn. Captan, chloroneb, metalaxyl, sodium azide and captafol inhibited mycorrhizal colonization and sporulation on citrus (Nemec, 1980). Occampo and Hayman (1980) conducted experiments with two crops of barely and maize and one crop of potato and found that AMF populations were decreased with benomyl especially in maize which are strongly mycorrhizal. In groundnut, soil drenching with PCNB, captan, captafol and mancozeb at the time of sowing inhibited colonization and sporulation by *Glomus mosseae* (Parvathi *et al.*, 1985 a).

Fitter and Nichols (1988) reported the reduction in AMF infection and 'P' inflow in pea due to benomyl treatment. Hwang (1988) showed that metalaxyl reduced the AMF colonization per root length of the seedlings of lucerne. Dodd and Jeffries (1989) showed that bavistin prevented spore germination of *Glomus mosseae* in wheat. Singh *et al.* (1990) found that AMF development in pigeonpea was inhibited by seed treatment with bavistin,

captafol and thiram. Jalali and Chhabra (1991) also proved that thiram was inhibitory to the development of *Glomus fasciculatum* in pearl millet than captafol and bavistin. Vitavax was more harmful to mycorrhizal development in *Vigna mungo* than bavistin (Nair *et al.*, 1991).

Habte *et al.* (1992) stated that the effectiveness of *Glomus aggregatum* in *Leucaena leucocephala* was completely suppressed at high levels of chlorothalonil. Fosetyl-Al and phosphonic acid inhibited colonization of different *Glomus* sp. in maize (Seymour *et al.*, 1994). The experiment conducted by Machado-Neto *et al.* (1994) in tomato revealed that mycorrhization was reduced due to application of benomyl at the rate of 100 g per kg seeds. Recently, Schreiner and Bethlenfalvy (1997) concluded that benomyl, PCNB and captan at 20 mg a. i. per kg soil inhibited spore germination of *Glomus etunicatum, G. mossea* and *Gigaspora rosea.*

Some of the fungicides do not exert any significant effect on mycorrhizal colonization and spore production. Tommerup and Briggs (1981) reported that carbendazim and captafol had no deleterious effect on germination and hyphal growth of *Acaulospora laevis, Glomus caledonium* and *Glomus monosporum.* Carbendazim had no inhibitory effect on root colonization and spore formation by different species of *Glomus* on soybean (Vyas *et al.*, 1990; Tiwari and Shukla, 1991). Plenchette and Perrin (1992) also proved that infection by *Glomus intraradices* on leek and wheat was not affected by most non-systemic fungicides. Benomyl and mancozeb applied on wheat also did not affect root colonization and spore number of AMF in soil (Cabello, 1994). Joseph (1997) showed that out of the different pesticides tested, thiram and carbofuran least affected the AMF

colonization and sporulation in ginger.

2.6.3 Insecticides and AMF

Bird *et al.* (1974) gave an initial mention of the striking increase in the mycorrhizal infection of cotton roots treated with the nematicide, DBCP. Half the recommended level of carbofuran increased the root colonization, chlamydospore number and inoculum potential of *Glomus fasciculatus* (Sreenivasa and Bagyaraj, 1989). Vijayalakshmi and Rao (1993) gave a further evidence for the stimulation of colonization of *Glomus* sp. of groundnut by monocrotophos.

Insecticides are also reported to have negative effect on AMF infection. Parvathi *et al.* (1985 b) showed that carbaryl, endosulfan, parathion and benomyl adversely affected colonization and sporulation of *Glomus mosseae* in groundnut. Phorate and carbofuran were found to have an adverse effect on *Glomus fasciculatum* in wheat (Gaur and Rana, 1990). Vijayalakshmi and Rao (1993) studied the effect of dimethoate and cypermethrin on *Glomus* sp. of groundnut and found that both the chemicals suppressed the AMF. The effect of soil application of carbofuran at recommended dose (2 kg ha^{-1}) enhanced AMF colonization and sporulation of *Glomus clarum* in groundnut (Venkateswarlu and Daft, 1995), while application of 5 kg ha^{-1} inhibited AMF infection. Endosulfan at 5 ppm also inhibited the growth of *Glomus* sp. Isobe *et al.* (1996) proved that dichlorvos, isoxathion, fenitrothion, endothiapepsin and malathion inhibited germination and mycelial growth of *Gigaspora margarita* in kidney beans.

2.6.4 Streptomycin and AMF

The effect of streptomycin in AMF colonization and sporulation has not been extensively studied. However, Schreiner and Koide (1993) observed that streptomycin application reduced the infection by *Glomus etunicatum* on lettuce. Sivaprasad (1995) opined that there was no considerable effect of streptomycin sulphate on the infection and spore production of AMF associated with sorghum.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Location and soil

All the experiments in the project were conducted under green house conditions at the Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. Lateritic red loam soil of Instructional Farm, Vellayani was used for the different experiments in pots of size 30 x 30 cm.(The nutrient status of the soil used is given in Appendix IV). The soil was sterilized by autoclaving at 1.02 kg cm^{-2} for 2 hours.

3.2 Maintenance of experimental plants

The plants in all the experiments were properly maintained by regular watering and weeding. The plants were fertilized using Ruakura plant nutrient solution (Smith *et al.*, 1983) 50 ml per pot at an interval of 10 days each for the first one month and subsequently at 15 days interval.

Composition of nutrient solution

Stock solution A

Magnesium Sulphate	-	1.11 g
Magnesium Chloride	-	0.5 g
Calcium Nitrate	-	8.39 g
Potassium Nitrate	-	1.13 g
Water	-	500 ml

Stock solution B

Sodium Chloride	-	0.165 g
Rock Phosphate	-	15 g

Potassium Sulphate	-	3.31 g
Sodium Sulphate	-	0.29 g
Water	-	500 ml

Stock solution C

(a) Boric acid	-	0.13 g in 10 ml water
(b) Manganese Sulphate	-	0.42 g in 0.1 N HCl and made up to 10 ml with water
(c) Zinc Chloride	-	0.12 g in 0.1 N HCl and made up to 10 ml with water
(d) Cupric nitrate	-	0.02 g in 0.1 HCl and made up to 10 ml with water
(e) Ammonium Molybdate	-	0.004 g in 10 ml water

1 ml each from items a, b, c, d and e were taken in a bottle and made up to 500 ml with water.

Final solution

300 ml each of stock solution A and B and 150 ml of solution C were taken and made up to 4.5 litres with water.

3.3 Planting material

Sorghum (*Sorghum vulgare* Pers. var-CSH-5) was used as the host plant for all the experiments in items 3.5,3.7,3.8 and 3.9. Seeds of sorghum obtained from National Seeds Corporation were surface sterilized with ethyl alcohol and allowed to germinate *in vitro*. Two to three seedlings were transplanted in the pots when they were eight days old, out of which only one

plant was retained.

3.4 Mycorrhizal inoculum and inoculation

Mixed inoculum consisting of *Glomus fasciculatum* and *Glomus etunicatum* maintained in pots using *Panicum maximum* as host at the Department of Plant Pathology, College of Agriculture, Vellayani was used for the study. The inoculum consisted of root bits, mycelial fragments and rhizosphere soil / vermiculite based substrate carrying 300-350 chlamydospores of AMF. Inoculation was performed by incorporating 30 g each of the inoculum with the substrate thoroughly mixed into each pot to a depth of about five cm.

3.5 Selection of suitable substrate for AMF inoculum production

The experiment was conducted to select the best substrate combination and was laid out in factorial CRD with 13 treatments and 3 replications during February - April 1998. Vermiculite was used as the basal medium along with different proportions of other substrates, *viz.*, perlite, sterile cowdung and sterile soil. The following were the different treatment combinations.

$T_1 = P_1C_1S_1$ = V - 1 kg

$T_2 = P_1C_1S_2$ = V - 900 g, S - 100 g

$T_3 = P_1C_2S_1$ = V - 950 g, C - 50 g

$T_4 = P_1C_2S_2$ = V - 850 g, S - 100 g, C - 50 g

$T_5 = P_1C_3S_1$ = V - 900 g, C - 100 g

$T_6 = P_1C_3S_2$ = V - 800 g, S - 100 g, C - 100 g

$T_7 = P_2C_1S_1$ = V - 800 g, P - 200g

$T_8 = P_2C_1S_2$ = V - 700 g, S - 100 g, P - 200 g

T_9 = $P_2C_2S_1$ = V - 750 g, C - 50 g, P - 200 g

T_{10} = $P_2C_2S_2$ = V - 650 g, S - 100 g, C - 50 g, P - 200 g

T_{11} = $P_2C_3S_1$ = V - 700 g, C - 100 g, P - 200 g

T_{12} = $P_2C_3S_2$ = V - 600 g, S - 100 g, C - 100 g, P - 200 g

T_{13} = Soil control

V = Vermiculite

P_1 = Perlite - '0' level per 100 g substrate

P_2 = Perlite 20 per cent "

S_1 = Soil '0' level "

S_2 = Soil 10 per cent "

C_1 = Cowdung '0' level "

C_2 = Cowdung 5 per cent "

C_3 = Cowdung 10 per cent "

Each pot was filled with one kg of the substrate combination. Observations were recorded on the following parameters.

3.5.1 Plant growth characteristics

Plant growth characteristics *viz.*, number of leaves and plant height were recorded at 30, 60 and 90 days after planting (DAP).

3.5.2 Dry matter production

The plants were pulled out after three months of growth and fresh weights of shoots were recorded. Fresh weights of roots were determined after thorough washing to remove the adhering substrate particles. The dry weights of shoots and roots were determined after oven drying to constant

weight at 60^0C.

3.5.3 Estimation of mycorrhizal colonization and intensity

The mycorrhizal colonization percentage in root samples was estimated on 30 and 90 days after planting following the procedure of Phillips and Hayman (1970). The root samples were cleaned free of soil particles, cut into one cm sized bits and fixed in FAA (Formaldehyde-Acetic acid - Alcohol - in 5:5:90 proportion). The roots were hydrolysed in 10 per cent potassium hydroxide at 100^0C for 10 - 15 minutes. The alkalinity of the samples was then neutralised by washing in one per cent hydrochloric acid and the root bits were stained with 0.05 per cent trypan blue in lactophenol (lactic acid - 20 ml, phenol - 20 ml glycerol - 40 ml and distilled water - 20 ml). The stained roots were arranged on a clean slide, pressed with needle and covered with cover slip and scanned under compound microscope for the presence of mycelium, vesicles and arbuscules of the AMF. The AMF colonization percentage was calculated as given below.

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

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

For estimating the intensity of AMF colonization the method evolved by Joseph (1997) was followed. One cm sized root bits from each sample were placed on a clean slide, the under surface of which was graduated into square columns of 2 mm size in the form of a grating by drawing six lengthwise lines

and sufficient number of cross lines across it. The portion of each root bit which falls in each column of the grating in a line (5 segments) was scanned in the microscope for observing mycorrhizal infection. Positive (+) or negative (-) signs were given for the presence or absence of mycorrhizal infection in each segment. The total number of positive and negative signs of ten roots bits were recorded and the percentage root segment AMF colonization was estimated using the formula,

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

The following intensity grades were assigned based on the root segment AMF colonization.

\+ = less than 25 % root segment AMF colonization

++ = 25 - 50 % root segment AMF colonization

+++ = 51 - 75 % root segment AMF colonization

++++ = > 75 % root segment AMF colonization

3.5.4 Estimation of mycorrhizal spore count

The extramatrical chlamydospores produced by the AMF were estimated following the wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Twenty five grams of the substrate was collected from each pot and made into a uniform suspension in 100 ml water by thoroughly stirring it. The suspension was then passed through a series of sieves ranging from 1000, 300, 250, 105 and 45 μ kept one below the other in the same order. The contents of the bottom two sieves were made into a suspension in water and transferred

to a nylon mesh (45µ) placed in a petridish. The petridish containing the nylon mesh with the spores was observed under stereomicroscope and the total AMF spore count was estimated.

3.6 Selection of suitable host plant for AMF inoculum production

The following host plants, *viz.*, guinea grass, setaria, congosignal and stylosanthes were evaluated alone and in combination with stylosanthes to identify the most suitable host combination for inoculum production. The substrate combination which was identified as the best for inoculum production in the previous experiment (vermiculite - 650 g, perlite - 200 g, cowdung - 50 g, soil - 100 g) was used as the substrate for the experiment. The experiment was conducted during June - August, 1998 by laying out in CRD with seven treatments and four replications. The treatment combinations were as follows :

T_1 - Guinea grass (*Panicum maximum* Jacq.)

T_2 - Setaria (*Setaria anceps* Staph.)

T_3 - Congosignal (*Brachiaria ruziziensis* Germian and Evrard)

T_4 - Stylosanthes (*Stylosanthes hamata* L.)

T_5 - Guinea grass + Stylosanthes

T_6 - Setaria + Stylosanthes

T_7 - Congosignal + Stylosanthes

3.6.1 Planting material

The seeds of guinea grass, setaria, congosignal and stylosanthes obtained from Kerala Livestock Development Board were used as the planting material. The seeds were surface sterilized using ethyl alcohol and sown in pots containing sterile soil and 15 - 20 days old seedlings were transplanted

into the pots containing selected substrate.

3.6.2 Observations recorded

Observations on the growth characteristics such as number of leaves / tillers and plant height, fresh and dry weights of shoots and roots, mycorrhizal colonization percentage, intensity and spore count were recorded as illustrated under 3.5.1,3.5.2,3.5.3 and 3.5.4. respectively.

3.7 Effect of plant growth regulators on AMF colonization and spore production

The different growth regulators evaluated for their influence on AMF colonization and spore production were indole acetic acid (IAA), indole butyric acid (IBA), naphthyl acetic acid (NAA), humic acid (12 %) and 2,4 dichlorophenoxy acetic acid (2,4-D). The experiment was laid out in CRD with 16 treatments replicated thrice using the selected substrate from experiment mentioned under 3.5. as the potting mixture during June - August, 1998. The following were the treatment combinations.

T_1 = IAA 25 mg l^{-1}

T_2 = IAA 50 mg l^{-1}

T_3 = IAA 100 mg l^{-1}

T_4 = IBA 25 mg l^{-1}

T_5 = IBA 50 mg l^{-1}

T_6 = IBA 100 mg l^{-1}

T_7 = NAA 25 mg l^{-1}

T_8 = NAA 50 mg l^{-1}

T_9 = NAA 100 mg l^{-1}

T_{10} = Humic acid 1000 µl l^{-1}

T_{11} = Humic acid 2000 µl l^{-1}

T_{12} = Humic acid 3000 µl l^{-1}

T_{13} = 2,4-D 2 mg l^{-1}

T_{14} = 2,4-D 4 mg l^{-1}

T_{15} = 2,4-D 6 mg l^{-1}

T_{16} = Control

3.7.1 Application of growth regulators

The growth regulatory substances were sprayed on the foliage twice, the first after 10 days of planting and the second after 30 days of the first spray. The plants were sprayed with the chemicals so as to cover the entire plant surface.

3.7.2 Observations recorded

Observations on number of leaves and plant height, fresh and dry weights of shoots and roots, mycorrhizal colonization percentage, intensity and spore count were recorded as mentioned in paragraphs 3.5.1, 3.5.2, 3.5.3 and 3.5.4 respectively.

3.8 Effect of plant stress inducers on AMF colonization and spore production

In order to evaluate the effect of different stress inducing substances on mycorrhizal colonization and spore production, an experiment was laid out during June-August, 1998 in CRD with 13 treatments replicated thrice. The following were the different treatment combinations :

T_1 = Ethrel 25 µl l^{-1}

T_2 = Ethrel 50 µl l^{-1}

T_3 = Ethrel 100 µl l^{-1}

T_4 = Abscissic acid (ABA) 0.5 mg l^{-1}

T_5 = Abscissic acid 1.0 mg l^{-1}

T_6 = Abscissic acid 2.0 mg l^{-1}

T_7 = Sodium chloride (NaCl) 0.5 %

T_8 = Sodium chloride 1.0 %

T_9 = Sodium chloride 1.5 %

T_{10} = Water stress on 15 days after planting (DAP)

T_{11} = Water stress on 45 days after planting

T_{12} = Water stress on 75 days after planting

T_{13} = Control

3.8.1 Application of stress inducers

The stress inducing substances were applied by spraying on the foliage 25 DAP except water stress. The water stress was given by stopping irrigation until wilting point and resuming it later.

3.8.2 Observations recorded

Observations on number of leaves and plant height, fresh and dry weights of shoots and roots, mycorrhizal colonization percentage, intensity and spore count were recorded as described in paragraphs 3.5.1, 3.5.2, 3.5.3 and 3.5.4. respectively.

3.9 Effect of plant protectants on AMF colonization and spore production

The effect of commonly used plant protectants on mycorrhizal colonization and spore production was tested by laying out an experiment in CRD with ten treatments and three replications during June - August, 1998. The following were the treatment details.

T_1 = Carbofuran 0.75 g / plant

T_2 = Carbofuran 1.5 g / plant

T_3 = Carbofuran 2.25 g / plant

T_4 = Metalaxyl 500 mg l^{-1}

T_5 = Metalaxyl 1000 mg l^{-1}

T_6 = Metalaxyl 2000 mg l^{-1}

T_7 = Streptomycin 500 mg l^{-1}

T_8 = Streptomycin 1000 mg l^{-1}

T_9 = Streptomycin 2000 mg l^{-1}

T_{10} = Control

3.9.1 Application of plant protectants

The plant protection chemicals at different concentrations were applied twice, the first being at the time of planting and the second after one month of first application.

3.9.2 Observations recorded

Observations on number of leaves and plant height, fresh and dry weights of shoots and roots, mycorrhizal colonization percentage, intensity and spore count were recorded as mentioned in paragraphs 3.5.1, 3.5.2, 3.5.3 and 3.5.4 respectively.

3.9.2a Estimation of the associated microflora

The populations of fungi, bacteria and actinomycetes in the substrates of different treatments were estimated using soil dilution plate technique (Timonin, 1940). One gram of the substrate sample was taken from each experimental pot and serial dilutions of 10^{-3}, 10^{-4} and 10^{-5} were made for estimating the populations of fungi, actinomycetes and bacteria respectively. One ml each of the different dilutions was pipetted out into petriplates containing 15 ml of the respective media (Martin's rose bengal agar, Kuster's agar and soil extract agar for fungi, actinomycetes and bacteria respectively) which was replicated thrice (Appendices I, II and III). The petriplates were carefully rotated for thorough mixing and incubated at room temperature for 2 days, 4 days and 6 days for bacteria, fungi and actinomycetes respectively. The colonies were counted by visual observation.

3.10 Statistical analysis

The data were analysed statistically by employing analysis of variance (Snedecor and Cochran, 1962).

RESULTS

4. RESULTS

4.1 Selection of substrate for inoculum production

4.1.1 Effect on plant growth characteristics

The results of the experiment on the effect of different substrate combinations on plant growth characteristics are presented in tables 1a, 1b and 1c. The data showed that perlite, cowdung and soil in different proportions in vermiculite based growth medium did not exert any significant influence on the growth of mycorrhizal sorghum plants (Table 1a). The data were further analysed to study the effect due to two factor interactions and the main effect of different substrates on growth enhancement. Results on two factor interactions showed that there was no significant effect due to interaction between perlite and cowdung and cowdung and soil (Table 1b). The study on the interaction between perlite and soil showed that significant increase in plant height was observed in perlite 20 per cent and soil 10 per cent (107.73 cm and 131.56 cm) and perlite 20 per cent, soil 0 combination (94.80 cm and 119.56 cm) as compared to perlite 0 and soil 0 combination (78.87 cm and 105.78 cm) and perlite 0, soil 10 per cent (69.99 cm and 82.92 cm) combination on 60 and 90 DAP, respectively.

Study on the main effects of different substrates indicated that perlite 20 per cent significantly enhanced the number of leaves (7.17) as compared to perlite 0 on 60 DAP (Table 1c). The plant heights on 60 and 90 DAP (101.27 cm and 125.56 cm respectively) due to perlite 20 per cent treatment were also significantly higher than that of perlite 0 treatment (74.43 cm and 94.34 cm).

