EFFECT OF AZOSPIRILLUM AND VA MYCORRHIZA ON THE GROWTH OF COCOA SEEDLINGS AND INCIDENCE OF SEEDLING BLIGHT DISEASE

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Effect of *Azospirillum* and VA mycorrhiza on the growth of cocoa seedlings and incidence of seedling blight disease"** 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 to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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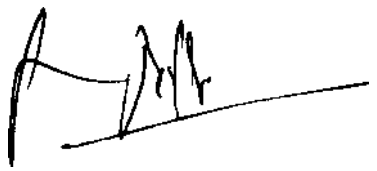
CERTIFICATE

Certified that this thesis, entitled **"Effect of *Azospirillum* and VA mycorrhiza on the growth of cocoa seedlings and incidence of seedling blight disease"** is a record of research work done independently by **Miss.Sunitha Anie Cheriyan,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.



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Dedicated to my loving parents

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INTRODUCTION

INTRODUCTION

Biofertilizers have emerged as a promising component of integrated nutrient supply system in Indian agriculture. Recently, the use of biofertilizer has attracted the attention of the small and large scale farmers because of its simple and cost effective nature. The current annual production of biofertilizers is in the range of 2000-2500 tonnes. Effective utilisation of biofertilizers will not only provide economic benefits, but also improve and maintain soil fertility and sustainability of the soil ecosystem.

Among the biofertilizers, *Azospirillum* and VA mycorrhiza have got greater importance in Indian agriculture. Use of *Azospirillum* and VA mycorrhiza is well accepted as efficient biofertilizers in many crops. *Azospirillum* is an associative nitrogen fixing bacterium and is also known to produce several growth promoting substances. Mycorrhiza is a symbiotic association between plant roots and fungal mycelia. VA mycorrhiza is associated as an obligate symbiont with majority of agricultural crops under a broad ecological range. VA mycorrhizal association improves plant growth and uptake of many nutrients mainly phosphorous. It is also known to have efficiency in checking many diseasesin crop plants.

The present study is taken up in cocoa which is one of the important cash crops of Kerala. It is a native of South America and it ranks third as a beverage crop in the world. Many diseases have been found in cocoa. Of several

diseases, seedling blight is an important nursery disease in cocoa. Major causal organism associated with this disease is *Phytophthora palmivora.* Now a days a lot of high yielding cocoa types which are tolerant to many diseases have been developed. Since cocoa is a cross pollinated plant, high yielding disease tolerant types are being multiplied by clonal propagation, mainly by budding. Budding is usually done on four to six months old root stock of cocoa itself. This time depends upon the growth and establishment of cocoa seedlings. If this period of four to six months required to get the root stock ready for budding could be reduced by the application of VA mycorrhiza and *Azospirillum*, cost of production and time required for production of budded plants will reduce substantially.

Considering all these beneficial effects of *Azospirillum* and VA mycorrhiza in the cultivation of cocoa, the present investigation was undertaken with following objectives.

i) To find out whether there is any synergistic effect for the combined inoculation of *Azospirillum* and VA mycorrhiza in improving the growth and establishment of cocoa seedlings.

ii) To find out the efficacy of the inoculants in imparting resistance to seedling blight disease.

REVIEW OF LITERATURE

Azospirillum is an associative nitrogen fixing bacterium, which was isolated for the first time by Beijerinck (1925). He named it as *Spirillum lipoferum*. Tarrand *et al.* (1978) revised the nomenclature of *Spirillum* and designated it as *Azospirillum*. Later Dobereiner *et al.* (1976) revealed that *Azospirillum* is a common inhabitant of tropical soils.

Kumari *et al.* (1976) reported the occurrence of *Azospirillum* in Indian soils. Govindan and Nair (1984) reported a fairly high population of *Azospirillum* in the rhizosphere and rhizoplane of cocoa. They reported that *A. lipoferum* was more prominent in cocoa rhizosphere. Govindan and Chandy (1985) first isolated the diazotroph *Azospirillum* from black pepper rhizosphere. Tilak *et al.* (1989) reported the occurrence of *Azospirillum* in VA mycorrhizal fungi, when surface sterilized spores of VA mycorrhizal fungi were inoculated with nitrogen free liquid medium containing malic acid and incubated under microaerophilic condition at 30°C.

Dobereiner and Day (1975) investigated *Azospirillum* as Gram negative, motile bacteria, generally vibrioid in shape. Okon *et al.* (1976a) found that when *Azospirillum* grown in nitrogen free medium, it forms subsurface pellicular growth indicating that their microaerophilic nature was due to the lack of oxygen protection mechanism for the nitrogenase enzyme and also found that

Azospirillum can grow as an aerob only when the medium was supplied with fixed nitrogen.

According to Okon *et al.* (1976b) unlike for the most other nitrogen fixing bacteria, sugars are poor substrates for *Azospirillum*. Neyra *et al.* (1977) and Baldani and Dobereiner (1980) reported the ability of *Azospirillum* strains to reduce nitrate to nitrite.

2.1 Response of crop plants to *Azospirillum* inoculation

Reynders and Vlassak (1982) studied on the response of winter wheat to *Azospirillum* inoculation. They found that inoculation increased the grain yield. *Azospirillum* inoculation along with the addition of fertilizers increased the yield in field grown wheat was reported by Rai and Gaur (1982). Subbian and Chamy (1984) reported that soil application with *Azospirillum* significantly increased the seed yield of sesame. A significant increase in root dry weight and grain yield was reported by Rai (1985) in *Panicum miliaceum* as a result of inoculation with *Azospirillum brasilense*. Govindan and Chandy (1985) studied the utilization of the diazotroph *Azospirillum* for inducing rooting in pepper cuttings. Inoculation of *Azospirillum* increased the number of roots per cutting, total length of root and root dry weight as compared to the control treatment. Besides 80 per cent of the inoculated cuttings also germinated when compared to only 40 per cent in untreated control.

In an experiment, Sarig *et al.* (1986) revealed that inoculation of naturally nodulated *Pisum sativum* L. (garden pea) with *Azospirillum* under green

house condition resulted in a significant increase in nodule number and also revealed that field inoculation of *Azospirillum* in garden pea and *Cicer arietinum* L. in winter wheat increased seed yield.

According to Schmidt *et al.* (1988) combined inoculation of *Rhizobium* and *Azospirillum* to seedlings of *Medicago sativa* grown in petridishes with sterile agar significantly increased the number of nodules, but the effects depended on the concentration of *Azospirillum* in the medium. They also observed that stimulating effect is due to the production of Indole Acetic Acid. *Azospirillum* inoculation along with the addition of nitrogen increased root dry weight in Pearlmillet (Joshi and Rao, 1989). Response of rice and wheat plants to *Azospirillum* inoculation was investigated by Sharma *et al.* (1989) and they reported a significant increase in grain yield following inoculation. Inoculation of *Azospirillum* or VA mycorrhizae increased the yield of onion bulbs (Gurubatham *et al.,* 1989). Bopiah and Khader (1989) obtained increase in plant height, shoot dry weight and root dry weight of black pepper by dipping the rooted cuttings in culture solution of *Azospirillum.*

Azospirillum inoculation increased tiller, height, dry matter, grain yield and fodder yield in Pearlmillet. This was observed by Wani *et al.* (1992). Zaady *et al.* (1993) studied the response of maize and oak seedlings inoculated with *Azospirillum brasilense* at a rate of 10 ml grown in synthetic medium containing fructose or malate as carbon source. They observed an increase in root surface area, root and leaf dry weight of maize after fifteen days following inoculation and also found that root dry weight was higher with fructose grown cells after forty

five days following inoculation. Same result was obtained with oak seedlings one month after inoculation.

Soil inoculation with *Azospirillum* at a rate of 2 kg per ha with the addition of NPK at the rate of 100, 125 and 25 kg/ha increased yield of cabbage (Jothi *et al.*, 1993). Yassin *et al.* (1994) found that root or soil inoculation of sweet potato with *Azospirillum* together with the addition of 26 kg N per ha increased the yield.

Devi *et al.* (1995) observed an increased germination percentage in chilli after seed treatment with *Azospirillum*. Seed treatment with *Azospirillum* at a rate of 2 kg/ha increased seed germination in bhendi. Plant height, root length and number of fruits per plant were highest with *Azospirillum* along with 50 per cent of the recommended N. Fruit yield was highest with both *Azospirillum* and 75 per cent of the recommended N (Sankaranarayanan *et al.*, 1995). Inoculation of bush pepper with *Azospirillum* increased the effect of growth at pH 6.0 and 8.0 (Varma, 1995). Mishra and Patjoshi (1995) obtained an increased fruit yield in okra after inoculation with *Azospirillum* alone and in combination with 50 per cent of the recommended NPK. Increased grain yield in rice was reported by Mishra and Sen (1996) in response to *Azospirillum* inoculation as seed treatment or foliar spray and also reported that foliar sprays are more effective than seed treatment. Sasikumar (1996) found that inoculation of rice with *Azospirillum* increased height, number of productive tiller and found beneficial influence on root parameters and yield. Swarupa (1996) reported that *Azospirillum brasilense* treatment significantly

increased plant height in coffee seedlings. *Azospirillum brasilense* inoculation increased dry matter yield in improved variety and hybrids of sorghum (Das *et al.*, 1997).

According to Arunkumar (1997) *Azospirillum* inoculation of amaranthus in combination with FYM and 75 per cent fertilizer nitrogen significantly increased plant height, root biomass, leaf area index and yield. But in brinjal inoculation along with 75 per cent nitrogen and FYM significantly increased yield, plant height and root biomass production.

2.2 Nitrogen fixation

Nitrogen fixing ability of *Azospirillum* has been reported by many workers. Dobereiner and Day (1975) reported that *Azospirillum* fixes nitrogen only under microaerophilic conditions. Kumari *et al.* (1976) confirmed nitrogen fixation by *Azospirillum* not only in microkjeldahl assay but also by the more definite methods of acetylene reduction assay and isotopic enrichment. Rao and Rao (1983) found that *Azospirillum* inoculation slightly increased the rhizosphere nitrogenase activity upto 88 days after sowing in upland rice and the effect was still greater after urea treatment. Significant increase in N yield as a result of *Azospirillum* inoculation was reported by Kapulnik *et al.* (1983). Monib *et al.* (1984) observed that in castor, *Azospirillum* was most effective among N-fixers for increasing plant and soil N. Inoculation of *Azospirillum lipoferum* under green house and field condition increased N yield in spring wheat was noticed by Mertens and Hess (1984). Kapulnik *et al.* (1985) observed that inoculation of wheat with

Azospirillum accumulated 20 per cent more N at the booting stage compared to uninoculated control.

Azospirillum brasilense inoculation in *Panicum* led to significant increase in associative nitrogen fixation (Rai, 1985). Pacovsky *et al.* (1985) noticed that inoculation of *Azospirillum brasilense* increased nitrogen assimilation by 25 per cent in sorghum. Maximum nitrogen fixation in wheat was reported by Padshetty *et al.* (1986) as a result of *Azospirillum* inoculation. Inoculation of rice seedlings with 10^6 colony forming units of *Azospirillum lipoferum* significantly increased N, P and K content of the plant. Joshi and Rao (1989) noticed increased nitrogenase activity in Pearlmillet in response to *Azospirillum brasilense* inoculation. Rao and Dass (1989) found that soil inoculation with pure cell suspension of *Azospirillum brasilense* or *Azotobacter chroococcum* resulted in growth enhancement of ber and pomegranate. They suggested that growth enhancement could be due to production of growth regulators and N fixation. High nitrogenase activity in carrot as a result of *Azospirillum brasilense* was observed by Nadkernichnaya *et al.* (1989). Subbiah (1990) noticed that application of *Azospirilllum brasilense* along with 50 per cent of the recommended N rate improved N use efficiency in tomato. Sasikumar (1996) reported that *Azospirillum* inoculation saved 25 per cent nitrogen in rice. Ratti and Janardhanan (1996) found that inoculation with *Azospirillum brasilense* significantly increased N content of leaf tissue in palmarosa.

2.3 Effect of VAM on crop plants

Mycorrhizae means a beneficial association between certain fungi and roots of higher plant. The term mycorrhizae was first coined by German botanist, Frank in the year 1885.

VA mycorrhiza was found to play an important role in improving the plant growth, nutrient uptake and yield. A study conducted by Bagyaraj and Sreeramulu (1982) found that inoculation of chilli with local isolate of VA mycorrhiza significantly increased growth, flowering and yield. They also found that inoculation along with the recommended level of P slightly increased the yield than the uninoculated plant given the full dose of phosphatic fertilizers. Zhenyao and Kaihen (1984) revealed that VA mycorrhizal inoculation along with rock phosphate showed vigorous growth, increased trunk diameter, and leaf area in seedlings of rough lemon.

Sivaprasad *et al.* (1984) collected root samples of cocoa from two gardens of Quilon district and reported that seven out of ten samples were mycorrhizal. The study revealed that VA mycorrhizal inoculation along with medium level of phosphorus application was more effective. Jalali and Thareja (1985) reported that mycorrhizae inoculation in nutrient rich soils significantly increased the growth parameters in *Cicer arietinum* compared with rock phosphate treatment alone.

Palipane and Bandara (1985) observed that inoculation with mycorrhizal root fragments resulted in increase of growth rate in coffee and cocoa

seedlings compared to uninoculated seedlings. In sterilized soils inoculation of *Calapogonium cacruleum* with *Glomus fasciculatum* increased dry weight (Ikram *et al.*, 1985). They also found that 2.1 times more P was required by uninoculated plants to achieve yields similar to the inoculated plants.

According to Sreeramulu and Bagyaraj (1986) nursery beds of chilli inoculated with *Glomus sp.* increased growth, flowering and yield. Tang and Chang (1986) revealed that citrus seedlings inoculated with VAM together with phosphate application gave best growth response and increase in fresh weight of aerial parts.

Khaliel and Elkhider (1987) studied that inoculation with VA mycorrhiza in low P soils resulted increased dry weight, number of nodes, lateral branches, leaves and higher percentage of survival in tomato. They also observed better growth response.

A pot culture experiment was conducted in green house by Sharma *et al.* (1988) to study the response of rice plants to VA mycorrhizal inoculation. They observed an increase in shoot and dry weight due to VA mycorrhizal inoculation. Sulochana *et al.* (1989) reported the effect of VA mycorrhizal fungi inoculation in sesame plant. They found an enhancement of growth as a result of VA mycorrhizal inoculation.

Widiastuti (1989) studied the effect of *Glomus fasciculatum* on the growth of cocoa seedlings. He observed that growth was greater in seedlings receiving triple super phosphate.

Cuenca et al. (1990) studied the effect of inoculation of cacao seedlings with introduced VA mycorrhizal fungi in soils treated with copper oxychloride or methyl bromide and with indigenous VA mycorrhizal fungi. Cacao seedlings responded well to indigenous VA mycorrhizal fungi which significantly increased height, dry weight and foliar uptake of P, Cu and Zn compared to introduced VA mycorrhizal fungi and sterile control.

Reena and Bagyaraj (1990) found that seedlings of *Tamarindus indicus* showed greater plant height, leaf number, stem girth and biomass due to VA mycorrhizal inoculation. Number of VAM species in the soil, percentage of root colonization and external hyphae were also higher.

According to Sivaprasad *et al.* (1990) inoculation with *Glomus fasciculatum* increased grain and straw yield in rice. At harvest they found an increased mycorrhizal infection. Raju *et al.* (1990) reported that sorghum roots colonized with *Glomus macrocarpum* enhanced plant growth. Jaizmevega *et al.* (1991) conducted a green house experiment to study the response of *Musa accuminata* to *Glomus mossea* inoculation. They observed an increase in fresh root weight and percentage of radicular infection due to mycorrhizae inoculation.

A study was conducted by Prabhakar *et al.* (1992) in groundnut grown in sterilized loam sandy soils inoculated with VA mycorrhiza. They found that VA mycorrhizal inoculation increased plant growth, number of flowers per plant, number of pods per plant and grain weight per plant. They also found that low levels of P was effective for VAM infection.

The response of chilli genotypes to VA mycorrhizal inoculation was investigated by Patil *et al.* (1992) and reported that inoculation resulted in early flowering, enhanced number of leaves, shoot length, root length, shoot dry weight and root dry weight compared to control. They also reported that growth response to VA mycorrhizal inoculation dependant on the plant genotype.

Morin *et al.* (1994) studied the effect of VA mycorrhizal inoculation in apple rootstock grown in soil containing high levels of extractable P under green house condition. They found that inoculation increased biomass of plants. Mycorrhizal inoculation also significantly increased the leaf surface area. All the mycorrhizal plant had similar percentage of root colonization. They also found that efficiency of mycorrhiza was associated with a layer of external hyphal net work but showed no relation to internal colonization.

Edathil *et al.* (1994) reported the maximum root colonization by native VA mycorrhiza at 45, 50 and 60 days after germination of brinjal, tomato, chilli respectively under field conditions and on 60^{th} day in pot culture.

Baon *et al.* (1994) conducted a pot culture experiment using sterilized soil to study the effect of VA mycorrhiza on barley. They found that VA mycorrhizal inoculation reduced the plant growth at low temperature. Sreenivasa (1994) reported that inoculation of chilli with VA mycorrhizal fungi together with the addition of P upto 56.2 kg/ha increased root colonization and sporulation.

