# SELECTION OF EFFICIENT VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI FOR INOCULATING *Acacia holosericea*, *Albizzia lebbeck* and *Tectona grandis*

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*Affectionately Dedicated to*

*My Beloved Parents*

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
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## CERTIFICATE

This is to certify that the thesis entitled "**SELECTION OF EFFICIENT VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI FOR INOCULATING *Acacia holosericea*, *Albizzia lebbeck* AND *Tectona grandis***" submitted by **Mr. KANAGAVEL RAJAN, S.** in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **AGRICULTURAL MICROBIOLOGY** to the University of Agricultural Sciences, Bangalore is a record of the research work carried out by him, under my guidance and supervision and that no part of the thesis has been submitted for the award of any degree, diploma, associateship, fellowship or similar titles.

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(S.KANAGAVEL RAJAN)

## CONTENTS

## List of tables

## List of figures

## LIST OF PLATES

# INTRODUCTION

# INTRODUCTION

Largely as a consequence of growing human and animal population there has been, world wide, an increase in deforestation and severe land degradation. Over centuries, cleaning of forests for fuel, farming and grazing has destroyed most of the vegetation. Excessive exploitation of the land has often led to desertification, a process that is being accelerated by the demand for scarce wood resources. In India alone the total desertified area is around 150 million hectares.

The consequence of acute shortage of food and fuel wood supply are well known. These necessitate intensive counter measures among which tree planting programmes are in the fore front, both for environmental protection and enhancement on the one hand and for renewable energy supplies, and other useful products like timber, resins, medicinal products, etc., on the other. Since the current annual rate of afforestation in India is increasing, a massive increase in planting activity is required before the turn of the century, much of it with species or in areas that are not traditionally planted (Burley, 1985).

Further, the high importance of these additional roles such as timber and fuel wood, of forests and trees has been recognized in the forestry sector policies of FAO, World

Bank, Asian Development Bank and other International and Bilateral Assistance Agencies (World Bank, 1978). Emphasis in National Development Programmes has changed from industrial plantation forestry towards local community development and this has been paralleled by a need for increased research (FAO, 1978). The priorities for such research were described by World Bank and FAO (1981) in a paper that was approved by the 17th Congress of International Union of Forestry Research Organization (IUFRO, 1982). Among the research priorities discussed, "Mycorrhizal and other Biological Relations", figured as an important top priority research. Tree species like Acacia holosericea, known for fodder and fuel wood production; Albizzia lebbeck and Tectona Grandis for high quality wood are given much importance in afforestation programmes now-a-days.

It is a well known fact that vesicular-arbuscular mycorrhizal (VAM) fungi play an important role in the uptake of mineral nutrients such as P and by providing other growth promoting substances. So far VAM fungal species are considered not to have any specificity towards different taxa of potential hosts. But recent studies indicate the existence of 'preferential associations' between a host and a fungus. Hence it is necessary to select a most suitable and efficient VAM fungus for individual tree species, to get

healthy, vigorously growing seedlings in the nursery. Acacia holosericea and Albizzia lebbeck are two important tree species used in afforestation programmes. These two species are mainly used as fuel wood and timber. Tectona grandis is an important tree species grown for its timber used for making furnitures. Hence a study was taken up to screen and select suitable and efficient VAM fungi for inoculations Acacia holosericea, Albizzia lebbeck and Tectona grandis in the nursery.

# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

## General account of Mycorrhiza

Botanically "mycorrhiza" is the mutualistic symbiosis between soil-borne fungi and roots of higher plants. The word mycorrhiza (fungus root, from the Greek: mykes (mushroom) and rhiza (root) was coined by Frank (1885)) to describe the union of a plant root and a fungus to form a single, morphological organ, in which the plant nourishes the fungus and the fungus the plant.

Two main types of mycorrhiza are distinguished today. They are ectomycorrhiza and endomycorrhiza. In ectomycorrhiza the fungus grows intercellularly in the cortex of the plant's roots (called as Hartig net), but never intracellurlarly. In endomycorrhiza the fungus grows inter and intracellularly and forms within the cortical cells specific fungal structures (Harley and Smith, 1983). Vesicular-arbuscular mycorrhiza (VAM), which comes under endomycorrhiza, is the most important and most widely distributed type of mycorrhiza. Since they form arbuscules (haustoria) like structures) and vesicles inside the root system, they are called vesicular-arbuscular mycorrhiza.

The mycorrhizal fungus is best considered as a far-reaching extension of the root system. A fine network of fungus hyphae explores and extracts nutrients from a volume

of soil far beyond the bounds of the root's capabilities. Many of these nutrients are translocated through the hyphal network to the mycorrhizae, where they are released to the roots for host utilization. In exchange, the host serves as primary energy source for the fungus, providing simple sugars and possibly other compounds derived from photosynthates.

**Occurrence and distribution of VAM fungi**

The VAM association is found in most plant families, so far examined, although it may be rare or absent in families such as Cruciferae, Chenopodiaceae, Caryophyllaceae and Cyperaceae (Hirrel et al., 1978). In addition to the widespread distribution of VAM throughout the plant kingdom, the association is geographically ubiquitous and occurs in plants growing in arctic, temperate and tropical regions. VAM occur over a broad ecological range from aquatic to desert environments (Mosse et al., 1981).

Fossil records suggest their presence in subterranean parts of the earlier land plants. Infact, VAM fungi are believed to have occured in 'Gondwanaland' before the continental drift, 125 million years ago (Trappe, 1977).

The first extensive survey of the occurrence of mycorrhizal associations in tropical plants was carried out by Janse (1896) in Java. He studied the Bryophytes,

vascular cryptograms, gymnosperms, monocotyledons and 38 species of woody dicotyledons. He found that 69 of the 75 species so examined, had characteristic endotrophic mycorrhizal associations. Despite this promising start, further interest was rather sporadic and the next entensive survey on the occurrence of mycorrhizal associations was made fifty years later by Johnston (1949). He examined 93 species, including 13 species of forest trees and observed that 80, including all the forest trees, possessed endotropic mycorrhizal associations.

After this period the informations on the occurrence of VAM were numerous and extensive. Redhead (1968) investigated the incidence of mycorrhizal associations in 51 tree species, indigenous to the lowland rain forests of Nigeria and also in 15 exotic tree species. All the exotic species and 44 of the indigenous species were found to have VAM association. Edmisten (1970) reported out of 32 principal forest species in Puerto Rico, 17 had VA mycorrhizal colonization. Shamsuddin (1979) reported that out of about 200 species of Malaysian forest trees, only one lacked mycorrhiza.

In India, Thaper and Khan (1985) assayed forest soils for occurrence and frequency of VA mycorrhizal fungi in 21 locations in 8 states in India. They reported 15 species belonging to 4 genera of Endogonaceae, i.e., Acaulospora,

Gigaspora, Glomus and Sclerocystis. Spores of Glomus macrocarpum were widely distributed followed by Sclerocystis spp., Gigaspora gigantea and Acaulospora sp. Moreover, new forest soils represented maximum number of species of Endogonaceae.

Sharma et al., (1986) in his report stated that all the tree species investigated in sub-tropical forest ecosystem of North India, were found to be mycorrhizal. The same authors reported in 1987 that tree species of Crypomeria japonica and Alnus mepalensis harboured Sclerocystis microcarpus and Gigaspora gregaria.

In a survey of 68 hardwood forest tree species, Brundrett and Kendrick (1987) reported that of the 68 species of plants examined, 71% were highly mycorrhizal, 9% had low level of VAM and 3% were non-mycorrhizal. In an another study conducted at Kerala, the highest colonizations was recorded in rubber (71.9%) followed by bread fruit (70.6%), coconut (62%), eucalyptus (59.4%) and sugar maple (19.9%) (Nair and Girija, 1988).

In a survey of 53 plant species from 30 families growing along the Madras Coast of India, most plants harboured VAM fungi. The dominant species were Glomus spp., and Entrophospora schenkii (Mohan Kumar et al., 1988).

increased the size of bacterial nodules and the proportion of nodulated plants among the leguminous plants.

Kormarik et al., (1982) tested eight hardwood tree species for its response to VAM. They observed that VAM inoculation increased the stem weight of seedlings by 2-80 fold over non-mycorrhizal controls, while the root weight of all the seedling were increased by 4-70 fold. In a similar experiment Furlan et al., (1983) observed that the Glomus sp. inoculted white ash tree seedlings grew better compared to uninoculated plants.

The effect of season on mycorrhizal colonization and sporulation by native mycorrhizal fungi was studied in two perennial tree hosts, viz., mango and leucaena. The results showed that maximum colonization and sporulation occured during winter (Nov. to Jan.) months. Summer months (Apr. to Jun.) were unfavourable for the proliferation of VAM in tropics. They also observed a positive correlation between relative humidity and mycorrhizal activity, but a negative correlation between temperature and mycorrhizal activity (Harinikumar and Bagyaraj, 1988).

Cruz et al., (1988) studied the effectiveness of four VAM fungi and Rhizobium in promoting the growth of three legumes in a P-deficient soil. They observed Glomus fasciculatum + Rhizobium were most effective for Acacia

Kiran Bala et al., (1989) examined 17 different plant species such as Acacia aneura, Azaradirachta indica, Bauhinia racemosa, Prosopis julifera, etc., and reported more than 50% root colonization by VAM fungi. The common fungal genera found were Glomus and Gigaspora.

Paredes and Leano (1993) examined the roots of Citrus mitis, Achras zapota, Sandoricum koetjape and Mangifera indica and found that VAM colonization was most abundant in these fruit trees.

Byra Reddy et al. (1994) in their studies reported the colonization of VA mycorrhizal fungi in 59 forest tree species belonging to 22 families. The intensity of colonization was high in 4 species, moderate in 23 species and low in 32 species.

**VAM fungi and tree growth**

The beneficial effects of inoculating forest tree nurseries with VAM fungi to improve plant growth are well known (Janos, 1983; Jeffries, 1987; Young, 1990; Bagyaraj, 1993).

Janos (1980) experimented 28 forestry species from a low land tropical rainforest region. Mycorrhizal inoculation of these forestry species improved the survival and seedling growth of 23 out of 28 species. VAM also

mangium and Scutellospora persica + Rhizobium for Albizzia falcataria and Glomus fasciculatum + Rhizobium for Acacia auriculiformis. Further, consistantly poor growth by uninoculated seedlings were observed. In an another experiment, it was observed that out of seven VAM fungi screened, a local isolate of Glomus mosseae was the best symbiont for Hawaiian giant cultivars of Leucaena leucocephala (Bagyaraj et al., 1989).

Jasper et al., (1989) recorded that the shoot dry weight of Acaulospora concurrens was increased by inoculating Glomus sp., Glomus fasciculatum, Scutellospora calospora and Acaulospora laevis. The dry weight of shoot increased up to 4 times in inoculated plants. The authors explained the increase in dry weight of shoot due to increased P uptake. Also the various mycorrhizal species differed in their ability to increase plant growth.

The effects of VAM inoculation on growth of cacao seedlings grown for 5 months in a nursery were studied by Cuenca et al., (1990). The results showed that cacao seedling responded well to indigenous VAM fungus, Scutellospora calospora. The fungus increased the plant height, dry weight, foliar uptake of P, Cu and Zn significantly in relation to the uninoculated control.

Michelsen and Rosendahl (1990) studied the effect of VAM fungi on growth and drought resistance of Acacia nilotica and Leucaena leucocephala seedlings with P fertilizer application for 12 weeks. The results showed that Acacia nilotica was more drought resistant than Leucaena leucocephala and VAM inoculation significantly increased the nodulation.

High levels of soluble phosphate applied with VAM fungi caused depressions at controlled condition in citrus plants (Antunes and Cardoso, 1991). The results showed significant increase in drymatter yields, and K contents due to VAM inoculation. But the increase was observed only at 0 and 50 PPM soluble P levels.

Reena and Bagyaraj (1990a) screened VAM fungi for selecting efficient inoculant fungi for two slow growing forest tree species, Acacia nilotica and Calliandra calothrysus. Out of the 13 VAM fungi tested, Glomus mosseae was found to be the best fungus for Acacia nilotica and Glomus velum for Calliandra calothrysus. In an another similar experiment the same authors (1990b) screened and selected an efficient fungus, Glomus margarita for inoculating Tamarindus indica. Notably the effect of VAM fungi differ in their efficiency and show specificity for various plant species (Jasper, 1989).

In a greenhouse experiment using unsterilized soil, inoculation with mixed species of Scutellospora and Glomus resulted in high drymatter, and stem diameter in cacao seedlings. Increase in P and Ca content of the shoot was also observed (Chulan and Martin, 1992). The authors also reported the dependency of cacao seedlings to VAM inoculation. In another study the growth response of rubber (Hevea brasiliensis) to VAM inoculation was assessed in two different sites containing indigenous mycorrhizal fungi. The seedling root stocks were inoculated with mixed VAM fungi belonging to the genus Glomus. The VAM inoculation increased the shoot drymatter up to 70% compared to the uninoculated control in the first site, whereas the shoot drymatter increase was only 29% in the second site (Ikram et al., 1992). This suggests the importance of indigenous mycorrhizal fungi on plant growth.

**Uptake of phosphorus and other mineral elements**

The beneficial effect of VAM on plant growth has mostly been attributed to an increase in the uptake of nutrients, especially phosphorus (Smith, 1980; Hetrick, 1989). Mycorrhizal fungi in association with plant roots seem likely to increase P uptake by more through exploration of soil volume thereby making 'positionally unavailable' nutrients 'available'. This is achieved by decreasing the

distance and increasing the surface area for absorption (Tinker, 1978).

Phosphorus in soil solution has been shown to reach plant roots mainly by the diffusion process. Sanders and Tinker (1973) concluded that the extensive hyphal growth of mycorrhiza effectively reduces the distance for diffusion and thereby increases the uptake. Hattingh et al., (1973) found that VAM fungal hyphae could intercept labelled P placed 27 cm from a mycorrhizal root, whereas it remained unavailable to non-mycorrhizal roots. Similarly when onion roots were prevented from entering the soil zone in which $^{32}P$ had been placed, Rhodes and Gerdemann (1975) observed that only mycorrhizal plants contained radio activity.

Owusu-Bennoah and Wild (1980) showed that the radius of the depletion zone for P around mycorrhizal onion roots was twice that of non-mycorrhizal roots. Calculations of Gerdemann (1968) estimated that mycorrhiza would increase P uptake 60 fold where diffusion limits the uptake, whereas the diffusion is not limiting uptake.

It has been observed that greater responses to mycorrhizal infection occured in coarse-rooted plant species than in fine rooted species (Crush, 1973; Hall, 1977), in high P adsorbing soil than in low P adsorbing soil (Sainz

and Arines, 1988) and in soils than in solution cultures (Howler, et al., 1982).

Experiments with phosphate labelled with $^{32}P$ indicated that the hyphae of VAM fungi obtain their extra phosphate from labile pool rather than by dissolving insoluble phosphate (Raj et al., 1981). Further, mycorrhizal roots on a unit weight basis absorbed much higher amounts of P than did non-mycorrhizal plants both in solution cultures (Cress et al, 1979) and in soils (Bolan et al., 1987). This suggests that mycorrhizal fungus hyphae have higher affinity for phosphate ions and lower threshold concentration for absorption than do plant roots.

In soils low in available P, mycorrhizal plants had higher rates of growth than non-mycorrhizal plants and increased concentrations of total P in tissues in early stages of plant development. The flow of P from soil into the roots of mycorrhizal plants is faster than into non-mycorrhizal plants (Smith, 1980; Hale and Sanders, 1982; Sanders and Tinker, 1983). The mechanism underlying the increased rate of uptake of P is the efficiency with which mycorrhizal roots exploit soil profile, with hyphae extending beyond the depletion zone surrounding the absorbing root and its root hairs (Tinker, 1975; Clarkson, 1985).

Bagyaraj and Manjunath (1980) observed that crops like cowpea, cotton and finger millet when inoculated with the VAM fungus Glomus fasciculatum, had higher phosphorus and zinc content. But inoculated plants did not differ significantly in magnesium content for uninoculated plants. Similarly, VAM fungi helped in the uptake of potassium, magnesium, copper, iron and manganese ions including phosphorus and zinc ions in groundnut (Krishna and Bagyaraj, 1984).

Pan and Cheng (1988) in a pot experiment with Leucaena leucocephala reported that VAM inoculated seedlings had significantly higher dry weight, nutrient uptake (N, P, K, Ca, Mg, Mn and Zn) compared to the control plants.

Manjunath et al., (1989) reported that mycorrhizal inoculation with Glomus aggregatum along with rock phosphate, significantly increased the uptake of phosphorus, copper, zinc and drymatter yield of Leucaena leucocephala plants. Faber et al., (1990) observed that with no supplemental Zn, mycorrhizal treatments had greater growth and zinc concentration than non-mycorrhizal treatments in corn. But there was no indication of nutrient interaction between Zn and P. Similarly citrus seedlings inoculated with Glomus etunicatum, had significantly increased plant P and K contents as well as drymatter yields.

Jayachandran et al, (1992) observed the plant growth and P uptake by non-mycorrhizal plants were improved by glycerophosphate and AMP, but amendment with phytic acid, RNA, ATP or CMP did not affect plant growth. In contrast, mycorrhizal plants benefitted from amendments with all the P sources, suggesting the capacity of VAM to mineralize organic phosphorus sources.

Rough lemon (Citrus jambhini hush) and Trifoliate orange (Poncirus trifoliata (L.), Raf.) inoculated with VAM showed higher shoot iron (Fe) concentration in comparison with non-mycorrhizal plants, suggesting the role by mycorrhiza in host Fe nutrition (Treeby, 1992).

**Uptake of Nitrogen**

The studies on the nitrogen uptake of VAM has received relatively little attention until recently, because elevated concentrations of N in tissues of mycorrhizal plants have rarely been observed, except in legumes in which indirect mycorrhizal effects on nodulation and $N_2$ fixation are involved (Smith et al., 1986).

Owusu-Bennoah and Mosse (1979) reported that mycorrhizal infection levels in lettuce decreased with increasing application levels of $NaNO_3$, $NH_4NO_3$, $(NH_4)_2SO_4$ and $Ca(NO_3)_2$. Brown et al., 1981 reported that application

of nitrate or ammonium level resulted in high levels of root colonization in sweet gum.

Chambers et al., (1980) found that mycorrhizal colonization reduced with application of high concentrations of inorganic nitrogen compound and this was generally greater with $NH_4^+$ than $NO_3^-$. This reduction in infection level was attributed to inhibition of pre-infection phase of the fungus. Many workes have reported increased fixation and plant growth by VAM (Manjunath and Bagyaraj, 1984; Barea et al., 1991; Azcon and Barea, 1992).

Harnel et al., (1991) observed that high $^{15}N$ transfer between mycorrhizal plants was not only associated with high mycelium density in soil but also with low soil microbial carbon, suggesting that the effect of mycorrhizal fungi (Glomus spp.) on soil microbial populations may be an important factor affecting N transfer between mycorrhizal plants.

Azcon and Barea (1992) reported that when the legume Medicago sativa L. was inoculated with both Rhizobium meliloti and VAM, the P and N concentrations were higher in VAM inoculated treatments than in uninoculated controls (or) Rhizobium alone inoculated treatment. This suggests a possible role of VAM in N uptake. Similarly Niemi and Eklund (1988) reported the increase in $N_2$ fixation and N content of two legume plants due to VAM inoculation.

**Water relations**

Moisture stress in plant is caused when absorption of water lags behind transpiration. Severe water stress causes cessation of growth and photosynthesis, disturbances of metabolic processes and finally death (Kramer, 1988).

Sieverding (1981) reported that the favourable effect of mycorrhiza on plant growth was more pronounced in the drier soil water regimes than wet normal regimes, particularly under conditions of restricted phosphorus uptake by plant roots and with less soil phosphorus available conditions. Nelson and Safir (1982) observed a greater fresh weight and dry weight of onion plants in pots which were colonized by Glomus etunicatum when exposed to several periods of soil water stress separated by periods of high water supply than non VAM onion plants. They attributed the better drought tolerance to better phosphorus nutrition.

Sieverding (1983) reported that shoot drymatter of Eupoterium oderatum fertilized with soluble (or) insoluble phosphate and inoculated with Glomus macrocarpum was higher than that of non-mycorrhizal plants fertilized with soluble phosphate. In driest condition the growth of mycorrhizal plants fertilized with insoluble phosphate was similar to that of non-mycorrhizal plants. The authors concluded that the better water utilization by mycorrhizal plants under

poor supply was the reason for better growth. Similarly Ellis et al., (1985) reported that Glomus fasciculatum inoculated wheat plants had greater leaf area, total plant and root weight than non-mycorrhizal plants when harvested one week after one severe stress period.

Kwapatha and Hall (1985) reported that in pot culture experiments mycorrhizal cowpea had greater drymatter and pod yield under limiting conditions of moisture and P. Further, mycorrhizal plant growth enhancement was positively correlated with soil water content at -1.5 M pa. They concluded that soil water status and mycorrhizal conditions interact in influencing plant growth.

Bethlenfalvay et al., (1987) found that increasing water stress enhanced that amount of extra matrical hyphae, and that VAM plants had lower permanent wilting point compared to non VAM plants. This lead to the conclusion that VAM plant roots have access to soil water which is not available to non VAM plants.

Bethlenfalvay et al., (1988) found that dry weights of soybean plants infected with Glomus mosseae were greater at severe stress than those of non-inoculated VAM plants. Growth enhancement of severely stressed VAM plants was attributed to increased uptake of water and P.

Michelsen and Rosendahl (1990) studied the effect of VAM on growth and drought resistance of Acacia nilotica and Leucaena leucocephala seedlings. They observed the differences between the plant species with respect to growth improvements caused by VAM inoculation and/or phosphorus fertilization under drought stress conditions. They hypothesized that VAM mediated drought resistance is purely a nutritional process, as no significant differences in the reduction of plant dry weight resulting from drought stress were found between phophorus fertilized and VAM inoculated plants.

Osonubi et al., (1991) in their experiments found that mycorrhizal inoculation of Albizzia lebbeck, Gliricidia sepium, Leucaena leucocephala and Acacia auriculiformis seedlings stimulated greater plant growth and drought tolerance. The drought tolerance was attributed to the improved accumulation of nutrients, especially phosphorus.

**VAM on plant growth**

Beneficial effects of VA mycorrhizal inoculation on a large number of plant species have already been well demonstrated (Mosse, 1981; Jeffries, 1987; Sieverding, 1991; Weber et al., 1993).

Janos (1980) in his experiments with plants grown in pots observed that VAM inoculation increased the seedling

growth of 23 out of 28 species from a low land tropical rain forest region. Mycorrhiza also increased the size of bacterial nodules and the proportion of nodulated plants among three leguminous species studied.

Plenchette et al., (1981) reported that the shoot length, leaf surface, stem diameter, root volume and dry weight of apple seedlings were significantly greater in the VAM inoculated treatment than the fertilized or unfertilized controls. But they did not observe any significant difference in foliar mineral content.

Furlan, et al., (1983) reported that the white ash seedling inoculated with VAM differed in their growth from control plants. They also observed that the growth differed between each fungal species. Similarly Lamar and Davery (1988) observed significant increase in height, root collar diameter, dry matter yield and P nutrition in VAM inoculated green ash seedlings compared to control plants.

Pan and Cheng (1988) observed that inoculating the legume tree Leucaena leucocephala with Glomus aggregatum increased the height, stem diameter, and nutrient uptake significantly compared to the uninoculated control plants. In another green house study, seedlings of sweetgum (Liquidambar styraciflua) and yellow poplar showed increased

growth when inoculated with Glomus macrocarpum and Glomus fasciculatum.

Bagyaraj et al., (1989) screened seven VAM fungi for its symbiotic response with four Hawaiian giant cultivars, $K_2$, $K_{28}$, $K_{67}$ and $K_{72}$ of Leucacna leucocephala. In their study they observed that inoculation significantly increased the drymatter and P content of plants in all the cultivars.

In a green house experiment with groundnut, Bell et al., (1989) observed both P and Zn nutrition are affected by VAM activity, but the dominant role of VAM was the uptake of P. Mycorrhizal plants responded to increasing P rate, attaining maximum drymatter at 120 Kg P $ha^{-1}$ in sterilized soil and at 30 kg P $ha^{-1}$ in non-sterile soil.

Henkel et al., (1989) in their experiment observed that inoculation of Agropyron smithii increased the shoot biomass and root infection when inoculated with indigenous VAM fungi. While the VAM fungi collected from a different site showed reduced activity.

Michelson and Rosendahl (1990) studied the effect of VAM fungi on growth and drought resistance of Acaia nilotica and Leucaena leucocephala seedlings in a glass house experiment. They observed the growth promoting effect of VAM fungi equalled the effect of phosphorus fertilization aftr 12 weeks. Differences between the two plant species

with respect to growth improvement due to inoculation and or phosphorus fertilization was also noticed.

In a similar study Acacia auriculiformis and Leucaena leucocephala were inoculated separately with Gigaspora margarita, Scutellospora persica and Sclerocystis clavispora in an autoclaved P-deficient soil. Among the three fungi it was observed G. margarita and S. persica were equally effective in promoting growth of both the trees. The height, stem diameter, and drymatter yield of both hosts inoculated with these two fungi were significantly greater than the uninoculated seedlings (Aggangan and Dela Cruz, 1991).

Osonubi et al., (1991) experimented with Acacia auriculiformis, Albizzia lebbeck, Gliricidia sepium and Leucaena leucocephala by inoculating with VAM fungal mixture consisting two Glomus and one Acaulospora species. The results showed that mycorrhizal inoculation stimulated greater plant growth, P and N uptake compared to the non-mycorrhizal plants in a low P soil.

**Interaction between beneficial soil organisms and VAM fungi**

The growth promoting effect of inoculation with beneficial soil micro organisms, VAM fungi alone or dual inoculation with both the symbionts is well documented

(Gianinazzi - Pearson and Diem, 1982; Gardner, 1986; Vejsadova et al., 1992; Sreenivasa and Krishnaraj, 1992).

Dual inoculation with Glomus fasciculatum and Rhizobium japonicum to soybeans significantly increased dry weight and nitrogen content of root (Bagyaraj et al, 1979) and total dryweight, nodule dryweight, nitrogenase and nitrate reductase activities (Carling et al., 1978).

Brown and Carr (1984) reported the effect of simultaneous inoculation of roots of lettuce seedlings with VAM endophytes and Azotobacter chroococcum. Dual inoculation increased yield of lettuce plants. Likewise, the beneficial effect of VAM was seen with Azospirillum brasilense (Padmavathi, 1992).

Roskoski et al., (1986) observed Rhizobium sp. and VAM endophytes, when dually inoculated, improved the growth of Acacia pennatula and Leucaena leucocephala significantly. Similarly, Dela Cruz et al., (1988) studied the effectiveness of four VA mycorrhizal fungi and Rhizobium in promoting growth of three legume tress viz., Acacia mangium, Albizzia falcataria, and Glomus fasciculatum + Rhizobium was most effective for Acacia mangium; Gigaspora margarita + Rhizobium was most effective for Albizzia aurculiformis. This study suggests the possible specificity among VAM fungi for various hosts.

Pot experiments were carried out by Young (1990) to determine the effects of single and mixed inoculations with phosphorus solubilizing bacteria and VAM fungi on the growth of Leucaena leucocephala, Acacia confusa, Acacia mangium and Liquidamber formosana in three subtropical soils. A synergistic effect of mixed inoculation on the growth was observed for all the three species. Also the results showed the beneficial effect to dual inoculation in soils with high unavailable phosphorus.

Azcon et al., (1990) reported that soil micro organisms, both bacteria and fungi help in the root colonization of VAM fungus Glomus mosseae. Similarly beneficial interaction of VAM fungi with soil rhizosphere bacteria was seen in oats (Will and Sylvia, 1990).

Similar to the interaction with Rhizobium, VAM fungi have a positive effect on $N^2$ fixing association between actinomycetes (Frankia) and non-legeminous plant species (Gardner, 1986; Fragga-Beddiar and Le-Tacon, 1990).

Vejsadova et al., (1992) studied the effect of two Bradyrhizobium japonicum strains and the effect of VAM on biomass production in soybean plants in a low P and N soil. The greatest nitrogenase activity, growth and yield occured in the presence of both the symbionts.

**Interaction between harmful microorganisms and VAM fungi**

The information on the control of root and shoot diseases by VAM fungi are contradictory (Bagyaraj, 1984). The mechanisms by which VAM fungi control pathogens are related to changes in the morphology or physiology of the host plant. Morphological alterations were reported to the lignification of cell walls, the production of other polysaccharides, and increased vascular systems of plant (Graham and Syvertsen, 1984).

Krishna and Bagyaraj (1983) studied the interaction between Glomus fasciculatum and the root pathogen Sclerotium rolfsii in groundnut. The results showed that the VAM fungus effectively reduced the number of sclerotia produced by the pathogen. So the VAM inoculated plants showed higher growth. Similarly, roots of sweet orange seedlings inoculated with Glomus fasciculatum and Phytophthora parasitica were more healthier and had higher biomass than that of roots infected with the pathogen alone (Davis and Menge, 1980).

Zambolim and Schenck, (1983) showed dual inoculation of soybean with the pathogen Rhizoctonia solani or Macrophomina phaseolina or Fusarium solani and the VAM fungus Glomus mosseae resulted in reduced mycorrhizal colonization. But the survival of plants was high in dual inoculated plants

when compared to the treatment in which pathogen alone was inoculated.

Rosendahl (1985) reported that the root rot pathogen Aphanomyces enteiches infecting pea plants was suppressed by Glomus fasciculatum. The interaction did not change even when the spore load of the pathogen was increased to 30 times.

Caron et al., (1986) reported that Glomus intraradices inoculation significantly reduced the population of Fusarium oxysporum f.sp. radius lycopersici of tomato. The reduction was found at all the P concentration.

Smith et al., (1986) suggested that mycorrhizal fungi can also increase the host tolerance to the nematode, Meloidogyne incognita in field conditions.

In a greenhouse study Chhabra et al., (1989) experimented the effect of VAM fungus Glomus fasciculatum on the three pathogens Helminthosporium maydis, Fusarium moniliforme and Acremonium kiliense. Among mycorrhiza treated plants F. moniliforme infected plants remained disease-free whereas the non-mycorrhizal plants developed disease symptoms. But there was no effect of mycorrhizal treatment on the development of diseases caused by A. kiliense and H. maydis. Similarly, Sreenivasa et al., (1992) in their study observed a significant inhibition of

sclerotial bodies in the VAM, Glomus fasciculatum and Glomus macrocarpum inoculated chilli plants.

**Mycorrhizal dependency**

Mycorrhizal dependency may be defined as the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility (Gerdemann, 1975). Based on their dependency the plants can be grouped into three categories: 1) Non-mycorrhizal; 2) Facultative mycotrophs and 3) Obligate mycotrophs.

Non-mycorrhizal plants are those which grow normally without forming mycorrhiza, even under low P conditions. The growth of these plants is not affected by mycorrhizal inoculation (Janos, 1975). Species such as Brassicaceae, Chenopodiaceae, Cyperaceae, etc., do not usually form mycorrhiza (Gerdemann, 1968). Facultative mycotrophs are those which are able to grow without mycorrhiza, but in which mycorrhizal colonization improves growth (Azcon and Ocampo, 1981). Obligate mycotrophs are those which can neither grow or survive without mycorrhiza (Janos, 1980).

In experiments with plants grown in pots, Janos (1980) observed VAM increased seedling growth of 23 out of 28 species from a low land tropical rain forest region having a wide range of dependencies. He noted large seeds are

advantageous to plants that depend on VAM because they provide mineral reserves upon awaiting infection. In otherwords, species that are least dependent on mycorrhizae have light seeds and colonize disturbed habitats.

In a mycorrhizal dependency study, four hardwood tree species, viz., Fraxinus pennsylvanica, Liquidamber styraciflua, Liriodendran tulipifera, and Plantanus occidentalis were treated with six VAM fungi, viz., Glomus mosseae, Glomus fasciculatum, Glomus etunicatum, Glomus macrocarpum, Glomus epigeum and Gigaspora margarita. Among these VAM fungi, Glomus macrocarpum inoculated tree species were highly dependent (Pope et al., 1983).

Saif (1987) observed the total uptake of all elements by non-mycorrhizal legumes and uptake of phosphorus, nitrogen and potassium by forage legumes were inversely correlated with mycorrhizal dependency.

Manjunath and Habte (1991) studied the extent to which root morphological characteristics depend on mycorrhiza. Among the two plants selected for study, one was moderately to very highly dependent Leucaena species and the other was marginally to moderately dependent Sesbania species. Usually the less dependent plants are characterized by higher root parameters such as mass, density, length, root hair incidence, diameter and total uptake; though the two

species were different morphologically, they were not consistently different from each other with respect to mycorrhizal colonization, P uptake and other parameters.

A study was conducted by Brejda et al., (1993) to determine the mycorrhizal dependency of 3 warm-season grasses, viz., Andropogon gerardii, Panicum virgatum and Calmovilfa longifolia. The plants were grown in sterilized soil. The results showed that increasing levels of P fertilizer from 5.4 to 27.0 mg/pot caused an initial increase, then dramtic decrease, in percentage colonization by G. deserticola but had no effect on percentage colonization by indigenous VAM fungi. Also mycorrhiza inoculated plants had a greater number of tillers, greater shoot weight, root weight and P concentration and a lower root/shoot ratio than uninoculated plants. The authors finally concluded that all the three grasses are highly mycorrhiza dependent.

**Selection of suitable VAM strains**

Many important hardwood forest trees are hosts for fungi that form VAM. Little work has been directed towards determining whether these fungi can be manipulated to improve seedling quality in forest nurseries. However, the work that has been done so far, indicates that these fungi can not only be a significant factor in quality seedling production . (Kabre, et al., 1982; Bagyaraj et al., 1989,

Vasanthakrishnan and Bagyaraj, 1990a), but also show preferential association (Sieverding, 1991; Reena and Bagyaraj, 1990b).

Kormanik et al., (1981) reported that the sweet gum seelings when inoculated with Glomus fasciculatum, Glomus spp. or a mixture of Glomus and Gigaspora showed significant increase in plant height, leaf number, root collar diameter, and total dry weight than those of uninoculated plants. Similarly, eight hardwood forest species were grown in fumigated soil without VAM fungi or in soil infested with either Glomus fasciculatus (GF), a mixture of Glomus mosseae and Glomus etunicatus (GM), or a mixture of several fungal species in the genera Glomus and Gigaspora (GG). In this experiment, although there were no significant differences among the VAM treatments, within tree species, differences among hosts were observed in the amount of hyphae, arbuscules, and vesicles produced by the fungi, which could be attributed to growth and development characteristics among hosts and VAM fungi (Kormanik, et al., 1982).

Kabre et al., (1982) observed that mycorrhizal seedling inoculated with Glomus spp. or with a mixture of Glomus and Gigaspora showed maximum response. Among the treatments a strain of Glomus mosseae proved to be an efficient fungus for Acer pseudoplantanus seedlings.

Glomus mosseae and Gigaspora margarita inoculated Sophora secudifolia plants increased fresh and dry weight of root and shoot, while inoculation with Glomus etunicatum and Glomus fasciculatum did not enhance seedling growth significantly (Strong and Davies, 1982). Similarly Kormanik (1985) observed that inoculating black walnut seedling with Gigaspora margarita was better when compared to either inoculating with Glomus fasciculatum or Glomus macrocarpum.

Furlan et al., (1983) in their experiment with white ash seedlings, inoculated five different species of endomycorrhizal fungi. Among the species tested, dry mass remained higher only in plants inoculated with Glomus epigeum, Glomus sp. No.3, and Glomus monosporum. Also they observed significant differences in growth between each fungal species used.

Aggangan et al., (1987) observed, among the four fungi viz., Glomus etunicatum, Glomus fasciculatum, Glomus macrocarpum and Glomus mosseae screened, Glomus etunicatum to be the best fungus for Acacia auriculiformis in an infertile soil. However, the percentage infection was similar in all the treatments (59-64 %). The uninoculated control plants had only 16 % mycorrhizal colonization. On the same line, in an another study, seven VAM fungi were screened for symbiotic response with four Hawaiian giant cultivars, $K_8$, $K_{28}$, $K_{67}$ and $K_{72}$ of Leucaena leucocephala.

Of the seven fungi screened, a local isolate Glomus mosseae was found to be the best mycorrhizal fungus for inoculation of Leucaena (Bagyaraj et al., 1989). Further, Chang and Shiucheen (1990) observed Glomus epigaeum to be the best fungus for citrus seedlings.

Reena and Bagyaraj (1990a) carried out an experiment to screen and select efficient VAM fungi for two slow growing forest tree species. Acacia nilotica and Calliandra calothrysus. The seedlings were inoculated with 13 different VAM fungi, obtained from various sources around the world. They observed, Acacia nilotica seedlings responded well to inoculation with Glomus mosseae whereas Calliandra calothrysus responded well to Glomus velum and Glomus merridum. The same authors (1990b) conducted another experiment to select an efficient VAM fungus for Tamarindus indica. In this experiment, Tamarindus indica responded best to inoculation with Gigaspora margarita.

The effect of introduced VAM fungi viz., Glomus spp., Acaulospora laevis, Scutellospora calospora and indigenous VAM fungi on the growth of cacao seedlings were studied by Cuenca et al., (1990). They observed that the seedlings responded well to the indigenous fungi, which was a mixture of VAM fungi including Scutellospora calospora.

Aggangan and Dela Cruz (1991) inoculated Acacia auriculiformis and Leucaena leucocephala with Gigaspora margarita, Scutellospora persica and Sclerocystis clavispora in an autoclaved P-deficient soil collected from degraded grassland. Among the 4 species of VAM fungi tested, Gigaspora margarita and S. persica were equally effective in promoting growth of both the tree species. S. clavispora was ineffective such that growth obtained through inoculation with this fungus was comparable with that of the uninoculated ones.

Chulan and Martin (1992) observed that inoculation of mixed species of Scutellospora and Glomus resulted in higher dry matter yield in vegetatively propagated Theobroma cacao than those inoculated with individual cultures.

**General information about the three tree species used in this study:**

1. Acacia holosericea

Acacia holosericea is a thornless and non-browsed legume tree from Australia suitable for fuelwood. It has the advantage of having the greatest adaptability to variations in soil type. Even in very salty soils its rate of survival was 84 % at 40 months. It was also the only species, among the other Acacias, which can tolerate water logged soils (Duke, 1981).

Acacia holosericea grows to a height of 6 to 10 m and performs satisfactorily with rainfall < 500 mm. The seedlings of this tree has high drought resistance during initial months. However it is sensitive to long periods of drought and does not coppice well. It also retains large phyllode mass during the dry season, whereas local Acacia spp. shed their leaves during this period.

It is mainly used as fuelwood, charcoal, fodder, shelter belt, in-land rehahilitation and as an ornamental plant. The major positive features of this multipurpose tree are,

1. good wood production, and characoal properties,
2. satisfactory adaptation to most soils and
3. a considerable production of phyllodes which is used as fodder.

This tree is used in forest plantation and also in rural afforestation as wind break and amenity plantation. This tree is being recommended for afforestation in India at present.

2. Albizzia lebbeck

It is one of the best known of the Indian trees as it is not only very comon in the forests almost all over the country but is extensively cultivated as an avenue and garden tree. It is a moderate sized or large deciduous

tree, with dark grey rather rough bark, with regular cracks appearing red or crimson inside (Allen and Allen, 1981).

Albizzia lebbeck is found even up to an elevation of 1500 m in the Himalayas. It prefers a good well-drained loams; but it is not very exacting. It can grow fairly well on laterite or black cotton soil, though it dislikes heavy clay. In the Andamans it thrives on calcareous soils. It grows in a variety of soils and climates, the rainfall varying from 635 mm to 2,540 mm. The mean monthly temperature varies from $5^{o}C$ to $45.6^{o}C$. It is fairly resistant to drought and frost.

On account of the hardy nature and fast rate of growth it is considered a useful species for afforestation work in the localities with rainfall as low as 760 mm. The sapwood of this tree is white and large, the heartwood dark brown. With lighter and darker streaks. The timber is strong, elastic and hard. It is used in India for buildings, furniture, boats, agricultural and other implements and structural purposes. In the Kolar Gold fields it is used as mine props in shafts and galleries. It has been passed as suitable for handles of tennis rackets. It is undoubtedly a fine decorative timber, suitable for frames and panels, and internal wood work in railway carriages.