Table 1 a. Effect of different substrates on growth characteristics of mycorrhizal sorghum plants (three factor interactions)

Perlite × cowdung × soil

Treatments		Number of leaves			Plant height (cm)		
		Days after planting			Days after planting		
		30	60	90	30	60	90
T_1	Perlite 0, Cowdung 0, Soil 0	4.67	6.33	8.00	21.63	63.63	89.33
T_2	Perlite 0, Cowdung 0, Soil 10 %	5.33	5.67	7.33	22.60	57.73	74.33
T_3	Perlite 0, Cowdung 5%, Soil 0	4.67	6.67	8.33	24.67	76.10	99.00
T_4	Perlite 0, Cowdung 5%, Soil 10 %	5.00	5.33	6.67	32.07	78.07	87.33
T_5	Perlite 0, Cowdung 10%, Soil 0	5.67	6.67	8.67	35.43	96.87	129.00
T_6	Perlite 0, Cowdung 10%, Soil 10%	4.33	5.67	7.33	28.27	74.17	87.07
T_7	Perlite 20g, Cowdung 0, Soil 0	4.00	7..33	8.33	21.63	90.07	125.00
T_8	Perlite 20g, Cowdung 0, Soil 10%	5.67	7.33	8.00	26.20	86.73	107.67
T_9	Perlite 20g, Cowdung 5%, Soil 0	5.33	7.67	8.67	28.03	100.67	124.00
T_{10}	Perlite 20g, Cowdung 5%, Soil 10%	5.33	6.66	9.33	37.00	126.30	155.33
T_{11}	Perlite 20g, Cowdung 10%, Soil 0	5.67	7.33	8.67	34.13	93.67	109.67
T_{12}	Perlite 20g, Cowdung 10%, Soil 10%	6.00	6.67	8.00	42.00	110.17	131.67
T_{13}	Soil control	5.00	7.33	8.00	45.33	93.70	95.37
	CD (0.05)	NS	NS	NS	NS	NS	NS

Table 1b. Effect of different substrates on growth characteristics of mycorrhizal sorghum plants (two factor interactions)

(i) Perlite × Cowdung

Treatments	Number of leaves			Plant height (cm)		
	Days after planting			Days after planting		
	30	60	90	30	60	90
Perlite 0, Cowdung 0	5.00	6.00	7.67	22.12	60.68	81.83
Perlite 0, Cowdung 5 %	4.83	6.00	7.50	28.37	77.08	93.17
Perlite 0, Cowdung 10 %	5.00	6.17	8.00	31.85	85.52	108.03
Perlite 20 %, Cowdung 0	4.83	7.33	8.17	23.92	88.40	116.33
Perlite 20 %, Cowdung 5 %	5.33	7.17	9.00	32.52	113.48	139.67
Perlite 20 %, Cowdung 10 %	5.83	7.00	8.33	38.07	101.92	120.67
CD (0.05)	NS	NS	NS	NS	NS	NS

(ii) Perlite × Soil

Perlite 0, Soil 0	5.00	6.56	8.33	27.24	78.87	105.78
Perlite 0, Soil 10 %	4.89	5.56	7.11	27.64	69.99	82.91
Perlite 20 %, Soil 0	5.00	7.44	8.56	27.93	94.80	119.56
Perlite 20 %, Soil 10 %	5.67	6.89	8.44	35.07	107.73	131.56
CD (0.05)	NS	NS	NS	NS	13.14	19.25

(iii) Cowdung × Soil

Cowdung 0, Soil 0	4.33	6.56	8.17	21.63	76.85	107.17
Cowdung 0, Soil 10 %	5.50	5.56	7.67	24.40	72.23	91.00
Cowdung 5 %, Soil 0	5.00	7.44	8.50	26.35	88.38	111.50
Cowdung 5 %, Soil 10 %	5.17	6.89	8.00	34.53	102.18	121.33
Cowdung 10 %, Soil 0	5.67	7.00	8.67	34.78	95.27	119.33
Cowdung 10 %, Soil 10 %	5.17	6.17	7.67	35.13	92.17	109.37
CD (0.05)	NS	NS	NS	NS	NS	NS

Table 1c. Effect of different substrates on growth characteristics mycorrhizal sorghum plants (main effects)

(i) Perlite

Treatments	Number of leaves			Plant height (cm)		
	Days after planting			Days after planting		
	30	60	90	30	60	90
Perlite 0	4.94	6.06	7.72	27.44	74.43	94.34
Perlite 20 %	5.33	7.17	8.50	31.50	101.27	125.56
CD (0.05)	NS	0.92	NS	NS	9.29	13.61

(ii) Cowdung

Treatments	Number of leaves			Plant height (cm)		
	Days after planting			Days after planting		
	30	60	90	30	60	90
Cowdung 0	4.92	6.67	7.92	23.02	74.54	99.08
Cowdung 5 %	5.08	6.58	8.25	30.44	95.28	116.42
Cowdung 10 %	5.42	6.58	8.17	34.96	93.72	114.35
CD (0.05)	NS	NS	NS	5.98	11.38	NS

(iii) Soil

Treatments	Number of leaves			Plant height (cm)		
	Days after planting			Days after planting		
	30	60	90	30	60	90
Soil 0	5.00	7.00	8.44	27.59	86.83	112.67
Soil 10 %	5.28	6.22	7.78	31.36	88.86	107.23
CD (0.05)	NS	NS	NS	NS	NS	NS

Similarly, cowdung 10 per cent significantly increased plant height on 30 DAP (34.96 cm) than plots without cowdung treatment (23.02 cm) while cowdung 5 per cent and 10 per cent significantly enhanced plant height on 60 DAP (95.28 cm and 93.72 cm respectively) as compared to treatment without cowdung (74.54 cm). However, the main effect of soil at different proportions was not significant in enhancing plant growth characteristics.

4.1.2 Effect on fresh and dry weights of shoots and roots

The effect of different substrate combinations on plant biomass was studied and the results are presented in tables 2a, 2b and 2c. The interaction of perlite, cowdung and soil at different combinations along with vermiculite did not produce any significant effect on fresh and dry weights of shoots and roots (Table 2a). The data on interaction between perlite and cowdung, perlite and soil and cowdung and soil are presented in table 2b. The two factor interactions too had no significant influence on fresh and dry weights of shoots and roots of mycorrhizal sorghum plants.

Data on the main effect of different substrates on fresh and dry weights of shoots and roots are presented in table 2c. The main effect of perlite 20 per cent was significantly superior in enhancing the fresh and dry weights of shoots and roots. The values of 118.06 g and 41.39 g of fresh and dry weights of shoots and 86.94 g and 13.72 g of fresh and dry weights of roots in perlite 20 per cent treatment as compared to 52.22 g, 15.56 g, 48.61 g and 7.08 g of fresh and dry weights of shoots and roots respectively of treatment without perlite amply illustrated it (Plates 1 and 2). The main effects due to either

Table 2a. Effect of different substrates on fresh and dry weights of shoots and roots of mycorrhizal sorghum plants (three factor interactions)

Perlite x Cowdung x Soil

Treatments		Weight of shoots (g)		Weight of roots (g)	
		Fresh weight	Dry weight	Fresh weight	Dry weight
T_1	Perlite 0, Cowdung 0, Soil 0	38.33	7.50	30.00	4.17
T_2	Perlite 0, Cowdung 0, Soil 10%	21.67	5.00	25.00	3.33
T_3	Perlite 0, Cowdung 5%, Soil 0	70.00	20.00	53.33	7.50
T_4	Perlite 0, Cowdung 5%, Soil 10%	35.00	14.17	50.00	7.50
T_5	Perlite 0, Cowdung 10%, Soil 0	88.33	26.67	71.67	10.00
T_6	Perlite 0, Cowdung 10%, Soil 10%	60.00	20.00	61.67	10.00
T_7	Perlite 20%, Cowdung 0, Soil 0	101.67	36.67	90.00	13.33
T_8	Perlite 20%, Cowdung 0, Soil 10%	95.00	23.33	63.33	9.17
T_9	Perlite 20%, Cowdung 5%, Soil 0	128.33	41.67	78.33	13.17
T_{10}	Perlite 20%, Cowdung 5%, Soil 10%	178.33	70.00	108.33	20.00
T_{11}	Perlite 20%, Cowdung 10%, Soil 0	93.33	35.00	65.00	10.00
T_{12}	Perlite 20g, Cowdung 10%, Soil 10%	111.67	41.67	116.67	16.67
T_{13}	Soil control	73.33	25.00	88.33	10.00
	CD (0.05)	NS	NS	NS	NS

Table 2b. Effect of different substrates on fresh and dry weights of shoots and roots of mycorrhizal sorghum plants (two factor interactions)

(i) Perlite × Cowdung

Treatments	Weight of shoots (g)		Weight of roots (g)	
	Fresh weight	Dry weight	Fresh weight	Dry weight
Perlite 0, Cowdung 0	30.00	6.25	27.50	3.75
Perlite 0, Cowdung 5 %	52.50	17.08	51.67	7.50
Perlite 0, Cowdung 10 %	74.17	23.33	66.67	10.00
Perlite 20 %, Cowdung 0	98.33	30.00	76.67	11.25
Perlite 20 %, Cowdung 5%	153.33	55.83	93.33	16.58
Perlite 20 %, Cowdung 10 %	102.50	38.33	90.83	13.33
CD (0.05)	NS	NS	NS	NS

(ii) Perlite × Soil

Perlite 0, Soil 0	65.56	18.06	51.67	7.22
Perlite 0, Soil 10 %	38.89	13.06	45.56	6.94
Perlite 20 %, Soil 0	107.78	37.78	77.78	12.17
Perlite 20 %, Soil 10 %	128.33	45.00	96.11	15.28
CD (0.05)	NS	NS	NS	NS

(iii) Cowdung × Soil

Cowdung 0, Soil 0	70.00	22.08	60.00	8.75
Cowdung 0, Soil 10 %	58.33	14.17	44.17	6.25
Cowdung 5 %, Soil 0	99.17	30.83	65.83	10.33
Cowdung 5 %, Soil 10 %	106.67	42.08	79.17	13.75
Cowdung 10 %, Soil 0	90.83	30.83	68.33	10.00
Cowdung 10 %, Soil 10 %	85.83	30.83	89.17	13.33
CD (0.05)	NS	NS	NS	NS

Table 2c. Effect of different substrates on fresh and dry weights of shoots and roots of mycorrhizal sorghum plants (main effects)

(i) Perlite

Treatments	Weight of shoots (g)		Weight of roots (g)	
	Fresh weight	Dry weight	Fresh weight	Dry weight
Perlite 0	52.22	15.56	48.61	7.08
Perlite 20 %	118.06	41.39	86.94	13.72
CD (0.05)	36.78	12.84	20.13	3.34

(ii) Cowdung

Cowdung 0	64.17	18.13	52.08	7.50
Cowdung 5%	102.92	36.46	72.50	12.04
Cowdung 10%	88.33	30.83	78.75	11.67
CD (0.05)	NS	NS	NS	NS

(iii) Soil

Soil 0	86.67	27.92	64.72	9.69
Soil 10 %	83.61	29.03	70.83	11.11
CD (0.05)	NS	NS	NS	NS

Plate 1 & 2 Increase in root volume of mycorrhizal sorghum due to the effect of perlite 20 per cent

P_1= Perlite 0 level per 100g substrate

P_2= Perlite 20 per cent "

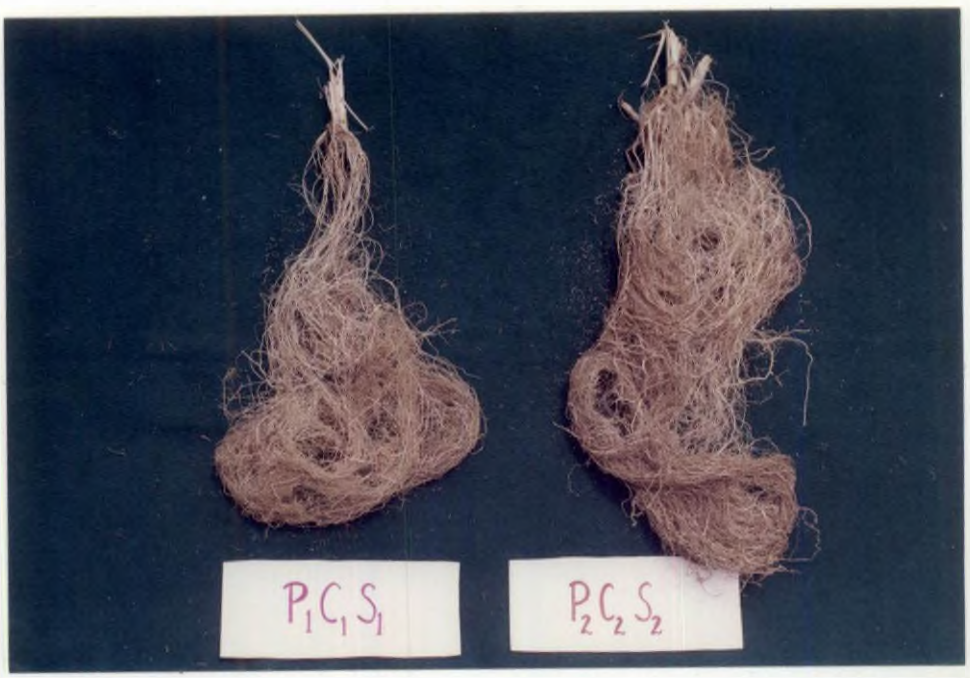
S_1= Soil 0 level "

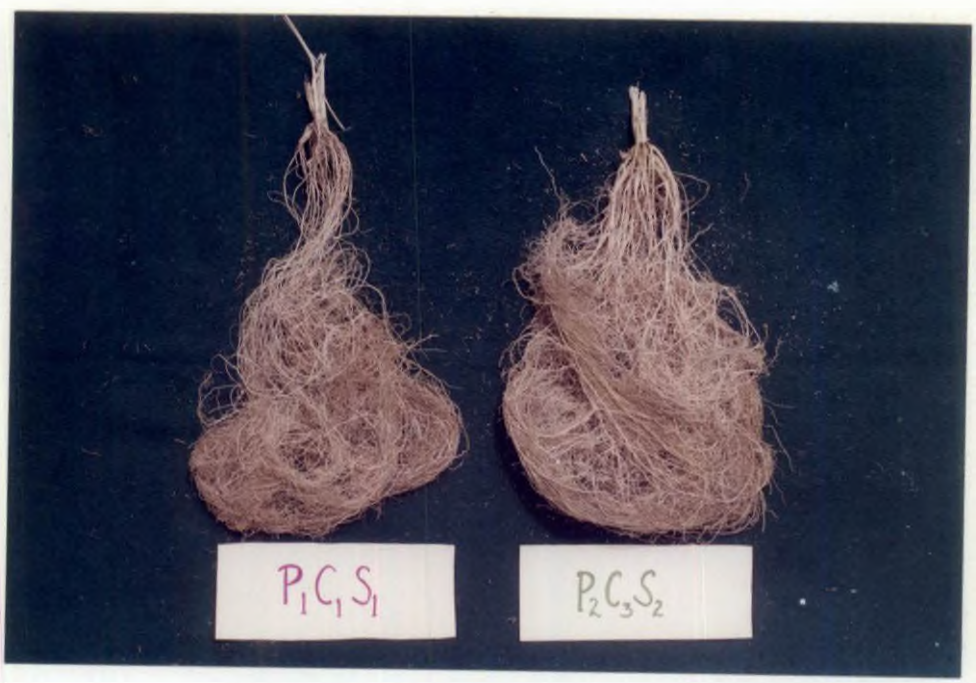
S_2= Soil 10 per cent "

C_1= Cowdung 0 level "

C_2= Cowdung 5 per cent "

C_3= Cowdung 10 per cent "


$P_1C_1S_1$
$P_2C_2S_2$


$P_1C_1S_1$
$P_2C_3S_2$

cowdung or soil were not significant in enhancing the fresh and dry weights of shoots and roots of mycorrhizal sorghum plants.

4.1.3 Effect on mycorrhizal colonization, intensity and spore production

Different substrate combinations did not exert statistically significant effect on mycorrhizal colonization in sorghum although very high mycorrhizal colonizations of more than 90 per cent were observed in all the different treatments on 90 DAP (Table 3a). Higher intensity of mycorrhizal colonization was also observed in all the treatments. However, significantly higher spore counts were produced in T_{10}, T_4, T_6 and T_9 treatments (1028.31, 819.92, 697.61 and 678.43 respectively) as compared to the soil control (580). The data further indicated that there was also significant reduction in spore count in certain treatments with maximum reduction in T_1 (259.74) followed by T_5 (359.81) and T_2 (379.82) accounting a percentage reduction of 44.78, 62.04 and 65.49 in the respective treatments as compared to control.

Analysis of the data on interaction due to perlite and cowdung in the experiment showed that although mycorrhizal colonization was uniformly higher in all the treatments, the interaction was found to be insignificant as in the case of three factor interactions of perlite, cowdung and soil (Table 3b). However, the interaction resulted in significantly higher spore count in perlite 20 per cent + cowdung 5 per cent combination and perlite 0 + cowdung 5 per cent combination when compared with that of the treatment with no perlite and cowdung. Maximum spore count of 844.30 was obtained in perlite 20 per cent + cowdung 5 per cent treated plots followed by 661.39 in perlite 0 + cowdung

Table 3a. Effect of different substrates on mycorrhizal root colonization, intensity and spore production in sorghum plants (three factor interactions)

Perlite × cowdung × soil

Treatments		AMF colonization (%) Days after planting				Intensity of colonization		Spore count (Per 25 g substrate)	
		30		90		30	90		
T_1	Perlite 0, Cowdung 0, Soil 0	74.05	(8.61)	98.66	(9.93)	+++	++++	259.74	(16.12)
T_2	Perlite 0, Cowdung 0, Soil 10%	78.19	(8.84)	96.09	(9.80)	+++	+++	379.82	(19.49)
T_3	Perlite 0, Cowdung 5%, Soil 0	74.95	(8.66)	97.06	(9.85)	++	+++	519.87	(22.80)
T_4	Perlite 0, Cowdung 5%, Soil 10%	72.89	(8.54)	92.19	(9.60)	++	+++	819.92	(28.63)
T_5	Perlite 0, Cowdung 10%, Soil 0	60.94	(7.81)	100.00	(10.00)	+	+++	359.81	(18.97)
T_6	Perlite 0, Cowdung 10%, Soil 10%	42.73	(6.54)	94.67	(9.73)	+	+++	697.61	(26.41)
T_7	Perlite 20%, Cowdung 0, Soil 0	73.38	(8.57)	100.00	(10.00)	++	++++	459.42	(21.43)
T_8	Perlite 20%, Cowdung 0, Soil 10%	82.96	(9.11)	97.19	(9.86)	+++	+++	586.63	(24.22)
T_9	Perlite 20%, Cowdung 5%, Soil 0	79.52	(8.92)	94.22	(9.71)	++	+++	678.43	(26.05)
T_{10}	Perlite 20%, Cowdung 5%, Soil 10%	64.15	(8.01)	95.39	(9.77)	+	++++	1028.31	(32.07)
T_{11}	Perlite 20%, Cowdung 10%, Soil 0	79.93	(8.94)	96.68	(9.83)	++	+++	519.87	(22.80)
T_{12}	Perlite 20%, Cowdung 10%, Soil 10%	79.31	(8.91)	90.30	(9.50)	+++	+++	496.63	(22.29)
T_{13}	Soil control	94.33	(9.71)	95.80	(9.78)	+++	+++	580.00	(24.07)
	CD (0.05)	NS		NS		-	-	95.91	(1.90)

Figures in parantheses indicate transformed values (square root transformation)

Table 3b. Effect of different substrates on mycorrhizal root colonization and spore production in sorghum plants (two factor interactions)

(i) Perlite x Cowdung

Treatments	AMF root colonization (%) Days after planting				Spore count (per 25g substrate)	
	30		90			
Perlite 0, Cowdung 0	76.70	(8.72)	97.37	(9.87)	316.94	(17.80)
Perlite 0, Cowdung 5 %	73.91	(8.60)	94.61	(9.73)	661.39	(25.72)
Perlite 0, Cowdung 10 %	51.43	(7.17)	97.31	(9.86)	514.86	(22.69)
Perlite 20 %, Cowdung 0	78.10	(8.84)	98.59	(9.93)	521.08	(22.83)
Perlite 20 %, Cowdung 5 %	71.63	(8.46)	94.80	(9.74)	844.30	(29.06)
Perlite 20 %, Cowdung 10 %	79.62	(8.92)	93.46	(9.67)	508.18	(22.54)
CD (0.05)	NS		NS		67.82	(1.34)

(ii) Perlite x Soil

Perlite 0, Soil 0	69.83	(8.36)	98.57	(9.93)	372.31	(19.30)
Perlite 0, Soil 10 %	63.56	(7.97)	94.31	(9.71)	617.28	(24.85)
Perlite 20 %, Soil 0	77.58	(8.81)	96.95	(9.85)	548.83	(23.43)
Perlite 20 %, Soil 10 %	75.25	(8.67)	94.27	(9.71)	685.97	(26.19)
CD (0.05)	NS		NS		55.37	(1.10)

(iii) Cowdung x Soil

Cowdung 0, Soil 0	73.72	(8.59)	99.33	(9.97)	352.51	(18.78)
Cowdung 0, Soil 10 %	80.56	(8.98)	96.64	(9.83)	477.63	(21.85)
Cowdung 5 %, Soil 0	77.22	(8.79)	95.63	(9.78)	596.51	(24.42)
Cowdung 5 %, Soil 10 %	68.45	(8.27)	93.78	(9.68)	921.17	(30.35)
Cowdung 10 %, Soil 0	70.11	(8.37)	98.33	(9.92)	436.17	(20.88)
Cowdung 10 %, Soil 10 %	59.62	(7.72)	92.47	(9.62)	592.86	(24.35)
CD (0.05)	NS		NS		67.82	(1.34)

Figures in parentheses indicate transformed values (Square root transformation)

5 per cent combination whereas it was only 316.94 in the non-treated plots. The spore counts due to cowdung 10 per cent application were significantly reduced (514.86 and 508.18) as compared to that of cowdung 5 per cent.

The interaction between perlite and soil did not exert any significant effect on AMF colonization at different growth stages of mycorrhizal sorghum. But, the spore count was significantly enhanced in the perlite + soil treated plots with the maximum being in perlite 20 per cent + soil 10 per cent treatment (685.97) followed by perlite 0 + soil 10 per cent (617.28) and perlite 20 per cent + soil 0 (548.83) treatments than that of untreated plots (372.31). The data further showed that perlite 20 per cent + soil 10 per cent and perlite 0 + soil 10 per cent combinations had significantly higher spore counts than perlite 20 per cent + soil 0 combination. The interaction between cowdung and soil also indicated that the mycorrhizal colonization was not significant due to the two factor interactions. Significantly higher spore counts were obtained in all the cowdung + soil treated plots with maximum being in cowdung 5 per cent + soil 10 per cent treatment (921.17) followed by cowdung 5 per cent + soil 0 (596.51), cowdung 10 per cent + soil 10 per cent (592.86), cowdung 0 + soil 10 per cent (477.63) and cowdung 10 per cent + soil 0 (436.17) as compared to the untreated plots (352.51). The two factor interaction of cowdung + soil also indicated that when cowdung was increased from 5 per cent to 10 per cent irrespective of the level of soil, the spore counts were significantly reduced from 596.51 to 436.17 and from 921.17 to 592.86.

The results of the analysis of the data on main effect of different substrates on mycorrhizal root colonization and spore production are as

detailed in table 3 c. Perlite 20 per cent significantly enhanced initial root colonization (76.41 per cent) and the spore count (615.49) when compared with perlite 0 level (66.65 per cent and 487.10 respectively). The root colonization was significantly higher on 30 DAP with cowdung 0 (77.10) and cowdung 5 per cent (72.77) as compared with that of cowdung 10 per cent level (64.76), whereas the spore count was significantly higher in all the cowdung treated plots with maximum of 750.06 in cowdung 5 per cent followed by 511.52 in cowdung 10 per cent treated plots when compared with 412.70 in untreated plots. The main effect due to application of soil did not exert significant influence on the mycorrhizal colonization. However, application of 10 per cent soil significantly enhanced the spore count (651.17) as compared to treatments without soil application (456.30).