Zhao and Li (1994) reported an increased plant height, number of leaves per plant, stem diameter and plant dry weight of sweet pepper inoculated with *Glomus* spp. They also observed that inoculation resulted in early flowering, increased cold resistance and prolonged the growing period. Thakur and Panwar (1995) reported higher drymatter production and seed yield in mungbean dually inoculated with *Glomus fasciculatum* and *Bradyrizobium* compared with either single inoculant.

Sonawane and Konde (1997) revealed that co-inoculation of VA mycorrhizal fungi and *Azospirillum* or *Azotobacter* resulted in higher root colonization and spore count and took less time for bud sprouting in grape vine. The leaf area was also significantly highest in the dual inoculated plants. Among *Azospirillum* and *Azotobacter, Azospirillum* found superior when used in conjunction with mixed culture of VA mycorrhiza.

Setua *et al.* (1998) studied the effect of inoculation of maize with VA mycorrhiza along with the addition of 30 kg P per ha per year in between the rows of two year old mulberry plants. They found that inoculation with addition of P

increased plant height, number of leaves per plant, leaf yield and leaf moisture. They also found a simultaneous reduction in use of 80 per cent phosphatic fertilizers per ha per year.

Sreeramulu and Bagyaraj (1998) conducted a net house experiment to study the growth response of cardamom seedlings to 13 different VA mycorrhizal fungi. They reported that seedlings inoculated with VA mycorrhizal fungi increased plant height, leaves, tillers and biomass than control. They also reported, among the different fungi used *Gigaspora margarita* and *Glomus macrosporum* exhibited significantly greater growth.

A significant increase in plant height, number of functional leaves, dry matter of plant, fresh and dry weight of bulbs, bulb diameter and N and P uptake were reported by Wani and Konde (1998) in garlic plants as a result of inoculation with *Glomus mosseae, Gigaspora margarita* and *Acaulospora calospora* and they found that *Glomus mosseae* was found to be effective. Inoculation of ber seedlings with VA mycorrhizal fungi singly or in combination significantly increased root and shoot dry weight (Shirsath *et al.*, 1998). According to George *et al.* (1998) inoculation with VA mycorrhiza increased yield of guinea grass compared with uninoculated control.

Hazarika *et al.* (1999) studied the effect of VA mycorrhiza on inoculation in black gram grown in sterilized or unsterilized soil with or without phosphatic fertilizers. They found that inoculation significantly increased plant

growth, dry matter, yield, spore density and percent root colonization compared to non mycorrhizal plants.

2.4 Effect of VA mycorrhiza in improving P uptake and uptake of other nutrients

VA mycorrhiza is found to enhance the plant growth by increasing the nutrient uptake. The uptake of phosphorus and other nutrient elements by VA mycorrhiza infected plants were first studied by Mosse (1957). Increased concentration of P and Zn in mycorrhizal inoculated cowpea, cotton and millets were reported by Bagyaraj and Manjunath (1980). Inoculation with VA mycorrhiza significantly increased P and Zn nutrition in green chilli was revealed by Bagyaraj and Sreeramulu (1982). Zenyao and Kaihen (1984) observed that inoculation of rough lemon with *Glomus* sp. along with the addition of rock phosphate increased P content.

Jayaratne *et al.* (1986) found that there was no significant difference in the uptake of nutrients by rubber plants inoculated with four species of mycorrhizal fungi. Pacovsky *et al.* (1986) revealed that presence of VA mycorrhiza fungus can decrease nutrient stress in environments limited in P, Zn, Cu and elements essential in N fixation.

An increase in P concentration of citrus seedlings due to the inoculation with VA mycorrhiza was reported by Tang and Chang (1986). VA mycorrhizal inoculation of rice plants grown under green house condition led to an increase in P and Zn content in shoots and roots (Sharma *et al.*, 1988).

Widiastuti (1989) studied the effect of *Glomus fasciculatum* on phosphorus uptake and growth of cocoa seedlings and reported that P uptake was greater in seedlings inoculated with *Glomus* sp. and triple super phosphate. Bhandari *et al.* (1990) observed that VA mycorrhizal association resulted in higher uptake of P and better utilization of N, Cu, Zn and S in some crops.

Increase in phosphate and Zn content in seedlings of *Tamarandus indicus* inoculated with VA mycorrhiza was studied by Reena and Bagyaraj (1990). Prabhakar *et al.* (1992) investigated an increased P concentration in groundnut as a result of VA mycorrhiza inoculation.

A study conducted by Durynina *et al.* (1993) on the effect of VA mycorrhiza along with the addition of super phosphate in spring wheat found an increase in P content in plants with VA mycorrhiza and super phosphate. They also found that inoculation had no effect on K uptake.

A significant increase in P uptake translocation and its subsequent transfer in maize plants inoculated with VA mycorrhiza was observed by Shnyreva and Kulaev (1994). They found that P was accumulated as low molecular organophosphorus compounds of acid insoluble fractions (RNAs). Inoculation of barley plants with *Glomus intraradius* increased specific P uptake (Baon *et al.*, 1994). They also observed a significant interaction between mycorrhiza and soil temperature for specific P uptake. Inoculation of sweet pepper with *Glomus spp.* increased P and N content was revealed by Zhao and Li (1994).

Bharadwaj and Dudeja (1998) reported that P uptake in pigeon pea was correlated with the number of VA mycorrhizal spores in soil. Sreeramlu and Bagyaraj (1998) reviewed that inoculation with VA mycorrhizal fungi increased nutrient uptake in cardamom seedlings.

Wani and Konde (1998) reported a significant increase in N and P uptake in garlic as a result of inoculation with VA mycorrhiza. Shirsath *et al.* (1998) found a significant increase in P uptake in ber seedlings inoculated with VA mycorrhiza singly or in combination.

2.5 Response of crop plants to dual inoculation of *Azospirillum* and VAM

Barea *et al.* (1983) studied the effect of interaction between *Azospirillum brasilense* and *Glomus mosseae* on the growth and nutrition of maize and rye grass. They found that application of *Azospirillum* to the mycorrhizal plants stimulated the development of VA mycorrhiza and improved plant growth at the last harvest of rye grass and maize plants also produced similar plant growth and N content and a high P content at the last harvest.

Rao *et al.* (1985a) reported that dual inoculation with *Glomus sp.* and *Azospirillum brasilense* significantly increased dry matter and grain yield of barley. Dual inoculation of pearlmillet with *Azospirillum brasilense* and VA mycorrhiza significantly increased dry matter content of shoot, root biomass and phosphorus uptake compared with uninoculated control (Rao *et al.*, 1985b). Pacovsky *et al.* (1985) found that combined inoculation of sorghum with

Azospirillum brasilense and VA mycorrhiza together with the addition of nutrient solution which did not contain N or P increased VA mycorrhizal colonization and biomass but N input due to *Azospirillum* was decreased.

Combined inoculation with *Azospirillum brasilense* and VA mycorrhizal fungi significantly increased growth, plant N and P contents, tuber weight and starch content in sweet potato was reported by Kandaswamy *et al.* (1988).

Tilak and Singh (1988) studied the effect of inoculation of pearlmillet with 3 species of VAM (*Acaulospora sp, Gigaspora margarita* or *Glomus fasciculatum*) alone or with *Azospirillum brasilense* or along with the addition of super phosphate (rock phosphate). They observed that soil inoculation with VAM species in the presence of either super phosphate or rock phosphate resulted in greater dry matter production and P uptake. They also observed that among the three VAM species *Glomus fasciculatum* either singly or in combination with *Azospirillum brasilense* gave better results compared to other two VAM species. Combined inoculation with *Azospirillum* and VAM increased bulb yields in onion as reported by Gurubatham *et al.* (1989).

Negi *et al.* (1990) revealed that combined inoculation of barley with *Azospirillum brasilense* and *Glomus versiforme* increased growth, grain yield, N and P uptake by roots, shoots and grains. They also found that amount of N_2 fixed was increased with increase in plant growth.

Dual inoculation of pearlmillet with *Azospirillum brasilense* and VA mycorrhiza together with the basal application of 20 kg N per ha resulted in highest grain yield of 2.71t per ha (Bar and Gautam, 1991).

Increased root dry weight and root volume was observed by Singh (1992) in some forage grasses in response to the combined inoculation of *Azospirillum brasilense* and *Glomus macrocarpum.* He also observed that arbuscule formation and sporulation were enhanced by the *Azospirillum brasilense* in some species of forage grasses.

Kumari and Balasubramanian (1993) conducted an experiment to study the effect of combined inoculation with VA mycorrhizal fungi and *Azospirillum brasilense* on growth and nutrient uptake by coffee seedlings. They found that inoculation significantly increased shoot length, root length and total dry weight of the plants. They also found a significant increase in N and P uptake and uptake of micronutrients like Fe, Cu, Zn and Mn and observed maximum VA mycorrhizal colonization of roots.

Subbiah (1994) observed some significant effects on nutrient uptake in chilli and bellary onion as a result of combined inoculation of *Azospirillum brasilense* and *Glomus fasciculatum* along with the addition of 50, 75 and 100 per cent of recommended N and 100 per cent of the recommended P.

Ratti and Janardhanan (1996) revealed that dual inoculation with *Glomus aggregatum* and *Azospirillum braslilense* significantly increased growth,

yield and oil content of palmarosa. Increased growth rate in *Casurina, Acacia* and *Eucalyptus* was reported by Balasubramanian and Ravichandran (1997) as a result of co-inoculation with VA mycorrhiza and *Azospirillum.*

Suguvanam *et al.* (1998) studied the influence of six VAM fungi and *Azospirillum* sp. individually or in combination on seedlings of *Tectona grandis.* Inoculation with individual VAM fungi and *Azospirillum* sp. increased shoot growth, biomass production, root colonization, tissue N, P and K concentration and reduced shoot root ratio compared with uninoculated control. They found that most significant responses were observed with *Gigaspora margarita* or *Glomus versiforme* in conjunction with *Azospirillum* sp.

2.6 VAM in disease control

Hedge and Rai (1984) reported that tomato plants showed resistance to damping off disease when inoculated with *Glomus fasciculatum.*

Bisht *et al.* (1985) reported that VA mycorrhiza formed by *Gigaspora calospora* excerted an inhibitory effect on the development of pigeon pea blight. Caron *et al.* (1986) observed reduction in root and population of *Fusarium oxysporum* in tomato following combined inoculation with VA mycorrhiza and *Fusarium oxysporum.*

Chakravarty and Mishra (1986) reported that wilting of *Cassia tora* caused by *Fusarium oxysporum* was reduced when root inoculated seedlings were transplanted to the pots containing soil inoculated with chlamydospores of *Glomus*

fasciculatum or *G. tenue*. They found that mycorrhizal fungi stimulated seedlings growth and reduced the *F. oxysporum* population in the rhizosphere.

Reddy *et al.* (1988) studied the effect of inoculation with VA mycorrhiza and *Fusarium oxysporum* f.sp. *ciceris* on wilt incidence in chickpea. They found that inoculation with *Fusarium* alone produced 35 per cent wilt and inoculation with VA mycorrhiza and *Fusarium* together produced 25 per cent wilt. Kobayashi (1990) reported that combined use of VA mycorrhiza and charcoal compost drastically reduced damping off caused by *Pythium splendens* or *Rhizoctonia solani* in two and three week old cucumber seedlings. They also reported that combined use of VA mycorrhiza and charcoal compost reduced the level of bacterial wilt of tomato caused by *Pseudomonas solanacearum*.

Suppression of *Sclerotium rolfsii* in chilli by VA mycorrhizal inoculation was reported by Sreenivasa *et al.* (1992). Sivaprasad (1993) reported the biocontrol of rhizome rot of ginger with *G. fasciculatum* and *G. mossea*. Iyer and Sundararaju (1993) investigated the interaction of AMF with *Pythium aphanidermatum* and *M. incognita*. They found that AMF, viz. *G. multicaule* and *G. fasciculatum* significantly enhanced growth of ginger where as the pathogen suppressed it. The AMF association was found to reduce the disease incidence and suggested that prior inoculation with AMF was effective in ameliorating the deleterious effect of the pathogen. Thomas *et al.* (1994) reported the biocontrol of damping off of cardamom caused by *F. moniliforme* and *R. solani* using AMF.

Sivaprasad *et al.* (1995) in a study reported that association of VA mycorrhiza in black pepper suppressed the foot rot disease incidence.

According to Joseph (1997) inoculation of ginger with two isolates of *Glomus* species along with *Trichoderma viridae* resulted in remarkable synergistic interaction in reducing the rhizome rot disease and enhancing biomass and yield. He also observed that single and dual inoculation of *Glomus* species and *Trichoderma viridae* significantly reduced the disease and enhanced plant growth and yield in the field also.

Sreeramulu *et al.* (1998) studied the efficiency of VA mycorrhiza (*Glomus fasciculatum*) and *Trichoderma harzianum* in controlling damping off (*Pythium aphanidermatum*) and black shank diseases (*Phytophthora parasitica* var. *nicotianae*) of tobacco seedlings under nursery condition. Results showed that dual inoculation of *G. fasciculatum* and *T. harzianum* was more effective in controlling both the disease than individual inoculation and resulted in better germination count and improved plant growth parameters.

2.7 Management of *Phytophthora* disease

A field evaluation of five fungicides was conducted by Reddy and Chandramohan (1984) found that all the fungicides reduced black pod incidence but the copper based fungicides performed best. Fenn and Coffee (1987) reported that application of potassium phosphonate or fosetyl Al as foliar spray or soil drench controlled the development of root rot of *Persea indica* naturally infested

with *Phytophthora cinnamomi*. Bhattacharyya *et al.* (1987) studied the effect of two formulations of oxadixyl (oxadixyl + mancozeb and oxadixyl + copper oxychloride) against *Phytophthora infestans* and found that two formulations were effective at 0.15 per cent and 0.2 per cent in decreasing disease incidence and increasing yield, but were ineffective in preventing tuber infection.

Suharban and Philip (1987) found that two sprays of one per cent Bordeaux mixture on the entire *Artocarpus* tree at two week intervals during ripening stage controlled the fruit rotting caused by *Phytophthora*. In lab and semicommercial experiments, fosetyl-Al was identified as a suitable commercial treatment for the control of brown rot disease on post harvest citrus fruits (Cohen *et al.*, 1987).

Patel and Patel (1988) reported that copper sulphate treatment was best for reducing the rotting of chiku fruits caused by *Phytophthora palmivora* followed by *Rhizopus stolonifer*, *Aspergillus niger* and yeast. Systemic fungicides like Alliette (fosetyl-Al) and metalaxyl had some curative effect against fruit rot in arecanut (Sastry and Hedge, 1988). Pereira *et al.* (1988) observed that application of metalaxyl + mancozeb and metalaxyl + copper oxychloride prevented an increase in infection by *Phytophthora capsici* in rubber plants. Field trials conducted by Gupta *et al.* (1988) noticed that Alliette (fosetyl-Al) and Bordeaux mixture controlled *Phytophthora palmivora* in betelvine.

Lee and Chung (1989) assessed the efficiency of metalaxyl-Mz and Alliette-F against *Phytophthora capsici in vitro* and *in vivo*. They observed

metalaxyl-Mz was more inhibitory to mycelial growth of *P. capsici* and Alliette-F was more inhibitory to zoosporangial formation. Thomas *et al.* (1989) tested 14 fungicides for the control of *Phytophthora meadii* on cardamom. Among 14 fungicides tested, the best control was given by 0.3 per cent Alliette 80 WP (fosetyl-Al) or one per cent Bordeaux mixture.

Application of potassium phosphonate as single preharvest spray or four post plant sprays controlled root rot and heart rot of pineapple caused by *Phytophthora cinnamomi* (Pegg *et al.*, 1990).

Trunk infection of cocoa plants with potassium phosphonate controlled black pod and stem canker was reported by Anderson and Guest (1990).

Nair and Sasikumaran (1991) tested 5 fungicides to control *Phytophthora palmivora* infection on *Piper nigrum*. They noticed that Bordeaux mixture gave the best control followed by metalaxyl, copper oxychloride and captafol and found Alliette was the least effective fungicide.

Effective management of seed bed diseases of tobacco caused by *Pythium aphanidermatum* and *Phytophthora palmivora* var. *nicotiana* was achieved by using combined application of organic ammendments with reduced rates of fungicides press mud incorporated in the beds two weeks before sowing, plus Blitox (copper oxychloride) at 0.2 per cent one day before sowing (Narendrappa *et al.*, 1992).

Singh and Singh (1996) observed the effectiveness of five spray fungicides for the control of *Phytophthora* infection on potato. They noticed all the treatments reduced the disease but 8 per cent metalaxyl + 64 per cent mancozeb was the most effective treatment.

A fungicidal trial was conducted by Ali *et al.* (1996) in 18 years old pepper plantation with *Grevillea robusta* as support trees to control foot rot using Bordeaux mixture (1%), copper oxychloride (0.1%), Ridomil (metalaxyl 0.1%) and Akomin (potassium phosphonate 0.2%) and found copper oxychloride was least effective treatment.

The use of copper oxychloride, mancozeb, metalaxyl, captafol, ziram and Bordeaux mixture significantly reduced *Phytophthora* leaf blight and increased corm yields of taro was reported by Das (1997).