The leaves and twigs form excellent fodder for camels and other cattle and the tree in several places is cultivated for this purpose. In Southern Kerala, it is planted in order to obtain leaf manure for paddy crops. The bark, leaves, flowers and seeds are used medicinally. A reddish brown gum is obtained from cracks in the bark. Further, Albizzia lebbeck is one of several species suitable as a host tree for lac.

A. lebbeck is suitable for afforestation in coastal, moderately dry and semi-arid regions. It is also recommended for dry hot forests with little rain fall.

3. Tectona grandis

Teak is one of the important timber trees found in tropical and sub-tropical countries such as India, Burma, Sri Lanka and Malaysia; it is also introduced to many parts of the world. The teak tree is indigenous to peninsular India and drier north eastern part of Java. Natural teak forests of India are confined mainly to the peninsular region.

In India several provenances of teak differing in growth characteristics, stem morphology, leaves and properties of timber have been recognized and classified into five ecotypes. They are very dry, dry, semi dry, moist, and very moist. Timber of teak from dry and very dry

areas exihit good figure and is useful for panelling and furniture.

Teak is a light demander; it does not tolerate light suppression at anytime and it requires overhead light and also from the sides for profuse growth and flowering. Only dominant trees produce profuse branching and flowering in the upper part of the crown and retain terminal vigorous growth after flowering. Seedlings also require 94% of light intensity for growth and development. Teak coppices and pollards vigorously. It is very sensitive to frost, but in upper Burma it extends atleast up to the latitude of $22.5^{o}$ and ascends to 3000 feet (Gupta, 1993).

Teak is well known world wide for its good quality wood. It is used for furniture which are resistant to insects and borers. It is good for panel work and other decorative works. In India it is used in buildings, ship building, frames, railway carriages and for structural purposes.

# MATERIAL AND METHODS

## MATERIALS AND METHODS

This study was done to screen several VAM fungi and to select the most efficient fungi for inoculating three forest tree species, viz., Acacia holosericea, Albizzia lebbeck and Tectona grandis.

The experiments were carried out in the green house of the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK campus, Bangalore.

### 3.1. Seed source

The Seeds of Acacia holosericea were collected from Madurai Kamaraj University, Madurai; the seeds of Albizzia lebbeck were collected from Forest Genetics and Tree Breeding Institute, Coimbatore and the stumps of Tectona grandis were collected from Forest office, Mudigere, Karnataka.

### 3.2. Nursery

The seeds of Acacia holosericea were sterilized with 5% sodium hypochlorite solution for 30 minutes and washed in sterile distilled water 3 times. After washing, the seeds were exposed to hot water (95 - 100$^{o}$C) for 5 minutes to break dormancy. The seeds were then sown in battery boxes (45 x 25 x 15 cms) containing sterilized soil: perlite mix (1:1 v/v).

The seeds of Albizzia lebbeck were sterilized with 5% sodium hypochlorite solution for 30 minutes, washed in sterile distilled water 3 times and sown in battery boxes (45 x 25 x 15 cms) containing sterilized soil: perlite mix (1:1 v/v).

The stumps of Tectona grandis were washed thoroughly with water, but not sterilized.

The seedling of Acacia holosericea and Albizzia lebbeck were maintained in the nursery for 60 days. During that time Ruakura nutrient solution at 100 ml per battery box was applied once in 15 days. The stumps of Tectona grandis were planted directly to the polybags.

### 3.3. The substrate

The experiments were carried out in a red sandy loam soil collected from the GKVK farm. The soil had a pH of 5.6 and contained 2.7 ppm available phosphate ($NH^3F$ + Hcl extractable) and an indigenous VA mycorrhizal population of 29 spores/25 ml of soil.

### 3.4. Transplanting

The 60 days old seedlings of Acacia holosericea and Albizzia lebbeck were transplanted to polybags of size 25 x 15 cm holding 1.7 kg unsterilized soil: sand: FYM mix (2:1:0.5 v/v). One seedling was planted per bag. Mycorrhizal inoculum (12,5000 I.P.) was applied to the

planting hole at a depth of about 5 cm deep just before transplanting. Control plants not inoculated with VA mycorrhiza received root pieces and substrate in which Rhodes grass (Chloris guyana) was grown without any mycorrhizal fungus.

The teak stumps were directly planted to the polybags of the the above mentioned size and contents.

For each treatment 20 replications were maintained. Later 10 uniform plants without much variation in each treatment were selected and rest were discarded, as variation is usually much in forest tree seedlings.

### 3.5. Mycorrhizal inoculum

The VA mycorrhizal fungi used in this study were maintained in a glass house as pot cultures using sterilized sand: soil mix (1:1 v/v) as the substrate and Rhodes grass as the host. The mixture consisting of the root system plus substrate, was finely chopped and air dried. The hyphae, spores and root segments present in the air dried soil served as inoculum. Each seedling was inoculated, at the time of transplanting, with cultures containing 12,500 infective propagules (I.P.). The inoculum potential of the cultures was estimated using the Most Probable Number (MPN) method described by Porter (1979). The VA mycorrhizal fungi and their quantity used in this study are given below.

| Sl. No. | Name | Source | Quantity used (in gms) (To give 12,500 I.P.) |
|---|---|---|---|
| 1. | Acaulospora laevis | Nedlands, Australia | 6.25 |
| 2. | Gigaspora margarita | ICRISAT, India | 10.5 |
| 3. | Glomus caledonicum | Nedlands, Australia | 7.0 |
| 4. | Glomus fasciculatum | California, U.S.A | 6.0 |
| 5. | Glomus intraradices | Bangalore, India | 1.8 |
| 6. | Glomus leptotichum | Bangalore, India | 3.44 |
| 7. | Glomus macrocarpum | Bangalore, India | 9.0 |
| 8. | Glomus mosseae | Bangalore, India | 1.47 |
| 9. | Scutellospora calospora | ICRISAT, India | 10.2 |

### 3.6. Maintenance

All the three tree species, viz., Acacia holosericea, Albizzia lebbeck and Tectona grandis were screened for their response to 9 different VA mycorrhizal fungi. Twenty replications were maintained for each treatment and only one seedling was maintained in each poly bag. The plants were maintained for 180 days after transplanting. During the growth period plants were watered depending on the need and supplied with Ruakura plant nutrient solution (without P) once in 15 days at 50 ml/bag (See Appendix 1).

At the end of the experiment out of 20 replications only 10 replications were used for further analysis and the

remaining plants which had overgrown or undergrown were eliminated.

### 3.7. Observations

The physical parameters like plant height and stem growth were taken periodically. While the leaf area, plant biomass, per cent soil aggregation, per cent mycorrhizal root colonization, number of mycorrhizal spores in root zone soil and phosphorus, zinc and copper concentrations of the shoot were taken after harvest as detailed below.

#### 3.7.1. Plant height

Plant height was measured from soil surface to the growing tip of the plant. Height was taken once in 30 days after transplanting for Acacia holosericea and Albizzia lebbeck and for Tectona grandis observations were taken once in 45 days after planting.

#### 3.7.2. Plant girth

The plant girth was measured 1 cm above the soil surface using vernier calipers.

#### 3.7.3. Leaf area

Leaf area was taken using the leaf area meter after harvest (180 days after transplanting).

### 3.7.4. Plant biomass

Shoot and root dry weights were taken after harvest by drying the plant samples at 60°C to a constant weight in a hot air oven.

### 3.7.5. Estimation of VAM fungal population in the root-zone soils

The soil samples collected from the root zone were air-dried and the spore population of VAM fungi were estimated following wet sieving and decanting method described by Gerdemann and Nicolson (1963).

#### 3.7.5.1. Wet-sieving and decanting method

For each soil sample, a representative portion of 25 g was suspended in a beaker of water and thoroughly stirred until all soil aggregates dispersed and formed a uniform suspension. The suspension was passed through a stack of test sieves with 1 mm, 450, 300, 250, 125, 105 and 45 microns. The residue was resuspended in water and decanted, repeating this at least six times for complete extraction of spores. Spores retained on 125, 105 and 45 micrometer sieves were pooled and spore suspension was made up to 100 ml for further quantification.

#### 3.7.5.2. Quantitative estimation of spores

The spore suspension obtained by wet-sieving and decanting method was used to quantify the population of VAM fungi in terms of number of spores in the soil.

The spore suspension was poured on a nylon mesh (pore size, 45 micrometer) and water was allowed to drain. The nylon mesh was placed on a grid-line petriplate and examined under stereoscopic microscope for recording the number of spores present on the nylon mesh. The final spore count was expressed as the number of spores/25 ml of soil.

### 3.7.6. Quantification of VAM in plant roots

Since the primary site of VAM to develop is in the cortical region of the terminal feeder roots, the fine terminal feeder roots were cautiously removed from the root and soil and washed thoroughly. The roots were preserved in formalin-acetic acid-alcohol (FAA) (90 : 5 : 5) solution.

#### 3.7.6.1. Staining roots

The root samples fixed in FAA were stained by the method described by Phillips and Hayman (1970).

The roots were treated with 10 % aqueous KOH for atleast 1 hour at 90$^{o}$C in a water bath. The KOH solution was decanted and the roots were bleached immediately with alkaline solution of hydrogen peroxide at room temperature. The bleaching period ranged from 10 to 30 minutes, depending on the size and colour intensity of the roots. The roots were rinsed under tap water to remove excess $H_2O_2$ . After rinsing, the roots were acidified with 1 % Hcl for 5 minutes for proper staining and the contents were decanted. The

root segments were then stained in 0.05 % trypan blue in lactoglycerol (Lactic acid, glycerol, and water in the ratio of 4 : 4 : 2 v/v/v)and heated gently for 10 minutes. The excess stain was removed by resuspending the root bits in lactoglycerol (without stain).

### 3.7.6.2. Grid-line intersect method

The stained roots were examined under stereo microscope for the presence of arbuscules, vesicles and intraradical hyphae of VAM fungi. The per cent root colonization by VAM fungi was determined following the grid-line intersect method (Giovannetti and Mosse, 1980). A grid of lines was marked in the bottom of the glass plate to form 1.27 cm squares.

The stained root sample was randomly spread on this glass plate. Horizontal and vertical grid-lines were scanned and the total number of roots intersecting grid-lines and the total number of intersections involving infected roots were recorded. The per cent root colonization was calculated by the formula.

$$\% \text{ infection} = \frac{\text{Total number of infected roots intersecting grid line}}{\text{Total number of roots intersecting grid lines}} \times 100$$

### 3.7.7. Estimation of soil aggregates less than 50 micrometer in size (Van Bavel, 1980)

The soil in which the plants were raised was used for determining soil aggregates. The method involved, measuring the concentration of 2 suspensions of the same soil, one of which was dispersed by standard dispersion procedure to give total silt plus clay, the other suspension was prepared by mild agitation of the sample in water, which gave the measures of unaggregated silt plus clay particles that is bound in water soluble aggregates of less than 50 micrometer.

The standard dispersion method involved the oxidation of all the organic matter present in the soil sample. Twenty gram of soil sample was taken in 600 ml beaker, to which 20 ml of 20 % $H_2O_2$ was added. It was placed on a waterbath of 60$^{o}$C with constant stirring till complete frothing stopped, after which the contents of the beaker were cooled. To this 10 ml of dispersing agents (35 % ammonium carbonate + 5 % sodium hexametaphosphate) was added and then stirred using a mechanical stirrer for 5 minutes. The entire content was then passed through 0.2 mm sieve to separate out the sand particles. The soil suspension was then transferred to a hydrometer jar and diluted with distilled water to 1000 ml. The cylinder was stoppered and inverted 2-3 times. Then the total silt and clay in the suspension was determined after 4 minutes 30 seconds using a

Bouyoucos hydrometer (1936). Similarly to measure the unbound silt and clay, 20 g of such sample was taken in a hydrometer jar. The jar was then inclined to a nearly horizontal position and shaken lightly to spread the sample over a distance of 10 or 12 cm, along the side of the jar. Distilled water was added slowly so as to favour wetting by capillarity rather than by flooding. When soil sample was completely wet, the suspension was diluted to 1000 ml, having taken care that the water did not fall directly into the soil. The soil was allowed to slake for 15 minutes. The cylinder was stoppered and gently inverted 20 times within a period of 40 seconds. After the required settling time, the unbound silt and clay in suspension was determined using a Bouyoucos hydrometer.

$$\text{Per cent aggregation} = \frac{\text{Wt. of total soil \& clay in dispersed suspension(g)} - \text{Wt. of soil \& clay in undispersed suspension(g)}}{\text{Wt. of total soil and clay in dispersed suspension (g)}} \times 100$$

### 3.7.8. Most probable number method for enumerating infective propagules of VAM fungi in soil

The number of infective propagules of mycorrhizal fungi in the root zone soil in polythene was bags determined by the (MPN) method of porter (1979).

Thirty grams of air-dried inoculum was used for ten fold series of soil dilutions up to $10^{-9}$ using 270 g sterilized sand: soil (1:1 v/v) mix per dilution. The diluent culture from $10^{-1}$ to $10^{-9}$ was filled to plant tubes (15 cm x 2.5 cm) at the rate of 50 g/tube in each dilution. For each dilution 5 replicate tubes were prepared. Onion seeds were sown in the plant tubes at the rate of 5 seeds/tube. Onion seedlings were grown in these tubes for 6 weeks in the green house. Later, roots were washed and stained with trypan blue in lactoglycerol (Phillips and Hayman, 1970). The presence or absence of VAM colonization was observed microscopically in all the replications in each dilution and scored positive or negative for mycorrhizal colonization. The most probable number of VAM propagules was then calculated using 3 appropriate dilutions and referring to MPN table (Alexander, 1965).

### 3.7.9. Phosphorus estimation

Plant phosphorus content was estimated by vanadomolybdate phosphoric yellow colour method described by Jackson (1971).

The dried, finely powdered plant samples of 500 mg each were digested in triacid mixture (nitric acid : perchloric acid : sulphuric acid : : 7 : 3 : 1) in 100 ml conical flasks. The flasks were kept on hot plate till the

cessation of white fumes. The flasks were then cooled and the volume was made up to 100 ml in volumetric flasks. From this, 10 ml aliquots were taken in 50 ml volumetric flasks and 10 ml of vanadomolybdic acid (see Appendix 3) added to this solution and the volume made up to 50 ml with distilled water. The intensity of yellow colour due to vanadomolybdate complex was read colorimetrically at 425 nm in a spectrophotometer (Spectronic 20). The phosphorus concentration was determined by comparing the absorbance with standard curve.

### 3.7.10. Zinc estimation

Zinc estimation was done using Atomic absorption spectrophotometer with Zn hollow cathode lamp set to a wavelength of 214 nm. The plant samples were digested as described for P estimation. Suitable aliquot dilutions were taken and read in an Atomic absorption spectrophotometer. The quantity of zinc in the sample was estimated by comparing with the standard and expressed as microgram/gram of plant sample. Zinc chloride solution was used for preparing the standard curve.

### 3.7.11. Copper estimation

Copper estimation was done by using Atomic absorption spectrophotometer with hollow cathode lamp specific for copper and the wavelength adjusted to 325 nm. The plant samples digested for P estimation were diluted and fed into

the Atomic absorption spectrophotometer (AAS). The quantity of copper in the sample was estimated by comparing with the standard and expressed as microgram/gram of plant sample. Copper sulphate solution was used for preparing the standard curve.

### 3.7.12. Sturdiness quotient

Sturdiness quotient reflects the spindly (or) stocky nature of the seedling. It is the ratio of height to diameter. Higher value denotes that the seedling is thin and lanky while the lower value denotes the sturdiness of a seedling.

Sturdiness quotient of the plant was calculated by the formula devised by Ritchie (1985).

$$\text{Sturdiness quotient} = \frac{\text{Height (cm)}}{\text{Diameter (mm)}}$$

### 3.7.13. Biovolume index

Biovolume index is a measure to calculate the total volume of a seedling based on its height and stem diameter. Both sturdiness quotient and biovolume index can be calculated without removing the seedlings for analysis.

Biovolume index of the plant was calculated by the formula suggested by Hatchell (1985).

$$\text{Biovolume index} = \text{Diameter (mm)}^2 \times \text{Height (cm)}$$

### 3.7.14. Quality index

Quality index is a measure to assess the quality of a seedling based on its height, stem diameter and dry biomass. Higher values denotes the better quality of the seedling.

Quality index was calculated as follows:

$$\text{Quality index} = \frac{\text{Seeding dry weight (g)}}{[\text{Height (cm)}/\text{Diameter (mm)}] + [\text{Top dry weight (g)}]}$$

## 3.8. Statistical analysis

The data were analysed using the completely randomized design, with the help of the computer (microvax system VAX/VMS, version 5.4, Digital Equipment Corporation, U.S.A.). The means were compared by Duncan's multiple range test at 5% level (Duncan, 1955).

# RESULTS

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The present study was conducted with an object of selecting efficient vesicular - arbuscular mycorrhizal fungi for inoculating three forest tree species, viz., Acacia holoserisea, Albizzia lebbeck and Tectona grandis. The results of this study are given below.

## Experiment 1

### 4.1. Selection of efficient VAM fungi for Acacia holosericea

#### 4.1.1. Influence of VAM on plant height

The effect of inoculation with different VAM fungi on plant height of Acacia holosericea at different stages of growth is given in Table 1. There was significant increase in plant height at all the stages.

On 90th day after transplanting (DAT), Glomus mosseae inoculation resulted in highest plant height (32.78 cm), followed by Gigaspora margarita (31.67 cm), Scutellospora calospora (31.54 cm), Glomus fasciculatum (30.94 cm) and Glomus caledonicum (30.42 cm). All the treatments were statistically significant, except Acaulospora laevis and Glomus intraradices which were on par with the uninoculated control (Fig.1).

On 120 DAT, plants inoculated with Glomus mosseae showed greater height (56.61 cm) than any other treatment,

**Table 1 Influence of different VAM fungi on plant height (cm) of *Acacia holosericea***

| VAM fungi | 90 DAS* | 120 DAS | 150 DAS | 180 DAS |
|---|---|---|---|---|
| Uninoculated control | 27.28$^{e}$ | 47.58$^{f}$ | 71.57$^{e}$ | 87.27$^{d}$ |
| *A. laevis* (Nedl.) | 28.18$^{e}$ | 48.67$^{ef}$ | 74.37$^{d}$ | 89.63$^{cd}$ |
| *Gi. margarita* (ICRISAT) | 31.67$^{d}$ | 51.51$^{d}$ | 78.34$^{ab}$ | 96.47$^{b}$ |
| *G. caledonicum* (Nedl.) | 30.42$^{c}$ | 51.51$^{d}$ | 77.49$^{bc}$ | 95.92$^{b}$ |
| *G. fasciculatum* (Riv.) | 30.94$^{bc}$ | 48.98$^{e}$ | 76.28$^{cd}$ | 95.56$^{b}$ |
| *G. intraradices* (Local) | 27.99$^{e}$ | 52.79$^{c}$ | 71.79$^{e}$ | 87.91$^{d}$ |
| *G. leptotichum* (Local) | 29.17$^{d}$ | 53.48$^{c}$ | 74.85$^{d}$ | 90.46$^{c}$ |
| *G. macrocarpum* (Local) | 29.43$^{d}$ | 53.91$^{bc}$ | 75.17$^{d}$ | 91.51$^{c}$ |
| *G. mosseae* (Local) | 32.78$^{a}$ | 56.61$^{a}$ | 79.84$^{a}$ | 99.54$^{a}$ |
| *S. calospora* (ICRISAT) | 31.54$^{b}$ | 54.79$^{b}$ | 78.91$^{ab}$ | 97.69$^{ab}$ |

Values with similar alphabets in each column do not differ significantly at P = 0.05%

* DAS - Days after sowing

Nedl. - Isolate from Nedlands, Australia
ICRISAT - Isolate from ICRISAT, Hyderabad
Riv. - Isolate from Riverside, U.S.A.
Local - Isolate from U.A.S., Bangalore

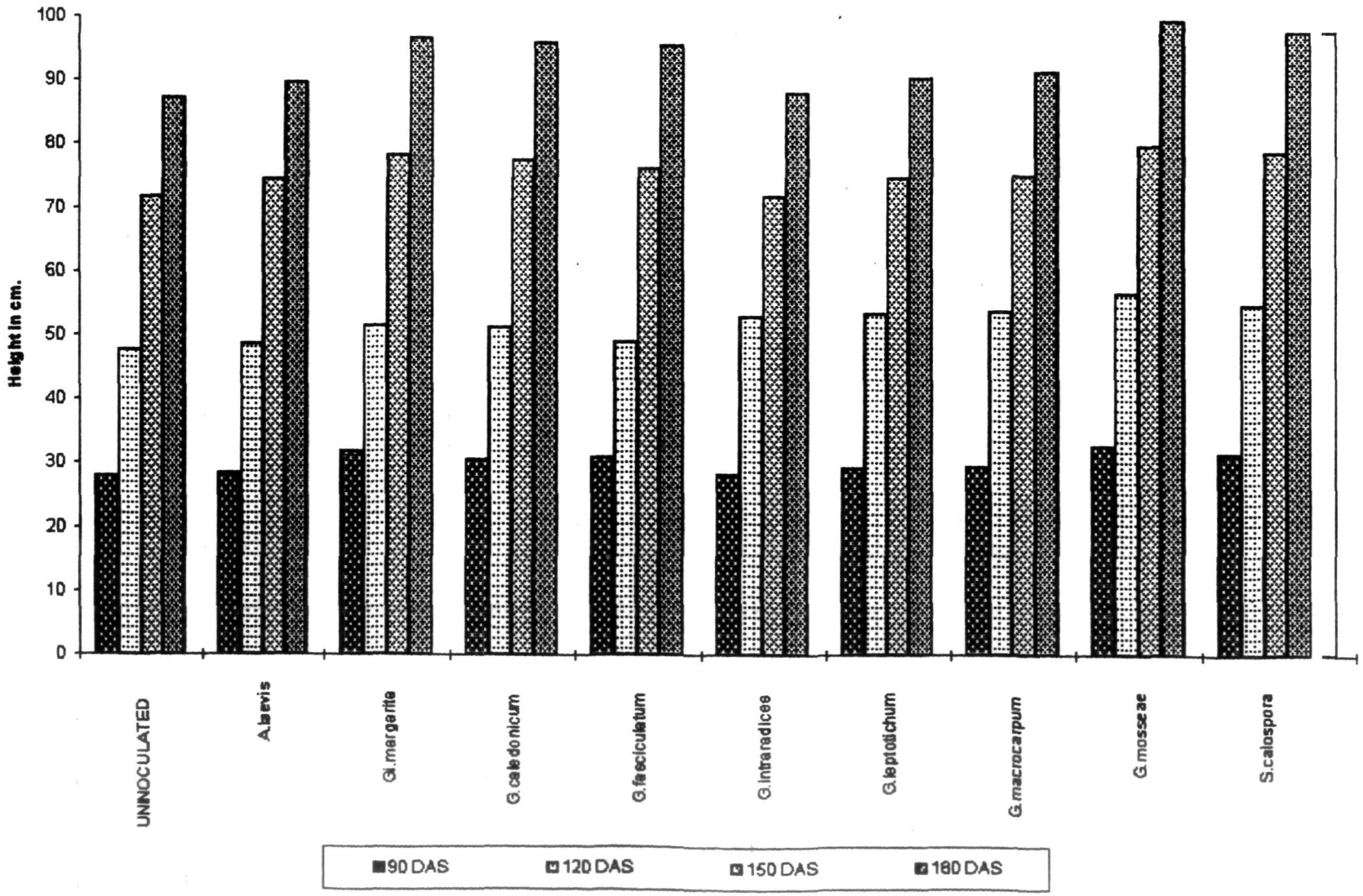
A. - *Acaulospora*
Gi. - *Gigaspora*
G. - *Glomus*
S. - *Scutellospora*

followed by Scutellospora calospora (54.79 cm), Glomus macrocarpum (53.91) and Glomus intraradices (52.79). Acaulospora laevis inoculated plants did not show any significant difference when compared to uninoculated control.

On 150 DAT, again Glomus mosseae inoculation resulted in highest plant height (79.8 cm) followed by Scutellospora calospora (78.91 cm), Gigaspora margarita (78.34 cm) and Glomus caledonicum (77.49 cm). But Glomus intraradices inoculated and uninoculated control plants were on par with each other.

On 180 DAT, plants inoculated with the VAM fungus Glomus mosseae was found to have higher plant height (99.54 cm) compared to other treatments, and it was on par with Scutellospora calospora inoculated treatment. Acaulospora laevis inoculated plant, recorded a height (89.63 cm) which was statistically on par with the control plants (87.27 cm). The other fungi in the order of performance in improving height of Acacia holosericea were Glomus caledonicum (95.92 cm), Glomus fasciculatum (95.56 cm) and Gigaspora margarita (96.47 cm). The height of plants inoculated with all these fungi were statistically on par with Scutellospora calospora treated plants.

Fig : 1. Influence of different VAM fungi on plant height (cm) of Acacia holosericea



**4.1.2. Influence of different VAM fungi on stem girth**

As shown in Table 2 all the treatments were statistically significant. On 90 DAT Glomus mosseae inoculated plants had highest stem girth (0.231 cm) followed by Gigaspora margarita (0.230 cm), Glomus caledonicum (0.229 cm), Glomus fasciculatum (0.266 cm), and Glomus leptotichum (0.225 cm) inoculated plants. All these treatments were statistically on par with each other. The uninoculated control plants had the lowest stem girth (0.210 cm).

On 120 DAT, the results showed plants treated with Glomus caledonicum (0.347 cm), Glomus mosseae (0.342 cm), Gigaspora margarita (0.342 cm) and Scutellospora calospora (0.341 cm) had stem girths which were on par with each other. Further, the treatments Acaulospora laevis, Glomus leptotichum and Glomus macrocarpum were not statistically significant from the uninoculated control treatment (Fig.2).

On 150 DAT, the plants inoculated with Glomus mosseae (0.468 cm), Scutellospora calospora (0.461 cm) and Gigaspora margarita (0.454 cm) were on par with each other, whereas the treatments Glomus macrocarpum, Glomus fasciculatum, Glomus intraradices, Glomus leptotichum and Acaulospora leaevis were not significantly different from the control plants.

**Table 2 Influence of different VAM fungi on stem girth (cm) of *Acacia holosericea***

| VAM fungi | 90 DAS | 120 DAS | 150 DAS | 180 DAS |
|---|---|---|---|---|
| Uninoculated control | $0.210^{d}$ | $0.319^{d}$ | $0.429^{e}$ | $0.529^{d}$ |
| *A. laevis* (Nedl.) | $0.223^{bc}$ | $0.324^{cd}$ | $0.432^{e}$ | $0.531^{d}$ |
| *Gi. margarita* (ICRISAT) | $0.230^{ab}$ | $0.342^{ab}$ | $0.454^{abc}$ | $0.561^{ab}$ |
| *G. caledonicum* (Nedl.) | $0.229^{ab}$ | $0.347^{a}$ | $0.449^{bcd}$ | $0.557^{ab}$ |
| *G. fasciculatum* (Riv.) | $0.226^{ab}$ | $0.334^{bc}$ | $0.440^{cde}$ | $0.552^{abc}$ |
| *G. intraradices* (Local) | $0.217^{c}$ | $0.322^{d}$ | $0.431^{e}$ | $0.530^{d}$ |
| *G. leptotichum* (Local) | $0.225^{ab}$ | $0.326^{cd}$ | $0.431^{e}$ | $0.534^{cd}$ |
| *G. macrocarpum* (Local) | $0.226^{ab}$ | $0.326^{cd}$ | $0.439^{de}$ | $0.547^{bcd}$ |
| *G. mosseae* (Local) | $0.231^{a}$ | $0.342^{ab}$ | $0.468^{a}$ | $0.571^{a}$ |
| *S. calospora* (ICRISAT) | $0.229^{ab}$ | $0.341^{ab}$ | $0.461^{ab}$ | $0.569^{a}$ |

Legend as in Table 1

**Fig : 2. Influence of different VAM fungi on stem girth (cm) of Acacia holosericea**

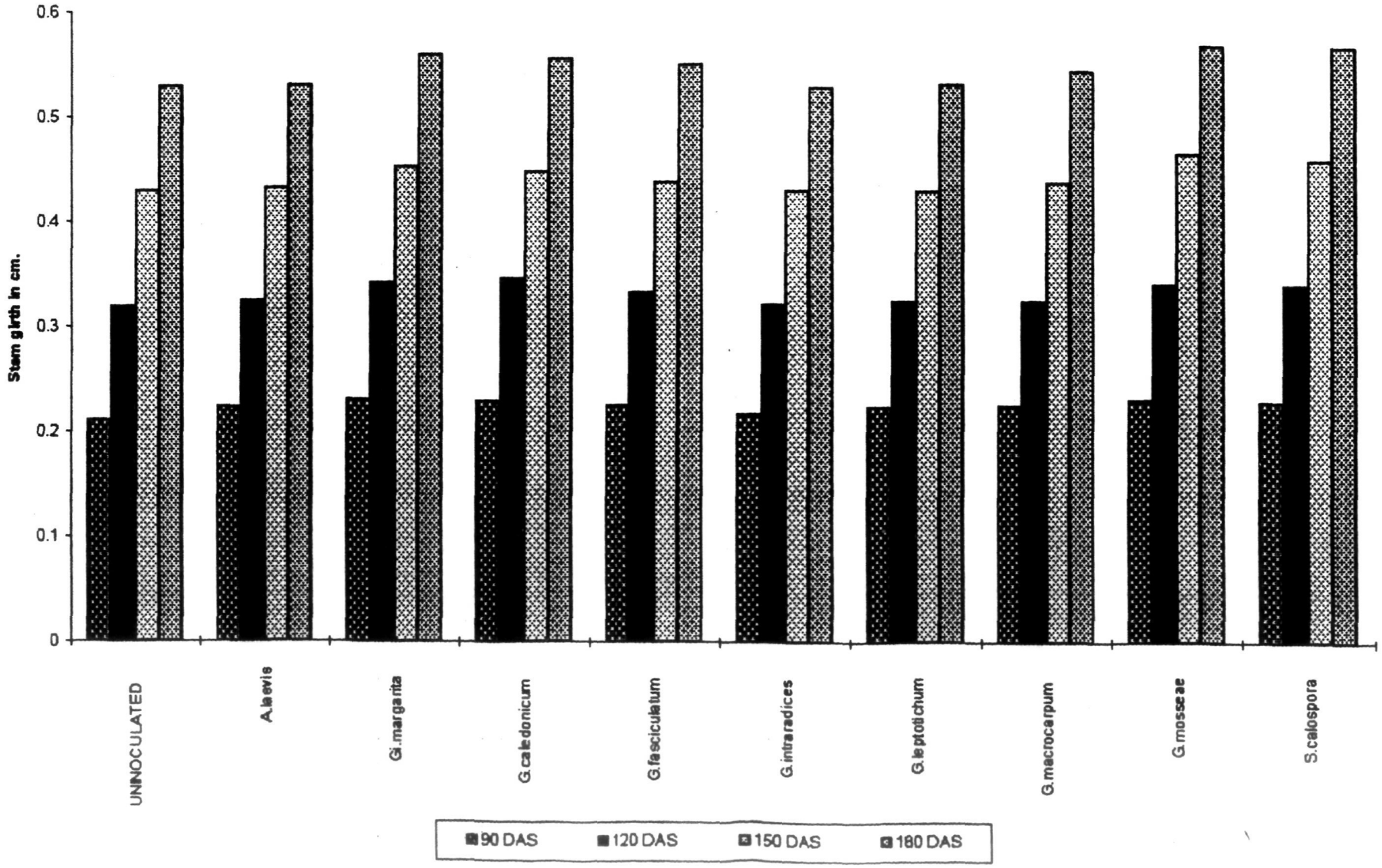


**Plate 1 & 2.** *Influence of Different VAM fungi on the growth of Acacia holosericea*

*C - UNINOCULATED CONTROL*

*1 - Glomus mosseae*

*2 - Scutellospora calospora*

*3 - Gigaspora margarita*

*4 - Glomus caledonicum*

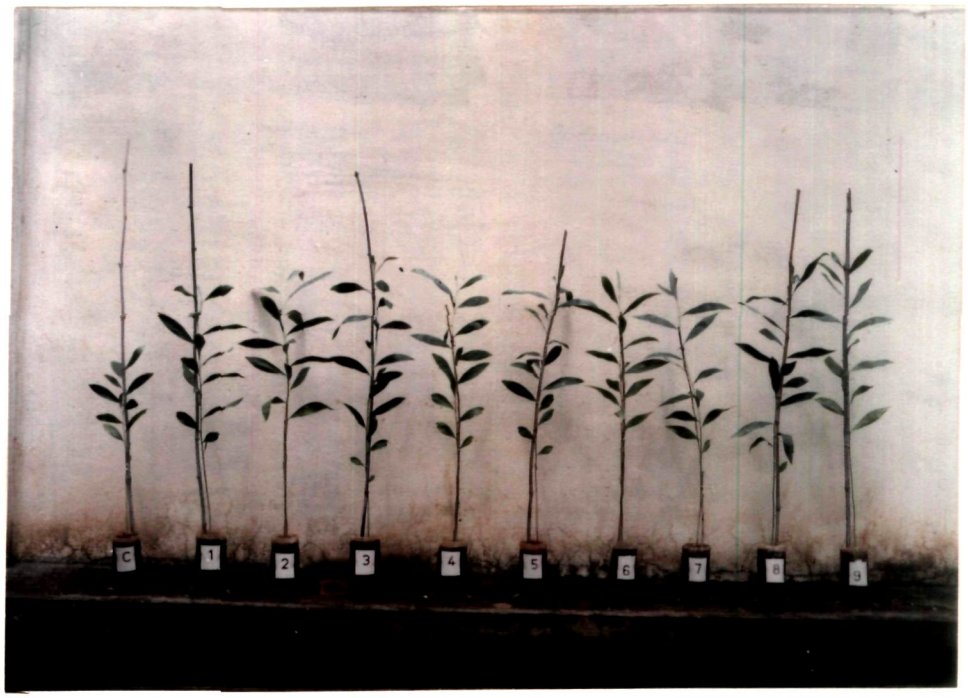
*5 - Glomus fasciculatum*

*6 - Glomus macrocarpum*

*7 - Glomus leptotichum*

*8 - Acaulospora laevis*

*9 - Glomus intraradices*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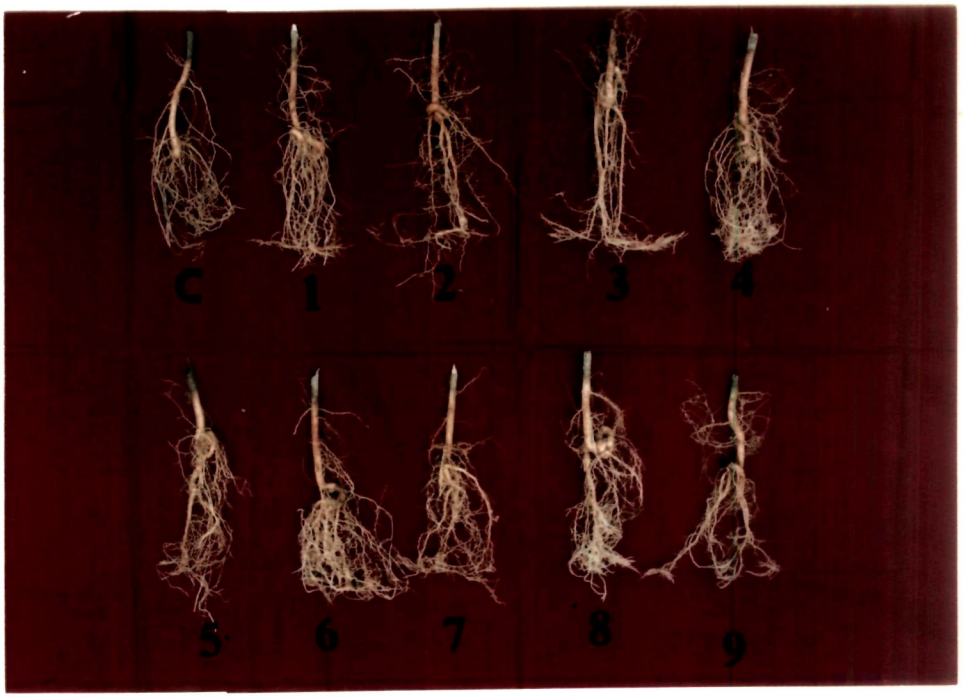
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On 180 DAT, the treatments Glomus mosseae (0.571 cm), Scutellospora calospora (0.569 cm), Gigaspora margarita (0.561 cm), Glomus caledonicum (0.557 cm) and Glomus fasciculatum (0.552 cm) had higher stem diameter and all of them were statistically on par with each other, whereas the remaining treatments were on par with the uninoculated control.

**4.1.3. Influence of different VAM fungi on plant biomass**

**Shoot biomass:** At harvest, the highest shoot biomass was observed in plants inoculated with Glomus mosseae (7.190 gm) followed by Scutellospora calospora (7.120 gm) and Gigaspora margarita (6.98 gm). All the three treatments were statistically on par with each other. On the other hand, the plants inoculated with Glomus leptotichum, Glomus intraradices and Acaulospora laevis did not show any significant increase over control (Table 3 and Fig.4).

**Root biomass:** The highest root dry weight was seen in plants inoculated with Glomus mosseae (3.920 g). The next best was Scutellospora calospora treated plants (3.800 g). Both these treatments were statistically on par with each other. But the plants inoculated with Glomus intraradices and Acaulospora laevis were not significantly different from the uninoculated control.