4.2 Selection of host plant for AMF inoculum production

4.2.1 The growth characteristics of host plants inoculated with AMF

The growth characters of the mycorrhizal plants alone and in combination with mycorrhizal stylosanthes were studied and the results are presented in table 4. When guinea grass was grown along with stylosanthes, there was a slight reduction with respect to the number of tillers produced by the plant. However, the plant height was remarkably increased at different stages of growth when grown along with stylosanthes (57.83, 60.15 and 62.18 cm on 30, 60 and 90 DAP as against 47.78, 52.4 and 54.55 cm when it was grown alone). There was a drastic reduction in the number of leaves and plant height of stylosanthes when grown along with guinea grass than grown alone.

Table 3c. Effect of different substrates on mycorrhizal root colonization and spore production in sorghum plants (main effects)

(i) Perlite

Treatments	Mycorrhizal root colonization (%) Days after planting				Spore count (per 25g substrate)	
	30		90			
Perlite 0	66.65	(8.15)	96.43	(9.82)	487.10	(22.07)
Perlite 20 %	76.41	(8.74)	95.61	(9.78)	615.49	(24.81)
CD (0.05)	5.49	(0.43)	NS		39.15	(0.77)

(ii) Cowdung

Cowdung 0	77.10	(8.78)	97.98	(9.90)	412.70	(20.32)
Cowdung 5 %	72.77	(8.53)	94.71	(9.73)	750.06	(27.39)
Cowdung 10 %	64.76	(8.05)	95.38	(9.77)	511.52	(22.62)
CD (0.05)	6.73	(0.53)	NS		47.95	(0.95)

(iii) Soil

Soil 0	73.65	(8.58)	97.76	(9.89)	456.30	(21.36)
Soil 10 %	69.28	(8.32)	94.29	(9.71)	651.17	(25.22)
CD (0.05)	NS		3.38	(0.17)	39.15	(0.77)

Figures in parantheses indicate transformed values (Square root transformation)

Table 4 Growth characteristics of host plants inoculated with AMF

Treatments		Number of tillers / leaves			Plant height (cm)		
		Days after planting			Days after planting		
		30	60	90	30	60	90
T_1	Guinea grass	9.25	10.50	11.50	47.78	52.40	54.55
T_2	Setaria	8.00	10.00	11.00	44.98	47.95	49.50
T_3	Congosignal	7.00	9.50	10.50	37.15	54.25	61.28
T_4	Stylosanthes	114.25	665.50	1437.00	27.95	38.43	42.58
T_5	Guinea grass + Stylosanthes	7.00 + 58.25	7.50 + 127.75	8.50 + 232.50	57.83 + 20.70	60.15 + 28.95	62.18 + 32.48
T_6	Setaria + Stylosanthes	8.50 + 77.25	10.00 + 170.75	11.00 + 277.50	44.08 + 22.00	48.00 + 26.90	50.20 + 31.73
T_7	Congosignal + Stylosanthes	7.25 + 66.75	9.50 + 137.25	11.75 + 198.25	52.48 + 24.05	62.13 + 28.68	76.68 + 31.10

The number of tillers produced by setaria and congosignal at different stages of growth were increased when they were grown along with stylosanthes than grown alone. There was not much difference in the plant height of setaria when grown alone and in combination with stylosanthes. However, plant height of congosignal was highly increased when grown along with stylosanthes (52.48, 62.13 and 76.68 cm) as against when grown alone (37.15, 54.25 and 61.28 cm) on 30, 60 and 90 DAP respectively. The growth characteristics of stylosanthes with respect to number of leaves and plant height were uniformly reduced when it was grown with other crops, but the reduction was comparatively less when it was grown with setaria.

4.2.2 Biomass production by different host plants inoculated with AMF

Data on fresh and dry weights of shoots and roots of different mycorrhizal host plants and their combination with stylosanthes are presented in table 5. The shoot fresh and dry weights of guinea grass were remarkably increased when grown along with stylosanthes (28.75 g and 6.25 g) as compared to its corresponding values when grown alone (23.13 g and 3.63 g). A similar remarkable increase in the root fresh and dry weights were also observed in the combined treatment of guinea grass and stylosanthes (63.13 g and 21.25 g) as against their sum of individual values (52.50 g and 10.38 g) of fresh and dry weights of roots of guinea grass and stylosanthes. In the congosignal and stylosanthes combined treatment also, a similar effect was observed. The shoot fresh and dry weights of congosignal were 39.38 g and 10.63 g in the combined treatment whereas the values were 25 g and 5 g in the individual

Table 5 Biomass production by different host plants inoculated with AMF

Treatments		Weight of shoots (g) Fresh weight	Dry weight	Weight of roots (g) Fresh weight	Dry weight
T_1	Guinea grass	23.13	3.63	40.00	8.13
T_2	Setaria	41.88	12.50	108.75	38.75
T_3	Congosignal	25.00	5.00	31.88	5.63
T_4	Stylosanthes	25.00	6.23	12.50	2.25
T_5	Guinea grass + Stylosanthes	28.75 + 8.75	6.25 + 1.63	63.13	21.25
T_6	Setaria + Stylosanthes	35.00 + 8.75	10.00 + 2.13	88.75	34.38
T_7	Congosignal + Stylosanthes	39.38 + 8.13	10.63 + 1.95	50.63	16.88

treatment. The same trend was reflected in the fresh and dry weights of roots also. The fresh and dry weights of roots were 50.63 g and 16.88 g in the combined treatment while the corresponding sum of individual values were 44.38 g and 7.88 g respectively. There was a decrease in the biomass production when setaria was grown along with stylosanthes. The shoot fresh and dry weights of setaria were 41.88 g and 12.5 g when grown alone, while the corresponding values were 35 g and 10 g in the combined treatment. The sum of root fresh and dry weights were also decreased from 121.25 g and 41.0 g when grown alone to 88.75 g and 34.38 g respectively in the combined treatment (Plates 3 and 4). In all the combined treatments, the fresh and dry weights of shoots of stylosanthes were remarkably reduced than when the plant was grown alone.

4.2.3 AMF colonization, intensity and spore production in different host plants

Among the combined treatments, maximum colonization of 95 and 100 per cent on 30 and 90 DAP were obtained in setaria + stylosanthes treatment (Table 6). The per cent mycorrhizal colonization of 71.65 and 88.96 per cent in the guinea grass treatment on 30 and 90 DAP was found to be decreased to 67.05 and 82.45 per cent respectively when it was grown in combination with stylosanthes. Conversely, the AMF colonization percentage was found to be increased when setaria as well as congosignal were grown along with stylosanthes than when grown alone. The AMF colonization percentage of stylosanthes was also found to be decreased when grown in combination with other hosts as compared with that of individual treatment. The intensity of

Plate 3 Root development in different mycorrhizal host plants

Plate 4 Root development in different mycorrhizal hosts in combination with stylosanthes


GUINEA GRASS
SETARIA
CONGOSIGNAL
STYLOSANTHES


GUINEA GRASS
+
STYLOSANTHES
SETARIA
+
STYLOSANTHES
CONGOSIGNAL
+
STYLOSANTHES

Table 6 AMF colonization, intensity and spore production in different host plants

Treatments		AMF colonizaiton (%)		Intensity of colonization		Spore count (per 25g substrate)
		30	90	30	90	
T_1	Guinea grass	71.65	88.96	++	+++	1137.50
T_2	Setaria	89.35	93.53	++	+++	1075.00
T_3	Congosignal	76.60	88.73	++	+++	1525.00
T_4	Stylosanthes	88.70	91.63	++++	++++	1625.00
T_5	Guinea grass + Stylosanthes	67.05 + 73.28	82.45 + 87.63	++ ++	+++ +++	1475.00
T_6	Setaria + Stylosanthes	95.00 + 73.68	100.00 + 83.30	+++ ++	+++ +++	987.50
T_7	Congosignal + Stylosanthes	72.05 + 73.30	100.00 + 81.73	++ ++	++++ ++	1612.50

colonization was found to be very high in the stylosanthes alone treatment. Among the individual treatments, stylosanthes had the highest spore count of 1625 followed by congosignal (1525) as against the least of 1075 of setaria. In the combined treatments, combination of congosignal + stylosanthes resulted in the production of maximum spore count of 1612.5 as against the least of 987.5 of setaria + stylosanthes treatment. The spore production was found to be remarkably increased from 1137.5 when guinea grass was used as the host plant to 1475 in the guinea grass + stylosanthes treatment.

4.3 Effect of plant growth regulators on AMF inoculum production

4.3.1 Effect on plant growth characteristics

The application of different plant growth regulators significantly influenced the plant growth characteristics at different stages of growth of mycorrhizal sorghum (Table 7). Application of 2,4-D 4 mg l^{-1} significantly enhanced the number of leaves (6) on 30 DAP followed by IAA 100 mg l^{-1}, humic acid 2000 µl l^{-1} and 2,4-D 2 mg l^{-1} (5.67 each) and IAA 50 mg l^{-1} and IBA 25 mg l^{-1} (5.33 each) as compared to that of control plants (4). All the different treatments produced more number of leaves than the control plants. On 60 DAP, significantly higher number of leaves were produced in treatments with IAA 50 mg l^{-1}, IBA 25 and 50 mg l^{-1} (8 each) as against control plants (6). The different treatments stimulated to produce more number of leaves on 60 DAP also. On subsequent growth stages (90 DAP), the effect of applications of different growth regulators did not exert significant effect to produce more number of leaves. With regard to plant height, application of humic acid 1000

Table 7 Effect of foliar application of growth regulators on growth characteristics of mycorrhizal sorghum plants

Treatments		Number of leaves			Plant height (cm)		
		Days after planting			Days after planting		
		30	60	90	30	60	90
T_1	IAA 25 mg l^{-1}	5.00	7.33	8.67	39.07	49.87	82.80
T_2	IAA 50 mg l^{-1}	5.33	8.00	10.67	48.00	53.73	77.53
T_3	IAA 100 mg l^{-1}	5.67	7.00	9.33	44.23	50.50	84.23
T_4	IBA 25 mg l^{-1}	5.33	8.00	8.67	46.67	54.03	94.07
T_5	IBA 50 mg l^{-1}	5.00	8.00	8.67	45.17	52.70	81.97
T_6	IBA 100 mg l^{-1}	5.00	7.33	8.67	38.50	49.40	84.60
T_7	NAA 25 mg l^{-1}	5.00	6.67	8.00	41.00	51.60	77.73
T_8	NAA 50 mg l^{-1}	4.67	7.33	9.00	41.33	49.93	83.33
T_9	NAA 100 mg l^{-1}	5.00	6.67	8.00	42.00	46.70	67.33
T_{10}	Humic acid 1000 µl l^{-1}	5.00	7.67	10.67	52.67	65.93	80.97
T_{11}	Humic acid 2000 µl l^{-1}	5.67	7.33	9.00	40.00	52.03	75.23
T_{12}	Humic acid 3000 µl l^{-1}	4.67	7.00	10.00	46.63	64.07	106.80
T_{13}	2,4-D 2 mg l^{-1}	5.67	7.33	9.33	45.00	59.27	93.53
T_{14}	2,4-D 4 mg l^{-1}	6.00	7.33	9.00	38.23	51.77	73.87
T_{15}	2,4-D 6 mg l^{-1}	5.00	6.67	8.00	27.00	49.07	65.93
T_{16}	Control	4.00	6.00	9.00	36.67	49.07	72.43
	C D (0.05)	1.27	1.83	NS	11.95	15.84	30.64

µl l^{-1} exerted significant effect in increasing plant height on 30 DAP and 60 DAP (52.67cm and 65.93 cm respectively) whereas in control plants the corresponding values were 36.67 cm and 49.07 cm respectively. At later stages of growth, stimulation of plant height was most significant in treatment with humic acid 3000 µl l^{-1}(106.80 cm) as compared to control (72.43 cm) (Plate 5). Most of the treatments tended to increase the plant height than the untreated plants. The effect of application of most of the plant growth regulators increased the growth characteristics at different stages of growth of the mycorrhizal plants.

4.3.2 Effect on fresh and dry weights of shoots and roots

The results indicated that application of humic acid 1000 µl l^{-1} produced significantly higher fresh and dry weights of shoot (41.67g and 19.0 g respectively) and dry root weight (6.67 g) when compared with the respective values of 21.67, 8.4 and 2.81 g in control plants (Table 8, Plate 6). The fresh weight of roots of different growth regulator treated plants were on par. The application of IAA 50 mg l^{-1} also induced a significantly higher root dry weight (5.83g) than control. No other treatments had any significant effect on the biomass of the mycorrhizal plants. In most of the treatments, the fresh and dry weights of shoots and roots were comparatively higher than that of the untreated plants.

4.3.3 Effect on AMF colonization, intensity and spore count

The data on the effect of plant growth regulators on AMF colonization,

Plate 5 Effect of humic acid 3000 µl l^{-1} on growth of mycorrhizal sorghum


CONTROL
HUMIC ACID
3000 ppm

Table 8 Effect of foliar application of growth regulators on fresh and dry weights of shoots and roots of mycorrhizal sorghum plants

Treatments		Weight of shoots (g)		Weight of roots (g)	
		Fresh weight	Dry weight	Fresh weight	Dry weight
T_1	IAA 25 mg l^{-1}	23.33	9.17	33.33	5.00
T_2	IAA 50 mg l^{-1}	25.83	10.17	49.17	5. 83
T_3	IAA 100 mg l^{-1}	23.83	10.00	45.00	5.00
T_4	IBA 25 mg l^{-1}	21.67	7.50	35.00	5.00
T_5	IBA 50 mg l^{-1}	21.67	10.00	33.33	3.33
T_6	IBA 100 mg l^{-1}	25.00	10.00	32.50	4.50
T_7	NAA 25 mg l^{-1}	23.33	8.33	30.00	4.67
T_8	NAA 50 mg l^{-1}	24.17	8.33	34.17	4.33
T_9	NAA 100 mg l^{-1}	20.17	9.17	29.17	5.00
T_{10}	Humic acid 1000 µl l^{-1}	41.67	19.00	55.00	6.67
T_{11}	Humic acid 2000 µl l^{-1}	20.83	7.50	32.50	3.83
T_{12}	Humic acid 3000 µl l^{-1}	29.00	13.33	31.67	4.17
T_{13}	2,4-D 2 mg l^{-1}	25.00	11.67	29.17	5.00
T_{14}	2,4-D 4 mg l^{-1}	23.33	8.33	27.50	4.67
T_{15}	2,4-D 6 mg l^{-1}	20.00	6.67	30.83	4.17
T_{16}	Control	21.67	8.40	30.60	2.81
	C D (0.05)	16.18	10.00	NS	2.57

Plate 6 Effect of humic acid 1000 µl l^{-1} on root development in mycorrhizal sorghum


CONTROL
HUMIC ACID
(1000 ppm)

intensity and spore count are presented in Table 9. The per cent mycorrhizal colonization in different treatments were not significant at different growth stages of the host plant when compared to control. A maximum of 100 per cent colonization was recorded in most of the treatments on 90 DAP. However, the mycorrhizal colonization in humic acid 1000 µl l^{-1} treatment was lower (94.43 per cent on 90 DAP) when compared to most of the treatments. The intensity of colonization was also remarkably very low in this treatment. The intensity of colonization was uniformly high or very high in all other treatments. There was no significant enhancement in spore production due to the application of different growth regulators. In contrast, the spore counts were significantly lower in plots treated with IAA 100 mg l^{-1} and NAA 25 mg l^{-1} (333.33 each), IBA 100 mg l^{-1} (416.67), IBA 50 mg l^{-1} (466.67), IAA 25 and 50 mg l^{-1}, humic acid 1000 µl l^{-1} (533.33 each), NAA 50 mg l^{-1} (583.33) and NAA 100 mg l^{-1} (616.67) than control (950).

4.4 Effect of stress inducers on AMF inoculum production

4.4.1 Effect on plant growth characteristics

Different stress inducing substances were tested to estimate their comparative effect on plant growth characteristics of mycorrhizal sorghum and the results are summarised in Table 10. The different stress inducing substances did not exert much significant effect on the number of leaves produced on 30 DAP. Subsequently, the effect was more significantly evidenced on 60 DAP. Significantly higher number of leaves were produced in treatments with ethrel 25 µl l^{-1}, ABA 0.5 mg l^{-1}, ABA 1.0 mg l^{-1}, ABA 2.0 mg l^{-1},

Table 9 Effect of foliar application of growth regulators on AMF colonization, intensity and spore production in sorghum plants

Treatments		AMF colonization (%)		Intensity of colonization		Spore count (per 25g substrate)
		Days after planting		Days after planting		
		30	90	30	90	
T_1	IAA 25 mg l^{-1}	84.43	100.00	++	++++	533.33
T_2	IAA 50 mg l^{-1}	86.47	100.00	++	+++	533.33
T_3	IAA 100 mg l^{-1}	89.97	100.00	+++	++++	333.33
T_4	IBA 25 mg l^{-1}	84.27	99.17	++	++++	1133.33
T_5	IBA 50 mg l^{-1}	83.30	100.00	+++	++++	466.67
T_6	IBA 100 mg l^{-1}	80.23	98.60	+++	++++	416.67
T_7	NAA 25 mg l^{-1}	81.53	100.00	+++	++++	333.333
T_8	NAA 50 mg l^{-1}	88.10	100.00	++	+++	583.33
T_9	NAA 100 mg l^{-1}	76.90	98.70	++	++++	616.67
T_{10}	Humic acid 1000 µl l^{-1}	73.87	94.43	+	++	533.33
T_{11}	Humic acid 2000 µl l^{-1}	87.90	100.00	++	++++	1250.00
T_{12}	Humic acid 3000 µl l^{-1}	83.33	100.00	++	++++	1066.67
T_{13}	2,4-D 2 mg l^{-1}	82.77	100.00	+++	++++	866.67
T_{14}	2,4-D 4 mg l^{-1}	90.67	100.00	+++	++++	933.33
T_{15}	2,4-D 6 mg l^{-1}	85.67	100.00	++	++++	1050.00
T_{16}	Control	86.33	98.70	++	+++	950.00
	C D (0.05)	NS	4.39	-	-	304.11

Table 10 Effect of foliar application of stress inducers on plant growth characteristics of mycorrhizal sorghum plants

Treatments		Number of leaves			Plant height (cm)		
		Days after planting			Days after planting		
		30	60	90	30	60	90
T_1	Ethrel 25 µl l^{-1}	4.33	8.67	12.67	48.73	78.73	101.70
T_2	Ethrel 50 µl l^{-1}	4.33	8.00	12.00	43.67	60.23	93.03
T_3	Ethrel 100 µl l^{-1}	4.00	6.67	10.00	42.23	63.73	95.90
T_4	ABA 0.5 mg l^{-1}	4.33	8.67	10.00	44.60	71.17	123.57
T_5	ABA 1.0 mg l^{-1}	4.67	8.67	10.67	44.33	70.07	98.67
T_6	ABA 2.0 mg l^{-1}	4.67	8.67	9.67	41.70	65.13	102.97
T_7	NaCl 0.5 %	4.33	8.33	10.00	41.23	62.07	96.00
T_8	NaCl 1.0 %	4.33	8.00	9.00	40.67	65.13	118.07
T_9	NaCl 1.5 %	4.67	8.67	10.00	43.87	67.33	108.27
T_{10}	Water stress on 15 D.A.P.	4.67	7.67	9.00	40.87	62.17	98.23
T_{11}	Water stress on 45 D.A.P.	4.33	6.67	10.67	37.70	52.30	85.10
T_{12}	Water stress on 75 D.A.P.	5.67	8.33	13.33	52.03	69.27	80.67
T_{13}	Control	5.00	6.33	8.67	41.70	57.83	102.83
	C.D. (0.05)	NS	1.96	3.36	NS	20.01	NS

NaCl 1.5 per cent (8.67 each), NaCl 0.5 per cent (8.33) and water stress on 75 DAP (8.33). In all the rest of the treatments also, more number of leaves were produced as compared to control (6.33). Most significant increase in number of leaves on 90 DAP was observed in water stress on 75 DAP treatment (13.33) followed by ethrel 25 µl l^{-1} (12.67) as compared to control plants (8.67) (Plate 7). In all other treatments also, number of leaves produced were comparatively higher than that of control. The different stress inducers tested did not exert much influence on the plant height during early stage of growth. The plant height in ethrel 25 µl l^{-1} treated plants were found to be significantly increased on 60 DAP (78.73 cm) while it was only 57.83 cm in the control plants. In most of the other treatments too, plant height was remarkably higher than the untreated plants on 60 DAP. During later stages of growth (90 DAP) also, the influence of different stress inducers were not significant in increasing plant height.

4.4.2 Effect on fresh and dry weights of shoots and roots

The different plant stress inducers were tested to find out their effect on fresh and dry weights of shoots and roots of mycorrhizal sorghum plants. The stress inducing substances could not make any significant influence on the dry matter production of the mycorrhizal plants (Table 11).

4.4.3 Effect on AMF colonization, intensity and spore count

The results of the data on application of stress inducers on AMF colonization, intensity and spore production are presented in Table 12.