Sitaramaiah and Hanuman (1997) studied the efficacy of metalaxyl, chlorothalonil, Bordeaux mixture, mancozeb and copper oxychloride applied as pre and post inoculation foliar sprays for the control of *Phytophthora capsici* on betelvine. They observed that none of the fungicides could completely inhibit lesion expansion with either application method. In general metalaxyl significantly reduced the stem lesion development.

Johri and Chaurasia (1998) reported that potassium phosphonate spray at monthly intervals efficiently controlled *Phytophthora palmivora* causing leaf rot and foot rot of *Piper betle*. Opoku *et al.* (1999) found that use of potassium

phosphonate as trunk injection in cocoa to control *Phytophthora* reduced the disease incidence by upto 60 per cent and increased the yield in some instances by 200 per cent or more.

According to Ali *et al.* (1999) three concentrations of potassium phosphonate (0.1, 1.0 and 5.0 g/litres) applied as either a single foliar spray or a single root drench at transplanting into pots containing pathogen infested sand or sand/peal, significantly reduced the incidence of *Phytophthora* root rot symptom in *Pinus radiata.*

Maheswari *et al.* (1999) tested the *in vitro* effect of nine fungicides against taro leaf blight pathogen *Phytophthora colocasiae* and found Ridomil Mz, Indofil M-45, Blitox 50 and Hill copper completely inhibited growth of *Phytophthora.*

Verma *et al.* (1999) reported that Bordeaux mixture was most effective against *Phytophthora citrophthora* causing blight in citrus followed by Blitox-50 (copper oxychloride), Indofil Z-78 and Indofil M-45 (mancozeb).

MATERIALS AND METHODS

MATERIALS AND METHODS

A study on the effect of *Azospirillum* and VA mycorrhiza on the growth of cocoa seedlings and incidence of seedling blight disease was conducted during 1998-2000 at the College of Horticulture, Vellanikkara. The details of the materials used and techniques adopted during the course of investigation are presented below.

3.1 Isolation of native *Azospirillum*

3.1.1 Root sample collection

Roots of cocoa plants were collected from the nursery of Cadbury KAU Co-operative Cocoa Research Project, College of Horticulture, Vellanikkara. Roots of about one mm diameter were collected along with a small quantity of soil around it in polyethylene bags to prevent drying of roots.

3.1.2 Isolation of *Azospirillum*

Cocoa roots collected from the nursery were washed in tap water to remove the adhering soil. The roots were then cut into bits of about one cm length. These root bits were surface sterilized with 0.1 per cent mercuric chloride solution for one minute and were washed in three changes of sterile water. These bits were then planted in sterilized nitrogen free bromothymol blue (NFb) medium in test tubes (Appendix-I). These test tubes were incubated at 37°C and were observed for the presence of subsurface, thin pellicular growth of *Azospirillum.*

3.2 Isolation of *Azospirillum* from commercial culture

About one gram of commercial inoculum was dispersed in sterile water prepared in test tube. Using an inoculation needle a loopful of the suspension was stabbed in semisolid malate medium in test tubes and was incubated at 37°C. These test tubes were observed for the subsurface thin pellicular growth of *Azospirillum*.

3.2.1 Commercial culture used

Commercial culture of *Azospirillum* was collected from Tamil Nadu Agricultural University, Coimbatore.

3.3 Purification of *Azospirillum*

Azospirillum was further purified by serial transfer of the white pellicular growth into fresh nitrogen free bromothymol blue medium. A loopful of the culture was stabbed into fresh NFb medium and incubated at 37°C for two days and then observed for the development of thin white pellicular growth.

3.4 Checking for purity

The purity of the culture was confirmed by studying various cultural and physiological characters.

3.4.1 Gram staining (Hucker and Conn, 1923)

Hucker's modification of gram staining was done. A smear of the *Azospirillum* was prepared on a clean glass slide. It was heat fixed over a flame by gentle intermittent heating and stained with Hucker's ammonium crystal violet

solution for one minute. Then it was washed in a gentle stream of running tap water. After washing, it was flooded with Gram's iodine solution for one minute, washed in running tap water and decolourised with 95 per cent ethanol. Then again washed in a gentle stream of running tap water. After washing, the slide was stained with safranin solution for one minute and the excess stain was washed off in tap water and dried. After drying, the slide was examined under light microscope for Gram reaction.

3.4.2 Growth on Okon's medium

A loopful of *Azospirillum* culture was taken from the white pellicular growth formed in NFb medium by using inoculation needle and dispersed in five ml sterile water. Again from this suspension, a loopful was taken and streaked on Okon's solid medium (Appendix II) in sterile petriplates. They were incubated at 37°C for five days and observed for the development of thin, dry, slightly convex colonies and change in colour of the medium to blue.

3.4.3 Growth on RC medium

A loopful of the *Azospirillum* culture was dispersed in five ml sterile water. This suspension was streaked on RC medium (Appendix III) and incubated at 37°C for four days. After incubation, they were observed for the development of scarlet bacterial colonies with rugose surface and undulating edges.

3.4.4 Growth on Potato Influsion Agar

A loopful of *Azospirillum* culture was taken and dispersed in five ml sterile water. Again a loopful was taken from this suspension and streaked on

Potato Infusion Agar (Appendix-IV) in sterilized petriplates. Then they were incubated at 37°C for seven days and observed for the pink and wrinkled bacterial colonies.

3.4.5 Physiological characterization

3.4.5.1 Acid production from glucose

Acid production from glucose by *Azospirillum* was tested using the medium having following composition.

Glucose	10.0 g
Peptone	2.0 g
$(NH_4)_2SO_4$	1.0 g
$MgSO_4$	1.0 g
$FeCl_3$	0.002 g
$MnSO_4$	0.002 g
Distilled water	1000.0 ml
(Bromothymol blue 2.0 ml) (Five per cent alcoholic solution)	
pH	7

The medium was prepared in test tubes. Each test tube contained about five ml medium. They were then autoclaved at 121°C for 20 minutes. To the sterilised medium, 0.1 ml of 48 hr old culture was inoculated and incubated at 37°C. These tubes were then observed for the change in colour of the medium.

3.4.5.2 Utilisation of carbon

Utilisation of various carbon sources by *Azospirillum* was tested by using NFb medium modified by excluding bromothymol blue and replacing malic

acid with one per cent carbon sources such as glucose, lactose, sucrose and mannose. Ten ml quantities of the media were transferred to test tube and sterilized in an autoclave at 115°C for 20 minutes.

A loopful of *Azospirillum* culture was used to inoculate the sterile media in test tubes. They were then incubated at 37°C for three days and observed for the development of thin, white, subsurface, pellicular growth.

3.4.5.3 Nitrate dissimilation test

The ability of *Azospirillum* to dissimilate nitrate was tested by using semi solid malate medium modified by adding five mM ammonium nitrate and 1.5 per cent agar. Five ml quantities of the medium were taken in test tubes and sterilized by autoclaving at 121°C for 20 minutes.

The sterilized tubes were inoculated with a loopful of *Azospirillum* culture and incubated at 37°C for five days and observed for the shredding of agar blocks which indicates the dissimilation of nitrate.

3.5 Maintenance of *Azospirillum*

Azospirillum culture was streaked on Okon's solid medium in petriplates, after purification and characterization. The single colony typical of *Azospirillum* was transferred to test tube slants of the same medium. Then they were stored in refrigerator at 4°C. The culture was maintained by periodic purification and subculturing into fresh test tube slants.

3.6 Mass production of *Azospirillum* for inoculation

For mass production of *Azospirillum* Okon's liquid medium was prepared in 250 ml conical flasks and was sterilized in an autoclave at 121°C for 20 minutes.

A suspension of the *Azospirillum* culture was prepared in sterilized water and was agitated vigorously to make it uniform. Five ml from this suspension was used to inoculare each of the 250 ml flask with Okon's medium. The inoculated flasks were shaken periodically at 37°C for three days. The contents of the flasks after incubation was applied to the seedlings at the rate of 100 ml per polyethylene bag.

3.7 Staining the roots for VAM

The roots collected from cocoa plants were observed for the mycorrhizal colonization by staining of roots. The staining was done by the method suggested by Phillips and Hayman (1970). The roots were first washed in tapwater to remove the soil particles adhering to it and then the roots were cut into small bits of about one cm length. They were then transferred into clean test tubes and fixed with FAA (Appendix V).

For staining, the root bits taken from FAA were initially softened by simmering in 10 per cent KOH at 90°C for one hour. The excess alkali was removed after cooling by repeated rinsing in tap water and then neutralised by two per cent HCl. The root bits were then stained by boiling with 0.05 per cent trypan

blue in lactophenol for three minutes. The excess stain was removed by clearing overnight in fresh lactophenol. The root bits were then examined for VAM infection under a light microscope.

3.8 Estimation of per cent VAM colonization

VAM infection percentage was calculated by the following formula.

$$\text{Per cent VAM colonization} = \frac{\text{Number of positive root segments}}{\text{Number of root segments observed}} \times 100$$

3.9 Isolation of spores of native VAM

Modified wet sieving and decanting method of Gerdemenn and Nicolson (1963) was adopted for the isolation of VA mycorrhiza spores from soil. Hundred gram of soil was collected from cocoa rhizosphere and was suspended in 1000 ml tap water. This was agitated vigorously to disperse all soil clumps. The supernatant was filtered after the heavier particle settled through a set of sieves of B.S.S. No.60 (250 micron), 150 (150 micron) and 350 (450 micron). The residue left behind was resuspended again in 1000 ml tap water. After settling down, the supernatant was passed through same set of sieves. This procedure was repeated three times in order to collect maximum number of spores from the soil. Finally, the materials present on each sieve was transferred to 100 ml beakers in a small volume of water and filtered through Whatman no.1 filter paper. The content of each filter paper was examined carefully under a stereo microscope for the typical VAM spores. Spores of uniform size and shape were transferred to moistened filter paper in petridishes.

3.10 Mass multiplication of VAM

Mass multiplication of VAM was done by inoculating in roots of maize (*Zea mays* L.) seedlings. Spores that are isolated from the cocoa rhizosphere were used for inoculation. VAM spores were placed at a depth of 5 cm in sterilized potting mixture containing sand and soil in the ratio 1:1 in polyethylene bags. Over this, the maize seeds were sown. The maize plants were grown for 60 days for the proper development of infected roots. Infected roots of these maize plants were used as the mycorrhizal inoculum for the experiments conducted during this investigation.

3.11 *Commercial VA mycorrhizal culture used*

Commercial VA mycorrhizal culture was collected from Tamil Nadu Agricultural University, Coimbatore. This was applied at the rate of 50 gm per polyethylene bag.

3.12 Experiment on the effect of individual and dual inoculation of *Azospirillum* and VA mycorrhiza in improving growth and establishment of cocoa seedlings.

This study was conducted in polyethylene bags of size 15 x 20 cm. It was laid out as a 3 x 3 factorial experiment in CRD with four replications. Five plants were maintained in each replication. The experiment was carried out during November to February. The potting mixture consisted of sand, soil and cow dung in the ratio 1:1:1. This potting mixture was fumigated with formaldehyde at a rate of one litre formaldehyde in twenty five litres of water. After fumigation, the potting mixture was covered with polyethylene sheet for seven days. After seven

days polyethylene sheet was removed and kept the fumigated soil exposed for some days. After that potting mixture was filled in polyethylene bags for sowing cocoa seeds.

Treatments

Azospirillum isolates - A

A_0 - No *Azospirillum* inoculation

A_1 - Inoculation of native *Azospirillum* culture

A_2 - Inoculation of commercial *Azospirillum* culture

VA mycorrhiza - M

M_0 - No VAM inoculation

M_1 - Inoculation of native VAM culture

M_2 - Inoculation of commercial VAM culture

Treatment combinations were as follows

A_0M_0	A_1M_0	A_2M_0
A_0M_1	A_1M_1	A_2M_1
A_0M_2	A_1M_2	A_2M_2

3.12.1 Cocoa seeds

Seeds of cocoa variety forestero procured from Cadbury KAU Co-operative Cocoa Research Project, College of Horticulture, Vellanikkara was used. Two seeds per bag were used for sowing.

3.12.2 Inoculation of VAM

Native VAM was mass multiplied as described earlier. Commercial VAM culture collected from Tamil Nadu Agricultural University was also used.

VAM inoculum was placed at a depth of 5 cm in the centre of polyethylene bags and over this seeds of cocoa were sown. Fifty gram VAM inoculum per polyethylene bag was used. VAM cultures were applied at the time of sowing.

3.12.3 Inoculation of *Azospirillum*

Native and commercial *Azospirillum* were mass multiplied as described earlier. They were applied four days after germination of seeds at a uniform rate of 100 ml per plant.

3.12.4 Maintenance of seedlings

Seedlings were maintained under green house condition and were irrigated regularly.

3.12.5 Observations

Biometric observations of the plants, viz. height of plant, leaf length breadth ratio, collar girth, number of leaves, fresh and dry weight of plant and fresh and dry weight of roots were recorded on 90th day. VAM infection per cent, N, P and K content of the plants were also recorded.

3.12.5.1 Height of plant

Height of plant was measured from soil level to the top most part of the plant.

3.12.5.2 Leaf length breadth ratio

Leaf length was taken from petiole to the tip of the leaf and breadth was taken across the leaf. Length breadth ratio was taken by dividing length by breadth.

3.12.5.3 Collar girth

Collar girth (diameter) was taken by using vernier calipers.

3.12.5.4 Number of leaves

Total number of leaves was taken by counting from oldest to youngest.

3.12.5.5 Fresh weight of plant top

Fresh weight of the plant top was taken immediately after uprooting and after seperating the root portion at the collar portion.

3.12.5.6 Fresh weight of root

After separating the root portion, root was washed with water, allowed to drain and the weight was taken with the help of electronic balance.

3.12.5.7 Dry weight of shoot and root

The dry weight of shoot and roots were taken after drying the samples to a constant weight at 60°C. Drying was done in a hot air oven.

3.12.5.8 VAM infection

VAM infection per cent was taken after staining of roots. Fifty root bits of about 1 cm length was observed for the presence of VAM. Presence or absence of VAM was noted in each root bit. Presence of either vesicles, arbuscules or even mycelium was recorded as positive.

3.12.5.9 Nitrogen content (Jackson, 1967)

Nitrogen content of the plant samples was determined by digesting 0.1 g of the sample in 5 ml of conc. H_2SO_4 using digesting mixture (K_2SO_4, $CuSO_4$ and Selenium powder in 100:10:1 ratio) in a digester till the solution becomes clear. After cooling to room temperature, the contents were made up to 100 ml using distilled water. Ten ml of the sample along with five ml of 40 per cent sodium hydroxide solution were steam distilled in a microkjeldahl distillation system. Ammonia liberated was collected in a receiver flask containing 20 ml of four per cent boric acid and two to three drops of methyl red-bromo cresol green indicator.

The distillate was titrated against 0.02 N sulphuric acid and from the titre value percentage of nitrogen was calculated using the formula

$$\text{Per cent nitrogen content} = \frac{V \times N \times V_t \times 0.014 \times 100}{V_2 \times W}$$

3.12.5.10 Phosphorus and potassium content (Jackson, 1967)

One gram of the produced sample was digested in diacid (Nitric acid and Perchloric acid 2:1 ratio) and the digest was made up to 100 ml. Five ml was taken from this and again made up to 25 ml to determine phosphorus content colorimetrically by vanadomolybdate phosphoric yellow colour method using Spectronic 20. An aliquot of one ml from the diacid was taken to read potassium content using flamephotometer.

3.13 Experiment on the efficiency of individual and dual inoculation of *Azospirillum* and VAM in improving the resistance of cocoa seedlings to seedling blight disease

This study was conducted in polyethylene bags of size 15 x 20 cm. Experiment was laid out in 4 x 4 factorial experiment in CRD with three replications. Five plants were maintained in each replication. The experiment was carried out during June to September The potting mixture consisted of sand, soil and cow dung in the ratio 1:1:1. This was fumigated as described earlier. Native VAM and *Azospirillum* were selected for these experiment, because of the superior performance of native isolates over other.

Treatments

Inoculants - I

I_0 - No inoculation

I_1 - Inoculation of native *Azospirillum*

I_2 - Inoculation of native VAM

I_3 - Inoculation of native *Azospirillum* and VAM

Chemical fungicides - F

F_0 - No fungicide application

F_1 - Application of copper oxychloride 0.3%

F_2 - Application of fosetyl Al 0.1%

F_3 - Application of potassium phosphonate 0.3%

Treatment combinations

I_0F_0	I_1F_0	I_2F_0	I_3F_0
I_0F_1	I_1F_1	I_2F_1	I_3F_1
I_0F_2	I_1F_2	I_2F_2	I_3F_2
I_0F_3	I_1F_3	I_2F_3	I_3F_3

3.13.1 Inoculation of *Azospirillum* and VAM

Native *Azospirillum* and VAM were applied as detailed in the previous experiment.

3.13.2 Isolation of *Phytophthora*

Phytophthora was isolated from the infected leaves of cocoa showing typical symptom of seedling blight disease. Infected portions were cut into small pieces and surface sterilized with 0.1 per cent mercuric chloride solution for one minute and washed in three changes of sterile water. The bits were then placed in sterile petri dishes containing potato dextrose agar (Appendix VI).