**Table 3 Influence of different VAM fungi on leaf area shoot and root dry weight of *Acacia holosericea***

| VAM fungi | Leaf area $cm^2$/plant | Dry weight (g/plant) | | |
|---|---|---|---|---|
| | | Shoot | Root | Total |
| Uninoculated control | 532.50$^{e}$ | 5.320$^{e}$ | 2.860$^{g}$ | 8.180$^{f}$ |
| *A. laevis* (Nedl.) | 578.25$^{cd}$ | 5.390$^{e}$ | 2.990$^{fg}$ | 8.380$^{f}$ |
| *Gi. margarita* (ICRISAT) | 578.10$^{cd}$ | 6.980$^{ab}$ | 3.670$^{b}$ | 10.650$^{bc}$ |
| *G. caledonicum* (Nedl.) | 621.18$^{a}$ | 6.920$^{bc}$ | 3.510$^{c}$ | 10.430$^{c}$ |
| *G. fasciculatum* (Riv.) | 580.71$^{bcd}$ | 6.710$^{c}$ | 3.320$^{d}$ | 10.020$^{d}$ |
| *G. intraradices* (Local) | 591.20$^{bc}$ | 5.300$^{e}$ | 2.910$^{fg}$ | 8.210$^{f}$ |
| *G. leptotichum* (Local) | 604.56$^{ab}$ | 5.400$^{e}$ | 3.020$^{f}$ | 8.420$^{f}$ |
| *G. macrocarpum* (Local) | 623.21$^{a}$ | 6.420$^{d}$ | 3.180$^{e}$ | 9.600$^{e}$ |
| *G. mosseae* (Local) | 582.78$^{bcd}$ | 7.180$^{a}$ | 3.920$^{a}$ | 11.110$^{a}$ |
| *S. calospora* (ICRISAT) | 562.15$^{d}$ | 7.120$^{ab}$ | 3.800$^{a}$ | 10.920$^{ab}$ |

Legend as in Table 1

Fig : 3. Influence of different VAM fungi on leaf area of Acacia holosericea

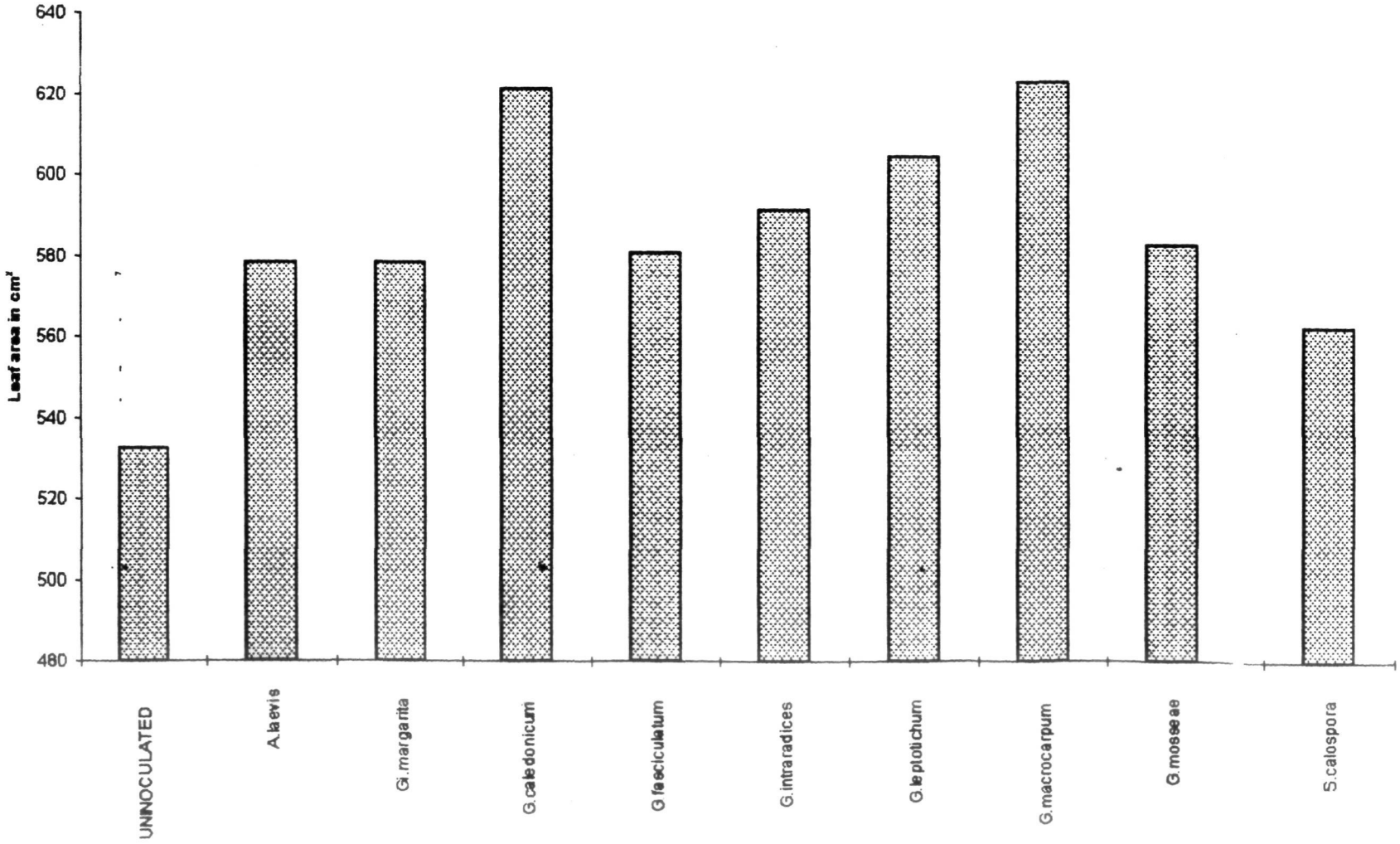
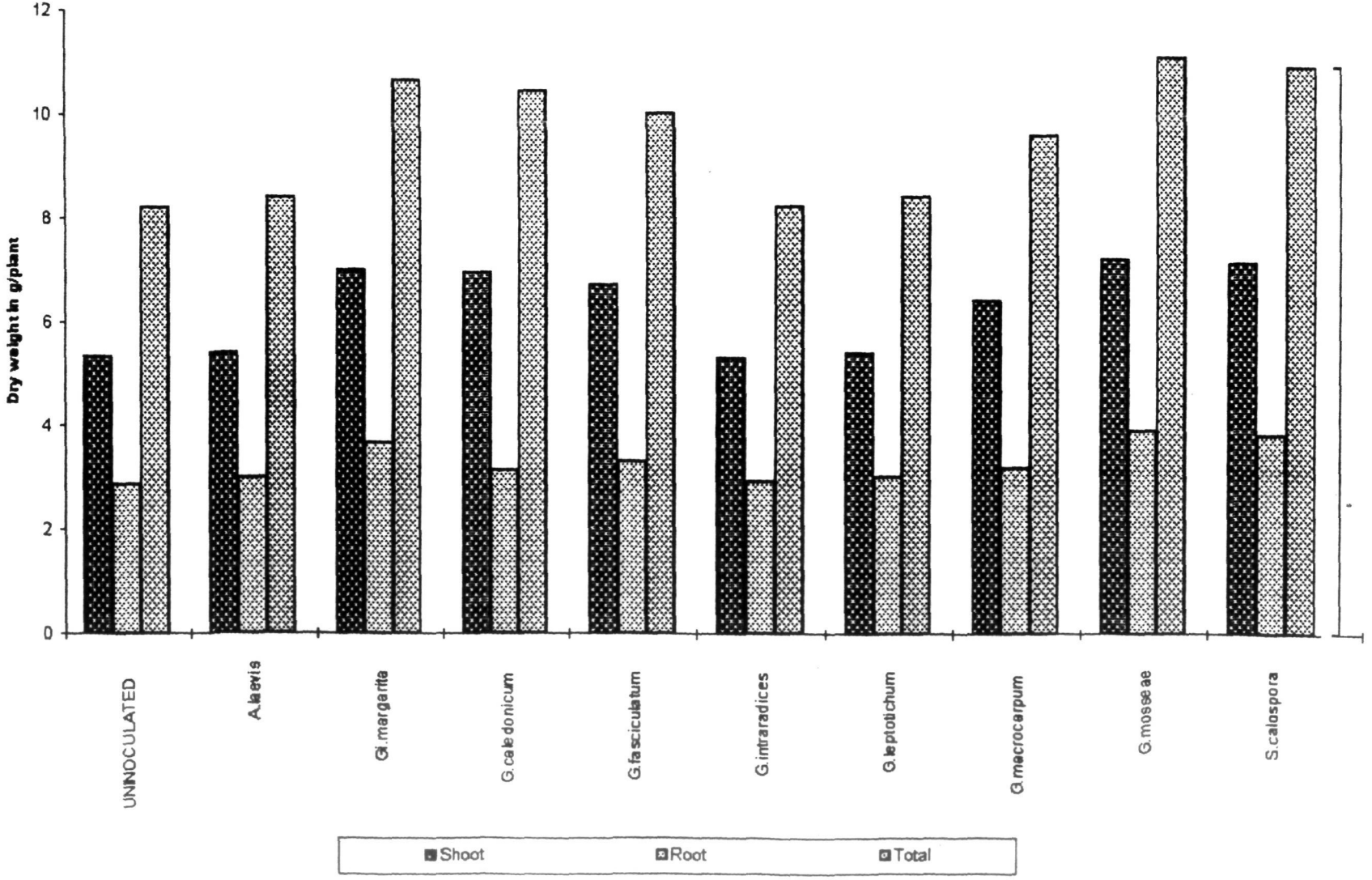


**Fig : 4. Influence of diffferent VAM fungi on shoot and root dry weight of Acacia holosericea**



**Total biomass:** The Glomus mosseae inoculated plants showed highest total biomass (11.11 g) followed by Scutellospora calospora (10.92g), Gigaspora margarita (10.65g) inoculated plants. The plants inoculated with Glomus leptotichum, Glomus intraradices and Acaulospora laevis were on par with the uninoculated control plants.

### 4.1.4. Influence of different VAM fungi on leaf area

The leaf area of Acacia holosericea treated with different VAM fungi was statistically significant (Table 3). The highest leaf area was found in plants treated with Glomus macrocarpum (623.21 $cm^2$) followed by Glomus caledonicum (621.18 $cm^2$) and Glomus leptotichum (604.50 $cm^2$). All these treatments were statistically on par with each other. The next best fungi were Glomus intraradices (591.20 $cm^2$), Glomus mosseae (582.78 $cm^2$) and Glomus fasciculatum (580.71 $cm^2$), which were also an par with each other. Compared to control all the treatments were highly significant (Fig.3).

### 4.1.5. Influence of VAM fungi on uptake of phosphorus by the host

As shown in the Table 5, the phosphorus concentration and content of shoot and root varied between different treatments.

**Table 4 Influence of different VAM fungi on phosphorus uptake by *Acacia holosericea***

| VAM fungi | P concentration (%) | | P content (mg/plant) | | |
|---|---|---|---|---|---|
| | Shoot | Root | Shoot | Root | Total |
| Uninoculated control | $0.132^{d}$ | $0.141^{d}$ | $7.02^{g}$ | $4.03^{h}$ | $11.05^{h}$ |
| *A. laevis* (Nedl.) | $0.175^{c}$ | $0.188^{c}$ | $9.43^{f}$ | $5.62^{g}$ | $15.05^{g}$ |
| *Gi. margarita* (ICRISAT) | $0.196^{ab}$ | $0.199^{bc}$ | $13.68^{bc}$ | $7.30^{c}$ | $20.98^{c}$ |
| *G. caledonicum* (Nedl.) | $0.192^{ab}$ | $0.199^{bc}$ | $13.27^{c}$ | $6.99^{d}$ | $20.26^{d}$ |
| *G. fasciculatum* (Riv.) | $0.191^{ab}$ | $0.192^{c}$ | $12.82^{d}$ | $6.37^{e}$ | $19.19^{e}$ |
| *G. intraradices* (Local) | $0.174^{c}$ | $0.189^{c}$ | $9.22^{f}$ | $5.50^{g}$ | $14.72^{g}$ |
| *G. leptotichum* (Local) | $0.172^{c}$ | $0.187^{c}$ | $9.29^{f}$ | $5.65^{g}$ | $14.94^{g}$ |
| *G. macrocarpum* (Local) | $0.188^{b}$ | $0.191^{c}$ | $12.09^{d}$ | $6.07^{f}$ | $18.16^{f}$ |
| *G. mosseae* (Local) | $0.202^{a}$ | $0.215^{a}$ | $14.52^{a}$ | $8.43^{a}$ | $22.95^{a}$ |
| *S. calospora* (ICRISAT) | $0.198^{ab}$ | $0.212^{ab}$ | $14.10^{ab}$ | $8.06^{b}$ | $22.16^{b}$ |

Legend as in Table 1

**Shoot phosphorus**

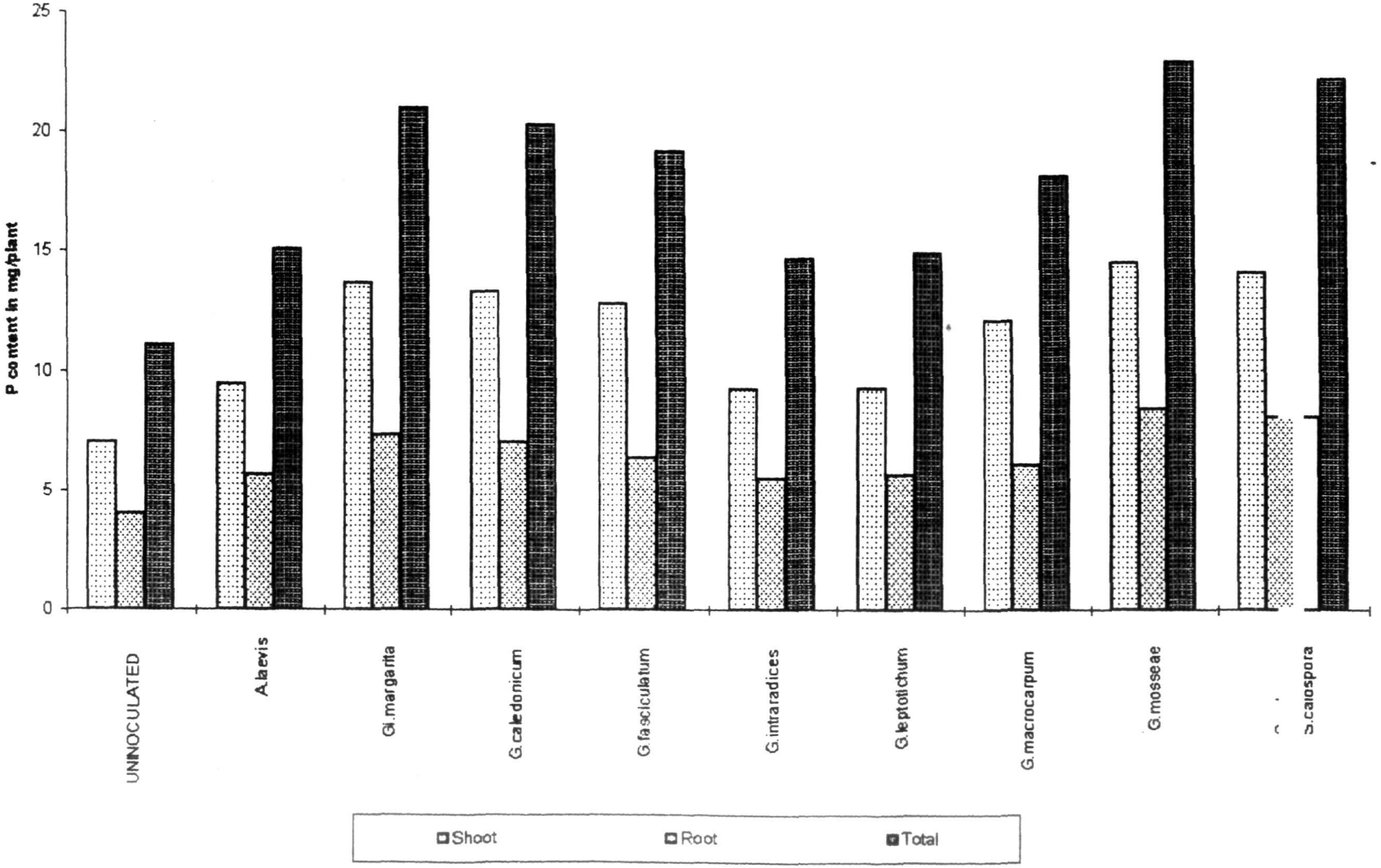
The difference in the shoot phosphorus content due to inoculation with VAM fungi was significantly different from that of the uninoculated plants (Table 4). Plants inoculated with Glomus mosseae had maximum P content (14.52 mg/pl) followed by Scutellospora calospora (14.10 mg/pl). Both the treatments were on par with each other. The control plants had the least P content of 7.02 mg/pl (Fig.5).

The Glomus mosseae inoculated plants had maximum P concentration (0.202 %) followed by Scutellospora calospora (0.198 %), Gigaspora margarita (0.196 %), Glomus caledonicum (0.192 %) and Glomus fasciculatum inoculated plants. All the above treatments were statistically on par with each other, although all the VAM inoculated treatments recorded significantly more shoot P concentration than the uninoculated control (0.132 %).

**Root phosphorus**

The Glomus mosseae inoculated plants showed higher root phosphorus content (8.430 mg/pl) followed by Scutellospora calospora (8.060 mg/pl) and Gigaspora margarita (7.30 mg/pl) treated plants, minimum was observed in the control plants (4.03 mg/pl). All the VAM inoculated treatments were significantly higher than the control treatment.

**Fig : 5. Influence of different VAM fungi on phosphorus uptake by Acacia holosericea**



The root phosphorus concentration was maximum in Glomus mosseae inoculated plants (0.215 %) followed by Scutellospora calospora inoculated plants (0.212 %) where as the least was observed in control plants (0.141 %).

**Total phosphorus**

The total phosphorus content of plants differed significantly with different mycorrhizal fungi used for inoculation. The uptake of phosphorus was maximum in Glomus mosseae inoculated plants (22.95 mg/pl) which was followed by Scutellospora margarita (22.16 mg/pl) and Gigaspora margarita (20.980 mg/pl) inoculated plants. All the treatments were significantly different from each other, except Acaulospora laevis, Glomus leptotichum and Glomus intraradices which were on par with each other (Table 4).

### 4.1.6. Influence of VAM fungi on uptake of zinc by the host

**Shoot zinc**

The shoot zinc content varied in plants inoculated with different VAM fungi (Table 5). The shoot zinc content was maximum in plants inoculated with Glomus mosseae (188.38 µg/pl) followed by Scutellospora calospora (178.71 µg/pl), Glomus caledonicum (175.08 µg/pl) and Gigaspora margarita (174.50 µg/pl) inoculated plants. When compared to uninoculated control all the inoculated treatments were significantly different (Fig.6).

**Table 5 Influence of different VAM fungi on zinc uptake by *Acacia holosericea***

| VAM fungi | Zn concentration (ppm) | | Zn content (µg/plant) | | |
|---|---|---|---|---|---|
| | Shoot | Root | Shoot | Root | Total |
| Uninoculated control | $19.1^{d}$ | $21.4^{e}$ | $101.61^{f}$ | $61.20^{h}$ | $162.81^{g}$ |
| *A. laevis* (Nedl.) | $22.7^{c}$ | $24.3^{cd}$ | $122.35^{e}$ | $72.66^{g}$ | $195.01^{f}$ |
| *Gi. margarita* (ICRISAT) | $25.0^{ab}$ | $26.2^{abc}$ | $174.50^{b}$ | $96.15^{c}$ | $270.65^{c}$ |
| *G. caledonicum* (Nedl.) | $25.3^{a}$ | $25.8^{abcd}$ | $175.08^{b}$ | $90.56^{d}$ | $265.64^{c}$ |
| *G. fasciculatum* (Riv.) | $23.4^{bc}$ | $25.4^{bcd}$ | $157.01^{c}$ | $84.33^{e}$ | $241.34^{d}$ |
| *G. intraradices* (Local) | $22.8^{c}$ | $23.9^{d}$ | $120.84^{e}$ | $69.55^{g}$ | $190.39^{f}$ |
| *G. leptotichum* (Local) | $22.7^{c}$ | $24.1^{d}$ | $122.58^{e}$ | $72.78^{g}$ | $195.36^{f}$ |
| *G. macrocarpum* (Local) | $22.9^{c}$ | $24.2^{cd}$ | $147.02^{d}$ | $76.96^{f}$ | $223.98^{e}$ |
| *G. mosseae* (Local) | $26.2^{a}$ | $27.5^{a}$ | $188.38^{a}$ | $107.80^{a}$ | $296.18^{a}$ |
| *S. calospora* (ICRISAT) | $25.1^{ab}$ | $26.9^{ab}$ | $178.71^{b}$ | $102.22^{b}$ | $280.96^{b}$ |

Legend as in Table 1

The shoot zinc concentration was maximum in Glomus mosseae inoculated plants (26.2 ppm), although the plants inoculated with Scutellospora calospora (25.1 ppm), Glomus caledonicum (25.3 ppm) and Gigaspora margarita (25.0 ppm) were on par with each other.

**Root zinc**

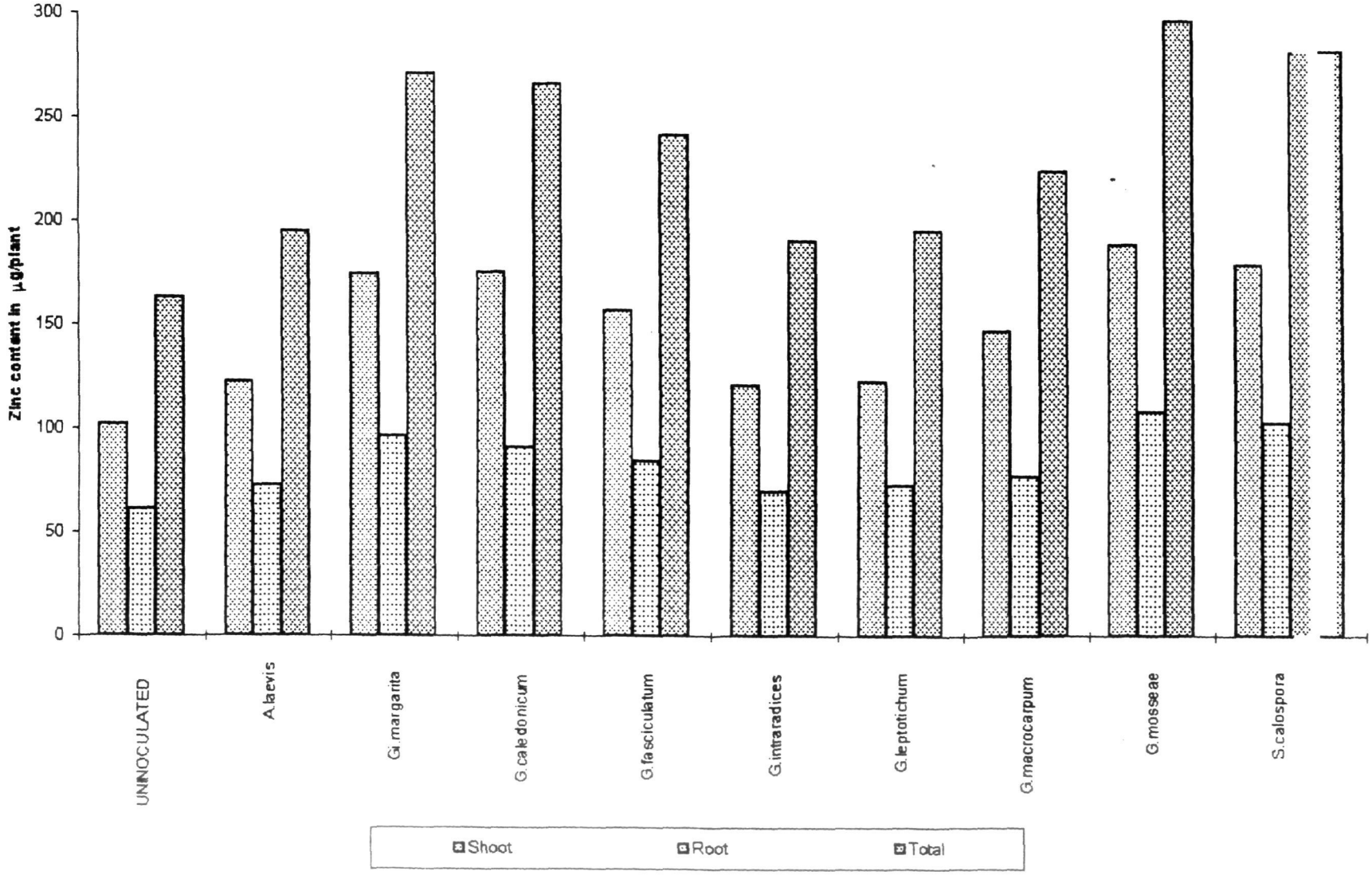
The root zinc content of all the inoculated plants were significantly higher than the control plants. Maximum content of 107.80 µg/pl was observed in Glomus mosseae inoculated plants, followed by Scutellospora calospora (102.22 µg/pl) and Gigaspora margarita (96.15 µg/pl) inoculated plants. Control plants had zinc content of 61.20 µg/pl.

The root zinc concentration did not show appreciable difference between treatments although the control plants accumulated significantly low zinc concentration (21.4 ppm). The Glomus mosseae inoculated plants recorded highest zinc concentration of 27.5 ppm, followed by Scutellospora calospora (26.9 ppm).

**Total zinc**

Maximum total zinc accumulation in the plant was observed in the Glomus mosseae inoculated plants (296.18 µg/pl), whereas the control plants accumulated the least zinc content of 162.81 µg/pl. The next best fungi

Fig : 6. Influence of different VAM fungi on Zinc uptake by Acacia holosericea



which accumulated more zinc were Scutellospora calospora (280.93 µg/pl), Gigaspora margarita (270.65 µg/pl) and Glomus caledonicum (265.64 µg/pl). The last two fungi were statistically on par with each other.

### 4.1.7. Influence of different VAM fungi on uptake of copper by the host

The effect of inoculation of different VAM fungi on uptake of copper is given in Table 6.

**Shoot copper**

The plants inoculated with Glomus mosseae recorded highest copper content (71.18 µg/pl) followed by those inoculated with Scutellospora calospora (69.78 µg/pl). Both were on par with each other (Fig.7). The next best fungi enhancing uptake of copper were Gigaspora margarita (68.40 µg/pl) and Glomus caledonicum (63.66 µg/pl). All the treatments were significantly higher than the uninoculated control.

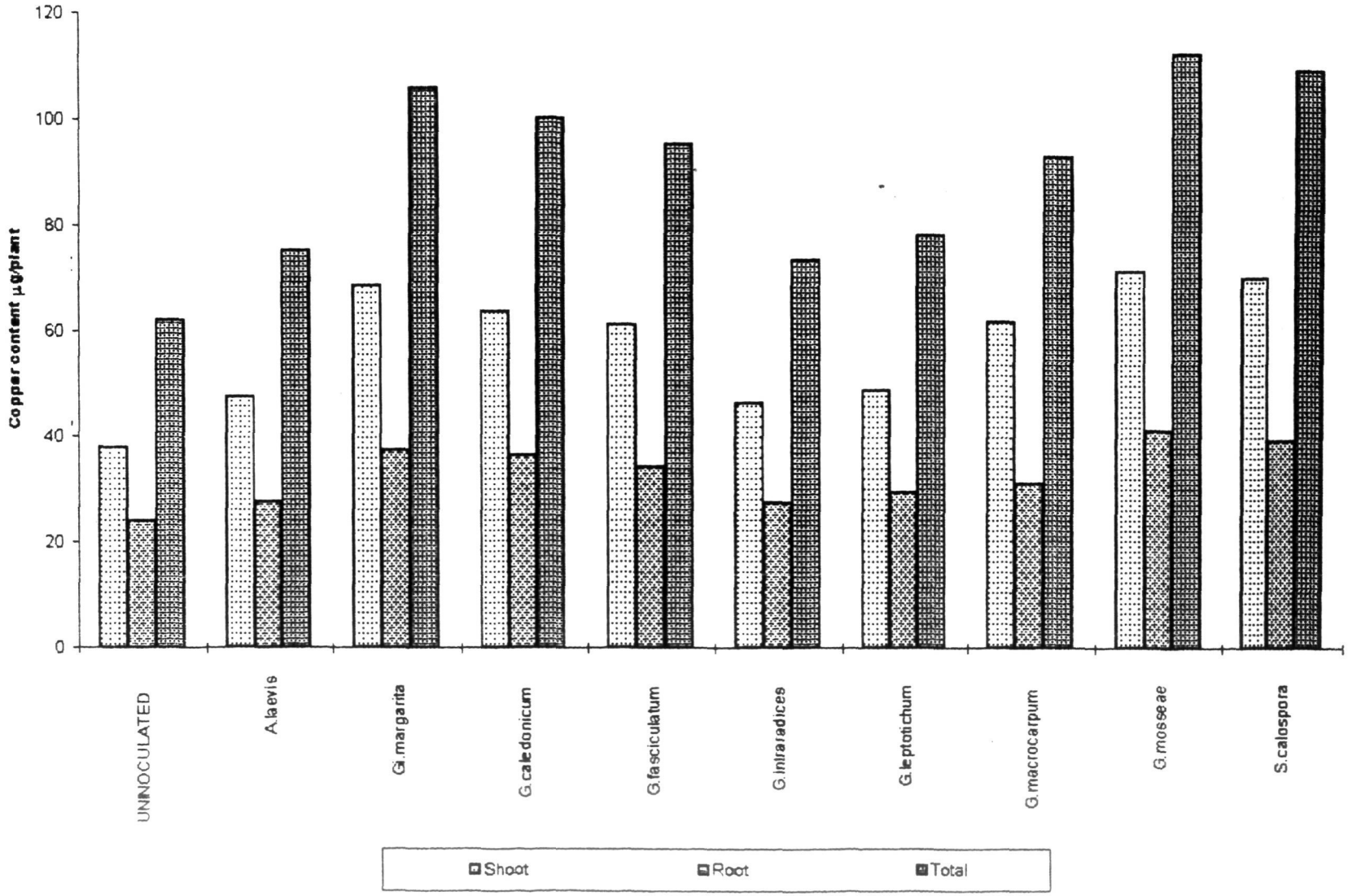
The shoot copper concentration was maximum in Glomus mosseae inoculated plants (9.9 ppm) followed by Scutellospora calospora (9.8 ppm) and Gigaspora margarita (9.8 ppm) inoculated plants. All the three treatments were on par with each other.

**Table 6 Influence of different VAM fungi on copper uptake by *Acacia holosericea***

| VAM fungi | Copper concentration (ppm) | | Copper content (µg/plant) | | |
|---|---|---|---|---|---|
| | Shoot | Root | Shoot | Root | Total |
| Uninoculated control | $7.1^{e}$ | $8.4^{c}$ | $37.77^{g}$ | $24.02^{h}$ | $61.79^{g}$ |
| *A. laevis* (Nedl.) | $8.8^{d}$ | $9.2^{b}$ | $47.43^{ef}$ | $27.51^{g}$ | $74.94^{ef}$ |
| *Gi. margarita* (ICRISAT) | $9.8^{ab}$ | $10.2^{a}$ | $68.40^{b}$ | $37.43^{c}$ | $105.83^{b}$ |
| *G. caledonicum* (Nedl.) | $9.2^{abcd}$ | $10.4^{a}$ | $63.66^{c}$ | $36.50^{c}$ | $100.16^{c}$ |
| *G. fasciculatum* (Riv.) | $9.1^{bcd}$ | $10.3^{a}$ | $61.06^{d}$ | $34.20^{d}$ | $95.26^{d}$ |
| *G. intraradices* (Local) | $8.7^{d}$ | $9.4^{b}$ | $46.11^{f}$ | $27.35^{g}$ | $73.46^{f}$ |
| *G. leptotichum* (Local) | $9.0^{cd}$ | $9.7^{ab}$ | $48.60^{e}$ | $29.29^{f}$ | $77.89^{e}$ |
| *G. macrocarpum* (Local) | $9.6^{abc}$ | $9.8^{ab}$ | $61.63^{cd}$ | $31.16^{e}$ | $92.79^{d}$ |
| *G. mosseae* (Local) | $9.9^{a}$ | $10.5^{a}$ | $71.18^{a}$ | $41.16^{a}$ | $112.34^{a}$ |
| *S. calospora* (ICRISAT) | $9.8^{ab}$ | $10.3^{a}$ | $69.78^{ab}$ | $39.14^{b}$ | $108.92^{b}$ |

Legend as in Table 1

Fig : 7. Influence of different VAM fungi on Copper uptake by Acacia holosericea



**Root copper**

The root copper content was found to be more in Glomus mosseae inoculated plants (41.16 µg/pl) while the plants inoculated with Scutellospora calospora, Gigaspora margarita and Glomus caledonicum had 38.14 µg/pl, 37.43 µg/pl and 36.50 µg/pl respectively.

The root copper concentration was maximum in Glomus mosseae inoculated plants (10.5 ppm) while all the treatments, except Acaulospora laevis and Glomus intraradices treated plants were on par with each other.

**Total copper**

The total copper content as shown in the table varied between treatments. The maximum total copper content was recorded in plants inoculated with Glomus mosseae (112.34 µg/pl) followed by plants inoculated with Scutellospora calospora (108.92 µg/pl), Gigaspora margarita (105.83 µg/pl) and Glomus caledonicum (100.15 µg/pl). The lowest copper content was observed in uninoculated control plants (61.79 µg/pl). Further, all the inoculated treatments were significantly higher than the uninoculated control.

### 4.1.8. Influence of VAM fungi on per cent mycorrhizal root colonization

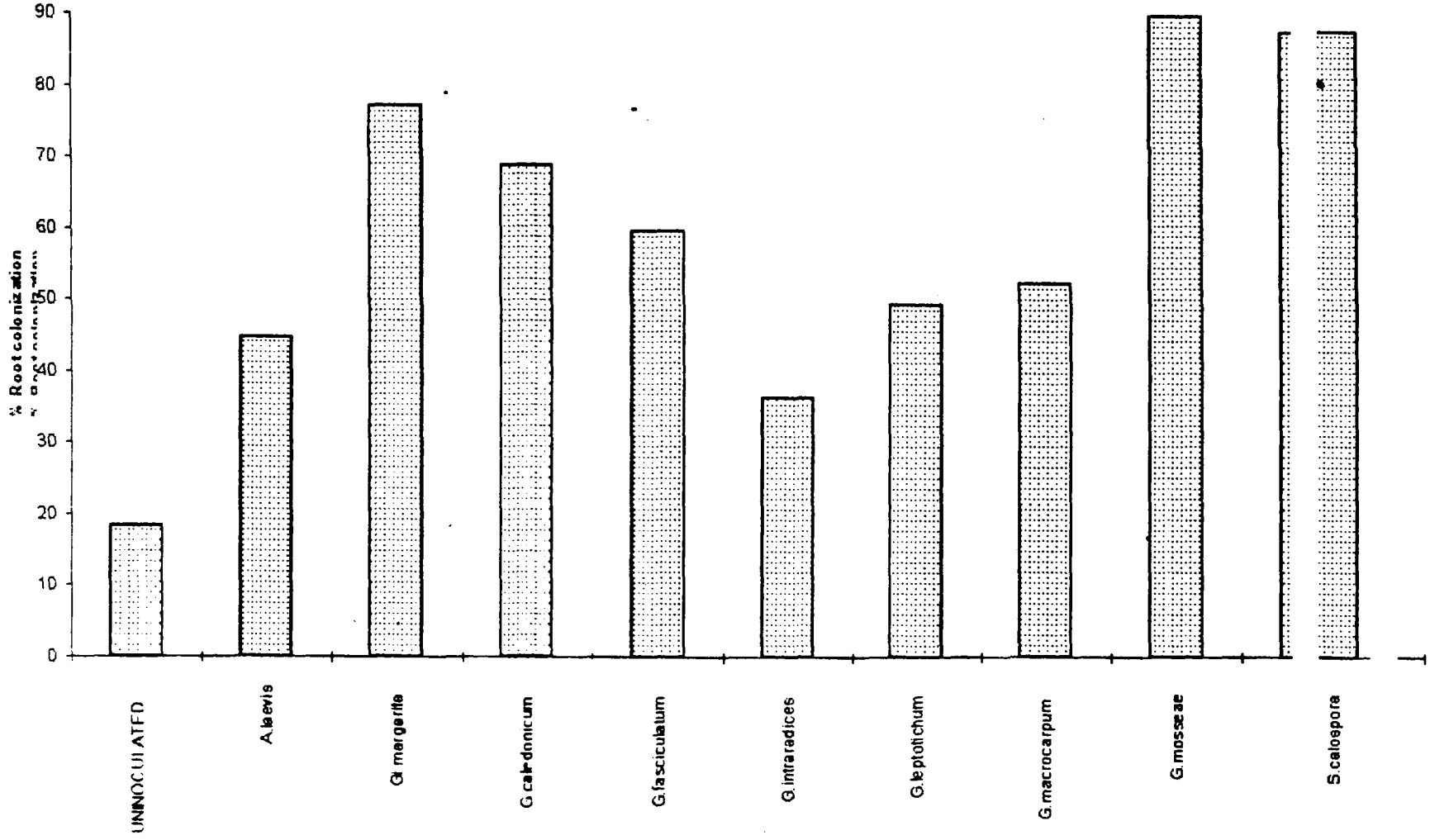
The per cent mycorrhizal root colonization was significantly higher in all the treatments compared to

**Table 7 Influence of different VAM fungi on mycorrhizal root colonization, spore numbers in soil and percent aggregation of soil planted with *Acacia holosericea***

| VAM fungi | % root colonization | Spore number/ 25 ml soil | % aggregation of soil |
|---|---|---|---|
| Uninoculated control | 18.310$^{h}$ | 29$^{i}$ | 18$^{h}$ |
| *A. laevis* (Nedl.) | 44.780$^{f}$ | 128$^{g}$ | 36$^{f}$ |
| *Gi. margarita* (ICRISAT) | 77.190$^{b}$ | 104$^{h}$ | 39$^{e}$ |
| *G. caledonicum* (Nedl.) | 68.720$^{c}$ | 136$^{f}$ | 48$^{b}$ |
| *G. fasciculatum* (Riv.) | 59.580$^{d}$ | 174$^{c}$ | 40$^{e}$ |
| *G. intraradices* (Local) | 36.250$^{g}$ | 162$^{d}$ | 42$^{d}$ |
| *G. leptotichum* (Local) | 49.280$^{e}$ | 189$^{b}$ | 45$^{c}$ |
| *G. macrocarpum* (Local) | 52.200$^{e}$ | 153$^{e}$ | 42$^{d}$ |
| *G. mosseae* (Local) | 89.620$^{a}$ | 207$^{a}$ | 52$^{a}$ |
| *S. calospora* ICRISAT) | 87.180$^{a}$ | 196$^{b}$ | 32$^{g}$ |

Legend as in Table 1

**Fig : 8. Influence of different VAM fungi on mycorrhizal root colonization of Acacia holoser icea**



control. Glomus mosseae inoculated plants showed the highest mycorrhizal root colonization (89.62 %) followed by Scutellospora calospora (87.18 %) both treatments being statistically on par with each other. The next best fungi were Gigaspora margarita (77.19 %) and Glomus caledonicum (68.72 %). The lowest per cent colonization was observed in uninoculated control which showed only 18.31 % colonization (Table 7 and Fig.8).

### 4.1.6. Influence of VAM fungi on number of mycorrhizal spores in root zone soil

The number of mycorrhizal spores present in 25 ml of root zone soil from different treatments is given in Table 6. All the inoculated treatments were significantly different from the uninoculated control. Glomus mosseae treated plants had maximum number of spores (207) followed by Scutellospora calospora (196), Glomus leptotichum (189), Glomus fasciculatum (174) and Glomus intraradices (162) treated plants. All the treatments were significantly different from each other. The uninoculated control plants had the minimum number of spores (29) (Fig.9).