Plate 7 Influence of water stress on growth of mycorrhizal sorghum


CONTROL
WATER STRESS
75th D O P

Table 11 Effect of foliar application of stress inducers on fresh and dry weights of shoots and roots of mycorrhizal sorghum plants

Treatments		Weight of shoots (g)		Weight of roots (g)	
		Fresh weight	Dry weight	Fresh weight	Dry weight
T_1	Ethrel 25 µl l^{-1}	40.83	9.17	38.33	7.83
T_2	Ethrel 50 µl l^{-1}	39.17	8.33	35.00	7.50
T_3	Ethrel 100 µl l^{-1}	42.50	9.17	25.83	5.83
T_4	ABA 0.5 mg l^{-1}	31.67	6.67	25.00	5.67
T_5	ABA 1.0 mg l^{-1}	27.50	5.00	26.67	5.83
T_6	ABA 2.0 mg l^{-1}	28.33	5.00	31.67	5.00
T_7	NaCl 0.5 %	28.33	5.00	21.67	4.33
T_8	NaCl 1.0 %	31.67	5.67	28.33	6.67
T_9	NaCl 1.5 %	41.67	7.50	27.50	6.67
T_{10}	Water stress on 15 D.A.P.	24.17	2.50	21.67	4.67
T_{11}	Water stress on 45 D.A.P.	30.00	5.33	21.67	5.17
T_{12}	Water stress on 75 D.A.P.	30.00	5.00	26.67	5.00
T_{13}	Control	26.67	5.83	31.67	5.83
	C.D. (0.05)	NS	NS	NS	NS

Table 12 Effect of foliar application of stress inducers on AMF colonization, intensity and spore production in sorghum plants

Treatments		AMF colonization (%)		Intensity of colonization		Spore count (per 25g substrate)
		Days after planting		Days after planting		
		30	90	30	90	
T_1	Ethrel 25 µl l^{-1}	83.80	96.73	++	+++	1083.33
T_2	Ethrel 50 µl l^{-1}	94.43	100.00	+++	++++	683.33
T_3	Ethrel 100 µl l^{-1}	88.40	97.53	++	+++	583.33
T_4	ABA 0.5 mg l^{-1}	95.47	100.00	++	++++	583.33
T_5	ABA 1.0 mg l^{-1}	90.47	100.00	++++	++++	683.33
T_6	ABA 2.0 mg l^{-1}	94.43	100.00	+++	++++	983.33
T_7	NaCl 0.5 %	88.57	100.00	+	++++	983.33
T_8	NaCl 1.0 %	84.10	100.00	++	++++	1016.67
T_9	NaCl 1.5 %	95.00	100.00	+++	++++	1233.33
T_{10}	Water stress on 15 D.A.P.	100.00	100.00	++	++++	1300.00
T_{11}	Water stress on 45 D.A.P.	88.33	100.00	++	++++	2300.00
T_{12}	Water stress on 75 D.A.P.	100.00	100.00	+++	++++	2500.00
T_{13}	Control	82.20	98.70	++	+++	616.67
	C.D. (0.05)	17.53	NS	-	-	343.98

Significantly higher levels of AMF colonization of 100 per cent was observed on 30 DAP with water stress on 15 and 75 DAP treatments as against control plants (82.20 per cent). On 90 DAP, most of the treatments induced 100 per cent AMF colonization and very high intensity of colonization. However, the intensity of colonization was found to be less in ethrel 25 µl l^{-1} and 100 µl l^{-1} treatments and control than the rest of the treatments. Significantly higher spore counts were observed in all the treatments which were subjected to water stress, in all the NaCl applied treatments and in ethrel 25 µl l^{-1} treatment with maximum being in water stress 75 DAP (2500) as compared to the control plants (616.67). The spore count was found to be progressively increased with increasing quantities of NaCl and with induction of water stress during later stages of plant growth. Conversely, the spore counts were tended to be decreased proportionately with increase in the quantity of ethrel (1083.33, 683.33 and 583.33 with ethrel 25 µl l^{-1}, 50 µl l^{-1} and 100 µl l^{-1} respectively). Application of lower doses of ABA did not have any significant effect on the number of spores produced as compared with control, while ABA 2 mg l^{-1} significantly enhanced the spore production (983.33).

4.5. Effect of plant protectants on AMF inoculum production

4.5.1 Effect on plant growth characteristics

The results of the experiment to test the effect of commonly used plant protectants on plant growth are presented in table 13. The effect of different plant protection chemicals tested were not significant on the number of leaves produced on 30 and 90 DAP. However, the effect of application of streptomycin 1000 mg l^{-1} and 2000 mg l^{-1} and metalaxyl 1000 mg l^{-1} significantly increased

Table 13 Effect of plant protectants on plant growth characteristics of mycorrhizal sorghum plants

Treatments		Number of leaves			Plant height (cm)		
		Days after planting			Days after planting		
		30	60	90	30	60	90
T_1	Carbofuran 0.75 g / plant	5.00	6.67	9.00	36.50	49.80	76.63
T_2	Carbofuran 1.5 g / plant	5.00	7.33	8.33	42.53	48.80	84.00
T_3	Carbofuran 2.25 g / plant	5.33	7.67	8.33	45.77	52.80	74.47
T_4	Metalaxyl 500 mg l^{-1}	5.00	7.00	8.67	35.17	45.33	63.70
T_5	Metalaxyl 1000 mg l^{-1}	6.00	8.67	9.33	47.60	60.23	92.53
T_6	Metalaxyl 2000 mg l^{-1}	5.33	7.67	8.67	43.37	56.57	78.57
T_7	Streptomycin 500 mg l^{-1}	5.00	7.33	8.33	44.13	54.57	85.57
T_8	Streptomycin 1000 mg l^{-1}	6.33	8.33	9.00	50.33	66.53	102.87
T_9	Streptomycin 2000 mg l^{-1}	5.33	8.33	9.67	46.20	62.57	110.97
T_{10}	Control	5.00	6.00	8.00	33.10	46.97	60.33
	C. D. (0.05)	NS	1.99	NS	14.97	18.13	35.99

number of leaves on 60 DAP with respective values of 8.33, 8.33 and 8.67 as compared to 6 of control plants. The effect of streptomycin was more pronounced at higher doses in enhancing the number of leaves produced at different growth stages of the plant. The response of mycorrhizal plants to the different plant protectants varied widely among treatments with respect to plant height. Most significant increase in plant height was observed in streptomycin 1000 mg l^{-1} treatment on 30 and 60 DAP (50.33 and 66.53 cm respectively) in contrast to control plants (33.10 and 46.97 cm respectively), while streptomycin 2000 mg l^{-1} treatment produced maximum plant height on 90 DAP (110.97 cm) which was followed by streptomycin 1000 mg l^{-1} (102.87) as compared to control (60.33 cm) (Plate 8).

4.5.2 Effect on fresh and dry weights of shoots and roots

Different plant protectants were applied on the mycorrhizal sorghum plants to test their effect on the fresh and dry weights of shoots and roots (Table 14). None of the chemicals had significant effect on the dry matter production of the mycorrhizal plants.

4.5.3 Effect on AMF colonization, intensity and spore production

Data revealed that plant protection chemicals did not significantly influence the AMF colonization (Table 15). The per cent AMF colonization was high in all the treatments with maximum of 100 in carbofuran 0.75 g per plant treatment. The intensity of colonization was also uniformly high in all the treatments with maximum in streptomycin 500 mg l^{-1} and 1000 mg l^{-1}. There

Plate 8 Effect of streptomycin 2000 mg l^{-1} on growth of mycorrhizal sorghum


STREPTOMYCIN
2000 ppm
CONTROL

Table 14 Effect of plant protectants on fresh and dry weights of shoots and roots of mycorrhizal sorghum plants

Treatments		Weight of shoots (g)		Weight of roots (g)	
		Fresh weight	Dry weight	Fresh weight	Dry weight
T_1	Carbofuran 0.75 g / plant	23.33	13.17	57.50	4.67
T_2	Carbofuran 1.5 g / plant	25.83	13.33	60.00	6.67
T_3	Carbofuran 2.25 g / plant	25.00	14.67	80.00	9.17
T_4	Metalaxyl 500 mg l^{-1}	20.83	11.00	58.33	5.00
T_5	Metalaxyl 1000 mg l^{-1}	40.83	20.83	96.67	8.67
T_6	Metalaxyl 2000 mg l^{-1}	22.50	11.83	65.67	6.17
T_7	Streptomycin 500 mg l^{-1}	22.50	13.50	37.67	5.17
T_8	Streptomycin 1000 mg l^{-1}	40.83	21.67	64.00	6.67
T_9	Streptomycin 2000 mg l^{-1}	40.00	21.83	55.00	7.50
T_{10}	Control	20.83	11.50	65.00	5.00
	C. D. (0.05)	NS	NS	NS	NS

Table 15 Effect of plant protectants on AMF colonization, intensity and spore production in sorghum plants

Treatments		AMF colonization (%)		Intensity of colonization		Spore count (per 25g substrate)
		Days after planting		Days after planting		
		30	90	30	90	
T_1	Carbofuran 0.75 g / plant	100.00	100.00	+++	+++	466.67
T_2	Carbofuran 1.5 g / plant	92.13	98.60	+++	+++	400.00
T_3	Carbofuran 2.25 g / plant	97.77	98.70	++	+++	333.33
T_4	Metalaxyl 500 mg l^{-1}	90.77	96.80	++	+++	466.67
T_5	Metalaxyl 1000 mg l^{-1}	91.37	98.70	++	+++	766.67
T_6	Metalaxyl 2000 mg l^{-1}	89.58	96.27	++	+++	800.00
T_7	Streptomycin 500 mg l^{-1}	87.73	96.27	++	++++	533.33
T_8	Streptomycin 1000 mg l^{-1}	88.67	96.67	+++	++++	533.33
T_9	Streptomycin 2000 mg l^{-1}	92.87	97.33	+++	+++	766.67
T_{10}	Control	90.97	95.70	+++	+++	1133.33
	C. D. (0.05)	NS	NS	-	-	356.93

was significant reduction in spore production due to the application of different plant protection chemicals, with the maximum reduction recorded in plots treated with carbofuran 2.25 g / plant (333.33) followed by carbofuran 1.5 g / plant (400) and carbofuran 0.75 g per plant (466.67) as against 1133.33 in untreated plots. The number of spores produced were progressively reduced at every increase in concentrations of carbofuran while the reverse happened with respect to metalaxyl and streptomycin. The spore production was progressively found to be increased from 466.67 in metalaxyl 500 mg l^{-1} treatment to 766.67 in metalaxyl 1000 mg l^{-1} and to 800 in metalaxyl 2000 mg l^{-1} treatments. A similar increase from 533.33 in streptomycin 500 and 1000 mg l^{-1} to 766.67 in streptomycin 2000 mg l^{-1} was noticed.

4.5.4 Effect on the associated microflora of the rhizosphre

The application of different plant protection chemicals showed a highly varying trend on its effect on the population of different microflora in the substrate (Table 16). The total fungal flora was not significantly affected due to different plant protectants. However, the data revealed that the fungal flora were comparatively higher in most of the treatments with maximum being in carbofuran 1.5 g per plant (11.49) followed by metalaxyl 2000 mg l^{-1} (10.48) as compared to the population in control (4.92). Application of metalaxyl at different doses tended to stimulate the fungal population in the substrate.

The population of actinomycetes was found to be significantly increased due to application of different plant protection chemicals except in metalaxyl 500 mg l^{-1} and 1000 mg l^{-1} treatment and carbofuran 2.25 g / plant (4.97, 2.05 and 5.05

Table 16 Effect of plant protectants on the associated microflora in the rhizosphere of mycorrhizal sorghum plants

Treatments		Fungi 10^3 / g	Actinomycetes 10^4 / g	Bacteria 10^5 / g
T_1	Carbofuran 0.75 g plant-^{1}	8.29 (3.05)	8.85 (3.14)	1.65 (1.47)
T_2	Carbofuran 1.5 g plant-^{1}	11.49 (3.53)	13.44 (3.80)	8.98 (3.16)
T_3	Carbofuran 2.25 g plant-^{1}	4.66 (2.38)	5.05 (2.46)	33.94 (5.91)
T_4	Metalaxyl 500 mg l^{-1}	5.81 (2.61)	4.97 (2.44)	10.03 (3.32)
T_5	Metalaxyl 1000 mg l^{-1}	5.27 (2.50)	2.05 (1.75)	5.73 (2.59)
T_6	Metalaxyl 2000 mg l^{-1}	10.48 (3.39)	17.33 (4.28)	19.51 (4.53)
T_7	Streptomycin 500 mg l^{-1}	5.63 (2.58)	16.83 (4.22)	6.99 (2.82)
T_8	Streptomycin 1000 mg l^{-1}	9.86 (3.30)	25.90 (5.19)	17.60 (4.31)
T_9	Streptomycin 2000 mg l^{-1}	6.58 (2.75)	11.20 (3.49)	1.74 (1.65)
T_{10}	Control	4.92 (2.43)	3.32 (2.08)	11.55 (3.54)
	CD (0.05)	NS	(1.22)	(1.72)

Figures in parantheses indicate transformed values (square root transformation)

respectively). Most significant increase in actinomycetes population was observed in streptomycin 1000 mg l^{-1} (25.90) followed by metalaxyl 2000 mg l^{-1} (17.33) and streptomycin 500 mg l^{-1} (16.83) as against 3.32 in control. Streptomycin application at 500 and 1000 mg l^{-1} was found to have stimulatory effect on the total populations of actinomycetes but at 2000 mg l^{-1}, the population was found to be reduced. A similar effect of reduction in actinomycete population was also found at higher concentration of carbofuran 2.25 g / plant. Although the population of actinomycetes was lower at low doses of metalaxyl (4.97 and 2.05), the population was significantly increased at higher concentration of metalaxyl 2000 mg l^{-1} (17.33).

An erratic trend on the population of bacteria with different doses of different plant protection chemicals tested was evident. The bacterial population was most significantly increased in carbofuran 2.25 g / plant treatment (33.94) while the population was significantly decreased in carbofuran 0.75 g / plant treatment (1.65) and in streptomycin 2000 mg l^{-1} treatment (1.74) as compared to control (11.55). The bacterial population was not significantly affected by the other treatments.

DISCUSSION

5. DISCUSSION

The lack of sufficient quantity of prime inoculum is the chief impediment in the adoption of AMF technology in sustainable agriculture. The present investigation was aimed at standardizing mass production techniques of AMF inoculum. Experiments were conducted to select suitable substrates and host plants for mass production of AMF and to study the effect of plant growth promoters, stress inducers and plant protectants on AMF colonization and spore production.

Soil was often used as the substrate for the AMF inoculum production. But, due to its high bulk density, efforts were made to find out other substances that can be used as effective substrates and carrier materials for the inoculum production. The present study to select the most suitable substrate combination for AMF mass inoculum production using sorghum as host plant showed that the different substrate combinations did not influence the plant growth characteristics and dry matter production of sorghum (Table 1a and 2a). The basal medium, vermiculite and the other substrate, perlite used in the experiment are inert materials and do not carry much nutrients. Hence, neither substantial growth enhancement nor biomass production is expected due to the different substrate combinations. Moreover, all the plants in the experiment were uniformly treated with nutrient solution during different growth stages. Analysis of the data further indicated that the interaction effect of perlite with soil significantly enhanced plant height mainly due to the perlite component rather than soil (Table 1b), while there was no significant effect due to interaction between perlite +

cowdung and cowdung + soil. The main effect of perlite also significantly enhanced growth characteristics in addition to significant increase in biomass production (Tables 1c and 2c) whereas main effect of soil or its interaction did not exert any significant influence on growth and biomass production. Significant enhancement of growth and biomass production due to the use of perlite-soilrite mix as substrates was obtained in rhodes grass (Sreenivasa and Bagyaraj, 1988a). Perlite, being low in organic matter and nutrient contents, is considered to be an ideal substrate for AMF mass production which permits improved aeration for AMF growth and spread (Wang *et al.*, 1993). Hence, it is inferred that growth enhancement and increase in biomass production noticed in perlite 20 per cent + cowdung 5 per cent + soil 10 per cent treatment is due to the effect of perlite. Significant increase in plant height was noticed due to the main effect of cowdung during the early stages of growth (Table 1c). It could be attributed to the availability of various nutrients in the organic manure (Cerna, 1980).

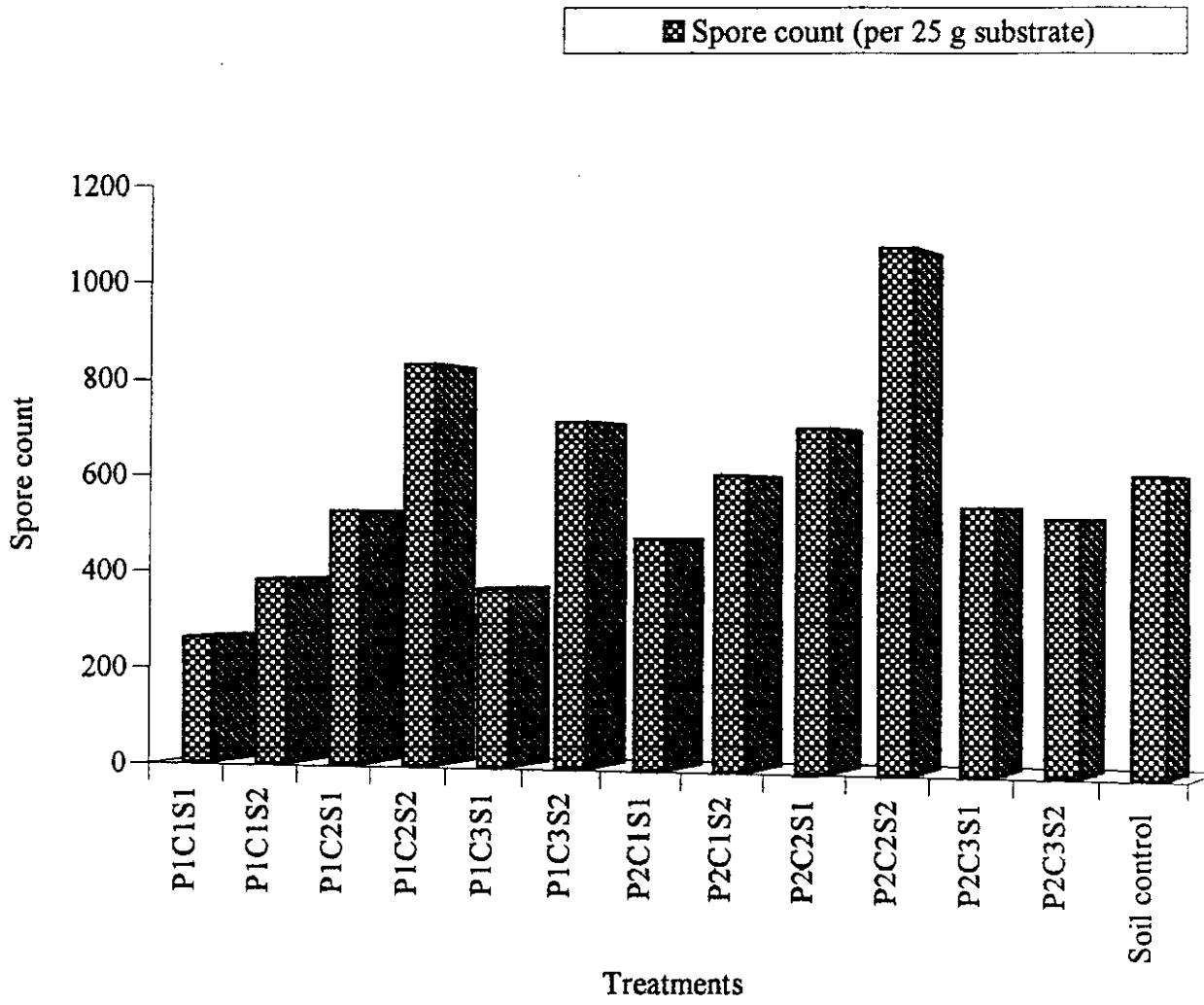
In all the substrate combinations a very high mycorrhizal colonization of above 90 percentage was observed which showed that the different substrate combinations did not exert much influence on mycorrhizal colonization and intensity. However, main effect of perlite 20 per cent significantly stimulated mycorrhizal colonization on 30 DAP, while the main effect of cowdung 10 per cent significantly reduced the per cent mycorrhizal colonization (Table 3c). Similar observations on the stimulatory effect of perlite (Sreenivasa and Bagyaraj, 1988; Mallesha *et al.*, 1992) and the inhibitory effect of cowdung at higher doses on mycorrhizal colonization (Sylvia, 1984; Aziz and Habte, 1988)

were reported.

The spore production was most significantly increased in perlite 20 per cent + cowdung 5 per cent + soil 10 per cent (1028.31) combination which accounts for an increase of 177.30 per cent over control (580) (Table 3a and Fig. 1). Significant increases in spore production were also noticed in the treatments, perlite 0 + cowdung 5 per cent + soil 10 per cent (819.92), perlite 0 + cowdung 10 per cent + soil 10 per cent (697.61) and perlite 20 per cent + cowdung 5 per cent + soil 0 (678.43). The data clearly showed the synergistic interaction effect of perlite, cowdung and soil in the right proportions in stimulating spore production. However, there was a reduction in spore production at higher doses of cowdung (at 10 per cent level), while at lower doses, it also stimulated the spore production probably due to the inhibition of mycorrhizal development consequent to higher nutrient availability. Further, the two factor interaction effect of perlite with minimal doses of cowdung significantly boosted the spore count (Table 3b(i)), while at higher doses of cowdung, the spore count showed a decreasing trend. Cowdung and soil interaction exerted maximum effect on spore production (921.17) (Table 3b (iii)). Similar positive interaction between soil and organic matter leading to higher AMF colonization and sporulation has been observed in sorghum (Zambolim *et al.*, 1992). The interaction due to perlite and soil also significantly enhanced spore production (685.97) as compared to the treatment without perlite and soil (372.31).

The main effect of perlite, cowdung and soil also significantly increased the spore production to varying extents. Maximum spore production was

Fig. 1 Effect of different substrates on AMF spore production in sorghum plants (three factor interaction)



achieved with cowdung 5.0 per cent which was followed by soil and perlite. The deleterious effect of cowdung on spore production at higher dose (10 per cent) was further evident here (Table 3c). The stimulatory effect of various substrates like perlite and soilrite on AMF spore production has been reported by various workers (Sreenivasa and Bagyaraj, 1988; Mallesha *et al.*, 1992; Rao *et al.*, 1995). Harinikumar and Bagyaraj (1989) observed that cowdung can promote AMF sporulation by enhancing root volume and the resultant proliferation of AMF in rhizosphere. But, when the level of cowdung was increased in the substrate, the nutrient availability was increased, particularly 'P'. Mycorrhizal colonization and spore production are greatly affected in 'P' rich soils (Aziz and Habte, 1988). Hence, the decrease in spore production at higher levels of cowdung might have been due to increased 'P' availability and the resultant suppression of sporulation. Soil is believed to be the ideal medium for AMF inoculum production and greatly favour AMF development and sporulation (Lakshman and Raghavendra, 1995). Its high bulk density prompted researchers to seek other substrates. The study emphasised the importance of inclusion of soil as an integral component of the substrate combination. The ideal substrate combination evolved in the present investigation for AMF inoculum production is perlite 20 per cent + cowdung 5 per cent + soil 10 per cent with 65 per cent vermiculite as the basal medium.