Dishes were incubated at room temperature when the growth of the fungus was visible, bits of mycelium were transferred to potato dextrose agar slants. Fungal cultures were maintained in potato dextrose agar slants. Periodic subculturing was done.

3.13.3 Pathogenicity test

Pathogenicity of the *Phytophthora* culture was confirmed by inoculating with culture discs on cocoa leaves. Culture disc was placed on the leaves and infection was observed after one day.

The pathogen *Phytophthora* was inoculated uniformly on one and a half month old cocoa seedlings by spraying with zoospore suspension to increase the inoculum load in the nursery for the incidence of seedling blight disease. Zoospore suspension was prepared by putting seven day old culture of the fungus in sterile

water for three days. Fungal mycelia were separated by filtering through a muslin cloth. From the remaining filterate zoospore suspenion was obtained.

One and a half month old cocoa seedlings were artificially inoculated with zoospore suspension by spraying the zoospore suspension uniformly on the foliage.

3.13.4 Spraying of fungicides

Fungicides viz. copper oxychloride, fosetyl Al and potassium phosphonate were sprayed at a dose of 0.3, 0.1 and 0.3 per cent respectively. Spraying was done only once. Fungicides were applied at the onset of symptom expression.

3.13.5 Observations

Biometric observations of the plants, viz. height of plant, leaf length breadth ratio, collar girth, number of leaves, fresh and dry weight of plant and fresh and dry weight of roots were recorded on 90^{th} day as described earlier. VAM infection per cent and disease score were also recorded.

3.13.5.1 Disease indexing

Disease index of each treatment was calculated by using a standard score chart developed by Prem, 1995.

Standard score chart

Disease score	Intensity of infection
0	No infection
1	Less than 12.5 per cent leaf area infected
3	13 to 25 per cent leaf area infected
5	26 to 50 per cent leaf area infected
7	51 to 75 per cent leaf area infected
9	More than 75 per cent leaf area infected
11	Defoliation
13	Mortality of the plant

Each treatment was compared with the score chart and a score was assigned. Disease index was calculated using the formula

$$\text{Disease index} = \frac{\Sigma \text{ disease rating} \times 100}{\text{Total number of leaves} \times \text{Maximum score}}$$

3.14 Statistical Analysis

The data on the above observations were analysed based on Das and Giri (1979).

RESULTS

RESULT

The results of the experiments are presented below.

The experiment was conducted using native *Azospirillum* isolated from cocoa roots collected from Cadbury KAU Cocoa Research Project, College of Horticulture, Vellanikkara. The commercial *Azospirillum* culture was also used which was procured from Tamil Nadu Agricultural University, Coimbatore. The primary isolation of native *Azospirillum* from the roots of cocoa appeared as thin, white, subsurface pellicular growth in nitrogen free bromothymol blue medium. The *Azospirillum* isolated from commercial culture was also used for the study. After the first isolation, the culture was purified by transferring to a series of fresh semisolid medium. The purity of the isolated culture was checked by conducting cultural and physiological characterization.

4.1 Cultural and Physiological characterization

The results of cultural and physiological characterization are presented in Table 1.

The Gram reaction was negative and the cells were vibriod in shape.

4.1.1 Growth on Okon's medium

The growth on Okon's medium was thin, dry, slightly convex colonies with a wavy surface and undulate margin. The characters were noted after incubation at 37°C for five days. Gradually the colour of the medium was changed to blue.

Table 1. Cultural and Physiological characters of *Azospirillum.*

1	Growth on NFb medium	- Thin white subsurface pellicular growth
2	Gram staining	- Negative
3	Growth on Okon's medium	- Colour of medium changed to blue
4	Growth on RC medium	- Scarlet colour with rugose surface and undulating edges
5	Growth on Potato infusionagar	- Pink, round to irregular, dense and wrinkled
6	Acid production from glucose	- Positive
7	Utilisation of carbon	

Glucose	Lactose	Sucrose	Mannose	Malic acid
Scanty	Scanty	Scanty	Scanty	Good

8	Nitrate dissimilation test	- Positive

4.1.2 Growth on RC medium

In RC medium, the colonies were round to irregular, surface was rugose and edges undulating. The colour of the colonies were scarlet.

4.1.3 Growth on Potato Infusion Agar

The growth of colonies on potato infusion agar was round and irregular. The colour was pink and colonies were dense and wrinkled.

4.1.4 Acid production from glucose

The change of colour of the medium from green to yellow after incubation for four days at 37°C indicated the production of acid from glucose under aerobic conditions.

4.1.5 Utilisation of carbon

Good growth of *Azospirillum* was seen in malate containing medium. Growth on glucose, lactose, sucrose and mannose containing media was scanty.

4.1.6 Nitrate dissimilation test

Shredding of agar blocks indicated the dissimilation of nitrate from NFb medium containing ammonium nitrate.

4.2 Staining roots for VAM infection

The stained roots were observed for VAM infection. The vesicles appeared oval or globular in shape with hyphal attachment. They were seen mostly

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Plate. 1a. VAM infection by native strains on cocoa root

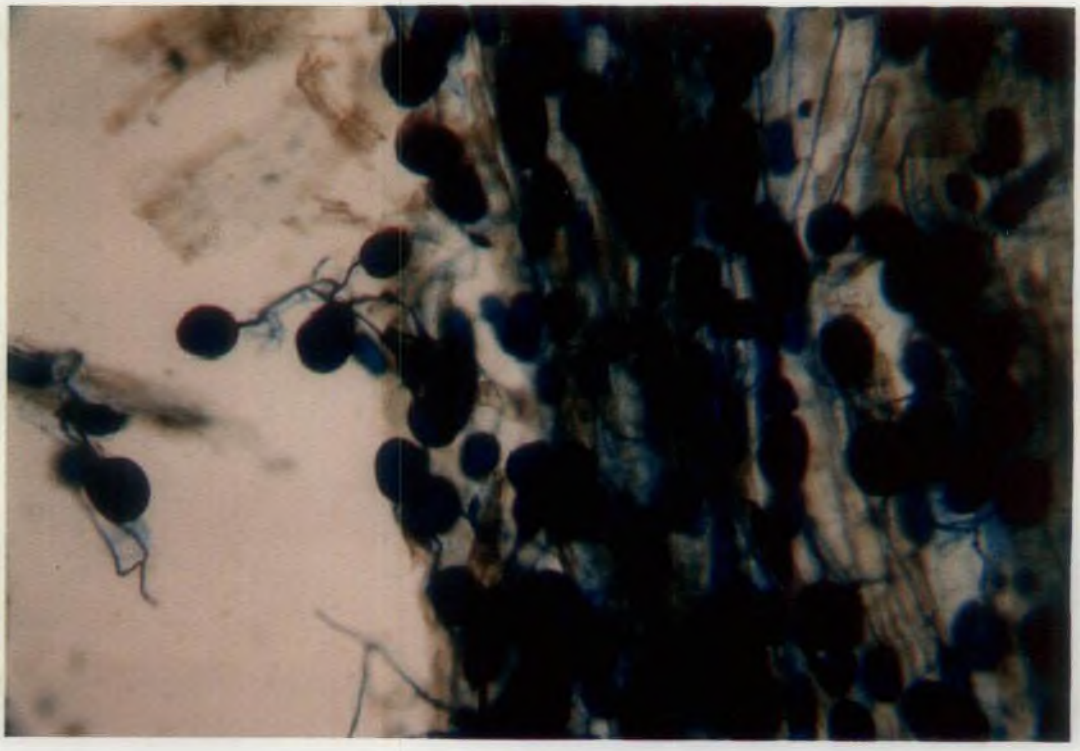


Plate. 1b. Vesicles of VAM fungi

in cortical cells. Cortical tissue also showed highly branched mycelium of the VAM fungi. Arbuscules appeared diffused, highly branched structures inside the cortical cells. The hypha, arbuscules and vesicles were stained blue (Plate 1a and 1b).

4.3 Experiment on the effect of individual and dual inoculation of *Azospirillum* and VA mycorrhiza in improving the growth and establishment of cocoa seedlings.

This experiment was conducted to study the effect of *Azospirillum* and VA mycorrhiza in improving the growth and establishment of cocoa seedlings. The results obtained are presented below.

4.3.1 Height of plant

The results on height of plant are presented in Table 2. Effects of *Azospirillum* inoculation significantly influenced the height of the plant. Both native and commercial *Azospirillum* treatments were significant in increasing the plant height over control. The maximum mean height of plant, 30.97 cm, was recorded in plants treated with native *Azospirillum* (A_1). Treatment A_2 gave a plant height of 30.6 cm. Mycorrhizal treatments also significantly increased the plant heights. Native VA mycorrhiza (M_1) gave an increased plant height of 31.06 cm. Plant height recorded in commercial VA mycorrhiza (M_2) was even lesser than control.

The interaction between *Azospirillum* and VA mycorrhiza had significant effect on plant height. *Azospirillum* treatment A_1 in combination with VA mycorrhiza M_1 recorded the maximum plant height of 31.98 cm, compared to

Table 2. Height of plant(cm)

	M_0	M_1	M_2	Mean
A_0	26.64	31.39	29.45	29.16
A_1	31.30	31.98	29.63	30.97
A_2	32.08	29.81	29.93	30.60
Mean	30.00	31.06	29.67	

CD (0.01) for A - 1.09
CD (0.01) for M - 1.09
CD (0.01) for AxM - 1.89

Table 3. Number of leaves

	M_0	M_1	M_2	Mean
A_0	14.88	19.45	20.60	18.11
A_1	21.35	21.75	20.53	21.21
A_2	22.70	19.35	19.58	20.54
Mean	19.64	20.18	20.33	

CD (0.01) for A - 1.46
M - NS
CD (0.01) for AxM - 2.52

Table 4. Leaf length breadth ratio

	M_0	M_1	M_2	Mean
A_0	2.57	2.55	2.68	2.59
A_1	2.68	2.66	2.67	2.67
A_2	2.76	2.71	2.65	2.70
Mean	2.67	2.64	2.66	

CD (0.01) for A - 0.07
M - NS
AxM - NS

Table 5. Collar girth (Diameter in mm)

	M_0	M_1	M_2	Mean
A_0	8.25	9.08	9.08	8.80
A_1	9.00	9.10	8.58	8.89
A_2	9.20	8.23	8.40	8.61
Mean	8.82	8.80	8.68	

A - NS
M - NS
CD (0.01) for AxM - 0.59

inoculation of *Azospirillum* alone. On the other hand, combination of A_1 with M_2 decreased the plant height than their single inoculation. Combined inoculation of A_2 with M_1 and M_2 resulted in decreased plant height compared to its single inoculation.

4.3.2 Number of leaves

Effect of *Azospirillum* treatment showed significant difference on number of leaves (Table 3). Isolates A_1 recorded a maximum mean number of leaves of 21.21. Isolate A_2 recorded a mean number of leaves of 20.54, superior than their control. VA mycorrhizal treatments did not vary significantly in their effects in increasing number of leaves. Treatment M_2 recorded a maximum mean number of leaves of 20.33 and minimum leaves were recorded in control (19.64). Effect of mycorrhizal inoculation did not show any significant influence in the number of leaves. However, commercial VA mycorrhiza (M_2) gave the highest number of leaves (20.33). Interaction effect of *Azospirillum* and VA mycorrhiza was also significant. Isolate A_1 in combination with M_1 gave the maximum number of leaves (21.75) in inoculated plants. In combined inoculation of isolates, A_1 with M_2, the mean number of leaves were less (20.53). Combined inoculation of A_2 with M_1 and M_2 resulted in decreased number of leaves compared to their individual inoculations.

4.3.3 Leaf length breadth ratio

The effect of treatments on leaf length breadth ratio is presented in Table 4. Individual inoculation of *Azospirillum* showed significant difference on

leaf length breadth ratio. Isolate A_2 gave significantly higher ratio of 2.70. This was statistically on par with A_1 also. VA mycorrhiza inoculation did not show any significance on leaf length breadth ratio. Both VA mycorrhizal treatments recorded a mean leaf length breadth ratio inferior to the uninoculated control.

Combined inoculation also did not show significance on leaf length breadth ratio. The maximum mean leaf length breadth ratio was observed in plants inoculated with A_2M_0 (2.76).

4.3.4 Collar girth

The results on the collar girth are presented in Table 5. Effect of individual inoculation of *Azospirillum* and VA mycorrhiza on collar girth was not significant. But, their interaction was significant. *Azospirillum* isolate A_1 recorded a maximum mean collar girth of 8.89 mm. Isolate A_2 recorded a collar girth (8.61 mm) inferior than their control (8.8 mm). Both the mycorrhizal treatments M_1 (8.8 mm) and M_2 (8.68 mm) found inferior than the control (8.82 mm) in improving collar girth. Dual inoculation of *Azospirillum* and VA mycorrhiza was significant in improving the collar girth. Dual inoculation of *Azospirillum* isolate A_1 and VA mycorrhiza M_1 resulted in higher mean collar girth of 9.10 mm compared with their individual inoculation. The maximum collar girth was found in A_2M_0 (9.2 mm). The other treatment combinations A_1M_2, A_2M_1 and A_2M_2 were found inferior than their individual inoculation.

4.3.5 Fresh weight of plant top

Fresh weight of plant tops are given in Table 6. Individual effect of *Azospirillum* was significant in increasing the fresh weight of plant top. *Azospirillum* isolate A_1 recorded the maximum mean fresh weight of 75.43 g. A_2 was also on par with A_1 and recorded 74.77 g. The individual inoculation of VA mycorrhiza did not show any significant difference on fresh weight of plant top. Both the treatments M_1 and M_2 were found inferior than the control (73.12 g).

Dual inoculation of *Azospirillum* and VA mycorrhiza showed significance on fresh weight of plant top. *Azospirillum* isolate A_2 with out VA mycorrhizal inoculation (A_2M_0) gave the highest mean fresh weight of 79.38 g. The least mean fresh weight of plant top was observed in control (62.8 g).

4.3.6 Dry weight of plant top

The effect of treatments on dry weight of plant top is presented in Table 7. Effect of *Azospirillum* inoculation was not significant on dry weight of plant top. However, both the isolates A_1 and A_2 showed higher mean dry weight of plant top compared to their control (23.28 g, 23.25 g respectively). Individual inoculation of VA mycorrhiza showed significant difference on dry weight of plant top. Maximum mean dry weight of 23.74 g was found in M_2, which was on par with the second ranking M_1.

Dual inoculation of *Azospirillum* and VA mycorrhiza had significance on dry weight of plant top. All combinations of dual inoculation was significantly

Table 6. Fresh weight of plant top (g)

	M_0	M_1	M_2	Mean
A_0	62.80	71.61	69.75	68.05
A_1	77.19	75.28	73.83	75.43
A_2	79.38	69.39	75.55	74.77
Mean	73.12	72.09	73.04	

CD (0.01) for A - 4.40
M - NS
CD (0.01) for AxM - 7.62

Table 7. Dry weight of plant top (g)

	M_0	M_1	M_2	Mean
A_0	18.53	23.33	24.26	22.04
A_1	22.49	24.03	23.32	23.28
A_2	23.21	22.91	23.65	23.25
Mean	21.41	23.42	23.74	

A - NS
CD (0.01) for M - 1.48
CD (0.01) for AxM - 2.58

Table 8. Fresh weight of root (g)

	M_0	M_1	M_2	Mean
A_0	18.68	23.60	23.03	21.79
A_1	27.88	25.06	23.97	25.64
A_2	23.70	25.58	22.95	23.41
Mean	23.42	24.11	23.11	

CD (0.01) for A - 1.89
M - NS
CD (0.01) for AxM - 3.29

Table 9. Dry weight of root (g)

	M_0	M_1	M_2	Mean
A_0	6.18	7.57	8.06	7.27
A_1	8.31	7.56	7.38	7.75
A_2	7.64	8.06	7.18	7.63
Mean	7.38	7.73	7.54	

CD (0.01) for A - 0.39
M - NS
AxM - NS

superior over control. Combination of treatment A_0 with VA mycorrhiza M_2 recorded maximum mean dry weight of plant top (24.26 g). The least dry weight of 18.53 g was noted in control plants.

4.3.7 Fresh weight of root

Table 8 shows the results on fresh weight of root. Individual inoculation of *Azospirillum* significantly influenced fresh weight of root. *Azospirillum* isolate A_1 recorded a maximum mean fresh weight of root (25.64 g). This was significantly superior over A_2 and control. In control, dry weight of roots was 21.79 g. Effect of VA mycorrhiza did not show any significant difference on fresh weight of root. VA mycorrhizal treatment M_1 gave higher mean fresh weight of root (24.11 g). VA mycorrhizal treatment M_2 (23.11 g) was found inferior even to the control (23.42 g).

The interaction of *Azospirillum* and VA mycorrhiza had significant effect on fresh weight of root. Inoculation of *Azospirillum* isolate A_2 in combination with VA mycorrhiza M_1 gave maximum mean fresh weight of 25.58 g compared to their individual inoculation. Control plants (A_0M_0) was inferior to all combinations of *Azospirillum* and VA mycorrhiza. In control plants fresh weight of roots was only 18.68 g.