### 4.1.10. Influence of VAM fungi on per cent soil aggregation

Table 7 shows the per cent soil aggregation as influenced by mycorrhizal inoculation with different VAM fungi. All the treatments were statistically significant. The highest per cent aggregation was observed in the soil

Fig : 9. Influence of different VAM fungi on mycorrhizal spore numbers in the root zone soil of Acacia holosericea

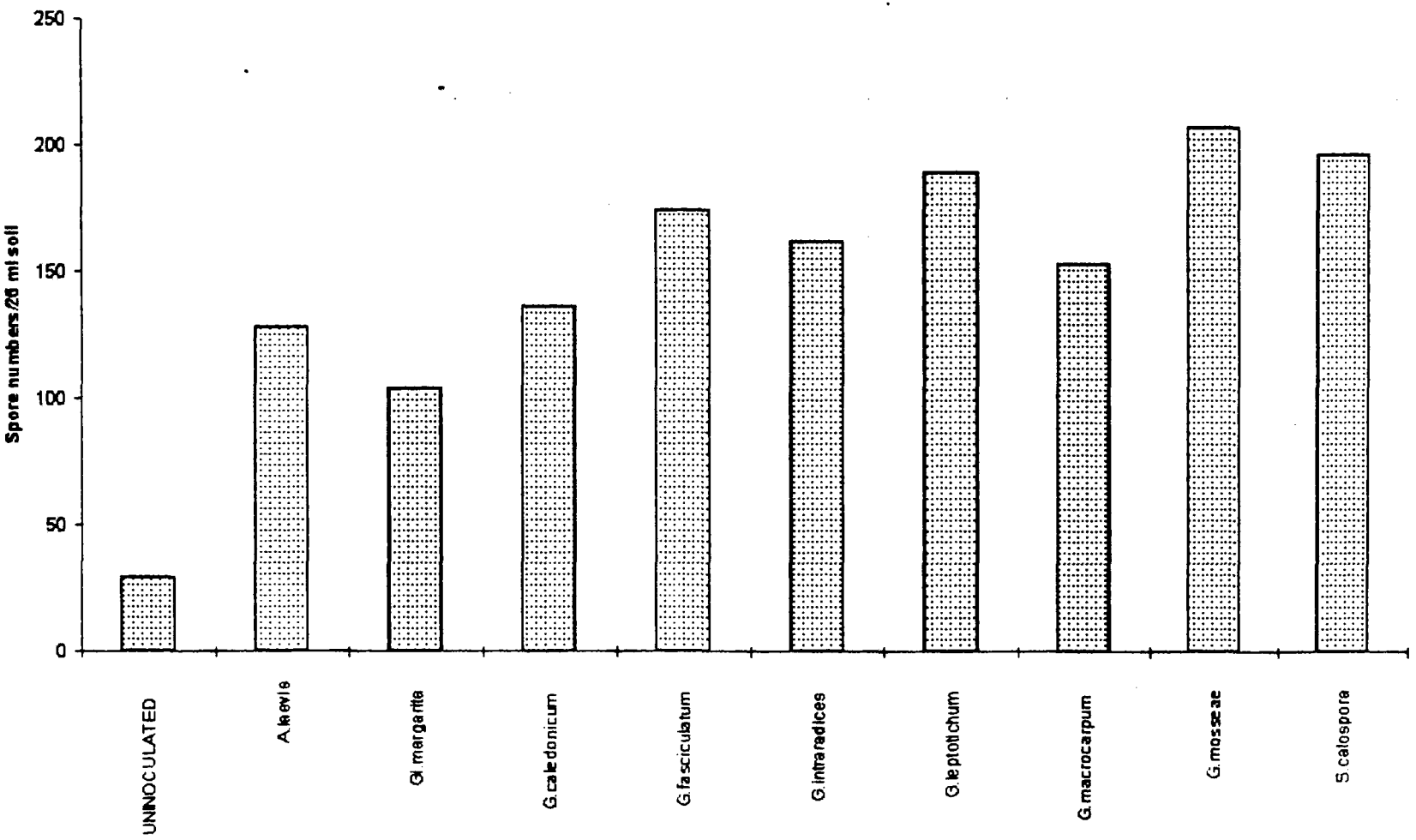
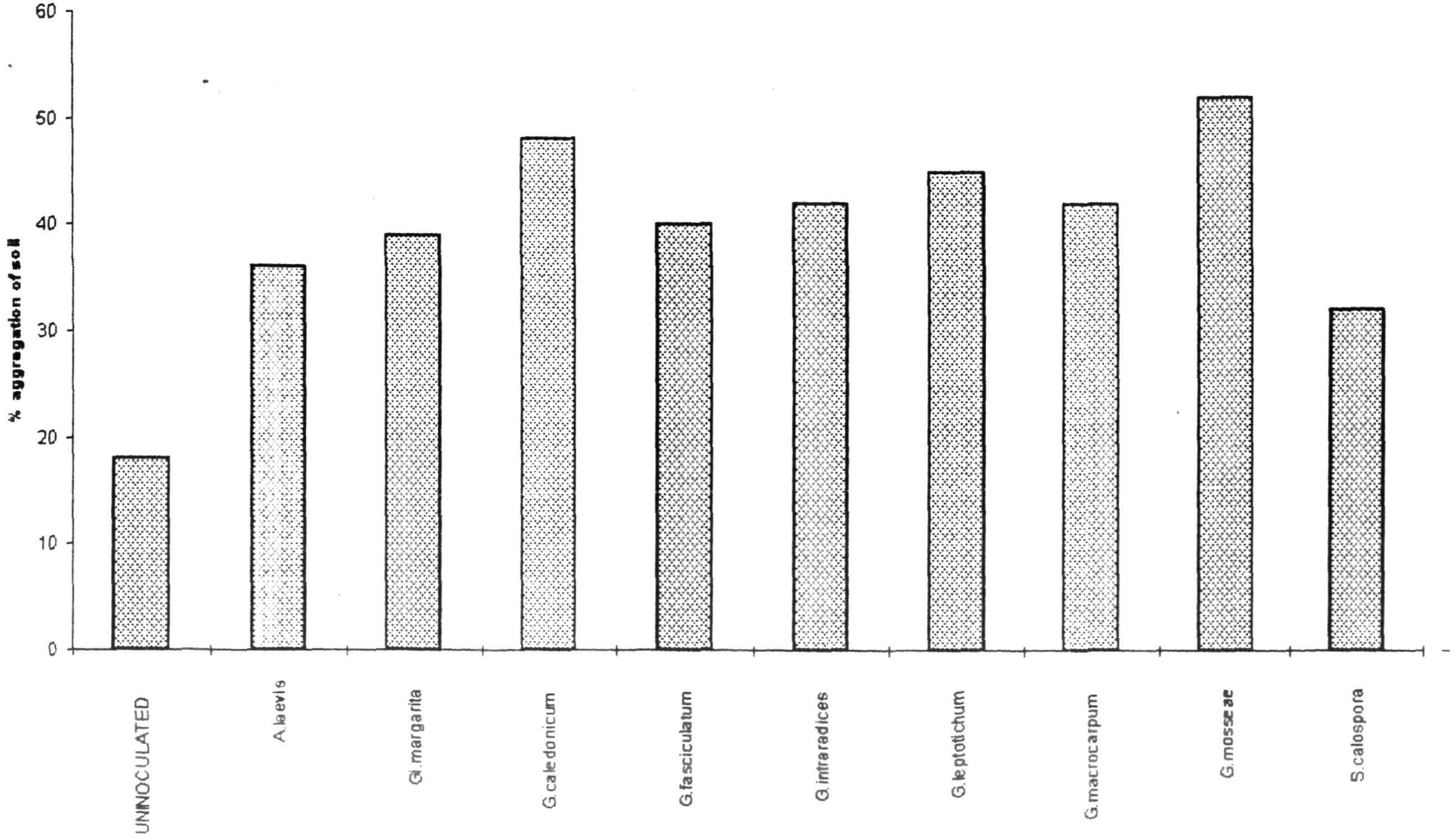


Fig : 10. Influence of different VAM fungi per cent aggregation of soil planted with Acacia holosericea



raised in the plants inoculated with Glomus mosseae (52 %) followed by Glomus caledonicum (48 %), Glomus leptotichum (45 %), Glomus macrocarpum (42 %) and Glomus intraradices (42 %) inoculated plants. The treatments Glomus macrocarpum and Glomus intraradices inoculated plants and Glomus fasciculatum and Gigaspora margarita inoculated plants were statistically on par with each other (Fig.10).

**4.1.11. Influence of VAM on sturdiness quotient**

The sturdiness quotient was more in Glomus mosseae inoculated plants (17.4) followed by plants inoculated with Glomus fasciculatum, Gigaspora margarita (17.2), Glomus caledonicum (17.2) and Scutellospora calospora (17.2) (Table 8 and Fig.11). As the values are absolute, statistical analysis was not done.

**4.1.12. Influence of VAM on biovolume index**

As shown in Table 8 biovolume index was more in Glomus mosseae inoculated plants (3245.4) followed by Scutellospora calospora (3162.8) and Gigaspora margarita (3036.1) treated plants while the uninoculated control plants had an index of 2442.2 (Fig.12).

**4.1.13. Influence of VAM on quality index**

Quality index was more in plants inoculated with Glomus mosseae (0.577) followed by Scutellospora calospora (0.574) and Gigaspora margarita (0.558) inoculated plants. The

**Table 8 Influence of different VAM fungi on sturdiness quotient (SQ) biovolume index (VI) and quality index (QI) of *Acacia holosericea***

| VAM fungi | Sturdiness quotient (SQ) | Biovolume index (VI) | Quality index (QI) |
|---|---|---|---|
| Uninoculated control | 16.4 | 2442.2 | 0.446 |
| *A. laevis* (Nedl.) | 16.9 | 2527.2 | 0.449 |
| *Gi. margarita* (ICRISAT) | 17.2 | 3036.1 | 0.558 |
| *G. caledonicum* (Nedl.) | 17.2 | 2975.9 | 0.544 |
| *G. fasciculatum* (Riv.) | 17.3 | 2678.7 | 0.518 |
| *G. intraradices* (Local) | 16.6 | 2469.4 | 0.446 |
| *G. leptotichum* (Local) | 16.9 | 2579.5 | 0.450 |
| *G. macrocarpum* (Local) | 16.7 | 2738.1 | 0.512 |
| *G. mosseae* (Local) | 17.4 | 3245.4 | 0.577 |
| *S. calospora* ICRISAT) | 17.2 | 3162.8 | 0.574 |

Legend as in Table 1

**Fig : 11. Influence of different VAM fungi on sturdiness quotient of Acacia holosericea**

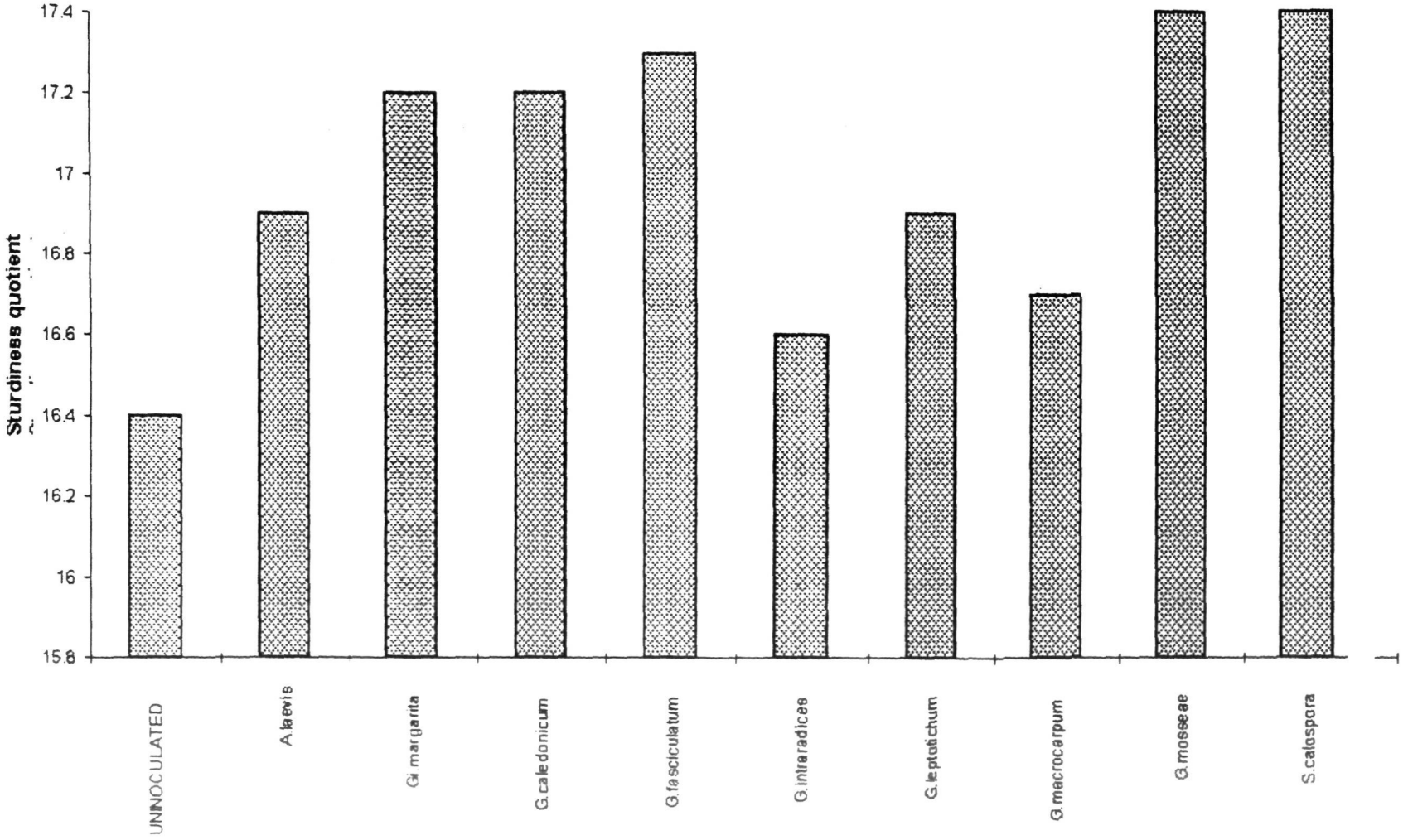


Fig :12. Influence of different VAM fungi on bio volume index of Acacia holosericea

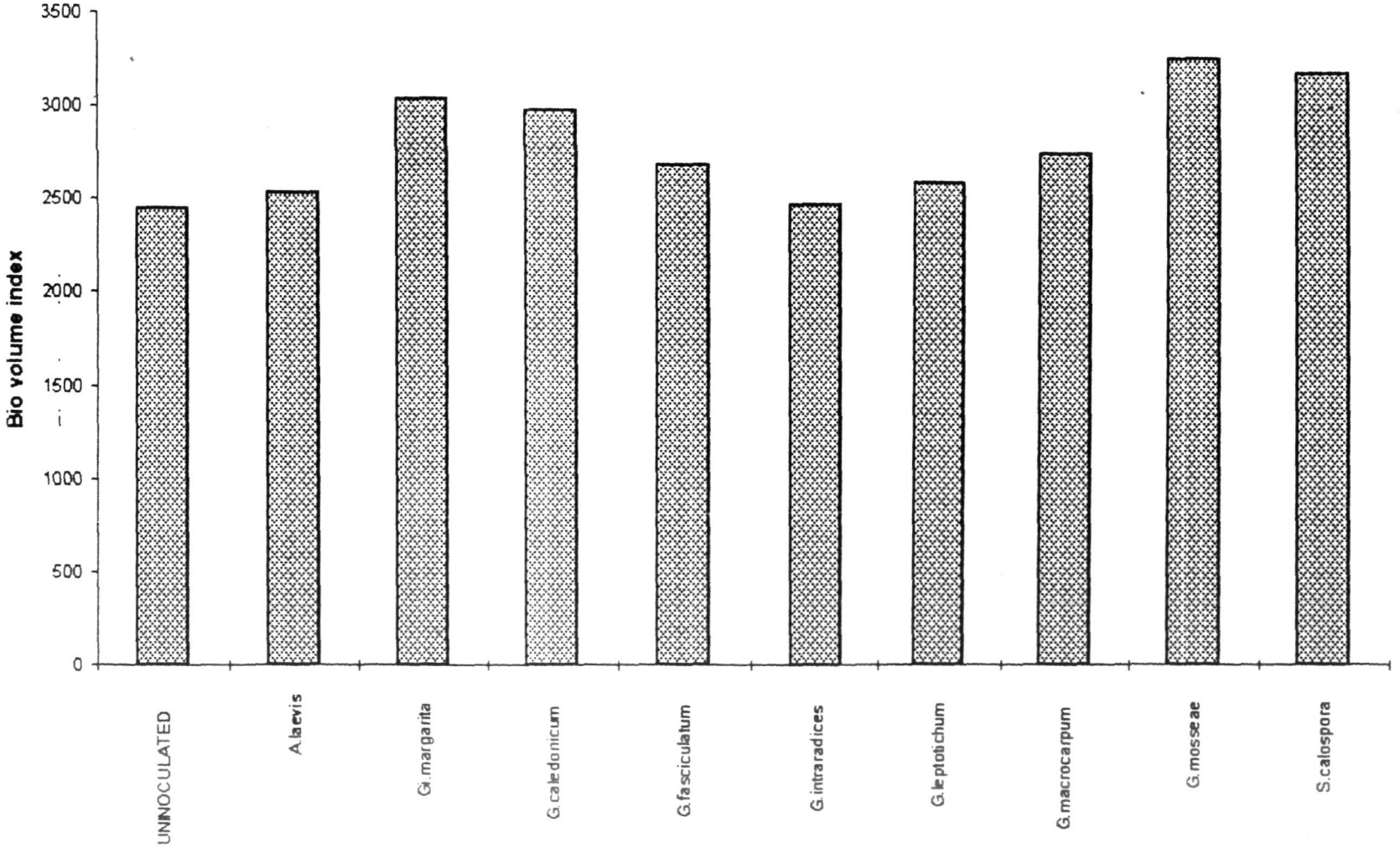
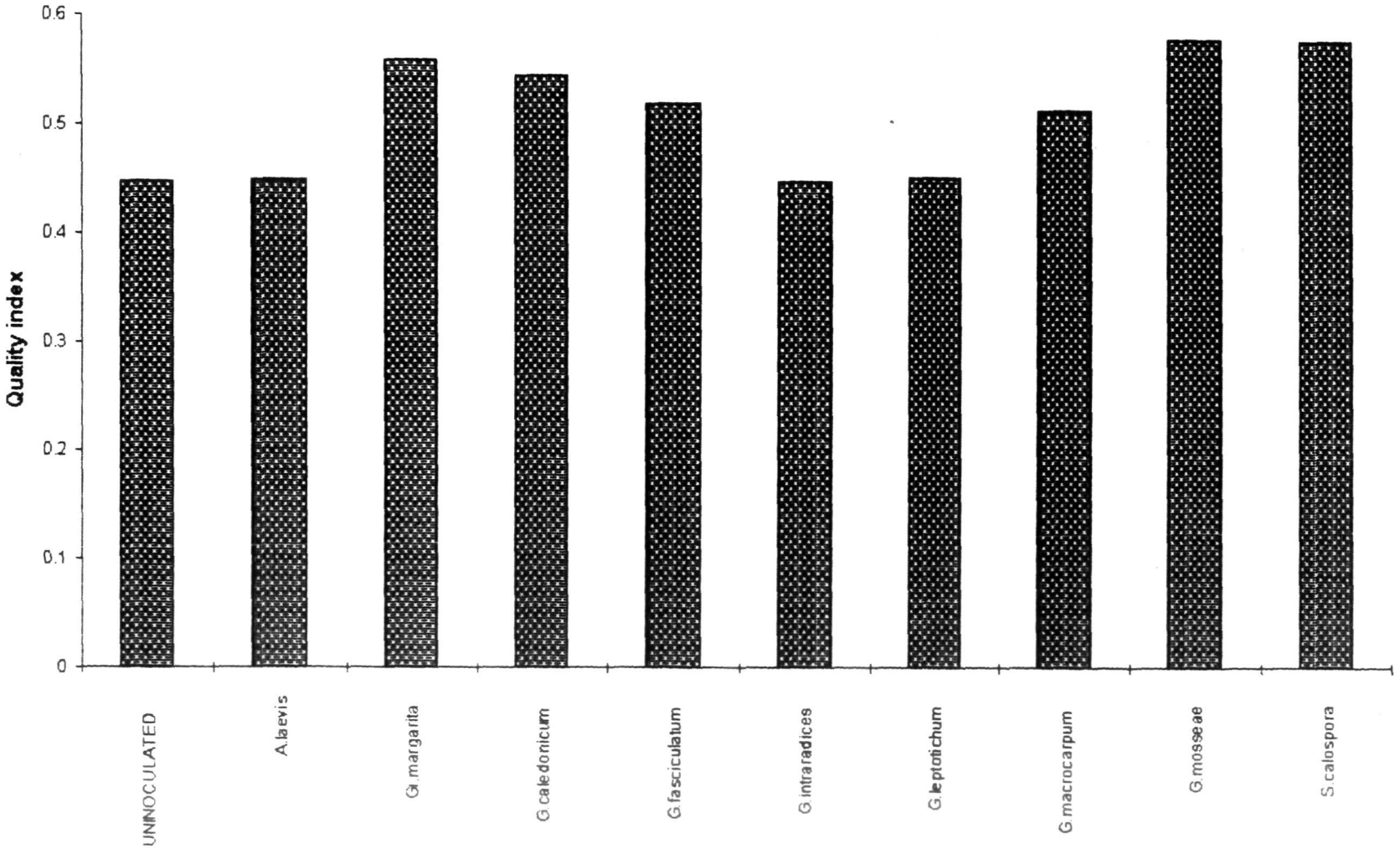


Fig : 13. Influence of different VAM fungi on quality index of Acacia holosericea



uninoculated control plants had a quality index of only 0.466 (Table 8 and Fig.13).

**Experiment 2**

## 4.2. Selection of efficient VAM fungi for Albizzia lebbeck

The result of different VAM fungi on plant growth and mycorrhizal parameters are given below.

### 4.2.1. Influence of VAM fungi on plant height

The plant height recorded on 90th, 120th, 180th days after transplanting were statistically significant.

As shown in Table 9, on 90 DAT, Glomus fasciculatum inoculated plants had maximum plant height of 34.16 cm, while the uninoculated control plants were only 23.40 cm. Glomus fasciculatum and Glomus macrocarpum inoculated plants were on par with each other, whereas the plants inoculated with Glomus mosseae, did not differ significantly from the uninoculated control (Fig.14).

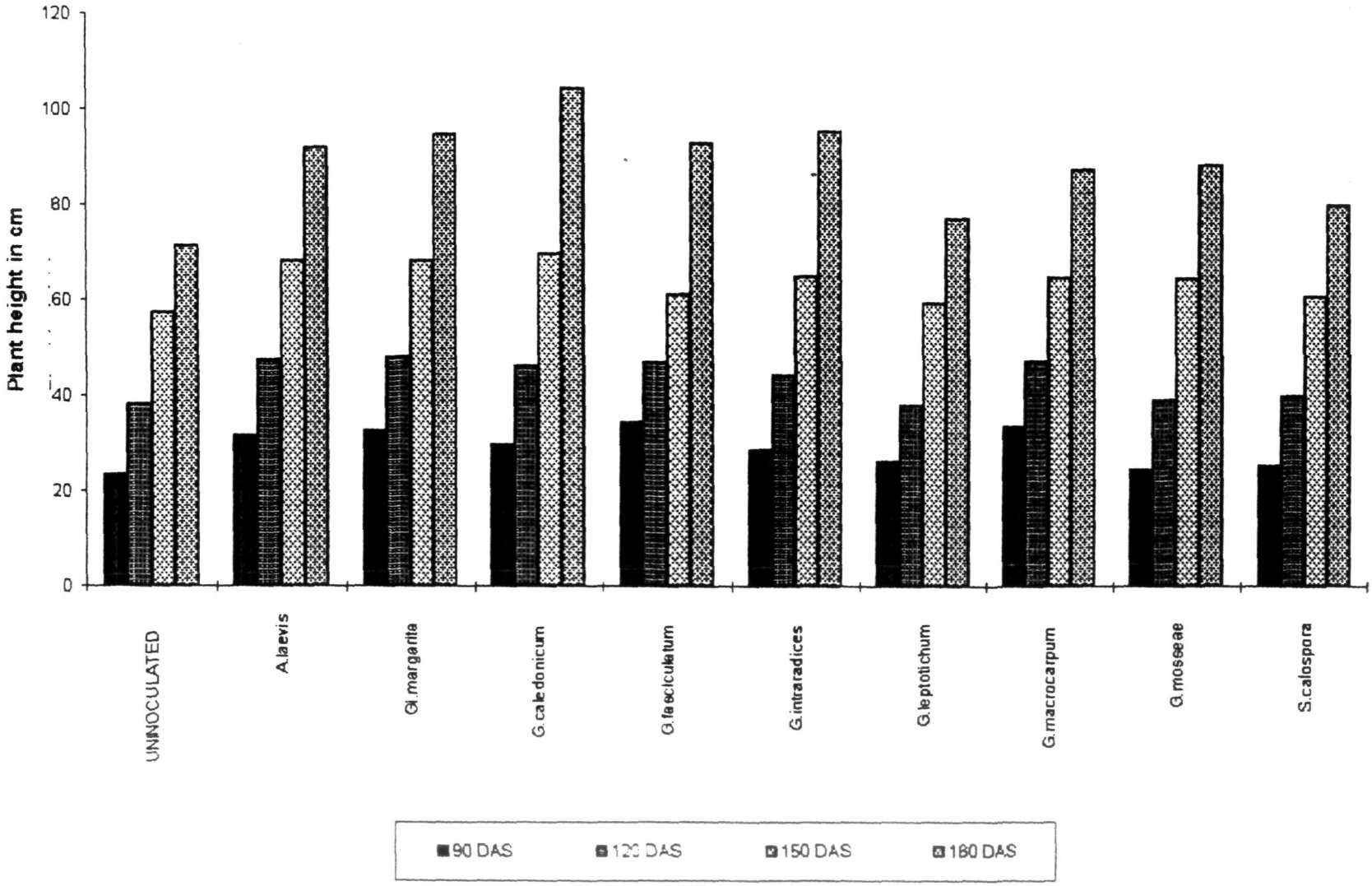
On 120 DAT, maximum plant height was observed in Gigaspora margarita inoculated plants (47.87 cm) followed by Acaulospora laevis (47.33 cm), Glomus macrocarpum (47.00 cm) and Glomus fasciculatum (46.80 cm) inoculated plants. All these four treatments were statistically on par with each other.

**Table 9 Influence of different VAM fungi on plant height (cm) of *Albizzia lebbeck***

| VAM fungi | 90 DAS* | 120 DAS | 150 DAS | 180 DAS |
|---|---|---|---|---|
| Uninoculated control | $23.40^{g}$ | $38.04^{e}$ | $57.31^{e}$ | $71.30^{e}$ |
| *A. laevis* (Nedl.) | $31.40$ | $47.33^{ab}$ | $68.27^{ab}$ | $92.17^{bc}$ |
| *Gi. margarita* (ICRISAT) | $32.55^{bc}$ | $47.87^{a}$ | $68.30^{ab}$ | $94.87^{b}$ |
| *G. caledonicum* (Nedl.) | $29.52^{d}$ | $46.13^{b}$ | $69.64^{a}$ | $104.46^{a}$ |
| *G. fasciculatum* (Riv.) | $34.16^{a}$ | $46.80^{ab}$ | $61.20^{cde}$ | $92.80^{bc}$ |
| *G. intraradices* (Local) | $28.56^{d}$ | $44.07^{c}$ | $64.93^{bc}$ | $95.45^{b}$ |
| *G. leptotichum* (Local) | $26.10^{e}$ | $37.89^{e}$ | $59.18^{e}$ | $76.99^{d}$ |
| *G. macrocarpum* (Local) | $33.37^{ab}$ | $47.00^{ab}$ | $64.77^{bc}$ | $87.37^{c}$ |
| *G. mosseae* (Local) | $24.30^{fg}$ | $38.79^{de}$ | $64.40^{bcd}$ | $88.09^{c}$ |
| *S. calospora* (ICRISAT) | $25.21^{ef}$ | $39.80^{d}$ | $60.42^{de}$ | $79.75^{d}$ |

Legend as in Table 1

Fig : 14. Influence of different VAM fungi on plant height (cm) of Albizzia lebbeck



On 150 DAT, Glomus caledonicum inoculated plants showed highest plant height (69.64 cm) followed by Gigaspora margarita (68.30 cm) and Acaulospora laevis (68.27 cm). The plants inoculated with Glomus fasciculatum (61.20 cm), Scutellospora calospora (60.42 cm) and Glomus leptotichum were on par with the uninoculated control plants.

Higher plant height was observed in plants inoculated with Glomus caledonicum (104.46 cm) which was significantly higher than the other treatments. The next best fungi were Glomus intraradices (95.45 cm), Gigaspora margarita (94.87 cm), Glomus fasciculatum (92.80 cm) and Acaulospora laevis (92.17 cm). All the four species were statistically on par with each other in improving plant height. All the inoculated plants were significantly different compared to uninoculated control.

### 4.2.2. Influence of VAM fungi on stem girth

Stem girth as influenced by different VAM fungi are given in Table 10.

On 90 DAT, Glomus leptotichum inoculated plants had highest stem girth (0.285 cm) followed by Glomus intraradices (0.279 cm), Acaulospora laevis (0.277 cm) and Scutellospora calospora, all the four treatments being on par with each other. But the stem girth of plants

Table 10 Influence of different VAM fungi on stem girth (cm) of *Albizzia lebbeck*

| VAM fungi | 90 DAS | 120 DAS | 150 DAS | 180 DAS |
|---|---|---|---|---|
| Uninoculated control | $0.254^{d}$ | $0.325^{d}$ | $0.456^{b}$ | $0.560^{c}$ |
| *A. laevis* (Nedl.) | $0.277^{ab}$ | $0.347^{c}$ | $0.470^{ab}$ | $0.598^{ab}$ |
| *Gi. margarita* (ICRISAT) | $0.257^{d}$ | $0.349^{bc}$ | $0.476^{ab}$ | $0.615^{a}$ |
| *G. caledonicum* (Nedl.) | $0.272^{bc}$ | $0.334^{d}$ | $0.465^{ab}$ | $0.600^{ab}$ |
| *G. fasciculatum* (Riv.) | $0.262^{cd}$ | $0.375^{a}$ | $0.487^{a}$ | $0.591^{ab}$ |
| *G. intraradices* (Local) | $0.279^{ab}$ | $0.360^{bc}$ | $0.483^{a}$ | $0.607^{a}$ |
| *G. leptotichum* (Local) | $0.285^{a}$ | $0.376^{a}$ | $0.489^{a}$ | $0.597^{a}$ |
| *G. macrocarpum* (Local) | $0.258^{d}$ | $0.387^{a}$ | $0.491^{a}$ | $0.616^{a}$ |
| *G. mosseae* (Local) | $0.261^{cd}$ | $0.362^{b}$ | $0.484^{a}$ | $0.587^{ab}$ |
| *S. calospora* (ICRISAT) | $0.276^{ab}$ | $0.350^{bc}$ | $0.478^{ab}$ | $0.591^{ab}$ |

Legend as in Table 1

Fig : 15. Influence of different VAM fungi on stem girth (cm) of Albizzia lebbeck

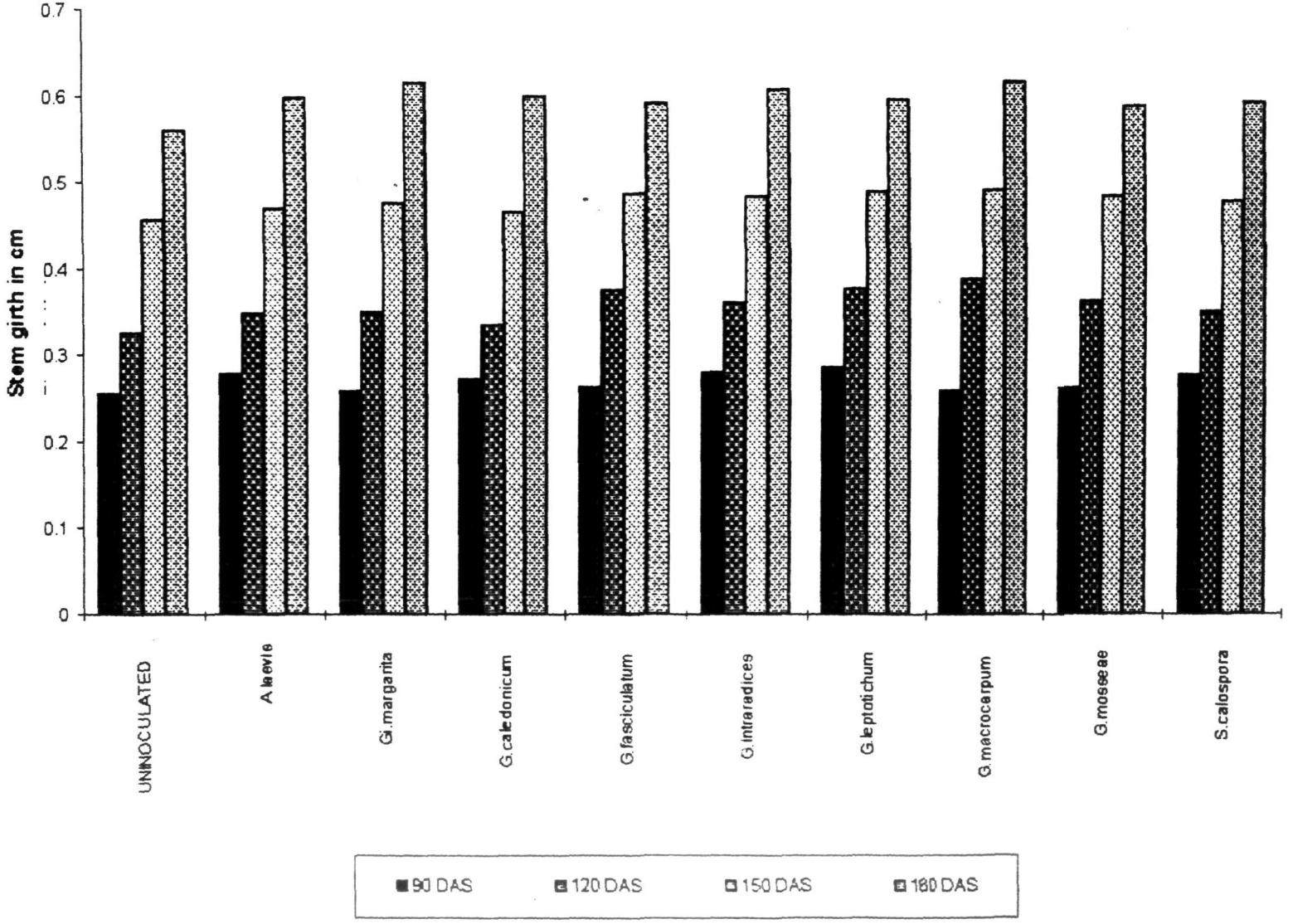


***Plate** 3 & 4. Influence of different VAM fungi on the growth of Albizzia lebbeck.*

*C - UNINOCULATED CONTROL*

*1 - Glomus macrocarpum*

*2 - Glomus caledonicum*

*3 - Glomus leptotichum*

*4 - Glomus intraradices*

*5 - Glomus mosseae*

*6 - Glomus fasciculatum*

*7 - Gigaspora margarita*

*8 - Scutellospora calospora*

*9 - Acaulospora laevis*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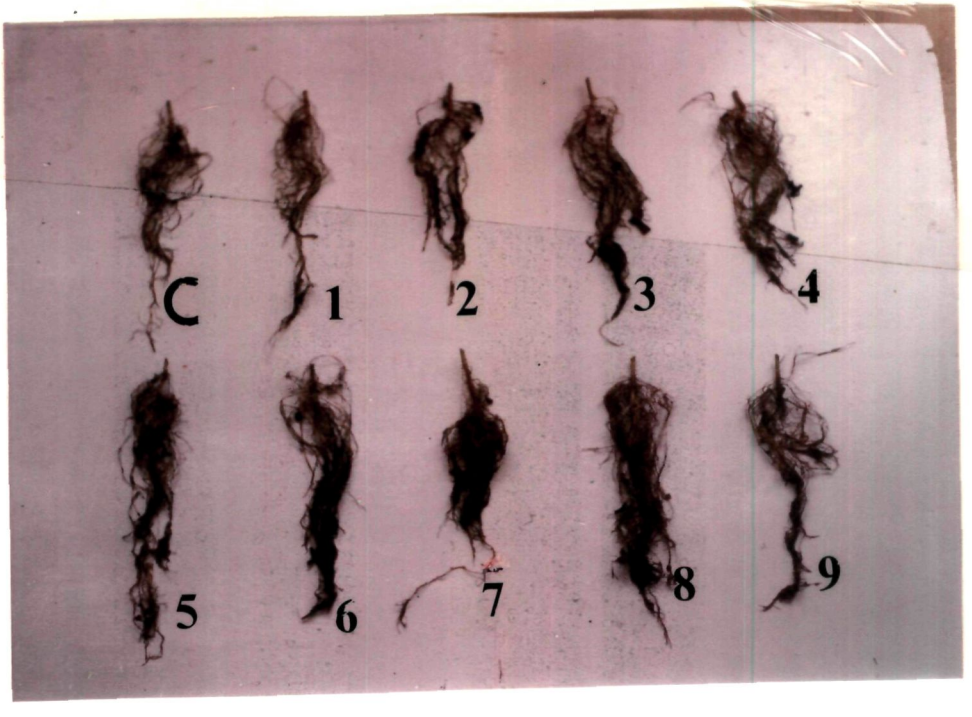
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inoculated with other fungi, excepting Glomus caledonicum did not differ significantly from control plants (Fig.15).

On 120 DAT, the maximum stem girth was seen in plants inoculated with Glomus macrocarpum (0.387 cm) followed by Glomus leptotichum (0.376 cm) and Glomus fasciculatum (0.375 cm). All these three fungi were statistically on par with each other (Table 10). However, the minimum stem girth occurred in uninoculated control plants (0.325 cm).

The stem girth of Glomus macrocarpum inoculated plants was maximum (0.491 cm) on 150 DAT. All the other treatments did not differ significantly from each other, excepting uninoculated control. Also the plants inoculated with Acaulospora leavis, Gigaspora margarita, Glomus caledonicum and Scutellospora calospora were statistically on par with the uninoculated control. At harvest, i.e., 180 DAT, the response of plants inoculated with different fungi were similar (Table 10).

### 4.2.3. Influence of VAM fungi on plant biomass

Table 11 gives the shoot, root and total biomass of plants at harvest.

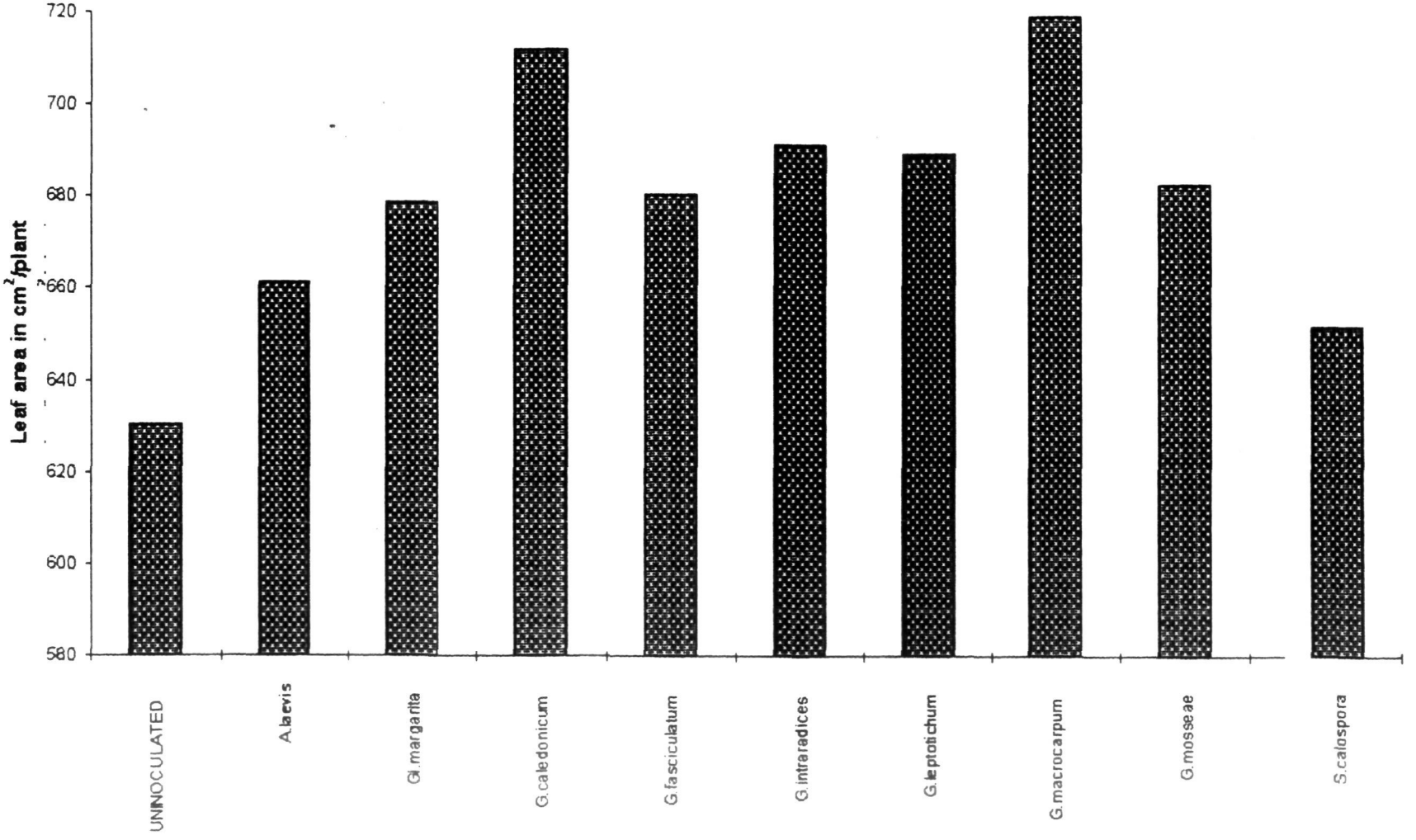
**Shoot biomass:** The maximum shoot dry weight of 8.83 g was found in plants inoculated with Glomus macrocarpum followed by Glomus caledonicum (8.75 g) and Glomus leptotichum (8.59 g), which were on par with each other. The lowest

**Table 11 Influence of different VAM fungi on leaf area, shoot and root dry weight of *Albizzia lebbeck***

| VAM fungi | Leaf area $cm^2$/plant | Dry weight (g/plant) | | |
|---|---|---|---|---|
| | | Shoot | Root | Total |
| Uninoculated control | $630.28^{e}$ | $6.58^{cd}$ | $3.01^{h}$ | $9.59^{g}$ |
| *A. laevis* (Nedl.) | $661.36^{cd}$ | $6.42^{cd}$ | $3.78^{fg}$ | $10.20^{ef}$ |
| *Gi. margarita* (ICRISAT) | $678.67^{cd}$ | $6.67^{c}$ | $3.70^{fg}$ | $10.37^{ef}$ |
| *G. caledonicum* (Nedl.) | $712.12^{ab}$ | $8.75^{a}$ | $4.96^{b}$ | $13.71^{b}$ |
| *G. fasciculatum* (Riv.) | $680.42^{cd}$ | $6.64^{c}$ | $3.82^{ef}$ | $10.46^{e}$ |
| *G. intraradices* (Local) | $691.27^{abc}$ | $7.26^{b}$ | $3.98^{de}$ | $11.24^{d}$ |
| *G. leptotichum* (Local) | $689.20^{abc}$ | $8.59^{a}$ | $4.21^{c}$ | $12.80^{c}$ |
| *G. macrocarpum* (Local) | $719.24^{a}$ | $8.83^{a}$ | $5.39^{a}$ | $14.22^{a}$ |
| *G. mosseae* (Local) | $682.59^{bcd}$ | $7.13^{b}$ | $4.08^{cd}$ | $11.21^{b}$ |
| *S. calospora* (ICRISAT) | $651.43^{de}$ | $6.33^{d}$ | $3.60^{g}$ | $9.93^{fg}$ |

Legend as in Table 1

Fig : 16. Influence of different VAM fungi on leaf area of Albizzia lebbeck



shoot dry weight was seen in uninoculated control plants (6.58 g). However, plants inoculated with Acaulospora laevis, Gigaspora margarita and Scutellospora calospora did not differ significantly from the control plants (Fig.17).