The comparative efficiency of guinea grass, setaria, congosignal and stylosanthes alone and the three grasses in combination with stylosanthes was tested for mass production of mycorrhizal inoculum. The growth characteristics of guinea grass (number of tillers and plant height) was slightly

reduced when stylosanthes was grown along with it (Table 4), while with setaria and congosignal the combination rather increased the growth characteristics of the two grasses. This may be due to the better nitrogen nutrition achieved through the nitrogen transfer from legume to non-leguminous grass. However, the growth of sytlosanthes was highly reduced when it was combined with the grasses probably due to the competition for nutrients other than nitrogen.

It is clear from the data that setaria is a good host for maximum biomass production since a large root volume is always preferred for an ideal AMF host plant (Jarstfer and Sylvia, 1992). Setaria has been suggested as a suitable host for AMF inoculum production by other workers too (Chulan and Omar, 1987 ; Sivaprasad, 1998). However, not withstanding its ability to produce higher root volume, in the present investigation, setaria alone or in combination with stylosanthes harboured significantly less number of spores (Table 6). Data on the biomass production of different mycorrhizal host plants in combination with stylosanthes indicated that the fresh and dry weights of shoots and roots were highly increased when guinea grass and congosignal were combined with stylosanthes while the biomass production was reduced when setaria was grown along with stylosanthes (Table 5).

Analysis of the suitability of these hosts for maximising spore counts indicated that of all the hosts and their combinations tested, maximum spore production was with stylosanthes (1625) followed by its combination with congosignal (1612.5) and congosignal alone (1525) (Table 6). This is in

agreement with the earlier finding by Singh (1992) who observed that out of the eight forage grasses tested, maximum spore production was achieved in the stylosanthes combination. However, the low root volume produced by the stylosanthes is an undesirable quality for using it as a host for commercial level inoculum production. In contrast, congosignal, in addition to its ability to produce significantly higher spore count as compared to setaria and guinea grass also produced good biomass. Guinea grass, though widely used as a host for AMF inoculum production (Bagyaraj and Manjunath, 1980), supported less spore production. Hence, congosignal which possesses the ability to produce higher spore count and a reasonable level of root volume can be combined with stylosanthes, which although recorded less root volume, harbours significantly higher number of spores. Thus, congosignal + stylosanthes was identified as the ideal host combination in the present study for maximising AMF colonization and spore production.

Growth regulator application showed a positive effect on the growth characteristics of mycorrhizal sorghum. The treatments with IAA 50 and 100 mg l^{-1}, IBA 25 and 50 mg l^{-1}, humic acid 2000 µl l^{-1} and 2,4-D 2 and 4 mg l^{-1} significantly increased the number of leaves during the early stages of plant growth (Table 7 and Fig. 2 and 3). The growth stimulating properties of these plant growth regulators, particularly during the early stages of plant growth are well documented (Salisbury and Ross, 1992). Dutra *et al.* (1996) and Souza *et al.* (1996a,1996b) observed that IBA had positive effect on the growth of mycorrhizal citrus seedlings. The growth stimulating properties of humic acid by increasing soil aggregation, aeration, permeability and by enabling the plants to

Fig. 2 Effect of growth regulators on number of leaves of mycorrhizal sorghum plants

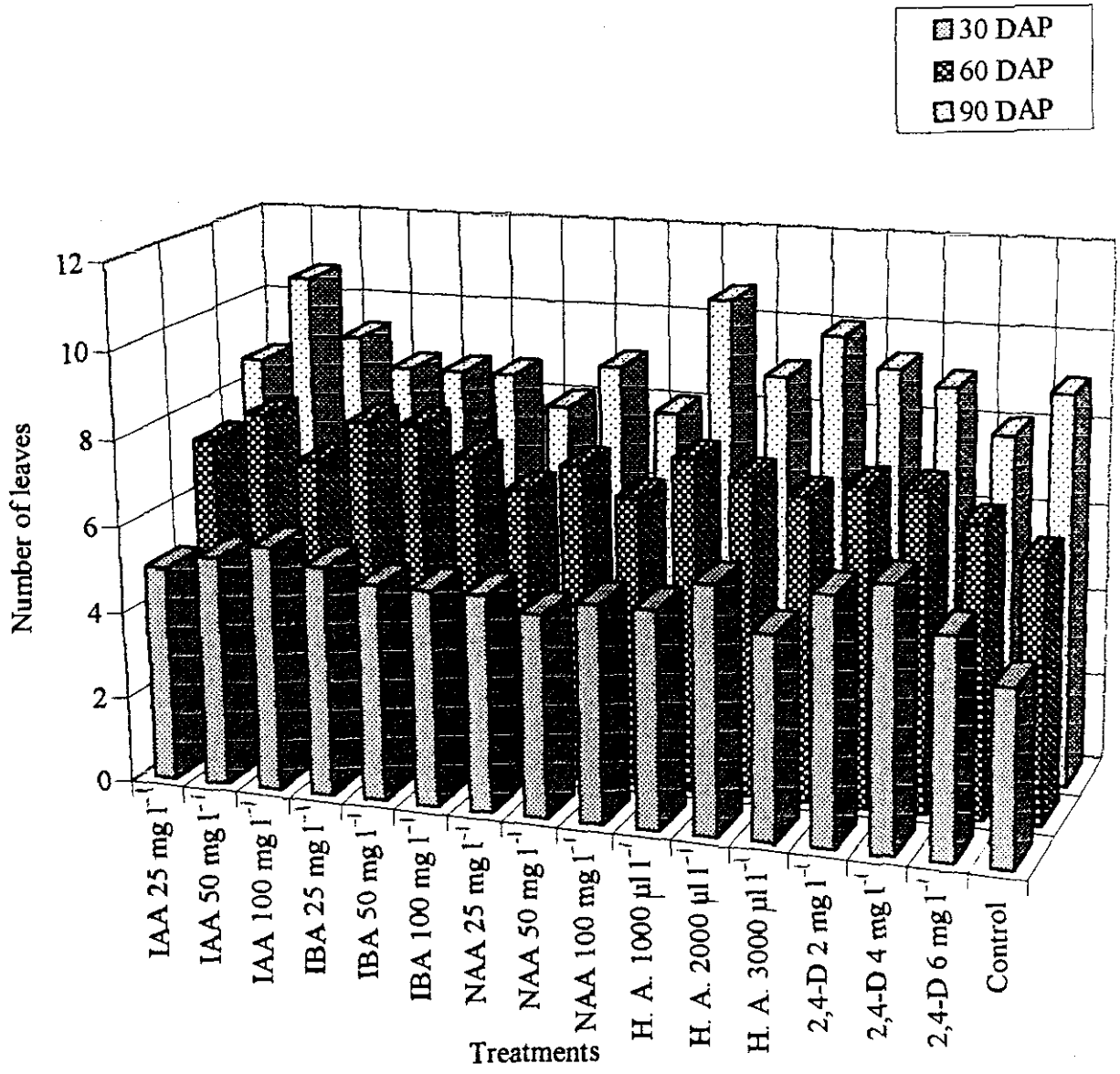
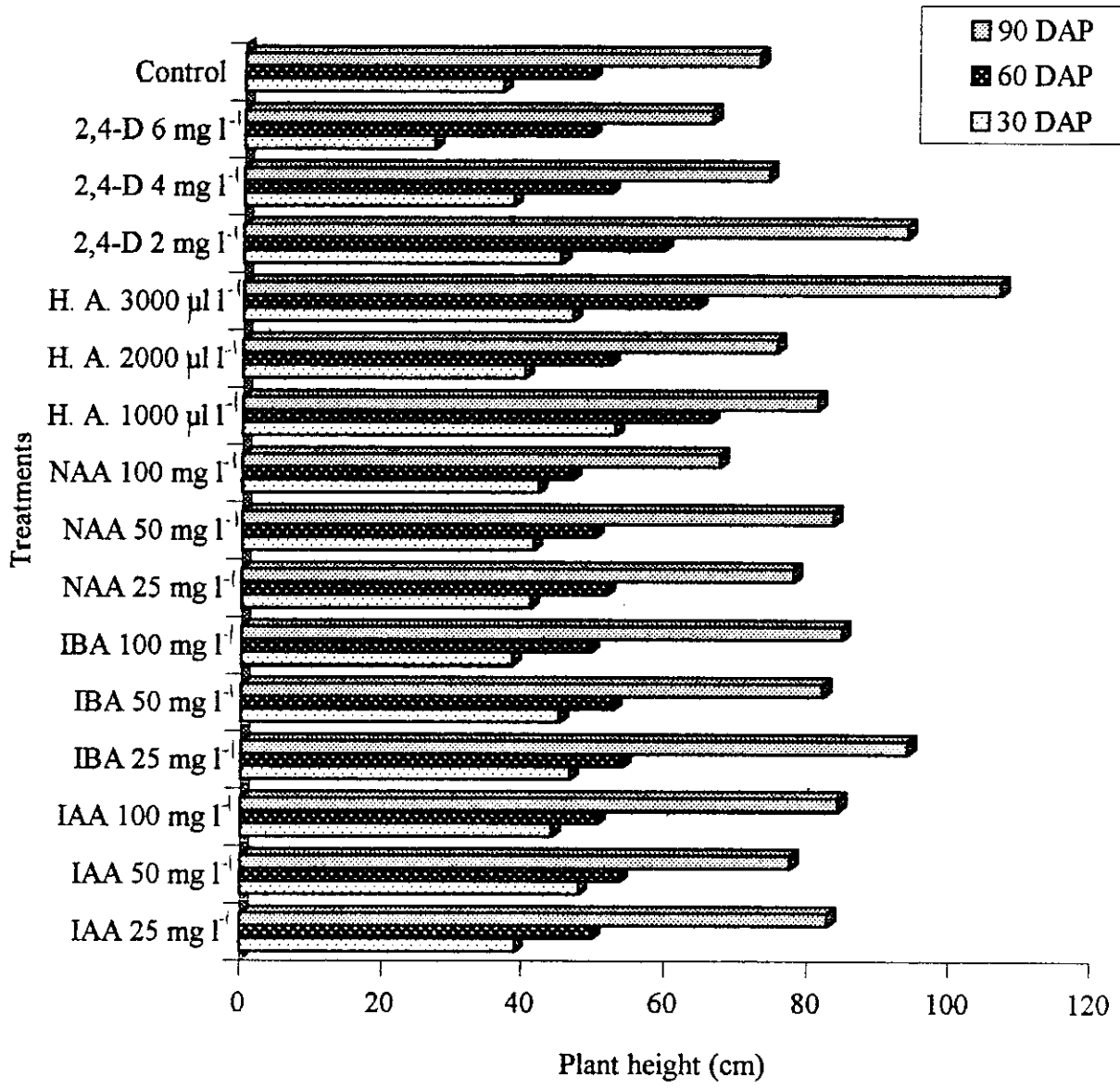


Fig. 3 Effect of growth regulators on plant height of mycorrhizal sorghum plants



utilize nutrients efficiently have been reported by Tan and Nopamornbodi (1979) and Muller-Wegener (1988).

With regard to biomass production, the superior effect of humic acid 1000 µl l^{-1} was evident. It significantly increased fresh and dry weights of shoots and roots consistently (Table 8). Higher doses of humic acid (above 2000 mg l^{-1}) had been postulated to be phytotoxic and strongly inhibited fresh and dry weights of shoots and roots of laurel plants (Vallini *et al.*, 1993). None of the other plant growth regulators tested showed any significant effect on biomass production ; perhaps the response of mycorrhizal plants to different growth regulators depends on the type of growth regulators, the AMF species and the host plants used. In the present study, humic acid 1000 µl l^{-1} produced the best response in mycorrhizal sorghum plants for plant growth and biomass production.

The per cent mycorrhizal colonization was found to be very high in most of the treatments including control (more than 98 per cent), although it was significantly reduced by humic acid 1000 µl $^{-1}$ (94.43 per cent) (Table 9 and Fig. 4). This could be attributed to higher root proliferation of the host as induced by the different growth regulators. However, there was no significant increase in spore production in any of the treatments (Fig. 5). The AMF spore count was found to be progressively decreased with increasing concentrations of IAA and IBA, while it was increased with increase in concentration of NAA and 2,4-D (Fig. 5). Firdaus *et al.* (1988) reported that AMF sporulation and infection was found to be reduced with increasing doses of IAA. The optimum concentration of humic acid for increasing AMF spore production was found

Fig. 4 Effect of growth regulators on AMF colonization in sorghum plants

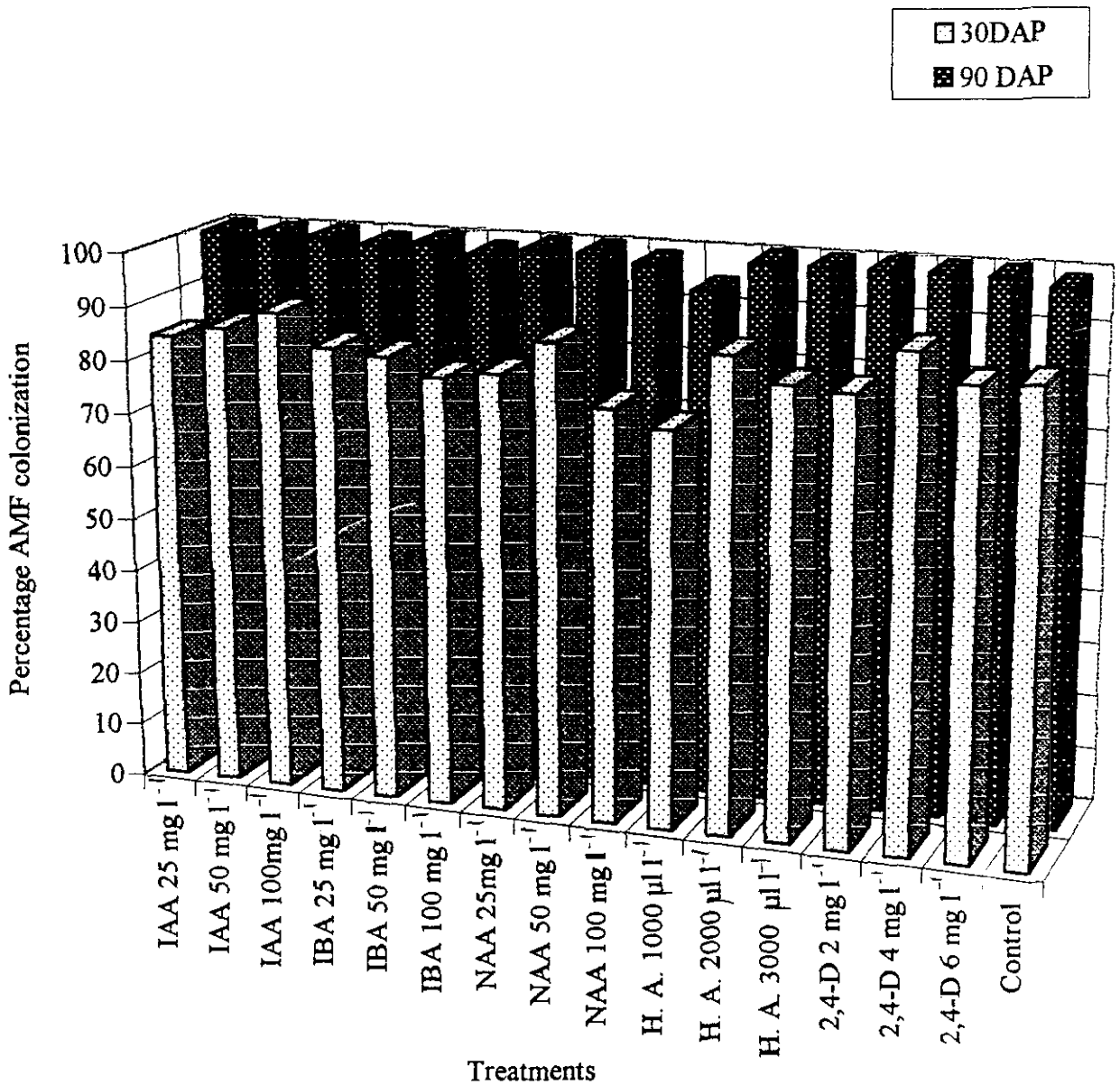
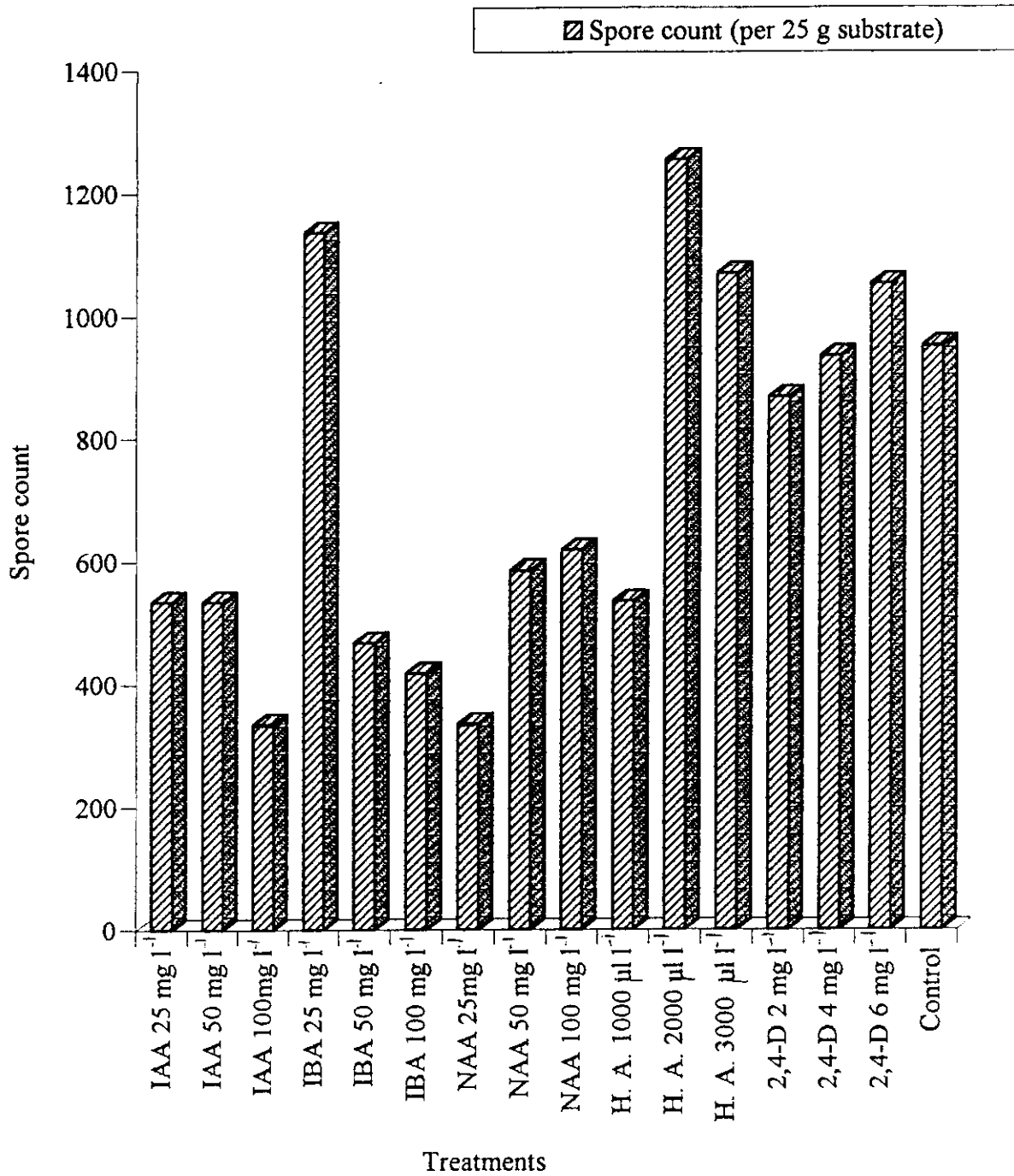


Fig. 5 Effect of growth regulators on AMF spore production in sorghum plants



to be 2000 µl l^{-1}. Detailed investigations are required to determine the optimum concentraiton at which other plant growth regulators promote AMF sporulation.

Vallini *et al.* (1993) observed that the AMF sporulation was not inhibited by increasing the doses of humic acid. He further pointed out that a slight depression of AMF infection was observed at a concentration of 3000 mg l^{-1} which could have possibly been mediated by the plant. However, it may be mentioned that studies on the effect of different growth regulators on inoculum production is sparse. The reduction in the spore count observed in some of the treatments could probably be due to the deleterious effect of the particular chemical. Conversely, certain growth regulators enhanced spore production with increase in doses which might be due to its inherent ability to promote plant growth and biomass, mycorrhizal colonization and spore production. Further detailed investigations are required to elucidate the specific effects of different growth regulators and the mechanisms involved in their response to mycorrhizal colonization and spore production.

Different stress inducing substances were tested in mycorrhizal sorghum plants to evaluate their response on AMF inoculum production. Significant increase in number of leaves was observed in the treatments, ethrel 25 µl l^{-1}, ABA 0.5, 1.0 and 2.0 mg l^{-1}, NaCl 0.5 and 1.5 per cent and water stress on 75 DAP on 60 days after planting (Table 10). On 90 DAP, the number of leaves were found to be significantly increased due to ethrel 25 µl l^{-1} and water stress on 75 DAP treatments. Plant height was significantly increased in ethrel 25 µl l^{-1} treatment on 60 DAP. No other treatments enhanced plant height

significantly during the different growth stages of the plant. Eventhough ethrel is commonly used as a flowering hormone, it can also promote shoot growth and elongation (Salisbury and Ross, 1992). However, ethrel is also reported to affect plant growth due to its activity on root formation and development (Azcon-Aguilar *et al.*, 1981).

The data further indicated that although there was a transient growth stimulation due to different stress inducers like ethrel, ABA, NaCl and water stress, the effect was not sustained throughout the life of the plant. This is truly reflected in the dry matter production of the mycorrhizal sorghum plants (Table 11). None of the treatments produced significantly higher fresh and dry weights of shoots and roots compared to control.