4.3.8 Dry weight of root

The results on dry weight of root are presented in Table 9. Effect of *Azospirillum* inoculation showed significant difference on dry weight of root. The

maximum mean dry weight of root was recorded in isolate A_1 (7.75 g). Isolate A_2 gave a mean dry weight of 7.63 g, higher than the control (7.27 g). Effect of VA mycorrhizal treatments on dry weight of root was not significant. However, the mean dry weight was higher than their respective control.

Interaction effect of *Azospirillum* and VA mycorrhiza on dry weight of root was not significant. Isolate A_2 in combination with VA mycorrhiza M_1 gave a mean dry weight of root higher than their single inoculation. Maximum dry weight of root was found in A_1M_0 (8.31 g).

4.3.9 Nitrogen content of plant

Nitrogen content of plants under different treatments are presented on Table 10. Individual effect of *Azospirillum* and VA mycorrhiza on nitrogen content of the plant was not significant. However isolate A_2 recorded a maximum nitrogen content of 2.55 per cent and was higher than the control. Among the VA mycorrhizal treatments, M_1 gave a maximum nitrogen content (2.43 %). However it was not statistically significant over control.

Combined effect of *Azospirillum* and VA mycorrhiza on nitrogen content was significant. Maximum nitrogen content of 2.65 per cent was recorded in *Azospirillum* isolate A_1 in combination with VA mycorrhiza M_1 compared to their individual inoculations. A_2M_0 also recorded the same value. All combinations involving A_0 (without *Azospirillum*) were significantly inferior to combination involving either A_1 or A_2.

Table 10. Nitrogen content of plant (percentage)

	M_0	M_1	M_2	Mean
A_0	2.01	2.09	2.08	2.06
A_1	2.43	2.65	2.44	2.51
A_2	2.65	2.56	2.44	2.55
Mean	2.36	2.43	2.32	

A - NS
M - NS
CD (0.01) for AxM - 0.48

Table 11. Phosphorus content of plant (percentage)

	M_0	M_1	M_2	Mean
A_0	0.14	0.16	0.15	0.15
A_1	0.14	0.17	0.15	0.15
A_2	0.14	0.16	0.16	0.15
Mean	0.14	0.16	0.15	

A - NS
CD (0.01) for M - 0.011
AxM - NS

Table 12. Potassium content of plant (percentage)

	M_0	M_1	M_2	Mean
A_0	2.19	2.49	2.39	2.36
A_1	2.90	2.69	2.62	2.74
A_2	2.75	2.86	2.88	2.83
Mean	2.61	2.68	2.63	

A - NS
M - NS
CD (0.01) for AxM - 0.14

Table 13. VAM infection percentage

	M_0	M_1	M_2	Mean
A_0	0.14(2.50)	0.84(55.50)	0.79(50.00)	0.59(36.00)
A_1	0.14(2.25)	1.09(77.75)	0.96(66.25)	0.73(48.75)
A_2	0.12(1.75)	1.08(77.25)	0.99(71.55)	0.73(50.18)
Mean	0.13(2.17)	1.001(70.17)	0.91(62.60)	

Data transformed. Original values given in parentheses.
A - NS
M - NS
AxM - NS

4.3.10 Phosphorus content of plant

The results on phosphorus content of plant are presented on Table 11. The effect of *Azospirillum* on phosphorus content was not significant. Both *Azospirillum* isolates were on par with their control and recorded the phosphorus content of 0.15 per cent. Effect of VA mycorrhiza on phosphorus content of plant was significant. VA mycorrhiza M_1 recorded a maximum phosphorus content of 0.16 per cent. This was on par with M_2 (0.15%) and was significantly superior to M_0 (0.14%).

Combined effect of *Azospirillum* and VA mycorrhiza on phosphorus content of plant was not significant. Even though it was not significant, combination of *Azospirillum* isolate A_1 with VA mycorrhiza M_1 gave the maximum phosphorus per cent (0.17%) compared to their individual inoculations. In control the phosphorus content was only 0.14 per cent.

4.3.11 Potassium content of plant

Table 12 shows the data on potassium content of plant. The effect of *Azospirillum* on potassium content was not significant. The *Azospirillum* isolate A_2 recorded a maximum potassium content of 2.83 per cent compared to A_1 and control. VA mycorrhizal treatment also showed no significant difference on potassium content. Treatment M_1 showed higher potassium content of 2.68 per cent.

Combined effect of *Azospirillum* and VA mycorrhiza on potassium content was significant. A_1 in combination with M_0 recorded the highest potassium content of 2.9 per cent. This was followed by A_2M_2 (2.88%) and A_2M_1 (2.86%).

4.3.12 VAM infection percentage

Effect of *Azospirillum*, VA mycorrhiza and their interactions did not show any significant difference with respect to VAM infection percentage (Table 13). Eventhough, mycorrhizal treatments were not significant, maximum VAM infection percentage was observed in M_1 (70.17). Mixed inoculation of *Azospirillum* isolate A_1 in combination with VA mycorrhiza M_1 gave higher VAM infection percentage than their single inoculation (A_1 M_1 77.75).

4.4 Experiment on the efficiency of individual and dual inoculation of *Azospirillum* and VAM in improving the resistance of cocoa seedlings to seedling blight disease

The results of this experiment are given below.

4.4.1 Height of plant

Result of the experiment revealed that there was no significant difference on the fungicidal treatments, inoculum treatments and their interactions (Table 14). However potassium phosphonate 0.3 per cent (F_3) treated plants were the tallest (21.77 cm). Control plants (F_0) recorded the lowest height (20.76 cm). Among the inoculants, combined inoculation of *Azospirillum* and VA mycorrhiza recorded the maximum height of 23.71 cm. All the inoculum treated plants were having much more heights than that was observed in uninoculated control (I_0,

Table 14. Height of plant (cm)

	F_0	F_1	F_2	F_3	Mean
I_0	16.87	17.60	17.40	17.53	17.35
I_1	20.31	21.20	20.35	22.07	20.98
I_2	21.57	23.41	22.25	23.45	22.67
I_3	24.31	22.97	23.54	24.01	23.71
Mean	20.76	21.29	20.89	21.77	

I - NS
F - NS
IxF - NS

Table 15. Number of leaves

	F_0	F_1	F_2	F_3	Mean
I_0	9.17	9.33	9.23	9.00	9.18
I_1	11.00	11.25	11.11	11.25	11.15
I_2	13.33	12.39	11.67	12.83	12.56
I_3	13.83	12.17	13.75	12.33	13.02
Mean	11.83	11.29	11.44	11.36	

I - NS
F - NS
IxF - NS

Table 16. Leaf length breadth ratio

	F_0	F_1	F_2	F_3	Mean
I_0	2.31	2.29	2.23	2.30	2.28
I_1	2.47	2.48	2.54	2.45	2.49
I_2	2.48	2.39	2.52	2.38	2.44
I_3	2.58	2.47	2.52	2.67	2.56
Mean	2.46	2.41	2.45	2.45	

I - NS
F - NS
IxF - NS

17.35 cm). Among interaction effects, plants received dual inoculation without any fungicide treatment (I_3F_0) recorded maximum height of 24.31 cm. The shortest plants were those which did not receive any inoculum treamtents or fungicide treatments (I_0F_0, 16.87 cm).

4.4.2 Number of leaves

With respect to inoculum treatment, fungicide treatment and their interaction effects there was no significant difference on number of leaves (Table 15).

Among the microbial inoculants maximum number of leaves were found in treatment I_3 (13.02). All the other treatments I_1 and I_2 had higher number of leaves than the control. Control plant recorded 9.18 leaves. All the fungicide treatments recorded lower number of leaves compared to the control. Among the interactions between inoculants and fungicides, treatment combination I_3F_0, recorded maximum number of leaves (13.83). The least number of leaves (9) were found in potassium phosphonate treated plants with out any inoculation.

4.4.3 Leaf length breadth ratio

Microbial inoculation, fungicide application and their combinations did not show any significant difference on leaf length breadth ratio (Table 16).

Dual inoculation of *Azospirillum* and VA mycorrhiza recorded a leaf length breadth ratio of 2.56 which was maximum. The minimum ratio among the inoculants were recorded in uninoculated control (2.28). Fungicide treatments did not show much variation in leaf length breadth ratio. The ratio was maximum in

control plants (2.46). The minimum ratio was found in copper oxychloride treated plants (F_1 2.41). This ratio was same in fosetyl Al and potassium phosphonate treated plants.

I_3F_3 showed maximum leaf length breadth ratio 2.67 among interaction effects. This was least in fosetyl Al treated plants without any inoculants (I_0F_2).

4.4.4 Collar girth

The results on collar girth are presented on Table 17. Dual inoculation of *Azospirillum* and VA mycorrhiza, recorded the maximum collar girth of 5.94 mm (I_3). Least collar girth was recorded in uninoculated control (5.17 mm). However, dual inoculation (I_3) could not rise to a statistically significant level. Between *Azospirillum* and VA mycorrhiza, the later performed better. Among fungicidal treatments potassium phosphonate was the best in improving collar girth (5.78 mm). Fosetyl Al treatment recorded the lowest collar girth of 5.62 mm. The treatment combination I_3F_3 recorded the maximum collar girth of 6.08 mm and the minimum collar girth was found in I_0F_1 (5.05 mm). Control I_0F_0 also recorded the next lowest value of 5.15 mm. The effects of fungicides, inoculants and their interactions did not rise to a significant level in improving the collar girth of the plant.

4.4.5 Fresh weight of plant top

Table 18 shows the data on fresh weight of plant top. Observations on this also failed to record any statistically significant differences among the treatments. However, the dual inoculation (I_3) of *Azospirillum* and VA mycorrhiza

Table 17. Collar girth (Diameter in mm)

	F_0	F_1	F_2	F_3	Mean
I_0	5.15	5.05	5.19	5.28	5.17
I_1	5.79	5.83	5.74	5.73	5.78
I_2	5.82	5.80	5.70	6.02	5.83
I_3	5.91	5.93	5.84	6.08	5.94
Mean	5.67	5.65	5.62	5.78	

I - NS
F - NS
IxF - NS

Table 18. Fresh weight of plant top (g)

	F_0	F_1	F_2	F_3	Mean
I_0	11.91	11.76	11.31	12.43	11.85
I_1	15.21	16.74	15.86	15.48	15.82
I_2	19.71	18.39	19.02	19.13	19.06
I_3	20.88	20.52	18.74	22.18	20.58
Mean	16.93	16.85	16.23	17.31	

I - NS
F - NS
IxF - NS

Table 19. Dry weight of plant top (g)

	F_0	F_1	F_2	F_3	Mean
I_0	4.14	4.13	3.89	4.22	4.09
I_1	5.01	5.42	5.27	5.05	5.19
I_2	6.21	6.24	5.69	7.38	6.38
I_3	6.34	7.11	6.16	7.39	6.75
Mean	5.42	5.72	5.26	6.01	

I - NS
F - NS
IxF - NS

recorded the wide difference from uninoculatd control I_0. I_3 recorded a fresh weight of 20.58 g. In control, it was only 11.85 g. I_2, VA mycorrhizal inoculation closely followed dual inoculation recording 19.06 g. Among fungicidal application potassium phosphonate was found to maximise fresh weight of plant top to 17.31 g. Fosetyl-Al application reduced the fresh weight of plant top to 16.23 g. It was even lesser than control (F_0). Except potassium phosphonate, the other two fungicides ie. copper oxychloride and fosetyl-Al decreased the fresh weight lower than that in control. Application of dual inoculants and potassium phosphonate together (I_3F_3) increased fresh weight of plant top to a maximum of 22.18 g. Among combinations, application of fosetyl-Al alone with out any inoculants (I_0F_2) recorded the minimum fresh weight of plant top (11.31 g).

4.4.6 Dry weight of plant top

Observations on this also did not show any significant differences among treatments and their combinations (Table 19). Result showed that the dual inoculation I_3 performed better than the other treatments in increasing the plant dry weight to a level of 6.75 g. I_0 recorded the minimum dry weight of 4.09 g. I_1 and I_2 ranked between I_3 and I_0. As in the case of fresh weight of plant top, dry weight also was maximum in plants treated with potassium phosphonate (6.01 g). The minimum was recorded in treatment with fosetyl Al (F_2 5.26 g) which was even lesser than untreated control. The treatment combinations also showed a trend compared to fresh weight of plant top. Treatment combination I_3F_3 recorded maximum dry weight of 7.39 g. The similarity of results of fresh weight and dry

weight showed that the dry weight of the plant was more or less uniform in various treatments.

4.4.7 Fresh weight of root

Results failed to record any significant differences among treatments in influencing fresh weight of root (Table 20). However, among the inoculum treatments, here also dual inoculation of VA mycorrhiza and *Azospirillum* (I_3) performed better in recording the highest fresh weight of 9.96 g. Uninoculated control recorded the minimum dry weight of 5.13 g. Between *Azospirillum* and VA mycorrhiza, the later was better in increasing the fresh weight of root (I_2 8.72 g, I_1 7.21 g).

The fungicide potassium phosphonate recorded the maximum of fresh weight of 8.15 g and the minimum was in control F_0.

The combination I_3F_3 performed best among other treatment combinations in improving the fresh weight of root of 10.19 g. The performance of all treatment combinations involving the dual inoculation of *Azospirillum* and VA mycorrhiza (I_3) performed better than other combinations. The minimum fresh weight of root was recorded in the control plants (I_0F_0, 4.45 g).

4.4.8 Dry weight of root

Results on dry weight of root are presented on Table 21. Inoculation treatments, fungicide treatments and their combinations did not produce any significant difference among treatments. Maximum dry weight of root was

Table 20. Fresh weight of root (g)

	F_0	F_1	F_2	F_3	Mean
I_0	4.45	5.01	5.73	5.34	5.13
I_1	6.85	6.48	7.93	7.56	7.21
I_2	8.04	8.69	8.64	9.52	8.72
I_3	10.18	9.38	10.07	10.19	9.96
Mean	7.38	7.39	8.09	8.15	

I - NS
F - NS
IxF - NS

Table 21. Dry weight of root (g)

	F_0	F_1	F_2	F_3	Mean
I_0	1.75	1.76	1.95	1.71	1.79
I_1	2.30	2.27	2.37	2.21	2.29
I_2	2.52	2.60	2.53	2.21	2.71
I_3	2.67	2.84	3.27	3.02	2.95
Mean	2.31	2.37	2.53	2.54	

I - NS
F - NS
IxF - NS

Table 22. VAM infection percentage

	F_0	F_1	F_2	F_3	Mean
I_0	0.19 (5.00)	0.14 (3.33)	0.21 (5.00)	0.22 (5.00)	0.19 (4.58)
I_1	0.11 (1.67)	0.21 (5.33)	0.14 (3.33)	0.19 (4.67)	0.16 (3.75)
I_2	1.07(77.00)	0.94(64.67)	0.93(64.33)	0.96(67.00)	0.98(68.25)
I_3	0.97(67.67)	1.04(74.33)	0.96(66.33)	0.93(64.00)	0.98(68.08)
Mean	0.59(37.84)	0.58(36.92)	0.56(34.75)	0.58(35.17)	

Data transformed. Original values given in parenthesis
I - NS
F - NS
IxF - NS

recorded in dual inoculated plants I_3 (2.95 g). The minimum was noted in uninoculated control. (I_0, 1.79g). However, this difference did not rise to a level of statistical significance.

The application of fungicide potassium phosphonate recorded the maximum dry weight of root, 2.54 g. Minimum dry weight was found in plants which did not receive any fungicide (F_0, 2.31 g).

Among the interaction between inoculants and fungicides, I_3F_2 recorded the maximum dry weight of (3.27 g). This was closely followed by I_3F_3 (3.02 g). The minimum dry weight of root was recorded in I_0F_3 where potassium phosphonate alone was applied without any inoculants. All the treatment combinations where, there was no inoculants, the dry weight was lower than 2 gm.

4.4.9 VAM infection percentage

Various treatments did not improve the VAM infection percentage to a significant level (Table 22). However, there was a marked difference between VA mycorrhizal inoculated plants and uninoculated plant in VAM infection percentage. The treatment I_2, VA mycorrhizal inoculation and I_3, VA mycorrhizal inoculation and *Azospirillum* inoculation, recorded a value of 0.98 where as in uninoculated control recorded only 0.19. Dual inoculation did not improved VAM infection than VA mycorrhizal inoculation alone.

Fungicide treatment did not seem to affect much as VAM infection percentage. The variation was minute. Uninoculated control recorded (F_0) the

maximum VAM infection of 0.59. All other fungicidal treatment slightly reduced the VAM infection percentage. Minimum infection was in plants treated with fosetyl-Al (0.56).

Among treatment combinations, VAM infection percentage was maximum in the combination I_2F_0 (1.07) where VA mycorrhiza was inoculated without any fungicidal treatment. Minimum VAM infection was in I_1F_0.

4.4.10 Disease Index before fungicide application

The data on the disease index is given in Table 23. The index of the disease incidence before the fungicide application (Plate 2a and 2b) was not significant among the inoculum treatments, fungicidal treatments and their interactions. However, disease was maximum in dual inoculated, plants and the disease was minimum in control plants. All together the natural disease index recorded a range of 3.99 to 8.48. But there was no, significant difference of treatment before any fungicide application.