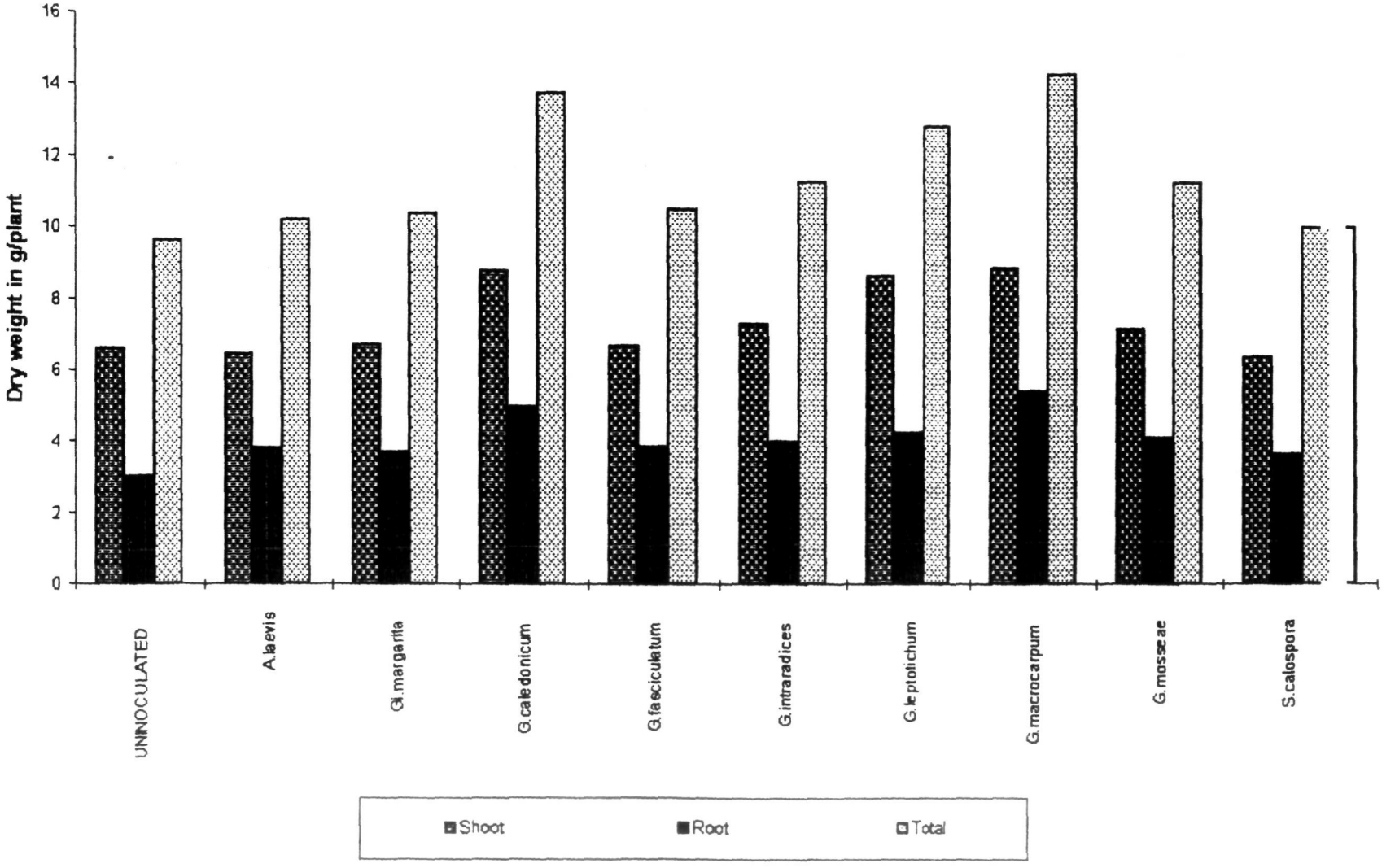
**Root biomass:** The root dry weight was maximum in plants inoculated with Glomus macrocarpum (5.39 g) which was significantly higher than other treatments. Further, plants inoculated with other fungi also had significantly higher biomass compared to the uninoculated control.

**Total biomass:** As shown in Table 11 Glomus macrocarpum inoculated plants had the highest dry weight (14.22 g), and it was significantly higher than all the other treatments. The next best fungi were Glomus caledonicum (13.71 g), Glomus leptotichum (12.80 g), Glomus intraradices (11.24 g) and Glomus mosseae (11.21 g). However, Scutellospora calospora inoculated plants did not differ significantly compared to uninoculated control plants.

### 4.2.4. Influence of VAM fungi on leaf area

The influence of different fungi on leaf area of Albizzia lebbeck is presented in Table 11. All the inoculated treatments had significantly more leaf area compared to the uninoculated treatment (Table 11 and Fig.16).

Fig : 17. Influence of different VAM fungi on shoot and root dry weight of Albizzia Lebbeck



At harvest, maximum leaf area was observed in plants inoculated with Glomus macrocarpum (719.24 $cm^2$) followed by Glomus caledonicum (712.12 $cm^2$), Glomus intraradices (691.27 $cm^2$) and Glomus leptotichum (689.29 $cm^2$). All these treatments were statistically on par with each other. Leaf area of plants inoculated with Scutellospora calospora did not differ significantly from the uninoculated control plants (Table 11).

### 4.2.5. Influence of VAM fungi on uptake of phosphorus by the host

The difference in the phosphorus concentration and content due to inoculation with VAM fungi was significantly more compared to uninoculated treatment (Table 12).

**Shoot phosphorus**

Maximum P content was observed in plants inoculated with Glomus macrocarpum (18.99 mg/pl) followed by Glomus caledonicum (18.29 mg/pl) and Glomus leptotichum, the latter two being on par with each other. The lowest P content was observed in uninoculated control plants (8.42 mg/pl). All the treatments were significantly higher compared to uninoculated control (Fig.18).

The shoot P concentration varied among the different treatments. Although the plants inoculated with Glomus macrocarpum showed highest P concentration (0.215), it was on par with the plants inoculated with Glomus caledonicum

Table 12 Influence of different VAM fungi on phosphorus uptake by *Albizzia lebbeck*

| VAM fungi | P concentration (%) | | P content (mg/plant) | | |
|---|---|---|---|---|---|
| | Shoot | Root | Shoot | Root | Total |
| Uninoculated control | $0.128^{e}$ | $0.136^{e}$ | $8.42^{g}$ | $4.09^{h}$ | $12.5^{h}$ |
| *A. laevis* (Nedl.) | $0.187^{cd}$ | $0.199^{cd}$ | $12.00^{f}$ | $7.52^{f}$ | $19.52^{f}$ |
| *Gi. margarita* (ICRISAT) | $0.195^{bcd}$ | $0.205^{bcd}$ | $13.01^{e}$ | $7.59^{f}$ | $20.60^{e}$ |
| *G. caledonicum* (Nedl.) | $0.209^{ab}$ | $0.220^{ab}$ | $18.29^{b}$ | $10.91^{b}$ | $29.20^{b}$ |
| *G. fasciculatum* (Riv.) | $0.193^{bcd}$ | $0.208^{bcd}$ | $12.82^{e}$ | $7.95^{e}$ | $20.77^{e}$ |
| *G. intraradices* Local) | $0.202^{abc}$ | $0.123^{abc}$ | $14.67^{c}$ | $8.48^{d}$ | $23.15^{d}$ |
| *G. leptotichum* (Local) | $0.207^{ab}$ | $0.211^{bc}$ | $17.78^{b}$ | $8.88^{c}$ | $26.66^{c}$ |
| *G. macrocarpum* (Local) | $0.215^{a}$ | $0.228^{a}$ | $18.99^{a}$ | $12.29^{a}$ | $31.28^{a}$ |
| *G. mosseae* (Local) | $0.196^{bcd}$ | $0.204^{bcd}$ | $13.97^{d}$ | $8.32^{d}$ | $22.29^{d}$ |
| *S. calospora* (ICRISAT) | $0.184^{d}$ | $0.193^{d}$ | $11.32^{f}$ | $6.95^{g}$ | $18.60^{g}$ |

Legend as in Table 1

(0.209), Glomus leptotichum (0.207) and Glomus intraradices. However, all the inoculated plants had significantly higher concentration of phosphorus compared to uninoculated plants.

**Root phosphorus**

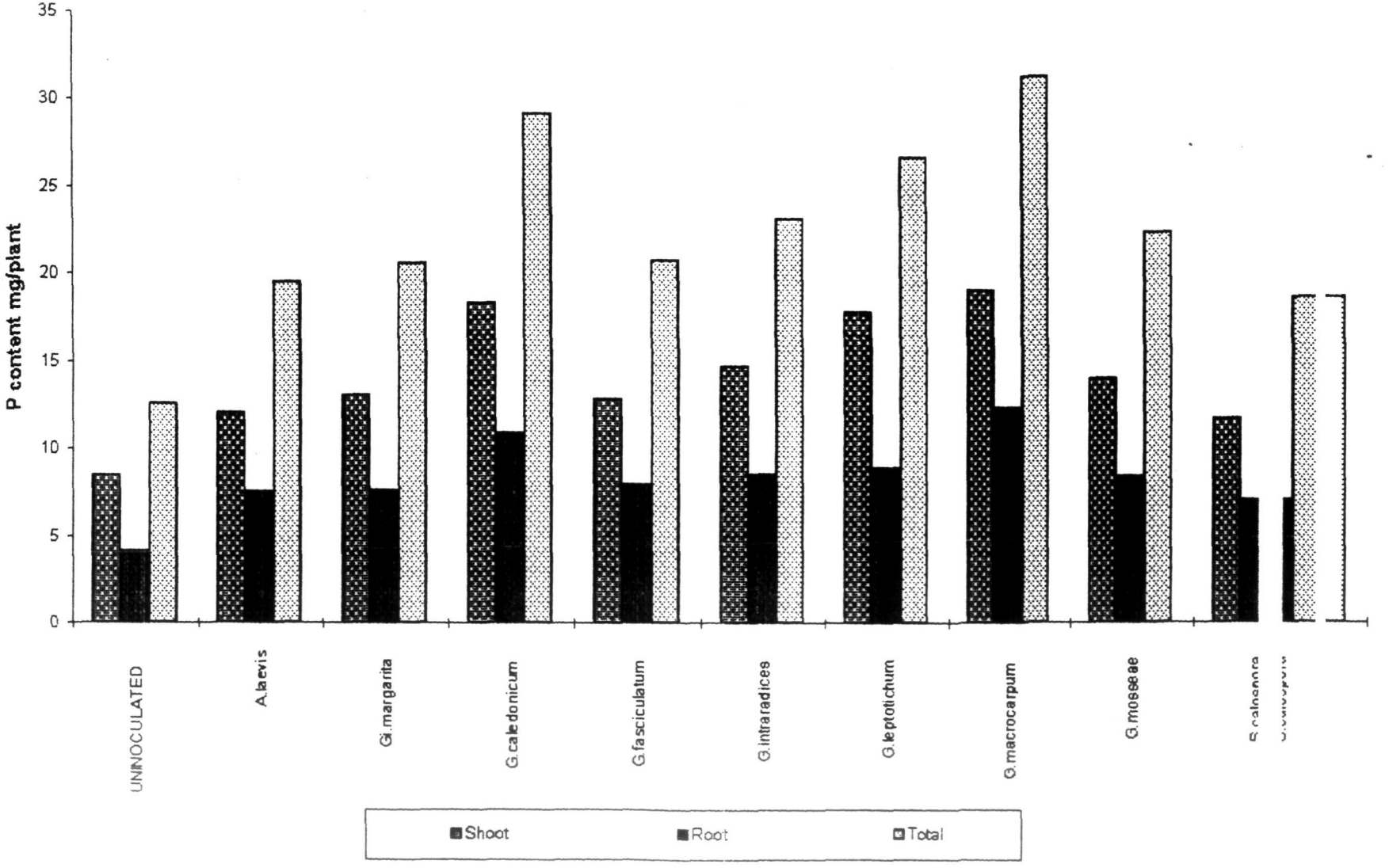
The root phosphorus content of inoculated plants was highly significant compared to control plants. Maximum P content was observed in plants inoculated with Glomus macrocarpum (12.29 mg/pl) followed by Glomus caledonicum (10.91 mg/pl), Glomus leptotichum (8.88 mg/pl), and Glomus intraradices (8.48 mg/pl). Lowest P content was observed in uninoculated control plants (4.09 mg/pl).

The root phosphorus concentration varied with the various treatments and the inoculated plants had significantly higher P concentration than the control plants. Maximum P concentration was observed in Glomus macrocarpum inoculated plants (0.204). It was on par with Glomus caledonicum (0.220) and Glomus intraradices (0.213) inoculated plants.

**Total phosphorus**

Inoculated plants had significantly higher P content compared to uninoculated plants. Maximum phosphorus content was seen in plants inoculated with Glomus macrocarpum (31.28 mg/pl) followed by Glomus caledonicum (29.20 mg/pl)

**Fig : 18. Influence of different VAM fungi on phosphorus uptake by Albizzia lebbeck**



and Glomus leptotichum (26.66 mg/pl). The lowest P uptake was observed in uninoculated control plants (12.51 mg/pl).

### 4.2.6. Influence of VAM fungi on uptake of zinc by the host

Table 13 shows the effect of different VAM fungi as the uptake of zinc by Albizzia lebbeck.

**Shoot zinc**

The zinc content was significantly higher in inoculated plants compared to uninoculated plants (Table 13). Plants inoculated with Glomus macrocarpum had significantly higher shoot zinc content (233.11 µg/pl). This was followed by Glomus caledonicum (223.13 µg/pl) and Glomus leptotichum (214.75 µg/pl). The shoot zinc content of all the inoculated treatments significantly differed from each other.

Higher zinc concentration was observed in plants inoculated with Glomus macrocarpum (26.4 ppm). All the inoculated treatments had high zinc concentration of the shoot compared to the uninoculated treatment (Fig.19).

**Root zinc**

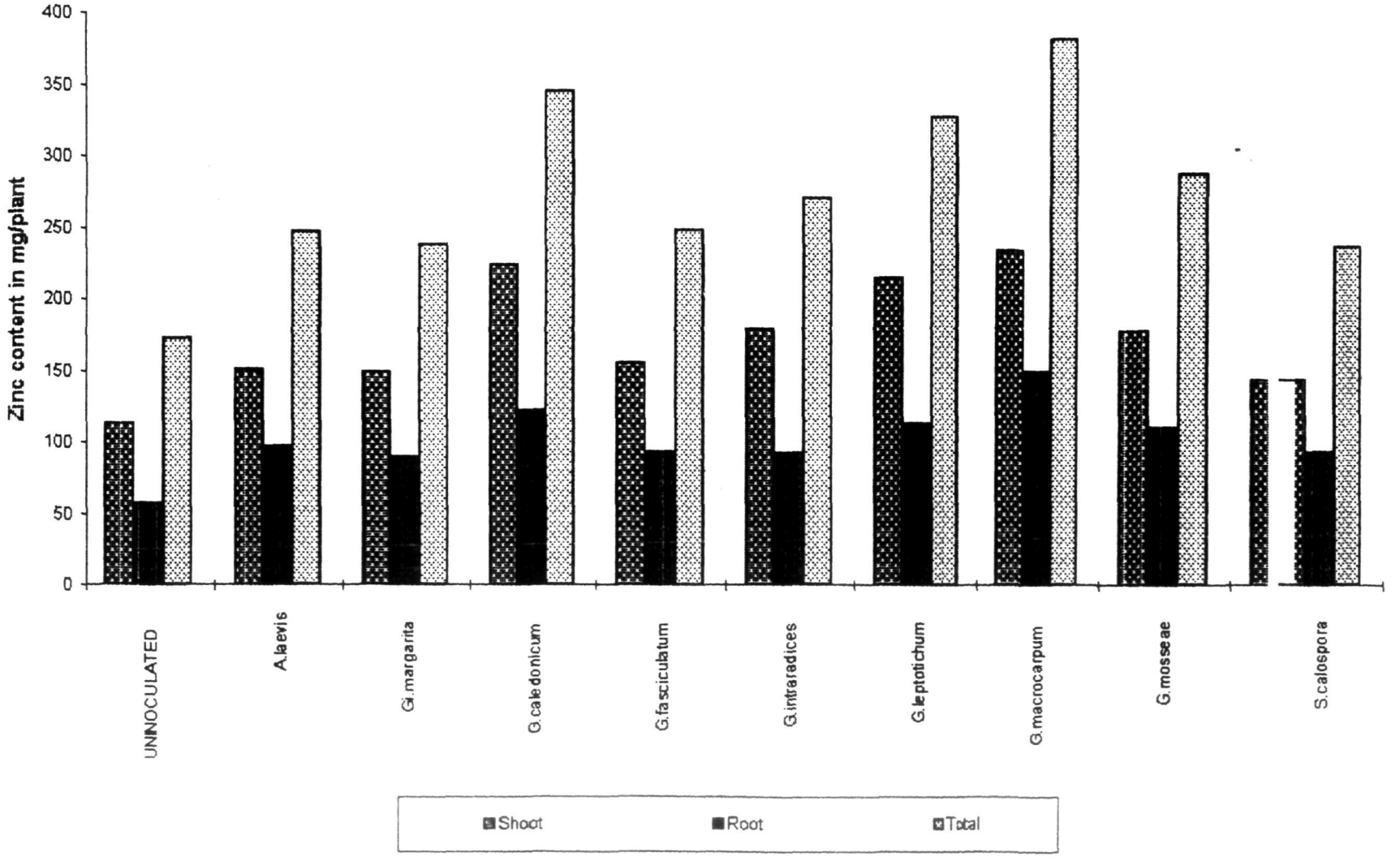
Plants inoculated with VAM fungi had higher zinc content compared to uninoculated control plants. The increase in zinc content was maximum in Glomus macrocarpum inoculated plants (148.26 ug/pl), while the uninoculated

**Table 13 Influence of different VAM fungi on zinc uptake by *Albizzia lebbeck***

| VAM fungi | Zn concentration (ppm) | | Zn content (µg/plant) | | |
|---|---|---|---|---|---|
| | Shoot | Root | Shoot | Root | Total |
| Uninoculated control | $17.1^{d}$ | $19.8^{e}$ | $112.52^{h}$ | $56.60^{g}$ | $172.12^{h}$ |
| *A. laevis* (Nedl.) | $23.5^{bc}$ | $25.4^{bc}$ | $150.87^{ef}$ | $96.01^{d}$ | $246.88^{f}$ |
| *Gi. margarita* (ICRISAT) | $22.3^{c}$ | $24.2^{cd}$ | $148.74^{fg}$ | $89.54^{f}$ | $238.28^{f}$ |
| *G. caledonicum* (Nedl.) | $25.2^{a}$ | $24.5^{cd}$ | $223.13^{b}$ | $121.52^{b}$ | $344.65^{b}$ |
| *G. fasciculatum* (Riv.) | $23.4^{bc}$ | $24.2^{cd}$ | $155.38^{e}$ | $92.44^{ef}$ | $247.82^{f}$ |
| *G. intraradices* (Local) | $24.6^{ab}$ | $23.1^{d}$ | $178.60^{d}$ | $91.94^{ef}$ | $270.54^{e}$ |
| *G. leptotichum* (Local) | $25.0^{ab}$ | $26.7^{ab}$ | $214.75^{c}$ | $112.41^{c}$ | $327.16^{c}$ |
| *G. macrocarpum* (Local) | $26.4^{a}$ | $27.5^{a}$ | $233.11^{a}$ | $148.25^{a}$ | $381.36^{a}$ |
| *G. mosseae* (Local) | $24.9^{ab}$ | $26.9^{ab}$ | $177.54^{d}$ | $109.76^{c}$ | $287.29^{d}$ |
| *S. calospora* (ICRISAT) | $22.6^{c}$ | $25.8^{abc}$ | $143.06^{g}$ | $92.88^{e}$ | $235.94^{g}$ |

Legend as in Table 1

**Fig : 19. Influence of different VAM fungi on zinc uptake by Albizzia lebbeck**



plants had minimum zinc (59.60 µg/pl). Next to Glomus macrocarpum inoculated plants, plants inoculated with Glomus caledonicum and Glomus leptotichum had more zinc content of 121.52 and 112.41 µg/pl respectively.

The zinc concentrations of inoculated plants were more compared to uninoculated plants although the response varied between the treatments. Maximum zinc concentration was observed in plants inoculated with Glomus macrocarpum (27.5 ppm) followed by plants inoculated with Glomus mosseae (26.9 ppm), Glomus leptotichum (26.7 ppm) and Scutellospora calospora (25.8 ppm). All the four treatments were on par with each other.

**Total zinc content**

Plants inoculated with Glomus macrocarpum had maximum zinc content (381.36 µg/pl) followed by Glomus caledonicum (347.65 µg/pl), Glomus leptotichum (327.16 µg/pl) and Glomus mosseae (287.29 µg/pl) inoculated plants (Fig. 19). The lowest total zinc content was observed in uninoculated plants (172.12 µg/pl).

### 4.2.7. Influence of VAM fungi on uptake of copper by the host

Uptake of copper by plants as influenced by different VAM fungi is given in Table 14.

**Table 14 Influence of different VAM fungi on copper uptake by *Albizzia lebbeck***

| VAM fungi | Copper concentration (ppm) | | Copper content (µg/plant) | | |
|---|---|---|---|---|---|
| | Shoot | Root | Shoot | Root | Total |
| Uninoculated control | $6.3^{f}$ | $7.1^{f}$ | $41.45^{g}$ | $21.37^{f}$ | $62.82^{g}$ |
| *A. laevis* (Nedl.) | $8.8^{de}$ | $7.6^{ef}$ | $56.50^{e}$ | $28.73^{e}$ | $85.23^{f}$ |
| *Gi. margarita* (ICRISAT) | $8.7^{de}$ | $8.9^{cd}$ | $58.03^{e}$ | $32.93^{e}$ | $90.96^{e}$ |
| *G. caledonicum* (Nedl.) | $10.5^{a}$ | $11.1^{a}$ | $91.88^{a}$ | $55.06^{a}$ | $146.94^{a}$ |
| *G. fasciculatum* (Riv.) | $8.5^{e}$ | $8.2^{de}$ | $56.44^{e}$ | $31.32^{d}$ | $87.76^{f}$ |
| *G. intraradices* (Local) | $9.9^{abc}$ | $8.4^{d}$ | $71.87^{c}$ | $33.43^{c}$ | $105.30^{d}$ |
| *G. leptotichum* (Local) | $9.4^{cd}$ | $10.5^{ab}$ | $80.75^{b}$ | $44.21^{b}$ | $124.96^{b}$ |
| *G. macrocarpum* (Local) | $10.2^{ab}$ | $10.2^{b}$ | $90.07^{a}$ | $54.98^{a}$ | $145.05^{a}$ |
| *G. mosseae* (Local) | $9.6^{bc}$ | $10.6^{ab}$ | $68.45^{d}$ | $43.25^{b}$ | $111.70^{c}$ |
| *S. calospora* (ICRISAT) | $8.2^{e}$ | $9.3^{c}$ | $51.91^{f}$ | $33.48^{c}$ | $85.39^{f}$ |

Legend as in Table 1

**Shoot copper**

In plants inoculated with Glomus caledonicum, the copper content was maximum (91.88 µg/pl), which was followed by Glomus macrocarpum (90.07 µg/pl) inoculated plants. Both were statistically on par with each other. The copper content was least in uninoculated plants (41.45 µg/pl) (Fig.20).

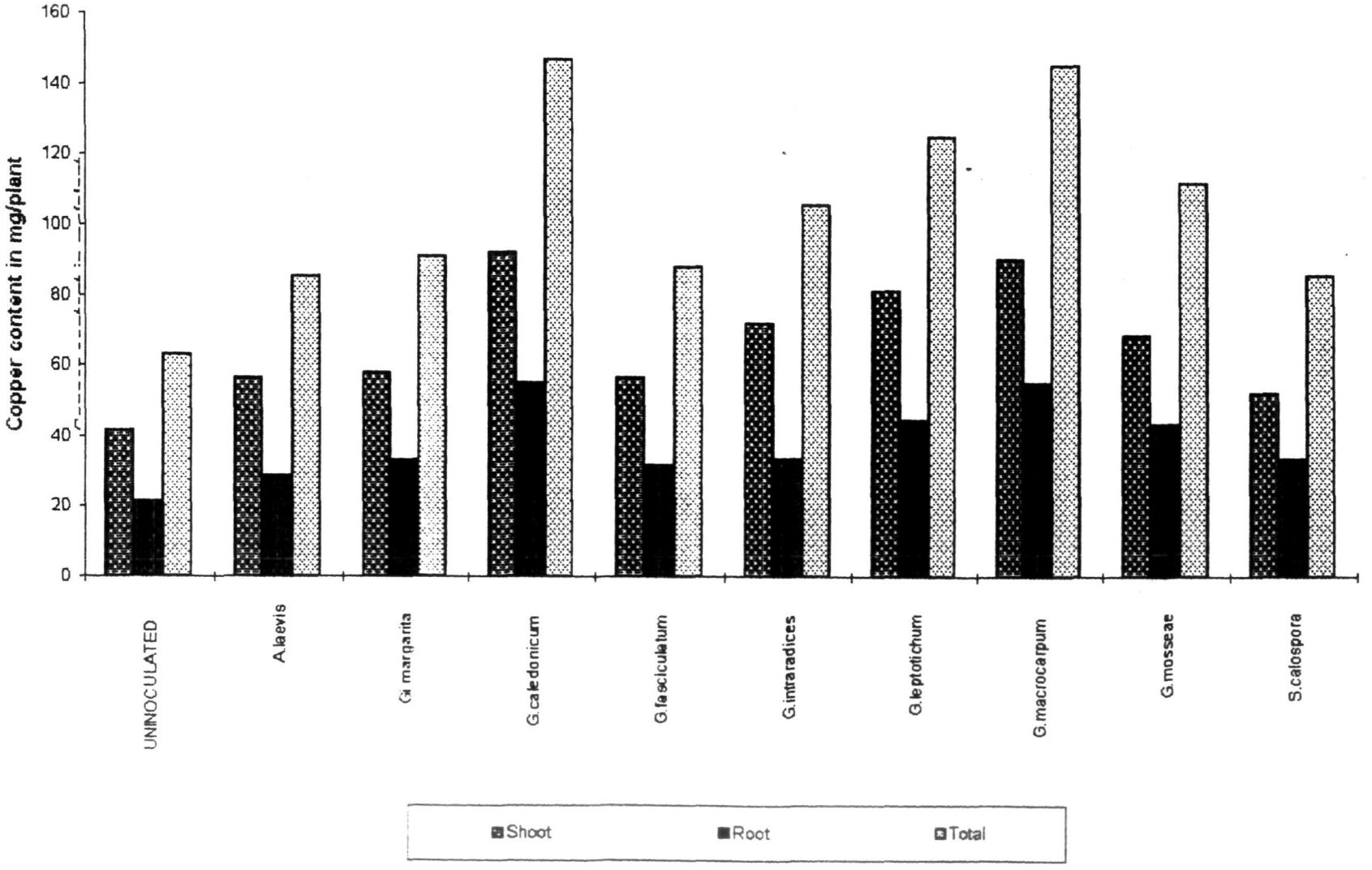
Higher copper concentration was observed in Glomus caledonicum (10.5 µg/pl) inoculated plants, followed by Glomus macrocarpum (10.2 µg/pl) and Glomus intraradices (9.5 µg/pl) inoculated plants. The concentration of copper in other VAM inoculated treatments varied, but all were significantly higher than the control.

**Root copper**

The copper content in the root in Glomus caledonicum inoculated plants was highest (55.06 µg/pl) closely followed by Glomus macrocarpum inoculated plants (54.98 µg/pl), although both were statistically on par with each other. Further, all the inoculated treatments differed significantly from the uninoculated control.

Plants inoculated with Glomus caledonicum had significantly higher copper concentration (11.1 ppm) followed by Glomus mosseae and Glomus macrocarpum inoculated

**Fig : 20. Influence of different VAM fungi on copper uptake by Albizzia lebbeck**



plants. All the inoculated treatments were significantly higher compared to uninoculated control.

**Total copper**

Significantly higher total copper content was recorded in Glomus caledonicum inoculated plants (146.94 µg/pl) followed by Glomus macrocarpum (145.05 µg/pl) treated plants. However, both the treatments were statistically on par with each other. The next best fungi enhancing copper uptake were Glomus mosseae (111.70 µg/pl) and Glomus intraradices (105.30 µg/pl). Lowest total copper content was observed in uninoculated control plants (62.82 ug/pl).

### 4.2.8. Influence of VAM fungi on per cent mycorrhizal root colonization

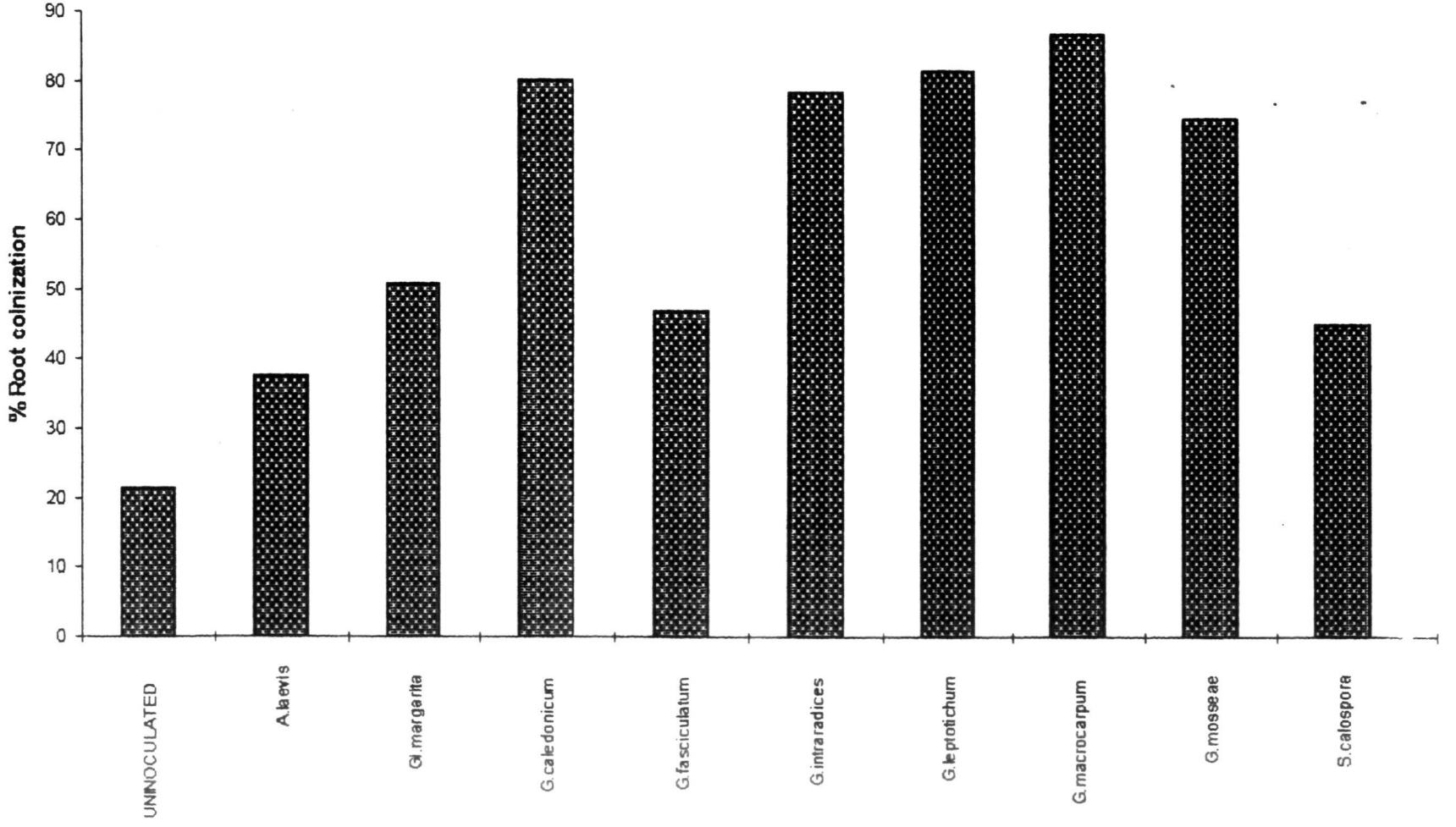
Plants inoculated with VAM fungi had significantly higher mycorrhizal root colonization compared to control plants. Maximum per cent root colonization was observed in plants inoculated with Glomus macrocarpum (86.78 %) and the least colonization was observed in uninoculated control plants (21.23 %). Plants inoculated with Glomus leptotichum, Glomus caledonicum and Glomus intraradices had 81.55 %, 80.21 % and 78.31 % colonization respectively. All the three treatments were statistically on par with each other (Table 15 and Fig.21).

**Table 15 Influence of different VAM fungi on mycorrhizal root colonization, spore numbers in soil and per cent aggregation of soil planted with Albizzia lebbeck**

| VAM fungi | % root colonization | Spore number/ 25 ml soil | % aggregation of soil |
|---|---|---|---|
| Uninoculated control | 21.23$^{g}$ | 48$^{h}$ | 22$^{g}$ |
| A. laevis (Nedl.) | 37.49$^{f}$ | 161$^{c}$ | 38$^{e}$ |
| Gi. margarita (ICRISAT) | 50.63$^{d}$ | 145$^{d}$ | 45$^{c}$ |
| G. caledonicum (Nedl.) | 80.21$^{b}$ | 113$^{e}$ | 47$^{b}$ |
| G. fasciculatum (Riv.) | 46.76$^{de}$ | 93$^{f}$ | 42$^{d}$ |
| G. intraradices (Local) | 78.31$^{bc}$ | 149$^{d}$ | 36$^{f}$ |
| G. leptotichum (Local) | 81.55$^{b}$ | 69$^{g}$ | 59$^{a}$ |
| G. macrocarpum (Local) | 86.78$^{a}$ | 171$^{b}$ | 48$^{b}$ |
| G. mosseae (Local) | 74.50$^{c}$ | 216$^{a}$ | 39$^{e}$ |
| S. calospora ICRISAT) | 44.93$^{e}$ | 87$^{f}$ | 36$^{f}$ |

Legend as in Table 1

**Fig : 21. Influence of different VAM fungi on per cent mycorrhizal root colonization of Albizzia lebbeck**



### 4.2.9. Influence of VAM fungi on number of mycorrhizal spores in the root zone soil

As shown in Table 15 all the treatments were statistically significant. Maximum spore load was observed in plants inoculated with Glomus mosseae (216) followed by Glomus macrocarpum (171), Acaulospora laevis (161) and Gigaspora margarita (145). The lowest number of spores was observed in uninoculated control (48). When compared to control, all the inoculated treatments were significantly different (Fig.22).

### 4.2.10. Influence of VAM fungi on per cent soil aggregation

Significantly higher per cent aggregation of soil was observed in all the inoculated treatments compared to the uninoculated treatment (Table 15). The per cent aggregation was more in plants inoculated with Glomus leptotichum (59 %) followed by Glomus macrocarpum (48 %), Gigaspora margarita (45 %) and Glomus fasciculatum (42 %). When compared to control all the inoculated treatment were significantly higher and in control the per cent aggregation was the lowest recording only 22.0 % (Table 15 and Fig.23).