The mycorrhizal colonization was found to be uniformly very high in all the treatments during the later stages of plant growth (Table 10). However, maximum colonization of 100 per cent was achieved in plants which were given water stress at early stages of growth (Fig. 6). The mycorrhizal spore production was significantly enhanced due to foliar application of different stress inducers at varying concentrations (Table 12). Water stress given at different stages of the plant growth most significantly enhanced the AMF spore production; the maximum enhancement being when water stress was given on 75 DAP (2500) (Fig. 7). Similar positive effects of water stress on mycorrhizal colonization and spore production were amply described by several workers (Baylis, 1969; Iqbal and Tauqir, 1982; Mahmood and Iqbal, 1982; Sieverding and Toro, 1988; Pai *et al.*, 1990; Simpson and Daft, 1990b; Sivaprasad, 1995). This could probably be attributed to the induction of

Fig. 6 Effect of stress inducers on AMF colonization in sorghum plants

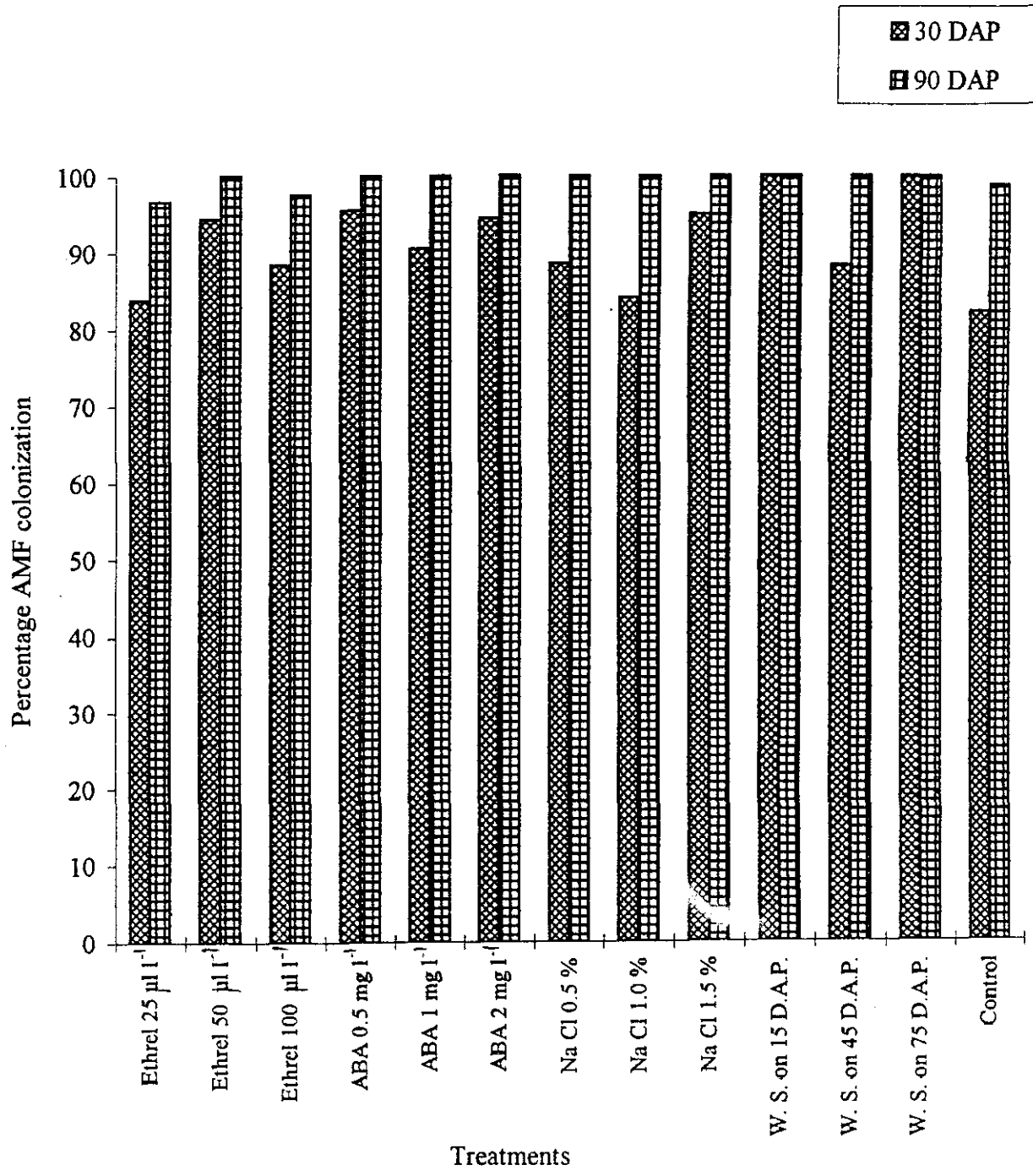
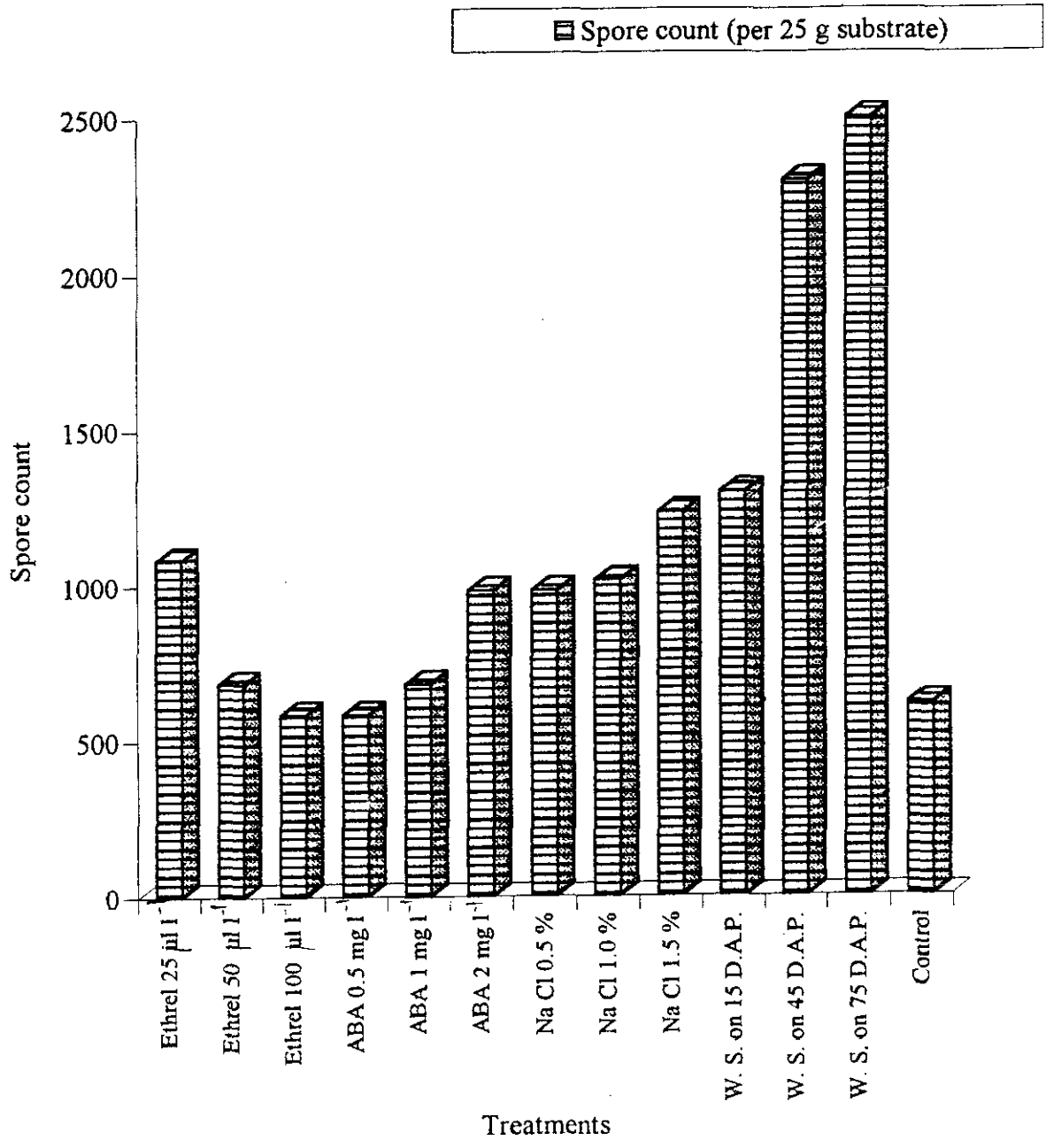


Fig. 7 Effect of stress inducers on AMF spore production in sorghum plants



senescence thereby triggering early sporulation (Ferguson and Woodhead, 1982) or the fungus might have reacted by increasing the production of propagules to survive in the stressed conditions.

The effect of NaCl also resulted in significant increase in spore production and there was a corresponding increase in the spore count with increasing concentrations of NaCl. This is in contrary to the earlier reports that NaCl inhibits the azygospore production, spore germination and hyphal growth of AMF (Hirrel, 1981 and Estaun, 1990). The exact mechanism of stimulated spore production due to NaCl obtained in the present investigation need to be investigated further for arriving at definite conclusions.

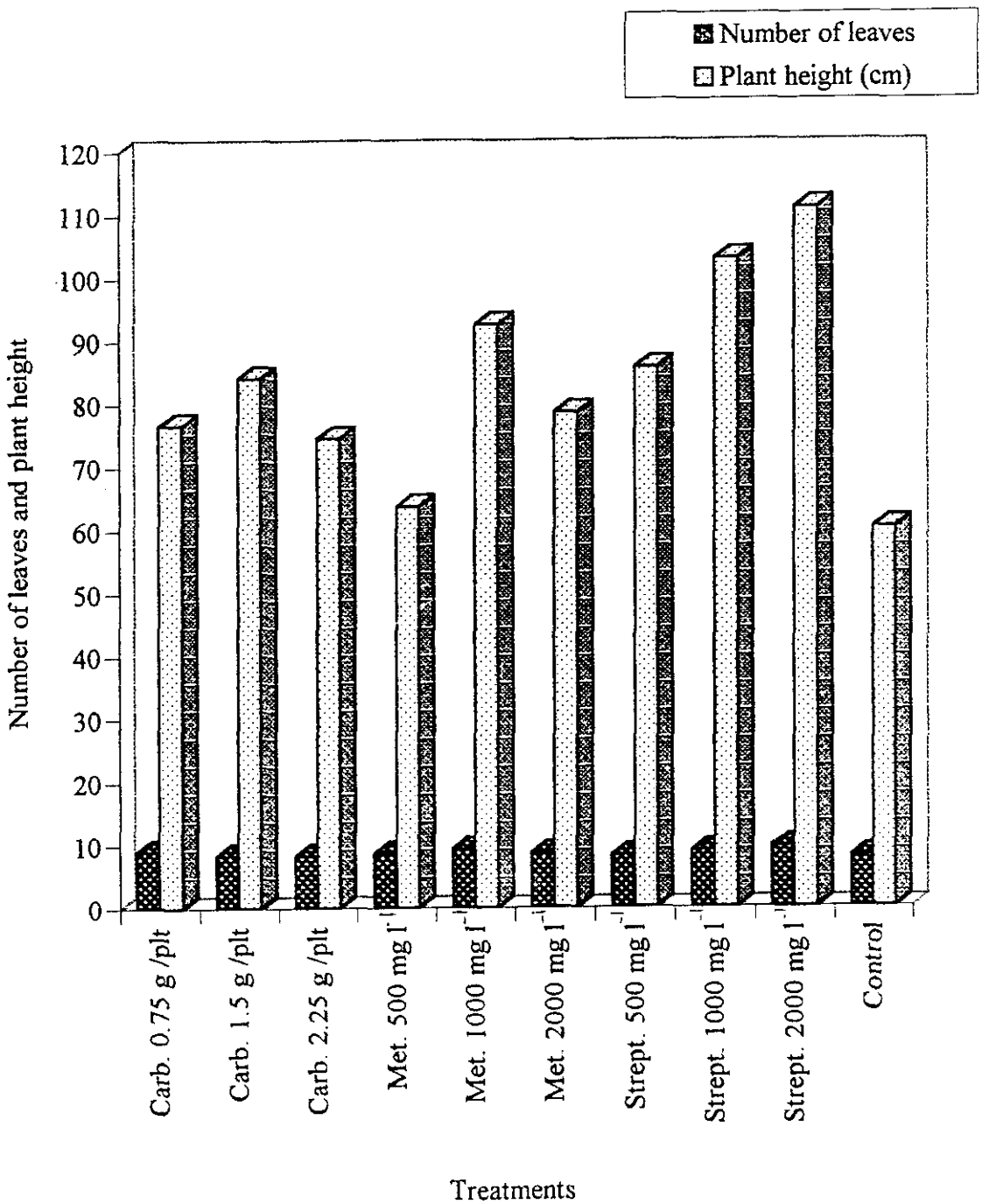
Spore production was also significantly enhanced due to ethrel 25 $\mu l\ l^{-1}$ treatment while its higher concentrations nullified this effect. Ishii *et al.* (1996) observed that very low levels of ethylene increased the sporulation and growth of AMF in trifoliate orange. However, at higher doses, it suppressed AMF formation due to the release of ethylene which could be an inhibitor of the fungal propagules in soil (Primrose, 1979 and Azcon-Aguilar *et al.*, 1981). These findings are in agreement with the present investigation also. The increase in AMF spore count could probably be achieved at a still lower concentration of ethrel. But, it is difficult to determine the critical concentration of ethrel since the ethylene released from it into the soil is degraded by the ethylene oxidising bacteria.

There was an increasing trend in the spore production with increase in the concentration of ABA and at the highest concentration tested (ABA 2 mg l^{-1}), the spore production was significantly increased. It is evident from the

experiment that the different stress inducers exerted significant influence on the spore production out of which the maximum was achieved with water stress induced at different growth stages of the plant. Hence, induction of water stress at later stages of growth of the host plant could be recommended as a standard practice for achieving maximum sporulation in AMF inoculum production systems. Information regarding the optimum concentrations of ethrel, ABA and NaCl for maximum spore production is not delineated in the present study and requires further investigations.

From the results of the study on the effect of plant protectants on growth characteristics of mycorrhizal plants, it is evident that metalaxyl 1000 mg l^{-1} exerted significant influence in increasing the leaf number, while streptomycin 1000 and 2000 mg l^{-1} significantly influenced the number of leaves and plant height (Table 13 and Fig. 8). It has been reported that AMF inoculation can improve plant growth, particularly when applied with metalaxyl, probably by reducing the pathogenic microorganisms in the rhizosphere of host plants (Afek *et al.*, 1991). Its growth enhancement effect was further reported in mycorrhizal groundnut by increasing production of nodules (Anusuya and Dhaneswari, 1995) and in mycorrhizal wheat (Shetty and Magu, 1997). The effect of streptomycin in significantly increasing the growth characteristics might be due to its antibiotic effect on phytopathogenic bacteria and stimulatory effect on other beneficial microflora in the rhizosphere. The data on the effect of plant protectants on associated microflora substantiate this inference in which the actinomycetes population was significantly higher than control (Table 16).

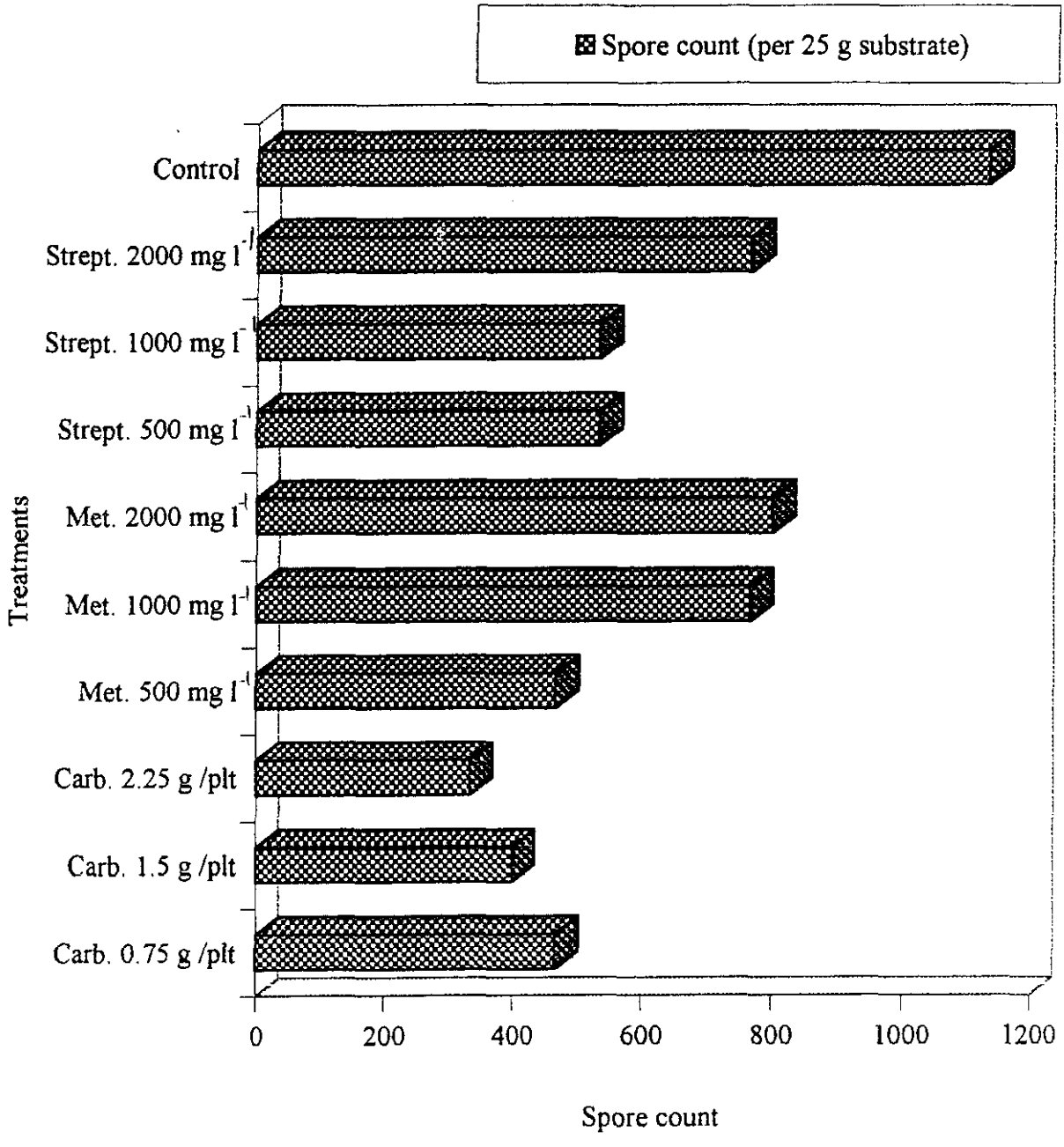
Fig. 8 Effect of plant protectants on growth characteristics of mycorrhizal sorghum plants



Eventhough carbofuran application has been reported to stimulate growth of mycorrhizal plants (Venkateswarlu and Daft, 1995), its effect was not significant on sorghum in the present investigation. The effect of plant protectants did not show any significant influence on the dry matter production of sorghum plants (Table 14). It has been reported by Venkateswarlu and Daft (1995) that plant protection chemicals especially carbofuran did not exert much influence on the shoot and root biomass of mychorrhizal plants.

No significant variation in AMF colonization was observed due to the application of different plant protectants (Table 15). But the intensity of AMF colonization was high in all the plant protectants treated plots and very high intensity of colonization was recorded in plants treated with streptomycin 500 and 1000 mg l^{-1}. This implied that the different chemicals tested did not affect the AMF colonization in sorghum. However, the application of different plant protectants significantly suppressed the AMF spore production (Fig. 9). Maximum reduction in spore count was observed in carbofuran 2.25 g treatment (333.33) while it was least in metalaxyl 2000 mg l^{-1} (800.00). The data further revealed that as the concentration of carbofuran was increased, the spore number was progressively decreased. In contrast, higher concentration of metalaxyl and streptomycin progressively stimulated AMF spore production. Several works indicated the inhibition of AMF sporulation by different plant protection chemicals. Higher levels of carbofuran are reported to be toxic to AMF sporulation (Gaur and Rana, 1990; Vankateswarlu and Daft, 1995). Application of metalaxyl 500 and 1000 mg l^{-1} drastically

Fig. 9 Effect of plant protectants on AMF spore production in sorghum plants

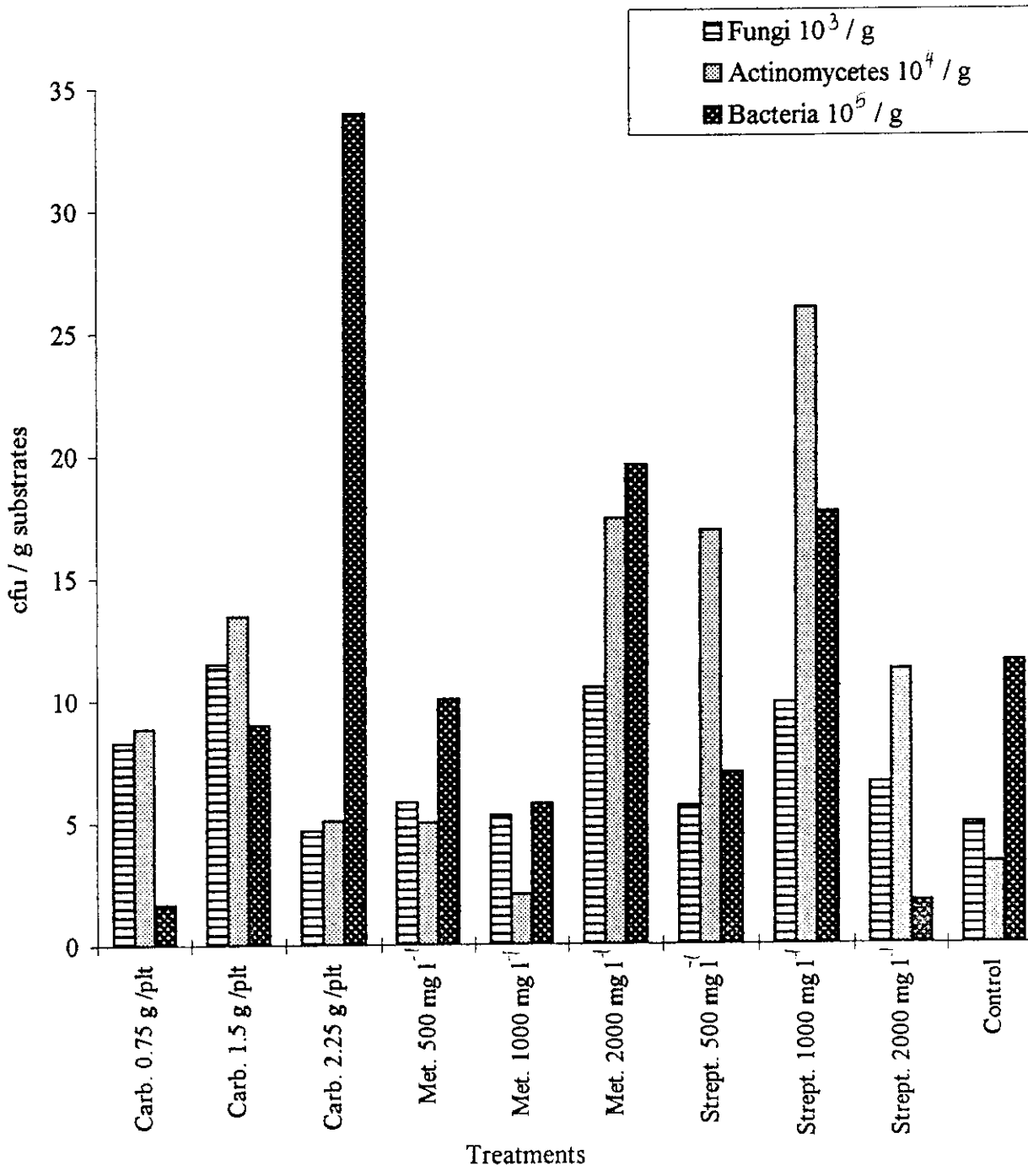


reduced AMF infection and spore production (Anusuya and Dhaneswari, 1995). There are also reports on the enhanced spore production due to metalaxyl (Seymour *et al.* 1994; Robert *et al.* 1995; Shetty and Magu, 1997). AMF spore production was found to be low at lower doses of streptomycin (Schreiner and Koide, 1993). The results of the present investigation supported these previous findings.