4.4.11 Disease index after fungicide application

The same table (Table 23) gives the data on disease index before and after fungicide application. Eventhough much change in the disease index was noted, it did not rise to a significant level. Maximum disease was recorded in dual inoculated plants I_3 (6.65) and the minimum was in control plants (4.71). However, this difference was not significant.

Plate. 2a. Seedling blight disease of cocoa

Plate. 2b. Seedling blight disease of cocoa (Close-up view)

Table 23. Disease index before fungicide application (Disease index after fungicide application given in parantheses)

	F_0	F_1	F_2	F_3	Mean
I_0	4.99 (5.26)	4.21 (3.73)	5.99 (5.78)	4.28 (4.05)	4.87 (4.71)
I_1	6.30 (6.59)	3.99 (4.56)	6.37 (6.85)	5.09 (5.07)	5.44 (5.77)
I_2	6.89 (6.28)	5.06 (6.97)	5.22 (4.08)	4.02 (2.79)	5.29 (5.05)
I_3	7.07 (6.56)	6.18 (5.95)	8.48 (7.51)	7.95 (6.58)	7.42 (6.65)
Mean	6.31 (6.17)	4.86 (5.30)	6.52 (6.05)	5.34 (4.62)	

Disease index before fungicide Application

I - NS
F - NS
IxF - NS

Disease index after fungicide application

I - NS
F - NS
IxF - NS

Table 24. Reduction in the disease index after fungicide application

	F_0	F_1	F_2	F_3	Mean
I_0	-0.27	0.48	0.21	0.23	0.16
I_1	-0.29	-0.57	-0.48	0.02	-0.33
I_2	0.61	-1.91	1.14	1.23	0.26
I_3	0.51	0.23	0.97	1.37	0.77
Mean	0.14	-0.44	0.47	0.72	

Among fungicidal treatments the minimum disease index was in plants treated with potassium phosphonate (4.62) maximum was in control (6.17). Among treatment combinations, the minimum disease was noted in I_2F_3 and the maximum was in I_3F_2 (7.51). All these variations did not differ significantly.

4.4.12 Reduction in the disease index after fungicide application

The data on the reduction in the disease index after fungicide application are given in Table 24. The inoculum treatments recorded a reduction in the disease index ranging from 0.16 to 0.77. Maximum reduction on the disease index was in dual inoculated plants (0.77). In *Azospirillum* treated plants, disease index was slightly increased after the fungicide application (I_1, -0.33). Among fungicidal treatments also a similar trend was noticed. All fungicidal treatments except copper oxychloride application recorded a remission of disease index which ranged from 0.14 to 0.72. Potassium phosphonate application (F_3, 0.72) recorded maximum reduction in disease index. Copper oxychloride application slightly increased (F_1, -0.44) the disease index after its application. In untreated control a slight (0.14) reduction in disease index was noted.

Among the interaction between inoculum and fungicides, maximum disease reduction was recorded in dual inoculated plants applied with potassium phosphonate (I_3F_3, 1.37). All the treatment combinations in which potassium phosphonate was applied showed reduction in disease index at various levels ranging from 0.02 - 1.37. Fosetyl Al recorded disease reduction in combination with VA mycorrhizal inoculation and also with dual inoculation.

Copper oxychloride application reduced the disease index when applied alone (I_0F_1) or with dual inoculation (I_3F_1). Another observation was that, VA mycorrhizal inoculation alone and dual inoculation alone without any fungicidal treatments reduced the disease index to 0.61 and 0.51 respectively. *Azospirillum* inoculation was found to increase the disease index in combination with copper oxychloride application and fosetyl-Al application. Only *Azospirillum* along with potassium phosphonate application (I_1F_3) was found to slightly reduce the disease index (0.02).

DISCUSSION

DISCUSSION

Now a days, greater emphasis is given to the use of *Azospirillum* and VA mycorrhiza in Indian agriculture. The beneficial effects of *Azospirillum* have already been reported in several crops by various workers (Reynders and Vlassak, 1982, Padshetty *et al.,* 1986, Joshi and Rao, 1989). The ability of VA mycorrhiza in the uptake of many nutrients particularly phosphorus and its effects on the control of many diseases are well established (Widiastuti, 1989, Bagyaraj and Manjunath, 1980, Baon *et al.,* 1994, Chakravarthy and Mishra, 1986). Considering the beneficial effects of *Asospirillum* and VA mycorrhiza, the present investigation was undertaken to study the response of cocoa seedlings to individual and dual inoculation of *Azospirillum* and VA mycorrhiza and to find out the efficacy of the inoculants in imparting resistance to seedling blight disease.

Native *Azospirillum* was isolated from the roots of cocoa seedlings. The isolate appeared as thin, white, subsurface pellicular growth in nitrogen free bromothymol blue medium as reported by Okon *et al.* (1976a). Isolate was Gram negative and vibrioid in shape as described by Dobereiner and Day (1975). In Okon's medium, growth of *Azospirillum* was found thin, dry, slightly convex colonies with a wavy surface and undulate margin as documented by Okon *et al.* (1977). In RC medium the colonies were round to irregular, the surface was rugose and edges undulating. The colour of the colonies were scarlet. Formation of this type of colonies in RC medium has been reported by Caceres (1982).

The *Azospirillum* isolate produced round irregular colonies with pink colour and were dense and wrinkled in potato infusion agar as described by Dobereiner *et al.* (1976).

Production of acid from glucose by *Azospirillum* was indicated by the change of colour of the medium from green to yellow and this was included in group I as reported by Tarrand *et al.* (1978).

Azospirillum isolate showed good growth in malate containing medium. Scanty growth was observed in glucose, lactose, sucrose and mannose containing media. The sugars were found to be poor source of carbon for *Azospirillum* according to Okon *et al.* (1976b).

Shredding of agar blocks indicated the dissimilation of nitrate by *Azospirillum* in NFb medium containing ammonium nitrate. This isolate could be nir^+ as documented by Neyra *et al.* (1977) and Baldani and Dobereiner (1980).

The infection of VA mycorrhiza in cocoa roots was observed by staining of the roots as described by Phillips and Hayman (1970). The Hyphae, arbuscules and vesicles were stained blue indicating the presence of VA mycorrhiza. The above results confirmed the genuiness of the cultures used for the study.

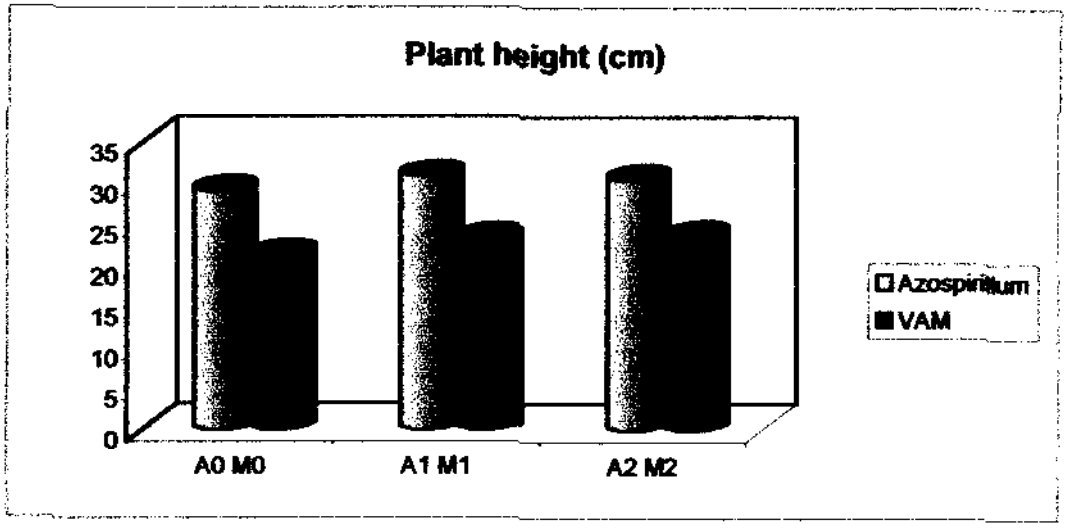
The analysis of the data on the experiment "the effect of individual and dual inoculation of *Azospirillum* and VA mycorrhiza in improving the growth and

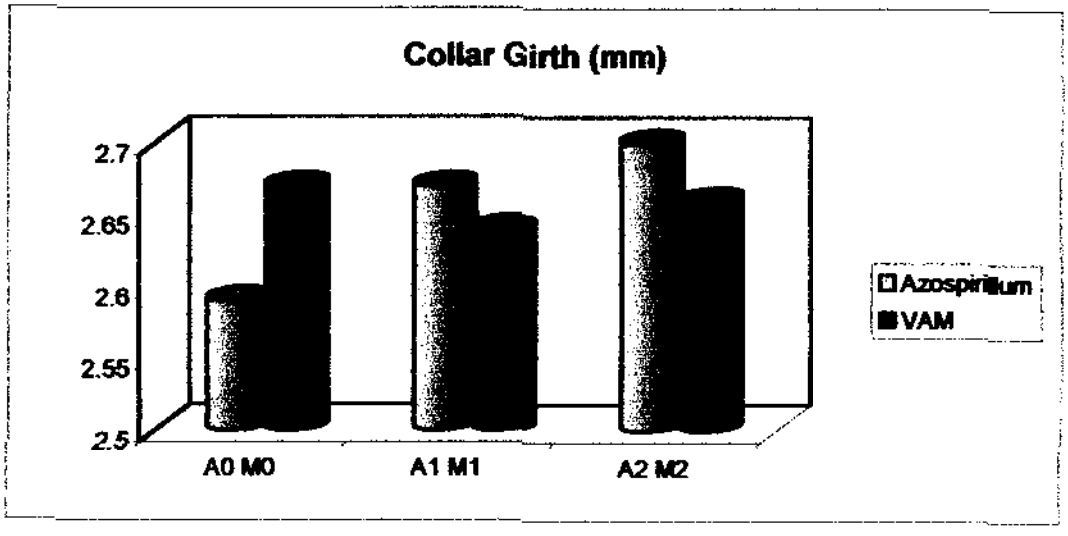
establishment of cocoa seedlings" revealed that treatments and their combinations were significant in improving many of the growth attributes of the cocoa seedlings. Both the height and number of leaves were significantly improved by the application of both native and commercial *Azospirillum* application over control. Thus the effect of *Azospirillum*, either native or commercial in improving the height and number of leaves is made evident. Between native and commercial *Azospirillum*, native isolate proved better over commercial culture (Fig.1). Native isolate A_1 recorded a height of 30.97 cm. Commercial culture inoculated plants and control plants recorded only 30.6 cm and 29.16 cm respectively. A similar trend in the efficiency of the cultures can be seen in the number of leaves also. The native isolate recorded a maximum number of 21.21 leaves closely followed by the commercial isolate. The efficiency of *Azospirillum* in improving the growth parameters is already reported by many workers (Bopiah and Khader, 1989, Wani *et al.*, 1992, Jothy *et al.*, 1993).

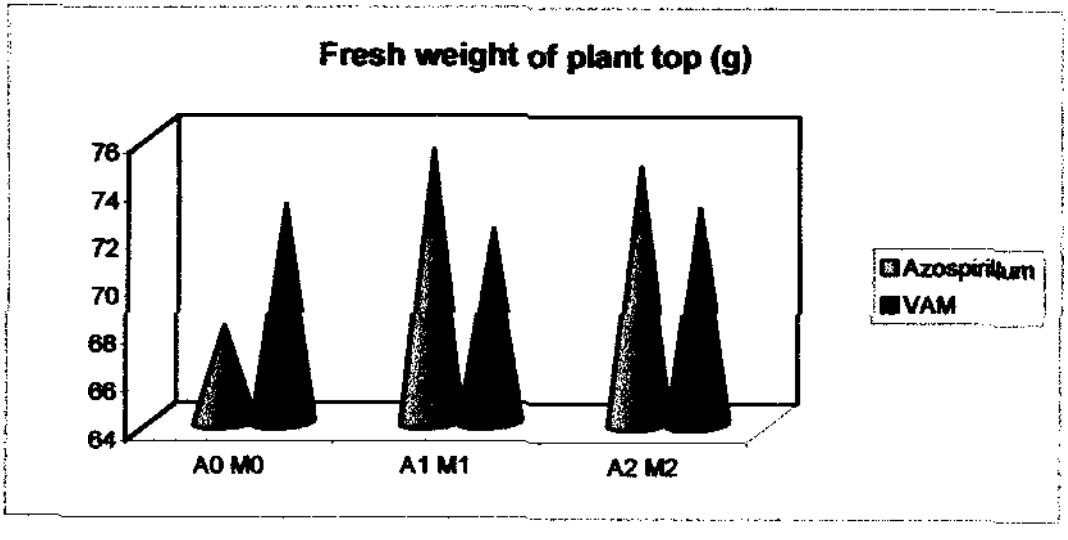
The local VA mycorrhizal isolate M_1 improved the plant height to a maximum of 31.06 cm while the commercial inoculant was even poorer than uninoculated control in contributing to the height of the plant. The superiority of local cultures over the introduced cultures was already reported by many workers (Cuenca *et al.*, 1990 and Edathil *et al.*, 1994).

Combined inoculation of local *Azospirillum* and VA mycorrhiza rank only second but it was on par with the best treatment combination. As far as

Fig. 1.


Plant height (cm)
35
30
25
20
15
10
5
0
A0 M0
A1 M1
A2 M2
Azospirillum
VAM


Collar Girth (mm)
2.7
2.65
2.6
2.55
2.5
A0 M0
A1 M1
A2 M2
Azospirillum
VAM


Fresh weight of plant top (g)
76
74
72
70
68
66
64
A0 M0
A1 M1
A2 M2
Azospirillum
VAM

influence on height of the plant is concerned, local isolate of both the inoculants and their combinations was found to increase this parameter to the maximum.

The effect of mycorrhiza on number of leaves was not significant. However, maximum number of leaves was noted in plants inoculated with commercial VA mycorrhiza. Eventhough, informations on effect of VA mycorrhiza on general growth of plant are available, their effect on number of leaves is scanty. Interaction effects also showed that mycorrhizal application does not play much role in increasing number of leaves. The treatment A_2M_0 recorded the maximum number of leaves. Thus the results showed that the local *Azospirillum* isolate significantly improved the number of leaves while the mycorrhizal application was not able to influence the number of leaves.

The length breadth ratio of the leaves is an indication of the shape of the leaf. A high ratio indicates the narrowness of the leaves and the low ratio indicates the round shape of the leaf. *Azospirillum* inoculation was effective in significantly influencing the leaf shape. The leaves of native *Azospirillum* inoculated plants were broader than commercial *Azospirillum* inoculated plants. Uninoculated plants produced plants with broadest leaves (A_0 2.59). Thus it become evident that *Azospirillum* inoculation influenced the leaf shape as a decisive factor. A review of the literature did not reveal much work on this aspect. Eventhough non significant, VA mycorrhiza was found to make the leaves more round than linear (M_0 2.67, M_1 2.64, M_2 2.66). Interaction effects did not affect the leaf shape significantly. Thus the data on length breadth ratio of the leaf indicated that

Azospirillum inoculation made the leaves more linear than the others. It can be presumed that the linear nature of the leaves of the inoculated plants is an indication of the efficiency of the inoculated *Azospirillum*.

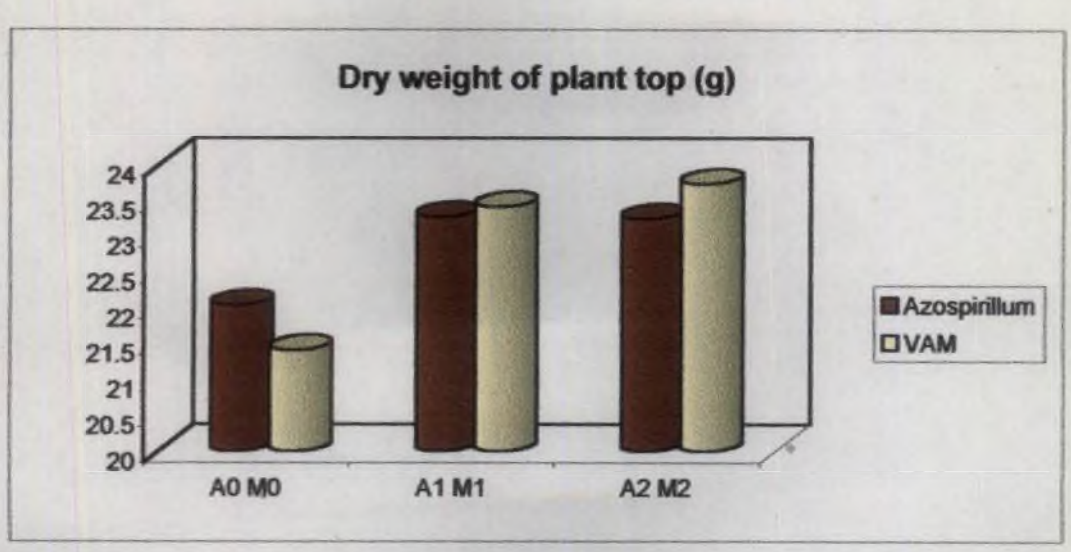
Collar girth which is an indication of the seedling vigour has not significantly influenced either by the individual application of *Azospirillum* or by mycorrhiza. But combined application significantly influenced the collar girth. The combination A_2M_0 recorded a collar girth of 9.2 mm. The minimum was in A_2M_1. This showed that commercial *Azospirillum* inoculant was effective in significantly increasing the collar girth, when applied alone (A_2M_0). But the efficiency was reduced when it was combined with the application of native VA mycorrhiza (A_2M_1). This result puts a question mark to the application of commercial *Azospirillum* along with VA mycorrhiza in improving the collar girth. Individual application of *Azospirillum* A_2 favoured the improvement in collar girth.