### 4.2.11. Influence of VAM fungi on sturdiness quotient

Maximum sturdiness quotient of the plant was observed in plants inoculated with Glomus caledonicum (17.41) followed by plants inoculated with Glomus intraradices (15.73) and Glomus fasciculatum. Among the inoculated

Fig : 22. Influence of different VAM fungi on mycorrhizal spore numbers in the root zone of Alb izzia Lebbeck

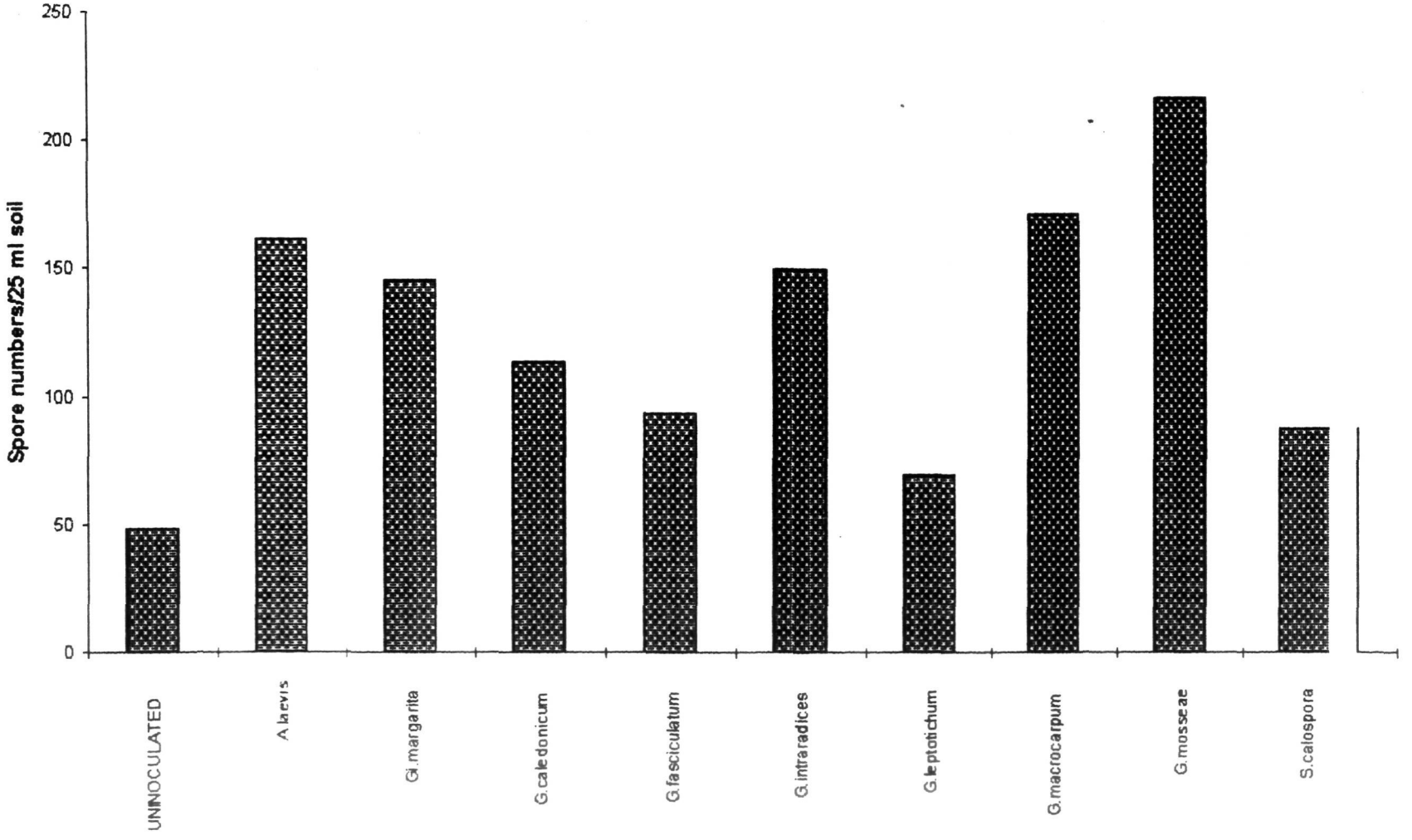
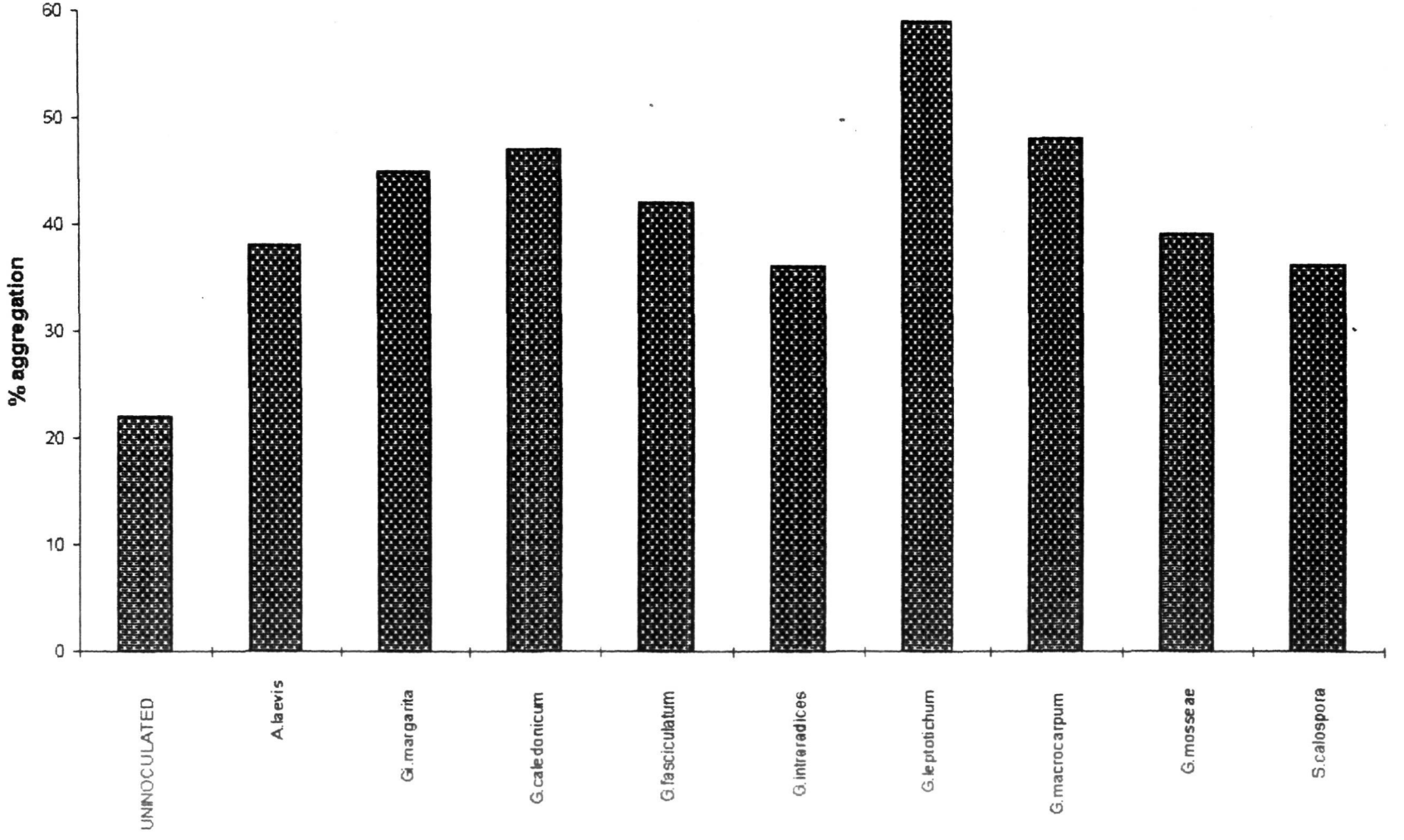


Fig : 23. Influence of different VAM fungi on per cent aggregation of soil planted with Albizzia lebbeck



plants, least sturdiness quotient was seen in seedlings treated with Glomus leptotichum. The uninoculated plants had a minimum sturdiness quotient of 12.73 (Table 16 and Fig.24).

**4.2.12. Influence of VAM fungi on biovolume index**

Biovolume index was in general more in inoculated plants than the uninoculated control plants. Maximum value was observed in plants inoculated with Glomus caledonicum (3760.56) followed by plants inoculated with Gigaspora margarita (3588.22) and Glomus intraradices (3516.85). In uninoculated control plants the biovolume index was 2235.95 (Table 16 and Fig.25).

**4.2.13. Influence of VAM fungi on quality index**

As shown in Table 16, maximum quality index was observed in plants inoculated with Glomus macrocarpum (0.899) followed by Glomus leptotichum (0.857) and Glomus caledonicum. The uninoculated control plants had an index value of 0.643. However, plants inoculated with Acaulospora laevis showed an index value (0.596) lower than the control plants (Fig.26).

Table 16 Influence of different VAM fungi on sturdiness quotient (SQ) biovolume index (VI) and quality index (QI) of *Albizzia lebbeck*

| VAM fungi | Sturdiness quotient (SQ) | Biovolume index (VI) | Quality index (QI) |
|---|---|---|---|
| Uninoculated control | 12.73 | 2235.97 | 0.643 |
| *A. laevis* (Nedl.) | 15.41 | 3296.04 | 0.596 |
| *Gi. margarita* (ICRISAT) | 15.43 | 3588.22 | 0.602 |
| *G. caledonicum* (Nedl.) | 17.41 | 3760.56 | 0.715 |
| *G. fasciculatum* (Riv.) | 15.70 | 3241.33 | 0.600 |
| *G. intraradices* (Local) | 15.73 | 3516.85 | 0.640 |
| *G. leptotichum* (Local) | 12.90 | 2743.99 | 0.857 |
| *G. macrocarpum* (Local) | 14.18 | 3315.31 | 0.899 |
| *G. mosseae* (Local) | 15.01 | 3035.31 | 0.669 |
| *S. calospora* ICRISAT) | 13.49 | 2785.52 | 0.651 |

Legend as in Table 1

Fig : 24. Influence of different VAM fungi on sturdiness quotient of Albizzia lebbeck

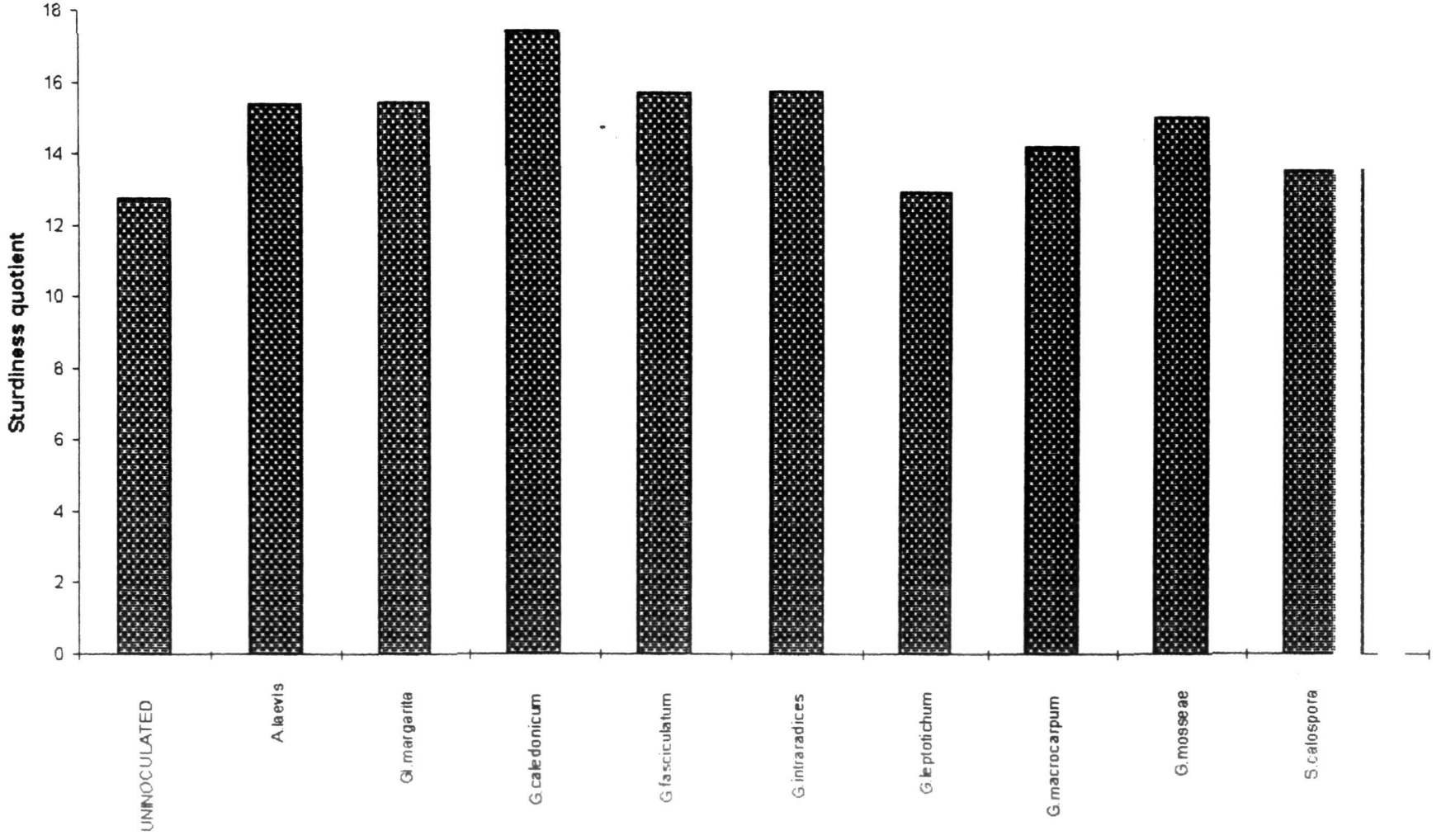


Fig : 25. Influence of different VAM fungi on bio volume index of Albizzia Lebbeck

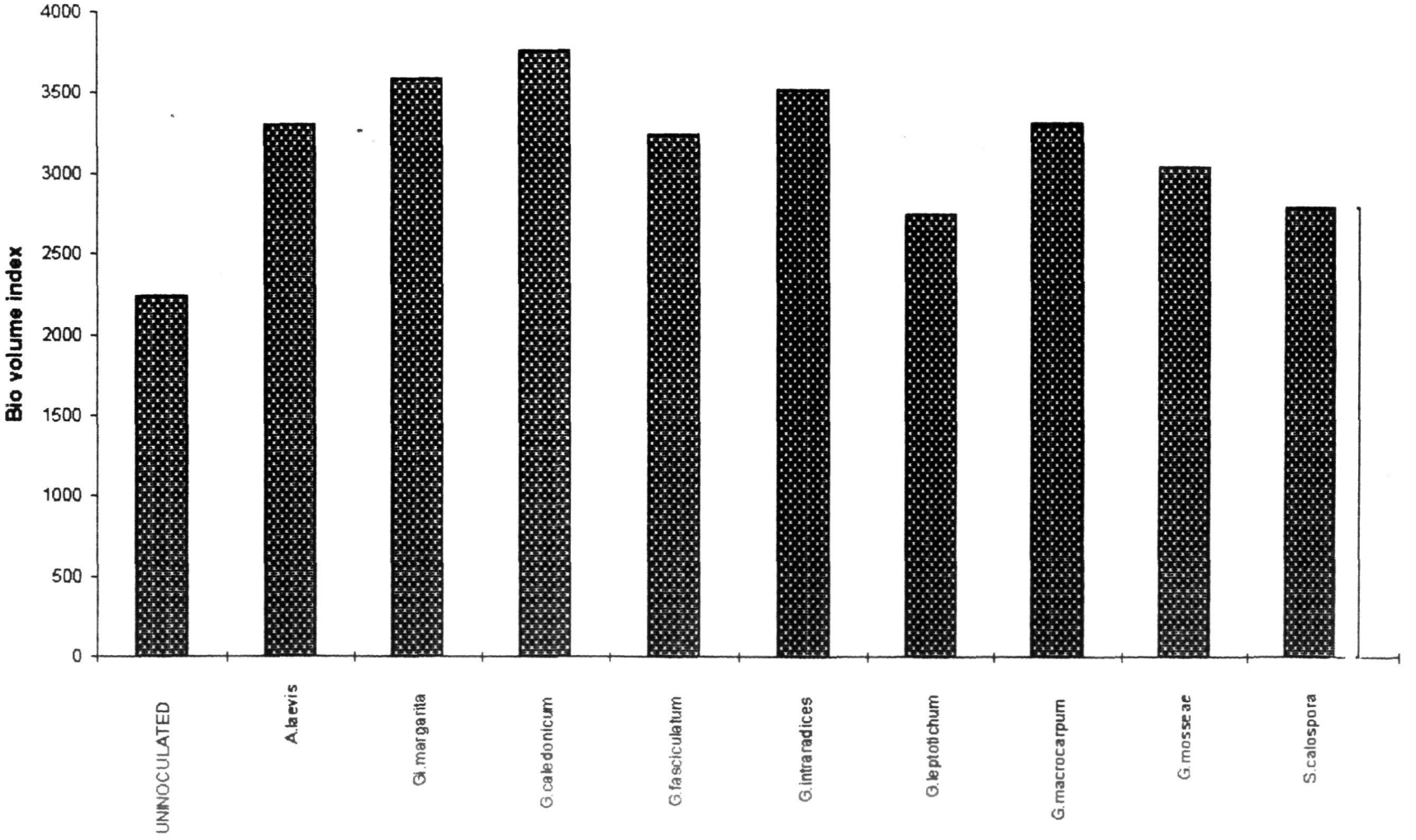
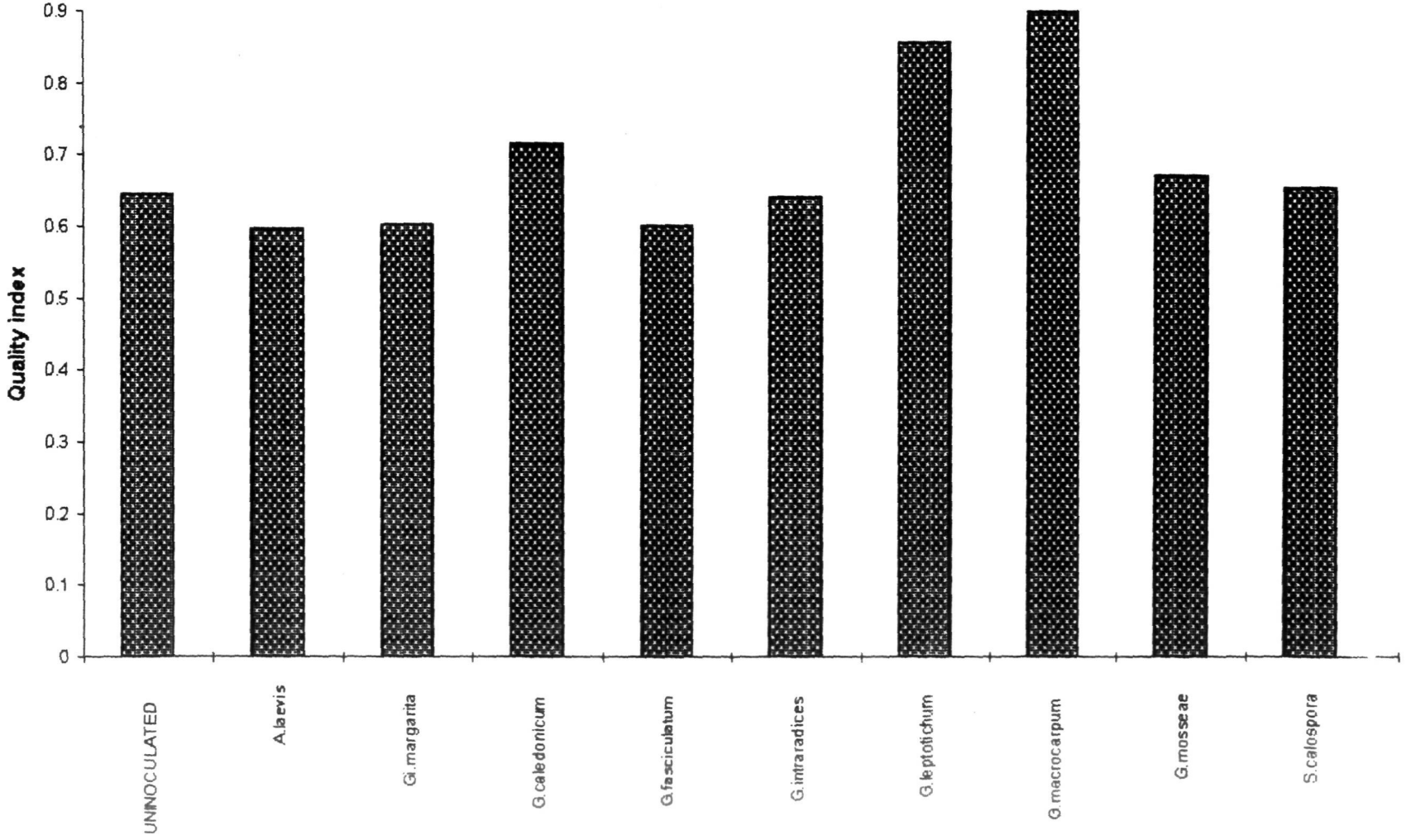


Fig : 26. Influence of different VAM fungi on quality index of Albizzia lebbeck



**Experiment 3**

## 4.3. Selection of efficient VAM fungi for Tectona grandis

### 4.3.1. Influence of VAM fungi on plant height

The effect of inoculation with different VAM fungi on plant height of Tectona grandis at different stages of growth is given in Table 17.

There was significant increase in plant height due to inoculation at all the stages of plant growth. On 45 DAT maximum plant height (22.49 cm) was observed in plants inoculated with Glomus mosseae. Uninoculated plants showed the least plant height (18.35 cm). Glomus caledonicum inoculated plants did not show any significant increase in plant height compared to uninoculated control. On 90 DAT, Glomus leptotichum inoculated plants had significantly more plant height (27.90 cm). No other inoculation treatment was significantly higher than the uninoculated control (Fig.27).

On 135 DAT, plants inoculated with the fungus Gigaspora margarita showed higher plant height, which was followed by plants inoculated with Glomus leptotichum (36.50 cm) and Glomus macrocarpum (35.64 cm). Plants inoculated with Glomus fasciculatum, Glomus caledonicum and Scutellospora calospora did not show any significant difference compared to uninoculated control. On the other hand, at harvest (180 DAT) maximum plant height was observed in plants

**Table 17 Influence of different VAM fungi on plant height (cm) of *Tectona grandis***

| VAM fungi | 45 DAT* | 90 DAT | 135 DAT | 180 DAT |
|---|---|---|---|---|
| Uninoculated control | 18.35$^{d}$ | 24.92$^{bcd}$ | 31.35$^{f}$ | 44.44$^{f}$ |
| *A. laevis* (Nedl.) | 19.74$^{c}$ | 24.65$^{cd}$ | 28.25$^{f}$ | 47.90$^{cde}$ |
| *Gi. margarita* (ICRISAT) | 22.12$^{a}$ | 26.54$^{ab}$ | 39.10$^{a}$ | 51.23$^{ab}$ |
| *G. caledonicum* (Nedl.) | 18.46$^{d}$ | 23.76$^{d}$ | 31.95$^{ef}$ | 45.48$^{ef}$ |
| *G. fasciculatum* (Riv.) | 20.24$^{c}$ | 25.36$^{bcd}$ | 32.94$^{def}$ | 45.48$^{ef}$ |
| *G. intraradices* (Local) | 20.32$^{c}$ | 25.20$^{bcd}$ | 34.47$^{bcd}$ | 48.07$^{cde}$ |
| *G. leptotichum* (Local) | 21.98$^{c}$ | 27.90$^{a}$ | 36.50$^{b}$ | 49.82$^{cde}$ |
| *G. macrocarpum* (Local) | 20.87$^{bc}$ | 25.64$^{bc}$ | 35.64$^{bc}$ | 53.62$^{a}$ |
| *G. mosseae* (Local) | 22.49$^{a}$ | 25.64$^{bc}$ | 34.04$^{cde}$ | 50.39$^{bc}$ |
| *S. calospora* (ICRISAT) | 19.73$^{c}$ | 24.67$^{cd}$ | 31.56$^{f}$ | 47.07$^{def}$ |

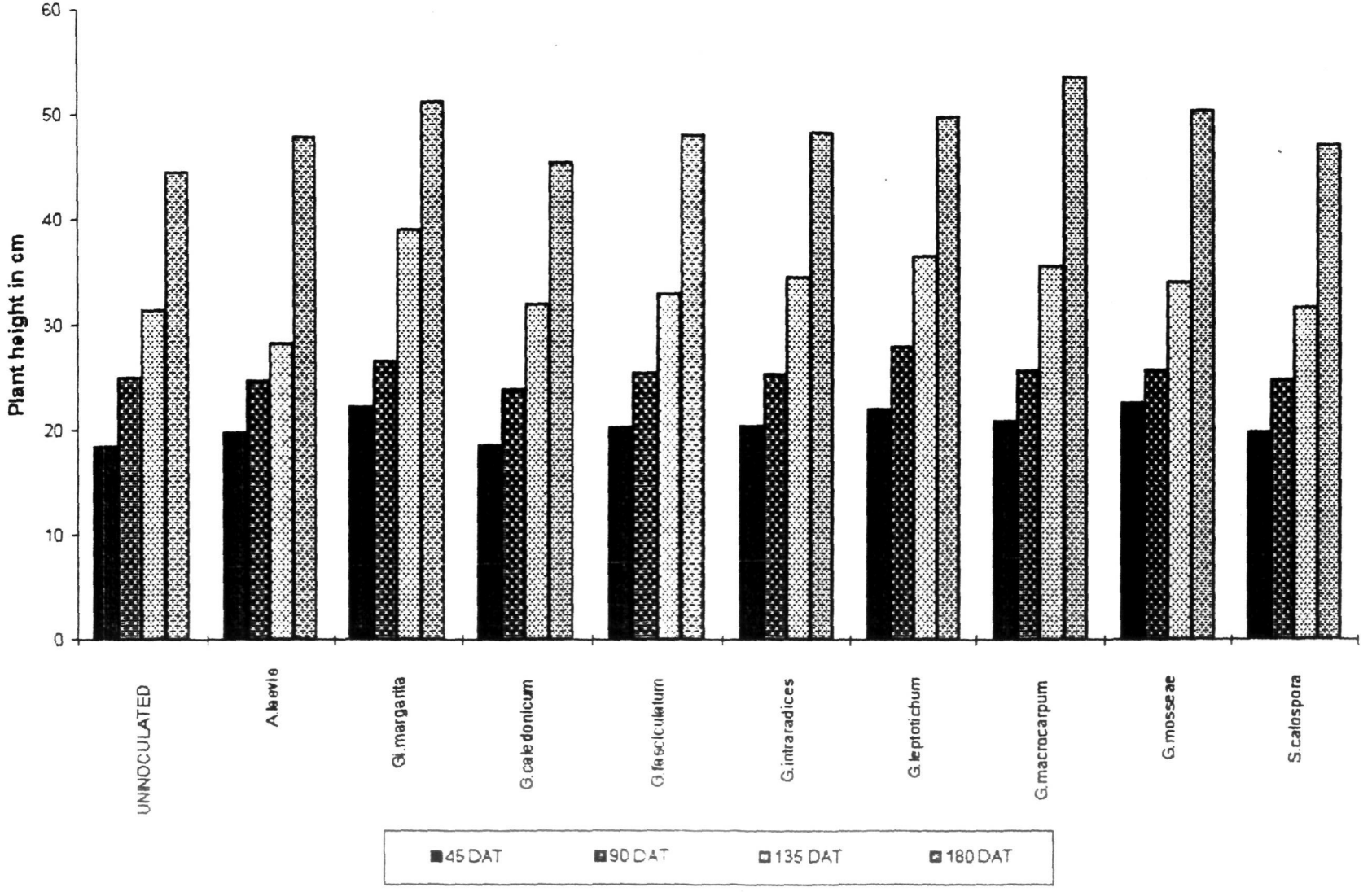
Values with similar alphabets in each column do not differ significantly at P = 0.05%

* DAT - Days after Transplanting

Nedl. - Isolate from Nedlands, Australia
ICRISAT - Isolate from ICRISAT, Hyderabad
Riv. - Isolate from Riverside, U.S.A.
Local - Isolate from U.A.S., Bangalore

A. - *Acaulospora*
Gi. - *Gigaspora*
G. - *Glomus*
S. - *Scutellospora*

Fig : 27. Influence of different VAM fungi on plant height (cm) of Tectona grandis



inoculated with Glomus macrocarpum (53.62 cm), followed by Gigaspora margarita (51.23 cm) and Glomus mosseae. Plants inoculated with Scutellospora calospora and Glomus caledonicum did not show any significant increase over uninoculated control.

### 4.3.2. Influence of VAM fungi on stem girth

The stem girth of the plants were recorded on 45th, 90th, 135th and 180th DAT. The values are presented in Table 18.

The stem girth of plants inoculated with Glomus leptotichum (0.285 cm), Glomus intraradices (0.279 cm), Acaulospora laevis (0.277 cm) and Scutellospora calospora (0.275) did not differ significantly among themselves although they were significantly higher than the control. On 45 DAT plants inoculated with Glomus macrocarpum (0.387 cm), Glomus leptotichum (0.376 cm) and Glomus fasciculatum (0.374 cm) did not differ significantly. But all the inoculated treatments, except Glomus caledonicum were significantly higher than the control (Fig.28).

On 135 DAT, the plants inoculated with all the VAM fungi except Glomus mosseae were on par with each other and were significantly higher than the uninoculated control. On 180 DAT the Glomus leptotichum inoculated plants recorded

Table 18 Influence of different VAM fungi on stem girth (cm) of *Tectona grandis*

| VAM fungi | 45 DAT | 90 DAT | 135 DAT | 180 DAT |
|---|---|---|---|---|
| Uninoculated control | 0.254$^{d}$ | 0.325$^{f}$ | 0.560$^{c}$ | 0.709$^{d}$ |
| *A. laevis* (Nedl.) | 0.277$^{ab}$ | 0.347$^{de}$ | 0.598$^{ab}$ | 0.717$^{d}$ |
| *Gi. margarita* (ICRISAT) | 0.257$^{d}$ | 0.349$^{cd}$ | 0.615$^{ab}$ | 0.834$^{b}$ |
| *G. caledonicum* (Nedl.) | 0.272$^{bc}$ | 0.334$^{ef}$ | 0.600$^{ab}$ | 0.822$^{b}$ |
| *G. fasciculatum* (Riv.) | 0.262$^{cd}$ | 0.374$^{ab}$ | 0.591$^{ab}$ | 0.843$^{ab}$ |
| *G. intraradices* (Local) | 0.279$^{ab}$ | 0.360$^{cd}$ | 0.607$^{ab}$ | 0.831$^{ab}$ |
| *G. leptotichum* (Local) | 0.258$^{d}$ | 0.387$^{a}$ | 0.616$^{a}$ | 0.868$^{b}$ |
| *G. macrocarpum* (Local) | 0.285$^{a}$ | 0.376$^{ab}$ | 0.597$^{ab}$ | 0.757$^{c}$ |
| *G. mosseae* (Local) | 0.261$^{cd}$ | 0.362$^{bc}$ | 0.585$^{bc}$ | 0.761$^{c}$ |
| *S. calospora* (ICRISAT) | 0.276$^{ab}$ | 0.350$^{cd}$ | 0.592$^{ab}$ | 0.720$^{d}$ |

Legend as in Table 17

Fig : 28. Influence of different VAM fungi on stem girth (cm) of Tectona grandis

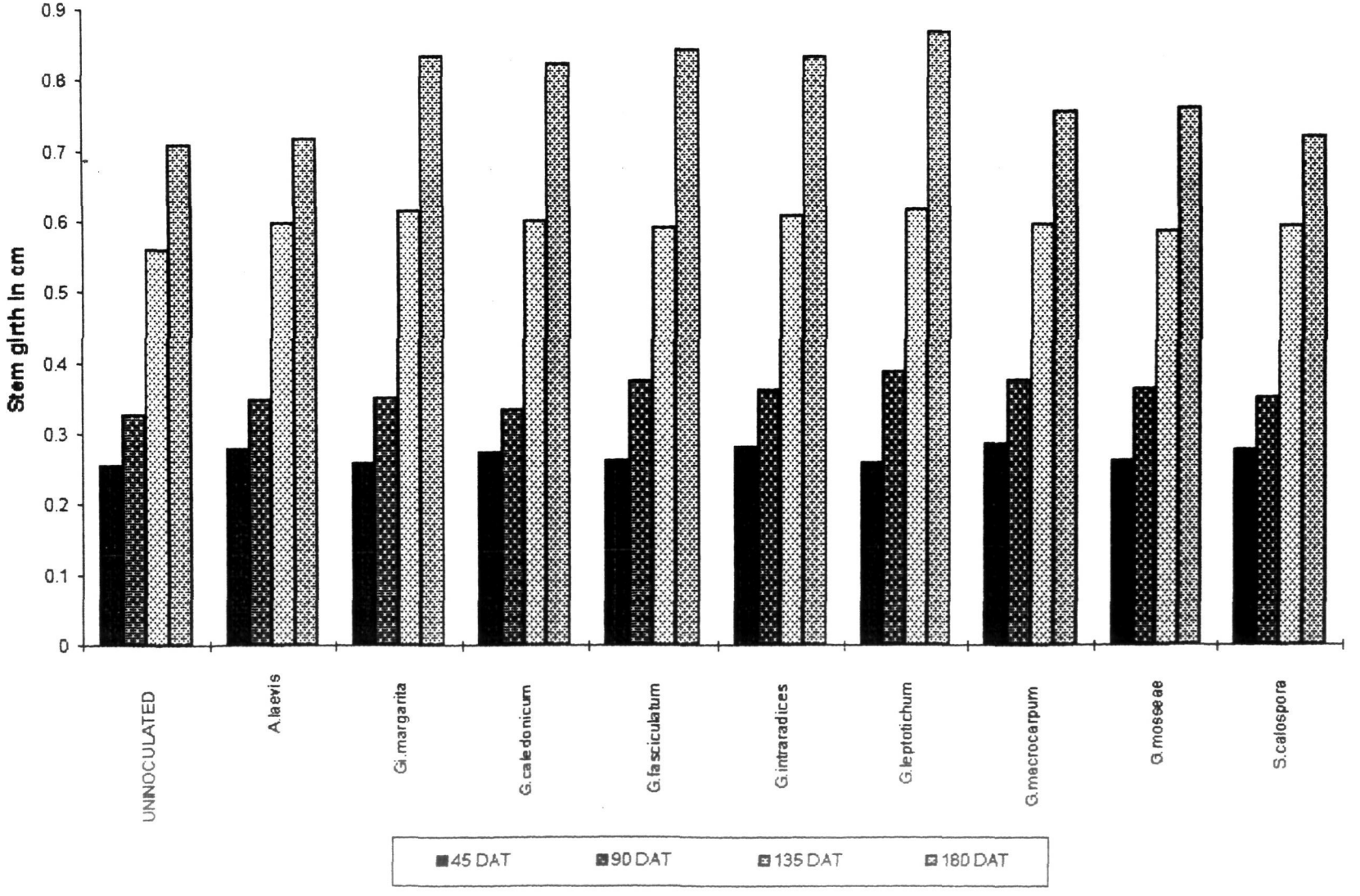


*Plate 5 & 6. Influence of VAM fungus on the growth of Tectona grandis.*

*1 & V - UNINOCULATED CONTROL*

*2 & I - INOCULATED WITH Glomus leptotichum*


U
I


1
2

highest stem diameter (0.868 cm). This was followed by plants inoculated with Glomus fasciculatum (0.843 cm), Gigaspora margarita (0.834), Glomus intraradices (0.831 cm) and Glomus caledonicum (0.822 cm), all the four being statistically on par with each other. Plants inoculated with Scutellospora calospora and Acaulospora laevis did not show significant increase in stem girth compared to control.

### 4.3.3. Influence of VAM fungi on plant biomass

The plant biomass as influenced by different VAM fungi at harvest (180 DAT) is given in Table 19.

**Shoot dry weight:** The shoot dry weight of Glomus leptotichum inoculated plants was the highest (13.64 g) closely followed by Gigaspora margarita (13.18 g) inoculated plants; both being statistically on par with each other. The next best fungi for improving shoot dry weight were Glomus macrocarpum (13.07 g), Glomus mosseae (12.89 g) and Glomus fasciculatum (12.71 g). All the inoculated treatments showed significantly more shoot dry weight compared to uninoculated control, which recorded the lowest dry weight of 9.76 g (Fig.30).

**Root dry weight:** Root dry weight was more in plants inoculated with Glomus leptotichum (8.38 g) although it was statistically on par with Glomus macrocarpum inoculated plants. All the inoculated treatments showed significantly

Table 19 Influence of different VAM fungi on leaf area, shoot and root dry weight of Tectona grandis

| VAM fungi | Leaf area | Dry weight (g/plant) | | |
|---|---|---|---|---|
| | $cm^2$/plant | Shoot | Root | Total |
| Uninoculated control | 975.22$^{g}$ | 9.76$^{e}$ | 5.07$^{h}$ | 14.83$^{g}$ |
| A. laevis (Nedl.) | 1213.47$^{e}$ | 11.03$^{c}$ | 6.03$^{f}$ | 17.06$^{e}$ |
| Gi. margarita (ICRISAT) | 1643.57$^{b}$ | 13.18$^{ab}$ | 7.05$^{bc}$ | 20.23$^{b}$ |
| G. caledonicum (Nldl.) | 1144.94$^{f}$ | 10.27$^{d}$ | 5.58$^{g}$ | 15.85$^{f}$ |
| G. f0sciculatum (Riv.) | 1350.70$^{d}$ | 12.71$^{b}$ | 6.67$^{de}$ | 19.38$^{cd}$ |
| G. intraradices (Local) | 1243.65$^{e}$ | 11.26$^{c}$ | 6.47$^{e}$ | 17.73$^{e}$ |
| G. leptotichum (Local) | 1829.98$^{a}$ | 13.64$^{a}$ | 8.38$^{a}$ | 22.02$^{a}$ |
| G. macrocarpum (Local) | 1612.73$^{b}$ | 13.07$^{b}$ | 8.33$^{a}$ | 21.40$^{a}$ |
| G. mosseae (Local) | 1407.56$^{c}$ | 12.89$^{b}$ | 7.16$^{b}$ | 20.05$^{bc}$ |
| S. calospora (ICRISAT) | 1328.77$^{d}$ | 11.85$^{c}$ | 6.79$^{cd}$ | 18.64$^{d}$ |

Legend as in Table 17

Fig : 29. Influence of different VAM fungi on leaf area of Tectona grandis

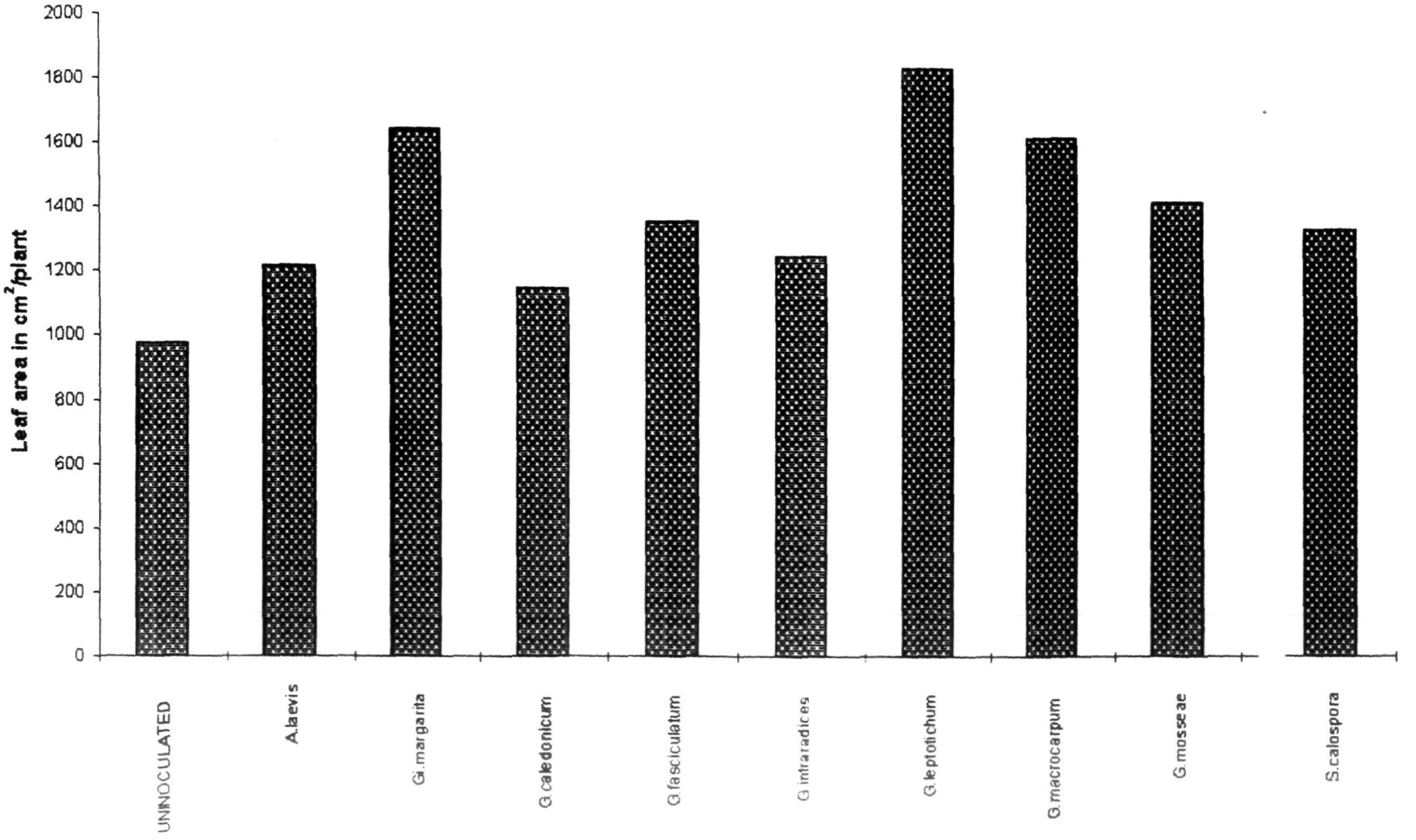
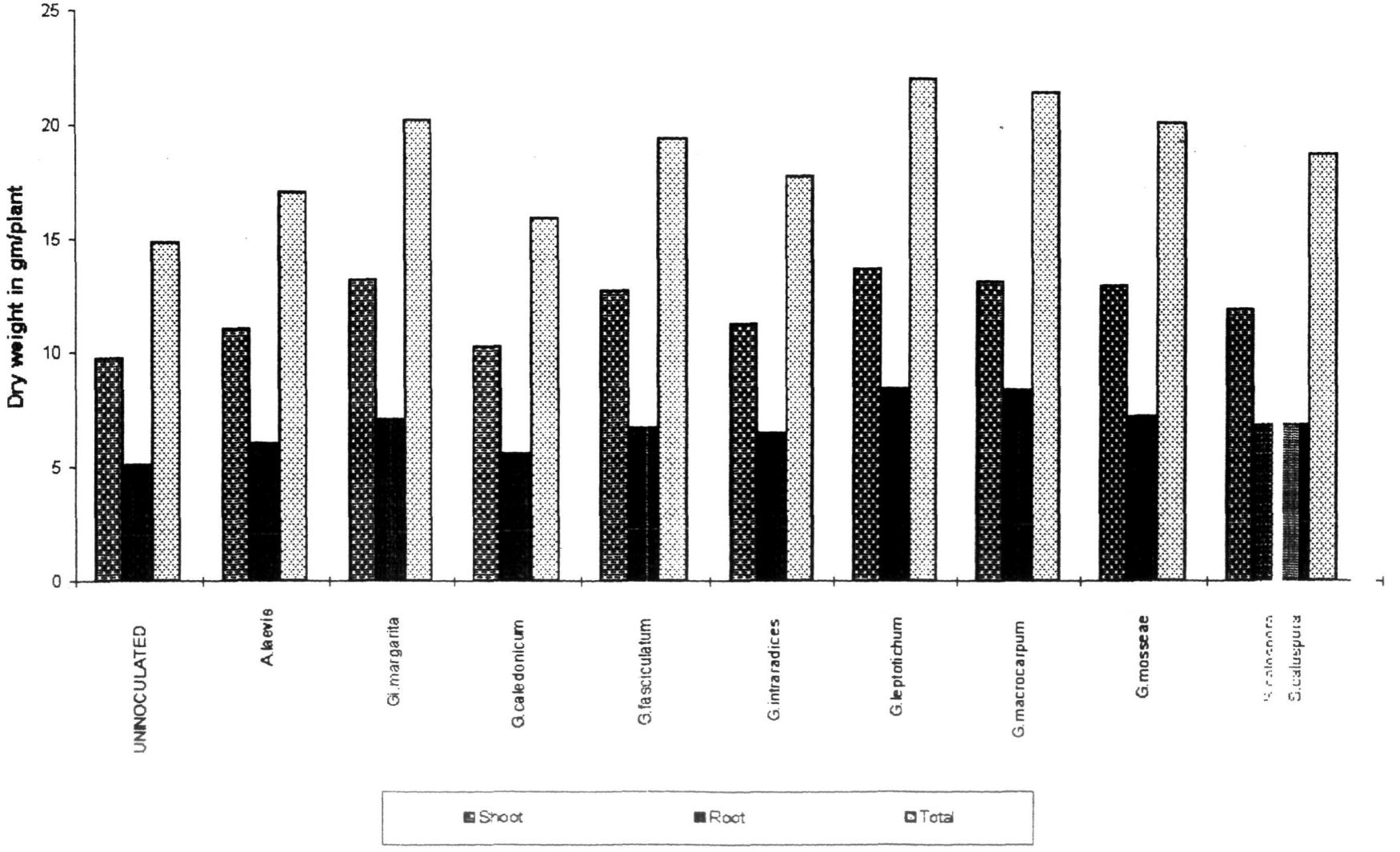


Fig : 30. Influence of different VAM fungi on shoot and root dry weight of Tectona grandis



more root dry weight compared to control. The control plants had the lowest dry weight of 5.07 g.