The analysis of the data on the effect of plant protectants on the associated microflora in the rhizosphere of mycorrhizal sorghum plants showed an erratic trend in the population of different microflora (Table 16 and Fig. 10). The plant protectants did not significantly influence the total fungal flora of the rhizosphere. This may either be due to the fact that when one plant protection chemical affect a particular group of fungal flora, other groups are stimulated to flourish and maintain the equilibrium; or the plant protection chemicals like carbofuran and streptomycin which are not reported to be fungitoxic might not be inhibitory to the fungal flora.

The population of actinomycetes were found to be significantly increased due to streptomycin treatment, metalaxyl 2000 mg l^{-1} and carbofuran 1.5 g $plant^{-1}$ while it was not significantly affected at lower doses of metalaxyl and lower and highest dose of carbofuran. The actinomycete population was temporarily increased at lower concentration of carbofuran which was not sustained at higher doses and inhibited its population. The reverse happened with metalaxyl. The population was low at lower concentration of the fungicide while its higher concentration stimulated the actinomycetes. Streptomycin significantly stimulated its population at all concentrations tested. Streptomycin, being an

Fig. 10 Effect of plant protectants on the associated microflora in the rhizosphere of mycorrhizal sorghum



antibiotic is inhibitory to bacterial population which might have indirectly favoured the proliferation of actinomycetes in the substrate.

The total bacterial population was found to be significantly reduced due to the effect of streptomycin 2000 mg l^{-1} and carbofuran 0.75 g $plant^{-1}$ while carbofuran 2.25 g $plant^{-1}$ significantly enhanced its population. This showed that streptomycin at higher concentration was deleterious to the total bacterial population in the rhizosphere of mycorrhizal sorghum, while higher doses of carbofuran stimulated it.

SUMMARY

6. SUMMARY

The present investigation was taken up for improving the techniques of the mass inoculum production of AMF.

Different substrate combinations were tested to determine their influence on plant growth and biomass of mycorrhizal sorghum and spore production of AMF. The study revealed that although the interaction of the different components *viz.*, perlite, cowdung and soil did not exert any significant influence on plant growth characteristics and biomass production, significant growth enhancement was noticed due to a two factor interaction between perlite and soil as well as the main effect of perlite and cowdung. The main effect of perlite was also significantly evident in enhancing the dry matter production. The substrate combination, perlite 20 per cent + cowdung 5 per cent + soil 10 per cent was found to be most ideal for maximum AMF spore production. The individual and the combined effect of these substrates contributed significantly in increasing the spore count. However, the spore production was found to be decreased when the concentration of the cowdung in the substrate combination was increased.

Study on the selection of suitable host plant for mycorrhizal inoculum production showed that growing stylosanthes along with setaria and congosignal increased the plant growth characteristics while with guinea grass it was reduced. The biomass production was found to be increased when guinea grass and congosignal were grown along with stylosanthes, while with setaria it was reduced. Although setaria was found to be the ideal host for

biomass production, it produced only less number of AMF spores. Maximum spore production was achieved in stylosanthes followed by its combination with congosignal and congosignal alone. It was evident from the experiment that congosignal + stylosanthes was the ideal host combination for maximum AMF colonization and spore production.

Study on the effect of different plant growth regulators showed that most of the growth regulators especially IAA, IBA, humic acid and 2,4-D stimulated significant growth enhancement at varying concentrations, whereas the biomass production was found to be greatly enhanced due to humic acid 1000 µl l^{-1} application. AMF colonization was found to be uniformly high in all the treatments with growth regulators and application of humic acid 2000 µl l^{-1} produced more number of AMF spores. Foliar application of humic acid 2000 µl l^{-1} was found to be suitable for increased AMF inoculum production.

Different stress inducing substances like ethrel, ABA, NaCl and water stress stimulated to produce significantly higher number of leaves in mycorrhizal sorghum plants. Water stress induced during different growth stages of plant resulted in very high spore production, the highest spore count of 2500 recorded when the experimental plants were water stressed on 75 DAP. Treatment with ethrel 25 µl l^{-1} and NaCl 0.5, 1 and 1.5 per cent and ABA 2 mg l^{-1} also produced significantly higher number of AMF spores.

Application of commonly used plant protectants indicated that metalaxyl (1000 mg l^{-1}) and streptomycin (1000 and 2000 mg l^{-1}) promoted plant growth characteristics significantly. While all the plant protection chemicals treated

plants exhibited very high AMF colonization percentage, their spore production was significantly decreased with maximum in carbofuran 2.25 g and minimum in metalaxyl 2000 mg l^{-1} treatment indicating that these chemicals are unsuitable for application on hosts intended for AMF inoculum production.

Estimation of the associated microflora in the rhizosphere of the plant protectants treated host plants indicated that none of the chemicals significantly altered the total fungal population, while population of actinomycetes was significantly increased with streptomycin treatment and population of bacteria was significantly reduced with streptomycin 2000 mg l^{-1} and carbofuran 0.75 g per plant.

The present study elucidated the ideal substrate and host combinations and identified stimulatory as well as inhibitory substances for maximisation of viable, infective propagules of AMF for commercial inoculum production.

REFERENCES

REFERENCES

*Aboul-Nasr, A. 1997. Inoculum production for vesicular-arbuscular mycorrhizal fungi on expanded clay. *Alexandria J. Agric. Res.* **42** : 169-176

Afek, U., Menge, J. A. and Johnson, E. L. V. 1990. Effect of *Pythium ultimum* and metalaxyl treatments on root length and mycorrhizal colonization of cotton, onion and pepper. *Plant Dis.* **74** : 117-120

Al-Raddad, A. M. 1995. Mass production of *Glomus mosseae* spores. *Mycorrhiza* **5** : 229-231

Alten, H. VON, Lindermann, A. and Schoenbeck, F. 1993. Stimulation of vesicular-arbuscular mycorrhizae by fungicides or rhizosphere bacteria. *Mycorrhiza* **2** : 167-173

Anusuya, D. and Dhaneswari, D. 1995. Effect of fungicides on nodulation and vesicular-arbuscular mycorrhizal development in groundnut. 1995. *Mycorrhizae : Biofertilizers for the Future* (Eds. Adholeya, A. and Singh, S.) *Proc. Third. Nat. Conf. on Mycorrhiza*. Tata Energy Research Institute, New Delhi. p. 141-143

Azcon, R., Azcon, G. DE., Aguilar, C. and Barea, J. M. 1978. Effects of plant hormones present in bacterial cultures on the formation and responses to VA endomycorrhiza. *New Phytol.* **80** : 359-364

Azcon-Aguilar, C., Rodriguez-Navarro, D. N. and Barea, J. M. 1981. Effects of ethrel on the formation and responses to VA mycorrhiza in *Medicago* and *Triticum*. *Plant Soil* **60** : 461-468

*Aziz, T. and Habte, M. 1988. Influence of organic residue on VAM symbiosis in *Leucaena leucocephala*. *Leucaena Res. Reports* **9** : 106-108

Backman, P. A. and Clark, E. M. 1977. Effect of carbofuran and other pesticides on vesicular-arbuscular mycorrhizae in peanuts. *Nematropica* 7 : 13-17

Bagyaraj, D. J. 1984. Biological interactions with VA mycorrhizal fungi. *VA Mycorrhiza* (Eds. Powell, C. L. and Bagyaraj, D. J.) CRC Press, Boca Raton, p. 131-153

Bagyaraj, D. J. and Manjunath, A. 1980. Selection of a suitable host for mass production of VA-mycorrhizal inoculum. *Plant Soil* **55** : 495-498

*Baltruschat, H. 1987. Evaluation of the suitability of expanded clay as a carrier material for VA mycorrhizal spores in field inoculation of maize. *Agnewandte Botanik* **61** : 163-169

Barea, J. M. and Azcon-Aguilar, C. 1982. Production of plant growth-regulating substances by the vesicular arbuscular mycorrhizal fungus *Glomus mosseae*. *Appl. Environ. Microbiol.* **43** : 810-813

Baylis, G. T. S. 1969. Host treatment and spore production by *Endogone*. *New Zealand. J. Bot.* **7** : 173-174

Baylis, G. T. S. 1971. Endogonaceous mycorrhizas synthesized in *Leptospermum* (Myrtaceae). *New Zealand J. Bot.* **9** : 293-296

Bertoldi, M. DE, Giovannetti, M., Griselli, M. and Rambelli, A. 1977. Effects of soil applications of benomyl and captan on the growth of onions and the occurrence of endophytic mycorrhizas and rhizosphere microbes. *Ann. appl. Biol.* **86** : 111-115

Bethlenfalvay, G. J., Thomas, R. S., Dakessian, S., Brown, M. S. and Ames, R. N. 1988. Mycorrhizae in stressed environments : effects on plant growth, endophyte development, soil stability and soil water. *Arid Lands : Today and Tomorrow* (Eds. Whitehead, E. E., Hutchinson, C. F., Timmermann, B. N and Varady, R. G.). Boulder, Colorado, USA. West View Press Inc. p. 1015-1029

Biermann, B. and Linderman, R. G. 1983. Use of vesicular arbuscular mycorrhizal roots, intraradical vesicles and extraradical vesicles as inoculum. *New Phytol.* 95 : 97-105

Bird, G. W., Rich, J. R. and Glover, S. U. 1974. Incresed endomycorrhizae of cotton roots in soil treated with nematicides. *Phytopath.* **64** : 48

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

*Boudarga, K. and Dexheimer, J. 1990. A simple method for culturing and multiplication of VA mycorrhizal fungi. *Agronomie* **10** : 417-422

Boyetchko, S. M. and Tewari, J. P. 1990. Root colonizaton of different hosts by the vesicular arbuscular mycorrhizal fungus *Glomus dimorphicum*. *Plant Soil* **129** : 131-136

Braunberger, P. G., Abott, L. K. and Robson, A. D. 1996. Infectivity of arbuscular mycorrhizal fungi after wetting and drying. *New Phytol.* **134** : 673-684

*Cabello, M. N. 1994. Effect of sequential application of fungicides on vesicular - arbuscular mycorrhizal fungi in field conditions. *Boletin Mycologico* **9** : 1-7

Calvet, C., Estaun, V. and Camprubi, A. 1993. Germination, early mycelial growth and infectivity of a vesicular-arbuscular mycorrhizal fungus in organic substrates. *Symbiosis* **14** : 405-411

*Caron, M. and Parent, S. 1988. Definition of a peat-lite medium for the use of vesicular-arbuscular mycorrhizae (VAM) in horticulture. *Acta Horticulturae* **221** : 289-294

Centre for mycorrhizal culture collection (CMCC), 1996. Effect of storage conditions on infectivity level of VA mycorrhizal fungi *Mycorrhiza News* **8** : 15

Cerligione, L. J., Liberta, A. E. and Anderson, R. C. 1988. Effects of soil moisture and soil sterilization on vesicular-arbuscular mycorrhizal colonization and growth of little bluestem (*Schizachyrium scoparium*) *Can. J. Bot.* **66** : 757-761

*Cerna, K. 1980. Effect of FYM and graduated ratio of mineral nutrients on yield formation in capsicum. *Sbarnik UVTIZ, Zahradnictive* **7** : 297-307

*Chulan, H. A. and Omar, M. 1987. Propagation and maintenance of VAM cultures. *Pertanika* **10** : 271-275

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

Daniels, B. A. and Trappe, J. M. 1980. Factors affecting spore germination in the vesicular-arbuscular mycorrhizal fungus, *Glomus epigaeus*. *Mycologia* **72** : 457-471

Declerck, S., Strullu, D. G. and Plenchette, C. 1996. *In vitro* mass production of the AMF, *Glomus versiforme* associated with Ri T-DNA transformed carrot roots. *Mycol. Res.* **100** (10) : 1237-1242

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

*Dehne, H. W. and Backhans, G. F. 1986. The use of vesicular-arbuscular mycorrhizal fungi in plant production. I. Inoculum production. *Zeitschrift fur Pflanzentrankheiten und Pflanzen Schutz.* **93** : 415-424

Dhillion, S. S. 1992. Evidence for host-mycorrhizal preference in native grassland species. *Mycol. Res.* **96** : 359-362

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

Duke, E. R., Johnson, C. R. and Koch, K. E. 1986. Accumulation of P, dry matter and betaine during NaCl stress of split citrus seedlings colonized with vesicular arbuscular mycorrhizal fungi on zero, one or two halves. *New Phytol.* **104** : 583-590

*Dutra, P. V., Abad, M., Almela, V. and Agusti, M. 1996. Auxin interaction with the vesicular arbuscular mycorrhizal fungus *Glomus intraradices* improves vegetative growth of two citrus root stocks. *Scientia Horticulture* **66** : 77-83

Estaun, M. V. 1990. Effects of sodium chloride and mannitol on germination and hyphal growth of the vesicular arbuscular mycorrhizal fungus, *Glomus mosseae*. *Agriculture, Ecosystems and Environment* **29** : 123-129

Ferguson, J. J. and Woodhead, S. H. 1982. Production of endomycorrhizal inoculum. A. Increase and maintenance of vesicular arbuscular mycorrhizal fungi. *Methods and Principles of Mycorrhizal Research* (Ed. Schenck, N. C.) American Phytopathological Society, St. Paul, p. 47-54

*Firdaus-E-Bareen, Iqbal, S. H. and Zain Ul-Abedin. 1988. Effect of IAA treatment of roots vesicular-arbuscular infections at various developmental stages of *Allium sativum* L. *Biologia Pakistan* **34** : 193-202

Fitter, A. H. and Nichols, R. 1988. The use of benomyl to control infection by vesicular-arbuscular mycorrhizal fungi. *New Phytol.* **110** : 201-206

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

Gaur, A. C. and Rana, J. P. S. 1990. Effect of insecticides on wheat crop inoculated with phosphate solubilising bacteria (PSB) and VAM fungi. *Trends in Mycorrhizal Research. Proc. Nat. Conf. Mycorrhiza.* Haryana Agric. Univ., Hisar, India, p. 190

Giovannetti, M., Schubert, A., Cravero, M. C. and Salutini, L. 1988. Spore production by the vesicular-arbuscular mycorrhizal fungus *Glomus monosporum* as related to host species, root colonization and plant growth enhancement. *Biol. Fertil. Soils* **6** : 120-124

Groth, D. E. and Martinson, C. A. 1983. Increased endomycorrhizal infection of maize and soybeans after soil treatment with metalaxyl. *Plant Dis.* **67** : 1377-1378

Gunze, C. M. B. and Hennessy, C. M. R. 1980. Effect of host-applied auxin on development of endomycorrhiza in cowpeas. *Trans. Br. Mycol. Soc.* **74** : 247-251

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

*Guzman-Plazola, R. A., Ferrera-Cerrato, R. and Etch-Evers, J. D. 1988. *Leucaena leucocephala,* a plant of high mycorrhizal dependence in acid soils. *Leucaena Res. Reports* **9** : 69-73

Habte, M., Aziz, T. and Yuen, J. E. 1992. Residual toxicity of soil - applied chlorothalonil in mycorrhizal symbiosis in *Leucaena leucocephala. Plant Soil* **140** : 263-268

Harinikumar, K. M. and Bagyaraj, D. J. 1989. Effect of cropping sequences, fertilizers and farmyard manure on vesicular-arbuscular mycorrhizal fungi in different crops over three consecutive seasons. *Biol. Fertil. Soils* **7** : 173-175

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

Hetrick, B. A. D. and Bloom, J. 1986. The influence of host plant on production and colonization ability of vesicular-arbuscular mycorrhizal spores. *Mycologia* **78** : 32-36

Hetrick, B. A. D., Bloom, J. and Feyerherm, S. M. 1985. Root colonization pattern of *Glomus epigaeum* in nine host species. *Mycologia* **77** : 825-828

Hirrel, M.C. 1981. The effect of sodium and chloride salts on the germination of *Gigaspora margarita. Mycologia* **73** : 610-617

*Hoflich, G. and Glante, F. 1991. Inoculum production and inoculation of effective VAM fungi. *Zentralblatt fur Mikrobiologie* **146** : 247-252

Hwang, S. F. 1988. Effects of VA mycorrhiza and metalaxyl on growth of alfalfa seedlings in soils from fields with 'alfalfa sickness' in Alberta. *Plant Dis.* **72** : 448-452

Iqbal, S. H. and Tauqir, S. 1982. The influence of soil mositure contents on VA endophytes of mycorrhizal cereals and subsequent production of endogonaceous spores by them. *Bull. Mycol.* **2** : 59-68

Ishii, T., Shrestha, Y. H., Matsumoto, I. and Kadoya, K. 1996. Effect of ethylene on the growth of vesicular-arbuscular mycorrhizal fungi and on the mycorrhizal formation of trifoliate orange roots. *J. Japanase Soc. Hort. Sci.* **65** : 525-529

Isobe, K., Fujii, H. and Tsuboki, Y. 1996. Effects of pesticides on spore germination and infection of kidney beans (*Phaseolus vulgaris* L.) of *Gigaspora margarita. Japanase J. Soil Sci.* and *Pl. Nutrition* **67** : 405-412

Jalali, B. L. and Chhabra, M. L. 1991. Effects of pesticides on vesicular-arbuscular mycorrhizal development and growth of pearl millet. *Indian Phytopath.* **44** : 314-318

*Jalaluddin, M. and Anwar, Q. M. K. 1991. VAM fungi in wheat and rice fields. *Pakistan J. Bot.* **23** : 115-122

Jarstfer, A. G. and Sylvia, D. M. 1992. Inoculum production and inoculation strategies for vesicular - arbuscular mycorrhizal fungi. *Soil Microbial Ecology* (Ed. Metting, F. B.). Marcel Dekker, New York, p. 369

*Jean, C. D. and Becerril, R. A. E. 1994. Effect of substrate on the development of sour orange trees inoculated with mycorrhizas. *Proc. Inter American Soc. Trop. Hort.* **38** : 90-94

Joseph, P. J. 1997. Management of rhizome rot and root-knot of ginger (*Zingiber officinale* R.) using VA mycorrhizal fungi and antagonists. Ph.D. thesis, Kerala Agricultural University, Thrissur. p. 192

*Kehri, H. K. and Chandra, S. 1990. VAM association in urd as affected by water stress condition in soil and foliar spray. *Acta Botanica Indica* **18** : 316-318

Lakshman, H. C. and Raghavendra, S. 1995. Effect of leaf litter and soil in different proportions on growth and biomass production in six timber tree species colonized with vesicular-arbuscular mycorrhizae. *Mycorrhizae : Biofertilizers for the Future* (Eds. Adholeya, A. and Singh, S.). *Proc. Third Nat. Conf. on Mycorrhiza*. p. 445-449

*Lee, S. S., Eom, A. H., Lee, O. H. and Kim, M. K. 1993. A study on the pot cultures of arbuscular mycorrhizal fungi in Korea. *Korean J. Mycol.* **21** : 38-50

Ludwing-Muller, J., Kaldorf, M., Sutter, E. G. and Epstein, E. 1997. Indole-3-butyric acid (IBA) is enhanced in young maize (*Zea mays* L.) roots colonized with the arbuscular mycorrhizal fungus *Glomus intraradices*. *Pl. Sci.* (Limerick) **125** : 153-162

Machado-Neto, J. G., Machado, J. O., Barreto, M., Palladini, L. A. and Matallo, M. B. 1994. Effect of benomyl on the formation of mycorrhiza in roots of Tomato (*Lycopersicon esculentum* Mill.). *Bull. Environ. Con. Toxicol.* **52** : 864-870

*Mahmood, T. and Iqbal, S. H. 1982. Influence of soil moisutre contents on VA mycorrhizal and pathogenic infection by *Rhizoctonia solani* in *Brassica napus*. *Pakistan J. Agric. Res.* **3** : 45-49

Mallesha, B. C., Bagyaraj, D. J. and Pai, G. 1992. Perlite-soilrite mix as a carrier for mycorrhiza and rhizobia to inoculate. *Leucaena leucocephala*. *Leucaena Res. Reports* **13** : 32-33

Manjunath, A. and Bagyaraj, D. J. 1981. Components of VA mycorrhizal inoculum and their effects on growth of onion. *New Phytol.* **87** : 355-361