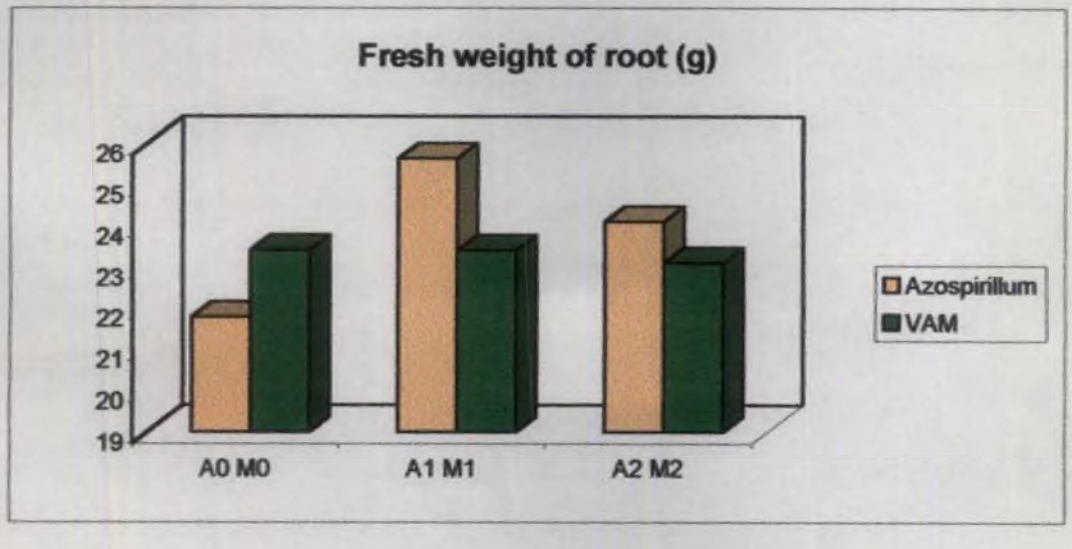
Collar girth being an important parameter in determining the seedling vigour, it became evident that *Azospirillum* inoculation is helpful in improving this parameter (Fig.1). The second ranking treatment combination A_1M_1 was also equally effective as the application of commercial *Azospirillum* alone in improving the collar girth of seedlings. The control plants recorded only 8.25 mm of collar girth, A_2M_0 and A_1M_1 treatment combinations recorded 9.2 and 9.1 mm respectively.

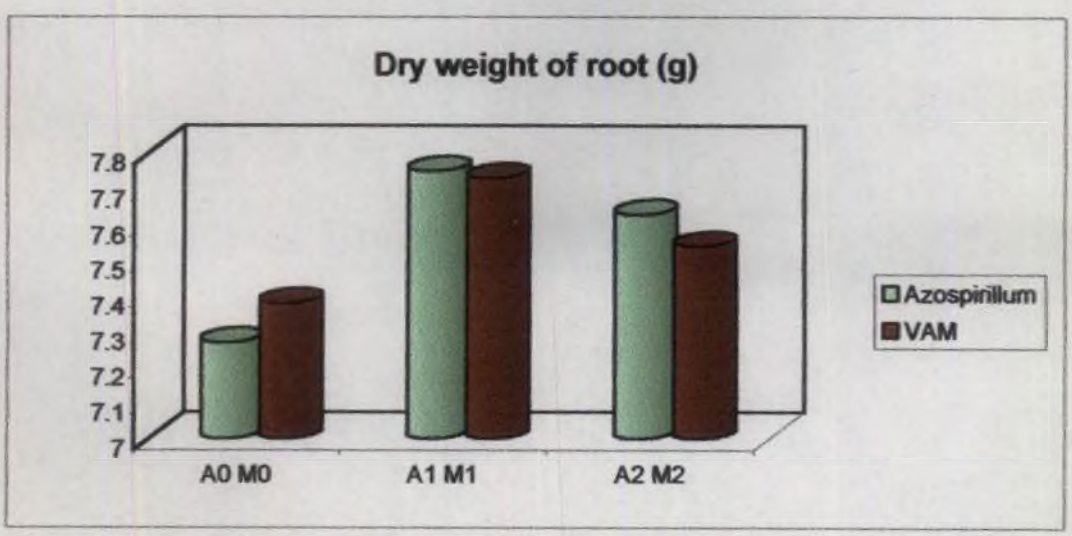
The data on the fresh weight of plant top clearly showed the beneficial effects of applying either native or commercial *Azospirillum* (Fig.1). Here also native *Azospirillum* was the best treatment in improving the fresh weight of plant top to a maximum of 75.43 g. In control plants it was only 68.05 g. Mycorrhizal inoculation was not significant in affecting the fresh weight of plant top. Interaction effects of *Azospirillum* and VA mycorrhiza was significant and the maximum fresh weight was recorded by A_2M_0 (79.38g). When the commercial *Azospirillum* A_2 was inoculated along with VA mycorrhiza, fresh weight was seen to reduce slightly. In collar girth also, such a trend was noticed. This points to a tendency for slight inhibition of the efficiency of both the inoculants when commercial *Azospirillum* was inoculated along with VA mycorrhiza applied together. But this inhibition did not reach to a statistically significant level.

Dry weight of plant top did not show a proportional value as that of the fresh weight of plant top (Fig.2). This showed that the different treatments and their combinations affected the dry matter content of the plants differently. When *Azospirillum* application did not show any effect, mycorrhizal application and dual inoculation showed significant effects on dry matter content. Commercial mycorrhizal application was found to provide the maximum dry weight of plant top to 23.74g. Moisture content of these plants were 67.5 per cent. In uninoculated plants (M_0) the dry weight was minimum and the moisture content of such plants were 70.7 per cent. This revealed that mycorrhizal application reduced the moisture content of the plant and made the plant more sturdy than succulent. This

Fig. 2.


Dry weight of plant top (g)
24
23.5
23
22.5
22
21.5
21
20.5
20
A0 M0
A1 M1
A2 M2
Azospirillum
VAM


Fresh weight of root (g)
26
25
24
23
22
21
20
19
A0 M0
A1 M1
A2 M2
Azospirillum
VAM


Dry weight of root (g)
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7
A0 M0
A1 M1
A2 M2
Azospirillum
VAM

may be the reason for improving the resistance to disease by mycorrhizal inoculated plants. Many workers reported increased resistance to plants against disease by mycorrhizal inoculation (Hedge and Rai, 1984, Sreenivasa *et al.*, 1992, Sivaprasad, 1993, Sivaprasad *et al.*, 1995). Maximum dry weight was recorded in A_0M_2 (24.26g). This was having a moisture content 65.2 per cent. In control plants (A_0M_0) the dry weight was 18.53 g with a moisture percentage of 70.5. This proves that application of commercial mycorrhiza inoculation improved the dry weight of plant top and made the plants more hardy with less moisture per cent. The mycorrhizal inoculation might have increased absorption of nutrients as was reported by many workers (Bagyaraj and Sreeramulu, 1982, Reena and Bagyaraj, 1990, Widiastuti, 1989, Wani and Konde, 1998) and contributed to the hardness of plant.

The data on the fresh weight of root showed the superiority of native *Azospirillum* inoculation by recording 25.64g of fresh weight (Fig.2). This was 17.6 per cent increase over control. This is another parameter which revealed the efficiency of native *Azospirillum* isolates over uninoculated control and even commercial *Azospirillum* cultures. In many cases it was reported that the native microorganisms performed better over introduced ones (Cuenca, *et al.*, 1990 and Edathil *et al.*, 1994). This may be due to highly adapted condition of native flora where it is available. Eventhough non significant, native mycorrhizal inoculation also showed the superiority over control and commercial inoculum (M_1 24.11 g, M_2 23.11 g and M_0 23.42 g). This also supports the above finding of the efficiency

of native isolates. Interaction effect also revealed that native isolate A_1 alone and in combination with M_1 and M_2 performed to the maximum. The data on fresh weight of root pointed out the need for the selection of locally adapted native strains of the microorganisms for better performance than any other commercial cultures.

Dry weight of root also followed similar trend. Maximum dry weight was recorded in A_1 (7.75 g) which was significant. The treatment combination A_1M_0 recorded a maximum of 8.31 g of root dry weight. The graphical representation reveals that the moisture content of the root did not vary much due to the treatments (Fig. 2). The inference that the native isolates performed better holds good as it is evident from the result on fresh weight of root also.

Both individual and dual inoculations did not increase the nitrogen content of the plant to a significant level. However it was slightly increased over uninoculated control. Native mycorrhiza was better in improving the nitrogen content of plant (M_1 2.43 per cent) than the commercial. But the commercial *Azospirillum* (A_2, 2.55 per cent) was better than native (Fig. 3). The effects of *Azospirillum* and VA mycorrhiza in improving the absorption of nitrogen is already established by many workers (Rao and Rao, 1983, Ratti and Janardhanan, 1996, Joshi and Rao, 1989, Bhandari *et al.,* 1990, Wani and Konde, 1998).

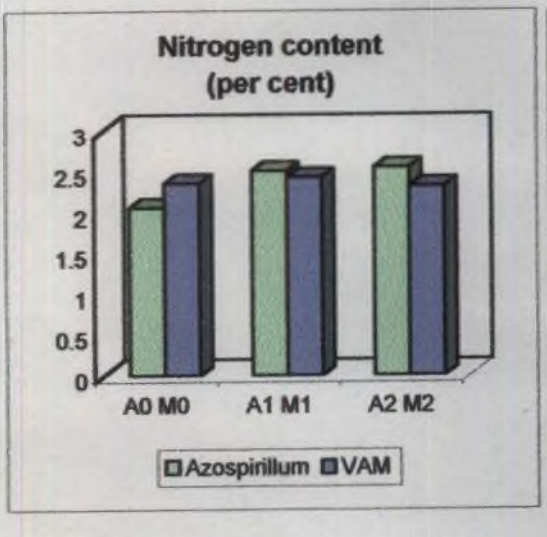
The dual inoculation of native isolates of both the organisms (A_1M_1, 2.65 per cent) increased nitrogen content of the plant to the maximum. This was

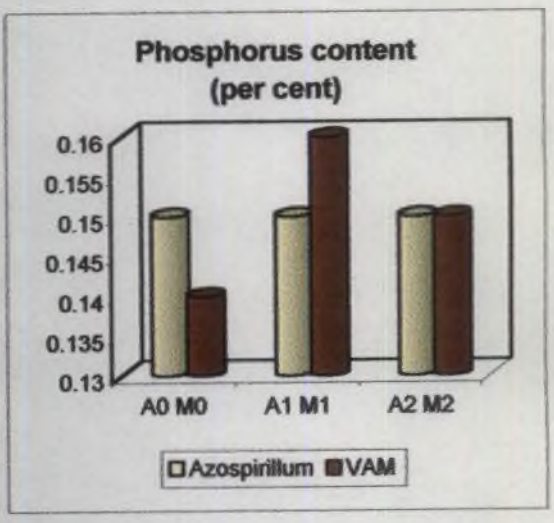
31.8 per cent increase over uninoculated control. This result also emphasised the superior performance of native isolates of both the organisms.

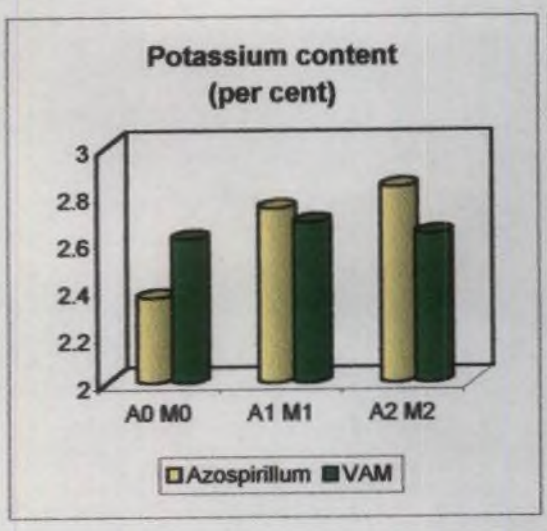
The effect of VA mycorrhiza in improving phosphorus uptake of crop plants is well established (Bagyaraj and Manjunath, 1980, Sharma *et al.*, 1988, Widiastuti, 1989, Shirsath *et al.*, 1998). In the present study it was the native VA mycorrhiza which increased the phosphorus content to the maximum of 0.16 per cent (Fig. 3). Both the inoculants M_1 and M_2 increased the phosphorus content of the plant than that present in M_0. *Azospirillum* inoculation and their combinations did not affect the phosphorus content. The increase in phosphorus content by the application of native VA mycorrhizal culture was 14.3 per cent over control plants. Based on the data, application of native isolates of VA mycorrhiza can be recommended for increasing the phosphorus content of cocoa seedlings.

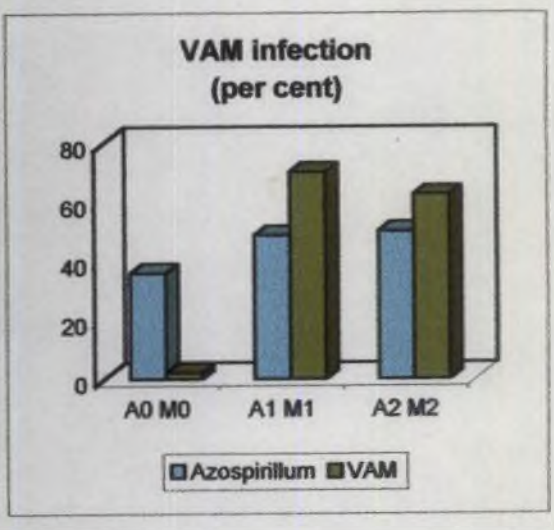
The absorption of potassium was found to be affected significantly by dual inoculation. Maximum potassium content was found in A_1M_0 followed by A_2M_2. Thus in this parameter dual application of commercial cultures of both the inoculants was found equally effective with native *Azospirillum* application alone. All the treatments and their combinations improved the potassium content of the plant over control. The difference in the efficiency among the inoculants was not significant. The data revealed that the strains of the microorganisms selected did not affect much in increasing the potassium content (Fig. 3).

Fig. 3.


Nitrogen content
(per cent)
3
2.5
2
1.5
1
0.5
0
A0 M0
A1 M1
A2 M2
Azospirillum
VAM


Phosphorus content
(per cent)
0.16
0.155
0.15
0.145
0.14
0.135
0.13
A0 M0
A1 M1
A2 M2
Azospirillum
VAM


Potassium content
(per cent)
3
2.8
2.6
2.4
2.2
2
A0 M0
A1 M1
A2 M2
Azospirillum
VAM


VAM infection
(per cent)
80
60
40
20
0
A0 M0
A1 M1
A2 M2
Azospirillum
VAM

The data on VAM infection percentage showed that the different treatments did not affect the VAM infection percentage to a significant level. However, maximum VAM infection percentage was found in native VA mycorrhizal inoculated plants (M_1 70.17) (Fig.3). In uninoculated plants, it was only 2.17 per cent. The commercial inoculant recorded only 62.6 per cent. Maximum VAM infection was found in combination A_1M_1 (77.75). This shows that combined inoculation of native isolates of *Azospirillum* and VA mycorrhiza influenced the mycorrhizal infection positively. So inoculation of VA mycorrhiza along with *Azospirillum* positively influenced the VAM infection percentage in cocoa seedlings. Beneficial effects of dual inoculations of VAM fungus with other microorganisms were reported by other workers (Kandaswamy *et al.*, 1988, Gurubatham *et al.*, 1989, Thakur and Panwar, 1995). So in this parameters also the native isolates are found to perform better.

An evaluation of the entire result showed that out of the 12 parameters observed, the native *Azospirillum* isolate A_1 ranked first in nine of the parameters. This proved the superiority of A_1 and it was selected for the next experiment. Similarly, the native mycorrhizal isolate M_1 showed superiority in seven of the parameters tested and thus it was also selected for the further experiment.

The next experiment was aimed to find out the efficiency of individual and dual inoculation of *Azospirillum* and VA mycorrhiza in improving the resistance of cocoa seedlings to seedling blight disease. The vigour of the plant as measured in various growth parameters were also recorded. The entire data

revealed that none of the treatments influenced the various growth parameters and disease index to a significant level. Combined inoculation of VA mycorrhiza and *Azospirillum* produced the maximum height of 23.71 cm. Individual inoculation was inferior than this. Fungicide treatments did not affect the height of the plant.

Synergistic effects of dual inoculation of microorganisms are reported by many workers (Negi *et al.,* 1990, Kumari and Balasubramanian, 1993, Ratti and Janardhanan, 1996, Suguvanam *et al.,* 1998). Synergistic effect was also found in the leaf length breadth ratio where the dual inoculation have an increased effort in making the leaves more linear (length breadth ratio 2.56). In uninoculated plants the leaves were broader than that in the inoculated plants (length breadth ratio 2.28). This results confirmed the results of previous experiment.