**Total biomass:** The total biomass of Glomus leptotichum inoculated plants was the highest (22.02 g) followed by Glomus macrocarpum (21.40 g), both the treatments being statistically on par with each other. The next best fungi in improving plant biomass were Gigaspora margarita (20.23 g), Glomus mosseae (20.05 g) and Glomus fasciculatum (19.39 g). The uninoculated control plants had the lowest biomass (14.39 g).

### 4.3.4. Influence of VAM fungi on leaf area

The effect of VAM fungal inoculation on leaf area was highly significant and the values are presented in Table 19. Glomus leptotichum inoculated plants had the maximum leaf area (1829.98 $cm^2$) followed by Gigaspora margarita (1643.56 $cm^2$), Glomus macrocarpum (1612.73 $cm^2$) and Glomus mosseae (1407.56 $cm^2$) treated plants. The uninoculated control plants had the minimum leaf area (975.22 $cm^2$) (Fig.29).

### 4.3.5. Influence of VAM fungi on uptake of phosphorus by the host

Table 20 shows the phosphorus concentration and content of the plant samples inoculated with different VAM fungi.

**Table 20 Influence of different VAM fungi on phosphorus uptake by *Tectona grandis***

| VAM fungi | P concentration (%) | | P content (mg/plant) | | |
|---|---|---|---|---|---|
| | Shoot | Root | Shoot | Root | Total |
| Uninoculated control | $0.131^{e}$ | $0.139^{e}$ | $12.79^{g}$ | $7.05^{h}$ | $19.84^{h}$ |
| *A. laevis* (Nedl.) | $0.178^{bcd}$ | $0.189^{cd}$ | $19.63^{e}$ | $11.40^{f}$ | $31.03^{f}$ |
| *Gi. margarita* (ICRISAT) | $0.192^{ab}$ | $0.206^{ab}$ | $25.31^{b}$ | $14.52^{c}$ | $39.83^{c}$ |
| *G. caledonicum* (Nedl.) | $0.172^{d}$ | $0.183^{d}$ | $17.66^{f}$ | $10.21^{g}$ | $27.87^{g}$ |
| *G. fasciculatum* (Riv.) | $0.184^{bcd}$ | $0.196^{bcd}$ | $23.39^{c}$ | $13.07^{e}$ | $36.46^{d}$ |
| *G. intraradices* (Local) | $0.177^{cd}$ | $0.182^{d}$ | $19.93^{e}$ | $11.78^{f}$ | $31.71^{f}$ |
| *G. leptotichum* (Local) | $0.202^{a}$ | $0.216^{a}$ | $27.55^{a}$ | $18.10^{a}$ | $45.65^{a}$ |
| *G. macrocarpum* (Local) | $0.190^{abc}$ | $0.203^{abc}$ | $24.83^{b}$ | $16.91^{b}$ | $41.74^{b}$ |
| *G. mosseae* (Local) | $0.182^{bcd}$ | $0.193^{bcd}$ | $23.46^{c}$ | $13.82^{d}$ | $37.28^{d}$ |
| *S. calospora* (ICRISAT) | $0.180^{bcd}$ | $0.196^{bcd}$ | $21.45^{d}$ | $13.30^{de}$ | $34.75^{e}$ |

Legend as in Table 17

**Shoot phosphorus**

The shoot phosphorus content was maximum in Glomus leptotichum inoculated plants (27.55 mg/pl) followed by Gigaspora margarita (25.31 mg/pl) and Glomus macrocarpum (924.83 mg/pl) inoculated plants, the two treatments being statistically on par with each other. All the other treatments were significantly higher than the uninoculated control (Fig.31).

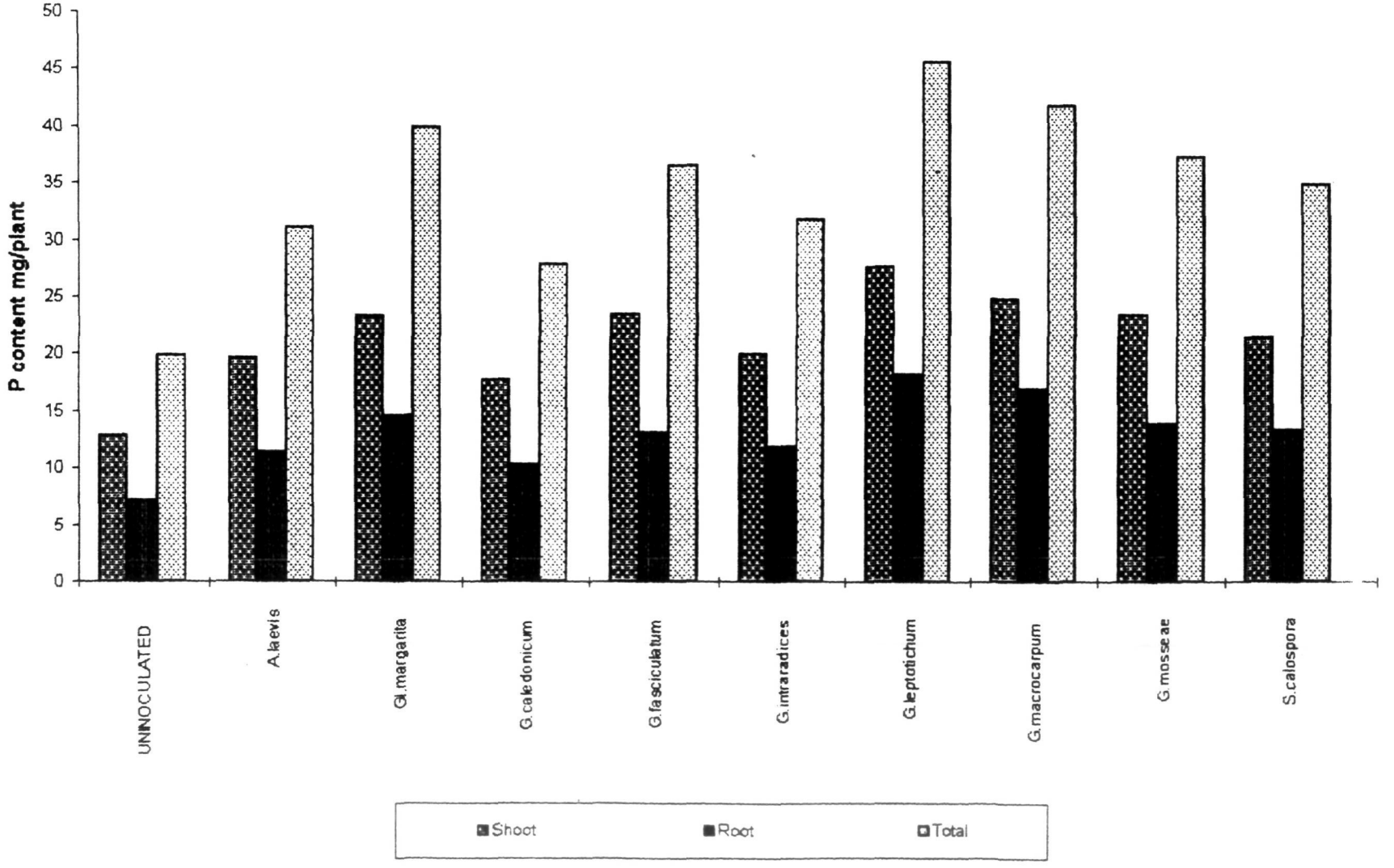
The shoot phosphorus concentrations of inoculated treatments were significantly higher than the uninoculated control. Maximum concentration was observed in Glomus leptotichum inoculated plants (0.202 %). However this treatment was statistically on par with Gigaspora margarita (0.192 %) and Glomus macrocarpum (0.190 %) inoculated treatments.

**Root phosphorus**

The root phosphorus content was high in Glomus leptotichum inoculated plants (18.10 mg/pl) followed by Glomus macrocarpum (16.91 mg/pl) and Gigaspora margarita (14.52 mg/pl) inoculated plants. The lowest was observed in control plants which recorded 7.05 mg/pl.

The root phosphorus concentration did not show much significance between treatments. However, all the inoculated treatments were significantly higher than the

Fig : 31. Influence of different VAM fungi on phosphrous by Tectona grandis



uninoculated control. The Glomus leptotichum inoculated plants had the highest root phosphorus concentration (0.211 %). This was followed by Gigaspora margarita (0.206 %) and Glomus macrocarpum (0.203 %) inoculated plants both being statistically on par with each other.

**Total phosphorus**

The total phosphorus content was maximum in plants inoculated with Glomus leptotichum followed by Glomus macrocarpum (41.74 mg/pl), Gigaspora margarita (39.83 mg/pl) and Glomus mosseae (37.28 mg/pl) treated plants. All the inoculated treatments were significantly different from the control treatment. The lowest total phosphorus content was recorded in control plants (19.84 mg/pl).

### 4.3.6. Influence of VAM fungi on uptake zinc by the host

Table 21 depicts the zinc content of both shoot and root of Tectona grandis as influenced by different VAM fungi. All the inoculated treatments had significantly higher zinc content than the control plants (Fig.32).

**Shoot zinc**

Inoculation with the VAM fungus Glomus leptotichum resulted in maximum zinc content (294.62 µg/pl) of the shoot followed by Glomus macrocarpum (275.78 µg/pl) and Glomus mosseae (261.67 µg/pl). All the treatments were significantly different from each other. The lowest zinc

Table 21 Influence of different VAM fungi on zinc uptake by *Tectona grandis*

| VAM fungi | Zn concentration (ppm) | | Zn content (ug/plant) | | |
|---|---|---|---|---|---|
| | Shoot | Root | Shoot | Root | Total |
| Uninoculated control | $14.7^{f}$ | $15.5^{f}$ | $143.47^{i}$ | $78.59^{g}$ | $222.06^{h}$ |
| *A. laevis* (Nedl.) | $17.4^{e}$ | $17.5^{de}$ | $191.92^{g}$ | $105.53^{e}$ | $297.45^{f}$ |
| *Gi. margarita* (ICRISAT) | $20.0^{bc}$ | $21.3^{ab}$ | $263.60^{e}$ | $150.17^{c}$ | $413.77^{c}$ |
| *G. caledonicum* (Nedl.) | $17.3^{e}$ | $16.8^{ef}$ | $177.67^{h}$ | $93.74^{f}$ | $271.41^{g}$ |
| *G. fasciculatum* (Riv.) | $19.7^{bcd}$ | $18.6^{cd}$ | $250.39^{d}$ | $124.06^{d}$ | $374.45^{d}$ |
| *G. intraradices* (Local) | $18.3^{de}$ | $20.1^{bc}$ | $206.06^{f}$ | $130.35^{d}$ | $336.11^{e}$ |
| *G. leptotichum* (Local) | $21.6^{a}$ | $21.4^{ab}$ | $294.62^{a}$ | $179.33^{a}$ | $473.95^{a}$ |
| *G. macrocarpum* (Local) | $21.1^{ab}$ | $20.0^{bc}$ | $275.78^{b}$ | $166.60^{b}$ | $442.38^{b}$ |
| *G. mosseae* (Local) | $20.3^{abc}$ | $21.8^{a}$ | $261.67^{c}$ | $156.09^{c}$ | $417.76^{c}$ |
| *S. calospora* (ICRISAT) | $19.5^{cd}$ | $22.3^{a}$ | $231.08^{e}$ | $151.42^{c}$ | $412.76^{c}$ |

Legend as in Table 17

content was present in uninoculated control plants (143.46 ug/pl).

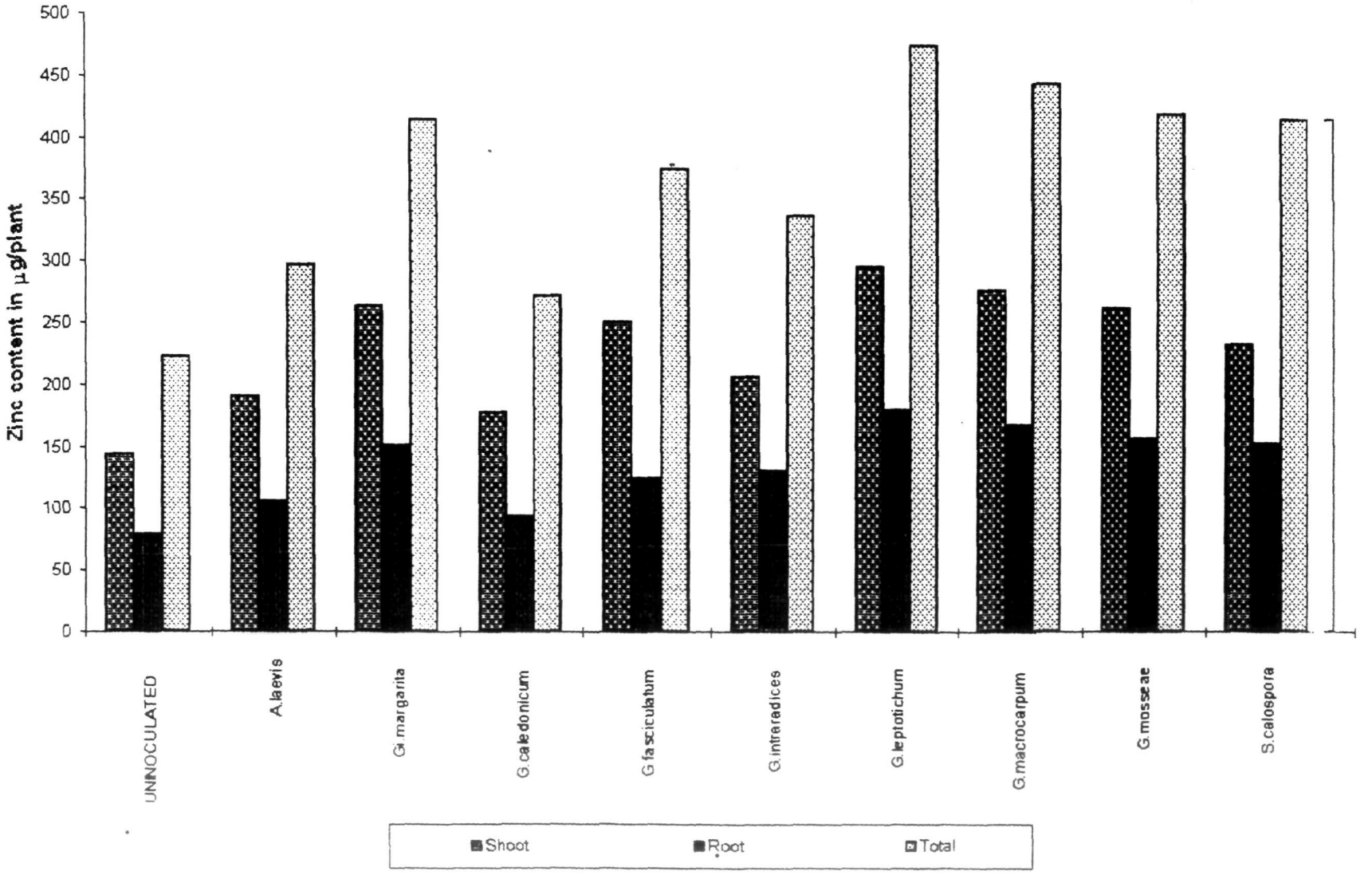
Highest concentration of 21.6 ppm zinc was recorded in plants inoculated with Glomus leptotichum (21.6 ppm) followed by Glomus macrocarpum (21.1 ppm) and Glomus mosseae (20.3 ppm) treated plants. All the three treatments were on par with each other.

**Root zinc**

The plants inoculated with Glomus leptotichum had the highest zinc content (179.33 µg/pl) compared to other treatments. The next best treatments were Glomus macrocarpum (166.60 µg/pl), Glomus mosseae (156.09 µg/pl) and Scutellospora calospora (151.42 ug/pl) inoculation. The control plants had the lowest root zinc content (78.59 µg/pl).

The concentration of zinc between treatments varied and most of the treatments were on par with each other. Highest concentration was found in plants inoculated with Glomus mosseae (21.8 ppm) followed by Glomus leptotichum (21.4 ppm) and Gigaspora margarita (21.3 ppm) inoculated plants. All the three treatments were statistically on par with each other.

Fig : 32. Influence of different VAM fungi on zinc uptake by Tectona grandis



**Total zinc**

The total zinc content was significantly higher in all the inoculated treatments compared to control plants. The highest total zinc content was observed in plants inoculated with Glomus leptotichum (473.95 µg/pl) and Scutellospora calospora (412.75 µg/pl) inoculated plants; the last three treatments being statistically on par with each other. However, all the inoculated treatments showed significantly more zinc content compared to uninoculated control which had the least content (222.0 µg/pl).

### 4.3.7. Influence of VAM fungi on uptake of copper by the host

The effect of various VAM fungi on copper uptake is shown in Table 22. All the inoculated treatments had significantly higher Cu content than the uninoculated control plants.

**Shoot copper**

The shoot copper content in Glomus leptotichum inoculated plants was more (138.54 µg/pl) followed by Glomus macrocarpum (122.75 µg/pl) and Gigaspora margarita (121.26 µg/pl) inoculated plants. The lowest copper content was found in control plants (60.5 µg/pl) (Fig.33).

The shoot copper concentration was more in plants inoculated with Glomus leptotichum (10.6 ppm) followed by Gigaspora margarita (9.2 ppm), Glomus macrocarpum (9.0 ppm)

**Table 22 Influence of different VAM fungi on copper uptake by *Tectona grandis***

| VAM fungi | Copper concentration (ppm) | | Copper content (µg/plant) | | |
|---|---|---|---|---|---|
| | Shoot | Root | Shoot | Root | Total |
| Uninoculated control | 6.2$^{f}$ | 6.7 | 60.50$^{h}$ | 33.97$^{h}$ | 94.47$^{i}$ |
| *A. laevis* (Nedl.) | 7.9$^{de}$ | 9.2 | 87.14$^{f}$ | 55.48$^{f}$ | 142.62$^{f}$ |
| *Gi. margarita* (ICRISAT) | 9.2$^{b}$ | 10.1 | 121.26$^{b}$ | 71.21$^{c}$ | 192.47$^{c}$ |
| *G. caledonicum* (Nedl.) | 7.5$^{e}$ | 8.5 | 77.03$^{g}$ | 47.43$^{g}$ | 124.46$^{h}$ |
| *G. fasciculatum* (Riv.) | 8.8$^{bc}$ | 8.3 | 77.44$^{g}$ | 55.36$^{f}$ | 132.80$^{g}$ |
| *G. intraradices* (Local) | 8.5$^{cd}$ | 10.1 | 95.71$^{e}$ | 65.35$^{d}$ | 161.06$^{e}$ |
| *G. leptotichum* (Local) | 10.6$^{a}$ | 10.4 | 138.54$^{a}$ | 96.63$^{a}$ | 235.17$^{a}$ |
| *G. macrocarpum* (Local) | 9.0$^{bc}$ | 11.6 | 122.76$^{b}$ | 87.15$^{b}$ | 209.91$^{b}$ |
| *G. mosseae* (Local) | 8.4$^{cd}$ | 9.4 | 108.28$^{c}$ | 67.30$^{d}$ | 175.58$^{d}$ |
| *S. calospora* (ICRISAT) | 8.7$^{bc}$ | 8.7 | 104.00$^{d}$ | 59.07$^{e}$ | 163.07$^{e}$ |

Legend as in Table 17

and Glomus fasciculatum (8.8 ppm) inoculated plants; the last three treatments being statistically on par with each other.

**Root copper**

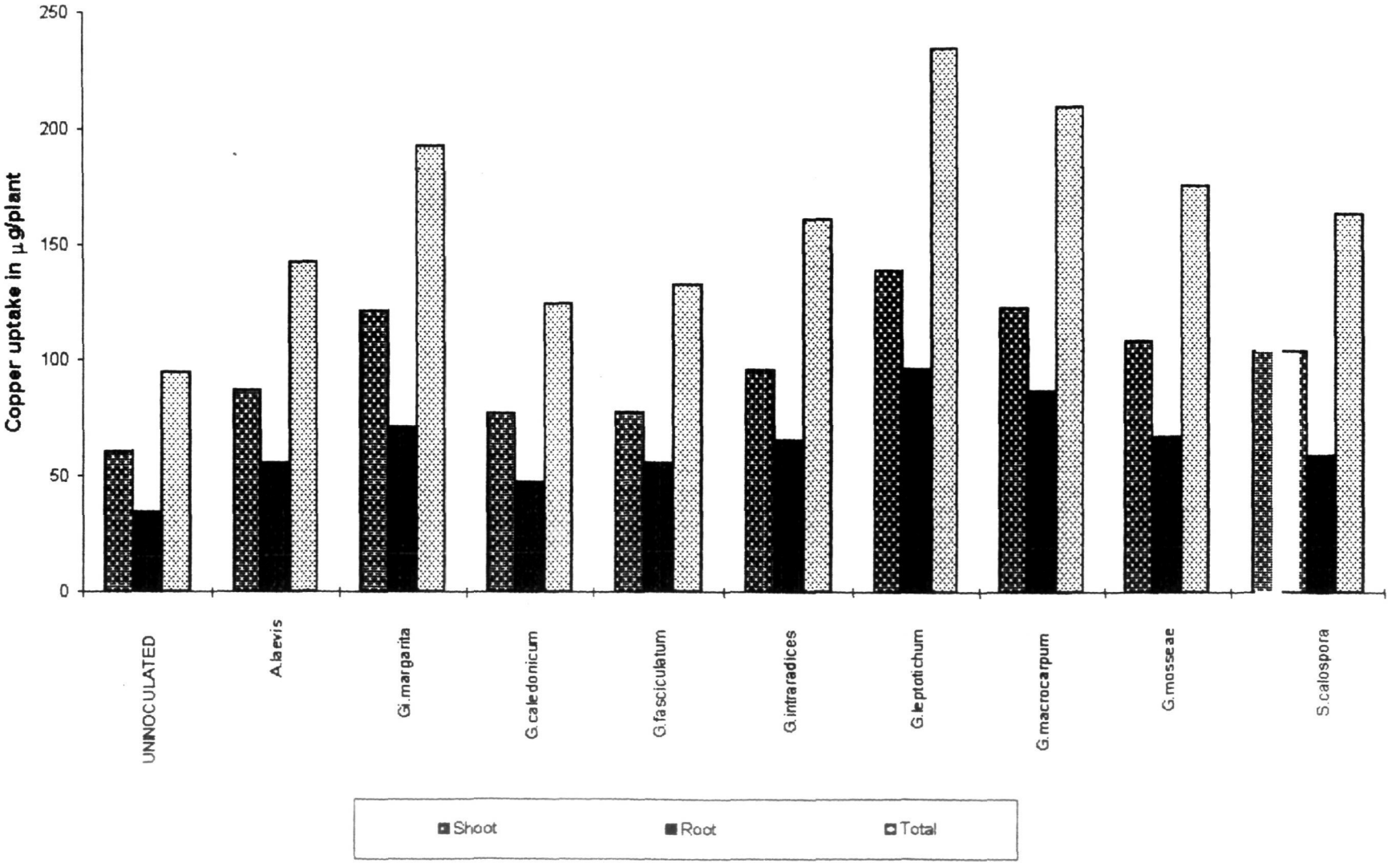
The root copper content was maximum in Glomus leptotichum inoculated plants (96.63 µg/pl). The other treatments which showed better uptake were Glomus macrocarpum (87.15 µg/pl) and Gigaspora margarita (71.21 µg/pl) inoculated plants. The uninoculated plants recorded the minimum copper content (33.97 µg/pl).

The highest root copper concentration was observed in the Glomus leptotichum inoculated plants (11.6 ppm ~~µg/pl~~), followed by Glomus macrocarpum (11.6 ppm), Gigaspora margarita (10.1 ppm) and Glomus intraradices (10.1 ppm) inoculated plants. Control plants had the least copper concentration (6.7 ppm) in the root.

**Total copper**

All the treatments were significantly different from each other, except Glomus intraradices and Scutellospora calospora inoculated treatments. Maximum copper uptake was observed in Glomus leptotichum inoculated plants (235.17 µg/pl) followed by Glomus macrocarpum (209.91 µg/pl), Gigaspora margarita (192.47 µg/pl) and Glomus mosseae (175.58 µg/pl) treated plants. The lowest

Fig : 33. Influence of different VAM fungi on copper uptake by Tectona grandis



copper content was recorded in uninoculated control plants (94.47 ug/pl).

### 4.3.8. Influence of VAM fungi on per cent mycorrhizal root colonization

Highest mycorrhizal colonization was observed in Gigaspora margarita inoculated plants (79.7 %) followed by plants treated with Glomus mosseae (74.2 %), Glomus macrocarpum (73.4 %) and Glomus leptotichum (66.7 %) treated plants. All the inoculated treatments showed significantly higher mycorrhizal root colonization compared to uninoculated control (19.1 %) (Table 23 and Fig.34).

### 4.3.9. Influence of VAM fungi on the number of mycorrhizal spores in the root zone soil

The number of mycorrhizal spores in the root zone soil was significantly more in all the VAM inoculated plants compared to uninoculated plants. Maximum number of mycorrhizal spores (284) was found in the root zone soil of plants inoculated with Glomus leptotichum followed by Glomus macrocarpum (243). The next best treatments were Glomus fasciculatum (201), Acaulospora laevis (192) and Gigaspora margarita (165) inoculated plants. The number of spores were significantly less (52) in the root zone of uninoculated plants (Table 23 and Fig.35).

Table 23 Influence of different VAM fungi on mycorrhizal root colonization, spore numbers in soil and percent aggregation of soil planted with *Tectona grandis*

| VAM fungi | % root colonization | Spore number/ 25 ml soil | % aggregation of soil |
|---|---|---|---|
| Uninoculated control | 19.1$^{g}$ | 52$^{g}$ | 20$^{h}$ |
| *A. laevis* (Nedl.) | 51.1$^{de}$ | 192$^{c}$ | 41$^{f}$ |
| *Gi. margarita* (ICRISAT) | 79.7$^{a}$ | 165$^{e}$ | 53$^{c}$ |
| *G. caledonicum* (Nedl.) | 48.8$^{ef}$ | 178$^{d}$ | 55$^{b}$ |
| *G. fasciculatum* (Riv.) | 52.5$^{d}$ | 201$^{c}$ | 52$^{c}$ |
| *G. intraradices* (Local) | 47.3$^{f}$ | 180$^{d}$ | 32$^{g}$ |
| *G. leptotichum* (Local) | 66.7$^{c}$ | 284$^{a}$ | 56$^{ab}$ |
| *G. macrocarpum* (Local) | 73.4$^{b}$ | 243$^{b}$ | 47$^{d}$ |
| *G. mosseae* (Local) | 74.2$^{b}$ | 161$^{e}$ | 57$^{a}$ |
| *S. calospora* ICRISAT) | 45.8$^{f}$ | 132$^{f}$ | 45$^{e}$ |

Legend as in Table 17

Fig : 34. Influence of different VAM fungi on mycorrhizal root colonization of Tectona grandis

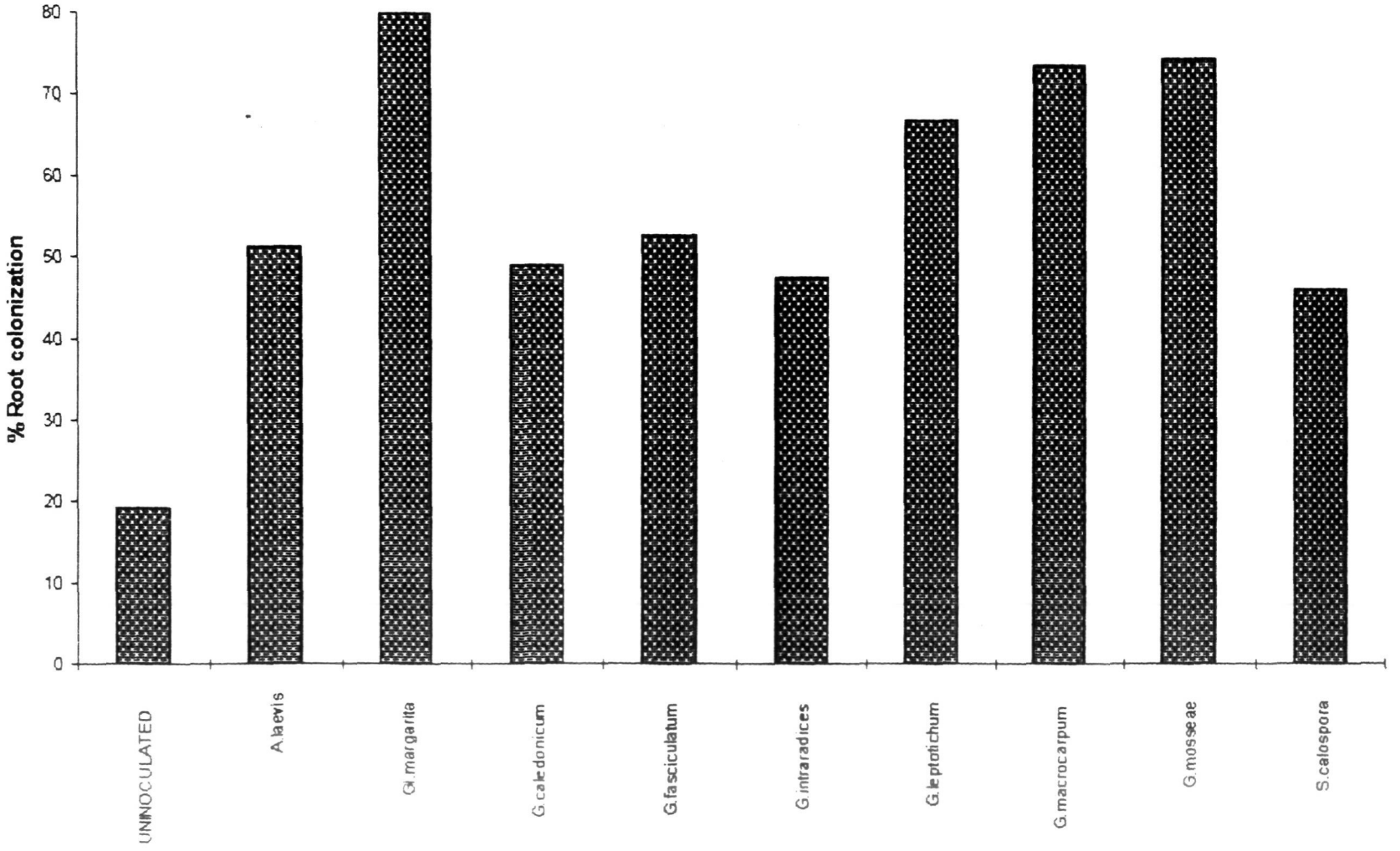


Fig : 35. Influence of different VAM fungi on spore numbers in the root zone soil of Tectona grandis

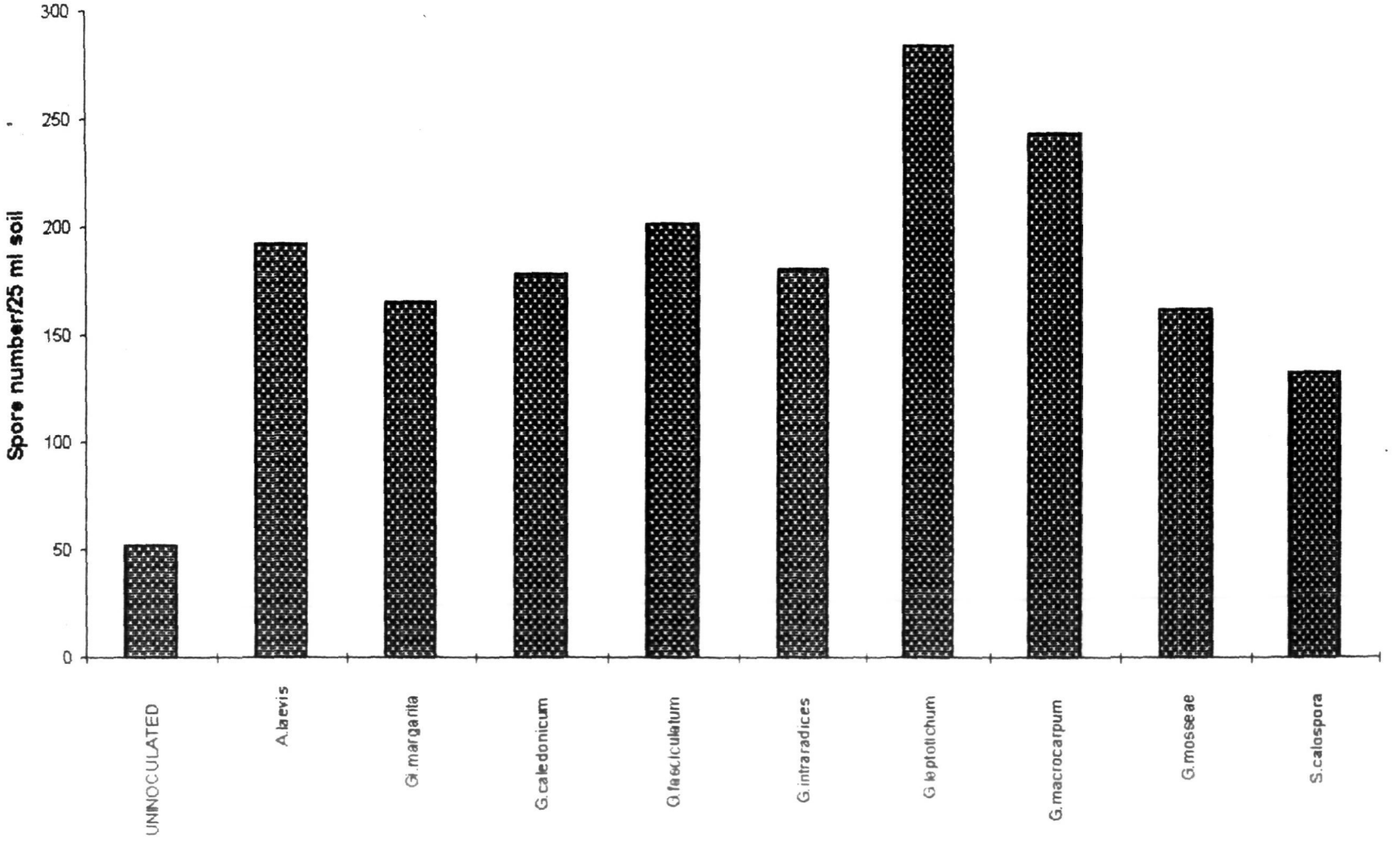
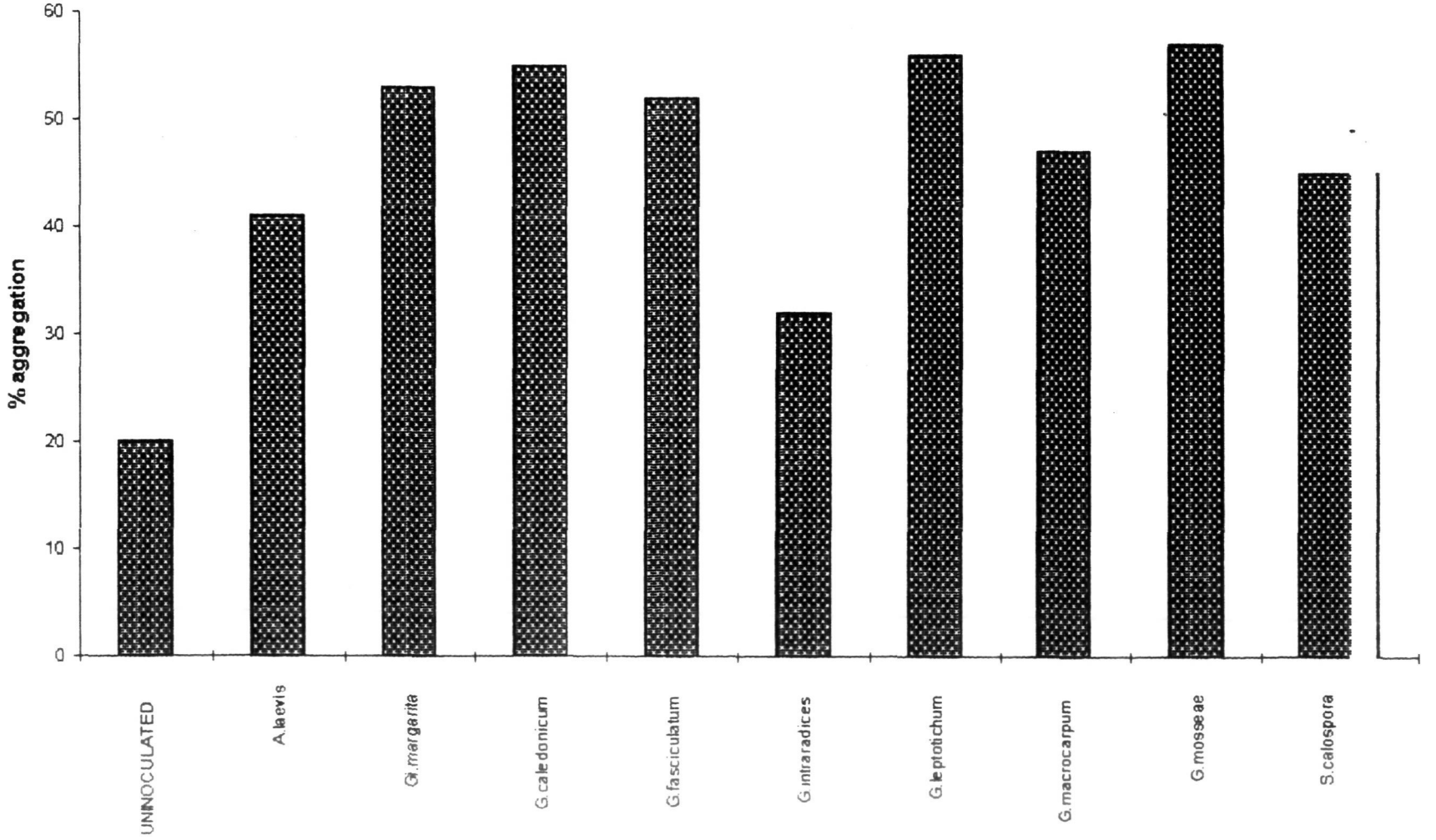


Fig : 36. Influence of different VAM fungi on per cent aggregation of soil planted with Tectona grandis



### 4.3.10. Influence of VAM fungi on per cent aggregation of soil

Table 23 shows the per cent aggregation of soil as influenced by inoculation with different VAM fungi. The highest per cent aggregation was found in soil inoculated with Glomus mosseae (57 %) followed by Glomus leptotichum (56 %), Glomus caledonicum (55 %), Gigaspora margarita (53 %) and Glomus fasciculatum (52 %). The per cent aggregation was lowest in uninoculated control which recorded only 20 % aggregation (Fig.36).