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

Marschner, H. 1995. Mineral Nutrition of High Plants. Academic Press, London, England

Mehrotra, M. D. 1996. Multiplication of VAMF on Paulownia - a veritable possibility. *Indian Forester* **122** : 858-860

Menge, J. A. 1983. Utilization of vesicular-arbuscular mycorrhizal fungi in agriculture. *Can. J. Bot.* **61** : 1015-1024

Menge, J. A. 1984. Inoculum production. *VA Mycorrhiza.* (Eds. Powell,C. L. and Bagyaraj, D. J). CRC Press, Boca Raton. p. 187-203

Mercy, M. A., Shrivashankar, G. and Bagyaraj, D. J. 1990. Mycorrhizal colonization in cowpea is host dependent and heritable. *Plant Soil* **121** : 292-294

Miller, R. M., Jarstfer, A. G. and Pillai, J. K. 1987. Biomass allocation in an *Agropyron smithii - Glomus Symbiosis. Amer. J. Bot.* **74** : 114-122

Morton, J. B. and Benny, G. L. 1990. Revised calssification 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. Phytopath.* **II** : 171-196

Muller-Wegener, U. 1988. Interaction of humic substances with biota. *Humic Substances and their Role in the Environment.* (Eds. Frimmel, F. H. and Christman, R. F). John Wiley and Sons, Chichester, New York. p. 192

Nadarajah, P. and Nawawi, A. 1990. Vesicular arbuscular mycorrhizal root colonization and spore production in maize inoculated with *Glomus fasciculatum. Trends in Mycorrhizal Research. Proc. Nat. Conf. on Mycorrhiza.* Haryana Agricultural University, Hisar, p. 196

Nair, S. K., Girija, V. K. and Kamala Nayar. 1991. Effect of certain plant protection chemicals on the incidence of VA mycorrhiza in black gram. *Agric. Res. J. Kerala* **29** : 61-62

Nelsen, C. E. and Safir, G. R. 1982. Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. *Planta* **154** : 407-413

Nemec, S. 1980. Effects of 11 fungicides on endomycorrhizal development in sour orange. *Can. J. Bot.* **58** : 522-526

Nicolson, T. H. 1960. Mycorrhiza in the graminae. II. Development in different habitats, particularly sand dunes. *Trans. Br. Mycol. Soc.* **43** : 132-145

Niemira, B. A., Safir, G. R., Hammerschmidt, R. and Bird, G. W. 1995. Production of pre-nuclear minitubers of potato with peat-based arbuscular mycorrhizal fungal inoculum. *Agron. J.* **87** : 942-946

Norman, N. O. and Linn, M. B. 1969. Deleterious effect of certain fungitoxicants on the formation of mycorrhiza on corn by *Endogone fasciculata* and on corn root development. *Phytopath.* **59** : 297-300

Occampo, J. A. and Hayman, D. S. 1980. Effects of pesticides on mycorrhiza in field - grown barely,maize and potatoes. *Trans. Br. Mycol. Soc.* **74** : 413-416

O' Keefe, D. M. and Sylvia, D. M. 1990. Mechanisms of the vesicular arbuscular mycorrhizal plant-growth response. *Handbook of Applied Mycology*, Vol. I. (Eds. Arora, D. K., Rai, B., Mukerji, K. G. and Knudsen, G. R.) Marcel Dekker, New York, p. 35-54

Pai, G., Bagyaraj, D. J. and Prasad, T. G. 1990. Mycorrhizal reproduction as influenced by moisture stress. *Current Trends in Mycorrhizal Research* (Eds. Jalali, B. L. and Chand, H.). *Proc. Nat. Conf. Mycorrhiza* Haryana Agric. Univ., Hisar, India, p. 25

Parvathi, K., Venkateswarlu, K. and Rao, A. S. 1985a. Toxicity of soil-applied fungicides and gypsum to the vesicular-arbuscular mycorrhizal fungus, *Glomus mosseae* in groundnut. *Can J. Bot.* **63** : 1673-1675

Parvathi, K., Venkateswarlu, K. and Rao, A. S. 1985b. Effects of pesticides on development of *Glomus mosseae* in groundnut. *Trans. Br. Mycol. Soc.* **84** : 29-33

*Perrin, R., Duvert, P. and Plenchette, C. 1988. Substrate receptiveness to mycorrhizal association : concepts, methods and applications. *Acta Horticulturae* **221** : 223-228

Pfleger, F. L., Steward, E. L. and Noyd, R. K. 1994. Role of VAM fungi in mine land revegetation. *Mycorrhizae and Plant Health.* (Eds. Pfleger, F. L. and Linderman, R.G.) APS, Minnesota, USA. p. 47-81

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

Plenchette, C. and Perrin, R. 1992. Evaluation in the greenhouse of the effects of fungicides on the development of mycorrhiza on leek and wheat. *Mycorrhiza* **1** : 59-62

Plenchette, C., Furlan, V. and Fortin, J. A. 1982. Effects of different endomycorrhizal fungi on five host plants grown on calcined montimorillonite clay. *J. Amer. Soc. Hortic. Sci.* **107** : 533-538

Price, N. S., Hussey, R. S. and Roncadori, R. W. 1990. Tolerance of cotton to *Meloidogyne incognita* as influenced by phosphorus fertilizer and a vesicular arbuscular mycorrhizal fungus. *Ann. appl. Biol.* **115** : 461-467

Primrose, S. B. 1979. Ethylene and agriculture : the role of the microbe. *J. appl. Bacteriol.* **46** : 1-25

Rao, V. P., Pawar, S. E. and Singh, S. N. 1995. Production and application of vesicular-arbuscular mycorrhizal inocula for sustainable agriculture. *Mycorrhizae : Biofertilizers for the Future* (Eds. Adholeya, A. and Singh, S). *Proc. Third Nat. Conf. Mycorrhiza.* Tata Energy Research Institute, New Delhi. p. 424-428

Robert, C. P. 1998. Management of foot rot of black pepper (*Piper nigrum* L.) with VA mycorrhizae and antagonists. Ph.D. thesis, Kerala Agricultural University, Thrissur p. 191

Robert, C. P., Sivaprasad, P., Mammootty, K.P. and Lekha, K. S. 1995. Effect of fungicides on vesicular-arbuscular mycorrhizal fungi under natural condiitons. *Mycorrhizae : Biofertilizers for the Future.* (Eds. Adholeya, A. and Singh, S.) *Proc. Third Nat. Conf. on Mycorrhiza.* Tata Energy Research Institute, New Delhi p. 137-146

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

Ryan, M. H., Chilvers, G. A. and Dumaresq, D. C. 1994. Colonization of wheat by VA mycorrhizal fungi was found to be higher on a farm managed in an organic manner than on a conventional manner. *Plant Soil* **160** : 33-40

Safir, G. R., Boyer, J. S. and Gerdemann, J. W. 1971. Mycorrhizal enhancement of water transport in soybean. *Science* **72** : 581-583

Safir, G. R., Boyer, J. S. and Gerdemann, J. W. 1972. Nutrient status and mycorrhizal enhancement of water transport in soybean. *Pl. Physiol.* **49** : 700-703

Saif, S. R. 1977. The influence of stage of host development on vesicular-arbuscular mycorrhizae and endogonaceous population in field grown vegetable crops I. Summer-grown crops. *New Phytol.* **79** : 341-348

Salisbury, B. F. and Ross, W. C. 1992. *Plant Physiology.* Wordsworth Publishing Co., California p. 366

Sanders, I. R. and Fitter, A. H. 1992. Evidence for differential responses between host-fungus combinations of VAM from a grassland. *Mycol. Res.* **96** : 415-419

*Schnitzler, W. H. and Michalsky, F. 1996. Tomatoes in different substrates with mycorrhiza. *Gemuse* (Munchen) **32** : 592-594

Schreiner, R. P., Bethlenfalvay, G. J. 1997. Mycorrhizae, biocides and biocontrol. 3. Effects of three different fungicides on developmental stages of 3 AM fungi. *Biol. Fertil. Soils* **24** : 18-26

Schreiner, R. P. and Koide, R. T. 1993. Streptomycin reduces plant response to mycorrhizal infection. *Soil Biol. Biochem.* **25** : 1131-1133

Seymour, N. P., Thompson, J. P. and Fiske, M. L. 1994. Phytotoxicity of fosetyl-Al and phosphonic acid to maize during production of vesicular-arbsuscular mycorrhizal inoculum. *Plant Dis.* **78** : 441-446

Shetty, P. K. and Magu, S. P. 1997. Influence of metalaxyl on *Glomus fasciculatum* associated with wheat (*Triticum aestivum*). *Curr. Sci.* **72** : 275-277

Sieverding, E. and Toro, T. S. 1988. Influence of soil water regimes on VA mycorrhiza. V. Performance of different VAM fungal species with cassava. *J. Agron. Crop. Sci.* **161** : 322-332

Simpson, D. and Daft, M. J. 1990a. Spore production and mycorrhizal development in various tropical crop hosts infected with *Glomus clarum*. *Plant Soil* **121** : 171-178

Simpson, D. and Daft, M. J. 1990b. Interactions between water stress and different mycorrhizal inocula on plant gowth and mycorrhizal development in maize and sorghum. *Plant Soil.* **121** : 179-186

*Singh, C. S. 1992. Mass inoculum production of vesicular arbuscular (VA) mycorrhizae. I. Selection of host in the presence of *Azospirillum brasilense*. *Zentralblatt fiir Mikrobiologie* **147** : 447-453

Singh, C. S. and Deepa JHA. 1994. Mass inoculum production of vesicular-arbuscular (VA) mycorrhizae : effect of various bacteriological media and fertilizer solutions. *Microbiol. Res.* **149** : 27-29

Singh, M. and Tilak, K.V. B. R. 1990. Response of different cultivars of sorghum (*S. vulgare*) to inoculation with *Glomus versiforme*. *Current Trends in Mycorrhizal Research* (Eds. Jalali, B. L. and Chang, H.). *Proc. Nat. Conf. on Mycorrhizae* Haryana Agricultural University, Hisar, p. 70

Singh, R. P., Chhabra, M. L. and Jalali, B. L. 1990. Pesticides-mycorrhiza interactions on the growth and development of pigeonpea (*Cajanus cajan*). *Trends in Mycorrhiza Research. Proc. Nat. Conf. Mycorrhiza.* Haryana Agricultural University, Hisar, p. 186

Sivaprasad, P. 1995. Evaluation of VA mycorrhiza as biocontrol agent against wilt disease of pepper (*Piper nigrum* L.). ICAR Project Report. Kerala Agricultural University, College of Agriculture, Vellayani, Thiruvananthapuram. p. 26-27

Sivaprasad, P. 1998. Management of root diseases of important spice crops of Kerala with VA mycorrhiza. DBT Project, Kerala Agricultural University, College of Agriculture, Vellayani, Thiruvananthapuram. p. 21-22

Sivaprasad, P., Ramesh, B., Mohanakumaran, N., Rajmohan, K. and Joseph, P. J. 1995. Vesicular-arbuscular mycorrhizae for the *ex vitro* establishment of tissue culture plantlets. *Mycorrhiza : Biofertilizers for the Future* (Eds. Adholeya, A. and Singh, S.) *Proc. Third Nat. Conf. on Mycorrhiza.* Tata Energy Research Institute, New Delhi. p. 281-283

Smith, G. S., Johnston, C. M. and Cornforth, I. S. 1983. Comparison of nutrient solutions for growth of plants in sand culture. *New Phytol.* 94 : 537-548

Snedecor, G. W. and Cochran, W. G. 1962. *Statistical Methods*. Oxford and IBH Publishing Co., New Delhi, p. 585

*Souza, E. S. DE, Burity, H. A., Santo, A. C. DoE. and Silva, M. L. R. B. DA. 1996. Alternative for arbuscular mycorrhizal fungi, inoculum production in aeroponic culture. *Pesquisa Agropecuaria Brasileira* **31** : 153-158

*Souza, P. V. D. DE., Fonforia, M. A., Berjon, M. A., Orenga, V. A. and De-Souza, P. V. D. 1996. Synthetic auxins and arbuscular mycorrhizal fungi interaction : influence on vegetative growth of sour orange. *Pesquisa Agropecuaria Guacha* **2** : 167-172

Spokes, J. R., Hayman, D. S. and Kandasamy, D. 1989. The effects of fungicide - coated seeds on the establishment of VA mycorrhizal infection. *Ann. appl. Biol.* **115** : 237-241

Spokes, J. R., Mac Donald, R. M. and Hayman, D. S. 1981. Effects of plant protection chemicals on vesicular-arbuscular mycorrhizas. *Pestic. Sci.* **12** : 346-350

Sreenivasa, M. N. and Bagyaraj, D. J. 1988a. Selection of a suitable substrate for mass multiplication of *Glomus fasiculatum*. *Plant Soil* **109** : 125-127

Sreenivasa, M. N. and Bagyaraj, D. J. 1988 b. Chloris gayana (Rhodes grass), a better host for the mass production of *Glomus fasiculatum* inoculum. Plant Soil **106** : 289-290

Sreenivasa, M. N. and Bagyaraj, D. J. 1989. Use of pesticides for mass production of vesicular-arbuscular mycorrhizal inoculum. *Plant Soil* **119** : 127-132

*Sreenivasa, M. N. and Rajasekhara, E. 1989. Influence of the stage of host development and genotypes on mycorrhizal colonization of field grown wheat. *Zentralbatt fur Mikrobiologie* **114** : 381-384

*Stevens, K. J., Peterson, R. L. 1995. The effet of water gradient on the vesicular-arbuscular mycorrhizal status of *Lythrum salicaria* L. (Purple loose strife). *Boletin Mycologico* **10** : 187-189

Sutt(, J. C. and Sheppard, B. R. 1976. Aggregation of sand-dune soil by endomycorrhizal fungi. *Can. J. Bot.* **54** : 326

Sylvia, D. M. 1984. Aerated-stream treatment to eliminate VA mycorrhizal fungi from soil. *Soil Biol. Biochem.* **16** : 675-676

Sylvia, D. M. and Hubbell, D. H. 1986. Growth and sporulation of vesicular arbuscular mycorrhizal fungi in aeroponic and membrane systems. *Symbiosis* **1** : 259-267

Tan, K. H. and Nopamornbodi, V. 1979. Effect of different levels of humic acids on nutrient content and growth of corn (*Zea mays* L.). *Plant Soil* **51** : 283-287

Tiwari, P.K. and Shukla, B. N. 1991. Influence of pesticides on *Glomus fasciculatum* and growth, development and nutrient uptake of soybean. *Indian J. Mycol. Pl. Pathol.* **21** : 49-55

Tommerup, I. C. and Briggs, G. G. 1981. Influence of agricultural chemicals on germination of vesicular-arbuscular endophyte spores. *Trans. Br. Mycol. Soc. 76* : 326-328

Trappe, J. M. 1987. Phylogenetic and ecological aspects of mycotrophy in the angiosperms from an evolutionary stand point. *Ecophysiology of VA Mycorrhizal Plants (Ed. Safir, G. R.) CRC Press, Boca Raton* p. 5-25

*Udaiyan, K., Sugavanam, V. and Manian,S. 1996. Effects of formaldehyde fumigation and fytolon drench on VAM fungi and nodulation in some leguminous forest tree seedlings in India. *Pertanika J. Trop. Agric. Sci.* **19** : 33-41

Vallini, G., Pera, A., Avio, L., Valdrighi, M. and Giovannetti, M. 1993. Influence of humic acids on laurel growth, associated rhizospheric microorgansims and mycorrhizal fungi. *Biol. Fertil. Soils* **16** : 1-4

Venkateswarlu, K. and Daft, M. J. 1995. Interaction effects of carbofuran and *Glomus clarum* on growth of groundnut. *Mycorrhizae : Biofertilizers for the Future* (Eds. Adholeya, A. and Singh, S.) *Proc. Third. Nat. Conf. Mycorrhiza.* Tata Energy Research Institute, New Delhi p. 124-127

*Vestberg, M. 1992. The effect of growth substrate and fertilizer on the growth and vesicular-arbuscular mycorrhizal infection of three hosts. *Agric. Sci. Finland* **1** : 95-105

Vestberg, M. and Uosukainen, M. 1992. A new method for producing VA-mycorrhiza inoculum in a soil-free substrate. *The Mycologist* **6** : 38

*Vijayalakshmi, M. and Rao, A. S. 1993. Effects of six insecticides and one fungicide on the development of VAM fungi in peanut. (*Arachis hypogeae* L.) *Zentralbatt fur Mikrobiologie* **148** : 60-65

Vilarino, A. and Sainz, M. J. 1997. Treatment of *Glomus mosseae* propagules with 50 per cent sucrose increase spore germination and inoculum potential. *Soil Biol. Biochem.* **29** : 1571-1573

Vyas, S. C. and Vyas, S. 1995. Influence of pesticides on mycorrhizal infection and chlamydospores formation in soybean (*Glycine max*). *Mycorrhizae : Biofertilizers for the Future* (Eds. Adholeya, A.and Singh, S.) *Proc. Third Nat. Conf. Mycorrhiza.* Tata Energy Reserach Institute, New Delhi p. 147-154

Vyas, S. C., Vyas, A., Mahajan, K. C. and Schroff, V. N. 1990. Effect of fungicides on mycorrhizal and rhizobial development of soybean. *Trend in Mycorrhiza Research. Proc. Nat. Conf. Mycorrhiza.* Haryana Agric. Univ., Hisar, p. 188

Wang, H., Parent, S., Gosselin, A. and Desjardins, Y. 1993. Vesicular arbuscular mycorrhizae peat-based substrates enhance symbiosis establishment and growth of three micropropagated species. *J. Amer. Soc. Hort. Sci.* **118** : 896-901

*Weritz, J., Ricken, B. and Hofner, W. 1992. Interactions of growth regulators and VA mycorrhizas in barley. *Angewandte Botanik* **66** : 115-120

Wilson, J. M. 1984. Competition for infection between vesicular-arbuscular mycorrhizal fungi. *New Phytol.* **97** : 427-43

Wood, T. 1984. Commercial production of VA mycorrhizal inoculum. Axenic versus non-axenic techniques. *Proc. Sixth North Am. Conf. Mycorrhizae* (Molina, R. Ed.) *Forest Res. Lab. Pub. Oregon.* USA p. 84

*Zambolim, L., Reis, M. A. DOS and Costa, L. M. DA. 1992. Substrates for increasing vesicular-arbuscular mycorrhizal inoculum of *Glomus etunicatum*. *Fitopatologia Brasileira* **17** : 28-31

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*Originals not seen

APPENDICES

APPENDIX - I

Martin's Rose-bengal Agar

Glucose	-	10.0 g
Peptone	-	5.0 g
KH_2PO_4	-	1.0 g
$MgSO_4$. 7 H_2O	-	0.05 g
Streptomycin	-	30.0 mg
Agar	-	15.0 mg
Rose-bengal	-	0.035 g
Distilled water	-	1000 ml

(The antibiotic is sterilized separately and added aseptically to the sterilized medium)

APPENDIX - II

Kuster's Agar

Glycerol	-	10.0 g
Caesin	-	0.3 g
$MgSO_4$	-	0.5 g
$FeSO_4$	-	0.1 g
KNO_3	-	2.0 g
NaCl	-	2.0 g
K_2HPO_4	-	0.5 g
$CaCO_3$	-	0.2 g
Agar	-	15.0 g
Distilled water	-	1000 ml

APPENDIX - III

Soil Extract Agar

Glucose	-	1.0 g
K_2HPO_4	-	0.5 g
Agar	-	15.0 g
Soil extract (stock)	-	100 ml
Tap water	-	900 ml

1000 g sieved garden soil is mixed with 100 ml tap water and steamed for 30 minutes. It is filtered through a double filter paper after adding a small amount of $CaCO_3$. 100 ml stock solution is added to 900 ml water with agar dissolved in it. Glucose is added prior to tubing.

APPENDIX IV

Nutrient Status of the soil used

Organic Carbon = 0.43 per cent

Available Nitrogen = 193 kg ha^{-1}

Available P_2O_5 = 47.3 kg ha^{-1}

Available K_2O = 94.8 kg ha^{-1}

Soil Composition

Coarse sand =16.7 per cent

Fine sand =31.3 per cent

Silt = 25.5 per cent

Clay = 26.5 per cent

Soil pH = 5.1

Water holding capacity = 21.5 per cent

Porosity = 32 per cent

Bulk density = 1.375 g cc^{-1}

STANDARDIZATION OF MASS PRODUCTION TECHNIQUE FOR VA MYCORRHIZA

By

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ABSTRACT OF THE THESIS
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ABSTRACT

Different methods to standardize the mass inoculum production techniques of AMF was attempted in the present investigation. Effort to identify suitable substrate combination indicated that a substrate combination consisting of basal medium of vermiculite 65 per cent, perlite 20 per cent, cowdung 5 per cent and soil 10 per cent as components was ideal for maximum AMF spore production as well as for plant growth.

Mycorrhizal setaria plant was found to be the ideal host for biomass production while maximum AMF spore count was obtained with stylosanthes, which possessed only less root volume. A host combination of congosignal and stylosanthes was found to be the ideal system for achieving maximum AMF colonization and spore production apart from increased plant growth and biomass.

Application of different plant growth regulators stimulated plant growth, biomass production and AMF spore production. Foliar application of humic acid 2000 $\mu l\ l^{-1}$ resulted in maximum increase in spore production and hence can be recommended for application in routine AMF inoculum production systems.

Induction of stress to the host plants using different stress inducing substances stimulated AMF sporulation. Water stress induced on 75 DAP resulted in maximum spore production. The stress inducers which increased spore production also include ethrel 25 $\mu l\ l^{-1}$, NaCl 0.5, 1.0, 1.5 per cent and ABA 2.0 $mg\ l^{-1}$ treatments.

Although the plant protection chemicals *viz*., metalaxyl (1000 mg l^{-1}) and streptomycin (1000 and 2000 mg l^{-1}) promoted plant growth, there was significant reduction in the AMF spore production and hence are unsuitable for application on hosts intended for AMF inoculum production.

The present study revealed that infective propagules of AMF in the inoculum could be increased by selecting substrates and host combinations suitable for inoculum production which could further be boosted up by applying selective growth promoting substances such as humic acid 2000 µl l^{-1} and inducing water stress during the later stages of plant growth.