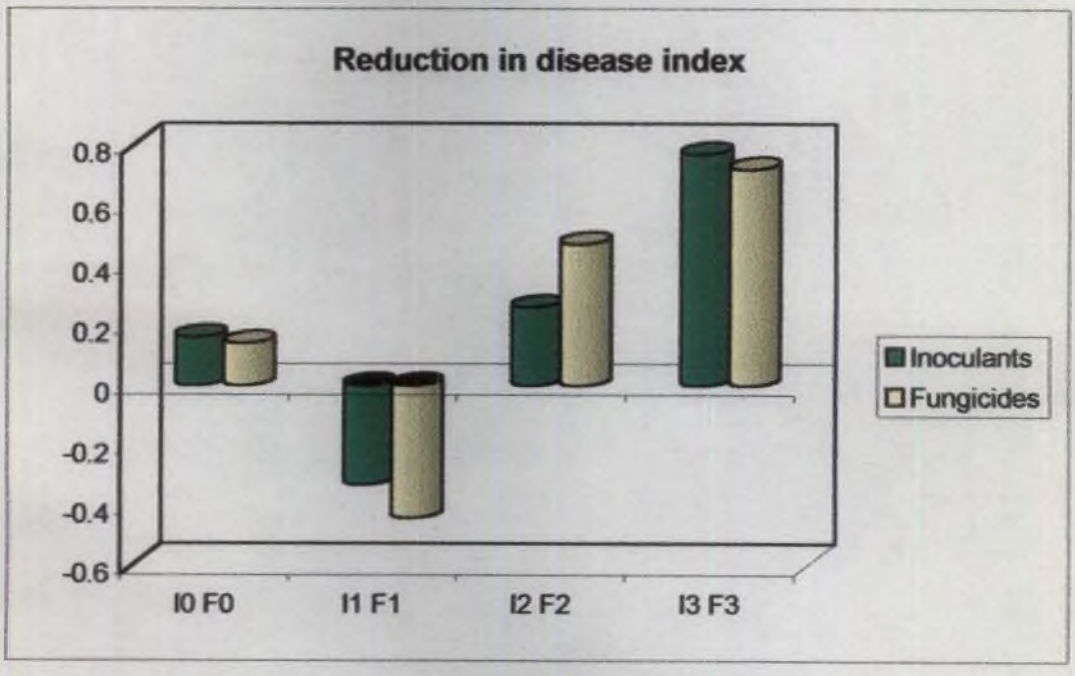
In case of collar girth dual inoculated plants recorded the highest collar girth of 5.94 mm. In control it was only 5.17 mm. The fungicides seem to have no direct influence on collar girth. When the dual inoculated plants were applied with potassium phosphonate the collar girth increased to 6.08 mm.

Fresh and dry weight of plant top also proved the synergistic effect of dual inoculation of *Azospirillum* and VA mycorrhiza than individual inoculations. There was an increase of 73.7 per cent as far as fresh weight of plant top was concerned as a result of dual inoculation over control. This increase was only 65 per cent for the dry weight of plant top. Variations in fresh weight and dry weight of plant top as a result of fungicide application were meagre. The results were

similar to the fresh weight and dry weight of root also. Fresh weight of dually inoculated roots were 9.96 g. It was an increase of 94.2 per cent over control. Dry weight of roots also showed synergistic effect as a result of dual inoculation. The fungicides did not show much effects on all the above characters. Mycorrhizal infection was drastically improved as a result of inoculation. But here the dual inoculation was not beneficial in imparting a synergistic effect. There was 416 per cent increase in VAM infection in inoculated plants compared to control. The need for artificial inoculation of VA mycorrhiza in increasing infection is an established fact.

The observations on the disease intensity before and after the treatment showed the positive effects of fungicide and inoculants in controlling the seedling blight disease. Dually inoculated plants showed a reduction in disease index to 0.77. In VA mycorrhiza treated plants the reduction in disease was 0.26. In *Azospirillum* inoculated plants the disease index was found to increase 0.33. Even with out any microbial treatments the natural reduction in disease index was 0.16. The efficiency of VA mycorrhiza in imparting the disease resistance is already reported by many workers (Hedge and Rai, 1984, Iyer and Sundararaju, 1993, Joseph, 1997). The present experiment with *Azospirillum* inoculation increased the disease index slightly, but when it was combined with VA mycorrhiza, there was a synergistic effect in controlling the cocoa seedling blight disease. Another observation was that the disease reduction by dual inoculation (0.77) was higher than that was achieved by the application of the fungicide, potassium phosphonate. Among the fungicides tested maximum reduction in disease index was found in

Fig. 4.


Reduction in disease index
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
I0 F0
I1 F1
I2 F2
I3 F3
Inoculants
Fungicides

application of 0.3 per cent potassium phosphonate (0.72). Many workers have already been reported the chemical control of *Phytophthora* using potassium phosphonate (Pegg *et al.*, 1990, Johri and Chaurasia, 1998, Ali *et al.*, 1999).

Application of fosetyl-Al was less efficient in controlling the disease than potassium phosphonate. The present study reveals that dual application is better than chemical treatments in checking the seedling blight disease (Fig. 4). However, this needs further confirmation since the disease incidence was generally low during the season. If fungicide treatment along with microbial inoculation is opted, the best treatment combination was found to be dual inoculation combined with application of potassium phosphonate (0.3 per cent). In this treatment combination the reduction in the disease index was 1.37.

The whole experiment revealed that native strains of *Azospirillum* and VA mycorrhiza performed better over commercial strains in improving the growth and establishment of cocoa seedlings and their combined inoculation had a synergistic effect in many growth parameters. For the control of seedling blight disease dual inoculation was proved to be efficient. Among the fungicides tested, application of potassium phosphonate 0.3 per cent performed better. So application of dual inoculants along with 0.3 per cent potassium phosphonate application was the best treatment combination in reducing the disease effectively.

SUMMARY

SUMMARY

An experiment was conducted on the "effect of *Azospirillum* and VA mycorrhiza on the growth of cocoa seedlings and incidence of seedling blight disease during 1998-2000 at the College of Horticulture, Vellanikkara. The objective of the study was to find out whether there was any synergistic effect for the combined inoculation of *Azospirillum* and VA mycorrhiza in improving growth and establishment of cocoa seedlings and to find out the efficiency of inoculants in imparting resistance to seedling blight disease. The results of the experiment are summarised below.

Native *Azospirillum* isolated from the roots of cocoa seedlings and commercial culture procured from Tamil Nadu Agricultural University, Coimbatore were used for the experiment. The cultures were purified and, cultural and physiological characterization were done. Similarly native and commercial VA mycorrhizal culture procured from Tamil Nadu Agricultural University were used. In the first experiment the individual and combined effects of these inoculants in improving growth parameters of cocoa seedlings were tested.

The results showed that most of the growth parameters were improved as a result of inoculation with *Azospirillum* and VA mycorrhiza. As far as height and number of leaves are concerned inoculation of native *Azospirillum* isolate proved better over commercial culture. Native isolate A_1 recorded a height of 30.97 cm. Similarly plant height was maximum (31.06 cm) in plants inoculated

with native VA mycorrhizal isolate. Combined inoculation of native *Azospirillum* and VA mycorrhiza ranked second but it was on par with the best treatment combination as far as the height of plant was concerned. Thus any sort of synergistic effect was not noticed as far as the height of plant was concerned. Results showed that local *Azospirillum* isolate significantly improved number of leaves while the mycorrhiza application did not influence the number of leaves. Inoculation of the microorganisms was found to influence the leaf shape (length breadth ratio). Uninoculated plants produced the broadest leaves (length breadth ratio 2.59). *Azospirillum* inoculation was found to make the leaves linear. VA mycorrhiza inoculation was found to make the leaves more broader than linear. Interaction effects was not found to affect the leaf shape. Generally, from the results it can be presumed that the linear nature of the leaves of the inoculated plants is an indication of the efficiency of inoculated *Azospirillum*.

The experiment showed that commercial *Azospirillum* inoculation was effective in significantly increasing collar girth (9.2 mm, A_2M_0) when applied alone but the efficiency was reduced when it was combined with the application of native VA mycorrhiza (A_2M_1, 8.23 mm). Dual inoculation of native *Azospirillum* and native VA mycorrhiza (A_1M_1, 9.1 mm) was equally effective as the application of commercial *Azospirillum* alone in improving the collar girth of cocoa seedlings. This proved the superiority of native isolates of both the microorganisms in improving the collar girth of cocoa seedlings.

Native *Azospirillum* was found to be the best treatment in improving the fresh weight of plant top to a maximum of 75.43 g. When commercial *Azospirillum* was inoculated along with VA mycorrhiza, fresh weight was seen to be reduced slightly. The different treatments and their combinations affected the dry matter content of the plant differently. It has shown that mycorrhiza application reduced the moisture content of the plant and make them more sturdy than succulent. In commercial mycorrhiza inoculated plants, moisture content was less than in control. Fresh weight of roots was the maximum in plants inoculated with native *Azospirillum* (25.64 g). Native VA mycorrhizal inoculation recorded the maximum fresh weight of root. Synergistic effect was also observed in improving the fresh weight of roots. Dry weight of root also followed a similar trend.

It was found that native mycorrhiza was better in improving nitrogen content of the plant (2.43 per cent) than the commercial VA mycorrhiza. But commercial *Azospirillum* (A_2, 2.55 per cent) was better than native in improving the nitrogen content. The maximum phosphorus content was seen in plants inoculated with native VA mycorrhiza (0.16 per cent). There was no synergistic effect. There was synergistic effect as far as potassium absorption was concerned. All the treatments and their combinations improved the potassium content of the plant. Native VA mycorrhizal inoculation was found to give maximum infection percentage of 70.17. Synergistic effect was also seen. Maximum VA mycorrhizal infection was found in the combination A_1M_1 (77.75).

The second experiment emphasised the control of cocoa seedling blight disease by the application of local isolates of VA mycorrhiza and *Azospirillum*. In *Azospirillum* inoculated plants, the disease index was found to increase slightly by 0.33. Synergistic effect was noticed in controlling the disease by dual inoculation. In dual inoculated plants, the reduction in disease index was 0.77. Another observation was that the disease reduction by dual inoculation was higher than that was achieved by the application of fungicide potassium phosphonate. Among the fungicide tested, application of 0.3 per cent potassium phosphonate was found to be the best.

The whole experiment revealed that the native strain of *Azospirillum* and VA mycorrhiza performed better over commercial strains in improving the growth and establishment of cocoa seedlings and their combined inoculation had a synergistic effect in many growth parameters. For control of seedling blight disease dual inoculation of VA mycorrhiza and *Azospirillum* was proved to be efficient.

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* Originals not seen

APPENDICES

APPENDIX-I

Composition of Nitrogen free bromothymol blue (NFb medium) (semisolid malate medium) (Dobereiner *et al.*, 1976)

Malic acid	5.0 g
KOH	4.0 g
K_2HPO_4	0.5 g
$FeSO_4.7H_2O$	0.5 g
$MnSO_4.H_2O$	0.01 g
$MgSO_4.7H_2O$	0.1 g
NaCl	0.02 g
$CaCl_2$	0.01 g
Na_2MoO_4	0.002 g
Agar	1.75 g
Distilled water	1000 ml
Bromothymol blue (0.5 per cent alcoholic solution)	2.0 ml
pH	7.0.

APPENDIX-II

Composition of Okon's medium (Okon *et al.*, 1977) as modified by Kumari *et al.* (1980).

a)	K_2HPO_4	6.0 g
	KH_2PO_4	4.0 g
	Distilled water	500.0 ml
b)	$MgSO_4.7H_2O$	0.2 g
	NaCl	0.1 g
	$CaCl_2$	0.02 g
	NH_4Cl	1.0 g
	Malic acid	5.0 g
	NaOH	3.0 g
	Yeast extract	0.05 g
	Na_2MoO_4	0.002 g
	$MnSO_4.H_2O$	0.001 g
	H_3BO_3	0.0014 g
	$Cu(NO_3)_2$	0.0004 g
	$ZnSO_4$	0.0021 g
	$FeCl_3$	0.002 g
	Agar	15.0 g
	Distilled water	500.0 ml
c)	Bromothymol blue (0.5 per cent alcoholic solution)	2.0 ml
	pH	7

APPENDIX-III

Composition of Rojo Congo (RC) medium (Caceres, 1982)

Malic acid	5.0 g
KOH	4.8 g
K_2HPO_4	0.5 g
$MgSO_4.7H_2O$	0.2 g
NaCl	0.1 g
Yeast extract	0.5 g
$FeCl_3.6H_2O$	0.15 g
Agar	20.0 g
Distilled water	1000.0 ml
Congo Red 1:400 aqueous solution)	15.0 ml
pH	7

APPENDIX-IV

Composition of Potato Influsion Agar

Potato	200.0 g
Malic acid	2.5 g
KOH	2.0 g
Sucrose	2.5 g
Agar	15.0 g
Vitamin solution	1.0 ml
Distilled water	1000.0 ml
pH	6.8

(Vitamin solution prepared by dissolving 10 mg biotin and 20 mg pyridoxin in 100 ml sterile distilled water).

APPENDIX-V

Composition of F.A.A.

Formalin (40%)	- 5 ml
Glacial acetic acid	- 5 ml
Ethanol (95%)	- 90 ml

APPENDIX-VI

Composition of Potato Dextrose Agar

Potato	- 200 g
Dextrose	- 20 g
Agar	- 20 g
Distilled water	- 1000 ml

EFFECT OF AZOSPIRILLUM AND VA MYCORRHIZA ON THE GROWTH OF COCOA SEEDLINGS AND INCIDENCE OF SEEDLING BLIGHT DISEASE

By

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ABSTRACT OF THE THESIS

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ABSTRACT

An experiment was conducted at the College of Horticulture, Vellanikkara on the "effect of *Azospirillum* and VA mycorrhiza on the growth of cocoa seedlings and incidence of seedling blight disease", during 1998-2000. The experiment was conducted using native isolates and commercial cultures of *Azospirillum* and VA mycorrhiza. The objective of the experiment was to find out whether there is any synergistic effect for the combined inoculation of *Azospirillum* and VA mycorrhiza in improving the growth and establishment of cocoa seedlings and to find out the efficacy of the inoculants in imparting resistance to seedling blight disease.

The first experiment showed that there was a positive influence for both VA mycorrhiza and *Azospirillum* in improving the growth parameters. The height and number of leaves were maximum in native *Azospirillum* inoculated plants. Native mycorrhizal inoculation recorded maximum height of plant while the number of leaves was maximum in plants inoculated with commercial VA mycorrhiza inoculant. Combined inoculation of native *Azospirillum* and VA mycorrhiza ranked only second but it was on par with the best treatment combination. The native isolates of both the inoculants and their combination was found to increase the height to the maximum. Maximum number of leaves was noted in plants inoculated with commercial VA mycorrhiza. VA mycorrhizal inoculation did not influence the number of leaves. The results on the leaf length

breadth ratio revealed that *Azospirillum* inoculation could influence the shape of leaves. It could be presumed that the linear nature of leaves is an indication on the efficiency of inoculated *Azospirillum*. Eventhough commercial *Azospirillum* was effective in improving the collar girth, the efficiency was slightly reduced when it was combined with native VA mycorrhiza. Commercial *Azospirillum* inoculation and the combination of native *Azospirillum* and native VA mycorrhiza were equally effective in improving the collar girth of cocoa seedlings to the maximum. The different treatments and their combinations affected the dry matter of the plant differently. VA mycorrhizal application was found to reduce the moisture content of the plant and make the plant more sturdy than succulent. This may be a factor that contributes to disease resistance of mycorrhizal plants. Due to the various treatments the moisture content of roots did not vary much.

Native mycorrhiza was found better in improving nitrogen content of the plant compared to commercial mycorrhiza. But commercial *Azospirillum* was better than native *Azospirillum* in improving the nitrogen content of the plant. Absorption of phosphorus was maximum (0.16%) in native VA mycorrhizal inoculated plants. Both the mycorrhizal inoculants were found to increase the phosphorus content of the plants. *Azospirillum* inoculation did not affect the phosphorus content of the plants. Dual inoculation of commercial cultures of both the organisms was found equally effective with native *Azospirillum* application alone in potassium absorption. VAM infection percentage was also maximum in

native VA mycorrhizal inoculated plants. Combined inoculation of native isolates of both the organisms influenced the mycorrhizal infection positively.

Out of the ten parameters observed, the native *Azospirillum* isolate A_1 ranked first in nine of the parameters. Similarly native mycorrhizal isolate M_1 showed superiority in seven of the parameters tested proving the superiority of native isolates over commercial inoculants.

In controlling the seedling blight disease of cocoa, it was found that dual inoculated plants showed a reduction in disease index by 0.77. In VA mycorrhiza treated plants reduction in disease index was 0.26. In *Azospirillum* treated plants there was a slight increase in disease index by 0.33. The disease reduction by dual inoculation was higher than that was achieved by the application of the fungicide potassium phosphonate. Among fungicides tested application of potassium phosphonate 0.3 per cent was better in controlling the seedling blight disease.