### 4.3.11. Influence of VAM fungi on sturdiness quotient

The effect of VAM fungi on sturdiness quotient of Tectona grandis is shown in Table 24. Glomus macrocarpum inoculated plants had a value of 7.08, followed by plants inoculated with Acaulospora laevis (6.68) and Glomus mosseae (6.62). Uninoculated control plants had a value of 6.27 (Table 24 and Fig.37).

### 4.3.12. Influence of VAM fungi on biovolume index

As shown in Table 24, maximum biovolume index was observed in plants inoculated with Glomus leptotichum (3753.56) followed by those inoculated with Gigaspora margarita (3563.33) and Glomus fasciculatum (3416.09). Uninoculated plants recorded a low biovolume index of 2233.9 (Table 24 and Fig.38).

**Table 24 Influence of different VAM fungi on sturdiness quotient (SQ), biovolume index (VI) and quality index (QI) of *Tectona grandis***

| VAM fungi | Sturdiness quotient (SQ) | Biovolume index (VI) | Quality index (QI) |
|---|---|---|---|
| Uninoculated control | 6.27 | 2233.90 | 1.809 |
| *A. laevis* (Nedl.) | 6.68 | 2462.49 | 2.004 |
| *Gi. margarita* (ICRISAT) | 6.14 | 3563.33 | 2.525 |
| *G. caledonicum* (Nedl.) | 5.53 | 3073.01 | 2.151 |
| *G. fasciculatum* (Riv.) | 5.70 | 3416.09 | 2.551 |
| *G. intraradices* (Local) | 5.81 | 3331.96 | 2.348 |
| *G. leptotichum* (Local) | 5.74 | 3753.56 | 2.989 |
| *G. macrocarpum* (Local) | 7.08 | 3072.69 | 2.474 |
| *G. mosseae* (Local) | 6.62 | 2918.19 | 2.381 |
| *S. calospora* ICRISAT) | 6.54 | 2440.11 | 2.250 |

Legend as in Table 17

Fig : 37. Influence of different VAM fungi on sturdiness quotient of Tectona grandis

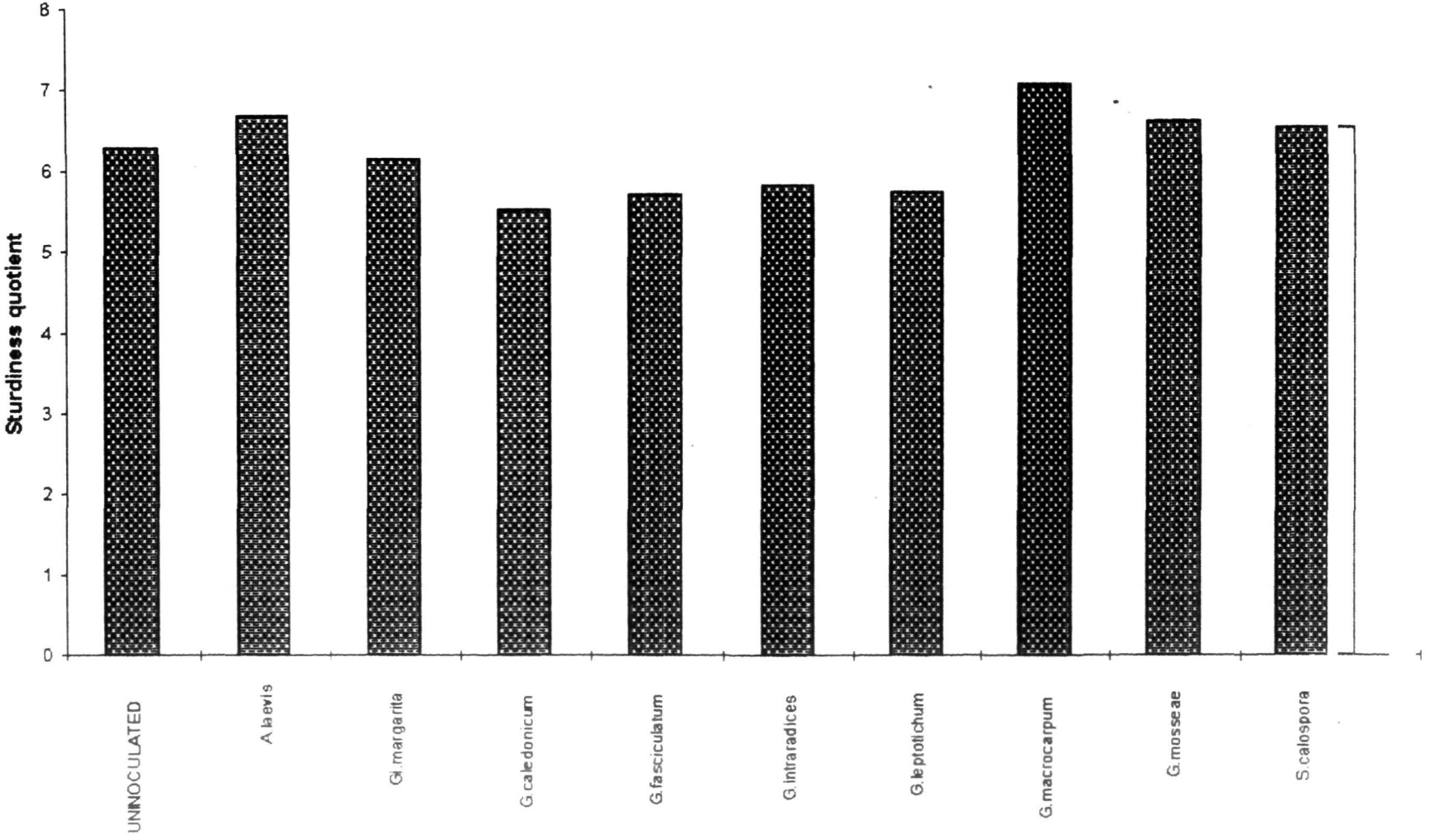
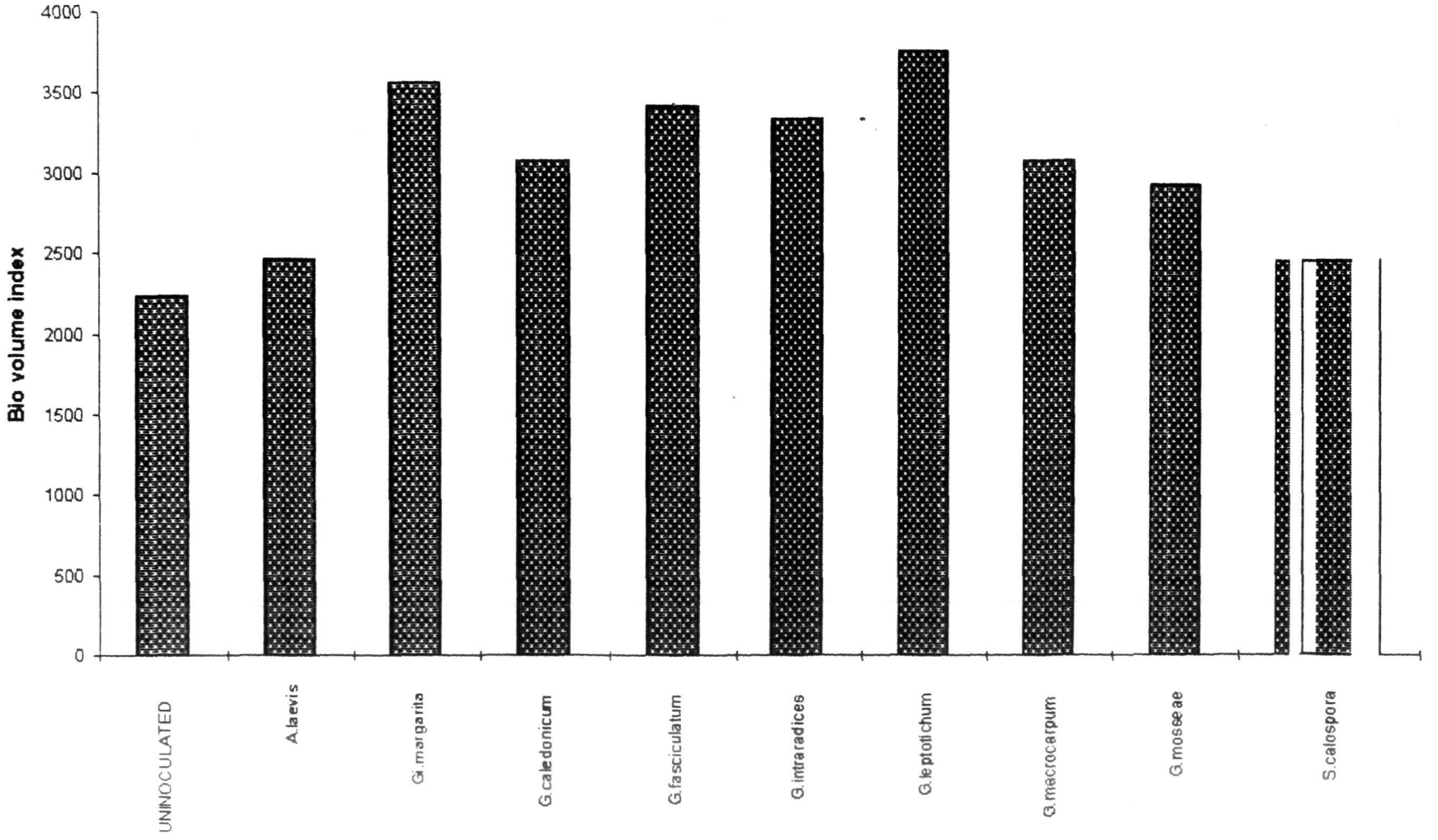


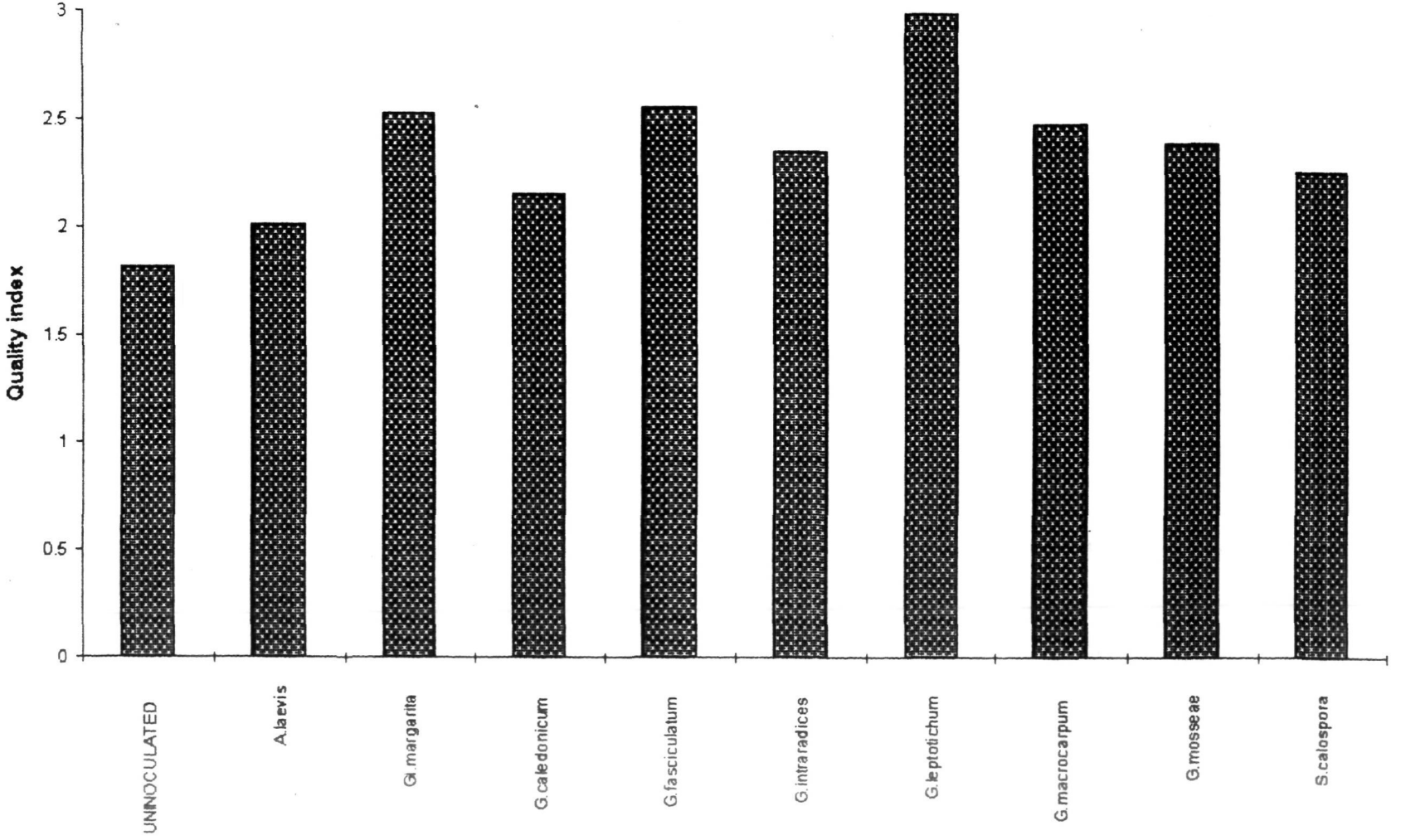
Fig : 38. Influence of different VAM fungi on bio volume index of Tectona grandis



**4.3.13. Influence of VAM fungi on quality index**

Maximum quality index value was observed in plants inoculated with Glomus leptotichum (2.989) followed by those treated with Glomus fasciculatum (2.551) and Gigaspora margarita (2.525). In uninoculated plants the value was 1.809 (Table 24 and Fig.39).

Fig : 39. Influence of different VAM fungi on quality index of Tectona grandis



# DISCUSSION

## DISCUSSION

Interest in VAM association stems largely from the observations that these fungi can increase uptake of mineral nutrients, especially phosphorus in conditions of low P availability (Bagyaraj, 1989; Manjunath et al., 1989), reduce susceptibility of plants to certain pathogens (Giovannetti, 1990), alter the water relations and photosynthetic activity of the plant (Brown and Bethlenfalvay, 1988), increase reproduction (Koide et al., 1988) and improve health and vigour of the seedlings (Reena and Bagyaraj, 1990).

Tropical forest trees commonly form mycorrhiza, Johnston (1949) in his studies found that 80 to 93 species of woody and herbaceous plants representing 33 families form VA mycorrhiza. In another experiment with plants grown in pots, VA mycorrhiza increased seedling growth of 23 of the 28 species from a tropical rain forest region (Janos, 1980). Their prevalence suggests that mycorrhiza may be of great importance for plant growth in mineral poor tropical soils (Bagyaraj, 1989). Although many crop plants are known to benefit because of this association (Jeffries, 1987), little work has been directed towards determining whether these fungi can be manipulated to improve the seedling quality of commercially and socially important hardwood trees (Bagyaraj, 1989). Further, it was believed earlier that the

VAM fungal species did not have any host specificity. But the recent studies have suggested the existence of host preference of the VAM symbionts of crop plants (Estaun et al., 1987; Louis and Lim, 1988; Bagyaraj et al., 1989) and tropical tree species (Reena and Bagyaraj, 1990a). "Preferential association" between a host and a particular VAM fungus may either be beneficial or crucial for plant production. It can be expected that the dominant fungus will infect the germinating plant first; thus the benefit of the VA mycorrhizal association to the plant may depend on whether a highly effective or a rather ineffective fungus is dominating the indigenous fungal community (Sieverding, 1988). Here the efficiency refers to the ability of the fungus to increase plant growth in a phosphate deficient soil and this depends on the ability to form extensive and well distributed hyphae in soil, to form extensive colonization in the root system, and to absorb P and other mineral nutrients from soil. In the present study an attempt was made to select efficient VAM fungi for inoculating three important tropical tree species viz., Acacia holosericea, Albizzia lebbeck and Tectona grandis.

## 5.1. Selection of efficient VAM fungi for Acacia holosericea

The seedlings inoculated with nine different VAM fungi showed varied response. In almost all the parameters

studied, inoculated plants showed significant difference compared to uninoculated control plants.

Inoculated plants grew significantly taller than the uninoculated plants. Plant height was 14.05 per cent more in plants inoculated with Glomus mosseae followed by Scutellospora calospora (11.9 per cent) and Gigaspora margarita (10.5 per cent) inoculated plants. Inoculated plants had significantly more stem girth compared to uninoculated plants. Glomus mosseae inoculated plants had 7.94 per cent more stem diameter compared to uninoculated control plants. Several workers have observed the effects of VAM fungi on plant growth (Abbott et al., 1989; Michelsen and Rosendahl, 1990). Dhillon (1992) suggested that differential VAM fungal responses according to the host species may result in selection pressures which favour certain specific fungus-host species combination.

Plant biomass is an important parameter which directly reflects the efficiency of a particular fungus. Lambert et al., (1979) noticed that one maize line grew better when infected with a mycorrhizal fungus and produced more dry weight at any concentration of P in plant tissue than did non-mycorrhizal plants. In the present study inoculation with VAM fungi resulted in significantly more plant biomass compared to uninoculated plants. The plant biomass was maximum in Glomus mosseae inoculated plants followed by

Scutellospora calospora and Gigaspora margarita inoculated plants. The increase in biomass was 53.82 per cent, 33.35 per cent and 30.2 per cent respectively. Jasper et al., (1989) reported that the dry weight of Acacia concurrens increased when separately inoculated with five different VAM fungi. The increase in shoot dry weight was up to four times the dry weight of uninoculated plants. Further Pope et al., (1983) in their experiment observed a 200 per cent increase in total dry weight of Platanus occidentalis inoculated with Glomus fasciculatum.

Maximum leaf area was observed in plants inoculated with Glomus macrocarpum where the increase was 17.03 per cent more over the control plants followed by Glomus macrocarpum and Glomus caledonicum and Glomus leptotichum. The plants inoculated with these two fungi had 11.67 per cent and 13.53 per cent more leaf area compared to the control plants. Similar observation was made by Kothari et al., (1990) working with maize where the inoculation with VAM fungi did not improve leaf area, however, the shoot dry weight was more than that of uninoculated control plants.

The beneficial effects of VA mycorrhiza on plant growth have often been related to the increase in the uptake of immobile nutrients, especially phosphorus (Manjunath et al., 1989). Mycorrhizal fungi in association with plant roots

increase phosphorus uptake by more thorough exploration of soil, by the external hyphae, beyond the root hair zone (Tinker, 1978). In the present study Glomus mosseae inoculated plants had 107.7 per cent more total phosphorus than the uninoculated control plants. Koide (1991) in his review discussed that the plants having higher biomass also had higher phosphorus content, eventhough Plenchette et al., (1983) in their studies showed that infection did not increase phosphorus uptake at higher phosphorus fertilization, although it did increase growth. The next best fungi Scutellospora calospora and Gigaspora margarita inoculated plants had 105 per cent and 89.97 per cent more total phosphorus content than the uninoculated control plants. Both these fungi also had higher root colonization and shoot biomass. More over, although the plants had higher P content in their shoots and roots, the actual P concentration did not differ significantly between most of the treatments. However, all the treatments had significantly higher phosphorus concentration than the uninoculated control plants. The same observation has also been made for copper and zinc content and concentration of VAM inoculated plants. This may be due to the enhanced uptake of other nutrients by mycorrhizal plants which might have caused a decrease in phosphorus concentration due to internal dilution (Bolan, 1991). More over, Bowen (1973) suggested that, although increased uptake may be a major

part of nutrient response in mycorrhizal association, it is not the only response, i.e., it is possible that a mycorrhizal plant may use its nutrients more efficiently than a non-mycorrhizal plant. This was further supported by Lambert et al. (1979). Thus in the present investigation, eventhough the phosphorus concentration between most of the inoculation treatments were not significantly different. The response of plants were better than the uninoculated control because of the above said reason.

Several workers have observed that the mycorrhizal fungi transport other mineral nutrients, such as zinc, copper, iron, etc. (Lambert et al., 1979; Gildon and Tinker, 1983; Treeby, 1992). The present results indicate that mycorrhizal colonization did enhance the uptake of the nutrients, zinc and copper, compared to uninoculated control. The plants inoculated with Glomus mosseae had more zinc content followed by plants inoculated with Scutellospora calospora and Gigaspora margarita. The increase in zinc content was 81.92 per cent, 72.55 per cent and 66.24 per cent respectively. Similarly the plants inoculated with Glomus mosseae had 81.81 per cent more copper content than the uninoculated control plants. This is in agreement with the observation of Krishna et al., (1984) where in they recorded increased uptake of the

nutrients zinc, copper, manganese and iron in the groundnut plants inoculated with Glomus fasciculatum.

Bolan (1991) suggested that the uptake of P is limited by the rate of diffusion of P in soil solution and not by the ability of the roots to absorb from low concentrations in soil solutions. The increase in phosphorus and other micronutrients by mycorrhizal colonization has been generally associated with a decrease in the distance that phosphate and other ions must diffuse to plant roots. In other words, the root colonization influences the phosphorus and other mineral uptake. In this study, VAM inoculation increased per cent root colonization and number of spores in the root zone soil. The influence of Glomus mosseae was highest on per cent root colonization and spore numbers, although Scutellospora calospora inoculated plants were also on par with the above treatment. Glomus mosseae inoculated plants had mycorrhizal colonization of 89.62 per cent while Scutellospora calospora inoculated plants had 87.18 per cent colonization. The uninoculated plants had much lower per cent mycorrhizal colonization and spore load, even though the soil was not sterilized. Similar observations were also made by Reena and Bagyaraj (1990b) in their studies with Calliandra calothrysus inoculated with 13 different VA mycorrhizal fungi. Nicolson (1972) showed that higher root colonization allows more fungal host contact and exchange of

nutrients, hence better plant growth. More over, the nitrogen transfer was associated with high mycelium density in soil (Harnel et al., 1991) and the pattern of root colonization for various fungal species is influenced by host genome (Boyetchko and Tewari, 1990). So naturally the fungus having higher root colonization will be better adapted and absorb more nutrients and thus better growth.

Soil aggregation is a measure of the amount of extra matricular hyphae, which in turn is related to the efficiency of the fungus (Abbott and Robson, 1985). In the present study the root zone soil of the plants inoculated with Glomus mosseae had 188.8 per cent more soil aggregation than the control. The next best fungi were Glomus caledonicum and Glomus leptotichum wherein the root zone soil of the inoculated plants had 166.67 per cent and 122.2 per cent more aggregation than the control. Thomas et al. (1986) noted that mycorrhizal onion roots significantly increased aggregation of silty clay loam soil, making it more porous compared to the uninoculated soil.

Seedling parameters such as sturdiness quotient, quality index and biovolume index are important indices in judging the quality of the seedlings and these calculations are routinely made by commercial forest growers (Hatchell, 1985; Ritchie, 1985). Since these calculations are based on the height, stem diameter and dry biomass, it very clearly

gives an indication about the quality of seedlings. In the present study the plants inoculated with Glomus mosseae was found to have maximum bio-volume index of 3245.4 and quality index of 0.5777. Higher bio-volume and quality index indicate better quality of the seedlings.

In this study the seedlings inoculated with Glomus mosseae had consistently shown higher root colonization, plant biomass, biovolume index and higher mineral content when compared to the plants inoculated with other fungi, suggesting that clear and specific relationship exists between a particular species of fungus and the plant. Giving weightage to quality index, but not neglecting the other parameters, Glomus mosseae was found to be the best fungus for inoculating Acacia holosericea while the next best fungus was Scutellospora calospora.

## 5.2. Selection of efficient VAM fungi for Albizzia lebbeck

In general, VAM inoculation improved plant growth, plant biomass and mineral content. The mycorrhizal parameters such as per cent root colonization, per cent aggregation and spore load were also higher in inoculated plants and significantly differed between inoculated treatments.

As discussed earlier, the VAM fungi are known to improve plant growth through improved P uptake and other

mechanisms (Manjunath and Habte, 1988; Bagyaraj, 1990; Jeffries, 1987). In the present study Albizzia lebbeck seedling responded well to all the VAM fungal inoculation. At the time of harvest the plant height was 46.51 per cent more in Glomus caledonicum inoculated plants compared to uninoculated control plants, while the next best fungus, Glomus intraradices, inoculated plants showed 33.87 per cent increased plant height than the control plants. Maximum stem girth was observed in Glomus macrocarpum treated plants followed by plants treated with Glomus caledonicum. But the inoculated treatments did not differ significantly from each other. Maronek et al., (1980) reported that Southern Magnolia inoculated with Glomus fasciculatum showed increased plant height. Reena and Bagyaraj (1990) in their studies with Tamarindus indica had shown the differential effect of 13 different VAM fungi on plant height and other parameters and clearly showed the dominance of Gigaspora margarita (ICRISAT) in improving the plant growth among the other 12 VA mycorrhizal fungi under the same growth conditions.

The plant biomass is an important parameter in selecting a fungus for its efficiency. Highest plant biomass was observed in plants inoculated with Glomus macrocarpum where increase in total biomass was 48.20 per cent followed by plants treated with Glomus caledonicum

where the increase was 42.96 per cent than the uninoculated control plants. The same trend was observed for both shoot and root biomass. Similar observations were made by Aggangan and Dela Cruz (1991) while doing experiments with Acacia auriculiformis and Leucaena leucocephala due to VA mycorrhizal inoculation. They observed that the fungus Gigaspora margarita increased the biomass considerably. Cuenca et al (1990) in their studies observed Glomus spp. inoculation resulted in the increased dry matter production of cacao seedlings. Maximum biomass was recorded in plants inoculated with Glomus macrocarpum in the present study. The plants inoculated with Glomus caledonicum, Glomus intraradices and Glomus leptotichum did not differ significantly from plants treated with Glomus macrocarpum. The plants inoculated with Glomus macrocarpum had 14.11 per cent more leaf area than the uninoculated control plants followed by the plants inoculated with Glomus caledonicum and Glomus intraradices. Plants inoculated with these two fungi had 12.99 per cent and 9.68 per cent more leaf area respectively compared to uninoculated control.

It is known that VAM inoculation increases the uptake of phosphorus and other nutrients such as copper and zinc (Jeffries, 1987) and the species of VAM fungi differ in their ability by which they increase nutrient uptake and plant growth (Bagyaraj, 1990). In this study the treatments

which had higher bio-mass also had higher phosphorus, zinc and copper content. Plants inoculated with Glomus macrocarpum had 150 per cent more P, 121.57 per cent more Zn and per cent more copper content than the uninoculated plants. Jalali and Thareja (1980) have shown that inoculating wheat with VAM fungi increases the phosphorus and other mineral contents. Mosse (1973) in her review cited reports indicating that variable responses have been found with reference to zinc content of the host when inoculated with different VAM fungi.

Per cent root colonization is an important measure for assessing the efficiency of a fungus. Sanders and Tinker (1973) observed that the rate of inflow of P into mycorrhizal roots was much higher than the uninoculated plants. They calculated the rate of inflow of P into hyphae to be six times the rate of the root hair. So an increase in absorption of P by mycorrhizal plants could be brought about by increased physical exploration of the soil. In this study, root colonization and per cent aggregation of soil was more in Glomus macrocarpum inoculated plants followed by Glomus leptotichum inoculated plants. The spore number was more in the root zone soil of plants inoculated with Glomus mosseae. However the root zone soil of plants inoculated with Glomus macrocarpum had 3.6 times more number of spores than the uninoculated root zone soil.

The seedling parameters viz. sturdiness quotient, biovolume index and quality index were more in plants inoculated with Glomus macrocarpum reflecting the quality of the forest tree seedlings. Quality index was maximum in plants inoculated with Glomus macrocarpum. The next higher quality index value was seen in plants treated with Glomus leptotichum.

Considering all the parameters and giving more emphasis to quality index, P content, per cent mycorrhizal root colonization and external hyphae in soil reflected by per cent aggregation, the best fungus for inoculating Albizzia lebbeck was Glomus macrocarpum and the next best fungus was Glomus leptotichum.

## 5.3. Selection of efficient VAM fungi for Tectona grandis

Plants inoculated with Glomus leptotichum showed 48.48 per cent more biomass than the uninoculated control, while the plants treated with Glomus macrocarpum and Gigaspora margarita had 44.30 per cent and 36.41 per cent more biomass respectively. However, the plants inoculated with Glomus macrocarpum showed highest plant height than the other treatments. But the stem girth and leaf area was maximum in plants inoculated with Glomus leptotichum. The increase in stem diameter was 22.43 per cent and leaf area was 87.65 per cent more than the control plants. Although the plants

inoculated with Glomus leptotichum did not show any superiority in plant height, the plants inoculated with the same fungus had maximum biomass, stem diameter, P content and per cent root colonization. This difference in height may be attributed to the inherent differences in the growth habit and phenology during the seedling stage (Dasappa, 1990). Further, the plants tend to increase more leaf area rather than height during initial stages. Plants inoculated with VAM fungi were significantly more taller than uninoculated control plants, except the plants inoculated with Scutellospora calospora and Glomus caledonicum.

Highest P content was observed in plants inoculated with Glomus leptotichum. The increase was observed both in shoot and root. The same fungus also resulted in the highest plant biomass and leaf area. This suggests that the plants having higher biomass also had higher P content. The next best fungi were Glomus macrocarpum and Gigaspora margarita; plants inoculated with these fungi had 130.09 per cent and 110.38 per cent more total P content respectively than the uninoculated control plants. Similarly the total zinc content was maximum in plants inoculated with Glomus leptotichum. The increase was 113.49 per cent more than the uninoculated control. The same trend was observed in the total copper content of the plants inoculated with VAM fungi. Glomus leptotichum inoculated plants had 122.20 per

cent more total copper than the uninoculated control plants. Similar observations were observed by other workers in different plants. Gilmore (1971) in his experiments observed that peach plants inoculated with VAM fungi recorded 2 to 3 times more zinc content than the uninoculated control plants.

Bolan et al. (1987) suggested that the increased uptake by the mycorrhizal plants is because of the exploration of the soil volume more thoroughly by mycorrhizal fungi. They also increased the rate of uptake of P from the sources by increasing the diffusion gradient either by a closer approach to the source or by achieving a low concentration of phosphate at the surface.

Regarding the per cent root colonization the plants showed varied response to inoculation. Maximum per cent colonization was observed in plants inoculated with Gigaspora margarita recording 79.7 per cent colonization while the next best fungi, Glomus mosseae and Glomus leptotichum, showed 74.2 per cent and 66.7 per cent colonization. As suggested earlier the pattern of root colonization for various fungal species is influenced by host genome (Boyetchko and Tewari, 1990). However the spore load in the root zone soil of plants inoculated with Glomus leptotichum had highest number of spores than any other treatments. It is a known fact that higher sporulation

allows more root colonization and thus more plant growth (Daft and Nicolson, 1972). Further, the per cent aggregation of soil was maximum in the root zone soil of Glomus mosseae inoculated plants. However it did not differ significantly from Glomus leptotichum inoculated plants. The aggregation in the root zone soil of Glomus leptotichum inoculated plants was 2.8 times more than the uninoculated control. This is because of the capacity of the mycorrhizal fungus to bind the soil particles together efficiently and thereby ensuring the stability of soil structure.

The quality of the seedlings are dependent on its sturdiness. The sturdiness quotient was less in plants inoculated with Glomus leptotichum which suggests better sturdiness of the seedling. Similarly the biovolume index, a measure to calculate the volume of the plant and vigour index which projects the vigour of the seedlings were maximum in the plants inoculated with Glomus leptotichum.

In all the above parameters plants inoculated with Glomus leptotichum had consistently shown more biomass, total P content, number of mycorrhizal spores in the root zone soil and per cent soil aggregation, indicating better proliferation of the fungal mycelium in soil. Further the sturdiness quotient, biovolume index and vigour index suggests that Glomus leptotichum inoculated plants would be better adapted when transplanted in the field site. Based

on this observations the best fungus for inoculating Tectona grandis is Glomus leptotichum. The next best fungus is Glomus macrocarpum.

The present investigation clearly brings out the existence of preferential association between the host and the fungus, and the need to screen and select suitable and efficient VAM fungi for various hosts. The present study brings out that the best fungus for inoculating Acacia holosericea nursery is Glomus mosseae, while for Albizzia lebbeck it is Glomus macrocarpum and for Tectona grandis it is Glomus leptotichum. These three tree species are normally raised in polybags or raised nursery beds and then planted in the field site. The amount of mycorrhizal inoculum required for treating nursery raised plants are extremely low compared to crops sown directly in the field. So it is quite feasible to inoculate the select VAM fungi into the nursery beds or containers used for raising nursery. Further the cost of producing or acquiring the VAM inoculum is very low (Sreenivasa and Bagyaraj, 1990). So the forest nursery growers can follow VAM inoculation as a routine practice to get healthy, better adapted and vigorously growing seedlings which will withstand transplant shock and establish better when planted in the field site.

# SUMMARY

## SUMMARY

The present study was undertaken to screen and select suitable and efficient vesicular arbuscular mycorrhizal fungi for three important forest tree species, viz., Acacia holosericea, Albizzia lebbeck and Tectona grandis. The nine different VAM fungi used in this study were Acaulospora laevis (Nedlands, Australia), Gigaspora margarita (ICRISAT, India), Glomus caledonicum (Nedlands, Australia), Glomus fasciculatum (Riverside, USA), Glomus intraradices (local), Glomus leptotichum (local), Glomus macrocarpum (local), Glomus mosseae (local) and Scutellospora calospora (ICRISAT, India). These cultures maintained in the germplasm bank of the Department of Agricultural Microbiology using Rhodes grass (Chloris guyana) as the host were used for inoculation. The forest tree seedlings were grown in polybags (25 x 15 cm) filled with unsterilized sand : soil : FYM in the ratio of 2:1:0.5. Inoculum at the rate of 12,500 I.P. was added per planting hole before transplanting.

The plant parameters observed were plant height, stem girth, leaf area, plant biomass, shoot and root phosphorus, zinc and copper contents. Mycorrhizal parameters like per cent soil aggregation, which is a measure of external mycorrhizal hyphae in soil, per cent mycorrhizal root colonization and number of mycorrhizal spores were also studied. The three important indices which reflect the

quality of forest tree seedlings viz. sturdiness quotient, volume index (biovolume) and quality index were also computed.

This study brought out the following conclusions:

(1) Existence of preferential association between a particular host and a VAM fungus.

(2) VAM inoculation improves plant growth and uptake of diffusion limited nutrients like phosphorus, zinc and copper.

(3) Mycorrhizal spore count, per cent root colonization and external hyphae are positively correlated with plant growth.

(4) The fungi differ in their ability to improve plant growth.

(5) There is a correlation between per cent root colonization and P content of plants.

Giving importance to quality index, plant biomass and total P content but not neglecting other characteristics, the best fungus for inoculating Acacia holosericea is Glomus mosseae, for Albizzia lebbeck it is Glomus macrocarpum and for Tectona grandis it is Glomus leptotichum.

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# APPENDICES

## APPENDIX - I

### Physical and Chemical Properties of the Substrate

| Sl.No. | Characteristics | Magnitude |
|---|---|---|
| I. a. | Soil texture | Red sandy loam |
| b. | Mechanical analysis | |
| i. | Coarse sand | 45.24 % |
| ii. | Find sand | 23.21 % |
| iii. | Silt | 9.73 % |
| iv. | Clay | 21.80 % |
| II. | Physico-chemical analysis | |
| i. | EC at 25$^{o}$C | 0.62 m.mho/cm |
| ii. | % Organic carbon | 0.48 |
| iii. | pH | 5.6 |
| iv. | Aval. P. (ppm) | 2.72 |
| v. | Aval. Fe (ppm) | 3.51 |
| vi. | Aval. Cu. (ppm) | 0.78 |
| vii. | Aval. Zn. (ppm) | 0.31 |

## APPENDIX - II

### Ruakura plant nutrient solution

### I. Major elements

| | | g/4.5 litres |
|---|---|---|
| Solution A: | $Mg(NO_3)_2.6H_2O$ | 22.25 |
| | $Ca(NO_3)_2.4H_2O$ | 75.50 |
| | $NH_4NO_3$ | 38.40 |
| | $KNO_3$ | 10.25 |
| Solution B: | Nacl | 1.50 |
| | * $KH_2PO_4$ | 12.00 |
| | * $K_2HPO_4$ | 7.40 |
| | $K_2SO_4$ | 29.783 |
| | $Na_2SO_4$ | 2.691 |

### II Minor nutrients

| | | | g/500 ml | |
|---|---|---|---|---|
| a) | Boron | $H_3BO_3$ | 6.3831 | Dissolve in 500 ml water |
| b) | Manganese | $MnCl_2.4H_2O$ | 20.2647 | Dissolve in 20.26 ml of 0.1 N Hcl make up to 500 ml with water |
| c) | Zinc | $ZnCl_2$ | 5.864 | Dissolve in 117.3 ml of 0.1 N Hcl and make up to 500 ml with water |

| | | | | |
|---|---|---|---|---|
| d) | Copper | $CuCl_2.2H_2O$ | 1.2073 | Dissolve in 12.07 ml of 0.1 N Hcl and make up to 500 ml with water |
| e) | Molybdenum | $(NH_4)_6Mo_7O_2.4H_2O$ | 0.2070 | Dissolve in 500 ml of water |
| f) | Cobalt | $CoCl_2.H_2O$ | 0.4088 | Dissolve in 500 ml of water |

Take 5 ml of each of a, b, c, d, e and f in a bottle and dilute to 2.5 litres with water.

**III iron** : Dissolve 13.5 g ferric citrate ($FeC_6H_5O_7.5H_2O$) in 199 ml of IN Hcl and dilute to 2.5 litres with water.

**To make up the solute**

Add 300 ml of A, 300 ml of B, 150 ml of II (diluted) and 22.5 ml of ferric citrate and make up the volume to 4.5 litres with water.

* For mycorrhizal plants $K_2HPO_4$ and $KH_2PO_4$ are not added while preparation.

## APPENDIX - III

### Vanadomolybdic acid

Solution A: Dissolve 258 of ammonium molybdate in 400 ml distilled water.

Solution B: Dissolve 1.25 g of ammonium metavanadate in 300 ml boiling water. Cool the solution B and add 250 ml of concentrated $HNO_3$ and again cool the solution to room temperature.

Pour solution A into solution B and dilute the mixture to 1 litre.